



Synthesis of the 3'-O-sulfated TF antigen with a TEG-N₃ linker for glycodendrimersomes preparation to study lectin binding

Mark Reihill, Hanyue Ma, Dennis Bengtsson and Stefan Oscarson*

Full Research Paper

Open Access

Address:
Centre for Synthesis and Chemical Biology, University College Dublin,
Belfield, Dublin 4, Ireland

Email:
Stefan Oscarson* - stefan.oscarson@ucd.ie

* Corresponding author

Keywords:
regioselective sulfation; thioglycoside donors; Thomsen–Friedenreich
antigen

Beilstein J. Org. Chem. **2024**, *20*, 173–180.
<https://doi.org/10.3762/bjoc.20.17>

Received: 07 November 2023

Accepted: 16 January 2024

Published: 30 January 2024

This article is part of the thematic issue "Chemical glycobiology".

Associate Editor: U. Westerlind



© 2024 Reihill et al.; licensee Beilstein-Institut.
License and terms: see end of document.

Abstract

The synthesis of gram quantities of the TF antigen (β -D-Gal-(1→3)- α -D-GalNAc) and its 3'-sulfated analogue with a TEG-N₃ spacer attached is described. The synthesis of the TF antigen comprises seven steps, from a known *N*-Troc-protected galactosamine donor, with an overall yield of 31%. Both the spacer (85%) and the galactose moiety (79%) were introduced using thioglycoside donors in NIS/AgOTf-promoted glycosylation reactions. The 3'-sulfate was finally introduced through tin activation in benzene/DMF followed by treatment with a sulfur trioxide–trimethylamine complex in a 66% yield.

Introduction

In a collaboration project with groups from Universities in Munich and Pennsylvania we are investigating carbohydrate–lectin interactions using programmable glycodendrimersomes based on synthetic glycans. We have earlier synthesized 2-[2-(2-azidoethoxy)ethoxy]ethyl (TEG-N₃) glycosides of lactose, 3'-Su-lactose and LacdiNAc (β -D-GalNAc-(1→4)- β -D-GlcNAc), which have then been used for production of the glycodendrimersomes and interaction studies with various galectins [1,2]. In the continuation of this collaboration, to investigate the binding of siglec-1 and the chimera of 3'-SuTF-binding siglecs and TF-binding galectin-3, TEG-N₃ glycosides of the TF antigen (β -D-Gal-(1→3)- α -D-GalNAc, **1**)

and its 3'-O-sulfated analogue (**2**, Figure 1) were required on a gram scale to allow efficient synthesis of the glycodendrimersomes. The TF antigen is presented on the surface of most human cancer cell types and its interaction with galectins 1 and 3 leads to tumour cell aggregation and promotes cancer metastasis [3-5]. The 3'-O-sulfated analogue is known to bind to siglecs 1, 4, and 8 [6] as well as galectin 4 [7,8], but its biological role is not that well investigated.

Compound **2** is a new compound but two syntheses of compound **1** have recently been reported, one using an enzymatic approach and a commercial α -TEG-N₃ GalNAc acceptor [9]

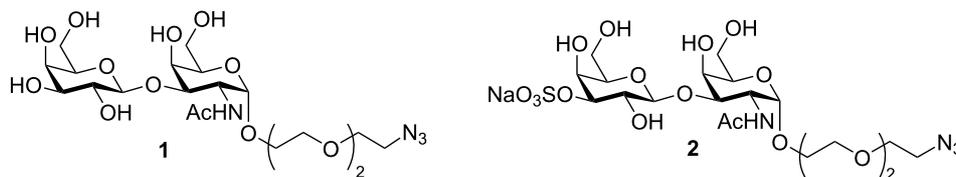


Figure 1: Structure of target compounds **1** and **2**.

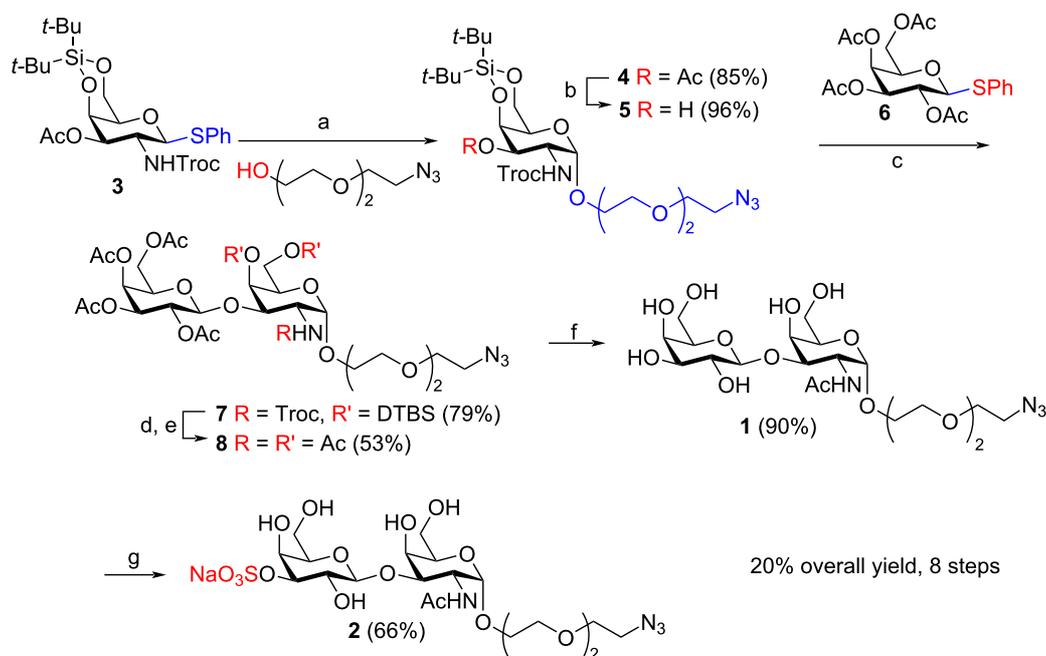
and one using glycosyl bromide donors and silver salt-promoted glycosylations [10].

Results and Discussion

To introduce the 2-[2-(2-chloroethoxy)ethoxy]ethyl (TEG-Cl) spacer both a Fischer synthesis starting from unprotected *N*-acetylgalactosamine and a Lewis acid-promoted reaction starting from per-acetylated galactosamine were initially tested. As reported [11], the Fischer synthesis gives low yields and α -selectivity. The Lewis acid-promoted reaction, which had worked well to produce β -linked TEG-spacer glycosides with per-acetylated lactose and 2-phthalimidoglucosamine [1,2] worked well with 2-chloroethanol as a spacer (68%, pure α) but failed with the TEG-Cl spacer [12], why we instead decided to use a thioglycoside donor to introduce the spacer. To ensure α -selectivity a di-*tert*-butylsilyl-4,6-acetal-protected donor, as developed by the Kiso group [13,14], was chosen. After some

initial testing the known *N*-Troc-protected donor **3** [15,16] (Scheme 1) was selected [17].

Since donor **3** possessed a Troc group, which contains 3 chlorine atoms, nucleophilic introduction of an azido group at this stage was predicted to be problematic. Therefore, the azido functionality was installed in the spacer before the glycosylation. Donor **3** underwent an NIS/AgOTf-promoted glycosylation with the TEG- N_3 acceptor [18], furnishing α -linked **4** in an 85% yield (Scheme 1). H-1 appeared as a doublet at 4.95 ppm with a *J* value of 3.6 Hz in the ^1H NMR spectrum proving the anomeric α -configuration. The presence of Troc-rotamers was also apparent, with a ratio of 19:6 being observed by ^1H NMR in CDCl_3 at 25 °C. Catalytic amounts of NaOMe (0.005 M) in MeOH were used to remove the acetate from compound **4**, taking care not to affect the Troc group, to afford acceptor **5** in a 96% yield.



Scheme 1: Synthesis of target compounds **1** and **2**. Key: a) NIS, AgOTf (20 mol %), 4 Å molecular sieves, CH_2Cl_2 , rt, 40 min, 85%; b) NaOMe (10 mol %), MeOH, rt, 4 h, 96%; c) NIS, AgOTf (19 mol %), AW-300 4 Å molecular sieves, CH_2Cl_2 , rt, 1 h, 79%; d) 1 M $\text{Bu}_4\text{NF}/\text{THF}$, THF, rt, 2 h, then HF-Py, rt, 3 h; e) $\text{Ac}_2\text{O}/\text{Py}$ (1:2, v/v), rt, 16 h, 53% over 3 steps; f) 1 M NaOMe/MeOH, MeOH, pH 10, rt, 1 h, 90%; g) Bu_2SnO , benzene/DMF (5:1, v/v), 125 °C, 24 h, then $\text{SO}_3\text{-NMe}_3$, DMF, rt, 72 h, then flash chromatography, then Dowex® 50WX4 (Na^+ form) resin, H_2O , rt, 16 h, 66%.

Earlier optimizations of the introduction of the β -linked galactose moiety using 2-azidoethyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy- α -D-galactopyranoside as acceptor showed an acetylated thioglycoside donor to be the best choice [12], surprisingly better than a benzoylated donor [19], why this donor was the first one tested also with the quite different acceptor **5**. An NIS/AgOTf-promoted glycosylation with donor **6** [20] yielded 79% of disaccharide **7**. Due to the presence of rotamers, NMR spectra of **7** proved to be difficult to analyse when data were recorded in CDCl₃. Changing the NMR solvent to CD₃OD greatly reduced the complexity of the spectra [21–23].

Since **7** possessed an azido group as part of the linker, removal of the Troc group under reductive conditions was ruled out due to probable chemoselectivity issues [24,25]. Interestingly, Jacquemard et al. outlined a useful, mild method for removing a range of carbamates using Bu₄NF in an article from 2004 [26]. As **7** contained a DTBS group, the possibility of removing both Troc and DTBS groups in a one-pot procedure was tested. Disaccharide **7** was therefore treated with 1 M Bu₄NF/THF and after 2 hours, full consumption of the starting material was observed by TLC. However, MALDI–TOF mass spectrometry (super-DHB matrix) revealed that only the Troc group had been removed, with the DTBS substituent proving to be stable under these conditions. Addition of a large excess of HF·Py (40 equiv) proved to be necessary to remove the bulky silyl group. After concentration, the crude product was acetylated (Ac₂O/Py, 1:2, v/v), furnishing per-acetylated compound **8** in a 53% yield over the 3 steps. Deacetylation of **8** with freshly prepared 1 M NaOMe/MeOH in MeOH at pH 10 furnished target **1** in a 90% yield.

Formation of a stannylidene acetal via tin-activation was employed to achieve selective 3'-*O*-sulfation of compound **1** [27], with a variety of conditions being attempted (Table 1). With a

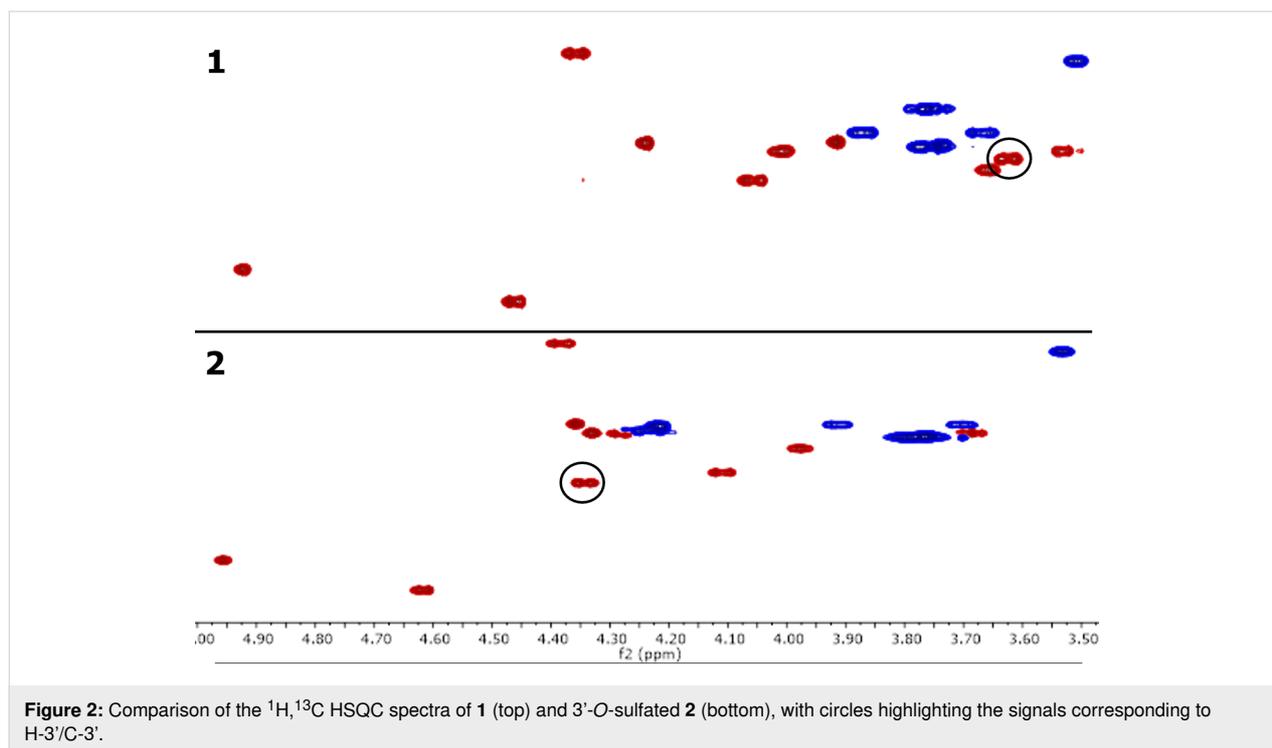
TEG-N₃ lactose compound, tin-activation was performed with Bu₂SnO in refluxing MeOH, followed by stirring with SO₃·NMe₃ in 1,4-dioxane to afford the 3'-*O*-sulfate in 65% yield [1]. Here, however, this choice of solvent in the sulfation step led to the material being insoluble and no reaction was observable by TLC. Changing the solvent of the sulfation reaction to DMF resulted in formation of a homogenous solution, but still no conversion to the sulfated product, even when the temperature was raised to 80 °C [28,29]. Switching the sulfating reagent to SO₃·Py or performing the reaction at 150 °C in a microwave did not improve the outcome [30,31].

Since there was no observable sulfation taking place, the tin-activation step was suspected to be the root of the problem. To rectify this, similar to Malleron et al., **1** was refluxed, in a Dean–Stark set-up, with Bu₂SnO in benzene/DMF (5:1, v/v) [32]. The solvent in the receiver was drained after 24 hours and the benzene was removed from the reaction mixture in vacuo. Sulfation was then performed through addition of SO₃·NMe₃ to the DMF solution. Consumption of **1** was observed by TLC after 72 hours and stirring with Dowex[®] 50WX4 (Na⁺ form) resin resulted in formation of target **2**. Purification by flash chromatography, however, led to isolation of a mixture of **2** and a tin-related impurity (*n*-butyl chain evident by NMR). Acetylation of this material followed by flash chromatography proved ineffective in removing the unwanted entity. To overcome this problem, flash chromatography was performed before stirring with the ion-exchange resin, with no apparent presence of tin impurities by NMR when the sequence was executed in this order and sulfated target **2** was obtained in a 66% yield on a one-gram scale. Comparing the ¹H, ¹³C HSQC spectra of compounds **1** and **2**, there is a clear downfield shift of the H-3'/C-3' signal from **1** to sulfated **2** (Figure 2). This showed that regioselective 3'-*O*-sulfation had been achieved, with HRMS also indicating that only one sulfate group was present.

Table 1: Summary of conditions attempted to achieve regioselective 3'-*O*-sulfation.

Tin-activation ^a			Sulfation ^a			
Solvent(s)	Temperature	Set-up	Sulfating reagent	Solvent	Temperature/conditions	Result/ yield
MeOH	95 °C	reflux	SO ₃ ·NMe ₃	1,4-dioxane	rt	material insoluble, no reaction
MeOH	95 °C	reflux	SO ₃ ·NMe ₃	DMF	rt–80 °C	no reaction
MeOH	95 °C	reflux	SO ₃ ·Py	DMF	80 °C	no reaction
MeOH	95 °C	reflux	SO ₃ ·Py	DMF	150 °C, microwave	no reaction
benzene/DMF (5:1, v/v)	125 °C	reflux, Dean–Stark	SO ₃ ·NMe ₃	DMF	rt	66%

^aTin-activation was performed with 1.2 equiv of Bu₂SnO in all cases for 16–24 h and sulfation reactions proceeded for 24–72 h.



Conclusion

An efficient synthesis of the important TF and 3'-Su-TF antigens equipped with a TEG- N_3 linker to allow formation of various conjugates has been developed for further interaction studies with lectins (galectins and siglecs). The synthesis of the 3'-Su-TF antigen **2** comprises eight steps from the known *N*-galactosamine donor **3**, where two of the steps, removal of the Troc- and DTBS protecting groups are performed in the same pot and the following acetylation without purification of the intermediate, why the synthesis is high-yielding (20% overall yield) and easily scalable (9 g of protected disaccharide **7** and 1 gram of target **2** were synthesized).

Experimental

General methods

All reactions containing air- and moisture-sensitive reagents were carried out under an inert atmosphere of nitrogen in oven-dried glassware with magnetic stirring. N_2 -flushed plastic syringes were used to transfer air- and moisture-sensitive reagents. All reactions were monitored by thin-layer chromatography (TLC) on Merck[®] DC-Alufolien plates precoated with silica gel 60 F₂₅₄. Visualisation was performed with UV-light (254 nm) fluorescence quenching, and/or by staining with an 8% H_2SO_4 dip (stock solution: 8 mL conc. H_2SO_4 , 92 mL EtOH), ninhydrin dip (stock solution: 0.3 g ninhydrin, 3 mL AcOH, 100 mL EtOH) and/or ceric ammonium molybdate dip (stock solution: 25 g ammonium molybdate tetrahydrate, 0.5 g $\text{Ce}(\text{SO}_4)_2$, 50 mL H_2SO_4 , 450 mL EtOH).

Chromatography

Silica gel flash chromatography was carried out using Davisil[®] LC60A (40–63 μm) silica gel or with automated flash chromatography systems, Buchi Reveleris[®] X2 (UV 200–500 nm and ELSD detection, Reveleris[®] silica cartiges 40 μm , Büchi Labortechnik AG[®]) and Biotage[®] SP4 HPFC (UV 200–500 nm, Biotage[®] SNAP KP-Sil 50 μm irregular silica, Biotage[®] AB).

Instrumentation

^1H NMR and ^{13}C NMR spectra were recorded on Varian Inova spectrometers at 25 °C in chloroform-*d* (CDCl_3), methanol-*d*₄ (CD_3OD), deuterium oxide (D_2O) or DMSO-*d*₆ ($(\text{CD}_3)_2\text{SO}$). ^1H NMR spectra were standardised against the residual solvent peak (CDCl_3 , $\delta = 7.26$ ppm; CD_3OD , $\delta = 3.31$ ppm; D_2O , $\delta = 4.79$ ppm; $(\text{CD}_3)_2\text{SO}$ $\delta = 2.50$ ppm); or internal trimethylsilane, $\delta = 0.00$ ppm). ^{13}C NMR spectra were standardised against the residual solvent peak (CDCl_3 , $\delta = 77.16$ ppm; CD_3OD , $\delta = 49.00$ ppm; $(\text{CD}_3)_2\text{SO}$ $\delta = 39.52$ ppm and ^{13}C NMR spectra recorded in D_2O are unreferenced. All ^{13}C NMR spectra are ^1H decoupled. All NMR data are represented as follows: chemical shift (δ ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, app d = apparent doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet), coupling constant in Hz, integration. Assignments were aided by homonuclear ^1H , ^1H (COSY, TOCSY) and ^1H , ^{13}C heteronuclear (HSQC, HMBC) two-dimensional correlation spectroscopies. ^{13}C chem-

ical shifts were reported to one decimal point unless an additional digit was required to distinguish overlapping peaks. Software for data processing: MestReNova, version 11.0.0–17609 (MestReLab Research S.L.). High-resolution mass spectrometry (HRMS) data were recorded on a Waters Micromass LCT LC–TOF instrument using electrospray ionisation (ESI) in positive mode. MALDI–TOF mass spectrometry data were recorded on a Scientific Analysis Instruments MALDI–TOF mass spectrometer in reflectron mode for oligosaccharides and in linear mode for glycoconjugates. Samples were prepared by pre-mixing 1 μ L of a solution containing the analyte with 20 μ L of a matrix solution (10 mg/mL, MeCN/H₂O, 1:1, v/v + 1% TFA), pipetting 1 μ L of the mixture onto the sample plate and drying under gentle heat from a heat gun. Optical rotations were recorded in a Perkin-Elmer polarimeter (Model 343) at the sodium D-line (589 nm) at 20 °C using a 1 dm cell. Samples were prepared at the concentration (g/100 mL) in the solvent indicated. Deprotected glycans were lyophilised using a freeze-dryer Alpha 1-2 LDplus (Christ Ltd): pressure: 0.055 mbar; ice-condenser temperature: –55 °C.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-O-acetyl-2-deoxy-4,6-O-di-tert-butylsilylene-2-(2'2'2'-trichloroethoxycarbonylamino)- α -D-galactopyranoside (4): Donor **3** [9,10] (9.3 g, 15 mmol) and the TEG-N₃ acceptor (synthesized as described in reference [12], but also commercially available, 3.9 g, 22 mmol) were placed under N₂ together and dissolved in dry CH₂Cl₂ (300 mL). 4 Å molecular sieves (10.2 g) were added and the resulting suspension was stirred at room temperature for 16 hours. NIS (6.66 g, 29.6 mmol) and AgOTf (760 mg, 2.96 mmol) were then added, and the reaction was stirred at room temperature for 40 minutes. The reaction was then quenched with Et₃N, filtered through Celite® and concentrated in vacuo. The resulting residue was taken up in EtOAc (700 mL) and washed with 10% aq Na₂S₂O₃ (700 mL), water (700 mL) and brine (700 mL). The organic phase was then dried over MgSO₄, filtered and reduced to dryness. Flash chromatography on silica gel (toluene \rightarrow toluene/EtOAc, 3:2) yielded **4** as an orange syrup (8.74 g, 85%). *R*_f = 0.4 (toluene/EtOAc, 7:3); [α]_D +92 (*c* 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.44 (d, *J* = 10.2 Hz, 1H, NH), 4.98 (dd, *J* = 11.1, 2.9 Hz, 1H, H-3), 4.95 (d, *J* = 3.6 Hz, 1H, H-1), 4.87 (d, *J* = 12.1 Hz, 1H, CH_{2(A)Troc}), 4.69–4.58 (m, 2H, H-4, CH_{2(B)Troc}), 4.49 (td, *J* = 10.6, 3.6 Hz, 1H, H-2), 4.26 (dd, *J* = 12.6, 2.2 Hz, 1H, H-6_(A)), 4.15 (dd, *J* = 12.5, 1.7 Hz, 1H, H-6_(B)), 3.90–3.77 (m, 2H, H-5, CH_{2(A)Linker}), 3.76–3.59 (m, 9H, CH_{2(B)Linker}, 4 \times CH_{2(Linker)}), 3.39 (t, *J* = 5.1 Hz, 2H, CH_{2(Linker)}), 2.07 (s, 3H, CH_{3(OAc)}), 1.08 (s, 9H, C(CH₃)_{3(DTBS)}), 1.02 (s, 9H, C(CH₃)_{3(DTBS)}); ¹³C NMR (126 MHz, CDCl₃) δ 171.3 (C=O_(OAc)), 154.6 (C=O_(Troc)), 98.5 (C-1), 95.8 (CCl_{3(Troc)}), 74.7 (CH_{2(Troc)}), 71.7 (C-3), 70.9 (CH_{2(Linker)}), 70.8 (CH_{2(Linker)}), 70.5 (C-4),

70.27 (CH_{2(Linker)}), 70.25 (CH_{2(Linker)}), 67.63 (CH_{2(Linker)}), 67.57 (C-5), 67.1 (C-6), 50.8 (CH_{2(Linker)}), 49.3 (C-2), 27.7 (C(CH₃)_{3(DTBS)}), 27.4 (C(CH₃)_{3(DTBS)}), 23.4 (C(CH₃)_{3(DTBS)}), 21.1 (CH_{3(OAc)}), 20.9 (C(CH₃)_{3(DTBS)}); HRESIMS *m/z*: [M + NH₄]⁺ calcd for C₂₅H₄₃Cl₃N₄O₁₀Si, 710.2158; found, 710.2158.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2-deoxy-4,6-O-di-tert-butylsilylene-2-(2'2'2'-trichloroethoxycarbonylamino)- α -D-galactopyranoside (5): Compound **4** (8.65 g, 12.5 mmol) was placed under N₂ and dissolved in dry MeOH (250 mL). NaOMe (68 mg, 1.3 mmol) was added, and the reaction was stirred at room temperature for 4 hours. The solution was then neutralised with Amberlite® IR120 (H⁺ form) resin, filtered and concentrated under reduced pressure. Flash chromatography on silica gel (toluene \rightarrow toluene/acetone, 7:3) yielded **5** as a gold-coloured syrup (7.83 g, 96%). *R*_f = 0.4 (toluene/EtOAc, 3:2); [α]_D +67 (*c* 1.0, CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ 5.58 (d, *J* = 9.8 Hz, 1H, NH), 4.93 (d, *J* = 3.6 Hz, 1H, H-1), 4.77 (d, *J* = 12.0 Hz, 1H, CH_{2(A)Troc}), 4.72 (d, *J* = 12.0 Hz, 1H, CH_{2(B)Troc}), 4.43 (d, *J* = 3.0 Hz, 1H, H-4), 4.28 (dd, *J* = 12.5, 2.2 Hz, 1H, H-6_(A)), 4.16 (m, 1H, H-6_(B)), 4.11 (dd, *J* = 10.1, 3.6 Hz, 1H, H-2), 3.87–3.79 (m, 2H, H-5, CH_{2(A)Linker}), 3.74 (dd, *J* = 11.4, 3.2 Hz, 1H, H-3), 3.70–3.63 (m, 9H, CH_{2(B)Linker}, 4 \times CH_{2(Linker)}), 3.43–3.34 (m, 2H, CH_{2(Linker)}), 2.53 (d, *J* = 11.8 Hz, 1H, OH), 1.07 (s, 9H, C(CH₃)_{3(DTBS)}), 1.05 (s, 9H, C(CH₃)_{3(DTBS)}); ¹³C NMR (126 MHz, CDCl₃) δ 155.4 (C=O_(Troc)), 98.6 (C-1), 95.7 (CCl_{3(Troc)}), 74.9 (CH_{2(Troc)}), 73.0 (C-4), 70.9 (CH_{2(Linker)}), 70.7 (CH_{2(Linker)}), 70.27 (CH_{2(Linker)}), 70.24 (CH_{2(Linker)}), 70.14 (C-3), 67.8 (C-5), 67.7 (CH_{2(Linker)}), 67.2 (C-6), 52.5 (C-2), 50.8 (CH_{2(Linker)}), 27.7 (C(CH₃)_{3(DTBS)}), 27.5 (C(CH₃)_{3(DTBS)}), 23.5 (C(CH₃)_{3(DTBS)}), 20.9 (C(CH₃)_{3(DTBS)}); HRESIMS *m/z*: [M + Na]⁺ calcd for C₂₃H₄₁Cl₃N₄O₉Si, 673.1606; found, 673.1605.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl-(1 \rightarrow 3)-2-deoxy-4,6-O-di-tert-butylsilylene-2-(2'2'2'-trichloroethoxycarbonylamino)- α -D-galactopyranoside (7): Donor **6** [16] (7.67 g, 17.4 mmol) and acceptor **5** (7.57 g, 11.6 mmol) were placed under N₂ together and dissolved in dry CH₂Cl₂ (230 mL). AW-300 4 Å molecular sieves (5.45 g) were added, and the resulting suspension was stirred at room temperature for 23 hours. NIS (5.23 g, 23.2 mmol) and AgOTf (577 mg, 2.25 mmol) were added, and the reaction was stirred at room temperature for 1 hour. Et₃N was then added until the pH became neutral, and the suspension was filtered through Celite®. The filtrate was then washed with water (400 mL), brine (400 mL), dried over MgSO₄, filtered and concentrated in vacuo. Compound **7** was isolated by flash chromatography on silica gel (toluene \rightarrow toluene/EtOAc, 1:4) as an orange foam (8.97 g, 79%). *R*_f = 0.5 (toluene/EtOAc, 3:7); [α]_D

+71 (c 1.0, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 5.40 (d, *J* = 3.4 Hz, 1H, H-4_{Gal}), 5.21 (m, 1H, H-2_{Gal}), 5.11 (dd, *J* = 10.4, 3.4 Hz, 1H, H-3_{Gal}), 5.05 (d, *J* = 12.2 Hz, 1H, CH₂(A)_{Troc}), 4.93 (d, *J* = 3.6 Hz, 1H, H-1_{GalNTroc}), 4.88 (d, *J* = 7.8 Hz, 1H, H-1_{Gal}), 4.79 (d, *J* = 2.8 Hz, 1H, H-4_{GalNTroc}), 4.58 (d, *J* = 12.2 Hz, 1H, CH₂(B)_{Troc}), 4.38 (dd, *J* = 11.1, 3.6 Hz, 1H, H-2_{GalNTroc}), 4.30 (m, 1H, H-6_(A)), 4.22–4.05 (m, 4H, H-5_{Gal}, H-6_(B), H-6_(A+B)), 3.96 (dd, *J* = 11.1, 2.8 Hz, 1H, H-3_{GalNTroc}), 3.90–3.79 (m, 2H, H-5_{GalNTroc}, CH₂(A)_{Linker}), 3.77–3.63 (m, 9H, CH₂(B)_{Linker}, 4 × CH₂(Linker)), 3.46–3.39 (m, 2H, CH₂(Linker)), 2.16 (s, 3H, CH₃(OAc)), 2.12 (s, 3H, CH₃(OAc)), 2.05 (s, 3H, CH₃(OAc)), 1.97 (s, 3H, CH₃(OAc)), 1.13–1.09 (m, 18H, 2 × C(CH₃)₃(DTBS)); ¹³C NMR (126 MHz, CD₃OD) δ 171.99 (C=O(OAc)), 171.93 (C=O(OAc)), 171.6 (C=O(OAc)), 171.4 (C=O(OAc)), 156.6 (C=O(Troc)), 103.8 (C-1_{Gal}), 99.6 (C-1_{GalNTroc}), 97.2 (CCl₃(Troc)), 79.3 (C-3_{GalNTroc}), 75.6 (CH₂(Troc)), 74.1 (C-4_{GalNTroc}), 72.6 (C-3_{Gal}), 71.8 (C-5_{Gal}), 71.52 (CH₂(Linker)), 71.49 (CH₂(Linker)), 71.1 (CH₂(Linker)), 70.5 (C-2_{Gal}), 69.0 (C-5_{GalNTroc}), 68.6 (C-4_{Gal}), 68.4 (C-6), 68.1 (CH₂(Linker)), 62.9 (C-6), 51.8 (CH₂(Linker)), 51.4 (C-2_{GalNTroc}), 28.2 (C(CH₃)₃(DTBS)), 28.0 (C(CH₃)₃(DTBS)), 24.3 (C(CH₃)₃(DTBS)), 21.7 (C(CH₃)₃(DTBS)), 21.0 (CH₃(OAc)), 20.6 (CH₃(OAc)), 20.51 (CH₃(OAc)), 20.48 (CH₃(OAc)). HRESIMS *m/z*: [M + NH₄]⁺ calcd for C₃₇H₅₉Cl₃N₄O₁₈Si, 998.3003; found, 998.3003.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-α-D-galactopyranoside [3] (8): Compound 7 (8.87 g, 9.03 mmol) was placed under N₂ and dissolved in dry THF (180 mL). 1 M Bu₄NF/THF (32 mL, 32 mmol) was added, and the reaction was stirred at room temperature. After 2 hours, the starting material had been consumed (judged by TLC) and HF·Py (70% HF, 9.5 mL, 370 mmol) was added. Stirring was continued at room temperature for a further 3 hours and the solution was then concentrated.

The crude was placed under N₂ and stirred at room temperature in Ac₂O/Py (180 mL, 1:2, v/v) for 16 hours. The solution was then reduced to dryness and purification by flash chromatography on silica gel (EtOAc→EtOAc/MeOH, 17:3) yielded 8 as a dark orange/brown syrup (3.82 g, 53% over 3 steps). *R*_f = 0.4 (EtOAc/MeOH, 19:1); [α]_D +76 (c 1.0, CH₃OH); ¹H NMR (500 MHz, CD₃OD) δ 5.42 (d, *J* = 3.1 Hz, 1H, H-4_{GalNAc}), 5.36 (dd, *J* = 3.4, 1.2 Hz, 1H, H-4_{Gal}), 5.06 (dd, *J* = 10.5, 3.4 Hz, 1H, H-3_{Gal}), 5.00 (dd, *J* = 10.5, 7.6 Hz, 1H, H-2_{Gal}), 4.83 (d, *J* = 3.8 Hz, 1H, H-1_{GalNAc}), 4.78 (d, *J* = 7.6 Hz, 1H, H-1_{Gal}), 4.43 (dd, *J* = 11.1, 3.6 Hz, 1H, H-2_{GalNAc}), 4.26 (m, 1H, H-5_{GalNAc}), 4.21–4.11 (m, 3H, H-6_(A+B)_{Gal}, H-6_(A)_{GalNAc}), 4.08 (dd, *J* = 11.1, 3.4 Hz, 1H, H-3_{GalNAc}), 4.04 (m, 1H, H-5_{Gal}), 3.97 (dd, *J* = 11.3, 7.3 Hz, 1H, H-6_(B)_{GalNAc}), 3.81 (m, 1H, CH₂(A)_{Linker}),

3.73–3.71 (m, 2H, CH₂(Linker)), 3.70–3.62 (m, 7H, 3 × CH₂(Linker), CH₂(B)_{Linker}), 3.42–3.36 (m, 2H, CH₂(Linker)), 2.14 (s, 3H, CH₃(Ac)), 2.11 (s, 3H, CH₃(Ac)), 2.06–2.02 (m, 9H, 3 × CH₃(Ac)), 1.99 (s, 3H, CH₃(Ac)), 1.93 (s, 3H, CH₃(Ac)); ¹³C NMR (126 MHz, CD₃OD) δ 173.1, 172.3, 172.08, 172.06, 172.04, 171.5, 171.2 (C=O(Ac)), 102.4 (C-1_{Gal}), 99.3 (C-1_{GalNAc}), 74.6 (C-3_{GalNAc}), 72.2 (C-3_{Gal}), 71.8 (CH₂(Linker)), 71.54 (C-5_{Gal}), 71.49 (CH₂(Linker)), 71.36 (CH₂(Linker)), 71.3 (CH₂(Linker)), 71.2 (C-4_{GalNAc}), 70.2 (C-2_{Gal}), 68.6 (C-4_{Gal}), 68.5 (C-5_{GalNAc}), 68.2 (CH₂(Linker)), 63.9 (C-6_{GalNAc}), 62.4 (C-6_{Gal}), 51.7 (CH₂(Linker)), 50.3 (C-2_{GalNAc}), 22.89, 20.82, 20.77, 20.73, 20.67, 20.50, 20.47 (CH₃(Ac)). As NMR spectra in the literature were recorded in CDCl₃ [3], NMR data are not comparable. HRESIMS *m/z*: [M + Na]⁺ calcd for C₃₂H₄₈N₄O₁₉; 815.2810; found; 815.2806.

2-[2-(2-Azidoethoxy)ethoxy]ethyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside [3,4] (1): Compound 8 (1.47 g, 1.85 mmol) was dissolved in MeOH (100 mL) and freshly prepared 1 M NaOMe/MeOH was added until the solution reached pH 10. The reaction was stirred at room temperature for 1 hour, then neutralised with Amberlite® IR120 (H⁺ form) resin. The resin was filtered off, washed with MeOH and the filtrate was concentrated in vacuo. After lyophilisation, 1 was obtained as a light brown/orange solid (900 mg, 90%) and required no further purification. *R*_f = 0.6 (EtOAc/MeOH, 2:3); [α]_D +76 (c 1.0, H₂O); ¹H NMR (500 MHz, D₂O) δ 4.92 (d, *J* = 3.7 Hz, 1H, H-1_{GalNAc}), 4.46 (d, *J* = 7.8 Hz, 1H, H-1_{Gal}), 4.36 (dd, *J* = 11.0, 3.7 Hz, 1H, H-2_{GalNAc}), 4.24 (d, *J* = 3.0 Hz, 1H, H-4_{GalNAc}), 4.06 (dd, *J* = 11.1, 3.1 Hz, 1H, H-3_{GalNAc}), 4.01 (m, 1H, H-5), 3.92 (d, *J* = 3.4 Hz, 1H, H-4_{Gal}), 3.87 (m, 1H, CH₂(A)_{Linker}), 3.81–3.71 (m, 12H, 2 × H-6_(A+B), 4 × CH₂(Linker)), 3.70–3.59 (m, 3H, H-3_{Gal}, H-5, CH₂(B)_{Linker}), 3.56–3.48 (m, 3H, H-2_{Gal}, CH₂(Linker)), 2.04 (s, 3H, CH₃(NHAc)); ¹³C NMR (126 MHz, D₂O) δ 174.5 (C=O(NHAc)), 104.7 (C-1_{Gal}), 97.4 (C-1_{GalNAc}), 77.3 (C-3_{GalNAc}), 74.9 (C-5), 72.5 (C-3_{Gal}), 70.64 (C-5), 70.54 (C-2_{Gal}), 69.7 (CH₂(Linker)), 69.51 (CH₂(Linker)), 69.46 (CH₂(Linker)), 69.2 (CH₂(Linker)), 68.7 (C-4_{GalNAc}), 68.5 (C-4_{Gal}), 66.5 (CH₂(Linker)), 61.1 (C-6), 60.9 (C-6), 50.1 (CH₂(Linker)), 48.5 (C-2_{GalNAc}), 22.0 (CH₃(NHAc)). NMR data match those reported in the literature [3,4]. HRESIMS *m/z*: [M + H]⁺ calcd for C₂₀H₃₆N₄O₁₃, 541.2357; found, 541.2354.

2-[2-(2-Azidoethoxy)ethoxy]ethyl 3-*O*-sulfo-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside sodium salt (2): Compound 1 (1.24 g, 2.29 mmol) and Bu₂SnO (645 mg, 2.75 mmol) were placed under N₂ together. Dry benzene/DMF (380 mL, 5:1, v/v) was added and the reaction was refluxed at 125 °C using a Dean–Stark apparatus. After 24 hours, the solvent in the receiver was drained, and the

benzene was removed from the reaction mixture in vacuo. $\text{SO}_3\cdot\text{NMe}_3$ (642 mg, 4.61 mmol) was then added to the DMF solution, and the reaction was stirred at room temperature. After 24 hours, an additional portion of $\text{SO}_3\cdot\text{NMe}_3$ (950 mg, 6.83 mmol) was added and stirring was continued at room temperature for a further 48 hours. The reaction mixture was then concentrated and flash chromatography on silica gel (EtOAc/MeOH, 1:0→0:1) yielded a yellow syrup, which was re-dissolved in H_2O (30 mL). Dowex® 50WX4 (Na^+ form) resin (1.28 g) was added, and the resulting suspension was stirred at room temperature for 16 hours. Filtration followed by concentration and lyophilisation of the filtrate yielded **2** as a pale-yellow foam (972 mg, 66%). $R_f = 0.3$ (EtOAc/MeOH, 3:2); $[\alpha]_D^{+76}$ (c 1.0, H_2O); $^1\text{H NMR}$ (500 MHz, D_2O) δ 4.95 (d, $J = 3.8$ Hz, 1H, H-1 $_{\text{GalNAc}}$), 4.62 (d, $J = 7.9$ Hz, 1H, H-1 $_{\text{Gal}}$), 4.41–4.31 (m, 4H, H-2 $_{\text{GalNAc}}$, H-3 $_{\text{Gal}}$, H-4 $_{\text{Gal}}$, H-4 $_{\text{GalNAc}}$), 4.30–4.16 (m, 5H, H-5, 2 × H-6 $_{(\text{A+B})}$), 4.11 (dd, $J = 11.1$, 3.1 Hz, 1H, H-3 $_{\text{GalNAc}}$), 3.97 (t, $J = 6.2$ Hz, 1H, H-5), 3.91 (m, 1H, CH₂(A)Linker), 3.85–3.62 (m, 10H, H-2 $_{\text{Gal}}$, CH₂(B)Linker, 4 × CH₂(Linker)), 3.56–3.50 (m, 2H, CH₂(Linker)), 2.05 (s, 3H, CH₃(NHAc)); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 174.5 (C=O(NHAc)), 104.2 (C-1 $_{\text{Gal}}$), 97.4 (C-1 $_{\text{GalNAc}}$), 79.9 (C-3 $_{\text{Gal}}$), 77.5 (C-3 $_{\text{GalNAc}}$), 72.1 (C-5), 69.6 (CH₂(Linker)), 69.47 (CH₂(Linker)), 69.38 (CH₂(Linker)), 69.2 (CH₂(Linker)), 68.8 (C-5), 68.55 (C-2 $_{\text{Gal}}$), 68.52 (C-4 $_{\text{GalNAc}}$), 68.3 (C-6), 66.9 (C-6), 66.7 (CH₂(Linker)), 66.5 (C-4 $_{\text{Gal}}$), 50.1 (CH₂(Linker)), 48.3 (C-2 $_{\text{GalNAc}}$), 22.0 (CH₃(NHAc)). HRESIMS m/z : $[\text{M} - \text{Na} + 2\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{37}\text{N}_4\text{O}_{16}\text{S}$, 621.1925; found, 621.1920.

Supporting Information

Supporting Information File 1

NMR spectra of compounds **1–5**, **7** and **8**.

[<https://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-20-17-S1.pdf>]

Acknowledgements

We thank Dr Yannick Ortin and Dr Jimmy Muldoon for NMR and MS support.

Funding

The authors kindly acknowledge Science Foundation Ireland awards 13/IA/1959 and 20/FFP-P/884 (to S.O) and a China Scholarship Council Ph.D. scholarship (to H. M).

ORCID® iDs

Mark Reihill - <https://orcid.org/0000-0003-3896-9346>

Hanyue Ma - <https://orcid.org/0009-0009-7307-0388>

Stefan Oscarson - <https://orcid.org/0000-0002-8273-4918>

References

- Xiao, Q.; Ludwig, A.-K.; Romanò, C.; Buzzacchera, I.; Sherman, S. E.; Vetro, M.; Vértesy, S.; Kaltner, H.; Reed, E. H.; Möller, M.; Wilson, C. J.; Hammer, D. A.; Oscarson, S.; Klein, M. L.; Gabius, H.-J.; Percec, V. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E2509–E2518. doi:10.1073/pnas.1720055115
- Ludwig, A.-K.; Michalak, M.; Xiao, Q.; Gilles, U.; Medrano, F. J.; Ma, H.; FitzGerald, F. G.; Hasley, W. D.; Melendez-Davila, A.; Liu, M.; Rahimi, K.; Kostina, N. Y.; Rodriguez-Emmenegger, C.; Möller, M.; Lindner, I.; Kaltner, H.; Cudic, M.; Reusch, D.; Kopitz, J.; Romero, A.; Oscarson, S.; Klein, M. L.; Gabius, H.-J.; Percec, V. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 2837–2842. doi:10.1073/pnas.1813515116
- Ju, T.; Otto, V. I.; Cummings, R. D. *Angew. Chem., Int. Ed.* **2011**, *50*, 1770–1791. doi:10.1002/anie.201002313
- Yu, L.-G. *Glycoconjugate J.* **2007**, *24*, 411–420. doi:10.1007/s10719-007-9034-3
- Zhao, Q.; Guo, X.; Nash, G. B.; Stone, P. C.; Hilken, J.; Rhodes, J. M.; Yu, L.-G. *Cancer Res.* **2009**, *69*, 6799–6806. doi:10.1158/0008-5472.can-09-1096
- Rapoport, E. M.; Pazynina, G. V.; Sablina, M. A.; Crocker, P. R.; Bovin, N. V. *Biochemistry (Moscow)* **2006**, *71*, 496–504. doi:10.1134/s0006297906050051
- Ideo, H.; Seko, A.; Ohkura, T.; Matta, K. L.; Yamashita, K. *Glycobiology* **2002**, *12*, 199–208. doi:10.1093/glycob/12.3.199
- Vokhmyanina, O. A.; Rapoport, E. M.; André, S.; Severov, V. V.; Ryzhov, I.; Pazynina, G. V.; Korchagina, E.; Gabius, H.-J.; Bovin, N. V. *Glycobiology* **2012**, *22*, 1207–1217. doi:10.1093/glycob/cws079
- Hoffmann, M.; Hayes, M. R.; Pietruszka, J.; Elling, L. *Glycoconjugate J.* **2020**, *37*, 457–470. doi:10.1007/s10719-020-09926-y
- Ou, C.; Li, C.; Feng, C.; Tong, X.; Vasta, G. R.; Wang, L.-X. *Bioorg. Med. Chem.* **2022**, *72*, 116974. doi:10.1016/j.bmc.2022.116974
- Sanki, A. K.; Mahal, L. K. *Synlett* **2006**, 455–459. doi:10.1055/s-2006-926264
- Ma, H. Synthesis of human Mucin-type glycans for interaction studies with bacterial and human lectins. Ph.D. Thesis, University College Dublin, Dublin, Ireland, 2019.
- Imamura, A.; Ando, H.; Korogi, S.; Tanabe, G.; Muraoka, O.; Ishida, H.; Kiso, M. *Tetrahedron Lett.* **2003**, *44*, 6725–6728. doi:10.1016/s0040-4039(03)01647-2
- Imamura, A.; Matsuzawa, N.; Sakai, S.; Udagawa, T.; Nakashima, S.; Ando, H.; Ishida, H.; Kiso, M. *J. Org. Chem.* **2016**, *81*, 9086–9104. doi:10.1021/acs.joc.6b01685
- Imamura, A.; Kimura, A.; Ando, H.; Ishida, H.; Kiso, M. *Chem. – Eur. J.* **2006**, *12*, 8862–8870. doi:10.1002/chem.200600832
- Komori, T.; Ando, T.; Imamura, A.; Li, Y.-T.; Ishida, H.; Kiso, M. *Glycoconjugate J.* **2008**, *25*, 647–661. doi:10.1007/s10719-008-9117-9
- Reihill, M. Synthesis of Glycans and Glycoconjugates to Assess Binding Properties of Bacterial and Human Lectins. Ph.D. Thesis, University College Dublin, Dublin, Ireland, 2020.
- Dakanali, M.; Do, T. H.; Horn, A.; Chongchivivat, A.; Jarusreni, T.; Lichlyter, D.; Guizzunti, G.; Haidekker, M. A.; Theodorakis, E. A. *Bioorg. Med. Chem.* **2012**, *20*, 4443–4450. doi:10.1016/j.bmc.2012.05.026
- Garegg, P. J.; Konradsson, P.; Kvarnström, I.; Norberg, T.; Svensson, S. C. T.; Wigiljus, B. *Acta Chem. Scand., Ser. B* **1985**, *39*, 569–577. doi:10.3891/acta.chem.scand.39b-0569
- Ohlsson, J.; Magnusson, G. *Carbohydr. Res.* **2000**, *329*, 49–55. doi:10.1016/s0008-6215(00)00154-3

21. Modarresi-Alam, A. R.; Najafi, P.; Rostamizadeh, M.; Keykha, H.; Bijanzadeh, H.-R.; Kleinpeter, E. *J. Org. Chem.* **2007**, *72*, 2208–2211. doi:10.1021/jo061301f
22. Rablen, P. R.; Miller, D. A.; Bullock, V. R.; Hutchinson, P. H.; Gorman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 218–226. doi:10.1021/ja982304f
23. Gamov, G. A.; Aleksandriiskii, V. V.; Sharnin, V. A. *J. Mol. Liq.* **2017**, *231*, 238–241. doi:10.1016/j.molliq.2017.01.078
24. Lin, W.; Zhang, X.; He, Z.; Jin, Y.; Gong, L.; Mi, A. *Synth. Commun.* **2002**, *32*, 3279–3284. doi:10.1081/scc-120014032
25. Tanimoto, H.; Kakiuchi, K. *Nat. Prod. Commun.* **2013**, *8*, 1021–1034.
26. Jacquemard, U.; Bénétteau, V.; Lefoix, M.; Routier, S.; Mérour, J.-Y.; Coudert, G. *Tetrahedron* **2004**, *60*, 10039–10047. doi:10.1016/j.tet.2004.07.071
27. Guilbert, B.; Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1994**, *35*, 6563–6566. doi:10.1016/s0040-4039(00)78273-6
28. Lubineau, A.; Alais, J.; Lemoine, R. *J. Carbohydr. Chem.* **2000**, *19*, 151–169. doi:10.1080/07328300008544072
29. Roy, R.; Cao, Y.; Kaltner, H.; Kottari, N.; Shiao, T. C.; Belkhadem, K.; André, S.; Manning, J. C.; Murphy, P. V.; Gabius, H.-J. *Histochem. Cell Biol.* **2017**, *147*, 285–301. doi:10.1007/s00418-016-1525-5
30. Xing, G.-W.; Wu, D.; Poles, M. A.; Horowitz, A.; Tsuji, M.; Ho, D. D.; Wong, C.-H. *Bioorg. Med. Chem.* **2005**, *13*, 2907–2916. doi:10.1016/j.bmc.2005.02.018
31. Raghuraman, A.; Riaz, M.; Hindle, M.; Desai, U. R. *Tetrahedron Lett.* **2007**, *48*, 6754–6758. doi:10.1016/j.tetlet.2007.07.100
32. Malleron, A.; Hersant, Y.; Narvor, C. L. *Carbohydr. Res.* **2006**, *341*, 29–34. doi:10.1016/j.carres.2005.10.004

License and Terms

This is an open access article licensed under the terms of the Beilstein-Institut Open Access License Agreement (<https://www.beilstein-journals.org/bjoc/terms>), which is identical to the Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0>). The reuse of material under this license requires that the author(s), source and license are credited. Third-party material in this article could be subject to other licenses (typically indicated in the credit line), and in this case, users are required to obtain permission from the license holder to reuse the material.

The definitive version of this article is the electronic one which can be found at:
<https://doi.org/10.3762/bjoc.20.17>