Organic & Biomolecular Chemistry

Supplementary Data for

Unexpected racemization and the hydrogen-deuterium exchange of the hydrogen at the α-carbon of proline analogs containing the 5-azoniaspiro[4.4]nonyl-group

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Keywords: Proline · Betaine · Hydrogen-Deuterium Exchange · Charged-tagged Peptides

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1. Synthetic details

a. Synthesis of H-γAbu-OEt·HCl[1]

A solution of 20.6 g (0.2 mol) of 4-aminobutyric acid (γAbu) in 140 mL of anhydrous ethanol in a 250 mL round bottom flask was prepared. After cooling down the solution in the ice-water bath, 20 mL (0.275 mol) of thionyl chloride were slowly added in 2 mL portions with a vigorous stirring. Next, the ice-water bath was removed and the solution was left at room temperature for additional 2 h. Ethanol was removed on a rotary evaporator and obtained colorless oil was recrystallized from hot ethanol; yield: 24.0 g (94%).

i. Analysis

^{1}H NMR (@500 MHz, CD_3CN, 300 K) δ: 1.22 (t, J = 7.1 Hz, 3H); 1.97–2.03 (m, 2H); 2.47 (t, J = 7.3 Hz, 2H); 2.98–3.04 (m, 2H); 4.10 (q, J = 7.1 Hz, 2H); 8.08 (s, 3H).

^{13}C{H} NMR (@125 MHz, CD_3CN, 300 K) δ: 14.5; 23.4; 31.9; 40.0; 61.4; 173.6

1. ^{1}H-NMR

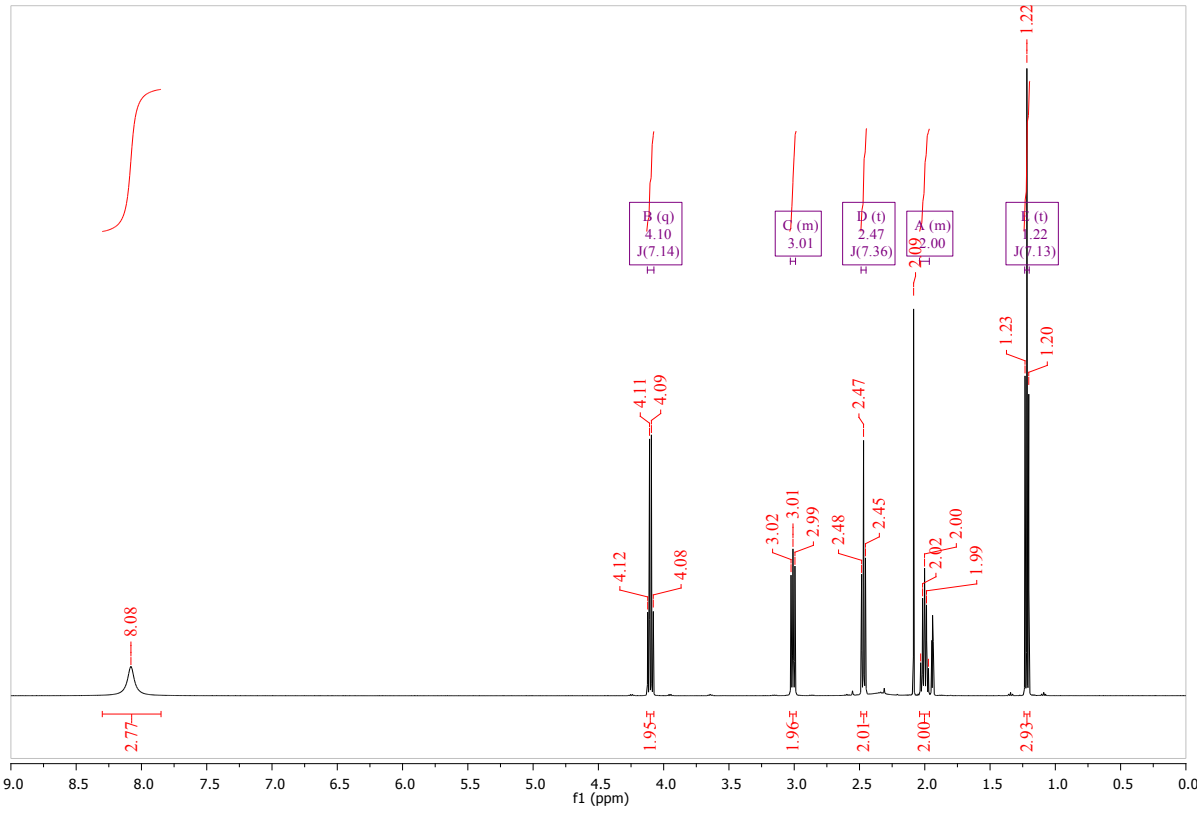


Figure 1S. ^{1}H NMR spectrum (@500 MHz, CD_3CN, 300 K, δ) of H-γAbu-OEt·HCl

2. ^{13}C-NMR

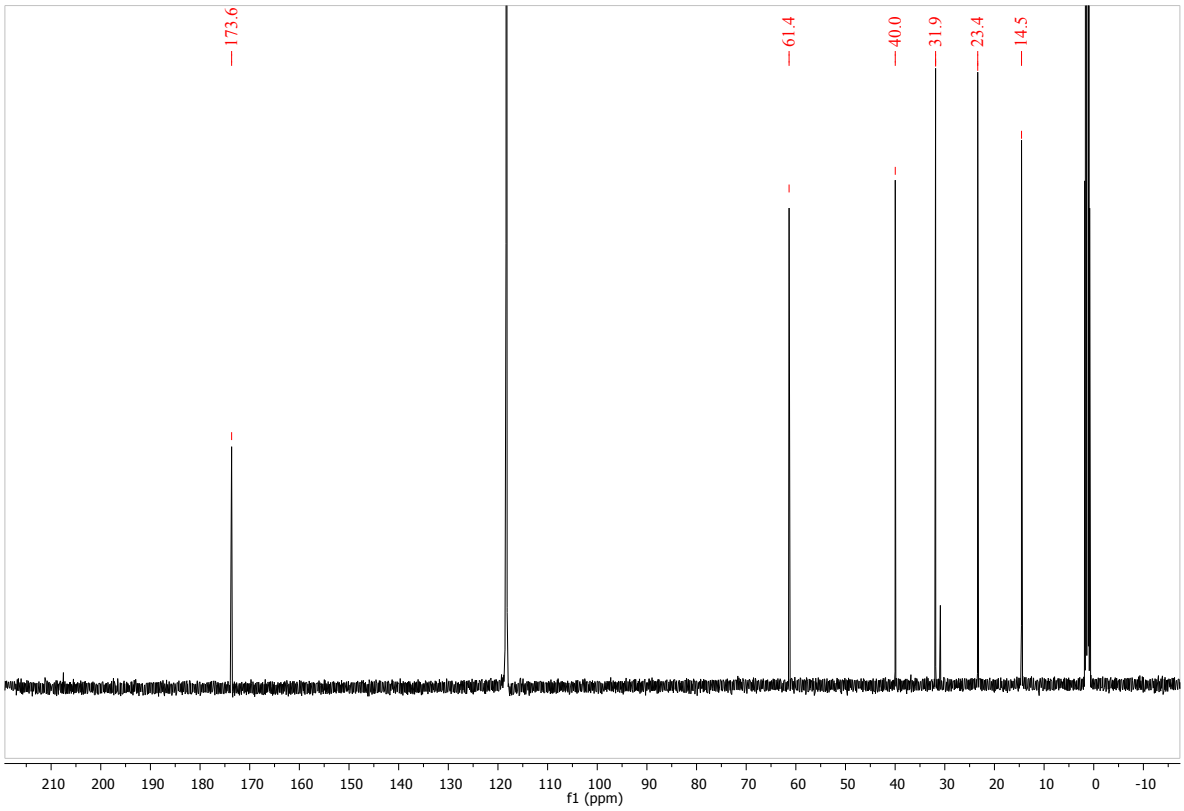
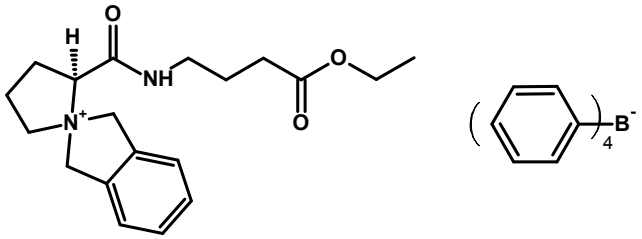


Figure 2S. ^{13}C{H} NMR spectrum (@125 MHz, CD_3CN, 300 K, δ) of H-γAbu-OEt·HCl

b. Synthesis of BASN$^+$-CO-γAbu-OEt·BPh_4



In a 100 mL round bottom flask chilled in the ice-water bath, a solution of Boc-Pro-OH (2.15 g, 10 mmol) and *N*-methylmorpholine (NMM, 1.10 mL, 10 mmol) in tetrahydrofuran (THF, 25 mL) was prepared. Then, isobuthyl chloroformate (1.34 mL, 10.5 mmol) was added with a vigorous stirring. Next, a solution of H-γAbu-OEt·HCl (1.67 g, 10 mmol) and triethylamine (TEA, 1.40 mL, 10 mmol) in DMF (10 mL) were added. Reaction proceeded for 1 h. The TEA·HCl salt was filtered off and the filtrate was concentrated. Obtained colorless oil was dissolved in 50 mL of ethyl acetate and washed with $KHSO_4$, diluted $NaHCO_3$, and H_2O. After removing the solvent on a rotary evaporator, the product was dried. Dipeptide Boc-Pro-γAbu-OEt was purified by flash chromatography using a Büchi Sepacore® flash system X50 (column – Sepacore® Silica 12 g; eluent – chloroform/methanol,

95:5 v/v); yield: 2.2 g (67%).[2] The Boc protecting group was removed with 10 mL of TFA/dichloromethane (DCM) (95:5, v/v); yield: 2.29 g (quantitative).

A benzo-5-azoniaspiro[4.4]nonyl-carbonyl group was obtained by mixing 1.02 g (3 mmol) of H-Pro-γAbu-OEt·CF_3COO^-, 0.79 g (3 mmol) of α,α'-dibromo-*o*-xylene and 1.24 g (9 mmol) of K_2CO_3 in 25 mL of acetonitrile for 24 h. After filtering off potassium carbonate, the filtrate was concentrated. Colorless oil was resuspended in a fresh portion of acetonitrile and chilled in the ice-water bath. An anion-exchange reaction was performed using 1.02 g (3 mmol) of sodium tetraphenylborate ($NaBPh_4$). Sodium bromide was filtered off on a filter funnel with a Celite® S pad and the product was dried under *vacuum*. Crystallization of $BASN^+$-CO-γAbu-OEt·BPh_4 was initiated by addition of methanol; yield: 0.33 g (17%).

i. Analysis

Boc-Pro-γAbu-OEt:

ESI–MS: *m/z* 351.199 $[M+Na]^+$; calc. for $C_{16}H_{28}N_2NaO_5$ $[M+Na]^+$: 351.189

^{1}H NMR (@500 MHz, $CDCl_3$, 300 K) δ: 1.24 (t, *J* = 7.1 Hz, 3H); 1,45 (s, 9H); 1.80–1.85 (m, 6H); 2.33 (t, *J* = 7.4 Hz, 2H); 3.26–3.48 (m, 4H); 4.12 (q, *J* = 7.1 Hz, 2H); 4.23 (1H).

^{13}C{H} NMR (@125 MHz, $CDCl_3$, 300 K) δ: 14.3; 24.1; 24.8; 28.2; 28.5; 31.7; 38.8; 47.2; 60.6; 61.4; 80.5; 155.9; 172.3; 173.3

1. ESI-MS

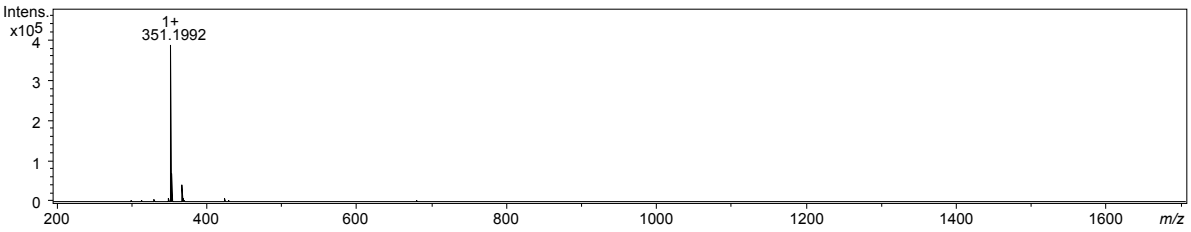


Figure 3S. ESI-MS spectrum of Boc-Pro-γAbu-OEt

2. ^{1}H-NMR

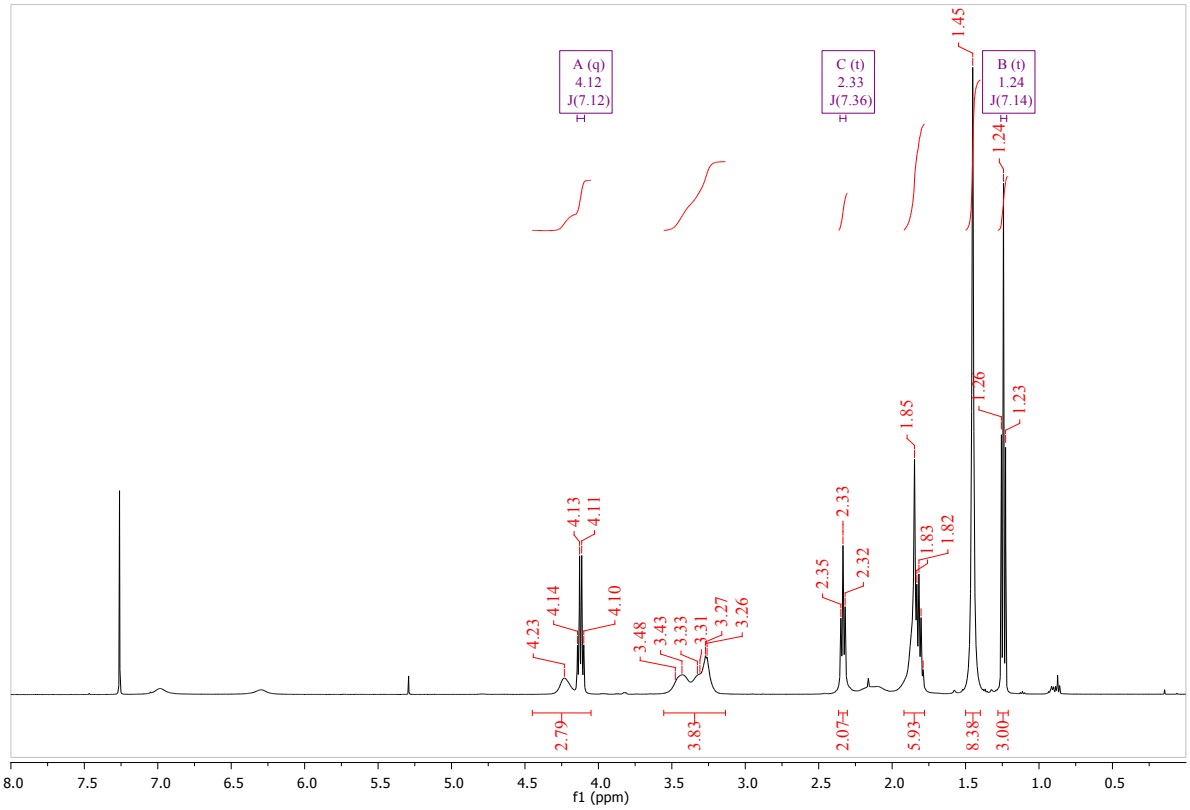


Figure 4S. ^{1}H NMR spectrum (@500 MHz, $CDCl_3$, 300 K, δ) of Boc-Pro-γAbu-OEt

3. $^{13}C\{H\}$-NMR

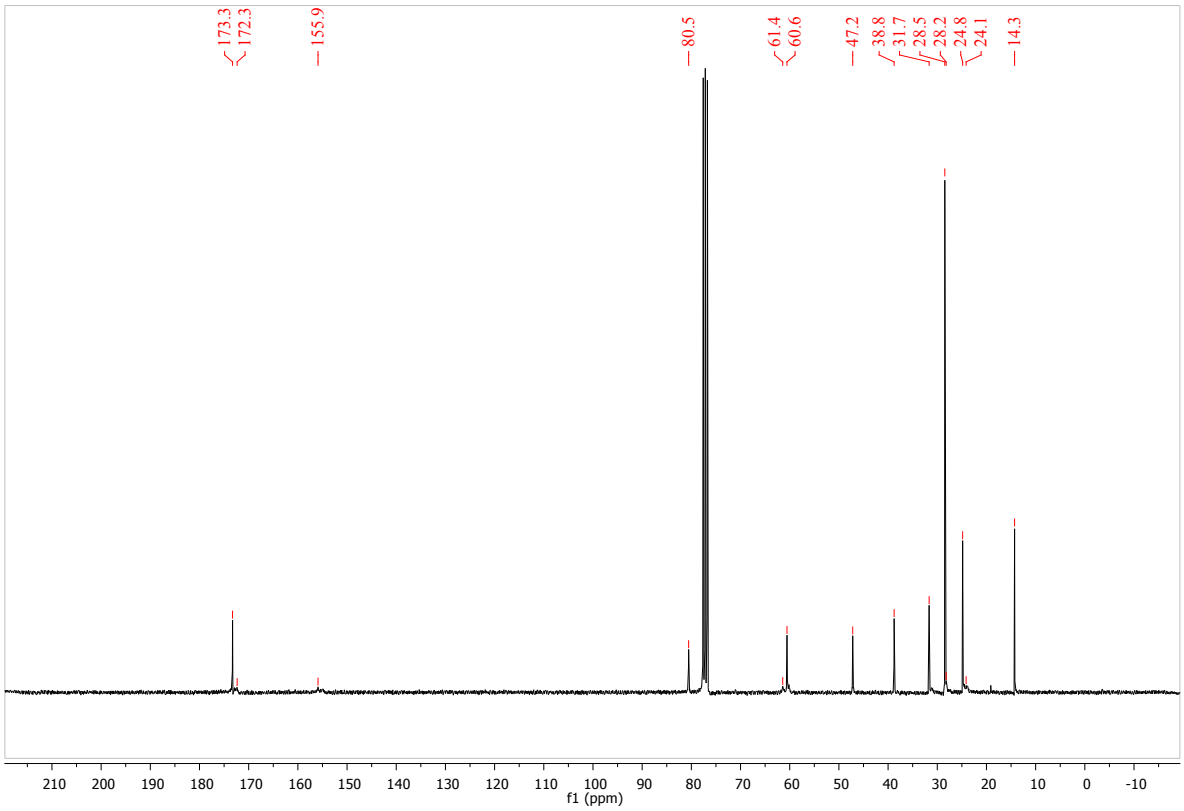


Figure 5S. $^{13}C\{H\}$ NMR spectrum (@125 MHz, $CDCl_3$, 300 K, δ) of Boc-Pro-γAbu-OEt

H-Pro-γAbu-OEt·CF_3COO^-:

ESI-MS: *m/z* 229.157 $[M+H]^+$; calc. for $C_{11}H_{21}N_2O_3$ $[M+H]^+$: 229.154

4. ESI-MS

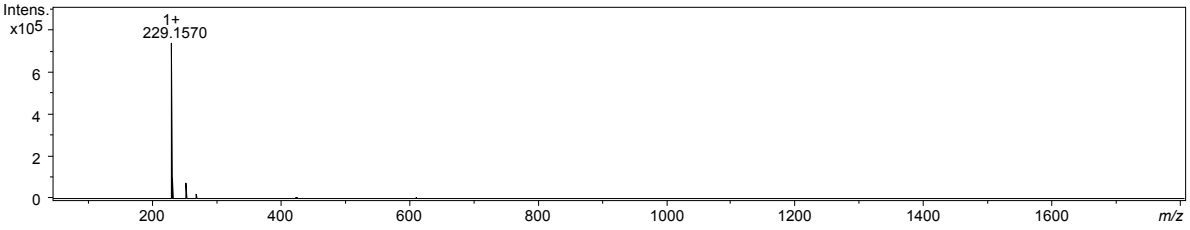


Figure 6S. ESI-MS spectrum of H-Pro-γAbu-OEt

$BASN^+$-CO-γAbu-OEt·BPh_4:

ESI-MS: *m/z* 331.207 $[M]^+$; calc. for $C_{19}H_{27}N_2O_3$ $[M]^+$: 331.201

^1H NMR (@500 MHz, CD_3CN, 300 K) δ: 1.22 (t, J = 7.1 Hz, 3H); 1.65–1.73 (m, 2H); 2.27 (t, J = 7.4 Hz, 2H); 2.47–2.55 (m, 1H); 3.10–3.20 (m, 2H); 3.26–3.61 (m, 1H); 3.90–3.95 (m, 1H); 4.09 (q, J = 7.1 Hz, 2H); 4.21–4.23 (m, 1H); 4.77 (dd, J = 14.3 Hz, 2H); 4.87 (dd, J =

14.4 Hz, 2H); 6.83–6.86 (m, 4H); 6.95 (s, 1H); 6.98–7.01 (m, 8H); 7.26–7.30 (m, 8H); 7.35–7.38 (m, 2H); 7.41–7.45 (m, 2H).

$^{13}C\{H\}$ NMR (@125 MHz, CD_3CN, 300 K) δ: 14.6; 21.1; 25.0; 27.3; 32.0; 39.7; 61.1; 65.1; 65.9; 69.6; 74.2; 122.8; 124.2; 124.3; 126.6; 126.6; 126.6; 126.6; 130.1; 130.2; 133.5; 134.3; 136.7; 136.7; 136.8; 136.8; 164.2; 164.6; 165.0; 165.4; 166.7; 173.8

5. ESI-MS

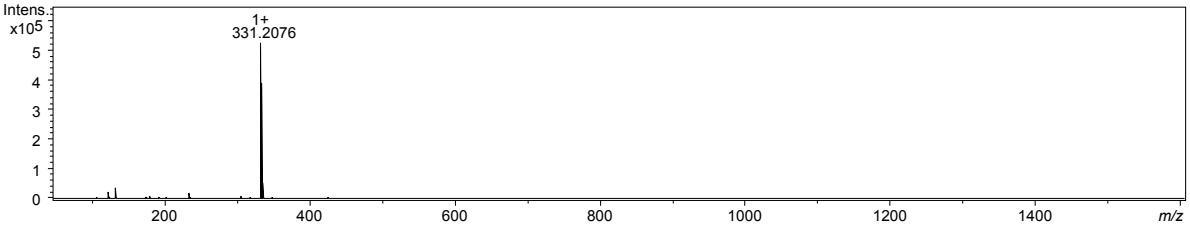


Figure 7S. ESI-MS spectrum of $BASN^+$-CO-γAbu-OEt

6. ^1H NMR

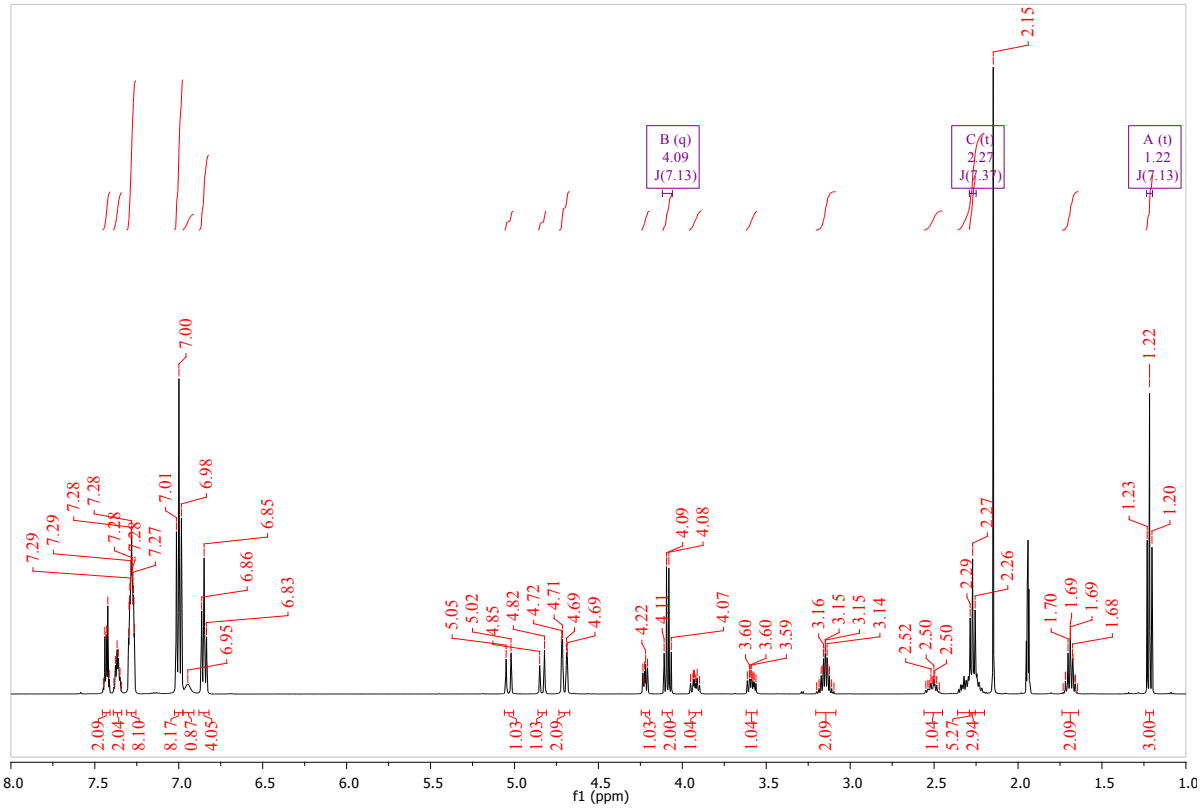


Figure 8S. ^1H NMR spectrum (@500 MHz, CD_3CN, 300 K, δ) of $BASN^+$-CO-γAbu-OEt·BPh_4

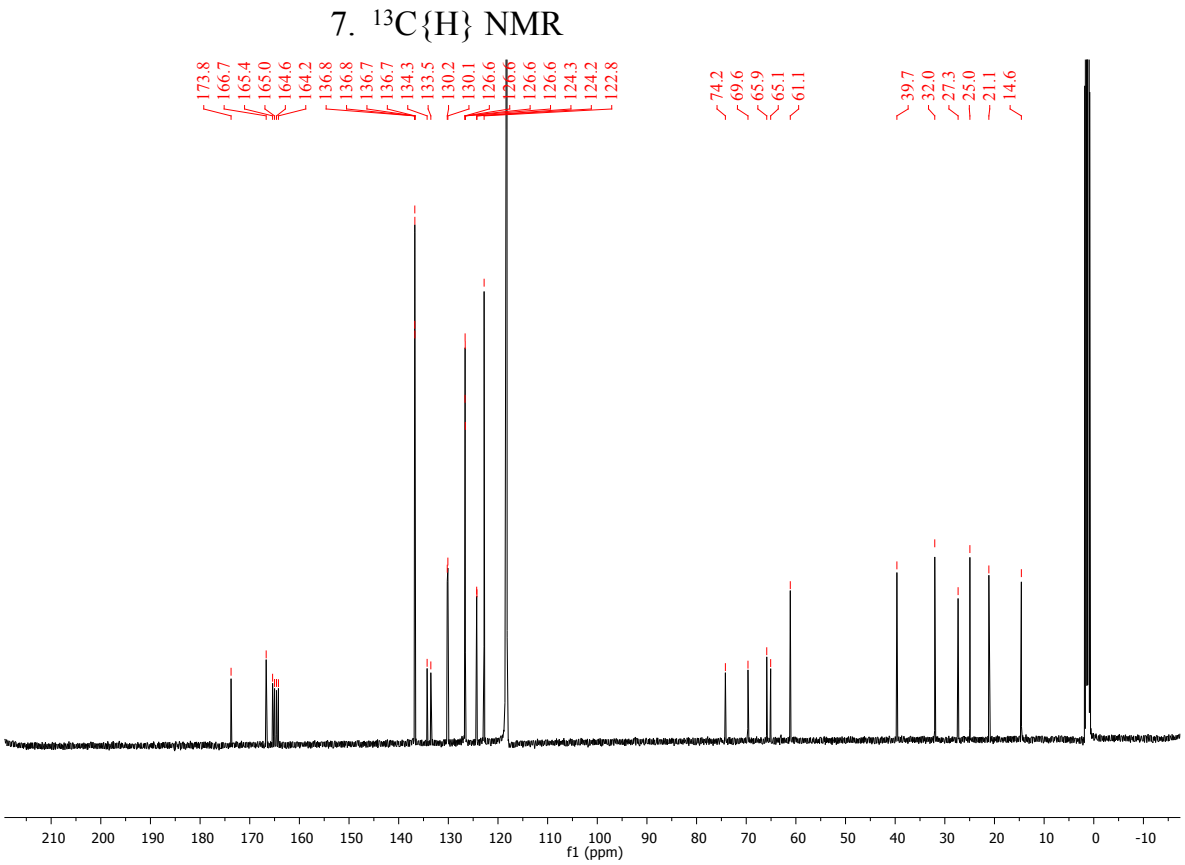


Figure 9S. ^{13}C{H} NMR spectrum (@125 MHz, CD_3CN, 300 K, δ) of BASN$^+$-CO-γAbu-OEt·BPh_4

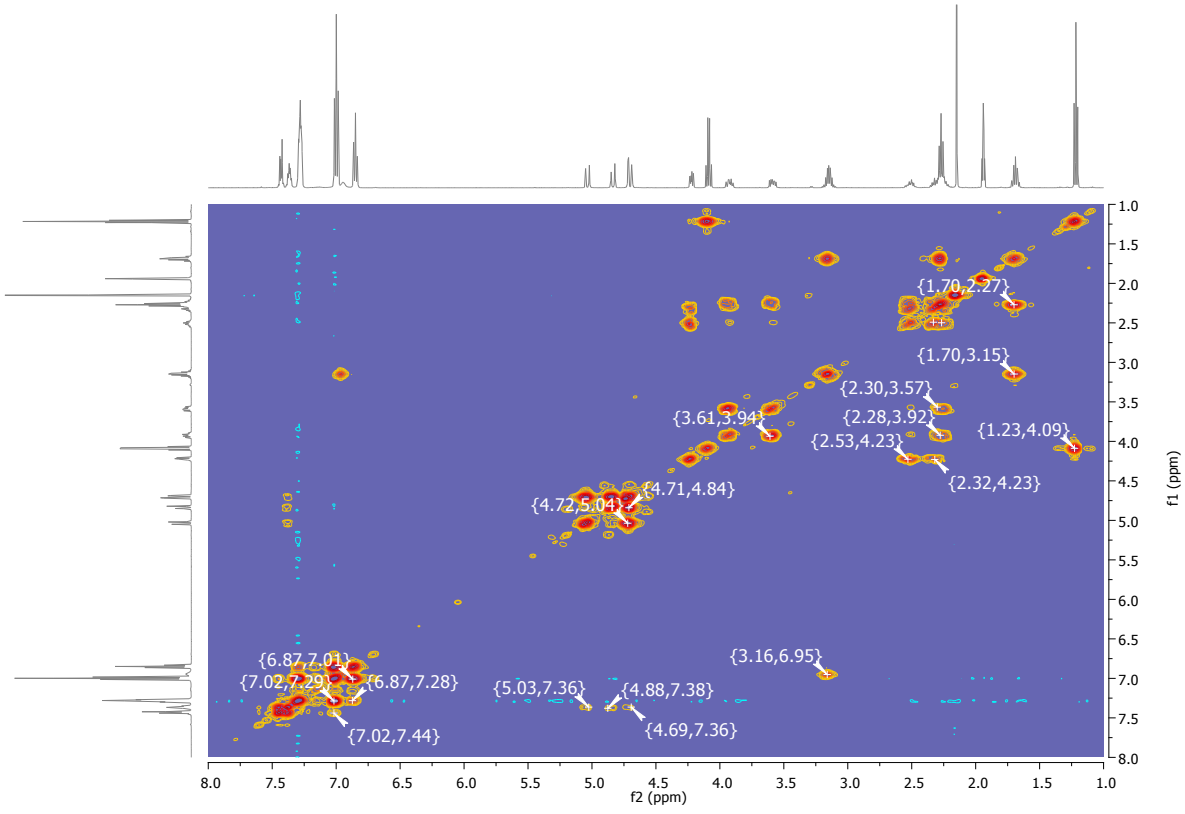


Figure 10S. COSY spectrum of BASN$^+$-CO-γAbu-OEt·BPh_4

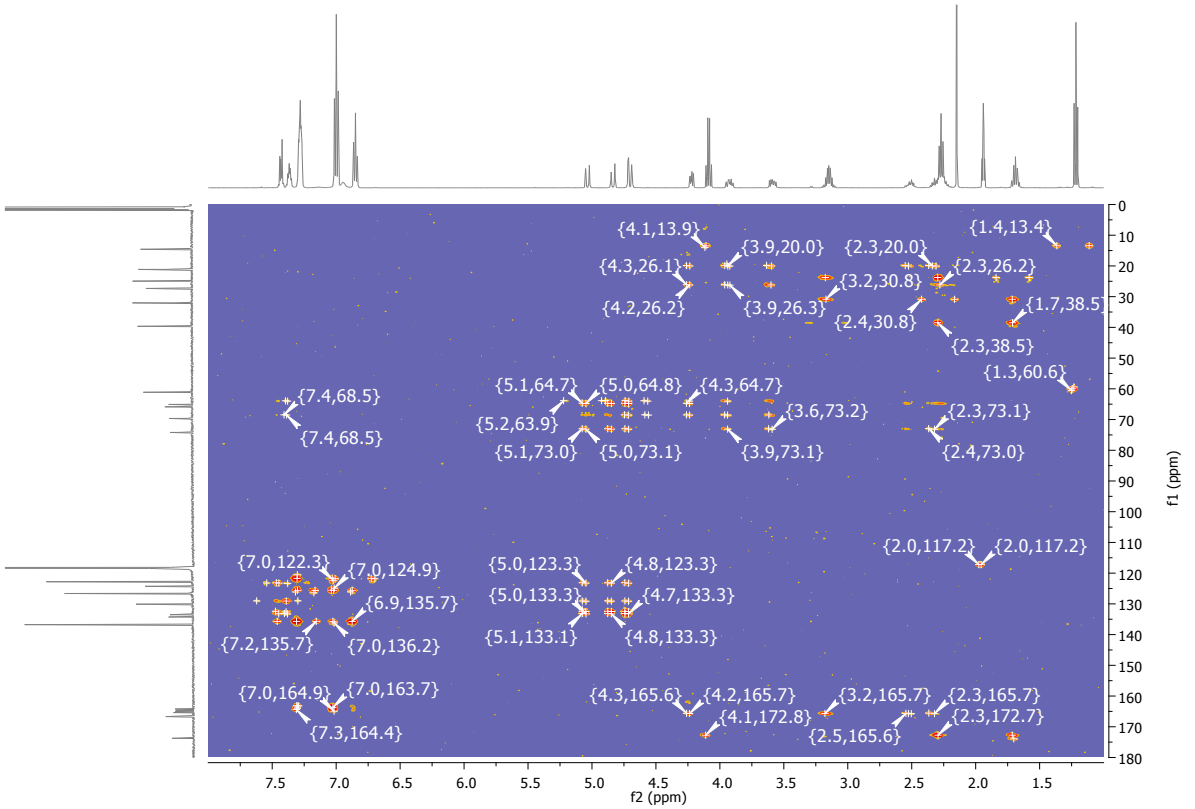


Figure 11S. HMBC spectrum of BASN⁺-CO-γAbu-OEt·BPh_4

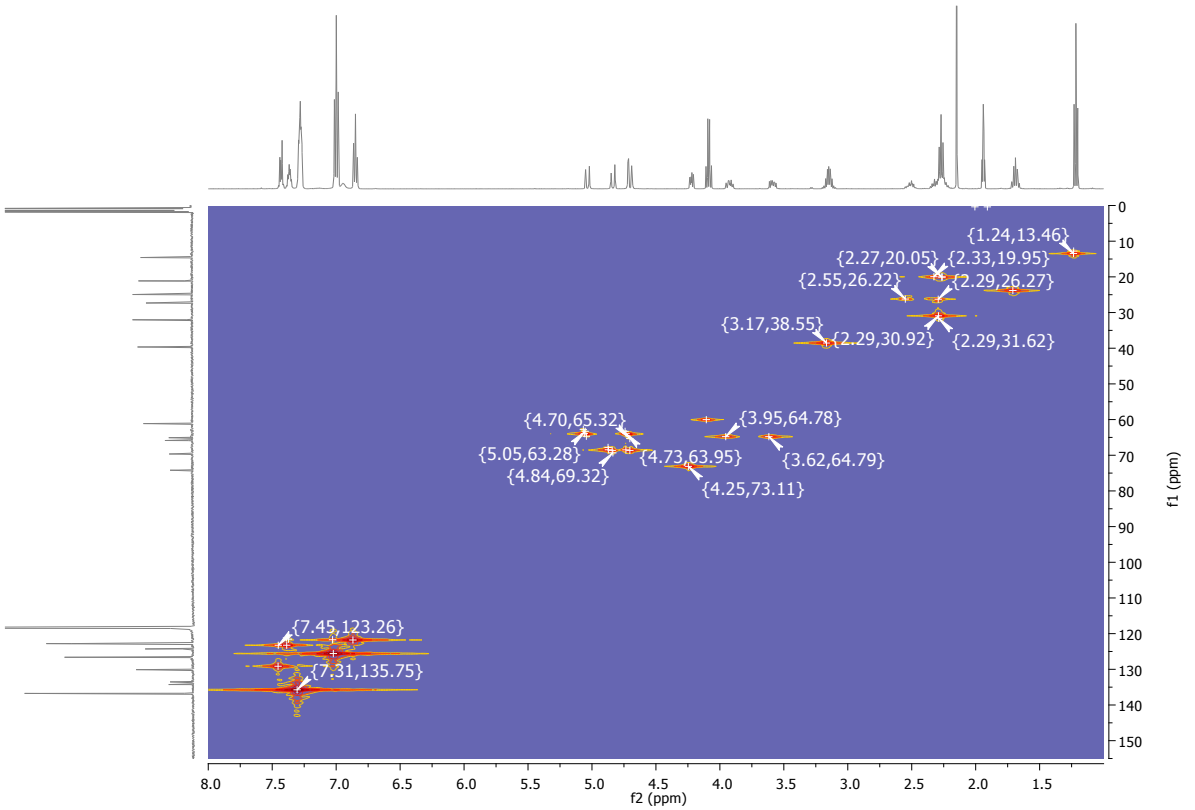


Figure 12S. HMQC spectrum of BASN⁺-CO-γAbu-OEt·BPh_4

c. Synthesis of 1-Boc-ethylenediamine[3]

In a 500 mL round bottom flask, 70 mL (1.05 mol) of 1,2-ethylenediamine and 100 mL of 1,4-dioxane were placed. A solution of 20.87 g (0.09 mol) of di-*tert*-butyl dicarbonate in 100 mL of 1,4-dioxane was added from a dropping funnel to the flask on a vigorous stirring in 2 h. The reaction mixture was left at room temperature for additional 22 h. 1,4-dioxane was removed on a rotary evaporator and 300 mL of water were added to obtain a colorless oil. Insoluble 1,4-di-Boc-ethylenediamine was filtered off. Aqueous solution was washed with DCM (4×250 mL). Organic fractions were dried with anhydrous magnesium sulfate and DCM was removed on a rotary evaporator; yield of colorless oil: 7.3 g (50%).

i. Analysis

1-Boc-ethylenediamine:

ESI-MS *m/z* 161.133 $[M+H]^+$; calc. for $C_7H_{16}N_2O_2$ $[M+H]^+$: 161.128

and *m/z* 105.070 $[M+H]^+$; calc. for $C_7H_{16}N_2O_2–C_4H_9$ $[M+H]^+$: 105.065

^{1}H NMR (@500 MHz, CD_3CN, 300 K) δ: 1.40 (s, 9H); 1.49 (s, 2H); 2.63 (t, J = 6.2 Hz, 2H); 3.01 (q, J = 6.1 Hz, 2H); 5.38 (bs, 1H).

^{13}C{H} NMR (@125 MHz, CD_3CN, 300 K) δ: 27.6; 41.7; 43.5; 78.0; 156.1

1. ESI-MS

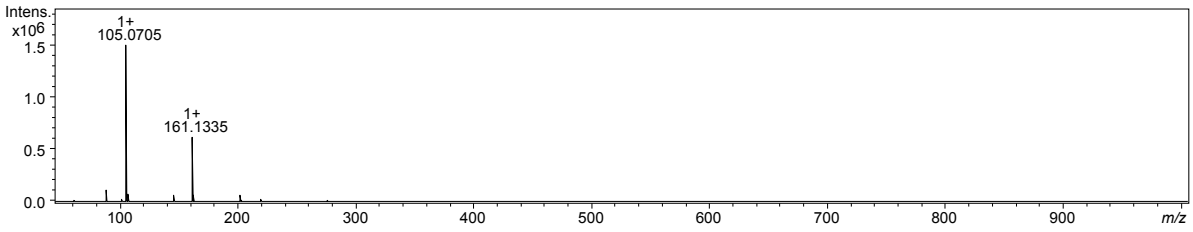


Figure 13S. ESI-MS spectrum of 1-Boc-ethylenediamine

2. ^{1}H NMR

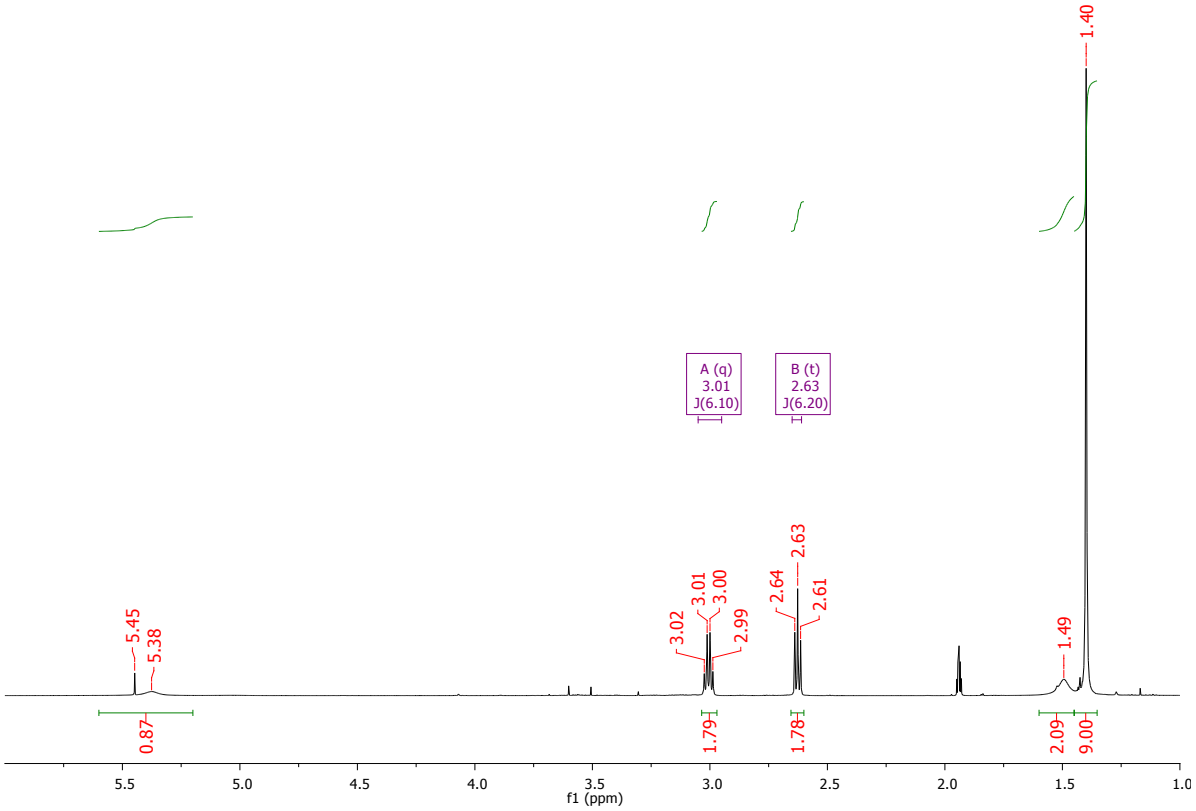


Figure 14S. ^{1}H NMR spectrum (@500 MHz, CD_3CN, 300 K, δ) of 1-Boc-ethylenediamine

3. $^{13}C\{H\}$ NMR

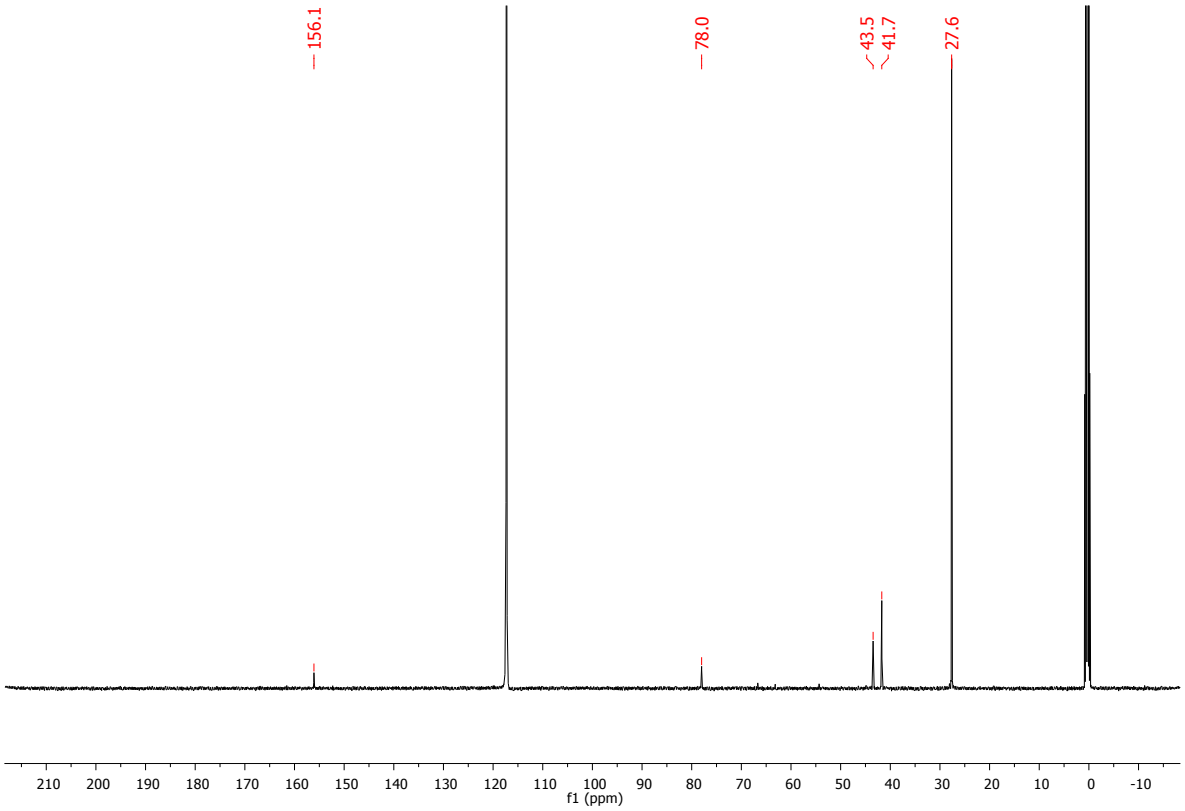
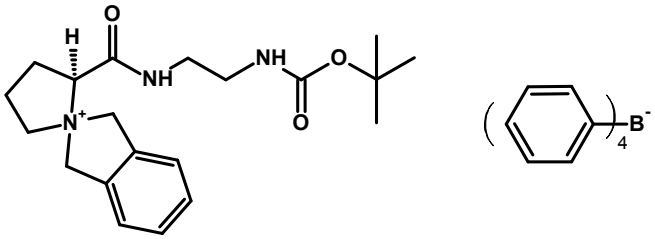


Figure 15S. $^{13}C\{H\}$ NMR spectrum (@125 MHz, CD_3CN, 300 K, δ) of 1-Boc-ethylenediamine

d. Synthesis of BASN$^+$-CO-ethylenediamine-*N*-Boc·BPh_4



In a 100 mL round bottom flask chilled in the ice-water bath, a solution of Z-Pro-OH (2.5 g, 10 mmol) and *N*-methylmorpholine (NMM) (1.10 mL, 10 mmol) in tetrahydrofuran (THF) (25 mL) was prepared. Then, isobuthyl chloroformate (1.34 mL, 10.5 mmol) was added on a vigorous stirring. Next, a solution of 1-Boc-ethylenediamine (1.6 g, 10 mmol) and triethylamine (TEA) (1.40 mL, 10 mmol) in DMF (10 mL) were added. After 5 minutes the TEA·HCl salt was filtered off and the filtrate was concentrated. Obtained colorless oil was washed with H_2O, diluted $NaHCO_3$, and H_2O and the product was dried. Z-Pro-ethylenediamine-*N*-Boc was purified by flash chromatography using a Büchi Sepacore® flash system X50 (column – Sepacore® Silica 12 g; eluent – chloroform/methanol 95:5, v/v); yield: 3.63 g (93%).[2] Z protecting group was removed by hydrogenolysis using 360 mg of Pd/C

(10%) in 50 mL of methanol for 4 h. Methanol was removed on a rotary evaporator and the obtained colorless oil was dried under *vacuum*; yield: 2.21 g (quantitative).

The benzo-5-azoniaspiro[4.4]nonyl-carbonyl group was obtained by mixing 0.77 g (3 mmol) of H-Pro-ethylenediamine-*N*-Boc, 0.79 g (3 mmol) α,α'-dibromo-*o*-xylene, and 1.24 g (9 mmol) K_2CO_3 in 25 mL of acetonitrile (24 h). After filtering off potassium carbonate, the filtrate was concentrated. A colorless oil was resuspended in a fresh portion of acetonitrile and chilled in the ice-water bath. An anion-exchange reaction was performed using 1.02 g (3 mmol) of sodium tetraphenylborate ($NaBPh_4$). Sodium bromide was filtered off on a filter funnel with a Celite® S pad and the product was dried under *vacuum*. Dissolving $BASN^+$-CO-ethylenediamine-*N*-Boc·BPh_4 in methanol afforded a white powder; yield: 0.3 g (15%).

i. Analysis

Z-Pro-ethylenediamine-*N*-Boc:

^{1}H NMR (@500 MHz, $CDCl_3$, 300 K) δ: 1.43 (s, 9H); 1.89–2.26 (m, 4H); 3.12–3.33 (m, 4H); 3.45–3.55 (m, 2H); 4.28–4.29 (m, 1H); 5.05–5.12 (m, 2H); 7.30–7.35 (m, 5H).

^{13}C{H} NMR (@125 MHz, $CDCl_3$, 300 K) δ: 24.7; 28.5; 29.0; 40.5; 47.2; 61.0; 67.5; 79.5; 128.1; 128.3; 128.7; 136.6; 156.2; 156.7; 172.5

1. ^{1}H NMR

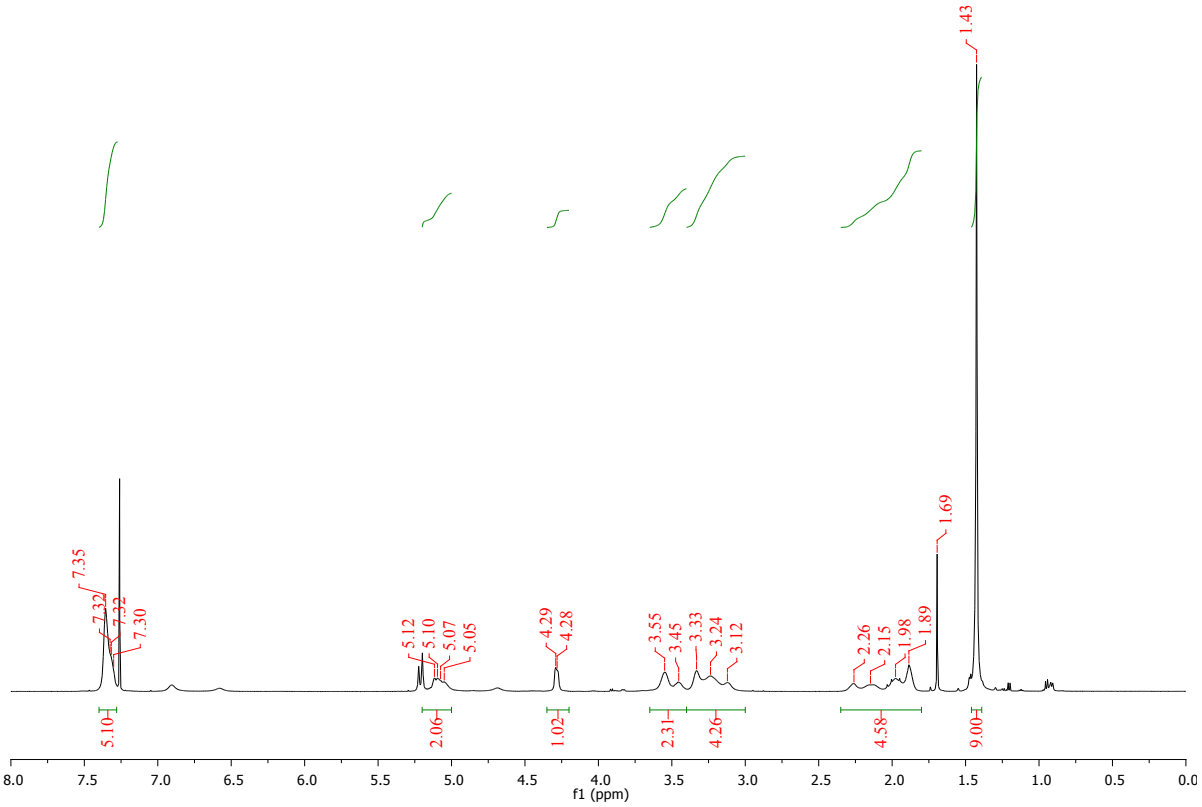


Figure 16S. ^{1}H NMR spectrum (@500 MHz, $CDCl_3$, 300 K, δ) of Z-Pro-ethylenediamine-*N*-Boc

2. ^{13}C NMR

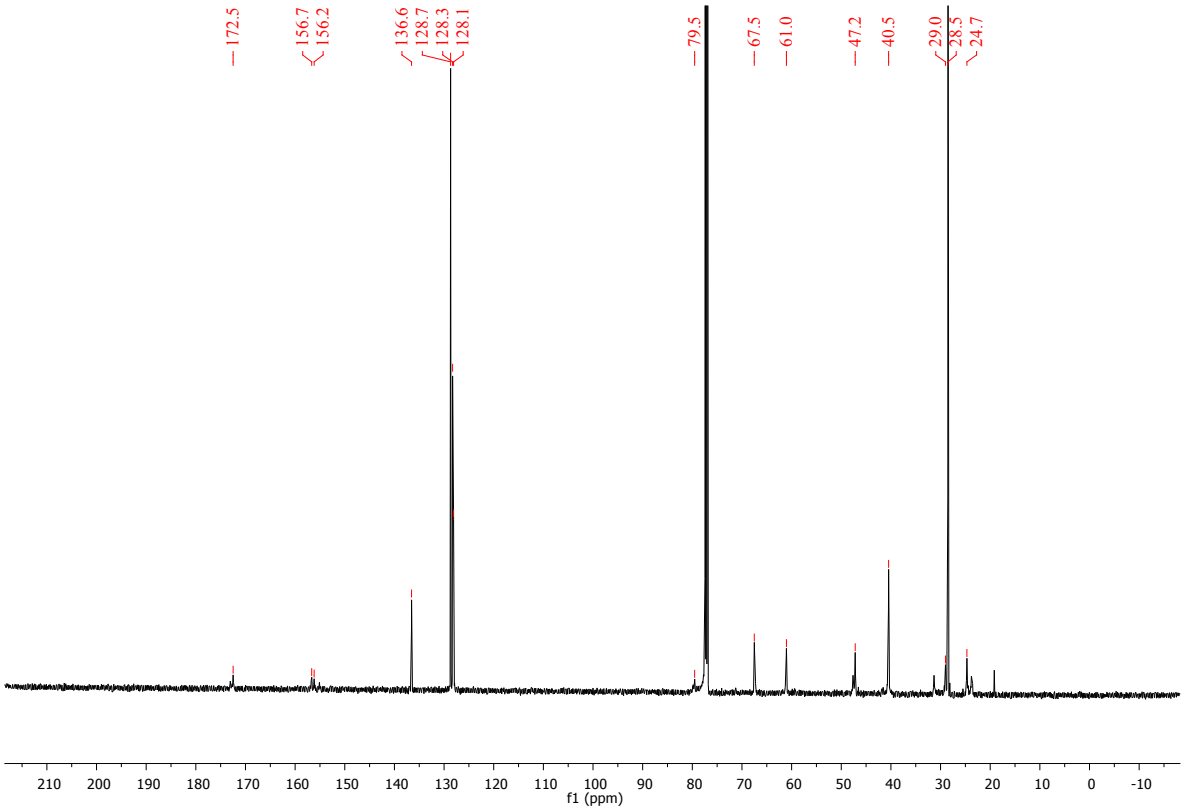


Figure 17S. ^{13}C{H} NMR spectrum (@125 MHz, $CDCl_3$, 300 K, δ) of Z-Pro-ethylenediamine-*N*-Boc

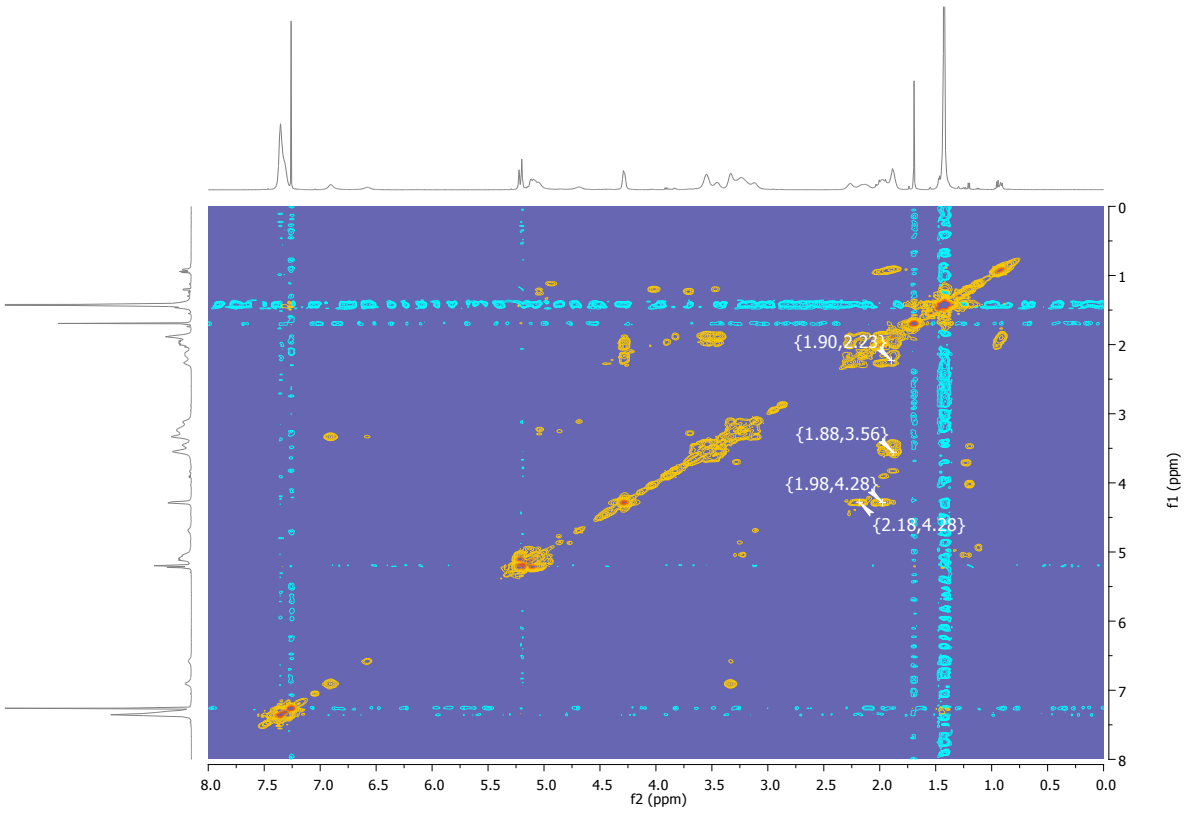


Figure 18S. COSY spectrum of Z-Pro-ethylenediamine-*N*-Boc

H-Pro-ethylenediamine-*N*-Boc:

ESI-MS: *m/z* 258.180 $[M+H]^+$; calc. for $C_{12}H_{23}N_3O_3$ $[M+H]^+$: 258.181

3. ESI-MS

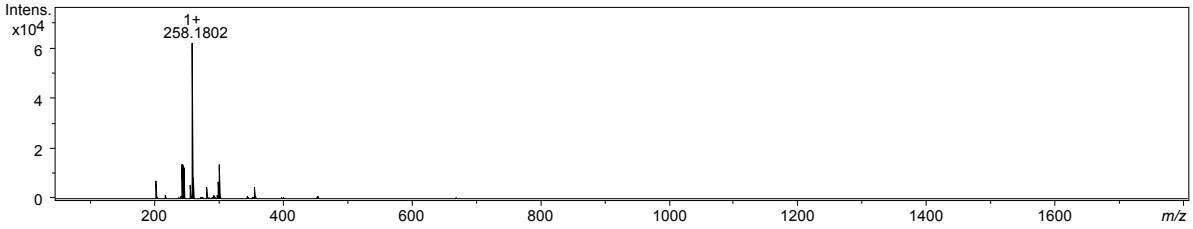


Figure 19S. ESI-MS spectrum of H-Pro-ethylenediamine-*N*-Boc

$BASN^+$-CO-ethylenediamine-*N*-Boc·BPh_4

ESI-MS: *m/z* 360.227 $[M]^+$; calc. for $C_{20}H_{30}N_3O_3$ $[M]^+$: 360.228

^1H NMR (@500 MHz, CD_3CN, 300 K) δ: 1.40 (s, 9H); 2.24–2.38 (m, 3H); 2.48–2.56 (m, 1H); 3.09–3.11 (m, 2H); 3.16–3.22 (m, 2H); 3.57–3.62 (m, 1H); 3.89–3.95 (m, 1H); 4.32 (dd, *J* = 6.0 Hz, 1H); 4.82 (dd, *J* = 14.5 Hz, 2H); 4.90 (dd, *J* = 14.4 Hz, 2H); 6.83–6.86 (m, 4H); 6.98–7.01 (m, 8H); 7.26–7.30 (m, 8H); 7.36–7.39 (m, 2H); 7.43–7.44 (m, 2H)

$^{13}C\{H\}$ NMR (@125 MHz, CD_3CN, 300 K) δ: 21.1; 27.3; 28.6; 40.2; 41.0; 65.1; 66.0; 69.6; 74.1; 79.6; 122.8; 124.3; 124.3; 126.6; 126.6; 126.6; 126.6; 127.9; 128.4; 128.6; 129.9; 130.1; 130.2; 130.5; 133.6; 134.3; 134.9; 136.7; 136.7; 136.8; 157.3; 164.2; 164.6; 165.0; 165.4; 167.0

4. ESI-MS

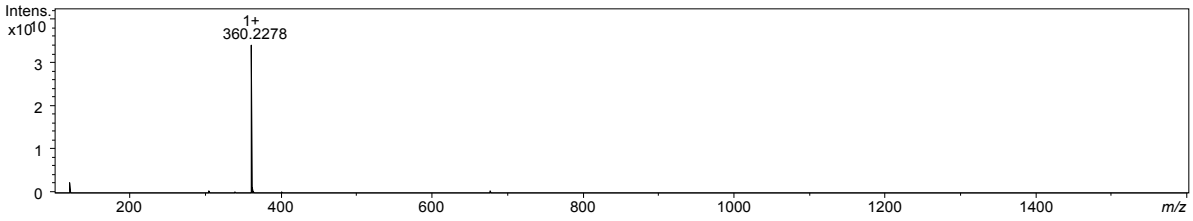


Figure 20S. ESI-MS spectrum of $BASN^+$-CO-ethylenediamine-*N*-Boc

5. ^{1}H NMR

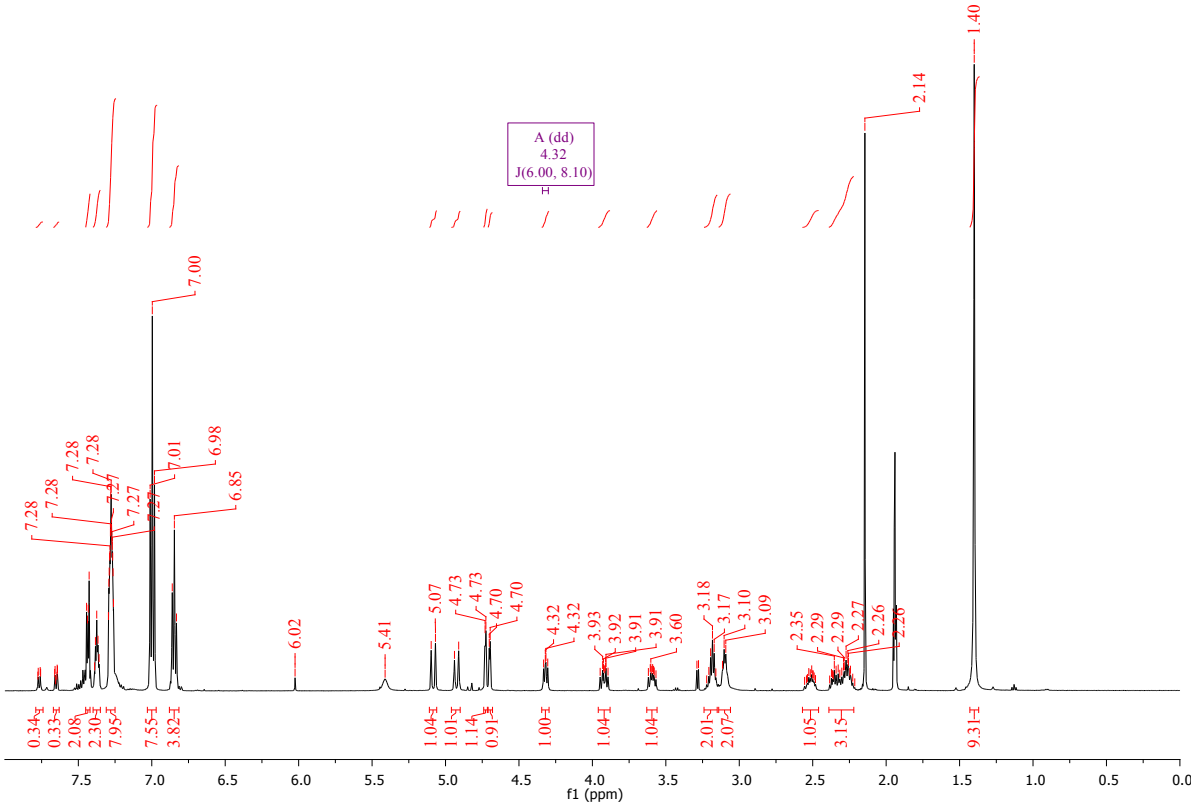


Figure 21S. ^{1}H NMR spectrum (@500 MHz, CD_3CN, 300 K, δ) of BASN^{+}-CO-ethylenediamine-*N*-Boc·BPh_4

6. $^{13}C\{H\}$ NMR

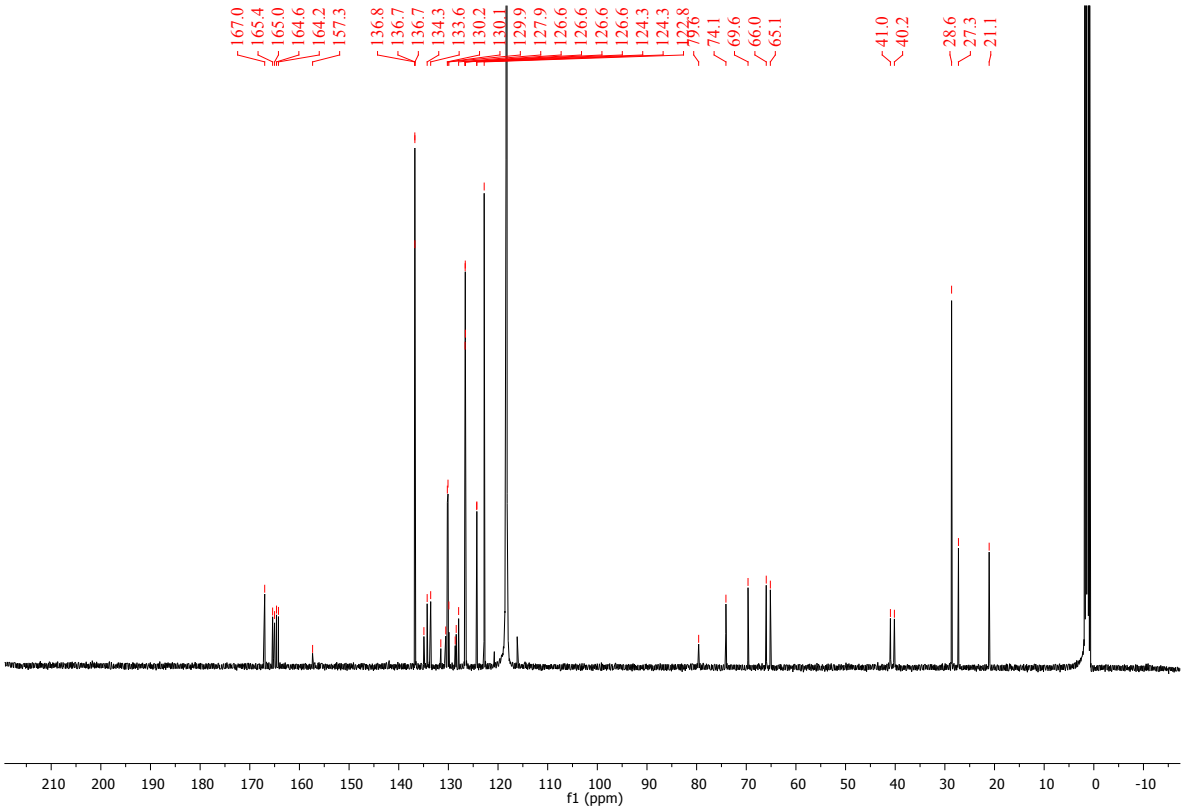


Figure 22S. $^{13}C\{H\}$ NMR spectrum (@125 MHz, CD_3CN, 300 K, δ) of BASN$^+$-CO-ethylenediamine-*N*-Boc·BPh_4

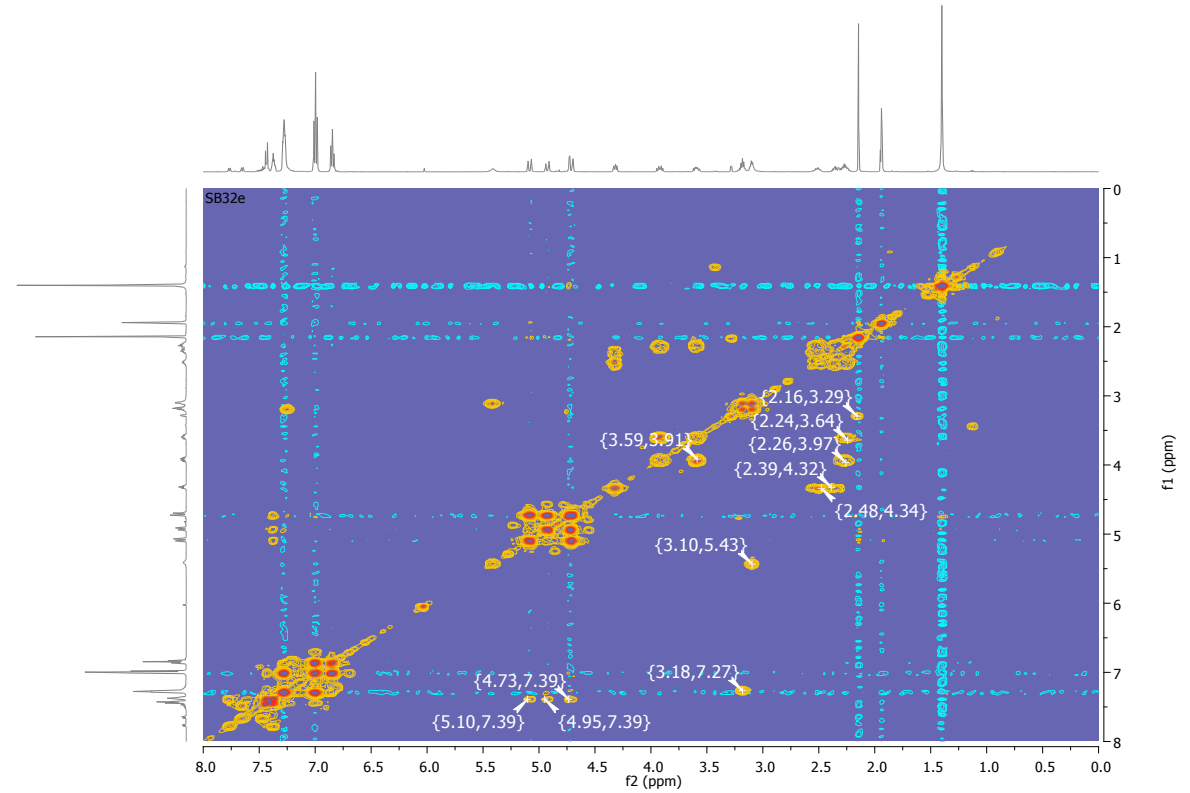


Figure 23S. COSY spectrum of BASN⁺-CO-ethylenediamine-*N*-Boc·BPh_4

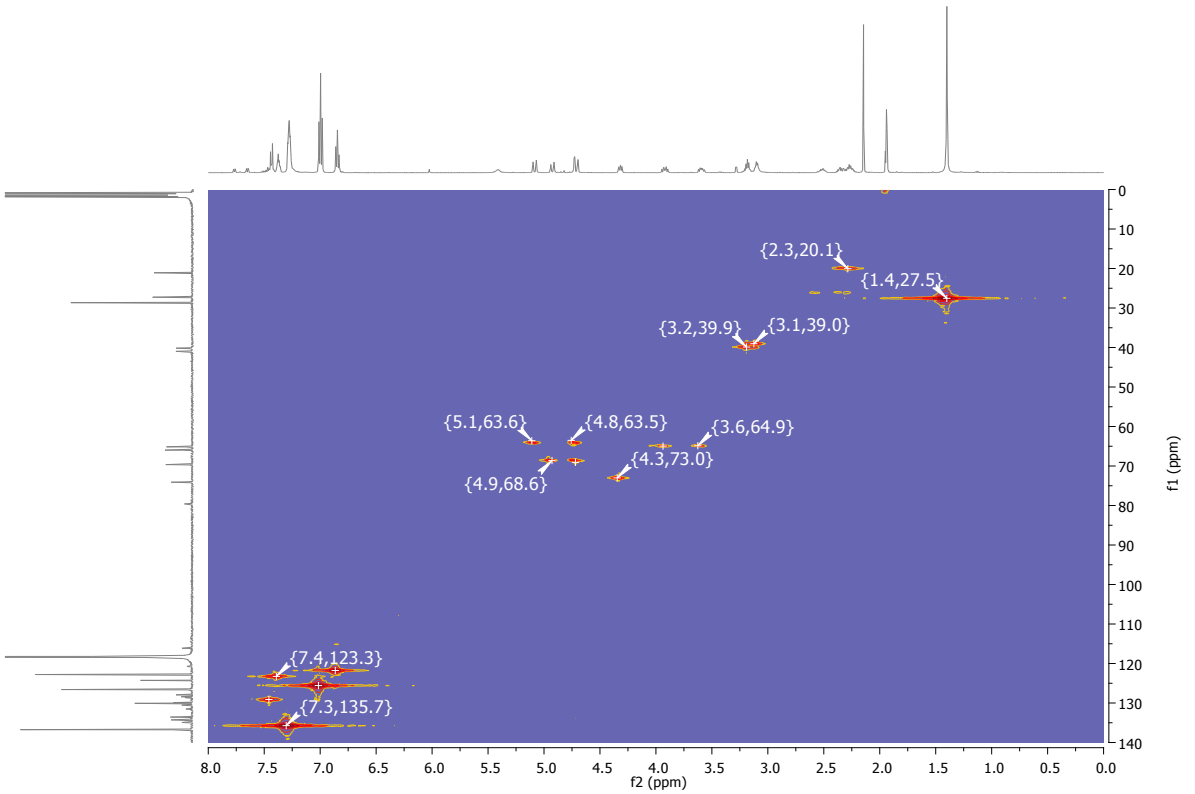


Figure 24S. HMQC spectrum of BASN⁺-CO-ethylenediamine-*N*-Boc·BPh_4

Table 1S. QAS-derivatized model peptides examined in this study

	Sequence	*m/z*	Charge	*m/z* calculated	Retention time [min]
1.	ASN^+–CO–DVYT–NH_2	647.339	$[M]^+$	647.339	12.2
2.	$BASN^+$–CO–DVYT–NH_2	695.339		695.339	14.6
3.	ASN^+–CO–SVWE–NH_2	670.356		670.355	16.4
4.	$BASN^+$–CO–SWVE–NH_2	718.354		718.355	18.6
5.	ASN^+–CO–MQIFVKT–OH	1017.580	$[M]^+$	1017.580	16.6
		509.294	$[M+H]^{2+}$	509.293	
6.	$BASN^+$–CO–MQIFVKT–OH	1065.579	$[M]^+$	1065.580	18.5
		533.294	$[M+H]^{2+}$	533.293	

2. ESI–MS spectra recorded during HDX in the 5–azoniaspiro[4.4]nonyl–carbonyl group

a. $BASN^+$–CO–DVYT–NH_2 → $BASN^+${D}–CO–DVYT–NH_2

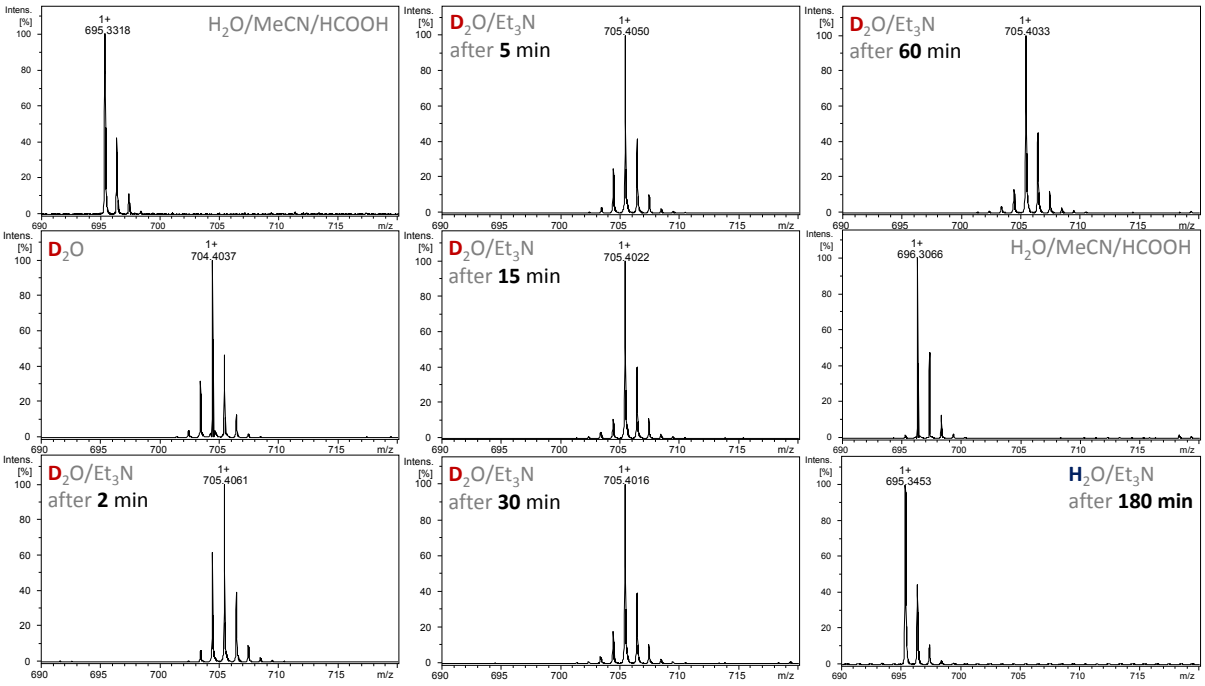


Figure 25S. ESI-MS spectra of the progress of the HDX exchange reaction in $BASN^+$–CO–DVYT–NH_2

b. $ASN^{+}–CO–SVWE–NH_2 \rightarrow ASN^{+}\{D\}–CO–SVWE–NH_2$

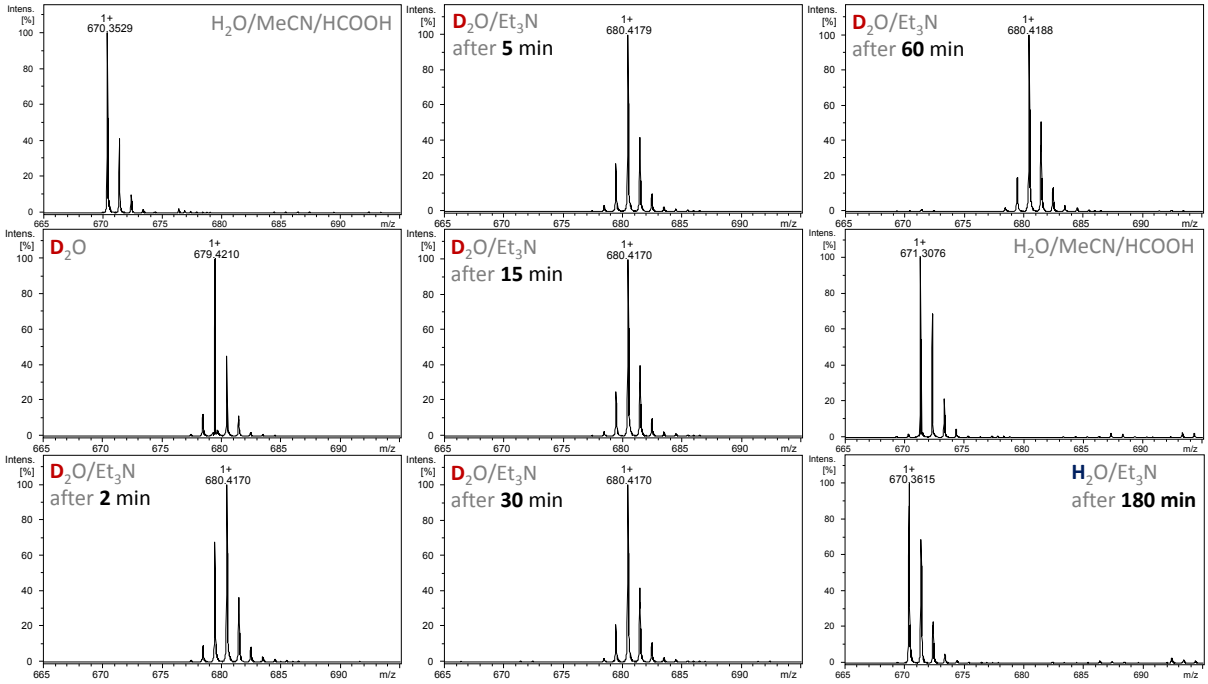


Figure 26S. ESI-MS spectra of the progress of the HDX exchange reaction in $ASN^{+}–CO–SWVE–NH_2$

c. $BASN^{+}–CO–SVWE–NH_2 \rightarrow BASN^{+}\{D\}–CO–SVWE–NH_2$

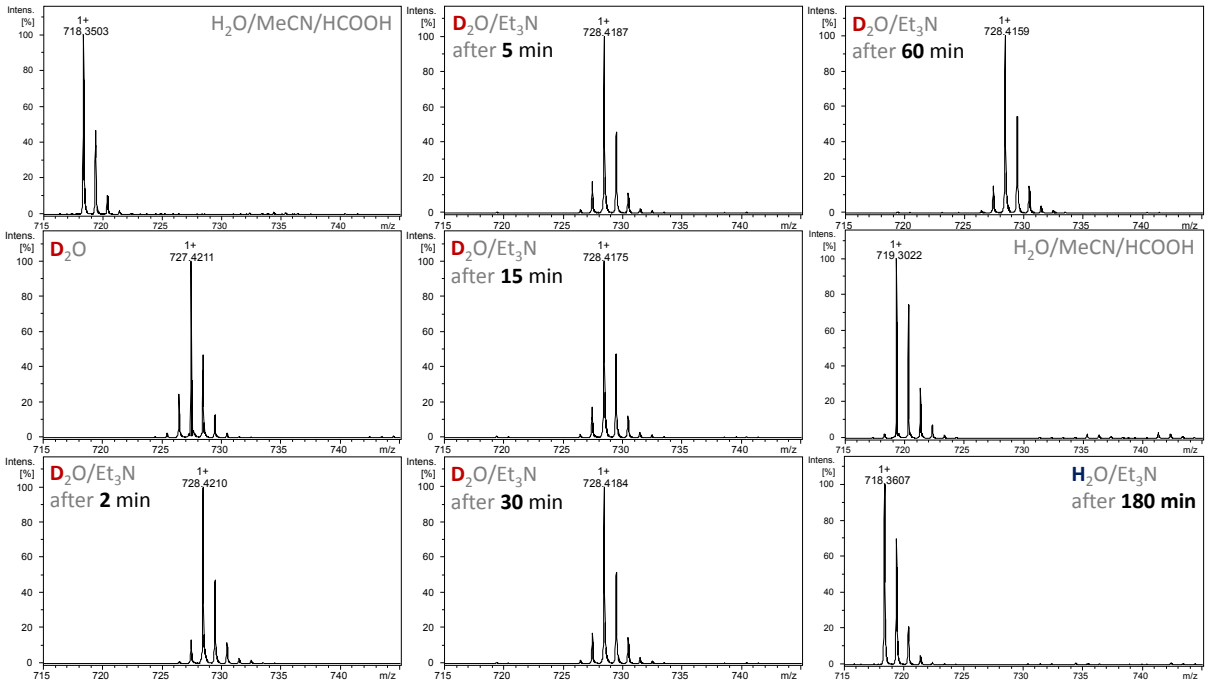


Figure 27S. ESI-MS spectra of the progress of the HDX exchange reaction in $BASN^{+}–CO–SWVE–NH_2$

d. ASN^{+}–CO–MQIFVKT–OH → ASN^{+}{**D**}–CO–MQIFVKT–OH

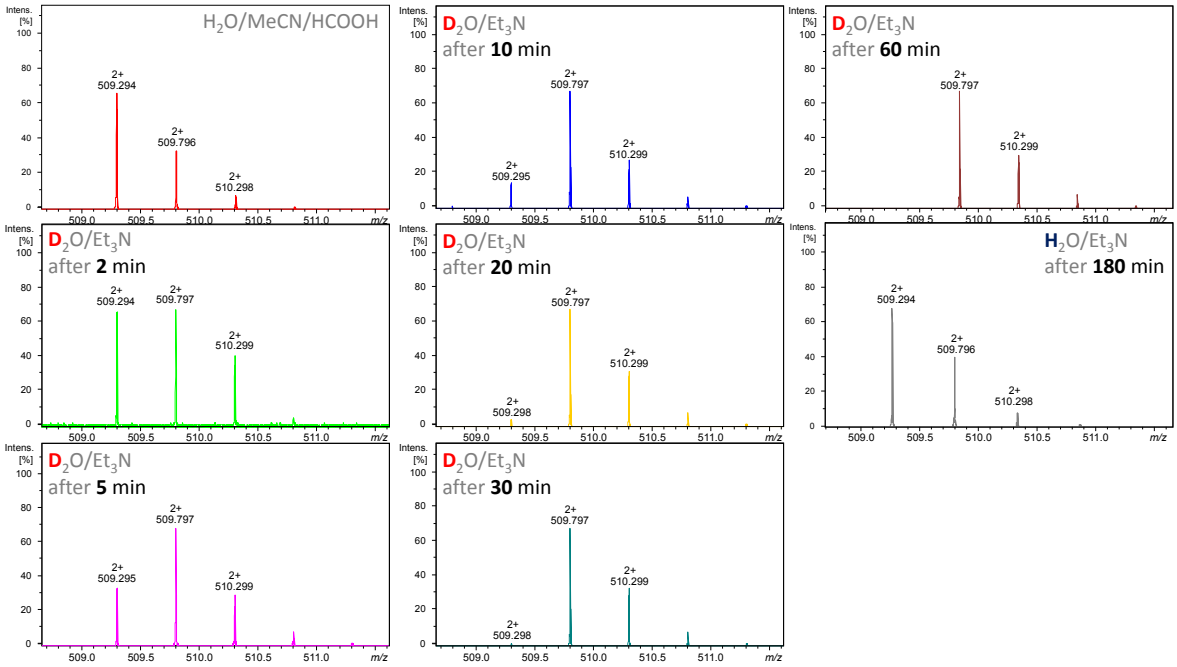


Figure 28S. ESI-MS spectra of the progress of the HDX exchange reaction in ASN^{+}–CO–MQIFVKT–OH. Before each MS measurement, a sample was lyophilized. Each ESI-MS spectrum was recorded using a mixture of water/acetonitrile (1:1) with 0.1% of formic acid

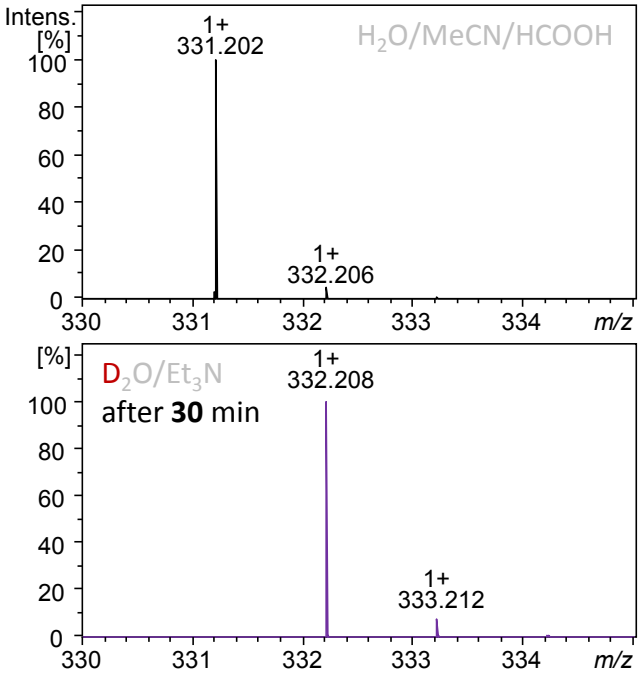


Figure 29S. ESI-MS spectra of the progress of the HDX exchange reaction in $BASN^{+}$-CO-γAbu-OEt. Before each MS measurement, a sample was lyophilized. Each ESI-MS spectrum was recorded using a mixture of water/acetonitrile (1:1) with 0.1% of formic acid

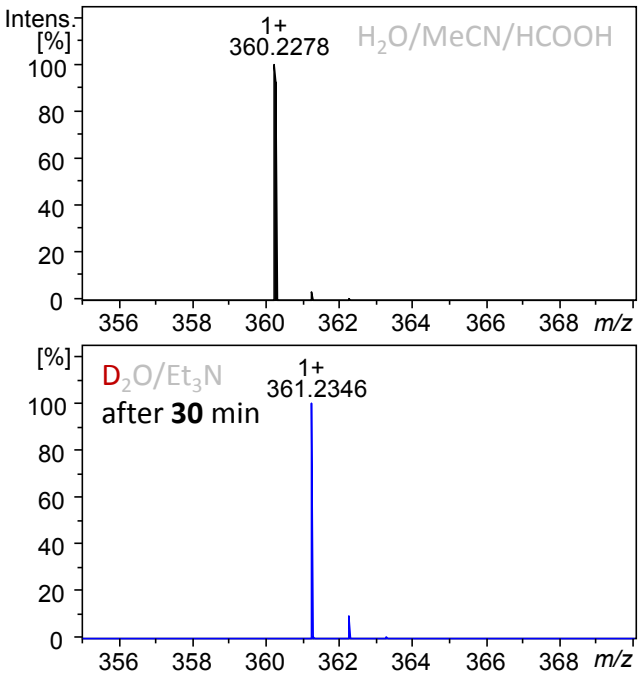
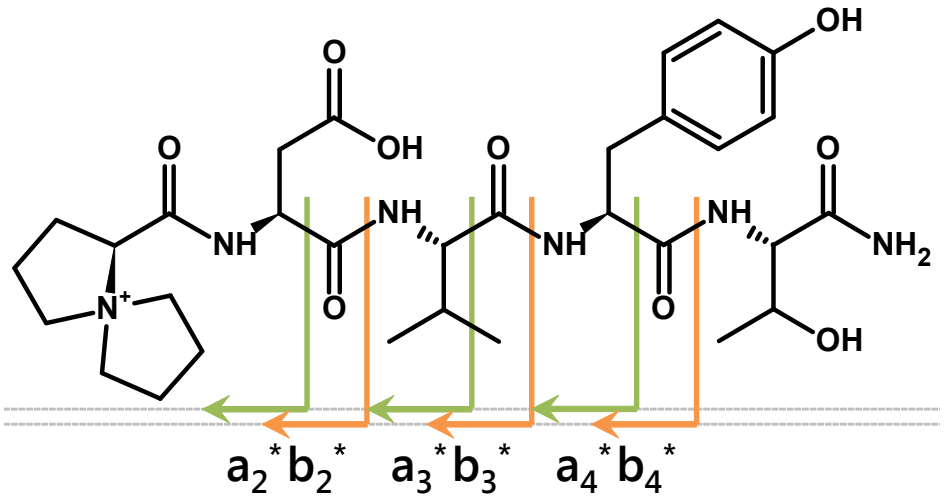


Figure 30S. ESI-MS spectra of the progress of the HDX exchange reaction in BASN⁺-CO-ethylenediamine-*N*-Boc. Before each MS measurement, a sample was lyophilized. Each ESI-MS spectrum was recorded using a mixture of water/acetonitrile (1:1) with 0.1% of formic acid

3. ESI–MS/MS spectra



Scheme 1S. Proposed nomenclature for a* and b*-type ions of the ASN⁺ (5-azoniaspiro[4.4]nonyl-carbonyl) and BASN⁺ (benzo-5-azoniaspiro[4.4]nonyl-carbonyl) groups.

a. $BASN^{+}–CO–DVYT–NH_2$

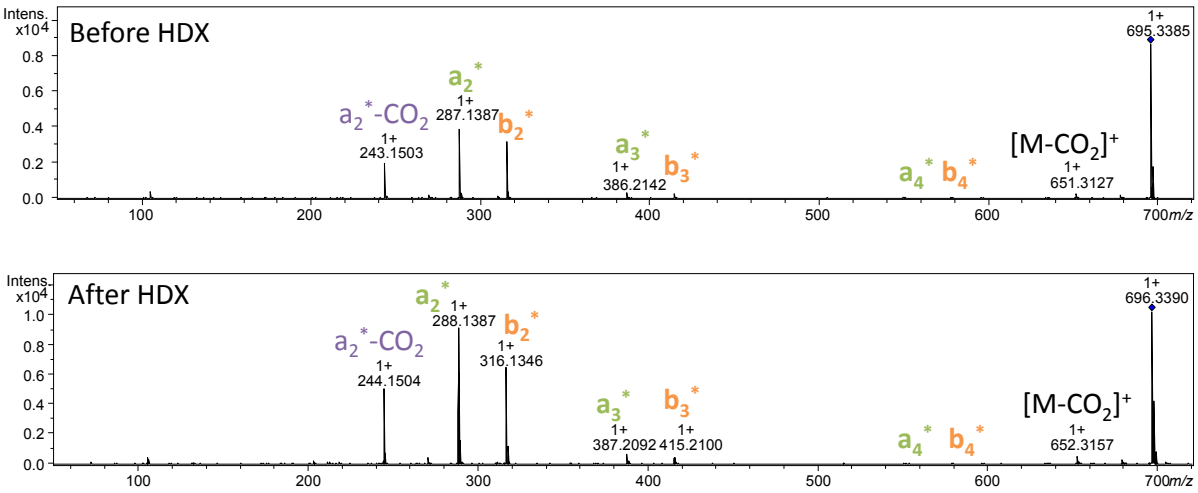


Figure 31S. ESI-MS/MS spectra of $BASN^{+}–CO–DVYT–NH_2$ before (collision energy: 30 V) and after (collision energy: 30 V) HDX

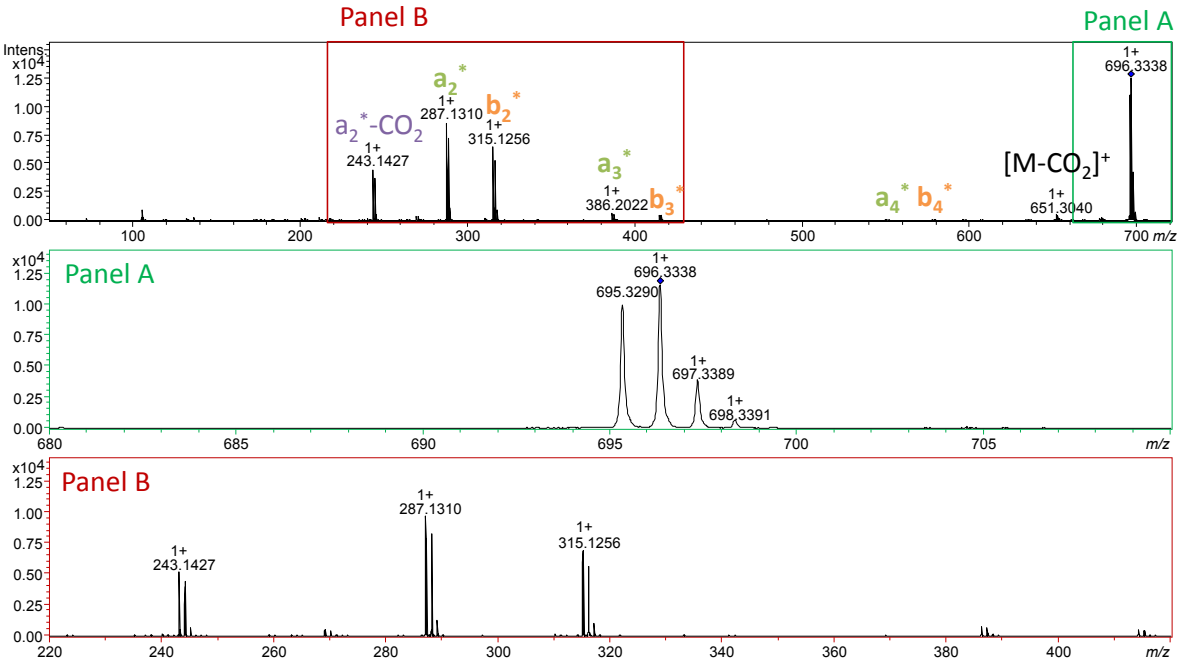


Figure 32S. ESI-MS/MS spectrum of a mixture of $BASN^{+}–CO–DVYT–NH_2$ and $BASN^{+}${**D**}–CO–DVYT–NH_2 (1:1). Collision energy: 30 V

b. $ASN^{+}–CO–SWVE–NH_2$

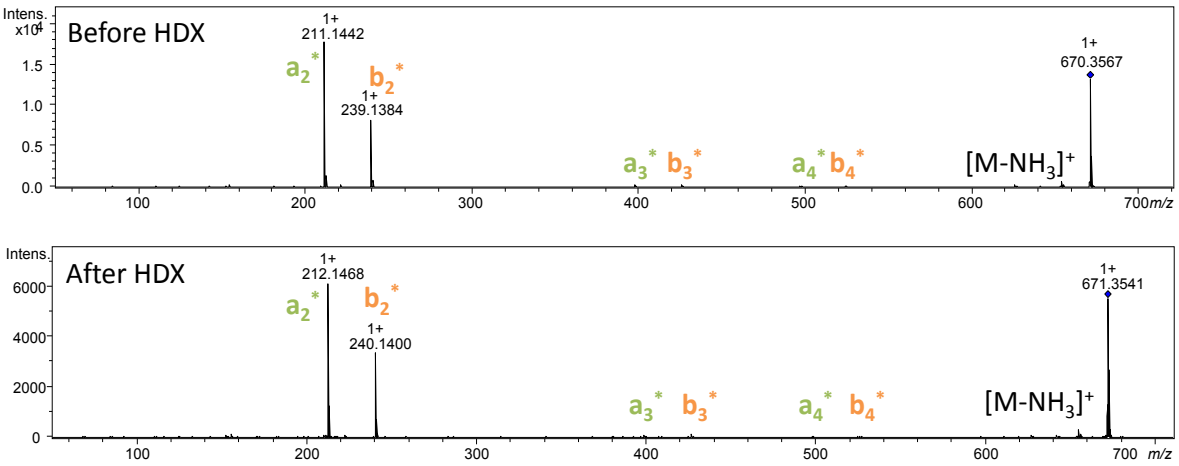


Figure 33S. ESI-MS/MS spectra of $ASN^{+}–CO–SWVE–NH_2$ before (collision energy: 30 V) and after (collision energy: 30 V) HDX

c. $BASN^{+}–CO–SWVE–NH_2$

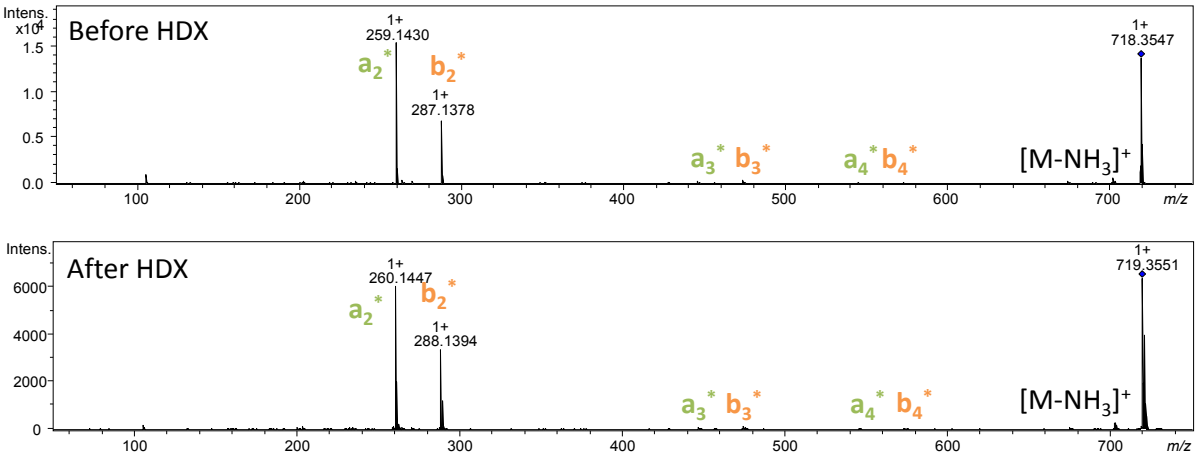


Figure 34S. ESI-MS/MS spectra of $BASN^{+}–CO–SWVE–NH_2$ before (collision energy: 30 V) and after (collision energy: 30 V) HDX

d. ASN^{+}–CO–MQIFVKT–OH

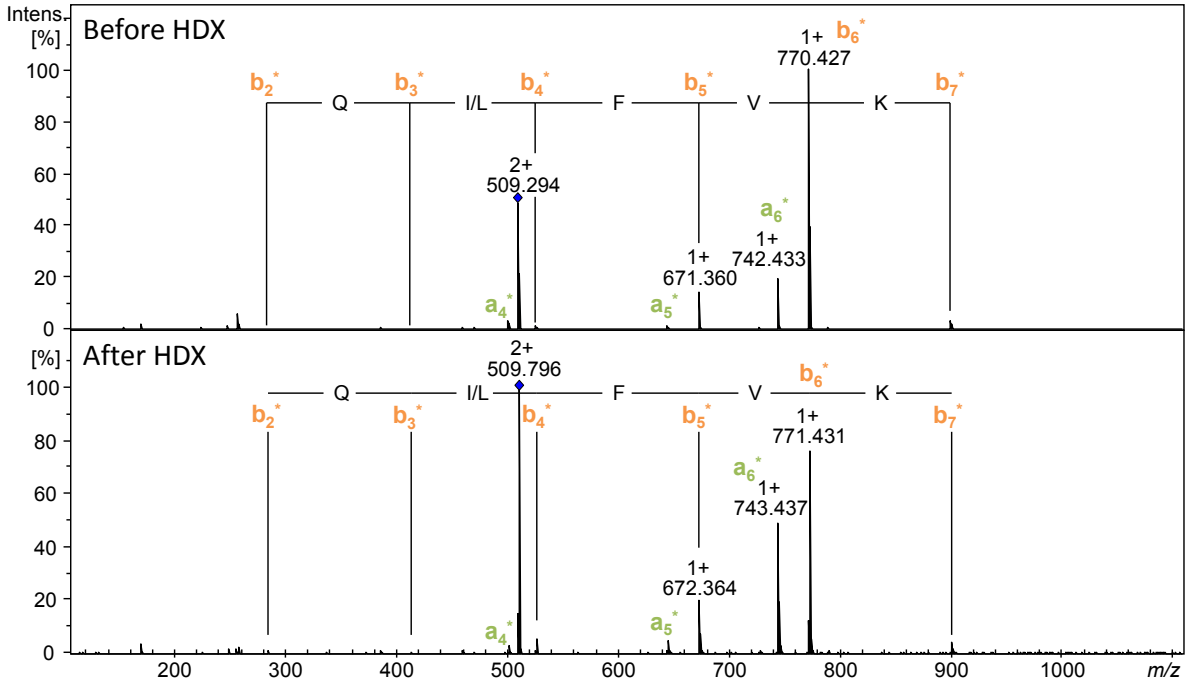


Figure 35S. ESI-MS/MS spectra of ASN^{+}–CO–MQIFVKT–NH_2 before (collision energy: 10 V) and after (collision energy: 12 V) HDX

e. $BASN^{+}$–CO–MQIFVKT–OH

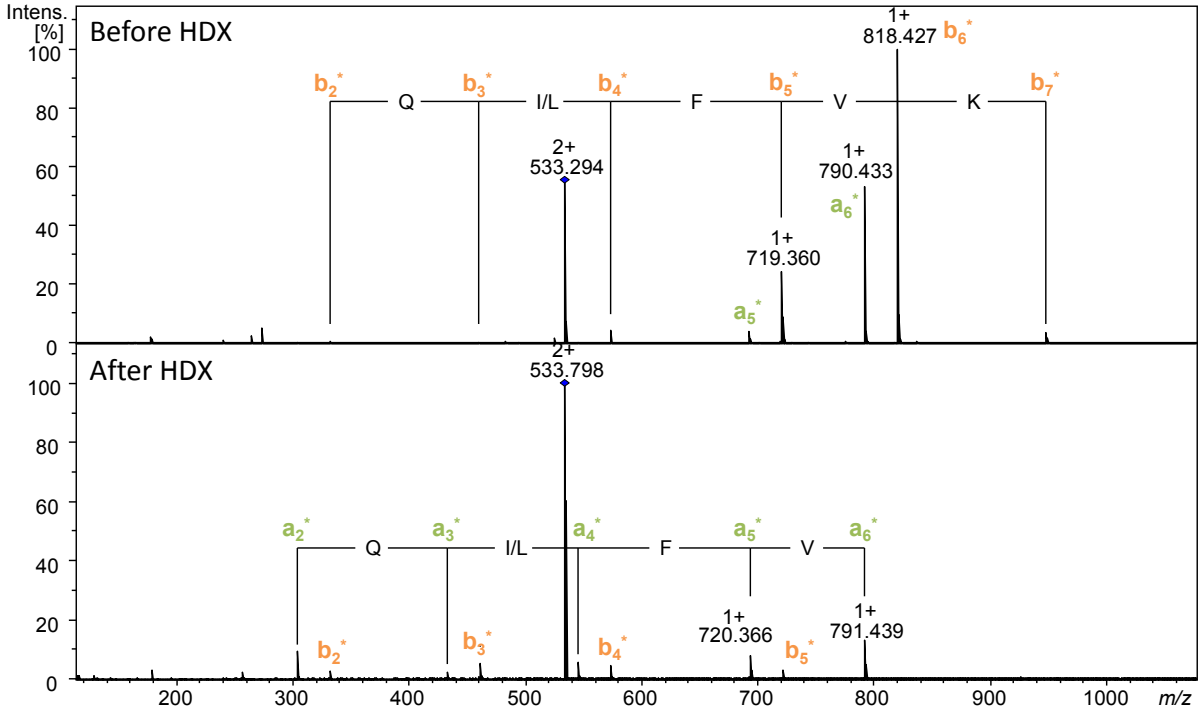


Figure 36S. ESI-MS/MS spectra of $BASN^{+}$–CO–MQIFVKT–NH_2 before (collision energy: 12 V) and after (collision energy: 22 V) HDX

4. NMR spectra recorded before and after HDX in the 5–azoniaspiro[4.4]nonyl–carbonyl group

   a. $ASN^{+}–CO–DVYT–NH_2$

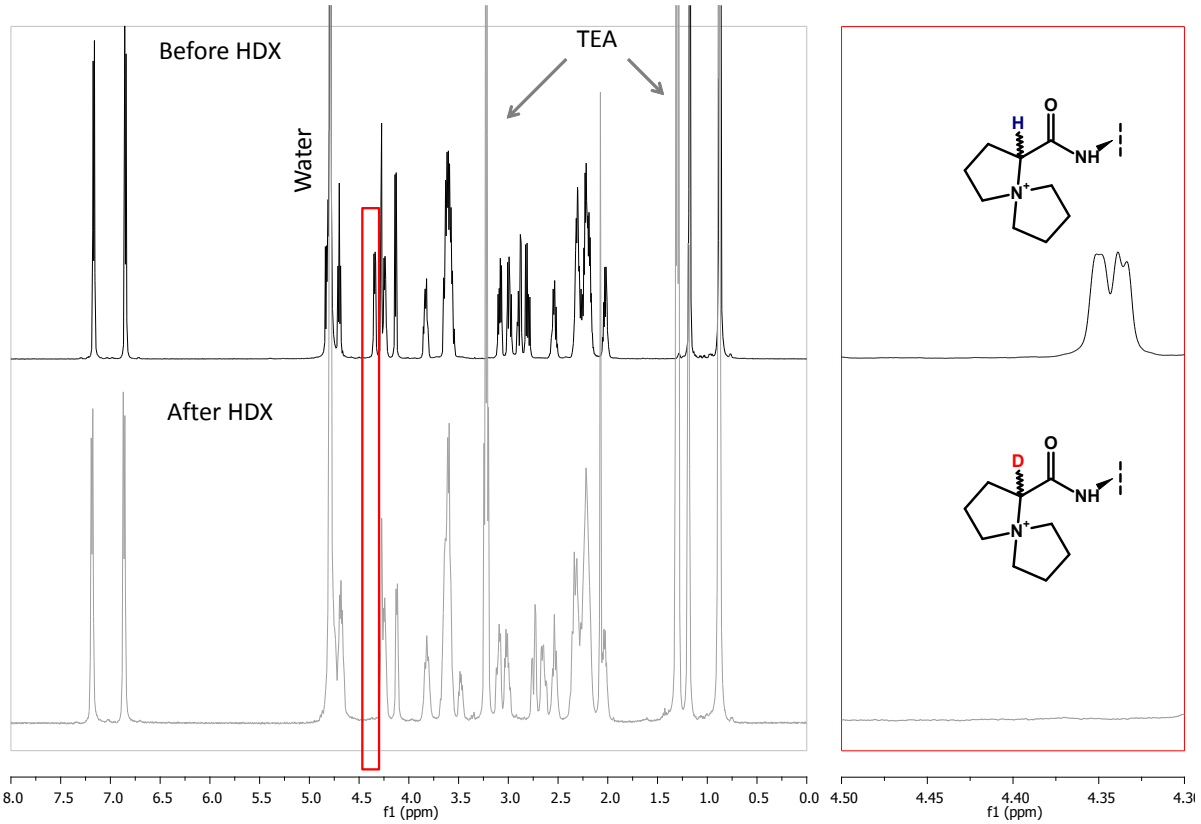


Figure 37S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of $ASN^{+}–CO–DVYT–NH_2$ before and after HDX. Red inset depicts disappearance of the signal of α-H (4.34 ppm) due to HDX in the 5-azoniaspiro[4.4]nonyl-carbonyl residue

b. $BASN^{+}–CO–DVYT–NH_2$

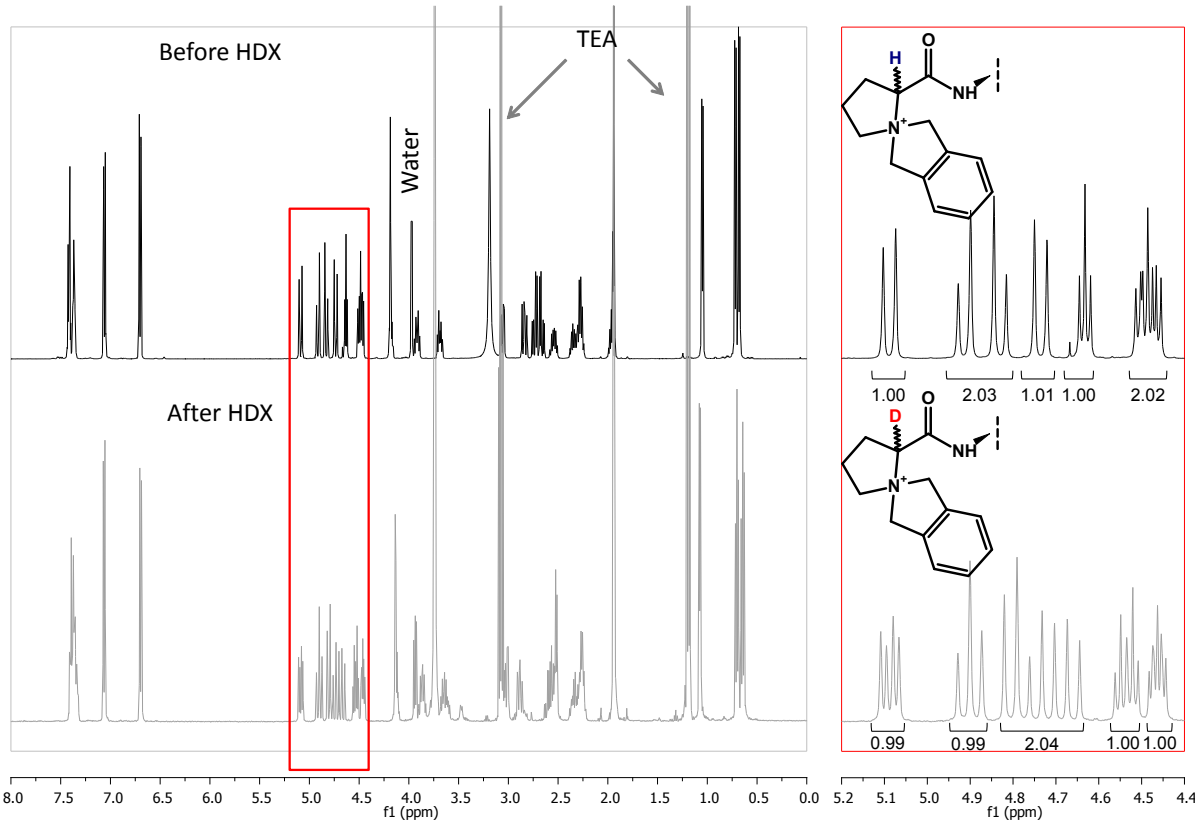


Figure 38S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of $BASN^{+}–CO–DVYT–NH_2$ before and after HDX. Red inset depicts disappearance of the signal of α-H (based on the integration of the signal at 4.49 ppm) due to HDX in the benzo-5-azoniaspiro[4.4]nonyl-carbonyl residue

c. $ASN^{+}–CO–SWVE–NH_2$

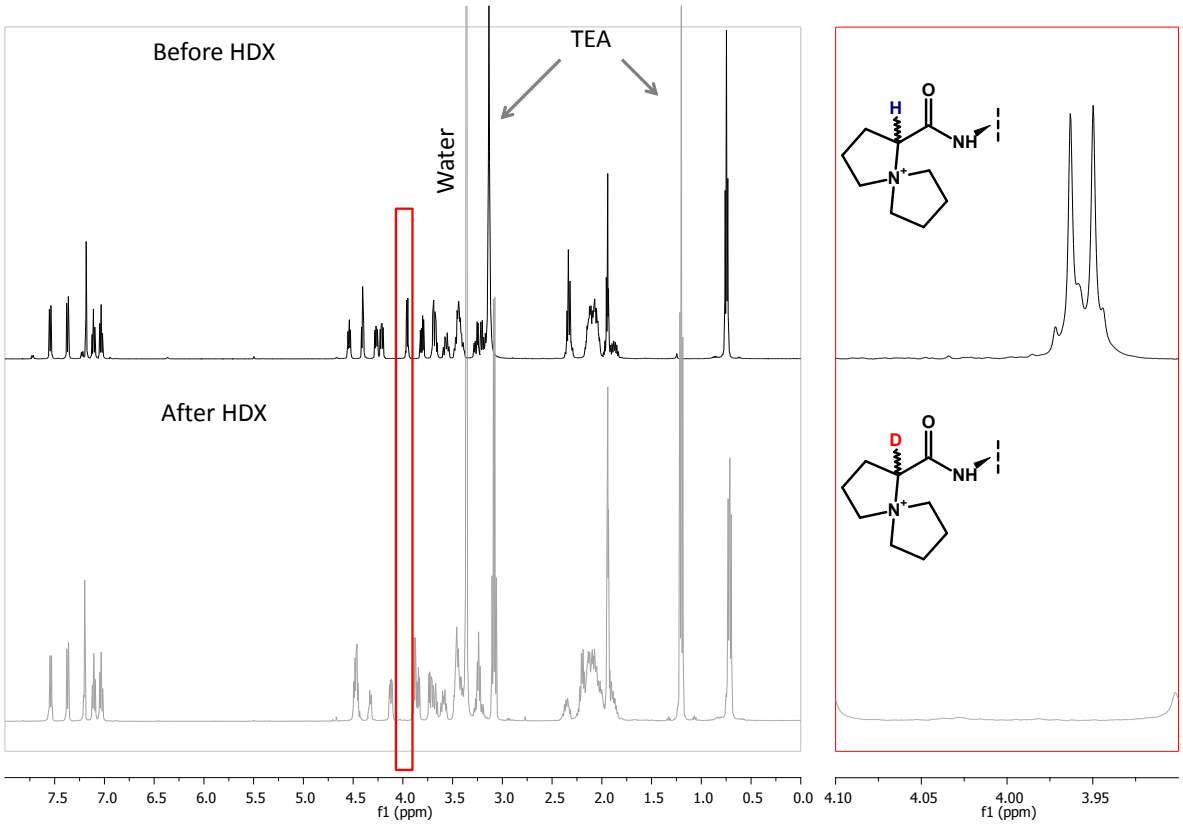


Figure 39S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of $ASN^{+}–CO–SWVE–NH_2$ before and after HDX. Red inset depicts disappearance of the signal of α-H (3.96 ppm) due to HDX in the 5-azoniaspiro[4.4]nonyl-carbonyl residue

d. $BASN^{+}–CO–SWVE–NH_2$

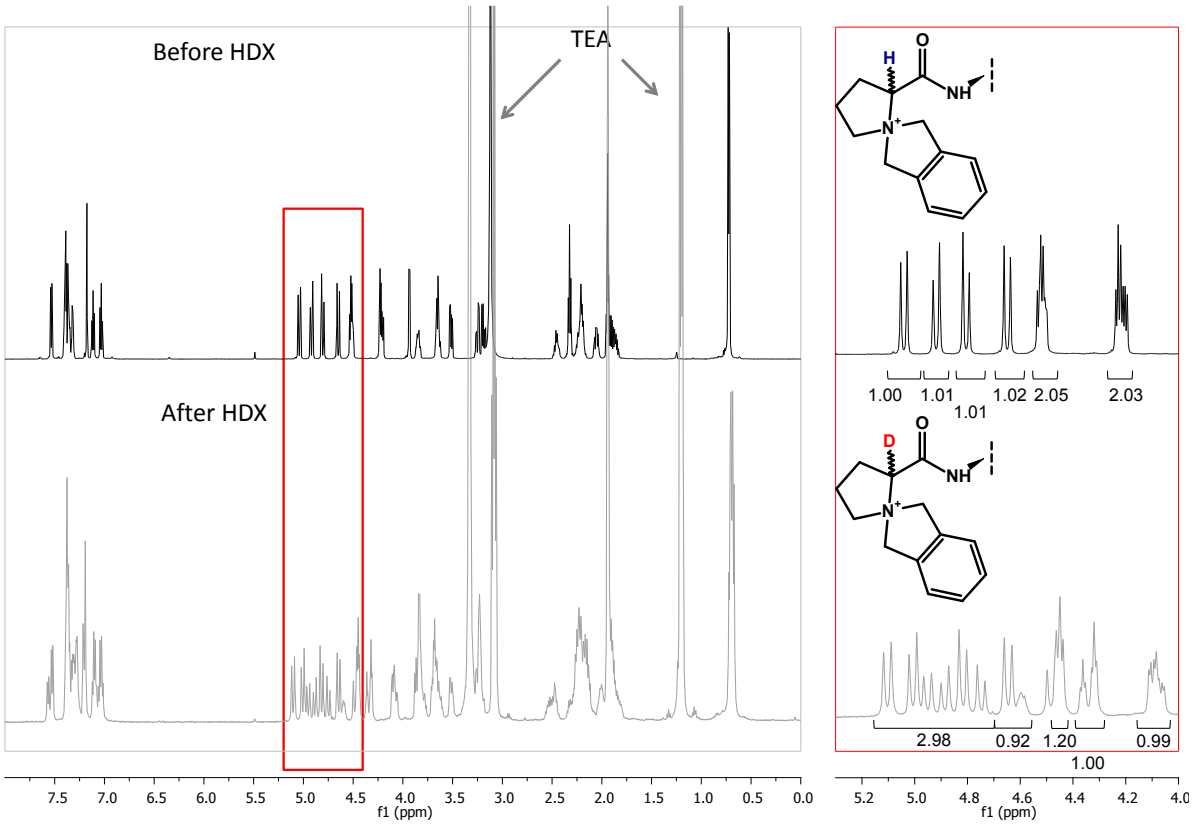


Figure 40S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of $BASN^{+}–CO–SWVE–NH_2$ before and after HDX. Red inset depicts disappearance of the signal of α-H (based on the integration of the signal at 4.10 ppm) due to HDX in the benzo-5-azoniaspiro[4.4]nonyl-carbonyl residue

e. $BASN^{+}–CO–\gamma Abu–OEt·BPh_4$

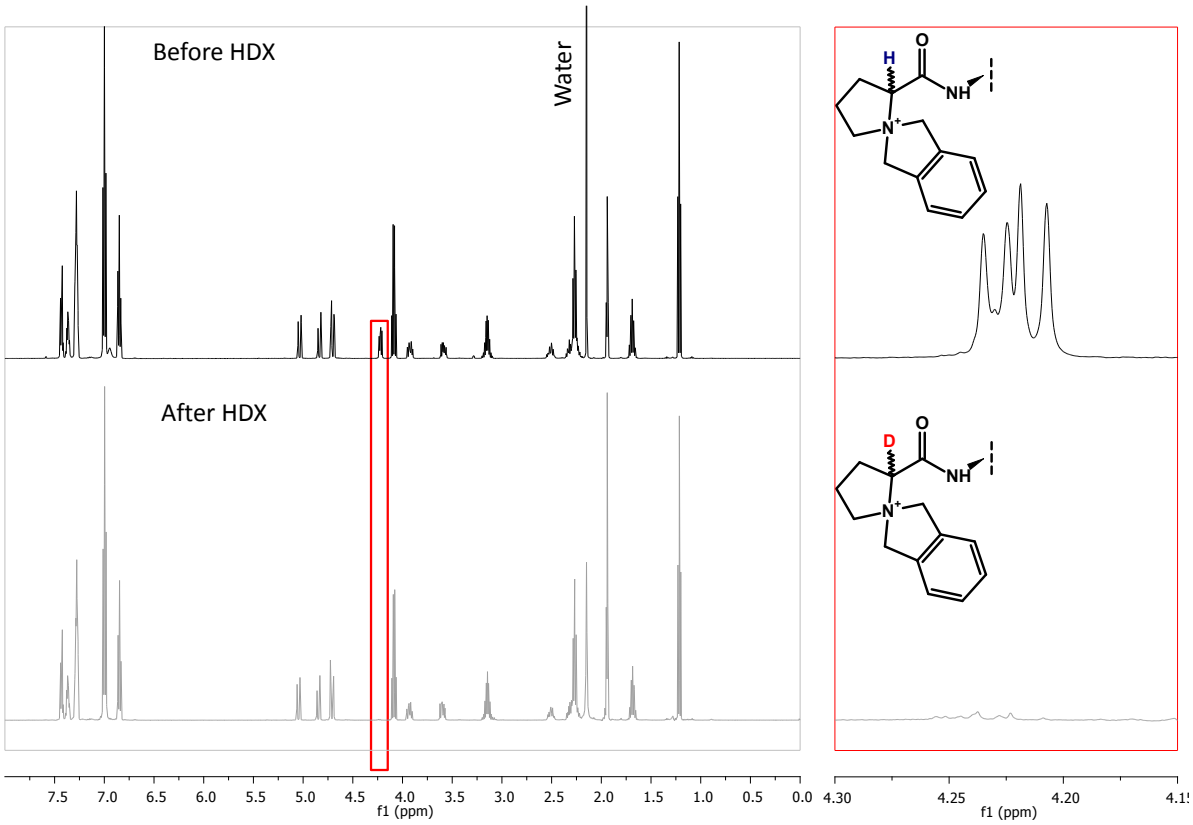


Figure 41S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of $BASN^{+}–CO–$ γAbu–OEt·BPh_4 before and after HDX. Red inset depicts disappearance of the signal of α-H (4.22 ppm) due to HDX in the benzo-5-azoniaspiro[4.4]nonyl-carbonyl residue

f. BASN^{+}-CO-ethylenediamine-*N*-Boc·BPh_4

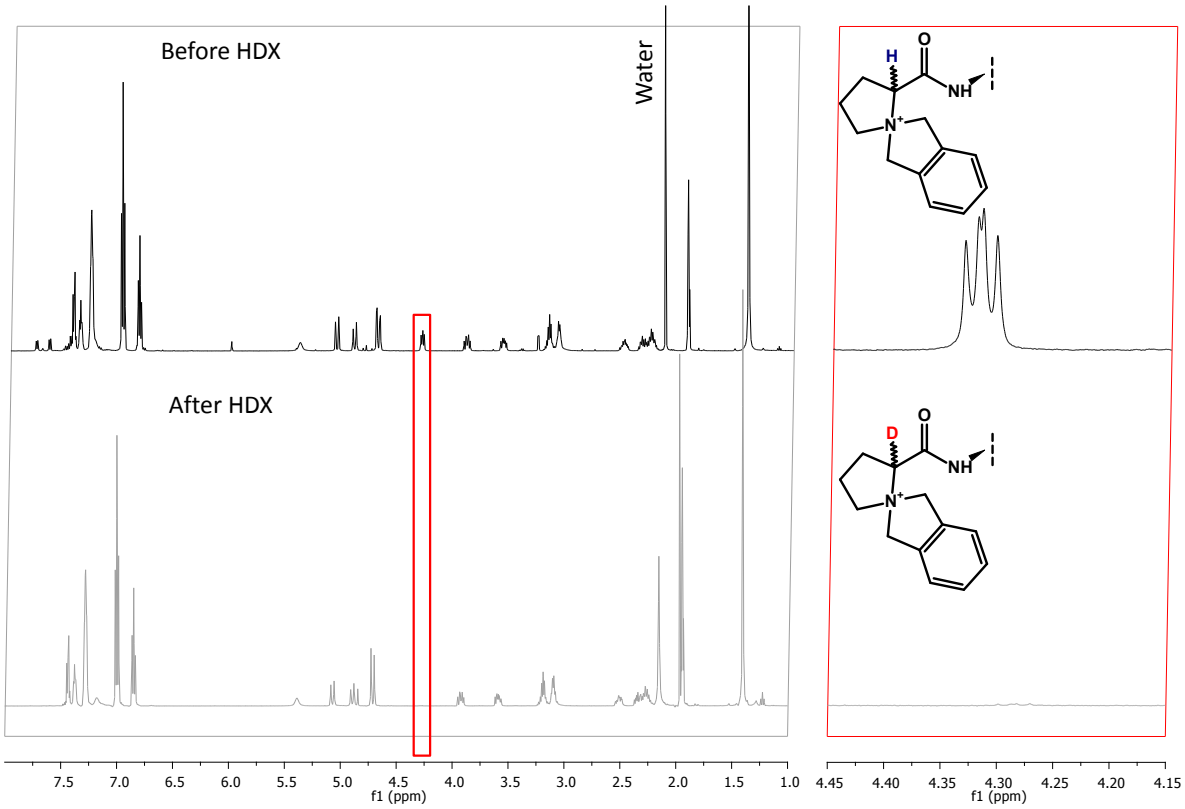


Figure 42S. ^{1}H-NMR (@500 MHz, CD_3CN, 300K) spectrum of BASN^{+}-CO-ethylenediamine-*N*-Boc·BPh_4 before and after HDX. Red inset depicts disappearance of the signal of α-H (4.22 ppm) due to HDX in the benzo-5-azoniaspiro[4.4]nonyl-carbonyl residue

5. X-ray crystal structure

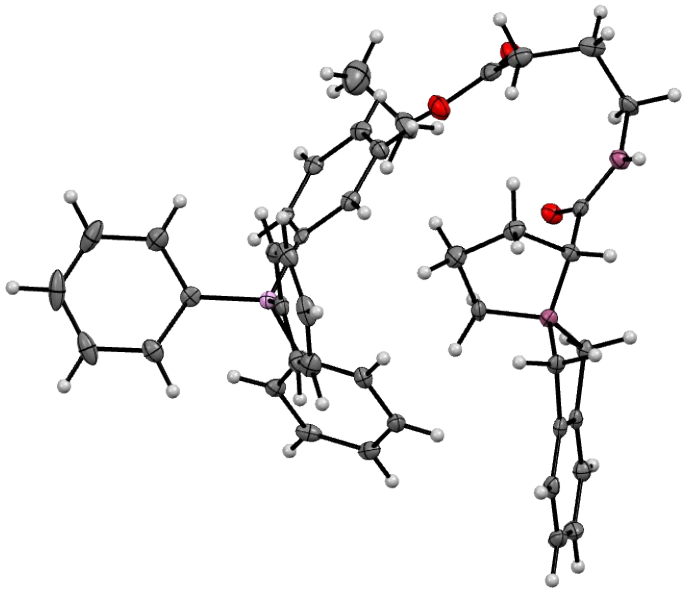


Figure 43S. X-ray crystal structure of BASN^{+}–CO– γAbu–OEt·BPh_4 along the a axis

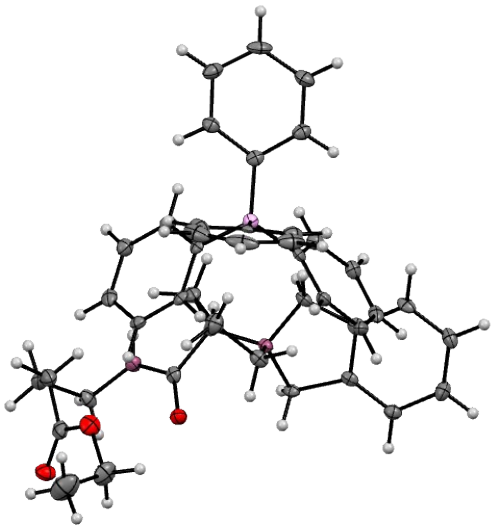


Figure 44S. X-ray crystal structure of the BASN^{+}–CO– γAbu–OEt·BPh_4 along the b axis

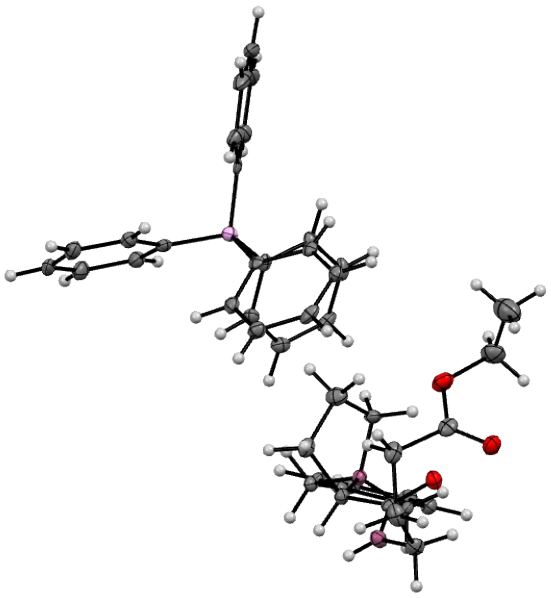


Figure 45S. X-ray crystal structure of the BASN^{+}–CO– γAbu–OEt·BPh_4 along the c axis

Identification code:	CCDC 1578235
Morphology:	Colorless plates
Crystal size (mm^3):	0.21 × 0.18 × 0.11
Empirical formula:	$C_{47}H_{42}BF_5N_2O_3$
Formula weight:	788.65

Unit cell dimensions	a (Å):	9.6421(8)
	b (Å):	18.6118(12)
	c (Å):	20.0924(14)
Volume (Å^3):		3605.72
Z:		4
Density $_{calculated}$ (g·cm^{-3}):		1.199
Crystal system:		Rhombohedral
Space group:		$P2_12_12_1$
λ (Å):		0.71073
Absorption coefficient μ (mm^{-1}):		0.074
Absorption correction:		Analytical
T_{min} / T_{max}:		0.0294 / 0.0721
T (K):		100(2)
ϑ range for data collection (°):		2.89 to 25.00
Index ranges:		–11 ≤ h ≤ 7; –22 ≤ k ≤ 10; –14≤ l ≤ 23
Reflections collected:		5785
Independent reflections:		4754 [R(int) =0.0465]
Parameters:		447
Goodness-of-fit on F^2:		1.016

6. ESI-MS/MS quantification experiments

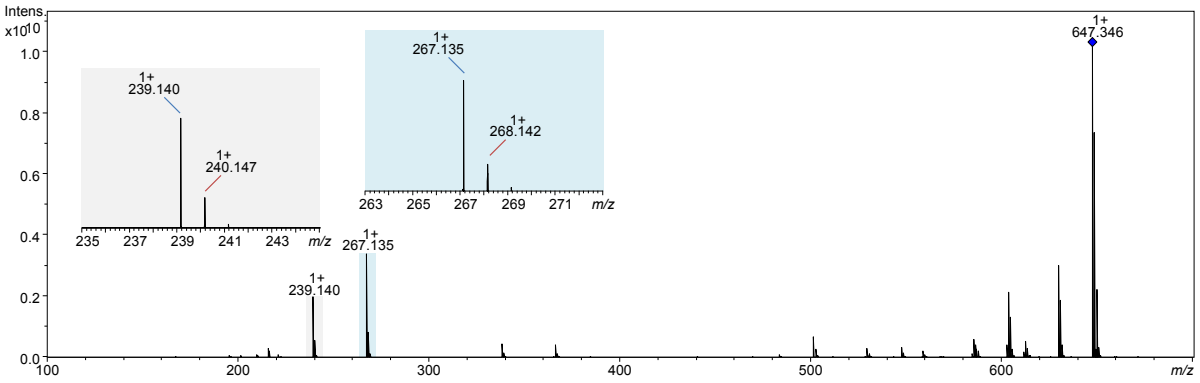


Figure 46S. ESI-MS/MS spectrum of 4:1 mixture of ASN^{+}-CO-DVYT-NH_2 and $ASN^{+}\{d_1\}$-CO-DVYT-NH_2 (parent ion m/z 647.346, collision energy 25 V).

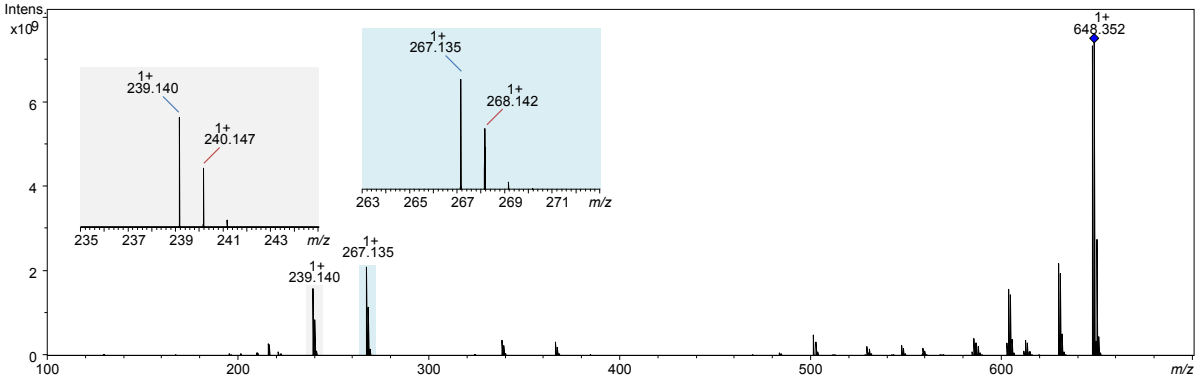


Figure 47S. ESI-MS/MS spectrum of 2:1 mixture of ASN^{+}-CO-DVYT-NH_2 and $ASN^{+}\{d_1\}$-CO-DVYT-NH_2 (parent ion m/z 648.352, collision energy 25 V).

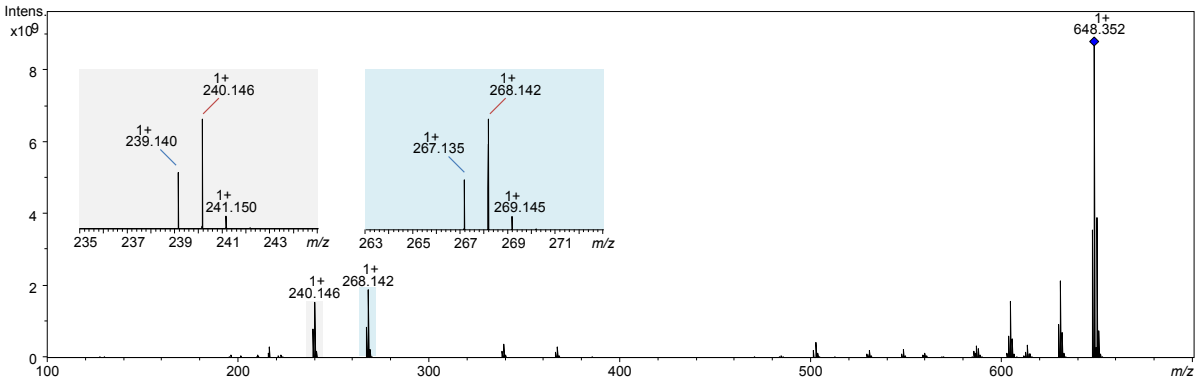


Figure 48S. ESI-MS/MS spectrum of 1:2 mixture of ASN^{+}-CO-DVYT-NH_2 and $ASN^{+}\{d_1\}$-CO-DVYT-NH_2 (parent ion m/z 648.352, collision energy 25 V).

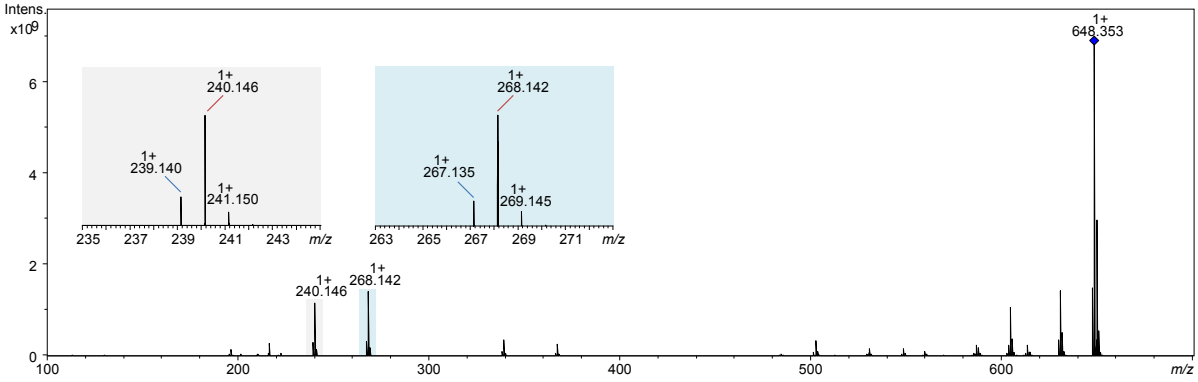


Figure 49S. ESI-MS/MS spectrum of 1:4 mixture of ASN^{+}-CO-DVYT-NH_2 and ASN^{+}{d_1}-CO-DVYT-NH_2 (parent ion m/z 648.353, collision energy 25 V).

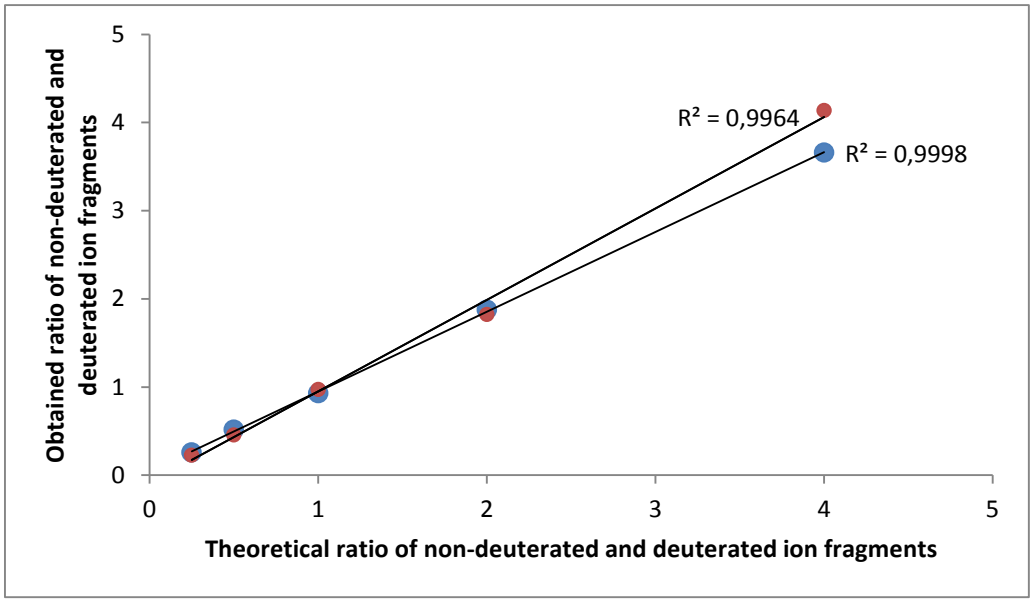


Figure 50S. The correlation between the theoretical and obtained ratios of non-deuterated and deuterated a_2^* (●) and b_2^* (●) ion fragments.

1 Guttmann S, Boissonnas RA (1958) Synthèse de la L–histidyl–L–phénylalanyl–L–arginyl–L–tryptophanyl–glycyl–ϵ–CBO–L–lysyl–L–prolyl–L–valylamide. *Helv Chem Acta* 41(6):1852–1867

2 Anderson GW, Zimmermann JE, Callahan FM, Reinvestigation of the mixed carbonic anhydride method of peptide synthesis. J Am Chem Soc 89(19):5012–5017

3 Reschela T, Koňáka C, Oupicky D, Seymour LW, Ulbrich K (2002) Physical properties and *in vitro* transfection efficiency of gene delivery vectors based on complexes of DNA with synthetic polycations. J Control Release 81(1-2):201–217