Chemistry–A European Journal
Research Article
doi.org/10.1002/chem.202400479
Chemistry Europe
European Chemical Societies Publishing
www.chemeurj.org

# Parametric Analysis of Donor Activation for Glycosylation Reactions

Mei-Huei Lin,[a, b] Yan-Ting Kuo,[a, b, c] José Danglad-Flores,[a] Eric T. Sletten,[a] and Peter H. Seeberger*[a, b]

The chemical synthesis of complex oligosaccharides relies on efficient and highly reproducible glycosylation reactions. The outcome of a glycosylation is contingent upon several environmental factors, such as temperature, acidity, the presence of residual moisture, as well as the steric, electronic, and conformational aspects of the reactants. Each glycosylation proceeds rapidly and with a high yield within a rather narrow temperature range. For better control over glycosylations and to ensure fast and reliable reactions, a systematic analysis of 18 glycosyl donors revealed the effect of reagent concentration, water content, protecting groups, and structure of the glycosyl donors on the activation temperature. With these insights, we parametrize the first step of the glycosylation reaction to be executed reliably and efficiently.

## Introduction

Glycosylation reactions have been explored since the seminal work of Fischer and Michael 130 years ago.[1–3] Conceptually, glycosylations are seemingly simple, as an activated leaving group at the anomeric position of an electrophilic glycosyl donor is displaced by a nucleophile, the glycosyl acceptor.[4] Yet, in practice, glycosylations are notoriously unreliable as properties of the reactants, such as stereochemistry, protecting group pattern, conformation, and leaving group, as well as the reaction conditions, including temperature, concentration, equivalents of activator, and residual water in the solvent influence the reactivity, selectivity, and efficiency of the reactions.[4,5] The effects of different factors concerning conditions and reactants on glycosylation selectivity and yield have been quantitated and among them, reaction temperature emerges as influential,[6–8] affecting both the rate of the desired reaction and the occurrence of undesired competing reactions.[9–14] Generally, it is accepted that glycosylations proceed rapidly within a relatively narrow temperature range. Below that temperature range, the reaction is sluggish, whereas, above that temperature range, side reactions begin to lower reaction efficiency. Therefore, glycosylations typically start at low temperatures (−78 °C) and slowly warm to ambient temperature over an ambiguous duration. In this way, the thermal condition that activates the glycosyl donor and subsequent attack of the nucleophile is reached without having to determine the exact temperature. This process is difficult to reproduce, results in unnecessarily long reaction times and side reactions, and requires excess reagents.[12,15] Variable-temperature (VT)-NMR has been employed to analyze reactive intermediates in glycosylation.[6,16] Identifying optimal temperatures for each glycosylation reaction would help suppress side reactions and optimizing both yield and glycosyl donor usage (Figure 1).

Glycosylations consist of two essential events that may occur most efficiently at two different temperatures: activation of the glycosyl donor and nucleophilic attack (Figure 1). Analyzing each step of a glycosylation is exceedingly pertinent for solid-phase oligosaccharide synthesis, as the activation reaction could occur in the bulk liquid-phase while the coupling to the dispersed solid support is restricted to less than 5% of the total reaction volume. In the context of solid phase synthesis, it is reasonable to assume that the activation step occurs virtually in absence of the desired glycosyl acceptor; Therefore, three general scenarios are proposed (Figure 1, bottom); i) extremely low temperature extends the life time of the glycosyl donor at the expense of process kinetics; ii) the optimal thermal conditions balance intermediate stability, mass transport towards the acceptors, and coupling rate;[17] while iii) elevated temperatures compromise the glycosyl donor before reaching the acceptor site. Therefore, a mismatch in reaction kinetics and diffusion to and within the solid-phase will drastically affect coupling efficiency. For the automated glycan assembly process it is useful to establish independently activation and coupling conditions to reflect the chemical and physical progression of the process.[15,18–20]

[a] M.-H. Lin, Y.-T. Kuo, J. Danglad-Flores, E. T. Sletten, P. H. Seeberger
Department of Biomolecular Systems
Max-Planck Institute of Colloids and Interfaces
Am Mühlenberg 1, 14476 Potsdam (Germany)
E-mail: Peter.Seeberger@mpikg.mpg.de

[b] M.-H. Lin, Y.-T. Kuo, P. H. Seeberger
Department of Chemistry and Biochemistry
Freie Universität Berlin
Arnimallee 22, 14195 Berlin (Germany)

[c] Y.-T. Kuo
GlycoUniverseGmbH&Co.KGaA
Am Mühlenberg 11, 14476 Potsdam (Germany)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202400479

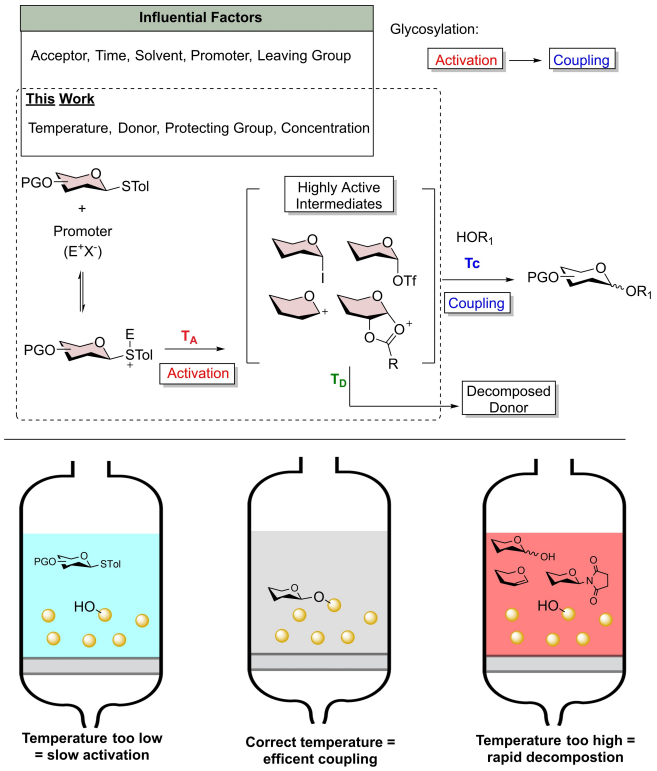


**Figure 1.** The work reported here focuses on the most important factors influencing glycosylation reactions. General mechanism of a glycosylation reaction involving thioglycoside glycosyl donors. PG = protecting group, $T_A$ = activation temperature, Tc = coupling temperature. $T_D$ = decomposition temperature. R = neighboring participating group. $R_1$ = glycosyl acceptor

Thioglycoside glycosyl donors are commonly used for AGA[21,22] and programmable one-pot reactions.[23,24] Due to the frequent use of this type of glycosyl donor, we investigated, through systematic isolation, the influence of reaction conditions and monosaccharide structural differences on the optimal glycosyl activation temperature in order to reduce deleterious side reactions and improve efficiency. In turn we see this as as a way to mitigate the need for use of excess donor in AGA, one of its major critiques.

Manual and semi-automated assays were used to determine the activation ($T_A$) and decomposition temperatures ($T_D$) of a set of thioglycoside monosaccharide glycosyl donors. Fundamental aspects that influence the temperature dependence of the activation process were deciphered to improve the reproducibility of the reactions. We then contrasted the findings to the concept of relative reactivity for further insights into the donor characteristics, temperature effects, and reactivity. The relative reactivity value (RRV) system serves as a valuable parameter for assessing the reactivity of tolyl thioglycosides.[25] These values are derived from a competitive high-performance liquid chromatography (HPLC) based experiment that involves a competition reaction between two glycosyl donors. The contrast between the assigned temperatures and their corresponding relative reactivity offered a potential correlation for quantitative estimation and conversion.[15,26]

## Results and Discussion

### Experimental Design

Manual and semi-automated assays were used to precisely measure the activation temperature for each glycosyl donor.[15] All necessary reagents are deposited in the reaction vessel for a single-temperature experiment without an exogenous nucleophile. The glycosyl donor is first added into the reaction vessel and cooled to the required temperature. Upon reaching the desired temperature, the activator solution was added to the solution of the glycosyl donor under constant agitation, and the mixture was left to react for five minutes. Next, the quenching solution is added, and the crude solution is transferred to another tube containing 10% aqueous sodium thiosulfate for quenching (for more details, see Supporting Information). This procedure was repeated at different temperatures using the same glycosyl donor.

The temperature of the reaction vessel was monitored in real-time using an internal K-type thermocouple in the automated system or a thermometer in case of manual experiments. Finally, $^1$H NMR was utilized to identify the activation temperature ($T_A$), defined as the highest temperature where the glycosyl donor remains unchanged in the reaction, and the decomposition temperature ($T_D$) of the glycosyl donor, the lowest reaction temperature where the glycosyl donor is consumed.

### Influence of Reaction Conditions on Activation of the Glycosyl Donor

In accordance with the Arrhenius equation, the reactant concentration, i.e. the amount of activator, acidity, and water concentration (Figure 2), will influence the glycosylation reaction rate at a given temperature. Increasing activator concentration enhances the production of side reactions, especially in the presence of a poor nucleophile or if activation occurs in the bulk solution-phase as for AGA.[4,11] Any residual water in the solvent of glycosylation reactions or other putative nucleophile (i.e. NIS) impacts glycosyl donor efficiency and reproducibility as it can trap and decompose activated glycosyl intermediates (Figure 2D).[27] The impact of these factors has been studied however in the context of the activation temperature has not been studied to date and may be one reason why glycosylation reactions are notoriously difficult to reproduce,[12] even when done in automation.

To investigate the influence of the concentration of the glycosyl donor on the activation temperature (Figure 2A), mannosyl thioglycoside **1** (90 mM) was activated with *N*-iodosuccinimide (NIS, 158 mM) and triflic acid (TfOH, 9 mM) between −8 and −2 °C ($T_A$ and $T_D$). Decreasing the concentration of **1** to 45 mM while maintaining the concentrations of all other reagents did not affect activation. However, there was a noticeable decrease in the activation temperature ($T_A$) as the glycosyl donor concentration was increased. Eventually, at

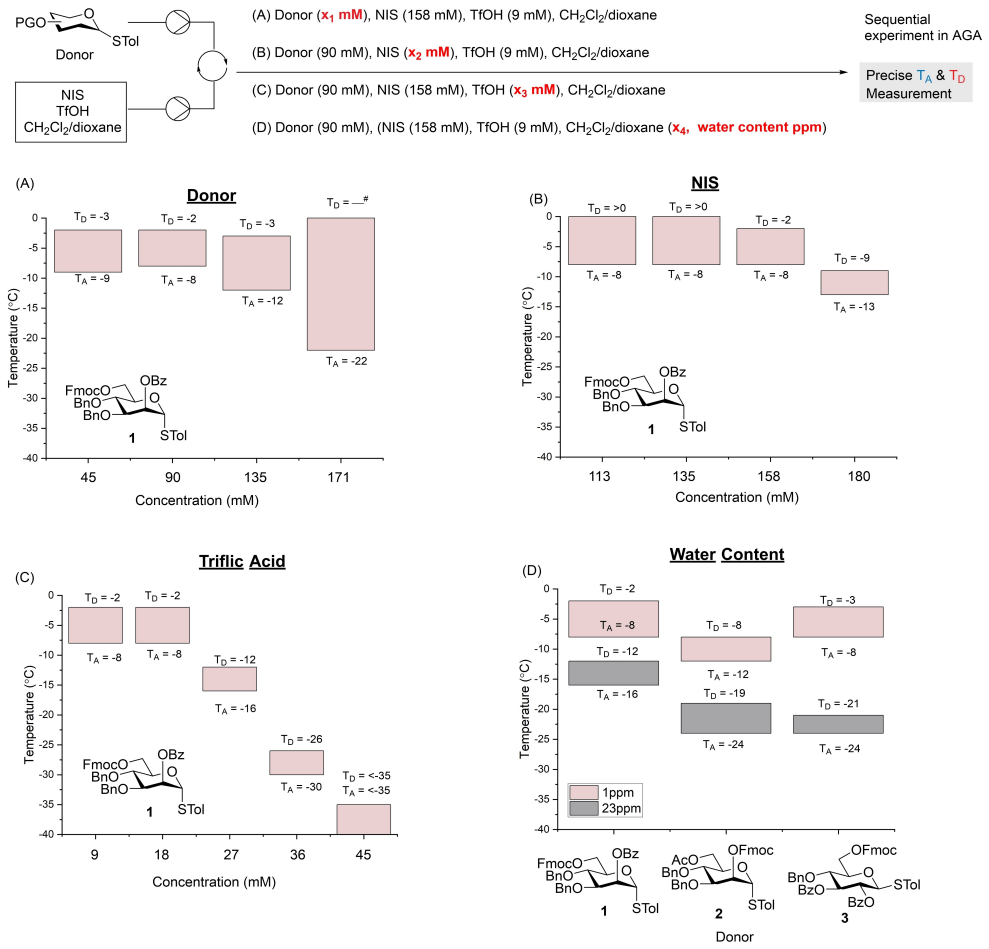


**Figure 2.** (A) Comparison of glycosyl donor concentration with activation and decomposition temperature of **1**. (B) Comparison of NIS concentration with activation and decomposition temperature of **1**. (C) Comparison of triflic acid concentration with activation and decomposition temperature of **1**. (D) Comparison between temperature outcomes of three different glycosyl donors **1**–**3** with a different water content of solvent. The gray bar represents 23 ppm water content, and the pink bar represents 1 ppm water content. The red $x_i$ denotes the variable. # no $T_D$ could be determined as NIS became the limiting reagent.

171 mM of **1**, no $T_D$ could be determined as NIS became the limiting reagent.

The reaction of the thioglycoside with NIS/TfOH converts the glycosyl donor into a highly reactive electrophile. Varying the NIS concentration from 113 to 158 mM had a marginal influence (Figure 2B), while at 180 mM NIS, the activation temperature was slightly lower. The triflate anion can serve as a nucleophile to form a transient covalent glycosyl triflate.[28,29] Therefore, the influence of changing concentrations of triflic acid on the activation temperature for glycosyl donor **1** was investigated (Figure 2C). Increasing the TfOH concentration accelerated the activation process and led to a decrease in activation temperature, potentially through increased formation of reactive intermediate(s).[11,12] With 9 or 18 mM of TfOH in the reaction, thioglycoside **1** was activated at higher temperatures ($T_A = -8\,°C$, $T_D = -2\,°C$), while at 45 mM TfOH, the glycosyl donor decomposed at $-35\,°C$.

Varying moisture levels in the solvent due to different vendors, water removal procedures, or even atmospheric humidity due to climate, season, and location can affect glycosylation performance.[30,31] This stems from its role as a competing nucleophile, leading to hydrolysis between the activated donor and water, generating hemiacetals and trehaloses.[9,27,32] Moisture typically is excluded by removing water from solvents before use. However, a rough estimation of the water content impact has practical value for reproducibility. To investigate the residual levels of water in the solvents on activation temperature, dichloromethane containing a measured water content of 23 ppm was compared to a solvent containing 1 ppm of water (Figure 2D). The temperature measurements were conducted using glycosyl donor **1**–**3**. A higher $T_A$ and $T_D$ were consistently observed with less water (1 ppm).[30] The activation temperature for mannose glycosyl donor **1** increased from $-16\,°C$ at 23 ppm by ten degrees in the presence of 1 ppm of water. The same trend was observed for mannoside **2** and glucoside **3**. The water concentration significantly influences the activation and decomposition temperature of the glycosyl donors. Increasing the moisture content can enhance the participation of water molecules from the organic solvent, acting as a nucleophile. The process traps the activated donors, and boosting the donor decomposition and reducing the temperature. Additionally, the reproducibility has

been evaluated with consistent low moisture control (1 ppm), and activation temperature remained at $T_A = \sim -8\,^{\circ}C$ and $T_D = \sim -2\,^{\circ}C$ (Figure S1 and S2). Semi-automated and manual batch reactions reproduced comparable activation temperatures (Figure S2).

### Structural Influence on Activation of Glycosyl Donors

Chemical glycan synthesis requires protecting groups to construct the desired linkages regioselectively.[21] These protecting groups significantly affect the reactivity of the glycosyl donors and the nucleophile.[25,33] While all C–O bonds are destabilizing to formation of an electrophilic center at the anomeric carbon, ester-type protecting groups tend to be more destabilizing than ether-type groups.[34–41] Protecting groups can change the intermediate conformation through steric impedance or neighboring and remote interaction, thereby regulating the reactivity and stereoselectivity of the glycosylation reaction.[42–45] A clear relation between activation temperature, glycosyl donor reactivity, and protecting groups remains to be defined.[33,46]

Thioglucoside and -glucosaminoside glycosyl donors **3**–**6** were compared to pinpoint the effect of the electronic contribution of protecting groups on the activation temperature (Figure 3).[4,22] As expected,[37] ester groups increased the $T_A$ of the glycosyl donor.[34,47–49] *N*-trichloroacetyl (NHTCA) protected glucosamine **6** required a lower temperature of activation when compared to glucoside **5**.[15,50,51] We postulate this occurs through enhancedanchimeric assistance of the amide or that the C–N bonds possess less destabilizing characteristics than C–O bonds.[52]

9-Fluorenylmethyloxycarbonyl carbonate (Fmoc), benzoate esters, and benzyl ethers are reliable temporary or permanent protecting groups widely used in AGA and solution phase oligosaccharide synthesis.[53] We then screened the effect of different positioning of the Fmoc group and different sugar types (glucose, mannose, galactose, and glucosamine) on the activation temperature (Figure 4). With Fmoc protection at the C6 hydroxyl (Figure 4A), the observed $T_A$ and $T_D$ decreases from mannose (Man) > glucose (Glc) > *N*-trichloroacetyl glucosamine (GlcNTCA) and galactose (Gal) in line with earlier findings.[15] When Fmoc is placed at the C4 position, the order changes slightly: Man > Glc > Gal > GlcNTCA (Figure 4B). Intriguingly, the order changes to Glc > GlcNTCA ≈ Man > Gal when the Fmoc is at the C3 position (Figure 4C), as there is a significant influence on GlcNTCA. Next, we examined the impact of varying the Fmoc position on the $T_A$ and $T_D$ of each sugar type. For mannose (Figure 4D), the observed $T_A$ and $T_D$ order from highest to lowest is 6-OFmoc > 4-OFmoc ≈ 3-OFmoc. However, in the cases of both Glc (Figure 4E) and GlcNTCA (Figure 4F) the order was 3-OFmoc > 6-OFmoc > 4-OFmoc. In the case of galactose (Figure 4G), the temperature trend follows 4-OFmoc > 3-OFmoc ≈ 6-OFmoc. The type of sugar considerably affects $T_A$ and $T_D$, whereas the position of Fmoc had a comparatively moderate influence.

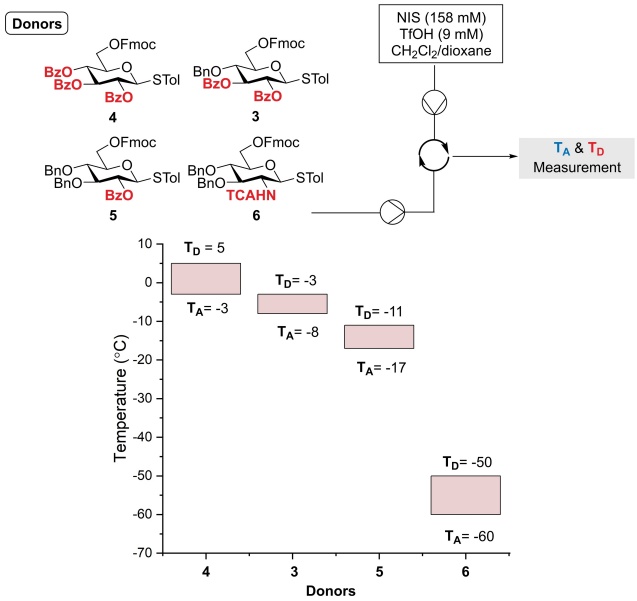


**Figure 3.** Influence of protecting groups on activation temperature of four glycosyl donors **3**–**6**.

### Comparison to the RRV System

Programmable one-pot syntheses rely on marked differences in the reactivity of the glycosyl donors used. Relative reactivity values (RRV) have been assigned to a host of thioglycoside glycosyl donors for the solution-phase method. The RRVs are determined via competitive HPLC-based experiments for many monosaccharides.[23,25,26,54]

For the 18 glycosyl donors, we investigated the correlation between RRVs and $T_A$ or $T_D$ (Figure 5 and Figure S3). Generally, the higher the RRV of a thioglycoside glycosyl donor, the lower the $T_A$ and $T_D$ in the order Man > Glc > GlcNTCA > Gal. This correlation also held for 3-Fmoc glucosamine **12**, an apparent singularity in the systematic study. The exceptionally high activation temperature (−18 °C) correlated well with the RRV of 40. This markedly different reactivity may result from additional stabilization or steric effects, but the exact reasons remain unclear.

## Conclusions

We present a detailed investigation into the factors that affect the activation temperature of different glycosyl donors, including reagent concentrations, protecting groups, and sugar moieties, for a better understanding and control of glycosylation reactions. Controlling the activator concentration and the water content is essential when selecting the optimal reaction temperature. Assigning activation ($T_A$) and decomposition temperature ($T_D$) for each glycosyl donor sets the basis for shorter, higher-yielding glycosylation reactions. Relative reactivity values already established for various thioglycoside

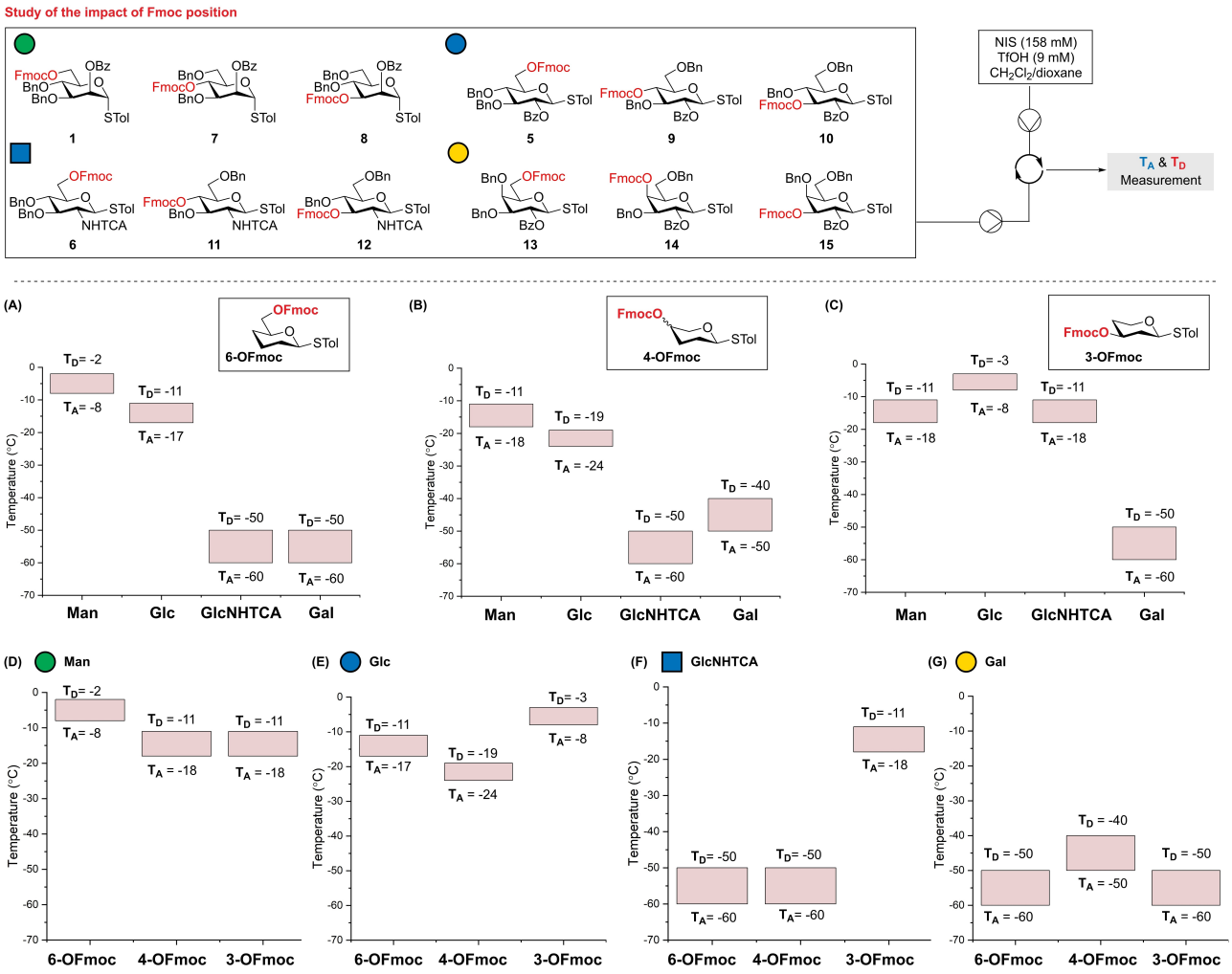


**Figure 4.** Sugar type and position of the Fmoc protecting group influence the activation temperature of thioglycoside glycosyl donors (A) 6-OFmoc (B) 4-OFmoc (C) 3-OFmoc (D) Mannose (E) Glucose (F) *N*-trichloroacetyl glucosamine (G) Galactose.

glycosyl donor for programmable one-pot syntheses guide the selection of reaction temperatures.

This study comprehensively evaluated the factors influencing the glycosyl donor but excluded the nucleophile. Therefore, future work will study the impact of nucleophiles on the outcome of glycosylation reactions.[55,56] Combining our activation temperature/reactivity charts with machine-learning techniques will eventually help establish glycosylation temperature settings and reduce the need for trial-and-error testing.

## Experimental Section

### Semi-automated Temperature Assay

A detailed description of the experimental setup can be found in the SI and in previous publications.[15] The system has four main channels: a donor, activator, quenching solution, and output line. All required reagents were automatically introduced into the reaction vessel for an isothermal experiment without a nucleophile. Thioglycoside donors were selected for the research as it is commonly utilized and can be easily activated with NIS and TfOH.[57] The general procedure involved four steps: glycosyl donor delivery, activator delivery, quenching, and NMR analysis. First, the glycosyl donor solution was introduced to the reaction vessel and cooled to the set temperature. Once the desired temperature was reached, 1 mL of activator solution was added to the donor solution and mixed by bubbling with Ar for 5 min. Subsequently, 1 mL of the 10% pyridine in DMF was added, and the crude solution was transferred to a supplementary external vessel containing 2 mL of quenching solution (10% aqueous sodium thiosulfate). The same procedure was then followed for each desired temperature with the same glycosyl donor. An internal thermocouple K-type monitored the real-time temperature of the solution. Finally, a proton NMR was utilized to identify both $T_A$ and $T_D$ of the glycosyl donor.

### Synthesis of Glycosyl Donors

A comprehensive description of the experimental setup and NMR spectrum is provided in the SI. Compound **7** can be synthesized from **S2**–**S9**,[58–60] while compound **8** can be synthesized from **S5**–**S15**.[61,62] Compound **4** can be synthesized from **S16**–**S2**,[43,63] and compound **5** from **S22**–**S25**.[64–66] Compound **10** can be synthesized from **S22**–**S30**,[67–69] and compound **12** from **S33**–**S38**.[70,71]

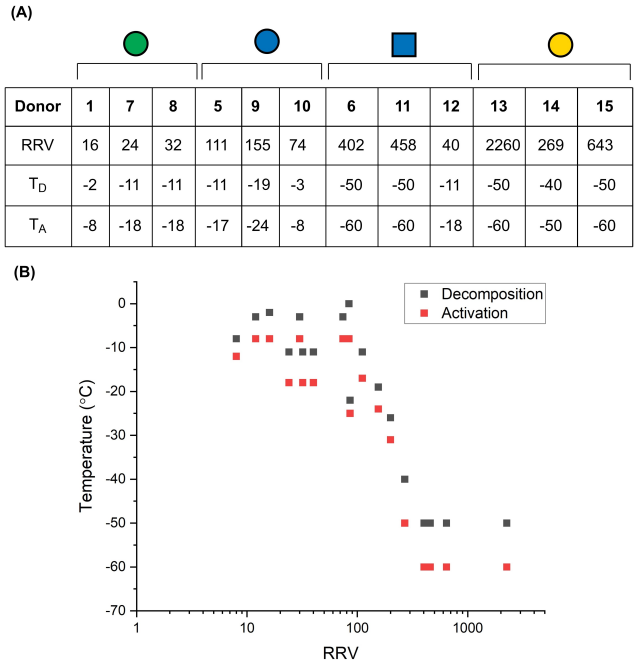


**Figure 5.** Comparison of thioglycoside glycosyl donor relative reactivity values and activation temperatures. (A) Table for experimental RRV, $T_A$, and $T_D$ of different thioglycosides. The relationship between the RRV and $T_D$ (B) The relationship between the RRV, $T_A$, and $T_D$ includes previously measured values. See Supporting Information for details.

## Acknowledgements

We gratefully acknowledge the Max-Planck Society for its generous financial support. Y.-T. K. acknowledges financial support from the European Union's Horizon2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 956758. Open Access funding enabled and organized by Projekt DEAL.

## Conflict of Interests

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:** Carbohydrates · Glycosylations · Thioglycoside · Protecting groups

[1] E. Fischer, *Ber. Dtsch. Chem. Ges.* **1893**, *26*, 2400–2412.
[2] E. Fischer, K. Delbrück, *Ber. Dtsch. Chem. Ges.* **1909**, *42*, 1476–1482.
[3] A. Michael, *Am. Chem. J.* **1879**, *1*, 305–312.
[4] P. O. Adero, H. Amarasekara, P. Wen, L. Bohe, D. Crich, *Chem. Rev.* **2018**, *118*, 8242–8284.
[5] C. J. Crawford, P. H. Seeberger, *Chem. Soc. Rev.* **2023**, *52*, 7773–7801.
[6] T. G. Frihed, M. Bols, C. M. Pedersen, *Chem. Rev.* **2015**, *115*, 4963–5013.
[7] D. Crich, *Acc. Chem. Res.* **2010**, *43*, 1144–1153.
[8] C.-W. Chang, M.-H. Lin, C.-C. Wang, *Chem. Eur. J.* **2021**, *27*, 2556–2568.
[9] S. Chatterjee, S. Moon, F. Hentschel, K. Gilmore, P. H. Seeberger, *J. Am. Chem. Soc.* **2018**, *140*, 11942–11953.
[10] M.-H. Lin, J. B. Wolf, E. T. Sletten, D. Cambié, J. Danglad-Flores, P. H. Seeberger, *ChemBioChem* **2023**, *24*, e202200607.
[11] H. H. Trinderup, S. M. Andersen, M. Heuckendorff, H. H. Jensen, *Eur. J. Org. Chem.* **2021**, 3251–3259.
[12] P. R. Andreana, D. Crich, *ACS Cent. Sci.* **2021**, *7*, 1454–1462.
[13] V. A. Demchenko, *Curr. Org. Chem.* **2003**, *7*, 35–79.
[14] S. Moon, S. Chatterjee, P. H. Seeberger, K. Gilmore, *Chem. Sci.* **2021**, *12*, 2931–2939.
[15] O. T. Tuck, E. T. Sletten, J. Danglad-Flores, P. H. Seeberger, *Angew. Chem. Int. Ed.* **2022**, *61*, e202115433.
[16] Y. Qiao, W. Ge, L. Jia, X. Hou, Y. Wang, C. M. Pedersen, *Chem. Commun.* **2016**, *52*, 11418–11421.
[17] S. Pinzón-López, M. Kraume, J. Danglad-Flores, P. H. Seeberger, *React. Chem. Eng.* **2023**, *8*, 2951–2962.
[18] C. M. Runnels, K. A. Lanier, J. K. Williams, J. C. Bowman, A. S. Petrov, N. V. Hud, L. D. Williams, *J. Mol. Evol.* **2018**, *86*, 598–610.
[19] R. B. Merrifield, *Science* **1965**, *150*, 178–185.
[20] P. H. Seeberger, *Acc. Chem. Res.* **2015**, *48*, 1450–1463.
[21] J. Danglad-Flores, S. Leichnitz, E. T. Sletten, A. Abragam Joseph, K. Bienert, K. Le Mai Hoang, P. H. Seeberger, *J. Am. Chem. Soc.* **2021**, *143*, 8893–8901.
[22] A. A. Joseph, A. Pardo-Vargas, P. H. Seeberger, *J. Am. Chem. Soc.* **2020**, *142*, 8561–8564.
[23] C.-W. Cheng, Y. Zhou, W.-H. Pan, S. Dey, C.-Y. Wu, W.-L. Hsu, C.-H. Wong, *Nat. Commun.* **2018**, *9*, 5202.
[24] C.-W. Cheng, C.-Y. Wu, W.-L. Hsu, C.-H. Wong, *Biochemistry* **2020**, *59*, 3078–3088.
[25] Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734–753.
[26] J.-C. Lee, W. A. Greenberg, C.-H. Wong, *Nat. Protoc.* **2007**, *1*, 3143.
[27] H. M. Christensen, S. Oscarson, H. H. Jensen, *Carbohydr. Res.* **2015**, *408*, 51–95.
[28] C.-W. Chang, M.-H. Lin, T.-Y. Chiang, C.-H. Wu, T.-C. Lin, C.-C. Wang, *Sci. Adv.* **2023**, *9*, eadk0531.
[29] D. Crich, S. Sun, *J. Am. Chem. Soc.* **1997**, *119*, 11217–11223.
[30] D. B. G. Williams, M. Lawton, *J. Org. Chem.* **2010**, *75*, 8351–8354.
[31] C. Raymond, T. Matthews, R. M. Horton, *Sci. Adv.* **2020**, *6*, eaaw1838.
[32] C.-W. Chang, M.-H. Lin, C.-H. Wu, T.-Y. Chiang, C.-C. Wang, *J. Org. Chem.* **2020**, *85*, 15945–15963.
[33] Y. Zhu, M. Delbianco, P. H. Seeberger, *J. Am. Chem. Soc.* **2021**, *143*, 9758–9768.
[34] B. Fraser-Reid, G. Anilkumar, L. G. Nair, L. Olsson, M. G. Martin, J. K. Daniels, *Isr. J. Chem.* **2000**, *40*, 255–262.
[35] K. Muru, C. Gauthier, *Chem. Rec.* **2021**, *21*, 2990–3004.
[36] Y. Zeng, Z. Wang, D. Whitfield, X. Huang, *J. Org. Chem.* **2008**, *73*, 7952–7962.
[37] D. R. Mootoo, P. Konradsson, U. Udodong, B. Fraser-Reid, *J. Am. Chem. Soc.* **1988**, *110*, 5583–5584.
[38] Z. Li, J. C. Gildersleeve, *Tetrahedron Lett.* **2007**, *48*, 559–562.
[39] D. Crich, M. Li, *Org. Lett.* **2007**, *9*, 4115–4118.
[40] A. A. Hettikankanamalage, R. Lassfolk, F. S. Ekholm, R. Leino, D. Crich, *Chem. Rev.* **2020**, *120*, 7104–7151.
[41] P. O. Adero, H. Amarasekara, P. Wen, L. Bohé, D. Crich, *Chem. Rev.* **2018**, *118*, 8242–8284.
[42] K. Greis, C. Kirschbaum, G. Fittolani, E. Mucha, R. Chang, G. von Helden, G. Meijer, M. Delbianco, P. H. Seeberger, K. Pagel, *Eur. J. Org. Chem.* **2022**, e202200255.
[43] M.-H. Lin, C.-W. Chang, T.-Y. Chiang, V. M. Dhurandhare, C.-C. Wang, *Org. Lett.* **2021**, *23*, 7313–7318.
[44] M. Marianski, E. Mucha, K. Greis, S. Moon, A. Pardo, C. Kirschbaum, D. A. Thomas, G. Meijer, G. von Helden, K. Gilmore, P. H. Seeberger, K. Pagel, *Angew. Chem. Int. Ed.* **2020**, *59*, 6166–6171.
[45] T. Hansen, H. Elferink, J. M. A. van Hengst, K. J. Houthuijs, W. A. Remmerswaal, A. Kromm, G. Berden, S. van der Vorm, A. M. Rijs, H. S. Overkleeft, D. V. Filippov, F. P. J. T. Rutjes, G. A. van der Marel, J. Martens, J. Oomens, J. D. C. Codée, T. J. Boltje, *Nat. Commun.* **2020**, *11*, 2664.
[46] K. Greis, S. Leichnitz, C. Kirschbaum, C.-W. Chang, M.-H. Lin, G. Meijer, G. von Helden, P. H. Seeberger, K. Pagel, *J. Am. Chem. Soc.* **2022**, *144*, 20258–20266.

[47] P. Grice, S. V. Ley, J. Pietruszka, H. M. I. Osborn, H. W. M. Priepke, S. L. Warriner, *Chem. Eur. J.* **1997**, *3*, 431–440.
[48] P. Konradsson, U. E. Udodong, B. Fraser-Reid, *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
[49] N. L. Douglas, S. V. Ley, U. Lücking, S. L. Warriner, *J. Chem. Soc. Perkin Trans. 1* **1998**, *1*, 51–66.
[50] M. Heuckendorff, C. M. Pedersen, M. Bols, *Org. Lett.* **2011**, *13*, 5956–5959.
[51] L. T. Poulsen, M. Heuckendorff, H. H. Jensen, *ACS Omega* **2018**, *3*, 7117–7123.
[52] R. P. Iczkowski, J. L. Margrave, *J. Am. Chem. Soc.* **1961**, *83*, 3547–3551.
[53] M. Guberman, P. H. Seeberger, *J. Am. Chem. Soc.* **2019**, *141*, 5581–5592.
[54] C.-W. Chang, C.-H. Wu, M.-H. Lin, P.-H. Liao, C.-C. Chang, H.-H. Chuang, S.-C. Lin, S. Lam, V. P. Verma, C.-P. Hsu, C.-C. Wang, *Angew. Chem. Int. Ed.* **2019**, *58*, 16775–16779.
[55] S. van der Vorm, T. Hansen, J. M. A. van Hengst, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, *Chem. Soc. Rev.* **2019**, *48*, 4688–4706.
[56] C. W. Chang, M. H. Lin, C. K. Chan, K. Y. Su, C. H. Wu, W. C. Lo, S. Lam, Y. T. Cheng, P. H. Liao, C. H. Wong, C. C. Wang, *Angew. Chem. Int. Ed.* **2021**, *60*, 12413–12423.
[57] R. Das, B. Mukhopadhyay, *ChemistryOpen* **2016**, *5*, 401–433.
[58] S. Escopy, Y. Singh, A. V. Demchenko, *Org. Biomol. Chem.* **2019**, *17*, 8379–8383.
[59] T. Polat, C.-H. Wong, *J. Am. Chem. Soc.* **2007**, *129*, 12795–12800.
[60] R. B. Zheng, S. A. F. Jégouzo, M. Joe, Y. Bai, H.-A. Tran, K. Shen, J. Saupe, L. Xia, M. F. Ahmed, Y.-H. Liu, P. S. Patil, A. Tripathi, S.-C. Hung, M. E. Taylor, T. L. Lowary, K. Drickamer, *ACS Chem. Biol.* **2017**, *12*, 2990–3002.
[61] Y. H. Lin, B. Ghosh, K.-K. Tony Mong, *Chem. Commun.* **2012**, *48*, 10910–10912.
[62] K.-K. Tony Mong, K.-S. Shiau, Y. H. Lin, K.-C. Cheng, C.-H. Lin, *Org. Biomol. Chem.* **2015**, *13*, 11550–11560.
[63] M. E. Giorgi, R. Lopez, R. Agusti, C. Marino, R. M. de Lederkremer, *Carbohydr. Res.* **2017**, *450*, 30–37.
[64] Y.-H. Wang, H.-W. Yeh, H.-W. Wang, C.-C. Yu, J.-H. Guh, D.-Z. Liu, P.-H. Liang, *Carbohydr. Res.* **2013**, *375*, 118–135.
[65] M. W. Weishaupt, S. Matthies, M. Hurevich, C. L. Pereira, H. S. Hahm, P. H. Seeberger, *Beilstein J. Org. Chem.* **2016**, *12*, 1440–1446.
[66] R. Yadav, S. Leviatan Ben-Arye, B. Subramani, V. Padler-Karavani, R. Kikkeri, *Org. Biomol. Chem.* **2016**, *14*, 10812–10815.
[67] X. Zou, C. Qin, C. L. Pereira, G. Tian, J. Hu, P. H. Seeberger, J. Yin, *Chem. Eur. J.* **2018**, *24*, 2868–2872.
[68] G. Liao, Z. Zhou, S. Burgula, J. Liao, C. Yuan, Q. Wu, Z. Guo, *Bioconjugate Chem.* **2015**, *26*, 466–476.
[69] G. Liao, S. Burgula, Z. Zhou, Z. Guo, *Eur. J. Org. Chem.* **2015**, 2942–2951.
[70] Z. Tu, H.-W. Hsieh, C.-M. Tsai, C.-W. Hsu, S.-G. Wang, K.-J. Wu, K.-I. Lin, C.-H. Lin, *Chem. Asian J.* **2013**, *8*, 1536–1550.
[71] X. Lu, M. N. Kamat, L. Huang, X. Huang, *J. Org. Chem.* **2009**, *74*, 7608–7617.

Manuscript received: February 2, 2024
Accepted manuscript online: March 28, 2024
Version of record online: April 17, 2024