Synthesis of an Isotopically Labeled Dilactam-Bridged Tetrapeptide

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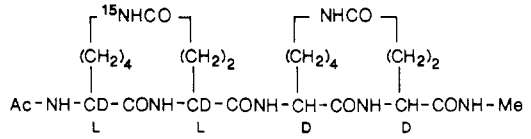
To aid in assigning the amide proton resonances in the NMR spectrum of the dilactam-bridged tetrapeptide derivative Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe, an isotopically labeled analogue was synthesized. The compound was deuteriated in the C_α position of the first two residues in the sequence (L-Lys and L-Glu) and also incorporated ^{15}N in the N-terminal lysine side chain. By labeling the peptide with these isotopes and comparing splitting patterns with those in the unlabeled analogue, unambiguous assignments of the amide NH's have been made. In the synthesis of the labeled tetrapeptide, the intermediate *N*-(benzyloxycarbonyl)-δ-[ε-^{15}N]amido-2-[α-^2H]-aminoadipic acid methyl ester (**23**) was prepared by two different routes. One synthetic route involved starting with a glutamic acid derivative and extending the side chain by one methylene unit via a modified Arndt–Eistert synthesis. An alternate route was carried out with D,L-2-aminoadipic acid as the starting material. The overall synthetic routes are compared. The isotopically labeled dilactam-bridged tetrapeptide was prepared by the fragment condensation of the appropriate dipeptides via an azide intermediate.

Introduction

A series of lactam-bridged model compounds have been synthesized in order to study properties of constrained peptides.[1] Complete assignments of the amides NH's in the proton NMR spectrum is essential for the examination of these molecules spectroscopically. Unambiguous assignment of the amide NH groups in the tetrapeptides[1] is complicated by the presence of identical (neglecting stereochemistry) residues in the molecules (i.e., two pairs of Lys and Glu residues). In order to assign the amide proton resonances in the NMR spectrum of the dilactam-bridged tetrapeptide Ac-L-Lys-L-Glu-D-Lys-D-Glu-NHMe, an isotopically labeled analogue was synthesized. The compound was deteriated in the C_α position of the first two residues in the sequence (L-Lys and L-Glu) and also incorporated ^{15}N in the N-terminal lysine side chain:



I

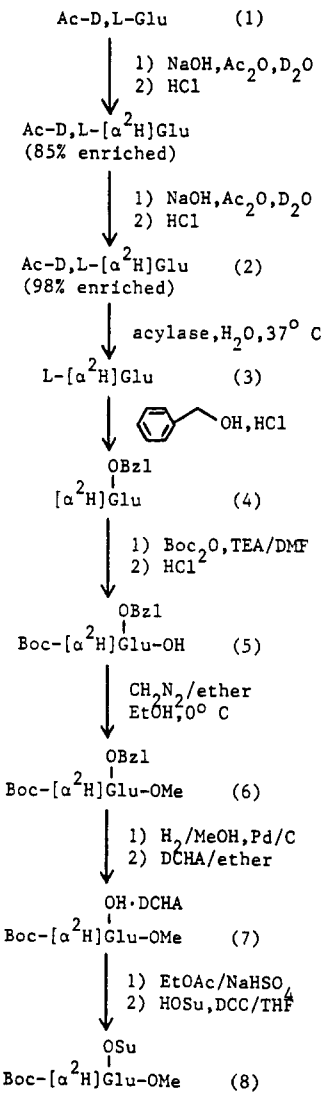
Results and Discussion

In order to differentiate the amide NH groups in the LLDD LysGluLysGlu molecule, selected positions were isotopically labeled. For this to be useful, it was necessary to observe changes in the splitting patterns of protons as compared to the proton NMR spectrum for the unlabeled compound. The rationale for designing compound I was, therefore, in terms of changing proton multiplicities as well as considering the level of synthetic difficulty.

For these reasons, the molecule was deteriated in the α-positions of L-Lys and L-Glu and was also labeled with ^{15}N in the L-Lys side chain. Deuterium (^2H) has a nuclear spin of 1 and, therefore, does not split the vicinal amide proton into a doublet in the proton NMR spectrum. The amide NH appears as a singlet.

In theory, the same reasoning would apply if the $C_\epsilon H_2$ of the L-Lys side chain were perdeuteriated. However, by inspection of the amide region in the proton NMR spectrum of the unlabeled analogue, the side-chain amide proton already appears as a singlet. In other words, deuteriating the adjacent methylene group would not be useful. Changing the splitting pattern of the side-chain NH was accomplished by incorporating ^{15}N in the L-Lys side chain. Since this isotope of nitrogen has a nuclear spin of $^1/_2$, the directly bonded proton is split into a doublet. As shown in Figure 1, by comparing splitting patterns of the amide proton resonances in the isotopically labeled tetrapeptide with those present in the unlabeled com-

Scheme I. Synthesis of Boc[α-^2H]Glu(OSu)OMe



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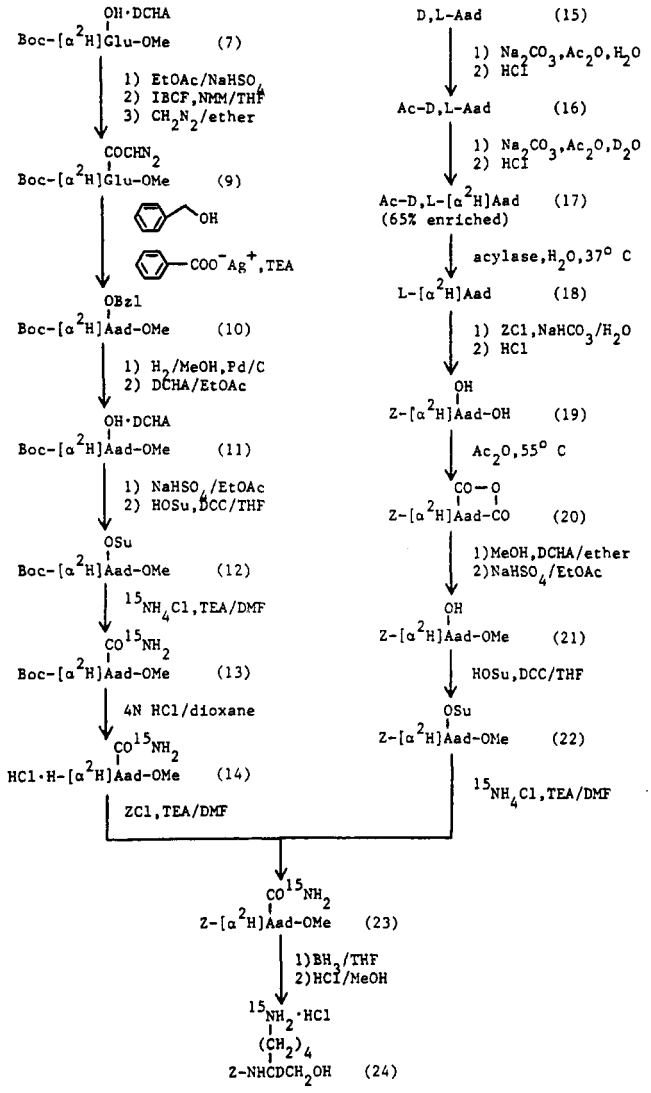
Scheme II. Synthesis of Ac-L-[ε-^{15}N,α-^{2}H]Lys-L-[α-^{2}H]Glu-L-Lys-D-Glu-NHMe



pound, unambiguous assignments were made.

The synthetic routes for the preparation of compound I are shown in Schemes I–III. Deuterium was incorporated in the α-position of the L-glutamic acid residue by treating racemic acetylglutamic acid (1) with acetic anhydride and deuterium oxide in the presence of sodium hydroxide.[2] The reaction proceeded via an oxazolone (or azylactone) intermediate and when acidified with hydrochloric acid gave the desired product with a level of deuterium incorporation of 85% as evidenced by proton NMR. After repeating this procedure, compound **2** was 98% enriched in deuterium at the α-carbon.

Selective cleavage of the amide bond in the acetylated L isomer by the enzyme acylase under physiological conditions (pH 7.5, T = 37 °C) generated optically pure α-deuteriated L-glutamic acid (**3**).[3] The side chain was protected via acid-catalyzed esterification with benzyl alcohol to give the corresponding benzyl ester **4**.[4] The α-amine of the benzyl ester was subsequently protected with the urethane-type protecting group *tert*-butyloxycarbonyl (Boc) by treating the zwitterion **4** with di-*tert*-butyl dicarbonate (Boc_2O) in an organic medium (*N,N*-

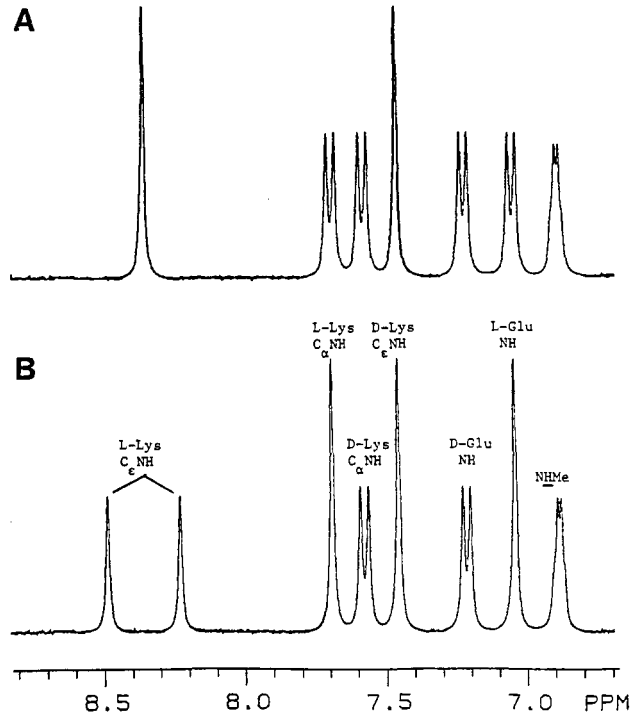
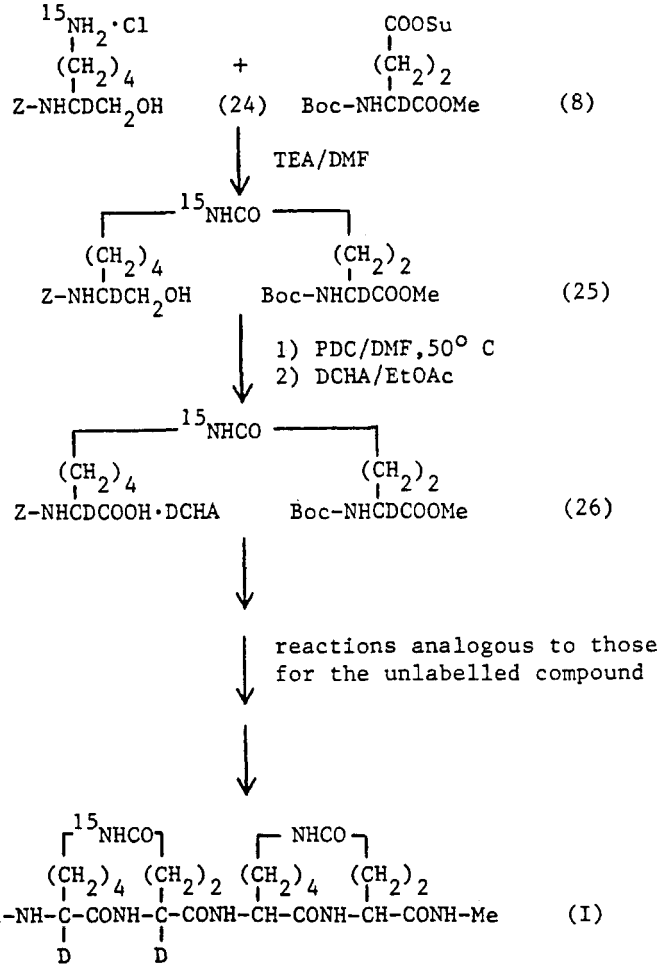


Figure 1. Comparison of the amide NH regions in the (A) LLDD LysGluLysGlu compound with its (B) isotopically labeled analogue. Spectrum was taken in DMSO-d_6 (4.5 mM) at 21 °C.

Scheme III. Synthesis of Z-[ε-^{15}N,α-^{2}H]Lysinol



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dimethylformamide) in the presence of triethylamine. Acidifying with hydrochloric acid gave the diprotected glutamic acid derivative **5**.

The fully protected glutamic acid derivative **6** was obtained by esterifying the *tert*-butyloxycarbonyl-protected free acid **5** with ethereal diazomethane in ethanol at 0 °C. The benzyl group was cleaved via hydrogenolysis in methanol using palladium (10%) adsorbed on carbon as the catalyst. The free acid was subsequently converted to its dicyclohexylamine (DCHA) salt (**7**). The free acid was liberated from its DCHA salt (**7**) by washing a suspension of the solid in ethyl acetate with aqueous sodium bisulfate solution. The resulting side-chain carboxyl group of glutamic acid was then activated (**8**) via the succinimide ester by reaction of the free acid with *N*-hydroxysuccinimide (HOSu) and the condensing reagent *N,N'*-dicyclohexylcarbodiimide (DCC) in tetrahydrofuran.

The amino alcohol **24** was synthesized by two different routes. One route involved the elongation of a glutamic acid derivative by one $-CH_2-$ group in order to generate a 2-aminoadipic acid (Aad) homologue. The other route involved preparation of the amino alcohol by using Aad as the starting material.

The DCHA salt **7** was suspended in ethyl acetate and washed with aqueous sodium bisulfate solution in order to generate the free acid. The mixed anhydride was formed by treating the free acid with isobutyl chloroformate (IBCF) in tetrahydrofuran. Subsequent addition of diazomethane in ether yielded the gold-colored diazomethyl ketone **9**.[5] The Aad benzyl ester derivative **10** was prepared by the Wolff rearrangement of the diazomethyl precursor **9** using silver benzoate in triethylamine in a solution of benzyl alcohol and tetrahydrofuran.[5]

The side-chain benzyl ester was cleaved by hydrogenolysis in methanol using 10% palladium on carbon as the catalyst. Treatment of the free acid with DCHA in ethyl acetate gave the diprotected DCHA salt **11**. Side-chain activation (**12**) was carried out by liberating the free acid from its DCHA salt (V11) as previously described and reacting it with HOSu and DCC in tetrahydrofuran. The ^{15}N-labeled side chain carboxamide **13** was generated by treating the succinimide ester **12** with ^{15}N ammonium chloride (99% enriched) in *N,N*-dimethylformamide in the presence of triethylamine. The Boc group was cleaved from the dilabeled compound **13** by treatment with the deprotecting reagent, 4 N hydrochloric acid in dioxane, giving the hydrochloride salt **14**.

The acetylated D,L-Aad **16** was obtained by reacting racemic Aad with acetic anhydride in water with sodium carbonate monohydrate as the base. After acidifying with hydrochloric acid and continuously extracting with ethyl acetate, the product **16** was obtained. Deuterium was incorporated at the α-carbon (**17**) by the same procedure used for the glutamic acid homologue **2** except that sodium carbonate monohydrate was used as the base rather than sodium hydroxide. When sodium hydroxide was used, the solution turned yellow and the product showed many spots on TLC. The level of deuterium incorporation was shown to be only 65% as evidenced by proton NMR. The procedure was repeated twice in order to obtain product 96% enriched in deuterium.

The acetyl group was cleaved in the L isomer by the procedure given for the glutamic acid homologue **3** resulting in optically pure deuteriated L-Aad acid **18**. The α-amine was protected by the benzyloxycarbonyl (Z) group by treating the zwitterion **18** with benzyl chloroformate (ZCl) and sodium bicarbonate in water. After acidifying with hydrochloric acid, the Z-protected diacid **19** was obtained. The cyclic anhydride **20** was synthesized by treating the diacid **19** with the dehydrating agent, acetic anhydride, at elevated temperature. Reaction of the cyclic anhydride **20** with methanol and DCHA generated the α- and δ-substituted methyl esters. Column chromatography was carried out in order to isolate the desired α-isomer **21**. The side chain was subsequently activated via the succinimide ester **22** by reacting the diprotected free acid **21** with HOSu and DCC in tetrahydrofuran.

The Z-protected carboxamide **23** was synthesized by two different routes. One synthetic route involved reacting the methyl ester hydrochloride **14** with benzyl chloroformate in the presence of triethylamine. The other route involved treating the succinimide ester **22** with ^{15}N ammonium chloride (99% enriched) in the presence of triethylamine. The side-chain C=O group of the dilabeled carboxamide **23** was subsequently reduced by using borane in tetrahydrofuran under refluxing conditions.[6-8] The borane complex was destroyed by addition of hydrochloric acid in methanol, giving the dilabeled amino alcohol **24**. Attempts were made at selectively reducing the side chain C=O in the presence of the C_α C=O group. Reactions were attempted by using the methyl ester[9] as well as the lithium salt[10] of the free carboxylic acid. In each case, the ester/carboxylate salt was reduced to the primary alcohol.

The overall yields for the alternate synthetic routes involving the DCHA salt **7** and racemic Aad **15** as starting materials were 14% and 13%, respectively. Although the overall yields are approximately equal, the former synthetic route is preferrable over the latter because each intermediate is easier to isolate.

The trilabeled dipeptide derivative **25** was prepared by reacting the amino alcohol **24** with the active ester **8** in *N,N*-dimethylformamide in the presence of triethylamine. The primary alcohol was then oxidized to the carboxylic acid by using pyridinium dichromate (PDC)[11] at elevated temperature. Treatment of the free acid with DCHA in ethyl acetate generated the DCHA salt **26**. The isotopically labeled tetrapeptide I was obtained by carrying out reactions completely analogous to those given for the unlabeled compound.[1] The proton NMR assignments for all isolated compounds are shown in Table I.

Experimental Section

Materials. The starting materials acetyl-D,L-glutamic acid and D,L-2-aminoadipic acid (Aad) were obtained from Sigma Chemical Co. and Aldrich Chemical Co., respectively. Benzyl chloroformate (technical grade, 95%), borane in tetrahydrofuran (1 M), isobutyl chloroformate, and deuterium oxide (Gold Label) were also purchased from Aldrich Chemical Co. Di-*tert*-butyl dicarbonate was obtained from Fluka, Inc. The enzyme porcine kidney acylase (type 1; Grade II) was purchased from Sigma Chemical Co. Ammonium chloride enriched in ^{15}N (99%) was obtained from ICN Biomedicals, Inc. (*N,N*-Dimethylamino)ethylamine was obtained from Pfaltz & Bauer, Inc. Tetrahydrofuran was purified by refluxing the stock over potassium for 2 h and testing for the presence of a blue color with benzophenone. The solvent was distilled, under nitrogen, and the fraction boiling at 65–66 °C (ca. 750 mm) was collected and stored under nitrogen. Column chromatography was carried out by using silica gel 60 (0.050–0.200

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Table I. Proton NMR Assignments of Isolated Intermediates in the Synthesis of Ac-L-[ϵ-^{15}N,α-^{2}H]Lys-L-[α-^{2}H]Glu-D-Lys-D-Glu-NHMe

	Glu				Asd/Lys								Z		Boc	ester	
compd[a]	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	$C_\epsilon H$	$C_\delta CO$-$^{15}NH_2$	$C_\epsilon{}^{15}NH$	Ph	CH_2	CH_3	CH_3	other
1	8.16 d (7.85)	4.20 m	1.97 m 1.79 m	2.31 m													12.40 s[b] 1.86 s[c]
2	8.16 s		1.97 m 1.79 m	2.31 m													12.40[b] 1.86 s[c]
6	7.31 s		1.96 m 1.81 m	2.44 m											1.34 s	3.61 s	7.36 s[d] 5.09 s[d]
7	7.55 s		1.86 m	2.69 m											1.3m s	3.61 s	1.10–1.25[f]
8	7.38 s		2.02 m 1.92 m	2.73 m											1.38 s	3.64 s	2.81[g]
11					7.28 s	2.13 m	1.64 m 1.80 m	2.60 m 1.53 m							1.40 s	3.62 s	1.00–1.25[f]
16					2.13 d (7.93)	4.14 m	1.69 m 1.54 m	1.54 m	2.71 t (7.21)								12.30[b] 1.85[c]
17					5.13 s		1.69 m 1.54 m	1.54 m	2.21 t (7.21)								12.30 s 1.85[c]
19					7.60 s		1.70 m 1.55 m	1.55 m	2.20 t (6.84)				7.3l s	5.03 s			12.3l s[b]
23					7.75 s		1.66 m	1.52 m	2.02 t (6.84)		7.23 d (74.9) 6.72 d (87.2)		7.36 s	5.03 s		3.63 s	
25	7.26 s	1.72 m	1.89 m (7.06)	2.11 t	6.96 s		2.98 s	1.50 m	1.27 m	1.27 m		7.78 d[h] (91.1)	7.35 s	5.00 s	1.37 s	3.61 m	
26	7.28 s		1.91 m	2.13 t (7.12)	7.32 m		2.97 m 2.88 m	1.56 m	1.31 m			7.80 d[h] (91.3)	7.34 s	5.00 s	1.37 s	2.6 s	1.10–1.25[f]
I	7.22[i]		2.04 m	2.23 m	7.68 s	2.65 m	1.35 m 1.60 m	1.35 m	3.58 m	3.51 m	8.34 d (91.2)						2.10 s[j] 6.88 d[k] (4.79)
	7.05 d (9.41)				7.57 d[i] (10.1)							7.46 m[i]					2.55 d[l] (4.32)

[a] Compound numbers correspond to those used in Schemes I–III; all spectra were taken at concentrations of 1 mg/0.5 mL DMSO-d_6 at 21 °C; coupling constants (J in hertz) are given in parentheses; see Experimental Section for further details. [b] COOH. [c] Acetyl CH_3. [d] Phenyl H's, benzyl ester. [e] Benzylic CH_2. [f] Dicyclohexyl H's. [g] Succinimide H's. [h] Doublet of triplets. [i] d isomer. [j] Acetyl CH_3. [k] NH of N-methylamide. [l] CH_3 of N-methylamide.

mm, 70–270 mesh ASTM) purchased from Brinkman, Inc. Analytical TLC plates were purchased from E. Merck: silica gel 60 F-254, 0.2-mm thickness, aluminum-backed. All isolated products were run in at least two solvent systems. The following chromatographic systems (by volume) were used: (1) ethyl acetate/acetic acid, 50:1; (2) chloroform/methanol, 10:1: (3) chloroform/methanol/acetic acid, 85:10:5; (4) 2,2,2-trifluoroethanol/acetic acid, 10:1; (5) 1-butanol/acetic acid/water, 3:1:1; (6) chloroform/methanol/acetic acid, 2:1:1. The TLC plates were developed with the following visualizing reagents: (a) ninhydrin, (b) *tert*-butyl hypochlorite/tolidine, (c) bromocresol green, and/or (d) ammonium sulfate/sulfuric acid/water as well as with (e) UV light (254 nm).

Instrumentation. Proton NMR spectra were obtained on a 360-MHz NMR (in the Fourier-transform mode) spectrometer built in-house from a continuous-wave Varian console equipped with an Oxford superconducting magnet and a Nicolet 1280 computer. The spectrometer is equipped with an extra frequency synthesizer for double irradiation (decoupling) experiments.

Peak positions are reported in parts per million (ppm) downfield from internal tetramethylsilane (TMS; Aldrich Chemical Co.). Coupling constants are expressed in hertz (Hz) and splitting patterns are abbreviated as s, singlet; d, doublet; t, triplet; m, unresolved multiplet. For assignments, spectra were taken using 99.9% dimethyl sulfoxide-d_6 (DMSO-d_6; MSD Isotopes).

Assignments were made on the basis of one-dimensional decoupling and two-dimensional homonuclear shift correlation[12] (COSY) and relayed coherence transfer[13,14] (RELCO) spectroscopy. Decoupling experiments were simply carried out by comparing splitting patterns of proton resonances in the normal, undecoupled spectrum with those present in the irradiated spectrum. Typical COSY and RELCO spectra were taken at concentrations of approximately 3–10 mM using 1K data points per spectrum for 128 spectra. The number of acquisitions was routinely 64 or 128 over a 1500-Hz spectral width. This corresponded to acquisition times of approximately 12 h.

Acetyl-D,L-[α-^{2}H]glutamic Acid (2). The deuteriated compound **2** was obtained, in part, by the method of Fujihara et al.[2]. The starting material, acetyl-D,L-glutamic acid (**1**; 16.5 g, 87.0 mmol), was dissolved in deuterium oxide (90.1 mL, 5000 mmol) containing sodium hydroxide (7.03 g, 176 mmol). Acetic anhydride (35 mL, 367 mmol) was then added, with extensive stirring, to the reaction mixture. The heterogeneous mixture was slowly raised to 50 °C by means of a water bath. Eventually the reaction mixture became homogeneous (pH slightly acidic) and was allowed to proceed for 16 h. The mixture was then cooled to 0 °C and concentrated hydrochloric acid added until pH 1. The solvents were removed under reduced pressure until the solution volume became approximately 50 mL. The solution was placed in the refrigerator overnight. The next day the white crystalline solid (12.8 g, 77%) was collected and washed with cold water, followed by acetone.

The product was 85% enriched in deuterium at the α-carbon as shown by ^{1}H NMR. The deuteriated product was then subjected to the same experimental procedure as previously mentioned to yield 98% enriched acetyl-D,L[α-^{2}H]glutamic acid (**2**): 9.91 g (60% overall); mp 180–181 °C (lit.[3] D,L-*N*-acetylglutamic-*N*,*2*,*3*,*3*,*4*,*4*-d_6 acid-d_2, mp 178–180 °C); R_f(**5c**) 0.47.

L-[α-^{2}H]Glutamic Acid (3). The product **3** was obtained, in part, by the method of Blomquist et al.[3] The deuteriated compound (**2**; 12.7 g, 66.9 mmol) was dissolved in 500 mL of water and adjusted to pH 7.5 with 2 N ammonium hydroxide. At room temperature, with vigorous stirring, acylase (0.78 g) was dissolved. The yellow-colored solution was then raised to 37 °C by means of a water bath. After 20 h decolorizing carbon was added and the temperature raised to 60 °C for 30 m. The carbon was then filtered through Celite and the filtrate adjusted to pH 3.22 with 5 N hydrochloric acid. The solvent was removed under reduced pressure to give a wet solid. After drying, in vacuo, overnight 100 mL of absolute ethanol/water (2/1, v/v) was added and the resulting mixture stirred for 30 min. The product **3** was collected and washed with absolute ethanol, warm acetone, and finally with ether: 4.82 g (98%); mp 196.5–198 °C (lit. mp 189–190.5[3], 198[15]); R_f(**5a**) 0.20; $[\alpha]^{20}_D$ (*c* 2.0, 5 N HCl) + 30.8° + (lit. +30.2°,[3] +28.9°[15]).

γ-Benzyl[α-^{2}H]glutamate (4). The benzyl ester **4** was synthesized, in part, by the method given in Greenstein et al.[4] A mixture of deuteriated glutamic acid (**3**; 10.0 g, 68.0 mmol), 17 mL of concentrated hydrochloric acid, and 68 mL of benzyl alcohol was refluxed until complete dissolution occurred (approximately 30 min). After an additional 10 min the reaction mixture was cooled to 0 °C and 550 mL of ether added. The mixture was placed in the refrigerator overnight. The next day the liquid was decanted and 50 mL of water added to the remaining solid. The aqueous solution of the benzyl ester hydrochloride was subsequently washed 2× with ether. The aqueous solution was then adjusted to pH 6.25 with concentrated ammonium hydroxide, at which time a solid appeared. After 2 h in the refrigerator, the solid was filtered and washed with cold water until the washes no longer formed a white precipitate when treated with aqueous silver nitrate. The solid was then recrystallized from boiling water. The product **4** was collected and washed with cold water: 4.84 g (30%); mp 181–182 °C (stock[16] 181–182 °C for protonated compound); R_f(**6a,e**) 0.25; $[\alpha]^{20}_D$ (*c* 1.0, 1 N HCl) +23.8° (stock[16] $[\alpha]^{22}_D$ (*c* 2.5, 1 N HCl) +27.7° for the protonated compound).

***N*-(*tert*-Butyloxycarbonyl)-γ-benzyl[α-^{2}H]glutamate (5).** To a solution of triethylamine (1.73 mL, 12.4 mmol) in 75 mL of *N*,*N*-dimethylformamide was added γ-benzyl[α-^{2}H]glutamate (**4**; 3.16 g, 11.3 mmol). After 15 min, di-*tert*-butyl dicarbonate (2.71 g, 12.4 mmol) was added to the slurry. Seventeen hours later the clear reaction mixture was concentrated under reduced pressure to give an oil. Water was added along with enough saturated sodium bicarbonate solution to dissolve the oil. The basic solution of the diprotected glutamic acid derivative was washed 3× with ether to remove any unreacted di-*tert*-butyl dicarbonate.

The aqueous solution was cooled to 0 °C and acidified with concentrated hydrochloric acid until pH 1. The oily mixture was subsequently extracted 3× with ethyl acetate. The ethyl acetate solution was washed 4× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated, under reduced pressure, to give an oil (**5**; 3.82 g, 100%; R_f(**1a,e**) 0.42) which was carried on to the next reaction step.

***N*-(*tert*-Butyloxycarbonyl)-γ-benzyl[α-^{2}H]glutamic Acid Methyl Ester (6).** To a solution of the diprotected free acid (**5**; 3.82 g, 11.3 mmol) dissolved in 50 mL of absolute ethanol at 0 °C was added diazomethane[17] in ether until the yellow color persisted. After 45 min acetic acid was added to destroy the excess diazomethane. The solvents were removed under reduced pressure to give an oil. A solid eventually formed after triturating the oil with hexanes/petroleum ether (1/1, v/v). The white solid was collected, washed with hexanes, and subsequently recrystallized from ethyl acetate/hexanes. The product **6** was collected and washed with hexanes: 3.71 g (93%); mp 41–42 °C (mp 40–41 °C[1] for the protonated analogue); R_f(**1a,b,e**) 0.66; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −16.5° (−16.0°[1] for the protonated analogue).

***N*-(*tert*-Butyloxycarbonyl)[α-^{2}H]glutamic Acid Methyl Ester, Dicyclohexylamine Salt (7).** Nitrogen was bubbled through a solution of the methyl ester (**6**; 3.66 g, 10.4 mmol) in 50 mL of methanol for 15 min. Palladium (10%) on carbon (0.5 g) was added and hydrogen introduced above the reaction mixture was filtered through Celite and the solvent removed under reduced pressure to give an oil (lit.[18] oil; R_f(**1a,b,c**) 0.51).

The oil was dissolved in 20 mL of anhydrous ether and cooled to 0 °C. Dicyclohexylamine (2.30 mL, 11.4 mmol) was added, with stirring. After 1 h the white solid was collected and washed with cold ether. The solid was recrystallized from chloroform/ether.

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(18) Anderson, J. C.; Barton, M. A.; Hardy, P. M.; Kenner, G. W.; Preston, J.; Sheppard, R. C. *J. Chem. Soc.* **1967**, 108–113.

The product 7 was collected and washed with cold ether: 4.56 g (99%); mp 168–169 °C (mp 167–168 °C[1] for the protonated analogue); R_f(3a,b,c)[19] 0.53; $[\alpha]^{20}_D$ (c, 1.0, MeOH) −12.7° (lit.[20] −13.0° for the protonated analogue).

***N*-(*tert*-Butyloxycarbonyl)-γ-succinimidyl[α-^{2}H]glutamic Acid Methyl Ester (8).** The dicyclohexylamine salt (7; 2.01 g, 4.55 mmol), 50 mL of ethyl acetate, and 20 mL of 2 M aqueous sodium bisulfate solution were added to a separatory funnel. The solid dissolved upon mixing. The aqueous layer was discarded, and the ethyl acetate solution was extracted with 2 M sodium bisulfate solution. The organic layer was washed 3× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was filtered and the filtrate concentrated under reduced pressure to give an oil.

The oil and *N*-hydroxysuccinimide (0.52 g, 4.55 mmol) were dissolved in 10 mL of tetrahydrofuran and cooled to 0 °C, and *N*,*N*′-dicyclohexylcarbodiimide (0.940 g, 4.55 mmol) was added. The reaction was allowed to warm to room temperature. After 24 h the reaction mixture was cooled to 0 °C, the *N*,*N*′-dicyclohexylurea filtered, and the solid washed with 40 mL of cold tetrahydrofuran. The filtrate was concentrated under reduced pressure to give an oil. Solid was obtained by crystallizing the oil from ethyl acetate/hexanes. The product 8 was collected and washed with hexanes: 1.42 g (87%); mp 118–119 °C (mp 118–119 °C[1] for the protonated analogue); R_f(1a,b,e) 0.54; $[\alpha]^{20}_D$ (c 1.0, MeOH) −15.6° (−15.6°[1] for the protonated analogue); mass spectrum, *m*/*e* 360 (M + 1). Anal. Calcd for $C_{15}{}^1H_{21}{}^2HN_2O_8$: C, 50.1; H, 6.5; N, 7.8. Found: C, 50.0; H, 6.4; N, 7.6.

***N*-(*tert*-Butyloxycarbonyl)-γ-(diazomethyl)[α-^{2}H]glutamic Acid Methyl Ester (9).** The methyl ester 9 was synthesized, in part, from the procedure given in Johnson et al.[5] The dicyclohexylamine salt (7; 4.50 g, 10.2 mmol) was liberated in the same manner as compound 7 given in the preparation of compound 8. The resulting oil was dried, in vacuo, over sodium hydroxide pellets for 12 h. The oil was then dissolved in 25 mL of purified tetrahydrofuran containing *N*-methylmorpholine (1.13 g, 10.2 mmol). After cooling to −30 °C by means of an acetone/dry ice bath, isobutyl chloroformate (1.32 mL, 10.2 mmol) dissolved in 10 mL of purified tetrahydrofuran was added slowly to the reaction mixture. After complete addition, 10 min elapsed before addition of 20 mL of ether (precooled to −75 °C). The *N*-methylmorpholine hydrochloride was filtered while the filtrate was collected into the receiving flask precooled to −50 °C. The filtrate was subsequently added to 125 mL of an ethereal solution of diazomethane[17] maintained at −20 °C. After 1 h the reaction mixture was brought to room temperature.

Twenty-one hours later the solvents were removed under reduced pressure to give a gold-colored oil. The oil was dissolved in 50 mL of ethyl acetate and subsequently washed 3× with saturated sodium bicarbonate solution, 3× with saturated sodium chloride solution, and finally dried over magnesium sulfate. The drying agent was filtered and the filtrate concentrated under reduced pressure to give a gold-colored oil (2.65 g, 92%; R_f(1a,e) 0.59). The product 9 was carried on to the next reaction step.

***N*-(*tert*-Butyloxycarbonyl)-δ-benzyl[α-^{2}H]aminoadipic Acid Methyl Ester (10).** The 2-aminoadipic acid derivative 10 was obtained, in part, from the procedure given in Johnson et al.[5] The *tert*-butyloxycarbonyl-protected methyl ester (9; 2.65 g, 9.29 mmol) was dissolved in 15 mL of tetrahydrofuran, followed by the addition of 15 mL of benzyl alcohol. After the temperature of the reaction mixture was raised to 35 °C, a solution of silver benzoate (1.06 g, 4.65 mmol) in triethylamine (15.0 mL, 10.8 mmol) was added dropwise, noting the evolution of nitrogen. After 18 h the reaction mixture was treated with decolorizing carbon for 30 min. The mixture was filtered through Celite and the solvents were removed under vacuum to give an oil. The oil was dissolved in 100 mL of ethyl acetate, and the solution washed 3× with 2 M sodium bisulfate solution and 3× with saturated sodium chloride solution and finally dried over magnesium sulfate. The drying agent was removed by filtration and the filtrate concentrated under reduced pressure to give an oil (10) which was carried on to the next reaction step.

***N*-(*tert*-Butyloxycarbonyl)[α-^{2}H]aminoadipic Acid Methyl Ester, Dicyclohexylamine Salt (11).** Nitrogen was bubbled through a solution of the triprotected aminoadipic acid derivative (10; 9.29 mmol) in 50 mL of methanol for 15 min. Palladium (10%) on carbon (0.5 g) was added and hydrogen introduced above the reaction mixture at atmospheric pressure. After 10 h the mixture was filtered through Celite and the solvent removed under reduced pressure to give a gold-colored oil.

The oil was dissolved in 50 mL of ethyl acetate and extracted 3× with saturated sodium bicarbonate solution. The basic washes were collected, cooled to 0 °C, and acidified to pH 1 with concentrated hydrochloric acid. The acid solution was then extracted 3× with 20-mL portions of ethyl acetate. The ethyl acetate extractions were combined, washed 4× with saturated sodium chloride solution, and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil. The oil was dissolved in 5 mL of ethyl acetate, cooled to 0 °C, and dicyclohexylamine (3.70 mL, 18.6 mmol) added, with stirring. After 15 min the mixture was placed in the freezer overnight. The product 11 was then collected and washed with ether: 2.33 g (55% for three reaction steps); mp 143–144 °C; R_f(1a)[19] 0.47; $[\alpha]^{20}_D$ (c 1.0, MeOH) −10.6°; mass spectrum, *m*/*e* 458 (M + 1). Anal. Calcd for $C_{24}{}^1H_{43}{}^2HN_2O_6$: C, 63.0; H, 9.9; N, 6.1. Found: C, 62.8; H, 9.6; N, 5.9.

***N*-(*tert*-Butyloxycarbonyl)-δ-succinimidyl[α-^{2}H]aminoadipic Acid Methyl Ester (12).** The dicyclohexylamine salt (11; 0.32 g, 0.69 mmol) was liberated in the same manner as compound 7 given in the preparation of compound 8. The resulting oil and *N*-hydroxysuccinimide (0.080 g, 0.69 mmol) were dissolved in 25 mL of tetrahydrofuran. At 0 °C, *N*,*N*′-dicyclohexylcarbodiimide (0.14 g, 0.69 mmol) was added and after 1 h the reaction mixture was allowed to warm to room temperature. Twenty-four hours later the mixture was cooled to 0 °C and the *N*,*N*′-dicyclohexylurea filtered. The filtrate was concentrated under reduced pressure to give an oil which was then dissolved in 50 mL of ethyl acetate. The organic solution was washed 3× with saturated sodium bicarbonate solution and 3× with saturated sodium chloride solution and finally dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil (0.25 g, 95%; R_f(3a,e) 0.56. The product 12 was carried on to the next reaction.

***N*-(*tert*-Butyloxycarbonyl)-δ-[ε-^{15}N]amido[α-^{2}H]aminoadipic Acid Methyl Ester (13).** To a solution of the protected succinimide ester (12; 0.25 g, 0.66 mmol) and triethylamine (202 μL, 1.45 mmol) in 15 mL of *N*,*N*-dimethylformamide was added, with vigorous stirring, ammonium chloride 99% enriched in ^{15}N (0.04 g, 0.72 mmol). After 19 h the solvent was removed under vacuum, leaving an oil which was dissolved in 25 mL of ethyl acetate. The organic solution was washed 3× with 2 M sodium bisulfate solution, 3× with saturated sodium bicarbonate solution, 3× with saturated sodium chloride solution and finally dried over magnesium sulfate. After filtering the drying agent, the filtrate was concentrated under reduced pressure to give an oil (13; 0.13 g; R_f(3a) 0.56) which was carried on to the next reaction step.

δ-[ε-^{15}N]Amido[α-^{2}H]aminoadipic Acid Methyl Ester, Hydrochloride Salt (14). To a flask containing the dilabeled methyl ester (13; 0.13 g, 0.50 mmol) was added 7 mL of 4 N hydrochloric acid in dioxane. After 2 h the solvent was removed under reduced pressure and the remaining oil dried, in vacuo, over sodium hydroxide pellets for 12 h. The oil (14; 0.10 g, 100%; R_f(6a) 0.36) was carried on to the next reaction step.

***N*-Acetyl-D,L-aminoadipic Acid (16).** To a mixture of D,L-aminoadipic acid (15; 9.95 g, 61.7 mmol) in 100 mL of water was slowly added sodium carbonate monohydrate (25.3 g, 204 mmol). The clear solution was cooled to 0 °C and acetic anhydride (11.6 mL, 123 mmol) added. After 1 h the reaction mixture, containing a small amount of white solid, was brought to room temperature for 24 h. The mixture was cooled to 0 °C and concentrated hydrochloric acid added *slowly* until pH 1. This acid solution was placed in a continuous liquid–liquid extraction apparatus and extracted for 3 days with ethyl acetate. The ethyl acetate solution was dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to yield an oil. After drying, in vacuo, overnight the oil was triturated by using petroleum ether. Eventually, a soft solid formed which was

(19) The compound decomposes on TLC plate; only the R_f of major spot is reported.

(20) Schroder, E.; Klieger, E. *Liebigs Ann. Chem.* **1964**, *673*, 196–207.

collected and washed with petroleum ether. After drying, in vacuo, for 2 h a clumpy solid (**16**) remained: 9.81 g (78%); mp 96–98 °C; R_f(**6c**) 0.49.

***N*-Acetyl-D,L-[α-^{2}H]aminoadipic Acid (17).** The deuteriated product **17** was obtained in the same manner as described in the synthesis of the glutamic acid homologue **2** except that sodium carbonate monohydrate was used as the base rather than sodium hydroxide. The product (**17**; 90%; mp 96–98 °C; R_f(**6a**) 0.49) was isolated by continuously extracting the acid solution with ethyl acetate as described in the previous procedure (synthesis of compound **16**). The product was 65% enriched in deuterium at the α-carbon as shown by proton NMR. The procedure was repeated two more times to obtain product 96% enriched in deuterium.

L-[α-^{2}H]Aminoadipic Acid (18). The product **18** was obtained by the procedure given in the synthesis of compound **3**: 70%; mp 198–199 °C dec (lit.[21] mp 205 °C dec); R_f(**6a**) 0.15; $[\alpha]^{20}_D$ (*c* 2.0, 5 N HCl) +22.2° (lit.[21] +22° for the protonated analogue).

***N*-(Benzyloxycarbonyl)[α-^{2}H]aminoadipic Acid (19).** Sodium bicarbonate (2.46 g, 29.3 mmol) was added to a solution of L-[α-^{2}H]aminoadipic acid (**18**; 1.05 g, 6.51 nmol) dissolved in 30 mL of water. The reaction mixture was cooled to 0 °C and benzyl chloroformate (1.02 mL, 7.17 mmol) added with extensive stirring. After 4 h the mixture was raised to room temperature for an additional 8 h. The reaction mixture was then extracted 2× with ether. The aqueous solution was cooled to 0 °C and acidified with concentrated hydrochloric acid until pH 1. The oily mixture was extracted 3× with ethyl acetate. The organic solution was washed 3× with 2 M sodium bisulfate solution and 4× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give a white solid. Ether was added and the product placed in the freezer overnight. The product **19** was collected and washed with cold ether: 1.44 g (75%); mp 122–124 °C; R_f(**1c,e**) 0.40; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −8.0°.

***N*-(Benzyloxycarbonyl)-[α-^{2}H]aminoadipic Acid Anhydride (20).** A mixture of the benzyloxycarbonyl-protected compound (**19**; 1.42 g, 4.81 mmol) and 75 mL of acetic anhydride were slowly heated to 50 °C. After 1 h at this temperature, the solvents were removed under reduced pressure to give an oil (1.32 g, 100%; R_f[19](**3c**) 0.44). The oil (**20**) was subsequently dried, in vacuo, over sodium hydroxide pellets and carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)[α-^{2}H]aminoadipic Acid Methyl Ester (21).** The product **21** was obtained, in part, by the procedure given in Klieger et al.[22] To a solution of the cyclic anhydride (**20**; 1.32 g, 4.8 mmol) dissolved in 28 mL of methanol/ether (1/3, v/v) was added dicyclohexylamine (1.3 mL, 6.34 mmol). After 24 h the solvents were removed under reduced pressure and the remaining oil was dissolved in 50 mL of ethyl acetate. The organic solution was washed 3× with 2 M sodium bisulfate solution and 4× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil. Column chromatography (column: *d* = 5 cm, *h* = 15 cm) was carried out by using ethyl acetate/acetic acid (100/0.75, v/v) as the eluent at a flow rate of 4 cm/min. The compound corresponding to the faster moving spot (on TLC) was collected and the solvents were removed under reduced pressure to give an oil (0.85 g, 57%; R_f(**1c**) 0.55). The product **21** was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)-δ-succinimidyl[α-^{2}H]aminoadipic Acid Methyl Ester (22).** The oil (**21**; 0.85 g, 2.8 mmol) from the previous reaction and *N*-hydroxysuccinimide (0.38 g, 3.3 mmol) were dissolved in 10 mL of tetrahydrofuran and cooled to 0 °C. *N,N'*-Dicyclohexylcarbodiimide (0.69 g, 3.3 mmol) was added and, after 1 h, the reaction mixture brought to room temperature. After 16 h the mixture was cooled to 0 °C and the *N,N'*-dicyclohexylurea removed. The filtrate was concentrated under reduced pressure to give an oil. The oil was dissolved in 35 mL of ethyl acetate and washed 3× with saturated sodium bicarbonate solution and 3× with saturated sodium chloride solution and dried over magnesium sulfate. The drying agent was filtered and the filtrate concentrated under reduced pressure to give an oil (**22**; 1.0 g, 89%; R_f(**1c,e**)[19] 0.57) which was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)-δ-[ε-^{15}N]amido[α-^{2}H]aminoadipic Acid Methyl Ester (23). Method 1.** To a solution of the methyl ester hydrochloride (**14**; 0.01 g, 0.50 mmol) and triethylamine (209 μL, 1.50 mmol) dissolved in 7 mL of *N,N*-dimethylformamide at 0 °C was added benzyl chloroformate (72 μL, 0.50 mmol). After 4 h the reaction mixture was raised to room temperature for 20 h. The solvent was removed under reduced pressure to give an oil. The oil was dissolved in 50 mL of ethyl acetate, washed 3× with 2 M sodium bisulfate solution, 3× with saturated sodium bicarbonate solution, and 4× with saturated sodium chloride solution, and dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil. The product **23** was obtained by crystallizing the oil from ethyl acetate/petroleum ether: 0.053 g (36% for two reaction steps), mp 94–95 °C; R_f (**3a,b,e**) 0.48; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −12.9°; mass spectrum, *m/e* 311 (M + 1). Anal. Calcd for $C_{15}{}^1H_{19}{}^2H^{14}N^{15}NO_5$: C, 58.1; H, 6.8; N, 9.4. Found: C, 58.1; H, 6.8; N, 9.3.

Method 2. To a solution of the active ester (**22**; 1.0 g, 2.48 mmol) and triethylamine (0.76 mL, 5.46 mmol) dissolved in 15 mL of *N,N*-dimethylformamide was added, with vigorous stirring, ammonium chloride 99% enriched in ^{15}N (0.15 g, 2.72 mmol). After 24 h the solvent was removed under reduced pressure to give an oil. The oil was dissolved in 50 mL of ethyl acetate, washed 3× with 2 M sodium bisulfate solution, 3× with saturated sodium bicarbonate solution, and 3× with saturated sodium chloride solution, and finally dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil. Column chromatography (column: *d* = 5 cm; *h* = 18 cm) was carried out by using ethyl acetate/acetic acid (1000/12.5, v/v) as the eluent at a flow rate of 2.5 cm/min. The compound whose R_f was 0.23 (**1a,b,e**) on TLC was isolated. The solvents were removed under reduced pressure to give a solid. The solid was recrystallized from ethyl acetate/petroleum ether. The product **23** was collected and washed with petroleum ether: 0.11 g (14% for four reaction steps); mp 94–95 °C; R_f(**3a,b,e**) 0.48; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −12.9°; mass spectrum, *m/e* 311 (M + 1). Anal. Calcd for $C_{15}{}^1H_{19}{}^2H^{14}N^{15}NO_5$: C, 58.1; H, 6.8; N, 9.4. Found: C, 58.0; H, 6.7; N, 9.3.

***N*-(Benzyloxycarbonyl)-[ε-^{15}N,α-^{2}H]lysinol, Hydrochloride Salt (24).** One molar borane in tetrahydrofuran (20 mL) was added to the dilabeled methyl ester (**23**; 0.067 g, 0.24 mmol) and the mixture refluxed for 3 h. The reaction mixture was cooled to room temperature and a solution of 10 mL of 4 N hydrochloric acid/dioxane in 25 mL of methanol was added. After refluxing the reaction mixture for 1 h, the solvents were removed under reduced pressure to give a white oil (**24**; R_f(**6a,e**) 0.67) which was carried on to the next reaction step.

***N*-(Benzyloxycarbonyl)-[ε-^{15}N,α-^{2}H]lysinol-*N'*-(*tert*-butyloxycarbonyl)[α-^{2}H]glutamic Acid Methyl Ester (25).** To a solution of the lysinol derivative **24** and triethylamine (50.5 μL, 0.362 mmol) dissolved in 5 mL of *N,N*-dimethylformamide was added the succinimide ester (**8**; 0.130 g, 0.362 mmol). After 24 h (*N,N*-dimethylamino)ethylamine (15 μL, 0.13 mmol) was added and the reaction stirred for an additional 1 h. The solvent was removed under reduced pressure to give an oil. The oil was dissolved in 25 mL of ethyl acetate, washed 3× with 2 M sodium bisulfate solution, 3× with saturated sodium bicarbonate solution, and 3× with saturated sodium chloride solution, and dried over magnesium sulfate. The drying agent was filtered and the filtrate concentrated under reduced pressure to give an oil. Column chromatography (column: *d* = 4 cm, *h* = 18 cm) was carried out by using ethyl acetate/acetic acid (1000/15, v/v) as the eluent at a flow rate of 2.5 cm/min. The compound whose R_f value (on TLC) was 0.21 (**1a,e**) was isolated. The solvent was removed under reduced pressure to give an oil (**25**): 0.064 g (53%); R_f(**1a,e**) 0.21.

***N*-(Benzyloxycarbonyl)-[ε-^{15}*N*,α-^{2}*H*']lysyl-*N'*-(*tert*-butyloxycarbonyl)[α-^{2}H]glutamic Acid Methyl Ester, Dicyclohexylamine Salt (26).** A solution of pyridinium dichromate[11] (0.50 g, 1.4 mmol) in 4 mL of *N,N*-dimethylformamide was added to the trilabeled dipeptide (**25**; 0.146 g, 0.285 mmol) and the reaction mixture heated to 50 °C by means of a water bath. After 24 h the reaction mixture was concentrated to a minimum and 25 mL of ethyl acetate added. The organic solution was washed

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(22) Klieger, E.; Gibian, H. *Liebigs Ann. Chem.* **1962**, *655*, 195–210.

3× with 2 M sodium bisulfate solution followed by successive water washings until the aqueous layer remained clear. The ethyl acetate layer was then dried over magnesium sulfate. The drying agent was removed and the filtrate concentrated under reduced pressure to give an oil. The oil was dissolved in 3 mL of ethyl acetate and cooled to 0 °C. With stirring, dicyclohexylamine (62.5 μL, 0.314 mmol) was added. Five minutes later 15 mL of ether was added and the mixture placed in the freezer overnight. The product **26** was collected and washed with cold ether: 0.141 g (70%); mp 123–124 °C (lit.[1] mp 122–123 °C for the protonated analogue); R_f(**3a,b,c,e**)[19] 0.48; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −1.4° (−1.3°[1] for the protonated analogue).

The following compounds were synthesized by the procedures given for the unlabeled analogues.[1]

***N*-(Benzyloxycarbonyl)-α-(pentachlorophenyl)[ε-^{15}N,α-^{2}H]lysyl-*N*′-(*tert*-butyloxycarbonyl)[α-^{2}H]glutamic Acid Methyl Ester.** Physical data: mp 154–155 °C; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −20.5° (mp 154–155 °C; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −20.6° for the protonated analogue).[1]

***N*-(Benzyloxycarbonyl)-α-(pentachlorophenyl)[ε-^{15}N,α-^{2}H]lysyl[α-^{2}H]glutamic Acid Methyl Ester, Hydrochloride Salt.** Physical data: mp 123–125 °C; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −4.9° (mp 123–125 °C; $[\alpha]^{20}_D$ (*c* 1.0, MeOH) −4.9° for the protonated analogue).[1]

***N*-(Benzyloxycarbonyl)-*cyclo*([ε-^{15}N,α-^{2}H]lysyl[α-^{2}H]-glutamyl)Methyl Ester.** Physical data: mp 232–234 °C dec; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −50.6° (mp 232–232.5 °C dec; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −50.5° for the protonated analogue);[1] mass spectrum, *m/e* 409 (M + 1). Anal. Calcd for $C_{20}{}^{1}H_{25}{}^{2}H_{2}{}^{14}N_{2}{}^{15}NO_6$: C, 58.8; H, 7.2; N, 10.5. Found: C, 58.7; H, 7.0; N, 10.4.

Acetyl-*cyclo*([ε-^{15}N,α-^{2}H]lysyl[α-^{2}H]glutamyl) Methyl Ester. Physical data: mp 291–293 °C dec; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −92.1° (mp 291–292 °C; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −92.0° for the protonated analogue).[1]

***N*-Acetyl-*cyclo*([ε-^{15}N,α-^{2}H]lysyl[α-^{2}H]glutamyl) Hydrazide.** Physical data: mp 276–278 °C dec; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −77.3° (mp 276–278 °C dec; $[\alpha]^{20}_D$ (*c* 1.0, TFE) −77.5°).[1]

***N*-Acetyl-*cyclo*(L-[ε-^{15}N,α-^{2}H]lysyl-L-[α-^{2}H]glutamyl)-*cyclo*(D-lysyl-D-glutamyl) *N*-Methylamide (I).** Physical data: mp >320 °C; $[\alpha]^{20}_D$ (*c* 0.5, TFE) −21.6° (mp > 320 °C; $[\alpha]^{20}_D$ (*c* 0.5, TFE) −21.8°);[1] mass spectrum, *m/e* 555 (M + 1). Anal. Calcd for $C_{25}{}^{1}H_{39}{}^{2}H_{2}{}^{14}N_{6}{}^{15}NO_7$: C, 54.1; H, 7.8; N, 17.9. Found: C, 53.9; H, 7.6; N, 17.6.

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(α-Haloalkyl)phosphonium Salts and Sulfur Nucleophiles: A New Type of Reaction Mechanism[1a,b]

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Reaction between (α-haloalkyl)phosphonium salts and some sulfur nucleophiles leads to the substitution product $Ph_3P^+CH_2SR\ X^-$. Evidence is presented that this substitution is not a normal S_N2 reaction and that it occurs through formation of a phosphonium ylide and a disulfide, reaction between them, and action of the resulting salt on the starting phosphonium salt. Then RSX and $Ph_3P{=}CH_2$ reenter the sequence, giving rise to a three-step chain nucleophilic substitution.

Recently is has been shown that aryl[2] and alkyl thiolates[3] can be converted into difluorinated thioethers by reaction with fluorinated polyhalomethane (eq 1). This conversion

$$RSNa + CF_2BrX \rightarrow RSCF_2Br + NaX \qquad (1)$$

$$R = R, Ar;\ X = Br, Cl$$

occurs via a chain reaction with the intermediate formation of difluorocarbene.[2] It has been extended (albeit in low yield) to some dialkyldithiocarbamyl anions in order to test the fungicidal activity of the resulting compounds.[4] In contrast, the corresponding reaction with several nonfluorinated polyhalomethanes led to thiocarbamoyl disulfides and/or sulfides only (eq 2). Evidently, these

$$R_2NC(S)SNa + \left\{\begin{matrix} CCl_4,\ BrCCl_3,\ CHCl_3,\ CHBr_3 \\ CCl_2{=}CCl_2,\ BrCH_2Cl \end{matrix}\right\} \rightarrow [R_2NC(S)]_2Z \qquad (2)$$

$$Z = SS, S$$

polyhalomethanes do not sustain the chain reaction that occurs with CF_2BrX, since the decomposition rate of, e.g., CX_3^- (X = Cl, Br) is much lower than that of CF_2X^-.[5] The products obtained, however, are clearly formed through the intermediate sulfenyl halide, e.g. eq 3, pointing out the

$$RS^- + BrCCl_3 \rightarrow CCl_3^- + RSBr \xrightarrow{RS^-} RSSR \qquad RSBr \xrightarrow{-S} RBr \xrightarrow{RS^-} RSR \qquad (3)$$

$$RS^- = R_2NC(S)S^-$$

remarkable tendency of the dialkyldithiocarbamyl anion to undergo halogenophilic attack,[6] which is a soft–soft interaction. This tendency is greater than that of alkyl[7] and aryl[8] thiolates, since it occurs even with $BrCH_2Cl$, which undergoes the normal substitution of bromine by heteroaromatic thiophenols.[9] It was therefore expected that reaction between (α-iodomethyl)triphenylphosphonium iodide (**1a**) and sodium *N,N*-dimethyldithiocarbamate (**2**) would lead to a phosphonium ylide. Actually, by stirring **1a** and **2** (1:1 ratio) in chloroform

(1) (a) Presented in part at the 12th International Symposium on the Organic Chemistry of Sulfur, Nijmegen, The Netherlands, June-July 1986; paper OB15. (b) Taken from the dissertations of Lucia Pozzi and Maria Grazia Ghezzi, required for their University Degree respectively in Pharmacy (1984) and Pharmaceutic Chemistry and Technology (1985), University of Milan.

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