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Cholic Acid as an Architectural Component in Biomimetic/Molecular Recognition Chemistry; Synthesis of the First "Cholaphanes".

Richard P. Bonar-Law and Anthony P. Davis*

Department of Chemistry, Trinity College, Dublin 2, Ireland

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Abstract *The readily available steroid cholic acid* (**1**) *has several advantages as a precursor for extended, preorganised molecular frameworks In the present work, full details are given for the conversion of* **1** *into "cholaphanes"* **2a,b**, *the first macrocyclic steroid derivatives intended for use in molecular recognition chemistry Diacetoxyketone* **11** *was prepared from methyl cholate* **8** *and treated with an arylmanganese reagent to give, after elimination of water and steroselective hydrogenation, monomer unit* **13** **2a** *was obtained by cyclodimerisation (up to 40% overall yield from* **8**) *and* **2b** *by subsequent deacetylation*

Introduction

There is growing interest in the construction of extended molecular frameworks with preorganised, usually convergent functionality, for applications in the fields of biomimetic and molecular recognition chemistry In this work there is an obvious need for rigid construction modules which can impose a high degree of organisation and predictability on the overall assembly The area has been dominated by the benzene ring, which may be interlinked in a wide variety of fashions,[1] but applications have also been found for aromatic heterocycles,[1c 1f,1j 2] alkynes,[1j,2g] carbohydrates,[3] and other saturated 6-membered rings [2a,4] In surveying this field a few years ago, we felt that one unit which might have been under-exploited was the steroid nucleus [5] Aside from having the necessary rigidity, it is relatively large so that, for example, it readily lends itself to incorporation in macrocycles able to encapsulate smaller molecules (*vide infra*) It is chiral (and available, of course, as a single enantiomer) and is of low symmetry, such that each proton and carbon can be distinguished (both for synthetic and spectroscopic purposes) Although it cannot provide the NMR shielding/deshielding effects which are valuable features of aromatic systems, it compensates by allowing

two orientations for a substituent at each carbon Finally, the importance of steroids in medicinal chemistry has resulted in an extensive background of relevant synthetic chemical information

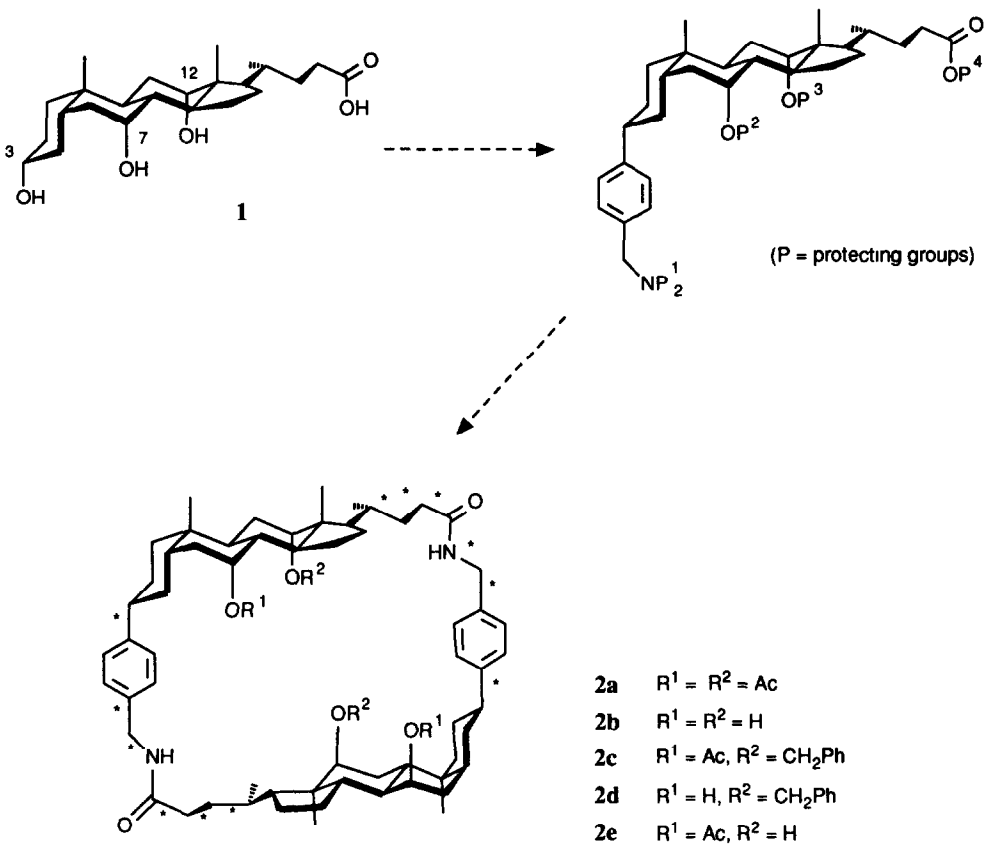
In order to realise the potential of the steroid nucleus, in biomimetic/molecular recognition chemistry, it was clear that we would need to (a) identify an inexpensive starting material with a sufficient degree of functionalisation to permit both its incorporation in larger frameworks and the attachment of "active" functionality, and (b) develop selective methods for elaborating the starting material in a controlled fashion In respect of the former, the 5β steroid cholic acid seemed especially promising It is produced on a large scale from cow bile and is very reasonably priced,[6] it has a useful degree of functionalisation which is evenly spaced around its periphery, and the 5β configuration gives it a curved profile which makes it a good starting point for frameworks with concave or toroidal surfaces Although three of the functional groups are chemically similar (in being secondary hydroxyls) the asymmetric nature of the steroid nucleus allows them to be distinguished The C3-OH, being equatorial, may be picked out by a variety of methods, and the two axial hydroxyls at C7 and C12 can be differentiated by selective acylations [7]

As a demonstration of our general idea, we decided to design and construct a macrocyclic framework in which two steroidal units would be connected head-to-tail *via* the (ubiquitous) benzene spacer For ease of synthesis we chose to retain the natural side chain of the cholic acid, but we felt it would be worthwhile making a direct connection between the aromatic ring and the steroid so as to limit the conformational options of the product An obvious tactic was to replace the 3α-OH with a *p*-(aminomethyl)phenyl unit, then cyclodimerise the resulting monomers by amide bond formation to give "cholaphanes" **2** (Scheme 1) Corey-Pauling-Koltun (CPK) molecular models suggested that these macrocycles would have a fair degree of flexibility due to rotation about the starred bonds, but that provided they chose open conformations they would surround a cavities of *ca* 40-50 Å^2 cross sectional area (sufficient to enclose a range of small-medium sized organic molecules) While it would be possible to utilise the 7,12α-OH's in a number of ways, we took the simplest option of protecting them so that they could be revealed in the final product Host molecules with preorganised, convergent hydroxyl groups are rare,[8] and we expected that **2** (R^1 and/or R^2 = H) would have interesting properties

In this and the following two papers,[9] we present full details of the synthesis of cholaphanes **2a-e**, and of an investigation of the solution structure of **2a** by NMR and molecular mechanics [10-12] Subsequent work has demonstrated that **2b** and **2d** do indeed have direct interest in molecular recognition chemistry, in that they are able to complex carbohydrate derivatives by H-bonding in a non-polar medium [13]

Results and Discussion

Introduction of Aryl Spacer Group The design of **2** was partly influenced by our realisation that the necessary transformation at C3 of the steroidal nucleus could probably be accomplished with reasonable ease As indicated in Scheme 2, it seemed likely that the stereochemistry of the aryl substituent could be controlled *via* catalytic hydrogenation of intermediate 2,3- and/or 3,4-alkenes Although such reactions are not especially stereoselective in simple monocyclic analogues,[14] the curved profile of projected intermediates **3** seemed almost certain to promote attack on the β-face Alkenes **3** should be accessible from 3-ketocholanes **4** *via* addition of an organometallic reagent **5** followed by elimination of the resulting alcohol(s) However, the involvement of an organometallic reagent in Scheme 2 raised two issues Firstly, it would be convenient to protect the carboxyl group of **1** by simple esterification, and also to be able to use acetyl protection on the 7

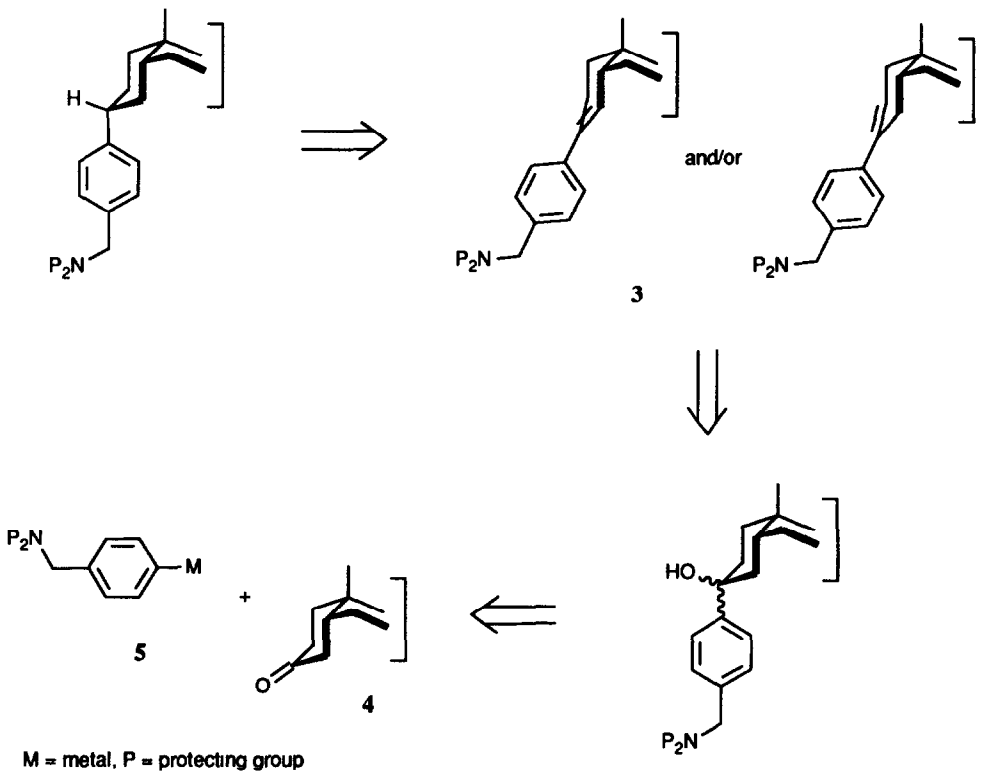


Scheme 1

and/or 12 hydroxyls (in particular, this would facilitate differentiation of the two positions) [7,9b] Thus a reagent which would add to ketones and not esters would be very advantageous Secondly, we would need to find an *N*-protecting group which would be compatible with the reagent

With regard to the former, we were directed towards organomanganese reagents by reports which indicated that they add smoothly to ketones at room temperature, but are essentially inert towards most esters [15,16] A number of preliminary experiments confirmed that arylmanganese reagents did indeed react in good yield with the ketone carbonyl of methyl 3-oxocholanoates without affecting the ester group Of the several variants on the method,[15c] the best results were given by arylmanganese iodides, derived from aryllithiums and manganous iodide, freshly prepared from iodine and manganese metal, with ether as solvent As expected, the product alcohols were quite sensitive to acidic conditions, readily eliminating water to give mixtures of regioisomeric Δ^2 and Δ^3 alkenes

Turning to the *N*-protecting moiety P_2 in reagent **5**, it would clearly need to be stable to (and incapable

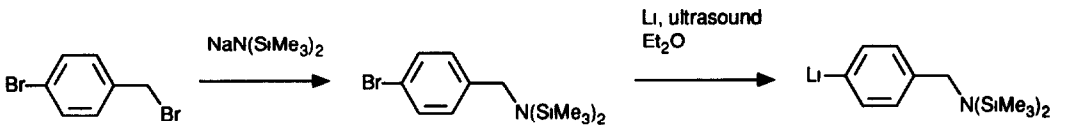


Scheme 2

of quenching) an organolithium reagent. On searching the literature we discovered that the options were rather few, the choice being between (a) silicon-based protecting groups which were unstable to acid and would not survive the work-up,[17] (b) incorporation of the N in a 2,5-dimethylpyrrole ring, which gives a unit with useful stability and physical properties, but requires vigorous conditions for reversal,[18] and (c) alkylation-dealkylation procedures which were unsuitable for various reasons [19] The best alternative appeared to be (a); although a silicon-based group would only afford temporary protection, it was hoped that a method could be found for separating the resulting amine from the manganese residues For reasons of cost and convenience we chose to use bis-trimethylsilyl[20] rather than the more specialised STABASE[17] group

Accordingly, the protected *p*-bromobenzylamine **6** was prepared from *p*-bromobenzyl bromide and sodium hexamethyldisilazide (Scheme 3).[21] **6** was found to be rather unstable but, provided it was freshly distilled, could be induced to react with lithium under the influence of sonication to give aryllithium **7** Model experiments with simple ketones confirmed the activity of **7**, and of the derived arylmanganese iodide

For our first "cholaphane" we chose the 7,12-diacetoxy ketone **11**[22] as the addition substrate Two methods were used for the preparation of **11** In the first, the equatorial 3α-OH group in methyl cholate **8** was selectively oxidised with $AgCO_3$/celite,[23] and the resulting ketone diacetylated In the second, shown in



6 7

Scheme 3

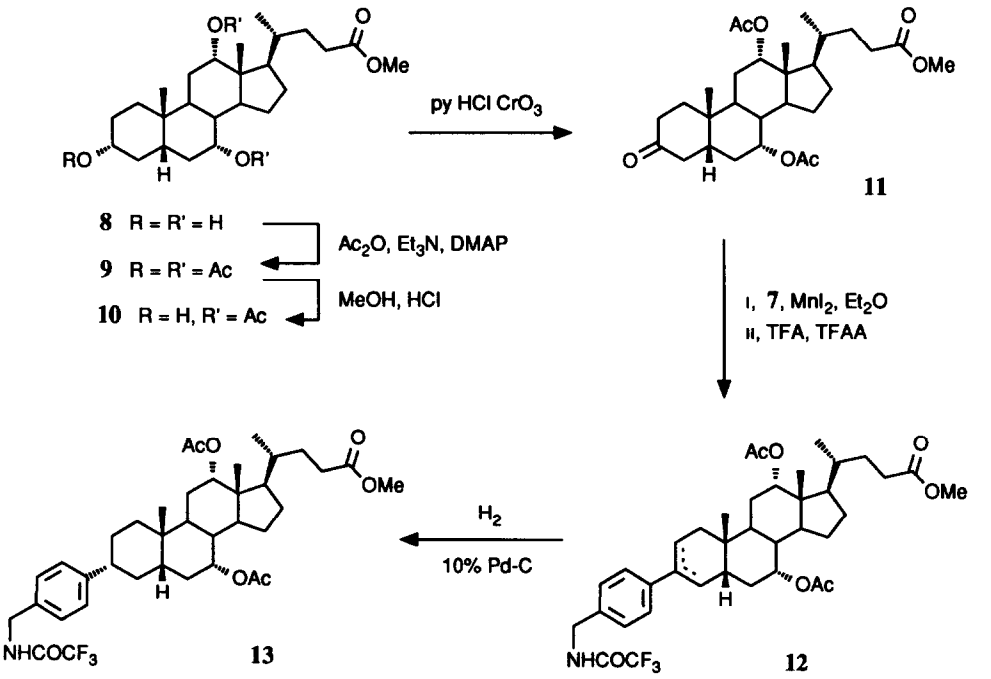
Scheme 4, **8** was triacetylated,[24] selectively deacetylated at position 3 with acidic methanol,[25] then oxidised with pyridinium chlorochromate Although less direct, the latter was very high yielding (95% overall) and the more convenient for large scale operation [26]

As shown in Scheme 4, treatment of **11** with the arylmanganese iodide derived from **7** in ether (room temperature, overnight) resulted, as expected, in clean attack at the 3-keto carbonyl We were pleased to find that addition of trifluoroacetic acid (TFA) to the reaction mixture, followed by trifluoroacetic anhydride (TFAA), caused elimination of water, *N*-desilylation and *N*-trifluoroacetylation *in situ* The resulting mixture of regioisomeric alkenes **12** was found to be unstable to prolonged storage and was generally hydrogenated immediately over 10% palladium on carbon As anticipated, the hydrogen was delivered to the β-faces of the double bonds[27] resulting in the α-arylated product **13** in 74% overall yield from **11** The product **13** was usually contaminated by a trace of biphenyl **14**, which had similar chromatographic properties to both **12** and **13**, but this was easily removed during subsequent steps

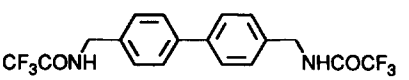
From Monomer to Cyclodimer; Cholaphanes 2a and 2b We felt that, in the first instance, it would be desirable to perform the cyclodimerisation of monomer unit **13** in a stepwise fashion Aside from the fact that a cleaner reaction might result, this would demonstrate the possibility of synthesising non-C_2-symmetric cholaphanes in a controlled fashion from two different precursors As shown in Scheme 5, **13** was (a) selectively deprotected at the amino terminus by treatment with aqueous ammonia, giving **15**, and (b) deprotected at both ends with base to give **16**, then reprotected at the amino terminus with t-butyl pyrocarbonate to give **17**

For the coupling of **15** and **17** we screened a variety of methods for amide bond formation The following reagents, which all gave excellent yields, were found to react at rates which increased in the order listed, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ),[28] dicyclohexylcarbodiimide (DCC) with *N*-hydroxybenzotriazole additive,[29] diphenylphosphoryl azide (DPPA),[30] and diethyl phosphorocyanidate (DEPC) [31] The latter reagent was thus the most convenient, and was also indicated for the macrocyclisations (where, given the high dilution required, reactivity would be at a premium)

When treated with DEPC and triethylamine in dichloromethane, **15** and **17** reacted to give the linear dimer **18** in 82% yield (Scheme 5) Deprotection of the acid (LiOH/THF/H_2O) then the amino group (TFA/$CHCl_3$) gave amino-acid **20** in 76% overall yield Cyclisation of **20** was successfully accomplished by treating a 3 5 mM solution in $CHCl_3$ with DEPC in the presence of solid K_2HPO_4 The product **2a** was isolated in 49% yield after chromatography and crystallisation from chloroform/methanol (the remaining material, apparently polymeric, forming a slow-moving band on chromatography) The crystals were quite



Scheme 4

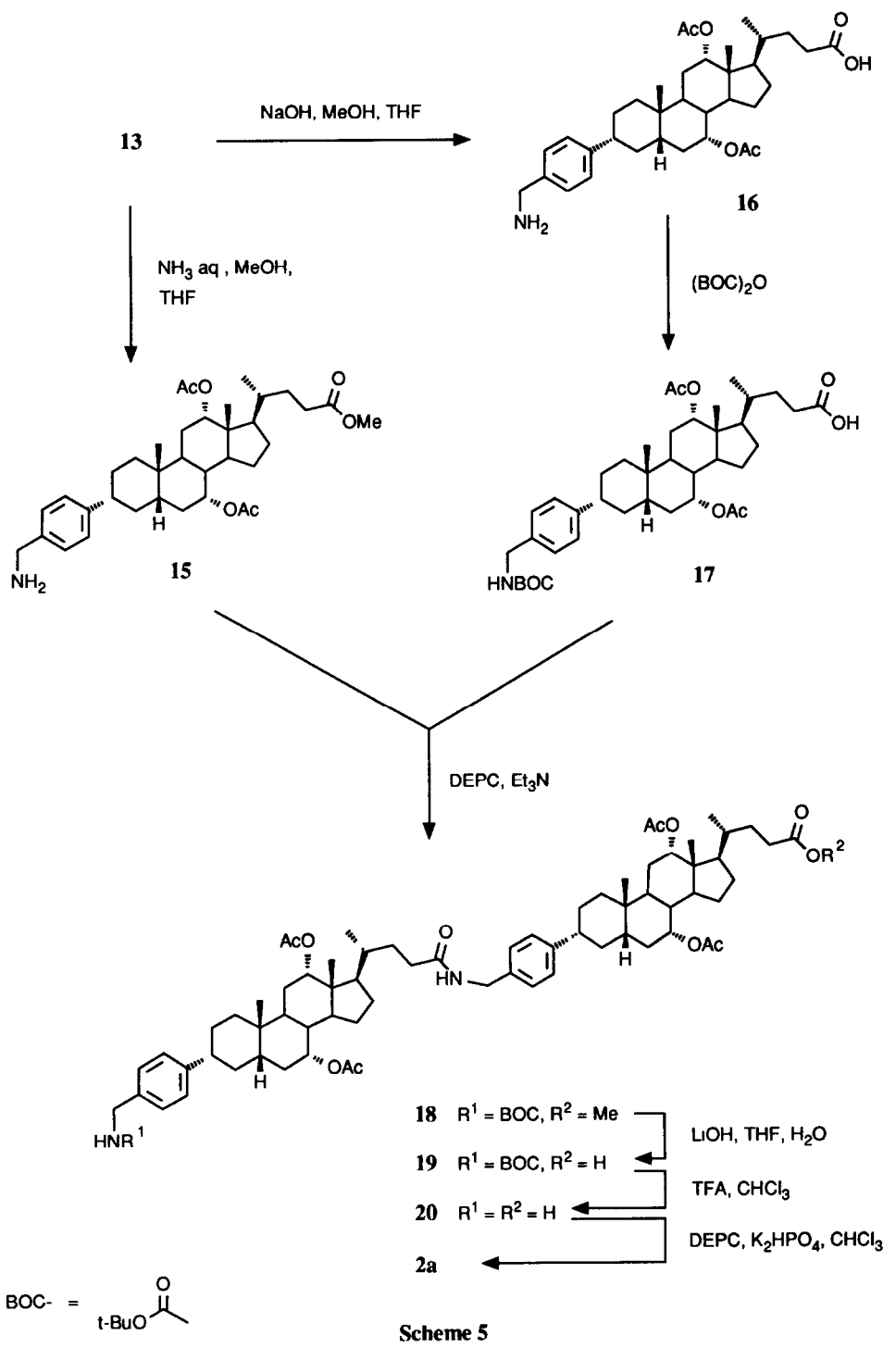


14

heavily solvated, becoming opaque on exposure to the atmosphere and retaining chloroform even after prolonged heating under a vacuum [32] Macrocycle **2a** was characterised by a full ^{1}H and ^{13}C NMR analysis (see following paper), mass spectrum (peaks at 1007, 947 and 887 corresponding to the loss of 2, 3 and 4 x CH_3CO_2H),[33] IR and microanalysis

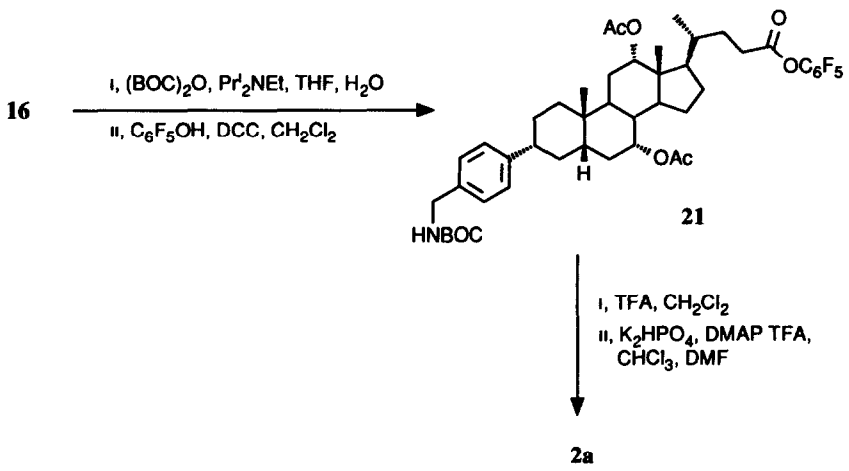
It is worth noting the use of an insoluble inorganic base to quench the acidic side-products formed during macrocyclisation Triethylamine gave poorer results It had previously been shown that $NaHCO_3$[34] and KH_2PO_4[35] are effective for cyclopeptide formation using DPPA In the present case the more basic K_2HPO_4 was required, possibly because of the lower acidity of HCN compared to HN_3

Having obtained **2a** *via* the stepwise route, we now explored the possibility of a direct cyclodimerisation of amino-acid **16** Treatment of **16** with DEPC under a range of conditions did indeed give **2a** in moderate yields, the best result (32% after crystallisation) being obtained from a 10 mM solution of **16** in $CHCl_3$-DMF (80 20) in the presence of K_2HPO_4 Although this procedure was quite convenient, an improved yield was clearly desirable The cyclisation of pentafluorophenyl amino acid esters had been


13
NaOH, MeOH, THF
16
NH3 aq , MeOH,
THF
(BOC)2O
15
17
DEPC, Et3N
18 R1 = BOC, R2 = Me
19 R1 = BOC, R2 = H
20 R1 = R2 = H
2a
LiOH, THF, H2O
TFA, CHCl3
DEPC, K2HPO4, CHCl3
BOC- =
t-BuO

Scheme 5

shown to be exceptionally effective in cyclopeptide synthesis[36] Accordingly **16** was converted into the *N*-BOC pentafluorophenyl (PFP) ester **21** (92% yield) as shown in Scheme 6 Treatment of **21** with TFA gave the corresponding alkylammonium trifluoroacetate We were pleased to find that a 1 4 mM solution of this salt in $CHCl_3$-DMF (86 14), on treatment with K_2HPO_4 and 4-dimethylaminopyridinium trifluoroacetate (DMAP TFA), gave **2a** in 65% crystalline yield



Scheme 6

Finally, in order to generate a cholaphane with active, inward-directed functionality, we needed to remove the *O*-acetyl protecting groups in **2a** Although they proved to be quite resilient, they could be hydrolysed cleanly by treatment with sodium hydroxide in THF-MeOH-H_2O (40°C for 24 h), allowing tetraol **2b** to be isolated in 90% crystalline yield Its structure was confirmed by the normal methods, and also by reacetylation to give **2a** using Ac_2O/pyridine/DMAP

While the synthesis of these first cholaphanes was not a trivial task, the net result was a process of quite satisfying efficiency Thus, considering the optimum procedures reported herein, the overall yield of cholaphane **2a** from methyl cholate **8** may be calculated to be 40% Incorporation of modifications developed for the synthesis of **2c-e**[9b] would probably raise this even further Given such effective synthetic methodology, it is reasonable to view the cholaphane framework as a starting point for more elaborate structures with various potential applications in the areas of biomimetic and molecular recognition chemistry

Experimental Section

General ^1H NMR spectra were recorded on Bruker WP 80 (80 MHz), Jeol JNM 270 (270 MHz) or Bruker MSL 300 (300 MHz) spectrometers TMS was used as the internal standard IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer Analytical thin layer chromatography (TLC) was performed on aluminium sheets coated with silica gel 60 (0.2 mm layer thickness) Steroidal compounds were visualised by charring over a flame Silica gel 60, 400-230 mesh (Merck) was used for flash

chromatography Reactions involving sonication were performed in a B & T laboratory ultrasonic cleaning bath Solvents were purified by standard procedures [37] For high-dilution reactions, dry DMF was obtained by standing over powdered BaO for one week, followed by fractional distillation at reduced pressure onto 4Å molecular sieves $CHCl_3$ was thoroughly washed with water to remove ethanol, then distilled from P_2O_5, eluting the distillate through a column of activated neutral alumina onto 4Å molecular sieves MnI_2 was obtained from ground Mn flakes and I_2 according to the published procedure[15c] as a cream, sometimes pinkish hygroscopic solid, which was stored in a dessiccator in the dark It was used within one week of preparation or made *in situ* for large-scale reactions

Methyl 7α,12α-*Diacetoxy*-3-*oxo*-*cholan*-24-*oate* (**11**) Methyl cholate (**8**)[7a] (52 g, 123 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (4 51 g, 37 mmol) were dissolved in dry dichloromethane (300 ml) A mixture of acetic anhydride (50 3 g, 493 mmol) and triethylamine (49 8 g, 493 mmol) was added dropwise with stirring Stirring was continued overnight, after which analysis by T L C indicated that the acetylation was complete Methanol (10 ml) was added to decompose excess acetic anhydride The mixture was concentrated by evaporation and partitioned between ether and 1M aqueous HCl The organic phase was washed with aqueous sodium hydrogen carbonate and brine, dried ($MgSO_4$) and evaporated to give methyl 3,7,12-triacetoxycholan-24-oate (**9**) as an oil (67 1 g) This material was dissolved in dry methanol (300 ml) and cooled in ice A solution of acetyl chloride (20 ml) in methanol (30 ml) was added dropwise with stirring over 1 h [25] The reaction vessel was removed from the ice and stirring was continued for 2 h The solvent was evaporated to give methyl 7α,12α-diacetoxy-3-hydroxycholan-24-oate (**10**) as an oil (61 g) A portion of this material (10 g, *ca* 19 7 mmol) was dissolved in dry dichloromethane (5 ml) and the solution added with stirring to a suspension of pyridinium chlorochromate (6 46 g, 30 mmol) in dichloromethane (40 ml), under a nitrogen atmosphere in a flask fitted with a condenser An exothermic reaction was observed The mixture was stirred for a total of 90 min, after which dry ether (40 ml) was added After a further hour, the supernatant liquor was removed and filtered through a short plug of silica gel The insoluble residue was washed with ether (3 x 40 ml), each extract being passed through the silica gel plug Silica gel (2 g) and dichloromethane (20 ml) were added to the residue After swirling the mixture, ether (20 ml) was added and the supernatant liquor was passed through the silica gel plug The plug was eluted with ether-dichloromethane (1 1, 20 ml) and the combined extracts were evaporated The residue was dissolved in a minimum volume of dichloromethane (*ca* 10 ml) and ether (40 ml) was added Crystallisation occurred to give **11**[22] (9 75 g, 95% overall from **8**) ^{1}H NMR (300 MHz, $CDCl_3$) δ 5 13 (br m, 1 H, 12β-H), 5 00 (br m, 1 H, 7β-H) 3 67 (s, 3 H, OMe), 2 99 (dd, *J* = 13 6, 15 2 Hz, 1 H, 4β-H), 2 12 (s, 3 H, OAc), 2 07 (s, 3 H, OAc), 1 02 (s, 3 H, 19-Me), 0 82 (d, *J* = 6 4 Hz, 3 H, 21-Me), 0 77 (s, 3 H, 18-Me)

p-*Bromo*-N,N-*bis*(*trimethylsilyl*)*benzylamine* (**6**) Hexamethyldisilazane (200 ml) was added to a suspension of sodium amide (20 g, 0 51 mol) in dry benzene (200 ml), and the mixture refluxed with efficient stirring for 9 h, under a slow flow of N_2 The benzene was removed by distillation through a fractionating column, hexamethyldisilazane (100 ml, distilled) was added to the hot residue, and the mixture was cooled to room temperature with maintenance of vigorous stirring Powdered *p*-bromobenzyl bromide (50 g, 0 2 mol) was added under a flow of N_2, and the pale-yellow suspension stirred for 2 d Dry hexane (500 ml) was added, and after standing for 12 h the supernatant liquid was decanted This process was repeated, the hexane extracts were combined and concentrated at reduced pressure, the residue was decanted from some yellow

precipitate, and excess hexamethyldisilazane was recovered by distillation at 12 mmHg (b p < 60 °C) The residual oil was distilled to give **6** as a pale yellow oil (50 43 g, 76%) b p 103-106 °C at 0 3 mmHg, IR (liq film) 1480, 1400, 1250, 1170, 1125, 1110 cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 39, 7 14 (ABq, J = 8 8 Hz, 4 H, ArH), 4 04 (br s, 2 H, CH_2) 0 08 (s, 18 H, 2 x $SiMe_3$) The decantation step in the workup may be replaced by filtration through a glass frit

p-*Lithio*-N,N-*bis(trimethylsilyl)benzylamine* (**7**) Bromide **6** (7 0 g, 0 021 mol) in Et_2O (50 ml) was added dropwise to freshly extruded lithium wire (0 7 g, 0 1 mmol) in dry Et_2O (15 ml) over 2 h During the addition the reaction flask was immersed in an ultrasonic bath, maintaining a gentle reflux The reaction mixture was further sonicated for 2 h Hydrolysis of a portion with aqueous acid, and back titration with aqueous base (methyl orange indicator; making allowance for amine protonation) indicated that the concentration of **7** was 0 3 M (81% conversion)

Methyl 7α,12α-*Diacetoxy*-3α-*[p-(trifluoroacetylaminomethyl)phenyl]-cholan*-24-*oate* (**13**) Organolithium **7** (0 52 M solution in Et_2O, 70 ml, 36 4 mmol) was added to a stirred suspension of MnI_2 (20 g, 66 mmol) in Et_2O (100 ml) at 0 °C, the ice-bath removed, and the dark-green suspension stirred for 30 min at room temperature After recooling to 0 °C, ketone **11** (7 5 g, 14 88 mmol) in CH_2Cl_2 (25 ml) was added in a slow stream with rapid stirring The mixture was sonicated for 15 min, and the brown suspension stirred overnight After cooling to 0 °C, TFA (8 5 ml, 110 mmol) was added dropwise, followed after 5 min by TFAA (30 ml) and CH_2Cl_2 (40 ml) After stirring for 5 h at room temperature, volatiles were removed by evaporation under reduced pressure, and a solution of the residue in CH_2Cl_2 was washed with 2 M aqueous HCl then 5% aqueous $NaHCO_3$, dried and evaporated The residue was chromatographed in batches using hexane-EtOAc (2 1) as eluant to give crude alkenes **12** as a foam (9 2 g in total) ^1H NMR (80 MHz, $CDCl_3$) δ 7 35, 7 23 (ABq, J = 8 5 Hz, 4 H, ArH), 6 65 (br, 1 H, NH), 5 95 and 5 82 (2 x br s, 0 4 H and 0 6 H, alkene 2-H and 4-H), 5 08 and 4 86 (2 x overlapping br s, 2 H, 12β-H and 7β-H), 4 51 (d, J = 6 Hz, 2 H, $NHCH_2$) 3 64 (s, 3 H, OMe), 2 03 (s, 3 H, OAc), 1 75 (s, 3 H, OAc), 1 02 (s, 3 H, 19-Me), 0 75 (s, 3 H, 18-Me) Biphenyl **14** was generally present as a minor contaminant ^1H NMR (300 MHz, $CDCl_3$) 7 59, 7 39 (ABq, J = 8 Hz, 8 H, ArH), 6 61 (br s, 2 H, NH), 4 59 (d, J = 6 Hz, 4 H, CH_2) The batches of **12** were hydrogenated immediately after chromatography using the general procedure **12** and 10% Pd-C (100 mg per gram of alkene) in EtOAc (15 ml per gram of alkene) was stirred under H_2 until gas absorption ceased (2-4 h) The suspension was filtered through a pad of silica gel, eluting with EtOAc, and solvent was evaporated under reduced pressure The combined crude hydrogenation products were stirred with hexane-CH_2Cl_2 (50 ml), undissolved biphenyl **14** was removed by filtration and the filtrate evaporated to a foam (7 9 g total) Slow crystallization from hexane-CH_2Cl_2 afforded **13** as prisms (1st crop 4 79 g, m p 197-200 °C, 2nd crop 2 94 g, m p 192-197 °C, total 74% based on **11**) ^1H NMR analysis indicated that the 2nd crop contained traces of biphenyl **14** (< 5%), the crude material was generally used directly in subsequent steps An analytical sample was obtained by crystallisation from hexane-CH_2Cl_2 m p 200-202 °C, $[\alpha]^{23}_D$ + 49° (c 1 0 in $CHCl_3$), IR (5% in $CHCl_3$) 3440 (NH), 1720 (br, C=O), 1600 cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 2 (br s, 4 H, ArH), 6 6 (br s, 1 H, NH), 5 1 (br t, 1 H, 12β-H), 4 92 (br m, 1 H, 7β-H), 4 49 (d, J = 5 5 Hz, 2 H, $NHCH_2$), 3 66 (s, 3 H, OMe), 2 11 (s, 3 H, OAc), 2 01 (s, 3 H, OAc), 0 97 (s, C-19 Me), 0 82 (d, J = 6 Hz, 3 H, 21-Me), 0 75 (s, 3 H, 18-Me) Anal Calcd for $C_{38}H_{52}NO_7F_3$ C, 65 97, H, 7 58, N, 2 03 Found C, 66 06, H, 7 66, N, 1 86

Methyl 3α-*(*p-*Aminomethyl)phenyl*-7α,12α-*diacetoxycholan*-24-*oate* (**15**) A solution of crude **13** (274 mg, 0 37 mmol, containing ~5% biphenyl **14**) in MeOH (2 5 ml), THF (3 ml) and conc aqueous NH_3 (35% w/w, 2 5 ml) was left for 2 5 d at room temperature in a stoppered flask Volatiles were removed by evaporation under reduced pressure, and the residue was partitioned between CH_2Cl_2 and 5% aqueous $NaHCO_3$ The aqueous layer was re-extracted with CH_2Cl_2, the combined organic layers dried, and the solvent evaporated Chromatography in CH_2Cl_2-MeOH (10 1) gave amine **15** as an oil (164 mg, 73%) IR (film from $CHCl_3$) 3200 (NH), 1720 (C=O) cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 31, 7 15 (ABq, *J* = 8 Hz, 4 H, ArH), 5 10 (br s, 1 H, 12β-H), 4 90 (br s, 1 H, 7β-H), 4 35 (br s, 2 H, NH_2), 3 89 (br s, 2 H, CH_2N), 3 66 (s, 3 H, OMe), 2 11 (s, 3 H, OAc), 2 10 (s, 3 H, OAc), 0 96 (s, 3 H, 19-Me), 0 80 (br d, *J* = 6 Hz, 3 H, 21-Me), 0 75 (s, 3 H, 18-Me) This material was used in subsequent experiments without further purification

3α-*(*p-*Aminomethylphenyl)*-7α,12α-*diacetoxycholan*-24-*oic acid* (**16**) A mixture of **13** (4 79 g, 6 93 mmol) and 1 08 M aqueous NaOH (20 ml, 21 6 mmol) in THF (30 ml) and MeOH (25 ml) was stirred for 5 h at room temperature, neutralized with 2 17 M aqueous HCl (6 75 ml, 14 65 mmol), and volatiles evaporated under reduced pressure Chromatography of the residue in $CHCl_3$-MeOH (7 3) grading to pure MeOH gave an amorphous solid which was dissolved in $CHCl_3$-MeOH (3 1, 20 ml), and filtered, washing with the same solvent mixture After evaporation of solvents the crude product was dissolved in MeOH (10 ml), and the stirred solution slowly diluted with H_2O (100 ml) The suspension was allowed to settle, the supernatant decanted, and the precipitate triturated with H_2O (30 ml), decanting the aqueous layer The remaining white solid was dried at 50 °C, 0 5 mmHg for 8 h, to give amino-acid **16** as a fine powder (3 56 g, 86%), m p 198-210 °C Processing the aqueous layers gave more amino-acid m p 195-208 °C (300 mg, combined yield ~95%) The above material was used for cyclodimerization reactions without further purification The chromatographic step could be omitted with little loss of purity An analytical sample was obtained by crystallization from DMF-H_2O m p 224-225 °C, IR ($CHCl_3$ mull) 3400 (br), 2650 (br), 2750 (br), 1720 (C=O) cm^{-1}, ^1H NMR (80 MHz, 10% CD_3OD in $CDCl_3$) δ 7 20 (br ABq, 4 H, ArH), 5 12 (br s, 1 H, 12β-H), 4 92 (br s, 1 H, 7β-H), 4 5-3 5 (br, 2 H, CH_2NH_2), 3 5-3 2 (br, CH_2NH_2 and CO_2H), 2 11 (s, 3 H, OAc), 2 01 (s, 3 H, OAc), 0 96 (s, 3 H, 19-Me), 0 82 (d, *J* = 6 Hz, 3 H, 21-Me), 0 76 (s, 3 H, 18-Me) Anal Calcd for $C_{35}H_{51}NO_6$ C, 72 25, H, 8 84, N, 2 41 Found C, 72 55, H, 8 87, N, 2 36

7α,12α-*Diacetoxy*-3α-*(*p-tert-*butoxycarbonylaminomethylphenyl)cholan*-24-*oic acid* (**17**) A mixture of crude **13** (338 mg, 0 44 mmol steroid, containing ~5% biphenyl **14**), 1 M aqueous LiOH (1 0 ml, 1 mmol), THF (5 ml) and H_2O (1 ml) was stirred at room temperature for 3 d Further 1 M aqueous LiOH (0 2 ml, 0 2 mmol) was added, and the mixture stirred for another 3 d, after which TLC in $CHCl_3$-MeOH (5 1) showed one major component The reaction mixture was neutralized with 2 M aqueous HCl, triethylamine (1 ml) was added, and the stirred homogenous solution was treated with di-*tert*-butyl pyrocarbonate (110 μl, 0 5 mmol) After 1 h at room temperature, the volatiles were removed by evaporation under reduced pressure A solution of the residue in CH_2Cl_2 was washed with 1 M aqueous H_3PO_4, then water, the organic layer was dried, and the solvent was evaporated Chromatography of the residue in CH_2Cl_2-MeOH (10 1) gave **17** as a foam (300 mg, 95%) IR (film from $CHCl_3$) 3520, 3450 (NH), 3500-2400 (br), 1700 (br, C=O) cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 21, 7 14 (ABq, *J* = 8 Hz, 4 H, ArH), 5 12 (br s, 1 H, 12β-H), 4 92 (br s, 2 H, 7β-H and NH), 4 28 (d, *J* = 6 Hz, 2 H, CH_2NH), 2 11 (s, 3 H, OAc), 2 01 (s, 3 H, OAc), 1 46 (s, 9 H, Bu^t), 0 97 (s, 3 H, 19-Me),

0 85 (br d, 3 H, 21-Me), 0 76 (s, 3 H, 18-Me)

Protected linear dimer **18** Diethyl phosphorocyanidate (DEPC) (50 μL, 0 33 mmol) was added to a stirred solution of acid **17** (200 mg, 0 293 mmol) and amine **15** (164 mg, 0 275 mmol) in dry CH_2Cl_2 (2 ml) containing triethylamine (85 μl, 0 61 mmol) at room temperature After 1 h, the reaction mixture was diluted with CH_2Cl_2, and washed with 1 M aqueous H_3PO_4 followed by 5% aqueous $NaHCO_3$, re-extracting the aqueous layers The combined organic layers were dried, and solvent removed by evaporation Chromatography of the residue in MeOH-$CHCl_3$ (2 98) afforded dimer **18** as a white solid (282 mg, 82% based on amine) IR ($CHCl_3$) 3450 (NH), 1710, 1670, 1600 cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 2 (m, 8 H, ArH), 5 68 (br t, J = 6 Hz, 1 H, $CH_2NHCOCH_2$), 5 1 (br s, 2 H, 12β-H), 4 9 (br s, 2 H, 7β-H), 4 81 (br s, 1 H, CH_2NHBOC), 4 39 (d, J = 6 Hz, 2 H, $CH_2NHCOCH_2$), 4 26 (d, J = 6 Hz, 2 H, CH_2NHBOC), 3 66 (s, 3 H, OMe), 2 1 (s, 6 H, 2 x OAc), 2 0 (s, 6 H, 2 x OAc), 1 46 (s, 9 H, Bu^t), 0 97 (br s, 6 H, 2 x 19-Me), 0 83 (br d, J = 6 Hz, 6 H, 2 x 21-Me), 0 75 (br s, 6 H, 2 x 18-Me) This material was contaminated by a small amount of a side product [^1H NMR δ 4 15 (q)], presumably derived from the DEPC However, it was used in the following experiment without further purification

Dimeric amino-acid **20** A mixture of protected linear dimer **18** (195 mg, 0 155 mmol) and 1 M aqueous LiOH (260 μL, 0 26 mmol) in THF (3 ml) and H_2O (0 5 ml) was stirred at room temperature for 12 h, then partitioned between $CHCl_3$ and 1 M aqueous H_3PO_4, re-extracting the aqueous layer with $CHCl_3$. The combined organic layers were dried and the solvent evaporated Chromatography of the residue in MeOH-$CHCl_3$ (2 98) afforded dimeric *N*-protected acid **19** (176 mg, 91%) as a white solid ^1H NMR (80 MHz, $CDCl_3$) δ 7 18 (m, 8 H, ArH), 5 72 (br t, J = 6 Hz, 1 H, $CH_2NHCOCH_2$), 5 11 (br s, 2 H, 2 x 12β-H), 4 92 (br s, 3 H, 2 x 7β-H and CH_2NHBOC), 4 40 (d, J = 6 Hz, 2 H, $CH_2NHCOCH_2$), 4 26 (d, J = 6 Hz, 2 H, CH_2NHBOC), 2 11 (s, 6 H, 2 x OAc), 2.01 (s, 6 H, 2 x OAc), 1 45 (s, 9 H, Bu^t), 0 97 (br s, 6 H, 2 x 19-Me), 0 85 (m, 6 H, 2 x 21-Me), 0 75 (br s, 6 H, 2 x 18-Me) TFA (0 5 ml) was added dropwise to a stirred solution of the above material (125 mg, 0 1 mmol) in dry $CHCl_3$ (2 ml) at 0 °C After standing for 1 h at room temperature, the solvent was evaporated under reduced pressure, the residue dissolved in MeOH (2 ml) and the mixture diluted with 1 M aqueous NaOH (50 ml) After neutralization with conc aqueous HCl the suspension was extracted with $CHCl_3$ (4 x 25 ml), the combined organic layers dried, and the solvent evaporated Chromatography of the residue in MeOH-$CHCl_3$ (5 95 → 20 80) afforded amino-acid **20** as a white solid (95 mg, 83%) IR ($CHCl_3$ mull) 3440, 3380, 3280 (NH), 1715 (C=O), 1650 cm^{-1}, ^1H NMR (80 MHz, 10% CD_3OD in $CDCl_3$) δ 7 3 - 7 1 (m, 8 H, ArH), 5 11 (br s, 2 H, 2 x 12β-H), 4 91 (br s, 2 H, 2 x 7β-H), 4 4 (s, 2 H, CH_2NH_2), 2 11 (s, 3 H, OAc), 2 10 (s, 3 H, OAc), 2 02 (s, 6 H, 2 x OAc), 0 97 (br s, 6 H, 2 x 19-Me), 0 83 (br m, 6 H, 2 x 21-Me), 0 75 (br s, 6 H, 2 x 19-Me)

Pentafluorophenyl 7α,12α-*Diacetoxy*-3α-(p-tert-*butoxycarbonylaminomethylphenyl*)-*cholan*-24-*oate* (**21**) A stirred solution of amino acid **16** (2 0 g, 3 27 mmol assuming 95% purity) in THF (20 ml), H_2O (10 ml) and di-isopropylethylamine (1 ml) was treated with di-*tert*-butyl pyrocarbonate (0 8 ml, 3 5 mmol) After 1 h the volatiles were removed by evaporation under reduced pressure and the residue partitioned between CH_2Cl_2 and 1 M aqueous H_3PO_4 The organic layer was washed with water, dried, and evaporated to leave crude *N*-protected acid **17** as a foam (2 30 g) A solution of the crude acid **17** (2 0 g, 2 94 mmol), dicyclohexylcarbodiimide (DCC) (700 mg, 3 4 mmol) and pentafluorophenol (675 mg, 3 66 mmol) in dry

CH_2Cl_2 (10 ml) was stirred for 12 h at room temperature The resulting suspension was filtered, the solid washed with CH_2Cl_2 (2 x 2 5 ml), and the filtrates evaporated under reduced pressure Chromatography of the residue in Et_2O-CH_2Cl_2 (2 98) gave pentafluorophenyl (PFP) ester **21** (2 286 g, 92% from **16**) as a colourless oil IR (liq film) 3460 (NH), 1775 (PFP ester C=O), 1705 (acetate C=O), 1600 cm^{-1}, ^1H NMR (80 MHz, $CDCl_3$) δ 7 17, 7 13 (ABq, J = 8 Hz, 4 H, ArH), 5 13 (br s, 1 H, 12β-H), 4 91 (br s, 1 H, 7β-H), 5 0 - 4 7 (br, 1 H, NH), 4 27 (d, J = 6 Hz, 2 H, CH_2NH)), 2 8 - 2 5 (m, 2 H), 2 12 (s, 3 H, OAc), 2 01 (s, 3 H, OAc), 1 49 (s, 9 H, Bu^t), 0.97 (s, 3 H, 19-Me), 0 78 (s, 3 H, 18-Me)

Macrocycle **2a** *by Cyclodimerisation of Amino-acid* **16** DEPC (0 3 ml, 1 98 mmol) was added to a stirred solution of amino-acid **16** (462 mg, 0 795 mmol) in dry $CHCl_3$ (60 ml) and dry DMF (23 ml) containing powdered, dried K_2HPO_4 (0 5 g, 2 87 mmol) After 7 d the resulting suspension was concentrated under reduced pressure (to 50 °C at 0 5 mmHg) The residue was partitioned between $CHCl_3$ and H_2O, re-extracting the aqueous layer with $CHCl_3$, the combined organic layers were dried, and the solvent was evaporated Chromatography in $CHCl_3$-EtOAc (3 2) gave a white solid (188 mg, R_f = 0 4 in column solvent) Slow crystallization from $CHCl_3$-MeOH (two crops) afforded **2a** as small prisms (150 7 mg, 32% assuming 0 5 $CHCl_3$ of crystallization, after drying for 2 h at 50 °C and 0 5 mmHg) A sample was prepared for analysis by further drying for 2 d at 80 °C, 0 5 mmHg m p > 300 °C, $[\alpha]_D^{23}$ + 87 6° (c = 1 0 in $CHCl_3$), MS, see text, IR (1% in $CHCl_3$) 3450 (NH), 1720 (ester C=O), 1660 (amide C=O), 1600 cm^{-1}, ^1H, ^{13}C NMR, see following paper in this journal Anal Calcd for $C_{70}H_{98}N_2O_{10}$ 0 5 $CHCl_3$ C, 71 32, H, 8 36 N, 2 36 Found C, 71 05, H, 8 63, N, 2 21

Macrocycle **2a** *by Cyclodimerization of Pentafluorophenyl Ester* **21** TFA (10 ml) was added to a stirred solution of PFP ester **21** (1 75 g, 2 06 mmol) in dry CH_2Cl_2 (10 ml) at 0 °C The ice-bath was removed, and after 1 h the volatiles were removed by evaporation under reduced pressure CCl_4 (10 ml) was added to the residue, and removed by evaporation under reduced pressure After repeating this procedure, the residue was dried for 2 h at 60 °C and 0 5 mmHg, and then dissolved in a mixture of dry $CHCl_3$ (1 25 L) and dry DMF (200 ml) To the stirred solution was added DMAP TFA (475 mg, 2 01 mmol) and dried, powdered K_2HPO_4 (1 75 g, 10 mmol) After 1 d more K_2HPO_4 (1 75 g) was added and the mixture stirred for 7 d The cloudy suspension was concentrated under reduced pressure (to 50 °C at 0 5 mmHg) and the residue partitioned between $CHCl_3$ and 1 M aqueous HCl The organic layer was washed with 5% aqueous $NaHCO_3$, dried, and the solvent evaporated to leave crude product (1 34 g) Crystallization from $CHCl_3$-MeOH gave **2a** as small prisms, (735 mg, after drying for 2 h at 50 °C and 0 5 mmHg) The mother liquor was chromatographed in $CHCl_3$-EtOAc (3 2) affording, after crystallization, a further 64 mg of **2a** (total 0 799 g, 65% assuming 0 5 x $CHCl_3$ of crystallization)

Macrocycle **2a** *- Cyclization of Dimeric Amino-acid* **20** DEPC (11 µl, 73 µmol) was added to a stirred suspension of dimeric amino-acid **20** (41 mg, 35 µmol) in dry $CHCl_3$ (10 ml) After 36 h, K_2HPO_4 (150 mg, 0 86 mmol) was added The mixture was stirred for 2 d, then diluted with $CHCl_3$ and washed with 1 M aqueous HCl followed by 5% aqueous $NaHCO_3$, reextracting the aqueous layers with $CHCl_3$ The combined organic layers were dried, and solvent evaporated Chromatography in $CHCl_3$-EtOAc (3 2) gave a white solid (25 mg) which was crystallized from $CHCl_3$-MeOH affording **2a** as small prisms (20 mg, 49% assuming 0 5 x $CHCl_3$ of crystallization, after drying for 2 h at 50 °C and 0 5 mmHg)

Macrocycle **2b** 1 M aqueous NaOH (10 ml) was added to a stirred solution of tetraacetate **2a** (343 mg, 0 289 mmol assuming 0 5 x $CHCl_3$ of crystallization) in THF (40 ml) and MeOH (10 ml), and the mixture heated at 40 °C for 24 h Organic solvents were removed by evaporation under reduced pressure, and the yellow suspension neutralized with conc aqueous HCl, and extracted with $CHCl_3$ (3 x 20 ml) The combined extracts were dried, and concentrated to ~10 ml Slow evaporation to ~2 5 ml afforded tetrahydroxy macrocycle **2b** as rafts of small prisms, (315 mg, 90% assuming 2 x $CHCl_3$ + 1 H_2O of crystallization, after drying for 2 h at 50 °C and 0 5 mmHg) m p > 300 °C, FAB MS 959 (MH^+), IR (nujol mull) 3670, 3610 ($CHCl_3$ of crystallization), 3350 (br), 1650 (amide C=O), 1550, 1515 cm^{-1}, ^1H NMR (270 MHz, ~0 5 mM in $CDCl_3$) δ 7 22, 7 16 (ABq, J = 8 24 Hz, 8 H, 2 x ArH), 5 78 (br t, 2 H, 2 x CH_2NH), 4 47,4 34 (*ABX*, J_{gem} = 15 Hz, J_{NH} = 5 and 6 Hz, 4 H, 2 x CH_2NH), 3 95 (br s, 2 H, 2 x 12β-H), 3 84 (brs, 2 H, 2 x 7β-H), 0 98 (br d, J = 6 Hz, 6 H, 2 x 21-Me), 0 95 (s, 6 H, 2 x 19-Me), 0 71 (s, 6 H, 2 x 18-Me). The analytical sample was prepared by drying for 2 d at 80 °C at 0 5 mmHg Anal Calcd for $C_{62}H_{90}N_2O_6$ 2 $CHCl_3$ H_2O C, 63 21, H, 7 79, N, 2 30 Found C, 63 43, H, 7 91, N, 2 13

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