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Synthesis of novel glycolipids derived from glycopyranosyl azides and *N*-(β-glycopyranosyl)azidoacetamides

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ABSTRACT

A general and expedient method based on a click reaction has been developed for the synthesis of novel glycolipids. The Cu(I) catalyzed [3+2] cycloaddition of several fully acetylated β- as well as α-D-glycopyranosyl azides, including the 1,6-diazide derived from D-glucose, with long chain alkyl propargyl ethers gave the respective 1,4-substituted 1,2,3-triazole derivatives in good yields. Treatment of fully acetylated *N*-(β-glycopyranosyl)azidoacetamides under similar conditions with alkyl propargyl ethers afforded the 1,2,3-triazolylacetamido derivatives in fairly good yields. Zemplen de-O-acetylation of all the fully acetylated derivatives furnished the free glycolipids in quantitative yields.

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Integral membrane proteins (IMPs) play vital roles in many important biological processes including bacterial resistance to antibiotics. Even after 20 years since the first determination of the crystal structure of an IMP in 1985, crystallization of this class of proteins remains a daunting task in X-ray crystallography of biomolecules.[1] Owing to their hydrophobicity and insolubility in aqueous medium, detergents are required for both solubilization and crystallization. Only a few classes of detergents have found general utility for crystallizing membrane proteins, and these are alkyl polyoxyethylenes, zwitterionic surfactants and carbohydrate-derived non-ionic surfactants.[2] The correct choice of detergent has been the key to success in membrane protein solubilization and crystallization. While it is imperative that a large library of detergents needs to be screened to identify the right candidate, only a limited number are commercially available.[1a] More importantly, there is a pressing need for designing new classes of structurally diverse detergents for efficient solubilization and crystallization of membrane proteins.

Synthetic glycolipids represent a major class of non-ionic detergents that are mild in nature and have a minimal influence on protein conformation. Termed as *green surfactants* in view of their preparation from naturally occurring renewable sources (sugars and fatty alcohols), very low toxicity and ready biodegradability, they are currently attracting significant attention.[3] The reactions employed earlier for the preparation of synthetic glycolipids, used in membrane protein studies, include O-/S-glycosidation of appropriately protected saccharides, reductive amination of lactose, selective N-acylation of free glycosylamines and O-alkylation and carbamoylation of selectively protected sugar derivatives. These methods, except the reductive amination and N-acylation, involve multiple protection and deprotection steps to ensure stereo- and regioselectivity, which often results in low overall yields and higher costs. Besides suffering from problems in product purification and scale-up, the procedure involving reductive amination destroys the pyranose/furanose ring structure, whereas the selective N-acylation[4] of free glycopyranosylamine is generally limited to the preparation of the β-anomer of the glycolipid. Selectively functionalized sugars carrying an azido group are very attractive chemical ligating agents in view of the stability of the azide functionality under a wide variety of reaction conditions and their utility in click chemistry.[5] We report herein on the development of a general and convenient method for the synthesis of novel and structurally diverse glycolipids.

The modification[6] of Huisgen's 1,3-dipolar cycloaddition has transformed the [3+2] cycloaddition between an alkyne and an organic azide into a regioselective and efficient method for chemical ligation. Applications of this elegant synthetic methodology to the preparation of diverse targets[7] including glycodendrimers, glycopolymers, glycopeptides and immobilization of carbohydrates onto solid surfaces have been reported.[8] In the present work, conjugation of the sugar moiety to the lipophilic chain was planned to be achieved by click reaction between two different azido derivatives of sugars and alkyl propargyl ethers. Acetylated glycopyranosyl azides[9] were chosen initially as versatile synthons for the present work as both the α- and the β-anomers could be readily prepared stereoselectively on large scale whilst a few are commercially available. The fully acetylated β-glycopyranosyl azides **1–7**, required for the current study, were obtained by the facile and near quantitative displacement of their corresponding α-chlorides[10] with NaN_3 in aqueous acetone at room temperature.[11]

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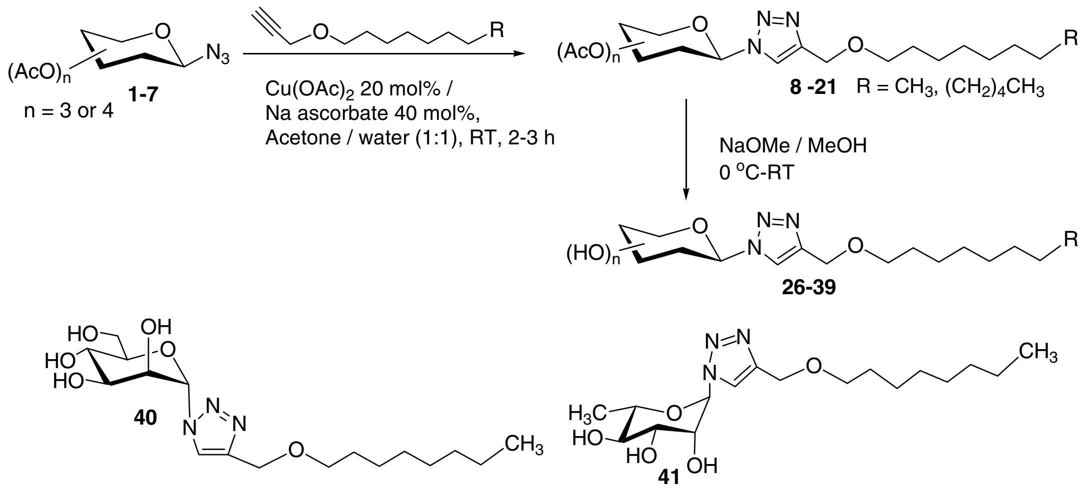


Scheme 1.

The initial click reaction was performed by reacting peracetylated β-D-glucopyranosyl azide (**1**) with *n*-octyl propargyl ether in the presence of Cu(I), which was generated in situ by the reduction of $Cu(OAc)_2$ with sodium ascorbate, using aqueous acetone as the solvent at room temperature (Scheme 1).[12] After complete consumption of the azide **1** in 2 h (TLC monitoring), the reaction mixture was worked-up and the crude product obtained was purified by flash column chromatography to afford the desired triazole **8** in almost quantitative yield. The ^{1}H NMR spectrum of **8** displayed a singlet at δ 7.78 assignable to the methine proton of the triazolyl ring of the 1,4-regioisomer. This assignment was further supported by a large and positive difference in the ^{13}C chemical shifts [$\Delta(\delta C4-\delta C5)$] of the two carbons of the triazole ring, as has been observed earlier in other triazole-linked compounds prepared by the click reaction.[13] Several other fully acetylated β-D-glycopyranosyl azides also underwent transformation to the corresponding triazolyl derivatives derived from octyl as well as dodecyl propargyl ether demonstrating the general applicability of the procedure (Scheme 1 and Table 1). The α-anomers of the fully acetylated D-mannopyranosyl azide, **22** and L-rhamnopyranosyl azide, **23** also served well as substrates in the click reaction yielding the glycolipids, **24** and **25**, derived from *n*-octyl propargyl ether in good yields. These structures formed by self-assembly of α- and β-anomers of synthetic glycolipids have been shown to depend on the configuration of the head group.[14] The ease with which both anomers can be prepared, as illustrated in the present work, is particularly useful in this regard. De-O-acetylation of the protected derivatives, **8**–**21** and **24–25**, was readily accomplished in near quantitative yield following Zemplen's method. All the novel protected and free glycolipids, **8**–**21**, **24**–**25**, and **26**–**39** in Scheme 1 (structures of **40** and **41**), have been fully characterized based on physical and spectral data.[15]

There has recently been an increasing interest in synthetic glycolipids carrying a carbohydrate group at both ends of a (long) hydrophobic chain, which are known as bolaamphiphiles.[16] Compounds such as **44** (Scheme 2) might also have interesting surfactant properties. Click Chemistry proved to be very effective in furnishing the novel target molecule **44** in fairly good overall yield. Reaction of the 1,6-diazide, **42**,[17] with 4 equiv of octyl propargyl ether in the presence of Cu(I) afforded the fully protected derivative **43**[18a] in 61% yield. This was converted quantitatively to the free lipid using NaOMe in methanol at room temperature. The ^{1}H NMR spectrum of **44**[18b] displayed two singlets at δ 7.70 and 7.50 assignable to the methine protons of the two triazolyl rings of the 1,4-regioisomer.

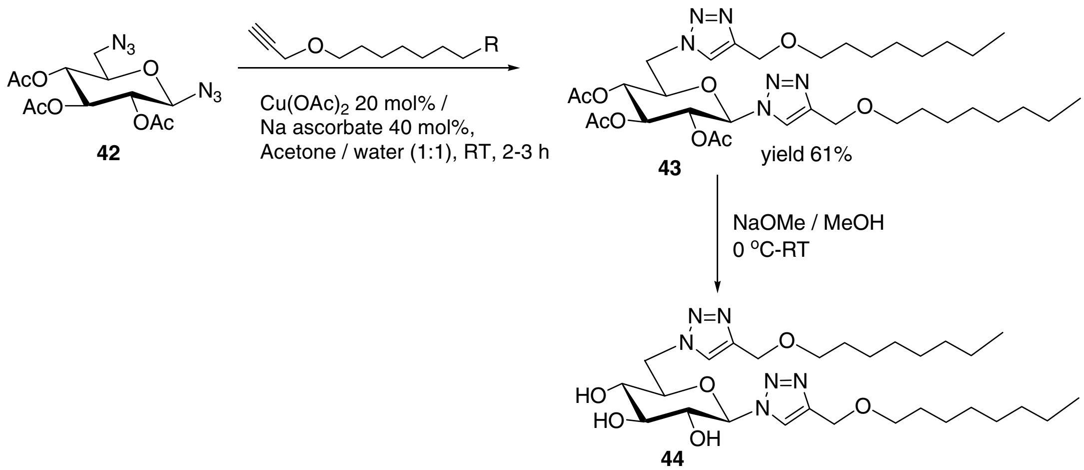
Table 1
Click reaction of fully acetylated glycopyranosyl azides with alkyl propargyl ethers

Entry	Sugar azide	Alkyl group	Product	Yield[a] (%)
1	Glcβ (**1**)	C_8H_{17}	**8**	98
2	Glcβ (**1**)	$C_{12}H_{25}$	**9**	83
3	Galβ (**2**)	C_8H_{17}	**10**	65
4	Galβ (**2**)	$C_{12}H_{25}$	**11**	60
5	Manβ (**3**)	C_8H_{17}	**12**	63
6	Manβ (**3**)	$C_{12}H_{25}$	**13**	76
7	GlcNAcβ (**4**)	C_8H_{17}	**14**	57
8	GlcNAcβ (**4**)	$C_{12}H_{25}$	**15**	73
9	L-Rhaβ (**5**)	C_8H_{17}	**16**	81
10	L-Rhaβ (**5**)	$C_{12}H_{25}$	**17**	94
11	Xylβ (**6**)	C_8H_{17}	**18**	66
12	Xylβ (**6**)	$C_{12}H_{25}$	**19**	88
13	Lacβ (**7**)	C_8H_{17}	**20**	61
14	Lacβ (**7**)	$C_{12}H_{25}$	**21**	64
15	Manα (**22**)	C_8H_{17}	**24**	64
16	L-Rhaα (**23**)	C_8H_{17}	**25**	91

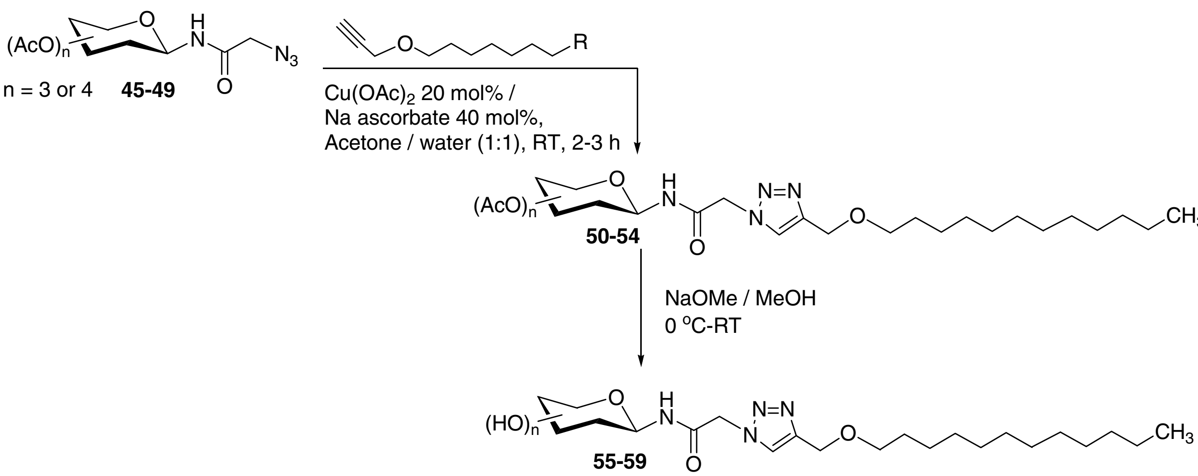
[a] Yield of isolated pure product.

N-(β-Glycopyranosyl)azidoacetamides are mimetics of the widely distributed GlcNAc-Asn linkage in glycoproteins. Their utility as valuable chemical ligating agents for the preparation of glycolipids is demonstrated here (Scheme 3 and Table 2). The relatively stable amide linker between the sugar and the lipid components is a unique feature of the novel targets, **55–59**. *N*-(β-Glycopyranosyl)azidoacetamides, **45**–**49**, prepared as reported earlier[19] starting from Glc, Gal, Man, GlcNAc, and Xyl were transformed by click chemistry to the fully acetylated triazolyl derivatives, **50–54**, in good yields, and were subsequently de-O-acetylated to furnish the free glycolipids, **55–59**, in quantitative yields. All the novel glycolipids, **50**–**59**, have been fully characterized based on their physical and spectral data.[20]

In summary, a number of novel glycolipids have been designed and synthesized using click chemistry. Besides their potential application in the isolation and crystallization of membrane proteins, these structurally well-defined non-ionic glycolipids might also be useful as solubilizers in pharmaceutical formulations.[21] Furthermore, the presence of a triazole ring connecting the sugar and the lipid part lends a valuable chromophoric handle for biophysical studies on glycolipid–protein interactions.



Scheme 2.



Scheme 3.

Table 2
Click reaction of fully acetylated *N*-(β-glycopyranosyl)azidoacetamides with *n*-dodecyl propargyl ethers

Entry	Sugar azide	Product	Yield[a] (%)
1	Glcβ (**45**)	**50**	70
2	Galβ (**46**)	**51**	82
3	Manβ (**47**)	**52**	80
4	GlcNAcβ (**48**)	**53**	73
5	Xylβ (**49**)	**54**	58

[a] Yield of isolated pure product.

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12. *General procedure for the preparation of peracetylated glycosyltriazolo lipids*: The azide (1 mmol) and alkyne (2 equiv) were dissolved in a 1:1 mixture (5:5 mL) of acetone and water in a 50 mL flask. To this solution, $Cu(OAc)_2$ (20 mol %, 1 M solution) and sodium ascorbate (40 mol %, 1 M solution) were added with stirring at room temperature. TLC analysis of the reaction mixture showed

complete disappearance of the azide after 0.5–2 h. Following removal of the solvent using a rotoevaporator, the residue was extracted with ethyl acetate (3 × 20 mL). The ethyl acetate solution was dried over anhydrous sodium sulfate and concentrated to dryness. The resulting syrupy product obtained was purified by flash column chromatography (10–50% ethyl acetate in hexane) over silica gel to furnish the title compounds.

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15. (a) *1-N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-4-(n-octyloxymethyl)-1,2,3-triazole* (**8**): Amorphous powder; mp 78–80 °C; $[\alpha]_D$ −19.5 (*c* 1, $CHCl_3$); IR (ν_{max}, cm^{-1}); 2931, 2358, 1749, 1708, 1421, 1362, 1220, 1093, 1038, 924, 599, 529; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.78 (s, 1H), 5.89 (d, 1H, *J* = 8.9 Hz, H-1), 5.50–5.38 (m, 2H, H-2, H-3), 5.25 (t, 1H, *J* = 9.7 Hz, H-4), 4.62 (s, 2H), 4.31 (dd, 1H, *J* = 5.0, 12.6 Hz, H-6a), 4.15 (dd, 1H, *J* = 1.8, 12.6 Hz, H-6b), 4.01 (m, 1H, H-5), 3.51 (t, 2H, *J* = 6.7 Hz), 2.10, 2.08, 2.05, 1.89 (4s, 12H, 4 × –COCH_3), 1.60 (m, 2H), 1.39–1.23 (m, 10H), 0.88 (t, 3H, *J* = 6.7 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.3, 169.7, 169.2, 168.7 (4 × –$COCH_3$), 146.4, 120.6, 85.7 (C-1), 75.1, 72.7, 71.0, 70.4, 67.8, 64.1, 61.6, 31.7, 29.6, 29.3, 29.1, 26.0, 22.5, 20.5, 20.4, 20.3, 20.0 (4 × –COCH_3), 14.0 (–CH_3); ESI MS: calcd for $C_{25}H_{39}N_3O_{10}Na$: 564.2534 $[M+Na]^+$. Found: 564.2533; (b) *1-N-(β-D-Glucopyranosyl)-4-(n-octyloxymethyl)-1,2,3-triazole* (**26**): Syrup; $[\alpha]_D$ −8.8 (*c* 0.3, MeOH); IR (ν_{max}, cm^{-1}): 3362, 2925, 2856, 1640, 1460, 1367, 1234, 1094, 1045, 899, 821, 512; ^1H NMR (D_2O, 400 MHz): δ 8.12 (s, 1H), 5.67 (d, 1H, *J* = 8.9 Hz, H-1), 4.50 (s, 2H), 3.94 (t, *J* = 9.0 Hz, 1H, H-2), 3.83–3.56 (m, 5H, H-3, H-4, H-5, H-6a, H-6b), 3.46 (m, 2H), 1.54 (m, 2H), 1.38–1.18 (m, 10H), 0.86 (m, 3H, –CH_3); ^{13}C NMR (D_2O, 100 MHz): δ 144.7, 123.9, 87.8 (C-1), 79.0, 76.4, 72.6, 71.0, 69.1, 63.5, 60.8, 32.0, 29.7, 29.5, 26.1, 23.5, 22.8, 14.0 (–CH_3); ESI MS: calcd for $C_{17}H_{31}N_3O_6Na$: 396.2101 $[M+Na]^+$. Found 396.2111.
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18. (a) *1,6-Di-N-[(4-n-octyloxymethyl)-1,2,3-triazolyl]-3,4,6-tri-O-acetyl-β-D-glucopyranose* (**43**): Amorphous powder; mp: 148–150 °C; $[\alpha]_D$ 16.0 (*c* 0.1, $CHCl_3$); IR (ν_{max}, cm^{-1}): 2922, 2853, 2361, 1743, 1456, 1372, 1301, 1255, 1219, 1132, 1114, 1095, 1071, 1041, 916, 907, 600; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.70 (s, 1H), 7.50 (s, 1H), 5.84 (d, 1H, *J* = 9.1 Hz, H-1), 5.48 (m, 1H, H-2), 5.42 (m, 1H, H-3), 5.06 (t, 1H, *J* = 9.6 Hz, H-4), 4.66 (dd, 1H, *J* = 2.4, 14.8 Hz, H-6b), 4.62 (s, 2H), 4.58 (s, 2H), 4.46 (dd, 1H, *J* = 7.6, 14.8 Hz, H-6a), 4.24 (m, 1H, H-5), 3.51 (m, 4H), 2.15, 2.05, 1.88 (3s, 9H, 3 × –COCH_3), 1.68–1.50 (m, 4H), 1.40–1.20 (m, 20H), 0.90–0.84 (m, 6H, 2 × –CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.8, 169.5, 168.7 (3 × –$COCH_3$), 146.4, 146.0, 123.7, 120.8, 85.4 (C-1), 75.5, 72.4, 71.1, 71.0, 69.9, 68.9, 64.1, 64.0, 50.4, 31.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.2, 29.2, 26.0, 22.6, 20.6, 20.4, 20.1, 14.0 (–CH_3); ESI MS: calcd for $C_{34}H_{57}N_6O_9$: 693.4192 $[M+H]^+$. Found 693.4187.; (b) *1,6-Di-N-[(4-n-octyloxymethyl)-1,2,3-triazolyl]-β-D-glucopyranose* (**44**): Syrup; $[\alpha]_D$ 10.0 (*c* 0.1, MeOH); IR (ν_{max}, cm^{-1}): 3286, 2922, 2853, 1741, 1464, 1379, 1301, 1236, 1117, 1092, 1047, 893, 827, 723; ^1H NMR (CD_3OD, 400 MHz): δ 7.97 (s, 1H), 7.74 (s, 1H), 5.58 (d, 1H, *J* = 9.1 Hz, H-1), 4.87 (dd, 1H, *J* = 2.6, 14.6 Hz, H-6a), 4.63 (m, 1H, H-6b), 4.59 (s, 2H), 4.53 (s, 2H), 3.97 (m, 1H, H-5), 3.89 (t, 1H, *J* = 9.0 Hz, H-2), 3.67–3.58 (m, 2H, H-3, H-4), 3.53 (t, 2H, *J* = 6.8 Hz, –OCH_2–), 3.47 (t, 2H, *J* = 6.8 Hz, –OCH_2–), 1.59 (m, 4H), 1.40–1.20 (m, 20H), 0.90 (m, 6H, 2 × –CH_3); ^{13}C NMR (CD_3OD, 100 MHz): δ 146.2, 146.0, 126.2, 124.2, 89.4, 78.7, 78.6, 74.0, 71.9, 71.8, 71.6, 64.6, 64.5, 52.0, 33.1, 33.0, 30.7, 30.6, 30.5, 30.4, 27.2, 27.1, 23.7, 14.5 (–CH_3); ESI MS: calcd for $C_{28}H_{50}N_6O_6Na$: 589.3687 $[M+Na]^+$. Found 589.3690.
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20. (a) *1-N-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyl)-(4-n-dodecyloxymethyl)-1,2,3-triazolylacetamide* (**50**): Amorphous powder; mp: 58–60 °C; $[\alpha]_D$ 10.0 (*c* 0.2, $CHCl_3$); IR (ν_{max}, cm^{-1}): 3411, 2924, 2855, 1708, 1553, 1422, 1363, 1221, 1092, 1039, 908, 734, 599, 529; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.66 (s, 1H), 6.91 (d, 1H, *J* = 8.8 Hz, –NH), 5.30 (t, 1H, *J* = 9.6 Hz, H-3), 5.22 (m, 1H, H-1), 5.13–4.97 (m, 3H, H-4, –CH_2–), 4.89 (m, 1H, H-2), 4.66 (s, 2H), 4.28 (dd, 1H, *J* = 4.4, 12.4 Hz, H-6a), 4.09 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6b), 3.82 (m, 1H, H-5), 3.54 (t, 2H, *J* = 6.8 Hz), 2.10, 2.04, 2.03, 2.02 (12H, 4 × –COCH_3), 1.60 (m, 2H), 1.38–1.23 (m, 18H), 0.89 (t, 3H, *J* = 6.8 Hz, –CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.8, 170.5, 169.8, 169.4 (4 × –$COCH_3$), 165.6, 146.6, 123.7, 78.5 (C-1), 73.8, 72.5, 71.1, 70.3, 68.1, 64.2, 61.5, 52.6, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 26.1, 22.6, 20.6, 20.5, 20.4, (4 × –COCH_3), 14.0 (–CH_3); ESI MS: calcd for $C_{31}H_{50}N_4O_{11}Na$: 677.3379 $[M+Na]^+$. Found 677.3374.; (b) *1-N-β-D-Glucopyranosyl-(4-n-dodecyloxymethyl)-1,2,3-triazolylacetamide* (**55**): Amorphous powder; mp: 130–135 °C; $[\alpha]_D$ 6.5 (*c* 0.1, MeOH); IR (ν_{max}, cm^{-1}): 3339, 2917, 2848, 2361, 2341, 1683, 1545, 1264, 1119, 1079, 1049, 720, 669, 631, 617, 575, 525; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.98 (d, 1H, –NH, D_2O exchangeable), 8.02 (s, 1H), 5.12 (m, 2H), 4.71 (t, 1H, *J* = 9.2 Hz, H-1), 4.48 (s, 2H), 3.62 (m, 1H, H-6a), 3.41 (m, 3H, –OCH_2–, H-6b), 3.25–3.05 (m, 4H, H-2, H-3, H-4 and H-5), 1.49 (m, 2H), 1.32–1.17 (m, 18H), 0.85 (t, 3H, *J* = 6.4 Hz, –CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 167.8, 145.8, 127.4, 81.6 (C-1), 80.6, 79.2, 74.5, 71.7, 71.4, 65.1, 62.7, 53.4, 33.2, 31.1, 31.0, 30.9, 30.9, 30.8, 30.6, 27.6, 24.0, 15.9 (–CH_3); ESI MS: calcd for $C_{23}H_{43}N_4O_7$: 487.3138 $[M+H]^+$. Found 487.3132.
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