Synthesis of Cyclo-bis[7α,12α-diacetoxy-3β-dicyanomethyl-3α-(4-methylenephenyl)cholanamide]; A Cholaphane with Reduced Flexibility and Externally-directed Functionality

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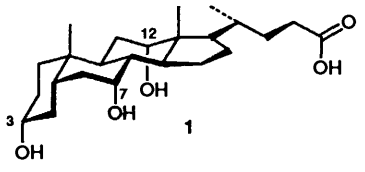
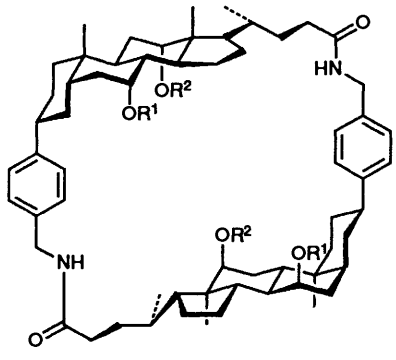
A second 'cholaphane' framework is manifested in the title compound **8**, which has been prepared from methyl 3α,7α,12α-triacetoxycholanoate **9** in 23% overall yield. The synthesis involves the Knoevenagel condensation of ketone **11** with malononitrile to give dicyanomethylene derivative **12**, equatorial-selective addition to the latter of an organocuprate derived from aryl bromide **15**, conversion of the resulting adduct into amino acid **22**, and cyclodimerisation. The framework of **8** has less conformational freedom and a better-defined cavity than the 'first-generation' cholaphanes **2**, and also bears externally-directed functionality.

During the past few years workers in this laboratory[1-3] and elsewhere[4] have been exploring the potential of cholic acid **1** as a starting material in biomimetic/molecular recognition chemistry. Among the factors which have stimulated this interest are (a) the low cost and ready availability of **1**, (b) its rigid steroidal skeleton, (c) its nicely spaced array of differentiable functionality, (d) its chirality and (e) its curved profile (facilitating the design of frameworks with concave or toroidal surfaces).

An early expression of the strategy was the design and synthesis of 'cholaphanes' **2**,[1] the first macrocyclic host





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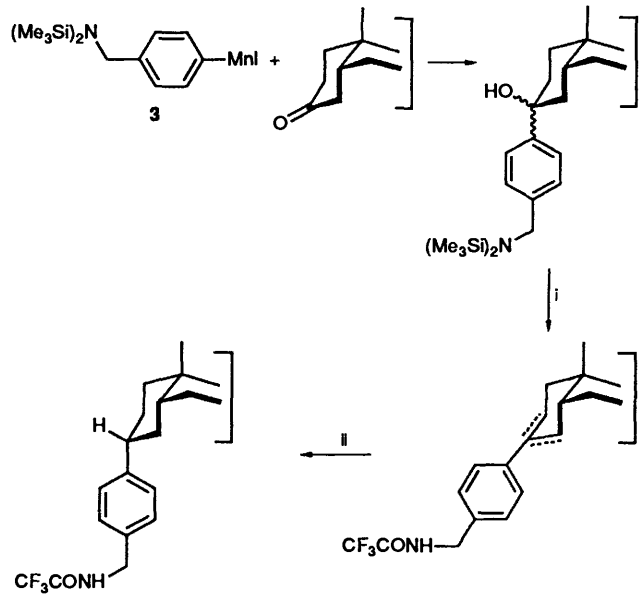
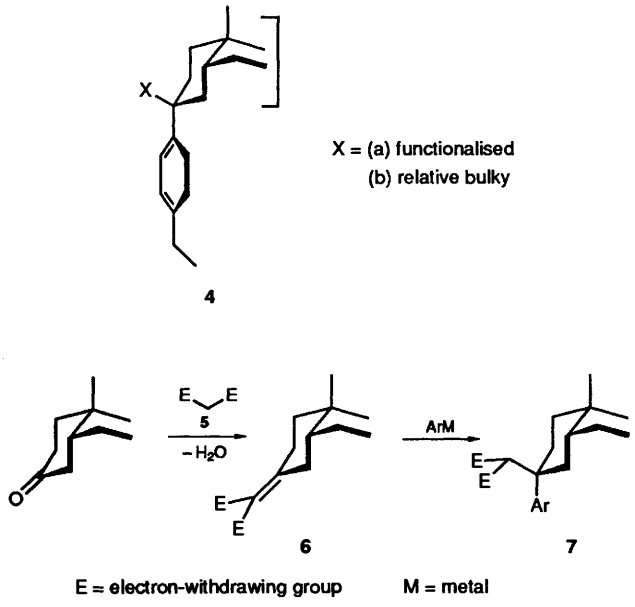
molecules based on cholic acid. Subsequent work demonstrated that cholaphanes **2** (R^1 = H, R^2 = H or CH_2Ph) are remarkable in acting as receptors for carbohydrate nuclei in organic solvents.[2] The key step in the synthesis of **2** from **1** was the introduction of a *p*-(aminomethyl)phenyl substituent in the 3α-position of the steroid nucleus. As shown in Scheme 1, this was achieved by the chemoselective addition of organomanganese reagent **3** to a 3-keto group followed by elimination and stereoselective hydrogenation from the (convex) β-face. Although successful, this sequence imposed a limitation on the final structure in that it precluded the possibility of a 3β-



Scheme 1 *Reagents and conditions:* i, CF_3CO_2H, $(CF_3CO)_2O$; ii, H_2, Pd–C

substituent. There were two reasons why the latter might be desirable. Firstly, it could provide *exo* directed functionality which could be used to assist solubilisation (either in water, by using ionic groups, or in organic solvents by attaching flexible hydrocarbon chains) or to attach the macrocycle to some other unit (*e.g.* a polymer). Secondly, a relatively bulky group would constrain the aromatic ring to take up a conformation roughly parallel to the C_2 axis of the macrocycle in contrast to the 'perpendicular' conformation favoured in cholaphanes **2** (*vide infra*). This would provide a better-defined cavity with an increased 'hydrocarbon surface', particularly important for the proposed move to water-soluble cholaphanes capable of hydrophobic binding. In addition, the loss of rotational freedom about the C(3)–aryl bond would somewhat reduce the overall flexibility of the cholaphane framework.

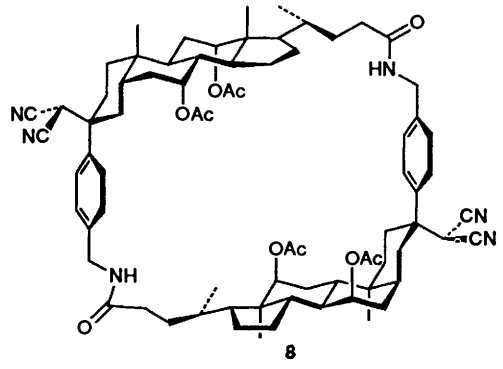
Our task was thus to identify a structural fragment **4** which could be developed from the 3-OH of cholic acid with the same chemo- and stereo-selectivity as had been obtained previously (Scheme 1). In particular, it would be convenient to protect the C-24 carboxy group of **1** as a methyl ester, so that reagents which would react with this group were to be avoided if possible. It seemed that an answer might lie in the sequence



Scheme 2

shown in Scheme 2. A Knoevenagel condensation[5] could be used to generate a highly electron-deficient alkene **6**, which should be subject to conjugate addition by an appropriate organometallic reagent (most probably an organocuprate) under conditions which would leave ester groups untouched. Precedent suggested that attack of the reagent should occur from the equatorial direction, giving the desired stereochemistry in adduct **7**.[6]

As described elsewhere,[7] model studies on 4-*tert*-butylcyclohexanone confirmed that this sequence could be carried through with excellent yields and selectivities for a number of Knoevenagel reagents **5**, using a higher-order cuprate[8] derived from phenylmagnesium bromide and CuCN as the aryl donor. In this paper we give details of the application of one of these methods (E = CN) to the synthesis of a 'second-generation' cholaphane **8** with externally-directed functionality and fewer conformational options than the earlier examples.



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Results and Discussion

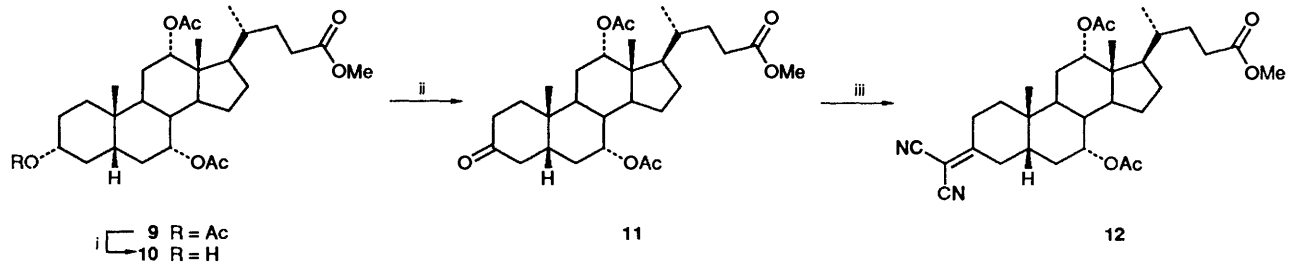
The model studies referred to above left one major question unanswered. While the new methodology was effective for introducing an unsubstituted phenyl group, it was not clear that it could be extended to the introduction of the *p*-(aminomethyl)-phenyl required for the synthesis of **8**. However, as the ketone **11** required as starting material for **8** was relatively accessible, it was decided to forgo further model investigations and work directly towards the macrocycle.

As shown in Scheme 3, ketone **11**[9] was prepared from methyl 3α,7α,12α-triacetoxycholanoate **9**[10] *via* selective acid-catalysed deacetylation of the equatorial 3α-OAc to give **10**,[11] followed by oxidation with sodium periodate and catalytic quantities of ruthenium trichloride, after the method of Sharpless and co-workers.[12] The procedure was quite simple to carry out on a large scale, giving ketone **11** in an overall yield of 82%. Condensation of **11** with malononitrile using the conditions developed by Mirek *et al.*[13] (catalysis by acetic acid and ammonium acetate in refluxing benzene, with azeotropic removal of water) proceeded smoothly, giving the dicyanomethylene derivative **12** in 93% yield after crystallisation.

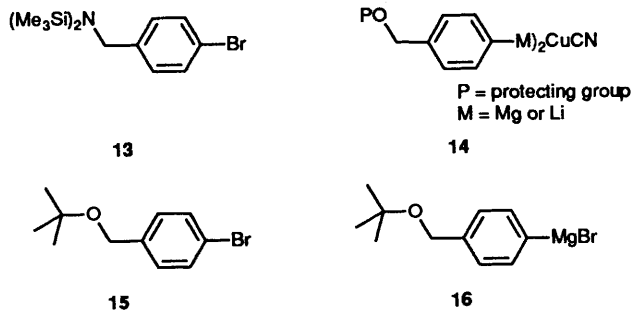
As shown in Scheme 1, the sequence employed for the 'first generation' cholaphanes **2** had relied on bis(trimethylsilyl) protection in the *p*-(aminomethyl)phenyl organometallic.[1] It seemed reasonable to suppose that the same tactic might work in the present case, the only difference being that the reagent would need to be a higher-order cuprate rather than an organomanganese derivative. Thus, the aryl bromide **13** was prepared from *p*-bromobenzyl bromide and sodium hexamethyldisilazide, and treated with magnesium in ether to give the corresponding Grignard reagent. In line with the model studies[7] the latter was combined with cuprous cyanide in the ratio 2:1, and an ethereal solution of dicyanomethylene derivative **12** was added to the mixture. Unfortunately, no addition product could be isolated. An analogous experiment employing the organolithium derived from **13**, with dimethoxyethane as solvent, was similarly unsuccessful.

It seemed quite likely that the problem was being caused by the lone pair on the nitrogen atom in these reagents which might be expected to have a high affinity for the copper and thus perturb the reactivity of the organometallic centre. Given the paucity of alternative *N*-protecting groups stable to strongly basic reagents,* and the presence of a free nitrogen lone pair in many of these alternatives, we decided to explore the 'safer' option of an oxygen-containing reagent **14**. Although perhaps long-winded, the substitution of the oxygen for a nitrogen at a later stage of the sequence seemed unlikely to cause serious problems.

* This issue is discussed in greater detail elsewhere, along with the development of a new silicon-based protecting group, the 'benzostabase' (BSB) group.[14] In the present work a higher-order cuprate derived from BSB-protected *p*-bromoaniline was also tested with dicyanomethylene derivative **12**, but was no more successful than the reagents derived from **13**.



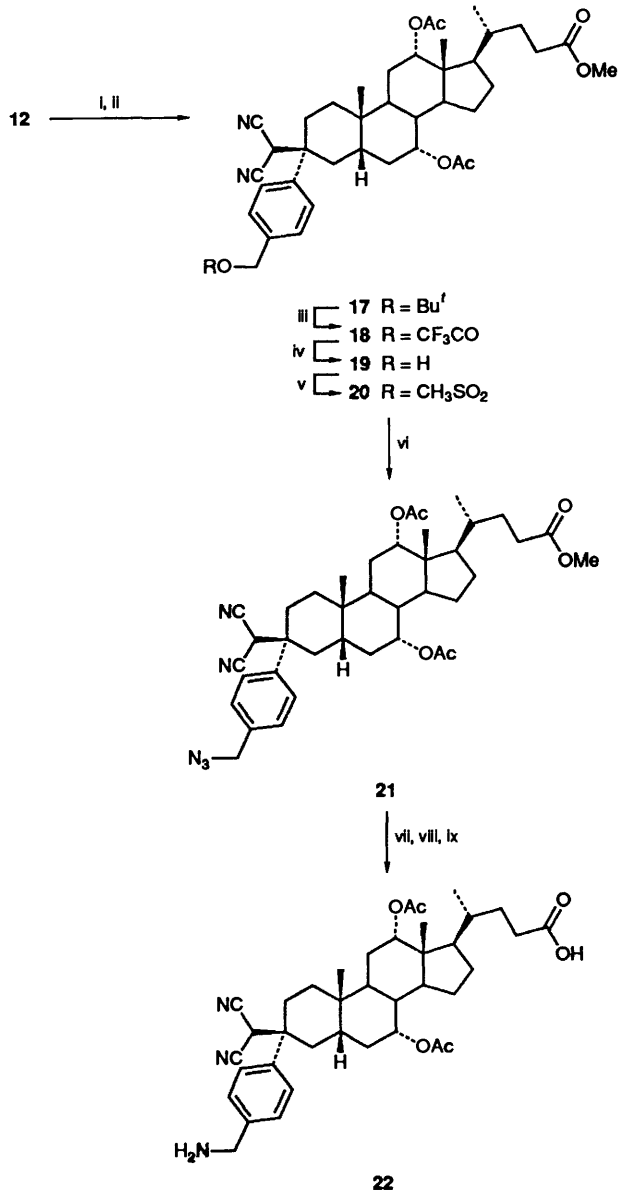
Scheme 3 *Reagents and conditions:* i, AcCl, CH_3OH; ii, $NaIO_4$, cat. $RuCl_3$, CH_3CN, CCl_4, H_2O; iii, $CH_2(CN)_2$, $NH_4^+OAc^-$, AcOH, C_6H_6



It appeared that a suitable *O*-protecting group P in reagent **14** would be *tert*-butyl, and we thus investigated the preparation of bromide **15** as starting material. The literature methods for this compound were not really satisfactory. The sulfuric acid-catalysed reaction of *p*-bromobenzyl alcohol and isobutene[15] had been reported to give only 54% yield (and was in any case inconvenient experimentally), and the reaction of *p*-bromobenzyl bromide with potassium *tert*-butoxide in DMF[16] (for which no yield was quoted in the original reference) gave poor results in our hands. However, a modification of the latter was developed, in which the potassium *tert*-butoxide was prepared from potassium metal and an excess of dry *tert*-butanol under sonication, the *p*-bromobenzyl bromide added and the sonication continued for several hours. This resulted in a 92% yield of bromide **15**, and gave material with a significantly higher melting point than previously recorded.

Attempts to convert bromide **15** into the corresponding organolithium derivative with lithium metal were unsuccessful, possibly because of deprotonation of the benzylic CH_2 by the organolithium once it was formed. However the Grignard reagent **16** was produced smoothly from **15** and magnesium in THF under sonication (30–60 min).* We were pleased to find that, as shown in Scheme 4, the higher-order cuprate derived from **16** and cuprous cyanide (ratio 2 : 1) reacted rapidly with **12** to give **17** in nearly quantitative yield. Only one stereoisomer could be detected, the structure being assigned by analogy with the model studies[7] (and confirmed by the X-ray crystal structure of the final product). The *tert*-butyl protecting group was removed with trifluoroacetic acid (CH_2Cl_2, 50 °C, 5.5 h) giving trifluoroacetate **18**, and the trifluoroacetate cleaved with aqueous ammonia (Et_2O, 30 min) to give alcohol **19** in 90% overall yield from dinitrile **12**.

Conversion of the benzylic hydroxy to an amino group was achieved *via* azide **21**. This could be formed directly from **19** using a Mitsunobu-type[17] procedure, following the method of Loibner and Zbiral[18] [Ph_3P, diethyl azodicarboxylate (DEAD), HN_3, benzene]. However, the process was not consistently successful, and a more satisfactory method (as shown in Scheme 4) involved conversion of alcohol **19** into the corresponding mesylate **20** followed by treatment with tetramethylguanidinium azide in chloroform.[19],† The mesylation step proved to be very sensitive to the exact procedure, the best results being obtained by addition of methanesulfonyl chloride to alcohol **19** in THF at 0 °C followed immediately by dropwise addition of di-isopropylethylamine. The overall conversion into azide **21** proceeded in a very satisfactory yield of



Scheme 4 *Reagents and conditions:* i, **16** (2 equiv.), CuCN (1 equiv.), THF; ii, NH_4Cl aq.; iii, CF_3CO_2H, CH_2Cl_2, 50 °C, 5½ h; iv, NH_3 aq., Et_2O, 30 min; v, CH_3SO_2Cl, Pr^i_2NEt, THF, 0 °C; vi, $(Me_2N)_2CNH_2^+N_3^-$, $CHCl_3$, 30 min; vii, Ph_3P, THF, CH_3OH, H_2O; viii, LiOH; ix, HCl aq.

93%. The azide could be reduced to the corresponding amine using catalytic hydrogenation or triphenylphosphine in wet THF.[22] The latter proved more successful, giving the amine in 85–88% yields. However, it was found to be preferable to perform the reduction and hydrolysis of the C-24 methyl ester concurrently in a one-pot procedure, giving amino acid **22** in 87% yield.

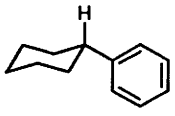
The remaining step in the synthesis of **8** was, of course, the cyclodimerisation of **22** *via* formation of two amide bonds. In the synthesis of **2** the analogous transformation had been accomplished using two methods.[1] Firstly, direct treatment of the amino acid with the condensing agent diethyl phosphorocyanidate [$(EtO)_2P(O)CN$, DEPC][23] in $CHCl_3$–DMF in the presence of K_2HPO_4 had given yields of (crystalline) macrocycle of up to 32%, and secondly a more lengthy (but more efficient) approach *via* an *N*-*tert*-butoxycarbonyl-protected pentafluorophenyl ester had given up to 55% yield. In the present work only the former was tried, a minor modification

* Reactions without sonication were much slower and gave complex product mixtures, as determined by aqueous quench followed by GC analysis.

† **CAUTION**: The interaction of azide ions and chlorinated solvents can lead to explosive side-products.[20] As part of a separate investigation we have found that, over a period of days at elevated temperatures, tetramethylguanidinium azide and chloroform can react to give significant quantities of a shock-sensitive, distillable liquid.[21]

being the use of THF as solvent. With an 8.6 mmol dm^{-3} concentration of amino acid **23**, the method resulted in a 42% crystalline yield of macrocycle **8**. On the basis of our earlier experience, it is quite likely that this yield could be improved by further investigations. Even so, the overall yield of **8** from ketone **11** was 28%. Not unexpectedly, for a rather rigid molecule of this size, the macrocycle was relatively insoluble in chloroform, dissolving to the extent of *ca.* 1 mg cm^{-3}. The ^1H NMR spectrum in $CDCl_3$ showed a feature which was also characteristic of the earlier series of cholaphanes **2**, namely the splitting of the benzylic protons into an AB quartet (further coupled to NH); the corresponding protons appeared as a singlet in the spectra of the acyclic precursors.

Final characterisation of **8** was provided by X-ray crystallography, as reported in our preliminary communication.[3] As expected, the structure showed the aryl spacer groups in an 'inward-facing' conformation (as illustrated in the formula), supporting our assumption that the *exo*-directed $CH(CN)_2$ groups would be bulky enough to favour such an orientation. However, in order to establish that the new linkage would have less freedom of rotation than that in **2**, it was necessary to resort to computational methodology. The MM2 molecular mechanics method, as implemented in MacroModel V3.1X, was applied to the model systems **23** and **24**. In the case



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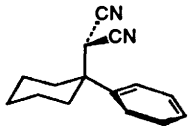

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of **23**, the favoured conformation was as shown, with the aromatic ring eclipsing the adjacent C–H. Rotation of the C–Ar bond through 60° in either direction exacted an energetic penalty of *ca.* 5.9 kJ mol^{-1}. A similar distortion of **24** from its preferred conformation (shown) was significantly more difficult, requiring *ca.* 13.4 kJ mol^{-1}.*

In conclusion, we have developed a practical and high-yielding synthesis of a second cholaphane framework with lower conformational freedom than the earlier system, a better-defined cavity and externally-directed functionality. As discussed elsewhere,[7] work on a model system has suggested that further modification of the dicyanomethyl group may not be practicable, so that the last of these 'advantages' may prove to be of limited worth. However, the other features are likely to be valuable in the development of, for example, new host molecules with more predictable complexation behaviour.

Experimental

^1H NMR spectra were recorded on a Bruker WP 80 spectrometer at 80 MHz or, where indicated, on a Bruker MSL 300 instrument at 300 MHz. $(CH_3)_4Si$ was used as the internal standard. *J*-Values are given in Hz. IR spectra were recorded on a Perkin-Elmer 883 spectrophotometer. Solvents were purified by standard procedures.[24] 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. Gas chromatography (GC) was performed using a Varian 3300 instrument fitted with a 25 m OV1 capillary column. Silica gel 60, 400–230 mesh (Merck) was used for flash chromatography and, where indicated, filtration of reaction products. Reactions involving sonication were performed in a B & T laboratory ultrasonic cleaning bath.

Methyl 7α,12α-Diacetoxy-3-oxocholan-24-oate **11**.—A solution of the triacetate **9**[10] (15.49 g, 28.2 mmol) in methanol (150 cm^3) was treated with acetyl chloride (10 cm^3).[11] After 15 min the mixture was evaporated under reduced pressure to give a white foam of the alcohol **10** (13.80 g) (pure by TLC). This material was then dissolved in acetonitrile–carbon tetrachloride (1:1; 100 cm^3) and stirred vigorously with sodium periodate (24.0 g, 112 mmol) in water containing ruthenium trichloride hydrate (150 mg, 0.7 mmol).[12] After 75 min the mixture was extracted with dichloromethane (250 cm^3) which was then evaporated under reduced pressure to give a grey powder (14.05 g). This was dissolved in and eluted with benzene–ethyl acetate (6:4), through a silica gel plug to give a white solid (12.99 g), which was crystallised from benzene to give ketone **11** (11.74 g, 82%), m.p. 189–190 °C (lit.,[9] 190–191 °C); TLC R_f 0.36 in hexane–ethyl acetate (1:1).

Methyl 7α,12α-Diacetoxy-3-dicyanomethylenecholan-24-oate **12**.—The ketone **11** (10.65 g, 21.1 mmol) and malononitrile (1.452 g, 21.97 mmol) were dissolved in dry benzene (23 cm^3) inside a 50 cm^3 round bottom flask fitted with a Dean and Stark apparatus. To this was added ammonium acetate (0.35 g) and glacial acetic acid (1 cm^3).[13] The reaction mixture was placed under an atmosphere of argon before being lowered into an oil-bath held at 100 °C. After 15 min, analysis by TLC implied the reaction was complete, so the mixture was allowed to cool to room temperature and then partitioned between chloroform–ether (1:1; 20 cm^3) and water (100 cm^3). After the aqueous layer had been removed some product crystallised out of the organic layer, so more chloroform (10 cm^3) was added to redissolve it. The organic layer was washed successively with water (2 × 50 cm^3) and brine (20 cm^3), and the solvent removed under reduced pressure. The crude product was crystallised from ethyl acetate to give white needles of the *unsaturated dinitrile* **12** (10.86 g, 93%), m.p. 217–218 °C (Found: C, 69.6; H, 7.7; N, 4.95. $C_{32}H_{44}N_2O_6$ requrires C, 69.54; H, 8.02; N, 5.07%); ν_{max}(film from THF)/cm^{-1} 2233 (conj CN), 1739 (CO) and 1597 (conj. alkene); $\delta_H(CDCl_3)$ 0.76 (3 H, s, 18-H), 0.81 (3 H, d, *J* 6, 21-H), 0.99 (3 H, s, 19-H), 2.09 (3 H, s. OCOMe), 2.10 (3 H, s, OCOMe) 3.66 (3 H, s, CO_2Me), 5.02 (1 H, m, 7β-H) and 5.12 (1 H, m, 12β-H); TLC R_f 0.52 in hexane–ethyl acetate (1:1).

tert-*Butyl* p-*Bromobenzyl Ether* **15**.—Fresh potassium wire (2.49 g, 89.2 mmol) was introduced into a flask containing dry *tert*-butanol (80 cm^3) under an atmosphere of argon. As the reaction proceeded the potassium reacted more slowly with the butanol, so after 45 min the flask was lowered into an ultrasonic bath and sonicated until the potassium had dissolved (15 min). Solid *p*-bromobenzyl bromide (18.60 g, 74.4 mmol) was added and the reaction mixture was sonicated for 2 h giving a milky white suspension. The flask was allowed to stand overnight, then sonicated for a further 6 h after which analysis by TLC implied that no *p*-bromobenzyl bromide remained. The reaction mixture was left standing for a further 24 h before being partitioned between ether (300 cm^3) and water (1.2 dm^3). The organic layer was washed with water (1 dm^3 then 2 × 500 cm^3) and then with brine (2 × 25 cm^3). The solution was filtered through sand and evaporated under reduce pressure to give 18.18 g of off-white crystals. This was distilled under vacuum to give a viscous liquid which on cooling gave *tert*-butyl ether **15** as a colourless crystalline solid (16.71 g, 92%), b.p. 89–91 °C at 0.2 mmHg; m.p. 58–59 °C (lit.,[15] 48–50 °C); $\delta_H(CDCl_3)$ 1.28 (9 H, s, *tert*-butyl), 4.38 (2 H, s, CH_2) and 7.20, 7.44 (4 H, ABq, J_{AB} 8.3, aromatic); TLC R_f 0.08 in hexane.

* Global minima were found using the Polak–Ribiere Conjugate Gradient (PRCG) algorithm. The energies of the other conformations were estimated after minimisation by Steepest Descents (SD), as the PRCG method merely relocated the global minima.

Methyl 7α,12α-*Diacetoxy*-3β-*dicyanomethyl*-3α-(p-*hydroxymethylphenyl*)-*cholan*-24-*oate* **19**.—Magnesium turnings (1.34 g, 55.1 mmol) were flame dried under vacuum (0.1 mmHg) and cooled under an atmosphere of argon. To this was added a solution of *tert*-butyl *p*-bromobenzyl ether **15** (9.73 g, 40.02 mmol) in dry THF (80 cm^3). The flask was lowered into an ultrasonic bath, the reaction was initiated with 1,2-dibromoethane (0.2 cm^3, 2.3 mmol) and the formation of the Grignard reagent was followed by GC, which implied that the reaction was *ca.* 98% complete after 30 min. The resultant solution was stirred in an ice bath, solid copper(I) cyanide (1.73 g, 19.3 mmol) was added under a flow of argon, and then the mixture was warmed to 10 °C. After waiting 15 min for all of the copper cyanide to dissolve, a solution of the unsaturated dinitrile **12** (9.84 g, 17.8 mmol) in dry THF (70 cm^3) was added. After approximately 5 min the reaction was quenched with a saturated solution of ammonium chloride (2 cm^3) and diluted with ether (50 cm^3). The slurry was filtered and the filtrate was evaporated under reduced pressure to give inorganic salts and an oil. This was partitioned between water and ether and the organic layer re-filtered and evaporated to give a yellow foam (15.5 g). The foam was triturated in hexane (30 cm^3), which extracted most of the *tert*-butyl benzyl ether from the insoluble product, leaving crude *tert*-butyl ether **17** (12.92 g, *ca.* 17.8 mmol).

The above material was dissolved in dichloromethane (18 cm^3) containing trifluoroacetic acid (10 cm^3, 70 mmol) and the solution was heated to 50 °C. After $5\frac{1}{2}$ h, analysis by TLC* implied that no *tert*-butyl ether **17** remained, a small amount of the desired alcohol **19** was present, and the majority of the material was the trifluoroacetate **18**. The reaction mixture was then partitioned between ether (60 cm^3) and aqueous ammonia (5 mol dm^{-3}, 200 cm^3) in a separating funnel. After 30 min analysis by TLC showed only the presence of the benzyl alcohol **19**. The aqueous layer was removed and the organic layer washed with water (2 × 100 cm^3) and brine (2 × 50 cm^3), and then passed through a silica gel plug (3 cm diameter × 4 cm length) and eluted with ethyl acetate (50 cm^3). This gave, on evaporation under reduced pressure, an off-white solid (11.24 g) which was dissolved in 1,1,1-trichloroethane and caused to precipitate by adding hexane–ether (1:1; 20 cm^3). The precipitate was washed with ether to give, after evacuation (0.1 mmHg; 3 h), the *benzyl alcohol* **19** (10.16 g), m.p. 109–111 °C (Found: C, 61.55; H, 7.0; N, 3.35. $C_{39}H_{52}N_2O_7$ with 1 molecule of CH_3CCl_3 requires C, 62.00; H, 6.98; N, 3.53%);† ν_{max}(film from THF)/cm^{-1} 3439 (OH), 2254w (CN) and 1740 (CO); $\delta_H(CDCl_3)$ 0.73 (3 H, s, 18-H), 0.77 (3 H, d, *J* 6.5, 21-H), 1.04 (3 H, s, 19-H), 2.00 (3 H, s, OCOMe), 2.01 (3 H, s, OCOMe), 3.64 (3 H, s, CO_2Me), 4.37 [1 H, s, $CH(CN)_2$], 4.75 (2 H, d, *J* 4.6, with D_2O shake goes to 2 H, s, benzyl), 4.96 (1 H, m, 7β-H), 5.05 (1 H, m, 12β-H) and 7.44, 7.57 (4 H, ABq, J_{AB} 11, aromatic).

Evaporation and flash chromatography [hexane–ethyl acetate (1:1)] of the mother liquor from the crystallisation gave more of the benzyl alcohol **19** (0.42 g), so that the total overall yield from unsaturated dinitrile **12** was 10.58 g (90%).

Methyl 7α,12α-*Diacetoxy*-3α-(p-*azidomethylphenyl*)-3β-*dicyanomethyl*-*cholan*-24-*oate* **21**.—A solution of the alcohol **19** (5.08 g, 7.69 mmol) in dry THF (50 cm^3) was stirred vigorously at 0 °C under an atmosphere of argon. To this was added methanesulfonyl chloride (2.4 cm^3, 31 mmol) immediately followed by diisopropylethylamine (2.7 cm^3, 15.5 mmol), dropwise over 3 min. The light yellow mixture was diluted with ether (50 cm^3) then washed with water (3 × 100 cm^3) and brine. Evaporation of the solvent under reduced pressure gave the methanesulfonate **20** (5.67 g) as a white foam. To this was added tetramethylguanidinium azide (2.93 g, 15.4 mmol), [19] and the resulting mixture was dissolved in chloroform (15 cm^3).‡ After 30 min the solution was diluted with ether (50 cm^3), washed with water (3 × 50 cm^3) then brine, and passed through a silica plug which was then eluted with ether–chloroform (1:1). Evaporation of the solvent gave the *azide* **21** (4.91 g, 93%). Crystallisation from ether afforded an analytical sample, m.p. 105–110 °C (Found: C, 67.75; H, 7.8; N, 9.75. $C_{39}H_{51}N_5O_6$ with 0.5 mol of H_2O requires C, 67.41; H, 7.54; N, 10.08);† ν_{max}(film from THF)/cm^{-1} 2252w (CN), 2101s (N_3), 1730br (CO) and 1600 (aromatic); $\delta_H(CDCl_3)$ 0.73 (3 H, s, 18-H), 0.77 (3 H, d, *J* 6.5, 21-H), 1.04 (3 H, s, 19-H), 1.98 (3 H, s, OCOMe), 2.00 (3 H, s, OCOMe), 3.64 (3 H, s, CO_2Me), 4.40 [1 H, s, $CH(CN)_2$], 4.42 (2 H, s, benzyl), 4.96 (1 H, m, 7β-H), 5.05 (1 H, m, 12β-H) and 7.40, 7.58 (4 H, ABq, J_{AB} 8.5, aromatic); TLC R_f 0.62 in hexane–ethyl acetate (1:1).

Cyclo-bis[7α,12α,-*diacetoxy*-3β-*dicyanomethyl*-3α-(4-*methylenephenyl*)*cholanamide*] **8**.—The azido ester **21** (1.868 g, 2.72 mmol) and triphenylphosphine (1.42 g, 5.44 mmol) were stirred in THF–methanol–water (2:2:1; 15 cm^3) for 1 h at room temperature. Lithium hydroxide monohydrate (0.457 g, 10.9 mmol) and THF–methanol–water (2:2:3; 7 cm^3) were added. After stirring for 1.5 h the mixture was neutralised with aqueous hydrochloric acid (2.18 cm^3; 5 mol dm^{-3}). Evaporation of the solvent under reduced pressure gave an off-white gum which was dissolved in chloroform–methanol (1:1) and put onto a silica gel column. Elution with chloroform washed out the triphenylphosphine oxide, and then elution with chloroform–methanol [(10:1) grading to (2:1)] gave amino acid **22** (1.264 g), followed by a mixture of **22** and lithium chloride. The lithium chloride was removed from the latter by washing a THF–chloroform solution of the mixture with water, after which evaporation and flash chromatography gave a further sample of amino acid **22** (0.280 g, 87% in total); ν_{max}(film from THF)/cm^{-1} 3440–2650br (NH), 2254w (CN) and 1735 (CO); TLC R_f 0.14 in chloroform–methanol (5:1).

To a solution of the above material (0.196 g, 0.30 mmol) in dry THF (35 cm^3), stirred under an atmosphere of argon, was added diethyl phosphorocyanidate (DEPC) [23] (115 mm^3, 0.759 mmol). After 10 min, dipotassium hydrogen phosphate [0.36 g, 2.0 mmol; previously flame dried under vacuum (0.1 mmHg), and cooled under argon] was added as a slurry in dry THF (5 cm^3) to the reaction mixture. After stirring for 12 days the solvent was removed under reduced pressure and the residue partitioned between water and chloroform. The solvent was evaporated from the organic layer under reduced pressure to give a viscous oil which was dissolved in a minimum volume of ethyl acetate–methanol and subjected to flash chromatography using hexane–ethyl acetate (1:5) as eluent. Evaporation of the solvent and recrystallisation of the product from chloroform–methanol–THF gave the *cholaphane* **8** (80 mg, 42%) as fine white needles, which became a fine white powder after evacuation (0.1 mmHg; 50 °C) for 6 h, m.p. 253–255 °C (Found:

* Both *tert*-butyl ether **17** and trifluoroacetate **18** had R_f 0.55 in hexane–ethyl acetate (1:1). However, TLC analysis was possible after treatment of samples with aqueous ammonia (as in the main procedure), converting **18** into alcohol **19** (R_f 0.30).

† Crystals of compounds with 3α-aryl-3β-dicyanomethyl substitution tended to be quite extensively solvated, as is often the case for rigid molecules with irregular structures. It was not generally possible to remove all the solvent before microanalysis.

‡ **CAUTION:** The interaction of azide ions and chlorinated solvents can lead to explosive side-products.[20] As part of a separate investigation we have found that, over a period of days at elevated temperatures, tetramethylguanidinium azide and chloroform can react to give significant quantities of a shock-sensitive, distillable liquid.[21]

C, 70.3; H, 7.85; N, 6.3. $C_{76}H_{98}N_6O_{10}$ with 2 mol of H_2O requires C, 70.67; H, 7.96; N, 6.51);* ν_{max}(film from THF)/cm^{-1} 3329 (NH), 2254w (CN), 1741 and 1663 (CO); δ_H(300 MHz; $CDCl_3$) 0.744 (6 H, s, 18-H), 0.778 (6 H, d, *J* 4.8, 21-H), 1.042 (6 H, s, 19-H), 1.986 (6 H, s, OCOMe), 2.000 (6 H, s, OCOMe), 2.344 (2 H, br, d, *J* 14, 4β-H), 2.530 (2 H, t, *J* 14, 4α-H), 4.366 [2 H, s, $CH(CN)_2$], 4.342 (2 H, dd, *J* 14, 6, CHN), 4.515 (2 H, dd, *J* 14, 6, CHN), 4.959 (2 H, m, 7β-H), 5.759 (2 H, t, *J* 6, NH) and 7.455, 7.536 (8 H, ABq, J_{AB} 8.4, aromatic); TLC R_f 0.45 in ethyl acetate.

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* Crystals of compounds with 3α-aryl-3β-dicyanomethyl substitution tended to be quite extensively solvated, as is often the case for rigid molecules with irregular structures. It was not generally possible to remove all the solvent before microanalysis.

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