Indium-Mediated Allylations of Unprotected Carbohydrates in Aqueous Media: A Short Synthesis of Sialic Acid

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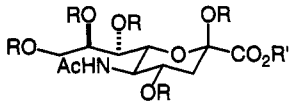
Received August 2, 1993

Background

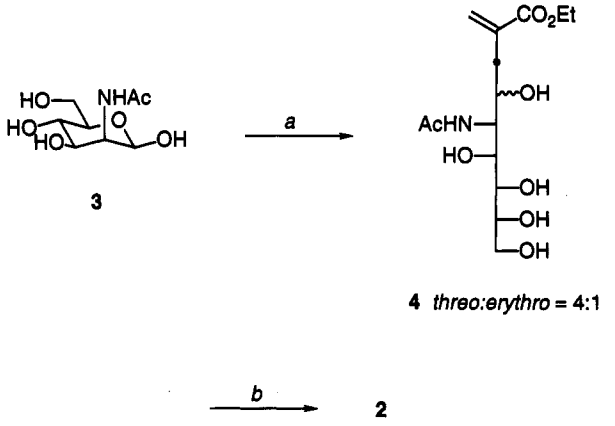
The carbohydrates on the surface of cells play a central role in cellular recognition events.[1] Sialic acid (Neu5Ac, 1), an element of gangliosides,[2] sialyl Lewis x,[3] and many other glycoconjugates,[4] is arguably the most important of these critical mediators of intercellular and cell–virion[1d,5] recognition. A recent review describes synthetic approaches to sialic acid and its derivatives.[6]



1 R = R' = H
2 R = Ac, R' = Et

Recent reports have established the potential of tin- and indium-mediated allylations of aliphatic aldehydes and ketones,[7] aldimines,[8] acid anhydrides,[9] and aldoses.[10,11] Our laboratory has focused on the development of protocols for the elaboration of unprotected aldoses to higher carbon sugars using tin or indium and allylic halides.[11] The reactions proceed in good yield and selectively form products containing a *threo* relationship between the newly generated hydroxyl group and that at C-2 of the aldose. The inability to allylate aldoses bearing an *N*-acetyl group at C-2 has limited the number of monosaccharides that are accessible with this methodology.[11] This paper describes the allylation of *N*-acetyl-β-D-mannosamine[12] (3) with a bromoacrylate and indium and the subsequent preparation of a protected form, 2, of sialic acid.

Scheme I[a]



[a] Key: (a) In, ethyl α-(bromomethyl)acrylate, 0.1 N HCl, EtOH, 40 °C, 90%; (b) O_3, MeOH, −78 °C; aqueous H_2O_2, HCO_2H, MeOH, −78 °C to rt; Ac_2O, C_5H_5N, DMAP, rt, 51%.

Discussion of Results

Heating a suspension of indium, or tin, *N*-acetyl-β-D-mannosamine (3), and ethyl α-(bromomethyl)acrylate[13] in aqueous ethanol, at temperatures less than or equal to 100 °C for up to 24 h, resulted in no detectable products derived from the addition of a carbon nucleophile to C-1 of 3. When the reaction was repeated in a mixture of ethanol and 0.1 N aqueous hydrochloric acid (6:1 v/v), the metal dissolved rapidly: the slow formation of an adduct identified as enoates 4 was observed in the reactions that included indium. We hypothesized that the major component of 4 possessed a *threo* relationship between the stereocenters at C-4 and C-5 based on the established *threo* diastereoselectivity of related transformations involving monosaccharides bearing a hydroxyl group at C-2;[11] this hypothesis was confirmed by conversion of this component to 2.

Application of optimized reaction conditions to 3 produced an inseparable mixture of enoates 4 (4:1 *threo*/*erythro*, based on the integration of the acetate singlets in the ^1H NMR spectrum) in 90% yield (see Scheme I). Ozonolysis of enoates 4, followed by oxidative decomposition of the ozonide,[14] and exhaustive acetylation provided protected β-sialoside 2 in 51% yield. Synthetic 2 was indistinguishable (^1H and ^{13}C NMR spectroscopy, mass spectrometry, and TLC in a number of solvents) from authentic material derived from sialic acid isolated from

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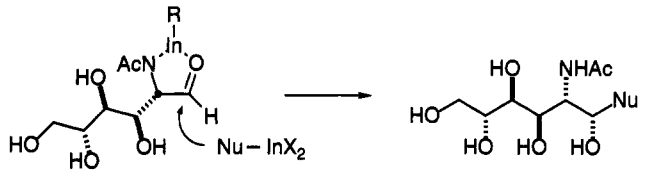

5 R = Nu or X *threo* product

Figure 1.

edible bird's nest.[15] The formation of **2** supported our hypothesis that the major product of the allylation of **3** was the *threo* diastereomer of **4**. This sequence has been carried out on a gram scale with no decrease in yield.

Figure 1 depicts our current hypothesis for the *threo* diastereoselectivity observed in the formation of **4**. Initial formation of Cram chelate **5** under the reaction conditions, followed by attack of the nucleophilic allylindium reagent at the activated carbonyl group, either inter- or intramolecularly, would selectively produce the *threo* diastereomer of the product. This simple model can also account for the *threo* diastereoselectivity observed previously in allylations of aldoses bearing a hydroxyl group at C-2.[11] The fact that the diastereoselectivity of the allylation reactions appears to be insensitive to the configuration at centers β to the carbonyl group, or more distant, leads us to discount, at this time, explanations that imply a role for these groups in the diastereoselectivity of these reactions.

Conclusion

We report a short synthesis of sialic acid (Neu5Ac, **1**) in a protected form, **2**, in two steps and 46% yield from *N*-acetyl-β-D-mannosamine (**3**). The synthesis is the result of the extension of our previously disclosed methodology for the tin- or indium-mediated allylation of unprotected carbohydrates in hydroxylic solvents to a pyranose bearing an *N*-acetyl group at C-2.

The practicality of this route to sialic acid compares favorably with that of previous nonenzymatic syntheses of sialic acid[6] and its isolation from bird's nest.[15] The enzymatic synthesis of sialic acid[16] is limited by the availability of Neu5Ac aldolase while the route we report is limited by the cost of indium and the production of indium salts as byproducts of the allylation reaction. We are exploring the use of alternative metals in this procedure and the development of a protocol for the regeneration of indium from its salts.

Our synthesis parallels the enzymatic synthesis of sialic acid[16] from **3** and sodium pyruvate and possesses the potential for great flexibility in the preparation of analogues of sialic acid. We are currently evaluating opportunities for the preparation of (1) *C*-glycosides of sialic acid, (2) stereochemical and structural analogues of sialic acid at positions other than C-4 and C-5, (3) ^{13}C-enriched sialic acid or analogues thereof, (4) N-5 analogues of sialic acid,[17] and (5) L-sialic acid. We will report progress in these areas in due course.

Experimental Section

Enoates 4. Indium (1.84 g, 16.0 mmol, powder) was added to a solution of *N*-acetyl-β-D-mannosamine monohydrate[12] (**3**) (957 mg, 4.0 mmol) and ethyl α-(bromomethyl)acrylate[13] (4.64 g, 24.0 mmol) in EtOH (24 mL) and 0.1 N HCl (4 mL). While being stirred vigorously in a stoppered flask, the reaction mixture was heated at 40 °C for 23 h. After being cooled to ambient temperature, the mixture was diluted with EtOH (75 mL) and filtered through a plug of Celite. The Celite plug was rinsed with EtOH (75 mL), and the combined filtrates were concentrated in vacuo. The residue was purified by SiO_2 chromatography (9:1 CH_2Cl_2–CH_3OH) to give **4** (1.21 g, 3.6 mmol, 90%, 4:1 *threo*/*erythro*): MS *m*/*z* 336 $(M + H)^+$; HRMS calcd for $C_{14}H_{26}NO_8$ $(M + H)^+$ 336.1659, found 336.1655. *threo*-**4**: ^1H NMR (500 MHz, CD_3OD) δ 6.22 (d, 1 H, J = 1.46 Hz), 5.67 (d, 1 H, J = 0.98 Hz), 4.31 (t, 1 H, J = 7.32 Hz), 4.19 (q, 2 H, J = 7.08 Hz), 3.87 (q, 2 H, J = 10.01 Hz), 3.77 (dd, J = 11.07 and 3.64 Hz), 3.72–3.69 (m, 1 H), 3.62 (dd, 1 H, J = 11.11 and 5.25 Hz), 3.42 (d, 1 H, J = 8.32 Hz), 2.48–2.45 (m, 2 H), 2.04 (s, 3 H), 1.29 (t, 3 H, J = 7.20 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 174.60, 168.67, 138.60, 128.35, 72.77, 71.46, 69.62, 68.52, 64.76, 61.96, 55.01, 38.07, 22.73, 14.50.

***per*-*O*-Acetylsialic Acid Ethyl Ester (2).** Ozone was bubbled through a solution of enoates **4** (100 mg, 298 μmol) in CH_3OH (10 mL) at −78 °C for 30 min. The reaction mixture was diluted with a solution prepared from H_2O (7 mL), HCO_2H (1 mL), and 30% H_2O_2 (2 mL). The cooling bath was removed, and the mixture was stirred at ambient temperature for 90 min in air and concentrated in vacuo. The residue was dissolved in pyridine (4 mL), and Ac_2O (1 mL) and DMAP (2.0 mg, 16 μmol) were added. After being stirred at ambient temperature for 13 h, the reaction mixture was diluted with $CHCl_3$ (50 mL), washed with 0.1 N HCl (2 × 25 mL) and brine (25 mL), diluted with *n*-heptane (50 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by SiO_2 chromatography (9:1 EtOAc–hexane) to give **2** (84 mg, 153 μmol, 51%): ^1H NMR (400 MHz, $CDCl_3$) δ 5.36 (dd, 1 H, J = 5.15 and 1.80 Hz), 5.24–5.21 (m, 2 H), 5.06–5.03 (m, 1 H), 4.45 (dd, 1 H, J = 12.45 and 2.57 Hz), 4.24–4.21 (m, 2 H), 4.15–4.10 (m, 3 H), 2.53 (dd, 1 H, J = 13.51 and 5.01 Hz), 2.13 (s, 3 H), 2.12 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 6 H), 1.88 (s, 3 H), 1.26 (t, 3 H, J = 7.15 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.94, 170.53, 170.19, 168.15, 165.60, 97.64, 72.85, 71.31, 68.34, 67.81, 62.40, 62.08, 49.24, 35.71, 23.12, 20.84, 20.78, 20.72, 13.74; MS *m*/*z* 570 $(M + Na)^+$; HRMS calcd for $C_{23}H_{33}NNaO_{14}$ $(M + Na)^+$ 570.1799, found 570.1816.

Acknowledgment. This work was supported by NIH Grant GM 30367. NMR spectra were obtained in the Harvard University NMR Laboratory which was supported in part by NIH Grant 1 S10 RR04870 and NSF Grant CHE 88 14019. Mass spectrometry was performed by Dr. A. Tyler and Ms. N. Niedowski. The Harvard University Mass Spectrometry Facility was supported by NSF Grant CHE 90 20042 and NIH Grant 1 S10 RR06716 01. We acknowledge a NIH/NCI postdoctoral fellowship to D.M.G.

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