Supporting Information

Tailor Made LasR Agonists Modulate Quorum Sensing in *Pseudomonas aeruginosa*

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SurfRx

We used SurfRx, developed by InSilicore Ltd., to retrieve pharmacophoric points from the LasR-C12 complex. First, we assigned atomic charges to the ligand and to the protein using MMFF94 and CHARMM force fields, respectively. We then derived Solvent Excluded Surface (SES) around each of the structures. Electrostatic potentials were calculated using Distance Dependent Dielectric (DDD) model for the ligand and Poisson-Boltzmann (PB) distribution model for the target, respectively. These potentials were separately mapped on their corresponding SES surfaces.

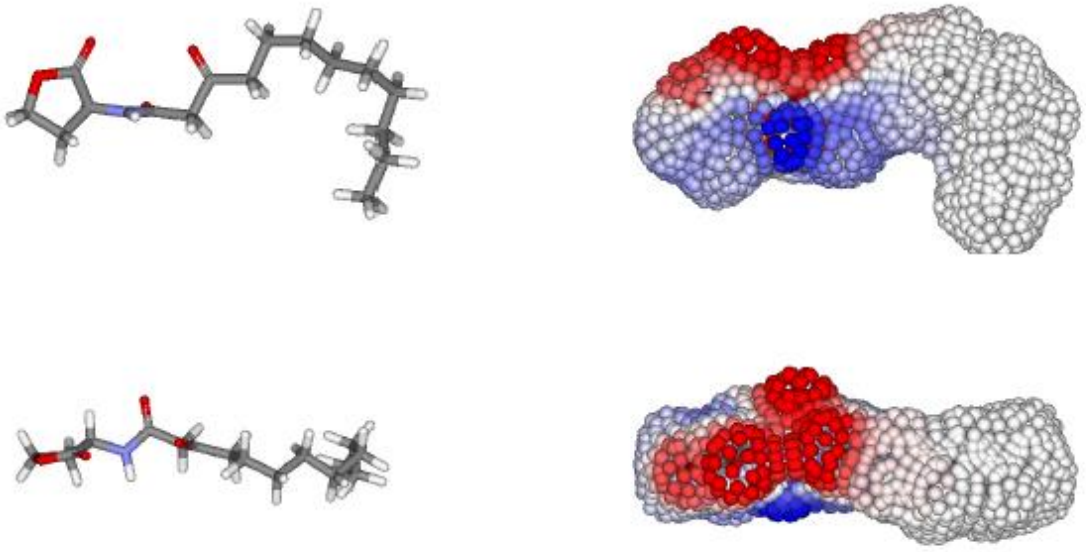


Fig. 1: Side and top views of the C12 ligand from PDB structure 3IX3 (left) and its SES colored by electrostatic potentials.

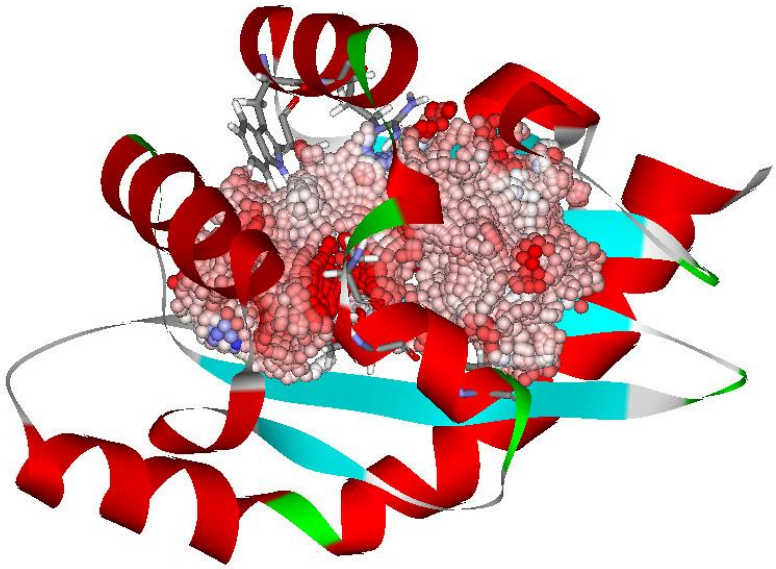


Fig. 2: LasR and its SES colored by electrostatic potentials.

Using the 'Compare' module of SurfRx we explored important interaction points between the target and the ligand. This was achieved by filtering the SES points based on energy cutoffs and using a partition labeling method to group them. Each group yielded a single pharmacophore point that stores information about the position, strength (weight), size (radius) and directionality of that group. A scoring function that is based on 3D Gaussian volume overlap was then used to determine the relative strength of interaction between ligand and target pharmacophores. Fig. 3 and 4, show pharmacophore points derived from the ligand and from the target, respectively, for negative (HBA), positive (HBD) or non-polar (PHOB) fields.

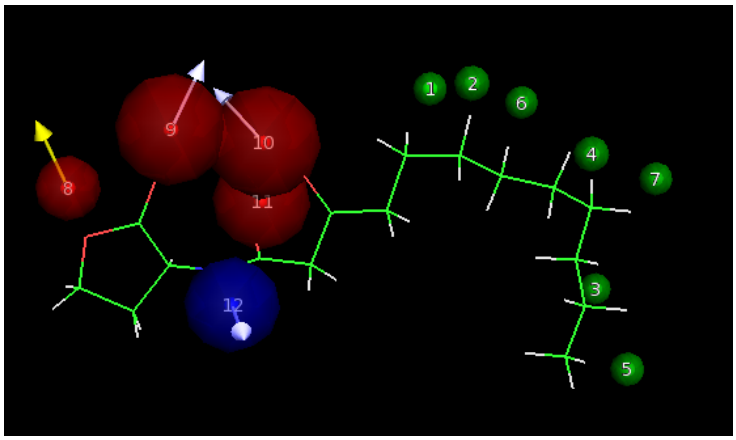


Fig. 3: C12 ligand and its derived pharmacophoric points. Size of points encodes the strength (weight) of the pharmacophores, arrows are given for polar points directionality. Colors: red (HBA), blue (HBD) and green (PHOB).

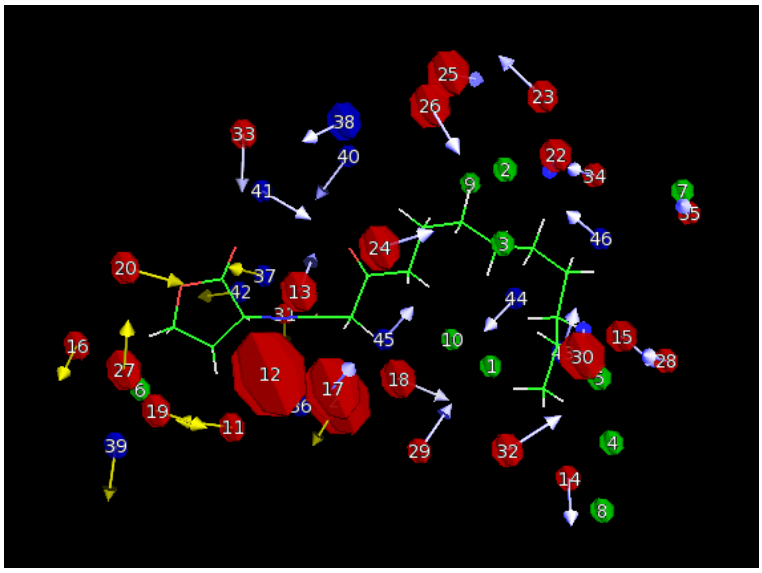


Fig. 4: LasR and its derived pharmacophoric points. Size of points encodes the strength (weight) of the pharmacophores, arrows are given for polar points directionality. Colors: red (HBA), blue (HBD) and green (PHOB).

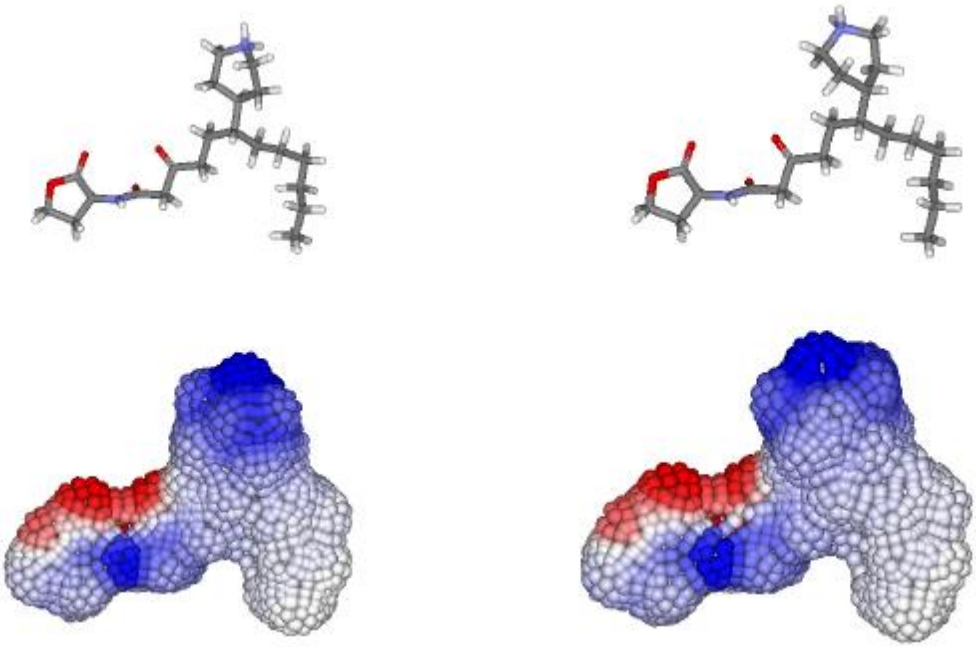


Fig. 5: (R)-3-oxo-N-((S)-2-oxotetrahydrofuran-3-yl)-6-(piperidin-4-yl)dodecanamide (C12-piperidine). Given are two possible conformers (top) and their corresponding SES maps (bottom).

Table 1: Summary of weight, radius and overlap score of the donor features and their conformers

Substitution (conformer)	HBD weight (kT)[a]	HBD radius (Å)[a]	HBA weight (kT)[a]	HBA radius (Å)[a]	Overlap score (local)[b]	Overlap score (global)[c]	Deviation (%)[d]
C12-HSL						578	
NH_2 (1)	9.4+9.0	8.0+3.5	15.0	2.4	338	916	0.00
NH_2 (2)	9.3+9.3	9.2+2.9	14.9	2.3	252	865	4.05
NH_2 (3)	8.9+8.9	8.9+3.5	14.2	2.5	77	654	0.15
OH (1)	11.1	3.3	14.8	2.7	4	609	4.43
OH (2)	11.1	3.1	13.4	3.7	-77	517	3.09
OH (3)	11.4	2.6	14.9	3.1	94	746	9.92
Phenol (1)	14.5+6.8	3.2+4.8	9.6+6.4	3.8+1.6	17	610	2.46
Phenol (2)	14.6+6.5	3.7+4.8	9.7+6.4	4.1+1.6	447	1006	1.85
Sulfonamide (1)	11+10.6	3.5+3.9	13.4+13.0	5.1+5.1	-686	-69	36.11
Sulfonamide (2)	11.4+8.5	4.0+4.0	13.1+11.8	4.9+5.2	-223	401	11.47
Urea (1)	12.7+12+10.0	3.6+3.5+3.7	15.9	4.5	432	1112	9.17
Urea (2)	12.5+12.4+8.5	3.3+3.0+3.6	14.3	5.0	-118	538	14.50
Amidine (1)	12.6+12.3+9.8	3.6+3.9+2.6	13.9	3.9	543	1113	0.71
Amidine (2)	12.3+12.2+10.4	3.7+3.5+3.0	14.3	3.6	311	891	0.22
Imidazole (1)	13.1+12.4+5.1	1.2+5.6+4.2	13.6	6.2	819	1393	0.29
Imidazole (2)	14.1+11.8+4.5	0.7+5.4+3.2	11.4	5.6	84	653	1.36
Aniline	10.9+10.8	9.5+3.5	8.6+8.5+6.8 +6.0	2.6+2.4+2.1 +0.3	424	1034	3.09
Piperidine (1)	22.1+12.1+12.0 +9.8+7.6	7.1+6.4+8.3+8.2 +3.7			2647	3033	5.95
Piperidine (2)	22.1+12.0+12.3 +10.4+7.7	7.1+7.7+6.9+7.6 +1.8			1584	2206	1.99

[a] - Multiple pharmacophore occurrences are separated by a plus sign. The order of features is kept between the weight and radius columns.
[b] - Local overlap score refers to the summation of strong individual overlap scores (|x| >10) resulting from interactions of pharmacophores corresponding to the ligand substituent part
[c] - Global overlap score refers to the summation of strong individual overlap scores (|x| >10) resulting from interactions of all ligand pharmacophores
[d] - Deviation is the % change of global score from the sum of local score and score of parent C12 compound

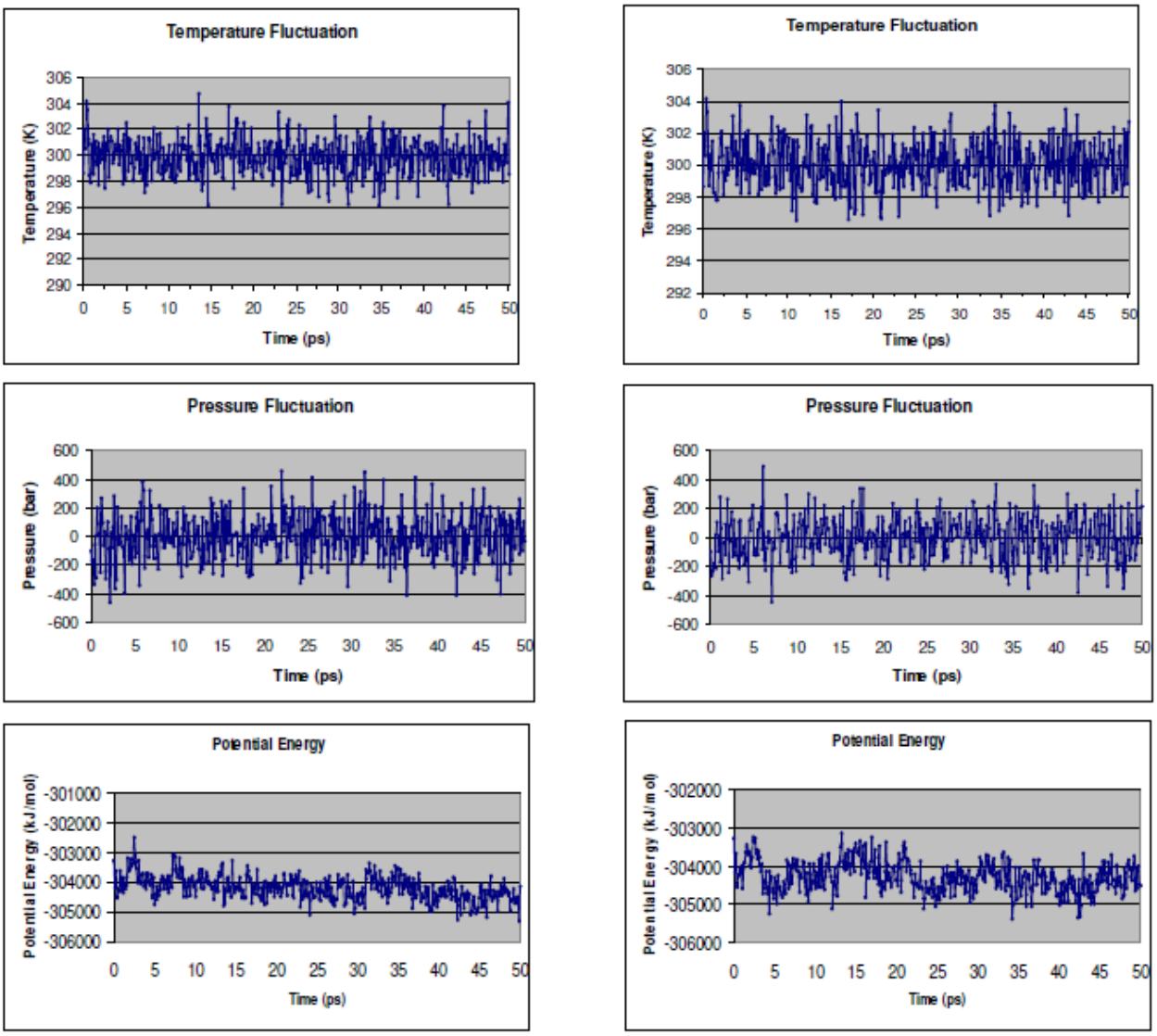


Fig. 6: Stability of MD runs for C12-piperidine. The temperature (top) and pressure (center) are stable along the MD simulation while potential energy (bottom) is converged. On the left are the parameters from the ligand restrained simulation, while on the right the unstrained simulation is shown.

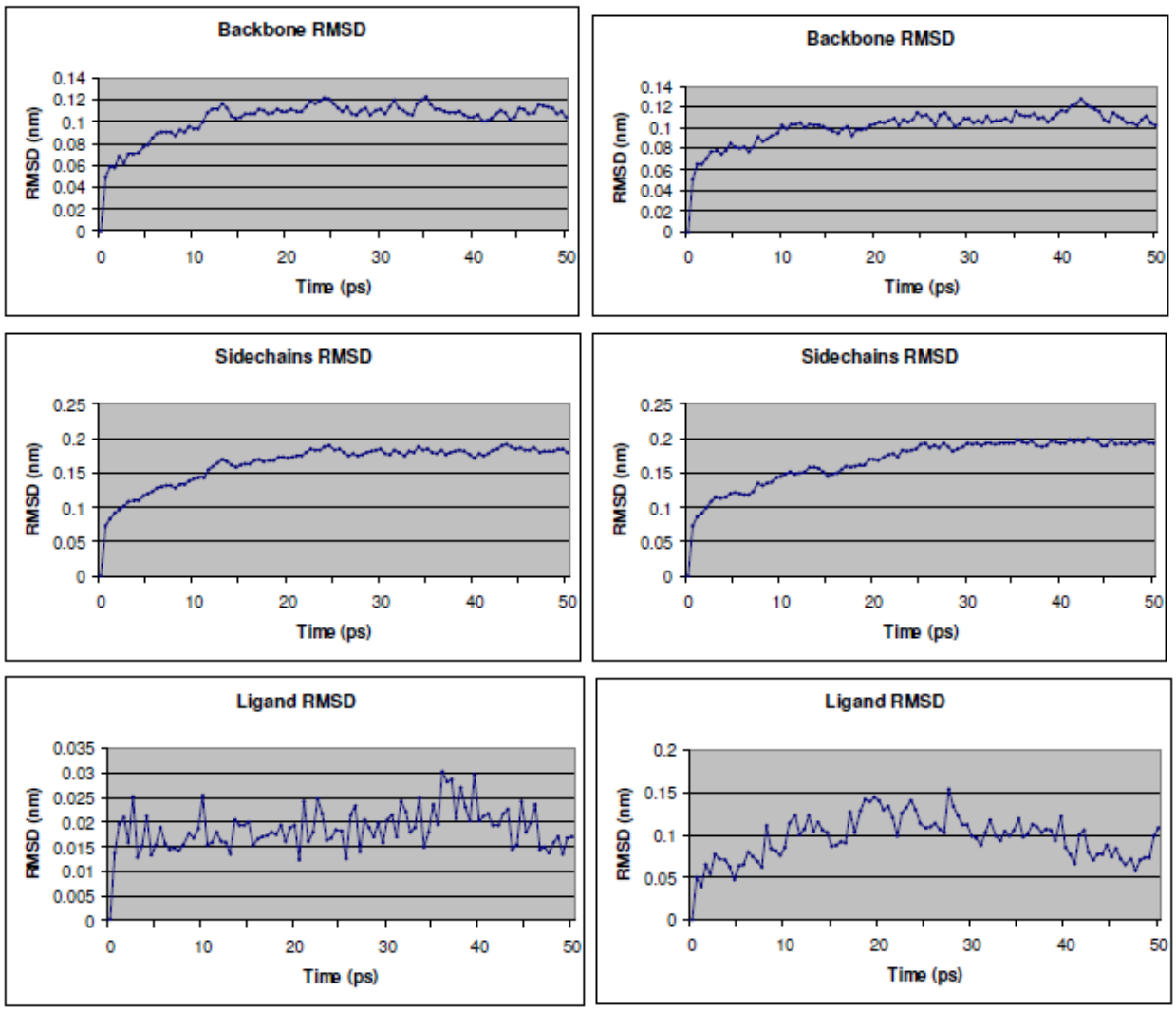


Fig. 7: Root mean square deviation (RMSD) of protein backbone (top), protein side chains (center), and ligand (bottom) in C12-piperidine MD simulations. In all cases a steady state is reached. On the left are the RMSD values from the ligand restrained simulation, while on the right the unstrained simulation is shown.

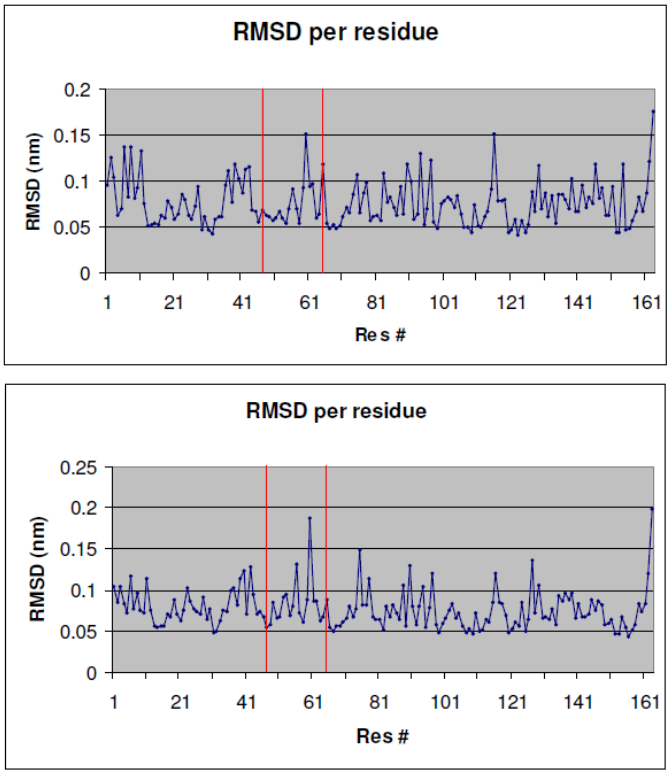


Fig. 8: RMSD per residue in C12-piperidine MD simulations. At the top are the parameters from the ligand restrained simulation, while at the bottom the unstrained simulation is given. The movements of Tyr47 (marked by the left red line) and Asp65 (marked by the right red line) during simulation are minor.

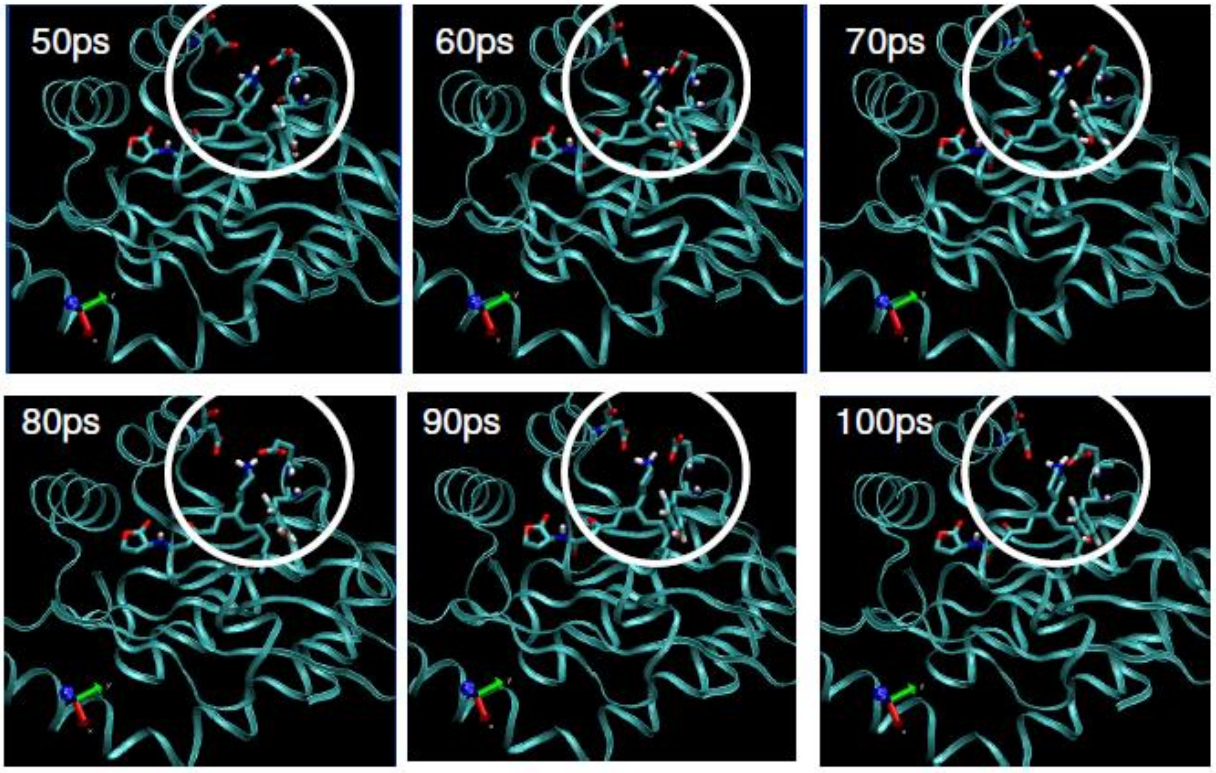
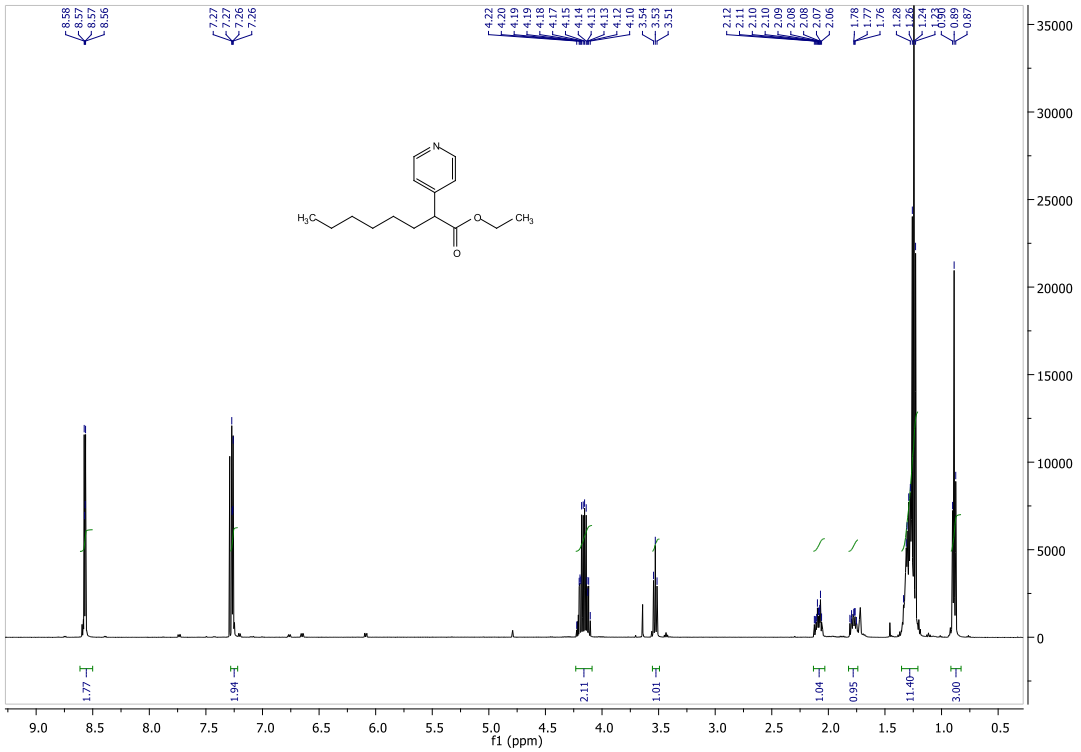
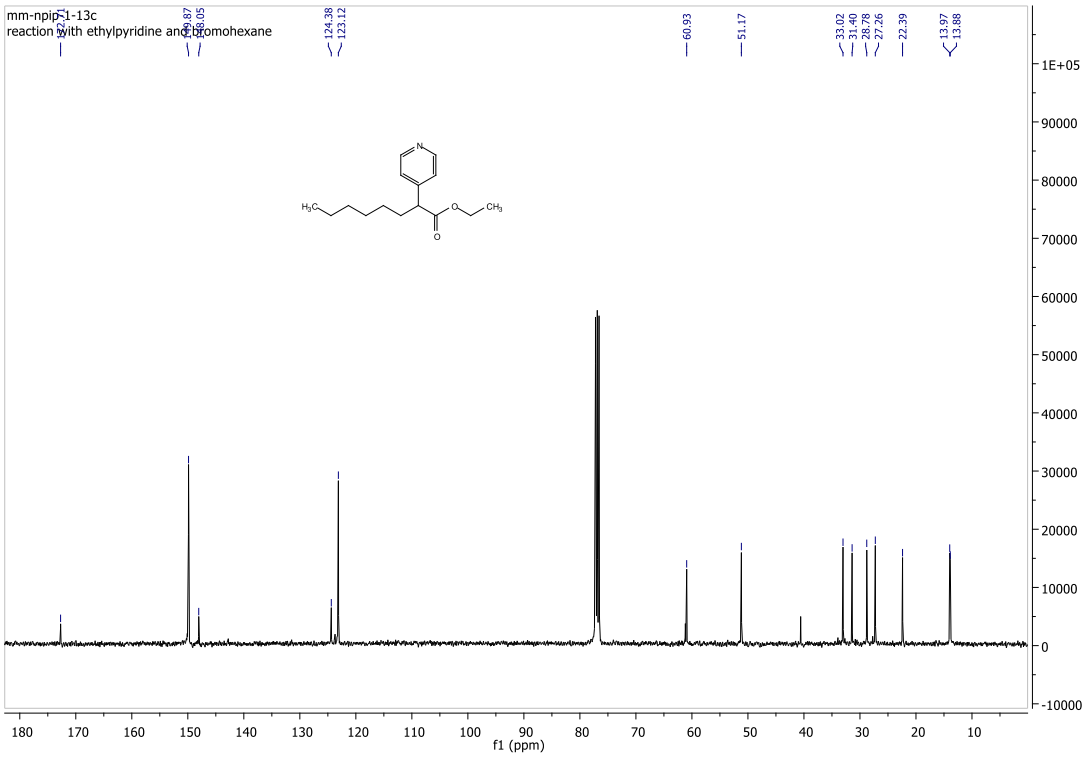


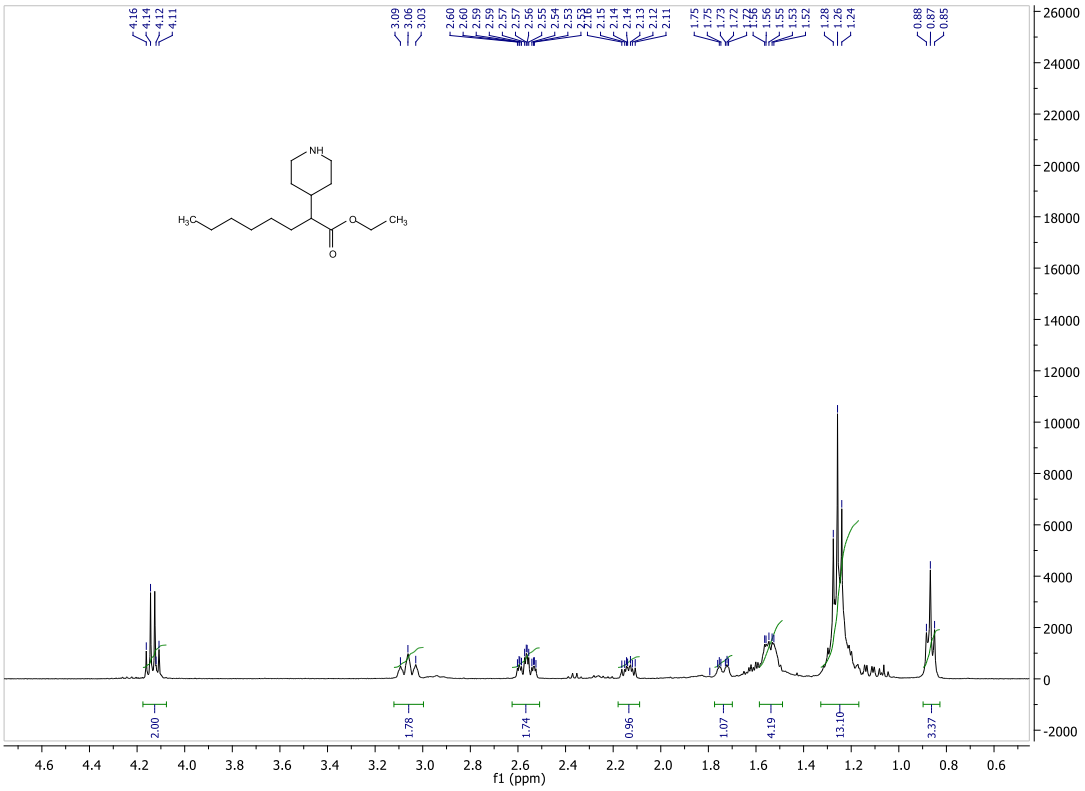
Fig. 9: Representative trajectories from advanced MD simulations of restrained C12-piperidine. The cavity accommodating the piperidine moiety is circled. Note minor movements in position of the piperidine ring and relevant residues residing in this cavity.



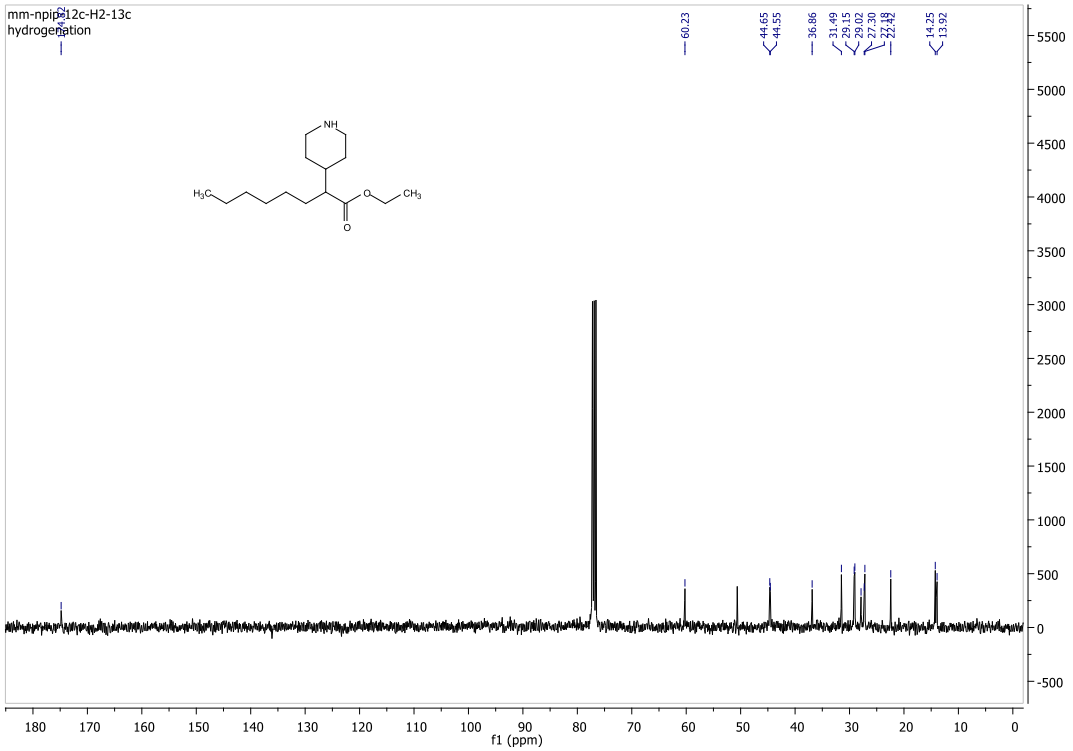
^{1}H-NMR spectrum of **1** in $CDCl_3$



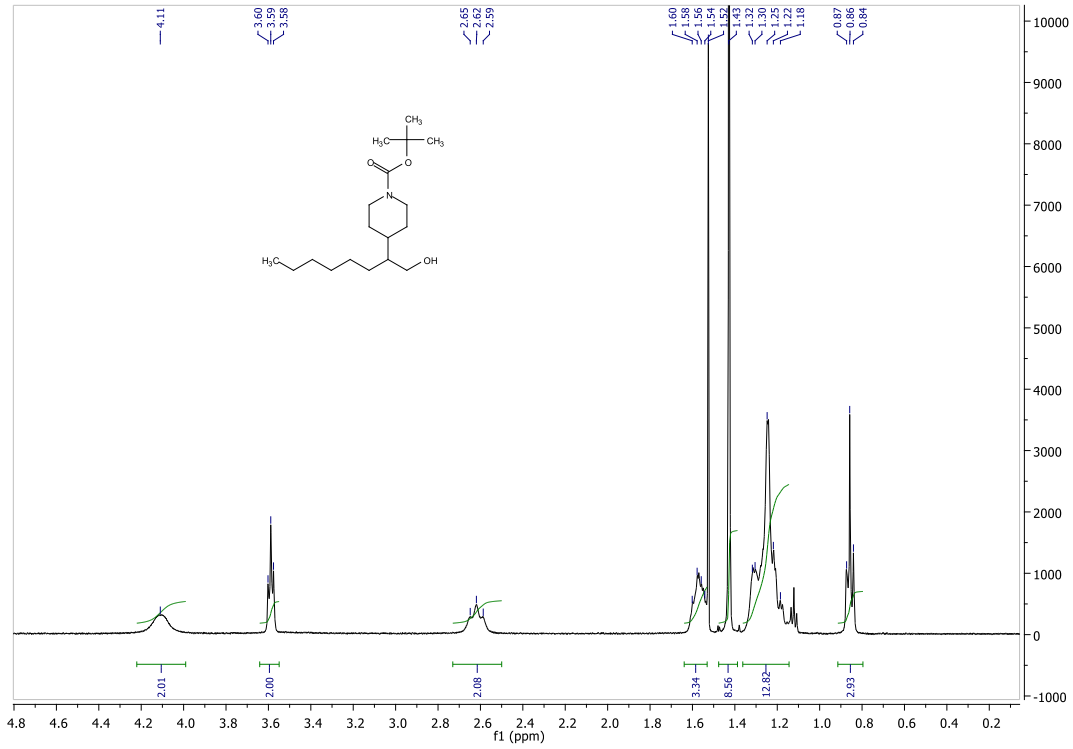
^{13}C NMR spectrum of **1** in $CDCl_3$



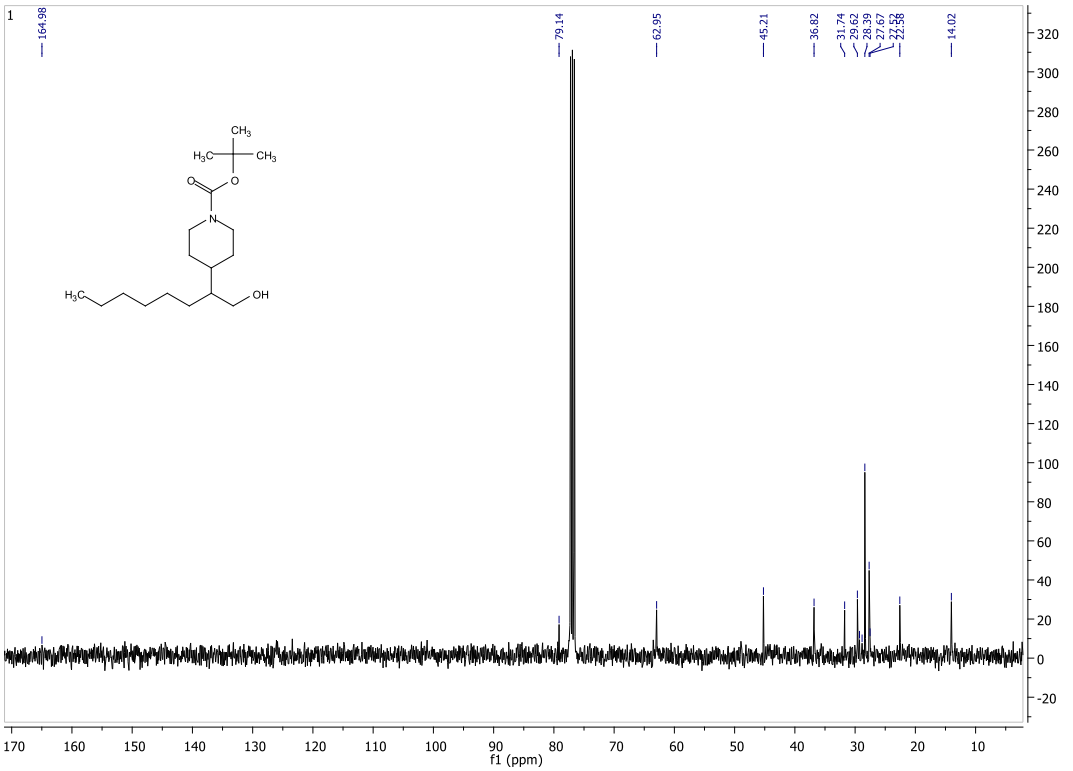
^{1}H NMR spectrum of **2** in $CDCl_3$



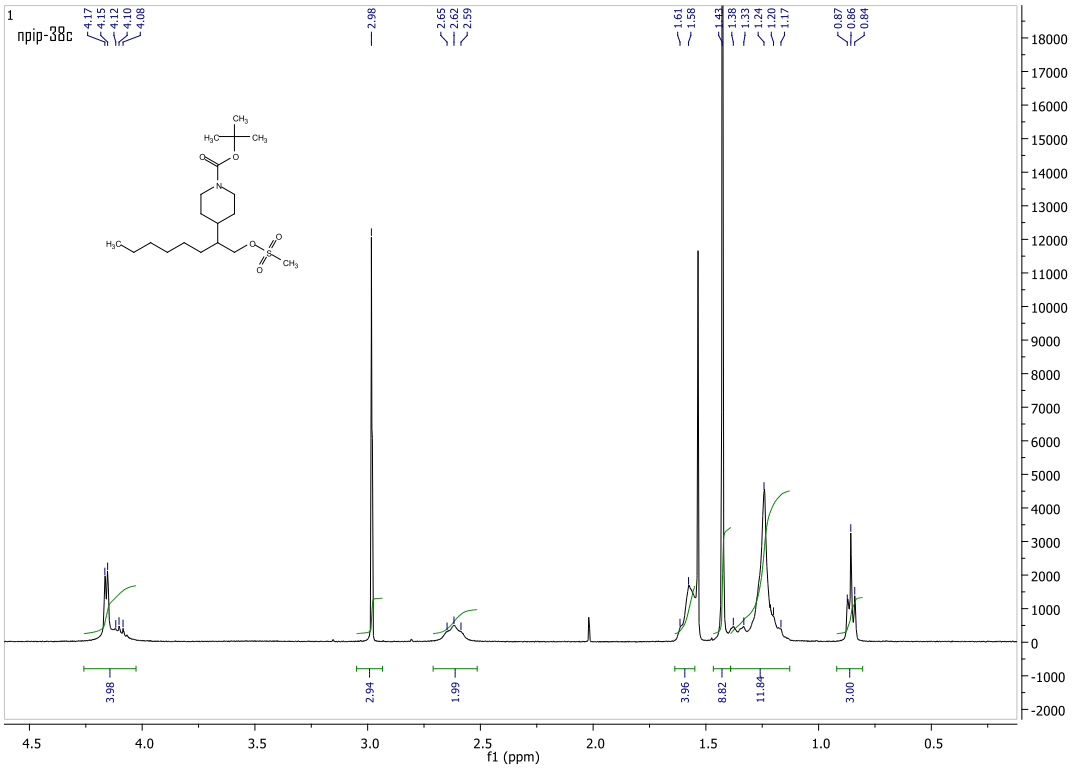
^{13}C NMR spectrum of **2** in $CDCl_3$



^{1}H NMR spectrum of **3** in $CDCl_3$



^{13}C NMR spectrum of **3** in $CDCl_3$



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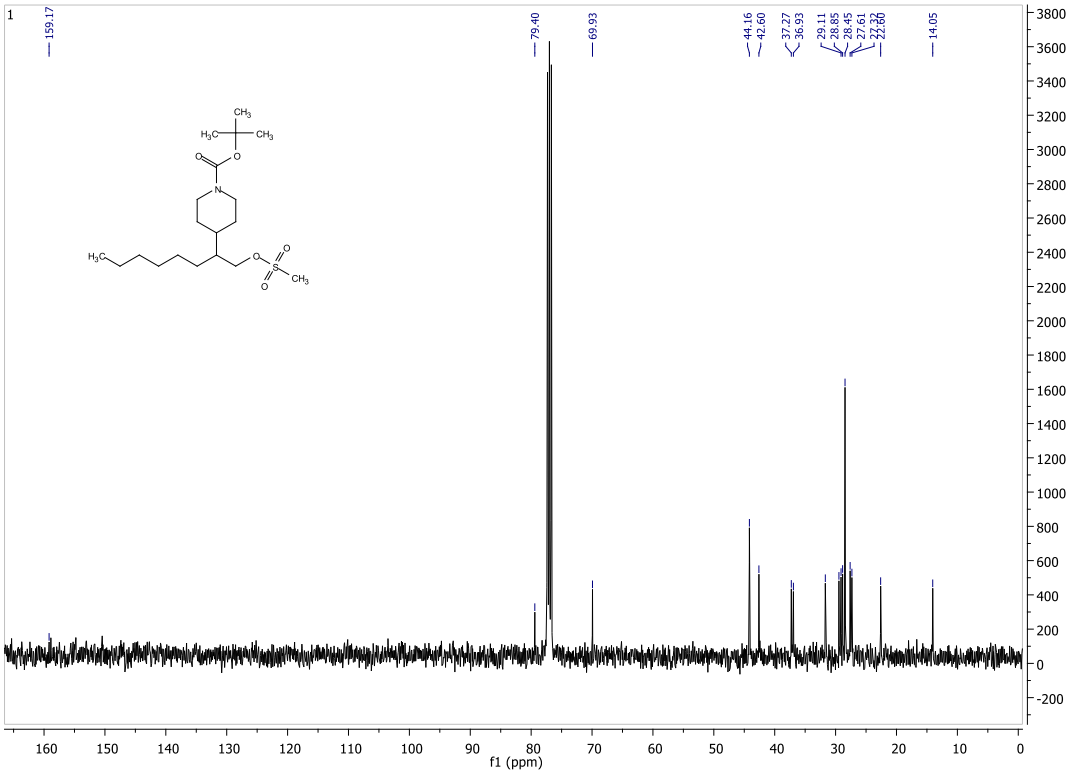
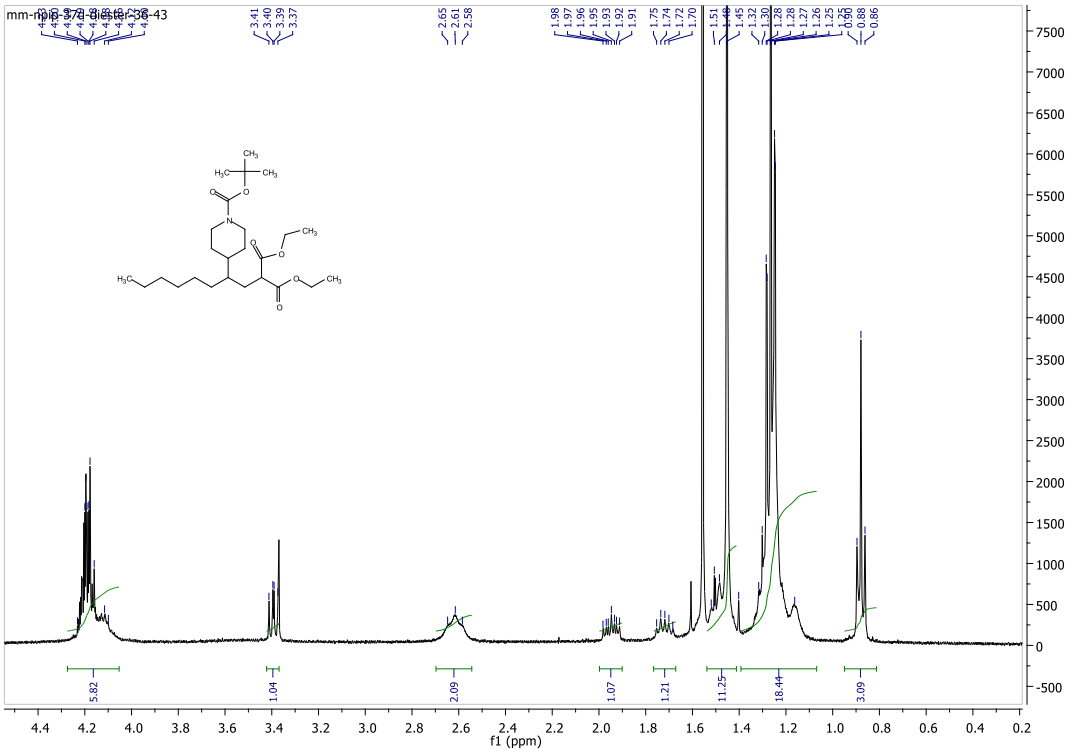
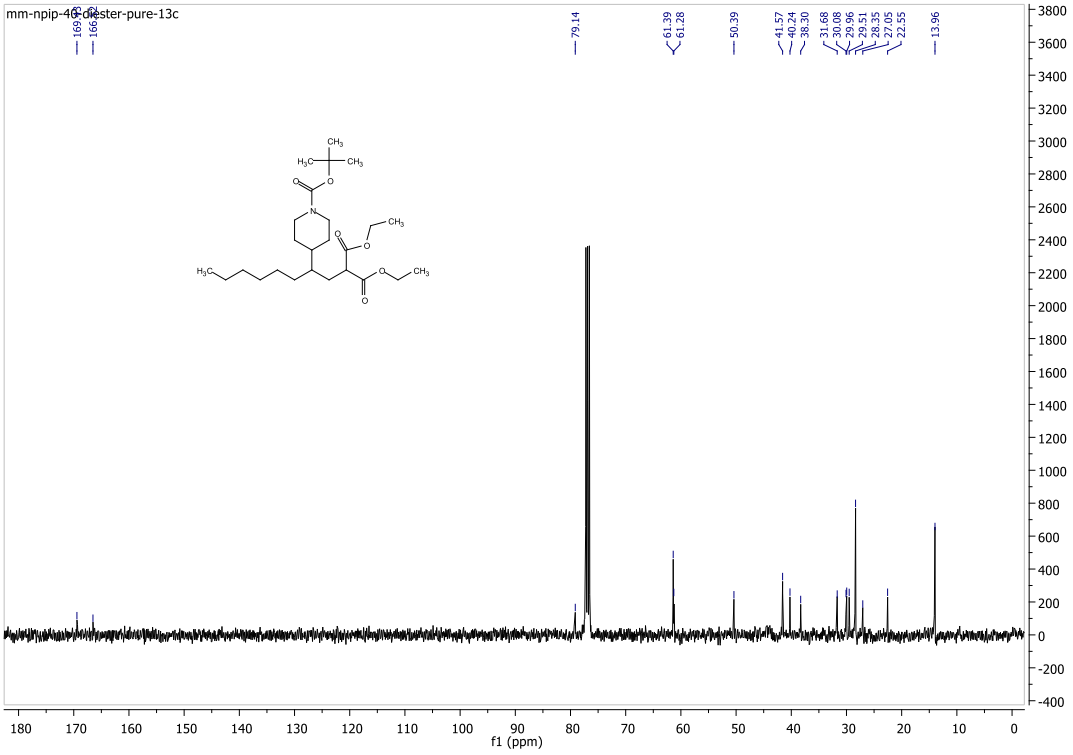


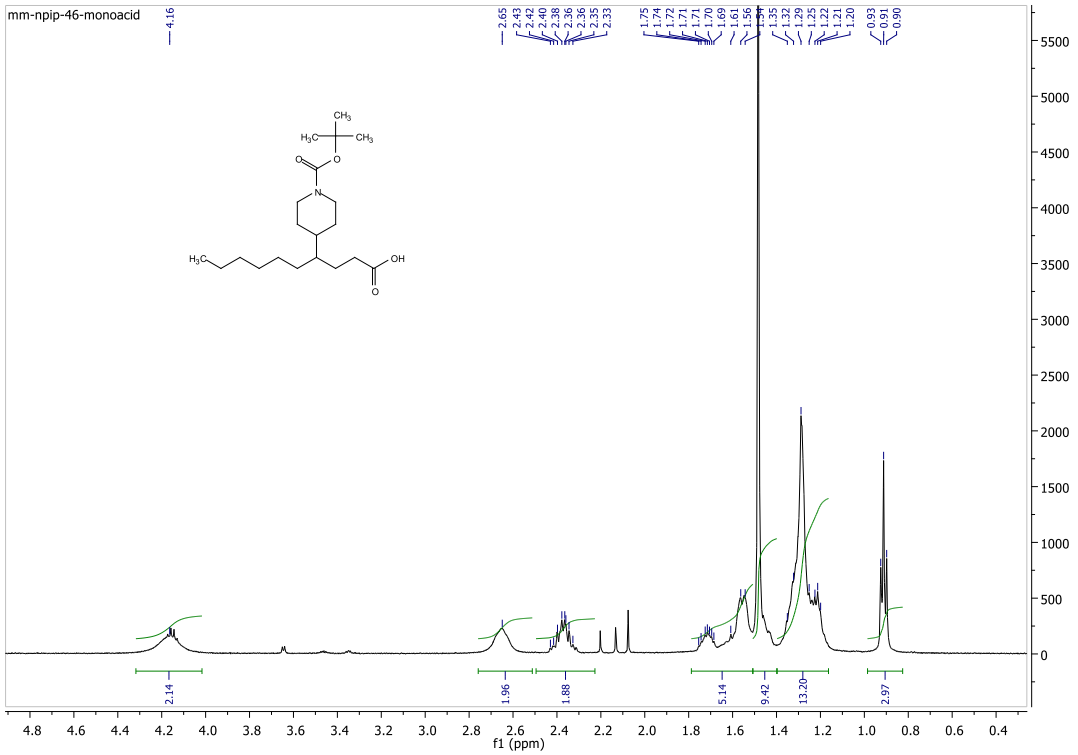
^{13}C NMR spectrum of **4** in $CDCl_3$



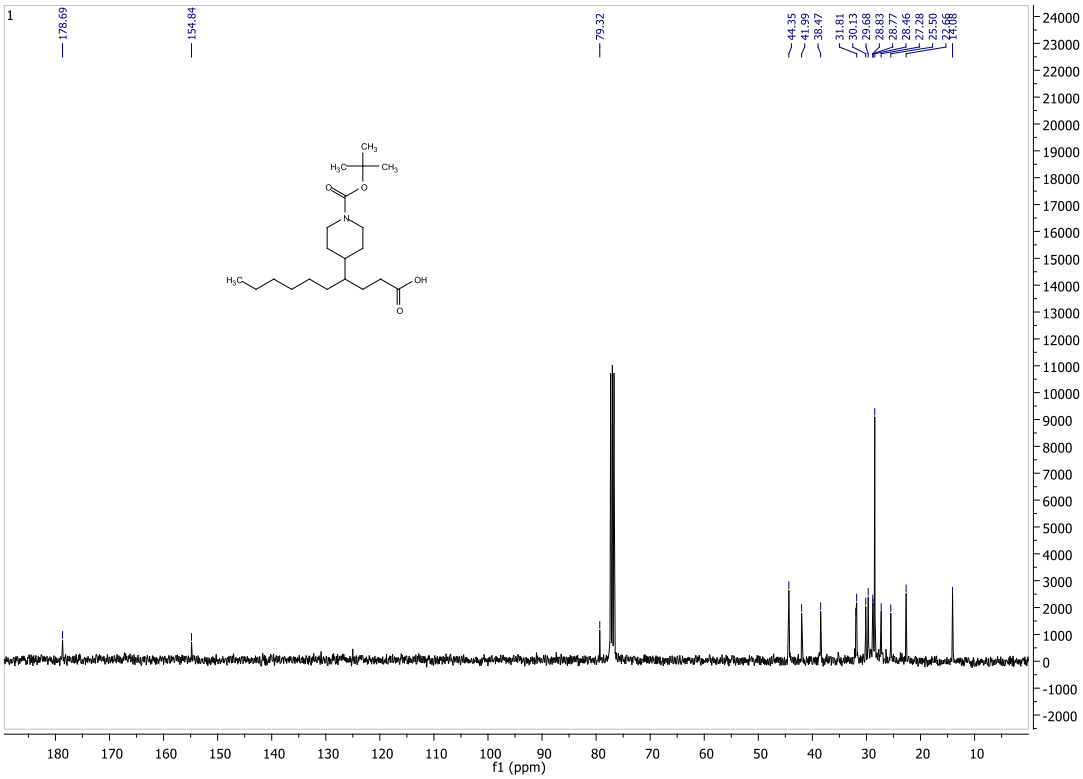
^{1}H NMR spectrum of **5** in $CDCl_3$



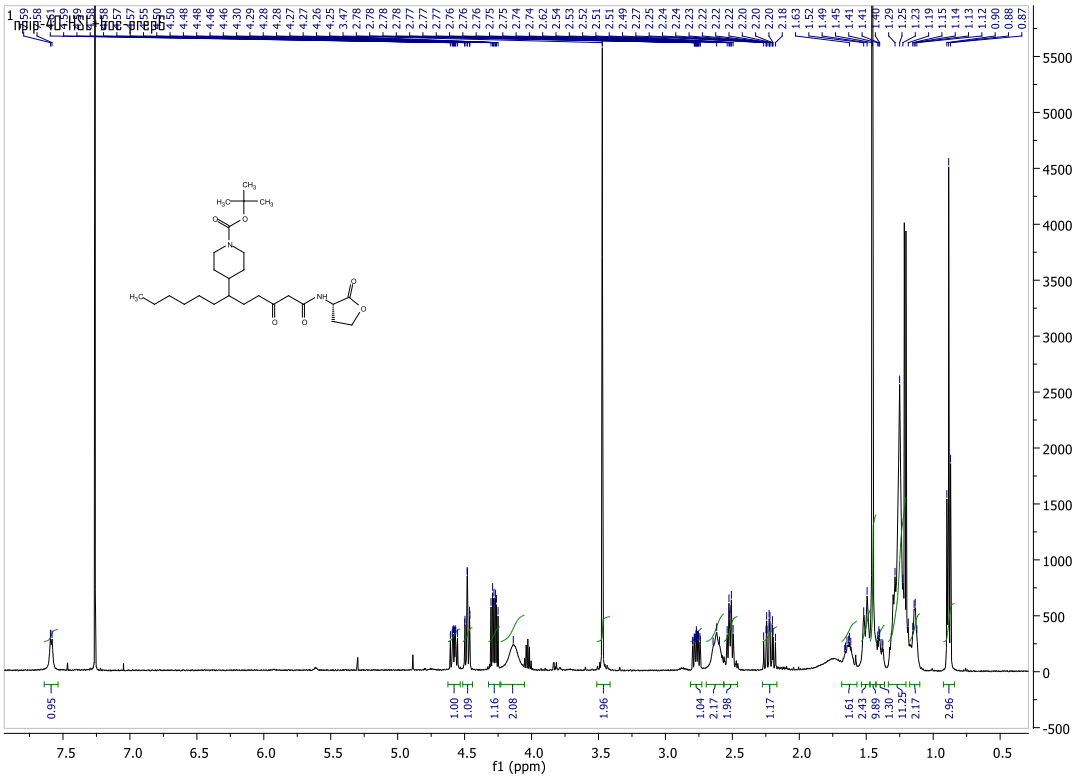
^{13}C NMR spectrum of **5** in $CDCl_3$



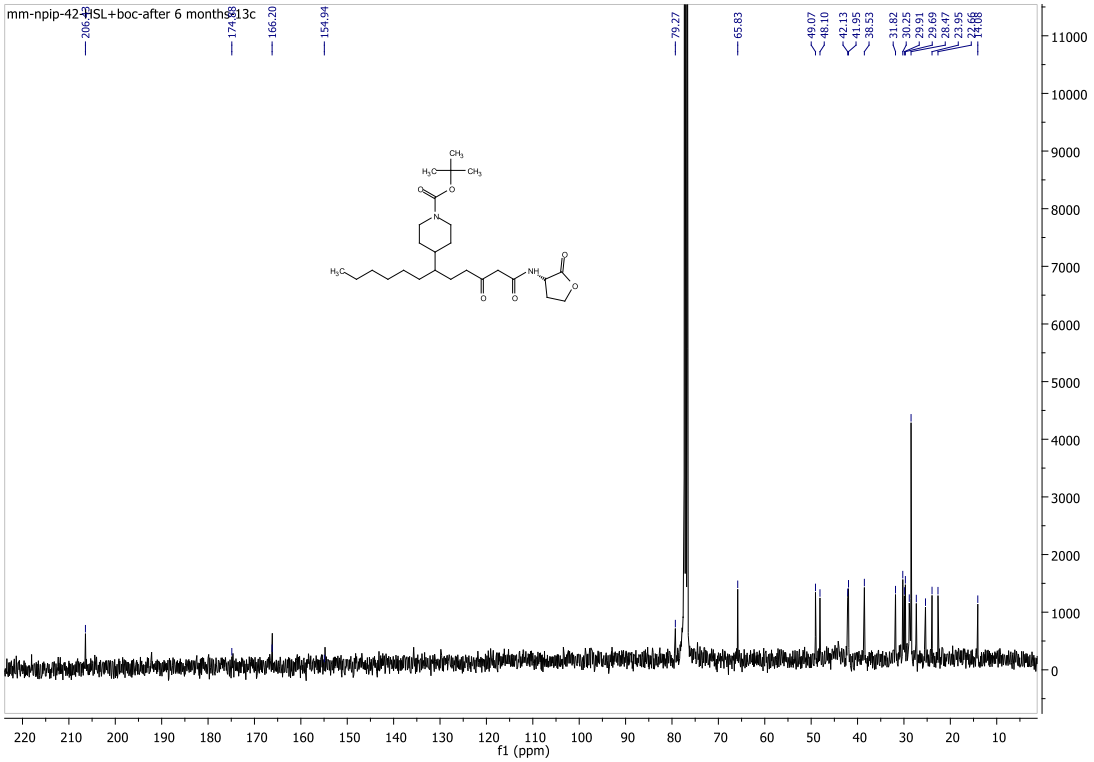
^{1}H NMR spectrum of **6** in $CDCl_3$



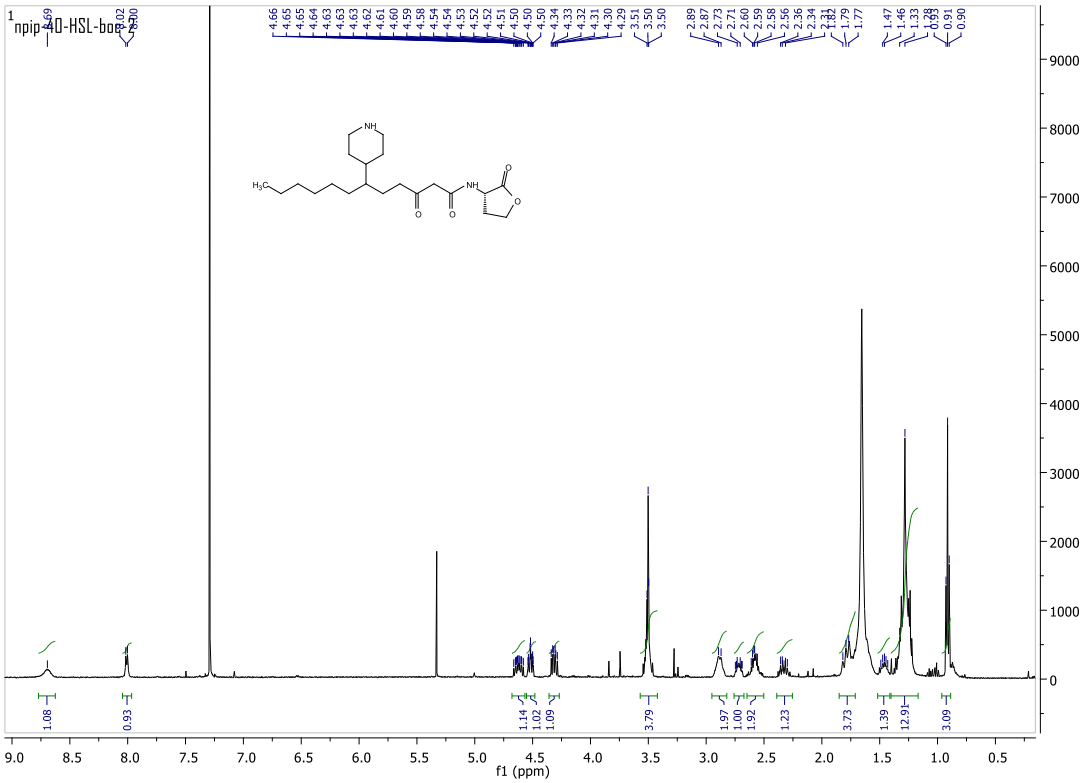
^{13}C NMR spectrum of **6** in $CDCl_3$



^{1}H NMR spectrum of **7** in $CDCl_3$



^{13}C NMR spectrum of **7** in $CDCl_3$



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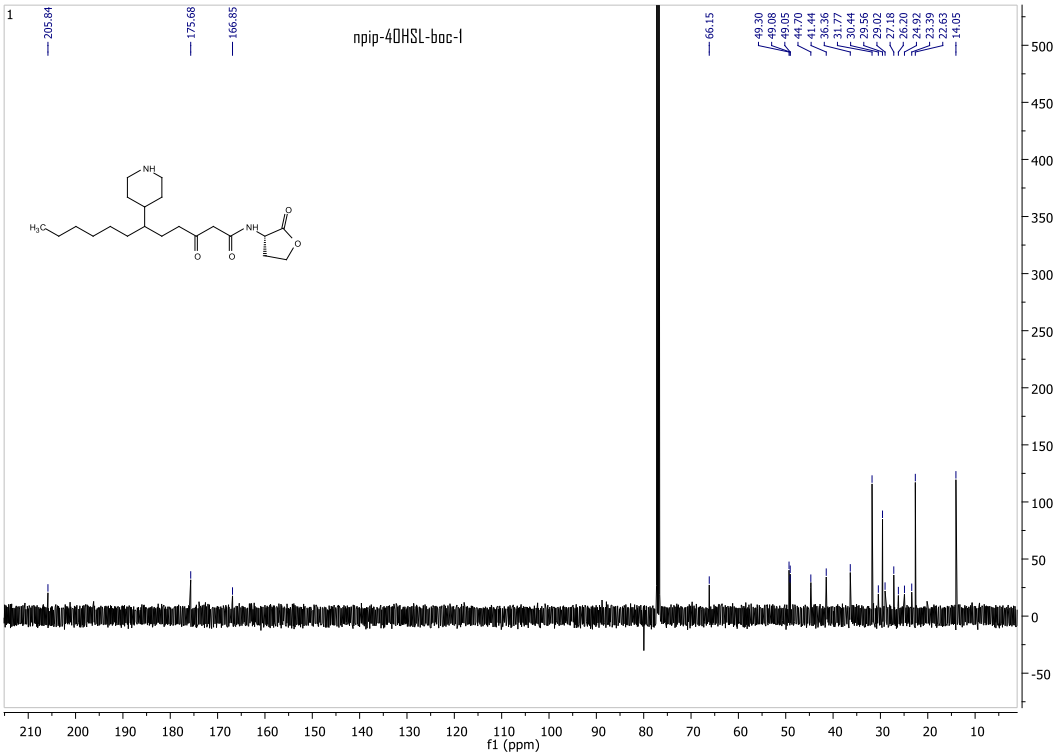

^{13}C NMR spectrum of **8** in $CDCl_3$

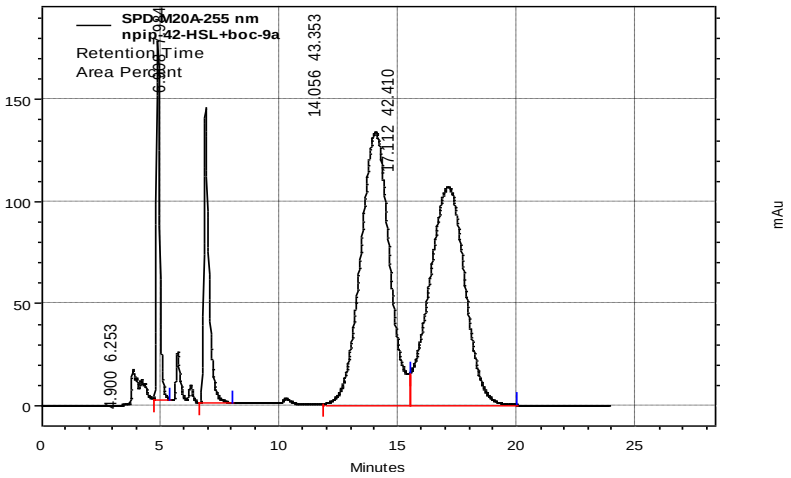

Figure 10: HPLC separation of diastereoisomers **7A** and **7B**. CHIRALPAK AD-H: 15% IPA in Hexane. 0.8ml/min, 20microliter, 254nm