178. Electrospray-Ionization Mass Spectrometry

Part III[1])

Acid-Catalyzed Isomerization of *N,N'*-Bis[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidines by the *Zip* Reaction[2])

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(21.VIII.96)

The electrospray tandem mass spectra (ESI-MS/MS) of the three *N,N'*-bis[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidines **1**–**3** displayed the same fragment-ion signals. These isomers could not be differentiated by ESI-MS/MS, since their fragmentation patterns are similar. (*E,E*)-*N*-(3-[^{15}N]Aminopropyl)-3,3'-bis(4-hydroxyphenyl)-*N,N'*-(butane-1,4-diyl)bis[prop-2-enamide] ([^{15}N(1)]-**1**) was synthesized in order to get further information about the fragmentation mechanisms. The comparison of the ESI-MS/MS of **1** and [^{15}N(1)]-**1** revealed a transamidation, the *Zip* reaction, under mass-spectral conditions of the $[\mathbf{1} + H]^+$ ions. Because of this reaction, the three isomers **1**–**3** could not be distinguished.

Introduction. – Mono-, di-, and trisubstituted spermidines (= *N*-(3-aminopropyl)butane-1,4-diamine) occur as derivatives of 4-hydroxycinnamic acid (= (*E*)-3-(4-hydroxyphenyl)prop-2-enoic acid) in the anthers of plants belonging to the plant families, *e.g.*, Fagaceae, Betulaceae, Juglandaceae [2–4], and Acanthaceae [5]. The polls of Acanthaceae plants are produced in small amounts only. Therefore, a purification of the rough extracts by high-performance liquid chromatography (HPLC) followed by electrospray-ionization mass spectrometry (ESI-MS) seems to be the most reliable analytical method for the identification of unknown compounds [6–8].

The *N,N'*-bis(4-hydroxycinnamoyl)spermidines (= *N,N'*-bis[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidines) can be substituted at the spermidine N-atoms, N(4) + N^8, N^1 + N^8, and N^1 + N(4) (compounds **1**–**3**, resp.). Identification of these polyamine derivatives is difficult, because a UV-light-induced isomerization of the C=C bonds takes place. Each compound isomerizes to the corresponding (*E/E*)-, (*E/Z*)-, (*Z/E*)-, and (*Z/Z*)-isomers [9] [10]. For this class of compounds, the commonly used methods for structure elucidation (fast-atom-bombardment mass spectrometry and NMR spectroscopy) failed [11].

In the context of the identification of products in plant extracts, the three *N,N'*-bis(4-hydroxycinnamoyl)spermidine derivatives **1**–**3** were investigated by means of ESI

[1]) Part II: [1].

[2]) Presented by *L.B.* at the '29. Diskussionstagung der Arbeitsgemeinschaft Massenspektrometrie der Deutschen Physikalischen Gesellschaft', on May 28–31, 1996, University of Bremen, Germany.

[3]) Part of the Ph. D. theses of *L.B.* and *W.H.*, University of Zürich, in preparation.

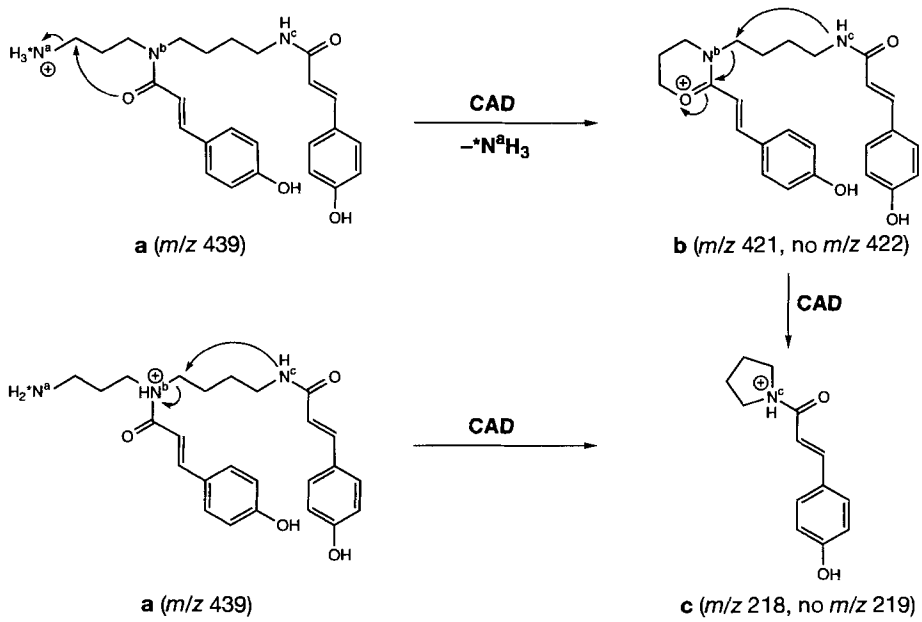
[4]) Part of the Diploma thesis of *C.F.S.*, University of Zürich, 1996.

tandem mass spectrometry (ESI-MS/MS). Since ESI-MS produces quasi-molecular ions with low fragmentation, the 'collision-induced decomposition' (CID) mass spectrometry at low activation energies (< 100 eV) was chosen as a suitable analytical method to obtain structural data [12] [13].

For further information concerning the fragmentation mechanisms of the *N,N'*-bis(4-hydroxycinnamoyl)spermidine derivatives, $^{15}N^a$-labeled-**1** was synthesized and studied.

Results and Discussion[5]). – *MS Studies.* To obtain structure information from the $[M + H]^+$ ions, ESI-MS/MS at low activation energies and using Ar as the collision gas generally yield abundant fragment ions by nonradical reactions [14–16]. The 'driving forces' for S_Ni-type fragmentations are the formation of stable ions and/or the loss of stable neutral fragments such as NH_3 (*e.g.*, oxonium in **b**, *m/z* 421, *Scheme 1*).

Scheme 1. *The Proposed Fragmentation Mechanisms Leading to the Unlabeled Fragments*



a (*m/z* 439) **b** (*m/z* 421, no *m/z* 422)

a (*m/z* 439) **c** (*m/z* 218, no *m/z* 219)

The ESI-MS/MS of the three *N,N'*-bis(4-hydroxycinnamoyl)spermidine isomers **1–3** are shown in *Fig. 1*. Although the position of the 3-(4-hydroxyphenyl)prop-2-enamide residues as well as the configuration of the C=C bonds were defined by the synthetic pathways and confirmed by ^{1}H-NMR and HPLC experiments [17], the fragment-ion signals in the three spectra appear at the same positions. The only difference lies in the relative intensity of these signals. This is of course not sufficient for differentiation of the three isomers.

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[5]) To facilitate the MS discussion, the three different N-atoms in spermidine are defined as N^a, N^b, and N^c.

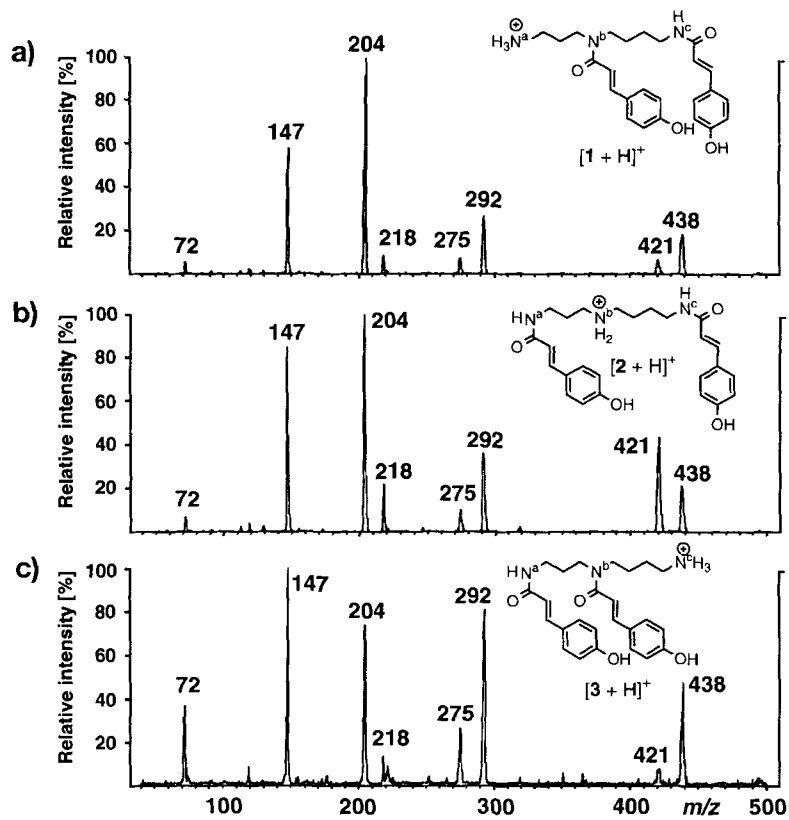


Fig. 1. *ESI-MS/MS of the* $[M + H]^+$ *ions* (*m/z* 438) *of* a) **1**, b) **2**, *and* c) **3**. All spectra were taken at 30-eV collision energy.

The labeled derivative [$^{15}N^1$]-**1** was synthesized to obtain some information about the fragmentation mechanisms. We expected that the comparison between the MS/MS of compounds **1** and [$^{15}N^1$]-**1** would explain why the three isomers **1**–**3** cannot be differentiated by means of ESI-MS/MS.

Fragmentation reactions observed in the MS/MS of compound **1** (*Fig. 2, a*) and of [$^{15}N^1$]-**1** (*Fig. 2, b*) are interpreted by analogy to the fragmentation mechanisms discussed by the mono-substituted [3-(4-hydroxyphenyl)prop-2-enoyl]spermidine derivatives [1]. Moreover, comparison of the two spectra given in *Fig. 2* indicates that two groups of signals are present, *i.e.*, those which are not shifted, and others shifted by 1 u.

A possible explanation for the structure of the fragment ions of which the signals remain unchanged is given in *Scheme 1*. The ion **b** (*m/z* 421, 422 not observed) is formed by loss of $^{15}NH_3$. A six-membered-ring structure can be postulated, because the positive charge of the oxonium ion is stabilized by delocalization. The ion structure of the second unchanged signal (*m/z* 218, 219 not observed) can be explained by the formation of the pyrrolidinium derivative **c**. This ion has two possible precursors: the quasi-molecular ion **a** (*m/z* 439) and the oxonium derivative **b** (*m/z* 421). The last fragment ions which remain

unchanged gave a signal at m/z 147 (no signal at m/z 148 was observed). They may have a carbonyl carbocation structure derived from the 3-(4-hydroxyphenyl)prop-2-enoyl residue and could arise from the amide-bond cleavage of the three ions **a**–**c**.

The MS/MS of the $[M + H]^+$ ions from compounds **1** and $[^{15}N^1]$-**1** yield fragment ions which also contain the N^a-atom. The signal of ion **d** in *Fig. 2, b* (m/z 293, *Scheme 2*), is shifted by 1 u compared to the corresponding signal (m/z 292) in *Fig. 2, a*. In both cases, the difference from the parent ion **a** is 146 u. This difference can be interpreted as the loss of one 3-(4-hydroxyphenyl)prop-2-enoyl residue (*Scheme 2*). By analogy to *N*-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide, the cleavage seems to be induced by neigh-

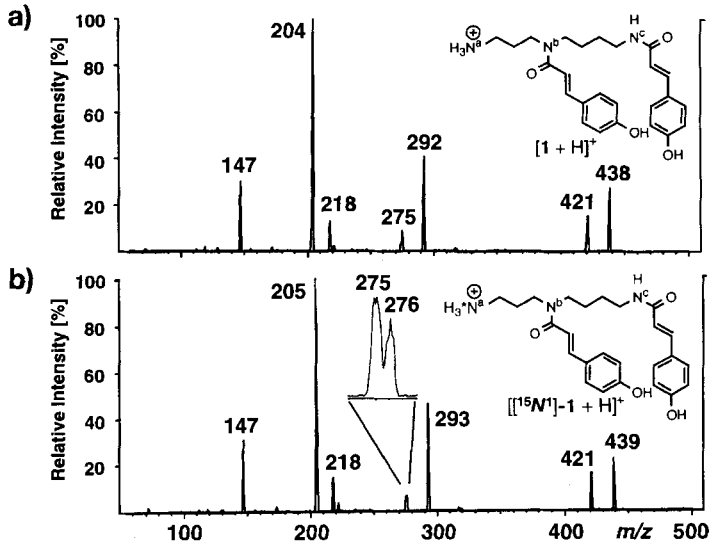
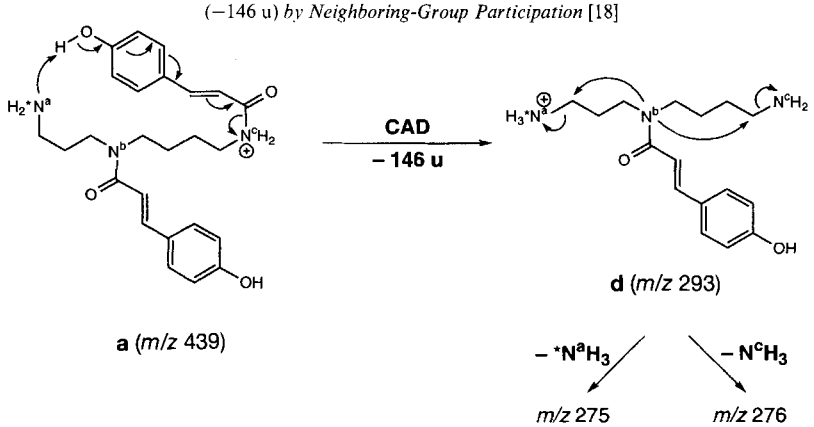


Fig. 2. *ESI-MS/MS* a) *of* $[1 + H]^+$ (m/z 438) *and* b) *of* $[[^{15}N^a]$-$1 + H]^+$ (m/z 439). The spectra were taken at 25-eV collision energy.

Scheme 2. *A Proposed Fragmentation Mechanism for the Loss of One 3-(4-Hydroxyphenyl)prop-2-enoyl Group* (−146 u) *by Neighboring-Group Participation* [18]



boring-group participation [18]. One phenolic proton is transferred from a 3-(4-hydroxyphenyl)prop-2-enoyl group to the basic primary amino group *via* a macrocyclic intermediate (14- or 19-membered). Due to the conjugation of the resulting phenolate with the enamide, the amide bond is cleaved, and ion **d** (*m/z* 293) is formed. The ion produced *via* the larger intermediate is shown. The proposed structure contains two primary amino groups. This seems to be in agreement with the possible loss of labeled ($\rightarrow m/z$ 275) or unlabeled ($\rightarrow m/z$ 276) ammonia. It is not possible to differentiate which of the two 3-(4-hydroxyphenyl)prop-2-enoyl groups is cleaved, because the *doublet* can be explained by the decomposition of both types of ions [1].

Comparison of the MS/MS of the $[\mathbf{1} + H]^+$ and $[^{15}N^a\text{-}\mathbf{1} + H]^+$ ions reveals a further pair of intense signals, differentiated by 1 u (*m/z* 204/205, see *Fig. 2, a* and *b*, resp.). These signals have a maximum of relative intensity at 25-eV collision energy. Considering their masses, these ions appear to be composed from the N^a-labeled 3-(4-hydroxyphenyl)prop-2-enamide and the trimethylene chain of the polyamine backbone. A direct structural formulation of ion **e** (*m/z* 205) on the basis of structure **1** is not possible. An acceptable solution, therefore, is to consider a transamidation, the *Zip* reaction, which leads to the ionized form of compound **2**. It is known from the chemistry in solution that strongly basic or acidic conditions can lead to the rearranged product [19]. Moreover, this reaction also proceeds thermally [20]. In ESI tandem mass spectrometry, all conditions favor a transamidation. The molecules are protonated in the gas phase, and, following this, their internal energy is increased by collision with neutral Ar-atoms. This reaction can, therefore, only take place under MS conditions. Compound $[^{15}N^a]$-**1** is a highly pure product with the structure given in *Scheme 4*. That means the sample did not contain any rearranged products such as **2** or **3** before ionization. This was confirmed by TLC (*Fluram*® as specific developer for NH_2 groups), ^{15}N-NMR, as well as ^{13}C-NMR spectra ($^1J(^{15}N,^{13}C)$ coupling constants).

The thermodynamically more stable, protonated secondary amide $[\mathbf{2} + H]^+$ is formed *via* a six-membered ring (*Scheme 3*). It is now possible to obtain the ion **e** (*m/z* 205), which contains only the labeled N-atom, by elimination of *N*-(4-aminobutyl)-3-(4-hydroxyphenyl)prop-2-enamide.

Isomerization in the other direction ($\mathbf{2} \rightarrow \mathbf{1}$) is not yet established, but its occurrence is highly probable. The most convincing argument for this reaction is the loss of ammonia (*m/z* 421, *Fig. 1, b*), which is only possible after isomerization[6]).

The same transamidations in the gas phase have been observed by the *N*-benzoylspermidine and by the *N*-[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidine isomers. A differentiation of the three isomers **1**–**3** by source-CID MS/MS, as accomplished by *Hu et al.* [1], could not be achieved.

To summarize, the ESI mass spectra of the three *N*,*N*′-bis[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]spermidines are very similar, because the compounds isomerize in the gas phase under conditions of MS measurements. By comparison of the spectra of **1** and $[^{15}N^a]$-**1**, it was shown that some fragments are formed from the unrearranged quasi-molecular ion **a** (*e.g.*, *m/z* 421 ($[M + H - NH_3]^+$)), but the ion assigned to the signal at *m/z* 205 (**e**) can only be produced from the rearranged molecular ion.

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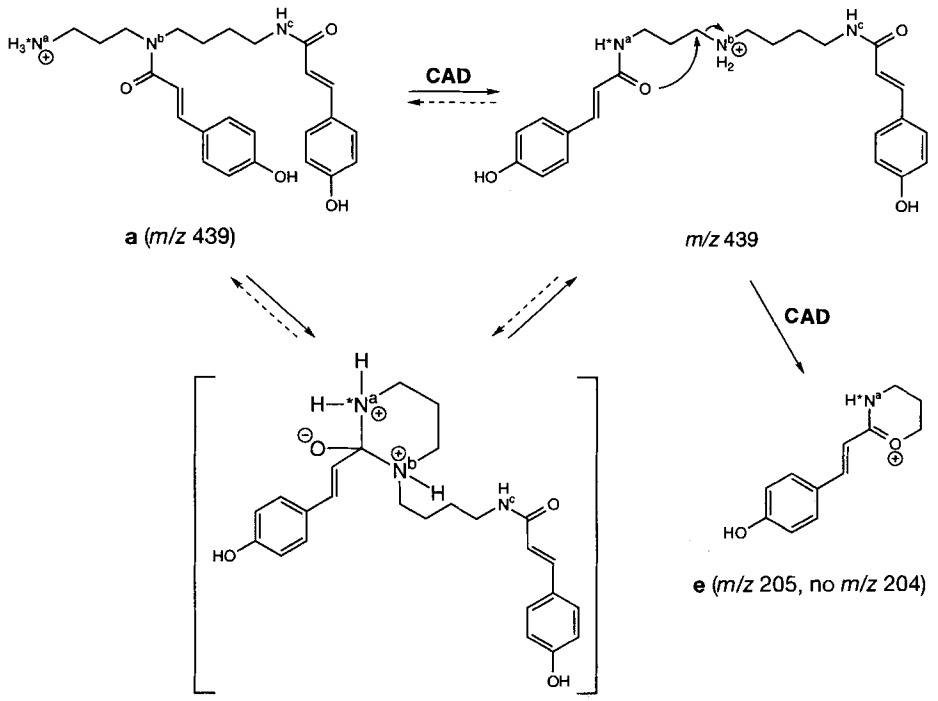
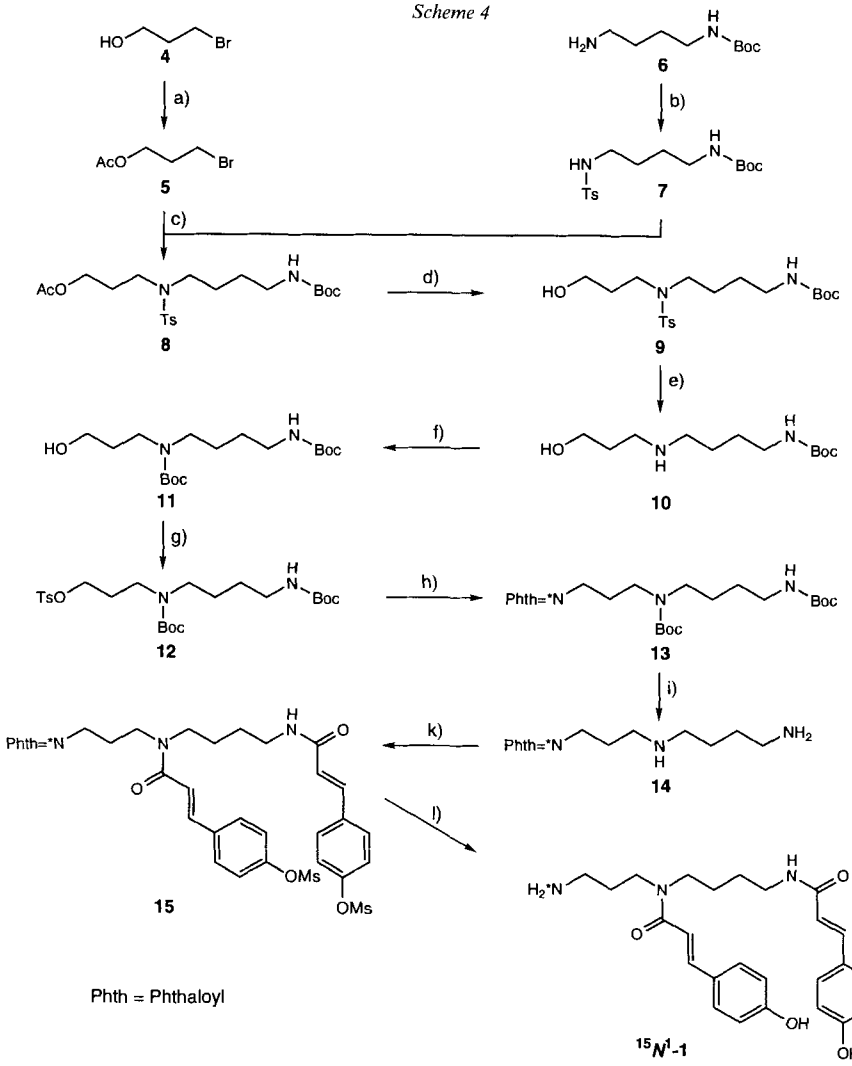
[6]) Some experiments are planned to determine which N-atom is eliminated from $[\mathbf{2} + H]^+$, and whether also $[\mathbf{2} + H]^+$ isomerizes to give $[\mathbf{3} + H]^+$.

Scheme 3. *Acid-Catalyzed Isomerization* (*Zip* Reaction) *in the Gas Phase*



Syntheses. For the introduction of the labeled N-atom, potassium (^{15}N)phthalimide was chosen, as the *Gabriel* synthesis is a well-known reaction for the introduction of a primary amine function (*Scheme 4*) [21]. Construction of the polyamine backbone was performed by *N*-alkylation. To avoid by-products, such as the dialkylated compounds, the asymmetrically disubstituted butane-1,4-diamine **7** was synthesized from **6** [22]. Compound **7** was then coupled with acetylated 3-bromopropan-1-ol (**4**) under strongly basic conditions (NaH; → **8**). After hydrolysis of **8** with aqueous NaOH in EtOH, **9** was obtained in 65% yield with respect to **5**. As the quantitative cleavage of the Ts group by electrolysis [23] has to be achieved before introduction of the phthaloyl group, the protecting group at N(4) has been exchanged for a Boc group (**9** → **10** → **11**). The primary alcohol derivative **11** was tosylated to obtain the precursor **12** for the *Gabriel* synthesis. Since **12** is not a very stable compound, it was not purified, and the labeled phthaloyl group was directly introduced to lead to **13** in good yield. After deprotection with CF_3COOH, the diamino derivative **14** was coupled with (*E*)-3-{4-[(methylsulfonyl)oxy]phenyl}prop-2-enoyl chloride [24] to yield the bis-acylated **15**. Activation of the unprotected 3-(4-hydroxyphenyl)prop-2-enoic acid with *N*,*N*′-dicyclohexylcarbodiimide [17] failed. The two final steps are the transformation of the phthalimide to the primary amine with hydrazine, followed *in situ* by deprotection of the 4-methylsulfonyl derivative with KOH in EtOH [24]. To avoid decomposition and rearrangement, [^{15}N^a]-**1** was immediately converted to the corresponding HCl salt.

Scheme 4



a) Ac_2O/cat. H_2SO_4, 78%. b) TsCl/Et_2O/4M aq. K_2CO_3, MeOH, 95%. c) NaH/DMF, 81%. d) EtOH/2M aq. NaOH soln., 61% [c) + d)]. e) Me_4NCl/EtOH/potential electrolysis, 98%. f) $(Boc)_2O/CH_2Cl_2$, 93%. g) TsCl/Et_3N/cat. 4-(dimethylamino)pyridine/CH_2Cl_2, 80%. h) Potassium (^{15}N)-phthalimide/DMF, 85%. i) $CF_3COOH/CHCl_3$, MeOH/1M aq. HCl soln., 82% [h) + i)]. k) Et_3N/AcOEt, (*E*)-3-{4-[(methylsulfonyl)-oxy]phenyl}prop-2-enoyl chloride, 81%. l) $N_2H_4 \cdot H_2O$/EtOH followed by KOH/EtOH, 2M aq. HCl soln., 84%.

The correct substitution pattern of [$^{15}N^a$]-**1** has been checked by TLC (*Fluram*® reagent as developer), ^{15}N-NMR, and ^{13}C-NMR spectroscopy ($^1J(^{15}N,^{13}C)$ coupling constants).

We are grateful to Mr. *M. Binder* and Mr. *D. Rentsch* for NMR measurements, to Mr. *N. Bild* and Dr. *A. Lorenzi-Riatsch* for recording the EI and CI mass spectra, to Mrs. *J. Kessler* for recording the IR spectra and elemental analysis, and to Mr. *A. Guggisberg* for technical assistance. This work was supported by the *Swiss National Science Foundation*.

Experimental Part

General. See [1]

1. *3-Bromopropyl Acetate* (**5**). To a soln. of 3-bromopropan-1-ol (**4**, 5.00 g, 36 mmol; *Fluka, purum*) was added dropwise 3.7 ml (4.0 g , 39 mmol) of Ac_2O. The mixture was treated with one drop of conc. H_2SO_4 and warmed up 1 h at 100°. The cooled soln. was diluted with ice-water (70 ml) and extracted with Et_2O. The extract was washed with sat. aq. $NaHCO_3$ (25 ml), dried ($MgSO_4$), evaporated, and the liquid residue distilled (100°/20 mbar): 5.08 g (28.1 mmol, 78%) of **5**. Fruity-smelling, colorless liquid. IR (film): 2975*s*, 2900*m*, 1740v*s* (br., C=O), 1470*m*, 1440*s*, 1385v*s*, 1365v*s*, 1240v*s* (br.), 1090*s*, 1035v*s*, 990*m*, 950*m*, 890*w*, 860*m*, 845*m*, 765*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.21 (*t*, J = 6.1, CH_2(1)); 3.47 (*t*, J = 6.6, CH_2(3)); 2.15 (*quint.*, J = 6.3, CH_2(2)); 2.06 (*s*, Me). ^{13}C-NMR ($CDCl_3$, 75 MHz): 170.88 (*c*, C=O); 62.19 (*t*, C(1)); 31.70 (*t*, C(2)); 29.36 (*t*, C(3)); 20.87 (*q*, Me).

2. tert-*Butyl* N-*(4-Aminobutyl)carbamate* (**6**). A vigorously stirred soln. of 16.37 g (75 mmol) of di(*tert*-butyl) dicarbonate ($(Boc)_2O$) in dioxane (200 ml) was treated dropwise at r.t. during 5 h with a soln. of 60.3 ml (52.89 g; 600 mmol) of butane-1,4-diamine (*Fluka, purum*) in dioxane (200 ml) and stirred at r.t. for further 60 h. The solvent was removed at 65°, the residue treated with H_2O (200 ml) and extracted with CH_2Cl_2 (7 × 150 ml). The org. phase was dried (Na_2SO_4), evaporated, and the oily residue distilled (120°/0.015 mbar): 13.94 g colorless oil, that contained butane-1,4-diamine (TLC). This oil was dried at r.t. (8 h) under high vacuum (h.v.): 12.38 g (65.8 mmol, 88%) of pure **6**. IR (film): 3350*s* (NH), 2970*s*, 2930*s*, 2860*s* 1695v*s* (C=O), 1525*s*, 1450*s*, 1390*s*, 1365v*s*, 1275*s*, 1250*s*, 1175v*s*, 1040*m*, 985*m*, 865*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.85 (br., BocNH); 3.12 (*m*, CH_2(1)); 2.71 (*t*, J = 6.6, CH_2(4)); 1.53–1.46 (*m*, CH_2(2), CH_2(3)); 1.44 (*s*, *t*-Bu); 1.28 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.08 (*s*, C=O); 78.97 (*s*, Me_3C); 41.85, 40.62 (2*t*, 2 NCH_2); 30.94, 27.52 (2*t*, C(2), C(3)); 28.46 (*s*, Me_3C).

3. tert-*Butyl* N-*{4-[(4-Toluylsulfonyl)amino]butyl}carbamate* (**7**). A soln. of 7.63 g (40 mmol) of TsCl (*Fluka, puriss*) in dry Et_2O (45 ml) was added dropwise at r.t. during 20 min to a stirred soln. of 7.53 g (40 mmol) of **6** in 4M aq. K_2CO_3 (60 ml). After 2 h at r.t., the Et_2O was evaporated, the mixture treated with MeOH (30 ml), warmed up 30 min at 50°; afterwards the MeOH was evaporated and the remaining aq. suspension extracted with AcOEt (100 ml). The org. layer was washed with H_2O (2 × 25 ml), dried (Na_2SO_4), concentrated to 10 ml, and crystallized by addition of hexane: 12.96 g of **7** (37.8 mmol, 95%). Colorless crystalline solid. M.p. 87.5–88.5°. IR (KBr): 3375*s*, 3275v*s*, 2960*m*, 2865*m*, 1690v*s* (C=O), 1595*w*, 1525v*s*, 1480*w*, 1445*m*, 1425*s*, 1390*m*, 1365*m*, 1320v*s*, 1275*s*, 1240*s*, 1180*s*, 1160v*s*, 1090*s*, 1070*s*, 1000*m*, 905*m*, 875*w*, 815*s*, 780*w*, 705*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.74, 7.30 (2*d*, J = 8.2, 4 arom. H); 5.10 (br., TsNH); 4.60 (br., BocNH); 3.05 (*q*, J = 6.0, $NHCH_2$); 2.93 (*q*, J = 6.3, $NHCH_2$); 2.42 (*s*, arom. Me); 1.50–1.47 (*m*, CH_2CH_2); 1.42 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.11 (*s*, C=O); 143.33, 137.02 (2*s*, 2 arom. C); 129.70, 127.09 (2*d*, 4 arom. CH); 79.23 (*s*, Me_3C); 42.82, 39.93 (2*t*, NCH_2); 28.41 (*q*, Me_3C); 27.18, 26.73 (2*t*, CH_2CH_2); 21.51 (*q*, arom. Me). ESI-MS: 365 (22, $[M + Na]^+$), 243 (100, $[M + H - CO_2 - (isobutene)]^+$). Anal. calc. for $C_{16}H_{26}N_2O_4S$ (342.46): C 56.12, H 7.65, N 8.18, S 9.36; found: C 56.07, H 7.33, N 8.21, S 9.51.

4. tert-*Butyl* N-*[8-Acetoxy-5-(4-toluylsulfonyl)-5-azaoctyl]carbamate* (**8**). A soln. of 8.56 g (25 mmol) of **7** in dry DMF (100 ml) was treated under Ar with 1.44 g (30 mmol) of NaH suspension (50% in white oil). The mixture was stirred at r.t. until the H_2 formation terminated (45 min). A soln. of 4.53 g (25 mmol) of **5** in dry DMF (50 ml) was then dropwise added within 30 min, the mixture stirred for further 2 h at r.t., and finally the solvent evaporated under h.v. The residue was treated with H_2O (150 ml) and sat. aq. $NaHCO_3$ (20 ml), extracted with CH_2Cl_2 (2 × 150 ml), the org. layers were dried (Na_2SO_4) and evaporated: 11.83 g of yellow oil. Crude **8** was used without purification for the following hydrolysis. A part of the crude product was purified (silica gel; hexane/Et_2O) for spectroscopic characterization. IR (film): 3390*w* (br., NH), 2975*m*, 2930*m*, 2865*w*, 1740*s* (C=O, ketone), 1710v*s* (C=O, carbamate), 1595*w*, 1515*s*, 1450*m*, 1390*m*, 1365*s*, 1340*s*, 1250v*s*, 1160v*s*, 1090*s*, 1045*m*, 865*w*, 815*w*, 725*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.68, 7.30 (2*d*, J = 8.1, 4 arom. H); 4.60 (br., BocNH); 4.07 (*t*, J = 6.2, CH_2O); 3.20–3.09 (*m*, 3 NCH_2); 2.42 (*s*, arom. Me); 2.04 (*s*, COMe); 1.87 (*quint.*, J = 7.0, CH_2CH_2OAc); 1.57–1.48 (*m*, CCH_2CH_2C); 1.44 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz): 170.84 (*s*, OCOMe); 155.92 (*s*, NHCOO); 143.21, 136.39 (2*s*, 2 arom. C); 129.61, 127.04 (2*d*, 4 arom. CH); 79.08 (*s*, Me_3C); 61.67 (*t*, CH_2O); 48.32, 45.43, 39.88 (3*t*, 3 NCH_2); 28.31 (*q*, Me_3C); 28.12, 27.16, 25.93 (3*t*, 3 CCH_2C); 21.38 (*q*, arom. Me); 20.78 (*q*, COMe). ESI-MS (+NaI): 465 (100, $[M + Na]^+$), 409 (30, $[M + Na - (isobutene)]^+$), 365 (9, $[M + Na - CO_2 - (isobutene)]^+$).

5. tert-*Butyl* N-*[8-Hydroxy-5-(4-toluylsulfonyl)-5-azaoctyl]carbamate* (**9**). A 2M aq. NaOH soln. (40 ml) was dropwise added to a soln. of 11.8 g (25 mmol) of **8** in dry EtOH (100 ml). The mixture was stirred for 2 h, then diluted with H_2O (50 ml), neutralized by addition of dry ice (pH 7–8), and finally the EtOH was evaporated. The remaining aq. suspension was extracted with CH_2Cl_2 (200 ml), the org. layers were dried (Na_2SO_4), evaporated,

and the residue purified by chromatography (silica gel, hexane/acetone 2:1): 6.08 g of **9** (15.2 mmol, 61% with respect to **5**). Colorless solid. IR (film): 3500 (sh, OH), 3390*s* (br., NH), 2970*s*, 2930v*s*, 2870*s*, 1710 (sh), 1690v*s* (C=O), 1595*w*, 1515*s*, 1455*m*, 1390*m*, 1365*s*, 1335v*s*, 1250*s*, 1160v*s*, 1090*s*, 1055*m*, 1020*m*, 910*w*, 865*w*, 815*m*, 725*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.68, 7.30 (2*d*, J = 8.1, 4 arom. H); 4.63 (br., BocNH); 3.73 (*t*, J = 5.7, CH_2O); 3.23 (*t*, J = 6.7, NCH_2); 3.14–3.09 (*m*, 2 NCH_2); 2.42 (*s*, arom. Me); 1.76 (*quint.*, J = 6.0, CH_2CH_2OH); 1.60–1.46 (*m*, CCH_2CH_2C); 1.44 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.09 (*s*, NHCOO); 143.36, 136.36 (2*s*, 2 arom. C); 129.75, 127.11 (2*d*, 4 arom. CH); 79.26 (*s*, Me_3C); 58.90 (*t*, CH_2O); 48.95, 45.41, 39.94 (3*t*, 3 NCH_2); 28.42 (*q*, Me_3C); 31.68, 27.35, 26.12 (3*t*, 3 CCH_2C); 21.49 (*q*, arom. Me). ESI-MS (+NaI): 423 (100, $[M + Na]^+$), 367 (25, $[M + Na - (isobutene)]^+$), 323 (6, $[M + Na - CO_2 - (isobutene)]^+$), 301 (16, $[M + H - CO_2 - (isobutene)]^+$).

6. tert-*Butyl* N-{*4-[(3-Hydroxypropyl)amino]butyl*}*carbamate* (**10**). The controlled-potential electrolysis of 606 mg (1.513 mmol) of **9** was carried out in a cylindrical, three-electrode, divided cell using an electronic potentiostat. Stirred Hg pool (area: 44 cm^2) adjusted to a −2.2 V; counter electrode: graphite rod. Reference electrode: SCE; SSE: 0.1M $Me_4N^+Cl^-$ in 94% EtOH (100 ml) as catholyte and anolyte. T = 5°; Ar atmosphere. After depletion of the electrolysis current to 10-mA background level (recorded i/t curve), the reaction was complete (Consumption: 312 Coulomb, theor. 292 Coulomb). The catholyte was evaporated to dryness, and the residue was purified by flash chromatography (aluminium oxide, *Merck* (standardized acc. to *Brockmann*), CH_2Cl_2/MeOH/25% NH_3 95:5:0.2) and evaporated to dryness under h.v.: 3.55 g of **10** (14.4 mmol, 98%). Pale-yellow solid. IR (film): 3320*s* (br., NH, OH), 2970*s*, 2930*s*, 2860*s*, 1690v*s* (C=O), 1525*s*, 1450*s*, 1390*s*, 1365v*s*, 1275*s*, 1250*s*, 1175v*s*, 1065*m*, 920*w*, 865*w*, 730*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.72 (br., BocNH); 3.80 (*t*, J = 5.6, CH_2O); 3.10 (m_c, NCH_2, NH, OH); 2.88 (*t*, J = 6.7, N–CH_2); 2.65 (m_c, NCH_2); 1.72 (*quint.*, J = 6.0, CH_2CH_2OH); 1.52 (m_c, CCH_2CH_2C); 1.44 (*s*, $C(CH_3)_3$). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.10 (*s*, NHCOO); 79.16 (*s*, Me_3C); 64.01 (*t*, CH_2O); 49.69, 49.24 (2*t*, 2 $CNHCH_2$); 40.33 (*t*-BocNHCH_2); 28.45 (*q*, Me_3C); 30.51, 27.78, 26.90 (3*t*, 3 CCH_2C). ESI-MS: 247 (100, $[M + H]^+$), 191 (42, $[M + H - (isobutene)]^+$).

7. *Di(*tert*-butyl)* N-*(3-Hydroxypropyl)*-N,N′-*(butane-1,4-diyl)bis[carbamate]* (**11**). A soln. of 3.40 g (15.6 mmol) of di(*tert*-butyl) dicarbonate in CH_2Cl_2 (15 ml) was dropwise added under cooling to a soln. of 3.2 g (13 mmol) of **10** in CH_2Cl_2 (50 ml). The mixture was then stirred for 2 h at r.t., the solvent evaporated to dryness under h.v., and the residue purified by chromatography (silica gel, hexane/AcOEt 1:2): 4.27 g (12.3 mmol, 93%) of **11**. Colorless solid. IR (film): 3460 (sh, OH), 3350*m* (br., NH); 2970*s*, 2930*s*, 2865*m*, 1690v*s* (br., C=O), 1525*s*, 1480*s*, 1450*m*, 1420*s*, 1390*m*, 1365v*s*, 1250*s*, 1170v*s*, 1060*m*, 1005*w*, 950*w*, 865*w*, 775*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.58 (br., BocNH); 3.56 (m_c, CH_2O); 3.35 (m_c, NCH_2); 3.14 (m_c, 2 NCH_2); 2.90 (br., OH); 1.67 (m_c, CH_2CH_2OH); 1.58–1.48 (*m*, CCH_2CH_2C); 1.46 (*s*, *t*-Bu); 1.44 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.03 (*s*, NHCOO); 80.05 (*s*, Me_3C); 79.22 (*s*, Me_3C); 58.36 (*t*, CH_2O); 46.75, 42.69, 40.18 (3*t*, 3 NCH_2); 28.43 (*q*, Me_3C); 30.70, 27.51, 25.75 (3*t*, 3 CCH_2C). ESI-MS (+NaI): 369 (100, $[M + Na]^+$), 247 (8, $[M + H - CO_2 - (isobutene)]^+$).

8. *Di(*tert*-butyl)* N-{*3-[(4-Toluylsulfonyl)oxy]propyl*}-N,N′-*(butane-1,4-diyl)bis[carbamate]* (**12**). A soln. of 1.39 g (4.00 mmol) of **11** in CH_2Cl_2 (20 ml) was treated at 0° with 1.1 ml (0.8 g, 8 mmol) of Et_3N and 50 mg of (dimethylamino)pyridine, and then a soln. of 1.14 g (6.00 mmol) of TsCl in CH_2Cl_2 (10 ml) slowly added dropwise. The mixture was stirred for 6 h at r.t., then evaporated to dryness, and the residue was taken up with 50 ml of CH_2Cl_2 and washed with 25 ml of a half sat. aq. $NaHCO_3$ soln. and with 25 ml of a sat. aq. NaCl soln. The org. layer was dried (Na_2SO_4), evaporated to dryness (h.v.), and the crude extract purified by chromatrography (silica gel, hexane/AcOEt 2:1): 1.60 g of **12** (3.19 mmol, 80%). Colorless oil, which decomposes slowly in the presence of H_2O. IR (film): 3350*w* (br., NH); 2970*s*, 2930*s* 2865*m*, 1690v*s* (br., C=O), 1595*m*, 1525*s*, 1495*s*, 1480*s*, 1450*m*, 1420*s*, 1390*m*, 1365v*s*, 1250*s*, 1175v*s*, 1125*m*, 1100*m*, 1035*m*, 1010*s*, 970*m*, 930*m*, 865*w*, 815*m*, 760*m*, 740*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.78, 7.35 (2*d*, J = 8.2, 4 arom. H); 4.8–4.2 (br., BocNH); 4.03 (*t*, J = 6.3, CH_2O); 3.19 (*t*, J = 7.0, NCH_2); 3.11 (*t*, J = 6.8, 2 NCH_2); 2.45 (*s*, arom. Me); 1.88 (m_c, CH_2CH_2OTs); 1.55–1.43 (*m*, CCH_2CH_2C); 1.44 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz): 156.05 (*s*, NHCOO); 155.41 (*s*, NHCOO); 144.83, 133.02 (2*s*, 2 arom. C); 129.88, 127.90 (2*d*, 4 arom. CH); 79.66 (*s*, Me_3C); 68.32 (*t*, CH_2O); 47.50, 43.76, 40.40 (3*t*, 3 NCH_2); 28.40 (*q*, Me_3C); 27.34, 25.68 (2*t*, 2 CCH_2C); 21.61 (*q*, arom. Me). ESI-MS (+NaI): 523 (100, $[M + Na]^+$), 423 (4, $[M + Na - CO_2 - (isobutene)]^+$), 401 (55, $[M + H - CO_2 - (isobutene)]^+$), 345 (32, $[M + H - CO_2 - 2(isobutene)]^+$), 301 (20, $[M + H - 2\,CO_2 - 2(isobutene)]^+$), 295 (62).

9. *Di(*tert*-butyl)* N-*(3-[^{15}N]Phthalimidopropyl)*-N,N′-*(butane-1,4-diyl)bis[carbamate]* (**13**). To a soln. of 1.40 g (2.79 mmol) of **12** in dry DMF (40 ml) was added 0.52 g (2.81 mmol) of potassium (^{15}N)phthalimide (*Fluka, purum*; 98 atom-% ^{15}N). The mixture was stirred for 48 h, the DMF evaporated under h.v., the residue taken up in $CHCl_3$ (50 ml) and washed with H_2O (50 ml). The aq. phase was washed again with $CHCl_3$, then the org. layers were combined and washed with sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated to give a colorless oil. Since

neither **12** could be detected in the ^{1}H-NMR spectrum, nor by-products on TLC, crude **13** was taken for the next step. A part of the crude product was purified by chromatography (silica gel, hexane/acetone 3:1) for spectroscopic characterizations.

Data of Crude **13**: IR ($CHCl_3$): 3450*w* (NH), 2975*m*, 2930*m*, 2865*w*, 1775*m* (C=O, imide), 1740 (sh), 1710*vs* (C=O, imide), 1685*vs* (C=O, carbamate), 1505*s*, 1470*s*, 1450*m*, 1435*m*, 1420*s*, 1390*s*, 1365*vs*, 1330*m*, 1245*m*, 1170*vs*, 1090*m*, 1025*m*, 910*s*, 890*w*, 865*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): 7.86–7.83 (*m*, 2 arom. H); 7.73–7.70 (*m*, 2 arom. H); 4.65 (br., BocNH); 3.69 (*t*, J = 7.3, $^{15}NCH_2$); 3.21 (m_c, 2 NCH_2); 3.12 (m_c, NCH_2); 1.91 (*quint.*, J = 7.2, $CH_2CH_2{}^{15}N$); 1.55–1.44 (*m*, CCH_2CH_2C); 1.43 (*s*, *t*-Bu); 1.42 (*s*, *t*-Bu). ^{13}C-NMR ($CDCl_3$, 75 MHz); 168.28 (*sd*, $^1J(^{15}N,^{13}C)$ = 12.1, 2 ^{15}NCO); 156.02 (*s*, NHCOO); 155.47 (*s*, NHCOO); 133.95 (*d*, 2 arom. CH); 132.13 (*sd*, $^2J(^{15}N,^{13}C)$ = 7.2, 2 arom. C); 123.23 (*d*, 2 arom. CH); 79.51 (*s*, Me_3C); 46.88, 45.00, 40.41 (3*t*, 3 NCH_2); 35.85 (*td*, $^1J(^{15}N,^{13}C)$ = 9.5, $^{15}NCH_2$); 28.42 (*q*, Me_3C); 27.41, 25.79 (2*t*, 2 CCH_2C). CI-MS: 477 (11, $[M + H]^+$), 378 (22), 377 (100, $[M + H - CO_2 -$ (isobutene)$]^+$), 321 (27, $[M + H - CO_2 - 2$(isobutene)$]^+$).

10. N-{*3-[(4-Aminobutyl)amino]propyl*}[^{15}*N*]*phthalimide* (**14**). A soln. of 1.291 g of crude **13** (2.32 mmol) in $CHCl_3$ was treated with 15 ml of CF_3COOH (*Fluka, puriss.*), stirred for 45 min at r.t. and evaporated to dryness. The residue was dissolved in MeOH (5 ml), treated with 1M aq. HCl soln. (10 ml) and evaporated to dryness. This procedure was repeated two times. The crude residue was crystallized with abs. EtOH (*ca.* 20 ml); 663 mg of **14**·2 HCl (1.90 mmol, 82% with respect to **12**). Colorless crystalline solid. M.p. 212–214°. IR (KBr): 3450*m* (br., NH), 3235*s*, 3195*s*, 2940*vs*, 2850*vs*, 2790*s*, 2740*s*, 2490*w*, 2420*w*, 1965*w*, 1770*s* (C=O, imide), 1705*vs* (C=O, imide), 1610*m*, 1550*m*, 1465*s*, 1455*s*, 1440*s*, 1410*s*, 1390*vs*, 1360*vs*, 1340*s*, 1315*s*, 1250*w*, 1215*w*, 1180*w*, 1150*w*, 1115*s*, 1085*m*, 1040*m*, 1025*m*, 1010*s*, 1005*s*, 955*w*, 920*w*, 895*w*, 880*s*, 855*m*, 795*w*, 765*m*, 750*m*, 720*vs*. ^{1}H-NMR (CD_3OD, 300 MHz): 7.92–7.79 (*m*, 4 arom. H); 3.81 (*td*, J = 6.6, $^2J(^{15}N,^1H)$ = 0.7, $^{15}NCH_2$); 3.14–3.06 (*m*, 2 NCH_2); 3.00 (*t*, J = 7.1, NCH_2); 2.12 (*quint.d*, J = 6.6, $J(^{15}N,^1H)$ = 2.5, $CH_2CH_2{}^{15}N$); 1.89–1.73 (*m*, CCH_2CH_2C). ^{13}C-NMR (CD_3OD, 75 MHz): 169.86 (*sd*, $^1J(^{15}N,^{13}C)$ = 12.9, 2 ^{15}NCO); 135.54 (*d*, 2 arom. CH); 133.33 (*sd*, $^2J(^{15}N,^{13}C)$ = 7.8, 2 arom. C); 124.26 (*d*, 2 arom. CH); 48.30, 46.82, 40.07 (3*t*, 3 NCH_2); 35.81 (*td*, $^1J(^{15}N,^{13}C)$ = 9.9, $^{15}NCH_2$); 26.79, 25.59, 24.30 (3*t*, 3 CCH_2C). ESI-MS: 277 (72, $[M + H]^+$), 260 (100, $[M + H - NH_3]^+$), 189 (39, $[M + H - H_2N(CH_2)_4NH_2]^+$).

11. *(E)-3-{4-[(Methylsulfonyl)oxy]phenyl}-*N*-{{[(E)-3-{4-[(methylsulfonyl)oxy]phenyl}prop-2-enoyl]-amino}butyl}-*N*-(3-[*^{15}*N]phthalimidopropyl)prop-2-enamide* (**15**). At 0° 0.95 ml (690 mg, 6.9 mmol) of Et_3N were added to a soln. of 562 mg (1.61 mmol) of **14**·2 HCl in dry AcOEt (20 ml) and stirred for 30 min. A soln. of 840 mg (3.24 mmol) of (*E*)-3-{4-[(methylsulfonyl)oxy]phenyl}prop-2-enoyl chloride in dry AcOEt (30 ml) was then added dropwise during 15 min. The suspension was then warmed up to r.t., stirred for 2.5 h. The colorless precipitate ($Et_3N \cdot HCl$) was filtered and washed thoroughly with AcOEt. After evaporation to dryness, the residue was purified by chromatography (silica gel, $CHCl_3$/MeOH 40:1): 945 mg of **15** (1.304 mmol, 81%). Colorless, voluminous spongy solid. IR (KBr): 3400*m*, 3290*m* (br., NH), 3025*w*, 2930*m*, 2860*w*, 1770*m* (C=O, imide), 1710*vs* (C=O, imide), 1645*s*, 1600*s*, 1540*m*, 1500*s*, 1430*m*, 1400*s*, 1365*vs*, 1205*s*, 1175*s*, 1150*vs*, 1105*w*, 1030*w*, 1015*m*, 975*s*, 870*vs*, 840*s*, 780*m*, 720*s*. ^{1}H-NMR ($CDCl_3$, 300 MHz, mixture of conformers; characteristic, sometimes doubled signals are given)[7]: 7.85–7.45 (*m*, 10 H); 7.26–7.23 (*m*, 4 arom. H); 6.90–6.74 (*m*, 1 olef. H, 0.5 NH); 6.52–6.38 (*m*, 1 olef. H, 0.5 NH); 3.78–3.72 (*m*, NCH_2); 3.57–3.43 (*m*, 3 NCH_2); 3.16 (*s*, 2 MeS); 2.12–1.97 (*m*, CH_2(3)); 1.78–1.59 (*m*, CH_2(7), CH_2(8)). ^{1}H-NMR (110°, (D_6)DMSO, 300 MHz, assignment with help of H,H-COSY): 7.83–7.80 (*m*, 4 Phth-H); 7.69 (*d*, J = 8.7, 2 CH(15')); 7.67 (br., NH); 7.61 (*d*, J = 8.9, 2 CH(15)); 7.46 (*d*, J = 15.5, CH(13')); 7.42 (*d*, J = 15.8, CH(13)); 7.34 (*d*, J = 8.7, 2 CH(16)); 7.32 (*d*, J = 8.7, 2 CH(16')); 7.00 (*d*, J = 15.4, CH(12')); 6.60 (*d*, J = 15.8, CH(12)); 3.68 (*td*, J = 7.1, $^2J(^{15}N,^1H)$ = 1.1, CH_2(2)); 3.54–3.46 ('*q*', CH_2(4), CH_2(6)); 3.34 (*s*, 2 MeS);

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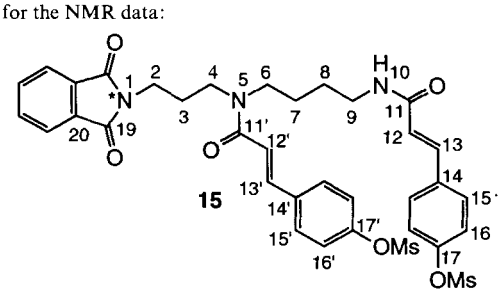
[7]) Atom numbering used for the NMR data:



3.26 (*q*, J = 6.4, CH_2(9)); 2.00 (*quint.d*, J = 7.2, $J(^{15}N,^1H)$ = 2.0, CH_2(3)); 1.65–1.52 (*m*, CH_2(7), CH_2(8)). ^{13}C-NMR ($CDCl_3$, 75 MHz, mixture of conformers; characteristic, sometimes doubled signals are given): 168.39 (*s*, 2 C(19)); 166.13, 165.73 (2*s*, C(11/11′)); 149.67, 149.66, 139.24, 132.05, 131.86 (5*s*, 6 arom. C); 141.34, 138.66, 134.37, 129.38, 129.28, 123.44, 123.26, 122.43, 122.06, 118.42 (10*d*, 12 arom. C, 4 olef. CH); 46.35, 44.71, 39.29 (3*t*, C(4/6/9)); 37.64 (*q*, 2 MeS); 35.39 (*t*, C(2)); 28.79, 27.20, 26.06 (3*t*, C(3/7/8)). ^{13}C-NMR (110°, (D_6)DMSO, 75 MHz): 167.09 (*sd*, $^1J(^{15}N,^{13}C)$ = 12.8, 2 C(19)); 164.79, 164.27 (2*s*, C(11/11′)); 149.10, 149.04 (2*s*, 2 C(17/17′)); 138.52, 136.14 (2*d*, C(13/13′)); 133.85, 133.77 (2*s*, C(14/14′)); 133.46, 128.59, 128.24, 122.14, 121.51, 121.39 (6*d*, 12 arom. CH); 131.20 (*sd*, $^2J(^{15}N,^{13}C)$ = 7.9, 2 C(20)); 123.31, 119.80 (2*d*, C(12/12′)); 45.84, 43.86, 37.95 (3*t*, C(4/6/9)); 37.37 (*q*, 2 MeS); 35.00 (*td*, $^1J(^{15}N,^{13}C)$ = 9.4, C(2)); 26.81, 26.04, 25.45 (3*t*, C(3/7/8)). ESI-MS (+NaI): 747 ($[M + Na]^+$).

12. *(E,E)-N(3-[^{15}N]Aminopropyl)-3,3′-bis(4-hydroxyphenyl)-N,N′-(butane-1,4-diyl)bis[prop-2-enamide]* ([$^{15}N^a$-**1**][8]). To a soln. of 807 mg of **15** (1.113 mmol) in EtOH (60 ml) was added 0.8 ml (16 mmol) of $N_2H_2 \cdot H_2O$ and stirred for 2 h at 40°. The precipitate was filtered, washed with EtOH (10 ml), and the filtrate was treated with a soln. of 2M KOH/EtOH (20 ml). The mixture was stirred for 6 h at r.t., acidified with conc. aq. HCl up to pH 5 (immediate precipitation of KCl) and then filtered. The filtrate was washed with dry EtOH and the combined org. phases evaporated to dryness. The residue was dissolved in warm EtOH (*ca.* 15 ml) and filtered again. The residue was dissolved in MeOH, treated with 3 g of silica gel and evaporated to dryness. The dry silica gel-product mixture was put in a column over 50 g of silica gel, chromatographed ($CHCl_3$/MeOH/25% NH_3 14:5:1), and the free amine $^{15}N^1$-**1** was converted to the HCl salt: 446 mg (0.939 mmol, 84%) of pure [$^{15}N^1$]-**1** · HCl pale yellow spongy solid. IR (KBr): 3400 (sh), 3050*s* (br.), 2940*s*, 1640*s*, 1605v*s*, 1585v*s*, 1515v*s*, 1480*s*, 1460*s*, 1435*s*, 1380*m*, 1275*s*, 1220*s*, 1170*s*, 1105*m*, 1080*m*, 980*m*, 945*w*, 830*s*. ^{1}H-NMR (CD_3OD, 300 MHz, mixture of conformers; characteristic, sometimes doubled signals are given): 7.58 (*d*, J = 15.3, NCOCH=C*H*); 7.51–7.37 (*m*, 5 H); 6.92–6.78 (*m*, 5 H); 6.45 (*d*, J = 15.7, NCOC*H*=CH); 3.69–3.49 (*m*, 2 NCH_2); 3.38 (*t*, J = 6.3, NCH_2); 2.94 (*t*, J = 6.8, NCH_2); 2.01–1.97 (*m*, $CH_2CH_2{}^{15}NH_2$); 1.80–1.65 (*m*, CCH_2CH_2C). ^{1}H-NMR (CD_3OD, 600 MHz, mixture of conformers; characteristic, sometimes doubled signals are given): 7.57 (*d*, J = 15.3, NCOCH=C*H*); 7.45 (*d*, J = 15.7, NCOCH=C*H*); 7.49, 7.38 (2*d*, J = 8.6, 4 C*H*=CHCOH); 6.86 (*d*, J = 15.3, NCOC*H*=CH); 6.80, 6.79 (2*d*, J = 8.6, 4 CH=C*H*COH); 6.44 (*d*, J = 15.7, NCOC*H*=CH); 3.59–3.55 ('*q*', 2 NCH_2); 3.37 (*t*, J = 6.6, $NHCH_2$); 2.92 (*t*, J = 6.8, $^{15}NCH_2$); 1.98 (*quint. d*, J = 6.7, $J(^{15}N,^1H)$ = 2.7, $CH_2CH_2{}^{15}NH_2$); 1.76–1.63 (*m*, CCH_2CH_2C). ^{15}N-NMR (CD_3OD, from ^{1}H,^{15}N-HMQC, 600 MHz; external ^{15}N reference (δ = 0 ppm): CH_3NO_2): −351.54. ^{13}C-NMR (CD_3OD, 75 MHz, mixture of conformers; characteristic, sometimes doubled signals are given): 170.17, 169.59 (2*s*, 2 CO); 166.93, 160.71 (2*s*, 2 C–OH); 145.25, 143.38 (2*d*, 2 NCOCH=*C*H); 131.14, 130.75 (2*d*, 4 arom. CH); 127.75, 127.56 (2*s*, 2 *C*CH=CH); 117.90, 114.21 (2*d*, 2 NCO*C*H=CH); 116.86, 116.81 (2*d*, 4 arom. CH); 48.91, 44.23, 40.06 (3*t*, 3 NCH_2); 38.16 (*td*, $^1J(^{15}N,^{13}C)$ = 4.8, $^{15}NH_2CH_2$); 27.82, 27.76, 26.99 (3*t*, 3 CCH_2C). ESI-MS: 461 (6, $[M + Na]^+$), 439 (100, $[M + H]^+$), 421 (8, $[M + H - {}^{15}NH_3]^+$).

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