Peptides Derived from Nucleoside β-Amino Acids form an Unusual 8-Helix

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Electronic supplementary information

Preparation of 3′-*N*-(9-fluorenylmethoxycarbonyl)-3′-amino-3′-deoxythymidyl-5'-carboxylic acid (3).

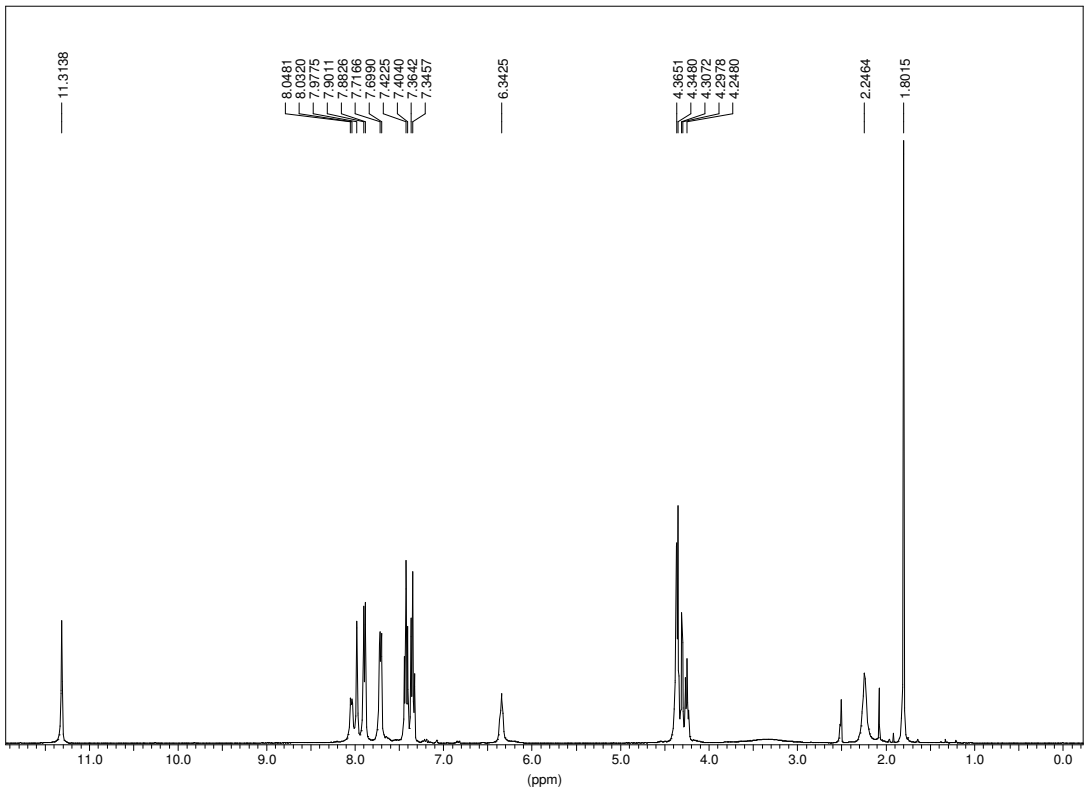
3'-*N*-(9-Fluorenylmethoxycarbonyl)-3'-amino-3′-deoxythymidine (**2**) (4.2 g, 9.1 mmol) was suspended in MeCN/H_2O (85 ml). To this suspension, TEMPO (285 mg, 1.8 mmol) and BAIB (6.45 g, 20 mmol) were added and the bright orange mixture stirred at ambient temperature. After 12 hours, a further portion of TEMPO (1.14 g, 7.3 mmol) and BAIB (2.93 g, 9.1 mmol) was added. After a further 12 hours stirring at ambient temperature, a pale brown precipitate was observed to have formed and the solution had separated into two distinct layers. The precipitate was isolated by filtration and washed with Et_2O. The remaining reaction mixture was left for a further 24 hours and a further crop of precipitate isolated and washed. Yield: 1.98 g, 4.1 mmol, 46 %.

δ^1H (400 MHz, MeOH-d_4) 1.80 (3H, s, CH_3 Thy), 2.25 (2H, bs, H2'), 4.25 (1H, pseudo-t, H4'), 4.30 (1H, pseudo-d, H3'), 4.35 (3H, pseudo-d, Fmoc H8, H9), 6.34 (1H, m, H1'), 7.38 (4H, dt, ArH), 7.70 (2H, d, ArH), 7.89 (2H, d, ArH), 7.98 (1H, s, H-6), 11.31 (bs, NH).

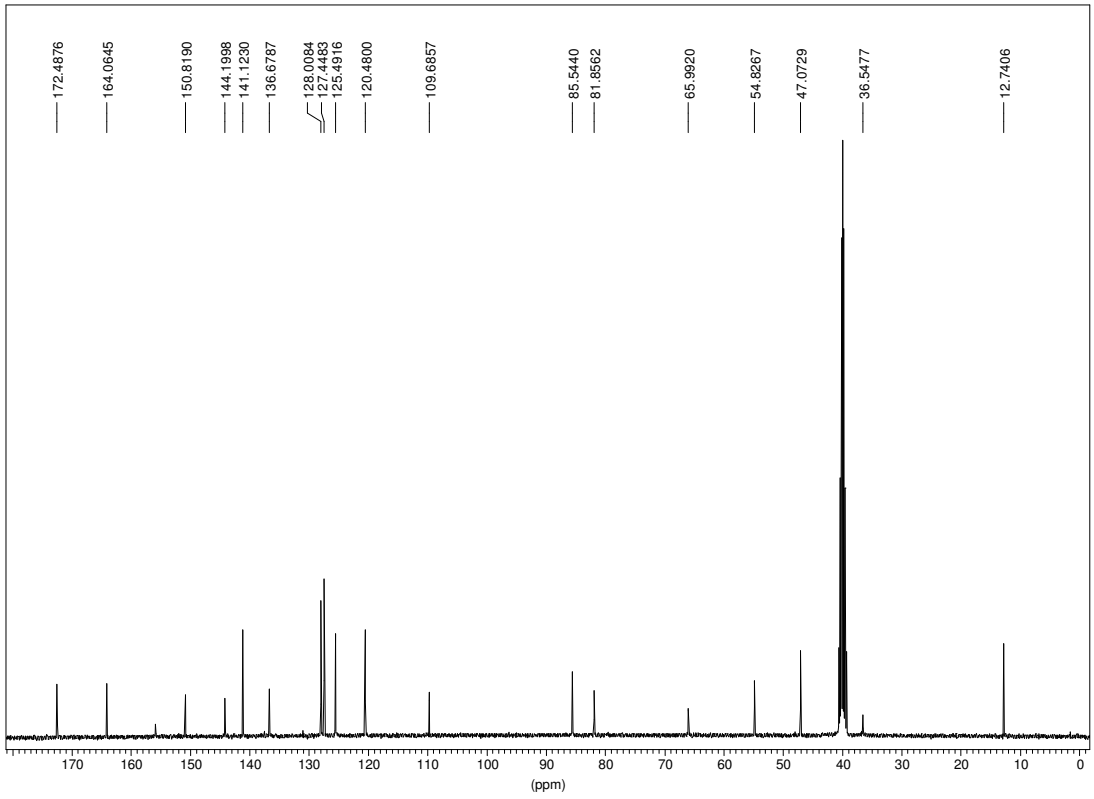
δ^{13}C (100 MHz, MeOH-d_4) 12.5 ($\underline{C}H_3$, Thy), 38.3 (C2'), 61.7 (C5'), 62.5 (C3'), 86.1 (C1'), 86.1 (C4'), 111.7 (C5), 138.1 (C6), 152.3 (C2), 166.4 (C4).

Analysis found C 62.60 H 4.78 N 8.68; $C_{25}H_{23}N_3O_7$ requires C 62.89 H 4.85 N 8.80.

Found m/z (ES^-) 476.1466 ($[M - H]^-$ 100%); $[C_{25}H_{23}N_3O_7 - H]^-$ requires 476.1548



^{1}H NMR Spectrum of Compound 3.



^{13}C NMR Spectrum of Compound 3.

Manual Solid-Phase Peptide Synthesis.

Assembly of the peptide: Peptides were synthesised manually in a batch procedure. *N*-Fmoc-9-amino-xanthe-3-yloxymethy-Sieber amide resin (0.71 mmol/g NHFmoc) was purchased from Novabiochem. Sieber amide resin (200 mg, 142 µmol NHFmoc) was swollen and the Fmoc group removed according to standard procedures (http://www.merckbiosciences.co.uk/docs/PROT/01-64-0014-001.pdf). To couple the first thymidine amino acid, (**3**) (17 mg, 35.5 mol) was dissolved in *N,N*-dimethylacetamide (DMAc) (178 µl) and 0.2 M HBTU in DMAc (178 µl) and disopropylethylamine (DIPEA) (6.8 µl, 39.0 µmol) were added to the solution which was then left for 60 s. The deprotected resin bed was then treated with this solution and agitated at ambient temperature for five hours. Following removal of the coupling solution the resin bed was washed with DMAc (x 5).

To cap unreacted sites, the capping solution (Ac_2O, DMAc, collidine, 1:8:1) was added to cover the resin bed and the vessel agitated for 5 minutes on an orbital shaker. To couple successive residues, reaction times were reduced to 30 min. Quantities for subsequent couplings were as follows: amino acid (**3**) (0.2 M, 500 µl), HBTU (0.2M, 500 µl), DIPEA (19 µl, 110 µmol). When all the required amino acid couplings had been performed, to obtain Fmoc off compounds the resin was subjected to a further deprotection step. To obtain Fmoc on compounds this extra deprotection was omitted from the procedure. Drying of the resin bed was achieved by washing with DMAc (x 5), DCM (x 5), MeOH (x 5), application of vacuum to the reaction vessel for 30 minutes and finally drying over silica gel in a vacuum desiccator for 24 hours.

Cleavage of oligomer from solid support: The dry resin was swelled in DCM for 30 minutes then washed three times with fresh DCM. The resin was covered with cleavage solution (1 % v/v TFA in DCM) and agitated for 2 minutes on an orbital shaker. The cleavage mixture was removed and the procedure repeated nine further times. Residual peptide was washed from the resin bed with DCM (x 3) and MeOH (x 3) then the combined cleavage solutions evaporated to dryness under reduced pressure and precipitated by the addition of H_2O. The suspension was centrifuged, the supernatant removed and fresh H_2O added. This was repeated 3 times prior to drying the peptide pellet under vacuum. Peptides were characterised by mass spectrometry and in some cases ir spectroscopy (Table 1).

Peptide	Molecular formula	Acc mass required	Acc mass found[a]	Ir data[b] (wavenumbers)
4	$C_{20}H_{26}N_7O_8^+$	492.1844	492.1843	3401, 1671
5	$C_{40}H_{48}N_{13}O_{16}Na^+$	988.3161	988.3182	-
6	$C_{55}H_{57}N_{13}O_{18}Na^+$	1210.3847	1210.3795	3411, 1665, 1642
7	$C_{80}H_{91}N_{25}O_{32}Na^+$	1936.616	1936.6069	-
8	$C_{95}H_{101}N_{25}O_{34}Na^+$	2158.684	2158.6863	-

Table 1. [a] High-resolution electrospray ionization mass spectrometry data for nucleoside β-peptides. Spectra were obtained in positive ionization mode from samples prepared in aqueous methanol and are accurate to within 5 ppm. [b] Ir data was obtained by evaporating methanolic solutions on NaCl plates.

NMR Experiments

^{1}H NMR spectra were recorded on either a Varian Unity Inova 500 or a Bruker DRX 500 using 5 mm indirect detection probes. NOESY spectra were acquired with 250 and 400 ms mixing times. TOCSY spectra were acquired with 80ms spin lock. Spectral widths of 6500 Hz were used and 2D data were acquired in 1k complex points (Varian) or 2k data points (Bruker). 256 pairs of t1 increments (Varian) or 512 t1 points were used. Data recorded on the Varian spectrometer were acquired using States hypercomplex phase cycling, those on the Bruker with TPPI. All 2D data were processed using a Gaussian window function (gaussian factor around 0.06 (Varian), 0.1 (Bruker) and linebroadening of around -5Hz. Spectra were imported to Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco) for preparation of the figures below.

Table 2. Chemical Shift assignments for (**6**) in d6-DMSO solution (ppm), 25 ^{0}C

Table 3. List of nOe's for (**6**).

Figure 1. Section of 1d 1H spectrum for tetramer (6) in d6-DMSO solution at 25 ^{0}C, recorded on a Varian Unity Inova 500.

Figure 2. Section of the TOCSY spectrum recorded for (6) in d6-DMSO solution at 25 C, acquired using a Varian Unity Inova 500. This section displays the NH to H1'/H3'/H4'/H2'/H2" connections, and H6 to Me connections.

Figure 3. Section of the NOESY spectrum recorded for (6) in d6-DMSO solution at 25 ^{0}C, acquired using a Varian Unity Inova 500. The mixing time was 250 ms. This section shows NH to H1'/H3'/H4' both inter-and intra-residue and H6 to H1'/H3' connections.

Figures 4, **5**, **7**, **8**. Show sections of the NOESY spectrum recorded for (7) in d5-pyridine at 5 ^{0}C on a Bruker DRX 500. The mixing time was 400ms.

Figure 6. Section of the TOCSY spectrum for (7) in d5-pyridine recorded at 5 ^{0}C on a Bruker DRX 500. This section displays the NH to H3'/H4' connections for comparison with Figure 5.

Figure 9. Numbering of sugar and base.

Figure 10. Some of the nOe connections used in determining the model for (**6**) shown in Figure 3 of manuscript.

Figure 11. DQF-COSY and TOCSY connections (note some overlap in chemical shifts).

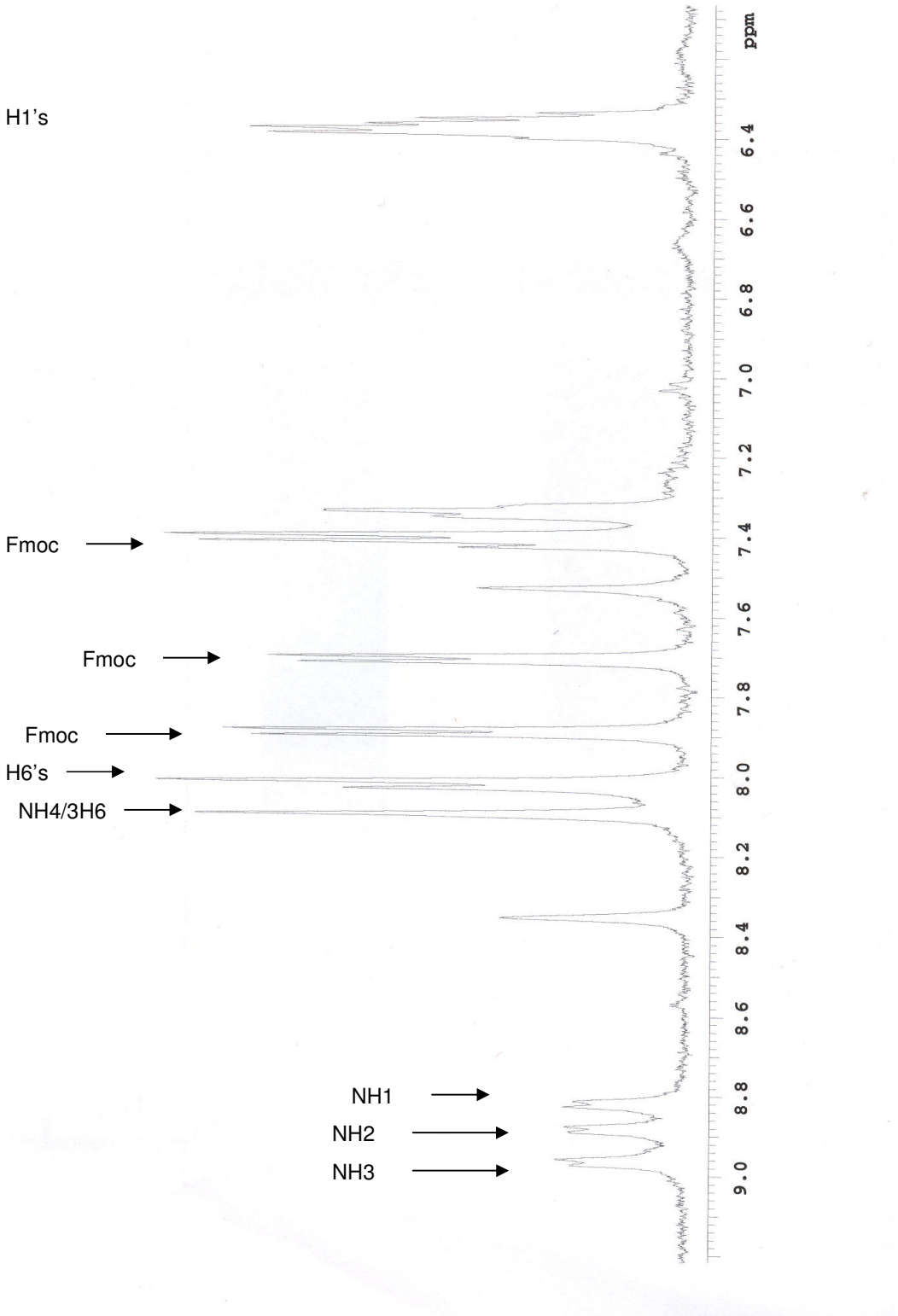
Table 2. Chemical Shift assignments for (**6**) in d6-DMSO solution (ppm), 25^0C

	NH	NH2	H1'	H2'/2"	H3'	H4'	H6	Me
1	8.85	7.6	6.48	2.41/2.33	4.6	4.35	n/a	1.9
2	8.92	n/a	6.33	2.46/2.39	4.64	4.38	8.21	1.9
3	8.94	n/a	6.33(5)	2.46(5)/2.34	4.66	4.39	8.06	1.9
4	8.13	n/a	6.32(5)	2.43/2.3	4.44	4.4	8.06	1.0

Table 3. List of nOe's for (**6**).

Residue	H-atom	Residue	H-atom	nOe
1	NH2	1	NH	Strong
1	H6	1	NH	Strong
1	H3'	2	NH	Weak
1	H1'	1	NH	Medium
1	H1'	1	H4'	Medium
1	NH	2	H3'	Medium
2	H1'	2	NH	Medium
2	H3'	2	NH	Strong
2	H6	2	Me	Medium
3	H3'	3	H6	Medium
3	NH	4	H4'	Medium
3	H6	2	H1'	Medium
3	H6	2	H3'	Weak
3	H1'	3	NH	Medium
3	H3'	3	NH	Medium
4	H1'	4	NH	Medium
4	H3'	3	NH	Medium
4	H3'	4	NH	Medium

All of the NH's displayed medium to strong nOe's to both their 2' and 2" protons. NOe's were also detected from H1' to their own H3' that were strong. Only the H1' of residue 1 displayed an nOe to its H4'.



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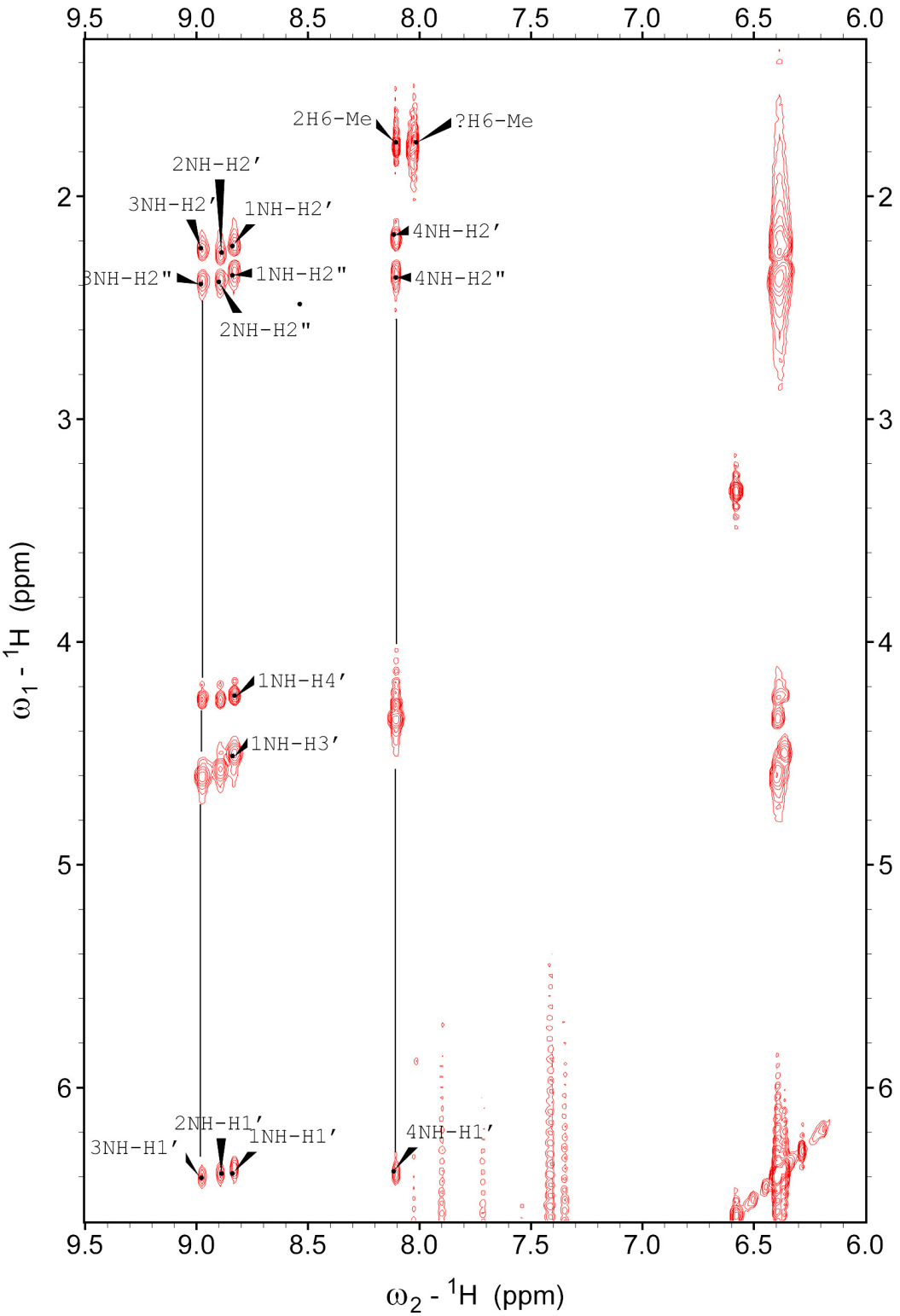
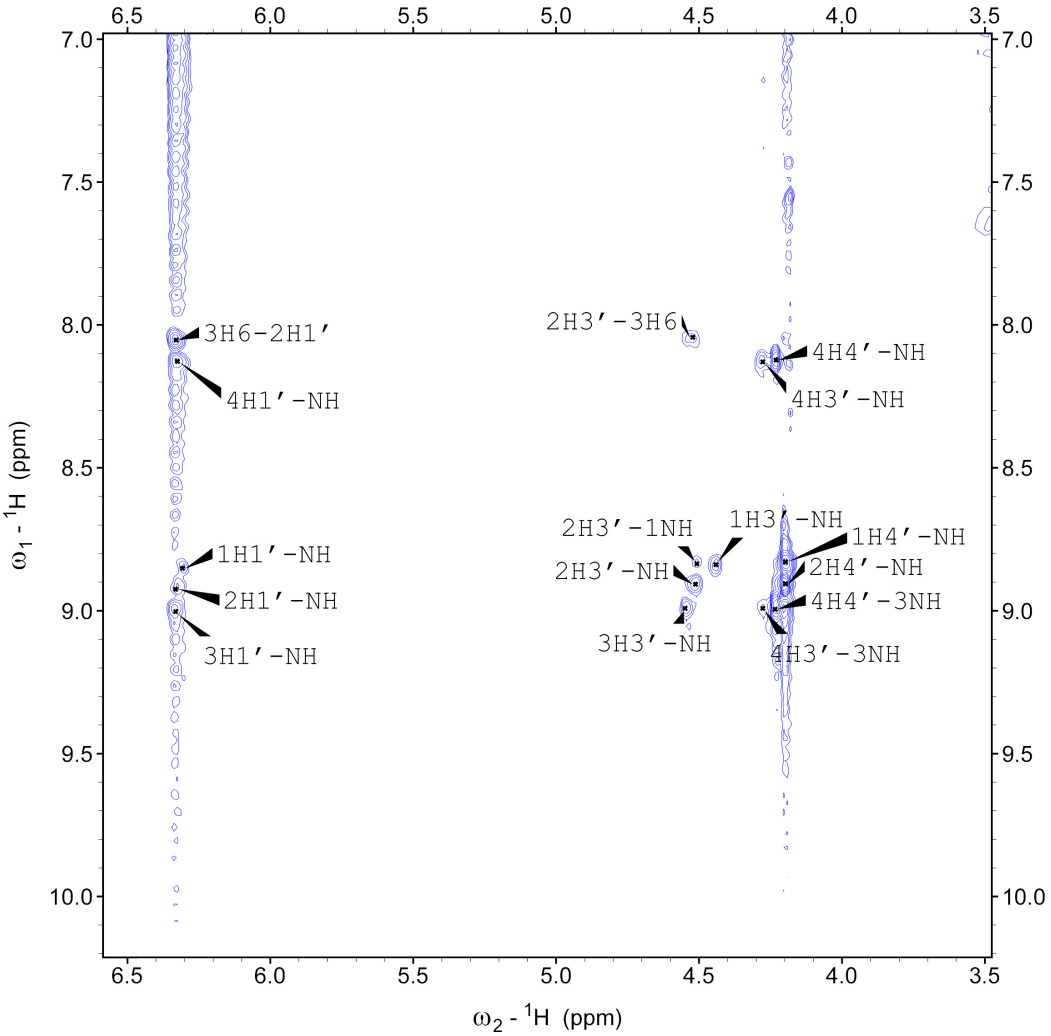


Figure 2. Section of the TOCSY spectrum recorded for (6) in d6-DMSO solution at 25 C, acquired using a Varian Unity Inova 500. This section displays the NH to H1'/H3'/H4'/H2'/H2" connections, and H6 to Me connections.



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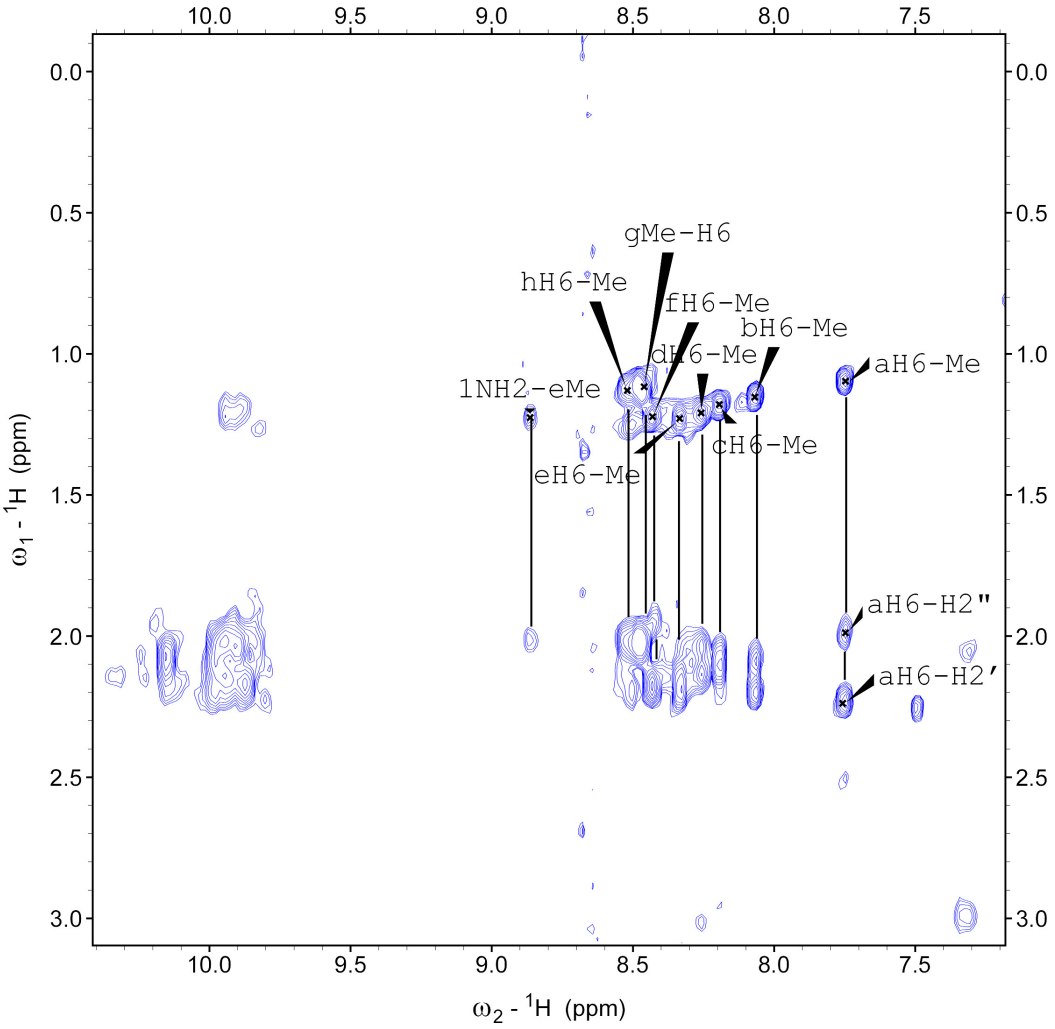


Figure 4. Sections of the NOESY spectrum recorded for (7) in d5-pyridine at 5 ^{0}C on a Bruker DRX 500. The mixing time was 400ms.

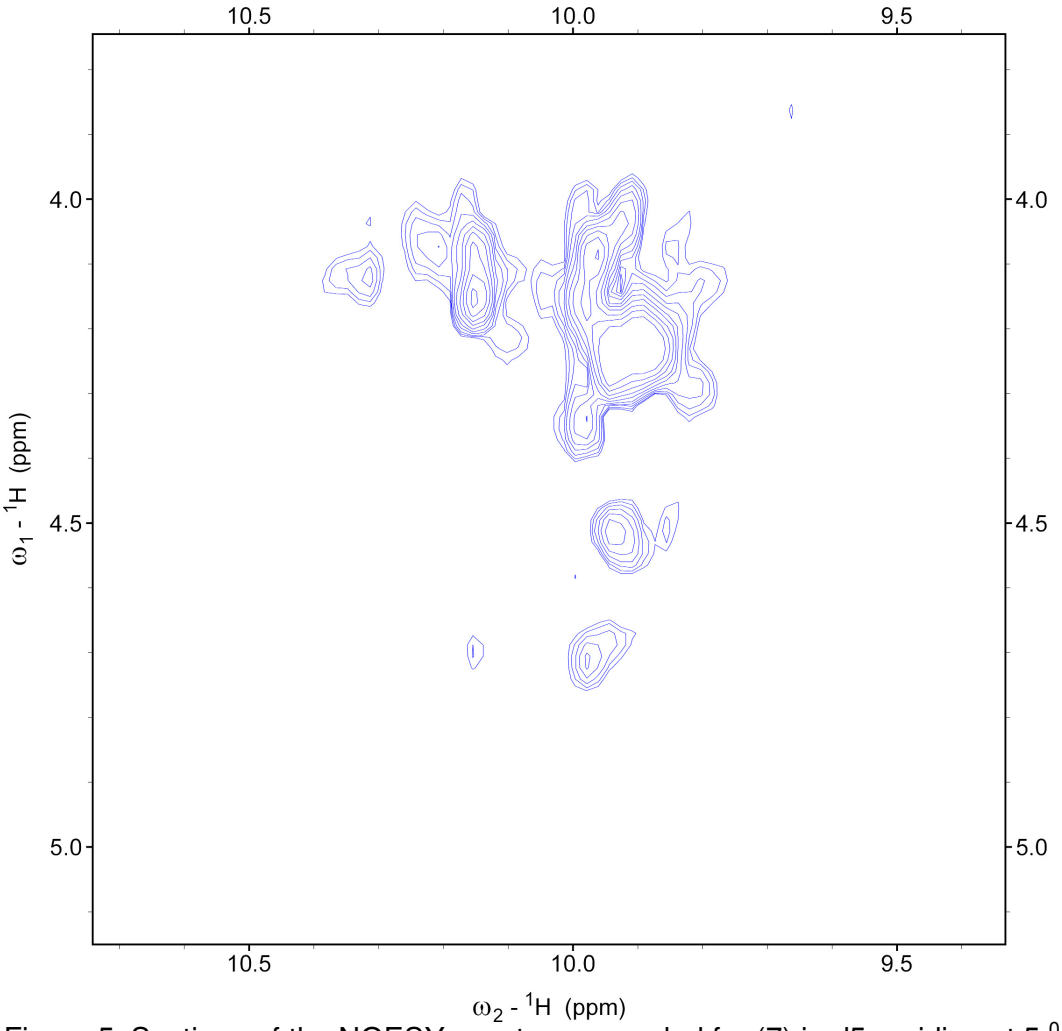
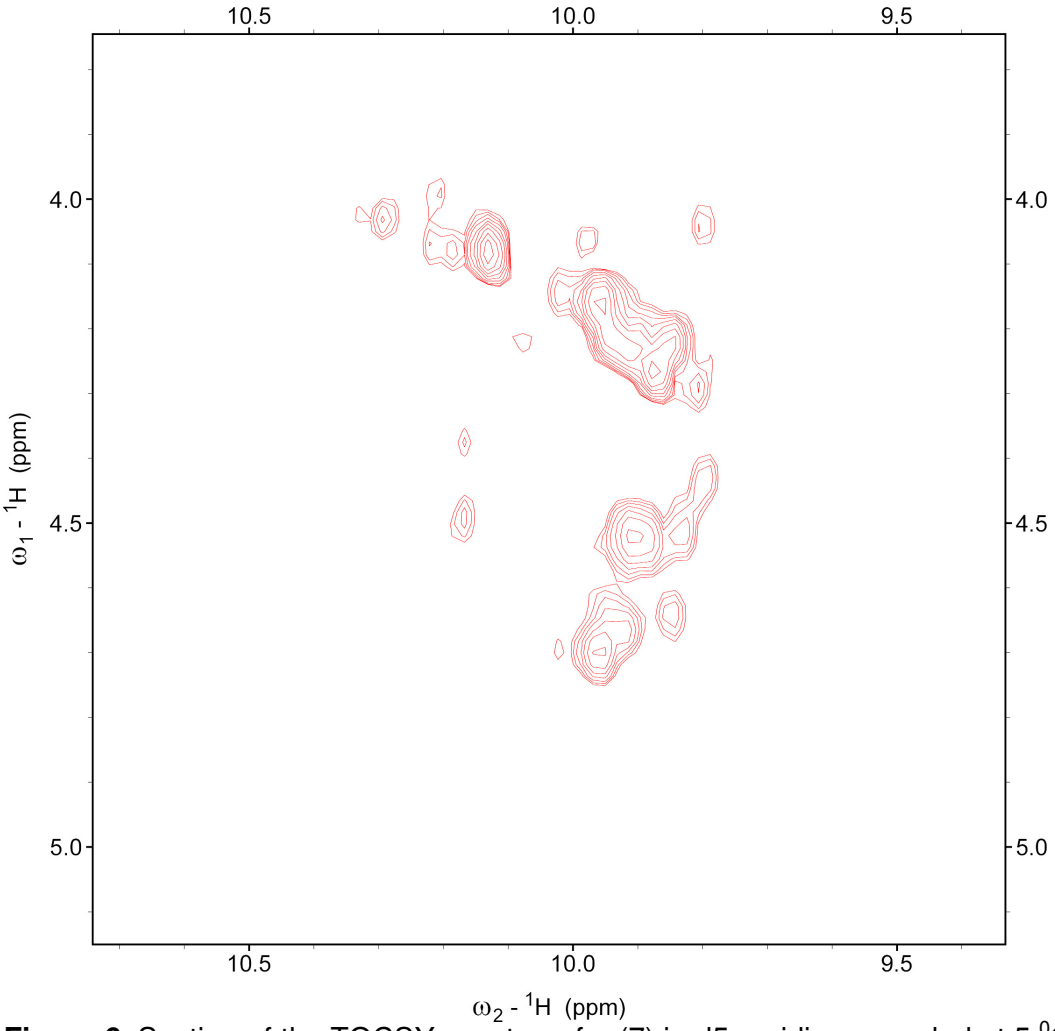


Figure 5. Sections of the NOESY spectrum recorded for (7) in d5-pyridine at 5 ^{0}C on a Bruker DRX 500. The mixing time was 400ms.



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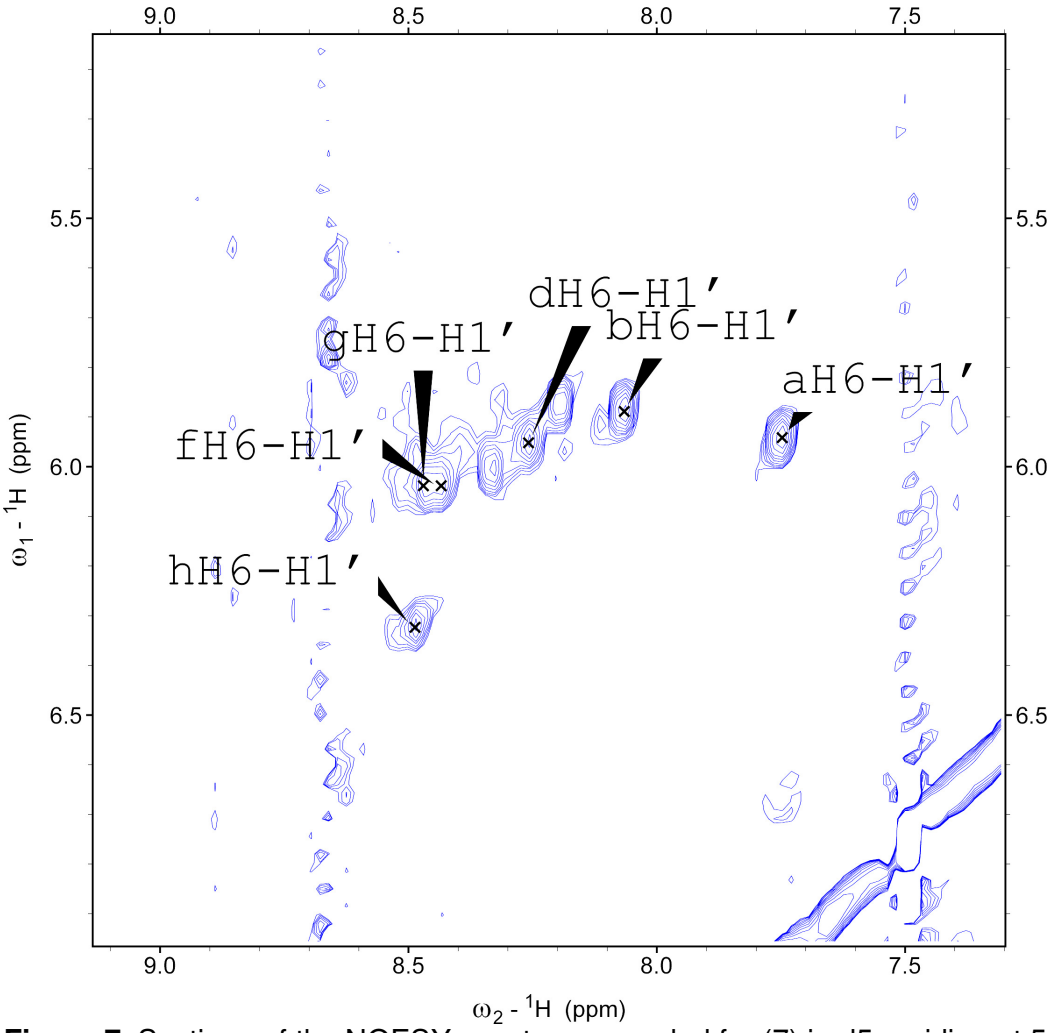


Figure 7. Sections of the NOESY spectrum recorded for (7) in d5-pyridine at 5 ^{0}C on a Bruker DRX 500. The mixing time was 400ms.

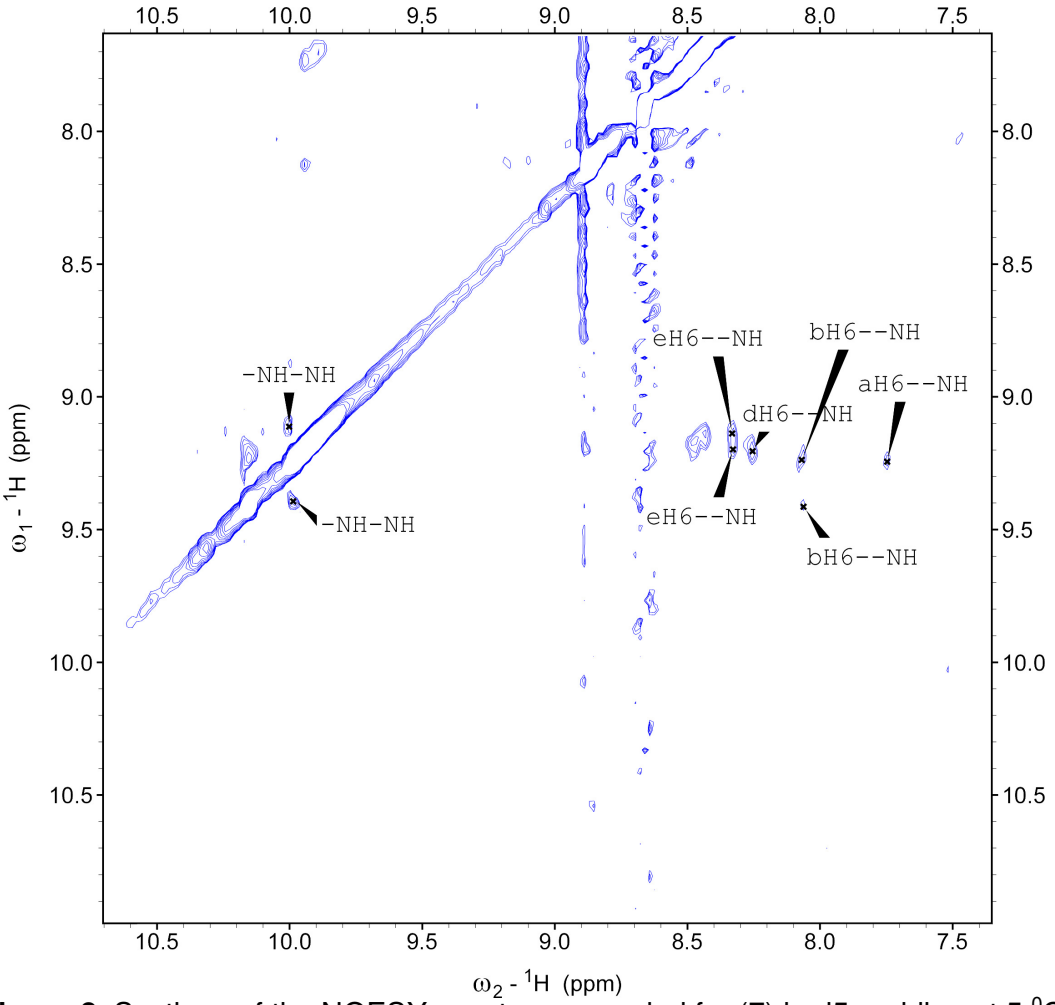


Figure 8. Sections of the NOESY spectrum recorded for (7) in d5-pyridine at 5 ^{0}C on a Bruker DRX 500. The mixing time was 400ms.

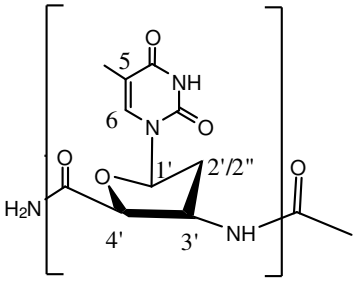
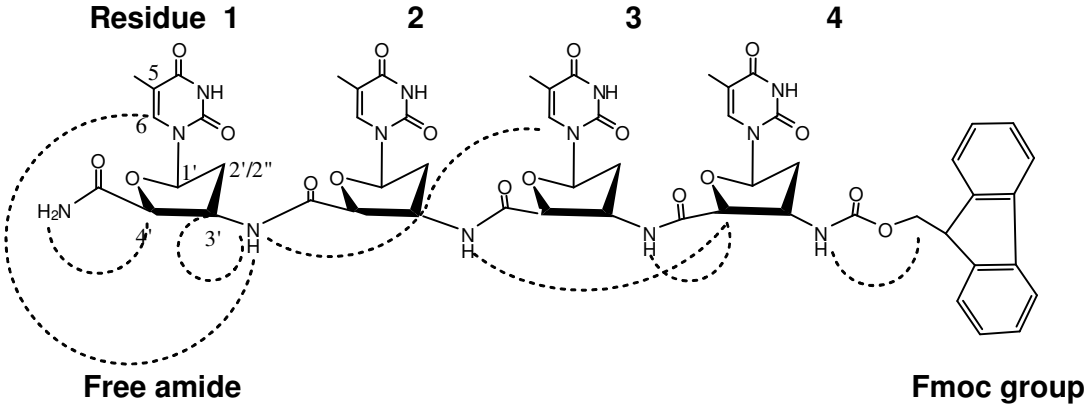


Figure 9. Numbering of sugar and base



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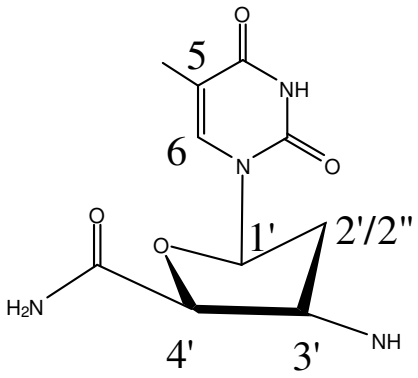

DQF-COSY – Connections

H1'-H2'
H1'-H2"
H2'-H2"
H2'-H3'
H2"-H3'
H3'-NH (of intervening amide)
H3'-H4'
Connections amongst the protons of the Fmoc group could also be detected.
H6 –Me (very weak)

TOCSY – Connections

All the short protons were correlated to each other including to the attached NH(of the intervening amide).
H6-Me – The H6's have similar chemical shifts and there is a small amount of resolution in the methyl shifts but not enough at room temperature in DMSO to unambiguously assign these.

Modelling of structure for tetramer (6)

Macro-Model (v90014) was used through the Masetro program (version 7.0.110) interface on a Linux platform. The tetramer was built within Maestro. Sugar puckers were loosely constrained around a 50/50 north:south pucker (as guided by H1'/H2' and H1'/H2" couplings observed in the monomer in the same solution conditions). Loose distance constraints were applied based on nOe observations (5 Å +/- 1.7 Å) and the amide links were constrained to remain trans. The structure generations were performed using $CHCl_3$ as solvent as the nearest alternative to DMSO. The tetramer was subjected to energy minimisation to find a local energy minimum. This low energy conformation was then used for conformational searching to find the global minimum, with the results used to seed the next run until no further low energy structures were found.

The minimization and conformational search was performed using the MMFF94s (Merck molecular force field static) force field which is a well parameterized force field for biopolymers. The PRCG (Polak-Ribiere conjugate gradient) method was used for the minimisation, with a maximum 2500 iterations performed, converging on the gradient with a 0.0500 threshold. The conformational search additionally used the torsional sampling method, MCMM (Monte Carlo minimisation method), run in batches of 1000 steps using the default settings. The final energy for the lowest energy conformation was -1431.98 $kJmol^{-}1$.
All of the distance constraints were applied loosely – set to either 5.0 A +/- 1.7 A.
Dihedral angels were constrained as follows (all +/- 10^0) (see text)
H1' – H2' 150^0
H1' – H2" 30^0
H3' – H2' -40^0
H3' – H2" 80^0
H3' – H4' -80^0

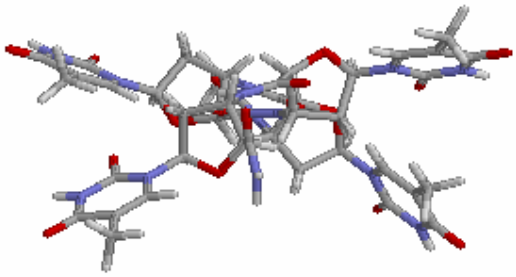

Figure 12. View along helix axis of structure generated using Macro-model and NMR constraints. The thymine rings 1 and 3 are on the right hand side, thymine rings 2 and 4 are on the left hand side.