Pergamon

Tetrahedron Letters, Vol. 37, No. 40, pp. 7307-7310, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01594-8

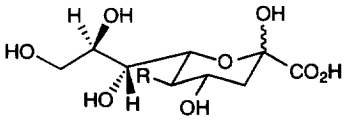
The First Synthesis of an Alkylmercury Containing *N*-Acetylneuraminic Acid Derivative

Milton J. Kiefel and Mark von Itzstein*

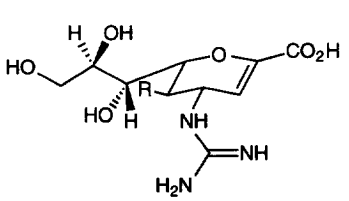
Department of Medicinal Chemistry, Victorian College of Pharmacy, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

Abstract: Structurally modified *N*-acetylneuraminic acids are important as potential substrates or inhibitors of sialic acid-recognising proteins as well as probes for the elucidation of sialic acid metabolism. This report describes the synthesis of a novel alkylmercury-containing *N*-acetylneuraminic acid derivative as a potential heavy-atom derivative for use in X-ray crystallographic studies.

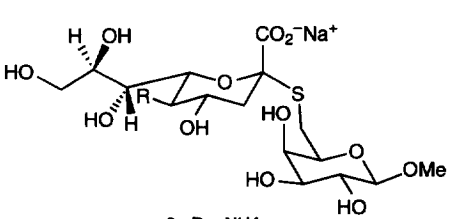
The importance of *N*-acetylneuraminic acid [Neu5Ac (**1**)] and related sialic acids as α-ketosidically-linked terminal units of many glycoproteins and glycolipids,[1,2] has resulted in a number of studies into the biological roles of such compounds, especially their importance in cell recognition phenomena.[1,3] As a consequence of these biological properties there has been a resurgence over recent years in the reported syntheses of structurally-modified sialic acids,[4] as potential substrates or inhibitors of sialic acid-recognising proteins, as well as probes for the elucidation of sialic acid metabolism. Our own efforts in this regard have resulted in the synthesis of the potent influenza virus sialidase inhibitor 4-deoxy-4-guanidino-Neu5Ac2en (**2**),[5] as well as the preparation of a variety of thiosialosides (e.g. **3**) as potential inhibitors of rotaviral infection.[6] The use of thiosialosides such as **3** as potential biological probes is of particular interest since it has been demonstrated[7] that such compounds are resistant to hydrolysis by influenza virus sialidase. Thiosialosides may therefore prove useful in studies aimed at elucidating information regarding structure-activity relationships between various sialic acid-recognising proteins and analogues or derivatives of *N*-acetylneuraminic acid.



1 R = NHAc



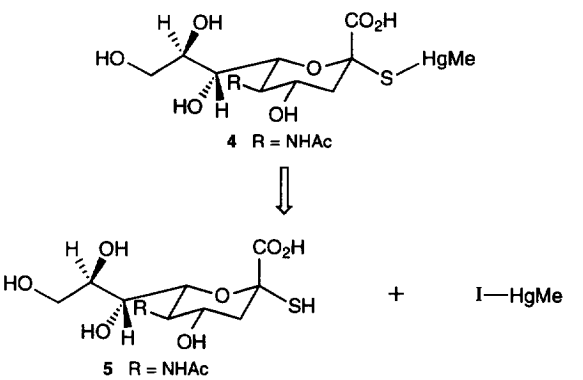
2 R = NHAc



3 R = NHAc

Whilst the use of a series of structurally related compounds as biological probes can be helpful in creating a model of the active site of a protein, definitive information about the nature of the binding pocket can best be obtained from X-ray crystallographic studies. Due to the size and structural complexity of proteins, it is often desirable to have the substrate or inhibitor bound in the active site during the acquisition of crystallographic data. Data gained in this way can be far more informative since it shows not only the gross structure of the active site, but also gives information regarding the types of interactions taking place between the substrate or inhibitor and the residues in the active site. The presence of a heavy atom near the active site during the acquisition of crystallographic data can be of further benefit, since it aids the interpretation of X-ray data.[8]

In view of this, and as part of our continued interest in the synthesis of thiosialosides,[6,9,10] we reasoned that an alkylmercury containing thiosialoside such as **4** would be ideally suited to use in X-ray crystallographic studies on sialic acid-recognising proteins, since it should be stable to hydrolysis and also contains a heavy atom. Our initial approach towards the synthesis of **4** is depicted in retrosynthetic terms in Scheme 1. Given the affinity of sulfur for mercury, and the substantial literature precedence for the preparation of alkylmercury mercaptides from the reaction between an alkylmercury halide and a thiol,[11] we felt that condensation between 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosonic acid (**5**)[12] and an alkylmercury halide would furnish the desired mercury containing thiosialoside **4**. Accordingly, a solution of the thiol **5** in MeOH was exposed to MeHgI[13] in the presence of aqueous NaOH (2M)[11b] at room temperature for 1 h gave a pale yellow solution containing a dark precipitate. Removal of the precipitate by filtration and examination of the filtrate by ^{1}H n.m.r. spectroscopy revealed that the product contained no methylmercury residue. Indeed the ^{1}H n.m.r. spectrum of the water soluble component of the reaction mixture was identical with the ^{1}H n.m.r. spectrum of Neu5Ac (**1**) itself.

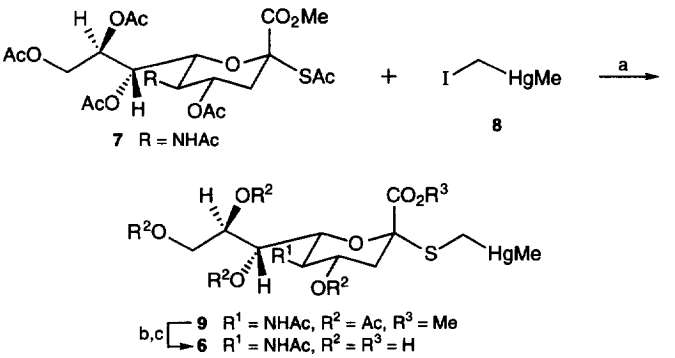


Scheme 1

The quantitative formation of Neu5Ac (**1**) from this reaction was indeed unexpected, and presumably occurs *via* the displacement of methylmercuric sulfide (dark precipitate) from **4** by hydroxide during the course of the reaction. This represents the only example, in our hands, of the hydrolysis of a thiosialoside under alkaline conditions, and must in part be due to the stability of the leaving group (methylmercuric sulfide) as well as the strength of the mercury–sulfur bond. Clearly a compound such as **4**, even if isolated, would be too unstable to serve as a useful compound for elucidating information about the active site of sialic acid-recognising proteins.

In order to circumvent the inherent instability of **4**, we felt that it would be desirable to insert a carbon spacer between the sulfur and the mercury atoms, resulting in a compound such as **6** which would hopefully be

more resistant to hydrolysis. We envisaged that **6** could be prepared from coupling between the known[9] 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 (**7**) and a monohaloalkylmercury(II) compound (eg. **8**), followed by deprotection (Scheme 2). Inspection of the literature revealed a simple method for the preparation of compounds like **8** *via* an alkylidene insertion reaction.[14] Thus, reaction of methylmercury iodide with ethereal diazomethane at –5 °C gave the thermally unstable methylene inserted product **8**. [**Caution:** monohaloalkylmercury(II) compounds are described[14] as "highly toxic and cause severe skin lesions even in dilute solutions".]



Scheme 2. (a) DMF, Et_2NH, 0 °C to RT, 15 h, 58%[9]; (b) NaOMe, MeOH, 0 °C to RT, 2 h; (c) NaOH (2M), MeOH, RT, 15 h, 74%.

To our delight, reaction between **7** and iodomethyl methylmercury (**8**) in *N,N*-DMF containing Et_2NH,[9] at 0 °C for 1 h followed by 15 h at room temperature, furnished the desired mercury containing thiosialoside **9** in 58% yield after chromatography (Scheme 2). The moderate yield may in part be due to some thermal decomposition of **8** during the course of the reaction. That the product from this coupling reaction was indeed **9** was confirmed by examination of its spectroscopic data.[15] In particular, a 3 H resonance at δ 0.47 in the ^{1}H n.m.r. spectrum of **9** exhibits a doublet symmetrically situated about the central methyl resonance, due to coupling with the ^{199}Hg nucleus (natural abundance 16.86%, $^2J_{Hg,H}$ = 131.7 Hz), consistent with the presence of a methylmercury residue.[11b,16] The positive electrospray mass spectrum of **9**[15] is also consistent with the proposed structure, showing a molecular ion of seven lines corresponding in intensity to the natural abundance of the mercury isotopes.

Treatment of **9** with NaOMe in MeOH followed by exposure to dilute aqueous NaOH (2M) provided the fully deprotected mercury containing thiosialoside **6**[17] in 74% yield after chromatography. Importantly, during the saponification process no formation of Neu5Ac (**1**) was detected (by t.l.c.) which suggests that the insertion of a methylene between the sulfur and mercury atoms has indeed resulted in a compound which is resistant to alkaline hydrolysis.

The preparation of the thiosialoside **9** represents the first reported synthesis of a mercury containing *N*-acetylneuraminic acid analogue. While the value of thiosialosides like **6** in assisting the acquisition and interpretation of X-ray crystallographic data on sialic acid-recognising proteins is still to be established, this report demonstrates the ease with which such compounds can be prepared. Studies directed towards further expanding the coupling of the 2-thioacetyl-Neu5Ac derivative **7** with glycosyl acceptors are continuing.

Acknowledgements: We wish to thank the Monash Research Fund and the Australian Research Council for financial support and Dr Jenny Wilson for help with n.m.r. acquisition.

References and Notes

1. (a) Corfield, A. P.; Schauer, R. *Sialic Acids, Chemistry, Metabolism and Function* in *Cell Biology Monographs*; Schauer, R., Ed., Springer Verlag: Wien, 1982; Vol. 10, pp 195–261. (b) Schauer, R. *Adv. Carbohydr. Chem. Biochem.*, **1982**, *40*, 131–234.
2. Horowitz, M. J.; Pigman, W. *The Glycoconjugates*; Academic Press: New York, 1978; Vol. I and II. (b) Tuppy, H.; Gottschalk, A. *Glycoproteins: Their Composition, Structure and Function*; Gottschalk, A., Ed., Elsevier: London, 1972; pp. 403–449.
3. (a) Reutter, W.; Köttgen, E.; Bauer, C.; Gerok, W. *Sialic Acids, Chemistry, Metabolism and Function* in *Cell Biology Monographs*; Schauer, R., Ed., Springer Verlag: Wien, 1982; Vol. 10, pp. 263–305. (b); Jeanloz, R. W.; Codington, J. F. *Biological Roles of Sialic Acids*; Rosenberg, A; Schengrund, C. L., Eds., Plenum Press: New York, 1976; pp. 201–227.
4. For recent reviews see: (a) Zbiral, E. *Carbohydrates: Synthetic Methods and Applications in Medicinal Chemistry*; Ogura, H.; Hasegawa, A.; Suami, T., Eds., VCH: New York, 1992; pp. 304–339; (b) von Itzstein, M.; Kiefel, M. J. *Carbohydrates: Targets for Rational Drug Design*; Witczak, Z. J.; Nieforth, K. A., Eds., MDI: New York, in press; (c) Wong, C.-H.; Whitesides, G. M., Eds., *Enzymes in Synthetic Organic Chemistry* in *Tetrahedron Organic Chemistry Series*; Baldwin, J. E.; Magnus, P. D., Eds., Elsevier: Oxford, 1994; pp. 215–219; (d) DeNinno, M. P. *Synthesis*, **1991**, 583–593.
5. von Itzstein, M.; Wu, W.-Y.; Jin, B. *Carbohydr. Res.*, **1994**, *259*, 301–305.
6. Kiefel, M. J.; Beisner, B.; Bennett, S.; Holmes, I. D.; von Itzstein, M. *J. Med. Chem.*, **1996**, *39*, 1314-1320.
7. (a) Pegg, M. S.; Wilson, J. C.; Kiefel, M. J.; Laver, W. G.; von Itzstein, M. unpublished results. (b) See for example: Suzuki, Y.; Sato, K.; Kiso, M.; Hasegawa, A. *Glycoconjugate J.*, **1990**, *7*, 349–356.
8. Glusker, J. P.; Trueblood, K. N. *Crystal Structure Analysis: A Primer*; Oxford University Press: New York, 1972.
9. Bennett, S.; von Itzstein, M.; Kiefel, M. J. *Carbohydr. Res.*, **1994**, *259*, 293–299.
10. (a) Smalec, B.; von Itzstein, M. *Carbohydr. Res.*, **1995**, *266*, 269–272. (b) Angus, D. I.; von Itzstein, M. *Carbohydr. Res.*, **1995**, *274*, 279–283.
11. See for example: (a) Makarova, L. G.; Nesmeyanov, A. N. *Methods in Elemento-Organic Chemistry*; Nesmeyanov, A. N.; Kocheshkov, K. A., Eds., North-Holland Publishing: Amsterdam, 1967; Vol. 4, and references therein; (b) Bach, R. D.; Weibel, A. T. *J. Am. Chem. Soc.*, **1976**, *98*, 6241–6249.
12. The thiol **5** was prepared by treatment of **7** under the same conditions as those described for the deprotection of **9** (see text).
13. Methylmercuric iodide was prepared by reaction of methylmagnesium iodide with mercuric chloride according to the procedure described in reference 11a.
14. Barluenga, J.; Campos, P. J.; Garcia-Martin, J. C.; Roy, M. A.; Asensio, G. *Synthesis*, **1979**, 893–896.
15. Compound **9** was obtained as an amorphous mass: m.p. 80-85 °C; $[\alpha]_D$ +24.2° (*c* 0.92, $CHCl_3$); IR (KBr) 1742, 1666, 1546, 1438, 1368, 1222 and 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.47 (s, 3 H, $^2J_{Hg,H}$ = 131.7 Hz, HgMe), 1.87 (s, 3 H, AcN), 1.88 (d, 1 H, $J_{10,10'}$ = 9.1 Hz, H-10), 1.95 (dd, 1 H, $J_{3a,3e}$ = 12.5, $J_{3a,4}$ = 11.7 Hz, H-3a), 2.02, 2.03, 2.13, 2.16 (4 × s, 4 × 3 H, 4 × AcO), 2.32 (d, 1 H, H-10'), 2.69 (dd, 1 H, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.78 (s, 3 H, CO_2Me), 3.80 (dd, 1 H, $J_{6,5}$ = 10.5, $J_{6,7}$ = 2.2 Hz, H-6), 4.04 (ddd, 1 H, $J_{5,4}$ = $J_{5,NH}$ = 10.5 Hz, H-5), 4.10 (dd, 1 H, $J_{9,9'}$ = 12.5, $J_{9,8}$ = 5.4 Hz, H-9), 4.35 (dd, 1 H, $J_{9',8}$ = 2.7 Hz, H-9'), 4.85 (ddd, 1 H, H-4), 5.13 (d, 1 H, NH), 5.33 (dd, 1 H, $J_{7,8}$ = 8.4 Hz, H-7), 5.42 (ddd, 1 H, H-8), assignments confirmed by COSY; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 16.1 (HgMe), 20.9, 21.3 [4 × OC(O)*Me*], 23.2 [NC(O)*Me*], 37.6 (C-3), 39.3 (C-10), 49.4 (C-5), 52.8 (CO_2*Me*), 62.4 (C-9), 67.6, 69.2, 70.4 (C-4,C-7,C-8), 74.3 (C-6), 86.0 (C-2), 167.9 (C-1), 170.1, 170.2, 170.3, 170.7, 171.0 [4 × *O*C(O)Me, N*C*(O)Me]; MS (Electrospray) *m/z* (relative intensity): 740 (19%), 739 (20), 738 (70), 737 (42), 736 (56), 735 (37), 734 (19) (all $[M+H]^+$), 680 (12), 679 (9), 678 (38), 677 (23), 676 (31), 675 (23), 674 (13) (all $[M-CO_2Me]^+$), 522 {$[(M+H)-SCH_2HgMe]^+$, 35}, 414 {$[M+H-(CO_2Me+SCH_2HgMe)]^+$, 100}. Anal. Found: C, 35.75; H, 4.53; N, 1.72; S, 4.50. $C_{22}H_{33}NO_{12}SHg$ requires: C, 35.89; H, 4.52; N, 1.90; S, 4.36%.
16. Singh, G.; Reddy, G. S. *J. Organomet. Chem.*, **1972**, *42*, 267–275.
17. Compound **6** was obtained as an amorphous mass: ^1H NMR (600 MHz, D_2O) δ 0.31 (s, 3 H, $^2J_{Hg,H}$ = 131.0 Hz, HgMe), 1.68 (dd, 1 H, $J_{3a,3e}$ = 12.5, $J_{3a,4}$ = 11.5 Hz, H-3a), 1.90 (d, 1 H, $J_{10,10'}$ = 9.4 Hz, H-10), 1.98 (s, 3 H, AcN), 2.10 (d, 1 H, H-10'), 2.71 (dd, 1 H, $J_{3e,4}$ = 4.8 Hz, H-3e), 3.51-3.53 (m, 2 H, H-6/H-7), 3.59 (dd, 1 H, $J_{9,9'}$ = 12.0, $J_{9,8}$ = 6.0 Hz, H-9), 3.62 (ddd, 1 H, $J_{4,5}$ = 10.2 Hz, H-4), 3.75 (dd, 1 H, $J_{5,6}$ 10.2 Hz, H-5), 3.81 (dd, 1 H, $J_{9',8}$ = 2.4 Hz, H-9'), 3.85 (ddd, 1 H, $J_{8,7}$ = 8.4 Hz, H-8), assignments confirmed by COSY.

(*Received in UK* 5 *June* 1996; *revised* 8 *August* 1996; *accepted* 15 *August* 1996)