International Journal of Mass Spectrometry 279 (2009) 176–181

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Short communication

ESI-MS characteristics of *N*-methylpyrrole polyamide/bis-cyclen conjugate

Chao Li [a], Chao Jiang [a], Ren-Zhong Qiao [a,*], Yu-Fen Zhao [b,*]

[a] *State Key Laboratory of Chemical Resource Engineering, Department of Pharmaceutical Engineering, Beijing University of Chemical Technology, Beijing 100029, China*
[b] *The Key Laboratory of Bio-organic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, China*

ARTICLE INFO

Article history:
Received 29 August 2008
Received in revised form 21 October 2008
Accepted 25 October 2008
Available online 5 November 2008

Keywords:
Polyamide
Cyclen
ESI/MS/MS
DNA
Ring contraction

ABSTRACT

The study of the dissociation of the protonated molecular species $[M+H]^+$ and selected fragment ions allowed proposals for the main fragmentation pathways of the title compound. The main fragmentation pathways occur by the cleavage of the C–CO bonds between *N*-methylpyrrole and carbonyl groups. Because of the introduction of bis-cyclen into the polyamide, the most striking feature of the MS/MS spectra is the prevalence of ring contractions. Electrospray ionization is proven to be a good method for the structural characterization and identification of these kinds of compounds.

1. Introduction

Polyamides containing *N*-methylpyrrole amino acids have attracted considerable attention from synthetic and biological chemists because they recognize and bind in the minor groove of predetermined DNA sequences with high affinity and specificity [1–5]. Azamacrocylic ligands such as 1,4,7,10-tetraazacyclododecane (cyclen) continue to receive considerable attention, and their metal complexes find widespread utility as a consequence of their robust and well-defined artificial nucleic acid chemistry [6–13]. The mass spectral fragmentation mechanisms of such compounds were reported in the past few years [14–16]. Moreover, Qiao et al. [17] have reported a new compound ($NO_2Py_4\gamma$-cyclen) as an artificial nuclease, and described the mass spectral characteristics of this compound. We are synthesizing various polyamides and small molecule conjugates as potent DNA cleavage agents in our lab. In this paper, the fragmentation of a new conjugate (bis-cyclen-Py_4Dp) was investigated using electrospray ionization mass spectrometry (ESI-MS) combined with tandem mass spectrometry (ESI-MS/MS).

2. Experimental

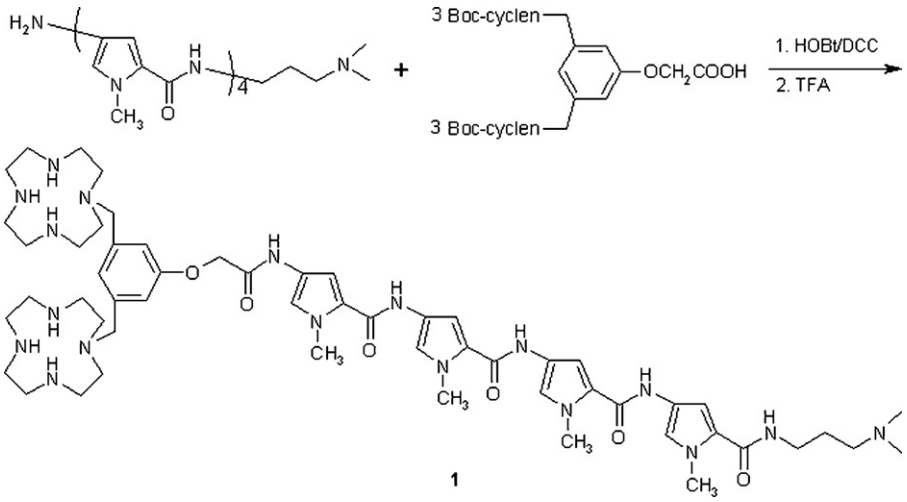
Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE-LC^{TM} ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z 6000. Nitrogen was used as drying gas at a flow rate of 4 L/min. The nebulizer pressure was 7.0 psi. The capillary was typically held at 4 kV and the source temperature was maintained at 300 °C. The instrument was operated at unit-mass resolution; calibration of m/z was performed using a standard ES-tuning-mix. Three scans were averaged for each spectrum. The samples, dissolved in methanol, were ionized by electrospray ionization and continuously infused into the ESI chamber by a Cole-Parmer 74900 syringe pump (Cole Parmer Instrument Company).

3. Results and discussion

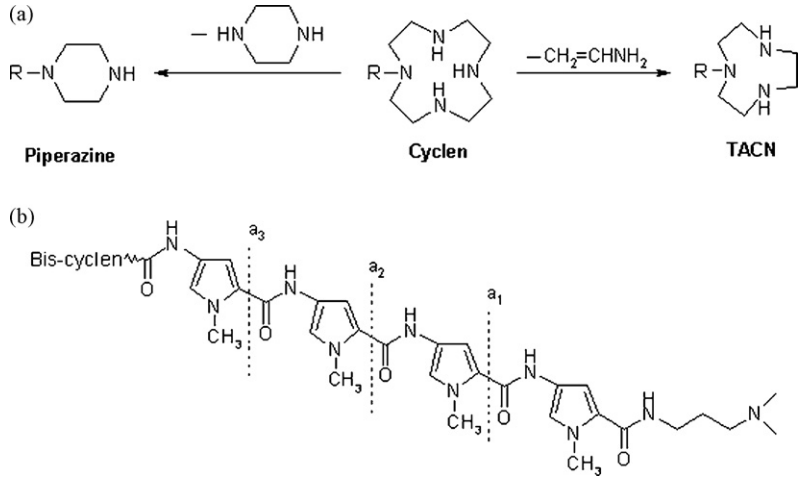
Compound 1 was prepared by a 1-hydroxybenzotriazole/dicyclohexylcarbodiimide (HOBt/DCC) mediated coupling reaction (Scheme 1), which has been characterized by accurate mass (m/z) measurement, ^{1}H NMR and ^{13}C NMR spectroscopy. The ability to induce DNA cleavage and recognize duplex DNA sequences has been an ongoing area of study in our group. The primary results showed this dinuclear Zn(II) complex was more effective in the cleavage of DNA under physiological conditions than the complex without polyamide.

* Corresponding authors. Tel.: +86 10 64413899; fax: +86 10 82728926.
E-mail address: qrz@mail.tsinghua.edu.cn (R.-Z. Qiao).

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.10.020



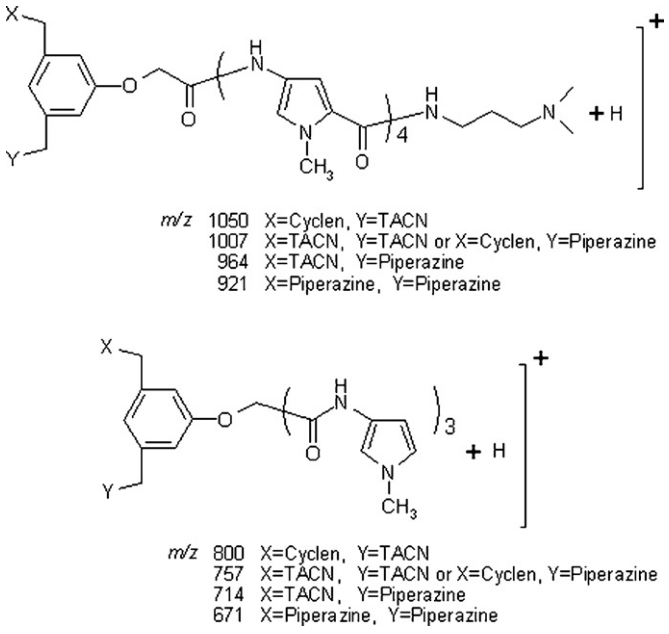
Scheme 1. The synthesis and structure of polyamide/bis-cyclen conjugate.



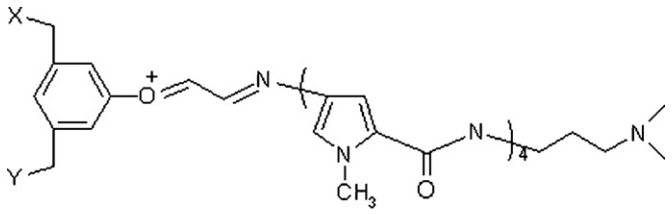
Scheme 2. Main fragmentation modes of polyamide and cyclen groups.

The ESI-MS^n spectra of compound 1 were obtained (Fig. 1). The most striking feature of the MS/MS spectra of the $[M+H]^+$ ions is the fragment ions a_1–a_3 at *m*/*z* 843, 721 and 599, all due to cleavage of the C–CO bond between *N*-methylpyrrole and carbonyl group. Because of the introduction of the bis-cyclen into the polyamide, the –NH of the cyclen group are liable to be protonated comparing with polyamide group. When the fragmentation of amide field occurred, the fragment with neutral terminal O=C=N (the *a* cleavage) is liable to form than fragments $C{\equiv}O^+$ or $CO–NH_2$ [16–19], as shown in Scheme 2b. 1,4,7-Triazacyclononane (TACN) and piperazine are formed by expelling a molecule of $CH_2{=}CHNH_2$ and piperazine from cyclen, respectively (Scheme 2a). For example, the formation of the ions at *m*/*z* 1050, 1007, 964 and 921 occurs by cyclen contractions from the precursor ion *m*/*z* 1093, and the ions *m*/*z* 800, 757, 714 and 671 correspond to cyclen contractions from the precursor ion *m*/*z* 843 (Scheme 3).

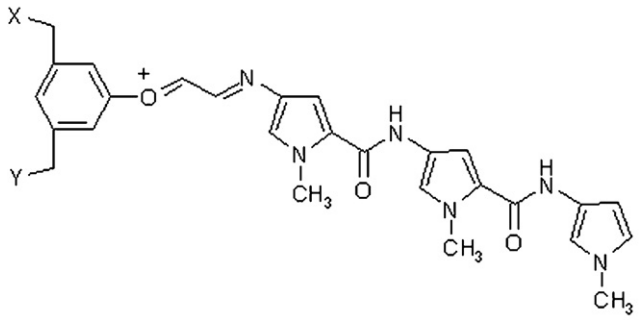
The other characteristic fragmentation pathway is that cyclen contractions accompany the loss of a neutral fragment (H_2O) with rearrangement of a hydrogen atom. The presence of common fragment ions *m*/*z* 1075, 1032, 989, 946, 903, 825, 782, 739, 696 and 653 elucidates the fragmentation pathways from their precursor ions (Scheme 4).



Scheme 3. Ring contractions of the ions at *m*/*z* 1093 and 843.

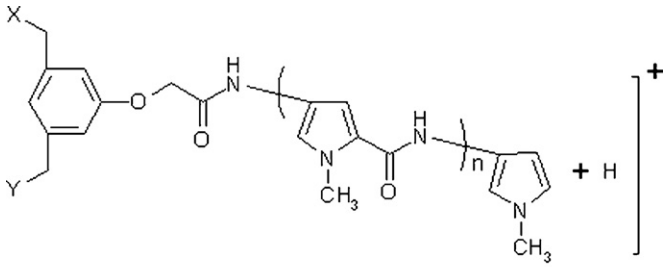


m/z 1075 X=Cyclen, Y=Cyclen
1032 X=Cyclen, Y=TACN
989 X=TACN, Y=TACN or X=Cyclen, Y=Piperazine
946 X=TACN, Y=Piperazine
903 X=Piperazine, Y=Piperazine



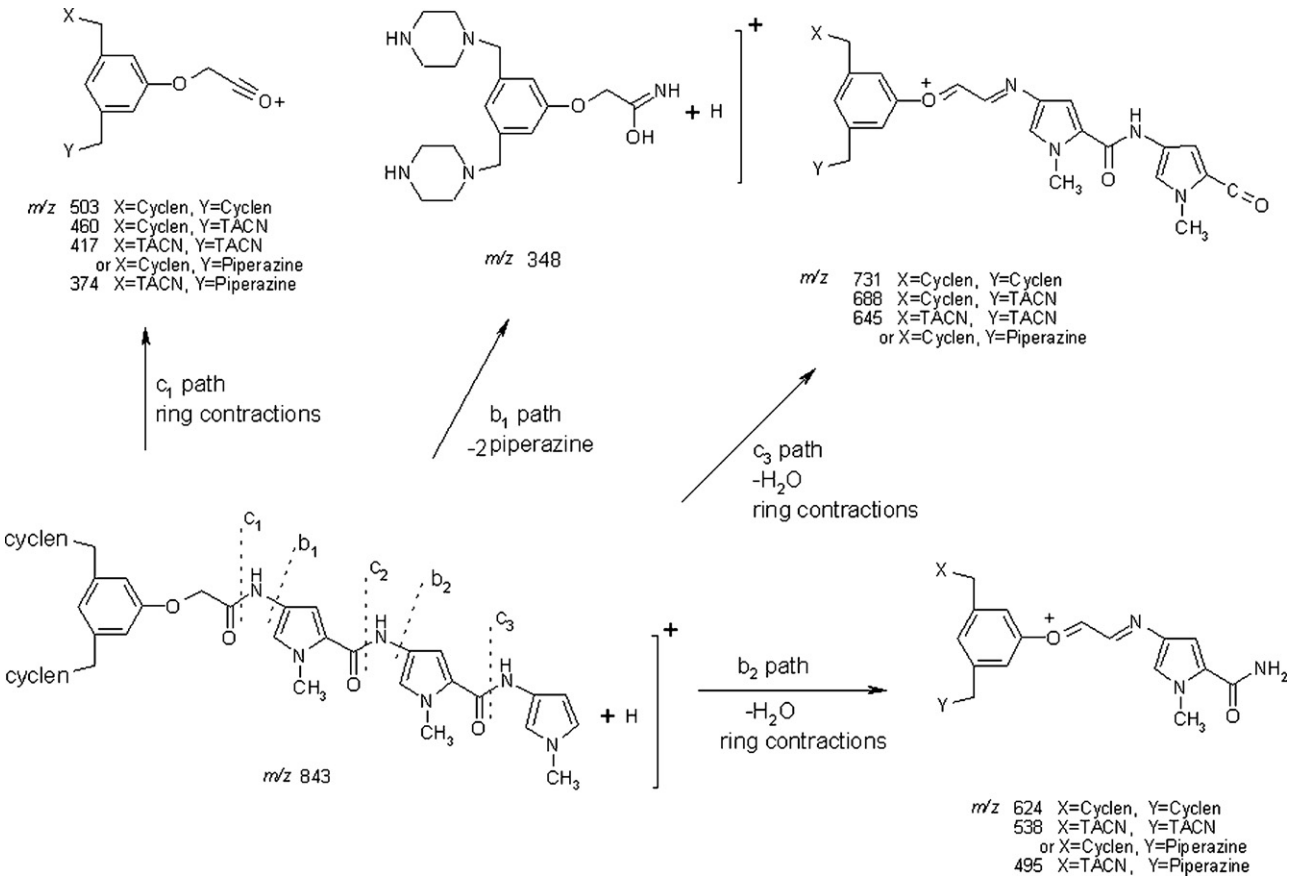
m/z 825 X=Cyclen, Y=Cyclen
782 X=Cyclen, Y=TACN
739 X=TACN, Y=TACN or X=Cyclen, Y=Piperazine
696 X=TACN, Y=Piperazine
653 X=Piperazine, Y=Piperazine

Scheme 4. The fragments of cyclen contractions and loss of H_2O.



m/z 843 n=2, X=Y=Cyclen
757 n=2, X=Y=TACN or X=Cyclen, Y=Piperazine
671 n=2, X=Y=Piperazine
721 n=1, X=Y=Cyclen
635 n=1, X=Y=TACN or X=Cyclen, Y=Piperazine
549 n=1, X=Y=Piperazine
599 n=0, X=Y=Cyclen
513 n=0, X=Y=TACN or X=Cyclen, Y=Piperazine
427 n=0, X=Y=Piperazine

Scheme 5. The fragments of cyclen contractions and the *a* pathway.



Scheme 6. ESI-MS^3 fragmentation pathway of the ion at *m/z* 843.

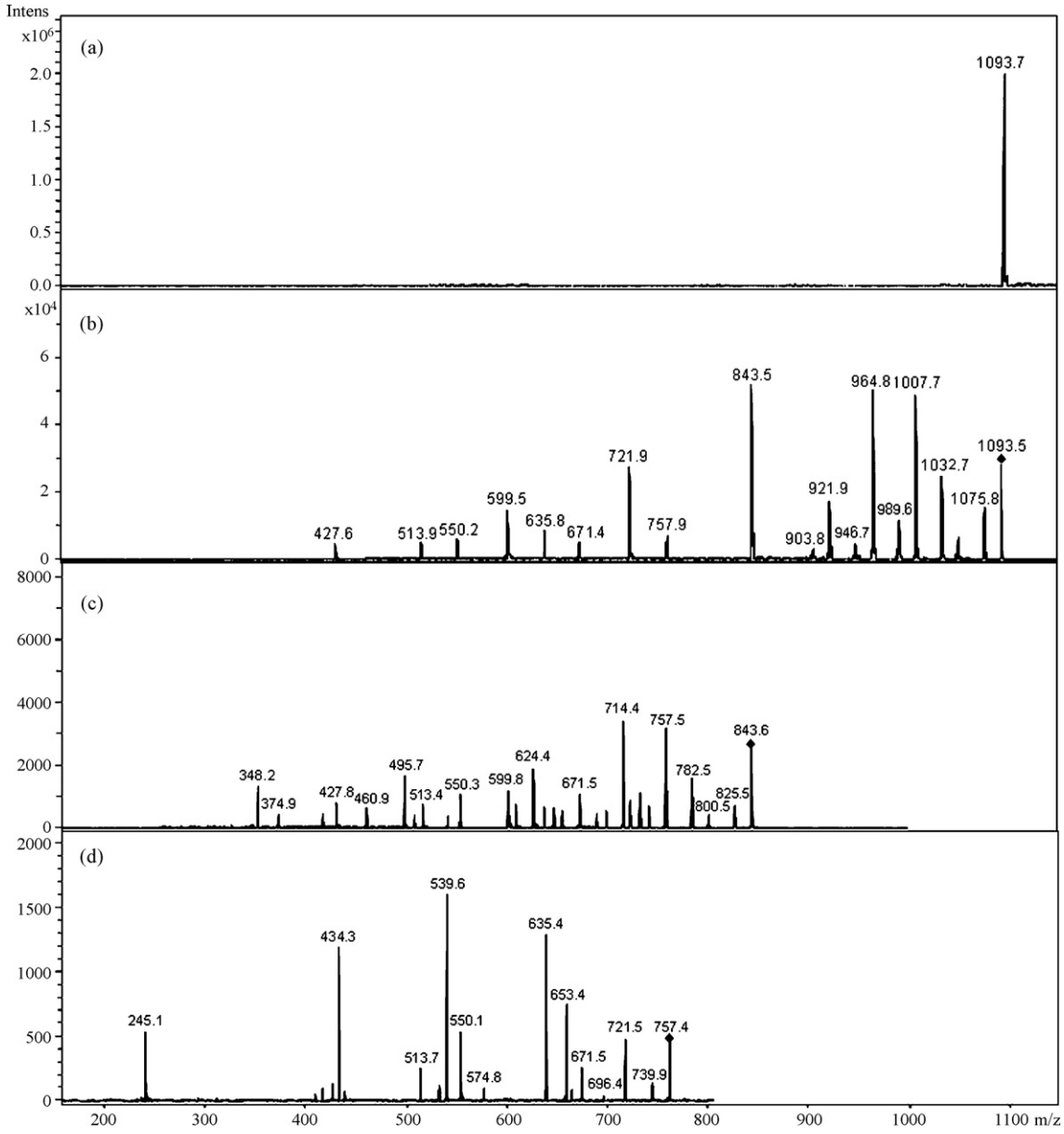
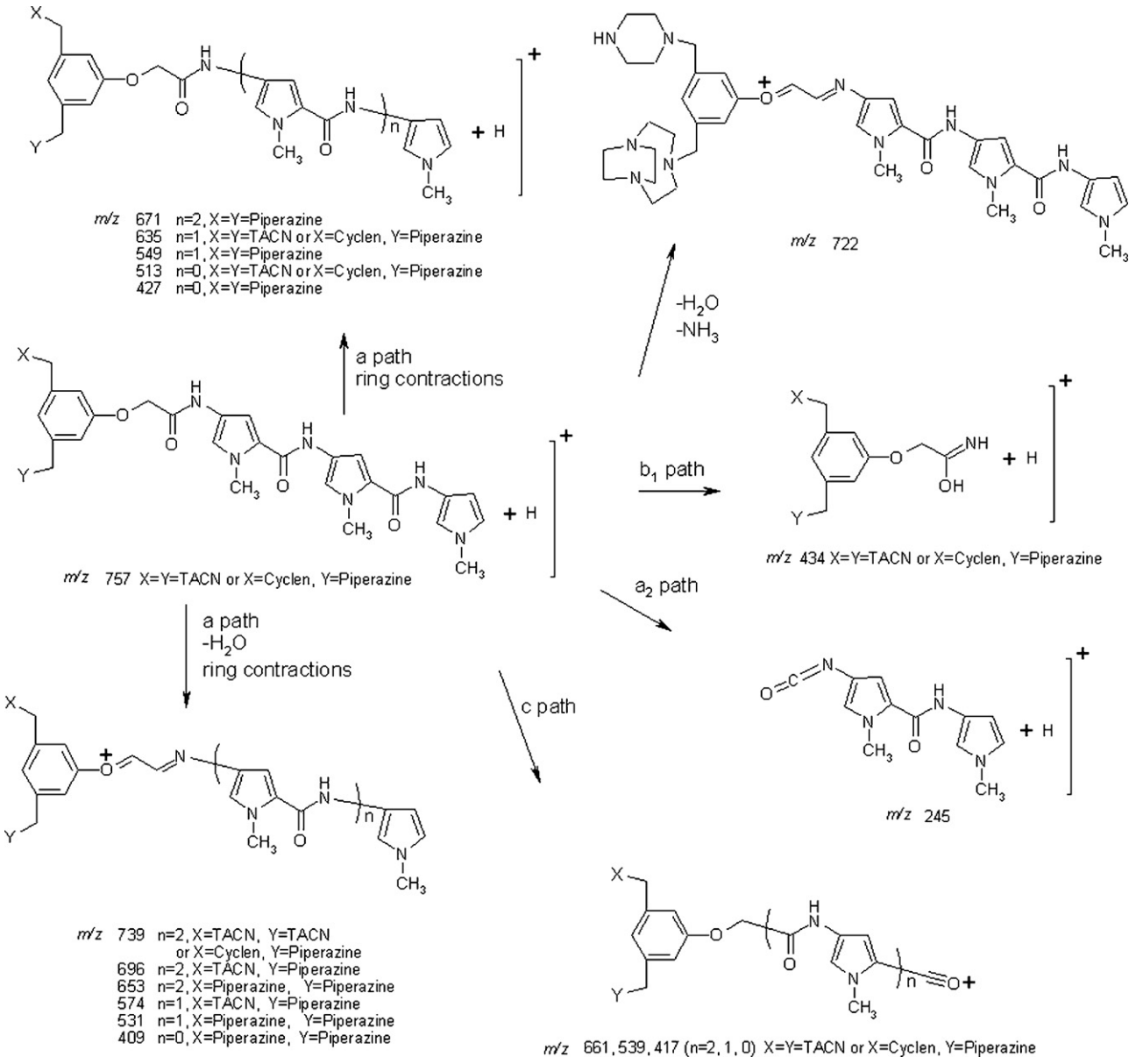


Fig. 1. The MS and MS^n spectra of compound 1.

Interestingly, the two cyclen groups of the various precursor ions undergo the same ring contraction reactions (X = Y), while simultaneously experiencing the cleavage of the C–CO bond between *N*-methylpyrrole and carbonyl group (the *a* pathway), as shown in Scheme 5. For example when $n = 2$, instead of the fragment ions m/z 800 ($n = 2$, X = cyclen, Y = TACN) and 714 ($n = 2$, X = TACN, Y = piperazine), the fragments in which both rings (X and Y) contract to TACN (or X = cyclen, Y = piperazine) or piperazine by expelling a molecule of $CH_2{=}CHNH_2$ (or a piperazine) or piperazine, respectively, are obtained. The same fragmentation mode can be observed for the precursor ions m/z 843 and 757. The ions m/z 721, 635, 549, 599, 513 and 427 as well as 671, 635, 549, 513 and 427 correspond to these fragmentation modes from precursor ions m/z 843 and 757, respectively.

In order to better understand the fragmentation mechanisms of polyamide/bis-cyclen conjugate, the ESI-MS data of the ion m/z 844 were recorded (not shown). The fragmentation pathways of the ion m/z 843, proposed in Scheme 6, can rationalize the spectra obtained. After the ring contractions from the precursor ion m/z 844, the fragmentation pathways involve the c_1 cleavage (m/z 503, 460, 417 and 374), b_1 cleavage (m/z 348), c_3 (m/z 731, 688 and 645) and b_2 (m/z 624, 538 and 495) cleavage and water loss with rearrangement a hydrogen atom. In addition, fragmentation pathways of the ion m/z 757 are shown in Scheme 7. The ions m/z 434 and 245 are produced by b_1 and a_2 cleavage, respectively. The ions m/z 661, 539 and 417 are generated by c cleavage. The ions m/z 739, 696, 653, 574, 531 and 409 from their precursor ion could result from the *a* cleavage, ring contractions and water loss with rearrangement a hydrogen atom. The ion m/z 722 is generated by the loss of H_2O and NH_3 with rearrangement a hydrogen atom from the precursor ion m/z 757 (when X = cyclen, Y = piperazine), and this fragmentation mode of NH_3 loss is consistent with the characteristic of compound ($NO_2Py_4\gamma$-cyclen) [17].



Scheme 7. ESI-MS^4 fragmentation pathway of the ion at m/z 757.

4. Conclusion

In this study, the ESI-MS^n data of polyamide/bis-cyclen conjugate provided abundant structural information. The main fragmentation pathways involve the cleavage of the C–CO bonds between rings and carbonyl groups (the *a* pathway) because of the introduction of bis-cyclen into the polyamide. The most striking feature of the MS/MS spectra are ring contraction reactions. The 1,4,7-triazacyclononane and piperazine moieties are formed by expelling a molecule of CH_2=$CHNH_2$ or piperazine, respectively, under ESI ionization conditions. The ESI-MS^n approach was proven to be a good method for the structural characterization and identification of this new class of DNA-recognizing compounds.

Acknowledgment

Support of this research by the National Nature Science Foundation of China is gratefully acknowledged (No. 20572008, No. 20732004 and No. 20872010).

References

[1] Q. Zhang, T.J. Dwyer, V. Tsui, D.A. Case, J. Cho, P.B. Dervan, D.E. Wemmer, J. Am. Chem. Soc 126 (2004) 7958.
[2] A. Blasko, K.A. Browne, T.C. Bruice, J. Am. Chem. Soc. 116 (1994) 3726.
[3] C.L. Kielkopf, S. White, J.W. Szewczyk, J.M. Turner, E.E. Baird, P.B. Dervan, D.C. Rees, Science 282 (1998)111.
[4] I.S. Blagbrough, E. Moya, S.P. Walford, Tetrahedron Lett. 37 (1996) 551.
[5] J.M. Gottesfeld, L. Neely, J.W. Trauger, E.E. Baird, P.B. Dervan, Nature 387 (1997) 202.
[6] W. Peng, P.Y. Liu, N. Jiang, H.H. Lin, G.L. Zhang, Y. Liu, X.Q. Yu, Bioorg. Chem. 33 (2005) 374.
[7] Y.G. Fang, J. Zhang, S.Y. Chen, N. Jiang, H.H. Lin, Y. Zhang, X.Q. Yu, Bioorg. Med. Chem. 15 (2007) 696.
[8] O. Wiest, C.B. Harrison, N.J. Saettel, R. Cibulka, M. Sax, B. Konig, J. Org. Chem. 69 (2004) 8183.
[9] S.J. Krivickas, E. Tamanini, M.H. Todd, M. Watkinson, J. Org. Chem. 72 (2007) 8280.
[10] K. Michaelis, M. Kalesse, Chem. Biochem. 1 (2001) 79.
[11] A.C. Benniston, P. Gunning, R.D. Peacock, J. Org. Chem. 70 (2005) 115.
[12] X.Y. Wang, J. Zhang, K. Li, N. Jiang, S.Y. Chen, H.H. Lin, Y. Huang, L.J. Ma, X.Q. Yu, Bioorg. Med. Chem. 14 (2006) 6745.
[13] L.J. Ma, G.L. Zhang, S.Y. Chen, B. Wu, J.S. You, C.Q. Xia, X.Q. Yu, J. Peptide Sci. 11 (2005) 812.

[14] Q.L. Li, J. Zhou, F.L. Tang, G. Yuan, Acta. Chim. Sinica 9 (2005) 834.
[15] Y. Ye, L.F. Cao, M.Y. Niu, X.C. Liao, Y.F. Zhao, Int. J. Mass Spectrom. 253 (2006) 141.
[16] C. Li, R.Z. Qiao, Y.F. Zhao, Int. J. Mass Spectrom. 270 (2008) 94.
[17] R.Z. Qiao, Z. Zhang, Y. Ju, C.Q. Xia, X.Q. Yu, Y.F. Zhao, Chin. J. Chem. 24 (2006) 923.
[18] H.H. Li, G. Yuan, Int. J. Mass Spectrom. 252 (2006) 54.
[19] Z.H. Ji, J.J. Li, J. Zhou, L.M. Hui, G. Yuan, S.M. Cai, J. Phys. Chem. B 109 (2005) 17162.