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Note

Synthesis of 7-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-4-methylcoumarin sodium salt: a fluorogenic substrate for sulfamidase

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Abstract

The title compound, useful for testing the efficacy of heparin sulfamidase, was synthesized in good yield starting from 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranosyl fluoride, reducing the azido group efficiently with $SnCl_2$–PhSH–Et_3N reagent and finally crystallizing the *N*-sulfated product from methanol after deacetylation.

Keywords: Heparin sulfamidase; Azido group; 4-Methylumbelliferyl

Mucopolysaccharidoses (MPS), a family of debilitating disorders affecting children, is caused by the deficiency of one or more enzymes required for stepwise degradation of glycosaminoglycans (GAGs).[1] A family of glycosidases and sulfatases catabolize specific GAGs in a particular sequence, usually starting from the nonreducing end of the chain. Subsequent enzymes can work only after the preceding enzymatic step has taken place. In effect, it requires four glycosidases, five sulfatases, and one non-hydrolytic transferase to degrade the substrate completely. Deficiency of any one enzyme in the cascade can cause severe physical and mental disorder. It is known that symptoms and severity of MPS can be reversed by enzyme replacement therapy. This has generated interest in the commercial production of these enzymes.

At Biomarin, we undertook production of MPS I (Hurler–Schei syndrome, deficiency of α-L-iduronidase),[1] MPS IIIA (Sanfilippo A, deficiency of heparin sulfamidase),[2–4] MPS IVA (Morquio A, deficiency of *N*-acetyl-D-galactosamine-6-sulfatase),[5,6] and MPS VI (Maroteaux–Lamy, deficiency of *N*-acetyl-D-galactosamine-4-sulfatase)[7] using recombinant techniques. In order to evaluate the efficacy, as well as for quality control purposes, relevant substrates were needed as their respective 4-methylumbelliferyl (4-MU) glycosides. In this communication, we describe an improved synthesis of 7-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-4-methyl coumarin, the recommended substrate for MPS IIIA.[8]

1. Results and discussion

Synthesis of 4-MU β-D-glycosides is well known.[9–13] However, the corresponding α-glycosides have been generally reported as either side products, or as minor components obtained during the synthesis of the β-glycosides. The title compound was prepared by selective sulfation of the free amine of 4-MU α-D-glucosaminide, obtained as a minor product during the synthesis of the corresponding 2-acetamido-2-deoxy-β-D-glycoside.[14] In Scheme 1, we chose 2-azido-2-deoxy derivatives of D-glucopyranose (**5**–**8**) with a non-participating group at C-2, as ideal glycosyl donors for higher α-selectivity. Several methods[16–24] are known for the synthesis of 2-azido-2-deoxy derivatives of D-glucopyranose. We utilized the procedure of Tailler et al.[15] for the synthesis of compound **2** from **1** and acetolyzed it to the tetraacetate **3** using 2% concentrated sulfuric acid in acetic

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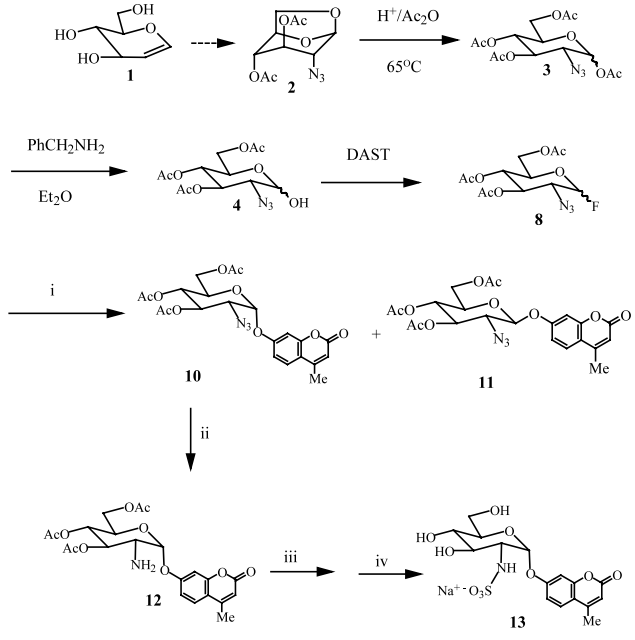
anhydride at 65 °C. Routine conversion of **3** into the corresponding 1-OH derivative **4** using benzylamine in diethyl ether and thence to the glycosyl donors, namely the 1-Cl (**5**), 1-Br (**6**), 1-trichloroacetamido (**7**), and 1-F (**8**) derivatives were accomplished using known reagents.[25–29] All glycosyl donors (**5**–**8**) were stable, could be purified by column chromatography and their ^{1}H NMR spectra established their structures (as α,β-mixtures). Attempts to prepare 4MU α-glycosides by reacting **5**–**7** with 7-hydroxy-4-methylcoumarin or its sodium salt in the presence of appropriate activating agents were unsuccessful. However, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-α,β-D-glucopyranosyl fluoride (**8**) and 7-*O*-trimethylsilyl-4-methylcoumarin (Me_3Si-MU) reacted smoothly in the presence of $BF_3{\cdot}Et_2O$ to give 4MU glycosides (**10** and **11**, total yield 72–75%, α/β:1.0/1.6). The ratio of α to β was unexpectedly poor as compared to that observed by Chiba et al.,[30] who used 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl bromide during the synthesis of α-D-glucosaminide-containing disaccharide fragments of heparin. Selective reduction of the azido group, a crucial step, could be accomplished with the reagent described by Bartra et al.[31] at decreased temperature (5–10 °C) using catalytic $SnCl_2$ (Scheme 1). A higher quantity of the tin(II) reagent, prolonged reaction, or higher temperature resulted in degradation and poor yields. At this stage, it was considered best to convert **12** directly into the sulfamido derivative and on to the final product **13**. Attempts to isolate **12** as its HCl salt



Scheme 1. DAST (diethylaminosulfur trifluoride); (i) 4MU-7-*O*-$SiMe_3$–$BF_3{\cdot}ET_2O$–CH_2C1_2; (ii) PhSh–Et_3N–$SnC1_2$ (cat.)–CH_3CN; (iii) pyridine·SO_3–ET_3N–DMF; (iv) NaOME–MeOH.

and then carry out sulfation and deacetylation, resulted in poor yields.

In summary, a convenient, practical synthetic route for the synthesis of sodium 7-*O*-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-4-methylcoumarin has been described. The reaction condition used to reduce the azido function with $Sn(SPh)_3^-$ is mild, well suited for the carbohydrate substrate, and produced the corresponding amine within a short time in good yield as compared with H_2S–pyridine[32].

2. Experimental

General methods.—Reactions were carried out under nitrogen in septum-sealed flasks, monitored by thin layer chromatography (TLC) on silica-coated aluminium plates (Silica Gel 60G F_{254}, E. Merck) and visualized under UV before and after charring. The spray reagent was 5% H_2SO_4 in EtOH. The solvent system (v/v) used for TLC and column chromatography was chosen from: I, 10:1:0.1 toluene–acetone–MeOH; II, 30:1:0.1 toluene–acetone–MeOH; III, 40:1:0.1 toluene–acetone–MeOH; IV, 20:1 toluene–EtOAc; V, 6:1 toluene–EtOAc; VI, 2:1 toluene–EtOAc; VII, 10:0.15 $CHCl_3$–MeOH; VIII, 30:0.15 $CHCl_3$–MeOH; IX, 40:0.15 $CHCl_3$–MeOH; X, 10:0.4 $CHCl_3$–MeOH; XI, 5:1 $CHCl_3$–5% aq MeOH; XII, 2:1 $CHCl_3$–5% aq MeOH. Prepacked silica gel columns (Isco Inc.) were used for all column chromatography. ^{1}H NMR spectra were run by Acorn NMR Inc., Livermore, CA, USA, using Jeol Eclipse 400, GE 360, or Nicolet NT 300 instruments, and decoupling experiments were carried out to assign chemical shifts of the ring protons unambiguously. Mass spectra were run at the mass spectrometry facility at UC Berkeley (College of Chemistry), elemental analyses were carried out by Desert Analytics (Tucson, AZ, USA), and specific rotations were measured at 25 °C using a Perkin–Elmer 241 polarimeter at Bay Analytical Laboratory, Inc., (Richmond, CA, USA). Solvents were evaporated under diminished pressure at a bath temperature of 40 °C or less. 7-*O*-Trimethylsilyl-4-methylcoumarin (Me_3Si-MU) was prepared by reacting the 7-hydroxy precursor with chlorotrimethylsilane in the presence of excess pyridine in refluxing CH_2Cl_2 (12 h). The reaction mixture was evaporated to dryness and the solid residue was thoroughly extracted with $CHCl_3$. Evaporation of the extract gave Me_3Si-MU, pure enough (by NMR) to be used directly in the coupling reaction.

7-O-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-α- and β-D-glucopyranosyl)-4-methylcoumarin (**10** and **11**).—Benzylamine (6.0 mL, 55 mM) was added to a solution of **3** (11.0 g, 29.5 mM) in Et_2O (120 mL) that was stirred at rt (25 °C). TLC (solvent I) showed complete conversion into **4** (R_f 0.28). Ether was evaporated off, the

residue dissolved in $CHCl_3$ (120 mL), and the solution was washed thoroughly with cold aq $NaHCO_3$ (saturated), and water consecutively, and dried (Na_2SO_4). Filtration and evaporation of the filtrate afforded crude product, which was purified by column chromatography (silica gel, 135 g) using solvent II (300 mL) and III (2L) consecutively to give pure **4** (8.3 g, yield 85%).

Compound **4** (6.0 g, 18.1 mM) was dissolved in THF (40 mL) and diethylaminosulfur trifluoride (DAST, 2.9 mL, 22 mM) was added to the stirred solution maintained at −30 °C. The cooling bath was removed and the reaction continued at rt until complete (1 h, TLC, solvent V, R_f product = 0.3). The mixture was cooled (−30 °C) and MeOH (5 mL) was added to decompose the excess of DAST. After 1 h (rt) of reaction with MeOH, the solvents were evaporated off, the syrupy residue was dissolved in $CHCl_3$ (100 mL) and the solution was washed with water, aq $NaHCO_3$ and water successively, and dried (Na_2SO_4). Filtration, evaporation of the filtrate, and column chromatography (silica gel 135 g, solvents V, 150 mL; VI, 1.2 L) afforded pure **8** (4.97 g, yield 82%). ^{1}H NMR indicated $\alpha/\beta = 1/5.5$.

Compound **8** (4.9 g, 14.7 mM) and Me_3Si-MU (6 g, 24 mM) were transferred into a reaction flask (200 mL), dried under high vacuum, and dissolved in CH_2Cl_2 (40 mL). The solution was cooled (5 °C) and a solution of $BF_3{\cdot}Et_2O$ (2.0 mL, 15.8 mM) in CH_2Cl_2 (4.0 mL) was added dropwise to the reaction flask with vigorous stirring. Reaction was continued at rt for 2 h, when TLC (solvent VII) indicated almost complete absence of the starting fluoride and appearance of two products (R_f 0.56, minor and R_f 0.43, major) and some degradation products. The mixture was diluted in the cold (5 °C) with $CHCl_3$ (20 mL), filtered, washed successively with water, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. Column chromatography (silica gel column, 200 g) using solvent VIII (200 mL), IX (1.2 L) and finally 15:0.15 (v/v) $CHCl_3$–MeOH (2.5 L) gave first **10** (α anomer, 1.4 g), followed by a mixture containing **10** and **11** (1.2 g, α/β: 1/1.6, by ^{1}H NMR) and finally **11** (β anomer, 2.5 g). Some unchanged **8** and its hydrolyzed product **4** (totaling 0.52 g) were also separated.

Analysis of **10**: mp 63–65 °C; $[\alpha]_{589}$ +214° (*c* 2.32, $CHCl_3$); NMR: δ_H ($CDCl_3$) 2.06–2.14 (3s, 9 H, 3 Ac), 2.42 (d, *J* 0.8 Hz, 3 H, CH_3 of coumarin), 3.58 (dd, 1 H, *J* 3.6, 10.8 Hz, H-2), 4.04 and 4.29 (2dd, 2 H, H-6,6′), 4.1 (m, 1 H, H-5), 5.16 (t, 1 H, *J* 9.8 Hz, H-4), 5.68 (d, 1 H, *J* 3.6 Hz, H-1), 5.69 (dd ~ t, 1 H, *J* 10.4 Hz, H-3), 6.21 (d, 1 H, *J* 0.8 Hz, H-3 in coumarin), 7.07 (dd, 1 H, *J* 2.6, 9.0 Hz, H-6 of coumarin), 7.14 (d, 1 H, *J* 2.4 Hz, H-8 of coumarin), 7.56 (d, 1 H, *J* 8.4 Hz, H-5 of coumarin); *m*/*z* 512 $[M + Na]^+$; M_W 489.4 for $C_{22}H_{23}N_3O_{10}$.

Analysis for **11**: mp 75–76 °C; $[\alpha]_{589}$ −19.6° (*c* 2.09, $CHCl_3$); NMR: δ_H ($CDCl_3$) 2.06–2.13 (3s, 9 H, 3Ac), 2.42 (d, 3 H, *J* 0.8 Hz, CH_3 of coumarin), 3.38 (dd ~ t, 1 H, *J* 10.0 Hz, H-2), 3.9 (m, 1 H, H-5), 4.17 and 4.31 (2dd, 2 H, H-6,6′), 5.02 (d, 1 H, *J* 8.4 Hz, H-1), 5.1 (2t overlapped, 2 H, *J* 10.0 Hz, H-3,4), 6.2 (d, 1 H, *J* 0.8 Hz, H-3 of coumarin), 6.9–7.01 (m, 2 H, H-6,8 of coumarin), 7.55 (d, *J* 9.2 Hz, H-5 of coumarin); *m*/*z* 512 $[M + Na]^+$; M_W 489.4 for $C_{22}H_{23}N_3O_{10}$.

Sodium 7-O-(2-deoxy-2-sulfamido-α-D-glucopyranosyl)-4-methylcoumarin (**13**).—Compound **11** (540 mg, 1.1 mM) was dissolved in MeCN (5 mL), cooled (5–8 °C), and a solution of benzenethiol (0.46 mL, 4.5 mM), Et_3N (0.46 mL, 3.3 mM), and tin(II) chloride (45 mg, 0.23 mM) in MeCN (3 mL) was transferred to it with vigorous stirring. TLC (solvent X) indicated conversion into product (R_f 0.33) within 1 h. Chloroform (40 mL) was added to the flask and the yellow solution transferred into a separatory funnel containing cold aq NaOH (0.5 M, 50 mL). After vigorous shaking, the colorless organic layer was separated, washed with ice cold water, dried (Na_2SO_4, 20–30 min), filtered, and the filtrate evaporated to dryness. Without further purification, the crude product was dissolved in a mixture of *N*,*N*-dimethylformamide (5 mL) and CH_2Cl_2 (2 mL) containing Et_3N (0.6 mL), and pyridine·SO_3 (0.48 g, 3 mM) was added. The reaction was continued at rt (90 min) until TLC (solvent XI) indicated complete conversion into a product (R_f 0.15). Methanol (5 mL) was added to decompose the excess pyridine·SO_3 and the mixture was stripped of all solvents azeotropically with toluene. The syrupy residue was dissolved in $CHCl_3$ (10 mL) containing $HCONMe_2$ (1–2%), loaded on a silica gel column (70 g) and eluted with solvents X (75 mL) and XI consecutively to give the crude sulfamido product as its triacetate (0.98 g). This was dissolved in MeOH (dry, 20 mL) and deacetylated at rt using NaOMe at pH ~8.0 (pH paper). TLC (solvent XII) indicated a clean conversion into a product (R_f 0.21) within 2 h. Aqueous MeOH (10%, 10 mL) was added to the flask and the solution shaken with IR-120 (Na^+) resin. Filtration and evaporation of the filtrate afforded a syrup, which was dissolved in MeOH (5 mL). Crystals separated, which were filtered and dried to give pure **13** (258 mg, 57%), mp 235–237 °C; $[\alpha]_{589}$ +121.4° (*c* 2.54, water); NMR: δ_H (D_2O) 2.44 (sharp d, 3 H, *J* 1.2 Hz, CH_3 of coumarin), 3.49 (dd, 1 H, *J* 3.2, 10.4 Hz, H-2), 3.63 (t, 1 H, *J* 10 Hz, H-4), 3.7–3.9 (m, 3 H, H-5,6,6′), 3.89 (dd ~ t, 1 H, *J* 10.4 Hz, H-3), 6.02 (d, 1 H, *J* 3.2 Hz, H-1), 6.24 (d, 1 H, *J* 1.2 Hz, H-3 of coumarin), 7.2 (m, 3 H, H-6,8 of coumarin), 7.72 (d, 1 H, *J* 7.2 Hz, H-5 of coumarin); *m*/*z* 416 $[M - Na]^-$. Anal. Calcd for $C_{16}H_{18}NNaO_{10}S$: C, 43.74; H, 4.13; N, 3.2; S, 7.3. Found: C, 43.76; H, 4.04; N, 3.5; S, 7.43.

Column chromatography of the mother liquor gave more product; total yield of **13**, 388 mg (79%).

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