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Synthesis of Thioglycoside-Based UDP-Sugar Analogues

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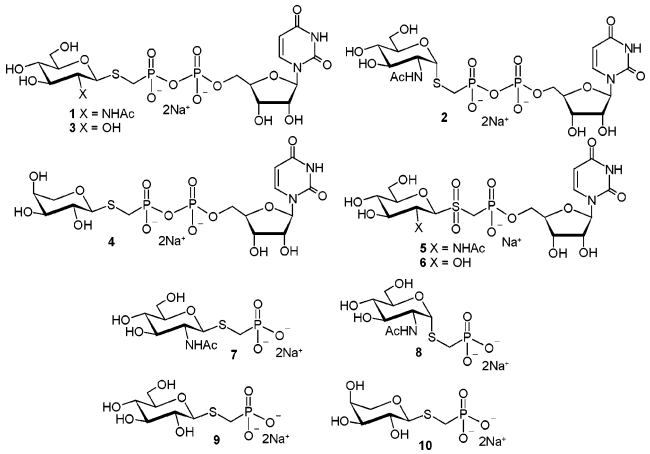
Abstract: Arbuzov reaction of *O*-acetyl-protected glycosylthiomethyl chlorides with triethyl phosphite and then phosphonate ethyl ester cleavage with trimethylsilyl bromide afforded glycosylthiomethyl phosphonates **13**, **18**, **22**, and **26**. These intermediates could be readily transformed into the *O*-deprotected phosphonates **7**−**10** and into title compounds **1**−**4**. Similarly, sulfonomethyl phosphonate moieties containing UDP-sugar analogues **5** and **6** were obtained.

Glycoside bond formation, following the Leloir pathway, is usually performed by transfer of an activated sugar from a nucleoside diphosphate sugar (NDP sugar) to a specific hydroxy group of the acceptor substrate. This important process in glycoconjugate synthesis is catalyzed by various glycosyltransferases, which require different NDP sugars as donor substrates. As glycoconjugates play a key role in numerous cell−cell recognition and interaction processes, the development of glycosyltransferase inhibitors is of great interest for the investigation of biochemical pathways and for therapeutic applications.[1] Therefore, several advances in the synthesis of glycosyltransferase inhibitors have been made and reviewed, recently.[2,3]

Furthermore, NDP sugars also occur as natural substrates in the biosynthesis of rare sugars. For example, the first step of the mammalian *N*-acetylneuraminic acid (Neu5Ac) biosynthesis is the isomerization of UDP-*N*-acetylglucosamine (UDP-GlcNAc) to *N*-acetylmannosamine (ManNAc), catalyzed by the UDP-GlcNAc 2-epimerase/ManNAc kinase.[4,5] This bifunctional enzyme has been found to catalyze the rate-limiting step in this biosynthetic pathway and therefore serves as the key regulator of cell surface sialylation.[6] Point mutations of this enzyme can result in diseases.[7,8]

SCHEME 1. UDP Sugar Analogues 1-6 and GTM-Phosphonates 7-10



As part of our ongoing program on the synthesis of glucosyltransferase,[9,10] galactosyltransferase,[11,12] and UDP-GlcNAc 2-epimerase inhibitors,[13,14] we would like to report here the synthesis of thioglycoside derived UDP-sugar derivatives **1**−**6** (Scheme 1) as potential substrate based inhibitors. In addition, the four glycosylthiomethyl (GTM) phosphonates **7**−**10** are also prepared to mimic the naturally occurring glycosyl phosphates, which play a vital role in the carbohydrate metabolism.[15]

Thioglycosides, in which the glycosidic oxygen atom has been replaced by a sulfur atom, are tolerated by most biological systems; moreover, this replacement increases the stability of the sugar−aglycon linkage against enzymatic cleavage as well as chemical degradation.[16] Therefore, the synthesis of *S*-linked glycoconjugates has been actively pursued in our laboratories.[17] Now the novel *S*-glycosidic UDP-GlcNAc analogues **1** and **2** were designed to mimic the natural UDP-GlcNAc by substituting the anomeric oxygen by a thiomethylene group. This design was also based on the fact that ground-state analogues of UDP-GlcNAc exhibit inhibitory activities.[18]

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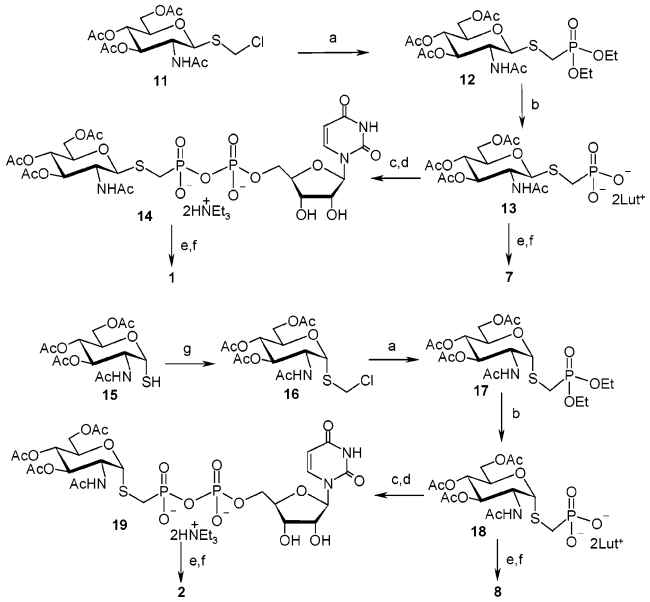
In addition, this substitution would provide valuable information about UDP-GlcNAc 2-epimerase by comparing the inhibitory activities of compounds **1** and **2**. Compounds **3** and **4** were designed to mimic glycosyl transfer with UDP-glucose and UDP-arabinose, respectively, and thus they should serve as glycosyltransferase inhibitors.[11] Recently, the sulfono group has been used as a phosphate surrogate to develop novel glycosyl transferase inhibitors, due to its biochemical stability and conceivably good membrane permeability.[19] Moreover, the partial negative charge located on the oxygen atom of the sulfono group lends itself to complex with the corresponding metal ion during the interaction with enzyme. As such, compounds **5** and **6**, in which sulfono phosphonates were chosen to replace the diphosphate linkage, were designed as potential UDP-GlcNAc 2-epimerase and glucosyltransferase inhibitors, respectively.

On the basis of the fact that the pyrophosphate-linked sugar-nucleoside conjugates were usually built up by coupling glycosyl phosphates with suitably activated nucleotides,[20] the corresponding GTM-phosphonates would be essential for the synthesis of the target compounds **1**−**4**. Recently, the sugar species GTM-Cl was developed in our laboratories and successfully applied to the synthesis of a new type of sugar-triazole derivative.[21] Also GTM-phosphonates should be accessible from GTM-Cl. To effect the transformation GTM-Cl **11**[21] (Scheme 2) was employed in the Arbuzov reaction with neat triethyl phosphite, and as hoped, the desired GTM-phosphonate **12** was produced in 78% yield after 2 days of refluxing. Importantly, the present chloride did not undergo any side reactions under the reaction conditions, as indicated by TLC. **12** was then treated with bromotrimethylsilane (TMSBr) in the presence of an excess of 2,6-lutidine,[13] leading smoothly to the deprotected phosphonate **13** as a lutidinium salt in quantitative yield, which could be used for the next step without purification. The ensuing coupling of **13** and UMP-morpholidate was conducted employing the reported procedure,[13,22] and expectedly, compound **14** was isolated as a triethylammonium salt in 42% yield after RP-HPLC (C18) purification. Subsequently, **14** was converted into target compound **1** by deacetylation with Et_3N in MeOH−H_2O followed by ion-exchange chromatography. Additionally, GTM-phosphonate **7** was also prepared from **13** by deacetylation and ion exchange, as shown in Scheme 2.

To synthesize the α-*S*-glycosidic UDP-GlcNAc mimetic **2**, α-GTM-Cl **16** was first prepared from the known α-GlcNAc thiol **15**[23] by reaction with dichloromethane, as described previously.[21] Subsequent treatment of **16** with triethyl phosphite gave rise to the desired GTM-phosphonate **17** in 69% yield, which followed the same route as above to reach the target molecule **2**. It is important to note that compound **2** is an interesting analogue of UDP-GlcNAc and could be a potent inhibitor of UDP-GlcNAc transferases[18] or UDP-GlcNAc 2-epimerase. Intermediate **18** (Scheme 2) was also converted into another GTM-phosphonate **8**, which should also be of great biological interest in view of the importance of α-GlcNAc-1-phosphate in a number of biological processes.[15a,d]

SCHEME 2[a]



[a] Reagents and conditions: (a) $P(OEt)_3$, 160 °C, 48 h, **12** (78%), **17** (69%); (b) TMSBr, 2,6-lutidine, CH_3CN, quant.; (c) UMP-morpholidate, tetrazole, py; (d) RP-HPLC (C18), **14** (42%), **19** (36%); (e) Et_3N/MeOH/H_2O (1:7:3); (f) Amberlite IR-120 Na^+-form, quant. over two steps; (g) DBU, CH_2Cl_2, 40%.

For the synthesis of the target structure **3**, glucosylthiomethyl chloride **20**[21] (Scheme 3) was used as starting material. In practice, **20** was first converted into GTM-phosphonate **21** by Arbuzov reaction with triethyl phosphite in 84% yield, which was then deprotected with TMSBr to give compound **22** as a lutidinium salt. Coupling of **22** and UMP-morpholidate in the presence of 1*H*-tetrazole led to compound **23** in 40% yield, which was further deacetylated and ion exchanged to furnish the target molecule **3**. On the other hand, direct deacetylation of **22** afforded the GTM-phophonate **9** after ion exchange chromatography. By the same route, target compound **4** was also successfully prepared from the corresponding GTM-Cl **24**,[21] as shown in Scheme 3, wherein intermediate **26** was transformed into GTM-phosphonate **10**.

For the synthesis of the sulfono-containing target compounds **5** and **6**, a different strategy was needed. First, thioglycosides **12** and **21** were oxidized to the corresponding sulfones **28** and **29** with mCPBA in 74% and 86% yields, respectively, as shown in Scheme 4. It should be noted here that attempts to synthesize **28** or **29** from the corresponding glycosylsulfonylmethyl chloride by Arbuzov reaction failed. Subsequent exposure of

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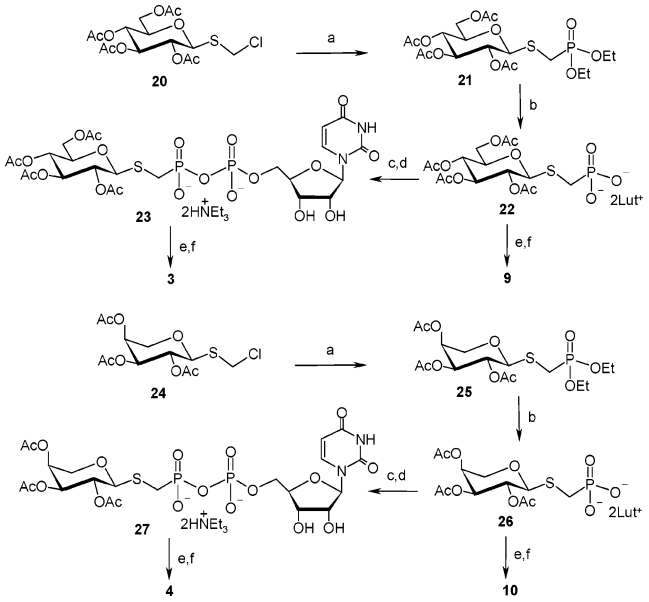
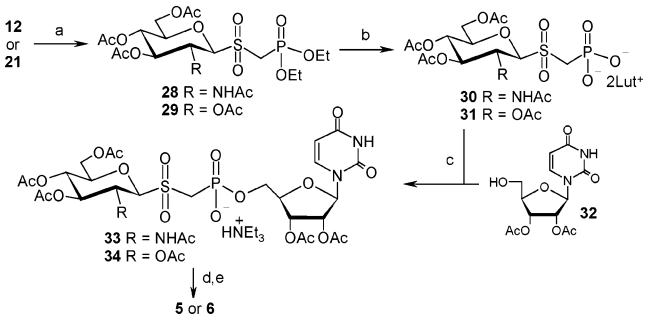
SCHEME 3[a]



[a] Reagents and conditions: (a) $P(OEt)_3$, 160 °C, 48 h, **21** (84%), **25** (80%); (b) TMSBr, 2,6-lutidine, CH_3CN, quant.; (c) UMP-morpholidate, tetrazole, Py; (d) RP-HPLC (C18), **23** (40%), **27** (45%); (e) Et_3N/MeOH/H_2O (1:7:3); (f) Amberlite IR-120 Na^+-form, quant.

SCHEME 4[a]



[a] Reagents and conditions: (a) mCPBA, Na_2HPO_4, CH_2Cl_2, **28** (74%), **29** (86%); (b) TMSBr, 2,6-lutidine, CH_3CN, quant.; (c) DCC, DMAP, Py, **33** (62%), **34** (67%); (d) Et_3N/MeOH/H_2O (1:7:3); (e) Amberlite IR-120 Na^+-form, quant.

28 and **29** to TMSBr in the presence of 2,6-lutidine quantitatively led to the corresponding sulfono phosphonate **30** and **31** as lutidinium salts, respectively. After drying under high vacuum, these salts were immediately coupled with 2′,3′-diacetyl uridine **32**,[24] and under the action of DCC the corresponding protected products **33** and **34** were isolated from the reaction mixtures in 62% and 67% yields, respectively. The removal of the acetyl groups from **33** and **34** was then conducted again by $Et_3N-MeOH-H_2O$, affording the corresponding desired target molecules **5** and **6** as triethylammonium salts, which were transformed into sodium salts by ion exchange chromatography.

In summary, from the new sugar species, GTM-Cl, new types of *S*-glycosidic sugar-nucleotide derivatives **1**−**6** were prepared as potential glycosyltransferase inhibitors and/or UDP-GlcNAc 2-epimerase inhibitors. The syntheses presented here could also be extended to the preparation of other potential glycosyltransferase inhibitors. In view of the ready access of the target molecules, together with their enhanced enzymatic and chemical stability, the design and synthesis of various novel structures is available. Testing of **1**−**6** as inhibitors of UDP-GlcNAc 2-epimerase is in progress. In addition, four GTM-phosphonates **7**−**10** were also synthesized to mimic the natural sugar 1-phosphate.

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Experimental Section

Disodium Uridin-5′-yl (2-Acetamido-2-deoxy-β-D-glucopyranosylthiomethylphosphono) Phosphate (1). A solution of **14** (20 mg, 20.7 μmol) in a mixture of $Et_3N-MeOH-H_2O$ (1:7:3, 2 mL) was stirred at room temperature overnight. The mixture was then concentrated in vacuo to give a residue, which was redissolved in a small amount of water and lyophilized to furnish the title compound as a triethylammonium salt. This salt was treated with ion-exchange resin (Na^+ form) to afford the desired pure product **1** as a white solid (14 mg, quant.). If necessary, the material can be further purified by precipitation from ethanol−water: ^{1}H NMR (600 MHz, D_2O) δ 7.69 (d, J = 7.8 Hz, 1H), 5.87 (d, J = 5.0 Hz, 1H), 5.80 (d, J = 7.7 Hz, 1H), 4.62 (overlapped with water peak, 1H), 4.19 (m, 2H), 4.10 (br s, 1H), 4.06 (m, 2H), 3.75 (d, J = 12.4 Hz, 1H), 3.60 (m, 2H), 3.39 (m, 1H), 3.32 (m, 2H), 2.87 (t, J = 13.7 Hz, 1H), 2.79 (t, J = 14.5 Hz, 1H), 1.90 (s, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.1, 162.4, 156.7, 140.1, 102.5, 87.9, 83.6, 82.3 (d, J = 8.9 Hz), 79.3, 74.7, 73.2, 69.2, 69.1, 64.3, 60.2, 54.1, 24.8 (d, J = 143.0 Hz), 21.6; ^{31}P NMR (243 MHz, D_2O) δ 11.8 (d, J = 26.1 Hz), −9.5 (d, J = 26.1 Hz); MALDI-MS (negative mode, ATT) m/z 636.3 [M − $2Na^+ + H^+]^-$.

Diethyl (3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosylthiomethyl) Phosphonate (12). GTM-Cl **11** (381 mg, 0.92 mmol) was refluxed with neat $P(OEt)_3$ (7.3 mL) at 160 °C for 48 h, and the excess of reagent was then removed under reduced pressure to give the crude product, which was purified by flash column chromatography (toluene/acetone, 5:1 → 1:1) to afford the title compound **12** (370 mg, 78%) as a white amorphous solid: $[\alpha]_D$ −55.9 (*c* 1.0 $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.71 (d, J = 9.0 Hz, 1H), 5.22 (t, J = 9.6 Hz, 1H), 5.04 (t, J = 9.8 Hz, 1H), 4.98 (d, J = 11.0 Hz, 1H), 4.21 (dd, J = 12.4, 4.6 Hz, 1H), 4.18−4.03 (m, 5H), 3.95 (t, J = 10.3 Hz, 1H), 3.69 (ddd, J = 10.0, 4.5, 2.3 Hz, 1H), 2.91 (dd, J = 15.6, 9.5 Hz, 1H), 2.73 (t, J = 14.5 Hz, 1H), 2.03 (s, 3H), 1.96 (s, 6H), 1.89 (s, 3H), 1.30 (t, J = 7.0 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.1, 169.9, 169.8, 168.8, 82.7 (d, J = 4.8 Hz, 1C), 75.1, 73.1, 68.2, 62.7 (d, J = 6.7 Hz, 1C), 62.1 (d, J = 6.7 Hz, 1C), 61.7, 52.2, 21.1 (d, J = 149.8 Hz, 1C), 20.04, 19.99, 15.81 (d, J = 5.7 Hz, 1C), 15.76 (d, J = 5.7 Hz, 1C); MALDI-MS (positive mode, DHB) m/z 536.3 $[M + Na^+]^+$, 552.3 $[M + K^+]^+$. Anal. Calcd for $C_{19}H_{32}NO_{11}PS$ (513.50): C, 44.44; H, 6.28; N, 2.73. Found: C, 44.21; H, 6.54; N, 2.41.

Bis-2,6-lutidinium (3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-glucopyranosylthiomethyl) Phosphonate (13). To a stirred solution of compound **12** (116 mg, 0.23 mmol) in dry CH_3CN (8.6 mL) was added 2,6-lutidine (0.28 mL, 2.4 mmol) under N_2. After cooling at 0 °C, TMSBr (0.24 mL, 1.9 mmol) was added dropwise to the mixture. The cooling bath was removed after 30 min, and the reaction was stirred at room temperature for 24 h, concentrated in vacuo, and azeotroped several times with dry CH_3CN to give **13** as a white solid in quantitative yield, which was used directly in the next step: ^{1}H NMR (250 MHz, CD_3OD) δ 8.34 (t, J = 7.9 Hz, 2H), 7.72 (d, J = 7.9 Hz, 4H), 5.17 (t, J = 9.8 Hz, 1H), 5.00 (t, J = 9.9 Hz, 1H), 4.89 (overlapped with water peak, 1H), 4.29 (dd, J = 12.4, 4.8 Hz, 1H), 4.13 (dd, J = 12.4, 2.2 Hz, 1H), 3.99 (t, J = 10.3 Hz, 1H), 3.82 (m, 1H), 2.94 (t, J = 13.1 Hz, 1H), 2.83 (t, J = 14.8 Hz, 1H), 2.76 (s, 12H), 2.06, 2.01, 1.98, 1.91 (4s, 12H); MALDI-MS (negative mode, ATT) m/z 456.5 $[M - 2Lut^+ + H^+]^-$.

Bistriethylammonium Uridin-5′-yl (3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-*β*-D-glucopyranosylthiomethylphosphono) Phosphate (14). The crude salt **13** (148 mg, 0.22 mmol) was coevaporated several times with dry pyridine. After addition of 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (165 mg, 0.24 mmol), the mixture was again coevaporated twice with dry pyridine, then dried under high vacuum for several hours. Subsequently, the solid was taken up in dry pyridine (6 mL) under N_2, and to this solution predried 1*H*-tetrazole (31 mg, 0.44 mmol) was added. After the solution was stirred at room temperature for 3 days, Hünigs base (0.17 mL, 0.99 mmol) and water (6 mL) were added, and then the solution was concentrated in vacuo. The residue was coevaporated again with the same amount of Hünigs base-water, dried under vacuum, and then purified by RP-HPLC (C18) (0.05 M triethylammonium bicarbonate (TEAB) buffer; gradient, 4−18% CH_3CN over 30 min; flow rate, 11 mL/min; t_R = 23 min). Compound **14** (89 mg, 42%) was obtained as a white solid: ^{1}H NMR (250 MHz, D_2O) δ 7.67 (d, J = 7.7 Hz, 1H), 5.80 (d, J = 4.2 Hz, 1H), 5.70 (d, J = 7.8 Hz, 1H), 5.00 (t, J = 9.7 Hz, 1H), 4.87 (t, J = 10.3 Hz, 2H), 4.21 (dd, J = 12.7, 3.3 Hz, 1H), 4.13 (m, 2H), 4.02 (m, 4H), 3.82 (t, J = 10.1 Hz, 2H), 2.82 (t, J = 13.0 Hz, 1H), 2.70 (t, J = 14.6 Hz, 1H), 1.90, 1.86, 1.82, 1.78 (4s, 12H); MALDI-MS (negative mode, ATT) m/z 762.4 [M − $2HNEt_3^+ + H^+]^-$.

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Supporting Information Available: Experimental details and analytical information for compounds **2**−**10**, **12**−**14**, **16**−**19**, **21**−**23**, **25**−**31**, **33**, and **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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