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Short Syntheses of Triamine Derivatives of Cholic Acid

Chunhong Li, Atiq-ur-Rehman, N. Kent Dalley and Paul B. Savage*

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

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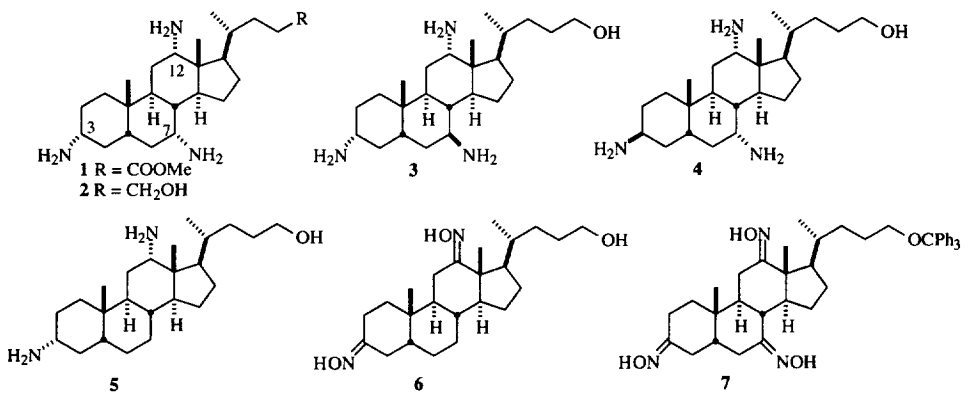
Abstract. Reduction of the trioxime derivative of dehydrocholic acid methyl ester with $NaBH_4$-$TiCl_4$ yielded triamine-derivatives of cholic acid. The major product from the reduction was the 3α,7α,12α diastereomer. Stereochemistry of the reduction products was determined via NMR spectroscopy and X-ray crystallography.

Cholic acid derivatives have been used as templates for combinatorial chemistry,[1] receptors for other molecules,[2] and antibacterial agents.[3] Recently, Davis and coworkers reported[4] the well planned synthesis of a new cholic acid derivative, a 'triamino-analogue' of methyl cholate (**1**) via a multistep procedure starting from cholic acid. We have been interested in compounds similar to **1** as they pertain to our development of new antibiotics and permeabilizers of the outer membranes of Gram-negative bacteria.[3a,b] Consequently, we have investigated short routes to cholic acid derivatives similar to **1** and have developed a method of preparing **2** in three steps from methyl cholate. This method gives **2** in a 32% overall yield from **8** and requires only one chromatographic separation which is straightforward. Triamines **3** and **4** are also formed in this procedure, but **2** is the predominant isomer formed.

Figure 1. Structures of cholic acid derivatives **1** – **7**.



Burrows and coworkers[5] reported the preparation of diamine **5** from the corresponding 3,12-bisoxime **6** by reduction using sodium in propanol. We attempted a similar reduction of 3,7,12-trisoxime **7** and found that a small amount of **2** was formed but that the major product was triamine **3** (the trityl group was removed during the reduction). Separation of the diastereomers formed in the reduction was possible using silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH).

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The ^1H NMR spectra of the triamines were very diagnostic in determining the stereochemistry of the reduced products. In particular, the numbers of axial and equatorial protons alpha to amines were very informative. These were distinguished by coupling constants (axial-axial couplings of > 7 Hz) and relative chemical shifts (equatorial protons were shifted down field from axial protons by > 0.7 ppm).

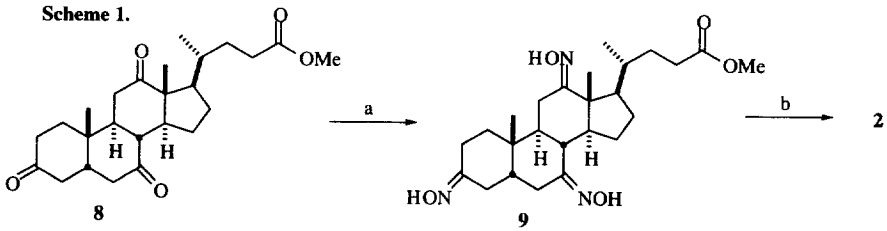
In the ^1H NMR spectrum of **3** we observed two resonances with axial-axial couplings, which were assigned as protons at C3 and C7. This assignment was made based on the fact that reduction of **6** with sodium in propanol gave 3α,12α diastereomer **5** as the primary product.[5] The principal difference between compounds **6** and **7** was substitution at C7. Provided that the stereoselectivity of the reduction of **6** and **7** with sodium in propanol was similar (i.e., that reduction gave primarily 3α,12α amines), the second axial proton should come from C7 with a β amine group. This conclusion is consistent with the outcome of the reduction of a 7-keto form of cholic acid with potassium in *t*-butanol reported by Davis and coworkers.[4] They found that the 7β alcohol was the major product of the reduction.

From the reduction of **7** with sodium in propanol, a small amount of **2** was isolated. The structure of **2** was tentatively assigned based on the observation of only one ^1H resonance coming from a proton alpha to an amine with axial-axial couplings.

Based upon the assumption that the reduction of **7** had yielded a small amount of **2**, we began investigating other reduction procedures that might produce **2** in greater amounts. As part of our efforts, we investigated reduction of trioxime **7** using combinations of sodium borohydride with various Lewis acids ($NiCl_2$,[6] MoO_3[6] and $TiCl_4$[7]) in 1,2-dimethoxyethane (glyme). Among these, $NaBH_4$-$TiCl_4$ was the only combination that after deprotection gave the stereoisomer tentatively assigned as **2** as the major triamine product. A minor amount of **3** was also isolated from the reaction mixture, but the primary triamine impurity from this reaction was a stereoisomer that exhibited only equatorial protons alpha to amines in the ^1H NMR spectrum. The only plausible assignment for this product was 3β,7α,12α diastereomer **4**.

After investigating a variety of conditions and substrates, we found a simple procedure to prepare **2**, which is shown in Scheme 1.[8] 5β-cholanic acid 3,7,12-trione methyl ester (**8**),[9] was treated with hydroxyl amine hydrochloride and sodium acetate in refluxing ethanol for 12 hr,[5] giving **9** in 97% yield. Reduction was effected by adding the oxime to a solution of $NaBH_4$-$TiCl_4$ in 1,2-dimethoxyethane and stirring the resulting mixture at room temperature for 24 h, followed by refluxing for 12 h. The reaction was quenched with ammonium hydroxide followed by treatment with hydrochloric acid. The mixture was then made basic with potassium hydroxide and the aqueous phase was extracted repeatedly with dichloromethane. Silica gel chromatography (CH_2Cl_2/MeOH/NH_4OH) of the concentrated extracts yielded **2** in 33% yield. Stereoisomers **3** and **4** were isolated in 8 and 14% yields, respectively.

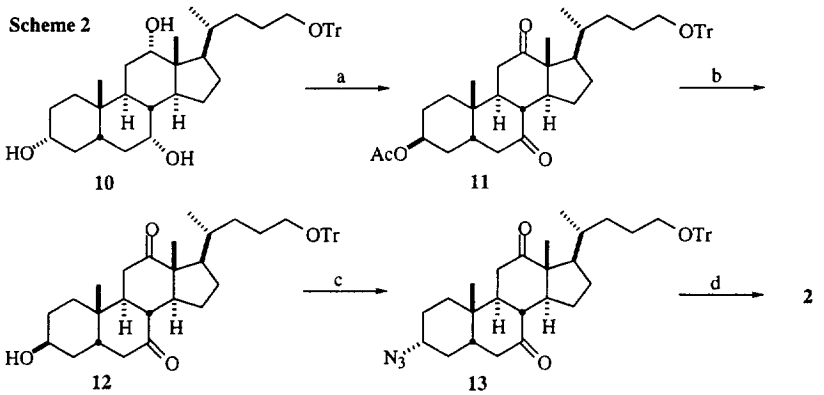
Scheme 1.



Reagents: a) NH_2OH-HCl, AcONa, EtOH (97%). b) $NaBH_4$ $TiCl_4$, glyme (33%).

In an attempt to improve the yield of **2** and as a means of confirming our stereochemical assignments, we prepared **2** via an alternate route (Scheme 2). Trityl ether **10**[3b] was treated with diisopropyldiazodicarboxylate, triphenyl phosphine and acetic acid to cause inversion at C-3. The resulting dialcohol was treated with pyridinium dichromate to give diketone **11** in 62% yield from **10**. The ester at C-3 was hydrolyzed with sodium hydroxide in methanol in 95% yield. The alcohol was mesylated and

immediately reacted with sodium azide to give **13** in 94% yield for the two steps. Diketone **13** was treated with hydroxylamine hydrochloride and sodium acetate and then reduced with $NaBH_4$-$TiCl_4$ as described above. Treatment of the triamines with acid during the work-up procedure resulted in hydrolysis of the trityl ether. Triamine **2** was the main product (35% yield) from the reduction, and as expected compound **4** was not detected.



Reagents: a) diisopropyldiazodicarboxylate, Ph_3P, AcOH, THF; PDC, 4 Å molecular sieves, CH_2Cl_2 (62%). b) NaOH, H_2O, MeOH, THF (95%). c) MsCl, Et_3N, CH_2Cl_2; NaN_3, DMSO (94%). d) NH_2OH•HCl, AcONa, EtOH; $NaBH_4$, $TiCl_4$, 1,2-dimethoxyethane; TsOH, MeOH; NaOH (35%).

Confirmation of the stereochemistry of the major product of the reduction of **9** came from the X-ray crystal structure of the tris*t*-butyloxycarbamate of **2** (**14** in Figure 2).[10] The crystals were grown from ethanol and water and included an equivalent of ethanol.

In summary, we have found that use of $NaBH_4$-$TiCl_4$ in the reduction of trioxime **9** in a key step yields a 'triamino-analog' of a reduced form of cholic acid (**2**) in only three steps from methyl cholate. This pathway, employing relatively inexpensive reagents, allows the rapid preparation of substantial quantities of a unique amphiphile. We have employed this method to prepare gram quantities of triamine **2** which we have incorporated into new permeabilizers of the outer membranes of Gram-negative bacteria.[11]

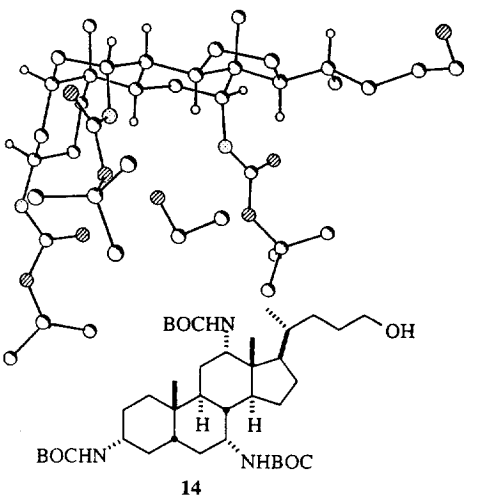


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Figure 2. Structure and X-ray crystal structure of **14**. Selected hydrogens have been omitted for clarity.

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8. Synthetic procedure for **2:** A 250 ml three neck flask was charged with glyme (100 ml), and to this was added **9** (1.00 g, 2.16 mmol) and sodium borohydride (2.11 g, 55.7 mmol). $TiCl_4$ (4.0 mL, 36.4 mmol) was added to the mixture slowly under nitrogen at 0° C. The resulting green mixture was stirred at room temperature for 24 h and then refluxed for another 12 h. The flask was cooled in an ice bath, and ammonium hydroxide (100 mL, 28-30% NH_3 in H_2O) was added. The resulting mixture was stirred for 6 hours at room temperature. Conc. HCl (60mL) was added slowly, and the acidic mixture was stirred for 8 h. The resulting suspension was made alkaline by adding solid KOH. The suspension was filtered and the solids were washed with MeOH. The combined filtrate and washings were combined and concentrated in vacuo. The resulting solid was suspended in 6% aqueous KOH (100 mL) and extracted with CH_2Cl_2 (4 x 75 mL). The combined extracts were dried over Na_2SO_4, and solvent was removed in vacuo to give 1.14 g of a white solid. The mixture was chromatographed on silica gel (CH_2Cl_2/MeOH/NH_4OH (28-30% NH_3 in H_2O) 10:5:1) giving **2** (0.282 g, 33% yield), **3** (0.066 g, 8% yield) and **4** (0.118 g, 14% yield). R_f values (CH_2Cl_2/MeOH/NH_4OH 10:5:1): **2:** 0.40; **3:** 0.49; **4:** 0.31. Characterization of **2:** m.p. 200–202°C; ^1H NMR (~10% $CDCl_3$ in CD_3OD, 300 MHz) δ 4.81 (bs, 7 H), 3.57–3.49 (m, 2 H), 3.14 (t, J = 3.2 Hz, 1 H), 2.97 (bs, 1 H), 2.55-2.50 (m, 1 H), 2.15–2.10 (m, 1 H), 1.95–1.83 (m, 3 H), 1.74–0.99 (series of multiplets, 20 H), 1.01 (d, J = 6.4 Hz, 3 H), 0.95 (s, 3 H), 0.79 (s, 3 H); $^{13}CNMR$ (~10% $CDCl_3$ in CD_3OD, 75 MHz) 63.28, 55.01, 52.39, 49.20, 48.69, 47.00, 43.24, 42.77, 41.03, 40.27, 36.82, 36.35, 35.75, 35.12, 32.77, 31.36, 30.10, 28.54, 27.88, 26.96, 24.35, 23.38, 18.18, 14.23; HRFAB-MS (thioglycerol + Na^+ matrix) *m/e*: ([M+H]$^+$) 392.3627 (100%); cacld. 392.3641.
9. Compound **8** can be prepared from methyl cholate by a variety of high yielding reactions. For examples see: (a) Pearson, A. J.; Chen, J.-S.; Han, G. R.; Hsu, S.-Y.; Ray, T. *J. Chem. Soc. Perkin Trans. 1* **1985**, 267. (b) Mitra, M. N.; Elliott, W. H. *J. Org. Chem.* **1968**, *33*, 175. (c) Takeda, K.; Igarashi, K. *J. Biochem. (Tokyo)* **1959**, *46*, 1313. We prepared **8** in near quantitative yield from methyl cholate and pyridinium dichromate.
10. Atomic coordinates, bond lengths and angles have been deposited with the Cambridge Crystallographic Data Center. The structure was solved, refined and displayed using Siemens SHELXTL PC Version 5.3 program package. All nonhydrogen atoms were refined anisotropically. Hydrogen atom positions for hydrogens bonded to carbon atoms were calculated. Hydrogen atoms bonded to oxygen and nitrogen atoms were found in the difference map. Hydrogens were refined using the riding model. X-ray source was graphite monochromated MoKα radiation, λ = 0.71073 Å. $C_{39}H_{69}N_3O_7 \cdot C_2H_5OH$, MW 738.09, monoclinic, $P2_1$, a = 11.448(2), b = 12.284 (2), c = 16.085 (3) Å, β = 95.20 (2)°, V = 2252.8 Å^3, 3071 data with 1971 > 2σ(I), R = 0.051, WR^2 = 0.099 with observed data, refined on F^2.
11. See the following Letter.