COMMUNICATION

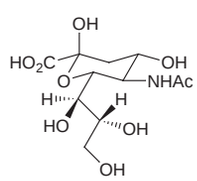
Diastereoselective synthesis of (−)-*N*-acetylneuraminic acid (Neu5Ac) from a non-carbohydrate source †

Martin Banwell,*[a] Chris De Savi[a] and Keith Watson[b]

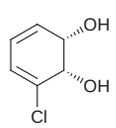
[a] *Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 0200, Australia*
[b] *Biota Chemistry Laboratory, Chemistry Department, Monash University, Clayton, Victoria 3168, Australia*

cis-1,2-Dihydrocatechol 2, a product of microbial oxidation of chlorobenzene, has been converted into a protected form, 17, of (−)-Neu5Ac (1) _via_ a fifteen step reaction sequence.

Sialic acids such as *N*-acetylneuraminic acid [(−)-Neu5Ac, **1**] have been implicated in a wide range of biologically important processes including cell-to-cell recognition, cell-adhesion, neural cell development and tumour metastasis.[1] These compounds also constitute a ligand class commonly recognised by many infectious pathogens such as viruses, bacteria and parasites. Consequently, there has been considerable interest in developing effective methods for the synthesis[2] of both sialic acids and analogues that would help elucidate their roles *in vivo*. Various enzymatic procedures including an aldolase-catalysed condensation of *N*-acetyl-D-mannosamine with pyruvic acid have been shown to provide useful quantities of compound **1**.[3] Elegant



1



2

chemical variations on this approach which use, in the penultimate step, 2-(metallomethyl)acrylates as pyruvate anion equivalents have been developed by the groups of Vasella,[4] Chan[5] and Whitesides.[6] We now report an enantiospecific and diastereoselective synthesis of a protected form of (−)-Neu5Ac that involves a related end game but which starts with the enantiopure *cis*-1,2-dihydrocatechol **2**,[7] a compound available in quantity *via* microbial oxidation of chlorobenzene. The only previous synthesis of the title compound from a non-carbohydrate source has been reported by Danishefsky *et al.*[8] who used a hetero-Diels–Alder reaction between a diene and an aldehyde to establish the pyranoid core. The present work was modelled on our recently disclosed[9] synthesis of KDN and should allow for the preparation of a wide range of ^{17}O-, ^{13}C- and/or ^{2}H-labelled (−)-Neu5Ac derivatives.

The reaction sequence leading from compound **2** to the protected form, **17**, of (−)-Neu5Ac is shown in Scheme 1. Thus, the acetonide derivative, **3**,[10] of **2** was converted into the azido alcohol **4** by established procedures.[11] The benzyl ether derivative, **5** ‡ {70%, $[\alpha]_D$ −113 (*c* 5.3)§}, of compound **4** was subjected to ozonolytic cleavage and a reductive work-up with $NaBH_4$ and in this way the diol **6** {70%, $[\alpha]_D$ +10 (*c* 1.0)} was obtained. Hydrogenolysis of the azido and benzyl ether moieties within compound **6** was achieved using dihydrogen in the presence of 10% palladium on carbon and the resulting aminotriol **7** was immediately subjected to reaction with benzyl bromide in the presence of K_2CO_3. The *N*,*N*-dibenzylated material **8** {90% from **6**, $[\alpha]_D$ −13 (*c* 4.7)} so-formed was treated with acetone and a trace of trifluoromethanesulfonic acid (TfOH) and the ensuing reaction produced bis-acetonide **9** {62%, $[\alpha]_D$ +59 (*c* 1.3)} which was identical with an authentic sample prepared from δ-gluconolactone.¶ Subjection of compound **9** to Swern oxidation conditions afforded the D-mannosamine derivative **10** {100%, $[\alpha]_D$ +52 (*c* 3.9)} which was condensed with the organozinc reagent derived from bromoacrylate **11** to give the *anti*-addition product **12** {85% at 90% conversion, $[\alpha]_D$ +5 (*c* 5.9)}.[12] Swern oxidation of this last compound then provided the highly unstable ketone **13** which was immediately reduced, in a diastereoselective fashion, with $NaBH_4$. The resulting alcohol was protected as the corresponding TMS ether **14** {60% from **12** at 67% conversion $[\alpha]_D$ +0.4 (*c* 2.3)}. Ozonolysis of the C–C double bond within alkene **14** could not be effected selectively because of competing oxidation of the *N*,*N*-dibenzyl moiety so the compound was reacted with AD-mix-α[13] and the resulting mixture of diastereoisomeric diols cleaved with lead tetraacetate to give the unstable ketone **15** (89%). Treatment of product **15** with methanolic HCl at room temperature for 18 h followed by acetylation of the crude reaction mixture afforded the nonulosonic acid derivative **16** {60%, $[\alpha]_D$ −51 (*c* 0.7)}. Debenzylation of the latter compound was readily achieved using formic acid–palladium black[14] and the intermediate amine acetylated to give the sialic acid derivative **17**[15] {75%, $[\alpha]_D$ −27 (*c* 1.0)}. This material was identical, in all respects, with an authentic sample {$[\alpha]_D$ −26 (*c* 1.4)} prepared from sialic acid according to the method[15] of Sinaÿ. Compound **17** is a versatile building block that has found considerable use[16] in the preparation of a wide range of sialic acid analogues.

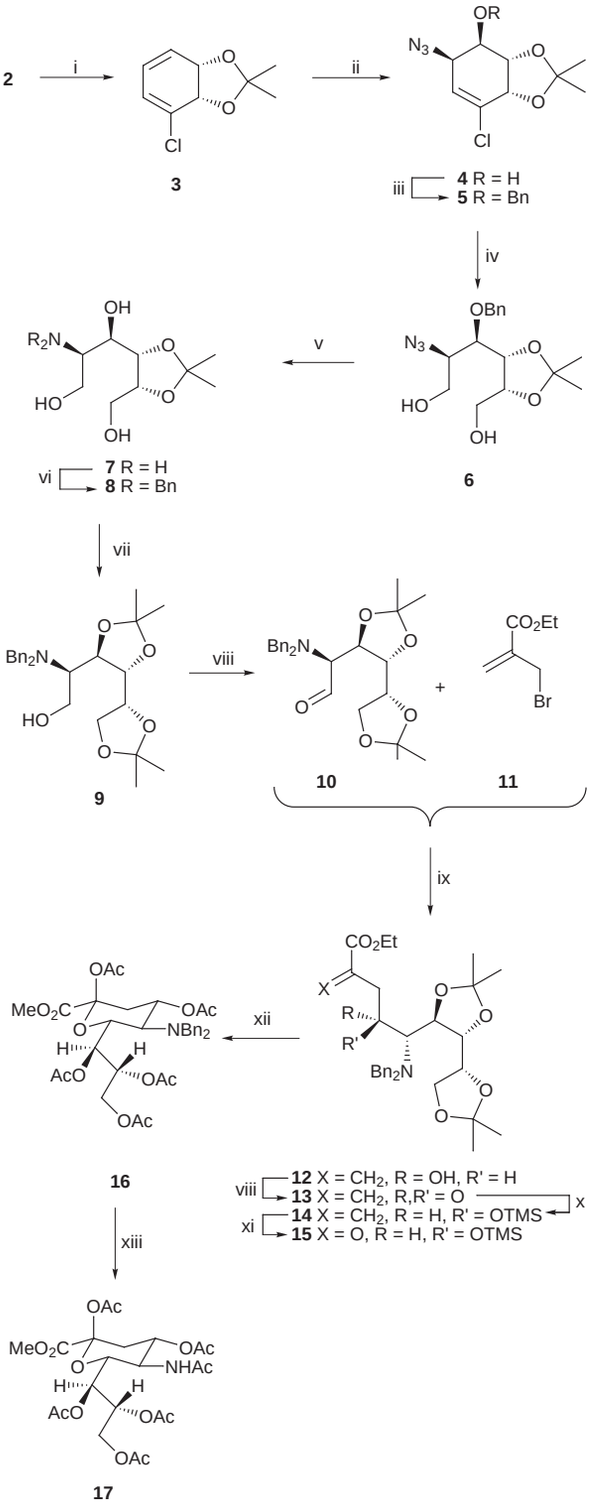
Experimental

Compound 6

A solution of compound **5** (1.30 g, 3.86 mmol) in methanol (20 ml) was cooled to −78 °C (dry-ice–acetone bath) and treated with a stream of ozone (*ca.* 40% ozone in oxygen) being produced by a Fischer Model 502 ozone generator. When the blue colour of ozone persisted and TLC analysis indicated that no starting material remained (*ca.* 0.66 h), the reaction mixture was purged with nitrogen for 0.5 h then warmed to 0 °C over 0.5 h. Sodium borohydride (1.00 g, 26 mmol) was added, in portions over 3 h, to the reaction mixture which was then warmed to 18 °C and treated with additional quantities of sodium borohydride (285 mg, 7.4 mmol). After a further 5.0 h, the reaction mixture was diluted with water (50 ml) then acidified (using 1 M aqueous HCl) to pH 3.0 and extracted with ethyl acetate (4 × 200 ml). The combined organic extracts were washed with brine (2 × 300 ml) then dried ($MgSO_4$), filtered and concen-

† The work described herein is the subject of a patent application (AIPO Patent Office Provisional Application No. PO8998, lodged September 5th, 1997).
‡ All new and stable compounds had spectroscopic data (IR, UV, NMR, mass spectrum) consistent with the assigned structure. Satisfactory combustion and/or high resolution mass spectral data were obtained for new compounds and/or suitable derivatives.
§ All optical rotations were determined in chloroform solution at 20 °C.

¶ Details of this synthesis will be disclosed shortly.



Scheme 1 *Reagents and conditions*: (i) see reference 10; (ii) see reference 11; (iii) NaH (1.1 mol equiv.), THF, 0 °C, 0.75 h then BnBr (1.3 mol equiv.), 0–18 °C, 4 h; (iv) O_3, MeOH, −78 to 0 °C, 0.05 h then $NaBH_4$ (9.0 mol equiv.), 18 °C, 7 h; (v) dihydrogen (50 psi), 10% Pd on C (20 wt%), MeOH, 18 °C, 16 h; (vi) BnBr (2.3 mol equiv.), K_2CO_3 (2.2 mol equiv.), 2:1 MeCN–H_2O, 60 °C, 16 h; (vii) Me_2CO, TfOH (cat.), 0 °C, 3 h; (viii) $(COCl)_2$ (1.2 mol equiv.), DMSO, CH_2Cl_2, −78 to 0 °C, 1 h then Et_3N (2.6 mol equiv.); (ix) Zn dust (1.2 mol equiv.), sat. aq. NH_4Cl, THF, 0 to 18 °C, 0.75 h; (x) $NaBH_4$ (6 mol equiv.), EtOH, −10 °C, 4 h then TMSCl (4.0 mol equiv.), HMDS (4.0 mol equiv.), pyridine, 0 to 18 °C, 19 h; (xi) AD-mix-α (2.2 mol equiv.), Bu^{t}OH, H_2O, 18 °C, 22 h then $Pb(OAc)_4$ (0.9 mol equiv.), $CaCO_3$ (11 mol equiv.), CH_2Cl_2, 18 °C, 0.33 h; (xii) 6% w/v HCl in MeOH, 18 °C, 18 h then Ac_2O (10 mol equiv.), DMAP (trace), pyridine, 18 °C, 20 h; (xiii) Pd black, 5% w/v HCO_2H in MeOH, 18 °C, 0.5 h then Ac_2O (10 mol equiv.), DMAP (trace), pyridine, 18 °C, 20 h. Bn = $C_6H_5CH_2$; DMAP = 4-(*N*,*N*-dimethylamino)pyridine; HMDS = hexamethyldisilazane.

trated under reduced pressure to give a light-yellow oil. Subjection of this material to flash chromatography (silica gel, 3:2 hexane–ethyl acetate elution) afforded, after concentration of the appropriate fractions (R_f 0.2), the azido diol **6** (920 mg, 70%) as a clear, colourless oil [Found: $(M - CH_3^{\bullet})^+$, 322.1408. $C_{16}H_{23}N_3O_5$ requires $(M - CH_3^{\bullet})^+$, 322.1403]; ν_{max}(NaCl)/cm^{-1} 3854 and 2101; δ_H(300 MHz, $CDCl_3$) 7.40–7.27 (5 H, m), 4.80 (1 H, d, *J* 11.2 Hz), 4.74 (1 H, d, *J* 11.2 Hz), 4.35 (1 H, t, *J* 6.1 Hz), 4.26 (1 H, q, *J* 6.1 Hz), 3.96 (1 H, m), 3.90–3.79 (2 H, complex m), 3.77–3.60 (3 H, complex m), 2.29 (1 H, br s), 2.07 (1 H, br s), 1.52 (3 H, s), 1.40 (3 H, s); δ_C(75 MHz, $CDCl_3$) 137.5 (C), 128.4 (CH), 128.0 (CH), 127.9 (CH), 108.6 (C), 78.2 (CH), 77.5 (CH), 76.7 (CH), 74.1 (CH_2), 64.5 (CH), 61.6 (2 × CH_2), 27.5 (CH_3), 25.4 (CH_3); *m/z* (EI, 70 eV) 322 [<1%, $(M - CH_3^{\bullet})^+$], 278 {21, $[M - (H_3C)_2CO - H^{\bullet}]^+$}, 91 (100).

Acknowledgements

We thank the Institute of Advanced Studies for financial support and the ARC for the provision of an APA (Industry) Scholarship to C. D. S. Dr Gregg Whited (Genencor International Inc.) is thanked for providing generous quantities of compound **2**.

References

1 M. von Itzstein and R. J. Thompson, *Top. Curr. Chem.*, 1997, **186**, 119 and references cited therein.
2 For the most recently reported chemical synthesis of (−)-Neu5Ac, *see* T. Takahashi, H. Tsukamoto, M. Kurosaki and H. Yamada, *Synlett*, 1997, 1065. For a useful review of earlier synthetic studies, *see* M. P. DeNinno, *Synthesis*, 1991, 583.
3 (*a*) C.-H. Wong and G. M. Whitesides, *Enzymes in Synthetic Organic Chemistry*, Tetrahedron Organic Chemistry Series Volume 12, Pergamon, Oxford, 1994 and references cited therein; (*b*) T. Sugai, A. Kuboki, S. Hiramatsu, H. Okazaki and H. Ohta, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 3581 and references cited therein.
4 F. Baumberger and A. Vasella, *Helv. Chim. Acta*, 1986, **69**, 1205.
5 T.-H. Chan and M.-C. Lee, *J. Org. Chem.*, 1995, **60**, 4228.
6 D. M. Gordon and G. M. Whitesides, *J. Org. Chem.*, 1993, **58**, 7937.
7 For reviews on the applications of *cis*-1,2-dihydrocatechols in synthesis, *see* (*a*) T. Hudlicky, D. A. Entwistle, K. K. Pitzer and A. J. Thorpe, *Chem. Rev.*, 1996, **96**, 1195; (*b*) T. Hudlicky and A. J. Thorpe, *Chem. Commun.*, 1996, 1993; (*c*) T. Hudlicky and J. W. Reed, in *Advances in Asymmetric Synthesis*, ed. A. Hassner, JAI, Greenwich, CT 1995, vol. 1, p. 271; (*d*) S. M. Brown and T. Hudlicky, in *Organic Synthesis: Theory and Applications*, ed. T. Hudlicky, JAI, Greenwich, CT 1993, vol. 2, 113; (*e*) H. A. J. Carless, *Tetrahedron: Asymmetry*, 1992, **3**, 795; (*f*) D. A. Widdowson, D. W. Ribbons and S. D. Thomas, *Janssen Chim. Acta*, 1990, 3.
8 S. J. Danishefsky, M. P. DeNinno and S. Chen, *J. Am. Chem. Soc.*, 1988, **110**, 3929.
9 M. Banwell, C. De Savi and K. Watson, *Chem. Commun.*, 1998, 1189.
10 T. Hudlicky, T. Nugent and W. Griffith, *J. Org. Chem.*, 1994, **59**, 7944.
11 T. C. Nugent and T. Hudlicky, *J. Org. Chem.*, 1998, **63**, 510.
12 For examples of related reactions, *see* (*a*) S. Hanessian, H. Park and R.-Y. Yang, *Synlett*, 1997, 351 and 353; (*b*) M. Banwell, C. De Savi, D. Hockless and K. Watson, *Chem. Commun.*, 1998, 645.
13 K. B. Sharpless, W. Amberg, Y. L. Bennani, G. A. Crispino, J. Hartung, K.-S. Jeong, H.-L. Kwong, K. Morikawa, Z.-M. Wang, D. Xu and X.-L. Zhang, *J. Org. Chem.*, 1992, **57**, 2768.
14 (*a*) B. ElAmin, G. M. Anantharamaiah, G. P. Royer and G. E. Means, *J. Org. Chem.*, 1979, **44**, 3442; (*b*) B. D. Gray and P. W. Jeffs, *J. Chem. Soc., Chem. Commun.*, 1987, 1329; (*c*) A. M. Diederich and D. M. Ryckman, *Tetrahedron Lett.*, 1993, **34**, 6169.
15 A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 1989, **190**, 317.
16 See, for example, (*a*) K. C. Nicolaou, C. W. Hummel and Y. Iwabuchi, *J. Am. Chem. Soc.*, 1992, **114**, 3126; (*b*) K. Higashi, S. Miyoshi, S. Nakabayashi, H. Yamada and Y. Ito, *Chem. Pharm. Bull.*, 1992, **40**, 2300; (*c*) T. Mukaiyama, T. Sasaki, E. Iwashita and K. Matsubara, *Chem. Lett.*, 1995, 455; (*d*) J. Scheigetz, R. Zamboni, M. A. Bernstein and B. Roy, *Org. Prep. Proced. Int.*, 1995, **27**, 637; (*e*) J. Gervay and T. Q. Gregar, *Tetrahedron Lett.*, 1997, **38**, 5921.

Paper 8/04524K
Received 15*th June* 1998
Accepted 24*th June* 1998