

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

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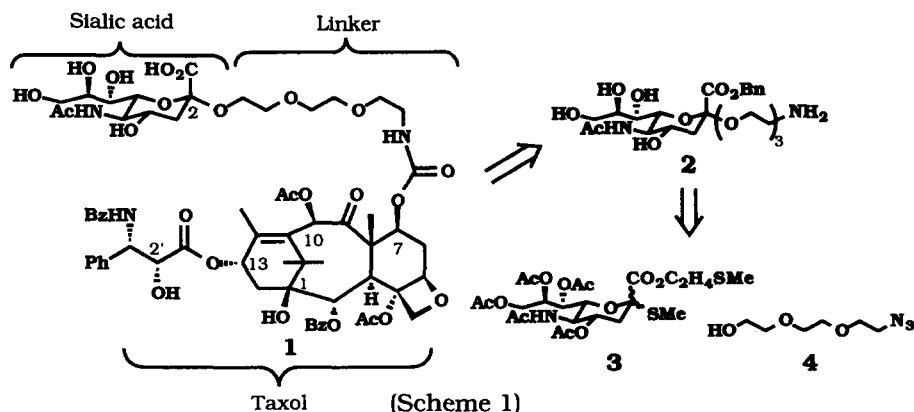
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Abstract: Glycosidation, using the methylthio derivative of *N*-acetylneuraminic acid **3**, of linker alcohol **4** in DME with "long-range participation" produced the α -glycosyl linkage with high stereoselectivity. The α -linked sialic acid **2**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

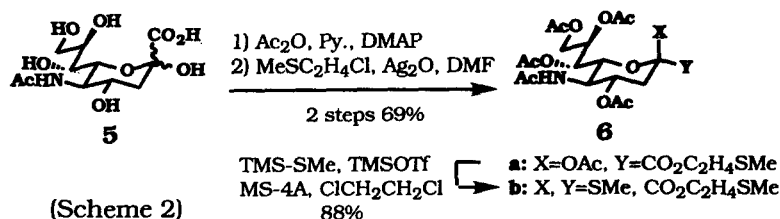
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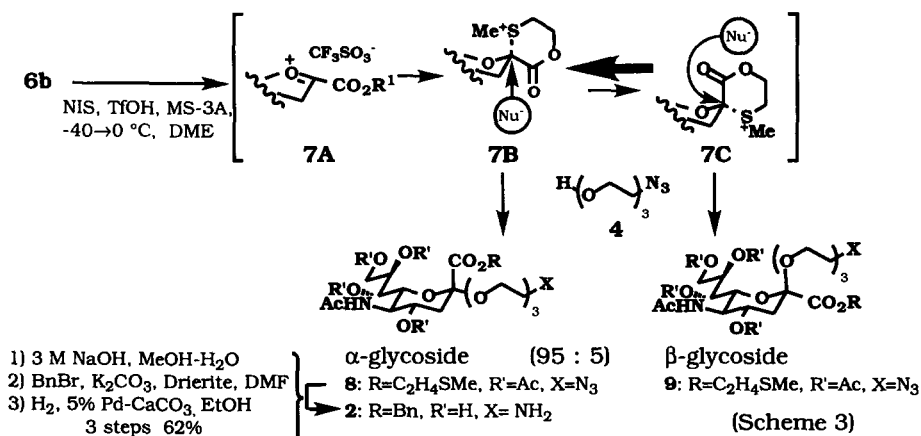
The unique diterpenoid taxol has potent anticancer and antileukemic properties, showing very promising activity in clinical trials particularly against ovarian and breast cancers.¹⁾ Among new generations of taxol-based antitumor agents with improved physical, chemical and biological profiles, the development 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).



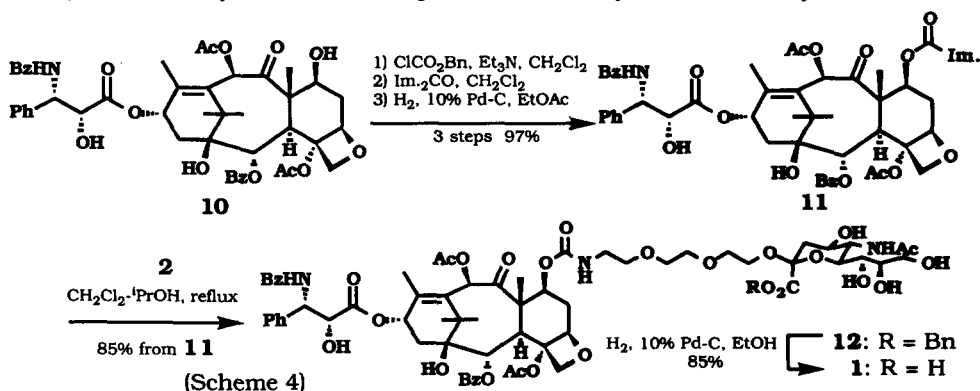
The prodrug **1** consists of taxol, sialic acid and 2-[2-(2-aminoethoxy)]ethoxy ethanol which are connected via the C7-carbamate of taxol with the C2- α -glycosidic bond of sialic acid. The design of hybrid **1**, rests on the ability of neuraminidase to allow for the regeneration of taxol or 7-triethylene 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 stereoselective formation of the α -glycoside linkage at C2 in sialic acid, since naturally occurring sialic acid conjugates universally exist in the thermodynamically unstable α -glycoside 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-NH₂ 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 / trifluoromethanesulfonic 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 anomeric 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 oxonium 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 -glycoside **8**.¹⁰⁾ Simultaneous hydrolysis of the acetates and ester groups in **8** with 3 M NaOH in MeOH / H₂O, esterification of the resulting acid with benzyl bromide in the presence of K₂CO₃ and Drierite in DMF and reduction of the terminal azide to the amine under hydrogenation (H₂ / 5% Pd-CaCO₃ / EtOH) gave the TEG-NH₂ α -glycoside **2** in 62% overall yield.





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 (H₂ / 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 CH₂Cl₂ (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⁾



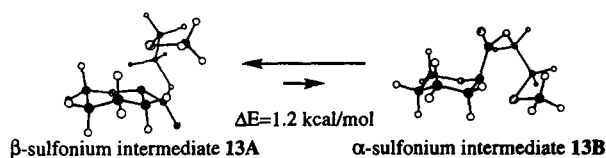
The aqueous solubility of the taxol-PEG-sialic acid derivative **1** was estimated using HPLC methodology. A 38 mM solution of **1** in methanol was used as a standard. The solubility of **1** was estimated by adding water in small portions to 5 mg of **1** until dissolution occurred. On the basis of 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 tubulin (1 mg/mL) obtained from porcine brain was incubated at 37 °C with **1**. The drug concentration that induces microtubule assembly by 50% (ED₅₀) was found to be 3.86 μ M (ED₅₀ for taxol; 0.86 μ M). In vitro testing of **1** employing

human lung carcinoma A427 and A427/VCR gave IC_{50} values of 1.0×10^{-7} M and 1.0×10^{-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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- 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**.



- 1**: $[\alpha]_D -19.0^\circ$ (c 0.48, MeOH). IR (KBr) 3408, 2930, 1720, 1636, 1535, 1372, 1244, 1107, 1067, 1025, 709. ^1H 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, $J_{13,14}=9.1$, $J_{\text{gem}}=15.3$ Hz, H-14), 2.14 (dd, 1H, $J_{13,14}=9.2$ Hz, H-14'), 2.13, 2.36 (2s, 6H, OAc), 2.47-2.64 (m, 1H, H-6 α), 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, $J_{2,3}=7.1$ Hz, H-3), 4.21 (s, 2H, H-20), 4.77 (d, 1H, $J_{2',3}=5.0$ Hz, H-2'), 4.98-5.01 (m, 1H, H-5), 5.48 (dd, 1H, $J_{6a,7}=7.4$, $J_{6b,7}=9.7$ Hz, 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): $\text{C}_{65}\text{H}_{81}\text{N}_3\text{O}_{26}\text{Na}$ $m/z=1342$ (M+Na).