

SUPPLEMENTARY MATERIAL

A theoretical and experimental case study of the hydrogen bonding predilection of S-methylcysteine

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S1. Synthesis

S1.1 General information

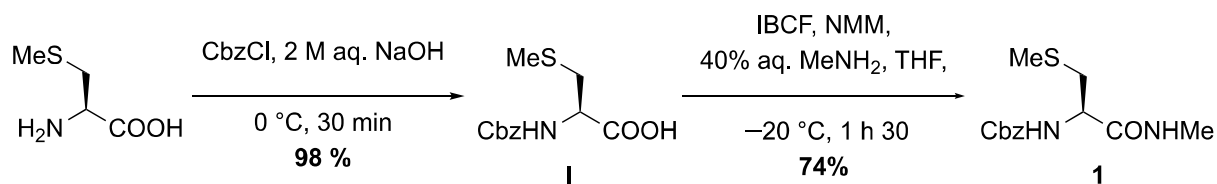
Materials and methods. *S*-methyl-L-cysteine was purchased from TCI; isobutyl chloroformate (IBCF) (98%), di-*tert*-butyl dicarbonate (Boc₂O) (99%) and trifluoroacetic acid (TFA) (peptide grade) were purchased from Fluorochem; benzyl chloroformate (CbzCl) (97 wt%) and triethylamine (extra pure) were purchased from Acros; magnesium sulfate (98%) was purchased from VWR Chemicals; *N*-methylmorpholine (NMM) (99%) and 40% aqueous methylamine were purchased from Sigma-Aldrich. These reagents were as supplied, except for NMM which was fractionally distilled (120 °C) from KOH and triethylamine which was fractionally distilled (90 °C) from KOH. Hydrochloric acid (36 wt%) and sodium hydroxide (98.5%) were purchased from Sigma-Aldrich, NaHCO₃ (99%) was purchased from Acros; these materials were used to make aqueous solutions. THF, when required dry, was distilled from sodium/benzophenone under argon. Other solvents (>99% purity) were purchased from VWR Chemicals or Carlo Erba and used as received (Abbreviation: PE = petroleum ether, boiling range 40-65 °C). Preparative flash chromatography was performed on silica gel columns (40-63 μm). Analytical thin-layer chromatography was carried out on commercial silica gel TLC plates of 0.25 mm thickness (Merck, Silica Gel 60F₂₅₄); after elution, the plates were visualized by fluorescence at 254 nm then revealed using acidic *p*-anisaldehyde solution (5% in EtOH), ninhydrin solution (14 mM in EtOH), or KMnO₄ solution (7.5% in water). Retention factors (R_f) are given for such TLC analyses.

Routine compound characterization. Melting points were measured in open capillary tubes on a Büchi B-540 apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1010 polarimeter using a 10 cm quartz cell; values for $[\alpha]_D^T$ (sodium D-line, temperature T) were obtained using solutions of concentration (c) in units of g/dL. ¹H and ¹³C NMR spectra were recorded at the indicated temperature on Bruker spectrometers operating at 360 MHz, 400 MHz or 600 MHz for ¹H and at 90 MHz or 100 MHz for ¹³C. For ¹H NMR spectra, chemical shifts (δ) are reported in parts per million (ppm) with reference to tetramethylsilane ($\delta = 0.00$ ppm) or residual protonated solvent ($\delta = 7.26$ ppm for CDCl₃, $\delta = 3.31$ ppm for CD₃OD) as internal standards. Splitting patterns for ¹H signals are designated as s (singlet), bs (broad singlet), bd (broad doublet), d (doublet), dd (double doublet), ddd (double double

doublet) or m (multiplet); coupling constants (J) are reported in hertz. For ^{13}C NMR spectra, chemical shifts (δ) are reported in parts per million (ppm) with reference to the deuterated solvent ($\delta = 77.2$ ppm for CDCl_3 , $\delta = 49.0$ ppm for CD_3OD) as an internal standard. Fourier-transform infrared (IR) spectra were recorded on a Perkin Elmer Spectrum Two spectrometer for neat samples (whether liquids or solids) using the ATR diamond accessory; maximum absorbances (ν) for significant bands are given in cm^{-1} . High-resolution mass spectrometry (HRMS) data were recorded on a Bruker MicroTOF-Q instrument using positive-mode electrospray ionization.

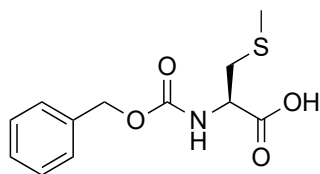
S1.2 Synthesis of Cbz-Cys(Me)-NHMe, **1**

Compound **1** was synthesized according to Scheme S1.



Scheme S1.

Cbz-S-methyl-L-cysteine I. To an ice-bath cooled solution of *S*-methyl-L-cysteine (2.00 g, 14.8 mmol, 1 eq.) in 2 M aqueous NaOH (25 mL, 50.0 mmol, 3.4 eq.) was added CbzCl (3.6 mL, 25.2 mmol, 1.7 eq.). The reaction mixture was stirred for 30 min at 0 °C. The mixture was washed with Et_2O (2×30 mL) and petroleum ether (30 mL). The remaining aqueous phase was acidified with 2 M HCl until pH 1-2 was reached then was extracted with EtOAc (6×40 mL). The combined organic layers were dried over MgSO_4 and concentrated under reduced pressure to give **I** (3.92 g, 98%) as a gold-colored oil which was used directly in the next step without further purification.



R_f 0.43 (EtOAc:MeOH, 1:1).

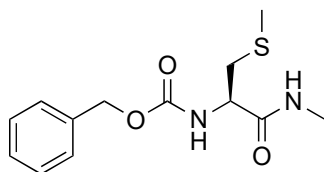
$^1\text{H NMR}$ (360 MHz, CD_3OD , 300 K) δ 7.41-7.22 (5H, m, CH^{Ar}), 5.11 (2H, s, CH_2), 4.39 (1H, dd, $^3J = 8.3$ Hz, $^3J = 4.8$ Hz, C^αH), 2.99 (1H, dd, $^3J = 4.7$ Hz, $^2J = 13.9$ Hz, $\text{C}^\beta\text{H}^{\text{a}}$), 2.89 (1H, dd, $^3J = 8.4$ Hz, $^2J = 13.9$ Hz, $\text{C}^\beta\text{H}^{\text{b}}$), 2.12 (s, 3H, CH_3).

$^{13}\text{C NMR}$ (90 MHz, CD_3OD , 300 K) δ 174.3 (amide CO), 158.5 (carbamate CO), 138.2 (C^{Ar}), 129.4, 129.0, 128.8 (3 CH^{Ar}), 67.7 (CH_2), 55.0 (C^α), 36.9 (C^β), 15.9 (CH_3).

$\text{IR } \nu$ 3322, 3033, 2920, 1696, 1512 cm^{-1} .

$\text{HRMS } m/z$ [$\text{M}+\text{Na}$] $^+$ calculated for [$\text{C}_{12}\text{H}_{15}\text{NNaO}_4\text{S}$] $^+$: 292.0608, found: 292.0614.

Cbz-S-methyl-L-cysteine-NHMe 1. To a cold (-20 °C) solution of **I** (500 mg, 1.86 mmol, 1 eq.) in dry THF (5 mL) under argon were added successively NMM (276 μL , 1.86 mmol, 1 eq.) and IBCF (241 μL , 1.86 mmol, 1 eq.). Following an activation period of 10 min at -20 °C, a solution of 40% aqueous methylamine (1.6 mL, 18.6 mmol, 10 eq.) in THF (2 mL) was added. The resulting mixture was stirred for 90 min at -20 °C then 5% aqueous NaHCO_3 (5 mL) was added. The resulting mixture was stirred for 1 h at room temperature then CH_2Cl_2 (20 mL) was added. The organic layer was collected, and the aqueous layer was extracted with more CH_2Cl_2 (5×20 mL). The combined organic layers were washed with 5% aqueous NaHCO_3 (2×5 mL), dried over MgSO_4 , filtered and concentrated under reduced pressure. Purification by flash chromatography (PE:EtOAc gradient from 7:3 to 0:1) gave **Cbz-S-methyl-L-cysteine-NHMe 1** as a white solid (388 mg, 74%).



R_f 0.23 (PE:EtOAc, 7:3).

Mp 151-152 °C.

$[\alpha]_D^{22}$ -3.41 (*c* 0.99, CHCl₃).

¹H NMR (600 MHz, CDCl₃, 273 K) δ 7.42-7.30 (5H, m, CH^{Ar}), 6.38 (1H, bs, NH²), 5.71 (1H, d, ³*J* = 5.6 Hz, NH¹), 5.12 (1H, d, ²*J* = 12.4 Hz, C^aH^{Cbz}), 5.09 (1H, d, ²*J* = 12.6 Hz, C^bH^{Cbz}), 4.32 (1H, ddd, ³*J* = 7.5 Hz, ³*J* = 6.5 Hz, ³*J* = 5.6 Hz, C^αH), 2.96 (2H, dd, ²*J* = 13.8 Hz, ³*J* = 7.5 Hz, C^βH^a), 2.83 (3H, d, ³*J* = 4.8 Hz, CH₃^{NHMe}), 2.81 (2H, dd, ²*J* = 13.8 Hz, ³*J* = 6.5 Hz, C^βH^b), 2.13 (3H, s, CH₃^{SMe}).

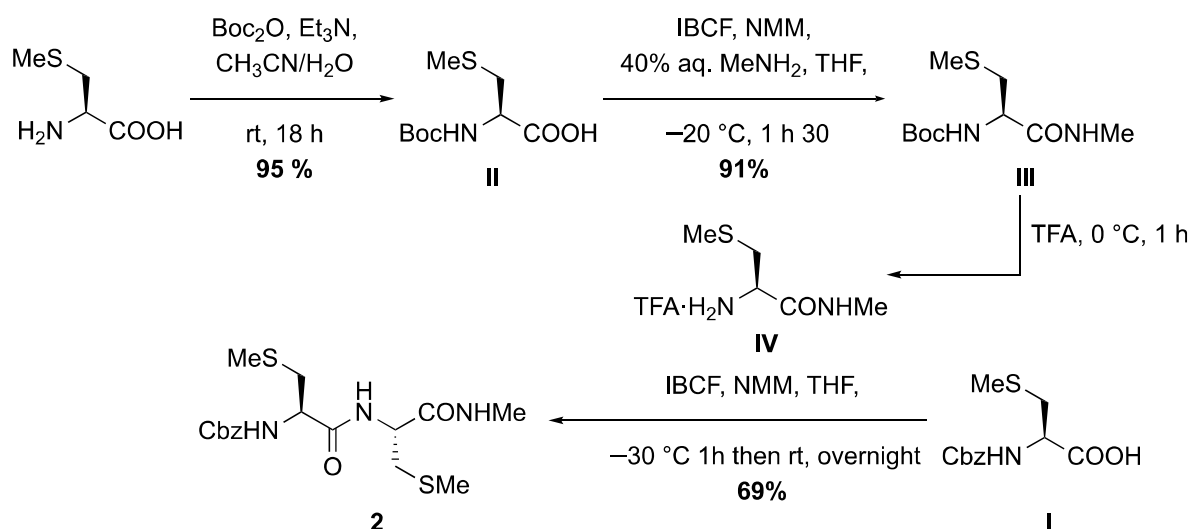
¹³C NMR (90 MHz, CDCl₃) δ 170.8 (amide CO), 156.1 (carbamate CO), 136.1 (C^{Ar}), 128.7, 128.5, 128.3 (3 CH^{Ar}), 67.4 (CH₂^{Cbz}), 54.0 (C^α), 36.7 (C^β), 26.6 (CH₃^{NHMe}), 15.9 (CH₃^{SMe}).

IR ν 3297, 2919, 1683, 1650, 1569, 1533 cm⁻¹.

HRMS *m/z* [M+Na]⁺ calculated for [C₁₃H₁₈N₂NaO₃S]⁺: 305.0923, found: 305.0930.

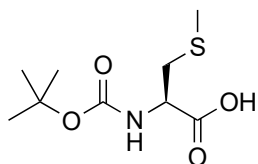
S1.3 Synthesis of Cbz-[Cys(Me)]₂-NHMe, 2

Compound **2** was synthesized according to Scheme S2.



Scheme S2.

Boc-*S*-methyl-L-cysteine II. To a solution of *S*-methyl-L-cysteine (3.15 g, 23.3 mmol, 1 eq.) in a 1:1 mixture of CH₃CN:H₂O (56 mL) was added trimethylamine (3.8 mL, 27.3 mmol, 1.17 eq.). A solution of Boc₂O (5.30 g, 24.2 mmol, 1.04 eq.) in CH₃CN (4 mL) was then added. The resulting mixture was stirred for 19 h at room temperature then CH₃CN was evaporated under reduced pressure. The aqueous layer was acidified with 1 M aqueous HCl solution until pH 1 was reached then was extracted with EtOAc (5 × 50 mL). The combined organic layers were washed successively with water (2 × 25 mL) and brine (1 × 25 mL), dried over MgSO₄ and concentrated under reduced pressure to give **II** (5.18 g, 95%) as a white solid which was used directly in the next step without further purification.



R_f 0.48 (EtOAc:MeOH 1:1).

Mp 72-74 °C [lit. 71-73 °C].¹

¹H NMR (400 MHz, CDCl₃, 300 K) δ 5.36 (1H, bs, NH), 4.61-4.43 (1H, m, C ^{α} H), 3.08-2.82 (2H, m, C ^{β} H), 2.16 (3H, s, CH₃^{SM e}), 1.46 (9H, s, CH₃^{Boc}).

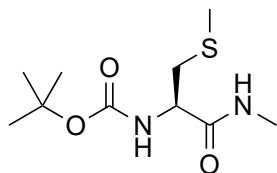
¹³C NMR (100 MHz, CDCl₃, 300 K) δ 174.7 (acid CO), 155.7 (carbamate CO), 80.9 (C^{Boc}), 53.0 (C ^{α}), 36.3 (C ^{β} H), 28.4 (CH₃^{Boc}), 16.4 (CH₃^{SM e}).

IR ν 3239, 2985, 2921, 1735, 1652 cm⁻¹.

HRMS m/z [M+Na]⁺ calculated for [C₉H₁₇NNaO₄S]⁺: 258.0770, found: 258.0771.

Boc-S-methyl-L-cysteine-NHMe III. To a cold (– 20 °C) solution of **II** (289 mg, 1.22 mmol, 1 eq.) in dry THF (3 mL) under argon were added successively NMM (134 μ L, 1.22 mmol, 1 eq.) and IBCF (158 μ L, 1.22 mmol, 1 eq.). Following an activation period of 10 min at – 20 °C, a solution of 40% aqueous methylamine (1.0 mL, 12.2 mmol, 10 eq.) in THF (1.7 mL) was added. The resulting mixture was stirred for 90 min at –20 °C then 5% aqueous NaHCO₃ (6 mL) was added. The resulting mixture was stirred for 1 h then CH₂Cl₂ (10 mL) was added. The organic layer was collected, and the aqueous layer was extracted with more CH₂Cl₂ (5 \times 10 mL). The combined organic layers were washed with 5% aqueous NaHCO₃ (2 \times 6 mL), dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by flash chromatography (PE:EtOAc, gradient from 1:1 to 0:1) gave **Boc-S-methyl-L-cysteine-NHMe III** as a white solid (277 mg, 91%).

¹ Ivanov, A. K.; Lavut, E. E.; Antonov, A. A.; Karel'skii, V. N. *Chem. Nat. Compd. (Engl. Transl.)*, **1989**, 25, 582-589.



R_f 0.67 (EtOAc).

Mp 84-86 °C.

$[\alpha]_D^{20}$ -1.05 (c 1.05, CHCl₃).

¹H NMR (360 MHz, CDCl₃, 300 K) δ 6.41 (1H, bs, NH²), 5.36 (1H, bs, NH¹), 4.23 (1H, ddd, ³ J = 7.0 Hz, ³ J = 6.4 Hz, ³ J = 5.4 Hz, C ^{α} H), 2.94 (1H, dd, ³ J = 13.9 Hz, ² J = 5.4 Hz, C ^{β} H^a), 2.84 (3H, d, ³ J = 4.9 Hz, CH₃^{NMe}), 2.81 (1H, dd, ³ J = 13.9 Hz, ² J = 7.0 Hz, C ^{β} H^b), 2.14 (3H, s, CH₃^{SMe}), 1.45 (9H, s, CH₃^{Boc}).

¹³C NMR (90 MHz, CDCl₃, 300 K) δ 171.3 (amide CO), 155.6 (carbamate CO), 80.6 (C^{Boc}), 53.7 (C ^{α}), 36.7 (C ^{β}), 28.5 (CH₃^{Boc}), 26.6 (CH₃^{NMe}), 16.0 (CH₃^{SMe}).

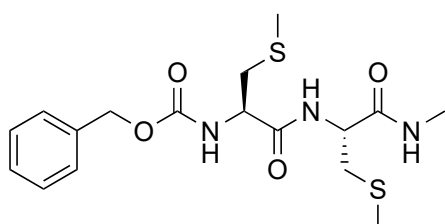
IR (neat) ν 3332, 2990, 2923, 1686, 1652, 1553, 1523 cm⁻¹.

HRMS [ESI(+)] m/z [M+Na]⁺ calculated for [C₁₀H₂₀N₂NaO₃S]⁺: 271.1083, found: 271.1087.

Cbz-(*S*-methyl-*L*-Cys)₂-NHMe 2. TFA (2.5 mL) was added dropwise to neat **III** (101 mg, 0.41 mmol, 1 eq.) held in an ice-bath cooled flask in such a way that the temperature of the developing solution remained at 0 °C. The resulting solution was then stirred for 1 h at room temperature then TFA was co-evaporated with CHCl₃ (4 × 10 mL) under reduced pressure to leave the TFA salt **IV** as a sticky solid which was used directly in the next reaction.

To a cold (-30 °C) solution of **I** (109 mg, 0.41 mmol, 1 eq.) in dry THF (5 mL) under argon were added successively NMM (115 μ L, 1.05 mmol, 2.56 eq.) and IBCF (57 μ L, 0.44 mmol, 1.07 eq.). The solution was stirred for 45 min just below -20 °C. A solution of TFA salt **IV** in dry THF (5 mL) was added and the recipient of **IV** was rinsed with more dry THF (0.5 mL)

and this was added too. The resulting mixture was stirred for 1 h just below $-30\text{ }^{\circ}\text{C}$ then was allowed to warm slowly to room temperature over 90 min. Volatiles were co-evaporated with CHCl_3 ($4 \times 10\text{ mL}$) under reduced pressure. The residue was partitioned between CH_2Cl_2 (15 mL) and 5% aqueous NaHCO_3 (6 mL). The organic layer was collected, and the aqueous layer was extracted with more CH_2Cl_2 ($6 \times 10\text{ mL}$). The combined organic layers were washed with 5% aqueous NaHCO_3 ($2 \times 10\text{ mL}$), dried over MgSO_4 , filtered and concentrated under reduced pressure. Purification by flash chromatography (PE:EtOAc, gradient from 7:3 to 0:1) gave **Cbz-(S-methyl-L-Cys)₂-NHMe 2** as a white solid (113 mg, 69%).



R_f 0.15 (PE:EtOAc, 1:1).

Mp 162-164 $^{\circ}\text{C}$.

$[\alpha]_D^{24}$ -26.4 (c 1.00, CHCl_3).

¹H NMR (600 MHz, CDCl_3 , 273 K) δ 7.43-7.30 (5H, m, CH^{Ar}), 7.16 (1H, bd, $^3J = 7.6\text{ Hz}$, NH^2), 6.91 (1H, bs, NH^3), 5.82 (1H, bd, $^3J = 5.5\text{ Hz}$, NH^1), 5.18 (1H, d, $^3J = 12.3\text{ Hz}$, $\text{C}^{\text{CbzH}^{\text{a}}}$), 5.09 (1H, d, $^3J = 12.2\text{ Hz}$, $\text{C}^{\text{CbzH}^{\text{b}}}$), 4.59 (1H, ddd, $^3J = 7.6\text{ Hz}$, $^3J = 6.1\text{ Hz}$, $^3J = 4.9\text{ Hz}$, $\text{C}^{\alpha(2)}\text{H}$), 4.33 (1H, ddd, $^3J = 6.8\text{ Hz}$, $^3J = 6.4\text{ Hz}$, $^3J = 5.5\text{ Hz}$, $\text{C}^{\alpha(1)}\text{H}$), 3.07 (1H, dd, $^2J = 14.3\text{ Hz}$, $^3J = 4.9\text{ Hz}$, 1H, $\text{C}^{\beta(2)}\text{H}^{\text{a}}$), 2.97 (1H, dd, $^2J = 13.8\text{ Hz}$, $^3J = 6.4\text{ Hz}$, $\text{C}^{\beta(1)}\text{H}^{\text{a}}$), 2.91 (1H, dd, $^2J = 13.8\text{ Hz}$, $^3J = 6.8\text{ Hz}$, $\text{C}^{\beta(1)}\text{H}^{\text{b}}$), 2.84 (1H, dd, $^2J = 14.3\text{ Hz}$, $^3J = 6.1\text{ Hz}$, $\text{C}^{\beta(2)}\text{H}^{\text{b}}$), 2.77 (3H, d, $^3J = 4.7\text{ Hz}$, CH_3^{NMe}), 2.14 (3H, s, $\text{CH}_3^{\text{SMe}(1)}$), 2.08 (3H, s, $\text{CH}_3^{\text{SMe}(2)}$).

¹³C NMR (90 MHz, CDCl_3 , 300 K) δ 170.5 (amide(1) CO), 170.4 (amide(2) CO), 156.5 (carbamate CO), 136.0 (C^{Ar}), 128.7, 128.5, 128.2 (3 CH^{Ar}), 67.5 (CH_2^{Cbz}), 54.3 ($\text{C}^{\alpha(1)}$), 52.3 ($\text{C}^{\alpha(2)}$), 36.4 ($\text{C}^{\beta(1)}$), 36.0 ($\text{C}^{\beta(2)}$), 26.5 (CH_3^{NMe}), 15.9 ($\text{CH}_3^{\text{SMe}(1)}$), 15.8 ($\text{CH}_3^{\text{SMe}(2)}$).

IR ν 3287, 2915, 1692, 1639, 1534 cm^{-1} .

HRMS m/z $[\text{M}+\text{Na}]^+$ calculated for $[\text{C}_{17}\text{H}_{25}\text{N}_3\text{NaO}_4\text{S}_2]^+$: 422.1160, found: 422.1177.

S1.4 Copies of ^1H and ^{13}C NMR spectra of 1 and 2

Cbz-Cys(Me)-NHMe 1

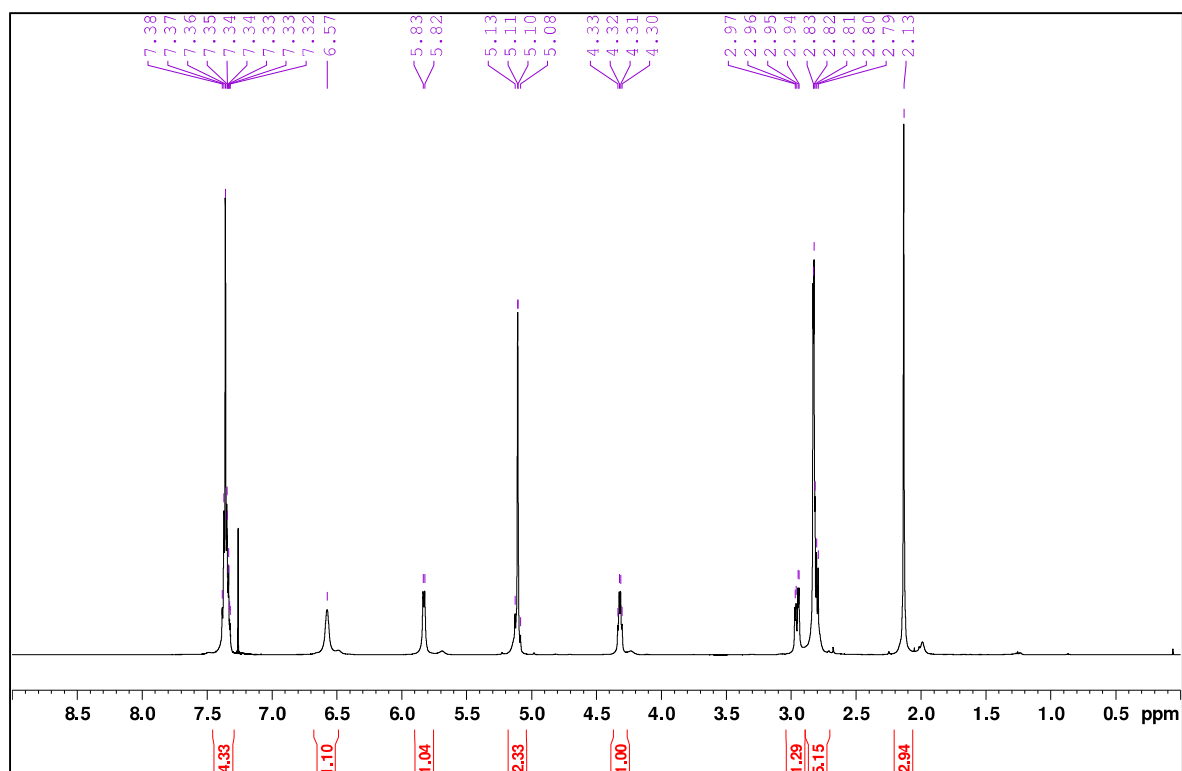
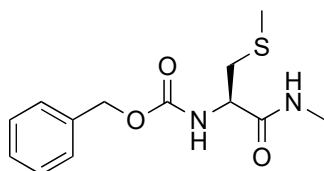


Fig. S1

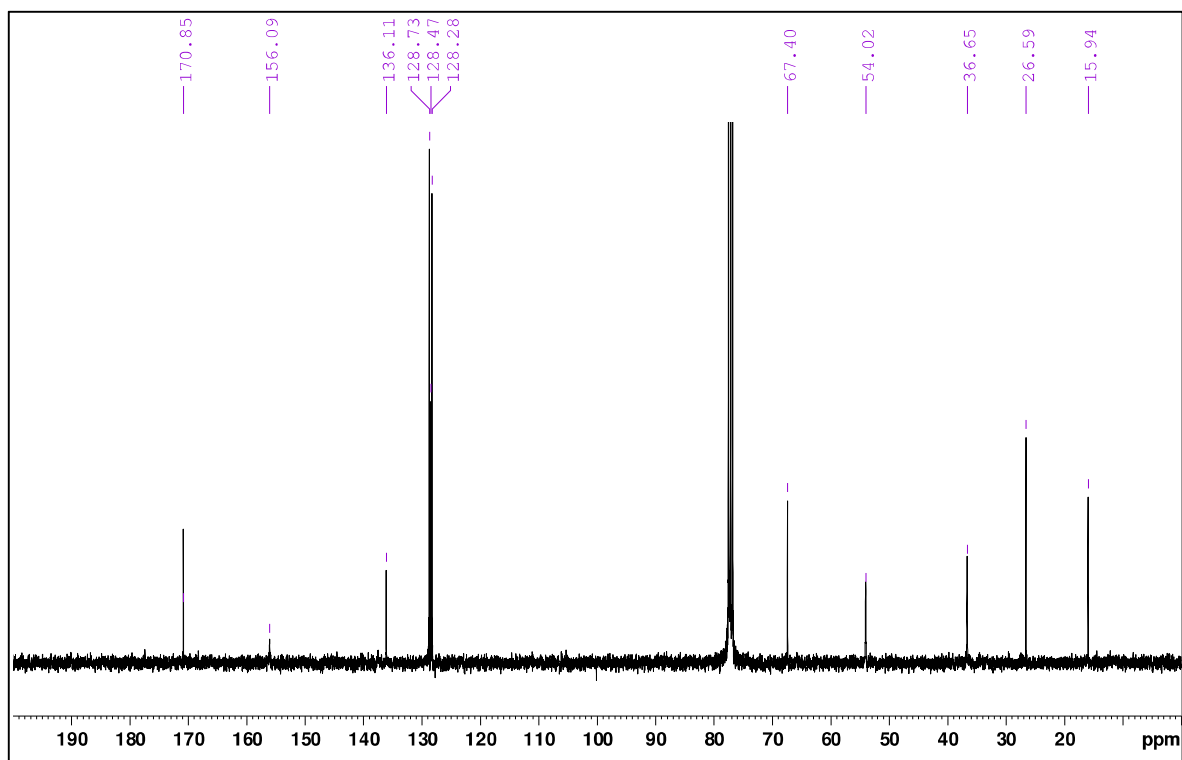
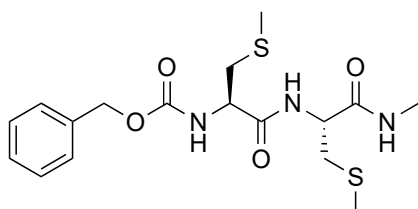


Fig. S2

Cbz-[(Cys(Me))₂-NHMe] 2



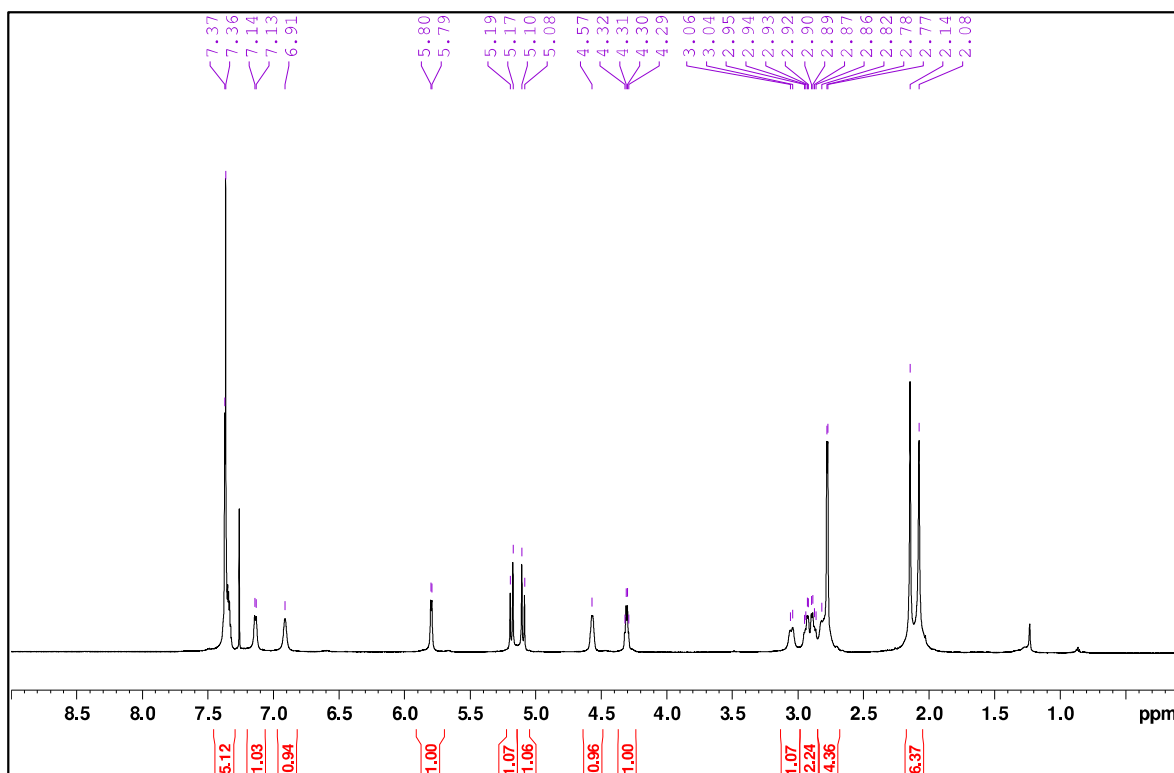


Fig S3

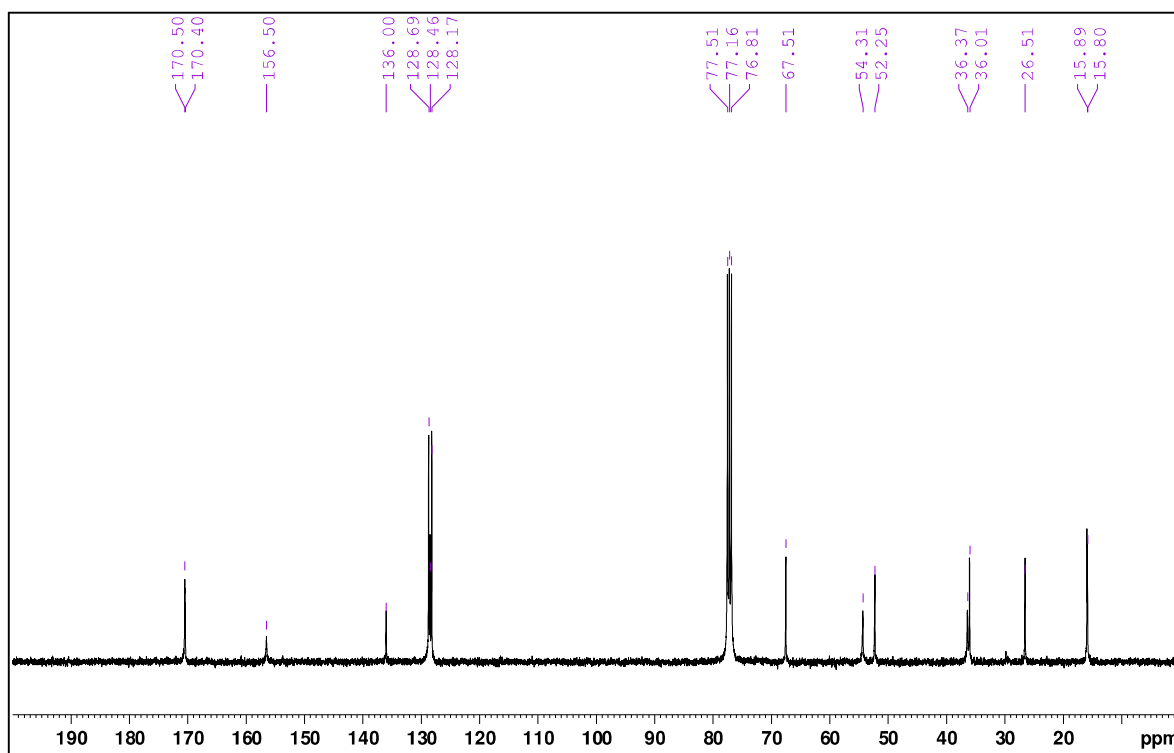


Fig S4.

S2. Conformational analysis

S2.1. Theoretical data on the lowest conformations of **1**

Table S1: Energetic parameters, frequencies (in cm^{-1} and decreasing order) and deviations of the lowest energy conformers of compound **1**: H-bond network, relative enthalpies (ΔH , 0 K) and Gibbs free energies (ΔG , 300 K) (both in kJ/mol.), theoretical vibrational frequencies (cm^{-1}) in the N-H stretch (amide A), amide I and amide II regions (in decreasing order) and average and maximum deviations ($\langle \delta \rangle$ and δ_{max} resp.) of the theoretical frequencies with respect to experimental frequencies recorded on A transition (the average deviation was calculated by considering all the 6 frequencies listed). These latter criteria can be used to guide the assignment, since typical discrepancy with experimental data is less than 20 cm^{-1} for free NH or $\text{NH}\cdots\text{OC}$ H-bonds but can amount up to $30\text{-}40 \text{ cm}^{-1}$ for $\text{NH}\cdots\text{S}$ bonds.² Such a criterion allows us to select small discrepancy structures, indicated here in bold in the two last columns. However, taking into account the intensity pattern, like in Fig. S2, allows us to restrict our preliminary assignment to sub-families 1-a and 1-c. Of course additional restrictions also result from energetic considerations, eventually leading to the assignment to the highlighted 1-a conformations.

² Biswal, H.S.; Gloaguen, E.; Loquais, Y.; Tardivel, B.; Mons, M. Strength of $\text{NH}\cdots\text{S}$ Hydrogen Bonds in Methionine Residues Revealed by Gas-Phase IR/UV Spectroscopy. *J. Phys. Chem. Lett.*, **2012**, *3*, 755-759. doi:10.1021/jz300207k

Conformers Conformations (Cbz rotamer)	Side chain orientation	Backbone type	ΔH (0K) (kJ/mol)	ΔG (300K) (kJ/mol)	Amide A		Amide I		Amide II		$\langle \delta \rangle$ rel. to A	δ_{\max} rel. to A
A					3423	3389	1729	1706.5	1550	1488.5		
B					3419	3389	1728.5	1705	1546.5	1486.5		
1-a (g-)	t,g+	5-6 γ	0	0	3438	3346	1725	1703	1555	1486	12	43
1-a (g+)	t,g+	5-6 γ	0.77	0.62	3433	3351	1726	1704	1552	1487	10	38
1-a (t)	t,g+	5-6 γ	3.26	1.51	3426	3342	1726	1703	1548	1475	12	47
1-b (t)	t,g+	cis-5-6 γ	3.25	5.58	3440	3350	1760	1708	1549	1438	23	51
1-b (g+)	t,g+	cis-5-6 γ	3.54	5.19	3449	3368	1754	1708	1542	1431	23	58
1-b (g-)	t,g+	cis-5-6 γ	7.63	7.99	3431	3362	1756	1708	1552	1439	19	50
1-c (g-)	t,g-	5-6 γ	6.69	6.14	3455	3417	1732	1706	1546	1488	11	32
1-c (g+)	t,g-	5-6 γ	6.83	6.55	3455	3427	1732	1706	1544	1481	15	38
1c (t)	t,g-	5-6 γ	9.74	6.78	3454	3421	1733	1705	1542	1489	13	32
1-d (g-)	t,t	5-6 γ	9.66	7.00	3417	3323	1733	1700	1555	1474	17	66
1-d (g+)	t,t	5-6 γ	10.84	10.33	3414	3327	1732	1700	1555	1473	17	62
1-d (t)	t,t	5-6 γ	13.41	9.20	3413	3326	1733	1700	1563	1467	20	63
2-a (g-)	g+,g+	cis-5 γ - π_{am}	0.31	3.86	3506	3424	1743	1704	1519	1434	37	83
2-a (g+)	g+,g+	cis-5 γ - π_{am}	9.09	5.26	3488	3425	1743	1707	1520	1432	34	65
2-a (t)	g+,g+	cis-5 γ - π_{am}	11.20	7.03	3490	3440	1748	1710	1542	1440	33	67
2-b (g-)	g+,g-	5 γ - π_{am}	6.78	6.08	3477	3409	1750	1713	1537	1476	21	54
2-b (g+)	g+,g-	5 γ - π_{am}	11.51	7.35	3474	3430	1751	1713	1528	1485	24	51
2-b (t)	g+,g-	5 γ - π_{am}	14.8	11.7	3482	3416	1752	1714	1533	1478	24	59
3-a (g+)	g+,g+	5 γ -7 $_L^N$	4.46	7.62	3395	3380	1717	1698	1563	1497	13	28
3-a (g-)	g+,g+	5 γ -7 $_L^N$	6.10	6.79	3364	3343	1711	1694	1559	1497	26	59
3-a (t)	g+,g+	5 γ -7 $_L^N$	8.44	6.76	3363	3341	1709	1693	1559	1500	27	60
3-b (g-)	g-,g-	5 γ -7 $_L^N$	5.49	7.90	3416	3384	1720	1706	1554	1497	6	9
3-b (g+)	g-,g-	5 γ -7 $_L^N$	6.88	5.75	3453	3357	1719	1700	1553	1502	16	32
3-b (t)	g-,g-	5 γ -7 $_L^N$	10.04	4.69	3440	3362	1719	1701	1551	1501	12	27
4-a (g+)	g-,g+	f-7 $_D^N$	7.83	12.10	3512	3348	1720	1697	1555	1517	30	89
4-a (g-)	g-,g+	f-7 $_D^N$	12.32	9.48	3514	3357	1721	1699	1561	1518	30	91
4-a (t)	g-,g+	f-7 $_D^N$	not a minimum		-	-	-	-	-	-	-	-
4-b (g+)	g-,g-	f-7 $_D^N$	11.85	10.18	3505	3340	1718	1696	1560	1517	32	82
4-b (g-)	g-,g-	f-7 $_D^N$	12.08	11.11	3511	3339	1718	1696	1567	1519	34	88
4-b (t)	g-,g-	f-7 $_D^N$	14.02	7.91	3506	3326	1718	1695	1557	1519	34	83

S2.2. Gas phase conformer-selective IR spectra of compound 1

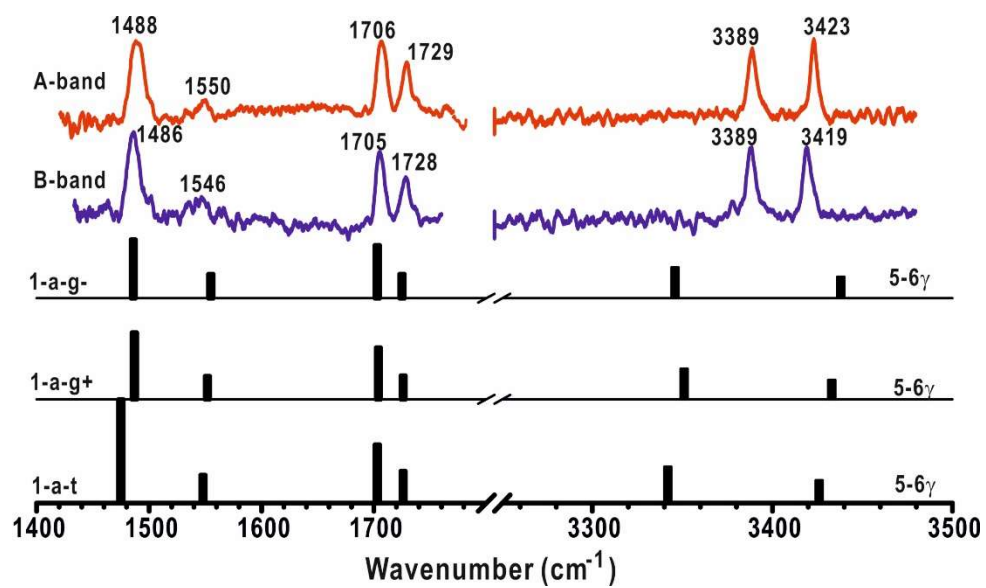


Fig. S5. Conformer-selective IR spectra of **1** from UV bands A and B together with the theoretical spectra of the three Cbz rotamers of sub-family 1-a.

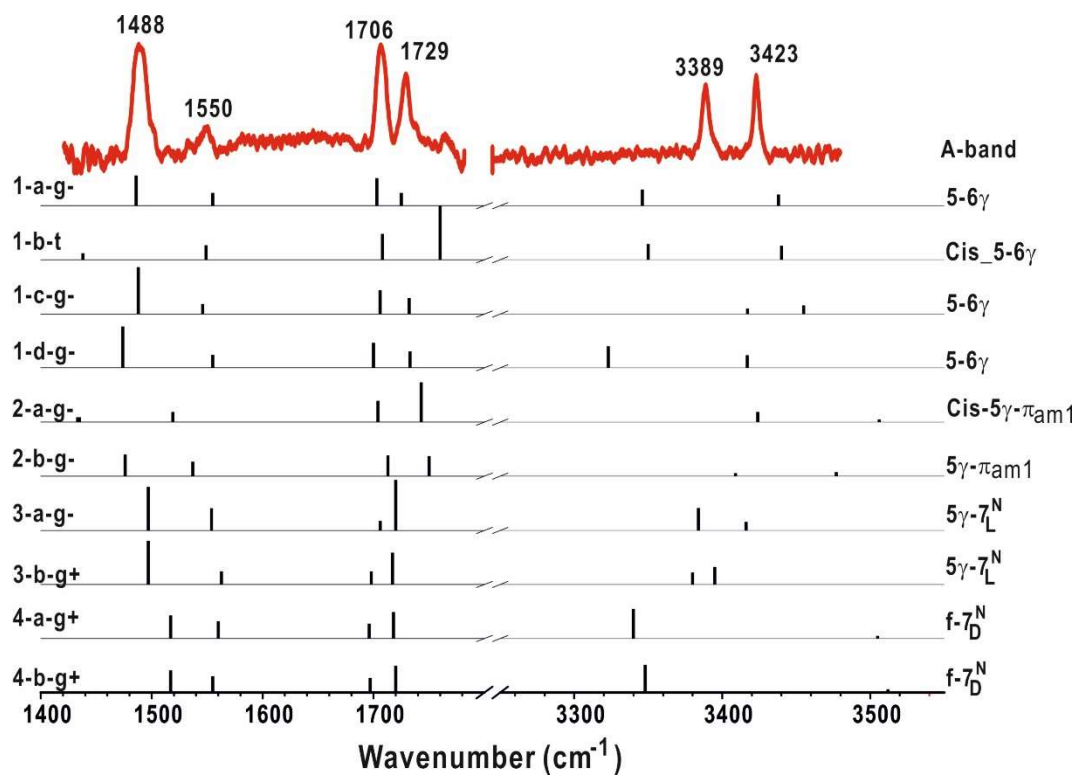


Fig. S6. Experimental spectra obtained on the UV band A of **1** (top) compared to the spectral diversity as illustrated by the theoretical spectra of the most stable Cbz rotamers of each sub-family (see Fig. 4).

S2.3. Comparison between gas phase and solution IR spectra of compound **1** and Cbz-Attc-NHMe.

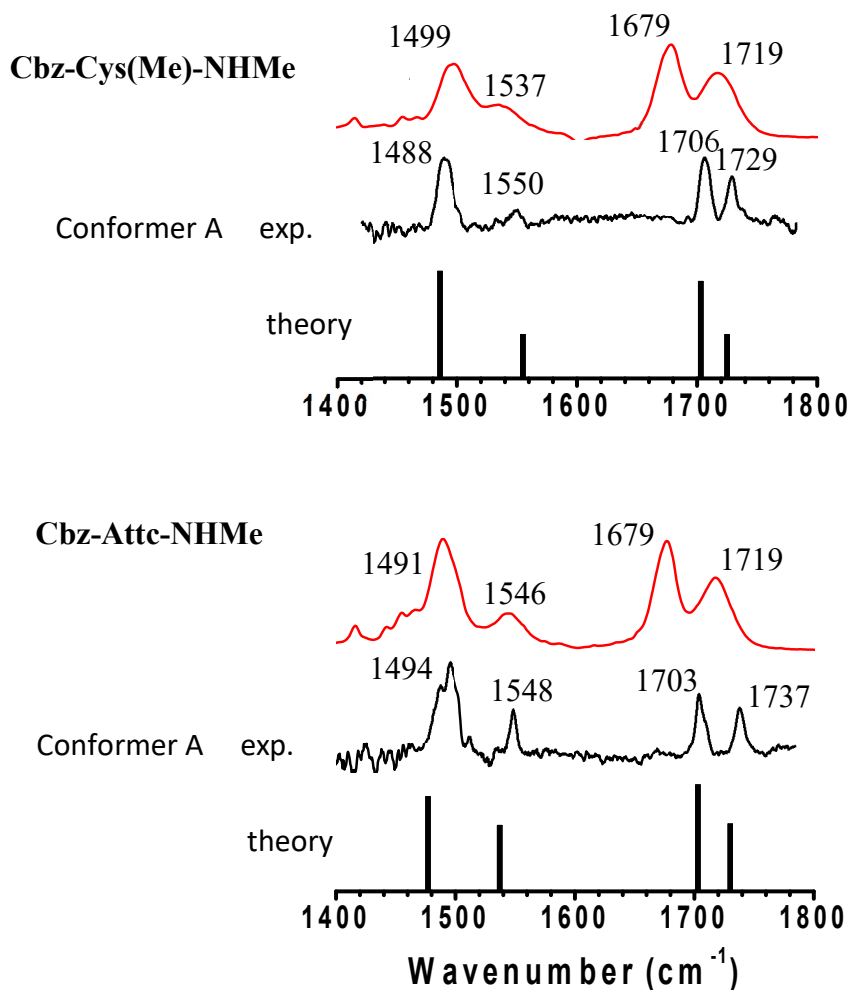


Fig. S7. Comparison of the amide I and II gas phase spectra (black) of the main conformer (A) of **1** and its Attc counterpart, with the corresponding solution spectra (red).

S2.4. Theoretical data on the lowest conformations of compound 2

Table S2. Energetic parameters, frequencies (cm⁻¹) and deviations of the lowest energy conformers of compound 2. Same caption details and comments as for Table S1.

Conformers /conformations (Cbz rotamer)	Backbone type	Side chains' orientations	$\Delta H(0K)$ (kJ/mol)	$\Delta G(300K)$ (kJ/mol)							A		B		C				
					Amide A			Amide I			Amide II			$\langle \delta \rangle$ rel. to A	δ_{max} rel. to A	$\langle \delta \rangle$ rel. to B	δ_{max} rel. to B	$\langle \delta \rangle$ rel. to C	δ_{max} rel. to C
A					3424	3415	3403	1722	1711	1697	1532	1505	1485						
B					3410	3391	3383	1730	1711	1698	1532	1502	1484						
C					3431	3399	3337	1762	1702	1664	1550	1517	1485						
l-a (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁻ /g ⁻	0.56	0	3473	3423	3404	1719	1711	1698	1538	1514	1486	9	50	16	63	26	68
l-b (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁺ /g ⁺	0	1.53	3420	3417	3405	1717	1712	1699	1523	1503	1486	3	8	10	26	26	68
l-c (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁻ /g ⁻ _g ⁺ /g ⁺	2.39	4.98	3417	3410	3392	1728	1713	1697	1535	1494	1485	5	11	6	19	22	55
l-d (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁺ /g ⁻	4.60	6.73	3434	3431	3424	1718	1712	1700	1540	1503	1485	8	21	15	41	26	87
l-e (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁻ /g ⁺	5.01	10.85	3481	3427	3422	1720	1716	1698	1537	1518	1486	13	58	21	71	30	86
l-b (t)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁺ /g ⁺	7.06	4.13	3425	3421	3409	1717	1710	1699	1527	1498	1482	4	7	11	30	26	73
l-f (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁻ /g ⁻ _g ⁻ /g ⁻	8.34	6.27	3458	3420	3395	1734	1712	1696	1537	1520	1487	9	35	14	48	22	59
l-g (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /a_g ⁻ /g ⁻	8.39	9.35	3442	3423	3412	1728	1720	1703	1524	1504	1488	8	18	14	32	27	76
l-c (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁻ /g ⁻ _g ⁺ /g ⁺	9.30	7.75	3412	3398	3387	1726	1712	1700	1540	1492	1479	9	17	5	10	21	50
l-h (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁻ /g ⁻ _g ⁺ /a	9.83	8.70	3406	3403	3393	1725	1716	1705	1538	1506	1482	7	18	6	12	23	56
l-b (g ⁻)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /g ⁺ _g ⁺ /g ⁺	10.05	5.12	3419	3412	3410	1713	1712	1700	1531	1501	1481	4	9	9	27	26	73
l-i (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	g ⁺ /a_g ⁺ /g ⁻	11.68	10.19	3448	3431	3422	1722	1718	1699	1536	1515	1493	10	24	18	40	28	85
l-j (g ⁺)	$5\gamma - 5\gamma/\pi_{am1} - 10/\pi_{am2}$	a/g ⁺ _g ⁻ /g ⁺	12.81	6.35	3448	3419	3414	1723	1717	1700	1527	1499	1488	7	25	14	38	28	78

Conformers / Conformations (Cbz rotamer)	Backbone type	Side chains' orientations	$\Delta H(0K)$ (kJ/mol)	$\Delta G(300K)$ (kJ/mol)										A		B		C	
					Amide A			Amide I			Amide II								
A					3424	3415	3403	1722	1711	1697	1532	1505	1485						
B					3410	3391	3383	1730	1711	1698	1532	1502	1484						
C					3431	3399	3337	1762	1702	1664	1550	1517	1485						
														$\langle \delta \rangle$ rel. to A	δ_{max} rel. to A	$\langle \delta \rangle$ rel. to B	δ_{max} rel. to B	$\langle \delta \rangle$ rel. to C	δ_{max} rel. to C
2-a (g+)	$5\gamma-\pi_{am1}/5\gamma-7$	g+,g+,g-,g+	7.38	10.76	3489	3431	3358	1743	1708	1671	1556	1514	1481	24	65	25	79	17	58
2-b (g+)	$5\gamma-\pi_{am1}/5\gamma-7$	g+,g+,g-,a	10.86	12.47	3473	3428	3325	1742	1710	1670	1562	1517	1482	26	78	27	63	15	42
2-c (g+)	$5\gamma-\pi_{am1}/5\gamma-7$	g+/g+ g-/g-	12.18	14.00	3443	3413	3322	1745	1712	1663	1559	1517	1490	23	81	24	61	9	17
2-c (g-)	$5\gamma-\pi_{am1}/5\gamma-7$	g+/g+ g-/g-	25.47	17.53	3432	3413	3334	1745	1717	1666	1554	1519	1492	20	69	22	49	7	17
2-c (t)	$5\gamma-\pi_{am1}/5\gamma-7$	g+/g+ g-/g-	27.80	16.95	3403	3394	3347	1754	1707	1688	1554	1514	1491	20	56	14	36	10	28
3-a (g+)	$5\gamma-5/\pi_{am1}-6\gamma$	g-/g- a/g+	20.24	12.87	3407	3400	3354	1746	1704	1689	1550	1479	1474	19	49	14	29	15	38
3-a (t)	$5\gamma-5/\pi_{am1}-6\gamma$	g-/g- a/g+	21.18	16.16	3410	3400	3357	1746	1704	1688	1549	1483	1473	18	46	13	26	15	34
3-a (g-)	$5\gamma-5/\pi_{am1}-6\gamma$	g-/g- a/g+	23.39	13.19	3404	3399	3349	1746	1706	1691	1548	1481	1471	20	54	14	34	15	36
4-a (g-)	$5-6\gamma/5\gamma-7$	a/g+ g+/g+	20.90	13.79	3450	3346	3287	1728	1710	1665	1552	1531	1482	33	116	30	96	20	53
4-a (g+)	$5-6\gamma/5\gamma-7$	a/g+ g+/g+	21.53	14.8	3449	3349	3285	1727	1710	1664	1554	1528	1480	33	118	30	98	20	51
4-a (t)	$5-6\gamma/5\gamma-7$	a/g+ g+/g+	25.16	16.92	3447	3352	3296	1728	1710	1665	1555	1538	1491	33	107	30	87	20	47
5-a (g-)	$5-5\gamma-\pi_{am2}$	a/g+ g+a	25.17	15.83	3459	3424	3357	1729	1710	1708	1533	1509	1491	13	46	15	49	21	44
5-a (g+)	$5-5\gamma-\pi_{am2}$	a/g+ g+a	26.05	17.05	3458	3420	3352	1729	1711	1709	1535	1508	1490	14	51	15	48	20	45
5-a (t)	$5-5\gamma-\pi_{am2}$	a/g+ g+a	28.16	16.03	3456	3421	3354	1729	1711	1709	1531	1491	1482	14	49	14	46	22	45

S2.5. Gas phase conformer-selective IR spectra of 2

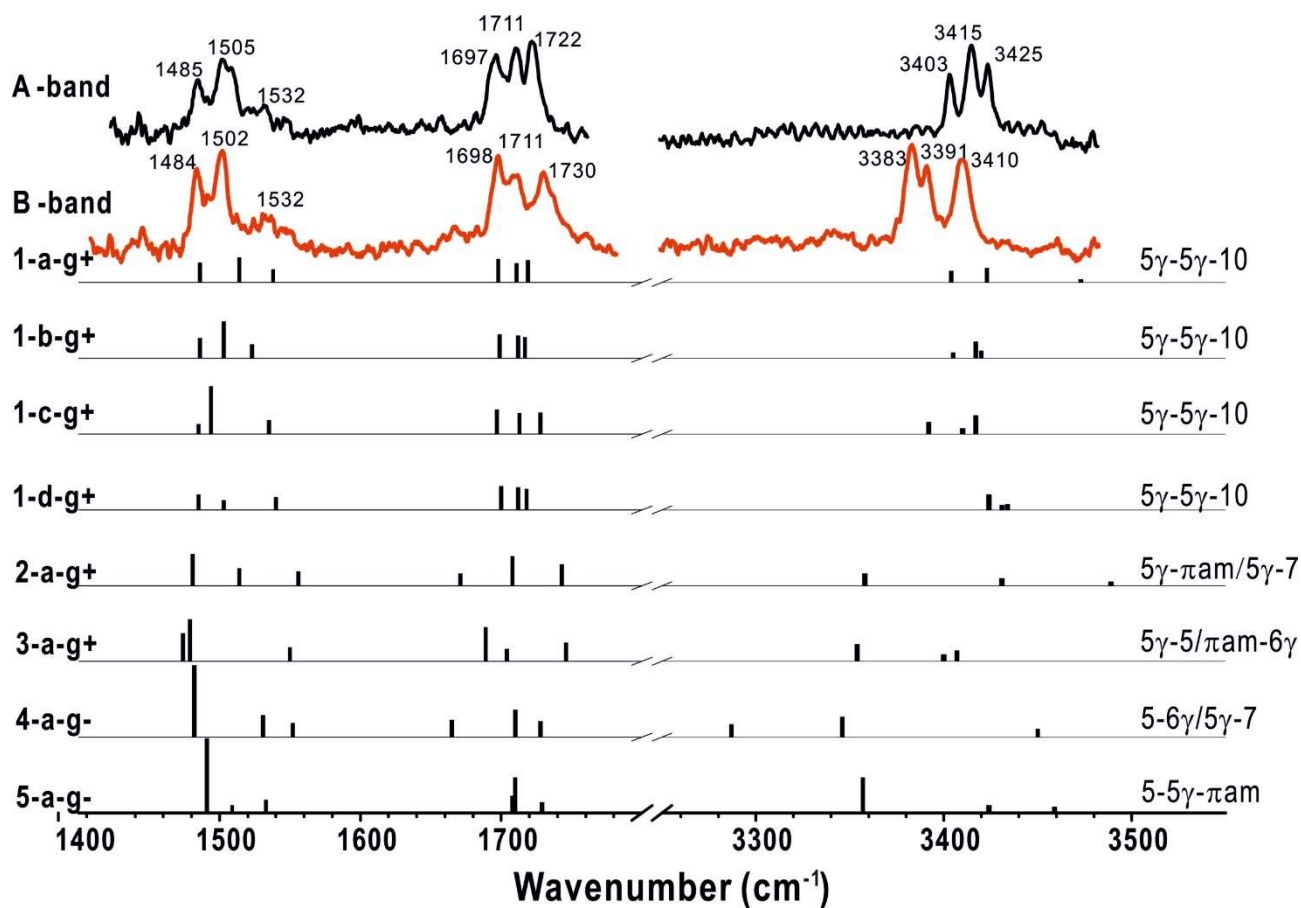


Fig. S8. *Top panel* Experimental conformer-specific IR spectra obtained by probing the UV bands A and B. *Lower panel* Theoretical IR spectra of the most stable conformer of selected backbone sub-families.

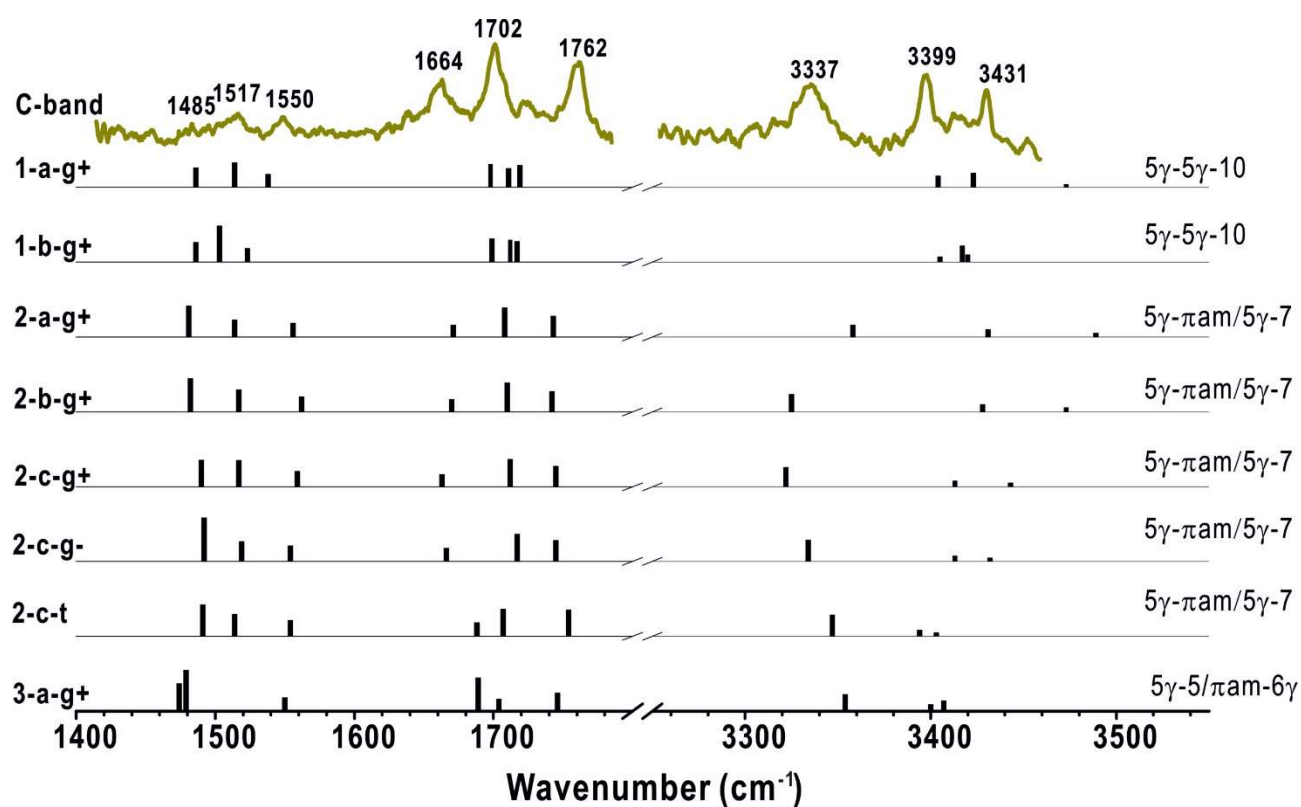


Fig. S9. *Above green line.* Experimental conformer specific IR/UV spectra obtained by probing the UV transition C. *Stick diagrams.* Theoretical IR spectra of the most stable conformer of selected backbone sub-families.

S2.6. Comparison between gas phase and solution IR spectra of **2** and Cbz-(Attc)₂-NHMe.

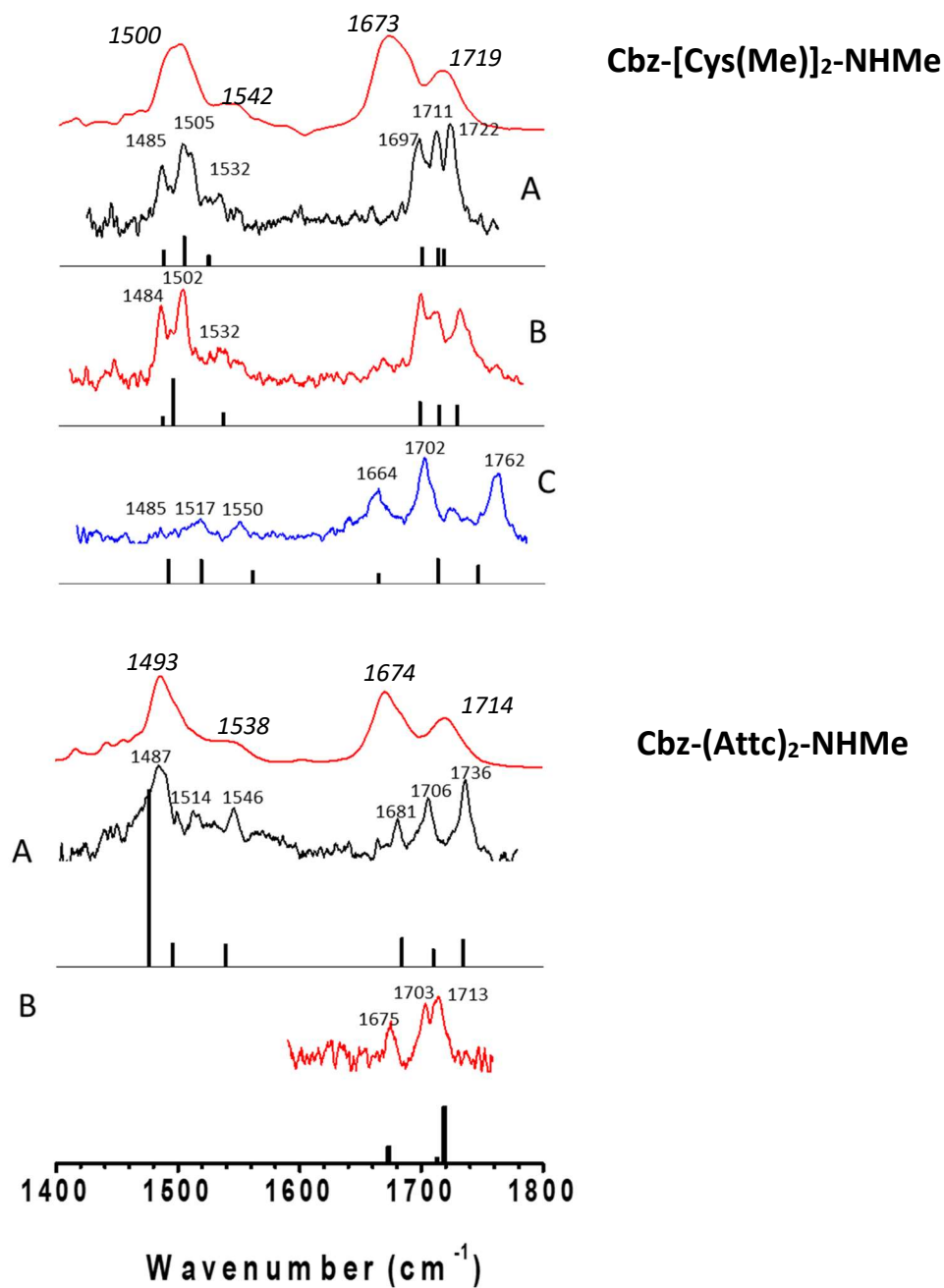


Fig. S10. Comparison of the amide I and II gas phase spectra of the main conformers (A, B and C) of **2** with that of conformers A and B of its Attc counterpart, with the corresponding solution spectra (top red spectra of each panel).

S3. ¹H NMR: DMSO-*d*₆ titration data

S3.1 Cbz-Cys(Me)-NHMe 1

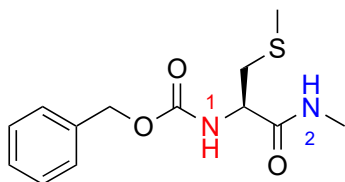


Table S3

	DMSO- <i>d</i> ₆ (% , v/v)												
NH	0	0.5	1	1.5	2	2.5	3	4	5	7.5	10	Δδ	
NH ¹	5.68	5.74	5.80	5.85	5.90	5.94	5.97	6.03	6.06	6.13	6.20	0.52	
NH ²	6.35	6.45	6.56	6.62	6.67	6.73	6.79	6.87	6.91	7.00	7.09	0.74	

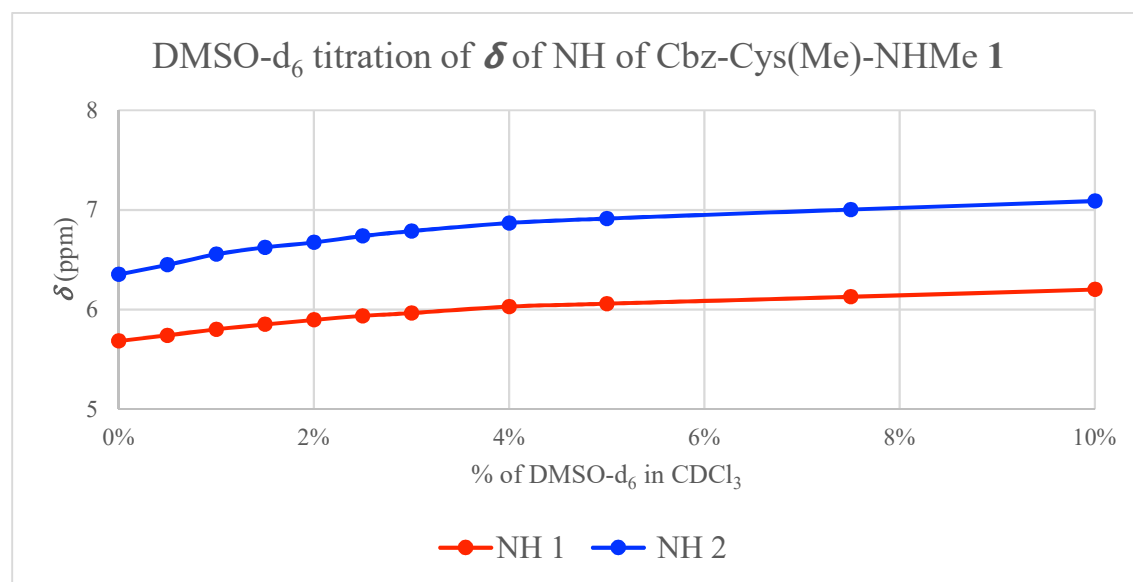


Fig. S11

S3.2 Cbz-[Cys(Me)]₂-NHMe 2

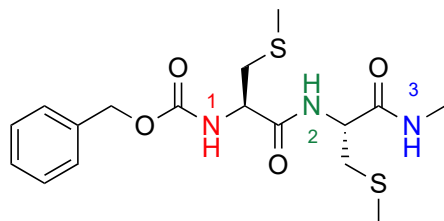


Table S4

NH	DMSO- <i>d</i> ₆ (% , v/v)											Δδ
	0	0.5	1	1.5	2	2.5	3	4	5	7.5	10	
NH ¹	5.70	5.79	5.86	5.91	5.97	6.01	6.06	6.13	6.18	6.29	6.36	0.66
NH ²	7.05	7.18	7.26	7.32	7.39	7.43	7.51	7.57	7.61	7.67	7.70	0.65
NH ³	6.77	6.82	6.88	6.91	6.94	6.96	6.99	7.03	7.05	7.10	7.13	0,36

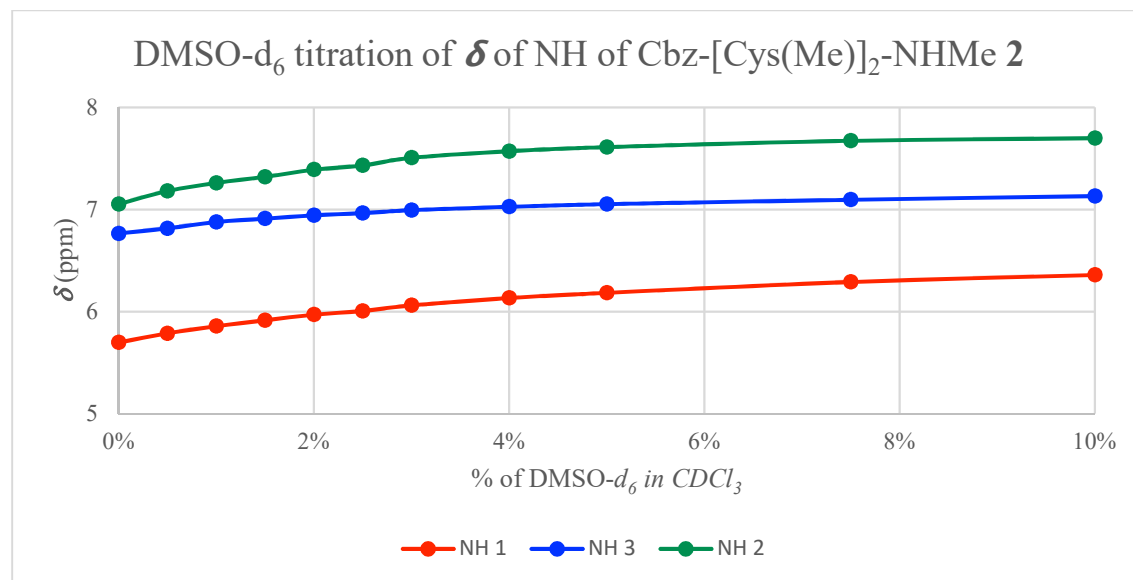


Fig. S12