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Glycosylated α-Azido Amino Acids: Versatile Intermediates in the Synthesis of Neoglycoconjugates

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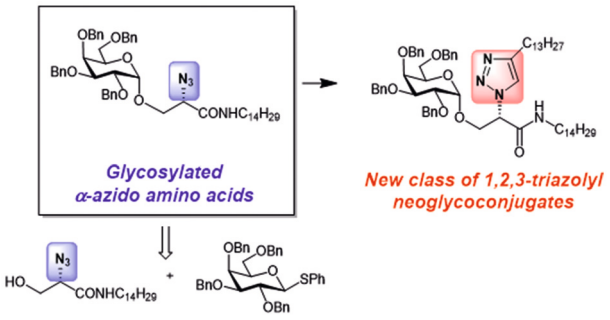

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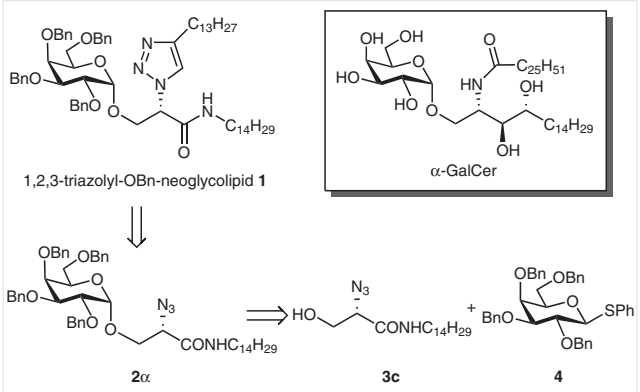
Abstract A series of glycosylated α-azido amino acids was synthesized as precursors for neoglycoconjugates, a class of important biomolecules for drug discovery, and sensor development. The synthetically challenging 1,2-*cis* α-galactosylated species described herein were designed as building blocks in the synthesis of analogues of α-galactosyl ceramide, a potent immunomodulator. A benzyl-protected 1,2,3-triazolyl α-galactosyl-L-serine derivative was prepared using copper azide alkyne cycloaddition to showcase the potential of glycosylated α-azido amino acids in neoglycoconjugate design.

Key words α-azido amino acid, glycosylation, neoglycoconjugates 1,2,3-triazolyl glycosides, CuAAC reaction

The copper azide–alkyne cycloaddition (CuAAC) has been used extensively in bioconjugation reactions of proteins, peptides, and glycans.[1–4] Thus, glycosyl azides have become useful intermediates in synthetic carbohydrate chemistry since they provide access to 1,2,3-triazole neoglycoconjugates via the CuAAC reaction.[5,6] Neoglycoconjugates are synthetic molecules with largely unexplored physiochemical properties and are finding interesting applications in many fields of glycobiology.[7,8] Many of the 1,2,3-triazole glycoconjugates reported are *N*-glycosides prepared from anomeric azides;[9] however, the azido group can also be inserted at other positions in the sugar or as part of the aglycone.[10] *O*-Glycosylated amino acids are important building blocks for the synthesis of glycopeptides and other glycoconjugates.[11–13] Rather surprisingly, very few examples of *O*-glycosylated α-azido amino acids have been reported[14–16] and even fewer of those refer to 1,2-*cis* glycosidic linkages.[17] In these reports the azido functional group is often installed temporarily as a masked amine and further functionalization by means of the CuAAC reaction has not yet been explored. Thus the synthesis of glycosylated α-azido amino acid derivatives would allow the inclusion of these intermediates in the tool box of glycoconjugation chemistry.

This work describes the synthesis of α-azido-L-serine glycosyl acceptors using different diazotransfer reagents and their reactions with orthogonally protected galactosyl donors to produce α- and β-galactosylated α-azido amino acid building blocks. As proof of concept of their potential in the preparation of neoglycoconjugates, the synthesis of the novel benzyl-protected 1,2,3-triazolyl analogue **1** of the immunostimulant glycosphingolipid α-galactosyl ceramide (α-GalCer) [18,19] was devised (Scheme 1). The remarkable biological activity of α-GalCer as an adjuvant in immunotherapies has prompted the synthesis of numerous structural analogues.[20,21] Some of these feature the 1,2,3-triazolyl motif, a good surrogate for amide bonds, which may improve physiological response.[22–24] Thus, the preparation of α-galactosyl glycolipid **1** was envisaged from α-galactosyl



Scheme 1 Synthetic precursors of 1,2,3-triazolyl analogue **1** of immunostimulant glycoshingolipid α-galactosyl ceramide (α-GalCer)

α-azido L-serine amide **2α**, obtained from the reaction of lipidic α-azido amide **3c** and thiophenyl donor **4**, in a highly convergent approach as shown in Figure 1.

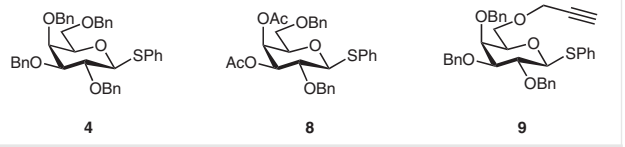
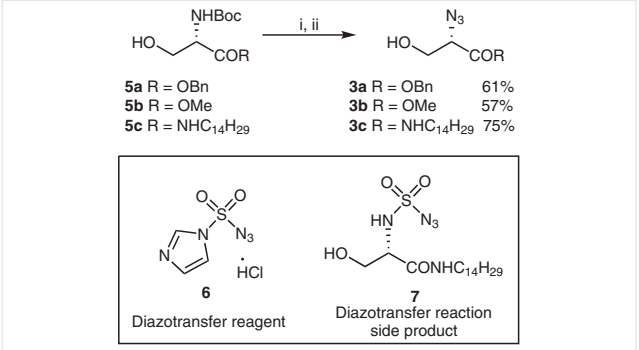


Figure 1 Galactosyl donors **4**, **8**, **9**

Firstly, the synthesis of L-serine α-azido glycosyl acceptors **3a–c** using diazo-transfer methodologies was investigated. Hence, benzyl and methyl α-azido esters **3a**[25] and **3b**,[16] respectively, were prepared from their corresponding *N*-Boc-protected derivatives **5a**[26] and **5b** as shown in Scheme 1. The reactions were carried out as one-pot procedures, initiated by the acidic removal of the carbamate protecting group followed by diazotransfer reaction with either trifluoromethanesulfonyl (triflyl) azide[27,28] or imidazole-1-sulfonyl azide **6** and Cu(II) catalysis in basic medium.[29] α-Azido esters **3a** and **3b** were obtained in 61% and 57% overall yields, respectively. In order to make the synthesis of galactosyl α-azido L-serine amide **2** more convergent, the direct conversion of lipidic amide **5c**[30] to the corresponding α-azido derivative **3c** was attempted. The conversion of amines into azides using diazotransfer methodologies has been extensively studied in amino acid esters[28] and peptides,[31] however, there is no literature precedent for this reaction in lipidic amino acid derivatives. We found that, if imidazole-1-sulfonyl azide **6** was used as the diazotransfer reagent, the reaction yield decreased significantly due to the formation of the side product **7**, which was isolated and identified (see Electronic Supporting Information, ESI). The optimized conditions for the synthesis of lipidic α-azido amide **3c** involved treatment with trifluoroacetic acid followed by diazotransfer reaction with triflyl azide in a mixture of 3:10:3 of water/methanol/dichloromethane to give **3c** in 75% yield (Scheme 2).

Galactosyl thiophenyl ethers **4**,[32] **8**,[33] and **9**[34] were selected as glycosyl donors suitable for 1,2-*cis* glycoside formation (Figure 1, see ESI for optimized synthetic procedures). Compounds **8** and **9** are functionalized with orthogonal protecting groups[35] and allow different reactivity regarding anomeric selectivity based on remote group participation compared to perbenzylated thioglycoside **4**. Selective functionalisation can also occur with these glycosides, leading to a greater variety of neoglyconjugate precursors. All donors feature a nonparticipating benzyl ether at the C-2 position to allow for the formation of the α-glycosylation product.

The glycosylation reaction of α-azido amino acids **3a–c** was then explored under a range of conditions (Table 1).[36] Thioglycoside activation was performed with *N*-iodosuccinimide (NIS) and triflic acid in all cases (except in the reaction for entry 5); however, the reaction temperature and solvent were varied.

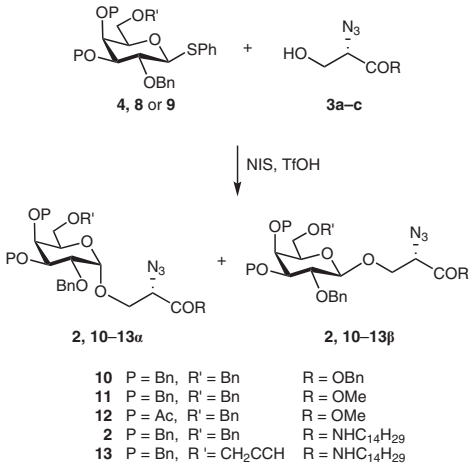


Scheme 2 *Reagents and conditions*: i) for **3a**,**b**: 1 M HCl, CH_2Cl_2, 18 h; for **3c**: TFA, CH_2Cl_2, 0 °C to rt, 18 h; ii) for **3a**: **6**, K_2CO_3, $CuSO_4{\cdot}5H_2O$, *t*-BuOH, rt, 6 h; 61% over two steps; for **3b**: TfN_3, NEt_3, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2/MeOH/H_2O (10:3:3), rt, 18 h; 57% over two steps; for **3c**: TfN_3, NEt_3, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2/MeOH/H_2O (10:3:3), rt, 18 h; 75% over two

Perbenzylated galactosyl donor **4** was reacted with L-serine α-azido benzyl ester **3a** in dichloromethane at room temperature, resulting in a mixture of α- and β-anomers (**10α** and **10β**) in a combined yield of 71% (Table 1, entry 1). The anomeric ratio could not be determined from the crude mixture due to overlapping of key signals in the ^{1}H NMR spectrum. Chromatographic separation only allowed the isolation of the α-anomer **10α** (26% yield). α-Azido methyl ester **3b** was reacted with galactosyl donor **4** using the same conditions, to give a 2.2:1 mixture of products (**11α**/**11β**) in 77% yield (Table 1, entry 2). Lowering the temperature (Table 1, entry 3) improved the yield to 84%, however the α-selectivity was reduced to a 1.8:1 α/β anomeric ratio. As anticipated, the stereoselective preference for the α-anomer **11α** was significantly increased when using THF as solvent (Table 1, entry 4) to give a 4:1 mixture of products **11α**/**11β** albeit in a moderate 53% yield. While the formation of glycosides with galactosyl donor **8** have been reported to occur with very high α-selectivity attributed to the influence of the remote acetyl protecting groups at the C-3 and C-4 positions,[31] the reaction of α-azido methyl ester **3b** with galactosyl donor **8** was only moderately selective, yielding a 2:1 mixture of **12α**/**12β** products in 63% yield (Table 1, entry 5). The galactosylation of lipidic α-azido amide **3c** with galactosyl donors **4** (Table 1, entry 6) and **9** (Table 1, entry 7) gave the corresponding products **2α**/**2β** and **13α**/**13β** in 63% and 80% yield, respectively, with a slight preference for the α-anomeric product (2.7:1 and 2.3:1 α/β anomeric ratio, respectively).

Glycosylated azido acid derivatives are biomolecules of interest for the synthesis of structurally novel glycoconjugates.[37] The galactosylated α-azido acid esters and amides reported herein can be easily prepared and are valuable synthetic intermediates in conjugation methodologies in-

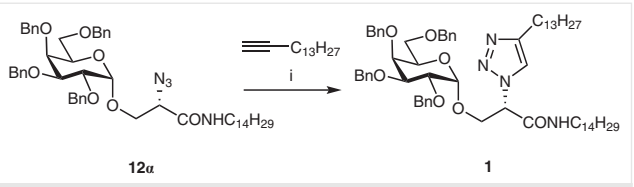
Table 1 Galactosylation Reactions of L-Serine α-Azido Amino Acid Derivatives **3a–c**



Entry	Acceptor	Donor	Product	Temp	Solvent	Yield (%)	α/β ratio[a]
1	**3a**	**4**	**10**	rt	CH_2Cl_2	71	–[b]
2	**3b**	**4**	**11**	rt	CH_2Cl_2	77	2.2:1
3	**3b**	**4**	**11**	–60 to –20 °C	CH_2Cl_2	84	1.8:1
4	**3b**	**4**	**11**	rt	THF	53	4:1
5[c]	**3b**	**8**	**12**	0 °C	CH_2Cl_2	63	2:1
6	**3c**	**4**	**2**	rt	THF	63	2.7:1
7	**3c**	**9**	**13**	rt	THF	80	2.3:1

[a] Anomeric ratio α/β of the product mixture was determined from the integration of key signals in the ^{1}H NMR spectrum.
[b] Anomeric ratio of the α/β product mixture could not be determined from the product mixture due to overlapping of key signals in the ^{1}H NMR spectrum.
[c] The promotor used in this reaction was TMSOTF in place of TfOH.

volving azido functionality, such as the CuAAC reaction.[38] Lipidic derivatives such as **2α**/**2β** serve as precursors to novel 1,2,3-triazolyl neoglycoconjugates. To validate potential applications in this regard, the synthesis of the protected L-serinyl analogue **1** of the immunostimulant glycosphingolipid α-GalCer was attempted as shown in Scheme 2. Thus, compound **2α** was reacted with 1-pentadecyne and copper(II) sulfate/sodium ascorbate catalysis to give perbenzylated galactosyl glycolipid **1** in 71% yield (Scheme 3). Furthermore, glycosides in which azides and alkynes are present simultaneously are becoming interesting molecular scaffolds that can lead to macrocyclic derivatives via intramolecular CuAAC reactions.[39–41] The possibility of applying such approach in the synthesis of macrocyclic neoglycolipids is presently being explored in our research group with galactosylated azido amides like **13α**/**13β**, which feature a propargyl ether at the C-6 position. Optimisation of the macrocyclization conditions is currently ongoing.



Scheme 3 *Reagents and conditions*: i) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, THF/MeOH/H_2O (2:2:1), rt, 18 h, 71%.

In summary, this work aims to highlight the potential of glycosylated α-azido acid derivatives as versatile intermediates in the synthesis of novel neoglycoconjugates. α-Azido L-serine glycosyl acceptors, including the novel lipidic amide **3c**, were prepared by diazotransfer reactions. The reactivity of these compounds in glycosylation reactions was demonstrated with a range of orthogonally protected galactosyl thiophenyl donors. As proof of concept for the suitability of these novel building blocks in glycoconjugate synthesis, galactosyl 1,2,3-triazolyl protected neoglycolipid **1** was readily prepared from 1,2-*cis*-galactosylated lipidic α-azido amide **2α**. Further investigations into the application of the glycosylated α-azido acid derivatives described herein are currently under way.

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Supporting Information

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(36) **Representative Procedure for Glycosylation of α-Azido Amino Acid Derivatives**
NIS (276 mg, 1.23 mmol) was added to a solution of thiophenyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (**4**, 388 mg, 0.61 mmol) and α-azido-L-serine tetradecyl amide (**3c**, 200 mg, 0.61 mmol) in anhydrous THF (6 mL) in the dark under $N_2$and at rt. TfOH (2 μL) was added, and the reaction mixture was stirred for 20 h. MeOH was added, and the solvent was removed in the rotary evaporator. The residue was diluted with CH_2Cl_2 (20 mL) and washed with 1 M aq $Na_2S_2O_4$ (20 mL) followed by brine (20 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to give a brown solid (α/β anomeric ratio of 2.7:1 was estimated from integration of ^{1}H NMR spectrum signals). The crude product was purified by flash column chromatography (PE to PE/EtOAc 5:1). Fractions containing glycosylated products **2α**/**2β** were recovered in a combined yield of 328 mg, 63%. The α-anomer product **2α** could be isolated as a white solid (81 mg, 35%): R_f = 0.13 (PE/EtOAc/toluene = 3:1:6); $[\alpha]_D^{25}$ +34.3 (*c*, 0.35 in CH_2Cl_2). IR (NaCl plate, CH_2Cl_2): ν_{max} = 3350.5, 2924.3, 2858.2, 2108.2, 1726.4, 1452.2, 1261.4 cm^{-1}. ^{1}H NMR (300 MHz): δ = 0.87 (t, *J* = 7.3 Hz, 3 H, CH_3), 1.25 (br s, 20 H, $(CH_2)_{10}CH_3$), 1.37–1.44 (m, 2 H, $NHCH_2CH_2(CH_2)_{10}CH_3$), 2.95–3.07 (m, 1 H, NHC*H*), 3.16–3.23 (m, 1 H, NHC*H*), 3.52–3.54 (m,2 H, H-6, H-6′), 3.68–3.76 (m, 1 H, H-β′), 3.90–3.96 (m, 3 H, H-3, H-4, H-5), 4.04–4.13 (m, 3 H, H-α, H-β, H-2), 4.38–4.95 (m, 8 H, CH_2Ph × 4), 4.87 (d, *J* = 3.1 Hz, 1 H, H-1), 6.59 (t, *J* = 5.0 Hz, 1 H, NH), 7.26–7.36 (m, 20 H, aromatics). ^{13}C NMR (75 MHz): δ = 14.1 (CH_3), 26.9, 29.3, 29.40, 29.45, 29.5, 29.63, 29.67, 29.7, 31.9 ($NHCH_2(CH_2)_{12}CH_3$), 39.5 ($NHCH_2(CH_2)_{12}CH_3$), 63.0 (C-α), 68.8 (C-6), 69.1 (C-β), 70.0 (C-4 or C-5), 73.1, 73.5, 74.7, 74.8, (CH_2Ph × 4), 74.9 (C-4 or C-5), 75.1 (C-2), 78.8 (C-3), 98.9 (C-1), 127.3, 127.4, 127.5, 127.6, 127.74, 127.76, 127.8, 127.9, 128.0, 128.23, 128.26, 128.32, 128.39, 128.4, 137.9, 138.4, 138.5, 138.6 (aromatics), 166.9 (CO). HRMS (ESI$^+$): *m/z* calcd for $C_{51}H_{68}N_4O_7H$ $[M + H]^+$: 849.519; found: 849.5161.
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