Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 17, pp. 2251-2254, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00410-1

THE EFFICIENT SYNTHESIS OF A BIS-GLYCOSYLATED STEROID DRUG TRANSPORT REAGENT: METHYL 3-β-AMINO-7α, 12α-DI(1'α-GLUCOSYL)-5β-CHOLATE (TC002)

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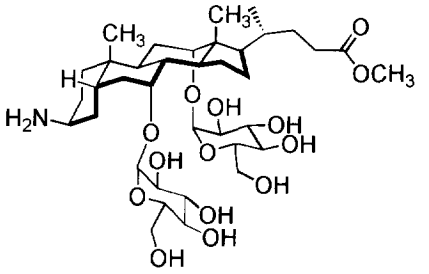
Abstract: The drug transport reagent, methyl 3-β-amino-7α, 12α-di(1'α-glucosyl)-5β-cholate (TC002) **1** was prepared in 15% overall yield from pentaacetyl glucose and methyl cholate. Pentaacetyl glucose was converted to glucosyl sulfoxide **2** in 56% overall yield. Methyl cholate was converted to methyl 3-β-azido cholate **3** in 67% yield. Bis-glycosylation of **3** with **2** followed by a single step reduction provided **1**. © 1997 Elsevier Science Ltd.

The development of both nontraditional drugs, such as peptides, proteins and oligonucleotides and many hydrophilic small molecule drugs has been hampered because of their inability to pass through membrane barriers. Recently, the discovery that facially amphiphilic glycosylated steroids facilitate the transport of both nontraditional drugs and hydrophilic small molecules drugs across membrane barriers has led to the development of methyl 3-β-amino-7α, 12α-di(1'α-glucosyl)-5β-cholate (TC002) as a general drug delivery reagent (Figure 1).[1] In our efforts to develop TC002 for clinical applications, we required an efficient large scale synthesis of this bis-glycosylated steroid. In this report, we describe an efficient large scale synthesis of TC002 thus making this material accessible for clinical study.

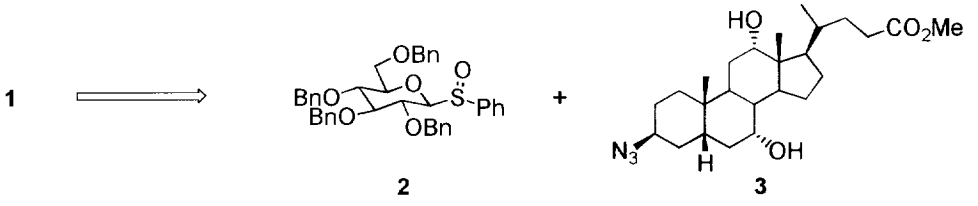


1 (TC002)

Figure 1

Earlier constructions of bis-glycosylated cholic acid derivatives proceeded by glycosylation of a C-3 benzoate protected methyl cholate; therefore, introduction of a C-3 β-amino functionality, as required for the preparation of **1**, necessitated benzoate removal in the presence of a base labile C-24 ester.[2] Such protecting group manipulations introduced additional unwanted synthetic steps resulting in reduced overall yield. Our approach for constructing TC002 relied on a convergent strategy where a C-3 amino functionalized cholic acid derivative would be simultaneously glycosylated at the C-7 and C-12 hydroxyls using sulfoxide glycosyl donor chemistry (Scheme 1).[2] We chose tetra-O-benzyl-β-D-glucosyl-1-phenylsulfoxide **2** as the protected glycosyl donor and methyl 3-β-azido cholate **3** as the amino functionalized steroid intermediate.

Scheme 1



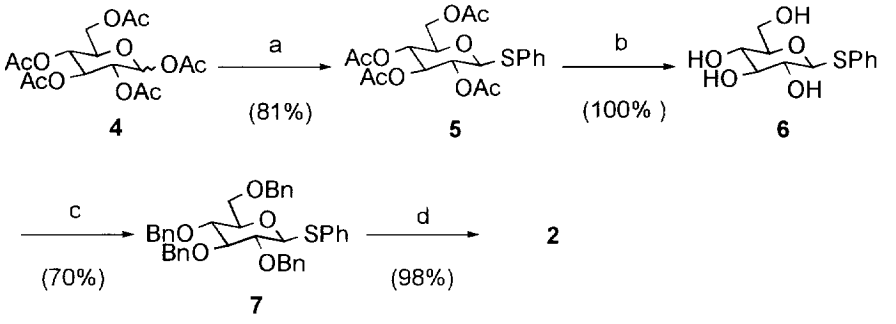
Our initial approach to the synthesis of glucosyl sulfoxide **2** began with commercially available tetra-O-benzyl-D-glucose ($4000/kg). Tetra-*O*-benzyl-D-glucose was converted in two steps to sulfoxide **2** by first introduction of an anomeric thiophenyl group (mixture of α- and β-anomers) using Bu_3P and $(PhS)_2$ followed by low temperature mCPBA oxidation.[2] Large scale execution of this approach proved problematic and expensive. Removal of Bu_3P required a tedious chormatographic purification step followed by crystallization from hexane. Crystallization provided the pure β-anomeric thioether, however, it resulted in loss of useable α-anomeric material. Low temperature mCPBA sulfide to sulfoxide oxidation was also incompatible with large scale synthesis thus making this initial approach unacceptable.

An alternative, highly efficient synthesis was developed for the large scale preparation of sulfoxide **2** (Scheme 2). Pentaacetyl glucose ($35/kg) **4** in the presence of BF_3-Et_2O and PhSH was converted exclusively to the β-thiophenyl ether derivative **5** in 81% yield after crystallization.[3] Removal of the acetate protecting groups using catalytic NaOMe followed by acidification with H^+ amberlite resin gave the thiophenyl glucose derivative **6** in quantitative yield. Without purification, **6** was converted in 70% yield after crystallization to the tetrabenzyl derivative **7** by reaction with benzyl chloride under phase transfer conditions.[4] Final oxidation to the sulfoxide **2** was accomplished in 98% yield using 30% $H_2O_2/Ac_2O/SiO_2$ at room temperature.[5] Sulfoxide **2** (15 kg) was prepared in 56% overall yield using this procedure.

The C-3 β-azido cholate derivative **3** (6 kg) was prepared as outlined in Scheme 3. Commercially available methyl cholate **9** was selectively tosylated on the C-3 hydroxyl group in 90% yield with tosyl chloride in pyridine. The tosylate **10** was then heated in DMSO with 1.5 equiv of NaN_3. After heating at 90 °C for 1.5 h, the reaction mixture was poured into ice water. The resulting precipitate was crystallized from methanol:H_2O (3:1) to give

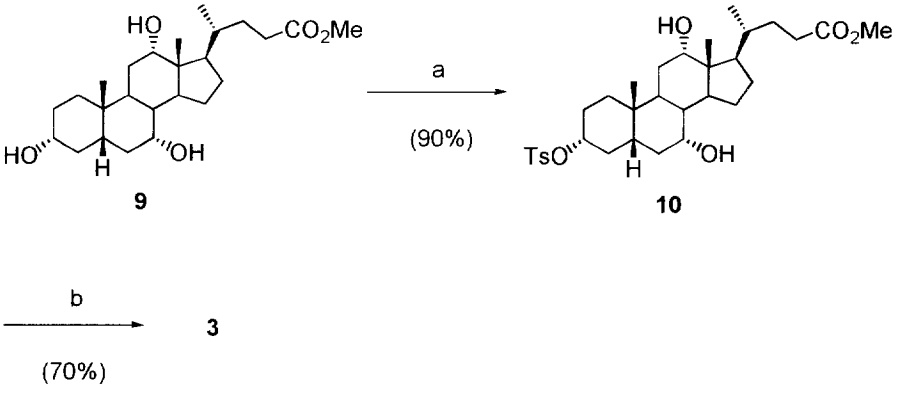
the azido cholate **3** in 70% yield. The use of DMSO in the azide displacement reaction was critical to the ease of product isolation.

Scheme 2



Reagents: (a) BF_3-Et_2O, PhSH, CH_2Cl_2, reflux, 2 h; (b) NaOMe (cat), 5 h, H^+ resin; (c) $[CH_3(CH_2)_3]_4N(HSO_4)$, BnCl, aq NaOH, CH_2Cl_2, 16 h, rt; (d) 30% H_2O_2, Ac_2O, SiO_2, CH_2Cl_2, 2 h, rt.

Scheme 3



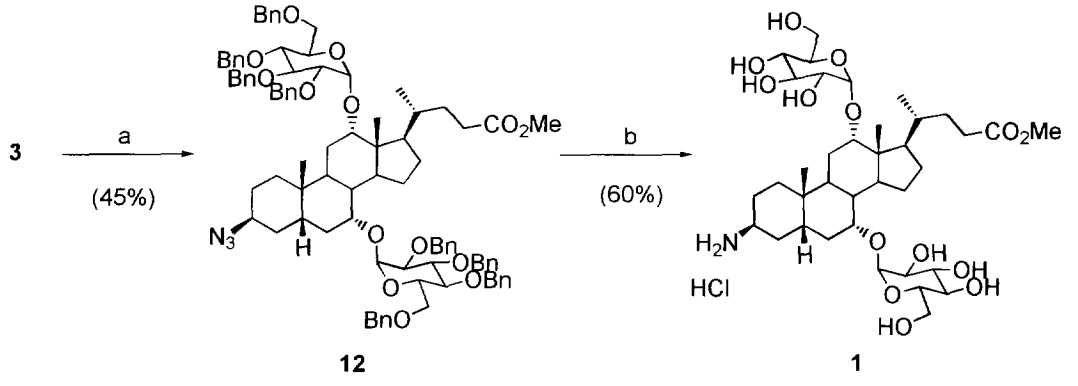
Reagents: (a) TsCl, pyridine, 10 °C, 16 h; (b) NaN_3, DMSO, 90 °C, 1.5 h.

Standard glycosylation conditions using anomeric sulfoxide glycosylation chemistry required that the reaction between **2** and **3** be carried out at -78 °C; however, this temperature was not compatible with standard process equipment.[6] Therefore, we investigated glycosylation at elevated temperatures. By careful examination of reagent stoichiometry and temperature, we were able to perform the glycosylation at -45°C to -30°C, a temperature range compatible with large scale synthesis. Bis-glycosylation of **3** (382 g) was accomplished in 45% crystallized yield by reacting **3** with 2.8 equiv. of **2** (1.7 kg), 1.1 equiv. of Tf_2O and 1.1 equiv. of 2,6-di-*t*-butyl-4-methylpyridine (DTBMP).

To complete the synthesis of **1** we needed to reduce the azide to the amine and remove 8 benzyl protecting groups. Initially, an efficient two step protocol was developed using Ra-Ni/H_2(g) in THF/MeOH to reduce the azide (98% yield) followed by debenzylation in the presence of 10% $Pd(OH)_2$/C, H_2 (g), MeOH, HCO_2H, 50 psi (60% yield). However, by modification of the solvent system (THF/MeOH, 3 mol HCl) and catalyst (10%

Pd/C), we were able in one step to reduce the azide and debenzylate to give **1** as its HCl salt in 60% yield after crystallization from methanol.

Scheme 4



Reagents: (a) **2**, Tf_2O, DTBMP, CH_2Cl_2, Tol, -45 to -30 °C, 1h; (b) 10%Pd/C, 3 mol HCl, THF:MeOH (1:1), H_2 (g) 50 psi, 24 h.

In conclusion, we have developed an efficient (15% overall yield) large scale synthesis of the facially amphiphilic bis-glycosylated steroid drug transport reagent TC002 (**1**), which is the first reported synthesis of this clinically important material. In addition, we have demonstrated for the first time the feasibility of large scale glycosylation using sulfoxide glycosylation chemistry for the preparation of medicinally important agents containing sugar units.

ACKNOWLEDGEMENT

We would like to thank Prof. Dan Kahne for helpful discussion, and T. Goetzen, W.C. Kokke and M. Christie of Caldron Process Chemistry for pilot plant scale-up of the azide displacement step.

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(*Received in USA* 17 *June* 1997; *accepted* 4 *August* 1997)