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University of Wollongong
NSW Australia

Synthetic approaches to bis-peptides attached on polynorbornane molecular scaffolds with well-defined relative positions and distances

by Muhong Shang [A], *Ronald N. Warrener* [B], *Douglas N. Butler* [B] *and Davor Margetić** [C]

[A] Department of Chemistry, University of Waterloo, 200 University Avenue West,Waterloo, Ontario N2L 3G1, Canada

[B] Intelligent Polymer Research Institute,University of Wollongong, Northfields Avenue,Wollongong, NSW, 2522, Australia

[C] Laboratory for Physical Organic Chemistry, Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia

(*) Corresponding author. Email: margetid@emma.irb.hr

Abstract. This paper describes novel synthetic approaches to polynorbornane molecular scaffolds substituted with peptides at various, well-defined positions. A library of norbornene building blocks with attached peptides was prepared. Alkene cyclobutane epoxide (ACE) coupling method was used as a key step reaction for connecting of two norbornene building blocks into bis-peptide scaffolds. Photodimerization of cyclobutene diesters offers alternative route to polynorbornane bis-peptides. Pyrrolo-peptides were used for preparation of peptide substituted 7-aza norbornenes. Unsymmetrical bis-peptide scaffolds were prepared by ACE coupling of peptide-norbornane epoxide with another norbornene-peptide block. Chemical elaboration of bridgehead dimethyl esters of ACE products or epoxide ACE reagents was also used for peptide attachment.

Introduction. Peptide like compounds with retained biological activity and improved pharmacokinetics are called peptidomimetics. Common approach towards the development of peptidomimetics is to use a molecular template or scaffold (often rigid heterocyclic ring systems) to which important pharmacophoric groups, such as amino acid side

chains, can be attached. Such compounds have the potential of being orally active, selective and metabolically stable. Non-native architectures in protein design and mimicry[1] often employ topological templates (synthetic devices that orient functional groups or structural units in well-defined spatial arrangements). Typically, template molecules represent structural motifs such as constrained polycyclic systems disposing selectively addressable functional groups. The use of templates exhibiting a predetermined backbone conformation for the selective attachment of functional sites (e.g. amino acid side-chains or peptides) is new approach in molecular recognition studies and peptide mimicry. Here conformationally constrained molecules are used as templates by geometrically fixing the first amino acid in the proper orientation for helix or b-sheet peptide initiation. As template molecules tetraphenyl porphyrin[3], 2,3,4-trisubstituted pyridine[3], substituted chromanones, isochromanones and diketopiperazines[4], calixarenes[5], constrained cyclic peptides[6,7], cyclodextrins[8], steroids[9], monosaccharides[10] and tetrahydrofuran[11] were used in literature.

Synthetic procedures for efficient production of series of conformationally more rigid structures (scaffolds) based on norbornene compounds were also developed. Non-peptidic norbornene scaffolds were used for protein-core mimetics[12], as inducers containing parallel peptides norbornene dicarboxylic acid[13] and 2,3-diazabicyclo[2.2.1]heptane constrained azapeptide templates.[14]

In our laboratories we have developed efficient synthetic strategies and methods for the attachment of amino acids and peptides to polynorbornane templates in well-defined relative positions by 'LEGO' BLOCK protocols.[15,16,17] Synthetically available attachment points identified on the polynorbornyl framework are the following: 1) bridgeheads, 2) end termini, 3) bridge and 4) *endo*- position (as schematically drawn in **Figure 1**). In this paper some novel synthetic strategies for peptide attachments to polynorbornenes have been reported.

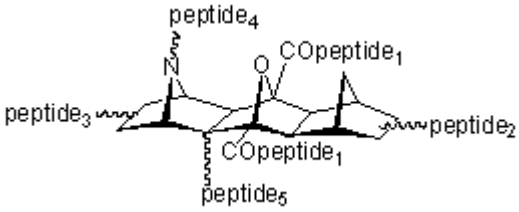
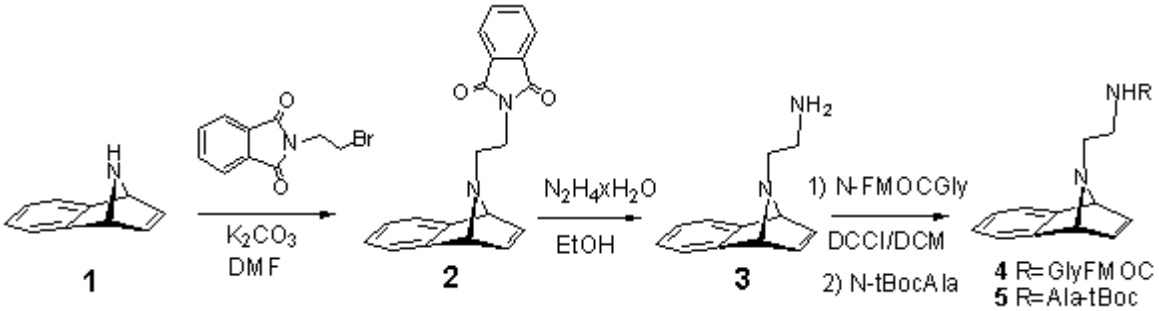


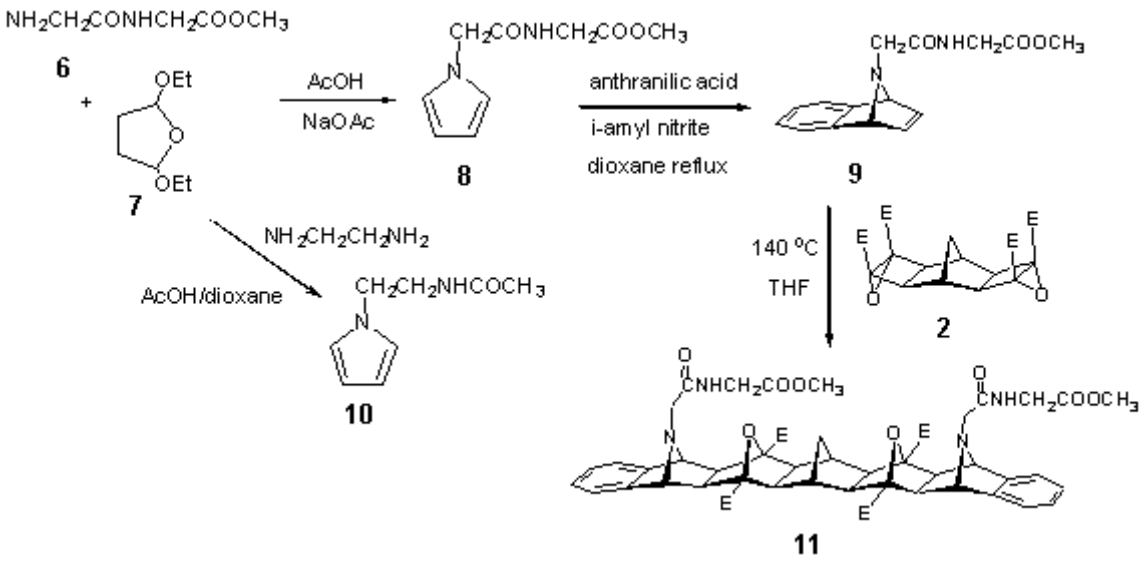
Figure 1

Aza bridge peptide attachment. This particular substitution offers, together of well-defined separation of two peptides, certain degree of flexibility due to the 7-aza bridge nitrogen inversion.[18] Derivatives of 7-azanorbornenes were used as synthetically the easiest access to attachment of peptides to the aza bridge. Thus, substitution of 7-azabenzonorbornadiene **1** with N-(2-bromoethyl)phthalimide in DMF afforded **2**, while deprotection of **2** with hydrazine hydrate[19] gave amine **3** (**Scheme 1**) in high yield. Standard dicyclohexyl carbodiimide (DCCI) peptide couplings with ester-protected aminoacids such as N-FMOCGly and N-tBocAla gave products **4** and **5**.[20]



Scheme 1

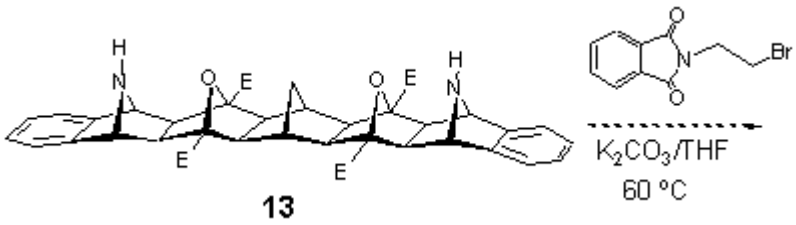
When substrates **4** and **5** were subjected to the ACE reaction with tetracyclic bis-epoxide **2** (in THF at 140 C, 2 hours) complex mixtures were produced, which was caused by decomposition of thermally unstable substrates.



Scheme 2

Analogous synthetic route to peptides attached on 7-azanorbornene skeleton employs as a key step Diels-Alder cycloaddition of appropriately substituted pyrroles such as **8** (**Scheme 2**). Standard condensation reaction of diethoxy tetrahydrofuran**[21]** **7** with methyl glycyl glycinate **6** and sodium acetate in acetic acid produced pyrrole **8** in 50 % yield. Methyl glycyl glycinate **6** was obtained by reaction of thionyl chloride and glycyl glycine in methanol**[22]** (60 %). Solution of substituted pyrrole **8** in dioxane was treated with anthranilic acid and isoamyl nitrite to afford 11 % of product **9**. Coupling of **9** with tetracyclic bis-epoxide **11** in dichloromethane by heating at 140 ^{o}C for 2 hours in a sealed glass tube produced bis-adduct **12** in 70 % yield.

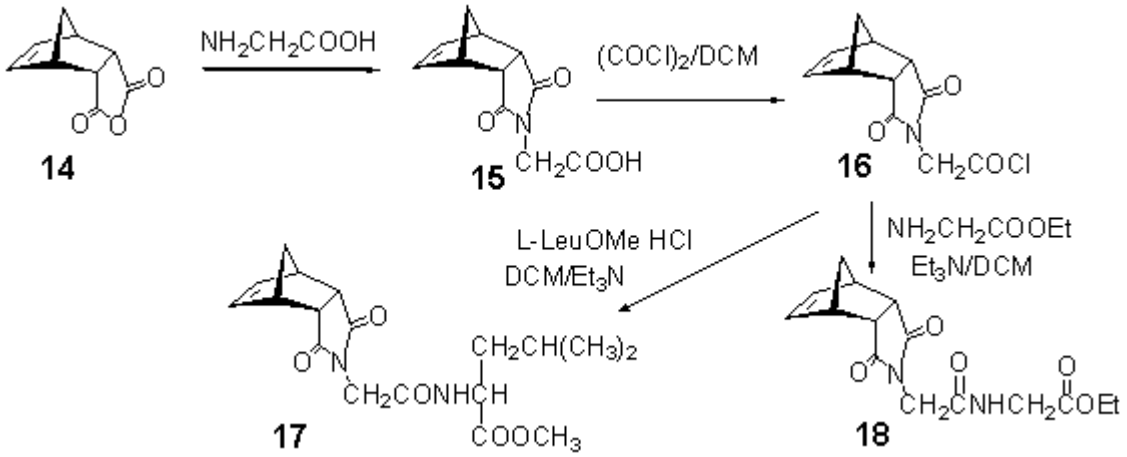
The other pyrrole **10** was used to obtain amine **3** (**Scheme 2**). Here, a mixture of 1,2-ethanediamine, acetic acid and diethoxy THF in dioxane was reacted to give almost pure product**[23]** (21 %). However, benzyne addition to this pyrrole was not satisfactory, either using standard anthranilic acid/isoamyl nitrite or amino benzotriazole/lead tetraacetate method.**[24]** Both reactions produced complex reaction mixtures, whose separations did not yield adduct.



Scheme 3

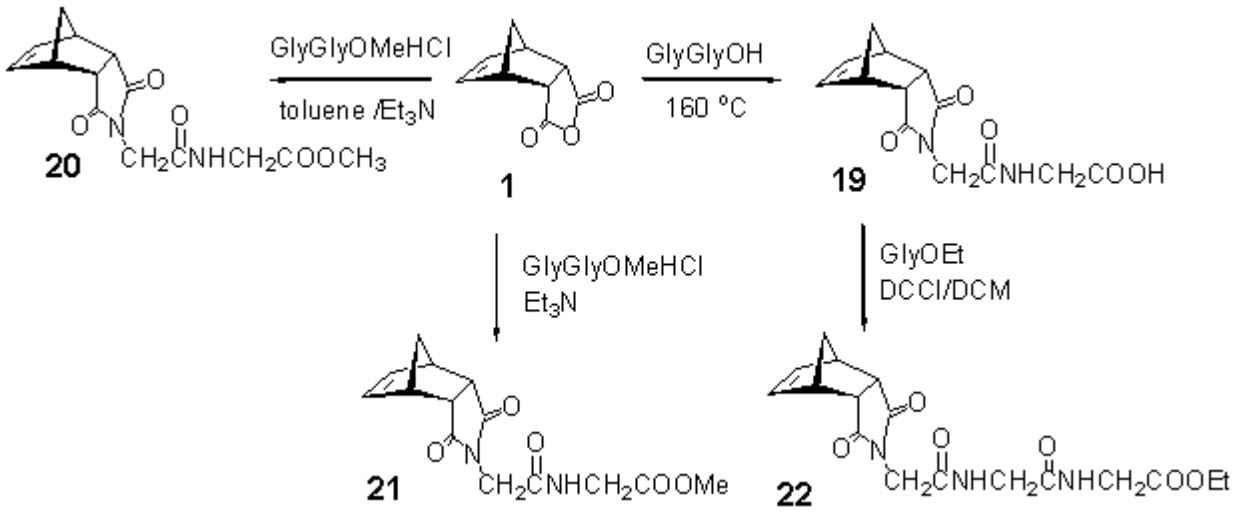
Finally, our attempts to substitute previously prepared polycyclic scaffold **13****[25]** by the direct alkylation with N-(2-bromoethyl)phthalimide, did not produce desired N-substituted polynorbornane (**Scheme 3**).

***Endo*-anhydride attachment.** In this chapter, we present synthetic work on building the library of norbornene-peptide blocks suitable for ACE coupling reaction. The *endo*- adduct of cyclopentadiene and maleic anhydride **2** was used for the peptide attachment point **(Scheme 4)**.



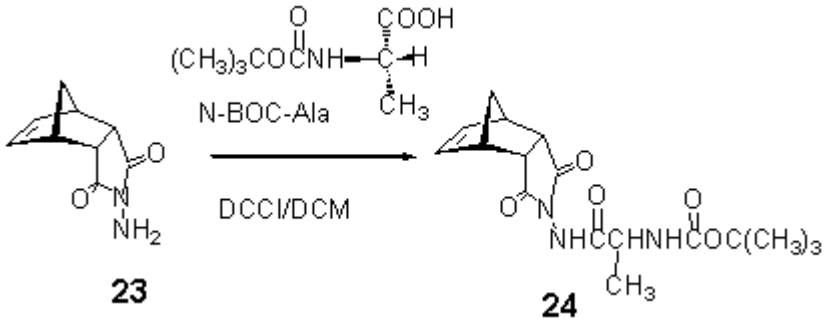
Scheme 4

To prepare imide **15**, different reaction conditions were used (**Scheme 4**): literature method **[26]** employs refluxing of anhydride **14**, glycine and magnesium sulphate in tetrahydrofuran for 15 hours (63 % yield). It was found that almost quantitative yield of **15** (96 %) could be obtained by melting mixture of equimolar amounts of anhydride **14** and glycine at 150 °C for half an hour. Acid **15** was further treated with oxalyl chloride and converted to chloride **16** by reflux (88 %).**[27]** This acid chloride **16** was used as convenient starting material for the synthesis of the desired peptides **17** and **18**. Thus, the reaction of compound **16** with the methyl ester of L-leucine gave monopeptide **17** (76 %), while reaction with ethyl ester of glycine gave **18** (42 %).



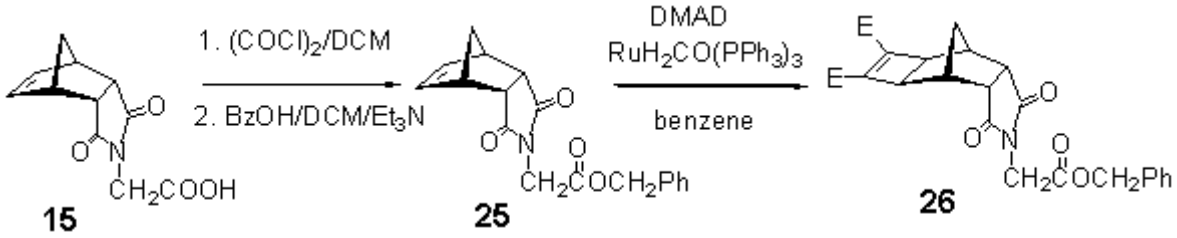
Scheme 5

Norbornene peptide BLOCKS could be directly prepared by condensation of anhydride **1** with corresponding amino acid or acid group protected aminoacid (**Scheme 5**). Thus, when anhydride **1** and glycyl glycinate were melted at 160 ^{o}C for 1 hour, 96 % yield of acid **19** was obtained. Methyl glycyl glycinate was condensed with anhydride **1** at toluene reflux to afford **20** in 60 % yield.**[25]** Similarly, reaction of **1** and glycyl glycinate ethyl ester hydrochloride at reflux gave norbornene block **21** in 49 % yield**[25]**. Dipeptide derivative **22** was prepared by DCCI coupling of monopeptide **19** with ethyl glycinate (53 %).**[28]**



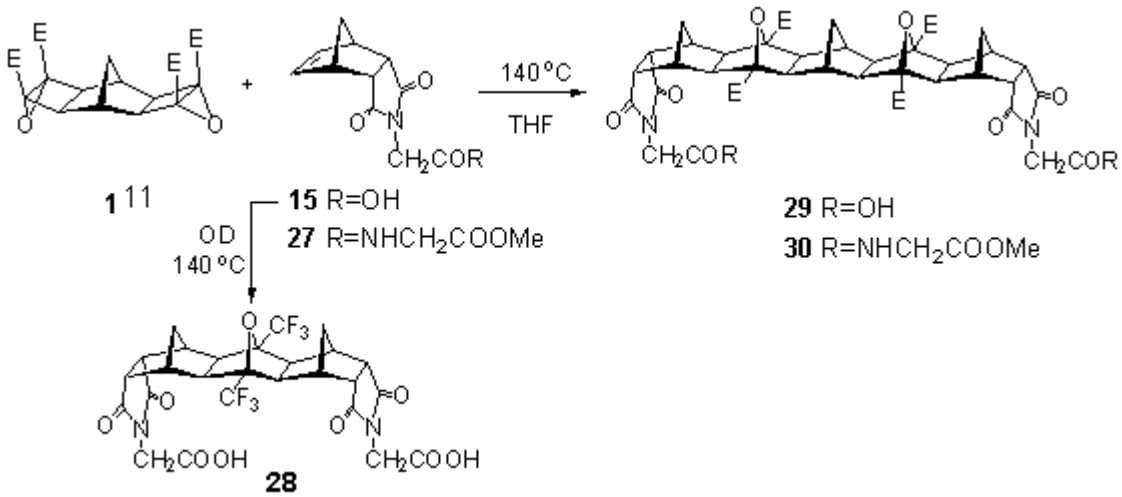
Scheme 6

Imido-acid **23** was selected as suitable building block for peptide coupling of aminoacids with protected amino group and norbornene blocks. Thus, reaction of N-BOC-Ala and imide **23**[29] in DCM facilitated with DCCI gave **24** in 72 % yield (**Scheme 6**).



Scheme 7

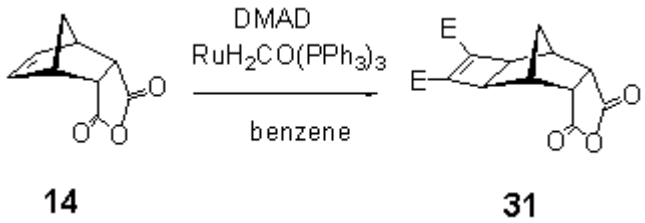
Benzyl ester **25** required for our synthetic work was prepared by two methods: by transformation of acid **15** into acid chloride and subsequent esterification with benzyl alcohol, and by condensation of acid with benzyl chloride using potassium carbonate in DMF (in 57 and 78 % yields, respectively) (**Scheme 7**). Benzyl ester **25** was further transformed into **26** by catalytic [2p+2p] Mitsudo reaction (dimethylacetylene dicarboxylate and $RuH_2CO(PPh_3)_3$ catalyst)[30] in benzene in 78 % yield.



Scheme 8

In a final reaction step, two norbornene peptide containing blocks (**15** and **27**) were joined by the double 1,3-dipolar ACE coupling reaction[31] with bis-epoxide **11** in THF and heating at 140 °C for 2 hours in a sealed glass tube (**Scheme 8**). In both reactions, symmetrical products were obtained. Bis-acid **29** was obtained in 70 %, while bis-peptide **30** in 62 % yield. Interestingly, it was found that *s*-tetrazine coupling of **27** did not produce desired

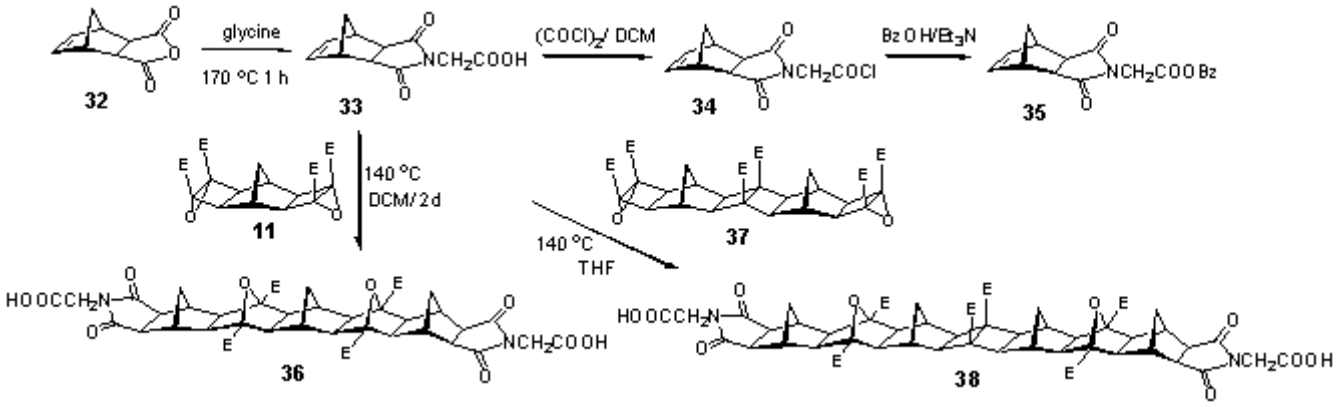
adduct. Smaller separation distance between two peptide units in polycycle **28** was achieved by 1,3,4-oxadiazole (OD) coupling[32] of acid **15** (at 140 ^{o}C for 2 days, 42 %).



Scheme 9

In contrast to benzyl ester **2**, (**in Scheme 7**), anhydride **14** poorly reacts with dimethyl acetylene dicarboxylate and $RuH_2CO(PPh_3)_3$ catalyst and yields only 11 % of product **31** (**Scheme 9**).

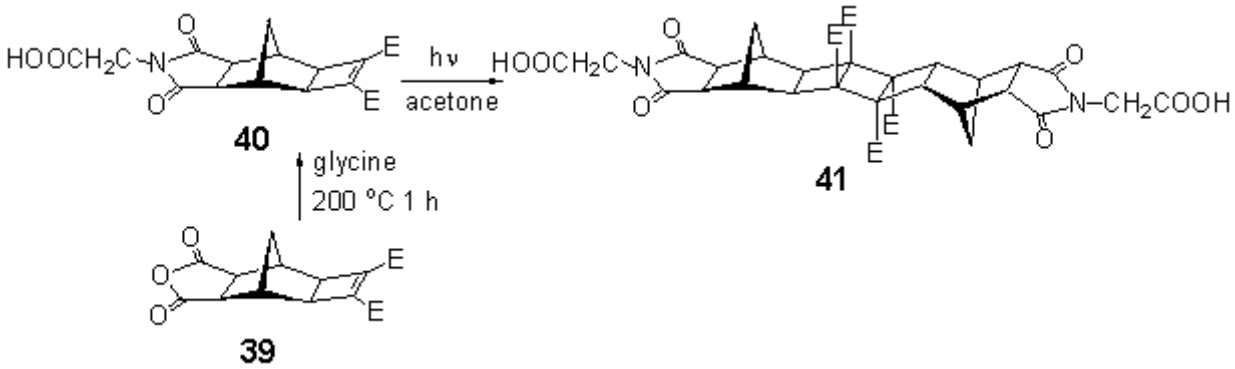
***Exo*-anhydride attachment.** Chemistry with *exo*- anhydride **32** is analogous to the work with *endo*- anhydride described above. As indicated by semiempirical AM1 modelling, the use of this anhydride enables preparation of polycylic scaffolds with longer CH_2-CH_2 geometrical separations.



Scheme 10

Acid **33** was prepared by heating of equimolar amounts of *exo*- norbornene dicarboxylic anhydride and glycine at 170 °C for 1 hour, while being stirred vigorously (in 89 % yield). Linear molecule **36** was prepared by the ACE coupling of **33** with bis-epoxide **11** in dichloromethane at 140 °C for 2 days in 97 % yield (**Scheme 10**). Longer scaffold **38** was prepared in similar manner using bis-epoxide **37** and heating in tetrahydrofuran at 140 ^{o}C for 2 hours in a stainless steel high pressure vessel (60 % yield). Furthermore, acid **33** was converted to benzyl ester **35** by *in situ* preparation of acid chloride using oxalyl chloride, followed by esterification with benzyl alcohol (29 %). Acid chloride **34** is stable substance and could be isolated after treatment of **33** with oxalyl chloride in 72 % yield.

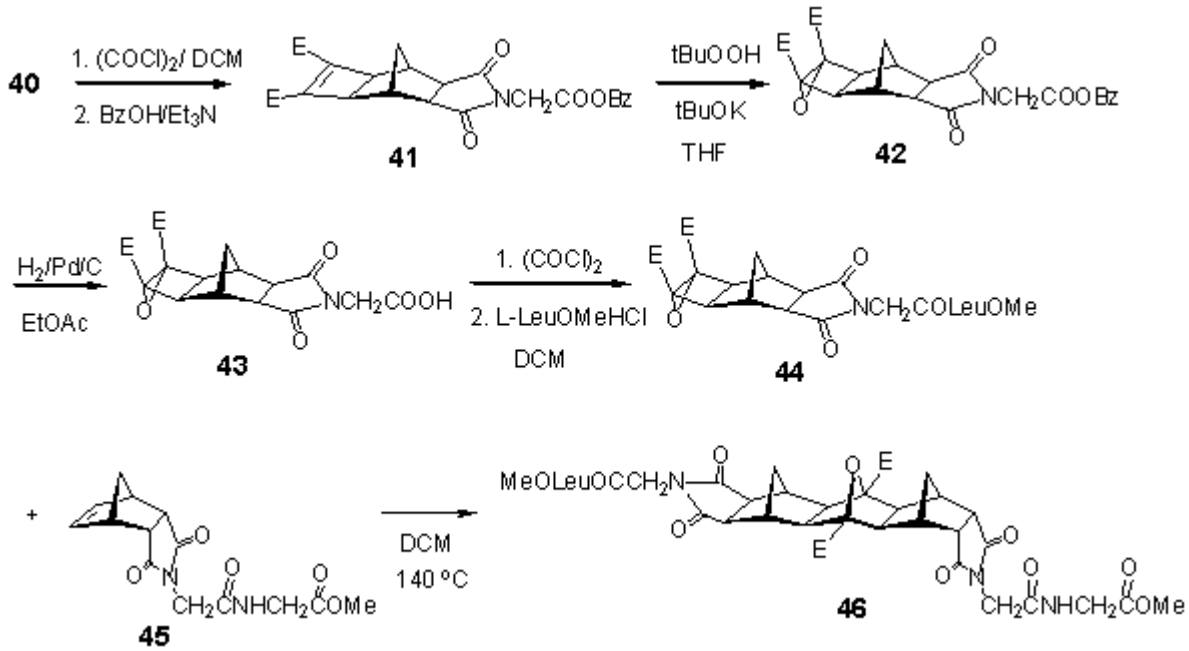
Photodimerisation



Scheme 11

Different CH_2-CH_2 separation distances than obtained in bis-adducts **12** and **38** could be easily achieved by [2p+2p] photodimerisation[33] of cyclobutene diester **40** in acetone after irradiation for 1 hour using 450 W medium pressure mercury lamp. In these reaction conditions, photodimer **41** was obtained in 25 % yield (**Scheme 11**). Linear precursor **39** was prepared by the melting reaction of anhydride **39** with glycine at 200 °C for 1 h in 80 % yield.

Mixed-unsymmetrical systems. ACE coupling method also offers an easy access to unsymmetrical polynorbornane systems, by 1,3-dipolar cycloaddition of norbornane epoxide (4p-component) with double bond (2p-component) of the second norbornene building block.[34] Both components are synthetised independently and joined in the final reaction step.



Scheme 12

Acid group of **40** was protected to make benzyl ester **41** in two reaction steps: preparation of acid chloride with oxalyl chloride, and esterification with benzyl alcohol in 61 % overall yield. This diester was further epoxydized to **42** using *t*-Bu-hydroperoxide, *t*-BuOK in THF (91 %). Debenzylation was achieved by catalytic hydrogenation in ethyl acetate with Pd/C in 80 % yield. Final synthetic step was attachment of L-LeuOMe onto **43** by treatment with oxalyl chloride followed by reaction with L-leucine methyl ester hydrochloride in 54 %. (Direct epoxydation of peptide ester attached to **40** did not yield any product). In a final reaction step, ACE coupling of norbornene **45** and

epoxide **44** in DCM were heated at 140 ^{o}C for two hours in a sealed glass tube to afford **46** in 54 % yield. Proton NMR spectrum of this compound is depicted in **Figure 2**.

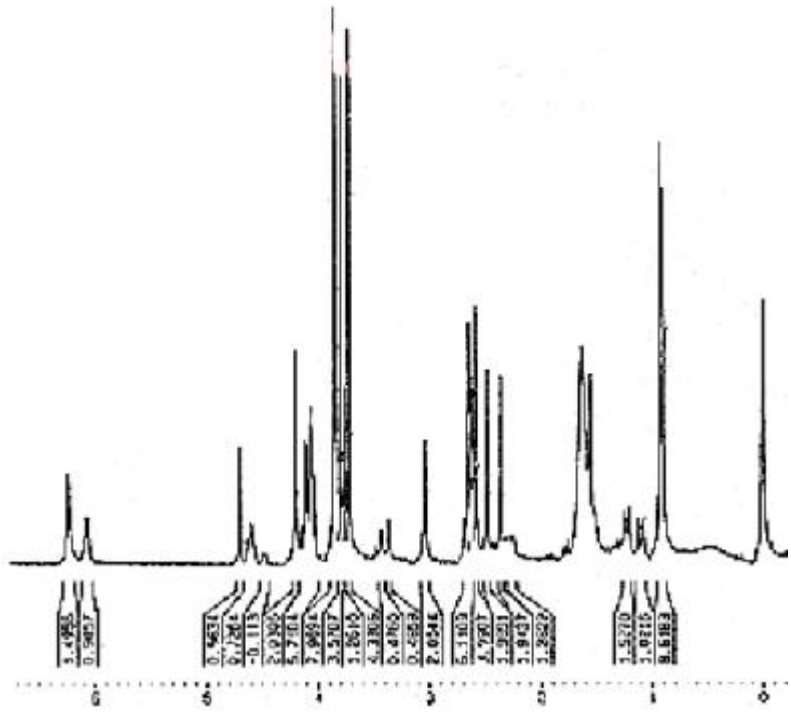


Figure 2. 1H-NMR spectrum of **46**

The CH_2-CH_2 distances of variety of bis-peptide scaffolds calculated by semiempirical AM1 method in Ångstroms are collected in **Figure 3**. These distances vary in length from 5.7 to 23.9 Å, illustrating the separation and orientation of bis-peptides attached to polynorbornane scaffolds.

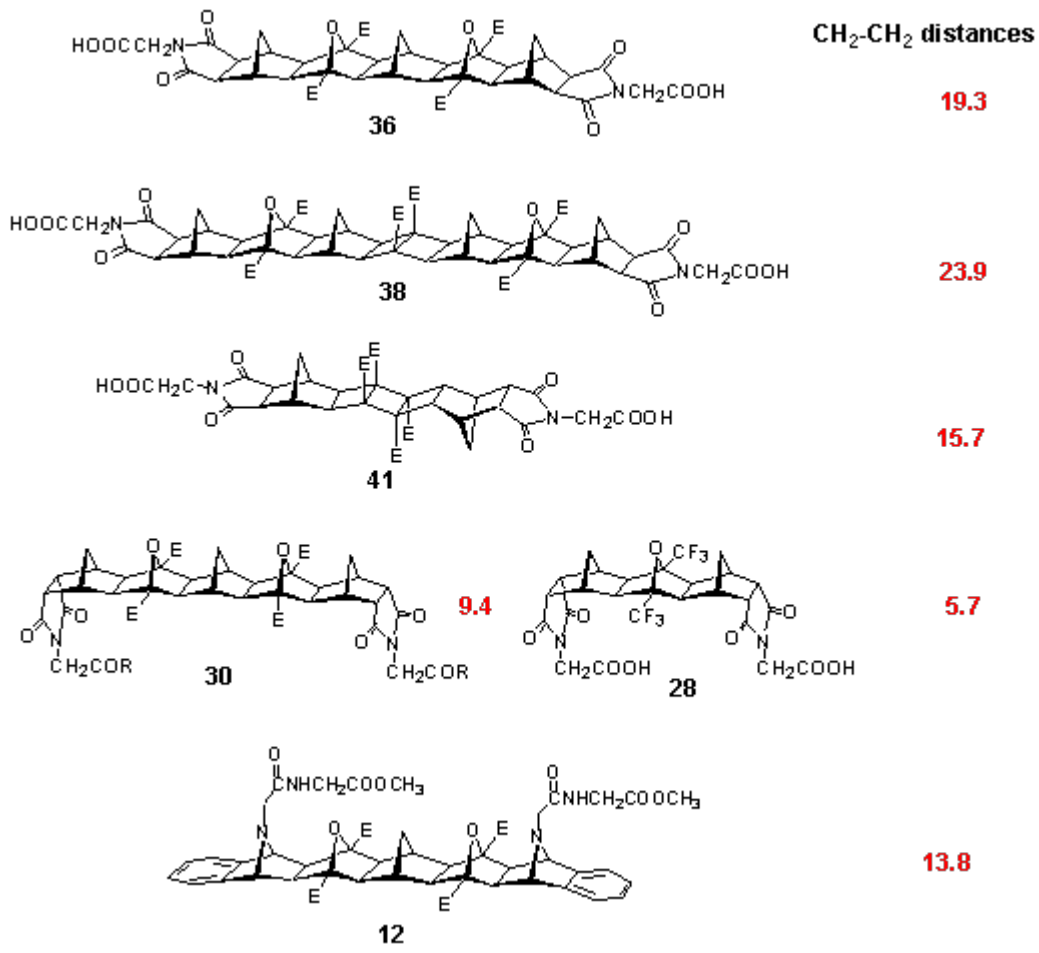
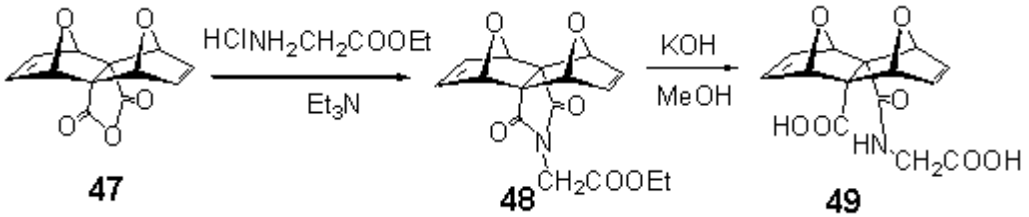


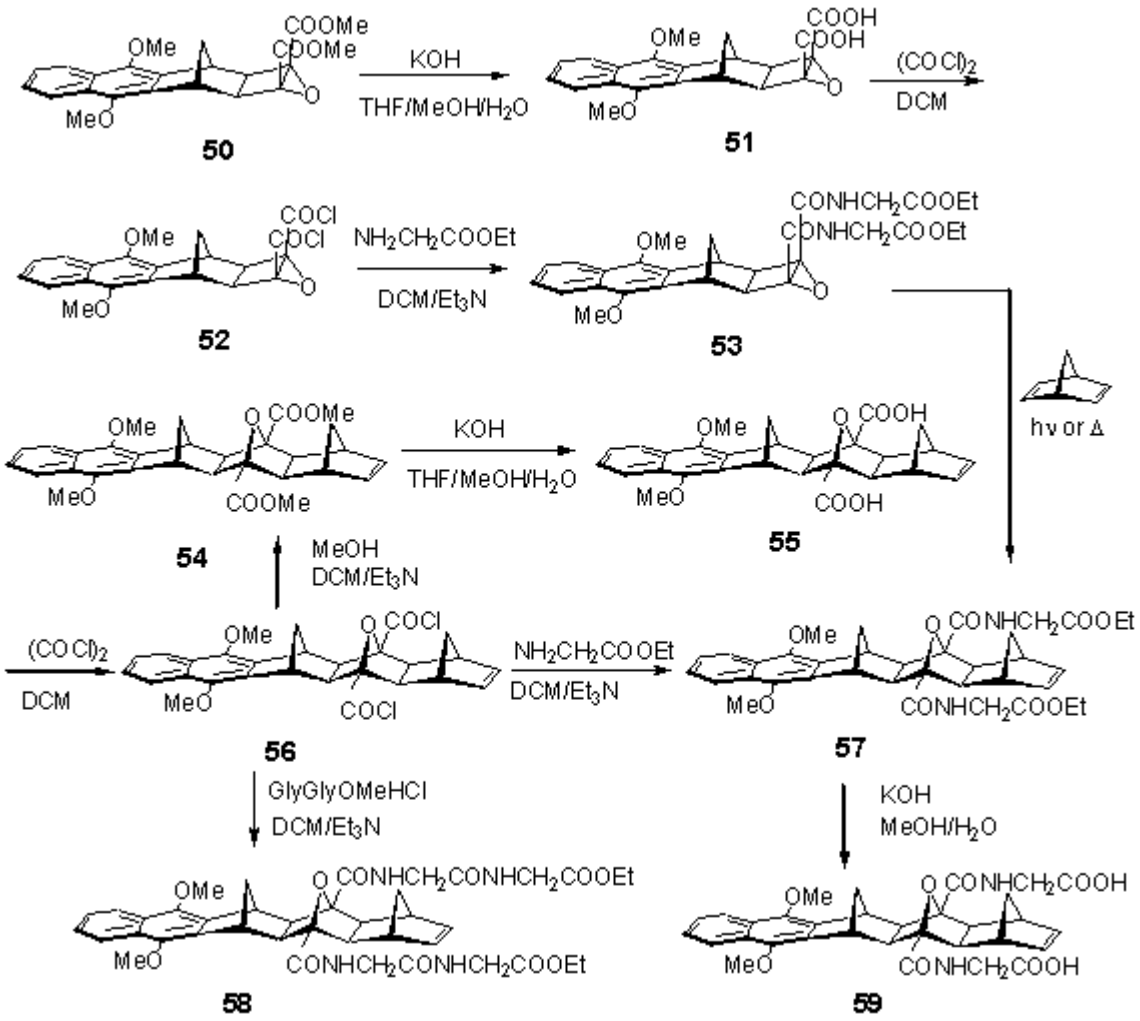
Figure 3

Other norbornene anhydrides considered for peptide elaboration include dioxanorbornene anhydride **47**[35], which was firstly converted to imidoester **48** by reaction with ethylglycinate (**Scheme 13**) and subsequently hydrolised to **49** in THF/MeOH, by treatment with aqueous solution of potassium hydroxide (in 41 %).



Scheme 13

Elaboration of bridgehead ester groups: p**re- and post-coupling peptide elaborations**. It was found that dimethyl ester epoxides such as **50** are quite stable and can be easily hydrolised in basic conditions to corresponding epoxide diacid **51** (90%) (**Scheme 14**). Further conversion of diacid **51**, is treatment with oxalyl chloride as efficient way to transformation to epoxy diacid chloride **52** (88 %), which readily reacts with various compounds such as amines or alcohols to give variety of substituted epoxides, in particular with ethyl glycinate to produce **53** (53 %). This reaction sequence allows preparation of variety of substituted epoxides, which were not accessible by elaboration of methyl esters at the cyclobutene stage, due to their instability in epoxidation conditions. Dipolar cycloaddition of epoxide **53** with norbornadiene, either thermally of photochemically induced gave adduct **57** (in 48 and 66 %, respectively).



Scheme 14

Reaction sequence analogous to the one described for pre- ACE coupling chemistry could be used to transform methyl esters of polycycle **54** at the 7-oxa bridgehead positions after ACE coupling, by base hydrolysis, formation of acid chloride by oxalyl chloride and reaction of so formed acid chloride to amides **57** and **58** or ester **54** (74, 53 and 82%, respectively). Furthermore, we have shown that **57** could be hydrolysed in basic conditions (potassium hydroxide) to diacid **59**, which serves as entering point for further polypeptide synthesis.

Conclusion. Polynorborene framework was used as a rigid scaffold for attachment of peptides in a precise and geometrically organized manner. ACE coupling method was the key step in preparation of symmetrical and unsymmetrical bis-peptides. Pyrrole substition offers different synthetic strategy to 7-azanorbornane peptide attachments. Bridgehead attached peptides could be obtained either by the pre- and the post- ACE coupling reactions.

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