2,3-Carbamate mannosamine glycosyl donors in glycosylation reactions of diacetone-D-glucose. An experimental and theoretical study

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Abstract: The role of the cyclic 2,3-*N*,*O*-carbamate protecting group in directing the selectivity of mannosylation reactions of diacetone-D-glucose, promoted by BSP/Tf_2O via α-triflate intermediates, has been investigated through a combined computational and experimental approach. DFT calculations were used to locate the transition states leading to the α or β anomers. These data indicate the preferential formation of the β–adduct with mannosyl donors either equipped with the 4,6-*O*-benzylidene protection or without it. The synthetic results confirmed this preference, showing in both cases an α/β selectivity of 4:6. This highlights a role for the 2,3-*N*,*O*-carbamate in sharp contrast with what described in the case of 2,3-*O*-carbonate mannosyl donors.

Keywords: carbohydrates; glycosylation; 1,2-*cis* glycosides; β-mannosides; DFT calculations.

1. Introduction

Glycosylation is still the most critical reaction in carbohydrate chemistry. In spite of significant advances, there is not a universal protocol that allows the formation of all types of glycosidic bonds, the main problem being stereoselectivity [1, 2]. The stereochemistry of a glycosylation reaction is influenced by a large number of different factors, which can make it in some cases hard to predict and difficult to control, especially when the synthetic targets are 1,2-*cis* glycosides [3]. An accurate design of the experimental conditions and of the structural elements of the donor allow a certain control on the mechanism of these reactions, which can range from a S_N2-like to a S_N1-like process, so influencing in some respect the stereochemistry of the products [4,7]. Unfortunately, a complete

control is far from reach. Similarly, several concerns affect the computational approaches aimed to predict the selectivity of the reaction or to rationalize the stereochemistry observed experimentally [8, 9].

Several well-established glycosylation procedures transform the original glycosyl donor into the corresponding anomeric triflate intermediate before the addition to the acceptor nucleophile. This strategy is largely used for β-mannoside synthesis [10]. Focusing on the donor structure, the strong influence of protecting groups on the stereochemical course of the glycosylation is well known. For example, the 4,6-*O*-benzylidene acetal, well explored by Crich, allows the obtainment of β–mannosides with noteworthy selectivity [11-15]. This effect is completely reversed by the introduction of an additional cyclic protecting group such as 2,3-*O*-carbonate, which induces α–selectivity [14, 16]. On the other hand, when the 3,4-*O*-carbonate cyclic protecting group is used, a moderate β–selectivity is again observed [17]. The effect of cyclic protecting groups in donors has been less studied in the synthesis of β-mannosamine glycosides, where the more reliable protocol is still the indirect approach through β-glucosylation followed by *gluco* to *manno* epimerization [18]. There is evidence that lower selectivities are observed in glycosylation of 2-azidomannosyl donors under Crich conditions, despite the presence of the benzylidene protecting group [19-20]. Advances in β-mannosylation cannot be generalized to mannosamine, and further studies are required to find new protocols [21-23].

In this framework, we were interested in the possible role of the 2,3-carbamate protecting group in directing the selectivity of the glycosylation of a mannosamine donor. Though an effect similar to that of 2,3-carbonate might be expected, with a preference for the α product, we hypothesized that differences in the reactants may translate into different stereochemical outcomes, leading to the less easily accessible β anomer. In this paper, we have studied the selectivity of the glycosylations with two 2,3-carbamate protected mannoside donors, phenylthio 4,6-*O*-benzylidene-2,3-carbamate-α-D-mannopyranoside **1** and its analogue **2** lacking the benzylidene protecting group on positions 4 and 6, respectively protected as acetate and benzyl ether (Figure1). The acceptor was diacetone-D-glucose **3**, a known glucosyl acceptor with a secondary unprotected hydroxyl group in position 3, commonly used to test glycosylation reactions. Before starting the experimental work, we have addressed the effect of the presence of the carbamate on the stereoselectivity of the glycosylations with a computational approach, based on the same theoretical model already used by Crich et al. to locate the transition state structures for glycosylations performed through the intermediacy of mannopyranosyl and glucopyranosyl triflates [8]. The computational study was performed on compounds **4** and **5**, corresponding to the α-triflate intermediates of simplified forms of donors **1** and **2**, better suited for computing (Scheme 1).

The results suggest a preference for the β adduct in the case of **4** and a net preference for the same anomer in the case of **5**. On this basis, we performed the synthetic work, to validate the computational results. Herein, we report all the theoretical and experimental investigations that highlight the role of the 2,3-carbamate protecting group of mannosamine glycosyl donors in determining the outcome of the glycosylation reaction.

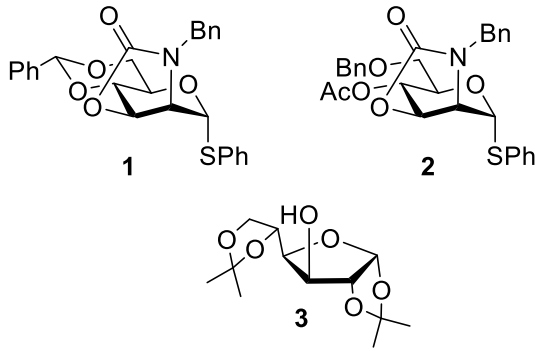
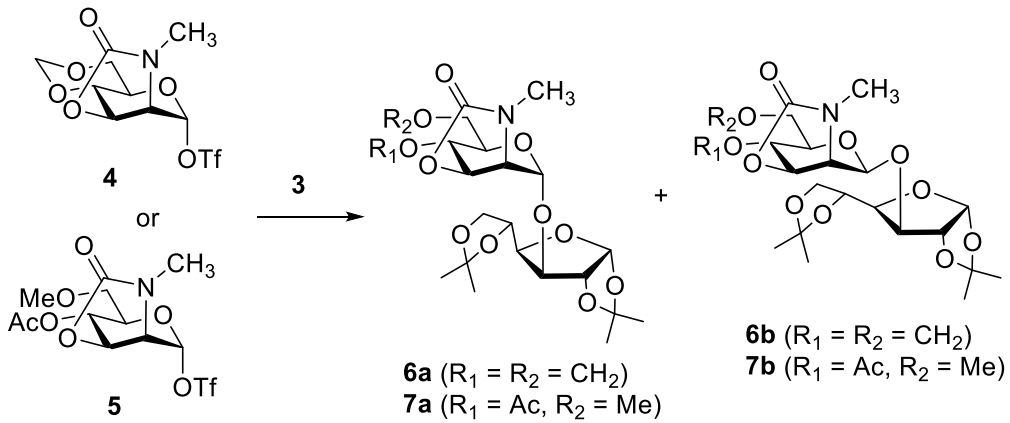


Figure 1. Structures of the 2,3-carbamate protected mannosamine donors **1** and **2**, and glucosyl acceptor **3**.

2. Results and Discussion

2.1 Computational studies

Density functional theory calculations of possible transition state structures for the reactions of glycosyl donors **4** and **5** and glycosyl acceptor **3** were carried out in dichloromethane by optimizations with the B3LYP functional at the 6-31G(d,p) level. The solvent effect was taken into account by using a self-consistent reaction field (SCRF) method, based on the polarisable continuum model (PCM), choosing dichloromethane as the solvent. With the optimized geometries, single-point energy calculations were performed using the B3LYP/6-311++G(3df,3pd) level still with the same solvent model. To avoid too long computational times, calculations were performed with the simplified models **4** and **5**, instead of **1** and **2**, respectively, in which the benzyl is replaced by methyl groups and benzylidene by methylidene (Scheme 1) [16].

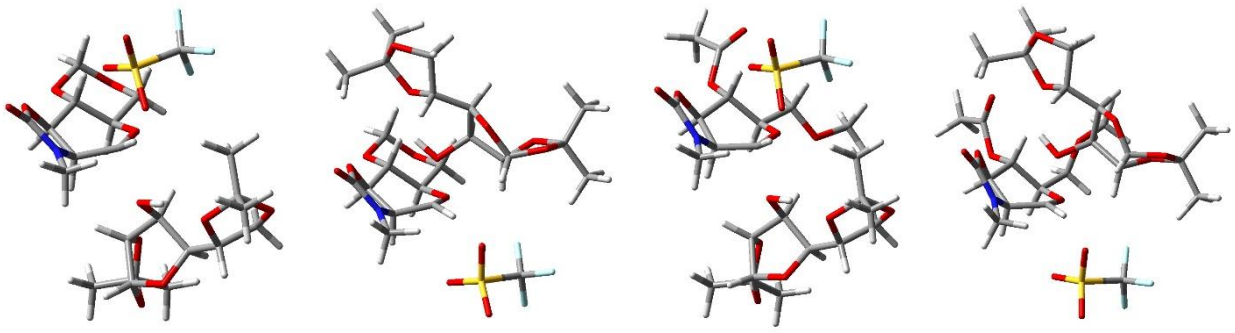


Scheme 1. Computationally studied glycosylation reactions of diacetone-D-glucose **3** with model mannosamine triflates **4** and **5**.

The reaction of the tricyclic glycosyl donor **4** with **3** was first investigated and several transition state structures were located which can give rise either to the α anomer **6a** or to the β anomer **6b** of the disaccharide product. The three-dimensional plots of the two lowest energy structures, **TS-4a** and **TS-4b**, are reported in Figure 2 together with their main geometrical features. There is a small difference in the Gibbs free energy of the two structures, 0.67 kcal/mol in favor of **TS-4b**, which corresponds to an α/β product ratio of 24:76 at room temperature and 17:83 at -60 °C.

A higher selectivity was predicted in the case of the reaction of the bicyclic glycosyl donor **5** with **3** which can result either in the α anomer **7a** or in the β anomer **7b** of the disaccharide product. The two lowest energy structures, **TS-5a** and **TS-5b** (Figure 2) show a Gibbs free energy difference higher than 2 kcal/mol with the latter one being favored. This predicts a very selective reaction with an α/β product ratio of 3:97 at room temperature and 1:99 at -60 °C.

The pyranose ring of the mannosamine donors adopts the same geometry in all the four TSs, which is similar to the $B_{2,5}$ conformation found by Crich in the TS for the reaction of isopropanol with 4,6-*O*-benzylidene mannosyl triflate leading to the β-glycoside product [8]. However, the presence of the additional ring determined by the 2,3-carbamate protection makes the pyranose ring closer to a $^{1}S_5$ skew rather than to a $B_{2,5}$ boat conformation. Moreover, the four TSs are highly unsymmetrical as the bond of the anomeric carbon to the incoming nucleophile is much shorter than that of the departing triflate (Figure 2). This suggests that they are late transition states where the bond of the anomeric carbon with the triflate is almost completely broken before the formation of the new bond with the nucleophile. When we looked for geometries showing the alternative $^{4}H_3$ conformation of the pyranose ring, described by Crich for the TS leading to the α-mannoside product [8], we were unable to locate them as, during the optimizations, the ring geometries always changed into the more stable $^{1}S_5$ conformation.



TS-4a
$\Delta\Delta G^{\ddagger}$ = 0.67 kcal/mol
r(C-ONu) = 1.982 Å
r(C-OTf) = 2.918 Å

TS-4b
$\Delta\Delta G^{\ddagger}$ = 0.00 kcal/mol
r(C-ONu) = 2.011 Å
r(C-OTf) = 2.936 Å

TS-5a
$\Delta\Delta G^{\ddagger}$ = 2.18 kcal/mol
r(C-ONu) = 1.941 Å
r(C-OTf) = 3.013 Å

TS-5b
$\Delta\Delta G^{\ddagger}$ = 0.00 kcal/mol
r(C-ONu) = 2.156 Å
r(C-OTf) = 2.762 Å

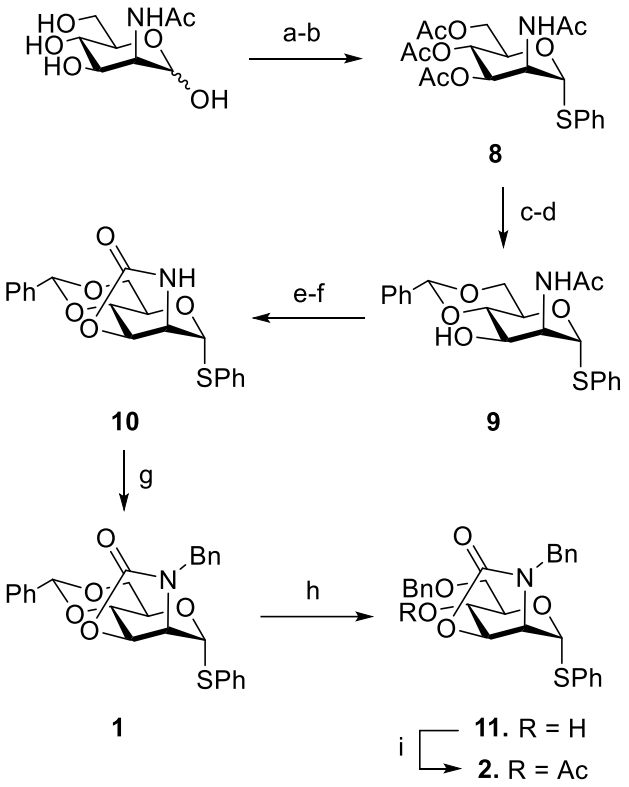
Figure 2. Three-dimensional plots of the lowest energy transition states for the reaction of diacetone-D-glucose **3** with mannosamine triflates **4** and **5**. For each transition state the relative free energy of activation and the lengths of the partial bonds to the leaving group, *r*(C-OTf), and to the nucleophile glucosyl acceptor **3**, *r*(C-ONu), are listed.

2.2 Chemistry

The synthesis of mannosamine donor **1** (Scheme 2) started with the peracetylation of *N*-acetyl-D-mannosamine, followed by glycosylation with thiophenol, mediated by boron trifluoride diethyl etherate, to obtain the corresponding α-phenylthio derivative **8** in 65% yield over two steps. Deacetylation under Zemplén conditions of compound **8**, followed by 4,6-*O*-benzylidene protection gave **9**. Then, the acetamido group of **9** was hydrolyzed under basic conditions to allow the subsequent formation of the 2,3-carbamate by reaction with *p*-nitrophenyl chloroformate and sodium hydrogen carbonate at reflux temperature. The tricyclic compound **10** was obtained smoothly in 70% yield over 4 steps. Intermediate **10** was finally transformed into donor **1** through a high yielding *N*-benzylation under standard conditions. The benzyl group was introduced onto the carbamate to reduce side-reactions at the carbamate nitrogen during glycosylation [24].

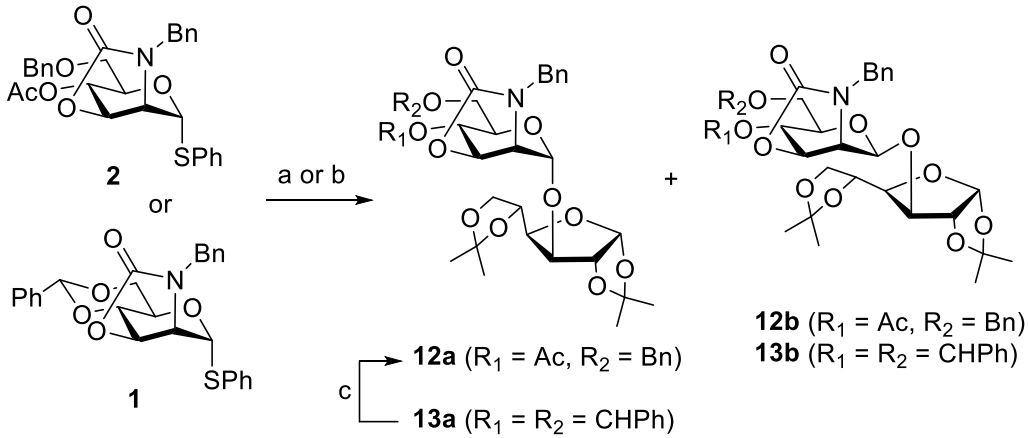
Mannosamine donor **2** was obtained starting from donor **1** through the regioselective reductive opening of the benzylidene acetal with triethylsilane/boron trifluoride-diethyl ether system to give compound **11** in 81% yield. Lastly, acetylation of the resultant free hydroxyl in position 4 of **11** afforded donor **2** in 96% yield.

The overall procedure for the obtainment of the desired 2,3-carbamate mannosamine donors **1** and **2** is efficient and applicable to gram scale synthesis.



Scheme 2. Synthesis of donor **1**. Reagents and conditions: a) Ac_2O, Pyridine; b) PhSH, $BF_3{\cdot}OEt_2$, DCE, 60°C, 65% *over 2 steps*; c) MeONa, MeOH; d) benzaldehyde dimethyl acetal, PTSA, CH_3CN; e) KOH, EtOH/H_2O, reflux; f) *p*-nitrophenyl chloroformate, $NaHCO_3$, H_2O, CH_3CN, reflux, 70% *over 4 steps*; g) BnBr, NaH, TBAI, DMF, 0°C to r.t., 93%; h) Et_3SiH, $BF_3{\cdot}OEt_2$, MS 4Å, CH_2Cl_2, 81%; i) Ac_2O, Pyridine, 96%. Abbreviations: DCE = dichloroethane; PTSA = *p*-Toluenesulfonic acid; TBAI = Tetrabutylammonium iodide; DMF = Dimethylformamide.

Based on the predictions obtained from DFT calculations, we started with the glycosylation between donor **2** and acceptor **3**.



Scheme 3. Glycosylations of the 2,3-carbamate protected mannosamine donors **1** and **2** with acceptor **3.** All reactions were conducted in DCM with 1.2 eq of the donors. Two different

activation systems were used for the glycosylations: a) **3**, BSP (1.5 eq.)/Tf_2O (1.5 eq.)/TTBP (3 eq.); b) **3**, NIS (1.5 eq.)/AgOTf (0.5 eq.). Other reaction conditions: c) i) Et_3SiH, $BF_3 \cdot OEt_2$, MS 4Å, CH_2Cl_2; ii) Ac_2O, Pyridine. NIS = N-Iodosuccinimide, BSP = 1-(Phenylsulfinyl)piperidine, TTBP = 2,4,6-Tri-*tert*-butylpyrimidine.

The 1-benzenesulfinyl piperidine (BSP)/triflic anhydride (Tf_2O) promotor system was initially evaluated. This system is known to readily activate armed and disarmed thioglycosides via glycosyl triflates at -60 °C in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) (Scheme 3) [14, 19, 25]. We performed the reaction at -40 °C, due to the low solubility of acceptor **3** at lower temperatures, pre-activating donor **2** before the addition of acceptor **3**. Unfortunately, we observed predominantly the decomposition of the thiol donor into more polar compounds, together with the formation of traceless amounts of the glycosylation product **12**, while most of acceptor **3** remained unaltered (Table 1 –entry 1). We then turned to the NIS/AgOTf thiophilic activator system [26]. Donor **2** was activated in-situ with AgOTf/NIS at -40 °C and smoothly condensed with acceptor **3** to give disaccharide **12** in 70% yields (Table 1 – entry 2). The glycosylation is highly reproducible, and the donor consumption is indicated by the color of the reaction that turns into a deep red. The analysis of the isolated products by ^1H NMR indicated the formation of a mixture of anomers **12a** and **12b** in a 4:6 ratio (Figure 3A), in favor of the β-anomer. The NMR spectrum did not allow an unequivocal attribution of the signals to the two anomers, as the mannosidic anomeric protons were both broad singlets. The configuration was tentatively established based on the assignments reported in the literature for azido-mannosides [27] where the anomeric proton of the alfa-anomer is in general downfield with respect to the β-one. This was further validated with chemical correlation (see below).

Unfortunately, though the preferential formation of the β-anomer predicted by theoretical calculations was confirmed by the experimental results, the observed selectivity was much lower than what was expected.

Table 1. Results of the glycosylations

entry	Donor	Activation system	Temp.	Time (min.)	Product (yield)	α/β ratio*
1	**2**	BSP/Tf_2O	- 40 °C	40’	**12** (-)	-
2	**2**	NIS/AgOTf	- 40 °C	90’	**12** (70%)	4:6

3	**1**	NIS/AgOTf	- 40 °C	90’	**13** (55%)	1:1
4	**1**	BSP/Tf_2O	- 40 °C	40’	**13** (57%)	4:6
5	**1**	BSP/Tf_2O	- 40 °C	15’	**13** (55%)	4:6
6	**1**	BSP/Tf_2O	- 40 °C	5’	**13** (59%)	4:6
7	**1**	BSP/ Tf_2O	- 60 °C	40’	**13** (27%)	4:6

* Anomeric ratio determined from an anomeric mixture by ^{1}H NMR analysis.

Thus, we turned to the glycosylations with the mannosamine tricyclic donor **1** (Scheme 3), which was initially coupled with acceptor **3** under the activation of NIS/AgOTf (Table 1 – entry 3). The glycosylation to disaccharide **13** proceeded smoothly in 55% yield, but without selectivity. So, we evaluated the BSP/Tf_2O promotor system which gave compound **13** in comparable yield, around 60%, but with a slight preference for one anomer (Table 1 – entry 4). Although both very small, the vicinal mannose coupling constants ($J_{1'\text{-}2'}$) of the two anomers **13a** and **13b** show a different coupling pattern, being one signal a broad singlet at 5.35 ppm and the other one a doublet (J = 3.0 Hz) at 5.02 ppm (Figure 3C). This suggests that the former signal can be assigned to the α-anomer and the latter to the β-one. This was confirmed by computation of theoretical coupling constants of α- and β-model compounds, reported in Figure 4, for which $J_{1,2}$ of 1.1 and 3.3 Hz, respectively, were predicted (Figure 4).

The stereochemical outcome of the glycosylation to disaccharide **13** mediated by BSP/Tf_2O (α/β 40:60) is in good agreement with the slightly more favourable data predicted by computing (α/β 24:76).

We wondered if the low selectivity was due to in-situ anomerization in the reaction conditions, and stopped the reaction immediately after the slow addition of the acceptor to the solution of the activated donor (5’) or after 15’ (Table 1 – entry 5-6). However, we always found the same 4:6 α/β anomeric ratio. The same when we performed the reaction at -60 °C: as expected, the yield was lower due to the poor solubility of the acceptor at this temperature, but the anomeric ratio was unaffected (Table 1 – entry 7).

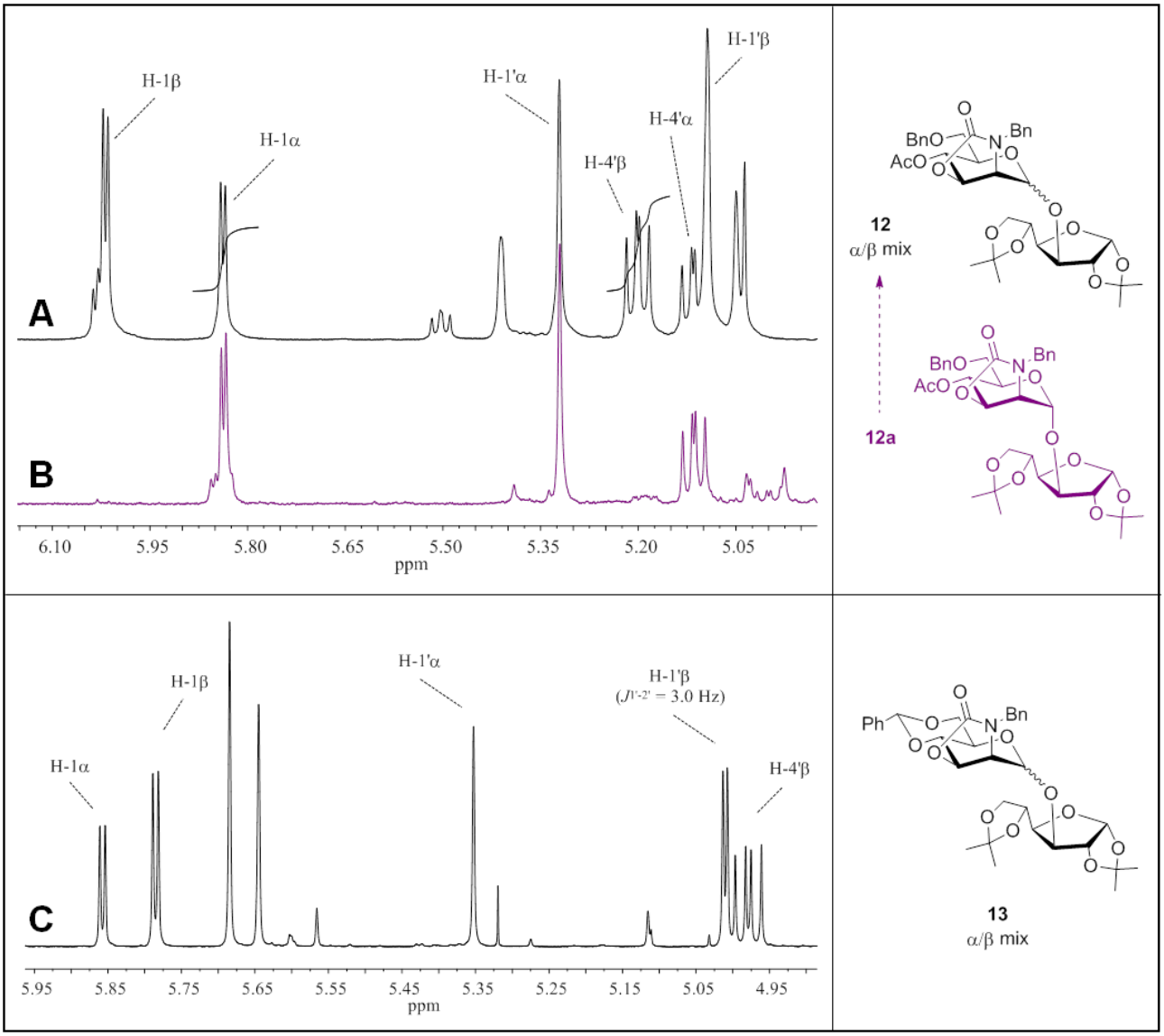
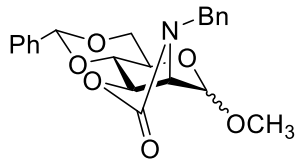


Figure 3. A) ^{1}H-NMR spectrum of the α/β mixture of disaccharide **12**; B) ^{1}H-NMR spectrum of the crude disaccharide **12a** obtained starting from **13a** after reductive benzylidene opening and acetylation, which has been used for chemical correlation; C) ^{1}H-NMR spectrum of the α/β mixture of disaccharide **13**.

Finally, we confirmed the configuration of the previously obtained **12a** and **12b** disaccharides by chemical correlation. After separation of anomers **13a** and **13b** by flash chromatography and complete ^{1}H-NMR characterization of the disaccharides, a pure amount of **13a** was transformed into **12a** by reductive benzylidene ring-opening, followed by acetylation of the 4'-OH. The ^{1}H-NMR of the crude product was compared with the spectrum of the **12a**/**12b** mixture, thus allowing to confirm the previous assignments (Figure 3B).

In summary, our study reports the effect of cyclic 2,3-*N*,*O*-carbamate protected systems on the stereoselectivity of mannosylations promoted by BSP/Tf_2O via α-triflate intermediates. DFT calculations on model systems indicate that the relative energies of the transition states leading to the alfa or beta anomers preferentially support the formation of the β-adduct. β-selectivity is expected for both donors **1** and **2**, and the results of the experimental reactions confirm this preference, within the limitation of model accuracy. Indeed, both benzylidene bearing donor **1** and bicyclic donor **2** gave glycosylation adducts in α/β ratio of 4:6. Even if the β-selectivity is moderate, we show that the presence of the 2,3-carbamate protecting group on mannosamine donor favors the formation of the β-adduct, either in the presence or not of the 4,6-*O*-benzylidene. This is in sharp contrast with what was described in the case of 2,3-*O*-carbonate mannosyl donors, where the presence of the 2,3-cyclic protecting group on 4,6-*O*-benzylidene-mannosides induced the exclusive formation of α-mannosides [14].

In addition, the β-selectivities herein described are similar to the ones reported in the case of 2-azidomannoside donors: the glycosylation of 4,6-*O*-benzylidene-2-azido-mannoside with acceptor **3** gives the disaccharide product in α/β ratio of 1:2 [27]. Our data support the notion that cyclic carbamate protected mannosaminyl donors can be considered a viable alternative to azido-mannosides in glycosylation strategies leading to mannosamine-containing oligosaccharides. Indeed, different naturally occurring oligosaccharides contain the 1,2-cis-linked β-mannosaminyl residue, such as for example, the bacterial capsular polysaccharides of *Staphylococcus aureus* type 5 [28] or *Streptococcus pneuomoniae* 19F and 19A [18]. The two new mannosamine donors developed in this study can be suitable building blocks for the synthesis of repeating unit oligomers of such bacterial species. In fact, besides the stereodirecting effect of the carbamate protecting group, mannosamine building blocks **1** and **2** can be regioselectively deprotected at C-4 to allow elongation to oligomers of these capsular polysaccharide bacterial species.



tricyclic model compounds	*calculated $J_{1,2}$ (Hz)*
α-OCH_3	1.1
β-OCH_3	3.3

Figure 4. $J_{1,2}$ coupling constant values of a tricyclic saccharidic model calculated by computational studies.

3. Experimental

3.1 General information

All chemicals were purchased from Sigma Aldrich (now owned by Merck KGaA) and used without any further purification unless otherwise described. All the reactions were performed under Argon atmosphere and using dry solvents, unless otherwise indicated. Dichloromethane (DCM), pyridine (Pyr) and triethylamine (TEA) were freshly distilled from CaH_2 prior to use. Dimethylformamide (DMF) and methanol were dried over activated molecular sieves.

Thin Layer Chromatography (TLC) was performed by using Silica gel on Merck TLC-PET foils precoated with a fluorescent indicator. Flash column chromatography was performed using a high-purity grade silica gel (SiO_2, high-purity grade (9385), pore size 60 Å, 230-400 mesh particle size) by Merck. The purity of all synthetized compounds was verified by nuclear magnetic resonance (NMR) analysis, using a 500 MHz Bruker FT-NMR AVANCE DRX500 spectrometer (pulsed field gradient, reverse broadband probe, ^{1}H at 500.13 MHz and ^{13}C at 125.77 MHz) at a sample temperature of 298K. High Resolution Mass Spectrometry (HRMS) was carried out on a high definition hybrid quadrupole/time-of-flight (QTof) mass spectrometer (Synapt G2Si system by Waters) equipped with electron-spry ionization (ESI) probe. A Thermo Quest Finnigan LCQ^{TM}DECA ion trap mass spectrometer, equipped with a Finnigan ESI interface, was used to perform Low Resolution Mass Spectrometry (LRMS). Optical rotation was measured at room temperature with a Perkin-Elmer 241 polarimeter (589 nm, D line from Na lamp). The synthetic sequence is reported at the best optimized scale.

3.2 Phenyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-1-thio-α-D-mannopyranoside (8)

Ac_2O (5 mL, 53 mmol) was added to a stirred suspension of *N*-acetyl-D-mannosamine (2.04 g, 9.23 mmol) in pyridine (10 mL) at 0°C. The mixture was gradually warmed to room temperature and stirred for 24 h. The reaction, turned into a clear solution, was concentrated under reduced pressure. The peracetylated intermediate (3.46 g, mixture of anomers, R_f = 0.34 in ethyl acetate) was obtained as white solid, and used in the next step without further purification.

Thiophenol (1.42 mL, 13.85 mmol) was added to a solution of the crude peracetylated intermediate in dichloroethane (45 mL). $BF_3{\cdot}Et_2O$ (2.30 mL, 18.46 mmol) was slowly added *via* dropping funnel, and the resulting mixture was heated at 60 °C. After 24 h, the reaction (turned into a deep purple solution) was cooled to room temperature, diluted with dichloromethane (30 mL) and washed with satd $NaHCO_3$ (3 x 50 mL, until basic pH). The aqueous phases were extracted with dichloromethane (80 mL) and the combined organic phases were washed with brine (50 mL), dried over sodium sulfate, and concentrated at reduced pressure. The crude (red oil) was purified by flash chromatography (hexane/ethyl acetate gradient, 35:65 to 25:75) to give compound **8** (2.67 g, 65%

two steps, R_f = 0.37 in hexane/ethyl acetate 3:7) as a white solid. *The spectroscopic data were in agreement with those reported in literature* [29].

3.3 Phenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside (9)

A 1 M solution of sodium methoxide in methanol (4.55 mL) was slowly added to a solution of compound **8** (2.67 g, 6.07 mmol) in methanol (60 mL). The reaction was stirred for 3 h at room temperature (TLC monitoring: disappearance of the starting material with hexane/ethyl acetate 3:7, product formation with ethyl acetate/methanol 6:1), then neutralized with an ion exchange resin (Dowex® 50WX8, H^+ form), filtered and concentrated. The deacetylated mannopyranoside [29] (1.88 g, R_f = 0.49 in ethyl acetate/methanol 6:1) was recovered as white foam, and used in the next step without any further purification.

To a solution of the crude mannopyranoside in acetonitrile (60 mL), benzaldehyde dimethyl acetal (2.2 mL, 14.92 mmol) and *p*-toluenesulfonic acid (0.23 g, 1.19 mmol) were added. The disappearance of the starting material was monitored by TLC, ethyl acetate/methanol 6:1. After 10 min, a white precipitate was formed, and the reaction was quenched by the addition of TEA and concentrated. Benzylidene **9** (4.3 g, crude) was recovered as an amorphous white solid and used directly in the next step without any further purification. A small aliquot (50 mg) of the crude was purified by flash chromatography for the spectroscopic characterizations of compound **9**. R_f = 0.76 (ethyl acetate/methanol 6:1) and 0.20 (hexane/ethyl acetate 25:75). $[\alpha]_D^{20}$ = +153 (*c* 0.2, Py). ^{1}H NMR (Py-d5, 500 MHz) δ 9.13 (d, $J_{2,NH}$ = 7.6 Hz, 1H, N*H*Ac), 7.98 (d, $J_{3,OH}$ = 4.1 Hz, 1H, O*H*), 7.68 – 7.14 (m, 10H, arom.), 6.15 (s, 1H, H-1), 5.53 (s, 1H, C*H*Ph), 5.40 (dd, $J_{2,NH}$ = 7.2, $J_{2,3}$ = 5.4 Hz, 1H, H-2), 4.83 – 4.76 (m, 1H, H-3), 4.71 (td, $J_{4,5}$ = 9.8, $J_{5,6a}$ = 4.8 Hz, 1H, H-5), 4.41 (t, $J_{4,5}$ = $J_{3,4}$ = 9.8 Hz, 1H, H-4), 4.28 (dd, $J_{6a,6b}$ = 10.2, $J_{5,6a}$ = 4.8 Hz, 1H, H-6a), 3.66 (t, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6b), 2.13 (s, 3H, NHCOC*H*$_3$). ^{13}C NMR (Py-d5, 126 MHz) δ 170.73 (NH*C*OCH$_3$), 138.29 (quat.), 134.64 (quat.), 129.28 – 126.86 (10C, arom.), 102.38 (*C*HPh), 88.89 (C-1), 80.38 (C-4), 68.51 (C-6), 66.87 (C-3), 65.28 (C-5), 56.08 (C-2), 22.78 (NHCO*C*H$_3$). HRMS (ESI+): *m/z* for $C_{21}H_{23}NO_5NaS$ calcd 424.1195 $[M+Na]^+$, found 424.1190.

3.4 Phenyl 2-amino-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-1-thio-α-D-mannopyranoside (10)

KOH (11.7 g, 209.0 mmol) was added to a stirred suspension of crude **9** (5.97 mmol) in aqueous ethanol (90 mL, EtOH/H_2O 5:1). The reaction mixture was heated under reflux for 24 h. The hot solution (turned into a deep brown mixture) was carefully poured into hot water (50 ml). After cooling to room temperature, the mixture was kept into an ice bath until the formation of an off-white precipitate was observed. The solid was filtered an a Hirsch funnel, washed with water and

dried under vacuum. The crude product de-acetylated intermediate (1.73 g, brown solid) was used in the next step without any further purification. R_f = 0.49 (ethyl acetate/methanol 6:1) and 0.10 (hexane/ethyl acetate 25:75), LRMS (ESI+): *m/z* for $C_{19}H_{21}NO_4SNa$ calcd 382.11 $[M+Na]^+$, found 360.0 $[M+H]^+$ (100%); 741.0 $[2M+Na]^+$ (55%).

To a suspension of the crude intermediate in acetonitrile (35 mL), 1 M aqueous $NaHCO_3$ (2.51 g, 29.85 mmol) was added, and the mixture was cooled to 0°C. A solution of *p*-nitrophenyl chloroformate (3.0 g, 14.92 mmol) in acetonitrile (25 ml) was then added dropwise to the reaction mixture. After 10 min, the reaction (turned from white into yellow) was gradually warmed to room temperature and stirred for 30 min. Heating at 80°C for 4 hours led to the disappearance of the starting material as revealed by TLC (disappearance of the starting material was followed in ethyl acetate/methanol 6:1, and product formation with hexane/ethyl acetate 1:1). After cooling to room temperature, the reaction mixture was diluted with ethyl acetate, washed with brine (2 x 75 mL), and the combined aqueous layers were extracted with ethyl acetate (100 mL). Then, the combined organic layers were dried over sodium sulfate, filtered, and evaporated. The crude product was purified by flash chromatography (hexane/ethyl acetate, 60:40) to give **10** (1.60 g, 70%, over 4 steps) as a white solid. R_f = 0.30 (hexane/ethyl acetate 6:4). $[\alpha]_D^{20}$ = +169 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.66 – 7.31 (m, 10H, arom.), 6.53 (s, 1H, N*H*), 5.60 (s, 1H, C*H*Ph), 5.57 (d, $J_{1,2}$ = 1.9 Hz, 1H, H-1), 4.83 (t, $J_{3,4}$ = $J_{2,3}$ = 7.8 Hz, 1H, H-3), 4.36 – 4.22 (m, 3H, H-2, H-5, H-6a), 4.06 (dd, $J_{4,5}$ = 9.8, $J_{3,4}$ = 7.8 Hz, 1H, H-4), 3.82 – 3.69 (m, 1H, H-6b). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.74 (O*C*ONH), 136.70 (1C, quat.), 132.92 (2C, arom.), 131.52 (1C, quat.), 129.33 – 126.17 (8C, arom.), 101.81 (*C*HPh), 83.75 (C-1), 78.80 (C-4), 75.02 (C-3), 68.57 (C-6), 61.46 (C-5), 56.18 (C-2). HRMS (ESI+): *m/z* for $C_{20}H_{19}NO_5NaS$ calcd 408.0882 $[M+Na]^+$, found 408.0878.

3.5 Phenyl 2-amino-2-N-benzyl-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-1-thio-α-D-mannopyranoside (1)

Benzyl bromide (1.5 mL, 12.3 mmol) was added to a stirred solution of compound **10** (1.58 g, 4.10 mmol) and tetrabutylammonium iodide TBAI (15 mg, 0.041 mmol) in DMF (40 mL). The mixture was cooled at 0°C, and NaH (60% in mineral oil, 0.82 g, 20.50 mmol) was added. The reaction was gradually warmed to room temperature, and monitored by TLC analysis (disappearance of starting material was followed in hexane/ethyl acetate 6:4, and product formation with toluene/ethyl acetate 95:5). After 90 min, the mixture was quenched by carefully addition of methanol, then diluted with ethyl acetate and washed with water (1 x 70 mL). The aqueous phase was extracted with ethyl acetate (3 x 40 mL), and the combined organics were washed with brine (70 mL), dried over sodium sulfate, filtered, and evaporated. Flash chromatography of the crude (toluene/ethyl acetate 95:5) gave compound **1** (1.81 g, 93%) as a white foam. R_f = 0.30 (toluene/ethyl acetate 95:5). $[\alpha]_D^{20}$

= +131 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.57 – 7.25 (m, 15H), 5.70 (s, 1H, H-1), 5.61 (s, 1H, C*H*Ph), 4.89 (d, *J* = 15.3 Hz, 1H, NC*H*HPh), 4.73 (t, $J_{2,3} = J_{3,4}$ = 7.8 Hz, 1H, H-3), 4.37 – 4.29 (m, 1H, H-5), 4.27 – 4.18 (m, 2H, H-6a, NCH*H*Ph), 4.00 – 3.92 (m, 2H, H-2, H-4), 3.76 (t, $J_{6a,6b} = J_{5,6b}$ = 10.2 Hz, 1H, H-6b). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.84 (O*C*ONBn), 136.68 – 131.97 (quat.), 129.40 – 126.22 (15C, arom.), 101.92 (*C*HPh), 82.03 (C-1), 79.33 (C-4), 72.12 (C-3), 68.42 (C-6), 61.19 (C-5), 58.54 (C-2), 47.00 (N*C*H_2Ph). HRMS (ESI+): *m/z* for $C_{27}H_{25}NO_5NaS$ calcd 498.1351 $[M+Na]^+$, found 498.1351.

3.6 Phenyl 2-amino-2-N-benzyl-6-O-benzyl-2,3-N,O-carbonyl-2-deoxy-1-thio-α-D-mannopyranoside (11)

A suspension of compound **1** (0.4 g, 0.84 mmol) and 4Å MS (0.4 g) in dichloromethane (17 mL) was stirred for 10 min at room temperature, then triethylsilane (1.25 mL, 8.40 mmol) was added After 0.5 h, a 0.5 M solution of $BF_3{\cdot}Et_2O$ (4.20 mL, 2.10 mmol) in dichloromethane was slowly added *via* dropping funnel to the reaction mixture. After 2 h, the reaction was quenched with 0.9 mL of TEA, diluted with dichloromethane, filtered over a Celite pad and concentrated *in vacuo*. The residue was purified by flash chromatography (toluene/ethyl acetate gradient, 85:15 to 70:30) to afford compound **11** (0.32 g, 81%) as a colourless oil. R_f = 0.26 (toluene/ethyl acetate 8:2). $[\alpha]_D^{20}$ = +51 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 – 7.14 (m, 15H, arom), 5.67 (s, 1H, H-1), 4.86 (d, *J* = 15.3 Hz, NC*H*HPh), 4.60 (d, *J* = 11.9 Hz, 1H, OC*H*HPh), 4.55 (t, $J_{2,3} = J_{3,4}$ = 7.6 Hz, 1H, H-3), 4.50 (d, *J* = 11.9 Hz, 1H, OCH*H*Ph), 4.32 – 4.24 (m, 1H, H-5), 4.18 (d, *J* = 15.3 Hz, 1H, NCH*H*Ph), 4.03 – 3.95 (m, 1H, H-4), 3.90 (dd, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 7.6 Hz, 1H, H-2), 3.73 (m, 2H, H-6), 3.17 (br s, 1H, O*H*). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.12 (O*C*ONBn), 137.65 – 132.35 (3C, quat.), 129.21 – 127.75 (15C, arom.), 81.74 (C-1), 76.48 (C-3), 73.60 (O*C*H_2Ph), 69.67 (C-5), 69.40 (C-4), 69.29 (C-6), 58.22 (C-2), 46.75 (N*C*H_2Ph). HRMS (ESI+): *m/z* for $C_{27}H_{27}NO_5NaS$ calcd 500.1508 $[M+Na]^+$, found 500.1520.

3.7 Phenyl 4-O-acetyl-2-amino-2-N-benzyl-6-O-benzyl-2,3-N,O-carbonyl-2-deoxy-1-thio-α-D-mannopyranoside (2)

Ac_2O (1.5 mL, 15.70 mmol) was added to a stirred solution of **11** (0.30 g, 0.63 mmol) in pyridine (5 mL). After 24 h, the reaction was concentrated under reduced pressure, and then dried under vacuum. Flash chromatography of the crude (hexane/ethyl acetate gradient, 75:25 to 65:35) afforded compound **2** (313 mg, 96%) as a colourless oil. R_f = 0.28 (hexane/ethyl acetate 75:25) and 0.49 (toluene/ethyl acetate 8:2). $[\alpha]_D^{20}$ = +61 (*c* 1.04, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 – 7.20 (m, 15H, arom.), 5.65 (*br*s, 1H, H-1), 5.20 (dd, $J_{3,4}$ = 7.5 Hz, $J_{4,5}$ = 9.6 Hz, 1H), 4.88 (d, *J* = 15.3 Hz, 1H, NC*H*HPh), 4.63 (t, $J_{2,3} = J_{3,4}$ = 7.5 Hz, 1H, H-3), 4.55 – 4.41 (m, 3H, OC*H*$_2$Ph, H-5), 4.19 (d, *J* = 15.3 Hz, 1H, NCH*H*Ph), 3.92 (dd, $J_{1,2}$ = 0.9 Hz, $J_{2,3}$ = 7.5 Hz, 1H, H-2), 3.62 – 3.53 (m,

2H, H-6a and H-6b), 2.08 (s, 3H, OCOC*H*$_3$). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.43 (O*C*OCH$_3$), 157.31 (O*C*ONBn), 137.65 (quat.), 134.45 (quat.), 132.79 (arom.), 132.23 (quat.), 129.22 – 127.73 (14C, arom.), 81.87 (C-1), 73.54 (O*C*H$_2$Ph), 73.39 (C-3), 69.28 (C-4), 69.18 (C-6), 68.88 (C-5), 58.03 (C-2), 46.76 (N*C*H$_2$Ph), 20.82 (OCO*C*H$_3$). HRMS (ESI+): *m/z* for $C_{29}H_{29}NO_6NaS$ calcd 542.1613 $[M+Na]^+$, found 542.1612.

3.8 4-O-Acetyl-2-amino-2-N-benzyl-6-O-benzyl-2,3-N,O-carbonyl-2-deoxy-1-thio-α/β-D-mannopyranosyl-(1→3)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (12)

D-Glucose diacetonide acceptor **3** (20 mg, 0.064 mmol), donor **2** (40 mg, 0.077 mmol) and freshly activated 4Å molecular sieves (60 mg) were suspended in dichloromethane (2 mL), stirred under argon for 10 min and then the mixture was cooled to -40 °C. After 10 min, N-iodosuccinimide (NIS, 22 mg, 0.096 mmol) and AgOTf (10 mg, 0.032 mmol) were quickly added and the reaction was monitored by TLC analysis (toluene/ethyl acetate 7:3). After 1.5 h, the reaction was quenched by the addition of TEA, diluted with dichloromethane, filtered over Celite, and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (toluene/ethyl acetate gradient, 80:20 to 70:30) to give product **12** (30 mg, 70%, colourless oil) as a 4:6 mixture of α/β anomers. The two anomers can be separated by flash chromatography (toluene/ethyl acetate gradient, 80:20 to 70:30 or hexane/ethyl acetate, 70:30). Alpha anomer (**12a**): R_f α = 0.37 (toluene/ethyl acetate 7:3). $[\alpha]_D^{20}$ = -2.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46 – 7.22 (m, 10H, arom.), 5.84 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.32 (s, 1H, H-1'), 5.11 (dd, $J_{3',4'}$ = 7.3 Hz, $J_{4',5'}$ = 9.8 Hz, 1H, H-4'), 4.75 (d, *J* = 15.4 Hz, 1H, NC*H*HPh), 4.71 (d, $J_{1,2}$= 3.6 Hz, 1H, H-2), 4.63 – 4.54 (m, 3H, H-3', OC*H*$_2$Ph), 4.28 (d, $J_{3,4}$ = 1.5 Hz, 1H, H-3), 4.25 (d, *J* = 15.4 Hz, 1H, NCH*H*Ph), 4.11 – 4.05 (m, 4H, H-4, H-5, 2 H-6), 4.00 – 3.94 (m, 1H, H-5'), 3.80 (d, $J_{2',3'}$ = 7.8 Hz, 1H, H-2'), 3.65 – 3.55 (m, 2H, 2 H-6'), 2.05 (s, 3H, OCOC*H*$_3$), 1.50 (s, 3H, C*H*$_3$ isopropylidene), 1.43 (s, 3H, C*H*$_3$ isopropylidene), 1.38 (s, 3H, C*H*$_3$ isopropylidene), 1.22 (s, 3H, C*H*$_3$ isopropylidene). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.39 (O*C*OCH$_3$), 157.65 (O*C*ONBn), 137.58 (quat.), 135.04 (quat.), 128.87 – 127.78 (10C, arom.), 112.06 (*C*CH$_3$), 109.63 (*C*C*H*$_3$), 105.17 (C-1), 95.76 (C-1'), 83.59 (C-2), 80.77 (C-3), 80.73 (C-4 or C-5), 73.73 (O*C*H$_2$Ph), 73.23 (C-3), 72.52 (C-4 or C-5), 69.24 (C-6'), 69.05 (C-4'), 68.10 (C-5'), 67.50 (C-6), 57.76 (C-2'), 47.13 (N*C*H$_2$Ph), 27.18 (C*C*H$_3$), 26.78 (C*C*H$_3$), 26.11 (C*C*H$_3$), 25.59 (C*C*H$_3$), 20.75 (OCO*C*H$_3$). HRMS (ESI+): *m/z* for $C_{35}H_{43}NO_{12}Na$ calcd 692.2683 $[M+Na]^+$, found 692.2687. Beta anomer (**12b**): R_f β = 0.44 (toluene/ethyl acetate 7:3). $[\alpha]_D^{20}$ = +27.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 – 7.15 (m, 10H, arom.), 6.02 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.20 (dd, 1H, $J_{3',4'}$ = 7.5 Hz, $J_{4',5'}$ = 9.8 Hz, H-4'), 5.09 (*br* s, 1H, H-1'), 4.80 (d, *J* = 15.3 Hz, 1H, NC*H*HPh), 4.64 – 4.50 (m, 4H, H-2, H-3', OC*H*$_2$Ph), 4.32 (dd, $J_{3,4}$ = 3.8 Hz, $J_{4,5}$ = 7.1 Hz, 1H, H-4), 4.25 – 4.16 (m, 2H, H-3, NCH*H*Ph), 3.98

– 3.91 (m, 1H, H-5'), 3.81 (dd, 1H, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 11.4 Hz, H-6a), 3.76 (*br* d, 1H, $J_{2',3'}$ = 8.0 Hz, H-2'), 3.71 (dd, $J_{5,6b}$ = 2.5 Hz, $J_{6a,6b}$ = 11.4, 1H, H-6b), 3.68 – 3.62 (m, 1H, H-5), 3.62 – 3.51 (m, 2H, 2 H-6'), 2.02 (s, 3H, OCOC*H*$_3$), 1.51 (s, 3H, C*H*$_3$ isopropylidene), 1.36 (s, 3H, C*H*$_3$ isopropylidene), 1.35 (s, 3H, C*H*$_3$ isopropylidene), 1.32 (s, 3H, C*H*$_3$ isopropylidene). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.39 (O*C*OCH$_3$), 157.69 (O*C*ONBn), 137.78 (quat.), 134.82 (quat.), 128.92 – 127.70 (10C, arom.), 112.25 (*C*CH$_3$), 106.40 (C-1), 100.99 (*C*CH$_3$), 94.78 (C-1'), 83.96 (C-2), 79.16 (C-4), 75.04 (C-3), 73.65, 73.52 (C-3', O*C*H$_2$Ph), 71.06 (C-5), 69.16 (C-6'), 69.02 (C-4'), 67.42 (2C, C-5' and C-6), 57.39 (C-2'), 47.05 (N*C*H$_2$Ph), 27.18 (C*C*H$_3$), 26.51 (C*C*H$_3$), 23.97 (C*C*H$_3$), 23.95 (C*C*H$_3$), 20.77 (OCO*C*H$_3$). HRMS (ESI+): *m/z* for $C_{35}H_{43}NO_{12}Na$ calcd 692.2683 $[M+Na]^+$, found 692.2677.

3.9 Phenyl 2-amino-2-N-benzyl-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-1-thio-α/β-D-mannopyranosyl-(1→3)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranoside (13)

A suspension of compound **1** (40 mg, 0.084 mmol), 1-(phenylsulfinyl)piperidine (BSP, 22 mg, 0.10 mmol), 2,4,6-tri-tert-butylpyrimidine (TTBP, 52 mg, 0.21 mmol) and 4Å MS (40 mg) in dichloromethane (3 mL) was stirred for 5 min at room temperature and then cooled to -40°C. After 10 min, Tf_2O (0.018 mL, 0.10 mmol) was added. Then, a solution of acceptor **3** (18 mg, 0.069 mmol) in dichloromethane (2 mL) was added dropwise. The reaction was monitored by TLC analysis (hexane/ethyl acetate 7:3, toluene/ethyl acetate 85:15). After 40 min, the reaction was quenched with TEA, diluted with dichloromethane, filtered over Celite, and the solvent evaporated under reduced pressure. Flash chromatography of the crude (toluene/ethyl acetate 85:15) afforded disaccharide **13** (16 mg, 60%) as a colourless oil in a 4:6 α/β mixture of anomers. The two anomers can be separated by flash chromatography (toluene/ethyl acetate gradient, 85:15). Alpha anomer (**13a**): R_f α = 0.26 (toluene/ethyl acetate 85:15). $[\alpha]_D^{20}$ = -13.3 (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 – 7.26 (m, 10H, arom.), 5.86 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.65 (s, 1H, C*H*Ph), 5.35 (*br* s, 1H, H-1'), 4.77 – 4.66 (m, 2H, NC*H*HPh, H-3'), 4.50 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-2), 4.46 – 4.37 (m, 1H, H-6a'), 4.31 (d, 1H, J = 15 Hz, NCH*H*Ph), 4.27 (*br* s, 1H, H-3), 4.17 – 4.03 (m, 4H, H-4, H-5, 2 H-6), 3.96 – 3.78 (m, 4H, H-2', H-4', H-6b', H-5'), 1.52 (s, 3H, C*H*$_3$ isopropylidene), 1.45 (s, 3H, C*H*$_3$ isopropylidene), 1.41 (s, 3H, C*H*$_3$ isopropylidene), 1.33 (s, 3H, C*H*$_3$ isopropylidene). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.26 (O*C*ONBn), 136.59 (quat.), 135.11 (quat.), 129.27 – 126.02 (10C, arom.), 112.26 (*C*CH$_3$), 109.75 (*C*CH$_3$), 105.17 (C-1), 101.79 (*C*HPh), 95.72 (C-1'), 84.11 (C-2), 80.65 (C-4 or C-5), 79.60 (C-3), 78.87 (C-4'), 72.53 (C-4 or C-5), 72.02 (C-3'), 68.59 (C-6'), 67.50 (C-6), 60.53 (C-5'), 58.40 (C-2'), 47.38 (N*C*H$_2$Ph), 27.29 (C*C*H$_3$), 26.78 (C*C*H$_3$), 26.28 (C*C*H$_3$), 25.66 (C*C*H$_3$). HRMS (ESI+): *m/z* for $C_{33}H_{39}NO_{11}Na$ calcd 648.2421 $[M+Na]^+$, found 648.2416. Beta anomer (**13b**): R_f β = 0.20 (toluene/ethyl acetate 85:15). $[\alpha]_D^{20}$ = -115 (*c* 0.2,

$CHCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 – 7.16 (m, 10H, arom.), 5.79 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 5.69 (s, 1H, C*H*Ph), 5.02 (d, 1H, $J_{1',2'}$ = 3.0 Hz, H-1'), 4.98 (dd, 1H, $J_{3',4'}$ = 7.1 Hz, $J_{4',5'}$ = 10.7 Hz, H-4'), 4.78 (d, *J* = 14.5 Hz, 1H, NC*H*HPh), 4.7 (dd, 1H, $J_{2',3'}$ = 7.1 Hz, $J_{3',4'}$ = 9.0 Hz, H-3'), 4.46 (d, *J* = 3.4 Hz, 1H, H-3), 4.41 – 4.36 (m, 1H, H-5), 4.98 (dd, 1H, $J_{5',6'a}$ = 5.0 Hz, $J_{6'a',6'b}$ = 10.3 Hz, H-6'a), 4.22 – 4.16 (m, 2H, H-2, H-6a), 4.16 – 4.10 (m, 2H, H-4, H-6b), 4.00 (d, *J* = 14.5 Hz, 1H, NCH*H*Ph), 3.96 (dd, 1H, $J_{1',2'}$ = 3.0 Hz, $J_{2',3'}$ = 9.0 Hz, H-2'), 3.92 (dd, 1H, $J_{5',6'b}$ = $J_{6'a',6'b}$ = 10.3 Hz, H-6'b), 3.71 – 3.64 (m, 1H, H-5'), 1.54 (s, 3H, CH_3 isopropylidene), 1.51 (s, 3H, CH_3 isopropylidene), 1.43 (s, *J* = 8.5 Hz, 3H, CH_3 isopropylidene), 1.36 (s, 3H, CH_3 isopropylidene). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 157.58 (O*C*ONBn), 136.78 (quat.), 135.33 (quat.), 129.21 – 125.94 (10C, arom.), 112.08 (*C*CH_3), 109.48 (*C*CH_3), 105.05 (C-1), 100.75 (*C*HPh), 90.89 (C-1'), 80.84 (C-2), 80.45 (C-4), 76.06 (C-3), 75.75 (C-4'), 72.86 (C-3'), 71.78 (C-5), 69.00 (C-6'), 67.70 (C-6), 64.48 (C-5'), 54.97 (C-2'), 47.32 (N*C*H_2Ph), 27.63 (C*C*H_3), 26.83 (C*C*H_3), 26.38 (C*C*H_3), 25.72 (C*C*H_3). HRMS (ESI+): *m/z* for $C_{33}H_{39}NO_{11}Na$ calcd 648.2421 $[M+Na]^+$, found 648.2420.

3.10 Computational details

The Gaussian 09 program package was used for all the optimizations of the transition states of the reaction between the glycosyl donors **4** and **5** and the glycosyl acceptor **3**. The structures were optimized using the BY3LYP functional at the 6-31G(d,p) level. All the optimization were performed in dichloromethane solvent, using the PCM as solvation model. Then, single-point energy calculations on the optimized geometries were performed using the B3LYP/6-311++G(3df,3pd) level still with the same solvent model. Frequency calculations at the BY3LYP/6-31G(d,p) gave the free energy correction which was added to the B3LYP/6-311++G(3df,3pd) single-point energy to give the free energy values used to determine the relative free energy of the transition states. Starting geometries were built taking into account the calculated TSs reported in the literature for the reaction of isopropanol with 4,6-O-methylidene protected mannosyl triflates [8] and introducing the appropriate structural modifications on both the glycosyl donor and acceptor. After a first optimization maintaining a fixed length (opt=modredundant option) for the partial bonds of the anomeric carbon to the leaving group and to the nucleophile, the free optimization of the TSs was performed. An extensive search of the conformational space was performed taking into account the orientation of the triflate as well as the nucleophile with respect to the pyranose ring and, in the case of **5**, the gg, gt, and tg orientation at the C5-C6 bond. In each of the four cases, a dozen of transition states were located and those reported in Figure 2 are the lowest energy structures. The calculated $J_{1,2}$ coupling constants were obtained using the nmr=spinspin option.

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Supporting Information: Copies of the ^{1}H and ^{13}C NMR spectra of all new compounds are given in the Supporting Information

References:

[1] D. Crich, Mechanism of a Chemical Glycosylation Reaction, Acc. Chem. Res., 43 (2010) 1144-1153.
[2] L.K. Mydock, A.V. Demchenko, Mechanism of chemical *O*-glycosylation: from early studies to recent discoveries, Org. Biomol. Chem., 8 (2010) 497-510.
[3] S.S. Nigudkar, A.V. Demchenko, Stereocontrolled 1,2-cis glycosylation as the driving force of progress in synthetic carbohydrate chemistry, Chem. Sci., 6 (2015) 2687-2704.
[4] P.O. Adero, H. Amarasekara, P. Wen, L. Bohé, D. Crich, The Experimental Evidence in Support of Glycosylation Mechanisms at the S_N1-S_N2 Interface, Chem. Rev., 118 (2018) 8242-8284.
[5] D. Crich, Methodology Development and Physical Organic Chemistry: A Powerful Combination for the Advancement of Glycochemistry, J. Org. Chem., 76 (2011) 9193-9209.
[6] S.G. Pistorio, J.P. Yasomanee, A.V. Demchenko, Hydrogen-Bond-Mediated Aglycone Delivery: Focus on OI-Mannosylation, Org. Lett., 16 (2014) 716-719.
[7] A.A.H. Abdel-Rahman, S. Jonke, E.S.H. El Ashry, R.R. Schmidt, Stereoselective Synthesis of β-D-Mannopyranosides with Reactive Mannopyranosyl Donors Possessing a Neighboring Electron-Withdrawing Group, Angew. Chem. Int. Ed., 41 (2002) 2972-2974.
[8] M. Huang, G.E. Garrett, N. Birlirakis, L. Bohé, D.A. Pratt, D. Crich, Dissecting the mechanisms of a class of chemical glycosylation using primary ^{13}C kinetic isotope effects, Nat. Chem., 4 (2012) 663-667.
[9] A. Franconetti, A. Ardá, J.L. Asensio, Y. Blériot, S. Thibaudeau, J. Jiménez-Barbero, Glycosyl Oxocarbenium Ions: Structure, Conformation, Reactivity, and Interactions, Acc. Chem. Res., 54 (2021) 2552-2564.
[10] D. Crich, S. Sun, Direct chemical synthesis of β-mannopyranosides and other glycosides via glycosyl triflates, Tetrahedron, 54 (1998) 8321-8348.
[11] D. Crich, S. Sun, Formation of β-Mannopyranosides of Primary Alcohols Using the Sulfoxide Method, J. Org. Chem., 61 (1996) 4506-4507.
[12] D. Crich, S. Sun, Direct Synthesis of β-Mannopyranosides by the Sulfoxide Method, J. Org. Chem., 62 (1997) 1198-1199.
[13] D. Crich, S. Sun, Direct Formation of β-Mannopyranosides and Other Hindered Glycosides from Thioglycosides, J. Am. Chem. Soc., 120 (1998) 435-436.
[14] D. Crich, M. Smith, 1-Benzenesulfinyl Piperidine/Trifluoromethanesulfonic Anhydride: A Potent Combination of Shelf-Stable Reagents for the Low-Temperature Conversion of Thioglycosides to Glycosyl Triflates and for the Formation of Diverse Glycosidic Linkages, J. Am. Chem. Soc., 123 (2001) 9015-9020.
[15] D. Crich, M. Smith, S-(4-Methoxyphenyl) Benzenethiosulfinate (MPBT)/Trifluoromethanesulfonic Anhydride: A Convenient System for the Generation of Glycosyl Triflates from Thioglycosides, Org. Lett., 2 (2000) 4067-4069.

[16] D. Crich, W. Cai, Z. Dai, Highly Diastereoselective α-Mannopyranosylation in the Absence of Participating Protecting Groups, J. Org. Chem., 65 (2000) 1291-1297.
[17] D. Crich, A.U. Vinod, J. Picione, The 3,4-*O*-Carbonate Protecting Group as a β-Directing Group in Rhamnopyranosylation in Both Homogeneous and Heterogeneous Glycosylations As Compared to the Chameleon-like 2,3-*O*-Carbonates, J. Org. Chem., 68 (2003) 8453-8458.
[18] L. Morelli, S. Fallarini, G. Lombardi, C. Colombo, L. Lay, F. Compostella, Synthesis and biological evaluation of a trisaccharide repeating unit derivative of Streptococcus pneumoniae 19A capsular polysaccharide, Bioorg. Med. Chem., 26 (2018) 5682-5690.
[19] J.D.C. Codée, R.E.J.N. Litjens, R. den Heeten, H.S. Overkleeft, J.H. van Boom, G.A. van der Marel, Ph_2SO/Tf_2O: a Powerful Promotor System in Chemoselective Glycosylations Using Thioglycosides, Org. Lett., 5 (2003) 1519-1522.
[20] R.E.J.N. Litjens, M.A. Leeuwenburgh, G.A. van der Marel, J.H. van Boom, A novel approach towards the stereoselective synthesis of 2-azido-2-deoxy-β-d-mannosides, Tetrahedron Lett., 42 (2001) 8693-8696.
[21] C. Alex, S. Visansirikul, A.V. Demchenko, A versatile approach to the synthesis of mannosamine glycosides, Org. Biomol. Chem., 18 (2020) 6682-6695.
[22] S. Nakamura, T. Tsuda, N. Suzuki, S. Hashimoto, Direct and stereoselective synthesis of 2-azido-2-deoxy-β-D-mannosides using the phosphate method, Heterocycles, 77 (2009) 843-848.
[23] B.R. Bhetuwal, F. Wu, S. Meng, J. Zhu, Stereoselective Synthesis of 2-Azido-2-deoxy-β-d-mannosides via Cs_2CO_3-Mediated Anomeric *O*-Alkylation with Primary Triflates: Synthesis of a Tetrasaccharide Fragment of Micrococcus luteus Teichuronic Acid, J. Org. Chem., 85 (2020) 16196-16206.
[24] S. Manabe, K. Ishii, Y. Ito, N-Benzyl-2,3-oxazolidinone as a Glycosyl Donor for Selective α-Glycosylation and One-Pot Oligosaccharide Synthesis Involving 1,2-*cis*-Glycosylation, J. Am. Chem. Soc., 128 (2006) 10666-10667.
[25] P. Wei, R.J. Kerns, Factors Affecting Stereocontrol during Glycosidation of 2,3-Oxazolidinone-Protected 1-Tolylthio-N-acetyl-D-glucosamine, J. Org. Chem., 70 (2005) 4195-4198.
[26] J.D.M. Olsson, L. Eriksson, M. Lahmann, S. Oscarson, Investigations of Glycosylation Reactions with 2-*N*-Acetyl-2*N*,3*O*-oxazolidinone-Protected Glucosamine Donors, J. Org. Chem., 73 (2008) 7181-7188.
[27] R.E.J.N. Litjens, L.J. van den Bos, J.D.C. Codée, R.J.B.H.N. van den Berg, H.S. Overkleeft, G.A. van der Marel, Sulfonium Triflate Mediated Glycosidations of Aryl 2-Azido-2-deoxy-1-thio-D-mannosides, Eur. J. Org. Chem.,(2005) 918-924.
[28] C. Jones Revised structures for the capsular polysaccharides from Staphylococcus aureus Types 5 and 8, components of novel glycoconjugate vaccines, Carb. Res., 340 (2005)1097-106.
[29] T. Buskas, P.J. Garegg, P. Konradsson, J.-L. Maloisel, Facile preparation of glycosyl donors for oligosaccharide synthesis: 2-azido-2-deoxyhexopyranosyl building blocks, Tetrahedron: Asymm., 5 (1994) 2187-2194.