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Orthogonally Protected Monosaccharide Building Blocks for Solid Phase Production of Diversity Oriented Libraries

Premraj Rajaratnam,[A] *Praveer Gupta,*[A] *Peter Katavic,*[A] *Krystle Kuipers,*[A] *Ngoc Huyh,*[A] *Sarah Ryan,*[A] *Tania Falzun,*[A] *Gerrald B. Tometzki,*[A] *Laurent Bornaghi,*[A] *Giang Le Thanh,*[A] *Giovanni Abbenante,*[A] *Ligong Liu,*[A] *Wim Meutermans,*[A] *Norbert Wimmer,*[A] *and Michael L. West*[A,B]

[A]Alchemia Limited, 3 Hi Tech Court, Eight Mile Plains, Brisbane, Qld 4113, Australia.
[B]Corresponding author. Email: mwest@alchemia.com.au

The large scale synthesis of three orthogonally protected monosaccharide scaffolds suitable for use in the solid phase preparation of large diversity libraries is presented. Scaffolds based on 2-amino-2-deoxy-D-glucopyranose, 2-amino-2-deoxy-D-allopyranose, and 2,4-diamino-2,4-dideoxy-D-galactopyranose were prepared in good yield and with minimal chromatographic purification from commercially available methyl 2-azido-2-deoxy-1-thio-β-D-glucopyranose and methyl 2-amino-2-deoxy-1-thio-β-D-glucopyranose.

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Introduction

With five functionalized chiral centres in a cyclic arrangement, monosaccharides represent versatile and compact scaffolds that provide the medicinal chemist plenty of scope to custom design molecules to a pharmacophore model.[1,2] Highly diverse molecules can be envisaged and new bioactives identified by appending suitable substituents at various positions on the monosaccharide scaffold.[3–6] For close to 20 years, this has been explored by many laboratories and that work is well reviewed.[7,8]

We have been interested in employing the sugar scaffold to produce libraries of systematic diversity.[9] In order to produce such libraries, the need for a series of orthogonally protected monosaccharide scaffolds was identified. It was apparent that these building blocks must be readily synthesized at the 100 g scale with potential to be prepared at the kilogram scale. The building blocks needed to be protected with groups that are stable to the wide variety of chemical conditions required to introduce and elaborate the substituent groups. Here we report the design and large scale synthesis of three such building blocks based on D-glucopyranose, D-allopyranose, and D-galactopyranose, that we have successfully used as the starting points in the production of our diversity oriented libraries.[10]

Results and Discussion

Building Block Design and Synthesis

It was anticipated that a series of large diversity libraries would be prepared from a small number of orthogonally protected monosaccharide scaffolds. Efficient production of these libraries required a solid phase approach necessitating one resin attachment position on the carbohydrate building block. The choice of the attachment position was governed by several other requisite characteristics of the target molecules within the library. The anomeric position in the target libraries always bears a substituent in order to prevent hemiacetal anomers and potential chain opening configurations of the final products, thus precluding the use of the anomeric position as a suitable attachment point to resin. An amino group at the *C*-2 position was used as this simplified the selection of the orthogonal protecting groups for the scaffold. The scaffolds were designed with the intention that the *C*-2 amine in the final products of the library would be substituted with groups such as acyl or carbamoyl moieties that restricted the rotation of the appended groups. Consequently, the *C*-2 position was unsuitable for attachment to the solid support. The *C*-3 and *C*-6 positions can be readily protected in a selective manner, leaving the least reactive position *C*-4 on 2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-allopyranose, which was used as the resin attachment point for orthogonally protected building blocks based on these monosaccharide scaffolds. The protecting groups, outlined in Table 1, were selected for the 2-amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-allopyranose building blocks, to allow regio-specific introduction of a wide variety of substituents. The thiomethyl glycoside[11] at *C*-1 allows direct introduction of a wide variety of substituents through glycosidation of a range of alcohols in solution phase before resin-loading.

A third monosaccharide scaffold was based on a 2,4-diamino-2,4-dideoxy-D-galactopyranose core. This scaffold contained a masked axial *C*-4-amino group and a protected equatorial *C*-2 amino group resulting in a building block containing two nitrogen atoms. The protecting groups selected for this building block were a thiomethyl glycoside for *C*-1, a 5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione (DTPM) for *C*-2, a benzoate ester for *C*-3, and an azide for *C*-4, with a masked resin-linking position at *C*-6 (Table 2). The resin linking position in the scaffold is masked with a *tert*-butyldiphenylsilyl ether to

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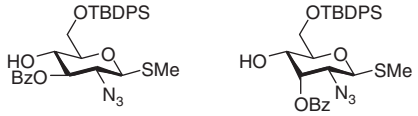
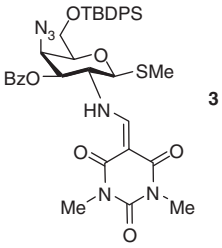
Table 1. 2-Amino-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-allopyranose building block design



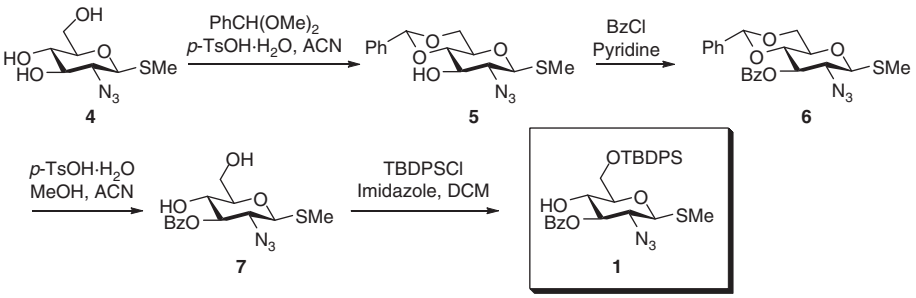
1 **2**

Order of reaction	Protecting or masking group	Chemistry for removal or conversion
1.	SMe – thiomethyl	Dimethyl(methylthio)-sulfonium trifluoromethanesulfonate in anhydrous diethyl ether
2.	Bz – benzoate ester	Sodium methoxide in anhydrous methanol
3.	TBDPS – *tert*-butyldiphenylsilyl ether	Pyridine/HF
4.	N_3 – azide	Dithiothreitol

Table 2. 2,4-Diamino-2,4-dideoxy-D-galactopyranose building block design



Order of reaction	Protecting or masking group	Chemistry for removal or conversion
1.	SMe – thiomethyl	Dimethyl(methylthio)-sulfonium trifluoromethanesulfonate in anhydrous diethyl ether
2.	TBDPS – *tert*-butyldiphenylsilyl ether	Pyridine/HF followed by immobilization on solid support
3.	Bz – benzoate ester	Sodium methoxide in anhydrous methanol
4.	DTPM – 5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione	Hydrazine hydrate in dichloromethane
5.	N_3 – azide	Dithiothreitol

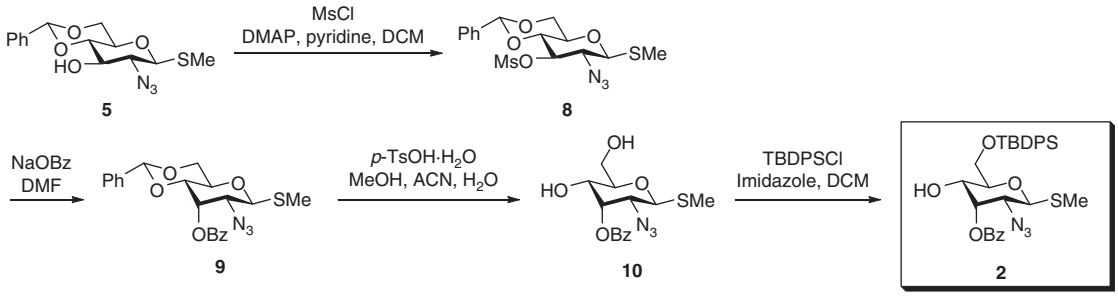


Scheme 1. Synthesis of building block 1.

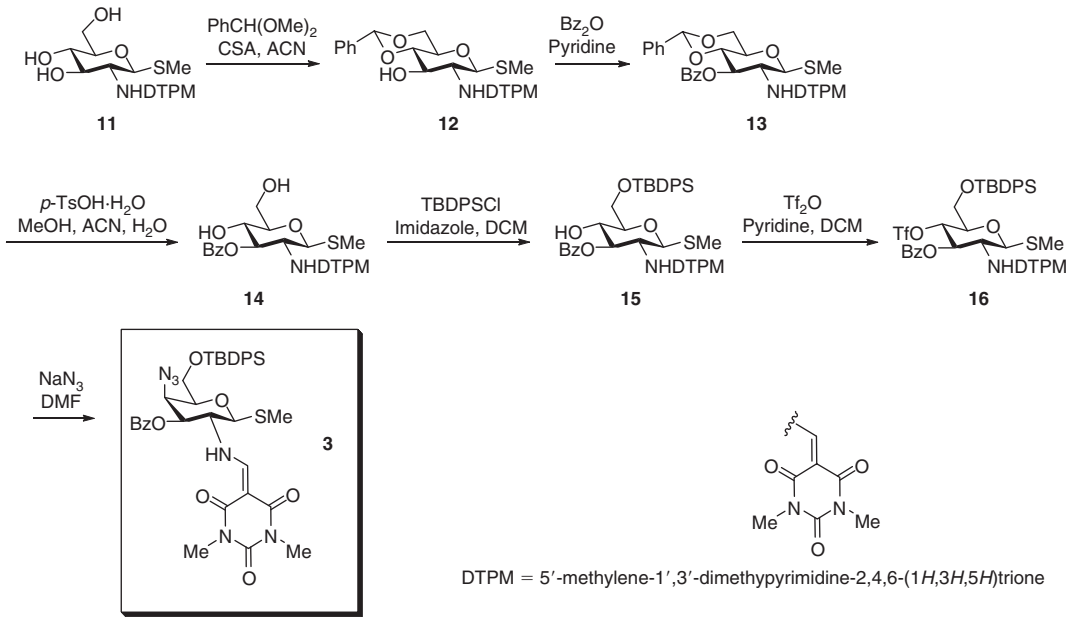
allow clean glycoside formation at the anomeric position. Once the anomeric glycosides are formed, the *tert*-butyldiphenylsilyl ether is removed with HF/pyridine to reveal the free hydroxyl group at *C*-6 ready for linking to the solid support.

The syntheses of the 2-amino-2-deoxy-D-glucopyranose building block **1**, and the 2-amino-2-deoxy-D-allopyranose building block **2** are shown in Schemes 1 and 2. These building blocks were prepared in solution and the procedures developed were amenable to large-scale synthesis.

The synthesis of building block **1** (Scheme 1) started with the benzylidene protection at *C*-4,6 of the commercially available methyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside **4**. Subsequently the hydroxyl group at *C*-3 of **5** was benzoylated using benzoyl chloride in pyridine to yield the fully protected D-glucopyranose **6**. The benzylidene deprotection of **6** was accomplished with 4-toluenesulfonic acid hydrate in methanol and acetonitrile to yield diol **7**. In the first purification of the synthesis, diol **7** was substantially purified by precipitation from a dichloromethane solution by addition of petroleum ether. Selective protection of the primary alcohol with *tert*-butyldiphenylchlorosilane in anhydrous dichloromethane furnished building block **1**, which was purified



Scheme 2. Synthesis of building block 2.



Scheme 3. Synthesis of building block 3.

by column chromatography on silica gel. Despite the need for a chromatographic purification, building block **1** was prepared on 100–200 g scale in 66% overall yield.

Building block **2** (Scheme 2) was prepared from benzylidene **5**. Mesylation of the *C*-3 hydroxyl group on **5** proceeded cleanly and the resultant mesylate **8** was used directly in the next reaction step. Mesylate displacement with inversion of stereochemistry at *C*-3 was achieved by reaction with sodium benzoate in *N*,*N*-dimethyl formamide at 140°C for 24 h to afford the benzoate-protected 2-azido-4,6-*O*-benzylidene-2-deoxy-D-allopyranose derivatived **9** as a black oil, which was effectively decolourized by the addition of activated charcoal. Removal of the benzylidene group from **9** by 4-toluenesulfonic acid hydrate in methanolic acetonitrile was followed by selective protection of the primary alcohol in **10** with *tert*-butyldiphenylchlorosilane giving building block **2**, which was purified by silica gel column chromatography. Building block **2** was prepared from intermediate **5** in 29% overall yield on 100 g scale.

Synthesis of the 2,4-diamino-2,4-dideoxy-D-galactoyranose based building block is described in Scheme 3. Methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-2-deoxy-1-thio-β-D-glucopyranoside **11** was prepared from commercially available methyl 2-amino-2-deoxy-1-thio-β-D-glucopyranoside by reaction with 5-((dimethylamino)methylene)-1,3-dimethylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione in methanol. The product **11** precipitated from the methanolic solution and was collected by vacuum filtration. Benzylidene formation was accomplished using the standard procedures described above, and the free hydroxyl group at *C*-3 of product **12** was benzoylated with benzoic anhydride in pyridine. Deprotection of benzylidene **13** and selective silylation of diol **14** gave intermediate **15**. The hydroxyl group at *C*-4 was then converted to a triflate **16** using triflic anhydride, and nucleophilic displacement of the triflate using sodium azide furnished the D-galactopyranose building block **3**, which was purified by silica gel chromatography. The D-galactopyranose derived building block **3** was prepared in 41% overall yield on a 100 g scale.

The described methods allow the preparation of three monosaccharide scaffolds on a large laboratory scale in good yield. Chromatographic purifications are restricted to simple separations, which are amenable to further scale-up. The scaffolds prepared in this manner have been employed in the preparation of large diversity libraries[12] and have been shown to be amenable to a wide array of solid phase chemistries. Additionally, the orthogonally protected scaffolds have been used in the solution phase preparation of gram quantities of biologically active compounds identified through screening of the diversity libraries.[12] Thus the building blocks described allow the medicinal chemist to rapidly prepare compounds from diversity library based discovery to pre-clinical assessment.

Table 3. Reactions monitored by reverse phase HPLC
Column: Zorbax SB-C18 5 μm 4.6 × 50 mm; flow rate: 1 mL min^{-1}; solvent A: acetonitrile/10 mM ammonium formate in water (1:9); solvent B: acetonitrile/10 mM ammonium formate in water (9:1), gradient cycle

Time [min]	%B
0	0
1	0
12	70
15	70
16	0
20	0

Materials and Methods

NMR spectra of intermediate compounds were recorded on a Varian AM300 spectrometer (^{1}H at 300 MHz and ^{13}C NMR spectra at 75 MHz). ^{1}D and ^{2}D NMR spectra of final products were recorded on a Varian spectrometer (^{1}H at 500 MHz, ^{13}C at 125 MHz, gCOSY at 500/125 MHz). Electrospray mass spectra were recorded on a micromass LCZ single quadrupole mass spectrometer. High resolution mass spectra were recorded on a Brucker microTOF-Q 70.

All chromatographic separations were performed by the 'dry column vacuum chromatography' technique[13] with silica gel 60 for flash chromatography 0.04–0.06 mm (230 to 400 mesh) purchased from Scharlau Chemie SA. An 11 cm diameter sinter funnel was employed.

Anhydrous solvents were purchased from Aldrich chemical company as Sure/Seal™ solvents. All other solvents were LR grade reagents supplied by Ajax.

Reactions were monitored by reverse phase HPLC under the following conditions (Table 3). Column: Zorbax SB-C18 5 μm 4.6 × 50 mm, flow rate: 1 mL min^{-1} solvent A: acetonitrile/10 mM ammonium formate (1:9) in water, solvent B: acetonitrile/10 mM ammonium formate in water (9:1), gradient cycle: $t = 0$ min 0% B; $t = 2$ min 0% B; $t = 12$ min 80% B; $t = 13$ min 100% B; $t = 15$ min 100% B; $t = 17$ min 0% B; $t = 20$ min 0% B.

Experimental

*Methyl 2-Azido-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**5**)*

Benzaldehyde dimethyl acetal (96 mL, 0.640 mol) was added to a solution of methyl 2-azido-2-deoxy-1-thio-β-D-glucopyranoside (**4**) (100 g, 0.425 mol) in dry acetonitrile (1.0 L) followed by *p*-toluenesulfonic acid monohydrate (670 mg, 3.5 mmol). The resulting solution (pH ∼4) was stirred at 60°C under nitrogen for 1.5 h then quenched by the dropwise addition of triethylamine until a pH of ∼8–9 was reached (∼10 mL). The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (DCM) (1.2 L). The organic phase was washed with brine (1.0 L) followed by water (2 × 1.0 L), dried over anhydrous magnesium sulphate, and filtered. The solvent was removed under reduced pressure and the crude product was evaporated from dry acetonitrile (2 × 600 mL) to give crude acetal (**5**) as a yellow solid (143.5 g). HPLC Rt 4.90 min. δ_H (300 MHz $CDCl_3$): 7.48 (4H, m, Ar-H), 5.50 (1H, s), 4.43 (1H, d, J 10.1, H_1), 4.34 (1H, dd, J 10.4, 6.8, H_4), 3.95 (2H, m, $H_{6,6'}$), 3.42 (1H, m), 3.38 (1H, m), 3.29 (1H, m), 2.37 (3H, s). *m/z* (ES MS) 324 $[M + H]^+$.

*Methyl 2-Azido-3-O-benzoyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**6**)*

Crude acetal (**5**) (143.5 g) was dissolved in anhydrous pyridine (1.0 L) and the resultant solution cooled in an ice water bath with stirring under nitrogen. Benzoyl chloride (60 mL, 0.517 mol) was added in a dropwise manner over 5 min to the solution of **5**. The resulting suspension was allowed to warm to room temperature and stirring continued for 2 h. After this time, water (20 mL) was added to the suspension and stirred for a further 1 h. Pyridine was removed under reduced pressure to give a yellow solid. The yellow solid was dissolved in DCM (1.2 L) and the organic phase was washed with 10% citric acid (3 × 800 mL), saturated sodium bicarbonate (2 × 800 mL), and brine (800 mL). The organic phase was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure to give the ester (**6**) as a white solid (172.5 g crude). HPLC Rt 7.18 min. δ_H (300 MHz $CDCl_3$): 8.18 & 7.70–7.37 (10H, m, Ar-H), 5.58 (1H, t, J 9.5), 5.52 (1H, s), 4.68 (1H, d, J 10.1, H_1), 4.43 (1H, dd, J 10.5, 6.8, H_4), 3.84 (1H, t, J 10., H_6), 3.74 (1H, t, J 10.0, $H_{6'}$), 3.63 (1H, m), 3.60 (1H, t, J 9.8), 2.41 (3H, s). *m/z* (ES MS) 428 $[M + H]^+$.

*Methyl 2-Azido-3-O-benzoyl-2-deoxy-1-thio-β-D-glucopyranoside (**7**)*

4-Toluenesulfonic acid monohydrate (700 mg, 3.68 mmol pH 4) was added to a solution of the benzoate (**6**) (172.5 g, crude) in methanol (1.2 L) and acetonitrile (400 mL). The resulting solution was heated at 60°C for 1 h then allowed to cool to room temperature. The reaction was neutralized with triethylamine and the solvent was removed under reduced pressure to give the diol (**7**) as a viscous yellow oil. The crude oil was dissolved in DCM (500 mL), washed with water (3 × 500 mL), and the organic layer evaporated under reduced pressure. The resultant oil was re-dissolved in DCM (80 mL) and (**7**) was precipitated by the portion wise addition of petroleum ether (500 mL). The precipitate was collected by vacuum filtration, dried under high vacuum to yield pure **7** (124.3 g, 86.2% over three steps). HPLC Rt 4.26 min. δ_H (300 MHz $CDCl_3$): 8.18 & 7.70–7.15 (5H, Ar-H), 5.18 (1H, t, J 9.5), 4.57 (1H, d, J 10.1, H_1), 3.92 (1H, m), 3.82 (1H, m), 3.74 (1H, t, J 9.5), 3.47 (2H, m), 2.36 (3H, s). *m/z* (ES MS) 340 $[M + H]^+$.

*Methyl 2-Azido-3-O-benzoyl-6-O-tert-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (**1**)*

The diol (**7**) (124 g, 0.366 mol) was dissolved in dry DCM (1.5 L) and the solution was stirred at room temperature under nitrogen. Imidazole (41 g, 0.602 mol) was added, followed by *tert*-butylchlorodiphenylsilane (TBDPS-Cl, 116 mL, 0.446 mol). The resulting mixture was heated at reflux for 2 h, and then allowed to cool to room temperature before further cooling in an ice water bath to effect precipitation of the imidazole salts. The salts were removed by vacuum filtration and the filtrate was washed with 10% citric acid (2 × 1.5 L) and water (1.5 L), and then dried over anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure to give a viscous oil (283.3 g), which was purified by column chromatography (silica gel 300 g, eluent: 5–20% EtOAc/petrol) to yield the desired product (**1**) 122.2 g (57.7%) from the initial chromatography and a further 40 g (19%) from re-chromatography of mixed fractions. The overall yield of (**1**) was 162.2 g (76.7%)

as a white solid on removal of solvents. HPLC Rt 7.19 min. impurity corresponding to methyl 2-azido-4-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside HPLC Rt 7.14 min. 0.2 area%. δ_H (500 MHz, $CDCl_3$): 8.12 (2H, d, Ar-H), 7.71 (4H, t, Ar-H), 7.62 (1H, t, Ar-H), 7.37–7.51 (8H, m, Ar-H), 5.19 (1H, t, $J_{H3,H4} = 9.4$, $J_{H2,H3}$ 9.4, H-3), 4.37 (1H, d, $J_{H1,H2}$ 10.1, H-1), 3.97 (2H, m, H-6a, H-6b), 3.92 (1H, t, H-4), 3.62 (1H, t, $J_{H2,H3}$ 9.98, H-2), 3.52 (1H, m, H-5), 3.10 (1H, bs, OH-4), 2.26 (3H, s, SMe), 1.07 (9H, s, tBu). δ_H (100.6 MHz, $CDCl_3$): 166.9 (C=O), 135.65, 135.57, 133.63, 132.93, 132.84, 130.02, 129.86, 129.15, 128.53, 127.78, 127.75 (all Ar-C), 84.27 (C-1), 79.47 (C-5), 78.21 (C-3), 70.60 (C-4), 64.12 (C-6), 63.48 (C-2), 26.81 (tBuC), 19.23 (tBuC), 12.35 (SMe). *m/z* (ES MS) 578 $[M + H]^+$. *m/z* (HRMS, ESI +ve) *m/z* $[M + Na]^+$ Anal. Calc. for $C_{30}H_{35}N_3NaO_5SSi$, 600.1964. Found: 600.1959.

*Methyl 2-Azido-4,6-O-benzylidene-2-deoxy-3-methanesulfonyl-1-thio-β-D-glucopyranoside (**8**)*

4-Dimethylaminopyridine (DMAP) (40.4 g, 331 mmol) and anhydrous pyridine (50 mL) were added to a pre-cooled (0°C) solution of the alcohol (**5**) (107 g of crude product prepared in the cognate preparation, ~331 mmol) in DCM (2 L). Methanesulfonyl chloride (31 mL, 402 mmol) was then added in a dropwise manner over 15 min. The resulting slurry was stirred at room temperature for 4 h and then water (1.1 L) was added and the suspension was stirred for a further 10 min. The organic phase was separated and washed with a cold HCl solution (0.54 M, 1.1 L) followed by water (2 × 1.1 L) before being dried over anhydrous magnesium sulfate. Removal of the magnesium sulfate by filtration and evaporation of the solvents under reduced pressure gave (**8**) as a white solid (118.5 g), which was used without further purification; HPLC Rt 5.34 min. *m/z* (ESMS) 402.06 $[M + H]^+$.

*Methyl 2-Azido-3-O-benzoyl-4,6-O-benzylidene-2-deoxy-1-thio-β-D-allopyranoside (**9**)*

The mesylate **(8)** (118.5 g, 295 mmol) and sodium benzoate (63.80 g, 443 mmol) in DMF (2 L) was stirred under nitrogen at 140°C for 24 h, then cooled to room temperature, and diluted with water (2 L). The product was extracted with chloroform (3 × 2 L) and the organic phase washed with water (2 × 4 L), brine (3 L), dried over anhydrous magnesium sulfate, filtered, and evaporated to give a thick black residue. This black residue was re-dissolved in EtOAc (2 L), heated to 73°C, and decoloured by the addition of activated charcoal (100 g) at this temperature. After filtration, the light yellow solution was evaporated under reduced pressure to give a yellow solid (**9**) (105 g), which was used directly for the next step without further purification. HPLC Rt 6.09 min. *m/z* (ESMS) 428 $[M + H]^+$, 491 $[M + Na^+CH_3CN]^+$.

*Methyl 2-Azido-3-O-benzoyl-2-deoxy-1-thio-β-D-allopyranoside (**10**)*

4-Toluenesulfonic acid monohydrate (4.67 g) was added to a solution of benzoate (**9**) (105 g) in acetonitrile (1100 mL), methanol (370 mL) and water (75 mL). The clear solution was stirred under nitrogen at 50°C for 11 h. The reaction mixture was cooled down in an ice bath and neutralized with triethylamine. The solvents were evaporated under reduced pressure and the residue was purified by column chromatography (silica gel 400 mL; eluent: EtOAc/Pet 1:2 to 1:1) to yield (**10**) as a pale yellow compound (41 g, 45% yield over three steps). HPLC Rt 4.30 min. *m/z* (ESMS) 340 $[M + H]^+$.

*Methyl 2-Azido-3-O-benzoyl-6-O-tert-butyldiphenylsilyl-2-deoxy-1-thio-β-D-allopyranoside (**2**)*

Diol (**10**) (40 g, 118 mmol) was dissolved in anhydrous DCM (2.5 L). Imidazole (9.62 g, 141 mmol) was added to the solution of diol (**10**), followed by *tert*-butyldiphenylchlorosilane (28.2 mL, 108 mmol). The mixture was refluxed at 40°C under nitrogen for 1 h. The reaction mixture was cooled to 0°C and stirred for a further 30 min during which time a fine white precipitate of imidazole hydrochloride settled out. The white imidazole salt was removed by filtration and rinsed with cold DCM (300 mL). The filtrate was washed with water (1 L) and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to give a dark yellow syrup, which was purified by column chromatography (silica gel 400 g; eluent petroleum ether 2 L; Pet/EtOAc 10:1 3 L; Pet/EtOAc 8:1 3 L; Pet/EtOAc 4:1 3 L) to give a white foam (45 g, 66%). The chromatographed product was further recrystallized with 2.5% EtOAc in hexane to yield (**2**) as pure white crystals. HPLC Rt 7.15 min. δ_H (500 MHz, $CDCl_3$): 8.07 (2H, d, *J* 7.7, Ar-H), 7.35–7.74 (13H, m, Ar-H), 5.19 (1H, t, $J_{H3,H4}$ 9.4, $J_{H2,H3}$ 9.4, H-3), 5.93 (1H, t, H-3), 4.83 (1H, d, $J_{H1,H2}$ 10.1, H-1), 4.03 (1H, dd, $J_{H3,H4}$ 2.5, $J_{H4,H5}$ 9.6, H-4), 3.95 (2H, m, H-6a, H-6b), 3.83 (1H, ddd, H-5), 3.57 (1H, dd, $J_{H2,H3}$ 10., H-2), 2.72 (1H, bs, 4-OH), 2.26 (3H, s, SMe), 1.06 (9H, s, tBu). δ_C (100.6 MHz, $CDCl_3$): 166.31 (C=O), 135.64, 135.56, 133.53, 132.94, 132.84, 129.93, 129.87, 129.38, 128.5, 127.774, 127.75 (all Ar-C), 81.14 (C-1), 76.21 (C-5), 71.98 (C-3), 68.48 (C-4), 64.31 (C-6), 60.43(C-2), 26.80 (tBuC), 19.23 (tBuC), 11.73 (SMe). *m/z* (ESMS) 578 $[M + H]^+$. *m/z* (HRMS, ESI +ve) $[M + Na]^+$ Anal. Calc. for $C_{30}H_{35}N_3NaO_5SSi$, 600.1964. Found: 600.1953.

5-((Dimethylamino)methylene)-1,3-dimethylpyrimidine-2,4,6-(1H,3H,5H)trione

N,*N*′-dimethylformamide dimethyl acetal (560 mL, 4.08 mol) was stirred at 0°C in chloroform (2 L). 1,3-Dimethylbarbituric acid (600 g, 3.76 mol) was dissolved in chloroform (3.3 L) and added to the reaction mixture dropwise over 1.5 h. When the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred until the reaction was judged to be complete by tlc (EtOAc/AcOH, 20:1), after approximately 2 h. The reaction mass was washed with cold water (3 × 4 L) followed by brine (2 L) and the organic layer dried over magnesium sulfate and filtered. The solvents were removed under vacuum to yield 5-[(dimethylamino)methylene]-1,3-dimethylpyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione (470 g, 58%).

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1H,3H,5H)trione]-2-deoxy-1-thio-β-D-glucopyranoside (**11**)*

Methyl 2-amino-2-deoxy-1-thio-β-D-glucopyranoside (355 g, 1.79 mol) was suspended in methanol (3 L) and the pH adjusted to ~9 by the addition of triethylamine. 5-[(Dimethylamino) methylene-1,3-dimethylpyrimidine]-2,4,6-(1*H*,3*H*,5*H*)trione (360 g, 1.70 mol) was dissolved in methanol (5 L) and added to the stirred solution of methyl 2-deoxy-2-amino-1-thio-β-D-glucopyranoside. The reaction was warmed to 50°C for 2 h after which time the solvents were removed under vacuum to furnish crude (**11**) suitable for use in the next stage.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-4,6-*O*-benzylidene-2-deoxy-1-thio-β-*D*-glucopyranoside (**12**)*

A mixture containing methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-2-deoxy-1-thio-β-D-glucopyranoside (**11**) (265 g, 0.706 mol), dimethyl benzaldehyde acetal (116 mL, 776 mmol), and (1*S*)-(+)-Camphor-10-sulfonic acid (53.44 g, 212 mmol) in acetonitrile (2.6 L) was stirred on the rotary evaporator (65°C bath temperature, 650 mbar) for 4 h. The mixture was then basified to pH 8 with Et_3N. Crude compound (**12**) (278 g) was taken directly to the next step without further purification. HPLC Rt 4.61 min. *m/z* (ESMS) 464 $[M + H]^+$, 927 $[2M + H]^+$.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-thio-β-*D*-glucopyranoside (**13**)*

A mixture of crude methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**12**) from the previous step (184 g) and benzoic anhydride (135 g, 0.597 mol) in anhydrous pyridine (720 mL) was stirred overnight at room temperature. The solvent was evaporated under reduced pressure, co-evaporated from toluene (2 × 400 mL), and the residue taken up in DCM (2 L). The organic phase was washed with 10% citric acid solution (2 × 1.4 L) and brine (1.6 L), dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a golden foam **13**, which was used directly in the following step. HPLC Rt 5.59 min. *m/z* (ESMS) 568 $[M + H]^+$, 1135 $[2M + H]^+$.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-2-deoxy-1-thio-β-*D*-glucopyranoside (**14**)*

The crude methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-thio-β-D-glucopyranoside (**13**) was dissolved in a mixture of acetonitrile (800 mL), methanol (400 mL), and water (40 mL). *p*-Toluenesulfonic acid (2.27 g, 12 mmol) was added and the mixture was heated for 3 h at 70°C. The solution was neutralized with triethylamine to pH 7 and the solvents removed under vacuum. The crude product was dissolved in ethyl acetate (1 L) and precipitated by the addition of petroleum ether (5 L). The precipitate was collected and crystallized from methanol:water (80:20, 4 L) to furnish the desired product **14**, which was collected by vacuum filtration and dried under vacuum. Yield (167 g, 88%, over three steps). HPLC Rt 3.98 min. *m/z* (ESMS) 480 $[M + H]^+$, 959 $[2M + H]^+$.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-6-*O*-tert-butyldiphenylsilyl-2-deoxy-1-thio-β-*D*-glucopyranoside (**15**)*

A mixture of methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-2-deoxy-1-thio-β-D-glucopyranoside (**14**) (42.179 g, 88.0 mmol) and imidazole (600 mg) in pyridine (100 mL) was stirred and heated at 120°C for 50 min before *tert*-butylchlorodiphenylsilane (32 mL, 120 mmol) was added portion-wise. The reaction mixture was stirred and heated for 1.5 h and then allowed to cool down to room temperature. The solvent was evaporated under reduced pressure and the residue was taken up in DCM (350 mL). The organic phase was washed with a 10% citric acid solution twice and then dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue purified by column chromatography (silica gel 200 g; eluent: petroleum ether 4 L, petroleum ether/DCM (1:1) 3 L, DCM 3 L, and 2% MeOH/DCM 3 L) to give the title compound (**15**) (49.63 g, 79%). HPLC Rt 6.88 min. *m/z* (ESMS) 718 $[M + H]^+$, 1435 $[2M + H]^+$.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-6-*O*-tert-butyldiphenylsilyl-2-deoxy-1-thio-4-*O*-trifluoromethanesulfonyl-β-*D*-glucopyranoside (**16**)*

A solution of methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (**15**) (99 g, 125 mmol) and pyridine (25 mL, 312 mmol) in anhydrous DCM (500 mL) was stirred under nitrogen and cooled in an iced water bath for 20 min. Triflic anhydride (36.7 mL, 218 mmol) was added dropwise over 10 min. The mixture was warmed to room temperature and stirring was continued for a further 30 min. The reaction mixture was diluted with DCM (2 L) and washed with 5% citric acid solution (2 × 1 L) followed by brine (1 L), dried over anhydrous magnesium sulphate, and then evaporated under reduced pressure to give the title compound (**16**) as a golden syrup (115.3 g). The crude residue **16** was used immediately in the following step without further purification. HPLC Rt 7.39 min. *m/z* (ESMS) 850 $[M + H]^+$, 1699 $[2M + H]^+$.

*Methyl 2-[Amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-4-azido-3-*O*-benzoyl-6-*O*-tert-butyldiphenylsilyl-2,4-dideoxy-1-thio-β-*D*-galactopyranoside (**3**)*

A suspension of methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-4-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside (**16**) (crude residue from previous reaction) and sodium azide (80.8 g, 1.24 mol) in anhydrous DMF (400 mL) was heated under nitrogen at 60°C for 16 h. The mixture was allowed to cool to room temperature, diluted with ethyl acetate (1.5 L) and washed with water (2 × 1 L) and brine (1 L). The organic phase was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a golden glass, which was dried under high vacuum to give the title compound (**3**) (94 g).

The crude product was dissolved in toluene and chromatographed on silica gel (300 g) eluting first with toluene (2 L) followed by toluene/ethyl acetate (20:1, 7.5 L) to yield pure **3** (62 g; 60.5% over 2 steps) as a white solid after solvent evaporation. HPLC Rt 7.31 min. δ_H (500 MHz, $CDCl_3$): 8.19 (1H, d, $J_{CH,NH}$ 13.6, CH=), 8.09 (2H, d, *J* 7.7, Ar-H), 7.16–7.75 (13H, m, Ar-H), 5.19 (1H, t, $J_{H3,H4}$ 9.4, $J_{H2,H3}$ 9.4, H-3), 4.37 (1H, d, $J_{H1,H2}$ 10.1, H-1), 5.49 (1H, dd, $J_{H2,H3}$ 10.3, H-3), 4.49 (1H, d, $J_{H1,H2}$ 10.0, H-1), 4.41 (1H, dd, $J_{H3,H4}$ 3.4, H-4), 3.80–3.95 (4H, m, H-2, H-5, H-6a, H-6b), 3.29 (3H, s, NCH_3), 3.28 (3H, s, NCH_3), 2.17 (3H, s, SMe), 1.10 (9H, s, tBu). δ_C (100.6 MHz, $CDCl_3$): 165.45 (all C=O), 164.92, 162.45, 159.55 (*C*H=C), 151.88 (C=O), 135.49, 134.04, 132.78, 132.60, 130.06, 130.02, 129.00, 128.73, 128.20, 128.11, 127.89 (all Ar-C), 92.28 (*C*=CH), 84.08 (C-1), 77.11 (C-5), 73.87 (C-3), 61.88 (C-6), 60.38 (C-2), 59.92(C-4), 27.78 (NMe), 27.16 (NMe), 26.84 (tBuC), 19.15 (tBuC), 11.90 (SMe). *m/z* (ESMS) 743 $[M + H]^+$, 1485 $[2M + H]^+$. *m/z* (HRMS, ESI +ve) $[M + Na]^+$ Anal. Calc. for $C_{37}H_{42}N_6NaO_7SSi$, 765.2503. Found: 765.2497. Two related impurities were identified in the final product by LCMS - methyl

2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-3-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-galactopyranoside (0.25%) and methyl 2-[amino-5′-methylene-1′,3′-dimethypyrimidine-2,4,6-(1*H*,3*H*,5*H*)trione]-4-azido-3-*O*-benzoyl-2,4-dideoxy-1-thio-β-D-galactopyranoside (0.2%).

Accessory Publication

gCOSY and HSQC 2D NMR spectra of final products are available on the Journal's website.

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