

Synthesis and Conformational Study of Water-Soluble, Rigid, Rod-like Oligopiperidines

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General Methods. All chemicals were purchased from Aldrich (St. Louis, MO).

Fmoc-4-piperidone was supplied by NeoMPS (Strasbourg, France). Analytical HPLC was run on a Varian instrument with a C18 column 5 μm (4.6×250 mm) from Vydac using a linear gradient of water with 0.1 % TFA (A) followed by acetonitrile containing 0.08 % TFA (B), at a flow rate of 1.2 mL/min (UV detection at 214 and 254 nm). NMR experiments were carried out on a Varian Inova 500 MHz. Analysis of 2D NMR data was performed using Varian VNMR 6.1B software. IR spectra were obtained using a Nicolet Nexus E.S.P. 670 FT-IR. Mass spectra were performed by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF) on a Perseptive Biosystems Voyager-DE PRO using α -cyano-4-hydroxycinnamic acid as a matrix.

1-(benzyloxycarbonyl)-4-piperidinone (2). *N*-(benzyloxycarbonyloxy)-succinimide (3.9 g, 15.7 mmol) in dioxane (50 mL) was added with stirring to an aqueous solution containing 4-piperidone monohydrate hydrochloride (3.6 g, 23.5 mmol) and Na_2CO_3 (3.3 g, 31.3 mmol). The reaction was allowed to proceed with stirring for 2 h at room temperature. The solution was evaporated and the product separated between ethyl acetate and water. The

organic phase was washed with water (2 x 100 mL), dried (MgSO₄), filtered and concentrated to yield **2** (3.3 g, 14.1 mmol, 90%). HPLC t_R 13.82 min (linear gradient, 0-100% B, 20 min); ¹H NMR (500 MHz, CD₃OD) δ 2.49 (t, J = 6.3 Hz, 4H), 3.83 (t, J = 6.3 Hz, 4H), 5.26 (s, 2H), 7.39-7.50 (m, 5H); ¹³C NMR (125 MHz, CD₃OD) δ 40.42, 41.43, 42.75, 67.45, 127.79, 127.94, 128.17, 128.51, 128.56, 136.90, 155.58; HRMS m/z found 234.1130 (M+H)⁺, calcd 234.1130.

N-[(benzyloxy)carbonyl]-4-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-piperidine (4). To a stirred solution of 1-(benzyloxycarbonyl)-4-piperidinone **2** (10 g, 42.9 mmol) and 1,4-dioxa-8-aza-spiro[4.5]decane **3** (5.5 mL, 42.9 mmol) in 1,2-dichloroethane (50 mL) at room temperature was added sodium triacetoxyborohydride (12.7 g, 60 mmol). The resulting solution was stirred at room temperature and was taken up in 0.1 N NaOH (100 mL), washed with water, dried over MgSO₄ and evaporated. The residue was chromatographed (SiO₂:AcOEt → 10% MeOH in AcOEt) to yield **4** (8.7 g, 24.1 mmol, 56%) as a white solid. HPLC t_R 12.22 min (linear gradient, 0-100% B, 20 min); ¹H NMR (500 MHz, CD₃OD) δ 1.38 (qd, J = 3.9 Hz, J = 12.2 Hz, 2H), 1.71 (t, J = 5.6 Hz, 4H), 1.85 (br d, J = 11.2 Hz, 2H); 2.52 (tt, J = 11.7 Hz, J = 3.4 Hz, 1H); 2.64 (t, J = 5.4 Hz, 4H), 2.80 (br s, 2H); 3.93 (s, 4H); 4.19 (br d, J = 13.7 Hz, 2H); 5.10 (s, 2H); 7.28-7.36 (m, 5H); ¹³C NMR (125 MHz, CD₃OD) δ 34.53, 43.44, 43.46, 46.83, 61.48, 64.06, 67.10, 106.77, 127.73, 127.95, 128.39, 136.99, 155.57; HRMS m/z found 361.2122 (M+H)⁺, calcd 361.2127.

4-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-piperidine (5). The compound **4** (2.4 g, 6.7 mmol) and 10% Pd/C (240 mg) were combined in ethanol (20 mL). This mixture was hydrogenated at room temp for 2h. The mixture was then filtered through Celite, the filter cake was washed with 2 x 10 mL of ethanol and the resulting solution was evaporated to yield **5** (1.5 g, 4.9 mmol, 98%). ¹H NMR (500 MHz, CD₃OD) δ 1.58 (qd, J = 2.9 Hz, J = 11.9 Hz, 2H), 1.75 (t, J = 5.6 Hz, 4H), 1.95 (br d, J = 12.7 Hz, 2H), 2.56 (tt, J = 11.2 Hz, J = 2.9 Hz,

1H), 2.65-2.73 (m, 2H), 2.70 (t, $J = 5.1$ Hz, 4H), 3.24 (br d, $J = 12.7$ Hz, 2H), 3.95 (s, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 27.30, 34.57, 44.71, 46.74, 60.66, 64.10, 106.82; HRMS m/z found 227.1765 ($\text{M}+\text{H}$) $^+$, calcd 227.1759.

***N*-[(benzyloxy)carbonyl]-4-Oxo-[1,4']bipiperidine (6).** The compound **4** (480 mg, 1.33 mmol) was treated with concentrated hydrochloric acid (14 mL) at 0 °C and then allowed to warm to room temperature. After 25 min, 50 mL of dichloromethane are added to the mixture at 0 °C, followed by aqueous NaOH (7 g) solution (10 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated. The residue was chromatographed ($\text{SiO}_2:\text{AcOEt} \rightarrow 10\%$ MeOH in AcOEt) to yield **6** (304 mg, 0.96 mmol, 72%) as a solid. HPLC t_{R} 11.46 min (linear gradient, 0-100% *B*, 20 min); ^1H NMR (500 MHz, CD_3OD) δ 1.35-1.54 (m, 2H), 1.74-1.82 (m, 2H), 1.84-1.94 (m, 2H); 2.43 (m, 2H); 2.54 (m, 3H); 2.85 (br s, 2H); 2.82 (m, 2H); 4.16-4.27 (m, 2H); 5.12 (s, 2H); 7.26-7.44 (m, 5H); ^{13}C NMR (125 MHz, CD_3OD) δ 34.99, 41.05, 43.48, 46.21, 48.69, 60.88, 61.58, 67.12, 127.78, 128.03, 128.48, 137.09, 155.53, 209.80; HRMS m/z found 317.1865 ($\text{M}+\text{H}$) $^+$, calcd 317.1865.

***N*-[(benzyloxy)carbonyl]-4-(1,4-Dioxa-8-aza-spiro[4.5]dec-8-yl)-[1,4';1',4'']terpiperidine (7).** To a stirred solution of **5** (138 mg, 0.61 mmol) and **6** (193 mg, 0.61 mmol) in 1,2-dichloroethane (15 mL) at room temperature was added sodium triacetoxyborohydride (181 mg, 0.83 mmol). The resulting solution was stirred at room temperature and was taken up in 0.1 N NaOH (30 mL), washed with water, dried over MgSO_4 and evaporated. The residue was chromatographed ($\text{SiO}_2:\text{AcOEt} \rightarrow 20\%$ MeOH in AcOEt) to yield **7** (225 mg, 0.43 mmol, 70%) as a white solid. HPLC t_{R} 11.18 min (linear gradient, 0-100% *B*, 20 min); ^1H NMR (500 MHz, CD_3OD) δ 1.38 (qd, $J = 3.9$ Hz, $J = 12.2$ Hz, 2H), 1.53 (qd, $J = 2.9$ Hz, $J = 12.2$ Hz, 2H), 1.55 (qd, $J = 3.4$ Hz, $J = 12.7$ Hz, 2H), 1.72 (t, $J = 5.8$ Hz, 4H), 1.88 (d, $J = 11.7$ Hz, 6H), 2.19 (t, $J = 11.7$ Hz, 4H), 2.25 (tt, $J = 11.7$ Hz, $J = 3.9$ Hz, 1H); 2.32 (tt, $J = 11.7$ Hz, $J = 3.9$ Hz, 1H); 2.46 (tt, $J = 11.5$ Hz, $J = 3.4$ Hz, 1H), 2.66 (t, $J =$

4.99 Hz, 4H), 2.82 (br s, 2H), 3.02 (br d, $J = 11.3$ Hz, 2H), 3.05 (br d, $J = 11.3$ Hz, 2H), 3.92 (s, 4H), 4.19 (br d, $J = 12.7$ Hz, 2H), 5.10 (s, 2H), 7.26-7.41 (m, 5H); ^{13}C NMR (125 MHz, CDCl_3) δ 155.38, 137.05, 128.69, 128.18, 128.07, 107.67, 67.25, 64.40, 62.53, 62.48, 61.99, 49.23, 47.10, 43.86, 35.48, 28.54; HRMS m/z found 527.3589 ($\text{M}+\text{H}$) $^+$, calcd 527.3597.

***N*-(benzyloxy)carbonyl-4-Methyl-[1,4']bipiperidine (S1).** To a stirred solution of 1-(benzyloxycarbonyl)-4-piperidinone **2** (1.17 g, 5 mmol) and 4-methylpiperidine (592 mg, 5 mmol) in 1,2-dichloroethane (15 mL) at room temperature was added sodium triacetoxymethylborohydride (1.48 g, 7 mmol). The resulting solution was stirred at room temperature and was taken up in 0.1 N NaOH (30 mL), washed with water, dried over MgSO_4 and evaporated. The residue was chromatographed (SiO_2 : AcOEt \rightarrow 10% MeOH in AcOEt) to yield **S1** (807 mg, 2.56 mmol, 51%) as an oil. HPLC t_R 12.90 min (linear gradient, 0-100% B, 20 min); ^1H NMR (500 MHz, CD_3OD) δ 0.97 (d, $J = 6.3$ Hz, 3H), 1.34 (qd, $J = 12.2$ Hz, $J = 3.9$ Hz, 2H), 1.50 (qd, $J = 12.2$ Hz, $J = 3.9$ Hz, 2H), 1.77 (br d, $J = 12.7$ Hz, 2H), 1.97 (br d, $J = 10.7$ Hz, 2H), 2.51 (td, $J = 12.2$ Hz, $J = 2$ Hz, 2H), 2.83 (tt, $J = 11.7$ Hz, $J = 3.9$ Hz, 1H), 2.85 (br s, 2H), 3.13 (d, $J = 11.7$ Hz, 2H), 4.25 (d, $J = 13.6$ Hz, 2H), 5.13 (s, 2H), 7.27-7.40 (m, 5H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.68, 23.06, 30.10, 32.89, 43.13, 49.33, 62.55, 67.20, 127.79, 128.02, 128.43, 136.95, 155.49; HRMS m/z found 317.2225 ($\text{M}+\text{H}$) $^+$, calcd 317.2229.

4-Methyl-[1,4']bipiperidine (S2). The compound **S1** (350 mg, 1.11 mmol) and 10% Pd/C (35 mg) were combined in ethanol (10 mL). This mixture was hydrogenated at room temp for 2h. The mixture was then filtered through Celite, the filter cake was washed with 2 x 10mL ethanol and the resulting solution was evaporated to yield **S2** (130 mg, 0.73 mmol, 64%) as a white solid. ^1H NMR (500 MHz, CD_3OD) δ 0.97 (d, $J = 6$ Hz, 3H), 1.29 (qd, $J =$

12.2 Hz, $J = 3.9$ Hz, 2H), 1.49 (br s, 1H), 1.71 (qd, $J = 12.7$ Hz, $J = 3.9$ Hz, 2H), 1.78 (d, $J = 13.7$ Hz, 2H), 2.12 (d, $J = 13.2$ Hz, 2H), 2.46 (tt, $J = 11.7$, $J = 3.4$ Hz, 2H), 2.82 (tt, $J = 11.2$ Hz, $J = 3$ Hz, 1H), 2.94 (td, $J = 12.7$, $J = 2.4$ Hz, 2H), 3.10 (br d, $J = 11.7$ Hz, 2H), 3.41 (br d, $J = 12.7$ Hz, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 20.45, 24.45, 29.53, 32.24, 43.21, 49.44, 60.26; HRMS m/z found 183.1870 ($\text{M}+\text{H}$) $^+$, calcd 183.1861.

General procedure for solid phase synthesis. The oligopiperidine was synthesized in Fmoc-tBu chemistry by the stepwise solid-phase methodology. Assembly of the protected peptide chains was carried out on a 25 μmol scale starting from Fmoc- β Ala-Wang resin. The Fmoc group was removed using 20% piperidine in DMF (1 \times 5 min, 1 \times 15 min) under nitrogen bubbling. The resin was then filtered and washed with DMF (6 \times 3 min). For each coupling step, a solution of the Fmoc-amino acid (10 equiv) and $\text{NaBH}(\text{OAc})_3$ (10 equiv) in 1,2-dichloroethane were added successively to the resin, and suspension was mixed for 60 min. A double coupling was performed systematically. After each coupling step, the resin was washed with MeOH (4 \times 3 min) and DMF (4 \times 3 min). After the removal of the last coupling step, the resin was washed with CH_2Cl_2 , ether and dried under nitrogen. Cleavage of the oligopiperidines from the resin were performed by treatment with a mixture of trifluoroacetic acid 95 % and water 5 %. After precipitation in cold diethyl ether and centrifugation, the oligopiperidines were solubilized and lyophilized. The crude oligomers derivative were finally purified by RP-HPLC (linear gradient, 0-80% B, 40min) and lyophilized.

Fmoc-(Pip) $_4$ - β Ala-OH (11). Overall yield after RP-HPLC purification and lyophilisation : 18%; HPLC t_{R} 12.99 min (linear gradient, 0-100% B, 20 min); HRMS m/z found 672.4125 ($\text{M}+\text{H}$) $^+$, calcd 672.4125.

Fmoc-(Pip)₆-βAla-OH (12). Overall yield after RP-HPLC purification and lyophilisation : 12%; HPLC t_R 12.94 min (linear gradient, 0-100% *B*, 20 min); HRMS *m/z* found 838.5598 (M+H)⁺, calcd 838.5595.

Fmoc-(Pip)₈-βAla-OH (13). Overall yield after RP-HPLC purification and lyophilisation : 10 %; HPLC t_R 12.81 min (linear gradient, 0-100% *B*, 20 min); HRMS *m/z* found 1004.7059 (M+H)⁺, calcd 1004.7065.

Fmoc-(Pip)₁₀-βAla-OH (14). Overall yield after RP-HPLC purification and lyophilisation : 4 %; HPLC t_R 12.78 min (linear gradient, 0-100% *B*, 20 min); HRMS *m/z* found 1170.8536 (M+H)⁺, calcd 1170.8535.

Molecular dynamics. The oligopiperidine structure **7** was obtained from a crystal structure (Figure 2), and used as the starting point for simulations. The AMBER 8 package was used for these simulations. In order to prepare the molecule for simulation, the ANTECHAMBER program from the AMBER package was used to assign GAFF (General AMBER Force Field) atom and bond types and force field parameters to all atoms, bonds and torsions in the molecule. The ANTECHAMBER program was also used to calculate and assign AM1-BCC partial charges to all atoms. Molecular dynamics simulations used the SANDER program with the GB/SA (Generalized Born) implicit solvent approximation. The PTRAJ program was used to extract several geometric values from each snapshot in the molecular dynamics trajectories. In order to determine how linear the molecule's geometry remained during each simulation, two metrics were used. The first is the length of the major axis, defined as the distance between two carbon atoms at the opposite ends of the molecule (Figure 9), which was measured for each snapshot in each MD trajectory. The second is the major axis angle, formed by the previously defined major axis carbon atoms, and a central nitrogen atom in the molecule (Figure 9). These two values characterize the overall geometry

of the molecule during the simulations, describing its tolerance for stretching, compressing and bending.

The distribution of the major axis lengths for all three temperature simulations are shown in the histograms in Figure 10. The distribution of the major axis bending angles for all three temperature simulations are shown in Figure 11, and the distribution statistics are shown in Table 1.

Crystallographic data. Crystallographic data were collected using a Bruker SMART CCD (charge coupled device) based diffractometer equipped with an Oxford Cryostream low-temperature apparatus operating at 193 K. A suitable crystal was chosen and mounted on a glass fiber using grease. Data were measured using omega scans of 0.3° per frame for 30 seconds, such that a hemisphere was collected. A total of 1271 frames were collected with a maximum resolution of 0.76 \AA . The first 50 frames were recollected at the end of data collection to monitor for decay. Cell parameters were retrieved using SMART¹ software and refined using SAINT on all observed reflections. Data reduction was performed using the SAINT software² which corrects for L_p and decay. The structures are solved by the direct method using the SHELXS-97³ program and refined by least squares method on F^2 , SHELXL-97,⁴ incorporated in SHELXTL-PC V 5.10.⁵ The structure was solved in the space group $Pbca$ (# 61) by analysis of systematic absences. All non-hydrogen atoms are refined anisotropically. Hydrogens were calculated by geometrical methods and refined as a riding model. The crystal showed evidence that it may be twinned. All aspects of this were evaluated and in our hands we could not find two appropriate twin matrices, and therefore left in the original cell and refined, but limited the data to 40 degrees 2θ . Expansion to the complete data gives R_1 value $\sim 11\%$. The crystal used for the diffraction study showed no decomposition during data collection. Drawing are done at 50% ellipsoids.

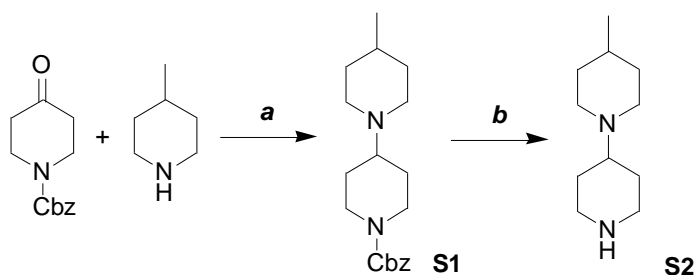
Acknowledgement. The CCD based x-ray diffractometer at Harvard University was purchased through NIH grant (1S10RR11937-01).

References

1. SMART V 5.050 (NT) *Software for the CCD Detector System*; Bruker Analytical X-ray Systems, Madison, WI (1998).
2. SAINT V 5.01 (NT) *Software for the CCD Detector System* Bruker Analytical X-ray Systems, Madison, WI (1998).
3. Sheldrick, G. M. SHELXS-90, *Program for the Solution of Crystal Structure*, University of Göttingen, Germany, 1990.
4. Sheldrick, G. M. SHELXL-97, *Program for the Refinement of Crystal Structure*, University of Göttingen, Germany, 1997.
5. SHELXTL 5.10 (PC-Version), *Program library for Structure Solution and Molecular Graphics*; Bruker Analytical X-ray Systems, Madison, WI (1998).

^a Obtained with graphite monochromated Mo K α ($\lambda = 0.71073 \text{ \AA}$) radiation. ^b $R1 = \sum ||F_o| - |F_c|| / \sum |F_o|$. ^c $wR2 = \{ \sum [w(F_o^2 - F_c^2)^2] / \{ \sum [w(F_o^2)] \} \}^{1/2}$.

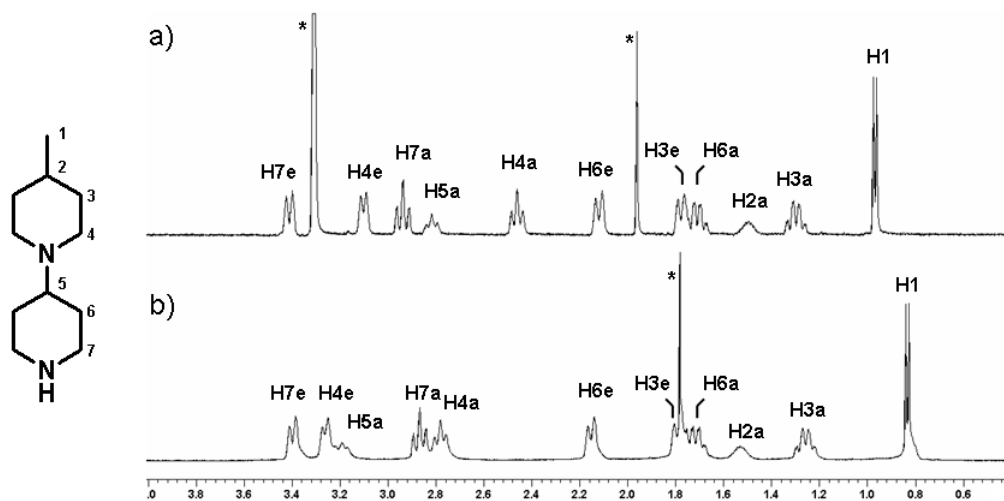
Supplemental Figure 1. Synthesis of 4-methylpiperidinopiperidine S2



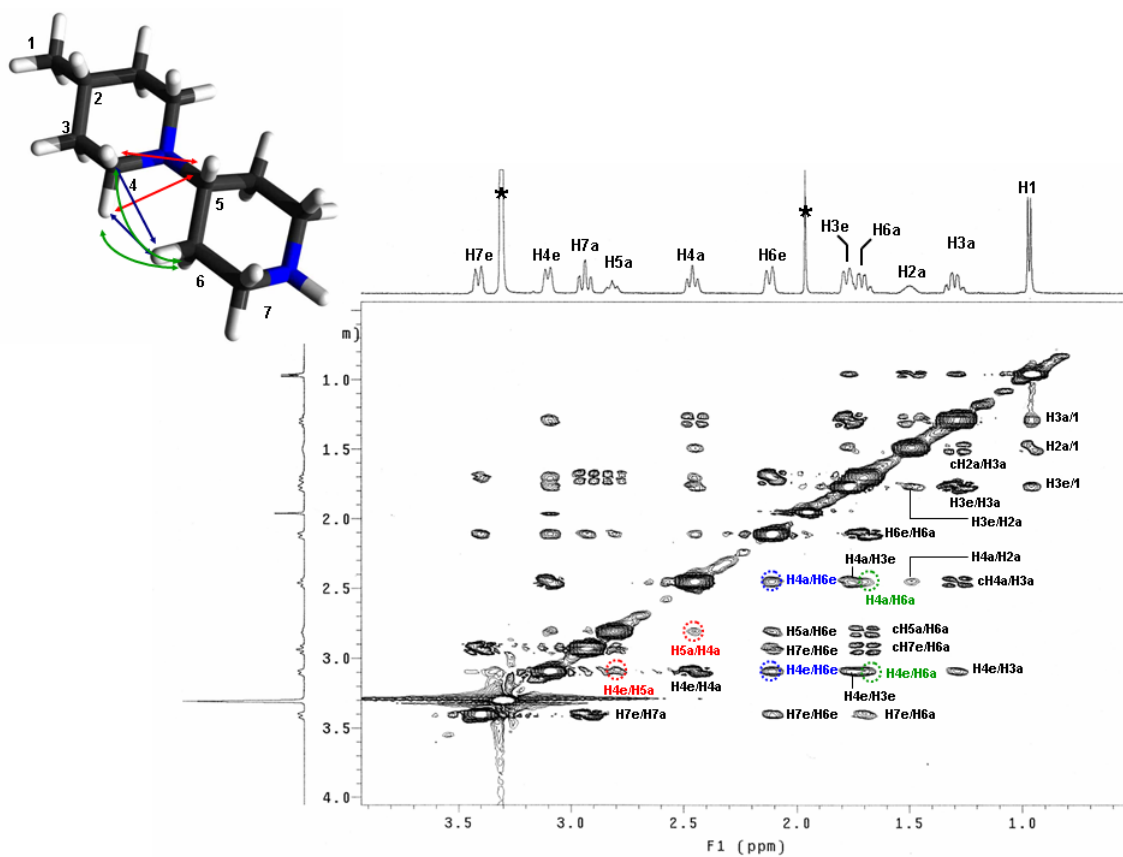
(a) NaBH(OAc)₃, 1,2-dichloroethane; (b) H₂, Pd/C, EtOH

Supplemental Figure 2. ^1H NMR spectra of *N*-methylpiperidinopiperidine **S2**

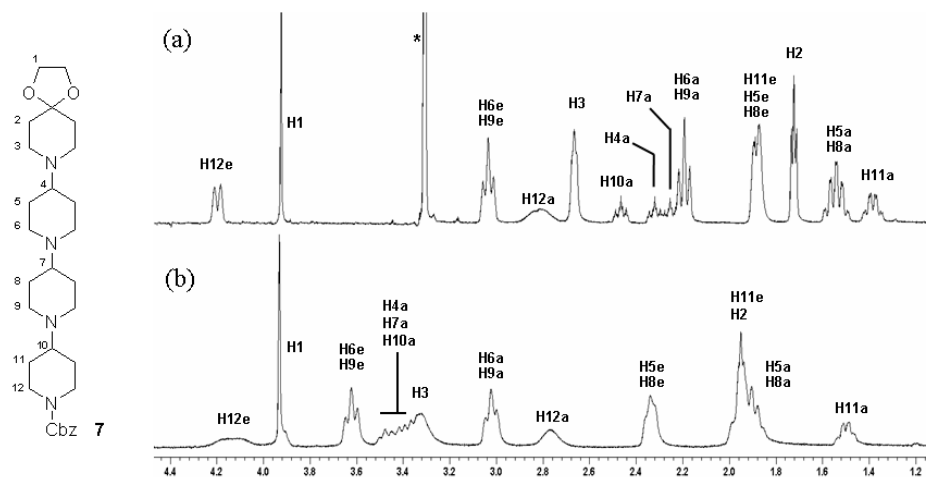
(a) in CD_3OD (2 mM) (b) in D_2O .



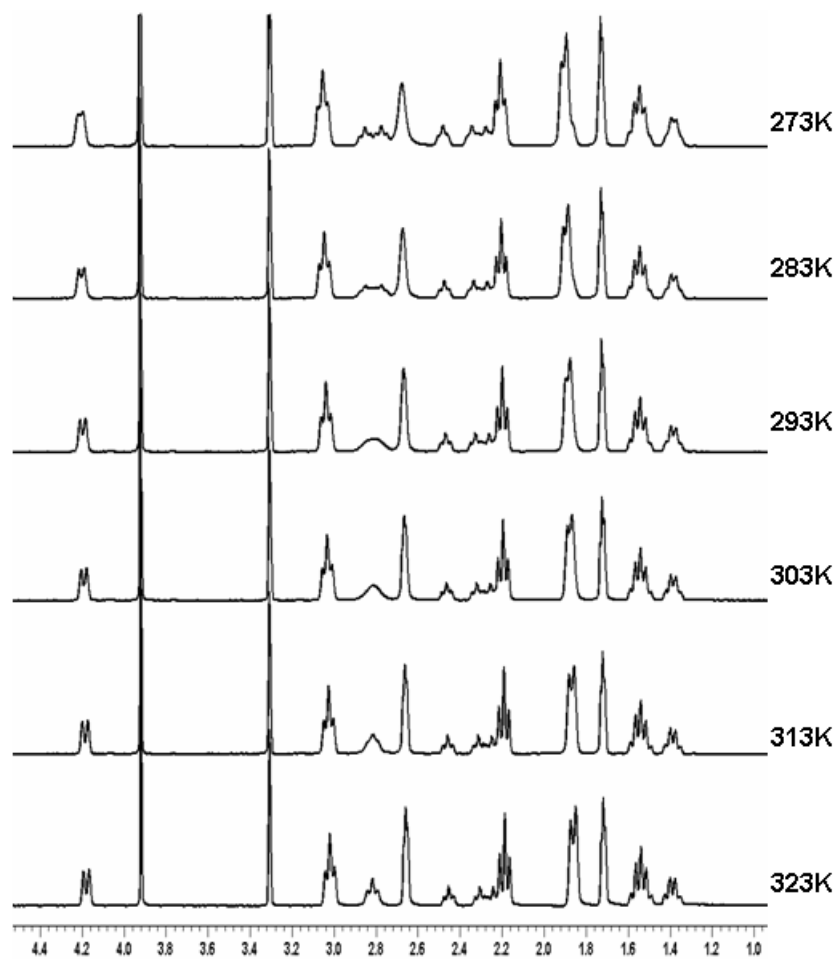
Supplemental Figure 3. 500 MHz NOESY of *N*-methylpiperidinopiperidine **S2** in CD₃OD (2 mM).



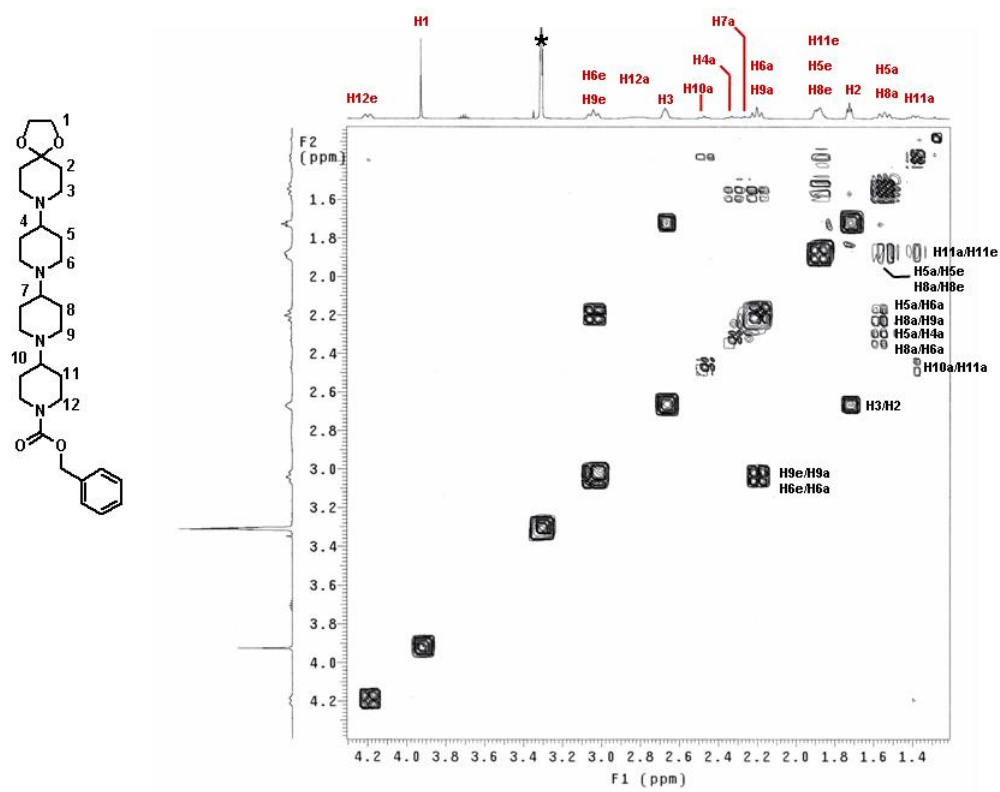
Supplemental Figure 4. 1D NMR spectrum of tetrapiperidine **7** (a) in CD₃OD (b) in D₂O + 4% (v/v) CD₃CO₂D.



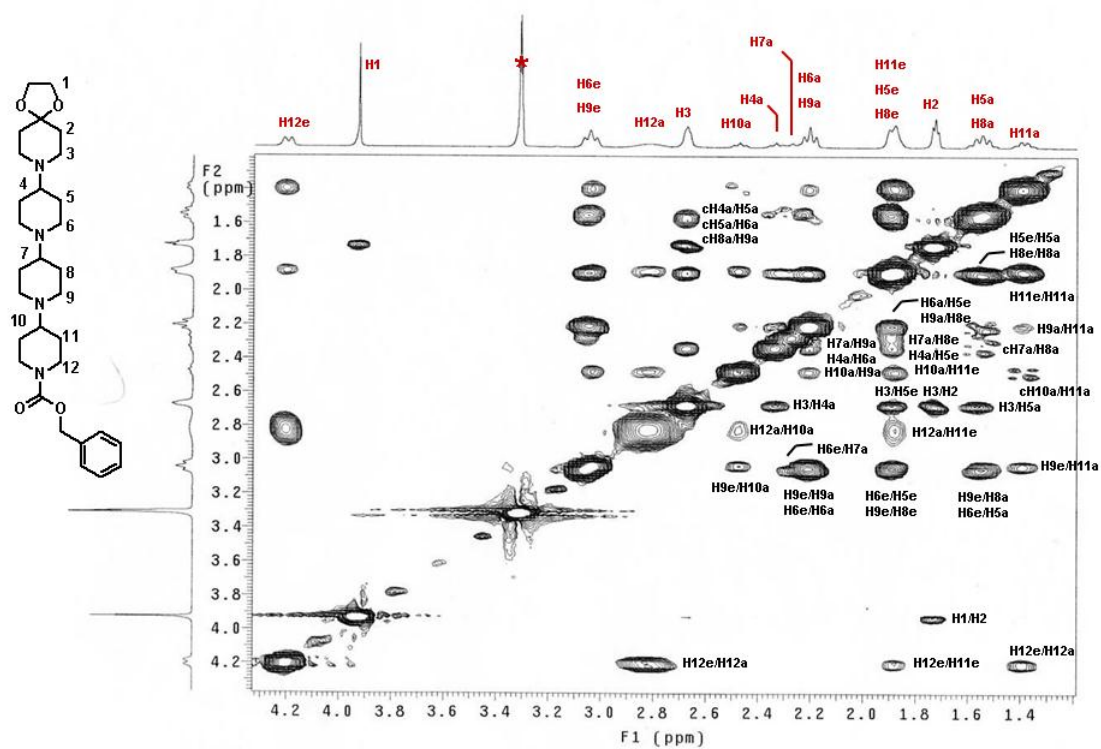
Supplemental Figure 5. Temperature-dependent NMR spectra of tetrapiperidine **7** in CD₃OD.



Supplemental Figure 6. 500 MHz COSY of tetrapiperidine **7** in CD₃OD.

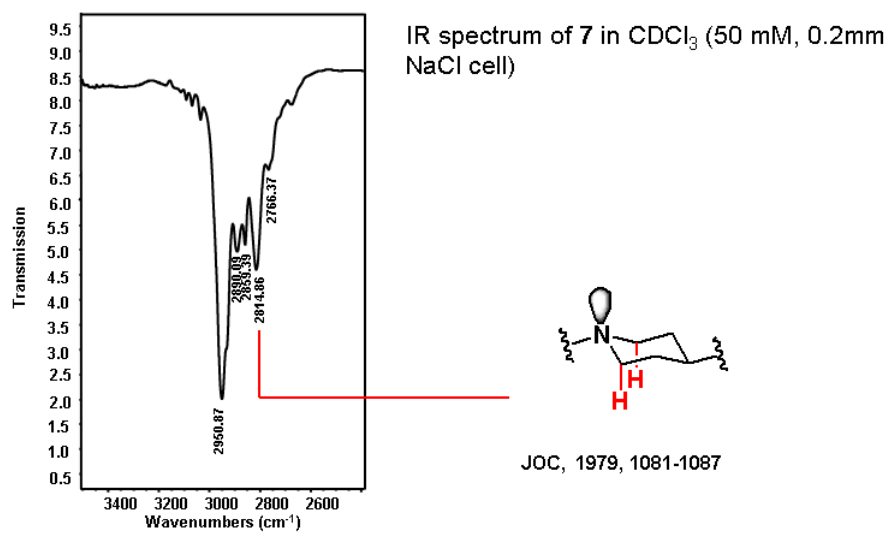


Supplemental Figure 7. 500 MHz NOESY of tetrapiperidine **7** in CD₃OD.

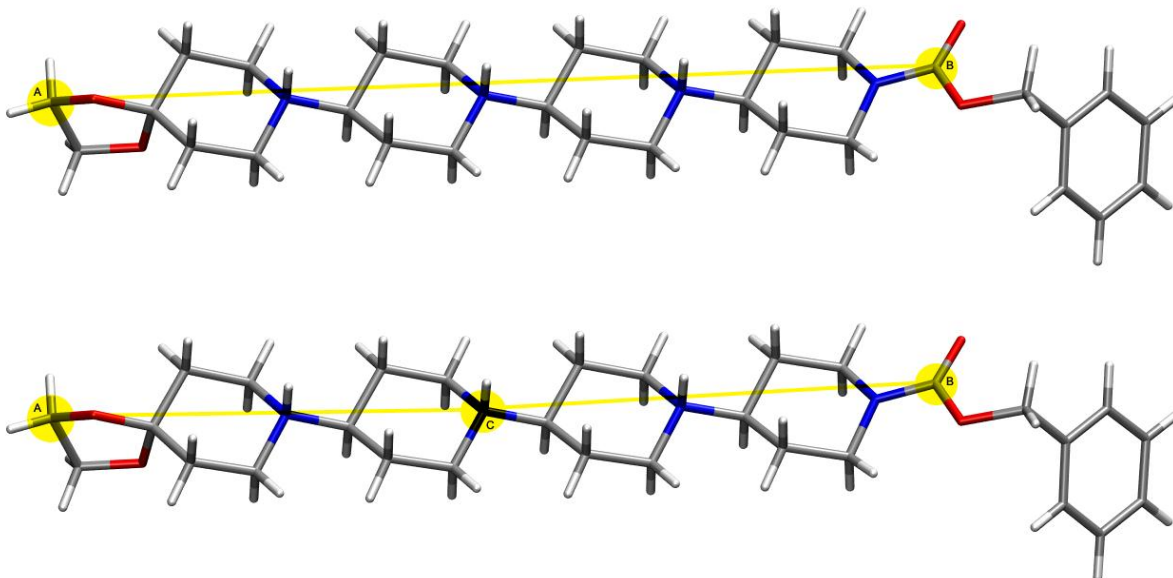


Supplemental Figure 8. IR analysis of tetrapiperidine **7**.

IR : Bohlmann band

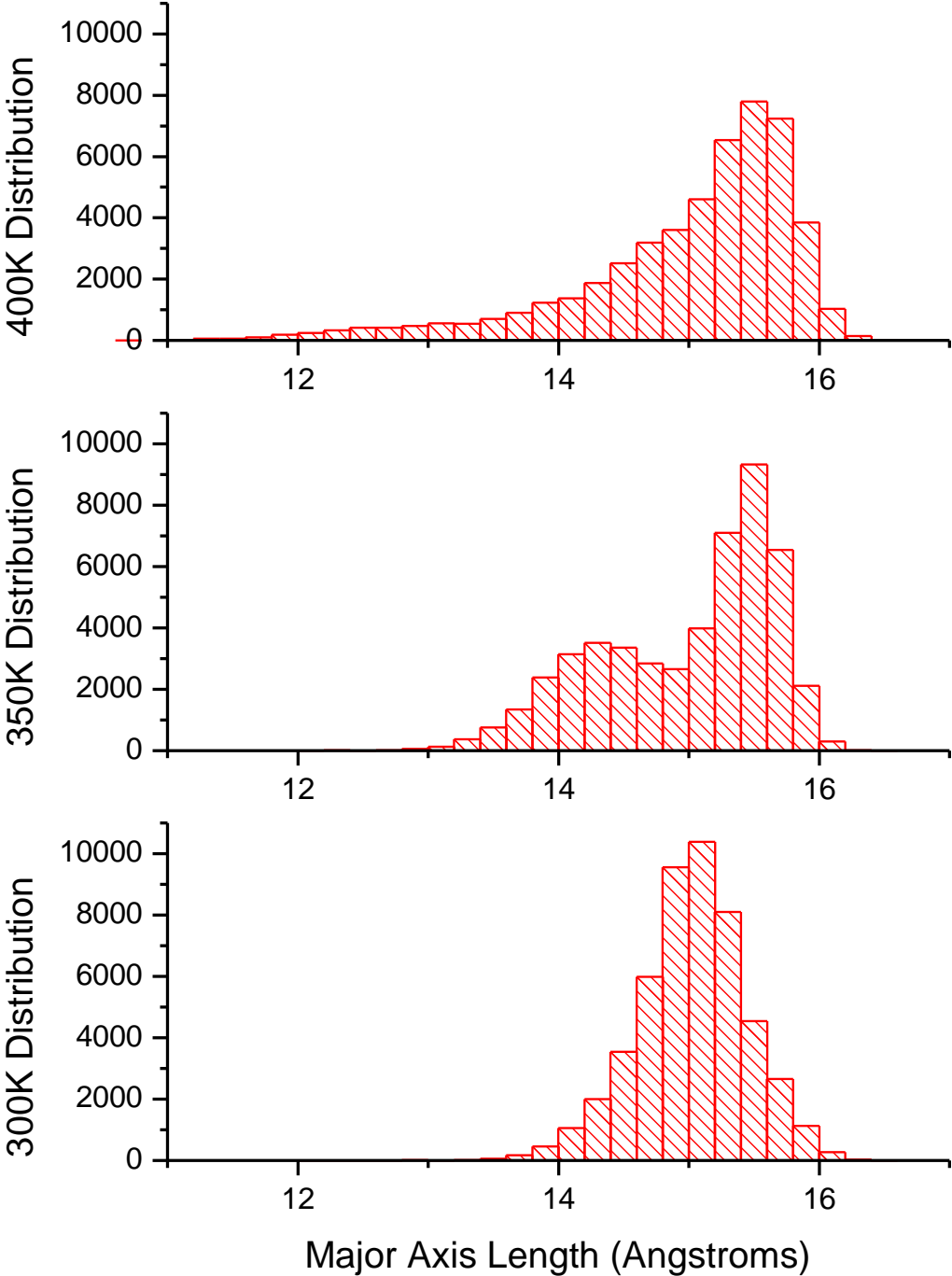


Supplemental Figure 9. Metrics used for simulations. The major axis length of the oligopiperidine structure (top). The major axis angle of the oligopiperidine structure (bottom).



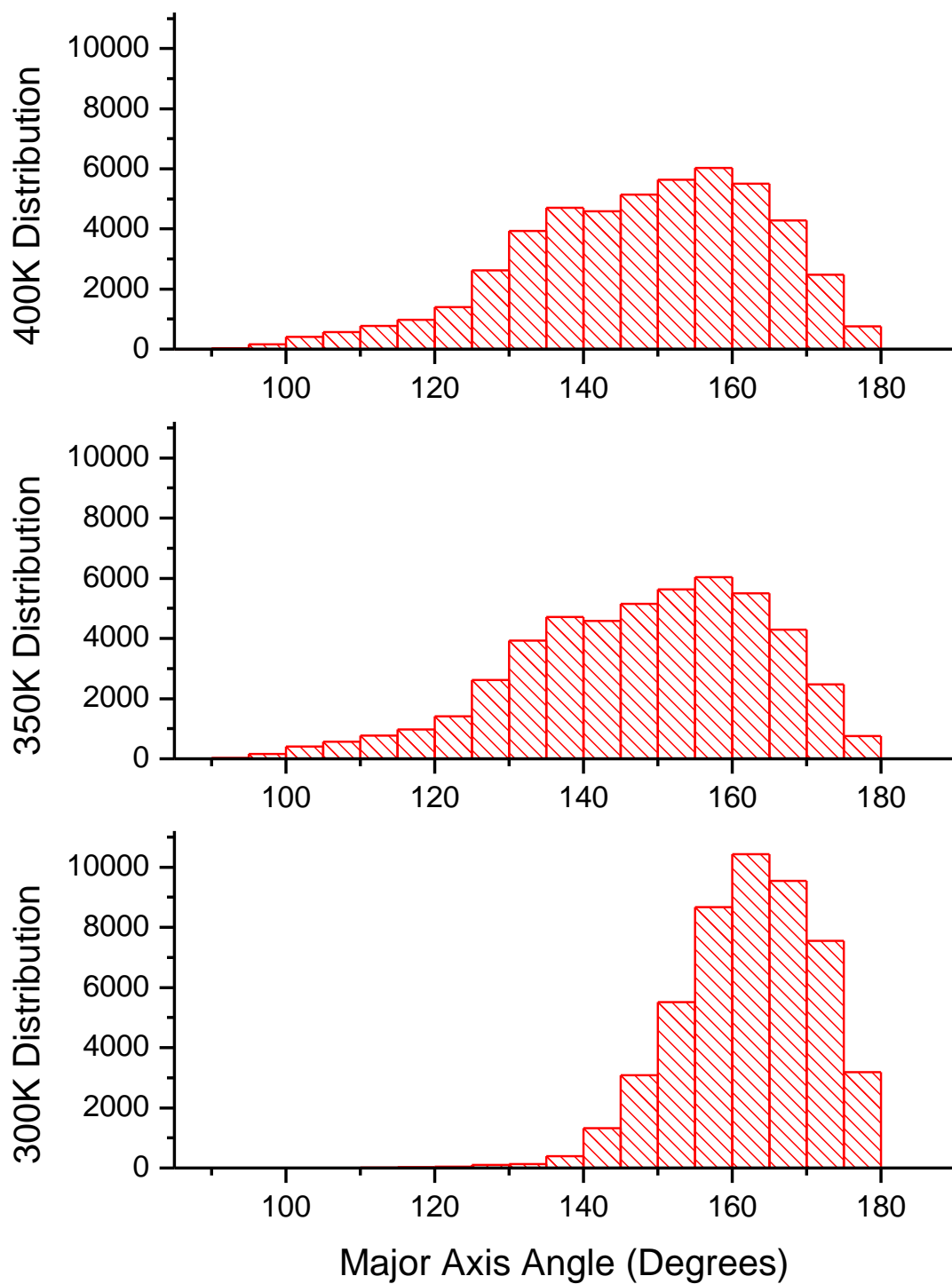
Supplemental Figure 10. Distribution of major axis lengths in simulations at 300 K, 350 K and 400 K.

Distributions of Major Axis Lengths
in MD Simulations at 300K, 350K & 400K



Supplemental Figure 11. Distribution of major axis angles in simulations at 300 K, 350 K and 400 K.

Distributions of Major Axis Angles in MD Simulations at 300K, 350K & 400K



Supplemental Table 1. Statistics of the major axis angle distributions

	Mean (degrees)	Std. Dev.
300K	162	9
350K	147	16
400K	147	16