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Synthesis of ^{13}C-dilabeled 4-coumaroylspermidines[1]

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Abstract

Five ^{13}C-dilabeled constitution isomers of 4-coumaroylspermidines were prepared in nine to eleven steps: N^1,4-di[(*E*)-4-coumaroyl]-(5,8-$^{13}C_2$)spermidine (**13b**), N^1-[(*E*)-4-coumaroyl]-(5,8-$^{13}C_2$)spermidine (**17b**), N^1,8-di[(*E*)-4-coumaroyl]-(1,4-$^{13}C_2$)spermidine (**20b**), N^4-[(*E*)-4-coumaroyl]-(1,4-$^{13}C_2$)spermidine (**24b**) and N^1,4,8-tri[(*E*)-4-coumaroyl]-(5,8-$^{13}C_2$)spermidine (**26**). The two ^{13}C-atoms were subsequently introduced using labeled potassium cyanide. The synthesis proceeds through stepwise construction of the polyamine backbone including protection and deprotection steps of the amino functions. Based on ^1H-^1H NOE interactions, the preliminary study of their binding reveals that **20b** binds to tRNA in the same way as spermidine does, whereas **24b** and **26** do not show any NOE effects with the tRNA protons.

Keywords: Alkaloids; Labeling; NMR; Polyamines

Introduction

Spermidine and spermine are natural polyamines widely distributed in the cells of numerous living organisms and have been shown to be critical for cell growth, carcinogenesis and neurotransmission [1]. In particular their hydroxycinnamamide derivatives (most often, 4-coumaroyl, caffeoyl, feruloyl and sinapoyl) have been extracted from many species of plants: although the mechanisms involved are not yet totally understood, their role in different processes (flowering, fruit-ripening, plant-senescence...) has been demonstrated [1]. Some of these polyamine conjugates can also be considered as precursors of macrocyclic polyamine alkaloids [1].

Recently, the binding site of spermine on tRNA could be localized by NMR experiments on the dilabeled compound [2]. Such studies aim a further understanding of the biological activity of these ubiquitous molecules and could facilitate the design of efficient analogues for the cancertherapy, for example.

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We propose a synthetical route to five dilabeled 4-coumaroylspermidines in order that their binding site on tRNA could be localized in the same way. From the seven possible 4-coumaroylspermidine derivatives, we just chose five that we found representative and because of the price of the labeling reagent: the trisubstituted **26**, two of the three disubstituted (**13b** and **20b**) and two of the three monosubstituted derivatives (**17b** and **24b**).

As for spermine itself [2], we want to use the carbons of the methylene protons next to the amino groups as the "spies" to follow the interactions with tRNA. The adopted strategy is based on ^{1}H-^{1}H NOE interactions, which would result from the possible spatial proximity of the dilabeled spermidine derivatives and the tRNA protons. We chose to label the carbons 5 and 8 of the spermidine chain, also because this special double labeling should permit further experiments: the di-^{13}C-labeled NMR method has already been used to study chain folding in molecules attached to receptor sites, antibodies, membranes and a host of other biologically important systems. Indeed these experiments are based upon the long-range coupling, ^{3}J, between two ^{13}C-atoms spaced four carbons apart [$^{13}C(1)$-C(2)-C(3)-$^{13}C(4)$] which depends on the dihedral angle about the C(2)-C(3) bond [3].

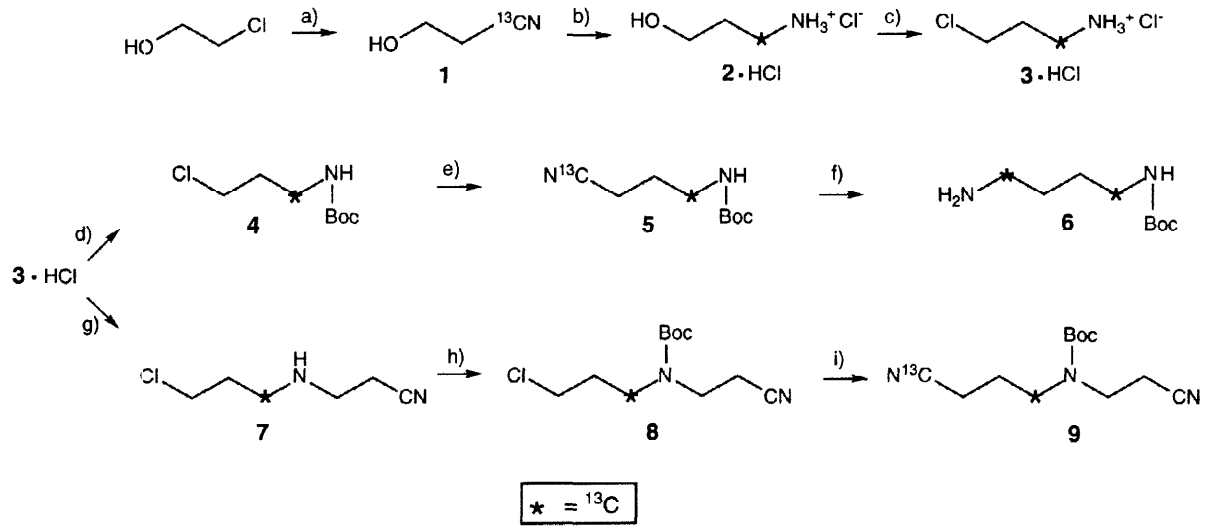
Results and Discussion

The labeling forced us to set up another strategy than the one used for the synthesis [4] of the non-labeled compounds. Depending on the isomer, their preparation starts from the commercial putrescine or spermidine: in our case we would have had to prepare the dilabeled equivalents. Moreover, the published synthesis took advantage of a statistical monoalkylation of the putrescine: this supposes a seven to eight excess of labeled putrescine, which was not conceivable! Finally, starting from 2-chloroethanol, introducing the two ^{13}C-atoms subsequently with labeled potassium cyanide (Scheme 1), we could prepare five derivatives carrying one, two or three 4-coumaroyl groups in nine to eleven steps. The synthesis proceeds through a stepwise construction of the polyamine skeleton including protection and deprotection steps of the amino functions.

As mentioned, the monoprotected putrescine and the spermidine were key intermediates in the synthesis of the non-labeled compounds. We then also chose to prepare the dilabeled analogs. (5,8-$^{13}C_2$)Spermidine (**21**) has already been prepared and studied [5] but the low yield partly due to the method used for its problematic isolation (e. g. paper chromatography) was not satisfactory for a multistep synthesis. Another limitating factor was that the labeling has had to be introduced at the first steps because the basic polyamine backbone should already carry it.

We thus introduced the two ^{13}C-atoms one by one by substitution of an halogen by potassium cyanide. The first labeling was run with a double excess of 2-chloroethanol compared to the quantity of labeled reagent [6]. The reduction of the nitrile **1** catalysed by platinum oxide [7] in methanolic hydrochloric acid afforded the amino alcohol **2** under the form of its hydrochloride which makes it easier to isolate by losing its volatility. The chlorination (of the hydroxy group) was carried out using thionyl chloride, and the chloropropanamine **3** could then again be isolated as its hydrochloride salt. The reverse of those two steps (first chlorination and then hydrogenation) has proved to be impossible as the hydrogenation of the 3-chloropropanenitrile causes hydrogenolysis: loss of the halogen. From this point, we could

obtain two different products: the *N*-protected **4** and the elongated **7** after the Michael addition of acrylonitrile [8]. This latter was treated analogously by protection with the Boc-group to **8**.



a) $K^{13}CN$, EtOH, H_2O, 5 h, reflux, (80%); b) PtO_2, MeOH, H_2O, HCl, H_2, 5 h, 25°C, 4.5 bar, (86%); c) $SOCl_2$, 1 h, 25°C, (83%); d) $(BOC)_2O$, $NaHCO_3$, NaCl, CH_2Cl_2, H_2O, 2 h, 25°C, (88%); e) $K^{13}CN$, NaI, EtOH, H_2O, overnight, reflux, (75%); f) Raney-Ni, 1 N NaOH, EtOH, H_2O, 20 h, 25°C, 40 psi, quantitative; g) CH_2=CHCN, Na, EtOH, 8 h, 25°C, (97%); h) $(BOC)_2O$, CH_2Cl_2, NEt_3, 90%; i) $K^{13}CN$, NaI, EtOH, H_2O, overnight, reflux, (68%). The yields in parentheses refer to the optimized reactions without labeling.

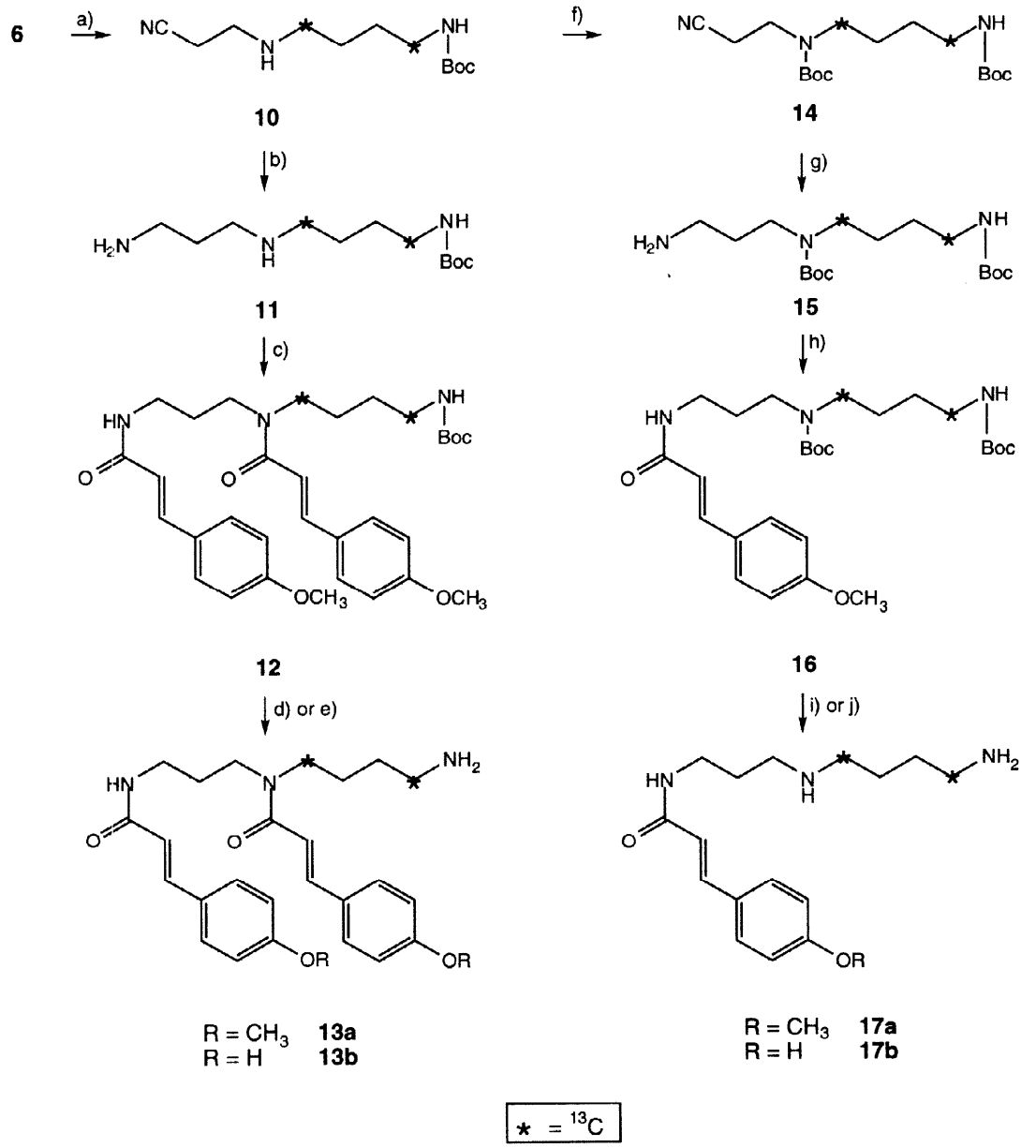
Scheme 1

The second ^{13}C-atom could then be introduced (**5** and **9**) in parallel. Raney nickel was the reagent of choice for hydrogenation of nitrile groups (one or two) in presence of the Boc-group sensitive to acid traces. We prepared on one side the mono-Boc putrescine **6** and on the other a mono-Boc spermidine **18** (Scheme 2).

Again after Michael addition of acrylonitrile on **6** to the nitrile **10**, introduction of an additional protecting group to **14** and hydrogenation to **15** or direct hydrogenation on Raney nickel to **11**, we could get two differently protected spermidine skeletons. One more pattern - a twice protected spermidine **22** after selective reaction of 2-{[(*tert*-butoxy)carbonyl]oxyamino}-2-phenylacetonitrile (BOC-ON) with the primary amino groups [9] - was obtained *via* the spermidine trihydrochloride **21** · 3 HCl (Scheme 3).

The last decisive step was the introduction of the 4-coumaroyl moieties: many methods were compared and tested on the more requiring substrate: the free spermidine because it supposes three amide bond formations at the same time. The published synthesis [4] of the non-labeled compounds makes use of the *N,N'*-dicyclohexylcarbodiimide (DCC) activation: but one can notice that the yield rapidly decreases while the number of 4-coumaroyl rests to be introduced increases. That method could even not be used for the preparation of the trisubstituted product.

The four methods we compared are all based on the same principle of activating the carboxylic function. Of course the free hydroxy group also had to be protected which would unavoidably induce side reactions.

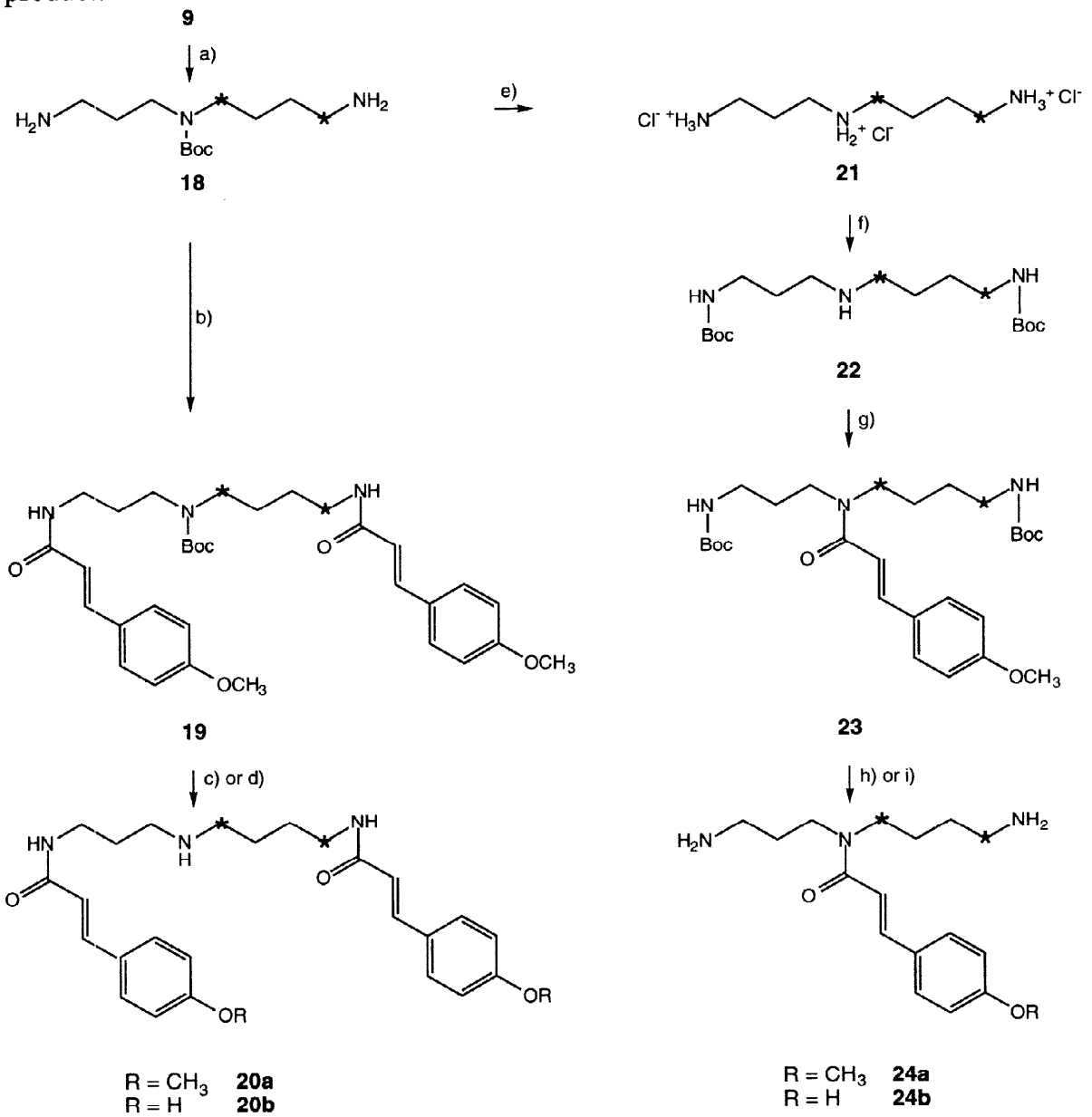


a) CH_2=CHCN, MeOH, 8 h, 25°C, 91%; b) Raney-Ni, 1 N NaOH, EtOH, H_2O, 20 h, 25°C, 40 psi, quantitative; c) (*E*)-3-(4-methoxyphenyl)-prop-2-enoyl chloride, NEt_3, AcOEt, overnight, 25°C, 92%; d) TFA, CH_2Cl_2, 40 min, 25°C, 99%; e) BBr_3, THF, 1 h, -78°C, quantitative; f) $(BOC)_2O$, CH_2Cl_2, 3 h, 25°C, (90%); g) Raney-Ni, 1 N NaOH, EtOH, H_2O, 20 h, 25°C, 40 psi, (86%); h) (*E*)-3-(4-methoxyphenyl)-prop-2-enoyl chloride, NEt_3, AcOEt, overnight, 25°C, 82%; i) TFA,CH_2Cl_2, 40 min, 25°C, 94%; j) BBr_3, THF, 1 h, -78°C, quantitative. The yields in parentheses refer to the optimized reactions without labeling.

Scheme 2

Our first trial which was with the Mukaiyama reagent (2-chloro-1-methyl-pyridinium iodide) [10], only led to the formation of the anhydride of the 4-methoxycinnamic acid and did not reveal any trace-formation of trisubstituted spermidine.

The second reagent was a sultone which previously showed great efficiency for the synthesis of maytenine (the N^1,8-dicinnamoylspermidine) [11]. Initially it was thought that an excess of this reagent could improve the 5 % yield reported for the trisubstituted spermidine side product.



a) Raney-Ni, 1 N NaOH, EtOH, H_2O, 24 h, 25°C, 40 psi, 87%; b) (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride, NEt_3, AcOEt, overnight, 25°C, 97%; c) TFA, CH_2Cl_2, 40 min, 25°C, quantitative; d) BBr_3, THF, 1 h, -78°C, 91%; e) 5 N aqueous HCl, quantitative; f) BOC-ON, THF, 8 h, 25°C, (80%); g) (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride, NEt_3, AcOEt, overnight, 25°C, 96%; h) TFA, CH_2Cl_2, 40 min, 25°C, 94%; i) BBr_3, THF, 1 h, -78°C, quantitative. The yields in parentheses refer to the optimized reactions without labeling.

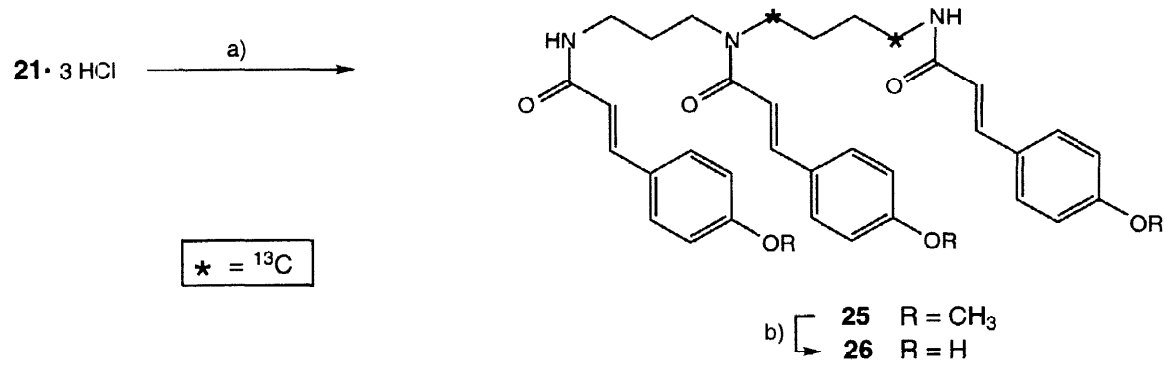
Scheme 3

However, even with an additional equivalent of the acid, only the pure di-4-methoxycinnamoylspermidine could be isolated; but this compound could be prepared more safely *via* the protected intermediate **18**.

An attempt with isobutylchloroformate both to protect the hydroxy and to activate the carboxyl group [12] seemed then to us quite promising as the di-4-coumaroylputrescine could be prepared in that way with high yield. Applied to spermidine we could not isolate any trace of the desired product from the complex resulting mixture.

For the synthesis of the trisubstituted non-labeled compound, *Hu et al.* [4] used the 4-mesyloxycinnamoyl chloride, that they had to prepare in three steps. We preferred to prepare the 4-methoxycinnamoyl chloride from its already commercially available corresponding acid. And finally after reaction in ethyl acetate in presence of triethylamine [13] we isolated 83 % of the expected product.

Among the known methods for dealkylation of arylethers, the cleavage with boron tribromide [14] was particularly well adapted to our case as it permits at the same time the deprotection of the amino groups from the Boc [15].



a) (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride, NEt_3, AcOEt, overnight, 25°C, (83%); b) BBr_3, THF, 1 h, -78°C, quantitative
The yield in parentheses refers to the optimized reactions without labeling.

Scheme 4

We then successively submitted the adequately Boc-protected spermidine (**11**, **15**, **18** and **22**) to amide formation with the acid chloride and afterwards either to trifluoroacetic acid for selective deprotection of the amino groups to obtain the 4-methoxycinnamoyl derivatives **13a**, **17a**, **20a** and **24a** or to BBr_3 for simultaneous deprotection of the hydroxy and the amino functions to the 4-coumaroyl compounds **13b**, **17b**, **20b**, **24b** and **26**. Although it was found that the reaction induces formation of mineral salt (noticeable by weighting the crude product after non-aqueous work-up), we most of the time preferred (based on "NMR- and TLC-purity") not to proceed to any further purification step taking into account the known instability of such free aminophenols and their possible isomerization [16] which could lead to bigger amount of the (*Z*)-derivative. The presence of the latter unnatural isomer could have unwished consequences when measuring the binding to tRNA.

Conclusion

Preliminary data [17] have revealed that **20b** binds to tRNA in the same way as spermidine (**21**) does, whereas **24b** and **26** do not show any NOE effects with the tRNA protons.

Experimental

Unless otherwise stated: all organic solvents were distilled prior to use. For the reactions, THF was dried over Na in presence of benzophenone. Chromatography: silica gel Merck 60 (40-63 μm); TLC on silica gel 60 F_{254}; spray reagents: K_2PtCl_6 (Schlittler reagent) in aqueous HCl for amines (brown, red, blue). $Ce(SO_4)_2$ in H_2SO_4 for amides (yellow). Fluram® reagent in Me_2CO for primary amines (fluorescence at 366 nm). Melting ranges (mp): Mettler FP-5/FP-52. In most cases, the mp of the final compounds could not be determined with accuracy because of their hygroscopicity. IR spectra (neat): Perkin-Elmer 781; data in $1/\lambda$ (cm^{-1}). ^{1}H-NMR: at 273 K and 300 MHz in $CDCl_3$; Bruker AC-300; or Bruker ARX-300, δ in ppm rel. to internal TMS (= 0.00 ppm), *J* in Hz. Coupling patterns are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet) and sym. (symmetrical). "cum" in the signal attribution corresponds invariably to a 4-coumaroyl or a 4-methoxycinnamoyl rest. In the cases of **13a**, **24a** and **24b**, we could simplify the spectra recording them at high temperature in DMSO. This was not possible in the case of **13b** and **26** where degradation of the compounds occurs. ^{13}C-NMR: at 75.6 MHz (Bruker ARX-300); δ in ppm rel. to $CDCl_3$ (= 77.0 ppm); multiplicities from DEPT (**d**istortionless **e**nhancement by **p**olarization **t**ransfer) experiments. $J^{13}_{C\text{-}C}$ refers to the coupling constant between a ^{13}C-labeled carbon atom and the isotopic fraction of a neighbouring ^{12}C atom. CI-MS (chemical ionization mass spectrometry) with NH_3 as the reactant gas; Finnigan SSQ 700 or Varian MAT 90; ESI-MS (electrospray ionization mass spectrometry): Finnigan TSQ 700; data in *m/z*. The (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride was prepared in 97% yield by reaction of 1 equivalent of oxalyl chloride in toluene with the commercially available corresponding acid.

3-Hydroxy-(1-^{13}C)propane-1-nitrile (1) [6]. A solution of K^{13}CN (2.04 g, 30.8 mmol) in water (5 mL) was added dropwise to a boiling solution of 2-chloroethanol (5.04 g, 4.19 mL, 62.6 mmol) and 10% mol NaI in 95% ethanol (30 mL). The mixture was heated at reflux for 5 h, and examined for completion. (A drop of the solution was tested with a 1 M solution of 4-nitrobenzaldehyde in DMSO. If a red-violet color is observed, there is unreacted CN^- ion left in the reaction, and reflux should be continued until a negative color test is observed.) The reaction mixture was evaporated, and to the resulting residue was added a 9:1 mixture of dichloromethane/ethanol. After filtration of NaCl, the solution was evaporated to dryness to get a red oil (1.97 g of crude **1**) which was used in the next step without further purification. IR: 3600, 3430, 2195, 1055. ^{1}H-NMR: 3.88 (*q*, *J* = 6.2, CH_2OH), 2.74 (*s* br., O*H*), 2.61 (*dt*, *J* = 9.6, 6.2, CH_2^{13}CN). ^{13}C-NMR: 117.1 (*s*, ^{13}*C*N). CI-MS: 90.1 (100, $[M + NH_4]^+$), 73.1 (12, $[M + 1]^+$).

3-Chloro-(1-^{13}C)propane-1-amine (3). A glass Parr pressure flask was charged with **1** (1.97 g, 27.3 mmol), platinum oxide catalyst (0.20 g, 0.88 mmol) and 32% aqueous HCl (7.1 mL) in

a 9:1 mixture of methanol/water (400 mL). The flask was placed under a hydrogen pressure of 4.5 bar and shaken 5 h at room temperature. The catalyst was filtered off through Celite® and the solvent was evaporated under reduced pressure yielding 2.65 g of a yellow oil [crude 3-amino-(3-^{13}C)propan-1-ol (**2** · HCl)]. Without further purification, at 25°C $SOCl_2$ (11.8 g, 7.20 mL, 99.0 mmol) was dropwise added to **2** · HCl. The reaction is very exothermic and after cooling down to room temperature the excess of reagent was evaporated *in vacuo* to give 2.54 g of crude **3** · HCl. *Rf* 0.15 [chloroform/methanol/ammonia water 25% 90:10:0.5]. ^{1}H-NMR (CD_3OD): 3.70 (*m q like*, CH_2Cl), 3.12 (*dt*, *J* = 143.1, 7.3, $^{13}CH_2NH_2$), 2.13 (*m* sym., $^{13}CH_2CH_2$). ^{13}C-NMR (CD_3OD): 36.9 (*t*, $^{13}CH_2$). CI-MS: 97.1 (30, [M(^{37}Cl) - HCl + 1]$^+$), 95.1 (100, [M(^{35}Cl) - HCl + 1]$^+$).

***tert*-Butyl *N*-[3-Chloro-(1-^{13}C)propyl]carbamate (4)** [18]. The hydrochloride of **3** (0.82 g, 6.24 mmol) was suspended in dichloromethane (12.5 mL), and an aqueous solution of $NaHCO_3$ (0.52 g, 6.24 mmol in 9.4 mL water) and NaCl (1.25 g, 21.4 mmol) was added at 0°C. After short stirring a solution of di(*tert*-butyl)dicarbonate (1.36 g, 6.24 mmol) in dichloromethane (3.1 mL) was added dropwise, and the mixture was stirred overnight at 25°C. It was then extracted with dichloromethane (3 x 15 mL). The organic phases were dried (Na_2SO_4) and evaporated to yield 1.31 g of a brown oil (crude **4**) which was used in the next step without further purification. *Rf* 0.80 [chloroform/methanol/ammonia water 25% 90:10:0.5]. IR: 3370, 3020, 1845, 1745, 1527, 1400, 1195, 1158, 1110, 867. ^{1}H-NMR: 4.67 (*s* br., N*H*Boc), 3.59 (*m* sym., CH_2Cl), 3.28 (*dq*, *J* = 138.5, 6.4, $^{13}CH_2NHBoc$), 1.97 (*m* sym., $^{13}CH_2CH_2$), 1.45 (*s*, *t*-Bu). ^{13}C-NMR: 37.9 (*t*, $^{13}CH_2$), 28.3 (*q*, $C(CH_3)_3$). CI-MS: 389.2 (8, [2 M + 1]$^+$), 236.2 (25), 214.2 (31, [M(^{37}Cl) + NH_4]$^+$), 212.2 (100, [M(^{35}Cl) + NH_4]$^+$), 197.2 (6, [M(^{37}Cl) + 1]$^+$), 195.2 (21, [M(^{35}Cl) + 1]$^+$), 158.1 (24, [M(^{37}Cl) - (isobutene) + NH_4]$^+$), 156.1 (79, [M(^{35}Cl) - (isobutene) + NH_4]$^+$).

{*tert*-Butyl *N*-[(3-^{13}C)Cyano](1-^{13}C)propyl}carbamate (5). A solution of K^{13}CN (0.44 g, 6.64 mmol) in water (1.3 mL) was added dropwise to a boiling solution of **4** (1.29 g, 6.64 mmol) and 10% mol NaI in 95% ethanol (6.6 mL). The mixture was heated at reflux overnight, and examined for completion (with a 1 M solution of 4-nitrobenzaldehyde in DMSO as above). The reaction mixture was evaporated to dryness. Water (50 mL) was added and the aqueous phase extracted with chloroform (3 x 50 mL). The organic phase was dried (Na_2SO_4) and evaporated. The residue (0.66 g) was passed through a column of silicagel eluted with a 8:2 diethylether/pentane mixture to yield **5** (0.28 g, 23%) of a very viscous slightly yellow liquid which solidifies at 0°C; mp 34.6-35.0°C. *Rf* 0.69 [chloroform/methanol/ammonia water 25% 90:10:0.5]. IR: 3005, 2390, 1710, 1200, 715, 662. ^{1}H-NMR: 4.65 (*s* br., N*H*Boc), 3.26 (*dq*, *J* = 138.3, 6.4, $^{13}CH_2NHBoc$), 2.45-2.31 (*m*, $CH_2{}^{13}CN$), 1.87 (*m* sym., $^{13}CH_2CH_2$), 1.45 (*s*, *t*-Bu). ^{13}C-NMR: 119.1 (*s*, ^{13}CN), 39.2 (*t*, $^{13}CH_2$), 28.3 (*q*, $C(CH_3)_3$). CI-MS: 204.2 (100, [M + NH_4]$^+$), 187.2 (76, [M + 1]$^+$).

{*tert*-Butyl *N*-[4-Amino-(1,4-$^{13}C_2$)butyl]}carbamate (6). At 25°C, to a 1 M solution of NaOH (1.19 g, 29.8 mmol) in 95% ethanol (30 mL) was added **5** (0.26 g, 1.38 mmol) solved in abs. ethanol (2.1 mL), and afterwards Raney-Ni (0.51 g). The mixture was continuously shaken at 25°C overnight under hydrogen (40 psi). After filtration through Celite®, the solution was

concentrated *in vacuo* to *ca.* 10 mL and diluted with water (60 mL). Extraction with chloroform (3 x 100 mL) followed by drying (Na_2SO_4) and evaporation gave **6** (0.28 g, quantitative) as a slightly yellow liquid. R_f 0.42 [chloroform/methanol/ammonia water 25% 78:19:3]. IR: 3450, 2970, 2930, 2860, 1710, 1495, 1365, 1165, 858. ^{1}H-NMR: 4.65 (*s* br., N*H*Boc), 3.18-3.06 (*dm*, J = 141.1, $^{13}CH_2$NHBoc), 2.71 (*dt*, J = 133.6, 6.8, $^{13}CH_2NH_2$), 1.57-1.41 [*m*, 15 H; incl. 1.44 (*s*, *t*-Bu), 1.40 (*s* br., NH_2)]. ^{13}C-NMR: 41.7 (*dt*, $J^{13}_{C,^{13}C}$ = 4.2, $^{13}CH_2$), 39.3 (*t*, CH_2), 28.3 (*q*, C(CH_3)$_3$). CI-MS: 381.4 (26, [2 M + 1]$^+$), 219.3 (23), 191.2 (100, [M + 1]$^+$), 135.1 (5, [M + 1 - (isobutene)]$^+$).

3-Amino-*N*-[(1-^{13}C)-3-chloropropyl]-propanenitrile (7). Under a nitrogen atmosphere at 0°C, **3** · HCl (1.68 g, 12.8 mmol) in ethanol (1.3 mL) was added to *in situ* prepared sodium ethanolate [0.30 g, 12.8 mmol of Na in ethanol (9.1 mL)]. After stirring, acrylonitrile (0.95 g, 1.18 mL, 18.1 mmol) in ethanol (1.3 mL) was added dropwise at 25°C and the mixture stirred overnight. Crude **7** (1.37 g) was obtained after evaporation *in vacuo* as a brown oil and used in the next step without further purification. R_f 0.58 [chloroform/methanol/ammonia water 25% 90:10:0.5]. IR: 2950, 2830, 2244, 1635, 1455, 1120. ^{1}H-NMR: 3.63 (*m* sym., CH_2Cl), 2.95 (*m* sym., CH_2NH), 2.81 (*dt*, J = 134.0, 6.6, $^{13}CH_2$NH), 2.53 (*m*, CH_2CN), 1.93 (*m* sym., $^{13}CH_2CH_2$), 1.69 (*s* br., N*H*). ^{13}C-NMR: 45.8 (*t*, $^{13}CH_2$). CI-MS: 150.1 (31, [M(^{37}Cl) + 1]$^+$), 148.1 (100, [M(^{35}Cl) + 1]$^+$).

{*tert*-Butyl *N*-[3-Chloro-(1-^{13}C)propyl]-*N*-(2-cyanoethyl)}carbamate (8). At 0°C a solution of $(Boc)_2O$ (2.0 g, 9.2 mmol) in dichloromethane (7.7 mL) was added within 10 min to a solution of **7** (1.35 g, 9.16 mmol) in dichloromethane (3.9 mL) with Et_3N (1.11 g, 1.53 mL, 11.0 mmol). The mixture was stirred overnight at 25°C. After addition of water (10 mL), extraction with chloroform (3 x 10 mL), drying (Na_2SO_4) and evaporation **8** (2.03 g, 90%) was obtained and used in the next step without further purification. R_f 0.71 [chloroform/methanol/ammonia water 25% 90:10:0.5]. IR: 2970, 2223, 1685, 1465, 1410, 1365, 1285, 1155, 1119. ^{1}H-NMR: 3.56 (*q*, J = 6.0, CH_2NBoc), 3.50 (*td*, J = 6.6, 3.5, CH_2Cl), 3.44 (*dt*, J = 138.0, 6.8, $^{13}CH_2$NBoc), 2.63 (*m* br., CH_2CN), 2.03 (*m* sym., $^{13}CH_2CH_2$), 1.48 (*s*, *t*-Bu). ^{13}C-NMR: 49.7, 47.1, 46.0, 45.6 (4 *t*, 4 CH_2), 28.2 (*q*, C(CH_3)$_3$). CI-MS: 267.2 (32, [M(^{37}Cl) + NH_4]$^+$), 265.2 (100, [M(^{35}Cl) + NH_4]$^+$), 250.2 (24, [M(^{37}Cl) + 1]$^+$), 248.2 (76, [M(^{35}Cl) + 1]$^+$), 211.1 (6, [M(^{37}Cl) - (isobutene) + NH_4]$^+$), 209.1 (19, [M(^{35}Cl) - (isobutene) + NH_4]$^+$), 194.1 (< 5, [M(^{37}Cl) - (isobutene) + 1]$^+$), 192.1 (11, [M(^{35}Cl) - (isobutene) + 1]$^+$), 173.2 (26), 168.2 (5), 148.1 (6, [M - Boc + 1]$^+$).

{*tert*-Butyl *N*-(2-Cyanoethyl)-*N*-[3-(^{13}C)cyano-(1-^{13}C)propyl]}carbamate (9). According to the procedure for **5**, starting from **8** (2.0 g, 8.1 mmol), K^{13}CN (0.64 g, 9.70 mmol) in water (1.6 mL) and 10% mol. NaI in 95% ethanol (8.1 mL), there was obtained **9** (0.71 g, 37%) as a yellowish oil after a column on silicagel eluted with a 3:2 diethyl ether/pentane mixture. R_f 0.33 [diethyl ether/pentane 4:1]. IR: 2970, 2245, 2190, 1690, 1465, 1410, 1367, 1160, 1125. ^{1}H-NMR: 3.48 (*dt*, J = 6.4, 3.6, CH_2NBoc), 3.43 (*dt*, J = 138.3, 6.9, $^{13}CH_2$NBoc), 2.64 (*m* br., CH_2CN), 2.39 (*m* sym., $CH_2{}^{13}$CN), 1.93 (*m* sym., $^{13}CH_2CH_2$), 1.49 (*s*, *t*-Bu). ^{13}C-NMR: 118.9 (*s*, ^{13}CN), 115.9 (*s*, CN), 81.3 (*s*, C(CH_3)$_3$), 47.2, 46.8 (2 *t*, $^{13}CH_2$), 28.2 (*q*, C(CH_3)$_3$), 14.6

(*dt*, $J^{13}_{C\text{-}C}$ = 56.6, CH_2). CI-MS: 258.3 (12, $[M + NH_4]^+$), 257.3 (100, $[M + NH_3]^+$), 240.3 (66, $[M + 1]^+$), 201.2 (18, [M - (isobutene) + $NH_3]^+$).

{*tert*-Butyl *N*-{4-[(2-Cyanoethyl)amino]-(1,4-$^{13}C_2$)butyl}}carbamate (10). At 25°C to a solution of **6** (0.25 g, 1.32 mmol) in abs. methanol (3.8 mL) acrylonitrile (0.01 g, 0.12 mL, 1.86 mmol) in 8.1 mL abs. methanol was added within 30 min. After overnight stirring, the mixture was evaporated to yield **10** (0.29 g, 91 %) as a yellowish oil used in the next step without further purification. R_f 0.84 [chloroform/methanol/ammonia water 25% 78:19:3]. IR: 3450, 2250, 1705, 1500, 1390, 1365, 1165. ^{1}H-NMR: 4.71 (*s* br., N*H*Boc), 3.1 (*dm*, *J* = 132.7, $^{13}CH_2$NHBoc), 2.98-2.80 (*m*, CH_2NH), 2.67 (*dt*, *J* = 139.7, 6.7, $^{13}CH_2$NH), 2.52 (*t*, *J* = 6.7, CH_2CN), 1.60-1.47 (*m*, 5 H), 1.44 (*s*, *t*-Bu). ^{13}C-NMR: 52.8, 48.6, 40.3 (3 *t*, $^{13}CH_2$), 28.3 (*q*, C(CH_3)$_3$). CI-MS: 244.3 (100, $[M + 1]^+$), 188.2 (28, [M - (isobutene) + 1]$^+$), 144.2 (12, [M - Boc + 1]$^+$).

{*tert*-Butyl *N*-{4-[(3-Aminopropyl)amino]-(1,4-$^{13}C_2$)butyl}}carbamate (11). According to the procedure for **6**, starting from **10** (0.15 g, 0.60 mmol) in abs. ethanol (0.9 mL), NaOH (1.19 g, 29.8 mmol) in 95% ethanol (30 mL), Raney-Ni (0.50 g), there was obtained **11** (0.17 g, quantitative) as a colorless oil. IR: 3450, 2930, 1707, 1500, 1365, 1163. ^{1}H-NMR: 4.90 (*s* br., N*H*Boc), 3.1 (*dm*, J = 139.6, $^{13}CH_2$NHBoc), 2.77 (*quint.*, *J* = 6.8, CH_2NH_2), 2.7 (*dm*, *J* = 138.1, $^{13}CH_2$NH), 2.67 (*td*, *J* = 7.1, 3.3, CH_2NH), 1.64 (*quint.*, *J* = 6.9, $CH_2CH_2CH_2$), 1.52 (*m* sym., 4 H), 1.44 (*s*, *t*-Bu), 1.34 (*s* br., 3 N*H*). ^{13}C-NMR: 55.3, 53.9 (2 *t*, CH_2), 49.7 (*dt*, $J^{13}_{C\text{-}^{13}C}$= 3.9, $^{13}CH_2$), 40.5 (*t*, $^{13}CH_2$), 28.5 (*q*, C(CH_3)$_3$). CI-MS: 248.3 ([M + 1]$^+$).

***tert*-Butyl *N*-{5-[(*E*)-3-(4-Methoxyphenyl)prop-2-enoyl]-8-{[(*E*)-3-(4-methoxyphenyl)prop-2-enoyl]amino}-5-[(1,4-$^{13}C_2$)azaoctyl]}carbamate (12).** At 0°C, under a nitrogen atmosphere, a solution of (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride (0.24 g, 1.22 mmol) in anhydrous ethyl acetate (10 mL) was added dropwise during 30 min to a solution of **11** (0.15 g, 0.61 mmol) and Et_3N (0.14 g, 1.37 mmol) in anhydrous ethyl acetate (5 mL) and stirred for 30 min. The suspension was then warmed up to room temperature, and stirred overnight protected from light. The mixture was evaporated *in vacuo*. Water (50 mL) was added and the residue was extracted with chloroform (3 x 50 mL) and dried (Na_2SO_4). After evaporation to dryness of the organic phase, the residue (0.32 g) was purified by chromatography (silica gel, chloroform/methanol 20:1): **12** (0.19 g, 54%) was isolated as a colorless voluminous spongy solid. IR: 3450, 2987, 2933, 1708, 1662, 1643, 1604, 1508, 1172, 1030, 980, 825. ^{1}H-NMR: 7.70 (*d*, *J* = 15.3, 1 olef. H), 7.57 (*d*, *J* = 15.6, 1 olef. H), 7.50 (*d*, *J* = 8.7, 2 arom. H), 7.46 (*d*, *J* = 8.8, 2 arom. H), 7.24 (*m* br.; N*H*cum), 6.91 (*d*, *J* = 8.7, 2 arom. H), 6.86 (*d*, *J* = 8.8, 2 arom. H), 6.72 (*d*, *J* = 15.3, 1 olef. H), 6.38 (*d*, *J* = 15.6, 1 olef. H), 4.62 (*s* br., N*H*Boc), 3.84 (*s*, OC*H*$_3$), 3.82 (*s*, OC*H*$_3$), 3.71-3.13 [*m*, 14 H; incl. 3.42 (*d*(*m t* like), *J* = 136.2, $^{13}CH_2$Ncum), 3.2 (*d*(*m q* like), *J* = 137.7, $^{13}CH_2$NHBoc)], 1.85-1.45 (*m*, 6 H), 1.43 (*s*, *t*-Bu). ^{13}C-NMR: 129.4, 129.2, 114.2, 114.1 (4 *d*, arom. *C*), 55.2 (*q*, OCH_3), 47.3 (*dt*, $J^{13}_{C\text{-}^{13}C}$= 4.1, $^{13}CH_2$), 46.1 (*t*, CH_2), 39.8 (*t*, $^{13}CH_2$), 28.3 (*q*, C(CH_3)$_3$). ESI-MS: 568.4 (67, $[M + 1]^+$), 512.4 (13, [M - (isobutene) + 1]$^+$), 468.3 (100, [M - Boc + 1]$^+$).

N^1,4-Di[(*E*)-4-methoxycinnamoyl]-(5,8-$^{13}C_2$)spermidine (= (*E*)-*N*-{4-[Amino-(1,4-$^{13}C_2$) butyl]-3,3'-bis(4-methoxyphenyl)-*N*,*N'*-(propane-1,3-diyl)}bis[prop-2-enamide]) (13a). A solution of **12** (90.8 mg, 0.16 mmol) in dichloromethane (10.4 mL) was treated with CF_3COOH (3.07 g, 26.9 mmol) and stirred for 40 min at 25°C under nitrogen. After evaporation to dryness, the residue was taken up in methanol (1.5 mL) and treated with 1 M aqueous HCl (2.5 mL). After evaporation to dryness, the acidic treatment was repeated twice. The residue was solved finally five times in ethanol (2 mL) and evaporated. After high vacuum drying **13a** · HCl (79.9 mg, 99%) was obtained as a yellowish solid. R_f 0.57 [chloroform/methanol/ammonia water 25% 78:19:3]. IR (KBr): 1645, 1635, 1605, 1515, 1255, 1175. ^{1}H-NMR (DMSO, 373 K): 7.88 (*s* br., NH_2), 7.56 (*d*, *J* = 8.5, 2 arom. H), 7.51-7.31 [*m*, 4 H; incl. 7.47 (*d*, *J* = 8.5, 2 arom. H)], 7.00-6.81 [*m*, 5 H; incl. 6.96 (*d*, *J* = 8.5, 2 arom. H)], 6.60-6.44 [*m*; incl. 6.51 (*d*, *J* = 15.8, 1 olef. H)], 3.82 (*s*, OCH_3), 3.78 (*s*, OCH_3), 3.46 (*d*(*m t* like), *J* = 135.8, $^{13}CH_2$Ncum), 3.61-3.44 (*m*, CH_2Ncum), 2.87 (*d*(*m t* like), *J* = 142.1, $^{13}CH_2NH_2$), 1.91-1.51 [*m*, 6 H; incl. 1.84 (q*uint.*, *J* = 7.0, $CH_2CH_2CH_2$)]. ^{13}C-NMR (CD_3OD): 130.7, 130.5 (2 *d*, 2 arom. *C*), 115.3, 55.8 (*q*, OCH_3), 48.8, 48.7 (2 *t*, CH_2), 46.9 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.4, $^{13}CH_2$), 40.3 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.4, $^{13}CH_2$). ESI-MS: 468.4 ([M + 1]$^+$).

N^1,4-Di[(*E*)-4-coumaroyl]-(5,8-$^{13}C_2$)spermidine (= (*E*)-*N*-{4-[Amino-(1,4-$^{13}C_2$)butyl]-3,3'-bis(4-hydroxyphenyl)-*N*,*N'*-(propane-1,3-diyl)}bis[prop-2-enamide]) (13b). Under a nitrogen atmosphere, a solution of **12** (90.8 mg, 0.16 mmol) in dichloromethane (2.4 mL) was cooled in a dry ice/acetone bath (-78°C) to which BBr3 (1 M in dichloromethane) (1.22 mL) was introduced and the mixture stirred for 30 min. The bath was then removed and the mixture was further stirred for 2 h. It was then evaporated *in vacuo* and the residue was solved in methanol (1 mL) and treated with 1 M aqueous HCl (2 mL). After evaporation to dryness, the acidic treatment was repeated twice. The residue was solved finally five times in ethanol (2 mL) and evaporated. After high vacuum drying **13b** · HCl (80.9 mg, quantitative) was obtained as a brown solid. R_f 0.17 [chloroform/methanol/ammonia water 25% 78:19:3]. IR (KBr): 1645, 1635, 1605, 1515, 1445, 1268, 1218, 1208, 1175, 975, 825. ^{1}H-NMR (CD_3OD): 7.63-7.38 (*m*, 6 H), 6.91-6.70 (*m*, 5 H), 6.45 (*d*, *J* = 15.7, 1 olef. H), 3.93-3.17 (*m*, incl. MeOH), 2.81-2.70 (*m*, 1 H), 2.02-1.60 (*m*, 2 + 4 H). ^{13}C-NMR (CD_3OD): 116.7 (*d*, arom. *C*), 48.8 (*t*, CH_2), 46.9 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.8, $^{13}CH_2$), 40.4 (*t*, CH_2). ESI-MS: 440.4 ([M - HCl + 1]$^+$).

Di(*tert*-butyl) *N*-(2-Cyanoethyl)-*N*,*N'*-[(1,4-$^{13}C_2$)butane-1,4-diyl]bis[carbamate] (14). At 25°C a solution of di(*tert*-butyl)dicarbonate (0.11 g, 0.50 mmol) in dichloromethane (3.7 mL) was dropwise added to a solution of **10** (0.12 g, 0.50 mmol) in dichloromethane (4.9 mL). The mixture was then stirred overnight at room temperature, the solvent evaporated to dryness to yield 0.18 g of a yellowish oil (crude **14**). R_f 0.91 [chloroform/methanol/ammonia water 25% 78:19:3]. IR: 3455, 2980, 2935, 2250, 1813, 1695, 1504, 1415, 1368, 1165, 1120, 1075. ^{1}H-NMR: 4.57 (*s* br., N*H*Boc), 3.55-3.42 (*m*, CH_2NBoc), 3.3 (*d* (*m t* like), *J* = 137.3, 2 H), 3.2 (*d* (*m q* like), *J* = 137.9, 2 H), 2.62 (*s* br., CH_2CN), 1.72-1.39 [*m*, 22 H; incl. 1.47, 1.44 (2 *s*, 2 *t*-Bu)]. ^{13}C-NMR: 52.7, 51.5, 48.3, 47.3, 40.1, 39.6 (6 *t*, CH_2), 28.4 (*q*, C(CH_3)$_3$). CI-MS: 361.4 (23, [M + NH_4]$^+$), 344.4 (100, [M + 1]$^+$), 288.3 (21, [M - (isobutene) + 1]$^+$), 244.3 (12, [M - Boc + 1]$^+$), 188.2 (20, [M - Boc - (isobutene) + 1]$^+$), 144.2 (12, [M - 2 Boc + 1]$^+$).

Di(*tert*-butyl) *N*-{3-{[(*E*)-3-(4-Methoxyphenyl)prop-2-enoyl]amino}propyl}-*N*,*N*'-[(1,4-$^{13}C_2$)butane-1,4-diyl]bis[carbamate] (16). According to the procedure for **6**, starting from **14** (0.16 g, 0.47 mmol) in abs. ethanol (0.7 mL), NaOH (1.21 g, 30.3 mmol) in 95% ethanol (30 mL) and Raney-Ni (0.50 g), there was obtained 0.13 g of a colorless oil [crude di(*tert*-butyl) *N*-(3-aminopropyl)-*N*,*N*'-[(1,4-$^{13}C_2$)butane-1,4-diyl]bis[carbamate] (**15**)]. Then according to the procedure for **12**, starting from the crude **15**, (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride (75 mg, 0.38 mmol) in anhydrous ethyl acetate (3.8 mL) and Et_3N (43 mg, 0.43 mmol) in anhydrous ethyl acetate (3 mL), there was obtained **16** (0.16 g, 82%) as a colorless voluminous spongy solid after chromatography (silica gel, chloroform/methanol 20:1). IR: 3450, 3360, 2980, 2936, 1705, 1663, 1604, 1509, 1417, 1365, 1250, 1170, 1030, 978, 825. ^{1}H-NMR: 7.57 (*d*, *J* = 15.6, 1 olef. H), 7.46 (*d*, *J* = 8.7, 2 arom. H), 6.98 (*s* br.; N*H*cum), 6.88 (*d*, *J* = 8.7, 2 arom. H), 6.33 (*d*, *J* = 15.6, 1 olef. H), 4.62 (*s* br., N*H*Boc), 3.82 (*s*, OC*H*$_3$), 3.34 (*m* sym., 4 H), 3.13 (*d m* sym., J = 127.4, 2 ^{13}C*H*$_2$), 1.72 (*m* sym., $CH_2CH_2CH_2$), 1.63-1.36 [*m*, 22 H; incl. 1.47, 1.44 (2 *s*, 2 *t*-Bu)]. ^{13}C-NMR: 129.2 (*d*, arom. *C*), 114.1 (d, *C*H=CHCON), 55.2 (*q*, O*C*H$_3$), 46.5 (*dt*, $J^{13}_{C,^{13}C}$ = 3.8, ^{13}*C*H$_2$), 40.0 (*t*, ^{13}*C*H$_2$), 28.4 (*q*, C(*C*H$_3$)$_3$). ESI-MS: 508.4 (29, [M + 1]$^+$), 452.3 (10, [M - (isobutene) + 1]$^+$), 408.4 (100, [M - Boc + 1]$^+$), 352.2 (31, [M - Boc - (isobutene) + 1]$^+$), 308.3 (13, [M - 2 Boc + 1]$^+$).

***N*^{1}-[(*E*)-4-Methoxycinnamoyl]-(5,8-$^{13}C_2$)spermidine (= (*E*)-*N*-{4-{[Amino-(1,4-$^{13}C_2$)butyl]amino}propyl}-3-(4-methoxyphenyl)-prop-2-enamide (17a).** According to the procedure for **13a**, starting from **16** (79.2 mg, 0.16 mmol) in dichloromethane (10.2 mL) and CF_3COOH (2.99 g, 26.2 mmol) and treated three times with 1 M aqueous HCl (2.5 mL) in methanol (1.7 mL), there was obtained **17a** · 2 HCl (55.9 mg, 94 %) as a white solid after high vacuum drying; mp 266.1-268.4°C. *R*f 0.33 [chloroform/methanol/ammonia water 25% 7:3:1]. IR (KBr): 3420, 2520, 2415, 1655, 1610, 1538, 1515, 1465, 1255, 1230, 1175, 1025, 970, 825. ^{1}H-NMR (CD_3OD): 7.57-7.45 (*m*, 3 H), 6.99-6.91 (*m*, 2 H), 6.50 (*d*, *J* = 15.8, 1 olef. H), 3.83 (*s*, OC*H*$_3$), 3.50-3.41 (*m t* like, C*H*$_2$NHcum), 3.11-3.02 (*m t* like, C*H*$_2$NH), 3.1 (*dm*, *J* = 142.4, 2 ^{13}C*H*$_2$), 2.05-1.72 (*m*, 2 + 4 H). ^{13}C-NMR (CD_3OD): 130.5 (*d*, arom. *C*), 115.4, 48.2, 48.1 (2 *t*, ^{13}*C*H$_2$), 40.0 (2 *t*, ^{13}*C*H$_2$). ESI-MS: 308.3 ([M - 2 HCl + 1]$^+$).

***N*^{1}-[(*E*)-4-Coumaroyl]-(5,8-$^{13}C_2$)spermidine (= (*E*)-*N*-{4-{[Amino-(1,4-$^{13}C_2$)butyl]amino}propyl}-3-(4-hydroxyphenyl)-prop-2-enamide (17b).** According to the procedure for **13b**, starting from **16** (79.2 mg, 0.16 mmol) in dichloromethane (1.7 mL), BBr_3 (1 M in dichloromethane) (0.83 mL) and treated three times with 1 M aqueous HCl (2 mL) in methanol (1 mL), there was obtained **17b** · 2 HCl (63.4 mg, quantitative) as a brown solid after high vacuum drying. *R*f 0.20 [chloroform/methanol/ammonia water 25% 7:3:1]. IR (KBr): 1655, 1605, 1580, 1535, 1515, 1470, 1435, 1270, 1218, 1170, 835. ^{1}H-NMR (CD_3OD): 7.51 (*d*, *J* = 15.8, 1 olef. H), 7.42 (*d*, *J* = 8.6, 2 arom. H), 6.80 (*d*, *J* = 8.6, 2 arom. H), 6.45 (*d*, *J* = 15.8, 1 olef. H), 3.51-3.41 (*m t* like, C*H*$_2$NHcum), 3.06 (*dm*, *J* = 143.4, 2 ^{13}C*H*$_2$), 3.12-3.01 (*m t* like, C*H*$_2$NH), 2.05-1.72 (*m*, 2 + 4 H). ^{13}C-NMR (CD_3OD): 48.6 (*t*, *C*H$_2$), 48.2, 48.1 (2 *t*, ^{13}*C*H$_2$), 40.2, 40.1 (2 *t*, ^{13}*C*H$_2$). ESI-MS: 294.4 ([M - 2 HCl + 1]$^+$).

***tert*-Butyl *N*-[4-Amino-(1,4-$^{13}C_2$)butyl]-*N*-(3-aminopropyl)carbamate (18).** According to the procedure for **6**, starting from **9** (0.68 g, 2.85 mmol) in abs. ethanol (8.6 mL), NaOH (2.74

g, 68.5 mmol) in 95% ethanol (68 mL) and Raney-Ni (0.57 g), there was obtained **18** (0.62 g, 87 %) as a colorless oil. IR: 3008, 2930, 1675, 1415, 1367, 1200, 1161, 715, 662. ^{1}H-NMR: 3.23 (*s* br., C*H*$_2$NBoc), 3.15 (*d* (*m* br.), J = 132.1, ^{13}C*H*$_2$NBoc), 2.71 (*dt*, *J* = 133.6, 7.0, ^{13}C*H*$_2$NH$_2$), 2.69 (*t*, *J* = 6.7, C*H*$_2$NH$_2$), 1.71-1.13 [m, 19 H; incl. 1.64 (*quint.*, *J* = 6.9, CH$_2$C*H*$_2$CH$_2$), 1.54 (*m* br., 2 H), 1.46 (*s*, *t*-Bu)]. ^{13}C-NMR: 79.2 (*s*, *C*(CH$_3$)$_3$), 60.9, 49.5 (*t*, *C*H$_2$), 46.7 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.5, ^{13}*C*H$_2$), 46.0, 45.6 (2 *t*, *C*H$_2$), 41.9 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.5, ^{13}*C*H$_2$), 28.4 (*q*, C(*C*H$_3$)$_3$). CI-MS: 495.5 (13, [2 M + 1]$^+$), 276.3 (30), 248.3 (100, [M + 1]$^+$), 148.2 (65, [M - Boc + 1]$^+$).

***tert*-Butyl *N*-{4-{[(*E*)-3-(4-Methoxyphenyl)prop-2-enoyl]amino}-(1,4-^{13}C$_2$)butyl}-*N*-{3-{[(*E*)-3-(4-methoxyphenyl)prop-2-enoyl]amino}propyl}carbamate (19).** According to the procedure for **12**, starting from **18** (0.17 g, 0.69 mmol) and Et$_3$N (0.16 g, 1.54 mmol) in anhydrous ethyl acetate (6 mL), a solution of (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride (0.27 g, 1.37 mmol) in anhydrous ethyl acetate (10 mL), there was obtained **19** (0.38 g, 97%) as a colorless voluminous spongy solid after chromatography (silica gel, CHCl3/MeOH 20:1). IR: 1660, 1603, 1510, 1250, 1172, 1030, 978, 825. ^{1}H-NMR: 7.75-7.36 (*m*, 6 H), 6.99-6.78 (*m*, 4 H), 6.44-6.23 (*m*, 2 H), 5.88 (*s* br., N*H*cum), 3.92-3.76 (*m*, 2 OC*H*$_3$), 3.56-3.25 (m, 4 H), 3.42 (*dm*, *J* = 137.9, ^{13}C*H*$_2$NHcum), 3.23 (*dm*, *J* = 132.6; ^{13}C*H*$_2$NBoc), 1.76 (*m* br.), 1.60 (*m* br., 4 H), 1.47 (*s*, *t*-Bu). ^{13}C-NMR: 129.2 (*d*, arom. *C*), 114.1, 55.2 (*s*, OCH$_3$), 46.8 (*t*, ^{13}*C*H$_2$), 45.8 (*t*, *C*H$_2$), 39.0 (*t*, ^{13}*C*H$_2$), 28.4 (*q*, C(*C*H$_3$)$_3$). ESI-MS: 568.4 (60, [M + 1]$^+$), 468.4 (100, [M - Boc + 1]$^+$).

***N*^{1},8-Di[(*E*)-4-methoxycinnamoyl]-(1,4-^{13}C$_2$)spermidine (= (*E*)-3,3'-Bis(4-methoxyphenyl)-*N*,*N*'-4-[(1,4-^{13}C$_2$)azaoctane-1,8-diyl]bis[prop-2-enamide]) (20a).** According to the procedure for **13a**, starting from **19** (0.10 g, 0.18 mmol) in dichloromethane (11.5 mL) and CF$_3$COOH (3.38 g, 29.6 mmol) and treated three times with 1 M aqueous HCl (2.5 mL) in methanol (1.5 mL), there was obtained **20a** · HCl (96.2 mg, quantitative) as a white solid after high vacuum drying; mp 209-213°C. *R*f 0.57 [chloroform/methanol/ammonia water 25% 78:19:3]. IR (KBr): 1652, 1600, 1515, 1255, 1175, 1025, 970, 828. ^{1}H-NMR (CD$_3$OD): 7.59-7.46 (*m*, 6 H), 6.99-6.87 (*m*, 4 H), 6.48 (*d*, *J* = 15.8, 1 olef. H), 3.82 (*s*, 2 OC*H*$_3$), 3.47-3.39 (*m t* like, C*H*$_2$NHcum), 3.38 (*d*(*m t* like), *J* = 138.3, ^{13}C*H*$_2$NHcum), 3.07 (*dm*, *J* = 143.2, ^{13}C*H*$_2$NH), 3.10-2.99 (*m t* like, C*H*$_2$NH), 2.02-1.89 (*m quint.* like, CH$_2$C*H*$_2$CH$_2$), 1.86-1.63 (*m* br., 4 H). ^{13}C-NMR (CD$_3$OD): 130.4 (*d*, arom. *C*), 115.3 (*d*, *C*H=CHCON), 55.8 (*s*, OCH$_3$), 48.5 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.5, ^{13}*C*H$_2$), 47.8 (*t*, *C*H$_2$), 39.5 (*dt*, $J^{13}_{C,}{}^{13}_{C}$ = 4.5, ^{13}*C*H$_2$). ESI-MS: 468.3 ([M - HCl + 1]$^+$).

***N*^{1},8-Di[(*E*)-4-coumaroyl]-(1,4-^{13}C$_2$)spermidine (= (*E*)-3,3'-Bis(4-hydroxyphenyl)-*N*,*N*'-4-[(1,4-^{13}C$_2$)azaoctane-1,8-diyl]bis[prop-2-enamide]) (20b).** According to the procedure for **13b**, starting from **19** (100 mg, 0.18 mmol) in dichloromethane (2.6 mL) and BBr$_3$ (1 M in CH$_2$Cl$_2$) (1.34 mL) and treated three times with 1 M aqueous HCl (2 mL) in methanol (1 mL), there was obtained 0.15 g of a brown solid after high vacuum drying. 70 mg of it were passed through a chromatography column under red light on silicagel eluted with a 78:19:3 mixture of chloroform/methanol/ammonia water 25% (*R*f 0.18). Pure **20b** · HCl (36.3 mg, 91% based on **19**) could finally be prepared as explained above by treatment with aqueous HCl in methanol;

mp 108-111°C. IR (KBr): 1660, 1645, 1605, 1515, 1220, 1170, 828. ^{1}H-NMR (CD_3OD): 7.49 (*dd*, *J* = 15.8, 4.5, 2 olef. H), 7.42 (*d*, *J* = 8.6, 4 arom. H), 6.80 (*dd*, *J* = 8.6, 1.6, 4 arom. H), 6.46 (*dd*, *J* = 15.8, 3.0, 2 olef. H), 3.50-3.39 (*m t* like, CH_2NHcum), 3.39 (*d*(*m t* like), *J* = 138.5, $^{13}CH_2$NHcum), 3.11-3.01 (*m t* like, CH_2NH), 3.07 (*d*(*m t* like), J = 143.3, $^{13}CH_2$NH), 2.03-1.93 (*m quint.* like, $CH_2CH_2CH_2$), 1.86-1.63 (*m* br., 4 H). ^{13}C-NMR (CD_3OD): 130.7, 116.7 (2 *d*, arom. *C*), 48.5 (*dt*, $J^{13}_{C-}{}^{13}_{C}$ = 4.3, $^{13}CH_2$), 39.7 (*dt*, $J^{13}_{C-}{}^{13}_{C}$ = 4.3, $^{13}CH_2$). ESI-MS: 440.3 ([M - HCl + 1]$^+$).

(1,4-$^{13}C_2$)Spermidine (= *N*-(3-Aminopropyl)-1,4-diamino-(1,4-$^{13}C_2$)butane) (21). At 25°C **18** (0.42 g, 1.69 mmol) was stirred overnight in 5 M aqueous HCl (10 mL). Water was then evaporated by azeotropic distillation with ethanol to afford **21** · 3 HCl (0.44 g, quantitative) as a white solid; mp 224.7-225.1°C. IR (KBr): 3420, 2510, 2450, 2410, 1600, 1515, 1455, 1400, 1355, 1265, 1235, 1155, 1055, 998, 880. ^{1}H-NMR (CD_3OD): 3.15 (*t*, *J* = 7.6, CH_2NH), 3.09 (*d*(*m t* like), *J* = 143.4, $^{13}CH_2$NH), 3.08 (*t*, *J* = 7.6, CH_2NH_2), 3.00 (*d t* , *J* = 142.8, 7.3, $^{13}CH_2NH_2$), 2.12 (*m quint.* like, $CH_2CH_2CH_2$), 1.81 (*m* sym., 4 H). ^{13}C-NMR (CD_3OD): 48.5, 48.4, 40.1, 40.0 (4 *t*, $^{13}CH_2$). CI-MS: 148.2 ([M - 3 HCl + 1]$^+$).

Di(*tert*-butyl) *N*,*N*'-[(1,4-$^{13}C_2$)-4-Azaoctane-1,8-diyl]bis[carbamate] (22). At 0°C, under a nitrogen atmosphere, **21** · 3 HCl (0.21 g, 0.82 mmol) was added to *in situ* prepared sodium methanolate (57 mg, 2.5 mmol of Na in 1.5 mL methanol). At 25°C, the solvent was evaporated using an oil pump. To the residue was added THF (2.5 mL), and to the mixture cooled at 0°C was finally dropped over 30 min a solution of 2-{[(*tert*-butoxy)carbonyl]oxyimino}-2-phenylacetonitrile (Boc-On, 0.44 g, 1.81 mmol) in THF (4 mL). The whole was then stirred at 25°C overnight protected from light. Most of the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate (20 mL). The organic phase was washed with 0.5 M aqueous NaOH (10 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated. The residue (0.33 g) was purified on a silica gel column eluted with a gradient from a 95:5:0.2 to a 7:3:1 mixture of chloroform/methanol/ammonia water 25 % to obtain **22** (0.11 g, 39%) as a pale yellow solid; mp 75.8-76.9°C. *Rf* 0.95 [chloroform/methanol/ammonia water 25 % 78:19:3]. IR: 3450, 2926, 1708, 1500, 1390, 1365, 1165. ^{1}H-NMR: 5.15 (*s* br., N*H*Boc), 4.83 (*s* br., N*H*Boc), 3.22 (*q*, *J* = 5.6, CH_2NHBoc), 3.12 (*d*(*m* sym.), *J* = 141.1, $^{13}CH_2$NHBoc), 2.71 (*td*, *J* = 6.3, 3.0, CH_2NH), 2.66 (*d*(*m t* like), *J* = 135.1, $^{13}CH_2$NH), 1.92 (*s* br., N*H*), 1.71 (*m* sym., $CH_2CH_2CH_2$), 1.56 (*m* br., 4 H), 1.44 (*s*, 2 *t*-Bu). ^{13}C-NMR: 169.8 (*s*, CO), 49.1 (*dt*, $J^{13}_{C-}{}^{13}_{C}$ = 3.8, $^{13}CH_2$), 40.2 (*t*, $^{13}CH_2$), 28.3 (*q*, $C(CH_3)_3$). ESI-MS: 348.3 (100, [M + 1]$^+$), 292.3 (28, [M - (isobutene) + 1]$^+$), 248.2 (7, [M - Boc + 1]$^+$), 236.2 (15, [M - 2 (isobutene) + 1]$^+$).

Di(*tert*-butyl) *N*,*N*'-{4-{[(*E*)-3-(4-methoxyphenyl)prop-2-enoyl]-(1,4-$^{13}C_2$)-4-azaoctane-1,8-diyl}bis[carbamate] (23). According to the procedure for **12**, starting from **22** (0.10 g, 0.29 mmol) and Et_3N (33 mg, 0.32 mmol) in anhydrous ethyl acetate (2.3 mL) and (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride (57 mg, 0.29 mmol) in anhydrous ethyl acetate (2.9 mL), there was obtained **23** (0.14 g, 96%) as a colorless voluminous spongy solid after chromatography (silica gel, CHCl3/MeOH 20:1). IR: 3450, 2987, 2930, 1707, 1643, 1600, 1503, 1365, 1248, 1170, 1030, 980, 823. ^{1}H-NMR: 7.66 (*d*, *J* = 15.3, 1 olef. H), 7.48 (*d*, *J* = 8.7, 2 arom. H), 6.90 (*d*, *J* = 8.7, 2 arom. H), 6.70 (*d*, *J* = 15.3, 1 olef. H), 5.52, 4.67, 4.58 (3 *s*

br., 2 N*H*Boc), 3.84 (*s*, OC*H*$_3$), 3.64 (*dm*, *J* = 136.4, ^{13}C*H*$_2$Ncum), 3.54-3.32 (*m*, C*H*$_2$Ncum), 3.22-3.03 (*m*, C*H*$_2$NHBoc), 3.13 (*dm*, *J* = 137.9, ^{13}C*H*$_2$NHBoc), 1.76-1.34 [*m*, 24 H; incl. 1.44, 1.43 (2 *s*, 2 *t*-Bu)]. ^{13}C-NMR: 114.6 (*d*, arom. C), 47.4 (*dt*, $J^{13}_{C,^{13}C}$ = 4.5, ^{13}CH$_2$), 46.2, 39.9 (2 *t*, ^{13}CH$_2$), 28.3 (*q*, C(*C*H$_3$)$_3$). ESI-MS: 508.3 (100, [M + 1]$^+$), 452.3 (33, [M - (isobutene) + 1]$^+$), 408.2 (40, [M - Boc + 1]$^+$), 352.2 (24, [M - Boc - (isobutene) + 1]$^+$), 308.2 (23, [M - 2 Boc + 1]$^+$).

N^4-[(*E*)-4-Methoxycinnamoyl]-(1,4-^{13}C$_2$)spermidine (= (*E*)-*N*-[4-{[Amino-(1,4-^{13}C$_2$)butyl]-*N*-(3-aminopropyl)-3-(4-methoxyphenyl)-prop-2-enamide (24a). According to the procedure for **13a**, starting from **23** (70.4 mg, 0.14 mmol) in dichloromethane (9.1 mL) and CF$_3$COOH (2.66 g, 23.3 mmol) and treated three times with 1 M aqueous HCl (2.2 mL) in methanol (1.5 mL), there was obtained **24a** · 2 HCl (49.6 mg, 94%) as a yellowish solid after high vacuum drying. *R*f 0.41 [chloroform/methanol/ammonia water 25% 7:3:1]. ^{1}H-NMR (DMSO, 363 K): 8.14 (*s* br., 2 N*H*$_2$), 7.63 (*d*, *J* = 8.7, 2 arom. H), 7.48 (*d*, *J* = 15.3, 1 olef. H), 6.96 (*d*, *J* = 8.7, 2 arom. H), 6.93 (*d*, *J* = 15.3, 1 olef. H), 3.82 (*s*, OC*H*$_3$), 3.62-3.51 (*m*, C*H*$_2$Ncum), 3.45 (*dt*, *J* = 137.0, 6.8, ^{13}C*H*$_2$Ncum), 2.86 (*t*, *J* = 7.2, C*H*$_2$NH$_2$), 2.85 (*d*(*m t* like), *J* = 141.8, ^{13}C*H*$_2$NH$_2$), 1.97 (*quint.*, *J* = 7.2, CH$_2$C*H*$_2$CH$_2$), 1.80-1.54 (*m*, 4 H). ^{13}C-NMR (CD$_3$OD): 48.4, 48.3 (2 *t*, ^{13}CH$_2$), 46.8 (*dt*, $J^{13}_{C,^{13}C}$ = 4.8, ^{13}CH$_2$), 40.5, 40.4 (2 *t*, ^{13}CH$_2$). ESI-MS: 308.3 ([M - 2 HCl + 1]$^+$).

N^4-[(*E*)-4-Coumaroyl]-(1,4-^{13}C$_2$)spermidine (= (*E*)-*N*-[4-{[Amino-(1,4-^{13}C$_2$)butyl]-*N*-(3-aminopropyl)-3-(4-hydroxyphenyl)-prop-2-enamide) (24b). According to the procedure for **13b**, starting from **23** (70.4 mg, 0.14 mmol) in dichloromethane (1.5 mL) and BBr$_3$ (1 M in CH$_2$Cl$_2$) (0.74 mL) and treated three times with 1 M aqueous HCl (2 mL) in methanol (1 mL), there was obtained **24b** · 2 HCl (53.9 mg, quantitative) as a brown solid after high vacuum drying. *R*f 0.25 [chloroform/methanol/ammonia water 25% 7:3:1]. ^{1}H-NMR (DMSO, 363 K): 7.86 (*s* br., 2 N*H*$_2$), 7.60 (*d*, *J* = 8.6, 2 arom. H), 7.55 (*d*, *J* = 15.3, 1 olef. H), 6.93 (*d*, *J* = 15.3, 1 olef. H), 6.92 (*d*, *J* = 8.6, 2 arom. H), 3.56 (*d*(*m t* like), *J* = 137.4, ^{13}C*H*$_2$Ncum), 3.68-3.52 (*m*, C*H*$_2$Ncum), 2.97 (*d*(*m* br.), *J* = 143.0, ^{13}C*H*$_2$NH$_2$), 2.97 (*m t* like, C*H*$_2$NH$_2$), 2.02 (*quint.*, *J* = 7.2, CH$_2$C*H*$_2$CH$_2$), 1.88-1.64 (*m*, 4 H). ^{13}C-NMR (CD$_3$OD): 131.1, 116.7 (2 *d*, arom. C), 48.4 (*t*, ^{13}CH$_2$), 46.9, 40.5, 40.4 (2 *t*, ^{13}CH$_2$). ESI-MS: 294.2 ([M - 2 HCl + 1]$^+$).

N^1,4,8-Tri[(*E*)-4-methoxycinnamoyl]-(5,8-^{13}C$_2$)spermidine (= (*E*)-4-[3-(4-Methoxyphenyl) prop-2-enoyl]-3,3'-bis(4-methoxyphenyl)-*N*,*N*'-[(1,4-^{13}C$_2$)-4-azaoctane-1,8-diyl]bis(prop-2-enamide) (25). At 0°C, under a nitrogen atmosphere, to a suspension of **21** · 3 HCl (0.12 g, 0.45 mmol) in anhydrous ethyl acetate (5 mL) was added Et$_3$N (0.30 g, 2.98 mmol) and stirred for 1 h. According to the procedure for **12**, after addition of a solution of (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride (0.27 g, 1.36 mmol) in anhydrous ethyl acetate (18 mL) there was obtained **25** (0.14 g, 50%) as a colorless voluminous spongy solid after chromatography (silica gel, CHCl3/MeOH 20:1; *R*f 0.36). IR: 3440, 3310, 2988, 2933, 2836, 1663, 1643, 1605, 1510, 1305, 1285, 1250, 1172, 1030, 980, 825. ^{1}H-NMR: 7.71 (*d*, *J* = 15.2, 1 olef. H), 7.57 (*dd*, *J* = 15.6, 2.7; 2 arom. H), 7.52-7.36 (*m*, 6 H), 7.18 (*m t* like, NHcum), 6.98-6.78 (*m*, 6 H), 6.73 (*d*, *J* = 15.2, 1 olef. H), 6.38 (*d*, *J* = 15.6, 1 olef. H), 6.27 (*d*, *J* = 15.5, 1 olef. H), 5.90 (*m* br., NHcum), 3.86-3.75 (*m*, 3 OC*H*$_3$), 3.64-3.55 (*m*, 2 H), 3.5 (*dm*, *J* = 137.7, 2

$^{13}CH_2$), 3.42-3.32 (*m q* like, 2 H), 2.05-1.50 (*m*, incl. H_2O). ^{13}C-NMR: 169.4 (*s*, CO), 129.3 (*d*, arom. C), 55.6 (*q*, OCH_3), 47.6 (*dt*, $J^{13}C,^{13}C$ = 4.0, $^{13}CH_2$), 46.0, 38.6 (*dt*, $J^{13}C,^{13}C$ = 4.0, $^{13}CH_2$). ESI-MS: 628.5 ([M + 1]$^+$).

N^1,4,8-Tri[(*E*)-4-coumaroyl]-(5,8-$^{13}C_2$)spermidine (= (*E*)-4-[3-(4-Hydroxyphenyl)prop-2-enoyl]-3,3'-bis(4-hydroxyphenyl)-*N*,*N*'-[(1,4-$^{13}C_2$)-4-azaoctane-1,8-diyl]bis(prop-2-enamide) (26). According to the procedure for **13b**, starting from **25** (97.7 mg, 0.16 mmol) in dichloromethane (2.9 mL) and BBr_3 (1 M in CH_2Cl_2) (1.54 mL), there was obtained after high vacuum drying **26** (98.3 mg, quantitative) as a brown solid. R_f 0.43 [chloroform/methanol/ammonia water 25% 78:19:3]. IR (KBr): 1645, 1635, 1600, 1515, 1445, 1260, 1210, 1165, 975, 825. ^{1}H-NMR (CD_3OD): 7.68-7.32 (*m*, 9 H), 6.96-6.68 (*m*, 7 H), 6.57-6.34 (*m*, 2 H), 3.93-3.25 (*m*, incl. MeOH), 3.15 (*m* sym., 1 H), 2.09-1.54 (*m*, 2 + 4 H). ^{13}C-NMR (CD_3OD): 117.3 (*d*, arom. C), 49.2, 47.8, 40.4, 40.1 (4 *t*, $^{13}CH_2$). ESI-MS: 586.4 ([M + 1]$^+$).

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