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PAPER

Development of highly stereoselective $GalN_3$ donors and their application in the chemical synthesis of precursors of Tn antigen†

George Ngoje, Janet Addae, Harpreet Kaur and Zhitao Li*

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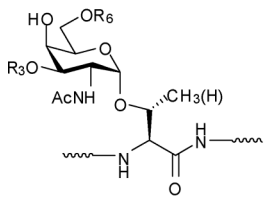
Introduction

α-GalNAc linkage is one of the most important glycosidic linkages in mucin-type *O*-glycans,[1] because all eight core structures are built upon the α-GalNAc-Ser(Thr) residues(Fig. 1).[2] Since abnormal expression of *O*-glycans is often associated with different stages of cancer, study of the physiologic role of these carbohydrates is very important. Many tumor associated carbohydrate antigen (TACA) have the common structure of α-GalNAc-Ser (or Thr), such as TF antigen, T antigen and Sialyl-T antigen. These antigens are interesting targets for developing new therapeutic and diagnostic tools for cancer treatments. Due to the difficulty of obtaining these molecules from natural sources in homogenous form and large quantity, development of highly selective reaction protocols to these structures is of particular interest for carbohydrate chemists and glycobiologists.[3–5]



Core 1: Gal β(1,3)--
Core 2: GlcNAc β(1,6)–
Gal β(1,3)–
Core 3: GlcNAc β(1,3)–
Core 4: GlcNAc β(1,6)–
GlcNAc β(1,3)--
Core 5: GalNAc α(1,3)--
Core 6: GlcNAc β(1,6)–
Core 7: GalNAc α(1,6)--
Core 8: Gal α(1,3)--

Fig. 1 Core structures of mucin type *O*-glycans.

Synthesis of α-GalNAc linkages, like many other 1,2-*cis* glycosidic linkages, is very challenging due to the lack of control on the stereochemistry outcome. General approach to form 1,2-*cis* glycosyidic linkage is through using non-participating group at 2-position to avoid anchimeric effect, which will favor the formation 1,2-*trans* linkages. In the case of forming α-GalNAc linkages, one of the most commonly used methods is to use 2-azido-2-deoxy-galactosyl ($GalN_3$) donors. However, in general, to achieve reasonable stereoselectivity, extensive optimization of the reaction conditions is necessary, because many factors are known to affect the ratio of α/β anomers and only the right combination of all factors can afford the optimum selectivity.[6–14] This optimization process can be very time-consuming and labor intensive, because many of these factors are not well understood and it is hard to predict their influence on the stereoselectivity. Development of robust protocol that can give the desired stereoselectivity (α-selectivity) without intensive optimization of the reaction conditions is of particular interest to carbohydrate chemists.

Our research group has been especially interested in the stereochemistry effect of protecting groups, because they are an indispensible part of carbohydrate chemistry and at the same time have a profound influence on the stereoselectivity of donors.[15–22] Our recent study indicated that the protecting groups, especially the acyl group, play a critical role for the stereoselectivity of $GalN_3$ donors.[23] More specifically, acetyl protecting groups at the 3- and 4-position of $GalN_3$ are most beneficial to the α-selectivity. The acetyl group at 6-position, on the other hand, has only very little or even opposite effect for the α-selectivity. Based on our theoretical study, this selectivity is potentially associated with remote participating effect of 3- and 4- acetyl groups, which has also been observed in Galactose donors before.[24] Another discovery is that the reaction temperature also affects the selectivity, *i.e.* higher temperature favors the α-selectivity. Based on this study, the ideal combination of protecting groups would be therefore acetyl groups at 3- and 4-positions and an electron-donating group at 6-position. In this paper, we report the rational design of two new $GalN_3$ donors that showed excellent α-selectivity and their applications in the synthesis of Tn antigen derivatives.

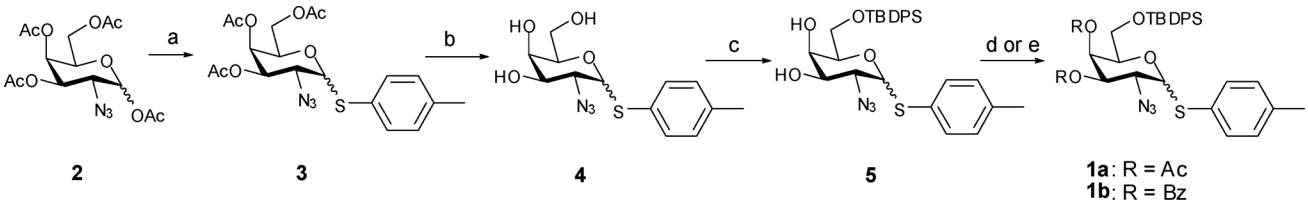
Results and discussion

Based on our study, we designed a new $GalN_3$ thioglycoside donor (compound **1a**, Scheme 1). Acetyl groups are used as protecting group at 3 and 4-positions. TBDPS group was used at the 6-position. This combination would afford the optimum α-selectivity in glycosylation reaction. The desired donor was then prepared from known compound **2** in four step through standard protecting group manipulations. Known compound **2** was synthesized from galactal through literature procedures.[25] Reaction of compound **2** with thiocresol with $BF_3{\cdot}Et_2O$ afforded the thioglycoside **3**, which was then deacetylated to afford intermediate **4**.[26] Selective protection of 6-OH with TBDPSCl gave compound **5**, which upon acetylation gave desired donor **1a** as an inseparable mixture of α/β anomers (close to 1 : 1 ratio).

Donor **1a** was then tested in glycosylation reaction with acceptor **6** using donor **3** as control (Scheme 2). The reactions were carried out in DCM, with TMSOTf/NIS as the promoter at 0 °C. When

Department of Chemistry, Binghamton University, Binghamton, NY, 13902

† Electronic supplementary information (ESI) available: ^1H and ^{13}C NMR of compounds **7, 8, 15, 16, 17, 19, 20, 21** and **23**. See DOI: 10.1039/c1ob05893b



Scheme 1 Synthesis of donor **1a** and **1b**.

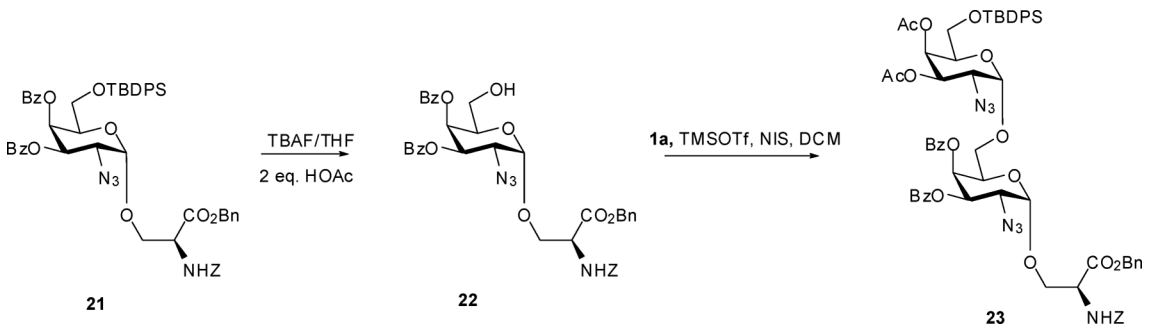
Scheme 2 Comparison between donor **1a** and **3**.

donor **3** was used, the reaction gave a 3 : 1 mixture of anomers in 86% yield, favoring α-product. When donor **1a** was used, the reaction gave almost exclusively α-product (> 20 : 1, with small amount of impurity that could be β-product but cannot be confirmed due to the low concentration) in high yield (99%). This experiment confirms the superior α-selectivity of this rationally designed donor.

This donor was then tested in a series of glycosylation reactions with various glycosyl acceptors (Table 1). In reaction with acceptor **9**, a secondary glucose acceptor with benzyl protection, α disaccharide was also isolated as the predominant product (> 15 : 1) in 77% yield (entry 2). The reaction with acceptor **10** gave only α-product at 68% yield (entry 3). The low reaction yields of acceptor **9** and **10** (entry 2 and 3) are believed to be associated with the lower reactivity of these acceptors, because unreacted acceptors were recovered from both reactions. Reaction with acceptor **11** also gave just α anomer (at 99% yield, entry 4). In all reactions with glycosyl acceptors, donor **1a** showed excellent stereoselectivity. It is noticeable that the reaction condition was arbitrarily set without any optimization, yet the stereoselectivity was excellent and the yields were also generally good.

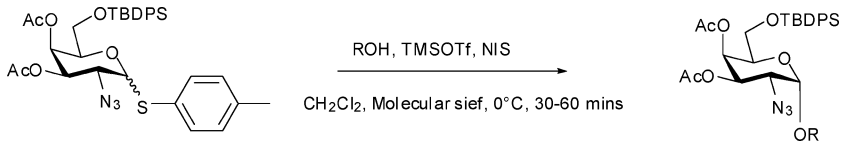
This donor was then tested in reaction with an L-serine acceptor **12** (Cbz protection on amino group, benzyl protection on carboxylic acid) to test the stereoselectivity in making Tn antigen derivatives. Under the general reaction conditions, the reaction afforded a mixture of both anomers. Even though the reaction highly favors the α product (5 : 1, entry 5), it was not satisfactory to us. Reaction at room temperature improved the selectivity (to 7 : 1, entry 6), but still less than perfect. To further improve the selectivity, a second donor was designed and prepared (compound **1b**, Scheme 1), which has benzoyl protecting groups at 3 and 4-positions, with the expectation that the benzoyl group could participate better than acetyl group and therefore improve the α-selectivity. In glycosylation with donor **1b**, the L-serine acceptor **12** did give only α product, which showed much satisfactory stereoselectivity (entry 7). A L-threonine acceptor (compound **13**) was also tested in the glycosylation, excellent α-selectivity (> 15 : 1) was obtained (entry 8). Since Fmoc is another commonly used protecting group for amino acid, an L-serine acceptor (compound **14**) with Fmoc protection was tested in the glycosylation. The reaction also afford predominantly α-product (> 15 : 1, entry 9).

The product formed from donor **1b** and serine acceptors can be easily converted to Tn antigen. They are also useful intermediates for the synthesis of the core structures of mucin type *O*-glycans, like core 6 and core 7. To demonstrate the application of these intermediates in synthesis of core structures, the TBDPS group of compound **21** was selectively removed to afford compound **22**, which gave a core 7 precursor (compound **23**) (Scheme 3)after glycosylation reaction with donor **1a** in good yield (78%) and high stereoselectivity (only α-product).



Scheme 3 Synthesis of a precursor to core 7.

Table 1 Results of glycosylations



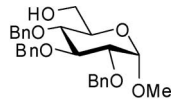
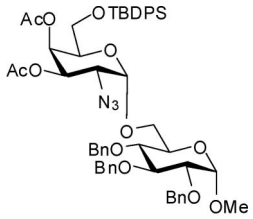
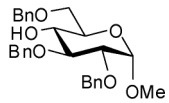
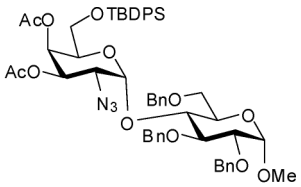
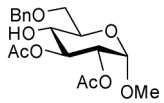
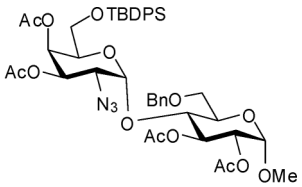
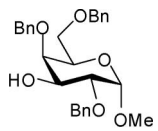
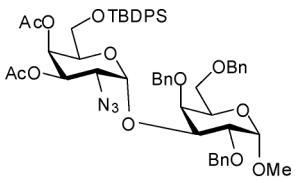
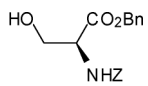
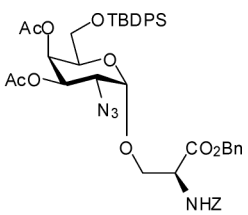
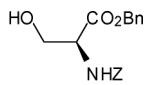
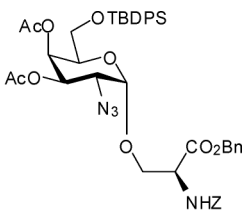
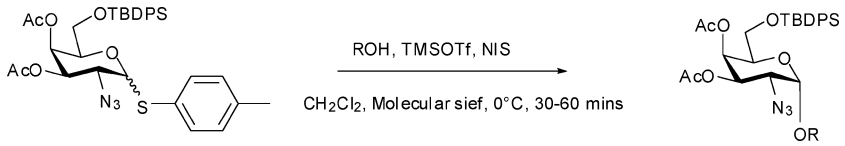
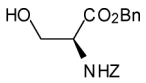
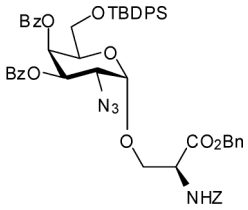
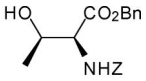
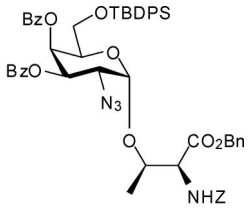
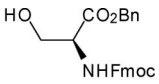
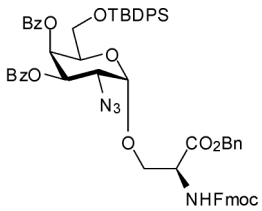
Entry	R–OH	Products	Yield[a]	α : β[b]
1	 **6**	 **8**	99%	20 : 1[c]
2	 **9**	 **15**	77%	15 : 1[c]
3	 **10**	 **16**	68%	α Only
4	 **11**	 **17**	99%	α Only
5	 **12**	 **18**	90%	5 : 1
6	 **12**	 **18**	90%	7 : 1[d]

Table 1 (*Contd.*)



Entry	R–OH	Products	Yield[a]	α : β[b]
7	 **12**	 **19**	70%	α only
8	 **13**	 **20**	65%	15 : 1[c]
9	 **14**	 **21**	71%	15 : 1[c]

Note:[a] Isolated yield. [b] Determined by ^{1}H NMR. [c] Small amount of impurity was observed, which is tentatively assigned as β anomer, but not confirmed due to limited quantity. [d] At room temperature.

In conclusion, two highly stereoselective $GalN_3$ donors were designed and synthesized based on a rational analysis of the protecting group-selectivity relationship. The glycosylation tests demonstrated that these donors are highly selective. Donor **1a** affords only α product in glycosylation reactions with glycosyl acceptors and also showed very high α-selectivity in glycosylation with serine acceptor. Donor **1b** gives only α product even in reactions with serine acceptor. This study not only provides a very accessible and highly selective donor for future research on stereoselective synthesis of α-GalNAc linkages, but also proves the concept that thorough study of the protecting group-selectivity relationship is extremely useful for the development of more efficient chemical synthesis of oligosaccharides.

Experimental

Materials and methods

Unless otherwise noted, reagents and materials were obtained from commercial suppliers and were used without further purification. TLC was performed on pre-coated glass plates (Silica Gel F_{254}). Spots were detected by visualization under UV lamp and/or by charring with *p*-anisaldehyde stain. All NMR data ware recorded on Bruker 360 MHz spectrometer. All ^{1}H NMR data was obtained at 360 MHz, all ^{13}C NMR data was obtained at 90 MHz. Proton and carbon chemical shifts are reported in parts per million (ppm) using $CDCl_3$ as an internal reference unless otherwise noted. Coupling constants (*J*) are reported in (Hz) and multiplicities are abbreviated as follows: (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broadened (br).

Typical procedure for the glycosylation reaction: donor (0.11 mmol, 1.1 equiv.) and acceptor (0.1 mmol, 1 equiv.) were dissolved in anhydrous dichloromethane (4 mL). Flame-dried molecular sieves was added. The mixture was stirred at room temperature for 30 mins and then cooled to 0 °C in ice bath. NIS (0.11 mmol, 1.1 equiv.) and TMSOTf (5 μL) was added. After 30–60 mins, the reaction was quenched with triethylamine (20 μL) and purified by flash chromatography.

2-Azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-galactopyranose-1-*O*-methylthioglycoside (5)

Compound **4** [27] (0.41 g, 1.32 mmoles, 1.0eq) was dissolved in dry DMF (15 ml) and imidazole(0.134 g, 2.0 mmoles,

1.5 mmoles) was added. While stirring at room temperature, *tert*-butyldiphenylchlorosilane was slowly added in duration of 15 min. The reaction mixture was then stirred overnight to completion. The reaction was then quenched with water (30 ml), extracted with dichloromethane(100 ml) and the resulting organic layer washed 3 × 50 ml distilled water, 1 × 50 ml brine, dried over $MgSO_4$ and then concentrated to give the crude oily product which was then purified through flash chromatography (0–50% EtOAc/hexanes) to give 0.58 g (80% yield) of **5** as a mixture of anomers. R_f 0.56 (1 : 1 EtOAc/hexanes:). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.82–7.66(m, 9H), 7.50–7.30(d, J = 7.7 Hz, 2H), 7.49–7.33(m, 16H), 7.09(d, J = 7.9 Hz, 2H), 7.05(d, J = 7.7 Hz, 2H), 5.6(d, J = 5.5 Hz, 1 Hz), 4.45–4.36(m, 2H), 4.25–4.16(m, 2H), 4.09(s, 1H), 4.05–3.80(m, 5H), 3.64–3.42(m, 4H), 3.32(br, 1H), 3.13(br, 1H), 2.34(s, 3H), 2.33(s, 3H), 1.12(s, 18H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 138.3, 137.6, 135.6, 135.5, 135.4, 133.4, 132.9, 132.7, 132.6, 130.0, 129.8, 129.7, 128.1, 127.9, 127.8, 88.9, 86.9, 77.8, 74.4, 70.4, 69.9, 69.1, 64.2, 64.1, 62.9, 61.3, 26.8, 21.1, 19.1. IR (cm^{-1}), 3450, 2932, 2112, 1428, 1258, 1113, 908.

2-Azido-3,4-di-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-galactopyranose-1-*O*-methylthioglycoside (1a)

To a stirred solution of the diol **5** (1.16 g, 2.12 mmoles, 1.0eq) in dichlomethane (15 ml) was added acetic anhydride (0.4 ml, 4.2 mmoles, 2.0eq), triethylamine(0.6 ml, 4.2 mmoles, 2.0eq) and catalytic DMAP and the reaction allowed to proceed for 1 h after which the the mixture was concentrated and the residue purified on flash chromatography(0–30% EtOAc/hexanes) to give the 3,4-di-*O*-Acetyl derivative **1a** (1.28 g, 75% yield). R_f 0.5 (1 : 5 EtOAc/hexanes). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.68–7.59(m, 9H), 7.53–7.28(m, 17H), 7.10(d, J = 8.1 Hz, 2H), 5.67(d, J = 3.0 Hz, 1 Hz), 5.59–5.48(m, 2H), 5.20(dd, J = 3.0, 10.7 Hz, 1H),4.91(dd, J = 3.4, 10.7 Hz, 1H), 4.65(t, J = 6.4 Hz, 1H), 4.45(d, J = 10.2 Hz, 1H), 4.25(dd, J = 5.1, 11.0 Hz, 1H) 3.85–3.70(m, 2H), 3.68–3.56(m, 4H), 2.34(s,3H), 2.29(s, 3H), 2.07(s, 3H), 2.04(s, 3H), 2.03(s, 3H), 2.00(s, 3H), 1.05(s, 18H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 169.6, 169.5, 169.4, 138.5, 138.1, 135.5, 133.6, 133.1, 129.8, 129.8, 129.6, 127.7, 87.7,86.8, 76.6, 73.3, 70.3, 69.8, 67.5, 66.6, 61.5, 61.3, 59.6, 58.4, 26.7, 21.0, 20.5, 20.4, 18.9. IR (cm^{-1}), 2932, 2113, 1750, 1368, 1214, 1081.

2-Azido-3,4-di-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-galactopyranose-1-*O*-methylthioglycoside (1b)

The diol **5** (0.54 g, 0.98 mmoles, 1.0eq) was dissolved in 10 ml of pyridine. Benzoyl chloride (0.34 ml, 2.95 mmoles, 3.0eq) was then added dropwise and the reaction stirred overnight. After dilution with 100 ml of dichloromethane, the reaction mixture was washed 2 × 50 ml distilled water, 2 × 50 ml 10% hydrochloric acid, 1 × 50 saturated $NaHCO_3$ then dried and purified on column chromatography to give **1b** (0.58 g, 78%yield). R_f 0.55 (1 : 3 EtOAc/hexanes). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.9(dd, J = 7.7, 15.8 Hz, 4H), 7.8(t, J = 8.5 Hz, 3H), 7.7–7.2(m, 34H),7.15(d, J = 8.1 Hz, 1H), 7.10(t, J = 7.3 Hz, 3H), 7.05(d, J = 8.1 Hz, 2H), 6.10(d, J = 3.0, 1H), 5.97(d, J = 3.0, 1H), 5.69(d, J = 5.9 Hz, 1H), 5.63(dd, J = 3.4, 11.1 Hz, 1H) 5.32(dd, J = 3.0, 10.7 Hz, 1H), 4.9(t, J = 7.3 Hz, 1H), 4.59–4.47(m, 2H), 3.90–3.82(m, 1H), 3.81–3.71(m, 1H)), 3.70–3.62(m, 2H), 3.72–3.64(m, 1H), 2.42(s, 3H), 2.31(s, 3H), 1.00(s, 18H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 172.1, 165.3, 165.2, 165.1, 164.9, 138.9, 138.2, 135.5, 135.4, 135.3, 134.9, 134.4, 133.7, 133.2, 133.1, 130.2, 129.8, 129.7, 129.6, 128.8, 128.5, 128.4, 128.3, 127.8, 127.6, 87.9, 85.9, 77.5, 73.9, 70.9, 70.1, 68.1, 67.2, 61.2, 59.9, 59.3, 26.6, 21.1, 18.9. IR (cm^{-1}), 2931, 2114, 1731, 1521, 1428, 1215, 1113.

Methyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-glucopyranoside (8)

R_f 0.64 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.62–7.25 (m, 25 H) 5.54 (d, J = 2.9 Hz, 1 H), 5.33 (dd, J = 11.2, 3.2 Hz, 1 H), 5.04–4.76 (m, 5 H), 4.67–4.60 (m, 3 H), 4.09–3.98 (m, 2 H), 3.80–3.47 (m, 8 H), 3.39 (s, 3 H), 2.05 (s, 3 H), 1.99 (s, 3 H), 1.02 (s, 9 H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 169.8, 138.2, 138.1, 135.49, 135.47, 129.8, 129.7, 128.4, 128.3, 128.0, 127.9, 127.82, 127.80, 127.7, 127.6, 127.5, 98.0, 97.9, 82.0, 80.0, 77.7, 75.7, 75.0, 73.3, 70.1, 69.1, 68.3, 67.7, 66.2, 61.6, 57.8, 55.1, 26.6, 20.6, 20.4, 19.0. HRMS (ESI-TOF) [MNa$^+$] calcd for $C_{54}H_{63}N_3O_{12}SiNa$ 996.4073, found 996.4072. IR (cm^{-1}), 2933, 2111, 1749, 1371, 1215, 1073, 701. UV/VIS, λ; 231 nm.

Methyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α- D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (15)

R_f 0.67 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.62–7.20 (m, 25 H), 5.71 (d, J = 4.0 Hz, 1 H), 5.57 (d, J = 1.8 Hz, 1 H), 5.33 (dd, J = 11.5, 2.9 Hz, 1 H), 5.10 (d, J = 10.8 Hz, 1 H), 4.84 (d, J = 11.1 Hz, 1 H), 4.69 (d, J = 11.9 Hz, 1 H), 4.60–4.57 (m, 2 H), 4.43–4.41 (m, 2 H), 4.08–4.03 (m, 2 H), 3.82–3.80 (m, 2 H), 3.68–3.48 (m, 5 H), 3.38 (s, 3 H), 2.05 (s, 3 H), 1.95 (s, 3 H), 1.00 (s, 9 H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 169.71, 169.67, 138.8, 137.9, 137.8, 135.6, 135.5, 132.9, 132.7, 129.72, 129.65, 128.4, 128.3, 128.22, 128.17, 128.04, 127.90, 127.86, 127.78, 127.75, 127.70, 127.64, 127.61, 127.53, 127.48, 127.44, 127.28, 127.21, 97.9, 97.5, 81.6, 80.5, 74.6, 74.4, 73.1, 69.4, 69.2, 69.1, 68.4, 67.4, 61.3, 57.6, 55.2, 26.7, 20.6, 20.5, 19.0. HRMS (ESI-TOF) [MH$^+$] calcd for $C_{54}H_{64}N_3O_{12}Si$ 974.4254, found 974.4244. IR (cm^{-1}), 2932, 2111, 1749, 1369, 1045, 701. UV/VIS, λ; 229 nm.

Methyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α- D-galactopyranosyl-(1→4)-2,3-di-*O*-acetyl-6-*O*-benzyl-α- D-glucopyranoside (16)

R_f 0.41 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.60–7.13 (m, 15 H), 5.60 (br, 1 H), 5.54 (t, J = 9.7 Hz, 1 H), 5.28 (dd, J = 7.2, 3.6 Hz, 1 H), 5.15 (d, J = 4.0 Hz, 1 H), 4.84 (d, J = 3.2 Hz, 1 H), 4.79 (dd, J = 5.8, 3.2 Hz, 1 h), 4.36 (m, 2 H), 4.11 (m, 1 H), 3.95 (t, J = 9.0 Hz, 1 H), 3.81 (br, 1 H), 3.78–3.49 (m, 5 H), 3.40 (s, 3 H), 2.04–1.97 (12 H), 1.00 (s, 9 H); ^{13}C NMR (90 MHz, $CDCl_3$): δ 170.3, 169.5, 137.8, 135.52, 135.48, 133.7, 132.8, 130.0, 129.8, 128.2, 127.70, 127.68, 127.44, 127.37, 99.3, 96.7, 75.8, 73.2, 72.0, 69.6, 69.4, 68.4, 68.3, 67.3, 61.1, 57.8, 55.2, 28.5, 26.6, 20.8, 20.7, 20.6, 20.4. HRMS (ESI-TOF) [MH$^+$] calcd for $C_{44}H_{56}N_3O_{14}Si$ 877.3526, found 877.3518. IR (cm^{-1}), 2933, 2112, 1751, 1646, 1238, 1044, 703. UV/VIS, λ; 230 nm.

Methyl 3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-α-D-galactopyranoside (17)

R_f 0.69 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ7.62–7.20 (m, 25 H), 5.61–5.57 (m, 2 H), 5.24 (d, *J* = 2.9 Hz, 1 H), 5.02 (d, *J* = 10.8 Hz, 1 H), 4.72 (d, *J* = 11.5 Hz, 1 H), 4.62–4.39 (m, 6 H), 4.15 (dd, *J* = 10.0, 2.5 Hz, 1 H), 4.00–3.96 (m, 2 H), 3.90–3.81 (m, 2 H), 3.68–3.64 (m, 1 H), 3.59–3.50 (m, 3 H), 3.27 (s, 3 H), 2.08 (s, 3 H), 2.03 (s, 3 H), 0.98 (s, 9 H); ^{13}C NMR (90 MHz, $CDCl_3$): *δ* 169.8, 169.6, 138.5, 138.2, 135.5, 133.0, 132.9, 129.6, 129.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.73, 127.70, 127.6, 127.52, 127.47, 98.4, 95.2, 75.7, 75.0, 74.9, 73.9, 73.40, 73.36, 69.0, 68.9, 68.81, 68.76, 68.74, 67.7, 61.1, 58.5, 55.1, 26.7, 26.6, 20.7, 20.6, 19.0. HRMS (ESI-TOF) [MNa^+] calcd for $C_{54}H_{63}N_3O_{12}SiNa$ 996.4073, found 996.4070. IR (cm^{-1}), 2931, 2115, 1752, 1428, 1110, 667. UV/VIS, λ; 228 nm.

***N*-Benzyloxy carbonyl-*O*-(2-azido-3,4-di-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-galactopyranosyl)-L-serine benzyl ester (18)**

R_f 0.50 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): *δ* 7.7–7.6 (m, 4H), 7.5–7.3, (m, 12H), 5.7 (d, *J* = 8.1, 1H) 5.7–5.6 (m, 1H), 5.55 (br, 1H), 5.50–5.52 (m, 1H), 5.48 (d, *J* = 2.9, 1H), 5.28 (d, *J* = 0.6, 2H), 5.26–5.18(m, 1H), 5.2–5.1 (m, 1H), 5.1 (s, 1H), 4.8 (d, *J* = 3.4 Hz, 1H), 4.76–4.74 (m, 1H), 4.6–4.5(m, 1H), 4.4–4.3(m, 1H),4.23(d, *J* = 7.88, 1H), 3.94–3.84(m, 1H), 3.7–3.6(m, 1H), 2.04(s,3H), 2.0(s, 3H), 1.03(s, 9H); ^{13}C NMR (90 MHz, $CDCl_3$): δ, 169.7, 169.6, 169.5, 169.3, 156.0, 136.0, 135.0, 132.8, 128.6, 128.5, 128.4, 128.1, 127.7, 102.5, 99.3, 73.3, 71.2, 69.7, 69.5, 68.2, 67.7, 67.3, 67.1, 67.0, 66.2, 61.2, 60.8, 57.6, 54.4, 34.6, 34.5, 31.5, 29.6, 29.0, 26.6, 23.5, 22.6, 20.6, 20.4, 18.9, 14.0, 11.34. IR (cm^{-1}), 2959, 2113, 1750, 1510, 1215, 1113. UV/VIS, λ; 229 nm.

***N*-(Benzyloxycarbonyl)-*O*-[3,4-di-*O*-benzoyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-galactopyranosyl]-L- serine benzyl ester (19)**

R_f 0.52 (1 : 2 EtOAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ8.0 (d, *J* = 8.0, 2 H), 7.9 (d, *J* = 7.3 Hz, 2 H), 7.7–7.6 (m, 4H), 7.6–7.4, (m, 5 H), 7.4–7.3 (m, 17 H), 7.1 (t, *J* = 7.3 Hz, 2 H), 6.0 (d, *J* = 2.6 Hz, 1 H), 5.7 (dd, *J* = 3.0,11 Hz, 1 H), 5.3–5.2 (m, 3 H), 5.1 (s, 1 H), 5.0(d, *J* = 3.4 Hz, 1 H), 4.7 (d, *J* = 8.1 Hz, 1 H), 4.2 (t, *J* = 6.8 Hz, 1 H), 4.2–4.1 (m, 1 H), 4.0 (dd, *J* = 3.0, 11.1 Hz, 1 H), 3.8–3.7(m, 3 H), 1.0(s, 9 H); ^{13}C NMR (90 MHz, CDCl3): δ, 169.6, 165.2, 165.0, 156.0, 136.0, 135.5, 135.4, 135.0, 133.4, 133.3, 133.2, 132.7, 132.5, 129.8, 129.7, 129.6, 129.2, 128.6, 128.5, 128.4, 128.2, 128.0, 127.7, 99.5, 69.9, 69.6, 68.9, 68.0, 67.0, 61.3, 58.3, 54.5, 26.6, 18.9; HRMS (ESI-TOF) [MH^+] calcd for $C_{54}H_{55}N_4O_{11}Si$ 963.3631, found 963.3621. IR (cm^{-1}), 2931, 2112.42, 1729, 1215, 1068. UV/VIS, λ; 231 nm.

***N*-(Benzyloxycarbonyl)-*O*-[3,4-di-*O*-benzoyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-galactopyranosyl]-L- threonine benzyl ester (20)**

R_f 0.58 (1 : 2 EtoAc/Hexanes), ^{1}H NMR (360 MHz, $CDCl_3$): δ7.9 (2d, *J* = 7.0, 6.9 Hz, each 2 H), 7.7–7.6 (m, 4 H), 7.–7.4, (m, 4 H), 7.4–7.3 (m, 22 H), 7.2 (t, *J* = 7.5 Hz, 2 H), 6.0 (d, *J* = 2.8 Hz, 1 H), 5.6 (dd, *J* = 3.2, 12 Hz, 1 H), 5.3–5.1 (m, 6H), 5.1 (s, 1 H), 5.0(d, *J* = 3.6 Hz, 1 H), 4.5 (br, 2H), 3.8 (dd, *J* = 3.2, 11 Hz, 1 H), 3.7 (d, *J* = 7.3 Hz, 2 H), 1.28 (d, *J* = 3.0 Hz, 3 H), 0.99(s, 9 H); ^{13}C NMR (90 MHz, CDCl3): δ, 170.0, 165.3, 165.0, 156.8, 136.2, 135.4, 135.3, 135.0, 134.9, 134.7, 133.2, 132.8, 132.7, 130.1, 129.7, 129.2, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 124.8, 99.2, 69.8, 69.3, 69.1, 68.8, 68.1, 67.8, 67.5, 67.2, 61.5, 59.9, 58.7, 29.6, 26.5, 25.7, 25.6, 18.9, 18.7; HRMS (ESI-TOF) [MH^+] calcd for $C_{55}H_{57}N_4O_{11}Si$ 977.3787, found 977.3778. IR (cm^{-1}), 2932, 2112, 1728, 1513, 1276, 1094, 909. UV/VIS, λ; 231 nm.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-[3,4-di-*O*-benzoyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-galactopyranosyl]-L-serine benzyl ester (21)**

R_f 0.54 (1 : 2 EtOAc/hexanes) ^{1}H NMR (360 MHz, $CDCl_3$): δ7.9 (2d, *J* = 7.7, 7.9 Hz, each 2 H), 7.8 (d, *J* = 7.5 Hz, 3 H), 7.7–7.5 (m, 7 H), 7.4–7.3, (m, 17 H), 7.1 (t, *J* = 7.2 Hz, 2 H), 4.5 (br, 2 H), 6.0 (d, *J* = 2.3 Hz, 1 H), 5.9–5.8 (m, 1 H), 5.7 (dd, *J* = 3.2, 11 Hz, 1 H), 5.4–5.1 (m, 4 H), 5.0(br, 1 H), 4.6 (br, 1 H), 4.5–4.3 (m, 3 H), 4.3–4.1 (m, 3 H), 4.0 (br, 1 H), 3.9–3.7(m, 4 H), 1.0(s, 9 H), ^{13}C NMR (90 MHz, CDCl3) δ, 169.6, 165.2, 165.0, 156.0, 143.7, 141.2, 135.5, 135.3, 135.0, 134.8, 133.2, 132.7, 132.5, 130.2, 129.8, 129.7, 129.5, 129.2, 128.6, 128.5, 128.2, 127.7, 127.6, 127.5, 127.0, 125.2, 119.8, 99.5, 69.9, 69.4, 68.8, 68.0, 67.7, 67.4, 61.3, 58.4, 54.5, 47.0, 29.6, 26.5, 25.6, 18.9; HRMS (ESI-TOF) [MH^+] calcd for $C_{61}H_{59}N_4O_{11}Si$ 1051.3944, found 1051.3941. IR (cm^{-1}), 2932, 2113, 1728, 1512, 1276, 1106, 909. UV/VIS, λ; 230 nm.

***N*-(Benzyloxycarbonyl)-*O*-[3,4-di-*O*-acetyl-2-azido-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-α-D-galactopyranosyl-(1→6)-3,4-di-*O*-benzoyl-2-azido-2-deoxy-α-D-galactopyranosyl]-L- serine benzyl ester (23)**

Compound **19** (0.12 g, 0.125 mmoles, 1.0eq) was dissolved in 5 ml of dry THF, followed by addition of TBAF in THF (140 µL, 0.48 mmoles, 3.8eq) and acetic acid (4.7 µL, 0.003 mmoles, 0.0833 mmoles, 0.66eq) at room temperature and the reaction stirred overnight. Upon completion, the reaction mixture was diluted with 50 ml of DCM washed with water (50 ml), $NaHCO_3$ (50 ml) and then water again (50 ml) then dried over $MgSO_4$, concentrated then purified on flash chromatography (10–35% EtOAc/hexanes) to afford 0.077 g (85% yield) of the alcohol **22**. R_f 0.31 (1 : 2 EtOAc/hexanes) ^{1}H NMR (360 MHz, $CDCl_3$) δ, 8.0, (d, *J* = 7.3 Hz, 2 H), 7.9 (d, *J* = 8.1 Hz, 2 H), 7.7–7.3 (m, 16 H), 6.0 (d, *J* = 7.7 Hz, 1 H), 5.7 (br, 1 H), 5.6 (dd, *J* = 3.0, 11 Hz, 1 H), 5.3–5.2 (m, 2 H), 5.1(s, 2 H), 5.0 (d, *J* = 3.0 Hz, 1 H), 4.6 (br, 1 H), 4.3–4.2 (m, 1 H), 4.1–4.0 (m, 2 H), 3.83(dd, *J* = 3.4, 11 Hz, 1 H), 3.7–3.6, (m, 1 H), 3.6–3.4, (m, 1 H). Acceptor **22** was glycosylated with donor **1a** under standard reaction conditions to afford compound **23**. R_f 0.63 (1 : 2 EtOAc/hexanes) ^{1}H NMR (360 MHz, $CDCl_3$): δ8.0 (d, *J* = 7.3 Hz, 2 H), 7.9 (d, *J* = 7.5 Hz, 3 H), 7.6 (t, *J* = 5.8 Hz, 4 H), 7.5 (t, *J* = 7.9 Hz, 1 H), 7.4–7.3 (m, 21 H), 4.5 (br, 2 H), 5.9 (d, *J* = 8.4 Hz, 1 H), 5.7 (d, *J* = 3.0 Hz, 1 H), 5.6 (s, 1 H), 5.3 (dd, *J* = 3.0, 11 Hz, 1 H), 5.3–5.2 (m, 2 H), 5.2 (d, *J* = 4.5 Hz, 2 H), 5.0 (d, *J* = 3.4 Hz, 1 H), 4.8 (d, *J* = 3.4 Hz, 1 H), 4.7 (br, 1 H), 4.3–4.1 (m, 5 H), 3.8–3.7(m, 2 H), 3.7–3.5 (m, 4 H), 2.0 (s, 3 H), 1.9 (s, 3 H), 0.97 (s, 9 H), ^{13}C NMR (90 MHz, $CDCl_3$) δ, 169.7, 165.2, 165.0, 156.0, 136.2, 135.5, 135.2, 133.5, 133.2, 132.9, 132.8,

129.7, 129.0, 128.6, 128.5, 128.4, 128.2, 128.0, 127.6, 99.0, 97.3, 69.3, 68.9, 68.8, 68.7, 68.6, 67.8, 67.6, 64.5, 67.1, 66.1, 61.3, 58.1, 57.6, 54.2, 29.6, 26.6, 20.6, 18.9; HRMS (ESI-TOF) [MH^+] calcd for $C_{64}H_{68}N_7O_{17}Si$ 1234.4435, found 1234.4446. IR (cm^{-1}), 2931, 2112, 1726, 1511, 1068, 909. UV/VIS, λ; 232 nm.

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