Carbohydrate Research, 208 (1990) 251–254
Elsevier Science Publishers B.V., Amsterdam

Note

Phase-transfer-catalyzed synthesis of a phenylseleno α-ketoside of *N*-acetylneuraminic acid, (phenyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-selenononulopyranosid)-onic acid; a new sialidase inhibitor

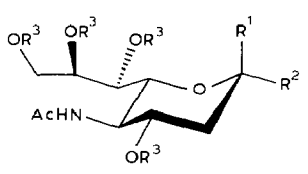
Jörg Rothermel and Hans Faillard*
Biochemie, Universität des Saarlandes, D-6600 Saarbrücken (F.R.G.)

(Received March 27th, 1990; accepted for publication, April 21st, 1990)

Selenium-substituted carbohydrates are known but rare compounds[1]. In sialic acid chemistry, they are gaining an increasing interest with regard to their use in stereocontrolled glycosidation reactions. Ito and Ogawa[2–4] reported a phenylseleno-substituted sialic acid derivative which they used in glycosidation reactions. They introduced a phenylseleno group at C-3 of sialic acid *via* the 2,3-dehydrosialic acid derivative. This group at C-3 was used as a stereocontrolling auxiliary for preparing α-ketosides of *N*-acetylneuraminic acid (**1**). Also other authors[5,6] reported glycosidation reactions with various selenium-substituted carbohydrates. However, up to now, no aryl or alkyl selenoketosides of sialic acids have been described.

We report herein the synthesis of a phenyl α-selenoketoside of *N*-acetylneuraminic acid (**1**). Because of the instability of free benzeneselenol, a special method of glycosidation was required. We succeeded in preparing the appropriate ketoside by use of phase-transfer catalysis, which we have already described as a very effective method for preparing aryl[7] α-ketosides and arylthio α-ketosides[8] of *N*-acetylneuraminic acid.

The reaction of **3** with benzeneselenol in the two-phase system, chloroform–0.5M aqueous sodium hydroxide, resulted in the peracetylated phenyl selenoglycoside **4** with high stereoselectivity, good yield, and in a very short reaction time. Whereas free selenophenol is very unstable, the ketoside **4** is a stable compound at room temperature. It crystallized easily from ethyl acetate–petrol ether. The α-D configuration of the



1 $R^1 = OH, R^2 = CO_2H, R^3 = H$
2 $R^1 = OH, R^2 = CO_2Me, R^3 = H$
3 $R^1 = Cl, R^2 = CO_2Me, R^3 = Ac$
4 $R^1 = CO_2Me, R^2 = SeC_6H_5, R^3 = Ac$
5 $R^1 = CO_2H, R^2 = SeC_6H_5, R^3 = H$

* To whom correspondence should be addressed.

0008-6215/90/$03.50

ketoside was ascertained from the chemical shifts[9] at δ 2.84 and 4.82 for H-3*e* and H-4 and the high positive rotation value[10] of +24.3°.

The protected ketoside **4** was *O*-deacetylated by the Zemplén procedure[11]. The hydrolysis of the methyl ester required up to 90 min in 0.1M aqueous sodium hydroxide. This long reaction time has been previously observed for thioketosides[8], whereas hydrolysis of the methyl ester group of ketosides is complete within 30 min[7]. Obviously the thio and the seleno substituents have a stabilizing effect on ester binding.

Deprotection of **4** gave, in good yield, **5** as a colorless, stable compound which also showed a chemical shift of δ 2.82 for H-3*e* that is characteristic for the α-D configuration, and a high positive rotation value of +45.5°.

Synthetic α-ketosides of *N*-acetylneuraminic acid are hydrolyzed by sialidases[7] which cleave the linkage of naturally occurring sialic acids. Synthetic α-ketosides of 2-thiosialic acid are stable towards sialidase treatment and are sialidase inhibitors[8,12,13]. When the seleno compound **5** was tested with *Clostridium perfringens* sialidase, it was not hydrolyzed but proved to be a sialidase inhibitor. The 4-methylumbelliferyl ketoside of *N*-acetylneuraminic acid, which was synthesized also by phase-transfer catalysis[7], was used as a hydrolyzable substrate in inhibition tests. A K_i-value of 1.0mM was obtained by Dixon plot[14] (Fig. 1), which showed that the phenyl selenoketoside **5** was a better inhibitor of *C. perfringens* sialidase than the thioketosides tested previously[8].

The synthesis of the selenoketoside of *N*-acetylneuraminic acid **5** demonstrated the effectiveness and the wide applicability of phase-transfer glycosidation. By this method even normally unstable aglycons, such as benzeneselenol, can be used in glycosidation reactions.

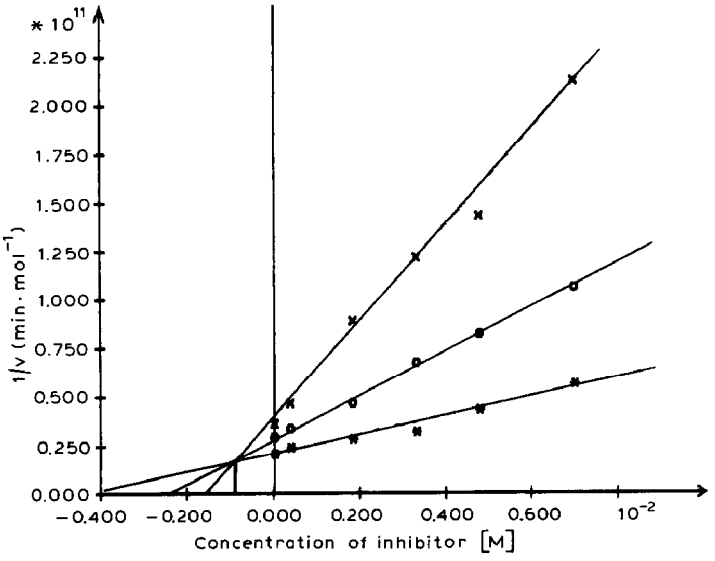


Fig. 1. Dixon-plot of the sialidase assay with the phenyl selenoketoside **5** as inhibitor and (4-methylumbelliferyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosid)uronic acid as substrate.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined with a Perkin–Elmer 241 automatic polarimeter. I.r. spectra were recorded with a Beckman Acculab 1-spectrometer and ^{1}H-n.m.r. spectra with a Bruker AM 400-spectrometer. T.l.c. was performed on silica gel (Merck 60 F_{254}) in ethyl acetate and 2:1:1 (v/v) propanol–butanol–water with detection by u.v. light or by charring with H_2SO_4. Column chromatography was conducted on silica gel (Merck 60). *N*-acetylneuraminic acid was isolated from edible birds nests. *Clostridium perfringens* neuraminidase (3 U·mg^{-1}) was obtained from Sigma Chemical Co. St. Louis, MO, USA. (4-Methylumbelliferyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-galacto-nonulopyranosid)onic acid was prepared by phase-transfer catalysis[7]. Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**2**) was prepared by the method of Kuhn *et al.*[15]. Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**3**) was prepared from **2** as previously described[7].

Methyl (phenyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-selenononulopyranosid)onate* (**4**) — To a solution of **3** (510 mg, 1.0 mmol) in chloroform (30 mL) was added benzene-selenol (470 mg, 3.0 mmol) dissolved in 0.5M aqueous NaOH (30 mL). The two-phase system was refluxed for 25 min in the presence of benzyltriethylammonium chloride (500 mg, 2.2 mmol) as phase-transfer catalyst. The organic layer was separated and washed with saturated NaCl solution, dried ($MgSO_4$), and concentrated to dryness under low pressure at room temperature. The residue was chromatographed on a silica gel column with ethyl acetate as eluent. The pure ketoside **4** crystallized from ethyl acetate–petrol ether. (yield 330–370 mg, 52–60%), m.p. 106–109°, $[\alpha]_D^{23}$ +24.3° (*c* 1.26, methanol), t.l.c. (ethyl acetate) R_f 0.35; ν_{max} 3450 (NH), 3020 (CH, arom.), 2980 (CH, aliph.), 1750, 1650 (C=O), 1540 (NH), 1430, 1370 (CH_3, CH_2), 1325, 1230 (COC, phenol), 1140, 1120, 1035, 955, 850, 740, and 690 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.88 (s, 3 H, NAc), 2.00, 2.06, 2.07, 2.12, (4 s, 12 H, 4 OAc), 2.05 (m, 1 H, H-3*a*), 2.84 (dd, 1 H, $J_{3e,4}$ 4.66, $J_{3e,3a}$ 12.9 Hz, H-3*e*), 3.56 (s, 3 H, $COCH_3$), 3.87 (dd, 1 H, H-6), 4.00 (dd, 1 H, H-5), 4.18 (dd, 1 H, H-9a), 4.39 (dd, 1 H, H-9b), 4.82 (m, 1 H, H-4), 5.25–5.30 (m, 2 H, H-7,8), 7.31–7.35 (m, 2 H, arom.), 7.41 (m, 1 H, arom.), and 7.63 (m, 1 H, arom.).

Anal. Calc. for $C_{26}H_{33}NO_{12}Se$: C, 49.53; H, 5.28; N, 2.22; Se, 12.52. Found: C, 49.21; H, 5.47; N, 2.03; Se, 12.14.

(Phenyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-*2-seleno-nonulopyranosid)onic acid* (**5**). — To a solution of **4** (315 mg, 0.5 mmol) in anhydrous methanol (35 mL) was added 0.1M methanolic sodium methoxide solution (1 mL). The mixture was stirred at room temperature for 2 h and Dowex 50W-X8 (H^+) cation-exchange resin was added to neutralize the base. The resin was filtered off and twice washed with methanol. The solvent was evaporated at room temperature and the residue dissolved in 0.1M aqueous NaOH solution (100 mL). After being stirred for 90 min at room temperature, the mixture was made neutral again with Dowex 50W-X8 (H^+) cation-exchange resin

and chromatographed with 2:1:1 (v/v) propanol–butanol–water on a silica gel column to yield **5** (180–190 mg, 80–85%) as a colorless powder, m.p. 141–145°, $[\alpha]_D^{23}$ +45.5° (*c* 1.3, methanol), t.l.c. (2:1:1, v/v, propanol–butanol–water) R_F 0.53; ν_{max} 3400–3300 (OH, NH), 3080 (CH, arom), 2950 (CH, aliph.), 1730, 1655 (C=O), 1600 (C=C, arom.), 1570 (NH), 1450, 1380 (CH_3, CH_2), 1330, 1290, 1240, 1205, 1140, 1040, 955, 750, and 695 cm^{-1}; ^{1}H-n.m.r. (D_2O): δ 1.94 (s, 3 H, NAc), 1.96 (m, 1 H, H-3*a*), 2.82 (dd, 1 H, $J_{3e,4}$ 4.24, $J_{3e,3a}$ 12.99 Hz, H-3*e*), 3.37–3.74 (m, 7 H, H-4,5,6,7,8,9a,9b), 7.36–7.50 (m, 3 H, arom.), and 7.61–7.66 (m, 2 H, arom.).

Anal. Calc. for $C_{17}H_{23}NO_9Se$: C, 43.97; H, 4.99; N, 3.02; Se, 17.01. Found: C, 43.61; H, 5.03; N, 2.87; Se, 16.73.

Enzyme assay. — Incubations with *Clostridium perfringens* sialidase were carried out in a total volume of 0.2 mL of 0.1M sodium acetate buffer (pH 5.5) containing 9mM $CaCl_2$ and 15mU of enzyme. Three concentrations of substrate [(methylumbelliferyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulopyranosid)uronic acid], 0.21, 0.43, and 0.87mM, and inhibitor concentrations from 0 to 6.95mM were used. The mixture was kept at 37° for 15 min and the hydrolysis stopped by addition of 0.1M Na_2CO_3 (0.8 mL, pH 11). Released 4-methylumbelliferone was detected by fluorescence spectrophotometry at 360 nm excitation and 450 nm emission. K_i values were determined by linear regression according to the Dixon method[14] (Fig. 1).

ACKNOWLEDGMENTS

The authors thank the Deutsche Forschungsgemeinschaft (Fa 45/4) and the Fonds der Chemischen Industrie for supporting this work, and Prof. Dr. M. Ashworth for reading the manuscript.

REFERENCES

1 A. L. Raymond, *Adv. Carbohydr. Chem. Biochem.*, 1 (1945) 129–145.
2 Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 28 (1987) 6221–6224.
3 Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 29 (1988) 3987–3990.
4 Y. Ito and T. Ogawa, *Tetrahedron*, 46 (1990) 89–102.
5 M. Perez and J.-M. Beau, *Tetrahedron Lett.*, 30 (1989) 75–78.
6 G. Jaurand, J.-M. Beau, and P. Sinaÿ, *J. Chem. Soc., Chem. Commun.*, (1982) 701–703.
7 J. Rothermel and H. Faillard, *Carbohydr. Res.*, 196 (1990) 29–40.
8 J. Rothermel and H. Faillard, *Biol. Chem. Hoppe-Seyler*, 370 (1989) 1077–1084.
9 J. Haverkamp, H. van Halbeek, L. Dorland, J. F. G. Vliegenthart, R. Pfeil, and R. Schauer, *Eur. J. Biochem.*, 122 (1982) 305–311.
10 V. Eschenfelder and R. Brossmer, *Glycoconjugate J.*, 4 (1987) 171–178.
11 G. Zemplén, *Ber. Deutsch. Chem. Ges.*, 59 (1926) 1254–1266.
12 I. M. Privalova and A. Ya. Khorlin, *Izv. Akad. Nauk. SSSR, Ser. Khim.*, (1969) 2785–2792.
13 A. Ya. Khorlin, I. M. Privalova, L. Y. Zakstelskaya, E. V. Molibog, and N. A. Evstigneeva, *FEBS Lett.*, 8 (1970) 17–19.
14 M. Dixon, *Biochem. J.*, 55 (1953) 170–171.
15 R. Kuhn, P. Lutz, and D. C. McDonald, *Chem. Ber.*, 99 (1966) 611–617.