Unravelling glycosylation reaction mechanisms

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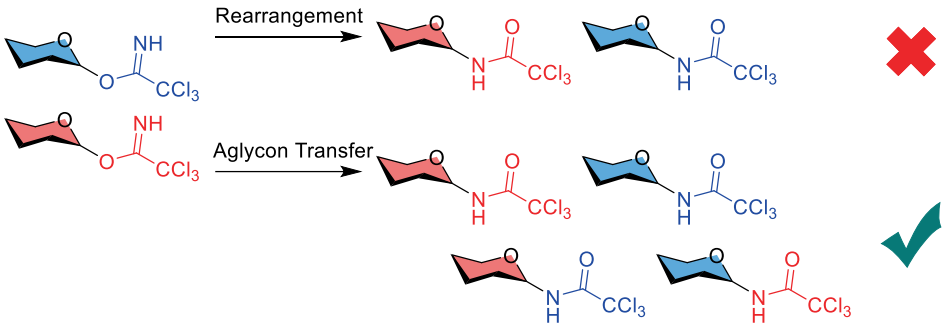
Chapter 2: “Rearrangement” of trichloroacetamide donors occurs through an intramolecular aglycon transfer mechanism

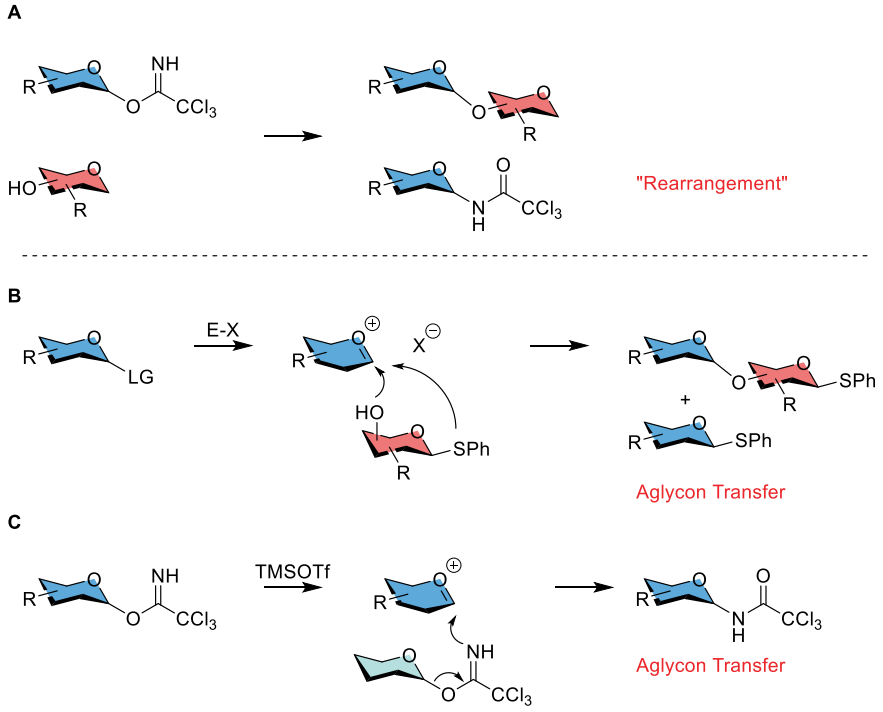


Published: van de Vrande, K. N. A.; Filippov, D. V.; Codée, J. D. C. *Org Lett* **2023**, *25* (33), 6128–6132. https://doi.org/10.1021/acs.orglett.3c02196.

Introduction

Glycosyl imidates have been one of the most popular glycosylating agents,[1] ever since their first conception by Sinaÿ and co-workers[2,3] and the introduction of the trichloroacetimidates by Schmidt and Michel.[4] Trichloroacetimidates have been applied in many ground-breaking syntheses of biologically relevant oligosaccharides and have been used on an industrial scale, as exemplified by the multi-kg scale production ArixtraTM, the synthetic heparin-type pentasaccharide anticoagulant.[5] Their popularity stems from the fact that they can be easily prepared from the corresponding lactol precursors and trichloroacetonitrile and rapidly activated using a catalytic amount of (Lewis)-acid. The high reactivity of trichloroacetamides, however, may lead to side reactions under glycosylation conditions and the formation of donor derived trichloroacetamides has been observed on many occasions, necessitating the use of an excess of expensive building blocks (See Scheme 1A).[6] The formation of the amide side product is often referred to as a "rearrangement" reaction, suggesting that it takes place through a unimolecular reaction.[6–9] To prevent the formation of the trichloroacetamide side product Schmidt and Toepfer have introduced the 'inverse glycosylation procedure'. In this procedure the acceptor and activator are pre-mixed before the (slow) addition of the donor as Schmidt and Toepfer reasoned that the formation of an 'acceptor-activator complex' in the absence of a donor would prevent donor decomposition.[8]

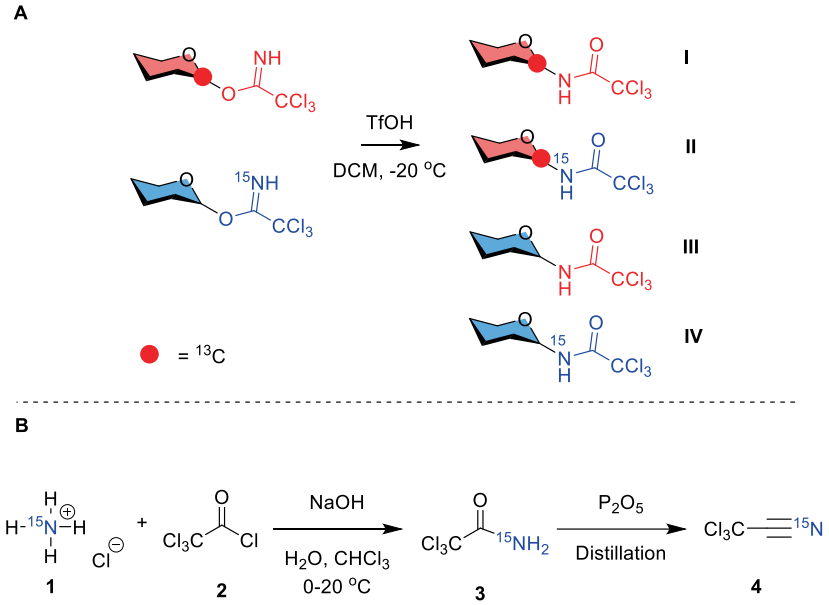
Thioglycosides are another very popular class of glycosyl donor glycosides because these are shelf-stable and can be activated in a selective manner using a range of soft electrophiles. This has led to the widespread use of these building blocks in chemoselective one-pot glycosylation procedures in which a thioglycoside building block having a free hydroxy group is used as an acceptor glycoside to generate a larger thioglycoside, that can immediately be used for the next glycosylation through activation of the thioacetal.[10] The nucleophilicity of the anomeric thiol, however, may lead to a reaction of the thioacetal with an activator donor, leading to the transfer of the thio-aglycon of the acceptor to the donor (Scheme 1B). This "aglycon transfer" process becomes important when the acceptor alcohol is relatively unreactive.[11] Other donor types can undergo similar side reactions and even the intermolecular transfer of an p-methoxy phenol group from an acceptor to an activated donor has been observed.[12]



Scheme 1: Possible mechanisms for the "rearrangement" of glycosyl imidates to glycosyl amides.

The wide-spread occurrence of intermolecular aglycon transfer reactions combined with the high nucleophilicity of the trichloroacetimidate imine functionality suggests that the trichloroacetamide side products in glycosylation reaction of trichloroacetamide donors may originate from an intermolecular aglycon transfer type process rather than an unimolecular rearrangement, as shown in Scheme 1C. To unravel the mechanism underlying the formation of trichloroacetamide side products, a series of cross-over experiments using $^{13}C/^{15}N$ labelled glycosyl imidate donors are described here.

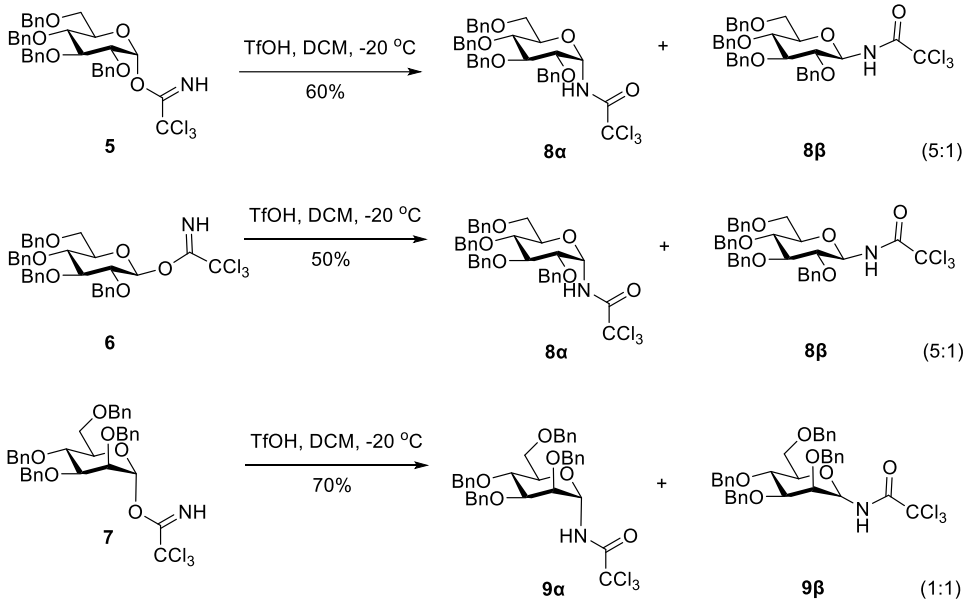
Results and Discussion



Scheme 2: (A) Starting materials and products of the $^{13}C/^{15}N$ exchange experiments. (B) Synthesis of ^{15}N labelled trichloroacetonitrile.

The experiment designed to differentiate between the intramolecular rearrangement and the intermolecular aglycon transfer mechanisms is depicted in Scheme 2A. Here two different isotopically labelled derivatives of the same donor molecule were used: one containing an anomeric ^{13}C-label and the other a ^{15}N-labelled imidate. This label could be obtained from ^{15}N-ammonium chloride **1** in two steps, as depicted in Scheme 2B. ^{15}N-Ammonium chloride was reacted with trichloroacetyl chloride **2**, to yield trichloroacetamide **3**,[13] which was subsequently distilled over phosphorus pentoxide to deliver ^{15}N-labelled trichloroacetonitrile **4**, which was used to synthesize the ^{15}N imidate donors.[14] The ^{13}C-labelled donors were synthesised from the commercially available mono-^{13}C-labelled monosaccharides following well-established procedures. The "rearrangement" experiment depicted in Scheme 2 started by preparing a 1 : 1 mixture of anomeric ^{13}C- and ^{15}N-labelled imidate donors. This mixture was subjected to 10 mol% of triflic acid in DCM for 30 minutes, during which the amide products were formed. An intramolecular mechanism would allow the generation of amides **I** and **IV**, where the initial label is retained, and exchange has not taken place (Scheme 2A). An intermolecular mechanism, on the other hand, can lead to a mixture of all four amides **I-IV**. Of the possible amides, compounds **II** and **III** can only be formed by intermolecular aglycon transfer. Of these amides, **II** can be used to report on the intermolecular aglycon transfer as it contains both the ^{13}C and the ^{15}N label and both labels are NMR-active nuclei.[15,16] For the experiments α- and β-configured glucosyl donors **5** and

6, as well as, α- mannosyl imidate **7** were employed, providing amide products **8** and **9**, respectively.



Scheme 3: Glycosyl imidate donors (**5-7**) and amide products (**8-9**).

As shown in Scheme 3, donors **5** and **6** yielded product **8α/β**, with an identical anomeric ratio of 5 : 1. Product **8α** obtained from **5** was isolated as a pure product and used for analysis.[17,18] Part of the ^{1}H NMR of **8α** is depicted in Figure 1A, zooming in on the NH- and H-1 region of the spectrum. For both peaks, a characteristic pattern can be seen, where a major middle peak is flanked by two smaller peaks. For both resonances, the middle peaks correspond to the protons that are not directly attached to either ^{15}N or ^{13}C, while the minor peaks are the protons directly coupled to ^{15}N or ^{13}C, having characteristic coupling constants of 92.3 Hz for ^{1}H-^{15}N and 164.8 Hz for ^{1}H-^{13}C. The integral of the middle peaks equals the total integral of the two flanking peaks. This indicates that in the product, 50% ^{13}C and 50% ^{15}N labels are incorporated.

In Figure 1B, the C-1 region of the ^{13}C NMR of **8α** is depicted. Here two different peaks for C-1 can be observed: a singlet at 77.15 ppm and a doublet at 77.14 ppm. For the doublet, a coupling of 10.2 Hz is observed, typical for a one-bond ^{13}C-^{15}N coupling.[16] This same coupling constant can be observed in the ^{15}N NMR spectrum of **8α** (Figure 1E). The small difference in chemical shift between the singlet and doublet observed in the ^{13}C spectrum originates from a ^{15}N isotope effect.[19] The resonances observed correspond to amide **I** (the singlet at 77.15 ppm) and amide **II** (the doublet at 77.14 ppm). Integration of the peaks shows that the singlet and doublet are present in equimolar amounts. This product ratio can only be obtained when an intermolecular reaction has taken place, where complete scrambling of the labels takes place.

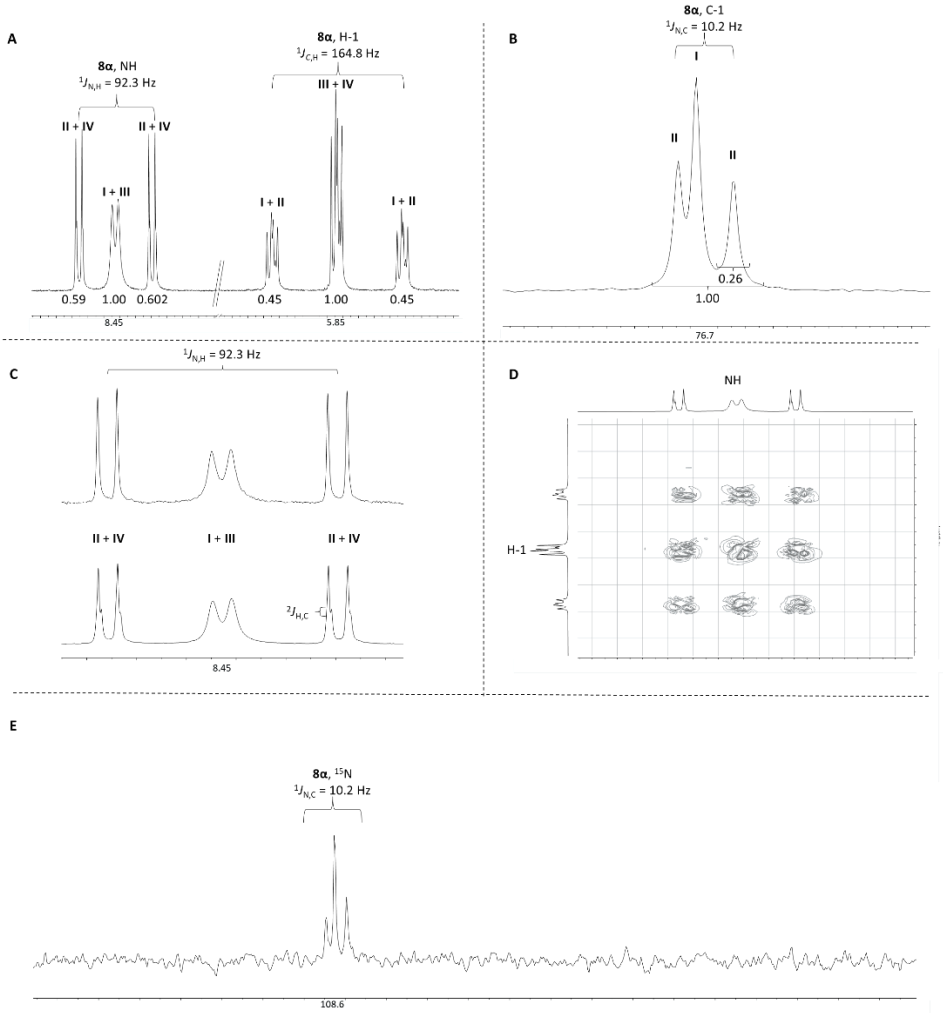
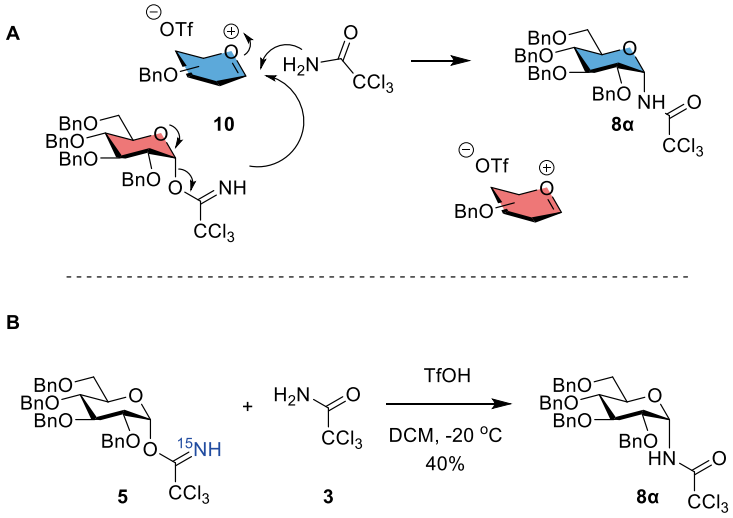


Figure 1: (A) ^{1}H NMR of **8α**, zoomed in onto the NH and H-1 region. (B) ^{13}C NMR of **8α**, zoomed in onto C-1. (C) (top) ^{13}C decoupled ^{1}H NMR of **8α**, zoomed in onto the NH region; (bottom) 'Normal' ^{1}H NMR. (D) COSY of **8α**, zoomed in onto the region between H-1 and NH. (E) ^{15}N NMR of **8α**.

Additional evidence for the formation of the distinctive amide **II** is provided by the spectra depicted in Figures 1C and 1D. In Figure 1A a small coupling of 2 Hz can be observed for the peaks split by ^{1}H-^{15}N coupling, which originates from the $^2J_{CH}$ coupling of the anomeric ^{13}C atom to the anomeric proton. In the ^{13}C-decoupled spectrum (Figure 1C), this coupling is absent turning the ddd-splitting into a dd peak. As the $^2J_{CH}$ is visible for the peaks that also couple to the ^{15}N atom this indicates that both labels are present in the same molecule. Furthermore, in the ^{1}H-^{1}H COSY spectrum of **8α** (Figure 1D), all resonances of the NH proton and H-1 correlate. Thus, the resonance of H1 that experiences a coupling to the ^{13}C correlates to the NH that is split by a ^{1}H-^{15}N coupling. Overall, these NMR experiments provide solid proof that the ^{13}C and ^{15}N labels are present in the same product and, thus,

that the glucosyl amides form through an intermolecular aglycon transfer mechanism (Scheme 1C), rejecting the intramolecular imidate rearrangement (Scheme 1A).

For the mannosyl imidate, similar results were obtained. The α-mannosyl imidate **7** (Scheme 3) provided both the α and β amides in a 1 : 1 ratio, and in both products, the ^{13}C and ^{15}N were incorporated, leading to relative integrals identical to those observed for the α-glucosyl amides.



Scheme 4: (A) Aglycon transfer from another trichloroacetimidate donor or from the generated trichloroacetamide. (B) Experiment designed to differentiate between these mechanisms.

While the experiments described above have excluded an intramolecular rearrangement mechanism, scrambling of the ^{13}C and ^{15}N labels may also occur in a bimolecular reaction, in which the trichloroacetamide (TCA) that is formed upon activation attacks an activated donor species. While the trichloroacetimidate should be significantly more nucleophilic than the amide, this process cannot be ruled out on the basis of the experiments described above.[6] Therefore, the experiment shown in Scheme 4 was designed: ^{15}N-labelled glucosyl imidate **5** was activated in the presence of either an equimolar amount or an excess of unlabelled TCA **3**. If an attack by TCA is a favourable pathway, a significant decrease of ^{15}N label in the product is to be observed.

The NH region of the NMR of **8α**, formed in these experiments, is shown in Figure 2, with the left panel showing the spectrum for the experiment with one equivalent TCA and the right panel for the one using three equivalents. The relative integrals for the double doublet of the ^{15}N-H versus the integral of the doublet of the ^{14}N-H peak, 0.72 vs 0.28 (1 equiv. TCA) and 0.70 vs 0.30 (3 equiv. TCA), indicate the main product to originate from aglycon transfer of the imidate, even when an excess of TCA is used as a competitor.

Finally, the potential activation of the ^{14}N-trichloroacetamidate **8α** under the acidic reaction conditions was examined as the expulsion of the trichloroacetamide from **8α** and addition of ^{15}N-TCA to the activated donor could lead to scrambling of the anomeric $^{14}N/^{15}N$ trichloroacetamides. Thus, ^{14}N-trichloroacetamidate **8α** was subjected to the acidic imidate

transfer conditions in the presence of ^{15}N TCA **3**. No uptake of ^{15}N could be observed in the product of this reaction, indicating that the anomeric trichloroacetamide is stable under the reaction conditions and the ^{14}N/^{15}N incorporation in **8α** the result of a glycosylation reaction under kinetic control.

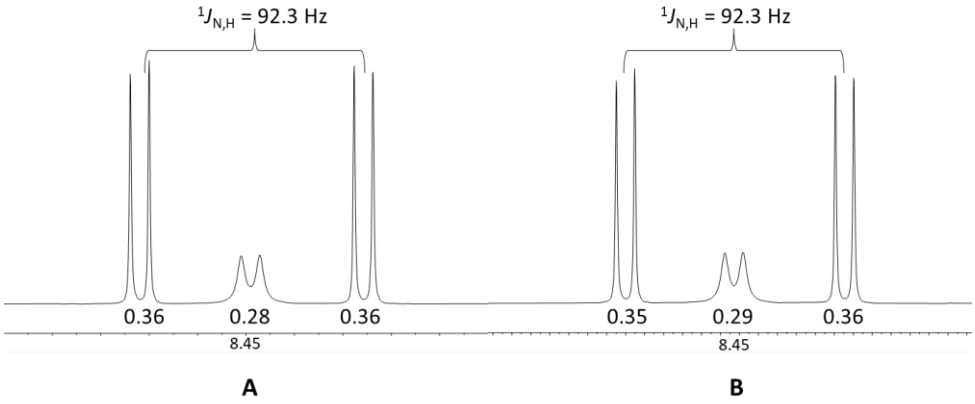


Figure 2: ^{1}H NMR of amide **8α**, zoomed in onto the NH range, formed after the experiment depicted in Scheme 4 with 1 (A) or 3 (B) equivalents of additional trichloroacetamide.

Conclusions

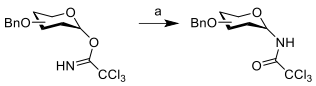
In conclusion, to differentiate between intramolecular rearrangement and intermolecular aglycon transfer reaction mechanisms to account for the formation of anomeric trichloroacetamides from trichloroacetimidate donors, ^{13}C and ^{15}N isotopic labelling experiments were used. These have unambiguously shown that the major route of anomeric trichloroacetamide formation follows the intermolecular aglycon transfer path, in which a glycosyl trichloroacetimidate attacks an activated donor species to produce another copy of an activated donor alongside the anomeric trichloroacetamide. This mechanism explains well the success of the 'inverse' procedure in which the amount of a reactive glycosyl donor in a glycosylation reaction with a poor nucleophile, is kept to a minimum. The detailed mechanistic insight described here will aid the further understanding of the glycosylation reaction and support the rational optimization of reaction conditions to enable the assembly of ever more complex oligosaccharides and glycoconjugates. [20]

Experimental

General Experimental Procedures

All chemicals were of commercial grade and used as received unless stated otherwise. Dichloromethane (DCM) and Dimethylformamide (DMF) were stored over activated 4 Å molecular sieves at least 18h before use. Flash column chromatography was performed on silica gel 60 Å (0.04 – 0.063 mm, Screening Devices B.V.). Thin layer chromatography (TLC) was conducted on TLC silica gel 60 plates (Kieselgel 60 F254, Merck) with UV detection (254 nm) and by spraying with a solution of $(NH_4)_6Mo_2O_{24}{\cdot}H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_2{\cdot}2H_2O$ (10 g/L) in 10% aqueous sulphuric acid followed by charring at 250 °C. High-resolution mass spectrometry (HRMS) was performed on a Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an electrospray ion source in positive-ion mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 °C) with resolution R = 60.000 at *m/z* 400 (mass range of 150-4000) and dioctylphalate (*m/z* = 391.28428) as lock mass. ^{1}H, ^{13}C and ^{15}N NMR spectra were recorded on Brucker AV-400, Brucker DMX-400 and Brucker AV-500 NMR instruments. Chemical shifts (δ) are given in part per million (ppm) relative to tetramethylsilane as an internal standard or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hertz (Hz). All presented 13C spectra are proton decoupled. Structural assignments were made with additional information from gCOSY, gHSQC, and gHMBC experiments. IR spectra were recorded on a Shimadzu FTIR-8300 IR spectrometer and are reported in cm^{-1}.

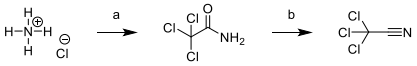
General Procedure for TfOH catalysed Imidate Rearrangement



Scheme S1: General procedure for the imidate to amide exchange. Reagents and conditions: a) TfOH (10 mol%), DCM, -20 °C.

A mixture containing the anomeric ^{13}C glycosyl donor (50 mg, 0.08 mmol, 0.5 eq) and the ^{15}N donor (50 mg, 0.08 mmol, 0.5 eq) was prepared and the ^{1}H, ^{13}C and ^{15}N NMR were measured. The sample was concentrated *in vacuo* and co-evaporated twice with dry toluene. The oil was dissolved in dry DCM (3 mL) and dried molsieves (3Å, rods) were added. The reaction mixture was cooled to -20 °C and TfOH (1.3 µL, 0.02 mmol, 0.1 eq) was added. After stirring at -20 °C for 30 min, the reaction was quenched with solid $NaHCO_3$ and allowed to warm to room temperature. The suspension was filtered over cotton and washed with saturated aqueous $NaHCO_3$ twice, once with water, dried over Na_2SO_4 and concentrated *in vacuo*. The crude ^{1}H, ^{13}C and ^{15}N NMR were measured before purification by flash column chromatography yielded the pure amide.

Reagent Synthesis



Scheme S2: Synthesis of ^{15}N labelled trichloroacetonitrile. Reagents and conditions: a) Trichloroacetyl chloride, NaOH, Chloroform, H_2O, 0-25 °C; b) P_2O_5, distillation.

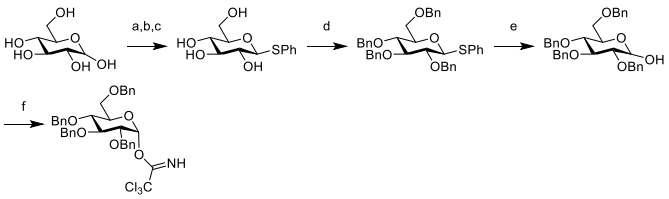
^{15}N Trichloroacetamide (3)

To a solution of trichloroacetyl chloride (10.3 mL, 93 mmol, 1.01 eq) in chloroform (250 mL) at 0 °C were added ^{15}N ammonium chloride (5.0 g, 92 mmol, 1.0 eq) and a solution of NaOH (7.63 g, 189 mmol, 2.06 eq) in water (70 mL). The reaction mixture was stirred at 0 °C for 15 min and then allowed to warm to r.t. in 1 h. The reaction was filtered over a glass filter and

the residue was washed with water and chloroform. The two layers of the filtrate were separated and the aqueous layer was extracted twice with Et_2O. The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by flash column chromatography (10-20% EtOAc in pentane) and combined with the solid residue to give the title compound as a white solid (7.01 g, 42.9 mmol, 47%). IR (thin film): 608, 744, 823, 1099, 1346, 1375, 1608, 1685, 3173, 3235, 3308, 3354. ^{1}H NMR (400 MHz, Acetone) δ 8.07 – 7.28 (br. s, 1H). ^{13}C NMR (101 MHz, Acetone) δ 163.8 (d, $^1J_{C,N}$ = 21.0 Hz, *C*=ONH$_2$), 93.7 (d, $^2J_{C,N}$ = 13.1 Hz, *C*Cl$_3$). ^{15}N NMR (41 MHz, Acetone) δ 92.6 HRMS (M+2ACN+H$^+$) 244.97761, found 244.97809.

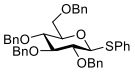
^{15}N Trichloro acetonitrile (4)

A mixture of ^{15}N trichloroacetamide (1.93 g, 11.8 mmol, 1.0 eq) and phosphorus pentoxide (3.5 g, 12.3 mmol, 1.04 eq) was mixed thoroughly. The mixture was heated with a heat gun and the formed liquid was collected by distillation using a short-path distillation setup. IR (thin film): 643, 744, 821, 1100, 1346, 1375, 1682, 3235. ^{13}C NMR (126 MHz, CDCl3) δ 113.2 (d, $^1J_{C,N}$ = 14.4 Hz, *C*N), 70.2 (d, $^2J_{C,N}$ = 4.0 Hz, *C*Cl$_3$) ^{15}N NMR (51 MHz, CDCl3) δ 255.8.



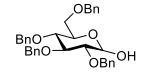
Scheme S3: Synthesis of the α-glucose imidate donor. Reagents and conditions: a) Ac_2O, $HClO_4$, r.t., overnight; b) PhSH, BF_3●Et_2O, DCM, reflux, overnight; c) NaOMe, MeOH, r.t., overnight; d) BnBr, NaH, DMF, 0-25 °C,; e) NBS, Acetone, H2O, 70 min., 0-25 °C; f) Trichloroacetonitrile, NaH, DCM, 2:40 h, 0-25 °C.

Phenyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucospyranoside (10)



To a solution of phenyl 1-thio-β-d-glucopyranoside (1.31 g, 4.97 mmol, 1.0 eq) (prepared according to literature[1]) in dry DMF (24 mL) at 0 °C were added NaH (60 w%, 0.93 g, 23 mmol, 4.8 eq) and BnBr (2.7 mL, 23 mmol, 4.8 eq). The reaction mixture was stirred at r.t. overnight. After completion, the reaction was cooled to 0 °C and quenched with MeOH. The crude was diluted with water and extracted trice with Et_2O. The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography of the crude (5-20% EtOAc in pentane) gave the title compound as a white solid (3.08 g, 4.97 mmol, *quant.*). IR (thin film): 644, 744, 821, 1100, 1346, 1375, 1611, 1682, 1694, 3235, ^{1}H NMR (400 MHz, CDCl3) δ 7.70 – 7.54 (m, 2H, CH$_{arom}$), 7.46 – 7.13 (m, 23H, CH$_{arom}$), 4.92 – 4.79 (m, 4H, CH$_2$ Bn), 4.73 (d, *J* = 10.2 Hz, 1H, CH$_2$ Bn), 4.67 (d, *J* = 9.8 Hz, 1H, H-1), 4.64 – 4.57 (m, 2H, CH$_2$ Bn), 4.54 (d, *J* = 12.0 Hz, 1H, CH$_2$ Bn), 3.84 – 3.59 (m, 4H, H-3, H-4, H-6), 3.58 – 3.46 (m, 2H, H-2, H-5) ^{13}C NMR (101 MHz, CDCl3) δ 138.5, 138.4, 138.1, 133.9 (C$_{q,\ arom}$), 132.1, 129.0, 128.6, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6 (CH$_{arom}$), 87.6 (C-1), 86.9 (C-3, C-4), 80.9 (C-2), 79.2 (C-5), 77.9 (C-3, C-4), 76.0, 75.6, 75.2, 73.5 (*C*H$_2$ Bn), 69.1 (C-6). HRMS (M+NH$_4^+$): cald. for $C_{39}{}^{13}CH_{40}O_5S_1NH_4$ 651.29683, found 651.29759. Spectra in agreement with literature.[22]

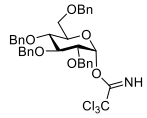
2,3,4,6-tetra-O-benzyl-D-glucospyranoside (11)



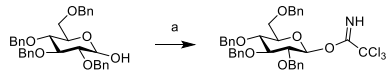
To a solution of phenyl 2,3,4,6-tetra-O-benzyl-1-thio-β-d-glucospyranoside (0.57 g, 0.9 mmol, 1.0 eq) in acetone (8 mL) and water (0.9 mL) at 0 °C was added NBS (0.48 g, 2.7 mmol, 3.0 eq).The reaction was stirred at r.t. for 70 min, after which it was quenched with saturated aqueous $Na_2S_2O_3$ and extracted trice with EtOAc. The combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in*

vacuo. Flash column chromatography (20-30% EtOAc in pentane) yielded the title compound as a white solid (0.35 g, 0.65 mmol, 71%). Data reported for a 2.5 : 1 α/β mixture: %). IR (thin film): 692, 731, 1053, 1356, 1452, 1584, 3027. ^{1}H NMR (400 MHz, CDCl3) δ 7.42 – 7.22 (m, 23H, CH_{arom}), 7.21 – 7.10 (m, 3H, CH_{arom}), 5.22 (dd, *J* = 3.1, 3.1 Hz, 1H, H-1α), 4.94 (dd, *J* = 10.8, 7.6 Hz, 2H, CH_2 Bn), 4.89 – 4.66 (m, 6H, CH_2 Bn, H-1β), 4.63 – 4.54 (m, 2H, CH_2 Bn), 4.48 (dd, *J* = 11.5, 3.2 Hz, 3H, CH_2 Bn), 4.03 (ddd, *J* = 10.1, 4.0, 2.1 Hz, 1H, H-5α), 3.97 (t, *J* = 9.3 Hz, 1H, H-4α), 3.74 – 3.51 (m, 6H, H-2α, H-3α, Hβ), 3.45 (d, *J* = 5.4 Hz, 0.4H, Hβ), 3.40 (dd, *J* = 9.1, 7.7 Hz, 0.4H, Hβ), 3.03 (d, *J* = 2.6 Hz, 1H, OH). ^{13}C NMR (101 MHz, CDCl3) δ 138.8, 138.3, 138.0 ($C_{q,\ arom}$), 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8 (CH_{arom}), 97.6 (C-1β), 91.5 (C-1α), 84.7, 83.2 (Cβ), 81.9 (C-5α), 80.1 (C-2, C-3α), 77.91, 77.8 (C-2, C-3α), 75.9, 75.2, 73.7, 73.6, 73.4 (CH_2 Bn), 70.5 (C-4α), 69.0 (C-6β), 68.7 (C-6α). HRMS ($M+NH_4^+$): cald. for $C_{33}{}^{13}C_1H_{36}O_6SNH_4$ 559.28837, found 559.28826. Spectra in agreement with literature.[23]

2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl trichloroacetimidate (5)

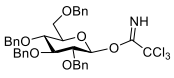


To a solution of 2,3,4,6-tetra-O-benzyl-d-glucospyranoside (150 mg, 0.28 mmol, 1.0 eq) in dry DCM (1.7 mL) were added trichloroacetonitrile (0.13 mL, 1.3 mmol, 4.6 eq) and NaH (60 w%, 1 mg, 0.03 mmol, 0.1 eq). The reaction mixture was stirred at r.t. for 10 min, after which it was cooled to 0 °C and additional NaH (60 w%, 14 mg, 0.36 mmol, 1.3 eq) was added. The reaction was allowed to warm to r.t. and stirred for an additional 2.5 h. The reaction mixture was filtered and concentrated *in vacuo*. Flash column chromatography (5-20% EtOAc in pentane) yielded the title compound as a clear oil (120-160 mg, 65-85%). Data reported for isotopically unlabelled compound: ^{1}H NMR (400 MHz, CDCl3) δ 8.58 (s, 1H, NH), 7.39 – 7.24 (m, 18H, CH_{arom}), 7.18 – 7.11 (m, 2H, CH_{arom}), 6.52 (d, *J* = 3.4 Hz, 1H, H-1), 4.96 (d, *J* = 11.0 Hz, 1H, CH_2 Bn), 4.84 (t, *J* = 10.4 Hz, 2H, CH_2 Bn), 4.78 – 4.65 (m, 2H, CH_2 Bn), 4.60 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.52 (d, *J* = 10.7 Hz, 1H, CH_2 Bn), 4.46 (d, *J* = 12.1 Hz, 1H, CH_2 Bn), 4.05 (t, *J* = 9.4 Hz, 1H, H-3), 3.99 (ddd, *J* = 10.2, 3.2, 1.9 Hz, 1H, H-5), 3.84 – 3.74 (m, 3H, H-2, H-4, H-6), 3.66 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6). ^{13}C NMR (101 MHz, CDCl3) δ 161.4 (C=N), 138.7, 138.1, 138.1, 137.9 ($C_{q,\ arom}$), 128.8, 128.8, 128.6, 128.5, 128.5, 128.5, 128.5, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7 (CH_{arom}), 94.5 (C-1), 81.5 (C-3), 79.4 (C-2, C-4), 76.9 (C-2, C-4), 75.8, 75.5, 73.6 (CH_2 Bn), 73.2 (C-5), 73.0 (CH_2 Bn), 68.1 (C-6), 30.4 (CCl_3). Characteristic peaks for the ^{13}C labelled compound: IR (thin film): 694, 736, 795, 1061. ^{1}H NMR (400 MHz, CDCl3) δ 6.52 (dd, *J* = 175.2, 3.4 Hz, 1H, H-1). Characteristic peaks for the ^{15}N labelled compound: IR (thin film): 695, 745, 800, 1024, 1064, 1086. ^{1}H NMR (400 MHz, CDCl3) δ 8.57 (d, J = 62.1 Hz, 1H, NH). ^{13}C NMR (101 MHz, CDCl3) δ 161.3 (d, *J* = 9.9 Hz, C=N), 94.4 (d, *J* = 3.5 Hz, C-1). ^{15}N NMR (41 MHz, CDCl3) δ 212.2. HRMS ($M+Na^+$): cald. for $C_{33}{}^{13}C_1H_{36}Cl_3NO_6SNa$ 707.15340, found 707.15393 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6Na$ 707.14708, found 707.14715 Spectra in agreement with literature.[24]



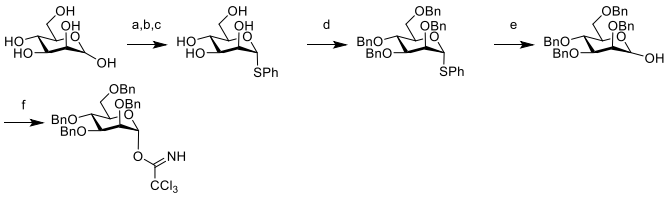
Scheme S4: Synthesis of β-glucose imidate donor. Reagents and conditions: a) Trichloroacetonitrile, K_2CO_3, DCM, r.t., overnight.

2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl trichloroacetimidate (6)

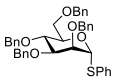


To a solution of 2,3,4,6-tetra-O-benzyl-d-glucospyranoside (0.53 g, 0.92 mmol, 1.0 eq) in dry DCM (7.5 mL) were added trichloroacetonitrile (0.8 mL, 7.8 mmol, 8.4 eq) and K_2CO_3 (0.55 g, 4.0 mmol, 4.3 eq). The reaction mixture was stirred overnight at r.t. After completion, the reaction mixture was filtered and concentrated *in vacuo*. Flash column chromatography (10-20% EtOAc in pentane + 1% Et_3N) yielded the title compound as a clear oil (440.9 mg, 0.64 mmol, 70%). Data reported for isotopically unlabelled compound: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1H, NH), 7.38 – 7.24 (m, 18H, CH_{arom}), 7.17 (m, 2H, CH_{arom}), 6.00 – 5.71 (m, 1H,

H-1), 4.94 (d, *J* = 10.8 Hz, 1H, CH_2 Bn), 4.91 (d, *J* = 11.0 Hz, 1H, CH_2 Bn), 4.82 (d, *J* = 10.8 Hz, 2H, CH_2 Bn), 4.76 (d, *J* = 10.8 Hz, 1H, CH_2 Bn), 4.62 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 4.58 (d, *J* = 10.8 Hz, 1H, CH_2 Bn), 4.54 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 3.75 (m, *J* = 2.7 Hz, 6H, H-2, H-3, H-4, H-5, H-6), 3.63 (m, 1H, H-3, H-4, H-5). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.3 (C=N), 138.6, 138.3, 138.1, 138.1 ($C_{q,\ arom}$), 128.5, 128.5, 128.5, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8 (CH_{arom}), 98.5 (C-1), 84.7, 81.1, 77.4, 76.0 (C-2, C-3, C-4, C-5), 75.8, 75.1, 75.1, 73.5 (CH_2 Bn), 68.3 (C-6). Characteristic peaks for the ^{13}C labelled compound: IR (thin film): 693, 731, 1044, 1073, 1286, 1362, 1452, 1496, 1674. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.10 – 5.53 (m, 1H, H-1). Characteristic peaks for the ^{15}N labelled compound: %). IR (thin film): 693, 731, 1025, 1056, 1209, 1287, 1452, 1496, 1655, 2863. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 61.5 Hz, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.3 (d, *J* = 9.9 Hz, C=N), 98.5 (d, *J* = 3.2 Hz, C-1). ^{15}N NMR (41 MHz, $CDCl_3$) δ 215.3. HRMS (M+Na$^+$): cald. for $C_{33}{}^{13}C_1H_{36}Cl_3NO_6SNa$ 707.15340, found 707.15339 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6Na$ 707.14708, found 707.14752. Spectra in agreement with literature.[24]



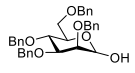
Scheme S5: Synthesis of the α-mannose imidate donor. Reagents and conditions: a) Ac_2O, $HClO_4$, r.t., overnight; b) PhSH, BF_3●Et_2O, DCM, r.t., overnight; c) NaOMe, MeOH, r.t., overnight; d) BnBr, NaH, DMF, 0-25 °C,; e) NBS, Acetone, H_2O, 70 min., 0-25 °C; f) Trichloroacetonitrile, NaH, DCM, 2:40 h, 0-25 °C.



Phenyl 2,3,4,6-tetra-O-benzyl-1-thio-α-D-mannopyranoside (12)

To a solution of phenyl 1-thio-α-d-mannopyranoside (1.98 g, 7.3 mmol, 1.0 eq) (prepared according to literature[22]) in dry DMF (34 mL) at 0 °C were added NaH (60 w%, 1.40 g, 35 mmol, 4.8 eq) and BnBr (42 mL, 35 mmol, 4.8 eq). The reaction mixture was stirred at room temperature overnight and afterwards quenched with MeOH and diluted with Et_2O. The mixture was washed trice with water and once with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (5-20% Et_2O in pentane) yielded the title compound as a white oil (4.26 g, 6.7 mmol, 92%). IR (thin film): 694, 728, 1015, 1054, 1075. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 – 7.40 (m, 2H, CH_{arom}), 7.39 – 7.16 (m, 23H, CH_{arom}), 5.62 (d, *J* = 1.8 Hz, 1H, H-1), 4.91 (d, *J* = 10.8 Hz, 1H, CH_2 Bn), 4.73 (d, *J* = 12.2 Hz, 1H, CH_2 Bn), 4.65 (d, *J* = 9.6 Hz, 1H, CH_2 Bn), 4.62 (d, *J* = 10.1 Hz, 1H, CH_2 Bn), 4.60 (d, *J* = 2.1 Hz, 2H, CH_2 Bn), 4.53 (d, *J* = 10.7 Hz, 1H, CH_2 Bn), 4.48 (d, *J* = 11.9 Hz, 1H, CH_2 Bn), 4.29 (ddd, *J* = 9.9, 5.0, 2.0 Hz, 1H, H-4), 4.07 (td, *J* = 9.5, 1.8 Hz, 1H, H-3), 4.00 (dd, *J* = 3.1, 1.7 Hz, 1H, H-2), 3.91 – 3.80 (m, 2H, H-5, H-6), 3.75 (dt, *J* = 10.9, 1.8 Hz, 1H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.5, 138.3, 138.0, 134.5 ($C_{q,\ arom}$), 131.7, 129.1, 128.5, 128.5, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.6, 127.5 (CH_{arom}), 85.8 (C-1), 80.3 (C-5), 76.3 (C-2), 75.3 (CH_2 Bn), 75.1 (C-3), 73.4 (CH_2 Bn), 72.9 (C-4), 72.2 (CH_2 Bn), 72.0 (CH_2 Bn), 69.3 (C-6). HRMS (M+NH_4^+): cald. for $C_{39}{}^{13}CH_{40}O_5SNH_4$ 651.29683, found 651.29621. Spectra in agreement with literature.[26]

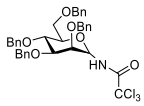
2,3,4,6-tetra-O-benzyl-D-mannospyranoside (13)



To a solution of Phenyl 2,3,4,6-tetra-O-benzyl-1-thio-α-d-mannopyranoside (0.51 g, 0.79 mmol, 1.0 eq) in acetone (7 mL) and water (0.7 mL) at 0 °C was added NBS (0.42 g, 2.4 mmol, 3.0 eq) and the reaction mixture was stirred at room temperature for 1h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ and extracted trice with EtOAc. The combined organic phase was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (10-30% EtOAc in pentane) yielded the title

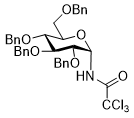
compound as a clear oil (0.37 g, 0.68 mmol, 87%). IR (thin film): 692, 731, 1020, 1053. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.22 (m, 18H, CH_{arom}), 7.20 – 7.11 (m, 2H, CH_{arom}), 5.25 (dd, *J* = 3.4, 1.9 Hz, 1H, H-1), 4.88 (d, *J* = 10.9 Hz, 1H, CH_2 Bn), 4.72 (d, *J* = 5.4 Hz, 2H, CH_2 Bn), 4.61 (s, 2H, CH_2 Bn), 4.55 (d, *J* = 8.9 Hz, 2H, CH_2 Bn), 4.52 – 4.46 (m, 1H, CH_2 Bn), 4.03 (ddd, *J* = 8.9, 6.3, 2.2 Hz, 1H, H-5), 3.95 (dd, *J* = 9.4, 3.1 Hz, 1H, H-3), 3.85 (t, *J* = 9.5 Hz, 1H, H-4), 3.79 (dd, *J* = 3.0, 2.0 Hz, 1H, H-2), 3.76 – 3.63 (m, 2H, H-6), 3.27 (d, *J* = 3.4 Hz, 1H, OH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.5, 138.5, 138.2 ($C_{q, arom}$), 128.5, 128.5, 128.5, 128.1, 128.0, 127.8, 127.7, 127.7 (CH_{arom}), 92.9 (C-1), 79.9 (C-3), 75.3 (C-4), 75.2 (CH_2 Bn), 74.9 (C-2), 73.4 (CH_2 Bn), 72.8 (CH_2 Bn), 72.3 (CH_2 Bn), 71.7 (C-5), 69.7 (C-6). HRMS (M+NH_4^+): cald. for $C_{33}{}^{13}CH_{36}O_6NH_4$ 559.28837, found 559.28814. Spectra in agreement with literature.[24]

2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl trichloroacetimidate (7)



To a solution of 2,3,4,6-tetra-O-benzyl-d-mannopyranoside (0.01 g, 0.19 mmol, 1.0 eq) and trichloro acetonitrile (0.1 mL, 0.85 mmol, 4.6 eq) in dry DCM (1.1 mL) was added NaH (60 w%, 1 mg, 0.02 mmol, 0.1 eq) and the reaction was stirred for 10 min. The reaction mixture was cooled to 0 °C and additional NaH (60 w%, 10 mg. 0.24 mmol, 1.3 eq) was added. The reaction was stirred for 2.5 h, filtered and concentrated *in vacuo*. Flash column chromatography (5-10% EtOAc in pentane + 1% Et_3N) yielded the title compound as a colourless oil. Data reported for isotopically unlabelled compound: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H, NH), 7.49 – 7.14 (m, 20H, CH_{arom}), 6.38 (d, *J* = 2.1 Hz, 1H, H-1), 4.90 (d, *J* = 10.6 Hz, 1H, CH_2 Bn), 4.76 (t, *J* = 2.4 Hz, 2H, CH_2 Bn), 4.73 – 4.48 (m, 5H, CH_2 Bn), 4.16 (t, *J* = 9.7 Hz, 1H, H-4), 4.01 – 3.90 (m, 2H, H-3, H-5), 3.87 (t, *J* = 2.6 Hz, 1H, H-2), 3.83 (dd, *J* = 11.1, 4.3 Hz, 1H, H-6), 3.72 (dd, *J* = 11.3, 2.1 Hz, 1H, H-6). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.5 (C=N), 138.3, 138.3, 138.1, 138.0 ($C_{q, arom}$), 128.8, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6 (CH_{arom}), 96.1 (C-1), 78.9 (C-3, C-4), 75.4 (CH_2 Bn), 74.9 (C-4), 74.2 (C-3, C-4), 73.6 (C-2), 73.3 (CH_2 Bn), 72.7 (CH_2 Bn), 72.4 (CH_2 Bn), 68.9 (C-6). Characteristic peaks for the anomeric ^{13}C labelled compound: IR (thin film): 692, 731, 792, 901, 958, 1025, 1054. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.37 (dd, *J* = 176.5, 2.0 Hz, 1H). Characteristic peaks for the ^{15}N labelled compound: IR (thin film): 692, 731, 794, 910, 960, 1025. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 61.7 Hz, 1H, NH). ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4 (d, *J* = 9.6 Hz, C=N). ^{15}N NMR (41 MHz, CDCl3) δ 213.5. HRMS (M+Na^+): cald. for $C_{35}{}^{13}CH_{36}Cl_3NO_6Na$ 707.15340, found 707.15396 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6Na$ 707.14708, found 707.14744. Spectra in agreement with literature.[24]

^{13}C / ^{15}N Exchange Experiment

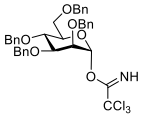


N-trichloroacetyl-2,3,4,6-tetra-O-benzyl-α-D-glucopyranosylamide (8)

Obtained after using the General Procedure for imidate transfer on both 2,3,4,6-tetra-O-benzyl-α-d-glucopyranosyl trichloroacetimidate and 2,3,4,6-tetra-O-benzyl-β-d-glucopyranosyl trichloroacetimidate. Flash column chromatography (5-15% EtOAc in pentane) yielded the title compound as a colourless oil (63 mg, 0.091 mmol, 60%, α/β > 20:1 for α donor; 48.8 mg, 0.071 mmol, 50%, α/β > 20:1 for β donor). Data reported for the α anomer: IR (thin film): 676, 695, 732, 819, 1067, 1453, 1495, 1728, 2869. ^{1}H NMR (400 MHz, Acetone) δ 8.46 (d, *J* = 7.7 Hz, 0.5H, ^{14}NH), 8.46 (ddd, *J* = 92.3, 7.7, 2.0 Hz, 0.5H, ^{15}NH), 7.48 – 7.24 (m, 20H, CH_{arom}), 5.87 (dd, *J* = 7.8, 5.5 Hz, 0.5H, ^{12}CH-1), 5.87 (ddd, *J* = 164.8, 7.8, 5.5 Hz, 0.5H, ^{13}CH-1), 4.93 (d, *J* = 11.3 Hz, 1H, CH_2 Bn), 4.85 (d, *J* = 11.0 Hz, 1H, CH_2 Bn), 4.80 (d, *J* = 11.3 Hz, 1H, CH_2 Bn), 4.75 (s, 2H, CH_2 Bn), 4.65 (d, *J* = 10.8 Hz, 1H, CH_2 Bn), 4.59 (d, *J* = 11.9 Hz, 1H, CH_2 Bn), 4.55 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.14 (t, *J* = 8.9 Hz, 1H, H-3), 3.97 (ddt, *J* = 9.2, 5.6, 1.7 Hz, 1H, H-2), 3.89 – 3.82 (m, 1H, H-5), 3.79 (dd, J = 11.0, 4.1 Hz, 1H, H-6), 3.71 (dd, J = 11.0, 1.9 Hz, 1H, H-6), 3.66 (dd, J = 9.8, 8.5 Hz, 1H, H-4). ^{13}C NMR (101 MHz, Acetone) δ 162.6 (C=O), 139.3, 138.8, 138.8, 138.2 ($C_{q,arom}$), 128.4, 128.3, 128.3, 128.1, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.4 (CH_{arom}), 102.5, 95.8, 94.1, 81.4 (C-3), 77.5 (C-2), 76.9 (C-1, 14N), 76.9 (d, J = 10.2 Hz, C-1, ^{15}N), 76.8 (C-5), 76.8 (CH_2 Bn), 74.8 (CH_2 Bn), 74.6 (CH_2 Bn), 72.9 (CH_2 Bn), 72.4 (C-4), 68.4 (C-6). Inverse gated ^{13}C NMR (101 MHz, Tol) δ 76.8 (s, C-1, 14N), 76.8 (d, *J* =

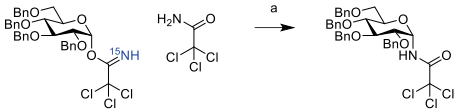
10.1 Hz, C-1, ^{15}N).^{15}N NMR (51 MHz, Acetone) δ 108.6 (NH, ^{12}C), 108.6 (d, J = 10.2 Hz, NH, ^{13}C). HRMS ($M+NH_4^+$): cald. for $C_{36}H_{36}Cl_3NO_6NH_4$ 701.19465, found 701.19482 cald. for $C_{35}{}^{13}CH_{36}Cl_3NO_6NH_4$ 702.19800, found 702.19769 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6NH_4$ 702.19168, found 702.19116 cald. for $C_{35}{}^{13}CH_{36}Cl_3{}^{15}NO_6NH_4$ 703.19504, found 703.19369. Spectra in agreement with literature.[27]

N-trichloroacetyl-2,3,4,6-tetra-O-benzyl-α/β-D-mannopyranosylamide (9)

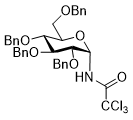


Obtained after using the General Procedure for imidate transfer on 2,3,4,6-tetra-O-benzyl-α-d-mannopyranosyl trichloroacetimidate. Flash column chromatography (20% Et_2O in pentane) yielded the title compound as a colourless oil (77.4 mg, 0.12 mmol, 77%, α/β = 1 : 1.6). IR (thin film): 695, 732, 819, 1025, 1090, 1453, 1495, 1724, 2864. ^{1}H NMR (400 MHz, Acetone) δ 8.80 (d, *J* = 8.4 Hz, 0.5H, ^{14}NHα), 8.80 (dd, *J* = 91.5, 8.4 Hz, 0.5H, ^{15}NHα), 7.88 (d, *J* = 8.8 Hz, 0.75H, ^{14}NHβ), 7.88 (dd, *J* = 96.0, 8.8 Hz, 0.75H, ^{15}NHβ), 7.51 – 7.22 (m, 50H, CH_{arom}), 5.75 (ddd, *J* = 163.8, 8.4, 5.8 Hz, 0.5H, ^{13}CH-1α), 5.74 (dd, *J* = 8.4, 5.8 Hz, 0.5H, ^{12}CH-1α), 5.38 (ddd, *J* = 158.3, 8.9, 1.7 Hz, 0.75H, ^{13}CH-1β), 5.31 (dd, *J* = 8.9, 1.7 Hz, 0.75H, ^{12}CH-1β), 5.08 (d, *J* = 11.5 Hz, 1H, CH_2 Bn), 4.99 – 4.48 (m, 19H, CH_2 Bn), 4.25 (t, *J* = 2.0 Hz, 1.5H, H-2β), 4.14 (m, 2H, H-2α,), 4.11 – 4.03 (m, 1H), 4.01 (d, *J* = 1.5 Hz, 2H), 3.96 – 3.90 (m, 1H), 3.85 – 3.77 (m, 2H), 3.77 – 3.73 (m, 2.5H, H-6), 3.64 (dtt, *J* = 7.2, 3.0, 1.5 Hz, 1.5H, H-5β). ^{13}C NMR (101 MHz, Acetone) δ 162.6 (^{14}N-C=Oα), 162.5 (d, *J* = 20.9 Hz, ^{15}N-C=Oα), 161.4 (^{14}N-C=Oβ), 161.4 (d, *J* = 20.8 Hz, ^{15}N-C=Oβ), 139.7, 139.6, 139.5, 139.5, 139.4, 139.3 ($C_{q,\ arom}$), 129.4, 129.2, 129.1, 129.1, 129.0, 129.0, 129.0, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 128.1 (CH_{arom}), 83.8 (C-5α), 79.4 (d, *J* = 13.9 Hz, ^{15}NC-1β), 79.4 (^{14}NC-1β), 78.0 (d, *J* = 11.1 Hz, ^{15}NC-1α), 78.0 (^{14}NC-1α), 77.2 (C-5β), 76.0 (C-2β), 75.9 (C-2α), 75.8 (CH_2 Bn), 75.7 (C-3α), 75.3, 75.1, 73.7 (CH_2 Bn), 73.6 (CH_2 Bn), 73.4 (CH_2 Bn), 73.3 (CH_2 Bn), 72.9 (CH_2 Bn), 72.8 (CH_2 Bn), 69.9 (C-6), 69.8 (C-6). Inverse gated ^{13}C NMR (101 MHz, Tol) δ 78.6 (s, C-1, 14Nβ), 78.6 (d, *J* = 14.1 Hz, C-1, ^{15}Nβ), 77.4 (s, C-1, 14Nα), 77.4 (d, *J* = 10.9 Hz, C-1, ^{15}Nα). ^{15}N NMR (41 MHz, Acetone) δ 114.5 (^{12}CNα), 114.5 (d, *J* = 11 Hz, ^{13}CNα), 114.1 (^{12}CNβ), 114.1 (d, *J* = 14 Hz, ^{13}CNβ). HRMS ($M+NH_4^+$): cald. for $C_{36}H_{36}Cl_3NO_6NH_4$ 701.19458, found 701.19465 cald. for $C_{35}{}^{13}CH_{36}Cl_3NO_6NH_4$ 702.19800, found 702.19740 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6NH_4$ 702.19168, found 702.19101 cald. for $C_{35}{}^{13}CH_{36}Cl_3{}^{15}NO_6NH_4$ 703.19504, found 703.19351. Spectra in agreement with literature.[27]

Mode of Attack Determination



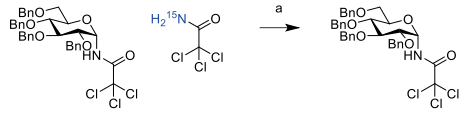
Scheme S6: Experiment to determine weather the nucleophile or the removed leaving group attacks. Reagents and conditions: a) TfOH (10 mol%), DCM, -20 °C.



N-trichloroacetyl-2,3,4,6-tetra-O-benzyl-α-D-glucopyranosylamide (8)

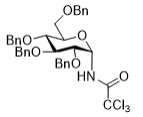
A mixture of ^{15}N-2,3,4,6-tetra-O-benzyl-α-d-glucopyranosyl trichloroacetimidate (100 mg, 0.15 mmol, 1.0 eq) and unlabelled tricholoacetamide (24 mg, 0.15 mmol, 1.0 eq or 72 mg, 0.44 mmol, 3.0 eq) was co-evaporated with dry toluene and dried molsieves (3 Å, rods) were added. The oil was dissolved in dry DCM (3 mL) and cooled to -20 °C. TfOH (1.3 µL, 0.02 mmol, 0.1 eq) was added and the reaction mixture was stirred at -20 °C for 30 min. The reaction was quenched with solid $NaHCO_3$ and allowed to warm to room temperature. The suspension was filtered over cotton and the filtrate was washed twice with saturated aqueous $NaHCO_3$ and once with water, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (5-15% EtOAc in pentane) yielded the title compound as a colourless oil (40 mg, 0.058 mmol, 40%). Data for the experiment with 1 equivalent of additional trichloracetamide: ^{1}H NMR (400 MHz, Acetone) δ 8.45 (d, *J* = 7.7 Hz, 0.28H, ^{14}NH), 8.44 (dd, *J* = 93.1, 7.7 Hz, 0.72H, ^{15}NH), 7.43 – 7.22 (m, 20H, CH_{arom}), 5.86 (dd, *J* = 7.7, 5.6 Hz, 1H, H-1), 4.92 (d, *J* = 11.2 Hz, 1H, CH_2 Bn), 4.83 (d, *J* = 10.9 Hz, 1H, CH_2 Bn), 4.79 (d, *J* = 11.3 Hz, 1H, CH_2 Bn), 4.73 (s, 2H, CH_2 Bn), 4.64 (d, *J* = 10.9 Hz, 1H, CH_2 Bn), 4.58 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.53 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.13 (dd, *J* = 9.3, 8.5 Hz, 1H, H-3), 3.99 – 3.92 (m, 1H, H-2), 3.84 (ddd, *J* = 9.8, 4.1, 1.9 Hz, 1H, H-5), 3.77 (dd, *J* = 11.0, 4.1 Hz, 1H, H-6), 3.69 (dd, *J* = 11.0, 1.9 Hz, 1H, H-6), 3.65 (dd, *J* = 9.9, 8.5 Hz, 1H, H-4). ^{13}C NMR (101 MHz, Acetone) δ 163.0 (^{14}N-C=O), 163.0 (d, *J* = 19.4 Hz, ^{15}N-C=O), 140.0, 139., 139.5, 138.9 ($C_{q,\ arom}$), 129.2, 129.0, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.2 (CH_{arom}), 81.9 (C-3), 79.1 (C-2), 78.3 (C-4), 77.7 (^{14}NC-1), 77.7 (d, *J* = 10.1 Hz, ^{15}NC-1) 75.6 (CH_2 Bn), 75.4 (CH_2 Bn), 73.7 (CH_2 Bn), 73.2 (CH_2 Bn), 73.0 (C-5), 69.7 (C-6). ^{15}N NMR (41 MHz, Acetone) δ 110.4. Characteristic peaks for the experiment with 3 equivalents of trichloroacetamide: ^{1}H NMR (400 MHz, Acetone) δ 8.45 (d, *J* = 7.7 Hz, 0.30H, ^{14}NH), 8.44 (dd, *J* = 92.2, 7.7 Hz, 0.70H, ^{15}NH). HRMS ($M+NH_4^+$): cald. for $C_{36}H_{36}Cl_3NO_6NH_4$ 701.19458, found 701.19465 cald. for $C_{36}H_{36}Cl_3{}^{15}NO_6NH_4$ 702.19168, found 702.19101. Spectra in agreement with literature.[27]

Reversibility Experiment



Scheme S7: Reaction used to determine the reversibility of the imidate transfer. Reagents and conditions: a) TfOH (10 mol%), DCM, -20 °C.

N-trichloroacetyl-2,3,4,6-tetra-O-benzyl-α-D-glucopyranosylamide (8)



A mixture of N-trichloroacetyl-2,3,4,6-tetra-O-benzyl-α-d-glucopyranosylamide (100 mg, 0.15 mmol, 1.0 eq) and ^{15}N trichloroacetamide (24 mg, 0.15 mmol, 1.0 eq) was co-evaporated with dry toluene and dissolved in dry DCM (3 mL). Dried molsieves (3 Å, rods) were added and the reaction mixture was cooled to -20 °C. TfOH (1.3 µL, 0.02 mmol, 0.1 eq) was added and the reaction was stirred at -20 °C for 30 min. The reaction was quenched with solid $NaHCO_3$ and filtered over cotton. The filtrate was washed twice with saturated aqueous $NaHCO_3$ and once with water, dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (5-15% EtOAc in pentane) yielded the title compound as a colourless oil (54 mg, 0.081 mmol, 55%). No ^{15}N incorporation was observed. ^{1}H NMR (400 MHz, Acetone) δ 8.45 (d, *J* = 7.7 Hz, 1H, NH), 7.43 – 7.21 (m, 20H, CH_{arom}), 5.87 (dd, *J* = 7.8, 5.6 Hz, 1H, h-1), 4.93 (d, *J* = 11.2 Hz, 1H, CH_2 Bn), 4.84 (d, *J* = 10.9 Hz, 1H, CH_2 Bn), 4.79 (d, *J* = 11.2 Hz, 1H, CH_2 Bn), 4.73 (s, 2H, CH_2 Bn), 4.64 (d, *J* = 10.9 Hz, 1H, CH_2 Bn), 4.58 (d, *J* = 12.0 Hz, 1H, CH_2 Bn), 4.53 (d, *J* = 11.9 Hz, 1H, CH_2 Bn), 4.14 (t, *J* = 8.9 Hz, 1H, H-3), 3.95 (dd, *J* = 9.3, 5.6 Hz, 1H, H-2), 3.84 (ddd, *J* = 9.9, 4.1, 1.9 Hz, 1H, H-5), 3.78 (dd, *J* = 11.0, 4.1 Hz, 1H, H-6), 3.70 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6), 3.65 (dd, *J* = 9.9, 8.6 Hz, 1H, H-4). ^{13}C NMR (101 MHz, Acetone) δ 163.0 (N-C=O), 140.0, 139.5, 139.5, 138.9 ($C_{q, arom}$), 129.1, 129.0, 129.0, 128.8, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.2 (CH_{arom}), 81.9 (C-3), 79.1 (C-2), 78.2 (C-4), 77.7 (C-1), 75.6 (CH_2 Bn), 75.3 (CH_2 Bn), 73.7 (CH_2 Bn), 73.2 (CH_2 Bn), 73.0 (C-5), 69.7 (C-6). HRMS (M+NH_4^+): cald. for $C_{36}H_{36}Cl_3NO_6NH_4$ 701.19458, found 701.19465. Spectra in agreement with literature.[27]

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