Thermodynamically-controlled cyclisation and interconversion of oligocholates: metal ion templated 'living' macrolactonisation

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A series of ester-linked macrocyclic oligomers (dimer–pentamer) of cholates equipped with a variety of recognition and reporter elements has been prepared (*a*) by conventional irreversible chemistry, and (*b*) using methoxide-catalysed transesterification under reversible equilibrium conditions. Templating by metal ions under these equilibrium conditions is also demonstrated. These results demonstrate the feasibility of creating a dynamic combinatorial library of receptors.

Macrocyclic molecules have been central to the emergence of supramolecular chemistry.[1] They have a defined cavity in which binding processes can take place as a result of the rigidity and spatial arrangement of functional groups imposed by the cyclic structure. Furthermore the synthetic challenge of such large ring systems has stimulated numerous new preparative approaches. In particular, templates have proved useful in the promotion of cyclisation reactions and they can dramatically improve yields in many systems.[2] Most reactions utilised in macrocycle syntheses have been performed under kinetic control, so the template has acted by bringing the two ends of a particular intermediate into close proximity, thus favouring their reaction. This effect results in an increase in the quantity of the product, the precursor to which was bound effectively by the template.

We, and others,[3] have been interested in carrying out such templating in a thermodynamic regime. This approach is potentially more versatile since the template will promote formation of the product that it binds best, rather than just accelerating the formation of a particular product. In a single component mixture such as that illustrated in Fig. 1, templating can bias the equilibrium between different sized cyclic oligomers C_n towards a particular product. If the idea is extended to the use of a mixture of several different building blocks, thermodynamic templating could lead to the generation of a mixed host that binds the template well (Fig. 2). In order to investigate this approach, we required an efficient thermodynamically-controlled method for the synthesis of macrocyclic molecules.

Many chemical reactions proceed under thermodynamic control and some of these have been utilised in macrocycle syntheses. Cyclic polyesters of small ring lactones have been extensively investigated by Seebach[4] and Roelens,[5] and a theoretical treatment of the product distribution expected in these systems has been developed by Mandolini.[6] Reversible imine formation has been used in the synthesis of many cyclam ionophores,[7] though it is not clear that the reversibility has been fully exploited in most cases. The reversible formation of imines has been used by Lindsey and Mauzerall in the synthesis of macrocyclic porphyrin–quinone adducts to give a high yield of the structurally most stable adduct.[8] Calix[4] arenes and calixresorcinarenes can be produced from larger ring analogues, demonstrating reversible bond formation in their synthesis.[9] Similarly, isolated α-, β- or γ-cyclodextrins are found to re-equilibrate to a mixture under the conditions of their formation, but enzymes are required for this process.[10] In most of these examples, the use of thermodynamic control in macrocyclisations has led to reactions generating a variety of products with a range of ring sizes.

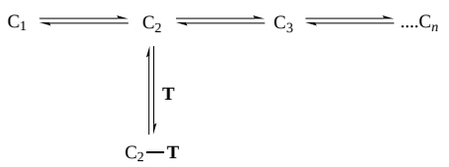


Fig. 1 Changing a cyclisation equilibrium using a thermodynamic template **T**

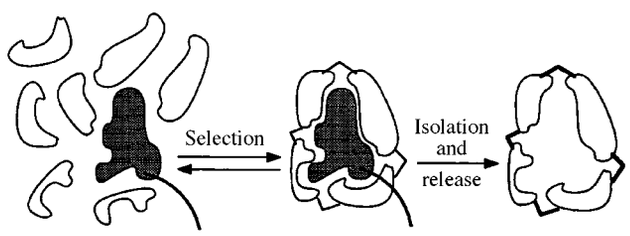


Fig. 2 Schematic picture of thermodynamic templating for host generation in a complex mixture of building blocks

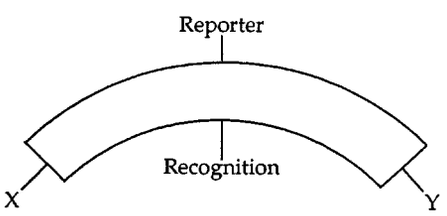


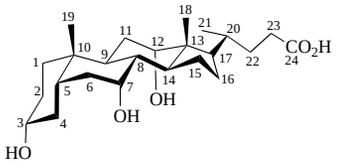
Fig. 3 A general building block for host generation

Strategy

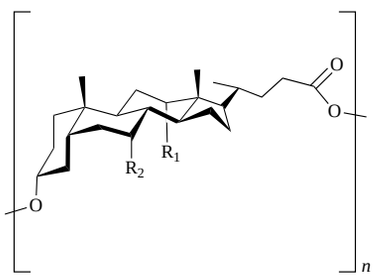
Considering the process in Fig. 2, a broad range of products will be formed if the mixture contains a variety of different building blocks. The utility of this approach will depend on the identification of the products, so it will be necessary for each building block to contain a unique spectroscopic reporter group. In addition to this, the building blocks should be relatively rigid and slightly curved, so as to define a cavity and they should contain a recognition element for interaction with the template or guest. Several possibilities can be imagined for this: an amide to hydrogen bond to a guest molecule; a charged group to attract an oppositely-charged site; or an aromatic group for π–π interactions. A cartoon picture of a suitable building block is shown in Fig. 3.

To realise these principles, we turned to derivatives of cholic acid. These bile acids have the general structure **1** and have the attractive feature of combining a rigid concave component with a flexible chain. Cholic acid derivatives have previously been

incorporated into several families of macrocycles[11] and these have been successful in the recognition and binding of sugar and metal ion guests.[12] Cyclocholates **2** were a particularly



Cholic acid **1**



Cyclocholates **2**

attractive target family for these studies since they consist of steroid units joined through ester linkages which can be generated under thermodynamic control by transesterification. Further, strategies for the selective protection of the three hydroxy groups have already been developed, so it is straightforward to synthesise differentially-functionalised steroid derivatives. We therefore envisaged preparing a series of cholate-based building blocks, each incorporating recognition and reporting elements. If each building block is furnished with an alcohol and a methyl ester, then transesterification should lead to the generation of cyclic products if methanol is removed from the mixture as shown in Scheme 1. From the mixture of cyclic products obtained, we hope to select a good binder by the action of a template.

In this paper we report the synthesis by conventional irreversible chemistry of a series of cyclocholates equipped with a variety of recognition and reporter elements; the exploration of reversible transesterification conditions; cyclocholate synthesis under these reversible conditions; and the influencing of the equilibrium using metal ions under thermodynamic conditions. Some of these results have previously been described in preliminary form.[13]

Results and discussion

Monomer synthesis

The general strategy for the synthesis of 7,12-differentially protected cholate derivatives was to begin by protecting the 3- and 7-hydroxy groups as acetates and then functionalise at the 12-position. After removal of the protecting groups, the 3-hydroxy group is more reactive than the 7-, so it can be re-protected allowing the 7-position to be functionalised. Removal of the protecting group then yields the 7-, 12-differentially functionalised 3-free alcohol methyl ester.

'MEM' monomer **10** has a polyether side chain as a recognition element and a benzyl group as a UV reporter. Starting from cholic acid **1**, the methyl ester **3** was prepared according to the method of Dias.[14] This was then acetylated in 60% yield at the 3- and 7-positions with acetic anhydride and pyridine at room temperature as described by Fieser and Rajagopalan to give **4**.[15] This material was next benzylated at the 12-position with benzyl 2,2,2-trichloroacetimidate[16] and trifluoromethanesulfonic acid to give methyl 12-benzyloxy-3,7-diacyloxycholanoate **5** in 62% yield.[17] Following basic hydrolysis of the two acetyl protecting groups and the methyl ester, the methyl ester was reformed by stirring in methanolic HCl. This gave the methyl 12-benzyloxy-3,7-dihydroxycholanoate **6** in 32% yield from diacetate **4** as shown in Scheme 2.

The benzyl ether **6** was next acetylated at the 3-position with acetic anhydride and pyridine to give the monoacetate **7** in 87% yield. Alcohol **7** was then alkylated with MEM chloride[18] and Hünig's base to form MEM ether **8** in 67% yield. Following basic hydrolysis of the 3-acetyl and the methyl ester groups, the methyl ester was reformed to yield the methyl 3-hydroxy-7-(2-methoxyethoxymethyl)-12-benzylcholanoate **10** in 52% overall yield from **6**. It is worth noting that the acid catalysed esterification step did not cause any significant decomposition of the acetal group.

The 'MEM monomer' **10** obtained was crystallised from diethyl ether and hexane. The crystal structure was solved and is shown in Fig. 4.[19] The crystals are monoclinic in space group $P2_1$ with two molecules in the unit cell. There are no particular interactions between the 7- and 12-side chains and no solvent is present in the lattice.

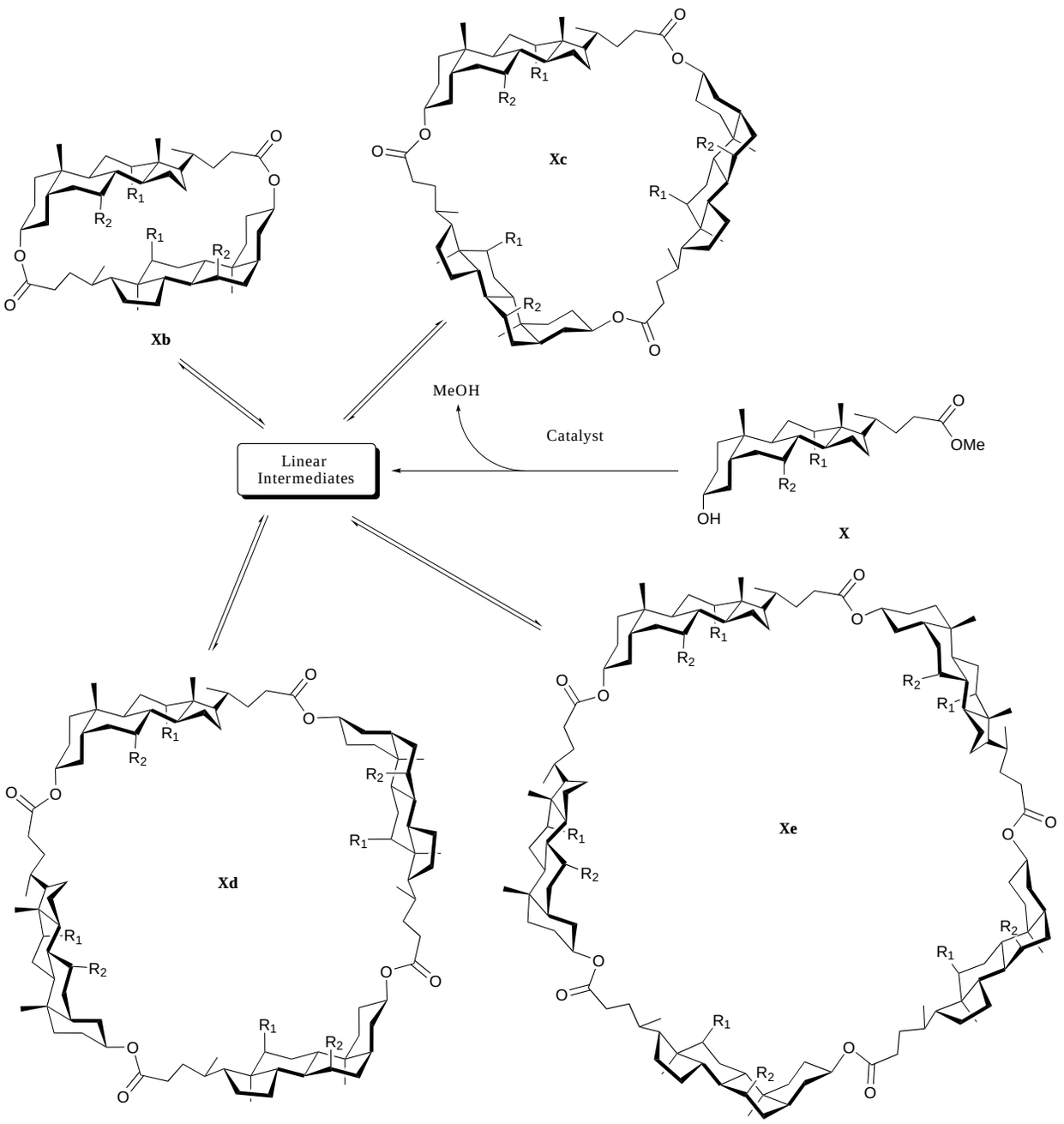
The 'bis(*p*-methoxybenzyl)' monomer **13** was synthesised as a control for the 'MEM' monomer. It has a different UV spectrum and no obvious metal-binding functionality. The 3-(ethyl carbonate) (or 'cathyl') derivative **11** was formed in 75% yield by addition of ethyl chloroformate and pyridine to methyl cholate **3** as described by Fieser.[20] The *p*-methoxybenzyl group was introduced by similar trichloroacetimidate methodology as described above. As the *p*-methoxybenzyl 2,2,2-trichloroacetimidate is not commercially available, it was synthesised from *p*-methoxybenzyl alcohol and trichloroacetonitrile by the method described by Cramer *et al.*[21] As found by Yonemitsu *et. al.*,[22] a weaker acid is required for the addition of this reagent to hydroxy groups. Catalysis with 5 mol% camphorsulfonic acid gave the dibenzylated product **12** in 75% yield as shown in Scheme 3. The final deprotection step furnished methyl 3-hydroxy-7,12-bis(*p*-methoxybenzyloxy)cholanoate **13** in 60% overall yield from **11**.

The 'deoxy' monomer **18** was synthesised as a further control with no binding sites and is shown in Scheme 4. Methyl deoxycholate was obtained from deoxycholic acid as described by Malik and Sharts[23] and the 3-hydroxy group was acetylated using the same procedure as in the cholate series above to give methyl 3-acyloxy-12-hydroxycholanoate **14** in 74% yield.[24] The *p*-phenylbenzyl group was also introduced *via* the 2,2,2-trichloroacetimidate route. The *p*-phenylbenzyl 2,2,2-trichloroacetimidate **15** was synthesised from *p*-phenylbenzyl alcohol and trichloroacetonitrile in 69% yield according to the method of Cramer *et al.*[21] Addition of this new reagent to the 3-protected deoxycholate **14** at room temperature with catalysis by trifluoromethanesulfonic acid (10 mol%) gave rise to smooth formation of the benzylated product **16** in 84% yield. Upon removal of the final protecting group, to give **17** and re-esterification, methyl 3-hydroxy-12-(*p*-phenyl)benzyloxycholanoate **18** was obtained in 56% overall yield from the 3-protected methyl deoxycholate **14**.

In order to synthesise the 'bis(BOM)' monomer **19** the 3-carbonate protected steroid **11** was alkylated with (benzyloxymethoxy)methyl chloride and Hünig's base according to the procedure of Stork and Isobe.[25] The dialkylated product was deprotected at the 3-position by addition of methanolic potassium methoxide, to yield the methyl 7,12-bis(benzyloxymethoxy)-3-hydroxycholanoate **19** in 58% yield from **11** after purification.

Kinetic syntheses of macrocyclic steroidal polyesters

Initially, cyclocholates of the 'MEM' **9**, 'bis(*p*-methoxybenzyl)' **20** and the 'deoxy' **17** cholic acids were all synthesised using the same procedure, shown in Scheme 5. The methyl ester was hydrolysed to the free acid by refluxing in THF and sodium



Scheme 1 An equilibrating mixture of cyclic steroid oligomers

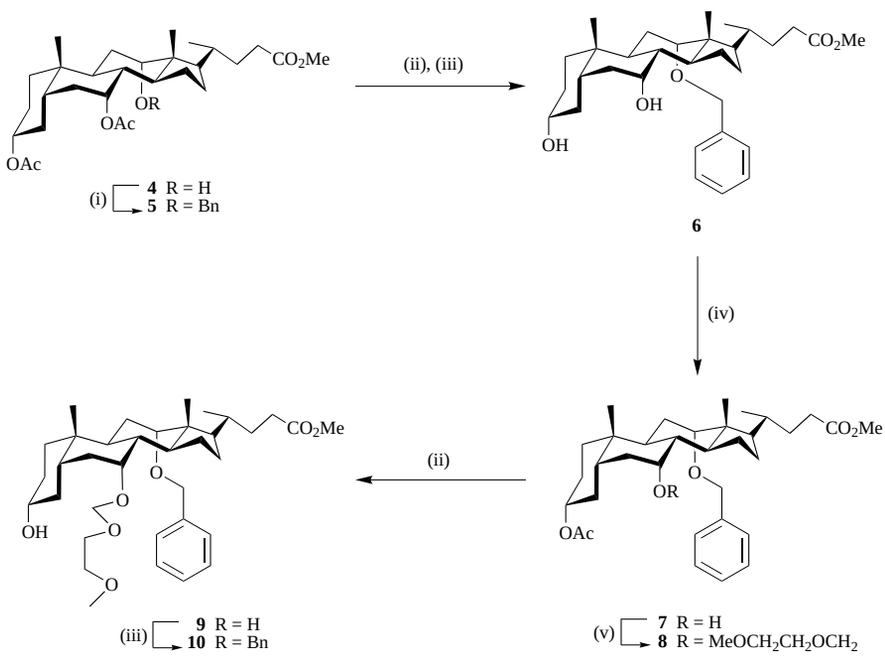
hydroxide and purified by flash column chromatography if necessary. Macrolactonisation was effected by an *in situ* variant[26] of the Yamaguchi cyclisation method.[27] The hydroxy acid at 15 mM concentration was stirred in dichloromethane in the presence of 4 Å molecular sieves and then 2,6-dichlorobenzoyl chloride and DMAP were added. After 24 h stirring at room temperature, the reaction was quenched. The different sized macrocyclic products were separated by flash column chromatography. Isolated yields are shown in Table 1. The nomenclature adopted is as follows: cyclisation of monomer acid **X** results in cyclic dimer **Xb**, cyclic trimer **Xc**, cyclic tetramer **Xd** and cyclic pentamer **Xe**. As found earlier,[26] the presence of both a 7- and a 12-substituent in the 'MEM' and the 'bis(*p*-methoxybenzyl)' monomers prevents formation of cyclic dimer and for all of the molecules, the cyclic trimer is the dominant product.

These macrocyclic polyesters were separable by flash column chromatography and the ring sizes were deduced from mass spectral data. The NMR spectra of the different macrocycles within a given series were essentially the same with only small variations in the ^{13}C and ^{1}H shifts at the 3-positions and virtually no variation in the ^{13}C shift of the 24-carbonyl carbon. The only exception to this was the 'deoxy' dimer **17b**. The carbonyl carbon was shifted downfield by 2 ppm and the aromatic region of the spectrum was more widely dispersed than in the other macrocycles.

Synthesis of the bis(benzyloxymethoxy) cyclic tetramer **27d** was carried out by the cyclisation of a linear dimer. To synthesise the linear dimer, suitable protocols were required for the protection of the 24-acid and the 3-hydroxy of the 'bis(BOM)' monomer **19**. The *tert*-butyldimethylsilyl group was found to be suitable for the protection of the 3-hydroxy group. No methods were found to be suitable for the selective cleavage of the methyl ester. In trial systems, lithium iodide in pyridine,[28] lithium iodide in DMF[29] and barium hydroxide in methanol[30] all failed to react at all with a steroidal monomer methyl ester, whilst potassium *tert*-butoxide in DMF cleaved every ester present within 30 s,[31] so a different protecting group was needed. 2,2,2-Trichloroethyl esters can be cleaved reductively with zinc powder, which can simply be filtered off after reaction, so this

Table 1 Isolated yields of cyclic products in the *kinetic* Yamaguchi macrolactonisation

Ring size	Dimer **Xb** (%)	Trimer **Xc** (%)	Tetramer **Xd** (%)	Pentamer **Xe** (%)
'MEM' **9**	0	17	17	10
'Deoxy' **17**	11	31	21	0
'Bis(*p*-methoxybenzyl)' **20**	0	11	8	5



Scheme 2 The synthesis of the 'MEM' monomer; (i) benzyl 2,2,2-trichloroacetimidate, CF_3SO_3H (ii) NaOH, THF (iii) MeOH, HCl (iv) Ac_2O, pyridine (v) MEM-Cl, Pr^i_2EtN

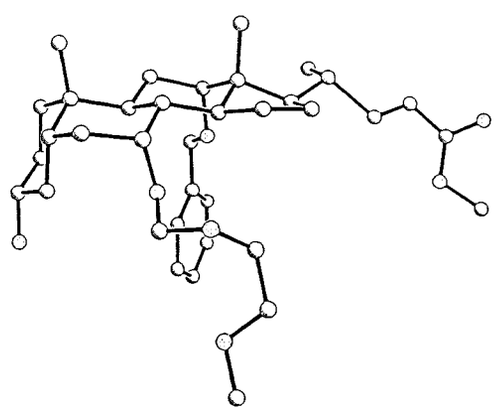


Fig. 4 The crystal structure of the 'MEM' monomer

was an attractive protection strategy.[32] Thus, the 2,2,2-trichloroethyl ester **22** with a free 3-hydroxy group was synthesised from the acid **21** by a Yamaguchi coupling in 94% yield.[27] The 3-TBDMS protected 24-free acid **24** was obtained from the methyl ester **23** and TBDMS chloride followed by hydrolysis of the methyl ester by refluxing in THF and sodium hydroxide in 63% overall yield.[33]

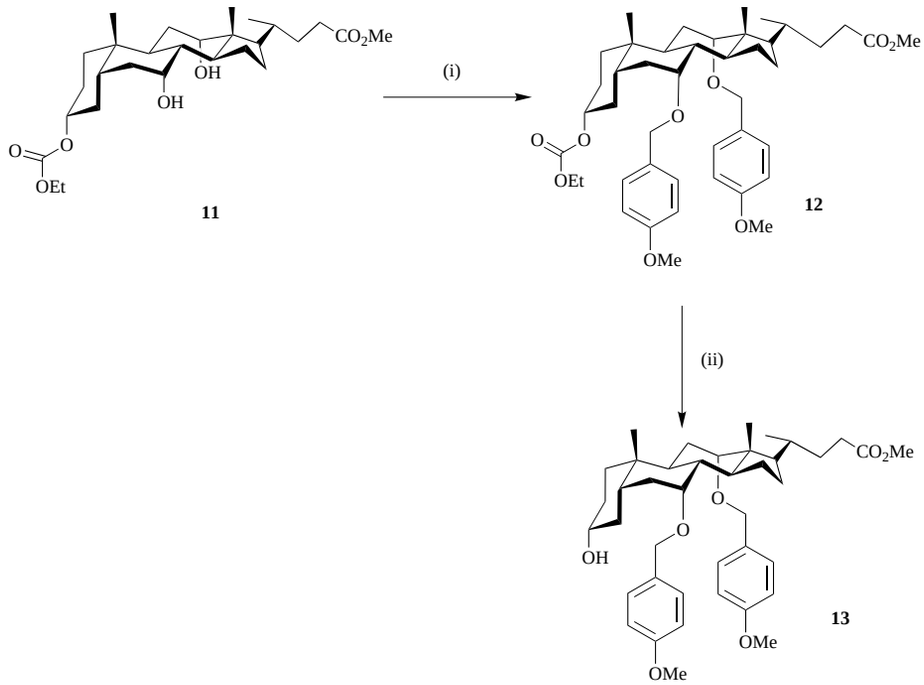
The two fragments were then coupled together by a Yamaguchi esterification to give the protected linear dimer **25** in 65% yield as shown in Scheme 6.[27] This was deprotected at the 3-position with aqueous HF in 93% yield to give **26**. No significant decomposition of the acetal was observed in this step. The 2,2,2-trichloroethyl ester was then removed by reaction with zinc powder in aqueous potassium phosphate buffer to give the free acid **27** in 85% yield. The linear dimer was then cyclised as shown using the modified Yamaguchi procedure described above. This yielded a small number of different macrocycles, the main product being cyclic tetramer **27d**, which was isolated in 37% yield.

Development of transesterification conditions for macrocyclisation

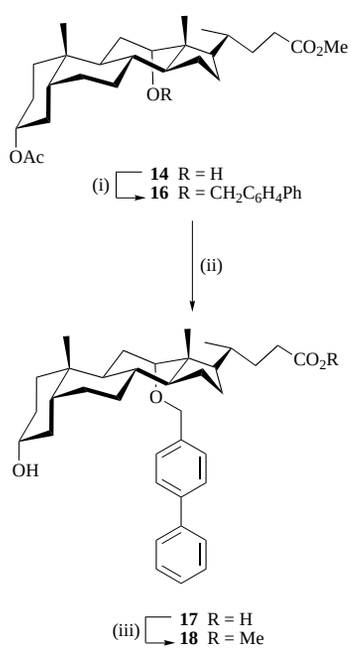
As described above, transesterification can produce an equilibrium distribution of products. This equilibrium can be driven to cyclic materials by removal of condensation products. If the starting material is a methyl ester, the condensation product is methanol which can be removed as an azeotrope with a suitable co-solvent. Toluene (bp 111 °C) is very effective for this as it forms an azeotrope with methanol (bp 65 °C) of composition 72.4 : 27.6 (methanol–toluene) at 63.7 °C. Azeotropic removal is more effective if the methanol is absorbed into cool 4 Å molecular sieves.[34] This was achieved in the Soxhlet-type apparatus shown in Fig. 5, which allows very small scale (<3 ml) reactions to be set up in a dry, inert atmosphere.

All of the cyclisation reactions were analysed by HPLC. Authentic samples of cyclic oligomers synthesised by the kinetic route described above were used to identify the peaks observed. Normal phase columns with solvent mixtures of hexane and propan-2-ol were used for the separations. By comparing the integrals of the peaks due to cyclic steroid oligomers (with a knowledge of the extinction coefficients), it was possible to calculate the ratio of the products in the mixture.

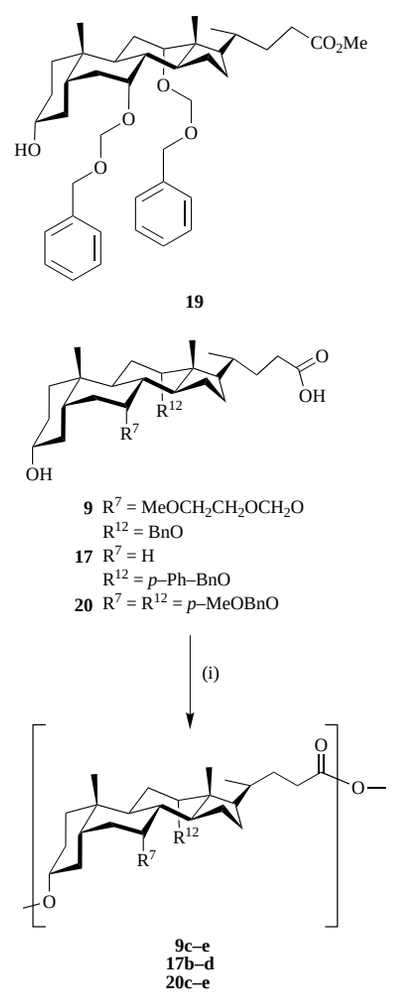
One of the main conclusions of the theoretical modelling of reversible cyclisations is the existence of a critical monomer concentration, above which any further material generates only polymeric products rather than macrocycles. Thus, it was important to keep solutions below the critical monomer concentration and to find conditions which would also be appropriate for use with several building blocks, so all reactions were carried out under fairly high dilution (5 mM). The catalyst of choice for the transesterification should fulfil two criteria: (i)



Scheme 3 The synthesis of the 'bis(*p*-methoxybenzyl)' monomer; (i) *p*-methoxybenzyl 2,2,2-trichloroacetimidate, camphorsulfonic acid (ii) KOMe, THF



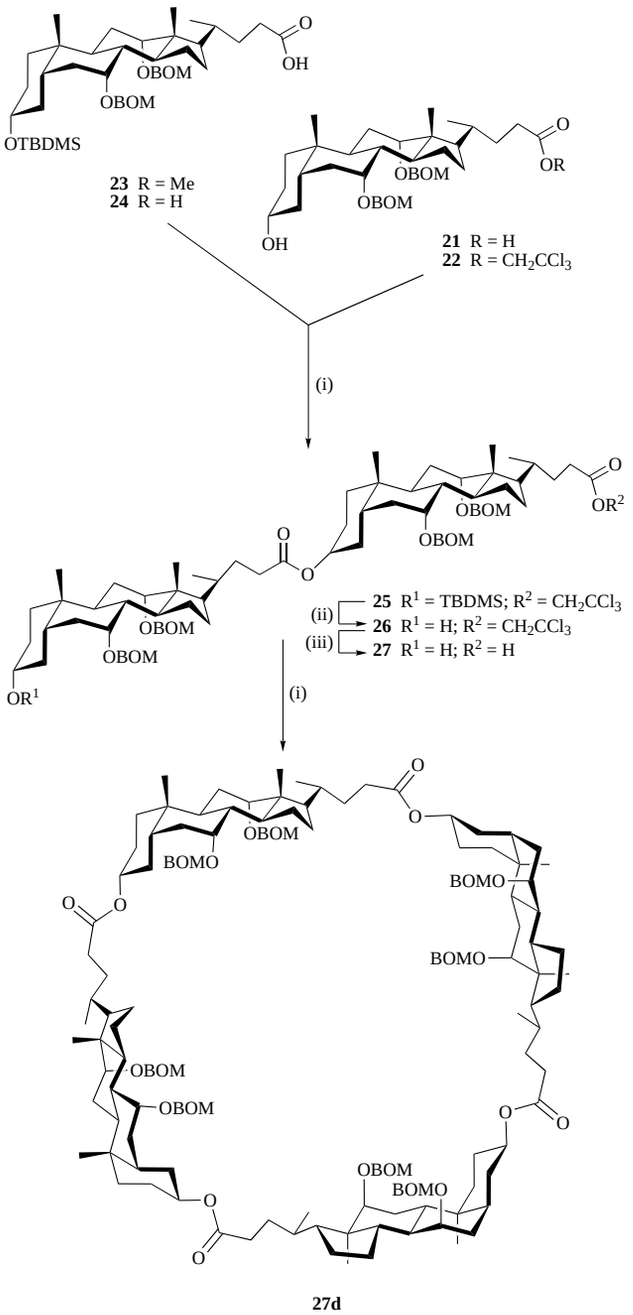
Scheme 4 The synthesis of the 'deoxy' monomer; (i) *p*-phenylbenzyl 2,2,2-trichloroacetimidate **15**, CF_3SO_3H (ii) NaOH, THF (iii) MeOH, HCl



Scheme 5 Kinetic synthesis of the cyclic oligomers (i) 2,6-dichlorobenzoyl chloride, DMAP

allow the monomers to explore many different combinations in a practical length of time (<30 min) even at relatively high dilution; (ii) not be too sensitive to steric hindrance and be able to cause reaction of a sterically more demanding preformed cyclic oligomer to a mixture. Catalysts were thus investigated for these two activities separately. For conversion of a preformed cyclic oligomer, a large quantity of a generally disfavoured product was required. The 'bis(BOM)' cyclic tetramer **27d** was employed because it was relatively easy to prepare as described above.

Acid catalysis has been used for a large number of transesterifications.[35,36] The effectiveness of toluene-*p*-sulfonic acid, trifluoromethanesulfonic acid and camphorsulfonic acid for the transesterification was investigated. None of these acids gave a satisfactory cyclisation of 'MEM' monomer **10** using 4 equiv. of acid and refluxing for 24 h. Camphorsulfonic acid did give some cyclic products from monomer **10** after 3 h and the cyclic 'bis(BOM)' tetramer **27d** was partially converted to cyclic trimer, but this was accompanied by decomposition. All of the other acids caused substantial decomposition of the reactants.



27d

Scheme 6 The synthesis of the 'bis(BOM)' tetramer; (i) 2,6 dichlorobenzoyl chloride, DMAP (ii) HF (aq.) (iii) Zn powder

Tetraalkyl titanates are effective agents for transesterification under essentially neutral conditions and are generally very soluble in organic solvents.[37] However, with either 1 or 10 equiv. of titanium isopropoxide, no transesterification was observed after 24 h reflux in toluene starting from the monomer or the tetramer. There were also no signs of decomposition. Pereyre *et al.* first found tributyltin alkoxides to be effective transesterification catalysts,[38] and the versatility of these reagents was improved by the development of distannoxane catalysts by Otera *et al.*[39] The reaction was attempted using the adduct between di-*n*-butyltin dichloride and di-*n*-butyltin oxide, using the adduct between 2,2-di-*n*-butyl-1,3,2-dioxastannolane and di-*n*-butyltin dichloride (also known as DOS/DTC) and using each of these individual components alone. The adduct between di-*n*-butyltin dichloride and di-*n*-butyltin oxide enabled the formation of some cyclic materials after extended reaction times in refluxing mesitylene (bp 163 °C) but this was thought to be too slow and rigorous for the purposes of this project.

A further commonly used method for transesterification is

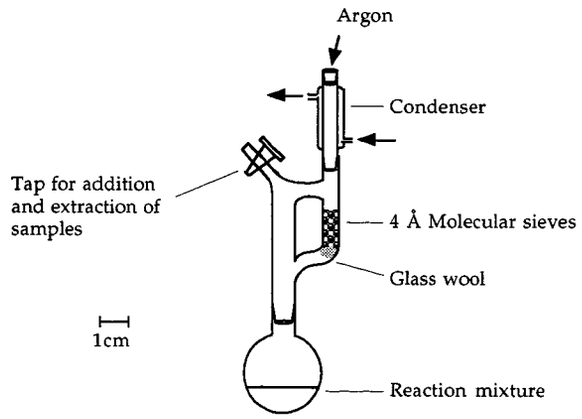


Fig. 5 The Soxhlet apparatus used for the transesterification reactions

base catalysis. Neither triethylamine nor 4-dimethylaminopyridine (2 equiv.) gave any reaction. Earlier experiments by Bonar-Law using sodium methoxide base as the tranesterification catalyst had shown that a 'bis(MOM)' protected cholate methyl ester could be cyclised to a mixture of cyclocholates although reversibility was not demonstrated.[40] Thus 10 mol% of a solution of potassium methoxide (freshly prepared from potassium and methanol) was added to dilute solution of the 'MEM' monomer **10** (5 mM). Samples were taken and after 30 min, virtually all of the material was found to have reacted to give cyclic products as investigated by HPLC. The final distribution of products by mass was trimer : tetramer : pentamer = 60 : 35 : 5.

However, the reaction was accompanied by the formation of a white precipitate in the flask (thought to be potassium alkoxide salts) and complete cessation of reaction. Additional monomer added to a cyclisation reaction that had already gone to completion (30 min) did not cyclise. Potassium methoxide had been effective in causing the cyclisation, but came out of solution as the methanol was removed. Thus, the distribution of products obtained was probably not a truly thermodynamic one as the reaction was not able to continue for a sufficient length of time.

For a true thermodynamic cyclisation, the product distribution obtained should be the same whether the starting material is monomer or a preformed cyclic oligomer. As a test of reversibility, preformed cyclic oligomers were subjected to the reaction conditions. When the catalyst was added as a solution in methanol, the amount of methanol solvent added to the reaction mixture was always greatly in excess over the amount of monomer present. Thus, after transesterification was initiated, early samples analysed by TLC and HPLC showed most (>80%) of the material present to be monomer methyl ester as the first process that occurred was the methanolysis of the cyclic oligomer. As further methanol was removed with time, the methyl esters recyclised to give a similar distribution (trimer : tetramer : pentamer = 50 : 35 : 15) to that found for reaction starting from the monomer **10**.

In order to find an alkoxide that would remain in solution, modified methoxide catalysts were investigated. Use of sodium methoxide and lithium methoxide gave similar results to the potassium methoxide reaction. Organic soluble salts (tetrabutylammonium halides and tetraphenylphosphonium halides) and ion binding agents (crown ethers and cryptands) were investigated as phase transfer catalysts. These generally caused the reaction to reach completion more quickly, but did not prevent formation of precipitates and crucially, as previously, the reaction stopped after the methanol had been removed. Pedersen had shown that a solution of potassium hydroxide can be prepared in toluene in the presence of dicyclohexyl-18-crown-6.[41] The procedure involved generating a crown–metal complex in methanol, adding toluene and then removing the methanol by several rounds of azeotropic distillation. The

Table 2 Equilibrium mass distributions for the 'MEM' monomer cyclisation at various concentrations

Concentration (mM)	Trimer **9c**	Tetramer **9d**	Pentamer **9e**	Higher cyclics	Polymer
1	85 ± 4	12 ± 2	3 ± 0.5	0	0
5	83 ± 2	12 ± 1	5 ± 0.5	0	0
10	81 ± 2	14 ± 2	5 ± 1	0	0
20	76 ± 3	13 ± 1	11 ± 1	0	0
50	71 ± 3	15 ± 2	10 ± 2	3 ± 1	0
80	72 ± 2	14 ± 1	9 ± 1	5 ± 1	0
500	43 ± 2	16 ± 2	8 ± 1	5 ± 1	28 ± 4

Table 3 The yields of cyclic products in the *thermodynamic* transesterification macrolactonisation

Monomer	Dimer (%)	Trimer (%)	Tetramer (%)	Pentamer (%)
'MEM' **10**	0	83 ± 2	12 ± 1	5 ± 0.5
'Bis(*p*-methoxybenzyl)' **13**	0	65 ± 2	24 ± 1	10 ± 1
'Deoxy' **18**	25 ± 3	53 ± 3	22 ± 3	0

authors reported that the naked hydroxide generated was extremely reactive and able to carry out aromatic nucleophilic substitutions. They also found that as methanol had been present as a solvent, 86% of the anions were in fact methoxide, rather than hydroxide.[42] Starting from potassium methoxide, a 0.06 M solution of the potassium methoxide–crown ether complex could be prepared using this procedure after filtration.

This catalyst solution was highly effective for transesterification to cyclocholates. Using 5 mol% of catalyst and 5 mM monomer, reaction to cyclic materials was complete after 10 min. Significantly, additional monomer added at a later time was also consumed and preformed cyclic tetramer **27d** was converted to the same mixture in a similar time. The use of different solvents (dichloromethane, hexane, acetonitrile, dioxane and mesitylene) in this procedure only brought about a deterioration in effectiveness of the catalyst.

Thermodynamic syntheses of macrocyclic steroidal polyesters

A thermodynamically-controlled oligomerisation should give rise to only cyclic products below the critical monomer concentration (cmc).[5] In a mixture of different building blocks, each will be expected to have a different cmc and the mixture itself will have its own cmc depending on its composition. Data obtained from a single component cyclisation should provide an estimate of the approximate concentration regime. As the amount of material contained in the cyclic portion of the products is expected to remain constant beyond the cmc, the mass of cyclic material present in a high concentration cyclics–polymer mixture will allow the cmc to be calculated. Accordingly, the cyclisation of the 'MEM' monomer **10** was carried out at a series of concentrations (Table 2). The products are all still cyclic up to 80 mM.

At a starting concentration of 500 mM, 72% of the material is contained in the cyclic portion, suggesting that the cmc is around 360 mM. This is merely a guide figure as it is derived from a single experiment and the peaks which were integrated overlap in the GPC (gel permeation chromatography) trace. With increasing concentration, there is a general decrease in the yield of cyclic trimer, but any other changes are comparable with experimental error.

The behaviour observed in these cyclisations is qualitatively similar to that predicted by the theory of Ercolani *et al.*[6] At low concentration, the mixture is composed entirely of cyclic material, whilst polymer is produced at higher concentration. The aspect of this work that does not fit with the model is the relatively narrow distribution of cyclic products observed. Even above the cmc, the amount of material contained in macrocycles greater than cyclic pentamer is very small. This may be due to a particular structural stability of these cyclic molecules. However, it is also likely that the behaviour of a rigid steroidal core connected to a flexible chain of only four carbon atoms cannot be modelled adequately by a simple system that assumes free rotation around all bonds. A more complex model based on rigid rods separated by short flexible chains might predict a distribution closer to that observed.

Utilising the transesterification conditions developed above, the various steroid 'monomers' were cyclised at a concentration of 5 mM. The distributions obtained are shown in Table 3. The distributions obtained are similar to those seen in the kinetically-controlled cyclisations described above. Again, the trimer is the major product in each case. The 'deoxy' monomer **18** is able to form cyclic dimer whilst the molecules with more bulky side chains are not.

As a demonstration that the reaction is genuinely proceeding under thermodynamic control, the catalyst was screened for its ability to convert a given *preformed* cyclic oligomer to the same thermodynamic product mixture as that obtained starting with the monomer. When catalyst (5 mol%) was added to previously isolated cyclic tetramer, the same mixture as obtained from monomer starting material was produced. No further change in the composition was observed after 10 min.

Metal ion templating of the cyclisation reactions

Following the finding that it was possible to cyclise the steroid derivatives efficiently by transesterification using a potassium methoxide–dicyclohexyl-18-crown-6 complex, the next goal was to attempt to influence the position of the equilibrium with an external agent.

As described above, the MEM side chain of monomer **10** should be able to bind to metal ions. Thus cyclisations were repeated in the presence of alkali metal salts. One equivalent of the alkali metal iodide was added to the monomer dissolved in toluene (5 mM) prior to injection of the catalyst (5 mol%). Samples were then removed at various times and analysed by HPLC. The errors deduced for the data obtained in these experiments are derived from multiple measurements. The distributions of cyclic products obtained are shown in Table 4. As seen in the table, the distribution is not significantly perturbed by any ions other than sodium. Addition of sodium shifts the distribution towards the larger rings, doubling the amount of cyclic tetramer produced, suggesting an interaction between the Na^+ ion and this macrocycle. Since the origin of this effect was not clear, control experiments were carried out using the other steroid building blocks.

The symmetrically-substituted cholate derivative **13** was designed to have a similar reporter group (now a *p*-methoxybenzyl group), but no obvious metal ion recognition functionality. The presence of substituents on both the 7- and the 12-positions prevented formation of cyclic dimer and a distribution similar to that in the 'MEM' series was obtained in the thermodynamic cyclisation. The distribution found and the effects of templates are shown in Table 4. The templating effects

Table 4 Metal ion templating of thermodynamic cyclisation reactions

'MEM' monomer cyclisation

Template	Trimer **9c** % mass	Tetramer **9d** % mass	Pentamer **9e** % mass
None	83 ± 2	12 ± 1	5 ± 0.5
LiI	76 ± 2	16 ± 1	7 ± 1
NaI	61 ± 3	24 ± 1	14 ± 1
KI	75 ± 3	15 ± 3	8 ± 1
CsI	81 ± 2	12 ± 1	5 ± 1

'Bis(*p*-methoxybenzyl)' monomer cyclisation

Template	Trimer **14c** % mass	Tetramer **14d** % mass	Pentamer **14e** % mass
None	65 ± 2	24 ± 1	10 ± 1
LiI	60 ± 3	25 ± 1	14 ± 1
NaI	47 ± 1	28 ± 1	25 ± 1
KI	65 ± 1	23 ± 2	12 ± 1
CsI	65 ± 3	24 ± 1	10 ± 1

'(*p*-Phenylbenzyl)' monomer cyclisation

Template	Dimer **19b** % mass	Trimer **19c** % mass	Tetramer **19d** % mass
None	25 ± 3	53 ± 3	22 ± 3
LiI	13 ± 2	59 ± 1	25 ± 3
NaI	25 ± 3	54 ± 1	21 ± 2
KI	32 ± 2	46 ± 2	17 ± 3
CsI	37 ± 2	48 ± 2	14 ± 3

observed for these molecules were very similar to those found in the 'MEM' case. Again, the distribution is not much affected by the metal ions except with sodium as the template. Here, the product distribution was shifted to larger rings, but this time the effect is almost exclusively to favour pentamer formation.

The deoxycholate derivative **18**, was also designed to have a similar reporter group to the 'MEM' monomer **10** (now included as a phenylbenzyl group), but no recognition functionality. With only a single side chain, the formation of sandwich complexes, which was possible for the 'bis(*p*-methoxybenzyl)' series, should not be possible, so the 'deoxy' monomer should be a better control for the cyclisations. The cyclisation of this unit differed from that of the 'MEM' and the 'bis(*p*-methoxybenzyl)' steroids in that the absence of a 7-substituent enabled formation of cyclic dimer. The same observation was made by Bonar-Law and Sanders in the kinetically-controlled cyclisation of similarly substituted cholate derivatives.[26] Despite the formation of dimer, cyclic trimer is still the most favoured product and the distribution is again quite narrow. The distribution found and the effects of templates are shown in Table 4. Surprisingly, cyclisation of 'deoxy' cholate **18** was also affected by the presence of metal ions and the effects were of a similar size to those seen with the 'MEM' monomer. The general trend observed was that the presence of small ions favoured the formation of large rings, whereas addition of the larger ions (caesium) shifted the distribution towards smaller rings. Again, the origin of the templating effect is not clear, but it is likely that it stems from a binding interaction of some kind.

Attempts were made to alter a preformed equilibrium distribution. Addition of a preformed equilibrium cyclisation product mixture to sodium iodide and repetition of the cyclisation protocol resulted in the product distribution shifting from one ratio to the other. The preformed equilibrium distribution was influenced by the template in the same way as a reaction beginning from monomer.

The templating effect observed in the 'MEM' series was an increase in yield of the cyclic tetramer and pentamer on addition of sodium. This effect could be rationalised as being the result of the MEM polyethers in the tetramer being able to interact effectively with sodium ions. If two MEM chains could wrap around a single sodium ion, coordinating it from five or six sites in a pseudo-crown cavity, then two sodium ions could bind to each tetramer. The observation that a similar outcome is obtained in the 'bis(*p*-methoxybenzyl)' cholate case, which lacks any obvious metal ion coordination sites, casts this simple picture into doubt. The result observed for the 'deoxy' series, whereby small ions favour formation of large rings, whilst large ions shift the distribution to smaller rings is also curious. A π–cation interaction appears an attractive explanation for this phenomenon, but such an effect might be expected to be quite small in a solvent such as toluene. We have investigated the metal ion binding properties of the cholate macrocycles using electrospray mass spectrometry and the observed trends are broadly reflected in the templated syntheses described here.

Conclusions

A method has been developed for the synthesis of macrocycles using transesterification under thermodynamic control, with the long term aim of creating a dynamic combinatorial library of receptors. We have called this process 'living macrolactonisation' because the equilibrium reaction mixture, which is macroscopically unchanging, actually contains a ferment of activity: individual cholate components are rapidly making and breaking bonds in ever changing relationships with new partners in a way that is reminiscent of the classical bullvalene prediction[43] but is all the more remarkable for being intermolecular. The requirements of such a reaction are stringent: it must be fast enough to reach equilibrium in a short time even when a mixture of starting materials is used and in the absence of excess alcohol. Thus, of the many catalysts available for transesterification, only few are sufficiently active to be appropriate for this application. It has been possible to template this thermodynamically-controlled cyclisation with metal ions. The equilibrium shift should reflect the relative abilities of the different macrocycles to bind the metal ions, and thus should result in selection of the best host. Essentially the same conditions have been applied to cinchona alkaloid and xanthene building blocks with the same efficiency.[44] Work is currently under way to extend the principle of thermodynamic templating to the more interesting binding of organic guests.

Experimental

NMR spectra were recorded on Bruker WM-250 or AM-400 spectrometers. Deuteriochloroform was deacidified before use by standing over anhydrous potassium carbonate. The following abbreviations indicate signal multiplicity: s = singlet; d = doublet; t = triplet; q = quartet; qn = quintet; sx = sextet; m = multiplet. In ^{13}C NMR spectra, Cq denotes a quaternary carbon, as deduced by a DEPT experiment. ^{13}C Assignments in spectra of cholic acid derivatives were made by comparison with literature values[45] and with the aid of HMBC and HMQC spectra. In ^{1}H NMR spectra, overlapping steroidal backbone resonances are not quoted. *J* Values are given in Hz. UV–visible absorption spectra were recorded on a Uvikon 810 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer and the spectra of solids were run as solutions in dichloromethane. Fast atom bombardment (FAB) mass spectra were obtained using a *m*-nitrobenzyl alcohol matrix on a Kratos MS-50 instrument. Experimental peak (mode) masses are compared with calculated mean masses. Microanalyses were carried out by the University Chemical Laboratory Microanalysis Department in Cambridge. Melting points were determined on a Reichert-Kofler block and are uncorrected.

Thin layer chromatography (TLC) was carried out on Kieselgel 60 F_{254} (Merck) 0.2 mm plates. Steroids were generally visualised with 5% phosphomolybdic acid in ethanol, otherwise in an iodine tank or by UV absorption. Flash column chrom-

atography was carried out according to the method of Still *et al.*[46] using Kieselgel 60 (Merck) 230–400 mesh and distilled solvents. All solvents were distilled prior to use. Dry solvents were freshly distilled from a drying agent (generally CaH_2) under an inert atmosphere. Organic extracts were dried with anhydrous magnesium sulfate.

All cholic acid derivatives are of the 5β series and 24-oates with the oxygen substituents in the 3-, 7- and 12-positions α orientated.

Methyl 12-benzyloxy-3,7-diacetoxycholanoate 5[17]

Benzyl 2,2,2-trichloroacetimidate (0.150 ml, 0.273 g, 1.30 mmol) was syringed into a solution of methyl 3,7-diacetoxy-12-hydroxycholanoate **4**[15] (330 mg, 0.65 mmol) in dry tetrachloromethane (7 ml) and cyclohexane (5 ml, distilled *ex* CaH_2) under argon and stirred for 15 min. Trifluoromethanesulfonic acid (0.020 ml, 0.034 g, 0.20 mmol) was syringed in and reaction was followed by TLC (chloroform–methanol, 20:1). After 3 h, the reaction appeared to have stopped with some starting material remaining, so a further equal portion of trifluoromethanesulfonic acid was added, resulting in complete reaction after a further 3 h. The reaction mixture was diluted with diethyl ether (30 ml) and then washed with aqueous NaOH (2 × 30 ml, 2.5 M) and water (30 ml), dried and the solvent was removed under reduced pressure to leave a yellow oil (0.370 g). A small amount of this compound was purified by flash column chromatography (hexane–ethyl acetate, 3:1) to yield *benzyl ether* **5** as a colourless oil. R_f 0.45 (hexane–ethyl acetate, 2:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1726 (CO_2Me, MeCO), 1648 (Ar); $\lambda_{max}(Et_2O)/nm$ 352.0, 357.5 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 300, 340); δ_H(400 MHz, $CDCl_3$) 0.69 (s, 3H, 18-Me), 0.88 (d, *J* 6.3, 3H, 21-Me), 0.92 (s, 3H, 19-Me), 2.01 (s, 3H, COMe), 2.04 (s, 3H, COMe), 3.64 (s, 3H, OMe), 3.69 (br s, 1H, 12βH), 4.37, 4.62 (ABq, *J* 12, 2H, CH_2Ph), 4.52 (tt, *J* 4.5, 11.3, 1H, 3βH), 4.87 (q, *J* 2.5, 1H, 7βH), 7.32–7.42 (m, 5H, Ar); δ_C(100 MHz, $CDCl_3$) 12.55 (18-Me), 17.52 (21-Me), 21.51, 21.69 (2 × CO*C*H_3), 22.68 (19-Me), 22.92 (15-CH_2), 23.12 (16-CH_2), 26.77 (2-CH_2), 27.44 (11-CH_2), 28.34 (9-CH), 30.96, 31.36 (22-CH_2, 23-CH_2), 34.45, 34.61, 34.86 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.19 (20-CH), 38.13 (8-CH), 40.98 (5-CH), 42.77 (14-CH), 46.17 (17-CH), 46.66 (13-Cq), 51.51 (OMe), 70.07 (*C*H_2Ph), 70.96 (7-CH), 74.07 (3-CH), 80.66 (12-CH), 127.01 (Ar *o*-CH), 127.19 (Ar *p*-CH), 128.29 (Ar *m*-CH), 139.64 (Ar Cq), 170.60, 170.76 (2 × *C*OMe), 174.76 (24-Cq); *m/z* (FAB) 597.5 $[M + H]^+$; found 597.3792, $C_{36}H_{53}O_7$ requires 597.3791.

Methyl 12-benzyloxy-3,7-dihydroxycholanoate 6[17]

Aqueous NaOH (3 ml, 2.5 M) was added to a stirred solution of methyl 12-benzyloxy-3,7-diacetoxycholanoate **5** (0.370 g, 0.62 mmol) in THF (10 ml) followed by methanol (2 ml). After stirring for 10 min at room temperature, the mixture was heated to reflux for 14 h after which time TLC (chloroform–methanol, 20:1) showed hydrolysis to be complete. The reaction mixture was then cooled to room temperature and neutralised with aqueous HCl (0.3 M). The volatile solvents were removed under reduced pressure and chloroform (20 ml) was added. The organic layer was washed with dilute aqueous HCl (3 × 15 ml, 0.3 M), dried and evaporated under reduced pressure to a yellow oil (0.284 g).

Methanolic HCl (3 ml, 12%) was added to a solution of this crude hydroxy acid in dry methanol (10 ml). After stirring for 12 h at room temperature, TLC (hexane–ethyl acetate, 2:1) showed reaction to be complete. After neutralising with aqueous NaOH (2.5 M), the methanol was removed under reduced pressure and diethyl ether (10 ml) was added. The organic layer was dried and evaporated under reduced pressure to a yellow oil (0.241 g). Flash column chromatography (light petroleum bp 40–60 °C–ethyl acetate, 3:2) provided *diol* **6** as a colourless oil (0.117 g, 40% from **4**). R_f 0.16 (hexane–ethyl acetate, 2:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3607 (OH), 1726 (CO_2Me), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 251.6, 257.6 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 340, 360); δ_H(400 MHz, $CDCl_3$) 0.71 (s, 3H, 18-Me), 0.89 (d, *J* 6.2, 3H, 21-Me), 0.89 (s, 3H, 19-Me), 3.42 (tt, *J* 4.5, 10.8, 1H, 3βH), 3.65 (s, 3H, OMe), 3.68 (br s, 1H, 12βH), 3.82 (br s, 1H, 7βH), 4.34, 4.60 (ABq, *J* 12, 2H, CH_2Ph), 7.24–7.38 (m, 5H, Ar); δ_C(100 MHz, $CDCl_3$) 12.55 (18-Me). 17.53 (21-Me), 21.72 (19-Me), 22.87 (16-CH_2), 23.14 (15-CH_2), 27.44 (11-CH_2), 28.36 (9-CH), 30.74, 30.96, 31.49 (2-CH_2, 22-CH_2, 23-CH_2), 34.41, 35.19 (1-CH_2, 6-CH_2, 10-Cq), 35.22 (20-CH), 38.17 (8-CH), 39.05 (4-CH_2), 41.19 (5-CH), 42.75 (14-CH), 46.14 (17-CH), 46.66 (13-Cq), 51.50 (OMe), 69.96 (*C*H_2Ph), 71.09, 71.78 (3-CH, 7-CH), 80.58 (12-CH), 127.12 (Ar *o*-CH), 127.19 (Ar *p*-CH), 128.31 (Ar *m*-CH), 139.70 (Ar Cq), 174.76 (24-Cq); *m/z* (FAB) 513.3 $[M + H]^+$; found 513.3582, $C_{32}H_{49}O_5$ requires 513.3567.

Methyl 3-acetoxy-12-benzyloxy-7-hydroxycholanoate 7

Acetic anhydride (0.10 ml, 0.10 g, 1.05 mmol) and pyridine (0.1 ml, 0.098 g, 1.24 mmol) were added to a solution of methyl 12-benzyloxy-3,7-dihydroxycholanoate **6** (95 mg, 0.19 mmol) in dry dichloromethane (2 ml) under an argon atmosphere at room temperature. After 48 h, TLC (hexane–ethyl acetate, 3:2) showed reaction to be complete and it was quenched by pouring into a mixture of aqueous H_2SO_4 (10 ml, 6 M) and diethyl ether (10 ml). The organic layer was washed with water (10 ml) and saturated aqueous $NaHCO_3$ (2 × 10 ml), dried and the solvent removed under reduced pressure to leave a white foam (90 mg, 88%). Flash column chromatography (hexane–ethyl acetate, 3:1) of a small amount of this material yielded a pure sample of *acetate* **7** that was analysed as follows. R_f 0.19 (hexane–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3608 (OH), 1726 (CO_2Me), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 254.5, 257.7 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 330, 360); δ_H(400 MHz, $CDCl_3$) 0.71 (s, 3H, 18-Me), 0.88 (d, *J* 6.3, 3H, 21-Me), 0.90 (s, 3H, 19-Me), 1.99 (s, 3H, $COCH_3$), 3.65 (s, 3H, OMe), 3.68 (br s, 1H, 12βH), 3.83 (br s, 1H, 7βH), 4.34, 4.60 (ABq, *J* 12, 2H, CH_2Ph), 4.53 (tt, *J* 4.5, 10.8, 1H, 3βH), 7.24–7.38 (m, 5H, Ar); δ_C(100 MHz, $CDCl_3$) 12.63 (18-Me), 17.52 (21-Me), 21.51 (19-Me), 22.72 (CO*C*H_3), 22.85 (16-CH_2), 23.23 (15-CH_2), 26.76 (11-CH_2), 27.06 (9-CH), 27.56 (2-CH_2), 30.99 (22-CH_2, 23-CH_2), 33.96, 34.72, 34.97, 35.01 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.27 (20-CH), 39.71 (8-CH), 41.23 (5-CH), 42.82 (14-CH), 46.21 (17-CH), 46.70 (13-Cq), 51.51 (OMe), 68.33 (7-CH), 70.27 (*C*H_2Ph), 74.28 (3-CH), 80.66 (12-CH), 127.25 (Ar *p*-CH), 127.30 (Ar *o*-CH), 128.31 (Ar *m*-CH), 139.39 (Ar Cq), 170.85 (*C*OMe), 174.78 (24-Cq); *m/z* (FAB) 555.4 $[M + H]^+$; found 555.3704, $C_{34}H_{51}O_6$ requires 555.3686.

12-Benzyloxy-3-hydroxy-7-(2-methoxyethoxymethoxy)-cholanoic acid 9

Diisopropylethylamine (0.230 ml, 0.143 g, 1.25 mmol) was added to a stirred solution of the crude acetate **7** (73 mg, 0.13 mmol) in dry dichloromethane (1 ml) at room temperature under argon. After 5 min, 2-methoxyethoxymethyl chloride (0.150 ml, 0.155 g, 1.25 mmol) was added and the mixture was heated to 50 °C for 14 h, after which time all starting material had disappeared by TLC (hexane–acetone, 4:1). The reaction mixture was diluted with diethyl ether (10 ml), washed with dilute aqueous HCl (10 ml, 0.1 M) and water (3 × 10 ml), dried and evaporated under reduced pressure to give a yellow residue (95 mg) which was purified by flash column chromatography (light petroleum bp 40–60 °C–ethyl acetate, 4:1) to give methyl 3-acetoxy-12-benzyloxy-7-(2-methoxyethoxymethoxy)cholanoate **8** as a colourless oil (70 mg, 85%).

Aqueous NaOH (2 ml, 2.5 M) and methanol (1 ml) were added to a stirred solution of the crude ester **8** (50 mg, 0.076 mmol) in THF (4 ml) and the mixture was then refluxed for 10 h, after which time, TLC (chloroform–methanol, 15:1) showed all the starting material to have reacted. The mixture was neutralised with aqueous HCl (*ca.* 10 ml, 0.3 M), the volatile solvents were evaporated under reduced pressure and diethyl

ether (10 ml) was added. The organic layer was then washed with water (3 × 10 ml), dried and the solvent evaporated under reduced pressure to leave the *hydroxy acid* **9** as white needle crystals (33 mg, 75%). R_f 0.21 (chloroform–methanol, 15:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3599 (OH), 3200 (COO–H), 1707 (CO_2H), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 257.6 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 400); δ_H(400 MHz, $CDCl_3$) 0.68 (s, 3H, 18-Me), 0.88 (d, *J* 7.0, 3H, 21-Me), 0.89 (s, 3H, 19-Me), 3.37 (s, 3H, $CH_2CH_2OCH_3$), 3.39 (br s, 1H, 3βH), 3.53 (t, *J* 4.6, 2H, OCH_2CH_2O), 3.61 (q, *J* 2.5, 1H, 7βH), 3.67 (br s, 12βH), 3.72 (t, *J* 4.8, 2H, OCH_2CH_2O), 4.35, 4.60 (ABq, *J* 12.0, 2H, CH_2Ph), 4.65, 4.77 (ABq, *J* 7.2, 2H, OCH_2O), 7.2–7.39 (m, 5H, Ar); δ_C(100 MHz, $CDCl_3$) 12.64 (18-Me), 17.54 (21-Me), 22.79 (19-Me), 22.79 (16-CH_2), 23.37 (15-CH_2), 27.47 (9-CH), 27.56 (11-CH_2), 30.84 (2-CH_2, 22-CH_2, 23-CH_2), 34.62, 35.30 (1-CH_2, 6-CH_2, 10-Cq), 35.30 (20-CH), 39.18 (4-CH_2), 39.59 (8-CH), 41.70 (5-CH), 42.33 (14-CH), 46.14 (17-CH), 46.48 (13-Cq), 59.09 (CH_2OMe), 67.69, 71.88 (OCH_2CH_2O), 70.00 (CH_2Ph), 72.07 (3-CH), 74.87 (7-CH), 80.71 (12-CH), 94.78 (OCH_2O), 127.14 (Ar *p*-CH), 127.24 (Ar *o*-CH), 128.27 (Ar *m*-CH), 139.71 (Ar Cq), 179.12 (24-Cq); *m/z* (FAB) 587.5 $[M + H]^+$; found 587.3934, $C_{35}H_{54}O_7$ requires 587.3948.

Methyl 12-benzyloxy-3-hydroxy-7-(2-methoxyethoxymethoxy)-cholanoate 10

12-Benzyloxy-3-hydroxy-7-(2-methoxyethoxymethoxy)cholanoic acid **9** (33 mg, 0.056 mmol) was dissolved in methanolic HCl (10 ml, 12%) and stirred for 1 h. After this time, TLC (hexane–ethyl acetate, 2:1) showed reaction to be complete, so the mixture was neutralised with NaOH (*ca*. 3 ml, 2.5 M) and the methanol removed under reduced pressure. A solution of the residue in diethyl ether (10 ml) was washed with water (3 × 10 ml, distilled), dried and the solvent evaporated under reduced pressure to leave a yellow oil (35 mg). Flash column chromatography (methanol–chloroform, 1:15) provided the *hydroxy ester* **10** as white needle like crystals (20 mg, 60%). R_f 0.22 (hexane–ethyl acetate, 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3607 (OH), 1731 (CO_2Me), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 217.1, 250.8, 258.0 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 5500, 250, 350); δ_H(400 MHz, $CDCl_3$) 0.68 (s, 3H, 18-Me), 0.88 (d, *J* 6.3, 3H, 21-Me), 0.90 (s, 3H, 19-Me), 3.37 (s, 3H, $CH_2CH_2OCH_3$), 3.39 (br s, 1H, 3βH), 3.53 (t, *J* 4.6, 2H, OCH_2CH_2O), 3.62 (q, *J* 2.5, 1H, 7βH), 3.65 (s, 3H, OMe), 3.67 (br s, 12βH), 3.72 (t, *J* 4.8, 2H, OCH_2CH_2O), 4.35, 4.59 (ABq, *J* 11.5, 2H, CH_2Ph), 4.65, 4.77 (ABq, *J* 7.1, 2H, OCH_2O), 7.2–7.39 (m, 5H, Ar); δ_C(100 MHz, $CDCl_3$) 12.64 (18-Me), 17.55 (21-Me), 22.79 (19-Me), 22.83 (16-CH_2), 23.74 (15-CH_2), 27.48 (11-CH_2), 27.58 (9-CH), 30.87, 30.94, 31.05 (2-CH_2, 22-CH_2, 23-CH_2), 34.63, 35.35 (1-CH_2, 6-CH_2, 10-Cq), 35.38 (20-CH), 39.22 (4-CH_2), 39.61 (8-CH), 41.72 (5-CH), 42.53 (14-CH), 46.17 (17-CH), 46.48 (13-Cq), 51.49 (OMe), 59.09 (CH_2OMe), 67.70, 71.91 (OCH_2CH_2O) 69.98 (CH_2Ph), 72.06 (3-CH), 74.80 (7-CH), 80.71 (12-CH), 94.75 (OCH_2O), 127.10 (Ar *p*-CH), 127.22 (Ar *o*-CH), 128.25 (Ar *m*-CH), 139.76 (Ar Cq), 174.82 (24-Cq); *m/z* (ES) 623 $[M + Na]^+$, 639 $[M + K]^+$; *m/z* (FAB) 601.6 $[M + H]^+$, 623.5 $[M + Na]^+$, 495.6 $[M - MEM]^+$, 387.3 $[M - MEM - Bn]^+$; found 623.3889, $C_{36}H_{56}O_7Na$ requires 623.3924.

Cyclic oligomers of 12-benzyloxy-3-hydroxy-7-(2-methoxyethoxymethoxy)cholanoic acid 9c–9e

12-Benzyloxy-3-hydroxy-7-(2-methoxyethoxymethoxy)cholanoic acid **9** (0.250 g, 0.426 mmol) and 4-dimethylaminopyridine (0.156 g, 1.28 mmol) were dissolved in dry dichloromethane (30 ml) at room temperature under argon in the presence of ground 4 Å molecular sieves (1 g, freshly activated). 2,6-Dichlorobenzoyl chloride (0.076 ml, 0.107 g, 0.512 mmol) was syringed in and the reaction was followed by TLC (hexane–acetone, 4:1). After 3 h, no starting material remained and three main products had formed, so the mixture was diluted with dichloromethane (20 ml), washed with aqueous HCl (3 × 50 ml, 0.3 M), dried and the solvent removed under reduced pressure to leave a yellow oil (0.250 g). The three major products were separated by flash column chromatography (hexane–acetone, 4:1).

Compound A (*cyclic trimer* 9c). Crystallised from hexane–diethyl ether (40 mg, 17%). Mp 209–210 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 217.0, 249.3, 257.9 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 16 200, 750, 975); δ_H(400 MHz, $CDCl_3$) 0.66 (s, 9H, 18-Me), 0.84 (d, *J* 6.35, 9H, 21-Me), 0.89 (s, 9H, 19-Me), 3.34 (s, 9H, $CH_2CH_2OCH_3$), 3.48 (t, *J* 4.6, 6H, OCH_2CH_2O), 3.63 (br s, 3H, 7βH), 3.67 (br s, 3H, 12βH), 3.72 (t, *J* 5.0, 6H, OCH_2CH_2O), 4.41, 4.63 (ABq, *J* 12.0, 6H, CH_2Ph), 4.56 (tt, *J* 11.2, 5.1, 3H, 3βH), 4.66, 4.79 (ABq, *J* 7.2, 6H, OCH_2O), 7.06 (t, *J* 7.4, 3H, *p*-Ar-H), 7.19 (t, *J* 7.7, 6H, *m*-Ar-H), 7.35 (d, *J* 7.4, 6H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.47 (18-Me), 17.42 (21-Me), 22.74 (19-Me), 23.15 (16-CH_2), 23.25 (15-CH_2), 26.59 (11-CH_2), 27.76 (9-CH), 30.06, 30.42, 31.35 (2-CH_2, 22-CH_2, 23-CH_2), 34.46, 35.07 (1-CH_2, 6-CH_2, 10-Cq, 4-CH_2), 34.85 (20-CH), 39.22 (8-CH), 41.34 (5-CH), 42.71 (14-CH), 44.47 (17-CH), 46.40 (13-Cq), 59.09 (CH_2OMe), 67.79, 71.83 (OCH_2CH_2O, OCH_2CH_2O), 70.10 (CH_2Ph), 73.78 (3-CH), 74.51 (7-CH), 80.43 (12-CH), 95.43 (OCH_2O), 127.21 (Ar *p*-CH), 127.33 (Ar *o*-CH), 128.18 (Ar *m*-CH), 139.41 (Ar Cq), 174.07 (24-Cq); *m/z* (FIB) 1728 $[M + Na]^+$, 1279 $[M - 2 \times MEM - 2 \times Bn]^+$, 1187 $[M - MEM - 2 \times Bn - C_4H_9O_2]^+$, 1079 $[M - MEM - 3 \times Bn - C_4H_9O_2]^+$.

Compound B (*cyclic tetramer* 9d). Crystallised from hexane–diethyl ether (40 mg, 17%). Mp 163–164 °C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1717 (CO_2Me), 1606 (Ar); $\lambda_{max}(Et_2O)/nm$ 217.1, 248.4, 258.0 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 22 000, 1000, 1400); δ_H(400 MHz, $CDCl_3$) 0.68 (s, 12H, 18-Me), 0.88 (d, *J* 6.1, 12H, 21-Me), 0.91 (s, 12H, 19-Me), 3.33 (s, 12H, $CH_2CH_2OCH_3$), 3.46 (t, *J* 4.5, 8H, OCH_2CH_2O), 3.62 (br s, 4H, 7βH), 3.65 (t, *J* 5.0, 8H, OCH_2-CH_2O), 3.67 (br s, 4H, 12βH), 4.36, 4.61 (ABq, *J* 12.1, 8H, CH_2Ph), 4.51 (tt, *J* 11.2, 4.0, 4H, 3βH), 4.63, 4.76 (ABq, *J* 7.0, 8H, OCH_2O), 7.20 (t, *J* 7.3, 4H, *p*-Ar-H), 7.30 (t, *J* 7.6, 8H, *m*-Ar-H), 7.38 (d, *J* 7.4, 8H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.63 (18-Me), 17.70 (21-Me), 22.74 (19-Me), 22.90 (16-CH_2), 23.34 (15-CH_2), 26.87 (11-CH_2), 27.44 (9-CH), 30.78, 31.12, 31.25 (2-CH_2, 22-CH_2, 23-CH_2), 34.71, 34.92, 35.04 (1-CH_2, 6-CH_2, 10-Cq, 4-CH_2), 34.81 (20-CH), 39.61 (8-CH), 41.47 (5-CH), 42.19 (14-CH), 45.61 (17-CH), 46.45 (13-Cq), 59.00 (CH_2OMe), 67.68, 71.81 (OCH_2CH_2O, OCH_2CH_2O), 70.82 (CH_2Ph), 74.31 (3-CH), 74.75 (7-CH), 80.78 (12-CH), 94.89 (OCH_2O), 127.08 (Ar *p*-CH, Ar *o*-CH), 128.18 (Ar *m*-CH), 139.68 (Ar Cq), 174.18 (24-Cq); *m/z* (FIB) 2296 $[M + Na]^+$, 1742 $[M - 3 \times MEM - 2 \times Bn]^+$, 1652 $[M - 2 \times MEM - 3 \times Bn - C_4H_9O_2]^+$, 1543 $[M - 2 \times MEM - 4 \times Bn - C_4H_9O_2]^+$.

Compound C (*cyclic pentamer* 9e). Yellow oil (25 mg, 10%). $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 217.0, 248.6, 258.2 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 27 500, 1250, 1700); δ_H(400 MHz, $CDCl_3$) 0.67 (s, 15H, 18-Me), 0.87 (d, *J* 6.1, 15H, 21-Me), 0.90 (s, 15H, 19-Me), 3.33 (s, 15H, $CH_2CH_2OCH_3$), 3.47 (t, *J* 4.5, 10H, OCH_2CH_2O), 3.61 (br s, 4H, 7βH), 3.66 (t, *J* 5.1, 10H, OCH_2CH_2O), 3.68 (br s, 5H, 12βH), 4.35, 4.60 (ABq, *J* 12.0, 10H, CH_2Ph), 4.52 (tt, *J* 4.0, 11.1, 4H, 3βH), 4.63, 4.75 (ABq, *J* 7.1, 10H, OCH_2O), 7.22 (t, *J* 7.3, 5H, *p*-Ar-H), 7.29 (t, *J* 7.3, 10H, *m*-Ar-H), 7.37 (d, *J* 7.3, 10H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.64 (18-Me), 17.61 (21-Me), 22.74 (19-Me), 22.90 (16-CH_2), 23.30 (15-CH_2), 26.60 (11-CH_2), 27.48 (9-CH), 30.71, 31.17 (2-CH_2, 22-CH_2, 23-CH_2), 34.07, 35.00 (1-CH_2, 6-CH_2, 10-Cq, 4-CH_2), 34.67 (20-CH), 39.55 (8-CH), 41.47 (5-CH), 42.26 (14-CH), 46.13 (17-CH), 46.77 (13-Cq), 59.01 (CH_2OMe), 67.66, 71.82 (OCH_2CH_2O, OCH_2CH_2O), 70.13 (CH_2Ph), 74.20 (3-CH), 74.82 (7-CH), 80.65 (12-CH), 94.83 (OCH_2O), 127.09 (Ar *p*-CH), 127.22 (Ar *o*-CH), 128.19 (Ar *m*-CH), 139.62 (Ar Cq), 174.03 (24-Cq); *m/z* (FAB) 2867 $[M + Na]^+$.

Methyl 3-ethoxycarbonyloxy-7,12-bis(*p*-methoxybenzyloxy)-cholanoate 12

p-Methoxybenzyl 2,2,2-trichloroacetimidate[21] (100 mg, 0.35 mmol) and methyl 3-ethoxycarbonyloxy-7,12-dihydroxy-cholanoate **11**[20] (50 mg, 0.10 mmol) were stirred in dry tetrachloromethane (1 ml) and cyclohexane (2 ml, distilled *ex* CaH_2) under argon and heated to 40 °C. Camphorsulfonic acid (4.6 mg, 0.02 mmol) was then added to the mixture and stirring was continued for 2.5 h. The reaction was quenched by pouring into pH 7 buffer (10 ml) and extracting into diethyl ether (3 × 10 ml), drying and removing solvent under reduced pressure. The crude product was purified by flash column chromatography (hexane–diethyl ether, gradient 4:1–3:1) to yield the *diether* **12** as a colourless oil (55 mg, 74%). R_f 0.16 (hexane–diethyl ether, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1733 (C=O), 1612 (Ar); $\lambda_{max}(Et_2O)/nm$ 274.2 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 3670); δ_H(400 MHz, $CDCl_3$) 0.64 (s, 3H, 18-Me), 0.85 (d, *J* 6.1, 3H, 21-Me), 0.91 (s, 3H, 19-Me), 1.27 (t, *J* 7, 3H, CH_2Me), 3.38 (q, *J* 4, 1H, 7βH), 3.62 (br s, 1H, 12βH), 3.64 (s, 3H, OMe), 3.79 (s, 6H, ArOCH_3), 4.13 (q, *J* 7.1, 2H, OCH_2), 4.15, 4.55 (ABq, *J* 11.6, 2H, ArOCH_2), 4.27, 4.51 (ABq, *J* 11.5, 2H, ArOCH_2), 4.37 (tt, *J* 4.2, 11.0, 1H, 3βH), 6.84 (d, *J* 7.1, 4H, *m*-Ar-H), 7.21 (d, *J* 7.0, 4H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.53 (18-Me), 14.26 (*Me*CH_2O) 17.53 (21-Me), 22.78 (19-Me), 22.99 (15-CH_2), 26.76 (2-CH_2), 27.53 (16-CH_2), 27.79 (9-CH), 28.63 (11-CH_2), 30.98, 31.07 (22-CH_2, 23-CH_2), 34.15, 34.72, 34.92 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.31 (20-CH), 39.82 (8-CH), 41.71 (5-CH), 42.33 (14-CH), 46.04 (13-Cq), 46.37 (17-CH), 51.24 (OMe), 55.24 (ArO*Me*), 63.46 (Me*C*H_2CO), 69.55, 69.74 (Ar*C*H_2O), 74.75 (7-CH), 78.33 (3-CH), 80.21 (12-CH), 113.50, 113.63 (Ar-CH), 128.59, 128.97 (Ar-CH), 131.71, 131.43 (Ar-C), 158.72 (Ar-C), 154.0 (O*C*OO), 174.87 (24-Cq); *m*/*z* (FAB) 735.9 $[M + H]^+$, 613.3 $[M - (OMe)Bn]^+$, 460.3 $[M - 2 \times (OMe)BnO]^+$; found 735.4488, $C_{44}H_{63}O_9$ requires 735.4472.

Methyl 3-hydroxy-7,12-bis(*p*-methoxybenzyloxy)cholanoate 13

Methyl 3-ethoxycarbonyloxy-7,12-bis(*p*-methoxybenzyloxy)-cholanoate **12** (30 mg, 0.038 mmol) was stirred in dry THF (1 ml) and a solution of potassium methoxide in methanol (0.5 ml, 0.5 M, 0.25 mmol) was added by cannula. After 2 h, TLC showed reaction to be complete and the mixture was poured into dilute aqueous HCl (10 ml, 3 M) and extracted into diethyl ether (2 × 10 ml). The organic extracts were then washed with water (3 × 10 ml), dried and concentrated under reduced pressure. The crude products were then purified by flash chromatography (toluene–ethyl acetate, 3:1) to yield the *hydroxy ester* **13** as a white foam (24 mg, 81%). R_f 0.35 (toluene–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3600 (OH), 1731 (CO_2Me), 1612 (Ar); $\lambda_{max}(Et_2O)/nm$ 273.4 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 4430); δ_H(400 MHz, $CDCl_3$) 0.67 (s, 3H, 18-Me), 0.89 (d, *J* 6.4, 3H, 21-Me), 0.89 (s, 3H, 19-Me), 3.32 (br s, 1H, 3βH), 3.41 (d, *J* 2.6, 1H, 7βH), 3.64 (br s, 1H, 12βH), 3.65 (s, 3H, OMe), 3.77 (s, 3H, ArOCH_3), 3.79 (s, 3H, ArOCH_3), 4.16, 4.56 (ABq, *J* 11.1, 2H, ArCH_2O), 4.31, 4.50 (ABq, *J* 11.8, 2H, ArCH_2O), 6.79 (d, *J* 8.6, 2H, *m*-Ar-H), 6.86 (d, *J* 8.6, 2H, *m*-Ar-H), 7.25 (d, *J* 8.6, 2H, *o*-Ar-H), 7.27 (d, *J* 8.6, 2H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.55 (18-Me), 17.56 (21-Me), 22.80 (19-Me), 22.90 (15-CH_2), 27.57 (16-CH_2), 27.85 (9-CH), 28.75 (11-CH_2), 30.85, 31.04, 31.13 (2-CH_2, 22-CH_2, 23-CH_2), 34.70, 35.40 (1-CH_2, 6-CH_2, 10-Cq), 35.34 (20-CH), 38.87 (4-CH_2), 39.88 (8-CH), 42.01 (5-CH), 42.43 (14-CH), 46.15 (17-CH), 46.43 (13-Cq), 51.51 (OMe), 55.28 (ArO*Me*), 69.21, 69.94 (Ar*C*H_2O), 72.10 (3-CH), 75.12 (7-CH), 80.38 (12-CH), 113.60 (Ar *m*-CH), 128.41, 129.12 (Ar *o*-CH), 131.53, 132.07 (Ar-*C*–CH_2), 158.65, 158.80 (Ar-*C*–OMe), 174.91 (24-Cq); *m*/*z* (FAB) 663.5 $[M + H]^+$, 541.7 $[M - (OMe)Bn]^+$, 387.3 $[M - 2 \times (OMe)Bn - OMe]^+$; found 662.4241, $C_{41}H_{58}O_7$ requires 662.4182.

3-Hydroxy-7,12-bis(*p*-methoxybenzyloxy)cholanoic acid 20

Methyl 3-hydroxy-7,12-bis(*p*-methoxybenzyloxy)cholanoate **13** (1.00 g, 1.51 mmol) was dissolved in THF (20 ml) and aqueous sodium hydroxide (20 ml, 2.5 M) and methanol (5 ml) were added. The mixture was then heated to reflux for 4 h after which TLC (chloroform–methanol, 15:1) indicated reaction to a single product. The mixture was then neutralised by addition of aqueous hydrochloric acid (*ca*. 15 ml, 3 M) and the volatile solvents were removed under reduced pressure, leaving an aqueous slurry. This was diluted with further water (50 ml) and the organic products were extracted into dichloromethane (3 × 50 ml), dried and evaporated under reduced pressure to yield the *hydroxy acid* **20** as a white powder which was pure by TLC (0.91 g, 93%). R_f 0.31 (chloroform–methanol, 15:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3599 (OH), 1707 (CO_2Me), 1612 (Ar); $\lambda_{max}(Et_2O)/nm$ 273.8 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 4120); δ_H(400 MHz, $CDCl_3$) 0.67 (s, 3H, 18-Me), 0.89 (d, *J* 4.7, 3H, 21-Me), 0.89 (s, 3H, 19-Me), 3.35 (tt, *J* 4.2, 10.6, 1H, 3βH), 3.41 (q, *J* 2.3, 1H, 7βH), 3.65 (br s, 1H, 12βH), 3.76 (s, 3H, ArOCH_3), 3.78 (s, 3H, $ArOCH_3$), 4.16, 4.56 (ABq, *J* 11.1, 2H, ArOCH_2), 4.31, 4.50 (ABq, *J* 11.8, 2H, ArOCH_2), 6.78 (d, *J* 8.7, 2H, *m*-Ar-H), 6.85 (d, *J* 8.6, 2H, *m*-Ar-H), 7.25 (d, *J* 8.6, 2H, *o*-Ar-H), 7.27 (d, *J* 8.6, 2H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.58 (18-Me), 17.55 (21-Me), 22.80 (19-Me), 22.93 (15-CH_2), 27.85 (9-CH), 27.56 (16-CH_2), 28.74 (11-CH_2), 30.77, 30.81, 31.10 (2-CH_2, 22-CH_2, 23-CH_2), 34.70, 35.39 (1-CH_2, 6-CH_2, 10-Cq), 35.30 (20-CH), 38.75 (4-CH_2), 39.87 (8-CH), 41.99 (5-CH), 42.47 (14-CH), 46.44 (13-Cq), 46.18 (17-CH), 55.27 (ArO*Me*), 69.28, 69.96 (Ar*C*H_2O), 72.11 (3-CH), 75.11 (7-CH), 80.40 (12-CH), 113.60 (Ar *m*-CH), 128.49, 129.18 (Ar *o*-CH), 131.46, 131.99 (Ar-*C*–CH_2), 158.68, 158.82 (Ar-*C*–OMe), 179.99 (24-Cq); *m*/*z* (FAB) 648.5 $[M]^+$, 527.7 $[M - (OMe)Bn]^+$; found 648.4023, $C_{41}H_{58}O_7$ requires 648.4026.

Cyclic oligomers of 3-hydroxy-7,12-bis(*p*-methoxybenzyloxy)-cholanoic acid 20c–20e

3-Hydroxy-7,12-bis(*p*-methoxybenzyloxy)cholanoic acid **20** (1.00 g, 1.54 mmol), 4-dimethylaminopyridine (546 mg, 4.62 mmol) and ground 4 Å molecular sieves (3 g, freshly activated) were stirred in dichloromethane (77 ml) for 30 min. 2,6-Dichlorobenzoyl chloride (0.235 ml, 386 mg, 1.85 mmol) was injected and stirring was continued for 48 h by which time three major products were visible by TLC (hexane–ethyl acetate, 3:1). The mixture was then diluted with further dichloromethane (100 ml) and washed with water (3 × 100 ml), dried and concentrated under reduced pressure. The reaction products were then separated by flash column chromatography (hexane–ethyl acetate, gradient 4:1–2:1).

Compound A (*cyclic trimer* 20c). Colourless oil (100 mg, 11%). R_f 0.51 (hexane–ethyl acetate, 2:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1719 (CO_2R), 1610 (Ar); $\lambda_{max}(Et_2O)/nm$ 274.9 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 11 500); δ_H(400 MHz, $CDCl_3$) 0.65 (s, 9H, 18-Me), 0.81 (d, *J* 6.6, 9H, 21-Me), 0.88 (s, 9H, 19-Me), 3.35 (q, *J* 2.1, 3H, 7βH), 3.46 (s, 9H, $ArOCH_3$), 3.58 (s, 9H, ArOCH_3), 3.58 (br s, 3H, 12βH), 4.15, 4.46 (ABq, *J* 11.2, 6H, ArOCH_2), 4.32, 4.53 (ABq, *J* 11.8, 6H, ArOCH_2), 4.57 (tt, *J* 4.6, 11.3, 3H, 3βH), 6.65 (d, *J* 8.6, 6H, *m*-Ar-H), 6.68 (d, *J* 8.6, 6H, *m*-Ar-H), 7.08 (d, *J* 8.5, 6H, *o*-Ar-H), 7.23 (d, *J* 8.5, 6H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.49 (18-Me), 17.42 (21-Me), 22.93 (19-Me), 23.07 (15-CH_2), 26.04 (9-CH), 27.90 (16-CH_2), 29.73 (11-CH_2), 30.22, 30.64 (2-CH_2, 22-CH_2, 23-CH_2), 34.13, 34.98, 35.56 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.10 (20-CH), 39.73 (8-CH), 41.66 (5-CH), 42.86 (14-CH), 44.49 (17-CH), 46.44 (13-Cq), 54.97, 55.03 (ArO*Me*), 69.85 (Ar*C*H_2O), 74.02, 74.14 (3-CH, 7-CH), 80.05 (12-CH), 113.55, 114.34 (Ar *m*-CH), 129.03, 130.03 (Ar *o*-CH), 130.63, 131.41 (Ar-*C*–CH_2), 158.83, 159.06 (Ar-*C*–OMe), 174.49 (24-Cq); *m*/*z* (FIB) 1913 $[M + Na]^+$, 1790 $[M - (OMe)Bn + Na]^+$, 1489 $[M - 3 \times (OMe)Bn - 2 \times OMe + Na]^+$, 1370 $[M - 4 \times (OMe)Bn - 2 \times OMe + Na]^+$.

Compound B (*cyclic tetramer* 20d). Colourless oil (80 mg, 8%). R_f 0.45 (hexane–ethyl acetate, 2:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1714 (CO_2R), 1611 (Ar); $\lambda_{max}(Et_2O)/nm$ 275.0 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 15 000); δ_H(400 MHz, $CDCl_3$) 0.64 (s, 12H, 18-Me), 0.88

(d, *J* 6.6, 12H, 21-Me), 0.89 (s, 12H, 19-Me), 3.34 (d, *J* 2.1, 4H, 7βH), 3.48 (s, 12H, ArOCH_3), 3.55 (s, 12H, ArOCH_3), 3.60 (br s, 4H, 12βH), 4.04, 4.47 (ABq, *J* 11.5, 8H, ArOCH_2), 4.31, 4.48 (ABq, *J* 11.7, 8H, ArOCH_2), 4.52 (tt, *J* 4.0, 11.1, 4H, 3βH), 6.65 (d, *J* 8.6, 8H, *m*-Ar-H), 6.71 (d, *J* 8.6, 8H, *m*-Ar-H), 7.11 (d, *J* 9.5, 8H, *o*-Ar-H), 7.23 (d, *J* 8.6, 8H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.59 (18-Me), 17.68 (21-Me), 22.90 (19-Me), 23.23 (15-CH_2), 26.89 (2-CH_2), 27.99 (9-CH), 27.62 (16-CH_2), 28.46 (11-CH_2), 31.40, 31.53 (22-CH_2, 23-CH_2), 34.33, 34.76, 35.03 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.23 (20-CH), 39.84 (8-CH), 41.79 (5-CH), 42.44 (14-CH), 45.67 (17-CH), 46.46 (13-Cq), 54.99, 55.10 (ArO*Me*), 69.70, 69.91 (Ar*C*H_2O), 74.29, 74.67 (3-CH, 7-CH), 80.41 (12-CH), 113.45, 113.57 (Ar *m*-CH), 128.62, 129.44 (Ar *o*-CH), 130.88, 131.52 (Ar-*C*–CH_2), 158.81, 158.90 (Ar-*C*–OMe), 174.43 (24-Cq); *m*/*z* (FIB) 2544 $[M + Na]^+$, 2426 $[M - (OMe)Bn + Na]^+$.

Compound C (*cyclic pentamer* 20e). Colourless oil (50 mg, 5%). R_f 0.35 (hexane–ethyl acetate, 2:1); $\nu_{max}(Et_2O)$/nm 1714 (CO_2R), 1611 (Ar); $\lambda_{max}(Et_2O)$/nm 275.0 (ε/dm^3 mol^{-1} cm^{-1} 19 500); δ_H(400 MHz, $CDCl_3$) 0.64 (s, 15H, 18-Me), 0.86 (d, *J* 5.7, 15H, 21-Me), 0.90 (s, 15H, 19-Me), 3.37 (br s, 5H, 7βH), 3.56 (s, 15H, ArOCH_3), 3.60 (s, 15H, ArOCH_3), 3.62 (br s, 5H, 12βH), 4.09, 4.48 (ABq, *J* 11.0, 10H, ArOCH_2), 4.27, 4.50 (ABq, *J* 11.6, 10H, ArOCH_2), 4.52 (br s, 5H, 3βH), 6.70 (d, *J* 8.6, 10H, *m*-Ar-H), 6.72 (d, *J* 8.6, 10H, *m*-Ar-H), 7.15 (d, *J* 9.5, 10H, *o*-Ar-H), 7.22 (d, *J* 8.6, 10H, *o*-Ar-H); δ_C(100 MHz, $CDCl_3$) 12.61 (18-Me), 17.65 (21-Me), 22.87 (19-Me), 23.27 (15-CH_2), 26.87 (16-CH_2), 27.94 (9-CH), 28.52 (11-CH_2), 31.31, 31.77 (2-CH_2, 22-CH_2, 23-CH_2), 34.27, 34.74, 35.02 (1-CH_2, 4-CH_2, 6-CH_2, 10-Cq), 35.19 (20-CH), 39.83 (8-CH), 41.75 (5-CH), 42.46 (14-CH), 46.08 (17-CH), 46.47 (13-Cq), 55.04, 55.11 (ArO*Me*), 69.68, 69.98 (Ar*C*H_2O), 74.22, 74.90 (3-CH, 7-CH), 80.32 (12-CH), 113.47, 113.59 (Ar *m*-CH), 128.68, 129.31 (Ar *o*-CH), 131.09, 131.60 (Ar-*C*–CH_2), 158.81, 158.83 (Ar-*C*–OMe), 174.29 (24-Cq); *m*/*z* (FIB) 2544 $[M + Na]^+$, 2426 $[M - (OMe)Bn + Na]^+$.

p-Phenylbenzyl 2,2,2-trichloroacetimidate 15

Sodium hydride powder (6.5 mg, 0.272 mmol) was added to *p*-phenylbenzyl alcohol (0.5 g, 2.72 mmol) in diethyl ether (1 ml). After stirring for 20 min, the effervescence ceased and the mixture was cooled to 0 °C. Trichloroacetonitrile (0.260 ml, 374 mg, 2.60 mmol) was then injected dropwise over 20 min, the flask was allowed to warm to room temperature and stirring was continued for 1 h. By this time, TLC (hexane–diethyl ether, 1:1) showed most of the starting material to have reacted to form a single product. The solvent was evaporated under reduced pressure and the products were dissolved in hexane and filtered to remove unreacted alcohol. After evaporating the solvent under reduced pressure, the 2,2,2-*trichloroacetimidate* **15** was obtained as a light yellow solid (0.62 g, 69%) (Found: C, 54.4; H, 3.8; Cl, 31.8; N, 4.1; $C_{15}H_{12}NOCl_3$ requires C, 54.82; H, 3.68; Cl, 32.4; N, 4.26%); R_f 0.66 (hexane–diethyl ether, 1:1); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3332 (NH), 1663 (Ar); $\lambda_{max}(Et_2O)$/nm 250.5 (ε/dm^3 mol^{-1} cm^{-1} 25 000); δ_H(400 MHz, $CDCl_3$) 5.39 (ArCH_2O), 7.36 (t, *J* 7.35, 1H, *p*-phenyl-H), 7.45 (t, *J* 7.32, 2H, *m*-phenyl-H), 7.51 (d, *J* 8.14, 2H, *o*-benzyl-H), 7.60 (d, *J* 7.19, 2H, *o*-phenyl-H), 7.62 (d, *J* 8.15, 2H, *m*-benzyl-H); δ_C(100 MHz, $CDCl_3$) 70.51 (Ar*C*H_2O), 91.7 (CCl_3), 127.12, 127.29, 128.19, 128.79 (*o*-, *m*-CHs), 127.43 (*p*-CH), 134.42 (Ar*C*-CH_2O), 140.66, 141.26 (Ar*C*C), 162.60 (C=N); *m*/*z* (FAB) 329 $[M + H]^+$; found 327.0006, $C_{15}H_{12}NO^{35}Cl_3$ requires 326.9984.

3-Hydroxy-12-(*p*-phenylbenzyloxy)cholanoic acid 17

Methyl 3-acetoxy-12-hydroxycholanoate **14**[24] (2.00 g, 4.35 mmol), *p*-phenylbenzyl 2,2,2-trichloroacetimidate **15** (1.97 g, 6.5 mmol) and 4 Å molecular sieves (2 g, freshly activated) were stirred in dry cyclohexane (40 ml) and dry carbon tetrachloride (20 ml) for 30 min. Trifluoromethanesulfonic acid (0.050 ml, 0.50 mmol) was then syringed in and stirring was continued for 1 h. TLC (hexane–acetone, 4:1) showed that reaction was not yet complete, so a further portion of trifluoromethanesulfonic acid (0.050 ml, 0.50 mmol) was added. Stirring was continued for a further 2 h by which time complete reaction to a single product had occurred, so the mixture was washed with aqueous sodium hydroxide (50 ml, 2.5 M) and water (2 × 25 ml), dried and the solvent removed under reduced pressure. Without purification, the yellow oil obtained was dissolved in THF (20 ml) and aqueous sodium hydroxide (20 ml, 2.5 M) and methanol (5 ml) were added. The mixture was then heated to reflux for 4 h after which TLC (chloroform–methanol, 15:1) indicated reaction to a single product. The mixture was then neutralised by addition of aqueous hydrochloric acid (*ca*. 15 ml, 3 M) and the volatile solvents were removed under reduced pressure, leaving an aqueous slurry. This was diluted with further water (50 ml) and the organic products were extracted into dichloromethane (3 × 50 ml), dried and evaporated under reduced pressure to yield the *hydroxy acid* **17** as a white powder (1.70 g, 73%) which was pure by TLC. R_f 0.42 (chloroform–methanol, 15:1); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3604 (OH), 3185 (COO–H), 1707 (CO_2H), 1605 (Ar); $\lambda_{max}(Et_2O)$/nm 250.3 (ε/dm^3 mol^{-1} cm^{-1} 24 000); δ_H(400 MHz, $CDCl_3$) 0.71 (s, 3H, 18-Me), 0.92 (d, *J* 4.7, 3H, 21-Me), 0.93 (s, 3H, 19-Me), 3.57 (tt, *J* 4.5, 10.8, 1H, 3βH), 3.70 (br s, 1H, 12βH), 7.34 (tt, *J* 2.0, 7.3, 1H, *p*-phenyl-H), 7.40–7.66 (m, 8H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.80 (18-Me), 17.59 (21-Me), 23.17 (15-CH_2), 23.34 (19-Me), 23.72 (16-CH_2), 26.06 (7-CH_2), 27.32 (11-CH_2), 27.61 (6-CH_2), 30.68 (23-CH_2), 30.78 (2-CH_2), 31.02 (22-CH_2), 33.74 (9-CH), 34.20 (10-Cq), 35.27 (20-CH), 35.35 (1-CH_2), 36.09 (8-CH), 36.54 (4-CH_2), 42.16 (5-CH), 46.31 (17-CH), 46.63 (13-Cq), 48.65 (14-CH), 70.05 (Ar*C*H_2O), 71.74 (3-CH), 81.22 (12-CH), 127.11, 127.16, 127.92, 128.79 (Ar *o*-, *m*-, *p*-CHs), 138.42 (Ar*C*CH_2O), 140.25, 141.08 (Ar*C*–C), 179.75 (24-Cq); *m*/*z* (FAB) 559.6 $[M + H]^+$; found 559.3854, $C_{37}H_{51}O_4$ requires 559.3902.

Methyl 3-hydroxy-12-(*p*-phenylbenzyloxy)cholanoate 18

3-Hydroxy-12-(*p*-phenylbenzyloxy)cholanoic acid **17** (1.83 g, 3.28 mmol) was dissolved in methanolic HCl (50 ml, 12%) and stirred for 2 h. After this time, TLC (hexane–ethyl acetate, 4:1) showed reaction to be complete, so the mixture was neutralised with aqueous NaOH (*ca*. 3 ml, 2.5 M) and the methanol was removed under reduced pressure. The residue was dissolved in dichloromethane (50 ml) and washed with water (3 × 50 ml, distilled), dried and the solvent was removed under reduced pressure. The light yellow product thus obtained was purified by flash column chromatography (hexane–ethyl acetate, gradient 5:1–3:1) to leave *hydroxy ester* **18** as a white solid (1.43 g, 80%) which resisted attempts at crystallization. R_f 0.18 (hexane–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3607 (OH), 1731 (CO_2Me), 1603 (Ar); $\lambda_{max}(Et_2O)$/nm 250.1 (ε/dm^3 mol^{-1} cm^{-1} 24 000); δ_H(400 MHz, $CDCl_3$) 0.71 (s, 3H, 18-Me), 0.92 (d, *J* 4.5, 3H, 21-Me), 0.92 (s, 3H, 19-Me), 3.57 (tt, *J* 4.6, 10.9, 1H, 3βH), 3.65 (s, 3H, OMe), 3.71 (br s, 1H, 12βH), 7.33 (tt, *J* 2.0, 7.4, 1H, *p*-phenyl-H), 7.40–7.63 (m, 8H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.80 (18-Me), 17.58 (21-Me), 23.11 (15-CH_2), 23.34 (19-Me), 23.76 (16-CH_2), 26.10 (7-CH_2), 27.24 (11-CH_2), 27.63 (6-CH_2), 30.76 (23-CH_2), 30.77 (2-CH_2), 31.04 (22-CH_2), 33.85 (9-CH), 34.21 (10-Cq), 35.35 (20-CH), 35.35 (1-CH_2), 36.11 (8-CH), 36.58 (4-CH_2), 42.18 (5-CH), 46.34 (17-CH), 46.63 (13-Cq), 48.85 (14-CH), 51.51 (OMe), 70.02 (Ar*C*H_2O), 71.89 (3-CH), 81.23 (12-CH), 127.13, 127.20, 127.89, 128.77 (Ar *o*-, *m*-, *p*-CHs), 138.45 (Ar*C*–CH_2O), 140.29, 141.04 (Ar*C*–C), 174.86 (24-Cq); *m*/*z* (FAB) 596 $[M + Na]^+$, 573.7 $[M + H]^+$; found 573.3944, $C_{38}H_{53}O_4$ requires 573.3988.

Cyclic oligomers of 3-hydroxy-12-(*p*-phenylbenzyloxy)cholanoic acid 17b–17d

3-Hydroxy-12-(*p*-phenylbenzyloxy)cholanoic acid **17** (0.10 g, 0.18 mmol), 4-dimethylaminopyridine (72.5 mg, 0.59 mmol) and ground 4 Å molecular sieves (2 g, freshly activated) were

stirred in dry toluene (90 ml) and heated to 80 °C for 2 h. 2,6-Dichlorobenzoyl chloride (0.032 ml, 45 mg, 0.216 mmol) was then injected and stirring was continued. After 48 h TLC (hexane–acetone, 3:1) showed the presence of three major products, so the mixture was cooled, washed with dilute aqueous HCl (2 × 50 ml) and water (50 ml) and the solvent removed under reduced pressure. The components of the colourless oil obtained were separated by flash chromatography (hexane–ethyl acetate, 20:1–10:1) and analysed as follows.

Compound A (*cyclic dimer* 17b). Isolated as a colourless oil (10 mg, 11%). R_f 0.44 (hexane–ethyl acetate, 10:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1603 (Ar); $\lambda_{max}(Et_2O)/nm$ 254.2 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 50 000); δ_H(400 MHz, $CDCl_3$) 0.64 (s, 6H, 18-Me), 0.66 (d, *J* 6.7, 6H, 21-Me), 0.98 (s, 6H, 19-Me), 3.57 (br s, 2H, 12βH), 3.91, 4.31 (ABq, *J* 13, 4H, ArCH_2O), 4.76 (tt, *J* 4.9, 9.7, 2H, 3βH), 7.12 (d, *J* 8.1, 4H, *o*-benzyl-H), 7.33 (d, *J* 8.1, 4H, *m*-benzyl-H), 7.35 (t, *J* 7.45, 2H, *p*-phenyl-H), 7.46 (t, *J* 7.6, 4H, *m*-phenyl-H), 7.55 (d, *J* 7.8, 4H, *o*-phenyl-H); δ_C(100 MHz, $CDCl_3$) 12.27 (18-Me), 17.45 (21-Me), 22.72 (15-CH_2), 23.41 (19-Me), 23.76 (16-CH_2), 26.95 (7-CH_2), 27.08 (11-CH_2), 27.62 (6-CH_2), 27.93 (2-CH_2), 28.09 (23-CH_2), 31.62 (22-CH_2), 32.14 (4-CH_2), 33.54 (9-CH), 34.75 (10-Cq), 34.95 (20-CH), 35.53 (1-CH_2), 36.47 (8-CH), 41.89 (5-CH), 43.31 (17-CH), 46.57 (13-Cq), 47.94 (14-CH), 69.11 (Ar*C*H_2O), 75.04 (3-CH), 81.64 (12-CH), 126.13, 126.46, 126.89, 128.80 (Ar *o*-, *m*-, *p*-CHs), 139.06, 139.56 (Ar*C*–C), 141.50 (Ar*C*–CH_2O), 176.24 (24-Cq); *m/z* (FAB) 1104.5 $[M + Na]^+$, 713.9 [M − 2 × *p*-phenylBn + $Na]^+$; found 1104.71 827, $C_{74}H_{96}O_6Na$ requires 1104.7138.

Compound B (*cyclic trimer* 17c). Isolated as a colourless oil (30 mg, 31%). R_f 0.31 (hexane–ethyl acetate, 10:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1603 (Ar); $\lambda_{max}(Et_2O)/nm$ 253.2 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 70 000); δ_H(400 MHz, $CDCl_3$) 0.66 (s, 9H, 18-Me), 0.77 (d, *J* 6.6, 9H, 21-Me), 0.97 (s, 9H, 19-Me), 3.60 (br s, 3H, 12βH), 4.26, 4.39 (ABq, *J* 12.1, 6H, ArCH_2O), 4.72 (tt, *J* 4.7, 11.2, 3H, 3βH), 7.23 (d, *J* 8.0, 6H, *o*-benzyl-H), 7.25–7.35 (m, 21H, *p*-phenyl-H, *m*-phenyl-H, *o*-phenyl-H, *m*-benzyl-H); δ_C(100 MHz, $CDCl_3$) 12.68 (18-Me), 17.55 (21-Me), 22.73 (15-CH_2), 23.36 (19-Me), 23.75 (16-CH_2), 26.85 (7-CH_2), 27.12 (6-CH_2), 27.20 (11-CH_2), 29.40 (2-CH_2), 30.04 (23-CH_2), 30.61 (22-CH_2), 31.96 (4-CH_2), 33.84 (9-CH), 34.24 (10-Cq), 34.70 (1-CH_2), 35.07 (20-CH), 36.06 (8-CH), 41.97 (5-CH), 44.69 (17-CH), 46.54 (13-Cq), 48.78 (14-CH), 70.40 (Ar*C*H_2O), 74.06 (3-CH), 81.04 (12-CH), 127.00, 128.27, 128.54 (Ar *o*-, *m*-, *p*-CHs), 138.40 (Ar*C*–CH_2O), 140.07, 140.89 (Ar*C*–C), 174.25 (24-Cq); *m/z* (FAB) 1645.7 $[M + Na]^+$, 1477.8 [M − Bn + $Na]^+$; found 1644.0791, $C_{111}H_{144}O_9Na$ requires 1644.0708.

Compound C (*cyclic tetramer* 17d). Isolated as a colourless oil (20 mg, 21%). R_f 0.25 (hexane–ethyl acetate, 10:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 253.0 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 92 000); δ_H(400 MHz, $CDCl_3$) 0.68 (s, 12H, 18-Me), 0.82 (d, *J* 6.7, 12H, 21-Me), 0.95 (s, 12H, 19-Me), 3.61 (br s, 4H, 12βH), 4.27, 4.40 (ABq, *J* 12.0, 8H, ArCH_2O), 4.81 (tt, *J* 4.8, 11.2, 4H, 3βH), 7.22 (d, *J* 8.0, 8H, *o*-benzyl-H), 7.26–7.36 (m, 28H, *p*-phenyl-H, *m*-phenyl-H, *o*-phenyl-H, *m*-benzyl-H); δ_C(100 MHz, $CDCl_3$) 12.75 (18-Me), 17.57 (21-Me), 22.81 (15-CH_2), 23.32 (19-Me), 23.78 (16-CH_2), 26.71 (7-CH_2), 27.21 (11-CH_2), 27.41 (6-CH_2), 29.60 (2-CH_2), 30.30 (23-CH_2), 31.10 (22-CH_2), 32.12 (4-CH_2), 33.81 (9-CH), 34.20 (10-Cq), 34.89 (1-CH_2), 35.20 (20-CH), 36.08 (8-CH), 42.10 (5-CH), 45.20 (17-CH), 46.56 (13-Cq), 48.81 (14-CH), 70.80 (Ar*C*H_2O), 73.82 (3-CH), 81.21 (12-CH), 127.06, 128.04, 128.73 (Ar *o*-, *m*-, *p*-CHs), 138.52 (Ar*C*–CH_2O), 140.36, 141.15 (Ar*C*–C), 174.25 (24-Cq); *m/z* (ES) 2184 $[M + Na]^+$, 2200 $[M + K]^+$.

Methyl 7,12-bis[(benzyloxy)methoxy]-3-hydroxycholanoate 19

Methyl 3-ethoxycarbonyloxy-7,12-dihydroxycholanoate **11**[20] (5.00 g, 10.2 mmol) was stirred in dry toluene (100 ml) and diisopropylethylamine (8.0 ml, 5.24 g, 40.6 mmol) and (benzyloxy)methoxymethyl chloride (4.00 ml, 6.35 g, 40.6 mmol) were syringed in. Stirring was continued for 5 min and the mixture was then heated to 90 °C. The reaction was followed by TLC (hexane–ethyl acetate, 3:1) showing an initial product (R_f 0.34) which disappeared as the reaction proceeded. The reaction appeared to have stopped after 19 h, so a further portion each of diisopropylethylamine (4.0 ml, 2.62 g, 20.3 mmol) and (benzyloxy)methoxymethyl chloride (4.00 ml, 6.35 g, 40.6 mmol) were syringed in, Reaction to a single main product (R_f 0.54) was deemed to be complete after a further 5 h and the solution was diluted with diethyl ether (100 ml) and washed with dilute aqueous HCl (200 ml, 1 M) and water (2 × 200 ml). The organic fraction was dried and the solvent removed under reduced pressure.

Without purification, this product was dissolved in dry THF (200 ml) and methanolic potassium methoxide solution (50 ml, 1.2 M) was added by cannula. This mixture was stirred for 1 h. TLC (hexane–ethyl acetate, 3:1) showed reaction to be complete so the mixture was neutralised with dilute aqueous HCl (*ca*. 20 ml, 3 M) and the volatile solvents were removed under reduced pressure. The aqueous slurry was diluted with water (100 ml), the products were extracted into diethyl ether (3 × 100 ml) and washed with water (50 ml). The resulting brown oil was purified by flash column chromatography (hexane–ethyl acetate, 2:1) to yield the *diether* **19** as a light yellow viscous oil (3.90 g, 58%). R_f 0.18 (hexane–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3600 (OH), 1731 (CO_2Me), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 251.8, 257.6, 263.7 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 400, 520, 420); δ_H(400 MHz, $CDCl_3$) 0.69 (s, 3H, 18-Me), 0.90 (s, 3H, 19-Me), 0.95 (d, *J* 6.5, 3H, 21-Me), 3.35 (tt, *J* 5.4, 10.8, 1H, 3βH), 3.65 (s, 3H, OMe), 3.70 (q, *J* 2.6, 1H, 7βH), 3.88 (br s, 1H, 12βH), 4.60–4.98 (4 × ABq, 8H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 10H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.56 (18-Me), 17.88 (21-Me), 22.79 (19-Me), 23.60 (15-CH_2), 25.07 (16-CH_2), 27.58 (9-CH), 27.72 (11-CH_2), 30.85, 31.03, 31.08, 31.17 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.79, 35.35 (1-CH_2, 6-CH_2), 35.56 (20-CH), 39.26 (4-CH_2), 39.67 (8-CH), 41.75 (5-CH), 42.11 (14-CH), 46.23 (17-CH), 46.41 (13-Cq), 51.47 (OMe), 70.07, 70.25 (Ar*C*H_2O), 72.02 (3-CH), 74.82 (7-CH), 79.94 (12-CH), 93.15, 94.15 (OCH_2O), 127.52, 127.57, 128.42 (Ar *o*-, *m*-, *p*-CHs), 138.23, 138.26 (Ar*C*–CH_2O), 174.70 (24-Cq); *m/z* (FAB) 685.7 $[M + Na]^+$, 663.5 $[M + H]^+$, 525.6 [M − BnOCH_2O + $Na]^+$, 507.6 [M − BnOCH_2O − H_2O + $Na]^+$, 417.4 [M − BnO-CH_2O − BnO + $Na]^+$, 399.3 [M − BnOCH_2O − BnO − H_2O + $Na]^+$, 387.3 [M − 2 × BnOCH_2O + $Na]^+$, 369.3 [M − 2 × BnOCH_2O − H_2O + $Na]^+$; found 685.4122, $C_{41}H_{58}O_7Na$ requires 685.4080.

Methyl 3-*tert*-butyldimethylsilyloxy-7,12-bis[(benzyloxy)-methoxy]cholanoate 23

Methyl 7,12-bis[(benzyloxy)methoxy]-3-hydroxycholanoate **19** (1.36 g, 2.10 mmol), 4-dimethylaminopyridine (0.040 g, 0.315 mmol), *tert*-butyldimethylsilyl chloride (0.380 g, 2.52 mmol) and ground 4 Å molecular sieves (2 g, freshly activated) were stirred in dry dichloromethane (50 ml) for 1 h. Triethylamine (0.42 ml, 0.303 g, 3.00 mmol, freshly distilled) was then injected and stirring was continued. After 20 h, TLC (hexane–ethyl acetate, 3:1) indicated that reaction was not yet complete and a further portion of *tert*-butyldimethylsilyl chloride (0.100 g, 0.66 mmol) was added and stirring was continued for a further 8 h. The reaction mixture was diluted with diethyl ether (100 ml) and washed with dilute aqueous H_2SO_4 (100 ml, 3 M) and water (2 × 100 ml) and then dried. After evaporation of the solvent under reduced pressure, the crude products were purified by flash column chromatography (hexane–toluene–ethyl acetate, 20:20:1) to yield the pure *silyl ether* **23** as a colourless oil (1.01 g, 63%). R_f 0.15 (hexane–toluene–ethyl acetate, 20:20:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1731 (CO_2Me), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 251.8, 257.6, 263.7 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 480, 550, 440); δ_H(400 MHz, $CDCl_3$) 0.00 (s, 6H, SiMe), 0.69 (s, 3H, 18-Me), 0.85 (s, 9H, Bu^t), 0.88 (s, 3H, 19-Me), 0.95 (d, *J* 6.3, 3H, 21-Me), 3.39 (tt, *J* 4.7, 10.7, 1H, 3βH), 3.65 (s, 3H, OMe), 3.67 (q, *J* 2.6, 1H,

7βH), 3.88 (br s, 1H, 12βH), 4.65–4.89 (4 × ABq, 8H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 10H, Ar-H); δ_C(100 MHz, $CDCl_3$) −4.60, −4.54 (2 × SiMe), 12.62 (18-Me), 17.98 (21-Me), 18.28 (Si*C*Me_3), 22.89 (19-Me), 23.56 (15-CH_2), 25.22 (16-CH_2), 25.95 (SiC*Me*$_3$), 27.76 (9-CH), 27.76 (11-CH_2), 31.03, 31.18, 31.27, 31.36 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.74, 35.48 (1-CH_2, 6-CH_2), 35.61 (20-CH), 39.65 (4-CH_2), 39.65 (8-CH), 41.91 (5-CH), 42.23 (14-CH), 46.19 (17-CH), 46.42 (13-Cq), 51.45 (OMe), 69.87, 70.12 (Ar*C*H_2O), 73.02 (3-CH), 75.38 (7-CH), 79.64 (12-CH), 93.98, 94.26 (2 × OCH_2O), 127.47, 127.61, 128.36 (Ar *o*-, *m*-, *p*-CHs), 138.25, 138.29 (Ar*C*–CH_2O), 174.69 (24-Cq); *m*/*z* (FAB) 800.1 $[M + Na]^+$, 776.1 $[M]^+$, 667.6 $[M - BnO]^+$, 637.6 $[M - BnOCH_2O]^+$, 531.7 $[M - BnOCH_2O - BnO]^+$, 501.7 $[M - 2 \times BnOCH_2O]^+$; found 776.5072, $C_{47}H_{72}SiO_7$ requires 776.5047.

3-*tert*-Butyldimethylsilyloxy-7,12-bis[(benzyloxy)methoxy]-cholanoic acid 24

Methyl 3-*tert*-butyldimethylsilyloxy-7,12-bis[(benzyloxy)methoxy]cholanoate **23** (1.01 g, 1.30 mmol) was stirred in THF (20 ml), aqueous NaOH (20 ml, 2.5 M) and methanol (2 ml) and heated to 50 °C. After 2 h, TLC (chloroform–methanol, 20:1) showed reaction to a single product to be complete, the mixture was neutralised with dilute aqueous HCl (*ca.* 20 ml, 3 M) and the volatile solvents were removed under reduced pressure. The products were dissolved in diethyl ether (50 ml) and washed with dilute aqueous HCl (50 ml, 1 M) and water (2 × 50 ml). After drying and removal of the solvent under reduced pressure, a light yellow oil was obtained that was purified by flash column chromatography (chloroform–methanol, 20:1) to yield the *acid* **24** as a colourless oil (0.860 g, 87%). R_f 0.50 (chloroform–methanol, 20:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3496 (OH), 1707 (CO_2H dimer), 1605 (Ar); $\lambda_{max}(Et_2O)$/nm 251.8, 257.5, 263.6 (ε/dm^3 mol^{-1} cm^{-1} 500, 530, 430); δ_H(400 MHz, $CDCl_3$) 0.01 (s, 6H, SiMe), 0.69 (s, 3H, 18-Me), 0.85 (s, 9H, Bu′-Me), 0.88 (s, 3H, 19-Me), 0.96 (d, *J* 6.2, 3H, 21-Me), 3.39 (tt, *J* 4.3, 10.6, 1H, 3βH), 3.66 (br s, 1H, 7βH), 3.88 (br s, 1H, 12βH), 4.62–4.91 (4 × ABq, 8H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 10H, Ar-H); δ_C(100 MHz, $CDCl_3$) −4.62, −4.56 (2 × SiMe), 12.61 (18-Me), 17.93 (21-Me), 18.27 (Si*C*Me_3), 22.87 (19-Me), 23.54 (15-CH_2), 25.20 (16-CH_2), 25.93 (SiC*Me*$_3$), 27.74 (9-CH), 27.74 (11-CH_2), 30.82, 31.00, 31.24, 31.36 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.72, 35.46 (1-CH_2, 6-CH_2), 35.56 (20-CH), 39.63 (4-CH_2), 39.63 (8-CH), 41.89 (5-CH), 42.20 (14-CH), 46.18 (17-CH), 46.41 (13-Cq), 69.85, 70.05 (Ar*C*H_2O), 73.01 (3-CH), 75.39 (7-CH), 79.63 (12-CH), 93.96, 94.26 (O*C*H_2O), 127.39, 127.43, 128.38 (Ar *o*-, *m*-, *p*-CHs), 138.21, 138.33 (Ar*C*–CH_2O), 179.10 (24-Cq); *m*/*z* (FAB) 786.1 $[M + Na]^+$, 693.7 $[M - BnO + Na]^+$, 671.6 $[M - BnO]^+$; found 786.4910, $C_{46}H_{70}SiO_7Na$ requires 786.4867.

2,2,2-Trichloroethyl 3-hydroxy-7,12-bis-[(benzyloxy)methoxy]-cholanoate 22

Methyl 7,12-bis[(benzyloxy)methoxy]-3-hydroxycholanoate **19** (1.032 g, 1.56 mmol) was dissolved in THF (10 ml), aqueous NaOH (10 ml, 2.5 M) and methanol (2 ml) and heated to reflux for 90 min. After this time, TLC (chloroform–methanol, 20:1) showed reaction to a single product (R_f 0.36) to be complete and the mixture was neutralised with dilute aqueous HCl (*ca.* 20 ml, 3 M). The volatile solvents were removed under reduced pressure and the products were dissolved in diethyl ether (50 ml) and washed with dilute aqueous HCl (50 ml, 1 M) and water (2 × 50 ml). After drying and removal of the solvent under reduced pressure, a light yellow oil was obtained (0.960 g) which was used without further purification.

The crude hydroxy acid, 4-dimethylaminopyridine (0.300 g, 2.35 mmol) and ground 4 Å molecular sieves (0.5 g, freshly activated) were stirred in dry toluene (8 ml) under argon for 20 min. 2,2,2-Trichloroethanol (0.225 ml, 0.335 g, 2.35 mmol) was injected and stirring was continued for 15 min before 2,6-dichlorobenzoyl chloride (0.290 ml, 375 mg, 1.79 mmol) was injected. After stirring for 16 h, TLC (hexane–ethyl acetate, 3:1) showed reaction to a single product to be almost complete, with a small amount of starting material remaining, so the mixture was diluted with diethyl ether (50 ml) and washed with dilute aqueous HCl (50 ml) and water (2 × 50 ml). After drying and removal of the solvent under reduced pressure, a yellow oil was obtained which was purified by flash column chromatography (hexane–ethyl acetate, 3:1) to yield the *trichloroethyl ester* **22** as a colourless viscous oil (1.021 g, 94%). R_f 0.18 (hexane–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3606 (OH), 1751 ($CO_2CH_2CCl_3$), 1605 (Ar); $\lambda_{max}(Et_2O)$/nm 251.8, 257.4, 263.1 (ε/dm^3 mol^{-1} cm^{-1} 500, 640, 550); δ_H(400 MHz, $CDCl_3$) 0.69 (s, 3H, 18-Me), 0.90 (s, 3H, 19-Me), 0.97 (d, *J* 6.2, 3H, 21-Me), 3.35 (tt, *J* 5.0, 10.6, 1H, 3βH), 3.70 (q, *J* 2.6, 1H, 7βH), 3.89 (br s, 1H, 12βH), 4.73 (s, 2H, CH_2CCl$_3$), 4.62–4.91 (4 × ABq, 8H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 10H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.58 (18-Me), 17.78 (21-Me), 22.78 (19-Me), 23.58 (15-CH_2), 25.03 (16-CH_2), 27.56 (9-CH), 27.71 (11-CH_2), 30.85, 31.03 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.79, 35.45 (1-CH_2, 6-CH_2), 35.34 (20-CH), 39.26 (4-CH_2), 39.67 (8-CH), 41.74 (5-CH), 42.11 (14-CH), 46.24 (17-CH), 46.43 (13-Cq), 70.11, 70.25 (Ar*C*H_2O), 72.01 (3-CH), 73.90 (*C*H_2CCl$_3$), 74.84 (7-CH), 79.95 (12-CH), 93.92, 94.15 (OCH_2O), 95.11 (CH_2CCl_3), 127.49, 127.58, 128.42 (Ar *o*-, *m*,-, *p*-CHs), 138.23 (Ar-*C*–CH_2O), 172.56 (24-Cq); *m*/*z* (FAB) 780.3 $[M + H]^+$, 643.7 $[M - BnOCH_2O]^+$, 504.4 $[M - 2 \times BnOCH_2O]^+$; found 779.3250, $C_{42}H_{58}O_7{}^{35}Cl_3$ requires 779.3231.

7,12-Bis[(benzyloxy)methoxy]-24-oxo-24-(2,2,2-trichloroethoxy)cholan-3-yl 7,12-bis[(benzyloxy)methoxy]-3-*tert*-butyldimethylsilyloxycholanoate 25

3-*tert*-Butyldimethylsilyloxy-7,12-bis[(benzyloxy)methoxy]-cholanoic acid **24** (0.476 g, 0.625 mmol), 2,2,2-trichloroethyl 3-hydroxy-7,12-bis[(benzyloxy)methoxy]cholonoate **22** (0.495 g, 0.634 mmol), 4-dimethylaminopyridine (0.016 g, 0.131 mmol), triethylamine (0.150 ml, 0.109 g, 0.108 mmol) and ground 4 Å molecular sieves (1 g, freshly activated) were stirred in dry dichloromethane (10 ml) under argon for 1 h. 2,6-Dichlorobenzoyl chloride (0.100 ml, 0.144 g, 0.688 mmol) was injected and the reaction soon turned a light yellow colour. After 90 min stirring at room temperature, TLC (hexane–ethyl acetate, 3:1) showed reaction to be complete, so the mixture was diluted with diethyl ether (100 ml) and washed with dilute aqueous HCl (100 ml, 3 M) and water (2 × 100 ml). After evaporation of the solvent under reduced pressure, the product was purified by flash column chromatography (hexane–diethyl ether, 3:1) to yield the *linear dimer* **25** as a colourless oil (0.620 g, 65%). R_f 0.70 (hexane–ethyl acetate, 3:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1751 ($CO_2CH_2CCl_3$), 1720 (CO_2R), 1604 (Ar); $\lambda_{max}(Et_2O)$/nm 251.7, 257.5, 263.5 (ε/dm^3 mol^{-1} cm^{-1} 1175, 1240, 1080); δ_H(400 MHz, $CDCl_3$) 0.00 (s, 6H, SiMe), 0.65 (s, 3H, 18-Me), 0.69 (s, 3H, 18-Me), 0.85 (s, 9H, Bu′), 0.87 (s, 3H, 19-Me), 0.91 (s, 3H, 19-Me), 0.92 (d, *J* 6.2, 3H, 21-Me), 0.97 (d, *J* 6.2, 3H, 21-Me), 3.39 (tt, *J* 5.2, 11.0, 1H, 3βH–OTBDMS), 3.64 (q, *J* 2.5, 1H, 7βH), 3.69 (q, *J* 2.3, 1H, 7βH), 3.85 (br s, 1H, 12βH), 3.89 (br s, 1H, 12βH), 4.54 (tt, *J* 4.7, 11.4, 1H, 3βH–OCO), 4.72 (s, 2H, CH_2CCl$_3$), 4.56–4.89 (4 × ABq, 16H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 20H, Ar-H); δ_C(100 MHz, $CDCl_3$) −4.59, −4.53 (2 × SiMe), 12.62 (18-Me), 17.84, 18.02 (21-Me), 18.30 (Si*C*Me_3), 22.78, 22.90 (19-Me), 23.52, 23.58 (15-CH_2), 25.13, 25.29 (16-CH_2), 25.96 (SiC*Me*$_3$), 27.00 (11-CH_2), 27.64, 27.78 (9-CH), 30.92, 31.06, 31.29, 31.47, 31.77 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.76, 34.80, 34.95 (1-CH_2, 6-CH_2), 35.51, 35.60 (20-CH), 39.68 (4-CH_2), 39.61, 39.68 (8-CH), 41.52, 41.93 (5-CH), 42.20 (14-CH), 46.24 (17-CH), 46.43 (13-Cq), 69.87, 69.95, 70.11, 70.28 (Ar*C*H_2O), 73.05 (3-CHOSi), 73.90 (*C*H_2CCl$_3$), 74.16 (3-*C*HOCO), 74.72, 75.52 (7-CH), 79.72, 79.79 (12-CH), 93.86, 93.99, 94.08, 94.37 (OCH_2O), 95.10 (CH_2CCl_3), 127.41, 127.46, 127.53, 127.62, 128.34, 128.37,

128.40 (Ar *o*-, *m*-, *p*-CHs), 138.09, 138.16, 138.28, 138.37 (Ar*C*–CH_2O), 172.53 (24-*C*qOCH_2Cl_3), 173.85 (24-*C*qOR); *m*/*z* (FAB) 1524.8 $[M + H]^+$; found 1522.9019, $C_{88}H_{125}O_{13}Si^{35}Cl_3$ requires 1522.9006.

7,12-Bis[(benzyloxy)methoxy]-24-oxo-24-(2,2,2-trichloroethoxy)cholan-3-yl 7,12-bis[(benzyloxy)methoxy]-3-hydroxycholanoate 26

The linear dimer silyl ether **25** (0.602 g, 0.395 mmol) was stirred in dry THF (25 ml) and cooled to 0 °C in an ice bath and aqueous HF (1.00 ml, 40%, 0.400 g, 20 mmol) was syringed in. After 30 min the reaction flask was removed from the ice bath and allowed to warm to room temperature. After 3 h, TLC showed all starting material to have reacted, so saturated aqueous sodium hydrogen carbonate was added (20 ml) and the volatile solvents were removed under reduced pressure. The residue was extracted into diethyl ether (2 × 50 ml), the extracts washed with water and dried. After evaporation of the solvent under reduced pressure, the crude product was purified by flash column chromatography (hexane–ethyl acetate, 2:1) to yield the *hydroxy ester* **26** as a white foam (516 mg, 93%). R_f 0.76 (hexane–ethyl acetate, 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3607 (OH), 1751 ($CO_2CH_2CCl_3$), 1719 (CO_2R), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 252.1, 257.7, 263.6 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 720, 900, 820); δ_H(400 MHz, $CDCl_3$) 0.66 (s, 3H, 18-Me), 0.70 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.91 (s, 3H, 19-Me), 0.92 (d, *J* 5.4, 3H, 21-Me), 0.97 (d, *J* 6.2, 3H, 21-Me), 3.35 (tt, *J* 4.8, 11.0, 1H, 3βH–OH), 3.67 (2 × q, *J* 2.5, 2H, 7βH), 3.85 (br s, 1H, 12βH), 3.89 (br s, 1H, 12βH), 4.55 (tt, *J* 4.7, 11.4, 1H, 3βH–OCO), 4.73 (s, 2H, C$H_2$$CCl_3$), 4.56–4.89 (4 × ABq, 16H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 20H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.54, 12.63 (18-Me), 17.84, 17.90 (21-Me), 22.79 (19-Me), 23.52, 23.60 (15-CH_2), 25.12 (16-CH_2), 27.01, 27.73 (11-CH_2), 27.57, 27.64 (9-CH), 30.76, 30.85, 31.06, 31.73 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.80, 34.95, 35.35 (1-CH_2, 6-CH_2), 35.53 (20-CH), 39.27 (4-CH_2), 39.60, 39.67 (9-CH), 41.52, 41.75 (5-CH), 42.07, 42.20 (14-CH), 46.27 (17-CH), 46.39, 46.44 (13-Cq), 69.95, 70.07, 70.23, 70.28 (ArCH_2O), 72.02 (3-CHOH), 73.90 (CH_2CCl_3), 74.18 (3-*C*HOCO), 74.77, 74.77 (7-CH), 79.79, 80.01 (12-CH), 93.85, 93.87, 94.23 (OCH_2O), 95.10 (CH_2CCl_3), 127.51, 127.53, 127.58, 127.62, 127.68, 128.42 (Ar *o*-, *m*-, *p*-CHs), 138.10, 138.16, 138.21, 138.28 (Ar*C*CH_2O), 172.54 (24-*C*q-OCH_2Cl_3), 173.86 (24-*C*qOR); *m*/*z* (FAB) 1432.5 $[M + Na]^+$, 1410.8 $[M + H]^+$; found 1408.7077, $C_{82}H_{111}O_{13}{}^{35}Cl_3$ requires 1408.7059.

7,12-Bis[(benzyloxy)methoxy]-24-hydroxy-24-oxocholan-3-yl 7,12-bis[(benzyloxy)methoxy]-3-hydroxycholanoate 27

The hydroxy ester **26** (0.480 mg, 0.340 mmol) was stirred in THF (10 ml) at room temperature and zinc dust (2.00 g, 30 mmol) and aqueous potassium phosphate buffer (2 ml, 1 M) were added. After stirring for 2 h, reaction appeared to have proceeded to around 75% completion by TLC (hexane–ethyl acetate, 1:1) and a further equal portion of zinc dust was added. After a further 2 h, no further reaction had occurred, so the zinc powder was filtered off and the solvent was evaporated under reduced pressure. The products were then dissolved in diethyl ether (50 ml), washed with water (2 × 50 ml), dried and the solvent removed under reduced pressure. After purification by flash column chromatography (hexane–ethyl acetate, 1:1), the *hydroxy acid* **29** was obtained as a white foam (325 mg, 75%). R_f 0.35 (hexane–ethyl acetate, 1:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3607 (OH), 3496 (COOH), 1713 (CO_2H, CO_2R), 1605 (Ar); $\lambda_{max}(Et_2O)/nm$ 251.9, 257.6, 263.5 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 680, 780, 620); δ_H(400 MHz, $CDCl_3$) 0.66 (s, 3H, 18-Me), 0.70 (s, 3H, 18-Me), 0.89 (s, 3H, 19-Me), 0.91 (s, 3H, 19-Me), 0.92 (d, *J* 5.4, 3H, 21-Me), 0.95 (d, *J* 6.3, 3H, 21-Me), 3.35 (tt, *J* 4.4, 11.1, 1H, 3βH–OH), 3.69 (2 × q, *J* 2.5, 2H, 7βH), 3.85 (br s, 1H, 12βH), 3.89 (br s, 1H, 12βH), 4.55 (tt, *J* 4.3, 11.3, 1H, 3βH–OCO), 4.56–4.89 (4 × ABq, 16H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 20H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.54, 12.62 (18-Me), 17.90 (21-Me), 22.78 (19-Me), 23.52, 23.60 (15-CH_2), 25.12 (16-CH_2), 27.00, 27.72 (11-CH_2), 27.56, 27.66 (9-CH), 30.78, 31.05, 31.11, 31.74 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.79, 34.94, 34.99, 35.34 (1-CH_2, 6-CH_2), 35.53 (20-CH), 39.21 (4-CH_2), 39.60, 39.67 (8-CH), 41.52, 41.74 (5-CH), 42.07, 42.20 (14-CH), 46.26 (17-CH), 46.39, 46.43 (13-Cq), 69.93, 70.07, 70.22, 70.26 (ArCH_2O), 72.05 (3-CHOH), 74.20 (3-*C*HOCO), 74.71, 74.96 (7-CH), 79.80, 80.03 (12-CH), 93.82, 93.98, 94.22 (OCH_2O), 127.52, 127.56, 127.63, 127.69, 128.42 (Ar *o*-, *m*-, *p*-CHs), 138.08, 138.15, 138.20, 138.26 (Ar-*C*–CH_2O), 179.38 (24-*C*qOCH_2Cl_3), 173.91 (24-*C*qOR); *m*/*z* (FAB) 1317.3 $[M + K]^+$, 1301.4 $[M + Na]^+$; found 1278.7953, $C_{80}H_{110}O_{13}$ requires 1278.7917.

Cyclic 7,12-bis[(benzyloxy)methoxy]cholanoate tetramer 27d

Linear dimeric hydroxy acid **27** (0.300 g, 0.235 mmol), 4-dimethylaminopyridine (0.107 g, 0.836 mmol) and ground 4 Å molecular sieves (1 g, freshly activated) were stirred in dry dichloromethane (120 ml) under argon at room temperature for 1 h and then 2,6-dichlorobenzoyl chloride (0.041 ml, 0.057 g, 0.274 mmol) was added by syringe. After 48 h, TLC (hexane–ethyl acetate, 2:1) showed reaction to one major and two minor products, so the mixture was washed with dilute aqueous HCl (100 ml, 3 M), saturated aqueous sodium hydrogen carbonate (100 ml) and water (100 ml) and dried. After evaporation of the solvent under reduced pressure, the products were purified by flash column chromatography (hexane–ethyl acetate, 4:1), to yield the *cyclic tetramer* **27d** as a white powder (110 mg, 37%). R_f 0.19 (hexane–ethyl acetate, 4:1); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 1718 (CO_2R), 1604 (Ar); $\lambda_{max}(Et_2O)/nm$ 251.4, 257.5, 263.5 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 1600, 1900, 1750); δ_H(400 MHz, $CDCl_3$) 0.67 (s, 12H, 18-Me), 0.92 (s, 12H, 19-Me), 0.94 (d, *J* 6.3, 12H, 21-Me), 3.67 (br s, 4H, 7βH), 3.86 (br s, 4H, 12βH), 4.54 (tt, *J* 4.3, 11.3, 4H, 3βH), 4.49–4.84 (4 × ABq, 32H, ArCH_2O, OCH_2O), 7.25–7.37 (m, 20H, Ar-H); δ_C(100 MHz, $CDCl_3$) 12.53 (18-Me), 18.03 (21-Me), 22.79 (19-Me), 23.51 (15-CH_2), 25.25 (16-CH_2), 27.07 (11-CH_2), 27.62 (9-CH), 31.01, 31.30, 31.60 (2-CH_2, 10-Cq, 22-CH_2, 23-CH_2), 34.86, 35.07, 35.17 (1-CH_2, 6-CH_2, 4-CH_2), 35.17 (20-CH), 39.69 (8-CH), 41.56 (5-CH), 42.10 (14-CH), 46.36 (17-CH), 46.36 (13-Cq), 69.96, 70.23 (ArCH_2O), 74.35 (3-CH), 75.12 (7-CH), 79.99 (12-CH), 94.08, 94.22 (OCH_2O), 127.55, 127.62, 128.41 (Ar *o*-, *m*-, *p*-CHs), 138.09 (Ar-*C*–CH_2O), 174.01 (24-Cq); *m*/*z* (FIB) 2544 $[M + Na]^+$, 2453 $[M - Bn + Na]^+$, 2423 $[M - BnOCH_2 + Na]^+$, 2407 $[M - BnOCH_2O + Na]^+$.

Preparation of dicyclohexyl-18-crown-6-potassium methoxide complex in toluene [38]

Dicyclohexyl-18-crown-6 (186 mg, 0.5 mmol) was dissolved in dry toluene (3 ml) and evaporated to dryness under reduced pressure and then placed under high vacuum for 10 min. Next, methanolic potassium methoxide solution (0.50 ml, 1.05 M, 0.52 mmol, freshly prepared by addition of potassium metal to dry methanol) was added and the crown ether dissolved. Toluene (1 ml) was then added and the volume was reduced to *ca.* 0.5 ml under reduced pressure. A further portion of toluene (1 ml) was then added and again evaporated to *ca.* 0.5 ml under reduced pressure. This process was repeated one further time to yield a dark yellow solution, with a small amount of precipitate on the walls of the flask. A final portion of toluene was then added and the whole solution was filtered under an inert atmosphere, to leave a light yellow solution. Water (1 ml) was added to a portion of this solution (0.5 ml) and the mixture was titrated with dilute hydrochloric acid (0.03 M, 1.0 ml) using phenolphthalein as indicator. The mixture was found to be 0.06 M.

Cyclisation protocol

'MEM' monomer **10** (15 mg, 25.0 μmol) was dissolved in dry toluene (5 ml) and refluxed through activated 4 Å molecular

Table 5 Solvent conditions employed in the HPLC separation of cyclocholate oligomers and the retention times of the various macrocyclic products

Molecules	Hexane–propan-2-ol ratio	Wavelengths monitored/nm	Dimer t_r/min	Trimer t_r/min	Tetramer t_r/min	Pentamer t_r/min
'MEM' cholates	80:20	211,215,258	—	4.10	5.53	7.42
'Bis(*p*-methoxybenzyl)' cholates	95:5	215,227,275	—	3.97	5.35	6.85
'Deoxy' cholates	98:2–92.8 in 12 min	215,226,252	2.96	3.11	4.16	—

sieves in the apparatus shown in Fig. 5. After the mixture had been refluxing for 30 min, potassium methoxide–dicyclohexyl-18-crown-6 complex in toluene (17.5 μl, 0.018 M, 0.32 μmol) was injected. Portions of reaction mixture (*ca*. 0.2 ml) were removed by syringe at intervals and injected into pH 7 buffer (1 ml) and diethyl ether (1 ml). After removal of the aqueous layer, the products were washed with water, dried with $MgSO_4$ and the solvent evaporated under a stream of argon. Samples were analysed by HPLC and TLC.

This procedure was followed for all the cyclisation reactions described, including those at different concentrations and those with templates present. When templates were used, there was always one equivalent of the metal ion per monomer unit: LiI (3.4 mg, 25.2 μmol), NaI (3.8 mg, 25.2 μmol), KI (4.2 mg, 25.2 μmol) and CsI (6.6 mg, 25.2 μmol).

Crystal structure determination for 10

$C_{36}H_{56}O_7$, $M_r = 600.81$, monoclinic, space group $P2_1$ (no. 4), $a = 13.377(4)$, $b = 9.278(2)$, $c = 13.856(3)$ Å, $\beta = 96.70(2)°$, $V = 1707.9(7)$ Å^3, $T = 293(2)$ K, $D_c = 1.168$ Mg m^{-3}, $Z = 2$, $F(000) = 656$, Cu-Kα radiation, $\lambda = 1.541\,80$ Å, μ(Cu-Kα) = 0.632 mm^{-1}. Crystal dimensions 0.50 × 0.30 × 0.20 mm.

2913 Reflections were recorded on a Rigaku AFC7R diffractometer in the range $3.21 < \theta < 60.05°$, and averaged to give 2723 reflections ($R_{int} = 0.0493$). The structure was solved by direct methods (SHELXS-86: TREF) and refined by full-matrix least-squares based on F^2 (SHELXL-93). H-atoms were placed in idealised positions and allowed to ride on the relevant heavy atom with independent isotropic vibrational parameters. $R_1 = 0.0753$ and $wR_2 = 0.1971$ for reflections with $I > 2\sigma(I)$ and $R_1 = 0.0801$ and $wR_2 = 0.2046$ for all data.

HPLC conditions

All of the HPLC analyses reported were carried out using a Hewlett Packard 1050 system with a 25 cm × 4 mm Spherisorb S5W normal phase column with simultaneous detection at several wavelengths by a Hewlett Packard HP 1050 Diode Array UV detector. The solvent flow rate was always 1 ml min^{-1} and 10 μl of sample was injected. The solvent mixture was varied depending on the molecules being analysed. The solvent conditions employed and the oligomer retention times using this system are given in Table 5.

Each experiment was repeated at least twice with a minimum of four samples being taken. Each sample was analysed at three different wavelengths. As the extinction coefficient per monomer unit in a given product is constant, the ratios of the HPLC integrals is equal to the mass ratios.

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