Synthesis of Nitrocyclitols Based on Enzymatic Aldol Reaction and Intramolecular Nitroaldol Reaction

Wen-Chih Chou, Christopher Fotsch, and Chi-Huey Wong*

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

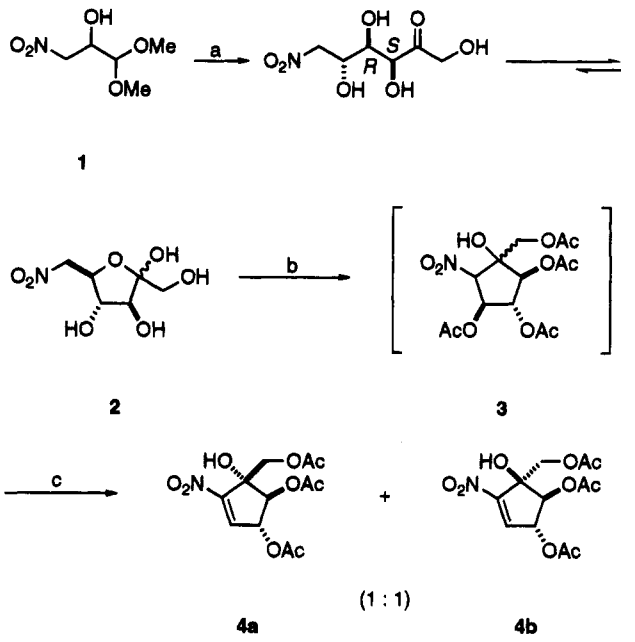
Received December 16, 1994

Enzymatic aldol addition reactions have become a useful method in organic synthesis.[1] FDP (fructose 1,6-diphosphate) aldolase, for example, has been used frequently in the synthesis of compounds such as rare ketose sugars,[2] thio-sugars,[3] and aza-sugars.[4] The enzyme accepts dihydroxyacetone phosphate (DHAP) and a variety of aldehydes to form optically active ketose phosphate with 3*S*,4*R* stereochemistry.[5] The resulting ketose phosphate can be further converted, depending on the aldehyde group used, to other sugar analogs or heterocyclic compounds. One interesting class of compounds which attracts our attention is biologically active polyhydroxylated carbocycles and aminocyclitols.[6] To date, only one example exists using FDP aldolase to form a chloroketose for the synthesis of a cyclitol.[7] We report here a new method for the synthesis of nitrocyclitols based on an FDP aldolase-catalyzed reaction with a nitroaldehyde combined with a nonenzymatic intramolecular nitro-aldol reaction.

Aldehydes which contain an α-hydroxy and a polar group at the β-position are normally very good substrates for FDP aldolase.[1] Nitroaldehyde **1** fulfills these requirements. The resulting nitroketose **2** can further undergo an intramolecular nitroaldol reaction[8] (the Henry reaction) to provide a carbocyclic compound, and the nitro group could be converted to other functional groups, e.g., amines by reduction or ketones through a Nef reaction.

Synthesis of nitroaldehyde **1** began with the nitroaldol addition of nitromethane to glyoxal dimethyl acetal.[9] The dimethyl acetal of **1** was hydrolyzed to give the nitroaldehyde as hydrate. Without isolating this product, it was treated with rabbit muscle FDP aldolase (Sigma) and DHAP[10] at pH 5.5.[11] After >95% of DHAP was consumed,[12] the pH was adjusted to 4.8 and sweet potato acid phosphatase (Sigma) was added to remove the phosphate group. The major product, 6-nitrofructose (**2**), was isolated as a mixture of anomers and a small amount of 6-nitrosorbose. When compound **2** was treated with Ac_2O and $BF_3{\cdot}OEt_2$, the nitrocyclopentenes **4a** and **4b** were obtained as a 1:1 mixture of diastereomers. Apparently, the nitro-sugar undergoes an intramolecular nitro-aldol condensation to give **3** followed by an elimination reaction.

Scheme 1[a]



[a] Reagents and conditions: (a) (i) aqueous HCl (18 mL), pH 1, 70 °C, 6 h; (ii) dihydroxyacetone phosphate (0.33 equiv, 1 mmol), FDP aldolase (160 U), pH 5.5, 24 °C, 13 h; (iii) phosphatase (100 U), pH 4.7, 37 °C, 12 h; (b) Ac_2O (5 mL), $BF_3{\cdot}Et_2O$ (0.2 mL); (c) Silica gel chromatography, 51% from **1**.

This procedure demonstrates the utility of the enzymatic aldol reaction for a short and efficient synthesis of nitrocyclitols (four steps, overall yield 51%). It is expected that nitroaldehydes will be accepted by various aldolases, and work is in progress to investigate the scope of this chemoenzymatic process and to further manipulate the nitro group.

Experimental Section

1,2-Dimethoxy-2-hydroxyl-3-nitropropane (1). To a flame-dried flask under Ar was added glyoxal dimethylacetal (1.2 g, 10 mmol) dissolved in 5.4 mL of CH_3NO_2 (100 mmol). Neutral

(1) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994; Chapter 4.

(2) (a) Wong, C.-H.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3199. (b) Durrwachter, J. R.; Drueckhammer, D. G.; Nozaki, K.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1986**, *108*, 7812. (c) Wong, C.-H.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493. (d) Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. (e) Bednarski, M. D.; Waldmann, H. J.; Whitesides, G. M. *Tetrahedron Lett.* **1986**, *27*, 5807.

(3) (a) Effenberger, F.; Straub, J. A.; Volker, N. *Liebigs Ann. Chem.* **1992**, 1297. (b) Schmid, W.; Heidlas, J.; Mathais, J. P.; Whitesides, G. M. *Liebigs Ann. Chem.* **1992**, 95. (c) Chou, W.-C.; Chen, L.; Fang, J.-M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 6191.

(4) (a) Ziegler, T.; Straub, A.; Effenberger, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 716. (b) Pederson, R. L.; Kim, M.-J.; Wong, C.-H. *Tetrahedron Lett.* **1988**, *29*, 4645. (c) von der Osten, C. H.; Sinskey, A. J.; Barbas, C. F., III; Pederson, R. L.; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1989**, *111*, 3924. (d) Straub, A.; Effenberger, F.; Fischer, P. *J. Org. Chem.* **1990**, *55*, 3926. (e) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182. (f) Wang, Y.-F.; Takaoka, Y.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1242.

(5) For a review see ref 1 and: Bednarski, M. D.; Simon, E. S.; Bischofberger, N.; Fessner, W.-D.; Kim, M.-J.; Lees, W.; Saito, T.; Waldmann, H.; Whitesides, G.-M. *J. Am. Chem. Soc.* **1989**, *111*, 627.

(6) (a) Aiyagi, T.; Yamamoto, T.; Kojiri, K.; Morishima, H.; Nagai, M.; Hamada, M.; Takechi, T.; Umezawa, H. *J. Antibiot.* **1989**, *42*, 883. (b) Farr, R. A.; Peet, N. P.; Kang, M. S. *Tetrahedron Lett.* **1990**, *31*, 7109. (c) Knapp, S.; Puradare, A.; Rupitz, K.; Withers, S. G. *J. Am. Chem. Soc.* **1994**, *116*, 7461. (d) Shiozaki, M.; Arai, M.; Kobayashi, Y.; Kasuya, A.; Miyamoto, S. *J. Org. Chem.* **1994**, *59*, 4450.

(7) Schmid, W.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 9670.

(8) For a review on the subject see: Rosini, G.; Ballini, R. *Synthesis* **1988**, 833.

(9) Hannesian, S.; Kloss, J. *Tetrahedron Lett.* **1985**, *26*, 1261.

(10) Pederson, R. L.; Esker, J.; Wong, C.-H. *Tetrahedron* **1991**, *47*, 2643. Jung, S.-H.; Jeong, J.-H.; Miller, P.; Wong, C.-H. *J. Org. Chem.* **1994**, *59*, 7182.

(11) At pH 6.7, nitroaldehyde **1** slowly decomposed under a retro-aldol reaction. Rabbit muscle FDP-aldolase exhibits ca. 50% of its maximum activity at pH 5.5, so twice the amount of enzyme was used in this reaction: Lebherz, H. G.; Rutter, W. J. *J. Biol. Chem.* **1973**, *248*, 1650.

(12) The consumption of DHAP is monitored by enzymatic assay. See: Bergmeyer, H. U. *Methods of Enzymatic Analysis*, 34th ed.; Verlag Chemie: Deerfield Beach, FL, 1984; Vol. 11, pp 146–147.

0022-3263/95/1960-2916$09.00/0

Al_2O_3 (4 g) was then added, and the suspension was vigorously stirred for 24 h at 20 °C. The solids were removed by vacuum filtration and washed with ca. 50 mL of THF. After concentration of the filtrate, a clear oil containing >90% of **1** (1.6 g, 94% yield) was isolated: ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.52 (brs, 1H, OH), 3.50 (s, 3H, OMe), 4.38 (m, 1H, H-2), 4.41–4.65 (m, 3H, H-3, H-1); ^{13}C NMR (63 MHz, $CDCl_3$) δ 55.67, 56.22, 68.97, 76.48, 104.2; IR (neat) 3442 (br), 2940, 2841, 1557, 1382, 1072 (br) cm^{-1}; MS (FAB, Na^+) *m/e* 188 ($M+Na^+$).

Compound **1** (70 mg, 0.42 mmol) was dissolved in DCl–D_2O (pD ~ 1) and heated to 52 °C for 48 h. The mixture was then heated to 70 °C for 12 h until the reaction was complete as determined by ^{1}H NMR. ^{1}H NMR (250 MHz, D_2O–DCl, pH 1) δ 4.35 (m, 1H, H-2), 4.80 (ABX, (partially underneath DOH peak) 2H, H-3), 5.11 (d, J = 4.0 Hz, 1H, H-1). A 0.47 M solution of this product (30 mL, 14 mmol) was combined with a 67 mM solution of DHAP (67 mL, 4.5 mmol), followed by addition of rabbit muscle aldolase (type IV, FDP aldolase [E.C. 4.1.2.13], 708 μL, 350 U. Sigma lot no. 110H9595) at pH 5.5. The mixture was stirred under Ar for 24 h until >90% of DHAP was consumed. The pH was then adjusted to 4.8 with 1 M HCl, and sweet potato acid phosphotase (type XA [E.C. 3.1.3.2], 1066 μL, 280 U, Sigma lot no. 12H7000) was added. After 48 h at ambient temperature, the pH was adjusted to 5.43. Water was then removed *in vacuo*, and the resulting solid was triturated three times with methanol and filtered through a 0.5-in. pad of Celite. The sample was concentrated *in vacuo* and absorbed onto 2.2 g of SiO_2 followed by flash chromatography (6:1:2% CH_2Cl_2–MeOH–H_2O and then 4:1:2% CH_2Cl_2–MeOH–H_2O) to yield 260 mg of a mixture of anomers, isomers, and possibly diastereomers of the expected product 6-nitrofructose (**2**) (1.2 mmol, 27% yield). The major chemical shifts are given here: ^{1}H NMR (400 MHz, D_2O) δ 3.62–3.80 (m, 3H, H-1 and H-6), 3.89 (dd, J = 8.6, 19 Hz, 1H, H-6), 4.42 (t, J = 8.1 Hz, 1H, H-5), 4.60 (d, J = 8.5 Hz, 1H, H-4), 4.94 (d, J = 8.5 Hz, 1H, H-3); ^{13}C NMR (101 MHz, D_2O, CH_3CN internal standard) δ 62.88, 73.43, 75.53, 80.85, 90.55, 97.19. The following peaks are for the α-anomer: 63.13, 73.69, 74.12, 77.12, 92.86; MS (FAB, Na^+) *m/z* 232 ($M + Na^+$). Part of the enzyme products apparently was further converted to the carbocyclic product 2-(hydroxymethyl)-1-nitro-2,3,4,5-tetrahydroxycyclopentane as indicated by the characteristic chemical shifts: ^{1}H NMR (400 MHz, D_2O) δ 3.62, 3.67 (AB quartet, J = 12.0 Hz, 2H, H-1), 3.95 (d, J = 6.1 Hz, 1H, H-6), 4.12 (t, J = 6.5 Hz, 1H, H-5), 4.87 (t, J = 6.8 Hz, 1H, H-4), 4.92 (d, J = 7.0 Hz, 1H, H-3); ^{13}C NMR (101 MHz, D_2O, CH_3CN internal standard) δ 63.38, 73.98, 76.95, 80.33, 90.76, 95.26; MS (FAB, Na^+) *m/z* 260. In an attempt to prepare the acetylated derivatives for further characterization, a mixture of unexpected products (**4a** + **4b**) was, however, obtained. We therefore decided to prepare the nitrocyclitols directed from the enzyme products without isolation of the intermediates. The procedure is as follows:

5-(Acetoxymethyl)-3,4-diacetoxycyclopent-1-en-5-ol (4a and 4b). An aqueous solution of **1** (495 mg, 3 mmol) was adjusted to pH 1.0 with HCl (the total volume was 18 mL) and heated to 70 °C for 6 h. This solution was adjusted to pH 5.5 with 2 N NaOH, followed by addition of DHAP (1 mmol) and FDP aldolase (160 U). The mixture was stirred at 24 °C under Ar for 13 h. The solution was adjusted to pH 4.7 with 2 N HCl and incubated at 37 °C with acid phosphatase (from sweet potatoes, type XA, 100 U) for 12 h. The reaction mixture was lyophilized to give a crude solid which was extracted with MeOH. Concentration of the MeOH solution under reduced pressure afforded a solid which was treated with Ac_2O (5 mL) and BF_3·Et_2O (0.2 mL) under Ar at 24 °C for 19 h. Evaporation of the excess Ac_2O followed by silica gel chromatography (EtOAc/hexane = 2:3) gave two diastereomers **4a** and **4b** (ratio 1:1) (160 mg, 51%). **4a**: TLC R_f = 0.30 (EtOAc/hexane, 2:3); $[\alpha]^{24}_D$ = −121.5° (*c* 0.89, EtOH); IR (neat) 3457, 3093, 1748, 1653, 1533 (NO_2), 1373, 1232, 1049 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.90 (s, 1H, OH), AB quartet (4.35, 4.42, J = 11.6 Hz, 2H), 5.47 (d, J = 6.0 Hz, 1H), 5.67 (dd, J = 2.0, 6.0 Hz, 1H), 7.04 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.56 (q), 20.64, (q, two CH_3), 62.56 (t), 73.29 (d), 79.08 (s), 84.01 (d), 134.99 (d), 150.09 (s), 169.74 (s), 170.10 (s), 170.88 (s); HRMS for $C_{12}H_{15}NO_9Na$ (M + Na) calcd 340.0645, found 340.0632. **4b**: TLC R_f = 0.26 (EtOAc/hexane, 2:3); $[\alpha]^{24}_D$ = −67.1° (*c* 0.73, EtOH); IR (neat) 3457, 3093, 1748, 1653, 1533 (NO_2), 1373, 1232, 1049 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.10 (s, 3H), 2.14 (s, 3H), 2.18 (s, 3H), 3.26 (s, 1H, OH), AB quartet (4.46, 4.50, J = 11.5 Hz, 2 H), 5.36 (d, J = 3.5 Hz, 1H), 5.80 (dd, J = 2.5, 3.5 Hz, 1H), 7.06 (d, J = 2.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.58 (q), 20.61 (q), 20.64 (q), 65.33 (t), 75.71 (d), 76.23 (d), 78.18 (s), 135.64 (d), 152.75 (s), 169.59 (s), 169.84 (s), 170.55 (s); HRMS for $C_{12}H_{15}NO_9Na$ (M + Na) calcd 340.0645, found 340.0640.

Acknowledgment. This research was supported by the NIH (GM44154).

Supplementary Material Available: The ^{1}H- and ^{13}C-NMR spectra for **4a** and **4b** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9421182