Pergamon

Tetrahedron Letters, Vol. 38, No. 15, pp. 2649-2652, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00442-5

Sialic Acid Aldolase-Catalyzed Condensation of Pyruvate and *N*-Substituted Mannosamine : A Useful Method for The Synthesis of *N*-Substituted Sialic Acids

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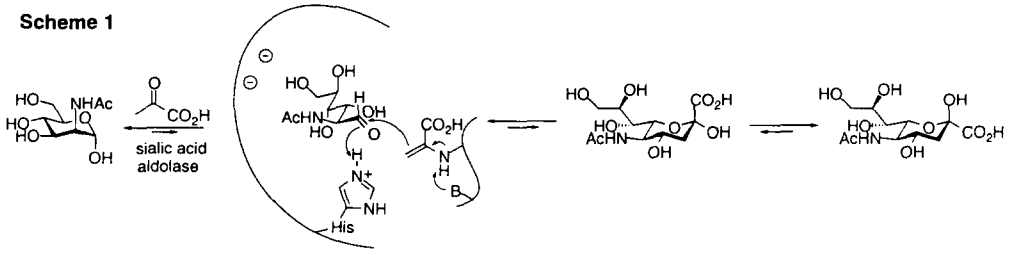
Abstract: Several *N*-substituted sialic acids were synthesized based on the sialic acid aldolase-catalyzed aldol condensation of *N*-substituted mannosamines and pyruvate. © 1997 Elsevier Science Ltd.

Sialic acids are often found as terminal components of many cell surface glycoproteins and glycolipids, and are essential in cell-cell communication, cell adhesion and receptor binding.[1] In order to investigate the function of sialic acids, various naturally occurring and modified sialic acids have recently been synthesized by both chemical and enzymatic methods.[2] The recent discovery that various CMP-sialic acids modified at C-5 are acceptable as donor substrates for sialyltransferases[3a] provides a new route to introduce a sialic acid probe to sialosides *in vitro* and perhaps *in vivo*. Although the synthesis of C-5 modified CMP-sialic acids is available via 5-deoxy-5-azido-sialic acid,[3b] an alternative method for the synthesis of various C-5 modified sialic acids may be desirable as certain of such compounds may be difficult to obtain by other means. Here we report the enzymatic synthesis of a number of C-5 modified sialic acids using sialic acid aldolase (N-acetylneuraminic acid aldolase, E. C. 4.1.3.3) from *microorganism* (available from Toyobo) as catalyst and *N*-substituted mannosamines as substrates.

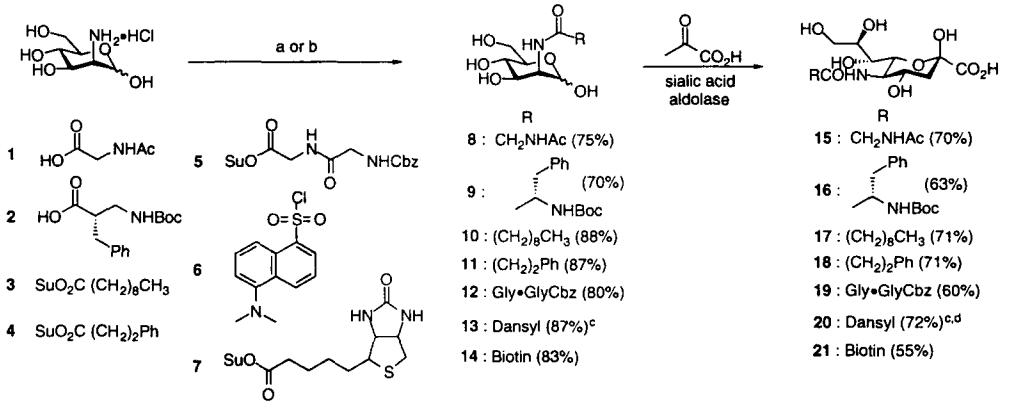
Scheme 1



The enzyme sialic acid aldolase catalyzes the reversible aldol reaction of *N*-acetyl-D-mannosamine (ManNAc) and pyruvate (Scheme 1).[4] The aldolase is a Schiff base-forming type I aldolase. An imidazole group in the active site is presumed to protonate the aldehyde group of the acceptor substrate[5] in reaction with the enamine (from the lysine residue in enzyme active site and pyruvate) complex to form a new C-C bond. With regard to its substrate specificity, sialic acid aldolase has been shown to be specific for pyruvate as

donor,[2d] but is flexible to a variety of the acceptor substrates.[6] Substitutions at C-4, C-5, or C-6 of ManNAc are acceptable. The configurations at C-4 and C-5 can be different from those of ManNAc.[2h] C-6 can be changed to various functionalities.[3b] A free hydroxyl at C-3 with the mannose configuration is, however, essential for a successful adol condensation. The configuration at C-2 is critical for the high reactivity of the substrate,[2g, 6] however, there is no study about the steric and substituent effects at C-2 position. We herein report our study on the use of C-2 modified mannosamine derivatives as acceptors for sialic acid aldolase.

Scheme 2



Keys: a) EDC, HOSu, DMF, 0.1 mM $NaHCO_3$. b) NEt_3, DMF, H_2O
c) sulfur directly attachs to nitrogen
d) yield based on 56% recovery of starting material.

The synthesis of C-2 modified mannosamine derivatives was carried out straightforwardly through coupling of a carboxyl group with mannosamine. The resulting products were purified by silica gel chromatography ($CHCl_3$/MeOH) to give compounds **8-14**.[7] Asymmetric aldol condensation of derivatives **8-14** and pyruvate mediated by sialic acid aldolase provides compound **15-21**.[8] All the C-2 modified mannosamine derivatives tested were good substrates for the aldolase providing a new useful route to various sialic acids with substituents (eg. amino acyl and peptidyl groups, dansyl group and biotin) at C-5 position which many be converted *in vivo* to sialyl conjugates for investigation of their biological functions.

Experimental

Synthesis of compounds 8-14. For compound **8** and **9**: A DMF solution (4 mL) of HOSu (1.2 mmol), EDC (1.2 mmol), and acid **1** or **2** (1.0 mmol) was stirred at 0 °C for 1 hr. D-Mannosamine in solution (1.3 mmol of $ManNH_2$•HCl, 0.1 mM $NaHCO_3$, pH 8.5) was then added and the mixture was allowed to react at rt for overnight. For compound **10-14**: A DMF solution (4 mL) of $ManNH_2$•HCl (1 mmol), compound **3-7** (1 mmol) and NEt_3 (3 mmol) was stirred at 0 °C for overnight. The solvent was evaporated and the residue was purified by chromatography ($CHCl_3$:MeOH=8:1) to afford the desired products **8-14** (70%-88%).
Enzymatic reaction : A 0.1 M solution of substrate (1 mmol) in a 0.05 M potassium phosphate buffer (pH 7.4) containing 1 mM dithiothreitol, sodium pyruvate (10 mmol), catalytic amounts of NaN_3, and 20 units of sialic acid aldolase was incubated at 37 °C for 3 days. The product was isolated by reverse-phase chromatography (LiChroprep® RP-18) eluted first with water (100 mL) and then with MeOH/H_2O (10%, 100

mL). Fractions containing the desirable product were pooled and freeze-dried to obtain the pure product (55%-72%).

Acknowledgments This work was financially supported by the NIH (GM44154)

References and Notes

1. Rosenberg, A., ed. *Biology of the Sialic Acids*; Plenum Press, New York, 1995.
2. For chemical synthesis, see (a) Julina, R.; Muller, I; Vasella, A.; Wyler, R. *Carbohydr. Res.* **1987**, *164*, 415. (b) Danishesky, S. J.; Deninno, M. P. *J. Org. Chem.* **1986**, *51*, 2615. (c) Choi, S.-K.; Lee, S.; whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 8739. For enzymatic synthesis, see (d) Kim, M.-J.; Hennen, W. J.; Sweers, H. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 6481. (e) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159. (f) Augé, C.; Bouxom, B.; Cavayé, B.; Gautheron, C. *Tetrahedron Lett.* **1989**, *30*, 2217. (g) David, S.; Malleron, A; Cavayé, B. *New J. Chem.* **1992**, *16*, 751. (h) Lin, C.-H.; Sugai, T.; Halcomb, R. L.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 10138. (i) Kragl, U.; Gödde, A.; Wandrey, C; Lubin, N.; Augé, C. *J. Chem. Soc. Perkin Trans. 1*, **1994**,119.
3. Groß, H. J.; Brossmer, B. *Glycoconjugate J.* **1995**, *12*, 739. (b) Liu, J. L.-C.; Shen, G.-J.; Ichikawa, Y.; Rutan, J. F.; Zapata, G.; Vann, W.F.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 3901.
4. (a) Comb, D. G.; Roseman, S. *J. Biol. Chem.* **1960**, *235*, 2529. (b) Brunett, P; Jourdian, G. W.; Roseman, S. *ibid.* **1962**, *237*, 2447. (c) Devries, G. H.; Binkley, S. B. *Arch. Biochem. biophys.* **1972**, *151*, 234. (c) Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong C.-H. *Chem. Rev.* **1996**, *96*, 443.
5. (a) Nees, S; Schauer, R.; Mayer, F.; Ehrlich, K. Z. *Physiol. Chem.* **1976**, *357*, 839. (B) Barnett, J. E. G.; Corina, D. L.; Rasool, G. *Biochem. J.* **1971**, *125*, 275.
6. (a) Fitz, W.; Schwark, J.-R., Wong, C.-H. *J. Org. Chem.* **1995**, *60*, 3663. (b) Zhou, P.; Salleh, H. M.; Honek, J. F. *J. Org. Chem.* **1993**, *58*, 264. (c) Augé, C.; Gautheron, C.; David, S. *Tetrahedron* **1990**, *46*, 201. (d) Halcomb, R. L.; Fitz, W.; Wong, C.-H. *Tetrahedron Asymmetry* **1994**, *5*, 2437.
7. Compound **8** (α and β mixures): ^{1}H NMR (500 MHz, D_2O) δ 5.10 (d, J = 1.5 Hz, $1H_a$), 5.00 (d, J = 1.5 Hz, $1H_b$), 4.31 (dd, J = 4.5, 1.5 Hz, $1H_b$), 4.02 (dd, J = 10.0, 4.5 Hz, $1H_b$), 3.99 (s, $2H_a$), 3.93 (s, $2H_b$), 3.85 (dd, J = 12.5, 2.5 Hz, $1H_b$), 3.84-3.80 (m, $4H_a$), 3.81 (m, $1H_b$), 3.77 (dd, J = 12.5, 5.0 Hz, $1H_b$), 3.58 (t, J = 9.5 Hz, $1H_a$), 3.48 (t, J = 10.0 Hz, $1H_b$), 2.03 (s, 3 H_a), 2.02 (s, 3 H_b); ^{13}C NMR (125 MHz, D_2O) δ 175.1, 173.0, 172.2, 93.3, 93.3, 72.5, 72.4, 69.3, 67.1, 66.9, 60.7, 54.6, 53.7, 49.3, 42.8, 22.1; HRMS calcd for $C_{10}H_{18}N_2O_7Na$ ($M+Na^+$) 301.1012, found 301.1012. Peracetylated data of compound **9**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34-7.22 (m, 5H), 6.42 (br, 1H), 5.73 (d, J = 1.5 Hz, 1H), 5.29 (dd, J = 10.0, 4.5 Hz, 1H), 5.10 (t, J = 10.0 Hz, 1H), 5.06 (br, 1H), 4.55 (ddd, J = 9.0, 4.5, 1.5 Hz, 1H), 4.33 (br, 1H), 4.18 (dd, J = 12.5, 4.5 Hz, 1H), 4.03 (dd, J = 12.5, 2.5 Hz, 1H), 3.97 (ddd, J = 10.0, 4.5, 2.5 Hz, 1H), 3.05 (dd, J = 13.5, 6.5 Hz, 1H), 3.03 (d, J = 13.5, 7.5 Hz, 1H), 2.16 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.6, 170.5, 170.0, 169.4, 168.0, 137.1, 128.1, 127.2, 91.3, 81.3, 70.0, 68.8, 65.3, 61.9, 56.1, 49.1, 27.7, 28.2, 20.8, 20.7, 20.7, 20.6; HRMS calcd for $C_{28}H_{38}N_2O_{12}Cs$ ($M+Cs^+$) 727.1479, found 727.1486. Compound **10** (α and β mixtures): ^{1}H NMR (500 MHz, D_2O) δ 4.87 (d, J = 1.5 Hz, $1H_a$), 4.76 (d, J = 1.6 Hz, $1H_b$), 4.27 (dd, J = 4.3, 1.5 Hz, $1H_a$), 4.16 (dd, J = 4.7, 1.6 Hz, $1H_b$), 3.88 (dd, J = 9.7, 4.7 Hz, $1H_b$), 3.74-3.70 (m, $2H_a$, $1H_b$), 3.67-3.64 (m, $1H_a$, $2H_b$), 3.53 (dd, J = 9.6, 4.3 Hz, $1H_a$), 3.47 (t, J = 9.7 Hz, $1H_b$), 3.37 (t, J = 9.6 Hz, $1H_a$), 2.18 (t, J = 7.9 Hz, $2H_a$), 2.17-2.13 (m, $2H_b$), 1.55-1.46 (m, $2H_a$, $2H_b$), 1.22-1.18 (m, $12H_a$, $12H_b$), 0.78 (t, J = 7.0 Hz, $3H_a$, $3H_b$); ^{13}C NMR (125 MHz, D_2O) δ 177.1, 95.0, 78.3, 74.6, 73.5, 70.6, 68.5, 68.1, 62.3, 62.1, 55.8, 55.0, 47.9, 37.2, 37.0, 33.0, 30.6, 30.5, 30.4, 30.3, 27.1, 27.0, 23.7, 14.4, 9.2; HRMS calcd for $C_{16}H_{31}NO_6Na$ ($M+Na^+$) 356.2049, found 356.2038. Compound **11** (α and β mixtures): ^{1}H NMR (500 MHz, D_2O) δ 7.27-7.14 (m, $5H_a$, $5H_b$), 4.88 (br, $1H_a$), 4.60 (br, $1H_b$), 4.41 (dd, J = 4.3, 1.5 Hz, $1H_b$), 4.27 (dd, J = 4.7, 1.5 Hz, $1H_a$), 3.98 (dd, J = 9.7, 4.7 Hz, $1H_a$), 3.87-3.83 (m, $3H_a$, $3H_b$), 3.64 (dd, J = 9.6, 4.3 Hz, $1H_b$), 3.54 (t, J = 9.7 Hz, $1H_a$), 3.44 (t, J = 9.6 Hz, $1H_b$), 2.96-2.90 (m, $2H_a$, $2H_b$), 2.62-2.52 (m, $2H_a$, $2H_b$); ^{13}C NMR (125 MHz, D_2O) δ 177.2, 176.0, 142.4, 129.5, 129.4, 127.2, 95.0, 95.0, 78.3, 74.6, 73.4, 70.7, 68.1, 68.5, 62.1, 62.2, 55.8, 55.1, 39.0, 38.8, 32.9, 30.7; HRMS calcd for $C_{15}H_{21}NO_6Cs$ ($M+Cs^+$) 444.0423, found 444.0406. Compound **12** (α and β mixtures): ^{1}H NMR (500 MHz, D_2O) δ 7.41-7.35 (m, $5H_a$, $5H_b$), 5.11 (s, $2H_a$, $2H_b$), 5.08 (br, $1H_a$), 4.99 (d, J = 1.4 Hz, $1H_b$), 4.44 (d, J = 3.6 Hz, $1H_a$), 4.31-4.30 (m, $1H_b$), 4.02-4.00 (m, $3H_a$, $1H_b$), 3.95-3.94 (m, $2H_b$), 3.86 (s, $2H_a$), 3.85 (s, $2H_a$, $2H_b$), 3.83-3.82 (m, $1H_a$), 3.80 (s, $2H_b$), 3.78-3.77 (m, $1H_b$), 3.57 (t, J = 9.8 Hz, $1H_b$), 3.47 (t, J = 9.8 Hz, $1H_a$); ^{13}C NMR (125 MHz, CD_3OD) δ 172.8, 171.9, 159.0, 137.9, 129.5, 129.0, 128.9, 94.6, 73.5, 70.7, 68.5, 62.3, 55.1, 49.8, 44.9, 43.4; positive electrospray forr $C_{18}H_{25}N_3O_9Na$ ($M+Na^+$) 450, found 450. Compound **13**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.55 (dt, J = 7.5, 1.0 Hz, 1H), 8.41 (dt, J = 8.7, 0.8 Hz, 1H), 8.30 (dd, J = 6.1, 1.3 Hz, 1H), 7.60-7.55 (m, 2H), 7.25 (dd, J = 7.5, 0.8 Hz, 1H), 4.59 (d, J = 1.6 Hz, 1H), 3.79 (dd, J = 9.8, 4.5 Hz, 1H), 3.78 (dd, J = 11.6, 4.2 Hz, 1H), 3.68 (dd, J = 11.6, 2.3 Hz, 1H), 3.65-3.61 (m, 1H),

3.53 (t, J = 9.8 Hz, 1H), 3.43 (dd, J = 4.5, 1.6 Hz, 1H), 3.34 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 153.2, 137.2, 131.3, 131.2, 131.0, 130.6, 129.2, 124.3, 120.8, 116.5, 94.7, 70.1, 68.5, 62.2, 58.4, 49.0, 45.8, 45.8; HRMS calcd for $C_{18}H_{24}N_2O_7SNa$ ($M+Na^+$) 435.1202, found 435.1187. Compound **14**: ^{1}H NMR (500 MHz, CD_3OD) δ 4.99 (d, J = 1.5 Hz, 1H), 4.48 (dd, J = 7.8, 5.0 Hz, 1H), 4.31 (dd, J = 7.8, 4.5 Hz, 1H), 4.28 (dd, J = 4.7, 1.5 Hz, 1H), 4.00 (dd, J = 9.7, 4.7 Hz, 1H), 3.85-3.81 (m, 1H), 3.78-3.74 (m, 2H), 3.58 (t, J = 9.7 Hz, 1H), 3.22-3.19 (m, 1H), 2.92 (dd, J = 12.8, 5.0 Hz, 1H), 2.29 (t, J = 7.4 Hz, 2H), 1.77-1.55 (m, 4H), 1.48-1.42 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 176.8, 175.2, 95.0, 73.5, 70.6, 68.5, 63.3, 62.2, 61.6, 57.0, 55.1, 41.0, 36.6, 29.7, 29.4, 26.8; HRMS calcd for $C_{16}H_{27}N_3O_7SCs$ ($M+Cs^+$) 538.0624, found 538.0630.

8. Compound **15**: ^{1}H NMR (500 MHz, D_2O) δ 4.04 (ddd, J = 12.5, 10.0, 5.0 Hz, 1H), 4.00 (d, J = 10.0 Hz, 1H), 3.93 (t, J = 10.0 Hz, $1H_b$), 3.92 (s, 2H), 3.81 (dd, J = 11.5, 2.5 Hz, 1H), 3.73 (ddd, J = 9.0, 7.0, 2.5 Hz, 1H), 3.58 (dd, J = 11.5, 7.0 Hz, 1H), 3.47 (d, J = 9.0 Hz, 1H), 2.34 (s, 2H), 2.18 (dd, J = 12.5, 5.0 Hz, 1H), 2.03 (s, 3H), 1.79 (t, J = 12.5 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 177.2, 175.3, 172.4, 96.7, 70.7, 70.3, 68.8, 67.4, 63.6, 52.7, 43.0, 39.7, 22.1; HRMS calcd for $C_{13}H_{22}N_2O_{10}Na$ ($M+Na^+$) 389.1172, found 389.1178. Compound **16**: ^{1}H NMR (500 MHz, D_2O) δ 7.25 (m, 5H), 4.26 (dd, J = 9.0, 6.0 Hz, 1H), 3.94 (d, J = 10.0 Hz, 1H), 3.91 (dt, J = 10.0, 4.5 Hz, 1H), 3.81 (t, J = 10.0, Hz, 1H),3 .75 (dd, J = 11.5, 2.5 Hz, 1H), 3.67 (ddd, J = 9.0, 6.0, 2.5 Hz, 1H), 3.49 (d, J = 11.5, 6.0 Hz, 1H), 3.38 (dd, J = 9.0, 1.0 Hz, 1H), 3.07 (dd, J = 14.0, 6.0 Hz, 1H), 2.83 (dd J = 12.4 Hz, 1H), 2.11 (dd, J = 12.0, 5.0 Hz, 1H), 1.71 (t, J = 12.0 Hz, H), 1.25 (s, 9H); ^{13}C NMR (125 MHz, D_2O) δ 177.1, 175.2, 173.6, 136.9, 129.6, 129.1, 127.4, 96.7, 81.8, 70.8, 70.4, 68.9, 67.2, 63.8, 56.7, 52.7, 39.8, 37.6, 27.8; HRMS calcd for $C_{23}H_{33}N_2O_{11}Cs_2$ ($M+2Cs^+$) 779.0193, found 779.0199. Compound **17**: ^{1}H NMR (500 MHz, D_2O) δ 3.83-3.77 (m, 1H), 3.78 (d, J = 10.2 Hz, 1H), 3.71 (t, J = 10.2 Hz, 1H), 3.65-3.63 (m, 1H), 3.57-3.55(m, 1H), 3.38 (dd, J = 11.8, 6.7 Hz, 1H), 3.28 (d, J = 8.9 Hz, 1H), 2.09 (t, J = 7.2 Hz, 1H), 2.01 (dd, J = 12.4, 4.7 Hz, 1H), 1.61 (t, J = 12.4 Hz, 1H), 1.40 (br, 2H), 1.40 (br, 2H), 1.10-1.07 (m, 12H), 0.65 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, D_2O) δ 180.5, 178.4, 96.8, 70.8, 69.1, 67.6, 63.8, 52.5, 39.9, 36.4, 31.5, 29.0, 28.7, 26.8, 25.8, 23.6, 22.4, 13,8; negative electrospray for $C_{19}H_{34}NO_9$ ($M-H^+$) 420, found 420. Compound **18**: ^{1}H NMR (500 MHz, D_2O) δ 7.25-7.22 (m, 2H), 7.17-7.15 (m, 3H), 3.83-3.77 (m, 1H), 3.73-3.67 (m, 2H), 3.55-3.53 (m, 2H), 3.31 (dd, J = 12.3, 7.9 Hz, 1H), 2.88-2.79 (m, 3H), 2.54-2.50 (m, 2H), 2.05 (dd, J = 12.8, 4.7 Hz, 1H), 1.63 (t, J = 12.8 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 177.1, 176.8, 140.9, 129.1, 128.8, 126.9, 96.9, 71.3, 70.6, 68.7, 67.5, 63.8, 52.4, 39.7, 37.7, 31.4; negative electrospray for $C_{18}H_{25}NO_9$ ($M-H^+$) 398, found 398. Compound **19**: ^{1}H NMR (500 MHz, D_2O) δ 7.40-7.35 (m, 5H), 5.14 (d, J = 12.4 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 4.07-4.03 (m, 1H), 4.02 (d, J = 10.3 Hz, 1H), 3.93 (s, 2H), 3.92 (t, J = 10.3 Hz, 1H), 3.83 (s, 2H), 3.79 (dd, J = 11.9, 2.1 Hz, 1H), 3.72-3.69 (m, 1H), 3.57 (dd, J = 11.9, 6.5 Hz, 1H), 3.44 (d, J = 9.1 Hz, 1H), , 2.17 (dd, J = 12.9, 4.7 Hz, 1H), 1.78 (t, J = 12.9 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 177.5, 173.9, 172.6, 159.5, 137.0, 129.6, 129.3, 128.7, 97.2, 71.2, 70.8, 69.3, 68.2, 67.9, 64.1, 53.2, 44.6, 43.3, 40.2; HRMS calcd for $C_{21}H_{29}N_3O_{12}Cs$ ($M+Cs^+$) 648.0806, found 648.0826. Compound **20**: ^{1}H NMR (500 MHz, D_2O) δ 8.40 (d, J = 8.6 Hz, 1H), 8.29-8.24 (m, 2H), 7.70-7.67 (m, 1H), 7.63 (dd, J = 8.6, 7.5 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 3.81 (d, J = 11.8 Hz, 1H), 3.81-3.77 (m, 1H), 3.42 (ddd, J = 9.4, 7.8, 2.7 Hz, 1H), 3.34 (dd, J = 11.8, 2.7 Hz, 1H), 3.25 (t, J = 10.1 Hz, 1H), 2.94-2.89 (m, 1H), 2.91 (s, 6H), 2.80 (dd, J = 11.8, 7.8 Hz, 1H), 2.00 (dd, J = 13.1, 5.0 Hz, 1H), 1.58 (t, J = 13.1 Hz, 1H); ^{13}C NMR (125 MHz, D_2O) δ 177.4, 136.2, 130.4, 130.0, 129.7, 129.3, 129.1, 125.2, 121.0, 117.1, 96.9, 71.2, 71.2, 69.4, 68.3, 64.4, 56.7, 46.0, 46.0, 40.4; HRMS calcd for $C_{21}H_{28}N_2O_{10}SNa$ ($M+Na^+$) 523.1362, found 523.1376. Compound **21**: ^{1}H NMR (500 MHz, D_2O) δ 4.56 (dd, J = 7.9, 5.0 Hz, 1H), 4.38 (dd, J = 7.9, 4.2 Hz, 1H), 3.96 (td, J = 10.7, 4.6 Hz, 1H), 3.93 (d, J = 10.1 Hz, 1H), 3.87 (t, J = 10.1 Hz, 1H), 3.79 (dd, J = 11.8, 2.7 Hz, 1H), 3.71 (ddd, J = 9.1, 6.6, 2.7 Hz, 1H), 3.55 (dd, J = 11.8, 6.6 Hz, 1H), 3.43 (d, J = 9.1 Hz, 1H), 3.32-3.30 (m, 1H), 2.95 (dd, J = 13.2, 5.0 Hz, 1H), 2.73 (t, J = 13.2 Hz, 1H), 2.28 (t, J = 7.3 Hz, 2H), 2.16 (dd, J = 12.9, 4.6 Hz, 1H), 1.77 (t, J = 12.9 Hz, 1H), 173-150 (m, 4H), 1.37 (quintet, J = 7.3 Hz, 2H); ^{13}C NMR (125 MHz, D_2O) δ 178.3, 177.5, 166.2, 97.2, 71.2, 71.0, 69.5, 68.0, 64.1, 62.8, 61.0, 56.1, 52.9, 40.5, 40.3, 36.5, 28.7, 28.4, 26.0; HRMS calcd for $C_{19}H_{31}N_3O_{10}SNa$ ($M+Na^+$) 516.1628, found 516.1640.

(*Received in USA* 24 *January* 1997; *accepted* 24 *February* 1997)