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Strategies and methods for the attachment of amino acids and peptides to chiral [*n*]polynorbornane templates

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A versatile synthesis of amino acid and peptide functionalised [*n*]polynorbornane scaffolds is described. The frameworks are constructed using the stereoselective and regioselective cycloaddition of suitably functionalised chiral cyclobutene epoxides with similar norbornenes. The strategies employed allow a range of topologies to be accessed and a number of regioselectively addressable linkage points to be accommodated.

Introduction

Macromolecules that exhibit a well-defined three-dimensional structure have proven useful as templates in a range of applications including peptidomimetics.[1] These scaffold-like molecules impart a specific geometry to any attached peptide loops or segments. Such TASP (Template Assembled Synthetic Peptides)[2] constructs have been successfully applied in small molecule recognition[3] and even protein surface recognition.[4] For successful peptide attachment, these molecules require selectively addressable points incorporated within their structure.

Polynorbornane frameworks are readily synthesised by means of the ACE (Alkene + Cyclobutene Epoxide) reaction.[5] This reaction involves the thermal ring opening of cyclobutene epoxides and subsequent 1,3-dipolar cycloaddition of the resulting carbonyl ylides to functionalised norbornenes. Such technology offers a versatile method for the synthesis of polynorbornane based frameworks of various geometries, both linear and bent.[6]

As polynorbornyl scaffolds inherently possess a high degree of structural rigidity they are ideally suited for use as molecular templates. Additionally, polynorbornyl templates offer, depending upon their design, the capacity to be chiral and hence may be synthesised as enantiomerically pure compounds. Whilst we have recently introduced the concept of [*n*]polynorbornane frameworks as rigid templates to which peptides can be attached in a regiospecific manner,[7] we herein report the precise protocol to assembling peptide chains on chiral [*n*]polynorbornane templates. Furthermore, the methodology outlined is equally well suited for attachment of other effectors *e.g.* DNA intercalators *via* peptide chains. †

Results and discussion

Several points on the [*n*]polynorbornyl framework have been identified as suitable for peptide attachment (see Fig. 1) and ideally, to complement traditional peptide synthetic methodology, these attachment points should be carboxylic acids or amines. With this in mind, the specific inclusion of such functional groups, in well defined spatial arrangements, to polynorbornane frameworks was pivotal to the current investigation.

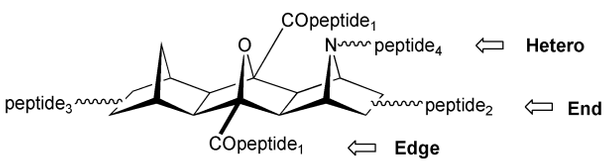

Fig. 1 Sites for attachment to [*n*]polynorbornanes.

Examination of a typical polynorbornyl framework[5] (Fig. 2) reveals that carboxy edge functionality is introduced, in the form of methyl esters, by means of the Mitsudo reaction of a norbornene with dimethylacetylenedicarboxylate (DMAD)[8] and as such is characteristic of ACE chemistry. Consequently, it was decided to exclusively adopt carboxylic acids as attachment points, and to use orthogonal protecting groups to enable these points to be selectively addressed. Whilst methyl esters are not ideal as an orthogonal protecting group, we have demonstrated previously[7] that the readily available di-*tert*-butylacetylenedicarboxylate (D^{t}BAD) is an efficient partner in the Mitsudo reaction. Such a reaction leads ultimately to [*n*]polynorbornanes with *tert*-butyl edge esters, which in turn offer the possibility of selective cleavage.

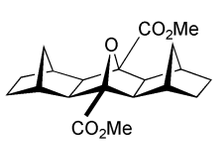

Fig. 2 Typical polynorbornyl framework.

Disconnection

To investigate the concept of regioselectively addressable groups, framework **1** (Fig. 3), that included both benzyl esters (end functionality) and *tert*-butyl esters (edge functionality) was designed—benzyl esters are well established as orthogonal to *tert*-butyl.[9] Retrosynthetic analysis identified the orthogonally protected epoxide **2** and norbornene *endo* ester **3** as necessary components. This latter building block was seen as readily obtainable from the Diels–Alder reaction of a suitable acrylate with cyclopentadiene. Such reactions are well documented, as is the *endo* preference of this cycloaddition.[10] For future

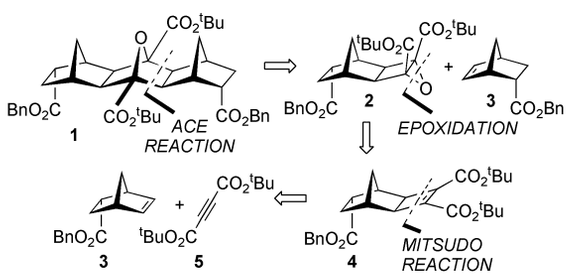

Fig. 3 Disconnection of proposed template.

† Such endeavours are presently underway and will be reported in due course.

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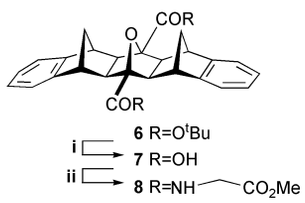
applications it was thought the likelihood of interaction between any attached peptide chains would be maximised if they were directed in this pseudo-axial *endo* orientation.

Edge substitution

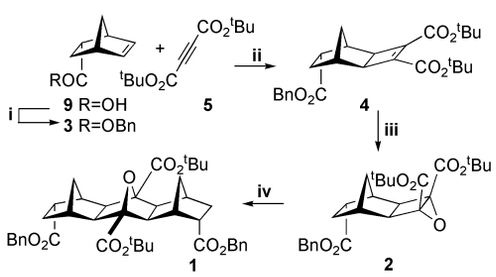
To explore the elaboration of the *tert*-butyl edge esters the synthesis of the model [*n*]polynorbornyl framework **8** (Scheme 1) was examined. The esters of **6** were cleanly cleaved with trifluoroacetic acid (TFA) in dichloromethane. Coupling of the resulting diacid **7** with glycine methyl ester using the combination of the water soluble 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) together with a catalytic amount of 1-hydroxy-1,2,3-benzotriazole (HOBt)[11] afforded the desired amino acid functionalised framework **8** in 67% yield.



Scheme 1 Edge functionalisation. *Reagents and conditions*: (i) 1 : 1 TFA–DCM, RT, 4 h; (ii) H-Gly-OMe, EDCI, HOBt, NEt_3, DMF, RT, 12 h.

End substitution and chirality

With the elaboration of *tert*-butyl esters established, we turned our attention to combining these with orthogonal end functionality. Benzyl ester **3** was synthesised from the readily available *endo*-norbornene carboxylic acid **9**,[12] by means of carbodiimide coupling with benzyl alcohol (Scheme 2).[13] The Mitsudo reaction of **3** with D^{t}BAD proceeded smoothly as did epoxidation of the product. The ACE reaction of this epoxide **2** was also successful although the ^{1}H NMR spectrum of the product **1** revealed *three* ^{t}Bu ester resonances.



Scheme 2 Synthesis of fully protected framework. *Reagents and conditions*: (i) BnOH, EDCI, DMAP, NEt_3, DCM, RT, 12 h; (ii) $RuH_2(CO)(PPh_3)$, D^{t}BAD, benzene, 80 °C, 24 h; (iii) TBHP, ^{t}BuOK, THF, RT, 12 h; (iv) **3**, Sealed tube, THF, 140 °C, 12 h.

It was recognised from the outset that three stereoisomers would be produced from the ACE reaction of a racemic *endo*-norbornene with an epoxide derived from such a norbornene (Fig. 4). Two chiral C_2v symmetric isomers (**I** and **III**) and a *meso* compound (**II**) are produced in a 1 : 1 ratio of **I&III** : **II**. For enantiomers **I** and **III** a single resonance was observed for the ^{t}Bu ester (δ_H = 1.46), however, the *meso* compound **II** revealed two distinct resonances for the ^{t}Bu esters (δ_H = 1.40 and δ_H = 1.52). In order to construct a framework in which the attachment points exhibit a well ordered and predetermined topography the synthesis had to be pursued using a single enantiomer of the norbornene *endo*-ester. Such optically pure material was available using the (2*S*)-norbornene *endo*-acid reported by Helmchen and coworkers.[14] When the synthesis was repeated using this optically pure norbornene no trace of the *meso* product could be detected by NMR spectroscopy indicating that indeed, **1** had been produced as a single enantiomer and was of the stereochemistry as indicated in Scheme 2. The use of chiral starting materials allowed a successful route to frameworks of type **III** to be developed and with it the controlled positioning of the attachment points had been achieved. From this point forward the racemic acid was used only for validating synthetic pathways prior to chiral framework construction.

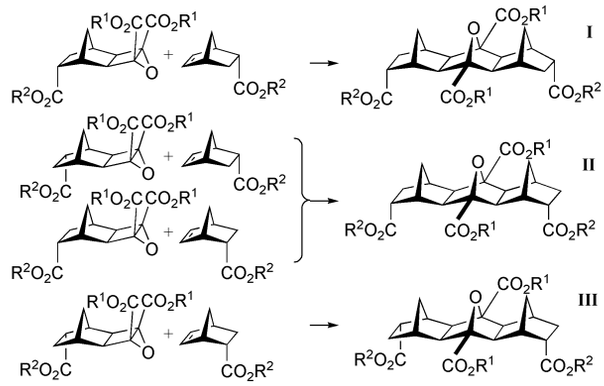


Fig. 4 Possible stereoisomers.

The benzyl esters were selectively removed from **1** by means of hydrogenation and the coupling reaction of the free acids with glycine methyl ester attempted. Unfortunately, a range of coupling reagents including carbodiimides,[11] PyBop[15] and EEDQ[16] failed to yield the desired product. This lack of reactivity is probably due to steric hindrance as it is known that polynorbornane frameworks are slightly curved in nature[6] and this exacerbates the known lesser reactivity of the *endo* position of norbornanes relative to the *exo* position.[17] Regardless of the cause, a new approach to end functionalisation was required.

Strategies

Our initial strategy had been to build the complete framework first and attach amino acids and peptides last (PATH A, Fig. 5). An alternative approach, one that also made use of the norbornene acid **3**, was to attach the amino acids or peptides first then construct the framework using the sequence of Mitsudo reaction, epoxidation and ACE reaction (PATH B) and this approach was subsequently evaluated.

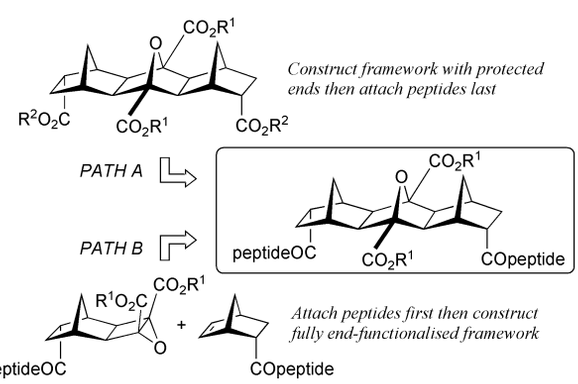
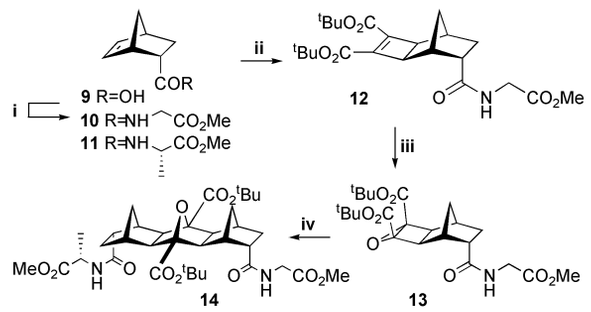


Fig. 5 Strategies for incorporating end functionality.

The carbodiimide mediated coupling of acid **9** with glycine methyl ester provided **10** in 76% unoptimised yield (Scheme 3). This derivative was successfully reacted under Mitsudo conditions followed by epoxidation to provide the desired oxirane **13**. This result reinforces our previous findings that an amide NH is tolerated if the cyclobutene to be epoxidised is activated by an ester function.[7] To our delight the ACE reaction of epoxide **13** with norbornene amino acid derivative **11** (synthesised as for **10** using alanine as the amino acid) provided the amino acid end functionalised framework **14** in 41% yield. This framework is an



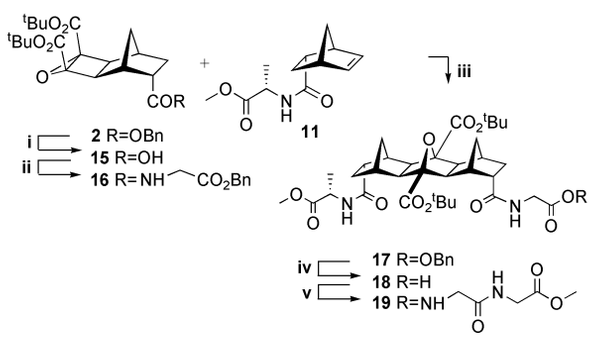
Scheme 3 End functionalisation using the 'attach first' strategy. *Reagents and conditions*: (i) H-Gly-OMe (or H-Ala-OMe for **11**), EDCI, HOBt, D^{i}PEA, DMF, RT, 12 h; (ii) **10**, $RuH_2(CO)(PPh_3)$, D^{t}BAD, benzene, 80 °C, 72 h; (iii) TBHP, ^{t}BuOK, THF, RT, 14 h; (iv) **11**, Sealed tube, 140 °C, DCM, 12 h.

example of the accommodation of three different groups: a glycine at one end, alanine at the other and *tert*-butyl groups at the edges.

Whilst this 'attach first' strategy was successful, there was sound rationale why the failed strategy (PATH A, Fig. 5) was the more appealing. Specifically, the high temperatures required in the thermal ACE cycloaddition were seen as incompatible with complex or valuable peptides which could be degraded or racemised.

Accordingly, we devised a third strategy that obviated steric concerns at the same time as minimising the risk of thermal damage to peptide side chains.

To this end, a short regioselectively addressable, amino acid spacer, attached to the [*n*]polynorbornane framework was required. Commencing with chiral epoxide **2**, the benzyl ester was removed cleanly by hydrogenation and the resulting acid coupled with glycine benzyl ester to afford **16** in 58% yield (Scheme 4).

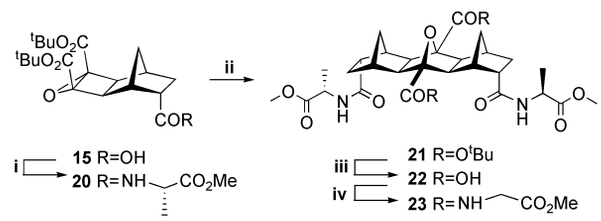


Scheme 4 Definitive strategy for adding end functionality. *Reagents and conditions*: (i) H_2, Pd/C, EtOAc, 24 h; (ii) H-Gly-OBn (or H-Ala-OMe for **17**), EDCI, HOBt, D^{i}PEA, DMF, RT, 12 h; (iii) Sealed tube, DCM, 140 °C, 12 h; (iv) H_2, Pd/C, EtOAc, 24 h; (v) H-Gly-Gly-OMe, EDCI, HOBt, D^{i}PEA, DMF, RT, 72 h.

Introduction of an amino acid in this manner is a further example of the flexibility of this methodology for framework construction. The ACE reaction of **16** with **11** produced framework **17** which had a selectively addressable point for attachment located a safe distance from the bulk of the norbornene. The crucial test for the elaboration of **17** *i.e.* deprotection and coupling with GlyGlyOMe to produce **19** was accomplished in 68% yield demonstrating conclusively that this was the method of choice for end functionalisation.

End and edge substitutions

With the successful synthesis of polynorbornane frameworks having edge substitution and others that had end substitution, our attention turned to a framework where both end and edge positions had been addressed with amino acids. The alanine end functionalised framework **21** (synthesised by the ACE reaction of norbornene **11** and epoxide **20**) was chosen for this purpose as the C_2 symmetric product would be easily recognised from its NMR spectrum. Indeed, stirring framework **21** with 1 : 1 TFA–DCM ovenight (Scheme 5) cleanly cleaved the *tert*-butyl esters then coupling of the diacid **22** with an excess of glycine methyl ester for 48 hours provided the complete peptidoframework **23** in 50% yield.



Scheme 5 Complete peptidoframework. *Reagents and conditions*: (i) H-Ala-OMe, EDCI, HOBt, D^{i}PEA, DMF, RT, 12 h; (ii) **11**, Sealed tube, DCM, 140 °C, 12 h; (iii) 1 : 1 TFA–DCM, RT, 12 h; (iv) H-Gly-OMe, EDCI, HOBt, D^{i}PEA, DMF, RT, 48 h.

Conclusion

In summary we have established the necessary protocols to synthesise chiral [*n*]polynorbornanes and to attach unlike amino acids at the ends and like amino acids on the edges of these frameworks. Furthermore we have shown how to extend small polypeptides at the ends of the template. We believe this approach has much to offer the field of peptidomimetics and we are presently exploring the construction of looped peptides using *N*- and *C*-terminals on chiral [*n*]polynorbornanes.

Experimental

NMR spectra were recorded on a Varian Unity Plus 300 MHz spectrometer. Electrospray mass spectra (ES) were obtained with a platform II single quadropole mass spectrometer (Micromass, Altrincham, UK). High resolution mass spectra (HRMS) were performed by the Centre for Molecular Architecture, Central Queensland University, Rockhampton. Melting points were performed on a Reichart hotstage microscope and are uncorrected. Optical rotations were performed on a Jasco DIP-1000 digital polarimeter using the Na D line (589 nm) and 95% EtOH as solvent and are given in 10^{-1} deg cm^2 g^{-1} (concentrations are reported as mg × mL^{-1}). Thin layer chromatography was performed on Merck Kieselgel 60 F_{254} plates. Column chromatography was performed using Merck Kieselgel 60 (70–230 mesh). All chromatography solvents were AR grade. Dichloromethane was distilled from CaH_2 and either used fresh or stored over 4 Å sieves. Anhydrous *N*,*N*-dimethylformamide was purchased from Aldrich Chemical Co. Tetrahydrofuran was freshly distilled from Na/benzophenone ketyl. Reagents were used as purchased except in the case of *tert*-butyl hydroperoxide (TBHP) which was prepared from commercial TBHP according to the method of Sharpless.[18]

Starting materials

Pure (±)-*endo*-norbornene-4-carboxylic acid **9** was obtained using iodolactonisation followed by zinc mediated elimination.[12] The (4*S*)-enantiomer of **9** was prepared by asymmetric synthesis according to the method of Helmchen and coworkers.[14] The general epoxidation procedure is our modification[7] of the TBHP–base reaction originally published by Meth-Cohn and coworkers.[19]

General epoxidation procedure

A solution of alkene (1.0 eq.) dissolved in freshly distilled THF (~10 mL : 100 mg substrate) under argon was cooled to 0 °C whereupon TBHP (3.04 M in toluene, 1.5 eq.) was added with stirring. The cold bath was maintained and after 10 min ^{t}BuOK

(0.3–0.5 eq.) was added in one portion. A slight yellow to orange colour indicated that the reaction had commenced. When TLC monitoring indicated the reaction was complete (4–12 hours), the reaction was quenched by addition of 10% Na_2SO_3 (1.5 eq.) and stirred for a further 10 min. This mix was subsequently partitioned between dichloromethane–H_2O (or chloroform–H_2O) and the organic phase separated. The aqueous phase was extracted twice more with dichloromethane (or chloroform) and the combined organics dried (Na_2SO_4), filtered and evaporated. Depending on the purity of the material obtained the product was either isolated by chromatography (EtOAc–hexane) and/or recrystallised (EtOAc–hexane).

(1α,2β,3α,10α,11β,12α,13β,14α,21α,22β)-24-Oxaoctacyclo-[10.10.1.1^{3,10}.1^{14,21}.0^{2,11}.0^{4,9}.0^{13,22}.0^{15,20}]pentacosa-4,6,8,15,17,19-hexaene-1,12-dicarboxylic acid 7

The *tert*-butyl ester framework **6** (38 mg, 0.07 mmol) was stirred in a 1 : 1 solution of TFA and dichloromethane for 4 hours. The solution was concentrated to dryness to provide 30 mg (~100%) of the title compound as an off white solid that was used directly in the next step; δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.35 (2H, d, J = 8.3, H23,25), 2.08 (4H, s, H2,11,13,22), 2.66 (2H, d, J = 9.5, H23,25), 3.24 (4H, s, H3,10,14,21), 6.92–6.95 (4H, m, ArH), 7.03–7.06 (4H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 43.05, 45.59, 55.41, 88.59, 120.67, 125.63, 148.33, 171.29.

1,12-Bis(methoxycarbonylmethylcarbamoyl)-(1α,2β,3α,10α,11β,12α,13β,14α,21α,22β)-24-oxaoctacyclo[10.10.1.1^{3,10}.1^{14,21}.0^{2,11}.0^{4,9}.0^{13,22}.0^{15,20}]pentacosa-4,6,8,15,17,19-hexaene 8

To a nitrogen flushed solution of diacid **7** (30 mg, 0.07 mmol), glycine methyl ester hydrochloride (20 mg, 0.16 mmol), triethylamine (16 mg, 0.022 mL, 0.16 mmol) in dry DMF (1.0 mL) the following were added in quick succession from pre-dried pretared screw cap vials: EDCI (30 mg, 0.16 mmol) and hydroxybenzatriazole (20 mg, 0.16 mmol). The reaction was allowed to stir overnight whereupon TLC indicated new material had formed. Workup consisted of removal of DMF under high vacuum, redissolving the crude material in chloroform (10 mL) and washing with dilute HCl (10 mL), sat $NaHCO_3$ (10 mL), and water (10 mL). Drying over $MgSO_4$, filtration and evaporation yielded a crude material that after column chromatography (1 : 1–1 : 0 EtOAc–hexane, R_f[1 : 1 EtOAc–hexane] = 0.25) provided the title compound as a waxy white solid. Yield 27 mg (67%); mp 130–133 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.46 (2H, d, J = 9.3, H23,25), 2.23 (4H, s, H2,11,13,22), 2.49 (2H, d, J = 9.3, H23,25), 3.33 (4H, s, H3,10,14,21), 3.83 (6H, s, CO_2Me Gly), 4.29 (4H, d, J = 5.6, CH_2 Gly), 6.91 (2H, t, J = 5.6, NH), 6.97–7.00 (4H, m ArH), 7.09–7.12 (4H, m ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 40.60, 43.55, 45.27, 52.55, 55.25, 90.91, 120.95, 125.80, 148.34, 169.19, 170.04; *m*/*z* (ES) 579.4 [$C_{32}H_{32}N_2O_7$ + Na]$^+$; Found 556.2202, $C_{32}H_{32}N_2O_7$ requires 556.2209.

Benzyl 2-*endo*-bicyclo[2.2.1]hept-5-enecarboxylate 3[13]

To a nitrogen flushed solution of bicyclo[2.2.1]hept-5-ene-2-*endo*-carboxylic acid **9**[12] (500 mg, 3.6 mmol), triethylamine (0.61 mL, 4.3 mmol) and benzyl alcohol (0.75 mL, 7.3 mmol) in dichloromethane (5.0 mL), EDCI (830 mg, 4.3 mmol) was added in one portion from a screw cap vial with stirring followed by DMAP (88 mg, 0.7 mmol) in an analogous manner. This reaction was stirred overnight whereupon the total volume was diluted to 20 mL using dichloromethane. The solution was washed with 1% HCl (20 mL) and the aqueous re-extracted with dichloromethane (10 mL). The combined organics were washed with H_2O and again the aqueous re-extracted with dichloromethane (10 mL). Total organics were dried (Na_2SO_4), filtered and evaporated with the residue being subject to chromatography using 1 : 20 EtOAc–hexane (R_f = 0.35) as eluent to yield a clear viscous oil. For (2*S*): Yield 690 mg (84%); $[\alpha]_D^{23.5}$ = −105 (c = 43.0); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.27 (1H, d, J = 8.3, H7*syn*), 1.42–1.49 (2H, m, H3*endo*, H7a), 1.92 (1H, ddd, J = 12.9, J = 9.0, J = 3.7, H3*exo*), 2.91 (1H, s, H4), 2.98–3.04 (1H, m, H2), 3.24 (1H, s, H1), 5.06 (1H, d, J = 12.5, C*Ha*HbAr), 5.09 (1H, d, J = 12.5, CHa*Hb*Ar), 5.88 (1H, dd, J = 5.9, J = 2.9, H6), 6.19 (1H, dd, J = 5.6, J = 3.2, H5), 7.29–7.40 (5H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 29.22, 42.55, 43.34, 45.78, 49.61, 65.98, 128.03, 128.47, 132.26, 136.31, 137.79, 174.57; Found 228.1148, $C_{15}H_{16}O_2$ requires 228.1150.

Di-*tert*-butyl 7β-benzyloxycarbonyl-(1α,2β,5β,6α)-tricyclo-[4.2.1.0^{2,5}]nona-3-ene-3,4-dicarboxylate 4

The reaction of norbornene **3** (677 mg, 3.0 mmol) with D^{t}BAD (680 mg, 3.0 mmol) using $RuH_2(CO)(PPh_3)$ (70 mg) in benzene (10 mL) was performed in accordance with the method of Mitsudo.[8] A reaction time of 24 hours was required and the crude product was purified by chromatography (1 : 20 EtOAc–hexane, R_f = 0.30) to afford the title compound as a white, crystalline, solid. For (7*S*): Yield 947 mg (70%);$[\alpha]_D^{23.3}$ = −10.3 (c = 61.0); mp 76–77 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.22 (1H, d, J = 11.0, H9*syn*), 1.47 (9H, s, $CO_2{}^t$Bu), 1.48 (9H, s, $CO_2{}^t$Bu), 1.41–1.65 (2H, m, H9a&H8*endo*), 1.82 (1H, ddd, J = 16.1, J = 11.5, J = 4.6, H8*exo*), 2.29 (1H, d, J = 4.2, H1), 2.61–2.63 (2H, m, H5&6), 2.90 (1H, d, J = 2.9, H2), 2.91 (1H, dt, J = 11.0, J = 4.6, H7), 5.10 (1H, d, J = 12.5, C*Ha*HbAr), 5.17 (1H, d, J = 12.5, CHa*Hb*Ar), 7.26–7.37 (5H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 28.03, 29.95, 32.02, 34.19, 37.68, 42.81, 45.06, 46.38, 66.15, 81.27, 81.36, 128.00, 128.05, 128.44, 136.08, 141.66, 142.37, 160.30, 160.43, 173.67; Found 455.2380, $C_{27}H_{34}O_6$ requires 455.2434.

Di-*tert*-butyl 8β-benzyloxycarbonyl-4-oxa-(1α,2β,3α,5α,6β,7α)-tetracyclo[5.2.1.0^{2,6}.0^{3,5}]decane-3,5-dicarboxylate 2

The alkene diester **4** (200 mg, 0.44 mmol) in dry THF (20 mL) was treated with TBHP (c = 3.0 M, 0.22 mL, 1.5 eq.) and MeLi (c = 1.4 M, 0.22 mL, 0.7 eq.) in accordance with the standard epoxidation method. A reaction time of 12 hours was required and the crude product was purified by chromatography (1 : 8 EtOAc–hexane, R_f = 0.35) to yield the title compound as a crystalline solid. For (8*S*): Yield 180 mg (87%); $[\alpha]_D^{22.5}$ = −8.45 (c = 21.8); mp 93–96 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.48 (10H, s, $CO_2{}^t$Bu), 1.45–1.50 (1H, obscured, H10), 1.55 (1H, dm, J = 12.9, H9*endo*), 1.78 (1H, ddd, J = 11.2, J = 11.2, J = 4.6, H9*exo*), 1.97 (1H, d, J = 11.9, H10), 2.29 (1H, d, J = 3.9, H6), 2.36 (1H, d, J = 3.9, H2), 2.73 (1H, d, J = 4.2, H1), 2.87 (1H, dt, J = 6.1, J = 4.4, H7), 3.03 (1H, d, J = 4.2, H6), 5.09 (1H, d, J = 12.5, C*Ha*HbAr), 5.13 (1H, d, J = 12.5, CHa*Hb*Ar), 7.30–7.36 (5H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 27.98, 29.99, 34.78, 36.97, 40.24, 45.12, 46.03, 49.55, 64.02, 64.15, 66.23, 82.81, 82.86, 128.01, 128.08, 128.15, 128.49, 135.95, 162.89, 173.38.

Di-*tert*-butyl 4β,11β-bis(benzyloxycarbonyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)-hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]-heptadeca-1,8-dicarboxylate 1

The alkene **3** (12 mg, 0.05 mmol) and epoxide **2** (25 mg, 0.05 mmol) were combined in a sealed tube with THF (0.5 mL) and heated overnight at 130 °C. The crude product was purified by chromatography (1 : 6 EtOAc–hexane, R_f = 0.25) to furnish the title compound as a waxy solid. For (4*S*,11*S*): Yield 22 mg (60%); $[\alpha]_D^{22.9}$ = −9.2 (c = 21.8); mp 89–91 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 0.96 (2H, d, J = 9.9, H15&17), 1.46 (18H, s, $CO_2{}^t$Bu), 1.52–1.62 (4H, m, H5α,5β,12α,12β), 1.93 (4H, dd, J = 9.9, J = 6.6, H2,7,9&14), 2.13 (2H, s, H6&13), 2.40 (2H, d, J = 9.9, H15&17), 2.48 (2H, d, J = 4.3, H3&10), 2.66–2.69 (2H, m, H4&11), 5.08 (2H, d, J = 12.5, C*Ha*HbAr), 5.24 (2H, d, J = 12.5, CHa*Hb*Ar), 7.32–7.39 (10H, m, Ph); δ_C(300 MHz, $CDCl_3$, Me_4Si) 28.17, 31.36, 35.75, 38.79, 42.07, 45.63, 50.49, 54.89, 66.37, 81.54, 89.54, 128.19, 128.55, 136.09, 167.78, 173.88; *m*/*z* (ES) 721.5 [$C_{42}H_{50}O_9$ + Na]$^+$.

2-*endo*-(Methoxycarbonylmethylcarbamoyl)bicyclo[2.2.1]hept-5-ene 10[10]

A nitrogen covered solution of 2-*endo*-bicyclo[2.2.1]hept-5-ene-carboxylic acid **9** (500 mg, 3.6 mmol) and glycine methyl ester hydrochloride (400 mg, 3.2 mmol) in DMF (10 mL) was cooled to 0 °C whereupon diisopropylethylamine (420 mg, 0.56 mL, 3.3 mmol), EDCI (700 mg, 3.7 mmol) and HOBt (430 mg, 3.2 mmol) were added. The reaction mixture was stirred overnight at room temperature whereupon TLC analysis indicated the reaction to be complete. Workup consisted of removal of DMF under high vacuum and partitioning of the residue between dichloromethane (30 mL) and 2% HCl (20 mL). Re-extraction of the aqueous using dichloromethane (2 × 20 mL) washing of the combined organics with water (20 mL) followed by drying ($MgSO_4$), filtering and evaporation yielded near pure product. Purification could be achieved using chromatography (3 : 7 EtOAc–hexane R_f = 0.40) or recrystallisation (Et_2O). Yield 670 mg (76%); mp 101–102 °C (Found: C 62.97, H 7.36, N 6.60. $C_{11}H_{15}NO_3$ requires C 63.14, H 7.23, N 6.69%); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.31 (1H, d, J = 8.3, H7a), 1.38 (1H, ddd, J = 12.0, J = 4.4, J = 2.7, H3*endo*), 1.47 (1H, dq, J = 8.3, J = 2.4, H7*syn*), 1.96 (1H, ddd, J = 12.0, J = 9.3, J = 3.7, H3*exo*), 2.93 (2H, m, H2&H4), 3.18 (1H, s, H1), 3.76 (3H, s, CO_2Me), 4.01 (2H, d, J = 5.1, CH_2 Gly), 5.88 (1H, br s, NH), 6.02 (1H, dd, J = 5.6, J = 2.7, H6), 6.25 (1H, dd, J = 5.6, J = 2.9, H5); δ_C(300 MHz, $CDCl_3$, Me_4Si) 29.87, 41.15, 42.68, 44.61, 46.19, 50.01, 52.31, 132.26, 137.87, 170.69, 174.50; Found 209.1053, $C_{11}H_{15}NO_3$ requires 209.1052.

2-*endo*-(1-Methoxycarbonylethylcarbamoyl)bicyclo[2.2.1]-hept-5-ene 11

Synthesised as described for **10** using alanine methyl ester. Purified using column chromatography (3 : 7 EtOAc–hexane R_f = 0.35). For (2*S*): Yield 84%; $[\alpha]_D^{20.8}$ = −142.3 (c = 80.1); mp 89–90 °C (sublimed > 80 °C); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.24 (1H, d, J = 8.3, H7a), 1.31 (3H, d, J = 7.1, CH(CH_3) Ala), 1.30–1.35 (1H, m, H3*endo*), 1.37 (1H, dq, J = 8.3, J = 2.0, H7*syn*), 1.86 (1H, ddd, J = 12.7, J = 9.3, J = 3.7, H3*exo*), 2.82–2.87 (2H, m, H2&H4), 3.12 (1H, s, H1), 3.68 (3H, s, CO_2Me), 4.55 (1H, quint, J = 7.3, C*H*(CH_3) Ala), 5.93 (1H, dd, J = 5.6, J = 2.7, H6), 6.04 (1H, brd, J = 6.6, NH), 6.16 (1H, dd, J = 5.6, J = 2.9, H5); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.22, 29.43, 42.54, 44.39, 46.03, 47.67, 49.78, 52.16, 132.05, 137.43, 173.56, 173.611; Found 223.1205, $C_{12}H_{17}NO_3$ requires 223.1208.

Di-*tert*-butyl 7β-methoxycarbonylmethylcarbamoyl-(1α,2β,5β,6α)-tricyclo[4.2.1.$0^{2,5}$]non-3-ene-3,4-dicarboxylate 12

The reaction of alkene **10** (40 mg, 0.19 mmol) with D^{t}BAD (60 mg, 0.26 mmol) using $RuH_2(CO)(PPh_3)$ (10 mg) in benzene (5.0 mL) was performed in accordance with the method of Mitsudo.[8] A reaction time of 72 hours was required and the crude product was purified by means of gradient chromatography (1 : 1–1 : 0 EtOAc–hexane, R_f[1 : 1 EtOAc–hexane] = 0.15) to afford the title compound as a viscous oil. For (7*S*): Yield 90 mg (98%); $[\alpha]_D^{25.0}$ = +2.4 (c = 18.6); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.25 (1H, d, J = 11.9, H9a), 1.48 (18H, s, $CO_2{}^tBu$), 1.56 (1H, d, J = 10.9, H9*syn*), 1.65 (1H, dm, J = 12.5, H8*endo*), 1.79 (1H, ddd, J = 15.6, J = 11.0, J = 4.6, H8*exo*), 2.29 (1H, d, J = 4.2, H1), 2.48 (1H, d, J = 4.2, H6), 2.72 (1H, d, J = 3.2, H5), 2.78–2.85 (1H, m, H7), 2.86 (1H, d, J = 3.2, H2), 3.75 (3H, s, CO_2Me), 4.05 (2H, m, CH_2 gly), 6.02 (1H, brt, NH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 28.10, 28.11, 29.54, 32.52, 34.21, 38.21, 41.30, 42.42, 46.07, 46.57, 52.30, 81.40, 81.46, 141.29, 142.29, 160.60, 160.73, 170.45, 172.98; m/z (ES) 893.6 $[C_{46}H_{66}N_2O_{14}+Na]^+$(dimer).

Di-*tert*-butyl 8β-(methoxycarbonylmethylcarbamoyl)-4-oxa-(1α,2β,3α,5α,6β,7α)-tetracyclo-[5.2.1.$0^{2,6}$.$0^{3,5}$]decane-3,5-dicarboxylate 13

A solution of alkene diester **12** (80 mg, 0.18 mmol) in dry THF (10.0 mL) was treated with TBHP (0.104 mL, 0.3 mmol) and ^{t}BuOK (14 mg, 0.12 mmol) in accordance with the standard method. A reaction time of 14 hours was required and the crude product was purified by chromatography (1 : 1 EtOAc–hexane R_f = 0.35) to yield the title compound as a slightly yellow coloured solid. For (8*S*): Yield 62 mg (70%); $[\alpha]_D^{23.6}$ = −2.46 (c = 14.4); mp 98–100 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.47 (18H, s, $CO_2{}^tBu$), 1.46–1.61 (2H, obscured, H10a&H9*endo*), 1.74 (1H, ddd, J = 12.7, J = 11.2, J = 4.6, H9*exo*), 1.98 (1H, d, J = 10.7, H10*syn*), 2.36 (1H, d, J = 3.9, H2), 2.46 (1H, d, J = 3.9, H6), 2.72–2.79 (2H, m, H1&H8), 2.93 (1H, d, J = 3.4, H7), 3.75 (3H, s, CO_2Me), 3.97 (1H, dd, J = 18.5, J = 5.4, C*Ha*Hb, Gly), 4.06 (1H, dd, J = 18.4, J = 5.2, CHa*Hb*, Gly), 6.02 (1H, brt, J = 4.9, NH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 27.99, 29.57, 35.20, 37.00, 40.68, 41.26, 45.59, 46.05, 49.61, 52.33, 63.92, 64.19, 82.78, 82.86, 162.99, 163.337, 170.45, 172.63; m/z (ES) 452.6 $[C_{23}H_{33}NO_8 + H]^+$.

Di-*tert*-butyl 4β-(methoxycarbonylmethylcarbamoyl)-11β-(1-methoxycarbonylethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)-hexacyclo[6.6.1.$1^{4,6}$.$1^{10,13}$.$0^{2,7}$.$0^{9,14}$]-heptadeca-1,8-dicarboxylate 14

Epoxide **13** (20 mg, 0.043 mmol) and alkene **11** (15 mg, 0.067 mmol) were combined in a sealed tube with dichloromethane (~0.5 mL) and heated at 130 °C for 12 hours whereupon the contents were concentrated to dryness and the crude product purified by gradient chromatography (1 : 1–1 : 0 EtOAc–hexane, R_f[1 : 1 EtOAc–hexane] = 0.15) to afford the title compound as a white solid. Yield 12 mg (41%); mp 118–120 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 0.99 (2H, d, J = 10.0, H15,17), 1.42 (3H, d, J = 7.3, CH(C*H*$_3$) Ala), 1.515 (9H, s, $CO_2{}^tBu$), 1.518 (9H, s, $CO_2{}^tBu$), 1.51–1.61 (4H, part. obscured, H5α,β,H12α,β), 2.08–2.21 (6H, m, H2,6,7,9,13,14), 2.33 (2H, s, H3,10), 2.42–2.49 (2H, m, H15,17), 2.54 (2H, dt, J = 10.5, J = 5.9, H4,11), 3.778 (3H, s, CO_2Me), 3.781 (3H, s, CO_2Me), 4.02 (1H, dd, J = 18.5, J = 4.9, C*Ha*Hb Gly), 4.15 (1H, dd, J = 18.3, J = 5.3, CHa*Hb* Gly), 4.60 (1H, quint, J = 7.1, C*H*(CH_3) Ala), 5.92 (1H, br t, J = 5.1, NH Gly), 5.97 (1H, d, J = 7.3, NH Ala); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.62, 28.17, 28.24, 30.87, 30.90, 36.15, 36.29, 38.82, 41.33, 42.46, 42.50, 46.61, 46.66, 48.11, 49.80, 49.82, 52.37, 52.44, 55.06, 55.14, 81.56, 89.61, 89.72, 168.04, 168.19, 170.49, 172.28, 172.98, 173.43.

Di-*tert*-butyl 7β-carboxylic acid-(1α,2β,3α,5α,6β,7α)-4-oxa-tetracyclo[5.2.1.$0^{2,6}$.$0^{3,5}$]decane-3,5-dicarboxylate 15

A solution of benzyl ester epoxide **2** (66 mg, 0.14 mmol) and 2% acetic acid (2 drops) in EtOAc (10 mL) was stirred under a hydrogen atmosphere in the presence of a 10% palladium on carbon catalyst (~10 mg) for 24 hours whereupon TLC indicated consumption of starting material. The reaction mixture was carefully filtered through Celite to remove the catalyst, the filter cake being washed thoroughly with EtOAc to ensure all product was collected. The solvent was removed under reduced pressure and the residue completely dried under high vacuum to provide a slightly impure material that was used directly in the next step. Yield 53 mg (~100%); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.48 (9H, s, $CO_2{}^tBu$), 1.49 (9H, s, $CO_2{}^tBu$), 1.48–1.57 (2H, obscured, H10a&H9*endo*), 1.80 (1H, ddd, J = 16.3, J = 11.5, J = 4.9, H9*exo*), 1.99 (1H, d, J = 10.0, H10*syn*), 2.36 (1H, d, J = 3.9, H6), 2.47 (1H, d, J = 3.7, H2), 2.75 (1H, d, J = 3.9, H1), 2.88 (1H, dt, J = 11.2, H8), 3.06 (1H, d, J = 3.7, H7); δ_C(300 MHz, $CDCl_3$, Me_4Si) 27.94, 29.86, 34.82, 36.89, 40.15, 44.81, 45.97, 49.47, 63.97, 64.06, 82.84, 82.94, 162.76, 162.97, 179.11; m/z (ES) 381.3 $[C_{20}H_{28}O_7 + H]^+$.

Di-*tert*-butyl 8β-(1-methoxycarbonylethylcarbamoyl)-4-oxa-(1α,2β,3α,5α,6β,7α)-tetracyclo[5.2.1.$0^{2,6}$.$0^{3,5}$]decane-3,5-dicarboxylate 20

A solution of epoxyacid **15** (53 mg, 0.14 mmol) and alanine methyl ester hydrochloride (25 mg, 0.18 mmol) in DMF

(2.0 mL) was prepared under nitrogen and to it diisopropylethyl amine (23 mg = 30 μl, 0.18 mmol), EDCI (40 mg, 0.21 mmol) and HOBt (5 mg) were added and the reaction allowed to stir overnight. The solvent was removed under high vacuum and the residue taken up in dichloromethane (10 mL) and washed with sat. citric acid (5.0 mL) followed by water (5.0 mL). The organic was dried (Na_2SO_4) filtered and evaporated to yield a product which when chromatographed (1 : 4 EtOAc–hexane R_f = 0.30) provided the title compound as a clear viscous oil. For (8*S*): Yield 20 mg (31%); $[\alpha]_D^{22.7}$ = −11.4 (*c* = 26.0); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.38 (3H, d, *J* = 7.1, CH(CH_3) Ala), 1.47 (18H, s, $CO_2{}^tBu$), 1.41–1.59 (2H, obscured m, H10a&H9*endo*), 1.72 (1H, ddd, *J* = 16.1, *J* = 12.7, *J* = 4.9, H9*exo*), 1.98 (1H, d, *J* = 10.5, H10*syn*), 2.35 (1H, d, *J* = 3.9, H2), 2.47 (1H, d, *J* = 3.9, H6), 2.68–2.75 (2H, m, H1&H8), 2.93 (1H, d, *J* = 3.4, H7), 3.74 (3H, s, CO_2Me), 4.52 (1H, quint, *J* = 7.3, C*H*(CH_3) Ala), 6.01 (1H, brd, *J* = 7.1, NH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.39, 28.00, 29.55, 35.20, 37.04, 40.66, 45.56, 46.12, 48.10, 49.59, 52.42, 64.00, 64.16, 82.74, 82.80, 163.05, 163.27, 171.99, 173.43; *m*/*z* (ES) 488.3 $[C_{24}H_{35}NO_8 + Na]^+$.

Di-*tert*-butyl 8β-(benzyloxycarbonylmethylcarbamoyl)-4-oxa-(1α,2β,3α,5α,6β,7α)-tetracyclo[5.2.1.0^{2,6}.0^{3,5}]decane-3,5-dicarboxylate 16

Synthesised as for **20** using glycine benzyl ester. Purified by column chromatography (1 : 3 EtOAc–hexane R_f = 0.35) to afford a colourless, slightly viscous oil. For (8*S*): Yield 58%; $[\alpha]_D^{21.3}$ = −6.6 (*c* = 30.7); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.47 (9H, s, $CO_2{}^tBu$), 1.48 (9H, s, $CO_2{}^tBu$), 1.47–1.48 (1H, obs, H10a), 1.58 (1H, ddd, *J* = 12.9, *J* = 5.4, *J* = 2.7, H9*endo*), 1.74 (1H, ddd, *J* = 12.7, *J* = 11.5, *J* = 4.6, H9*exo*), 1.99 (1H, d, *J* = 10.5, H10*syn*), 2.36 (1H, d, *J* = 3.9, H2), 2.47 (1H, d, *J* = 3.9, H6), 2.72–2.79 (2H, m, H1, H8), 2.94 (1H, d, *J* = 3.4, H7), 4.00 (1H, dd, *J* = 18.6, *J* = 4.9, CH_AH_B), 4.11 (1H, dd, *J* = 18.6, *J* = 4.9, CH_AH_B), 5.18 (2H, s, CH_2Ar), 6.02 (1H, t, *J* = 5.1, NH), 7.30–7.40 (5H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 27.99, 29.59, 35.20, 36.99, 40.66, 41.44, 45.59, 46.06, 49.61, 63.92, 64.18, 67.20, 82.78, 82.85, 128.38, 128.52, 128.61, 135.05, 163.00, 163.33, 169.88, 172.63.

Di-*tert*-butyl 4β-(benzyloxycarbonylmethylcarbamoyl)-11β-(1-methoxycarbonylethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)-hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}] heptadeca-1,8-dicarboxylate 17

Epoxide **16** (40 mg, 0.076 mmol) and alkene **11** (40 mg, 0.17 mmol) were combined in a sealed tube with dichloromethane (~0.5 mL) and heated at 130 °C for 12 hours. The tube was subsequently cracked and the contents concentrated to dryness to afford a crude product that was purified by chromatography (1 : 1 EtOAc–hexane R_f = 0.15) to yield the title compound as a glassy solid. For (4*S*,11*S*): Yield 41 mg (70%); $[\alpha]_D^{21.0}$ = −29.6 (*c* = 26.7); mp 107–109 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 0.99 (2H, d, *J* = 9.3, H15,H17), 1.41 (3H, d, *J* = 7.1, CH(CH_3) Ala), 1.49 (9H, s, $CO_2{}^tBu$), 1.52 (9H, s, $CO_2{}^tBu$), 1.45–1.61 (4H, m, H5α,H5β,H12α,H12β), 2.08–2.21 (6H, m, H2,H6,H7,H9,H13,H14), 2.33 (2H, s, H3,H10), 2.42–2.59 (4H, m, H15,H17,H4,H11), 3.78 (3H, s, CO_2Me), 4.04 (1H, dd, *J* = 18.6, *J* = 4.6, CH_aH$_b$ gly), 4.21 (1H, dd, *J* = 18.6, *J* = 5.4, CH$_a$$H_b$ gly), 4.60 (1H, quint, *J* = 7.1, C*H*(CH_3) Ala), 5.21 (2H, s, CH_2Ar), 5.92 (1H, t, *J* = 4.9, NH gly), 5.97 (1H, d, *J* = 7.1, NH Ala), 7.34–7.40 (5H, m, ArH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.64, 28.17, 28.23, 30.85, 30.90, 36.17, 36.30, 38.83, 41.49, 42.49, 46.66, 48.11, 49.80, 52.46, 55.06, 55.10, 67.21, 81.56, 81.58, 89.62, 89.73, 128.39, 128.52, 128.63, 135.15, 168.06, 168.18, 169.97, 172.30, 173.00, 173.44.

Di-*tert*-butyl 4β-(1-carboxymethylcarbamoyl)-11β-(1-methoxycarbonylethylcarbamoyl)-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)-16-oxahexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]heptadeca-1,8-dicarboxylate 18

A solution of benzyl ester **17** (40 mg, 0.022 mmol) and 1 M acetic acid (2 drops) in EtOAc (20 mL) was stirred for 24 hours with a catalytic amount of palladium on carbon (< 5 mg) under an atmosphere of hydrogen. After 24 hours TLC analysis of the reaction mixture indicated that all starting material had been consumed. Workup consisted of removal of catalyst by filtration (Whatman no. 1), followed by concentration *in vacuo* that provided a crude product which, when examined by NMR displayed no benzyl resonances. This material was used directly in the next step without further purification. Yield 28 mg (79%); δ_H(300 MHz, CD_3OD, Me_4Si) 1.02 (1H, d, *J* = 8.8), 1.03 (1H, d, *J* = 9.8), 1.38 (3H, d, *J* = 7.1, CH(CH_3) Ala), 1.54 (9H, s, $CO_2{}^tBu$), 1.56 (9H, s, $CO_2{}^tBu$), 1.49–1.61 (4H, m), 2.08–2.18 (4H, m), 2.26–2.29 (2H, m), 2.33–2.38 (2H, m), 2.41–2.45 (2H, m), 2.58–2.69 (2H, m), 3.76 (3H, s, CO_2Me), 3.87–3.92 (2H, m, CH_2 gly), 4.32 (1H, q, *J* = 7.3, C*H*(CH_3) Ala), 8.10 (1H, brt, NH), 8.19 (1H, brd, NH); δ_C(300 MHz, CD_3OD, Me_4Si) 18.23, 21.61, 29.34, 30.38, 32.28, 32.37, 37.69, 37.90, 41.27, 42.94, 45.27, 47.91, 48.28, 49.01, 50.63, 50.71, 52.15, 52.22, 53.63, 57.33, 57.39, 61.84, 67.85, 84.05, 84.15, 91.96, 92.13, 170.91, 170.92, 173.73, 175.32, 176.23, 176.82.

Di-*tert*-butyl 4β-[1-{[(methoxycarbonylmethylcarbamoyl)-methyl]carbamoyl}methylcarbamoyl]-11β (1-methoxycarbonyl-ethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α, 14β)-hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]heptadeca-1,8-dicarboxylate 19

A solution of acid **18** (20 mg, 0.030 mmol), glycyl-glycine methyl ester (100 mg, 0.68 mmol) and diisopropylethylamine (105 mg, 0.14 mL, 0.81 mmol) in dry DMF (2.5 mL) was prepared under nitrogen and to it EDCI (200 mg, 1.0 mmol) and HOBt (10 mg) were added in quick succession from screw cap vials. The reaction mixture was subsequently stirred for 72 hours. Workup consisted of removal of solvent under high vacuum, redissolving the residue in dichloromethane (10 mL) and washing of this organic solution with saturated citric acid (2 × 5 mL) followed by water (5 mL). The combined organics were set aside and the combined aqueous extracted with a second portion of dichloromethane (10 mL), the total organics combined, dried (Na_2SO_4), filtered and evaporated to yield a crude that when subject to chromatography (10 : 99 : 1 MeOH–EtOAc–NH_4OH R_f = 0.15) provided the title compound as a waxy white solid. For (4*S*,11*S*): Yield 16 mg (68%); $[\alpha]_D^{23.1}$ = −23.8 (*c* = 10.4); mp 198–200 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 0.97 (2H, d, *J* = 9.5, H15, H17), 1.39 (3H, d, *J* = 7.0, CH(CH_3) Ala), 1.48 (9H, s, $CO_2{}^tBu$), 1.50 (9H, s, $CO_2{}^tBu$), 1.44–1.56 (4H, m, H5α,H5β,H12α,H12β), 2.05 (1H, d, *J* = 6.9, H7), 2.08 (3H, br s, H6,H9,H14), 2.12 (1H, d, *J* = 3.7, H13), 2.18 (1H, d, *J* = 6.6, H2), 2.31 (1H, d, *J* = 4.0, H3), 2.35–2.36 (2H, m, H15,H10), 2.40 (1H, d, *J* = 10.3, H17), 2.47–2.52 (1H, m, H4), 2.59 (1H, dt, *J* = 10.9, *J* = 4.8, H11), 3.72 (3H, s, CO_2Me), 3.75 (3H, s, CO_2Me), 3.99–4.05 (6H, m, CH_2 × 3 gly × 3), 4.56 (1H, quint, *J* = 7.3, C*H*(CH_3) Ala), 6.04 (1H, d, *J* = 7.3, NH Ala), 6.71 (1H, t, *J* = 5.5, NH gly), 7.15 (1H, t, *J* = 5.1, NH gly), 7.23 (1H, t, *J* = 5.1, NH gly); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.63, 28.24, 28.33, 30.61, 30.92, 36.13, 36.31, 38.92, 38.96, 41.26, 42.64, 42.86, 43.10, 43.49, 46.66, 46.76, 48.25, 49.91, 49.99, 52.42, 52.52, 55.15, 55.56, 81.87, 82.26, 89.65, 90.01, 168.19, 169.03, 169.50, 170.20, 170.27, 172.48, 173.46, 174.18; *m*/*z* (ES) 789.5 $[C_{39}H_{56}N_4O_{13} + H]^+$; Found 788.3841 $C_{39}H_{56}N_4O_{13}$ requires 788.3844.

Di-*tert*-butyl 4β,11β-bis(1-methoxycarbonylethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)-hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]heptadeca-1,8-dicarboxylate 21

Epoxide **20** (30 mg, 0.06 mmol) and alkene **11** (20 mg, 0.09 mmol) were combined in a sealed tube with dichloromethane

(~0.5 mL) and heated for 6 hours at 140 °C. The tube was subsequently cracked and the contents concentrated to dryness to produce a crude product that was purified by gradient chromatography (1 : 1 EtOAc–hexane–1 : 0 EtOAc–hexane, R_f[1 : 0 EtOAc–hexane] = 0.75) to afford a white solid. For (4*S*,11*S*): Yield 47 mg (90%); $[\alpha]_D^{25} = -50.7$ ($c = 21.2$); mp 122–125 °C; δ_H(300 MHz, $CDCl_3$, Me_4Si) 0.97 (2H, d, J = 9.8, H15,H17), 1.40 (6H, d, J = 7.1, CH(CH_3) Ala), 1.51 (18H, s, $CO_2{}^tBu$), 1.50–1.59 (4H, obscured m, H5α,H5β,H12α,H12β), 2.08 (2H, d, J = 6.6, H7,H14), 2.12 (2H, d, J = 3.9, H6,H13), 2.19 (2H, d, J = 6.6, H2,H9), 2.31 (2H, d, J = 4.1, H3,H10), 2.42 (2H, d, J = 8.9, H15,H17), 2.49 (2H, dt, J = 10.5, J = 4.6, H4,H11), 3.77 (s, 6H, CO_2Me), 4.59 (2H, q, J = 7.1, CH(CH$_3$) Ala), 5.99 (2H, d, J = 7.1, NH); δ_C(300 MHz, $CDCl_3$, Me_4Si) 18.6, 28.14, 30.87, 36.27, 38.80, 42.46, 46.60, 48.08, 49.75, 53.43, 55.03, 81.59, 89.64, 168.14, 172.27, 173.44; *m*/*z* (ES) 711.6 $[C_{36}H_{52}N_2O_{11} + Na]^+$.

4β,11β-Bis(1-methoxycarbonylethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β,8α,9β,10α,13α,14β)hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]-heptadeca-1,8-dicarboxylic acid 22

A 1 : 1 solution of TFA in dry dichloromethane (1.0 mL) was added quickly in one portion to a nitrogen flushed round bottom flask containing the alanyl-alanine-di-*tert*-butyl ester framework **20** (40 mg, 0.058 mmol) and a magnetic stirrer. The resultant solution was stirred under a nitrogen atmosphere overnight whereupon TLC analysis indicated complete consumption of starting material. Solvent was removed *in vacuo* and complete dryness achieved using high vacuum. For (4*S*,11*S*): Yield 27 mg (81%); $[\alpha]_D^{26} = -57.1$ ($c = 24.0$); mp 270 °C (140 °C polymerisation, 170 °C partial decomposition with odour, 240–270 °C slow decolourisation and decomposition); δ_H(300 MHz, CD_3OD, Me_4Si) 1.02 (2H, d, J = 9.8, H15,H17), 1.36 (6H, d, J = 7.3, CH(CH_3) Ala), 1.50 (2H, td, J = 11.2, J = 4.2, H5α,H12α), 1.56–1.62 (2H, m, H5β,H12β), 2.14–2.16 (4H, m, H6,H7,H13,H14), 2.32 (2H, d, J = 6.6, H2,H9), 2.39 (2H, d, J = 9.0, H15,H17), 2.49 (2H, d, J = 4.2, H3,H10), 2.60 (2H, dt, J = 11.0, J = 4.6, H4,H11), 3.76 (6H, s, CO_2Me), 4.38 (2H, q, J = 7.3, CH(CH$_3$) Ala); δ_C(300 MHz, CD_3OD, Me_4Si) 17.25, 31.53, 36.99, 40.51, 44.33, 47.11, 49.61, 51.31, 52.83, 56.55, 172.43, 174.72, 175.28; *m*/*z* (ES) 577.3 $[C_{28}H_{36}N_2O_{11} + H]^+$.

1,8-Bis(methoxycarbonyl-methylcarbamoyl)-4β,11β-bis(1-methoxycarbonylethylcarbamoyl)-16-oxa-(1α,2β,3α,6α,7β, 8α,9β,10α,13α,14β)-hexacyclo[6.6.1.1^{4,6}.1^{10,13}.0^{2,7}.0^{9,14}]heptadecane 23

A solution of the preceding diacid **22** (27 mg, 0.047 mmol), glycine methyl ester hydrochloride (25 mg, 0.20 mmol), diisopropylethylamine (15 mg, 0.020 mL, 0.12 mmol) in dry DMF (1.0 mL) was prepared under nitrogen and to this solution the following were added successively from screw cap vials: EDCI (35 mg, 0.18 mmol) and HOBt (5 mg). The reaction was subsequently stirred at room temperature for a total of 48 hours. Workup consisted of removal of solvent under high vacuum, redissolving the residue in dichloromethane (10 mL). This organic solution was washed with saturated citric acid (2 × 5 mL) and water (5 mL). The combined organics were set aside and the combined aqueous extracted with a second portion of dichloromethane (10 mL), the total organics combined, dried (Na_2SO_4), filtered and evaporated to afford a crude that when subject to gradient chromatography (1 : 1 EtOAc–hexane–1 : 10 MeOH–EtOAc, R_f[1 : 10 MeOH–EtOAc] = 0.35) afforded the title compound as a waxy solid. For (4*S*,11*S*): Yield 17 mg (50%); mp 104–106 °C; $[\alpha]_D^{23.5} = -20.5$ (10.5); δ_H(300 MHz, $CDCl_3$, Me_4Si) 1.04 (2H, d, J = 10.3, H15,H17), 1.36 (6H, d, J = 7.3, CH(CH_3) Ala), 1.57 (2H, td, J = 12.2, J = 4.6, H5α,H12α), 1.70 (2H, dd, J = 12.4, J = 2.7, H5β,H12β), 2.13 (2H, d, J = 5.9, H7,H14), 2.26 (4H, m, H2,H9,H6,H13), 2.34 (2H, d, J = 10.0, H15,H17), 2.54–2.58 (2H, m, H4,H11), 2.69 (2H, brs, H3,H10), 3.75 (6H, s, CO_2Me), 3.82 (6H, s, CO_2Me), 4.50–4.67 (6H, brm, CH_2 gly, CH(CH$_3$) Ala); δ_C(300 MHz, CD_3OD, Me_4Si) 13.27, 25.84, 31.88, 34.08, 35.42, 36.98, 41.76, 43.13, 45.55, 47.90, 48.34, 51.22, 86.55, 164.83, 166.7, 168.45; *m*/*z* (ES) 719.4 $[C_{34}H_{46}N_4O_{13}+H]^+$; Found 718.3050, $C_{34}H_{46}N_4O_{13}$ requires 718.3061.

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