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EMERGING AREA

When the nine-membered enediynes play hide and seek

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The lack of stability of the 9-membered enediynes not associated with an apoprotein may explain the low number of isolated natural compounds containing this core. To overcome such a problem, particular attention should be paid during the process of extraction and isolation of secondary metabolites, especially from microorganisms such as actinomycetes in order to identify the non-cycloaromatized derivatives.

Introduction

Since their discoveries, enediyne natural products have received considerable attention because they combine impressive antibiotic and antitumoral activities with unique molecular structures.[1] Neocarzinostatin **1**, a nine-membered enediyne, was first isolated in 1965 as a chromoprotein from a culture of *Streptomyces carzinostaticus* and first biological evaluations have presented broad-spectrum antibiotic activity. More recently antiproliferative activity has been shown against numerous tumor cell lines. During that time, a mechanism of action has been hypothesized to account for its biological profile. It was proposed that this enediyne chromoprotein delivers the neocarzinostatin chromophore **2** within the minor groove of the target cell's DNA and after a series of reactions leads to a 1,4-benzenoid diradical *via* the cycloaromatization of the enediyne moiety (Fig. 1).[2]

Since the isolation of **1** and the structural determination of **2** in the mid-1980s, other enediyne compounds, such as calicheamicin **3**, esperamicin **4** and dynemicin **5** for example, have been isolated as secondary metabolites produced from soil and marine microorganisms, fully characterized and evaluated in terms of biological activities. In general, these valuable derivatives can be categorized into two types of enediynes: the 10-membered enediynes including anthraquinonic derivatives and the 9-membered ones (Fig. 2).

While the 10-membered enediynes are stable enough to be isolated,[3] the 9-membered enediynes without their apoprotein undergo spontaneous cycloaromatization leading to an exclusive isolation of aromatized derivatives. To our knowledge, only the 9-membered enediyne antibiotic N1999A2 **12**, isolated from *Streptomyces* sp. AJ9493, is stable and has been identified as a non-chromoprotein.[4] It is probably fairly speculative to propose a definitive explanation to justify the difference of stability between the 10-membered and 9-membered enediynes (except **12**). To date, Nicolaou has suggested that spontaneous Bergman cycloaromatization should occur when the distance of the acetylene carbon atoms is in the range of 3.31–3.20 Å. In contrast, both Snyder and Magnus proposed that differential molecular strain in the ground state and transition state is the commanding element for ring closure.[5]

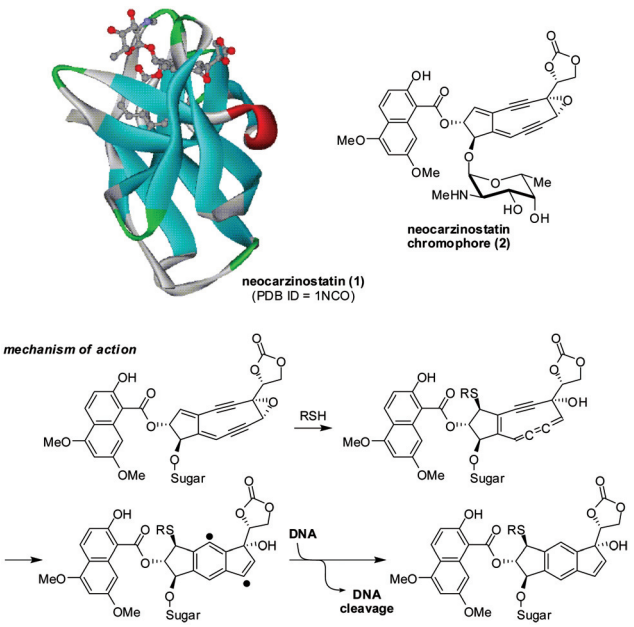

Fig. 1 Structures of neocarzinostatin (**1**) and chromophore (**2**) and mechanism of action with attack of a thiol group.

Putative and masked enediynes

Recently, cyclopenta[*a*]indene substrates have been isolated from marine actinomycetes *Salinispora tropica*,[6] *Salinispora pacifica*[7]

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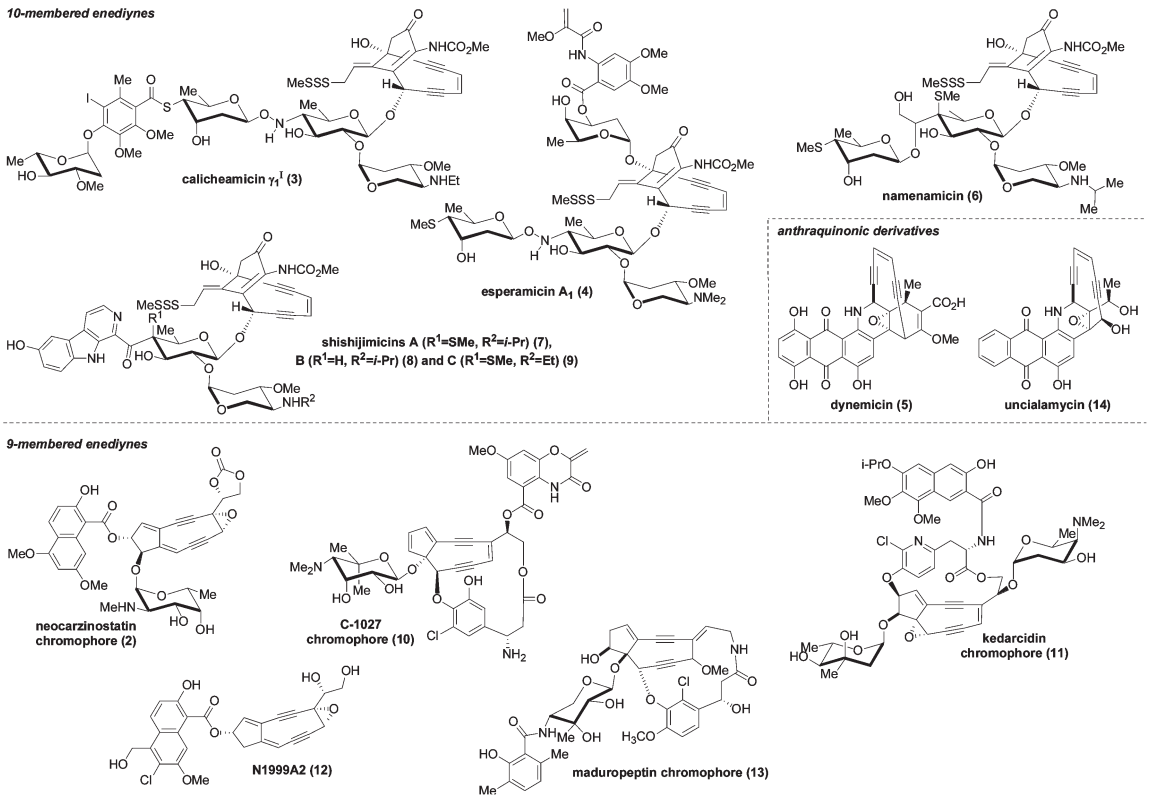


Fig. 2 Naturally occurring enediynes.

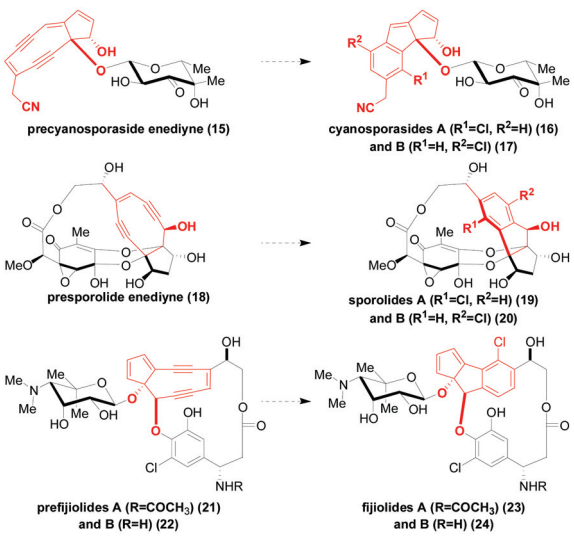
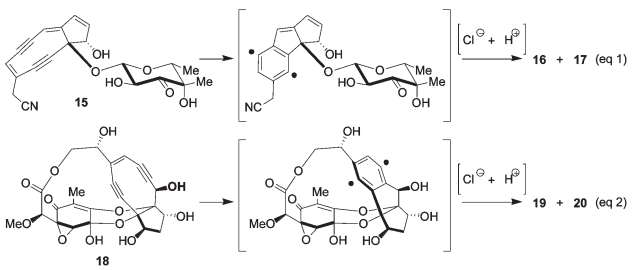


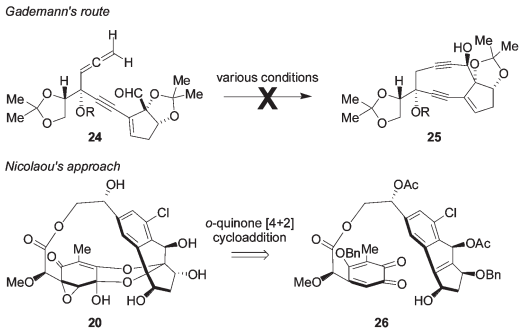
Fig. 3 Putative enediyne derivatives.

and strain CNS-653[8] whose structures derive probably from enediyne precursors. The isolation of these unsaturated carbon backbones was considered as the result of the rapid cyclization of a 9-membered enediyne core, once separated from their apoprotein with direct incorporation of a chlorine atom (Fig. 3).

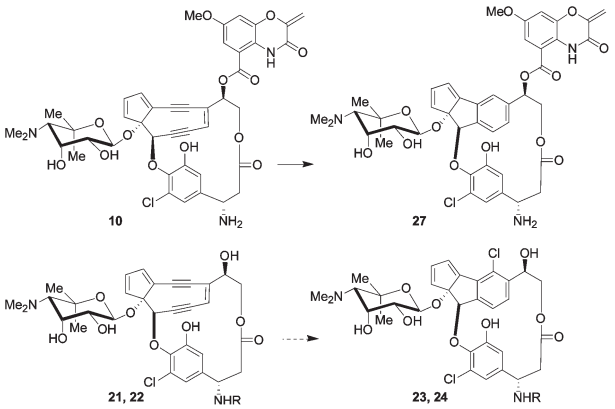
During the last decade, Fenical and co-workers thus studied extensively secondary metabolites isolated from strains of the genus *Salinispora*. Three distinct species have been identified: *S. arenicola*, *S. tropica* and *S. pacifica*. In 2005, they reported the isolation and characterization of two unprecedented halogenated macrolides, sporolides A **19** and B **20** from *S. tropica*. One year later, the same group isolated two new compounds, cyanosporasides A **16** and B **17**, as a 1 : 1 mixture, from *S. pacifica*. These latter possess a cyclopenta[*a*]indene framework similar to the products coming from the cycloaromatization of enediyne moieties, *e.g.* neocarzinostatin, C-1027, *etc.* Based on this hypothesis, the authors presumed that **16** and **17** could also be formed from a common enediyne precursor **15**, suggesting that these compounds were the result of a Bergman reaction with trapping of the diradical intermediate by a chlorine atom and a hydrogen one (Scheme 1 – eqn (1)). This biosynthesis suggestion has been strongly supported after a mechanistic study of the formation of chloroaromatic compounds *via* nucleophilic addition of chloride to the *para*-benzyne derived from an enediyne.[9] A biosynthesis of the sporolides A **19** and B **20** has been also proposed after identification of the responsible gene cluster



Scheme 1 Bergman cycloaromatization.



Scheme 2 Gademann's route toward the total synthesis of sporolides and Nicolaou's retrosynthetic approach.



Scheme 3 C-1027 and fijiolides A and B.

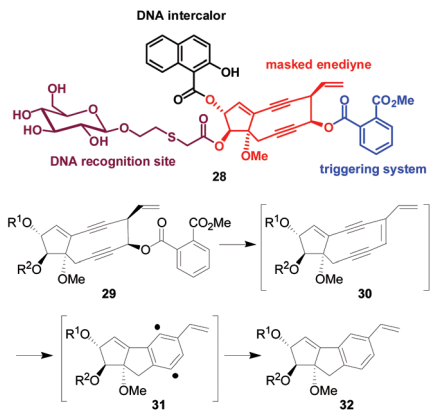


Fig. 4 Masked enediyne analogues.

by Moore and co-workers.[6d,10] The formation of the chlorinated cyclopenta[*a*]indene has been also achieved after a Bergman cycloaromatization from a non-identified enediyne **18** (Scheme 1 – eqn (2)).[6d,10] On the basis of these studies, Gademann[11] and more recently Smith[12] proposed synthetic studies to prepare the sporolides, and Nicolaou disclosed the first total synthesis of sporolide B **20** and 9-*epi*-sporolide B.[13]

Gademann and co-workers concentrated their efforts on the exploration of the enediyne route in order to build the cyclopenta[*a*]indene moiety. Whatever the precursor of enediynes synthesized, the authors have not succeeded in their tentative effort to form the 9-membered enediyne due to their unsuccessful attempts to isomerize the allene **25** into a terminal alkyne (Scheme 2). Unlike Gademann, Nicolaou, as well as Smith, focused their retrosynthetic analysis toward a non-biomimetic approach, the chlorocyclopenta[*a*]indene part being prepared directly.

In 2010, fijiolides A **23** and B **24** were isolated from a marine-derived bacterium of the genus *Nocardiopsis* collected from the Beqa Lagoon, Fiji.[8] Due to their chlorocyclopenta[*a*]indene core these marine natural compounds look like the above mentioned derivatives, and with the exception of the chlorine and the benzoxazine moiety they are very close to the cycloaromatized C-1027 **27** (Scheme 3). This is why fijiolides A **23** and B **24**, considered also as putative enediynes, would be probably formed after a Bergman rearrangement from **21** and **22** followed by chlorine trapping. Because fijiolides are cycloaromatized derivatives, they do not display significant antibiotic activities unlike the chromoprotein C-1027 including **10** which possess antitumor and antibiotic activities.

The discovery that unstable enediyne compounds easily transformed into less active molecules like the above mentioned also indicates that some enediynes might have been missed during the classical extraction process. This recurrent problem of stability of enediyne derivatives combined with the small quantity of material available by extraction from microbial cultures are crucial bottlenecks for the development of these biological valuable compounds as drugs. A genome scanning method indicated the wide occurrence of enediyne biosynthetic loci among actinomycetes and highlighted their expression variability according to the growth conditions.[14] Careful attention must be also paid to the choice of solvent extraction and to chromatographic processes used for the isolation of these enediynes to avoid Bergman cycloaromatization. For this purpose, combined with optimized culture and extraction procedures, the total synthesis appears as an essential tool in order to have ample quantities of products and more stable enediyne analogues of the natural compounds. To that end, Tanaka's group designed some 1,5-diyne derivatives as masked enediyne equivalents of neocarzinostatin chromophore **2**, kedarcidin chromophore **11**, and C-1027 **10**. After preparation of an enediyne-like model and having given the proof of the concept, they embarked on the synthesis of masked enediynes possessing DNA intercalator and sugar moieties such as **28** (Fig. 4). Elimination of the triggering system in a weakly basic buffered solution (pH 7.5) leads to the highly reactive 9-membered enediyne **30** which undergoes a spontaneous Bergman cycloaromatization to afford the 1,4-benzoid diradical **31**. As the well-known 9-membered enediyne chromophore, the masked enediyne **28** possesses all the requirements to cleave DNA.[15]

Conclusion

Enediyne compounds are remarkable active natural products which own a unique and versatile core. Their low number could be improved by the use of the genome mining approach which has already underlined the importance of the growth conditions for their expression but also by taking into account their stability. This latter depends on the size of the enediyne-ring, the

9-membered one requiring an apoprotein to be stable. To overcome such a problem, particular attention should be paid during the isolation process. The strategy reported here, to develop masked enediyne analogues, is an important alternative to overcome this issue and should help to design new stable compounds.

Notes and references

1 P. R. Hamann, J. Upeslacis and D. B. Borders, in *Anticancers Agents from Natural Products*, ed. G. M. Cragg, G. G. I. Kingston and D. J. Newman, CRC Press, 2nd edn, 2011, pp. 575–621.

2 (*a*) N. Ishida, K. Miyazaki, K. Kumagai and M. Rikimaru, *J. Antibiot. Ser. A*, 1965, **18**, 68–76; (*b*) K. Edo, M. Mizugaki, Y. Koide, H. Seto, K. Furihata, N. Otake and N. Ishida, *Tetrahedron Lett.*, 1985, **26**, 331–334; (*c*) J. R. Baker, D. N. Woolfson, F. W. Muskett, R. G. Stoneman, M. D. Urbaniak and S. Caddick, *ChemBioChem*, 2007, **8**, 704–717; (*d*) H.-W. Chi, Y.-C. Chien, C.-Y. Liu, C.-J. Tseng, Y.-J. Lee, J.-L. Chan, Y.-R. Chu and D.-H. Chin, *Chem.–Eur. J.*, 2011, **17**, 1493–1506.

3 (*a*) S. J. Danishefsky and M. D. Shair, *J. Org. Chem.*, 1996, **61**, 16–44; (*b*) G. A. Ellestad, *Chirality*, 2011, **23**, 660–671.

4 (*a*) T. Ando, M. Ishii, T. Kajiura, T. Kameyama, K. Miwa and Y. Sugiura, *Tetrahedron Lett.*, 1998, **39**, 6495–6498; (*b*) N. Miyagawa, D. Sasaki, M. Matsuoka, M. Imanishi, T. Ando and Y. Sugiura, *Biochem. Biophys. Res. Commun.*, 2003, **306**, 87–92; (*c*) S. Kobayashi and M. Hirama, *J. Synth. Org. Chem., Jpn.*, 2004, **62**, 184–193.

5 (*a*) K. C. Nicolaou, G. Zuccarello, Y. Ogawa, E. J. Schweiger and T. Kumazawa, *J. Am. Chem. Soc.*, 1988, **110**, 4866–4868; (*b*) P. Magnus and P. A. Carter, *J. Am. Chem. Soc.*, 1988, **110**, 1626–1628; (*c*) J. P. Snyder, *J. Am. Chem. Soc.*, 1989, **111**, 7630–7632; (*d*) J. P. Snyder, *J. Am. Chem. Soc.*, 1990, **112**, 5367–5369.

6 (*a*) G. O. Buchanan, P. G. Williams, R. H. Feling, C. A. Kauffman, P. R. Jensen and W. Fenical, *Org. Lett.*, 2005, **7**, 2731–2734; (*b*) P. R. Jensen, P. G. Williams, D.-C. Oh, L. Ziegler and W. Fenical, *Appl. Environ. Microbiol.*, 2007, **73**, 1146–1152; (*c*) D. W. Udwary, L. Ziegler, R. N. Asolkar, V. Sing, A. Lapidus, W. Fenical, P. R. Jensen and B. S. Moore, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 10376–10381; (*d*) M. Nett and B. S. Moore, *Pure Appl. Chem.*, 2009, **81**, 1075–1084.

7 D.-C. Oh, P. G. Williams, C. A. Kauffman, P. R. Jensen and W. Fenical, *Org. Lett.*, 2006, **8**, 1021–1024.

8 S.-J. Nam, S. P. Gaudêncio, C. A. Kauffman, P. R. Jensen, T. P. Kondratyuk, L. E. Marler, J. M. Pezzuto and W. Fenical, *J. Nat. Prod.*, 2010, **73**, 1080–1086.

9 C. L. Perrin, B. L. Rodgers and J. M. O'Connor, *J. Am. Chem. Soc.*, 2007, **129**, 4795–4799.

10 R. P. McGlinchey, M. Nett and B. S. Moore, *J. Am. Chem. Soc.*, 2008, **130**, 2406–2407.

11 (*a*) J.-Y. Wach and K. Gademann, *Synlett*, 2009, 2849–2851; (*b*) S. Bonazzi, M. Binaghi, C. Fellay, J.-Y. Wach and K. Gademann, *Synthesis*, 2010, 631–642.

12 J. A. Gladding, J. P. Bacci, S. A. Shaw and A. B. Smith, *Tetrahedron*, 2011, **67**, 6697–6706.

13 (*a*) K. C. Nicolaou, J. Wang and Y. Tang, *Angew. Chem., Int. Ed.*, 2008, **47**, 1432–1435; (*b*) K. C. Nicolaou, Y. Tang and J. Wang, *Angew. Chem., Int. Ed.*, 2003, **48**, 3449–3453; (*c*) P. Li and D. Menche, *Angew. Chem., Int. Ed.*, 2009, **48**, 5078–5080; (*d*) K. C. Nicolaou, J. Wang, Y. Tang and L. Botta, *J. Am. Chem. Soc.*, 2010, **132**, 11350–11363.

14 E. Zazopoulos, K. Huang, A. Staffa, W. Liu, B. O. Bachmann, K. Nonaka, J. Ahlert, J. S. Thorson, B. Shen and C. M. Farnet, *Nat. Biotechnol.*, 2003, **21**, 187–190.

15 (*a*) T. Takahashi, H. Tanaka, H. Yamada, T. Matsumoto and Y. Sugiura, *Angew. Chem., Int. Ed.*, 1996, **35**, 1835–1837; (*b*) T. Takahashi, H. Tanaka, H. Yamada, T. Matsumoto and Y. Sugiura, *Angew. Chem., Int. Ed.*, 1997, **36**, 1524–1526; (*c*) T. Takahashi, H. Tanaka, A. Matsuda, T. Doi and H. Yamada, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3299–3302; (*d*) T. Takahashi, H. Tanaka, A. Matsuda, T. Doi, H. Yamada, T. Matsumoto, D. Sasaki and Y. Sugiura, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3303–3306; (*e*) T. Takahashi, H. Tanaka, T. Doi and H. Yamada, *J. Synth. Org. Chem., Jpn.*, 1998, **56**, 987–999; (*f*) Y. Tanaka, S. Fuse, H. Tanaka, T. Doi and T. Takahashi, *Org. Process Res. Dev.*, 2009, **13**, 1111–1121.