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SYNTHESIS OF (2*R*,4*S*,5*S*)-5-ACETAMIDO-4-HYDROXY-PIPECOLINIC ACID AS A POTENTIAL INHIBITOR OF SIALIDASES

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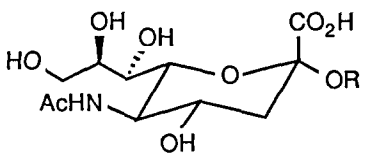
Summary: (2*R*,4*S*,5*S*)-5-Acetamido-4-hydroxy-pipecolinic acid (**2**), a weak inhibitor of sialidases, was synthesized from *N*-BOC-D-glucosamine.

Sialidases[1] play important roles in human and animal metabolism[2] and during viral infections by orthomyxoviruses[3]. Selective inhibition of sialidases might prove useful both for biochemical studies and for clinical applications. One of the structurally simplest inhibitors may be **2**, possessing a basic nitrogen (similarly to nojirimycin[4] and analogous piperidine derivatives[5] which are strong competitive inhibitors of α- and/or β-glycosidases) and - in the $^2C_5$ conformation - an axial carboxy group as it is present in the naturally occurring α-D-glycosides **1** of *N*-acetyl-neuraminic acid[2]. The piperidine **2** appeared to be accessible from 2-amino-2-deoxy-D-glucose via an intramolecular substitution at C(6) by the C(2)-amino group and the introduction of an acetamido group with inversion of the configuration at C(5).



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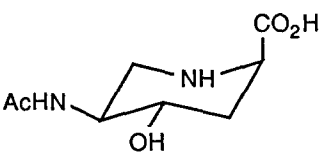

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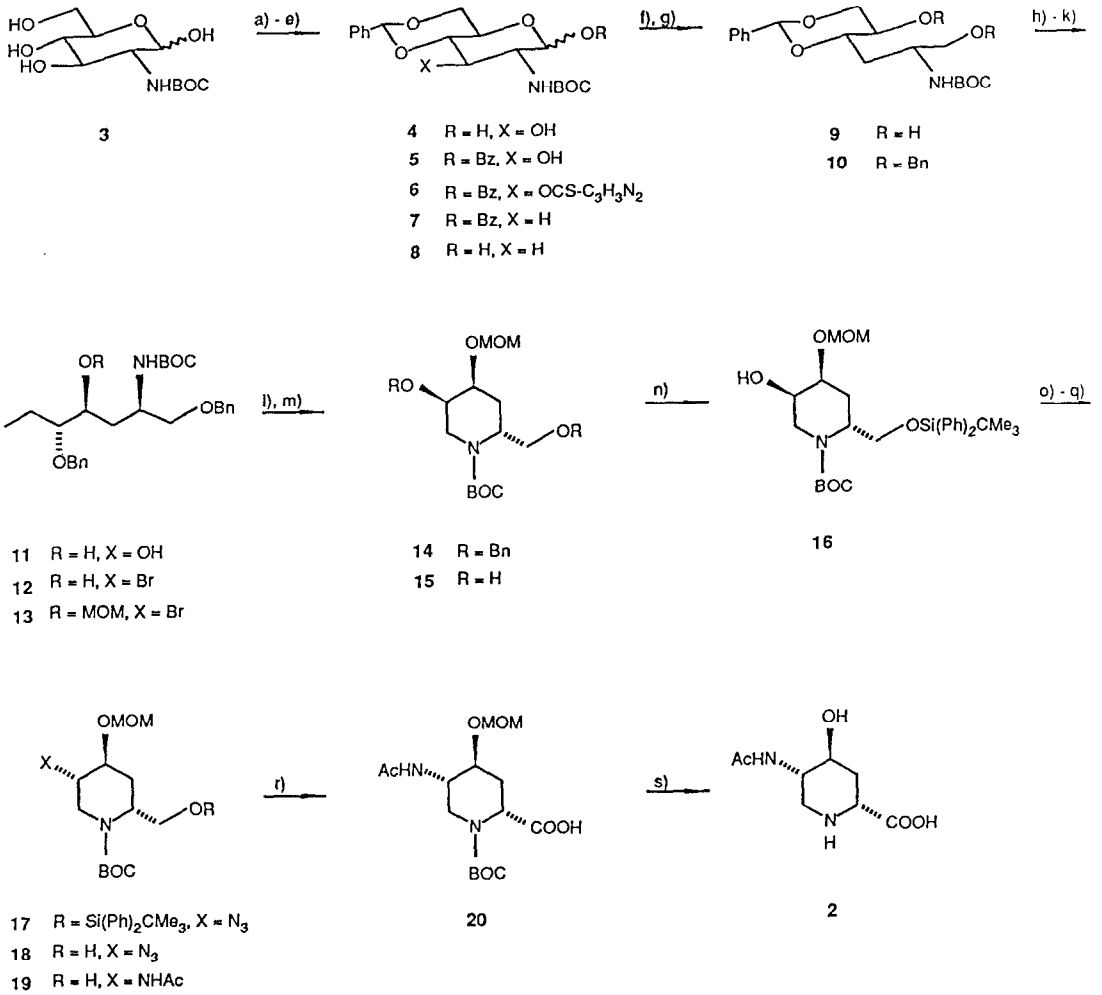
Benzylidenation[6] and selective benzoylation at -78° of the easily accessible, protected glucosamine **3**[7] (see Scheme) gave a mixture of the anomers 5α and 5β[8] which were separated by MPLC (5α: m.p. 218-219°, $[\alpha]_D$ = +114.3°; 5β: m.p. 198-199°, $[\alpha]_D$ = -60.7°)[9]. The alcohols 5α and 5β were deoxygenated[10] at C(3) *via* the thio-

Scheme



a) $PhCHO/ZnCl_2$, 4 h r.t. (62%); b) 1 eq. LDA in THF/HMPT, -78°, 1 eq. PhCOCl, -78° -> r.t. (76%); c) 2 eq. TCDI in CH_2ClCH_2Cl, 2.5 h reflux (96%); d) Bu_3SnH in toluene, 2.5 h reflux (93%); e) 1.5 eq. NaOMe in MeOH, 0.5 h 0° (86%); f) $NaBH_4$ in THF/MeOH, 0.5 h 0° (95%); g) BnBr and $BaO/Ba(OH)_2 \cdot 8H_2O$ in DMF, 40 h r.t. (78%); h) 0.05*M* HCl in MeOH, 40 min. r.t. (88%); i) 2.4 eq. $P(Ph)_3$/1.4 eq. CBr_4 in pyridine, 3 d r.t. (81%); k) $ClCH_2OMe/(Me_2CH)_2NH$ in CH_2Cl_2/AcOEt, -10° -> r.t., 68 h r.t. (93%); l) 1.5 eq. $KOC(CH_3)_3$ in THF, 3 h 0° (quant.); m) cat. H_2/Pd (10% on C) in EtOH, r.t. (quant.); n) 1.2 eq. TBDPSCl and 3 eq. imidazole in DMF, 16 h r.t. (94%); o) 1.5 eq. $P(Ph)_3$/1.5 eq. DEAD/1.5 eq. HN_3 in benzene, 1 h r.t. (95%); p) 1.2 eq. TBAF·$3H_2O$ in THF, 2 h r.t. (92%); q) cat. H_2/Pd (black) in EtOH, then 1.2 eq. Ac_2O, 20 min. r.t. (79%); r) $RuO_2 \cdot H_2O/NaIO_4$ in $CH_3CN/CCl_4/H_2O$, 1 h r.t. (98%); s) TFA, 45 min. r.t., then Amberlite IRA-93 (85%).

carbonyl imidazolides **6** (m.p. 203-205°, $[\alpha]_D$ = +107.2°). Crystallization of the reduction product from $CHCl_3$/MeOH afforded the pure α-D-*ribo* pyranose 7α (m.p. 208°, $[\alpha]_D$ = +111.9°). The esters **7** were transesterified to give a 2:1 mixture of 8α and 8β. Reduction of the hemiacetal, benzylation[11] of both hydroxyl groups, and hydrolysis gave the diol **11** ($[\alpha]_D$ = -11.1°) which, upon treatment with $P(Ph)_3$ and CBr_4[12], led to the primary bromide **12** (m.p. 81-82°, $[\alpha]_D$ = -19.8°). Under mildly basic conditions (Huenig's base), the bromoalcohol **12** was transformed[13] into the acetal **13** (m.p. 62-63°, $[\alpha]_D$ = +7.4°) and hence, under strongly basic conditions ($KOC(CH_3)_3$ in THF) into the fully protected piperidine **14** (m.p. 42°, $[\alpha]_D$ = -31.1°). The secondary alcohol **16** ($[\alpha]_D$ = +14.4°) was obtained by hydrogenolysis and selective silylation. Mitsunobu reaction[14] gave the azide **17** ($[\alpha]_D$ = -1.3°) with complete inversion of configuration. Deprotection of the primary hydroxyl group and reduction of the azide (H_2, Pd) followed by N-acetylation gave the hydroxyamide **19** (m.p. 138-140°, $[\alpha]_D$ = +76.0°) which was oxidized (RuO_4)[15] to the acid **20** (m.p. 118-120°, $[\alpha]_D$ = +74.1°). After cleavage of the protective groups by TFA, the aminoacid **2**[16] (overall yield from **3**: 9.5%) was purified by ion exchange chromatography on Amberlite IRA-93.

The conformation of the zwitterionic salt **2** was deduced from ^{1}H-NMR spectroscopy[16]. The coupling constants $J_{3ax,4}$, $J_{4,5}$, and $J_{5,6ax}$ suggest a 2:1 equilibrium between the desired $^2C_5$ and the $^5C_2$ conformation, assuming J = 11.5 Hz for a 1,2-diaxial and J = 1.5 Hz for a 1,2-diequatorial arrangement. The pipecolinic acid **2** is indeed a weak competitive inhibitor of bacterial sialidases, inhibiting the enzyme from *Vibrio cholerae* (purchased from Behringwerke) by 50% at 10^{-2}M and 29% at 10^{-3}M concentrations, and the sialidase from *Arthrobacter ureafaciens* (CalBiochem) by 84% at 10^{-2}M and 37% at 10^{-3}M. In contrast, sialidase from Fowl plague virus, kindly donated by Prof. *R. Rott*, Giessen, is not inhibited, and the same applies to sialidases from animal origin (*e.g.* from the starfish *Asterias rubens*[17] and bovine testis[18]). The enzyme assays were performed in 0.1 ml of 100 mM acetate buffer, pH 5.5 (starfish enzyme), pH 4.9 (*A. ureafaciens* and virus enzymes), and pH 4.5 (testis sialidase), containing 0.1 mU sialidase, various concentrations of the inhibitor and 0.2 mM 4-methylumbelliferyl α-glycoside of **1** as substrate, at 37° for 15 min[18]. In the case of *V. cholerae* sialidase, the incubation buffer consisted of 50 mM acetate, 154 mM NaCl and 9 mM $CaCl_2$ (pH 5.5). At 2.5 mM $CaCl_2$ and 8 mM **2** the inhibition of *V. cholerae* sialidase increases from 8% to 33% between pH 5.5 and pH 7.4 in acetate-maleate buffer (50 mM each).

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8 All new products gave satisfactory spectroscopical and analytical data.

9 If not otherwise stated, the optical rotations were measured at 25° ($CHCl_3$, c = 0.8-1.2).

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16 M.p. 267-269°, $[\alpha]_D$ = +34.6° (H_2O), R_F 0.15 (BuOH/H_2O/AcOH 4:1:1). - ^{1}H-NMR (400 MHz, D_2O): 4.09 (*dd*, H-C(2)), 3.97 (br. *td*, H-C(5)), 3.81 (br. *td*, H-C(4)), 3.44 (*dd*, H_{eq}-C(6)), 3.26 (*dd*, H_{ax}-C(6)), 2.44 (*ddd*, H_{eq}-C(3)), 2.06 (*ddd*, H_{ax}-C(3)); $J_{2,3ax}$ = 4.9, $J_{2,3eq}$ = 6.1, $J_{3ax,3eq}$ = 14.6, $J_{3ax,4}$ = 8.5, $J_{3eq,4}$ = 3.6, $J_{4,5}$ = 8.0, $J_{5,6ax}$ = 8.2, $J_{5,6eq}$ = 4.0, $J_{6ax,6eq}$ = 13.2 Hz. - ^{13}C-NMR (50 MHz, D_2O): 174.72 (*s*, NHAc), 172.88 (*s*, COO), 65.49 (*d*, C(4)), 54.96 (*d*, C(2)), 49.03 (*d*, C(5)), 42.45 (*t*, C(6)), 31.03 (*t*, C(3)), 22.38 (*q*, NHAc). - IR (KBr): 3600-2010, 1660, 1635, 1555, 1450, 1395, 1310 cm^{-1}. - CI-MS: 203 (M^+ + 1).

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