Regio- and Stereo-selectively Deuteriated Sialyl Glycerolipids for Dynamic Studies by ^2H NMR Spectroscopy†

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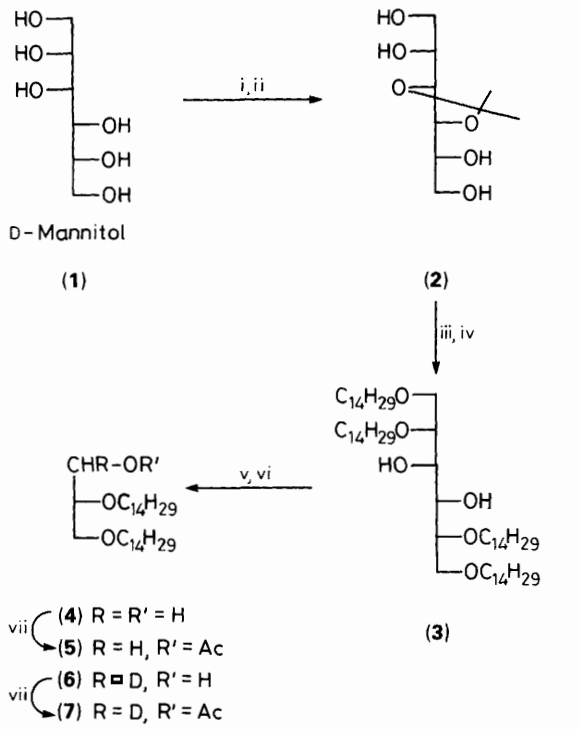
Unlabelled and deuterium labelled [3-$^2H_1$]1,2-di-*O*-tetradecyl-sn-glycerol were prepared from D-mannitol through the intermediacy of 3,4-isopropylidene-D-mannitol; regio- and stereo-selective mono- and bis-deuteriation of sialic acid under base catalysed enolization gave [3ax-$^2H_1$]- and [3ax,3eq-$^2H_2$]-sialic acids which were glycosidated with the glycerolipids after derivatization.

^2H NMR spectroscopy is a powerful, non-destructive technique for studying the orientation and motional properties of molecules in an anisotropic environment, *e.g.*, lipid molecules in biological membranes.[1] The application of ^2H NMR spectroscopy to glycolipids has yielded information on the orientation, ordering, and dynamic properties of both mono- and di-saccharide head groups.[2,3] Sialic acid (**8**) has been sought as an excellent candidate for ^2H NMR studies because its ubiquitous presence at the penultimate non-reducing ends of glycoproteins and glycolipids has been associated with a number of biological and immunological phenomena.[4] For instance, sialyloligosaccharides have been identified as receptors for the human influenza virus[5] and as oncogenic markers.[6] The present study extends the previous studies[2] to sialic acid glycerolipids labelled on either the sialic acid or on the glycerol residues. The syntheses of the non-deuteriated (**4**) and (**9**) and the selectively deuteriated (**6**), (**10**), and (**11**) glycerolipids and sialic acid analogues are depicted in Schemes 1 and 2 respectively.

The strategy involved in the synthesis of the known[7] 1,2-di-*O*-tetradecyl-sn-glycerol (**4**) differed from the previous one[7] in that the key intermediate was 3,4-isopropylidene-D-mannitol[8] (**2**) [m.p. 85.2—86.6 °C, $[\alpha]_D^{23}$ + 30.2° (H_2O)]‡ instead of the more usual (*R*)-2,3-*O*-isopropylideneglyceraldehyde. The Scheme 1 depicted herein uses one less step[7] and avoids the manipulation of oily intermediates. Thus, D-mannitol was transformed into the crystalline tetrol (**2**) following a sequence of tris-acetonation/kinetic de-acetonation[8] (58% overall). Alkylation of the tetrol (**2**) with tetradecyl bromide [NaH, *N*,*N*-dimethylformamide (DMF), 76%] gave pure acetonide (**3**) after silica gel chromatography. Acid

† A preliminary account of this work has been presented at the Japanese–German Symposium on Sialic Acids, Berlin, May 18—21, 1988.

‡ All compounds had satisfactory analyses and spectroscopic characteristics.



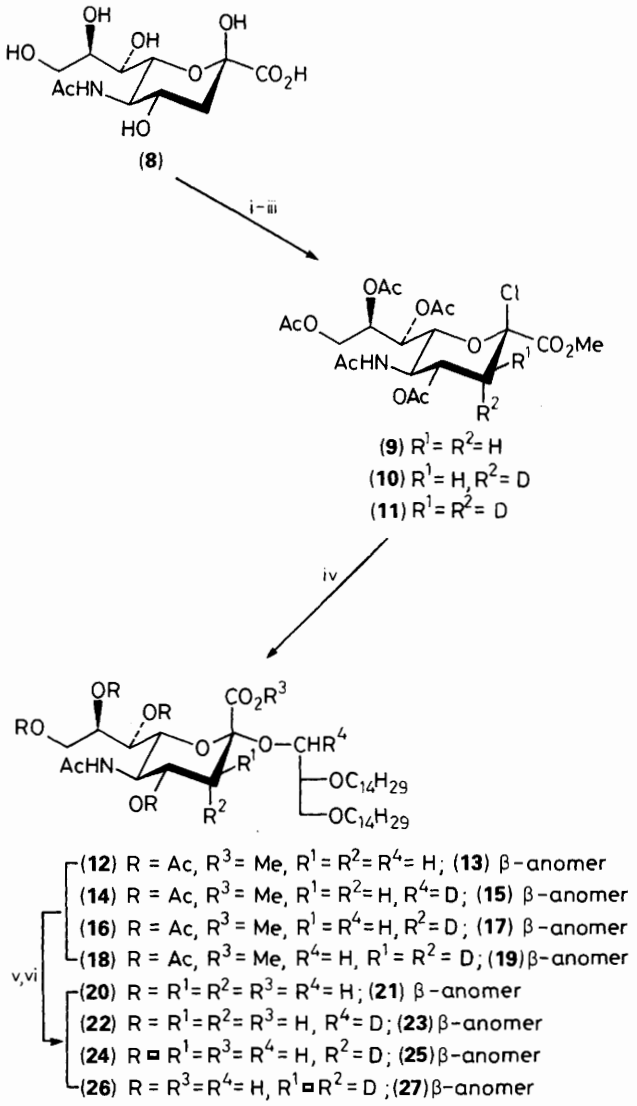
Scheme 1. *Reagents and conditions:* i, H_2SO_4, acetone, HOAc, 25 °C, 20 h, 67%; ii, 70% $HOAc_{aq}$, 40 °C, 1.75 h, 86%; iii, $Me(CH_2)_{13}Br$, NaH, DMF, 25 °C, 48 h, 76%; iv, HCl (1 M), MeOH, $CHCl_3$ (1:4:6 v/v), reflux, 4 days, 91%; v, H_5IO_6, Et_2O, 25 °C, 20 h, 88%; vi, $NaBH_4$ or $NaBD_4$, MeOH, 25 °C, 6 h, 93%; vii, Ac_2O, pyridine, 25 °C, 1 h, 99%.

hydrolysis of the isopropylidene group furnished the crystalline diol (**3**) (91%, m.p. 41.3—42.2 °C, $[\alpha]_D^{23}$ −8.1°). Periodic acid cleavage of the vicinal diol in ether afforded the aldehyde (88%, m.p. 28.0—29.6 °C, $[\alpha]_D^{23}$ +9.4°) which was then reduced with $NaBH_4$ or $NaBD_4$ to give unlabelled (**4**) and [3-$^2H_1$] labelled 1,2-di-*O*-tetradecyl-sn-glycerol (**6**), respectively (93%, m.p. 42.0—42.6 °C, $[\alpha]_D^{23}$ −9.3°). Compound (**4**) has the same physical properties as the compound prepared previously by a different route.[7]

To establish whether diastereofacial stereoselectivity occurred to an appreciable extent during borodeuteride reduction, alcohols (**4**) (for comparison) and (**6**) were subjected to acetylation (99% yield). As expected, both pro-(*R*) and pro-(*S*) H-3 protons exhibited a downfield shift (~0.6 p.p.m.) in their ^1H NMR spectra (200 MHz), which permitted their characterization. These protons were highly overlapped in the δ ~3.35—3.75 region for (**4**) and (**6**). Two signals of equal intensity appeared as a doublet of doublets at δ 4.05 (J_{gem} 11.6, $J_{2,3}$ 5.6 Hz) and 4.17 (J_{gem} 11.6, $J_{2,3}$ 4.1 Hz) for (**5**) while these signals converged to two doublets of almost identical intensity (1.05 : 1) at δ 4.05 ($J_{2,3}$ 5.6 Hz) and 4.17 ($J_{2,3}$ 4.1 Hz) for the deuteriated analogue (**7**). These results were indicative of no strong preferential stereochemical induction.[9]

In order to favour anti-Cram (chelation) diastereoselectivity during the reduction of the aldehyde precursor, zinc borodeuteride was used instead of sodium borodeuteride [$Zn(BD_4)_2$, Et_2O, 25 °C].§ In this case, the level of induction was 62 : 38 (1.6 : 1) in favour of a selective deuteriation at the pro-(*R*) C-3 position (tentative assignment).[9] Thus, one signal

§ No improvement in tetrahydrofuran (THF) and at lower temperature.



Scheme 2. *Reagents and conditions:* i, NaOD, D_2O, 25 °C, 3.5 h for (**10**), 48 h for (**11**), pD 11.6; ii, MeOH, Dowex 50-X8 (H^+), 25 °C, 24 h, 95%; iii, AcCl, HOAc, 25 °C, 48 h, >95%, iv, $Hg(CN)_2$, $HgBr_2$, (**4**) or (**6**), CH_2Cl_2, 4Å molecular sieves, 25 °C, 48 h, 37% α, 25% β; v, NaOMe, MeOH, 25 °C, 4.5 h, 95%; vi, NaOH (0.1 M), THF (4 : 1 v/v), 25 °C, 4.5 h, 90%.

appeared at δ 4.05 [d, $J_{2,3}$ 3.9 Hz, 1H, 1 $H_{3\text{-pro-}(S)}$] and one at δ 4.17 [d, $J_{2,3}$ 5.6 Hz, 0.63 H, $H_{3\text{-pro-}(R)}$].

We then turned our attention to the deuteriated sialic acid precursors (**10**) and (**11**) (Scheme 2). The regio- and stereoselective deuterium incorporations in sialic acid (**8**) were performed on a preparative scale (~500 mg) following a slight modification (H^+ resins neutralization) of literature procedures.[10] Hence, base catalysed enolization of the H-3 protons (H-3ax, δ 1.83; H-3eq, δ 2.21) of (**8**) confirmed previous kinetic measurements[10] [k(H-3ax) : k(H-3e) 21.4 : 1] and afforded quantitative yields of [H-3ax-$^2H_1$] sialic acid (pD 11.6, 25 °C, 3.5 h) which was ~92% deuteriated. Selective removal of the H-3ax proton was evidenced by the absence of its signal at δ 1.83 and by the appearance of the H-3eq signal as a doublet. As is evident from the observed coupling constants, the geminal coupling (12.7 Hz) was missing. A similar treatment of (**8**) (pD 11.6, 25 °C, 48 h) afforded fully deuteriated (>95%) [H-3ax,H-3eq-$^2H_2$] sialic acid.

The unlabelled and deuterium labelled sialic acids were then transformed into their respective glycosyl donors (**9**)—(**11**) [m.p. 91.1—93.1 °C (sint'd), 105 °C (melt), $[\alpha]_D^{23}$ −64°] following the two step procedures that one of us originally proposed (H^+, MeOH then AcCl, HOAc; >90% overall).[11] This simplified procedure is noteworthy in the light of recent literature confusion concerning its preparation in three steps.[12]

Attempts to glycosylate (**4**) and (**6**) with the glycosyl donors (**9**)—(**11**) under conditions different from those previously published[13] for the non-labelled analogue (**12**) were unsuccessful. Some preliminary successes with the use of silver salicylate were not reproducible, and thus mercuric cyanide and mercuric bromide were finally adopted as suitable catalysts.[13] Glycosidations as previously described by Ogawa and Sugimoto[13] afforded the α-anomers (**12**)—(**18**) as the major products (37% yield) together with the β-anomers (**13**)—(**19**) (25% yield) which were separated by flash chromatography. The α-anomers (**12**)—(**18**) had $[\alpha]_D^{23}$ −8.6° ($CHCl_3$) and showed characteristic ^1H NMR spectra with H-3eq signals [except (**18**)] at δ 2.55. The H-3eq signals of the β-anomers (**13**)—(**19**) {$[\alpha]_D^{23}$ −15.2° ($CHCl_3$)} have chemical shifts of δ 2.41 [except for (**17**) and (**19**)], indicative of their anomeric configurations in accord with well established empirical rules.[14] Zemplen deacetylation (NaOMe, MeOH) afforded semi-crystalline glycoside methyl esters of (**20**)—(**26**) {95%, m.p. 106 °C (sint'd), 139—140° (melt), $[\alpha]_D^{23}$ + 0.4°} which were then transformed into the sodium salts of (**20**)—(**26**) ($[\alpha]_D^{23}$ + 10.4°, MeOH; m.p. 138—138.5°; 90% yield) after saponification [NaOH (0.1 M), THF]. All α-glycosides (**20**)—(**26**) showed intense (base peak) negative FAB MS at 774(M^-) for (**20**), 775 for (**22**) and (**24**), and 776 (**26**) for $C_{42}H_{80}NO_{11}$ ($H_{79}D$ or $H_{78}D_2$).

Preliminary ^2H NMR studies on (**26**) under conditions previously described for other glycerolipids[2] showed quadrupolar splitting $\Delta\nu_Q$ of ~18 kHz (50 °C). The spectra were indicative of molecules undergoing axially symmetric anisotropic motion. Partially relaxed spectra of (**26**) at 30 °C revealed a null point at a delay time of <5 ms, indicating a spin–lattice relaxation time T_1 of <7 ms. This suggests a head group mobility comparable with other glycerolipids, but reduced compared to phospholipids.

In conclusion, sialic acid containing glycerolipids having regio- and stereo-selectively incorporated deuterium labels are good models of cell membranes for dynamic studies by ^2H NMR spectroscopy.

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