View Article Online / Journal Homepage / Table of Contents for this issue

Design and synthesis of enediyne-based peptide with selective peptide-cleaving activity†

Snigdha Roy and Amit Basak*

Received (in Cambridge, UK) 12th November 2009, Accepted 17th December 2009
First published as an Advance Article on the web 25th January 2010
DOI: 10.1039/b923814j

Published on 25 January 2010. Downloaded by Temple University on 28/10/2014 00:06:31.

A hybrid peptide–enediyne molecule was synthesised and shown to undergo selective intramolecular peptide chain cleavage by the 1,4-diyl radical, the potential intermediate of the enediyne system.

Some naturally occurring enediynes are protein complexes, the non-protein (*i.e.* enediyne) component of which has proteolytic activity. In the case of kedarcidin, the non-protein enediyne component recognises and cleaves histones.[1,2] The enediyne component of esperamicin has also been shown to cause proteolysis and to have antitumour activity.[3] Diradicals, generated from the reactive enediyne moiety, abstract hydrogen from proteins,[4] including at backbone positions, resulting in a captodatively stabilized radical as an intermediate,[5] whose fate depends on the environment. It may interact with molecular oxygen and the resulting peroxy radical ultimately leads to the cleavage of the peptide. Alternatively, the peptide radical may cross-link or form an adduct with another radical source. Inspired by this finding, we intended to synthesize a novel enediyne–peptide hybrid with peptide cleavage activity at tailor-made positions. For this we needed a molecular scaffold where the basic template contains two long parallel chains in the cisoid form. One of these is a polypeptide chain while the other carries the enediyne frame connected to the template *via* a suitable linker (Fig. 1). The cisoid conformation of these two chains is essential to bring the enediyne moiety closer to the peptide chain. In order to achieve such an arrangement, we have selected linkers that can participate in hydrogen bonding with the peptide chain. The purpose is to cut the peptide chain selectively *via* the aid of the 1,4-diyl radicals. Based upon our earlier experience with H-bonded enediyne containing scaffolds,[6,7] we have designed and synthesized a novel class of peptide–enediyne conjugate **3** (Fig. 2) and subsequently demonstrated its selective cleavage ability of the peptide moiety by mass spectrometric studies. A peptide **4** with a similar scaffold also followed a similar cleavage pathway, thus supporting selectivity in the degradation pathway.

Our first template, based on the azobenzene moiety, relied upon the photochemically or thermally induced reversible *E*–*Z*-isomerization[8,9] that would have offered a new handle to trigger cleavage activity. Thus the molecule **1** was first synthesized and its half-life in the *Z* form was measured by

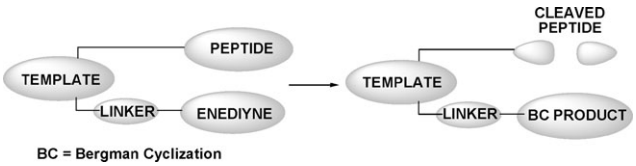


Fig. 1 Design of peptide cleaving agent.

first converting the *E* to the *Z* form by photoirradiation (365 nm) followed by a thermal re-isomerization experiment. The half life for the re-isomerization was found to be ~12 h at room temperature. Since generation of diradicals from aryl fused enediynes requires temperatures higher than 60 °C, a half life of 12 h at ~30 °C is not sufficient to keep the two arms of the azo compound in the *cis* form. This result prompted us to prepare the corresponding more reactive aliphatic enediyne-based compound **2** following the same synthetic protocol as for compound **1**. It was found to be extremely reactive and could be converted to the *Z*-form (half life ~12 h) by photoirradiation. Upon incubation of a d_6-benzene solution of compound **2** in predominant *Z* form, fragmentation of the peptide chain could be observed, as revealed by the appearance of peaks having smaller *m*/*z* values in the ESI mass spectrum recorded on the incubation mixture. However, the fragmentations could not be explained properly and the pattern did not match that proposed by Jones and Warner[10] or Hirama *et al.*[11] for the degradation of peptides or proteins (Scheme 1).

After the inconclusive results with the azo-based compounds, we decided to change the basic template to a 1,2-dialkynyl benzene in order to avoid the problem of thermal re-isomerization. The reason for choosing the two alkyne moieties

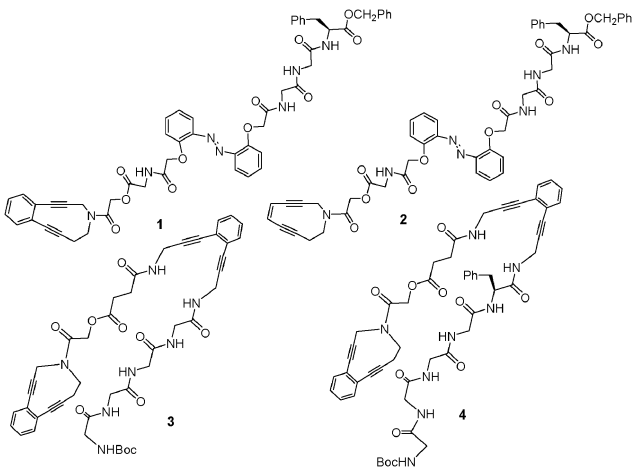


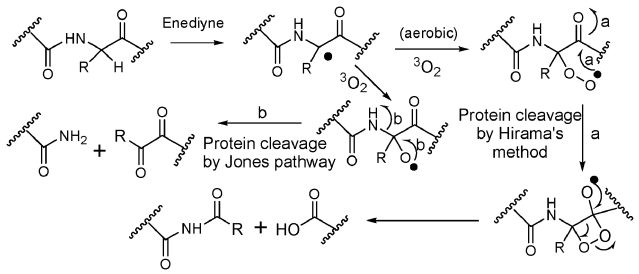
Fig. 2 Target enediyne–peptide hybrids.

Indian Institute of Technology, Chemistry, IIT Main Road, Kharagpur, West Bengal 721302, India.
E-mail: absk@chem.iitkgp.ernet.in

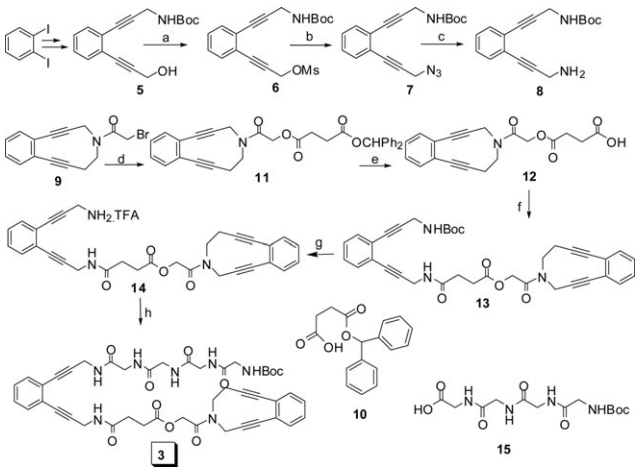
† Electronic supplementary information (ESI) available: Synthesis of **1**, **2** and **4**, ^{1}H, ^{13}C NMR of new compounds, VT NMR, COSY, DSC and MS spectra. See DOI: 10.1039/b923814j

View Article Online

Published on 25 January 2010. Downloaded by Temple University on 28/10/2014 00:06:31.



Scheme 1 Generation and fate of C-centered peptide radicals.



Scheme 2 Synthesis of enediyne–peptide hybrid **3**. Reagents and conditions: (a) MsCl, Et_3N, DCM, 0 °C, 15 min, 96%; (b) NaN_3, DMF, rt. 7 h, 67%; (c) PPh_3, THF, H_2O, rt, 10 h, 70%; (d) **10**, Cs_2CO_3, DMF, rt, 10 h, 59%; (e) TFA, anisole, DCM, 0 °C to rt, 1 h; (f) **8**, EDC·HCl, DMAP, DCM, rt, 10 h, 50%; (g) TFA, DCM, 0 °C to rt, 1.5 h; (h) **15**, EDC·HCl, DMAP, DCM, 0 °C to rt, 10 h, 41%.

in the template was to allow more space between the adjacent chains so that these do not suffer severe steric crowding. A succinoyl moiety was used as a linker to connect the enediyne while the tetrapeptide (Boc-gly-gly-gly-gly) was connected directly to the aminopropargyl arm. The synthesis, although quite straightforward, involved several steps and was accomplished starting from 1,2-diiodobenzene (Scheme 2). The compound **3** was characterized by NMR and mass spectroscopic studies. It showed an onset temperature of ~65 °C for Bergman cyclization as indicated by DSC.[12]

To examine the self fragmentation of **3**, we incubated its solution in d_6-benzene (500 μL) containing 4 drops of DMSO (for solubility) at 65 °C for 10 days. The degradation of the peptide side chain by the diradical formed by the cyclisation of enediyne was confirmed by MALDI-TOF-MS which showed two strong new peaks at *m*/*z* 672.5 and 688.5, corresponding to molecular ions (+Na) for **19** and **20**, respectively. The mass spectrum was recorded in ESI mode which also showed the presence of **19** and **20** in the incubation mixture (appearance of peaks at *m*/*z* 649 and 665 respectively). ESI-MS-MS studies on molecular ion peaks at 856 (molecular ion for **3** + Na), 665 and 649 revealed that all are separate entities. The MS on the molecular ion for the starting enediyne gave peaks at 800 and 756. The MS analysis on 665 and 649 produced peaks that are shown in Fig. 3 along with possible structures.

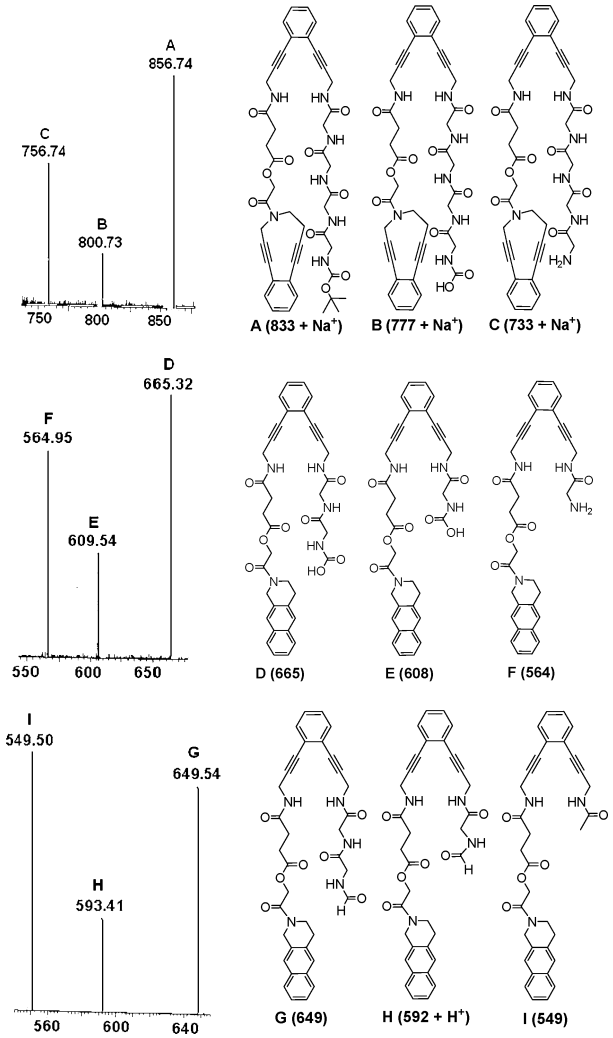


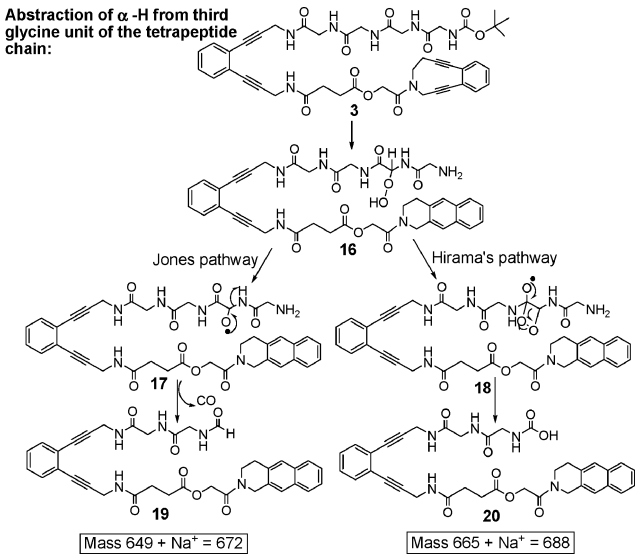
Fig. 3 MS-MS spectra.

The formation of **19** and **20** could be explained if one of the radical centres in the diradical abstracts the α-H from the third glycine (gly-gly-**gly**-gly-Boc) which then reacts with molecular oxygen. The resulting peroxy radical then undergoes degradation following pathways proposed by Jones and Warner[10] and Hirama *et al.*[11] to generate the fragments **19** and **20** respectively. The fragmentation pathway is shown in Scheme 3. If similar abstraction had taken place from the second glycine (gly-**gly**-gly-gly-Boc), peaks at *m*/*z* 615 and 631 were expected which were not present in the mass spectrum (Scheme 4). Similarly, the absence of peaks at *m*/*z* 729 and *m*/*z* 745 also ruled out the degradation from abstraction of the H from the terminal glycine. To reinforce this concept of selective cleavage, a pentapeptide–enediyne hybrid **4** was also synthesized and was subjected to the same incubation conditions. It also showed production of fragments *via* Jones and Hirama pathways, as indicated by the appearance of peaks at *m*/*z* 764 and 780 in the MALDI spectrum (Scheme 5). The exact reason for this preference to abstract an α-H from the third glycine unit may be due to its proximity to the radical (generated *via* BC). This has been supported by the energy minimized structure of the starting enediyne (included in the ESI†) which showed the proximity of the terminal acetylenic carbon and the α-H of the third glycine unit.

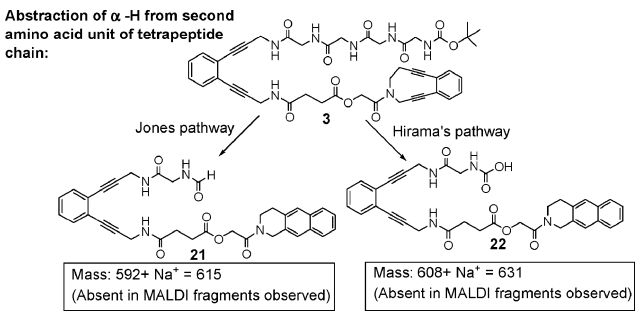
 This journal is © The Royal Society of Chemistry 2010

View Article Online

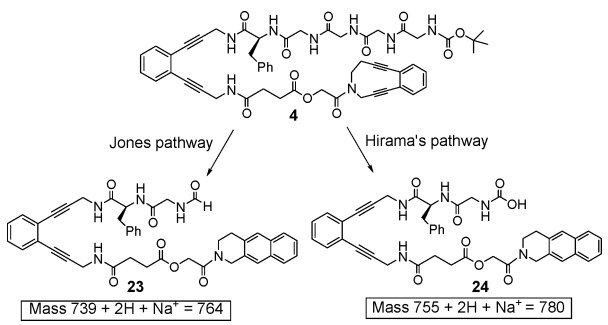
Published on 25 January 2010. Downloaded by Temple University on 28/10/2014 00:06:31.



Scheme 3 Mass fragmentation for abstraction of H at C-3.



Scheme 4 Possible fragmentation for abstraction of H at C-2.



Scheme 5 Abstraction of α-H from third glycine unit of the pentapeptide chain of compound **4**.

In conclusion, we have successfully accomplished the synthesis of enediyne–peptide hybrids which showed selective peptide chain cleavage by the 1,4-diyl radical. This reveals the possibility of selective protein cleavage *via* a similar strategy. Current studies are aimed towards that direction.

The authors AB and SR are grateful to CSIR, Government of India, for research funding and a fellowship respectively. DST is thanked for the funds for a 400 MHz NMR facility under the IRPHA program.

Notes and references

1 K. Iida and M. Hirama, *J. Am. Chem. Soc.*, 1994, **116**, 10310.
2 (*a*) N. Zein, P. Reiss, M. Bernatowicz and M. Bolgar, *Chem. Biol.*, 1995, **2**, 451; (*b*) N. Zein, A. M. Casazza, T. W. Doyle, J. E. Leet, D. R. Schroeder, W. Solomon and S. G. Nadler, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 8009; (*c*) N. Zein, W. Solomon, K. L. Colson and D. R. Schroeder, *Biochemistry*, 1995, **34**, 11591.
3 G. B. Jones, G. W. Plourde II and J. M. Wright, *Org. Lett.*, 2000, **2**, 811.
4 (*a*) P. Chen, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1478; (*b*) G. B. Jones and F. S. Fouad, *Curr. Pharm. Des.*, 2002, **8**, 2415; (*c*) Y. Zhang, T. Irshaidat, H. Wang, K. V. Waynant, H. Wang and J. W. Herndon, *J. Organomet. Chem.*, 2008, **693**, 3337.
5 (*a*) I. K. Chu, J. Zhao, M. Xu, S. O. Siu, A. C. Hopkinson and K. W. M. Siu, *J. Am. Chem. Soc.*, 2008, **130**, 7862; (*b*) D. Moran, R. Jacob, G. P. F. Wood, M. L. Coote, M. J. Davies, R. A. J. O'Hair, C. J. Easton and L. Radom, *Helv. Chim. Acta*, 2006, **89**, 2254; (*c*) J. J. Brocks, F. M. Welle, H. Beckhaus and C. Richardt, *Tetrahedron Lett.*, 1997, **38**, 7721; (*d*) A. C. Hopkinson, *Mass Spectrom. Rev.*, 2009, **28**, 655.
6 (*a*) A. Basak, S. S. Bag and H. M. M. Bdour, *Chem. Commun.*, 2003, 2614; (*b*) A. Basak, S. S. Bag and A. Basak, *Bioorg. Med. Chem.*, 2005, **13**, 4096.
7 A. Basak, K. R. Rudra, S. S. Bag and A. Basak, *J. Chem. Soc., Perkin Trans. 1*, 2002, 1805.
8 (*a*) L. Rahman, S. Kumar, C. Tschierske, G. Israel, D. Ster and G. Hegde, *Liq. Cryst.*, 2009, **36**, 397; (*b*) M. Xu, L. Chen, Y. Zhou, T. Yi, F. Li and C. Huang, *J. Colloid Interface Sci.*, 2008, **326**, 496; (*c*) U. A. Hrozhyk, S. V. Serak, N. V. Tabiryan and T. J. Bunning, *Adv. Funct. Mater.*, 2007, **17**, 1735; (*d*) C. Bang, A. Shishido and T. Ikeda, *Macromol. Rapid Commun.*, 2007, **28**, 1040; (*e*) Y. Zong, J. Ruehe and W. Knoll, *Responsive Polym. Mater.*, 2006, 50; (*f*) G. Galli and E. Chiellini, *Liq. Cryst.*, 2006, **33**, 1297.
9 (*a*) M. Kar and A. Basak, *Chem. Rev.*, 2007, **107**, 2861; (*b*) A. Basak, H. M. Bdour, J. C. Shain, S. Mandal, K. R. Rudra and S. Nag, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 1321; (*c*) A. Okamoto, K. Tanabe, T. Inasaki and I. Saito, *Angew. Chem., Int. Ed.*, 2003, **42**, 2502; (*d*) A. Dussy, C. Meyer, E. Quennet, T. A. Bickle, B. Giese and A. Marx, *ChemBioChem*, 2002, **3**, 54.
10 G. B. Jones and P. M. Warner, *J. Am. Chem. Soc.*, 2001, **123**, 2134.
11 M. Hirama, K. Akiyama, T. Tanaka, T. Noda, K. Iida, I. Sato, R. Hanaishi, S. F. Ishisaka, M. Ishiguro, T. Otani and J. E. Leet, *J. Am. Chem. Soc.*, 2000, **122**, 720.
12 (*a*) T. A. Zeidan, S. V. Kovalenko, M. Manoharan and I. V. Alabugin, *J. Org. Chem.*, 2006, **71**, 962; (*b*) M. Kar, A. Basak and M. Bhattacharjee, *Tetrahedron Lett.*, 2006, **47**, 117; (*c*) A. Basak, U. K. Khamrai and U. Mallik, *Chem. Commun.*, 1996, 749–750; (*d*) A. Basak and J. Shain, *Tetrahedron Lett.*, 1998, **39**, 1623.