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Preparation of a Protected Triamino Analogue of Cholic Acid and Sequential Incorporation of Amino Acids in Solution and on a Solid Support

Xiao-Ti Zhou, Atiq-ur Rehman, Chunhong Li, and Paul B. Savage*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602

savage@byu.edu

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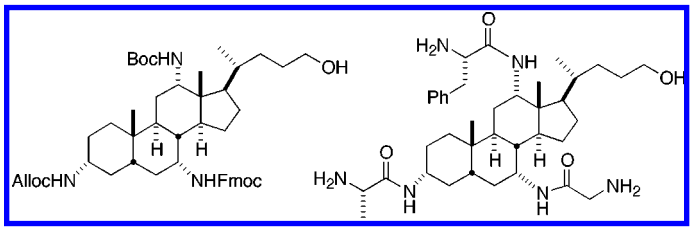
ABSTRACT



We have prepared a triamine derivative of cholic acid with protecting groups on the amines that allow sequential amide formation. The triamine was formed from 3α,7α,12α-trihydroxycholan-24-ol with good stereoselectivity. Sequential removal of the amine protecting groups and amide formation was achieved in high-yielding steps and was performed in solution and on a solid support.

Steroid nuclei have been used extensively in peptide mimicry,[1] in the preparation of receptors for various molecules,[2] and in the development of antimicrobial agents.[3] Cholic acid has attracted significant attention primarily due to the orientation of its three hydroxyl groups on one face of the steroid, and many derivatives of cholic acid have been prepared. To increase the facial amphiphilicity of cholic acid, Davis and co-workers[4] have prepared a triamino analogue of cholic acid in which each of the hydroxyl groups in the molecule was replaced with an amine group (with retention of stereochemistry). We have used a similar triamine derivative of cholic acid to prepare antimicrobial agents.[5] Recently, Davis and co-workers reported the preparation of a diamine derivative of cholic acid suited for combinatorial chemistry.[6] The compound offers three potentially reactive sites directly on the steroid nucleus: two amine groups and a hydroxyl group. In our work with amino acid functionalized cholic acid derivatives, we have found that attachment of amino acid groups to cholic acid via ester linkages can yield compounds that are not stable under mildly basic conditions.[7] To avoid stability problems with ester groups, we have

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prepared an appropriately protected triamine derivative of cholic acid (**1**; Figure 1) that allows sequential amide

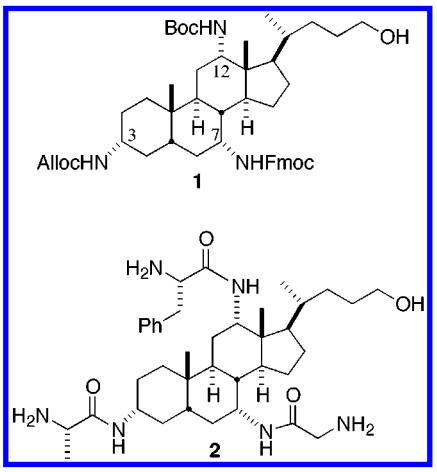


Figure 1. Structures of tricarbamate **1** and triamide **2**.

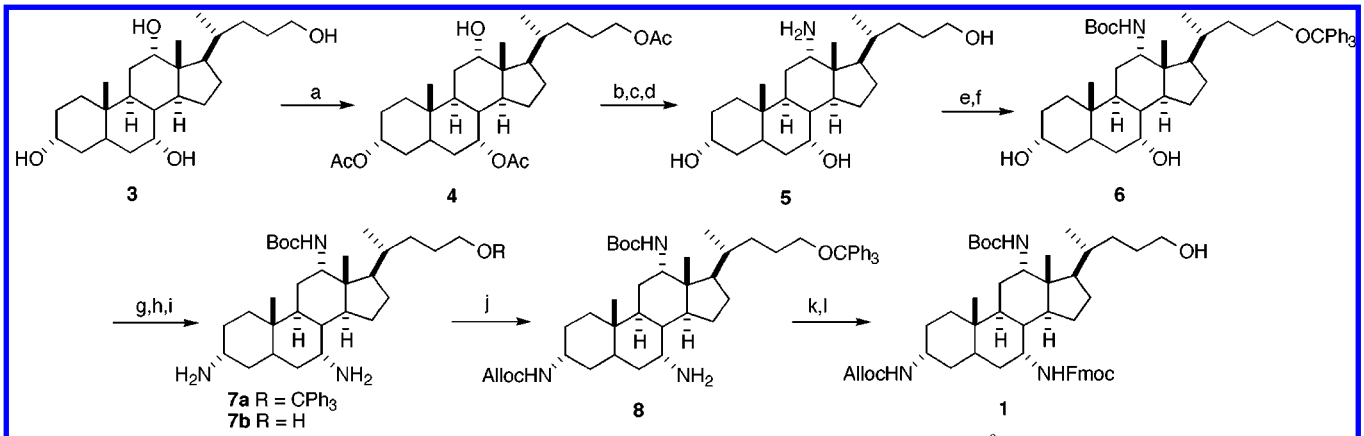
formation at C-3, C-7, and C-12 on the steroid, yielding triamides such as **2**. In addition, we have shown that sequential deprotection of the three amine groups and amide formation can be accomplished on a solid support.

In our earlier work on the synthesis of a triamine derivative of cholic acid,[8] the amine groups were incorporated in a single step. However, to provide for sequential amide formation with the amine groups, orthogonal protection of the amine groups was required. Therefore, it was necessary to introduce the amine groups in separate steps. On the steroid nucleus, functionality at C-7 and C-12 is more hindered than that at C-3. Consequently, we reasoned that if we could differentiate functional groups at C-7 and C-12, reactions at C-3 would be much more rapid than at either of the two former positions and differentiation of groups at C-7 (or C-12) and C-3 would be straightforward.

To differentiate C-7 and C-12 and to initiate the preparation of **1**, triacetate **4** was formed in good yield (Scheme 1). Oxidation of the C-12 alcohol, oxime formation, and reduction using sodium in *n*-propanol[9] gave amine **5**. The stereochemistry at C-12 was confirmed via ^{1}H NMR; i.e., the proton α to the amine was clearly equatorial. The C-12 amine was protected as the (*tert*-butyloxy)carbamate, and a trityl ether was formed from the primary alcohol. Oxidation of the remaining alcohol groups on **6**, oxime formation, and reduction gave a mixture of **7a** and **7b**, which were readily separable. Stereochemical assignments of **7a** and **7b** were made on the basis of ^{1}H NMR comparisons to previously prepared compounds.[8] This step was the lowest yielding of the series. Nevertheless, because of the ease by which **6** was prepared and because of the high yields of subsequent steps, it was possible to bring gram quantities of material through this step. After isolation of **7a**, a variety of protecting groups were used in attempts to differentiate the amine groups at C-3 and C-7. Some protecting groups, including Fmoc chloride and trimethylsilylethoxychloroformate, offered little selectivity between these amines. Allyloxychloroformate (Alloc chloride) proved to selectively react with the amine at C-3. Protection of the remaining amine group with Fmoc chloride and deprotection of the primary alcohol yielded **1**.

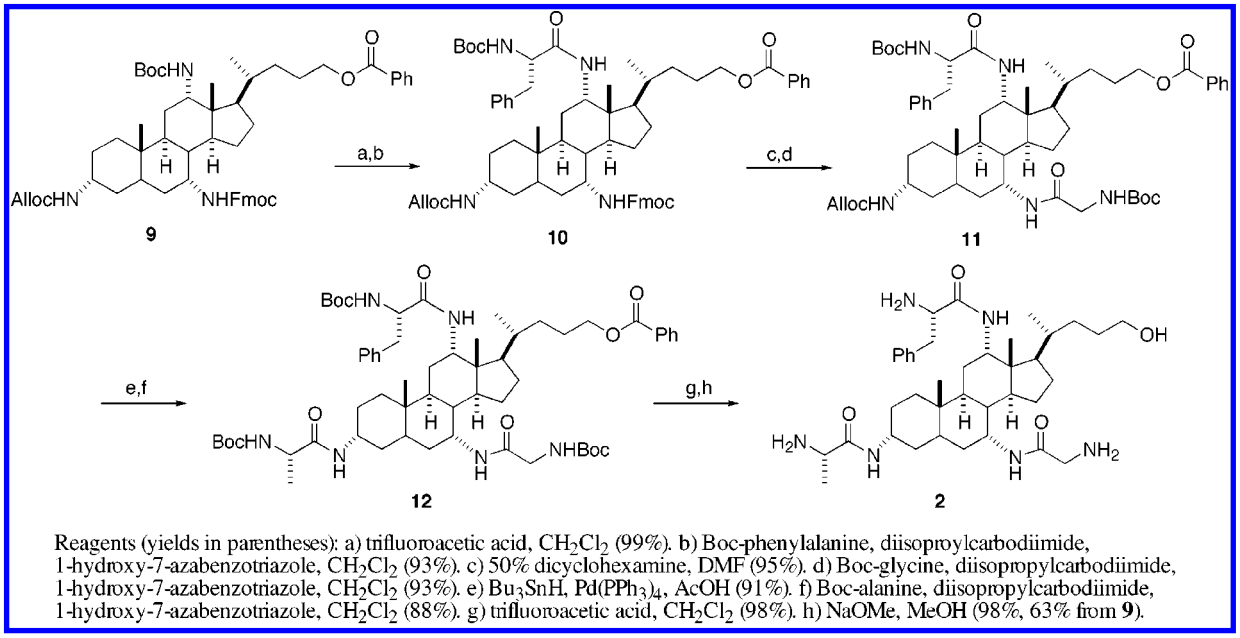
To verify that the three protecting groups could be removed independently of one another and in the presence of an ester group at C-24, we prepared triamide **2** in solution (Scheme 2). The benzoyl ester of **1** (**9**) was formed in anticipation of immobilization of **1** on a solid support via an ester linkage. Because we planned on using alcohol **1** in solid-phase synthesis, it was important that the yield of each step was optimized, and all yields of the reactions in Scheme 2 were near or above 90%. We elected to use Boc-protected amino acids; consequently, it was necessary to first deprotect the amine at C-12, followed by amide formation with Boc-

Scheme 1. Preparation of Tricarbamate **1**



Reagents (yields in parentheses): a) Ac_2O, DMAP, Et_3N, CH_2Cl_2 (87% yield). b) pyridinium dichromate, 4 Å sieves, CH_2Cl_2 (99%). c) NH_2OH•HCl, NaOAc, 95% EtOH (~100%). d) *n*-PrOH, Na° (95%). e) $(Boc)_2O$, Et_3N, CH_2Cl_2 (93%). f) TrCl, DMAP, Et_3N (78%). (g) pyridinium dichromate, 4 Å sieves, CH_2Cl_2 (97%). h) NH_2OH•HCl, NaOAc, 95% EtOH (~100%). i) PtO_2, AcOH, H_2 (**7a:** 38%; **7b:** 30%). j) AllocCl, Py, THF (89%). k) FmocCl, CH_2Cl_2, DIEA (94%). l) *p*-TsOH, CH_2Cl_2 (84%).

Scheme 2. Preparation of Triamide **2**



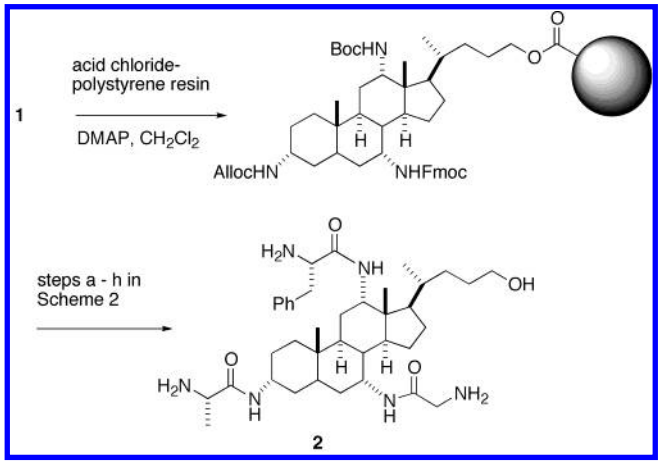
Reagents (yields in parentheses): a) trifluoroacetic acid, CH_2Cl_2 (99%). b) Boc-phenylalanine, diisoproylcarbodiimide, 1-hydroxy-7-azabenzotriazole, CH_2Cl_2 (93%). c) 50% dicyclohexamine, DMF (95%). d) Boc-glycine, diisopropylcarbodiimide, 1-hydroxy-7-azabenzotriazole, CH_2Cl_2 (93%). e) Bu_3SnH, $Pd(PPh_3)_4$, AcOH (91%). f) Boc-alanine, diisopropylcarbodiimide, 1-hydroxy-7-azabenzotriazole, CH_2Cl_2 (88%). g) trifluoroacetic acid, CH_2Cl_2 (98%). h) NaOMe, MeOH (98%, 63% from **9**).

phenylalanine, giving **10**. Deprotection of the amine at C-7 in the presence of a benzoyl ester proved challenging. Many of the nitrogen bases typically used to remove Fmoc groups cleaved the ester. We found, however, that 50% dicyclohexylamine in DMF effectively removed the Fmoc group without cleaving the benzoyl ester. After the Fmoc group was removed, Boc-glycine was incorporated. Deprotection of the amine at C-3 and amide formation with Boc-alanine yielded **12**. The amino acids were deprotected followed by cleavage of the ester, giving **2** in 63% overall yield from **9**. In contrast to cholic acid derivatives in which amino acids were attached to the steroid via ester linkages, triamide **2** was stable under basic conditions.

A potentially important consideration is that while only Boc-protected amino acids were used to form the amides on the steroid scaffolding, the orthogonality of the nitrogen protecting groups on **1** is expected to allow incorporation of a Boc-protected amino acid at C-12, an Fmoc-protected amino acid at C-7, and an Alloc-protected amino acid at C-3. Subsequently, these protecting groups could be removed in turn and additional amino acids could be incorporated. Thus, three peptide chains of designed or random sequence could be incrementally built upon a cholic acid scaffolding.

To verify that preparation of triamides could be accomplished on a solid support, compound **1** was coupled to an acid chloride containing resin (Scheme 3). The loading was ~33% of the theoretical amount, and methyl esters were formed from the remaining acid groups. Subsequently, the sequence described in Scheme 2 was followed. At the

Scheme 3. Preparation of Triamide **2** via Solid-Phase Synthesis



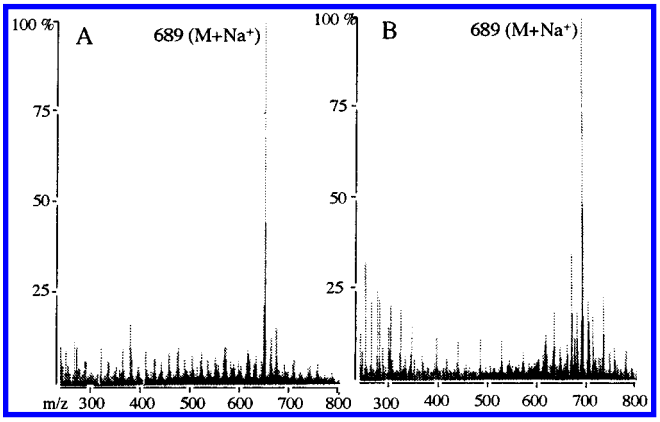


Figure 2. FAB-mass spectra (thioglycerol/Na^+ matrix) of **2:** (A) spectrum of the purified compound prepared in solution (Scheme 2); (B) spectrum of **2** prepared on a solid support without purification (Scheme 3).

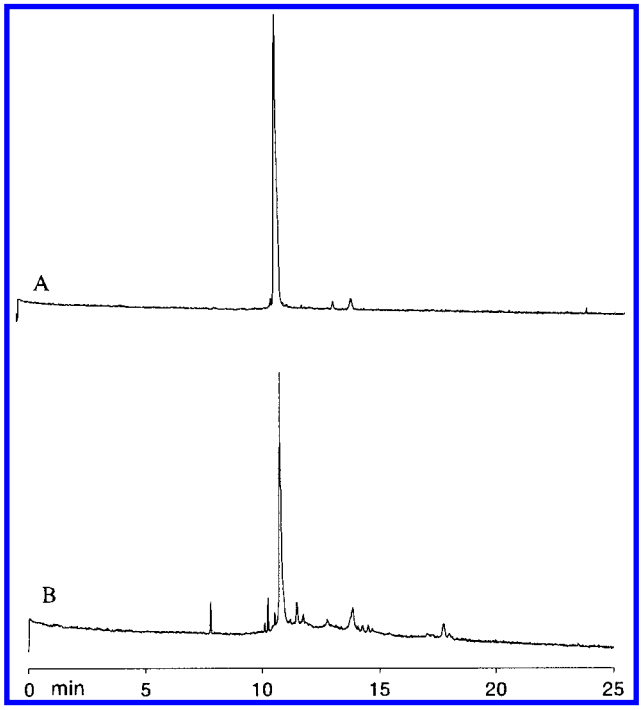


Figure 3. (A) Electropherogram of the purified compound prepared in solution (Scheme 2). (B) Electropherogram of **2** prepared on a solid support without purification (Scheme 3).

conclusion of each step, a small amount of the resin was removed and the steroid was cleaved from the resin. TLC was used to monitor the progress of the synthesis. After incorporation of the three amino acids and cleavage of the product from the resin, the resulting compound was compared to purified **2**. The products from solution and solid-phase syntheses were identical on TLC, and **2** was clearly the major product of the solid-phase synthesis. FAB-MS confirmed that **2** was formed (Figure 2), and capillary electrophoresis demonstrated that **2** was the major product of the solid-phase sequence (Figure 3).

Compound **1** offers a means of sequentially forming robust amide bonds to C-3, C-7, and C-12 on a cholic acid nucleus. We have demonstrated that three different groups can be added to the cholic acid scaffolding via solution synthesis or on a solid support. In addition, C-24 can be functionalized or used to link the compound to a solid support. We anticipate that this compound will facilitate designed syntheses of novel small molecule receptors formed from three peptide strands. It may also be possible to use **1** as the nucleus of peptide triple-helix bundles. We are currently using the compounds in combinatorial synthesis of receptors for selected small-molecule targets, and our results will be published in due course.

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Supporting Information Available: Experimental details for the preparation of **1**−**12** and capillary electrophoresis. This material is available free of charge via the Internet at http://pubs.acs.org.

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