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CARBOHYDRATE RESEARCH

Note

A facile synthesis of a useful 5-*N*-substituted-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid from 2-acetamido-2-deoxy-D-glucose

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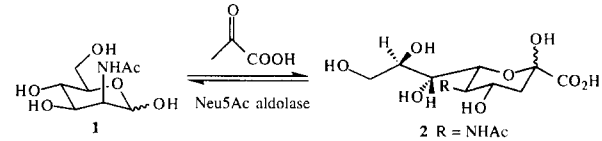
Abstract

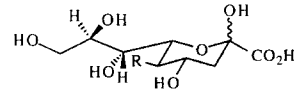
The chemoenzymic synthesis of the sialic acid Neu5Boc from the commercially-available carbohydrate *N*-acetyl-D-glucosamine is presented. A basic resin-catalysed epimerisation of *N*-acetyl-D-glucosamine to *N*-acetyl-D-mannosamine is also discussed. © 1997 Elsevier Science Ltd.

Keywords: Chemoenzymic synthesis; Sialic acid; *N*-acetyl-D-hexosamine; Epimerisation

The enzyme Neu5Ac aldolase (E.C. 4.1.3.3) catalyses a reversible aldol condensation between 2-acetamido-2-deoxy-D-mannose (*N*-acetyl-D-mannosamine, ManNAc, **1**) and pyruvic acid to give 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Neu5Ac, **2**) [1]. Neu5Ac aldolase is readily available from several species of bacteria and is also found in mammalian tissues [2,3]. Over the past several years the enzyme has been extensively used in the synthesis of both natural and unnatural sialic acids [4–14]. As part of our investigations into sialic acid-metabolising enzymes such as Neu5Ac aldolase [13], Kdn-sialidase [14] and influenza virus sialidase [15–17], we required an economical and readily available source of the functionalised neuraminic acid, 5-*tert*-butyloxycarbonylamino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Neu5Boc, **3**). This together with our interest in enzyme synthesis has led us to investigate the chemoenzymic synthesis of Neu5Boc (**3**).



1 2 R = NHAc



3 R = NHBoc

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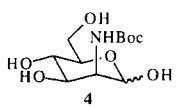
One approach to the synthesis of **3** requires the regioselective manipulation of ManNAc (**1**) to afford *N*-*tert*-butyloxycarbonyl-D-mannosamine (**4**), which then can be used as a substrate for Neu5Ac aldolase. However, given the high commercial cost of **1**, we felt that a less expensive starting material would be more appropriate, as our aim was to develop a multigram synthesis of Neu5Boc (**3**). A suitable alternative is provided with *N*-acetyl-D-glucosamine (**5**). Although epimerase-catalysed isomerisation of *N*-acetyl-D-glucosamine (**5**) to **1** has been reported [18,19], the availability and cost of the epimerase makes it somewhat unattractive. A number of literature methods for the base-catalysed epimerisation of **5** to **1** are available [20–22]; however, these procedures have been limited by low yields and are considered problematic because of the presence of salts. Therefore, we sought an alternative methodology which would avoid the limitations associated with the published procedures.



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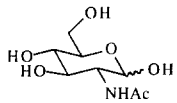

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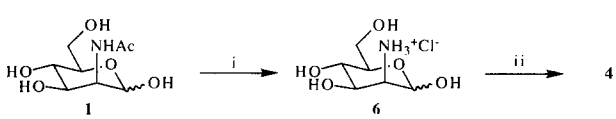
Accordingly, we have found that the basic resin, Amberlite IRA-400 (OH^-) in a continuous-flow system smoothly catalysed the epimerisation of GlcNAc (**5**) to the desired ManNAc (**1**). By exploiting the solubility differences between **1** and **5** in ethanol, a quick and useful purification method was achieved which provided **1** contaminated with ~ 10% of the starting material. Although the percentage conversion per cycle is only modest, the recyclable nature of this simple methodology facilitates conversion of large quantities of GlcNAc (**5**) to ManNAc (**1**) in good overall yield.

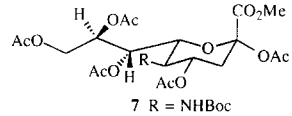
In a two-step, one-pot reaction, regiospecific manipulation of ManNAc (**1**) with 2 M hydrochloric acid, followed by exposure of the intermediate **6** to *tert*-butyloxycarbonyl anhydride, gave **4** with in good yield and high purity (> 90% by ^1H NMR spectroscopy) after workup (Scheme 1). The contaminant



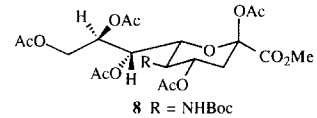
Scheme 1. Reagents and conditions: (i) 2 M HCl reflux, 16 h; (ii) *tert*-butyloxycarbonyl anhydride, H_2O, EtOH, $NaHCO_3$, room temperature, overnight.

is presumed to be the corresponding *gluco* epimer of **4** that originates from the minor GlcNAc contaminant in the ManNAc used.

The synthesis of **3** was readily achieved by exposure of **4** (used without further purification) to Neu5Ac aldolase in the presence of excess sodium pyruvate using membrane-enclosed enzyme catalysis [23]. This afforded the anomeric mixture of 5-*tert*-butyloxycarbonylamino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (**3**) in 67% yield. At equilibrium in D_2O, only the β anomer of **3** was observed by ^1H NMR spectroscopy. This is understandable given that the β anomer is presumably more thermodynamically stable compared to the α anomer [13,14]. For characterisation purposes the anomeric mixture was readily converted to the corresponding peracetylated methyl esters **7** and **8** under usual conditions [15,24].

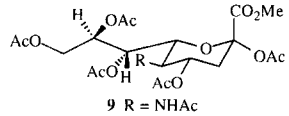


7 R = NHBoc

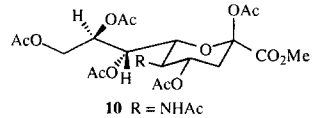


8 R = NHBoc

Not unexpectedly, the resonances due to H_{3ax} in both the α and β per-*O*-acetylated methyl esters **7** and **8**, respectively, were found to be under the *O*-acetyl resonances. The anomers **7** and **8** were fully assigned by comparison of the chemical shifts of H-4, H-7 and H-8 with the corresponding α and β anomers of the per-*O*-acetyl Neu5Ac methyl esters (**9** and **10**, respectively) reported by Marra and Sinaÿ [24].



9 R = NHAc



10 R = NHAc

In conclusion, we have successfully synthesised 5-*tert*-butyloxycarbonylamino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid using a combination of base-catalysed epimerisation, followed by a two-step, one-pot reaction and enzymic synthesis. This compound provides an alternatively C-5 protected neuraminic acid that will be useful in the further derivatisation of this position, and the process nicely complements the introduction of a CBz group using a similar strategy [25]. Moreover these results also suggest that it is possible to synthesise sialic acids functionalised at C-5 on a multigram scale by regioselective manip-

ulation of the readily available 2-acetamino-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) using minimal protecting group chemistry.

1. Experimental

General.—Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Microanalysis were performed by the Chemical and Micro Analytical Services Pty. Ltd., Belmont, Victoria, Australia.

Preparation of N-*acetyl*-D-*mannosamine* (**1**).—A solution of *N*-acetyl-D-glucosamine (100 g, 45.25 mmol) in deionised H_2O (450 mL) was passed through a column containing Amberlite IRA-400 (OH^-) resin (100 g) at a flow rate of 0.1 mL per minute. The eluant was neutralised to pH between 6 and 7 with Dowex 50 × 8 (H^+) resin and freeze dried to afford a white amorphous mass. The amorphous mass was refluxed in absolute EtOH (400 mL) for 0.5 h and left at 5 °C overnight. The resulting suspension was filtered, and the filter cake was found to be unreacted starting material (86 g). The filtrate was concentrated to give **1** (10.25 g, 73% based on reacted starting material) as a white amorphous mass contaminated with ~ 10% starting material. Both ^1H and ^{13}C NMR spectral data was in good agreement with commercial samples. This material was used without further purification.

Synthesis of N - tert - *butyloxycarbonyl* - D - *mannosamine* (**4**).—A solution of **1** (2 g, 8.36 mmol) in 2 M HCl (20 mL) was refluxed for 16 h and then stirred with active carbon Darco G-60 (0.2 g) for 30 min and filtered. The colourless filtrate was evaporated to dryness to afford the intermediate mannosamine salt **6** (1.72 g). To **6** (1.08 g, 5.02 mmol) in a mixture of H_2O (7 mL), EtOH (18 mL) and $NaHCO_3$ (0.9 g, 10.71 mmol) was added *tert*-butyloxycarbonyl anhydride (2.3 g, 10.55 mmol) in EtOH (5 mL). The resultant mixture was stirred at room temperature overnight, then concentrated to remove EtOH. The residue was diluted with H_2O (5 mL) and washed with hexane (3 × 10 mL). The aqueous layer was extracted with butanol (2 × 20 mL). The organic extracts were combined, washed with H_2O (2 × 4 mL) and concentrated. The residue was dissolved in H_2O and lyophilised to give **4** (1.04 g, 74%) as a white amorphous mass of 90% purity (by ^1H NMR). Ratio of α:β anomers by ^1H NMR was 3:2, respectively. ^1H NMR (D_2O) δ 1.41 (s, 9 H, Bu^t), 3.25–4.11 (m, 6 H, H-2, H-3, H-4, H-5, H-6), 4.93 (s, 0.4 H, H-1 β anomer), 5.08 (s, 0.6 H, H-1 α anomer); FABMS: 280 [$(M + 1)^+$, 45%], 262 (33), 206 (100), 163 (91). This material was used without further purification.

Synthesis of 5 - tert - *butyloxycarbonylamino* - *3,5* - *dideoxy*-D-*glycero*-α,β-D-*galacto*-*2*-*nonulosonic acid* (**3**).—To a solution containing **4** (1.04 g, 3.71 mmol), sodium pyruvate (4.77 g, 43.36 mmol) and sodium azide (20 mg) in H_2O (20 mL) at pH 7.8 was placed a dialysis bag containing Neu5Ac aldolase (10 U) and bovine serum albumin (25 mg) dissolved in the above-mentioned solution. The resultant mixture was stirred at 35–40 °C for 5 days. The solution was extracted with butanol (2 × 20 mL), and the aqueous layer was acidified to pH 3 with Dowex 50 × 8 (H^+) resin. The suspension was filtered, and the filtrate diluted with H_2O (30 mL), then eluted through an ion-exchange column containing AGI × 2 (formate) resin (100 mL). The resin was washed with H_2O (700 mL) and eluted with 1 M HCOOH. Fractions containing **3** were detected by TLC (1:5:1, EtOAc–2-propanol–H_2O, R_f 0.4), combined and then concentrated. The residue was dissolved in H_2O, treated with Darco G-60 (50 mg), filtered and lyophilised. The resultant amorphous mass was recrystallised with 50% aqueous MeOH to afford **3** (0.92 g, 67%): mp 106–108 °C; ^1H NMR (D_2O) of β anomer δ 1.45 (s, 9 H, Bu^t), 1.82 (dd, 1 H, $J_{3ax,3eq}$ 12.9 Hz, $J_{3ax,4}$ 12.4 Hz, H-3ax), 2.25 (dd, 1 H, $J_{3eq,3ax}$ 12.9 Hz, $J_{3eq,4}$ 4.8 Hz, H-3eq), 3.57–3.72 (m, 3 H, H-5, H-7, H-9a), 3.79–3.84 (m, 2 H, H-8, H-9b), 3.93–4.04 (m, 2 H, H-4, H-6); ^{13}C NMR (D_2O) δ 28.9 [$C(CH_3)_3$], 40.6 (C-3), 54.4 (C-5), 64.1 (C-9), 68.2 (C-4), 69.3 (C-7), 72.1 (C-6), 72.5 (C-8), 82.5 [$C(CH_3)_3$], 98.1 (C-2), 159.3 [C(O)NH], 178.0 (C-1); FABMS: 367 ($M^+ + 1$).

Synthesis of methyl 2,4,7,8,9-penta-O-*acetyl*-*5*-tert-*butyloxycarbonylamino*-*3,5*-*dideoxy*-D -*glycero*-α,β-D-*galacto* - *2* - *nonulopyranosonate* (**7** and **8**).—To a solution of **3** (125 mg, 0.34 mmol) in anhydrous MeOH (6 mL) was added $SOCl_2$ (20 μL) and trimethylorthoformate (0.15 mL) under N_2 [13]. The resultant mixture was stirred for 25 h and concentrated. The residue was dissolved in H_2O and lyophilised to afford a white amorphous mass. The amorphous mass was treated with Ac_2O (2 mL) and pyridine (4 mL) according to the procedure described by Marra and Sinaÿ [24]. After 48 h, it was concentrated and chromatographed (2:3, EtOAc–hexane) to afford **7** (30 mg, 15%) and **8** (78 mg, 39%) as an amorphous mass; ^1H NMR ($CDCl_3$) of **7** δ 1.40 (s, 9 H, Bu^t), 2.03, 2.04, 2.07, 2.09, 2.10 (5 × s, 15 H, 5 × OAc), 2.58 (dd, 1 H, $J_{3eq,4}$ 4.8 Hz, $J_{3eq,3ax}$ 13.0

Hz, H-3eq), 3.76 (s, 3 H, MeO), 3.79–3.82 (m, 1 H, H-5), 4.06 (dd, 1 H, $J_{9a,8}$ 5.6 Hz, $J_{9a,9b}$ 12.5 Hz, H-9a), 4.28 (dd, 1 H, $J_{9b,8}$ 2.7 Hz, $J_{9b,9a}$ 12.5 Hz, H-9b), 4.36 (d, 1 H, $J_{NH,5}$ 10.4 Hz, NH), 4.61 (dd, 1 H, $J_{6,7}$ 2.2 Hz, $J_{6,5}$ 10.8 Hz, H-6), 5.00 (ddd, 1 H, $J_{4,3ea}$ 4.8 Hz, $J_{4,3ax}$ 11.4 Hz, $J_{4,5}$ 10.5 Hz, H-4), 5.20 (ddd, 1 H, $J_{8,9b}$ 2.7, $J_{8,9a}$ 5.6 Hz, $J_{8,7}$ 7.8 Hz, H-8), 5.45 (dd, 1 H, $J_{7,6}$ 2.2 Hz, $J_{7,8}$ 7.8 Hz, H-7); ^{1}H NMR ($CDCl_3$) of **8** [2] δ 1.41 (s, 9 H, Bu^t), 2.04, 2.05, 2.07 (3 × s, 9 H, 3 × OAc), 2.15 (s, 6 H, 2 × OAc), 2.57 (dd, 1 H, $J_{3eq,4}$ 4.9 Hz, $J_{3eq,3ax}$ 13.4 Hz, H-3eq), 3.79 (s, 3 H, MeO), 3.86 (t, 1 H, $J_{5,4} = J_{5,6} = J_{5,NH} = 10.4$ Hz, H-5), 4.04 (dd, 1 H, $J_{6,7}$ 2.2 Hz, $J_{6,5}$ 10.4 Hz, H-6), 4.12 (dd, 1 H, $J_{9a,8}$ 6.5 Hz, $J_{9a,9b}$ 12.4 Hz, H-9a), 4.32 (d, 1 H, $J_{NH,5}$ 10.4 Hz), 4.49 (dd, 1 H, $J_{9b,8}$ 2.6 Hz, $J_{9b,9a}$ 12.4 Hz, H-9b), 5.11 (ddd, 1 H, $J_{8,9b}$ 2.6 Hz, $J_{8,7}$ 5.4 Hz, $J_{8,9a}$ 6.5 Hz, H-8), 5.16–5.23 (m, 1 H, H-4), 5.47 (dd, 1 H, $J_{7,6}$ 2.4 Hz, $J_{7,8}$ 5.5 Hz, H-7); FABMS: 416 (100%), 374 (21%), 356 (28), 198 (50), 192 (54); Anal. Calcd for $C_{25}H_{37}NO_{15}$: C, 50.8; H, 6.3; N, 2.4. Found: C, 50.8; H, 6.4; N, 2.3.

Acknowledgements

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[2] Assignments confirmed by DQF–COSY.