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The "Triamino-analogue" of Methyl Cholate; A Facial Amphiphile and Scaffold with Potential for Combinatorial and Molecular Recognition Chemistry

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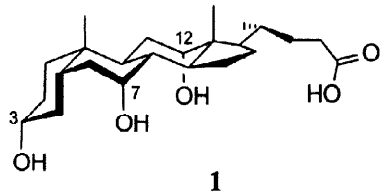
Abstract: The triamino steroid **2** is synthesized from cholic acid (**1**), and found to possess little tendency to aggregate at pH 5 - 6 in aqueous solution. **2** and/or related derivatives are expected to find use in the synthesis of receptors and combinatorial libraries, and as "contrafacial amphiphiles" for drug delivery across cell membranes.

Cholic acid (**1**), and its deoxy analogues, have been variously exploited for the construction of synthetic receptors,[1] novel amphiphiles,[2] and scaffolds for the assembly of combinatorial libraries.[3] In all these applications, there are significant advantages to the replacement of hydroxyl by amino functionality. Amino groups can be derivatised rapidly and quantitatively, retain H-bond donor capabilities after acylation, and are highly hydrophilic when protonated.[4] Herein we report the first synthesis of **2**, a tris-deoxa-tris-aza analogue of methyl cholate, and the preliminary characterisation of its solution properties at neutral and acidic pH. In particular we highlight its potential as a "facial amphiphile", with enforced hydrophobic and hydrophilic surfaces which may confer useful recognition and transport properties in biphasic media.



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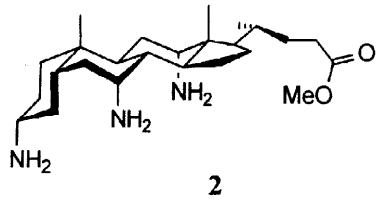

2

Our synthetic route to **2** is summarised in the Scheme. Where possible the OH → NH_2 conversions were performed by double displacement, to maximise certainty of configurational assignments and minimise the need to separate diastereomeric mixtures of highly polar polyamine derivatives. Ketodiol **3**, was available from **1** *via* an efficient literature procedure.[5] Reduction with potassium in *tert*-amyl alcohol, after the method of Giordano et al.,[6] yielded 7-*epi*-cholic acid (**4**) along with a trace (*ca.* 5%) of **1**. Esterification, followed by selective acetylation of the equatorial hydroxyl groups, gave diacetate **5**. Removal of the unwanted 7α diastereomer at this point was aided by the slower rate of acylation of its (axial) 7-OH group. Oxidation of the 12-OH, selective deacetylation at position 3,[7] and Mitsunobu inversion gave intermediate **6** in 24%

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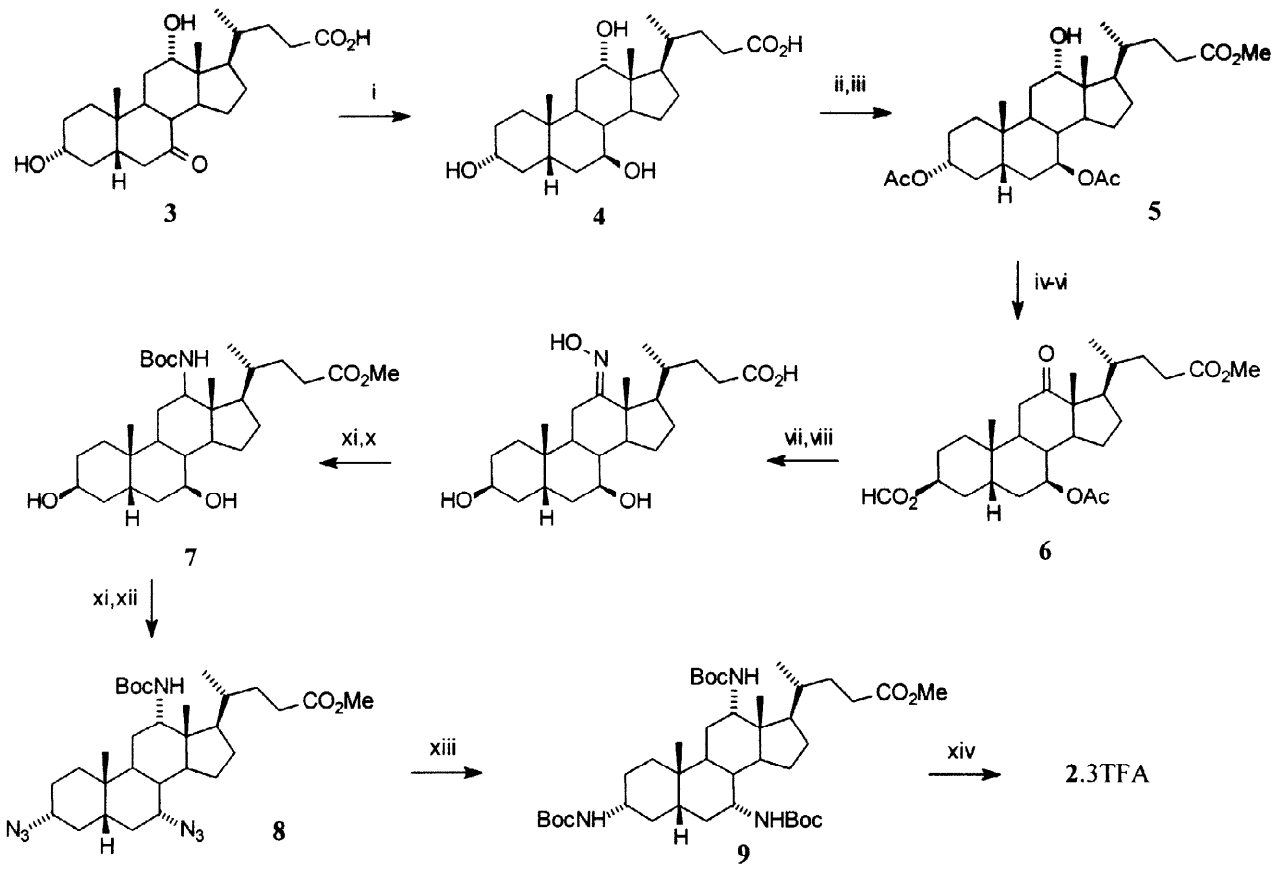
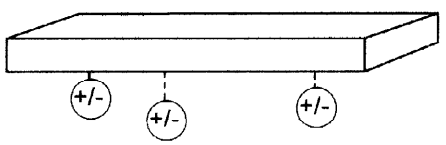


Scheme. *Reagents and conditions*: i, K, *tert*-amyl alcohol, reflux; ii, MeOH, H_2SO_4; iii, Ac_2O, py, room temp., 8 h; iv, $Na_2Cr_2O_7$, AcOH; v, MeOH, AcCl; vi, Ph_3P, DEAD, HCO_2H, THF; vii, H_2NOH, NaOAc, MeOH; viii, NaOH, MeOH; ix, Na, EtOH, then $(Boc)_2O$; x, CsF, MeI, DMF; xi, MsCl, Et_3N, CH_2Cl_2; xii, NaN_3, DMPU, 80 °C, 10 d; xiii, PtO_2, H_2, $(Boc)_2O$; xiv, TFA, CH_2Cl_2.

overall yield from **1**. The first of the amino groups, at C12, was now introduced by oximation, ester hydrolysis and Na/EtOH reduction.[8] After protection of the free amino and carboxyl groups, the 12-carbamates **7** were isolated as an 85:15 mixture of epimers (32% from **6**). Mesylation followed by azide displacement[9] gave **8** as the major product, separable from its 12-epimer by chromatography and clearly identifiable from the ^{1}H NMR coupling pattern of the (equatorial) C(12)*H* (dt, J = 9 and 3 Hz). Reduction of the azides with *in situ* carbamoylation yielded tris-carbamate **9**,[10] which could be deprotected to give **2** as necessary.

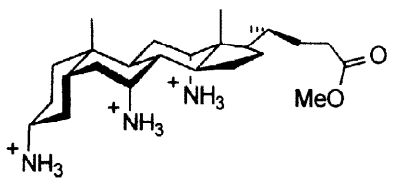
Although **9** contains identical N-protecting groups, it should be noted that a second group, orthogonal to Boc, could have been introduced after the reduction of **8**. Selective derivatisation of the (equatorial) 3α-NH_2 could probably be accomplished at this stage, allowing a third protection method to be used at position 7. The synthesis in the Scheme may therefore be adaptable to an analogue of **9** with differential *N*-protection, independently addressable at all three centres, and thus a versatile starting material for combinatorial libraries[3b] or asymmetrically substituted podand-type receptors.

Amphiphiles of unusual geometry have been subjects of recent research in several laboratories.[2,11] In particular, Gellman has suggested that "contrafacial amphiphiles" **10** may be geometrically unsuited to forming micelles, unlikely to self-associate in aqueous solution, and thus available to act as vehicles for drug delivery across cell membranes.[2d] Though sometimes considered a natural prototype for facial amphiphiles, cholic acid (**1**) is imperfect in this respect. While the steroidal faces are clearly differentiated, its most hydrophilic centre is the side-chain carboxylate, on which it relies for water solubility (as, apparently, do the glycosylated analogues studied by Kahne and co-workers[2b]). *In contrast, 2 lacks a highly polar side-chain, but should possess far greater hydrophilicity on the α-face of the steroid at acid or neutral pH.*



10

Cf.



2.3H$^+$

Preliminary studies on **2** in aqueous solution suggested aggregation properties consistent with Gellman's proposal. A ^{1}H NMR study following the 3β, 7β and 12β protons at variable pH (2-12) confirmed that all three nitrogens remain almost completely protonated up to pH ~ 7.[12] At pH 5-6, the triamine was soluble to at least 1 M. ^{1}H NMR spectra taken at concentrations between 80 and 0.3 mM at this pH were well-resolved and essentially similar, suggesting that triprotonated **2** is monomeric over at least this range. To obtain an estimate of the critical aggregation concentration (CAC), we investigated the solubilisation by **2** of the hydrophobic dye Orange OT, again at pH 5-6. This method had previously been applied to **1** at pH 7, yielding CAC's of ~0.01 M.[2b] In the case of **2**, solutions in contact with Orange OT remained colourless at concentrations up to ~ 0.45 M, after which small amounts of the dye began to dissolve. An accurate determination was precluded by the high concentrations required and limited quantity of amphiphile available. However the data clearly suggest a CAC in the same region (0.35 - 0.5 M) as those reported by Gellman for bis-anionic dibenzobarrelene facial amphiphiles,[13] and well above any concentration which might reasonably be attained *in vivo*.

In conclusion we have synthesized the "triaza-analogue" of the cholic acid acid nucleus, one of the steroidal residues most widespread in Nature. Its unusual amphiphilic profile may find use in drug delivery systems, especially for anionic agents, in addition to any inherent biological activity.[14] The positioning of three co-directed amino groups on a rigid steroidal skeleton, also furnished with a functionalised side chain, suggests further applications in receptor design and combinatorial chemistry.

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6. Giordano, C.; Perdoncin, G.; Castaldi, G. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 499.
7. While both 3α and 7β positions are equatorial, it seems that the former is slightly more exposed. A small amount of diol formed by over-reaction could be bis-acetylated for recycling.
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10. *Selected data for* **9**: ^{1}H NMR [300 MHz, $(CD_3)_2CO$] δ 0.84 (s, 3H, 18-Me), 0.92 (d, 3H, *J* 6.3 Hz, 21-Me), 0.98 (s, 3H, 19-Me), 1.40 (s, 27H, 3 x $C(CH_3)_3$), 3.34 (br. m, 1H, 3β-H), 3.60 (s, 3H, CO_2Me), 3.66 (br. m, 1H, 7β-H), 4.03 (br. m, 1H, 12β-H); ^{13}C NMR [$(CD_3)_2CO$] δ 13.7 (C-18), 17.9 (C-19), 22.9 (C-21), 23.5 (C-15), 27.5, 27.7, 28.3 ($C(CH_3)_3$), 28.5 (2 x $C(CH_3)_3$), 29.2 (C-9), 31.2 (C-22), 31.3 (C-23), 32.5 (C-6), 35.1 (C-10), 35.3 (C-20), 36.3, 36.6, 37.4 (C-8), 42.6, 45.0, 45.5 (C-13), 47.8, 48.8, 51.2 (CO_2CH_3), 53.7, 78.0 (C-3), 78.1 (C-7,12), 155.4 (NCO), 155.7 (NCO), 155.8 (NCO), 174.1 (CO_2CH_3); υ_{max} (film from DCM) cm^{-1} 3400 (NH), 1711(CO), 1170; Anal. Found: C, 67.07; H, 9.76; N 5.91. $C_{41}H_{71}N_3O_8$ requires C, 67.09; H, 9.75; N, 5.72%.
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