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Note

Towards the synthesis of sialic acid-based *Salmonella typhimurium* sialidase inhibitors ☆

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The synthesis and biological evaluation of sialic acid analogues, in particular influenza virus sialidase inhibitors, has been of great interest to us in recent times [1–4]. Although the X-ray crystal structure of *Salmonella typhimurium* sialidase has been recently reported [5], little information has been published concerning the inhibition of this sialidase by sialic acid analogues [6]. Our interest in the synthesis of bacterial and viral sialidase inhibitors has led us to investigate the synthesis of potential inhibitors of *S. typhimurium* sialidase.

We have previously reported the synthesis of a number of *S*-sialylsaccharides [7] and *S*-sialylnucleosides [8] which use methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**1**) as a key intermediate. *S*-Linked sialosides are thought to be metabolically stable to sialidases. We have now applied our previously described methodology [7] towards the synthesis of a number of novel *S*-sialoside analogues that have served as useful probes in the development of inhibitors against *Salmonella typhimurium* sialidase. Both previous data [6] and the data described here clearly show that Neu5Ac2en (**2**), a well-known sialidase inhibitor and useful benchmark [2], has a significantly higher K_i value ($\sim 10^{-4}$ M) against *S. typhimurium* sialidase when compared with other bacterial or viral sialidases [2]. The most logical explanation for this is that the transition state for the reaction catalysed by *S. typhimurium* sialidase does not resemble the proposed transition state for the reaction catalysed by influenza virus sialidase [9]. The postulated sialosyl cation

☆ Dedicated to Professor Stephen Angyal on the occasion of his 80th birthday.

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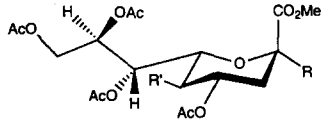
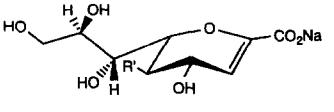
transition state in the reaction catalysed by influenza virus sialidase [10] is mimicked, to some extent, by the planar features around C-2 and C-3 and the ring oxygen in **2**.

Indeed, this notion of an alternative transition state is in complete agreement with the conclusion of Guo and Sinnott where it has been postulated that the reaction catalysed by *S. typhimurium* sialidase proceeds through a single transition state that closely resembles the ground-state $^2C_5$ chair conformation [11].

Compounds **6**, **7** and **8**, contain sulfur-linked hydrophobic aglycon units. This provided us with an opportunity to investigate, in part, the nature of the *S. typhimurium* sialidase aglycon-binding region. Under our standard assay conditions [2], all of these probes, compounds **6–8**, were found to have weak inhibition ($\geq$ 1 mM). The explanation for these observations is presumably that the aglycon-binding region does not accommodate an aglycon unit that is either hydrophobic in nature and/or is sterically demanding. It is noteworthy that naturally occurring substrates such as fetuin or sialyllactose have significantly more hydrophilic aglycon units.



R' = NHAc **1** R = SAc

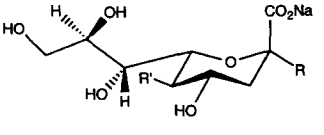


2 R' = NHAc

3 R = S

4 R = S (S)

5 R = S

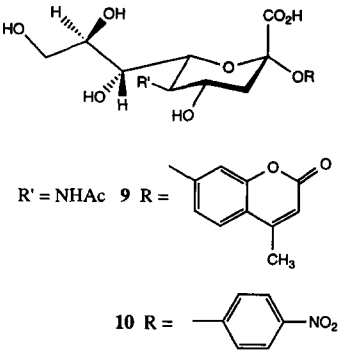


R' = NHAc **6** R = S

7 R = S (S)

8 R = S

However, given that the synthetic sialosides 4-(methylcoumarin-7-yl) 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonic acid (**9**) and *p*-nitrophenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosidonic acid (**10**) are considered good substrates for this enzyme and that the aglycon units of these compounds are also rather hydrophobic, there must be some additional influence on the affinity of these compounds as a result of the replacement of the anomeric oxygen by sulfur. Indeed, our preliminary molecular modelling studies suggest that the *S. typhimurium* sialidase aglycon binding region does contain a number of hydrophilic amino acid residues and may not accommodate sulfur as well as it does oxygen [12].



1. Experimental

General.—^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer. Mass spectra were obtained using a Jeol JMS-DX 300 mass spectrometer. Optical rotations were measured at 25°C using a JASCO DIP-370 polarimeter. All reagents and solvents were distilled prior to use. Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**1**) and methyl (2-propenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**5**) were prepared according to published procedures (see refs [13] and [7], respectively).

Preparation of sodium 2-ethylbutyl 5-acetamido-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**6**).—To a solution of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (**1**) (800 mg, 1.46 mmol) and 2-ethyl-1-bromobutane (0.193 mL, 1.38 mmol) in dry DMF (5 mL) was added dry diethylamine (2 mL) at room temperature under an atmosphere of nitrogen. After stirring for 4 h, the diethylamine was removed in vacuo, and the remaining solution was diluted with EtOAc (50 mL), initially washed with 1 N HCl (30 mL) and then with water (2 × 30 mL). The organic layer was then dried (Na_2SO_4) and concentrated under reduced pressure to yield a residue that was passed down a column of silica gel (4:1, EtOAc–hexane). Removal of the solvent yielded methyl (2-ethylbutyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**3**) as a white solid (700 mg, 80%): $[\alpha]_D$ +24.5°C 0.01, $CHCl_3$); ^{1}H NMR data (300 MHz, $CDCl_3$): alkyl unit-δ 0.83 (t, 6 H, H-4), 1.33 (bs, 5 H, H-3, H-2), 2.67 (m, 2 H, H-1); Neu5Ac unit-δ 1.84 (s, 3 H, AcN), 2.00, 2.01, 2.10, 2.12 (4 s, 12 H, 4 Ac), 2.48 (dd, 1 H, $J_{3e,3a}$ 11.9, $J_{3e,4}$ 4.3 Hz, H-3e), 3.76 (s, 3 H, CO_2CH_3), 3.82 (dd, 1 H, $J_{6,7}$ 1.7, $J_{6,5}$ 10.7 Hz, H-6), 4.01 (ddd, 1 H, $J_{5,6} = J_{5,4} = J_{5,NH}$ = 10.3 Hz H-5), 4.09 (dd, 1 H, $J_{9,8}$ 4.4, $J_{9,9'}$ 12.3 Hz H-9), 4.29 (dd, 1 H, $J_{9,9'}$ 12.2, $J_{8,9}$ 2.3 Hz, H-9'), 4.82 (ddd, 1 H, $J_{4,5}$ $J_{4,3a}$ 10.5 $J_{4,3e}$ 4.4 Hz, H-4), 5.20 (d, 1 H, J_{NH}, 5 10.0 Hz, NH), 5.28 (m, 1 H, H-7), 5.30 (m, 1 H, H-8). ^{13}C NMR data (75 MHz, $CDCl_3$): alkyl unit-δ 10.6, 10.7 (C-4), 25.1, 25.2 (C-3), 32.4 (C-1), 40.5

(C-2); Neu5Ac unit-δ 20.8, 21.1 [4 OC(O)Me], 23.1 (NH*Ac*), 38.2 (C-3), 49.4 (C-5), 52.8 (CO_2*Me*), 62.2 (C-9), 67.5, 69.1, 69.8 (C-4, C-7, C-8), 74.2 (C-6), 83.2 (C-2), 168.5 (C-1), 170.1, 170.6, 170.9, [4 O*C*(O)Me]. FABMS: 592 $(M+1)^+$, 532 $(M^+ - CO_2CH_3)$

To a stirred solution of compound **3** (286 mg, 4.9 mmol) in anhydrous methanol (4 mL) was added sodium methoxide [1 mL of a solution of Na (20 mg) in anhydrous methanol (3 mL)] at room temperature under an inert atmosphere. Upon completion (TLC) the solvent was removed (in vacuo) to yield an amorphous mass, to which was added water (4 mL). The pH of this solution was adjusted to 14 with (0.1 M) NaOH and stirred overnight. Dowex®-50W X 8 (H^+) resin was then added to bring the pH to 3.0, and the solution was immediately filtered through Celite. The mixture was then freeze dried to yield the title compound **6** as an amorphous mass that was subjected to chromatography (silica gel, 7:2:1 EtOAc–MeOH–H_2O) to afford **6** (173 mg, 85%): $[\alpha]_D$ +18.0° (*c* 0.01, H_2O); ^{1}H NMR data (300 MHz, D_2O): alkyl unit-δ 0.90 (t, 6 H, H-4), 1.42 (bm, 4 H, H-3), 1.52 (m, 1 H, $J_{2,1'}$ 12.2 $J_{2,1}$ 6.0 Hz, H-2), 2.66 (dd, 1 H, $J_{1,2}$ 6.8 $J_{1,1'}$ 12.3 Hz, H-1), 2.85 (d, 1 H, $J_{1,1'}$ 5.5 Hz H-1′); Neu5Ac unit-δ 1.78 (dd, 1 H, $J_{3a,3e}$ 12.0, $J_{3a,4}$ 11.8 Hz, H-3a), 2.09 (s, 3 H, AcN), 2.85 (dd 1 H, $J_{3e,4}$ 5.0 $J_{3e,3a}$, 12.1 Hz, H-3e), 3.63–3.80 (m, 4 H), 3.84–3.95 (m, 3H). ^{13}C NMR data (75 MHz, D_2O): alkyl unit-δ 12.51, 12.81 (C-4), 27.18, 27.53 (C-3), 35.36 (C-1), 42.78 (C-2); Neu5Ac unit-δ 24.56 [NHC(O)*Me*], 43.54 (C-3), 54.32 (C-5), 65.10 (C-9), 70.69 (C-4), 70.94 (C-7), 74.44 (C-8), 77.33 (C-6), 87.76 (C-2), 176.38 (C1), 177.60 [NH*C*(O)Me]; FABMS 410 $(M+1)^+$; HRMS calculated for $C_{17}H_{32}NO_8S$: 410.1848; found 410.1834.

In a similar manner were prepared the following compounds:

Sodium (2S)-2-methylbutyl 5-acetamido-2,3,5-trideoxy-2-thio-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**7**).—Methyl [(2*S*)-2-methylbutyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-thio-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (**4**) was prepared in a similar manner to compound **3**. Thus, treatment of methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-2,3,5-trideoxy-2-thio-*glycero*-α-D-*galacto*-2-nonulopyranosonate (300 mg, 0.55 mmol) with 2-methyl-1-bromobutane (78 mg, 0.52 mmol) gave **4** in 86% yield after chromatography (silica gel, 7:2:1 EtOAc–MeOH–H_2O) as an amorphous white solid: $[\alpha]_D$ +134.2° (*c* 0.01, $CHCl_3$); ^{1}H NMR data (300 MHz, $CDCl_3$): alkyl unit-δ 0.87 (t, 6 H, H-4), 0.95 (t, 3 H, H-3), 1.23 (m, 1 H, H-3′), 1.45 (m, 2 H, H-2,3) 2.57 (ddd, 2 H, H-1); Neu5Ac unit-δ 1.87 (s, 3 H, AcN), 2.02, 2.03, 2.13, 2.15 (4 s, 12 H, 4 Ac), 2.72 (dd, 1 H, $J_{3e,3a}$ 11.4, $J_{3e,4}$ 4.6 Hz, H-3e), 3.80 (s, 3 H, CO_2CH_3), 3.85 (dd, 1 H, $J_{6,7}$ 1.7, $J_{6,5}$ 10.7 Hz, H-6), 4.05 (ddd, 1 H, $J_{5,6} = J_{5,4} = J_{5,NH}$ = 10.3 Hz H-5), 4.13 (dd 1 H, $J_{9,8}$ 4.4, $J_{9,9'}$ 12.2 Hz H-9), 4.30 (dd, 1 H, $J_{9,9'}$ 11.9, $J_{8,9}$ 2.1 Hz, H-9′), 4.84 (ddd, 1 H, $J_{4,5} = J_{4,3a}$ 10.7, $J_{4,3e}$ 4.3 Hz, H-4), 5.12 (d, 1 H, $J_{NH,5}$ 10.6 Hz, NH), 5.33 (bm, 2 H, H-7, H-8); ^{13}C NMR data (75 MHz, $CDCl_3$): alkyl unit-δ 1.3 (C-4), 18.1 (C-3), 28.2 (C-3), 34.9 (C-2), 35.5 (C-1); Neu5Ac unit-δ 21.3, 21.0 [4 OC(O)*Me*], 38.3 (C-3), 49.4 (C-5), 53.2 (CO_2*Me*), 62.4 (C-9), 67.7, 69.3, 69.9 (C-4, C-7, C-8), 83.2 (C-2), 168.7 (C-1). 170.3, 170.8, 171.1 [4 O*C*(O)Me]; FABMS: 578 ($(M+1)^+$, 50%), 518 (69), 474, (35), 414 (100).

Compound **4** was deprotected as described above for **6** to give **7** in 75% yield after chromatography (silica gel, 7:2:1 EtOAc–MeOH–H_20) as an amorphous white solid: $[\alpha]_D$ +31.6° (*c* 0.01, H_2O); ^{1}H NMR data (300 MHz, D_2O): alkyl unit-δ 0.78 (t, 3 H,

H-4), 0.86 (d, 3H, H-3′), 1.09 (hept, 1 H, $J_{3',4} = J_{3',2} = J_{3',3}$ 7.3 Hz, H-3′), 1.36 (hept, 1 H, $J_{3,4} = J_{3,2} = J_{3,3'}$ 6.5 Hz, H-3), 1.55 (pent, 1 H, $J_{2,3'} = J_{2,1} = J_{2,3}$ 6.5 Hz, H-2), 2.55 (dd, 2 H, $J_{1,2}$ 6.5 Hz, H-1); Neu5Ac unit-δ 1.69 (t, 1 H, $J_{3a,3e}$ 12.3 Hz, H-3a), 1.95 (s, 3 H, AcN), 2.72 (dd, 1 H, $J_{3e,3e}$ 12.8, $J_{3e,4}$ 4.6 Hz, H-3e), 3.48–3.65 (m, 4 H), 3.70–3.81 (m, 3 H). ^{13}C NMR data (75 MHz, D_2O): alkyl unit-δ 13.1 (C-4), 21.0 (C-3), 30.6 (C-3), 36.9 (C-1), 36.8 (C-2); Neu5Ac unit-δ 24.7 [NHC(O)*Me*], 43.6 (C-3), 54.4 (C-5), 65.3 (C-9), 70.9 (C-4), 71.0 (C-7), 74.5 (C-8), 77.4 (C-6), 87.8 (C-2), 176.5 (C-1), 177.70 [NH*C*(O)Me]; FABMS 396 $(M + 1)^+$; HRMS calculated for $C_{16}H_{30}NO_8S$: 396.1692; found 396.1697.

Preparation of sodium 2-propenyl 5-acetamido-2,3,5-trideoxy-2-thio-D-glycero-α-D-galacto-*2-nonulopyranosidonic acid* (**8**).—Compound **8** was cleanly prepared from **5** under the same reaction conditions described for **6** in 65% yield after chromatography (reversed-phase silica gel, water) as an amorphous white solid: $[\alpha]_D$ +27.0° (*c* 0.01, H_2O); ^{1}H NMR data (300 MHz, D_2O): allyl unit-δ 3.36 (d, 2 H, $J_{1,1'}$ 6 Hz H-1,1′), 5.03 (d, 1 H, $J_{3,2}$ 10.3 Hz H-3 *cis*), 5.15 (d, 1 H, $J_{3,2}$ 16.9 Hz, H-3*trans*), 5.73–5.84 (m, 1 H, H-2); Neu5Ac unit-δ 1.77 (t, 1 H, $J_{3a,3e}$ 12.7 Hz, H-3a), 1.95 (s, 3 H, AcN), 2.72 (dd, 1 H, $J_{3e,3a}$ 12.7, $J_{3e,4}$ 5 Hz, H-3e), 3.40–3.60 (m, 4 H), 3.68–3.76 (m, 3H). ^{13}C NMR data (75 MHz, D_2O): allyl unit-δ 34.8 (C-1), 120.7 (C-3), 136.3 (C-2); Neu5Ac unit-δ 24.6 [NHC(O)*Me*], 42.8 (C-3), 54.4 (C-5), 65.4 (C-9), 70.2 (C-4), 71.1 (C-7), 73.8 (C-8), 77.5 (C-6), 86.3 (C-2), 174.45 (C-1′), 177.60 [NH*C*(O)Me]; FABMS 366 $(M + 1)^+$; HRMS calculated for $C_{14}H_{24}NO_8S$: 366.1223; found 366.1227.

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