Synthesis of α-Galactosyl Ceramide Analogues with an α-Triazole at the Anomeric Carbon

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ABSTRACT

The synthesis of 1,2,3-triazole containing analogues of α-GalCer and galacturonic acid containing *Sphingomonous* cell wall antigens is described. Anomerisation was used to provide the required α-glycosyl azide precursor. Copper azide-alkyne cycloaddition (CuAAC) generated the α-triazole linkage.

1. Introduction

The α-galactosylceramide (α-GalCer, KRN7000) **1** and several other structurally related bacterial glycolipids such as the glucuronic acid containing **2** interact with natural killer T (NKT) cells when bound to the glycoprotein CD1d (Fig 1.).[1,2] This recognition event promotes an immune response leading to the production of T helper 1 (Th1) and T helper 2 (Th2) cytokines, such as interferon-γ (IFN-γ) and interleukin 4 (IL-4).[3] The production of Th1 cytokines assists against various types of infection, whereas Th2 cytokines assist with the alleviation of autoimmune diseases. The Th1 and Th2 cytokines tend to inhibit each other's biological functions and α-GalCer stimulates the production of high levels of these cytokines. However, there is a lack of discrepancy between the Th1 and Th2 response to α-GalCer and many glycolipid antigens have been synthesized in efforts to generate bias. Structural modifications investigated to date have included varying of the sugar moiety, as well as the polar portion of the ceramide, the lipid chain and the nature and configuration of the anomeric linkage. Some analogues have shown promising results. Wong and co-workers have introduced a fluorinated biaryl ether derivative **3**, which showed enhanced Th1 cytokine production.[4] Glycosidic bond modifications have been investigated for reasons which include limiting the degradation of glycolipid antigens by glycosidases, and with a view to investigating whether such derivatives can display bias towards the production of the Th1 or Th2 cytokines. Modifications at the anomeric position have included synthesis of *S*-, and *C*-glycolipids and oxime derivatives. There is continuing interest in the synthesis of analogues.[5]

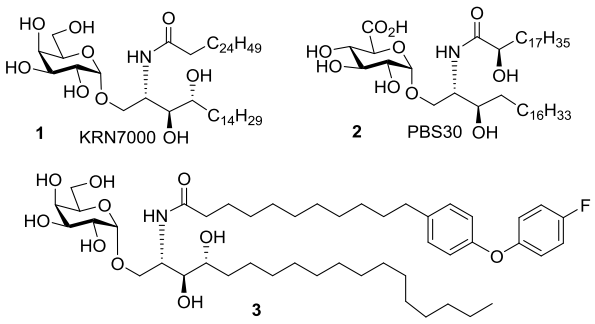


Fig 1. Glycosphingolipid antigens **1-3**

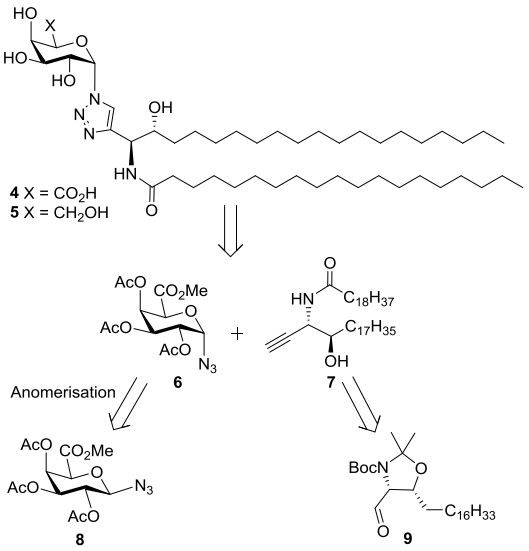
Glycosyl triazoles have been of increased interest in recent years with such neoglycoconjugates being evaluated as lectin binding agents.[6] Despite this research, variants of the glycosphingolipids which have an α-1,2,3-triazole linkage at the anomeric position (glycosyl triazole) have not been synthesized to date and they would be of interest to prepare. While glycosyl azides have been used as intermediates in preparation of carbohydrate derivatives, work has focused mostly on β-azides with there being few investigations using α-glycosyl azides to date. In the case of these immunostimulatory glycolipids, having the α-configuration at the anomeric carbon seems to be very important. The insertion of a triazole between the anomeric carbon and the carbon atom adjacent to the lipid nitrogen atom alters the spatial orientation between the sphinogolipid and the saccharide. A simple molecular modeling comparison between **2**

and **5** indicated that the distance between the carbon adjacent to to the carbon atom adjacent to the lipid nitrogen and the anomeric carbon increases by ~1.2 Å. The triazole atoms potentially have interactions with the CD1d receptor. We describe herein the preparation of the galacturonic acid and galactose derivatives **4** and **5**.

2. Results and Discussion

2.1 Synthesis

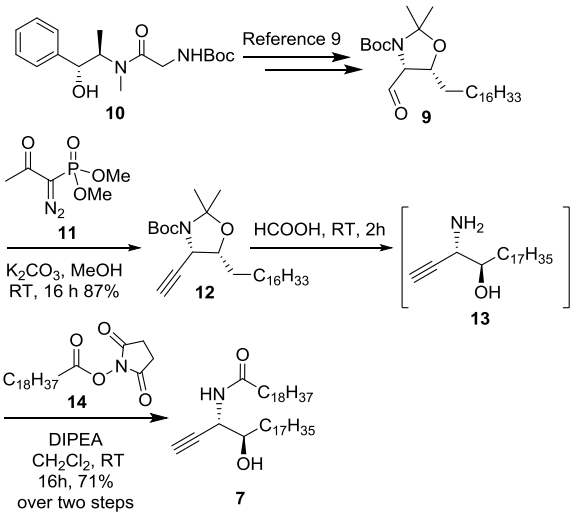
The retrosynthetic analysis of **4** and **5** is shown in Scheme 1. It was envisaged that the 1,4-triazole glycolipid **4** would be generated by the copper azide-alkyne cycloaddition reaction (CuAAC)[7] between α-glycosyl azide **6**[8] and alkyne **7**. In turn, the alkyne **7** would be generated from aldehyde **9**. We envisaged that the anomerisation of the β-azide **8** to give the required α-azide precursor **6**.



Scheme 1. Retrosynthetic analysis of glycolipids with an α-triazole

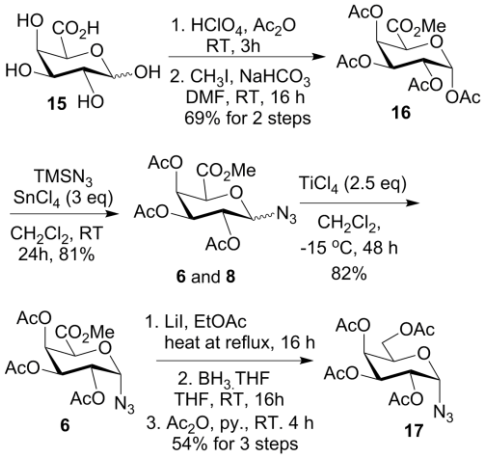
The synthesis began with the preparation of **7** (Scheme 2). The pseudoephedrine chiral auxiliary **10** was used as described previously for the preparation of the aldehyde **9**.[9] The Ohira-Bestmann modification of the Seyferth-Gilbert homologation has provided mild conditions for the preparation of terminal alkynes from aldehydes.[10] Reaction of **9** with the Ohira-Bestmann reagent **11** provided the alkyne **12** in 87% yield. Treatment of **12** with formic acid[11] for 2 h removed both the oxazolidine and Boc protecting groups and gave the sphinganine **13** which was immediately reacted with succinate **14**[12] to give the desired glycolipid precursor **7**. Overall the lipid **7** was obtained in 9 steps from the glycinamide **10**.

The synthesis of α-glycosyl azide **6** was next carried out (Scheme 3) by a procedure modified from that in the recent literature.[8] Firstly, the methyl ester **16** was prepared in 2 steps from galacturonic acid **15** as previously described.[8] Next the introduction of the azide at the anomeric center using $SnCl_4$ (3 eq.) and $TMSN_3$ (2.5 eq.)[13] was investigated in order to try to effect glycosidation and anomerisation in one pot. The exchange of the acetate for azide can usually be effected in the presence of 0.5 eq of $SnCl_4$. In this case the higher amount of $SnCl_4$ was needed in order to ensure full conversion of **16** to glycosyl azide. This glycosidation provided a mixture of 4:1 mixture of anomers **6** and **8**, which could not be separated. Lewis acids such $SnCl_4$ and $TiCl_4$ have been shown to promote chelate induced anomerisation in uronic acid *O*-, *S*- and N_3 glycoside derivatives and these reactions can proceed in high yield and selectivity for the α-anomer.



Scheme 2. Synthesis of alkyne **7**

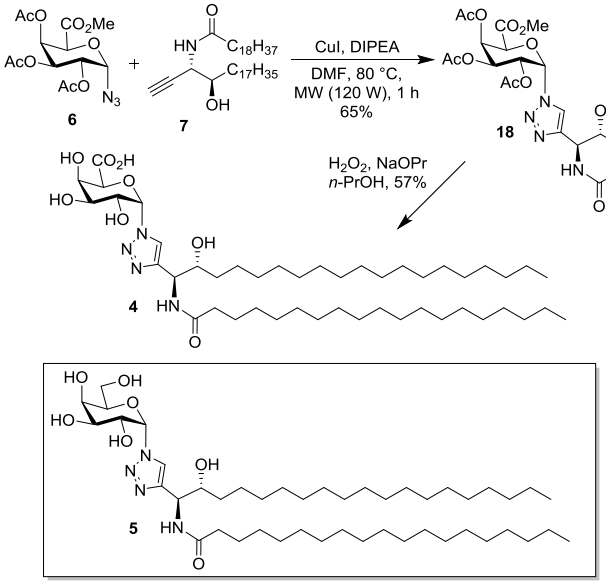
Coordination of the pyranose ring oxygen and the C-5 carbonyl to the Lewis acid increases the anomerisation rate, promoting endocyclic cleavage leading to the more thermodynamically favored α-anomer in high selectivity. Hence the formation of the α-azide as the major anomer is due to Sn(IV) promoted anomerisation of the initially formed β-azide **8**.[8] In order to further increase the proportion of the α-azide, the mixture was treated with $TiCl_4$ (2.5 fold excess) as the α:β ratio in such anomerisation reactions can be influenced by the concentration of $TiCl_4$. The effect of $TiCl_4$ on anomerisation ratio can be explained by coordination of this more active Lewis acid to the anomeric azide, increasing the electron withdrawing nature of the anomeric substituent and thus enhancing the anomeric effect. The α:β ratio was not just dependent on $TiCl_4$ concentration but also on temperature and the α-selectivity was found to be higher at -15°C than at room temperature. Next, the selective cleavage of methyl ester **6** to its carboxylic acid derivative was achieved by heating with LiI in EtOAc at reflux.[14] Chemoselective reduction of the acid with BH_3.THF followed by acetylation successfully provided the galactosyl azide **17**.[15]



Scheme 3. Synthesis of α-azides **6** and **17**

With the glycosyl azides **6** and **17** in hand the synthesis of the triazole linkage was then explored. The copper catalysed version of the Huisgen azide-alkyne cycloaddition has been an important

advance in the preparation of 1,2,3-triazoles and this reaction usually provides the 1,4-isomer in a highly regioselective manner. The glycolipid **18** was prepared as depicted in Scheme 4. On using microwave conditions described by Opatz and co-workers, **18** was obtained in 65% yield.[16] The reaction was highly selective, giving only the 1,4 isomer. Removal of the protecting groups from **18** then gave target compound **4**. As previously described for the deprotection of uronic acid derivatives, the removal of the protecting groups was achieved using hydroperoxide generated in *n*-propanol from sodium propoxide and hydrogen peroxide.[17] The use of methoxide, which is more basic than peroxide, can lead to the elimination of acetic acid and the formation of the undesired saturated compound. The synthesis of the 1,4-triazole galactose derivative **5** was achieved by a similar sequence from the azide **17**. The CuAAC reaction from **17** provided the triazole in 68% yield and subsequent removal of protecting groups gave **5** in 86% yield.



Scheme 4. Synthesis of 1,4-triazoles

3. Conclusion

To conclude, the synthesis of α-glycolipids based on galactose and galacturonic acid, and with a triazole at the anomeric position has been achieved. The route included anomerisation to generate the α-azide of galacturonic acid, which was subsequently converted to the galactose derivative. Microwave assisted CuAAC from these α-azides led to the generation of the desired triazole linkage. Consequently the evaluation of the immunostimulatory potential of such glycolipids will be of interest.

3. Experimental section

3.1. General

Optical rotations were determined at the sodium D line at 20°C. NMR spectra were recorded at 500 & 600 MHz. Chemical shifts are reported relative to internal Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 7.26), pyridine-d_5 (δ 8.74, 7.58, 7.22), $(CD_3)_2SO$ (δ 2.50) for ^1H and Me_4Si in $CDCl_3$ (δ 0.0) or $CDCl_3$ (δ 77.0), pyridine-d_5 (δ 150.0, 136.0, 124.0) and $(CD_3)_2SO$ (δ 39.5) for ^{13}C. ^1H NMR signals were assigned with the aid of COSY and ^{13}C NMR signals were assigned with the aid of DEPT, gHSQCAD and/or gHMBCAD. Coupling constants are reported in Hertz. The IR spectra were recorded using thin film with an ATR attachment. High resolution mass spectra were recorded using electrospray TOF spectrometry. Silica gel used had a pore size 60 Å, particle size 40-60 μm 230-400 mesh particle size. Dichloromethane, methanol, THF and DMF solvents were used as obtained from a Pure Solv™ Solvent Purification System. DMA, ethyl acetate and acetone were used as obtained from commercial suppliers. Reagents were used as obtained from commercial suppliers.

3.2. (4S,5R)-t-Butyl-5-heptadecyl-2,2-dimethyl-4-(prop-1-enyl)-oxazolidine-3-carboxylate

This alkene precursor to aldehyde **9** was synthesised according to previously reported procedures detailed in ref. 9. Analytical data: IR (film) cm^{-1}: 2923, 2854, 1698, 1456, 1383, 1375, 1364, 1251, 1178, 1073; ^1H NMR (500 MHz, $CDCl_3$, 1:1 mixture of *E*&*Z* isomers) δ 5.57 (1H, dq, *J* 13.1, 6.3, H-2), 5.47 (1H, dq, *J* 13.1, 6.4), 5.26 (1H, dd, *J* 8.8, 1.7), 5.23 (1H, dd, *J* 8.8, 1.7), 4.17 (1H, t, *J* 6.9), 4.00 (1H, dd, *J* 9.1, 5.0), 3.89 (2H, q, *J* 6.2), 1.65 (3H, d, *J* 1.6 Hz, CH_3), 1.64 (3H, d, *J* 1.7, CH_3), 1.55 (2H, s), 1.50 (2H, s), 1.44 (5H, s), 1.41 (7H, s), 1.34 (9H, s), 1.18 (51H, s), 0.81 (6H, t, *J* 6.9, each CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.9, 129.0, 128.8, 126.6, 126.3, 92.7, 92.3, 79.8, 79.1, 76.8, 62.6, 62.2, 56.6, 31.9, 29.7 (3s), 29.6 (2), 29.5, 29.4, 29.2, 28.5, 27.3, 25.8, 25.6, 24.9, 23.8, 22.7, 17.8, 17.7, 14.1; ESI-HRMS calcd for $C_{30}H_{57}NO_3Na$ 502.4236, found *m/z* 502.4235 $[M+Na]^+$.

3.3. (4S,5R)-t-Butyl-5-heptadecyl-4-(formyl)-2,2 dimethyloxazolidine-3-carboxylate (9)

To a solution of alkene described above (194 mg, 0.41 mmol) in 10:1 acetone-water (3.9 mL) were added, 2,6-lutidine (0.1 mL. 0.82 mmol), 4-methylmorpholine-*N*-oxide (72 mg, 0.615 mmol) and a catalytic amount of osmium tetroxide (0.1 mL of 2.5% in *t*-BuOH, 2 mol%). The solution was stirred vigorously for 2 days. Then $PhI(OAc)_2$ (264 mg, 0.82 mmol) was added and the mixture stirred for a further 3 h. The reaction was quenched with aq sodium thiosulphate and then extracted into EtOAc (x 2). The combined organic portions were washed with water, brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 30:1) provided **9** (117 mg, 61%) as a colourless oil; IR (film) cm^{-1}: 2923, 2854, 1738, 1712, 1466, 1366, 1256, 1176, 1112; ^1H NMR (500 MHz, 3:2 mixture of rotamers, the asterisk* denotes the signals of the minor rotamer, $CDCl_3$) δ 9.51* (1H, d, *J* 3.0, H-1), 9.44 (1H, d, *J* 4.0, H-1), 4.19 – 4.11 (1H, m, H-3), 3.99 (1H, dd, *J* 6.6, 3.9, H-2), 1.66 (3H, s, CH_3), 1.60* (3H, s, CH_3), 1.50 (5H, s, overlapping signals, CH_2 & CH_3), 1.46* (3H, s, CH_3), 1.43* (9H, s, *t*-Bu), 1.33 (9H, s, *t*-Bu), 1.19 (48H, s, each CH_2 & CH_2*), 0.81 (5H, t, *J* 6.9, each CH_3 & CH_3*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 200.2, 200.0* (C=O), 152.5*, 151.4 (C=O), 94.4, 93.7* (each isopropyl C), 81.1*, 80.8 (each *t*-Bu C), 77.2*, 76.4 (*C*HO), 67.6, 67.5* (*C*HN), 31.9, 29.8, 29.7 (3s), 29.6, 29.5, 29.4 (3s) (each CH_2), 28.3*, 28.3, 27.5*, 26.7, 26.2*, 26.1, 24.8*, 23.8 (each CH_3), 22.7 (CH_2), 14.1 (CH_3); ESI-HRMS calcd for $C_{28}H_{53}NO_4Na$ 490.3872, found *m/z* 490.3889 $[M+Na]^+$.

3.4. (4S,5R)-t-Butyl-5-heptadecyl-4-(ethynyl)-2,2-dimethyloxazolidine-3-carboxylate (12)

Under an argon atmosphere, aldehyde **9** (111 mg, 0.24 mmol) and the Ohira-Bestmann reagent **11** (0.1 mL, 0.72 mmol) were taken up in dry methanol (1.1 mL) and cooled to 0 °C. Potassium carbonate (133 mg, 0.96 mmol) was added and the reaction mixture was stirred at 0 °C for 1 h, warmed to room temperature and stirred for a further 16 h. The reaction was diluted with satd NH_4Cl and then extracted into EtOAc (x 2). The combined organic layers were dried over Na_2SO_4 and the solvent removed

under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 30:1) provided the title compound **12** (96 mg, 87%) as a colourless oil; $[\alpha]_D$ +23 (*c* 1, $CHCl_3$); IR (film) cm^{-1}: 3313, 2923, 2854, 1704, 1457, 1375, 1365, 1246, 1176, 1142, 1089; ^1H NMR (500 MHz, 2.5:2 rotamer ratio, the asterisk denotes the signals of the minor rotamer, $CDCl_3$) δ 4.50* (1H, dd, *J* 5.1, 2.1, H-2), 4.35 (1H, dd, *J* 5.1, 2.1, H-2), 3.91 – 3.86 (2H, m, overlapping signals, H-3* & H-3), 2.24* (1H, d, *J* 2.2, H-1), 2.22 (1H, d, *J* 2.1, H-1), 1.77 – 1.64 (4H, m), 1.57 (3H, s, CH_3), 1.53* (3H, s, CH_3), 1.49* (3H, s, CH_3), 1.44 (3H, s, CH_3), 1.43* (9H, s, *t*-Bu), 1.42 (9H, s, *t*-Bu), 1.19 (60H, s, each CH_2 & CH_2*), 0.81 (6H, t, *J* 6.9, CH_3 & CH_3*); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.8,* 151.3 (C=O), 93.8, 93.3* (each isopropyl C) 80.8*, 80.2 (each *t*-Bu C), 79.9, 79.5* (-*C*≡CH), 76.1, 75.8* (*C*HO), 72.8*, 72.3 (-C≡*C*H), 52.2, 52.0* (*C*HN), 31.9, 30.3, 30.1, 29.7 (3s), 29.6, 29.5, 29.4 (2s) (each CH_2), 28.4, 27.5*, 26.5, 25.6, 25.5*, 25.3*, 24.4 (each CH_3), 22.7 (CH_2), 14.1 (CH_3); ESI-HRMS calcd for $C_{29}H_{53}NO_3Na$ 486.3923, found *m/z* 486.3945 $[M+Na]^+$.

3.5. 2,5-Dioxopyrrolidin-1-yl nonadecanoate (14)

N-Hydroxy-succinimide (0.27 g, 2.34 mmol) and EDC (0.45 g, 2.34 mmol) were added to a solution of nonadecanoic acid (0.7 g, 2.34 mmol) in CH_2Cl_2 (46 mL). After stirring the mixture overnight, the solvent was concentrated under reduced pressure and the resulting residue dissolved in CH_2Cl_2. The solution was washed with water, dried over Na_2SO_4 and solvent removed under reduced pressure to provide the title compound **14** (0.84 g, 90%) as a white solid. The compound was used without further purification; ^1H NMR (500 MHz, $CDCl_3$) δ 2.83 (4H, s, O=CC*H*$_2$C*H*$_2$C=O), 2.60 (2H, t, *J* 7.5, CH_2C=O), 1.78-1.69 (2H, m, CH_2), 1.45-1.35 (2H, m, CH_2), 1.25 (28H, s, each CH_2), 0.88 (3H, t, *J* 6.9, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 168.7 (each C=O), 31.9, 31.0, 29.7 (2s), 29.6 (2s), 29.4, 29.1, 28.8, 25.6, 24.6, 22.7 (each CH_2), 14.1 (CH_3).

3.6. N-((3S,4R)-4-Hydroxyhenicos-1-yn-3-yl)nonadecanamide (7)

Alkyne **12** (90 mg, 0.19 mmol) was taken up in formic acid (6 mL) and stirred vigorously for 2 h. Toluene (15 mL) was added and the solvents were removed under reduced pressure. The resulting residue was taken up in water and basified with solid $NaHCO_3$, extracted into CH_2Cl_2, dried over Na_2SO_4 and solvent removed under reduced pressure to give intermediate amine **13.** This was taken up in CH_2Cl_2 (6 mL) and DIPEA (0.12 mL, 0.68 mmol) was added. To this was added a solution of **14** (192 mg, 0.49 mmol) in CH_2Cl_2 (3 mL) and the mixture stirred for 24 h. Upon completion the reaction mixture was diluted with CH_2Cl_2, washed with satd aq $NaHCO_3$ and then extracted into CH_2Cl_2 (x2). The combined organic layers were washed with water, brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc, 4:1) provided the title compound **7** (83 mg, 71% over two steps) as a white solid; $[\alpha]_D$ +5.5 (*c* 0.8, $CHCl_3$); R_f 0.3 (cyclohexane EtOAc 3:1); mp 86-90 °C; IR (film) cm^{-1}: 3341 br, 3228, 2917, 2849, 1631, 1532, 1472; ^1H NMR (500 MHz, $CDCl_3$) δ 6.07 (1H, d, *J* 8.5, NH), 4.81 (1H, dt, *J* 8.5, 2.6, H-3), 3.62 (1H, td, *J* 6.7, 2.8, H-4), 2.30 (1H, d, *J* 2.4, H-1), 2.20 (2H, t, *J* 7.7, CH_2), 1.67-1.56 (4H, overlapping signals, CH_2), 1.53-1.42 (1H, m, CH(*H*)), 1.34-1.21 (59 H, overlapping signals, CH_2), 0.88 (6H, t, *J* 6.7, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6 (C=O), 79.7 (*C*≡CH), 73.8 (C-4), 73.3 (C≡*C*H), 46.9 (C-3), 36.8, 34.5, 32.1, 29.9 (3s), 29.8, 29.7 (2s), 29.6, 29.5 (2s), 29.4, 25.7 (2s), 22.9 (each CH_2), 14.3 (CH_3); ESI-HRMS calcd. for $C_{40}H_{78}NO_2$ 604.6033, found *m/z* 604.6025 $[M+H]^+$.

3.7. 1,2,3,4-Tetra-O-acetyl-α-D-galactopyranosiduronic acid, methyl ester (16).

To a stirred solution of $HClO_4$ (220 µL) in Ac_2O (57 mL) at 0°C was added D-galacturonic acid monohydrate **15** (10 g, 52 mmol) in one portion. The reaction was warmed to room temperature and then stirred for 3 h. The reaction was then re-cooled to 0 °C and MeOH was added cautiously. After stirring for a further 30 min the reaction was partitioned between EtOAc and H_2O. The aq layer was extracted into EtOAc (x 2) and the combined organic layers were washed with water, brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The resulting residue was azeotroped several times with toluene to remove acetic acid. Once removed the residue was taken up in DMF (58 mL). Sodium hydrogen carbonate (8.3 g, 100 mmol) and iodomethane (8.1 mL, 130 mmol) were added and the solution was stirred overnight. The reaction was diluted with H_2O and extracted into EtOAc. The combined organic layers were washed with H_2O, brine, dried over $NaSO_4$, filtered and solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) provided the title compound **16** (13.5 g, 69% over two steps) as a white solid; NMR data (^1H and ^{13}C) were in good agreement with the reported literature data;[17] R_f 0.18 (petroleum ether-EtOAc 2:1); m.p. 135-145 °C; IR (film) cm^{-1}: 2966, 1747, 1440, 1371, 1208, 1068, 942; ^1H NMR (500 MHz, $CDCl_3$) δ 6.52 (1H, d, *J* 2.6 Hz, H-1), 5.84-5.78 (1H, m, H-4), 5.42 – 5.36 (2H, overlapping signals, H-2, H-3), 4.75 (1H, d, *J* 1.4 Hz, H-5), 3.77 (3H, s, OMe), 2.16 (3H, s), 2.12 (3H, s), 2.02 (6H, s) (each OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 169.6 (2s), 168.4, 166.5 (each C=O), 89.6 (C-1), 70.7 (C-5), 68.6 (C-4), 66.9, 66.0 (C-2 and C-3), 52.8 (OMe), 20.8, 20.6, 20.5 (2s) (each OAc); ESI-HRMS calcd for $C_{15}H_{20}O_{10}Na$ 399.0903, found *m/z* 399.0916 $[M+Na]^+$.

3.8. 1-Azido-1-deoxy-2,3,4-tri-O-acetyl-D-galactopyranuronic acid, methyl ester (6&8)

Methyl ester **16** (10 g, 26.6 mmol) and trimethylsilyl azide (8.7 mL, 67 mmol) were taken up in dry CH_2Cl_2 (100 mL) and cooled to 0 °C. Tin (IV) chloride (9.4 mL, 80 mmol) was added dropwise. The resulting solution was warmed to room temperature and stirred for 24 h. The mixture was diluted with CH_2Cl_2 washed with 1M $KHSO_4$, satd. $NaHCO_3$, water and brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) provided the title compound **8** (5.92 g, 62 %) as a mixture of anomers (α:β, 4:1); NMR data for both anomers (^1H and ^{13}C) were in good agreement with the reported literature data;[8] IR (film) cm^{-1}: 2959, 2119, 1744, 1439, 1371, 1207, 1129, 1063; ^1H NMR (500 MHz, $CDCl_3$) δ 5.76 (1H, d, *J* 3.9, H-1α), 5.75 (1H, dd, *J* 3.1, 1.6, H-4α), 5.72 (1H, dd, *J* 3.4, 1.4, H-4β), 5.28 (1H, dd, *J* 10.9, 3.0, H-3α), 5.24 (1H, dd, *J* 10.7, 3.9, H-2α), 5.18 (1H, dd, *J* 10.4, 8.7, H-2β), 5.08 (1H, dd, *J* 10.2, 3.5, H-3β), 4.76 (1H, d, *J* 1.6 Hz, H-5α), 4.67 (1H, d, *J* 8.7, H-1β), 4.39 (1H, d, *J* 1.4, H-5β), 3.76 (3H, s, OMe), 3.75 (3H, s, OMe), 2.11 (6H, s), 2.10 (3H, s), 2.09 (3H, s), 2.08 (3H, s), 1.99 (3H, s, each OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.9, 169.8, 169.7, 169.6, 169.5, 169.1, 166.6, 165.8 (each C=O), 88.4 (C-1β), 86.9 (C-1 α), 74.0 (C-5β), 70.3 (C-3β), 70.1 (C-5α) , 68.6 (C-4α), 68.0 (C-4β), 67.6 (C-2β), 66.9, 66.7 (C-2α and C-3α), 52.9 (OMe), 52.8 (OMe), 20.6 (2s), 20.5 (3s), 20.4 (each OAc); ESI-HRMS calcd. for $C_{13}H_{17}N_3O_9Na$ 382.0862, found *m/z* 382.0869 $[M+Na]^+$.

3.9. 1-Azido-1-deoxy-2,3,4-tri-O-acetyl-α-D-galactopyranuronic acid, methyl ester (6)

To a stirred solution of the mixure of anomers **6** and **8** (1.2 g,

3.34 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C was added 1M $TiCl_4$ in CH_2Cl_2, (8.35 mL) dropwise. The solution was stirred at 0 °C for 10 minutes and then placed in a fridge freezer at -15 °C for 48 h. The reaction mixture was then diluted with CH_2Cl_2, washed twice with sat. $NaHCO_3$, water, brine, dried over $NaSO_4$ and solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) provided the title compound **6** (987 mg, 82%) as a white foam; NMR data (^{1}H and ^{13}C) were in good agreement with the reported literature data;[8] R_f 0.54 (cyclohexane-EtOAc 1:1); m.p. 107-110 °C; IR (film) cm^{-1}: 2959, 2114, 1768, 1744, 1441, 1368, 1250, 1213, 1057; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.78 (1H, d, *J* 3.9 Hz, H-1), 5.77 (1H, dd, *J* 3.0, 1.6 Hz, H-4), 5.30 (1H, dd, *J* 10.8, 3.1 Hz, H-3), 5.26 (1H, dd, *J* 10.7, 3.9 Hz, H-2), 4.78 (1H, d, *J* 1.5 Hz, H-5), 3.77 (3H, s, OMe), 2.12 (3H, s), 2.11 (3H, s), 2.01 (3H, s) (each OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.0, 169.7, 169.6, 166.6 (each C=O), 86.0 (C-1), 70.1 (C-5), 68.7 (C-4), 66.9, 66.8 (C-2 and C-3), 52.9 (OMe), 20.6 (2s), 20.5 (each OAc); ESI-HRMS calcd for $C_{15}H_{17}N_3O_9Na$ 382.0862, found *m/z* 382.0864 $[M+Na]^+$.

3.10. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl azide (17)

Lithium iodide (1.6 g, 8.3 mmol) was added to a solution of methyl ester **6** in anhydrous EtOAc and the reaction mixture was heated at reflux for 16 h. Upon cooling the reaction mixture was quenched with 10% HCl and the aqueous layer extracted into EtOAc (x 2). The combined organic portions were washed with satd $Na_2S_2O_3$, dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford the carboxylic acid intermediate (397 mg, 83%) as a yellow solid. This compound was used in the next step without further purification; m.p. 171-173 °C; IR (film) cm^{-1}: 3484 (br), 2998, 2121, 1744, 1678, 1619, 1371, 1212, 1122; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.85 – 5.76 (2H, overlapping signals, H-1 and H-4), 5.39 – 5.10 (3H, overlapping signals, H-2, H-3 and OH), 4.82 (1H, s, H-5), 2.12 (6H, s each OAc), 2.00 (3H, s, OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 169.8, 169.8, 168.8 (each C=O), 86.9 (C-1), 69.8 (C-5), 68.5 (C-4), 66.8 (C-2 or C-3), 66.7 (C-2 or C-3), 20.6, 20.5, 20.5 (each OAc); ESI-HRMS calcd. for $C_{12}H_{14}N_3O_9$ 344.0730, found *m/z* 344.0733 $[M-H]^-$. This acid intermediate (100 mg, 0.29 mmol) was taken up in dry THF (3 mL) and cooled to 0 °C. To this was added BF_3.THF complex (0.9 mL of a 1.0 M solution in THF, 0.9 mmol) dropwise. The reaction was warmed to room temperature and stirred overnight. Methanol was added and the solvents were removed under reduced pressure. The crude residue was taken up in Ac_2O (2 mL) and pyridine (2 mL) and stirred for a further 4 hours. The reaction was diluted with EtOAc, washed with 1M HCl, water, brine, dried over Na_2SO_4 and solvent removed under reduced pressure. Flash chromatography of the residue (petroleum ether-EtOAc 2:1) provided the title compound **17** (70 mg, 65% over two steps) as a crystalline solid; NMR data (^{1}H and ^{13}C) were in good agreement with the reported literature data;[15] $[\alpha]_D$ +180 (*c* 1.0, $CHCl_3$); R_f 0.3 (cyclohexane-EtOAc 2:1); m.p. 77-78 °C; IR (film) cm^{-1}: 2981, 2118, 1744, 1373, 1207, 1122, 1064; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.66 (1H, d, *J* 4.0, H-1), 5.46 (1H, dd, *J* 3.2, 1.3, H-4), 5.25 (1H, dd, *J* 10.8, 3.1, H-3), 5.20 (1H, dd, *J* 10.7, 4.1, H-2), 4.36 (1H, td, *J* 6.9, 1.2, H-5), 4.16 – 4.09 (2H, overlapping signals, H-6a & H-6b), 2.14 (3H, s), 2.11 (3H, s), 2.06 (3H, s), 1.99 (3H, s) (each OAc); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 170.1, 170.0, 169.8 (each C=O), 86.7 (C-1), 68.5 (C-5), 67.6 (C-4), 67.4, 67.2 (C-3 & C-2), 20.6 (4s, each OAc); ESI-HRMS calcd. for $C_{14}H_{19}N_3O_9Na$ 396.1019, found *m/z* 396.1036 $[M+Na]^+$.

3.11. N-((1*S*,2*R*)-2-Hydroxy-1-(1-(5-(*S*)-methoxycarbonyl-2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (18)

Alkyne **7** (15 mg, 0.025 mmol) was dissolved in *N, N*-dimethylformamide (1.5 mL). After addition of azide **6** (14 mg, 0.038 mmol), copper(I)iodide (1 mg, 0.005 mmol) and *N,N*-diisopropyl-ethylamine (9 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H_2O (3 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Flash chromatography of the residue (cyclohexane-EtOAc 2:1) provided the title compound **18** (15 mg, 65%) as a white solid; $[\alpha]_D$ +58 (*c* 1.3, $CHCl_3$); R_f 0.3 (cyclohexane-EtOAc 1:1); IR (film) cm^{-1}: 3298 br, 2918, 2850, 1752, 1651, 1467, 1371, 1215, 1072, 720; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (1H, br s, triazole-H), 6.65 (1H, d, *J* 7.9, NH), 6.54 (1H, d, *J* 5.9, H-1), 6.13 (1H, dd, *J* 10.6, 3.5, H-3), 5.97 (1H, dd, *J* 3.5, 1.6, H-4), 5.52 (1H, dd, *J* 10.5, 5.9, H-2), 5.19 (1H, d, *J* 1.6, H-5), 5.09 (1H, dd, *J* 8.0, 3.1, H-1'), 3.87 (1H, brs, H-2'), 3.74 (3H, s, OMe), 2.18 (2H, t, *J* 7.7, CH_2), 2.15, 2.02 (3H, s) (each OAc), 1.91 (1H, br s, OH) 1.84 (3H, s, OAc), 1.67-1.52 (2H, overlapping signals, CH_2), 1.51-1.44 (1H, overlapping signals, CH_2), 1.37-1.16 (60H, overlapping signals, each CH_2), 0.88 (6H, t, *J* 6.8, each CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 169.2, 169.5, 169.4, 166.5 (each C=O), 145.0 (triazole, CH=*C*), 125.6 (triazole *C*H), 81.7 (C-1), 74.4 (C-2'), 72.3 (C-5), 68.5 (C-4), 67.2 (C-3), 66.8 (C-2), 52.8 (OMe), 48.8 (C-1'), 36.6, 34.6, 31.9, 29.7 (2s), 29.6 (3S), 29.5 (2s), 29.3 (2s), 25.8, 25.5, 22.7 (each CH_2), 20.6, 20.5, 20.2, 14.1 (each CH_3); ESI-HRMS calcd for $C_{53}H_{93}N_4O_{11}$ 961.6841, found *m/z* 961.6847 $[M-H]^-$.

3.12. N-((1*S*,2*R*)-2-Hydroxy-1-(1-(5-(*S*)-hydroxycarbonyl-β-L-arabinopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (4)

The protected lipid **18** (13.6 mg, 14 µmol) was dissolved in *n*-PrOH (3.4 mL) and H_2O_2 (0.34 mL of 30% aq solution). To this was added *n*-PrONa/*n*-PrOH (850 µL of an 0.1 M solution in *n*-PrOH, , 85 µmol) dropwise at a rate of 100 µL/h. After addition was completed the reaction was stirred for a further 1 h. Water (9 mL) was added and the solution centrifuged at 15000 rpm for 15 min. The supernatant was removed and the precipitate treated a further two times with water. The precipitate was lyophilised to provide the title compound **4** (6.6 mg, 57%) as a white solid; ^{1}H NMR (600 MHz, CD_3CO_2D-DMSO-D_6, 60 °C) δ 7.92 (1H, s, triazole-*H*), 6.22 (1H, d, *J* 5.2, H-1), 4.99 (1H, d, *J* 5.9 Hz, H-1'), 4.51 (1H, d, *J* 2.3 Hz, H-5), 4.27 – 4.23 (2H, overlapping signals H-3 & H-4), 4.14 (1H, dd, *J* 8.7, 5.2, H-2), 3.70 (1H, p, *J* 4.4, 3.8, H-2'), 2.14 – 2.05 (2H, overlapping signals, CH_2), 1.49 – 1.41 (2H, overlapping signals, CH_2), 1.39 – 1.31 (1H, overlapping signals, CH_2), 1.16 (61 H, s, each CH_2), 0.76 (6H, t, *J* 6.9, each CH_3); ^{13}C NMR (150 MHz, CD_3CO_2D-DMSO-D_6, 60 °C) δ 174.3, 170.9 (each C=O), 146.0 (triazole, CH=*C*), 126.3 (triazole, *C*H=C), 85.7 (C-1), 74.6 (C-5), 73.6 (C-2'), 70.6 (C-3 or C-4), 70.2 (C-3 or C-4), 68.5 (C-2), 51.3 (C-1'), 36.7, 34.4, 32.5, 30.2 (4s), 30.1, 26.5, 26.2, 23.2 (each CH_2), 14.6 (CH_3); ESI-HRMS calcd. for $C_{46}H_{85}N_4O_8$ 821.6367, found *m/z* 821.6379 $[M-H]^-$.

3.13. N-((1*S*,2*R*)-2-hydroxy-1-(1-(α-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)nonadecyl)nonadecanamide (5)

Alkyne **7** (10 mg, 0.017 mmol) was dissolved in *N,N*-dimethylformamide (0.5 mL). After addition of azide **17** (9.3 mg, 0.025 mmol), copper(I)iodide (1 mg, 0.005 mmol) and *N,N*-diisopropyl-ethylamine (9 µL), the reaction mixture was placed in a microwave reactor for 60 min, at 80 °C and 120 W. The resulting solution was diluted with EtOAc and washed with H_2O (3 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent removed under reduced pressure. Flash chromatography

of the residue (cyclohexane-EtOAc 2:1) provided the protected glycolipid (11 mg, 68%) as a white solid; $[\alpha]_D$ +45 (c 0.9, $CHCl_3$); R_f 0.4 (cyclohexane-EtOAc 1:1); IR (film) cm^{-1}: 3337 br, 2917, 2850, 1747, 1631, 1530, 1371, 1217, 1070, 915, 729; ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (1H, s, triazole-H), 6.67 (1H, d, *J* 8.0, NH), 6.38 (1H, d, *J* 6.0, H-1), 6.10 (1H, dd, *J* 10.7, 3.5, H-3), 5.66 (1H, dd, *J* 3.4, 1.2, H-4), 5.48 (1H, dd, *J* 10.7, 6.0, H-2), 5.08 (1H, dd, *J* 8.0, 3.2, H-1'), 4.69 (1H, td, *J* 6.6, 1.3, H-5), 4.11 (1H, dd, *J* 11.4, 6.8, H-6a), 4.05 (1H, dd, *J* 11.4, 6.4, H-6b), 3.87 (1H, ddt, *J* 9.8, 7.1, 4.1, H-2'), 3.49 (1H, d, *J* 10.1, OH), 2.20 – 2.16 (5H, overlapping signals, CH_2 & OAc), 2.01 (3H, s), 2.00 (3H, s), 1.83 (3H, s, each OAc), 1.59 (2H, s, CH_2), 1.51 – 1.45 (1H, m, CH(*H*)), 1.33 – 1.18 (61H, overlapping signals, each CH_2), 0.88 (6H, t, *J* 6.9, 2 x CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 170.3, 170.1, 170.0, 169.5 (each C=O), 144.8 (triazole, CH=*C*), 125.5 (triazole, *C*H=C), 81.9 (C-1), 74.4 (C-2'), 70.7 (C-5), 67.6 (C-3), 67.4 (C-4), 67.2 (C-2), 61.1 (C-6), 48.7 (C-1'), 36.6, 34.6, 31.9, 29.7 (3s), 29.6 (3s), 29.5, 29.4, 29.3 (2s), 25.8, 25.5, 22.7 (each CH_2), 20.6 (3s), 20.2, 14.1 (each CH_3); ESI-HRMS calcd. for $C_{54}H_{95}N_4O_{11}$ 975.6997, found *m/z* 975.7020 $[M-H]^-$. The protected lipid (7 mg, 7.2 μmol) was dissolved in *n*-PrOH (1 mL) and H_2O_2 (30%, 0.12 mL). To this was added *n*-PrONa (430 μL of an 0.1 M solution in n-PrOH, 43 μmol,) dropwise at a rate of 100 μL/h. After the addition was completed the reaction was stirred for a further 1 h. Water (4 mL) was then added and the solution centrifuged at 15000 rpm for 15 min. The supernatant was removed and the precipitate treated a further two times with water. The precipitate was lyophilised to provide the title compound **5** (5 mg, 86%) as a white solid; ^1H NMR (600 MHz, pyridine-d_5) δ 9.01 (1H, d, *J* 8.9, NH), 8.71 (1H, s, triazole H), 7.64 (1H, br s, OH), 6.99 (1H, br s, OH), 6.86 (1H, d, *J* 4.7, H-1), 6.80 (1H, d, *J* 4.2, OH), 6.54 (2H, br s, *J* 6.6, each OH), 6.08 (1H, dd, *J* 8.8, 5.0, H-1'), 5.24-5.14 (2H, m, overlapping signals, H-2 & H-3), 4.89 (1H, td, *J* 5.9, 1.5, H-5), 4.83 (1H, s, H-4), 4.50 (1H, td, *J* 7.7, 5.9, 3.4, H-2'), 4.47 – 4.43 (1H, m, H-6a), 4.37 (1H, dt, *J* 10.8, 5.0, H-6b), 2.50-2.38 (2H, m, CH_2), 1.96 – 1.76 (5H, overlapping signals, CH_2), 1.59 (1H, tdd, *J* 13.1, 10.6, 9.2, 5.3, CH(*H*)), 1.33 – 1.23 (58 H, overlapping signals, each CH_2), 0.88 (6H, t, *J* 6.9, 2 x CH_3); ^{13}C NMR (150 MHz, pyridine-d_5) δ 172.7 (C=O), 146.4 (triazole *C*), 126.3 (triazole, *C*H), 87.5 (C-1), 77.2 (C-5), 73.8 (C-2'), 71.6 (C-2 or C-3), 70.2 (C-4), 69.5 (C-2 or C-3), 62.0 (C-6), 51.8 (C-1'), 36.8, 35.1, 32.3, 30.2 (2s), 30.1 (2s), 30.0, 29.9 (2s), 29.8, 26.8, 26.5, 23.1 (each CH_2), 14.4 (CH_3); ESI-HRMS calcd. for $C_{46}H_{87}N_4O_7$ 807.6575, found *m/z* 807.6579 $[M-H]^-$.

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References and notes

[1] Mattner, J.; DeBord, K. L.; Ismail, N.; Goff, R. D.; Cantu, C.; Zhou, D.; Saint-Mezard, P.; Wang, V.; Gao, Y.; Hoebe, K.; Schneewind, O.; Walker, D.; Beutler, B.; Teyton, L.; Savage, P.; Bendelac, A. *Nature* **2005**, *434*, 525-529.

[2] Kinjo, Y.; Wu, D.; Kim, G.; Xing, G. W.; Poles, M. A.; Ho, D. D.; Tsuji, M.; Kawahara, K.; Wong, C. H.; Kronenberg, M. *Nature* **2005**, *434*, 520-525

[3] Kronenberg, M. *Annual Review of Immunology* **2005**, *23*, 877-900.

[4] (a) Lin, K.H.; Liang, J.J.; Huang, W.I.; Lin-Chu, S.Y.; Su, C.Y.; Lee, Y.L.; Jan, J.T.; Lin, Y.L.; Cheng, Y.S.E.; Wong, C.H. *Antimicrob. Agents. Chemother*. **2010**, *54*, 4129–4136. (b) Lia, X.; Fujiob, M.; Imamurab, M.; Wub, D.; Vasana, S.; Wong, C.H.; Hoa, D.D.; Tsujia, M. *Proc. Natl. Acad. Sci.* **2010**, *107*, 13010–13015.

[5] (a) Dere, R.T.; Zhu, X. *Org. Lett.* **2008**, *10*, 4641–4644. (b) Blauvelt, M.L.; Khalili, M.; Jaung, W.; Paulsen, J.; Anderson, A.C.; Wilson, S.B.; Howell, A.R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6374–6376. (c) Fujii, S.; Shimizu, K.; Hemmi, H.; Fukui, M.; Bonito, A.J.; Chen, G.; Franck, R.W.; Tsuji, M.; Steinman R. M. *Proc. Natl. Acad. Sci.* **2006**, 103, 11252-11257. (d) Chen,W.; Xia, C.; Cai, L.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3859-3862.

[6] (a) Pérez-Balderas, F.; Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asín, J. A.; Isac-García, J.; Santoyo-González, F. *Org. Lett.* **2003**, *5*, 1951-1954. (b) Morvan, F.; Meyer, A.; Jochum, A.; Sabin, C.; Chevolot, Y.; Imberty, A.; Praly, J.-P.; Vasseur, J.-J.; Souteyrand, E.; Vidal, S. *Bioconugate. Chem.* **2007**, *18*, 1637-1643. (c) Wang, G.-N.; Andre, S.; Gabius, H.-J.; Murphy, P. V. *Org. Biomol. Chem.* **2012**, *10*, 6893-6907. (d) Campo, V. L.; Carvalho, I.; Da Silva, C. H. T. P.; Schenkman, S.; Hill, L.; Nepogodiev, S. A.; Field, R. A. *Chem. Sci.* **2010**, *1*, 507-514. (e) Papadopoulos, A.; Shiao, T. C.; Roy, R. *Mol. Pharmaceutics.* **2011**, *9*, 394-403. (f) Kuijpers, B. H. M.; Groothuys, S.; Keereweer, A. R.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; van Delft, F. L.; Rutjes, F. P. J. T. *Org. Lett.* **2004**, *6*, 3123-3126. (g) André, S.; Jarikote, D. V.; Yan, D.; Vincenz, L.; Wang, G.-N.; Kaltner, H.; Murphy, P.V.; Gabius, H.-J. *Bioorg. Med. Chem. Lett.* **2012**, 22, 313-318.

[7] (a) Meldal, M.; Tornoe, C. M.; *Chem. Rev.*, **2008**, *108*, 2952-3015. (b) Kolb, H. C.; Finn M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.*, **2001**, 40, 2004-2021. (c) Dedola, S.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 1006-1017.

[8] Farrell, M.; Zhou, J.; Murphy, P. V. *Chem. Eur. J.* **2013**, *19*, 14836–14851.

[9] O'Reilly, C.; Murphy, P. V. *Org. Lett.* **2011**, *13*, 5168-5171. Analytical data, previously not reported for **9** is provided in the experimental section.

[10] (a) Ohira, S. *Synth. Commun.* **1989**, *19*, 561-564 (b) Mueller, S., Liepold, B., Roth, G. J., Bestmann, H. J. *Synlett.* **1996**, 521-522.

[11] Long, R. M.; Lagisetti, C.; Coates, R. M.; Croteau, R. B. *Arch. Biochem. Biophys.* **2008**, *477*, 384-389.

[12] Howarth, N. M.; Lindsell, W. E.; Murray, E.; Preston, P. N. *Tetrahedron.* **2005**, *61*, 8875-8887.

[13] Tosin, M.; Murphy, P. V. *Org. Lett.* **2002**, *4*, 3675-3678.

[14] Mayato, C.; Dorta, R. L.; Vazquez, J. T. *Tetrahedron Lett.* **2008**, *49*, 1396-1398.

[15] The spectra of **17** were in good agreement with previously reported data; Capicciotti, Szilágyi, L.; Györgydeák, Z. *Carbohydr. Res.* **1985**, *143*, 21-41.

[16] Wiebe, C.; Schlemmer, C.; Weck, S.; Opatz, T. *Chem. Commun.* **2011**, *47*, 9212-9214.

[17] Vogel, C.; Boye, H.; Kristen, H. *Journal für Praktische Chemie* **1990**, *332*, 28-36.