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DESIGN AND SYNTHESIS OF A WATER-SOLUBLE TAXOL ANALOGUE : TAXOL-SIALYL CONJUGATE

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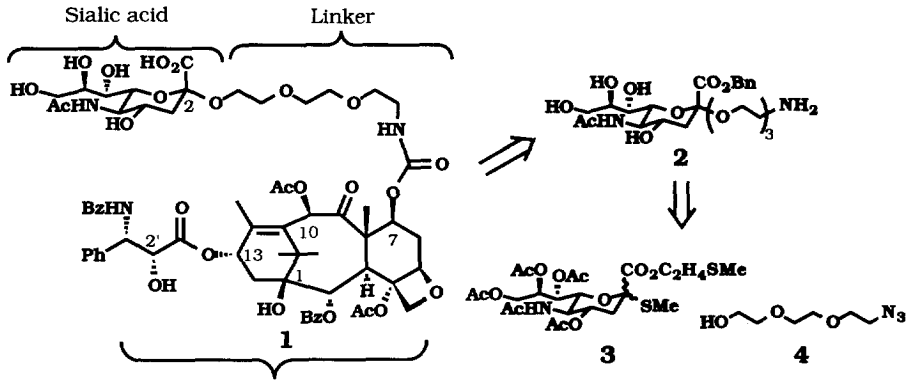
Abstract: Glycosidation, using the methylthio derivative of *N*-acetylneuraminic acid **3**, of linker alcohol **4** in DME with "long-range participation" produced the α-glycosyl linkage with high stereoselectivity. The α-linked sialic acid **2** was introduced in taxol without protection of the alcohol functionality in sialic acid.

The unique diterpenoid taxol has potent anticancer and antileukemic properties, showing very promising activity in clinical trials paticularly against ovarian and breast cancers.[1] Among new generations of taxol-based antitumor agents with improved physical, chemical and biological profiles, the development of a water-soluble analogue or prodrug of taxol is currently an important goal.[2] Previous studies on the structure-activity relationships of taxol and its derivatives showed that the C13-side chain including the C2'-hydroxy group is critical for maintaining the biological properties of taxol,[3] whereas the C7- and C10-hydroxy groups are less sensitive.[4] A number of derivatives carrying groups at C2', C7, or both hydroxyl groups have been synthesized. For example, the C7-polyethylene glycol derivative of taxol (7-PEG taxol) has been shown to produce a highly water-soluble derivative that still maintains a cytotoxic profile.[5] *N*-acetylneuraminic acid (Neu5Ac; sialic acid) conjugates, including glycoproteins and gangliosides, play an essential role in biological molecular recognition processes, such as cell adhesion and differentiation phenomena.[6] It is also well known that the activity of neuraminidase is greatly enhanced at the surface of cancer cells.[7] In this communication, we report the design and synthesis of a water-soluble "taxol-sialic acid" hybrid **1** as a potential neuraminidase cleavable prodrug (Scheme 1).



(Scheme 1)

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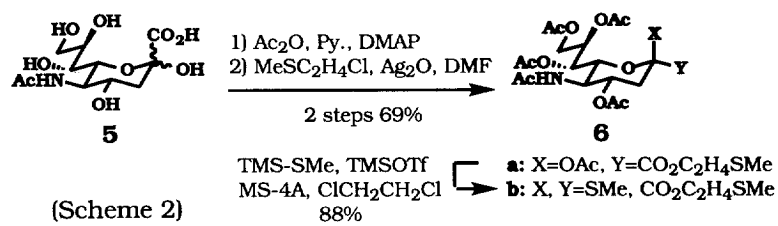
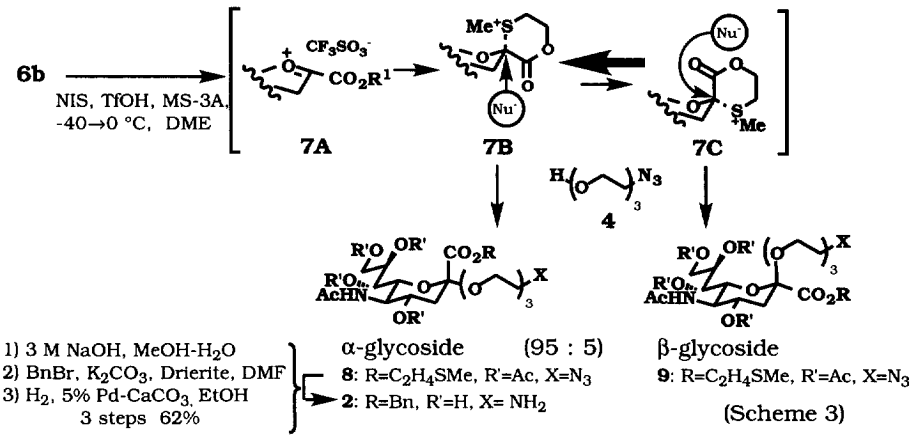
The prodrug **1** consists of taxol, sialic acid and 2-[2-(2-aminoethoxy)]ethoxy ethanol which are connected via the C7-carbamate of taxol with the C2-α-glycosidic bond of sialic acid. The design of hybrid **1**, rests on the ability of neuraminidase to allow for the regeneration of taxol or 7-triethylene glycol taxol (7-TEG taxol) from **1** through enzymatic hydrolysis. The TEG group, linking the taxol and sialic acid, is introduced in order to improve the water solubility of the molecule. To achieve the synthesis of **1**, three major obstacles have to be overcome. The first one is the regioselective introduction of a suitable linker at C7 in taxol. The second one is the setereoselective formation of the α-gylcoside linkage at C2 in sialic acid, since naturally occurring sialic acid conjugates universally exist in the thermodynamically unstable α-gylcoside conformation. The third one is to introduce the sialyl conjugate, i.e. **2**, to the taxol skeleton without protection of all the hydroxyl groups in sialic acid. For the first and third problems, we used a relatively stable C7-carbonylimidazole **11**, which allows for facile introduction of the α-linked sialic acid **2** to the taxol skeleton without protection of the alcohols in sialic acid. For the formation of the α-glycosidic bond, an anomeric mixture (α:β=1:1) of 2-thioglycoside **3** was used as the glycosyl donor, whose 2-methylthioethyl ester was designed to stabilize the newly formed oxonium intermediate via long-range participation as shown in **7B** and **7C**.

The TEG-NH_2 α-glycoside **2** was prepared from *N*-acetylneuraminic acid **5**. Protection of the pentahydroxy groups in **5** with acetic anhydride (Py. / DMAP) and esterification of the remaining acid with 2-chloroethyl methyl sulfide in the presence of silver (I) oxide in DMF gave 2-methylthioethyl ester **6a** in 69% yield (Scheme 2). Thioglycosidation of **6a** with (methylthio)trimethylsilane, promoted by trimethylsilyl trifluoromethanesulfonate (TMSOTf), at 50 °C for 6 h in 1,2-dichloroethane gave a 50 : 50 mixture of α- and β-methyl thioglycosides **6b** in 88% yield.[8] Glycosidation of **6b** with 2-[2-(2-azidoethoxy)ethoxy] ethanol (**4**) as a glycosyl acceptor proceeded smoothly upon activation with N-iodosuccinimide / trifluoromethansulfonic acid (NIS / TfOH)[9] at -40 °C in DME to afford a 95 : 5 mixture of α- and β-glycosides **8** and **9** in 45% yield (Scheme 3). The 2,3-dehydro derivative, produced by β-elimination of the amomeric acetate, was also formed in this reaction. The high α-selectivity in the glycosidation can be rationalized through the following tentative mechanism. Activation of thioglycoside **6b** with NIS / TfOH should initially lead to the oxonim intermediate **7A** which can be stabilized through the effect of long-range participation of the ester side chain as shown **7B** and **7C**. If the glycosidation reaction is of the S_N2 type as shown in Scheme 2, the thermodynamically stable β-sulfonium intermediate **7B** should provide the α-gylcoside **8**.[10] Simultaneous hydrolysis of the acetates and ester groups in **8** with 3 M NaOH in MeOH / H_2O, esterification of the resulting acid with benzyl bromide in the presence of K_2CO_3 and Drierite in DMF and reduction of the terminal azide to the amine under hydrogenation (H_2 / 5% Pd-$CaCO_3$ / EtOH) gave the TEG-NH_2 α-glycoside **2** in 62% overall yield.

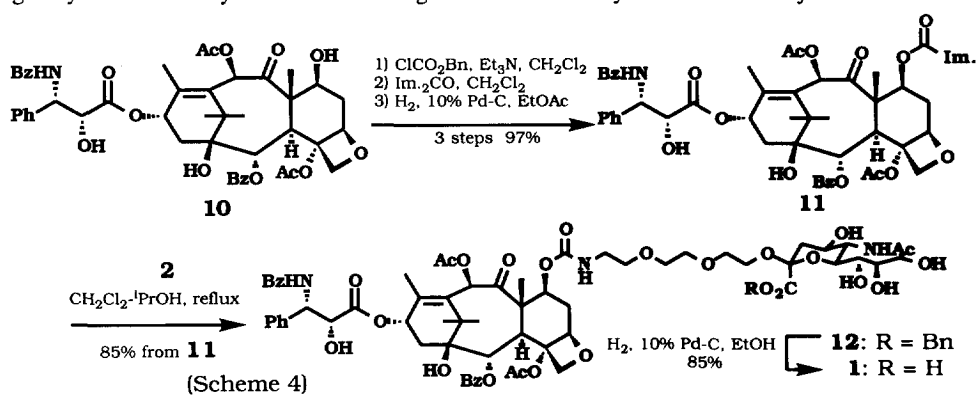


(Scheme 2)



(Scheme 3)

Based on the different reactivities of the three hydroxy groups in **10** towards acylation, the C7-carbamate was introduced as a linker in the following way (Scheme 4). The most reactive C2'-hydroxy group was protected with benzyl chloroformate in the presence of triethylamine to give the C2'-benzyl carbonate (BOC) regioselectively. Then the C7-secondary alcohol was also protected with 1,1'-carbonyldiimidazole and finally the BOC group at C2' was removed selectively under hydrogenation (H_2 / 10% Pd-C / EtOAc) to provide the C7-carbonylimidazole **11** in 97% overall yield from **10**. Coupling of **11** with the conjugated amine **2** in a mixture of 2-propanol and CH_2Cl_2 (3 : 2) at reflux resulted in the formation of the taxol-sialic acid hybrid **12** in 85% yield without aminolysis of the acetate and benzoate group in **11**. Thus we could introduce the sialic acid moiety to the taxol skeleton without protection of the sialic acid hydroxyl functionality. Hydrogenolysis of the benzyl ester **12** in EtOH gave the free carboxylic acid **1** in 85% yield.[11]



(Scheme 4)

The aqueous solubility of the taxol-PEG-sialic acid derivative **1** was estimated using HPLC methodology. A 38 mM solution of **1** in methanol was used as a standard. The solubility of **1** was estimated by adding water in small portions to 5 mg of **1** until dissolution occurred. On the basis of the UV absorption [254 nm] of the standard solution (38 mM in MeOH), the solubility of **1** was calculated to be 28 mg/mL. Then the hybrid **1** was subjected to two types of biological evaluation; microtubule binding and cytotoxicity assay. The effect of **1** on microtubule assembly in the absence of GTP was studied. The crude tublin (1 mg/mL) obtained from porcine brain was incubated at 37 °C with **1**. The drug concentration that induces microtubule assembly by 50% (ED_{50}) was found to be 3.86 μM (ED_{50} for taxol; 0.86 μM). In vitro testing of **1** employing

human lung carcinoma A427 and A427/VCR gave IC_{50} values of 1.0 x 10^{-7} M and 1.0 x 10^{-6} M, respectively. The use of sialic acid derivative to modify the 7-position of taxol has been shown to produce a water soluble derivative that still maintains binding affinity to microtubule and a cytotoxic profile.

In summary, we have reported a synthesis of the taxol-sialic acid hybrid **1** as a potential neuraminidase cleavable prodrug, as well as a method for stereoselective formation of α-glycosidic linkage in sialic acid using the concept of "long-range participation".

References and Notes

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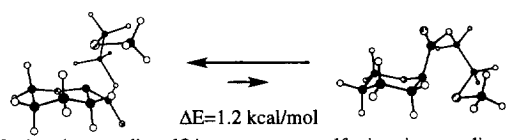
10) Application of PM3 calculations to the α- and β-sulfonium intermediates (all hydroxy groups and side chain at C5 in sialic acid were replaced by hydrogens for simplicity of calculations) revealed that the energy of β-sulfonium intermediate **13A** was calculated to be 1.2 kcal/mol more stable than that of α-sulfonium intermediate **13B**.



β-sulfonium intermediate **13A** α-sulfonium intermediate **13B**

11) **1:** $[\alpha]_D$ -19.0° (c 0.48, MeOH). IR (KBr) 3408, 2930, 1720, 1636, 1535, 1372, 1244, 1107, 1067, 1025, 709. ^{1}H NMR (270 MHz, CD_3OD) δ 1.14, 1.16 (2s, 6H, Me-16,17), 1.58-1.71 (m, 1H, H-6b), 1.77-1.86 (m, 1H, H-3ax.), 1.77, 1.96, 2.00 (3s, 9H, Me-19,18,NAc), 2.04 (dd, 1H, $J_{13,14}$=9.1,Jgem=15.3Hz, H-14), 2.14 (dd, 1H, $J_{13,14'}$=9.2Hz, H-14'), 2.13, 2.36 (2s, 6H, OAc), 2.47-2.64 (m, 1H, H-6α), 2.80-2.85 (m, 1H, H-3eq.), 3.27-3.92 (m, 19H, H-a,b,c,d,e,f,(Neu5Ac-4,5,6,7,8,9,9')), 3.93 (d, 1H, $J_{2,3}$=7.1Hz, H-3), 4.21 (s, 2H, H-20), 4.77 (d, 1H, $J_{2',3}$=5.0Hz, H-2'), 4.98-5.01 (m, 1H, H-5), 5.48 (dd, 1H, $J_{6a,7}$=7.4,$J_{6b,7}$=9.7Hz, H-7), 5.65 (d, 1H, H-2), 5.66 (d, 1H, H-3'), 6.16 (dd, 1H, H-13), 6.46 (s, 1H, H-10), 7.26-8.12 (m, 15H, Ar). MS (FAB, NBA): $C_{65}H_{81}N_3O_{26}Na$ m/z=1342 (M+Na).