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Short communication

Characteristic fragmentation behaviors of novel dithiocarbamic acid esters studied by electrospray ionization tandem mass spectrometry

Xueling Hou [a], Yaowu Sha [b,**], Xin Wang [a], Zemei Ge [a], Runtao Li [a,*]

[a] *School of Pharmaceutical Sciences, Health Science Center, Peking University, Beijing 100083, China*
[b] *The Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, China*

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Abstract

The fragmentation patterns of a novel kind of dithiocarbamic acid esters with excellent anticancer activity were analyzed by positive ion electrospray ionization mass spectrometry in conjunction with tandem mass spectrometry (ESI/MS^n). The fragmentation patterns of sodium adduct ions $[M + Na]^+$ were characterized with elimination of hydrogen cyanide and most of their counterparts could be observed. The fragmentation patterns of protonated molecular ions $[M + H]^+$ were characterized with single bond cleavage between thiocarbonyl group and nitrogen atom, and between thiocarbonyl group and sulfur atom. The piperazine moiety of the molecular of $[M + H]^+$ favors a rearrangement to expel azirane, and the suggested rearrangement mechanism is consistent with experimental observations.

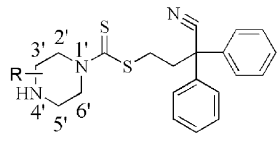
Keywords: Mass spectrometry; Dithiocarbamic acid ester; Fragmentation pathway

1. Introduction

Accompanying the development of industry and the increase of environmental pollution, the incidence of cancer in the population has increased strikingly in the past few decades. Hence, the need for innovative treatments for various cancers is becoming more urgent. Despite major breakthroughs in many areas of modern medicine, significant challenges to the successful treatment of cancer still remain because of the difficulty in discovering novel agents that can selectively kill tumor cells or inhibit their proliferation without causing general toxicity. Dithiocarbamic acid esters exhibit a variety of valuable biological effects, including antibacterial activity [1,2], antifungal activity [3], the ability to chelate heavy metals [4,5] and to function as NO scavengers [6]. Recently, we discovered several kinds of dithiocarbamic acid esters possessing significant anticancer activity [7,8]. One of the compounds, 4-methyl-piperazine-1-carbodithioic acid 3-cyano-3, 3-diphenyl-propyl ester (**1B**), showed excellent in vitro and in vivo anticancer activity and very low toxicity [9]. With **1B** as the lead compound, a series of derivatives with formula **1** were synthesized, and some of them showed potential anticancer activity [10].

To the best of our knowledge, the studies of dithiocarbamic acid esters have usually focused on their pharmaceutical activity [11–13], with a few papers reporting their mass spectrometric behavior [14]. As an extention of our previous works [15–17], we report the fragmentation patterns of a series of dithiocarbamic acid esters under electrospray ionization tandem mass spectrometry conditions.

1



Compd.	1A	1B	1C	1D	1E
R	H	4'-Me	3'-Me	3',5'-Me_2	3',6'-Me_2

* Corresponding author. Tel.: +86 10 82801504; fax: +86 10 62346154.
** Corresponding author.
E-mail addresses: shayw@tsinghua.edu.cn (Y. Sha), lirt@mail.bjmu.edu.cn (R. Li).

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2. Experimental

2.1. Preparation of samples

The dithiocarbamic acid esters **1** were synthesized in our laboratory and their structures were confirmed using ^{1}H NMR, ^{13}C NMR, ESI-MS and elemental analysis. The detailed synthetic process and biological activities will be reported elsewhere.

2.2. Mass spectrometric conditions

The ESI mass spectra were recorded on a Bruker ESQUIRE-LC^{TM} ESI ion trap spectrometer equipped with a gas nebulizer probe, and is capable of analyzing ions up to m/z 6000. The MS^{n} spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the precursor ions of interest. The experiments were performed in positive ion mode using the following experimental conditions: drying gas, nitrogen, at a flow rate of 4 L/min; nebulizer pressure, 7 psi; capillary voltage, 4 kV; heated capillary temperature, 300 °C. The samples, dissolved in methanol, were ionized by ESI and continuously infused into the ESI chamber at a flow rate of 27 μL/min using a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). Five scans were averaged for each spectrum.

Table 1
Main ions and relative intensities of **1A**–**1E** in ESI/MS spectra

Compound	m/z (% relative intensity)		
	$[M+Na]^{+}$	$[M+H]^{+}$	**12**
1A	404 (100)	382 (10)	129 (26)
1B	418 (100)	396 (12)	143 (27)
1C	418 (100)	396 (12)	143 (27)
1D	432 (100)	410 (10)	157 (15)
1E	432 (100)	410 (15)	157 (23)

3. Results and discussions

3.1. ESI mass spectra

The characteristic fragmentations in the ESI mass spectra of this series of dithiocarbamic acid esters are summarized in Table 1. For most compounds of formula **1**, only three

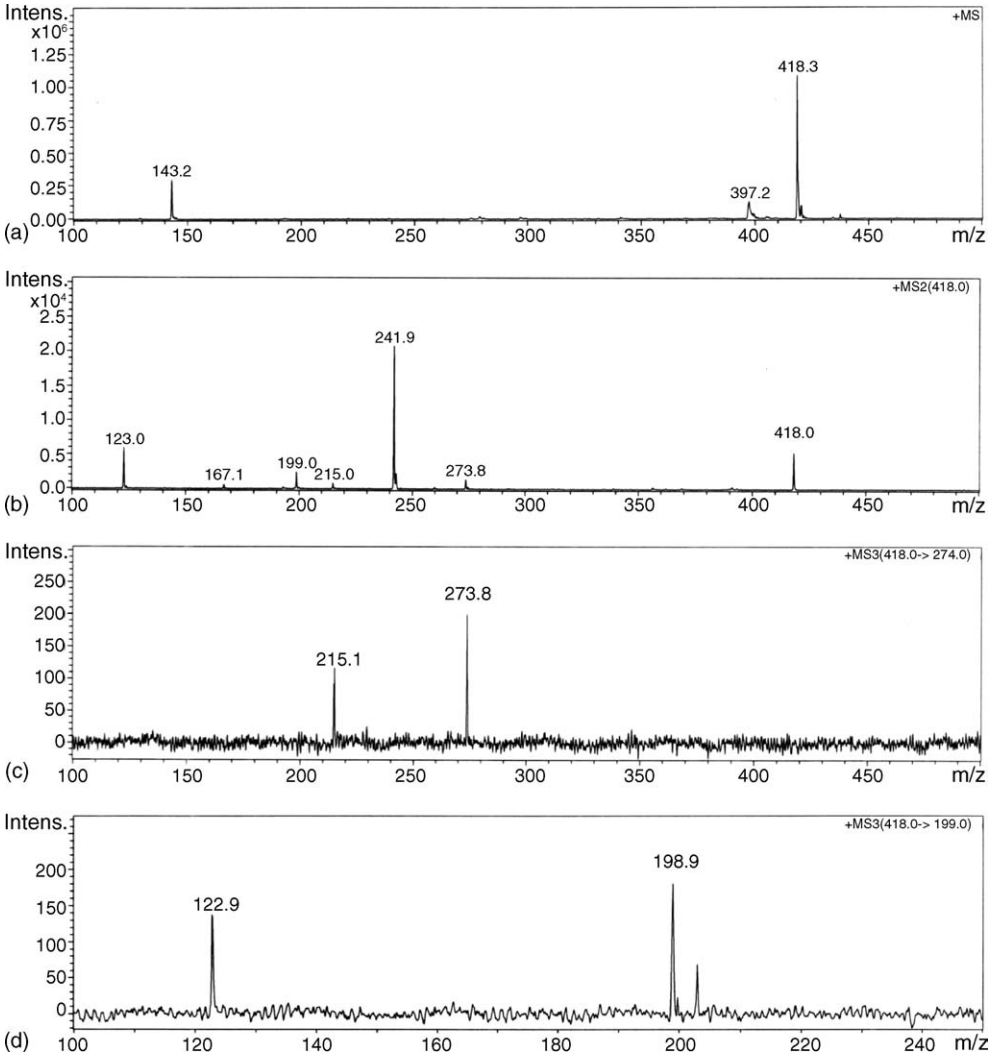


Fig. 1. (a) ESI/MS spectrum of **1C**. (b) ESI/MS^{2} spectrum of [**1C** + Na]$^{+}$. (c) ESI/MS^{3} spectrum of fragment ion **4**–**5**. (d) ESI/MS^{3} spectrum of fragment ion **6**–**8**.

Table 2
ESI/MS^2 data of sodium adduct ions $[M + Na]^+$

Compound	*m/z* (% relative intensity)							
	$[M + Na]^+$	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1A	404 (23)	377 (1)	274 (7)	242 (100)	215 (1)	185 (12)	153 (1)	109 (0)
1B	418 (9)	391 (4)	274 (9)	242 (100)	215 (6)	199 (28)	167 (3)	123 (32)
1C	418 (19)	391 (1)	274 (8)	242 (100)	215 (5)	199 (11)	167 (2)	123 (24)
1D	432 (50)	405 (1)	274 (8)	242 (100)	215 (5)	213 (16)	181 (3)	137 (58)
1E	432 (28)	405 (1)	274 (24)	242 (100)	215 (10)	213 (4)	181 (13)	137 (65)

main peaks were observed in the ESI/MS spectra, corresponding to sodium adduct ions $[M + Na]^+$, protonated molecular ions $[M + H]^+$ and piperazinylthioformic cation **12**. The sodium adduct ions were always observed to the base peaks. The ESI/MS spectrum of compound **1C** is shown in Fig. 1a as a representative spectrum for this series of compounds.

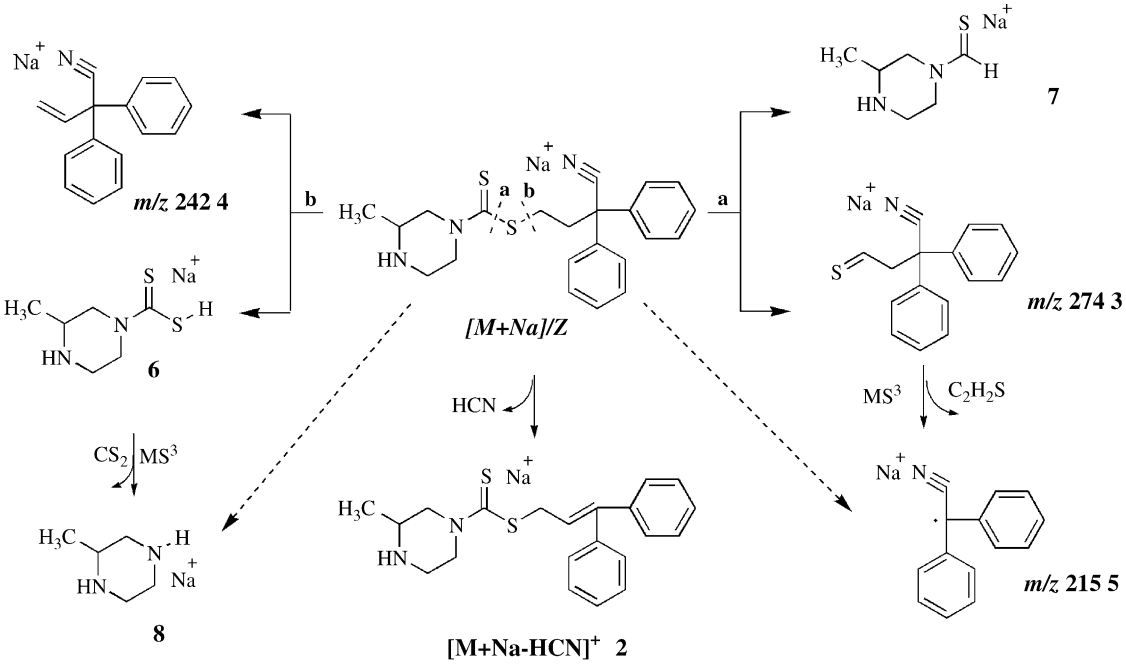
3.2. ESI/MS^n spectra of sodium adduct ions $[M + Na]^+$

In order to identify and characterize the detailed fragmentation patterns, the ESI/MS^2 spectra of sodium adduct ions $[M + Na]^+$ were recorded (Fig. 1b for **1C**), and the results are listed in Table 2. Various fragmentation pathways are summarized in Scheme 1. There were several dominant fragment ion signals in the ESI/MS^2 spectra of the precursor $[M + Na]^+$ ions. Firstly, the sodium adduct molecular ions $[M + Na]^+$ afforded the fragment ions $[M + Na\text{-}HCN]^+$ **2** by elimination of hydrogen cyanide (HCN). Secondly, the ESI/MS spectra showed bond rupture between the thiocarbonyl group and sulfur atom that resulted in complementary ions at *m/z* 274 (**3**) and alkylpiperazinylthioformaldehyde adduct ions **7**. Other complementary ions at *m/z* 242 (**4**) and the alkylpiperazinyldithioformic acid sodium adduct ion **6** were derived from bond scission between sulfur atom and methylene carbon. Both pairs of ions could be attributed to elimination reactions. These five sodium adduct fragmentation ions were formed directly from precursor sodium adduct ions $[M + Na]^+$.

In order to identify the fragmentation pathways of other ions, the ESI/MS^3 spectra of the ion at *m/z* 274 (**3**) and alkylpiperazinyldithioformic acid sodium adduct ion **6** were recorded. When the fragment ion at *m/z* 274 (**3**) was isolated, the corresponding ion at *m/z* 215 (**5**) was observed as the only fragment ion in the MS^3 spectrum (Fig. 1c for **1C**). Similarly, alkylpiperazinyl sodium adduct ions **8** accounted for the single peak when sodium adduct fragment ions **6** were isolated and fragmented in the ESI/MS^3 spectra (Fig. 1d for **1C**).

3.3. ESI/MS^n analysis of protonated molecular ions $[M + H]^+$

Compared with the ESI/MS^2 spectra of the sodium adduct ions $[M + Na]^+$, the protonated molecular ions $[M + H]^+$ gave some different fragmentation patterns in the ESI/MS^2 spectra, as shown in Fig. 2a for **1C**. Their fragmentation patterns are



Scheme 1. Fragmentation patterns of sodium adduct ion $[M + Na]^+$.

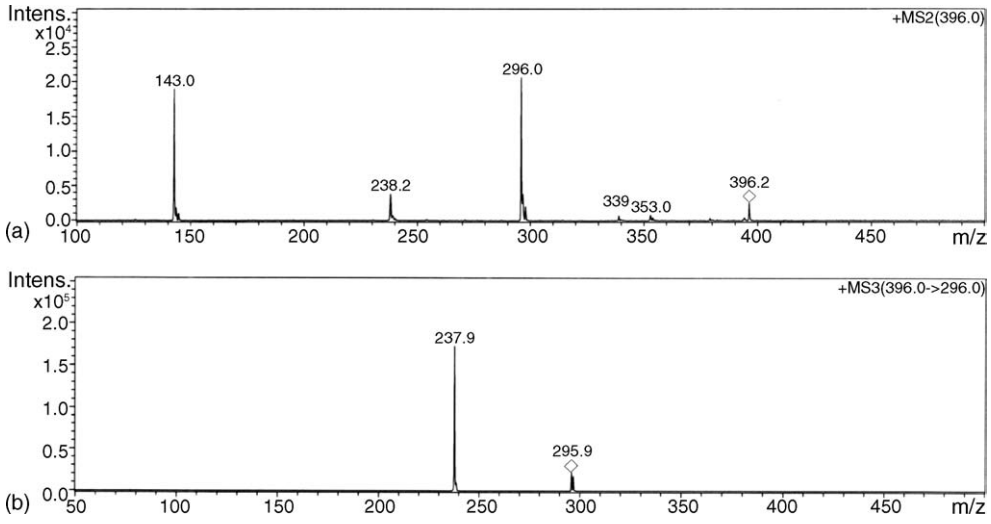
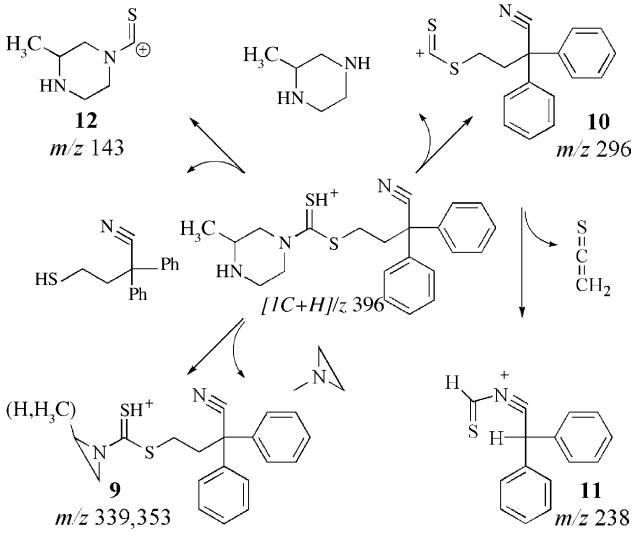


Fig. 2. (a) ESI/MS^2 spectrum of [**1C** + H]$^+$. (b) ESI/MS^3 spectrum of fragment ion **10**–**11**.



Scheme 2. Fragmentation patterns of protonated molecular ion [**1C** + H]$^+$.

shown in Scheme 2 and the corresponding data are summarized in Table 3.

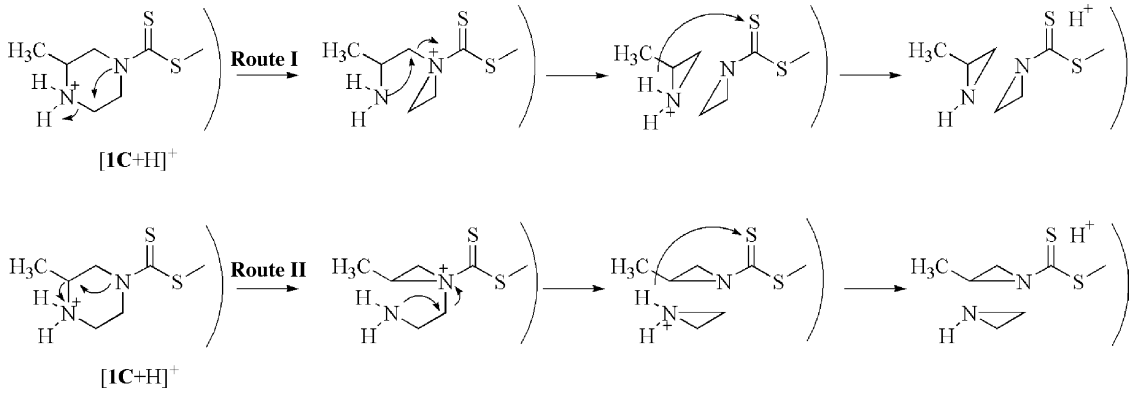
The protonated molecular ions [M + H]$^+$ displayed three fragmentation pathways. The bond rupture between thiocarbonyl and nitrogen resulted in the fragment ion at m/z 296 (**10**), corresponding to loss of 3-methylpiperazine. Further investigation of the ion at m/z 296 in the ESI/MS^3 spectrum showed only one ion (m/z 238 (**11**)), which was also observed in the ESI/MS spectra of [M + H]$^+$, being generated by loss of thioketene, S=C=CH$_2$ (Fig. 2b for **1C**).

Interestingly, breaking of the bond between thiocarbonyl and sulfur produced the alkylpiperazinyl thiocarbonyl cation **12**, which was also observed in the ESI mass spectrum of **1**. This observation proved that the fragment ions **12** in the ESI mass spectrum originated through fragmentation of the protonated molecular ion.

In particular, the piperazine moiety of the molecular ions [M + H]$^+$ fragments easily to generate the ions at m/z 353 and 339 (**9**) by losing azirane or 2-methylazirane under ESI/MS^2 conditions. Corresponding rearrangement mechanisms are suggested in Scheme 3. There are two rearrangement routes (I and II) for substituted piperazine derivatives. In Route I, the lone electron pair on the amide nitrogen attacks the unsubstituted α-carbon of the opposite ammonium, resulting in the ring opening of piperazine and the transformation of positive charge from the ammonium nitrogen to the thioamide nitrogen. A similar rearrangement eliminates azirane and leads to the fragment ion at m/z 339. In Route II, the lone electron pair on the amide nitrogen attacks the substituted α-carbon of the opposite ammonium nitrogen and generates the fragment ion at m/z 353 through a similar rearrangement mechanism.

Table 3
ESI/MS^2 spectral data of protonated molecular ions [M + H]$^+$

Compound	m/z (% relative intensity)				
	[M + H]$^+$	**9**	**10**	**11**	**12**
1A	382 (27)	339 (1)	296 (100)	238 (8)	129 (24)
1B	396 (10)	339 (2)	296 (72)	238 (41)	143 (100)
1C	396 (12)	339 (3); 353 (4)	296 (100)	238 (18)	143 (92)
1D	410 (8)	353 (6)	296 (93)	238 (14)	157 (100)
1E	410 (8)	353 (2)	296 (23)	238 (5)	157 (100)



Scheme 3. Proposed rearrangement mechanism of **1C**.

4. Conclusion

Electrospray ionization tandem mass spectra of five dithiocarbamic acid esters with excellent anticancer activities have been studied and their representative fragmentation pathways have been rationalized and supported by tandem mass spectrometry experiments. There were some interesting findings from the experimental results. In the ESI mass spectra, there were three dominant ions $[M + Na]^+$, $[M + H]^+$ and piperazinylthioformic cations **12**, which were derived from $[M + H]^+$. Further study of ESI/MS^2 spectra showed that protonated molecular ions $[M + H]^+$ had different fragmentation pathways from those of the sodium adduct ions. In terms of $[M + Na]^+$ ions, the bonds linking the thiocarbonyl group and the sulfur atom, sulfur atom and the methenylene carbon were easier to dissociate than other bonds and produced two pairs of complementary ions. In the case of the protonated molecules $[M + H]^+$, the bond dissociation usually happened between thiocarbonyl and sulfur or thiocarbonyl and nitrogen; while, the piperazine ring favors a rearrangement to expel azirane. The proposed rearrangement mechanism is consistent with experimental observations. These results will be helpful for the study of fragmentation pathways of dithiocarbamic acid esters under ESI/MS conditions and will allow us to easily identify specific dithiocarbamic acid esters during synthesis. In addition, the results will further the understanding of the metabolic pathways and pharmacokinetic properties of these compounds.

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References

[1] T. Aboul-Fadl, A. El-Shorbagi, Eur. J. Med. Chem. 31 (1996) 165.
[2] H. Imamura, N. Ohtake, H. Jona, A. Shimizu, M. Moriya, H. Sato, Y. Sugimoto, C. Ikeura, H. Kiyonaga, M. Nakano, R. Hagano, S. Abe, K. Yamada, T. Hashizume, H. Morishima, Bioorg. Med. Chem. Lett. 10 (2000) 109.
[3] C. Len, A.S. Boulognemerlot, D. Posted, J. Agric. Food Chem. 44 (1996) 2856.
[4] S. Hidaka, T. Funakoshi, H. Shimada, M. Tsuruoka, S. Kojima, J. Appl. Toxicol. 15 (1995) 267.
[5] S.K. Tandon, S. Singh, V.K. Jain, S. Prasad, Fundam. Appl. Toxicol. 31 (1996) 141.
[6] C.S. Lai, V.P. Vassilev, T.M. Wang, US 5,847,004 (1998).
[7] B.G. Guo, Z.M. Ge, T.M. Cheng, R.T. Li, Acta Pharmacol. Sin. 36 (2001) 185.
[8] Z.M. Ge, R.T. Li, T.M. Cheng, J.R. Cui, Arch. Pharm. Pharm. Med. Chem. 334 (2001) 173.
[9] W. Guo, F.X. Ran, R.Q. Wang, J.R. Cui, R.T. Li, T.M. Cheng, Z.M. Ge, Chin. J. Clin. Pharmacol. Ther. 9 (2004) 59.
[10] By in vitro evaluation of anticancer activity against HL-60 and Bel-7402 cell lines, the listed compounds showed 100% growth inhibition at concentration of 130 μM.
[11] V.F. Plyusnin, V.P. Grivin, S.V. Larionov, Coord. Chem. Rev. 159 (1997) 121.
[12] P.J. Nieuwenhuizen, Appl. Catal. A: Gen. 207 (2001) 55.
[13] (a) R.T.A. Mayadunne, E. Rizzardo, J. Chiefari, Y.K. Chong, G. Moad, S.H. Thang, Macromolecules 32 (1999) 6977;
(b) C. Schilli, M.G.L. Rfer, A.H.E. Müller, Macromolecules 35 (2002) 6819.
[14] J. Szafranek, G. Blotny, P. Vouros, Tetrahedron 34 (1978) 2763.
[15] (a) X. Wang, Y.W. Sha, R.T. Li, Rapid Commun. Mass Spectrom. 18 (2004) 829;
(b) X. Wang, Y.W. Sha, R.T. Li, Int. J. Mass Spectrom. 235 (2004) 111.
[16] (a) X. Wang, F.L. Gao, H.B. Piao, T.M. Cheng, R.T. Li, Bioorg. Med. Chem. Lett. 13 (2003) 1729;
(b) F.L. Gao, X. Wang, H.M. Zhang, T.M. Cheng, R.T. Li, Bioorg. Med. Chem. Lett. 13 (2003) 1535.
[17] (a) L. Shen, Y.W. Sha, X.Y. Hong, Rapid Commun. Mass Spectrom. 18 (2004) 1534;
(b) R. Adden, P. Mischnick, Int. J. Mass Spectrom. 242 (2005) 6663;
(c) M. Schäfer, C. Schmuck, L. Geiger, M.J. Chalmers, C.L. Hendrickson, A.G. Marshall, Int. J. Mass Spectrom. 237 (2004) 33;
(d) W. Tüting, R. Adden, P. Mischnick, Int. J. Mass Spectrom. 232 (2004) 107;
(e) J.K. Zhu, Z.Y. Shi, Int. J. Mass Spectrom. 226 (2003) 369;
(f) M.M. Kish, C. Wesdemiotis, Int. J. Mass Spectrom. 227 (2003) 191;
(g) J. Bunch, M.R. Clench, D.S. Richards, Rapid Commun. Mass Spectrom. 18 (2004) 3051;
(h) X.S. Miao, R.E. March, C.D. Metcalfe, Rapid Commun. Mass Spectrom. 17 (2003) 149.