Pergamon

Tetrahedron Letters 39 (1998) 509–512

TETRAHEDRON LETTERS

Synthesis of Phosphonate Analogues of CMP-Neu5Ac Determination of α(2-6)-Sialyltransferase Inhibition[1]

Bernd Müller, Thomas J. Martin, Christoph Schaub, and Richard R. Schmidt*

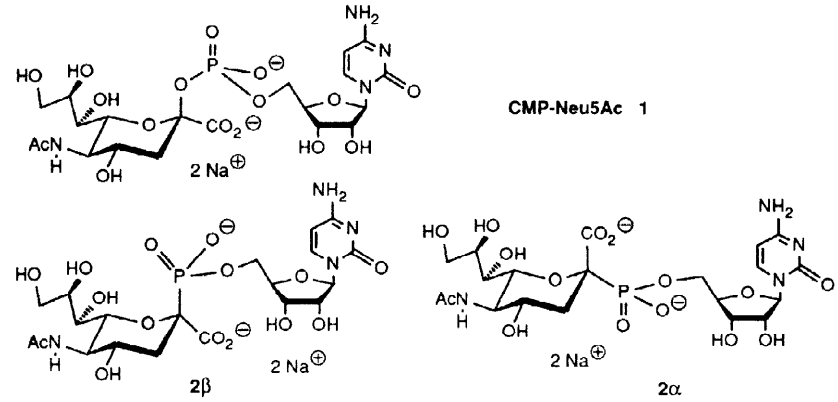
Fakultät für Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany

Received 23 October 1997; accepted 6 November 1997

Abstract: Treatment of sialyl phosphites with TMSOTf as catalyst affords α- and β-sialyl phosphonates. This phospshite/phosphonate-exchange reaction was employed for the synthesis of sialylphosphonate dialkyl esters **5a**α-**5c**α and **5a**β-**5c**β. Monodemethylation of **5c**α and **5c**β, linkage to 5'-O-unprotected cytidine derivative **7**, and then deprotection furnished the phosphonate analogue **2**β of CMP-Neu5Ac and the corresponding α-isomer **2**α, respectively. Their structures could be assigned and inhibition of α(2-6)-sialyltransferase was determined.

Sialylation at the nonreducing end of glycoconjugates is an important biological process which plays a role in various biological events, such as cell adhesion and inflammation.[2] Additionally, several reports indicate that there is a correlation between cell surface sialic acid content or sialyltransferase activity and the growth[3] or metastatic potential of tumor cells.[4,5] The sialylation is carried out with the help of sialyltransferases which use CMP-Neu5Ac (Scheme 1, **1**) as glycosyl donor.[6] Therefore, the biological function of the various sialylated oligosaccharide epitopes could best be investigated with sialyltransferase inhibitors.[7-9]

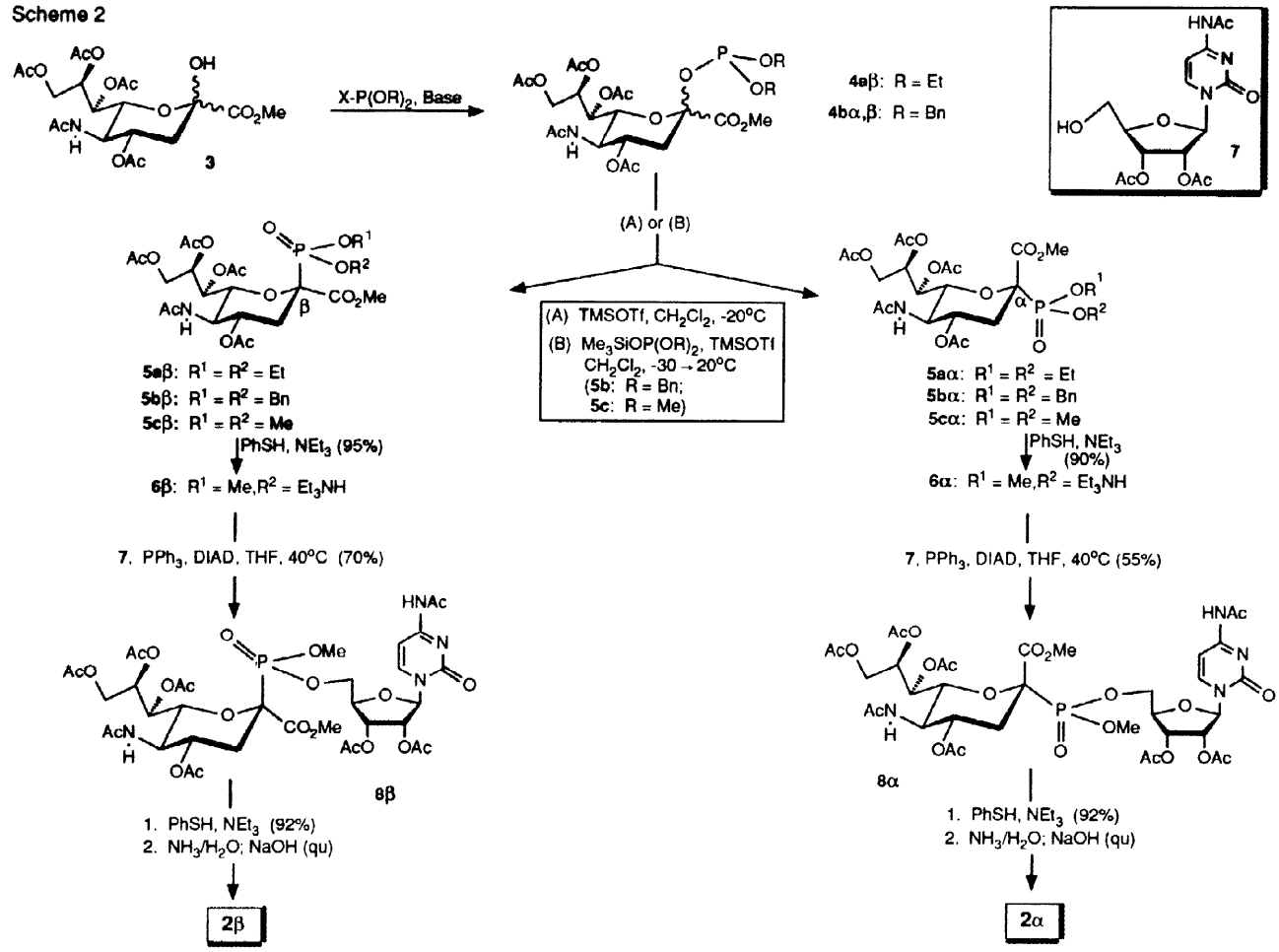
Scheme 1



Substrate analogous inhibitors may be readily accessible by generating the corresponding phosphonate **2**β. The synthesis of this compound was recently claimed,[10] based on a method which we have previously investigated.[11] As outlined in Scheme 2, it consists of a phosphite/phosphonate exchange reaction (transformation of **4** into **5**). This reaction was detected as side reaction of sialylations with sialyl phosphites when low-reactive acceptors were employed.[11] Thus, when sialic acid derivative **3**[12] was transformed into diethyl phosphite **4a**β[11,13] or into dibenzyl phosphites **4b**α,β,[14] on treatment with TMSOTf alone (Method A) or with TMSOTf in the presence of dialkyl-trimethylsilylphosphite (Method B) not only the β-phosphonates **5a**β and **5b**β but (mainly as major products) also the α-phosphonates **5a**α and **5b**α, respectively, were obtained (**5a**α:**5a**β = 1:1; **5b**α:**5b**β = 3:2).[11] The structural assignments could be based on $^{31}P/^{1}H$- and $^{13}C/^{1}H$-

100040-4039/98/$19.00
PII: S0040-4039(97)10625-6

Scheme 2



correlations as shown in Table 1 and their comparison with NMR data obtained for structurally related moieties.[15] Inspection of the NMR data of the compounds claimed to be **2β**[10] and **5cβ**[10] indicates, that instead the α-isomers **2α** and **5cα** were obtained ($^3H_{ax,P}$ = 11.8 and 11.7 Hz). (Table 1)

Table 1. Typical NMR Data of Compounds **5aα-5cα, 5aβ-5cβ**

Compound	$^3H_{eq,P}$ [Hz]	$^3H_{ax,P}$ [Hz]	$^3H_{ax,C\text{-}1}$ [Hz]	$^3H_{eq\text{-}C\text{-}1}$ [Hz]	$^3H_{3ax,4}$ [Hz]	$^3H_{3eq,4}$ [Hz]	$^2H_{ax,3eq}$ [Hz]
5aα	1	12.5	8.5	n.d.[a]	11.8	4.7	13.1
5aβ	5.0	35.5	0	n.d.	n.d.	4.8	13.9
5bα	1	13.0	8.5	3.0	13.0	3.5	13.0
5bβ	5.0	35.5	4.0	0	13.0	5.0	13.0
5cα[b]	1	11.7	n.d.	n.d.	11.7	3.6	13.0
5cβ	6.5	33.9	n.d.	n.d.	13.3	4.8	14.0
2α[b]	0	11.8	n.d.	n.d.	11.8	4.7	12.8
2β	7.0	30.0	n.d.	n.d.	10.9	7.0	12.9

[a] n.d. = not determined

[b] see ref. 10

Therefore, based on the above described methodology[11] we prepared from **4b** with dimethyltrimethylsilyl phosphite (3 equivalents) in the presents of TMSOTf as catalyst (0.5 equivalents) (Method B)

phosphonates **5**cα,β (87%, α:β = 2:1), which could be separated by silica gel chromatography (toluene/acetone, 2:1). For the subsequent transformations of each of the compounds, essentially a previously reported protocol was employed.[10,16] Monodemethylation of the phosphonate moiety was performed by treatment with $PhSH/NEt_3$ in THF[17] leading to **6**α and **6**β in practically quantitative yields. Condensation with 2',3'-di-O-acetyl-N-acetyl-cytidine **7**[16,18] under Mitsunobu conditions[19] (PPh_3/diisopropyl azodicarboxylate) afforded fully protected phosphonates **8**α (70%) and **8**β (55%), respectively, each as two diastereoisomers due to the stereogenic phosphorous atom. Ensuing O-demethylation of the phosphonate group, again by treatment with $PhSH/NEt_3$, afforded triethylammonium salts, expectedly as single isomers. Deacetylation could be readily performed by treatment with NaOMe/MeOH (Zemplén conditions) and the methyl ester was cleaved by treatment with aqueous NaOH (0.01 N for **2**β, 0.5 N for **2**α). Neutralization with Amberlite IR 120 (H^+-form) followed by ion exchange chromatography (Amberlite IR 120, Na^+-form) afforded target molecules **2**α[10] and **2**β[21] in high yields. The NMR data of **2**α were in accordance to those previously published.[10] The NMR data found for **2**β (Table 1 and ref. 21) are in agreement with the configurational assignment.

Sialyltransferase inhibition of **2**α and **2**β was investigated with α(2-6)-sialyltransferase obtained from rat liver (EC 2.4.99.1) with the help of a recently introduced assay system.[7] Both compounds exhibited competitive inhibition; K_i = 250 μM (± 20) for **2**β; K_i = 780 μM (± 70) for **2**α. As expected, **2**β shows higher enzyme affinity than **2**α and its binding to α(2-6)-sialyltransferase is less than one order of magnitude lower than the natural sialyl donor CMP-Neu5Ac (K_M = 45 μM).[7,20] Competitive binding of both anomers is supported by recent results[22] which indicate some tolerance of the enzyme to structural modifications at the anomeric site.

References and Notes

1. This work was supported by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie*.– C.S. is grateful for a stipend from the *Fonds der Chemischen Industrie*.

2. Phillips, M.; Nudelman, E.; Gaeta, F.C.A.; Perzez, M.; Singhal, A.K.; Hakomori, S.; Paulson, J.C. *Science* **1990**, *250*, 1130-1132; Waltz, G.; Arufto, A.; Kalanus, W.; Bevilaqua, M.; Seed, B. *Science* **1990**, *250*, 1132-1135; Rosenberg, A. in *Biology of Sialic Acids*, Plenum Press, New York, **1995.**

3. Bernacki, R.J. *Science* **1977**, *195*, 577-580.

4. Fogel, M.; Altvogt, P.; Schirrmacher, V. *J. Exp. Med.* **1983**, *157*, 371-376.

5. Dennis, J.W. in *Cell Surface Carbohydrates and Cell Development* (Fukuda, M.; ed.), CRC Press, Boca Raton, **1992**, pp. 174-175.

6. Harduin-Leepers, A.; Recchi, M.A.; Delannoy, P. *Glycobiology* **1995**, *5*, 741-758.

7. Schaub, C.; Müller, B.; Schmidt, R.R. *Glycoconjugate J.* **1997**, *14*, in press; and ref. therein.

8. Kijima, I.; Ezawa, K.; Toyoshima, S.; Furuhata, K.; Ogura, H.; Osawa, T. *Chem. Pharm. Bull.* **1982**, *30* 3278-3283; Kleineidam, R.G.; Schmelzer, T.; Schwarz, R.T.; Schomer, R. *Glycoconjugate J.* **1997**, *14*, 57-66.

9. Hashimoto, H.; Sato, K.; Wakabayashi, T.; Kodama, H.; Kajihara, Y. *Carbohydr. Res.* **1993**, *247*, 179-193.

10. Imamura, M.; Hashimoto, H. *Tetrahedron Lett.* **1996**, *37*, 1451-1454.

11. Martin, T.J. *Dissertation*, Universität Konstanz, **1994**; Schmidt, R.R., Lecture XVIth Int. Carbohydr. Symp., Paris, July **1992**.

12. Kuhn, R.; Lutz, P.; MacDonald, M.L. *Chem. Ber.* **1966**, *99*, 611-617.

13. Martin, T.J.; Schmidt, R.R. *Tetrahedron Lett.* **1992**, *33*, 6123-6126; Martin, T.J.; Brescello, R.; Toepfer, A.; Schmidt, R.R. *Glycoconjugate J.* **1993**, *10*, 16-25.

14. Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am Chem. Soc.* **1992**, *114*, 8748-8750; Sim, M.M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**,*115*, 2260-2267.

15. Benezra, C. *J. Am. Chem. Soc.* **1973**, *95*, 6890-6894; Benezra, C.; Ourisson, G. *Bull. Soc. Chim. France* **1966**, 1825-1827; Vysotskii, V.I.; Skobun, A.S.; Tilichenko, M.N. *J. Gen. Chem.* **1973**, *48*, 2216-2220.

16. Müller, B. Untersuchungen zur Dissertation, Universität Konstanz, **1996**; Amann, F. *Dissertation*, Universität Konstanz, **1997**.

17. Daub, G.W.; Van Tamelen, E.E. *J. Am. Chem. Soc.* **1977**, *99*, 3526-3528.

18. Zielinski, W.S.; Smrt, J.; Béranek, J. *Coll. Czech. Chem. Commun.* **1974**, *39*, 3560-3563; Schaub, C. *Diplomarbeit*, Universität Konstanz, **1995**.

19. Norbeck, D.W. *J. Org. Chem.* **1987**, *52*, 2174-2179; Campbell, D.A. *J. Org. Chem.* **1992**, *57*, 6331-6335; Malachowski, W.P.; Coward, J.K. *J. Org. Chem.* **1994**, *59*, 7616-7624.

20. Gross, H.J.; Rose, U.; Krause, J.M.; Paulson, J.C.; Schmid, K.; Feeney, R.E.; Brossmer, R. *Biochemistry* **1989**, *28*, 7386-7392.

21. 2β: ^{1}H NMR (600 MHz, D_2O, *Me*OH = 3.35): δ 8.04 (d, 1 H, $J_{6,5}$ = 7.6 Hz, H-6), 6.19 (d, 1 H, H-5), 6.04 (d, 1 H, $J_{1',2'}$ = 4.4 Hz, H-1'), 4.36-4.34 (m, 3 H, $J_{6",5"}$ = 9.9 Hz, H-2', H-3', H-6"), 4.26 (m, 2 H, H-4', H-5'a), 4.20-4.18 (m, 2 H, $J_{4",3"eq}$ = 7 Hz, $J_{4",3"ax}$ = 10.9 Hz, $J_{4",5b"}$ = 9.9 Hz, H-5'b, H-4"), 4.03 (m, 1 H, $J_{8",9"b}$ = 9 Hz, $J_{8",9"a}$ = 2 Hz, $J_{8",9"b}$ = 6.5 Hz, H-8"), 3.91 (dd, 1 H, $J_{9"a,9"b}$ = 11.1 Hz, H-9"a), 3.84 (dd, 1 H, H-5"), 3.69 (dd, 1 H, H-9"b), 3.51 (d, 1 H, H-7"), 2.81 (ddd, 1 H, $J_{3"eq,3"ax}$ = 12.9 Hz, $J_{3"eq,P}$ = 7 Hz, H-3"eq), 2.08 (s, 3 H, N-Ac), 1.85 (ddd, 1 H, $J_{3"ax,P}$ = 30.0 Hz). ^{31}P NMR (109.25 MHz, D_2O) δ 17.5.

22. Amann, F.; Schaub, C.; Müller, B.; Schmidt, R.R., manuscript in preparation.