Synthesis of Fluorinated Amino Acids and Their Derivatives in Flow

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Stella Vukelić

From Zagreb, Croatia

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I hereby declare that this PhD thesis was prepared autonomously. Content, quotes or images previously published by third parties are indicated by referring to the original work.
The work presented in this dissertation resulted in the following publications:

- Stella Vukelić, Kerry Gilmore, Beate Koksch, Peter H. Seeberger, "A Sustainable Semi-Continuous Flow Synthesis of Hydantoins", *Eur. J. Of Chem. A*, **2016**, DOI: 10.1002/chem.201602609.
- Stella Vukelić, Johann Moschner, Susanne Huhmann, Rita Fernandes, Allison Ann Berger, Beate Koksch, "Synthesis of side-chain fluorinated amino acids and their effect on biophysical properties of peptides and proteins in "Modern Synthetic Routes and Reactivity of Fluorinated Compounds", H. Groult, F. Leroux & A. Tressaud, Eds., *Elsevier*, in press.
- Stella Vukelić, Dmitry B. Ushakov, Kerry Gilmore, Beate Koksch, Peter H. Seeberger, "Flow Synthesis of Fluorinated Amino Acids", *Eur. J. Org. Chem*. **2015**, 14, 3036-3039.

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To my grandad

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The true sign of intelligence
is not knowledge but imagination
Albert Einstein

List of Publications

Scientific Publications and Book Chapters

- Stella Vukelić, Kerry Gilmore, Beate Koksch, Peter H. Seeberger, "A Sustainable Semi-Continuous Flow Synthesis of Hydantoins", *Eur. J. Of Chem. A*, 2016, DOI: 10.1002/chem.201602609.

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- Stella Vukelić, Dmitry B. Ushakov, Kerry Gilmore, Beate Koksch, Peter H. Seeberger, "Flow Synthesis of Fluorinated Amino Acids", *Eur. J. Org. Chem.* **2015**, *14*, 3036-3039.
  - Featured in an interview by ChemistryViews: http://www.chemistryviews.org/details/ezine/7956531/FlowSynthesis_of_Fluorinated_Amino_Acids.html

- Kerry Gilmore, Stella Vukelić, D. Tyler McQuade, Beate Koksch, Peter H. Seeberger, **"**Continuous Reductions and Reductive Aminations Using Solid $NaBH_4$", *Org. Process Res. Dev.* **2014**, *18*, 1771-1776.
  - Featured in C&E News (cen.acs.org) 24.11.2014 "Sodium Borohydride Goes With The Flow", November 24, 2014 (92(47)).

- Jyotirmoy Maity, Ulla I. M. Gerling, Stella Vukelić, Andreas Schäfer, Beate Koksch, "Proline-glutamate chimera's side chain conformation directs the type of β-hairpin structure", *Amino Acids* **2014**, *46*, 177-186.

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Table of Contents

List of Abbreviations

PET	Positron Emission Tomography
NMR	Nuclear Magnetic Resonance
SAR	Structure And Reactivity
Boc	*tert*-Butyloxycarbonyl group
CbZ	Carboxybenzyl
TMSCl	Trimethylsilyl chloride
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBT	Hydroxybenzotriazole
CDI	1,1'-Carbonyldiimidazole
DMAP	4-Dimethylamino pyridine
Et_3N	Triethylamine
Boc_2O	Di-tert-butyl dicarbonate
HFA $2H_2O$	Hexafluoroacetone trihydrate
DMSO	Dimethylsulfoxide
DCM	Dichloromethane
DBU-HFA salt	2,3,4,6,7,8,9,10-octahydro-1H-pyrimido[1,2-a]azepin-5-ium 1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-olate
Dap	2,3-diamino propionic acid
TBAC	Tetrabutylammonium chloride
t-BuOK	Potassium tert-butoxide
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EDA	Ethyldiazo acetate
THF	Tetrahydrofurane
TLC	Thin Layer Chromatography
$TMSCF_3$	Trimethyl(trifluoromethyl)silane
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
DMA	Dimethylacetamide
MeCN	Acetonitrile
$[Ru(bpy)_3]Cl_2$	Tris(bipyridine)ruthenium(II) chloride
Chloranil	Tetrachloro-1,4-benzoquinone
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
NaOAc	Sodium acetate
MeOH	Methanol
Deoxofluor	Bis(2-methoxyethyl)aminosulfur trifluoride
DAST	Diethylaminosulfur trifluoride
TPP	5,10,15,20-Tetraphenylporphin
TBAF	Tetrabutylammonium fluoride
TMSCN	Trimethylsilyl cyanide
DEA	Diethanol amine
2-MeTHF	2-methyltetrahydrofuran
TFA	Trifluoroacetic acid
$Zn(OTf)_2$	Zink (II) triflate
TBAT	Tetrabutylammonium difluorotriphenylsilicate
$Cu(OAc)_2$	copper (II) acetate
TBHP	tert-Butyl hydroperoxide
BOX	(4S,4'S,5R,5'R)-2,2'-(propane-2,2-diyl)bis(4,5-diphenyl-4,5-dihydrooxazole)
Pa	picolinamido group

$[Ru(bpy)_3]$ $(PF_6)_2$	Tris(2,2′-bipyridine)ruthenium(II) hexafluorophosphate
DTBP	Di-tert-butyl peroxide
$[Co(dmgH)_2pyCl]$,	(dmgH = dimethylglyoxime and py = pyridine)
PMP	*p*-metoxyphenyl
AgOTf	Silver trifluoromethanesulfonate
$Mn(OAc)_3$ $2H_2O$	Manganese (III) acetate dehydrate
LED lamp	Light emitting diode
DEAE	Diethylaminoethyl cellulose

Summary

The incorporation of fluorine or fluorinated moieties into organic molecules has influenced the development of various fields including material science, diagnostics, Positron Emission Tomography (radiotracers labeled with ^{18}F nuclei), ^{19}F NMR-based biological studies, synthetic organic chemistry (synthesis of fluorinated organic molecules as well as fluorinated amino acids), structure and reactivity studies of fluorinated proteins, as well as ^{19}F magnetic resonance imaging (MRI).

One application of interest is the replacement of natural amino acids within peptide/protein models by fluorinated versions, as the unique properties of fluorine influence the peptide's/protein's properties and secondary structure. In some cases, the presence of fluorine can increase the catabolic or thermal stability of the peptide and alter its folding pattern. Despite the fact that these scaffolds show great potential for manipulation – facilitating investigation and the control of complex biochemical processes – investigations are hindered due to limited access to fluorinated amino acids. The currently available synthetic methods suffer from multiple synthetic steps, often poor yields, toxic reagents, purifications, expensive starting materials, and protecting group manipulations.

To facilitate access to fluorinated amino acids, a convenient, two-step semi-continuous process was developed. By combining a photooxidative cyanation with an acid-mediated hydrolysis, my process allows for fluorinated amino acids to be accessed directly from the broad pool of primary, unprotected amines, without the need for isolation of any intermediate. During the optimization of the photooxidative cyanation process, it was shown that oxygen gas can be replaced by air without compromising the yield, and that a greener solvent (2-MeTHF) can be used as an alternative to THF. The semi-continuous process exploits two major benefits of flow chemistry – efficient photochemistry, used to synthesize amino nitriles, and use of a hazardous reagent (30% HCl) above its boiling to hydrolyze amino nitriles to amino acids. The process requires no purification and can be easily scaled.

Modular nature of the developed process was utilized to set the stage for the expansion of this methodology to access enantiopure fluorinated amino acids. To pursue these target structures, enzymatic transformation of fluorinated amino nitriles to amino acids in batch was optimized as part of a collaborative effort with Almac®. A packed-bed reactor was designed to adjust this process to flow conditions and preliminary tests to determine the product formation were run.

The modular flow approach to access fluorinated amino acids was divergently expanded to the synthesis of hydantoins. Branching from the synthetically valuable α-amino nitrile, a biphasic carboxylation/rearrangement was realized to generate a range of hydantoins – a heterocycle found in a number of biologically relevant arenas. In this process, the higher surface-to-volume ratio in gas-liquid

reactions under flow conditions facilitates the employment of CO_2, which was trapped by the crude amino nitriles to form hydantoins. A mixed solvent system was used for the addition of CO_2 in order to combine the photooxidative cyanation reaction with the carboxylation as well as to increase the solubility of CO_2. The semi-continuous process requires no purification and uses green reagents and solvents to deliver hydantoin scaffolds starting from the amines.

This thesis also describes my efforts towards developing a method for the direct incorporation of a CF_3 group into the α-position of protected amino acids. This is the first example of the direct α-trifluoromethylation of an amino acid and presents facile access to another group of fluorinated amino acids.

Zusammenfassung

Der Einbau von Fluoratomen oder fluorierten Gruppen in organische Moleküle hat die Entwicklung von verschiedenen Gebieten, einschließlich der Materialwissenschaft, Diagnostik, Positronenemissionstomographie (Radiotracer mit ^{18}F Kernen gekennzeichnet), ^{19}F NMR-basierte biologische Studien, synthetischen organischen Chemie (Synthese von fluorierten organischen Molekülen sowie fluorierte Aminosäuren), Struktur und Reaktivität Studien fluorierter Proteine sowie ^{19}F Magnetresonanztomographie (MRI), beeinflusst.

Eine interessante Anwendung ist der Austausch von natürlichen Aminosäuren in Peptiden/Proteinen mit fluorierten Aminosäauren. Die einzigartigen Eigenschaften von Fluor beeinflussen die Eigenschaften des Peptids/Proteins und dessen Sekundärstruktur. In einigen Fällen kann das Vorhandensein von Fluor die katabolische oder thermische Stabilität des Peptids erhöhen und dessen Faltungsmuster beeinflussen. Obwohl diese Verbindungen ein großes Potenzial für die Untersuchung und Kontrolle komplexer biochemischer Prozesse haben, sind solche Studien aufgrund der begrenzten Verfügbarkeit von fluorierten Aminosäuren limitert. Die derzeit verfügbaren Synthesemethoden benötgen meist mehreren Syntheseschritte, haben häufig schlechte Ausbeuten und benötigen toxische Reagenzien, aufwändige Aufreinigungsmethoden, teure Ausgangsmaterialien sowie Schutzgruppenchemie.

Um den Zugang zu fluorierte Aminosäuren zu erleichtern wurde ein bequemer, zweistufiger halbkontinuierlicher Prozess entwickelt. Durch die Kombination einer photooxidative Cyanierung mit einer sauren Hydrolyse ermöglicht dieser Prozess direkten Zugang zu fluorierte Aminosäuren aus dem breiten Pool an primären, ungeschützten Aminen ohne die Isolierung von Zwischenprodukten. Bei der Optimierung der photooxidative Cyanierung wurde gezeigt, dass gasförmiger Sauerstoff durch Luft ersetzt werden kann ohne die Ausbeute zu beeinflussen. Weiters konnte ein grünes Lösungsmittel (2-MeTHF) als Alternative zu THF eingesetzt werden. Der halbkontinuierliche Prozess nutzt zwei große Vorteile Flow Chemie: Zum einen wird durch dieses Technologie die Effizienz der photochemischen Aminotrilisyntheses gesteigert und zum anderen konnte die nachfolgende Hydrolyse durch Verwendung eines gefährlichen Reagenz (30% HCl) oberhalb seines Siedepunkts intensiviert werden. Der Prozess benötigt keine Aufreinigungsschritte und kann leicht skaliert werden.

Die modulare Natur dieses Prozess wurde verwendet um diese Methode zur Herstellung von enantiomerenreinen fluorierten Aminosäuren zu Ermöglichen. Um diese Zielstrukturen zu synthetizieren wurde die enzymatische Umwandlung von fluorierten Aminonitrilen zu Aminosäuren im Batch in kollaboration mit Almac® optimiert. Für eine kontinuirliche Anwendung wurde ein Festbettreaktor enwickelt welcher in ersten Vorversuchen vielversprechende Ergebnisse erzielte.

Der modulare Ansatz, der Zugriff zu fluorierten Aminosäuren bietet wurde auf eine divergente Synthese von Hydantoinen erweitert. Dazu wurden die synthetisch wertvollen α-Aminonitrile mit einer zweiphasigen Carboxylierung/Umlagerungsreaktion gekoppelt um eine Palette der biologisch relevanten Heterocyclen zu erzeugen. In diesem Prozess wird das höhere Oberflächen-zu-Volumen-Verhältnis bei Gas-Flüssig-Reaktionen in Durchflussreaktoren ausgenutzt. Dies ermöglicht die effiziente Verwendung von CO_2, um Hydantoine von Aminonitrilen zu synthetisieren. Hierbei wurde ein Lösungsmittelgemisch für die CO_2-Zugabe benutzt, um die photooxidative Cyanierungsreaktion mit der Carboxylierung zu verbinden. Das verwendete Lösungsmittelgemisch bietet auch eine erhöhte CO_2 Löslichkeit in der Reaktionslösung. Der semi-kontinuierlichen Prozess benötigt keine Aufreinung und verwendet grüne Reagenzien und Lösungsmittel um Aminen in Hydantoine umzuwandeln.

Diese Dissertation beschreibt auch meine Bemühungen um einen Prozess zum direkten Einbau einer CF_3-Gruppe in der α-Position von geschützten Aminosäuren zu entwickeln. Dies ist das erste Beispiel für die direkte α-Trifluormethylierung einer Aminosäure und stellt einen einfachen Zugang zu einer anderen Gruppe von fluorierten Aminosäuren dar.

1. General Significance of Fluorination of Organic Molecules

The importance of fluorinated compounds in the development of various research fields is ever-growing. These unique, yet interconnected research fields involve material chemistry,[1-4] diagnostics, (PET, radiotracers labeled with ^{18}F nuclei)[5-6] ^{19}F NMR biological studies,[7-8] synthetic organic chemistry (synthesis of fluorinated organic molecules as well as fluorinated amino acids),[9-12] SAR studies of fluorinated proteins[13-16] and ^{19}F magnetic resonance imaging (MRI).[17]

Research fields where fluorination made the biggest impact are agrochemistry[18-19] and medicinal chemistry.[20-23] The impact of fluorine and fluorinated groups on agrochemicals and pharmaceuticals arises from the strength of the C–F bond, being the strongest bond in organic chemistry and fluorine's size, exchanging F for H yields minimal steric perturbations and leads to stable derivatives.[24] Perfluorination (*e.g.* XCF_3) usually increases lipophilicity and thus bioavailability of molecules passing cellular membranes[25] and site specific incorporation of a fluorine atom or a fluorinated group can be used to protect against or suppress *in vivo* metabolism *e.g.* P450 oxidations.[25] In addition the polarity of the C–F bond,[26-27] and associated dipoles can influence conformational changes relative to the hydrogen analogue and can lead to improved target binding. Fluorine's electron withdrawing effect can be used for adjusting the acidity through H-bonding of adjacent protic functional groups, *e.g.* OH, NRH, CO_2H, *etc*.[19]

Consequently, the introduction of a fluorine atom into a molecular scaffold can have diverse impacts which help to promote development of lead compounds and their performance value. Despite the certain degree of predictability in designing biologically active fluorinated compounds, many derivatives still need to be synthesized in a trial and error process until the molecule with the desired properties is obtained.[28]

1.1 Fluorinated Amino Acids and Their Significance

Since the first report of a fluorinated amino acid,[29] it was hypothesized that fluorine's properties might be used to improve natural peptides and proteins, and increase their potential as active pharmaceuticals.[15] The introduction of fluorinated aromatic amino acids into proteins has proven beneficial in therapeutic proteins and vaccine studies by increasing their shelf life.[30] Incorporation of fluorinated amino acids into peptide-based vaccines has also improved their catabolic stability which was remarkable because of the low bioavailability of the antigenic peptides at the target position.[31] Moreover, fluorinated amino acids can also be used to differentiate the role of aromatic acids in peripheral membrane protein and integral membrane protein interactions due to the change in the polarity of the fluorinated aromatic ring with respect to the native variant, and it can destabilize

cation-π interactions.[32] An alternative application of these fluorinated analogues is to study protein structure and function. In the past, protein engineering was limited to naturally occurring amino acids. However, increased availability of fluorinated derivatives of amino acids has lead to their increased use in in protein engineering and functional studies.[31, 33]

There are two ways to incorporate fluorinated amino acids into proteins: residue specific and site specific. The method of incorporation is chosen based on the nature of the study and the purpose of the peptide synthesis. Site specific incorporation is used to study the conformational changes of peptides and to detect protein-ligand interaction by ^{19}F NMR spectroscopy.[34] This is a powerful tool for understanding protein structure, stability and function. Incorporation of fluorine can affect protein thermal stability,[35-40] binding within the peptide,[36, 39-40] the kinetics of forming the secondary structure[15] and folding of the peptide.[41-43] Fluorinated amino acids also alter the enzymatic activity and help in understanding the mechanistic processes.[44] Residue specific incorporation of unnatural amino acids allows for tests of proteomic and genetic code hypotheses. If the organisms succeed in adapting to unuatural amino acids incorporated, this might lead to new genetic codes and possible development of organisms which could survive solely on fluorinated amino acids. It also enables labelling of proteins, elucidation of biochemical mechanisms and development of biomaterials.[45] As mentioned in the beginning of this section, fluorinated amino acids can be incorporated into the peptides globally or site specifically and this can be performed by solid phase peptide synthesis, chemoselective peptide ligation,[46] non-natural protein expression[47] as well as protein semi-synthesis. However, the challenge of non-natural peptide and protein synthesis is mostly hindered by the availability of suitable fluorinated building blocks for solid phase peptide synthesis or their compatibility with the protein expression machinery.[15]

1.2 Fluorine in Natural Products

Around 5000 different natural products containing bromine, iodine and chlorine are known.[48] On the contrary, fluorine has been identifed in only 13 secondary metabolites eight of which are ω-homologues of long chain fatty acids found as co-metabolites in the seeds of the same plant. Thus only 6 discrete fluorinated natural products have been isolated if the fatty acid homologues are omitted.[49] The first isolated fluorinated organic compound was fluoroacetate, a metabolite of the Southern African plant *Dichapetalum cymosum*.[49] In 1986 the last fluorinated structurally novel, natural product 4-fluorothreonine was isolated from the bacterium *Streptomyces cattleya*.[50] If we take fluorine's chemical properties into account, this scarcity of fluorine in natural compounds is more understandable when compared to chlorine, bromine and iodine. Most of Earth's fluorine is 'trapped' in the form of the insoluble salts fluorspar (CaF_2) and cryolite (Na_3AlF_6), which limits its accessability for biological systems.[25] For example sea water contains 1.3 p.p.m. fluoride and 19,000 p.p.m.

chloride, which may help to explain why fluorine's biochemistry hasn't made much progress.[51] Nature incorporates halogen atoms enzymatically using three possible enzymatic mechanisms. Enzymatic halogenation processes usually proced through a hypohalous intermediate, a species which is produced by vanadium dependent halogenases and H_2O_2. The oxidation potential of fluorine is 3.06 V, the highest in the halogen group (vs. −1.36 V (Cl), −1.07 V (Br) and −0.54 V (I)) which doesn't allow for its participation in this reaction. The second pathway of enzymatic incorporation of halogens includes a nucleophilic opening of epoxide intermediates with halides; however, fluorine has an extraordinarily high hydration energy [117 kcal/mol vs. 84 kcal/mol (Cl), 78 kcal/mol (Br) and 68 kcal/mol (I)],[52] it is therefore a poor nucleophile in an aqueous biological environment. The least common pathway for biological halogenation involves radicals such as the biosynthesis of trichloromethylcontaining barbamide.[53] Fluorine could undergo a similar mechanism but its radical is difficult to generate and its high reactivity would reduce regio/stereoselectivity of the fluorination. Esentially, as demonstrated by O' Hagan and coworkers, nature synthesizes the known 13 fluorinated organic products from the same precursor 5′-fluoro-5′-deoxyadenosine produced by fluorinase (Figure 1.1)[51] and all of these compounds are structural derivatives of carboxylic acids.

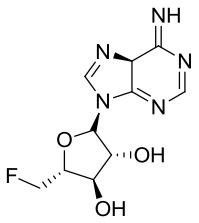


Figure 1.1 5'-fluoro-5'-deoxyadenosine.

Taking into account that C−F bond is one of the strongest chemical bonds, it can be concluded that its formation/cleavage in a biological manner requires highly activated intermediates which are difficult to generate under biological conditions.[26] Due to the unique properties of organic molecules and in order to expand the available pool of fluorinated organic compounds, methods for overcoming these synthetic hurdles are highly sought after.

Therefore, in the next chapter synthetic strategies commonly employed to access fluorinated amino acids and an overview of the existing methods with their advantages and drawbacks will be discussed.

Bibliography

[1] Y. Liang, D. Feng, Y. Wu, S.-T. Tsai, G. Li, C. Ray, L. Yu, *Journal of the American Chemical Society* **2009**, *131*, 7792.
[2] H. J. Son, W. Wang, T. Xu, Y. Liang, Y. Wu, G. Li, L. Yu, *Journal of the American Chemical Society* **2011**, *133*, 1885.
[3] A. C. Stuart, J. R. Tumbleston, H. Zhou, W. Li, S. Liu, H. Ade, W. You, *Journal of the American Chemical Society* **2013**, *135*, 1806.
[4] J. Heier, Free University of Berlin (Berlin), **2010**.
[5] A. F. Brooks, J. J. Topczewski, N. Ichiishi, M. S. Sanford, P. J. H. Scott, *Chemical Science* **2014**, *5*, 4545.
[6] S. L. Lisheng Cai, Victor W. Pike, *European Journal of Organic Chemistry* **2008**, *2008*, 2853.
[7] H. Chen, S. Viel, F. Ziarelli, L. Peng, *Chemical Society Reviews* **2013**, *42*, 7971.
[8] E. N. G. Marsh, Y. Suzuki, *ACS Chemical Biology* **2014**, *9*, 1242.
[9] C. D. C. R. Smits, K. Burger, B. Koksch, *Chemical Society Reviews* **2008**, *37*, 1727.
[10] X.-L. Qiu, F.-L. Qing, *European Journal of Organic Chemistry* **2011**, *2011*, 3261.
[11] K. Mikami, S. Fustero, M. Sánchez-Roselló, J. L. Aceña, V. Soloshonok, A. Sorochinsky, *Synthesis* **2011**, *2011*, 3045.
[12] J. L. Aceña, A. E. Sorochinsky, V. A. Soloshonok, *Synthesis* **2012**, *44*, 1591.
[13] N. C. Yoder, K. Kumar, *Chemical Society Reviews* **2002**, *31*, 335.
[14] C. Jäckel, B. Koksch, *European Journal of Organic Chemistry* **2005**, *2005*, 4483.
[15] M. Salwiczek, E. K. Nyakatura, U. I. M. Gerling, S. Ye, B. Koksch, *Chemical Society Reviews* **2012**, *41*, 2135.
[16] E. N. G. Marsh, *Accounts of Chemical Research* **2014**, *47*, 2878.
[17] I. Tirotta, V. Dichiarante, C. Pigliacelli, G. Cavallo, G. Terraneo, F. B. Bombelli, P. Metrangolo, G. Resnati, *Chemical Reviews* **2015**, *115*, 1106.
[18] P. Jeschke, *ChemBioChem* **2004**, *5*, 570.
[19] P. Jeschke, *Pest Management Science* **2010**, *66*, 10.
[20] C. Isanbor, D. O'Hagan, *Journal of Fluorine Chemistry* **2006**, *127*, 303.
[21] J.-P. Bégué, D. Bonnet-Delpon, *Journal of Fluorine Chemistry* **2006**, *127*, 992.
[22] K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881.
[23] D. O'Hagan, *Journal of Fluorine Chemistry* **2010**, *131*, 1071.
[24] T. Fujiwara, D. O'Hagan, *Journal of Fluorine Chemistry* **2014**, *167*, 16.
[25] J. Wang, M. Sánchez-Roselló, J. L. Aceña, C. del Pozo, A. E. Sorochinsky, S. Fustero, V. A. Soloshonok, H. Liu, *Chemical Reviews* **2014**, *114*, 2432.
[26] D. O'Hagan, *Chemical Society Reviews* **2008**, *37*, 308.
[27] J. D. Dunitz, *ChemBioChem* **2004**, *5*, 614.
[28] Y. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J. L. Aceña, V. A. Soloshonok, K. Izawa, H. Liu, *Chemical Reviews* **2016**, *116*, 422.
[29] R. Filler, H. Novar, *The Journal of Organic Chemistry* **1960**, *25*, 733.
[30] N. Budisa, W. Wenger, B. Wiltschi, *Molecular BioSystems* **2010**, *6*, 1630.
[31] M. Gómez-Nuñez, K. J. Haro, T. Dao, D. Chau, A. Won, S. Escobar-Alvarez, V. Zakhaleva, T. Korontsvit, D. Y. Gin, D. A. Scheinberg, *PLoS ONE* **2008**, *3*, e3938.
[32] A. G. Tao He, Stephen J. Eyles, Yan-Jiun Lee, Wenshe R. Liu, Jiangyun Wang, Jianmin Gao and Mary F. Roberts, *The Journal of Biological Chemistry* **2015**, *290*, 19334.
[33] C. Noren, S. Anthony-Cahill, M. Griffith, P. Schultz, *Science* **1989**, *244*, 182.
[34] N. K. Mishra, A. K. Urick, S. W. J. Ember, E. Schönbrunn, W. C. Pomerantz, *ACS Chemical Biology* **2014**, *9*, 2755.
[35] M. Salwiczek, S. Samsonov, T. Vagt, E. Nyakatura, E. Fleige, J. Numata, H. Cölfen, M. T. Pisabarro, B. Koksch, *Chemistry – A European Journal* **2009**, *15*, 7628.
[36] B. Bilgiçer, A. Fichera, K. Kumar, *Journal of the American Chemical Society* **2001**, *123*, 4393.

[37] Y. Tang, G. Ghirlanda, W. A. Petka, T. Nakajima, W. F. DeGrado, D. A. Tirrell, *Angewandte Chemie International Edition* **2001**, *40*, 1494.
[38] Y. Tang, G. Ghirlanda, N. Vaidehi, J. Kua, D. T. Mainz, W. A. Goddard, W. F. DeGrado, D. A. Tirrell, *Biochemistry* **2001**, *40*, 2790.
[39] T. Vagt, E. Nyakatura, M. Salwiczek, C. Jackel, B. Koksch, *Organic & Biomolecular Chemistry* **2010**, *8*, 1382.
[40] A. Niemz, D. A. Tirrell, *Journal of the American Chemical Society* **2001**, *123*, 7407.
[41] U. I. M. Gerling, M. Salwiczek, C. D. Cadicamo, H. Erdbrink, C. Czekelius, S. L. Grage, P. Wadhwani, A. S. Ulrich, M. Behrends, G. Haufe, B. Koksch, *Chemical Science* **2014**, *5*, 819.
[42] E. K. Nyakatura, O. Reimann, T. Vagt, M. Salwiczek, B. Koksch, *RSC Advances* **2013**, *3*, 6319.
[43] A. Volonterio, S. Bellosta, F. Bravin, M. C. Bellucci, L. Bruché, G. Colombo, L. Malpezzi, S. Mazzini, S. V. Meille, M. Meli, C. Ramírez de Arellano, M. Zanda, *Chemistry – A European Journal* **2003**, *9*, 4510.
[44] S. Ye, B. Loll, A. A. Berger, U. Mulow, C. Alings, M. C. Wahl, B. Koksch, *Chemical Science* **2015**, *6*, 5246.
[45] J. A. Johnson, Y. Y. Lu, J. A. Van Deventer, D. A. Tirrell, *Current opinion in chemical biology* **2010**, *14*, 774.
[46] C. P. R. Hackenberger, D. Schwarzer, *Angewandte Chemie International Edition* **2008**, *47*, 10030.
[47] T. S. Young, P. G. Schultz, *The Journal of Biological Chemistry* **2010**, *285*, 11039.
[48] G. W. Gribble, *Journal of Chemical Education* **2004**, *81*, 1441.
[49] D. O'Hagan, D. B. Harper, *Journal of Fluorine Chemistry* **1999**, *100*, 127.
[50] M. M. Sanada, T.; Iwadare, S.; Williamson, J. M.; Arison, B. H.; Smith, J. L.; Douglas, A. W.; Liesch, J. M.; Inamine, E., *The Journal of Antibiotics*

1986, *39*, 259.
[51] D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton, C. D. Murphy, *Nature* **2002**, *416*, 279.
[52] R.-H. Fan, Y.-G. Zhou, W.-X. Zhang, X.-L. Hou, L.-X. Dai, *The Journal of Organic Chemistry* **2004**, *69*, 335.
[53] N. Sitachitta, J. Rossi, M. A. Roberts, W. H. Gerwick, M. D. Fletcher, C. L. Willis, *Journal of the American Chemical Society* **1998**, *120*, 7131.

2. Synthesis of Fluorinated Amino Acids

Approaches to the synthesis of fluorinated amino acids can be divided into synthesis using the methodology of the amino acid chemistry and the one that exploits the chemistry of organofluorine compounds.[1-2] These two approaches are sometimes referred to as a classical and modern approach, respectively. It is not always easy to draw a strict line between these two approaches.[1] In the following subchapter (2.1) different classical methods for synthesis of fluorinated amino acids will be examined. After introducing the classical methods, synthetic approaches to fluorinated amino acid synthesis that use the chemistry of organofluorine compounds will be reviewed (Figure 2.1).

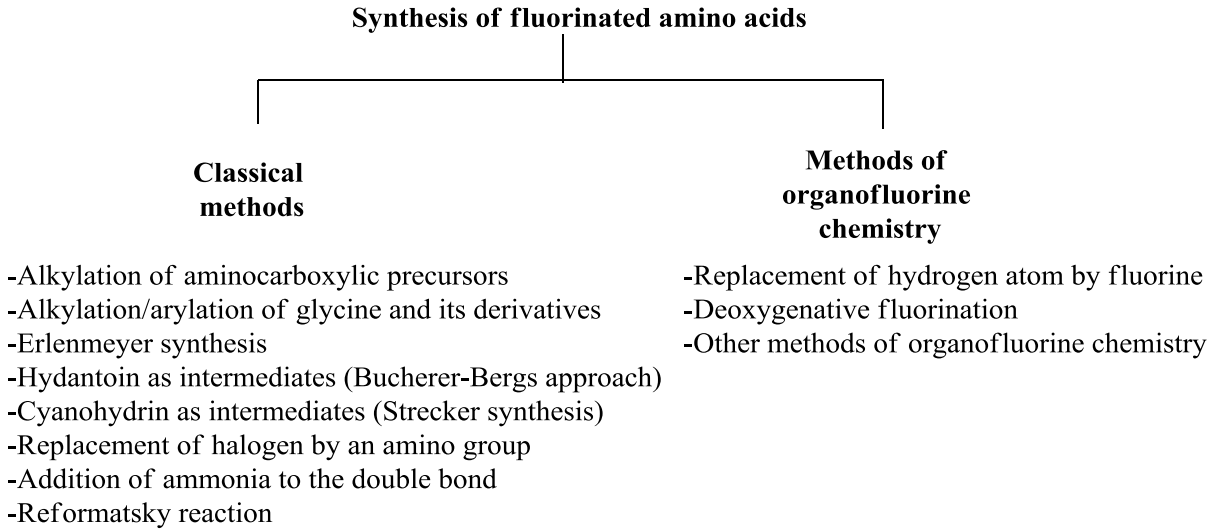


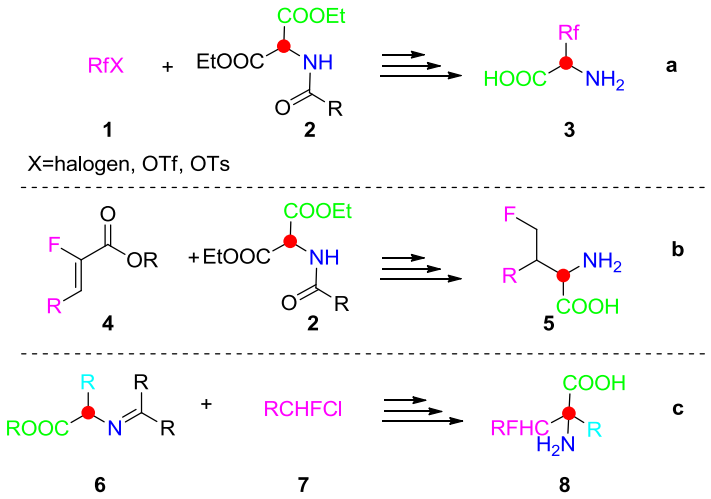
Figure 2.1 Overview of the methods used for synthesis of fluorinated amino acids.

2.1. Synthesis of Fluorinated Amino Acids Using Classical Methods of Amino Acids Synthesis

Classical methodology for the synthesis of amino acids can be applied to the synthesis of fluorinated amino acids due to the similar reactivity of fluorinated and non-fluorinated variants and the chemical inertness of the C−F bond.[1-2] When the reaction center is two or more C−C bonds away from the fluorine or fluorine containing groups, the electronic effect hardly influences the course of the reaction. Nonetheless, when the high electronegativity of fluorine causes a change in the polarity of the functional groups and bonds in the vicinity, it can reduce the reactivity of some fluorinated compounds (especially polyfluorinated ones). Additionally, C−F bonds in the β- and γ- position to the carbonyl group can sometimes be unexpectedly labile.[1-2] In these cases the applicability of the classical methods to the synthesis of fluorinated amino acids[2] is limited.

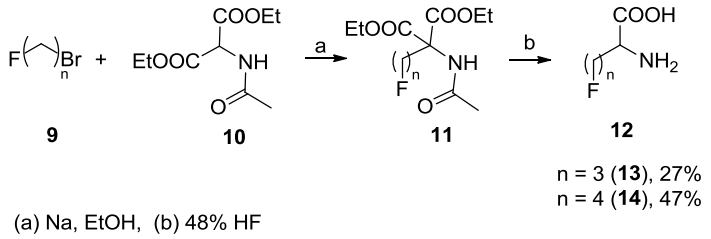
2.1.1 Alkylation of Amino Carboxylic Precursors (2-Acylaminomalonic Ester or Schiff Base)

Alkylation of a masked amino acid synthon is considered to be a facile approach to amino acid synthesis. Alkylation can be achieved in three similar ways (Scheme 2.2): a) alkylation of 2-acylamino malonic esters, b) extension of this approach to Michael acceptors (instead of alkyl groups) and c) examples where a Schiff base is used instead of the 2-acylamino malonic ester (See Scheme 2.2 below).



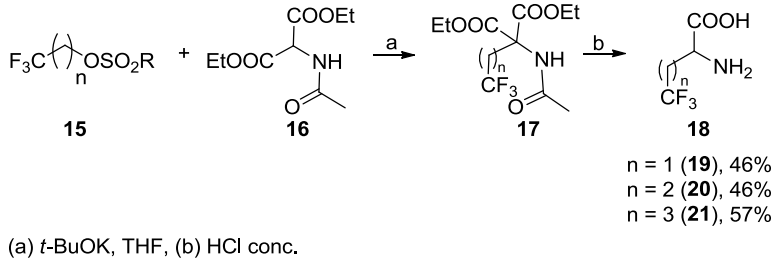
Scheme 2.2 Methods of alkylation of amino carboxylic precursors
(a) Alkylation of 2-acylamino malonic acid esters
(b) Michael addition
(c) Schiff base alkylation

The alkylation of 2-acylaminomalonic ester **2** under basic conditions is a common method for the synthesis of amino acids after subsequent hydrolysis and decarboxylation. It has been extensively applied to the synthesis of its fluorinated derivatives. Alkyl halides and alkyl triflates containing fluorine are the most common electrophiles used. 2-Amino-5-fluoropentanoic acid (5-fluoronorvaline) (**13**) and 2-amino-6-fluorohexanoic acid (6-fluoronorleucine) (**14**) were prepared using this approach (Scheme 2.3).[3]



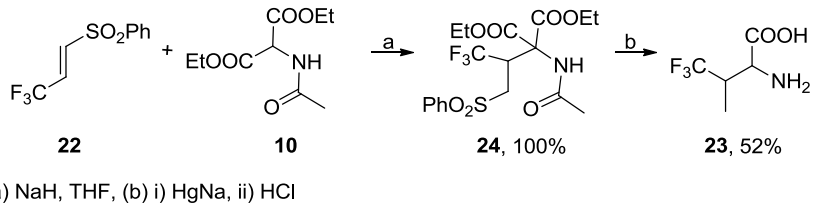
Scheme 2.3 Alkylation of 2-aminomalonic ester with fluorinated alkyl bromides to access monofluorinated amino acids.

Alkyl triflate can also be used instead of alkyl bromide, Tsushima used this approach to synthesize 2-amino-4,4,4-trifluorobuanoic acid[4] (**19**), 5,5,5-trifluoronorvaline (**20**) and 6,6,6-trifluoronorleucine (**21**) (Scheme 2.4).



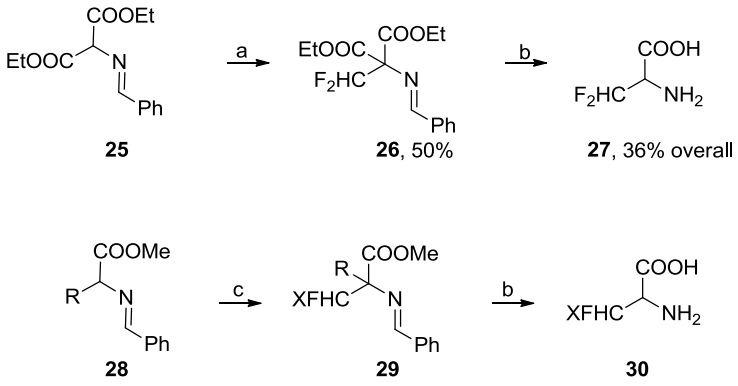
Scheme 2.4 Alkylation of 2-aminomalonic ester with fluorinated alkyl triflates to access trifluoromethylated amino acids.

The second approach involves the alkylation of acetamidomalonic ester **10** via the Michael addition to activated olefins **22** exemplified in the synthesis of 4,4,4-trifluorovaline (**23**)[5] (Scheme 2.5). Here, the fluorinated amino acid is obtained by condensation of acetamidomalonic ester **10** with the activated alkene containing benzenesulfonyl group **22.** This group is then removed using sodium amalgam.[6] Hydrolysis of the resulting product **24** gives **23** (Scheme 2.5).



Scheme 2.5 Michael addition to acetamidomalonic ester as a synthetic starting point towards 4,4,4-trifluorovaline (**23**).

The third alkylation approach includes alkylation of the Schiff bases **26** and **29** formed from aminomalonic esters[7] and various 2-aminomonocarboxylic esters (Scheme 2.6).[7-10] In this way 2-amino-3,3-difluoropropanoic acid (**27**) and its chlorinated variants **30** were obtained.

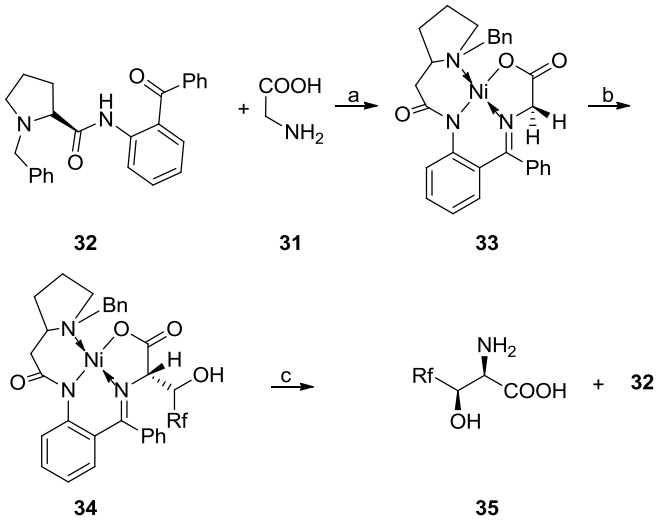


Scheme 2.6 Alkylation of Schiff's base to access fluorinated amino acids **27** and **30.**

Despite the fact that these three classical alkylation methods offer access to various fluorinated amino acids they suffer from some drawbacks. Significant product decomposition arises from hydroxyl substitution of the fluorine containing side chain during hydrolysis and decarboxylation of the alkylated amino malonic ester intermediate. Additionaly, due to the strong bases used in the Michael reaction, numerous undesirable side reactions are common, leading to complex mixtures that are difficult to separate.[11-12] Finally, it should be noted that preparation of the starting materials is non-trivial and tends to be inconvenient and time consuming in preparatively useful scales.

2.1.2 Alkylation/arylation of Glycine and Its Derivatives

The second classic method for fluorinated amino acid synthesis is the Ni(II) complex approach. Similar to the alkylation of 2-acylaminomalonic esters, an electrophile (the side-chain of the amino acid) is added to glycine (**31**) or its derivatives which are trapped within a Ni(II) complex. In Scheme 2.7 synthesis of enantiomerically pure fluorine-containing *β*-phenylserines **35** and *β*-alkylserines **35** is shown.[2, 13]



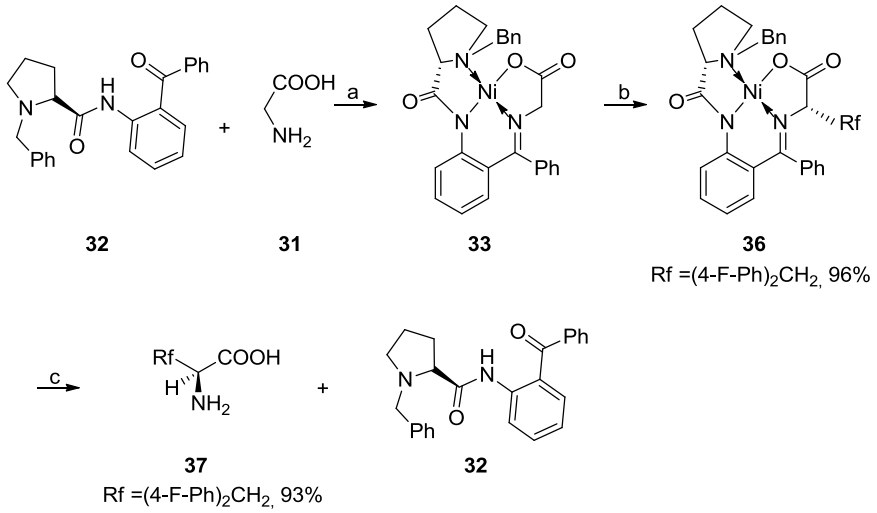
Rf = 2-FC_6H_4, 4-FC_6H_4, 2-$(CHF_2O)C_6H_4$, 4-$(CHF_2O)C_6H_4$, 2-$CF_3C_6H_4$, 4-$(CF_3O)C_6H_4$, 3-F-4-$MeOC_6H_3$, CF_3, $H(CF_2)_6$, 70-82%

Rf = 2-FC_6H_4, 4-FC_6H_4, 2-$(CHF_2O)C_6H_4$, 4-$(CHF_2O)C_6H_4$, 2-$CF_3C_6H_4$, 4-$(CF_3O)C_6H_4$, 3-F-4-$MeOC_6H_3$, CF_3; (2*S*,3*S*), $H(CF_2)_6$; (2*S*,3*S*), 74-95%

(a) $Ni(OAc)_2$, MeONa/MeOH (b) RfCHO, NaOAc (c) HCl, MeOH

Scheme 2.7 Synthesis of enantiomerically pure fluorine-containing *β*-phenylserines **35.**

The aforementioned Ni(II) complex of the glycine Schiff base can be synthesized from commercially available *(S)*-2-*N*-(*N'*-benzylprolyl)-o-aminobenzophenone (**32**), glycine and nickel nitrate with sodium methoxide.[2, 13] Hydroxyalkylation of the complex **33** with fluorinated aldehydes in the presence of sodium methoxide proceeds with high stereoselectivity forming complex **34** (Scheme 2.7). Addition of hydrochloric acid to the Ni(II) complexes **34** liberates fluorinated serines and the chiral auxiliary **32** can be recovered (Scheme 2.7). Soloshonok and co-workers have shown that in addition to aldehydes it is possible to use fluorinated benzyl chlorides for introduction of fluorinated benzyl side chains to alanine or glycine through the Ni(II) complex (Scheme 2.8).[14] Subsequent acid hydrolysis produces fluorinated phenylalanines[15] or α-methylphenylalanines in excellent yields.



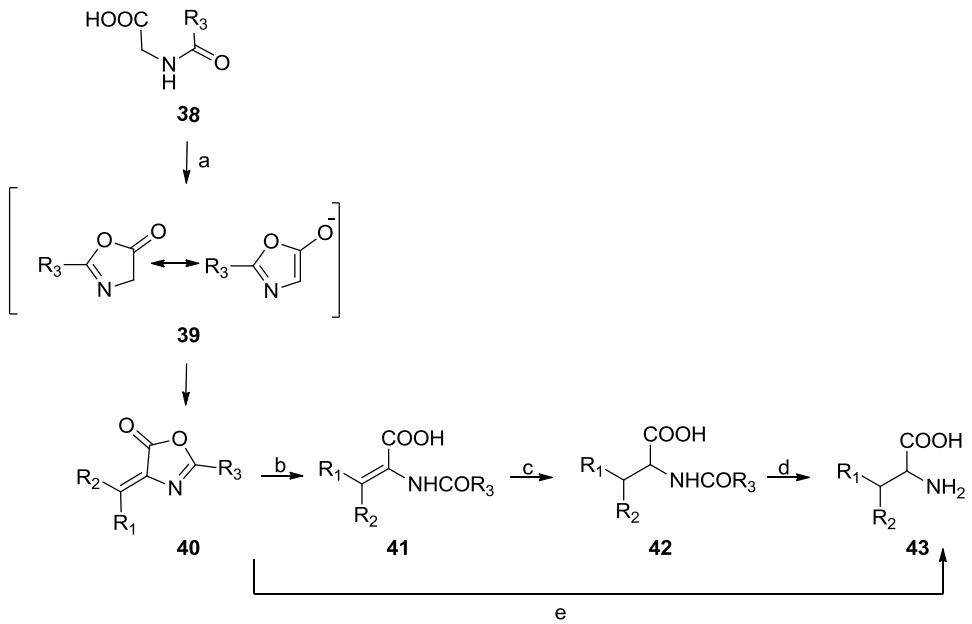
(a) $Ni(NO_3)_2$, NaOMe, (b) $(4\text{-F-Ph})_2CH_2Cl$, *t*-BuOK, MeCN (c) 2 M HCl

Scheme 2.8 Synthesis of enantiomerically pure fluorinated amino acid **37.**

Although interesting, the described method cannot be considered atom-economical. The molecular weights of the Ni(II) complexes are large and amount to approximately 80% of the total weight of the product mixture.[16]

2.1.3 Azlactone Synthesis (Erlenmeyer Synthesis)

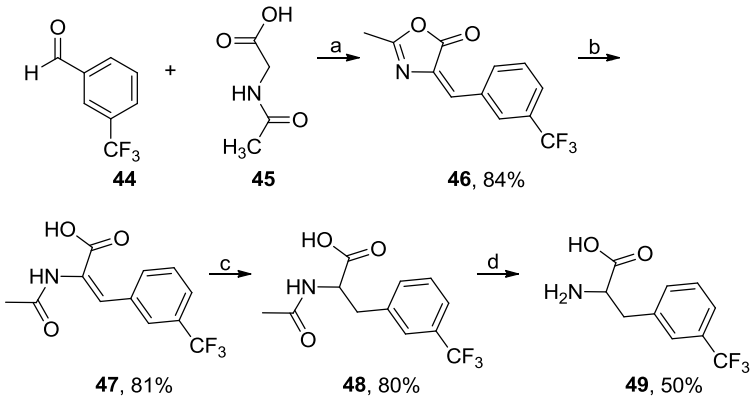
The third approach to the synthesis of amino acids is Erlenmeyer synthesis (Scheme 2.9). This method has been widely exploited in the preparation of fluorinated aromatic amino acid analogues. In contrast to the first two synthetic approaches, the Erlenmeyer method includes cyclization in the first step. The classical example of azlactone synthesis is the reaction between hippuric acid **38** and aromatic aldehyde in the presence of base usually under reflux conditions in acetic anhydride (Scheme 2.9).[17] From compound **40**, the Erlenmeyer synthesis can follow one of two routes;[1] gradual, stepwise opening of the azlactone ring (b→c→d) or direct reduction to the desired amino acid (e) (Scheme 2.9).



(a) 1, $NaOAc/Ac_2O$, R_1COR_2, (b) base or acid (c) H_2, Pd/C, (d) H_3O^+ (e) HI/P (red)/Ac_2O

Scheme 2.9 Synthesis of amino acids through azlactone.

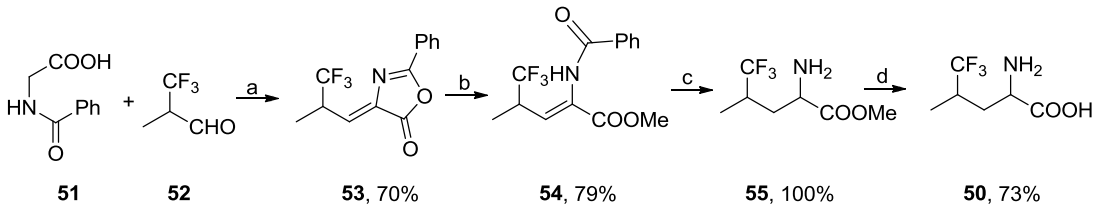
The Erlenmeyer azlactone method was applied in the synthesis of all three monofluorophenylalanines (*o*-, *m*- and *p*-).[1, 18-20] Condensation of fluorinated benzyladehyde **44** with N-acetyl **45** leads to 2,4-disubstituted 5-oxazolone **46**, which is converted into the desired *m*-trifluoropheynlalanine (**49**) by oxazolone ring opening, reduction and hydrolysis (Scheme 2.10).[21] Aside from monofluorophenylalanines, many polyfluorinated analogues of phenylalanine[1, 22-24] were prepared using the same approach. Recently, a one-pot Erlenmeyer synthesis of poly-fluorinated amino acid derivatives was reported.[25] Here, the Erlenmeyer synthesis of azlactone was combined with reduction by HI / P (red) without isolation to give the desired derivatives in higher or comparable yields to those achieved by multiple step synthesis.



(a) Ac_2O, NaOAc, (b) acetone/H_2O, (c) H_2, Pd/C, (d) HCl

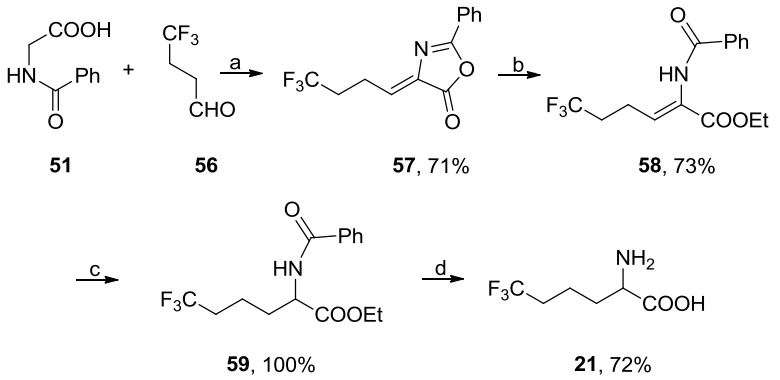
Scheme 2.10 Synthesis of *m*-trifluoromethyl phenylalanine (**49**).

However, there are not many reported examples of syntheses of fluorinated aliphatic amino acids that utilize this classical method. In fact, the application of this method in preparation of non-fluorinated amino acids is also poor. Chandrasekhar *et al.* suggest that the success of the azlactone formation depends on the aromaticity of the azlactone aninon intermediate **39**[26] This highlights the problem of stability of aliphatic aldehydes under the Erlenmeyer reaction conditions,[26] which is the reason Erlenenmeyer synthesis generally fails in the case of aliphatic aldehydes. Despite the instability of aliphatic aldehydes under the reaction conditions and the reported aromaticity requirement, the synthesis of a set of fluorinated aliphatic amino acids was achieved using a slightly modified Erlenmeyer method. In the synthesis of 5,5,5-trifluoroleucine (**50**) and 6,6,6-trifluoronorleucine (**21**), the classical conditions for azlactone formation (sodium acetate) were substituted by Pb(II) acetate or Zn(II)acetate (Scheme 2.11).[25, 27] After lead acetate and acetic anhydride catalyzed azlactone formation of **53** and **57** from their corresponding aldehydes **52** and **56** and hippuric acid[28-29] the azlactones could be converted into 5,5,5-trifluoroleucine (**50**) and 6,6,6-trifluoronorleucine (**21**) by reduction and subsequent hydrolysis, or by direct HI / P reduction (Scheme 2.11).



51 **52** **53**, 70% **54**, 79% **55**, 100% **50**, 73%

(a) $Pb(OAc)_2x3H_2O$, Ac_2O, THF, (b) EtOH, NaOEt, (c) H_2, Pd/C, (d) HCl



51 **56** **57**, 71% **58**, 73%

59, 100% **21**, 72%

(a) $Pb(OAc)_2x3H_2O$, Ac_2O, THF, (b) MeOH, NEt_3, (c) H_2, Pd/C, (d) 6 N HCl

Scheme 2.11 Synthesis of of 5,5,5-trifluoroleucine (**50**) and 6,6,6-trifluoronorleucine (**121**) through azlactone intermediate.

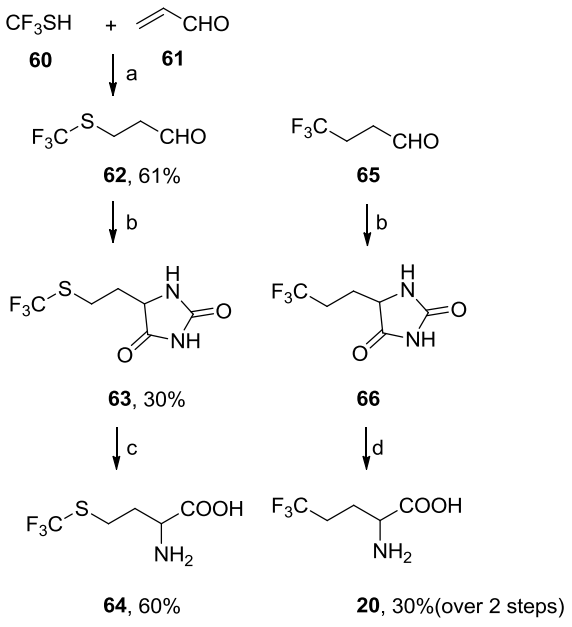
Due to the dependence of the Erlenmeyer synthesis on the aromaticity of the intermediate azlactone anion and the instability of aliphatic aldehydes under these conditions, this method results in

a limited scope and in general lower yield for aliphatic fluorinated amino acids.[30] In these cases, unfortunately toxic $Pb(OAc)_2$ is necessary to enable azlactone formation.

2.1.4 Synthesis of Amino Acids Through Hydantoin Intermediates (Bucherer Bergs Approach)

The synthesis of fluorinated amino acids via a hydantoin intermediate is an alternative classical approach which, in addition to Erlenmeyer synthesis, affords amino acids with one additional carbon unit when compared to the starting material. Usually for the purpose of amino acid synthesis the Bucherer-Bergs approach is used to access hydantoins which are opened under acidic conditions to yield the desired amino acid.

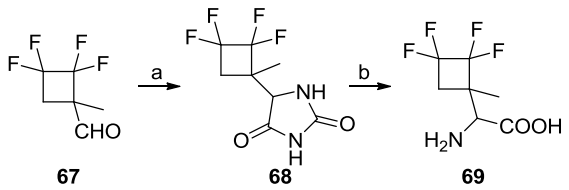
This approach was applied in the synthesis of trifluoromethionine (**64**)[31] and 5,5,5-trifluoronorvaline (**20**)[32] (Scheme 2.12). In both cases an aldehyde was the starting material, however, in the trifluoromethionine synthesis, trifluoromethyl thiol (**60**) was coupled to acrolein to give **63**. Bucherer-Bergs conditions were applied to aldehydes **62** and **65** and resulted in hydantoins **63** and **66** which, under acidic conditions, provide trifluoromethionine (**64**) and 5,5,5-trifluoronorvaline (**20**).



(a) $Cu(OAc)_2$, $CHCl_3$, (b) NaCN, $(NH_4)_2CO_3$, (c) i) NaOH,H_2O, ii) HCl to pH=6, MeOH, (d) H_3O^+

Scheme 2.12 Synthesis of trifluoromethionine (**64**) and 5,5,5-trifluoronorvaline (**20**) through hydantoin intermediate.

This method can offer facile access to unusual fluorinated amino acids, *e.g.* ones bearing a tetrafluorocyclobutyl substituent.[33] Tetrafluorocyclobuytl substituted aldehyde **67** gives hydantoin **68** when Bucherer-Bergs conditions are applied. Hydrolysis under acidic conditions affords amino acid **69** (Scheme 2.13).

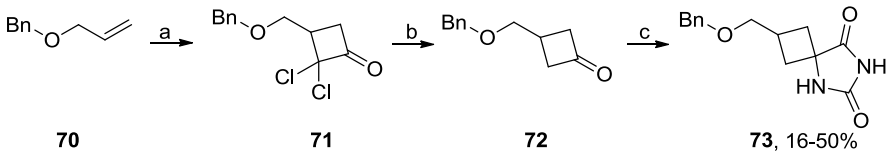


67 **68** **69**

(a) NaCN, $(NH_4)_2CO_3$, (b) 60% H_2SO_4

Scheme 2.13 Synthesis of tetrafluorocyclobuty amino acid **69** through hydantoin intermediate.

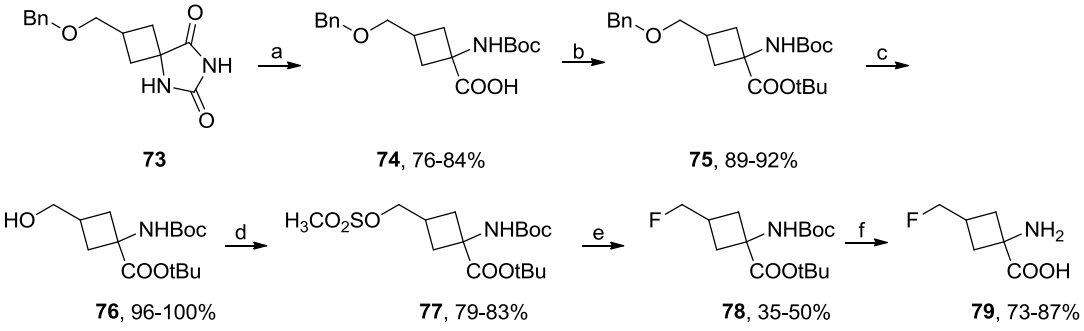
The hydantoin approach has been exploited in the synthesis of some unnatural ^{18}F labelled fluorinated amino acids. Using allyl benzyl ether **70**, hydantoin **73** was prepared in three steps (Scheme 2.14) and subsequently hydrolysed. The resulting crude amino acid was Boc and *t*-butyl protected to yield **75**. Deprotection of the benzyl ether affords the corresponding alcohol **76** (Scheme 2.15). Mesylation of the hydroxy group, and fluorination using TBAF furnishes protected fluorinated amino acid **78**. Deprotection of **78** yielded 1-amino-3-fluoromethyl-cyclobutane-1-carboxylic acid − FMACBC (**79**). Similarly, when intermediate **83** was treated with $K^{18}F$, [^{18}F]−FMACBC **79** was obtained (See Scheme 2.16).[34] Other cyclic fluorinated amino acids were prepared using the hydantoin approach.[35] Problems such as low solubilty of starting aldehydes or ketones, sublimation of ammonium carbonate, handeling of KCN and purification of the products are the main drawbacks of this approach.[36]



70 **71** **72** **73**, 16-50%

(a) CCl_3COCl, $POCl_3$, Zn-Cu couple, (b) Zn, AcOH, (c) $(NH_4)_2CO_3$, KCN, NH_4Cl

Scheme 2.14 Synthesis of hydantoin precursor **82.**

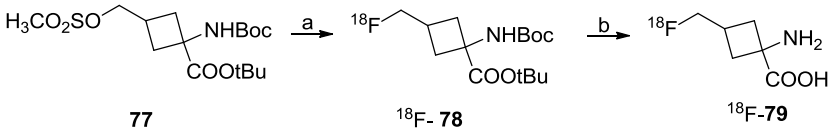


73 **74**, 76-84% **75**, 89-92%

76, 96-100% **77**, 79-83% **78**, 35-50% **79**, 73-87%

(a) i) 3 N NaOH, ii) Boc_2O, MeOH, Et_3N, (b) $Cl_3CC(=NH)OtBu$, DCM, (c) H_2, 10% Pd/C, MeOH, (d) $MeSO_2Cl$, 2,6-lutidine, DCM, (e) TBAF, THF, (f) 3 N HCl, MeOH

Scheme 2.15 Synthesis of FMACBC (**79**).



77 ^{18}F- **78** ^{18}F-**79**

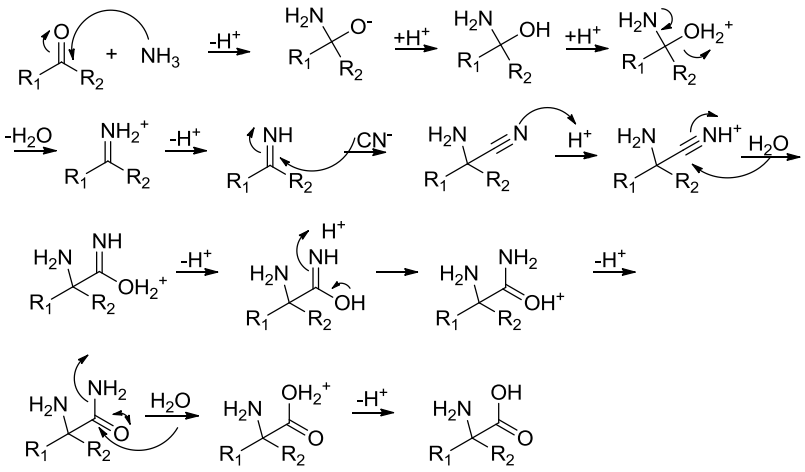
(a) K^{18}F, $K_{2,2,2,}$ MeCN, (b) 6 N HCl

Scheme 2.16 Synthesis of [^{18}F] − FMACBC (^{18}F-**79**).

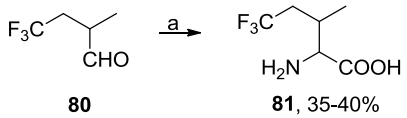
2.1.5. Cyanohydrin Synthesis (Strecker Reaction)

An additional approach that involves the addition of one carbon atom to the starting material is the Strecker synthesis.

The Strecker reaction involves nucleophilic addition of a cyanide group to an *in situ* formed imine. The resulting amino nitrile is subsequently hydrolyzed to afford an amino acid (Scheme 2.16). For example the Strecker route was exploited in the synthesis of an amino acid with a terminal CF_3-group, 5,5,5-trifluoroisoleucine **81** (See Scheme 2.17).[37]



Scheme 2.16 Mechanism of amino acid formation by Strecker reaction.

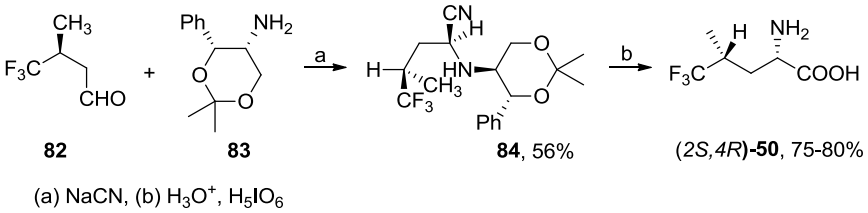


80 **81**, 35-40%

(a) i) KCN, NH_4Cl/ NH_4OH, ii) H_3O^+

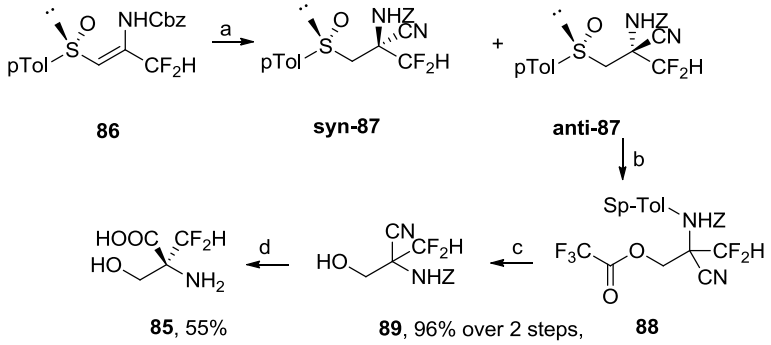
Scheme 2.17 Synthesis of trifluoroisoleucine **81** by Strecker reaction.

After the initial applicability of the Strecker reaction in the synthesis of trifluoromethylated racemic amino acid was demonstated, Wenings *et al.* applied similar conditions to the asymmetric synthesis of (2*S*,4*R*)- and (2*S*,4*S*)-5,5,5-trifluoroleucine (**50**). The asymmetry was a result of utilizing optically pure starting materials **82** and **83** (Scheme 2.18).[38]



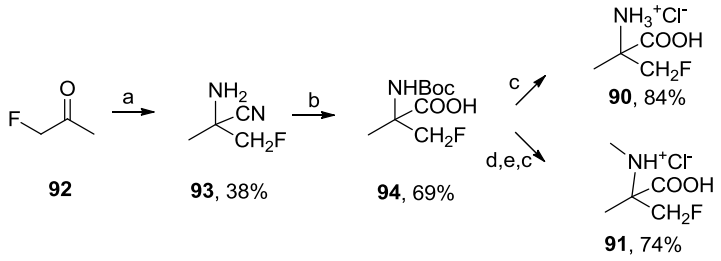
Scheme 2.18 Application of Strecker reaction for synthesis of (2*S*,4*R*)-5,5,5-trifluoroleucine (**50**).

Further utilization of this approach for the asymmetric synthesis of fluorinated amino acids was demonstrated through the synthesis of difluorinated amino acid, (*S*)-*α*-difluoromethylserine (**85**) in four steps from *N*-Cbz-*α*-fluoroalkyl-*β*-sulfinylenamine (**86**) (Scheme 2.19).[39] Nucleophilic addition of potassium cyanide to **86** results in amino nitrile **87**. Removal of this sulfinyl group from the anti-product **87**, followed by introduction of the oxygen functionality using a Pummerer reaction produced **88**. Hydrolysis of the trifluoroacetate and reduction of the sulfinyl moiety in a one-pot procedure yields the *β*-hydroxy-*α*-aminonitrile **89** which is hydrolysed to give the enantiomerically pure (*S*)-*α*-difluoromethylserine (**85**).[40]



Scheme 2.19 Synthesis of (*S*)-*α*-difluoromethylserine (**85**) by Strecker reaction from enantiomerically pure starting materials.

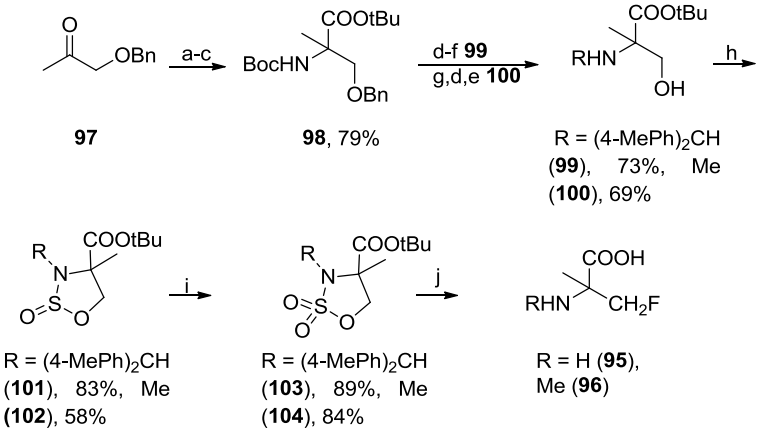
In 2002, Strecker reaction found an interesting application in the synthesis of candidates for novel radiopharmaceuticals.[41] 2-Amino-3-fluoro-2-methylpropanoic acid (FMAP, **90**) and 3-fluoro-2-methyl-2-(methylamino)propanoic acid (*N*–MeFMAP, **91**) could be easily accessed employing Strecker conditions (See Scheme 2.20).[41]



(a) KCN, NH_4Cl, H_2O, (b) HCl, Boc_2O, MeOH, Et_3N, (c) HCl aq., (d) $Cl_3CC(=NH)OtBu$, DCM, (e) NaH, DMF, MeI

Scheme 2.20 Synthesis of 2-Amino-3-fluoro-2-methylpropanoic acid (FMAP, **90**) and 3-fluoro-2-methyl-2-(methylamino)propanoic acid *(N*–MeFMAP, **91** using Strecker approach).

However, this strategy could not be applied to the radiosyntheses of [^{18}F]–**95** and [^{18}F]–**96**. Therefore, cyclic sulphamidates were utilized as the main precursors in this synthesis (Scheme 2.21).[42-45] The α-methyl serine derivative **98** was a common intermediate in the synthesis of both [^{18}F]–**95** and [^{18}F]–**96**. Aminoalcohols **99** and **100** were synthesized in two different ways (Scheme 2.21). The latter were subsequently reacted with $SOCl_2$ and gave the cyclic sulphamidites **101** and **102**. $NaIO_4$ and ruthenium (IV) oxide were used to oxidize **101** and **102**. Radiolabelling with [^{18}F]–HF and hydrolysis provided [^{18}F]–**95** and [^{18}F]–**96**.[41]



(a) $(NH_4)_2CO_3$, KCN, NH_4Cl, EtOH, H_2O, (b) 5 N NaOH, Boc_2O, MeOH, Et_3N, (c) $Cl_3CC(=NH)OtBu$, DCM, (d) 10% Pd/C,H_2, MeOH, (e) p-TsOH*H_2O, EtOH, (f) DMBCl, Et_3N, DCM, (g) NaH, DMF, MeI, (h) SO_2Cl, Et_3N, toluene or DCM, (i) $NaIO_4$, cat. RuO_2, H_2O, MeCN, (j) ^{18}F-HF, $K_{2,2,2}$, K_2CO_3, MeCN, 6 N HCl

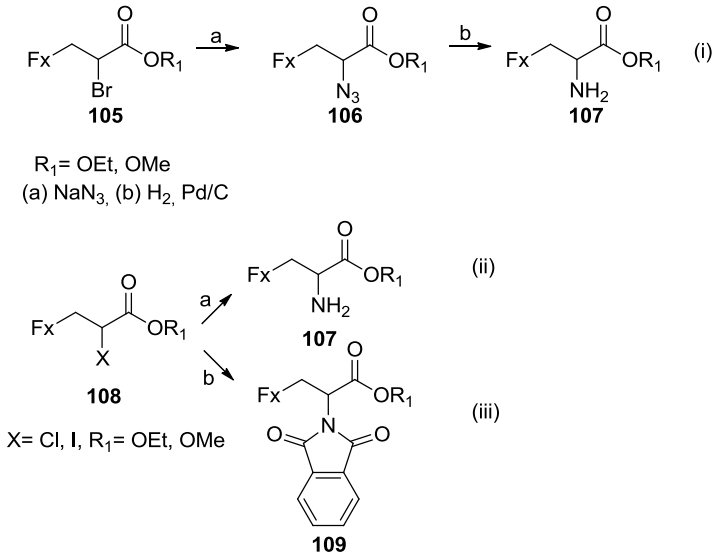
Scheme 2.21 Synthesis of 2-Amino-3-[^{18}F]-fluoro-2-methylpropanoic acid (FMAP, **95**) and 3[^{18}F]-fluoro-2-methyl-2-(methylamino)propanoic acid (*N*–MeFMAP, **96**).

Furthermore, the Strecker synthesis is mostly exploited for the synthesis of α-trifluoromethylated amino acids and more examples of such transformations can be found in the Chapter 3. The major limitations of the Strecker reaction are related to the instability of imines or the

instability of the starting aldehydes. Additionally, KCN, which was initially used in aqueous solutions, is toxic and produces a highly alkaline reaction mixture, resulting in a limited substrate scope.[46]

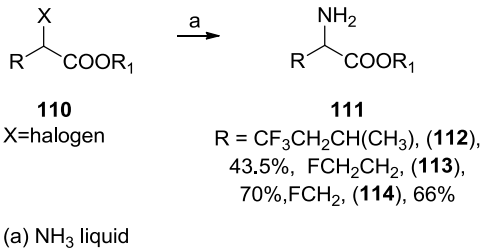
2.1.6. Replacement of Halogen Group by an Amino Group

Replacement of α-halogen in α-halogenated carboxylic acids by an amino group is classical method used in fluorinated amino acid synthesis. Usually, a two-step process is employed. Fluorine-containing α-bromocarboxylic acid or ester is reacted with sodium azide and the azido group is subsequently reduced to an amino group (Scheme 2.22). Alternative options are the direct aminolysis of fluorine-substituted α-chloro- or α-iodocarboxylic acids or their reaction with potassium phthalimide (Scheme 2.22). Experimental conditions are dictated by the availability of the fluorinated starting materials and also by the stability of the functional groups present in the molecule to the conditions applied.[2]



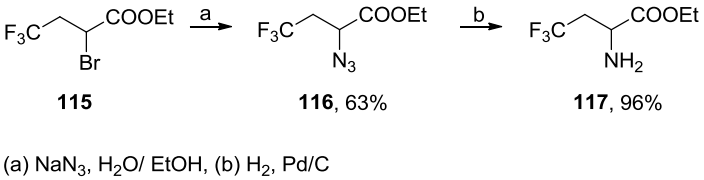
Scheme 2.22 Replacement of halogen by amino group; (i) azide approach, (ii) direct introduction of amino group and (iii) phtalimide method.

This method of direct aminolysis of the α-halogenated acid **110** was employed in the synthesis of 5,5,5-trifluoroisoleucine (**112**),[37] 4-fluoro-2-aminobutyric acid (**113**) and 3-fluorovaline (**114**) (Scheme 2.23).[47]



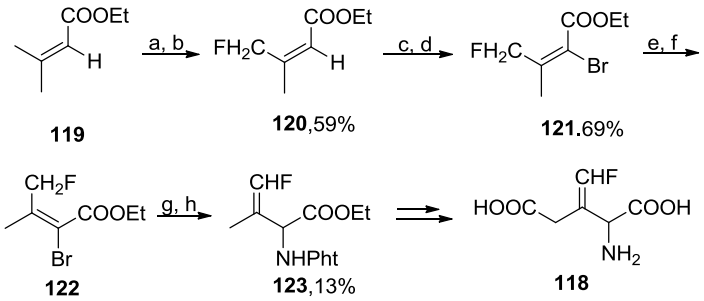
Scheme 2.23 Synthesis of fluorinated amino acid **111** through direct aminolysis.

At the same time, a similar method via an intermediate azide became routine in the syntheses of fluorinated amino acids.[48-50] The azide intermediate route is exemplified in the synthesis of an ethyl ester of 4,4,4-trifluorobutyric acid (**117**) (Scheme 2.24).[50]



Scheme 2.24 Synthesis of an ethyl ester of 4,4,4-trifluorobutyric acid (**117**) by azide approach.

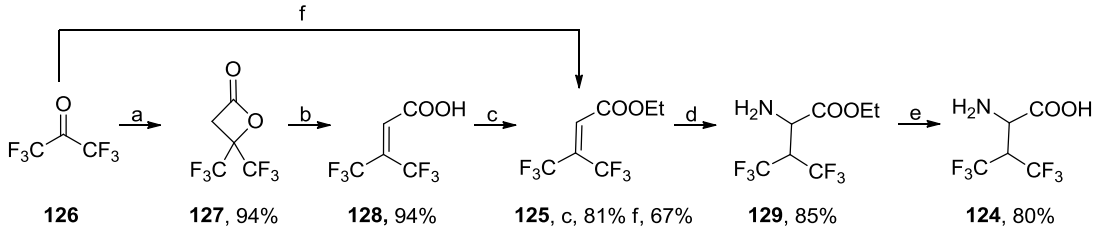
An alternative to direct aminolysis of the *α*-halogenated ester and the azide approach, as mentioned earlier, is the introduction of amino moiety by using phthalimide. One of the examples where this method proved useful is in the synthesis of (E)-3-fluoromethyleneglutamic acid (**118**)[51] (Scheme 2.25). Mc Donald *et al* used a non-fluorinated starting material **119** and introduced fluorine by halogen exchange after bromine introduction by NBS. A second bromination affords **121**. After deconjugation, **123** gives exclusively the E isomer **122**. Bromine displacement by NH_3 and successive deprotection of phthalimide gave **123**, which is a precursor in the synthesis of **118** (Scheme 2.25).



Scheme 2.25 Synthesis of fluorinated amino acid **118** using the phthalimide method.

2.1.7 Addition of Ammonia or its Derivatives to the Double Bond of Acrylate Systems

Installation of the amino group to a fluorinated amino acid by using ammonia can also be achieved by its addition to *α,β*-unsaturated esters. The simplest example is adding ammonia or its derivatives to a molecule of acrylic acid. This approach is commonly used to synthesize the derivatives of hexafluorovaline and *α*-trifluoromethyl-*β*-alanine due to the high electrophilicity of the double bond in the initial unsaturated acid. The first reported synthesis of hexafluorovaline[2] (**124**) on a preparative scale was achieved by addition of ammonia to ethyl-*β,β* bis(trifluoromethyl)acrylate (**125**), which was obtained in three steps from hexafluoroacetone (**126**) and ketene or by the Wittig reaction (Scheme 2.26).[52]

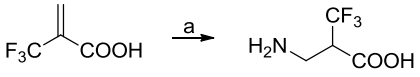


126 **127**, 94% **128,** 94% **125**, c, 81% f, 67% **129**, 85% **124**, 80%

(a) CH_2CO, (b) H_2SO_4, (c) EtOH, (d) NH_3, (e) HCl-H_2O, (f) Ph_3P=CHCOOEt

Scheme 2.26 Synthesis of hexafluorovaline (**124**) by addition of ammonia to an acrylate system.

Addition of the ammonia to the double bond can, in addition, be expanded to the synthesis of trifluoromethylated *β*-amino acids, which is exemplified in the synthesis of α-trifluoromethyl-β-alanine. Ojima and co-workers have shown that using gaseous ammonia can afford **130** in quantitave yield (Scheme 2.27).[53]

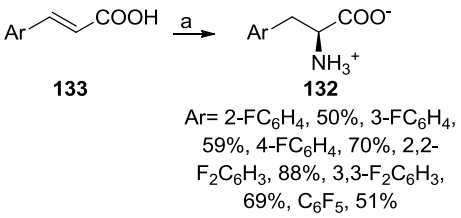


131 **130**, 100%

(a) NH_3 (g)

Scheme 2.27 Synthesis of *α*-trifluoromethyl-*β*-alanine (**130**).

A more recent and very interesting alternative to these traditional batch methods exploits very efficient enzyme chemistry. Moreover, ammonia can be added to *α,β*-unsaturated esters enzymatically, using ammonia lyases. This was demonstrated by the synthesis of phenylalanines **132** with different fluorination patterns in the aromatic ring (Scheme 2.28).[54]

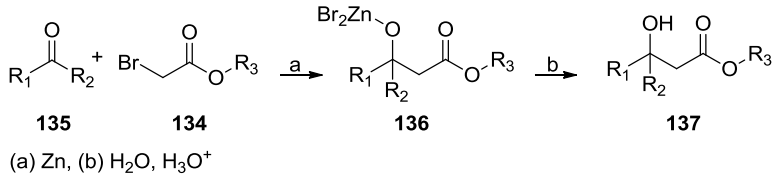


Scheme 2.28 Enzymatic introduction of amino group to access fluorinated amino acids **132**.

As shown in the examples above, the addition of ammonia to unsaturated esters is advantageous for the synthesis of fluorinated aliphatic amino acids, if the unsaturated carboxylic acid used is highly fluorinated. A high degree of fluorination increases electrophilicity and enables an easier attack of the amino group on the desired α-position.[2] Chemical addition of the amino group to the unsaturated bond is not suitable for synthesis of aromatic fluorinated amino acids. The vicinity of the aromatic rings does not allow for the existence of the double bond; enzymatic approaches do offer an alternative but these are very substrate specific.

2.1.8. Reformatsky Reaction

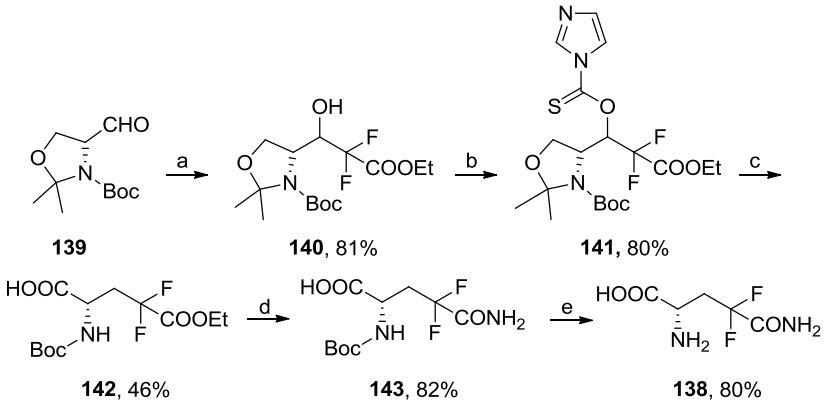
As opposed to the approaches disscused above, Reformatsky reaction allows for the introduction of two carbon atoms, *i.e.* carbonyl group carrying additional carbon. Reformatsky reaction (sometimes spelled Reformatskii reaction) is a transformation in which aldehydes or ketones react with α-halo esters, using a metallic zinc to form β-hydroxy-esters. The organozinc reagent, also called a 'Reformatsky enolate', is prepared by condensation of an α-halo ester **134** with zinc dust. Reformatsky enolates are less reactive than lithium enolates or Grignard reagents that hinder the nucleophilic addition to the ester group (Scheme 2.29).



Scheme 2.29 Reformatsky reaction.

The above mentioned transformation, was a key step in the asymmetric synthesis of 4,4-difluoroglutamine (**138**, Scheme 2.30). Garner aldehyde **139** was subjected to Reformatsky conditions in THF in the presence of ultrasonic waves which resulted in alcohol **140** as a diastereoisomeric mixture. The mixture was transformed into imidazolylthiocarbonates **141**. Deoxygenation of **141** and immediate oxidation using a stoichiometric amount of H_5IO_6 with catalytic CrO_3 gave **142**.

Aminolysis of the ester **142** with 28% aqueous NH_3 and subsequent hydrolysis by TFA gives the desired L-4,4-difluoroglutamine (**138**) (Scheme 2.30).[55]



(a) $BrCF_2COOEt$, Zn, ultrasound, THF, (b) thiocarbonyldiimidazole, 1,2-dichloroethane, (c) i) Et_3SiH, Bz_2O_2, ii) CrO_3 cat., H_5IO_6, MeCN, (d) 28% aq. NH_3, 1 N $KHSO_4$ (e) TFA

Scheme 2.30 Synthesis of 4,4-difluoroglutamine (**138**) with Reformatsky reaction as a key step.

Reformatsky reaction catalyzed by Zn has some drawbacks; one of them being the high reaction temperature. The reaction must be carried out in boiling THF in order to be high yielding. The other drawback is activation of the Zn metal (acid wash and/or addition of TMSCl).[56] Additionally, this reaction is heterogeneous and thus more difficult to regulate.[57-58] The reproducibility of the classical Reformatsky reaction is quite low and it offers access only to the thermodynamic products.[59] Other problems of the Reformatsky reaction are low yields due to the enolization of the Reformatsky reagent and many associated side reactions; including the self-condensation of carbonyl compounds and α-bromoester,[60] elimination of α-bromoester[60] and retro-Reformatsky fragmentation.[61] A small variation in the ester moiety affects the reactivity of the α-bromoester.[62] For example, the methyl and ethyl α-bromoesters react regularly, whereas bulkier isopropyl, *t*-butyl and neopentyl esters are inert.

2.2 Methods of Organofluorine Chemistry for the Synthesis of Fluorine-Containing Amino Acids

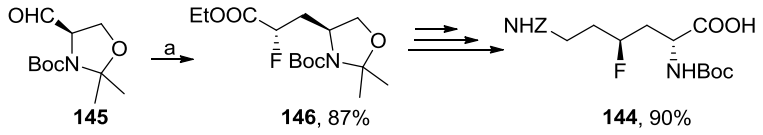
After exhaustively reviewing different classical methods utilized in the synthesis of fluorinated amino acids, in this subchapter another perspective of the synthesis of fluorinated amino acids will be examined. At this point, the focus will be shifted towards methods used in the synthesis of organofluorine compounds which are applied to the synthesis of fluorinated amino acids. Many new fluorinated organic compounds found their application as synthons in the preparation of fluorinated amino acids. Moreover, new fluorinating agents, by which fluorine or fluorine-containing groups can be introduced into the molecule by fluorination of one of the intermediates in the synthesis or directly to a side chain of non-fluorinated amino acid are available on the market.[63-77] By using these fluorinating reagents, numerous fluorinated analogues of amino acids which were not accessible by

methods of classical chemistry are now available. Perhaps, the biggest advantage of the direct electrophilic fluorinations is the complete retention of stereochemistry as well as the fact that some of the functional groups do not need protection in this process. However, these reactions proceed with a lack of regioselectivity.[1-2]

In the next three subchapters, methods of the direct incorporation for fluorine will be discussed, starting with the substitution of hydrogen by fluorine, methods of deoxygenative fluorination and ending with other methods of organofluorine chemistry used for synthesis of fluorinated amino acids.

2.2.1 Replacement of the Hydrogen Atom by Fluorine

In the literature, examples of aliphatic fluorinated amino acids, obtained by direct fluorination are scarce. This is probably due to the fact that aliphatic amino acids are far less stable towards fluorine incorporation than the aromatic ones. Another reason for the low exploitation of this method for fluorinated amino acid synthesis lies in the high number of reactive C−C bonds, leading to reactions with low regioselectivity.[2] Direct introduction of fluorine to the molecule by replacement of the hydrogen atom can be perfomed using various fluorine sources (HF, $FClO_3$, XeF_2, CF_3OF).[78] In 2003, fluorinated amino acid **144** was synthesized according to Scheme 2.31.[79]

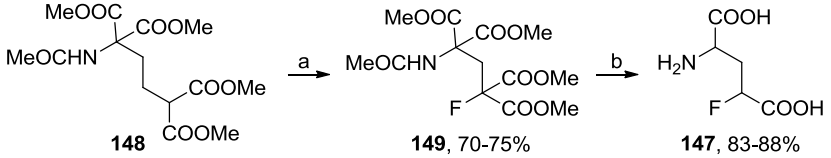


145 **146**, 87% **144**, 90%

(a) i) triethyl 2-fluoro-2-phosphonoacetate, NaH, ii) H_2, Pd/C, EtOH

Scheme 2.31 Schematic overview of access to Cbz protected fluorinated amino acid **144** by direct fluorination.

A further example of synthesis of fluorinated amino acids carrying a functional group in the side chain by direct fluorination is 4-fluoroglutamic acid (**147**) synthesis (Scheme 2.32). The γ-position of tetramethyl 2-acetamido-2,4-dicarboxylglutarate (**148**) was fluorinated using perchloryl fluoride. This was followed by hydrolysis and decarboxylation to the desired product (Scheme 2.32).[80-81]



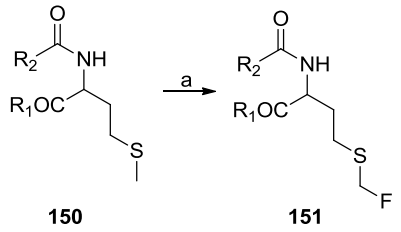
148 **149**, 70-75% **147**, 83-88%

(a) $FClO_3$, NaOEt, (b) HCl

Scheme 2.32 Synthesis of 4-fluoroglutamic acid **147** by direct fluorination of **148**.

Janzen *et al.*[82] have on the other hand reported using XeF_2 for direct fluorination of the methionine derivative **150**. This finding that XeF_2 is suitable for α-fluorination of sulfides might be a

useful technique in labelling other alkylthio derivatives.[82] No overfluorination was observed (See Scheme 2.33).



150 **151**

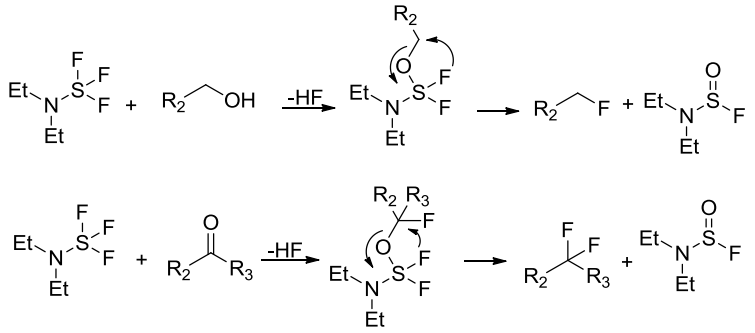
R_1 = OMe, R_2 = OtBu, CH_2Ph
(a) XeF_2, MeCN

Scheme 2.33 Direct introduction of fluorine into methionine using XeF_2.

As already mentioned, this is also one of the most straightforward routes to enantiomerically pure fluorinated amino acids. This methodology avoids multistep synthesis and is very useful in preparing ^{18}F-fluorinated amino acids by using elemental fluorine, acetyl hypofluorite or XeF_2.[2] Using the direct incorporation of fluorine by acetyl hypofluorite enantiomerically pure (*S*)-3-fluorotyrosine, (*S*)-monofluorophenylalanine and ^{18}F labelled (*S*)−[3-^{18}F]−fluorotyrosine have been obtained.[83] Ring fluorination of aromatic amino acids can also be achieved by elemental fluorine. Thus, treatment of a solution of tyrosine in hydrofluoric acid with N_2-diluted fluorine gas yields 3-fluorotyrosine.[84]

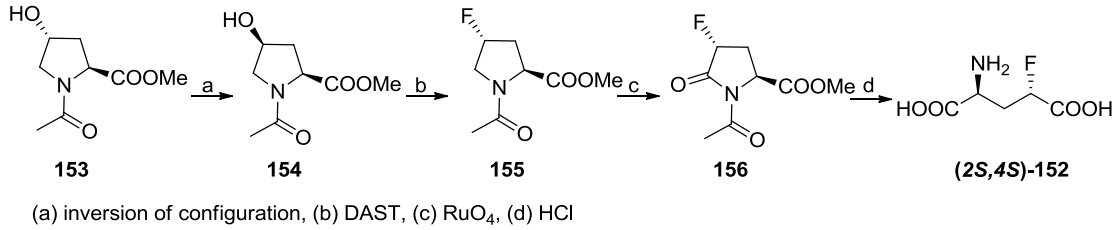
2.2.2 Deoxygenative Fluorination

An alternative approach to the direct introduction of fluorine is the reaction of deoxygenative fluorination. Fluorinating reagents that derived from SF_4, such as DAST and Deoxofluor, are widely used deoxygenative fluorination reagents for alcohols, aldehydes, ketones, and carboxylic acids without preactivation and offer a straightforward and easy access to organic fluorides from readily available starting materials.[85] These aminosulfurane derivatives have evolved to several generations,[86] and their synthetic applications have been a topic of research of many groups.[87-93] Recently, new reagents, XtalFluor E and XtalFluor-M as well as 4-tert-butyl-2,6-dimethylphenylsulfur trifluoride (Fluolead),[94-98] were developed as a new generation of selective fluorinating agents that show relatively high thermal stability and robustness. These nucleophilic fluorinating agents function so that an alkoxyaminosulfur difluoride intermediate is first formed, followed by a nucleophilic attack of the fluoride onto the carbon to give the fluorinated product (Scheme 2.34). The driving force for this reaction is the strong affinity of the sulfur atom toward oxygen (S−O bond 124 kcal/mol vs S−F bond 82 kcal/mol).[99]



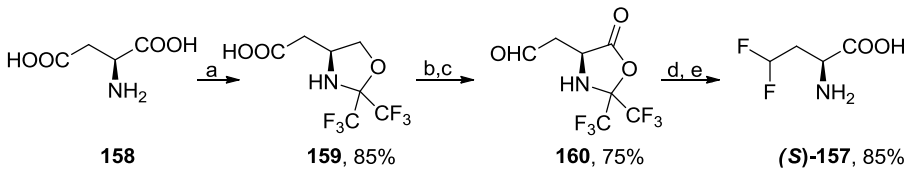
Scheme 2.34 Mechanism of DAST fluorination.

Despite the price and the instability of deoxyfluorinating reagents DAST and Deoxofluor tend to be thermally unstable, they are commonly used in the synthesis of fluorinated amino acids. One example where DAST was used to introduce fluorine is in the transformation of hydroxyproline into (2*S*,4*S*)-4-fluoroglutamic acid (**152**, Scheme 2.35).[100] Fluorodehydroxylation of (−)-*N*-acetyl-*trans*-4-hydroxy-(*S*)-proline methyl ester **153** using DAST gave optically active methyl ester of 4-fluoroproline (**154**) whose oxidation and subsequent hydrolysis yields (2*S*,4*S*)-4-fluoroglutamic acid (**152**) (Scheme 2.35).



Scheme 2.35 Synthesis of (2*S*,4*S*)-4-fluoroglutamic acid **152** by direct fluorination using DAST.

This fluorination method can also be applied to the compounds bearing a carbonyl group which can be difluorinated or trifluorinated (carbonyl vs. carboxyl group). Following are two examples where HFA was used to simultaneously protect amino and carboxylic group of an amino acid whereas an additional carbonyl group in the side chain was transformed into a fluorinated moiety using DAST. Aspartic acid was the starting material for synthesis of (*S*)-2-amino-4,4-difluorobutanoic acid (**157**). HFA protection offered the possibility to reduce the carboxylic group in the side chain while retaining the α-carboxylic group. Subsequent fluorination using DAST gave (*S*)-2-amino-4,4-difluorobutanoic acid (**157**). [101]

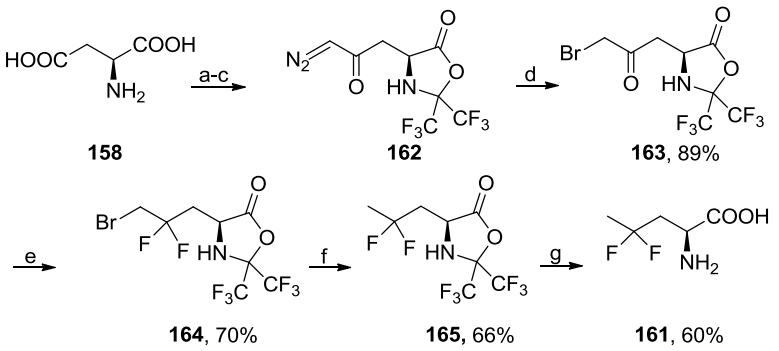


158 **159**, 85% **160**, 75% ***(S)*-157**, 85%

(a) Hexafluoroacetone, (b) $SOCl_2$, (c) $Pd/BaSO_4$, (d) DAST, (e) H_2O / *i*-PrOH

Scheme 2.36 Synthesis of (*S*)-2-amino-4,4-difluorobutanoic acid (**157**) by using DAST for introduction of fluorine to the side chain.

The broad applicability of the HFA protection method in combination with DAST for fluorine introduction is mirrored in the fact that fluorine can be incorporated into a non-terminal position as well.[102] The synthesis of (*S*)-2-amino-4,4-difluoropentanoic acid (**161**) required a slightly different approach where carboxylic group in the ω-position was converted to the δ-carbonyl group which offers acces to deoxyfluorination (Scheme 2.37).



158 **162** **163**, 89%

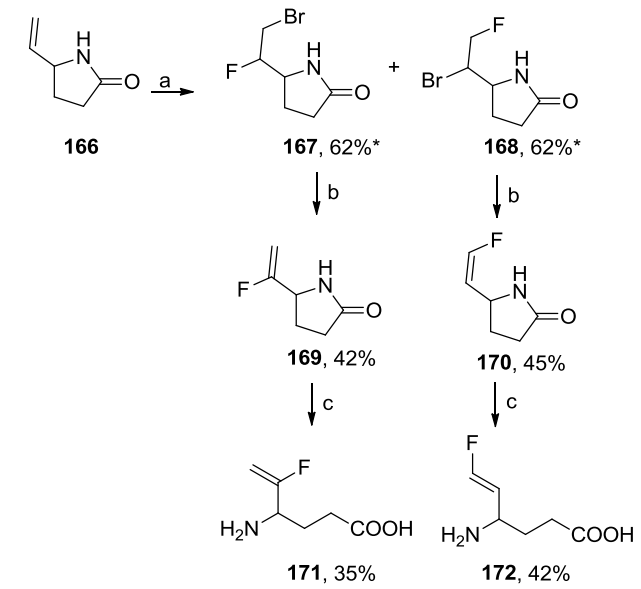
164, 70% **165,** 66% **161**, 60%

(a) Hexafluoroacetone, (b) $SOCl_2$, (c) CH_2N_2, (d) HBr, (e) DAST, (f) Bu_3SnH/AIBN, (g) H_2O/*i*-PrOH

Scheme 2.37 Synthesis of (*S*)-2-amino-4,4-difluoropentanoic acid (**161**).

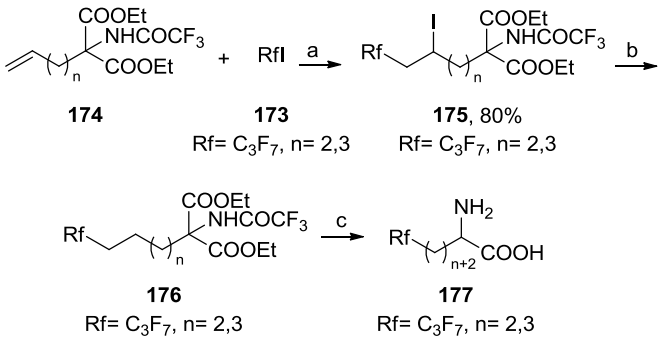
2.2.3 Application of Other Methods of Organofluorine Chemistry to the Synthesis of Fluorinated Amino Acids

Apart from the substitution of hydrogen by fluorine and deoxygenative fluorination, fluorine introduction can be carried out by the addition of fluorine (or fluorinated moieties) to the double bond. For instance, using fluorinating reagents, fluorine can be introduced to the terminal double bond in the molecule (Scheme 2.38 and 2.39). Similar simultaneous addition, in this case of fluorine and bromine atoms, to 5-vinylpyrrolid-2-one (**166**) gives 5-(bromofluoroethyl) pyrrolidones **167** and **168**. Dehydrobromination of compounds **167** and **168** and subsequent hydrolysis exposes γ-amino-ω-fluorovinylbutyric acids **169** and **170** (Scheme 2.38).[103] Reaction of perfluoroalkyl iodides with alkenic acids exhibiting a terminal C=C bond[104] became a general method for synthesis of long perfluorinated amino acids.[105] The addition of perfluoropropyl iodide **173** to **174** in the presence of the radical initiator azo-bis(isobutyronitrile) gives the products **175** which after reduction and deprotection afford **177** (Scheme 2.39).



(a) HF, pyridine, NBS, *167+168, (b) *t*-BuOK,THF, (c) 1 N HCl

Scheme 2.38 Synthesis of fluorinated amino acids **171** and **172** by introduction of fluorine using HF pyridine.



(a) ABN, (b) Zn/HCl, (c) NaOH

Scheme 2.39 Synthesis of fluorinated amino acids **177** through radical addition of perfluorinated side chain.

Bibliography

[1] V. Tolman, *Amino Acids* **1996**, *11*, 15.
[2] P. K. Valerii, A. S. Vadim, *Russian Chemical Reviews* **1991**, *60*, 850.
[3] M. Raasch, *The Journal of Organic Chemistry* **1958**, *23*, 1567.
[4] T. Tsushima, K. Kawada, S. Ishihara, N. Uchida, O. Shiratori, J. Higaki, M. Hirata, *Tetrahedron* **1988**, *44*, 5375.
[5] T. Taguchi, G. Tomizawa, M. Nakajima, Y. Kobayashi, *Chemical & Pharaceultical Bulletin* **1985**, *33*, 4077.
[6] B. M. Trost, H. C. Arndt, P. E. Strege, T. R. Verhoeven, *Tetrahedron Letters* **1976**, *17*, 3477.
[7] K. K. Tadahiko Tsushima, Osamu Shiratori, and Naomi Uchida, *Heterocycles* **1985**, *23*, 45.
[8] P. Bey, J. P. Vevert, *Tetrahedron Letters* **1978**, *19*, 1215.
[9] P. Bey, J. B. Ducep, D. Schirlin, *Tetrahedron Letters* **1984**, *25*, 5657.
[10] P. Bey, J. P. Vevert, V. Van Dorsselaer, M. Kolb, *The Journal of Organic Chemistry* **1979**, *44*, 2732.
[11] S. Kobayashi, *Synlett* **1994**, *1994*, 689.
[12] E. D. Bergmann, D. Ginsburg, R. Pappo, in *Organic Reactions*, John Wiley & Sons, Inc., **2004**.
[13] V. A. Soloshonok, V. P. Kukhar, S. V. Galushko, N. Y. Svistunova, D. V. Avilov, N. A. Kuz'mina, N. I. Raevski, Y. T. Struchkov, A. P. Pysarevsky, Y. N. Belokon, *Journal of the Chemical Society, Perkin Transactions 1* **1993**, 3143.
[14] V. P. Kukhar, Y. N. Belokon, V. A. Soloshonok, N. Y. Svistunova, A. B. Rozhenko, N. A. Kuz'mina, *Synthesis* **1993**, *1993*, 117.
[15] V. A. Soloshonok, T. Ono, *Journal of Fluorine Chemistry* **2009**, *130*, 547.
[16] A. E. Sorochinsky, J. L. Aceña, H. Moriwaki, T. Sato, V. A. Soloshonok, *Amino Acids* **2013**, *45*, 691.
[17] T. Rosen, in *In Comprehensive Organic Synthesis, Vol. 2* (Ed.: B. M. F. Trost, I.; Heathcock, C. H.), Pergamon, Oxford, **1991**, pp. 402.
[18] G. Schiemann, W. Roselius, *Berichte der deutschen chemischen Gesellschaft (A and B Series)* **1932**, *65*, 1439.
[19] G. Schiemann, W. Winkelmüller, *Journal für Praktische Chemie* **1932**, *135*, 101.
[20] E. L. Bennett, C. Niemann, *Journal of the American Chemical Society* **1950**, *72*, 1800.
[21] R. Filler, H. Novar, *The Journal of Organic Chemistry* **1960**, *25*, 733.
[22] V. P. M. G. V. Shishikin, *Zhurnal Obschchei Khimii* **1966**, *36*, 660.
[23] R. Filler, N. R. Ayyangar, W. Gustowski, H. H. Kang, *The Journal of Organic Chemistry* **1969**, *34*, 534.
[24] A. T. Prudchenko, *Izv Sib Otd Akad Nauk SSSR, Ser Khim Nauk* **1970**, *14*, 95.
[25] D. J. C. Alexander V. Sameta, A. C. Buchanan & Andrei A. Gakha, *Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry* **2002**, *32*, 941.
[26] S. Chandrasekhar, P. Karri, *Tetrahedron Letters* **2006**, *47*, 5763.
[27] R. R. E. Baltazzi, *Chemistry and Industry* **1954**, 191.
[28] in *US P. 4370504*.
[29] I. Ojima, *L'Actualite Chemique* **1987**, 171.
[30] S. Chandrasekhar, P. Karri, *Tetrahedron Letters* **2007**, *48*, 785.
[31] R. Dannley, R. Taborsky, *The Journal of Organic Chemistry* **1957**, *22*, 1275.
[32] H. M. Walborsky, M. Baum, D. F. Loncrini, *Journal of the American Chemical Society* **1955**, *77*, 3637.
[33] in *US P. 2662915*.
[34] L. Martarello, J. McConathy, V. M. Camp, E. J. Malveaux, N. E. Simpson, C. P. Simpson, J. J. Olson, G. D. Bowers, M. M. Goodman, *Journal of Medicinal Chemistry* **2002**, *45*, 2250.

[35] J. A. Monn, M. J. Valli, S. M. Massey, R. A. Wright, C. R. Salhoff, B. G. Johnson, T. Howe, C. A. Alt, G. A. Rhodes, R. L. Robey, K. R. Griffey, J. P. Tizzano, M. J. Kallman, D. R. Helton, D. D. Schoepp, *Journal of Medicinal Chemistry* **1997**, *40*, 528.
[36] J. L. Monteiro, B. Pieber, A. G. Corrêa, C. O. Kappe, *Synlett* **2016**, *27*, 83.
[37] N. Muller, *Journal of Fluorine Chemistry* **1987**, *36*, 163.
[38] K. Weinges, E. Kromm, *Liebigs Annalen der Chemie* **1985**, *1985*, 90.
[39] P. Bravo, S. Capelli, S. V. Meille, P. Seresini, A. Volonterio, M. Zanda, *Tetrahedron: Asymmetry* **1996**, *7*, 2321.
[40] T. Kitazume, J. T. Lin, T. Yamazaki, *Tetrahedron: Asymmetry* **1991**, *2*, 235.
[41] J. McConathy, L. Martarello, E. J. Malveaux, V. M. Camp, N. E. Simpson, C. P. Simpson, G. D. Bowers, J. J. Olson, M. M. Goodman, *Journal of Medicinal Chemistry* **2002**, *45*, 2240.
[42] J. J. Posakony, T. J. Tewson, *Synthesis* **2002**, *2002*, 0766.
[43] M. E. V. Dort, Y.-W. Jung, P. S. Sherman, M. R. Kilbourn, D. M. Wieland, *Journal of Medicinal Chemistry* **1995**, *38*, 810.
[44] D. M. Wieland, M. R. Kilbourn, D. J. Yang, E. Laborde, D. L. Gildersleeve, M. E. van Dort, J. L. Pirat, B. J. Ciliax, A. B. Young, *International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes* **1988**, *39*, 1219.
[45] L. T. Boulton, H. Thijs Stock, J. Raphy, D. C. Horwell, *Journal of the Chemical Society, Perkin Transactions 1* **1999**, 1421.
[46] S. Dhruva Kumar, Anil Saini, and Jagir S. Sandhu, *Ra Sayan Journal of Chemistry* **2008**, *1*, 639.
[47] H. Lettré, U. Wölcke, *Justus Liebigs Annalen der Chemie* **1967**, *708*, 75.
[48] J. Lazar, W. A. Sheppard, *Journal of Medicinal Chemistry* **1968**, *11*, 138.
[49] D. F. Loncrini, H. M. Walborsky, *Journal of Medicinal Chemistry* **1964**, *7*, 369.
[50] H. M. Walborsky, M. E. Baum, *The Journal of Organic Chemistry* **1956**, *21*, 538.
[51] I. A. McDonald, M. G. Palfreyman, M. Jung, P. Bey, *Tetrahedron Letters* **1985**, *26*, 4091.
[52] W. H. Vine, K.-H. Hsieh, G. R. Marshall, *Journal of Medicinal Chemistry* **1981**, *24*, 1043.
[53] I. Ojima, K. Kato, K. Nakahashi, T. Fuchikami, M. Fujita, *The Journal of Organic Chemistry* **1989**, *54*, 4511.
[54] A. Gloge, J. Zoń, Á. Kövári, L. Poppe, J. Rétey, *Chemistry – A European Journal* **2000**, *6*, 3386.
[55] P. Meffre, R. H. Dave, J. Leroy, B. Badet, *Tetrahedron Letters* **2001**, *42*, 8625.
[56] *Organometallics in Synthesis, The Manual, Vol. 2*, John Wiley & Sons Ltd., England, **2002**.
[57] A. Conan, S. Sibille, J. Perichon, *The Journal of Organic Chemistry* **1991**, *56*, 2018.
[58] J. Dekker, P. H. M. Budzelaar, J. Boersma, G. J. M. Van der Kerk, A. J. Spek, *Organometallics* **1984**, *3*, 1403.
[59] L. Wessjohann, T. Gabriel, *The Journal of Organic Chemistry* **1997**, *62*, 3772.
[60] J. F. Ruppert, J. D. White, *The Journal of Organic Chemistry* **1974**, *39*, 269.
[61] M.-X. Zhao, M.-X. Wang, C.-Y. Yu, Z.-T. Huang, G. W. J. Fleet, *The Journal of Organic Chemistry* **2004**, *69*, 997.
[62] M. S. Newman, F. J. Evans, *Journal of the American Chemical Society* **1955**, *77*, 946.
[63] K. Uneyama, *Organofluorine Chemistry*, Blackwell Publishing Ltd,, Oxford, **2006**.
[64] P. Kirsch, in *Modern Fluoroorganic Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA, **2005**, pp. 299.
[65] T. Hiyama, *Organofluorine Compounds*, **2000**.
[66] R. D. Chambers, *Fluorine in Organic Chemistry*, Blackwell Publishing Ltd., **2004**.
[67] J.-P. Bégué, D. Bonnet-Delpon, *Bioorganic and Medicinal Chemistry of Fluorine*, John Wiley & Sons, Inc., **2007**.
[68] *Fluorine in Pharmaceutical and Medicinal Chemistry* Imperial College Press, London, **2012**.
[69] (Ed.: I. Ojima), John Wiley & Sons, Ltd, **2009**, pp. 1.
[70] G. W. Gribble, *Chemical Society Reviews* **1999**, *28*, 335.
[71] M. G. Campbell, T. Ritter, *Organic Process Research & Development* **2014**, *18*, 474.
[72] T. Fuchigami, *Electrochemistry* **2013**, *81*, 913.
[73] C. Hollingworth, V. Gouverneur, *Chemical Communications* **2012**, *48*, 2929.
[74] U. Hennecke, *Angewandte Chemie International Edition* **2012**, *51*, 4532.

[75] T. Furuya, A. S. Kamlet, T. Ritter, *Nature* **2011**, *473*, 470.
[76] S. Lectard, Y. Hamashima, M. Sodeoka, *Advanced Synthesis & Catalysis* **2010**, *352*, 2708.
[77] V. V. Grushin, *Accounts of Chemical Research* **2010**, *43*, 160.
[78] in *US P. 4427661*.
[79] R. V. Weatherman, *Organic & Biomolecular Chemistry* **2003**, *1*, 3257.
[80] V. Tolman, K. Vereš, *Tetrahedron Letters* **1966**, *7*, 3909.
[81] K. V. V Tolman, *Collect. Czech. Chem. Commun.* **1967**, *32*, 4460.
[82] A. F. Janzen, P. M. C. Wang, A. E. Lemire, *Journal of Fluorine Chemistry* **1983**, *22*, 557.
[83] M. Murakami, K. Takahashi, Y. Kondo, S. Mizusawa, H. Nakamichi, H. Sasaki, E. Hagami, H. Iida, I. Kanno, S. Miura, K. Uemura, *Journal of Labelled Compounds and Radiopharmaceuticals* **1988**, *25*, 773.
[84] Y. T. Y. s. V P Kukhar, I I Gerus, M T Kolycheva, *Russian Chemical Reviews* **1991**, *60*, 1050.
[85] C. Ni, M. Hu, J. Hu, *Chemical Reviews* **2015**, *115*, 765.
[86] N. Al-Maharik, O'Hagan, D. , *Aldrichimica Acta* **2011**, *44*, 65.
[87] R. P. Singh, J. n. M. Shreeve, *Synthesis* **2002**, *2002*, 2561.
[88] K. A. Jolliffe, *Australian Journal of Chemistry* **2001**, *54*, 75.
[89] M. Hudlicky, *Org. React.* **1987**, *35*, 513.
[90] I. D. Haidy Ferret, Domingo Gomez Pardo,, a. J. C. Luc Van Hijfte, *Arkivoc* **2010**, *8*, 126.
[91] W. C. Smith, *Angewandte Chemie International Edition in English* **1962**, *1*, 467.
[92] J. A. Wilkinson, *Chemical Reviews* **1992**, *92*, 505.
[93] T.-X. Metro, B. Duthion, D. Gomez Pardo, J. Cossy, *Chemical Society Reviews* **2010**, *39*, 89.
[94] T. Umemoto, R. P. Singh, Y. Xu, N. Saito, *Journal of the American Chemical Society* **2010**, *132*, 18199.
[95] in *U.S. Patent 7265247*.
[96] in *U.S. Patent 7381846*.
[97] R. P. Singh, T. Umemoto, *The Journal of Organic Chemistry* **2011**, *76*, 3113.
[98] W. Xu, H. Martinez, W. R. Dolbier Jr, *Journal of Fluorine Chemistry* **2011**, *132*, 482.
[99] Y.-R. Luo, *CRC Handbook of Chemistry and Physics,, Vol. 89*, CRC Press/Taylor and Francis, Florida, USA, **2009**.
[100] M. Hudlický, *Journal of Fluorine Chemistry* **1993**, *60*, 193.
[101] D. Winkler, K. Burger, *Synthesis* **1996**, *1996*, 1419.
[102] S. N. Osipov, T. Lange, P. Tsouker, J. Spengler, L. Hennig, B. Koksch, S. Berger, S. M. El-Kousy, K. Burger, *Synthesis* **2004**, *2004*, 1821.
[103] M. Kolb, J. Barth, J. G. Heydt, M. J. Jung, *Journal of Medicinal Chemistry* **1987**, *30*, 267.
[104] N. O. Brace, *The Journal of Organic Chemistry* **1962**, *27*, 4491.
[105] N. O. Brace, *The Journal of Organic Chemistry* **1967**, *32*, 430.

3. Synthesis of Specific Classes of Fluorinated Amino Acids: α-CF_3 Amino Acids and Optically Active Amino Acids

After revising the available methods for synthesis of fluorinated amino acids in general, this subchapter focus will be brought to the synthetic strategies for two specific classes of fluorinated amino acids, namely, α-CF_3 amino acids and enantiomerically pure fluorinated amino acids. The first part of this chapter deals with the significance and synthesis of α-trifluoromethyl amino acids while the second part is oriented towards the access to optically active fluorinated amino acids with the emphasis on the enzymatic synthetic approach.

3.1 Synthesis of α-CF_3 Amino Acids and Their Significance

α-Trifluoromethylated amino acids belong to a class of α,α-dialkylated amino acids. Alkylation of the α-position of amino acids results in a significant steric constraint. The reason for this lies in the perturbation of dihedral angles between all functional groups attached to the quaternary carbon. When incorporated into peptides, these amino acids cause conformational changes in peptides and proteins.[1-3] One advantage of the incorporation of an amino acid with such a steric constraint is the possibility to design peptides with unique tertiary structure. In addition to the different steric demand,[4-7] electronic and electrostatic[5-7] characteristics of the trifluoromethyl group also offer the unique set of features which can influence the properties of the newly synthesized fluorinated peptide variant.[8-9] Incorporation of these amino acids into certain peptides can cause a retardation of the proteolytic degradation,[9] augmentation of *in vivo* absorption as well as drug permeability through certain body barriers[10] and enhancement of thermal stability of peptides.[11] Furthermore, α-trifluoromethylated amino acids can offer insight into peptide secondary and tertiary structures by ^{19}F NMR studies.[12]

Nonetheless, the fluorine effect depends on the position of the CF_3 substituent in a peptide.[13-15] For instance, trifluoromethyl group in aromatic systems can only be hydrolysed by strong acid. However, a trifluoromethyl group on a carbon atom attached to acidic hydrogen atoms such as in 3,3,3-trifluoroalanine is unstable in basic medium. pH higer than 7.0 causes a degradation of trifluoromethyl group to give the corresponding carboxylate.[16-17] Incorporation of trifluoromethyl groups, increases the chemical stability of molecules due to the high bond strength, as an example; the C−C-bond in 1,1,1-trifluoroethane or hexafluoroethane is 59 and 42 kJ more stable when compared to ethane.[18-19]

With the aforementioned benefits in mind, it comes as no surprise that this molecule is an interesting and valuable target. Synthetic approaches to this molecule include direct fluorination or using a fluorinated synthon.[20] Although, direct fluorination can offer a faster and more straightforward way to the target molecules, it is often difficult to control the regio- and stereoselctivity of its incorporation. Additionally, other functional groups in the molecule, if not protected, can be affected in an undesired way. Fluorinated reagents employed for this purpose are expensive, toxic, corrosive, and sometimes explosive. Therefore, using fluorinated synthones remains the attractive alternative to introduce fluorine and perfluoroalkyl groups into organic molecules.[20]

When looking at the molecule of *α*-trifluoromethylated amino acid and thinking in terms of fluorinated synthons, four logical disconnections follow (Figure 3.1). Every retrosynthetic pathway presents the introduction of a single functional group (carboxylic group (a), amino group (b), the alkyl/aryl chain (c) or the (d) (Figure 3.1). In the next four subchapters, every mentioned disconnection (Figure 3.1) will be examined separately, as a specific synthetic pathway to the desired taget molecule.

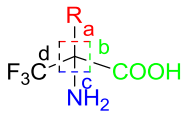
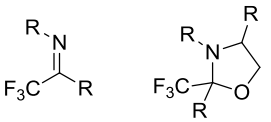


Figure 3.1 Crucial disconnections of *α*-trifluoromethylated amino acids.

3.1.1 Introduction of Carboxylic Moiety

Herein, the incorporation of the carboxylic moiety will be discussed, specifically, introduction of the carboxylic moiety through the Strecker reaction and alkoxycarbonylation. The Strecker reaction, described in the chapter 2.1.5 (Scheme 2.16)[21] is one of the most used methods for preparing *α*-amino acids. It uses the reactivity of *in situ* formed imines with cyanide in the presence of an amine hydrochloride to form an *α*-aminonitrile which is subsequently hydrolyzed to yield the desired *α*-amino acid (Scheme 2.16). When trifluoromethylated ketones are subjected to Strecker reaction, direct addition of the nitrile produces a very stable cyanohydrin. The competition between the cyanide attack on the imine or the ketone results in low yields. It is for this reason that the classical Strecker reaction for the synthesis[22] of *α*-trifluoromethyl-*α*-amino acids is significantly limited. On the other hand, modifications of this synthetic strategy could offer useful approaches to the desired compounds.

The typical Strecker reaction, proceeds through an imine intermediate **178** but reaction can also proceed through the oxazolidinone intermediate **179** (Figure 3.2). This intermediate can be formed from trifluoromethylated ketones.[23] Cyanide addition to the described intermediates is found to be enhanced by the presence of a Lewis acid and by the electron-withdrawing trifluoromethyl group in the *α*-position.[24-25]

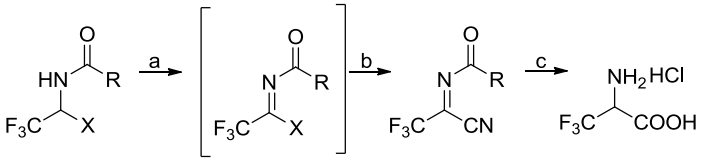


178 **179**

imines **178**, oxazolidinones **179**

Figure 3.2 Typical precursors in the Strecker synthesis of amino acids.

Some syntheses of *α*-trifluoromethylated amino acids that involve the imine type intermediate **178** will be discussed in more detail.[26-32] The first synthesis of hydrochloric salt of *α*-trifluoromethyl substituted amino acid, 3,3,3-trifluoroalanine (**180**)[16, 26, 28] was reported by hydrocyanation of electrophilic *N*-acyl trifluoroacetaldimines **181** as intermediates[16] and subsequent hydrolysis (Scheme 3.3). *N*-Acylimines of fluoral **181** show polymerization tendency, especially at elevated temperatures which is the reason they are generated *in situ* from stabile crystalline *N*-acyl-1-chloro-2,2,2-trifluoromethyl amines **182**.[33]

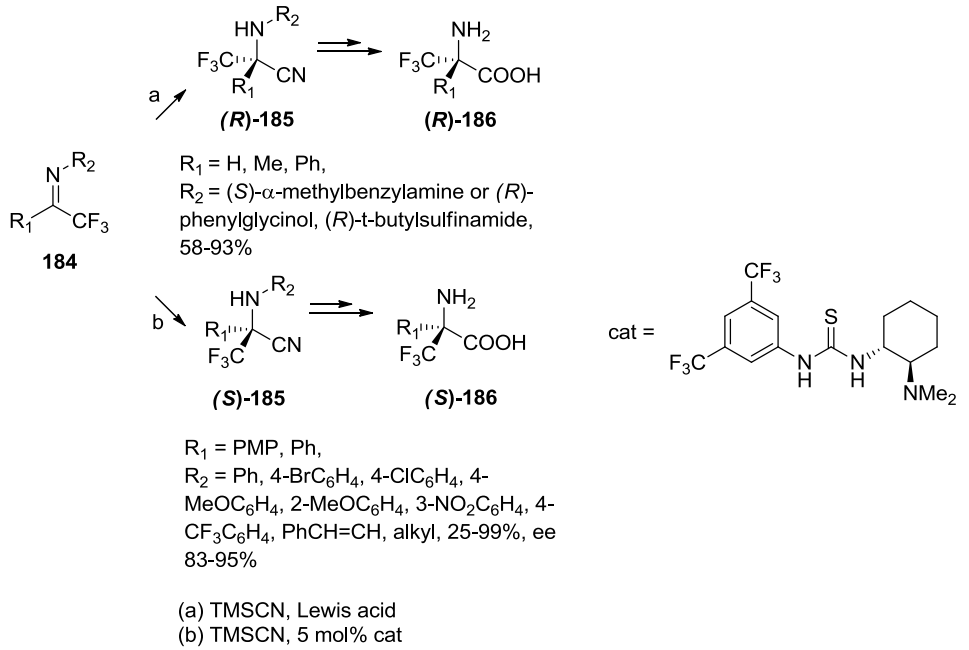


182 **181**, 50-80% **183**, 81-98% **180**, 65-81%

R= CH_3, Ph, X= Cl, Br, SOOEt,
(a) Et_3N, (b) HCN, (c) 6 N HCl, AcOH

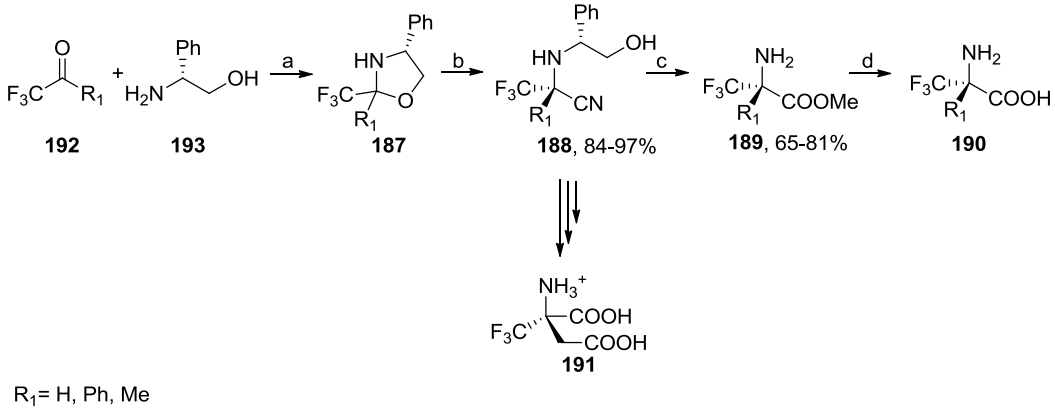
Scheme 3.3 Synthesis of 3,3,3-trifluoroalanine (**180**) by Strecker approach.

A Strecker reaction involving an imine intermediate was also used for the asymmetric synthesis of fluorinated amino acids. Using this method, chirality can be introduced into the molecule in two ways. One option is to have a chiral center present in the imine intermediate **184** (usually as a substituent on the nitrogen) (a, Scheme 3.4). Intermediate **184** was synthesized by the reaction of fluoral hemiacetal or *α*-trifluoromethyl ketones and (*S*)-*α*-methylbenzylamine or (*R)*-phenylglycinol and their derivatives or *(R)*-*t*-butylsulfinamide.[29, 34-35] The second option is a chiral catalyst which introduces the cyanide in an enantioselective fashion into a non-chiral intermediate **184** (b, Scheme 3.4).[32] Subsequent hydrolysis of the chiral amino nitrile and deprotection thereof furnishes the desired trifluoromethylated amino acid.[29, 34]



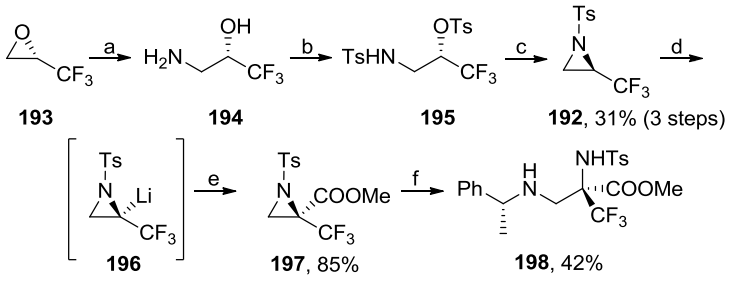
Scheme 3.4 Introduction of chirality to the target amino acid; chiral starting material or chiral catalyst approach.

Correspondingly, oxazolidinones **178** (Figure 3.2), also one of the intermediates in the synthesis of *α*-trifluoromethylated amino acids,[29, 34] can be accessed from similar starting materials used for imine synthesis (Scheme 3.5).[29] **187** can likewise be trapped by TMSCN in a Strecker-type reaction to give the amino nitrile intermediate **188** (Scheme 3.5) in better diastereoselectivities than in the case of imine intermediate.[29] **188** was conveniently converted into corresponding *α*-trifluoromethylated amino acid **190** by deprotecting the amino group and hydrolyis of the methyl ester (Scheme 3.5).[29, 34] Brigaud *et al.* expanded this method of amino nitrile formation[29] to the synthesis of enantiopure (*S*)- and (*R*)-*α*-trifluoromethyl-aspartic acid **191** (Scheme 3.5).



Scheme 3.5 Synthesis of trifluoromethylated amino acid **190** and access to enantiopure (*S*)- and (*R*)-α-trifluoromethyl-aspartic acid **191**.

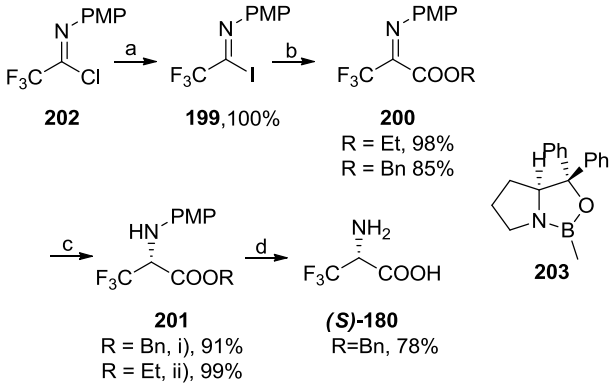
A complementary approach to introduction of the carbonyl group to the trifluoromethylated synthon is alkoxycarbonylation of *α*-trifluoromethylated aziridine and *N*-aryl substituted trifluoroacetoimidoyl iodides.[30, 36-37] Yamauchi *et al.* discovered that *α*-trifluoromethylated aziridine **192** could be prepared starting from optically pure 2,3-epoxy-1,1,1-trifluoropropane (**193**, Scheme 3.6). Deprotonation of **192** and reaction with ethylchloroformate yields an enantiomerically pure alkoxycarbonylation product **197**. *α*-Trifluoromethylated aziridines are highly reactive towards ring opening by nucleophiles and find its purpose in preparation of quaternary chiral *α*-trifluoromethylated amino acid derivatives **198** as shown below.



(a) NH_3 (aq), (b) TsCl, pyridine, (c) NaH, (d) *n*-BuLi, (e) MeOCOCl, (f) NaH, (*S*)-$PhCHMeNH_2$

Scheme 3.6 Introduction of carboxylic group to the trifluoromethylated aziridine to access α-trifluoromethylated amino acid **198**.

As mentioned,[37] alkoxycarbonylation can be performed on *N*-aryl substituted trifluoroacetoimidoyl iodides **199**. These substrates in a palladium catalyzed carboxylation reaction give the corresponding imine **200** (Scheme 3.8). After **200** was converted to *α*-imino trifluoropyruvate derivative **201**, the desired amino acid can be synthesized by applying specific conditions[31, 38-39] (Scheme 3.7). The nature of the *N*-substituent on the imine and the required alcohol affect the yield of the reaction. When DMF or 1,3-dimethyl-2-imidazolidinone were used as additives,[30] the *tert*-butoxycarbonylation of **199** could be performed which is a great advantage since this group can be removed using mild conditions.

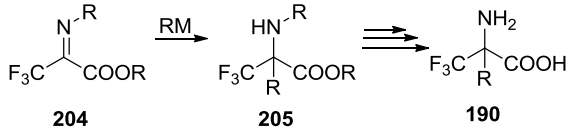


(a) NaI, (b) $Pd(dba)_3CHCl_3$, CO, ROH, K_2CO_3, (c) i) cateholborane oxaborilidine **203** or ii) $Pd(OCOCF_3)_2$, (*R*)-BINAP, H_2, CF_3CH_2OH, (d) i) CAN, ii) H_2, Pd/C, MeOH

Scheme 3.7 Alkoxycarbonylation of *N*-aryl substituted trifluoroacetoimidoyl iodides.

3.1.2 Alkylation of the Fluorinated Substrate

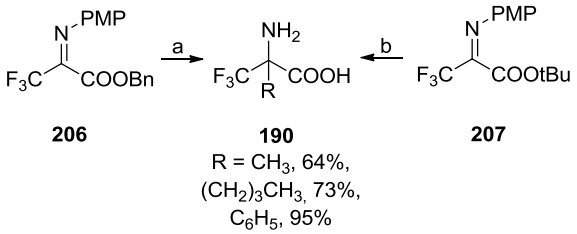
There is a variety of alkylation procedures for *N*-protected imines **204** of fluorinated pyruvates that yield *α*-trifluoromethylated amino acids of high structural diversity (Scheme 3.8).[40-41] This allows for the introduction of various saturated, unsaturated or even functionalized side chains using different organometallic derivatives or catalysts (Mg, Li, Zn, Cd, Ti, Pd). Grignard reagents are commonly used nucleophiles because they tolerate a variety of functional groups, including unsaturated bonds, and metalorganic moieties like organosilicon, organotin, and organocobalt substructures,[42-43] which can be used to further give multifunctional *α*-trifluoromethylated amino acids.[44] This approach also allows the incorporation of the Boc- and Cbz-group.



R= alkyl/aryl, M= Mg, Li, Zn, Cd, Ti, Pd

Scheme 3.8 Synthesis of *α*-trifluoromethylated amino acids by alkylation of fluorinated imine.

Specifically, alkylation of the imine **206** and **207** and subsequent removal of the protecting groups can afford *α*-trifluoromethylated-alanine, *α*-trifluoromethylated-phenylglycine and *α*-trifluoromethylated-norleucine (Scheme 3.9).[30, 37]



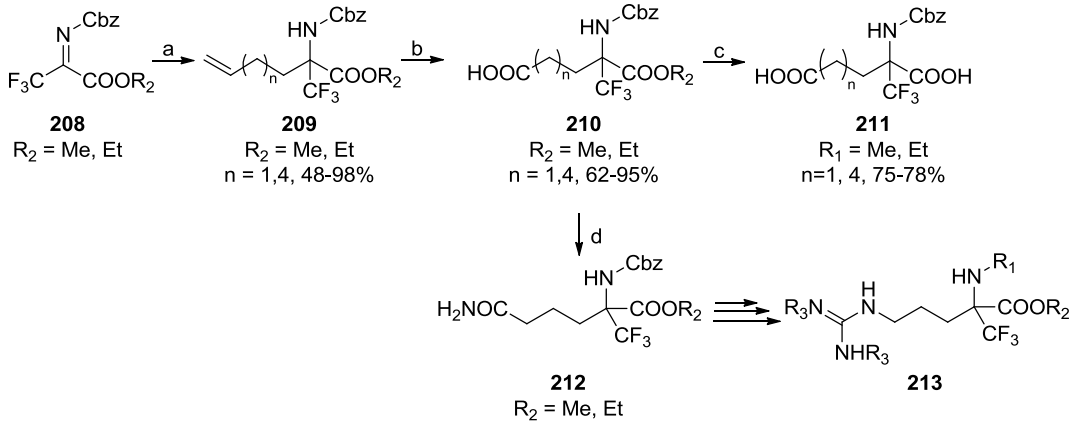
206 **190** **207**

R = CH_3, 64%,
$(CH_2)_3CH_3$, 73%,
C_6H_5, 95%

(a) i) MeLi or BuLi, ii) CAN, ii) H_2, Pd/C, (b) i) PhLi, ii) CAN, iii) HCl

Scheme 3.9 Access to *α*-trifluoromethylated amino acids **190** by alkylation of trifluorinated imines.

Changing the degree of saturation in the alkylating reagent allows for the synthesis of more complex and functionalized *α*-trifluoromethylated amino acids, such as *α*-trifluoromethylaspartate and *α*-trifluoromethyl-2-aminoheptanedioic acid (*α*-TfmPim).[45] Simple oxidation of the double bond gives the carboxylic group at the *ω*-position. If hydrolyzed, compound **210** furnishes *α*-trifluoromethylaspartate **211** and *α*-trifluoromethyl-2-aminoheptanedioic acid (**211**, *α*-TfmPim). Further chemical transformation affords *α*-trifluoromethylated arginine (**213**) (Scheme 3.10).[46]



208 R_2 = Me, Et

209 R_2 = Me, Et, n = 1,4, 48-98%

210 R_2 = Me, Et, n = 1,4, 62-95%

211 R_1 = Me, Et, n=1, 4, 75-78%

212 R_2 = Me, Et

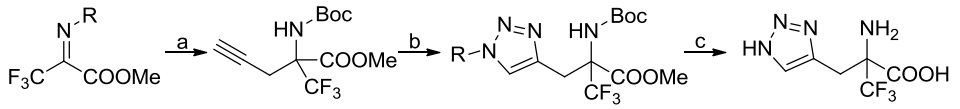
213

(a) $CH{=}CH(CH_2)_nMgBr$,n = 1,4, (b) $KMnO_4$, (c) i) NaOH, ii) H_2, Pd/C, (d) C_6F_5OH, pyridine, DCC, NH_4OH

Scheme 3.10 Synthesis of *α*-trifluoromethylaspartate and α-trifluoromethyl-2-aminoheptanedioic acid (*α*-TfmPim) and access to α-trifluoromethylated arginine (**213**).

Going even further, an alkyne can also be used as an alkylating agent for the *α*-trifluoromethylated pyruvate; this is exensively utilized in the synthesis of *α*-trifluromethylated amino acids. For instance, in the synthesis of *α*-trifluoromethylated-azahistidine analogues **214**[47] (Scheme 3.11 A) a simple alkyne group was attached to the *N*-protected trifluorinated pyruvate and later treated with azide in a click reaction resulting in a triazole functionality. In addition, a more complex *ω*-aminoalkynyl group (using $LiCCCH_2N(TMS)_2$), was introduced as a side chain, in the synthesis of *α*-trifluroomethylornithine (**218**) (Scheme 3.11 B).[48] The adduct **220**, was cyclized to 2-piperidone derivative **221** after hydrogenation of the triple bond (Scheme 3.11 B). Simultaneous cleavage of the

Boc group and ring opening upon treatment with HCl gave α-trifluoromethylated-ornithine hydrochloride (**218**).

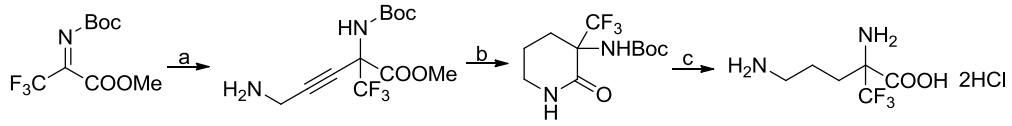


215 **216** **217** **214**

R= Z, CH_2OPiv, 41-74% R= Z, CH_2OPiv, 80-92% R= Z, CH_2OPiv, 94%

(a) CH_2=C=CHMgBr, (b) RN_3, $CuSO_4$, Na-ascorbate, (c) i) KOH, ii) H_2, Pd/C

A



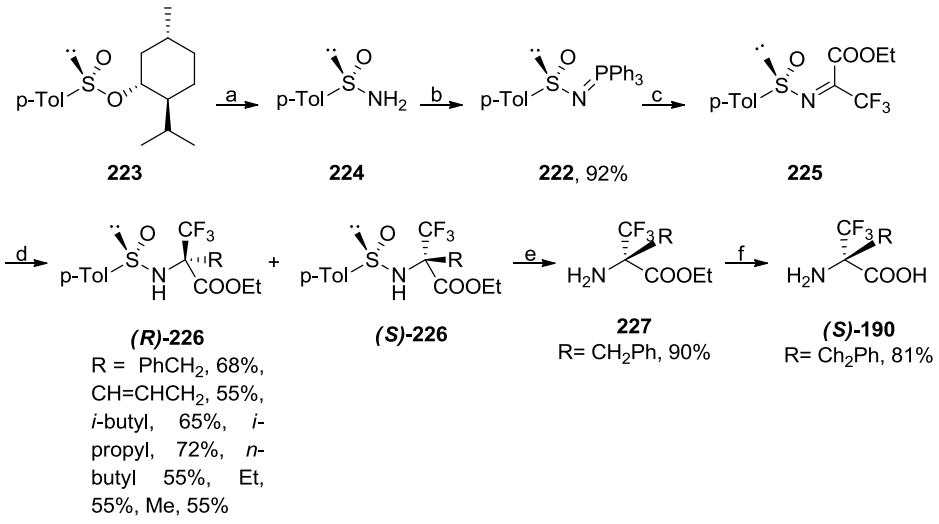
219 **220**, 91% **221**, 96% **218**, 97%

(a) i) $LiCCCH_2N(TMS)_2$, ii) HCl/H_2O, (b) H_2, Pd/C, (c) 6 N HCl

B

Scheme 3.11 A Synthesis of α-CF_3-azahistidine analogue **214**, **B** Synthesis of trifluoromethylornitine **218**

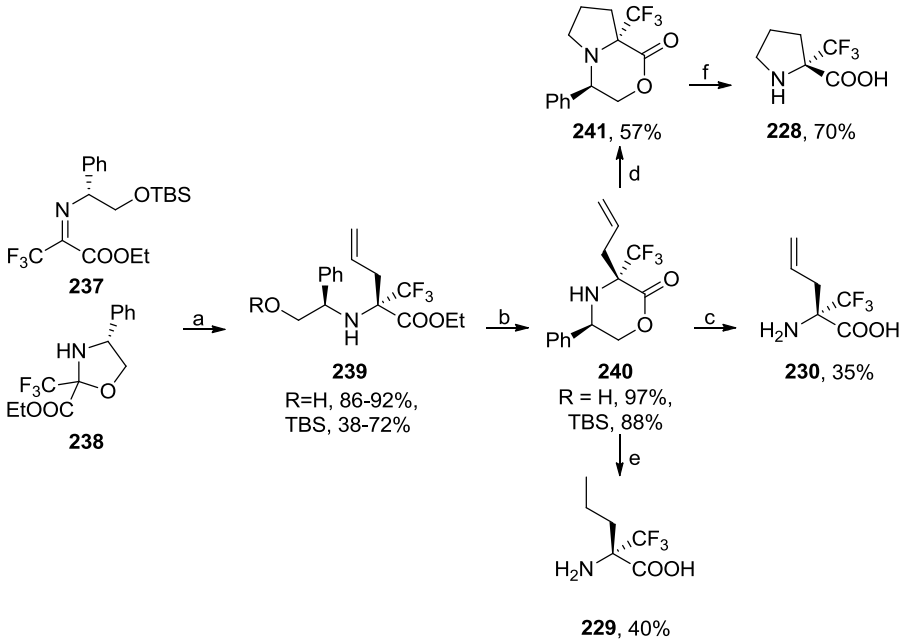
Up to this point, a Cbz or Boc protected imine of α-trifluoromethylated pyruvate has been presented and discussed. However, mounting a chiral sulfinyl group as a protecting and directing group on the nitrogen affords synthesis of optically active α-trifluoromethylated amino acids.[49-53] These derivatives are not prone to hydrolysis as the corresponding *N*-acyl and *N*-alkoxycarbonyl derivatives and they allow for the recovery of the chiral auxiliary as menthyl sulfinate. These compounds were synthesized by the aza-Wittig reaction from the chiral Staudinger reagent **222**, which is obtained from the Davis sulfinamide **224**, and ethyl trifluoropyruvate (Scheme 3.12). Sulfinimines **225** were coupled to different Grignard reagents and a variety of nonracemic amino acid derivatives was obtained. Diastereoselectivity depended on the nature of Grignard's reagent where usually more sterically hindered nucleophiles gave higher enantiomeric excess.



(a) LiHDMS, NH_4Cl/H_2O, (b) PPh_3, DEAD, (c) $CF_3COCOOEt$, (d) i) RMgCl, THF, ii) NH_4Cl, H_2O, (e) i) separation, ii) TFA, (f) i) 0.5 N KOH, $MeOH/H_2O$ ii) Dowex 50-W

Scheme 3.12 Synthesis of *α*-trifluoromethylated amino acid **190** by using a chiral sulfinyl imine **225**.

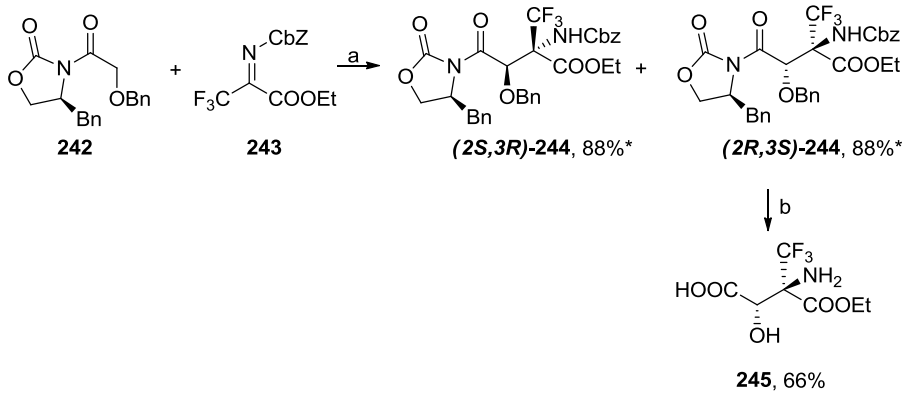
In analogy with the introduction of a carboxylic moiety to the molecule of *α*-trifluromethylated amino acid, where the key intermediate amino nitrile could be synthesized by cyanation of imine or alternatively oxazolidine (Scheme 3.5), introduction of the alkyl chain to the molecule of *α*-trifluoromethylated amino acid can also be performed by alkylation of oxazolidine. In the case of the synthesis of optically pure *(S)*-*α*-trifluoromethylproline (**228**), (*S*)-*α*-trifluoromethylnorvaline (**229**) and (*S*)-*α*-trifluroomethylallylglycine (**230**) Lewis acid promoted diastereoselective allylation of chiral *α*-trifluoromethylimine **237** or oxazolidine **238**[54] was conducted (Scheme 3.13). The allylation of the oxazolidine intermediate **238** was higher yielding when compared to the allylation of the imine **237** (Scheme 3.13), after cyclization of **239** to morpholines **240**, **240** could be converted to *(S)*-α-trifluoromethylallylglycine (**230**) and *(S)*-*α*-trifluoromethylnorvaline (**229**) (*α*-TfmNva), while the diastereomeric mixture was used for the synthesis of *(S)*-*α*-trifluoromethylproline (**228**). Hydroboration of the double bond, introduction of OH moiety using peroxide with installation of a good leaving group mesylate, followed by a ring closure yielded the pyrrolidine ring (bicyclic compound **241**). Removal of the chiral auxilary gave the enantiomers of *α*-trifluoromethylproline (**228**).



(a) CH_2=$CHCH_2SiMe_3$, Lewis acid, (b) R=H, PTSA, R=TBS, TBAF, (c) i) separation, ii) LiOH, iii) $Pb(OAc)_4$, iv) 3 N HCl, v) Dowex, (d) i) 9-BBN, ii) H_2O_2, NaOAc, iii) MsCl, Et_3N, iv) separation, (e) i) separation, ii) H_2, $Pd(OH)_2$, iii) 1N HCl, iv) Dowex, (f) i) H_2, PdOH, ii) 3 N HCl, iii) Dowex

Scheme 3.13 Access to to (*S*)- *α*-trifluoromethylallylglycine (**230**) and (*S*)-*α*-trifluoromethylnorvaline (**229**) (*α*-TfmNva (S)-*α*-trifluoromethylproline (**228**) by alkyation of imine **237** and oxazolidine **238**.

Furthermore, Zanda *et al.* reported on a commercially available *(S)*-(*α*-benzyloxy)acetyl 2-oxazolidinone (**242**) as a chiral moiety that can afford diastereomerically enriched product **244** (Scheme 3.14). Thus, Evans oxazolidinone is a strategy commonly used in selective aldol synthesis. Product (2*S,*3*S*)-**244** could be transformed to D-erythro-*α*-trifluoromethylated-*β*-hydroxy aspartate **245** by removal of the chiral auxiliary.[55] The value of this procedure is that it furnishes a D-*α*-trifluoromethylated amino acid.

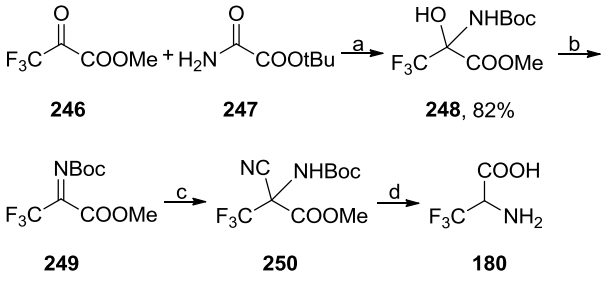


(a) $TiCl_4$, DIPEA, *overal isolated yield, (2*R*, 3*S*)-**244** major, (b) i) LiOH, H_2O_2, ii) H_2, $Pd(OH)_2$

Scheme 3.14 Synthetic entry towards to D-erythro-*α*-trifluoromethylated-*β*-hydroxy aspartate (**245**) by alkylation of **242**.

3.1.3 Amination of the Fluorinated Synthon

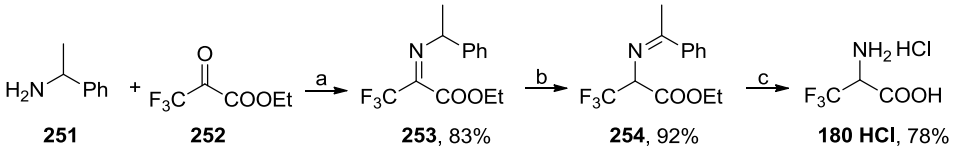
The third retrosynthetic approach via the amino group detachment to the fluorinated synthon is the next logical disconnection of the target molecule (Figure 3.1). Construction of this C−N bond is usually achieved through amination of fluorinated pyruvates, ketones and their derivatives which leads to imine formation. These imines can be further reduced and transformed to give the desired amino acids. Introduction of stereocenters usually arises from the use of chiral reducing agents or can be inherent with the use of chiral substrates. Alkylation of these imines is one way to get to the desired substrates (described in the chapter 3.1.2). The reaction between trifluoropyruvates and *N*-alkyl imines is not straightforward due to the difficulty of hemiaminal dehydration. Consequently, this is frequently done in a stepwise fashion.[56] For example, trifluoropyruvate (**246**) with **247** delivers a hemiaminal **248**, which has to be treated with trifluoroacetic anhydride to give the desired imine **249** (Scheme 3.15). After cyanation of the imine **249**, the resulting amino nitrile **250** is decarboxylated and deprotected by hydrogen chloride to *α*-trifluoromethylated glycine (**180**).



(a) 82%, (b) TFAA, pyridine, (c) TMSCN, (d) i) HCl, ii) Et_3N

Scheme 3.15 Synthesis of *α*-trifluoromethylated glycine (**180**) by direct amination of fluorinated synthon **246** and subsequent Strecker reaction.

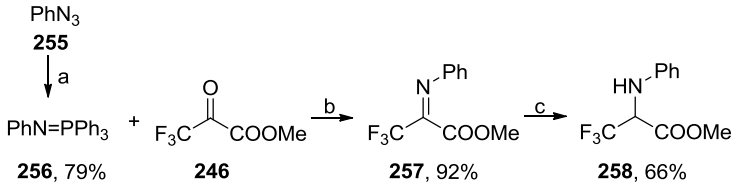
This methodology was further developed by the exchange of **247** by 1-phenyl-ethylamine (**251**) (Scheme 3.16)[57] which enabled direct transamination of the *α*-ketotrifluorocarboxylic ester (**252**) to the corresponding Schiff base **253** (Scheme 3.16). After consequent base catalyzed [1,3]-proton shift reaction (PSR), an isomeric ketamine **254** which can be hydrolyzed to afford *α*-trifluoromethyl glycine (**180**, Scheme 3.16).



(a) *p*-TsOH, (b) (1,3)-PSR, Et_3N, (c) i) 1 N HCl, ii) HCl conc.

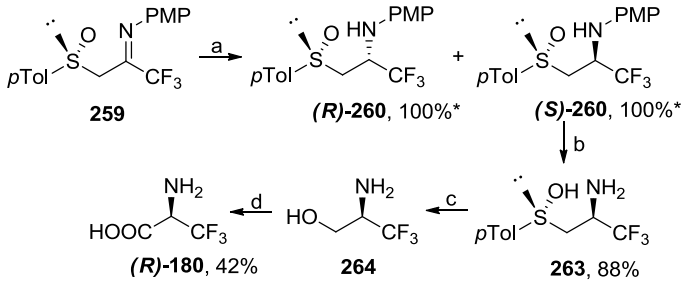
Scheme 3.16 Synthesis of *α*-trifluoromethyl glycine **180** by direct amination of **252**.

The C−N bond can also be constructed by the aza-Wittig reaction, condensing iminophosphorane **256** with methyl-trifluoromethylpyruvate **246**[58] where imine **257** is formed (Scheme 3.17) This imine can later be reduced to the amino ester **258** using zinc metal (Scheme 3.17).



Scheme 3.17 Synthesis of **258** through aza-Wittig reaction.

An alternative entry towards enantiomerically enriched *α*-trifluoromethylglycine **180** is using a chiral auxiliary instead of chiral reducing agents. As aforementioned, trifluoromethyl(arylsulfinyl)methyl imine (**259**) can be obtained from aza-Wittig reaction between *N*-aryl iminophosphoranes and chiral *γ*-trifluoro-*β*-ketosulfoxides.[59] The stereocenter in the starting material **259** ensures that the reduction by sodium borohydride gives the corresponding amine **260** in an enantioselective fashion (Scheme 3.18). After separation of the diastereomers, the *(S)*-amine **260** is transformed to enantiomerically pure *(R)*-*α*-trilfuoromethylglycine **180**.



Scheme 3.18 Synthesis of trifluoromethylglycine (**180**) by using a chiral auxiliary.

Examined syntheses of *α*-trifluoromethyl amino acids offer an approach to various analogues thereof however, they also employ multiple reaction steps and are not atom economical. The most straightforward way to access this class of fluorinated amino acids would be by direct functionalization of *α*-position of the amino acid. This would reduce the number of synthetic steps and avoid protection and deprotection during the synthetic process. In the literature direct CF_3 functionalization of the *α*-position of the amino acid has not been described.

3.2 Synthesis of Enantiomerically Pure Amino Acids- Enzymatic Approach

Next, an insight in the synthesis of another specific class of fluorinated amino acids will be given. The following subchapter will provide an insight in the synthesis of enantiopure fluorinated amino acids with the emphasis on the enzymatic synthetic approach.

3.2.1 Synthesis of Enantiomerically Pure Fluorinated Amino Acid, Standard Approach

Several research groups are focused on development of asymmetric syntheses for the preparation of fluorinated α-amino acids.[60-68] The usual approach to synthesis of mentioned compounds utilizes stereoselective alkylation of an enantiopure auxiliary.[69-74] For instance, an imine which contains the chiral protecting group to direct the stereochemistry in the Strecker reaction[29, 35, 75] Variation of that approach is starting from an enantiomerically pure coumpound, where stereochemistry is already set.[76] In addition, the use of the Ni(II) chiral complex represents another pathway of interest[70, 72] as well as employing chiral catalyst to induce stereoselectivity.[32, 49, 77-79] However, mentioned methods have downsides associated with expensive catalysts[80] and chiral auxiliaries or complexity and number of synthetic steps.

3.2.2 Enzymatic Synthesis of Amino Acids on an Industrial Scale

Awareness of the increasing environmental pollution has resulted in the development of atom-economical, environmentally friendly and greener chemical processes.[81-83] Cleaner production processes, renewable energy and reuse of the resources as well as treatment of waste and the produced amount thereof are some of the new chemical challenges. 25 years ago, the green chemistry concept emerged, based on the concepts of atom economy and the ratio of waste produced/product obtained.[81-84]

Catalytic processes can and should be a substitute for stoichiometric reactions because of their higher efficiency. The fact that more than 80% of the produced chemicals today are made using catalytic processes shows the importance of catalysis research.[82, 84]

Catalytic reactions should also be fast, selective and easily scaled-up. As a special field of catalytic reactions, biocatalytic transformations are environmentally acceptable, efficient and meet the standards of green chemistry (cost-efficiency, waste reduction, energy consumption).[81, 83-85]

The fact that enzymes can catalyze some of the most complex chemical reactions under mild conditions with very high specificity for the substrate reflects in widespread industrial use of enzymes in the textile, pharmaceutical and fine chemical industries. Industrially, amino acids are produced in

four basic ways: chemical synthesis (including asymmetric synthesis), extraction, fermentation, and enzymatic routes.[86] The classical chemical synthesis is applied to produce either the achiral glycine or racemic amino acids, for instance D,L-methionine. To access L-amino acids by this route, chemical synthesis has to be combined with a resolution step. Some cases of L-amino acid production include prochiral precursors and enzymes as chiral catalysts. There are many known catalytic asymmetric syntheses for many L-amino acids and their D-analogues, however, only a few are applied on industrial scale.[86] The mentioned extraction process offers access to almost all the proteinogenic L-amino acids by isolation from protein hydrolysates. In this procedure protein rich products are used as starting materials (keratin, feathers, bloodmeal or technical gelatin) (Fig. 3.19).

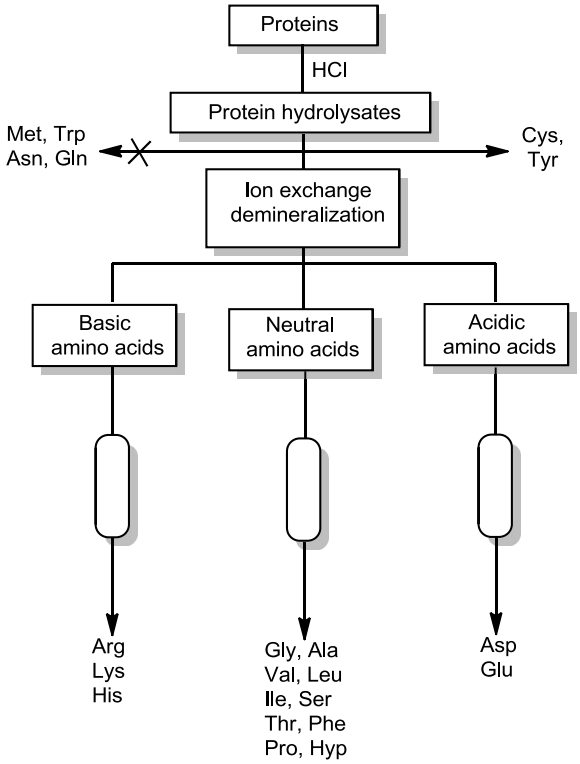
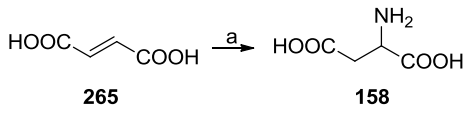


Figure 3.19 Extraction of amino acids.

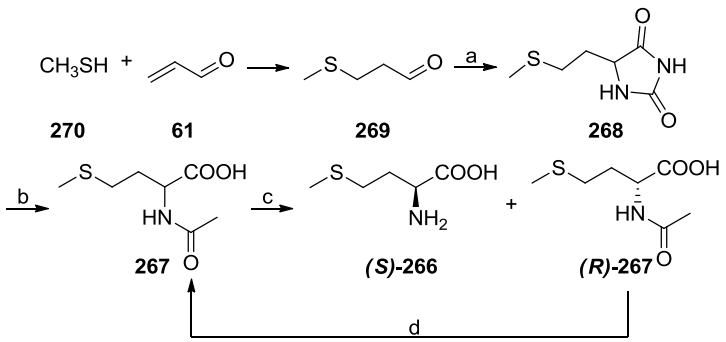
On the other hand, there are some examples of an enzymatic industrial amino acid production. Perhaps, the best example is the development of an enzymatic industrial process for aspartate production. An L-aspartate ammonia lyase catalyzed (L-aspartate ammonia lyase, EC 4.3.1.1) addition of ammonia to fumaric acid[87] affords the product in higher yield, productivity of the process is higher and the amount of byproducts is reduced (Scheme 3.20). This offers easy separation of L-aspartic acid from the reaction mixture by crystallization.



a) NH_3, L-aspartate ammnoia lyase

Scheme 3.20 L-aspartate ammonia lyase catalyzed synthesis of aspartate.

Later, a continuous production of L-aspartic acid employing immobilized L-aspartate ammonia lyase isolated from *Escherichia coli* was first commercialized in Japan.[88] The first industrial application of immobilized microbial cells in a fixed-bed reactor took place in 1973 with a large scale production of aspartate conducted by an immobilized cell system containing *E. coli* cells entrapped in polyacrylamide gel lattice.[89] This process was developed further and improved by immobilization of the cells in *k*-carrageenan, which afforded increased operational stability that enabled biocatalyst half-lives of almost two years.[90] L-aspartic acid became an importatnt intermediate in the manufacture of the dipeptide sweetener aspartame, methyl ester of L-aspartyl- L-phenylalanine, therefore the enzymatic production was further improved throughout the immobilization of *Escherichia coli* strains using polyurethane[91] or polyethylenimine, glass fiber support. Although, industrial production of aspartate evolved tremendously it is not the only example of an amino acid that is produced enzymatically on the industrial scale. L- Methionine (*(S)*-**266**) and valine are also produced using enzymatic transformations. However, both in the production of L-methionine (*(S)*-**266**) and L-valine, enzymes are only employed for the resolution of racemic *N*-acetyl-methionine (**267**) and *N*-acetyl-valine. In the case of methionine acylase from *Aspergillus oryzae* is used in a continuously operated fixed-bed or enzyme membrane reactor.[92] Alternatively the production of methionine from D,L-5-(2-methylthioethyl)hydantoin **268** utilizes growing cells of *Pseudomonas sp.* strain (Scheme 3.21).[93] It is evident that enzymes are exploited in industry as catalysts due to their ability to be recycled and reused and their incredible stereospecificity. Therefore, the enzymatic synthetic entries towards fluorinated amino acids are not surprising. These will be described in more detail in the next subschapter.

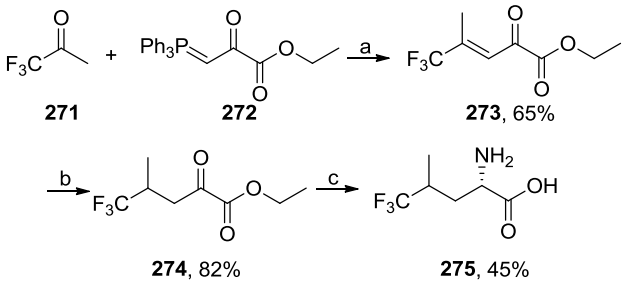


(a) HCN, NH_3, CO_2, (b) i) OH^-, ii) Ac_2O, (c) Aminoacylase **I** EC 3.5.1.14, (d) racemization

Scheme 3.21 Degussa production of methionine.

3.2.3 Synthesis of Amino Acids by Exploiting Enzymatic Processes

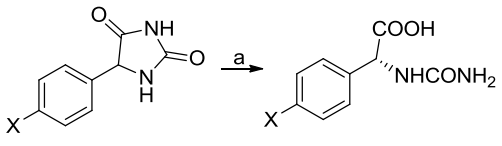
Although enzymes are mostly substrate specific and as fluorinated amino acids are not naturally occurring substrates, there is no general enzymatic method for their synthesis. Fortunatelly, not all the enzymes are completely substrate specific, and, moreover, they can be engineered to accept different substrates. Consequently, the synthesis of fluorinated amino acids can be performed enzymatically. The following examples describe three different types of enzymes that were employed in fluorinated amino acid synthesis. Phenylalanine dehydrogenase/formate dehydrogenase system was a system used in the synthesis of hexafluoroleucine, tetrafluoroleucine[94] and 5,5,5-trifluoroleucine.[95] Enzymatic system afforded the enantioselective introduction of the amino group into the molecule (Scheme 3.22).



(a) THF, (b) H_2, Pd/C, THF, (c) i) 1 M Na_2CO_3, ii) Phenylalanine dehydrogenase, formate dehydrogenase, NADH, 1.5 M $HCOONH_4$, pH 8.5

Scheme 3.22 Synthesis of 5,5,5-trifluoroleucine **275** by Phenylalanine dehydrogenase/formate dehydrogenase system.

A slightly different approach was used for synthesis of *N*-carbamoyl-D-*p*-fluorophenylglycine and *N*-carbamoyl-D-*p*-trifluoromethylphenylglycine (Scheme 3.23). Here, hydantoinase catalyzed the conversion of the initial hydantoin **276** to the desired fluorinated aromatic amino acids (Scheme 3.23).[96]



276
X=F, CF_3,

277
100% conv., 98% ee

(a) D-hydantoinase, pH 9

Scheme 3.23 Synthesis of *N*-carbamoyl-D-*p*-fluorophenylglycine and *N*-carbamoyl-D-*p*-trifluoromethylphenylglycine using hydantoinase.

A separate group of enzymes used for fluorinated amino acid synthesis are esterases. In the synthesis of (*S*)-*γ*-fluoroleucine ethyl ester, the stereoselectivity is introduced using Novozyme 435.[97] Resolution of trifluoroethylglycine by acylase was also a key step to access the enantiomerically pure compound.[98]

A final easy approach to enantiomerically pure fluorinated amino acids is using nitrilases, enzymes that catalyze the hydrolysis of nitrile group to a carboxylic group. These were employed in the synthesis of fluorophenylglycine.[99] After giving an overview of general synthesis of fluorinated amino acids and focusing on synthesis of two specific classes thereof (α-trifluoromethyl amino acids and optically active fluorinated amino acids), in the next chapter hydantoins, important derivatives of amino acids will be brought to the spotlight.

Bibliography

[1] C. Toniolo, M. Crisma, F. Formaggio, C. Peggion, *Peptide Science* **2001**, *60*, 396.
[2] C. Toniolo, F. Formaggio, B. Kaptein, Q. B. Broxterman, *Synlett* **2006**, *2006*, 1295.
[3] J. L. Aceña, A. E. Sorochinsky, V. A. Soloshonok, *Synthesis* **2012**, *44*, 1591.
[4] P. Bravo, A. Farina, M. Frigerio, S. V. Meille, F. Viani, V. Soloshonok, *Tetrahedron: Asymmetry* **1994**, *5*, 987.
[5] V. A. Soloshonok, T. Hayashi, K. Ishikawa, N. Nagashima, *Tetrahedron Letters* **1994**, *35*, 1055.
[6] V. A. Soloshonok, A. D. Kacharov, D. V. Avilov, T. Hayashi, *Tetrahedron Letters* **1996**, *37*, 7845.
[7] V. A. Soloshonok, A. G. Kirilenko, N. A. Fokina, V. P. Kukhar, S. V. Galushko, V. K. Švedas, G. Resnati, *Tetrahedron: Asymmetry* **1994**, *5*, 1225.
[8] M. Zanda, *New Journal of Chemistry* **2004**, *28*, 1401.
[9] B. K. Rene Smits, *Current Topics in Medicinal Chemistry* **2006**, *6*, 1483.
[10] A. G. a. M. J. J. J. T. Welch, in *General Features of Biological Activity of Fluorinated Amino Acids*, John Wiley & Sons, Chichester, **1995**, pp. 311.
[11] Y. Tang, G. Ghirlanda, W. A. Petka, T. Nakajima, W. F. DeGrado, D. A. Tirrell, *Angewandte Chemie International Edition* **2001**, *40*, 1494.
[12] D. Maisch, P. Wadhwani, S. Afonin, C. Böttcher, B. Koksch, A. S. Ulrich, *Journal of the American Chemical Society* **2009**, *131*, 15596.
[13] V. Asante, J. Mortier, G. Wolber, B. Koksch, *Amino Acids* **2014**, *46*, 2733.
[14] R. Csuk, B. Siewert, J. Wiemann, *Bioorganic & Medicinal Chemistry Letters* **2013**, *23*, 3542.
[15] C. Jäckel, M. Salwiczek, B. Koksch, *Angewandte Chemie International Edition* **2006**, *45*, 4198.
[16] F. Weygand, W. Steglich, I. Lengyel, F. Fraunberger, A. Maierhofer, W. Oettmeier, *Chemische Berichte* **1966**, *99*, 1944.
[17] Ü. Ü. B. Koksch, H.-D. Jakubke, *Pharmazie* **1997**, *52*, 74.
[18] A. S. Rodgers, W. G. F. Ford, *International Journal of Chemical Kinetics* **1973**, *5*, 965.
[19] A. S. R. S. S. Chen, J. Chao, R. C. Wilhoit and B. J. Zwolinski, *J. Phys. Chem. Ref. Data* **1975**, *4*, 441.
[20] M. Schlosser, *Tetrahedron* **1978**, *34*, 3.
[21] A. Strecker, *Justus Liebigs Annalen der Chemie* **1850**, *75*, 27.
[22] M. Koos, H. S. Mosher, *Tetrahedron* **1993**, *49*, 1541.
[23] J.-P. Begue, D. Bonnet-Delpon, B. Crousse, J. Legros, *Chemical Society Reviews* **2005**, *34*, 562.
[24] Y. Xu, W. R. Dolbier, *The Journal of Organic Chemistry* **2000**, *65*, 2134.
[25] T. Fuchigami, S. Ichikawa, *The Journal of Organic Chemistry* **1994**, *59*, 607.
[26] W. Steglich, H. U. Heininger, H. Dworschak, F. Weygand, *Angewandte Chemie International Edition in English* **1967**, *6*, 807.
[27] F. Weygand, K. Burger, K. Engelhardt, *Chemische Berichte* **1966**, *99*, 1461.
[28] F. Weygand, W. Steglich, *Chemische Berichte* **1965**, *98*, 487.
[29] F. Huguenot, T. Brigaud, *The Journal of Organic Chemistry* **2006**, *71*, 7075.
[30] H. Amii, Y. Kishikawa, K. Kageyama, K. Uneyama, *The Journal of Organic Chemistry* **2000**, *65*, 3404.
[31] H. Abe, H. Amii, K. Uneyama, *Organic Letters* **2001**, *3*, 313.
[32] D. Enders, K. Gottfried, G. Raabe, *Advanced Synthesis & Catalysis* **2010**, *352*, 3147.
[33] N. N. Sergeeva, A. S. Golubev, L. Hennig, K. Burger, *Synthesis* **2002**, *2002*, 2579.
[34] N. Lebouvier, C. Laroche, F. Huguenot, T. Brigaud, *Tetrahedron Letters* **2002**, *43*, 2827.
[35] H. Wang, X. Zhao, Y. Li, L. Lu, *Organic Letters* **2006**, *8*, 1379.
[36] Y. Yamauchi, T. Kawate, H. Itahashi, T. Katagiri, K. Uneyama, *Tetrahedron Letters* **2003**, *44*, 6319.
[37] H. Watanabe, Y. Hashizume, K. Uneyama, *Tetrahedron Letters* **1992**, *33*, 4333.
[38] T. Sakai, F. Yan, S. Kashino, K. Uneyama, *Tetrahedron* **1996**, *52*, 233.
[39] T. Sakai, F. Yan, K. Uneyama, *Synlett* **1995**, *1995*, 753.

[40] S. N. Osipov, A. Golubev, N. Sewald, A. F. Kolomiets, A. V. Fokin, K. Burger, *Synlett* **1995**, *1995*, 1269.
[41] N. S. a. K. Burger, in *Fluorine-containing Amino Acids* (Ed.: V. P. K. a. V. A. Soloshonok), Wiley & Sons, Chichester, **1995**.
[42] K. Burger, N. Sewald, *Synthesis* **1990**, *1990*, 115.
[43] N. Sewald, K. Gaa, K. Burger, *Heteroatom Chemistry* **1993**, *4*, 253.
[44] S. N. Osipov, A. S. Golubev, N. Sewald, T. Michel, A. F. Kolomiets, A. V. Fokin, K. Burger, *The Journal of Organic Chemistry* **1996**, *61*, 7521.
[45] K. B. a. K. Gaa, *Chem.-Ztg.* **1990**, *114*, 101.
[46] M. Moroni, B. Koksch, S. N. Osipov, M. Crucianelli, M. Frigerio, P. Bravo, K. Burger, *The Journal of Organic Chemistry* **2001**, *66*, 130.
[47] G. T. Shchetnikov, A. S. Peregudov, S. N. Osipov, *Synlett* **2007**, *2007*, 0136.
[48] S. N. Osipov, A. S. Golubev, N. Sewald, K. Burger, *Tetrahedron Letters* **1997**, *38*, 5965.
[49] R. Husmann, E. Sugiono, S. Mersmann, G. Raabe, M. Rueping, C. Bolm, *Organic Letters* **2011**, *13*, 1044.
[50] A. Asensio, P. Bravo, M. Crucianelli, A. Farina, S. Fustero, Juan G. Soler, Stefano V. Meille, W. Panzeri, F. Viani, A. Volonterio, M. Zanda, *European Journal of Organic Chemistry* **2001**, *2001*, 1449.
[51] P. Bravo, M. Crucianelli, B. Vergani, M. Zanda, *Tetrahedron Letters* **1998**, *39*, 7771.
[52] M. Crucianelli, F. D. Angelis, F. Lazzaro, L. Malpezzi, A. Volonterio, M. Zanda, *Journal of Fluorine Chemistry* **2004**, *125*, 573.
[53] F. Lazzaro, M. Crucianelli, F. De Angelis, M. Frigerio, L. Malpezzi, A. Volonterio, M. Zanda, *Tetrahedron: Asymmetry* **2004**, *15*, 889.
[54] G. Chaume, M.-C. Van Severen, S. Marinkovic, T. Brigaud, *Organic Letters* **2006**, *8*, 6123.
[55] P. Bravo, S. Fustero, M. Guidetti, A. Volonterio, M. Zanda, *The Journal of Organic Chemistry* **1999**, *64*, 8731.
[56] W. Steglich, K. Burger, M. Dürr, E. Burgis, *Chemische Berichte* **1974**, *107*, 1488.
[57] V. A. Soloshonok, V. P. Kukhar, *Tetrahedron* **1997**, *53*, 8307.
[58] R. S. Foster, H. Adams, H. Jakobi, J. P. A. Harrity, *The Journal of Organic Chemistry* **2013**, *78*, 4049.
[59] P. Bravo, S. Capelli, S. V. Meille, P. Seresini, A. Volonterio, M. Zanda, *Tetrahedron: Asymmetry* **1996**, *7*, 2321.
[60] A. E. Sorochinsky, J. L. Aceña, H. Moriwaki, T. Sato, V. Soloshonok, *Amino Acids* **2013**, *45*, 1017.
[61] A. E. Sorochinsky, J. L. Aceña, H. Moriwaki, T. Sato, V. A. Soloshonok, *Amino Acids* **2013**, *45*, 691.
[62] J. L. Aceña, A. E. Sorochinsky, H. Moriwaki, T. Sato, V. A. Soloshonok, *Journal of Fluorine Chemistry* **2013**, *155*, 21.
[63] G. B. a. M. C. R. Saladino, *Mini-Reviews in Medicinal Chemistry* **2012**, *12*, 277.
[64] X.-L. Qiu, F.-L. Qing, *European Journal of Organic Chemistry* **2011**, *2011*, 3261.
[65] K. Mikami, S. Fustero, M. Sánchez-Roselló, J. L. Aceña, V. Soloshonok, A. Sorochinsky, *Synthesis* **2011**, *2011*, 3045.
[66] A. E. Sorochinsky, V. A. Soloshonok, *Journal of Fluorine Chemistry* **2010**, *131*, 127.
[67] A. Tarui, K. Sato, M. Omote, I. Kumadaki, A. Ando, *Advanced Synthesis & Catalysis* **2010**, *352*, 2733.
[68] J. Luis Acena, A. Simon-Fuentes, S. Fustero, *Current Organic Chemistry* **2010**, *14*, 928.
[69] X. Zhang, W. Ni, W. A. van der Donk, *The Journal of Organic Chemistry* **2005**, *70*, 6685.
[70] J. Wang, D. Lin, S. Zhou, X. Ding, V. A. Soloshonok, H. Liu, *The Journal of Organic Chemistry* **2011**, *76*, 684.
[71] J. A. Pigza, T. Quach, T. F. Molinski, *The Journal of Organic Chemistry* **2009**, *74*, 5510.
[72] D. Houck, J. Luis Aceña, V. A. Soloshonok, *Helvetica Chimica Acta* **2012**, *95*, 2672.
[73] T. Yajima, H. Nagano, *Organic Letters* **2007**, *9*, 2513.
[74] H. Erdbrink, I. Peuser, U. I. M. Gerling, D. Lentz, B. Koksch, C. Czekelius, *Organic & Biomolecular Chemistry* **2012**, *10*, 8583.

[75] M. Abid, L. Teixeira, B. Török, *Organic Letters* **2008**, *10*, 933.
[76] K. Okuda, T. Hirota, D. A. Kingery, H. Nagasawa, *The Journal of Organic Chemistry* **2009**, *74*, 2609.
[77] D. Enders, Z.-X. Chen, G. Raabe, *Synthesis* **2005**, *2005*, 306.
[78] Y.-L. Liu, T.-D. Shi, F. Zhou, X.-L. Zhao, X. Wang, J. Zhou, *Organic Letters* **2011**, *13*, 3826.
[79] G. Huang, J. Yang, X. Zhang, *Chemical Communications* **2011**, *47*, 5587.
[80] F. Drouet, A. F. M. Noisier, C. S. Harris, D. P. Furkert, M. A. Brimble, *European Journal of Organic Chemistry* **2014**, *2014*, 1195.
[81] P. T. Anastas, J. C. Warner, *Green chemistry: theory and practice*, Oxford university press, **2000**.
[82] R. Noyori, *Nat Chem* **2009**, *1*.
[83] J. C. W. P. T. Anastas, in *Green Chemistry: Theory and Practice, Oxford University Press:*, Oxford University Press, New York, **1998**.
[84] P. T. Anastas, M. M. Kirchhoff, *Accounts of Chemical Research* **2002**, *35*, 686.
[85] R. Wohlgemuth, *Current Opinion in Biotechnology* **2010**, *21*, 713.
[86] I. G. K. Drauz, A. Kleeman, H. P. Krimmer, W. Leuchtenberger, C. Weckbecker, in *Ullman's Encyclopedia of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co. KGaA,, Weinheim, **2012**.
[87] T. T. I. Chibata, T. Sato, *Aspartic Acid, Vol. 3*, Pergamn, Oxford, **1985**.
[88] Y. Yokote, S. Maeda, H. Yabushita, S. Noguchi, K. Kimura, H. Samejima, *Journal of Solid Phase Biochemistry*, *3*, 247.
[89] T. Sato, T. Mori, T. Tosa, I. Chibata, M. Furui, K. Yamashita, A. Sumi, *Biotechnology and Bioengineering* **1975**, *17*, 1797.
[90] I. Chibata, T. Tosa, T. Sato, *Applied Microbiology* **1974**, *27*, 878.
[91] M. C. Fusee, W. E. Swann, G. J. Calton, *Applied and Environmental Microbiology* **1981**, *42*, 672.
[92] U. P. W. Leuchtenberger, in *Enzymes in Industry, Production and Applications* (Ed.: W. Gerhartz), VCH, Weinheim, Germany **1990**.
[93] K. W. Takahiro Ishikawa, Yukuo Mukohara, Shinobu Kobayashi & Hiroaki Nakamura, *Bioscience, Biotechnology, and Biochemistry* **1993**, *57*, 133.
[94] H.-P. Chiu, R. P. Cheng, *Organic Letters* **2007**, *9*, 5517.
[95] H. Biava, N. Budisa, *Tetrahedron Letters* **2013**, *54*, 3662.
[96] M. B. Arcuri, S. J. Sabino, O. A. C. Antunes, E. G. Oestreicher, *Journal of Fluorine Chemistry* **2003**, *121*, 55.
[97] J. Limanto, A. Shafiee, P. N. Devine, V. Upadhyay, R. A. Desmond, B. R. Foster, D. R. Gauthier, R. A. Reamer, R. P. Volante, *The Journal of Organic Chemistry* **2005**, *70*, 2372.
[98] T. Tsushima, K. Kawada, S. Ishihara, N. Uchida, O. Shiratori, J. Higaki, M. Hirata, *Tetrahedron* **1988**, *44*, 5375.
[99] J. A. Chaplin, M. D. Levin, B. Morgan, N. Farid, J. Li, Z. Zhu, J. McQuaid, L. W. Nicholson, C. A. Rand, M. J. Burk, *Tetrahedron: Asymmetry* **2004**, *15*, 2793.

4. Synthesis of Hydantoins and Their Significance

One specific synthetic pathway to hydantoins was described earlier (Bucherer-Bergs synthesis, chapter 2.1.4.) where hydantoins were discussed as precursors to fluorinated amino acids (classical approach, chapter 2.1.4), however they are also very important derivatives of amino acids and a class of heterocycles with diverse biological and medicinal potential.[1-2] This core is present in a variety of pharmacologically active compounds and is reported to act as antiarrhythmics (*e.g.* azimilide),[3-4] anticonvulsants (*e.g.* phenytoin),[5-7] antitumor compounds,[8] antibacterial agents (*e.g.* nitrofurantoin) and skeletal muscle relaxant (*e.g.* dantrium). Some derivatives of hydantoins are active as aldose reductase inhibitors,[9] and anti androgens[10] (*e.g.* nilutamide). Additionally, the hydantoin moiety is also present in several new leads in current drug candidates such as small-molecule inhibitors of cell division,[11-12] discovery of the PDE5 inhibitor tadalafil[13] and nonsteroidal androgen receptor antagonists or agonists.[14]

Hydantoins and their derivatives are naturally occurring compounds and can be found mostly in marine organisms and in bacteria. For instance, aplysinopsins, alkaloids extracted from sponges and corals, show cytotoxic properties.[15-17] In contrast, hydantocidin shows herbicidal and plant regulatory growth activity through the inhibition of adenyl succinate synthetase.[18-19] Synthetically, hydantoins are considered to be important precursors of amino acids, via either acid- or base- mediated hydrolysis.[20]

Since hydantoins show a plethora of interesting biological properties and are therefore thoroughly exploited in medicine, agriculture and synthetic chemistry, diverse synthetic strategies to access them were developed. In the next subchapter most exploited synthetic pathways that lead to hydantoins are going to be discussed.

In the Figure 4.1 several possible disconnections of the hydantoin molecule are shown.[21] Pathway A shows hydantoin formation from ureas and carbonyl compounds (Fig. 4.1 A). N-1 and N-3 unsubstituted hydantoins can be generated according to the Bucherer- Bergs method by the reaction of a carbonyl compound with inorganic cyanide, a second nitrogen and a carbonyl unit can be introduced using ammonium carbonate (Fig. 4.1 B). Read-like reactions between amino acids (esters) and inorganic isocyanates afford hydantoins with an unsubstituted N-3 position (Fig. 4.1 C). The use of alkyl or aryl isocyanates results in a substitution at nitrogen N-3 (Fig 4.1 D). Amino amides (Fig. 4.1 E) already contain four atom needed for the hydantoin ring, and the introduction of a C-1 unit can close the hydantoin ring. When α-halogen amides are coupled with inorganic iso(thio)cyanates (Fig. 4.1 F) N-1 unsubstituted hydantoins are generated.

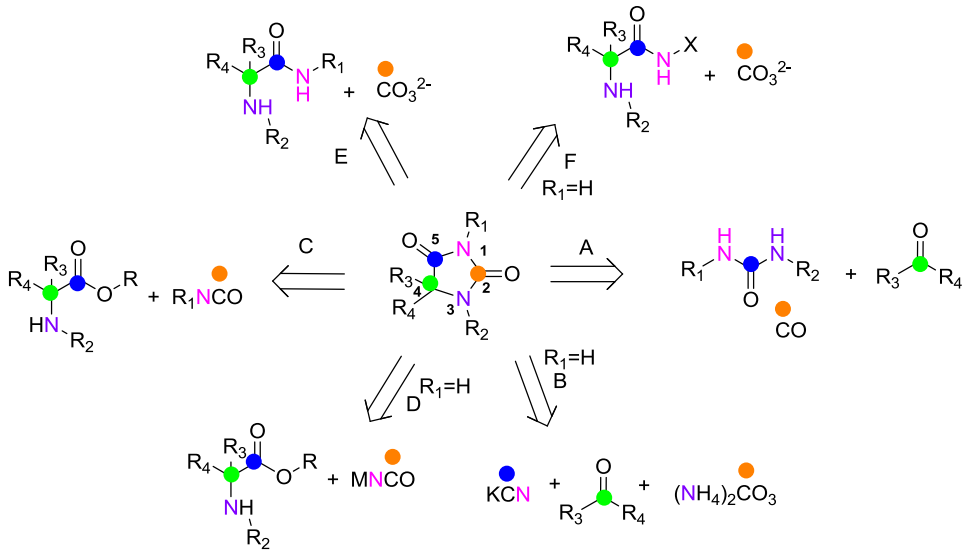


Figure 4.1. Synthetic strategies and building blocks to access hydantoins.

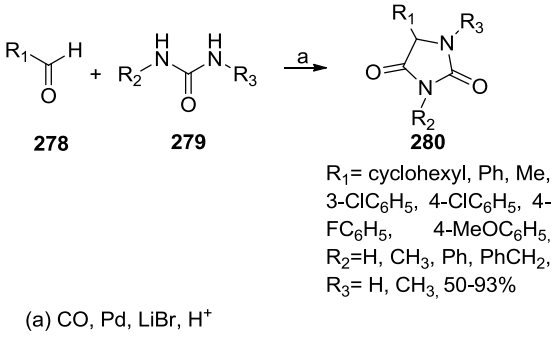
In the next few subschapters mentioned approaches to hydantoin synthesis are going to be discussed in more details and are going to be reinforced by representative examples.

4.1 From Carbonyl Compounds and Ureas

Herein, access to hydantoins from carbonyl compounds and ureas will be discussed in two separate parts; the approach that uses monocarbonyl compounds as starting materials and the approach in which dicarbonyl compounds are used for hydantoins synthesis.

4.1.1 From Monocarbonyl Compounds or Carbon Dioxide and Ureas

Beller *et al.* reported on a one-pot synthesis for the preparation of hydantoins by palladium catalyzed[22] reaction of aldehydes **278** with various ureas **279** and carbon monoxide to yield mono-, di- and trisubstituted hydantoins **280** (Scheme 4.2).

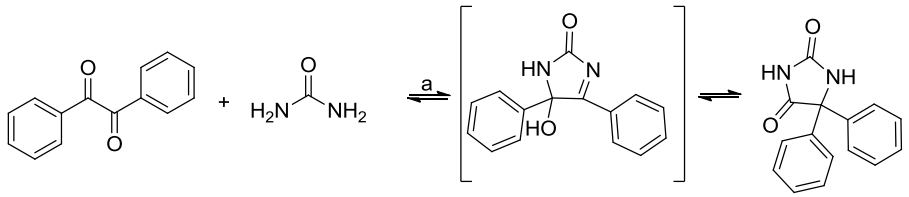


Scheme 4.2 Preparation of hydantoins **280** from ureas **279**, aldehydes **278** and CO.

4.1.2 From *α*-Dicarbonyl Compounds and Ureas

Blitz synthesis of hydantoins is usually used to access phenytoin (**281**).[23] In a base catalyzed reaction benzil (**282**) is activated with urea (**283**), and subsequent shift of an aryl group furnishes hydantoin moiety (Scheme 4.3).

Mechanism of Blitz synthesis of hydantoin formation was also investigated by mass and NMR spectroscopy with ^{13}C labelled benzil derivative.[24-25]

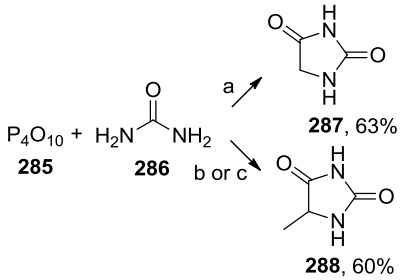


283 **282** **284** **281**,36%

(a) KOH, DMSO, heat

Scheme 4.3 Investigation of mechanism of Blitz synthesis.

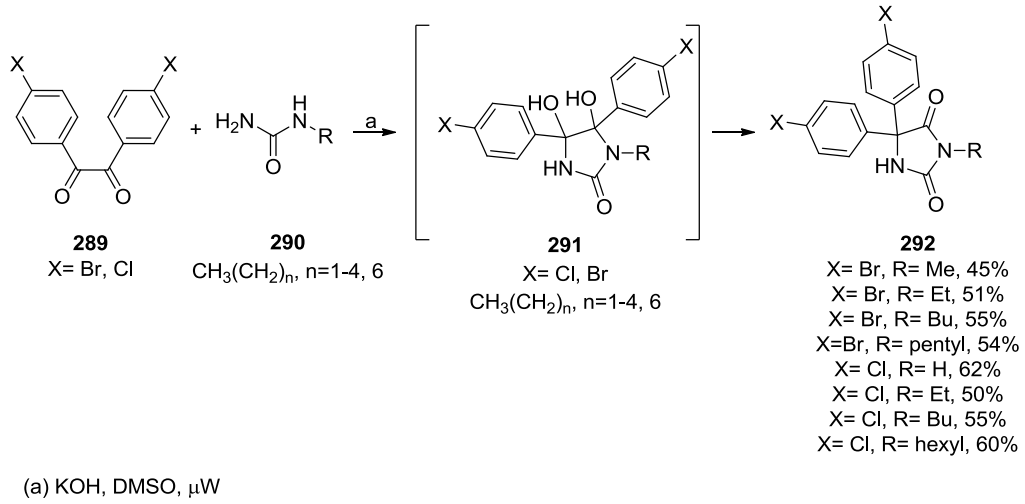
It was noticed that the presence of solid acids[26] or phosphoric anhydride (**285**)[20] promotes the coupling between urea derivatives and dicarbonyl compounds. Moreover, Micheletti *et al.*[20] formed hydantoins within 10 minutes from glyoxal and its derivative with urea (**286**) in the presence of phosphoric anhydride (**285**, Scheme 4.4). In addition to described Micheletti's approach[20] solid acids (Dowex activated with sulfuric acid) were also reported to promote the direct synthesis of hydantoin.[26]



(a) H_2O, CHOCHO, (b) H_2O, $CHOCOCH_3$, (c) H_2O, $CHOCHOHCH_2OH$

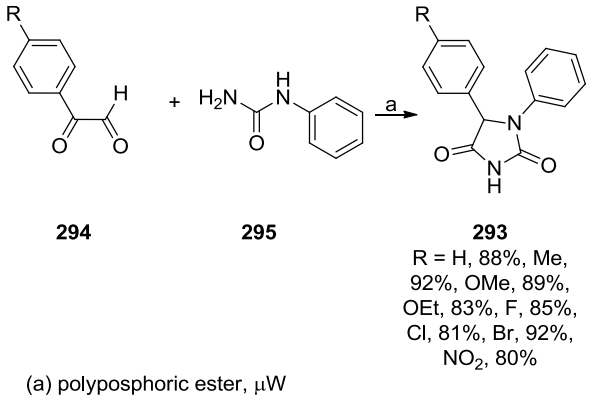
Scheme 4.4 Phosphoric acid catalyzed synthesis of hydantoins.

As an alternative to the acid catalyzed variants, microwave-assisted synthesis was found to improve yield and shorten reaction time. Irradiation of alkaline mixture of benzils **289** and alkyl ureas **290** in DMSO with 750 W microwave pulses yielded phenytoin derivatives **292** (Scheme 4.5).[23, 27-28]



Scheme 4.5 μW assisted synthesis of hydantoins **292**.

Finally, Paul and co-workers[29] combined the microwave-assisted approach with polyphosphoric esters mediators in the synthesis of disubstituted hydantoins **293** from arylglyoxals **294** with phenylurea **295** (Scheme 4.6).



Scheme 4.6 Combined μW assisted approach and polyphosphoric ester-catalyzed synthesis of **293**.

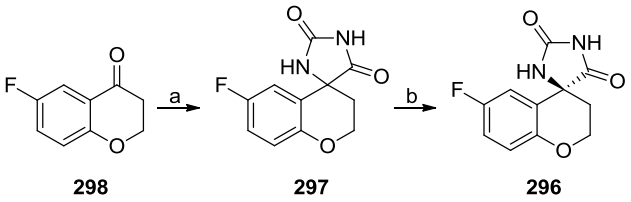
As discussed, carbonyl compounds are, in combination with ureas, extensively exploited in the synthesis of hydantoins. Moreover, the next approach to hydantoin synthesis, which will be examined in the next subchapter and was mentioned in the Synthesis of fluorinated amino acids (Section 2.1.4) also utilizes carbonyl compounds, but in combination with different reagents.

4.2 Methods based on Bucherer-Bergs Synthesis

Introduced in chapter 2.1.4, in the context of an important synthetic pathway to a valuable precursor in amino acid synthesis, Bucherer-Bergs hydantoin synthesis (Scheme 4.1 B) includes a reaction between a carbonyl compound, potassium cyanide and ammonium carbonate. These standard conditions remained unchanged and are still currently a method of choice to synthesize hydantoins for

a broad range of applications.[30-33] Herein, the applicability of this method in hydantoin synthesis will be described.

Utilizing this method, various spirohydantoins were accessed, such as sorbinil **296**, an aldose reductase inhibitor which was prepared starting from benzopyranone[34] **298** (Scheme 4.7) or PET ligand for tumor detection which was accessed through hydantoin **297** (Scheme 4.7).[35]

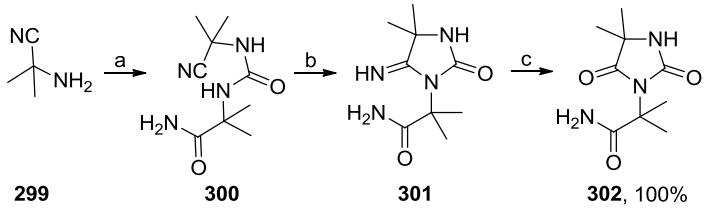


(a) KCN, $(NH_4)_2CO_3$, EtOH, H_2O, (b) i) Brucin, ii) HCl

Scheme 4.7 Synthesis of hydantoin **296** using Bucherer-Bergs approach.

Although this synthesis offers access to some hydantoins, it is marred with various problems, the first being the requirement of a very polar solvent system used for this reaction, which, although useful for solubilizing ammonium carbonate, limits the starting materials to polar aldehydes and ketones. Furthermore, the volatility of ammonium and CO_2 means they need to be used in excess. Additionally, due to its significant toxicity, heating a cyanide solution is a safety hazard.

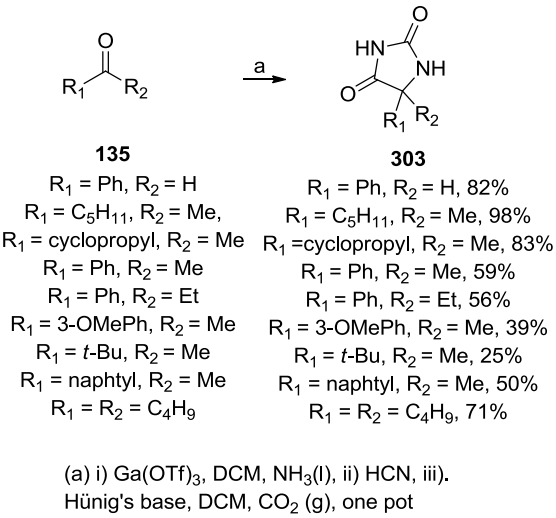
In order to solve some of the listed problems, reaction conditions and reagents themselves were modified in various ways. More precisely, Uhrich[36] and O'Brien[37] used CO_2 to treat α-amino nitrile **299** in order to obtain disubstituted urea **300**, which, after the cyclization in water at room temperature and hydrolization of the imino compound **301**, gives hydantoin **302** (Scheme 4.8).



(a) CO_2, (b) UV, H_2O, (c) H_2O

Scheme 4.8 Synthesis of hydantoins by treating amino nitriles **302** by CO_2.

Later, this strategy was advanced to a one-pot synthesis when hydantoins were accessed using CO_2. In this one-pot process, the Conway group used gallium(III) triflate to catalyze the amino nitrile formation from corresponding ketones or aldehydes and CO_2 to close the hydantoin ring (Scheme 4.9).[38]



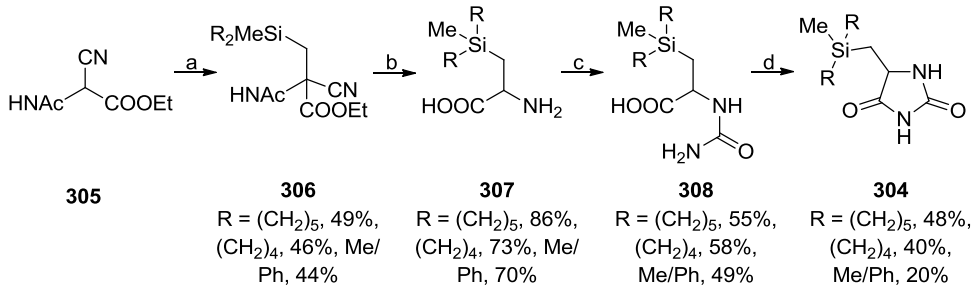
Scheme 4.9 One-pot Bucherer-Bergs synthesis of hydantoins.

The second approach, which was used to circumvent some of the problems associated with the Bucherer-Bergs method, is to use modern technologies. It was shown that ultrasonication, microreactor technology and microwave assisted synthesis can accelerate hydantoin formation via Bucherer-Bergs method.[39-40]

4.3 Methods Based on the Read Synthesis

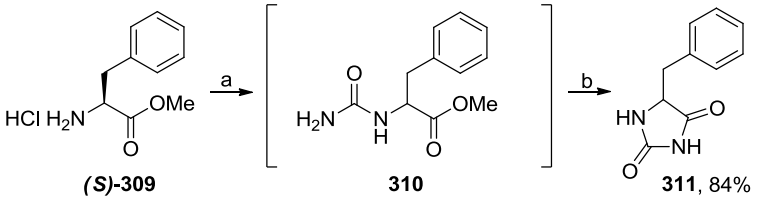
The Read synthesis of hydantoins, as opposed to the previous two methods discussed, uses 'masked' carbonyl synthons. Here, an amino acid is treated with KOCN that gives an intermediate which is concomitantly cyclized in the presence of acid to yield hydantoins (Scheme 4.1 C).

Using this approach, silicon containing hydantoins **304**[41] were synthesized. Starting from **305**, silicon group was added to obtain **306**. Decarboxylation of **306** furnished **307**, which was reacted with KOCN to give the precursor **308**. **308** was subsequently cyclized under acidic conditions to yield the desired hydantoin **304** (Scheme 4.10).



Scheme 4.10 Access to hydantoins through the Read synthesis.

In addition to the utilization of cyanate under classical chemical synthesis conditions, a mechanochemical effort using KOCN to access hydantoins was recently reported.[42] The ester salt derivative of amino acid **309** was mixed with KOCN and water, using the procedure of ball-milling, to yield an urea precursor **310** which was closed under basic conditions to give a hydantoin moiety **311** (Scheme 4.11).

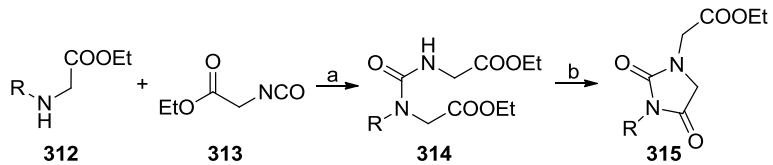


(a) KOCN, H_2O, ball milling, (b) KOH, ball milling

Scheme 4.11 Mechanochemical entry towards synthesis of hydantoin **311**.

4.4 Synthesis of Hydantoins from Amino Acids or Esters and Isocyanates

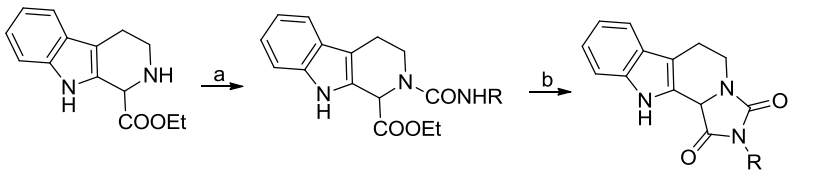
Hydantoins can also be easily accessed via an isocyanate starting material (Scheme 4.1 D). Here, a derivative of amino acid **312** is reacted with isocyanate **313** to give a substituted urea derivative **314** which subsequently cyclizes to form the desired hydantoin **315** (Scheme 4.12).[43-44] This was a key step in the synthesis of integrin GP IIb/IIIa[43-44] and aldose reductase inhibitors.[45]



(a) Et_2O or EtOAc, (b) HCl

Scheme 4.12 Synthesis of hydantoins **315** starting from isocyanate.

Moreover, this approach has proven to be robust and it could be used even when amino acids were a part of different polycyclic ring systems[46-49] for instance, tetrahydro-βcarboline-1-carboxylic acid (Scheme 4.13).[50] An isocyanate is added to the nitrogen on **316** and subsequent cyclization of the compound **317** gives the desired bicyclic hydantoin **318** (Scheme 4.13).



316 **317** **318**

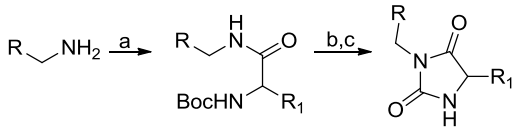
R = Ph, 66%, Et, 61% R = Ph, 56%, Et, 37%

(a) RNCO, MeOH, (b) EtOH, HCl

Scheme 4.13 Synthesis of bicyclic hydantoins **318**.

4.5 From Amino Amides and Carbonic Acid Derivatives

If the hydantoin molecule is disconnected according to the pathway E, Scheme 4.1, an amino acid amide is the starting material. By coupling Boc protected amino acids and primary amines **322**, an amide of an amino acid **323** can be obtained. This amino acid **323** after deprotection and cyclization with carbonyldiimidazole (CDI) affords hydantoins **324** (Scheme 4.14).[51-52]

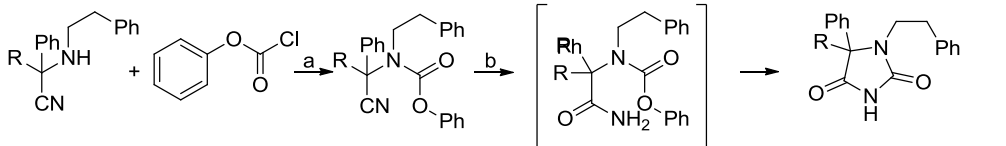


322 **323** **324**

(a) N-Boc amino acid, HOBT, EDC, DCM, (b) TFA, (c) CDI, Hunig's base,THF

Scheme 4.14 Synthesis of hydantoins **324** from amino amides.

Moreover, amino acid amides[53] can also be subjected to phenyl chloroformate to yield a product with an additional CO unit **325**, where subsequent cyclocondensation furnishes hydantoin **326**. A similar cyclization of *α*-cyano amide **327** with excess of basic hydrogen peroxide gives hydantoins **326** via a carbamate intermediate **329** (Scheme 4.15).[54]



327 **328** **325** **329** **326**

R = H, 58%, Me, 11% R = H, 58%, Me, 11% R = H, 58%, Me, 11%

(a) C_5H_5N, (b) 30% H_2O_2, K_2CO_3, DMSO

Scheme 4.15 Synthesis of hydantoin **326** by cyclization of *α*-cyano amide **325**.

The earlier approaches to access hydantoins have various benefits however they are also tied to many drawbacks. In the discussed methods, starting materials are used which have to be synthesized (*α*-cyano amides, Scheme 4.16) prior to hydantoin synthesis. Coupling of dicarbonyl

compounds and ureas is limited by the available starting materials and by the reactivity thereof. Although there have been attempts to improve the reaction conditions by employing catalysts and MW irradiation, no general method was developed. Additionally, the last two approaches discussed are substrate specific and can not be applied as a general method for hydantoin synthesis.

To conclude, a more efficient method to access hydantoins is required. Ideally starting materials should be commercially available and the operating procedure should be safe and efficient.

Bibliography

[1] A. Alizadeh, E. Sheikhi, *Tetrahedron Letters* **2007**, *48*, 4887.
[2] D. R. Anderson, N. C. Faibish, P. Beak, *Journal of the American Chemical Society* **1999**, *121*, 7553.
[3] M. Matsukura, Y. Daiku, K. Ueda, S. Tanaka, T. Igarashi, N. Minami, *Chemical & Pharmaceutical Bulletin* **1992**, *40*, 1823.
[4] I. Bélai, *Tetrahedron Letters* **2003**, *44*, 7475.
[5] J. Bosch, T. Roca, J. Domènech, M. Suriol, *Bioorganic & Medicinal Chemistry Letters* **1999**, *9*, 1859.
[6] T. Anger, D. J. Madge, M. Mulla, D. Riddall, *Journal of Medicinal Chemistry* **2001**, *44*, 115.
[7] C. W. Bazil, *Current Treatment Options in Neurology*, *6*, 339.
[8] R. F. Struck, M. C. Kirk, L. S. Rice, W. J. Suling, *Journal of Medicinal Chemistry* **1986**, *29*, 1319.
[9] R. Sarges, R. C. Schnur, J. L. Belletire, M. J. Peterson, *Journal of Medicinal Chemistry* **1988**, *31*, 230.
[10] G. Teutsch, F. Goubet, T. Battmann, A. Bonfils, F. Bouchoux, E. Cerede, D. Gofflo, M. Gaillard-Kelly, D. Philibert, *The Journal of Steroid Biochemistry and Molecular Biology* **1994**, *48*, 111.
[11] S. Hotha, J. C. Yarrow, J. G. Yang, S. Garrett, K. V. Renduchintala, T. U. Mayer, T. M. Kapoor, *Angewandte Chemie International Edition* **2003**, *42*, 2379.
[12] B. Maiti, K. Chanda, C.-M. Sun, *Organic Letters* **2009**, *11*, 4826.
[13] A. Daugan, P. Grondin, C. Ruault, A.-C. Le Monnier de Gouville, H. Coste, J. M. Linget, J. Kirilovsky, F. Hyafil, R. Labaudinière, *Journal of Medicinal Chemistry* **2003**, *46*, 4533.
[14] K. Tachibana, I. Imaoka, T. Shiraishi, H. Yoshino, M. Nakamura, M. Ohta, H. Kawata, K. Taniguchi, N. Ishikura, T. Tsunenari, H. Saito, M. Nagamuta, T. Nakagawa, K. Takanashi, E. Onuma, H. Sato, *Chemical and Pharmaceutical Bulletin* **2008**, *56*, 1555.
[15] G. Guella, I. Mancini, H. Zibrowius, F. Pietra, *Helvetica Chimica Acta* **1988**, *71*, 773.
[16] J. M. Chezal, G. Delmas, S. Mavel, H. Elakmaoui, J. Métin, A. Diez, Y. Blache, A. Gueiffier, M. Rubiralta, J. C. Teulade, O. Chavignon, *The Journal of Organic Chemistry* **1997**, *62*, 4085.
[17] L. Selič, R. Jakše, K. Lampič, L. Golič, S. Golič-Grdadolnik, B. Stanovnik, *Helvetica Chimica Acta* **2000**, *83*, 2802.
[18] A. Renard, J. Lhomme, M. Kotera, *The Journal of Organic Chemistry* **2002**, *67*, 1302.
[19] M. W. Walter, *Natural Product Reports* **2002**, *19*, 278.
[20] G. Baccolini, C. Boga, C. Delpivo, G. Micheletti, *Tetrahedron Letters* **2011**, *52*, 1713.
[21] M. G. M. Meusel, *Organic Preparations and Procedures International: The New*

Journal for Organic Synthesis **2004**, *36*, 391.
[22] M. Beller, M. Eckert, W. A. Moradi, H. Neumann, *Angewandte Chemie International Edition* **1999**, *38*, 1454.
[23] G. G. Muccioli, J. H. Poupaert, J. Wouters, B. Norberg, W. Poppitz, G. K. E. Scriba, D. M. Lambert, *Tetrahedron* **2003**, *59*, 1301.
[24] G. Schwenker, H. Guo, *Archiv der Pharmazie* **1993**, *326*, 45.
[25] G. Schwenker, H. Guo, S. Bernhart, *Archiv der Pharmazie* **1992**, *325*, 779.
[26] C. Cativiela, J. M. Fraile, J. I. García, B. Lázaro, J. A. Mayoral, A. Pallarés, *Applied Catalysis A: General* **2002**, *224*, 153.
[27] G. G. Muccioli, J. Wouters, J. H. Poupaert, B. Norberg, W. Poppitz, G. K. E. Scriba, D. M. Lambert, *Organic Letters* **2003**, *5*, 3599.
[28] S. C. P. D. D. Rishipathak, D. P. Belsare, *Asian Journal of Organic Chemistry* **2007**, *19*, 5450.
[29] S. Paul, M. Gupta, R. Gupta, A. Loupy, *Synthesis* **2002**, *2002*, 0075.
[30] J. P. Rizzi, R. C. Schnur, N. J. Hutson, K. G. Kraus, P. R. Kelbaugh, *Journal of Medicinal Chemistry* **1989**, *32*, 1208.
[31] C. Shibuya, S. Ouchi, *Agricultural and Biological Chemistry* **1988**, *52*, 589.

[32] C. Lamberth, S. Blarer, *Synlett* **1994**, *1994*, 489.
[33] K. Lavrador, D. Guillerm, G. Guillerm, *Bioorganic & Medicinal Chemistry Letters* **1998**, *8*, 1629.
[34] R. Sarges, J. Bordner, B. W. Dominy, M. J. Peterson, E. B. Whipple, *Journal of Medicinal Chemistry* **1985**, *28*, 1716.
[35] J. McConathy, L. Martarello, E. J. Malveaux, V. M. Camp, N. E. Simpson, C. P. Simpson, G. D. Bowers, J. J. Olson, M. M. Goodman, *Journal of Medicinal Chemistry* **2002**, *45*, 2240.
[36] E. O. K. Uhrich , J. Worman, *Synthetic Communications* **1986**, *16*, 1387.
[37] J. J. W. E. S. O. Richard A. O'brien *Synthetic Communications* **1992**, *22*, 823.
[38] R. G. Murray, D. M. Whitehead, F. Le Strat, S. J. Conway, *Organic & Biomolecular Chemistry* **2008**, *6*, 988.
[39] S. C. P. a. D. P. B. D. D. Rishipatak, *Asian Journal of Chemistry* **2007**, *19*, 5450.
[40] J. L. Monteiro, B. Pieber, A. G. Corrêa, C. O. Kappe, *Synlett* **2016**, *27*, 83.
[41] R. J. Smith, S. Bratovanov, S. Bienz, *Tetrahedron* **1997**, *53*, 13695.
[42] L. Konnert, B. Reneaud, R. M. de Figueiredo, J.-M. Campagne, F. Lamaty, J. Martinez, E. Colacino, *The Journal of Organic Chemistry* **2014**, *79*, 10132.
[43] A. Peyman, V. Wehner, J. Knolle, H. U. Stilz, G. Breipohl, K.-H. Scheunemann, D. Carniato, J.-M. Ruxer, J.-F. Gourvest, T. R. Gadek, S. Bodary, *Bioorganic & Medicinal Chemistry Letters* **2000**, *10*, 179.
[44] H. U. Stilz, B. Jablonka, M. Just, J. Knolle, E. F. Paulus, G. Zoller, *Journal of Medicinal Chemistry* **1996**, *39*, 2118.
[45] T. Kotani, Y. Nagaki, A. Ishii, Y. Konishi, H. Yago, S. Suehiro, N. Okukado, K. Okamoto, *Journal of Medicinal Chemistry* **1997**, *40*, 684.
[46] T. Kobayashi, H. Fujieda, Y. Murakami, T. Nakamura, K. Ono, S.-y. Yamamoto, H. Kato, *Bulletin of the Chemical Society of Japan* **1994**, *67*, 3082.
[47] Y.-D. Gong, H.-Y. Sohn, M. J. Kurth, *The Journal of Organic Chemistry* **1998**, *63*, 4854.
[48] Z. Szakonyi, F. Fülöp, D. Tourwé, N. De Kimpe, *The Journal of Organic Chemistry* **2002**, *67*, 2192.
[49] J. Charton, S. Delarue, S. Vendeville, M.-A. Debreu-Fontaine, S. Girault-Mizzi, C. Sergheraert, *Tetrahedron Letters* **2001**, *42*, 7559.
[50] M. Solymár, M. Palkó, T. Martinek, F. Fülöp, *Monatshefte für Chemie / Chemical Monthly*, *133*, 1423.
[51] D. Kim, L. Wang, C. G. Caldwell, P. Chen, P. E. Finke, B. Oates, M. MacCoss, S. G. Mills, L. Malkowitz, S. L. Gould, J. A. DeMartino, M. S. Springer, D. Hazuda, M. Miller, J. Kessler, R. Danzeisen, G. Carver, A. Carella, K. Holmes, J. Lineberger, W. A. Schleif, E. A. Emini, *Bioorganic & Medicinal Chemistry Letters* **2001**, *11*, 3099.
[52] Y. Nomoto, H. Takai, T. Hirata, M. Teranishi, T. Ohno, K. Kubo, *Chemical & Pharmaceutical Bulletin* **1990**, *38*, 3014.
[53] J.-i. Yamaguchi, M. Harada, T. Kondo, T. Noda, T. Suyama, *Chemistry Letters* **2003**, *32*, 372.
[54] J.-P. Leblanc, H. W. Gibson, *The Journal of Organic Chemistry* **1994**, *59*, 1072.

5. Aim

As a part of our continuing interest in the incorporation of fluorinated amino acids into coiled-coil peptide models in order to study the conformational changes and the properties of the fluorinated variants, we are currently creating new methodologies to more easily access a broader scope of fluorinated amino acids. The objective of this thesis is to develop a methodology which would enable facile, straightforward, and safe access to these building blocks. Current synthetic approaches towards fluorinated amino acids entail numerous drawbacks such as long reaction times, the handling of toxic materials, and numerous synthetic steps requiring purification. These drawbacks can be circumvented by exploiting continuous flow chemistry. Building on the process of photooxidative cyanation of amines in flow, a safe and rapid method to access fluorinated amino acids is to be developed, thereby, providing racemates in good to excellent yield. This photooxidative cyanation also is to be combined with enzymatic transformations to obtain enantiomerically pure fluorinated amino acids, and carboxylation to provide hydantoins, derivatives of amino acids. In addition to approaches in which fluorinated synthons are used for the synthesis of fluorinated amino acids, a novel methodology is to be introduced which results in the trifluoromethylation of protected amino acids, providing access to α-trifluoromethylated amino acids.

6. Applied Methods

The main part of this thesis is focused on developing more efficient synthetic procedures to access fluorinated amino acids and their derivatives. In order to achieve this, an alternative to the classical methods of synthetic organic chemistry was explored. Since the experimental efforts described in this dissertation were largely centered around flow chemistry this methodology is summarized below.

6.1 Introduction to Flow Chemistry

A reaction run "in flow" is a chemical reaction run in a continuously flowing stream as opposed to the more traditional batch production. To be more specific, pumps move fluid into a tube, and at the joint of two or more tubes, the fluids contact one another. If these fluids are reactive, a reaction takes place. Flow chemistry is a well-established technique often applied when large quantities of a given material are being manufactured. However, this term was only taken over recently for its application on a laboratory scale.[1]

Continuous reactors usually consist of tubing and are manufactured from materials such as stainless steel, glass or polymers. The reactive components are mixed by means of diffusion, if the diameter of the reactor is small *e.g.* less than 1 mm ; such setups are also referred to as microreactors. The residence time of the reagents in the reactor (*i.e.,* the amount of time the reagents spend in the reactor) can be calculated from the volume of the reactor and the flow rate according to the equation 6.1:

$$\text{Residence Time} = \text{Reactor Volume} / \text{Flow Rate} \qquad \textbf{(Eq 6.1.)}$$

Therefore, to achieve a longer residence time, reagents can be pumped more slowly and/or a larger volume reactor can be used. Production rates can vary from nano-liters to liters per minute.

Flow reactors offer several advantages. They enable better control over heat transfer in the reaction mixture compared to the batch process. When performing the reaction a big portion of the reaction mixture, but only in a form of a thin layer, is in contact with channel walls. This enables rapid dissipation of heat in the reaction and at the same time offers rapid addition of heat to the reaction mixture for the same reason. Faster heat transfer in microreactors offers the possibility of "green" processing, which enables transformations in the absence of solvent.[2] Higher temperatures and pressures also accelerate certain reactions, for instance, catalytic hydrogenation[3] and aza Diels–Alder[4] reactions. More rapid heat transfer and uniform temperature control in microreactors also enable exploration of reactions that use supercritical solvents.[2]

In addition, the large portion of the reaction mixture (but at the same time thin layer of it) in contact with channel walls has a higher surface area-to-volume ratio, leading to an increase in photon-flux for photochemical reactions. Namely, the design of the flow photoreactors enables the homogeneous exposure of the reaction mixture to the light source.

Another advantage of the flow reactor is precise control over mixing. The degree of mixing often influences the outcome of a reaction. Conversion and selectivity of the reaction are determined by the quality of mixing for reactions where rate of reaction is higher than the speed of mixing. Even in slow reactions, mixing inhomogeneitie and corresponding spatial and temporal variations in reaction conditions can result in lower yields. Reactions in batch reactors exhibit different mixing mechanisms when compared to ones in microfluidic systems.[2] Microreactors offer a surface area, in both homogeneous and heterogeneous reactions, that is at least, two orders of magnitude greater than batch, which corresponds an improvement in the performance of mass transfer-limited reactions of at least two orders of magnitude. In microreactors the reduction of the diffusion length is crucial for fast mixing since the mixing time is proportional to the square of the diffusion length. In many micromixers, which are important parts of microreactors, the reactant flow is split into many lamination segments to shorten the mixing time.

Heterogeneous reactions also benefit from flow conditions. Gas-liquid reactions benefit from a larger contact area between the gas and liquid phases which ensures better mass transfer between the two phases. Similarly, running a biphasic reaction, in which one phase is solid, under flow conditions provides a larger interface between the solid (often used as a packed bed reactor) and the liquid or gas phase.

Moreover, applying microreactor technology in organic synthesis also offers better control over hazardous reagents (*e.g.,* corrosive gases or liquids) and high pressure/temperature conditions in which the size of the reactor prevents thermal runaways and explosions.

Finally, multistep reactions in flow can be especially beneficial if the reaction intermediates are unstable, toxic, or sensitive to air since they will be created *in situ* and consumed immediately upon formation. What is more, purification can be coupled with the reaction in flow as one of the steps in multistep syntheses.

Scaling up an optimized reaction can be achieved quickly with little or no process development work, by either changing the reactor volume or by running several reactors in parallel.
However, as with any new technique/technology, a positive or negative hyperbole has to be avoided. Flow reactors are not a ‘magic bullet’ but they can be an excellent complement to traditional batch reactors.[5-6]

6.2 Increased Surface Area-to-Volume Ratio and Flow Photochemistry

As mentioned above, increased surface area-to-volume ratio results in better heat and mass transfer and increased photon flux for photochemical reactions. Photochemistry includes physical and chemical processes in which the absorption of photons enables the desired chemical transformation (often at or close to room temperature and under atmospheric pressure).[7] The application of these mild and sustainable conditions and reagents offers products which are rarely accessible by applying thermal conditions. Performing chemical reactions by using light of an appropriate wavelength is a step towards the implementation of green chemistry.[8-9] Photochemical reactions can proceed, depending on the reaction parameters via two different reaction paths:[10] photochemical or photocatalytic. In the case of a photochemical reaction, the compound that absorbs the light is also the reagent that undergoes a structural or chemical conversion, such as a bond cleavage and/or bond formation. In contrast, in a photocatalytic reaction, a molecular sensitizer (*e.g.,* Rose Bengal) or bulk material (*e.g.,* TiO_2) absorbs light and subsequently transfers energy/electrons to a reagent which then participated in the desired reaction. After transfer, the light-absorbing catalyst returns to its electronic ground state.[11] The absorption of light is expressed by the Lambert–Beer law:

$$A = -log\frac{I}{I0} = \varepsilon\lambda \cdot c \cdot l \qquad \textbf{(Eq.6.2.)}$$

Absorbance ( ) is the negative logarithm of the intensity of the transmitted light divided by the intensity of the incident light. This law states that the light intensity I_0 decreases along the path length l (cm) through the solution, depending on the molar decadic extinction coefficient ε_λ (L mol^{-1} cm^{-1}) and the molar concentration c (mol L^{-1}). ε_λ is a specific constant, that describes the ability of a given molecule to absorb light of a specific wavelength. Strongly absorbing molecules have a high molar extinction coefficient at the given wavelength.[11]

As illustrated by the following examples, effective transmission of light through a conventional glass flask is limited. In Figure 6.3 three graphs are plotted with different progression stressing the impact of concentration and extinction coefficient on the light transmission depending on the path length. The first, green-blue graph represents a solution of riboflavin tetraacetate (0.1 mM) with approx. ε_λ = 12,500 L mol^{-1} cm^{-1} (in acetonitrile:water, 1:1, v/v, measured at 446 nm).[12] From the graph it can be concluded that the light must pass 8 mm through the solution in order to achieve 90% absorbance (corresponding to a residual transmission of 10%).[11] If the concentration of the solution is increased to 0.5 mM the path length which absorbs the equivalent amount of light shortens to 1.6 mm (light-blue graph, Figure 6.3). Therefore the light will be absorbed mainly by the components of the reaction solution that are 1.6 mm away from the light source.[11] Moreover, if light-absorbing materials are used which have a molar extinction coefficient greater than 300,000 L mol^{-1} cm^{-1} (*e.g.*, tetraphenylporphyrin, measured at 420 nm), the light path length decreases even more, to

approximately 10 µm at 90% absorbance (dark-blue curve).[11] In this case, the incident light will be mainly absorbed only by the components of the solution that are10 µm away from the light source, whereas deeper layers cannot be irradiated. One potential solution to this problem lies in using a more powerful light source; however, this might lead to an increase in side product formation or reagent/product decomposition by overirradiation.[11]

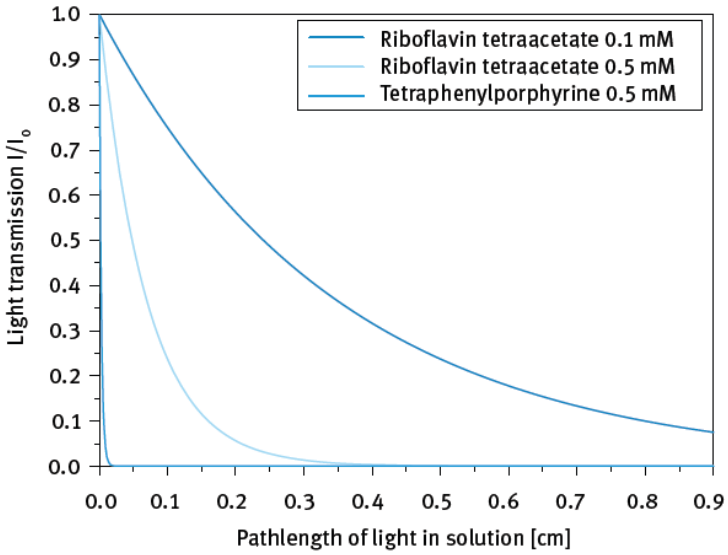


Figure 6.3. Plotting the Lambert–Beer law for different concentrations of riboflavin tetraacetate (green blue, light blue) or with the extinction coefficient of tetraphenylporphyrin at 0.5 mM (dark blue), reproduced with permission[11]

Besides photochemical decomposition by intense irradiation and the formation of side products, the electronic excitation of a molecule can lead to strong non-radiative deactivation during which vibrational energy is transferred via collisions with other molecules (usually solvent) to the environment. This process results in heating of the solution.[13]

Applying flow chemistry can address some of the problems related to both irradiation depth and heat removal. One important advantage of microstructured flow technology is the utilization of the complete volume of a batch vessel that can be transported continuously in the form of a thin film or streamed phase inside a channel or a tube. The micrometer-sized diameter and the structure of the tubes used define the thickness of the volumetric flow of the reaction solution.[14-18] In this type of reactor, the small width allows for the complete and homogeneous irradiation of the reaction solution. In addition, this setup also offers the possibility of using a liquid film containing the highly concentrated light-absorbing material or a light absorber with a very high extinction coefficient, for example Rose Bengal with $\varepsilon_\lambda > 100{,}000$ L mol^{-1} cm^{-1}.[11] The second advantage of using microreactors to perform photochemical reactions is the possibility of controlling the exact time of irradiation by adjusting the rate of the liquid flow, therefore preventing the formation of the side products.[11] The

third advantage of a microreactor flow photo-setup is full control of the heat generated during a photochemical reaction. Utilizing small volumes of the reaction solution inside a microchannel results in the ratio between the contact area of the channel walls and the appropriate solution volume being very large compared to a conventional flask.[11] Therefore, the thermal equilibration between the portion of hot reaction solution in the center of the microchannel and the cold channel wall occurs much faster than is the case in a 10 mL flask reaction.[11] This efficient temperature control diminishes or avoids side product formation due to "hotspots".

The design of the flow reactor for photochemistry mainly depends on the choice of the light source.[11] With the recent expansion of the flow photochemistry field a variety of custom-built reactor designs has emerged. Chip-based reactors, falling film reactors and capillary-based reactors are the most prominent examples.[11] Chip-based reactors are often made of glass or polymeric materials. One example of this type of reactors is the one made from quartz to allow the transmission of UV light.[11] Here the linear channel has an overall length of 50 mm, a width of 500 μm and a possible depth of 10 μm to 500 μm which offers control over the irradiated reaction solution volume (Figure 6.4).[11]

Figure 6.4. Quartz glass microreactor with a single microchannel filled with methylene blue solution. Adapted with permission[11]

The falling film reactor was initiated by Griesbeck *et al.* with a XeCl excimer lamp (60 cm in length, 3 kW) as the central light source surrounded by a glass barrel.[19] The reaction solution is pumped from a cooled reservoir to the upper edge of this glass barrel from which it runs down, driven by gravity, along the surface. The reactor design is suitable for the irradiation of larger reactant quantities. Depending on the solvent, using this concept of a falling film reactor, a liquid film with thicknesses in the range of a millimeter can be produced.[11] However, the majority of flow reactors for photochemistry reactions are custom-built using any available flow chemistry equipment present on the market.[11] In literature, there are many examples that describe unconventional reactor designs. Light-transparent tubes are wrapped around glass parts or devices and cooled by air streams or cooling baths. Although the described reactors are functional, they are inadequate for numbering-up or scaling-up processes. A more suitable reactor for photochemical synthesis on a larger scale was designed by

Booker-Milburn *et al*.[20] UV/Vis-transparent fluorinated ethylene propylene (FEP) tubing was tightly wrapped around the immersion well (Figure 6.5). The radial emission of the central Hg vapor lamp inside the immersion well provided homogeneous illumination of the reaction solution inside the FEP tubing.[20] Modern versions of this reactor use high-power LEDs which produce less heat and circumvent the need to cool the reaction.

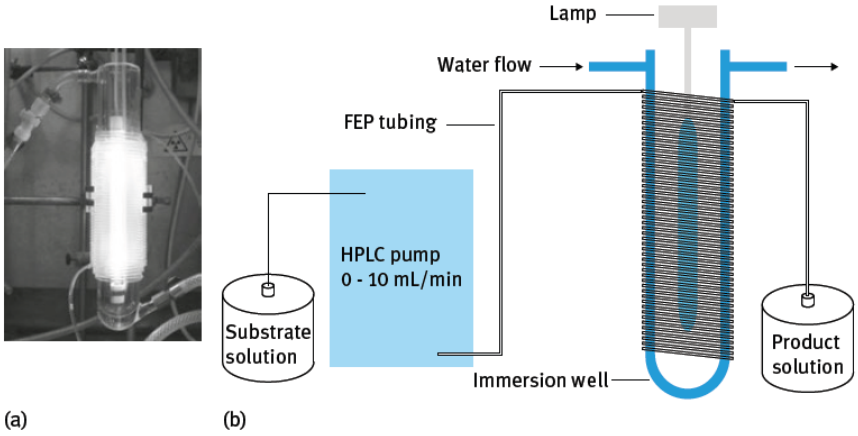


Figure 6.5. (a) FEP-based flow reactor with a mercury vapor lamp burning in the center of an immersion well. Adapted with permission[11]; (b): a schematic illustration of continuous flow photochemical reactor based on an FEP capillary wrapped around an immersion well. Adapted with permission[11]

6.3 In-House Designed Flow Photoreactor

With all the benefits of performing the photoreaction in flow in mind, a flow photoreactor[21] was used for amino nitrile formation. The setup for flow photoreaction used is shown in the Figures 6.6 and 6.7 and the corresponding engineering diagram is shown in Figure 6.8. The Vapourtec R2+ system was used for continuous flow photosynthesis. In an ETFE T-mixer (IDEX Health and Science) substrate solution was mixed with oxygen (99.995%, H_2O < 3.0 ppm*mol; ALPHAGAZ™ 1 O_2; Werk DEF 2 Krefeld-Gellep), which was delivered through a check valve from an oxygen gas tank. The gas pressure was regulated to 20 bars and the flow adjusted by a mass-flow controller (Brooks, SLA5800). This solution was then pumped through the photoreactor, consisting of FEP tubing (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter) of 9.5 mL volume wrapped in two layers around a glass plate (7cm × 9 cm) (Figure 6.6). A 30 cm long piece of tubing was used to connect the T-mixer with a 0.5 mL precooling loop, placed directly before the photoreactor. The LED module (OSA Opto Light, OLM-018 B, 420 nm emission wavelength, 12 W,

36 V) was mounted in front of this photoreactor at a distance of 3 cm. The photoreactor was immersed in an acetone bath and cooled with an immersion cooler (Huber, TC100E-F-NR). A 30 cm long piece of tubing connected the outlet of the photoreactor with a 7 bar backpressure regulator. The collected solution was concentrated on a rotary evaporator (Büchi R-210) and further purified by column chromatography.

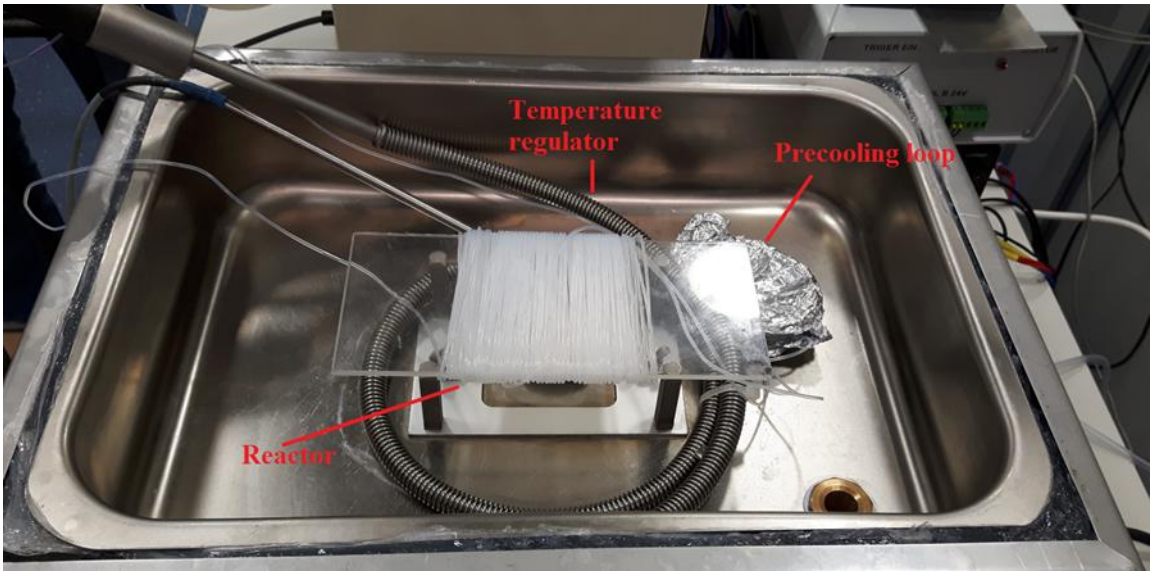


Figure 6.6. The in-house-built flow photoreactor

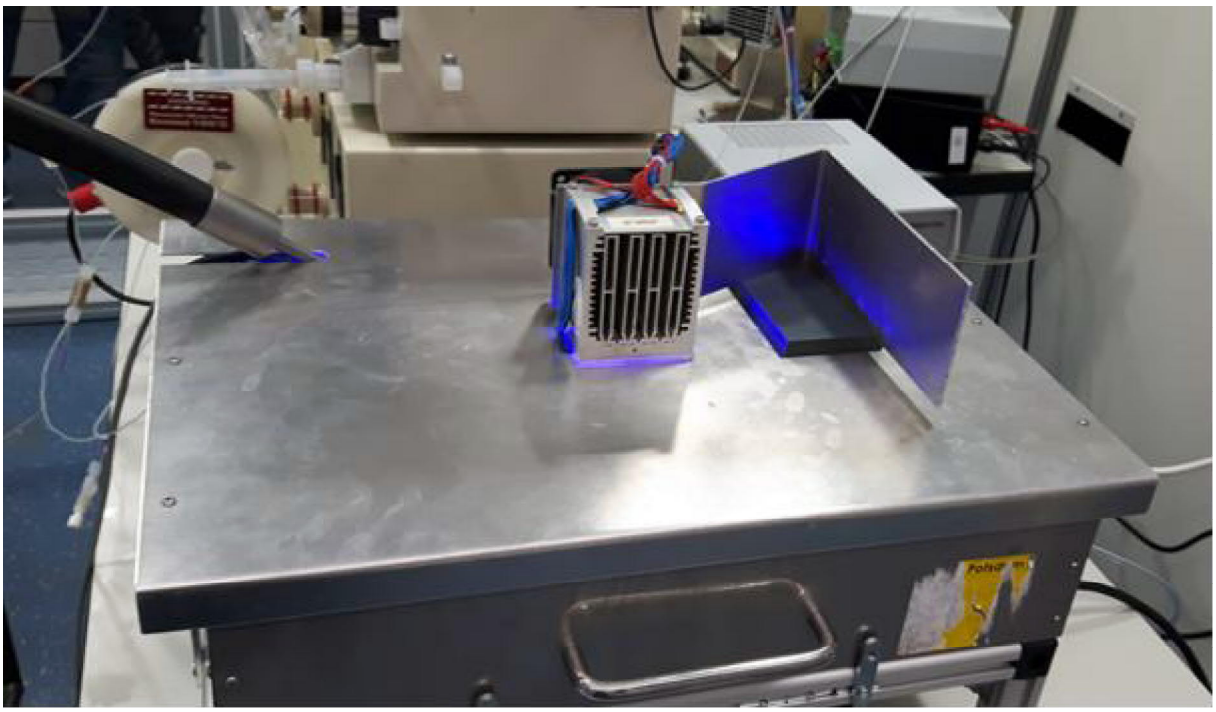


Figure 6.7. The in-house-built flow photoreactor

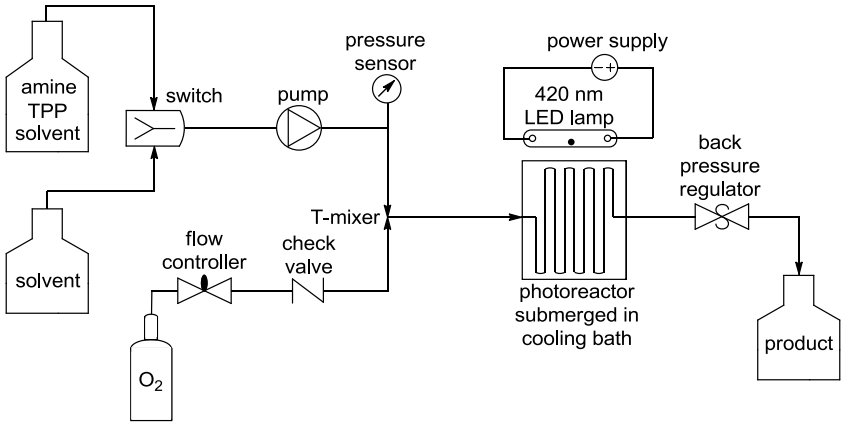


Figure 6.8. Engineering diagram of the in-house built flow photoreactor

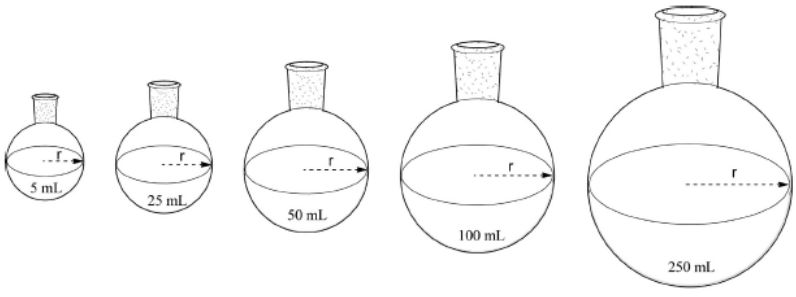
6.4 Heterogeneous Gas-Liquid Reactions In Flow

The use of gases in a modern synthetic laboratory must be highly controlled. This is especially true for very toxic and corrosive gases; in fact, in these cases dedicated high-pressure facility rooms are typically built to enable access to gas-based transformations. The reason for this lies in the fact that when conventional synthetic equipment is used, leakages are very difficult to prevent. Usually, pressurized gas reactions are supervised by specially trained personnel and are conducted in the presence of specialized gas and/or pressure detectors. Moreover, scale-up of high-pressure reactions is usually restricted to avoid the risks of gas leakage and explosions.[22] Namely, in the higher volume reactors, working with flammable gases can lead to explosive ignition due to the unexpected sudden influx of air or following the leakage of the flammable gas.

On the other hand, gases are attractive reagents for a variety of chemical transformations due to the fact that their concentration in the reaction mixture can be increased by a simple increase in the pressure and the excess can be removed simply by venting. Establishing the stoichiometry of a gas-liquid reaction by solubilizing sufficient quantities of the gas in the reaction medium is a limiting factor of most of the reactions involving gaseous reagents because the of solubility of some of the gases such as CO is low.[22]

Hence, many reactions involving gases require high concentrations of the gas in order to proceed at an acceptable rate, and this concentration is approximately proportional to the pressure (Boyle's law for gas-phase, Henry's law for solutions).[23] Regardless of the high pressures applied, the concentration of the dissolved gas decreases as the temperature increases, therefore, an increase in the pressure must follow to obtain the same amount of the gas in the solution at elevated temperature. Continuous flow technology offers numerous advantages when compared to traditional batch synthesis.[24-32] To start with, the high heat and mass transfer rates possible using small-channeled fluidic systems allow for the application of a wider range of conditions, many of which can not be

applied to classical batch reactors. A high interfacial area (a), the contact area between the gas and the liquid in gas-liquid reactions, is essential for an efficient mass transfer rate. Batch reactions carried out in a traditional round-bottom flask have significantly lower a values which decrease with an increase in the size of the flask (Figure 6.9.).[22]



Radius r (m)				
0.014	0.021	0.025	0.033	0.043
Interfacial area a (m^2m^{-3})				
107	71	60	46	35

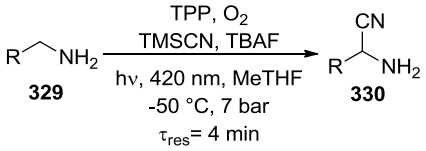
Figure 6.9. Qualitative measure of interfacial area (a) for round-bottom flasks when the liquid is static.[22]

Upon stirring a reaction, the vortex formed increases the interfacial area, which also depends on the speed of mixing. In Table 6.10, interfacial areas for different reactor types are shown. Much larger interfacial areas for certain reactor types are evident, e.g. microchannel reactors (a = 3,400 to 18,000 m^2 m^{-3}), with a maximum interfacial value of 18,000 m^2m^{-3} for a 300 μm × 100 μm microchannel.[33] In conclusion, delivery of the gases in flow can be controlled by regulating the flow volumes and by the application of higher internal pressures within the flow system to increase dissolution of the gas.[22]

Table 6.10. Published interfacial area for different gas-liquid contactors[33]

Type of contactor	a (m^2m^{-3})
Bubble columns	50-600
Couette-Taylor flow reactor	200-1200
Impinging jet absorbers	90-2050
Packed columns, concurrent	10-1700
Packed columns, counter current	10-350
Spray column	75-170
Static mixers	100-1000
Stirred tank	100-2000
Tube reactors, horizontal and coiled	50-700
Tube reactors, vertical	100-2000
Gas-liquid microchannel contactor	3400-18000

The reaction of photooxidative cyanation (Scheme 6.11.) was conducted using the photoreactor described above (Figures 6.6, 6.7 and 6.8). Here the oxidation of amines to imines was coupled to cyanide addition to the imine. Oxygen (or air) is used as an oxidant in this transformation. Oxidations carried out by molecular oxygen or air pose severe safety risks and process challenges. The above mentioned transformations are exothermic and the produced heat in the reaction can be difficult to dissipate which causes reductions in selectivity and product quality under these non-isothermal conditions.[34] As oxidations are, in fine-chemical manufacturing, mostly carried out under elevated pressures and temperatures, the possibility of spontaneous ignition is high. To prevent explosions, large-scale applications in conventional batch reactors usually take place with the oxygen concentration below the limiting oxygen concentration (LOC)[35], achieved by mixing the gaseous oxidant with an inert gas (*e.g.* N_2) to dilute the oxygen/solvent vapor. When a LOC is used, this concentration is too low for successful transformation under optimal temperature/pressure conditions which then have to be adjusted accordingly. Performing the oxidation at non-optimal temperatures and pressures results in relatively slow and inefficient processes.



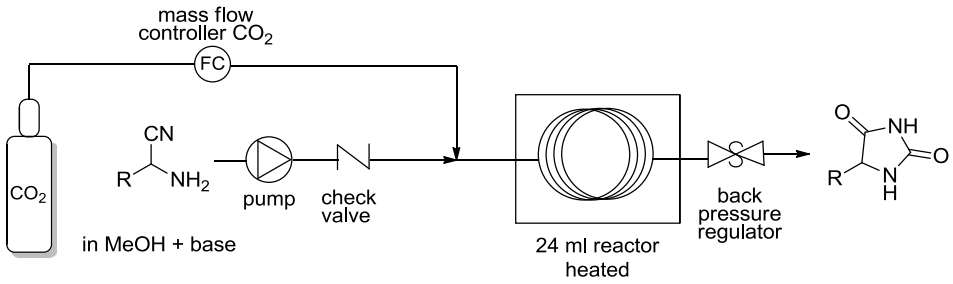
Scheme 6.11. Photooxidative cyanation of amines to obtain α-amino nitriles

By using continuous flow (micro)reactor technology the above-mentioned safety hazards of simultaneously working at high-temperature/high-pressure regimes ("novel process windows") can be addressed.[24, 26-27, 36-38] As mentioned in the first section of this chapter, exothermic reactions are easily controlled by very good mass and heat transfer which makes this technique an ideal tool for hazardous chemical processes.[39] Moreover, the excellent temperature control, the small volumes, and the small channel dimensions diminish the potential for explosion propagation inside the reactor.[40-41] Specifically, biphasic gas/liquid reactions such as aerobic oxidations can benefit from flow micro(reactor) technology due to fast mixing and a significantly larger interfacial area between the liquid and the gaseous phase[36] which was recognized when the reaction of photooxidative cyanation was carried out.

6.5 Reactor for CO_2 addition to α-Amino Nitriles

The advantages of the gas-liquid reactions mentioned above, such as the larger interfacial area between phases, control over the reaction temperature, and the possibility of running reactions under high pressure were also exploited in the carboxylation of amino nitriles by CO_2 gas. The carboxylation

reaction was performed using the designed reactor depicted below (Scheme 6.12). Here, a 24 mL reactor consisting of FEP tubing, immersed in a heated water bath, heated between 80 °C and 110 °C. In an ETFE T-mixer (IDEX Health and Science) substrate solution (amino nitrile and Hünig's base) was mixed with carbon dioxide (Air liquide®, ISO 14175-C1-C), which was delivered through a check valve from a carbon dioxide gas tank. The gas pressure was regulated to 20 bars and the flow adjusted by a mass flow controller (Brooks, SLA5800). This solution was then pumped through the 24 mL reactor. A 30 cm long piece of tubing connected the outlet of the reactor with a 7 bar backpressure regulator.



Scheme 6.12. Engineering diagram of reactor for carboxylation

6.6 General Features of Fixed Bed Reactors

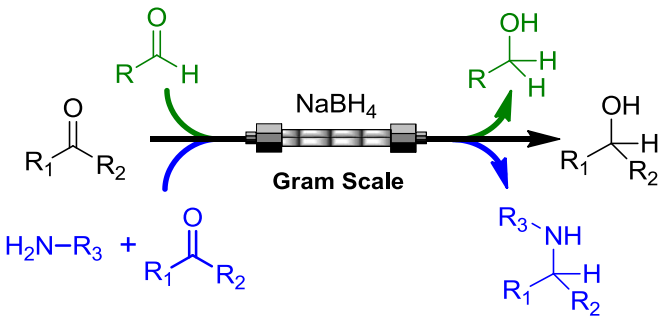
Fixed bed reactors are exploited in industrial processing, where a tube filled with solid particles of different sizes and shapes represents a reactor, and the ends of the cartridges are closed with small pore size filters in order to keep the catalyst material in the cartridge throughout the operation (Scheme 6.13).[1] Fixed bed reactors can be used either as fixed-bed catalysts or as fixed-bed reagents.

The efficiency of catalysis depends on the particle size of the catalyst and the loading (amount of catalyst per amount of bulk material/solid support). The reaction typically takes place on the surface of the catalyst, therefore catalyst beds with high surface-to-volume ratios result in better conversions per weight of catalyst. Well-defined particle size and active surface are very important. A catalyst bed with very small particles tends to cause a high-pressure drop in the system and can leach into the solution through the filter.[1] On the other hand, large particles result in low conversions due to the small surface-to-volume ratio.

Scheme 6.13. Commercially available Omnifit cartridge. Aadapted with permission.[42]

Heterogeneous catalysis offers many advantages. For example, no separation of the immobilized reagent/catalyst from the reaction mixture is needed and the reusability of the catalyst meets the economical efficiency and environmental requirements of green chemistry. In addition, variation in the surface area of the catalyst can lead to an increase in the rate of the reaction, and, due to the immediate removal of the product, catalyst poisoning and side reactions are minimized. A number of commercially available supported catalysts exist.[1]

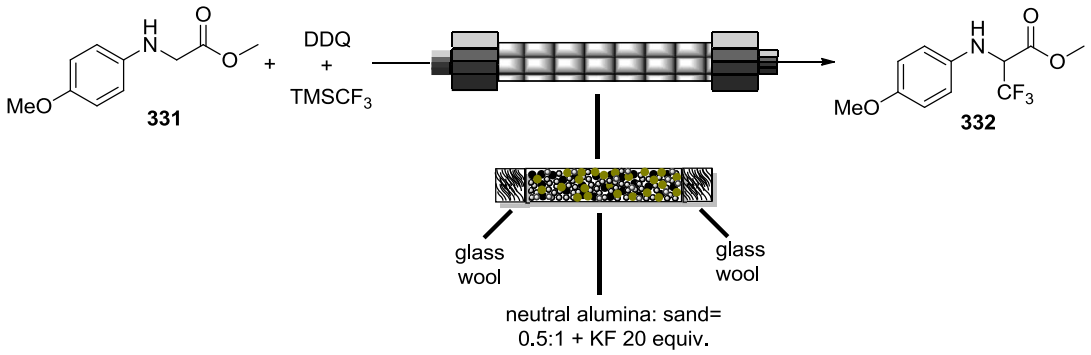
On the other hand, packed bed reagents, although fairly similar to packed-bed catalysts, display certain different properties. Their main advantage lies in the slow leaching[43] of the reagent into the reaction mixture which affords only certain amounts of the reagent in the mixture at any given time. One example of the successful use of a fixed-bed reagent is a $NaBH_4$/celite/LiCl column[44] developed for the reduction of ketones, aldehydes and *in situ* formed imines. Using specified additives and co-solvents, the reductions were accelerated and additional stabilization of the column was achieved.[44] The column leaches active borohydride in amounts stoichiometric with respect to the reductant.



Scheme 6.14. Reduction process in which a packed-bed reagent is used.

6.7 Fixed-Bed Reagent for Trifluoromethylation of Glycine Derivatives

A fixed-bed reactor was designed for CF_3 addition to the protected glycine ester (Scheme 6.15.). FEP tubing (7.94 mm) O.D. x (0.635 mm) I.D., connected in-line using 7.94 mm ETFE flangeless ferrules, 7.94 mm ½-20 Peek flangeless nuts and female 5/16-24 to male 1/14-28 KEL-F® (PCTFE) adapters was adjusted to serve as a cartridge. The average mass of the column was 1.17 g (calculated from the mass of the column and density of DMA as a solvent V=1.25 mL). The described cartridge was filled with a mixture of sand and neutral alumina in the ratio of 1:0.5 and KF (numbers of equivalents adjusted with respect to the starting material) in a way that the total mass of the dry filling was 2.3 g. The fixed-bed reactor was connected with a syringe pump by a 10 cm FEP tube (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter) and at its outlet there was a 10 cm FEP tube (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter). The reaction mixture (starting material, $TMSCF_3$, DDQ in DMAc) was fed into the packed bed using a syringe pump (Harvard PHD 2000 Infuse/Withdraw).



Scheme 6.15. Fixed-bed column consisting of a mixture of neutral alumina, sand, and KF for trifluoromethylation of glycine derivatives

6.8 Packed-Bed Reactor for Enzymatic Transformation

A fixed bed reactor was designed for the biotransformation of amino nitriles to the respective enantiopure amino acids (Scheme 6.16.). FEP tubing (7.94 mm) O.D. x (0.635 mm) I.D., connected in-line using 7.94 mm ETFE flangeless ferrules, 7.94 mm ½-20 Peek flangeless nuts and female 5/16-24 to male 1/14-28 KEL-F® (PCTFE) adapters was adjusted to serve as a cartridge. The described cartridge was filled with immobilized enzyme (750 mg, HA 403 S beads containing enzyme) and to the ends of the column, glass wool plugs were put in place to prevent leaching of the packing material (Figure 6.16). The column volume was determined to be 1.5 mL (calculated from the column mass and density of water). The substrate in the assay (*p*-$FC_6H_5CHNH_2CN$ in 10% MeOH in phosphate buffer pH 7.0) was fed onto the packed bed using a syringe pump (Harvard PHD 2000 Infuse/Withdraw). The fixed bed reactor was connected to a syringe pump by a 10 cm FEP tube

(IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter) and at its outlet there was a 10 cm FEP outlet tube (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter).

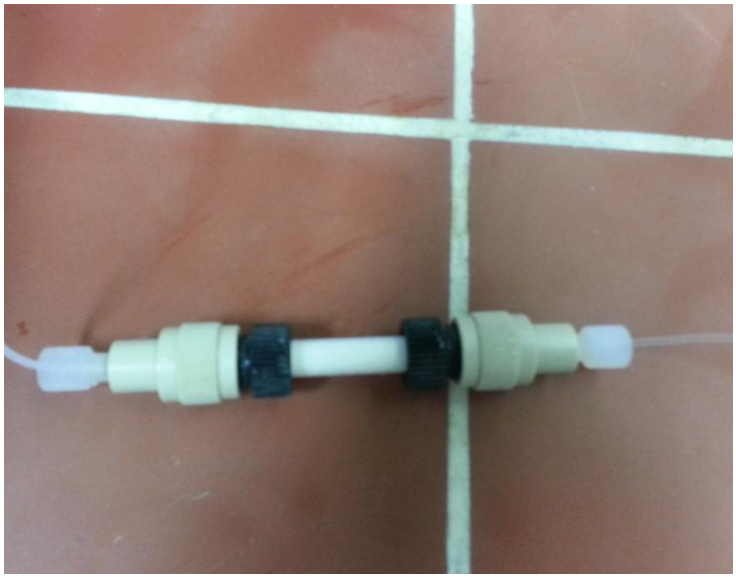


Figure 6.16 Column for enzymatic transformation

Bibliography

[1] T. G. M. Fekete, in *Flow Chemistry, Vol. 2* (Ed.: V. H. F. Darvas , G. Dorman), De Gruyter Textbook, **2014**.
[2] R. L. Hartman, J. P. McMullen, K. F. Jensen, *Angewandte Chemie International Edition* **2011**, *50*, 7502.
[3] J. Kobayashi, Y. Mori, S. Kobayashi, *Chemical Communications* **2005**, 2567.
[4] F. Benito-Lopez, W. Verboom, M. Kakuta, J. G. E. Gardeniers, R. J. M. Egberink, E. R. Oosterbroek, A. van den Berg, D. N. Reinhoudt, *Chemical Communications* **2005**, 2857.
[5] N. Kockmann, M. Gottsponer, B. Zimmermann, D. M. Roberge, *Chemistry – A European Journal* **2008**, *14*, 7470.
[6] D. M. Roberge, B. Zimmermann, F. Rainone, M. Gottsponer, M. Eyholzer, N. Kockmann, *Organic Process Research & Development* **2008**, *12*, 905.
[7] W. J. Klán P, *Photochemistry of organic compounds from concepts to practice.* , John Wiley & Sons Ldt, Chichester,West Sussex, UK, **2009**.
[8] B. P. Mason, K. E. Price, J. L. Steinbacher, A. R. Bogdan, D. T. McQuade, *Chemical Reviews* **2007**, *107*, 2300.
[9] R. A. Sheldon, *Chemical Society Reviews* **2012**, *41*, 1437.
[10] M. Fagnoni, D. Dondi, D. Ravelli, A. Albini, *Chemical Reviews* **2007**, *107*, 2725.
[11] T. H. Rehm, in *Flow Chemistry, Vol. 2* (Ed.: F. Darvas, Hessel, Volker, Dorman, György), Walter de Gruyter GmbH, Germany, **2014**.
[12] U. Megerle, M. Wenninger, R.-J. Kutta, R. Lechner, B. Konig, B. Dick, E. Riedle, *Physical Chemistry Chemical Physics* **2011**, *13*, 8869.
[13] J. d. P. Paul Atkins, *Atkins' Physical Chemistry*, Oxford University Press, Oxford, UK, **2010**.
[14] M. Oelgemöller, O. Shvydkiv, *Molecules* **2011**, *16*.
[15] T. Van Gerven, G. Mul, J. Moulijn, A. Stankiewicz, *Chemical Engineering and Processing: Process Intensification* **2007**, *46*, 781.
[16] E. E. Coyle, M. Oelgemoller, *Photochemical & Photobiological Sciences* **2008**, *7*, 1313.
[17] M. Oelgemoeller, *Chemical Engineering & Technology* **2012**, *35*, 1144.
[18] J. P. Knowles, L. D. Elliott, K. I. Booker-Milburn, *Beilstein Journal of Organic Chemistry* **2012**, *8*, 2025.
[19] A. G. Griesbeck, N. Maptue, S. Bondock, M. Oelgemoller, *Photochemical & Photobiological Sciences* **2003**, *2*, 450.
[20] B. D. A. Hook, W. Dohle, P. R. Hirst, M. Pickworth, M. B. Berry, K. I. Booker-Milburn, *The Journal of Organic Chemistry* **2005**, *70*, 7558.
[21] D. B. Ushakov, K. Gilmore, D. Kopetzki, D. T. McQuade, P. H. Seeberger, *Angewandte Chemie International Edition* **2014**, *53*, 557.
[22] C. J. Mallia, I. R. Baxendale, *Organic Process Research & Development* **2016**, *20*, 327.
[23] M. O'Brien, N. Taylor, A. Polyzos, I. R. Baxendale, S. V. Ley, *Chemical Science* **2011**, *2*, 1250.
[24] D. T. McQuade, P. H. Seeberger, *The Journal of Organic Chemistry* **2013**, *78*, 6384.
[25] I. R. Baxendale, *Journal of Chemical Technology & Biotechnology* **2013**, *88*, 519.
[26] J. C. Pastre, D. L. Browne, S. V. Ley, *Chemical Society Reviews* **2013**, *42*, 8849.
[27] C. Wiles, P. Watts, *Green Chemistry* **2012**, *14*, 38.
[28] N. G. Anderson, *Organic Process Research & Development* **2012**, *16*, 852.
[29] J. Wegner, S. Ceylan, A. Kirschning, *Advanced Synthesis & Catalysis* **2012**, *354*, 17.
[30] L. Malet-Sanz, F. Susanne, *Journal of Medicinal Chemistry* **2012**, *55*, 4062.
[31] J. Wegner, S. Ceylan, A. Kirschning, *Chemical Communications* **2011**, *47*, 4583.
[32] M. Baumann, I. R. Baxendale, *Beilstein Journal of Organic Chemistry* **2015**, *11*, 1194.
[33] J. Yue, G. Chen, Q. Yuan, L. Luo, Y. Gonthier, *Chemical Engineering Science* **2007**, *62*, 2096.
[34] B. Pieber, C. O. Kappe, in *Organometallic Flow Chemistry*, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1.

[35] P. M. Osterberg, J. K. Niemeier, C. J. Welch, J. M. Hawkins, J. R. Martinelli, T. E. Johnson, T. W. Root, S. S. Stahl, *Organic Process Research & Development* **2015**, *19*, 1537.
[36] V. Hessel, D. Kralisch, N. Kockmann, T. Noël, Q. Wang, *ChemSusChem* **2013**, *6*, 746.
[37] L. Vaccaro, D. Lanari, A. Marrocchi, G. Strappaveccia, *Green Chemistry* **2014**, *16*, 3680.
[38] K. F. Jensen, B. J. Reizman, S. G. Newman, *Lab on a Chip* **2014**, *14*, 3206.
[39] B. Gutmann, D. Cantillo, C. O. Kappe, *Angewandte Chemie International Edition* **2015**, *54*, 6688.
[40] K. Jähnisch, V. Hessel, H. Löwe, M. Baerns, *Angewandte Chemie International Edition* **2004**, *43*, 406.
[41] T. Inoue, M. A. Schmidt, K. F. Jensen, *Industrial & Engineering Chemistry Research* **2007**, *46*, 1153.
[42] *Vol. 2016*, Kinesis, http://kinesis.co.uk/media/catalog/product/cache/1/small_image/210x/9df78eab33525d08d6e5fb8d27136e95/0/0/006EZ-06-05-FF_0.jpg, **2016**.
[43] T. Noël, T. J. Maimone, S. L. Buchwald, *Angewandte Chemie International Edition* **2011**, *50*, 8900.
[44] K. Gilmore, S. Vukelić, D. T. McQuade, B. Koksch, P. H. Seeberger, *Organic Process Research & Development* **2014**, *18*, 1771.

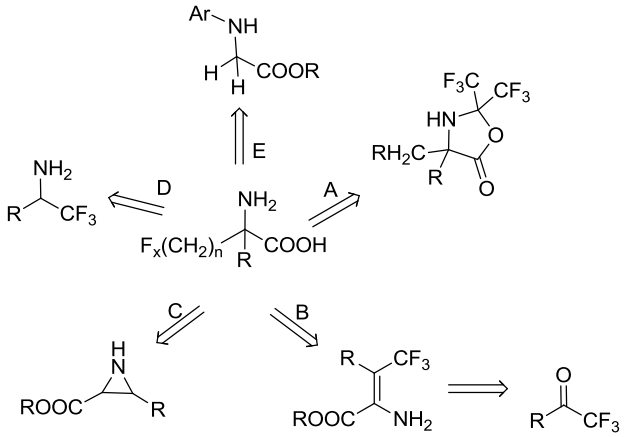
7. Results and Discussion:

New Approaches to Synthesis of Fluorinated Amino Acids

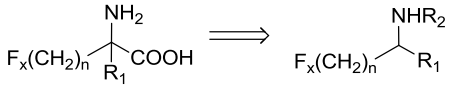
In this chapter and the following one new synthetic approaches towards amino acid synthesis will be discussed. Inspired by earlier research in the field we envisioned the development of new synthetic strategies towards a variety of fluorinated amino acids, α-trifluoromethylated amino acids, as well as variants bearing one or multiple fluorine atoms in the side chain.

Being aware of the two major approaches to the synthesis of fluorinated amino acids, the fluorinated building block approach and the direct incorporation of fluorine,[1-2] we first decided to pursue the more obvious, albeit more challenging, direct fluorination/trifluoromethylation approach. Using this approach we wanted to introduce fluorine into the β- or ω- position of the side chain. Our initial strategy for the direct incorporation of fluorine included a protected, non-fluorinated amino acid as a starting material (Scheme 7.1 A). HFA was identified as an appropriate protecting group, which offers simultaneous protection of both the amino and carboxylic moieties of an amino acid. As the installation of hexafluoroacetone (HFA) protection proved challenging, an alternative synthetic approach, which included exploiting HFA trihydrate for production of the key precursor, a trifluoromethylated ketone was tested (Scheme 7.1 B). The major advantage of this approach was the use of HFA trihydrate salt to avoid HFA gas formation, thereby offering a convenient and safer pathway for the direct incorporation of the trifluoromethyl group into the side chain, and access to β-trifluoromethylated amino acids. However, this mentioned methodology for trifluoromethyl anion production suffered from low reproducibility, which was an indicator that it would not be the best strategy.

Aziridine was next recognized as a new key intermediate (Scheme 7.1 C). A description of its low-yielding synthesis and initial attempts to nucleophilically open the aziridine ring using a fluorinated nucleophile follow. Unfortunately, none of these strategies towards the synthesis of trifluorinated amines yielded the desired product. However, although we were not successful, the lessons learned along the way finally lead us to a more straightforward retrosynthetic approach; namely the direct incorporation of the trifluoromethyl group into the α- position of an amino acid (Scheme 7.1 E).



Scheme 7.1. Retrosynthesis of fluorinated amino acids via direct introduction of fluorine.



Scheme 7.2. Retrosynthesis of fluorinated amino acids by using fluorinated building blocks approach.

Additionally, the classical approach to amino acid synthesis was exploited, Chapter 2.1; recognizing the value of the Strecker reaction for amino acid synthesis, we wanted to synthesize fluorinated amino acids by a fluorinated building block approach. A semi-continuous process for the synthesis of racemic fluorinated amino acids from fluorinated amines was developed and will be described in Chapter 7 (Scheme 7.2). This methodology was expanded to the synthesis of enantiopure fluorinated amino acids (Chapter 8) and amino acid derivatives (hydantoins, described in Chapter 9). Consequently, asymmetric enzymatic synthesis of fluorinated amino acids and the synthesis of hydantoins will be described.

To begin with, in the next four subchapters, the retrosynthetic pathways that include the direct trifluoromethylation shown in Scheme 7.1 will be described in more detail. An initial approach in which the strategy of fluorination of an HFA-protected amino acid was explored (Scheme 7.1 A) will be discussed first. Following is the disconnection of the desired target molecule that led to the trifluoromethylated ketone as a key synthon (Scheme 7.1 B) and the efforts to synthesize this precursor will be discussed. Further retrosynthetic reasoning that led to aziridine (Scheme 7.1 C) will be discussed and the attempts to form this valuable precursor together with the encountered problems will be described. An alternative to the trifluoromethylated ketone approach was a trifluoromethylated amine (Scheme 7.1 D); this synthesis will also be depicted. Finally, the description of successful direct trifluoromethylation of the α-position of the amino acid which allows for the direct incorporation of the CF_3 group at the α-position, in one step will be presented (Scheme 7.1 E).

7.1. HFA Protection Synthetic Strategy

As mentioned, the incorporation of fluorine into the side chain was chosen as the fastest and easiest route to side-chain fluorinated amino acids. Moreover, site selective transformation of readily available amino acids is a known strategy that leads to desired targets.[3] In general, site selective incorporation of fluorine into the side chain of an amino acid requires prior protection of the 1-carboxy and the amino groups. This is usually accomplished in a stepwise fashion; one chemical functionality is protected in one synthetic step (Figure 7.3 A). However, a protecting group that offers a simultaneous protection of amino and carboxylic functions is of great interest. By forming a heterocyclic (mostly five-membered) system (Figure 7.3 B) both moieties can be protected in a bidentate fashion.[3]

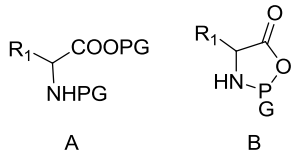
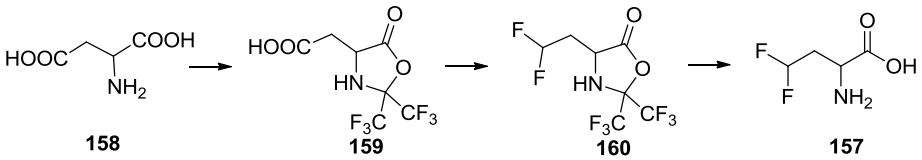


Figure 7.3. Modes of protection of amino acids.

Moreover, oxazolidinones obtained from protected amino acids and formaldehyde are used for site selective transformations of amino acids, as demonstrated in the preparation of *N*-*α*-aryl glutamines from Glu[4] and Dap from Asp[5] or syntheses of *N*-methylamine acids.[6] In comparison to formaldehyde, the use of HFA as a bidentate protecting group for the amino acids, is more synthetically attractive. Not only does this reagent protect both the 1-carboxy group and the amino group but it also simultaneously activates the 1-carboxy group toward nucleophiles, which is of great value in peptide synthesis; the need for activation of carboxylic group for peptide coupling is thereby avoided.[3] As our intention was to incorporate synthesized fluorinated amino acids into peptides, we planned on following Burger's synthesis (Scheme 7.4), to use HFA for protection of carboxylic and amino groups of aspartate, and later to perform fluorination at the *ω*-position of the HFA protected amino acid intermediate.[7]

Since HFA is commercially available as a gas (bp -28 °C) and is reported to be highly toxic, corrosive, teratogenic, very harmful upon inhalation or ingestion;[3] it is not a reagent which is desirable to work with. Consequently, a method to generate HFA gas *in situ* was developed. According to the literature[8] HFA gas is usually obtained by the addition of HFA trihydrate to H_2SO_4 (80-100 °C) using a custom-made setup (Figure 7.5). HFA trihydrate is added using a dropping funnel to boiling sulfuric acid, a second flask with boiling sulfuric acid is used as an extra drying agent, as HFA is extremely hygroscopic due to the presence of two CF_3 groups attached to the carbonyl group, which, by inductive effect withdraw electrons from it and make it susceptible to nucleophilic attack. The resulting dried HFA gas is introduced into a flask with amino acid in DMSO in order to perform

the protection reaction. To ensure safe working conditions, a condenser is placed above the flask to re-condense the gas, to prevent it from 'escaping' the reaction flask and to increase its concentration in the flask containing the amino acid. Extra gas produced is released into the fume hood after passage through two drying flasks.



Scheme 7.4. Synthetic sequence to access 2-amino-4,4-difluorobutanoic acid **157**.[7]

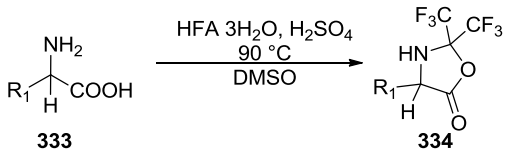
As can be easily noticed there are many significant difficulties with this method: introduction of HFA trihydrate via dropping funnel, two flasks with boiling sulfuric acid and the release of extra gas into the fume hood. Additionally, gas-liquid reactions in batch in general suffer from poor yields due to the small contact surface area between the two phases. Taking into account all these weaknesses and the expectation that the following step would be a direct fluorination, we realized the benefit of flow chemistry for this synthetic process and decided to develop the process of HFA amino acid protection directly in flow.[9-15] Thus a flow setup that could replace the custom-made glassware setup was designed. A syringe pump was introduced to add the HFA trihydrate to a boiling H_2SO_4 solution in a more controlled manner (Figure 7.6). In the setup, after the flask containing H_2SO_4, a cooling loop was introduced to collect the HFA gas (bp -28°C) and a tube was installed to bubble it directly into the reaction mixture increasing, the surface area-to-volume ratio. After the reaction flask, a trap for gas excess was introduced in order to remove it from the reaction mixture in a more controlled fashion (Figure 5.6).

Figure 7.5. Classical setup for HFA gas production and its addition to the amino acid
(a) Cooled condenser which keeps HFA in the reaction flask, (b) Reaction flask with amino acid in DMSO, (c) Dropping funnel with HFA trihydrate, (d) Flask 1 with H_2SO_4, (e) Flask 2 with H_2SO_4

Figure 7.6. Modernized setup for HFA gas production and its addition to the amino acid, (a) Reaction flask with amino acid in DMSO, (b) Cooling loop for condensation of HFA, (c) Flask with H_2SO_4, (d) Syrinige which contains HFA trihydrate

Using the described flow setup, reaction of protection of amino acid by HFA was performed; however, due to the low solubility of amino acids in DMSO, yields, obtained by NMR were low to moderate (Table 7.7). The greater solubility of polar aspartate in DMSO is reflected in the higher yield, 71%, when compared to serine and valine. These compounds, unfortunately, could not be isolated. Removing DMSO from the products proved very challenging, as well as the fact that the mass during isolation decreased rapidly.

Table 7.7 Protection of amino acid using HFA trihydrate using flow setup.



333 **334**

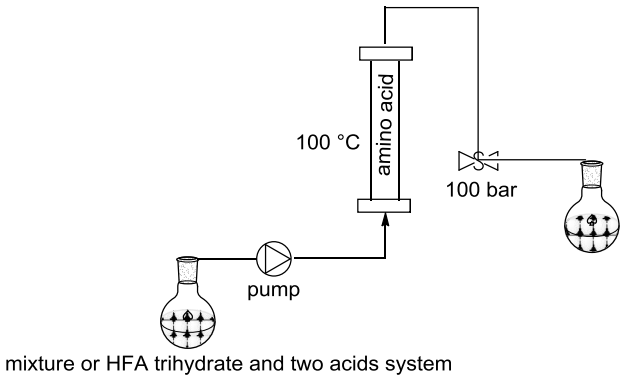
Entry[a]	Amino acid	NMR yield (%)
1	Aspartate	71
2	Serine	56
3	Valine	31

[a] Reaction conditions: amino acid (1.5 M in DMSO), 30 mmol of HFA trihydrate added at 77.7 µL/min, H_2SO_4 100 mL, heated to 90 °C, mesitylene used as internal standard

Due to the only partial success of the current flow process an alternative flow setup was designed. The focus was switched to a packed bed reactor. Here, we utilized a bench-top hydrogenation reactor from ThalesNano®, the "H-cube". This reactor combines continuous flow microchemistry with hydrogen generation and involves flowing reagents through a disposable packed catalyst cartridge under high temperatures and pressures. In order to adapt this tool to our requirements, we filled the column with amino acid and hypothesized that a mixture of acetic acid and either TFA or acetic anhydride (H_2SO_4 is not compatible with the machine design) when flowed through the amino acid packed column at 100 °C and under 100 bar would afford the desired protected amino acid (Scheme 7.8). We expected that a mixture of two acids would, at a given pressure and temperature, dehydrate the HFA trihydrate and produce the desired HFA gas, which would react *in situ* with the packed bed amino acid. The packed bed offers a greater surface that could react with the produced HFA gas and thus afford the desired product in higher yield. However, we obtained no product, with the amino acid column remaining intact and unreacted.

Although we were met with some small successes, the greater number of difficulties prevailed and it was decided that this synthetic pathway, installing HFA as a protecting group, should be

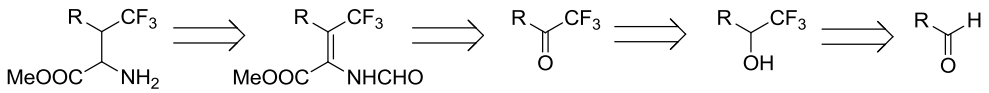
dismissed. The more efficient approach of direct trifluoromethylation was determined to be a more attractive strategy to access fluorinated amino acids.



Scheme 7.8 Design of the flow setup for amino acid protection.

7.2 Synthetic Approach to Fluorinated Amino Acids Through Trifluoromethylated Ketone

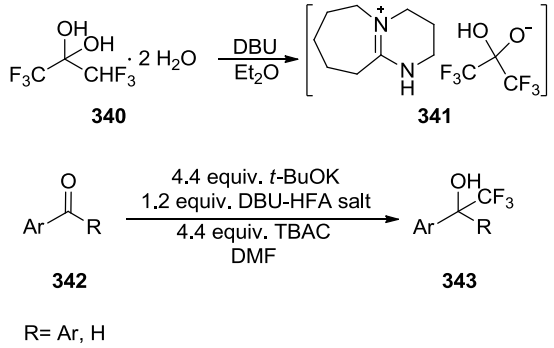
In our pursuit of the direct incorporation of a fluorine/trifluoromethyl group into the side chain of an amino acid, the synthesis of *β*-trifluoromethylated amino acid was envisioned (Scheme 7.1 B). As shown in the Scheme 7.1 B, inspired by the work of Daigneault *et al.,*[16] the following retrosynthetic strategy for *β*-trifluoromethylated amino acids was considered (Scheme 7.9). Synthesis starts with the trifluoromethylation of an aldehyde, and subsequent oxidation of the resulting alcohol to ketone is followed by Schölkopf's method[17] to obtain a formyl-protected alkene. This formyl group prevents racemization upon deprotection.[18-19] The key step in Daigneault's synthesis is a stereoselective hydrogenation with [((*R*)-trichickenfootphos)Rh(cod)]BF_4 catalyst which offers a direct approach to the enantiomerically pure amino acid. This method offers a pathway to various aliphatic and aromatic trifluoromethylated amino acids.



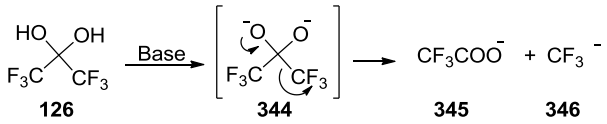
Scheme 7.9 Retrosynthesis of desired ester of fluorinated amino acids.

Based on the recent literature various trifluoromethylating reagents are available: $TMSCF_3$, $CF_3B(OMe)_3K$, $(EtO)_2POCF_3$ and HCF_3[20-25] for the trifluoromethylation of an aldehyde group. Nevertheless, Colby's triflouromethylating agent **341**[26] represents an efficient, interesting, novel and straightforward alternative to trifluoromethylated alcohols (Scheme 7.10). We chose this trifluoromethylating reagent due to its affordability and the ease of synthesis of the CF_3 salt which takes place just upon mixing of the reagents, therefore it does not require complicated pre-synthesis.

Additionally, it is an interesting way to utilize HFA trihydrate for the generation of the trifluoromethyl anion (CF_3^-), avoiding the formation of HFA gas. Colby *et al.* use HFA trihydrate in combination with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to form the salt **341** (Scheme 7.10) which upon reaction with potassium tertbutoxide releases CF_3^- (Scheme 7.11)[26] (confirmed by ^{19}F NMR fragmentation studies). DMF is used as a solvent in order to stabilize the formed CF_3^- by formation of a hemiaminal intermediate which acts as the trifluoromethylating reagent.[27-28]



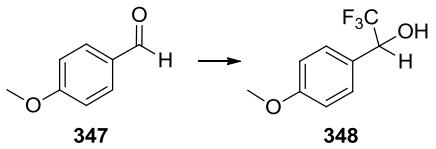
Scheme 7.10. Trifluoromethylation using a DBU-HFA salt.



Scheme 7.11. Formation of CF_3^-.

When a 0.12 M solution of aldehyde was reacted with tetrabutylammonium chloride (TBAC), *t*-BuOK and DBU-HFA salt at low temperatures, (Table 7.12) the trifluoromethylated alcohol of anisaldehyde was obtained in maximum 61% yield (Table 7.12) with extremely low reproducibility. This is due to the sensitivity of the DBU-HFA salt to air and moisture. Unfortunately, optimization of the number of salt equivalents, the temperature, the base and the solvent (Table 7.12) does not affect the yield (Table 7.12). Although we were satisfied to obtain our desired product in moderate yield, this reaction suffers from extremely low reproducibility with yields varying from 0 to 60% under identical reaction conditions (Table 7.13). With our goal of a robust and useful process in mind, we were again forced to change our synthetic route to an alternative key intermediate, aziridine.

Table 7.12 Optimization of trifluoromethylation of anisaldehyde.

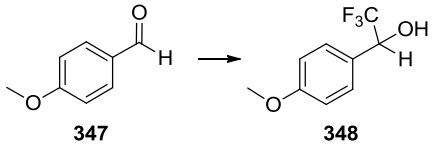


347 **348**

Entry[a]	TBAC (equiv.)	*t*-BuOK (equiv.)	DBU-HFA Salt (equiv.)	Temperature (°C)	Solvent	Conversion (%)
1	6.6	6.6	2.4	-30	DMF	61
2	6.6	6.6	1.6	-30	DMF	31
3	6.6	6.6	1	-30	DMF	14
4	10	10	5	-30	DMF	0
5	8.8	8.8	2.4	-30	DMF	0
6	4.4	4.4	2.4	-15	DMF	55
7	4.4	4.4	2.4	-15	DMF/THF	23
8	4.4	4.4	2.4	-15	DMF	60
9	4.4	4.4	2.4	-15	DMF	30
10	8.8	4.4	2.4	-15	DMF	40
11	4.4	8.8	2.4	-15	DMF	40
12	8.8	8.8	2.4	-15	DMF	55

[a] Reaction conditions: *t*-BuOK and TBAC in 2 ml of solvent, ice bath, DBU-HFA salt added. Stirred for 30 min, aldehyde 0.024 ml (0.240 mmol) added, stirred for 1.5 h, reaction ran under argon, yield determined by mesitylene as internal standard.

Table 7.13. Reproducibility of trifluoromethylation of anisaldehyde.



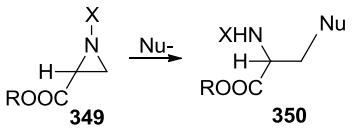
347 **348**

Entry	TBAC (equiv.)	*t*-BuOK (equiv.)	DBU-HFA Salt (equiv.)	Temperature (°C)	Solvent	Conversion (%)
1	4.4	4.4	2.4	-15	DMF	0
2	4.4	4.4	2.4	-15	DMF	0
3	4.4	4.4	2.4	-15	DMF	17
4	4.4	4.4	2.4	-15	DMF	55
5	4.4	4.4	2.4	-15	DMF	60
6	4.4	4.4	2.4	-15	DMF	55
7	4.4	4.4	2.4	-15	DMF	33
12	4.4	4.4	2.4	-15	DMF	60
13	4.4	4.4	2.4	-15	DMF	30
16	4.4	4.4	2.4	-15	DMF	15

Reaction conditions: *t*-BuOK and TBAC in 2 ml of DMF, ice bath, DBU-HFA salt added. Stirred for half an hour, aldehyde 0.024 ml (0.240 mmol) added, stirred for 1.5 h, glassware was oven dried, reaction ran under argon, conversion determined by mesitylene as an internal standard.

7.3 Attempt to Synthesize Fluorinated Amino Acids Through Aziridines as Intermediates

In an effort to access *β*-trifluoromethylated/fluorinated amino acids by direct incorporation of a fluorine/trifluoromethyl group into the side chain, we slightly modified our retrosynthesis and identified the aziridine as a key intermediate (Scheme 7.1 C). Aziridine formation and subsequent opening is an established pathway to access amino acids.[29-31] A general synthetic entry to amino acids is to open a suitably substituted aziridine-2-carboxylate **349** with a variety of nucleophiles as shown in Scheme 7.14. Reaction at the *β*-carbon atom would give the desired protected *α*-amino acids **350**. Regiospecific ring opening at the *β*-carbon atom of such an aziridine ester to yield the more useful product **350** has been successfully achieved using heteronucleophiles such as thiols,[32-38] [32] alcohols,[37-47] amines[48-51] and halides.[37-38, 52]

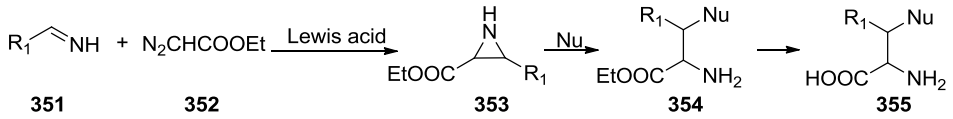


349 **350**

X= Ts, alkyl, aryl, Nu= RSH, ROH, RNH_2, X

Scheme 7.14 Opening of the aziridine ring.

To access side chain fluorinated amino acids, we envisioned the addition of a CF_3^- or F^- to the *β*- position of the aziridine (Scheme 7.15). Although the nucleophilic ring opening of aziridine using F^-[53] as well as carbon nucleophiles[53] was previously reported, to best of our knowledge nucleophilic attack using CF_3^- was not tested.



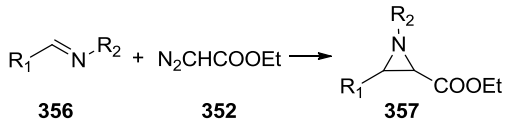
351 **352** **353** **354** **355**

Nu= F, CF_3
R_1= alkyl, aryl

Scheme 7.15 Envisioned synthesis of fluorinated amino acids through aziridines.

In view of the fact that most fluoride sources (pyridine·$(HF)_x$, $BF_3.Et_2O$, F_2) are toxic or difficult to handle we once again wanted to take advantage of the inherent safety by adapting this synthesis to a better controlled and contained flow method. The first step of the new strategy involved development of a synthetic route to aziridines. Classical methods to obtain aziridines are ring closure of amino alcohols,[54-57] ring opening of epoxides using sodium azide[56, 58] and the addition of haloester enolates to *N*-(trimethylsilyl) imines.[59] The formation of aziridines from imines and ethyl diazoacetate[60-63] (Scheme 7.16) was immediately attractive to us due to its direct approach and readily

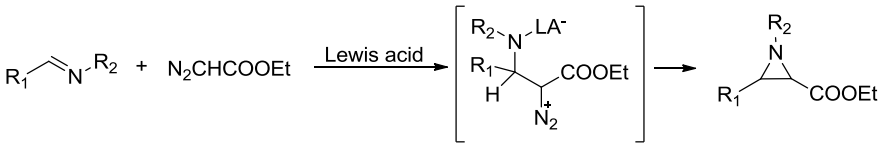
available starting materials. Additionally, our group has had previous success in producing imines from the corresponding amines in a very rapid flow process involving photoooxidation.[64]



356 **352** **357**

Scheme 7.16 Model reaction for aziridine formation.

To increase the rate of the reaction, we also considered the addition of Lewis acids. Their presence in the reaction mixture ensures drawing the electrons away from the C—N bond by coordination to this nitrogen, which facilitates nucleophilic attack on the electrophilic carbon and enables the subsequent ring closure to yield the aziridine (Scheme 7.17).[65-66]

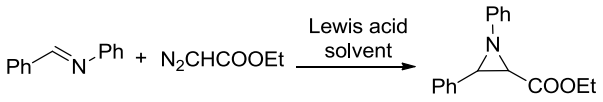


356 **352** **358** **359**

Scheme 7.17 Lewis acid assisted aziridine formation.

Using our model substrate **360** we began the optimization of the reaction conditions. After testing CuI in combination with $AgBF_4$ (Entries 1 and 2, Table 7.18) in different solvents, $CuCl_2$ (Entry 3) and $Yb(OTf)_3$ (Entry 5) as Lewis acids' we finally observed product formation when we used $Zn(OTf)_2$ (Entry 6).[61] Nonetheless, using $BF_3 \cdot Et_2O$ proved as a more successfull strategy for formation of aziridine in our hands (Entries 7-9, 11). Further optimization by increasing the temperature (Entry 8) and reaction time (Entry 9), led to the best yield (58%) for aziridine formation.

Table 7.18 Optimization of aziridine formation in batch.



360 352 361

Entry[a]	Lewis Acid	Reaction time (h)	Temperature (°C)	Solvent	Yield (%)[e]
1[a]	CuI/ $AgBF_4$	2	ice	DCM	0
2[a]	CuI/ $AgBF_4$	2	ice	MeCN	0
3[b]	$CuCl_2$/10mol%	2	r.t.	acetone	0
4[b]	$CuCl_2$/30 mol%	2	r.t.	acetone	0
5[d]	$Yb(OTf)_2$	2	r.t.	DCM	0
6[a]	$Zn(OTf)_2$	2	r.t.	DCM	20
7[c]	BF_3Et_2O	1	r.t.	hexane	30
8[c]	BF_3Et_2O	1	40	hexane	45
9[c]	BF_3Et_2O	3	40	hexane	58
10[c]	$BF_3.Et_2O$, 40 mol%	1	40	hexane	n/a[f]
11[c]	BF_3Et_2O	5	40	hexane	41

[a] 0.5 equiv. of ethyl diazoacetate is used (with respect to the imine (1 mmol, 0.33 M), 20 mol% of CuI/$AgBF_4$. [b] Reaction conditions: imine (0.3 mmol, 0.1 M), ethyl diazoacetate (1.0 equiv.), Lewis acid, under Ar for 1 h, [c] Reaction conditions 0.2 mmol imine, 0.2 M, $BF_3 \cdot Et_2O$ (10 mol%) added, heated (if applicable), 0.2 mmol ethyl diazoacetate added and stirred, [d] 0.38 mmol of ethyl diazoacetate, 1.5 equiv. of imine, reaction conditions:[61], [e] Determined by NMR using an internal standard, [f] Complex mixture obtained

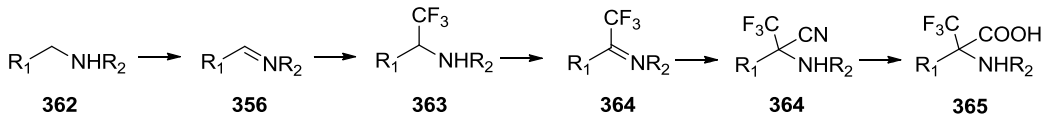
With a moderate yielding aziridine synthesis in hand, we next turned to the ring opening step by fluoride. Reports on nucleophilic opening of the aziridine ring using halides are frequent,[53] however, those involving the fluoride ion are few in number. The reason lies in the low acidity of hydrogen fluoride which can require the presence of a Lewis acid for aziridine opening.[67] TBAF[68-69] pyridine·$(HF)_x$[70-71] and $BF_3.Et_2O$[72] have also been employed in the ring opening of aziridines.

We tested this strategy under a variety of conditions employing pyridine·$(HF)_x$ and $BF_3 \cdot Et_2O$ as cheap fluoride sources. However, all reactions resulted in complex mixtures, from which isolation was difficult.

7.4 Synthetic Approach to *α*-trifluoromethylated Amino Acids through Trifluoromethylated Imine

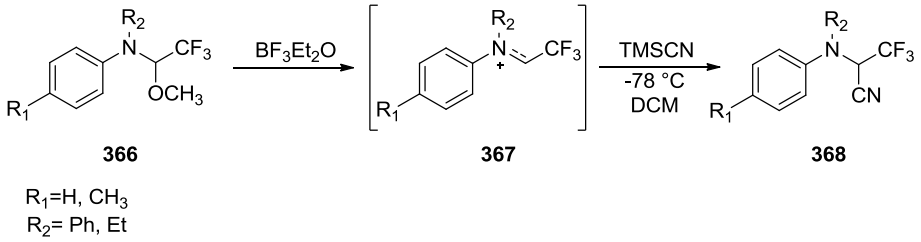
α-Trifluoromethylated amino acid synthesis with direct incorporation of a trifluoromethyl moiety or fluorine to the amino acid molecule was planned as an alternative to the classical approach to synthesis of α-CF_3 amino acids. The usual way to access these molecules is starting from trifluoromethylated/fluorinated building blocks (see Chapter 3.1), which makes the direct trifluoromethylation/fluorination a challenge. One of the possible disconnections (if using the direct trifluoromethylation approach) gives the *α*-CF_3 amine as a synthon (Scheme 7.1 D). Simultaneously, in

the Seeberger laboratory, a simple method for oxidation of primary amines to imines was developed,[64] which represented a suitable way to access our desired key precursor **364** (Scheme 7.19) for our envisioned synthesis of fluorinated amino acids (Scheme 7.19). By photooxidation of the amine, imine **356** is obtained and it is a good electrophile suitable for attack of the CF_3^- group which would give α-CF_3 amine **364**.[20, 73-75] We hypothesized that by second photooxidation of the α-CF_3 amine **364**, we would get the α-CF_3 imine **35** which could be trapped by a cyanide nucleophile in a Strecker-type reaction to give the desired product **37** (Scheme 7.19).



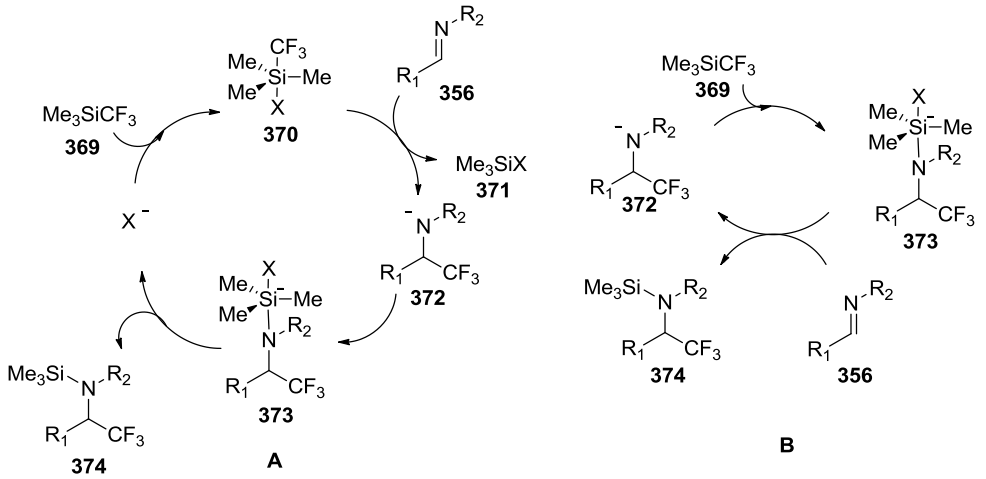
Scheme 7.19 Envisioned synthesis of fluorinated amino acids through imines.

The photooxidative cyanation of amines to amino nitriles using singlet oxygen includes the formation of cation **367** (Scheme 7.20) and subsequent cyanation of the latter to form amino nitrile **368**. Generation of a carbocation bearing an α-trifluoromethyl substituent is quite difficult due to the high electronegativity of this group. To the best of our knowledge, the only example of the Strecker reaction using the α-CF_3 imine is the one where α,α-methoxylated, trifluoromethylated amines were used as starting materials.[76] The methoxy moiety is a good leaving group and by treating the amine with the Lewis acid, α-trifluoromethylated amine is produced and can be trapped by TMSCN (Scheme 7.20). However, this method requires anodic methoxylation of various types of *N*-(fluoroethyl)amines to obtain the starting materials, an electrochemical method not available in every chemical laboratory.[76]



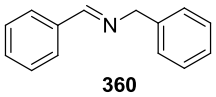
Scheme 7.20 Cyanation of α-CF_3 amines[76]

Advantage of our envisioned method over the ones available is the commercial availability of starting materials. The key step in the synthesis is trifluoromethylation of the imine **356** (Scheme 7.19)[20, 73-75] which when using $TMSCF_3$ (Ruppert-Prakash reagent) can follow two catalytic cycles[77] (Scheme 7.21). Silane in $TMSCF_3$ **369** is activated by F^- which enables the transfer of the CF_3 group to the C—N bond. Amide anion **372** can regenerate the Lewis base activator (A, Scheme 7.21) or catalytically promote the reaction (B, Scheme 7.21).[77]



Scheme 7.21 Mechanism for trifluoromethylation of imines[77], A-regeneration of Lewis base activator, B- **372** as catalytically active species

To establish the nucleophilic trifluoromethylation step, attack on the model imine **360** (Figure 7.22) was attempted using $TMSCF_3$ as a source of CF_3^- and tetrabutylammonium fluoride (TBAF) as an activator of $TMSCF_3$, however, no trifluoromethylation product was observed. Since it was reported that the presence of KHF_2/TFA enhances the CF_3 addition to the imines,[78] these additives were also tested, however; again, product formation did not occur.



360

Figure 7.22 Substrate used for fluorination.

We re-considered the choice of our model substrate. The reactivity of the imines towards nucleophilic addition can be increased by careful choice of the substituents on the nitrogen. Electron-withdrawing groups on the imine nitrogen or imine C=N bond, being part of the strained, small ring (Figure 7.23, strained) stabilize the negative charge formed upon nucleophilic attack (Intermediate **372**, Scheme 7.21), this; in turn stabilizes the negative charge and thus enables trifluoromethylation of intermediate **372** (Figure 7.21).[77] Conventional Schiff bases with an aromatic group on the nitrogen are poorly reactive, whereas imines with alkyl or benzyl group have mostly been shown to be completely inert (Figure 7.23).[77] Taking this into account, a more activated substrate was synthesized, the electron-withdrawing SO_2Ph group increases the electrophilicity of the imine carbon. Trifluoromethylation was carried out using TBAF as an activator but only a trace amount of product was detected. After increasing the electrophilicity of the substrate and failing to perform the trifluoromethylation, we decided to change the activator. A similar substrate[79] bearing a tolyl group instead of SO_2Ph, was successfully trifluoromethylated using tetrabutylammonium difluorotriphenylsilicate (TBAT) as an activator of $TMSCF_3$. In our hands, TBAT[79] as an activator

gave the product in 47% (Entry 5, Table 7.24). Although we found a better activator for the Ruppert-Prakash reagent, its price (10118.7 euros/mol) deemed this transformation inefficient (Table 7.24).

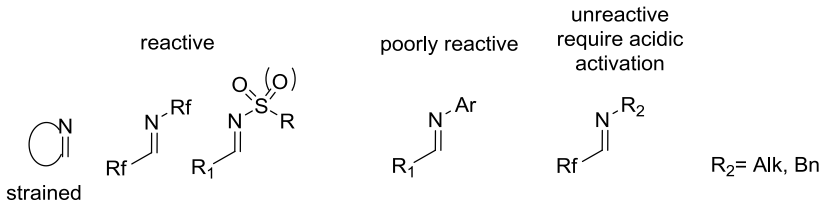
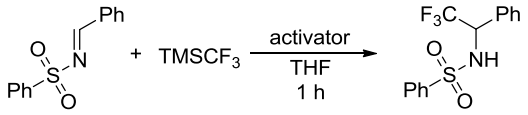


Figure 7.23 Reactivity of the imines in the process of nucleophilic addition.

Table 7.24 Optimization of CF_3 addition to imine.



Entry[a]	Activator	Temperature (°C)	Solvent	Yield (%)[b]
1	TBAF	-20	THF	trace amount
2	TBAF	0-5	THF	trace amount
3	TBAF	r.t.	THF	trace amount
4	TBAF	40	THF	trace amount
5	TBAT	0-5	THF	47

[a] Reaction conditions: imine (0.04 M in THF), $TMSCF_3$ (1.3 equiv.), activator (1.3 equiv.) in THF, under argon for 1 h, [b]NMR yield using mesytilene as an internal standard.

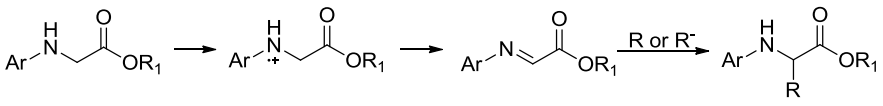
Despite the only partial success of strategies to directly introduce a fluorine atom/trifluoromethyl group, understanding of the reactivity and handling of HFA was gained. In addition, we obtained insight into the reactivity of aziridines and aldehydes towards trifluoromethylation/fluorination by the chosen trifluoromethyl/fluorine source. And finally, we realized that trifluoromethylation of imines is not a straightforward approach, instead special activators and a careful design of the substrate are required. Undeterred by the difficulties encountered to this point, we once again returned to our drawing board and sought a more attractive and robust synthetic route to access the desired side chain fluorinated/trifluoromethylated products. In the next subchapter (7.5) efforts towards the shortest and most direct route to our target, direct α-trifluoromethylation of protected amino acid will be described.

7.5 Trifluoromethylation of Protected Amino Acids

General synthetic methods to access nonnatural α-amino acids or to modify natural amino acids are desirable. Moreover, the direct α-functionalization of natural peptides uses the existing structure to offer fast access to diverse new peptides. Available methods used for the α-functionalization of amino acid derivatives or amides include: functionalization of α-carbanions (formed by deprotonation using a strong base),[80-81] radical α-bromination by *N*-bromosuccinimide,[82-83] UV photolysis in the presence of di-*tert*-butyl peroxide (DTBP),[84] Claisen rearrangement[85-89] and a ruthenium-catalyzed α-oxygenation.[90-93] Inspite of the various methods of classical C–C bond formation and modern accomplishments of transition metal catalysis, C–C bond fomation reactions use the pre-functionalized starting material which requires additional synthetic steps. Often, the pre-formed functional groups are simultaneously 'lost'. This repetitive pre-functionalization and deprotection together with isolation and purification, decrease the overall material efficiency in the synthesis of complex organic molecules and, at the same time, increase chemical waste.[94] As the complexity of the molecule increases, the material efficiency decreases. Chemical reactions, in which various chemical bonds in widely available natural resources, petroleum, natural gas, biomass, N_2, CO_2, O_2, water, and others can be selectively transformed directly without affecting other bonds and without the need for complicated pre-activations became a focus of interest.[94] These reactions could help to increase efficiency of the synthetic procedures. Transition metal-catalyzed C–H bond activation followed by C–C bond formation has thus attracted interest in recent years.[94] Outstanding progress has been made in this field which allows access to complex compounds more rapidly. For instance, the substrates capable of undergoing oxidative activation are the C−H bonds in the α-position to a tertiary nitrogen atom[95-114] tetrahydroisoquinolines, benzylic ethers and diphenylmethanes.[115] Therefore, it does not come as a surprise that selective oxidative functionalizaton of α-amino acid derivatives has gained significant attention[116-129] since Li's[116-117] efficient strategy to activate the sp^3 C-H bond adjacent to the nitrogen atom of glycine derivatives using the *tert*-butyl hydroperoxide (TBHP) as an oxidant and CuBr as a catalyst. Huang's group expanded a similar methodology in which a $Cu(OAc)_2$/pyrrolidine/TBHP-catalyzed coupling reaction for the oxidative functionalization of glycine esters with cyclic and acyclic ketones was developed.[118] Wang's group, on the other hand, used 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) as an oxidant and described a $Cu(OTf)_2$/BOX/DDQ-catalyzed asymmetric coupling reaction of glycine esters with α-substituted β-ketoesters.[120] More recently, Rueping's group showed that, in addition to existing oxidants, oxidation can be carried out by using photoredox chemistry and they developed a relay catalysis for the functionalization of glycine esters combining visible-light photoredox and Lewis acid catalysis.[122] Similar methodology was used to couple β-ketoesters to glycine esters, but here, $Cu(OTf)_2$ was used to form a nucleophile from the β-ketoester with $Ru(bpy)_3Cl_2$ and air was used for activation of the

glycine ester.[130] In an effort to extend the use of CDC (Cross-Dehydrogenative Coupling) reactions to the synthesis of α,α-disubstituted amino acids, You and co-workers used PA as a directing group at the N-terminus of the glycine derivative to afford the formation and subsequent reaction of the necessary ketimine intermediate. Different substituted indoles could be reacted with phenylalanine ester derivatives leading to the coupling products.[123] Yuan and co-workers also used indoles as nucleophiles and coupled them to the glycine esters using CuCl as a catalyst and oxygen as a terminal oxidant.[128] Another effort to expand the scope of coupling products resulted in a DTBP mediated α-alkylation of α-amino esters by alkanes, proceeding through dual sp^3 C–H bond cleavage.[121] Recently, Wu's group developed a C–H functionalization of α-amino acids without the use of any oxidant or base, using $Ru(bpy)_3(PF_6)_2$ and $Co(dmgH)_2pyCl$ as a photosensitizer and a catalyst, respectively, a variety of glycine esters with β-ketoesters or indole derivatives can be quantitatively converted into the desired cross-coupling products and hydrogen (H_2).[129] This methodology was expanded to the synthesis of substituted quinolines from glycine esters with alkenes or alkynes by a one-pot dehydrogenative Povarov/oxidation tandem process also developed by Mancheno's group[125] ($FeCl_3$/TEMPO salt), Hu's group[131] ($FeCl_3$/DTBP) and Wang's group[124] (triarylaminium salt/$InCl_3/O_2$).

The above mentioned transformations of glycine derivatives are proposed to proceed through a single electron transfer (SET) oxidation mechanism (Scheme 7.25).[116-118, 120-121, 123-130, 132] Furthermore, Rueping's work[122] offers evidence for a radical cation-induced mechanism since photosensitized electron transfer (PET) is a reliable method for generating radical cations of a variety of ionizable substrates.[133]



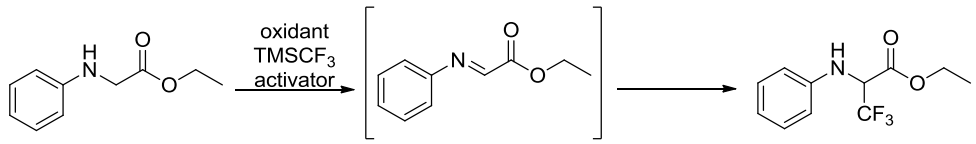
Scheme 7.25 nucleophilic addition to the α-position of protected amino acid.

Despite their common mechanism, from the above examples of C–H activation of glycine ester derivatives it is evident that these can be oxidized using a variety of reagents, and that the intermediate can be trapped by various nucleophiles, affording in one step an α-functionalized amino acid. Although there is an example of oxidative CF_3 addition to benzylic amines,[134] no trifluoromethylation of amino acid-like substrates was reported. As α-CF_3 amino acids are an interesting target (as previously stated in the chapter 3.1) we sought a way to combine the known data about activation of α- C–H bond in amino acid-type derivatives with their α-CF_3 group functionalization. Our goal was to expand the range of non-proteinogenic, fluorinated amino acids available by developing a new methodology for the introduction of the CF_3 group to the α-position of amino acids, and possibly even extend it to selective trifluoromethylation of peptides.

This methodology would have the advantage of reactions with low economic and ecological impacts and would allow the formation of C–C bonds without prefunctionalization of coupling partners. Additionally, it would significantly shorten the synthetic route to the desired materials.

7.5.1 Optimization of CF_3 Addition to PMP-Protected Glycine Ester

We envisioned *α*-C–H bond activation in an oxidative manner; the substrate **367** is oxidized to the corresponding imine **368** which is trapped *in situ* by nucleophilic attack of CF_3^- (Scheme 7.26), CF_3^- would be generated *in situ* using Ruppert-Prakash reagent and an activator. To demonstrate *α*-CF_3 addition, an ester substrate of aryl protected Gly **367** was chosen since the previous studies[116-118, 120-126, 128-131] had shown that the aryl group allows for the oxidation of the amine and subsequent addition of a nucleophile to the *α*-position. Optimization of the reaction conditions will be described in detail in the next subchapters.



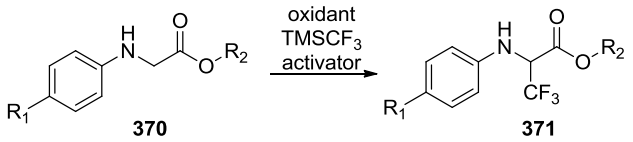
367 **368** **369**

Scheme 7.26 CF_3 addition to glycine ester derivative.

7.5.1.1 Oxidative C-H Activation of α-Position of PMP Gly Ester Under $TMSCF_3$ Conditions

As mentioned above, we chose the Rupert-Prakash reagent **369** (Scheme 7.21) as the trifluoromethylating agent therefore we examined several oxidation conditions using our substrate to determine which conditions would be compatible with subsequent CF_3 addition. Using *N*-phenylglycine ester **370** (Table 7.27) as a substrate we applied various oxidants under CF_3 conditions; namely, benzoyl peroxide, chloranil and DDQ were tested with $TMSCF_3$. In addition, $Ru(bpy)_3Cl_2$ was tested as a photosensitizer (Entries 6,7, Table 7.27). It was hypothesized that peroxides, DDQ or the photosensitizers would oxidize our substrate to the corresponding imine, which would facilitate the addition of CF_3 to the *in situ* formed imine (Scheme 7.26). Yet, trifluoromethylated product was obtained only using benzoyl peroxide, chloranil and DDQ as oxidants. Since DDQ conditions proved to be the most efficient, it was chosen as an optimal oxidant for our further optimization (Entry 5, Table 7.27).

Table 7.27 Optimization of CF_3 addition to protected amino acid, oxidant screen.



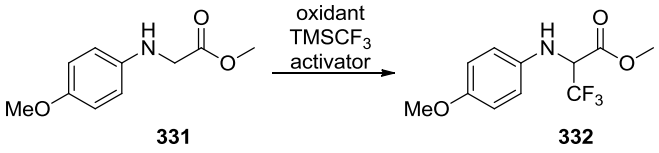
370 R_1 = MeO, R_2 = Me **331**; R_1 = H, R_2 = Et **367**

371 R_1 = MeO, R_2 = Me **332**; R_1 = H, R_2 = Et **369**

Entry	Oxidant/catalyst	Solvent	Product (%)	Reaction time (h)	Reaction temperature (°C)
1[a]	BPO[b]	MeCN	1	Overnight	r.t.
2[a]	Benzoquinone	MeCN	0	Overnight	r.t.
3[a]	DDQ	MeCN	20	Overnight	r.t.
4[c]	chloranil	DMA	25	5	r.t.
5	DDQ	DMA	58	4	Ice bath
6[c]	Rubpy, TEMPO, $(NH_4)_2S_2O_8$	DMA	Trace amounts	Overnight	r.t.
7[c]	Rubpy, TEMPO, CCl_3Br	DMA	1	Overnight	r.t.

[a] Conditions: *p*-MeOPhNHCH$_2$COOEt (0.2 M in solvent)1.3 equiv. of oxidant, 3 equiv of KF and 3 equiv. of $TMSCF_3$ (2 M solution in THF), stirred under inert atmosphere. [b] at 40 °C, 4 % of product is detected, at 6 equiv. of $TMSCF_3$/ KF 5% of product is detected. [c] Conditions: *p*-MeOPhNHCH$_2$COOEt (0.1 M in solvent)1.3 equiv. of oxidant, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, inert atmosphere.

After the initial oxidant screening, starting material for further optimization was changed. According to the literature, the presence of the methoxy group in the *p*-position of the aromatic ring promotes the oxidation reaction (Scheme 7.28).[118]



331 332

Scheme 7.28 Optimization of CF_3 addition to glycine ester derivative.

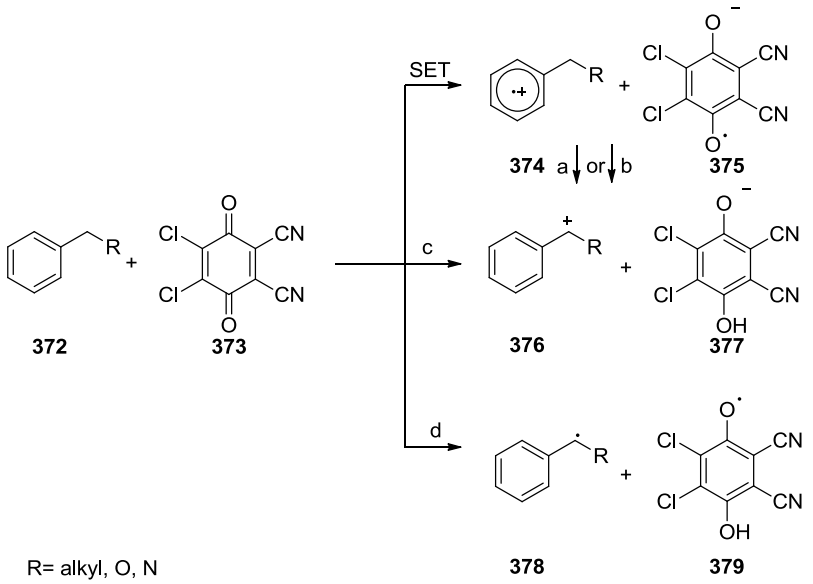
Quinones play an important role as oxidizing reagents in organic chemistry and are hydrogen acceptors in biological processes.[135] Quinones with high reduction potential, like DDQ, have been extensively used in organic synthesis for the oxidation of organic compounds. Despite their widespread use, mechanistic details of these processes are still controversial.[136-144] In general, there are four most plausible scenarios for C–H activation by DDQ (Scheme 7.29):[144-146]

(a) Electron transfer from the substrate to DDQ to generate the radical cation of the substrate and the radical anion of DDQ, followed by hydrogen atom transfer to generate the substrate cation and [DDQ-H], a two-step process (Scheme 7.29)

(b) Electron transfer from the substrate to DDQ to generate the radical cation of the substrate and the radical anion of DDQ, followed by proton transfer and another SET to afford substrate cation and [DDQ-H] (Scheme 7.29)

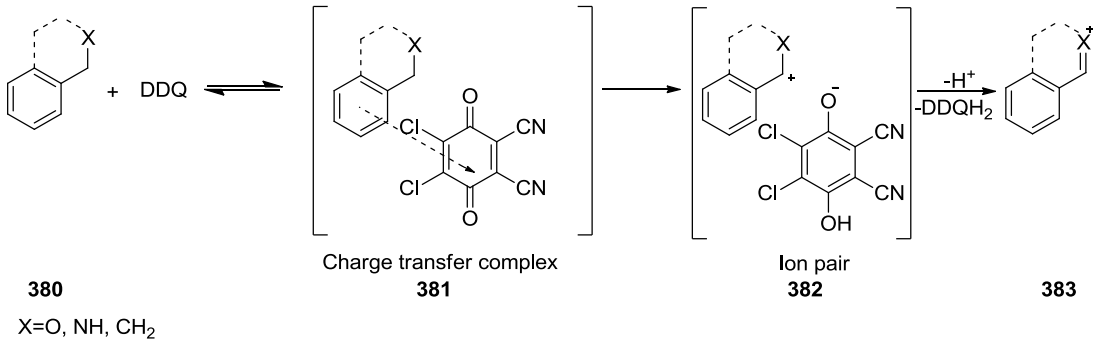
(c) Hydride transfer from substrate to DDQ generating the substrate cation and $[DDQ\text{-}H]^-$; a single step (Scheme 7.29)

(d) Hydrogen atom transfer from substrate to DDQ generating the substrate radical and $[DDQ\text{-}H]^{\cdot}$; a single step (Scheme 7.29).



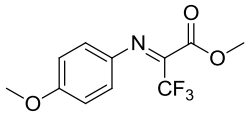
Scheme 7.29 Possible mechanistic pathways of H abstraction from toluene by DDQ.[144, 146]

Oxidation of the substrate most likely occurs by hydride transfer from substrate to the quinone forming an ion pair product (Scheme 7.30). The specific mechanism of hydride abstraction has been the subject of some controversy and discussion. [144-146]



Scheme 7.30 General mechanism of DDQ oxidation.

Hence, it was hypothesized that our chosen substrate, PMP-protected glycine ester **331** undergoes oxidation with DDQ and CF_3^- is subsequently added to the iminium ion. In addition, we observed additional product formation. In the ^{19}F NMR spectrum, a peak at -75.15 ppm was observed. It was isolated and identified as the over-oxidation product **384 (**Figure 7.33). From here on, in this text, it will be addressed as byproduct **384**.



384

Figure 7.33 Byproduct of the trifluoromethylation of PMPprotected glycine ester, methyl 3,3,3-trifluoro-2-((4-methoxyphenyl)imino)propanoate (**384**).

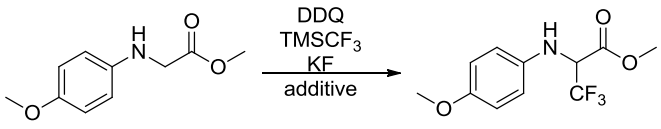
7.5.1.2 Optimization of Reaction Conditions

It is known that in the presence of Cu(I) or Cu(0) in stoichiometric[147] or catalytic amounts.[148] a '$CuCF_3$' species is formed[148-149] which can be used *in situ* to perform aromatic trifluoromethylation reactions,[147] trifluoromethylation of carbonyl compounds[150] or even the trifluoromethylation of the benzylic position adjacent to nitrogen in amines.[134] Based on Li's research,[134] we hypothesized that the addition of Cu(I) or Cu(0) to our system might facilitate the formation of '$CuCF_3$' and push the reaction towards the full conversion to the desired trifluoromethylated product.

Initially, CuI was chosen and the reaction is performed in MeCN (Entry 1, Table 7.31), however; when compared to the non-catalyzed reaction (Entry 6, Table 7.31) no significant difference in the yield was observed. Furthermore, we tested CuCl (Entry 2) and Cu(I) species in the presence of more complex ligands (Entries 3, 4, 5) but no significant difference in the yield of the desired product was observed.

In order to increase the conversion to the desired product, the electrophilicity of the imine could be increased by employing a Lewis acid[116, 118, 122-123] thereby facilitating faster addition of the nucleophile. In that sense addition of Cu Lewis' acids (mentioned above), $Sc(OTf)_3$ (Entry 7) and AgOTf (Entries 8 and 9) in DMA were tested and when compared to the non-catalyzed reaction (Entry 10) no increase in product formation was observed. Based on the literature data, AgOTf in stoichiometric amounts facilitates the formation of $AgCF_3$,[151] which could mediate CF_3 addition to the imine and thus our desired product formation. In the case of AgOTf the reaction yield varied due to the labourious work-up (multiple washes to remove the Ag reagent).

Table 7.31 Optimization of CF_3 addition to protected amino acid, additive screen.



331 **332**

Entry	Additive	NMR Yield product (%)	NMR Yield Byproduct 384 (%)	Time (h)	Solvent	Temperature
1[a]	CuI, 5 mol%	28	6	5	MeCN	r.t.
2[a]	CuCl, 5 mol%	30	9	5	MeCN	r.t.
3[a]	CuI+phenantroline, 5 mol%	27	6	5	MeCN	r.t.
4[a]	MeCN Cu(I)BF_4, 5 mol%	28	6	5	MeCN	r.t.
5[a]	chloro(1,3-bis(2,6-diisopropylphenyl) imidazol-2-ylidene)copper(I), 5 mol%	24	6	5	MeCN	r.t.
6[a]	no	26	5	5	MeCN	r.t.
7[c]	$Sc(OTf)_3$ 10 mol%	51	20	4	DMA	Ice bath
8[d]	$Ag(OTf)_3$ 3 equiv.	22		4	DMA	Ice bath
9[d]	$Ag(OTf)_3$ 3 equiv.	47		4	DMA	Ice bath
10	no	58	10	4	DMA	Ice bath

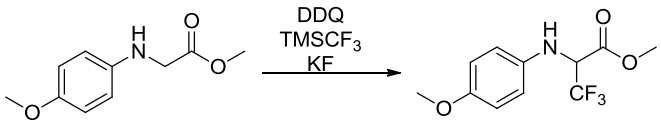
Conditions: [a] *p*-$MeOPhNHCH_2COOEt$ (0.2 M in solvent), 5 mol% of additive 1.3 equiv. of DDQ, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, stirred in inert atmosphere. [b] *p*-$MeOPhNHCH_2COOEt$ (0.2 M in solvent), 2 mol% of additive 1.3 equiv. of DDQ, 3 equiv. of KF and 3 equiv. of $TMSCF_3$,stirred in inert atmosphere, [c] *p*-$MeOPhNHCH_2COOEt$ (0.2 M in solvent), 10 mol% of additive 1.3 equiv. of DDQ, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, stirred in inert atmosphere. [d] *p*-$MeOPhNHCH_2COOEt$ (0.2 M in solvent), 3 equiv. of additive 1.3 equiv. of DDQ, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, stirred in inert atmosphere.

We identified MeCN and DMA as solvents that enhance product formation (See Entries 4 and 5, Table 7.32) while EtOAc, hexane and toluene yield low or no conversion to the trifluoromethylated protected amino acid (Entries 1-3, Table 7.32). Together with the desired product, as mentioned, formation of byproduct **384** was observed. We reasoned that the addition of the second, less polar solvent might slow down byproduct formation by slowing down the oxidation step and faciliting the CF_3 addition to yield the target compound (Table 7.32). Increase in the yield, when compared to the

reaction in pure MeCN, was observed only when toluene was used as a co-solvent (Entry 11). With this finding, we tested and increased amount of DMA with toluene (Entry 12, Table 7.32) which did not affect the yield significantly. On the other hand, we also explored the possibility of a mixed solvent system with MeCN (Entries 13-15, Table 7.32). When reaction mixtures in MeCN and DMA were compared it was observed that the reaction proceeds faster in MeCN, but yields more byproducts.

In conclusion, polar, aprotic solvents, like DMA enhance product formation which supports our hypothesis about the reaction proceeding via an imine intermediate that is trapped by the CF_3^- nucleophile. Formation of the imine is immediate and quantitative upon the addition of DDQ to the starting material in polar aprotic solvents: NMR was performed right after mixing the amine and DDQ in DMA.

Table 7.32 Optimization of CF_3 addition to protected amino acid, solvent screen.



331 **332**

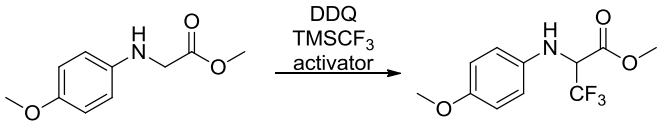
Entry[a]	Solvent	Product (%)	Time (h)	Temperature (°C)
1	EtOAc	1	5	Ice bath
2	hexane	0	5	Ice bath
3	toluene	0	5	Ice bath
4	MeCN	25	5	r.t.
5	DMA	58	4	Ice bath
6	20% DMA/chloroform	0	5	Ice bath
7	20% DMA/chlorobenzene	0	5	Ice bath
8	20% DMA/dioxane	4	5	Ice bath
9	20% DMA/ EtOAc	5	5	Ice bath
10	20 % DMA/DCM	28	5	Ice bath
11	20% DMA/toluene	36	5	Ice bath
12	50% DMA/toluene	40	4	Ice bath
13	20% DMSO//MeCN	20	5	r.t.
14	20% DMF/ MeCN	25	5	r.t.
15	20% DMA/ MeCN	38	5	r.t.

[a] *p*-MeOPhNHCH$_2$COOEt (0.2 M in solvent), 1.3 equiv. of DDQ, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, stirred in inert atmosphere.

KF is used in the system to activate $TMSCF_3$; F^- coordinates to Si to make a transient complex which expels CF_3^- (Scheme 7.21).[20] Considerable effort has been devoted to the development of

different catalytic systems for the activation of the Ruppert-Prakash reagent and these include nucleophilic initiators such as the fluoride anion (CsF, TBAF, TBAT), alkoxide (*t*-BuOK), amine *N*-oxide (Me_3NO), acetate (LiOAc), *N*-heterocyclic carbenes (NHC), phosphine ($P(tBu)_3$), as well as electrophilic initiators such as Lewis acids (TiF_4/DMF, $Cu(OAc)_2$/dppe/toluene).[152] The most commonly used activators for $TMSCF_3$ in the trifluoromethylation of imines are CsF, TBAT and TBAF.[20, 79, 153] Employing CsF gave a lower yield of the product (Entry 1 and 2, Table 7.33) whereas TBAT and TBAF (Entries 3 and 4, Table 7.33) did not yield the desired product under applied reaction conditions. We hypothesized that lower yield when employing CsF (in comparison to KF) is obtained because CsF is more hygroscopic, eventhough the reaction was performed in dry conditions in inert atmosphere, H_2O was present in the system which hinders the addition of CF_3 to the imine (CF_3H is formed). After the performed activator screening it was obvious that KF, our first choice, is the best activator for $TMSCF_3$ in our system.

Table 7.33 Optimization of CF_3 addition to protected amino acid, activator screen.



331 **332**

Entry[a]	Activator	Solvent	Product (%)	Time (h)	Temperature (°C)
1	CsF	DMA	23	4	Ice bath
2	KF	DMA	58	4	Ice bath
3	TBAT	DMA	0	4	Ice bath
4	TBAF[b]	DMA	0	4	Ice bath

[a] p-MeOPhNHCH$_2$COOEt (0.2 M in DMA), 3 equiv. of activator 1.3 equiv. of DDQ, 3 equiv. of $TMSCF_3$, stirred in inert atmosphere, [b] 1 M solution in THF.

7.5.2 Adapting the CF_3 Addition to Flow Conditions

KF is only partially soluble in DMA and MeCN, therefore a white precipitate is observed at the bottom of the flask. As we obtained a maximum yield of 58% for the desired product together with imine, hypothesis was that the reaction might be limited by the solubility of KF. With the intention of increasing conversion to the desired product, packed bed reactor was developed. In that way, we would solve the solubility issue with KF and increase the contact surface area between the KF and the reactants. To decrease the amount of KF used in our packed bed we decided to use an inert porous material as a column packing medium. We started off by using neutral alumina, expecting that a change in the pH of the reaction mixture might influence the conversion to the desired product. Increasing in the retention time at 10 equivalents of KF did not influence the conversion to the desired product (Entry 2, Table 7.34). In addition, decreasing in the number of KF equivalents did not change

the yield significantly (Entry 3). Increasing the KF equivalent to 20 brought a slight increase in the conversion to the desired product (Entry 4); however, further increases in the KF equivalents did not result in increase in the yield (Entry 6). By changing the solvent to a mixture of DMA and MeCN (Entry 5) the yield slightly decreased and when the reaction time was decreased to 3 minutes (Entry 7) the yield stayed the same as at the 15 minute residence time. The change in the column composition (Entries 8 and 9) did not affect the yield significantly either, even when basic alumina, silica gel or molecular sieves were used instead of neutral alumina as packing material (Entries 10, 11 or 12); the yield of the desired product was still approximately 40%. Ultimately, a decrease of the reaction temperature to -9 ˚C (Entry 15) was successful and pushed the yield to 55%. Neither shortening the retention time (Entry 16) nor using MeCN and DMA as a mixed solvent system (Entry 18) had any influence on the yield of the desired product. We also hypothesized that the product may be formed in higher yield, but may decompose over time partially due to the pH of the reaction mixture, and the presence of DDQ. Therefore, we performed the reaction by trapping the product in an acidic or basic mixture upon exiting the column (Entries 19 and 20) but observed no change in the yield.

Table 7.34 Optimization of CF_3 addition to PMP protected glycine ester using a packed-bed reactor

Entry[a]	Column composition	Solvent	KF/ equiv.	Temperature	Reaction time (min)	%Product
1	1:1-sand:neutral alumina	DMA	10	r.t.	15	32
2	1:1-sand:neutral alumina	DMA	10	r.t.	30	29
3	1:1-sand:neutral alumina	DMA	5	r.t.	16	31
4	1:1-sand:neutral alumina	DMA	20	r.t.	17	41
5	1:1-sand:neutral alumina	DMA:MeCN=1:1	10	r.t.	14	23
6	1:1-sand:neutral alumina	DMA	30	r.t.	15	36

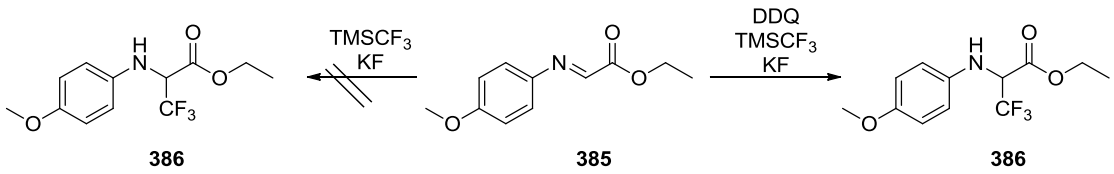
7	1:1-sand:neutral alumina	DMA	20	r.t.	3	35
8	1.5:0.5-sand:neutral alumina	DMA	20	r.t.	3	39
9	1.5:0.25-sand:neutral alumina	DMA	20	r.t.	1	30
10	1.5:0.5-sand-basic alumina	DMA	20	r.t.	1	40
11	1.5:0.5-sand-silica gel	DMA	20	r.t.	1	40
12	1.5:0.5-sand-molecular sieves	DMA	20	r.t.	1	43
13	1.5:0.5-sand:neutral alumina	DMA	20	Ice bath	1	44
14	1.5:0.5-sand:neutral alumina	DMA	20	-9	1	44
15	1.5:0.5-sand:neutral alumina	DMA	20	-9	3	55
16	1.5:0.5-sand:neutral alumina	DMA	20	-9	0,1	40
17	1.5:0.5-sand:neutral alumina	DMA	20	-9	10	54
18	1.5:0.5-sand:neutral alumina	1:1= DMA: MeCN	20	-9	3	33
19	1.5:0.5-sand:neutral	Quench: 10% MeCOOH in	20	-9	3	49

	alumina	DMA				
	1.5:0.5-	Quench:				
20	sand:neutral	10%Et_3N in	20	-9	3	49
	alumina	DMA				

[a] *p*-MeOPhNHCH$_2$COOEt, (0.2 M in DMA), 1.3 equiv. DDQ, 3 equiv. TMSCF$_3$, work-up with NaHCO$_3$ aq. solution and EtOAc, for more details about packed bed column, see Applied Methods.

7.5.3 Mechanistic Studies

In order to test the hypothesis about the mechanism of the reaction, a control experiment was carried out. The imine of the glycine ethyl ester substrate was subjected to TMSCF$_3$ and KF in DMA, and no product **386** was observed. (Scheme 7.35) When the same reaction was carried out in the presence of DDQ (1.3 equiv.), product **386** was detected (Scheme 7.35).



Scheme 7.35 Addition of CF$_3$ to the *α*-position

This may indicate that the reaction does not proceed via the neutral imine intermediate. Puzzled by the aforementioned results, our next hypothesis was that the reaction could proceed through a radical mechanism. On this basis we decided to test Mn(OAc)$_3$·2H$_2$O instead of DDQ as an oxidant. Mn(OAc)$_3$ is an important radical oxidant in organic synthesis for the formation of C-C bonds.[154] It is usually used to initiate free radical reactions on mono, and 1,3-dicarbonyl compounds.[155] In our case the product is formed when 1.3 equiv. of Mn(OAc)$_3$·2H$_2$O is used; however, the yield is lower (14%) than in the presence of DDQ. This is not direct proof of a radical mechanism taking place. Moreover, when a mixture of DDQ (1 equiv.) and Mn(OAc)$_3$·2H$_2$O (20 mol%) is used, the product is obtained in 52% yield, yet when the ratio is slightly changed, (0.9 equiv. DDQ and 30 mol% Mn(OAc)$_3$·2H$_2$O) the yield dropped (38%).

New method for α-trifluoromethylation of protected amino acids was developed. Using this transformation in a batch reactor 58% of the product was formed. This process was adjusted to flow conditions using a packed-bed reactor. The flow process was optimized and the yield of 55% of the product was achieved.

Outlook

Further tests are needed to confirm the mechanism of this transformation. Radical traps, such as TEMPO could be employed to determine if the radical mechanism is taking place. Future work

should also focus on applying the developed system to other type of substrates such as amides instead of esters. If this is successful, trifluoromethylation of peptides could be tested as well.

Bibliography

[1] P. K. Valerii, A. S. Vadim, *Russian Chemical Reviews* **1991**, *60*, 850.
[2] C. D. C. R. Smits, K. Burger, B. Koksch, *Chemical Society Reviews* **2008**, *37*, 1727.
[3] J. Spengler, C. Böttcher, F. Albericio, K. Burger, *Chemical Reviews* **2006**, *106*, 4728.
[4] C. S. Esslinger, K. A. Cybulski, J. F. Rhoderick, *Bioorganic & Medicinal Chemistry* **2005**, *13*, 1111.
[5] B. K. Rene Smits, *Current Topics in Medicinal Chemistry* **2006**, *6*, 1483.
[6] A. G. a. M. J. J. J. T. Welch, in *General Features of Biological Activity of Fluorinated Amino Acids*, John Wiley & Sons, Chichester, **1995**, pp. 311.
[7] D. Winkler, K. Burger, *Synthesis* **1996**, *1996*, 1419.
[8] F. Weygand, K. Burger, K. Engelhardt, *Chemische Berichte* **1966**, *99*, 1461.
[9] M. Baumann, I. R. Baxendale, S. V. Ley, N. Nikbin, C. D. Smith, *Organic & Biomolecular Chemistry* **2008**, *6*, 1587.
[10] J.-i. Yoshida, in *Flash Chemistry*, John Wiley & Sons, Ltd, **2008**, pp. i.
[11] C. D. Smith, I. R. Baxendale, S. Lanners, J. J. Hayward, S. C. Smith, S. V. Ley, *Organic & Biomolecular Chemistry* **2007**, *5*, 1559.
[12] C. H. Hornung, M. R. Mackley, I. R. Baxendale, S. V. Ley, *Organic Process Research & Development* **2007**, *11*, 399.
[13] N. Nikbin, M. Ladlow, S. V. Ley, *Organic Process Research & Development* **2007**, *11*, 458.
[14] V. Hessel, S. Hardt, H. Löwe, in *Chemical Micro Process Engineering*, Wiley-VCH Verlag GmbH & Co. KGaA, **2005**, pp. i.
[15] V. Hessel, H. Löwe, A. Müller, G. Kolb, in *Chemical Micro Process Engineering*, Wiley-VCH Verlag GmbH & Co. KGaA, **2005**, pp. 182.
[16] C. Benhaim, L. Bouchard, G. Pelletier, J. Sellstedt, L. Kristofova, S. Daigneault, *Organic Letters* **2010**, *12*, 2008.
[17] D. Enders, Z.-X. Chen, G. Raabe, *Synthesis* **2005**, *2005*, 306.
[18] L. Panella, A. M. Aleixandre, G. J. Kruidhof, J. Robertus, B. L. Feringa, J. G. de Vries, A. J. Minnaard, *The Journal of Organic Chemistry* **2006**, *71*, 2026.
[19] J. C. Sheehan, D.-D. H. Yang, *Journal of the American Chemical Society* **1958**, *80*, 1154.
[20] C. A. Gloria Rubiales, Eduardo Martínez de Marigorta and Francisco Palacios *Arkivoc* **2014**, *2*, 362.
[21] K. Iwanami, T. Oriyama, *Synlett* **2006**, *2006*, 0112.
[22] V. V. Levin, A. D. Dilman, P. A. Belyakov, M. I. Struchkova, V. A. Tartakovsky, *Tetrahedron Letters* **2011**, *52*, 281.
[23] P. Cherkupally, P. Beier, *Tetrahedron Letters* **2010**, *51*, 252.
[24] G. K. S. Prakash, P. V. Jog, P. T. D. Batamack, G. A. Olah, *Science* **2012**, *338*, 1324.
[25] Z. Gonda, S. Kovács, C. Wéber, T. Gáti, A. Mészáros, A. Kotschy, Z. Novák, *Organic Letters* **2014**, *16*, 4268.
[26] M. V. Riofski, A. D. Hart, D. A. Colby, *Organic Letters* **2013**, *15*, 208.
[27] T. Billard, Bernard R. Langlois, G. Blond, *European Journal of Organic Chemistry* **2001**, *2001*, 1467.
[28] J. Joubert, S. Roussel, C. Christophe, T. Billard, B. R. Langlois, T. Vidal, *Angewandte Chemie International Edition* **2003**, *42*, 3133.
[29] K. J. M. Beresford, N. J. Church, D. W. Young, *Organic & Biomolecular Chemistry* **2006**, *4*, 2888.
[30] N. J. Church, D. W. Young, *Tetrahedron Letters* **1995**, *36*, 151.
[31] T. Katagiri, Y. Katayama, M. Taeda, T. Ohshima, N. Iguchi, K. Uneyama, *The Journal of Organic Chemistry* **2011**, *76*, 9305.
[32] Z. Bernstein, D. Ben-Ishai, *Tetrahedron* **1977**, *33*, 881.

[33] S. K. Wakamiya Tateaki, Shiba Tetsuo, Nakajima Kiichiro, Neya Masahiro, Okawa Kenji, *Bulletin of the Chemical Society of Japan* **1982**, *55*, 3878.
[34] K. Nakajima, H. Oda, K. Okawa, *Bulletin of the Chemical Society of Japan* **1983**, *56*, 520.
[35] R. J. Parry, M. V. Naidu, *Tetrahedron Letters* **1983**, *24*, 1133.
[36] J. E. Baldwin, R. M. Adlington, N. Moss, N. G. Robinson, *Journal of the Chemical Society, Chemical Communications* **1987**, 1664.
[37] B. S. Axelsson, K. J. O'Toole, P. A. Spencer, D. W. Young, *Journal of the Chemical Society, Chemical Communications* **1991**, 1085.
[38] B. S. Axelsson, K. J. O'Toole, P. A. Spencer, D. W. Young, *Journal of the Chemical Society, Perkin Transactions 1* **1994**, 807.
[39] K. Harada, I. Nakamura, *Journal of the Chemical Society, Chemical Communications* **1978**, 522.
[40] H. K. Nakamura Isao, *Chemistry Letters* **1979**, *8*, 313.
[41] N. K. Tanaka Takumi, Maeda Toru, Nakamura Akihiro, Hayashi, Noboru, Okawa Kenji, *Bulletin of the Chemical Society of Japan* **1979**, *52*, 3579.
[42] T. Tanaka, K. Nakajima, K. Okawa, *Bulletin of the Chemical Society of Japan* **1980**, *53*, 1352.
[43] K. Okawa, K. Nakajima, T. Tanaka, *Journal of Heterocyclic Chemistry* **1980**, *17*, 1815.
[44] K. Okawa, M. Yuki, T. Tanaka, *Chemistry Letters* **1979**, *8*, 1085.
[45] K. Nakajima, M. Neya, S. Yamada, K. Okawa, *Bulletin of the Chemical Society of Japan* **1982**, *55*, 3049.
[46] K. Nakajima, T. Tanaka, M. Neya, K. Okawa, *Bulletin of the Chemical Society of Japan* **1982**, *55*, 3237.
[47] J. Bodenan, J. Chanet-Ray, R. Vessiere, *Synthesis* **1992**, *1992*, 288.
[48] T. Shiba, T. Ukita, K. Mizuno, T. Teshima, T. Wakamiya, *Tetrahedron Letters* **1977**, *18*, 2681.
[49] T. Wakamiya, K. Mizuno, T. Ukita, T. Teshima, T. Shiba, *Bulletin of the Chemical Society of Japan* **1978**, *51*, 850.
[50] K. Nakajima, T. Tanaka, K. Morita, K. Okawa, *Bulletin of the Chemical Society of Japan* **1980**, *53*, 283.
[51] N. Shigematsu, H. Setoi, I. Uchida, T. Shibata, H. Terano, M. Hashimotop, *Tetrahedron Letters* **1988**, *29*, 5147.
[52] T. N. Wade, F. Gaymard, R. Guedj, *Tetrahedron Letters* **1979**, *20*, 2681.
[53] X. E. Hu, *Tetrahedron* **2004**, *60*, 2701.
[54] J. W. Kelly, N. L. Anderson, S. A. Evans, *The Journal of Organic Chemistry* **1986**, *51*, 95.
[55] D. Tanner, *Angewandte Chemie International Edition in English* **1994**, *33*, 599.
[56] D. Tanner, P. Somfai, *Tetrahedron Letters* **1987**, *28*, 1211.
[57] E. Kuyl-Yeheskiely, M. Lodder, G. A. van der Marel, J. H. van Boom, *Tetrahedron Letters* **1992**, *33*, 3013.
[58] J. Legters, L. Thijs, B. Zwanenburg, *Tetrahedron Letters* **1989**, *30*, 4881.
[59] G. Cainelli, M. Panunzio, D. Giacomini, *Tetrahedron Letters* **1991**, *32*, 121.
[60] K. Juhl, R. G. Hazell, K. Anker Jorgensen, *Journal of the Chemical Society, Perkin Transactions 1* **1999**, 2293.
[61] K. G. Rasmussen, K. Anker Jorgensen, *Journal of the Chemical Society, Perkin Transactions 1* **1997**, 1287.
[62] K. G. J. Rasmussen, Karl Anker, *Journal of the Chemical Society, Chemical Communications* **1995**, 1401.
[63] L. Casarrubios, J. A. Pérez, M. Brookhart, J. L. Templeton, *The Journal of Organic Chemistry* **1996**, *61*, 8358.
[64] D. B. Ushakov, K. Gilmore, D. Kopetzki, D. T. McQuade, P. H. Seeberger, *Angewandte Chemie International Edition* **2014**, *53*, 557.
[65] W. Xie, J. Fang, J. Li, P. G. Wang, *Tetrahedron* **1999**, *55*, 12929.
[66] A. Mazumdar, Z. Xue, M. F. Mayer, *Synlett* **2007**, *2007*, 2025.
[67] V. A. Petrov, *Journal of Fluorine Chemistry* **2000**, *106*, 25.
[68] A. Dureault, I. Tranchepain, J. C. Depezay, *The Journal of Organic Chemistry* **1989**, *54*, 5324.
[69] R.-H. Fan, Y.-G. Zhou, W.-X. Zhang, X.-L. Hou, L.-X. Dai, *The Journal of Organic Chemistry* **2004**, *69*, 335.

[70] G. R. Ayi Ayicoue I., *Journal of the Chemical Society, Perkin Transactions 1* **1983**, 2045.
[71] T. N. Wade, *The Journal of Organic Chemistry* **1980**, *45*, 5328.
[72] C.-H. Ding, L.-X. Dai, X.-L. Hou, *Synlett* **2004**, *2004*, 2218.
[73] W. Xu, W. R. Dolbier, *The Journal of Organic Chemistry* **2005**, *70*, 4741.
[74] L. Bernardi, E. Indrigo, S. Pollicino, A. Ricci, *Chemical Communications* **2012**, *48*, 1428.
[75] J.-C. Blazejewski, E. Anselmi, M. P. Wilmshurst, *Tetrahedron Letters* **1999**, *40*, 5475.
[76] T. Fuchigami, S. Ichikawa, *The Journal of Organic Chemistry* **1994**, *59*, 607.
[77] A. D. Dilman, V. V. Levin, *European Journal of Organic Chemistry* **2011**, *2011*, 831.
[78] V. V. Levin, A. D. Dilman, P. A. Belyakov, M. I. Struchkova, V. A. Tartakovsky, *European Journal of Organic Chemistry* **2008**, *2008*, 5226.
[79] G. K. S. Prakash, M. Mandal, G. A. Olah, *Synlett* **2001**, *2001*, 77.
[80] P. Beak, W. J. Zajdel, D. B. Reitz, *Chemical Reviews* **1984**, *84*, 471.
[81] A. I. Meyers, *Aldrichimica Acta* **1985**, *18*, 59.
[82] C. J. Easton, C. A. Hutton, G. Rositano, E. W. Tan, *The Journal of Organic Chemistry* **1991**, *56*, 5614.
[83] C. J. Easton, I. M. Scharfbillig, E. Wui Tan, *Tetrahedron Letters* **1988**, *29*, 1565.
[84] H. S. Knowles, K. Hunt, A. F. Parsons, *Tetrahedron Letters* **2000**, *41*, 7121.
[85] B. Kübel, G. Höfle, W. Steglich, *Angewandte Chemie International Edition in English* **1975**, *14*, 58.
[86] K. Burger, K. Geith, K. Gaa, *Angewandte Chemie International Edition in English* **1988**, *27*, 848.
[87] R. E. Ireland, R. H. Mueller, A. K. Willard, *Journal of the American Chemical Society* **1976**, *98*, 2868.
[88] P. A. Bartlett, J. F. Barstow, *The Journal of Organic Chemistry* **1982**, *47*, 3933.
[89] U. Kazmaier, H. Mues, A. Krebs, *Chemistry – A European Journal* **2002**, *8*, 1850.
[90] S. Murahashi, T. Naota, T. Kuwabara, T. Saito, H. Kumobayashi, S. Akutagawa, *Journal of the American Chemical Society* **1990**, *112*, 7820.
[91] S.-I. Murahashi, A. Mitani, K. Kitao, *Tetrahedron Letters* **2000**, *41*, 10245.
[92] T. Naota, T. Nakato, S.-I. Murahashi, *Tetrahedron Letters* **1990**, *31*, 7475.
[93] G. Cainelli, M. DaCol, P. Galletti, D. Giacomini, *Synlett* **1997**, *1997*, 923.
[94] S. A. Girard, T. Knauber, C.-J. Li, in *From C-H to C-C Bonds: Cross-Dehydrogenative-Coupling*, The Royal Society of Chemistry, **2015**, pp. 1.
[95] S.-I. Murahashi, N. Komiya, H. Terai, T. Nakae, *Journal of the American Chemical Society* **2003**, *125*, 15312.
[96] S. Doye, *Angewandte Chemie International Edition* **2001**, *40*, 3351.
[97] S. Sakaguchi, T. Kubo, Y. Ishii, *Angewandte Chemie International Edition* **2001**, *40*, 2534.
[98] A. T. Chatani Naoto, Ikeda Tsutomu, Yorimitsu, Shuhei, K. F. Ishii Yutaka, Murai Shinji, *Journal of the American Chemical Society* **2000**, *122*, 12882.
[99] N. Chatani, T. Asaumi, S. Yorimitsu, T. Ikeda, F. Kakiuchi, S. Murai, *Journal of the American Chemical Society* **2001**, *123*, 10935.
[100] C.-H. Jun, *Chemical Communications* **1998**, 1405.
[101] B. DeBoef, S. J. Pastine, D. Sames, *Journal of the American Chemical Society* **2004**, *126*, 6556.
[102] H. M. L. Davies, C. Venkataramani, T. Hansen, D. W. Hopper, *Journal of the American Chemical Society* **2003**, *125*, 6462.
[103] T. Yoshimitsu, Y. Arano, H. Nagaoka, *Journal of the American Chemical Society* **2005**, *127*, 11610.
[104] T. Maruyama, S. Suga, J.-i. Yoshida, *Journal of the American Chemical Society* **2005**, *127*, 7324.
[105] S. Suga, T. Nishida, D. Yamada, A. Nagaki, J.-i. Yoshida, *Journal of the American Chemical Society* **2004**, *126*, 14338.
[106] S. Suga, S. Suzuki, J.-i. Yoshida, *Journal of the American Chemical Society* **2002**, *124*, 30.
[107] J.-i. Yoshida, S. Suga, S. Suzuki, N. Kinomura, A. Yamamoto, K. Fujiwara, *Journal of the American Chemical Society* **1999**, *121*, 9546.
[108] C. S. Yi, S. Y. Yun, I. A. Guzei, *Organometallics* **2004**, *23*, 5392.
[109] Z. Li, D. S. Bohle, C.-J. Li, *Proceedings of the National Academy of Sciences* **2006**, *103*, 8928.

[110] Z. Li, C.-J. Li, *Organic Letters* **2004**, *6*, 4997.
[111] Z. Li, C.-J. Li, *Journal of the American Chemical Society* **2005**, *127*, 6968.
[112] Z. Li, C.-J. Li, *Journal of the American Chemical Society* **2005**, *127*, 3672.
[113] Z. Li, C.-J. Li, *European Journal of Organic Chemistry* **2005**, *2005*, 3173.
[114] Z. Li, C.-J. Li, *Journal of the American Chemical Society* **2006**, *128*, 56.
[115] A. F. M. Noisier, M. A. Brimble, *Chemical Reviews* **2014**, *114*, 8775.
[116] L. Zhao, O. Baslé, C.-J. Li, *Proceedings of the National Academy of Sciences* **2009**, *106*, 4106.
[117] L. Zhao, C.-J. Li, *Angewandte Chemie International Edition* **2008**, *47*, 7075.
[118] J. Xie, Z.-Z. Huang, *Angewandte Chemie International Edition* **2010**, *49*, 10181.
[119] Z. Q. Liu, M. Zhou, X. H. Zhang, J. M. Xu, Y. P. Xue, Y. G. Zheng, *J Mol Microbiol Biotechnol* **2012**, *22*.
[120] G. Zhang, Y. Zhang, R. Wang, *Angewandte Chemie International Edition* **2011**, *50*, 10429.
[121] H. Peng, J.-T. Yu, Y. Jiang, H. Yang, J. Cheng, *The Journal of Organic Chemistry* **2014**, *79*, 9847.
[122] S. Zhu, M. Rueping, *Chemical Communications* **2012**, *48*, 11960.
[123] K. Li, G. Tan, J. Huang, F. Song, J. You, *Angewandte Chemie International Edition* **2013**, *52*, 12942.
[124] X. Jia, F. Peng, C. Qing, C. Huo, X. Wang, *Organic Letters* **2012**, *14*, 4030.
[125] H. Richter, O. García Mancheño, *Organic Letters* **2011**, *13*, 6066.
[126] Z.-Q. Wang, M. Hu, X.-C. Huang, L.-B. Gong, Y.-X. Xie, J.-H. Li, *The Journal of Organic Chemistry* **2012**, *77*, 8705.
[127] C. Huo, C. Wang, C. Sun, X. Jia, X. Wang, W. Chang, M. Wu, *Advanced Synthesis & Catalysis* **2013**, *355*, 1911.
[128] C. Huo, C. Wang, M. Wu, X. Jia, H. Xie, Y. Yuan, *Advanced Synthesis & Catalysis* **2014**, *356*, 411.
[129] X.-W. Gao, Q.-Y. Meng, J.-X. Li, J.-J. Zhong, T. Lei, X.-B. Li, C.-H. Tung, L.-Z. Wu, *ACS Catalysis* **2015**, *5*, 2391.
[130] X.-W. Gao, Q.-Y. Meng, M. Xiang, B. Chen, K. Feng, C.-H. Tung, L.-Z. Wu, *Advanced Synthesis & Catalysis* **2013**, *355*, 2158.
[131] P. Liu, Z. Wang, J. Lin, X. Hu, *European Journal of Organic Chemistry* **2012**, *2012*, 1583.
[132] G. Liu, *Organic & Biomolecular Chemistry* **2012**, *10*, 6243.
[133] A. G. Griesbeck, N. Hoffmann, K.-d. Warzecha, *Accounts of Chemical Research* **2007**, *40*, 128.
[134] H. Mitsudera, C.-J. Li, *Tetrahedron Letters* **2011**, *52*, 1898.
[135] W. Lai, C. Li, H. Chen, S. Shaik, *Angewandte Chemie International Edition* **2012**, *51*, 5556.
[136] B. M. Trost, *Journal of the American Chemical Society* **1967**, *89*, 1847.
[137] P. Mueller, J. Rocek, *Journal of the American Chemical Society* **1972**, *94*, 2716.
[138] F. Stoos, J. Rocek, *Journal of the American Chemical Society* **1972**, *94*, 2719.
[139] R. P. Thummel, W. E. Cravey, D. B. Cantu, *The Journal of Organic Chemistry* **1980**, *45*, 1633.
[140] B. W. Carlson, L. L. Miller, *Journal of the American Chemical Society* **1985**, *107*, 479.
[141] K. M. Zaman, S. Yamamoto, N. Nishimura, J. Maruta, S. Fukuzumi, *Journal of the American Chemical Society* **1994**, *116*, 12099.
[142] C. Rüchardt, M. Gerst, J. Ebenhoch, *Angewandte Chemie International Edition in English* **1997**, *36*, 1406.
[143] F. Wurche, W. Sicking, R. Sustmann, F.-G. Klärner, C. Rüchardt, *Chemistry – A European Journal* **2004**, *10*, 2707.
[144] V. S. Batista, R. H. Crabtree, S. J. Konezny, O. R. Luca, J. M. Praetorius, *New Journal of Chemistry* **2012**, *36*, 1141.
[145] H. H. Jung, P. E. Floreancig, *Tetrahedron* **2009**, *65*, 10830.
[146] O. R. Luca, T. Wang, S. J. Konezny, V. S. Batista, R. H. Crabtree, *New Journal of Chemistry* **2011**, *35*, 998.
[147] H. Urata, T. Fuchikami, *Tetrahedron Letters* **1991**, *32*, 91.
[148] M. Oishi, H. Kondo, H. Amii, *Chemical Communications* **2009**, 1909.
[149] V. C. R. McLoughlin, J. Thrower, *Tetrahedron* **1969**, *25*, 5921.
[150] S. Wu, W. Zeng, Q. Wang, F.-X. Chen, *Organic & Biomolecular Chemistry* **2012**, *10*, 9334.

[151] J.-B. Liu, X.-H. Xu, F.-L. Qing, *Organic Letters* **2015**, *17*, 5048.
[152] J.-A. Ma, D. Cahard, *Journal of Fluorine Chemistry* **2007**, *128*, 975.
[153] A. S. Pilcher, H. L. Ammon, P. DeShong, *Journal of the American Chemical Society* **1995**, *117*, 5166.
[154] M. Yılmaz, E. V. B. Yılmaz, A. T. Pekel, *Helvetica Chimica Acta* **2011**, *94*, 2027.
[155] B. B. Snider, *Tetrahedron* **2009**, *65*, 10738.

8. Results and Discussion:

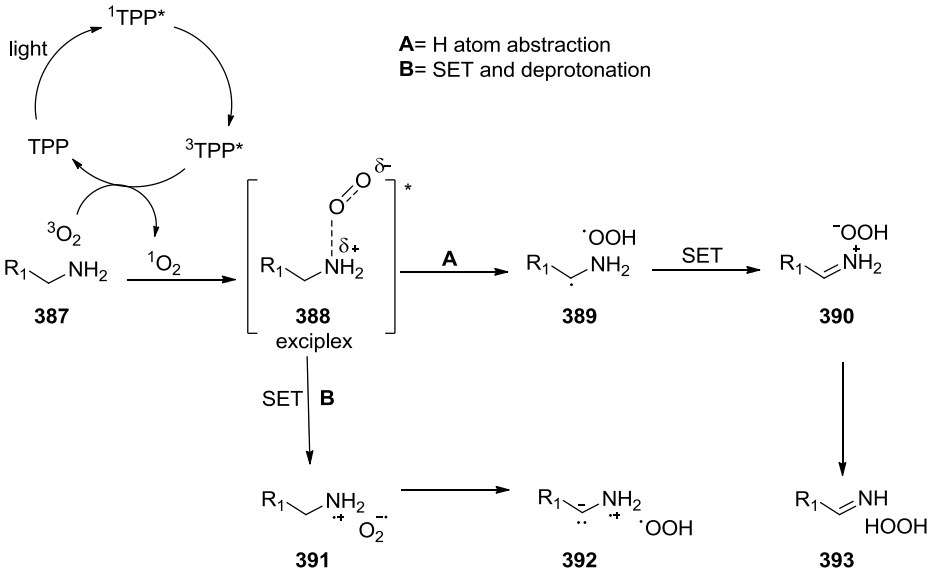
Synthesis of Fluorinated Amino Acids and Their Derivatives Using Fluorinated Building Blocks

This chapter has been modified in part from the following publication *Flow Synthesis of Fluorinated α-Amino Acids,* S. Vukelić, D.B. Ushakov, K. Gilmore, B. Koksch and P.H. Seeberger*, Eur. J. Org. Chem*, 2015, **14**, 3036-3039.

As depicted in the Scheme 7.2 an alternative approach to fluorinated amino acids is using fluorinated building blocks and applying the classical methods for synthesis of fluorinated amino acids, such as the Strecker reaction. The Strecker reaction is the most exploited and protecting group-free route to access amino nitriles. Subsequent hydrolysis of amino nitriles leads to amino acids. Nevertheless, as already mentioned, Strecker reaction suffers from drawbacks related to the preparation of primary imines[1-4] such as the need to work under anhydrous conditions and the reactivity of aldehydes. Aldimines themselves are unstable, resulting in nitrile and enamine formation as well as polymerization. Using the fast and clean method for the direct oxidation, oxidation of primary amines to imines can be done in a flow photoreactor.[5] These valuable intermediates can be trapped *in situ* to give α-cyanoepoxides[6] and α-amino nitriles.[5] Formed α-amino nitriles can be converted to amino acids by boiling it for 48 h in the HCl. However, in the next subchapters a semi-continuous synthesis of racemic fluorinated amino acids, starting from fluorinated amines will be described, as well as the possiblity of expanding this methodology to access enantiopure fluorinated amino acids.

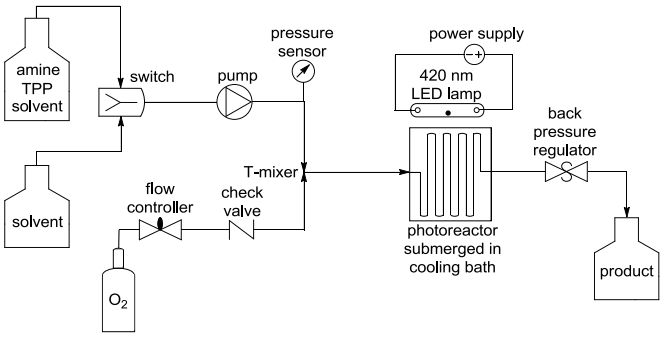
8.1.1 Oxidation of Amines

Selective oxidation of amines to imines represents an easy way to access these chemical compounds. Direct oxidation can usually proceed only with benzylamines and using expensive stoichiometric oxidants,[7-11] or complex metal catalysts.[12] On the other hand, singlet oxygen is an attractive oxidant for amine oxidation because it is affordable and highly atom-economical, it does not have a high negative environmental impact and at the same time it can be used for amine oxidation (Scheme 6.1).[13-15] Singlet oxygen can be activated in different ways. For instance, Che *et al.* reported on activating oxygen by TPP to oxidate secondary benzylamines to the corresponding imines (Scheme 8.1), they coupled this reaction to Ugi-type reactions in a batch reactor.[16] When organogold (III) complex was used to activate singlet oxygen, oxidative cyanation of tetrahydroisoquinolines gave α-aminonitriles in high yields.[17] Yet, singlet oxygen is difficult to generate and handle which is why it is not broadly used for imine formation.



Scheme 8.1 Generation of singlet oxygen using TPP as a sensitizor

In the Seeberger laboratory, a variable-temperature flow photoreactor for the efficient generation of $^1O_2$ was developed.[13] It was later modified by adding an LED lamp (420 nm) and TPP was introduced as the photosensitizer.[5, 18] The setup consists of a reactor from FEP tubing wrapped around a glass plate and immersed in an acetone bath. The bath is closed and LED lamp is installed right above the reactor to provide light. Using a chiller, temperature of the acetone bath, and therefore of the reaction, can be controlled (Scheme 8.2).[5]

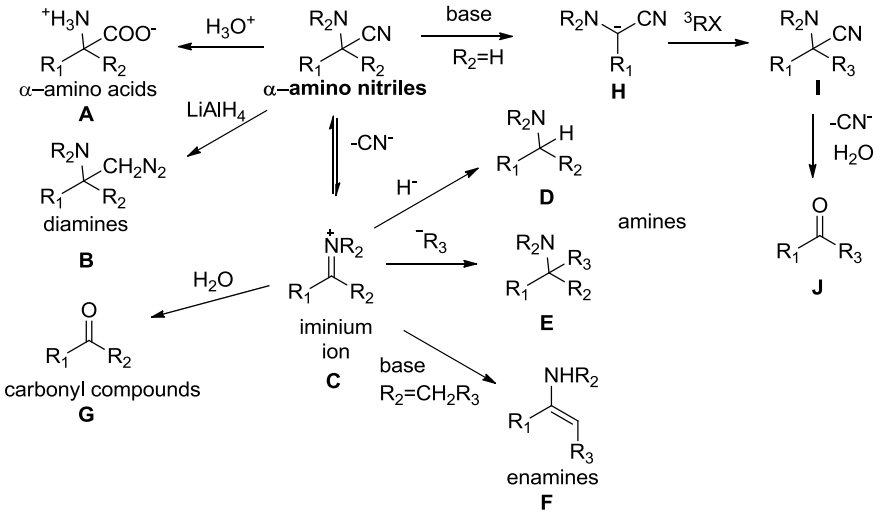


Scheme 8.2 Design of the photosensitizer[5]

8.1.2 Synthesis of Amino Nitriles and Their Significance

The described photooxidation module (Scheme 8.2) was used for continuous aerobic oxidative cyanation of amines. Namely, imines synthesized using the setup (Scheme 8.2) were trapped *in situ* by trimethylsilyl cyanide (TMSCN) to yield amino nitriles, TBAF was used to promote the nucleophilicity of the cyanide and the temperature was kept at -50 °C to prevent oxidative coupling (starting material amine can act as a nucleophile and add to the formed imine). Primary amino nitriles were isolated without oxidative coupling.[5] These chemical compounds are important intermediates

for the synthesis of a wide variety of amino acids, amides, diamines, hydantoins and nitrogen-containing heterocycles (Scheme 8.3).[19-20]



Scheme 8.3 Reactivity of α-amino nitriles.[19]

One mode of amino nitrile reactivity is via the nitrile group. The nitrile group can be hydrolyzed to give *α*-amino acids. Alternatively, it can be reduced using lithium aluminium hydride to 1,2-diamines (**B**)[19] (Scheme 8.3).

α-Amino nitriles contain "masked" iminium ions, whereby loss of cyanide anion under a variety of conditions (*e.g.* use of silver salts, copper salts, Brønsted or Lewis acids and by thermolysis) yields an intermediate iminium ion (**C**). This opens a pathway to substituted amines (**D** and **E**). Tautomerization of intermediate iminium ion gives the corresponding enamine (**F**) or if it undergoes hydrolysis the corresponding carbonyl compound (**G**) is formed (Scheme 8.3).[19]

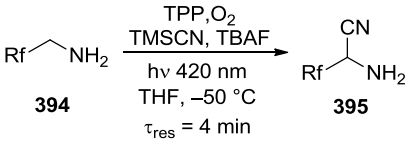
A third mode of reactivity is a reverse in polarity (Umpolung) at the *α*-carbon. It is possible to deprotonate *α*-amino nitrile at *α*-position (R^2 = H) by a strong base. The carbanion (**H**) is able to nucleophilically attack an electrophile which results in formation of amino nitrile (**I**). Amino nitrile (**I**) can undergo any of the aforementioned transformations (Scheme 8.3).[19]

8.1.3 Optimization of Reaction Conditions for Amino Nitriles Synthesis

A previously developed procedure for photooxidative cyanation of primary amines[5] was used. A 0.1 M solution of 4-fluorobenzylamine with 2.5 equiv. TMSCN, 10 mol% TBAF, and 0.02 mol% TPP in THF (1 mL min^{-1}) was mixed with oxygen gas (10 mL min^{-1}) using a T-mixer prior to entering the photoreactor which was cooled to -50 °C. After a residence time of four minutes, the desired *α*-amino nitrile was obtained in 76% yield. Increasing the amount of TMSCN to 3.5 equiv., resulted in increased (94%) yield with complete consumption of starting material.

Benzyl and homobenzyl α-amino nitriles bearing various degrees of fluorination were obtained when optimized conditions were applied. Monofluorinated benzylamines (Entries 1-3, Table 8.6) give moderate to high yields, the *ortho* substituted derivative (Entry 3, Table 8.6) is low yielding presumably due to the slower rate of oxidation for this derivative.[21] Yields of 75-89% were observed for the homobenzyl species (Entries 10-12, Table 8.6). Multiple fluorine atoms (Entries 7 and 8, Table 8.6) or trifluoromethyl groups (Entries 4-6, Table 8.6) on the aromatic ring give poor to moderate yields of the corresponding *α*-amino nitriles, even at decreased temperatures.[5]

Table 8.6 Substrate scope for photooxidative cyanation.



Entry[a]	R	Yield, (%)[b]
1	4-F-C_6H_4	70(94)[c]
2	3-F-C_6H_4	88
3	2-F-C_6H_4	61
4[d]	4-CF_3-C_6H_4	27
5[d]	3-CF_3-C_6H_4	52
6[d]	2-CF_3-C_6H_4	36
7	3,4-di-F-C_6H_3	55
8	3,5-di-F-C_6H_3	40
9	4-CH_2FO-C_6H_4	55
10	4-F-$C_6H_4CH_2$	89
11	3-F-$C_6H_4CH_2$	79
12	2-F-$C_6H_4CH_2$	75
13	$CF_3CH_2CH_2$	60

[a] Reaction conditions: amine (0.1 M in THF), TMSCN (3.5 equiv.), TBAF (0.14 equiv.) TPP (0.02 mol %), O_2 (3-5 mL/min), LED 420 nm, –50 °C, τ_{res}=5 min, 7 bar BPR, [b] Isolated, [c] ^{1}H NMR yield using mesitylene as an internal standard, [d] Reaction was run at –60 °C.

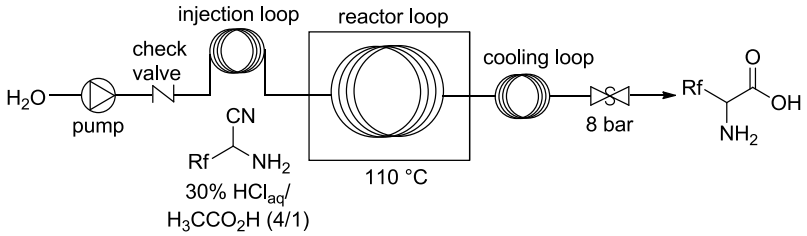
8.1.4 Hydrolysis of Amino Nitriles to Amino Acids

Since we were able to successfully synthesize fluorinated amino nitriles, we pursued further our desired product, fluorinated amino acids. Hydrolysis of the amino nitrile under acidic conditions[22] was attempted in flow. One advantage of flow chemistry over traditional batch procedures is the

ability to use solvents, such as the required 30% HCl_{aq}, well above their boiling point.[23] After the solvent from the photooxidation reaction was removed, the *α*-amino nitriles were found to be only partially soluble in this acidic solution (30% HCl). While alcoholic cosolvents, such as 2-propanol or *n*-butanol, help to homogenize the solutions, the corresponding *α*-amino esters were obtained, together with the desired amino acid in the ratio 5:1. A small screen of solvent mixtures revealed acetic acid in 30% HCl_{aq} [1:4(v/v)] as an optimal mixture for the synthesis of amino acids.

A 0.1 M, solution of 4-fluorobenzyl amino nitrile in 30% HCl_{aq} [1:4(v/v)] was, with the help of the injection loop, passed through a 22 mL reactor (70 °C) at 8 bar pressure. After a 37 minute residence time, nearly full conversion of the *α*-amino nitrile is observed. However, due to incomplete hydrolysis, formation of the amide is observed (Entry 1, Table 8.7). Increasing the temperature to 110 °C gives full conversion to the desired *α*-amino acid with no intermediate amide (Entry 3, Table 8.7). Decreasing the residence time results in incomplete conversion (Entries 4 and 5, Table 8.7).

Table 8.7 Optimization of hydrolysis of amino nitrile in flow.



Entry[a]	Temperature (°C)	τ_{res} (min)	Ratio of Products[b]		
			Amino Acid	Amide	Amine Salt
1	70	37	1.0	2.7	0.1
2	90	37	1.0	0.1	0.0
3	110	37	1.0	0.0	0.0
4	110	18	1.0	0.15	0.0
5	110	9	1.0	0.54	0.0

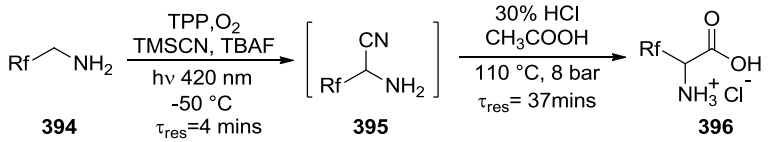
[a] Reaction conditions: α-amino nitrile (0.1 M in CH_3COOH/30% HCl_{aq} (1/4 v/v)), [b] Determined by ^{19}F NMR.

8.1.5 Semi-Continous Process

Following the optimization of both separate processes (photooxidative cyanation and hydrolysis of amino nitrile in flow), was the development of a semi-continuous process connecting these two. A fully semi-continuous process was developed. To mediate the transition between the two processes, solvent for the photooxidative cyanation had to be changed to 2-MeTHF which allowed the extraction of water-soluble byproducts from the first step, the TBAF used for activation of TMSCN in the photooxidative cyanation was removed in this way. After removing the solvent from the photooxidation reaction *in vacuo*, the crude material was dissolved in a 4:1 mixture of 30% HCl_{aq}/acetic acid to provide a 0.1 M solution with small amounts of precipitate, which were easily filtered. Good yields were observed for the two-step process, affording benzylic (Table 8.8, Entries 2

and 4) and homobenzylic (Entry 1, Table 8.8) fluorinated α-amino acids in 60-67%. The lower yield for the *m*-CF_3 derivative (Entry 3, Table 8.8) is presumably because of inefficient photooxidative cyanation (Entry 5, Table 8.8). The true advantage of the described process is shown in Entry 5, Table 8.8. Namely, when the mentioned aliphatic structure was subjected to photooxidative cyanation, the desired amino nitrile was obtained. However, when the chromatographic purification was carried out, the product decomposed on the column. By using our developed semi-continuous two-step process, we were able to isolate the corresponding amino acid thereof, circumventing the isolation of the unstable intermediate.

Table 8.8 Scope of the semi-continuous two-step process for synthesis of fluorinated amino acids.



394 **395** **396**

Entry[a]	R	Yield (%)
1	4-F-$C_6H_4CH_2$	64
2	4-F-C_6H_4	67
3	4-CF_3-C_6H_4	24[b]
4	3,4-di-F-C_6H_3	60
5	$CF_3CH_2CH_2$	63[b]

[a] For the full experimental details, see Experimental Section,
[b] Average yield over two runs.

8.2 Access to Enantiomerically Pure Amino Acids: Enzymatic Resolution of Amino Nitriles

As an extension of our semi-continuous synthesis of fluorinated amino acids, the development of a synthetic strategy which would offer access to enantiopure fluorinated amino acids was envisioned. As discussed in Chapter 3.1, there are several synthetic strategies that offer access to enantiopure amino acids. As the shortest and the most straightforward route we have chosen an enzymatic transformation.[24]

8.2.1 Benefits and Drawbacks of Enzymatic Approach

Enzymes are widely exploited in industry because they offer access to enantiomerically pure products usually at room temperature, under neutral aqueous conditions, and without protection of functional groups.[25] In organic synthesis they are used as stand-alone catalysts in reactions or combined with other enzymes or chemical reagents to achieve the desired transformations. This class

of catalysts results in stereo- and regiochemically defined products with great acceleration of the reaction rate (typically 10^5 to 10^8 fold).[25] However, problems regarding the use of enzymes remain; they are relatively unstable, isolation is pricy, it is difficult to recover active enzymes from the reaction mixture and certain enzymes require co-factors which need to be regenerated.[26] Some of these drawbacks can be circumvented by using the whole cells; this, however, is not a perfect solution and suffers from occurence of additional enzymatic reactions.[27]

8.2.2 Immobilization of Enzymes

Despite the drawbacks connected to enzymatic transformations, there are different ways of using enzymes for biocatalytic transformations; using them as individual molecules in solution, as aggregates (with other entities) and immobilized (attached at the surface). Using immobilized enzymes can be one way to overcome their instability. Immobilized enzymes are enzymes attached to an inert, insoluble material with retention of their activity. Immobilization holds the enzyme in place throughout the reaction, and, in an industrial context, offers easier reactor operation and product separation. It also enables the catalyst to be reused and gives the possibility of using a wider range of reactors. In addition immobilization can lead to an increase in the resistance of enzymes to changes in the pH and temperature. However, when working on immobilized enzymes the resistance of the support matrix against mechanical stress should be taken into account. Mechanical stirring batch reactors can damage the support surface, leading to protein leaching and a loss of recyclability.[28] Immobilization can also cause the complete loss or a reduction in activity of the enzyme; it also poses an additional cost and limits diffusion.[26]

That is why properties of the support matrices are of crucial factors for the activity of the immobilized enzyme. Matrix should be physically resistant to compression, hydrophilic, inert towards enzymes, easily derivatized, biocompatible, resistant to microbes and inexpensive. Based on chemical content, supports can be organic or inorganic. Organic supports may be composed of natural or synthetic polymers[26] (See Table 8.9).

Table 8.9 Overview of types of immobilization matrices for enzymes[26]

Organic				**Inorganic**	
Natural polymers			**Synthetic polymers**	**Natural minerals**	**Processed materials**
Polysaccharides:	Proteins:	Carbon	Polystyrene	Betonite	Glass
Cellulose	Collagen		Polyacrylate	Silica	Metals
Dextrans	Albumin		Polymethacrylate		Controlled pores metal oxides
Agarose			Polyacrylamide		
Agar			Polyamides		
Chitin			Vinyl		
Alginate			Allyl-polymers		

According to the type of interactions enzymes can be divided into irreversibly and reversibly immobilized. Irreversible immobilization is a type of binding in which, in order to detach the enzyme from the support, either the activity of the enzyme or the support must be sacrified. Some examples of irreversible immobilization are covalent coupling, entrapment (micro-encapsulation) and cross-linking. On the other hand, reversible immobilization includes adsorption (non-specific adsorption, ionic binding, hydrophobic adsorption and affinity binding), chelation, metal binding or immobilization through disulfide bond formation.[26] Porous matrices are the support of choice because the greater surface area enables higher enzyme loading and protection of the immobilized enzyme; therefore, most industrial applications utilize organic matrices.[26] The first industrial application of immobilized enzymes was the one used aminoacylase from *Aspergillus oryzae* for the resolution of synthetic racemic D,L amino acids.[29]

8.2.3 Continuous Flow Biocatalyzed Process

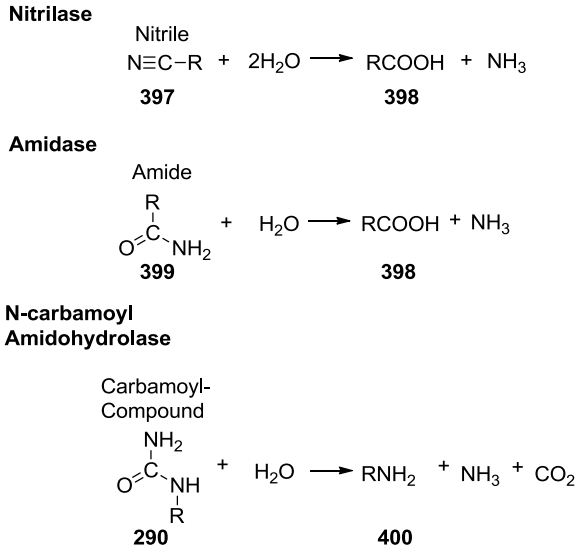
The aforementioned benefits of enzymatic synthesis and the progress in immobilization of enzymes[30-31] have shed light onto continuous flow processes as an important tool to develop new reaction methodologies. Correspondingly, a tremendous amount of work has entered the literature that relates to the continuous flow biocatalyzed process,[32-43] and it has been recognized that this technology improves enzymatic reactions.[44] This is directly connected to the numerous types of equipment that are now on the market and the industrial requirement to develop new technologies for industrial purposes. The most preferred type of reactors for biocatalytic continuous processes is the packed bed reactor. Ideally, reagents are converted into products in just one cycle, avoiding the recycling conditions where reagents pass throughout the packed bed for several times until full conversion is obtained.[28]

8.2.4 Nitrilases- Mechanism of Action

Considering all the benefits of enzymatic synthesis and flow chemistry discussed above, we decided to combine the two approaches for reaching the enantiopure targets, fluorinated amino acids. Several enzymatic transformations to access amino acids enantioselectively are available (Chapter 3.2). As we began to strive towards the development of a continuous flow process to afford enantiopure fluorinated amino acids and we already had a fast and elegant method for accessing racemic amino nitriles in hand.[5] We sought for an enzymatic transformation that would convert the nitrile group to a carboxylic group enantioselectively and in that way introduce chirality into the molecule. Hydrolysis of non-peptide C–N bonds is catalyzed by C–N hydrolases (superfamily of nitrilases).[45] In the more recent literature, the term "nitrilase superfamily" is often used instead of "C–N hydrolase"[45] because many members of this superfamily were assigned as "nitrilases" or "nitrilase-

like" due to their sequence similarity to known nitrilase sequences. Today it is clear that many of these proteins are not nitrilases, often their exact enzymatic function is still unknown.

C–N hydrolases can be divided into 13 different branches based on sequence identity and catalytic activity. As an example, three different types of C–N hydrolases, namely *nitrilases*, *aliphatic amidases*, and *carbamoyl amidohydrolases* and the reactions they typically catalyze are shown in Scheme 8.10. Release of ammonia is usually associated with C–N hydrolase reactions (Scheme 8.10).



Scheme 8.10 Catalytic activity of C-N hydrolases.

The described enzymatically catalyzed nitrile hydrolysis can follow two mechanistically different pathways; two-step hydrolysis, catalyzed by nitrile hydratase and a co-expressed amidase, or direct hydrolysis, catalyzed by nitrilase (See Figure 8.11).[46]

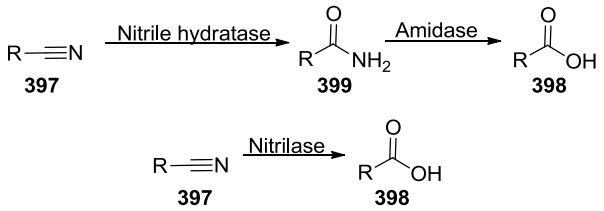


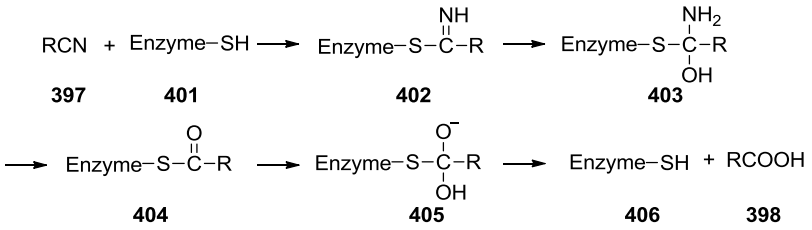
Figure 8.11 Enzymatic hydrolysis of nitriles.

Nitrilases are the branch of the superfamily that is best characterized[47] and comprises of nitrilases (as mentioned), cyanide hydratase and cyanide dehydratase. Although similar in primary structure, these enzymes show different catalytic activities. Nitrilase catalyze hydration of nitriles to the corresponding acids and can be used with wide variety of substrates, whereas, cyanide hydratase and cyanide dehydratase can be used efficiently only with HCN and produce amides or acids products, respectively.[47] A catalytic triad containing glutamic acid, lysine and cysteine is characteristic for all members of the nitrilase superfamily, whereas a conserved cysteine, tryptophan and glutamic acid

motif in addition to the cysteine residue of the catalytic triad characterize only the nitrilase branch (Figure 8.12). Nitrilases are thiol enzymes, their catalytic activity comes from the sulfhydryl group.[48] Accordingly, catalytic activity can be promoted by thiol-reducing agents (dithiothreitol) and inhibited by thiol binding metal ions ($CuSO_4$, $AgNO_3$).[49]

Figure 8.12 Catalytic triad in nitrilases, reproduced with the presmission from,[50-51] crystal structure of the putative Nit active site determined by crystallization of NitFhit peptide of *C. Elegans*.

In the 1960s it was postulated that one of the two SH groups in the active site of the nitrilase itself acts as a nucleophile and attacks the nitrile to give an imine. The imine is subsequently hydrolyzed into an acyl moiety and NH_3 is released as a byproduct. The acyl-enzyme complex is hydrolyzed by H_2O to give the carboxylic acid and regenerate the enzyme (Scheme 8.13).[50]



Scheme 8.13 Mechanism of nitrile hydrolysis by nitrilases.[50]

Although it was previously stated that the difference between nitrilase and the nitrile hydratase/amidase system is that the latter goes through amide formation to give the amino acid, recent studies have shown that amides can also be produced by nitrilases acting on a nitrile substrate. It is hypothesized that this occurs when the enzyme-substrate complex (Scheme 8.13) breaks after the addition of the first water molecule; the addition of the second water molecule is 'late'.[52]

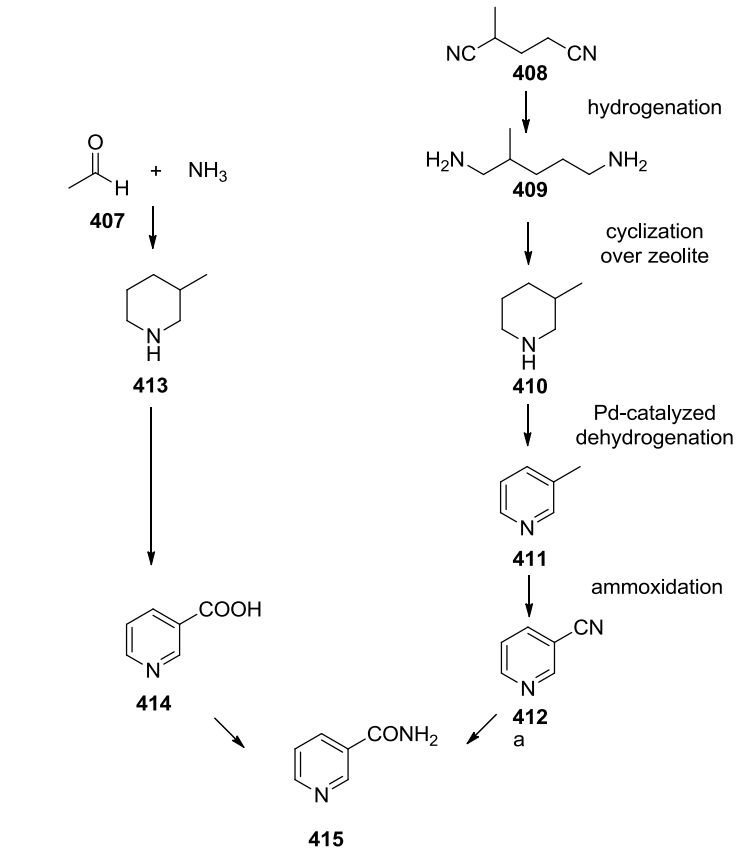
Despite the similar mechanistic pathway, some of the nitrilases are very substrate specific whereas others have a broad substrate range. According to substrate specificity, they can be divided into aliphatic, aromatic and heterocyclic nitrilases and arylacetonitrilases.[45, 47, 53] There are various

examples of enzymatic nitrile hydrolysis in the literature exploiting isolated enzymes or whole cells.[54-59] In the next subchapter an overview of the industrial applications of nitrilases is given.

8.2.5 Nitrilases and Nitrile Hydratases in Industry

The main industrial application of nitrilases is in the production of carboxylic acids. In this subchapter three examples of industrial processes based on nitrilases (both nitrilases and nitrile hydratases) will be described.

Perhaps the biggest industrial application of nitrilases is their employment in the production of nicotinic acid, a procedure patented by Lonza®. Nicotinic acid and nicotinamide are common forms of vitamin B. Nicotinamide (vitamin B3) is used as a vitamin supplement for food and animal feed. Its industrial production (3500 t/a) invloves nitrile hydratase (whole cells of *Rhodococcus erythropolis*). Lonza® process includes four catalytic reactions (Figure 8.14). The first three steps are conducted at a temperature above 300 ˚C in the gas phase using chemical catalysts. By introducing biocatalysis in the last step, instead of chemical alkaline hydrolysis of 3-cyanopyridine, complete selectivity (as opposed to 4% of nicotinic acid byproduct; 96% yield when compared to the chemical production) and the elimination of acid or base from the production process was achieved.[60-61] In this step 3-cyanopyridine in buffer is continously fed into the reactor with *Rhodococcous rhodochrorus* J1 cells, containing nitrile hydratase, which are immobilised in polyacrylamide. Subsequently, decolorisation of the crude nicotinamide solution and nanoflitration is performed to remove the bioburden. After filtration, a white, granular, free-flowing, non-caking solid of nicotinamide is isolated. This high-yielding biotransformation is run in a continous fashion at low temperature and atmospheric pressure with recycling of ammonia and hydrogen, using only one solvent throught the entire process, which makes it a safer and greener process.[60-61]



Scheme 8.14 New Lonza nicotin amide process.

Another industrial process which involves nitrilase is the production of mandelic acid. Mandelic acid is used as an optical resolving agent and is an intermediate in the syntehsis of pharmaceuticals and agrochemicals. BASF® uses kinetic resolution of racemic mandelonitrile (Figure 6.15) to produce mandelic acid in multitons per year. Nitrilase [E. coli JM109 (pDHE19.2)] efficiently catalyzes the hydrolysis of the (*R*)-enantiomer **416** with high conversion and excellent enantioselectivities, up to >99% ee. By adjusting the pH, the remaining enantiomer in the resolution is re-racemized in the presence of cyanide ions due to an equilibrium between hydroxy nitrile and aldehyde which pushes the yield of the reaction to full conversion.[62-63]

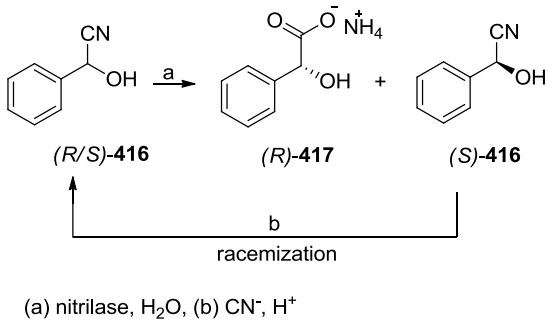


Figure 8.15 Resolution of mandelonitrile (BASF®).[62-63]

Moreover, Nitto chemicals® established a biotransformation as a main step in production of acrylamide in which nitrile hydratase-containing cells are immobilized with polyacrylamide gel. Acrylamide is used in the production of synthetic fibers and flocculant agents and it is one of the most important commodity chemicals. If enzymatic processes for the production of acrylamide are compared to the coventional process that use copper-salt catalysts, it is obvious that the step of recovering the remaining acrylonitrile and removal of the copper can be omittted due to fact that the yield of the biocatalytic transformation approaches 100%. Mild conditions, low reaction temperature (under 10 °C) and use of immobilized cells repeatedly, makes the biotransformation process simpler and more economical solution (Figure 8.16).[64]

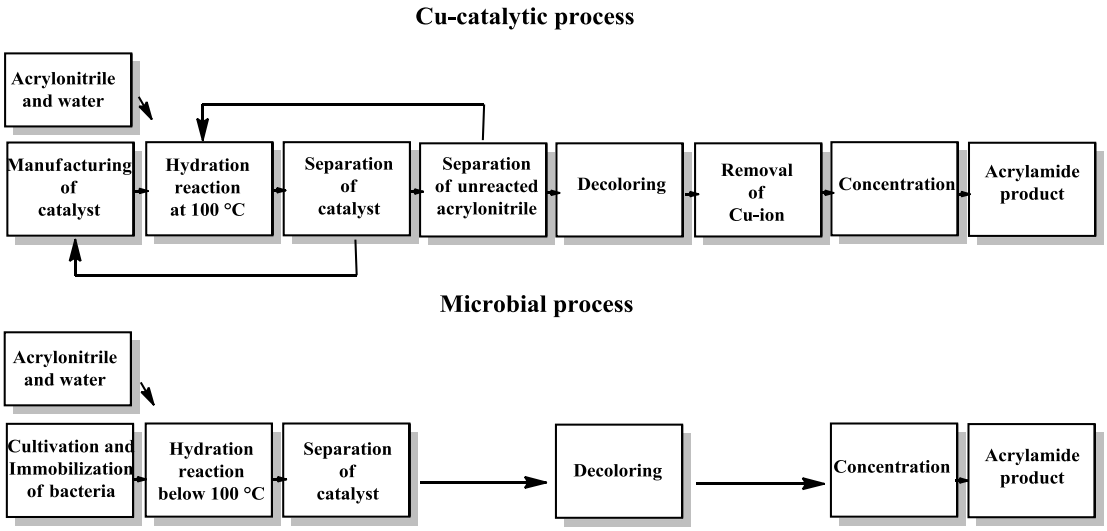
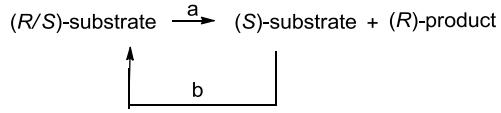


Figure 8.16 Comparison of microbial and conventional process for acrylamide, adapted[64]

8.2.6 Nitrilase Catalyzed Hydrolysis of Amino Nitrile

As previously discussed, enzymatic resolution is an established pathway to enantiopure products, through which, starting from the racemic substrate mixture, a single enantiomer is obtained. One disadvantage of this system is that it can provide a maximum of 50% of the desired product (kinetic resolution). This could be circumvented by using the unwanted enantiomer of the starting

material; namely by its racemization. Thus racemization would provide 'more' substrate for the enzyme and product yield would increase. This process is referred to as "dynamic kinetic resolution" (Scheme 8.17), and can theoretically provide a single enantiomer (99% enantiomeric excess (ee) or greater) in 100% yield in cases where a highly efficient way of racemizing the starting material is combined with a highly enantioselective enzyme.[65]

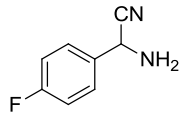


(a) Enzyme, max 50% yield, (b) recycling

Scheme 8.17 (*R*)-Selective enzymatic resolution with recycling of unreacted (*S*)-substrate.

A continuous process connecting photooxidation and enzymatic transformation was established as a direct way of accessing enantiopure amino acids from amines. This continuous process connects the short and efficient photooxidative cyanation with a green biocatalytic transformation to yield a single enantiomer of amino acid. Nitrilase was chosen for this transformation. It had been reported that an organism containing recombinant nitrilase was effectively used in a form of a packed bed.[66] Moreover, nitrilases had been reported to catalyze the desired transformation in batch using our desired fluorinated substrate (Figure 8.18);[67] however, the authors reported a problem with reproducibility and byproduct formation. Despite the reported problems, we decided to try the biotransformation in a continuous flow setup using nitrilases obtained from Almac®.

16 nitrilases were screened against our target substrate **418** (Figure 8.18) at a 10 mM substrate concentration with 1.6 mg of cell lysate containing nitrilase; nitrilase 115 was the only enzyme that showed enzymatic activity against the desired substrate (Figure 8.18).



418

Figure 8.18 Substrate used to screen Almac® nitrilases.

The desired enzymatic activity was confirmed by HPLC and mass spectrometry. In addition, the biocatalytic transformation was run on a larger scale (30 mg of the amino nitrile as starting material) and the carboxylic acid product was identified using NMR spectroscopy.

In the described experiments, the biocatalyst was used in the form of a cell lysate. Taking advantage of the fact that a reduction in amount of cell lysate used (hence the enzyme itself), led to a reduction in the rate of the enzymatic reaction, the ee at different time points was determined. The goal was to determine a time point at which the enzyme stops being selective for one of the enantiomers and starts converting the other enantiomer of the starting material to the undesired enantiomer of the

product. Reaction conditions for the chosen substrate were optimized in batch conditions,[67] by adjusting the ratio of substrate to catalyst. Using nitrilase 115 we reached the highest yield and the highest ee at pH 7.0 and 37 °C.

It was previously reported that the addition of KCN enhances the stability of the starting material;[67] however, when added to our system, no difference was observed. As described in the Section 8.2.5, most of the commercial scale processes that use nitrilases are performed using immobilized enzymes. With the aforementioned benefits of enzyme immobilization, an overview of the methods and materials used for immobilization of nitrilases follows. The immobilization methods can be roughly divided into: entrapment, cross-linking, adsorption, and covalent bonding. The immobilization materials include polyvinyl alcohol, DEAE cellulose, alginate (Sr^{2+}, Ba^{2+}, Na^{+}, Al^{3+}, Ca^{2+}), alumina, carrageenan gels, and polyacrylamide.[68-70]

Immobilization of nitrilase 115 on four different types of resin (ECFR, HA 403 S, Purolite D and EC HFA) was carried out. Supported enzymes were tested in batch under optimal conditions (10 mM substrate concentration, 75 mg of supported enzyme, 1 mL of phosphate buffer, pH 7.0, 37 °C) and it was found that Purolite D and EC HFA supports show lower enantioselectivity when compared with ECFR and HA 403 S supports.

Extinction coefficient at 261 nm of amino nitriles (starting materials) and amino acids (products) were determined by UV/Vis spectroscopy. Standard reaction was performed using immobilized enzyme at 37 ˚C and pH 7.0 at 10 mM substrate concentration and 75 mg of supported enzyme for 15 minutes. Temperature, pH and amount of enzyme were varied to test whether the activity of the supported enzyme could be modulated (Tables 8.19A and 8.19B).

Table 8.19A Optimization of enzymatic reaction using NIT 155 supported on ECFR

ECFR		**Yield**		**Conversion**		**ee**	
		A	B	A	B	A	B
Temperature	37	57	34	41	20	62	38
	34	27	16	41	24	63	37
	40	16[a]	17	22	24	63	37
	50	40	17	27	11	70	30
pH	6.2	46	33	43	31	58	42
	9.0	52	36	44	31	59	41
	11.0	16	8,	13	7	65	34
Special condition	60 mg E/40 °C	57	28	32	20	67	33
	150 mg E/ 37°C	27	22	41	34	55	45

*A enantiomer of the product with shorter retention time, B enantiomer of the product with longer retention time, SM-starting material.

Table 8.19B Optimization of enzymatic reaction using NIT 155 supported on HA 403 S

HA 403 S		Yield		Conversion		ee	
		A	B	A	B	A	B
Temperature	37	32.	11	23	44	76	25
	34	23	7	25	8	76	24
	40	21	6	24	7	78	22
	50	10	4	12	5	72	28
pH	6.2	49	14	33	10	78	22
	9.0	45	13	34	10	78	22
	11.0	12	4	12	4	76	24
Special condition	60 mg E/40 °C	20	6	16	5	77	23
	150 mg E/ 37°C	29	15	4	2	66	34

*A enantiomer of the product with shorter retention time, B enantiomer of the product with longer retention time, SM-starting material.

The following observations were made using the immobilized enzymes: ECFR-supported enzyme shows higher yields and conversions than HA 403 S-supported enzyme after 15 min in all tested conditions (Table 8.19A and 8.19B).

HA 403 S-supported enzyme shows higher enantioselectivity (ee) than ECFR-supported enzyme under all tested conditions. The ee for ECFR-supported enzyme is between 59% and 67%, (Table 8.19A), whereas, the ee of HA 403 S-supported enzyme is between 76% and 78% (Table 8.19A). Under ECFR-supported enzyme reaction conditions pH 7.0 and 37 ˚C and 60 mg of supported enzyme at 40 ˚C were the optimal conditions (Table 8.19B, yield, conversion and ee were compared).

For HA 403-supported enzyme, pH 6.2 and pH 9.0 were the optimal conditions (Table 6.19B, yield, conversion and ee were compared).

Outlook

After optimizing the enzymatic reaction in batch reactor using immobilized enzyme, next step was to adjust this reaction to flow conditions. Initial attempts to develop a packed-bed flow reactor were carried out (for more details on the packed bed reactor for enzymatic transformations see chapter 6). In the future, sepabeads HA 403 S or purolite ECFR, depending on the choice of immobilization matrix, should be mixed with the immobilized cell lysate to increase the packed-bed volume. Moreover, residence time and substrate concentration should be optimized and it should be tested whether quenching agent is needed or if MeOH can be used to elute the product completely from the packed bed reactor. Reusability of the enzymatic packed bed reactor should also be investigated. In addition, the enzymatic process should also be examined for other fluorinated amino nitrile substrates. Finally, if applicable, dynamic kinetic resolution conditions for the enzymatic process should be established to increase the yield of the product over 50%.

Bibliography

[1] S. Nieto, J. M. Dragna, E. V. Anslyn, *Chemistry – A European Journal* **2010**, *16*, 227.
[2] L. R. Reddy, M. Prashad, *Chemical Communications* **2010**, *46*, 222.
[3] R. S. Varma, R. Dahiya, S. Kumar, *Tetrahedron Letters* **1997**, *38*, 2039.
[4] B. Miriyala, S. Bhattacharyya, J. S. Williamson, *Tetrahedron* **2004**, *60*, 1463.
[5] D. B. Ushakov, K. Gilmore, D. Kopetzki, D. T. McQuade, P. H. Seeberger, *Angewandte Chemie International Edition* **2014**, *53*, 557.
[6] D. B. Ushakov, K. Gilmore, P. H. Seeberger, *Chemical Communications* **2014**, *50*, 12649.
[7] K. C. Nicolaou, C. J. N. Mathison, T. Montagnon, *Angewandte Chemie* **2003**, *115*, 4211.
[8] K. C. Nicolaou, C. J. N. Mathison, T. Montagnon, *Journal of the American Chemical Society* **2004**, *126*, 5192.
[9] P. Müller, D. M. Gilabert, *Tetrahedron* **1988**, *44*, 7171.
[10] K. A. Mukaiyama Teruaki, Fukuda Yoshio, Matsuo Jun-ichi, *Chemistry Letters* **2001**, *30*, 390.
[11] X.-F. Wu, A. Petrosyan, T. V. Ghochikyan, A. S. Saghyan, P. Langer, *Tetrahedron Letters* **2013**, *54*, 3158.
[12] R. D. Patil, S. Adimurthy, *Asian Journal of Organic Chemistry* **2013**, *2*, 726.
[13] F. Lévesque, P. H. Seeberger, *Organic Letters* **2011**, *13*, 5008.
[14] C. Schweitzer, R. Schmidt, *Chemical Reviews* **2003**, *103*, 1685.
[15] N. Hoffmann, *Chemical Reviews* **2008**, *108*, 1052.
[16] G. Jiang, J. Chen, J.-S. Huang, C.-M. Che, *Organic Letters* **2009**, *11*, 4568.
[17] W.-P. To, G. S.-M. Tong, W. Lu, C. Ma, J. Liu, A. L.-F. Chow, C.-M. Che, *Angewandte Chemie International Edition* **2012**, *51*, 2654.
[18] D. Kopetzki, F. Lévesque, P. H. Seeberger, *Chemistry – A European Journal* **2013**, *19*, 5450.
[19] D. Enders, J. P. Shilvock, *Chemical Society Reviews* **2000**, *29*, 359.
[20] T. Opatz, *Synthesis* **2009**, *2009*, 1941.
[21] D. B. Ushakov , M. B. Plutschack , K. Gilmore, P. H. Seeberger *Chemistry – A European Journal* **2015**, *21*, 6528.
[22] B. F. M. E. C. Kendall, *Org.Synth.* **1929**, *9*.
[23] B. Reichart, G. Tekautz, C. O. Kappe, *Organic Process Research & Development* **2013**, *17*, 152.
[24] *Asymmetric Synthesis and Application of α-Amino Acids, Vol. 2*, ACS, **2009**.
[25] K. M. Koeller, C.-H. Wong, *Nature* **2001**, *409*, 232.
[26] F. B.-V. B. M. Brena, in *Methods in Biotechnology, Immobilization of enzymes and Cells,* (Ed.: J. M. Guisan), Humana Press.
[27] M. Ueda, A. Tanaka, **2000**, *90*, 125.
[28] I. Itabaiana Jr, L. S. de Mariz e Miranda, R. O. M. A. de Souza, *Journal of Molecular Catalysis B: Enzymatic* **2013**, *85–86*, 1.
[29] I. Chibata, T. Tosa, T. Sato, *Applied Microbiology* **1974**, *27*, 878.
[30] A. Rosevear, *Journal of Chemical Technology and Biotechnology. Biotechnology* **1984**, *34*, 127.
[31] U. T. Bornscheuer, *Angewandte Chemie International Edition* **2003**, *42*, 3336.
[32] R. Yuryev, S. Strompen, A. Liese, *Beilstein Journal of Organic Chemistry* **2011**, *7*, 1449.
[33] I. Itabaiana, Jr, I. C. R. Leal, L. S. M. Miranda, R. O. M. A. de Souza, *Journal of Flow Chemistry* **2013**, *3*, 122.
[34] B. S. Yadav, U. Rani, S. S. Dhamija, P. Nigam, D. Singh, *Journal of Basic Microbiology* **1996**, *36*, 205.
[35] H. M. S. Milagre, C. D. F. Milagre, P. J. S. Moran, M. H. A. Santana, J. A. R. Rodrigues, *Enzyme and Microbial Technology* **2005**, *37*, 121.
[36] W. G. Birolli, I. M. Ferreira, N. Alvarenga, D. d. A. Santos, I. L. de Matos, J. V. Comasseto, A. L. M. Porto, *Biotechnology Advances* **2015**, *33*, 481.
[37] R. Wendhausen Jr, P. J. S. Moran, I. Joekes, J. A. R. Rodrigues, *Journal of Molecular Catalysis B: Enzymatic* **1998**, *5*, 69.

[38] I. Rodríguez-Ruiz, E. Masvidal-Codina, T. N. Ackermann, A. Llobera, *Microfluidics and Nanofluidics* **2014**, *18*, 1277.
[39] J. Madarász, D. Németh, J. Bakos, L. Gubicza, P. Bakonyi, *Journal of Cleaner Production* **2015**, *93*, 140.
[40] I. Itabaiana, F. K. Sutili, S. G. F. Leite, K. M. Goncalves, Y. Cordeiro, I. C. R. Leal, L. S. M. Miranda, M. Ojeda, R. Luque, R. O. M. A. de Souza, *Green Chemistry* **2013**, *15*, 518.
[41] D.-T. Tran, Y.-J. Lin, C.-L. Chen, J.-S. Chang, *Applied Energy* **2014**, *126*, 151.
[42] B.-P. Wu, Q. Wen, H. Xu, Z. Yang, *Journal of Molecular Catalysis B: Enzymatic* **2014**, *101*, 101.
[43] L. Schönstein, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2013**, *24*, 202.
[44] N. N. Rao, S. Lütz, K. Würges, D. Minör, *Organic Process Research & Development* **2009**, *13*, 607.
[45] C. Brenner, *Curr Opin Struct Biol* **2002**, *12*.
[46] D. E. Robertson, J. A. Chaplin, G. DeSantis, M. Podar, M. Madden, E. Chi, T. Richardson, A. Milan, M. Miller, D. P. Weiner, K. Wong, J. McQuaid, B. Farwell, L. A. Preston, X. Tan, M. A. Snead, M. Keller, E. Mathur, P. L. Kretz, M. J. Burk, J. M. Short, *Appl Environ Microbiol* **2004**, *70*.
[47] C. O'Reilly, P. D. Turner, *Journal of Applied Microbiology* **2003**, *95*, 1161.
[48] M. Podar, J. R. Eads, T. H. Richardson, *BMC Evolutionary Biology* **2005**, *5*, 1.
[49] N. Layh, J. Parratt, A. Willetts, *J Mol Catal B: Enzym* **1998**, *5*.
[50] J.-S. Gong, Z.-M. Lu, H. Li, J.-S. Shi, Z.-M. Zhou, Z.-H. Xu, *Microbial Cell Factories* **2012**, *11*, 1.
[51] H. C. Pace, S. C. Hodawadekar, A. Draganescu, J. Huang, P. Bieganowski, Y. Pekarsky, C. M. Croce, C. Brenner, *Current Biology* **2000**, *10*, 907.
[52] M. Piotrowski, S. Schönfelder, E. W. Weiler, *Journal of Biological Chemistry* **2001**, *276*, 2616.
[53] H. C. Pace, C. Brenner, *Genome Biol* **2001**, *2*.
[54] M. K. S. Vink, R. Wijtmans, C. Reisinger, R. J. F. van den Berg, C. A. Schortinghuis, H. Schwab, H. E. Schoemaker, F. P. J. T. Rutjes, *Biotechnology Journal* **2006**, *1*, 569.
[55] D. Brady, A. Beeton, J. Zeevaart, C. Kgaje, F. Rantwijk, R. A. Sheldon, *Applied Microbiology and Biotechnology* **2003**, *64*, 76.
[56] D. Brady, A. Beeton, J. Zeevaart, C. Kgaje, F. Rantwijk, R. A. Sheldon, *Appl Microbiol Biotechnol* **2004**, *64*.
[57] M. A. Wegman, U. Heinemann, F. van Rantwijk, A. Stolz, R. A. Sheldon, *Journal of Molecular Catalysis B: Enzymatic* **2001**, *11*, 249.
[58] T. C. Bhalla, A. Miura, A. Wakamoto, Y. Ohba, K. Furuhashi, *Appl Microbiol Biotechnol* **1992**, *37*.
[59] M.-X. Wang, *Accounts of Chemical Research* **2015**, *48*, 602.
[60] N. M. Shaw, K. T. Robins, A. Kiener, *Adv Synth Catal* **2003**, *345*, 425.
[61] H. W. W.Aehle, C. Schultz, H. Gröger, C. Dinkel, K. Drautz, in *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley, Weinheim, **2012**.
[62] Hans E. Schoemaker, *Advanced Synthesis & Catalysis* **2001**, *343*, 751.
[63] A. Schmid, J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258.
[64] H. Y. T. Nagasawa, *Pure and Applied Chemistry* **1990**, *62*, 1141.
[65] M.-J. Kim, J. Park, Y. K. Choi, in *Multi-Step Enzyme Catalysis*, Wiley-VCH Verlag GmbH & Co. KGaA, **2008**, pp. 1.
[66] L.-Q. Jin, Z.-T. Li, Z.-Q. Liu, Y.-G. Zheng, Y.-C. Shen, *Journal of Industrial Microbiology & Biotechnology* **2014**, *41*, 1479.
[67] J. A. Chaplin, M. D. Levin, B. Morgan, N. Farid, J. Li, Z. Zhu, J. McQuaid, L. W. Nicholson, C. A. Rand, M. J. Burk, *Tetrahedron: Asymmetry* **2004**, *15*, 2793.
[68] P. Kaul, A. Banerjee, U. C. Banerjee, *Biomacromolecules* **2006**, *7*.
[69] J. Dias, R. Rezende, V. Linard, *Appl Microbiol Biotechnol* **2001**, *56*.
[70] Z. Q. Liu, M. Zhou, X. H. Zhang, J. M. Xu, Y. P. Xue, Y. G. Zheng, *J Mol Microbiol Biotechnol* **2012**, *22*.

9. Results and Discussion:

Synthesis of Amino Acid Derivatives

This chapter has been modified in part from the following publication *A Sustainable, Semi-Continuous Flow Synthesis of Hydantoins*, S. Vukelić, B. Koksch, P. H. Seeberger and K. Gilmore, *Chem. A Eur. J.*, DOI: 10.1002/chem.201602609.

Expanding the methodology to amino acid derivatives is another aspect of the work described in this dissertation. Hydantoins were chosen as valuable targets (for more details on their use see Section 4.). Here a two-step procedure for synthesis of hydantoins is described. The potential for elegantly forming amino nitriles by photooxidative cyanation using oxygen gas is combined with the fact these products can be easily derivatized to yield hydantoins using readily available and inexpensive CO_2 gas. This entire continuous process was enhanced by flow conditions since in biphasic (gas/liquid) reactions local concentration of the gaseous reagent is increased when compared to a batch reaction which increases the rate of the reaction.

9.1 Flow Synthesis of Hydantoins

Gases are attractive reagents for a variety of chemical transformations because unreacted population can be simply removed from the reaction vessel by venting. On the other hand, to provide an excess of the reagent, a simple increase in pressure is enough to push more reagent into the reaction vessel and the reaction toward product formation. Reactions involving high pressure and toxic, flammable and corrosive gases require the use of special equipment or specially built facilities and extra safety regulations. To circumvent these problems regarding the gas-liquid reactions, continuous flow techniques are being applied.

As mentioned above, due to the small size of the reactor, working at elevated pressures is not a safety hazard, the risk of explosions inside the reactor is reduced and higher operational range of pressures and temperatures is available.[1-2] Moreover, applying high-pressure conditions ensures a larger interface area for mass transfer between the two phases. Partial pressure of the gas, and thus its solubility in the liquid phase increases. In case the gas used is one of the reagents, raising the pressure is one way to increase its concentration in the reaction mixture. In addition, continuous flow devices allow for more rapid screening of reaction conditions in biphasic gas/liquid reactions compared to pressurized autoclave systems.[1]

Since the global emission of CO_2 in 2014 was 35.9 Gt, it is a freely available gas and is an atom-economical source of a carbonyl group, it is also non-toxic. Hence, there is a great interest in expanding green synthetic procedures using the CO_2 synthon to access organic

compounds,[3] and it does not come as a surprise that it was previously employed in the synthesis of hydantoins.[4] We have shown that we are able to access amino nitriles readily from available amines, and wanted to broaden that methodology to hydantoin synthesis.

Multiphase reactions, particularly, gas/liquid reactions, can be significantly accelerated using continuous flow technology. Enlarged interfacial areas combined with pressurization enable an increase in gas solubility. Kappe and co-workers have shown they can significantly accelerate (32 minute reaction time) Bucherer-Bergs reaction in mesoflow when the water/ethyl acetate mixture was taken to 120 °C at 20 bar pressure.[5] However, issues associated with α-aminonitrile formation from carbonyls in water remained. Flow photochemistry involving the sustainable, and atom-economical oxidant singlet oxygen ($^1O_2$) has proven very fruitful. It was shown that this short-lived reactive intermediate can be utilized to rapidly transform natural extracts and to oxidize primary and secondary amines. When combined with amine oxidations, the imine generated upon photooxidation can be trapped by a cyanide source to generate α-aminonitriles. By coupling this photooxidative cyanation module to a second gas/liquid reaction with CO_2, the potential pool of starting materials for hydantoin synthesis could be expanded to aromatic and aliphatic amines.

9.1.1 Design of Flow Setup for Hydantoin Synthesis

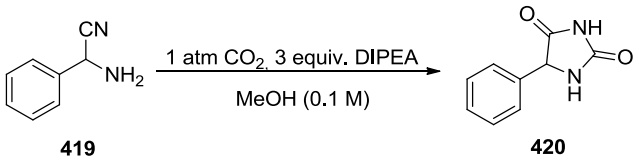
A convenient reactor consisting of FEP tubing was designed. CO_2 gas, flow rate of which was controlled using MFC, was mixed with a liquid reaction mixture using a T-mixer, and the heterogeneous mixture was passed through a 24 mL heated reactor. The system was pressurized using a 6 bar BPR (Figure 9.2).

Flow gas-liquid reaction is far more efficient than the same reaction in batch conditions, because more efficient mixing of the phases using a T-mixer is achieved and by pressurizing the system we obtain greater proportion of the gas dissolved in solution during the reaction than is the case under batch conditions.

In contrast the same reaction in batch suffers from a low surface-to-volume ratio between the gas and liquid phases which prevents mixing of the two phases and thus requires longer reaction times because of the lack of available CO_2 gas in solution.[4]

9.1.2 Optimization of Reaction Conditions

Initially we tested CO_2 addition to amino nitriles in batch using a balloon filled with CO_2. Solvent screen was performed with 2-amino-2-phenylacetonitrile as a substrate (See Scheme 9.1).



Scheme 9.1 Model reaction for hydantoin formation.

Solvents such as THF, DCM, MeOH, ethyl acetate and acetonitrile were tested. Reaction between amino nitriles and CO_2 affords the hydantoin product in all of the screened solvents; nevertheless, THF affords products with impurities. Although all the screened solvents proved to be suitable for the desired reaction, MeOH was the only exception from the solubility issue, namely, the hydantoin product is soluble only in MeOH which is why it became the solvent of choice for adapting this process to flow. Our reactor design is shown in Figure 9.2

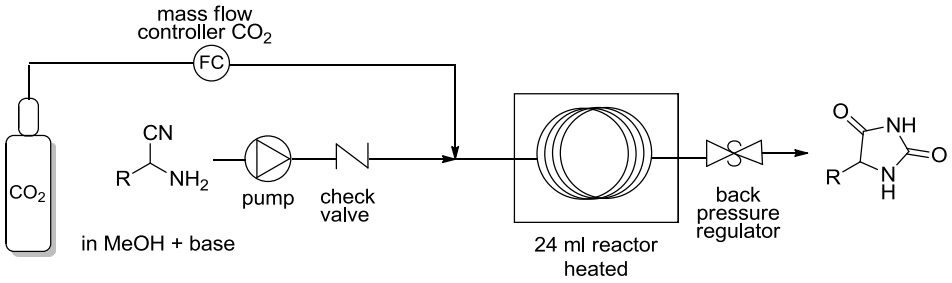
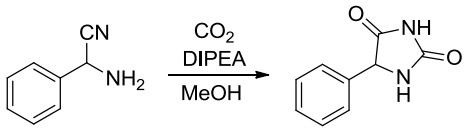


Figure 9.2 Reactor for hydantoin synthesis.

We started with a 10 mL reactor and residence time of 10 minutes using a 0.1 M solution of amino nitrile with 3 equiv. of Hünig's base at room temperature (Table 9.3). Increasing the temperature to 50 °C (Entry 3, Table 9.3) leads to more than a two-fold increase in conversion (from 5% to 13%), and increasing the number of CO_2 equivalents (Entry 4, Table 9.3) leads to a significant increase (43%). Pressurizing the system, increasing the temperature of the reaction and using a larger reactor (longer residence time) enables full conversion (Entry 8, Table 9.3). Raising the temperature of the reaction, has the greatest impact on conversion to the product, whereas pressure plays a minor role. When considering the mechanism of hydantoin formation (Scheme 9.4) it is possible to speculate that higher temperatures increase the rate of ring closure. Reducing the amount of base (two versus three equivalents, Entry 9, Table 9.3) was attempted but starting material was observed (63% conversion) compared to full conversion.

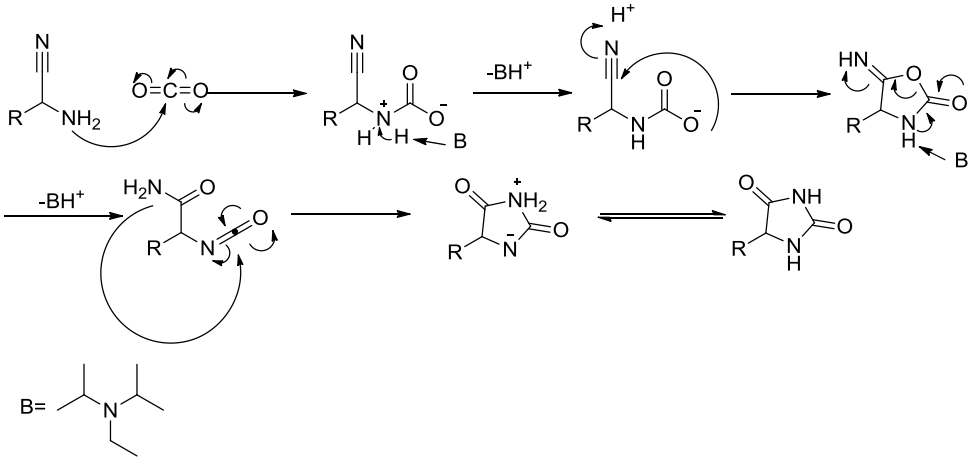
Table 9.3 Optimization of CO_2 addition to amino nitriles



419 420

Entry[a]	Pressure (bar)	Residence time (min)	CO_2		Temperature (°C)	Conversion (%)[b]
			Flow Rate (mL min^{-1})	Equivalents		
1	0.1	10	2	0.8	rt	5
2	1.8	10	2	0.8	rt	5
3	1.8	10	2	0.8	50	13
4	1.8	10	6	2.5	50	43
5	3.4	10	6	2.5	70	81
6	5	10	6	2.5	70	83
7	5	10	6	2.5	80	90
8[c]	7.5	20	6	2.5	80	100
9[c,d]	7.5	20	6	2.5	50	63

[a]α-Amino nitrile **3** (0.1 M in MeOH), Hünig's base (3 equiv.), solution flow rate (1 mL min^{-1}), τ_{res} = 10 min, reactor size 10 mL,[b] Determined by ^{1}H NMR. [c] τ_{res} = 20 min, reactor size 24 mL, [c] Hünig's base 2 equiv.



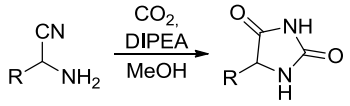
Scheme 9.4 Mechanism of hydantoin formation.

9.1.3 Connecting Photooxidative Cyanation to CO_2 Addition and Substrate scope of the Reaction

After optimization of the reaction conditions (Entry 8, Table 9.3), the scope and limitations of this biphasic transformation were explored using benzylic, homobenzylic and aliphatic *α*-amino nitriles. Benzylic substrates were higher yielding (78-98%) in the CO_2 addition reaction (Entry 1 and 5, Table 9.5) than the aliphatic substrates: cyclopentylamino nitrile (30%) and neopentylamino nitrile (23%) have poor yields in the CO_2 addition reaction (Entry 8 and 9,

Table 9.5). From Table 9.5 it is obvious that this method is efficient for benzylic substrates while it is limited for the substrates with steric hinderance at the alpha position. Fluorination in the aromatic ring does not influence the rate of hydantoin formation.

Table 9.5 Substrate scope for the optimized reaction of hydantoin formation.



330 421

Entry[a]	R	Yield (%)[b]
1	4-F-C_6H_4-	78
2	C_6H_5-	74
3	4-$CH_3OC_6H_4$	38
4	3-$FC_6H_3CH_2$	76
5	3-$CF_3C_6H_4$	98
6	$C_6H_5CH_2$	77
7	$C_6H_5CH_2CH_2$	58
8	$(CH_3)_3C$	23
9	$(CH_2)_4$	30

[a] α-Amino nitrile (0.1 M, in MeOH), Hünig's base (3 equiv.), CO_2 (6 mL min^{-1}), 80 °C, τ_{res} = 20 min, 6 bar BPR [b] Isolated yield after crystallization from EtOH:H_2O= 1:1.

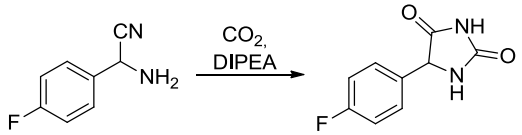
As with our previous work,[6] we were looking for a way to connect the carbonylation reaction to the photooxidative cyanation, due to the instability of amino nitrile intermediates. Photooxidative cyanation was found to be robust and could be performed equally well in DCM, THF and 2-MeTHF with no change in the yield,[6-7] the subsequent carbonylation would ideally be performed in one of the listed solvents. However, the hydantoin product was poorly soluble in DCM and THF, as mentioned previously. Therefore, a process was envisioned where the THF solution exiting the photoreactor (containing our intermediate) could be mixed with an equal volume of MeOH containing the base. This mixed solution could then be treated with CO_2.

Unfortunately, initial screening of the CO_2 addition reaction to pure *α*-amino nitrile in a 1:1 THF:MeOH solution showed a significant drop in conversion (62%) to the desired product when compared to pure MeOH (Entry 1, Table 9.6). Prolonging the reaction time (Entry 2, Table 9.6) by decreasing the flow rate of both the gas and the solution did not affect the conversion (62%). However, increasing the amount of CO_2 (Entry 3, Table 9.6) and the temperature (Entry 4, Table 9.6) leads to an increase in conversion, to 75 and 100%, respectively. By decreasing the amount of CO_2 (Entry 5, Table 9.6), we still observed the full conversion to the desired product.

After optimizing the reaction of CO_2 addition in the mixed solvent system, we addressed the problem of purification which proved to be problematic due to the similar solubilities of side products from the photooxidation reaction (TBAF) and the hydantoin product. Namely, TBAF, used for activation of TMSCN in photooxidative cyanation is soluble in a water/EtOH system, which was used for crystallization of the hydantoin products. By washing the concentrated

reaction mixture with DCM, clean hydantoin product could be obtained; however, hydantoins are partially soluble in DCM, causing partial loss of the product. Partial solubility of hydantoins in organic solvents was observed previously by the Kappe group.[5] We have encountered difficulties previously with connecting the photooxidative cyanation process[6] with the process of hydrolysis but were able to address this by using 2-MeTHF, which offered aqueous extraction of the byproducts from the photooxidative cyanation reaction prior to the CO_2 addition reaction. Taking the crude reaction through the described process, upon drying, pure material was obtained.

Table 9.6 Optimization of the reaction of hydantoin formation in a mixed solvent system



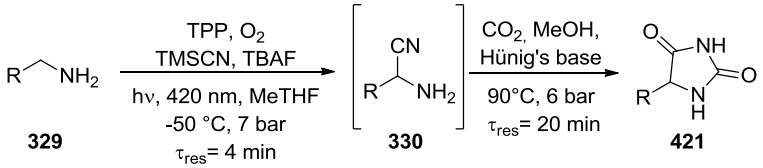
418 **422**

Entry[a]	Flow rate of the reaction mixture (mL min^{-1})	Flow rate of the gas (mL min^{-1})	Temperature (°C)	Conversion[b] (%)
1	1	6	80	62
2	0.75[c]	5	80	62
3	1	10[d]	80	75
4	1	10[d]	90	100
5	1	8	90	100

[a] α-Amino nitrile (0.1M in solvent) mixed with Hünig's base (3 equiv.) in 1/1 THF/MeOH, [b] Determined by ^{1}H NMR. For full reaction details, see Experimental Section, [c]τ_{res}=28 min [d]τ_{res}=17 min

Using this continuous process, we accessed hydantoins (Table 9.10) starting from amines without isolation of the unstable amino nitrile intermediate. The value of this continuous process is reflected in Entry 3 of Table 9.10 where a fluorinated aliphatic amine is directly converted to the corresponding hydantoin, bypassing the intermediate amino nitrile which decomposes upon isolation. The continuous process described here requires no chromatography.

Table 9.10 Substrate scope for the continuous two-step process.



Entry	R	Yield[c] (%)
1[a]	4-F-C_6H_4-	65
2[b]	C_6H_5-	84
3[a]	$CF_3CH_2CH_2$	52

[a] Amine (0.1 M in MeTHF), TMSCN (3.5 equiv.), TBAF (0.14 equiv.), TPP (0.02 mol %), O_2 (6 mL min^{-1}), LED 420 nm, –50 °C, τr_{es} = 4 min, 7 bar BPR. Crude reaction mixture (1 mL min^{-1}) ,3 equiv. Hünig's base in MeOH (1 mL min^{-1}). Crude reaction mixture (1 mL min^{-1}) ,CO_2 gas (8 mL min^{-1}) 90 °C, τ_{res} = 20 min, 6 bar BPR, [b] TMSCN 2.5 equiv., [c] Isolated yield.

In conclusion, a novel and green approach to the synthesis of hydantoins has been developed using consecutive biphasic gas/liquid reactions in continuous flow. The two-step process transforms primary amines using highly atom economical (O_2: oxidant, CO_2: carbonyl source) and traceless reagents (light) in environmentally friendly solvents (MeOH and 2-MeTHF). Using this approach, a variety of benzylic and aliphatic unprotected hydantoins could be obtained with no chromatographic purification.

Bibliography

[1] B. Pieber, C. O. Kappe, in *Organometallic Flow Chemistry*, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1.
[2] M. Brzozowski, M. O'Brien, S. V. Ley, A. Polyzos, *Accounts of Chemical Research* **2015**, *48*, 349.
[3] M. Cokoja, C. Bruckmeier, B. Rieger, W. A. Herrmann, F. E. Kühn, *Angewandte Chemie International Edition* **2011**, *50*, 8510.
[4] R. G. Murray, D. M. Whitehead, F. Le Strat, S. J. Conway, *Organic & Biomolecular Chemistry* **2008**, *6*, 988.
[5] J. L. Monteiro, B. Pieber, A. G. Corrêa, C. O. Kappe, *Synlett* **2016**, *27*, 83.
[6] S. Vukelić, D. B. Ushakov, K. Gilmore, B. Koksch, P. H. Seeberger, *European Journal of Organic Chemistry* **2015**, *2015*, 3036.
[7] D. B. Ushakov, K. Gilmore, D. Kopetzki, D. T. McQuade, P. H. Seeberger, *Angewandte Chemie International Edition* **2014**, *53*, 557.

10. Conclusions and Outlook

Fluorinated amino acids are interesting targets in the context of drug delivery and protein science. As a result of even one fluorine atom present, the amino acid can change its properties tremendously. Although fluorine has a size similar to hydrogen, the difference in electronegativity is responsible for the changes in the stability and the molecular interactions when these molecules are incorporated into peptides/ proteins. While the properties of fluorine can be exploited to modulate the stability and structure of peptide models in a desired direction, these can also complicate the synthesis of fluorinated molecules.

As discussed, the synthesis of fluorinated amino acids and their derivatives can be approached from two different angles, using available fluorinated starting materials or introducing the fluorine atom directly into the molecule. We pursued both strategies. First, the methodology for synthesis of racemic fluorinated amino acids was developed and expanded to the synthesis of hydantoins. The foundation was also set for the enantioselective synthesis of fluorinated amino acids in flow starting from fluorinated synthons. Secondly, our pursuit for the development of a methodology for the direct trifluoromethylation of amino acids has also borne fruit.

Using the continuous flow photooxidative cyanation process as a scaffold to construct the key intermediate amino nitriles, we developed a two-step semi-continuous process which offers easy and rapid access to racemic fluorinated amino acids starting from fluorinated amines. In this process the benefits of using hazardous reagents (30% HCl) above their boiling point under flow conditions were exploited to hydrolize amino nitriles to amino acids. The process requires no purification and is easily scaled, making it interesting for industrial applications. Keeping in mind that industry tends to shy away from processes involving hazardous oxygen gas due to safety regulations, we tested air in the reaction of photooxidative cyanation and showed that oxygen can be replaced by air without affecting the yield. The scope of this process could be broadened by increasing the pool of available fluorinated amines, and the development of a flow process to access fluorinated amines could be a future direction.

Photooxidative cyanation was also combined with a heterogeneous gas-liquid flow reaction to form hydantoins. The greater surface-to-volume ratio in gas-liquid reactions under flow conditions enabled the use of CO_2 as a readily available and abundant source of a carbonyl group. To make use of carbon-dioxide, a mixed solvent system was used for this reaction– necessary to combine the photooxidative cyanation reaction and to solubilize CO_2. The developed semi-continuous process requires no purification. What makes this process particularly interesting is the use of green reagents and solvents to deliver these useful scaffolds. Future work in this field should be oriented towards

expanding this methodology to N-substituted hydantoins: if secondary amines are used as starting materials, and the process of CO_2 addition to secondary α-amino nitriles is optimized, this methodology would offer access to these building blocks. Hydantoins are active pharmaceutical ingredients of various medicine, have found their application in material science and agriculture, they can also be exploited to synthesize enantiopure amino acids.

Inspired by the successful transformation of α-amino nitriles to racemic fluorinated amino acids, we wished to expand our efforts towards the synthesis of enantiopure fluorinated species. To pursue these targets, we have optimized the biotransformation of fluorinated amino nitrile to amino acid in batch in a collaborative effort with Almac.® We also designed and ran preliminary tests on a packed-bed reactor to adapt this process to flow conditions. Further efforts will be oriented toward adapting and optimizing the described biotransformation to flow conditions. More specifically, the volume of the designed packed bed reactor needs to be increased in order to prolong the residence time. This could be done by mixing the immobilized enzyme with the non-functionalized support used for the immobilization. After the process is optimized in flow, it should be elucidated whether the acid quench is required upon completion of the enzymatic reaction. If this is not the case, reusability of the enzymatic packed bed should be determined. In addition, racemization of the undesired enantiomer of amino nitrile could be initiated by developing conditions for dynamic kinetic resolution, which would allow for the desired fluorinated amino acid to be obtained in yields greater than 50%. After determining the scope and limitations of this enzymatic transformation, this flow process could be combined with photooxidative cyanation and a continuous two-step process for synthesis of enantiomerically pure fluorinated amino acids could be realized.

With the goal of the direct incorporation of a trifluoromethyl group into an amino acid, a method was developed which affords the addition of a CF_3 group into the α-position of an N-PMP protected glycine ester. Using an oxidant and the Ruppert-Prakash reagent, it is possible to add the CF_3 group to the alpha position in a single step. At this time, the mechanism of the developed transformation is unclear and mechanistic studies are being conducted to elucidate it.

As the incorporation of fluorinated amino acids into peptides is sometimes hindered by the presence of fluorine in the molecule (*e.g.* reduced nucleophilicity of the amino group influences the efficiency during peptide synthesis), it may be of interest to study the developed α-trifluoromethylation process using short-chain peptides as substrates. If the process could be optimized for the N-PMP protected glycine amide substrates, it could potentially be used for the trifluoromethylation of peptides. Ideally, if the process would be selective for the α-trifluoromethylation of N-terminal amino acid (PMP-protected amine should be oxidized more readily than the one participating in the amide bond) this could be beneficial for the ^{18}F labelling of peptides.

In conclusion, this dissertation demonstrates that combining photooxidative cyanation to two different flow modules (hydrolysis and CO_2 addition) can enable access to two different classes of molecules (racemic amino acids and hydantoins). Moreover, future optimization of the biocatalytic process may offer a third flow module which would, by combining with photooxidative cyanation reaction, yield enantiopure fluorinated amino acids. It has also been shown that trifluoromethylation of the α-position of N-PMP protected glycine esters can be carried out in a single step and adapted to flow conditions by designing an appropriate packed bed reactor. Further studies should explore the scope of this process by employing amides and ketones/aldehydes as substrates.

11. Experimental Section

11.1 General Experimental Data

Commercial grade solvents and reagents were used unless stated otherwise. Anhydrous solvents were obtained from a Dry Solvent System (Waters, Milford, USA). Solvents for chromatography were of technical grade. Sensitive reactions were carried out in oven-dried glassware and under an argon atmosphere. Molecular sieves were activated by heating under high vacuum prior to use. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates pre-coated with silica gel of 0.25 mm thickness (Macherey-Nagel, Düren, Germany) and on Pre-coated TLC-sheets ALUGRAM Xtra SIL G/UV, aluminium plates pre-coated with silica gel of 0.20 mm thickness (Macherey-Nagel, Germany). Spots were visualized by staining with ninhydrin or an aqueous solution of potassium permanganate. Flash chromatography was performed on Fluka technical grade silica gel (230-400 mesh) and using Reveleris® X2 flash chromatography system (Grace discovery science) with Macherey-Nagel silica gel 60M (0.04–0.063 mm) used for dry loading. Commercially available (Reveleris) column: Silica 12 g, 40 μm with a flow rate of eluent 28 mL/min. ELSD and UV (254 and 280 nm) detectors were used with low detector sensitivity at 30 mV and 0.05 AU threshold detection respectively. Chromatographic columns were reused after washing with acetone/methanol (from 100:0 to 95:5 over 7.5 column volumes). Solvents were removed under reduced pressure using a rotary evaporator (Büchi R-210) and high vacuum (<1 mbar). ^{1}H, ^{13}C, ^{19}F and two-dimensional NMR spectra were measured using Varian 400-MR spectrometer or a Varian 600 spectrometer (both Agilent, Santa Clara, USA) at 298 K. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ 7.26 in ^{1}H and 77.16 in ^{13}C NMR; MeOD: δ 3.31 in ^{1}H and 49.00 in ^{13}C NMR; DMSO-d_6: δ 2.50 in ^{1}H NMR and 39.52 in ^{13}C NMR).

The following abbreviations are used to indicate peak multiplicities: s singlet; bs broad singlet, d doublet; dd doublet of doublets; ddd doublet of doublet of doublets, dddd doublet of doublet of doublet of doublets, br d broad doublet, br dd broad doublet of doublets, t triplet; tt triplet of triplets, q quartet, m multiplet.

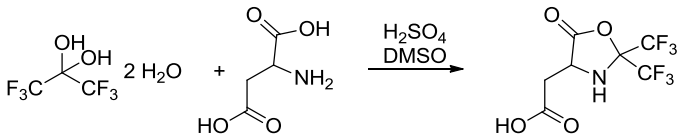
Coupling constants (J) are reported in Hertz (Hz). NMR spectra were evaluated using MestreNova 6.2 (MestreLab Research SSL, Santiago de Compostella, Spain). High resolution mass spectrometry by electrospray ionization (ESI-HRMS) was performed at Freie Universität Berlin, Mass Spectrometry Core Facility, with a 6210 ESI-TOF mass spectrometer (Agilent). Low resolution mass spectrometry was performed on a Mass Spectrometer Agilent 1100 LC MSD Model G1946D (LC-MS). Schemes were prepared using ChemBioDraw Ultra 12.0.2 (Cambridgesoft, Waltham, USA).

11.2 General Information for the Flow Apparatus for HFA Protection of Amino Acids

$HFA{\cdot}2H_2O$ was introduced into a three-neck flask (100 mL) with 50 mL of concentrated H_2SO_4 using a syringe pump Harvard Pump PhD Elite 2000. Three neck-flask was connected to a 10 mL loop (fluorinated ethylene–propylene copolymer (FEP) tubing (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter) cooled in a dry ice/acetone bath by a 30 cm FEP tubing via an in house made connection (plastic 1 mL syringe connected with plastic vacuum tube). After the 10 mL cooling loop, 30 cm FEP tubing was used to connect the setup to the two-neck flask containing an amino acid in DMSO. Setup was further connected with an oil bubbler and two traps with 50 cm FEP tubing. First trap contained silica gel blue (Sigma Aldrich) and the second one contained water (See Figure 11.1 below).

Figure 11.1. Flow apparatus for HFA protection of amino acids

2-(5-oxo-2,2-bis(trifluoromethyl)oxazolidin-4-yl)acetic acid[1]



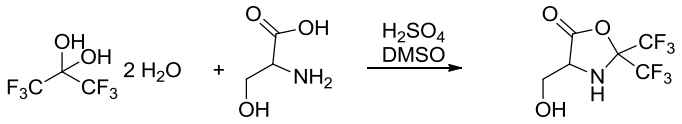
$HFA{\cdot}2H_2O$ (3.5 mL, 30 mmol) was introduced into a three-neck flask with 50 mL concentrated H_2SO_4 using a syringe pump Harvard Pump 11 Elite. Three-neck flask was connected to a 10 mL loop cooled in a dry ice/acetone bath. After the cooling loop, tubing was used to connect the setup to the two necked flask with aspartate (0.80 g, 6.0 mmol) in DMSO (4 mL, 1.5 M). Setup was further connected by tubing

with an oil bubbler and two traps. First trap contained silica gel blue (Sigma Aldrich) and the second one contained water. Yield of the product, 71%, was determined using ^{1}H NMR (mesytilene as internal standard).

Work up: Reaction mixture mixed with 2 mL ice cold water, extracted with DCM 3 x (V = 24 mL), aqueous layer was washed 3 x (V = 12 mL) with DCM, all organic layers washed 4 x with ice cold water (V = 8 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Clean product couldn't be obtained after the work up, product not isolated.

Peaks of the product identified in the impure product[1]: ^{1}H NMR (400 MHz, $CDCl_3$): 3.00-3.02 (m, 1H), 3.05-3.06(m, 1H), 3.59 (bs, 1H), 4.37 (m, 1H); ^{19}F (376 MHz, $CDCl_3$):-–80.94 (q, 3F, J = 8.5Hz), -80.06 (q, 3F, J = 8.5Hz).

4-(hydroxymethyl)-2,2-bis(trifluoromethyl)oxazolidin-5-one[2]

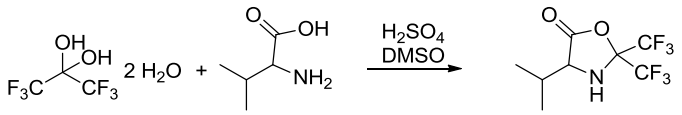


$HFA{\cdot}2H_2O$ (3.5 mL, 30 mmol) was introduced into a three-neck flask with 50 mL concentrated H_2SO_4 using a syringe pump Harvard Pump 11 Elite. Three-neck flask was connected to a 10 mL loop cooled in a dry ice/acetone bath. After the cooling loop, tubing was used to connect the setup to the two necked flask with serine (0.63 g, 6.0 mmol) in DMSO (4 mL, 1.5 M). Setup was further connected by tubing with an oil bubbler and two traps. First trap contained silica gel (blue) and the second one contained water. Yield of the product, 56%, was determined using ^{1}H NMR (mesytilene as internal standard).

Work up: Reaction mixture mixed with 24 mL of ice cold water, extracted with 3 x 24 mL DCM, aqueous layer washed 3 x 12 mL DCM, all organic layers washed with 4 x 8 mL ice cold water, dried over $MgSO_4$ and evaporated *in vacuo*. Silica gel added and stirred for 1.5 h at room temperature. Filtered and evaporated *in vacuo*. Clean product could not be obtained.

Peaks of the product identified in the impure product: ^{1}H NMR (400 MHz, $CDCl_3$): 1.97 (m, 1H), 3.35-3.88 (m, 1H), 3.99-4.02 (m, 1H); ^{19}F (376 MHz, $CDCl_3$): -80.39 (m, 3F), -80.30 (m, 3F).

4-isopropyl-2,2-bis(trifluoromethyl)oxazolidin-5-one[2]

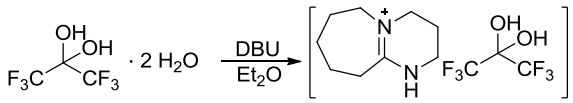


$HFA{\cdot}2H_2O$ (3.5 mL, 30 mmol) was introduced into a three-neck flask with 50 mL concentrated H_2SO_4 using a syringe pump Harvard Pump 11 Elite. Three-neck flask was connected to a 10 mL loop cooled

in a dry ice/acetone bath. After the cooling loop, tubing was used to connect the setup to the two-neck flask with valine (0.70 g, 6.0 mmol) in DMSO (4 mL, 1.5 M). Setup was further connected by tubing with an oil bubbler and two traps. First trap contained silica gel (blue) and the second one contained water. Yield of the product, 31%, was determined using ^{1}H NMR (mesytilene as internal standard).
Work up: Reaction mixture mixed with 24 mL ice cold water, extracted with 3 x 24 mL DCM, aqueous layer washed 3 x 12 mL DCM, all organic layers washed with 4 x 8 mL with ice cold water, dried over $MgSO_4$ and evaporated *in vacuo*.
^{1}H NMR (400 MHz, $CDCl_3$): 0.99 (d, 3H), 1.07 (d, 3H), 2.11-2.19 (m, 1H), 2.84 (bs, 1H), 3.82-3.84 (m, 1H); ^{19}F (376 MHz, $CDCl_3$): -80.39 (q, 3F, J=8.7 Hz), -80.03 (q, 3F, J = 8.7 Hz).

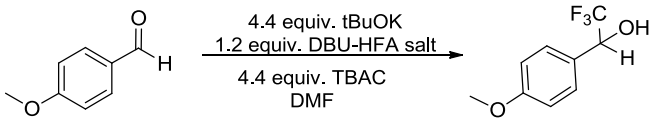
11.3 Synthesis of Precursors to Access Fluorinated Amino Acids via Fluorinated Ketones and Aziridines

Hydrate-1,8-diaza-bicyclo[5.4.0]undec-7-ene salt[3]



To a solution of hexafluoroacetone tri-hydrate (0.84 mL, 7.2 mmol) in Et_2O (6 mL) DBU (1 mL, 7.2 mmol) was added. A colorless precipitate formed immediately, was filtered, washed with Et_2O (3 × 2 mL), and dried to provide the product as a colorless solid (2.1 g, 85%).
^{1}H NMR (400 MHz, $CDCl_3$): 1.64-1.66 (m, 2H), 1.70-1.73 (m, 4H), 2.79-2.81 (m, 2H), 3.3 (t, 2H, J = 5.8 Hz), 3.34-3.41 (m, 4H), 5.94 (bs, 3H); ^{19}F (376 MHz, $CDCl_3$): -85.27 (s, 6H).
Spectral data match the literature.[3]

2,2,2-trifluoro-1-(4-methoxyphenyl)ethanol[3]

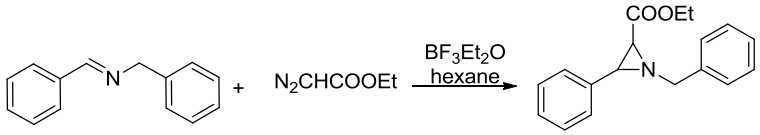


To a –30 °C mixture of *t*-BuOK (147 mg, 1.31 mmol) and TBAC (366 mg, 1.31 mmol) in anhydrous DMF (3.0 mL) DBU-HFA salt (168 mg, 0.918 mmol) was added. After 30 min at –30 °C, *p*-anisaldehyde (24 µL, 0.2 mmol) was added. After stirring for 1.5 h, the reaction mixture warmed to rt, saturated aqueous NH_4Cl (5 mL) was added, and the resulting mixture was extracted with EtOAc (3 ×

10 mL). The combined organics were dried over $NaSO_4$ and concentrated under reduced pressure. 60% yield was determined by ^{19}F NMR using $CF_3C_6H_5$ as an internal standard.

Product was not isolated. Peaks of the product identified in the crude reaction mixture[4]: ^1H ($CDCl_3$, 400 MHz): 3.81 (s, 3H), 4.95 (q, 1H, J = 6.7 Hz), 6.91 (d, 2H, J = 8.8 Hz), 7.39 (d, 2H, J = 8.8 Hz); ^{19}F NMR (376 MHz, $CDCl_3$): -78.58 (d, J = 6.7 Hz).

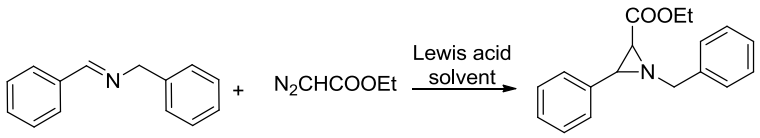
Ethyl 1,3-diphenylaziridine-2-carboxylate



N-benzylidene-1-phenylmethanamine (21 μL, 0.2 mmol) was weighed in a flame dried tube, it was evacuated and back-filled with Ar twice, hexane (1 mL) was added followed by the BF_3Et_2O (0.02 mmol). Reaction mixture was heated to 40 °C and EDA (36 mg, 0.2 mmol) was added. Reaction was stopped after 3 h and the reaction mixture was analyzed by NMR using mesytilene as internal standard.

Peaks of the product identified in the crude reaction mixture[5]: ^1H NMR (400 MHz, $CDCl_3$): 0.99 (t, 3H, J = 7.1 Hz), 3.21 (d, 1H, J = 6.8 Hz), 3.61 (d, J = 6.7 Hz, 1H), 3.97-4.09 (m, 2H), 7.06-7.09 (m, 2H), 7.27-7.36 (m, 7H), 7.51-7.53 (m, 2H).

General procedures for other reactions including different Lewis acids ($Yb(OTf)_2$, $Zn(OTf)_2$, $CuCl_2$)[5]:



Lewis acid (amount stated in a Table 11.2 below) and *N*-benzylidene-1-phenylmethanamine (21 μL, 0.2 mmol) were weighed in a flame dried tube, it was evacuated and back filled with Ar twice, solvent (1 mL) was added. EDA (36 mg, 0.2 mmol) was added and it was stirred at the r.t. The reaction mixture was analyzed by ^1H NMR using mesytilene as internal standard. Product was not isolated.

General procedure for synthesis of aziridine in the presence of $AgBF_4$, Entries 1 and 2[6], Table 11.2:

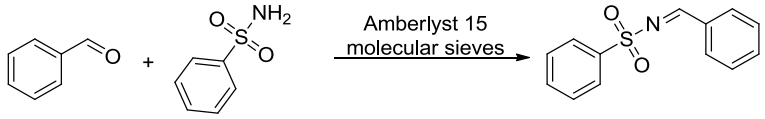
$AgBF_4$ and CuI were weighed in a flame dried microwave tube, it was evacuated and back filled with Ar twice. Solvent was added, reaction mixture was cooled using an ice bath and it was stirred. Afterwards EDA and *N*-benzylidene-1-phenylmethanamine in solvent were added. Prior to addition both of the solutions were evacuated and backfilled with Ar. After 2 h reaction was stopped. The reaction mixture was analyzed by ^{1}H NMR using mesytilene as internal standard.

Table 11.2 Optimization of aziridine formation using different Lewis acids

Entry[a]	Lewis Acid	Reaction time (h)	Temperature (°C)	Solvent	Yield (%)[e]
1[a]	CuI/ $AgBF_4$	2	ice	DCM	0
2[a]	CuI/ $AgBF_4$	2	ice	MeCN	0
3[b]	$CuCl_2$/10mol%	2	r.t.	acetone	0
4[b]	$CuCl_2$/30 mol%	2	r.t.	acetone	0
5[d]	$Yb(OTf)_2$	2	r.t.	DCM	0
6[a]	$Zn(OTf)_2$	2	r.t.	DCM	20
7[c]	BF_3 Et_2O	1	r.t.	hexane	30
8[c]	BF_3 Et_2O	1	40	hexane	45
9[c]	BF_3 Et_2O	3	40	hexane	58
10[c]	BF_3Et_2O, 40 mol%	1	40	hexane	n/a[f]
11[c]	BF_3 Et_2O	5	40	hexane	41

[a] 0.5 equiv. of EDA is used (with respect to the imine (1 mmol, 0.33M), 20 mol% of CuI/$AgBF_4$, [b] Reaction conditions: imine (0.3 mmol, 0.1 M), diazo-reagent (1.0 equiv.), Lewis acid, under Ar for 1 h, [c] Reaction conditions 0.2 mmol imine, 0.2 M, BF_3 Et_2O (10 mol%) added, heated (if applicable), 0.2 mmol ethyl diazoacetate added and stirred, [d] 0.38 mmol of ethyl diazoacetate, 1.5 equiv. of imine, [e] Determined by NMR using an internal standard, [f] Complex mixture obtained.

***N*-benzylidenebenzenesulfonamide[7]**

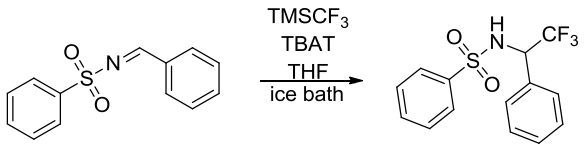


Using Dean Stark apparatus, benzenesulfonamide (6.17 g, 39.3 mmol) and benzaldehyde (4.0 ml, 39.3 mmol) were heated to 130 °C in in the presence of 4 Å molecular sieves (6 g) and Amberlyst 15 (0.08 g) in dry toluene (33 mL) for 24 h. It was filtered and the solvent was evaporated *in vacuo*. Product obtained as yellow solid (7.3 g, 76%).

^{1}H NMR (400 MHz, $CDCl_3$): 7.24-7.28 (m, 3H), 7.41-7.44 (m, 2H), 7.49-7.51 (m, 3H), 7.92-7.95 (m, 2H), 8.48 (s, 1H).

Spectral data match the literature.[8]

***N*-(2,2,2-trifluoro-1-phenylethyl)benzenesulfonamide**



N-benzylidenebenzenesulfonamide (98 mg, 0.4 mmol) and $TMSCF_3$ (0.26 mL, 2M solution in THF, 0.520 mmol) mixture prepared in dry THF (5 mL). In a flame dried tube TBAT (259 mg, 0.48 mmol) weighed, evacuated, backfilled with argon and THF (2 mL) added. Cooled in an ice bath. Solution of *N*-benzylidenebenzenesulfonamide and $TMSCF_3$ slowly added using a syringe. Stirred in an ice bath for 45 min, saturated NH_4Cl aq. sol. (4 mL) added and warmed to r.t. Extraction 3 x with EtOAc. 47% yield was determined by ^{19}F NMR using C_6F_6 as internal standard.

Asignment of the impure product: ^1H NMR (400 MHz, $CDCl_3$): 1.26 (aliphatic impurity),1.6 (s, H_2O), 4.94 (q, 1H, J = 7.8 Hz), 5.58 (d, 1H, J = 9.1 Hz), 7.16-7.19 (m, 2H), 7.24-7.32 (m, 3H), 7.35-7.39 (m, 2H), 7.47-7.51(m, 1H), 7.71-7.74 (m, 2H); ^{19}F (376 MHz, $CDCl_3$): -73.95 (d, 3F, J=7.8 Hz).

Isolation and further characterization of the product are in progress (^{13}C NMR and mass spectrum).

11.4 General Procedure for Trifluoromethylation of Glycine Derivatives Using Different Oxidants

In an oven dried flask, methyl 2-((4-methoxyphenyl)amino)acetate (39 mg, 0.2 mmol), KF (35 mg, 0.6 mmol), and oxidant (59 mg, 0.26 mmol) were weighed and solvent (1 mL) was added. The flask was evacuated and backfilled with N_2, $TMSCF_3$ (0.85 g, 0.6 mmol) was added. Reaction was stirred. The reaction mixture was analyzed by ^{19}F NMR using $CF_3C_6H_5$ as internal standard. KF used was dried in the oven prior to use (48h).

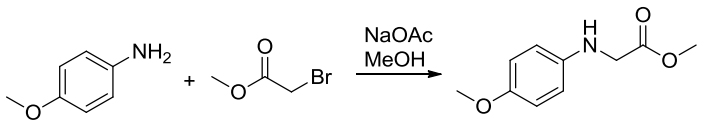
Reaction conditions for certain oxidant are given in a Table 11.3 below.

Table 11.3 Trifluoromethylation of glycine derivatives using different oxidants

Entry	Oxidant/catalyst	Solvent	Product (%)	Reaction time (h)	Reaction Temperature (°C)
1[a]	BPO[b]	MeCN	1	Overnight	r.t.
2[a]	Benzoquinone	MeCN	0	Overnight	r.t.
3[a]	DDQ	MeCN	20	Overnight	r.t.
4[c]	chloranil	DMAc	25	5	r.t.
5	DDQ	DMA	58	4	Ice bath
6[c]	Rubpy, TEMPO, $(NH_4)_2S_2O_8$	DMA	Trace amounts	Overnight	r.t.
7[c]	Rubpy, TEMPO, CCl_3Br	DMA	1	Overnight	r.t.

[a] Conditions: 0.2 mmol amine ($PhNHCH_2COOEt$), 1.3 equiv. of oxidant, 3 equiv. of KF and 3 equiv. of $TMSCF_3$ (2 M solution in THF, 1 ml of solvent),[b] at 40 °C 4 % of product is detected, at 6 equiv. of $TMSCF_3$/KF 5% of product is detected,[c] Conditions: 0.2 mmol (*p*-$MeOPhNHCH_2COOEt$), 1.3 equiv. of oxidant, 3 equiv. of KF and 3 equiv. of $TMSCF_3$, 2 ml of solvent)

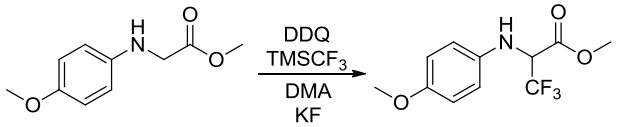
Methyl 2-((4-methoxyphenyl)amino)acetate[9]



2-Bromoacetyl bromide (4.73 mL, 50 mmol), NaOAc (4.10 g, 50 mmol) and *p*-anisidine (6.16 g, 50 mmol) were mixed in MeOH (100 mL) and heated overnight under N_2 at 70°C. Reaction mixture was filtered. Solvent was evaporated in vacuo. Recrystallation was done from DCM / hexanes to afford the pure product 2-(4-methoxyphenylamino)-*N*-methylacetamide (7.30 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): 3.74 (s, 1H), 3.77 (s, 3H), 3.88 (s, 2H), 6.58-6.60 (m, 2H), 6.77-6.81 (m, 2H); ^{13}C (400 MHz, $CDCl_3$): 46.80, 52.35, 55.85, 114.57, 115.02, 141.19, 152.82, 171.99.; HRMS (ESI): ($M+H^+$) calcd: 196.0969, found: 196.0975.

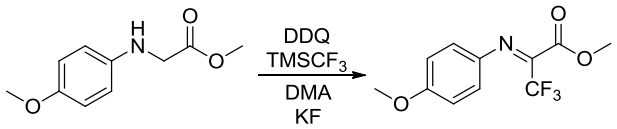
Methyl 3,3,3-trifluoro-2-((4-methoxyphenyl)amino)propanoate



In an oven dried flask, methyl 2-((4-methoxyphenyl)amino)acetate (39 mg, 0.2 mmol), KF (35 mg, 0.6 mmol) and DDQ (59 mg, 0.26 mmol) were weighed and DMA (1 mL) was added. KF used was dried in the oven prior to use (48 h at 110 °C). The flask was evacuated and backfilled with N_2, $TMSCF_3$ (89 μL, 0.6 mmol) was added. Reaction was stirred for 4 h in an ice bath. 58% yield by ^{19}F NMR using $CF_3C_6H_5$ as internal standard was determined. Work up was done by extraxtion of the crude reaction mixture with EtOAc (3 x 10 mL), organic layers were connected and concentrated. Column chromatography (hexane-EtOAc = 0-100%) afforded product as a solid (20 mg, 38%).

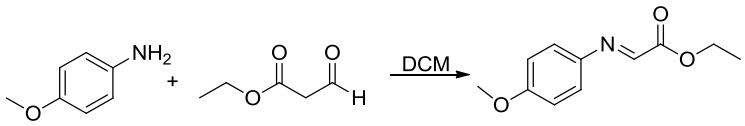
^1H NMR (400 MHz, $CDCl_3$): 3.75 (s, 1H), 3.85 (s, 3H), 3.88 (s, 2H), 4.28 (bs, NH), 4.48 (q, 1H, J = 7.0 Hz), 6.69-6.71 (m, 2H), 6.79-6.81 (m, 2H); ^{13}C (400 MHz, $CDCl_3$): 53.70, 55.80, 61.10 (q, J = 31.3 Hz), 115.05, 116.34, 123.41 (q, J = 283.1 Hz), 139.09, 154.11, 167.24; ^{19}F NMR (376 MHz, $CDCl_3$): -72.76 Hz (d, J = 7.0 Hz), HRMS ($M+H^+$) calcd: 264.0843, found: 264.0873.

Methyl 3,3,3-trifluoro-2-((4-methoxyphenyl)imino)propanoate



This product was obtained as the side product in the previous reaction as a result of over oxidation.
^1H NMR (400 MHz, $CDCl_3$): 3.85 (s, 3H), 4.12 (q, 1H, J = 7.2 Hz), 5.80 (s, 1H), 6.93-6.97 (m, 2H), 7.29-7.33 (m, 2H), 8.05 (s, 1 H); ^{19}F NMR (376 MHz, $CDCl_3$): -75.16 Hz (s, 3F).*
*Further characterization is in progress; high resolution mass spectrum and ^{13}C NMR spectrum will be measured.

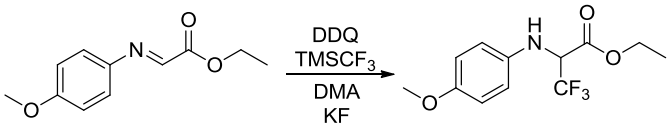
Ethyl 2-((4-methoxyphenyl)imino)acetate



Ethylglyoxalate (6 mL, 27 mmol, 50 vol% in toluene) was added to a stirred solution of *p*-anisidine (3.32 g, 27 mmol) in dry DCM (50 mL) at room temperature. After two hours reaction time, activated 4 Å molecular sieves (2 grams) were added and the mixture was stirred for additional three hours. After the reaction was run to completion as detected by TLC, the solution was filtered through a plug of Celite®, solvent removed *in vacuo*. Additionally dried by concentrating with toluene. Product obtained as a viscous orange oil (4.97 g, 89%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (t, *J* = 7.11,1H), 3.84 (s, 3H), 3.88 (s, 2H), 4.41 (d, 2H, *J* = 7.1 Hz), 6.93 (d, 2H, *J* = 8.5 Hz), 7.36 (d, 2H, *J* = 8.6 Hz), 7.94 (s, 1H); ^{13}C (400 MHz, $CDCl_3$): 14.21, 55.48, 61.88, 114.50, 123.59, 141.35, 147.98, 160.49, 163.58.
Spectral data match the literature.[10]

Ethyl 3,3,3-trifluoro-2-((4-methoxyphenyl)amino)propanoate



In an oven dried flask, ethyl 2-((4-methoxyphenyl)imino)acetate (39 mg, 0.2 mmol), KF (35 mg, 0.6 mmol) and DDQ (59 mg, 0.26 mmol) were weighed and DMA (1 mL) was added. The flask was evacuated and backfilled with N_2. Reaction mixture was cooled in an ice bath, $TMSCF_3$ (89 µL, 0.6 mmol) was added. Reaction was stirred for 4 h. The reaction mixture was analyzed by ^{19}F NMR (with trifluorotoluene as internal standard). A mixture of products is obtained. Preparative TLC was run in EtOAc:hexane = 1:1. Product and unknown compound were isolated in a single fraction and could not be separated.
^{1}H NMR (400 MHz, $CDCl_3$): 1.31 (t, *J* = 7.1, 3H), 1.57 (bs, water), 3.75 (s, 3H), 3.82 (s, unidentified), 4.30 (m, 2H), 4.45 (m, 1H), 6.70 (d, 2H, *J* = 8.9 Hz), 6.80 (d, 2H, *J* = 8.9 Hz), ^{19}F (376 MHz, $CDCl_3$): -72.70 (q, 3F, *J* = 6.9 Hz), ^{13}C (400 MHz, $CDCl_3$):13.93, 55.64, 61.04 (q, *J* = 31.1 Hz), 62.91, 114.88, 116.14, 123.32 (q, *J* = 283.1 Hz*), 139.04, 153.89, 116.50, HRMS ($M+H^+$) calcd: 278.0999, found: 278.1020.
*Second coupling constant could not be determined because of the signal-to-noise ratio.

11.5 General Procedure for Synthesis of Methyl 3,3,3-trifluoro-2-((4-methoxyphenyl)amino)propanoate in a fixed Bed Reactor

In an oven dried flask, methyl 2-((4-methoxyphenyl)amino)acetate (39 mg, 0.2 mmol), DDQ (59 mg, 0.26 mmol) were weighed and DMA (1 mL) was added. The flask was evacuated and backfilled with N_2 and $TMSCF_3$ (89 µL, 0.6 mmol) was added. Reaction mixture was fed onto the fixed bed reactor (for more details about the fixed bed reactor see chapter 6, Applied Methods, 6.5 Fixed Bed Reactor for Triluoromethylation of Glycine Derivatives) using Harvard PHD 2000 syringe pump. Packed bed was flushed with three column volumes of DMA. Reaction mixture was collected and analyzed by ^{19}F NMR using $CF_3C_6H_5$ as internal standard.

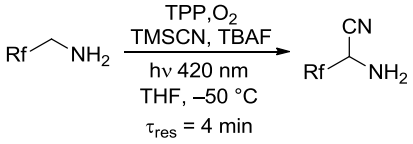
11.6 General Information for the Photooxidative Cyanation Process

Vapourtech R series R2+ was used for continuous flow photosynthesis. For more details on the on the flow apparatus (photoreactor) see Chapter 6. Applied Methods, 6.3 In House Designed Flow Photoreactor. Oxygen used for photooxidation reaction was 99.995%, H_2O <3.0 ppm*mol; ALPHAGAZ™ 1 O_2; Werk DEF 2 Krefeld-Gellep and it was delivered through a check valve from an oxygen gas tank. Gas pressure was regulated to 20 bar and the flow adjusted by a gas-flow controller (Influx, SV1B5-AI05).

The LED module OSA Opto Light, OLM-018 B, 420 nm emission wavelength, 12 W was used as a light source in the photooxidation reaction.

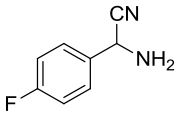
Cryostat Huber, TC100E-F-NR was used to cool the acetone bath during the photooxidative cyanation reactions.

11.7 General Procedure for Synthesis of Fluorinated α-Amino Nitriles



TMSCN (3.5 equiv.) was added to the solution of amine (1 mM) and TPP (1 mg per 5 mL) in THF, followed by addition of a 1 M solution of TBAF in THF (4 mol% based on TMSCN). The resulting solution was mixed with oxygen gas (solution flow rate 1.0 mL min^{-1}) and pumped through the photoreactor. Gas flow rate was adjusted such that the residence time was 4 min. The solvent was removed i*n vacuo* and the residue was purified by column chromatography. In the case of CF_3 substituted aromatics, work-up with aqueous $Na_2S_2O_3$ (sat.) was done prior to removing the solvent *in vacuo*.

2-Amino-2-(4-fluorophenyl)acetonitrile

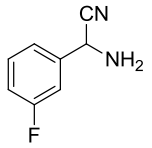


A solution of 4-fluorophenyl)methanamine (0.457 mL, 4 mmol), TMSCN (1.751 mL, 14 mmol), TBAF (0.560 mL, 0.56 mmol) and TPP (8 mg, 0.0128 mmol) in THF (40 mL) was pumped through the

photoreactor at –50 °C. Flash chromatography of the residue after solvent evaporation (DCM to 2% MeOH in DCM) afforded the product as light yellow crystals (422 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.94 (bs, 2H), 4.90 (s, 1H), 7.10 (t, *J* = 8.5 Hz, 2H), 7.50–7.54 (m, 2H), ^{19}F NMR (376 MHz, $CDCl_3$): –112.49 (dddd, J=18.8, 15.0, 15.0, 11.3, 7.5 Hz); ^{13}C (100 MHz, $CDCl_3$): 46.7, 116.2 (d, *J* = 21.9 Hz), 120.9, 128.7 (d, *J* = 8.4 Hz), 132.2, 163.0 (d, *J* = 248.2 Hz). Data is in agreement with those previously reported in the literature.[11]

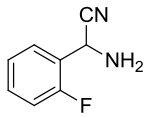
2-Amino-2-(3-fluorophenyl)acetonitrile



A solution of (3-fluorophenyl)methanamine (0.41 mL, 1 mmol), TMSCN (0.438 mL, 3.5 mmol), TBAF (0.140 mL, 0.14 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL) was pumped through the photoreactor at –50 °C. Flash chromatography of the residue after solvent evaporation (hexane: EtOAc1:10 to DCM and then to 1% MeOH in DCM afforded the product as a yellow oil (133 mg, 88%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.98 (bs, 2H), 4.91 (s, 1H), 7.08 (t, *J* = 8.3 Hz, 1H), 7.26–7.29 (m, 1H), 7.32–7.34 (m, 1H) 7.39 (dd, *J* = 4.3, 3.3 Hz, 1H); ^{19}F NMR (376 MHz, $CDCl_3$): –111.41 (ddd, J= 11.3, 11.28, 7.5 Hz); ^{13}C (100 MHz, $CDCl_3$): 46.8, 114.0 (d, *J* = 23.2 Hz), 116.2 (d, *J* = 21.1 Hz), 120.6, 122.4 (d, *J* = 3.1 Hz), 130.9 (d, *J*= 8.2 Hz), 138.7 (d, *J* = 7.3 Hz), 161.6 (d, *J* = 247.7 Hz); HRMS (ESI): ($M + H^+$) calcd 151.0667, found 151.0682.

2-Amino-2-(2-fluorophenyl)acetonitrile

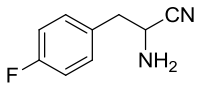


Solution of 2-fluorophenyl)methanamine (0.110 mL, 0.960 mmol), TMSCN (0.421 mL, 3.36 mmol), TBAF (0.134 mL, 0.134 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL) was pumped through the photoreactor at –50 °C. Flash chromatography of the residue after solvent evaporation (hexane:ethylacetate 10:1 to hexane:ethylacetate 4:1 to dichloromethane and then to 1% methanol in dichloromethane) afforded the product as a yellow oil (88.1 mg, 61%).

^{1}H NMR (400 MHz, $CDCl_3$): 2.02 (br s, 2H), 5.10 (s, 1H), 7.12 (ddd, *J* = 10.2, 8.3, 1.1 Hz, 1H), 7.21 (ddd, *J* = 7.6, 7.6, 1.1 Hz), 7.39 (dddd, *J*= 11.3 , 5.5 , 5.5 3.8 Hz), 7.53 (ddd, *J* = 7.6, 7.6, 1.6 Hz, 1H);

^{19}F NMR (376 MHz, $CDCl_3$): –118.26 (ddd, J = 10.4, 7.5, 5.4 Hz); ^{13}C (100 MHz, $CDCl_3$): 42.2 (d, J = 4.3 Hz,), 116.2 (d, J = 20.9 Hz), 120.0, 124.3 (d, J = 13.5 Hz), 125.0 (d, J = 3.1 Hz), 128.2 (d, J = 3.1 Hz), 131.2 (d, J = 8.3 Hz), 160.0 (d, J = 248.8 Hz); HRMS (ESI): ($M + Na^+$) calcd 173.0486, found 173.0507.

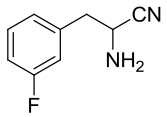
2-Amino-3-(4-fluorophenyl)propanenitrile



A solution of 2-(4-fluorophenyl)ethanamine (0.131 mL, 1 mmol), TMSCN (0.438 mL, 3.5 mmol), TBAF (0.140 mL, 0.140 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL, 0.1 M) was pumped through the photoreactor (7.5 mL) temperature –50 °C. Flash chromatography of the residue after solvent evaporation (dichloromethane to 1% methanol in dichloromethane) afforded the product as a yellow oil (145.7 mg, 89%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.65 (br s, 2H), 2.99 (ddd, J =11.3, 11.3, 3.8 Hz, 2H), 3.89 (dd, J = 6.9, 6.1 Hz, 1H), 7.03 (dd, J = 8.7, 8.7 Hz, 2H), 7.26 (dd, J = 8.6, 5.4 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$): –114.85 (dddd, J = 8.7, 8.7, 5.3, 5.3 Hz); ^{13}C (100 MHz, $CDCl_3$): 40.3, 44.7 (d, J = 1.5 Hz), 115.6 (d, J = 21.4 Hz), 121.5, 131.3 (d, J = 8.1 Hz), 162.4 (d, J = 246.0 Hz); HRMS (ESI): ($M + H^+$) calcd 165.0823, found 165.0826.

2-Amino-3-(3-fluorophenyl)propanenitrile

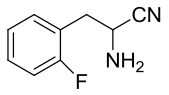


A solution of 2-(3-fluorophenyl)ethanamine (0.131 mL, 1 mmol), TMSCN (0.438 mL, 3.5 mmol), TBAF (0.140 mL, 0.140 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL, 0.1 M) was pumped though the photoreactor at –50 °C. Flash chromatography of the residue after solvent evaporation (dichloromethane to 1% methanol in dichloromethane) afforded the product as a yellow oil (129.0 mg, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.64 (br s, 2H), 3.03 (app ddd, J = 12.0, 12.0, 4.0 Hz, 2H), 3.94 (dd, J = 6.5, 6.5 Hz), 6.99–7.03 (m, 2H, 1H), 7.09 (br d, J = 7.9 Hz, 1H), 7.30–7.36 (m, 2H); ^{19}F NMR (376 MHz, $CDCl_3$): –112.50 (ddd, J = 9.0, 9.0, 5.9 Hz); ^{13}C (100 MHz, $CDCl_3$): 40.9, 44.5 (d, J = 1.7 Hz),

114.8 (d, J = 21.0 Hz), 116.7(d, J = 21.4 Hz), 121.4, 163.0 (d, J = 246.8 Hz); HRMS (ESI): ($M + H^+$) calcd 165.0823, found 165.0819.

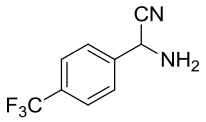
2-Amino-3-(2-fluorophenyl)propanenitrile



A solution of 2-(2-fluorophenyl)ethanamine (0.131 mL, 1 mmol), TMSCN (0.438 mL, 3.5 mmol), TBAF (0.140 mL, 0.140 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL) was pumped through the photoreactor at –50 °C. Flash chromatography of the residue after solvent evaporation (dichloromethane to 2% methanol in dichloromethane) afforded the product as yellow oil (123.8 mg, 75%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.66 (br s, 2H), 3.09 (app ddd, J = 12.0, 12.0, 4.0 Hz, 2H), 3.94 (dd, J = 7.1, 7.1 Hz), 7.08 (dd, J = 9.1, 9.1 Hz, 1H), 7.14 (dd, J = 7.5, 7.5 Hz 1H), 7.27–7.32 (m, 2H); ^{19}F NMR (376 MHz, $CDCl_3$): –117.44 (ddd, J = 9.8, 6.9, 5.8 Hz); ^{13}C (100 MHz, $CDCl_3$): 35.2 (d, J = 1.7 Hz), 44.0, 115.8 (d, J = 21.9 Hz), 121.4, 122.4 (d, J = 15.4 Hz), 124.6 (d, J = 3.7 Hz), 129.8 (d, J = 8.2 Hz), 131.9 (d, J = 4.3 Hz), 161.4 (d, J = 246.1 Hz); HRMS (ESI): ($M + H^+$) calcd 165.0823, found 165.0819.

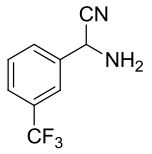
2-Amino-2-(4-(trifluoromethyl)phenyl)acetonitrile



A solution of (4-(trifluoromethyl)phenyl)methanamine (0.201 mL, 1.412 mmol), TMSCN (0.618mL, 4.94 mmol), TBAF (0.395 mL, 0.395 mmol) and TPP (3 mg, 0.0048 mmol) in THF (14.1 mL) was pumped through the photoreactor at –60 °C. The mixture after photoreactor was diluted with dichloromethane (10 mL) and extracted with aqueous $Na_2S_2O_3$ (sat), before it was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography of the residue using Reveleris® X2 flash chromatography system (hexane/dichlomethane from 100:0 to 0:100 over 20 column volumes) afforded the product as an orange oil (75 mg, 27%).

^{1}H NMR (400 MHz, $CDCl_3$): 2.0 (br s, 2H), 4.98 (s, 1H,), 7.70 (br s, 4H); ^{19}F NMR (376 MHz, $CDCl_3$): –69.79 (s); ^{13}C (100 MHz, $CDCl_3$): 47.0, 120.4, 123.9 (q, J = 272.5 Hz), 126.2 (q, J = 3.8 Hz), 127.3, 131.5 (q, J = 32.8 Hz), 140.0. HRMS (ESI): ($M + H^+$) calcd 201.0635, found 201.0636.

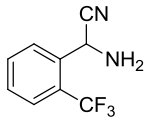
2-Amino-2-(3-(trifluoromethyl)phenyl)acetonitrile



A solution of (3-(trifluoromethyl)phenyl)methanamine (0.308 mL, 2.150 mmol), TMSCN (0.941mL, 7.53 mmol), TBAF (0.301 ml, 0.301 mmol) and TPP (4 mg, 0.0064 mmol) in THF (21.5 mL) was pumped through the photoreactor at –60 °C. The mixture after photoreactor was diluted with dichloromethane (10 mL) and extracted with aqueous $Na_2S_2O_3$ (sat.), before it was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography of the residue using Reveleris® X2 flash chromatography system (from 0-100 hexane to dichloromethane over 5 column volumes, 100% dichloromethane for 5 column volumes, from 0-100 dichloromethane to acetone over 3.5 column volumes) afforded the product as an orange-ish oil (223 mg, 52%).

^{1}H NMR (400 MHz, $CDCl_3$): 2.0 (br s, 2H), 4.99 (s, 1H), 7.57 (dd, J = 7.8, 7.8 Hz, 1H), 7.66 (br d, J = 7.8, 7.8 Hz, 1H), 7.77 (br d, J = 7.8, 7.8 Hz, 1H), 7.84 (br s, 1H); ^{19}F NMR (376 MHz, $CDCl_3$): –62.7(s); ^{13}C (100 MHz, $CDCl_3$): 47.0, 120.4, 123.8 (q, J = 3.8 Hz), 123.9 (q, J = 272.5 Hz), 126.1 (q, J = 3.7 Hz), 129.8, 130.2, 131.7 (q, J = 32.5 Hz), 137.3; HRMS (ESI): ($M + H^+$) calcd 201.0635, found 201.0643.

2-Amino-2-(2-(trifluoromethyl)phenyl)acetonitrile

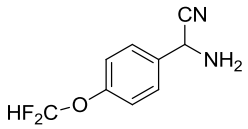


A solution of (2-(trifluoromethyl)phenyl)methanamine (0.228 mL, 1.627 mmol), TMSCN (0.712 mL, 5.70 mmol), TBAF (0.228 mL, 0.228 mmol) and TPP (3 mg, 0.0040 mmol) in THF (16.3 mL) was pumped through the photoreactor at –60 °C. The mixture after photoreactor was diluted with dichloromethane (10 mL) and extracted with aqueous $Na_2S_2O_3$ (sat.) (15 mL) before it was dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography of the residue using Reveleris® X2 flash chromatography system (from 0-100 hexane to dichloromethane over 5 column volumes, 100% dichloromethane for 5 column volumes, from 0-100 dichloromethane to acetone over 3.5 column volumes) afforded the product as an orange oil (117 mg, 36%).

^{1}H NMR (400 MHz, $CDCl_3$): 1.96 (br s, 2H), 5.28 (s, 1H), 7.52 (dd, J = 7.7, 7.7 Hz, 1H), 7.69 (dd, J = 16.2, 7.9 Hz, 2H), 7.89 (br d, J = 7.8, 1H); ^{19}F NMR (376 MHz, $CDCl_3$): –58.61(s); ^{13}C (100 MHz,

$CDCl_3$): 44.0 (q, *J* = 2.8 Hz), 120.2, 124.0 (q, *J* = 273.9 Hz), 126.7 (q, *J* = 5.6 Hz), 127.6 (q, *J* = 30.8 Hz), 129.0, 129.5, 133.3, 135.8; HRMS (ESI): ($M + Na^+$) calcd 223.0454, found 223.0457.

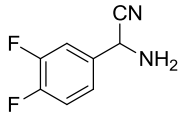
2-Amino-2-(4-difluoromethoxy)phenyl)acetonitrile



A solution of (4-(difluoromethoxy)phenyl)methanamine (0.154 mL, 1.062 mmol), TMSCN (0.438 mL, 3.5 mmol), TBAF (0.140 mL, 0.14 mmol) and TPP (2 mg, 0.0032 mmol) in THF (10 mL) was pumped through the photoreactor –at –50 °C. Residence time is 4 min. Flash chromatography of the residue after solvent evaporation using Reveleris® X2 flash chromatography system (from 0-100 hexane to dichloromethane over 5 column volumes, 100% dichloromethane for 5 column volumes, from 0-100 dichloromethane to acetone over 3.5 column volumes) afforded the product as a yellow oil (116 mg, 55%).

^{1}H NMR (400 MHz, CD_3OD): 5.01 (s, 1H), 6.86 (dd, *J* = 74.2, 74.2 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 8.4, 2H); ^{19}F NMR (376 MHz, CD_3OD): –83.75 (d, *J* = 73.8 Hz); ^{13}C (100 MHz, CD_3OD): 47.2 , 117.62 (t, *J* = 258.1 Hz), 120.5, 122.0, 129.7, 135.7, 153.1; HRMS (ESI): (M+-HCN) calcd 172.0574, found 172.0588.

2-Amino-2-(3,4-difluorophenyl)acetonitrile)

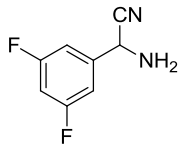


A solution of (3,4-difluorophenyl)methanamine (0.237 mL, 2.0 mmol), TMSCN (0.876 mL, 7.0 mmol), TBAF (0.280 mL, 0.28 mmol) and TPP (4 mg, 0.0064 mmol) in THF (20 mL) was pumped through the photoreactor at –60 °C. Flash chromatography of the residue after solvent evaporation using Reveleris® X2 flash chromatography system (from 0-100 hexane to dichloromethane over 5 column volumes, 100% dichloromethane for 5 column volumes, from 0-100 dichloromethane to acetone over 3.5 column volumes) afforded the product as a yellow oil (185 mg, 55%).

^{1}H NMR (400 MHz, $CDCl_3$): 2.00 (br s, 2H), 4.87 (s,1H), 7.15–7.21 (m, 1H), 7.25–7.28 (m, 1H), 7.38 (ddd, *J* = 10.2, 7.3, 2.2 Hz, 1H); ^{19}F NMR (376 MHz, CD_3OD): –136.95—137.06 (m), –135.85—135.95 (m); ^{13}C (100 MHz, CD_3OD): 46.3 (d, *J* = 1.6 Hz), 116.09 (d, *J* = 18.8 Hz), 117.85 (d, *J* = 17.8

Hz), 120.5, 122.9 (dd, *J* = 6.7, 3.7 Hz), 133.3 (dd, *J* = 5.5, 3.8 Hz), 150.6 (dd *J* = 250.2, 10.1, Hz), 150.4 Hz (dd *J* = 250.1, 10.5, Hz); HRMS (ESI): ($M + Na^+$) calcd 191.0397, found 191.0434.

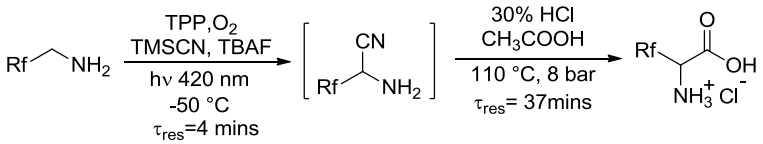
2-Amino-2-(3, 5-difluorophenyl)acetonitrile



A solution of (3,5-difluorophenyl)methanamine (0.237 mL, 2.0 mmol), TMSCN (0.876 mL, 7.0 mmol), TBAF (0.280 mL, 0.28 mmol) and TPP (4 mg, 0.0064 mmol) in THF (20 mL, 0.1 M) was pumped through the photoreactor at –60 °C. Flash chromatography of the residue after solvent evaporation using Reveleris® X2 flash chromatography system (from 0-100 hexane to dichloromethane over 5 column volumes, 100% dichloromethane for 5 column volumes, from 0-100 dichloromethane to acetone over 3.5 column volumes) afforded the product as a yellow oil (136 mg, 40%).

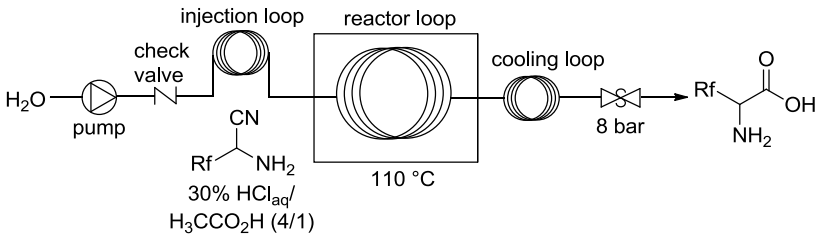
^{1}H NMR (400 MHz, CD_3OD): 5.05 (s,1H), 6.98 (dddd, *J* = 9.0, 9.0, 2.2, 2.2 Hz, 1H), 7.19 (app ddd, *J* = 6.7, 6.7, 1.9 Hz, 2H); ^{19}F NMR (376 MHz, CD_3OD): –110.55 (br dd, J = 8.39 Hz, 8.39 Hz); ^{13}C (100 MHz, CD_3OD): 104.9 (dd, *J* = 25.8, 25.8 Hz), 111.1 (dd, J = 7.46, 7.46 Hz), 121.4, 143.1 (dd, *J* = 9.3, 9.3 Hz), 164.61 (dd, *J* = 248.3, 12.8 Hz). HRMS (ESI): ($M + H^+$) calcd 169.0572, found 169.0575.

11.8 General Procedure for Synthesis of Fluorinated Amino Acids



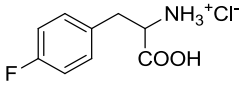
2-MeTHF was used as a solvent for the synthesis of fluorinated amino nitriles using the set-up described above. The reaction mixture, collected after the photoreactor, was washed with water (3 × 20 mL) and organic solvent was removed *in vacuo*. The crude material was dissolved in 1.2 mL of acetic acid; 3.5 mL of 30% aqueous HCl was added followed by sonication for 2 min and filtration. The precipitate was washed with 2.5 mL of 30% HCl. An injection loop (6 mL, fluorinated ethylene–propylene copolymer (FEP) tubing (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter) was filled with filtrate and the solution was passed through a 22 mL reactor (fluorinated ethylene–propylene copolymer (FEP) tubing (IDEX Health and Science, natural color, 1.57 mm outer diameter, 0.76 mm inner diameter), using Knauer pump Smartline 100 at 0.6 mL min^{-1}. Reactor was heated to 110 ˚C (Scheme 11.5 below) and at the end of the reactor, back pressure regulator was set

(Syrris FRX pressure). Solvent was removed *in vacuo* to afford the desired amino acid salt as a white solid.



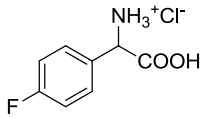
Scheme 11.5 Flow apparatus for synthesis of fluorinated amino acids

2-Amino-3-(4-fluorophenyl)propanoic acid hydrochloride salt



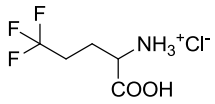
2-(4-fluorophenyl)ethanamine (0.122 mL, 0.933 mmol) gave the product (156 mg, 67% over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): 3.18 (dd, J = 14.5, 7.2 Hz, 1H) 3.27-3.31 (m, 1H), 4.25 (dd, J = 6.2, 6.2 Hz, 1H), 7.09 (t, J = 8.6 Hz, 2H), 7.33 (dd, J = 7.9 Hz, 5.6 Hz); ^{19}F NMR (376 MHz, CD_3OD): –116.99—117.01 (m); ^{13}C (100 MHz, CD_3OD): 36.4, 55.1, 116.7 (d, J = 21.7 Hz), 131.53 (d, J = 3.2 Hz), 132.43 (d, J = 8.2 Hz), 163.74 (d, J = 244.7 Hz), 171.00; HRMS (ESI): (M+ H^+) calcd 184.0769, found 184.0786.

2-Amino-2-(4-fluorophenyl)acetic acid hydrochloride salt



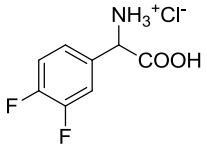
(4-fluorophenyl)methanamine (0.123 mL, 1.080 mmol) gave amthe product (143 mg, 64% over 2 steps). ^{1}H NMR (400 MHz, CD_3OD): 7.22 (dd, J = 8.2, 8.2 Hz, 2H), 7.57–7.59 (m, 2H); ^{19}F NMR (376 MHz, CD_3OD): –113.1—113.1 (m, 1F); ^{13}C (100 MHz, CD_3OD): 56.8, 117.2 (d, J = 22.2 Hz), 129.9 (d, J = 3.2 Hz), 131.7 (d, J = 8.6 Hz), 164.7 (d, J = 248.1 Hz), 170.5; HRMS (ESI): (M + H^+) calcd 170.0612, found 170.0709.

2-Amino-5,5,5-trifluoropentanoic acid hydrochloride salt



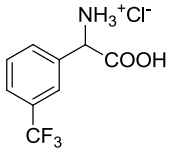
4,4,4-trifluorobutan-1-amine (0.111 mL, 1.184 mmol) gave the product (150 mg, 61% over 2 steps).
^{1}H NMR (400 MHz, CD_3OD): 2.11–2.26 (m, 2H), 2.35–2.57 (m, 2H), 4.13 (dd, J = 5.4 Hz), 8.62 (br s, 1H); ^{19}F NMR (376 MHz, CD_3OD): –68.3 (t, J = 10.5 Hz); ^{13}C (100 MHz, CD_3OD); 24.2, 30.08 (q, J = 29.7 Hz), 52.6, 129.8 (q, J = 275.4 Hz), 170.8; HRMS (ESI): ($M + H^+$) calcd 170.0429, found 170.0411.

2-Amino-2-(3,4-difluorophenyl)acetic acid hydrochloride salt



(3,4-difluorophenyl)methanamine (0.106 mL, 0.9 mmol) gave the product (120 mg, 60% over 2 steps).
^{1}H NMR (400 MHz, CD_3OD): 5.21 (s, 1H), 7.39–7.43 (m, 2H), 7.49–7.53 (dd, J = 9.0, 9.0 Hz, 1H); ^{19}F NMR (376 MHz, CD_3OD): –138.35 (t, J = 8.6 Hz); ^{13}C (100 MHz, CD_3OD): 56.5, 118.8 (dd, J = 14.9, 4.9 Hz), 119.4 (t, J = 9.3 Hz), 126.5 (t, J = 5.3 Hz), 131.1 (t, J = 4.8 Hz), 151.6 (dd, J = 250.6 Hz, 14.8 Hz), 152.3 (dd, J = 251.8 Hz; 14.2 Hz), 170.1; HRMS (ESI): ($M + H^+$) calcd 188.0518, found 188.0613.

2-Amino-2-(3-(trifluoromethyl)phenyl)acetic acid hydrochloride salt



(3-(trifluoromethyl)phenyl)methanamine (0.118 mL, 0.826 mmol) gave the product (110 mg, 50% over 2 steps).
^{1}H NMR (400 MHz, CD_3OD): 5.31 (s, 1H), 7.70–7.74 (m, 1H), 7.79–7.83 (m, 2H), 7.88 (br s, 1H); ^{19}F NMR (376 MHz, CD_3OD): –64.25 (br s); ^{13}C (100 MHz, CD_3OD): 57.0, 126.5 (q, J = 271.6 Hz), 126.1 (q, J = 3.9 Hz), 127.7 (q, J = 3.4 Hz), 131.5, 132.5 (q, J = 32.6 Hz), 135.1, 170.1; HRMS (ESI): ($M + H^+$) calcd 218.0429, found 218.0393.

11.9 Enzymatic Reactions in Batch Using Cell Lysate: General Information

Assay; 10% MeOH in phosphate buffer (if not stated otherwise, pH 7.0). Phosphate buffer was prepared according to the Sigma Aldrich.[12] Nitrilases in a form of a cell lysate and immobilized on four different supports were obtained from Almac®

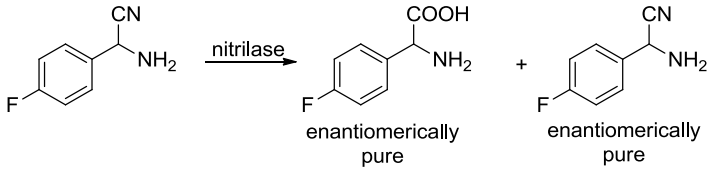
Shaker, Eppendorf AG

HPLC 1, VWR Hitachi, Elite LaChrome and HPLC 2, Agilent Technologies 1200 Series with chiral column: CHIRALPAK® ZWIX(-) (250 x 4.0mm, 3μm), were used for analysis, HPLC method: isocratic, mobile phase: MeOH:H_2O = 98:2 + 50 mM HCOOH + 25 mM DEA. Samples were filtered prior to HPLC analysis using sample filters, injection volume is 200 μL. UV measurements were performed on a UV spectrophotometer Shimadzu UV-mini-1240 using a 1 cm cuvette. Mass spectrometry was performed on a Mass Spectrometer Agilent 1100 LC MSD Model G1946D,

Preparation of substrate in assay solution with KCN:

2-amino-2-(4-fluorophenyl)acetonitrile (375.4 mg, 2.5 mmol) and KCN (165.3 mg, 2.5 mmol) were solubilized in assay (25 mL of MeOH and 225 mL of phosphate buffer pH 7.7).

Qualitative studies



Initial screening of 16 different nitrilases to determine the activity against the desired substrate:

1 mL of substrate solution with KCN in assay was added to 1.6 mg of cell lysate (containing nitrilase) and the reaction was shaken 300 rpm at 37 °C for total of 72 h. Reaction was quenched with 20 μL of conc. HCl and diluted with 2 mL of MeOH. Centrifuge 15 min 3200 rpm. Supernatant collected. Samples were analyzed by HPLC after 2 h, 5 h, 8 h, 24 h, 48 h and 72 h reaction time. HPLC chromatograms were obtained using HPLC 1.

Below is a chromatogram of a reaction ran with NIT 115 after 72 h (Figure 11.6) and a chromatogram of a mixture containing racemic product, 2-amino-2-(4-fluorophenyl)acetic acid and starting material, 2-amino-2-(4-fluorophenyl)acetonitrile.

Figure 11.6. Chromatogram of NIT 115 sample after 72 h

Figure 11.7. Chromatogram of starting material and the racemic product r.t. 6.26 min shows starting material: 2-amino-2-(4-fluorophenyl)acetonitrile, r.t. 8.97 min and 9.79 min show the two enantiomers of the product 2-amino-2-(4-fluorophenyl)acetic acid.

Comparison of the enzymatic reaction in presence of KCN and without KCN

Preparation of substrate solution without KCN:

2-amino-2-(4-fluorophenyl)acetonitrile (37.54 mg, 0.25 mmol) was solubilized in 2.5 mL of MeOH and 22.5 mL of phosphate buffer pH 7.7.

Enzymatic reaction in the presence of KCN and without KCN-comparison

0.3 mg of cell lysate (containing NIT 115), 10 mM substrate conc. (1 mL, pH 7), 1 equiv. of KCN (with respect to the substrate, if stated that cyanide is present), shaken for total of 5 h at 300 rpm at 37 °C. Quenched with 20 μL of HCl conc., diluted with 2 mL of MeOH, centrifuged 15 min at 3200 rpm. Supernatant analyzed by HPLC. It seemed that presence of KCN does not influence the outcome of the reaction (Figure 11.8- 11.11). HPLC chormatograms were obtained using HPLC 2.

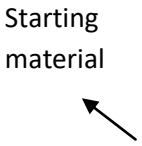


Figure 11.8. Chromatogram of NIT 115 catalyzed reaction after 1 h, without KCN

Figure 11.9. Chromatogram of NIT 115 catalyzed reaction after 1 h, in the presence of KCN

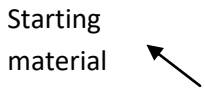


Figure 11.10. Chromatogram of NIT 115 catalyzed reaction after 5 h, in the presence of KCN

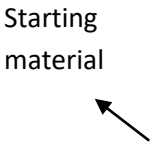


Figure 11.11. Chromatogram of NIT 115 catalyzed reaction after 5 h, without KCN

Identification of the product by NMR

2-amino-2-(4-fluorophenyl)acetonitrile (30 mg, 0.2 mmol), solubilized in 20 mL of assay and added to 28.9 mg cell lysate, 37 °C, shaker 300 rpm, 4 h. Quenched with 400 µL of HCl conc. and diluted with 40 mL of MeOH, centrifuged 15 min at 3200 rpm. Solvent removed *in vacuo*. 75% conversion to the desired product was determined by ^{19}F NMR.

In the crude mixture ^{1}H NMR and ^{19}F NMR peaks of desired product were identified: ^{1}H NMR (400 MHz, CD_3OD): 5.07 (s, 1H), 7.19–7.23 (m, 2H), 7.52-7.55 (m, 2H); ^{19}F NMR (376 MHz, CD_3OD): –113.16 (m, 1F), MS: (M^+) calcd 170.0, found: 170.0.
In the ^{19}F NMR additional unidentified peaks observed: -112.75, -113.08, -116.02.
-116.31 ppm peak of starting material identified.

Quantitative studies

UV measurements for determining the extinction coefficient of 2-amino-2-(4-fluorophenyl)acetonitrile and 2-Amino-2-(4-fluorophenyl)acetic acid

Extinction coefficient of 2-amino-2-(4-fluorophenyl)acetonitrile (starting material) and 2-Amino-2-(4-fluorophenyl)acetic acid hydrochloride (product) were determined at 261 nm by UV measurements.

Determination of extinction coefficient for the *(S)*-2-Amino-2-(4-fluorophenyl)acetic acid :
4.48 mg of (*S)*-2-Amino-2-(4-fluorophenyl)acetic acid (Sigma Aldrich) solubilized in 10 mL of 4% HCl in MeOH; serial dilution; factor 1.3. Eight samples prepared with decreasing concentration, UV spectrum measured (Figure 11.12).

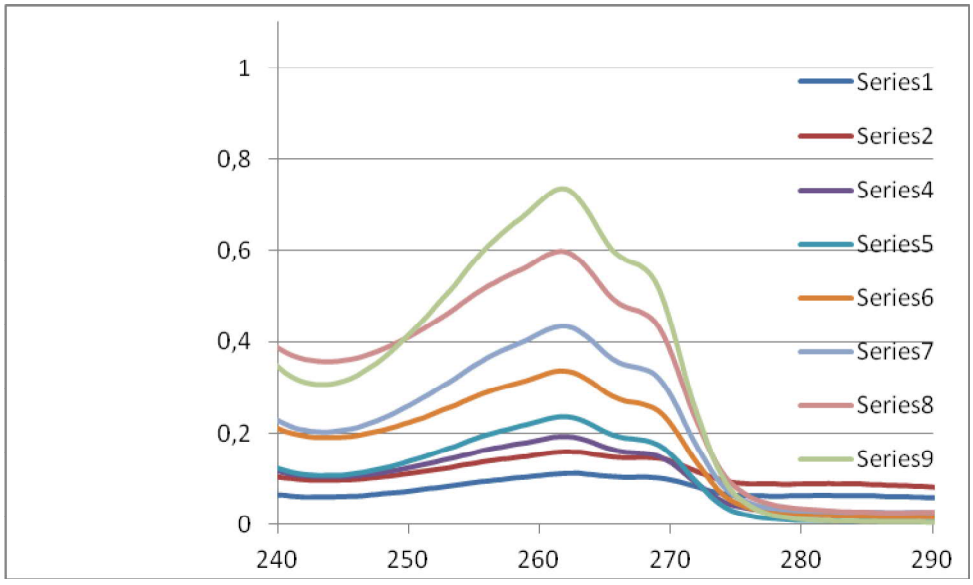


Figure 11.12 Dependence of absorbance of solution of 2-Amino-2-(4-fluorophenyl)acetic acid in 4% HCl in MeOH on the wave length, Series1-Series 9 (Serial dilution).

Table 11.13 Dependence of the absorbance of the concentration at 261 nm for 2-Amino-2-(4-fluorophenyl)acetic acid.

Concentration (2-Amino-2-(4-fluorophenyl)acetic acid) (mM)	A at 261 nm
0.27	0.135
0.35	0.192
0.63	0.217
0.84	0.249
1.12	0.376
1.49	0.432
1.99	0.656
2.654	0.749

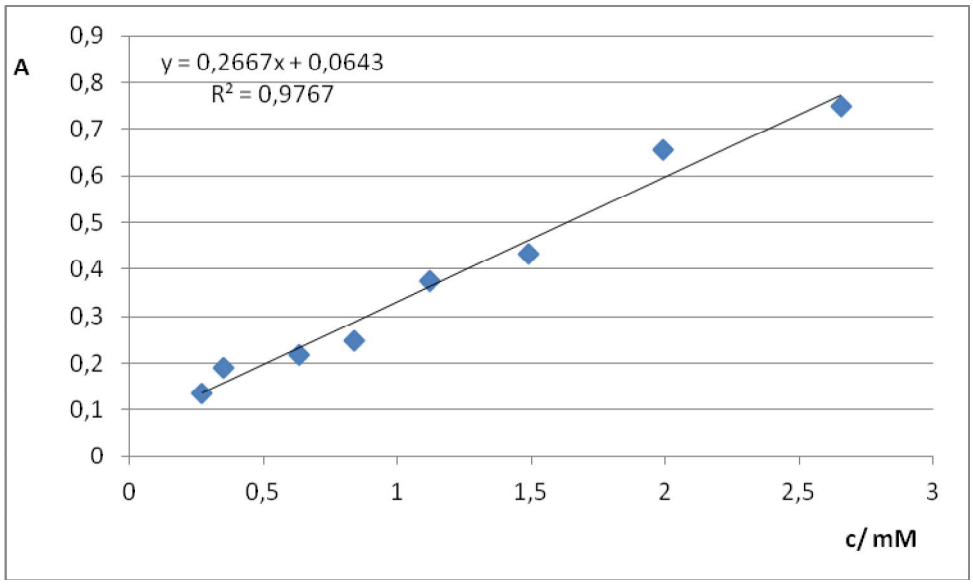


Figure 11.14 Dependence of absorption of 2-Amino-2-(4-fluorophenyl)acetic acid on the concentration at 261 nm.

Graphical interpretation (Figure 11.14) of the dependence of the absorption on the concentration at 261 nm gave the extinction coefficient of 0.2667 mM^{-1} cm^{-1}according to the Lambert-Beer's law (Figure 11.14).

Determination of extinction coefficient for the 2-amino-2-(4-fluorophenyl)acetonitrile:

0.8 mg of 2-amino-2-(4-fluorophenyl)acetonitrile was solubilized in 10 mL of MeOH; serial dilution; factor 1.1, 9 samples prepared with decreasing concentration, UV spectrum measured (Figure 11.15).

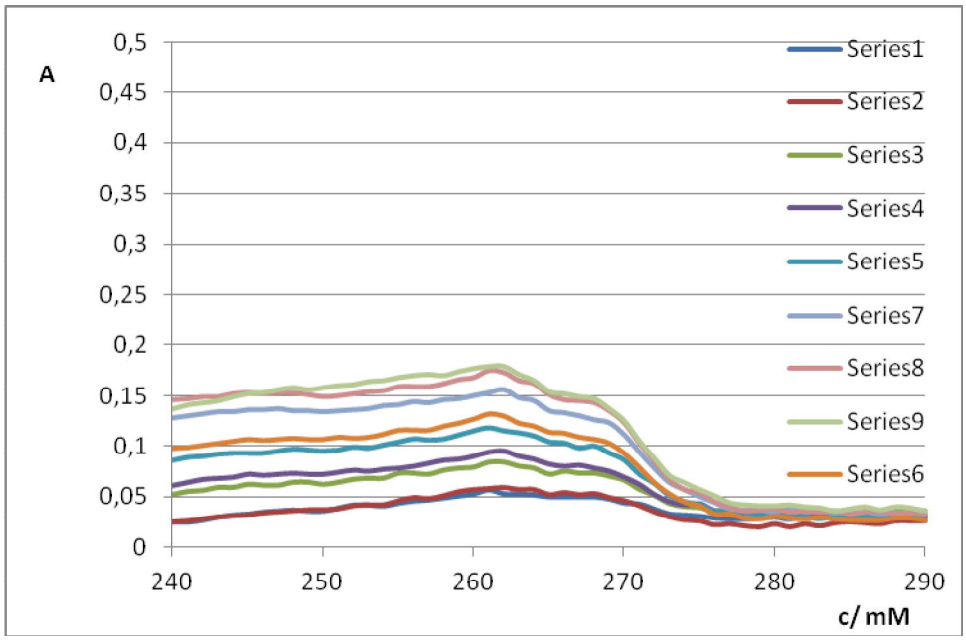


Figure 11.15 Dependence of absorbance of solution of 2-amino-2-(4-fluorophenyl)acetonitrile in in MeOH on the wave length, Series1-Series 9 (Serial dilution).

Table 11.16 Change of the absorbance according to the concentration at 261 nm for 2-amino-2-(4-fluorophenyl)acetonitrile solution in MeOH.

Concentration (2-amino-2-(4-fluorophenyl)acetonitrile) (mM)	**A at 261 nm**
0.15	0.056
0.20	0.063
0.25	0.100
0.30	0.120
0.35	0.159
0.40	0.183
0.45	0.207
0.50	0.230
0.53	0.253

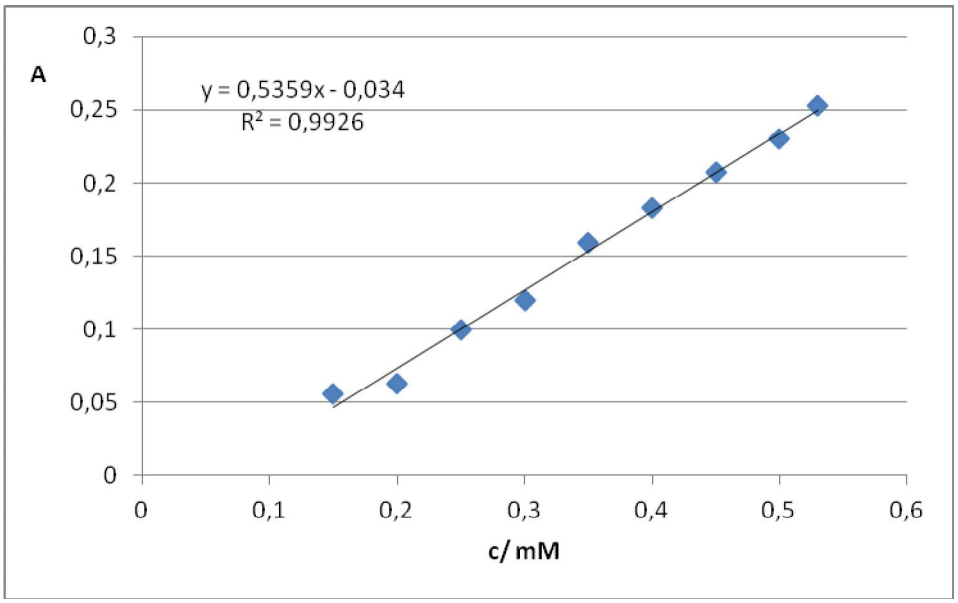


Figure 11.17 Dependence of absorption 2-amino-2-(4-fluorophenyl)acetonitrile on the concentration at 261 nm.

Graphical interpretation of dependence of the absorption on the concentration at 261 nm gave an extinction coefficient of 0.5359 mM^{-1} cm^{-1} according to the Lambert-Beer's law (Figure 11.17).

Immobilized Enzymes Tests

Immobilization of nitrilase 115 on four different types of resin (ECFR, HA 403 S, Purolite D and EC HFA) was carried out by Almac®. Supported enzymes were tested in batch under optimal conditions (10 mM substrate concentration (1 mL), 75 mg of supported enzyme, pH 7, 37 °C) for total of 8 h (Figures 11.18-11.25). It is clear that HA 403 S and ECFR supported enzymes show cleaner chromatogtrams and less starting materials (Figures 11.22-11.25). HPLC chromatograms were obtained using HPLC 2.

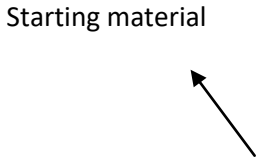
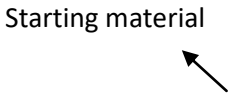


Figure 11.18 Enzymatic reaction using EC HFA-supported enzyme, reaction time 15 min
EC HFA rt 8.499 min, A=709.6; rt 9.244 min, A=432.9; rt 10.107 min, A=531.9.



FFigure 11.19 Enzymatic reaction using EC HFA-supported enzyme, reaction time 8 h
EC HFA rt 9.061 min, A=914.5; rt 9.679 min, A=124.8; rt 9.831 min, A=147.6.

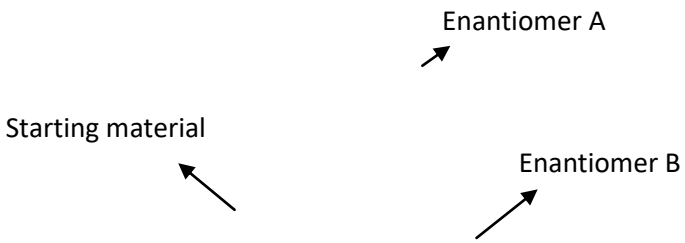


Figure 11.20 Enzymatic reaction using ECFR-supported enzyme, reaction time 15 min
ECFR rt 9.002 min, A=1045.6; rt 9.861 min, A=757.5; additional rt 9.438 min, A=82.1.

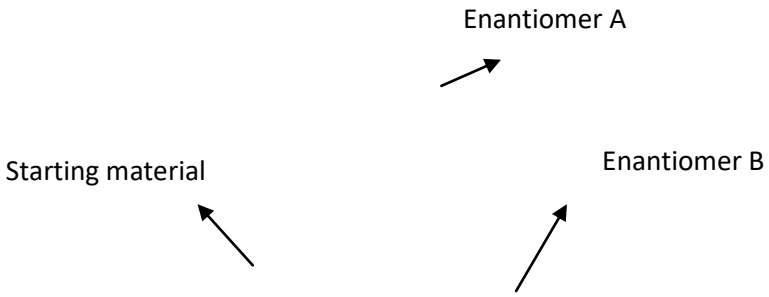
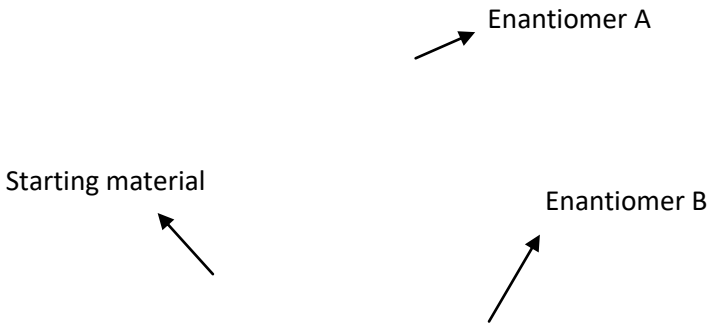


Figure 11.21 Enzymatic reaction using ECFR-supported enzyme, reaction time 8 h
ECFR rt 9.137 min, A=1584.4; rt 9.783 min, A=636.2; rt 10.06 min, A= 374.3.



11.22 Enzymatic reaction using HA 403 S-supported enzyme, reaction time 15 min
HA 403 S rt 9.090 min, A=782.1, rt 9.800 min, A=437.0.

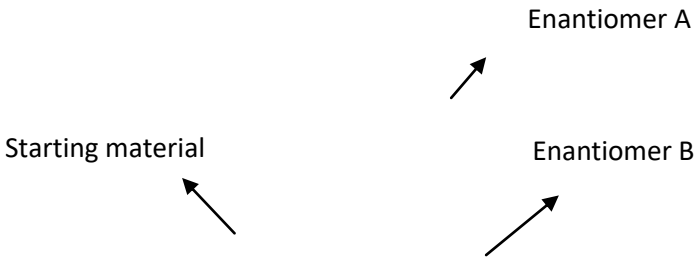


Figure 11.23 Enzymatic reaction using HA 403 S-supported enzyme, reaction time 8 h
HA 403 S rt 9.090 min, A=782.1, rt 9.800 min, A=437.0.

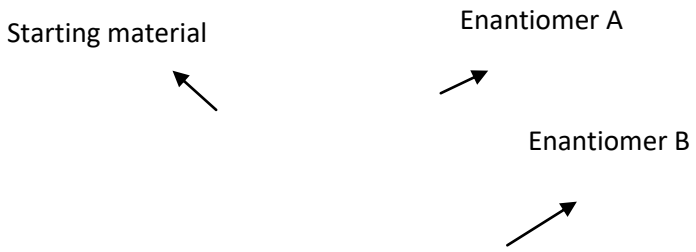


Figure 11.24 Enzymatic reaction using Purolite D-supported enzyme, reaction time 15 min Purolite D, rt 9.214 min, A=1156.6, rt 10.212 min, A=475.5.

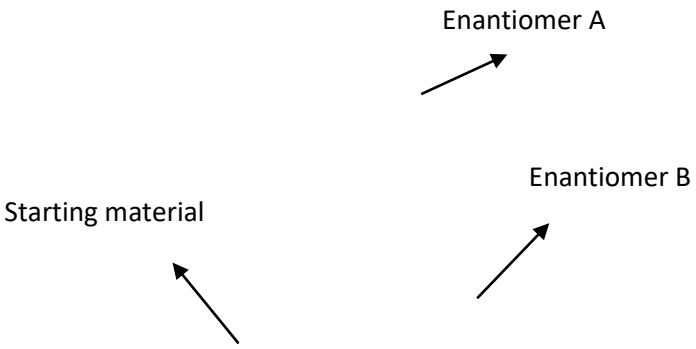


Figure 11.25 Enzymatic reaction using Purolite D-supported enzyme, reaction time 8 h Purolite D, rt 9.098 min, A=1393.7, rt 9.752 min, A=766.4.

Due to the fact that HA 403 S and ECFR supported enzymes showed higher conversion, they were further used to optimize the reaction conditions. Tests ran for optimization of ee and yield for ECFR and HA 403 S are summarized in the Tables 11.26 and 11.27 below.

Table 11.26 Optimization of ee and yield for ECFR supported nitrilase.

ECFR		Yield		Conversion		ee	
		A	B	A	B	A	B
Temperature	37	57	34	41	20	62	38
	34	27	16	41	24	63	37
	40	16[a]	17	22	24	35	37
	50	40	17	27	11	70	30
pH	6	46	33	43	31	58	42
	9	52	36	44	31	59	41
	11	16	8	13	7	65	34
Special conditions	60 mg E/40 °C 150 mg E/ 37°C	57	28	32	20	67	33
		27	22	41	34	55	45

*A enantiomer of the product with shorter retention time, B enantiomer of the product with longer retention time, SM-starting material, E supported enzyme

Table 11.27 Optimization of ee and yield for HA 403 S supported nitrilase.

HA 403 S		Yield		Conversion		ee	
		A	B	A	B	A	B
Temperature	37	32	11	23	44	76	25
	34	23	7	25	8	76	24
	40	21	6	24	7	78	22
	50	10	4	12	5	72	28
pH	6	49	14	33	10	78	22
	9	45	13	34	10	78	22
	11	12	4	12	4	76	24
Special conditions	60 mg E/40 °C 150 mg E/ 37°C	20	6	16	5	77	23
		29	15	4	2	66	34

*A enantiomer of the product with shorter retention time, B enantiomer of the product with longer retention time, SM-starting material, E supported enzyme

Enantiomers of the product 2-Amino-2-(4-fluorophenyl)acetic acid were named A and B in the Tables 11.26 and 11.27 above because their absolute stereochemistry was not determined.

Yields and conversions were determined based on the extinction coefficient that was calculated from UV measurements according to the following equations:

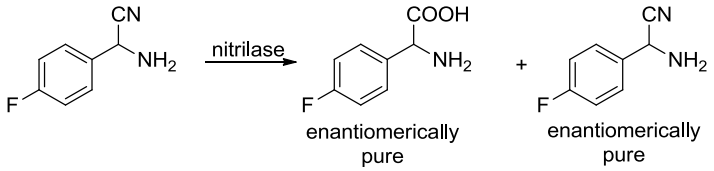
$$Conversion = \frac{\frac{A_A}{\varepsilon_P}}{\frac{A_A}{\varepsilon_P} + \frac{A_B}{\varepsilon_P} + \frac{A_{SM}}{\varepsilon_{SM}}}$$

$$Yield = \frac{\frac{A_{A/B}}{A_P} \cdot c_P}{c_P}$$

A_A = area of enantiomer A of the product, A_B = area of enantiomer B of the product and A_{SM} = area of the starting material, A_P = area of the standard product solution, c_P = concentration of the standard product solution. ε_P = extinction coefficient of the product, ε_{SM} = extinction coefficient of the starting material.

Areas correspond to the areas in HPLC chromatograms.

Enzymatic transformation in flow

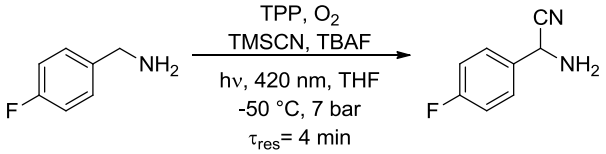


Packed bed reagent described in the Chapter 6. Applied Methods (subchapter 6.8) was used to perform this transformation. Reaction mixture (22.5 mg substrate in the 15 mL of assay) was flowed onto a packed-bed reactor (V = 1.5 mL) at 0.1 mL min^{-1} using a Harvard PHD 2000 (Withdraw / Infuse) syringe pump (t_{res} = 15 min). Column was washed with three column volumes of assay and finally with 1 column volume of MeOH. Four fractions were collected; pre-wash (assay used to wet the column), reaction mixture, wash after the reaction mixture (one column volume), wash after the reaction mixture (two column volumes) and MeOH. Weak signal of the product peak was identified in the ^{19}F NMR in the reaction mixture fraction.

11.10 General Procedure for the Synthesis of *α*-Amino Nitriles Used in Further Hydantoin Synthesis

Details on the flow apparatus (photoreactor) for synthesis of α-amino nitriles can be found in 6. Applied Methods, In House Designed Flow Photoreactor. For the synthesis of amino nitriles used in hydantoin synthesis, mass flow controller Brooks, SLA5800 was used.
Photooxidative cyanation process was optimized for Brooks SLA5800 mass flow controller (See Table 10.22 below).

Table 11.28 Optimization of photooxidative cyanation using Brooks SLA5800 MFC.

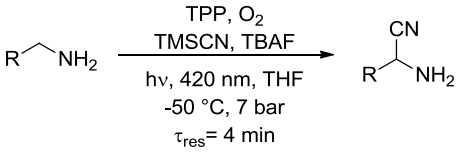


Entry[a]	O_2 flow (ml min^{-1})	Reaction mixture flow (ml min^{-1})	Conversion %
1	2	1	44%
2	4	1	73%
3	6	1	100%

[a] Amine (0.1M in THF), TMSCN (3.5 equiv.), TBAF (0.14 equiv.), TPP (0.02 mol %), O_2, LED 420 nm, –50 °C, 7 bar BPR.

TMSCN (3.5 equiv.) was added to the solution of amine (0.1 M) and TPP (1 mg per 5 mL) in THF, followed by addition of a 1M solution of TBAF in THF (4 mol% based on TMSCN). The resulting solution was mixed with oxygen gas (solution flow rate 1.0 mL min^{-1}) and pumped through the photoreactor (Described in more details in chapter 6. Applied Methods, 6.3 In House Designed Flow Photoreactor). The gas flow rate was adjusted to 6.0 mL min^{-1}. The solvent was removed *in vacuo* and the residue was purified by column chromatography. Amino nitriles synthesized are in the Table 11.29 below. Spectra of the known compounds match the literature data.

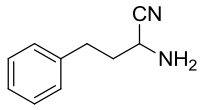
Table 11.29 Photooxidative cyanation of amines



Entry	R
1[a]	4-F-C_6H_4-C
2[b]	C_6H_5-
3	4-$CH_3OC_6H_4$
4[a]	3-$FC_6H_3CH_2$
5[a]	3-$CF_3C_6H_4$
6[b]	$C_6H_5CH_2$
7	$C_6H_5CH_2CH_2$
8[b]	$(CH_3)_3C$
9[b]	$(CH_2)_4$

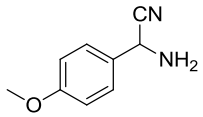
[a] For non-fluorinated substrates 2.5 equiv. of TMSCN was used [b] Spectrum matches the literature data[13-14]

2-amino-4-phenylbutanenitrile



^{1}H NMR (400 MHz, $CDCl_3$): 1.76 (bs, 2H,), 2.07 (q, 2H, *J* = 7.4 Hz), 2.85 (m, 2H), 7.20-7.24 (m, 3H), 7.29-7.33 (m, 2H) ^{13}C (100 MHz, $CDCl_3$): 31.69, 36.92, 42.72, 122.14, 126.61, 128.56, 128.79, 139.88 HRMS (ESI): ($M + H^+$): calcd 161.1000, found 161.1079.

2-amino-2-(4-methoxyphenyl)acetonitrile



^{1}H NMR (400 MHz, $CDCl_3$): 1.98 (bs, 2H), 3.82 (s, 3H), 4.87 (s, 1H), 6.93 (d, *J* = 7.5 Hz, 2H), 7.45 (d, *J* =8.1 Hz, 2H) ^{13}C (100 MHz, $CDCl_3$): 46.89, 55.52, 114.53, 121.26, 128.09, 128.55, 160.18, HRMS (ESI): ($M + H^+$) calcd 163.0793, found 163.0878.

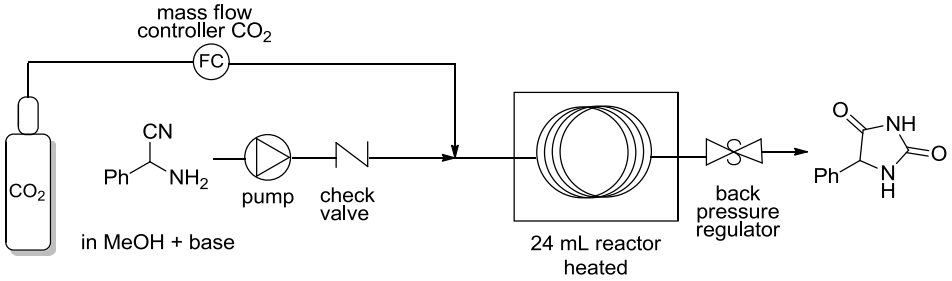
General information for synthesis of hydantoins in flow

Vapourtech R series R2+ was used for continuous flow CO_2 addition reaction.

CO_2 gas used was purchased from Air Liquide®, ALIGAL 2, 99,9 Vol.%, $H_2O \leq 50$ ppm.

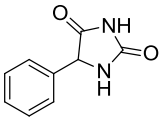
11.11 General Procedure for Synthesis of Hydantoins from α-Amino Nitriles

Hünig's base (3 equiv.) was added to the solution of amino nitrile (0.1 M) in MeOH. The resulting solution (flow rate 1.0 mL min^{-1}) was mixed with carbon dioxide gas (flow rate 6 ml min^{-1}). The solution then entered the 24 ml reactor heated to 80 °C, residence time 20 min (See Scheme 11.30 below, for more details about the designed reactor see chapter 6 Applied Methods, 6.5 Reactor for CO_2 addition to α-amino nitriles). The solvent was removed *in vacuo* and the residue was purified by crystallization from EtOH/ H_2O (1:1).



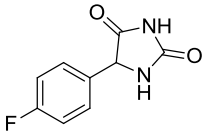
Scheme 11.30 Flow apparatus for synthesis of hydantoins from α-amino nitriles.

5-phenylimidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.97 mL Hünig's base, 19 mL MeOH). Reaction was run in triplicate and the average yield was 74%.
^{1}H NMR (400 MHz, DMSO-d_6): 5.16 (s, 1H, H1), 7.31-7.42 (m, 5H), 8.39 (s, 1H), 10.77 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 61.24, 126.07, 126.7, 128.27, 157.53, 174.22; ^{19}F NMR (376 MHz, DMSO-d_6): -114.18 (tt, 1F, J = 9.1 Hz, 5.4 Hz); HRMS (ESI): (M^+) calcd 176.0586, found: 176.0659.

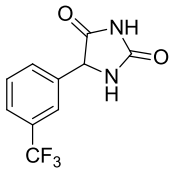
5-(4-fluorophenyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.85 mL Hünig's base, 17 mL MeOH). Reaction was run in duplicate and the average yield is 78%.

^{1}H NMR (400 MHz, DMSO-d_6): 5.19 (s, 1H), 7.20-7.24 (m, 2H), 7.34-7.37 (m, 2H), 8.39 (s, 1H), 10.70 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 60.43, 115.49 (d, J = 21.7 Hz), 127.75 (d, J = 8.3 Hz), 132.23 (d, J = 3.0 Hz), 157.41, 161.97 (d, J = 244.15 Hz), 174.10; ^{19}F NMR (376 MHz, DMSO-d_6): -114.18 (tt, 1F, J = 9.1 Hz, 5.4 Hz); HRMS (ESI): (M + H^+) calcd: 195.0565, found: 195.0570.

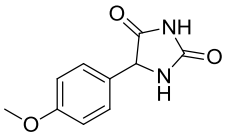
5-(3-(trifluoromethyl)phenyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.64 mL Hünig's base, 13 mL MeOH). Reaction was run in duplicate and the average yield is 76%.

^{1}H NMR (400 MHz, DMSO-d_6): 5.36 (s, 1H), 7.66-7.69 (m, 3H, H4'), 7.73-7.75 (m, 1H), 8.48 (s, 1H), 10.90 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 60.50, 123.24 (q, J = 3.87 Hz, 124.11 (q, J = 272.46 Hz), 125.07 (q, J = 3.87 Hz), 129.29 (q, J = 31.79 Hz), 129.92, 130.74, 137.42, 157.48, 173.66; ^{19}F NMR (376 MHz, DMSO-d_6): -61.14 (s, 3F): HRMS (ESI): (M + Na^+) calcd 267.0352, found 267.2672

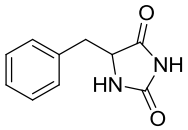
5-(4-methoxyphenyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.79 mL Hünig's base, 16 mL MeOH). Reaction was run in triplicate and the average yield is 38%.

^{1}H NMR (400 MHz, DMSO-d_6): 3.73 (s, 3H), 5.07 (s, 1H), 6.94 (d, 2H, J = 7.8 Hz), 7.22 (d, 2H, J = 8.1 Hz), 8.31 (s, 1H), 10.71 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 55.18, 60.69, 114.05, 127.94, 127.98, 157.42, 159.22, 174.52, HRMS(ESI): (M+H^+): calcd 207.0765, found 207.0763.

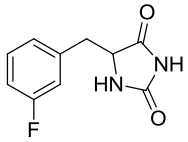
5-benzylimidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.90 mL Hünig's base, 17 mL MeOH). Reaction was run in triplicate and the average yield is 77%.

^{1}H NMR (400 MHz, DMSO-d_6): 2.91-2.93 (m, 2H), 4.31-4.34 (m, 1H), 7.17-7.31 (m, 5H), 7.91 (s, 1H), 10.41 (bs, 1H); ^{13}C (100 MHz, DMSO-d_6): 36.42, 58.39, 126.65, 128.08, 129.73, 135.62, 157.11, 175.18, HRMS (ESI): ($M+H^+$): calcd 191.0816, found 191.0816.

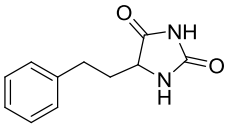
5-(3-fluorobenzyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.88 mL Hünig's base, 17 mL MeOH). Reaction was run in triplicate and the average yield is 76%.

^{1}H NMR (400 MHz, DMSO-d_6): 2.94 (app ddd, 2H, J = 4.8, 4.8, 13.9 Hz), 4.33-4.36 (m, 1H), 6.99-7.08 (m, 3H), 7.29-7.35 (m, 1H), 7.92 (s, 1H), 10.25 (bs, 1H); ^{13}C (100 MHz, DMSO-d_6): 36.12, 58.18, 113.54 (d, J = 20.74 Hz), 116.41 (d, J = 21.07 Hz), 125.86 (d, J = 2.67 Hz), 129.99 (d, J = 8.45 Hz), 138.60 (d, J = 7.65 Hz), 157.13, 161.87 (d, J = 242.8 Hz), 175.09; ^{19}F (DMSO-d_6): -113.95-113.88 (m, 1F); HRMS (ESI): ($M+H^+$) calcd 209.0721, found 209.0725.

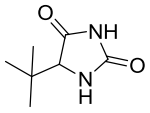
5-phenethylimidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 0.80 mL Hünig's base, 16 mL MeOH). Reaction was run in triplicate and the average yield is 58%.

^{1}H NMR (400 MHz, DMSO-d_6): 1.72-1.82 (m, 1H), 1.89-1.97 (m, 1H), 2.64 (t, 2H, J = 7.9 Hz), 3.93-3.95 (m, 1H), 7.17-7.22 (m, 3H), 7.27-7.31 (m, 2H), 8.11 (s, 1H) 10.64 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 30.44, 33.44, 57.01, 126.02, 128.36, 128.44, 140.87, 157.48, 175.94, HRMS (ESI): ($M+H^+$) calcd: 205.0977, found: 205.0979.

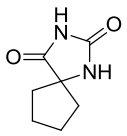
5-(tert-butyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (250 mg substrate, 1.15 mL Hünig's base, 23 mL MeOH). Reaction was run in triplicate and the average yield is 23%.

^{1}H NMR (400 MHz, DMSO-d_6): 0.91 (s, 9H), 3.67 (s, 1H), 7.93 (s, 1H), 10.52 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 23.52, 33.97, 65.88, 157.60, 179.35; HRMS (ESI): ($M+H^+$) calcd 157.0972, found 157.0980.

1,3-diazaspiro[4.4]nonane-2,4-dione

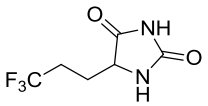


Substrate was synthesized according to the general procedure (250 mg substrate, 1.16 mL Hünig's base, 23 mL MeOH). Reaction was run in triplicate and the average yield is 30%.
^{1}H NMR (400 MHz, DMSO-d_6): 1.65-1.72 (m, 2H), 1.89-1.92 (m, 6H), 8.15 (s, 1H), 10.54 (s, 1H); ^{13}C (100 MHz, DMSO-d_6): 24.67, 37.14, 68.29, 156.28, 179.35.
These data are in agreement with those previously reported in the literature.[15]

11.12 General Procedure for Continuous Flow Process to Access Hydantoins from Amines

TMSCN (3.5 equiv. for fluorinated amines, 2.5 equiv. for non-fluorinated amines) was added to the solution of amine (0.1 M) and TPP (1 mg per 5 mL) in MeTHF, followed by addition of a 1 M solution of TBAF in THF (4 mol% based on TMSCN). The resulting solution was mixed with oxygen gas (solution flow rate 1.0 mL min^{-1}) and pumped through the photoreactor. The gas flow rate was adjusted to 6.0 mL min^{-1}. Hünig's base (3 equiv. with respect to the amine) in MeOH (1:1 volume with photooxidative cyanation crude mixture) at 1 mL min^{-1} was mixed with the crude mixture after photooxidative cyanation in a 50 cm FEP tube. The resulting solution was mixed with carbon dioxide gas (solution flow rate 1.0 mL min^{-1}) and pumped through the 24 mL reactor heated to 90 °C. The gas flow rate was adjusted to 8 mL min^{-1}. The solvent was removed *in vacuo* (water bath heated to 60 °C) and dried under vacuum overnight using an oil bath heated to 60 °C.

5-(3,3,3-trifluoropropyl)imidazolidine-2,4-dione



Substrate was synthesized according to the general procedure (127 mg substrate, 0.438 mL TMSCN, 0.150 mL TBAF (2 M solution in THF, 10 mL MeTHF, 0.510 mL Hünig's base, 10 mL MeOH).[1]H NMR (400 MHz, DMSO-d_6): 1.63-1.73 (m, 1H), 1.81-1.90 (m, 1H), 2.26-2.39 (m, 2H), 4.04-4.08 (m, 1H), 7.99 (s, 1H), 10.71 (bs, 1H); [13]C (100 MHz, DMSO-d_6): 24.58, 29.17 (q, J = 28.4 Hz), 56.51, 127.65 (q, J = 275.8 Hz), 157.70, 175.73; [19] F (376 MHz, DMSO-d_6): -65.03 ppm (t, 3F, J =10.87 Hz); HRMS (ESI): ($M+Na^+$) calcd 219.0358 , found: 219.0369

Bibliography

[1] J. Heier, Free University of Berlin (Berlin), **2010**.
[2] F. Weygand, K. Burger, K. Engelhardt, *Chemische Berichte* **1966**, *99*, 1461.
[3] M. V. Riofski, A. D. Hart, D. A. Colby, *Organic Letters* **2013**, *15*, 208.
[4] B. t. Folléas, I. Marek, J.-F. Normant, L. Saint-Jalmes, *Tetrahedron* **2000**, *56*, 275.
[5] K. G. Rasmussen, K. Anker Jorgensen, *Journal of the Chemical Society, Perkin Transactions 1* **1997**, 1287.
[6] S.-H. Lee, T.-D. Han, K. Yu, K.-H. Ahn, *Bull Korean Chem. Soc* **2001**, *22*, 449.
[7] B.-C. Chen, F. A. Davis, in *Encyclopedia of Reagents for Organic Synthesis*, John Wiley & Sons, Ltd, **2001**.
[8] A. Z. A. Hasaninejad, H. Sharghi, M. Shekouhya, *ARKIVOC* **2008**, *11*, 64.
[9] L. Zhao, C.-J. Li, *Angewandte Chemie International Edition* **2008**, *47*, 7075.
[10] E. B. Robert C. Simon, J. H. Schrittwieser, J. H. Sattler, J. Pietruszka, K. Faber, W. Kroutil, *Chemical Communications* **2014**, *50*, 15669.
[11] D. Landini, M. Penso, *The Journal of Organic Chemistry* **1991**, *56*, 420.
[12] S. Aldrich*, Vol. 2016*, http://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html, **2016**.
[13] S. Vukelić, D. B. Ushakov, K. Gilmore, B. Koksch, P. H. Seeberger, *European Journal of Organic Chemistry* **2015**, *2015*, 3036.
[14] D. B. Ushakov, K. Gilmore, D. Kopetzki, D. T. McQuade, P. H. Seeberger, *Angewandte Chemie International Edition* **2014**, *53*, 557.
[15] I. A. Rivero, E. A. Reynoso-Soto, A. Ochoa-Terán, *Arkivoc* **2011**, *2*, 260.

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