177. Electrospray-Ionization Mass Spectrometry

Part 2[1])

Neighboring-Group Participation in the Mass-Spectral Decomposition of 4-Hydroxycinnamoyl-spermidines

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The three mono substituted *N*-[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidines **1**–**3** have been studied by positive-ion electrospray-ionization tandem mass spectrometry (ESI-MS/MS). Because of the neighboring-group participation, the MS/MS of $[1 + H]^+$ and $[2 + H]^+$ are essentially similar, while compound **3** can be easily distinguished from **1** and **2** because of the characteristic ions at *m/z* 218. However, with the source collision-induced dissociation (source-CID) MS/MS technique, the compounds **1** and **2** can be unambiguously distinguished by the signal of the pyrrolidinium ion (*m/z* 72) from their daughter ion (*m/z* 275). The source-CID MS/MS of the labeled compound *N*-(4-aminobutyl)-*N*-(3-aminopropyl)-*N*-[3-(4-hydroxyphenyl)prop-2-en[^{15}N]amide] ([^{15}N(4)]-**2**) provide more information on the decomposition mechanisms and proved the occurrence of a partial transamidation reaction **2**→**1** during the measurement.

Introduction. – The remarkable increase in information on the biochemical properties of polyamines and the awareness that inhibition of the enzymes involved in their biochemical pathways leads to valuable pharmacological agents, making these compounds important tools for the organic chemists [2]. Although the sporadic identification of the derivatives formed from the reaction of amines and 3-(4-hydroxyphenyl)prop-2-enoic acid (= 4-hydroxycinnamic acid) in various plants has been reported for many years, it is only recently that the widespread nature and potential significance of these amides has been recognized [3] [4]. Since polyamine derivatives often appear as inseparable mixtures of minute amounts, electrospray-ionization mass spectrometry (ESI-MS) is generally used to investigate this class of compounds, especially due to the possibility of the on-line coupling with a HPLC system [5–7]. To fully benefit from the potential of tandem mass spectrometry, it is important to comprehend the fragmentation mechanism of compounds under investigation, when this technique is used. Furthermore, informations on the decomposition reactions can be achieved by the application of the source-CID MS/MS technique [8] [9]. Herein, a MS/MS of the compound is produced in the ion source, and the thus generated ions can be further investigated by usual MS/MS analysis. Recently, the synthesis of all seven (*E*)-*N*-[3-(4-hydroxyphenyl)prop-2-enoyl]spermidines has been accomplished [10]. The aim of this investigation is the mass-spectral identification of these compounds (for the mass-spectral behavior of the corresponding *N*,*N*-bis-(4-hydroxycinnamoyl)spermidines, see [11]).

[1]) Part 1: [1].

[2]) Part of planned Ph.D. thesis of *W. H.*, Universität Zürich.

Results and Discussion[3]) – The MS/MS of the three isomeric *N*-[3-(4-hydroxyphenyl)prop-2-enoyl]spermidines **1**–**3** acquired under the same conditions are reproduced in the *Fig. 1,a*, *Fig. 2,a*, and *Fig. 4,a*, respectively. All of them are quite similar, and the intense signals appear at the same *m/z* values. Furthermore, **1** and **2** show essentially the same fragment ions (*Fig. 1,a*, and *Fig. 2,a*, resp.). In general, similar or nearly identical mass spectra of isomeric compounds can arise *i*) from the same fragmentation pathways; *ii*) from different pathways leading to the ions with the same *m/z* values; *iii*) by fragmentation of only one isomer, which is formed by rearrangement of the starting materials [12]. To explain this mass-spectral behavior, the labeled compound [^{15}N(4)]-**2** was synthesized and investigated as well.

Fragmentations of N-{*3-[(4-Aminobutyl)amino]propyl*}*-3-(4-hydroxyphenyl)prop-2-enamide* (**1**). The MS/MS behavior of [**1** + H]$^+$ (*Fig. 1,a*) is summarized in *Scheme 1*. It

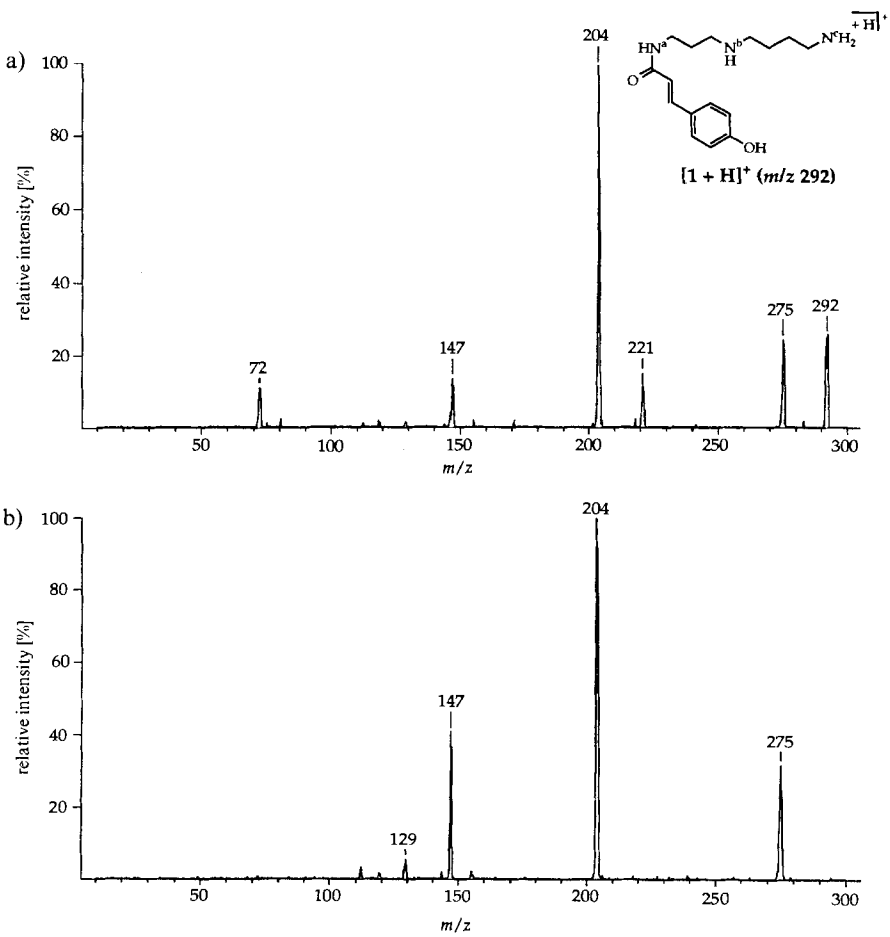
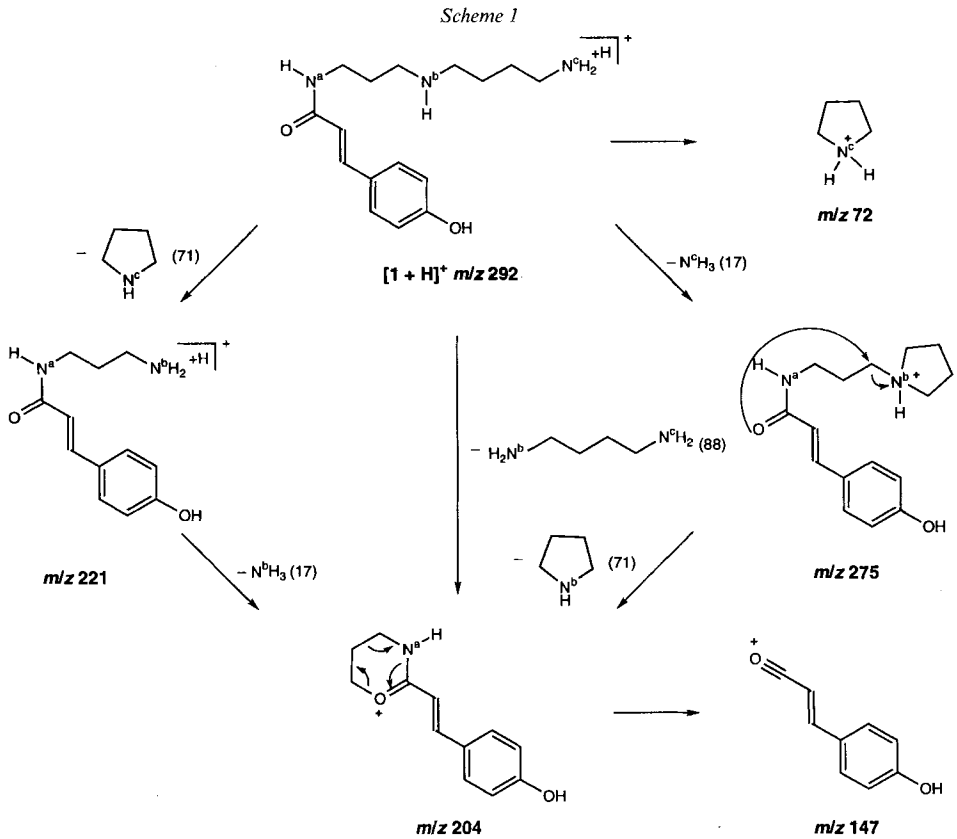


Fig. 1. a) *ESI-MS for* [**1** + *H*]$^+$ (*m/z* 292). b) *ESI-MS/MS/MS for its daughter ion* m/z *275*

[3]) To facilitate the mass-spectral discussion, the three different N-atoms in spermidine (= *N*-(3-aminopropyl)butane-1,4-diamine) are defined as N^a, N^b, and N^c.

is reasonable to attribute the decomposition of the compound to intramolecular substitution reactions. All of the fragment ions can be explained by the formation of cyclic structures, either in the ions or in the expelled neutral molecules.

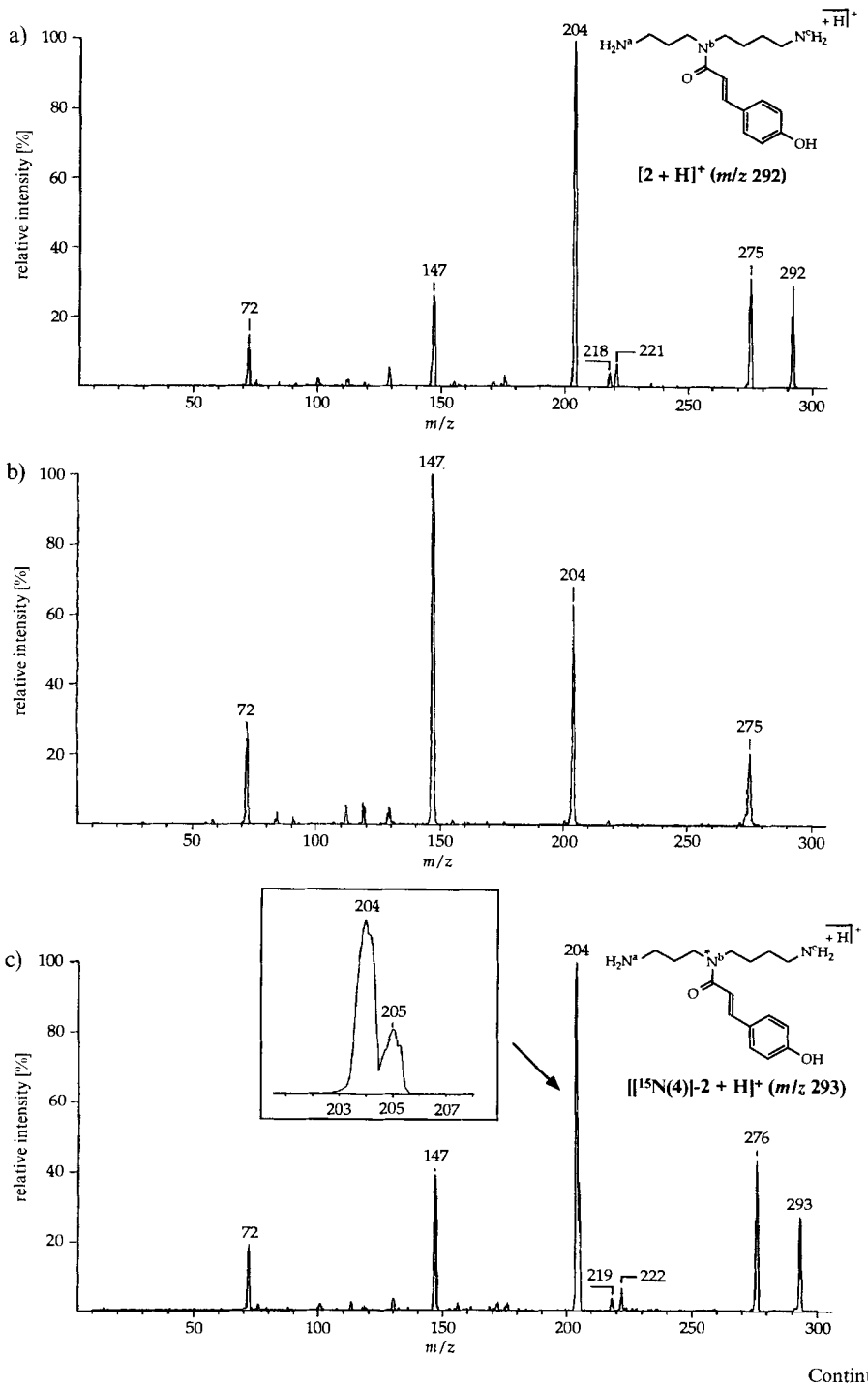
Scheme 1



The structures of the ions assigned to the signals at m/z 275 and 221 are readily interpreted in terms of expulsion of neutral amine fragments from $[M + H]^+$. Of special interest is the fragment ion corresponding to the signal m/z 204 with the highest relative intensity (loss of 88 Da), since it should have a stable structure containing the trimethylene part from the spermidine unit. The resonance-stabilized six-membered ring structure appears more reasonable than the alternative four-membered one. The ions attributed to the signal at m/z 147 can be interpreted as arising from the cleavage of the amide bond.

The absence of the signals at m/z 72 in the source-CID MS/MS of the $[M + H - NH_3]^+$ ion (m/z 275) indirectly supports the proposed structure for the ions assigned to the signal at m/z 275 (*Fig. 1,b*).

Fragmentations of N-*(4-Aminobutyl)*-N-*(3-aminopropyl)*-*3*-*(4-hydroxyphenyl)prop-2-enamide* (**2**). The fragment ions observed in MS/MS of the $[M + H]^+$ ion of **2** (*Fig. 2,a*) are summarized in *Scheme 2* and appear to be essentially similar to those found in the spectrum of **1**.


a)
relative intensity [%]
m/z
204
147
72
275
292
218
221
[2 + H]+ (m/z 292)
b)
147
204
72
275
c)
204
205
203 205 207
147
72
276
293
219
222
[[15N(4)]-2 + H]+ (m/z 293)

Continued

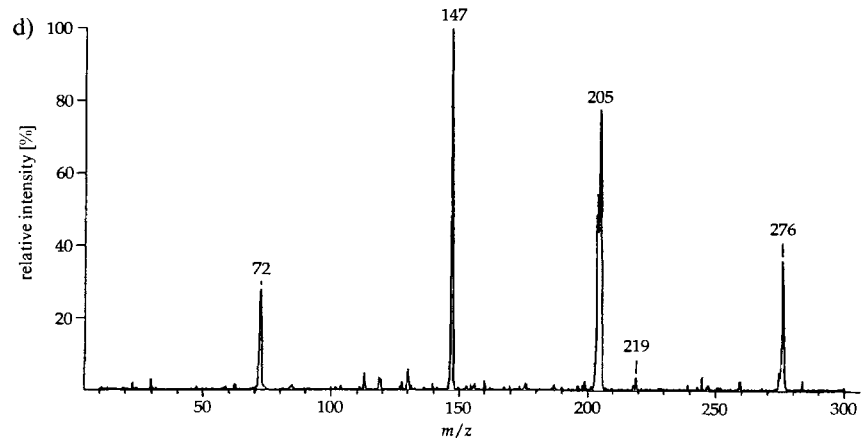


Fig. 2. a) *ESI-MS/MS for* $[2 + H]^+$ (*m/z* 292). b) *ESI-MS/MS/MS for its daughter ion* m/z *275*. c) *ESI-MS/MS for* $[[^{15}N(4)]\text{-}2 + H]^+$ (*m/z* 293). d) *ESI-MS/MS/MS for its daughter ion* m/z *276*

The fragment ions of compounds **1** and **2** might be considered as being formed *via* similar pathways or even by the same pathways. Considering the structural difference of both compounds, it can be assumed that compound **2** is transformed partially into its isomer **1**, *via* a six-membered-ring intermediate, prior to its mass-spectral decomposition. To confirm this hypothesis, [^{15}N(4)]-**2** was investigated. Its synthesis will be discussed later.

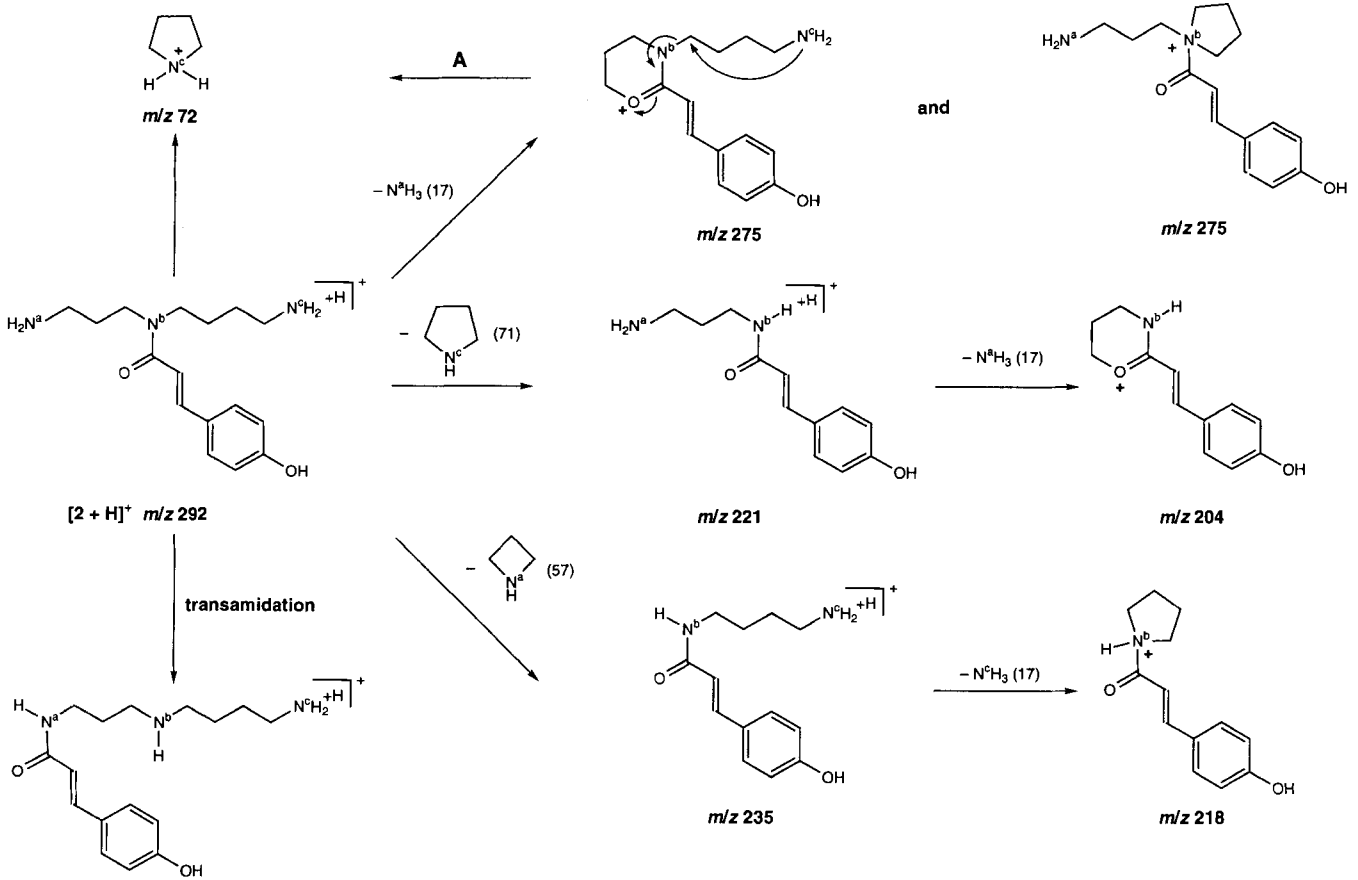
The signal at *m/z* 204 seems to be an ideal basis to confirm this assumption. According to *Scheme 1*, the ions with the signal at *m/z* 204, formed from **1**, contain the N^a-atom; thus the signal will not be shifted to *m/z* 205, in case N^b is labeled by ^{15}N. However, the signal at *m/z* 204 corresponds to the ions arising from **2** and containing the N^b-atom only. (*Scheme 2*), if no isomerization between compound **1** and **2** takes place. In case of ^{15}N^b-labeled **2**, this signal is expected at *m/z* 205 only. As it is shown in *Fig. 2,c*, the signal at *m/z* 204 appears in the MS/MS of the labeled isomer as a *doublet* of the signals at *m/z* 204 and 205. It indicates that a transamidation (**2**→**1**, or $[\mathbf{2} + H]^+ \rightarrow [\mathbf{1} + H]^+$) partly occurrs during the measurement.

Further measurements with the [^{15}N(4)]-**2** provide more information on the other decomposition mechanisms. The observation that the ratio of ion intensities of the ions with *m/z* 205 and 204 ($I(205)/I(204) = 27\%$), which are derived from daughter ions with *m/z* 222, was independent of the collision energy can be explained by two energetically identical structures of the ions corresponding to the signal at *m/z* 222 (*Scheme 3*).

However, the $I(205)/I(204)$ ratio for the same ions derived from the daughter ion with *m/z* 276 was found to be strongly dependent on the collision energy (*Fig. 3,a*): it decreases significantly when the energy is increased.

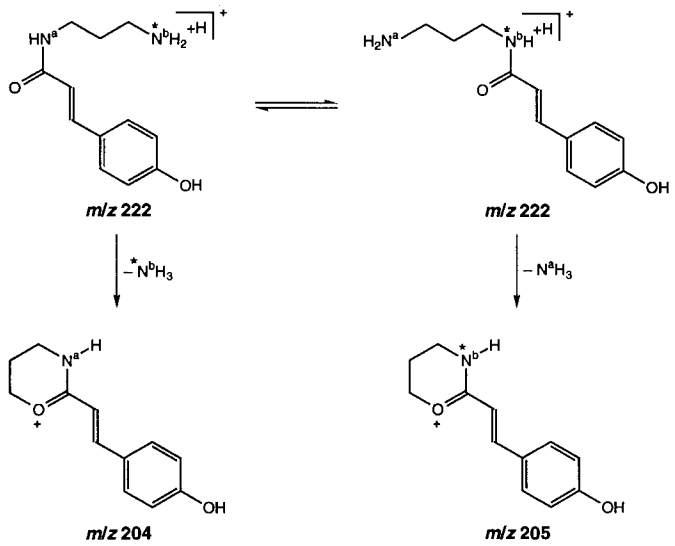
Since the daughter ions corresponding to *m/z* 276 cannot undergo transamidation, it might suggest that *Pathway A* is favored over *Pathway B*, when the collision energy is increased. *Pathway A* corresponds to the intramolecular substitution of N^c-atom at the

Scheme 2



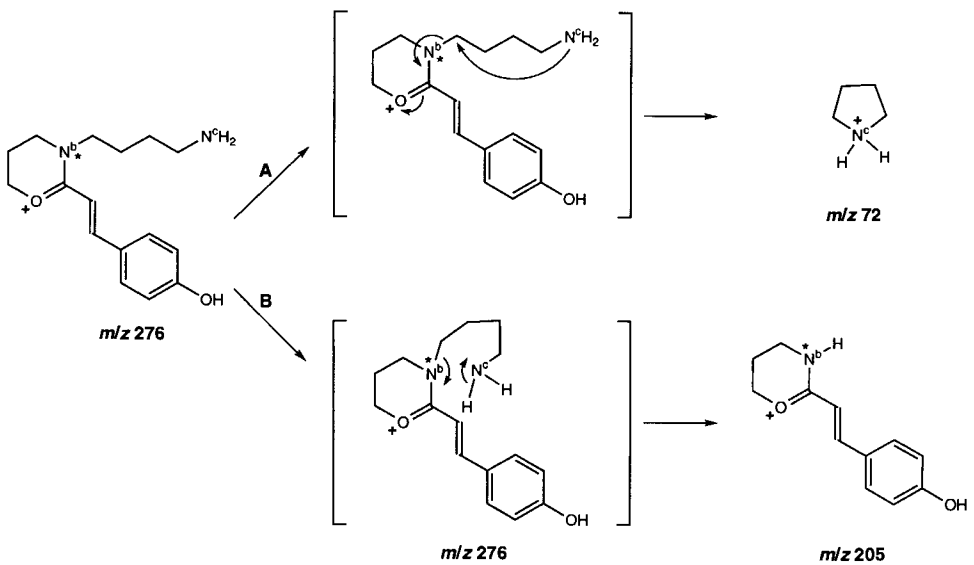
[1 + H]$^+$ *m/z* 292; for further fragmentation, see *Scheme 1*.

Scheme 3



C-atom adjacent to N^b and formation of the pyrrolidinium ion corresponding to *m/z* 72, while the alternative *Pathway B* represents a H-shift *via* a four-membered transition state (*Scheme 4*).

Scheme 4



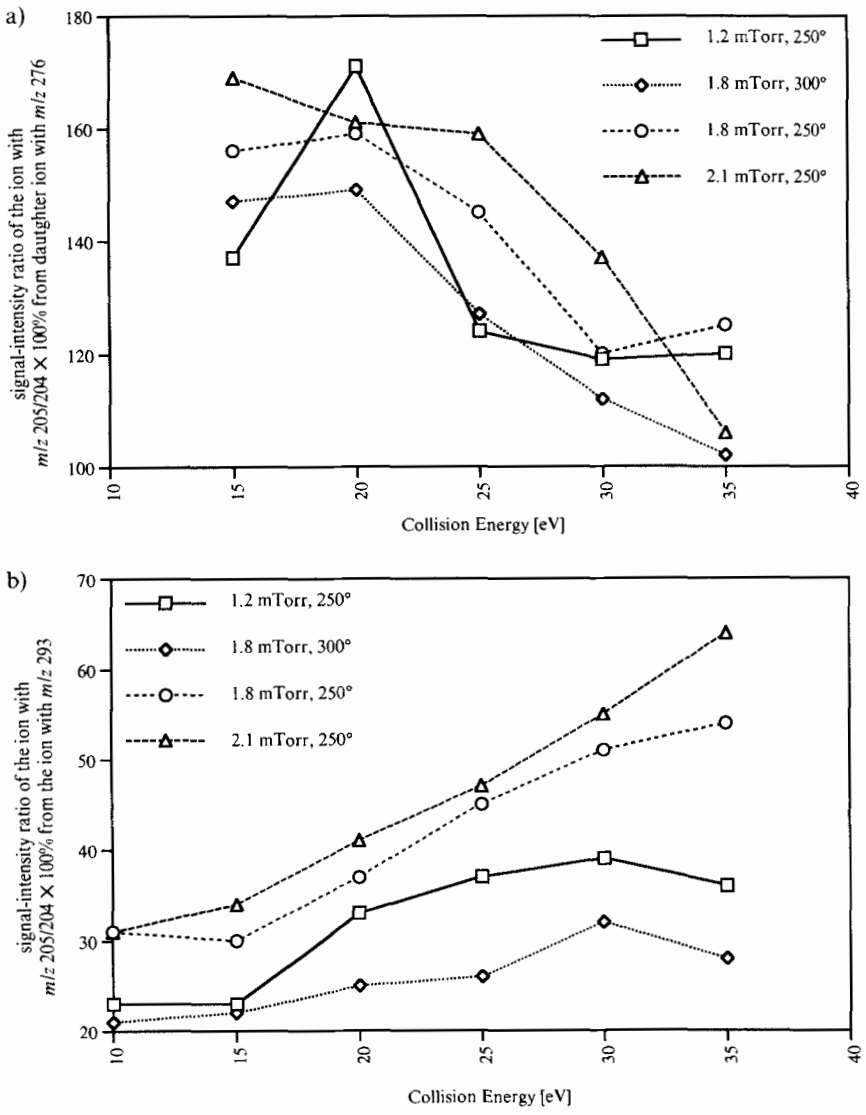


Fig. 3. a) *Signal-intensity ratio of the ion with* m/z *205/204 from daughter ion with* m/z *276*. b) *Intensity ratio of the ion with* m/z *205/204 from the ion with* m/z *293*

The signal-intensity ratio $I(205)/I(204)$ for the ions derived from ions corresponding to m/z 293 was also strongly dependent on the collision energy, but the ratio increased with increasing energy (*Fig. 3,b*). This observation can be explained with the preferred formations of smaller ions during the decomposition of the ions corresponding to m/z 293 (the quasi '[[^{15}N(4)]-labeled-**1** + H]$^+$') at higher energies, circumventing the pathways leading to the ions with the signal at m/z 204.

After having rationalized the similarity of the MS/MS from compound **1** and **2**, it is easier to find a way to distinguish the two isomers from each other. Since the transamidation of **1** does not occur quantitatively to compound **2** during the measurement, and since there are two possible structures of the ion corresponding to *m/z* 275 derived directly from **2**, it is predictable that [pyrrolidine + H]$^+$ (*m/z* 72) could be observed in the source-CID MS/MS of the daughter ions assigned to *m/z* 275 from **2** (*Pathway A*, *Scheme 2*), which is in contrast to **1** (*Fig. 1,b* and *Fig. 2,b*). This peak offers a way to differentiate compound **2** from **1**, even though the MS/MS of their $[M + H]^+$ ions are almost identical.

Fragmentations of N-{*4-[(3-Aminopropyl)amino]butyl*}*-3-(4-hydroxyphenyl)prop-2-enamide* (**3**). The mass spectra of compounds **1** and **2** can be easily distinguished from that of compound **3** according to the characteristic signals at *m/z* 218.

The fragmentations observed in the MS/MS spectrum of the $[M + H]^+$ ions of compound **3** (*Fig. 4,a*) are depicted in *Scheme 5*. The ion assigned to *m/z* 218 represents the loss of the neutral propane-1,3-diamine from M^+. Of special interest in the mass spectrum of compound **3** is the signal at *m/z* 204, which is unexpected. Because there is no direct pathway from compound **3** to the ion corresponding to the signal at *m/z* 204, it might only be rationalized as the result of a transamidation. However, this isomerization (a secondary amide is tranformed to a tertiary amide *via* a seven-membered ring) is energetically disfavored [13]. The presence of this signal indirectly supports, therefore, the proposed resonance-stabilized structure leading to the signal at *m/z* 204.

No pyrrolidinium ion (*m/z* 72) can directly be formed from the molecular ion of compound **3**. The ion corresponding to *m/z* 72, arisen from the daughter ion assigned to *m/z* 275 (*Fig. 4,b*), which, on the basis of the discussion above, indicates the presence of the compound **2**, gives a further evidence of the proposed transamidation mechanism *via* a seven-membered ring. It is possible that the ions undergo one further transamidation from **2** to **1** (discussed above) *via* a six-membered ring.

In the MS/MS of [**3** + H]$^+$ after a H/D exchange experiment (*Fig. 4,c*), a small signal at *m/z* 152 was recognized as the corresponding one at *m/z* 146 in the undeuterated case. Accordingly, this ion must possess six exchangeable protons, indicating that this ion should be formed from the spermidine backbone. The mechanism of the formation of the ions corresponding to *m/z* 146 has been already studied in detail [1]. In case of compound **3**, formation of the ion assigned to *m/z* 146 becomes remarkable, since there are only subordinate possibilities for the formation of the energetically favored five- and six-membered rings. Furthermore, all the relevant peaks are shifted in the coresponding spectra of the D_5-labeled derivative. This is considered to be an important evidence for the structures proposed in this paper.

It must be emphasized that there are no equilibria among the compounds **1**, **2**, and **3** during the measurements. Neither the signal at *m/z* 218 in the MS/MS of [**1** + H]$^+$ nor the signal at *m/z* 72 in the source-CID MS/MS of [**1** + H − NH_3]$^+$ (*m/z* 275) was observed (*Fig. 1*), which is, on the basis of the discussion above, characteristic for the presence of compound **3** and **2**, respectively. The transamidations take place only in one direction, as it is shown in *Scheme 5*.

*Synthesis of the [^{15}N(4)]-***2**. The synthesis of [^{15}N(4)]-**2** shown in *Scheme 6* started with 2-aminobutan-1-ol (**4**), protected with the Boc group, followed by conversion of the

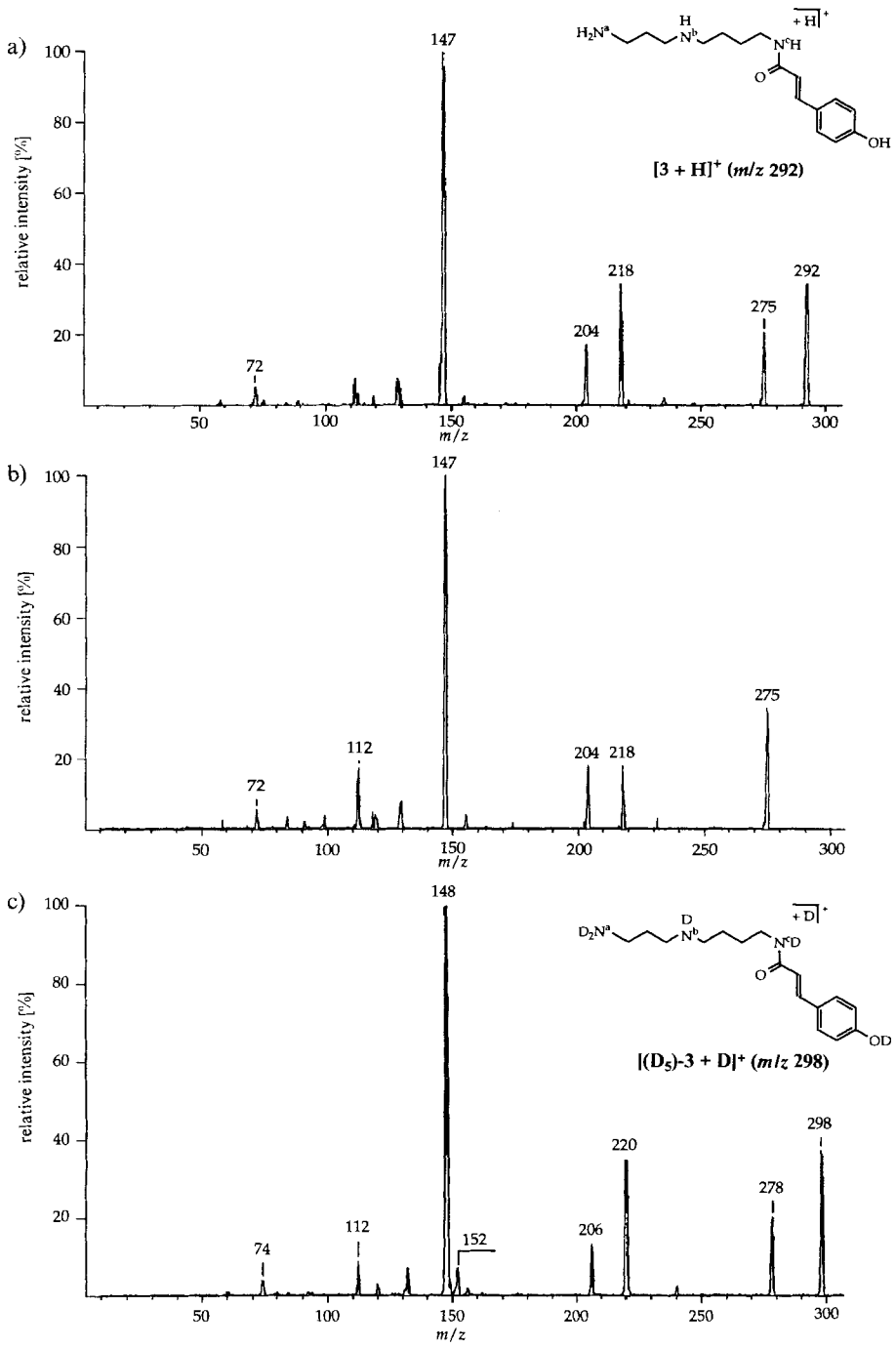
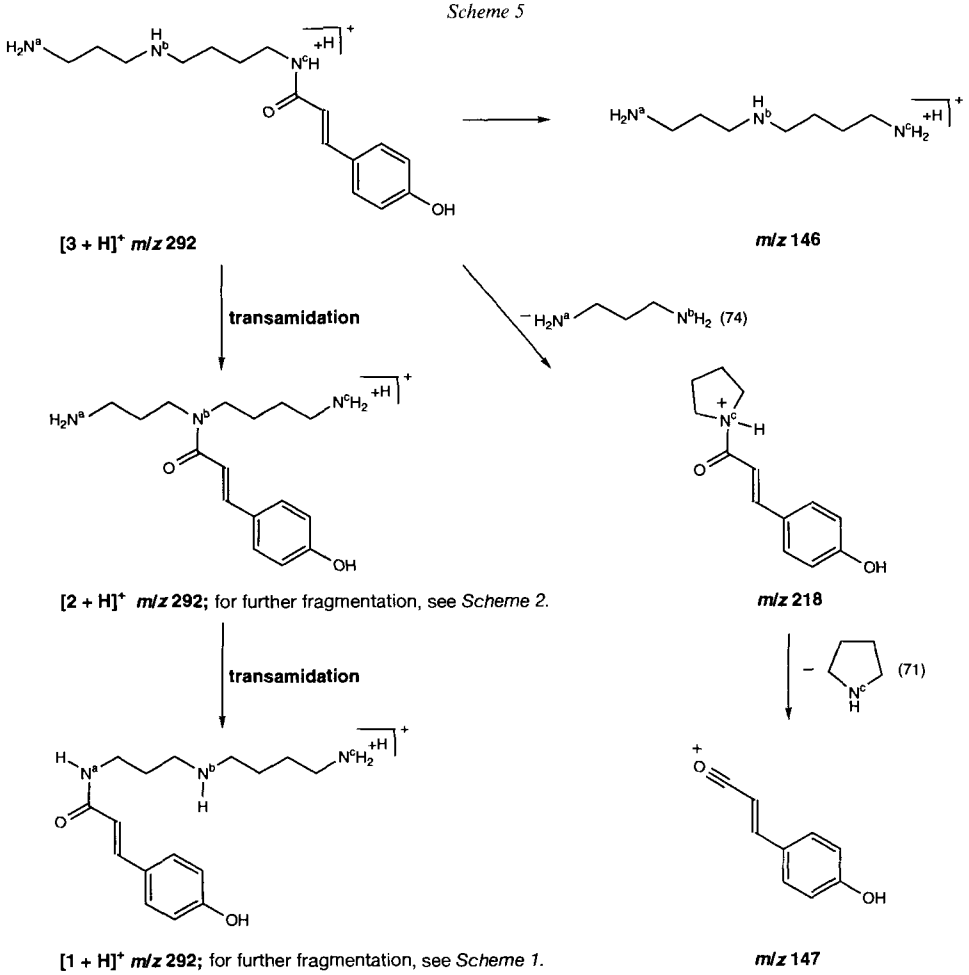


Fig. 4. a) *ESI-MS/MS for* $[3 + H]^+$ (*m/z* 292). b) *ESI-MS/MS/MS for its daughter ion with* m/z *275.* c) *ESI-MS/MS for* $[(D_5)\text{-}3 + H]^+$ (*m/z* 298)

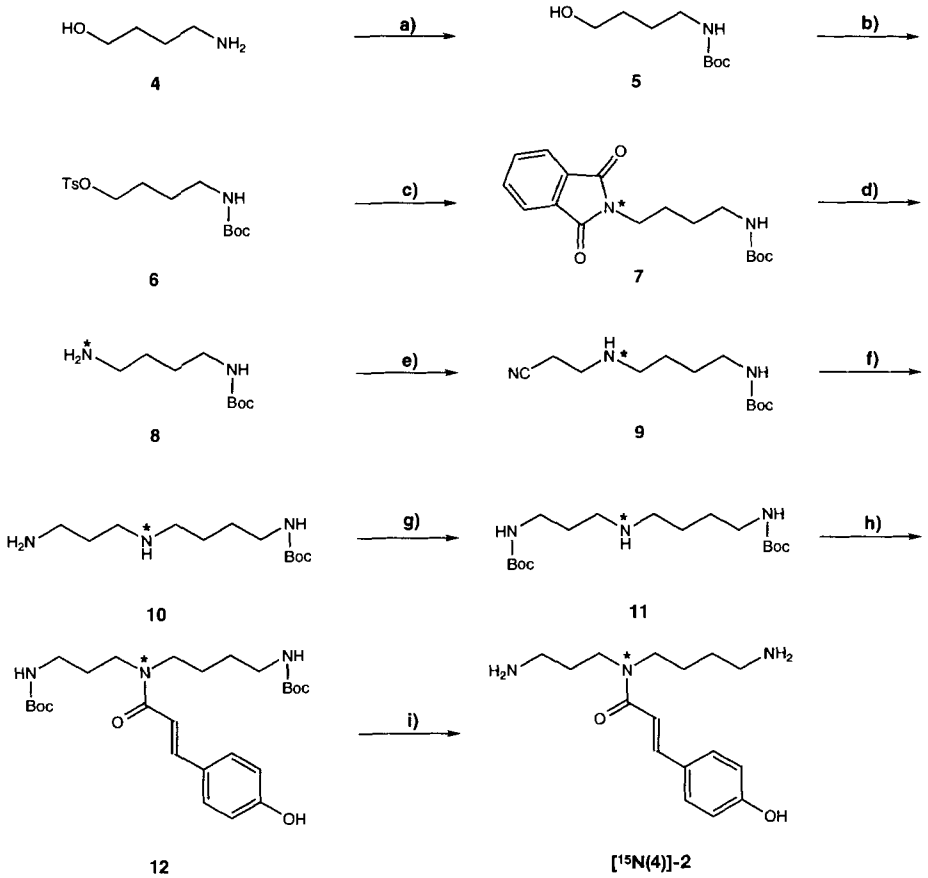
Scheme 5



free OH group to the toluene-4-sulfonate **6** [14]. Compound **6** was converted, in 97% yield, to the phthalimide **7** using potassium (^{15}N)phthalimide [15], and subsequent phthalimide removal (hydrazine/methanol) led to the desired monoprotected diamine **8**.

Reaction of the diamine **8** with acrylonitrile, followed by reduction with *Raney*-Ni, gave the triamine **10** [16], which was treated with 2-{[(*tert*-butoxy)carbonyl]oxyimino}-2-phenylacetonitrile (Boc-On) to yield the diprotected spermidine derivative **11** [17] [18]. The coupling of **11** with (*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid (= 4-hydroxycinnamic acid) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) gave **12**. Finally, the removal of both Boc groups with CF_3COOH afforded [^{15}N(4)]-**2** in an overall yield of 27%. However, according to the ^{1}H-NMR spectrum, we found that [^{15}N(4)]-**2** underwent (*E*/*Z*)-isomerization during the workup. This (*E*/*Z*)-isomerization and rotational isomerization (hindered rotation about the N(4)-amide bond) led to a highly complex ^{13}C-NMR spectrum, which could not be unambiguously interpreted. This behavior is currently under investigation.

Scheme 6



a) $(Boc)_2O/CH_2Cl_2$, 82%. b) TsCl, Et_3N, and DMAP/CH_2Cl_2, 80%. c) $K^{15}NPhth/DMF$, 97%. d) $N_2H_4 \cdot H_2O$/MeOH, 94%. e) CH_2=CHCN/MeOH, 92%. f) *Raney*-Ni, H_2/1N NaOH in 95% EtOH, 91%. g) Boc-On, Et_3N/THF, 70%. h) (*E*)-3-(4-Hydroxyphenyl)prop-2-enoic acid, DCC/THF, 79%. i) CF_3COOH/CH_2Cl_2, 1N aq. HCl, 95%.

To conclude, we have demonstrated that the constitutional isomers **1**, **2**, and **3** with very similar mass spectra (ESI/MS) can be distinguished by MS/MS and source-CID MS/MS methods. Because of the neighboring-group participation, **1** and **2** displayed the same fragment ions in the MS/MS, since a transamidation (shift of the carbonyl substituent from N^b to N^a) took place. According to the MS/MS of $^{15}N(4)$-labeled compound **2**, it was established that the transamidation did not occur quantitatively during the measurement. It should be pointed out that compounds **1** and **3** give almost identical ^{1}H-NMR spectra and HPLC diagrams. It is, therefore, very difficult to differentiate one from the other by NMR techniques. However, they can be distinguished easily on the basis of the signal at m/z 218 from compound **3** using MS/MS technique.

This work was supported by the *Swiss National Science Foundation* which is gratefully acknowledged.

Experimental Part

General. M.p.: *Mettler FP-5.* IR: in cm^{-1}, in $CHCl_3$; *Perkin-Elmer 781* spectrophotometer, in cm^{-1}. ^{1}H-NMR: at 300 MHz; *Bruker ARX-300/AM-300.* ^{13}C-NMR: at 75.5 MHz, *AM-300.* Chemical shifts in ppm in $CDCl_3$, unless otherwise stated. EI- and CI-MS: *Finnigan-SSQ 700* or *Finnigan-MAT 90*; *m/z* (rel. %); Reaktand-Gas: NH_3.

All the investigated compounds were dissolved in MeOH (HPLC-grade, *Scharlau*, Barcelona, Spain) at a concentration of *ca.* 5 nmol/ml. For deuteration experiments, CD_3OD (*Cambridge Isotope Laboratories*, Andover, MA, USA) was used. ESI/MS Investigations were carried out with a triple stage quadrupole instrument (*Finnigan-TSQ 700*, San José, CA, USA), equipped with a combined *Finnigan* Atmospheric Pressure Ion (API) source. The solns. were continuously introduced into the source with a syringe infusion pump (*Harvard Instruments*, Southnatick, MA; USA) at a flow rate of 5 µl/min. The resolution was adjusted at a peak width of 0.7 to 0.8 µ at half peak height for both scanning quadrupoles. The scan rate was set at 100 to 600 μs^{-1} for MS/MS experiments; 20 scans were averaged. The capillary was held at 200 or 300°, sheath gas pressure at 8 psi. For MS/MS experiments, Ar was used as collision gas with a pressure of 1.2 to 2.1 m Torr. The collision voltage was varied between −20 and −25 eV until the relative intensity of the quasimolecular ion signal decreased to *ca.* 35%. Source-CID spectra were obtained by applying an additional voltage of −5 to −10 eV at the octapole lens until the source-CID spectra showed similar intensities at the same *m/z* values as the MS/MS spectra of the quasimolecular ions. Source-CID fragments were further investigated in MS/MS experiments with a variation of the collision voltage between −10 and −50 eV, depending on the voltage applied at the octapole and on the quality and information of the obtained spectra.

1. tert-*Butyl* N-*(4-Hydroxybutyl)carbamate* (**5**). A soln. of $(Boc)_2O$ (5.25 g, 24.0 mmol) in CH_2Cl_2 (20 ml) was added within 10 min to a soln. of 4-aminobutan-1-ol (**4**, 2.13 g, 23.9 mmol) in CH_2Cl_2 (10 ml) with Et_3N (4 ml, 28.8 mmol). The mixture was stirred for 3 h. The residue, obtained on concentration, was purified on a silica-gel column (CH_2Cl_2/MeOH 10:1 (*v/v*)), to obtain 3.72 g (82%) of **5**. Colorless oil which crystallized at 4°. M.p. 31.1–32.8°. IR (KBr): 3350*s* (br.), 2975*s*, 2926*s*, 2868*s*, 1695*s*, 1530*s*, 1452*m*, 1392*m*, 1368*s*, 1279*s*, 1251*s*, 1170*s*, 1058*m*, 1040*m*. ^{1}H-NMR: 4.62 (br. *s*, BocNH); 3.67 (*t*, *J* = 6.0, CH_2(4)); 3.15 (*t*, *J* = 6.7, CH_2(1)); 1.64–1.54 (*m*, 5 H); 1.44 (*s*, *t*-Bu). ^{13}C-NMR: 156.21 (*s*, CO); 79.28 (*s*, Me_3C); 62.73, 40.51, 29.75 (3*t*, 3 C); 28.44 (*q*, Me_3C); 26.62 (*t*, 1 C). ESI-MS: 212 ($[M + Na]^+$). CI-MS: 190 (4, $[M + 1]^+$), 134 (100, $[M + 1 - (isobutene)]^+$), 116 (8, $[M + 1 - (isobutene) - H_2O]^+$), 90 (7, $[M + 1 - (isobutene) - CO_2]^+$). Anal. calc. for $C_9H_{19}NO_3$ (189.25): C 57.12, H 10.12, N 7.45; found: C 57.13, H 10.17, N 7.53.

2. tert-*Butyl* N-*{4-[(4-Toluylsulfonyl)oxy]butyl}carbamate* (**6**). To a stirred soln. of **5** (1.89 g, 10.0 mmol) in CH_2Cl_2 (30.0 ml), Et_3N (3 ml, 21.5 mmol), 4-(dimethylamino)pyridine (DMAP; 120 mg, 1.1 mmol), and TsCl (2.29 g, 12.0 mmol) were added sequentially at 0°, and the stirring was continued for 2 h at r.t. The mixture was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to leave a pale-yellow oil. The oil was purified by chromatography on a silica-gel column (AcOEt/hexane 2:1 (*v/v*)) to leave the pure **6** (2.74 g, 80%) as a colorless oil, which slowly crystallized at 4°. M.p. 50.9–51.8°. IR (KBr): 3042*s*, 3002*m*, 2972*s*, 2940*m*, 2870*m*, 1713*s*, 1598*m*, 1528*s*, 1478*m*, 1455*m*, 1367*s*, 1348*s*, 1271*s*, 1258*s*, 1240*s*, 1193*s*, 1170*s*, 1098*m*, 1037*m*, 968*s*, 955*s*, 870*s*, 855*s*, 820*s*. ^{1}H-NMR: 7.77 (*d*, *J* = 8.2, 2 H); 7.33 (*d*, *J* = 8.2, 2 H); 4.52 (br. *s*, BocNH); 4.03 (*t*, *J* = 6.3, CH_2(4)); 3.09 (*t*, *J* = 6.6, CH_2(1)); 2.44 (*s*, MeC_6H_4); 1.71–1.62 (*m*, 2 H); 1.54–1.45 (*m*, 2 H); 1.41 (*s*, *t*-Bu). ^{13}C-NMR: 156.96 (*s*, CO); 145.79, 134.12 (2*s*, 2 C); 130.88, 128.88 (2*d*, 4 C); 80.27 (*s*, Me_3C); 71.13 (*d*, C(4)); 40.86 (*t*, C(1)); 29.40 (*q*, Me_3); 27.22 (*t*, C(2), C(3)); 22.64 (*q*, MeC_6H_4). ESI-MS: 366 ($[M + Na]^+$). CI-MS: 288 (5, $[M + 1 - (isobutene)]^+$), 172 (13, $[M + 1 - TsOH]^+$), 116 (100, $[M + 1 - (isobutene) - TsOH]^+$). Anal. calc. for $C_{16}H_{25}NO_5S$ (343.44): C 55.96, H 7.34; found: C 55.70, H 7.57.

3. tert-*Butyl* N-*(4-[^{15}N]Phthalimidobutyl)carbamate* (**7**). A mixture of **6** (688 mg, 2.0 mmol) and potassium (^{15}N)phthalimide (372 mg, 2.0 mmol) in DMF (25 ml) was stirred at 20° for 3 d. After evaporation of the solvent under reduced pressure, 10 ml of 5% aq. HCl soln. was added to the residue and extracted with CH_2Cl_2 (4 × 15 ml). The extract was dried (Na_2SO_4) and the solvent removed, yielding **7** (619 mg, 97%). Colorless solid. M.p. 104.5–105.9°. IR: 3450, 2975, 2925, 1769, 1710*s*, 1610, 1508*s*, 1390*s*, 1368*s*, 1352*s*, 1330, 1240, 1170*s*, 1038, 865. ^{1}H-NMR: 7.84 (*dd*, *J* = 5.4, 3.0, 2 H); 7.22 (*dd*, *J* = 5.4, 3.0, 2 H); 3.71 (*td*, *J* = 7.0, $^2J(^{15}N,H) = 0.9$, CH_2(4)); 3.18–3.08 (*m*, CH_2(1)); 1.73–1.44 (*m*, 4 H); 1.43 (*s*, *t*-Bu). ^{13}C-NMR: 166.0 (*s*, $^1J(^{15}N,C) = 12.8$, CO of phthalimide); 153.58 (*s*, CO of Boc); 131.55 (*d*, 2 arom. C); 129.71 (*s*, 2 arom. C); 120.84 (*d*, 2 arom. C); 76.78 (*s*, Me_3C); 37.74 (*t*, C(1)); 35.15 (*t*, $^1J(^{15}N,C) = 9.1$, C(4)); 26.04 (*q*, Me_3C); 25.09 (*t*, C(2)); 23.60 (*t*, C(3)). CI-MS: 337 (4, $[M + 1 + NH_3]^+$), 320 (10, $M + 1]^+$), 281 (100, $[M + 1 + NH_3 - (isobutene)]^+$), 264 (10, $[M + 1 - (isobutene)]^+$), 220 (65, $[M + 1 - (isobutene) - CO_2]^+$). EI-MS: 246 (40), 219 (27), 218 (100), 200 (16), 190 (11), 162 (25), 161 (92), 150 (16), 149 (11), 148 (50), 131 (12), 105 (15), 104 (44), 77 (13), 76 (31), 70 (97), 57 (100), 44 (17), 41 (23).

4. tert-*Butyl* N-*(4-[^{15}N]Aminobutyl)carbamate* (**8**). To a soln. of the **7** (477 mg, 1.5 mmol) in MeOH (15 ml), $N_2H_4 \cdot H_2O$ (135 mg, 2.7 mmol) was added, and the mixture was stirred under reflux. After 2 h, it was cooled to r.t., filtered, and evaporated under reduced pressure. To the residue 10 ml of 10% aq. NaOH soln. were added, and the aq. layer was extracted with CH_2Cl_2 (4 × 15 ml), and the combined org. extracts were dried (Na_2SO_4) and evaporated to afford 266 mg (94%) of **8**. Colorless oil. IR: 3343*s*, 2960*s*, 2920*s*, 2852*s*, 1700*s*, 1687*s*, 1568*s*, 1544*s*, 1525, 1474*s*, 1448*s*, 1386*s*, 1360*s*, 1270*s*, 1246*s*, 1170*s*, 1037, 864. ^{1}H-NMR: 4.78 (br. *s*, BocNH); 3.32–3.17 (*m*, CH_2(1)); 2.71 (*t*, *J* = 6.3, CH_2(4)); 1.52–1.46 (*m*, 4 H); 1.44 (*s*, *t*-Bu); 1.33 (br. *s*, NH_2). ^{13}C-NMR: 156.06 (*s*, CO of Boc); 79.00 (*s*, Me_3C); 41.82 (*t*, $^1J(^{15}N,C) = 3.9$, C(4)); 40.46 (*t*, C(1)); 30.90 (*t*, C(3)); 28.45 (*q*, Me_3C); 27.52 (*t*, C(2)). EI-MS: 189 (M^+), 133 (38), 116 (27), 114 (15), 103 (24), 98 (16), 74 (28), 71 (39), 70 (26), 59 (20), 57 (100), 55 (12), 46 (40), 41 (46).

5. tert-*Butyl* N-{*4-[(2-Cyanoethyl)[^{15}N]amino]butyl*}*carbamate* (**9**). Under N_2 atmosphere, a soln. of acrylonitrile (65 mg, 1.22 mmol) in MeOH (10 ml) was added to a MeOH soln. (10 ml) containing **8** (230 mg, 1.22 mmol) within 30 min and allowed to stirred for 10 h. The residue, obtained on concentration, was purified on a silica-gel column (AcOEt/MeOH 4:3 (*v*/*v*)), to obtain 271 mg (92%) of **9**. Colorless oil. IR: 3448*s*, 2968*s*, 2920*s*, 1702*s*, 1505*s*, 1450, 1388*s*, 1362*s*, 1245*s*, 1163*s*, 1045*s*, 858*s*, 657*s*. ^{1}H-NMR: 4.78 (br. *s*, BocNH); 3.22–2.99 (*m*, 2 H); 2.92 (*t*, *J* = 6.6, 2 H); 2.66 (*t*, *J* = 6.6, 2 H); 2.52 (*td*, *J* = 6.6, 2.5, 2 H); 1.58–1.49 (*m*, 5 H); 1.44 (*s*, *t*-Bu). ^{13}C-NMR: 156.04 (*s*, CO of Boc); 118.69 (*s*, CN); 79.09 (*s*, Me_3C); 48.72 (*t*, $^1J(^{15}N,C) = 5.0$, 1 C); 45.03 (*t*, $^1J(^{15}N,C) = 5.0$, 1 C); 41.80 (*t*, 1 C); 28.45 (*q*, Me_3C); 27.52, 27.27, 18.75 (3*t*, 3 C). ESI: 243 ($[M + 1]^+$).

6. tert-*Butyl* N-{*4-[(3-Aminopropyl)[^{15}N]amino]butyl*}*carbamate* (**10**). To a soln. of **9** (254 mg, 1.05 mmol) and NaOH (600 mg) in 15 ml of 95% EtOH, 300 mg of *Raney*-Ni were added, and hydrogenated under 50 psi of H_2 for 20 h. The catalyst was then filtered, washed well with MeOH, and the filtrate was concentrated. The residue was taken up into 10% aq. NaOH (10 ml), and the product was extracted into CH_2Cl_2 (4 × 15 ml), dried (Na_2SO_4), and evaporated to afford 235 mg (91%) of **10**. Colorless oil. IR: 3291*s*, 2962*s*, 2920*s*, 2853*s*, 1709*s*, 1688*s*, 1559, 1544, 1526*s*, 1475, 1449, 1386, 1360*s*, 1270, 1247*s*, 1170*s*, 1132*s*, 1034*s*, 864*s*. ^{1}H-NMR: 4.91 (br. *s*, BocNH); 3.12–2.96 (*m*, BocNHCH_2); 2.77 (*t*, *J* = 6.8, 2 H); 2.71–2.59 (*m*, 4 H); 1.63 (*quint.*, *J* = 6.8, 2 H); 1.51–1.46 (*m*, 7 H); 1.44 (*s*, *t*-Bu). ^{13}C-NMR: 155.92 (*s*, CO of Boc); 78.80 (*s*, Me_3C); 49.50 (*t*, $^1J(^{15}N,C) = 4.1$, 1 C); 47.75 (*t*, $^1J(^{15}N,C) = 3.6$, 1 C); 41.68, 40.45, 33.60 (3*t*, 3 C); 28.32 (*q*, Me_3C); 27.80, 27.30 (2*t*, 2 C). EI-MS: 246 (M^+), 219 (5), 202 (11), 173 (27), 156 (21), 146 (18), 140 (30), 134 (11), 133 (10), 116 (10), 114 (19), 112 (14), 99 (13), 88 (50), 85 (52), 84 (11), 74 (18), 73 (12), 72 (22), 71 (43) 70 (38), 59 (24), 58 (23), 57 (100), 56 (14), 55 (13), 46 (15), 46 (96), 44 (36), 43 (20), 42 (50), 41 (37).

7. *Di(*tert-*butyl)* N,N′-*(4-[^{15}N]Azaoctane-1,8-diyl)bis[carbamate]* (**11**). Et_3N (0.2 ml, 1.4 mmol) and 2-{[(*tert*-butoxy)carbonyl]oxyimino}-2-phenylacetonitrile (Boc-On, 148 mg, 0.60 mmol) in THF (15 ml) were added to a soln. of **10** (146 mg, 0.59 mmol) in THF (10 ml) at 0° within 3 h, and the resulting soln. was stirred at r.t. for 14 h. Most of the solvent was removed *i.v.*, and the residue was dissolved in AcOEt (20 ml). The org. phase was washed with 10 ml of 5% aq. NaOH and 10 ml of H_2O, dried ($MgSO_4$), and evaporated. The residue was purified on a silica-gel column (AcOEt/MeOH 4:3 (*v*/*v*)), to obtain 144 mg (70%) of **11**. Pale-yellow solid. IR: 3452, 3018*s*, 2978, 2978, 2932, 1708*s*, 1507*s*, 1478, 1454, 1392, 1369*s*, 1265, 1245, 1223*s*, 1208*s*, 1168*s*, 926, 790*s*, 754*s*, 670*s*, 663*s*. ^{1}H-NMR: 5.18, 4.74 (2 br. *s*, 2 BocNH); 3.16–2.90 (*m*, CH_2(1), CH_2(8)); 2.61–2.38 (*m*, CH_2(3), CH_2(5)); 1.60–1.44 (*m*, 7 H); 1.38 (*s*, 2 *t*-Bu). ^{13}C-NMR: 156.05, 155.95 (2*s*, 2 CO); 78.92 (*s*, 2 Me_2C); 49.20 (*t*, $^1J(^{15}N,C) = 4.1$, 1 C); 47.41 (*t*, $^1J(^{15}N,C) = 3.6$, 1 C); 40.31, 38.94, 29.66 (3*t*, 3 C); 28.33 (*q*, Me_3C); 27.70, 27.07 (2*t*, 2 C). ESI: 347 ($[M + 1]^+$).

8. *Di(*tert-*butyl)* N,N′-{*4-[(E)-3-(4-Hydroxyphenyl)prop-2-enoyl]-4-[^{15}N]azaoctane-1,8-diyl*}*bis[carbamate]* (**12**). A soln. of *N*,*N*′-dicyclohexylcarbodiimid (DCC, 62 mg, 0.30 mmol) and (*E*)-4-(hydroxyphenyl)prop-2-enoic acid (= 4-hydroxycinnamic acid, 45 mg, 0.27 mmol) in THF (10 ml) was added to a soln. of **11** (90 mg, 0.26 mmol) in THF (5 ml) under N_2 atmosphere within 30 min and stirred for 20 h. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified on a silica-gel column (CH_2Cl_2/MeOH 9:1 (*v*/*v*)), to obtain 101 mg (79%) of **12**. Colorless solid. IR: 3200 (br.), 2965, 2927, 2860, 1700*s*, 1640*s*, 1605*s*, 1584*s*, 1508*s*, 1500*s*, 1440, 1390, 1366*s*, 1270, 1247*s*, 1164*s*, 980, 908*s*, 825*s*. ^{1}H-NMR: 7.62 (*d*, *J* = 15.3, 1 olef. H); 7.35 (*d*, *J* = 8.3, 2 arom. H); 6.88 (*d*, *J* = 8.3, 2 arom. H); 6.61 (*d*, *J* = 15.3, 1 olef. H); 4.98–4.72 (br. *s*, 2 BocNH); 3.58–3.35 (*m*, CH_2(3), CH_2(5)); 3.22–3.07 (*m*, CH_2(1), CH_2(8)); 1.98–1.50 (*m*, 7 H); 1.44 (*s*, 2 *t*-Bu). ^{13}C-NMR: 167.58 (*d*, $^1J(^{15}N,C) = 16.2$, CO); 158.60 (*s*, 1 C); 156.29 (*s*, 2 CO of Boc); 143.39 (*d*, 1 C); 129.67 (*d*, 2 arom. C); 127.08 (*s*, 1 C); 116.02 (*d*, 2 arom. C); 113.76 (*d*, 1 C); 79.58, 79.16 (2*s*, 2 Me_3C); 47.59, 43.54, 40.08, 37.47 (4*t*, 4 C); 28.45 (*q*, 2 Me_3C); 27.68, 27.46, 26.80 (3*t*, 3 C). ESI-MS: 493 ($[M + 1]^+$).

9. *(E)-N-(4-Aminobutyl)-N-(3-aminopropyl)-3-(4-hydroxyphenyl)prop-2-en[^{15}N]amide* ([^{15}N(4)]-**2**). A CH_2Cl_2 (5 ml) soln. containing **12** (70 mg, 0.141 mmol) was added under Ar atmosphere to CF_3COOH (2 ml). The mixture was stirred for 2 h at ambient temp. and the solvent removed *i.v.* The residue was dissolved in 2 ml of 5% aq. HCl with *ca.* 5 ml of EtOH, and the soln. was concentrated *i.v.* This process was repeated for three times to yield 45 mg (95%) of ^{15}N(4)-**2**·2 HCl. Pale-yellow solid. IR ([^{15}N(4)]-**2**·2 HCl, KBr): 3350 (br.), 3210*s*, 3111*s*, 3000*s*, 2760, 1640*s*, 1610*s*, 1585*s*, 1514*s*, 1482*s*, 1455, 1448*s*, 1435*s*, 1352, 1316, 1289, 1265*s*, 1221*s*, 1199, 1167, 1152*s*, 1128, 1112, 1102, 1040, 980*s*, 880*s*, 832*s*, 818, 790, 755, 731. ^{1}H-NMR (CD_3OD, 5 H exchanged): 7.60, 7.58 (2*d*, J = 15.3, 1 olef. H); 7.51 (*d*, J = 8.6, 2 arom. H); 6.88 (*d*, J = 15.3, 1 olef. H); 6.81 (*d*, J = 8.6, 2 arom. H); 3.69–3.51 (*m*, 4 H); 3.06–2.91 (*m*, 4 H); 1.99–1.95 (*m*, 2 H); 1.85–1.60 (*m*, 4 H). ESI-MS: 293 ([M + 1]$^+$).

REFERENCES

[1] L. Bigler, M. Hesse, *J. Am. Soc. Mass Spectrom.* **1995,** *6,* 634.
[2] P.P. McCann, A.E. Pegg, A. Sjoerdsma, 'Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies', Academic Press, San Diego, 1987.
[3] D. Strack, U. Eilert, V. Wray, J. Wolff, H. Jaggy, *Phytochemistry* **1990,** *29,* 2893.
[4] B. Meurer, V. Wray, R. Wiermann, D. Strack, *Phytochemistry* **1988,** *29,* 839.
[5] C. Werner, W. Hu, A. Lorenzi-Riatsch, M. Hesse, *Phytochemistry* **1995,** *40,* 461.
[6] M. Bokern, L. Witte, V. Wray, M. Nimtz, B. Meurer-Grimes, *Phytochemistry* **1995,** *39,* 1371.
[7] E. de Hoffmann, *J. Mass Spectrom.* **1996,** *31,* 129.
[8] G. Hopfgartner, W. Vetter, W. Meister, H. Ramuz, *J. Mass Spectrom.* **1996,** *31,* 69.
[9] M. Claeys, H. Van den Heuvel, S. Chen, P.J. Derrick, F.A. Mellon, K.R. Price, *J. Am. Soc. Mass Spectrom.* **1996,** *7,* 173.
[10] W. Hu, M. Hesse, *Helv. Chim. Acta* **1996,** *79,* 548.
[11] L. Bigler, C.F. Schnider, W. Hu, M. Hesse, *Helv. Chim. Acta* **1996,** *79,* 2152.
[12] H. Bosshardt, M. Hesse, *Angew. Chem.* **1974,** *86,* 256; *ibid. Int. Ed.* **1974,** *13,* 252.
[13] A. Guggisberg, B. Dabrowski, U. Kramer, C. Heidelberger, M. Hesse, H. Schmid, *Helv. Chim. Acta* **1978,** *61,* 1039.
[14] S. Takano, T. Sugihara, K. Ogasawara, *Heterocycles* **1990,** *31,* 1721.
[15] F. Effenberger, U. Stelzer, *Angew. Chem.* **1991,** *103,* 866; *ibid. Int. Ed.* **1991,** *30,* 873.
[16] R.J. Bergeron, J.S. McManis, *J. Org. Chem.* **1988,** *53,* 3108.
[17] W.J. Fiedler, M. Hesse, *Helv. Chim. Acta* **1993,** *76,* 1511.
[18] T.L. Huang, S.A. Dreder, V.A. Manneh, J.W. Blankenship, D.S. Fries, *J. Med. Chem.* **1992,** *35,* 2414.