Supporting information

Steroidomimetic tetrahydroisoquinolines for the design of new microtubule disruptors

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1) General Methods for Synthesis.

Unless otherwise stated, HPLC grade solvents were used and commercial reagents and starting materials were used without further purification. Sulfamoyl chloride was prepared by an adaptation of the method of Appel and Berger[1] and was stored at 4 °C under positive N_2 pressure as a solution in toluene, as described by Woo *et al.*[2] Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminium sheets silica gel 60 F254, Art. No. 5554). Product(s) and starting materials(s) were detected by either viewing under UV light or treatment with an ethanolic solution of phosphomolybdic acid followed by heating. Flash column chromatography was performed on glass columns packed with silica gel (Sorbsil/Matrex C60) or on ISCO CombiFlash Rf Automated Flash Chromatography System run RediSep Rf disposable flash columns. Nuclear

magnetic resonance spectra were recorded on either a Jeol Delta 270 MHz or Varian Mercury VX 400 MHz spectrometer and in deuteriochloroform unless stated otherwise. ^{1}H NMR spectra were recorded at 270 MHz or 400 MHz with shifts reported in parts per million (ppm, δ) relative to residual chloroform (δ_H = 7.26 ppm) or residual DMSO (δ_H = 2.50 ppm). Coupling constants, *J*, are reported in hertz. ^{13}C NMR spectra were recorded at 100.6 MHz with the central peak of chloroform (δ_C = 77.16 ppm) or DMSO (δ_C = 39.52 ppm) as internal standard. The following abbreviations are used to describe resonances in ^{1}H NMR spectra: br, broad; s, singlet; d, doublet; dd, double doublet; q, quartet; m, multiplet; t, triplet. HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. For chromatographic conditions, refer to the experimental data of individual compound. All biologically tested compounds attained a purity level of 95% or above by HPLC. LC-MS analysis was performed on a Waters 2790 Alliance linked up with a ZQ MicroMass spectrometer and a Waters 996 PDA detector. Atmospheric pressure chemical ionisation (APCI) or electrospray (ES) high resolution mass spectra were recorded on a Bruker microTOF Focus. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points (mp) were determined using a Stanford Research Systems Optimelt MPA100 automated melting point system and are uncorrected.

References

1) Appel, R.; Berger, G. Uber das Hydrazidosulfamid (On hydrazidosulfamide.) Chem. Ber. ***1958,*** *91, 1339-1341.*

2) Woo, L. W. L.; Lightowler, M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Heteroatom-substituted analogues of the active-site directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit estrone sulphatase by a different mechanism. J. Steroid Biochem. Mol. Biol. ***1996****, 57, 79-88.*

*2) Syntheses of Compounds **8c** and **9c**.*

2-Benzyloxy-1-methoxy-4-((*E*)-2-nitroprop-1-enyl)benzene 2b. Compound **1** (8.0 g, 33.1 mmol), ammonium acetate (2.55 g, 33.1 mmol) and nitroethane (120 mL, 1.67 mol) were stirred at 120 °C for 22h. The reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was dissolved in EtOAc (200 mL), washed with water (40 mL) and brine (2 x 40 mL), then dried ($MgSO_4$), filtered and concentrated *in vacuo*. Crystallisation from hot ethanol afforded compound **2b** as yellow crystals (6.19 g, 63%) mp 102-104 °C. δ_H 2.27 (3H, s), 3.94 (3H, s), 5.19 (2H, s), 6.91 (1H, d, *J* 2.0), 6.94 (1H, d, *J* 8.4), 7.05 (1H, dd, *J* 8.4, 2.0), 7.30-7.43 (4H, m), 7.97 (1H, s). LC/MS (APCI-): *m/z* 297.93 (M^--H).

2-Benzyloxy-1-methoxy-4-(2-nitropropyl)benzene A. Finely powdered sodium borohydride (1.52 g, 40.2 mmol) was covered with ethanol (20 mL) and a solution of compound **2b** (6.0 g, 20.1 mmol) in THF (40 mL) was added at 0 °C over 0.5h. The reaction mixture was stirred at 0 °C for 1h and at rt for 0.5h. HCl (2M, 20 mL) was added very carefully and the reaction mixture was then extracted with EtOAc (3 x 100 mL). The combined organics were washed with water (60 mL) and brine (60 mL), then dried ($MgSO_4$) and evaporated. Purification by flash column chromatography (hexane/EtOAc 4:1) afforded compound **A** as a pale green solid (3.35 g, 55%). δ_H 1.44 (3H, d, *J* 6.7), 2.87 (1H, dd, *J* 14.1, 6.9), 3.19 (1H, dd, *J* 14.1, 7.4), 3.85 (3H, s), 4.65 (1H, sept, *J* 6.9), 5.12 (2H, s), 6.65-6.71 (2H, m), 6.81 (1H, d, *J* 8.2), 7.25-7.43 (5H, m). LC/MS (APCI-): *m/z* 300.01 (M^--H).

1-(3-Benzyloxy-4-methoxyphenyl)propan-2-amine B. Raney nickel (50% slurry in water, 3.0 g) was washed in presence of a magnetic stirring bar with methanol (3 x 5 mL). Compound **A** (3.05 g, 10.1 mmol) in methanol (70 mL) was then introduced and the reaction mixture was cooled to 0 °C. Hydrazine hydrate (2.53 g, 50.5 mmol) was added in a dropwise manner at which stage the reaction was brought to 40 °C for 18h. After cooling to rt the reaction mixture was filtered through celite, washed with methanol (4 x 50 mL) and concentrated *in vacuo*. Purification by flash column

chromatography EtOAc/methanol gradient) afforded compound **B** as a pale yellow oil (2.074 g, 76%). δ_H 1.04 (3H, d, *J* 6.4), 2.36 (1H, dd, *J* 13.3, 8.2), 2.58 (1H, dd, *J* 13.3, 5.2), 2.98-3.10 (1H, m), 3.86 (3H, s), 5.13 (2H, s), 6.67-6.83 (3H, m), 7.25-7.43 (5H, m). LC/MS (ES+): *m/z* 272.19 (M^++H).

***N*-(1-(3-Benzyloxy-4-methoxyphenyl)propan-2-yl)acetamide 3b.** Compound **B** (2.07 g, 7.6 mmol) was dissolved in DCM (20 mL) and TEA (1.6 mL, 11.4 mmol). Acetic anhydride was then added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 1h and at rt for 23h. Water (30 mL) was added carefully and the mixture extracted with DCM (4 x 30 mL). The combined organics were washed with brine (30 mL), then dried ($MgSO_4$), filtered and concentrated in vacuo to afford compound **3b** as a white powder (2.268 g, 95%); mp 139-142 °C. δ_H 0.99 (3H, d, *J* 6.7), 1.91 (3H, s), 2.57 (1H, dd, *J* 13.6, 7.2), 2.70 (1H, dd, *J* 13.6, 5.7), 3.85 (3H, s), 4.08-4.22 (1H, m), 5.12 (2H, s), 5.17-5.23 (1H, m), 6.68-6.71 (2H, m), 6.79-6.82 (1H, m), 7.24-7.44 (5H, m). LC/MS (APCI-): *m/z* 312.29 (M^--H).

2-Acetyl-6-benzyloxy-7-methoxy-3-methyl-1,2,3,4-tetrahydroisoquinoline 7. Compound **3b** (2.21 g, 7.04 mmol) was treated with paraformaldehyde (6.32 g, 210 mmol) and *p*-TSA (120 mg, 0.64 mmol in toluene (55 mL) at 120 °C for 22h. The reaction mixture was cooled to rt, filtered and evaporated. EtOAc (60 mL) was added and the organic layer was washed with water (3 x 50 mL), brine (50 mL) then dried ($MgSO_4$) and evaporated to afford compound **7** as a colourless oil (2.15 g, 94%). δ_H 1.04 and 1.12 (3H, 2d, *J* 7.2 and 6.7), 2.14 and 2.17 (3H, 2s), 2.38-2.51 (1H, m), 2.89-3.06 (1H, m), 3.84 and 3.85 (3H, 2s), 4.06-4.60 (2H, m), 4.96-5.14 (1H, m), 5.08 and 5.10 (2H, 2s), 6.61 and 6.64 (1H, 2s), 6.62 and 6.67 (1H, 2s), 7.28-7.44 (5H, m). LC/MS (ES+): *m/z* 348.19 (M^++Na).

6-Benzyloxy-7-methoxy-3-methyl-1,2,3,4-tetrahydroisoquinoline C. Compound **7** (3.16 g, 9.7 mmol) was treated with potassium hydroxide (5.44 g, 97.0 mmol) in ethanol (36 mL) and water (12 mL) at 120 °C for 66h. The reaction mixture was then cooled to rt and concentrated. Water (30 mL)

was then added and the mixture was then extracted with DCM (3 x 50 mL). The combined organics were washed with brine (50 mL), then dried ($MgSO_4$) and evaporated to afford compound **C** as a beige solid (2.51 g, 91%) mp 110-112 °C. δ_H 1.20 (3H, d, *J* 6.4 Hz), 1.78 (1H, s, br), 2.35 (1H, dd, *J* 15.8, 10.6), 2.60 (1H, dd, *J* 15.8, 3.7), 2.88-3.01 (1H, m), 3.82 (3H, s), 3.93 (1H, d, *J* 15.6), 4.03 (1H, d, *J* 15.6), 5.09 (2H, s), 6.53 (1H, s), 6.56 (1H, s), 7.23-7.44 (5H, m). LC/MS (ES+): *m/z* 284.13 (M^++H).

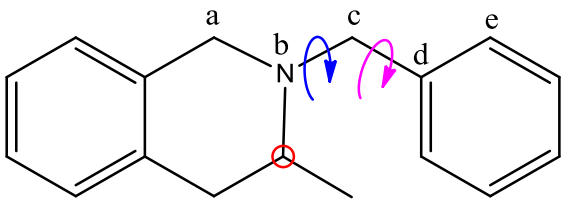
6-Benzyloxy-7-methoxy-2-(3-methoxybenzyl)-3-methyl-1,2,3,4-tetrahydroisoquinoline D. Compound **C** (300 mg, 1.1 mmol) was treated with 3-methoxybenzyl chloride (0.18 mL, 1.3 mmol) and TEA (0.30 mL, 2.1 mmol) in ethanol (3.0 mL) at 130 °C for 1.5h under microwave irradiation. The mixture was then evaporated and the residues dissolved in EtOAc (30 mL). The solution was then washed with brine (30 mL), dried, and evaporated. Flash column chromatography (hexane/EtOAc gradient) afforded **D** as a colourless oil (357 mg, 84%). δ_H 1.12 (3H, d, *J* 6.4), 2.47 (1H, dd, *J* 16.1, 5.7), 2.86 (1H, dd, *J* 16.1, 4.9), 3.01-3.11 (1H, m), 3.55 (2H, t, *J* 13.4), 3.76-3.81 (2H, m), 3.80 (6H, s), 5.10 (2H, s), 6.47 (1H, s), 6.60 (1H, s), 6.79 (1H, ddd, *J* 8.2, 2.5, 1.0), 6.94-6.97 (2H, m), 7.15-7.45 (6H, m). LC/MS (ES+): *m/z* 404.25 (M^++H).

6-Hydroxy-7-methoxy-2-(3-methoxybenzyl)-3-methyl-1,2,3,4-tetrahydroisoquinoline 8c. Pd/C (10%, 33 mg) was covered with ethanol (6 mL) and compound **D** (330 mg, 0.82 mmol) was added as solution in THF (6 mL). The reaction mixture was degassed then placed under hydrogen at rt for 0.75h before filtering through celite. The filtrate was evaporated and purified by flash column chromatography (hexane/EtOAc 2:1) to give an oil which crystallized from hexane to afford compound **8c** as a pale yellow solid (118 mg, 46%); mp 100-105 °C. δ_H 1.12 (3H, d, *J* 6.7), 2.49 (1H, dd, *J* 16.1, 5.8), 2.89 (1H, dd, *J* 16.1, 4.8), 3.01-3.12 (1H, m), 3.48-3.74 (4H, m), 3.78 (3H, s), 3.79 (3H, s), 5.42 (1H, s), 6.42 (1H, s), 6.63 (1H, s), 6.77-6.81 (1H, m), 6.93-6.96 (2H, m), 7.21 (1H, d, *J* 8.2). LC/MS (ES+): *m/z* 314.18 (M^++H). HRMS (ES+): *m/z* found 314.1748; $C_{19}H_{24}NO_3^+$ (M^++H) requires 314.1751.

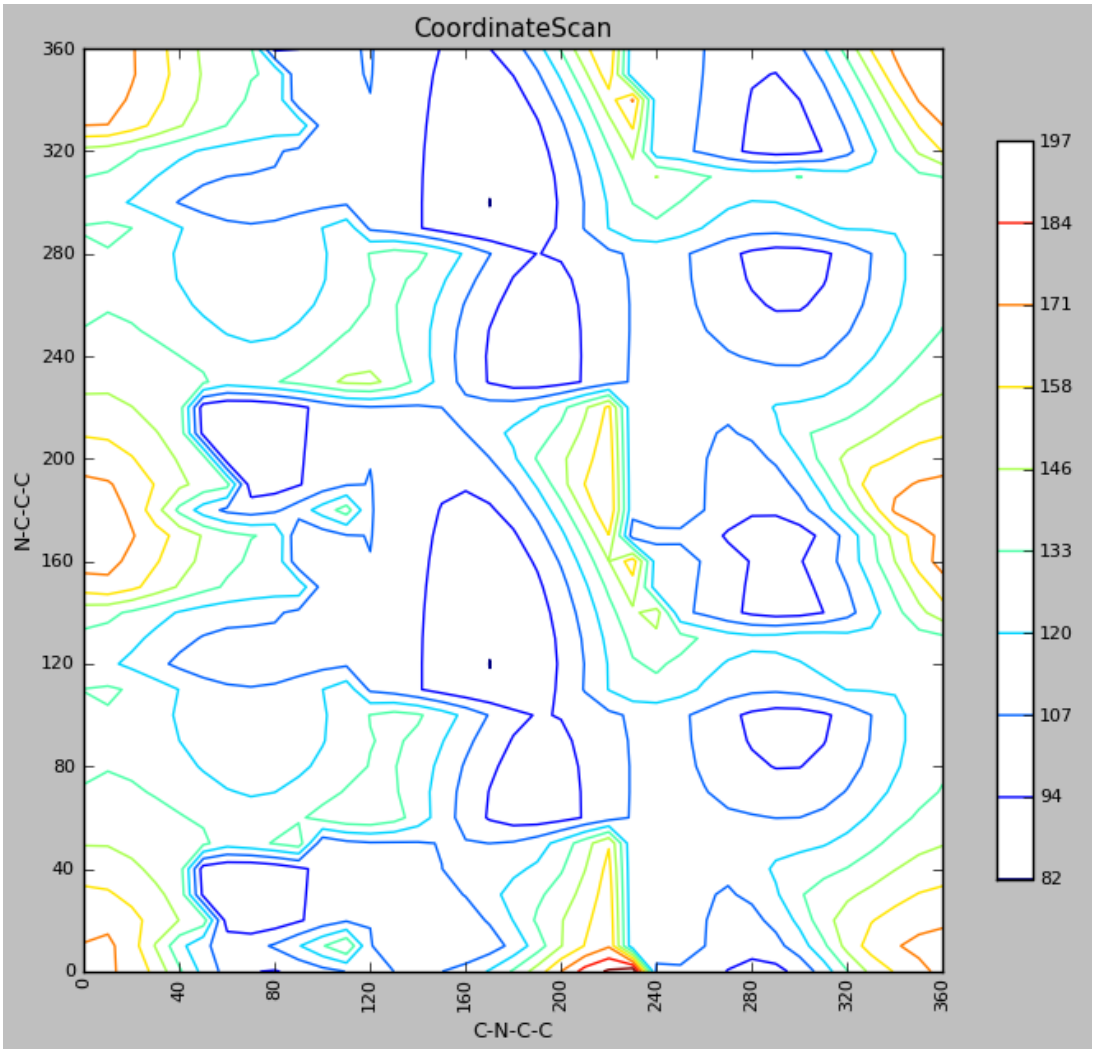
7-Methoxy-2-(3-methoxybenzyl)-3-methyl-6-*O*-sulfamoyl-1,2,3,4-tetrahydroisoquinoline 9c.

Sulfamoyl chloride (0.5M in toluene, 2.8 mL, 1.4 mmol) was concentrated *in vacuo* and cooled to 0 °C until the sulfamoyl chloride solidified. DMA (1.0 mL) was added and the resulting solution was added directly to **8c** (88 mg, 0.28 mmol) at 0 °C under nitrogen. The reaction mixture was stirred at rt for 16h. Sodium bicarbonate (saturated, 50 mL) was added and the mixture was extracted with EtOAc (100 mL). The organic layer was washed repeatedly with water (50 mL portions), then brine, then dried ($MgSO_4$) and evaporated. Flash column chromatography (hexane to EtOAc) afforded compound **9c** as a pale yellow solid (56 mg, 51%); mp 154-156 °C. δ_H (DMSO-d_6) 1.06 (3H, d, *J* 6.5), 2.44-2.51 (1H, m), 2.89 (1H, dd, *J* 16.0, 4.6), 3.01-3.07 (1H, m), 3.46-3.60 (4H, m), 3.70 (3H, s), 3.73 (3H, s), 6.79 (1H, s), 6.82 (1H, dd, *J* 7.2, 2.0), 6.89-6.94 (2H, m), 7.02 (1H, s), 7.24 (1H, t, *J* 8.0), 7.85 (2H, s, br). LC/MS (ES+): *m/z* 393.10 (M^++H).

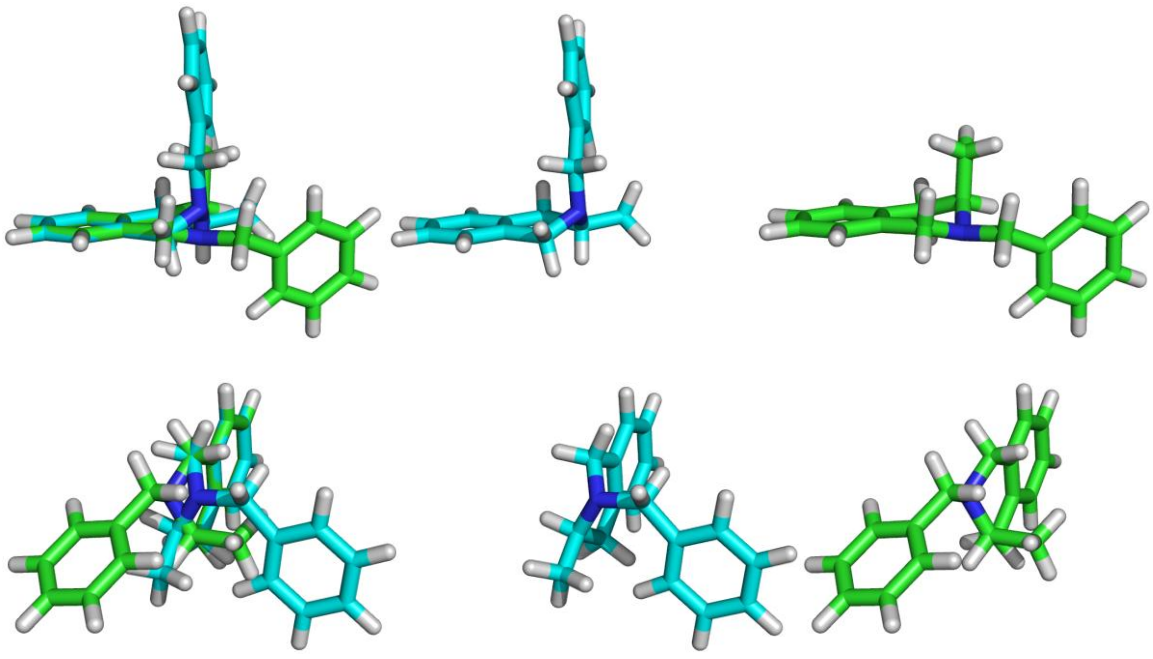
Modeling the compounds.



The Schrödinger software (http://www.schrodinger.com) was used for the modelling work. The molecules were built using the Build tools in Maestro, and then minimised. The Coordinate Scan component of the MacroModel package was used to rotate the following bonds and calculate the energies of the resultant molecules: the dihedral angle a-b-c-d, indicated by the blue arrow, was rotated through 360 degrees in steps of 10 degrees; and, the dihedral angle b-c-d-e, indicated by the purple arrow, was independently rotated through 360 degrees in steps of 10 degrees. The energy for each conformer was calculated. The C-3 carbon circled in red is chiral: the results for the *R*-isomer are presented below – those obtained for the *S*-isomer are comparable.



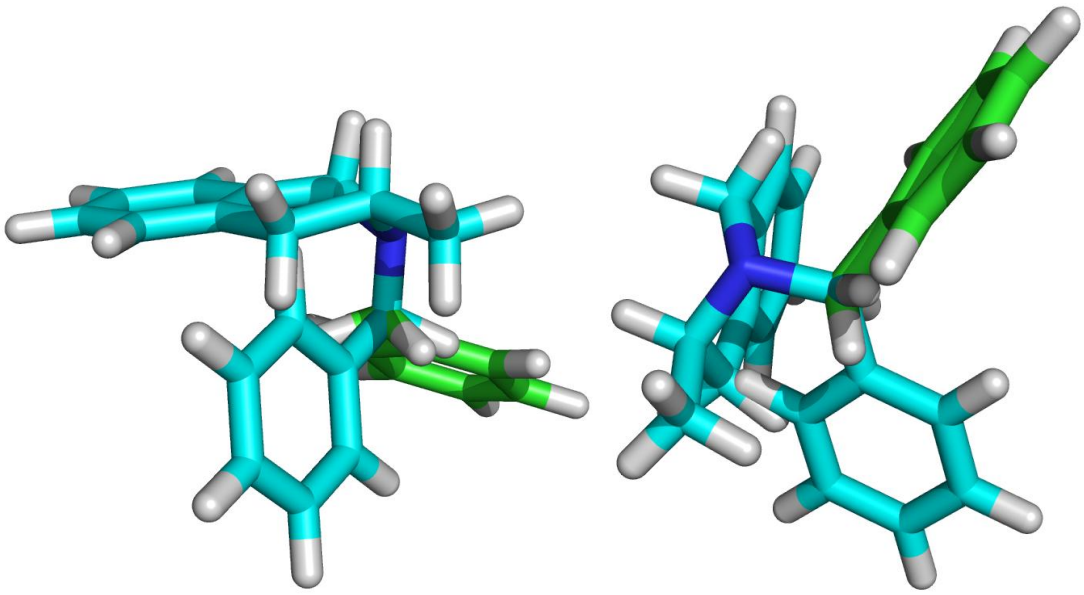
The energy plot of the Coordinate Scan is shown above. The minimum energy conformation, shown below in green, has a total energy of 81.466 kJ/mol, and the maximum energy conformation, shown below in cyan, has a total energy of 209.481 kJ/mol.



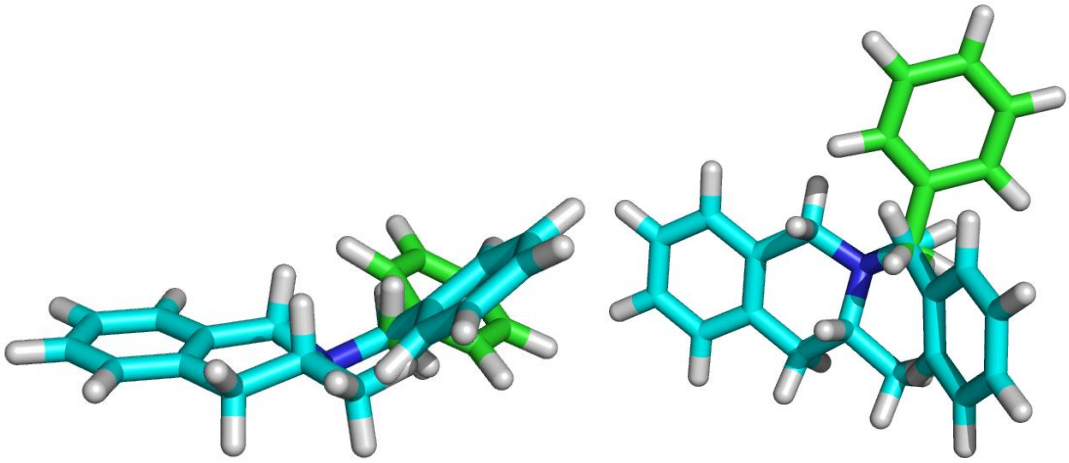
One problem with the Coordinate Scan is that after each rotation the molecule is put through a brief minimization procedure. This results in the THIQ core of the molecule moving, specifically the nitrogen is in different positions in the minimum and maximum energy conformations. To look at the minimum and maximum energy conformations without movement of the THIQ core a range of structures have been built and their energies calculated using the Current Energy component of the MacroModel package. The Current Energy calculation was performed without any constraints or restraints. For the molecules above this results in calculated energy values similar to those derived from the Coordinate Scan, as shown in the Table below.

	Coordinate Scan Energy Calculation (kJ/mol)	Current Energy Calculation (kJ/mol)
Minimum Energy Conformation	81.466	81.471
Maximum Energy Conformation	209.481	207.886

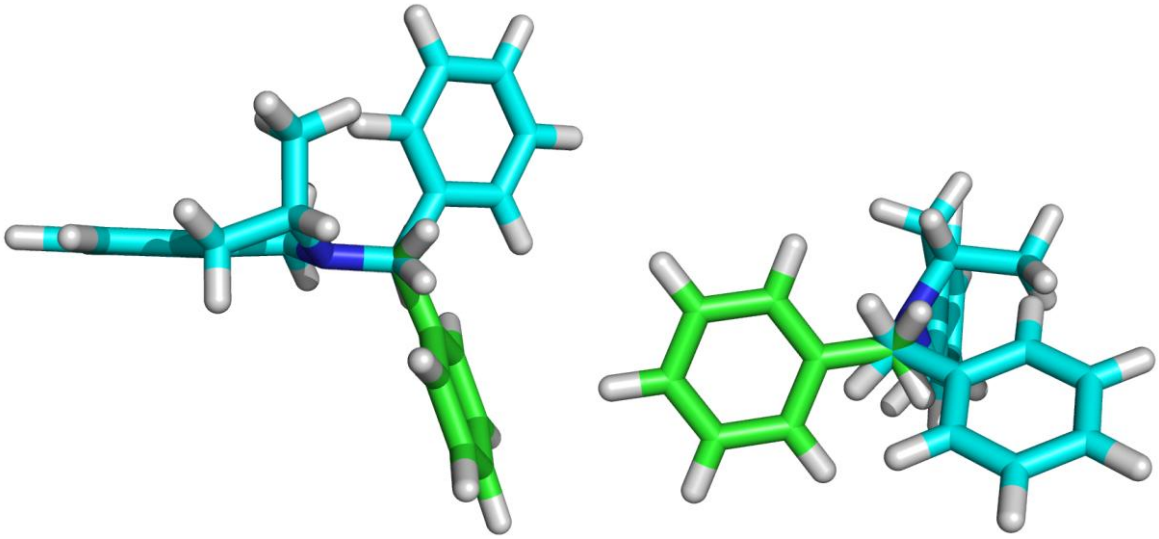
In the figures below the minimum energy conformations are in green and the maximum energy conformations are in cyan. In this approach the lone pair on the nitrogen can be construed as being a fourth functional group attached to the nitrogen, thus making the nitrogen chiral. For clarity the stereochemistry of the nitrogen and the methyl group are listed below. The calculated maximum energies are much higher because clashes have been deliberately introduced.



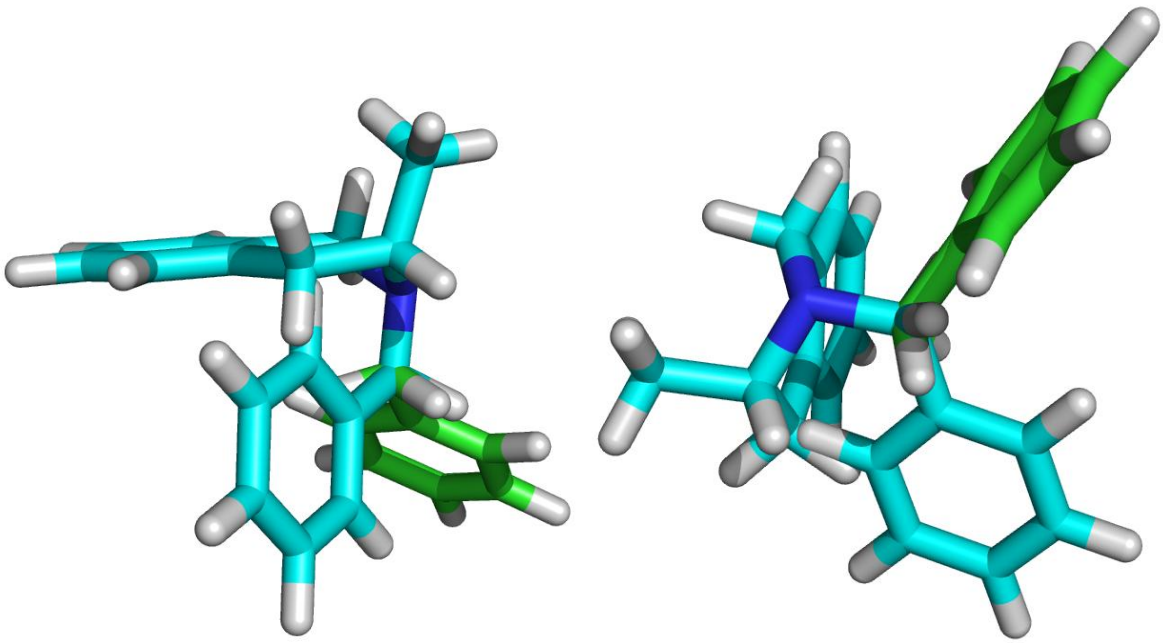
Mol101 & Mol01a. Nitrogen – ***S***. Methyl – ***R***.
Minimum E = 87.499 kJ/mol.
Maximum E = 25039.605 kJ/mol.



Mol02 & Mol02a. Nitrogen – ***R***. Methyl – ***R***.
Minimum E = 127.004 kJ/mol.
Maximum E = 10420.247 kJ/mol.



Mol03 & Mol03a. Nitrogen – ***R***. Methyl – ***S***.
Minimum E = 113.826 kJ/mol.
Maximum E = 4578.335 kJ/mol.



Mol04 & Mol04a. Nitrogen – ***S***. Methyl – ***S***.
Minimum E = 94.117 kJ/mol.
Maximum E = 27402.256 kJ/mol.