Supporting Information for

Macrocyclic β-Sheet Peptides that Inhibit Aggregation of a Tau-Protein-Derived Hexapeptide

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Full Citation for Reference 50

Yu, L. P.; Edalji, R.; Harlan, J. E.; Holzman, T. F.; Lopez, A. P.; Labkovsky, B.; Hillen, H.; Barghorn, S.; Ebert, U.; Richardson, P. L.; Miesbauer, L.; Solomon, L.; Bartley, D.; Walter, K.; Johnson, R. W.; Hajduk, P. J.; Olejniczak, E. T. *Biochemistry* **2009**, *48*, 1870-1877.

Figures S1 and S2: Two independent experiments, each in triplicate, showing the effect of macrocycle 1a on AcPHF6 aggregation.

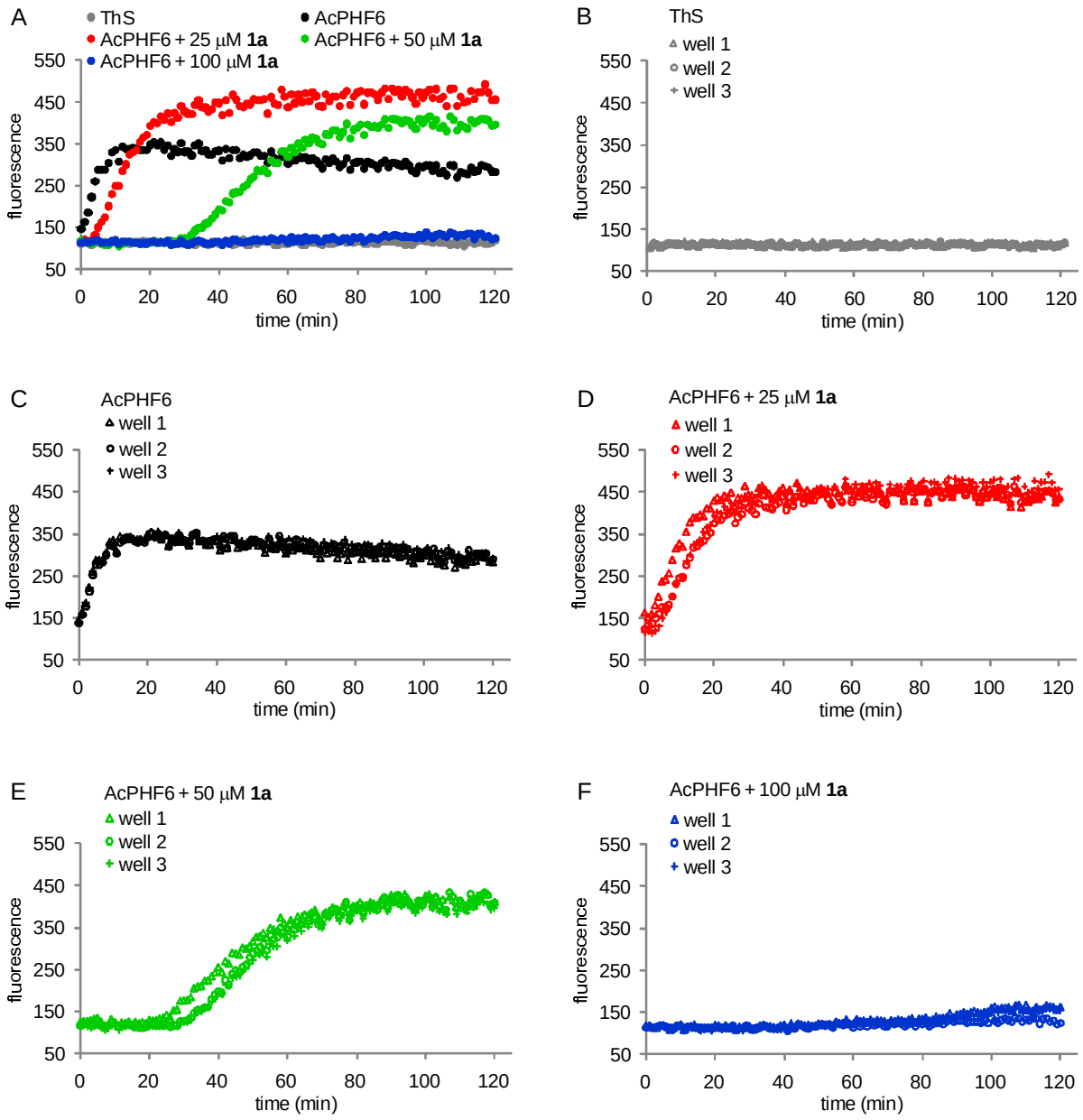


Figure S1. AcPHF6 aggregation and inhibition of AcPHF6 measured by ThS fluorescence (Experiment 1). (A) Aggregation of 100 μM AcPHF6 in the absence and presence of 25, 50, and 100 μM of macrocycle **1a** (Figure 7B in the paper). (B) Triplicate runs associated with ThS background fluorescence. (C) Triplicate runs associated with aggregation of 100 μM AcPHF6. (D) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 25 μM **1a**. (E) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 50 μM **1a**. (F) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 100 μM **1a**.

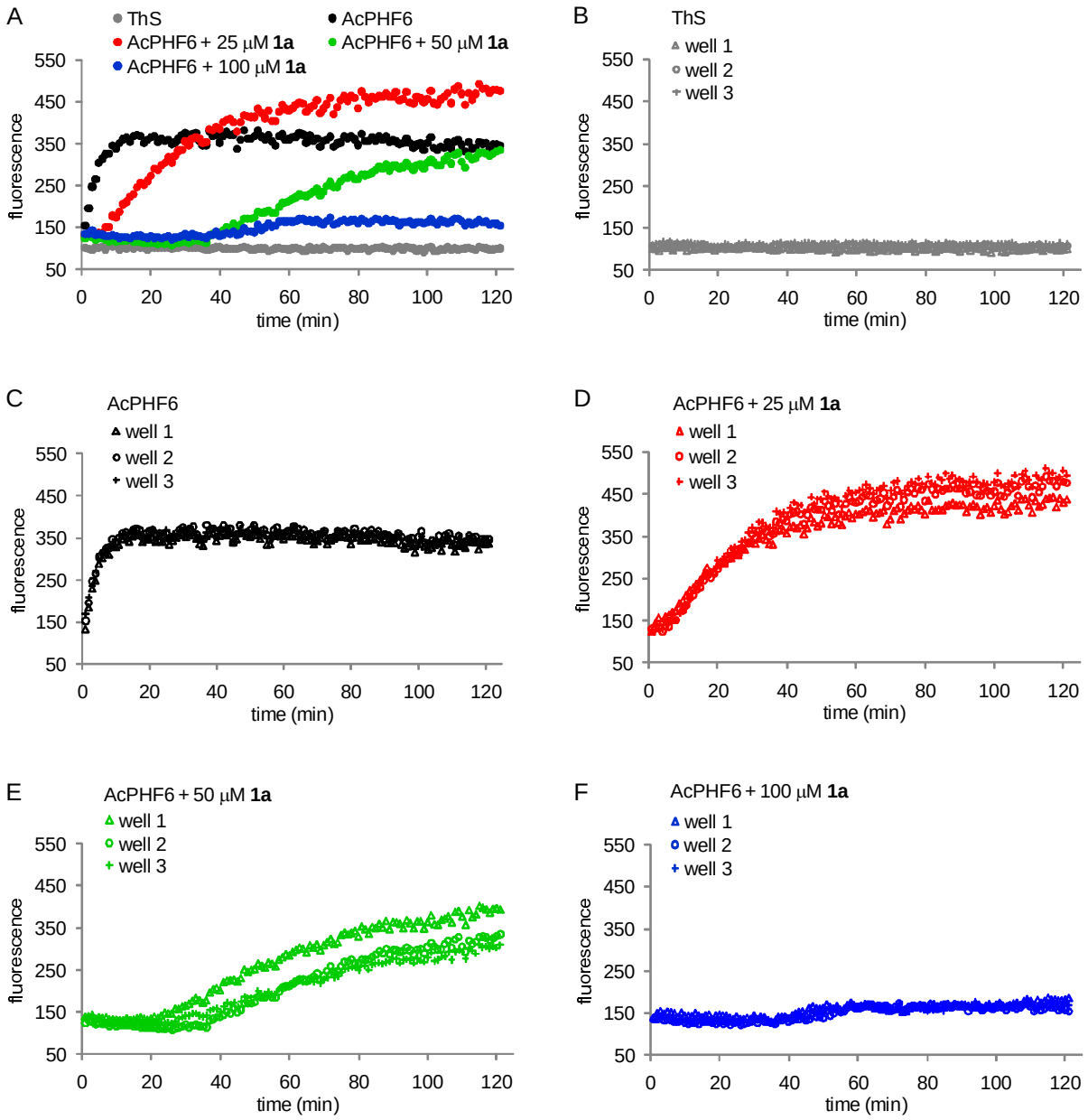


Figure S2. AcPHF6 aggregation and inhibition of AcPHF6 measured by ThS fluorescence (Experiment 2). (A) Aggregation of 100 μM AcPHF6 in the absence and presence of 25, 50, and 100 μM of macrocycle **1a**. (B) Triplicate runs associated with ThS background fluorescence. (C) Triplicate runs associated with aggregation of 100 μM AcPHF6. (D) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 25 μM **1a**. (E) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 50 μM **1a**. (F) Triplicate runs associated with aggregation of 100 μM AcPHF6 + 100 μM **1a**.

^{1}H NMR Spectroscopy Experiments

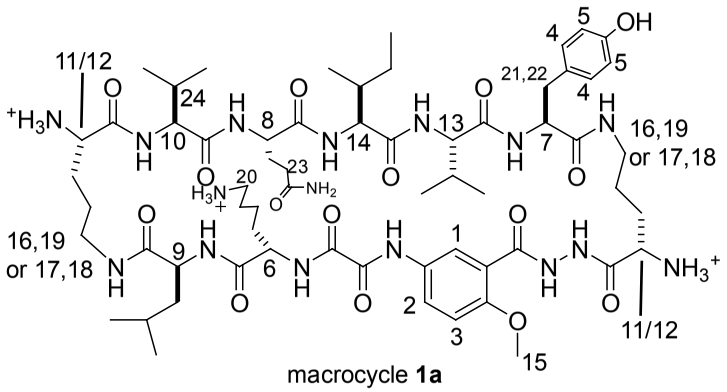
^{1}H NMR studies were conducted at 500 MHz (Brüker Avance) or 600 MHz (Brüker Avance). All peptides were studied at 2 mM in D_2O at 298 K (1D, TOCSY, and ROESY). Macrocycles **1a** and **1c** were also studied at 2 mM in D_2O at 279 K (1D and ROESY) to facilitate observing the NOE cross-peaks that are close to the HOD peak at 298 K. Peptides **1d**, **1e**, and **2a** were also studied at 2 mM in 9:1 H_2O/D_2O at 298 K or 279 K (1D, TOCSY, and ROESY) to obtain sequence-specific assignments of their residues. A 150-ms mixing time was used for TOCSY experiments. A 200- or 250-ms mixing time was used for ROESY experiments. NMR data were processed with the Brüker XWIN-NMR software. Chemical shifts were calibrated with the internal standard DSA.[1]

Table S1. Chemical shifts of the α-protons of R_1-R_7 of macrocycles **1** and **5** and chemical shifts of the α-protons of R_1-R_5 of acyclic peptides **2**.

peptide	chemical shifts of the α-protons (ppm)						
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1a	4.27 (V)	4.39 (Q)	4.12 (I)	4.12 (V)	4.48 (Y)	4.54 (K)	4.35 (L)
1b	4.64 (Q)	4.09 (I)	4.28 (V)	4.81 (Y)	4.40 (K)	4.34 (L)	4.51 (K)
1c	4.43 (V)	4.44 (Q)	4.29 (I)	4.27 (V)	4.62 (Y)	4.56 (L)	4.48 (K)
1d	4.33 (V)	4.37 (Q)	4.17 (I)	4.05 (V)	4.52 (Y)	4.59 (K)	4.17 (V)
1e	4.39 (V)	4.42 (Q)	4.19 (I)	4.23 (V)	4.57 (Y)	4.39 (V)	4.42 (K)
1f	4.30 (V)	4.41 (Q)	4.16 (I)	4.15 (V)	4.52 (Y)	4.60 (R)	4.39 (L)
1g	4.46 (V)	4.45 (Q)	4.32 (I)	4.28 (V)	4.64 (Y)	4.56 (L)	4.53 (R)
5a	4.19 (V)	4.42 (Q)	4.04 (I)	4.06 (V)	4.49 (Y)	4.45 (dK)	4.39 (dL)
5c	4.16 (V)	4.44 (Q)	4.10 (I)	4.11 (V)	4.53 (Y)	4.58 (dL)	4.34 (dK)
2a	4.07 (V)	4.38 (Q)	4.09 (I)	4.14 (V)	4.41 (Y)	--	--
2b	4.40 (Q)	4.08 (I)	4.07 (V)	4.55 (Y)	4.15 (K)	--	--

[1] Nowick, J. S.; Khakshoor, O.; Hashemzadeh, M.; Brower, J. O. *Org. Lett.* **2003**, *5*, 3511-3513.

Macrocycle **1a**, 1H NMR spectrum, 2 mM in D_2O with DSA, 25°C, 500 MHz



macrocycle **1a**

*: alternate conformation

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F2P -1.029 ppm
F2 -514.86 Hz
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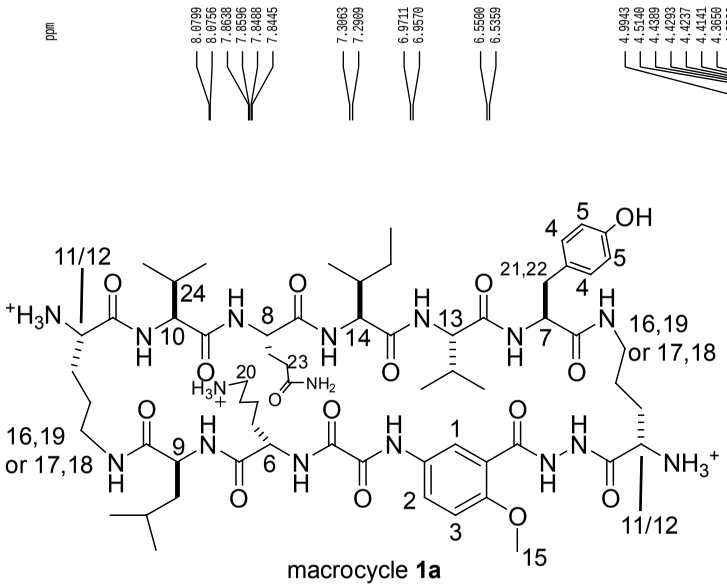
Macrocycle **1a**, 1H NMR spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz



*: alternate conformation

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TE	279.0 K
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F2 - Processing parameter

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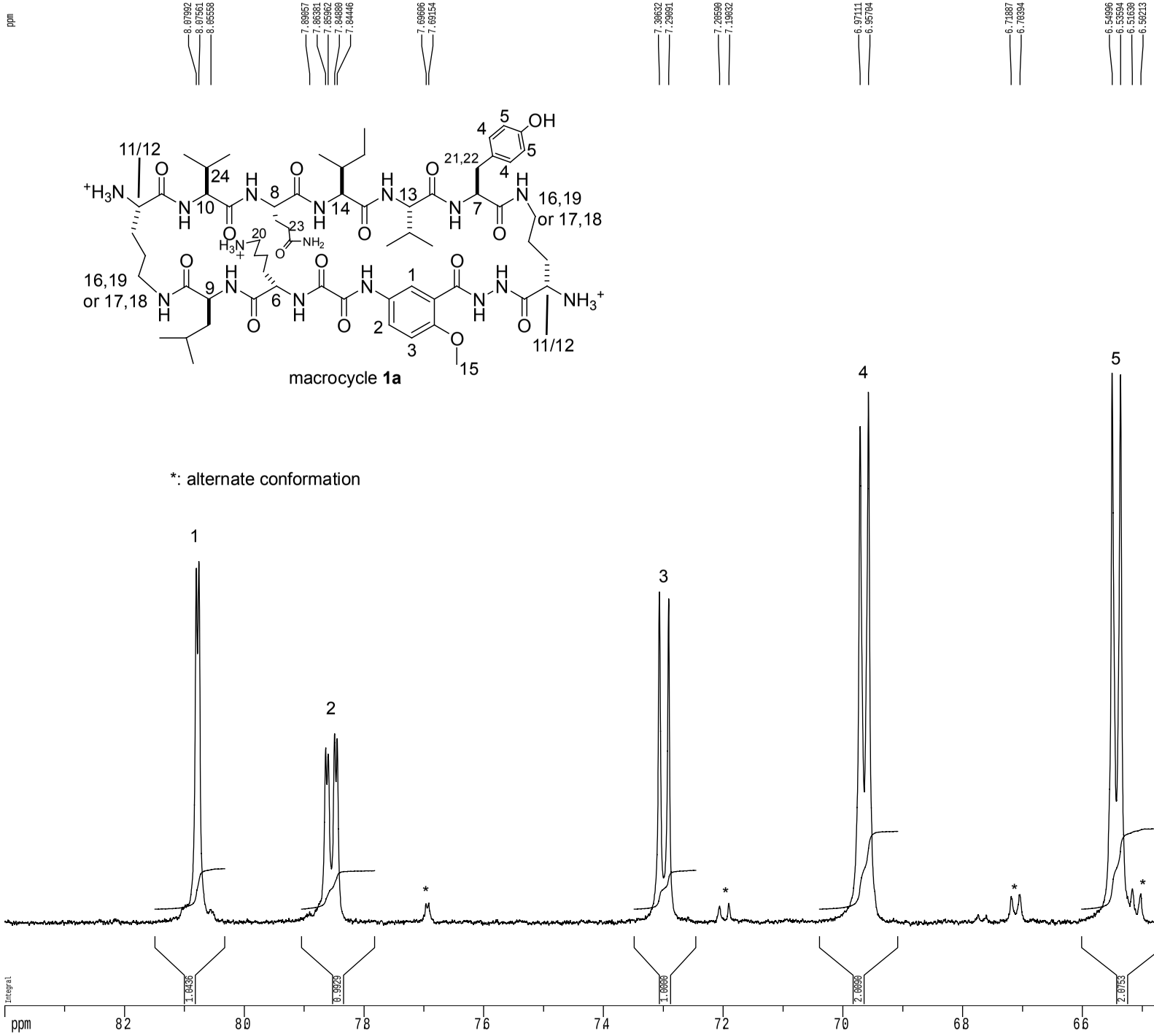
1D NMR plot parameters

CX	22.80 cm
CY	60.00 cm
F1P	9.000 ppm
F1	5401.17 Hz
F2P	-0.500 ppm
F2	-300.06 Hz
PPMCM	0.41667 ppm/cm
HZCM	250.05412 Hz/cm

Macrocycle **1a**, 1H NMR spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz

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======== CHANNEL f1 ========
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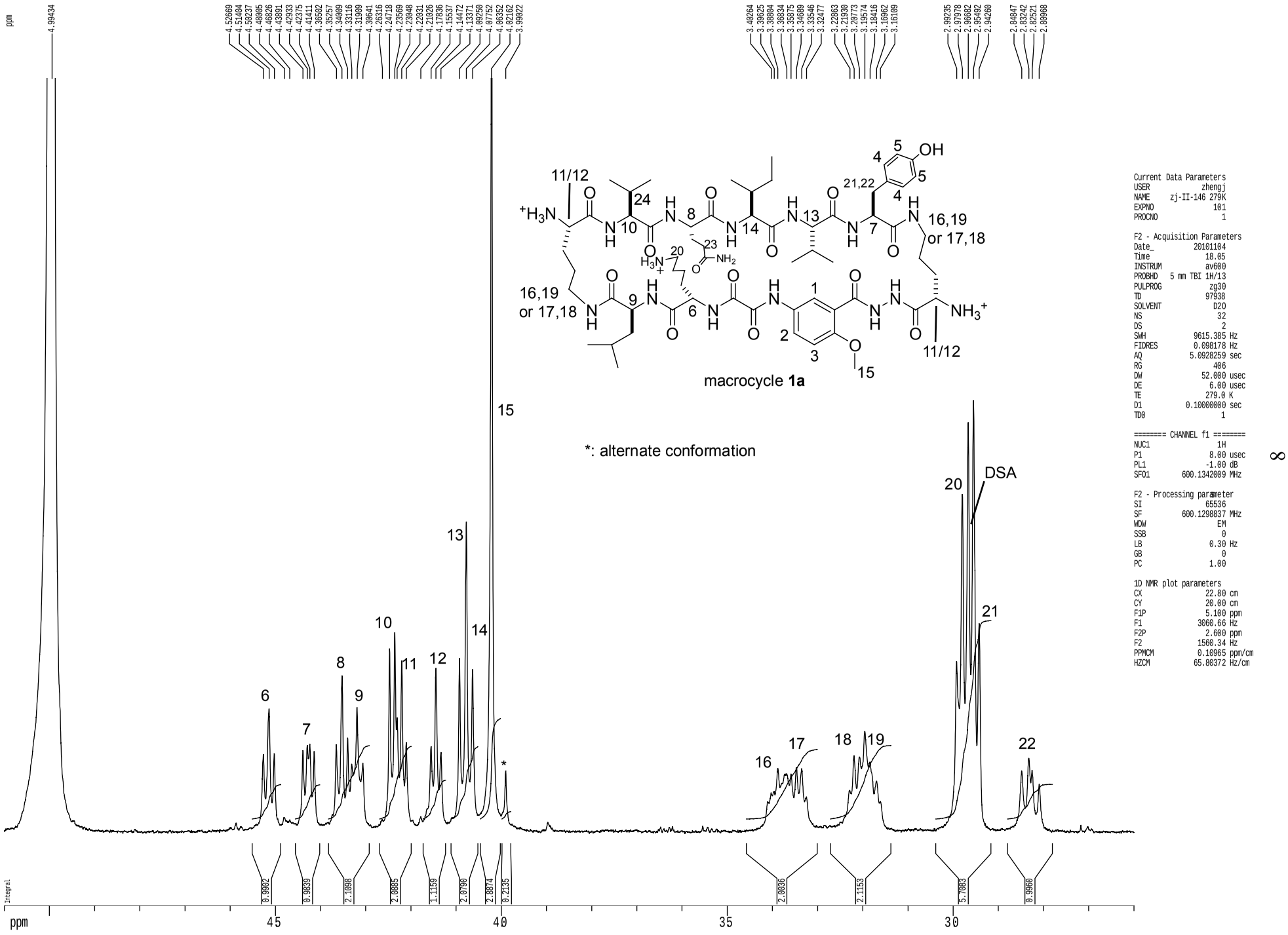
Macrocycle **1a**, 1H NMR spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz

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SOLVENT             D2O
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DS                    2
SWH            9615.385 Hz
FIDRES        0.098178 Hz
AQ            5.0928259 sec
RG                  406
DW               52.000 usec
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======== CHANNEL f1 ========
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1D NMR plot parameters
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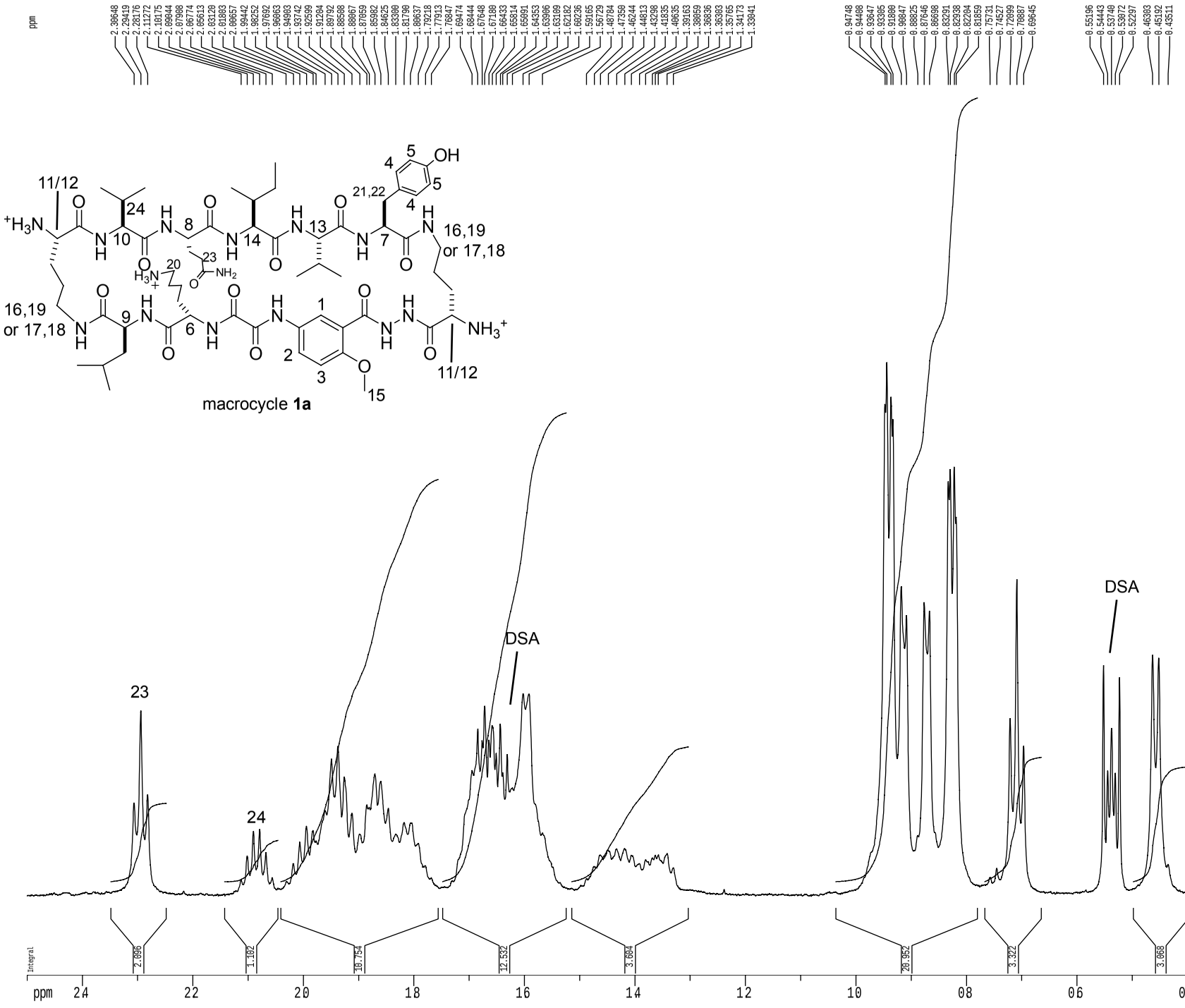
Macrocycle **1a**, 1H NMR spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz

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======== CHANNEL f1 ========
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F2 - Processing parameter
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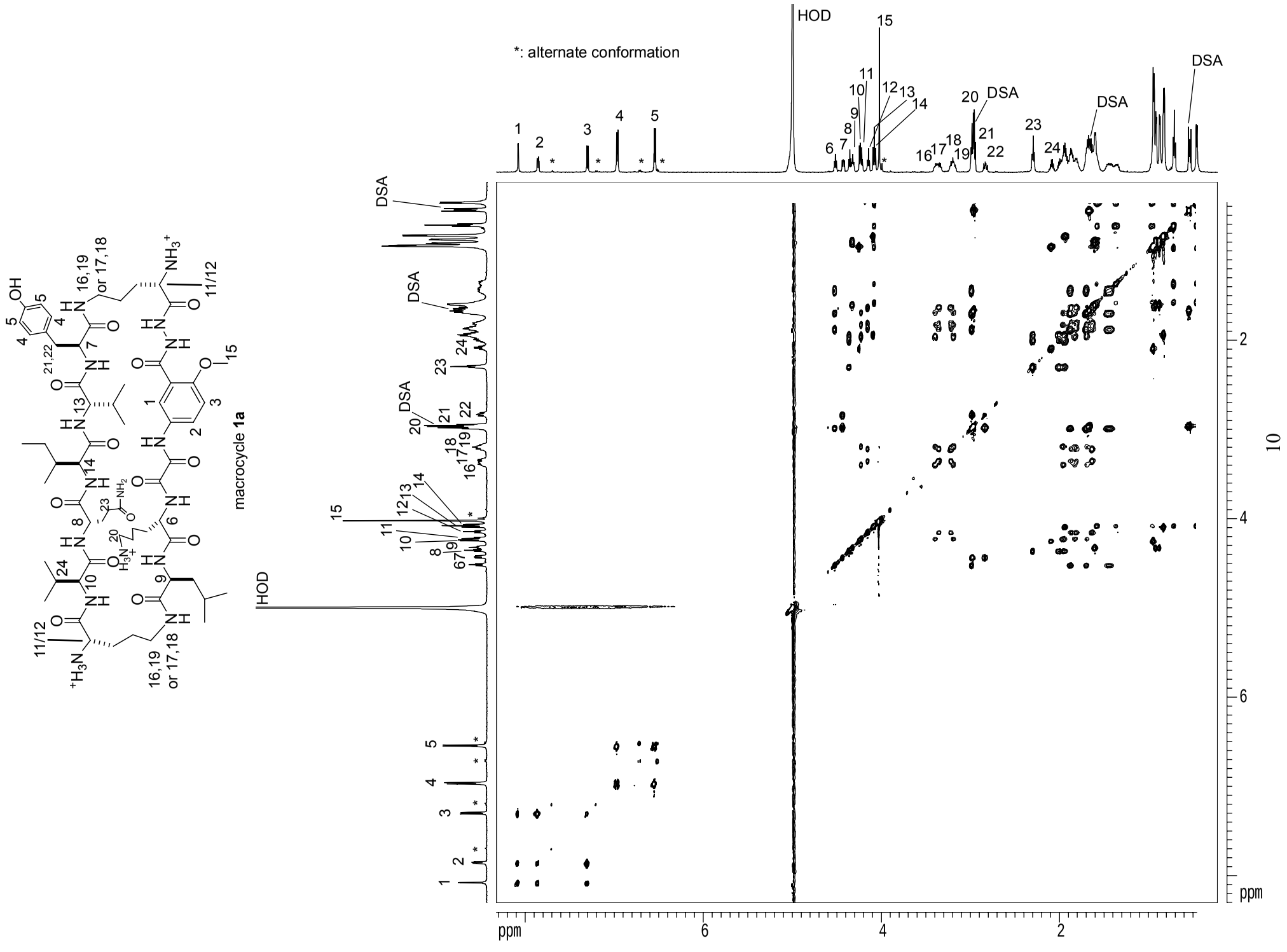
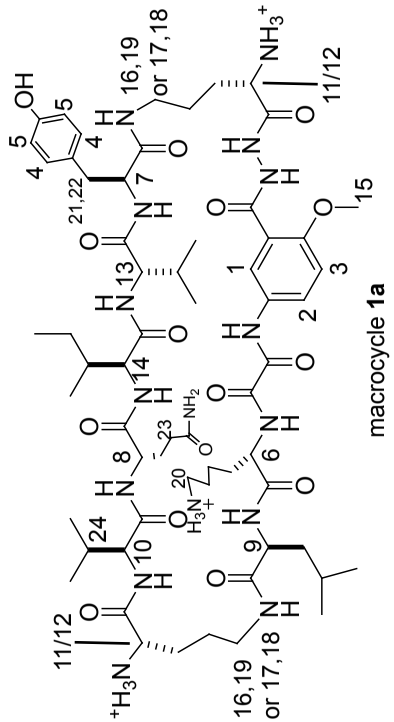
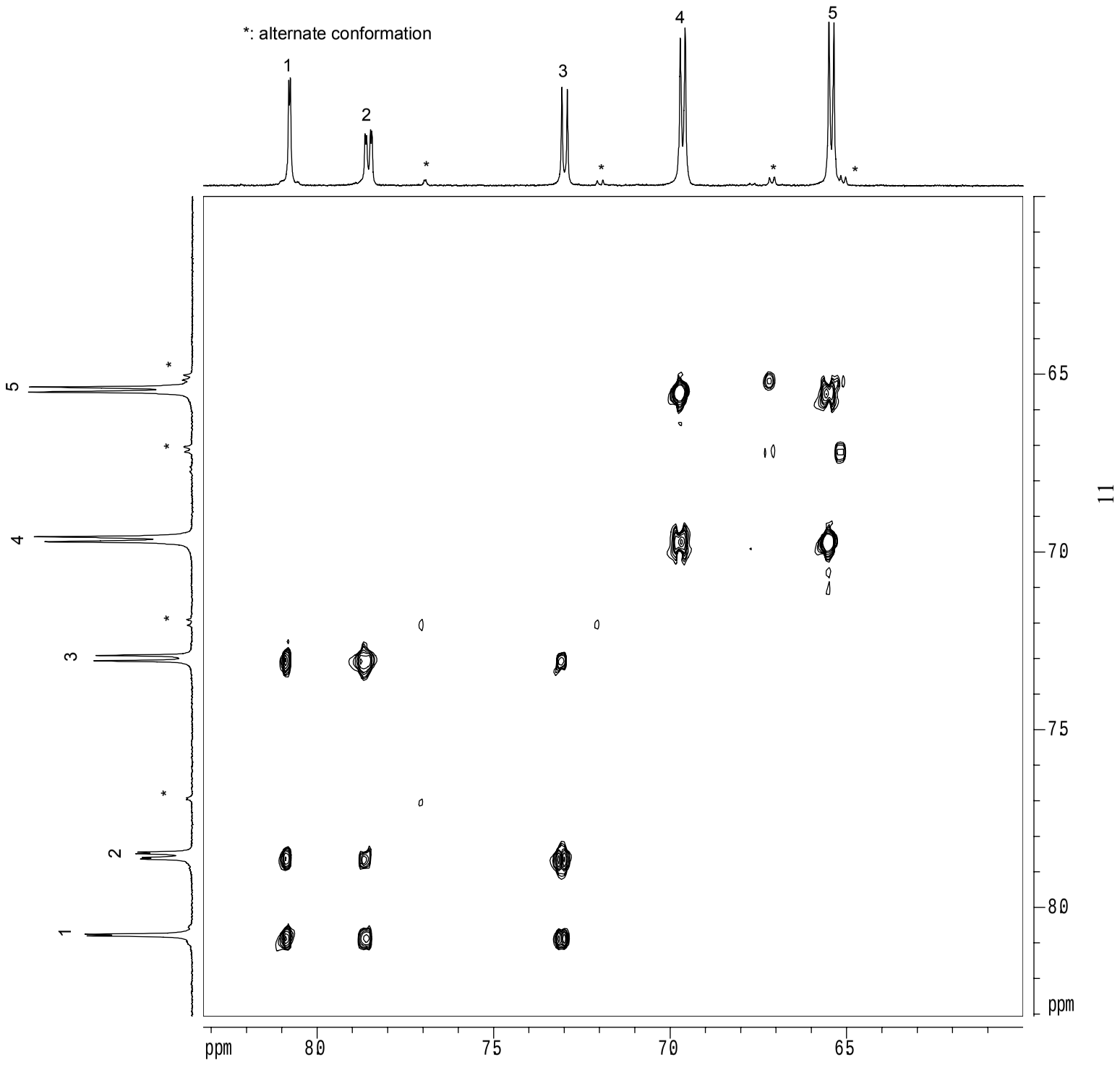
Macrocycle **1a**, TOCSY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 150 ms mixing time

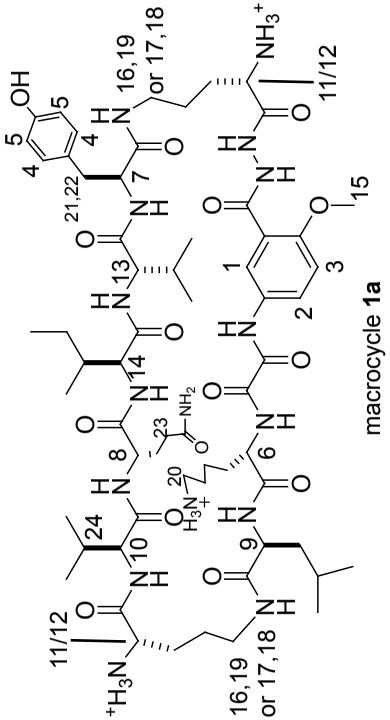


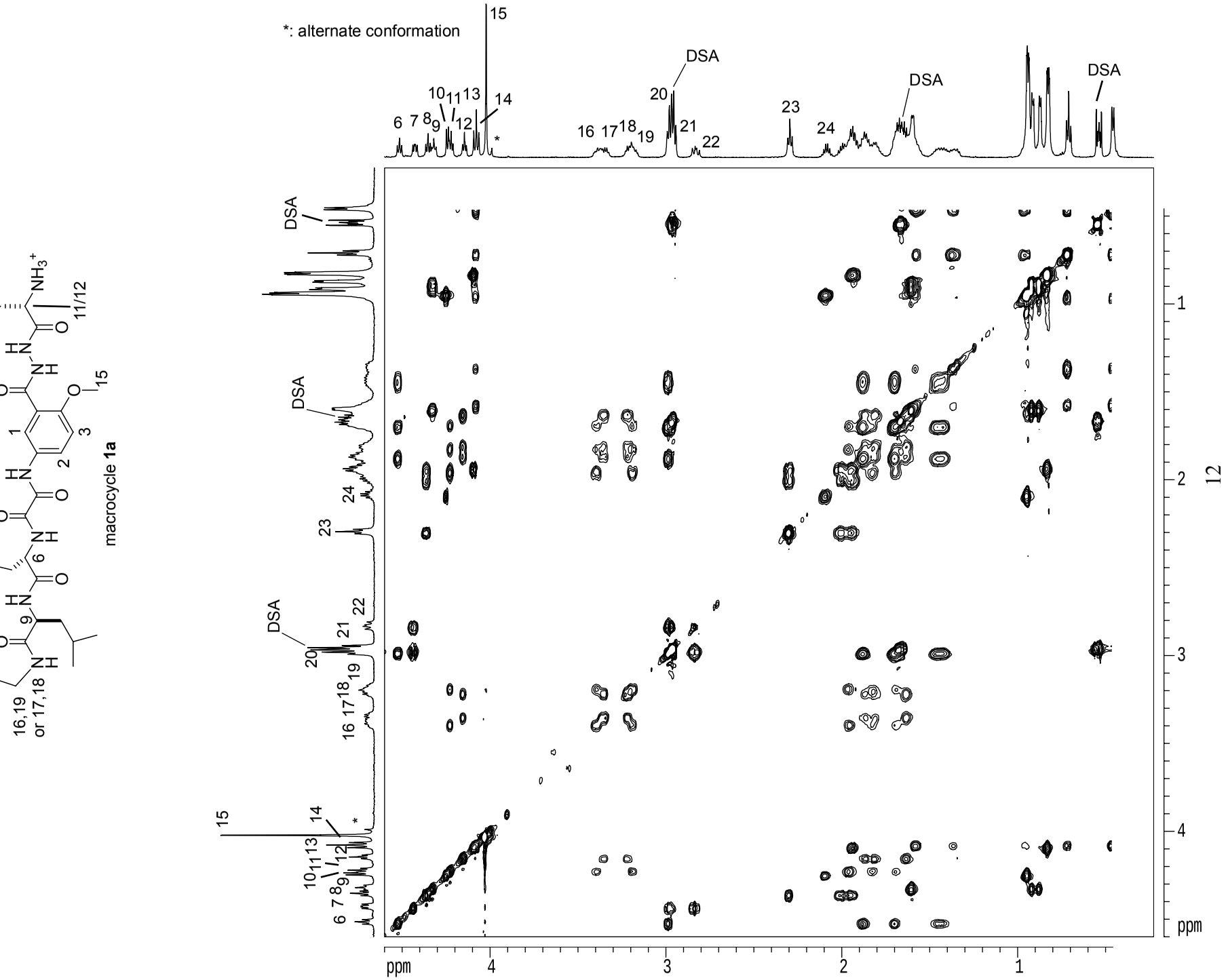
Macrocycle **1a**, TOCSY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 150 ms mixing time





Macrocycle **1a**, TOCSY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 150 ms mixing time





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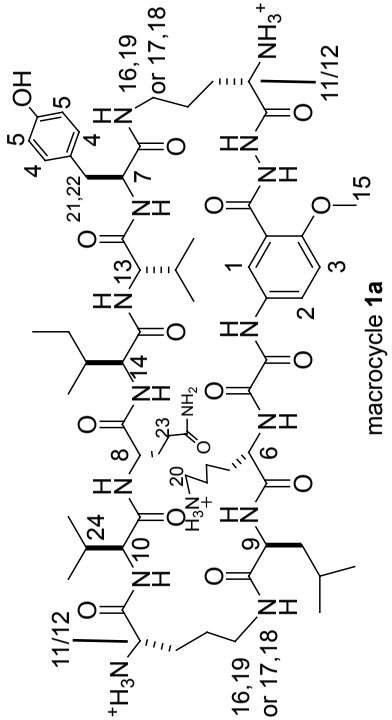
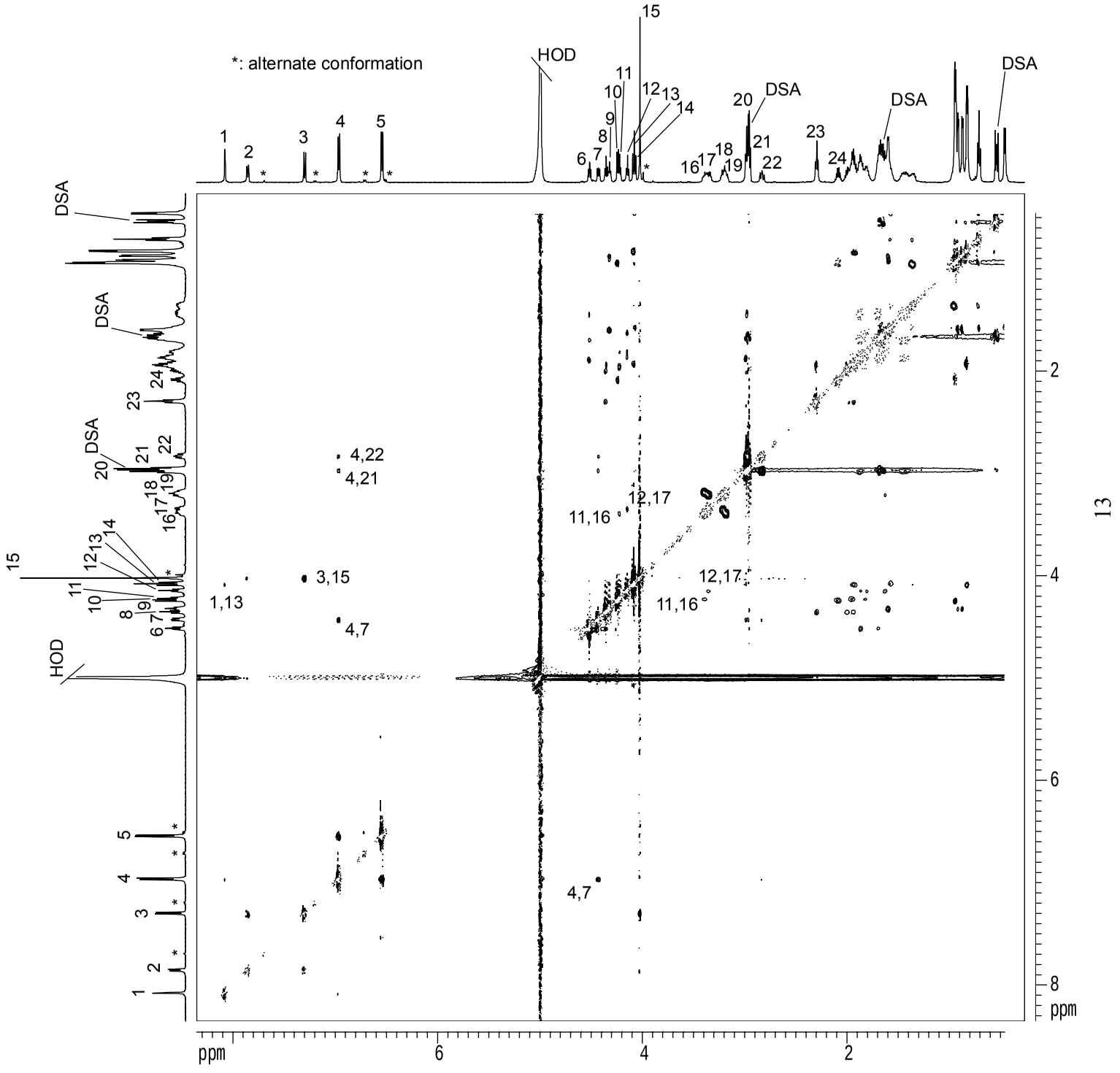
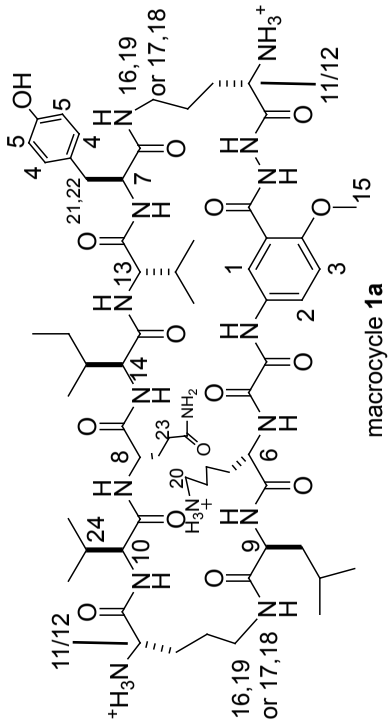
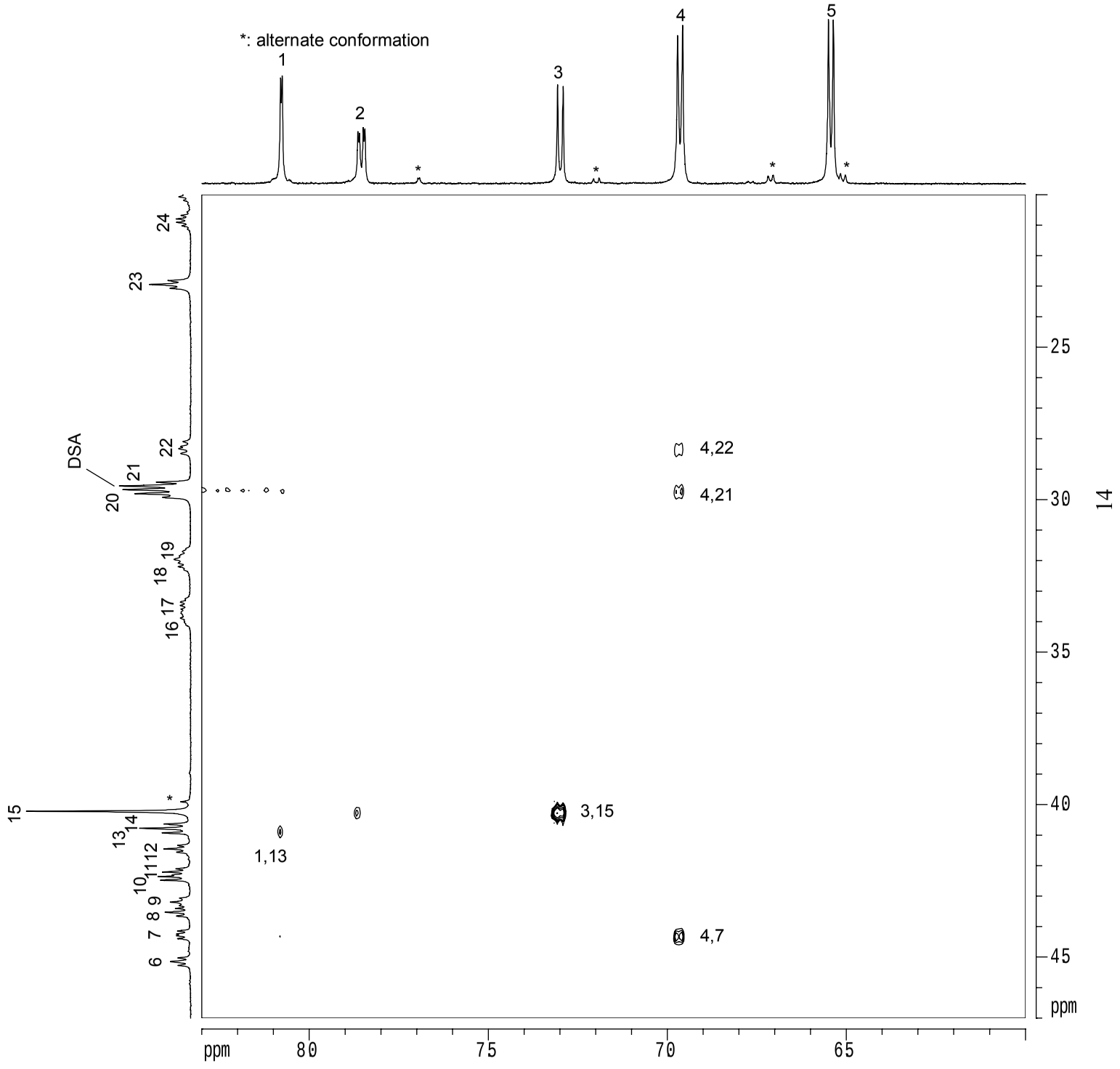


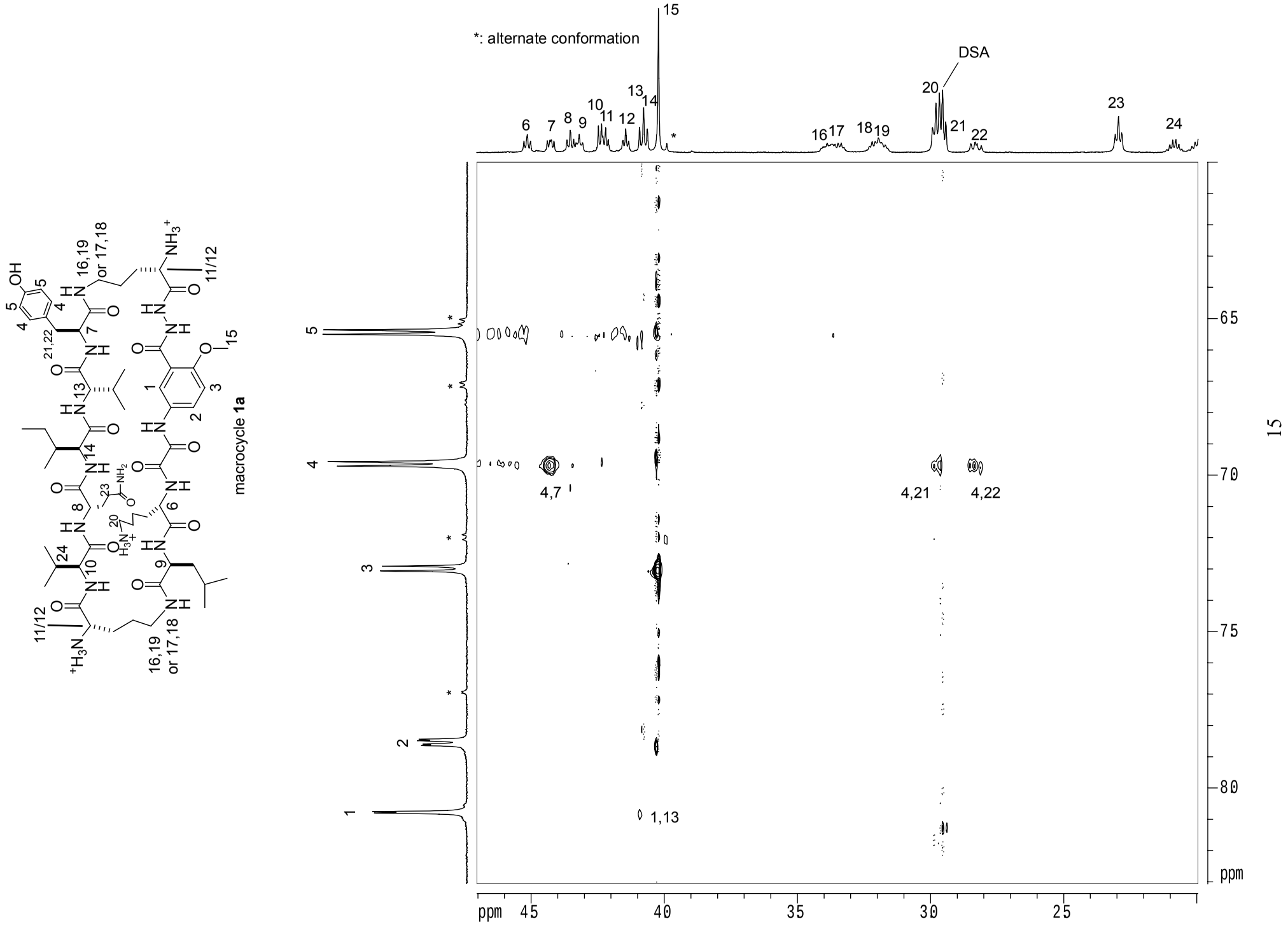


Macrocycle **1a**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time

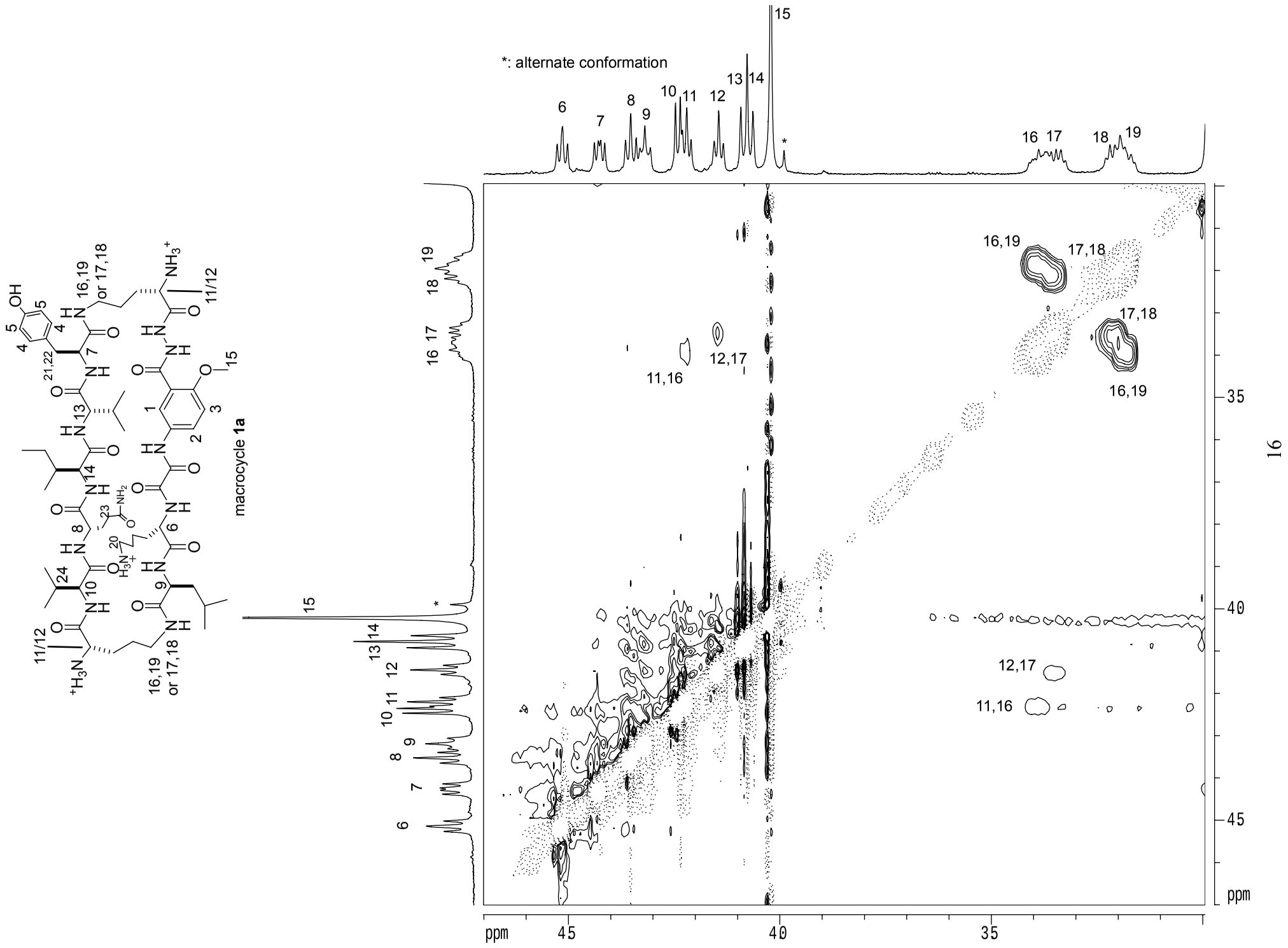




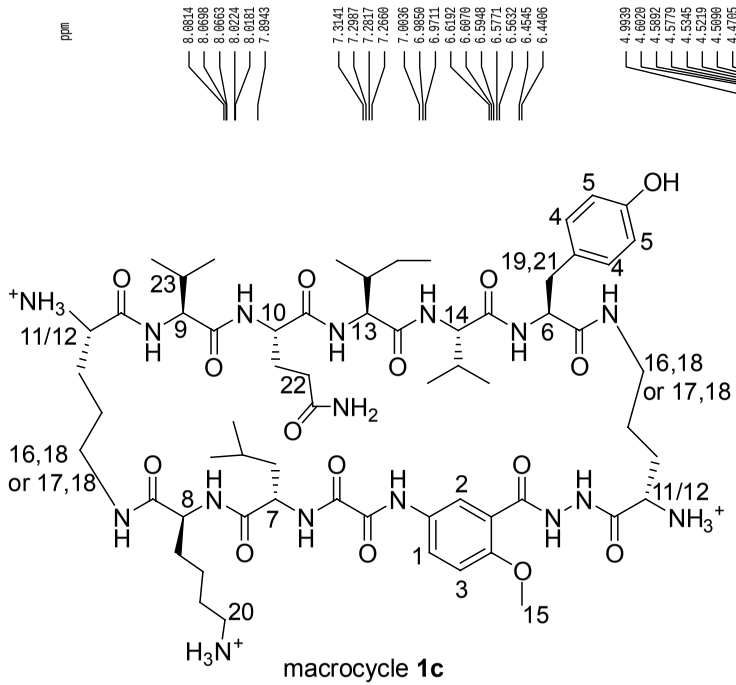
Macrocycle **1a**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time



Macrocycle **1a**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time



Macrocycle **1c**, 1H NMR spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz



macrocycle **1c**

*: alternate conformation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DE                6.00 usec
TE               279.0 K
D1            0.10000000 sec
TD0                    1

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F2 - Processing parameter
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F1              5401.17 Hz
F2P              -0.500 ppm
F2              -300.06 Hz
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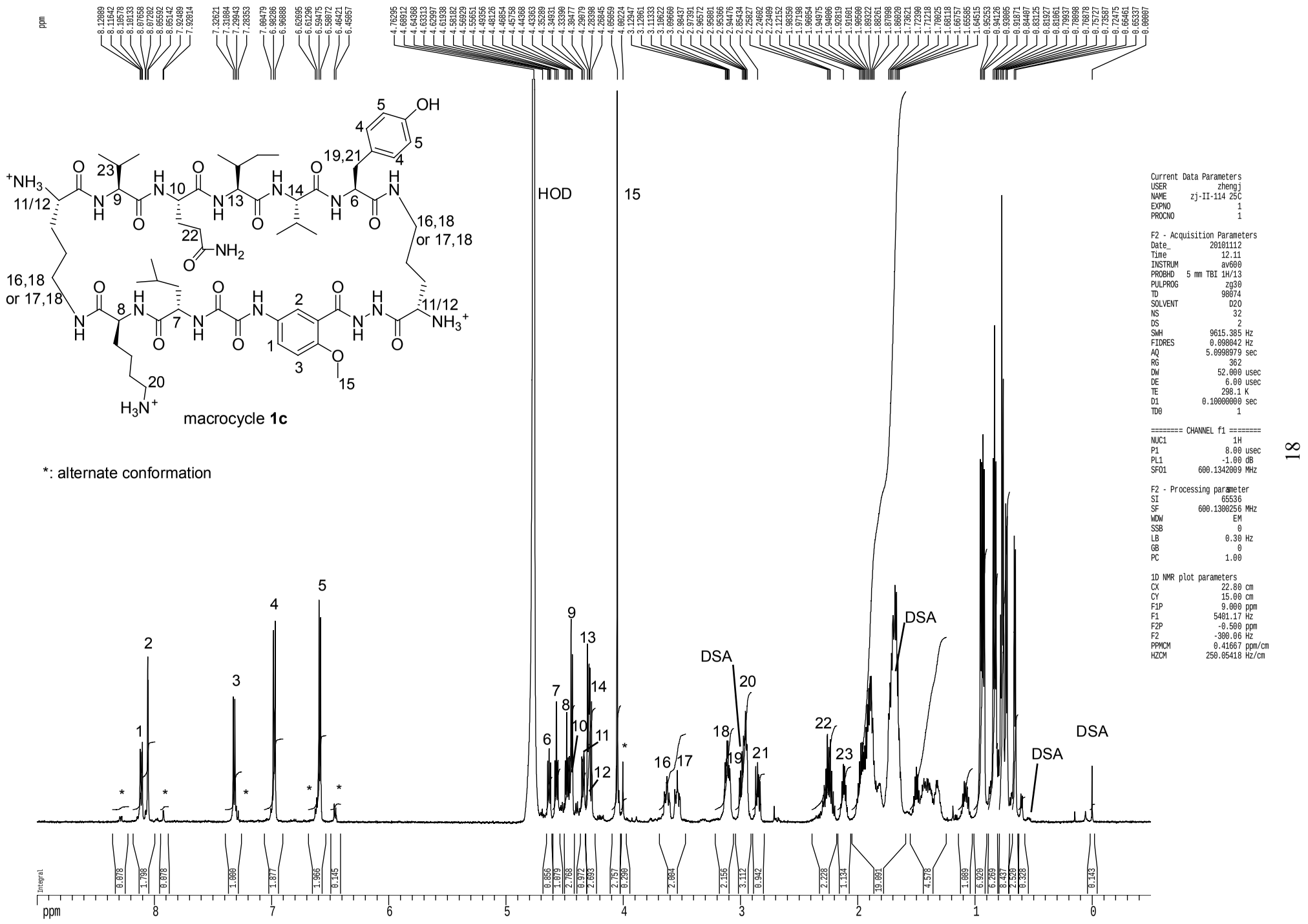
Macrocycle **1c**, 1H NMR spectrum, 2 mM in D_2O with DSA, 25°C, 600 MHz

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RG                 362
DW              52.000 usec
DE               6.00 usec
TE              298.1 K
D1           0.10000000 sec
TD0                  1

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1D NMR plot parameters
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F1             5401.17 Hz
F2P              -0.500 ppm
F2             -300.06 Hz
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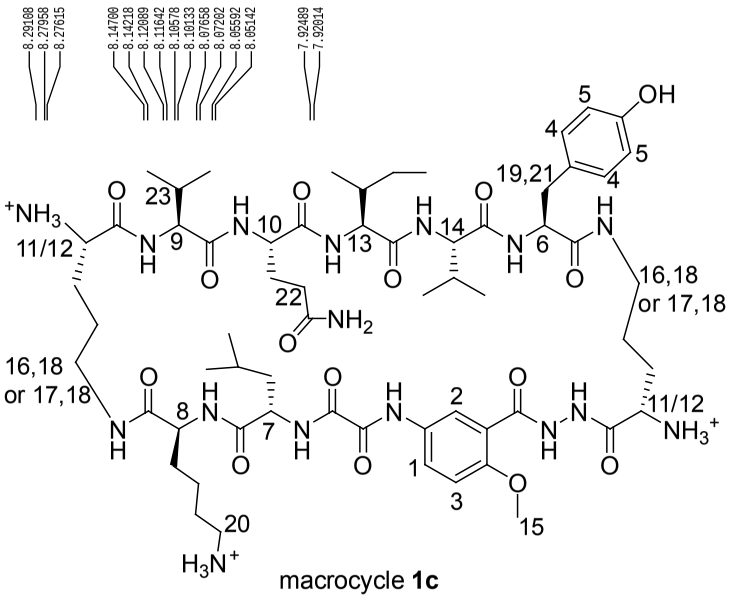
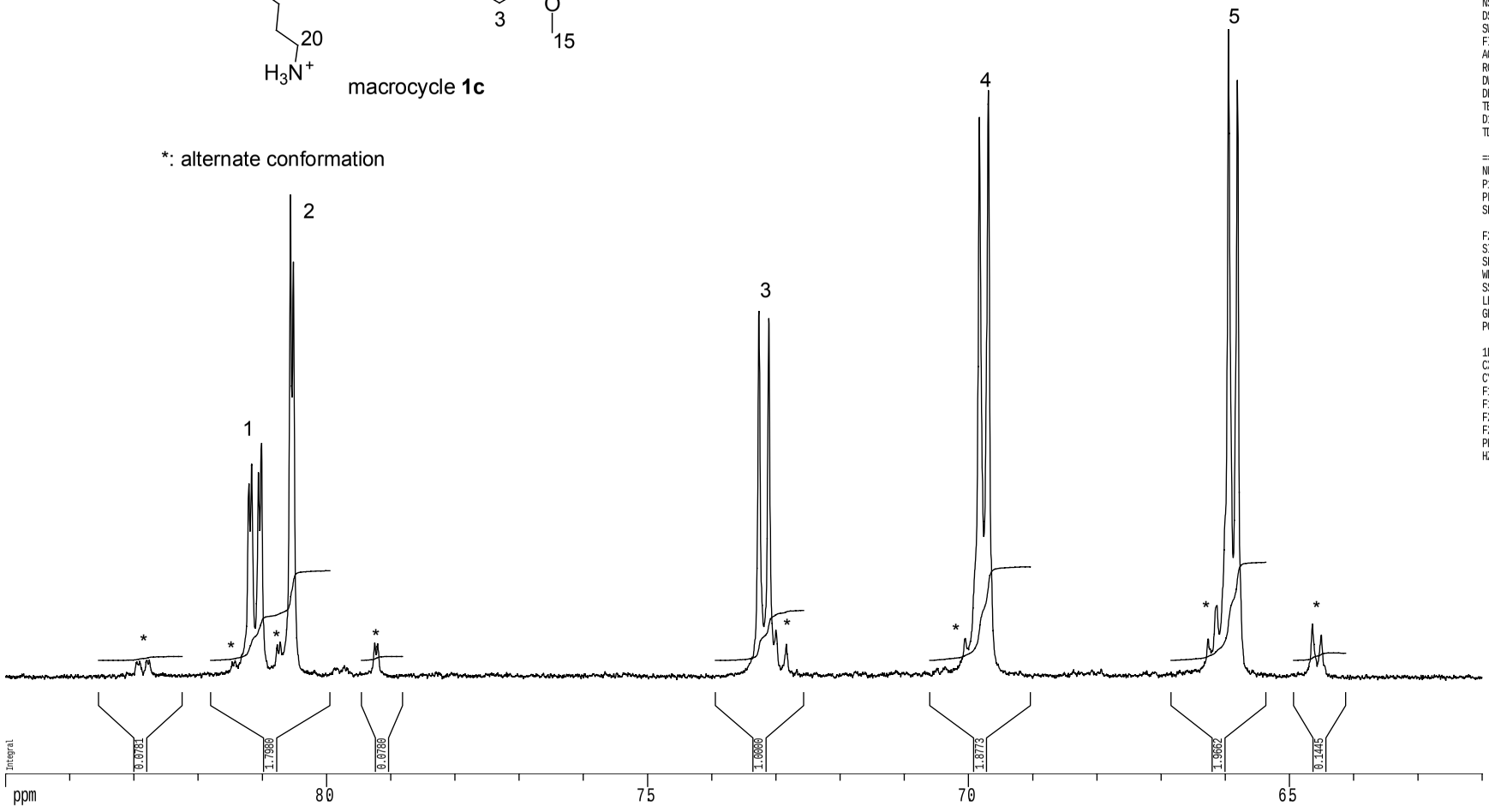
Macrocycle **1c**, 1H NMR spectrum, 2 mM in D_2O with DSA, 25°C, 600 MHz



macrocycle **1c**

*: alternate conformation

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TE               298.1 K
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======== CHANNEL f1 =======
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PL1              -1.00 dB
SFO1         600.1342009 MHz

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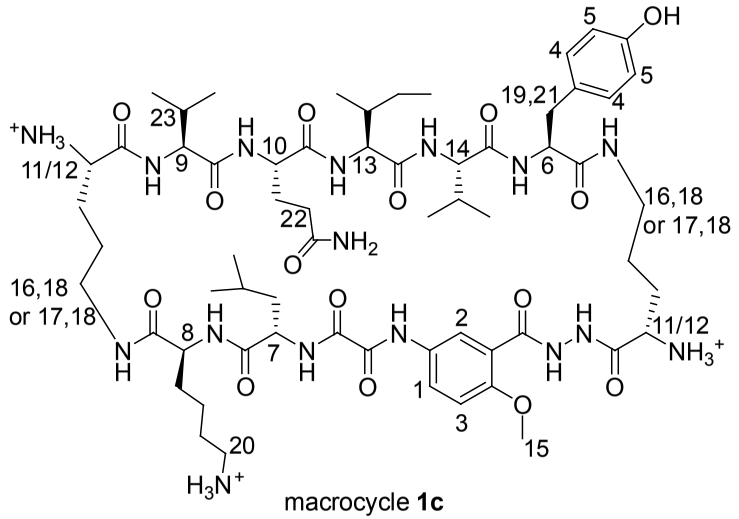
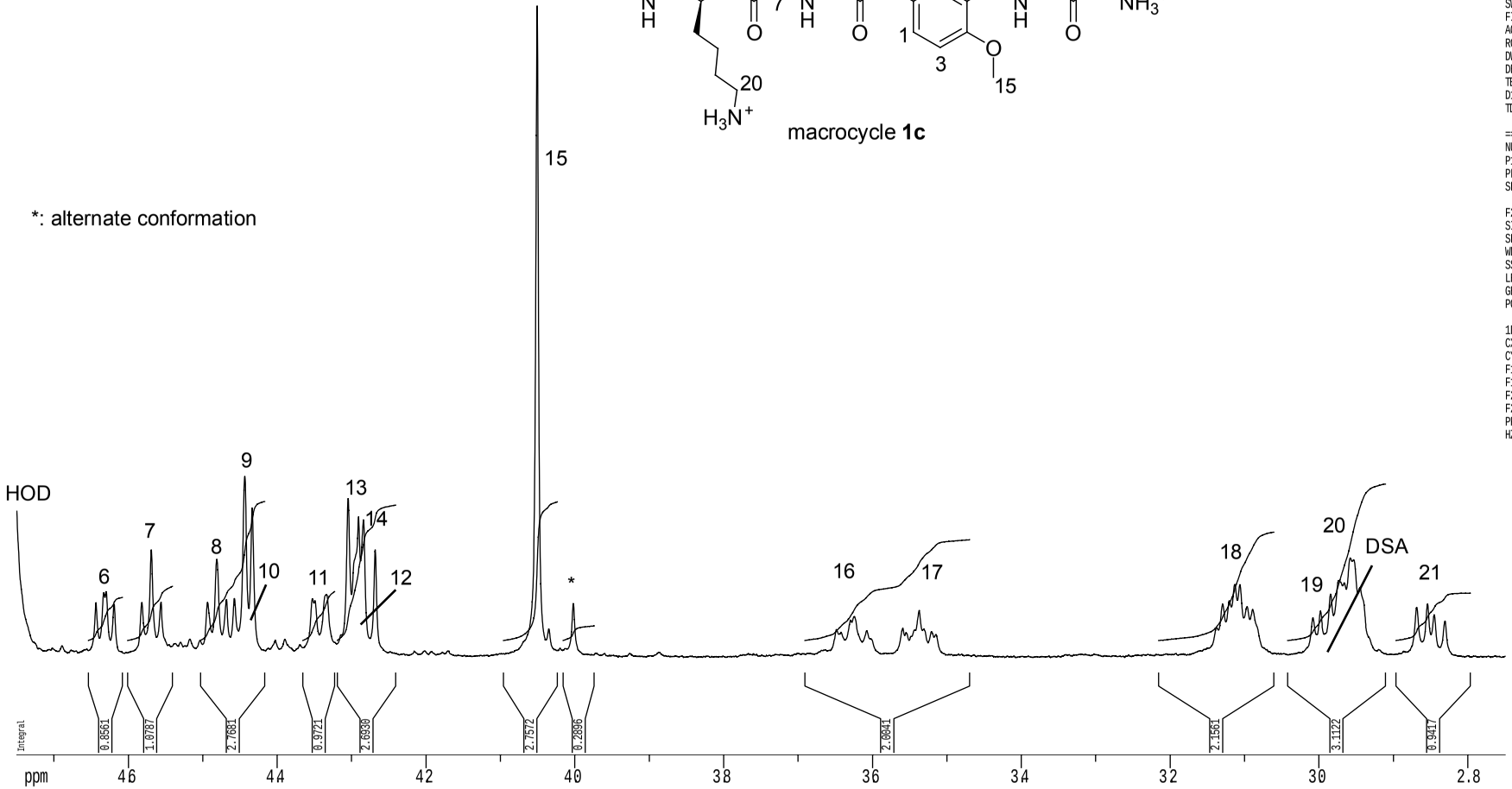
```

Macrocycle **1c**, 1H NMR spectrum, 2 mM in D_2O with DSA, 25°C, 600 MHz



macrocycle **1c**

*: alternate conformation

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Current Data Parameters
USER              zhengj
NAME          zj-II-114 25C
EXPNO                    1
PROCNO                   1

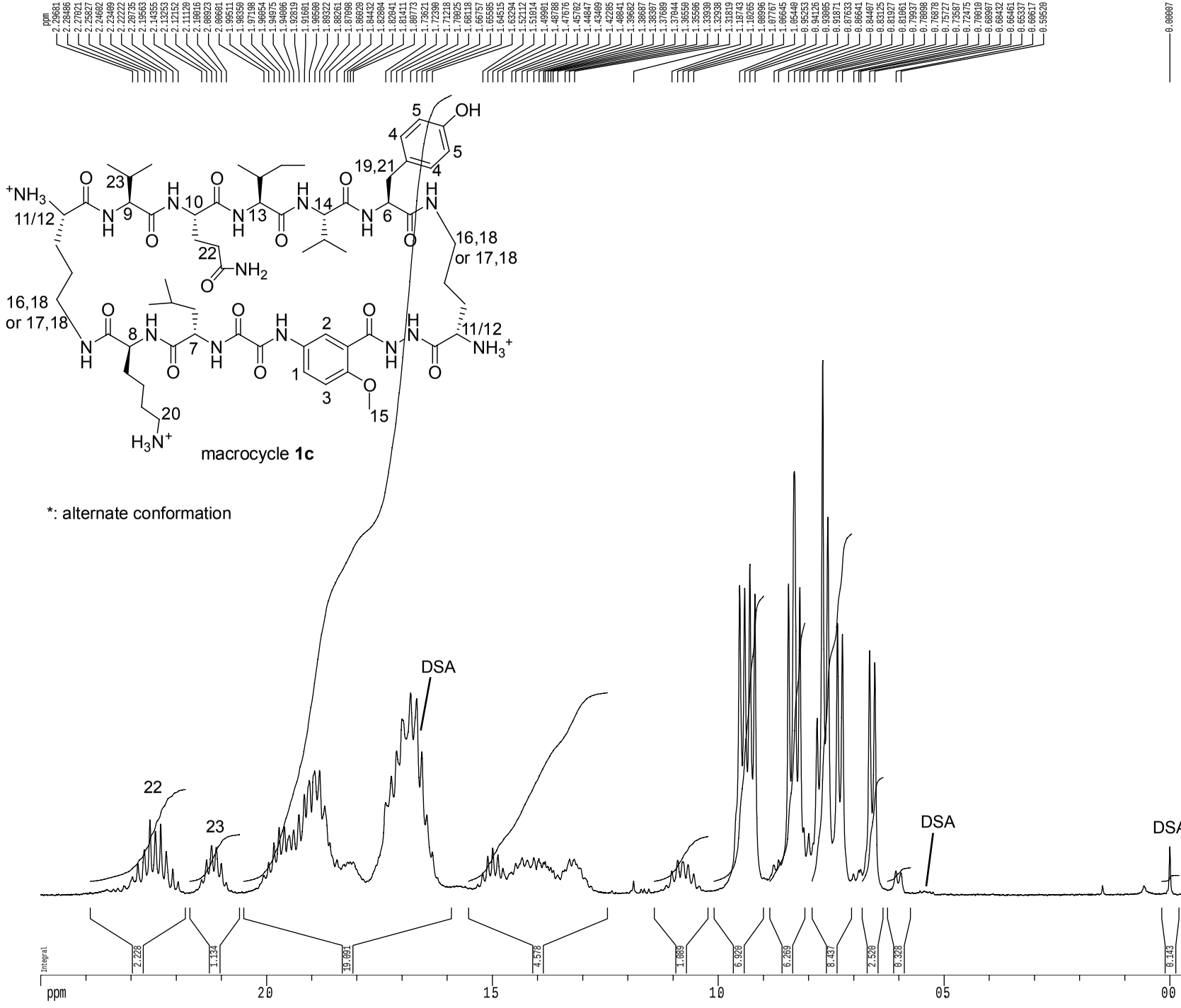
F2 - Acquisition Parameters
Date_           20101112
Time               12.11
INSTRUM            av600
PROBHD   5 mm TBI 1H/13
PULPROG             zg30
TD                 98074
SOLVENT              D2O
NS                    32
DS                     2
SWH             9615.385 Hz
FIDRES          0.098042 Hz
AQ              5.0998979 sec
RG                   362
DW                52.000 usec
DE                 6.00 usec
TE                298.1 K
D1             0.10000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 8.00 usec
PL1               -1.00 dB
SFO1         600.1342009 MHz

F2 - Processing parameter
SI                 65536
SF           600.1300256 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00

1D NMR plot parameters
CX                 22.80 cm
CY                 10.00 cm
F1P                4.750 ppm
F1               2850.62 Hz
F2P                2.750 ppm
F2               1650.36 Hz
PPMCM            0.08772 ppm/cm
HZCM            52.64299 Hz/cm
```

Macrocycle **1c**, 1H NMR spectrum, 2 mM in D_2O with DSA, 25°C, 600 MHz




```
Current Data Parameters
USER                zhengj
NAME          zj-II-114 25C
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_            20101112
Time                12.11
INSTRUM             av600
PROBHD   5 mm TBI 1H/13
PULPROG              zg30
TD                  98074
SOLVENT               D2O
NS                     32
DS                      2
SWH              9615.385 Hz
FIDRES           0.098042 Hz
AQ               5.0998979 sec
RG                    362
DW                 52.000 usec
DE                  6.00 usec
TE                 298.1 K
D1              0.10000000 sec
TD0                     1

======== CHANNEL f1 =======
NUC1                   1H
P1                  8.00 usec
PL1                -1.00 dB
SFO1          600.1342009 MHz

F2 - Processing parameter
SI                  65536
SF            600.1300256 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00

1D NMR plot parameters
CX                  22.80 cm
CY                  10.00 cm
F1P                 2.500 ppm
F1                1500.33 Hz
F2P                -0.200 ppm
F2                -120.03 Hz
PPMCM             0.11842 ppm/cm
HZCM             71.06803 Hz/cm
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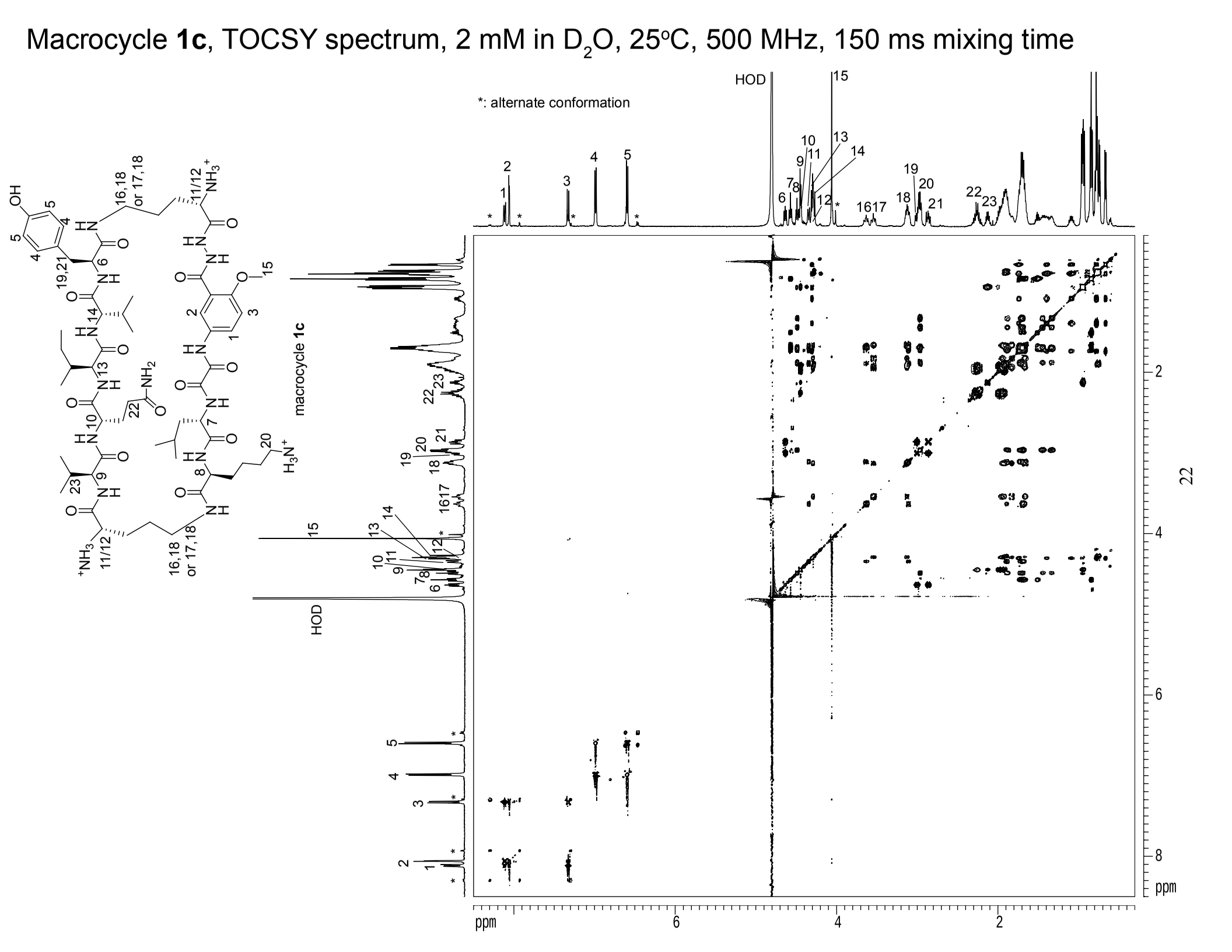
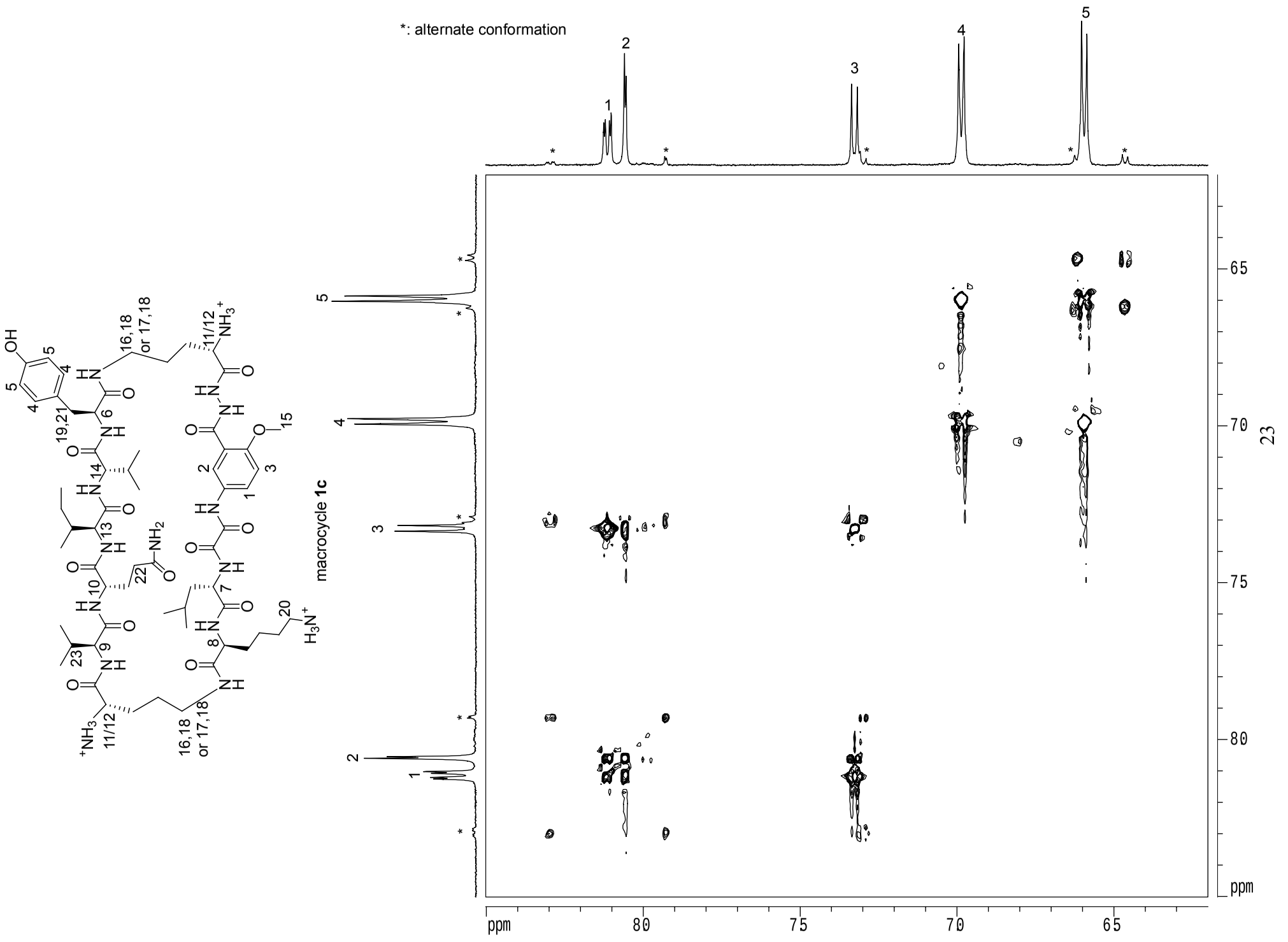
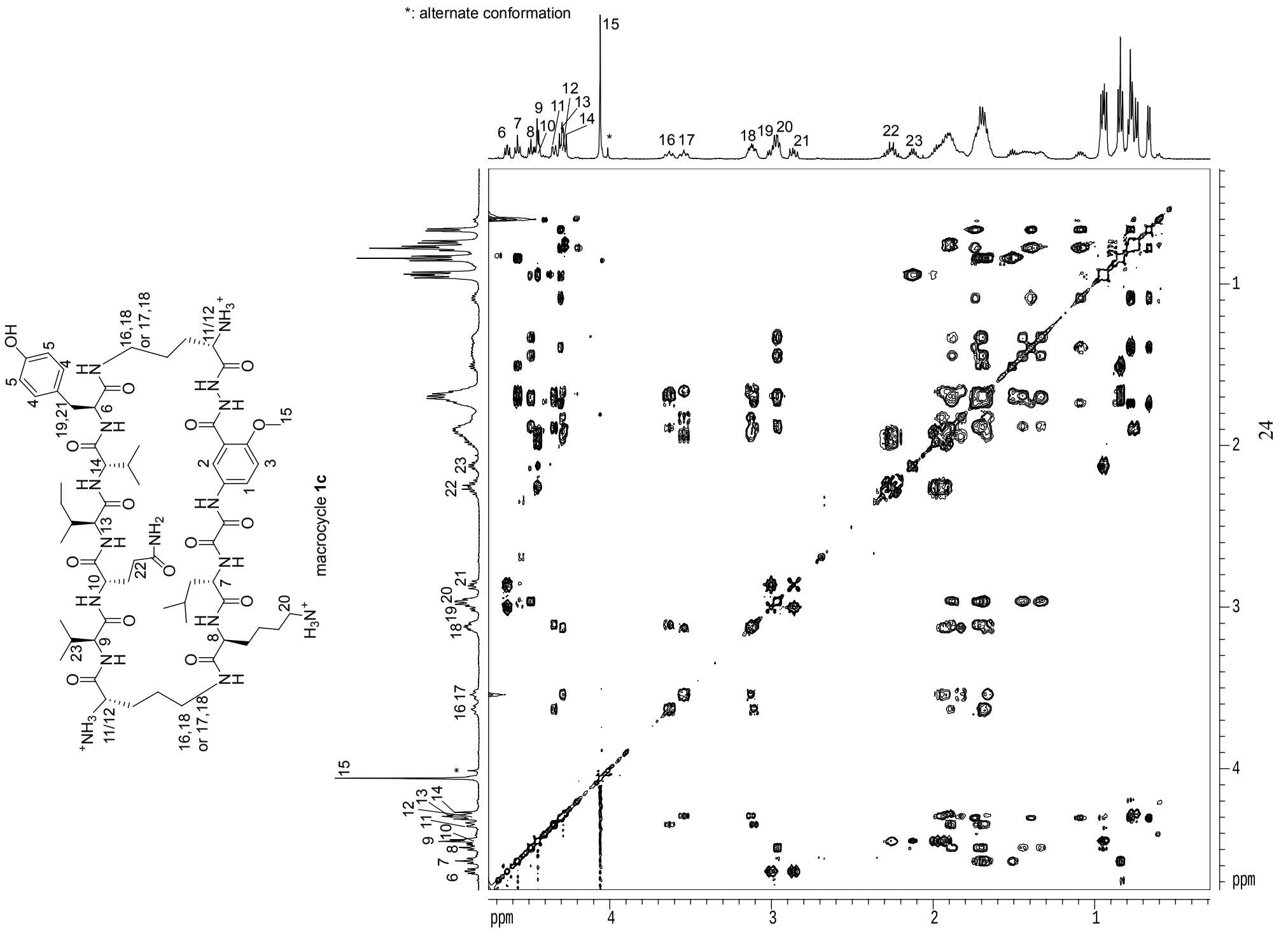
Macrocycle **1c**, TOCSY spectrum, 2 mM in D_2O, 25°C, 500 MHz, 150 ms mixing time



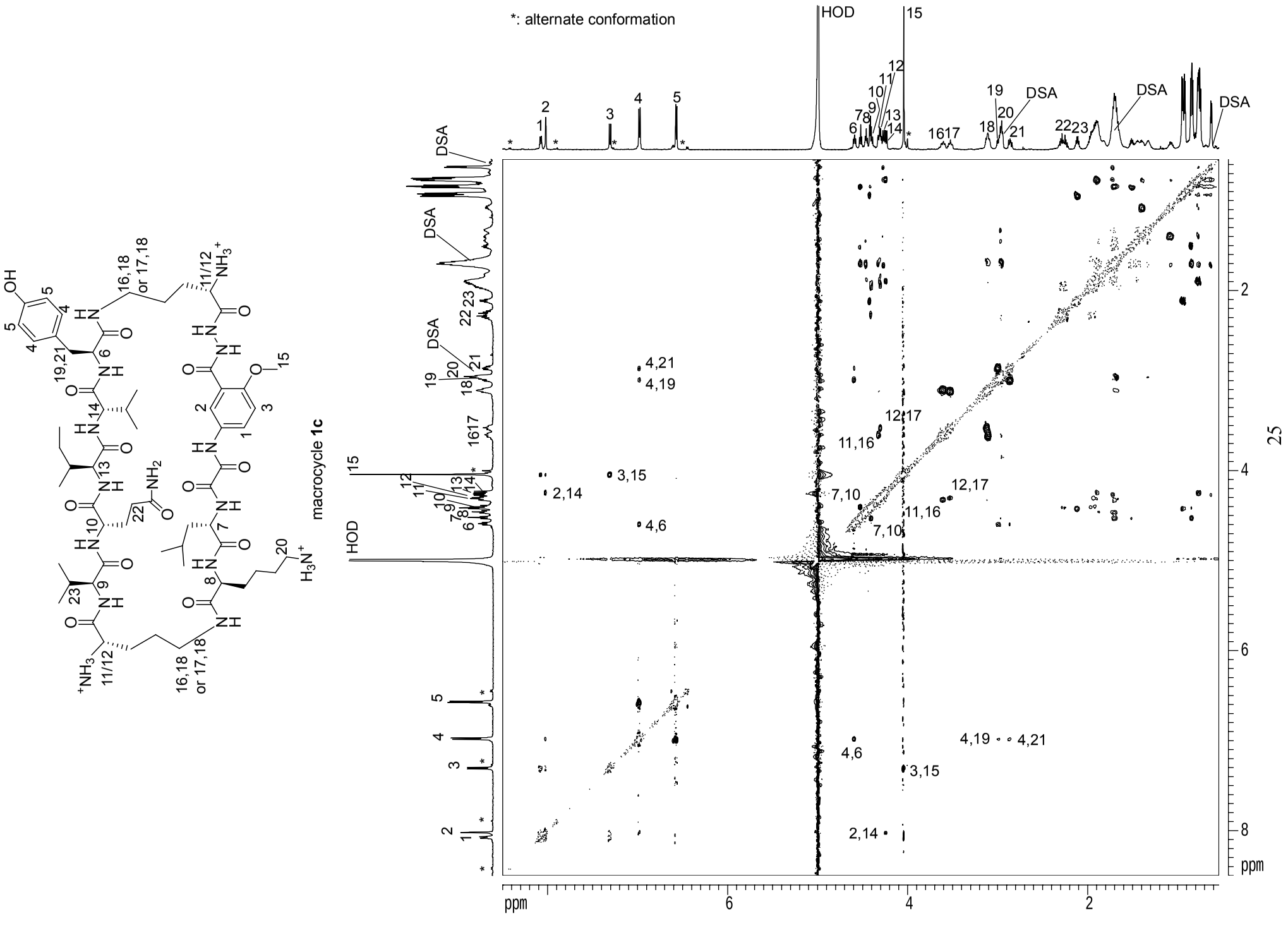
Macrocycle **1c**, TOCSY spectrum, 2 mM in D_2O, 25°C, 500 MHz, 150 ms mixing time



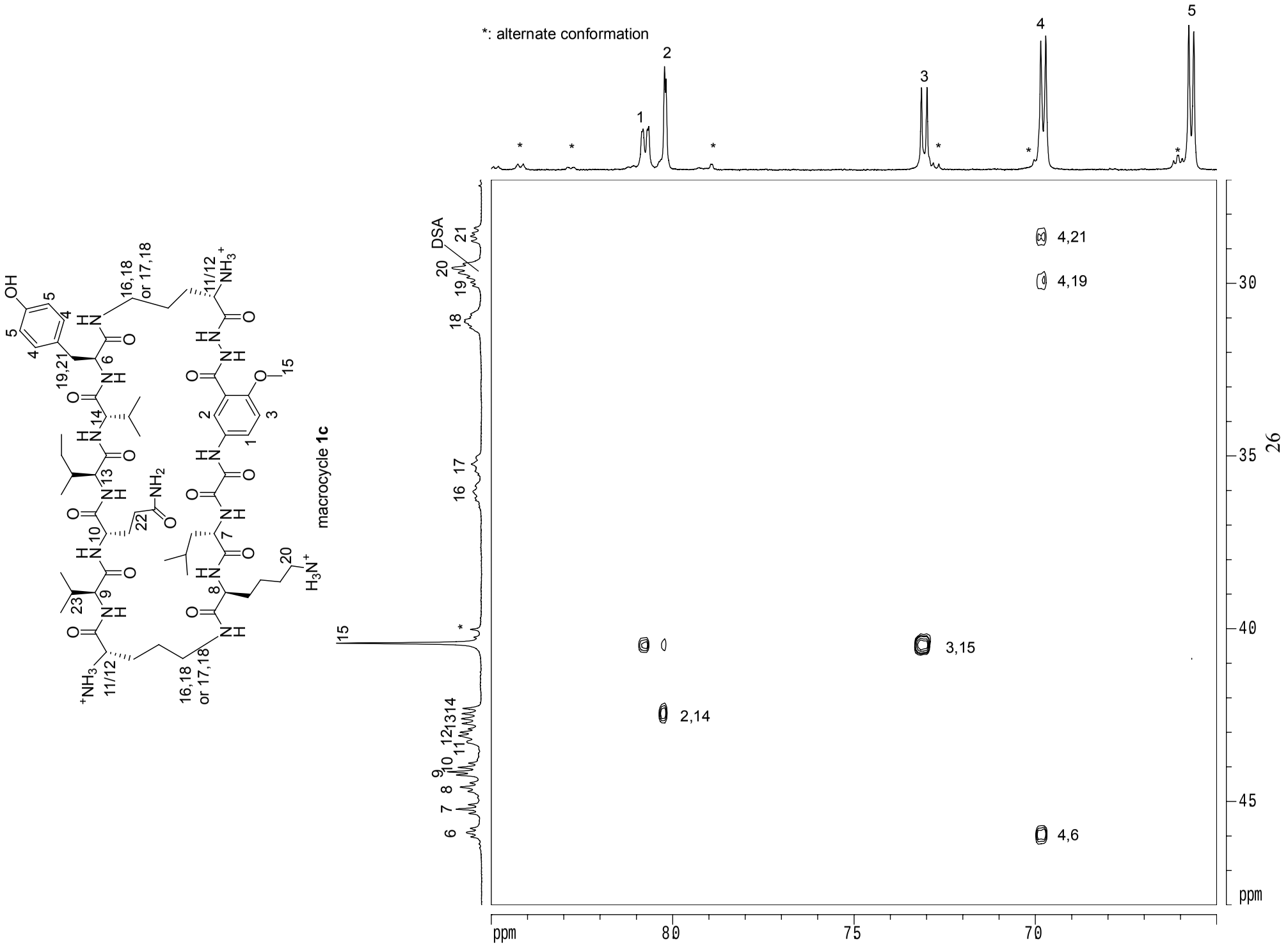
Macrocycle **1c**, TOCSY spectrum, 2 mM in D_2O, 25°C, 500 MHz, 150 ms mixing time



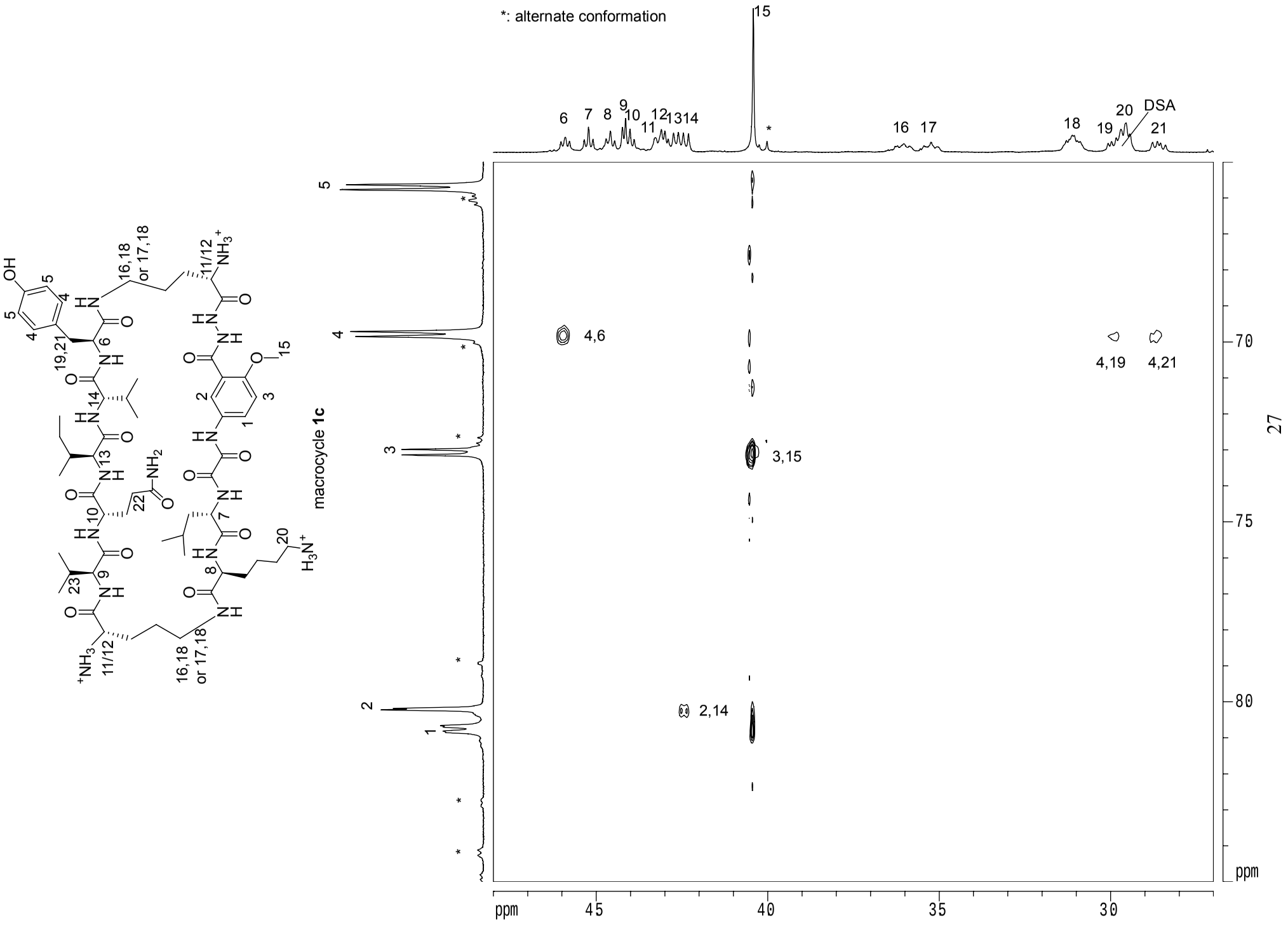
Macrocycle **1c**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time



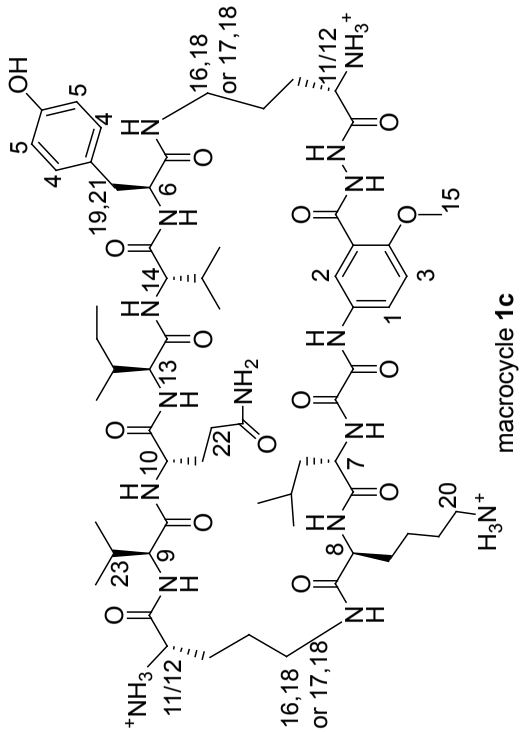
Macrocycle **1c**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time



Macrocycle **1c**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time

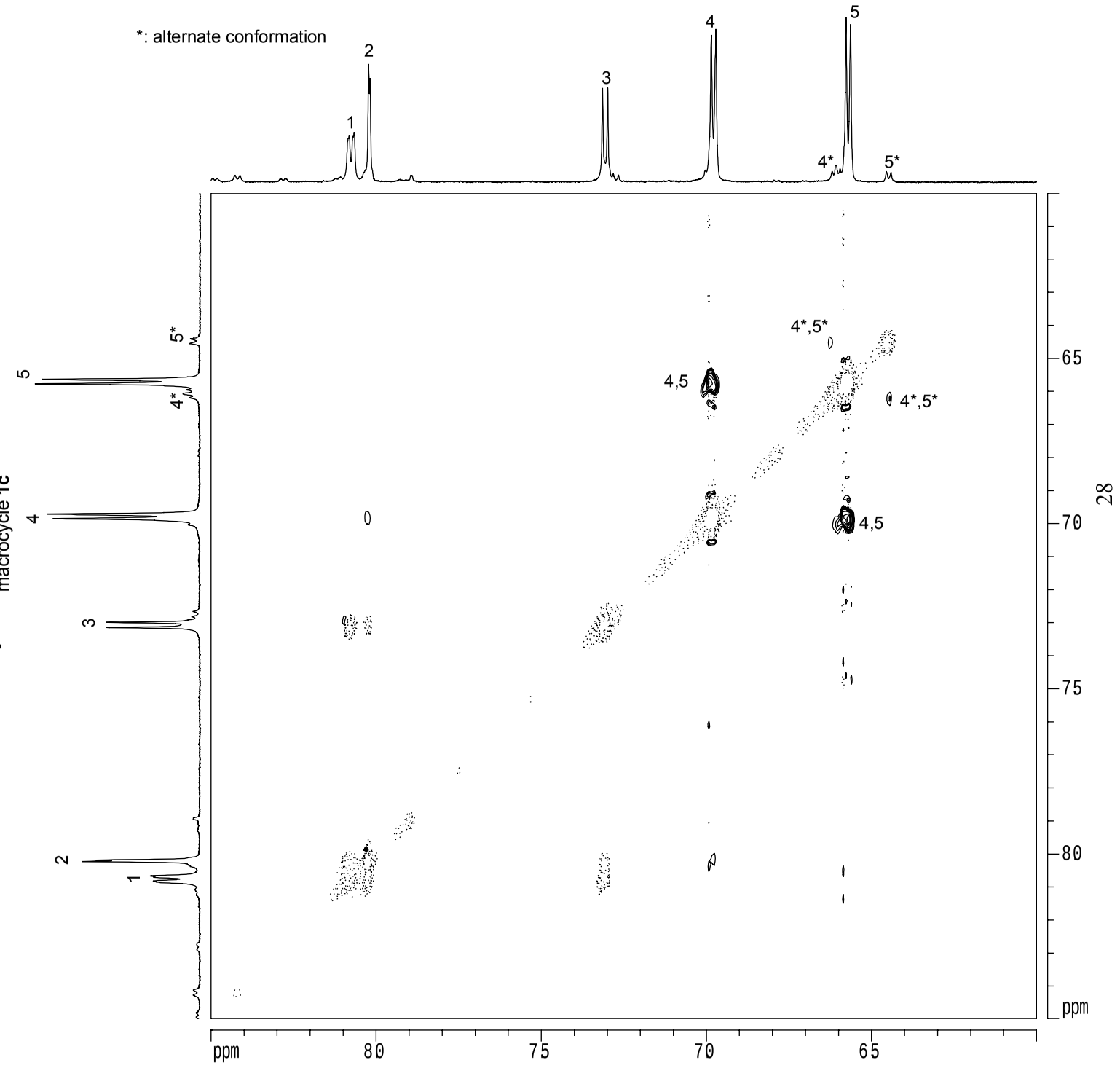


Macrocycle **1c**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time



*: alternate conformation

macrocycle **1c**



Macrocycle **1c**, ROESY spectrum, 2 mM in D_2O with DSA, 6°C, 600 MHz, 250 ms mixing time