

Supporting Information for:

*perfect*BASH: Band-selective homo-nuclear decoupling in peptides and peptidomimetics

Julian Ilgen¹, Lukas Kaltschnee^{1,2}, Christina M. Thiele^{1,*}

1: Clemens-Schöpf-Institut für Organische Chemie und Biochemie, Technische Universität Darmstadt, Alarich-Weiss-Straße 16, D-64287 Darmstadt, Germany, cthiele@thielelab.de

2: current address: Max-Planck-Institut für Biophysikalische Chemie, Am Faßberg 11, D-37077 Göttingen, Germany

Table of Contents

1	Experiments on the Elastin fragment GVG(VPGVG) ₃	3
1.1	Elastin sample and experimental setups	3
1.2	Conventional ¹ H spectra	3
1.3	¹ H-experiments with <i>perfectBASH</i> band selective decoupling	3
1.4	<i>perfectBASH</i> spectra of the elastin fragment GVG(VPGVG) ₃	5
2	Cyclosporine A	8
2.1	NOESY vs. EASY-ROESY	8
2.2	Cyclosporine A spin system used for simulations	9
2.3	Pulse design	11
2.4	Simulated and experimental spectra	12
2.5	Simulated spectra with further tested pulse shapes	14
3	Experiments on the hexameric oligourea sample	16
3.1	Experimental section	16
3.1.1	1D ¹ H-HOBS	16
3.1.2	Interferogram-based acquisition	16
3.2	Resulting spectra	17
4	F1- <i>perfectBASH</i> CLIP-COSY and relayed-CLIP-COSY	20
5	Bruker pulse sequence codes	22
5.1	1D ¹ H-experiment with <i>perfectBASH</i> decoupling	22
5.2	RESET-compatible 1D- <i>perfectBASH</i> sequence	26
5.3	1D ¹ H-HOBS sequence	30
5.4	2D TOCSY with F1- <i>perfectBASH</i> homonuclear decoupling	33
5.5	2D NOESY with F1- <i>perfectBASH</i> homonuclear decoupling	38
5.6	2D EASY-ROESY with F1- <i>perfectBASH</i> homonuclear decoupling	42
5.7	2D CLIP-COSY with F1- <i>perfectBASH</i> homonuclear decoupling	47
5.8	2D CLIP-COSY relayed (n=2) with F1- <i>perfectBASH</i> homonuclear decoupling	51
6	References	55

1 Experiments on the Elastin fragment GVG(VPGVG)₃

1.1 Elastin sample and experimental setups

The sample contains the elastin fragment GVG(VPGVG)₃ (10% w/w) dissolved in a 50mM sodium phosphate buffer pH 7 in H₂O/D₂O 80:20[1].

Experiments were done on a Bruker Avance III system with 600.3 MHz proton base frequency, equipped with a 5 mm triple-resonance broadband inverse probe (¹H, ²H, ³¹P, BB) with z-gradient. The maximum z-gradient strength was experimentally determined to be $(0.494 \pm 0.007) \text{ T m}^{-1}$ using a stimulated echo experiment on a doped water sample[2].

A 90° proton pulse with duration of 11.1 μs was used.

Gradients used for coherence selection had durations of 1 ms and smoothed square shape (SMSQ) digitized using 100 points. All gradients strengths are given as a fraction of the maximum amplitude and were followed by a recovery delay of 200 μs.

All bandwidths given for the pulse shapes used for frequency selective refocusing were calculated with the Bruker Shape Tool of TopSpin 3.2 using the “*Calculate Bandwidth for Refocusing –My*” option. The numbers given are calculated by default for 70% of the maximum refocusing profile. Note, however, that at least 95% of the maximum refocusing profile is recommended to obtain clean homonuclear decoupled spectra using the *perfectBASH* scheme. The pulses used herein were adjusted to fulfill this criterion.

1.2 Conventional ¹H spectra

The ¹H spectrum in Figure 1-1a) was acquired using the *zgesgp* pulse programs from the Bruker pulse sequence library. Excitation sculpting was executed with 2.5 ms long soft rectangular pulses (Squa100.1000) with 4.68 ppm offset and flanked in each case by gradients with 31% and 11% strength, respectively.

1.3 ¹H-experiments with *perfectBASH* band selective decoupling

1D ¹H-*perfectBASH* experiments were acquired with the pulse sequence in the SI chapter 5.1 including an option for solvent presaturation during the relaxation delay. In all experiments this option was checked to attenuate the strong water signal. Water was presaturated for 1 s, the offset was set on 4.697 ppm and the RF-power was $4.5 \cdot 10^{-5} \text{ W}$. All spectra were measured at 300 K.

The spectra were collected with 10 kHz spectral width and the interferogram based acquisition mode using 32 data-chunks of 20 ms duration.

Several ^1H -*perfect*BASH spectra of the elastin sample were acquired applying different selective refocusing pulses. For the spectra in Figure 1-1 c), Figure 1-2 b), Figure 1-3 b) and Figure 1-4 a) a 2.6 ms long twofold phase-modulated RSnob pulse with two offsets was used. The phase-modulated pulse, digitized in 10000 points with two offset frequencies at 0 Hz (=SPOFFS) and 2500 Hz (=SPOFFS + 2500 Hz) was created using Bruker ShapeTool of TopSpin 3.2 with the “*Multiple Phase Modulation*” option. The quality of the frequency selective refocusing was checked with a gradient selected selective spin echo (*selgpse*) and is shown in Figure 1-1 b). The offset of the selective pulse was set to 3.99 ppm.

The spectra in Figure 1-2 a) and Figure 1-4 b) were acquired using a 1.6 ms long ReBurp pulse (3600 Hz bandwidth, 5.98 ppm offset) and a 4.5 ms long ReBurp pulse (1290 Hz bandwidth, 3.92 ppm offset), respectively. Coherence selection was enforced in all experiments with gradients of $G_1=33\%$ strength in the first echo block and $G_2=82\%$ in the second echo block respectively. 8 transients per data-chunk were accumulated and a relaxation delay of 2 s was used.

For FID reconstruction the *pshift* AU from the Manchester NMR Methodology Group web pages (<http://nmr.chemistry.manchester.ac.uk>) was used yielding a 646.8 ms FID, which was zero filled to 32768 complex points and multiplied with an exponential apodization function (1 Hz line broadening) before Fourier transformation.

1.4 *perfectBASH* spectra of the elastin fragment GVG(VPGVG)₃

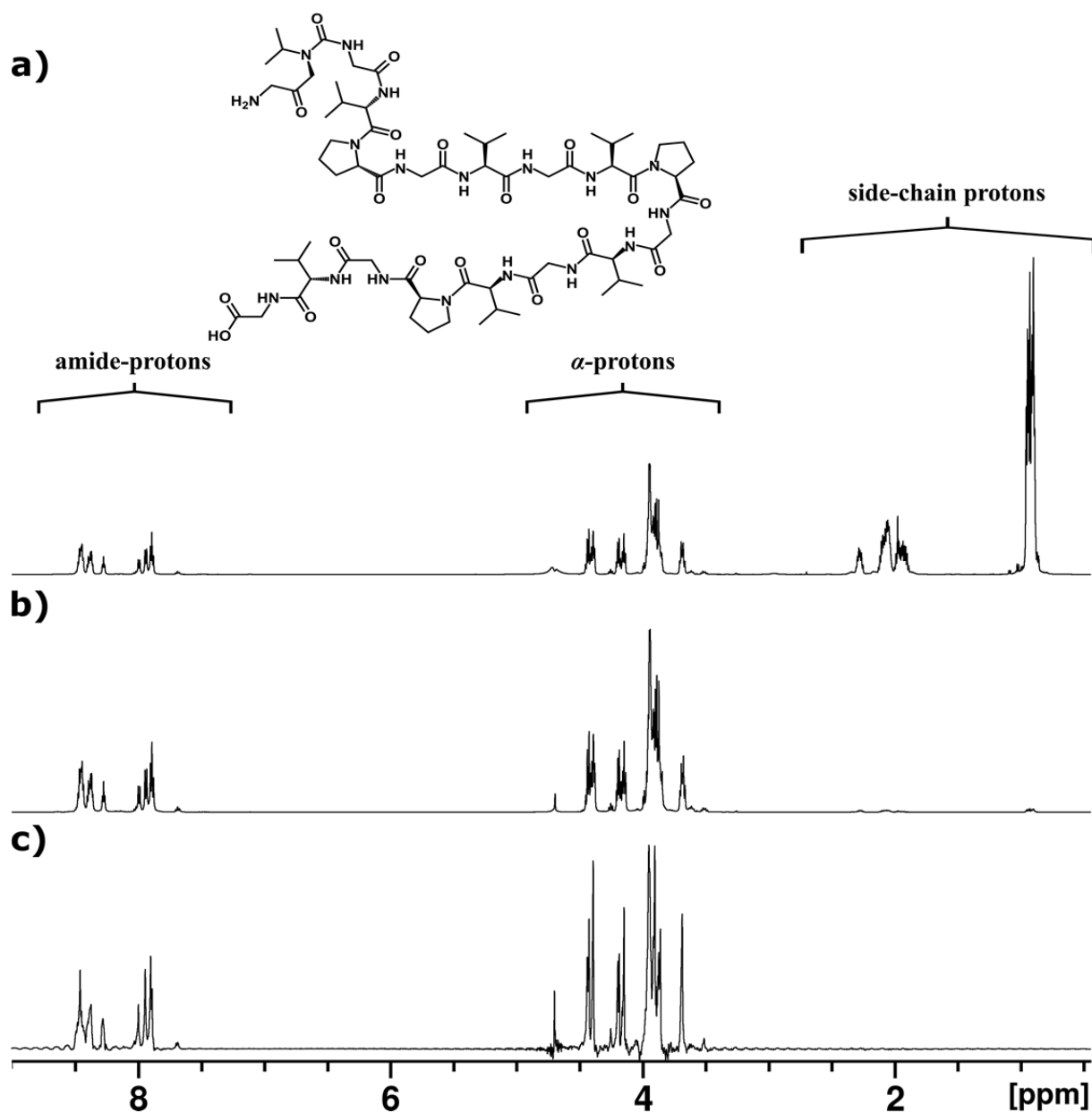


Figure 1-1: Proton spectra of the elastin fragment GVG(VPGVG)₃ obtained with (a) conventional ¹H (600.3 MHz proton base frequency), (b) a gradient selected selective spin echo using a 2.6 ms twofold phase-modulated RSnob refocusing pulse and (c) 1D ¹H-*perfectBASH* using a 2.6 ms two-fold phase-modulated RSnob refocusing pulse.

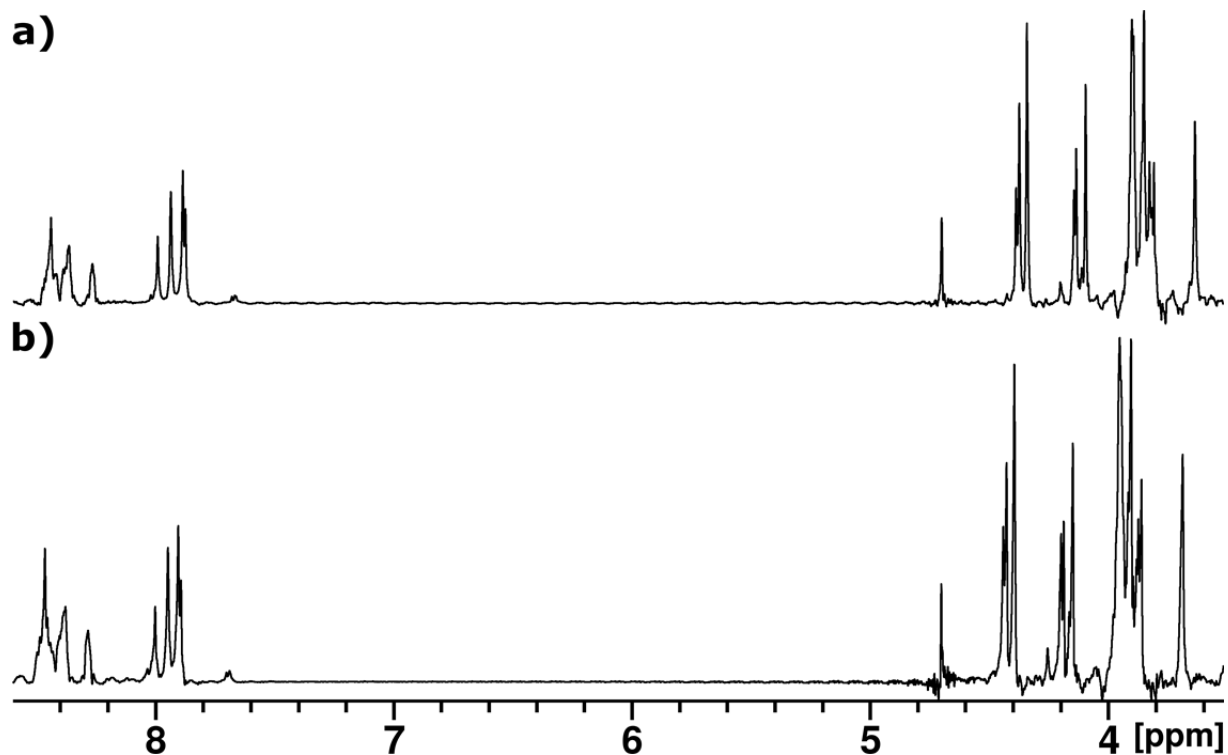


Figure 1-2: 1D ^1H -*perfectBASH* spectra of the elastin fragment $\text{GVG}(\text{VPGVG})_3$ obtained with (a) a 1.6 ms ReBurp refocusing pulse and (b) a 2.6 ms two-fold phase-modulated RSnob refocusing pulse. All spectra were obtained with 600.3 MHz proton base frequency.

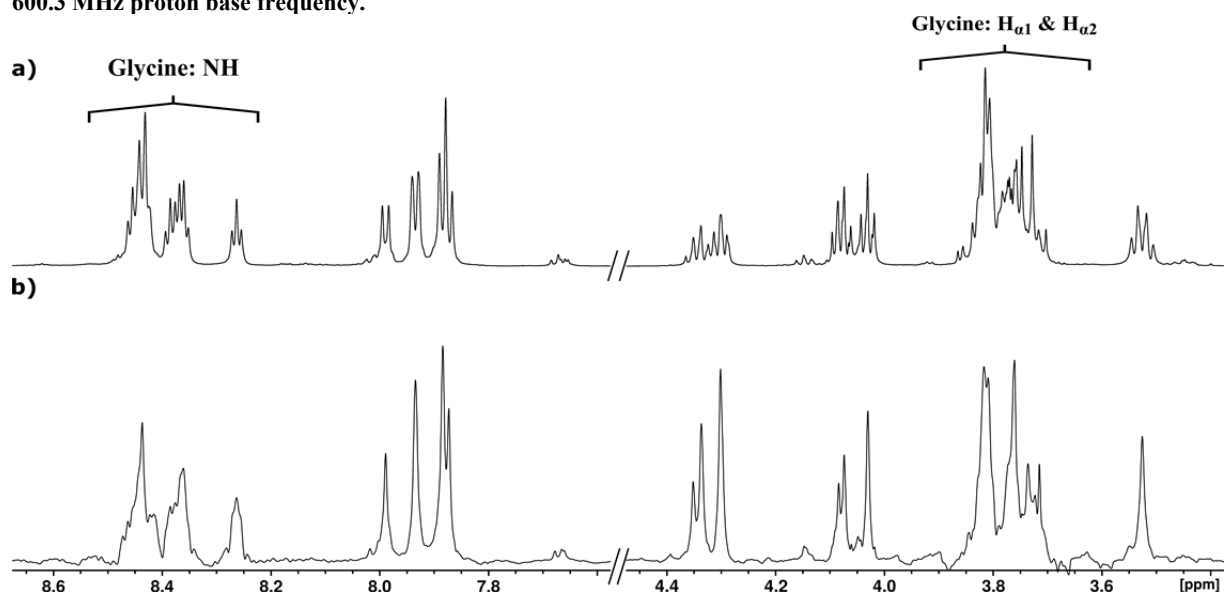


Figure 1-3: Expansions of the amide- and α -proton region of (a) the conventional ^1H -spectrum (600.3 MHz proton base frequency) and (b) the 1D ^1H -*perfectBASH* spectrum, illustrating the incomplete homonuclear decoupling of the amide- and α -protons of glycine.

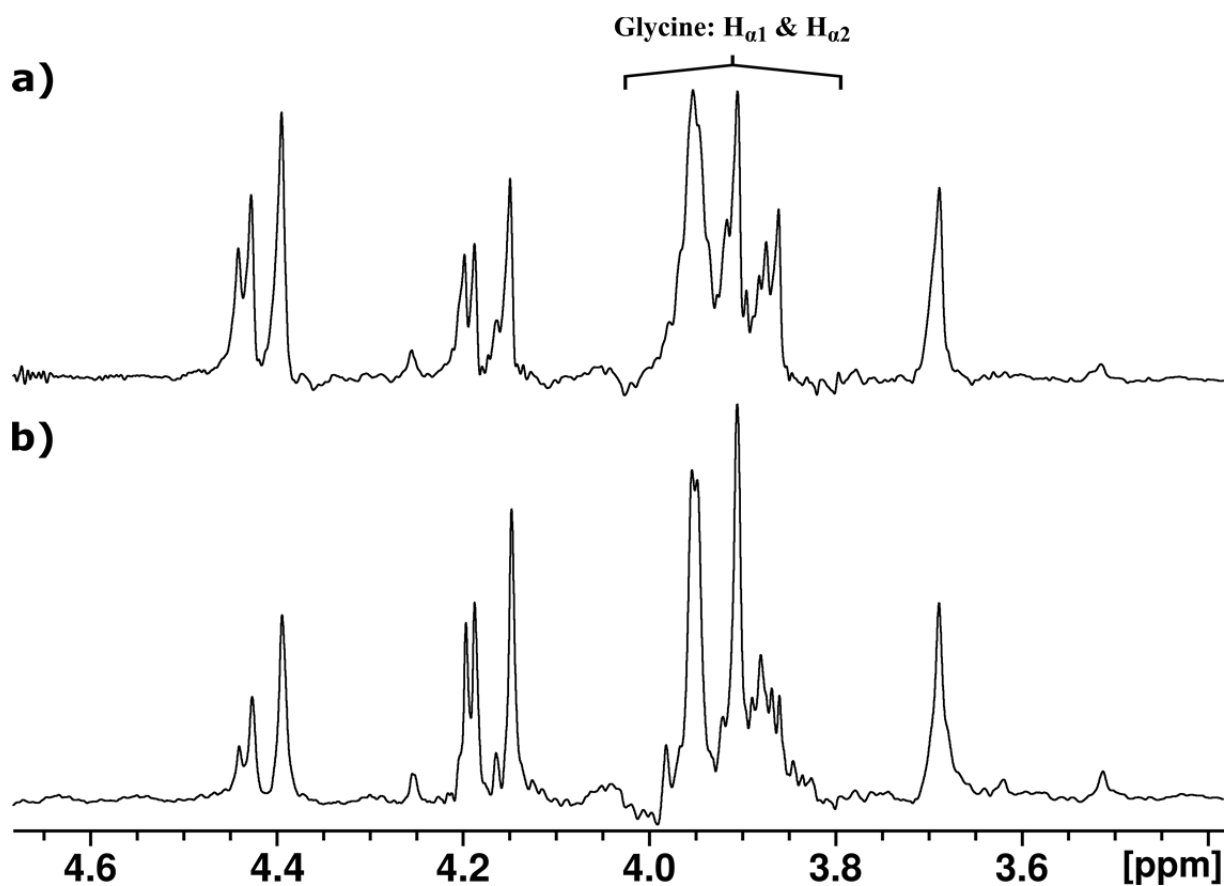


Figure 1-4: 1D ^1H -*perfectBASH* spectra obtained with (a) a 2.6 ms twofold phase-modulated RSnob refocusing pulse and (b) a 4.5 ms ReBurrp refocusing pulse (1290 Hz bandwidth, 3.92 ppm offset). All spectra were obtained with 600.3 MHz proton base frequency. In the spectrum (a) the amide- as well as the α -proton region is band selectively homonuclear decoupled, hence the diastereotopic and mutually coupled α -protons of the glycine amino acids are not fully decoupled. In spectrum (b) only the α -proton region is band selectively homonuclear decoupled using the *perfectBASH* sequence. The mutually coupled α -protons of glycine should be decoupled in this case and should appear as singlets. However the quality of the spectrum is only marginally improved due to the clustered and strongly coupled nature of the glycine α -protons.

2 Cyclosporine A

2.1 NOESY vs. EASY-ROESY

Under the chosen measurement conditions (700.17 MHz and 300 K) the longitudinal nuclear overhauser effect of the cyclosporine A sample seems to be near the zero crossing. This is illustrated with a zero-quantum filtered NOESY (*noesygpphs*) spectrum (Figure 2-1), which shows only little cross peaks with comparably low intensity. In contrast, the EASY-ROESY (*roesyadjsphpr*) spectrum (Figure 2-2) shows significantly more cross peaks.

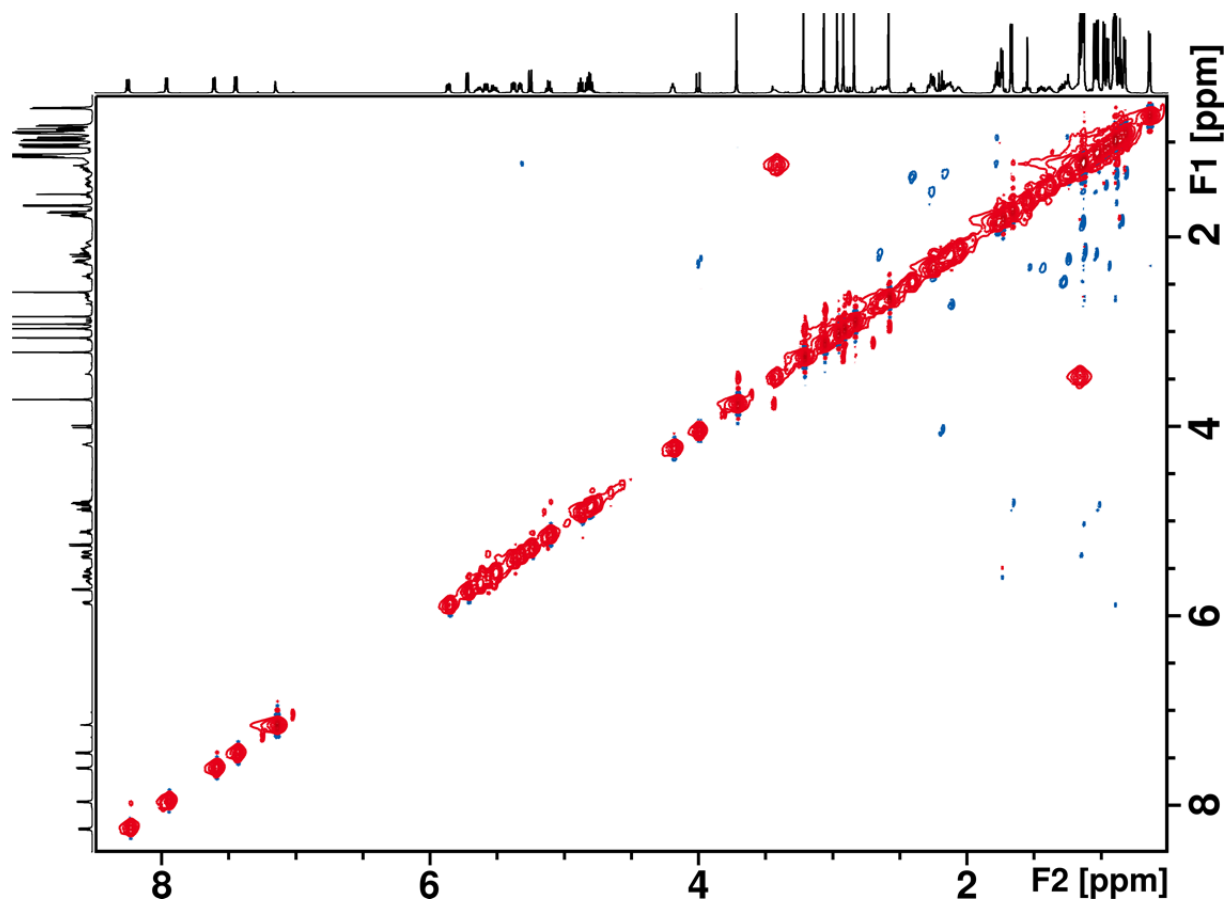


Figure 2-1: Zero-Quantum filtered NOESY of cyclosporine A in benzene-*d*₆, obtained at 700.17 MHz and 300 ms mixing time.

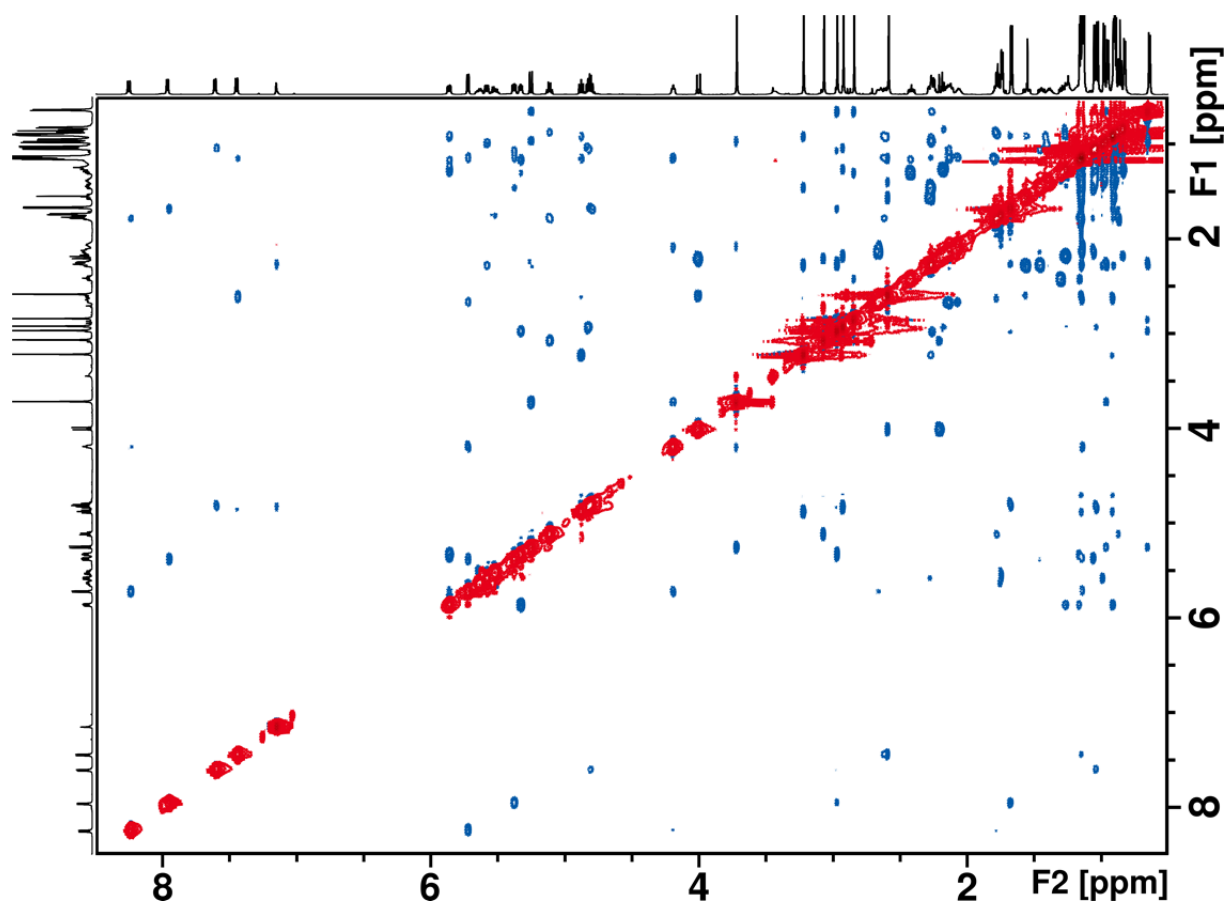


Figure 2-2: EASY-ROESY of cyclosporine A in benzene- d_6 , obtained at 700.17 MHz and 300 ms mixing-time (45° spin-lock angle, 5000 Hz RF-amplitude).

2.2 Cyclosporine A spin system used for simulations

The reduced spin system used for the simulations of cyclosporine A only contains the amide-, α -, N -methyl- and some side chain protons with chemical shifts close to the α -proton region. Chemical shifts were extracted from a 1D ^1H -PSYCHE[3] spectrum and a ^1H , ^{13}C -HSQC (*hsqcetgpsp.2*).

J -coupling constants were determined from the conventional proton spectrum. To treat the effects of relaxation during the experiment at least phenomenologically, T_2 -rates were estimated for the sites considered, and also fed into the simulation program. For a rough estimate of T_2 , we assumed that $T_2 \approx T_1$ be valid. We are well aware, that this is by no means a rigorous treatment of the effects of relaxation during the pulse sequence. The T_1 -relaxation times were determined with a series of inversion recovery experiments (*t1ir*), using seven delays (5 ms, 10 ms, 50 ms, 500 ms, 1.25 s, 4 s and 30 s) and subsequent fitting of the integrals. The spectral parameters for the spin system input file are listed in the table below. A simulated proton spectrum of the reduced spin system is shown in Figure 2-3.

Proton assignment		Chemical Shift δ / ppm	J -coupling constant ${}^nJ_{ij}$ / Hz	Relaxation times $T_1 \approx T_2$ / ms
1 MeBmt	α	5.7216	${}^3J_{\alpha,\beta} = 7.61$	587.4
	β	4.1917		584.7
	NMe	3.7166	--	690.0
	ϵ	5.6394	${}^3J_{\epsilon,\zeta} = 14.9$	1154.0
	ζ	5.5211		1343.0
2 Abu	α	5.1125	${}^3J_{\alpha,\text{NH}} = 9.76$	880.6
	NH	8.2483		479.0
3 Sar	α	4.0027	--	415.3
	NMe	3.0664		851.7
4 MeLeu	α	5.5827	--	842.0
	NMe	2.9671		743.3
5 Val	α	4.8763	${}^3J_{\alpha,\beta} = 9.92, {}^3J_{\alpha,\text{NH}} = 8.66$	827.0
	β	2.6128		504.7
	NH	7.4459		599.6
6 MeLeu	α	5.3782	--	549.0
	NMe	3.2182		728.5
7 Ala	α	4.8011	${}^3J_{\alpha,\text{NH}} = 7.30$	1063.0
	NH	7.9611		471.0
8 Ala	α	4.8251	${}^3J_{\alpha,\text{NH}} = 7.81$	1063.0
	NH	7.6066		687.0
9 MeLeu	α	5.8634	--	421.9
	NMe	2.9212		790.2
10 MeLeu	α	5.3298	--	416.0
	NMe	2.8419		907.8
11 MeVal	α	5.2528	--	794.5
	NMe	2.5853		685.0

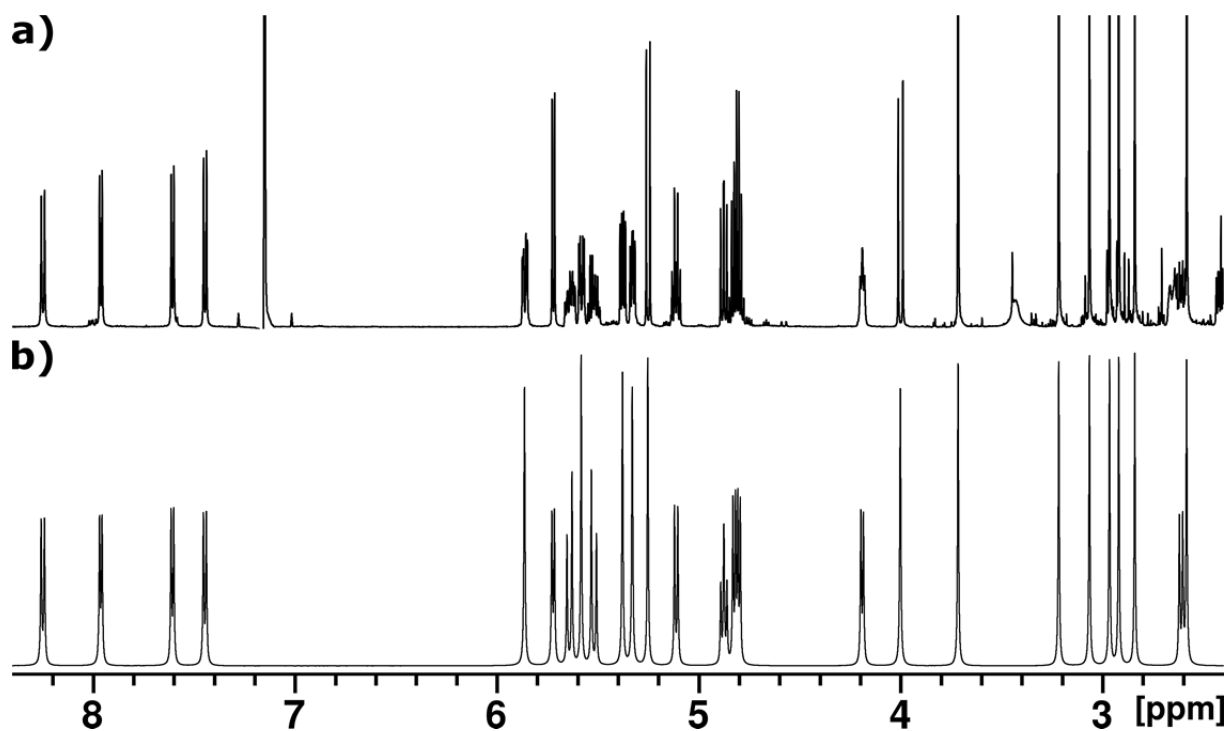


Figure 2-3: Proton spectra of cyclosporine A in benzene-*d*₆ obtained (a) experimentally (600.3 MHz proton base frequency, 300 K) and (b) via simulation using the Bruker NMRsim package and the reduced cyclosporine A system described above.

2.3 Pulse design

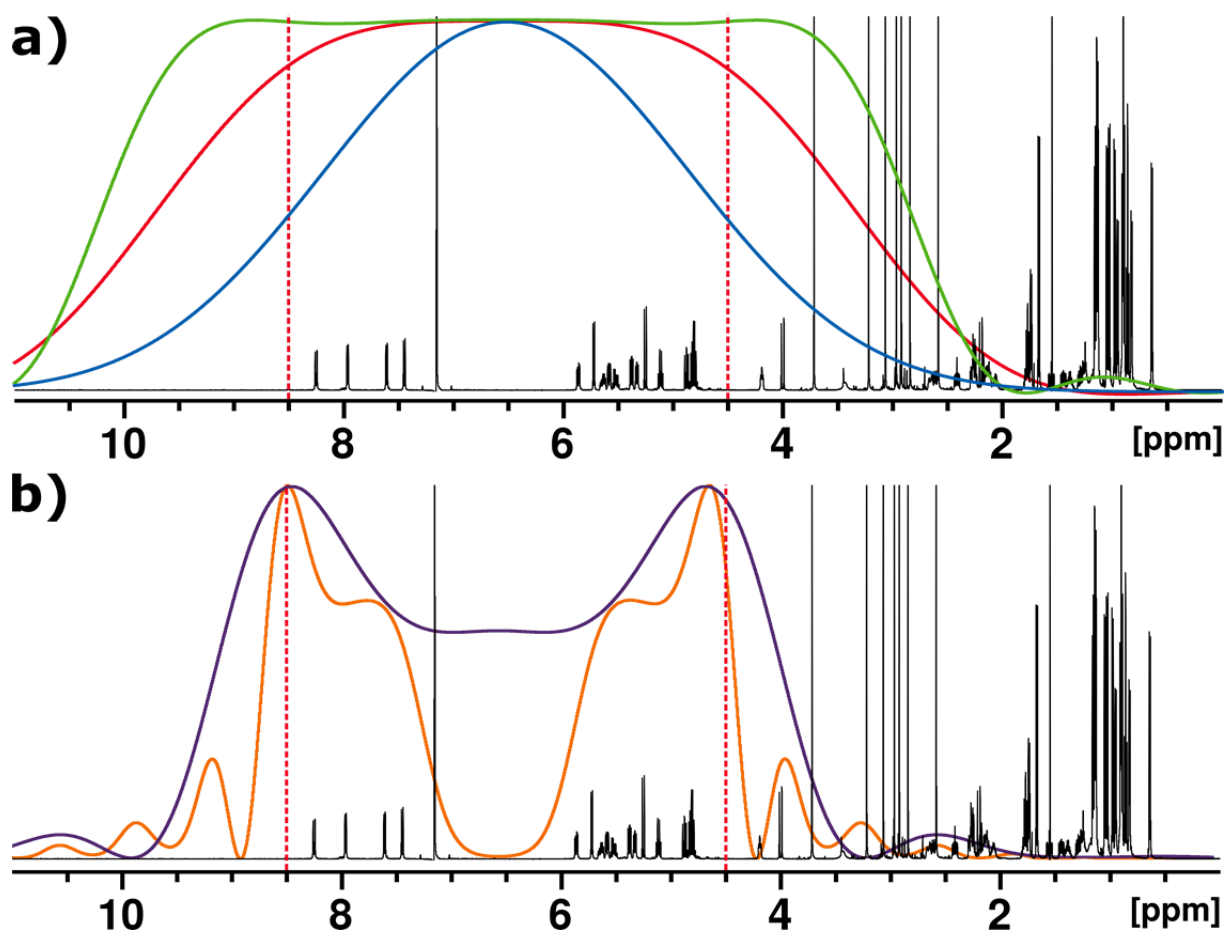


Figure 2-4: Simulated refocusing profiles of tested selective refocusing pulses potentially usable for *perfectBASH* band selective homonuclear decoupling of the amide- and α -protons in cyclosporine A. In (a) profiles for a 400 μ s Gaussian refocusing pulse (blue), a 800 μ s RSnob refocusing pulse (red) and 1.49 ms ReBurp (green). In (b) simulated refocusing profile for a two-fold phase modulated RSnob-refocusing pulse (purple) or ReBurp-refocusing pulse (orange) with two different offsets at 5.32 ppm in the α -H-region and 7.79 ppm in the amide-region of cyclosporine A. For better clarity, the profiles were calculated for inversion, but do not differ substantially from the case of refocusing. The profiles were simulated for 600.3 MHz proton base frequency.

2.4 Simulated and experimental spectra

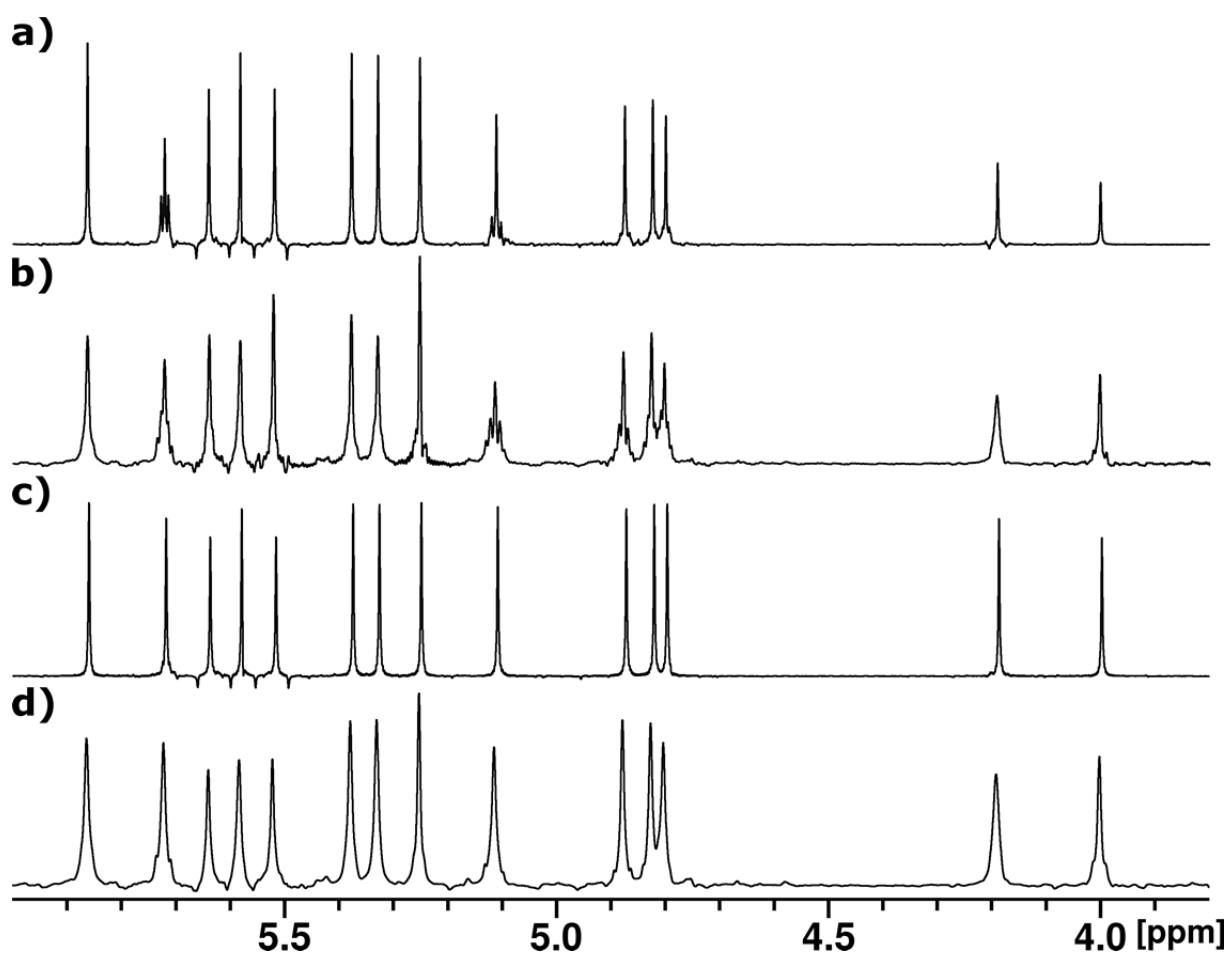


Figure 2-5: Comparison between experimental (b and d) and simulated (a and c) 1D ^1H -perfectBASH spectra of the α -proton region of cyclosporine A. In (a) and (b) a $800\ \mu\text{s}$ RSnob refocusing pulse (2920 Hz bandwidth, 6.52 ppm offset) was used for selective refocusing, whereas in (c) and (d) a 1.49 ms ReBurp refocusing pulse (3900 Hz bandwidth, 6.52 ppm offset) was applied. All spectra were obtained with 600.3 MHz proton base frequency.

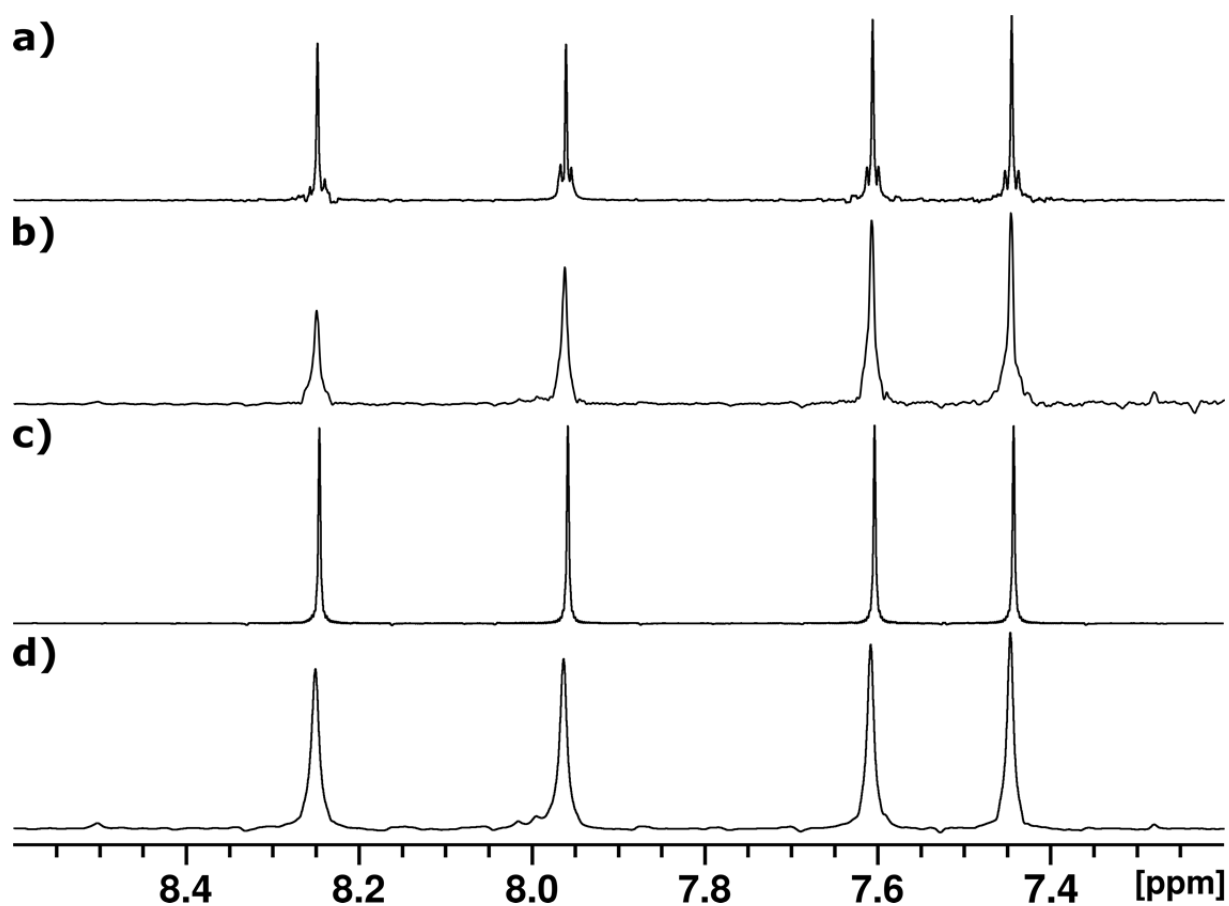


Figure 2-6: Comparison between experimental (b and d) and simulated (a and c) 1D ^1H -*perfect*BASH spectra of the amide-proton region of cyclosporine A. In (a) and (b) a $800\ \mu\text{s}$ RSnob refocusing pulse (2920 Hz bandwidth, 6.52 ppm offset) was used for selective refocusing, whereas in (c) and (d) a 1.49 ms ReBurp refocusing pulse (3900 Hz bandwidth, 6.52 ppm offset) was applied. All spectra were obtained with 600.3 MHz proton base frequency.

2.5 Simulated spectra with further tested pulse shapes

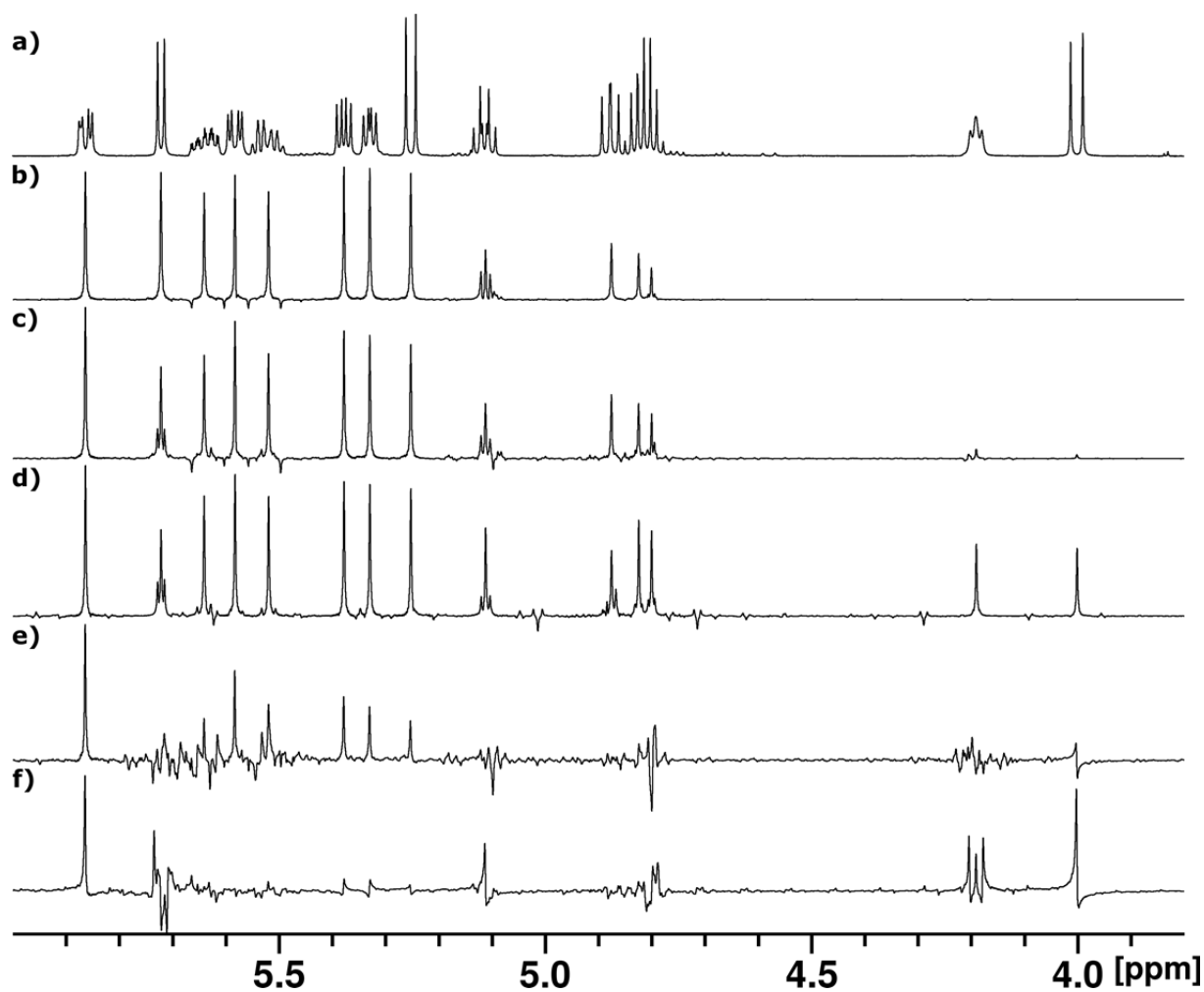


Figure 2-7: 1D ^1H -spectrum (a) and simulated (b – f) 1D ^1H -perfectBASH spectra of the α -proton region of cyclosporine A. The spectra were obtained using (b) a 2.6 ms ReBurp refocusing pulse (2236 Hz bandwidth), (c) a 1.1 ms RSnob refocusing pulse (2120 Hz bandwidth), (d) a 400 μs Gaussian refocusing pulse (2205 Hz bandwidth), (e) a 1.5 ms twofold phase-modulated RSnob pulse with two offsets at 5.32 ppm (α -proton region) and 7.79 ppm (amide-proton region) and (f) a 5 ms twofold phase-modulated ReBurp pulse with two offsets at 5.32 ppm (α -proton region) and 7.79 ppm (amide-proton region). The refocusing profiles of the last two pulses are shown in Figure 2-4b. All spectra were obtained with 600.3 MHz proton base frequency.

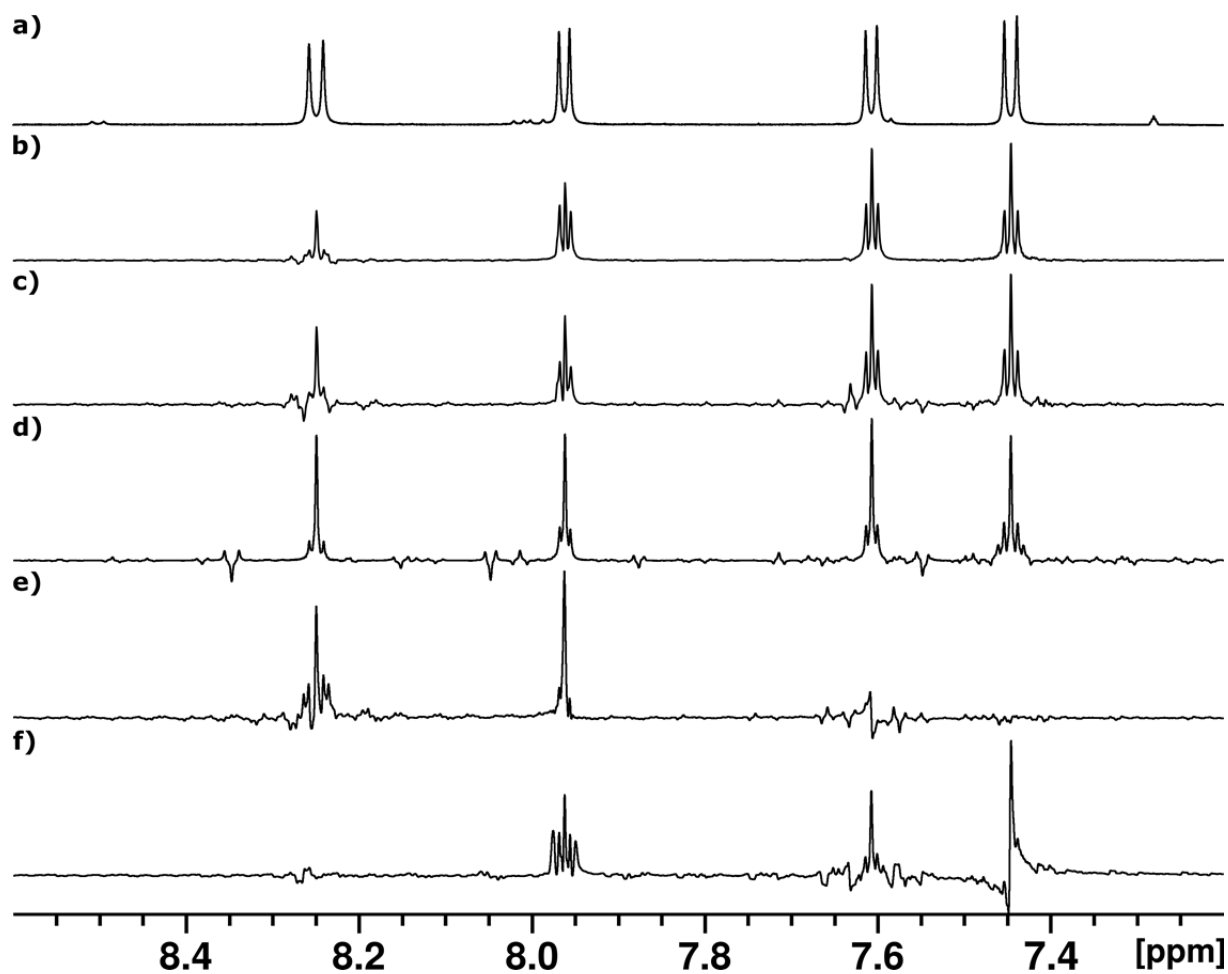


Figure 2-8: 1D ¹H-spectrum (a) and simulated (b – f) 1D ¹H-*perfect*BASH spectra of the amide-proton region of cyclosporine A. The spectra were obtained using (b) a 2.6 ms ReBurp refocusing pulse (2236 Hz bandwidth), (c) a 1.1 ms RSnob refocusing pulse (2120 Hz bandwidth), (d) a 400 μs Gaussian refocusing pulse (2205 Hz bandwidth), (e) a 1.5 ms twofold phase-modulated RSnob pulse with two offsets at 5.32 ppm (α -proton region) and 7.79 ppm (amide-proton region) and (f) a 5 ms twofold phase-modulated ReBurp pulse with two offsets at 5.32 ppm (α -proton region) and 7.79 ppm (amide-proton region). The refocusing profiles of the last two pulses are shown in Figure 2-4b. All spectra were obtained with 600.3 MHz proton base frequency.

3 Experiments on the hexameric oligourea sample

3.1 Experimental section

Experiments were done on a Bruker Avance III with 700.17 MHz proton base frequency, equipped with a QCI probe (^1H , ^{13}C , ^{19}F , ^{15}N) with z-gradient (0.53 T m^{-1} maximum gradient strength). Sample temperature was regulated at 320 K using a BCU-Xtreme unit. Temperature calibration was performed using an ethyleneglycol- d_6 sample. The 90° proton pulse duration was $6.99 \mu\text{s}$.

Gradients used for coherence selection had durations of 1 ms and smoothed square shape (SMSQ) digitized using 100 points. All gradients strengths are given as a fraction of the maximum amplitude and were followed by a recovery delay of $200 \mu\text{s}$.

The band selective homonuclear decoupled spectra of the β -proton and α_I -proton region independently were acquired with the 1D ^1H -HOBS scheme and with a band selective Zangger-Sterk type interferogram-based acquisition[4].

3.1.1 1D ^1H -HOBS

1D ^1H -HOBS spectra were acquired using the 1D ^1H -HOBS sequence in chapter 5.3. The strong water signal was presaturated with continuous low power irradiation lasting for 1 s, the offset was set on 4.61 ppm.

Both spectra were collected with 5 kHz spectral width and 12798 complex points (640 ms acquisition time). For HOBS decoupling during acquisition the FID was cut into 32 blocks of 20 ms duration separated by the decoupling pulses. The band selective decoupling of the β -proton region (Figure 3-2b) was performed with a ReBurp refocusing pulse with a duration of 17 ms (345 Hz bandwidth) and 4.67 ppm offset. The α_I -proton region (Figure 3-3b) was decoupled using a 15 ms ReBurp refocusing pulse (390 Hz bandwidth) with 4.12 ppm offset.

Gradients for coherence selection in the selective refocusing element (G_1) before acquisition had durations of 1 ms and 17% gradient strength, the gradients during real-time decoupling had durations of $500 \mu\text{s}$ and strengths of $G_2=7 \%$ and $G_3=5 \%$, respectively.

32 transients were accumulated and a relaxation delay of 2 s was used. The acquired FIDs were zero filled to 65536 complex points and multiplied with an exponential apodization function (1 Hz line broadening) before Fourier transformation.

3.1.2 Interferogram-based acquisition

The Zangger-Sterk type interferogram-based acquisition was performed with the Zangger-Sterk pulse sequence used in the PEPSIE paper[5]. The FID was acquired in 32 data-chunks with duration of 20 ms each. Band selective decoupling of the β -proton region (Figure 3-2c) was performed with a ReBurp refocusing pulse with a duration of 17 ms (345 Hz bandwidth) and 4.67 ppm offset. The α_I -proton region (Figure 3-3c) was decoupled using a 15 ms ReBurp refocusing pulse (390 Hz bandwidth) with

4.12 ppm offset. The gradient for coherence selection had gradient strength of 83%, the slice-selection gradient was switched off. 32 transients were accumulated and a relaxation delay of 2 s was used. For FID reconstruction the *pshift* AU from the Manchester NMR Methodology Group web pages (<http://nmr.chemistry.manchester.ac.uk>) was used yielding a 646.8 ms FID, which was zero filled to 65536 complex points and multiplied with an exponential apodization function (0.3 Hz line broadening) before Fourier transformation.

3.2 Resulting spectra

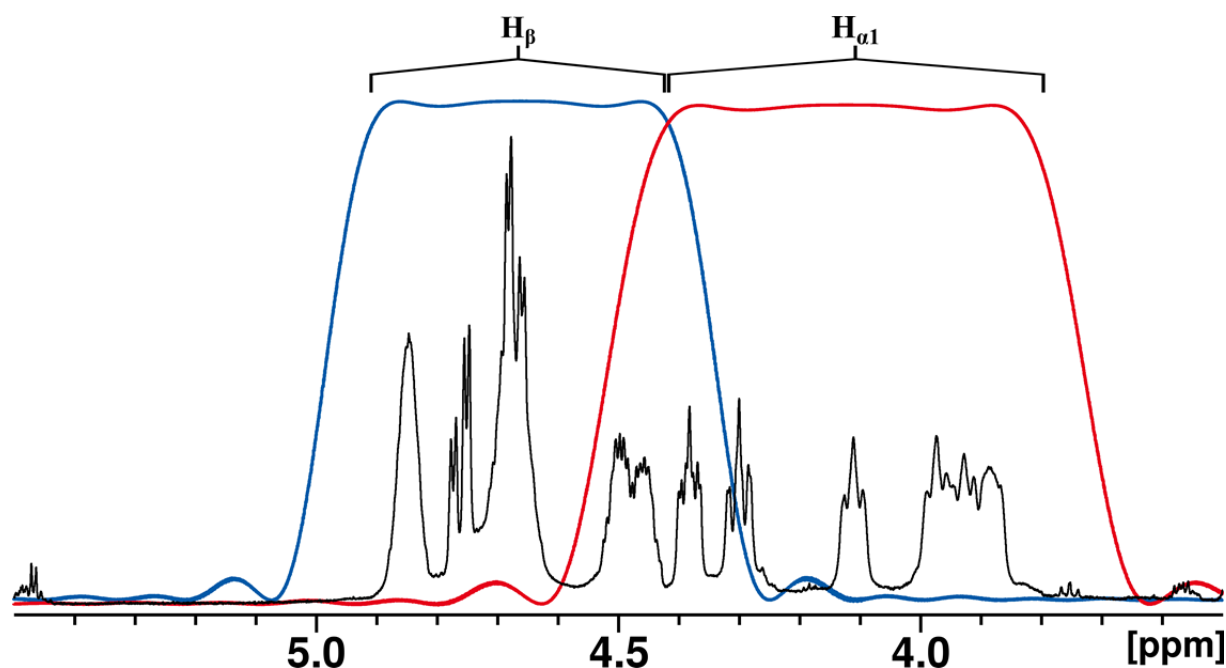


Figure 3-1: Simulated refocusing profiles of a 17 ms ReBurp refocusing pulse (345 Hz bandwidth) with 4.67 ppm offset for band selective decoupling of the β -proton region (blue profile) and of a 15 ms ReBurp refocusing pulse (390 Hz bandwidth) with 4.12 ppm offset for band selective decoupling of the α_1 -proton region (red profile). Probably there is partial co-refocusing of the other proton group (β - and α_1 -protons, respectively) at the edges of the refocusing profile. For better clarity, the profiles were calculated for inversion, but do not differ substantially from the case of refocusing. The profiles were simulated for 700.17 MHz proton base frequency.

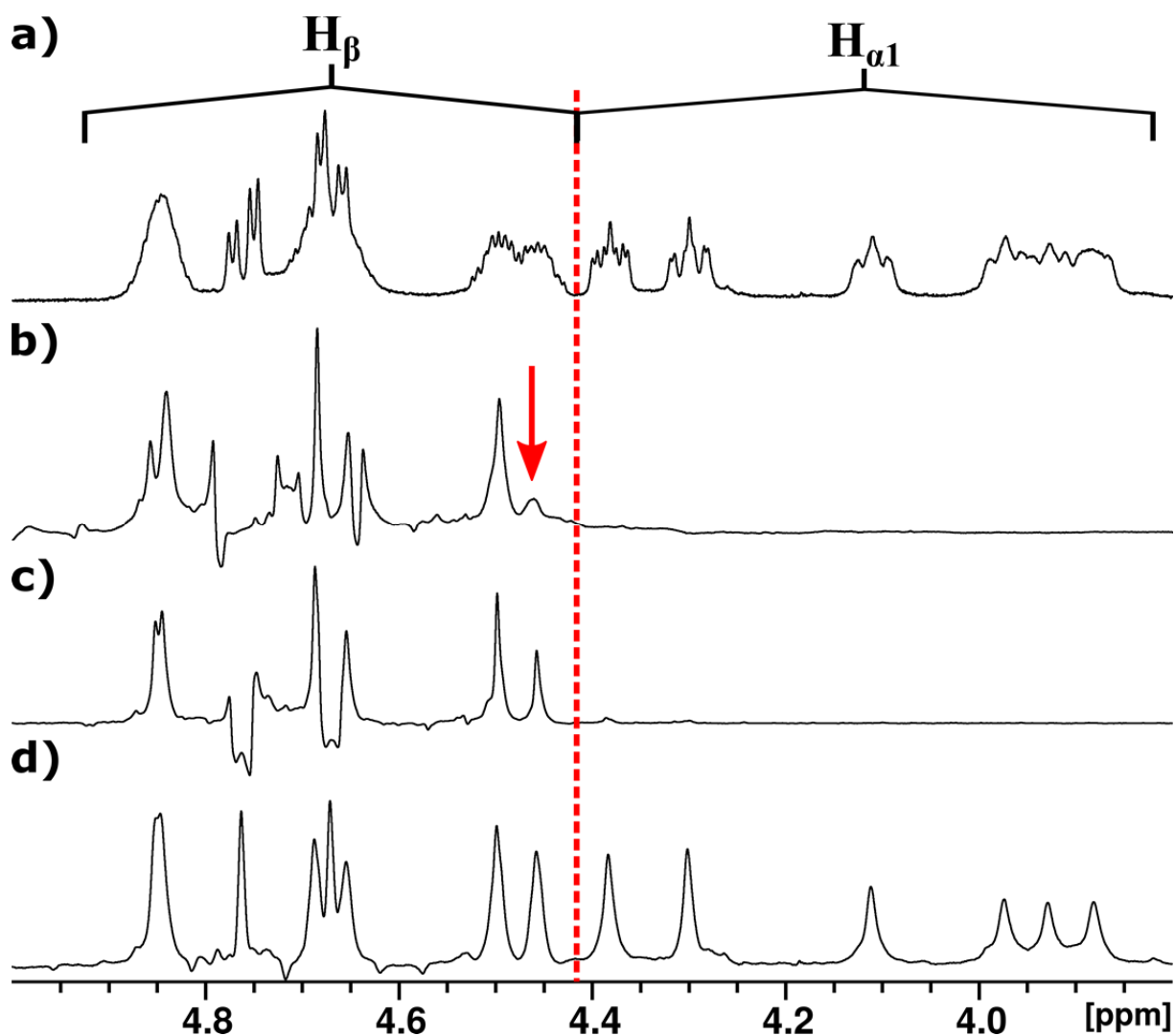


Figure 3-2: Spectra of the hexameric oligourea with homonuclear decoupling of the β -proton region, obtained using (a) conventional ^1H (700.17 MHz proton base frequency), (b) HOBS, (c) band selective homonuclear decoupling using the Zangger-Sterk type interferogram-based acquisition and (d) *perfectBASH*. For selective refocusing a 17 ms ReBurp refocusing pulse (345 Hz bandwidth) with 4.67 ppm offset was used in (b) and (c), in spectrum (d) a 4.5 ms Reburp refocusing pulse (1290 Hz bandwidth) with 4.37 ppm offset was applied. Spectrum (b) shows broadened lines and significant distortions, which result from the long interruptions of the FID (≈ 17 ms) in the real-time acquisition scheme. Using the band selective variant of the Zangger-Sterk type interferogram-based acquisition the homonuclear decoupling quality is improved significantly. Nevertheless, the distortions in spectrum (c) result from two mutually coupled protons of the benzylic protecting group.

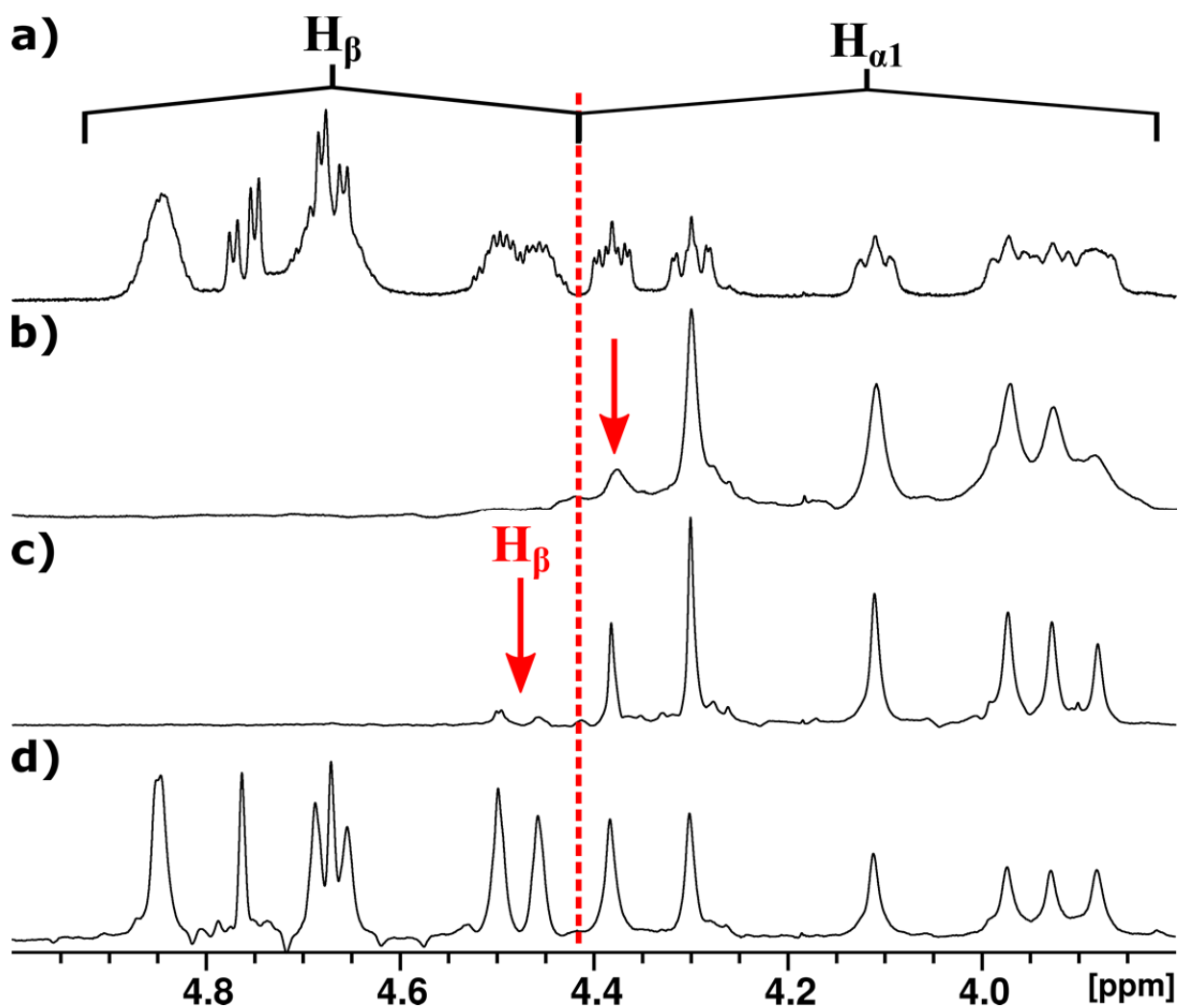


Figure 3-3: Spectra of the hexameric oligourea with homonuclear decoupling of the α -proton region, obtained using (a) conventional ^1H (700.17 MHz proton base frequency), (b) HOBS, (c) band selective homonuclear decoupling using the Zangger-Sterk type interferogram-based acquisition and (d) *perfectBASH*. For selective refocusing a 15 ms ReBurp refocusing pulse (390 Hz bandwidth) with 4.12 ppm offset was used in (b) and (c), in spectrum (d) a 4.5 ms Reburp refocusing pulse (1290 Hz bandwidth) with 4.37 ppm offset was applied. Spectrum (b) shows similar distortions like spectrum (b) in Figure 3-2. This can be solved using the band selective variant of the Zangger-Sterk type interferogram-based acquisition. However, in the present case partial co-refocusing of β -protons happens.

4 *F1-perfect*BASH CLIP-COSY and relayed-CLIP-COSY

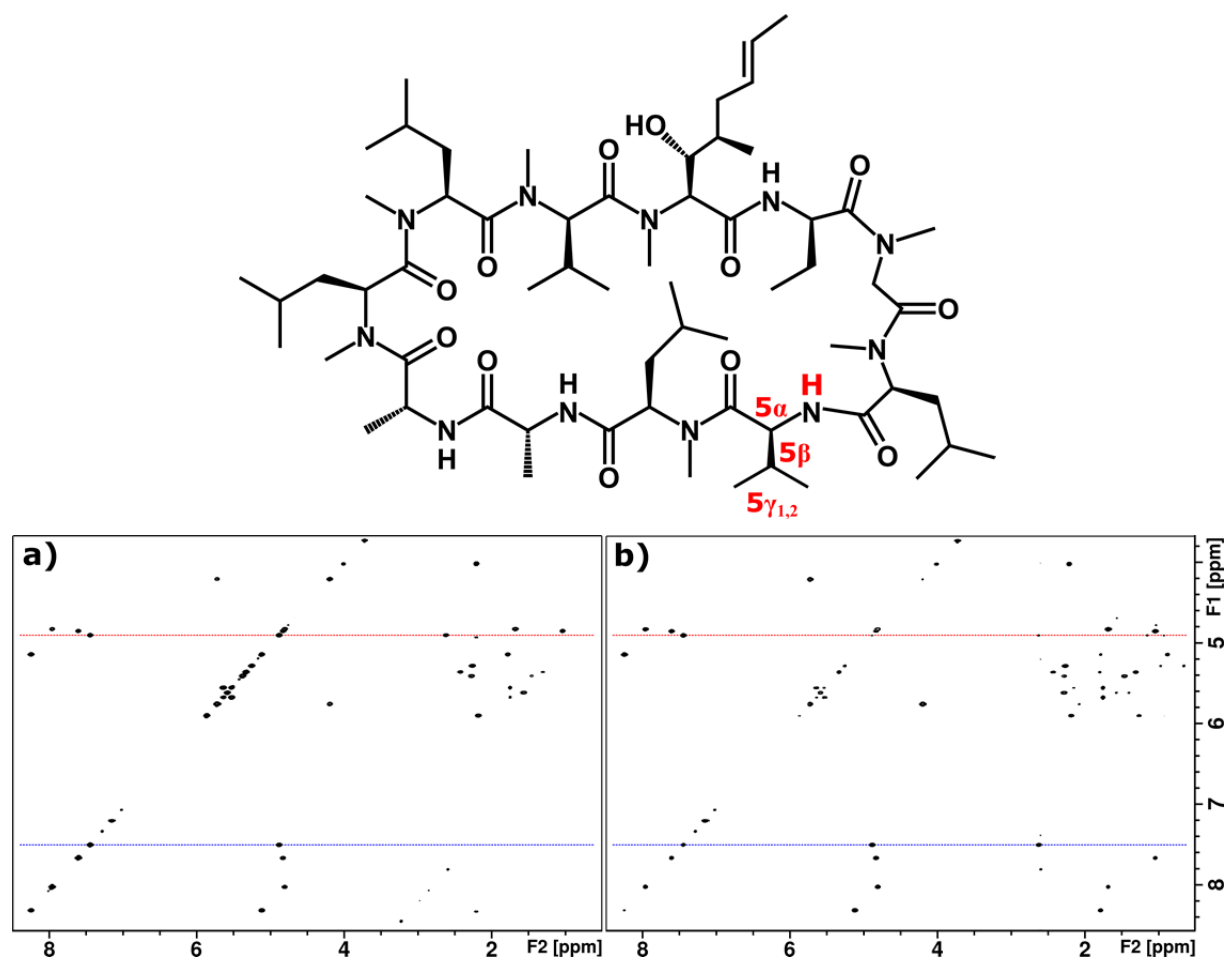
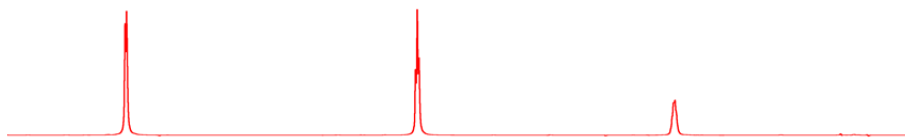


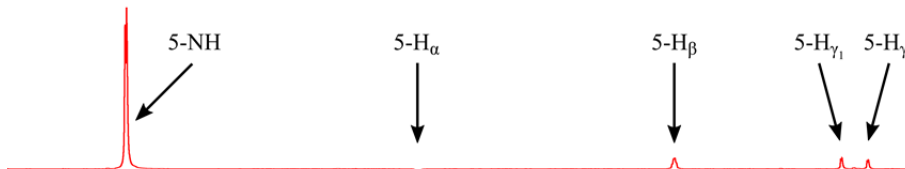
Figure 4-1: *F1-perfect*BASH CLIP-COSY (a) and *F1-perfect*BASH relayed-CLIP-COSY (b; with two transfer steps) of the backbone proton region of cyclosporine A. The dotted lines indicate the positions, at which the *F2*-traces shown in Figure 4-2 have been extracted. The red trace belongs to the α -proton of amino acid 5-valine, as indicated in the structure above. The blue trace belongs to the amide-proton of the same amino acid. Both positive and negative contours are shown in black, to avoid confusion stemming from the signal sign changes expected in the case of the relayed experiment. The spectra were obtained with 600.3 MHz proton base frequency.

a)

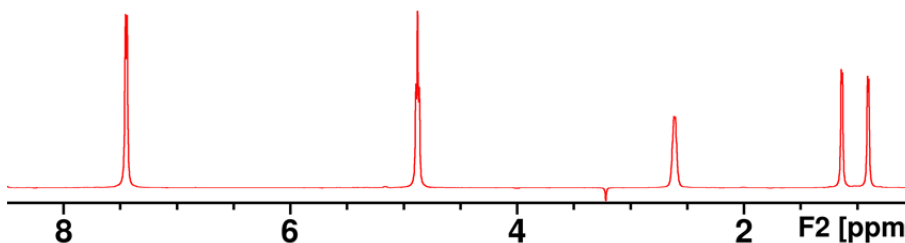
F1-PerfectBASH CLIP-COSY



F1-PerfectBASH relayed CLIP-COSY

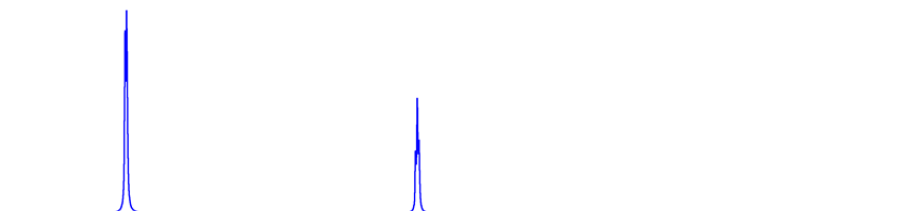


F1-PerfectBASH TOCSY

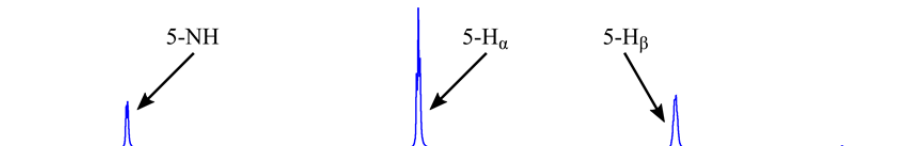


b)

F1-PerfectBASH CLIP-COSY



F1-PerfectBASH relayed CLIP-COSY



F1-PerfectBASH TOCSY

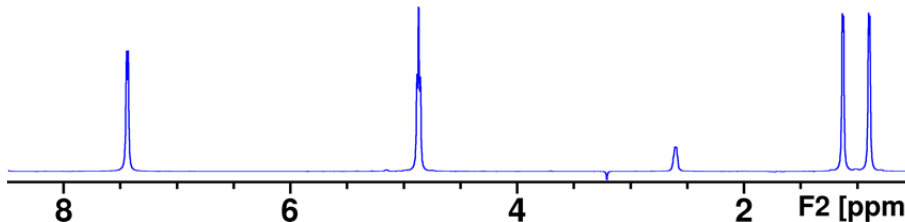


Figure 4-2: Comparison of extracted F2-traces of the (a, red) 5 α - and (b, blue) 5NH-proton from the *F1-perfectBASH* CLIP-COSY, the *F1-perfectBASH* relayed-CLIP-COSY (two transfer steps) and the *F1-perfectBASH* TOCSY (Figure 5 in the main text). In (a) the CLIP-COSY delivers only two correlations from the 5 α -proton to the 5 β - and 5NH-proton, respectively. The relayed-CLIP-COSY shows two extra correlation stemming from a two-step transfer originating from the 5 α -proton to the two methyl groups 5 γ_1 and 5 γ_2 , whereas the TOCSY exhibit all correlations from the whole spin system of amino acid 5-valine. The traces were extracted from spectra, which were obtained with 600.3 MHz proton base frequency.

5 Bruker pulse sequence codes

5.1 1D ¹H-experiment with *perfectBASH* decoupling

```
; 1D 1H-PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; band selective homonuclear decoupling using frequency selective pulses incorporated in Perfect-Echo
; pulse sequence is based on 1D 1H-PEPSIE
; interferogramm based aquisition mode in pseudo direct dimension
; J is refocussed at centre of chunk
; option for solvent presaturation during relaxation delay
; presaturation offset defined via cnst40 [ppm]
; data can be reconstructed using the "pshift" macro available at http://nmr.chemistry.manchester.ac.uk
; avance-version (12/01/11)
;
;
; Relevant papers:
;(1) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(2) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(3) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(4) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Grad.incl>

define delay tauA
define delay tauB
define delay tauC

"d11=30m"
"d12=20u"
"in0=inf1/2"
"tauA=in0+(dw*2*cnst4)+de"
"p2=p1*2.0"
```

```

"tauB=in0/2-p16-d16-10u"
"tauC=in0/2+10u+(dw*2*cnst4)+de"

"cnst21=cnst20*bf1"                ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs2=cnst22"

#ifdef CWPR                        ; solvent presaturation
"d18=d1-d17"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else
#endif /*CWPR*/

;;start pulsedsequence;;
1 ze
2 d11

3 d12

#ifdef CWPR                        ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1                  ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12 p19:f1                          ; set power level on f1-channel for presaturation

d18                                ; residual relaxation delay

d17 cw:f1 ph29                      ; solvent presaturation
4u do:f1

d12 fq=0:f1                          ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1                          ; reset power level on f1-channel

#else                                ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 (p1 ph1):f1                        ; 90 degree pulse excitation

10u

d0                                ; Incremented delay

tauA p10:f1                          ; power switching f1-channel

p16:gp1
d16

(p12:sp2 ph2:r):f1                  ; first frequency selective refocusing pulse

p16:gp1
d16

tauA p11:f1                          ; power switching f1-channel

d0                                ; Incremented delay

10u

5 (p1 ph3):f1                        ; 90 degree pulse for perfect echo J-removal

```

```

d0                ; incremented delay

tauB

10u

p16:gp2*0.5      ; CTP, +0.5
d16

(p2 ph4):f1      ; hard 180 degree pulse

tauC p10:f1      ; power switching f1-channel

p16:gp2*-0.5     ; CTP, -0.5
d16

(p12:sp2 ph5:r):f1          ; second frequency selective refocusing pulse

p16:gp2*-1.0      ; CTP, -1
d16 p11:f1        ; power switching f1-channel

10u BLKGRAD

d0                ; incremented delay

6 go=2 ph31
30u
d11 mc #0 to 2 F1QF(caldel(d0, +in0))

exit

;Phase Cycling
;ph1                ; Hard 90
;ph2                ; First selective 180
;ph3                ; Hard 90
;ph4                ; Hard 180
;ph5                ; Second selective 180
;ph29               ; CW solvent presaturation
;ph31               ; Receiver

ph1= 0 2
ph2= 1 1 2 2 3 3 0 0
ph3= 1 3
ph4= 0
ph5= 0
ph29=0
ph31=2 0 0 2 2 0 0 2

;ph31= ph1 + ph2*2 + ph3*0 + ph4*2 + ph5*2          ; Receiver

;p1 : f1 channel - 90 degree high power pulse
;p2: f1 channel - high power 180 pulse width
;p12: duration of selective 180 pulse
;p16: CTP gradient pulse width

;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for continuous wave for solvent presaturation

;sp2: selective pulse power level
;spoffs2: selective pulse offset
;spnam2: file name for selective pulse [ReBurp.1000]

;gpz1: CTP gradient 20-50% first echo
;gpz2: CTP gradient 50-90% second echo
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100

;d0: incremented delay, set initial value to 0 s
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O

```

[30 msec]


```
;d12: delay for power switching           [20 usec]
;d16: gradient recovery delay
;d17: delay for solvent presaturation
;d18: reduced relaxation delay
;td1: number of chunks to acquire

;NS: number of scans
;DS: number of dummy scans
;cnst4: number of points to drop at the beginning of each FID
;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]
;cnst42: difference for frequency switching on f1-channel
;
;FnMODE: QF

;preprocessor-flags-start
; CWPR: presaturation of solvent at beginning of pulsedsequence
; option -DCWPR (eda: ZGOPTNS)
;preprocessor-flags-end
```

5.2 RESET-compatible 1D-*perfectBASH* sequence

```
; 1D 1H-PERFECT-BASH FOR RESET-PROCESSING
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; band selective homonuclear decoupling using frequency selective pulses incorporated in Perfect-Echo
; pulse sequence based on 1D 1H-PEPSIE
; interferogramm based aquisition mode in pseudo indirect dimension
; J is refocussed at centre of chunk
; option for solvent presaturation during relaxation delay
; presaturation offset defined via cnst40 [ppm]
; Data can be reconstructed using the Bruker "proc_reset" AU-program, details see Ref. (5)
; avance-version (12/01/11)
;
;
; Relevant papers:
;(1) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(2) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(3) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(4) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;(5) P. Sakhaii, B. Haase and W. Bermel, J. Magn. Reson.; 2009, 199, 192-198
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=
;
;
#include <Avance.incl>
#include <Grad.incl>

define delay tauA
define delay tauB
define delay tauC

"i0=dw*131"
"tauA=i0+(dw*2*130)+de"
"p2=p1*2.0"
"tauB=i0/2-p16-d16-10u"
"tauC=i0/2+10u+(dw*2*130)+de"
"l29=2*131*td1"

"cnst21=cnst20*bf1" ; offset calculation for frequency selective refocusing
```

```

"cnst22=cnst21-01"
"spoffs2=cnst22"

#ifdef CWPR                ; solvent presaturation
"d18=d1-d17"
"cnst41=cnst40*bf1"
"cnst42=cnst41-01"
#else
#endif /*CWPR*/

;;start pulsesequence;;
1 ze
2 d11

3 d12

#ifdef CWPR                ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1          ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12 pl9:f1                ; set power level on f1-channel for presaturation

d18                        ; residual relaxation delay

d17 cw:f1 ph29            ; solvent presaturation
4u do:f1

d12 fq=0:f1                ; reset frequency on f1-channel [fq=SF01]

d12 pl1:f1                ; reset power level on f1-channel

#else                        ; no solvent presaturation
d1
d12 pl1:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 (p1 ph1):f1              ; 90 degree pulse excitation

10u

d0                        ; Incremented delay

tauA p10:f1                ; power switching f1-channel

p16:gp1
d16

(p12:sp2 ph2:r):f1        ; first frequency selective refocusing pulse

p16:gp1
d16

tauA pl1:f1                ; power switching f1-channel

d0                        ; Incremented delay

10u

5 (p1 ph3):f1              ; 90 degree pulse for perfect echo J-removal

d0                        ; Incremented delay

tauB

```

```

10u

p16:gp2*0.5          ; CTP, +0.5
d16

(p2 ph4):f1          ; hard 180 degree pulse

tauC p10:f1          ; power switching f1-channel

p16:gp2*-0.5        ; CTP, -0.5
d16

(p12:sp2 ph5:r):f1          ; second frequency selective refocusing pulse

p16:gp2*-1.0        ; CTP, -1
d16 p11:f1          ; power switching f1-channel

10u BLKGRAD

d0          ; incremented delay

6 go=2 ph31
30u
d11 mc #0 to 2 F1QF(caldel(d0, +in0))

exit

;Phase Cycling
;ph1          ; Hard 90
;ph2          ; First selective 180
;ph3          ; Hard 90
;ph4          ; Hard 180
;ph5          ; Second selective 180
;ph29         ; CW solvent presaturation
;ph31         ; Receiver

ph1= 0 2
ph2= 1 1 2 2 3 3 0 0
ph3= 1 3
ph4= 0
ph5= 0
ph29=0
ph31=2 0 0 2 2 0 0 2

;ph31= ph1 + ph2*2 + ph3*0 + ph4*2 + ph5*2          ; Receiver

;p1 : f1 channel - 90 degree high power pulse
;p2: f1 channel - high power 180 pulse width
;p12: duration of selective 180 pulse
;p16: CTP gradient pulse width

;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for continuous wave for solvent presaturation

;sp2: selective pulse power level
;spoffs2: selective pulse offset
;spnam2: file name for selective pulse [ReBurp.1000]

;gpz1: CTP gradient 20-50% first echo
;gpz2: CTP gradient 50-90% second echo
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100

;d0: incremented delay, set initial value to 0 s
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O          [30 msec]
;d12: delay for power switching   [20 usec]
;d16: gradient recovery delay
;d17: delay for solvent presaturation

```

```
;d18: reduced relaxation delay

;cnst4: number of points to drop at the beginning of each FID
;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]
;cnst42: difference for frequency switching on f1-channel
;l29: total number of points in reconstructed FID
;l30: number of complex points at the beginning not to be included in reconstruction
;l31: number of complex points along the acquisition dimension per block block length about 8 to 10ms
;in0: increment for d0 ; dw*l31
;td1: number of chunks to acquire

;NS: number of scans
;DS: number of dummy scans
;
;FnMODE: QF

;preprocessor-flags-start
; CWPR: presaturation of solvent at beginning of pulsedsequence
; option -DCWPR (eda: ZGOTINS)
;preprocessor-flags-end
```

5.3 1D ¹H-HOBS sequence

```
;1D 1H-HOBS
;
;avance-version (14/07/25)
;1D sequence
;bandselective homodecoupling during aquisition
;based on HOBS sequence from Ref. (1)
;including presaturation during relaxation delay
;presaturation offset defined with cnst40
;calculation of offsets for selective refocusing pulses
;
;
;;Literature;;
;(1) L. Castañar, P. Nolis A. Virgili & T. Parella, Chem. Eur. J. 19, 17283-17286 (2013)
;(2) J. Ying, J. Roche & A. Bax, J. Magn. Reson. 241, 97-102 (2014)
;
;$CLASS=HighRes
;$DIM=1D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>

;p2=p1*2"
;d11=30m"
;d12=20u"

;p29=300u"
;d3=de+12u"

"d62=aq/(10*2)" ; duration of one "real-time" chunk
"d63=d62/2" ; half duration for first chunk

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d17"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else
#endif /*CWPR*/

"COUNTER=(trunc((cnst31/100)*10))+1"
"l31=10+COUNTER"

"spoff35=bf1*(cnst21/1000000)-o1" ; offset for selective refocusing pulse

"acqt0=-p1*2/PI"

dwellmode explicit

1 ze
2 d11

3 d12

#ifdef CWPR ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1 ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]
```

```

d12 p19:f1          ; set power level on f1-channel for presaturation

d18                ; residual relaxation delay

d17 cw:f1 ph29     ; solvent presaturation
4u do:f1

d12 fq=0:f1       ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1        ; reset power level on f1-channel

#else              ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

(p1 ph1):f1

d3

p28:gp1
d16 p10:f1

(p46:sp35 ph1):f1
12u

p28:gp1
d16 p10:f1

ACQ_START(ph30,ph31) ;total delay here=de

0.1u REC_UNBLK
0.05u DWL_CLK_ON
d63:r
0.05u DWL_CLK_OFF
0.1u REC_BLK

4 p29:gp2
d16 p11:f1
(p2 ph2):f1
p29:gp2
d16

p29:gp3
d16 p10:f1
5u
(p46:sp35 ph3):f1
5u
p29:gp3
d16

0.1u REC_UNBLK
0.05u DWL_CLK_ON
d62:r
0.05u DWL_CLK_OFF
0.1u REC_BLK

p29:gp2
d16 p11:f1
(p2 ph2):f1
p29:gp2
d16 p10:f1

p29:gp3
d16
5u
(p46:sp35 ph3):f1

```

```

5u
p29:gp3
d16

0.1u REC_UNBLK
0.05u DWL_CLK_ON
d62:r
0.05u DWL_CLK_OFF
0.1u REC_BLK

10 to 4 times 131

d62

rcyc=2
30m mc #0 to 2 F0(zd)
exit

ph1=0 2 2 0 1 3 3 1
ph2=0 2
ph3=0 2
ph29=0 0 0 0 0 0 0 0 ; Contineous_wave_water_presaturation
ph30=0
ph31=0 2 2 0 1 3 3 1

;p1 : f1 channel - high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p28: duration of CTP gradient pulse 1
;p29: duration of CTP gradient pulse 2
;p46: f1 channel - duration of 180 degree shaped pulse
;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for contineous wave for water presaturation

;sp35: f1 channel - region selective refocusing pulse [RSnob, ReBurp]

;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d17: delay for solvent presaturation
;d18: reduced relaxation delay
;d62: length of block between decoupling pulses : = aq/10 [< 20-25 msec]
;d63: = d62/2

;cnst21: chemical shift for selective pulse (offset, in ppm)
;cnst31: = v9, random variation of +/- v9 %
;cnst40: solvent offset [ppm]
;cnst42: difference for frequency switching on f1-channel

;10 : number of blocks during acquisition time adjust to get d62 as required
;ns: 1 * n, total number of scans: NS * TD0
;ds: 4

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpz1: 17%
;gpz2: 7%
;gpz3: 5%

;preprocessor-flags-start
;LABEL_CWPR: presaturation of solvent at beginning of pulsedsequence
; option -DCWPR (eda: ZGOPTNS)
;preprocessor-flags-end

```


5.4 2D TOCSY with F1-*perfectBASH* homonuclear decoupling

```
; 2D TOCSY WITH HOMODECOUPLING IN F1 USING PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; with DIPSI-2 for isotropic mixing
; zero-quantum filtration before and after isotropic mixing
; phase sensitive
; band selective homonuclear decoupling using frequency selective pulses incorporated in Perfect-Echo
; homodecoupling scheme based on PEPSIE and F1-PSYCHE-TOCSY from Ref. (5)
; J is refocussed at the beginning of mixing
; option for solvent presaturation during relaxation delay possible in zg-options
; presaturation offset defined via cnst40 [ppm]
; avance-version (12/01/11)
;
;
; Relevant Papers:
;(1) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(2) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(3) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(4) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;(5) Foroozandeh, M.; Adams, R. W.; Nilsson, M.; Morris, G. A. J. Am. Chem. Soc. 2014, 136, 11867.
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Delay.incl>
#include <Grad.incl>

define delay tauA

;;Calculations isotropic mixing and zero-quantum filtration;;
"FACTOR1=(d9/(p6*115.112))/2+0.5"
"l1=FACTOR1*2"
"d12=20u"
"p21=p11"
"p22=p12"
"d11=30m"

;;Calculations PerfectBASH;;
"p2=p1*2.0" ; 180 high power pulse
"in0=inf1/2"
```

```

"d0=0u" ; incremented delay for chemical shift evolution during homodecoupling-block
"tauA=p16+d16"
"cnst21=cnst20*bf1" ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs40=cnst22"

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d19"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else

#endif /*CWPR*/

;;start pulsesequence;;
1 ze
2 d11
3 d12

#ifdef CWPR ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1 ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12 p19:f1 ; set power level on f1-channel for presaturation

d18 ; residual relaxation delay

d19 cw:f1 ph29 ; solvent presaturation
4u do:f1

d12 fq=0:f1 ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1 ; reset power level on f1-channel

#else ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 p1 ph1 ; 90 degree excitation pulse

5u ; beginning of decoupling using PerfectBASH
5u p10:f1 ; power switching f1-channel

d0 ; Incremented delay

tauA

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

(p40:sp40 ph5:r):f1 ; first selective refocusing pulse

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

tauA p11:f1 ; power switching f1-channel

d0 ; Incremented delay

10u

5 (p1 ph6):f1 ; 90 high power pulse for perfect echo J-removal

d0 ; Incremented delay

```

```

p16:gp2          ; CTP 2
dl6              ; gradient recovery delay

(p2 ph7):f1      ; 180 high power pulse

10u

p16:gp2          ; CTP 2
dl6              ; gradient recovery delay

p16:gp3          ; CTP 3
dl6              ; gradient recovery delay

(p40:sp40 ph8:r):f1 ; second selective refocusing pulse
dl6              ;gradient recovery delay

p16:gp3          ; CTP 3
dl6

10u pl1:f1       ; power switching f1-channel

d0              ; incremented delay F1-dimension

6 pl ph2         ; 90 degree pulse at begin of isotropic mixing step

5u pl0:f1

(center (p21:gp11) (pl1:sp1 ph4):f1 ) ; first z-filter element
dl7              ; gradient recovery delay

5u pl10:f1

7 p6*3.556 ph23          ; begin isotropic mixing using dipsi-2
p6*4.556 ph25
p6*3.222 ph23
p6*3.167 ph25
p6*0.333 ph23
p6*2.722 ph25
p6*4.167 ph23
p6*2.944 ph25
p6*4.111 ph23
p6*3.556 ph25
p6*4.556 ph23
p6*3.222 ph25
p6*3.167 ph23
p6*0.333 ph25
p6*2.722 ph23
p6*4.167 ph25
p6*2.944 ph23
p6*4.111 ph25
p6*3.556 ph23
p6*4.556 ph23
p6*3.222 ph25
p6*3.167 ph23
p6*0.333 ph25
p6*2.722 ph23
p6*4.167 ph25
p6*2.944 ph23
p6*4.111 ph25
p6*3.556 ph23
p6*4.556 ph25
p6*3.222 ph23
p6*3.167 ph25
p6*0.333 ph23
p6*2.722 ph25
p6*4.167 ph23
p6*2.944 ph25
p6*4.111 ph23
lo to 7 times 11

```

```

5u p10:f1

p17:gp4          ; purge gradient
d17              ; gradient recovery delay

( center (p22:gp12) (p12:sp2 ph4):f1 )          ; second z-filter element
d17              ; gradient recovery delay

50u BLKGRAD
5u p11:f1

8 p1 ph3          ; 90 degree pulse at end of isotropic mixing step

go=2 ph31
d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph5, +90) & calph(ph6, +90) & calph(ph7, +90) & calph(ph8, +90), caldel(d0,
+in0))

exit

ph1= 0 2          ; Hard 90 excitation
ph2= 0 0 0 0 2 2 2 2 ; Hard 90 before mixing
ph3= 0 0 2 2      ; Hard 90 after mixing
ph4= 0            ; adiabatic 180 (z-filter element)
ph5= 1 1 3 3      ; First selective refocusing pulse
ph6= 1 3          ; Hard 90 Perfect-Echo J-removal
ph7= 0            ; Hard 180
ph8= 0            ; Second selective refocusing pulse
ph23=3            ; dispsi-2
ph25=1            ; dispsi-2
ph29=0            ; CW-presaturation
ph31=0 2 2 0 2 0 0 2 ; receiver

;p1: high power 90 pulse width
;p2: high power 180 pulse width
;p6: 90 degree low power pulse
;p11: duration of first ZQC dephasing element
;p12: duration of second ZQC dephasing element
;p16: duration of CTP gradient
;p17: duration of CTP gradient
;p21: duration ZQC dephasing gradient
;p22: duration ZQC dephasing gradient
;p40: duration of selective 180 pulse

;p11: f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for presaturation
;p110: DIPSI-2 power

;sp1: first adiabatic 180 pulse power level
;spoffs1: first adiabatic 180 pulse offset (0 Hz)
;sp2: second adiabatic 180 pulse power level
;spoffs2: second adiabatic 180 pulse offset (0 Hz)
;sp40: selective refocusing pulse
;spw40 : RF power of selective 180 pulse
;spnam40: file name for selective 180 pulse

;d0 : incremented delay
;d1 : relaxation delay
;d9 : TOCSY mixing time
;d16: gradient recovery delay, homodecoupling
;d17: gradient recovery delay, mixing step
;d18: residual relaxation delay
;d19: delay for solvent presaturation

;gpz1: CTP gradient
;gpz2: CTP gradient
;gpz3: CTP gradient

```

```
;gpz4: Purge gradient
;gpz11: ZQC gradient 1-3%
;gpz12: ZQC gradient 1-3 %
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam11: RECT.1
;gpnam12: RECT.1

;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]
;cnst42: difference for frequency switching on f1-channel

;l1 : loop for DIPSI cycle
;in0 : 1/(2 * SW) = DW
;NS : number of scans 8*n
;DS : number of dummy scans 16
;td1 : number of t1 increments
;MC2 : TPPI or States-TPPI

;preprocessor-flags-start
;LABEL_CWPR: presaturation of solvent at beginning of pulsesequence
; option -DCWPR (eda: ZGOPTNS)
;preprocessor-flags-end
```

5.5 2D NOESY with F1-*perfectBASH* homonuclear decoupling

```
; 2D NOESY WITH HOMODECOUPLING IN F1 USING PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; 2D homonuclear correlation via dipolar coupling
; dipolar coupling may be due to noe or chemical exchange
; zero-quantum filtration during mixing
; phase sensitive
; band selective homonuclear decoupling using frequency selective pulses incorporated in Perfect-Echo
; homodecoupling scheme based on PEPSIE and F1-PSYCHE-TOCSY from Ref. (7)
; J is refocussed at the beginning of mixing
; option for solvent presaturation during relaxation delay possible in zg-options
; presaturation offset defined via cnst40 [ppm]
; avance-version (12/01/11)
;
;
; Relevant Papers:
;(1) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(2) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(3) J.A. Aguilar, S. Faulkner, M. Nilsson and G.A. Morris, Angew. Chem. Int. Ed.; 2010, 49, 3901-3903.
;(4) G.A. Morris, J.A. Aguilar, R. Evans, S. Haiber and M. Nilsson, J. Am. Chem. Soc.; 2010, 132, 12770 - 12772.
;(5) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(6) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;(7) Foroozandeh, M.; Adams, R. W.; Nilsson, M.; Morris, G. A. J. Am. Chem. Soc. 2014, 136, 11867.
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Grad.incl>

define delay tauA
define delay TAU

"d11=30m"
"d12=20u"

;;Definition NOESY mixing;;
"TAU=d8-(p32+p16+d16*2+20u)"
;p11=p32" ; adiabatic pulse as long as ZQC dephasing gradient
```

```

;;Calculations PerfectBASH;;
"p2=p1*2.0" ; 180 high power pulse
"in0=inf1/2"
"d0=0u" ; incremented delay for chemical shift evolution during homodecoupling-block
"tauA=p16+d16"
"cnst21=cnst20*bf1" ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs40=cnst22"

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d19"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else

#endif /*CWPR*/

;;begin pulsedsequence;;

1 ze
2 d11
3 d12
4u

#ifdef CWPR ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42:f1 ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12

d18 p19:f1 ; residual relaxation delay + set power level on f1-channel for presaturation

d19 cw:f1 ph29 ; solvent presaturation
4u do:f1

d12 fq=0:f1 ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1 ; reset power level on f1-channel

#else ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 p1 ph1 ; 90 high power excitation pulse

5u
5u p10:f1 ; power switching f1-channel

d0 ; Incremented delay

tauA

p17:gp1 ; CTP 1
d17 ; gradient recovery delay

(p40:sp40 ph5:r):f1 ; first selective refocusing pulse

p17:gp1 ; CTP 1
d17 ; gradient recovery delay

tauA p11:f1 ; power switching f1-channel

```

```

d0 ; Incremented delay

10u

5 (p1 ph6):f1 ; 90 high power pulse for perfect echo J-removal

d0 ; Incremented delay

p17:gp2 ; CTP 2
d17 ; gradient recovery delay

(p2 ph4):f1 ; 180 high power pulse
10u

p17:gp2 ; CTP 2
d17 ; gradient recovery delay

p17:gp3 ; CTP 3
d17 p10:f1 ; gradient recovery delay + power switching f1-channel

(p40:sp40 ph4:r):f1 ; second selective refocusing pulse
d17 ;gradient recovery delay

p17:gp3 ; CTP 3
d17

10u p11:f1 ; power switching f1-channel

d0 ; incremented delay

(p1 ph2) ; 90 high power pulse at beginning of NOESY mixing time

10u p10:f1 ; power switching f1-channel

(center(p32:sp29 ph4:r):f1 (p11:gp11)) ; Thrippleton-Keeler z-filter element
d16 ; gradient recovery delay

p16:gp4 ; purge gradient
d16 p11:f1 ; gradient recovery delay and power switching f1-channel

10u BLKGRAD
TAU ; mixing time

(p1 ph3) ; 90 high power pulse at end of NOESY mixing time

go=2 ph31
d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph5, +90) & calph(ph6, +90) & calph(ph4, +90), caldel(d0, +in0))
exit

;phase cycling
ph1=0 2 ; Hard 90 Excitation
ph2=0 0 0 0 0 0 0 2 2 2 2 2 2 2 2 ; Hard 90 Begin NOESY mixing
ph3=0 0 0 0 2 2 2 2 ; Hard 90 End NOESY mixing
ph4=0 ; adiabatic 180 zFilter & Hard 180 & 2nd sel. 180
ph5=1 1 3 3 ; 1st sel. 180
ph6=1 3 1 3 ; Hard 90 Perfect Echo
ph29=0 ; CW_Presaturation
ph31=2 0 2 0 0 2 0 2 0 2 0 2 0 2 0 2 0 ; Receiver

;p1 : f1 channel - 90 degree high power pulse
;p2: f1 channel - 180 degree pulse width
;p11: duration of ZQC dephasing gradient
;p16: purge gradient pulse during NOESY mixing time [5m]
;p17: homospoil/gradient pulse during F1-homodecoupling [1m]
;p32: f1 channel - 180 degree shaped pulse (adiabatic)
;p40: duration of selective 180 pulse

;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for presaturation

```



```

;sp29: f1 channel - shaped pulse (adiabatic)
;spw29: RF power of adiabatic 180 pulse
;spnam29: file name for adiabatic 180 pulse
;sp40: f1-channel - selective 180 pulse
;spw40 : RF power of selective 180 pulse
;spnam40: file name for selective 180 pulse

;gpz1: CTP gradient (20-50%) decoupling block in F1-dimension
;gpz2: CTP gradient (20-50%) decoupling block in F1-dimension
;gpz3: CTP gradient (20-50%) decoupling block in F1-dimension
;gpz4: purge gradient NOESY mixing time          [40%]
;gpz11: ZQC dephasing gradient (ca. 11%)
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam11: RECT.1

;d0: incremented delay (2D)
;d1: relaxation delay: 1-5 * T1
;d8: mixing time
;d11: delay for disk I/O                [30 msec]
;d12: delay for power switching         [20 usec]
;d16: delay for homospoil/gradient recovery
;d17: delay for homospoil/gradient recovery in F1-dimension
;d18: reduced relaxation delay
;d19: delay for solvent presaturation

;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]
;cnst42: difference for frequency switching on f1-channel

;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;NS : 8*n
;DS : 16
;td1 : number of t1 increments
;FnMODE: States-TPPI, TPPI, States or QSEQ

;preprocessor-flags-start
;LABEL_CWPR: presaturation of solvent at beginning of pulsesequence
; option -DLABEL_CWPR (eda: ZGOPTNS)
;preprocessor-flags-end

;for sweepwidth of adiabatic shape and adjusting gpz11:
;see supplementary material of M.J. Thrippleton & J. Keeler, Angew. Chem. Int. Ed. 42, 3938-3941 (2003)

```

5.6 2D EASY-ROESY with F1-*perfectBASH* homonuclear decoupling

```
; 2D EASY-ROESY WITH HOMODECOUPLING IN F1 USING PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; jump-symmetrized with adiabatic spinlocks for mixing
; correction to ensure symmetrically shifted offsets for spin-locking
; midpoint of symmetric offset shifting of spin-lock defined via cnst32 [ppm] and should be set center of 1H spectrum
; phase sensitive
; band selective homonuclear decoupling using frequency selective pulses incorporated in Perfect-Echo
; homodecoupling scheme based on PEPSIE and F1-PSYCHE-ROESY from Ref. (5)
; J is refocussed at the beginning of mixing
; option for solvent presaturation during relaxation delay possible in zg-options
; presaturation offset defined via cnst40 [ppm]
; avance-version (12/01/11)
;
;
; Relevant Papers:
;(1) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(2) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(3) C.M. Thiele, K. Petzold & J. Schleucher, Chem. Eur. J. 15, 585-588 (2009)
;(4) J. Schleucher, J. Quant, S. Glaser & C. Griesinger, J. Magn. Reson A 112, 144-151 (1995)
;(3) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(4) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;(5) Procházková, E., Kolmer, A., Ilgen, J., Schwab, M., Kaltschnee, L., Fredersdorf, M., Schmidts, V., Wende, R. C.,
;Schreiner, P. R., Thiele, C. M., Angew. Chem. Int. Ed. 2016, 55, 15754-15759
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Grad.incl>

define pulse P_SL
define delay tauA

"d11=30m"
"d12=20u"

;;calculations for ROESY-Spinlock;;
"cnst24=1000000.0*tan((cnst28*2*PI)/360.0)/(dw*4)"
"if ( cnst24 > 6500 ) {cnst25 = 6400.0;} else {cnst25 = cnst24;}"
```

```

"if ( cnst24 > 6500 ) {cnst29 = atan(cnst25*4*dw/1000000.0)*360.0/(2*PI);} else {cnst29=cnst28;}"
"if (cnst26<cnst25) {cnst27=cnst25;} else {if (cnst26>6500) {cnst27=6400;} else {cnst27=cnst26;}}"

"cnst30=abs(cnst27/tan((cnst29*2*PI)/360.0))"      ; requested offset shifting for low- and high-field SL, if offset o1 is 1H-
spectrum center

"cnst33=o1-(cnst32*bf1)"                          ; difference between PerfectBASH offset and 1H-spectrum center, to allow
symmetrical spin-locking, cnst32 defines the midpoint of symmetrical offset shifting!!

"cnst34=cnst30+cnst33"                            ; correction for lowfield SL offset
"cnst35=cnst30-cnst33"                            ; correction for highfield SL offset

define list<frequency> roesylist={sfo hz, 0.0, -cnst34, cnst35, 0}

"p30=1000000.0/(cnst27*4)"
"cnst31= (p30/p1) * (p30/p1)"
"spw10=plw1/cnst31"
"spw12=plw1/cnst31"
"spw13=plw1/cnst31"
"spw16=plw1/cnst31"
"spw17=plw1/cnst31"
"cnst23=cnst30+cnst31+p30"
"p41=1m"
"P_SL=p15/2"
"spoff10=0"
"spoff12=0"
"spoff13=0"
"spoff16=0"
"spoff17=0"

;;Calculations PerfectBASH;;
"p2=p1*2.0"                                       ; 180 high power pulse
"in0=inf1/2"
"d0=0u"                                           ; incremented delay for chemical shift evolution during homodecoupling-block
"tauA=p16+d16"
"cnst21=cnst20*bf1"                              ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs40=cnst22"

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d19"
"cnst41=cnst40*bf1"                              ; changed according to use ppm values for solvent offset
"cnst42=cnst41-o1"
#else
#endif /*CWPR*/

;; start pulsesequence ;;
1 ze
2 d11
3 d12 roesylist:f1
4u roesylist.inc

#ifdef CWPR                                       ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42:f1                                ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12

d18 p19:f1                                       ; residual relaxation delay + set power level on f1-channel for presaturation

d19 cw:f1 ph29                                   ; solvent presaturation
4u do:f1

d12 fq=0:f1                                       ; reset frequency on f1-channel [fq=SF01]

```

```

    d12 p11:f1                ; reset power level on f1-channel

#else                          ; no solvent presaturation
    d1
    d12 p11:f1
#endif /*CWPR*/

    50u UNBLKGRAD

4 p1 ph1                      ; 90 high power excitation pulse
    5u
    5u p10:f1                 ; power switching f1-channel

    d0                        ; Incremented delay

    tauA

    p17:gp3                   ; CTP 3
    d17                       ; gradient recovery delay

    (p40:sp40 ph2:r):f1       ; first selective refocusing pulse

    p17:gp3                   ; CTP 3
    d17                       ; gradient recovery delay

    tauA p11:f1               ; power switching f1-channel

    d0                        ; Incremented delay

    10u

5 (p1 ph3):f1                 ; 90 high power pulse for perfect echo J-removal

    d0                        ; Incremented delay

    p17:gp4                   ; CTP 4
    d17                       ; gradient recovery delay

    (p2 ph4):f1               ; 180 high power pulse

    10u

    p17:gp4                   ; CTP 4
    d17                       ; gradient recovery delay

    p17:gp5                   ; CTP 5
    d17 p10:f1                ; gradient recovery delay + power switching f1-channel

    (p40:sp40 ph5:r):f1       ; second selective refocusing pulse

    p17:gp5                   ; CTP 5
    d17

    10u p11:f1                ; power switching f1-channel

    d0                        ; Incremented delay

6 (p1 ph6):f1                 ; 90 high power pulse before ROESY mixing

    4u

    p16:gp1                   ; purge gradient
    d16 roesylist:f1          ; gradient recovery delay
    4u roesylist.inc

#   ifdef AV2
    (p41:sp12 ph6):f1         ; adiabatic ramp up (lowfield, positive offset)
    3u
    (P_SL:sp10 ph6):f1        ; low field spinlock
    3u

```

```

(p41:sp13 ph6):f1 ; adiabatic ramp down (lowfield, positive offset)
# else
(p41:sp12 ph6):f1
(P_SL:sp10 ph6):f1
(p41:sp13 ph6):f1
# endif /*AV2*/

4u
4u roesylist:f1
4u roesylist.inc

# ifdef AV2
(p41:sp16 ph6):f1 ; adiabatic ramp up (highfield, negative offset)
3u
(P_SL:sp10 ph6):f1 ; high field spinlock
3u
(p41:sp17 ph6):f1 ; adiabatic ramp down (highfield, negative offset)
# else
(p41:sp16 ph6):f1
(P_SL:sp10 ph6):f1
(p41:sp17 ph6):f1
# endif /*AV2*/

4u

p16:gp2 ; purge gradient
d16 roesylist:f1 ; gradient recovery delay

4u roesylist.inc
4u p11:f1
4u BLKGRAD

(p1 ph7) ; 90 high power pulse after ROESY mixing

go=2 ph31
d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph2, +90) & calph(ph3, +90) & calph(ph4, +90) & calph(ph5, +90), caldel(d0,
+in0))
exit

ph1= 0 2 ; Hard 90 excitation
ph2= 1 1 1 1 2 2 2 2 ; First selective 180
ph3= 1 3 1 3 ; Hard 90 Perfect Echo
ph4= 0 ; Hard 180
ph5= 0 ; Second selective 180
ph6= 0 ; Hard 90 ROESY mixing begin
ph7= 2 2 0 0 3 3 1 1 ; Hard 90 ROESY mixing end
ph29=0 ; CW presaturation
ph31=0 2 2 0 3 1 1 3 ; Receiver

;p1 : f1 channel - 90 degree high power pulse
;p2: high power 180 pulse width
;p15: f1 channel - pulse for ROESY spinlock
;p16: purge gradient pulse during ROESY mixing time [1m]
;p17: homospoil/gradient pulse during F1-homoddecoupling [1m]
;p30: f1 channel - 90 degree pulse at sp10
;p35: f1 channel - 90 degree pulse at sp40
;p40: duration of selective 180 pulse
;p41: f1 channel - shaped pulse for adiabatic ramp [1m]

;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for presaturation

;P_SL: f1 channel - pulse width for low- and highfield spinlock
;sp10: f1 channel - shaped pulse for ROESY-spinlock (= p11 + cnst31)
;spnam10: Squa100.1000
;sp12: f1 channel - shaped pulse for adiabatic ramp down (lowfield, positive offset) (= p11 + cnst31)
;spnam12: Gaussramp+down.1
;sp13: f1 channel - shaped pulse for adiabatic ramp up (lowfield, positive offset) (= p11 + cnst31)
;spnam13: Gaussramp+up.1
;sp16: f1 channel - shaped pulse for adiabatic ramp down (highfield, negative offset) (= p11 + cnst31)

```

```

;spnam16: Gaussramp-down.1
;sp17: f1 channel - shaped pulse for adiabatic ramp up (highfield, negative offset) (= p11 + cnst31)
;spnam17: Gaussramp-up.1
;sp40: f1-channel - selective 180 pulse
;spw40 : RF power of selective 180 pulse
;spnam40: file name for selective 180 pulse

;gpz1: purge gradient 31% ROESY element
;gpz2: purge gradient 11% ROESY element
;gpz3: CTP gradient (20-50%) homodecoupling F1-dimension
;gpz4: CTP gradient (20-50%) homodecoupling F1-dimension
;gpz5: CTP gradient (20-50%) homodecoupling F1-dimension
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam4: SMSQ10.100
;gpnam5: SMSQ10.100

;d0 : incremented delay (2D)
;d1 : relaxation delay; 1-5 * T1
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d17: delay for homospoil/gradient recovery in F1-dimension
;d18: reduced relaxation delay
;d19: delay for solvent presaturation

;cnst20: offset for selective refocusing [ppm]
;cnst23; (for display purpose only)
;cnst24: min. RF field strength to make sure that the carrier is shifted
; to the edge of the spectrum
;cnst25: reduced min. RF field strength in case an upper limit of 6.5kHz is exceeded
; (set to 6.4kHz), this leads to a recalculation of the tilt angle (cnst29)
;cnst26: requested RF field strength (gammaB1) for ROESY spinlock
; reduced to 6.4kHz if an upper limit of 6.5kHz is exceeded
;cnst27: used RF field strength (gammaB1) for ROESY spinlock
;cnst28: requested tilt angle for ROESY spinlock (between axis of spinlock and z-axis) [45 degree]
;cnst29: used tilt angle for ROESY spinlock (between axis of spinlock and z-axis)
;cnst30: low and highfield offset,
; calculated from gammaB1 (cnst27) for tilt angle (cnst29)
;cnst31: difference in power level (dB) for spinlock relative to p11
;cnst40: solvent offset [ppm]
;cnst41: spectrum center [Hz]
;cnst42: difference for frequency switching on f1-channel

;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 1
;NS : 8*n
;DS : 16
;td1 : number of t1 increments
;FnMODE: States-TPPI, TPPI, States or QSEQ

;preprocessor-flags-start
;CWPR: presaturation of solvent at beginning of pulsesequence
; option -DCWPR (eda: ZGOPINS)
;preprocessor-flags-end

```

5.7 2D CLIP-COSY with F1-*perfectBASH* homonuclear decoupling

```
; 2D CLIP-COSY WITH HOMODECOUPLING IN F1 USING PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavorably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; Clean In-phase COSY
; 2D H,H-correlation using in-phase transfer
; based on the CLIP-COSY pulse sequence from Ref. (1)
; phase sensitive
; band selective homonuclear decoupling in F1 using frequency selective pulses incorporated in Perfect-Echo
; J is refocussed at the beginning of mixing
; option for solvent presaturation during relaxation delay possible in zg-options
; presaturation offset defined via cnst40 [ppm]
; avance-version (12/01/11)
;
;
; Relevant Papers:
;(1) M. R. Koos, G. Kummerloewe, L. Kaltschnee, C. M. Thiele and B. Luy, Angew. Chem. Int. Ed. 2016, 55, 7655.
;(2) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(3) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(4) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;(5) L. Kaltschnee, A. Kolmer, I. Timari, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Köver, G. A. Morris and C. M. Thiele,
; Chem. Commun.; 2014, 50, 15702 - 15705
;
;
; $CLASS=HighRes
; $DIM=2D
; $TYPE=
; $SUBTYPE=
; $COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>

define delay tauA

;;CLIP-COSY statements;;
"p2=p1*2"
"d6=1s/(cnst1*4)" ; coupling evolution delay in mixing step
"d11=30m"
"d12=20u"
"p35=p32"
"p36=p33"

;;Calculations PerfectBASH;;
"p2=p1*2.0" ; 180 high power pulse
"in0=infl/2"
```

```

"d0=0u" ; incremented delay for chemical shift evolution during homodecoupling-block
"tauA=p16+d16"
"cnst21=cnst20*bf1" ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs40=cnst22"

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d19"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else

#endif /*CWPR*/

;;begin pulsesequence;;
1 ze
2 d11
3 d12
4u

#ifdef CWPR ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1 ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]

d12 p19:f1 ; set power level on f1-channel for presaturation

d18 ; residual relaxation delay

d19 cw:f1 ph29 ; solvent presaturation
4u do:f1

d12 fq=0:f1 ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1 ; reset power level on f1-channel

#else ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 p1 ph1 ; 90 high power excitation pulse

5u
5u p10:f1 ; power switching f1-channel

d0 ; Incremented delay

tauA

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

(p40:sp40 ph10:r):f1 ; first selective refocusing pulse

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

tauA p11:f1 ; power switching f1-channel

d0 ; Incremented delay

10u

5 (p1 ph11):f1 ; 90 high power pulse for perfect echo J-removal

```



```

d0                ; Incremented delay

p16:gp2          ; CTP 2
d16              ; gradient recovery delay

(p2 ph12):f1     ; 180 high power pulse

10u

p16:gp2          ; CTP 2
d16              ; gradient recovery delay

p16:gp3          ; CTP 3
d16 p10:f1       ; gradient recovery delay + power switching f1-channel

(p40:sp40 ph12:r):f1 ; second selective refocusing pulse

p16:gp3          ; CTP 3
d16              ; gradient recovery delay

10u p11:f1       ; power switching f1-channel

d0                ; incremented delay

6 p1 ph7         ; first Thrippleton-Keeler-filter

5u p10:f1
(center(p35:gp11) (p32:sp28 ph8):f1)
5u
d16 p11:f1

7 p1 ph2         ; begin in-phase COSY mixing

d6
p2 ph3
d6
p1 ph4
d6
p2 ph3
d6

8 p1 ph5         ; second Thrippleton-Keeler-Filter

5u p10:f1
(center(p36:gp12) (p33:sp29 ph9):f1)
5u
d16 p11:f1

p16:gp13         ; purge gradient
5u
d16 BLKGRAD

9 p1 ph6

go=2 ph31
d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph10, +90) & calph(ph11, +90) & calph(ph12, +90) , caldel(d0, +in0))
exit

;phase cycling
ph1=0 2          ; 90 excitation
ph2=2 2 2 2 0 0 0 0 ; 90 begin mixing
ph3=0           ; 180 mixing element
ph4=1          ; 90 mixing element
ph5=0 0 2 2    ; 90 end mixing and begin second z-filter
ph6=0          ; 90 begin acquisition
ph7=0          ; 90 begin first z-filter
ph8=0          ; 180 adiabatic first z-filter
ph9=2          ; 180 adiabatic second z-filter
ph10=1 1 1 1 2 2 2 2 ; 180 first selective refocusing
ph11=1 3 1 3   ; 90 Perfect-Echo

```

```

ph12=0                ; 180 hard + second selective refocusing
ph29=0                ; CW presaturation
ph31=0 2 2 0 0 2 2 0 ; receiver

;p1 : 90 degree high power pulse
;p2 : 180 degree high power pulse
;p16: homospoil/gradient pulse
;p32: 180 degree adiabatic pulse for ZQC-dephasing
;p33: 180 degree adiabatic pulse for ZQC-dephasing
;p35: duration ZQC-dephasing gradient
;p36: duration ZQC-dephasing gradient
;p40: duration of selective refocusing pulse

;p10: zero power
;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for presaturation

;sp28: adiabatic 180 pulse
;sp29: adiabatic 180 pulse
;spnam28: file name for adiabatic 180 pulse
;spnam29: file name for adiabatic 180 pulse
;sp40: selective 180 pulse
;spw40: RF power of selective 180 pulse
;spnam40: file name for selective 180 pulse

;d0: incremented delay
;d1: relaxation delay: 1-5*T1
;d6: 1/(4J(HH))
;d11: delay for disk I/O                [30 msec]
;d12: delay for power switching          [20 usec]
;d16: delay for homospoil/gradient recovery
;d18: reduced relaxation delay
;d19: delay for solvent presaturation

;for z-only gradients:
;gpz1: CTP gradient
;gpz2: CTP gradient
;gpz3: CTP gradient
;gpz11: z-filter 7.2%
;gpz12: z-filter -7.5%
;gpz13: purge gradient -17.9%

;use gradient files:
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam11: RECT.1
;gpnam12: RECT.1
;gpnam13: SMSQ10.100

;cnst1: > J(HH) [30Hz]
;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]

;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 2
;NS: 8*n
;DS: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEQ

;preprocessor-flags-start
;CWPR: presaturation of solvent at beginning of pulsesequenece
; option -DCWPR (eda: ZGOPTNS)
;preprocessor-flags-end

```

5.8 2D CLIP-COSY relayed (n=2) with F1-*perfectBASH* homonuclear decoupling

```
; RELAYED CLIP-COSY WITH F1-HOMODECOUPLING USING PERFECT-BASH
;
; This pulse sequence is part of the paper:
; perfectBASH: Band selective homonuclear decoupling in peptides and peptidomimetics"
; Authors: Julian Ilgen, Lukas Kaltschnee, Christina M. Thiele
;
;
; Julian Ilgen and Lukas Kaltschnee
; Technical University Darmstadt
; Avance II+/III Version
; Topspin 3.x
;
;
; Description and Comments:
; The pulse sequence has been coded for test purposes only and may contain errors.
; It does contain arguments that can lead to hardware damages if acquisition parameters
; are set unfavourably. The functionality of the pulse sequence itself may differ
; depending on the hardware as well as the software used to execute it. Functionality
; on differing systems cannot be granted.
; Any use of this pulse sequence on a spectrometer is at your own risk!
; By using this pulse sequence or any modification of it in any published material
; you agree to acknowledge the above-mentioned publication.
;
; Clean In-phase COSY
; 2D H,H-correlation using in-phase transfer
; relayed CLIP-COSY with two steps
; pulse sequence is based on the CLIP-COSY relayed from Ref. (2)
; phase sensitive
; band selective homonuclear decoupling in F1 using frequency selective pulses incorporated in Perfect-Echo
; J is refocussed at the beginning of mixing
; option for solvent presaturation during relaxation delay possible in zg-options
; presaturation offset defined via cnst40 [ppm]
; avance-version (12/01/11)
;
;
; Relevant Papers:
;(1) M. R. Koos, G. Kummerloewe, L. Kaltschnee, C. M. Thiele and B. Luy, Angew. Chem. Int. Ed. 2016, 55, 7655.
;(2) T. Gyöngyösi, I. Timári, J. Haller, B. Luy, K. E. Kövér, ChemPlusChem 2018, 83(1), 53-60
;(3) J. Ilgen, L. Kaltschnee, C. M. Thiele, J. Magn. Reson. 2018, 286, 18-29
;(4) K. Zangger and H. Sterk, J. Magn. Reson., 1997, 124, 486-489.
;(5) J.A. Aguilar, M. Nilsson, G. Bodenhausen and G.A. Morris, Chem. Commun.; 2012, 48, 811-813.
;
;$CLASS=HighRes
;$DIM=2D
;$TYPE=
;$SUBTYPE=
;$COMMENT=

#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>
#include <De.incl>

define delay tauA

;;CLIP-COSY statements;;
"p2=p1*2"
"d6=1s/(cnst1*4)" ; coupling evolution delay in mixing step
"d11=30m"
"d12=20u"
"p35=p32"
"p36=p33"

;;Definitions for PerfectBASH;;
"in0=infl/2"
```

```

"d0=0u" ; incremented delay for chemical shift evolution during homodecoupling-block
"tauA=p16+d16"
"cnst21=cnst20*bf1" ; offset calculation for frequency selective refocusing
"cnst22=cnst21-o1"
"spoffs40=cnst22"

;;Calculations solvent presaturation;;
#ifdef CWPR
"d18=d1-d19"
"cnst41=cnst40*bf1"
"cnst42=cnst41-o1"
#else

#endif /*CWPR*/

;;begin pulsesequence;;
1 ze
2 d11
3 d12
4u

#ifdef CWPR ; begin of solvent presaturation

d12 fq=0:f1

d12 fq=cnst42 :f1 ; set frequency on f1-channel to solvent shift for presaturation [fq=SF01+cnst42]
d12 p19:f1 ; set power level on f1-channel for presaturation

d18 ; residual relaxation delay

d19 cw:f1 ph29 ; solvent presaturation
4u do:f1

d12 fq=0:f1 ; reset frequency on f1-channel [fq=SF01]

d12 p11:f1 ; reset power level on f1-channel

#else ; no solvent presaturation
d1
d12 p11:f1
#endif /*CWPR*/

50u UNBLKGRAD

4 p1 ph1 ; 90 high power excitation pulse

5u
5u p10:f1 ; power switching f1-channel

d0 ; Incremented delay

tauA

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

(p40:sp40 ph10:r):f1 ; first selective refocusing pulse

p16:gp1 ; CTP 1
d16 ; gradient recovery delay

tauA p11:f1 ; power switching f1-channel

d0 ; Incremented delay

10u

5 (p1 ph11):f1 ; 90 high power pulse for perfect echo J-removal

d0 ; Incremented delay

```

```

p16:gp2          ; CTP 2
d16              ; gradient recovery delay

(p2 ph12):f1     ; 180 high power pulse

10u
p16:gp2          ; CTP 2
d16              ; gradient recovery delay

p16:gp3          ; CTP 3
d16 p10:f1       ; gradient recovery delay + power switching f1-channel

(p40:sp40 ph12:r):f1 ; second selective refocusing pulse

p16:gp3          ; CTP 3
d16

10u p11:f1       ; power switching f1-channel

d0              ; Incremented delay

6 p1 ph7         ; first Thrippleton-Keeler-filter

5u p10:f1
(center(p35:gp11) (p32:sp28 ph8):f1)
5u
d16 p11:f1

7 p1 ph2         ; begin in-phase COSY mixing

d6              ; 1st transfer
p2 ph3
d6
p1 ph3
d6
p2 ph4
d6
d6              ; 2nd transfer (relay)
p2 ph13
d6
p1 ph13
d6
p2 ph4
d6

8 p1 ph5         ; second Thrippleton-Keeler-filter

5u p10:f1
(center(p36:gp12) (p33:sp29 ph9):f1)
5u
d16 p11:f1

p16:gp13         ; purge gradient
5u
d16 BLKGRAD

9 p1 ph6

go=2 ph31
d11 mc #0 to 2 F1PH(calph(ph1, +90) & calph(ph10, +90) & calph(ph11, +90) & calph(ph12, +90) , caldel(d0, +in0))
exit

;phase cycling
ph1=0 2          ; 90 excitation
ph2=2 2 2 2 0 0 0 0 ; 90 begin mixing
ph3= 1 3        ; 180 & 90 first mixing element
ph4= 3 2        ; 180 both mixing elements
ph13= 1 1 3 3   ; 180 & 90 second mixing element
ph5=0 0 2 2     ; 90 end mixing and begin second z-filter

```

```

ph6=0 ; 90 begin acquisition
ph7=0 ; 90 begin first z-filter
ph8=0 ; 180 adiabatic first z-filter
ph9=2 ; 180 adiabatic second z-filter
ph10=1 1 1 1 2 2 2 2 ; first selective refocusing pulse
ph11=1 3 1 3 ; 90 Perfect-Echo J-removal
ph12=0 ; 180 hard + second selective refocusing pulse
ph29=0 ; CW presaturation
ph31=0 2 2 0 0 2 2 0 ; receiver

;p1 : 90 degree high power pulse
;p2 : 180 degree high power pulse
;p16: homospoil/gradient pulse
;p32: 180 degree adiabatic pulse for ZQC-dephasing
;p33: 180 degree adiabatic pulse for ZQC-dephasing
;p35: duration z-filter dephasing gradient
;p36: duration z-filter dephasing gradient
;p40: duration of selective refocusing pulse

;p10: zero power
;p11 : f1 channel - power level for pulse (default)
;p19 : f1 channel - power level for presaturation

;sp28: adiabatic dephasing pulse
;sp29: adiabatic dephasing pulse
;spnam28: file name for adiabatic 180 pulse
;spnam29: file name for adiabatic 180 pulse
;sp40: selective refocusing pulse
;spw40: RF power of selective 180 pulse
;spnam40: file name for selective 180 pulse [ReBurp.1000]

;d0: incremented delay
;d1: relaxation delay: 1-5*T1
;d6: 1/(4J(HH))
;d11: delay for disk I/O [30 msec]
;d12: delay for power switching [20 usec]
;d16: delay for homospoil/gradient recovery
;d18: reduced relaxation delay
;d19: delay for solvent presaturation

;for z-only gradients:
;gpz1: CTP gradient
;gpz2: CTP gradient
;gpz3: CTP gradient
;gpz11: z-filter 7.2%
;gpz12: z-filter -7.5%
;gpz13: purge gradient -17.9%
;gpnam1: SMSQ10.100
;gpnam2: SMSQ10.100
;gpnam3: SMSQ10.100
;gpnam11: RECT.1
;gpnam12: RECT.1
;gpnam13: SMSQ10.100

;cnst1: > J(HH) [30Hz]
;cnst20: offset for selective refocusing [ppm]
;cnst40: solvent offset [ppm]

;inf1: 1/SW = 2 * DW
;in0: 1/(1 * SW) = 2 * DW
;nd0: 2
;NS: 8*n
;DS: 16
;td1: number of experiments
;FnMODE: States-TPPI, TPPI, States or QSEQ

;preprocessor-flags-start
;CWPR: presaturation of solvent at beginning of pulsesequence
; option -DCWPR (eda: ZGOTINS)
;preprocessor-flags-end

```

6 References

- [1] Susann Weißheit, Marie Kahse, Kerstin Kämpf, Alesia Tietze, Michael Vogel, Roland Winter, C. M. Thiele, Elastin-like Peptide in Confinement: FT-IR and NMR T1 relaxation data, *Z. Phys. Chem.*, under revision.
- [2] L. Kaltschnee, A. Kolmer, I. Timári, V. Schmidts, R. W. Adams, M. Nilsson, K. E. Kövér, G. A. Morris, C. M. Thiele, "Perfecting" pure shift HSQC: full homodecoupling for accurate and precise determination of heteronuclear couplings, *Chem. Commun.*, 50 (2014) 15702-15705.
- [3] M. Foroozandeh, R. W. Adams, N. J. Meharry, D. Jeannerat, M. Nilsson, G. A. Morris, Ultrahigh-resolution NMR spectroscopy, *Angew. Chem. Int. Ed.*, 53 (2014) 6990-6992.
- [4] K. Zangger, H. Sterk, Homonuclear broadband-decoupled NMR spectra, *J. Magn. Reson.*, 124 (1997) 486-489.
- [5] J. Ilgen, L. Kaltschnee, C. M. Thiele, A pure shift experiment with increased sensitivity and superior performance for strongly coupled systems, *J. Magn. Reson.*, 286 (2018) 18-29.