Supporting Information

Potent Inhibitors of the Hepatitis C Virus NS3 Protease:

Design and Synthesis of Macrocyclic Substrate-Based b-Strand Mimics

Nathalie Goudreau, Christian Brochu, Dale R. Cameron, Jean-Simon Duceppe, Anne-Marie Faucher, Jean-Marie Ferland, Chantal Grand-Maître, Martin Poirier, Bruno Simoneau, Youla S. Tsantrizos*

General

NMR: ^{1}H NMR spectra of all synthetic intermediates and products were obtained at 23 ^{o}C on a 400 MHz NMR spectrometer and the chemical shifts are given in ppm, referenced to the internal deuterated solvent.

Purification, determination of homogeneity and biological testing of ligands: All compounds evaluated *in vitro* biological assays were purified by semi-preparative C_{18} reversed-phase HPLC on a Whatman Partisil 10 ODS-3 column using a linear gradient from 5% aqueous CH_3CN (containing 0.06% TFA) to 100% CH_3CN (containing 0.06% TFA) to >98% homogeneity based on HPLC (λ = 220 nm). IC_{50} determinations were carried out in the *in vitro* enzymatic assays as previously reported using NS3-NS4A heterodimer protein of genotype 1b.[17] The values reported in Scheme 6 are the average of a minimum of three independent determinations.

L-Allylglycine methyl ester hydrochloride (**5b**) ^{1}H NMR (DMSO- d_6), δ 2.59 (dd, *J*= 7 Hz, 2H), 3.73 (s, 3H), 4.12 (dd, *J*= 7 Hz, 1H), 5.21-5.15 (m, 2H), 5.82-5.72 (m, 1H), 8.57 (bs, 3 H). ES^+ MS *m/z* 130 $(M+H)^+$

L-S-Allylcysteine methyl ester hydrochloride (**6b**) ES^+ MS *m/z* 176 $(M+H)^+$; ES^- MS *m/z* 174 $(M-H)^-$

(2S,4R)-4-(Quinolin-4-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (**15a**) ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H), 2.32-2.42 (m, 1H), 2.62-2.71 (m, 1H), 3.72 (s, 3H), 3.68-3.79 (m, 2H), 4.09-4.18 (m, 1H), 4.44-4.50 (dd, *J* = 8.5 Hz, 1H), 5.35 (bs, 1H), 7.07 (d, *J* = 5.1 Hz, 1H), 7.56 (dd, *J* = 7.5 Hz, 1H), 7.75 (dd, *J* = 7.5 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 8.13 (dd, *J* = 7.6 Hz, 1H), 8.73 (d, *J* = 5.1 Hz, 1H). ES^+ MS *m/z* 359.6 $(MH)^+$.

(2S,4R)-4-(7-Methoxy-quinolin-4-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-methyl ester (**15b**) ^{1}H NMR ($CDCl_3$, rotamers in ~2:1 ratio): δ (major rotamer) 1.44 (s, 9H), 2.35-2.42 (m, 1H), 2.65-2.74 (m, 1H), 3.78 (s, 3H), 3.95 (s, 3H), 3.89-3.95 (m, 2H), 4.51 (dd, *J* = 8 Hz, 1H), 5.15 (bs, 1H), 6.55 (m, 1H), 7.1 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.36 (d, *J* = 2.6 Hz, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 8.65 (d, *J* = 5.1 Hz, 1H). ES^+ MS *m/z* 389.4 $(MH)^+$.

(2S)-*N*-Boc-2-amino-6-heptenoic acid (**20a**) ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.45-1.57 (m, 2H), 1.62-1.73 (m, 1H), 1.82-1.92 (m, 1H), 2.05-2.12 (m, 2H), 4.32 (bs, 1H), 4.98 (dm, *J* = 10.2 Hz, 1H), 5.10 (dm, *J* = 17.0 Hz, 1H), 5.66-5.84 (tdd, *J* = 6.7, 10.2 , 17.0 Hz, 1H). ES^+ MS *m/z* 244.2 $(M+H)^+$.

Dipeptides **21a,b**. The hydrochloride salts of the *D*-allylglycine methyl ester was coupled to the *L*-Boc-proline fragment **15c** following the standard protocol. In each case, the product was purified by flash column chromatography (using 50% EtOAc in hexane as the eluent) to obtain the corresponding dipeptide as a white solid in ~90-95% yield.

Dipeptide **21a** ^{1}H NMR (DMSO-d_6), mixture of rotamers in 2:1 ratio: δ (major rotamer) 1.32 (s, 9H), 2.25-2.26 (m, 1H), 2.40-2.30 (m, 1H), 2.43-2.50 (m, 1H), 2.65-2.50 (m, 1H), 3.62 (s, 3H), 3.82-3.70 (m, 2H), 3.92 (s, 3H), 4.48-4.38 (m, 2H), 5.04 (d, *J*= 11.0 Hz, 1H), 5.39 (d, *J*= 17.0 Hz, 1H), 5.53 (br s, 1H), 5.78-5.65 (m, 1H), 7.17 (dd, *J*= 3.0, 9.0 Hz, 1H), 7.39 (d, *J*= 2.0 Hz, 1H), 7.44 (s, 1H), 7.57-7.46 (m, 3H), 7.95-7.92 (m, 1H), 8.32-8.24 (m, 2H), 8.35 (d, *J*= 9.0 Hz, 1H). ES^+ MS *m/z* 576 $(M+H)^+$; ES^- MS *m/z* 574 $(M-H)^-$

Dipeptide **21b** ^{1}H NMR (DMSO-d_6), mixture of rotamers in 2:1 ratio: δ (major rotamer) 1.34 (s, 9H), 2.17-2.28 (m, 1H), 2.48-2.36 (m, 2H), 2.61-2.52 (m, 1H), 3.63 (s, 3H), 3.75 (dd, *J*= 4.0,

12.0 Hz, 1H), 3.80 (d, *J*= 12 Hz, 1H), 3.93 (s, 3H), 4.38-4.30 (m, 1H), 4.51-4.40 (m, 1H), 5.05 (d, *J*= 10.0 Hz, 1H), 5.13 (dd, *J*= 2.0, 16.0 Hz, 1H), 5.53 (bs, 1H), 5.84-5.72 (m, 1H), 7.18, (dd, *J*= 3.0, 10.0 Hz, 1H), 7.40 (d, *J*= 3 Hz, 1H), 7.46 (s, 1H), 7.57-7.47 (m, 3H), 7.95 (d, *J*= 10.0 Hz, 1H), 8.27 (d, *J*= 7.0 Hz, 2H), 8.35 (d, *J*= 7.0 Hz, NH). ES^+ MS *m/z* 576 $(M+H)^+$; ES^- MS *m/z* 574 $(M-H^-)$

Dipeptides **22a,b**. The hydrochloride salts of **6a** and **6b** were independently coupled to the *L*-Boc-proline fragment **15b** following the standard protocol. In each case, the product was purified by flash column chromatography (using a solvent gradient from 3% to 8% MeOH in EtOAc) to isolate each of the dipeptides **22a** and **22b** as colorless oils (~85% yield).

Dipeptide **22a** ^1H NMR ($CDCl_3$), mixture of rotamers in ~1:5 ratio: d (major rotamer) 1.46 (s, 9H), ~2.4 (br 1H), 2.86-2.91 (dd, *J* = 6.0 , 14.0 Hz, 1H), 2.95-3.00 (m, 2H), 3.12-3.18 (m, 2H), 3.75-3.84 (m, 4H), 3.84-3.93 (m, 1H), 3.94 (s, 3H), 4.54-4.57 (m, 1H), 4.80 (bs, 1H), 5.05-5.22 (m, 3H), 5.71-5.81 (m, 1H), 6.64 (bs, 1H), 7.12-7.14 (dd, *J* = 2.6 , 9.2 Hz, 1H), 7.36 (d, *J* = 2.6 Hz, 1H), 7.66 (bs, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 8.66 (d, *J* = 5.1 Hz, 1H). ES^+ MS *m/z* 546.3 $(M+H)^+$; ES^- MS *m/z* 544.2 $(M-H)^-$.

Dipeptide **22b** ^1H NMR ($CDCl_3$), mixture of rotamers in ~1:5 ratio: d (major rotamer) 1.49 (s, 9H), ~2.4 (br 1H), 2.80 (m, 1H), 2.86-2.91 (dd, J = 6.0 , 14.0 Hz, 1H), 2.95-3.00 (m, 1H), 3.12-3.18 (m, 2H), 3.75-3.84 (m, 5H), 3.94 (s, 3H), ~4.65 (bs, 1H), 4.80 (bs, 1H), 5.05-5.22 (m, 3H), 5.71-5.81 (m, 1H), 6.64 (bs, 1H), 7.12-7.14 (dd, J = 2.6 , 9.2 Hz, 1H), 7.36 (d, J = 2.6 Hz, 1H), 7.66 (bs, 1H), 7.99 (d, J = 9.2 Hz, 1H), 8.66 (d, J = 5.1 Hz, 1H). ES^+ MS *m/z* 546.3 $(M+H)^+$; ES^- MS *m/z* 544.2 $(M-H)^-$.

Compounds **23b** ^{1}H NMR ($CDCl_3$) mixture of rotamers (~1:9 ratio): δ (major rotamer) 1.15-1.28 (m, 1H), 1.42 (s, 18H), 1.43-1.75 (m, 4H), 2.26-2.20 (m, 2H), 2.38 (br, 1H), 2.92 (br, 1H), 3.69-3.75 (m, 2H), 3.94 (s, 3H), 4.52 (br, 1H), 4.95 (d, *J* = 10.2 Hz, 1H), 5.00 (d, *J* = 17.2 Hz, 1H), 5.29 (br, 1H), 5.74-5.83 (m, 1H), 7.03 (br, 1H), 7.08 (dd, *J* = 2.3, 9.2 Hz, 1H), 7.42-7.51 (m, 5H), 7.95 (d, *J* = 9.2 Hz, 1H), 8.03 (bd, *J* = 7 Hz, 2H). ES^+ MS *m/z* 658.3 $(M+H)^+$; ES^- MS *m/z* 656.2 $(M-H)^-$.

Dipeptide methyl ester **25b**. The *N*-Boc and *t*-butyl ester moieties of compound **23b** were simultaneously hydrolysed using the same procedure as described above for **23a**. In this case, the free acid was directly converted to the corresponding proline hydrochloride salt methyl ester **25b**, which was used dirrectly in the synthesis of tripeptide dienes without purification. **25b**·HCl salt ^{1}H NMR ($CDCl_3$) mixture of rotamers in (~1:9 ratio): δ (major rotamer) 1.28-1.34 (m, 1H), 1.49-1.66 (m, 4H), 2.02-2.15 (m, 2H), 2.27-2.35 (m, 1H), 2.81-2.89 (m, 1H), 3.46-3.50 (m, 1H), 3.62 (s, 3H), 3.67-3.78 (m, 1H), 3.78-3.88 (m, 1H), 3.99 (s, 3H), 4.49-4.62 (m, 1H), 4.94 (dd, *J* = 1.0, 10.2 Hz, 1H), 5.02 (dd, *J* = 1.4, 18.8 Hz, 1H), 5.80-5.92 (m, 1H), 5.99 (bs, 1H), 7.44 (d, *J* = 8.9 Hz, 1H), 7.65-7.76 (m, 4H), 8.07 (bs, 1H), 8.27 (bd, *J* = 7.3 Hz, 2H), 8.57 (d, *J* = 8.9 Hz, 1H), 9.03 (bs, 1H). ES^+ MS *m/z* 516.2 $(M+H)^+$

Dipeptide **27** Peptide coupling between the enantiomerically enriched (>98% ee)[37b] ethyl ester of vinyl ACCA (1R, 2S)-**9** and the P2 building block **15f**, under the usual peptide coupling conditions, led to the isolation of the corresponding *N*-Boc-protected dipeptide **27**. ^{1}H NMR (DMSO-d_6) mixture of rotamers in (~1:15 ratio): δ (major rotamer) 1.16 (t, *J* = 7.3 Hz, 3H), 1.28-1.30 (m, 1H), 1.35 (s, 9H), 1.65-1.69 (m, 1H), 2.13-2.18 (m, 1H), 2.25-2.34 (m, 1H), 2.57-

2.65 (m, 1H), 3.80 (br, 2H), 3.93 (s, 3H), 4.00-4.09 (q, *J* = 7.3 Hz, 2H), 4.27-4.33 (m, 1H), 5.11 (d, *J* = 8.9 Hz, 1H), 5.27 (d, *J* = 16.2 Hz, 1H), 5.56 (br, 1H), 5.63-5.73 (m, 1H), 7.18 (dd, *J* = 2.3 , 8.9 Hz, 1H), 7.39 (d, *J* = 2.3 Hz, 1H), 7.46 (s, 1H), 7.49-7.56 (m, 3H), 7.94 (d, *J* = 8.9, 1H), 8.27 (d, *J* = 7.0 Hz, 2H), 8.74 (s, 1H). ES^+ MS *m/z* 602.3 $(M+H)^+$; ES^- MS *m/z* 600.2 $(M-H)^-$.

Diene **28a** ^1H NMR ($CDCl_3$) δ 1.40 (s, 9H), 1.25-1.45 (m, 6H), 1.60-1.69 (m, 1H), 1.70-1.80 (m, 1H), 1.95-2.06 (m, 2H), 2.35-2.47 (m, 1H), 2.47-2.55 (m, 1H), 2.55-2.65 (m, 1H), 2.92-3.00 (m, 1H), 3.72 (s, 3H), 3.96 (s, 3H), 3.94-3.98 (m, 1H), 4.31 (bd, *J*= 12 Hz, 1H), 4.45-4.52 (m, 1H), 4.62 (dd, *J*= 12, 7 Hz, 1H), 4.89-4.93 (m, 1H), 4.92 (d, *J*= 10 Hz, 1H), 4.97 (dd, *J*= 2, 17 Hz, 1H), 5.12-5.19 (m, 2H), 5.40 (bs, 1H), 5.63-5.85 (m, 2H), 7.04-7.11 (m, 2H), 7.39 (bd, *J*= 8Hz, 1H), 7.43-7.54 (m, 5H), 8.02-8.09 (m, 3H). ES^+ MS *m/z* 729.5 $(M+H)^+$; ES^- MS *m/z* 727.3 $(M-H)^-$.

Diene **28b** ^1H NMR ($CDCl_3$), δ 1.39 (s, 9H), 1.22-1.41 (m, 6H), 1.55-1.62 (m, 1H), 1.68-1.75 (m, 1H), 2.00-2.05 (m, 2H), 2.44-2.61 (m, 3H), 2.84-2.92 (m, 1H), 3.74 (s, 3H), 3.96 (s, 3H), 3.96-4.00-(m, 1H), 4.30-4.35 (m, 1H), 4.43-4.50 (m, 1H), 4.61 (dd, *J*= 8 Hz, 1H), 4.80-4.85 (m, 1H), 4.91-5.01- (m, 2H), 5.12-5.18 (m, 2H), 5.41 (bs, 1H), 5.64-5.83 (m, 2H), 7.04-7.11 (m, 3H), 7.43-7.55 (m, 5H), 8.01-8.07 (m, 3H). ES^+ MS *m/z* 729.5 $(M+H)^+$; ES^- MS *m/z* 727.3 $(M-H)^-$.

Diene **29a** ^1H NMR ($CDCl_3$): δ 1.38 (s, 9H), 1.48-1.68 (m, 5H), 1.75-1.85 (m, 1H), 2.05-2.13 (m, 1H), 2.33-2.42 (m, 1H), 2.90-3.00 (m, 2H), 3.15-3.18 (m, 2H), 3.75 (s, 3H), 3.94 (s, 3H), ~3.95 (bs, 1H), 4.26 (d, *J* = 11 Hz, 1H), 4.45-4.52 (m, 1H), 4.70-4.75 (m, 1H), 4.91-5.04 (m, 3H), 5.11-5.20 (m, 3H), 5.28-5.35 (m, 1H), 5.71-5.83 (m, 2H), 6.65 (d, *J* = 5.4 Hz, 1H), 7.12 (dd,

J = 2.5 , 9.2 Hz, 1H), 7.36 (d, *J* = 2.2 Hz, 1H), 7.56 (d, *J* = 6.7 Hz, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 8.67 (d, *J* = 5.1 Hz, 1H). ES^+ MS *m/z* 671.3 $(M+H)^+$; ES^- MS *m/z* 669.3 $(M-H)^-$.

Diene **29b** ^1H NMR ($CDCl_3$): δ 1.38 (s, 9H), 1.49-1.64 (m, 5H), 1.75-1.82 (m, 1H), 2.05-2.12 (m, 2H), 2.35-2.45 (m, 1H), 2.92-2.98 (m, 1H), 3.11-3.19 (m, 2H), 3.77 (s, 3H), 3.94 (s, 3H), ~3.98 (bs, 1H), 4.28 (d, *J* = 9.5 Hz, 1H), 4.45-4.52 (m, 1H), 4.70-4.75 (m, 1H), 4.82-4.90 (m, 1H), 4.96-5.05 (m, 2H), 5.12-5.17 (m, 3H), 5.28-5.35 (m, 1H), 5.71-5.83 (m, 2H), 6.64 (d, *J* = 5.2 Hz, 1H), 7.12 (dd, *J* = 2.5 , 9.2 Hz, 1H), 7.36 (m, 2H), 8.03 (d, *J* = 9.0 Hz, 1H), 8.67 (d, *J* = 5.3 Hz, 1H). ES^+ MS *m/z* 671.3 $(M+H)^+$; ES^- MS *m/z* 669.3 $(M-H)^-$.

Ligand **29c** ^1H NMR (DMSO-d_6): δ 1.17 (s, 9H), 1.32-1.64 (m, 4H), 1.96-2.05 (m, 2H), 2.25-2.35 (m, 1H), 2.50-2.54 (m, 2H), 2.85 (dd, *J* = 4.8, 13.6 Hz, 1H), 3.15 (d, *J* = 7.0 Hz, 2H), 3.90 (bd, *J* = 10.1 Hz, 1H), 3.97 (s, 3H), 4.08-4.15 (m, 1H), 4.41-4.46 (m, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 4.65 (dd, *J* = 8.3Hz, 1H), 4.94-5.14 (m, 4H), 5.63 (bs, 1H), 5.69-5.83 (m, 2H), 7.12 (d, *J* = 6.8 Hz, 1H), 7.32 (d, *J* = 8.9 Hz, 1H), 7.42 (bs, 2H), 8.26 (d, *J* = 8.3 Hz, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 9.02 (d, *J* = 5.7 Hz, 1H). FAB HRMS *m/z* found: 657.295780 $(M+H)^+$; calculated for $C_{33}H_{45}N_4O_8S$: 657.295812

Ligand **29d** ^1H NMR (DMSO-d_6): δ 1.19 (s, 9H), 1.40-1.65 (m, 4H), 1.95-2.05 (m, 2H), 2.25-2.35 (m, 2H), 2.65-2.82 (m, 2H), 3.15-3.25 (m, 2H), 3.85-3.95 (m, 1H), 3.97 (s, 3H), 4.05-4.15 (m, 1H), 4.37-4.42 (dd, *J* = 6.8 , 13.5 Hz, 1H), 4.5 (d, *J* = 11 Hz, 1H), 4.66 (dd, *J* = 8.8 Hz, 1H), 4.95-5.20 (m, 4H), 5.61 (br, 1H), 5.69-5.80 (m, 2H), 7.13 (d, *J* = 7.7 Hz, 1H), 7.28-7.35 (m, 1H), 7.35-7.45 (m, 1H), 7.41 (s, 1H), 8.27 (d, *J* = 6.4 Hz, 1H), 8.29 (d, *J* = 9.0 Hz, 1H), 8.99 (br, 1H),

12.8 (br, 1H). FAB HRMS *m/z* found: 657.295780 $(M+H)^+$; calculated for $C_{33}H_{45}N_4O_8S$: 657.295812

Diene **30c** ^{1}H NMR ($CDCl_3$) rotamers in ~10:1 ratio): δ (major rotamer) 1.41 (s, 9H), 1.48-1.60 (m, 5H), 1.60-1.72 (m, 4H), 2.05-2.20 (m, 4H), 2.35-2.45 (m, 1H), 2.95-3.55 (m, 1H), 3.67 (s, 3H), 3.73-3.83 (m, 1H), 3.92-3.98 (m, 1H), 3.96 (s, 3H), 4.25-4.32 (m, 1H) 4.45-4.55 (m, 1H), 4.78-4.85 (m, 1H), 4.93-5.06 (m, 4H), 5.12-5.30 (m, 1H), 5.43 (bs, 1H), 5.73-5.85 (m, 2H), 7.03-7.12 (m, 2H), 7.43-7.54 (m, 6H), 8.01-8.07 (m, 3H). ES^+ MS *m/z* 741.4 $(M+H)^+$; ES^- MS *m/z* 739.3 $(M-H)^-$

Dienes **31** ^{1}H NMR ($CDCl_3$, mixture of rotamers in ~1:10 ratio): δ (major rotamer) 1.22 (t, *J* = 7.3 Hz, 3H), 1.30-1.50 (m, 7H), 1.41 (s, 9H), 1.56-1.64 (m, 1H), 1.72-1.81 (m, 1H), 1.85-1.91 (m, 1H), 1.98- 2.05 (m, 2H), 2.15 (dd, *J* = 8.5 , 17.4 Hz, 1H), 2.36-2.45 (m, 1H), 2.93-3.02 (m, 1H), 3.91-3.95 (m, 1H), 3.96 (s, 3H), 4.16 (q, *J* = 7.3 Hz, 2H), 4.30 (bd, *J* = 10.5 Hz, 1H), 4.41-4.48 (m, 1H), 4.82-4.87 (m, 1H), 4.88-5.00 (m, 2H), 5.07-5.15 (m, 2H), 5.27-5.34 (m, 1H), 5.44 (bs, 1H), 5.70-5.80 (m, 2H), 7.05-7.13 (m, 2H), 7.43-7.58 (m, 5H), 8.00-8.09 (m, 3H). ES^+ MS *m/z* 755.3 $(M+H)^+$; ES^- MS *m/z* 753.3 $(M-H)^-$.

Macrocyclic inhibitor **32b** ^{1}H NMR (DMSO-d_6) δ 1.10 (s, 9H), 1.16-1.26 (m, 1H), 1.42-1.60 (m, 2H), 1.68-1.82 (m, 2H), 2.08-2.15 (m, 1H), 2.43- ~2.5 (m, 3H), 2.67 (dd, *J* = 10.5 , 13.4 Hz, 1H), 3.00 (dd, *J* = 7.0 , 14.1 Hz, 1H), 3.10 (dd, *J* = 8.0 , 14.2 Hz, 1H), 3.87 (bd, *J* = 8.9 Hz, 1H), 3.97 (s, 3H), 3.90-4.03 (m, 1H), 4.28 (ddd, *J* = 3.2, 8.6 , 10.5 Hz, 1H), 4.60 (bd, *J* = 12.1 Hz, 1H), 4.75 (dd, *J* = 8.0 Hz, 1H), 5.18-5.25 (m, 1H), 5.48-5.53 (m, 1H), 5.7 (bs, 1H), 7.13 (d, *J* =

5.4 Hz, 1H), 7.32 (dd, *J* = 2.7 , 9.2 Hz, 1H), 7.40 (d, *J* = 2.7 Hz, 1H), 7.45 (d, *J* = 6.7 Hz, 1H), 8.29 (d, *J* = 9.2 Hz, 1H), 8.51 (d, *J* = 8.9 Hz, 1H), 9.04 (d, *J* = 6.7 Hz, 1H). FAB HRMS *m/z* found $(M+H)^+$ 629.264410; calculated for $C_{31}H_{41}N_4O_8S_1$ 629.264512

Methyl ester **39a** After RCM of diene **28a** under the usual reaction conditions, the mixture of methyl ester olefins obtained (**38**, Z:E ration of 1:1) were reduced under catalytic hydrogenation conditions to give the saturated ester **39a**. ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H), 1.23-1.41 (m, 7H), 1.55-1.72 (m, 4H), 1.80-2.10 (m, 3H), 2.47-2.57 (m, 1H), 2.92-3.04 (m, 1H), 3.72 (s, 3H), 3.72-3.75 (m, 1H), 4.09 (s, 3H), 4.14-4.25 (m, 1H), 4.33-4.41 (m, 1H), 4.49-4.62 (m, 2H), 5.00 (bs, 1H), 5.17 (d, *J*= 8 Hz, 1H), 5.61-5.74 (m, 1H), 6.80-7.20 (m, 2H), 7.23 (bs, 1H), 7.57 (bs, 4H), 8.11 (d, *J*= 9 Hz, 1H), 8.26 (bs, 2H), 8.97 (s, 1H). ES^+ MS *m/z* 703 $(M+H)^+$; ES^- MS *m/z* 701 $(M-H)^-$.

Methyl ester **41a** After RCM of diene **28b** under the usual reaction conditions, the mixture of methyl ester olefins obtained (**40**, Z:E ration of 1:14) were reduced under catalytic hydrogenation conditions to give the saturated ester **41a**. ^{1}H NMR ($CDCl_3$), δ 1.23-1.38 (m, 4H), 1.40 (s, 9H), 1.43-1.48 (m, 2H), 1.53-1.62 (m, 1H), 1.90-1.99 (m, 1H), 2.00-2.20 (m, 2H), 2.3-2.43 (m, 2H), 2.63-2.72 (m, 1H), 3.06-3.15 (m, 1H), 3.76 (s, 3H), 3.87-3.94 (m, 1H), 3.95 (s, 3H), 4.28 (bd, *J* = 10.0 Hz, 1H), 4.52-4.64 (m, 2H), 5.02 (dd, *J* = 5.1, 7.3 Hz, 1H), 5.23-5.53 (m, 4H), 7.07 (s, 1H), 7.10 (dd, *J* = 2.6, 8.9 Hz, 1H), 7.44 (d, *J* = 2.4 Hz, 1H), 7.44-7.55 (m, 3H), 7.68 (d, *J* = 7.9 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 2H). ES^+ MS *m/z* 703.4 $(M+H)^+$; ES^- MS *m/z* 701.4 $(M-H)^-$

Macrocyclic inhibitor **41b** The saturated macrocyclic methyl ester **41a** was saponified and purified by C_{18} reversed phase HPLC to afford compound **41b** as a white solid (>99% homogeneity by HPLC). ^{1}H NMR (DMSO-d_6), δ 1.01-1.55 (m, 13H), 1.53 (s, 9H), 1.60-1.65 (m, 1H), 1.70-1.90 (m, 2H), 2.50-2.55 (m, 2H), 3.93-3.96 (m, 1H), 3.96 (s, 3H), 4.12 (m, 1H), 4.42-4.47 (m, 1H), 4.60 (bd, *J*= 13 Hz, 1H), 4.78 (t, *J*= 8 Hz, 1H), 5.77 (br s, 1H), 7.05 (d, *J*= 7 Hz, 1H), 7.23 (bs, 1H), 7.51 (bs, 1H), 7.59-7.70 (m, 4H), 8.15-8.30 (m, 3H), 8.33 (d, J= 9 Hz, 1H). FAB HRMS *m/z* found: 689.355260 $(M+H)^+$; calculated for $C_{38}H_{49}N_4O_8$: 689.355040.

Ligand **42** ^{1}H NMR (DMSO-d_6, mixture of rotamers in ~1:10 ratio): δ (major rotamer) 0.86 (2t, *J* = 6.6 , 7.2 Hz, 6H), 1.22 (s, 9H), 1.30-1.40 (m, 9H), 1.40-1.50 (m, 1H), 1.50-1.60 (m, 1H), 1.60-1.69 (m, 1H), 2.20-2.30 (m, 1H), 2.48-2.60 (m, 1H), 3.87 (d, *J* = 9.7 Hz, 1H), 3.96 (s, 3H), 4.14-4.18 (m, 2H), 4.49 (brd, *J* = 11.4 Hz, 1H), 4.61 (dd, *J* = 8.3 Hz, 1H), 5.72 (brs, 1H), 7.07 (d, *J* = 7.0 Hz, 1H), 7.20 (br, 1H), 7.48 (s, 1H), 7.63 (br, 4H), 8.12 (d, *J* = 7.7 Hz, 1H), 8.20 (br, 3H). FAB HRMS *m/z* found $(M+H)^+$ 691.371000; calculated for $C_{38}H_{51}N_4O_8$ 691.370690

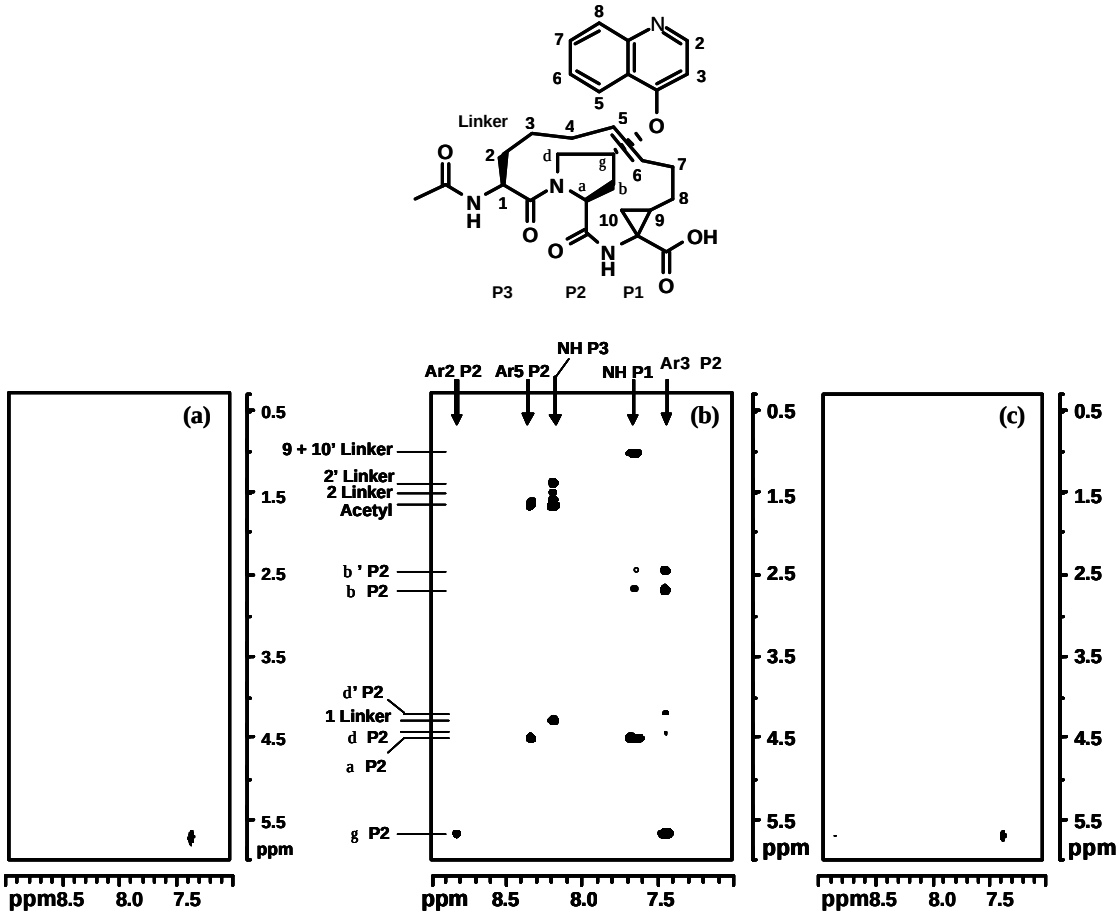
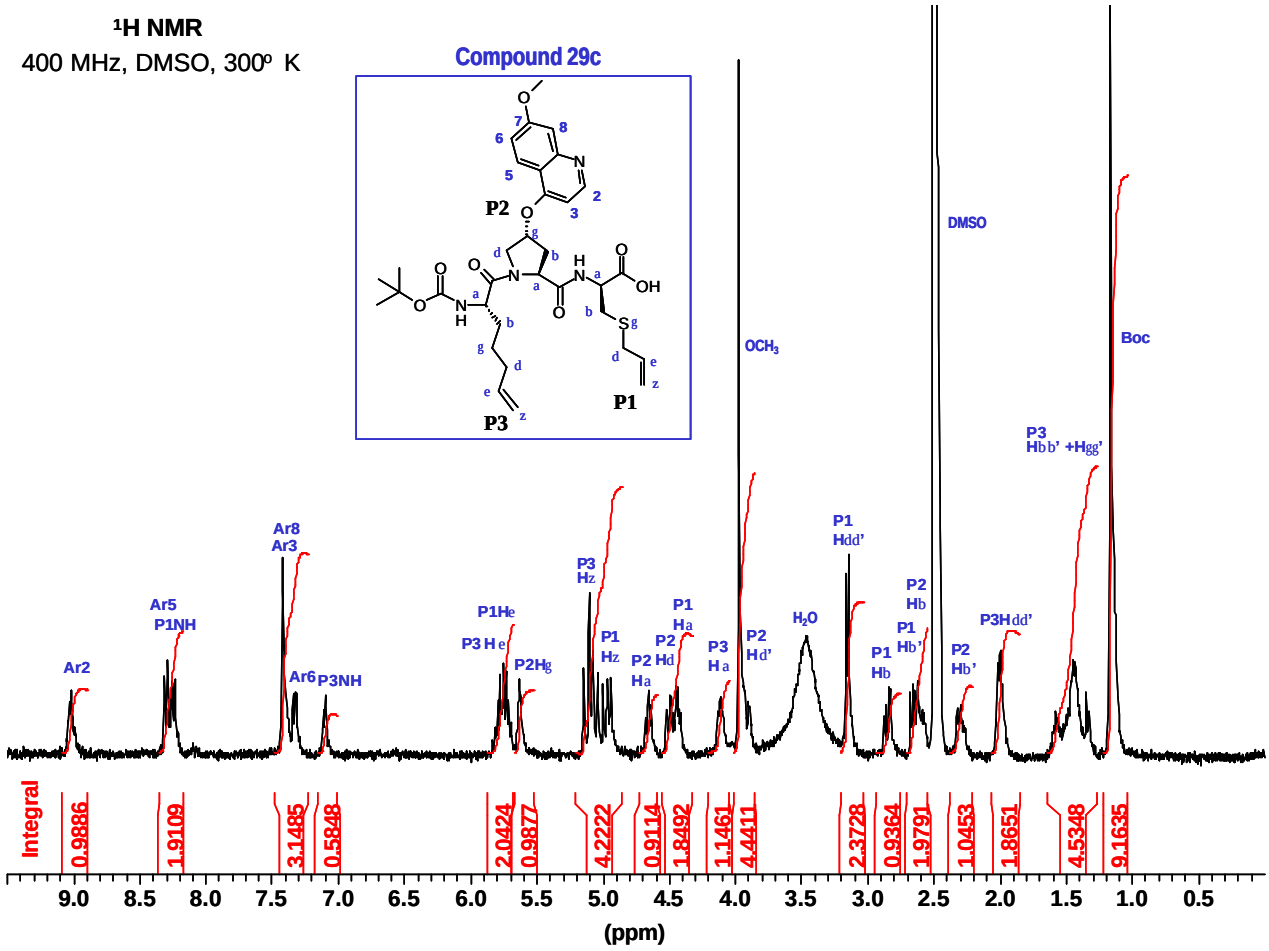


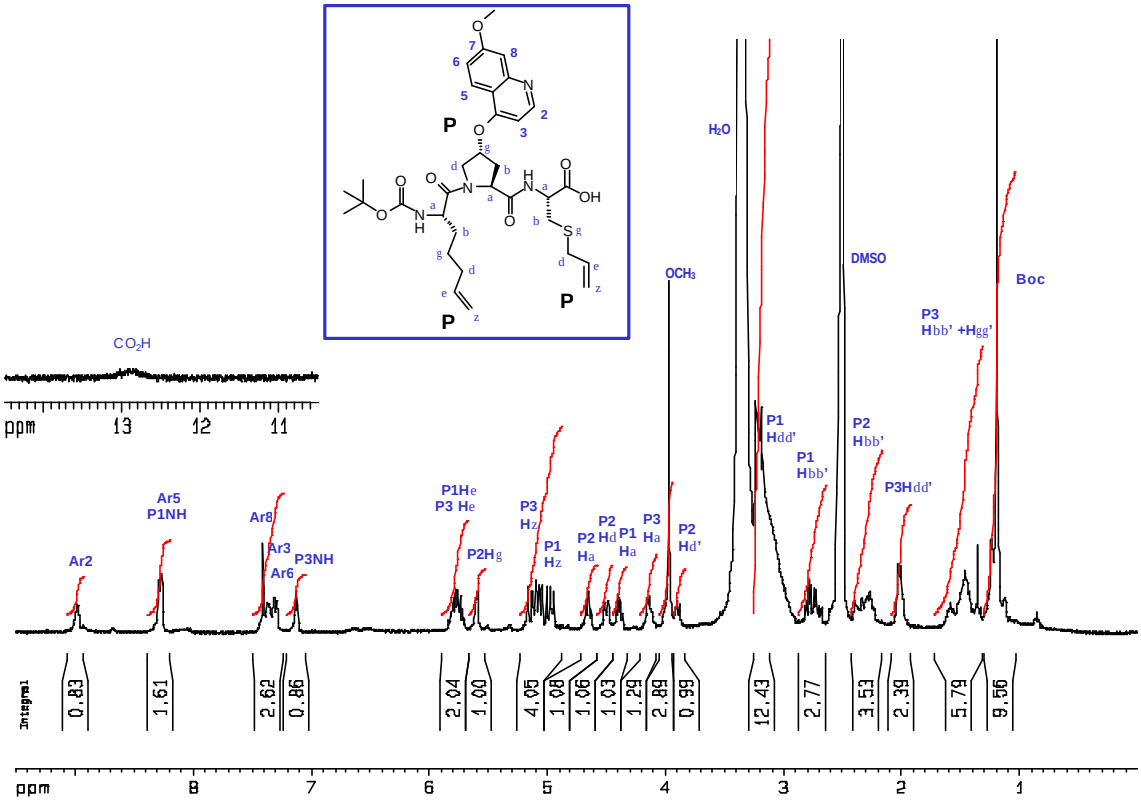
Figure 1. Section of the 2D ^{1}H NMR spectra of inhibitor **33c**: **a)** NOESY spectrum (200 ms mixing time) of free **33c**; **b)** NOESY spectrum (200 ms mixing time) of **33c** in the presence of NS3 protease BK poly Lys (**33c**:NS3 ratio of 22:1);[14] **c)** NOESY spectrum (200 ms mixing time) of **33c** in the presence of NS3 protease and hexapeptide **43** (**33c**:NS3:**43** ratio of 22:1:2.5). The resonance assignments for all three panels are indicated in panel **(b)**.


¹H NMR
400 MHz, DMSO, 300º K
Compound 29c
P2
P1
P3
Ar2
Ar5
P1NH
Ar8
Ar3
Ar6
P3NH
P1He
P3 He
P2Hg
P3
Hz
P1
Hz
P2
Ha
P2
Hd
P1
Ha
P3
Ha
OCH3
P2
Hd'
H2O
P1
Hdd'
P1
Hb
P2
Hb
P1
Hb'
DMSO
P2
Hb'
P3Hdd'
P3
Hbb' +Hgg'
Boc
Integral
0.9886
1.9109
3.1485
0.5848
2.0424
0.9877
4.2222
0.9114
1.8492
1.1461
4.4411
2.3728
0.9364
1.9791
1.0453
1.8651
4.5348
9.1635
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
(ppm)

^{1}H NMR

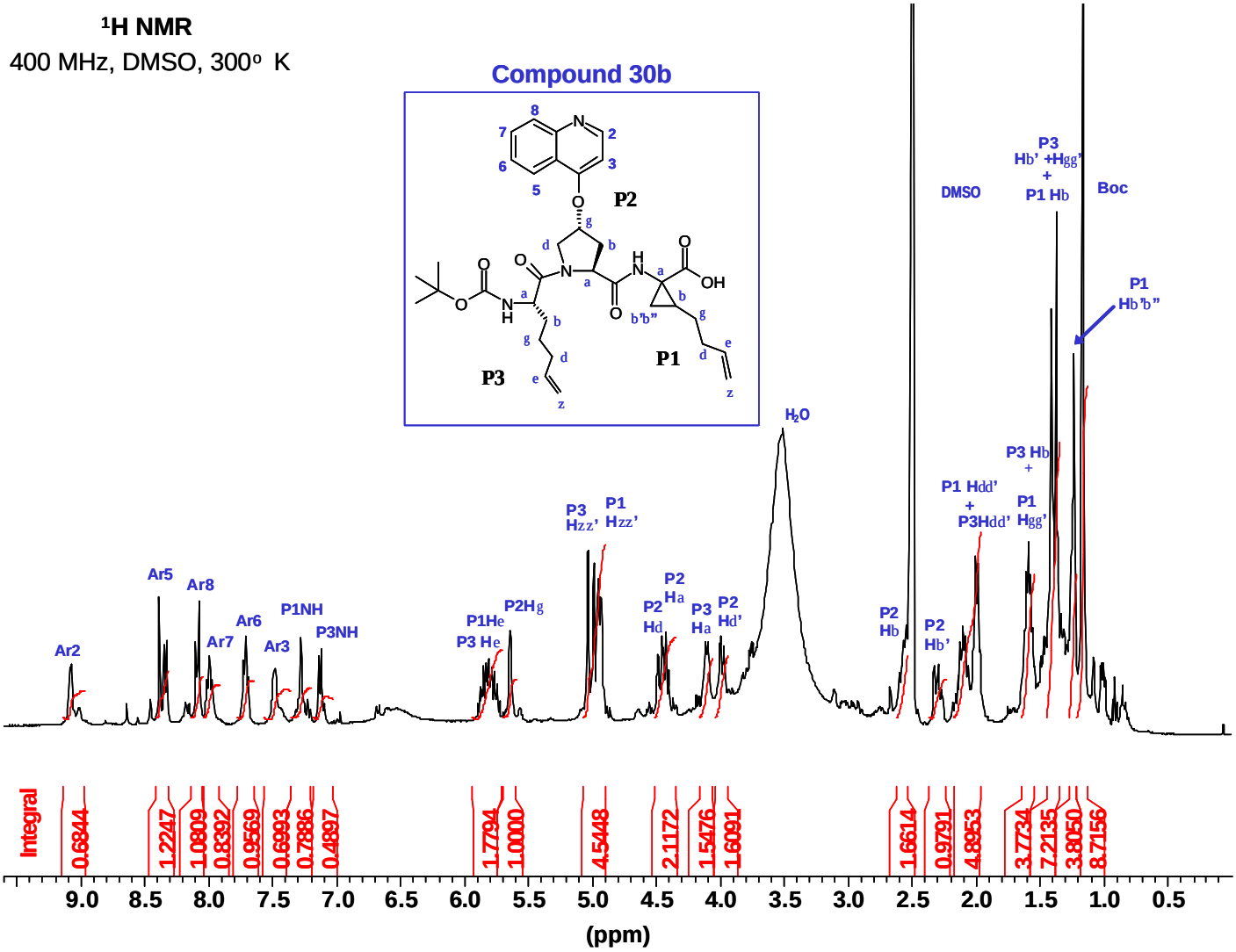
400 MHz, DMSO, 300^o K

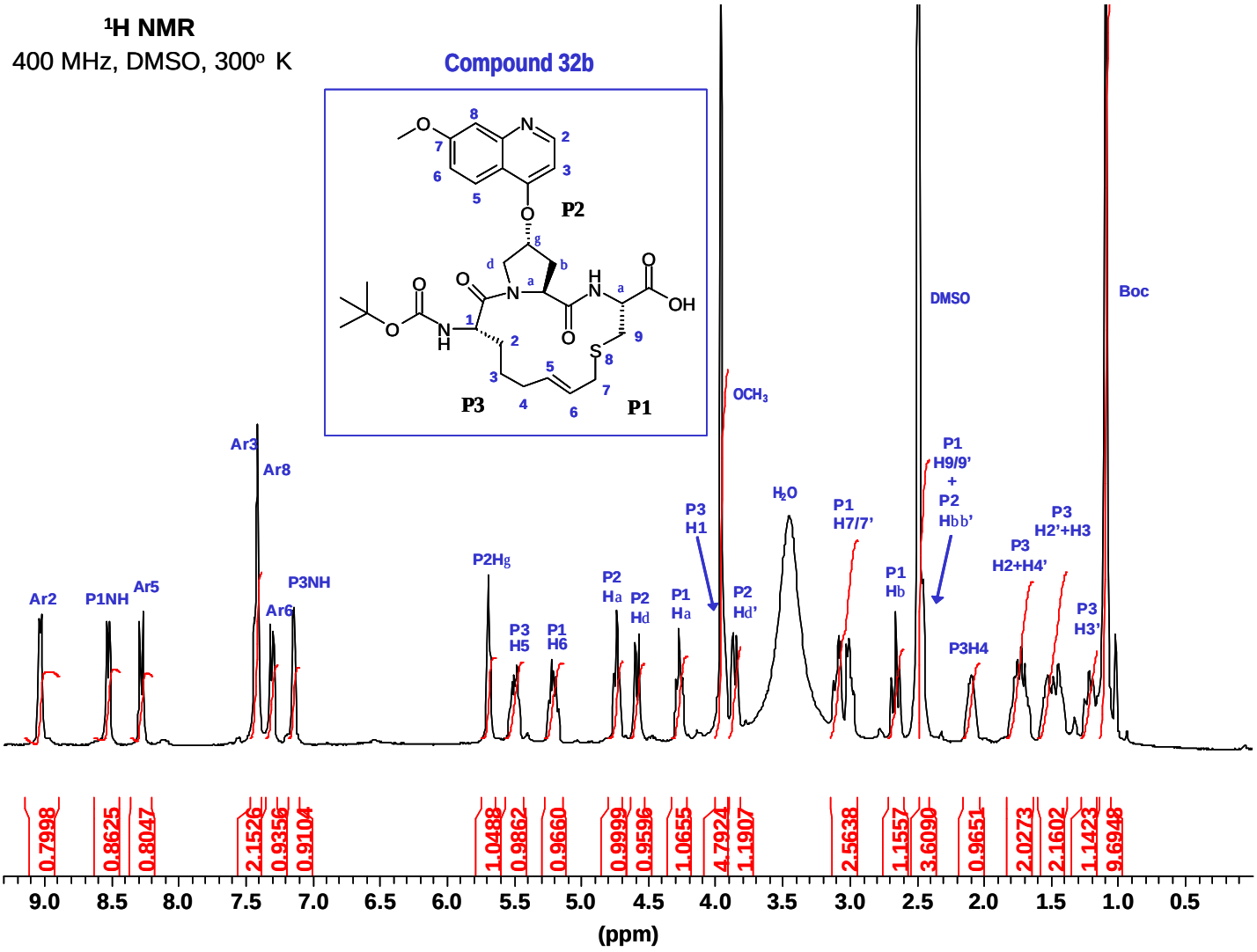
Compound 29d



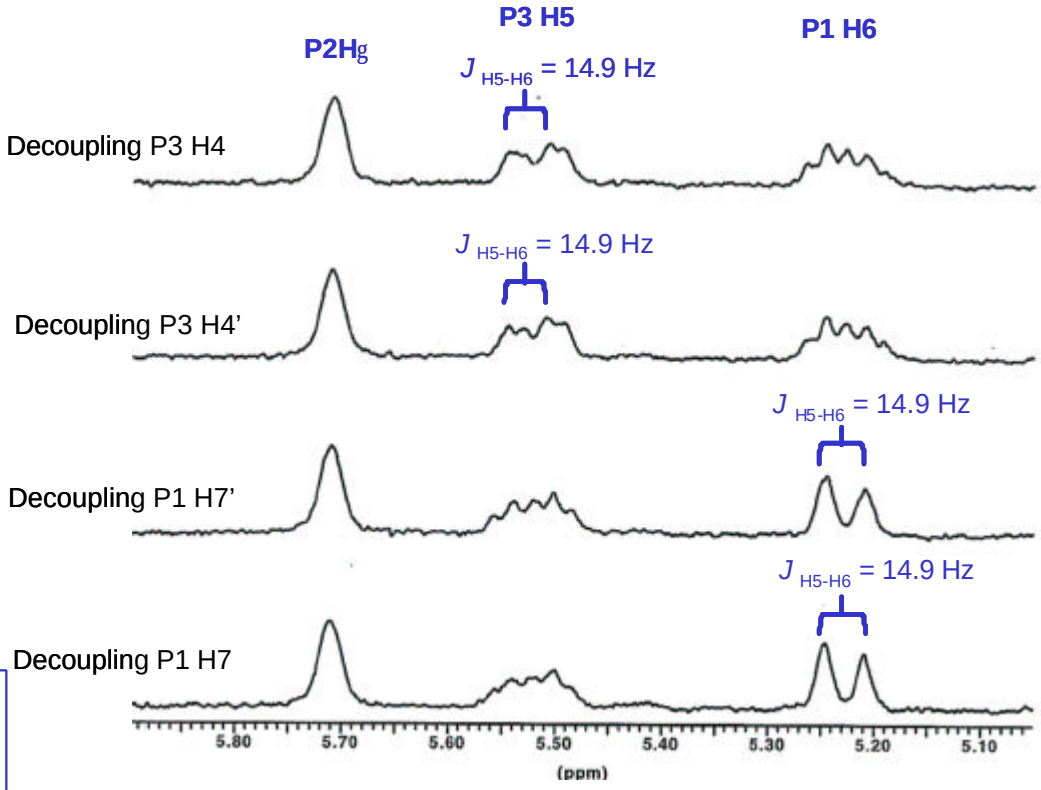
^{1}H NMR

400 MHz, DMSO, 300^o K

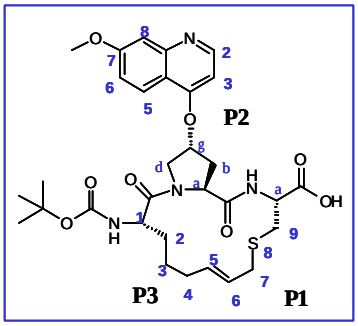



1H NMR
400 MHz, DMSO, 300º K
Compound 32b
P2
P3
P1
Ar2
P1NH
Ar5
Ar3
Ar8
Ar6
P3NH
P2Hg
P3 H5
P1 H6
P2 Ha
P2 Hd
P1 Ha
P3 H1
OCH3
P2 Hd'
H2O
P1 H7/7'
P1 Hb
DMSO
P1 H9/9' + P2 Hbb'
P3H4
P3 H2+H4'
P3 H2'+H3
P3 H3'
Boc
0.7998
0.8625
0.8047
2.1526
0.9356
0.9104
1.0488
0.9862
0.9660
0.9999
0.9596
1.0655
4.7924
1.1907
2.5638
1.1557
3.6090
0.9651
2.0273
2.1602
1.1423
9.6948
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

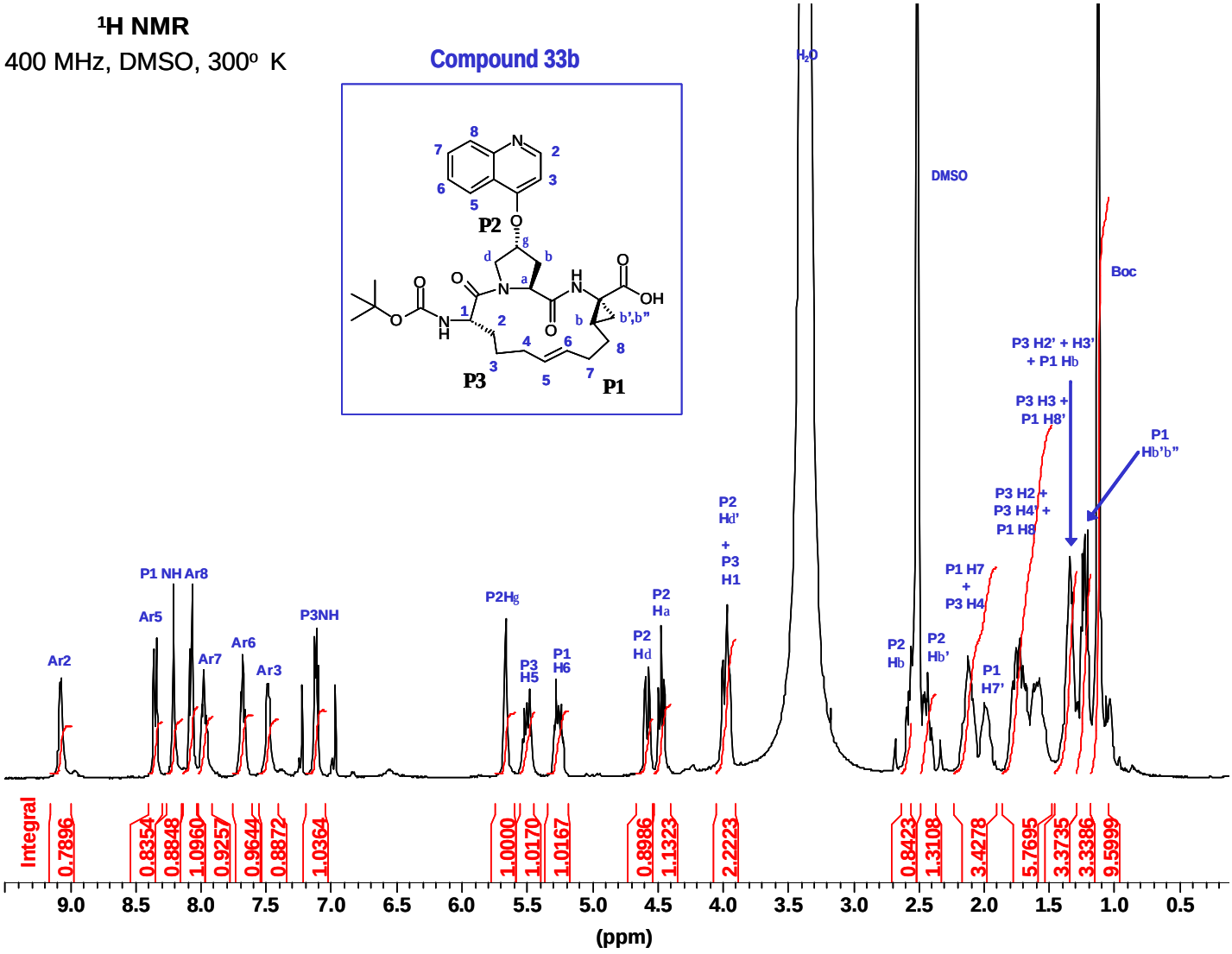
^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300^o K



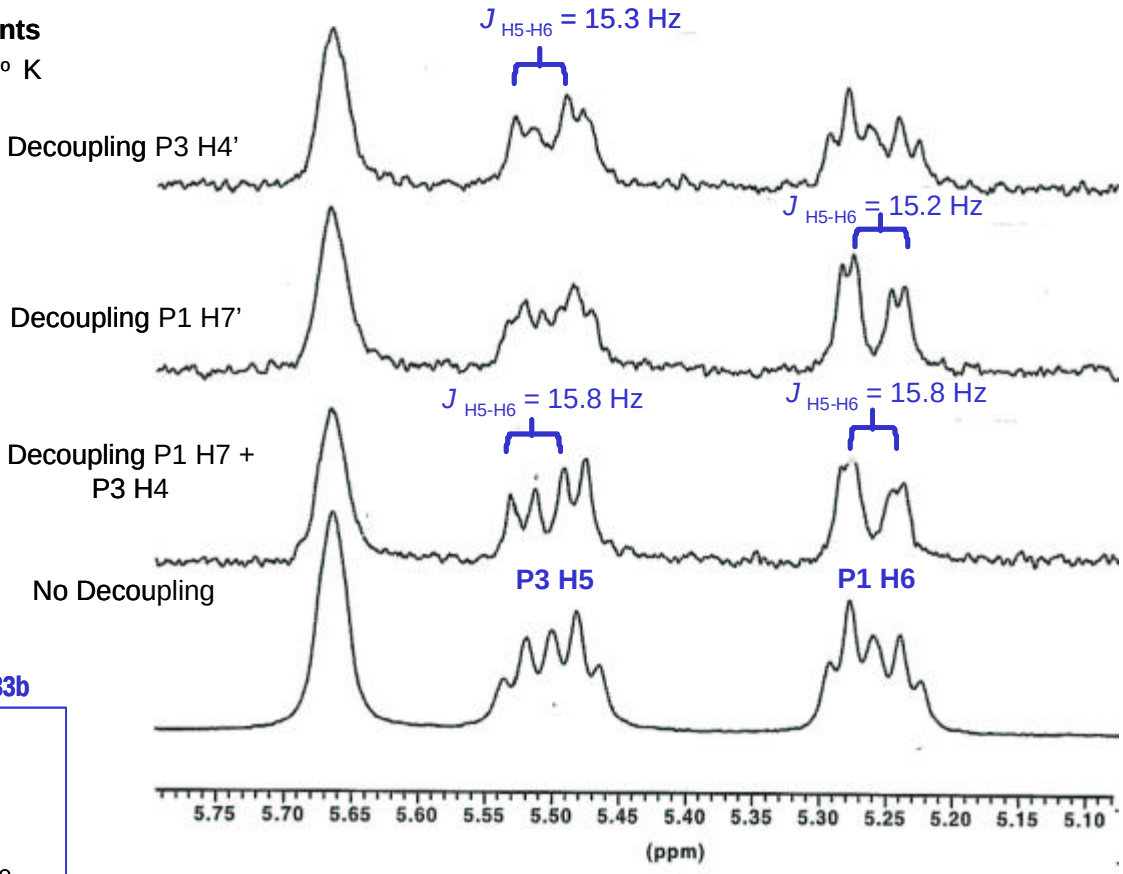
Compound 32b



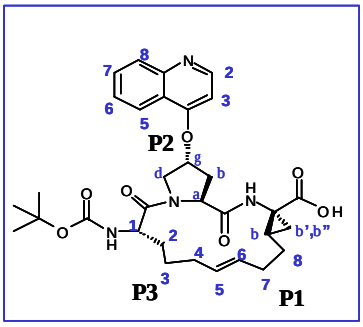
J_{H5-H6} = 14.9 Hz → ***trans***


1H NMR
400 MHz, DMSO, 300° K
Compound 33b
P2
P3
P1
H2O
DMSO
Boc
P3 H2' + H3' + P1 Hb
P3 H3 + P1 H8'
P1 Hb'b"
P3 H2 + P3 H4' + P1 H8
P1 H7 + P3 H4
P1 H7'
P2 Hb
P2 Hb'
P2 Hd' + P3 H1
P2 Ha
P2 Hd
P3 H5
P1 H6
P2Hg
P3NH
Ar2
Ar5
P1 NH Ar8
Ar7
Ar6
Ar3
Integral
0.7896
0.8354
0.8848
1.0960
0.9257
0.9644
0.8872
1.0364
1.0000
1.0170
1.0167
0.8986
1.1323
2.2223
0.8423
1.3108
3.4278
5.7695
3.3735
3.3386
9.5999
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

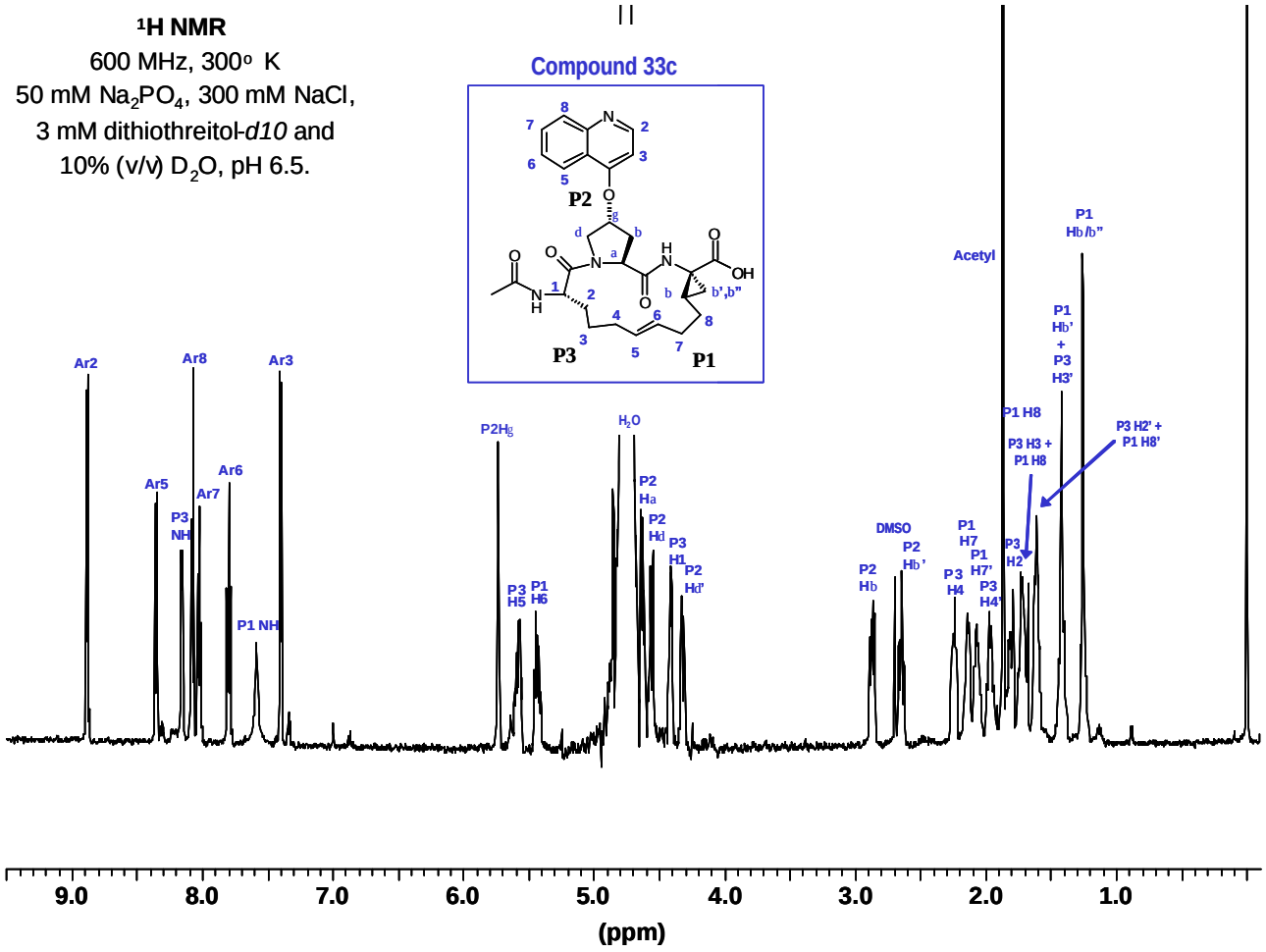
^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300° K



Compound 33b

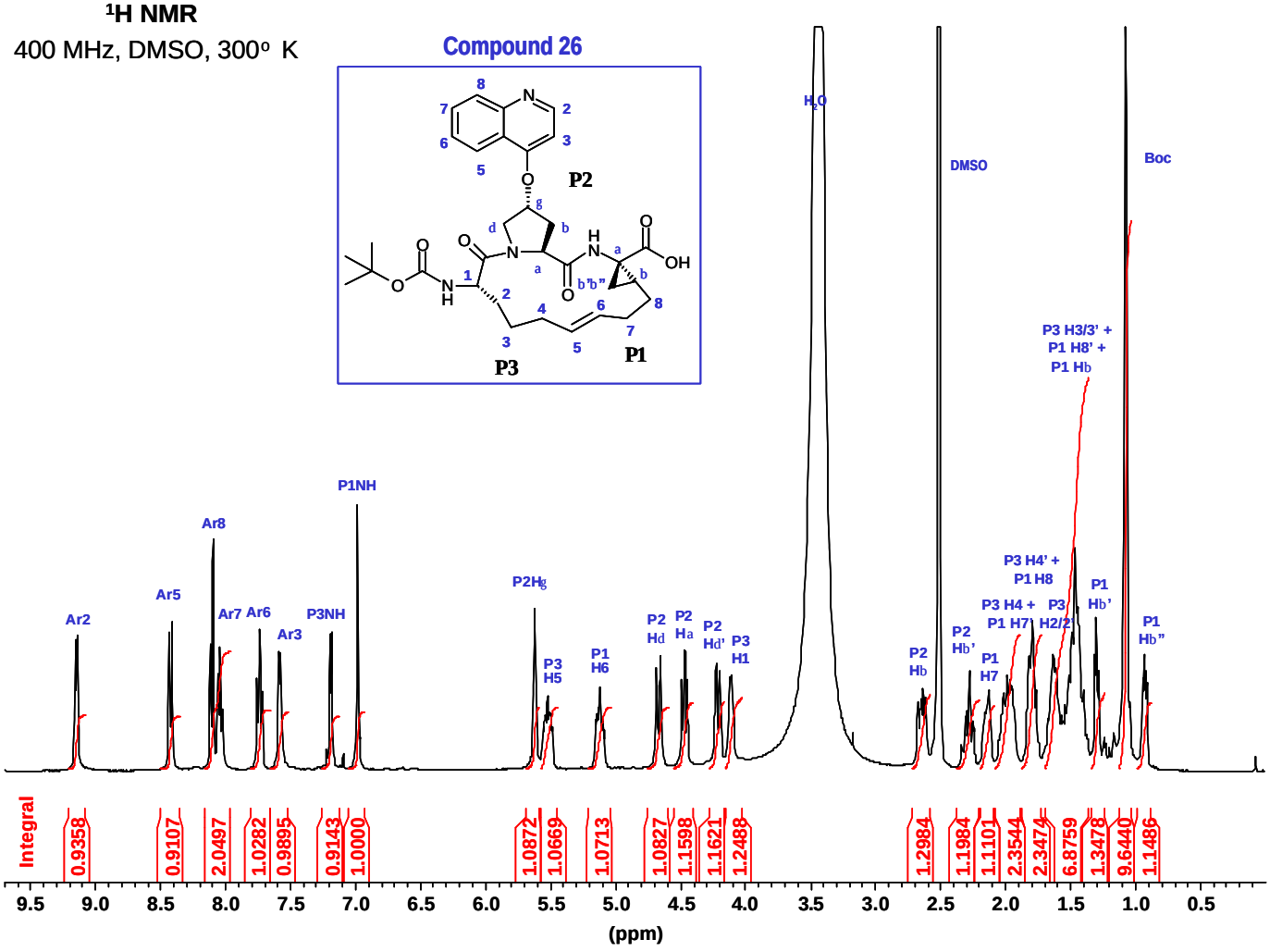


J_{H5-H6} = 15.5 Hz → ***trans***

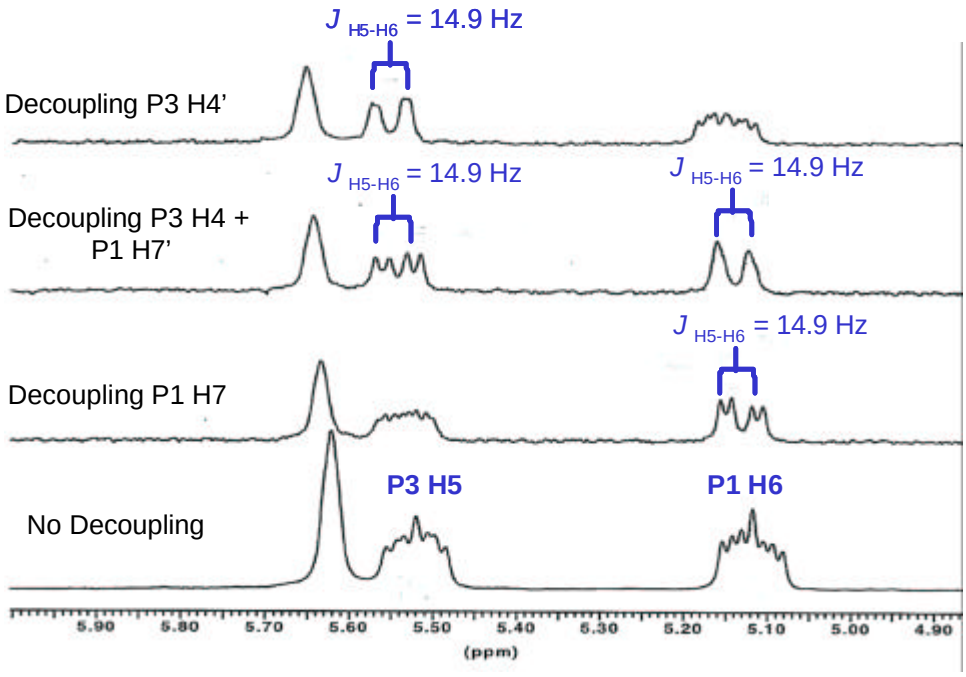

¹H NMR
600 MHz, 300° K
50 mM Na2PO4, 300 mM NaCl,
3 mM dithiothreitol-d10 and
10% (v/v) D2O, pH 6.5.
Compound 33c
P2
P3
P1
Ar2
Ar8
Ar3
Ar5
Ar7
Ar6
P3 NH
P1 NH
P2Hg
P3 H5
P1 H6
H2O
P2 Ha
P2 Hd'
P3 H1
DMSO
P2 Hb
P2 Hb'
P3 H4
P1 H7
P1 H7'
P3 H4'
P3 H2
P1 H8
P3 H3 + P1 H8
P3 H2' + P1 H8'
Acetyl
P1 Hb/b"
P1 Hb' + P3 H3'
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
(ppm)

^{1}H NMR

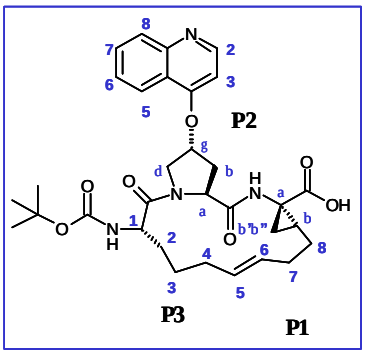
400 MHz, DMSO, 300^o K



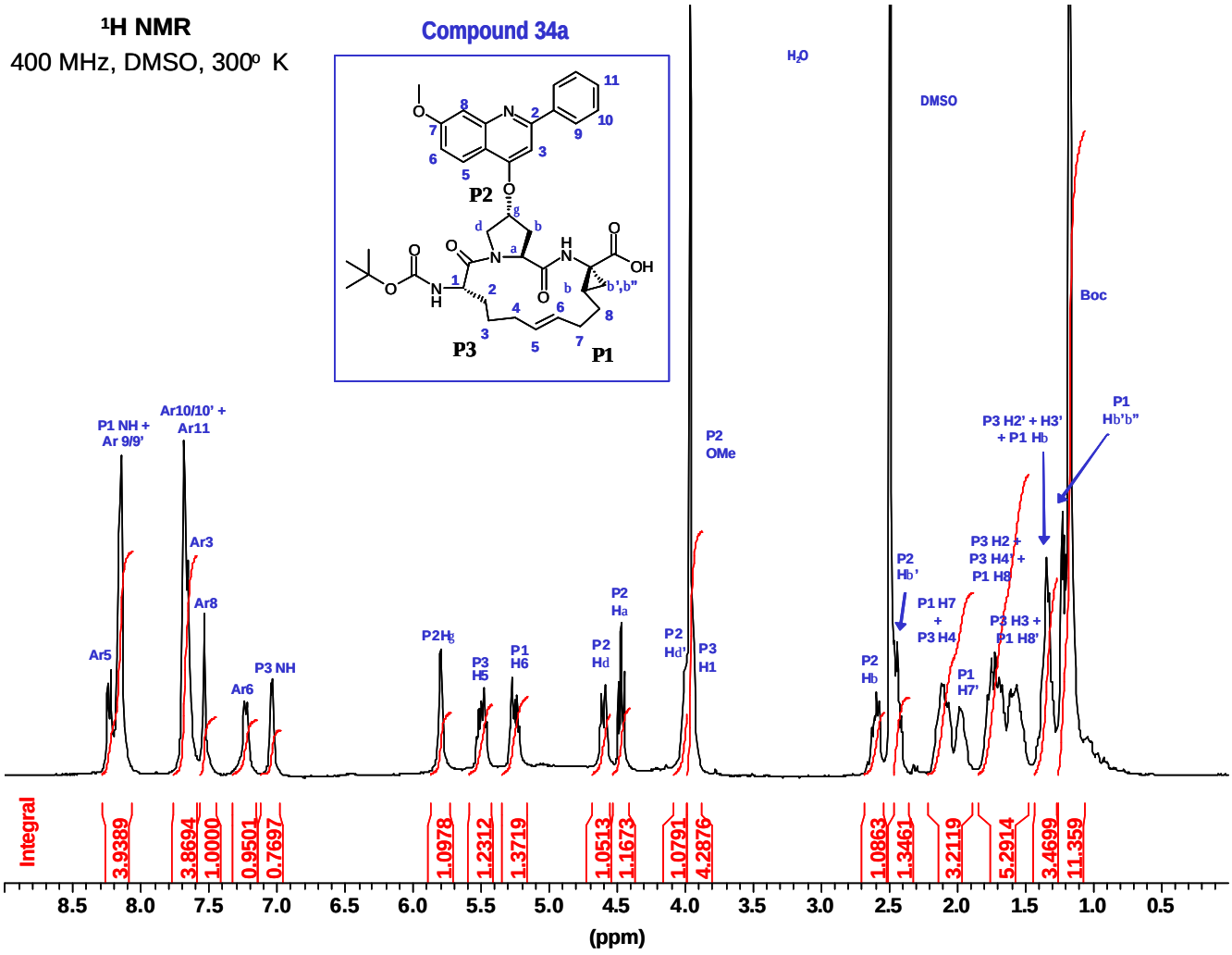
^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300^o K



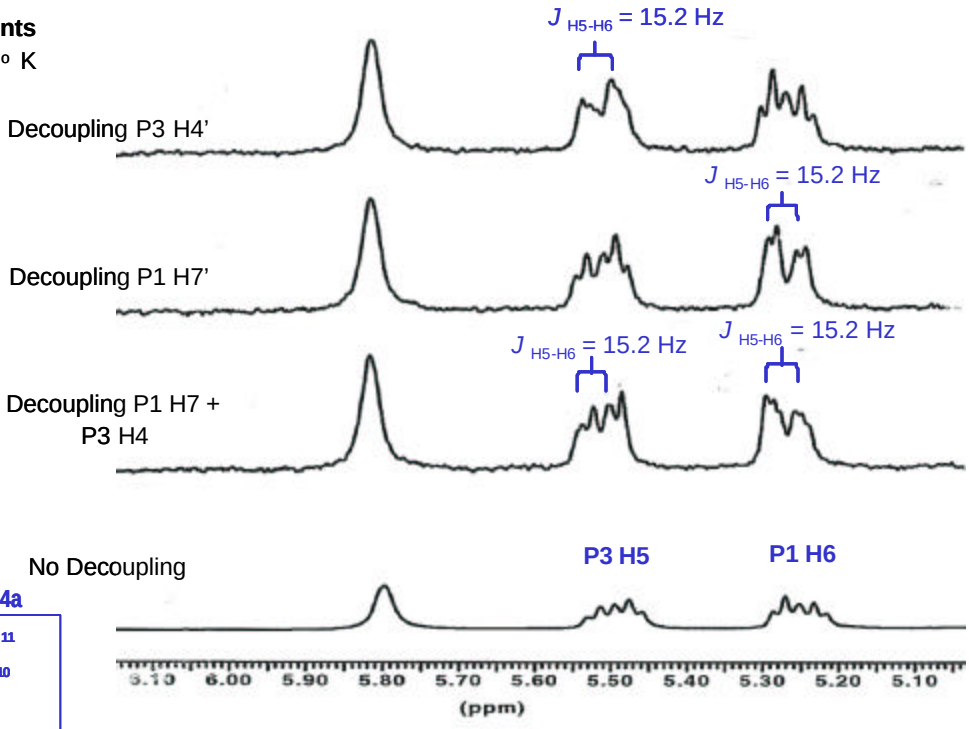
Compound 26



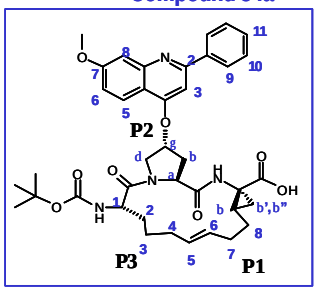
J_{H5-H6} = 14.9 Hz → ***trans***


¹H NMR
400 MHz, DMSO, 300º K
Compound 34a
P2
P3
P1
H2O
DMSO
Boc
P1 NH + Ar 9/9'
Ar10/10' + Ar11
Ar3
Ar8
Ar5
Ar6
P3 NH
P2Hg
P3 H5
P1 H6
P2 Ha
P2 Hd
P2 Hd'
P3 H1
P2 OMe
P2 Hb
P2 Hb'
P1 H7 + P3 H4
P1 H7'
P3 H2 + P3 H4' + P1 H8
P3 H3 + P1 H8'
P3 H2' + H3' + P1 Hb
P1 Hb'b"
Integral
3.9389
3.8694
1.0000
0.9501
0.7697
1.0978
1.2312
1.3719
1.0513
1.1673
1.0791
4.2876
1.0863
1.3461
3.2119
5.2914
3.4699
11.359
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
(ppm)

^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300° K



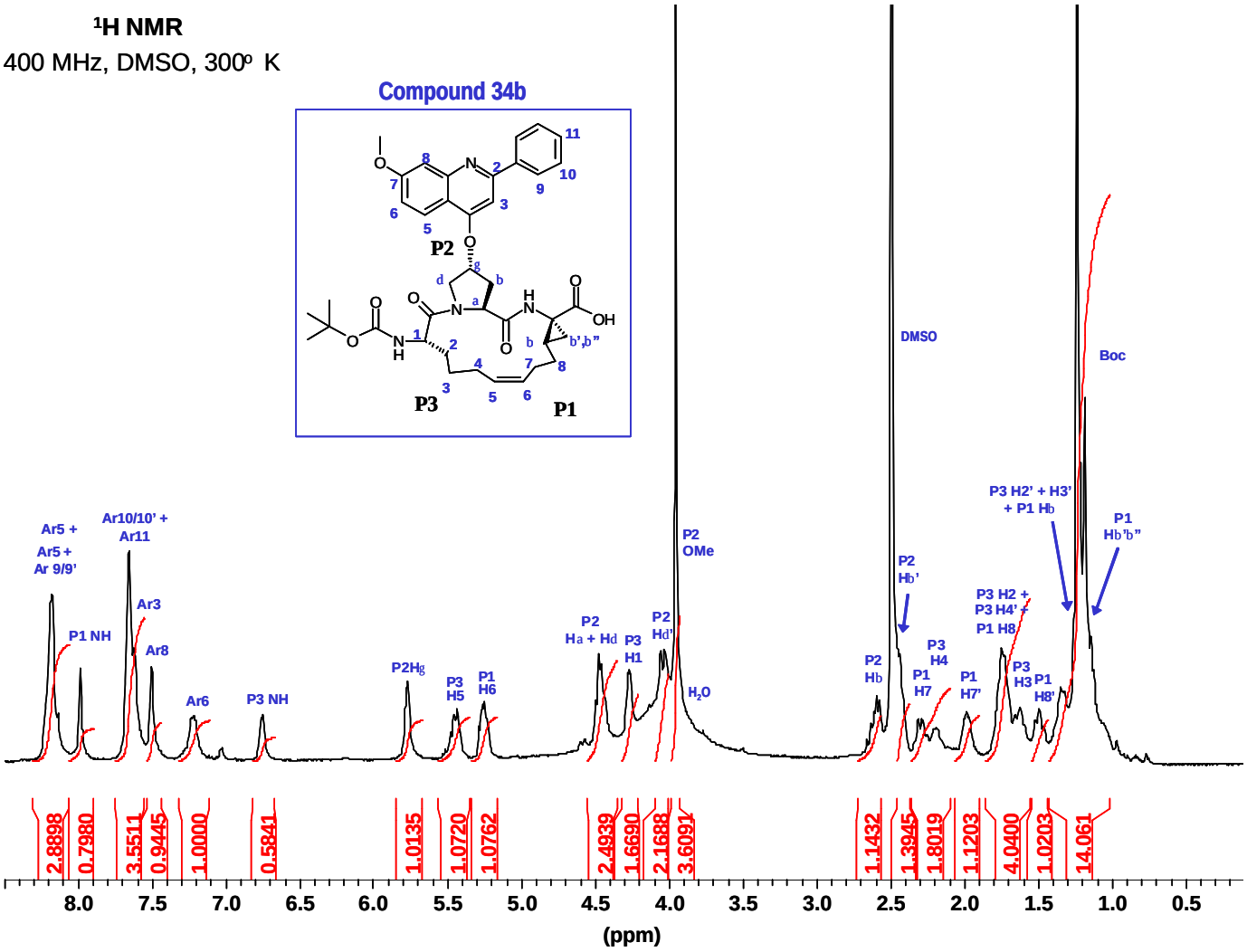
Compound 34a



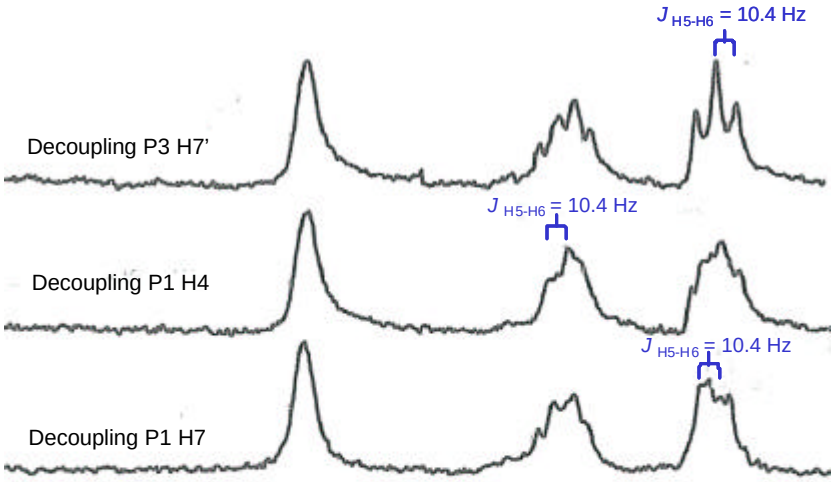
J_{H5-H6} = 15.2 Hz → ***trans***

^{1}H NMR

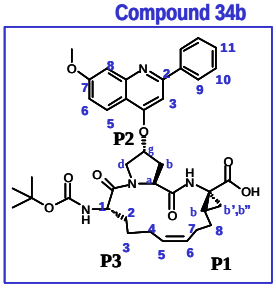
400 MHz, DMSO, 300^o K

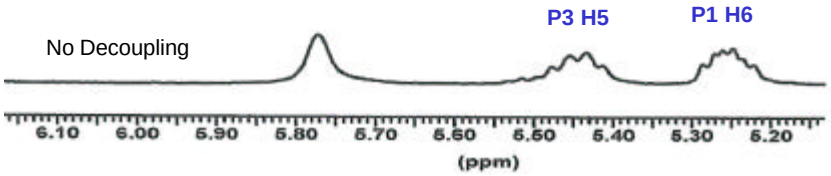


^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300^o K



Compound 34b

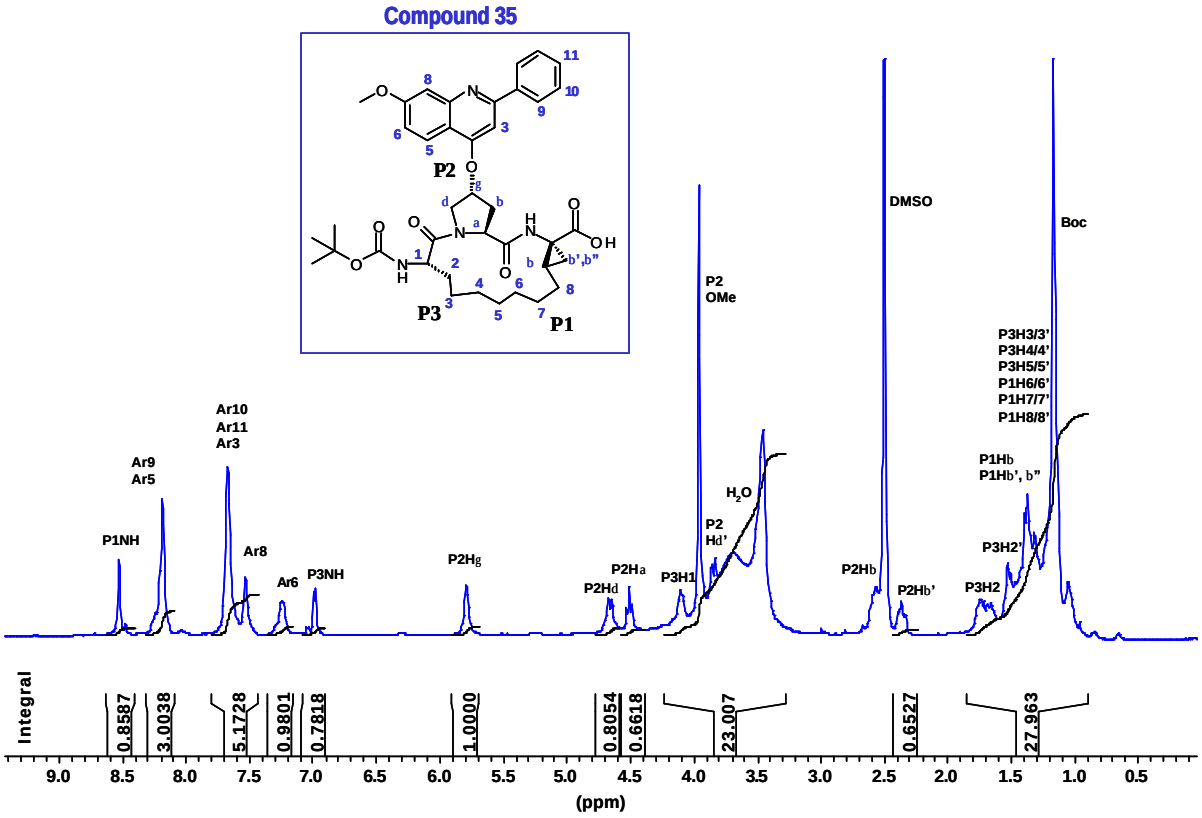




J_{H5-H6} = 10.4 Hz → ***cis***

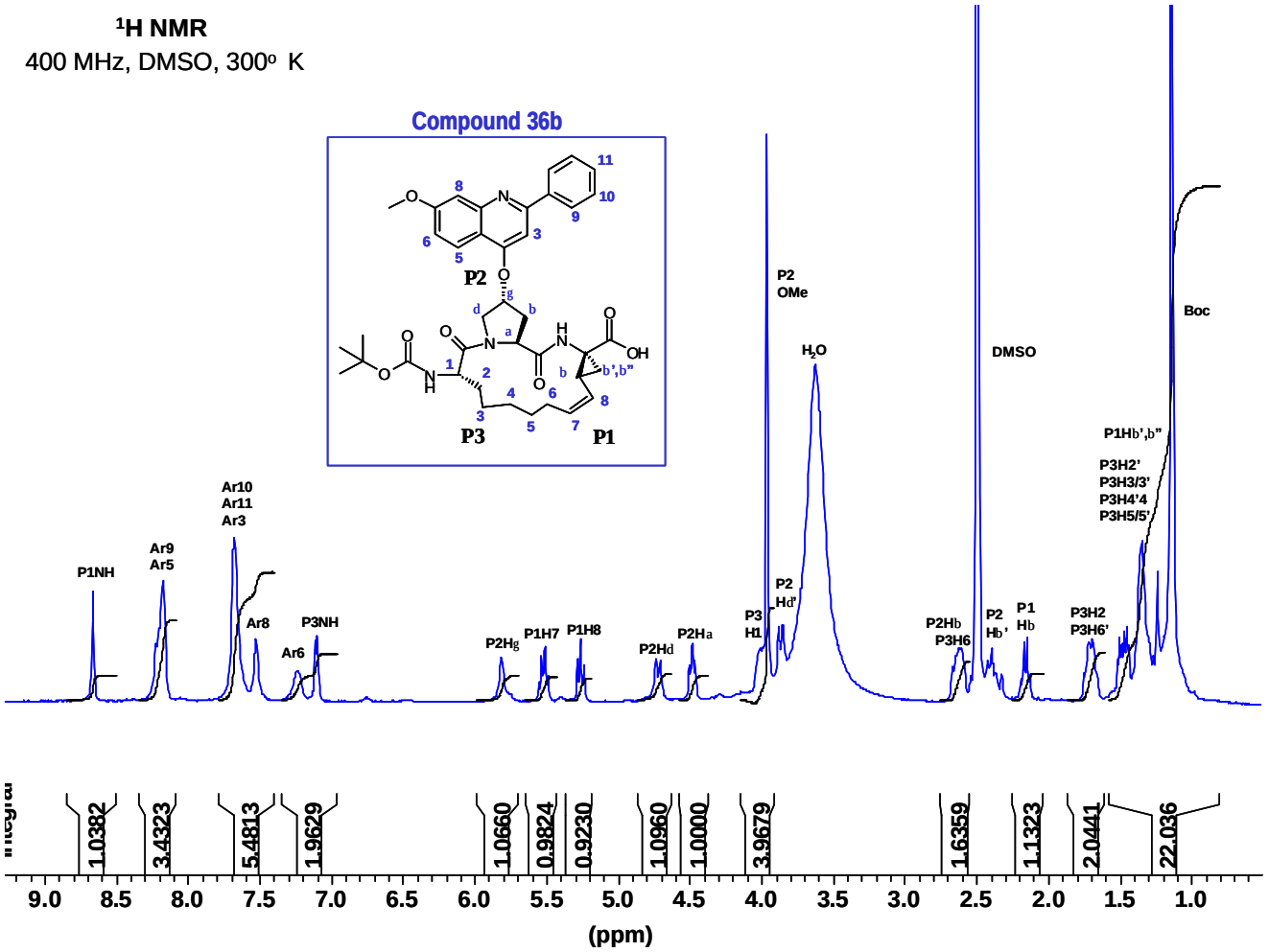
^{1}H NMR

400 MHz, DMSO, 300^{o} K

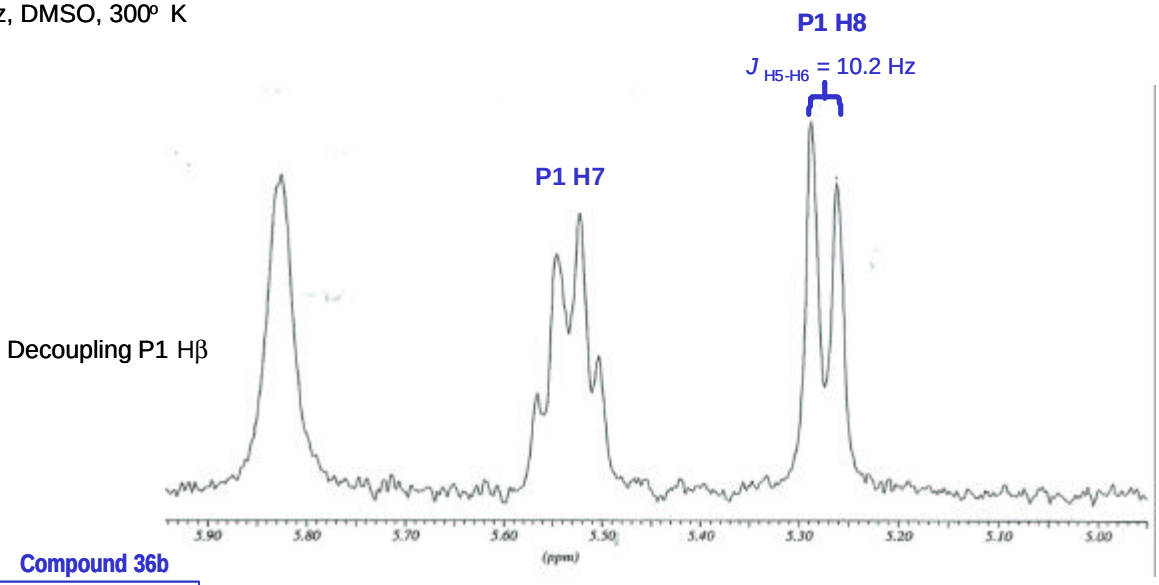


^{1}H NMR

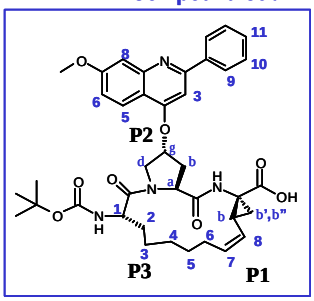
400 MHz, DMSO, 300^o K



^{1}H NMR
Decoupling experiments
400 MHz, DMSO, 300º K



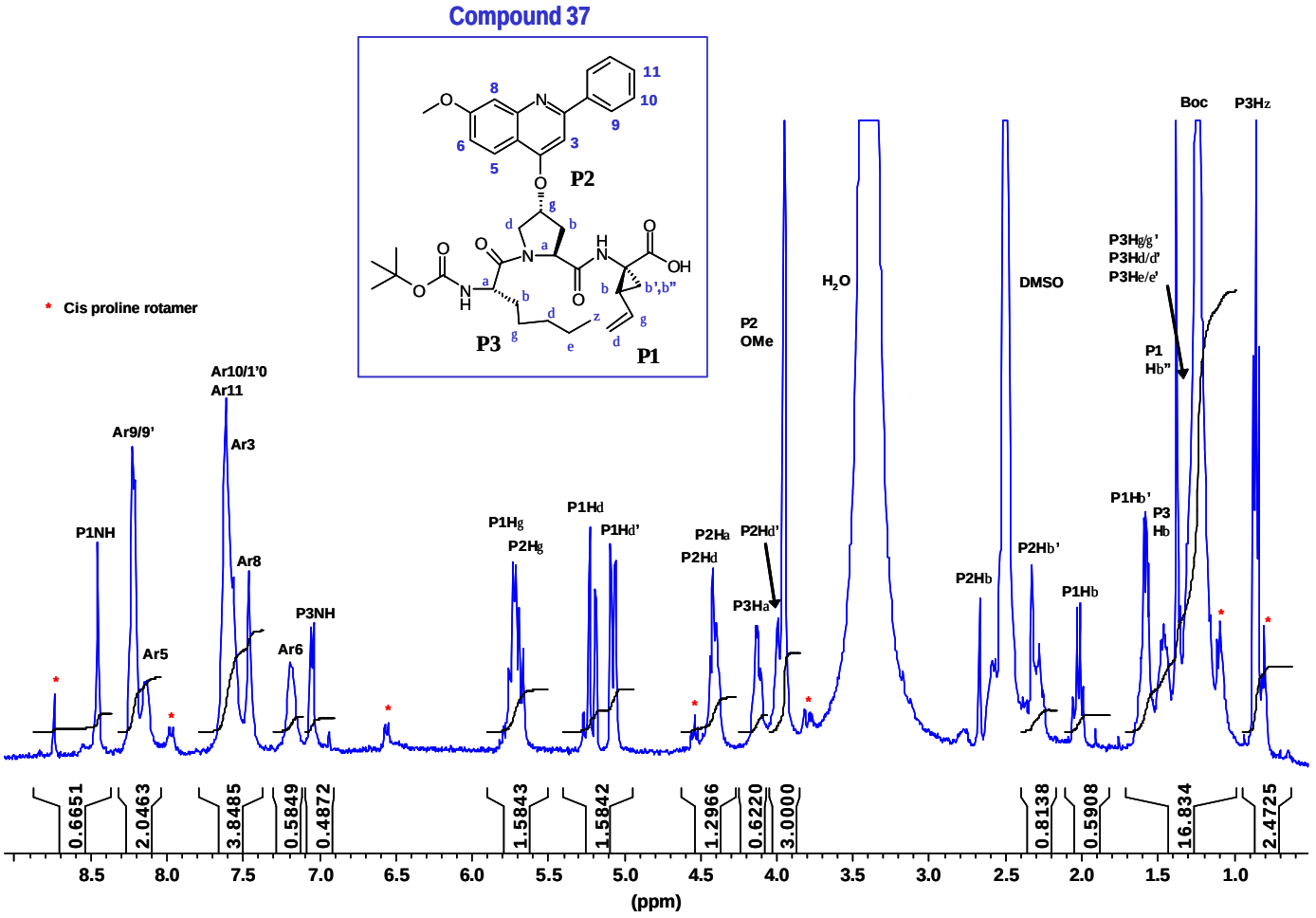
Compound 36b



J_{H5-H6} = 10.2 Hz → ***cis***

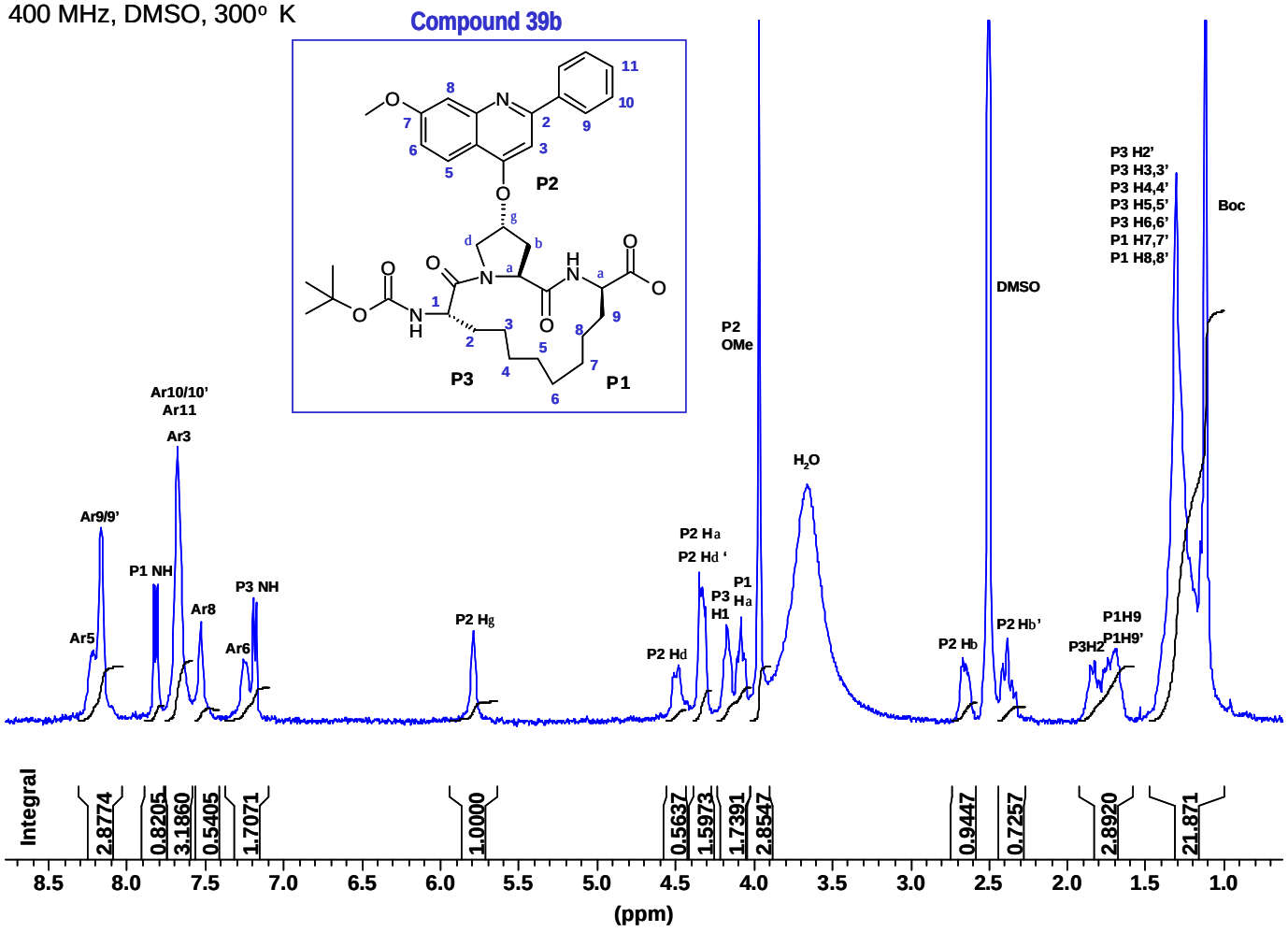
^{1}H NMR

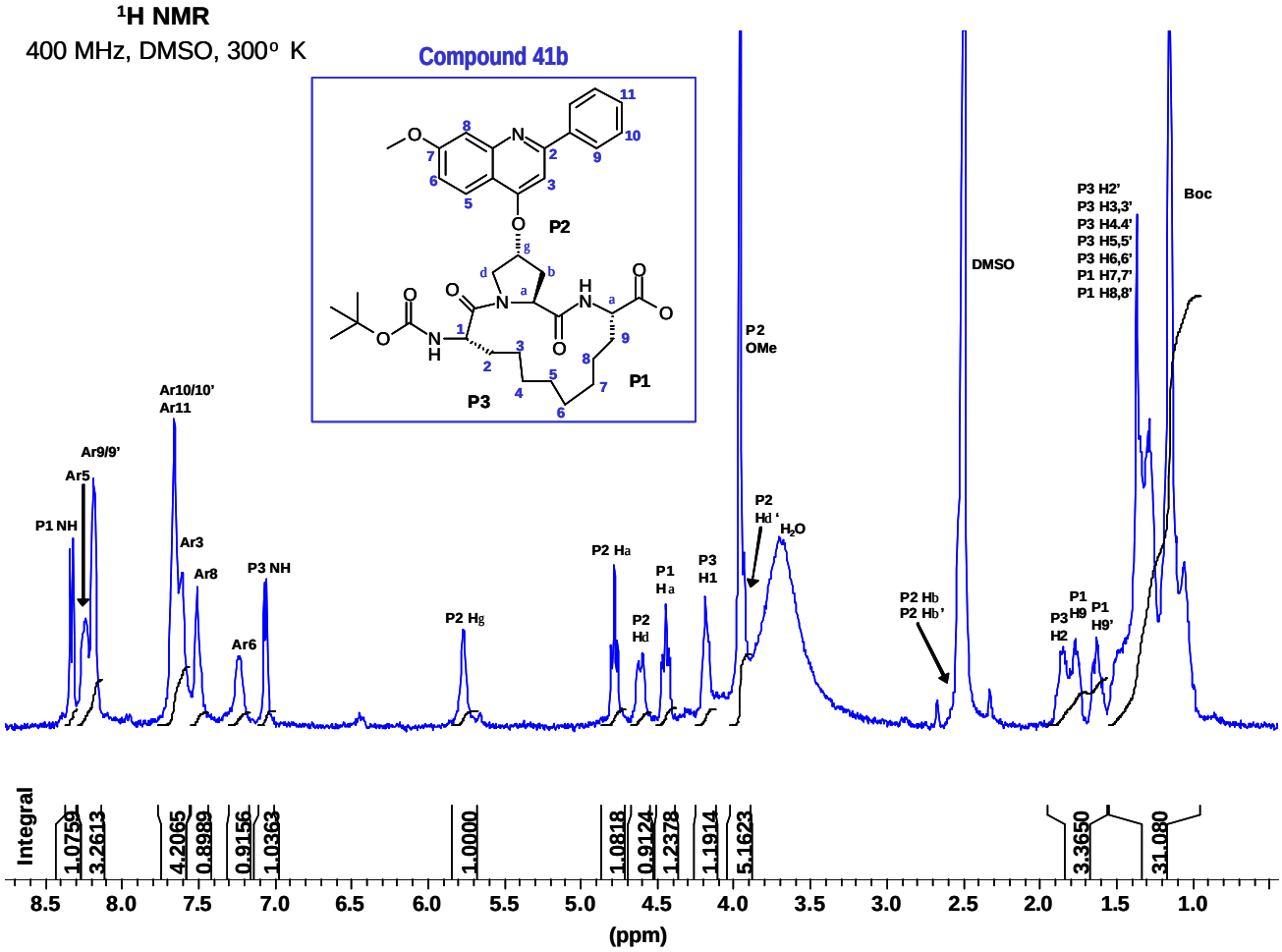
400 MHz, DMSO, 300^{o} K



^{1}H NMR

400 MHz, DMSO, 300^{o} K




1H NMR
400 MHz, DMSO, 300° K
Compound 41b
P2
P3
P1
P1 NH
Ar5
Ar9/9'
Ar10/10'
Ar11
Ar3
Ar8
P3 NH
Ar6
P2 Hg
P2 Ha
P2 Hd
P1 Ha
P3 H1
P2 OMe
P2 Hd ' H2O
P2 Hb
P2 Hb'
DMSO
P3 H2'
P3 H3,3'
P3 H4.4'
P3 H5,5'
P3 H6,6'
P1 H7,7'
P1 H8,8'
Boc
P3 H2
P1 H9
P1 H9'
Integral
1.0759
3.2613
4.2065
0.8989
0.9156
1.0363
1.0000
1.0818
0.9124
1.2378
1.1914
5.1623
3.3650
31.080
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
(ppm)

^{1}H NMR

400 MHz, DMSO, 300^o K