Synthesis, reactivity and conformational preferences of novel enediynyl peptides: a possible scaffold for β-sheet capping turns

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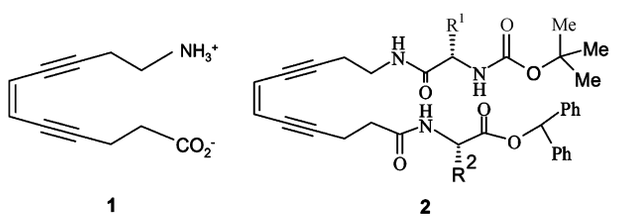
Novel enediynyl tripeptides **2(a–c)** in fully protected forms have been prepared *via* a sequence of palladium(0)-based Sonogashira coupling. The thermal reactivity of these peptides was shown to be dependent upon the nature of the side chain in the amino acids. Analysis of the CD-spectra of these peptides as well as the variation of chemical shifts with temperature revealed the presence of a β-sheet nucleating conformation in equilibrium with a conformation induced by H-bond formation between the CO and NH belonging to the enediynyl amino acid.

Introduction

Enediynes have drawn unprecedented interest amongst the scientific community because of their cytotoxic activity and possible use as anticancer drugs.[1] All studies so far have been concentrated on their synthesis and evaluation of chemical as well as biological activity. The special structural feature of *Z*-enediynes that caught our attention is the type of reverse-turn associated with the two acetylenic arms. One can consider making enediynyl amino acid containing peptides, which may be forced to adopt typical conformational motifs. The structural motif in which we were interested was the β-sheet nucleating entity. This constitutes a well studied subset of the reverse turn and is a common feature in biologically active peptides and globular proteins. The sheet capping turns[2] are widely believed to act as a molecular recognition site for many biological processes. There have been several reports of aromatic/heteroaromatic based amino acid templates used as sheet-capping turn mimetics.[3] Our aim was to incorporate the enediynyl amino acid **1** into peptides **2** (Fig. 1) and then find



1 **2**

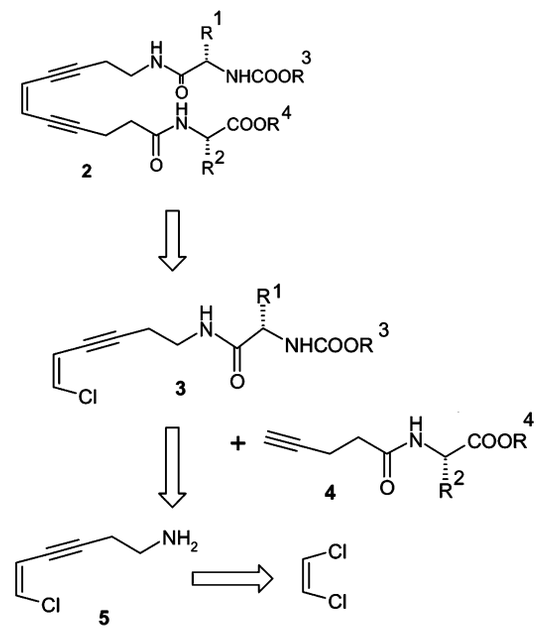
Fig. 1

out the conformational preferences by Differential Scanning Calorimetry (DSC), NMR and CD-measurements. Our long term goal is to use these compounds as anticancer devices with the peptide providing the molecular recognition and the enediynes the anticancer effect.

Results and discussion

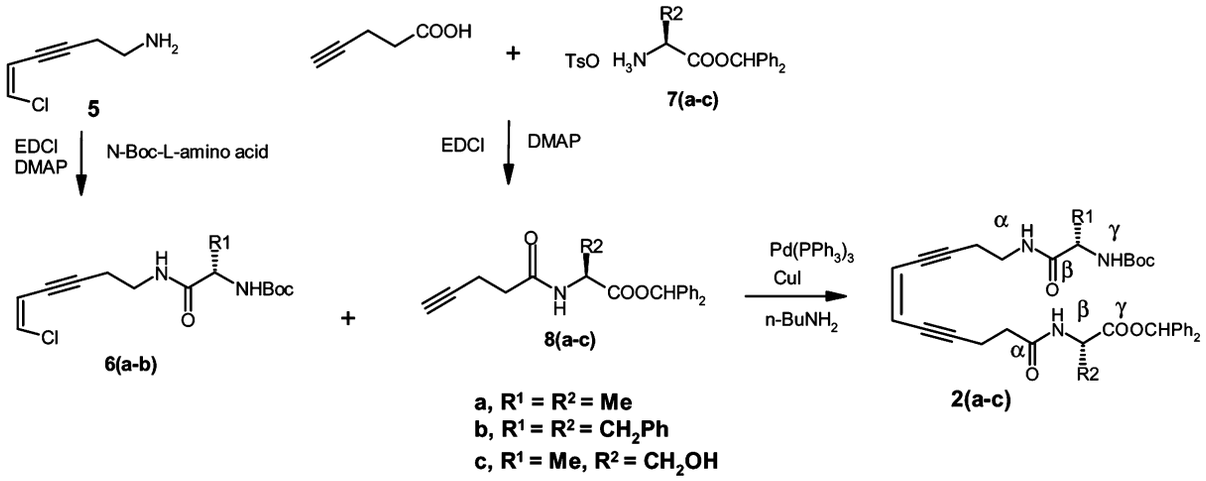
Synthesis of the enediynyl peptides

The synthesis of the enediynyl peptides required consideration of the following points: a) stability of peptide bonds under the conditions of Pd(0)-based Sonogashira coupling[4] which employs excesses of strong bases like *n*-butylamine that may cause transamidation and b) the possibility of the racemization at the α-center during Pd(0)-coupling. Since enyne coupling with *cis*-dichloroethylene can be accomplished under ambient conditions,[5] we decided to synthesize the *cis*-dichloroethylene based enediynyl peptides **2(a–c)**. The use of 1,2-dibromo-benzene as the backbone for enediyne was not considered because of the vigorous conditions[6] necessary for the Sonogashira coupling (neat base as solvent and refluxing conditions). The retrosynthetic analysis of the tripeptides is shown below (Scheme 1).



Scheme 1

In accordance with the retrosynthetic strategy, the amine **5** was prepared according to our published procedure.[6] It was then coupled to *N*-t-Boc amino acids in the presence of EDCI,[7] to produce one arm of the target peptide **6(a–b)**. At this juncture we had two options: either to couple the enediyne with pent-5-ynoate and then deprotect the ester followed by coupling with a carboxy-protected amino acid or to couple with an already prepared monopeptide. Because of the problem of selective deprotection (hydrogenolysis or acid-catalyzed



Scheme 2

methods are not acceptable), we had chosen the second option. Thus, the acetylenic amides **8(a–c)** were synthesized *via* standard protection, deprotection and coupling. These were then successfully coupled to the eneyne **6(a–b)** to give the protected tripeptides **2(a–c)** in very good yields and without any racemization (Scheme 2). The ^{13}C and ^{1}H NMR were extremely clean, no extra peak was seen, thus demonstrating that there was practically no racemization during Sonogashira coupling.

The DSC behaviour of the enediynyl peptides

The thermal reactivity of the enediynyl peptides in the protected form was first determined by DSC.[8] It has now become routine practice to follow the starting temperature for Bergman Cyclization (BC) by DSC. As the molecules are heated, beyond a certain temperature diradicals are generated which polymerize and hence show an exothermic peak. The DSC is taken in the solid state and the resulting product is a polymer. It is reasonable to assume that the start of exothermic rise is a reflection of the onset of Bergman Cyclization. The comparison of onset temperatures for BC for the peptides can be regarded as an indication of the relative closeness of the two acetylenic arms. The peptides, namely *N*-t-Boc-Ala-ED-Ala ($OCHPh_2$) **2a** and *N*-t-Boc-Phe-ED-Phe ($OCHPh_2$) **2b**, showed a clear exothermic rise. The thermal behaviour of the other peptide, *N*-t-Boc-Ala-ED-Ser ($OCHPh_2$) **2c** was not very informative as there was no sharp rise in the DSC curve. In the case of alanine-based peptide **2a**, the onset temperature for Bergman Cyclization (BC)[9] was found to be at ~180 °C whereas the corresponding temperature for the phenylalanine-based peptide **2b** was ~135 °C (Fig. 2). Thus, BC is more facile in peptide **2b** than in the peptide **2a**, and this may indicate that the acetylenic arms in peptide **2b** are closer together. The free peptide NH_3^+-Phe-ED-Phe-COO^- (**2d**) obtained by deprotection of **2b** with TFA, showed an onset temperature for BC starting at ~130 °C. There was practically no perturbation of the kinetics of BC upon deprotection in contrast with the cases of enediynyl amino acids previously prepared in our laboratory[10] (Scheme 3). This type of behaviour was not unexpected as the free amine and the carboxylic acid are far apart in these molecules and therefore produce no perceptible effect on the cyclization kinetics even if there are additional electrostatic or H-bond interactions.

Conformational studies of enediynyl peptides by CD and variable temperature NMR

To have an idea about the preferred conformations, Circular Dichroism (CD)[11] spectra of the fully protected peptides were then recorded. Although the peptides were small, the CD spectra (shown in Fig. 3) did reveal some noticeable features.

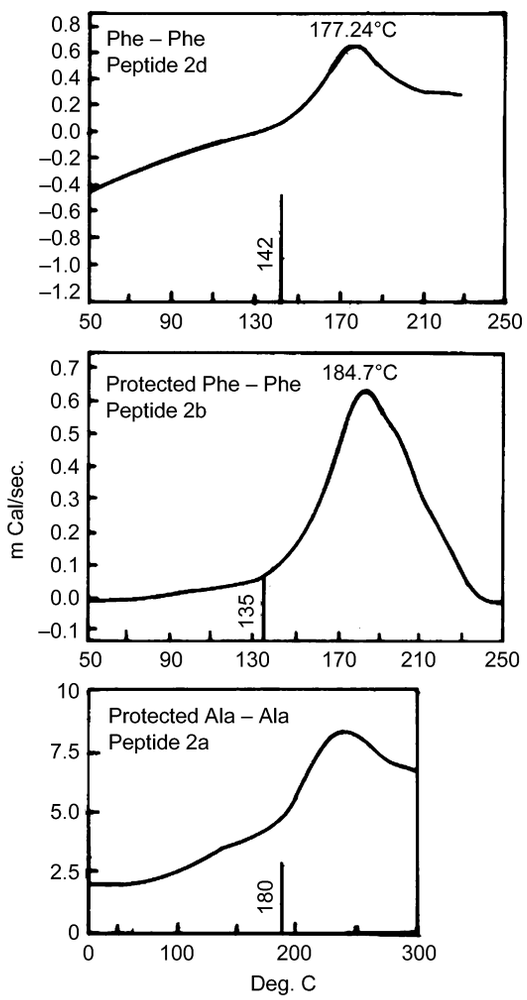
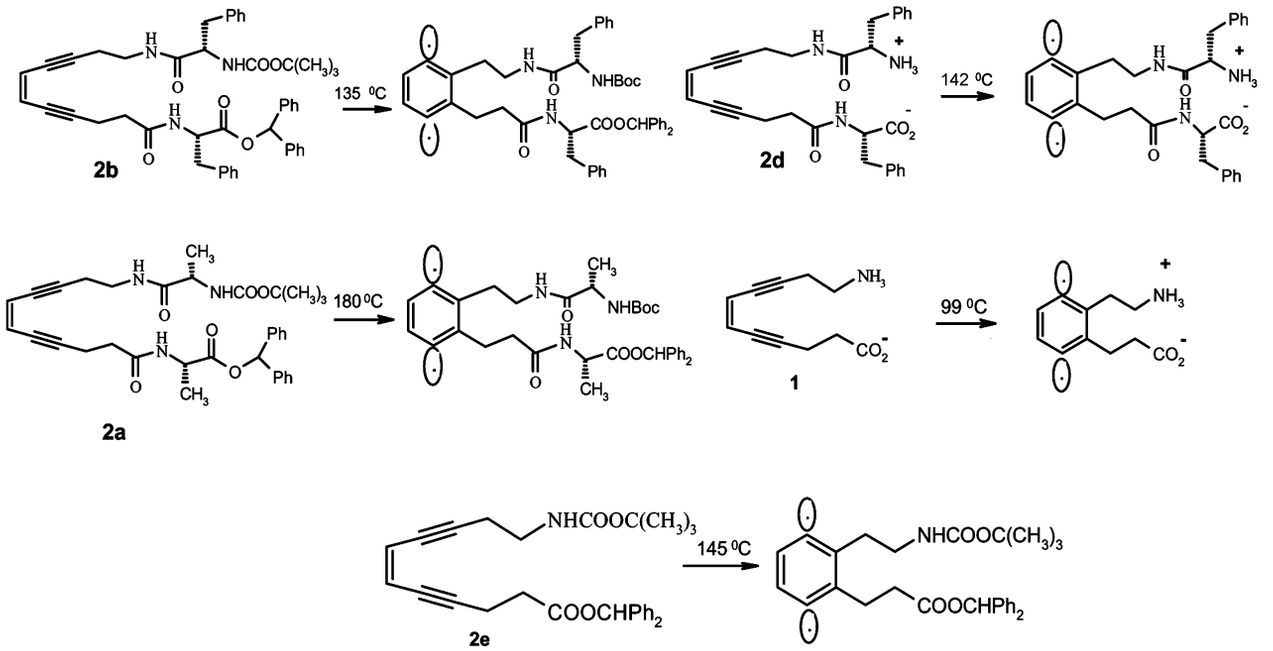


Fig. 2 DSC curve of the enediynyl peptides.

As can be seen in Fig. 3, there was clear indication of the presence of a β-sheet capping type conformation in all these peptides. This is apparent from the appearance of the minima at ~211 nm. However, along with the minima, there were also maxima at ~192 nm which are typical of α-helices of a peptide. Although our peptides are too small to adopt any fully helical structure, the appearance of this maxima, probably indicates the presence of a conformation for all the peptides in solution which involves H-bond formation between the N–H and amide carbonyl, with both functionalities belonging to the enediyne moiety. The minima at ~211 nm probably indicate the presence



Scheme 3

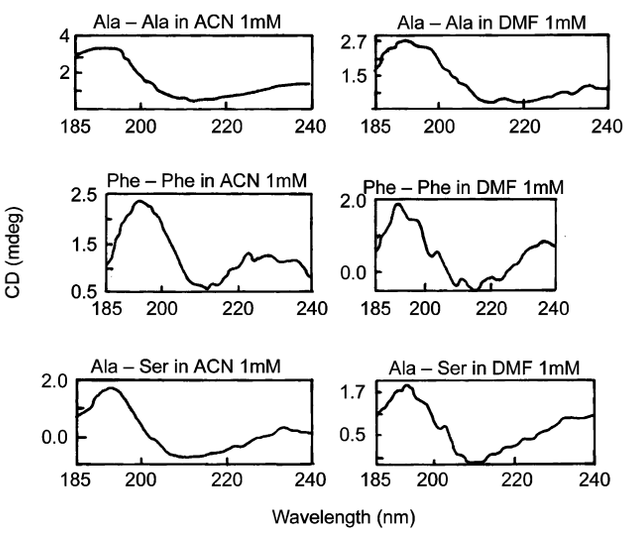


Fig. 3 CD-spectra of enediynyl peptides.

of a conformation formed by a H-bond between the β-NH and the α-carbonyl as well as between the γ-NH and the γ-carbonyl in equilibrium with conformation induced by H-bond formation between CO and NH belonging to the enediynyl amino acid [*vide* structure **2(a–c)**].

Variable temperature ^{1}H-NMR was next used to probe intramolecular H-bonding in the peptides. Previous NMR studies on various amides have demonstrated that intramolecularly hydrogen bonded N–H's exhibit a relatively large temperature dependence of the shift ($\Delta\delta/\Delta T$) relative to free N–H's which show a small temperature dependence of chemical shift.

The temperature dependence[12] of the amide protons, including the one protected as a carbamate, was recorded in the temperature range 25–60 °C in d_6-DMSO (Fig. 4). The variation of chemical shift ($\Delta\delta/\Delta T$) for all the three types of N–H's in the three peptides is shown in (Table 1).

From the data in Table 1, the following conclusions can be drawn: a) in all the peptides, the N–H's showed medium to high temperature dependence of chemical shift ($\Delta\delta/\Delta T$). b) The highest $\Delta\delta/\Delta T$ was observed from the carbamate N–H indicating weak intramolecular H-bonding and c) in all the peptides, the β-N–H's showed a slightly weaker intramolecular H-bonding compared with the α-N–H's.

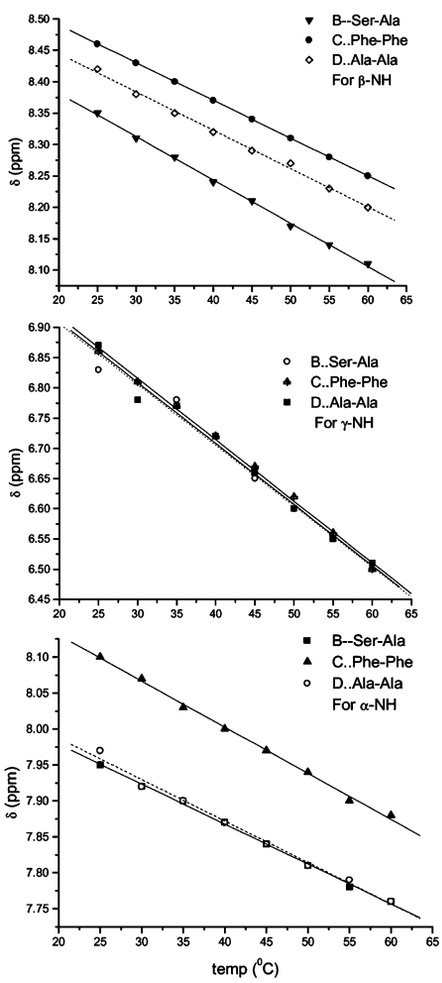


Fig. 4 Plot of chemical shift of α,β,γ-NH *vs.* temperature.

In view of the above data, we propose that there is significant proportion of a β-sheet-like conformation which was indicated by both the CD-measurements and the generally comparable ($\Delta\delta/\Delta T$) values for the chemical shifts of β and γ-N–H's. However, the results also indicate the presence of other conformations, in particular, the α-NH values indicate intra-

Table 1 Variation of chemical shift with temperature ($\Delta\delta/\Delta T$)

Peptide	α-NH ($\Delta\delta/\Delta T$) (ppb)	β-NH ($\Delta\delta/\Delta T$) (ppb)	γ-NH ($\Delta\delta/\Delta T$) (ppb)
2a (AA)	−5.5	−6.0	−9.9
2b (PP)	−5.5	−6.1	−10.2
2c (AS)	−6.4	−6.9	−10.0

molecularly H-bonded. The variable temperature experiments indicate that the conformation resembling a β-sheet capping type motif is quite significant. A representation is shown in Fig. 5.

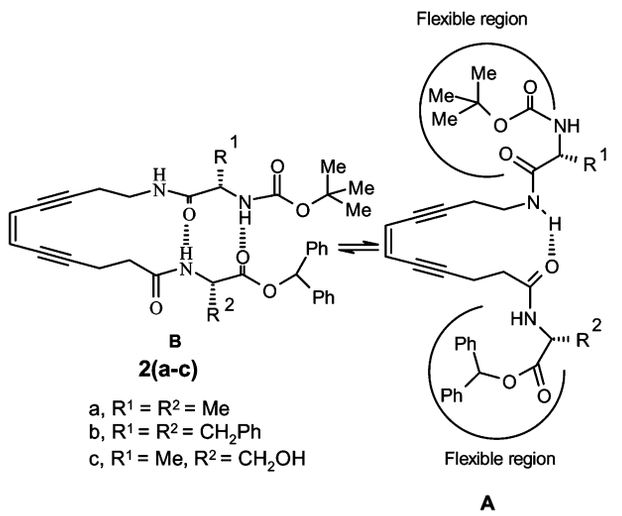


Fig. 5

Thus, our studies confirm that intramolecular H-bond formation in enediynyl amino acid containing peptides can act as a scaffold to nucleate the formation of β-sheet like structures. In the future, our laboratory will be involved in synthesizing much larger peptides, including cyclic peptides to make use of this unique character of enediynes.

Experimental

General

IR spectra were recorded on Perkin Elmer model 883 using KBr pellets for solids and neat for liquids. The characteristic peaks are expressed in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AC 200 spectrometer (unless mentioned otherwise). Melting points (mp) were recorded on a Toshniwal hot-coil stage melting point apparatus and are uncorrected. DSC measurements were done using a NETZSCH 40 Thermal Analyzer Instrument. Mass spectra were obtained from CRIM (Clinical Research Institute of Montreal), Canada, CDRI. Variable temperature NMR spectra were recorded in a Bruker 300 instrument at IICB, Calcutta. Columns were prepared with silica gel (60 to 120 mesh, S. D. Fine chemicals). All the reactions were carried out under argon/nitrogen atmosphere.

N-(6-Chlorohex-5-en-3-ynyl)-*N′*-*tert*-butyloxycarbonyl-L-phenylalaninamide (6b)

To a solution of *N*-*tert*-butyloxycarbonyl-L-phenylalanine (306 mg, 1.15 mmol), in dry CH_2Cl_2 (15 ml), 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDCI) (175 mg, 0.918 mmol) was added and the reaction mixture was stirred for 1 h at 0 °C. The amine **5** (100 mg, 0.772 mmol), dissolved in CH_2Cl_2 (1 ml), was added followed by DMAP (125 mg, 0.926 mmol) and the mixture was stirred for another 3 h at room temperature. After partitioning between CH_2Cl_2 and water (50 ml each), the organic layer was dried over Na_2SO_4 and evaporated. From the oily residue, the title compound **6b** was isolated pure by chromatography (Si-gel, hexane–EtOAc = 4 : 1); Yield: 60%; State: white solid; mp 128 °C; ν_{max} (KBr): 3345, 2980, 2931, 2272, 1689, 1650, 1167 cm^{-1}; δ_H: 1.61 (9H, s, *tert*-butyl-*H*), 2.43–2.50 (2H, m, $NHCH_2CH_2$), 2.94–3.23 (2H, m, CH_2Ph), 3.31–3.43 (2H, m, $NHCH_2$), 4.53–4.57 (1H, m, H_α), 5.70 (1H, br s, N*H*Boc), 5.77–5.82 (1H, dt, J = 2.06, 2.07 Hz, ClCH=C*H*), 6.35 (1H, d, J = 7.43 Hz, ClC*H*=CH), 6.73 (1H, br s, $NHCH_2$), 7.18–7.37 (5H, m, Ar-*H*); Mass (EI) *m*/*z* 378, 376 (M^+).

N-(6-Chlorohex-5-en-3-ynyl)-*N′*-*tert*-butyloxycarbonyl-L-alaninamide (6a)

The compound **6a** was prepared following the same procedure as described for **6b**. Yield: 65%; State: white solid; mp 138 °C; ν_{max} (KBr): 3313, 2217, 1650, 1567, 1522, 1452, 1368, 1250 cm^{-1}; δ_H: 1.35 (3H, d, J = 7.08 Hz, CH_3), 1.43 (9H, s, *tert*-butyl-*H*), 2.55–2.63 (2H, ddd, J = 2.03, 2.05, 2.02 Hz, $NHCH_2CH_2$), 3.40–3.50 (2H, q, J = 3.54 Hz, $NHCH_2$), 4.11–4.18 (1H, m, H_α), 5.01 (1H, br s, N*H*Boc), 5.81–5.86 (1H, dt, J = 2.06, 2.07 Hz, ClCH=C*H*), 6.33 (1H, d, J = 7.36 Hz, ClC*H*=CH), 6.53 (1H, br s, $NHCH_2$); Mass (EI) *m*/*z* 300 (M^+).

General procedure for the synthesis of *N*-(pent-4-ynoyl)-L-amino acid benzhydryl esters 8(a–c)

To a solution of tosylate salt **7(a–c)** (4.51 mmol) in CH_2Cl_2 (15 ml), pent-4-ynoic acid (530 mg, 5.42 mmol) was added followed by EDCI (1 g, 4.96 mmol). The whole reaction mixture was cooled to 0 °C and stirred for 15 min. After that it was allowed to warm to room temperature. 1-Hydroxybenzotriazole (HOBT) (731 mg, 5.41 mmol) and DMAP (5.42 mmol) were added and stirred for another 3 h. The reaction mixture was then partitioned between CH_2Cl_2 and water (50 ml each). The organic layer was dried and evaporated to leave a residue from which the title compound **8(a–c)** was isolated by column chromatography (Si-gel, hexane–EtOAc = 1 : 1).

***N*-(Pent-4-ynoyl)-L-serine benzhydryl ester (8c).** Yield 65%; State: white solid; mp 145 °C; ν_{max} (KBr): 2120, 1750, 1612, 930 cm^{-1}; δ_H: 1.97 (1H, t, J = 2.30 Hz, C*H*C), 2.43–2.54 (4H, m, $COCH_2CH_2$), 3.86–4.05 (2H, m, CH_2OH), 4.75–4.82 (1H, m, H_α), 6.79 (1H, d, J = 7.49 Hz, N*H*), 6.90 (1H, s, C*H*Ph_2), 7.33–7.39 (10H, m, Ar-*H*); δ_C: 14.65, 34.93, 54.87, 63.14, 69.48, 78.55, 82.69, 126.88, 127.07, 128.12, 128.19, 128.56, 139.35, 169.61, 171.43; Mass (EI) *m*/*z* 351 (M^+).

***N*-(Pent-4-ynoyl)-L-alanine benzhydryl ester (8a).** Yield: 70%; State: white solid; mp 128 °C; ν_{max} (KBr): 2120, 1745, 1656, 1450, 1262 cm^{-1}; δ_H: 1.44 (3H, d, J = 7.04 Hz, CH_3), 1.96 (1H, t, J = 2.42 Hz, C*H*C), 2.36–2.44 (2H, m, $COCH_2CH_2$), 2.47–2.54 (2H, m, $COCH_2$), 4.71–4.79 (1H, m, H_α), 6.24 (1H, d, J = 6.82 Hz, N*H*), 6.87 (1H, s, C*H*Ph_2), 7.27–7.42 (10H, m, Ar-*H*); δ_C: 14.62, 18.43, 35.02, 48.19, 69.35, 78.06, 82.67, 126.89, 126.95, 128.08, 128.52, 139.31, 139.50, 170.35, 172.03; Mass (EI) *m*/*z* 335 (M^+).

***N*-(Pent-4-ynoyl)-L-phenylalanine benzhydryl ester (8b).** Yield: 72%; State: white solid; mp 175 °C; ν_{max} (KBr): 2372, 1723, 1644, 1500 cm^{-1}; δ_H: 1.91 (1H, t, J = 2.39 Hz, C*H*C), 2.35–2.49 (4H, m, $COCH_2CH_2$), 3.12–3.16 (2H, dd, J = 2.0, 1.39 Hz, CH_2Ph), 5.03–5.07 (1H, m, H_α), 6.09 (1H, d, J = 7.67 Hz, N*H*),

6.85 (1H, s, C*H*Ph_2), 6.86–7.37 (15H, m, Ar-*H*); δ_C: 8.91, 29.42, 32.00, 47.40, 63.71, 72.51, 77.00, 121.17, 121.28, 121.94, 122.33, 122.59, 122.75, 122.81, 122.90, 123.68, 129.70, 133.58, 164.68, 164.92; Mass (EI) *m*/*z* 411 (M^+).

General procedure for the synthesis of protected enediynyl tripeptides 2(a–c)

To a degassed solution of the eneynes **6(a–b)** (0.398 mmol) in dry benzene (10 ml), $Pd(PPh_3)_4$ (18.3 mg, 0.015 mmol) and n-$BuNH_2$ (120 µl, 1.592 mmol) were added and stirred for 5 min. Then CuI (15.12 mg, 0.0796 mmol) was added and stirred for another 20 min after which a solution of the compound **8(a–c)** (245 mg, 0.597 mmol) in benzene (1 ml) was added dropwise and the reaction mixture was stirred at 40 °C for 3 h. Then EtOAc (10 ml) and saturated NH_4Cl solution (10 ml) were added and stirred for a further 10 min. The organic layer was washed with water (3 × 20 ml), dried and evaporated. The title compounds **2(a–c)** were purified by chromatography (Si-gel, hexane–EtOAc = 1 : 1).

***N-tert*-Butyloxycarbonyl-L-alaninylaminoundec-5-ene-3,7-diynoyl-L-alanine benzhydryl ester (2a).** Yield: 60%; State: viscous oil; ν_{max} (neat): 2275, 1567, 1522, 1368 cm^{-1}; δ_H: 1.36 (3H, d, *J* = 7.0 Hz, CH_3), 1.42 (9H, s, *tert*-butyl-*H*), 1.44 (3H, d, *J* = 7.9 Hz, CH_3), 2.48–2.60 (4H, m, 2 × CC$H_2$$CH_2$), 2.60–2.76 (2H, t, *J* = 3.86 Hz, COCH_2), 3.37–3.48 (2H, m, NCH_2), 4.14 (1H, m, CHNHBoc), 4.72–4.80 (1H, m, CHCOOCHPh$_2$), 5.30 (1H, br s, NHBoc), 5.71 (2H, m, C*H*=C*H*), 6.72 (1H, br s, N*H*CH_2), 6.86 (1H, s, C*H*Ph_2), 6.92 (1H, t, *J* = 7.2 Hz, CH_2CON*H*), 7.31–7.35 (10H, m, Ar-*H*); δ_C: 15.87, 18.13, 18.52, 20.47, 28.27, 35.02, 36.53, 48.08, 49.99, 76.04, 76.39, 77.32, 78.90, 79.66, 79.90, 94.20, 118.96, 119.68, 126.93, 128.01, 128.49, 139.39, 139.66, 155.46, 170.73, 172.14, 173.05; Mass (EI) *m*/*z* 599 (M^+); HRMS calculated for $C_{35}H_{41}N_3O_6$ 599.2997 found 599.2999.

***N-tert*-Butyloxycarbonyl-L-phenylalaninylaminoundec-5-ene-3,7-diynoyl-L-phenylalanine benzhydryl ester (2b).** Yield: 55%; State: viscous oil; δ_{max} (neat): 2334, 1737, 1076 cm^{-1}; δ_H: 1.38 (9H, s, *tert*-butyl-*H*), 2.43–2.51 (4H, m, 2 × CCH_2), 2.59–2.71 (2H, m, COCH_2), 2.94–3.27 (4H, m, 2 × CH_2Ph), 3.31–3.41 (2H, m, NCH_2), 4.28–4.32 (1H, m, C*H*NHBoc), 5.00–5.10 (1H, m, C*H*COOCHPh$_2$), 5.35 (1H, br s, N*H*Boc), 5.69 (2H, m, C*H*=C*H*), 6.27 (1H, br s, N*H*CH_2), 6.65 (1H, t, *J* = 7.19 Hz, CON*H*CH), 6.93 (1H, s, C*H*Ph_2), 6.88–7.37 (20H, m, Ar-H); δ_C: 15.88, 20.51, 28.28, 35.18, 37.88, 38.52, 38.77, 53.26, 78.16, 78.92, 79.75, 94.15, 118.97, 119.68, 126.87, 127.63, 128.00, 128.24, 128.43, 128.50, 128.58, 129.28, 129.36, 135.65, 136.79, 139.37, 155.38, 170.60, 170.84, 171.42; Mass (EI) *m*/*z* 751 (M^+); HRMS calculated for $C_{47}H_{49}N_3O_6$ 751.3624 found 751.3628.

***N-tert*-Butyloxycarbonyl-L-alaninylaminoundec-5-ene-3,7-diynoyl-L-serine benzhydryl ester (2c).** Yield: 65%; State: viscous oil; ν_{max}(neat): 2216, 1731, 1522 cm^{-1}; δ_H: 1.30 (3H, d, *J* = 4.40 Hz, CH_3), 1.42 (9H, s, *tert*-butyl-*H*), 2.55–2.62 (4H, m, 2 × CCH_2), 2.70–2.73 (2H, m, COCH_2), 3.38–3.50 (2H, m, NCH_2), 3.98–4.13 (3H, m, OCH_2 & CH_3C*H*), 4.79–4.85 (1H, m, OCH_2C*H*), 5.23 (1H, d, *J* = 7.57 Hz, N*H*Boc), 5.72 (2H, m, C*H*=C*H*), 6.80 (1H, br s, N*H*CH_2), 6.89 (1H, s, C*H*Ph_2), 7.12 (1H, t, *J* = 7.87 Hz, OCH_2CHN*H*), 7.32–7.35 (10H, m, Ar-*H*); δ_C: 15.78, 18.33, 20.36, 28.23, 35.18, 38.71, 54.99, 63.07, 78.34, 78.96, 79.72, 80.25, 94.00, 119.05, 119.81, 126.86, 127.11, 127.96, 128.08, 128.48, 128.63, 139.35, 139.51, 155.56, 169.76, 171.34, 173.53; Mass (EI) *m*/*z* 615 (M^+); HRMS calculated for $C_{35}H_{41}N_3O_7$ 615.2946 found 615.2950.

Synthesis of 11-aminoundec-6-ene-4,8-diyn-1-oic acid (1)

A solution of *N-tert*-butyloxycarbonyl 11-aminoundec-6-ene-4,8-diyn-1-oic acid diphenylmethyl ester (**2e**)[10] (50 mg) and trifluoroacetic acid (150 µl) in dry dichloromethane (6 ml) was stirred for one hour at 0 °C. The solvent was evaporated. Benzene was added and evaporated. The benzene re-evaporation process was repeated three times. The title compound was precipitated with ether as a brown solid; yield: 53%; mp 128 °C (dec.); δ_H (acetone-d_6) 2.56 (2H, t, *J* = 5.87 Hz, CH_2CO), 2.65 (2H, t, *J* = 6.95 Hz, CH_2CH_2CO), 2.76 (2H, t, *J* = 6.41 Hz, CH_2N), 3.52 (2H, br s, CH_2CH_2N), 5.80–5.89 (2H, m, CH=CH); Mass (FAB) *m*/*z* 191 (M^+).

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References

1 (*a*) K. C. Nicolaou and W. M. Dai, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1387; (*b*) K. C. Nicolaou, W. M. Dai, S. C. Tsay, V. A. Estevez and W. Wrasidlo, *Science.*, 1992, **256**, 1172; (*c*) K. C. Nicolaou, *Chem. Br.*, 1994, 33; (*d*) M. E. Maier, *Synlett*, 1995, 13; (*e*) J. W. Grissom, G. U. Gunawardena, D. Klingberg and D. Huang, *Tetrahedron*, 1996, **52**, 6453.
2 (*a*) D. S. Kemp, T. P. Curran, J. G. Boyd and T. Allen, *J. Org. Chem.*, 1991, **56**, 6683; (*b*) D. S. Kemp, D. E. Blanchard and C. C. Muendel, in *Peptides: Chemistry, Structure, Biology*, eds. J. Smith and J. Rivier, ESCOM, Lieden, 1992, 319; (*c*) R. Hirschmann, *Angew. Chem., Int. Ed. Engl.*, 1991, **56**, 6683; R. A. Wiley and D. H. Rich, *Med. Res. Rev.*, 1993, **13**, 327.
3 (*a*) K. Y. Tsang, H. Diaz, N. Graciani and J. W. Kelly, *J. Am. Chem. Soc.*, 1994, **116**, 3988; (*b*) D. S. Kemp and Z. Q. Li, *Tetrahedron Lett.*, 1995, **36**, 4179.
4 K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Lett.*, 1975, **17**, 4467.
5 J. C. Shain, U. K. Khamrai and A. Basak, *Tetrahedron Lett.*, 1997, **38**, 6067.
6 R. Singh and G. Just, *Tetrahedron Lett.*, 1990, **31**, 185.
7 J. C. Sheehan and S. L. Ledis, *J. Am. Chem. Soc.*, 1973, 875.
8 K. Burkhead and H. Rutters, *Tetrahedron. Lett.*, 1994, **35**, 2337.
9 (*a*) R. P. Jones and R. G. Bergman, *J. Am. Chem. Soc.*, 1972, **94**, 660; (*b*) R. G. Bergman, *Acc. Chem. Res.*, 1973, **6**, 25; (*c*) T. P. Lockhart, P. B. Comita and R. G. Bergman, *J. Am. Chem. Soc.*, 1981, **103**, 4082; (*d*) T. P. Lockhart, P. B. Comita and R. G. Bergman, *J. Am. Chem. Soc.*, 1981, **103**, 4091.
10 H. M. Bdour, Ph.D thesis, Indian Institute of Technology, Kharagpur, 2000.
11 (*a*) D. L. Minor and P. S. Kim, *Nature*, 1994, **367**, 660; (*c*) T. E. Creighton, *Proteins: Structure and Molecular Properties*, 2nd edn. W. H. Freeman and Co, New York, 1993, pp. 187; (*d*) M. Kahn, *Synlett*, 1993, **821**, 12.
12 (*a*) H. Diaz, J. R. Espina and J. W. Kelly, *J. Am. Chem. Soc.*, 1992, **114**, 8316; (*b*) K. Kim and J. P. Germanas, *J. Org. Chem.*, 1997, **62**, 2853.