Appendices

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A1. Structure of amino acids

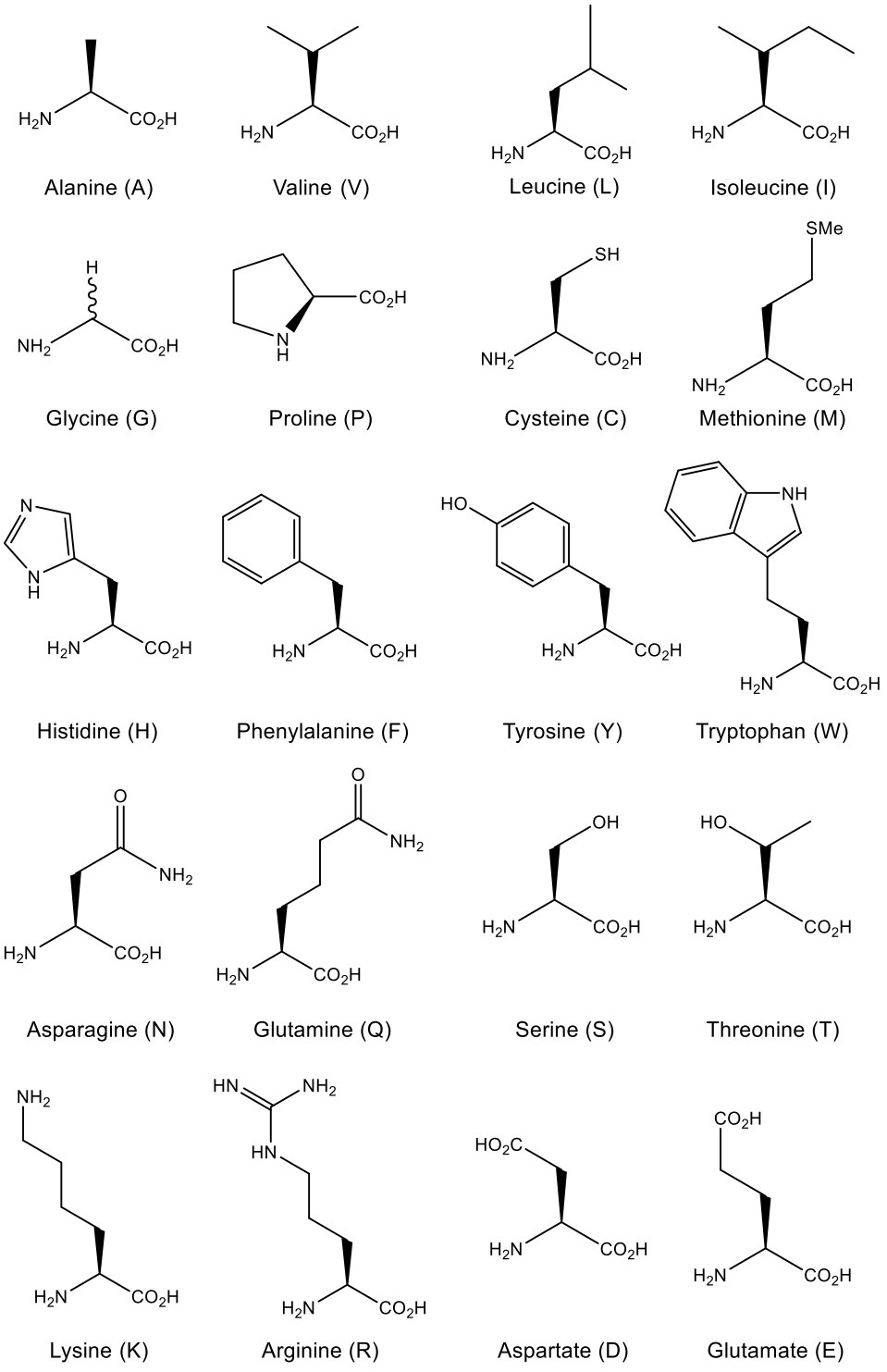


Figure A1.1 Structures of 20 natural amino acids.

A2. Mutagenesis and $P450_{BM3}$ variants

A2.1 List of $P450_{BM3}$ variants

Table A2.1 Variants and their mutations in the **initial library**.

No.	Variants	Mutations
1	WT	–
2	A330P	A330P
3	GVQ	A74G/F87V/L188Q
4	K19	H171L/Q307H/N319Y
5	K19/F87V	F87V/H171L/Q307H/N319Y
6	K19/F87V/E267V	F87V/H171L/E267V/Q307H/N319Y
7	K19/F87A/A82M/I263G	A82M/F87A/H171L/I263G/Q307H/N319Y
8	K19/F87V/A328I	F87V/H171L/Q307H/N319Y/A328I
9	K19/F87A/F81W	F81W/F87A/H171L/Q307H/N319Y
10	K19/F87A/I263A	F87A/H171L/I263A/Q307H/N319Y
11	K19/F87V/Q403P	F87V/H171L/Q307H/N319Y/Q403P
12	KU3/A330P/A328I	N239H/I259V/A276T/A328I/A330P
13	KU3/A330P/S72W	S72W/N239H/I259V/A276T/A330P
14	KU3/A330P/V78I	V78I/N239H/I259V/A276T/A330P
15	RLYF/K19	R47L/Y51F/H171L/Q307H/N319Y
16	RLYF/K19/F87A/A184I	R47L/Y51F/F87A/H171L/A184I/Q307H/N319Y
17	RLYF/K19/F87A/A328I	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
18	RLYF/K19/F87A	R47L/Y51F/F87A/H171L/Q307H/N319Y
19	RP/H171L	R47L/Y51F/H171L/I401P
20	RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
21	RT2/S72G/A330W	R47L/Y51F/S72G/A191T/N239H/I259V/A276T/A330W/L353I
22	RT2/A330P/V78I/A184I	R47L/Y51F/V78I/A184I/A191T/N239H/I259V/A276T/A330P/L353I
23	KU3/A330P	N239H/I259V/A276T/A330P
24	RP/H171L/I263G	R47L/Y51F/H171L/I263G/I401P
25	A82M/A330P	A82M/A330P
26	I263A/A330P	I263A/A330P
27	F81W/T260A	F81W/T260A
28	GV/A184I	A74G/F87V/A184I
29	GVQ/A264G	A74G/F87V/L188Q/A264G
30	K19/F87V/A264G	F87V/H171L/A264G/Q307H/N319Y
31	K19/F87V/E267V V78I	V78I/F87V/H171L/E267V/Q307H/N319Y
32	K19/F87A/A82M/E267F	A82M/F87A/H171L/E267F/Q307H/N319Y
33	RLYF/K19/F87A/A328I/E267F	R47L/Y51F/F87A/H171L/E267F/Q307H/N319Y/A328I
34	RLYF/K19/F87A/A328L	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328L
35	RLYF/K19/F87A/A328I/S72W	R47L/Y51F/S72W/F87A/H171L/Q307H/N319Y/A328I
36	RLYF/K19/F87A/A328I/V78I	R47L/Y51F/V78I/F87A/H171L/Q307H/N319Y/A328I
37	RL YF/IR	R47L/Y51F/I263R
38	RLYF/K19/F87A/A328F	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328F
39	RLYF/K19/F87A/I263G	R47L/Y51F/F87A/H171L/I263G/Q307H/N319Y
40	RP/A82W/I263A	R47L/Y51F/A82W/I263A/I401P
41	RP/A82M/I263A	R47L/Y51F/A82M/I263A/I401P
42	RP/F87V	R47L/Y51F/F87V/I401P
43	RP/F87V/V78I	R47L/Y51F/V78I/F87V/I401P
44	RP/H171L/I263G A184I	R47L/Y51F/H171L/A184I/I263G/I401P
45	RT2/H171L/I263G/A330W	R47L/Y51F/H171L/A191T/N239H/I259V/I263G/A276T/L353I/A330W
46	RP/H171L/I263G/F87V/V78I	R47L/Y51F/V78I/F87V/H171L/I263G/I401P
47	RP/V78I/E267V	R47L/Y51F/V78I/E267V/I401P
48	RT2/S72W/A330W	R47L/Y51F/S72W/A191T/N239H/I259V/A276T/A330W/L353I

Table A2.2 Variants and their mutations in the **refined library**.

No.	Variants	Mutations
1	WT	–
2	A330P	A330P
3	I263G	I263G
4	GIQ/I263G	A74G/F87I/L188Q/I263G
5	GLQ/I263G/A328G	A74G/F87L/L188Q/I263G/A328G
6	GQ/I263G/A328L	A74G/L188Q/I263G/A328L
7	GV/A184I	A74G/F87V/A184I
8	GV/A184I/A328G	A74G/F87V/A184I/A328G
9	GV/A184I/I263G	A74G/F87V/A184I/I263G
10	GV/A184I/I263G/A328G	A74G/F87V/A184I/I263G/A328G
11	GVQ	A74G/F87V/L188Q
12	GVQ/A330W	A74G/F87V/L188Q/A330W
13	GVQ/I263A/A328G	A74G/F87V/L188Q/I263A/A328G
14	GVQ/I263G	A74G/F87V/L188Q/I263G
15	GVQ/I263G/V78L	A74G/V78L/F87V/L188Q/I263G
16	K19	H171L/Q307H/N319Y
17	K19/F87A/A82M/I263G	A82M/F87A/H171L/I263G/Q307H/N319Y
18	K19/F87A/F81W	F81W/F87A/H171L/Q307H/N319Y
19	K19/F87A/I263A	F87A/H171L/I263A/Q307H/N319Y
20	K19/F87V	F87V/H171L/Q307H/N319Y
21	K19/F87V/A328I	F87V/H171L/Q307H/N319Y/A328I
22	K19/F87V/E267V	F87V/H171L/E267V/Q307H/N319Y
23	K19/F87V/I263G	F87V/H171L/I263G/Q307H/N319Y
24	K19/F87V/Q403P	F87V/H171L/Q307H/N319Y/Q403P
25	KU3/A330P/A328I	N239H/I259V/A276T/A328I/A330P
26	KU3/A330P/I263G	N239H/I259V/I263G/A276T/A330P
27	KU3/A330P/S72W	S72W/N239H/I259V/A276T/A330P
28	KU3/A330P/V78I	V78I/N239H/I259V/A276T/A330P
29	R19	R47L/Y51F/H171L/Q307H/N319Y
30	R19/A330W/S72A	R47L/Y51F/S72A/H171L/Q307H/N319Y/A330W
31	R19/F87A	R47L/Y51F/F87A/H171L/Q307H/N319Y
32	R19/F87A/A184I	R47L/Y51F/F87A/H171L/A184I/Q307H/N319Y
33	R19/F87A/A328I	R47L/Y51F/F87A/H171L/Q307H/N319Y/A328I
34	R19/F87I	R47L/Y51F/F87I/H171L/Q307H/N319Y
35	RLYF/H171L/I263G	R47L/Y51F/H171L/I263G
36	RLYF/H171L/I263G/A330W	R47L/Y51F/H171L/I263G/A330W
37	RLYF/H171L/I263G/A330W/L437S	R47L/Y51F/H171L/I263G/A330W/L437S
38	RLYF/H171L/I263G/L437LF	R47L/Y51F/H171L/I263G/L437LF
39	RLYF/H171L/I263R	R47L/Y51F/H171L/I263R
40	RLYF/I263G/A330P	R47L/Y51F/I263G/A330P
41	RT2	R47L/Y51F/A191T/N239H/I259V/A276T/L353I
42	RT2/A330P	R47L/Y51F/A191T/N239H/I259V/A276T/A330P/L353I
43	RT2/A330P/V78I/A184I	R47L/Y51F/V78I/A184I/A191T/N239H/I259V/A276T/A330P/L353I
44	RT2/S72G/A330W/I263G	R47L/Y51F/S72G/A191T/N239H/I259V/I263G/A276T/A330W/L353I
45	RP/H171L	R47L/Y51F/H171L/I401P
46	RP/H171L/I263G	R47L/Y51F/H171L/I263G/I401P
47	RP/H171L/I263G/F87V/V78I	R47L/Y51F/V78I/F87V/H171L/I263G/I401P
48	RP/V78I/E267V	R47L/Y51F/V78I/E267V/I401P

Table A2.3 Variants generated by molecular docking guided mutagenesis and their mutations.

No.	Variants	Mutations
1	GV/A184I/I263G/A328G/S72F	S72F/A74G/F87V/A184I/I263G/A328G
2	GV/A184I/I263G/A328G/S72M	S72M/A74G/F87V/A184I/I263G/A328G
3	GV/A184I/I263G/A328G/L75F	A74G/L75F/F87V/A184I/I263G/A328G
4	GV/A184I/I263G/A328G/L75M	A74G/L75M/F87V/A184I/I263G/A328G
5	GV/A184I/I263G/A328G/A330F	A74G/F87V/A184I/I263G/A328G/A330F
6	GL/A184I/I263G/A328G	A74G/F87L/A184I/I263G/A328G
7	GI/A184I/I263G/A328G	A74G/F87I/A184I/I263G/A328G
8	GL/A184I/I263G/A328G/L75F	A74G/L75F/F87L/A184I/I263G/A328G
9	GL/A184I/I263G/A328G/L75M	A74G/L75M/F87L/A184I/I263G/A328G
10	GL/A184I/I263G/A328G/S72F	S72F/A74G/F87L/A184I/I263G/A328G
11	GL/A184I/I263G/A328G/S72M	S72M/A74G/F87L/A184I/I263G/A328G
12	GL/A184I/I263G/A328G/A330F	A74G/F87L/A184I/I263G/A328G/A330F
13	GI/A184I/I263G/A328G/L75F	A74G/L75F/F87I/A184I/I263G/A328G
14	GI/A184I/I263G/A328G/L75M	A74G/L75M/F87I/A184I/I263G/A328G
15	GI/A184I/I263G/A328G/S72F	S72F/A74G/F87I/A184I/I263G/A328G
16	GI/A184I/I263G/A328G/S72M	S72M/A74G/F87I/A184I/I263G/A328G
17	GI/A184I/I263G/A328G/A330F	A74G/F87I/A184I/I263G/A328G/A330F
18	GL/A184I/I263G/A328G/L75M/A330F	A74G/L75M/F87L/A184I/I263G/A328G/A330F
19	GL/A184I/I263G/A328G/S72F/A330F	S72F/A74G/F87L/A184I/I263G/A328G/A330F
20	GL/A184I/I263G/A328G/S72F/L188M	S72F/A74G/F87L/A184I/L188M/I263G/A328G
21	GL/A184I/I263G/A328G/S72F/L188F	S72F/A74G/F87L/A184I/L188F/I263G/A328G
22	GV/A184I/I263G/A328G/S72F/V26M	V26M/S72F/A74G/F87V/A184I/I263G/A328G
23	GV/A184I/I263G/A328G/S72F/L188F	S72F/A74G/F87V/A184I/L188F/I263G/A328G
24	GV/A184I/I263G/A328G/S72F/V26M/L188F	V26M/S72F/A74G/F87V/A184I/L188F/I263G/A328G
25	GV/A184I/I263G/A328G/S72F/S332F	S72F/A74G/F87V/A184I/I263G/A328G/S332F
26	GV/A184I/I263G/A328G/S72F/V26M/S332F	V26M/S72F/A74G/F87V/A184I/I263G/A328G/S332F
27	GV/I263G/A328G/S72F/L181F	S72F/A74G/F87V/L181F/I263G/A328G
28	GV/A184I/I263V/A328G/S72F	S72F/A74G/F87V/A184I/I263V/A328G
29	GV/A184I/I263A/A328G/S72F	S72F/A74G/F87V/A184I/I263A/A328G
30	GV/A184I/I263G/A328G/S72F/S332L	S72F/A74G/F87V/A184I/I263G/A328G/S332L
31	RLYF/H171L/I263G/A330W/L437S/V78F	R47L/Y51F/V78F/H171L/I263G/A330W/L437S
32	RLYF/H171L/I263G/A330W/L437S/V26M	V26M/R47L/Y51F/H171L/I263G/A330W/L437S
33	RLYF/H171L/I263G/A330W/L437S/V26L	V26L/R47L/Y51F/H171L/I263G/A330W/L437S
34	RLYF/H171L/I263F/A330W/L437S	R47L/Y51F/H171L/I263F/A330W/L437S
35	GQ/I263G/A328L/S72M	S72M/A74G/L188Q/I263G/A328L
36	GQ/I263G/A328L/L75F	A74G/L75F/L188Q/I263G/A328L
37	GQ/I263G/A328L/L75M	A74G/L75M/L188Q/I263G/A328L
38	GQ/I263G/A328L/S332F	A74G/L188Q/I263G/A328L/S332F
39	GQ/I263G/A328L/S332L	A74G/L188Q/I263G/A328L/S332L
40	GQ/I263G/A328L/M354W	A74G/L188Q/I263G/A328L/M354W
41	GQ/I263G/A328L/V78F/S332F	A74G/V78F/L188Q/I263G/A328L/S332F
42	GQ/I263G/A328L/L75F/S332F	A74G/L75F/L188Q/I263G/A328L/S332F
43	GQ/I263G/A328L/L75F/S332F/L188F	A74G/L75F/L188F/I263G/A328L/S332F
44	GQ/I263G/A328L/L75F/S332F/L437LS	A74G/L75F/L188Q/I263G/A328L/S332F/L437LS
45	GQ/I263G/A328L/L75F/A330L	A74G/L75F/L188Q/I263G/A328L/A330L
46	GQ/I263G/A328L/L75F/S72M	S72M/A74G/L75F/L188Q/I263G/A328L
47	GQ/I263G/A328L/L75F/S72M/S332F	S72M/A74G/L75F/L188Q/I263G/A328L/S332F
48	GQ/I263G/A328L/L75F/S332F/A330L	A74G/L75F/L188Q/I263G/A328L/A330L/S332F
49	GQ/I263G/A328L/L75F/S332F/A330I	A74G/L75F/L188Q/I263G/A328L/A330I/S332F
50	GQ/I263G/A328L/L75F/S72M/A330L	S72M/A74G/L75F/L188Q/I263G/A328L/A330L
51	GQ/I263G/A328L/L75F/S72F/A330L	S72F/A74G/L75F/L188Q/I263G/A328L/A330L

Table A2.3 content		
No.	**Variants**	**Mutations**
52	GQ/I263G/A328L/T260L	A74G/L188Q/I263G/A328L/T260L
53	GQ/I263G/A328L/T260I	A74G/L188Q/I263G/A328L/T260I
54	GQ/I263G/A328L/T260L/A82M	A74G/L188Q/I263G/A328L/T260L/A82M
55	GQ/I263G/A328L/T260L/S332F	A74G/L188Q/I263G/A328L/T260L/S332F
56	GQ/I263G/A328L/T260L/S332F/A82M	A74G/L188Q/I263G/A328L/T260L/S332F/A82M
57	GQ/I263G/T260L/A330L	A74G/L188Q/I263G/T260L/A330L
58	GQ/I263G/T260L/A330F	A74G/L188Q/I263G/T260L/A330F
59	GQ/I263G/T260L/A330F/A82M	A74G/L188Q/I263G/T260L/A330F/A82M
60	R19/S72A/A330W/L188F	R47L/Y51F/H171L/Q307H/N319Y/S72A/A330W/L188F
61	R19/S72A/A330W/S332L	R47L/Y51F/H171L/Q307H/N319Y/S72A/A330W/S332L
62	R19/S72A/A330W/S332F	R47L/Y51F/H171L/Q307H/N319Y/S72A/A330W/S332F
63	R19/S72A/A330W/L181F	R47L/Y51F/H171L/Q307H/N319Y/S72A/A330W/L181F
64	KU3/A330P/S72W/V26F	S72W/N239H/I259V/A276T/A330P/V26F
65	KU3/A330P/S72W/V26L	S72W/N239H/I259V/A276T/A330P/V26L
66	KU3/A330P/S72W/L181F	S72W/N239H/I259V/A276T/A330P/L181F
67	KU3/A330P/S72W/I263F	S72W/N239H/I259V/A276T/A330P/I263F
68	KU3/A330P/S72W/A264F	S72W/N239H/I259V/A276T/A330P/A264F
69	KU3/A330P/S72W/T438L	S72W/N239H/I259V/A276T/A330P/T438L
70	KU3/A330P/S72W/T438F	S72W/N239H/I259V/A276T/A330P/T438F

Table A2.4 Sequence of primers used for site-directed mutagenesis.

No.	Primers	Sequence
1	F87L Forward	GACGGGTTATTAACAAGCTGGACGCATGAA
	F87L Reverse	CCAGCTTGTTAATAACCCGTCTCCTGCAAA
2	F87I Forward	CGTGATTTTGCAGGAGACGGGTTAATTACAAGCTGG
	F87I Reverse	TTTTTCATGCGTCCAGCTTGTAATTAACCCGTCTCC
3	A74G/S72F Forward	GATAAAAACTTATTTCAAGGGCTTAAATTTGTACGTGATTTTGCAGG
	A74G/S72F Reverse	ACGTACAAATTTAAGCCCTTGAAATAAGTTTTTATCAAAGCGTGATTCAT
4	A74G/S72M Forward	GATAAAAACTTAATGCAAGGGCTTAAATTTGTACGTGATTTTGCAGG
	A74G/S72M Reverse	ACGTACAAATTTAAGCCCTTGCATTAAGTTTTTATCAAAGCGTGATTCAT
5	A74G/L75F Forward	AGTCAAGGGTTTAAATTTGTACGTGATTTTGCAGGAGA
	A74G/L75F Reverse	TACAAATTTAAACCCTTGACTTAAGTTTTTATCAAAGCGTGATTCATCG
6	A74G/L75M Forward	AGTCAAGGGATGAAATTTGTACGTGATTTTGCAGGAGACGG
	A74G/L75M Reverse	ACGTACAAATTTCATCCCTTGACTTAAGTTTTTATCAAAGCGTGATTCAT
7	A328G/A330F Forward	TGGCCAACTGGTCCTTTCTTTTCCCTATATGCAAAAGAAGATAC
	A328G/A330F Reverse	TAGGGAAAAGAAAGGACCAGTTGGCCATAAGCGCAGCG
8	A328G/A330M Forward	TGGCCAACTGGTCCTATGTTTTCCCTATATGCAAAAGAAGA
	A328G/A330M Rev.	TAGGGAAAACATAGGACCAGTTGGCCATAAGCGCAGCG
9	V26F Forward	GATAAACCGTTTCAAGCTTTGATGAAAATTGCGGATG
	V26F Reverse	CAAAGCTTGAAACGGTTTATCTGTGTTTAATAACG
10	T88F Forward	GACGGGTTATTTTTCAGCTGGACGCATGAAAAAAATTGG
	T88F Reverse	CGTCCAGCTGAAAAATAACCCGTCTCCTGCAAAATC
11	L188F Forward	ATGAACAAGTTTCAGCGAGCAAATCCAGACGACCC
	L188F Reverse	TGCTCGCTGAAACTTGTTCATTGCTTCATCCAGTGC
12	L181F Forward	GTCCGTGCATTCGATGAAGCAATGAACAAGCTGCAG
	L181F Reverse	TGCTTCATCGAATGCACGGACCATACTTGTAATAAATGGATG
13	T438F Forward	GAAACTTTATTCTTAAAACCTGAAGGCTTTGTGGTAAAAGC
	T438F Reverse	AGGTTTTAAGAATAAAGTTTCTTTAATATCCAGCTCGTAGTTTG
14	T438L Forward	GAAACTTTATTGTTAAAACCTGAAGGCTTTGTGGTAAAAG
	T438L Reverse	AGGTTTTAACAATAAAGTTTCTTTAATATCCAGCTCGTAGT
15	A184I/L188F Forward	CTGGATGAAATAATGAACAAGTTTCAGCGAGCAAATCCAGACGACCCAG
	A184I/L188F Reverse	TGCTCGCTGAAACTTGTTCATTATTTCATCCAGTGCACGGACCATAC
16	A184I/L188M Forward	CTGGATGAAATAATGAACAAGATGCAGCGAGCAAATCCAGACGAC
	A184I/L188M Reverse	TGCTCGCTGCATCTTGTTCATTATTTCATCCAGTGCACGGAC
17	A184I/L188W Forward	CTGGATGAAATAATGAACAAGTGGCAGCGAGCAAATCCAGACGACCCAG
	A184I/L188W Reverse	TGCTCGCTGCCACTTGTTCATTATTTCATCCAGTGCACGGACCATAC
18	I263G/T260L Forward	CAAATTATTCTATTCTTAGGTGCGGGACACGAAACAACAAGTGGTC
	I263G/T260L Reverse	GTGTCCCGCACCTAAGAATAGAATAATTTGATAGCGAATGTTCTCG
19	I263G/T260I Forward	CAAATTATTATCTTCTTAGGTGCGGGACACGAAACAACAAGTGGTC
	I263G/T260I Reverse	GTGTCCCGCACCTAAGAAGATAATAATTTGATAGCGAATGTTCTCG
20	I263G/T260F Forward	CAAATTATTTTCTTCTTAGGTGCGGGACACGAAACAACAAGTGGTC
	I263G/T260F Reverse	GTGTCCCGCACCTAAGAAGAAAATAATTTGATAGCGAATGTTCTCG
21	A328G/A330L Forward	TGGCCAACTGGTCCTCTGTTTTCCCTATATGCAAAAGAAG
	A328G/A330L Reverse	TAGGGAAAACAGAGGACCAGTTGGCCATAAGCGCAGC
22	A328G/A330I Forward	TGGCCAACTGGTCCTATCTTTTCCCTATATGCAAAAGAAGATACGG
	A328G/A330I Reverse	TAGGGAAAAGATAGGACCAGTTGGCCATAAGCGCAGCGCTTC
23	A328L/S332L Forward	TGGCCAACTCTTCCTGCGTTTCTCCTATATGCAAAAGAAGATACGG
	A328L/S332L Reverse	TGCATATAGGAGAAACGCAGGAAGAGTTGGCCATAAGCGCAG
24	A328L/S332F Forward	TGGCCAACTCTTCCTGCGTTTTTTCTATATGCAAAAGAAGATACGGTG
	A328L/S332F Reverse	TGCATATAGAAAAAACGCAGGAAGAGTTGGCCATAAGCGCAGC
25	A328L/S332W Forward	TGGCCAACTCTTCCTGCGTTTTGGCTATATGCAAAAGAAGATACGGTG
	A328L/S332W Reverse	TGCATATAGCCAAAACGCAGGAAGAGTTGGCCATAAGCGCAGC
26	A330I Forward	CCAACTGCTCCTATTTTTTCCCTATATGCAAAAGAAGATACGGTGC
	A330I Reverse	CATATAGGGAAAAAATAGGAGCAGTTGGCCATAAGCGCAGC

Table A2.4 content		
No.	**Primers**	**Sequence**
27	A330V Forward	CCAACTGCTCCTGTTTTTTCCCTATATGCAAAAGAAGATACGGTGC
	A330V Reverse	CATATAGGGAAAAAACAGGAGCAGTTGGCCATAAGCGCAGC
28	A330F Forward	CCAACTGCTCCTTTTTTTTCCCTATATGCAAAAGAAGATACGGTGC
	A330F Reverse	CATATAGGGAAAAAAAAGGAGCAGTTGGCCATAAGCGCAGC
29	A82M Forward	AGCGCTTAAATTTGTACGTGATTTTATGGGAGACGGGTTATT
	A82M Reverse	CCCGTCTCCCATAAAATCACGTACAAATTTAAGCGCTTGAC
30	A82F Forward	GTACGTGATTTTTTCGGAGACGGGTTATTTACAAGC
	A82F Reverse	CCCGTCTCCGAAAAAATCACGTACAAATTTAAG
31	A82L Forward	GTACGTGATTTTTTAGGAGACGGGTTATTTACAAGCTGGACG
	A82L Reverse	CCCGTCTCCTAAAAAATCACGTACAAATTTAAGCGCTTG
32	V78L Forward	CGCTTAAATTTCTACGTGATTTTGCAGGAG
	V78L Reverse	GCAAAATCACGTAGAAATTTAAGCGCTTGA
33	V78F Forward	CGCTTAAATTTTTTCGTGATTTTGCAGGAG
	V78F Reverse	CTGCAAAATCACGAAAAAATTTAAGCGCTT
34	V78M Forward	CGCTTAAATTTATGCGTGATTTTGCAGGAG
	V78M Reverse	GCAAAATCACGCATAAATTTAAGCGCTTGA

A3. Stereochemistry determination

A3.1 Specific rotation measurements

Table A3.1. Specific rotation data for cyclic amine oxidation products. ee values were from chiral phase GC.

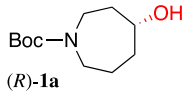
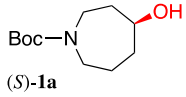
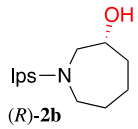
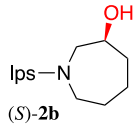
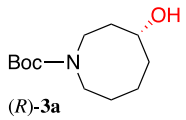
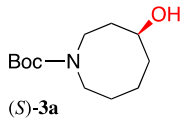
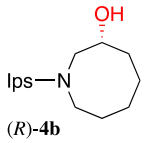
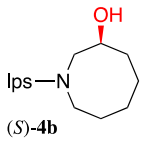
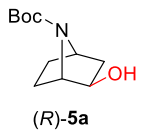
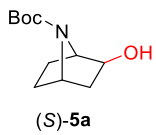
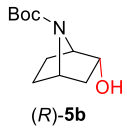
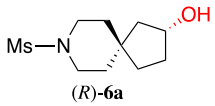
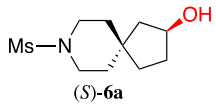
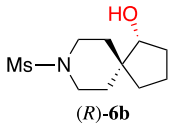
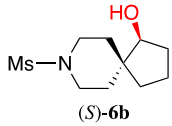
Product	Variant	*ee*	$[\alpha]_D^{25}$	Solvent	Literature data
 (*R*)-**1a**	RLYF H171L I263G A330W	90%	+5.2	$CHCl_3$	+5.7 [26]
 (*S*)-**1a**	KU3 A330P S72W	84%	–5.0	$CHCl_3$	–6.7 [26]
 (*R*)-**2b**	VQ S72G A330W	96% [a]	–6.2	$CHCl_3$	—
 (*S*)-**2b**	GL A184I I263G A328G S72F L188M	92% [a]	+5.7	$CHCl_3$	—
 (*R*)-**3a**	RLYF I263G A330P	98% [b]	+11.9	$CHCl_3$	—
 (*S*)-**3a**	KU3 A330P S72W	77% [b]	–9.2	$CHCl_3$	—
 (*R*)-**4b**	R19 S72G A330W	99% [c]	+13.3	$CHCl_3$	—
 (*S*)-**4b**	GV A184I I263G A328G S72F S332F	92%	–12.2	CHCl3	—
 (*R*)-**5a**	R19 F87I	99%	+20.8	CH_2Cl_2	+21.1 [27]
 (*S*)-**5a**	GL A184I I263G A328G	90%	–19.7	CH_2Cl_2	–21.6 [27]

Table A3.1 content

Product	Variant	*ee*	$[\alpha]_D^{25}$	Solvent	Literature data
 (*R*)-**5b**	R19 S72G A330W	80%	–4.1	$CHCl_3$	–3.8 [28]
 (*S*)-**5b**	GQ I263G T260L A330F	90%	+4.8	$CHCl_3$	—
 (*R*)-**6a**	K19 F87V A328I	88%	+2.4	$CHCl_3$	—
 (*S*)-**6a**	RP H171L I263G	77% [d]	–1.8	$CHCl_3$	—
 (*R*)-**6b**	—	—	—	—	—
 (*S*)-**6b**	VQ S72G A330W	95%	–17.4	CH_2Cl_2	—

[a] Absolute configuration inferred by conversion of a Boc-derivative with known absolute configuration to the Ips-derivative.
[b] Absolute configuration tentatively assigned by comparison with *N*-Boc-azepane oxidation products with known absolute configuration.
[c] Absolute configuration determined by single crystal X-ray crystallography.
[d] Absolute configuration tentatively assigned by Mosher ester analysis.

A3.2 Single crystal X-ray crystal structure determination

Table A3.2. Crystal structure determination data for **(*R*)-7c**.

Empirical formula	$C_{10}\ H_{21}\ N\ O_3\ S$	
Formula weight	235.35	
Temperature	150 K	
Crystal system / Space group	Orthorhombic / $P\ 2_1\ 2_1\ 2_1$	
Unit cell dimensions	a = 5.66273(4) Å	α= 90°.
	b = 11.31439(9) Å	β= 90°.
	c = 18.48434(15) Å	γ = 90°.
Volume	1184.298(16) $Å^3$	
	Rint = 0.022	
Data / restraints / parameters	2465 / 0 / 138	
Final R indices [I>2sigma(I)]	R1 = 0.0191, wR2 = 0.0527	
Absolute structure parameter	0.009(10)	
Extinction coefficient	65(4)	
Absolute structure parameter	0.009(10)	

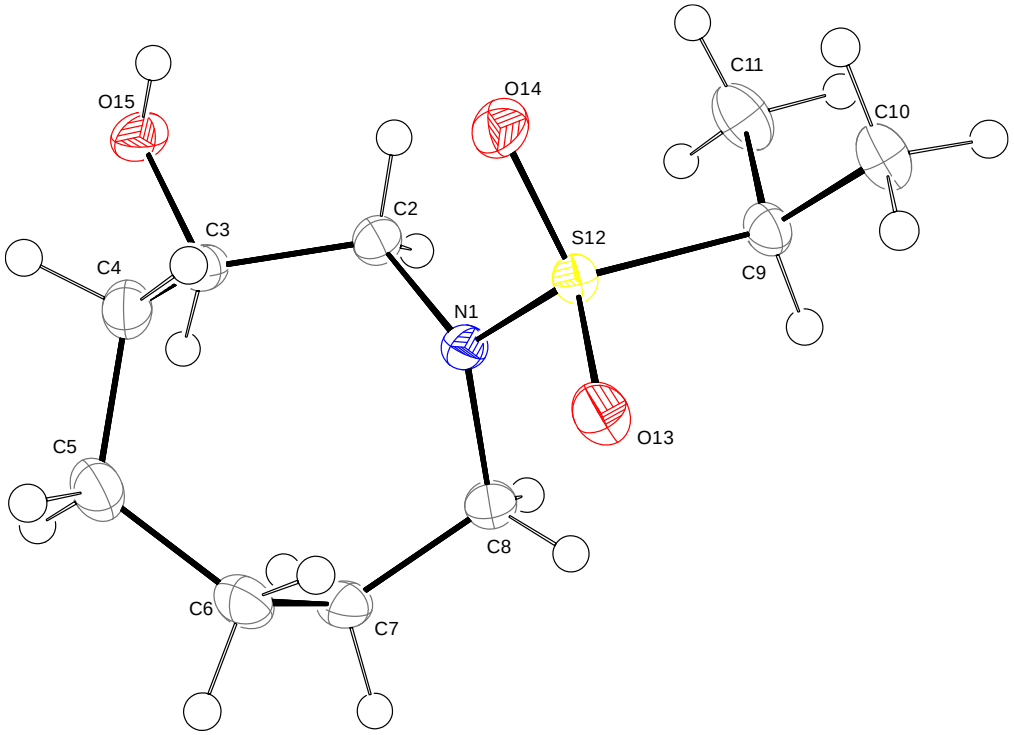


Figure A3.1. Ortep drawing of (*R*)-7c. Thermal ellipsoids shown at 50% probability.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 2159261. Copies of the data can be obtained, free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.

Table A3.3. Crystal structure determination data for **(*S*)-10a**.

Empirical formula	$C_{10}\ H_{19}\ N\ O_3\ S$	
Formula weight	233.33	
Temperature	150 K	
Crystal system / Space group	Orthorhombic / $P\ 2_1\ 2_1\ 2_1$	
Unit cell dimensions	a = 5.88210(10) Å	α= 90°.
	b = 10.5364(2) Å	β= 90°.
	c = 18.3923(3) Å	γ = 90°.
Volume	1139.88(3) $Å^3$	
	Rint = 0.064	
Data / restraints / parameters	2399 / 76 / 153	
Final R indices [I>2sigma(I)]	R1 = 0.0355, wR2 = 0.0979	
Absolute structure parameter	-0.012(7)	

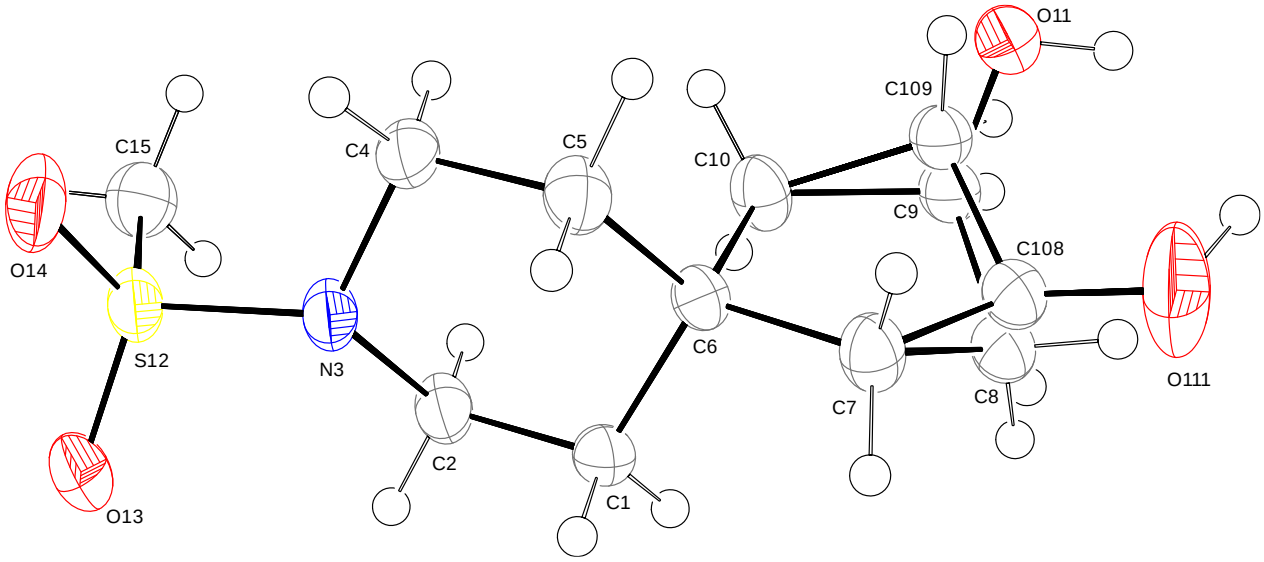


Figure A3.2. Ortep drawing of (*S*)-10a. Thermal ellipsoids shown at 50% probability. Any disorder is off-set with 100 in the numbering scheme.

This structure has been solved in $P\ 2_1\ 2_1\ 2_1$, which allows for absolute structure determination. As the structure was solved and refined it became obvious that C8, C9 and O11 could be in two orientations. The occupancy of the main component was refined to 0.832(4) [Atoms C8, C9 and C11] whereas the minor component was refined to 0.168(4) [Atoms C108, C109 and O111].

Bayesian analysis of the Bijvoet pairs gave the Hooft y parameter as -0.011(7) and the probability that the structure was the correct hand was >99.9% given that the crystal is enantiopure.

Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 2159262. Copies of the data can be obtained, free of charge via http://www.ccdc.cam.ac.uk/data_request/cif.

A3.3 Mosher ester analysis

A3.3.1 Mosher ester analysis for product 10a

Table A3.4. $\Delta\delta^{RS}$ data for the (*R*)- and (*S*)-8-(methylsulfonyl)-8-azaspiro[4.5]decan-1-yl Mosher esters **10a**-(*R*)-ester and **10a**-(*S*)-ester. The alcohol **10a** was isolated from a reaction with the variant RP/H171L/I263G. δ (***R***)- or (***S***)-**ester** are the chemical shifts of protons in the ^{1}H NMR of the (*R*)- or (*S*)-ester. $\Delta\delta^{RS}$ is calculated by δ *R*-ester **–** δ *S*-ester. Predicted $\Delta\delta^{RS}$ is the direction of the shift of the δ (***R***)**-ester** relative to δ (***S***)**-ester** for each proton under analysis. The results suggested a (2*S*) configuration for the alcohol **10a** from the variant RP/H171L/I263G. This assignment was supported by the crystal structure (Fig. A3.2, Appendices **A3.2**).

Proton	δ (**R**)**-ester** (ppm)	δ (**S**)**-ester** (ppm)	$\Delta\delta^{RS}$ (ppm)	Predicted $\Delta\delta^{RS}$	
				(2R)-**10a**	(2S)-**10a**
1'	1.65	1.71	–0.06	>0	<0
1"	1.82	1.85	–0.03	>0	<0
2	5.44	5.42	+0.02	<0	>0
3'	1.90	1.84	+0.06	<0	>0
3"	2.06	2.05	+0.01	<0	NSD*
4'	1.56	1.54	+0.02	NSD*	>0
4"	1.61	1.54	+0.07	NSD*	>0
6	1.55	1.56	–0.01	>0	<0
10	1.55	1.55	0	<0	NSD*

*NSD: No significant difference

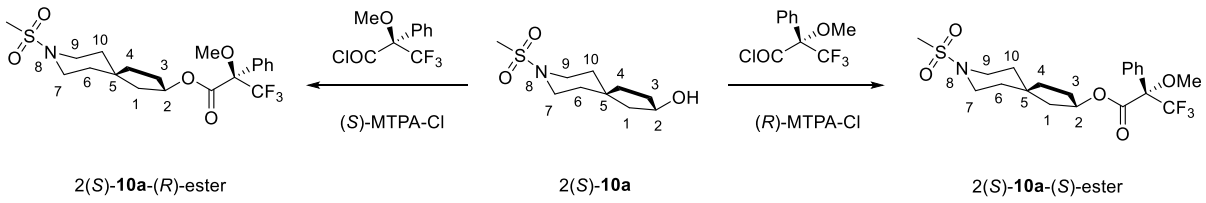
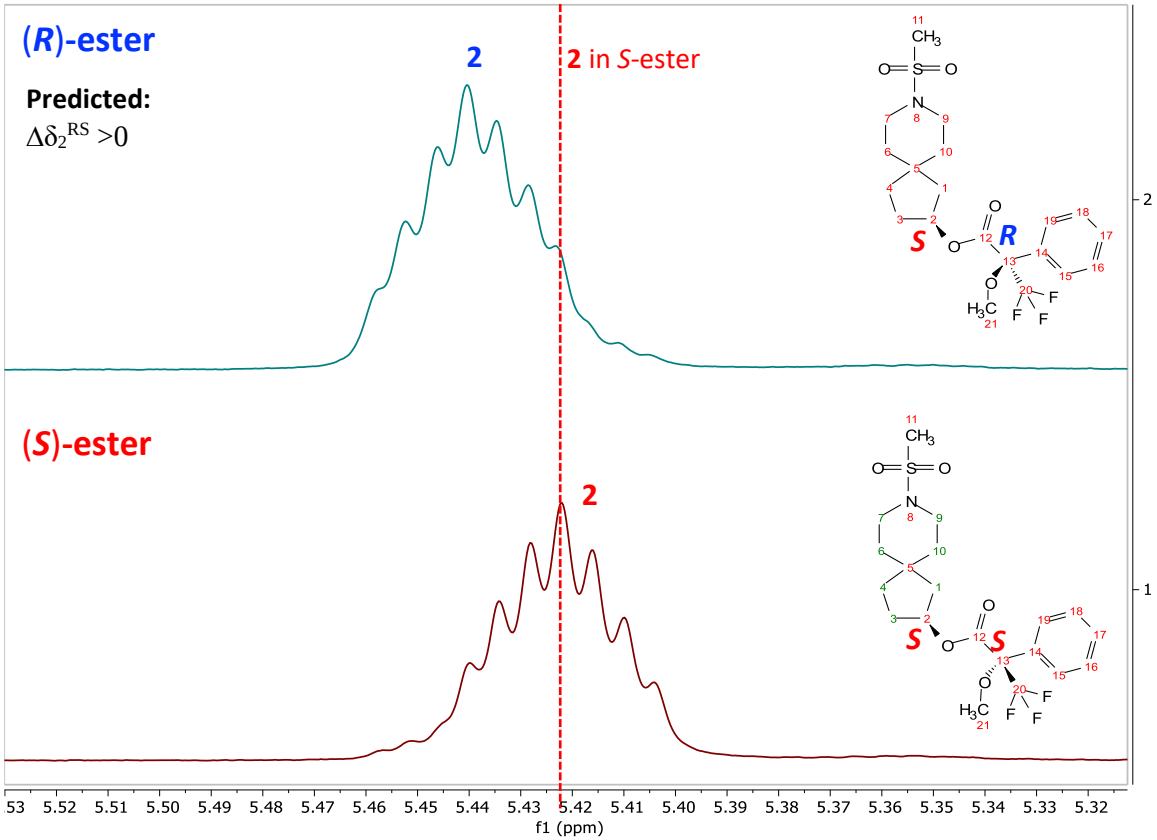


Figure A3.3. Conformations used for the analysis of the predicted $\Delta\delta^{RS}$ values of protons in the (*R*)- and (*S*)-esters, based on a 2(*S*) configuration for alcohol **10a**.



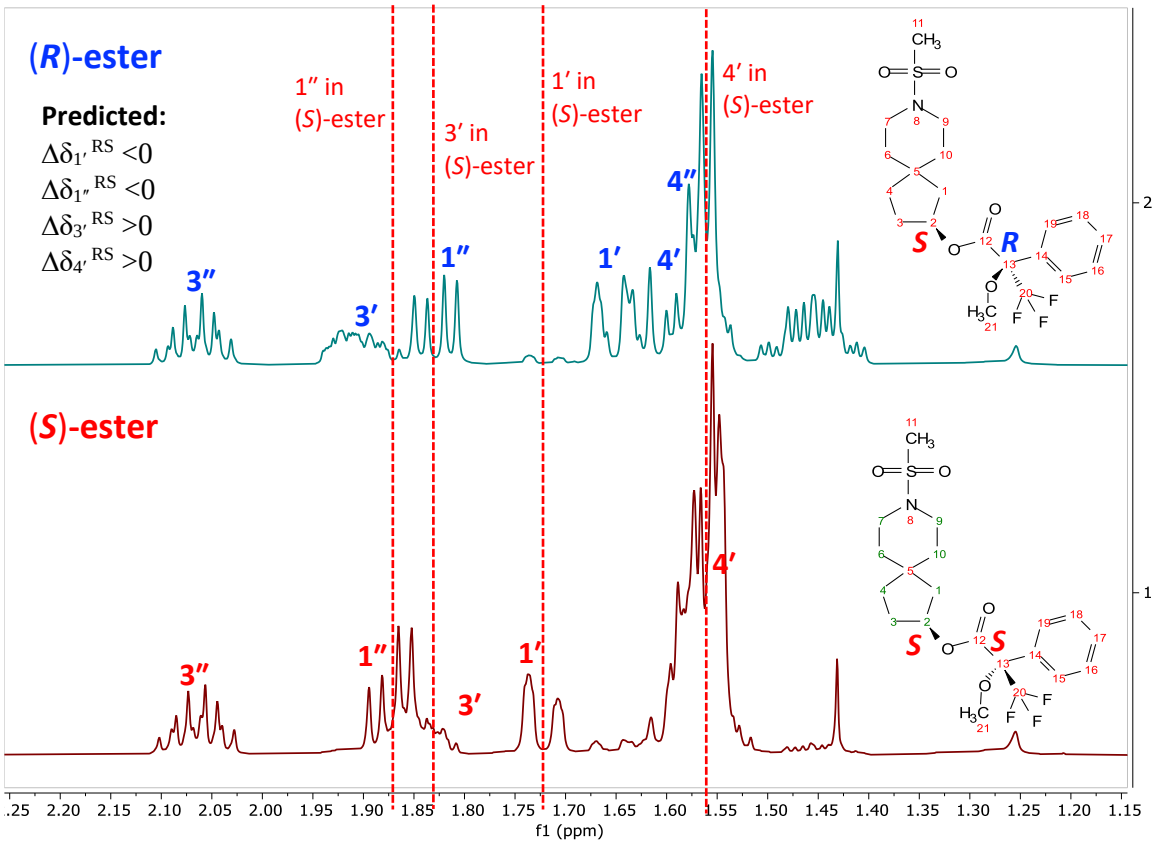


Figure A3.4. ^{1}H NMR spectra of **10a**-*R*-ester and **10a**-*S*-ester.

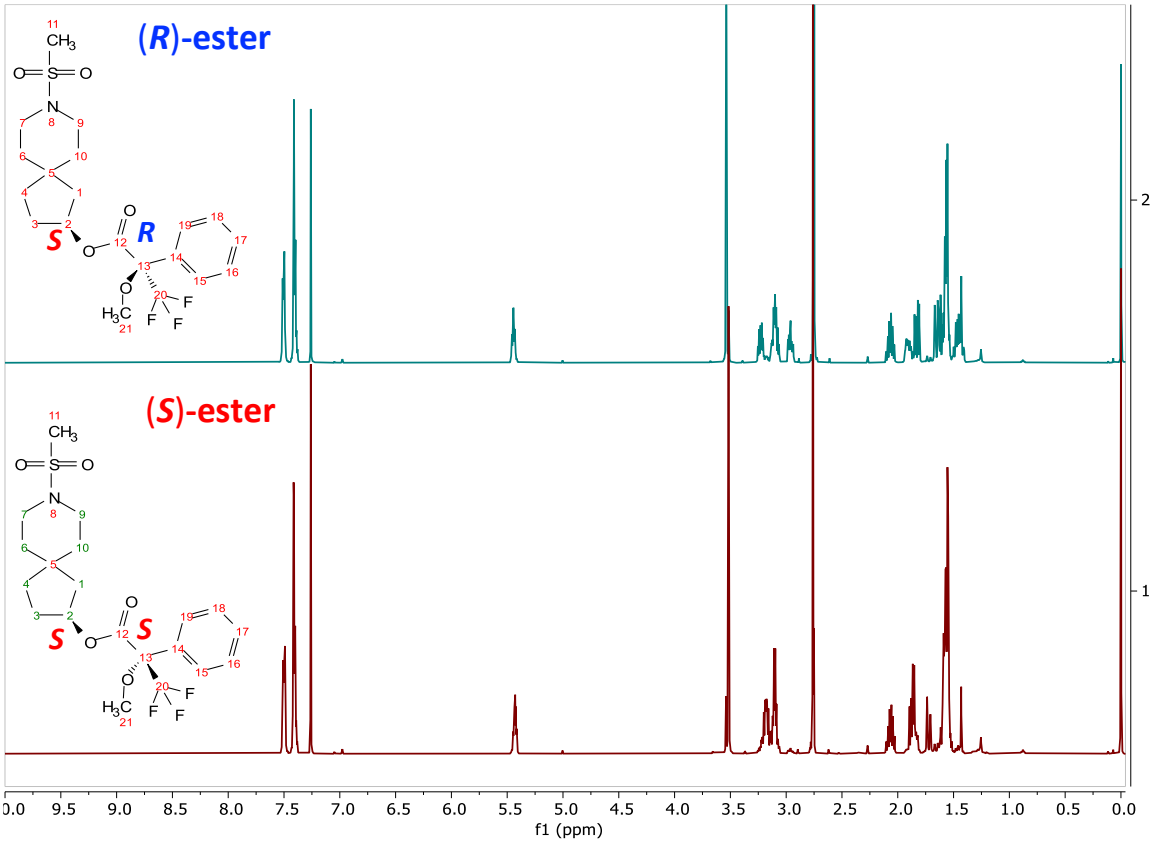


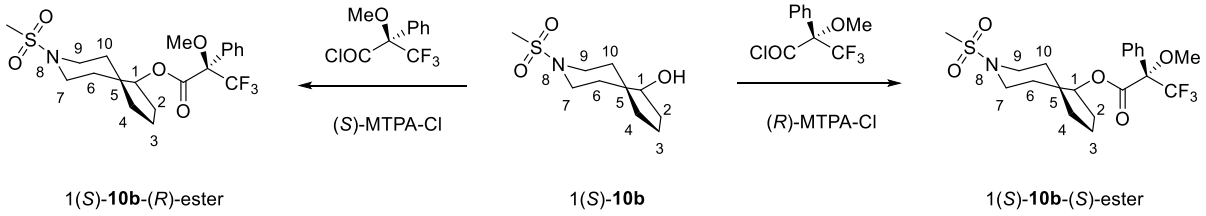
Figure A3.5. ^{1}H NMR spectra of **10a**-*R*-ester and **10a**-*S*-ester.

A3.3.1 Mosher ester analysis for product 10b

Table A3.5. $\Delta\delta^{RS}$ data for the (*R*)- and (*S*)-8-(methylsulfonyl)-8-azaspiro[4.5]decan-1-yl Mosher esters **10b**-(*R*)-ester and **10b**-(*S*)-ester. The alcohol **10b** was isolated from a reaction with the variant VQ/S72G/A330W. δ ***R***- or ***S*-ester** are the chemical shifts of protons in the ^{1}H NMR of the (*R*)- or (*S*)-ester. $\Delta\delta^{RS}$ is calculated by δ *R*-ester **–** δ *S*-ester. Predicted $\Delta\delta^{RS}$ is the direction of the shift of the δ (***R*)-ester** relative to δ (***S*)-ester** for each proton under analysis. The analysis suggested a (1*S*) configuration for the alcohol **10b** from the variant VQ/S72G/A330W. However, this assignment is tentative because no other data are available to support this configuration.

Proton	δ (**R**)-**ester** (ppm)	δ (**S**)-**ester** (ppm)	$\Delta\delta^{RS}$ (ppm)	Predicted $\Delta\delta^{RS}$	
				1(R)-**10b**	1(S)-**10b**
1	5.03	5.08	–0.05	NSD*	<0
2'	1.72	1.80	–0.08	>0	<0
2"	2.12	2.18	–0.06	>0	<0
6'	1.53	1.46	+0.07	<0	>0
6"	1.65	1.57	+0.08	<0	>0
7'	2.89	2.90	–0.01	NSD*	NSD*
7"	3.32	3.18	+0.14	<0	>0
9'	3.03	3.07	–0.04	NSD*	NSD*
9"	3.43	3.33	+0.10	<0	>0

*NSD: No significant difference



1(*S*)-**10b**-(*R*)-ester 1(*S*)-**10b** 1(*S*)-**10b**-(*S*)-ester

Figure A3.6. Conformations used for the analysis of the predicted $\Delta\delta^{RS}$ values of protons in (*R*)- and (*S*)-esters, based on a 1(*S*) configuration for alcohol **10b**.

A4. GC analysis and product selectivity

A4.1 GC analysis

A4.1.1 GC conditions and product retention times

Gas chromatographic (GC) analyses were carried out with a ThermoFisher Scientific Trace 1300 instrument equipped with a flame ionisation detector (FID) and an AI1310 autosampler using a DB-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technology, UK) or for chiral-phase analysis a Cyclosil-B fused silica column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, Agilent Technology, UK) using helium as the carrier gas at a flow rate of 1.5 mL/min. For normal-phase analyses, both the injector and the FID were held at 250 °C. For chiral-phase analyses, the injector was held at 200 °C and the FID at 250 °C.

For normal-phase GC analysis of the oxidation products of *N*-Boc-azepane, **1**, the oven temperature was held at 160 °C for 2 min then raised at 20 °C/min to 250 °C and held for 0.5 min. Retention times for compounds are: *N*-Boc-azepane (**1**), 2.92 min, *N*-Boc-4-hydroxyazepane (**1a**), 4.10 min, *N*-Boc-3-hydroxyazepane (**1b**), 3.84 min. For chiral-phase analysis, the oven temperature was held at 80 °C for 2 min then raised at 0.75 °C/min to 150 °C and held for 20 min. Retention times for compounds are: *N*-Boc-azepane (**1**), 49.84 min, *N*-Boc-(4*R*)-hydroxyazepane [(*R*)-**1a**], 99.33 min, *N*-Boc-(4*S*)-hydroxyazepane [(*S*)-**1a**], 98.68 min.

For normal-phase GC analysis of the oxidation products of *N*-Ips-azepane, **5**, the oven temperature was held at 180 °C for 1 min then raised at 15 °C/min to 260 °C and held for 0.5 min. Retention times for compounds are: *N*-Ips-azepane (**5**), 2.99 min, *N*-Ips-4-hydroxyazepane (**5a**), 4.02 min, *N*-Ips-3-hydroxyazepane (**5c**), 3.77 min. For chiral-phase analysis, the oven temperature was held at 100 °C for 2 min then raised at 1 °C/min to 170 °C and held for 30 min. Retention times for compounds are: *N*-Ips-azepane (**5**), 55.13 min, *N*-Ips-(3*R*)-hydroxyazepane [(*R*)-**5c**], 84.42 min, *N*-Boc-(3*S*)-hydroxyazepane [(*S*)-**5c**], 85.03 min.

For normal-phase GC analysis of the oxidation products of *N*-Boc-azocane, **6**, the oven temperature was held at 140 °C for 1 min, then raised at 10 °C/min to 200 °C and held for 1 min. Retention times for compounds are: *N*-Boc-azocane (**6**), 3.71 min, *N*-Boc-4-hydroxyazocane (**6b**), 5.25 min. For chiral analysis, the oven temperature was held at 60 °C for 2 min, then raised at 1 °C/min to 160 °C and held for 15 min. Retention times for compounds are: *N*-Boc-azocane (**6**), 69.44 min, *N*-Boc-(4*R*)-hydroxyazocane [(*R*)-**6b**], 104.40 min, *N*-Boc-(4*S*)-hydroxyazocane [(*S*)-**6b**], 103.65 min.

For normal-phase GC analysis of the oxidation products of *N*-Ips-azocane, **7**, the oven temperature was held at 180 °C for 1 min then raised at 20 °C/min to 300 °C and held for 2 min. Retention times for compounds are: *N*-Ips-azocane (**7**), 3.32 min, *N*-Ips-4-hydroxyazocane (**7b**), 4.22 min, *N*-Ips-3-

hydroxyazocane (**7c**), 4.07 min. For chiral analysis, the oven temperature was held at 110 °C for 2 min then raised at 1 °C/min to 175 °C and held for 30 min. Retention times for compounds are: *N*-Ips-azocane (**7**), 55.90 min, *N*-Ips-(3*R*)-hydroxyazocane [(*R*)-**7c**], 88.11 min, *N*-Boc-(3*S*)-hydroxyazocane [(*S*)-**7c**], 88.89 min.

For normal-phase GC analysis of the oxidation products of *N*-Boc-7-azabicyclo[2.2.1]heptane, **8**, the oven temperature was held at 140 °C for 2 min then raised at 20 °C/min to 240 °C. Retention times for compounds are: *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**), 3.48 min, *N*-Boc-2-*exo*-hydroxy-7-azabicyclo[2.2.1]heptane (**8a**), 4.53 min, *N*-Boc-2-*endo*-hydroxy-7-azabicyclo[2.2.1]heptane (**8b**), 4.87 min. For chiral-phase analysis of the *exo*-alcohol product **8a**, the oven temperature was held at 80 °C for 2 min then raised at 1 °C/min to 160 °C and held for 30 min. Retention times for compounds are: *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**), 38.75 min, *N*-Boc-2(*R*)-*exo*-hydroxy-7-azabicyclo[2.2.1]-heptane [(*R*)-**8a**], 67.58 min, *N*-Boc-2(*S*)-*exo*-hydroxy-7-azabicyclo[2.2.1]heptane [(*S*)-**8a**], 68.75 min. For chiral-phase analysis of the *endo*-alcohol product **8b**, the oven temperature was held at 60 °C for 2 min then raised at 1 °C/min to 160 °C and held for 40 min. Retention times for compounds are: *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**), 56.70 min, *N*-Boc-*O*-TMS-2(*R*)-*endo*-hydroxy-7-azabicyclo[2.2.1]-heptane [*O*-TMS-(*R*)-**8b**], 79.45 min, *N*-Boc-*O*-TMS-2(*S*)-*endo*-hydroxy-7-azabicyclo[2.2.1]heptane [*O*-TMS-(*S*)-**8b**], 79.69 min.

For normal-phase GC analysis of the oxidation products of *N*-Ms-8-azaspiro[4.5]decane, **10**, the oven temperature was held at 220 °C for 2 min, then raised at 20 °C/min to 290 °C and held for 0.5 min. Retention times for compounds are: *N*-Ms-8-azaspiro[4.5]decane (**10**), 2.69 min, *N*-Ms-2-hydroxy-8-azaspiro[4.5]decane (**10a**), 3.59 min, *N*-Ms-1-hydroxy-8-azaspiro[4.5]decane (**10b**), 3.63 min.

A4.1.2 GC traces for major products

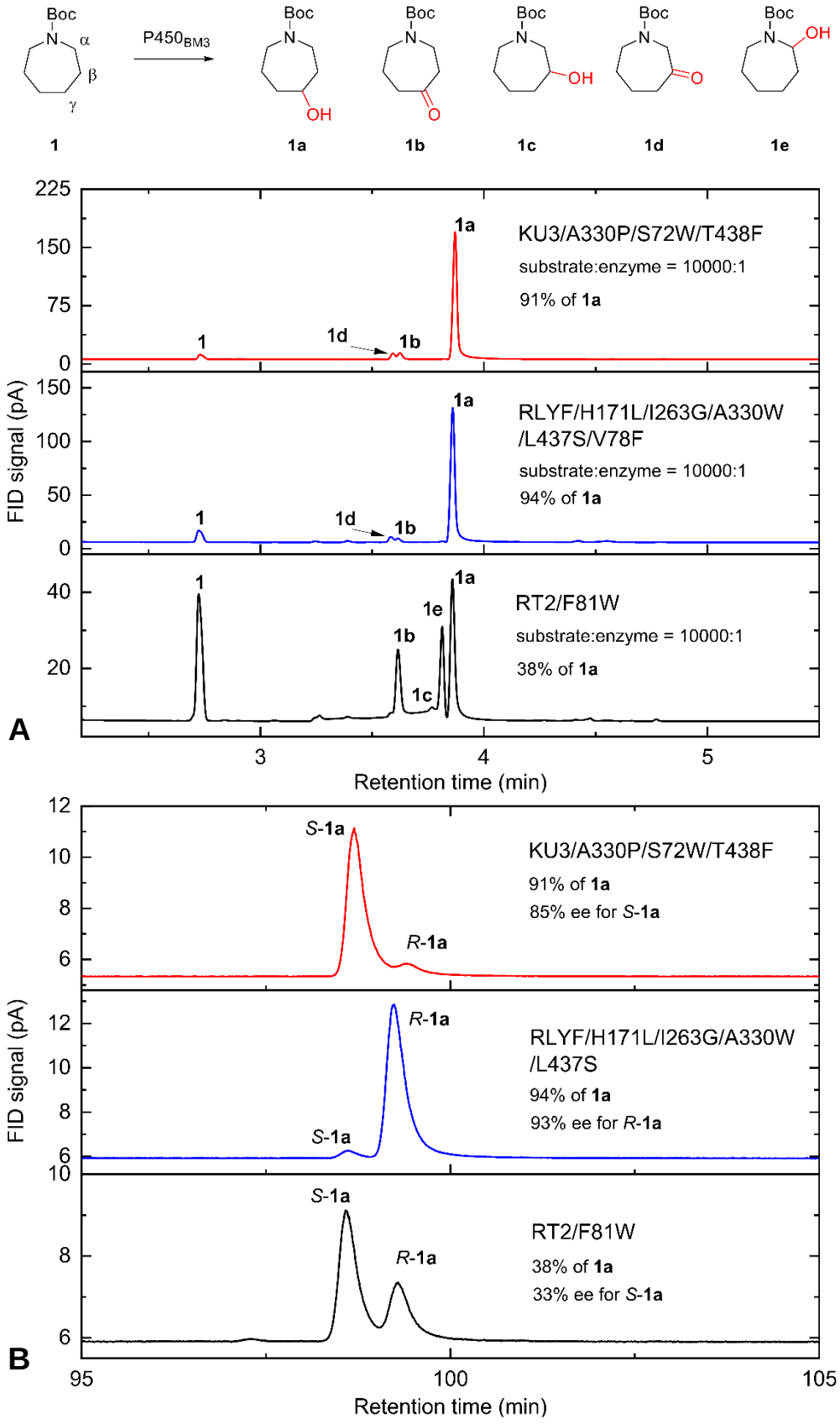


Figure A4.1. GC analysis of variants with the highest regio- and enantio-selectivity for the γ alcohol **1a** of *N*-Boc-azepane: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

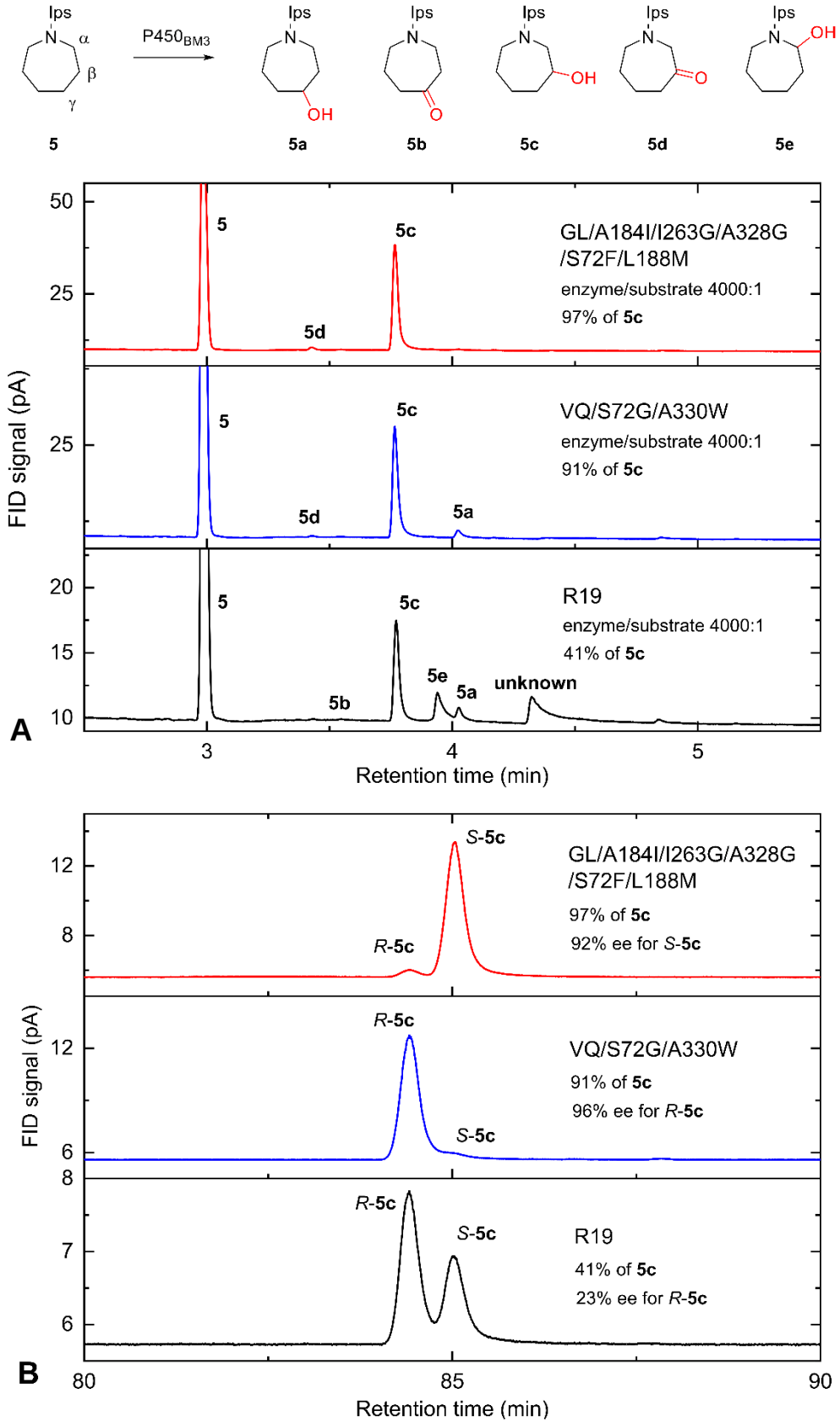


Figure A4.2. GC analysis of variants with the highest regio- and enantio-selectivity for the β alcohol **5c** of *N*-Ips-azepane: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

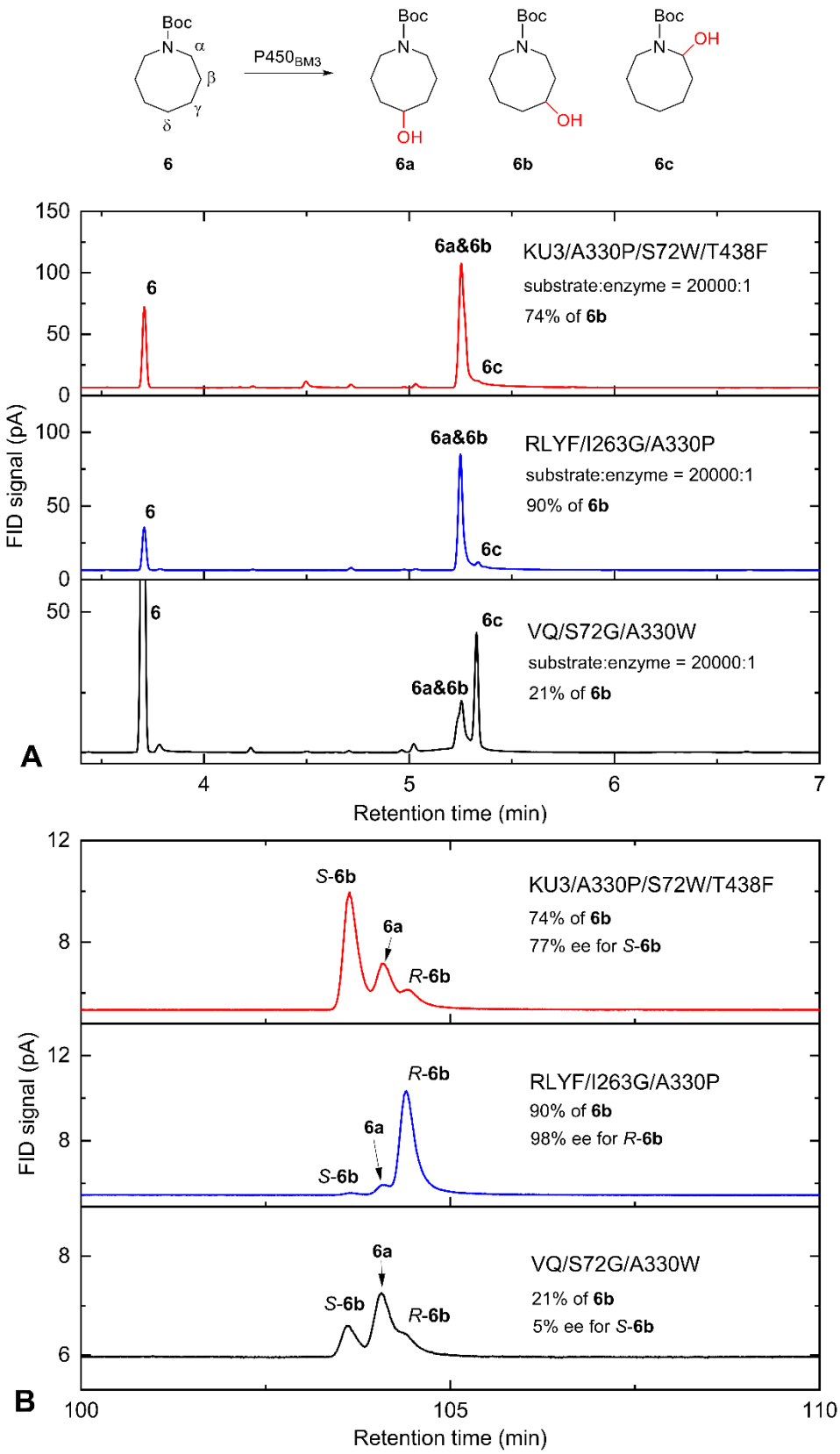


Figure A4.3. GC analysis of variants with the highest regio- and enantio-selectivity for the γ alcohol **6b** of *N*-Boc-azocane: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

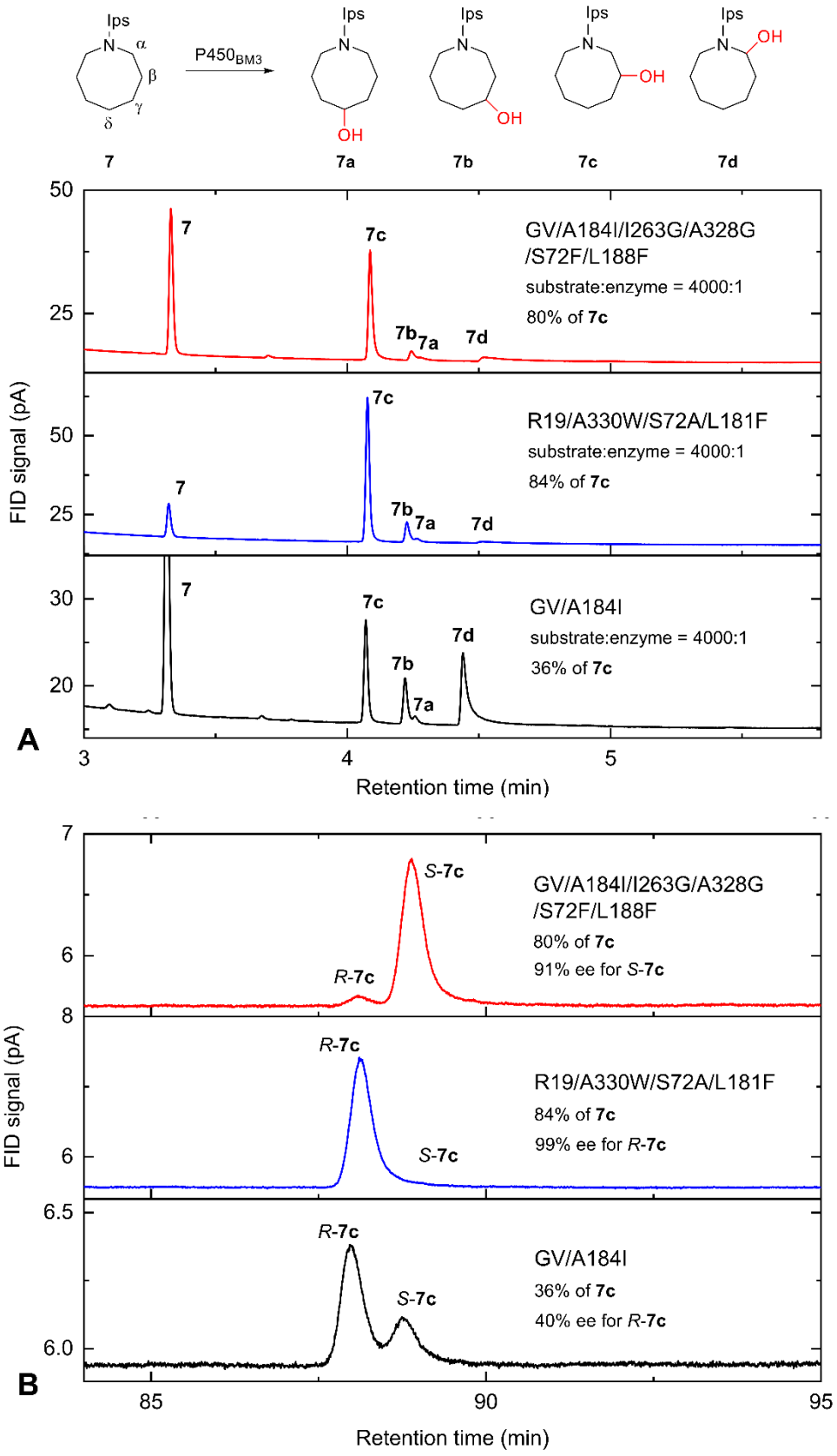


Figure A4.4. GC analysis of variants with the highest regio- and enantio-selectivity for the β alcohol **7c** of *N*-Ips-azocane: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

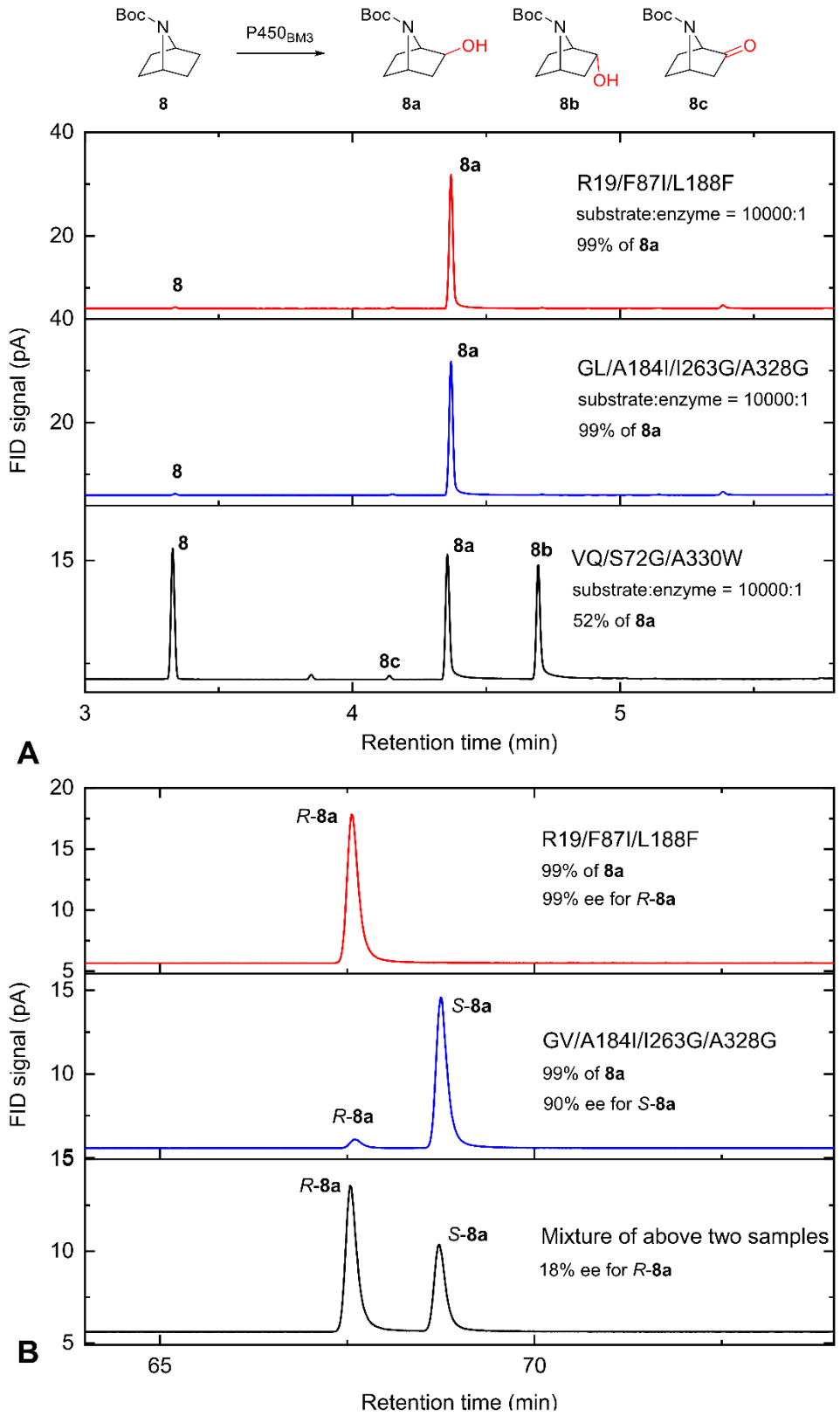


Figure A4.5. GC analysis of variants with the highest regio- and enantio-selectivity for the *exo* alcohol **8a** of **8**: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

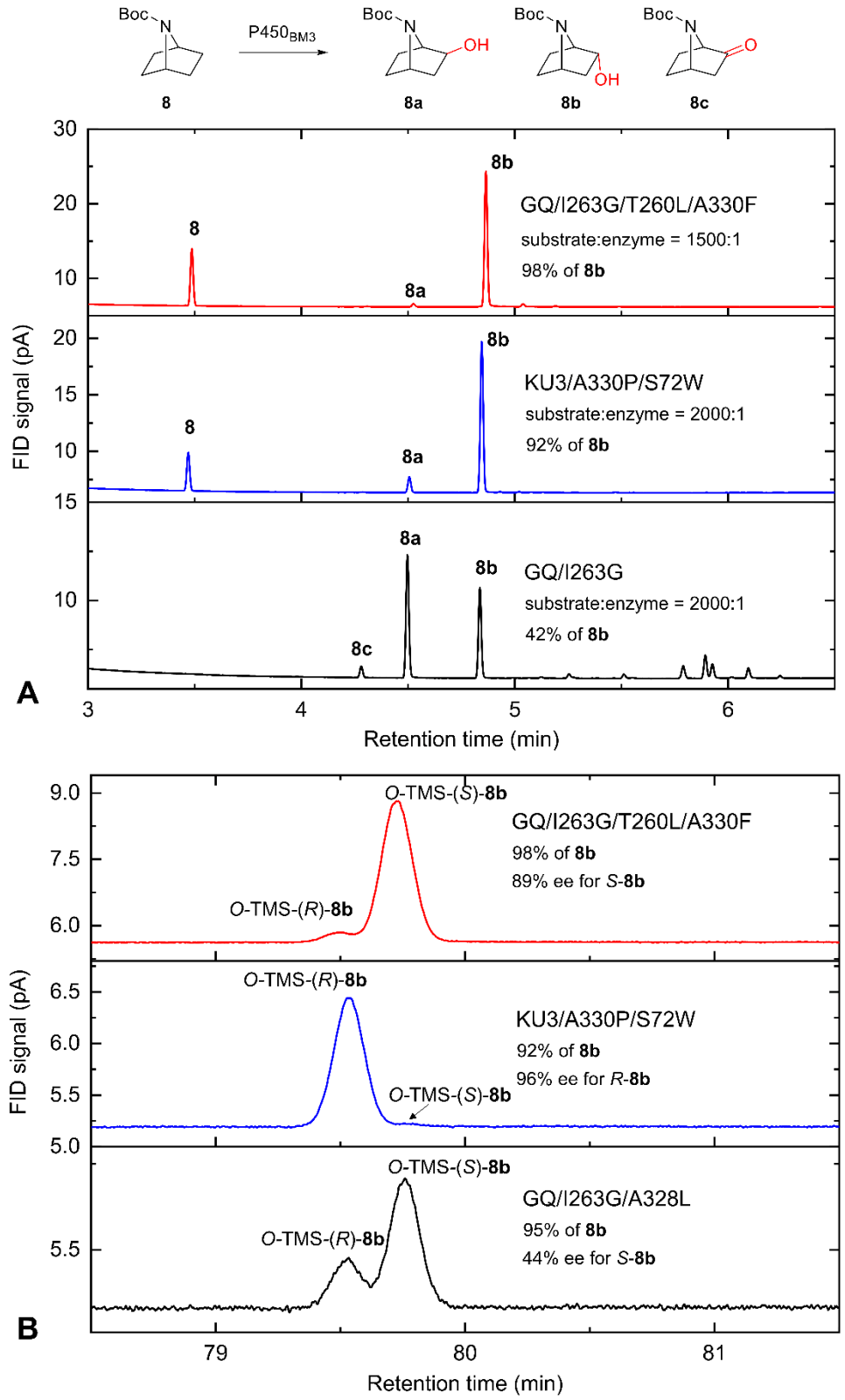


Figure A4.6. GC analysis of variants with the highest regio- and enantio-selectivity for the *endo* alcohol **8b** of **8**: **A**. chromatograms from a normal phase column; **B**. chromatograms from a chiral phase column.

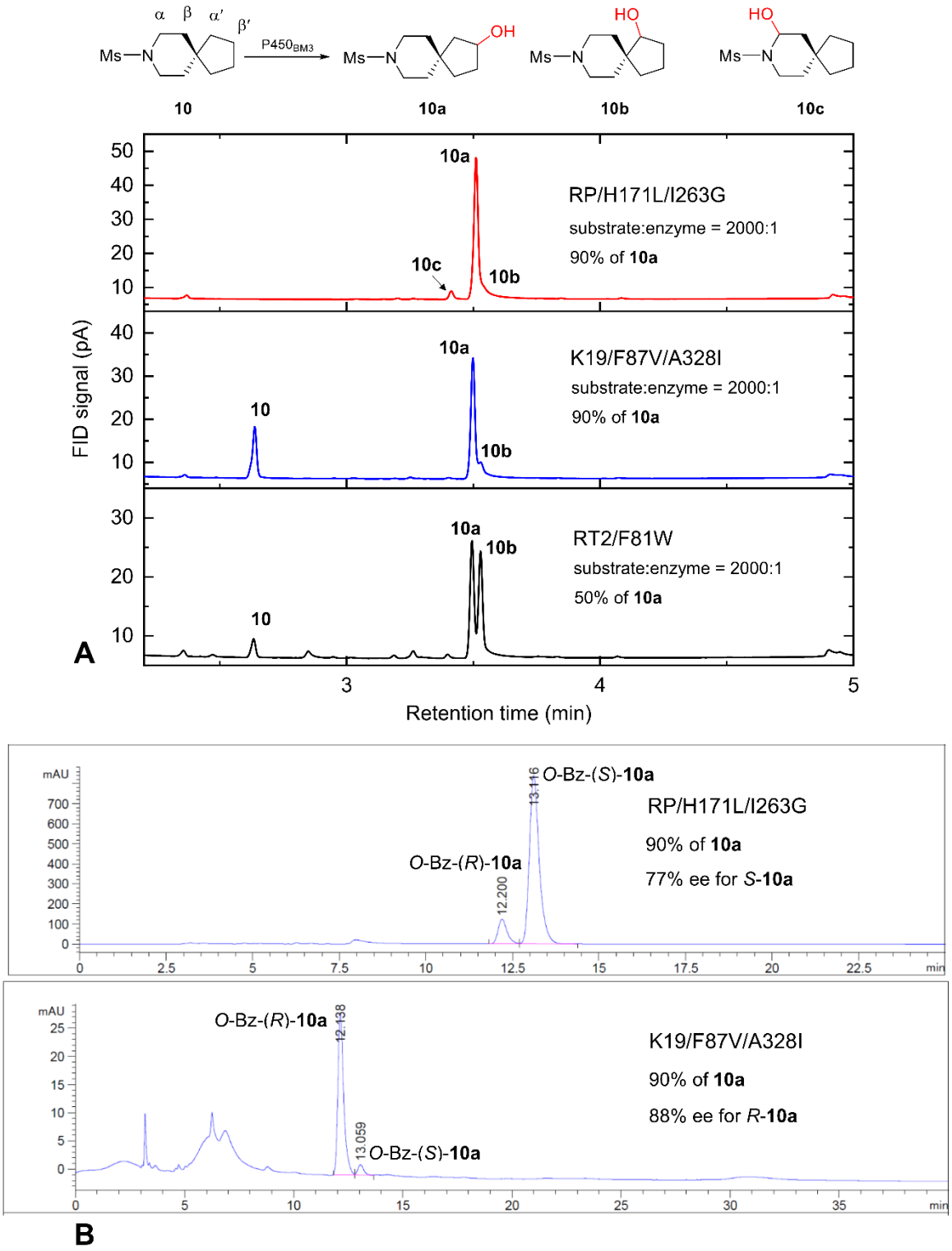


Figure A4.7. GC and chiral-phase HPLC analysis of variants with the highest regio- and enantio-selectivity for the β′ alcohol **10a** of **10**: **A**. GC chromatograms from a normal phase column; **B**. HPLC chromatograms from an AD-H column with hexane : isopropyl alcohol = 4:1 as the mobile phase, flow rate = 1 mL/min, run time = 40 min. Retention times for products: *N*-Ms-*O*-Bz-2(*R*)-hydroxy-8-azaspiro[4.5]decane (*O*-Bz-(*R*)-**10a**), 12.2 min, *N*-Ms-*O*-Bz-2(*S*)-hydroxy-8-azaspiro[4.5]decane (*O*-Bz-(*S*)-**10a**), 13.1 min.

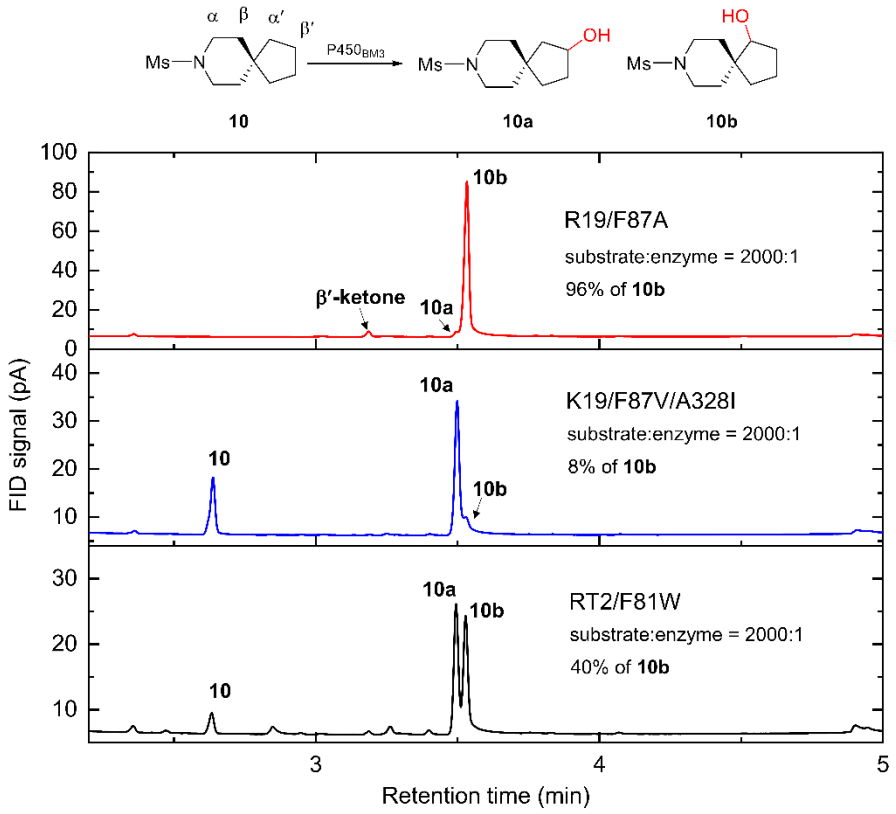
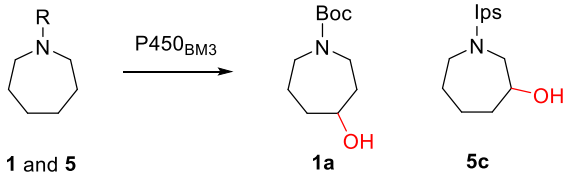


Figure A4.8. GC analysis of variants with the highest regioselectivity for the α′ alcohol **10b** of **10** from a normal phase column.

A4.2 Selectivity data for major products

A4.2.1 Selectivity data for azepane derivatives

Table A4.1. Activity and selectivity of selected $P450_{BM3}$ variants in the screening panel for the oxidation of *N*-Boc-azepane (**1**) to 4-hydroxy-*N*-Boc-azepane (**1a**), and of *N*-Ips-azepane (**2**) to 3-hydroxy-*N*-Ips-azepane (**5c**). TON refers to the total turnover number for the formation of the enantiomer in a reaction with a substrate-to-enzyme ratio of 2000:1.



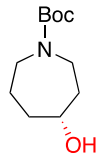
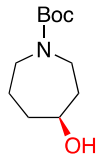
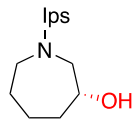
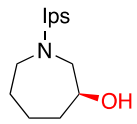
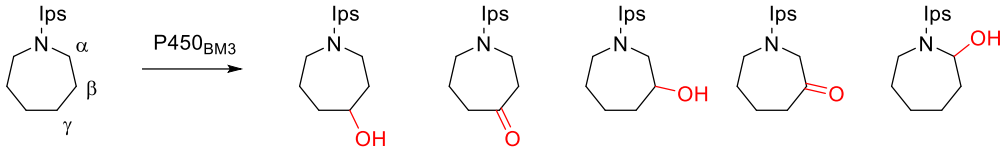
Product	Variant	Conversion	Regioselectivity	*ee*	TON
 (*R*)-**1a** (*R*)-4-hydroxy-*N*-Boc-azepane	**RLYF/H171L/I263G/A330W/L437S**	**80%**	**90%**	**90%**	**1370**
	RLYF/H171L/I263G/L437LF	91%	86%	83%	1430
	RP/H171L/I263G	96%	70%	80%	1210
	RT2/S72G/I263G/A330W	86%	75%	79%	1160
	KU3/I263G/A330P	84%	79%	43%	950
	RLYF/I263G/A330P	95%	75%	42%	1010
	RLYF/H171L/I263G/A330P	98%	64%	20%	750
 (*S*)-**1a** (*S*)-4-hydroxy-*N*-Boc-azepane	**KU3/S72W/A330P**	**96%**	**90%**	**84%**	**1590**
	KU3/A330P	85%	92%	81%	1420
	RT2/A330P	95%	93%	82%	1610
	KU3/I263V/A330P	30%	94%	82%	510
	RT2/V78I/A184I/A330P	86%	85%	82%	1330
	RT2/A330W	93%	50%	60%	750
 (*R*)-**5c** (*R*)-3-hydroxy-*N*-Ips-azepane	**VQ/S72G/A330W**	**60%**	**91%**	**96%**	**1070**
	R19/S72G/A330W	33%	45%	94%	280
	R19/A330W	45%	29%	91%	250
	VQ/S72P/A330W	36%	93%	90%	630
	GVQ/A330W	20%	82%	90%	310
	RP/F87V	42%	64%	82%	480
	GVQ	45%	67%	75%	530
 (*S*)-**5c** (*S*)-3-hydroxy-*N*-Ips-azepane	GV/A184I	47%	50%	30%	300
	GV/A184I/A328G	32%	16%	45%	75
	GVQ/I263G	35%	83%	60%	460
	K19/F87V/I263G	43%	65%	60%	440
	GV/A184I/I263G	48%	50%	60%	380
	GVQ/I263A/A328G	15%	65%	81%	180
	GV/A184I/I263G/A328G	**22%**	**76%**	**79%**	**290**

Table A4.2 Docking-guided mutagenesis of $P450_{BM3}$ for the oxidation of *N*-Ips-azepane (**5**) to the (*S*)-β alcohol (*S*)-3-hydroxy-*N*-Ips-azepane, (*S*)-**5c**, with GV/A184I/I263G/A328G as the precursor variant. The substrate-to-enzyme ratio was 1000:1 unless indicated otherwise. TON: total turnover number for the formation of (*S*)-**5c** in reactions with a substrate-to-enzyme ratio of 1000:1 unless stated otherwise; Product percentages may not sum to 100% due to minor side product(s).

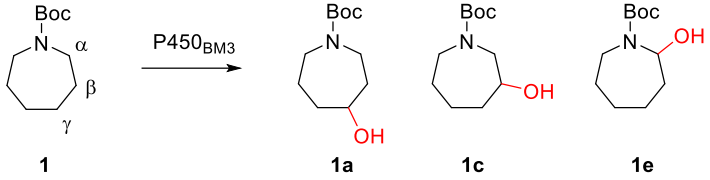


5 **5a** **5b** **5c** **5d** **5e**

Variants	Conversion	(*S*)-3-hydroxy-*N*-Ips-azepane, (*S*)-5c	TON for (*S*)-5c	5d	5a	5e
Base variant						
GV/A184I/I263G/A328G	**43%**	**76% (79% *ee*)**	**290**	**3%**	**7%**	**15%**
Additional mutations						
F87L	82%	94% (88% *ee*)	720	6%	–	–
F87I	66%	89% (74% *ee*)	510	7%	4%	–
L75F	98%	46% (83% *ee*)	410	3%	8%	42%
L75M	65%	49% (82% *ee*)	290	6%	7%	43%
S72F	98%	49% (81% *ee*)	430	8%	14%	20%
S72M	82%	45% (85% *ee*)	340	2%	5%	48%
A330F	88% [a]	43% (79% *ee*)	1350	2%	36%	9%
F87L/L75M	82%	91% (91% *ee*)	710	5%	–	4%
F87L/S72F	52%	96% (91% *ee*)	480	–	–	–
F87L/S72M	26%	95% (88% *ee*)	220	–	–	–
F87L/A330F	40% [a]	97% (87% *ee*)	1450	2%	–	–
F87I/L75F	44%	78% (86% *ee*)	330	2%	2%	18%
F87I/L75M	60%	77% (88% *ee*)	430	4%	4%	14%
F87I/S72F	90%	77% (84% *ee*)	640	9%	3%	8%
F87I/S72M	60%	82% (82% *ee*)	450	3%	6%	6%
F87L/S72F/A330F	40% [a]	98% (88% *ee*)	1470	–	–	–
F87L/S72F/L188M	**40% [a]**	**97% (92% *ee*)**	**1490**	**–**	**–**	**2%**
F87L/S72F/L188F	25% [a]	97% (92% *ee*)	930	–	–	3%

[a] Substrate-to-enzyme ratio = 4000:1.

Table A4.3 Docking-guided mutagenesis of $P450_{BM3}$ for the oxidation of *N*-Boc-azepane (**1**) to the (*R*)-γ alcohol (*R*)-4-hydroxy-*N*-Boc-azepane, (*R*)-**1a**, with RLYF/H171L/I263G/A328G/A330W/L437S as the precursor variant. The substrate:enzyme ratio was 20000:1. β=O: 4-oxo-*N*-Boc-azepane; γ-ol: 4-hydroxy-*N*-Boc-azepane. TON: total turnover number for the formation of (*R*)-**1a** in reactions with a substrate-to-enzyme ratio of 20000:1. Product percentages may not sum to 100% due to minor side product(s).



1 **1a** **1c** **1e**

Variants	Conversion	(*R*)-4-hydroxy-*N*-Boc-azepane (*R*)-1a	TON for (*R*)-1a	1c	1e
Base variant					
RLYF/H171L/I263G/A328G/A330W/L437S	**60%**	**90% (90% ee)**	**10300**	**8%**	**2%**
Additional mutations					
V78F	**80%**	**94% (93% ee)**	**14500**	**2%**	**4%**
I263F	50%	90% (91% ee)	8600	6%	4%
V26M	65%	87% (85% ee)	10500	7%	5%
V26L	55%	90% (88% ee)	9300	8%	2%

A4.2.2 Selectivity data for azocane derivatives

Table A4.4. Activity and selectivity of selected $P450_{BM3}$ variants in the screening panel for the oxidation of *N*-Boc-azocane (**6**) to 4-hydroxy-*N*-Boc-azocane (**6b**), and of *N*-Ips-azocane (**7**) to 5-hydroxy-*N*-Ips-azocane (**7a**) and 3-hydroxy-*N*-Ips-azocane (**7c**). TON refers to the total turnover number for the formation of the enantiomer in a reaction with a substrate-to-enzyme ratio of 2000:1 for **6** and 1000:1 for **7**.

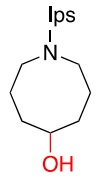
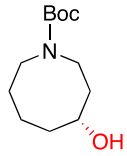
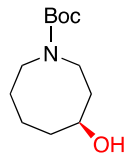
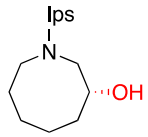
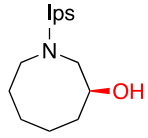
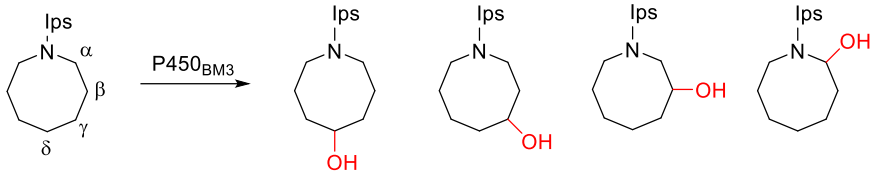
Products	Variants	Conversion	Regioselectivity	*ee*	TON
 **7a** 5-hydroxy-*N*-Ips-azocane	**R19/I263G/A328L**	**95%**	**95%**	**–**	**900**
 (*R*)-**6b** (*R*)-4-hydroxy-*N*-Boc-azocane	**RLYF/I263G/A330P**	**100%**	**90%**	**98%**	**1780**
	RT2/S72G/I263G/A330W	90%	85%	98%	1800
	RP/H171L/S72W/I263G	95%	75%	98%	1520
	RP/H171L/I263G	99%	67%	97%	1310
	KU3/I263G/A330P	99%	85%	96%	1580
	RLYF/H171L/I263G/A330P	100%	80%	96%	1570
	RLYF/H171L/I263G/A330W/L437S	100%	51%	83%	930
	RT2/S72G/I263S/A330W	80%	80%	82%	1170
 (*S*)-**6b** (*S*)-4-hydroxy-*N*-Boc-azocane	KU3/S72G/A330P	50%	82%	60%	660
	R19/S72G/A330W	50%	35%	65%	290
	KU3/A330P	95%	75%	70%	1210
	R19/F87I	95%	45%	80%	770
	KU3/S72W/A330P	**100%**	**74%**	**77%**	**1240**
 (*R*)-**7c** (*R*)-3-hydroxy-*N*-Ips-azocane	R19/S72P/A330W	40%	86%	99%	340
	R19/S72A/A330W	**40%**	**85%**	**99%**	**340**
	R19/S72G/A330W	40%	80%	99%	320
	GVQ/A330W	83%	34%	90%	270
	GVQ/I263G	75%	66%	20%	300
	GV/A184I/I263G	90%	40%	18%	210
 (*S*)-**7c** (*S*)-3-hydroxy-*N*-Ips-azocane	GVQ/I263A/A328G	80%	75%	71%	510
	GV/A184I/I263G/A328G	**30%**	**76%**	**77%**	**180**

Table A4.5. Docking-guided mutagenesis for the formation of the (*S*)-β alcohol (*S*)-3-hydroxy-*N*-Ips-azocane, (*S*)-**7c**, with GV/A184I/I263G/A328G as the precursor variant. TON: total turnover number for the formation of (*S*)-**7c** in a reaction with a substrate-to-enzyme ratio as specified below; β=O: 3-oxo-*N*-Ips-azocane; Product percentages may not sum to 100% due to minor side product(s).



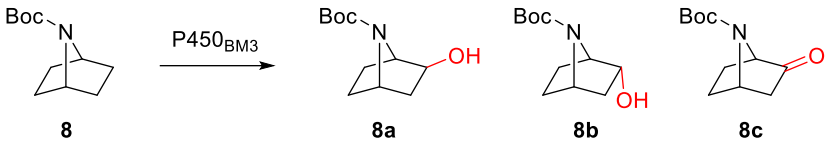
7 **7a** **7b** **7c** **7d**

Variants	Conversion	(*S*)-3-hydroxy-*N*-Ips-azocane (*S*)-7c	TON for (*S*)-7c	β=O	7b	7a	7d
Base variant							
GV/A184I/I263G/A328G	**27% [a]**	**76% (77% ee)**	**180**	**–**	**13%**	**7%**	**2%**
Additional mutations							
F87L	67% [a]	86% (55% ee)	450	5%	–	8%	–
S72F	46% [a]	73% (90% ee)	320	2%	9%	6%	10%
S72M	57% [a]	49% (ee: ND)	–	–	10%	6%	35%
L75M	60% [a]	48% (ee: ND)	–	–	27%	–	25%
F87L/S72F	40% [a]	91% (66% ee)	300	–	8%	–	–
S72F/L188F	**65% [b]**	**80% (91% ee)**	**1000**	**2%**	**5%**	**4%**	**9%**
S72F/S332F	68% [b]	70% (95% ee)	930	3%	9%	3%	15%
S72F/I263V	43% [b]	80% (92% ee)	660	–	5%	5%	10%
S72F/L188F/V26L	16% [b]	85% (89% ee)	260	–	5%	4%	6%

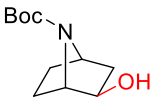
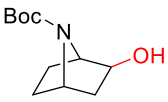
[a] Substrate-to-enzyme ratio =1000:1.
[b] Substrate-to-enzyme ratio =2000:1.

A4.2.3 Selectivity data for 7-azabicyclo[2.2.1]heptane derivatives

Table A4.6. Activity and selectivity of selected $P450_{BM3}$ variants in the screening panel for the oxidation of *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**) to *exo*-2-hydroxy-*N*-Boc-7-azabicyclo[2.2.1]heptane (**8a**) and *endo*-2-hydroxy-*N*-Boc-7-azabicyclo[2.2.1]heptane (**8b**). TON refers to the total turnover number for the formation of the enantiomer in a reaction with a substrate-to-enzyme ratio of 2000:1.



8 **8a** **8b** **8c**

Product	Variant	Conversion	% 8a	*ee*	TON
 (*R*)-**8a** (*R*)-*exo*-2-hydroxy-*N*-Boc-7-azabicyclo-[2.2.1]heptane	**R19/F87I**	**99%**	**>99%**	**>99%**	**2000**
	K19/F87V/E267V	99%	89%	99%	1750
	K19/F87V/A264G	85%	45%	97%	750
	GVQ	99%	68%	99%	1340
	GVQ/A328G	60%	51%	86%	570
	GVQ/A330W	90%	41%	99%	740
	GLQ/I263G/A328F	99%	100%	97%	1930
	GIQ/I263G	90%	99%	90%	1860
	GIQ/I263G/A328G	95%	93%	26%	1110
 (*S*)-**8a** (*S*)-*exo*-2-hydroxy-*N*-Boc-7-azabicyclo-[2.2.1]heptane	**GVQ/I263G/A328G**	**99%**	**99%**	**86%**	**1840**
	GLQ/I263G	99%	99%	56%	1530
	R19/F87A/A328L	75%	17%	63%	210
	K19/F87A/F81W	90%	67%	99%	1200

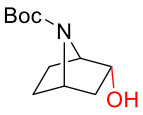
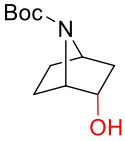
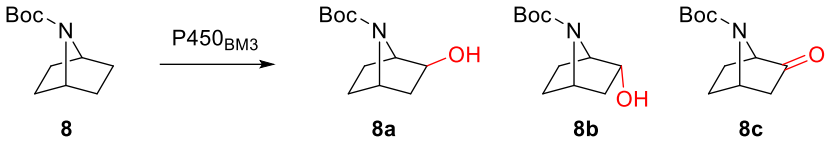
Product	Variant	Conversion	% 8a	*ee*	TON
 (*R*)-**8b** (*R*)-*endo*-2-hydroxy-*N*-Boc-7-azabicyclo-[2.2.1]heptane	GVQ/I263V/A328L	96%	97%	57%	1460
	R19/A330W	90%	95%	68%	1440
	R19/S72A/A330W	95%	95%	76%	1590
	GVQ/I263A/A328L	98%	98%	77%	1770
	R19/S72G/A330W	95%	97%	80%	1660
	KU3/S72W/A330P	**98%**	**93%**	**96%**	**1790**
 (*S*)-**8b** (*S*)-*endo*-2-hydroxy-*N*-Boc-7-azabicyclo-[2.2.1]heptane	**GQ/I263G/A328L**	**18%**	**95%**	**44%**	**240**

Table A4.7. Docking-guided mutagenesis for the oxidation of *N*-Boc-7-azabicyclo[2.2.1]heptane, **8**, to the (*S*)-*endo* alcohol (*S*)-*endo*-2-hydroxy-*N*-Boc-7-azabicyclo[2.2.1]heptane, (*S*)-**8b**, with GQ/I263G/A328L as the precursor variant. TON: total turnover number for the formation of (*S*)-**8b** in a reaction with a substrate-to-enzyme ratio as specified below.



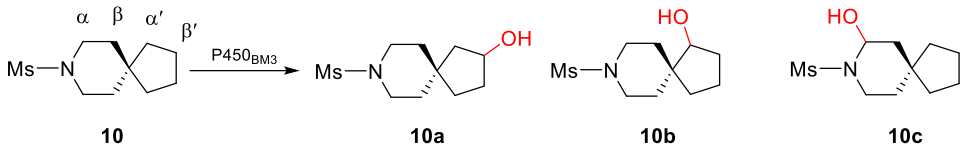
8 **8a** **8b** **8c**

Variants	Conversion	% 8b	*ee* for (*S*)-8b	TON for (*S*)-8b	% 8a	%8c
Base variant						
GQ/I263G/A328L	**18%** [a]	**95%**	**44%**	**240**	**5%**	–
Additional mutations						
L75F	31% [a]	96%	60%	480	4%	–
S332F	28% [a]	96%	52%	410	4%	–
L75F/S332F	30% [a]	84%	71%	430	12%	4%
T260L	48% [b]	97%	76%	410	3%	–
T260L/A82M	63% [a]	95%	79%	1070	3%	2%
T260L/S332F	24% [b]	96%	86%	210	4%	–
T260L/A330F	**92%** [b]	**98%**	**88%**	**850**	**2%**	–
T260L/A330F/A82M	49% [a]	98%	82%	870	2%	–

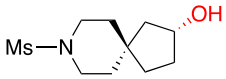
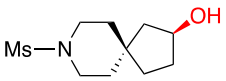
[a] Substrate-to-enzyme ratio = 2000:1.
[b] Substrate-to-enzyme ratio = 1000:1.

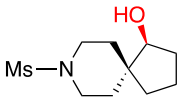
A4.2.4 Selectivity data for 8-azaspiro[4.5]decane derivatives

Table S10. Activity and selectivity of selected $P450_{BM3}$ variants in the screening panel for the oxidation of *N*-Ms-8-azaspiro[4.5]decane (**10**) to *N*-Ms-8-azaspiro[4.5]decan-2-ol (**10a**) and *N*-Ms-8-azaspiro-[4.5]decan-1-ol (**10b**). TON refers to the total turnover number for the formation of the enantiomer in a reaction with a substrate-to-enzyme ratio of 2000:1.



10 **10a** **10b** **10c**

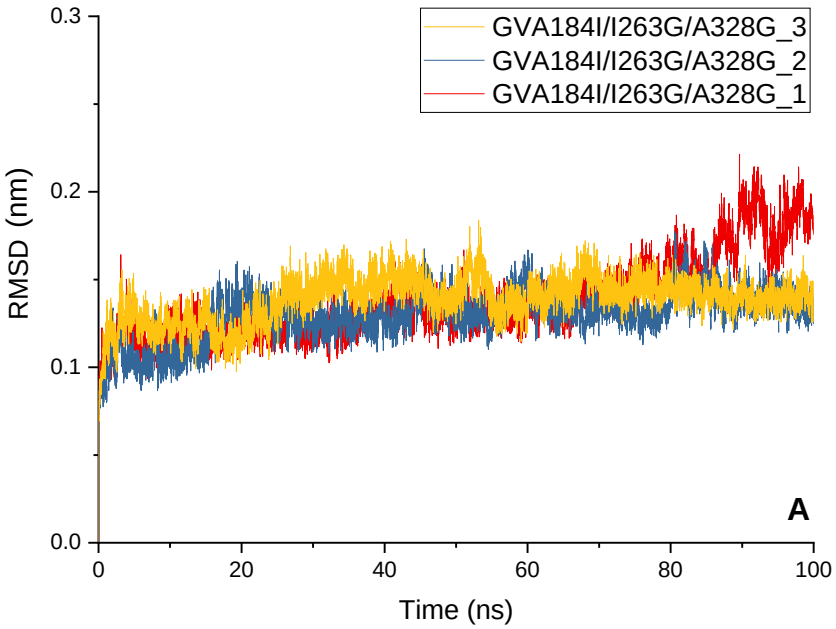
Product	Variants	Conv.	% 10a	*ee*	TON	10b	10c
 (*R*)-**10a** (*R*)-*N*-Ms-8-azaspiro-[4.5]decan-2-ol	K19/F87V/A328I	50%	90%	88%	850	8%	2%
	R19/F87A/A328F	50%	96%	–	1000[a]	4%	–
	GVQ/I263G	95%	90%	–	1900[a]	7%	3%
	K19/F87V/A328V	40%	72%	–	800[a]	20%	8%
 (*S*)-**10a** (*S*)-*N*-Ms-8-azaspiro-[4.5]decan-2-ol	GVQ/I263G/V78L	60%	68%	–	1200[a]	25%	7%
	R19/F87A/A328L	40%	92%	70%	630	6%	2%
	RP/H171L/I263G	85%	90%	77%	1350	7%	3%

Product	Variants	Conv.	% 10b	*ee*	TON	10a	10c
 (*S*)-**10b** (*S*)-*N*-Ms-8-azaspiro-[4.5]decan-1-ol	VQ/S72G/A330W	100%	84%	95%	1640		
	R19/F87A/A82M/A330W	73%	97%	96%	1390		
	R19/F87A/F81W	55%	95%	94%	1010		
	R19/F87A	100%	96%	95%	1900		
	GVQ	85%	95%	95%	1580		
	RP/V78I/E267V	100%	92%	96%	1800		

[a] Based on substrate conversion.

A5. MD simulation and molecular docking

A5.1 Docking study of *N*-Ips-azepane 5



	Cluster population
Replica 1	C1 (4323) C2 (3190) C3 (1263)
Replica 2	C1 (9043) C2 (606)
Replica 3	C1 (7751) C2 (2023)

B

Figure A5.1. MD-simulation outputs for GV/A184I/I263G/A328G. **(A)** The RMSD plot for Cα atoms of 3 replicas of GV/A184I/I263G/A328G during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

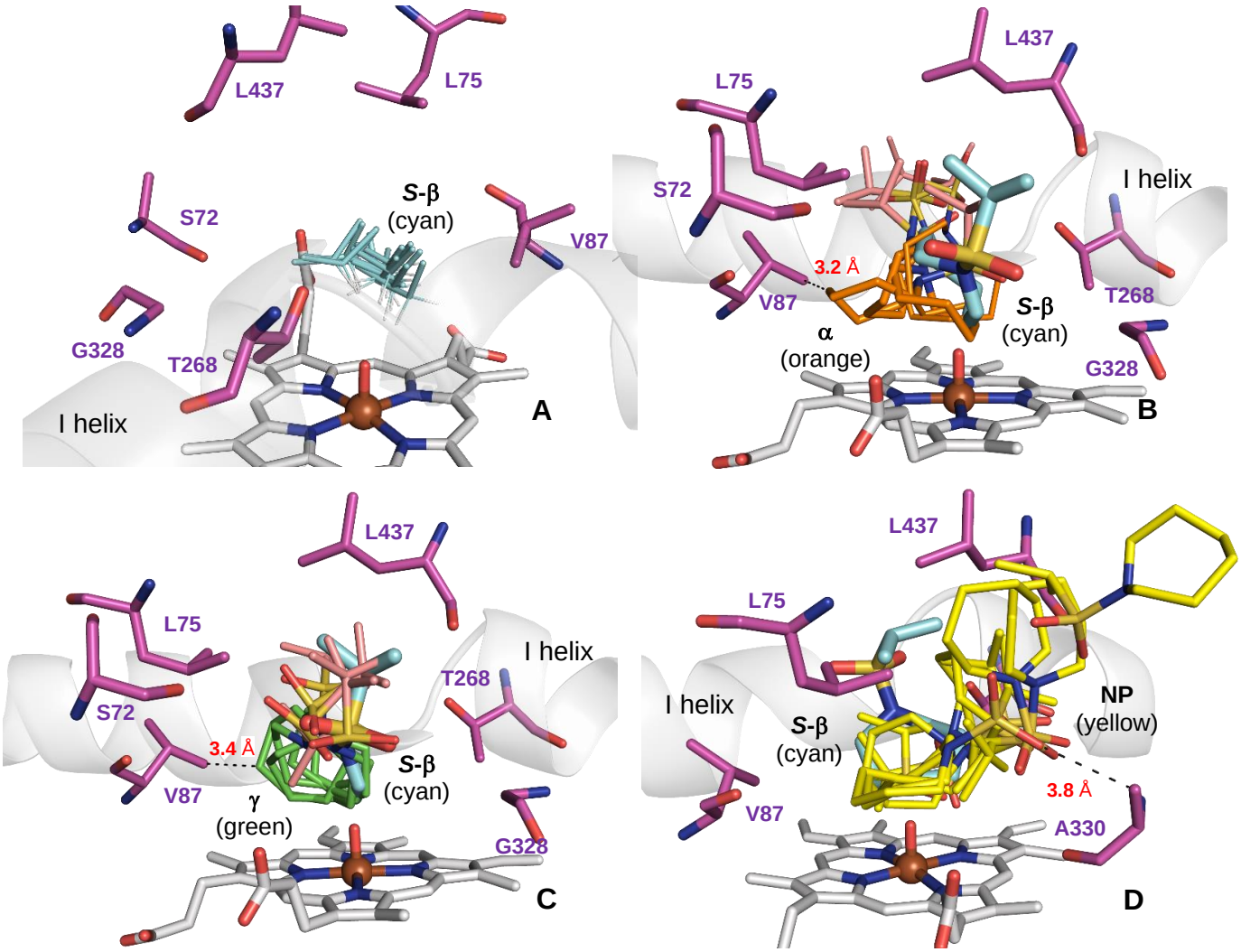
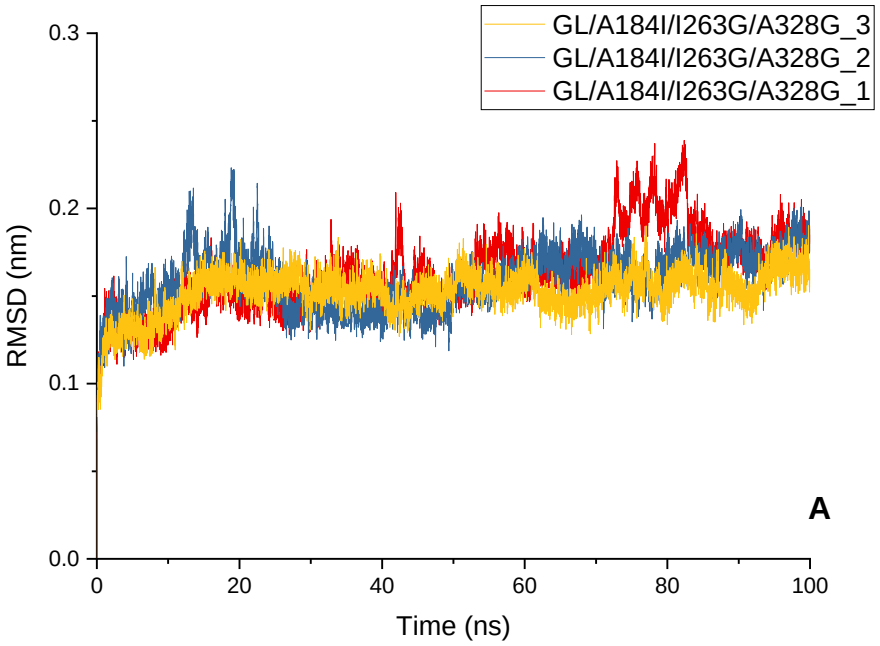


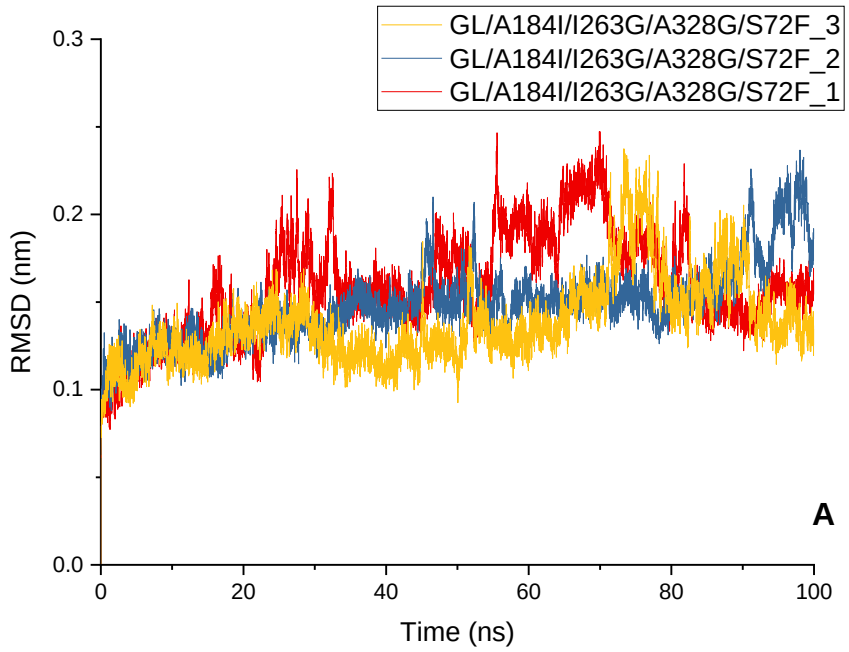
Figure A5.2. Analysis of results from the docking of *N*-Ips-azepane, **2**, with the GV/A184I/I263G/A328G variant. **(A)** The positions of the pro-(*S*) hydrogens on C3 of the azepane ring of the (*S*)-□ poses. **(B)** The (*S*)-β poses (cyan) overlaid with α poses; mutations F87L/I, S72F/M and L75F/M could destabilise α poses without greatly affecting the (*S*)-β poses and therefore improve the regioselectivity for the 3-alcohol **2b**. **(C)** The (*S*)-β poses (cyan) overlaid with γ poses; mutations F87L/I, S72F/M and L75F/M could destabilise the γ poses without affecting the (*S*)-β poses and therefore improve the regioselectivity for the 3-alcohol **2b**. **(D)** The (*S*)-β poses (cyan) overlaid with non-productive (NP) poses; mutations A330F/M could destabilise the NP poses and therefore increase the proportion of productive poses and improve enzymatic activity.



	Cluster population
Replica 1	C1 (4790) C2 (1701) C3 (1336)
Replica 2	C1 (5532) C2 (2078) C2 (988)
Replica 3	C1 (8686) C2 (961)

B

Figure A5.3. MD-simulation outputs for GL/A184I/I263G/A328G. **(A)** The RMSD plot for Cα atoms of 3 replicas of GL/A184I/I263G/A328G during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).



	Cluster population
Replica 1	C1 (3131) C2 (1944) C3 (1715)
Replica 2	C1 (4805) C2 (1885) C3 (1461)
Replica 3	C1 (6399) C2 (1606) C3 (769)

B

Figure A5.4. MD-simulation outputs for GL/A184I/I263G/A328G/S72F. **(A)** The RMSD plot for Cα atoms of 3 replicas of GL/A184I/I263G/A328G/S72F during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

A5.2 Docking study of N-Ips-azocane 7

A5.2.1 The (*S*)-β alcohol product

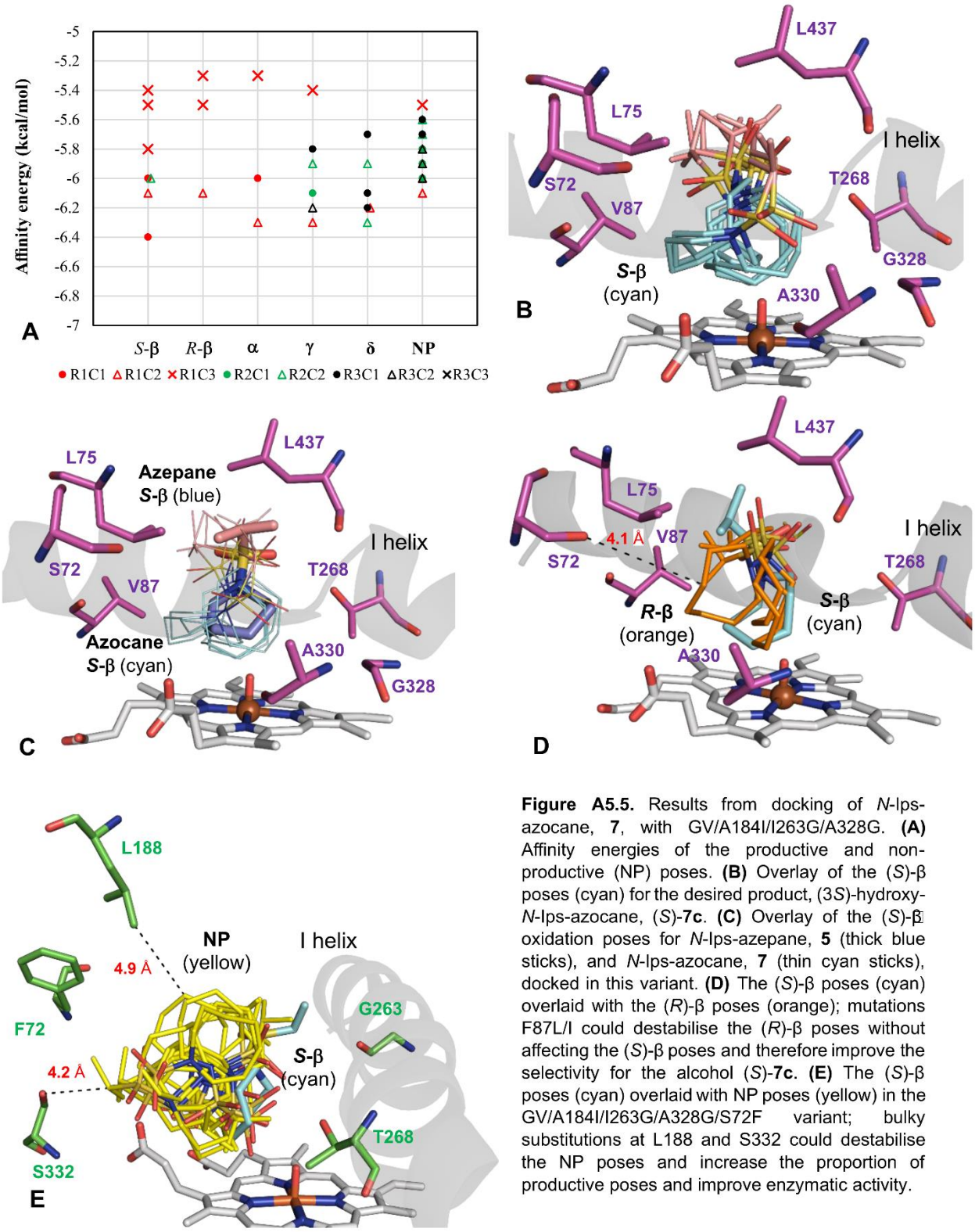
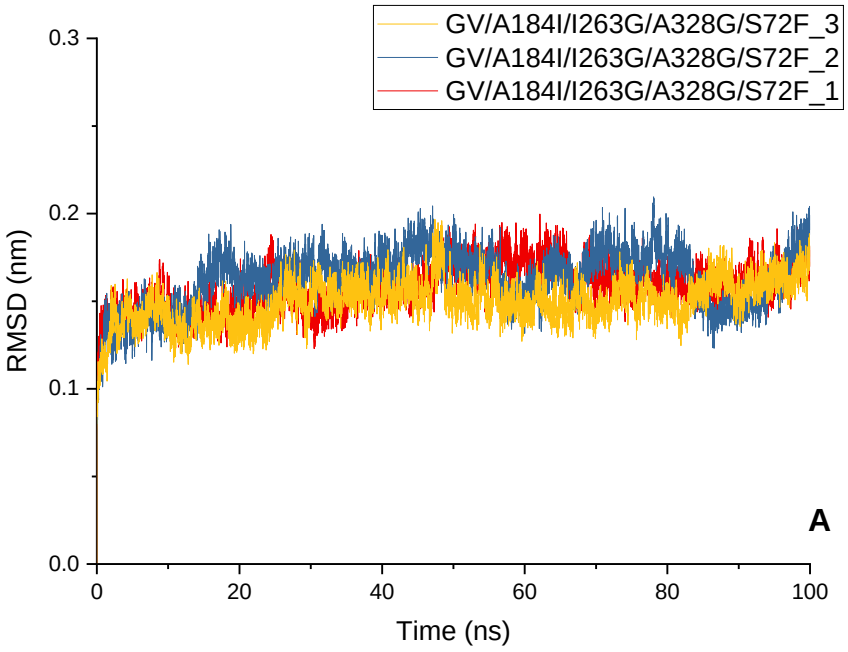


Figure A5.5. Results from docking of *N*-Ips-azocane, **7**, with GV/A184I/I263G/A328G. **(A)** Affinity energies of the productive and non-productive (NP) poses. **(B)** Overlay of the (*S*)-β poses (cyan) for the desired product, (3*S*)-hydroxy-*N*-Ips-azocane, (*S*)-**7c**. **(C)** Overlay of the (*S*)-β oxidation poses for *N*-Ips-azepane, **5** (thick blue sticks), and *N*-Ips-azocane, **7** (thin cyan sticks), docked in this variant. **(D)** The (*S*)-β poses (cyan) overlaid with the (*R*)-β poses (orange); mutations F87L/I could destabilise the (*R*)-β poses without affecting the (*S*)-β poses and therefore improve the selectivity for the alcohol (*S*)-**7c**. **(E)** The (*S*)-β poses (cyan) overlaid with NP poses (yellow) in the GV/A184I/I263G/A328G/S72F variant; bulky substitutions at L188 and S332 could destabilise the NP poses and increase the proportion of productive poses and improve enzymatic activity.

Figure A5.5. Results from docking of *N*-Ips-azocane, **7**, with GV/A184I/I263G/A328G.

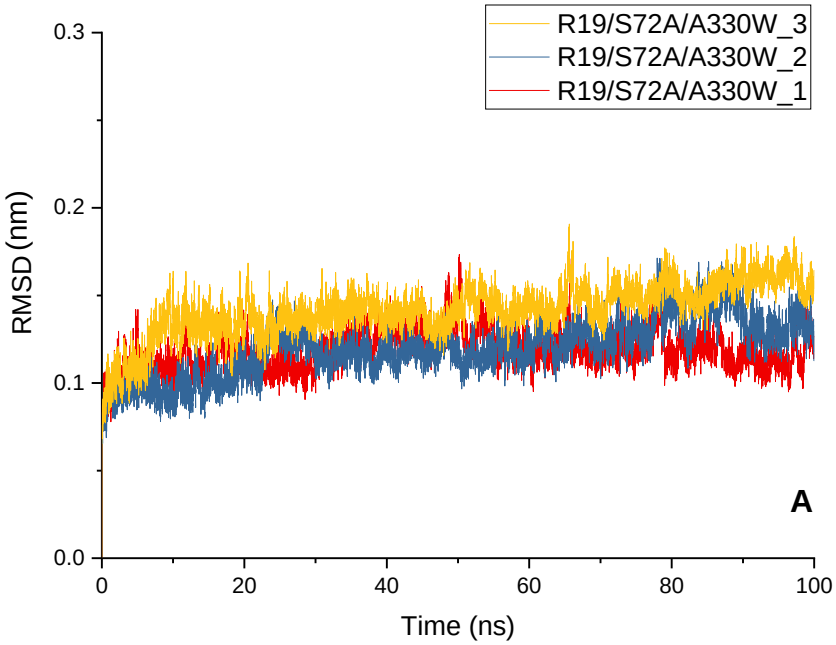


	Cluster population
Replica 1	C1 (5949) C2 (2536) C3 (873)
Replica 2	C1 (4775) C2 (3224) C2 (925)
Replica 3	C1 (6849) C2 (1365) C3 (782)

B

Figure A5.6. MD-simulation outputs for GV/A184I/I263G/A328G/S72F. **(A)** The RMSD plot for Cα atoms of 3 replicas of GV/A184I/I263G/A328G/S72F during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

A5.2.2 The (*R*)-β alcohol product



	Cluster population
Replica 1	C1 (7568) C2 (1226) C3 (519)
Replica 2	C1 (6328) C2 (1968) C3 (1404)
Replica 3	C1 (7010) C2 (1162) C3 (523)

B

Figure A5.7. MD-simulation outputs for R19/S72A/A330W. **(A)** The RMSD plot for Cα atoms of 3 replicas of R19/S72A/A330W during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

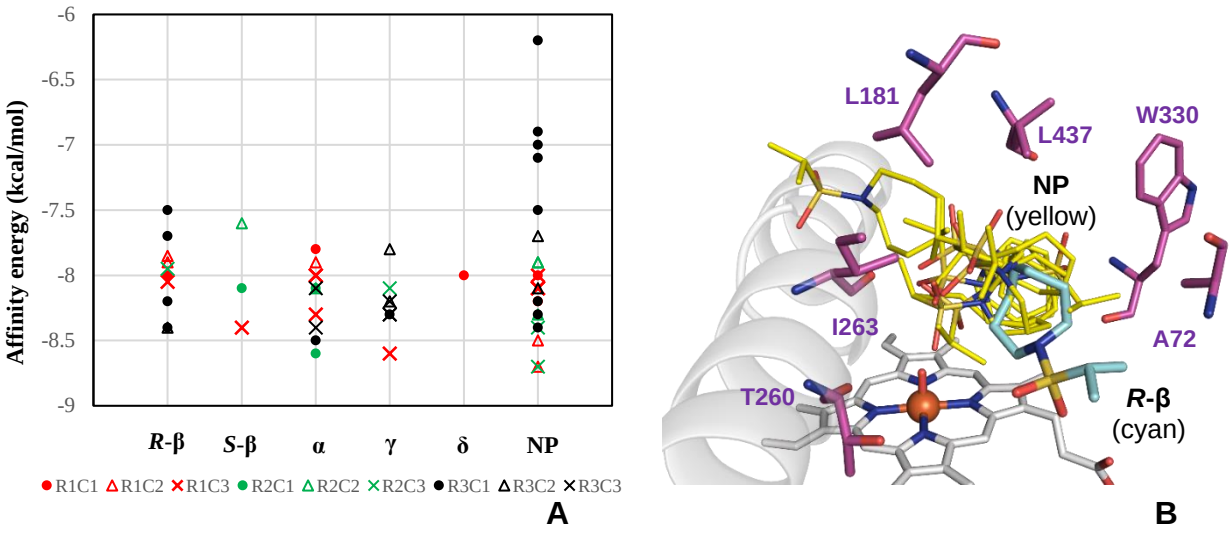
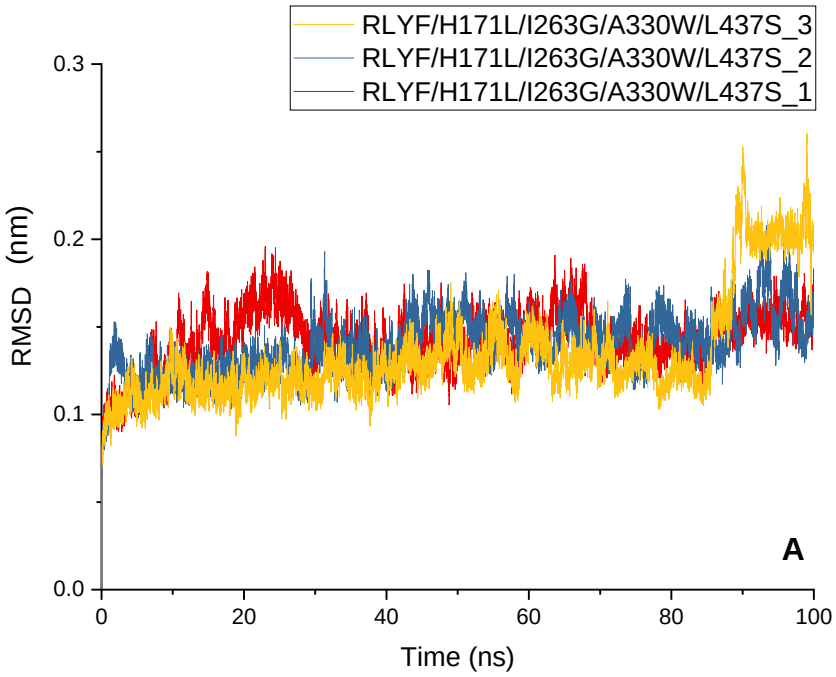


Figure A5.8. Analysis of results from the docking of *N*-Ips-azocane, **4**, with the R19/S72A/A330W variant. **(A)** Affinity energies of the productive and non-productive (NP) poses. **(B)** The (*R*)-β poses (cyan) overlaid with the NP poses (yellow); bulky substitutions at L181 could destabilise the NP poses and therefore increase the proportion of productive poses and improve enzymatic activity.

A5.3 Docking study of *N*-Boc-azepane 1

A5.3.1 The (*R*)-γ alcohol product



	Cluster population
Replica 1	C1 (5721) C2 (1728) C3 (1610)
Replica 2	C1 (8203) C2 (925) C3 (625)
Replica 3	C1 (6358) C2 (2440) C3 (521)

B

Figure A5.9. MD-simulation outputs for RLYF/H171L/I263G/A330W/L437S. **(A)** The RMSD plot for Cα atoms of 3 replicas of RLYF/H171L/I263G/A330W/L437S during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

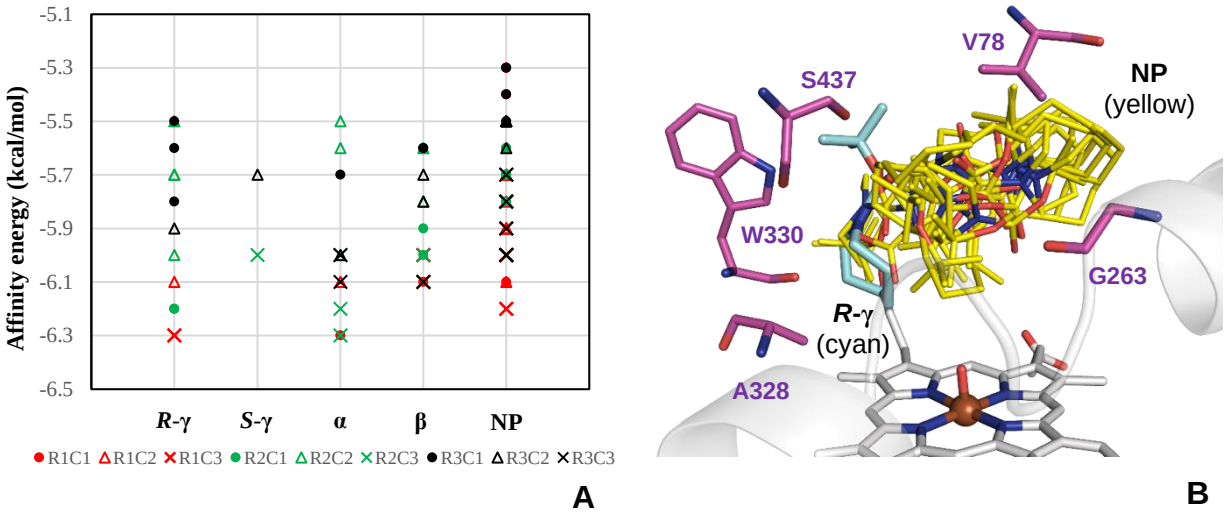
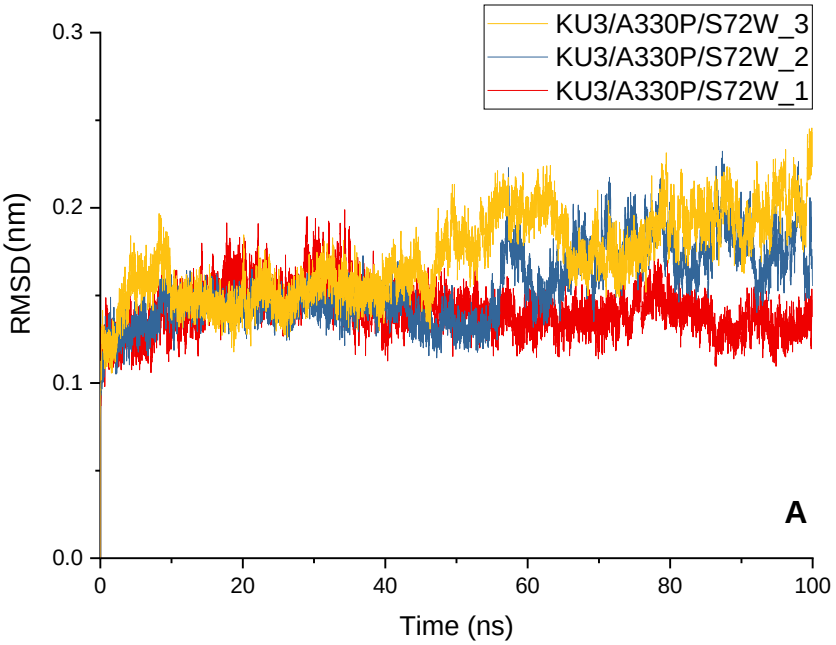


Figure A5.10. Analysis of results from the docking of *N*-Boc-azepane, **1**, with the variant RLYF/H171L/I263G/A330W/L437S. **(A)** Affinity energies of the productive and non-productive (NP) poses. **(B)** The (*R*)-γ poses (cyan) overlaid with the NP poses (yellow); bulky substitutions at V78 could destabilise the NP poses and therefore increase the proportion of productive poses and improve enzymatic activity.

A5.3.2 The (*S*)-γ alcohol product



	Cluster population
Replica 1	C1 (6316) C2 (2093) C3 (783)
Replica 2	C1 (6049) C2 (2513) C3 (583)
Replica 3	C1 (3982) C2 (2103) C3 (1572)

B

Figure A5.11. MD-simulation outputs for KU3/A330P/S72W. **(A)** The RMSD plot for Cα atoms of 3 replicas of KU3/A330P/S72W during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

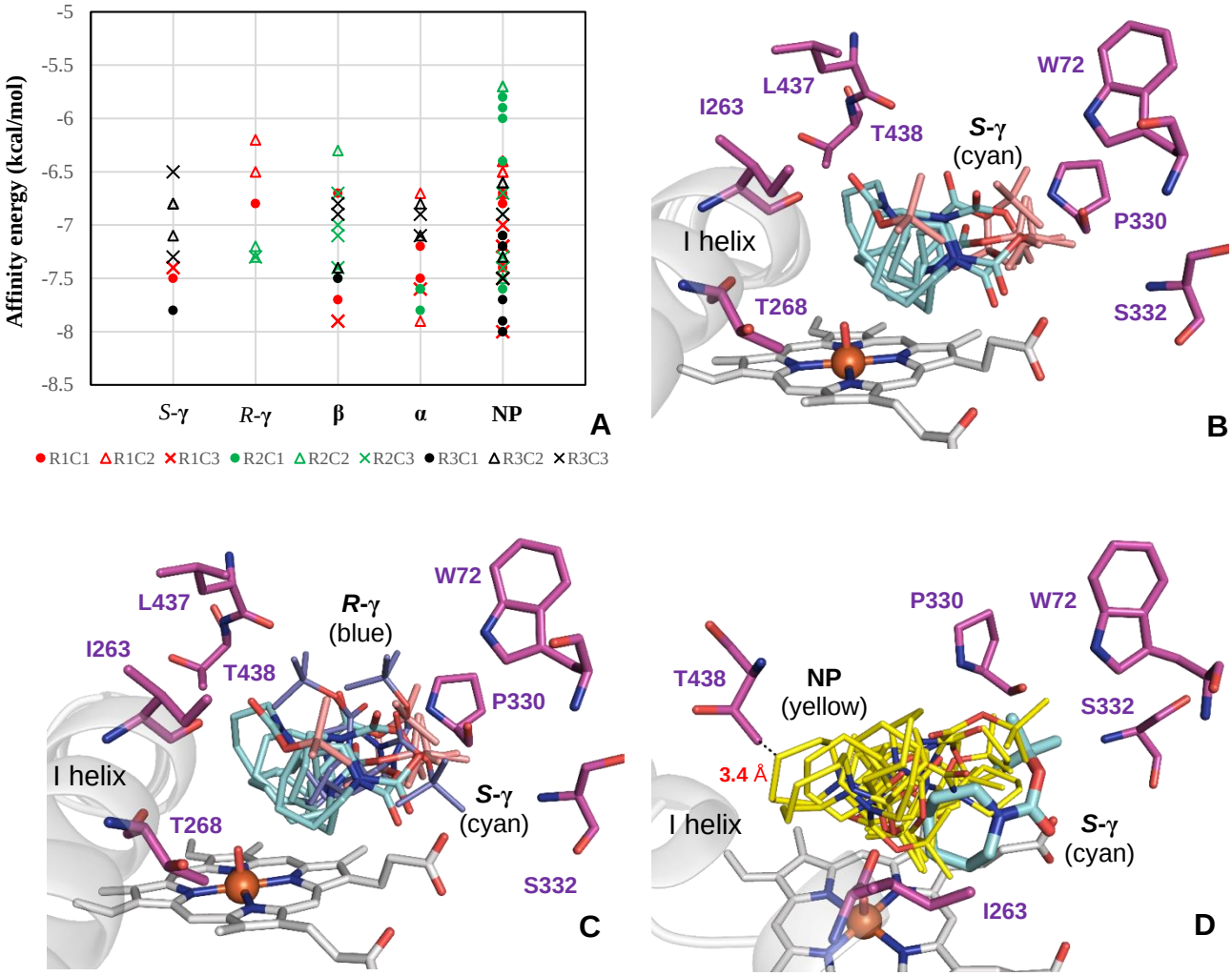
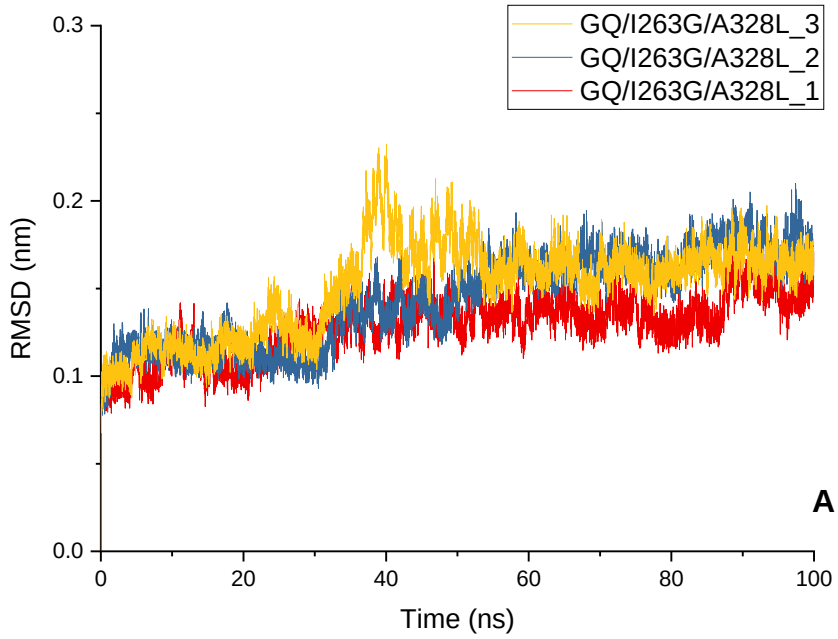


Figure A5.12. Analysis of results from the docking of *N*-Boc-azepane, **1**, with the KU3/A330P/S72W variant. **(A)** Affinity energies of the productive and non-productive (NP) poses. **(B)** Overlay of the (*S*)-γ poses (cyan) that would lead to the desired product, 4(*S*)-hydroxy-*N*-Boc-azepane, (*S*)-**1a**. **(C)** The (*S*)-γ poses (cyan) overlaid with the (*R*)-γ poses (blue); mutations to improve enantioselectivity of (*S*)-**1a** are difficult to propose due to the overlap of (*S*)-γ- and (*R*)-γ poses. **(D)** The (*S*)-γ poses (cyan) overlaid with the NP poses (yellow); bulky substitutions at T438 could destabilise the NP poses and therefore increase the proportion of productive poses and improve enzymatic activity.

A5.4 Docking study of *N*-Boc-7-azabicyclo[2.2.1]heptane 8



	Cluster population
Replica 1	C1 (6804) C2 (1984) C3 (783)
Replica 2	C1 (4807) C2 (2869) C2 (1132)
Replica 3	C1 (3186) C2 (2644) C3 (1642)

B

Figure A5.13. MD-simulation outputs for GQ/I263G/A328L. **(A)** The RMSD plot for Cα atoms of 3 replicas of GQ/I263G/A328L during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

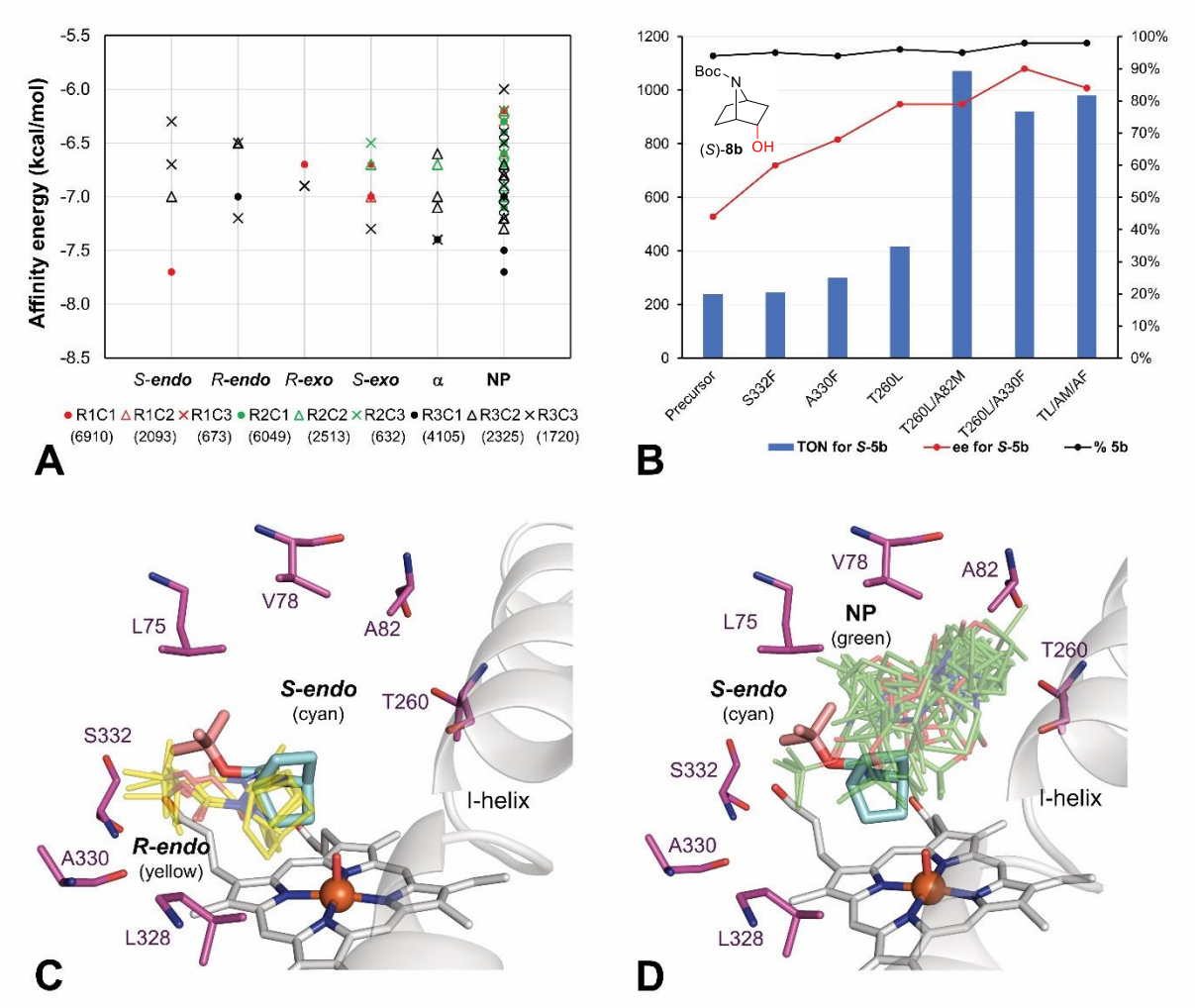
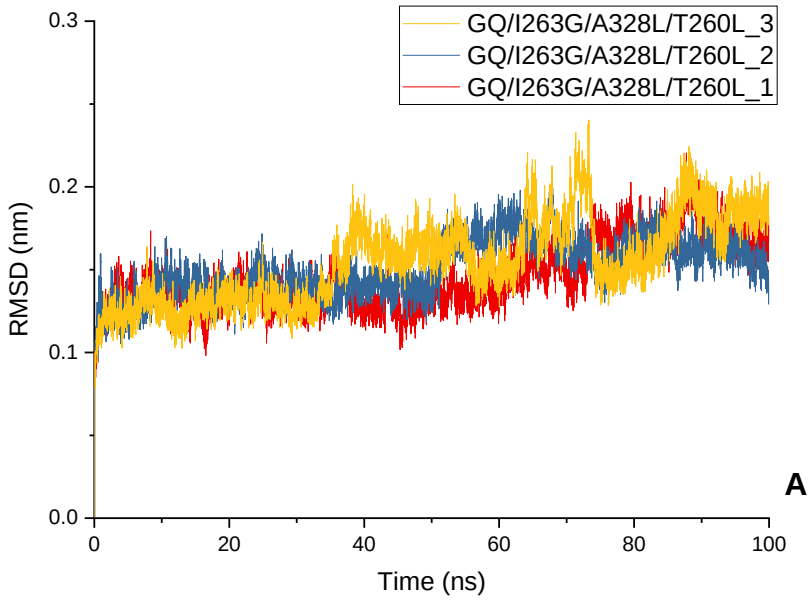


Figure A5.14. Iterative docking guide mutagenesis (IDGM) study for *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**), aiming to improve selectivity for the *S-endo* alcohol *S*-**8b**. **(A)** The distribution of output poses and their affinity energies from docking analysis on **8** with the MD-simulated structure of GQ/I263G/A328L; RxCy: simulated cluster y of replica x; Numbers below RxCy are the cluster sizes. **(B)** TON and ee for *S*-**8b** of selected variants generated in the ICGM process; Bars (blue) represents TON for *S*-**8b**; Dots (black) represent regioselectivity for **8b**; Dots (red) represents ee for *S*-**8b**; Precursor: GQ/I263G/A328L; S332F: GQ/I263G/A328L/S332F; A330F: GQ/I263G/A328L/A330F, *etc.*, TL/AM/AF: GQ/I263G/A328L/**T260L**/**A82M**/**A330F**. **(C)** Lowest energy *S-endo* pose (cyan) overlaid with *R-endo* poses (light yellow) from docking with GQ/I263G/A328L; Mutations S332F/M and A330F/M are estimated to be able to destabilise *R-endo* poses while maintaining the *S-endo*-pose and therefore improve *ee* for *S*-**8b**. **(D)** Lowest energy *S-endo* pose (cyan) overlaid with NP poses (light green) from docking with GQ/I263G/A328L; Mutations T260L/M and A82F/M are estimated to be able to destabilise NP poses while maintaining the *S-endo*-pose and therefore improve TON for *S*-**8b**.



	Cluster population
Replica 1	C1 (2914) C2 (2040) C3 (1081)
Replica 2	C1 (6846) C2 (1726) C2 (1264)
Replica 3	C1 (3511) C2 (3206) C3 (1572)

B

Figure A5.15. MD-simulation outputs for GQ/I263G/A328L/T260L. **(A)** The RMSD plot for Cα atoms of 3 replicas of GQ/I263G/A328L/T260L during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

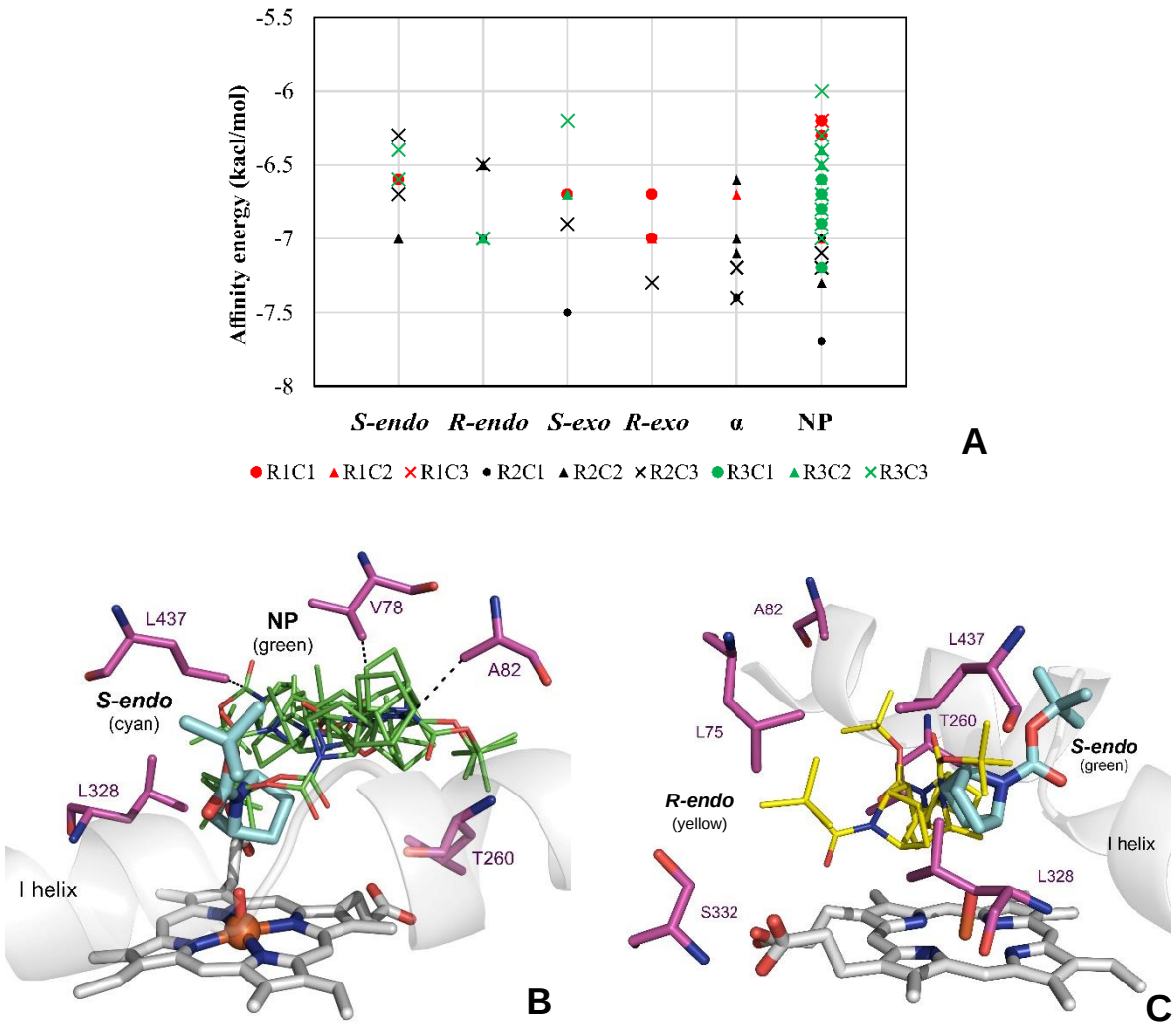
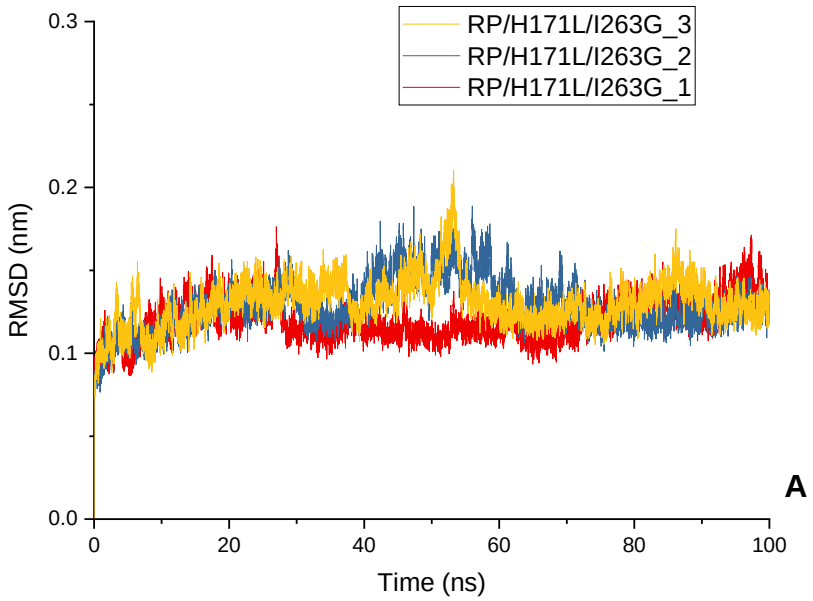


Figure A5.16. Analysis of results from the docking of *N*-Boc-7-azabicyclo[2.2.1]heptane (**8**), with the GQ/I263G/A328L/T260L variant. **(A)** Affinity energies of the productive and non-productive (NP) poses. **(B)** Overlay of the (*S*)-*endo* pose (cyan) with the NP poses (green), mutations at A82 and V78 might disfavour the NP poses and improve enzymatic activity. **(C)** Overlay of the (*S*)-*endo* pose (cyan) with the (*R*)-*endo* poses (yellow), mutations at S332 and A330 might disfavour the (*R*)-*endo* poses and improve enantioselectivity.

A5.5 Docking study of *N*-Ms-8-azaspiro[5.4]decane 10



A

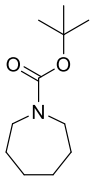
	Cluster population
Replica 1	C1 (7021) C2 (2228) C3 (503)
Replica 2	C1 (6860) C2 (1262) C2 (900)
Replica 3	C1 (6560) C2 (1310) C3 (859)

B

Figure A5.17. MD-simulation outputs for RP/H171L/I263G. **(A)** The RMSD plot for Cα atoms of 3 replicas of RP/H171L/I263G during 100 ns MD simulation runs. **(B)** Size of clusters from a total of 10000 snapshot structures in each replica simulation, with a cluster size cut-off of 500 (5% of the total).

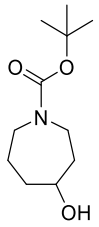
A6. Product information

1-(*tert*-Butoxycarbonyl)azepane (1)



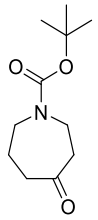
Based on the procedure of Dong *et al*.,[29] to a stirred solution of azepane (2.0 g, 20 mmol.) and 4-(*N*,*N*-dimethylamino)pyridine (0.2 g, 2 mmol.) in dichloromethane (100 mL) at 0 °C was added dropwise a solution of di-*tert*-butyl dicarbonate (6.6 g, 30 mmol.) in dichloromethane (20 mL). The reaction mixture was stirred at 0 °C for 10 min, warmed to room temperature and stirred for 24 h at which time the reaction was complete (TLC and GC). The solution was washed with 120 mL saturated aq $NaHCO_3$ solution and then 120 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **1** as a colourless oil (2.9 g, 73%). R_f 0.79 (30% ethyl acetate in petroleum ether). (Mixture of rotamers) **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.33 (t, *J* = 6.0 Hz, 2H), 3.27 (t, *J* = 6.0 Hz, 2H), 1.67 – 1.55 (m, 4H), 1.48 (m, 4H), 1.41 (s, 9H). (Mixture of rotamers) **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.7, 78.9, 47.0, 46.6, 28.6, 28.5, 27.5, 26.9. NMR spectroscopic data are consistent with those reported in the literature.[30] **HRMS** (ESI+): Calc'd for $C_{11}H_{21}NNaO_2^+$ $[M+Na]^+$: 222.1465, found: 222.1465.

1-(*tert*-Butoxycarbonyl)-4-hydroxyazepane (1a)



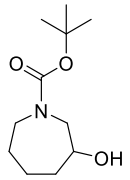
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the RLYF/H171L/I263G variant showing 70% regioselectivity for **1a** and 80% *ee* for (*R*)-**1a** (1 μM), substrate **1** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford alcohol **1a** as a colourless oil (20 mg, 58%). R_f 0.2 (30% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **1a** with the RLYF/H171L/I263G/A330W/L437S variant (91% of **1a** and 90% *ee* for (*R*)-**1a**). Alcohol (*R*)-**1a** was isolated as a colourless oil (15 mg, 45%). $[\alpha]_D^{25}$ +5.2 (*c* = 1 g/100 mL, $CHCl_3$). The (*S*)-enantiomer was isolated from a reaction with the KU3/A330P/S72W variant (90% of **1a** and 85% *ee* for (*S*)-**1a**) as a colourless oil (12 mg, 35%). $[\alpha]_D^{25}$ –5.0 (*c* = 1 g/100 mL, $CHCl_3$). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.87 (ddt, *J* = 12.5, 8.5, 4.0 Hz, 1H), 3.51 – 3.19 (m, 4H), 2.02 – 1.50 (m, 7H), 1.45 (s, 9H). (Mixture of rotamers) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.7, 79.4, 71.2/70.9, 46.7/45.8, 41.5/41.1, 37.5/37.4, 35.7/35.4, 28.6, 22.7/22.2. NMR spectroscopic data are consistent with those reported in the literature.[26] **HRMS** (ESI+): Calc'd for $C_{11}H_{21}NNaO_3^+$ $[M+Na]^+$: 238.1414, found: 238.1413.

1-(*tert*-Butoxycarbonyl)-3-oxo-azepane (1b)



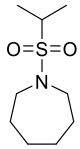
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the RLYF/H171L/I263G/A330P variant showing 19% regioselectivity for **1b** (1 μM), substrate **1** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 85% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford alcohol **1a** as a white solid (7 mg, 16%). R_f 0.22 (30% ethyl acetate in petroleum ether). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.61 – 3.51 (m, 4H), 2.67 – 2.58 (m, 4H), 1.81 – 1.73 (m, 2H), 1.44 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 211.8, 154.4, 80.0, 49.6, 48.9, 43.9, 43.5, 43.2, 43.0, 28.4, 25.8, 25.6. NMR spectroscopic data are consistent with those reported in the literature.[26] **HRMS** (ESI+): Calc'd for $C_{11}H_{21}NNaO_3^+$ $[M+Na]^+$: 236.1257, found: 236.1257.

1-(*tert*-Butoxycarbonyl)-3-hydroxyazepane (1c)



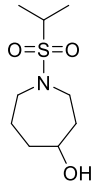
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the RT2/S72G/A330W variant showing 50% regioselectivity for **1b** and 80% *ee* for (*R*)-**1b** (1 μM), substrate **1** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 80% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine , dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford alcohol **1b** as a white solid (6 mg, 36%). R_f 0.24 (30% ethyl acetate in petroleum ether). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.95 (tt, *J* = 11.5, 5.5 Hz, 1H), 3.85 – 3.41 (m, 2H), 3.40 – 3.30 (m, 1H), 3.24 (dd, *J* = 15.0, 4.0 Hz, 1H), 2.98 (ddd, *J* = 14.0, 8.5, 5.5 Hz, 1H), 1.91 – 1.83 (m, 1H), 1.78 – 1.62 (m, 3H), 1.59 – 1.53 (m, 1H), 1.47 (s, 9H), 1.40 – 1.30 (m, 1H). (Mixture of rotamers) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 157.9, 80.1, 71.4/70.9, 53.9/53.5, 49.0/48.0, 36.6/36.0, 29.1, 28.6, 28.2, 21.1. NMR spectroscopic data are consistent with those reported in the literature.[26] **HRMS** (ESI+): Calc'd for $C_{11}H_{21}NNaO_3^+$ $[M+Na]^+$: 238.1414, found: 238.1414.

1-(Isopropylsulfonyl)azepane (5)



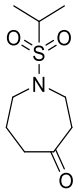
Based on the procedure of Park *et al*.,[31] to a stirred solution of azepane (2.0 g, 20 mmol) and triethylamine (8.3 g, 60 mmol) in dichloromethane (90 mL) at 0 °C was added dropwise a solution of isopropylsulfonyl chloride (5.7 g, 40 mmol) in dichloromethane (10 mL). The reaction mixture was warmed to room temperature and stirred for 4 h, after which time the reaction had completed (TLC and GC). The solution was washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **2** as a light yellow oil (1.8 g, 44%). R_f 0.6 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.35 (t, *J* = 6.0 Hz, 4H), 3.19 (sept, *J* = 7.0 Hz, 1H), 1.78 – 1.69 (m, 4H), 1.65 – 1.59 (m, 4H), 1.32 (d, *J* = 7.0 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 52.7, 49.1, 30.1, 27.0, 16.9. **HRMS** (ESI+): Calc'd for $C_{11}H_{21}NNaO_3^+$ $[M+Na]^+$: 228.1019, found: 228.1020.

1-(Isopropylsulfonyl)-4-hydroxyazepane (5a)



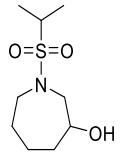
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the RP/H171L/I263G variant showing 94% regioselectivity for **5a** and 95% *ee* for (*R*)-**5a** (1 μM), substrate **5** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 85% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford alcohol **5a** as a colourless oil (14 mg, 75%). R_f 0.08 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.99 (tt, *J* = 7.5, 3.0 Hz, 1H), 3.51 – 3.39 (m, 2H), 3.38 – 3.28 (m, 2H), 3.20 (sept, *J* = 7.0 Hz, 1H), 2.09 – 2.01 (m, 1H), 2.00 – 1.87 (m, 2H), 1.86 – 1.77 (m, 2H), 1.72 – 1.63 (m, 1H), 1.32 (d, *J* = 7.0 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 70.2, 52.8, 48.4, 43.6, 38.9, 34.9, 23.4, 16.8. **HRMS** (ESI+): Calc'd for $C_9H_{19}NNaO_3S^+$ $[M+Na]^+$: 244.0978, found: 244.0977.

1-(Isopropylsulfonyl)-4-oxo-azepane (5b)



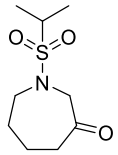
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the RP/H171L/I263G/S72W variant showing 25% regioselectivity for **5b** (1 μM), substrate **5** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford alcohol **5b** as a colourless oil (9 mg, 20%). R_f 0.12 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.55 (dt, *J* = 21.2, 5.4 Hz, 4H), 3.20 (hept, *J* = 6.9 Hz, 1H), 2.76 – 2.63 (m, 4H), 1.95 – 1.85 (m, 2H), 1.34 (d, *J* = 6.9 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 210.7, 53.5, 51.4, 46.1, 45.1, 42.7, 26.0, 16.7. **HRMS** (ESI+): Calc'd for $C_9H_{17}NNaO_3S^+$ $[M+Na]^+$: 242.0821, found: 242.0822.

1-(Isopropylsulfonyl)-3-hydroxyazepane (5c)



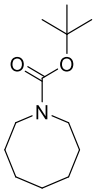
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the GV/A184I variant showing 50% regioselectivity for **5c** and 30% *ee* for (*S*)-**5c** (1 μM), substrate **5** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford alcohol **5c** as a colourless oil (16 mg, 40%). R_f 0.1 (30% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **5c** with the VQ/S72G/A330W variant (91% of **5c** and 96% *ee* for (*R*)-**5c**). Alcohol (*R*)-**5c** was isolated as a colourless oil (21 mg, 53%). $[\alpha]_D^{25}$ –6.2 (*c* = 1 g/100 mL, $CHCl_3$, 25 °C). The (*S*)-enantiomer was isolated from a reaction with the GL/A184I/I263G/A328G/S72F/L188M variant (97% of **5c** and 92% *ee* for (*S*)-**5c**) as a colourless oil (14 mg, 35%). $[\alpha]_D^{25}$ +5.7 (*c* = 1 g/100 mL, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.87 (dq, *J* = 7.5, 5.0 Hz, 1H), 3.54 – 3.32 (m, 3H), 3.22 (sept, *J* = 7.0 Hz, 1H), 3.21 – 3.13 (m, 1H), 2.87 (s, 1H), 1.92 (dddd, *J* = 14.0, 9.0, 5.0, 1.5 Hz, 1H), 1.82 – 1.68 (m, 1H), 1.67 – 1.57 (m, 1H), 1.48 – 1.36 (m, 1H), 1.31 (d, *J* = 7.0 Hz, 3H), 1.30 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 70.5, 55.9, 52.9, 51.1, 35.8, 30.5, 20.7, 16.8. **HRMS** (ESI+): Calc'd for $C_9H_{19}NNaO_3S^+$ $[M+Na]^+$: 244.0978, found: 244.0977.

1-(Isopropylsulfonyl)-3-hydroxyazepane (5d)



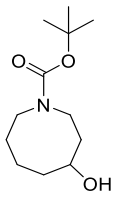
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the GV/A184I variant showing 23% regioselectivity for **5d** (1 μM), substrate **5** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford alcohol **5d** as a colourless oil (8 mg, 18%). R_f 0.14 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.86 (s, 2H), 3.35 (t, *J* = 5.4 Hz, 2H), 3.18 (hept, *J* = 6.8 Hz, 1H), 2.75 – 2.68 (m, 2H), 1.88 (dt, *J* = 10.8, 5.4 Hz, 2H), 1.68 (ddt, *J* = 12.0, 8.5, 4.8 Hz, 2H), 1.29 (d, *J* = 6.8 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 210.7, 59.3, 53.6, 51.9, 42.1, 31.3, 23.5, 16.7. **HRMS** (ESI+): Calc'd for $C_9H_{19}NNaO_3S^+$ $[M+Na]^+$: 242.0821, found: 242.0821.

1-(*tert*-Butoxycarbonyl)azocane (6)



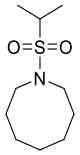
Based on the procedure of Dong *et al*.,[29] to a stirred solution of azocane (1.1 g, 10 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.1 g, 1 mmol) in dichloromethane (50 mL) at 0 °C was added dropwise a solution of di-*tert*-butyl dicarbonate (3.3 g, 15 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at 0 °C for 10 min then warmed to room temperature and stirred for 24 h after which time the reaction had completed (TLC and GC). The solution was washed with 60 mL saturated aq $NaHCO_3$ solution and then 60 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **6** as a colourless oil (1.7 g, 80%). R_f 0.65 (30% ethyl acetate in petroleum ether). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.33 (t, *J* = 6.0 Hz, 2H), 3.26 (t, *J* = 6.0 Hz, 2H), 1.71 – 1.59 (m, 4H), 1.59 – 1.48 (m, 6H), 1.45 (s, 9H). (Mixture of rotamers) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.7, 79.0, 48.1, 47.6, 28.7, 27.6, 27.2, 26.4, 26.2, 25.1. **HRMS** (ESI+): Calc'd for $C_{12}H_{23}NNaO_2^+$ $[M+Na]^+$: 236.1621, found: 236.1621.

1-(*tert*-Butoxycarbonyl)-4-hydroxyazocane (6a)



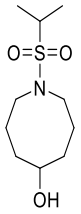
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the RLYF/H171L/I263G variant showing 67% regioselectivity for **6a** and 95% *ee* for (*R*)-**6a** (1 μM), substrate **6** (2 mM, 1 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 92% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The solution was washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford alcohol **6a** as a colourless oil (10 mg, 47%). R_f 0.15 (30% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **6a** with the RLYF/I263G/A330P variant (87% of **6a** and 98% *ee* for (*R*)-**6a**). Alcohol (*R*)-**6a** was isolated as a colourless oil (13 mg, 61%). $[\alpha]_D^{25}$ +11.9 (*c* = 1 g/100 mL, $CHCl_3$). The (*S*)-enantiomer was isolated from a reaction with the KU3/A330P/S72W variant (74% of **6a** and 77% *ee* for (*S*)-**6a**) as a colourless oil (12 mg, 56%). $[\alpha]_D^{25}$ –9.2 (*c* = 1 g/ 100mL, $CHCl_3$). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.92 – 3.80 (m, 1H), 3.66 – 3.08 (m, 4H), 1.98 – 1.76 (m, 3H), 1.76 – 1.51 (m, 5H), 1.47 (s, 9H). (Mixture of rotamers) **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.8/155.3, 79.5, 71.4/70.9, 47.4/46.7, 44.0/43.3, 36.6/35.8, 35.3, 33.4, 28.7, 28.0/27.0, 22.4/21.1. **HRMS** (ESI+): Calc'd for $C_{12}H_{23}NNaO_3^+$ $[M+Na]^+$: 252.1570, found: 252.1570.

1-(Isopropylsulfonyl)azocane (7)



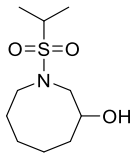
Based on the procedure of Park *et al*.,[31] to a stirred solution of azocane (0.6 g, 5 mmol) and triethylamine (2.1 g, 15 mmol) in dichloromethane (23 mL) at 0 °C was added dropwise a solution of isopropylsulfonyl chloride (1.4 g, 10 mmol) in dichloromethane (2 mL). The reaction mixture was warmed to room temperature and stirred for 4 h after which time the reaction had completed (TLC and GC). The solution was washed with 25 mL saturated aq $NaHCO_3$ solution and then 25 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **7** as a colourless oil (0.3 g, 27%). R_f 0.5 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.30 (t, *J* = 5.5 Hz, 4H), 3.24 (sept, *J* = 7.0 Hz, 1H), 1.75 – 1.66 (m, 4H), 1.65 – 1.59 (m, 6H), 1.31 (d, *J* = 7.0 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 52.1, 49.8, 28.4, 27.1, 24.7, 16.9. **HRMS** (ESI+): Calc'd for $C_{10}H_{21}NNaO_2S^+$ $[M+Na]^+$: 242.1185, found: 242.1184.

1-(Isopropylsulfonyl)-5-hydroxyazocane (7a)



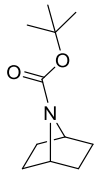
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the R19/I263G/A328L variant showing 95% regioselectivity for **7a** (1 μM), substrate **7** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (15% ethyl acetate in petroleum ether) to afford alcohol **7a** as a colourless oil (15 mg, 80%). R_f 0.15 (30% ethyl acetate in petroleum ether). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.07 (tt, *J* = 7.5, 3.5 Hz, 1H), 3.40 (ddd, *J* = 14.0, 7.5 Hz, 5.0 Hz, 2H), 3.24 (sept, *J* = 7.0 Hz, 1H), 3.25 – 3.16 (m, 2H), 1.97 – 1.64 (m, 8H), 1.33 (d, *J* = 7.0 Hz, 6H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 70.8, 52.4, 49.8, 33.2, 24.6, 16.9. **HRMS** (ESI+): Calc'd for $C_{10}H_{21}NNaO_3S^+$ $[M+Na]^+$: 258.1134, found: 258.1135.

1-(Isopropylsulfonyl)-3-hydroxyazocane (7c)



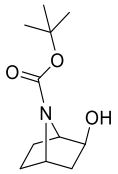
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the GVQ/A330W variant showing 34% regioselectivity for **7c** and 90% *ee* for (*R*)-**7c** (1 μM), substrate **7** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 80% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (15% ethyl acetate in petroleum ether) to afford alcohol **7c** as a colourless oil (4 mg, 21%). R_f 0.09 (30% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **7c** with the R19/S72G/A330W variant (85% of **7c** and 99% *ee* for (*R*)-**7c**). Alcohol (*R*)-**7c** was isolated as a colourless oil (10 mg, 54%). $[\alpha]_D^{25}$ +13.3 (*c* = 1 g/100 mL, $CHCl_3$). The (*S*)-enantiomer was isolated from a reaction with the GV/A184I/I263G/A328G/S72F/S332F variant (75% of **7c** and 92% *ee* for (*S*)-**7c**) as a colourless oil (12 mg, 63%). $[\alpha]_D^{25}$ –12.2 (*c* = 1 g/100 mL, $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.76 (dq, *J* = 8.5, 4.5 Hz, 1H), 3.58 – 3.51 (m, 1H), 3.46 (dd, *J* = 15.5, 4.5 Hz, 2H), 3.33 (sept, *J* = 7.0 Hz, 1H), 3.08 (ddd, *J* = 13.5, 8.0, 3.0 Hz, 1H), 1.89 – 1.66 (m, 6H), 1.61 – 1.47 (m, 2H), 1.36 (d, *J* = 7.0 Hz, 3H), 1.35 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 70.9, 55.3, 52.1, 51.9, 32.2, 27.2, 25.8, 22.7, 16.9, 16.9. **HRMS** (ESI+): Calc'd for $C_{10}H_{21}NNaO_3S^+$ $[M+Na]^+$: 258.1134, found: 258.1135.

7-(*tert*-Butoxycarbonyl)-7-azabicyclo[2.2.1]heptane (8)



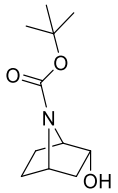
Based on the procedure of Dong *et al*.,[29] to a stirred aolution of 7-azabicyclo[2.2.1]heptane (1.0 g, 10 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.1 g, 1 mmol) in dichloromethane (50 mL) at 0 °C was added. The flask was placed on ice and di-*tert*-butyl dicarbonate (3.3 g, 15 mmol) dissolved in 10 mL of dichloromethane was then added dropwise. The reaction mixture was stirred on ice for 10 mins and then warmed to room temperature and left for 24 h. After 24 h, the reaction had proceeded to completion (TLC and GC). The solution was washed with 60 mL saturated aq $NaHCO_3$ solution and then 60 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **8** as a colourless oil (1.4 g, 71%). R_f 0.30 (10% ethyl acetate in petroleum ether). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 4.21 – 4.11 (m, 2H), 1.82 – 1.65 (m, 4H), 1.43 (s, 9H), 1.37 (d, *J* = 7.5 Hz, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 155.9, 79.3, 56.3, 29.7, 28.4. NMR spectroscopic data are consistent with those reported in the literature.[32] **HRMS** (ESI+): Calc'd for $C_{11}H_{19}NNaO_2^+$ $[M+Na]^+$: 220.1308, found: 220.1310.

7-(*tert*-Butoxycarbonyl)-2-*exo*-hydroxy-7-azabicyclo[2.2.1]heptane (8a)



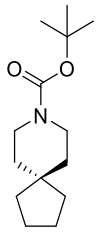
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the K19/F87A/I263G variant showing 90% regioselectivity for **8a** and 90% *ee* for (*R*)-**8a** (1 μM), substrate **8** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 95% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (15% ethyl acetate in petroleum ether) to afford alcohol **8a** as a colourless oil (15 mg, 79%). R_f 0.30 (50% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **8a** with the R19/F87I variant (99% of **8a** and 99% *ee* for (*R*)-**8a**). Alcohol (*R*)-**8a** was isolated as a colourless oil (17 mg, 89%). $[\alpha]_D^{25}$ +20.8 (*c* = 1 g/100 mL, CH_2Cl_2). The (*S*)-enantiomer was isolated from a reaction with the GL/A184I/I263G/A328G variant (99% of **8a** and 90% *ee* for (*S*)-**8a**) as a colourless oil (14 mg, 74%). $[\alpha]_D^{25}$ –19.7 (*c* = 1 g/100 mL, CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.23 (td, *J* = 5.0, 1.0 Hz, 1H), 4.11 (d, *J* = 5.5 Hz, 1H), 3.86 (dd, *J* = 7.0, 2.0 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.83 (dd, *J* = 13.0, 7.0 Hz, 1H), 1.80 – 1.68 (m, 1H), 1.68 – 1.55 (m, 2H), 1.45 (s, 9H), 1.30 – 1.20 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 156.8, 80.1, 74.5, 63.3, 55.6, 42.7, 28.4, 28.3, 24.1. NMR spectroscopic data are consistent with those reported in the literature.[27] **HRMS** (ESI+): Calc'd for $C_{11}H_{19}NNaO_3^+$ $[M+Na]^+$: 236.1257, found: 236.1258.

7-(*tert*-Butoxycarbonyl)-2-*endo*-hydroxy-7-azabicyclo[2.2.1]heptane (5b)



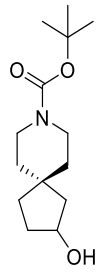
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the R19/A330W variant showing 90% regioselectivity for **8b** and 68% *ee* for (*R*)-**8b** (1 μM), substrate **5** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 91% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (15% ethyl acetate in petroleum ether) to afford alcohol **8b** as a colourless oil (16 mg, 83%). R_f 0.40 (50% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **8b** with the R19/S72G/A330W variant (95% of **8b** and 80% *ee* for (*R*)-**8b**). Alcohol (*R*)-**8b** was isolated as a colourless oil (14 mg, 71%). $[\alpha]_D^{25}$ –4.1 (*c* = 1 g/100 mL, $CHCl_3$). The (*S*)-enantiomer was isolated from a reaction with the GQ/I263G/T260L/A330F variant (98% of **8b** and 88% *ee* for (*S*)-**8b**) as a colourless oil (13 mg, 66%). $[\alpha]_D^{25}$ +4.8 (*c* = 1 g/100 mL, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.35 (dddd, *J* = 10.0, 4.5, 3.5, 1.5 Hz, 1H), 4.15 – 4.10 (m, 2H), 2.27 – 2.11 (m, 2H), 1.78 (ttd, *J* = 12.5, 5.0, 3.0 Hz, 1H), 1.68 – 1.58 (m, 1H), 1.52 (ddd, *J* = 12.0, 9.0, 4.0 Hz, 1H), 1.44 (s, 9H), 1.05 (dd, *J* = 12.5, 3.5 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.8, 79.8, 71.0, 60.1, 57.5, 39.4, 29.9, 28.4, 20.8. NMR spectroscopic data are consistent with those reported in the literature.[27] **HRMS** (ESI+): Calc'd for $C_{11}H_{19}NNaO_3^+$ $[M+Na]^+$: 236.1257, found: 236.1260.

8-(*tert*-Butoxycarbonyl)-8-azaspiro[4.5]decane (9)



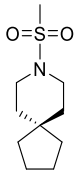
Based on the procedure of Dong *et al*.,[29] to a stirred solution of azocane (0.7 g, 5 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (0.1 g, 1 mmol) in dichloromethane (50 mL) at 0 °C was added dropwise a solution of di-*tert*-butyl dicarbonate (3.3 g, 15 mmol) in dichloromethane (10 mL). The reaction mixture was stirred at 0 °C for 10 min then warmed to room temperature and stirred for 24 h after which time the reaction had completed (TLC and GC). The solution was washed with 60 mL saturated aq $NaHCO_3$ solution and then 60 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (5% ethyl acetate in petroleum ether) to afford substrate **9** as a white solid (0.8 g, 67%). R_f 0.7 (30% ethyl acetate in petroleum ether). (Mixture of rotamers) **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.39 – 3.32 (m, 4H), 1.61 (td, *J* = 6.3, 5.4, 2.4 Hz, 4H), 1.45 (s, 9H), 1.40 (dd, *J* = 12.8, 6.7 Hz, 8H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.0, 79.1, 41.0, 37.6, 37.3, 28.5, 24.3. **HRMS** (ESI+): Calc'd for $C_{10}H_{19}NNaO_2S^+$ $[M+Na]^+$: 262.1778, found: 262.1776.

8-(*tert*-Butoxycarbonyl)-2-hydroxy-8-azaspiro[4.5]decane (9a)



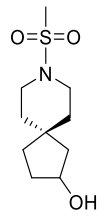
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the RT2 variant showing 60% regioselectivity for **9a** (1 μM), substrate **9** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (40% ethyl acetate in petroleum ether) to afford alcohol **9a** as a light yellow solid (12 mg, 47%). R_f 0.4 (80% ethyl acetate in petroleum ether). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.36 (td, *J* = 6.1, 3.0 Hz, 1H), 3.43 – 3.36 (m, 2H), 3.32 (ddt, *J* = 13.5, 6.8, 3.3 Hz, 2H), 1.97 – 1.86 (m, 1H), 1.80 (dd, *J* = 13.7, 6.4 Hz, 1H), 1.72 – 1.62 (m, 2H), 1.54 (ddd, *J* = 15.2, 7.3, 4.5 Hz, 3H), 1.49 (d, *J* = 5.0 Hz, 1H), 1.44 (s, 9H), 1.37 (t, *J* = 5.9 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 155.0, 79.2, 73.4, 47.1, 41.2, 40.2, 38.8, 37.9, 35.4, 34.3, 28.5.

8-(Methylsulfonyl)-8-azaspiro[4.5]decane (10)



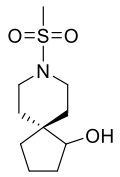
Based on the procedure of Park *et al*.,[31] to a stirred solution of 8-azaspiro[4.5]decane (0.7 g, 5 mmol) and triethylamine (2 mL, 15 mmol) in dichloromethane (23 mL) at 0 °C was added dropwise a solution of methylsulfonyl chloride (1.4 g, 10 mmol) in dichloromethane (2 mL). The reaction mixture was warmed to room temperature and stirred for 8 h after which time the reaction had completed (TLC and GC). The solution was washed with 27 mL saturated aq $NaHCO_3$ solution and then 27 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (10% ethyl acetate in petroleum ether) to afford substrate **10** as a white solid (0.8 g, 76%). R_f 0.30 (20% ethyl acetate in petroleum ether). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 3.18 (t, *J* = 5.5 Hz, 4H), 2.76 (s, 3H), 1.63 (quin, *J* = 3.5 Hz, 4H), 1.56 (t, *J* = 5.5 Hz, 4H), 1.43 (quin, *J* = 3.5 Hz, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 43.9, 40.5, 37.6, 37.0, 34.6, 24.4. **HRMS** (ESI+): Calc'd for $C_{10}H_{19}NNaO_2S^+$ $[M+Na]^+$: 240.1029, found: 240.1030.

8-(Methylsulfonyl)-2-hydroxy-8-azaspiro[4.5]decane (10a)



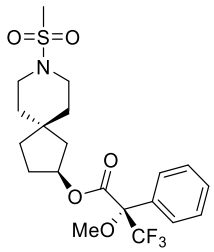
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 50 mL) containing the RP/H171L/I263G/A184I variant showing 85% regioselectivity for **10a** and 70% *ee* for (*S*)-**10a** (1 μM), substrate **10** (2 mM, 0.5 mL of 200 mM methanol stock, 0.1 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The organic extracts were combined and washed with 50 mL saturated aq $NaHCO_3$ solution and then 50 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (40% ethyl acetate in petroleum ether) to afford alcohol **10a** as a white solid (13 mg, 62%). R_f 0.20 (80% ethyl acetate in petroleum ether). The same procedure was applied for the (*R*)-enantiomer of **10a** with the K19/F87V/A328I variant (90% of **10a** and 88% *ee* for (*R*)-**10a**). Alcohol (*R*)-**10a** was isolated as a white solid (14 mg, 67%). $[\alpha]_D^{25}$ +5.8 (*c* = 1 g/100 mL, $CHCl_3$). The (*S*)-enantiomer was isolated from a reaction with the RP/H171L/I263G variant (90% of **10a** and 77% *ee* for (*S*)-**10a**) as a white solid (12 mg, 58%). $[\alpha]_D^{25}$ –5.1 (*c* = 1 g/100 mL, $CHCl_3$). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 4.38 (tt, *J* = 5.5, 4.0 Hz, 1H), 3.26 – 3.11 (m, 4H), 2.77 (s, 3H), 1.97 – 1.86 (m, 1H), 1.85 – 1.63 (m, 5H), 1.62 – 1.44 (m, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 73.5, 47.2, 43.9, 43.7, 39.7, 38.5, 37.7, 35.5, 34.8, 34.5. **HRMS** (ESI+): Calc'd for $C_{10}H_{19}NNaO_3S^+$ $[M+Na]^+$: 256.0978, found: 256.0977.

8-(Methylsulfonyl)-1-hydroxy-8-azaspiro[4.5]decane (10b)



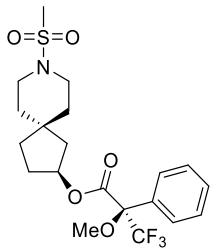
Preparative scale reactions were conducted at ambient temperature over 16 h in phosphate buffer (pH 7.9, 200 mM, 100 mL) containing the VQ/S72G/A330W variant showing 84% regioselectivity for **10b** and 95% *ee* for (*S*)-**10b** (1 μM), substrate **6** (2 mM, 1 mL of 200 mM methanol stock, 0.2 mmol) and $NADP^+$ (4 μM); glucose (100 mM) and glucose dehydrogenase (2 U/mL) were included to regenerate the NADPH cofactor. After 16 h, the reaction had proceeded to 90% conversion (GC). The reaction mixture was extracted with ethyl acetate (3 × 100 mL). The organic extracts were combined and washed with 100 mL saturated aq $NaHCO_3$ solution and then 100 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (40% ethyl acetate in petroleum ether) to afford alcohol **10b** as a light yellow oil (30 mg, 67%). R_f 0.29 (80% ethyl acetate in petroleum ether). The same procedure was applied for the (*S*)-enantiomer of **10b** with the R19/F87A variant (95% of **10b** and 95% *ee* for (*S*)-**10b**). Alcohol (*S*)-**10b** was isolated as a light yellow oil (25 mg, 56%). $[\alpha]_D^{25}$ –17.4 (*c* = 1 g/100 mL, CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_3OD) δ 3.76 (t, *J* = 5.5 Hz, 1H), 3.54 – 3.43 (m, 2H), 3.34 (quin, *J* = 1.5 Hz, 1H), 2.99 (dddd, *J* = 12.0, 10.5, 9.0, 3.0 Hz, 2H), 2.85 (s, 3H), 2.06 – 1.96 (m, 1H), 1.87 – 1.76 (m, 2H), 1.75 – 1.59 (m, 4H), 1.59 – 1.50 (m, 2H), 1.41 (dddd, *J* = 13.5, 5.0, 3.0, 2.0 Hz, 1H). **^{13}C NMR** (126 MHz, CD_3OD) δ 80.3, 44.9, 44.5, 44.3, 35.6, 34.3, 33.3, 32.9, 30.9, 20.7. **HRMS** (ESI+): Calc'd for $C_{10}H_{19}NNaO_3S^+$ $[M+Na]^+$: 256.0978, found: 256.0979.

8-(Methylsulfonyl)-8-azaspiro[4.5]decan-(2*S*)-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (10c)



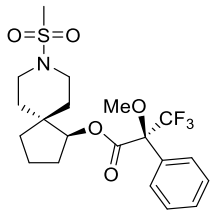
Based on the procedure of Hoye *et al*.,[33] to a stirred solution of alcohol **10a** (10 mg, 0.04 mmol) isolated from reaction with the RP/H171L/I263G variant, and dry pyridine (10 μL, 0.12 mmol) in anhydrous dichloromethane (1 mL) in a screw-capped 10 mL glass vial was added dropwise (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (15 μL, 0.08 mmol). The vial was capped and the reaction mixture stirred at ambient temperature for 2 h after which time the reaction had completed (TLC and GC). The solution was washed with 1 mL saturated aq $NaHCO_3$ solution and then 1 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to afford ester **10c** as a colourless oil (9 mg, 50%). R_f 0.20 (50% ethyl acetate in petroleum ether). The *ee* for product **10a** from the RP/H171L/I263G variant was determined as 77% by ^{1}H NMR analysis of **10c**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 7.5, 3.0 Hz, 2H), 7.43 – 7.38 (m, 3H), 5.43 (qt, *J* = 6.0, 3.0 Hz, 1H), 3.54/3.52 (s, 3H), 3.24 – 3.15 (m, 2H), 3.14 – 3.06 (m, 2H), 2.76/2,75 (s, 3H), 2.06 (dtd, *J* = 14.5, 8.5, 6.0 Hz, 1H), 1.94 – 1.81 (m, 2H), 1.72 (ddd, *J* = 14.5, 3.0, 1.5 Hz, 1H), 1.64 – 1.52 (m, 7H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.1, 132.3, 129.8, 128.6, 127.4, 123.4 (q, *J* = 289.0 Hz), 84.5 (q, *J* = 28.5 Hz), 78.9, 55.4, 43.9, 43.7, 43.5, 40.0, 37.4, 37.2, 35.3, 34.9, 31.3. **HRMS** (ESI+): Calc'd for $C_{20}H_{26}F_3NNaO_5S^+$ $[M+Na]^+$: 472.1376, found: 472.1375

8-(Methylsulfonyl)-8-azaspiro[4.5]decan-(2*S*)-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (10d)



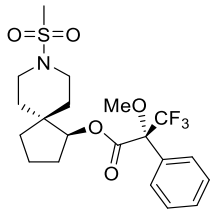
Based on the procedure of Hoye *et al*.,[33] product **10a** (10 mg, 0.04 mmol.), isolated from reaction with the RP/H171L/I263G variant, was mixed with dry pyridine (10 μL, 0.12 mmol) in a screw-capped 10 mL glass vial charged with a magnetic stirring bar. 1 mL of anhydrous dichloromethane was then added to the mixture. The (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (15 μL, 0.08 mmol) was then added dropwise. The vial was then capped and the reaction mixture stirred at ambient temperature for 2 h. After 2 h, the reaction had proceeded to completion (TLC and GC). The solution was washed with 1 mL saturated aq $NaHCO_3$ solution and then 1 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to afford ester **10d** as a colourless oil (10 mg, 57%). R_f 0.20 (50% ethyl acetate in petroleum ether). The *ee* for product **10a** from the RP/H171L/I263G variant was determined as 77% by ^{1}H NMR of **10d**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 – 7.48 (m, 2H), 7.40 (qd, *J* = 4.5, 1.5 Hz, 3H), 5.44 (qt, *J* = 5.5, 3.0 Hz, 1H), 3.54/3.52 (s, 3H), 3.22 (tt, *J* = 16.5, 8.5 Hz, 1H), 3.15 – 3.05 (m, 2H), 2.96 (ddd, *J* = 12.0, 8.0, 4.0 Hz, 1H), 2.76/2.75 (s, 3H), 2.07 (dtd, *J* = 14.5, 8.5, 6.0 Hz, 1H), 1.96 – 1.79 (m, 2H), 1.76 – 1.52 (m, 6H), 1.52 – 1.39 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.1, 132.4, 130.0, 128.6, 127.4, 123.4 (q, *J* = 289.0 Hz), 84.4 (q, *J* = 28.0 Hz), 78.9, 55.5, 43.9, 43.7, 40.0, 37.5, 37.1, 35.8, 34.8, 31.2. **HRMS** (ESI+): Calc'd for $C_{20}H_{26}F_3NNaO_5S^+$ $[M+Na]^+$: 472.1376, found: 472.1376.

8-(Methylsulfonyl)-8-azaspiro[4.5]decan-(1*S*)-yl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (10e)



Based on the procedure of Hoye *et al.*,[33] product **10b** (10 mg, 0.04 mmol), isolated from reaction with the R19/F87A variant, was mixed with dry pyridine (10 μL, 0.12 mmol) in a screw-capped 10 mL glass vial charged with a magnetic stirring bar. 1 mL of anhydrous dichloromethane was then added to the mixture. The (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (15 μL, 0.08 mmol) was then added dropwise. The vial was then capped and the reaction mixture stirred at ambient temperature for 2 h. After 2 h, the reaction had proceeded to completion (TLC and GC). The solution was washed with 1mL saturated aq $NaHCO_3$ solution and then 1 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to afford ester **10e** as a colourless oil (10 mg, 56%). R_f 0.25 (50% ethyl acetate in petroleum ether). The *ee* for product **10b** from the R19/F87A variant was determined as 95% by ^{1}H NMR of **10e**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 7.0, 2.5 Hz, 2H), 7.45 – 7.36 (m, 3H), 5.09 (dd, *J* = 5.5, 2.5 Hz, 1H), 3.56/3.49 (s, 3H), 3.38 – 3.29 (m, 1H), 3.18 (dt, *J* = 11.0, 5.0 Hz, 1H), 3.07 (ddd, *J* = 12.5, 9.0, 3.5 Hz, 1H), 2.91 (ddd, *J* = 12.5, 9.0, 3.5 Hz, 1H), 2.76/2.72 (s, 3H), 2.22 – 2.12 (m, 1H), 1.87 – 1.52 (m, 8H), 1.51 – 1.40 (m, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.2, 132.5, 129.9, 128.6, 127.2, 123.3 (q, *J* = 289.0 Hz), 84.3 (q, *J* = 28.0 Hz), 83.8, 55.6, 44.2, 43.4, 42.8, 35.0, 34.0, 33.5, 30.5, 30.4, 20.4. **HRMS** (ESI+): Calc'd for $C_{20}H_{26}F_3NNaO_5S^+$ [M+Na$^+$]: 472.1376, found: 472.1373.

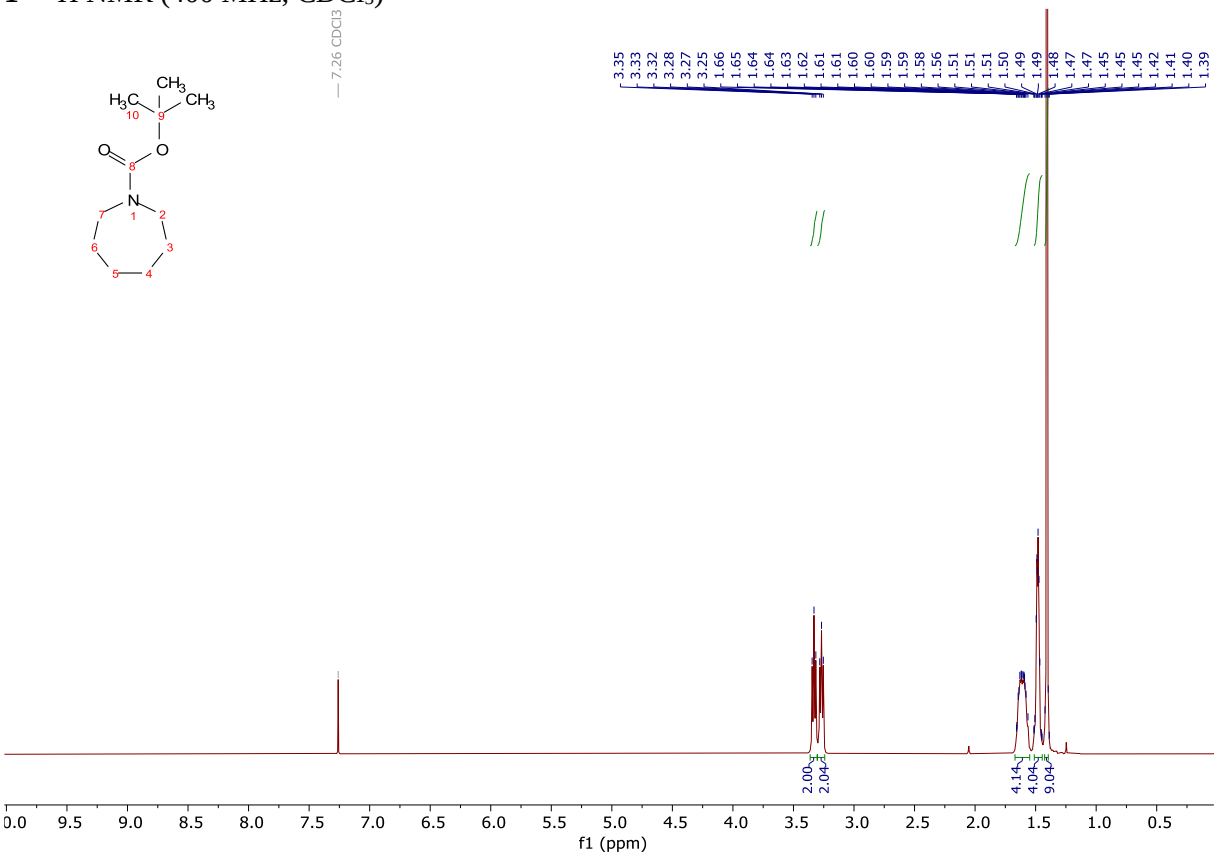
8-(Methylsulfonyl)-8-azaspiro[4.5]decan-(1*S*)-yl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (10f)



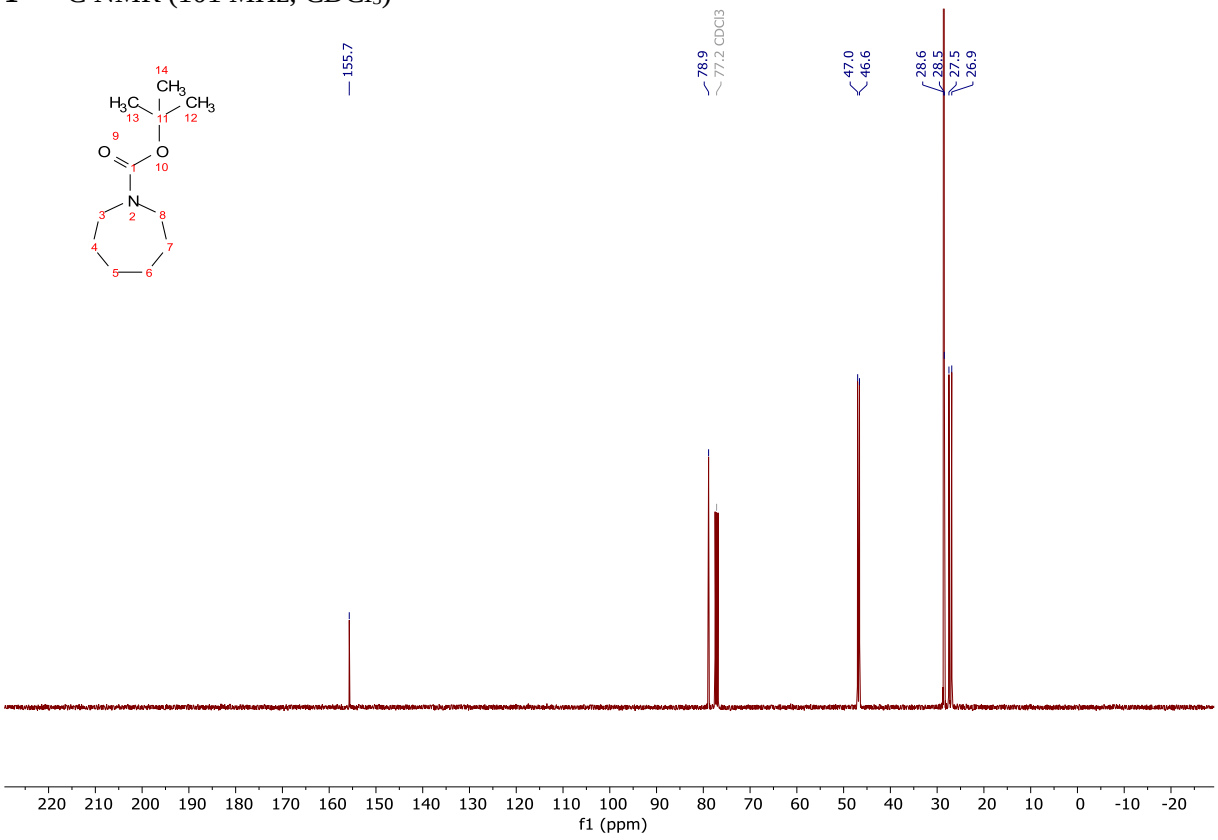
Based on the procedure of Hoye *et al.*,[33] product **10b** (10 mg, 0.04 mmol), isolated from reaction with the R19/F87A variant, was mixed with dry pyridine (10 μL, 0.12 mmol) in a screw-capped 10 mL glass vial charged with a magnetic stirring bar. 1 mL of anhydrous dichloromethane was then added to the mixture. The (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (15 μL, 0.08 mmol) was then added dropwise. The vial was then capped and the reaction mixture stirred at ambient temperature for 2 h. After 2 h, the reaction had proceeded to completion (TLC and GC). The solution was washed with 1 mL saturated aq $NaHCO_3$ solution and then 1 mL brine, dried over Na_2SO_4, filtered, and the solvent removed by rotary evaporation. The mixture was purified by silica gel column chromatography (30% ethyl acetate in petroleum ether) to afford ester **10f** as a colourless oil (11 mg, 62%). R_f 0.25 (50% ethyl acetate in petroleum ether). The *ee* for product **10b** from the R19/F87A variant was determined as 95% by ^{1}H NMR of **10f**. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 7.0, 3.0 Hz, 2H), 7.42 (dd, *J* = 5.0, 2.0 Hz, 2H), 5.03 (dd, *J* = 5.5, 2.5 Hz, 1H), 3.56/3.49 (s, 3H), 3.36 – 3.27 (m, 1H), 3.03 (ddd, *J* = 12.5, 9.5, 3.5 Hz, 1H), 2.90 (ddd, *J* = 12.5, 10.0, 3.5 Hz, 1H), 2.76/2.72 (s, 3H), 2.20 – 2.08 (m, 1H), 1.79 – 1.43 (m, 10H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 166.3, 132.1, 130.0, 128.7, 127.6, 123.3 (q, *J* = 288.5 Hz), 84.7 (q, *J* = 28.0 Hz), 84.4, 55.4, 44.1, 43.5, 42.8, 35.0, 33.9, 33.0, 30.7, 30.5, 30.3, 20.3. **HRMS** (ESI+): Calc'd for $C_{20}H_{26}F_3NNaO_5S^+$ $[M+Na]^+$: 472.1376, found: 472.1375.

A7. NMR spectra

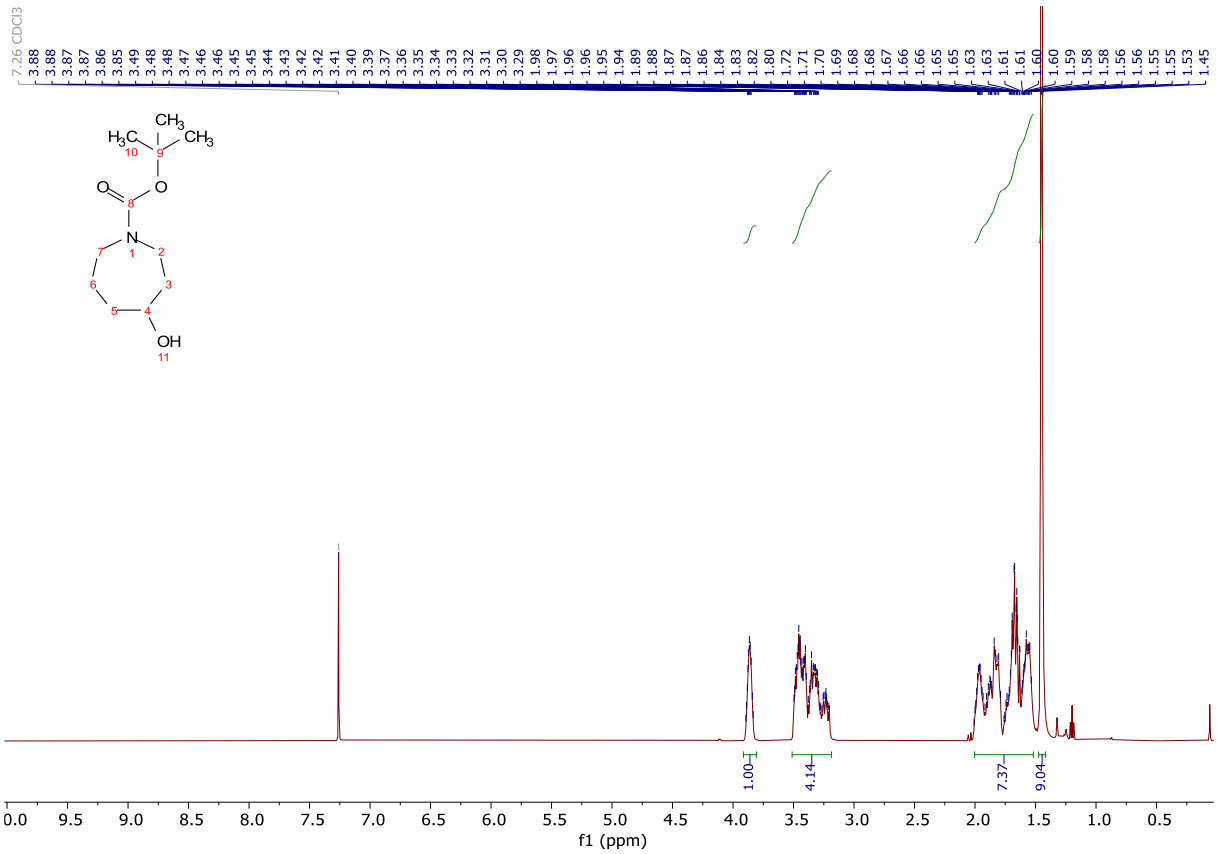
1 – ^{1}H NMR (400 MHz, $CDCl_3$)



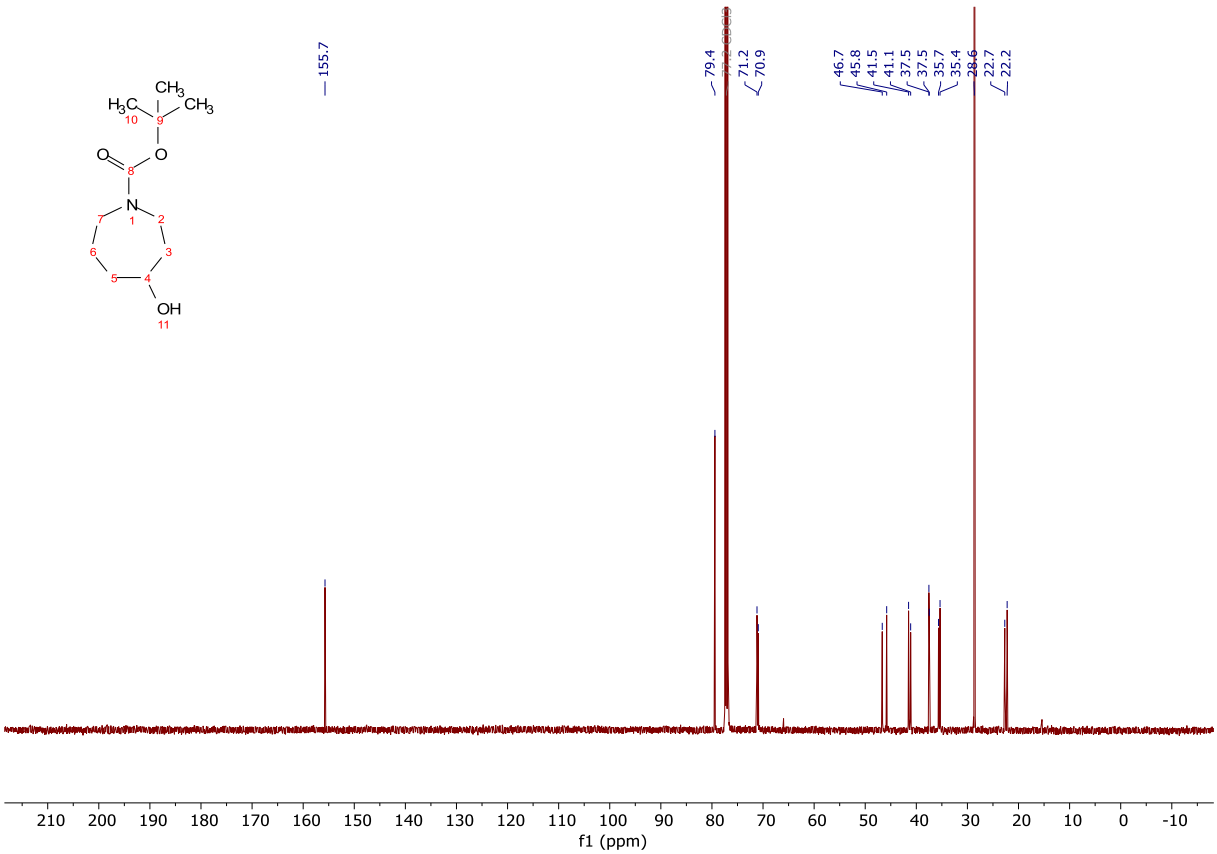
1 – ^{13}C NMR (101 MHz, $CDCl_3$)



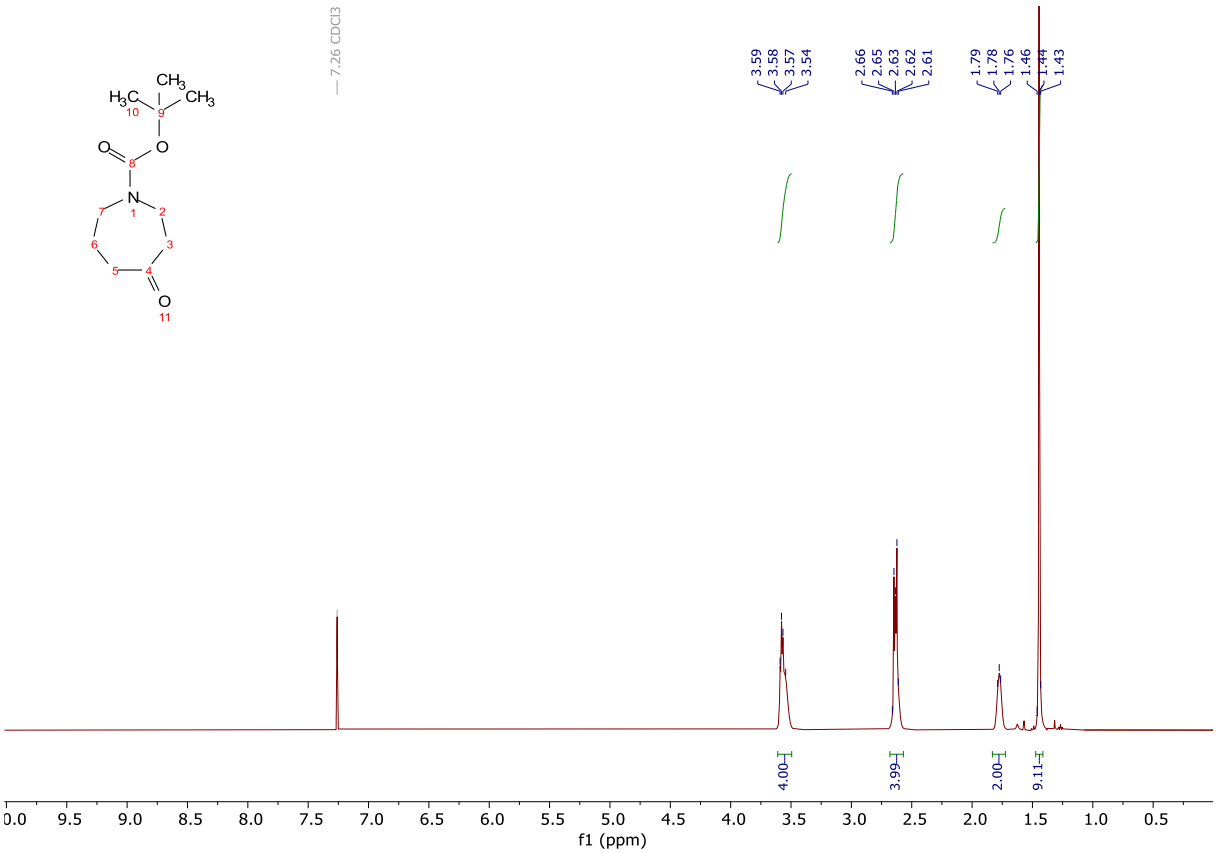
1a - ^{1}H NMR (500 MHz, $CDCl_3$)



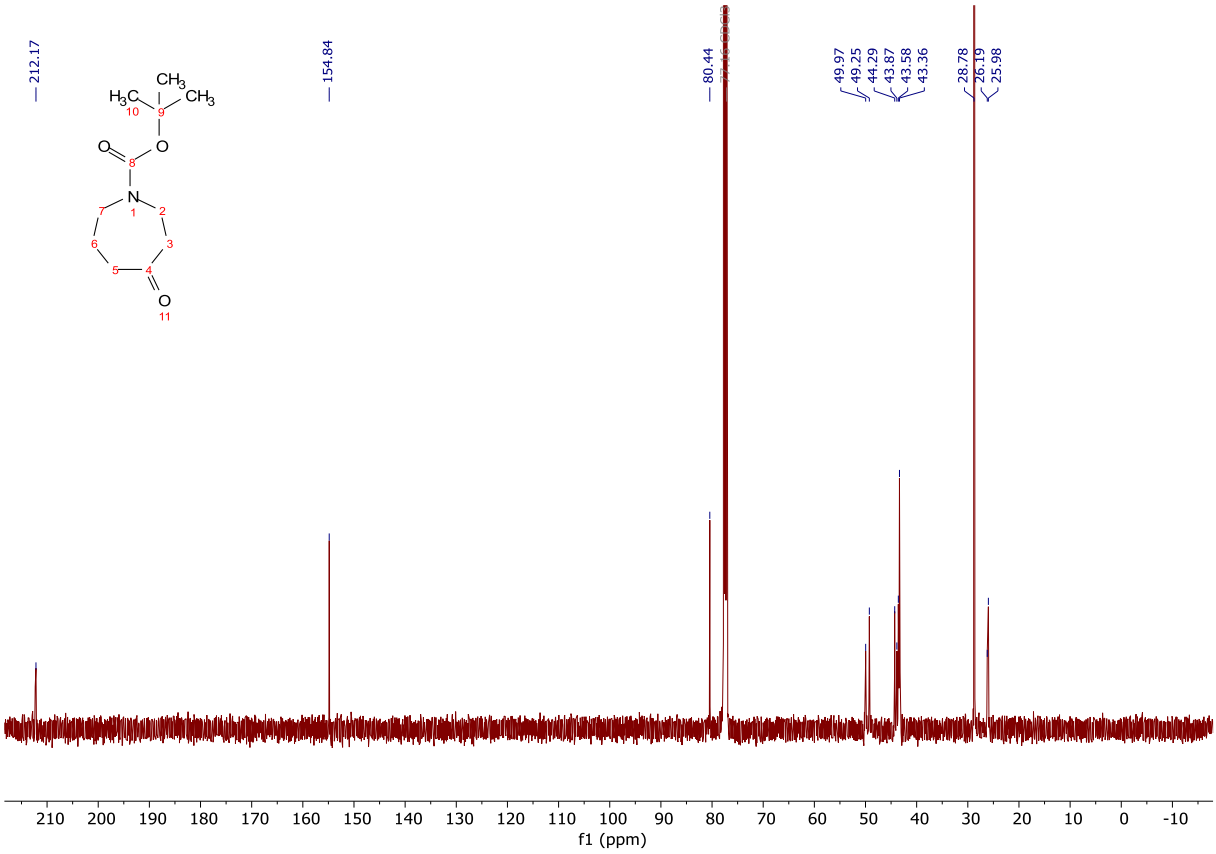
1a - ^{13}C NMR (126 MHz, $CDCl_3$)



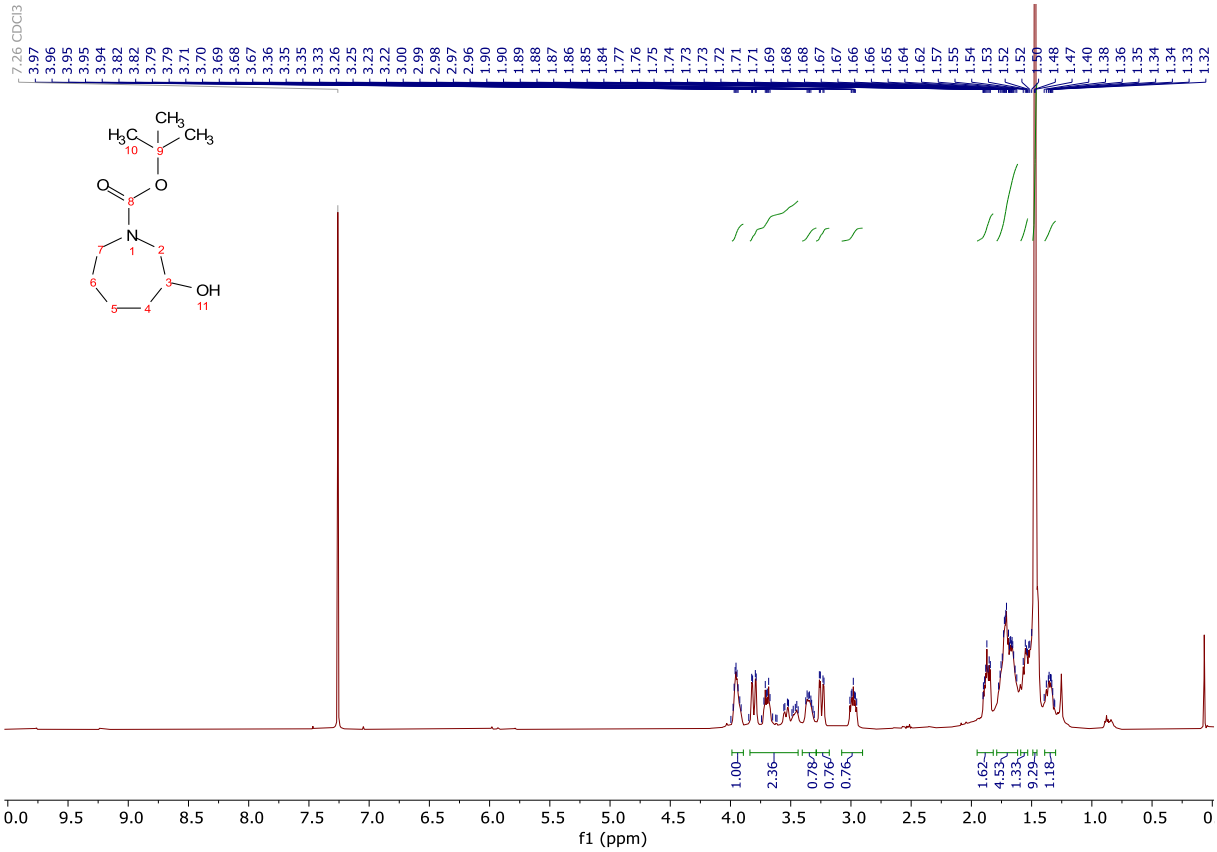
1b - ^{1}H NMR (500 MHz, $CDCl_3$)



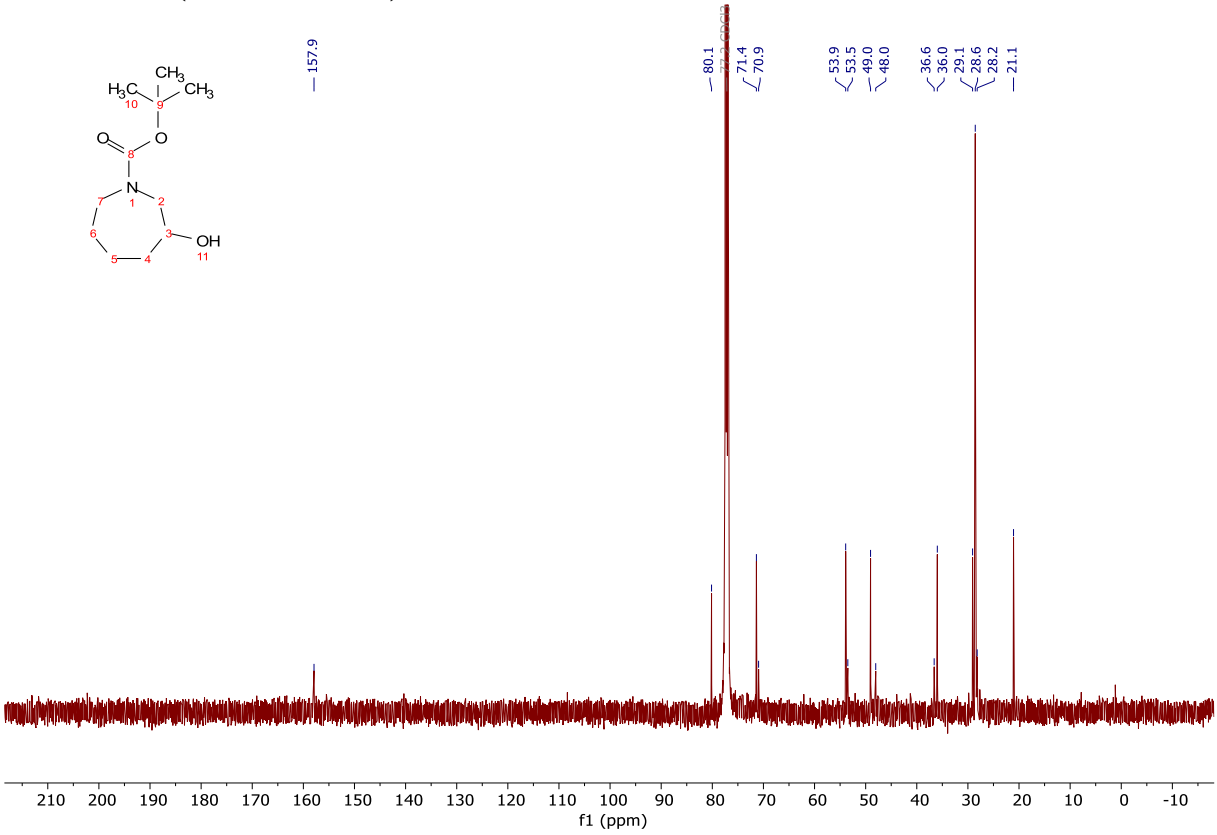
1b - ^{13}C NMR (126 MHz, $CDCl_3$)



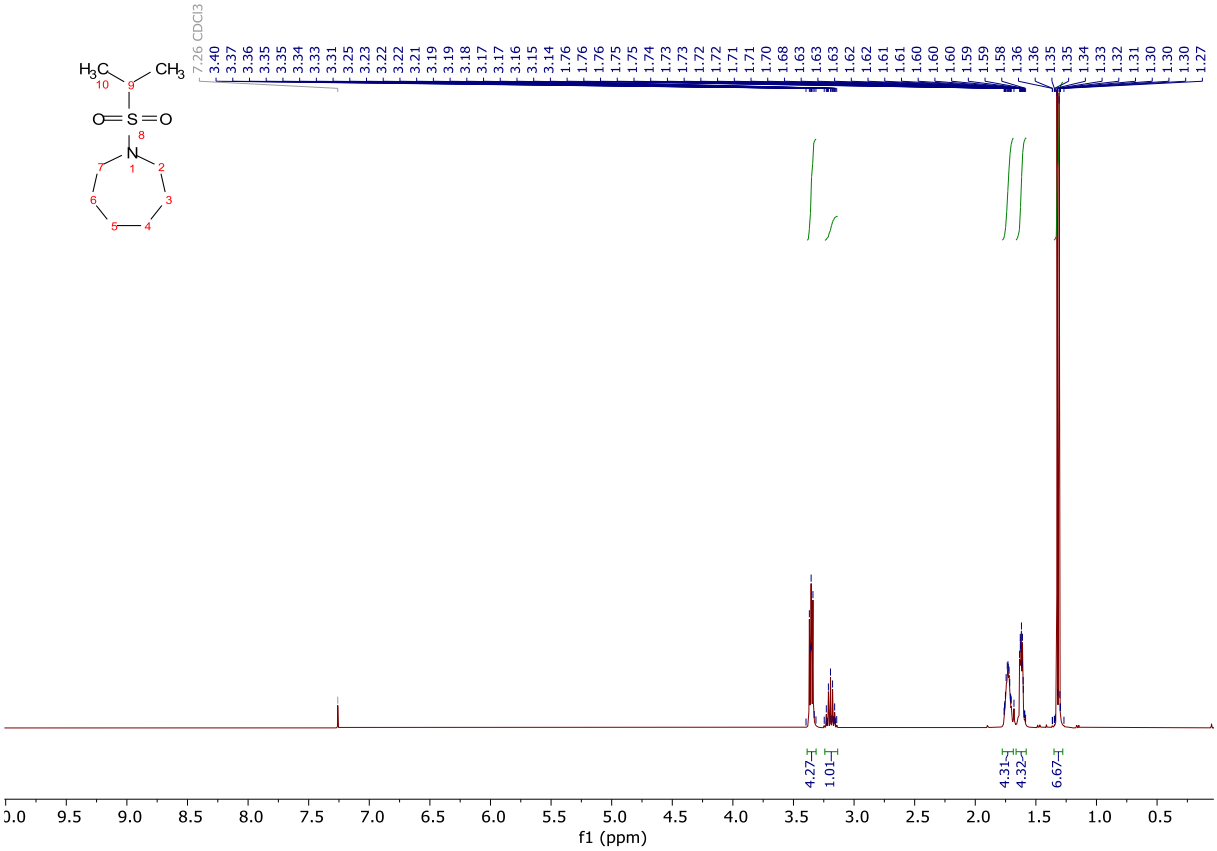
1c - ^{1}H NMR (500 MHz, $CDCl_3$)



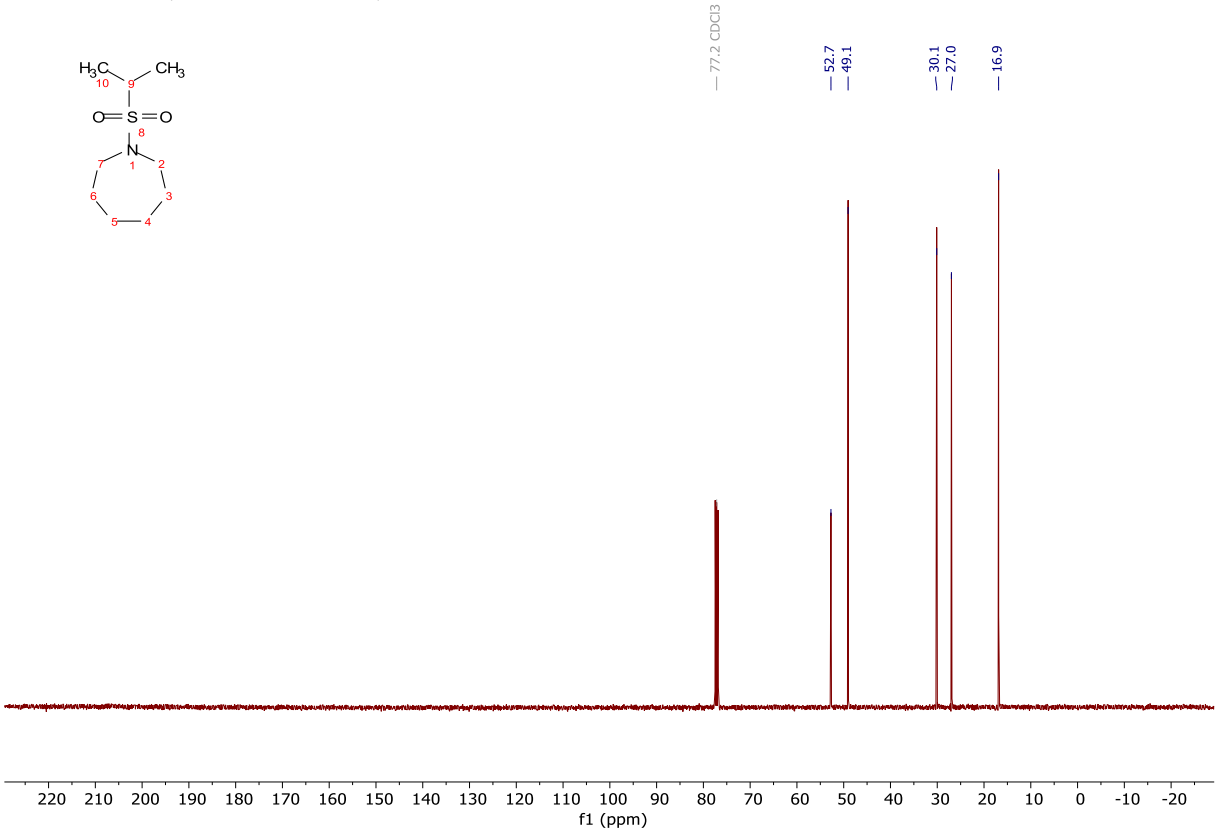
1c - ^{13}C NMR (126 MHz, $CDCl_3$)



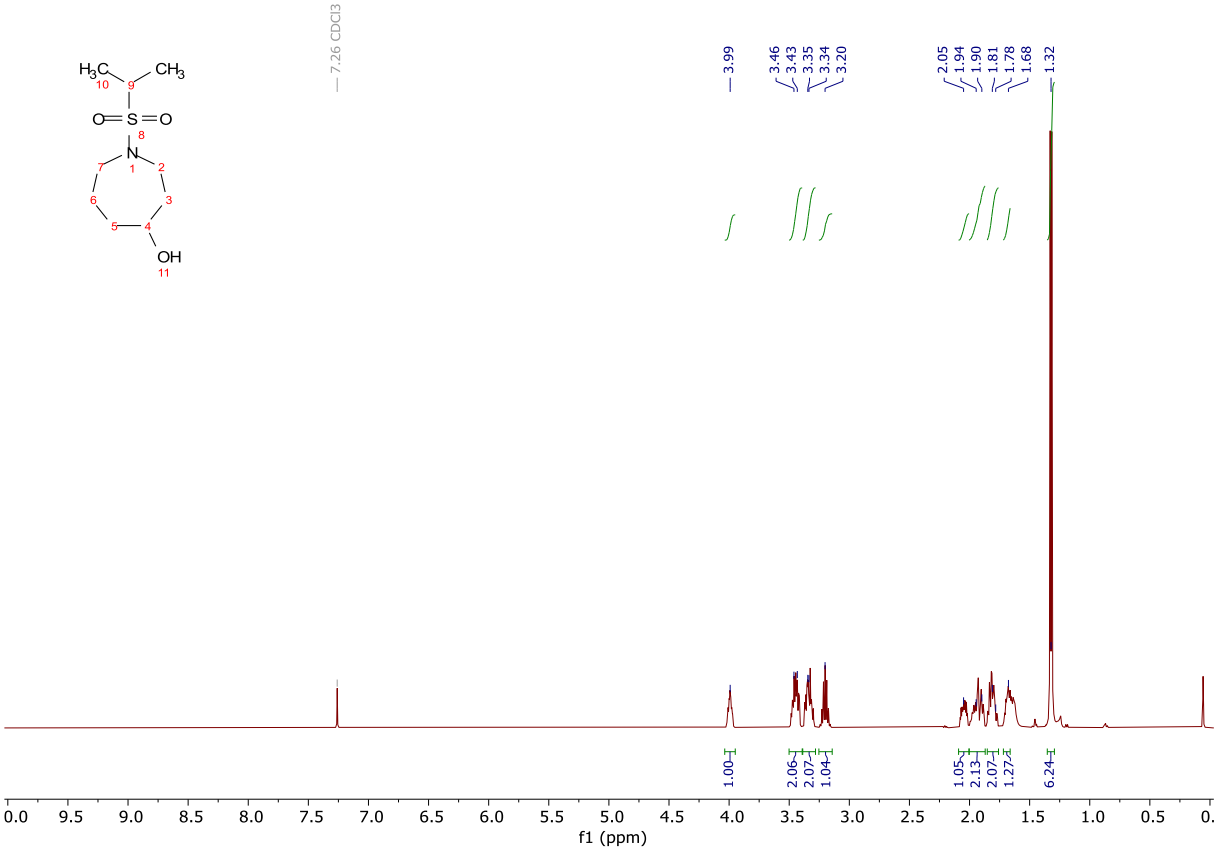
5 - ^{1}H NMR (400 MHz, $CDCl_3$)



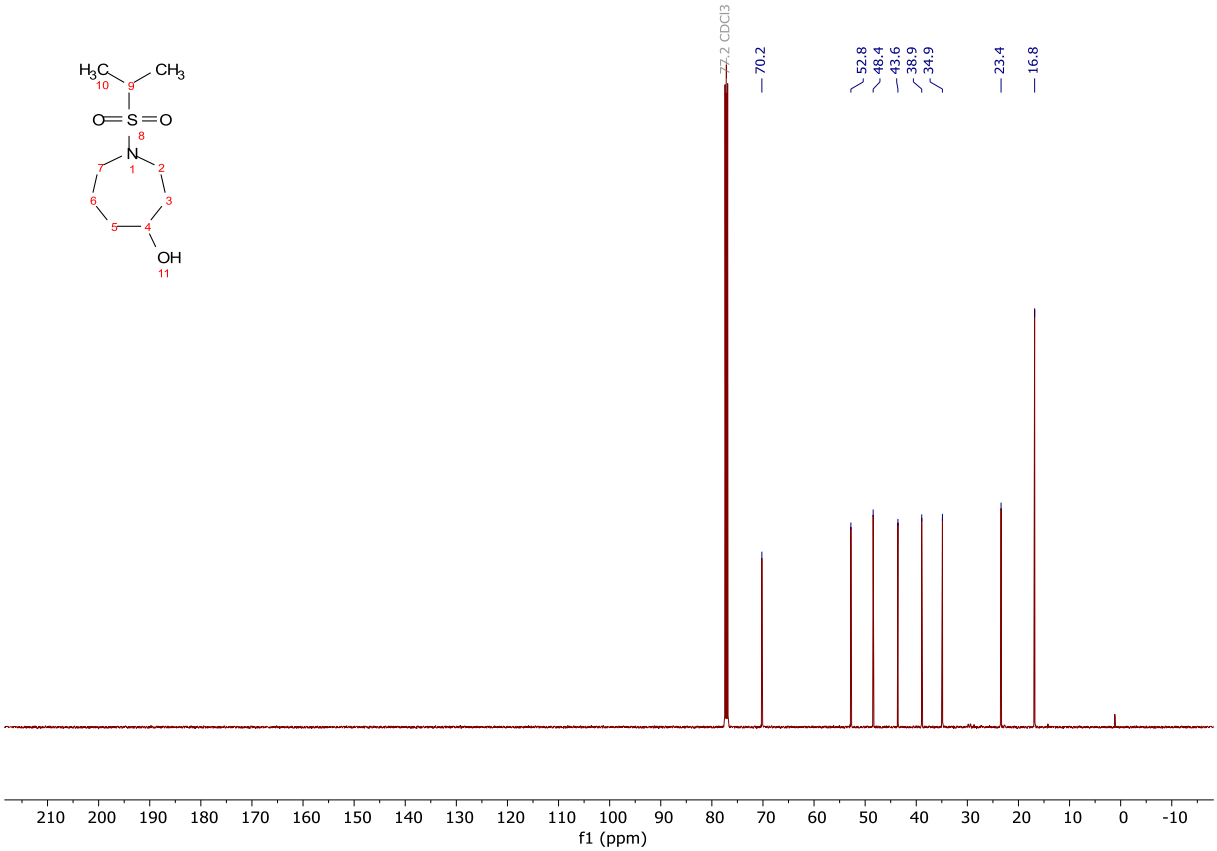
5 - ^{13}C NMR (101 MHz, $CDCl_3$)



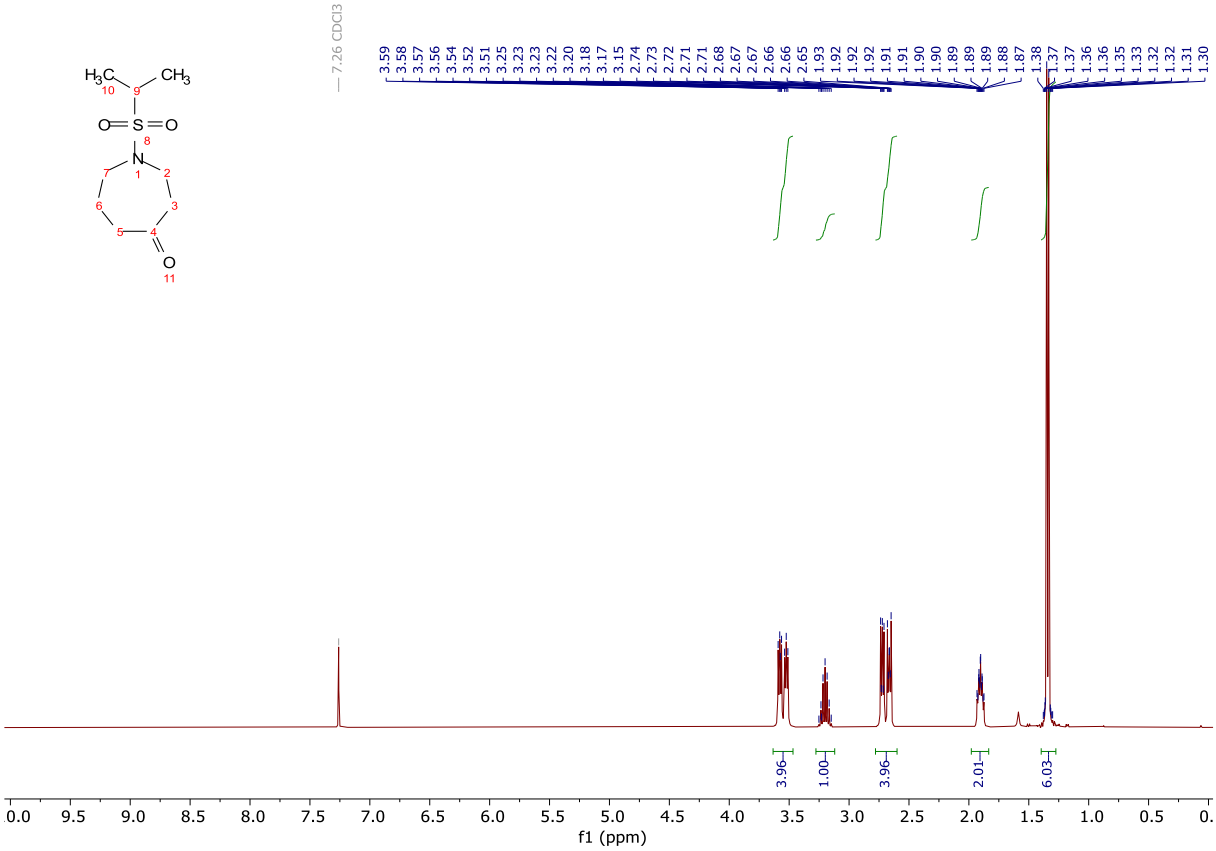
5a - ^{1}H NMR (500 MHz, $CDCl_3$)



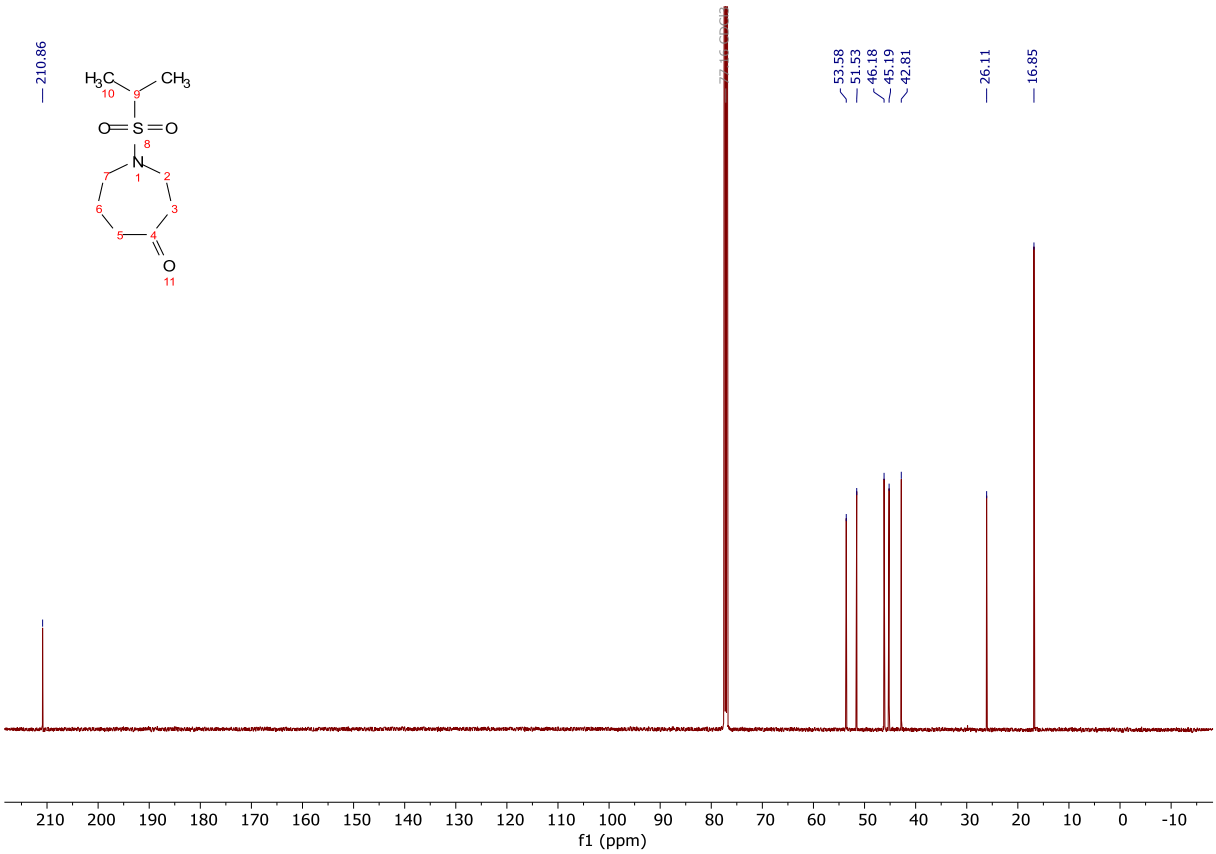
5a - ^{13}C NMR (126 MHz, $CDCl_3$)



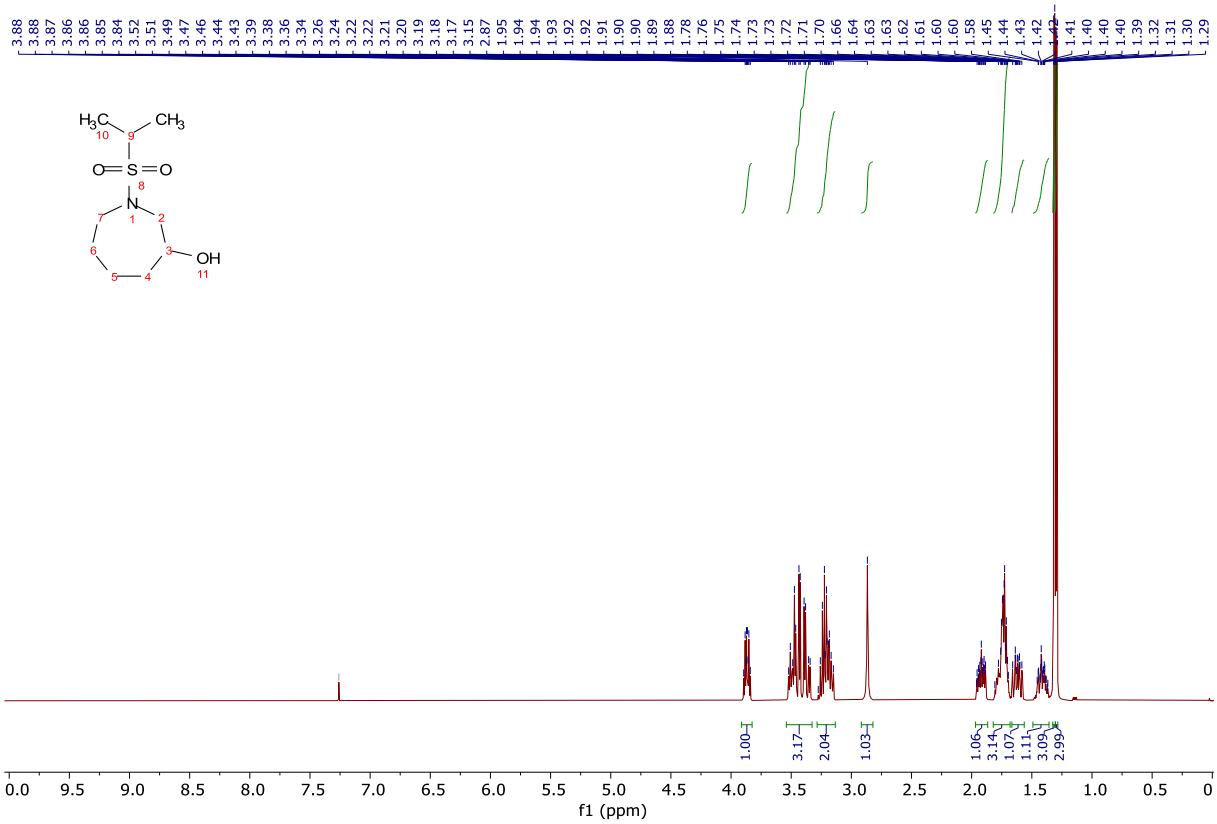
5b - ^{1}H NMR (400 MHz, $CDCl_3$)



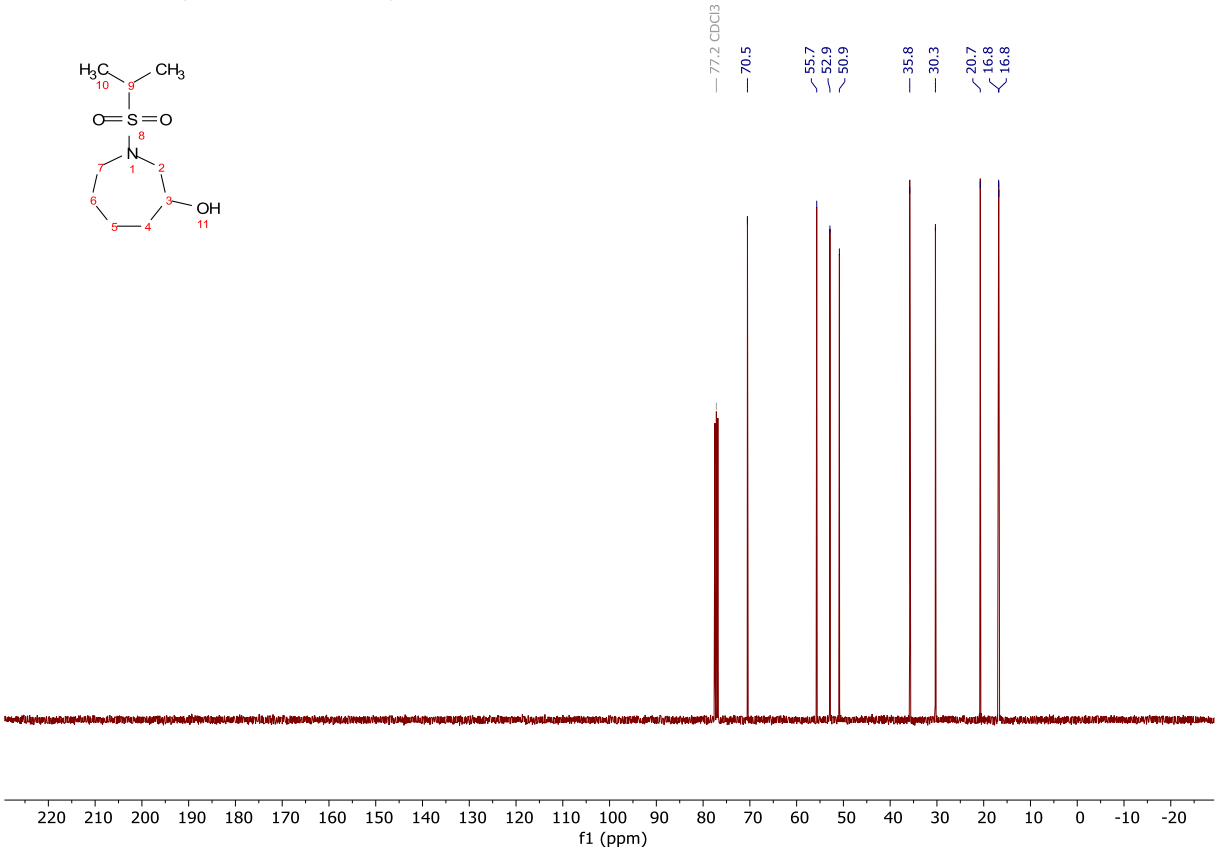
5b - ^{13}C NMR (126 MHz, $CDCl_3$)



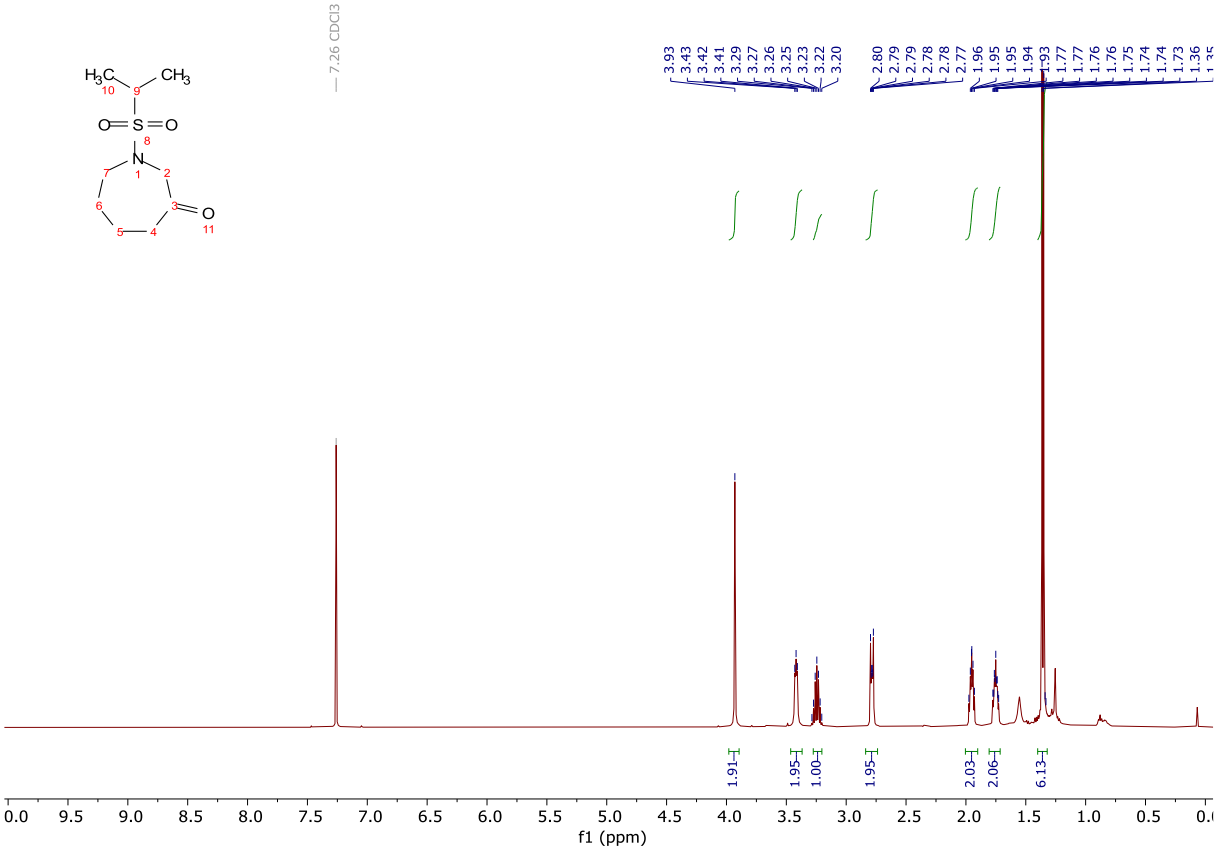
5c - ^{1}H NMR (400 MHz, $CDCl_3$)



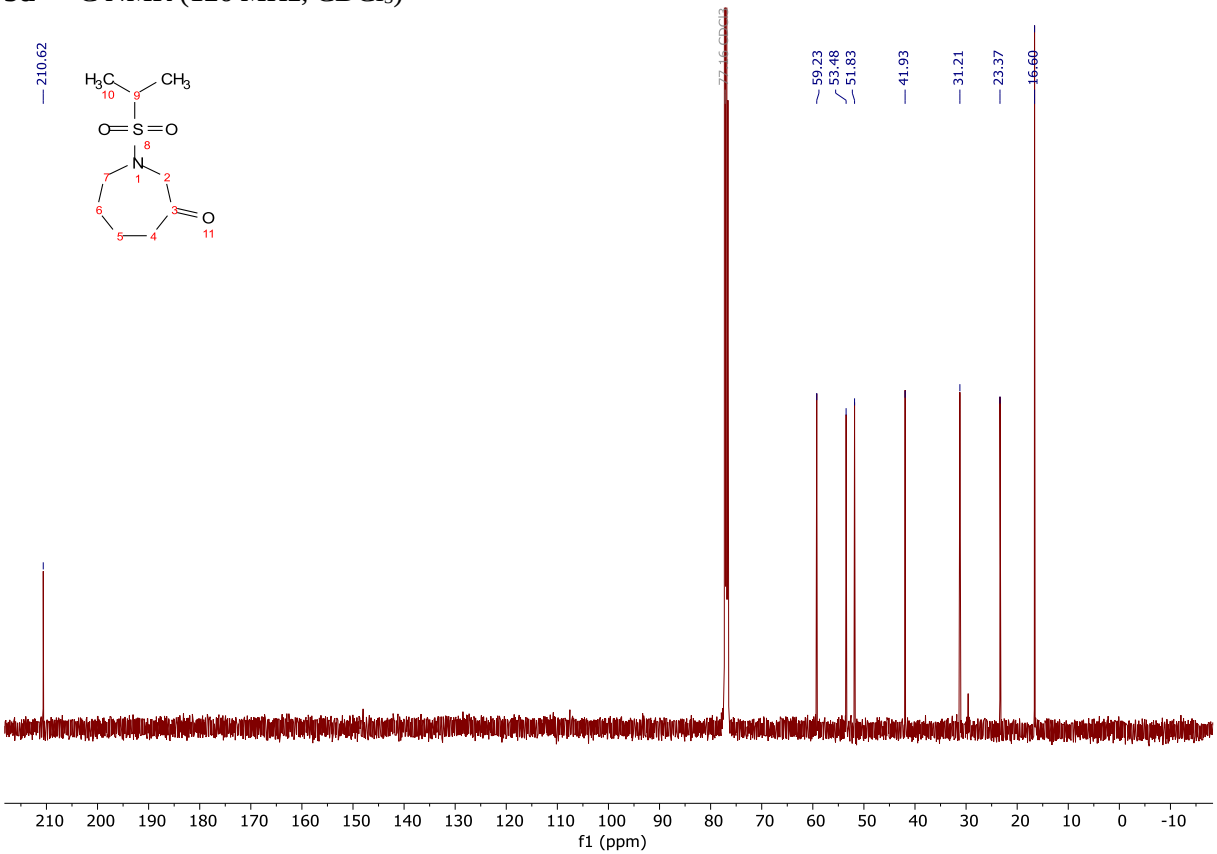
5c - ^{13}C NMR (101 MHz, $CDCl_3$)



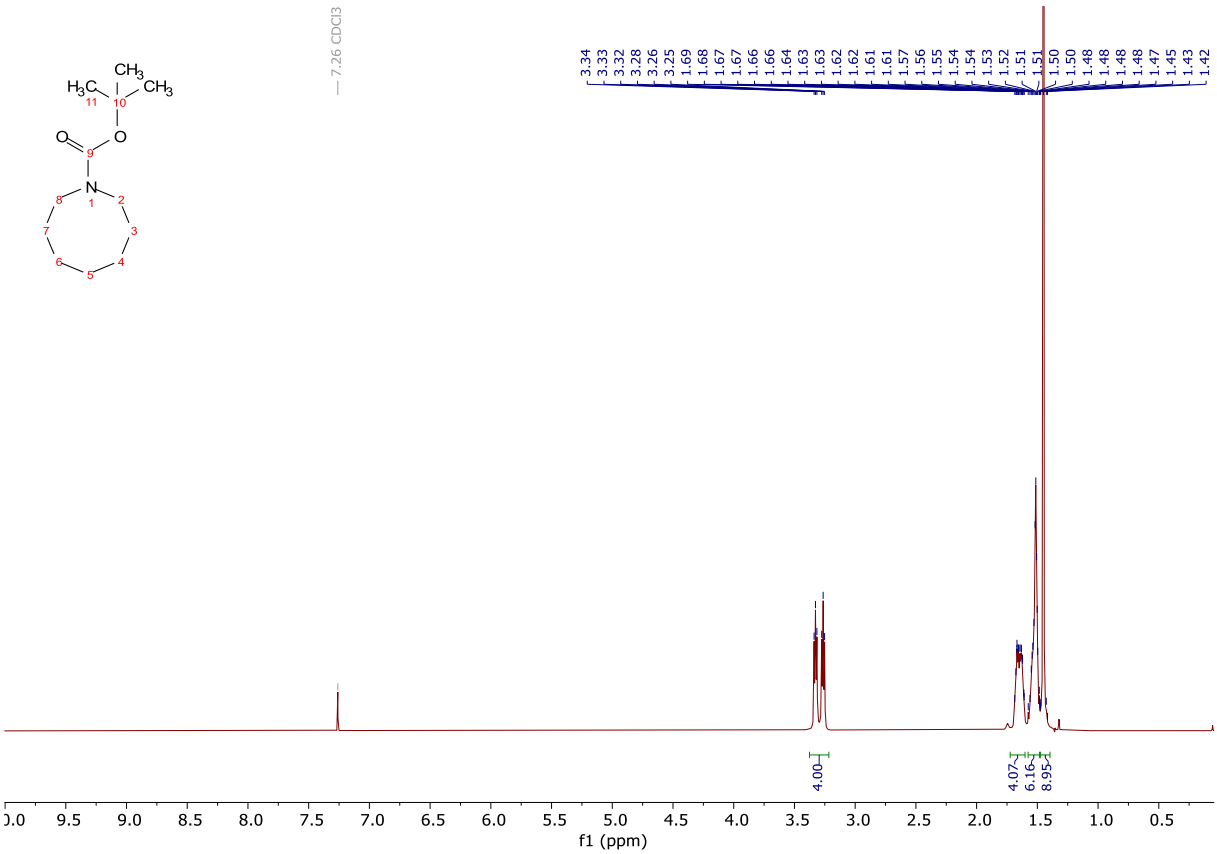
5d - ^{1}H NMR (500 MHz, $CDCl_3$)



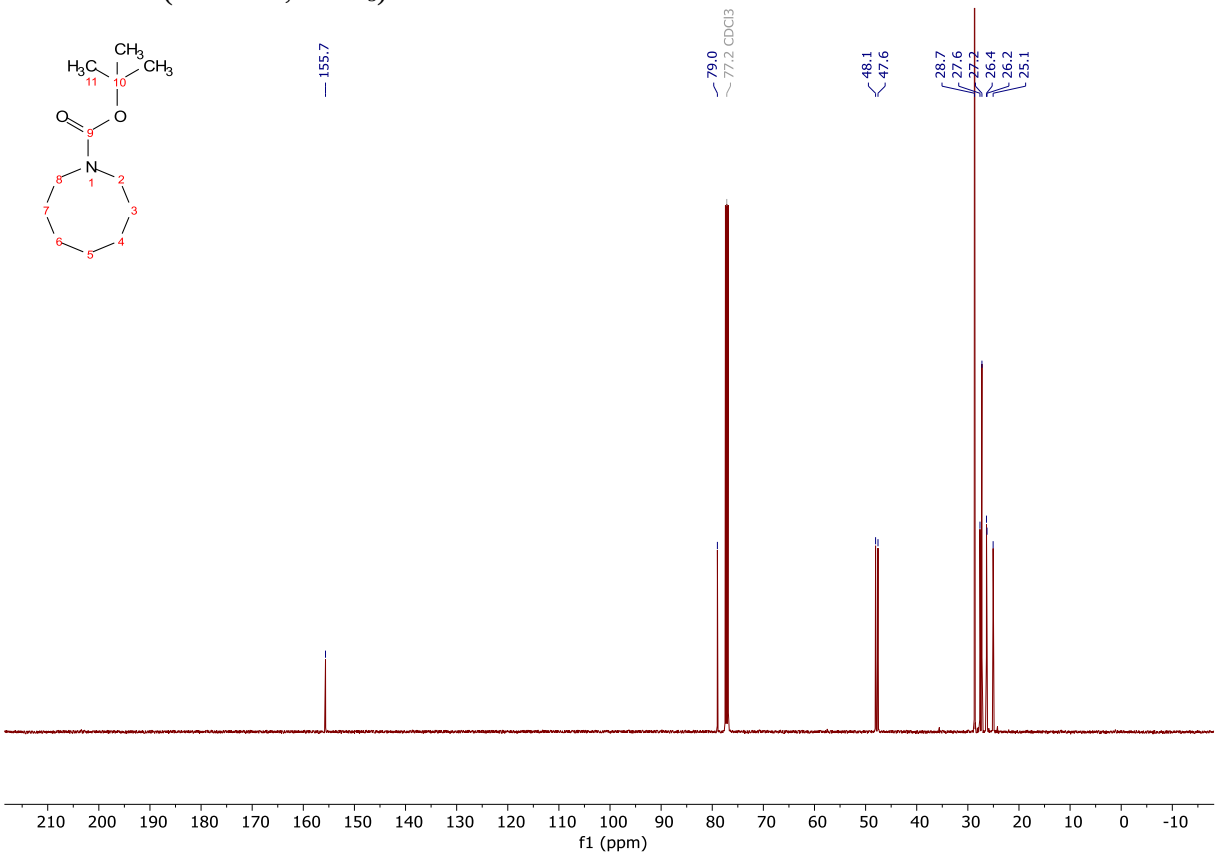
5d - ^{13}C NMR (126 MHz, $CDCl_3$)



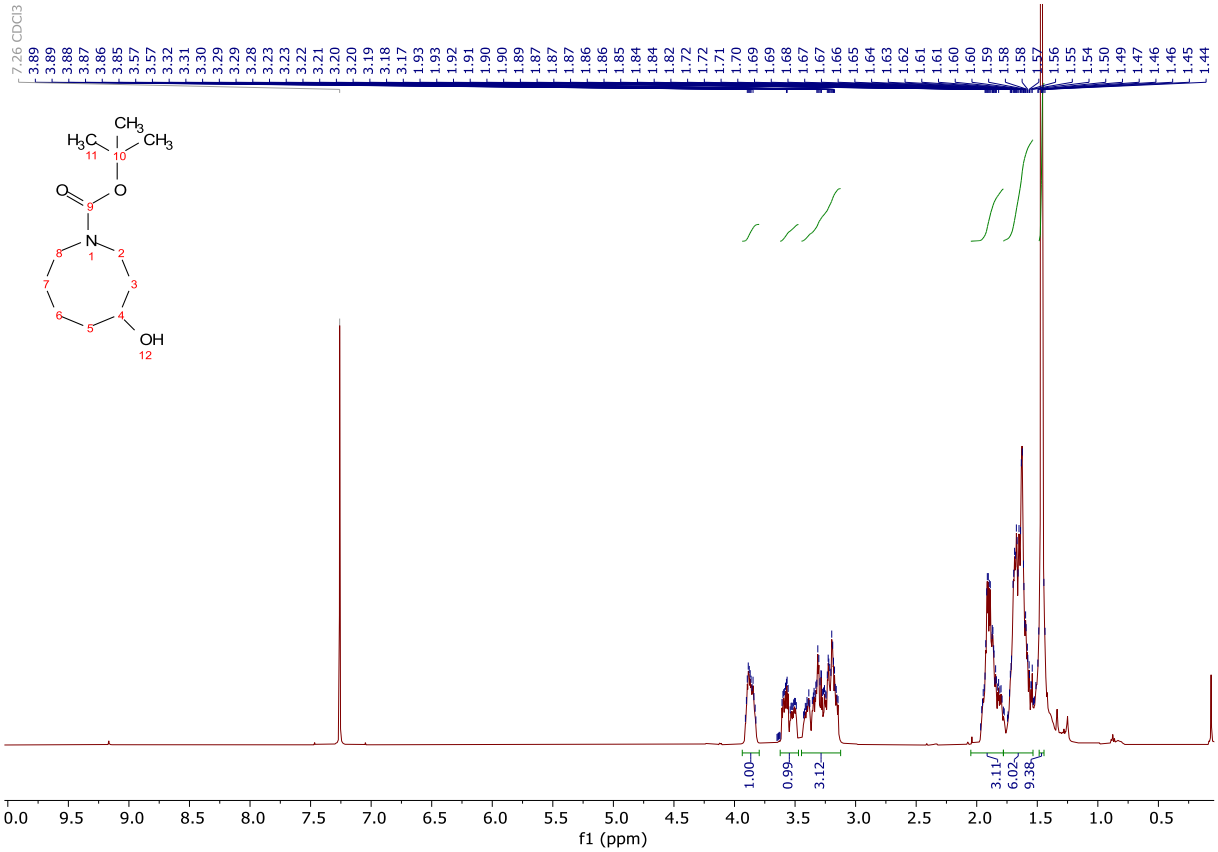
6 - ^{1}H NMR (500 MHz, $CDCl_3$)



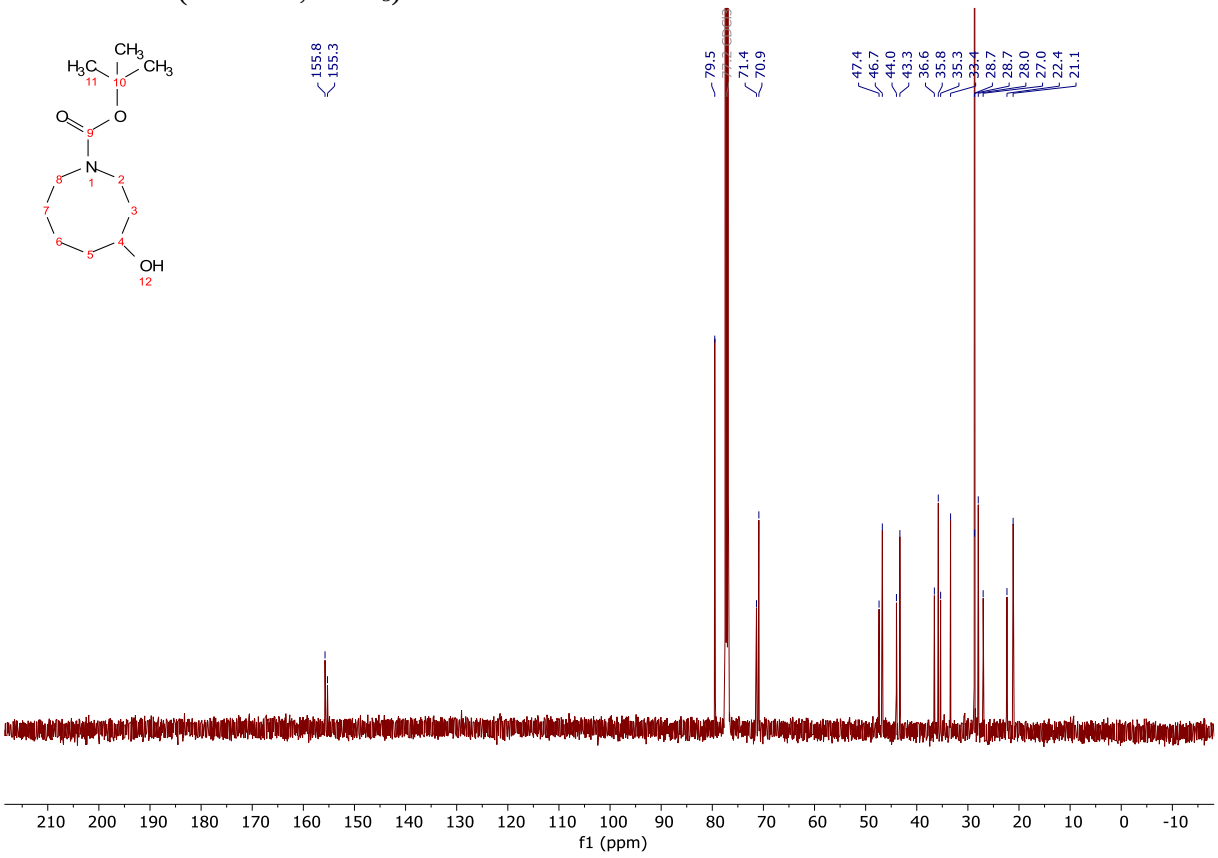
6 - ^{13}C NMR (126 MHz, $CDCl_3$)



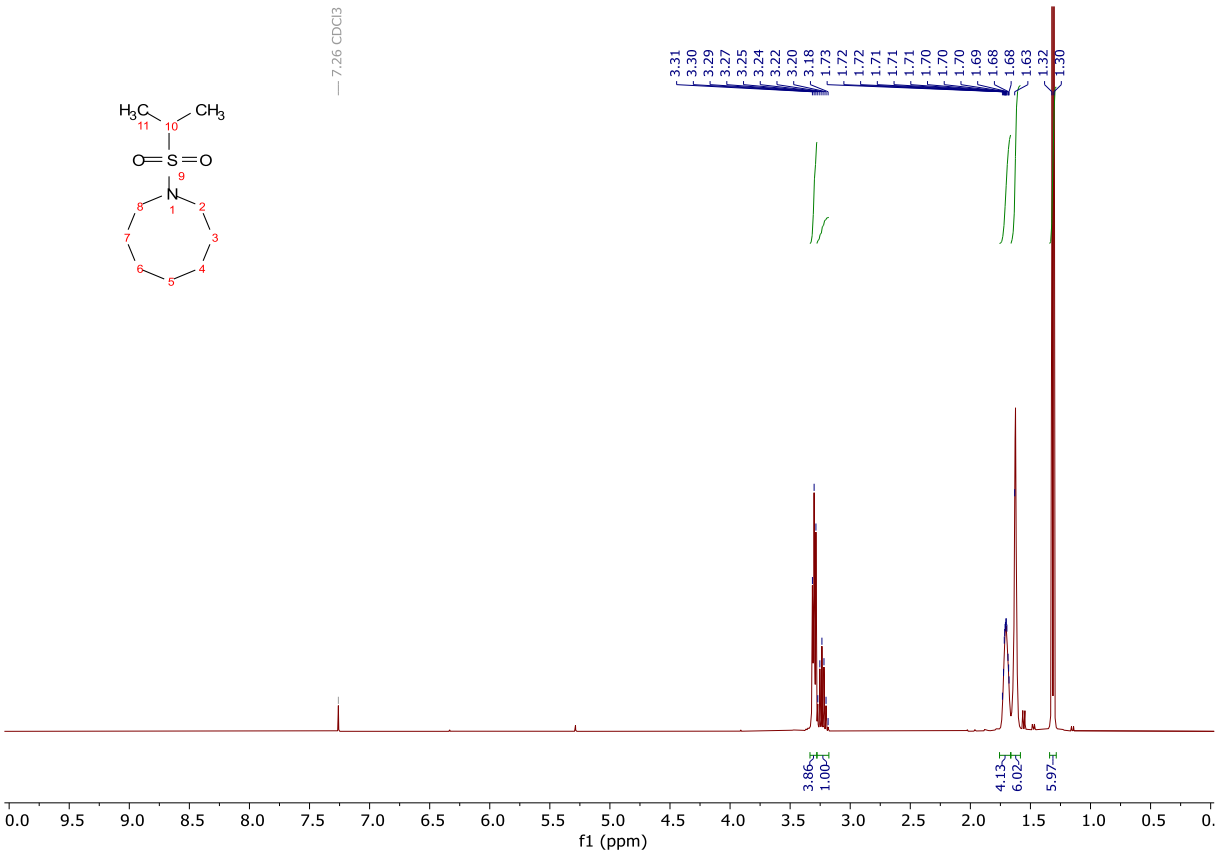
6a - ^{1}H NMR (500 MHz, $CDCl_3$)



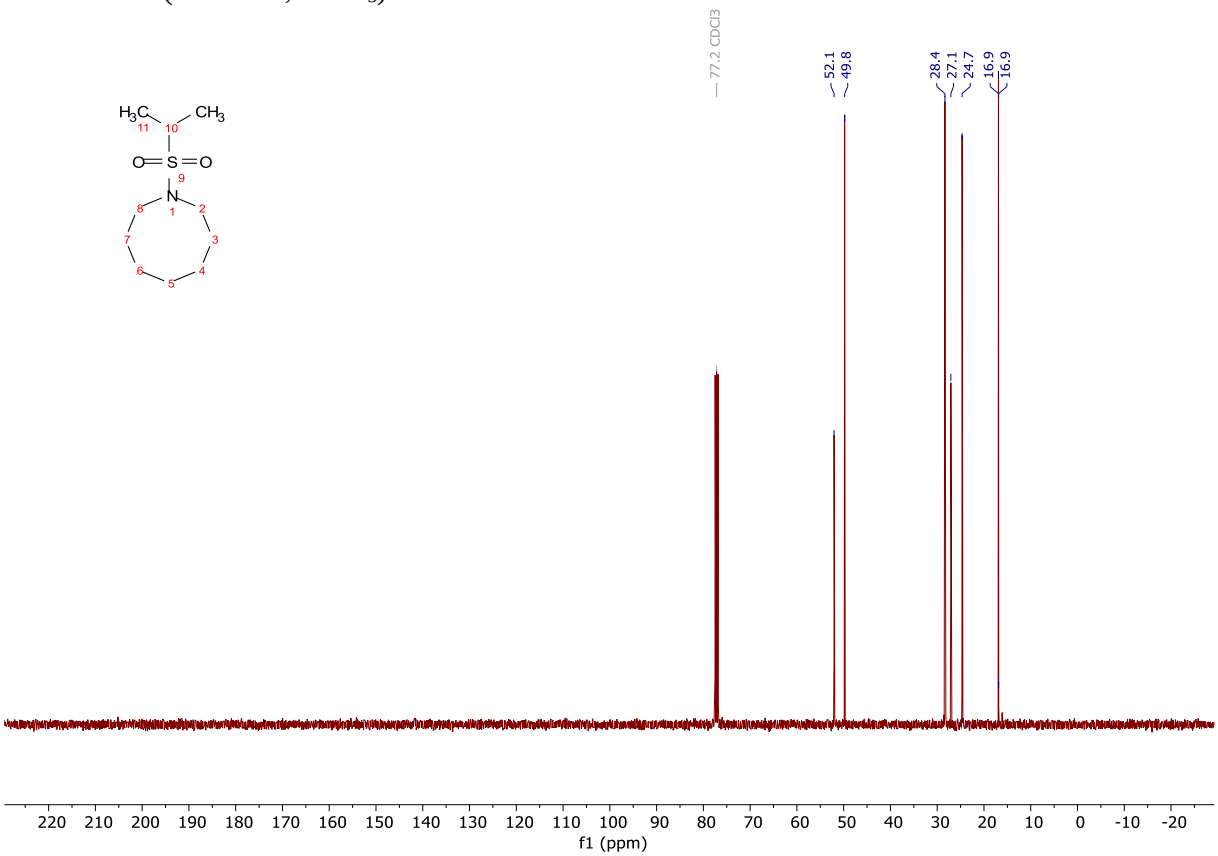
6a - ^{13}C NMR (126 MHz, $CDCl_3$)



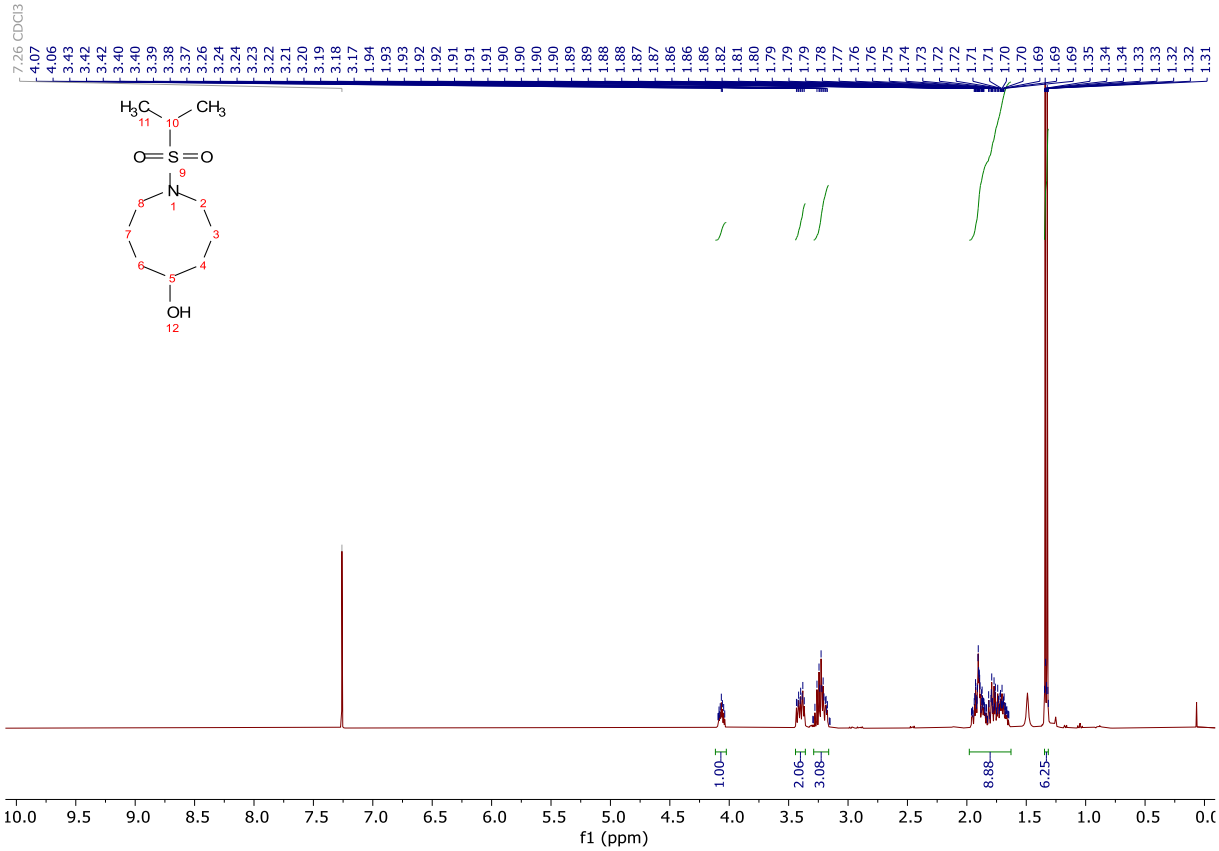
7 - ^{1}H NMR (500 MHz, $CDCl_3$)



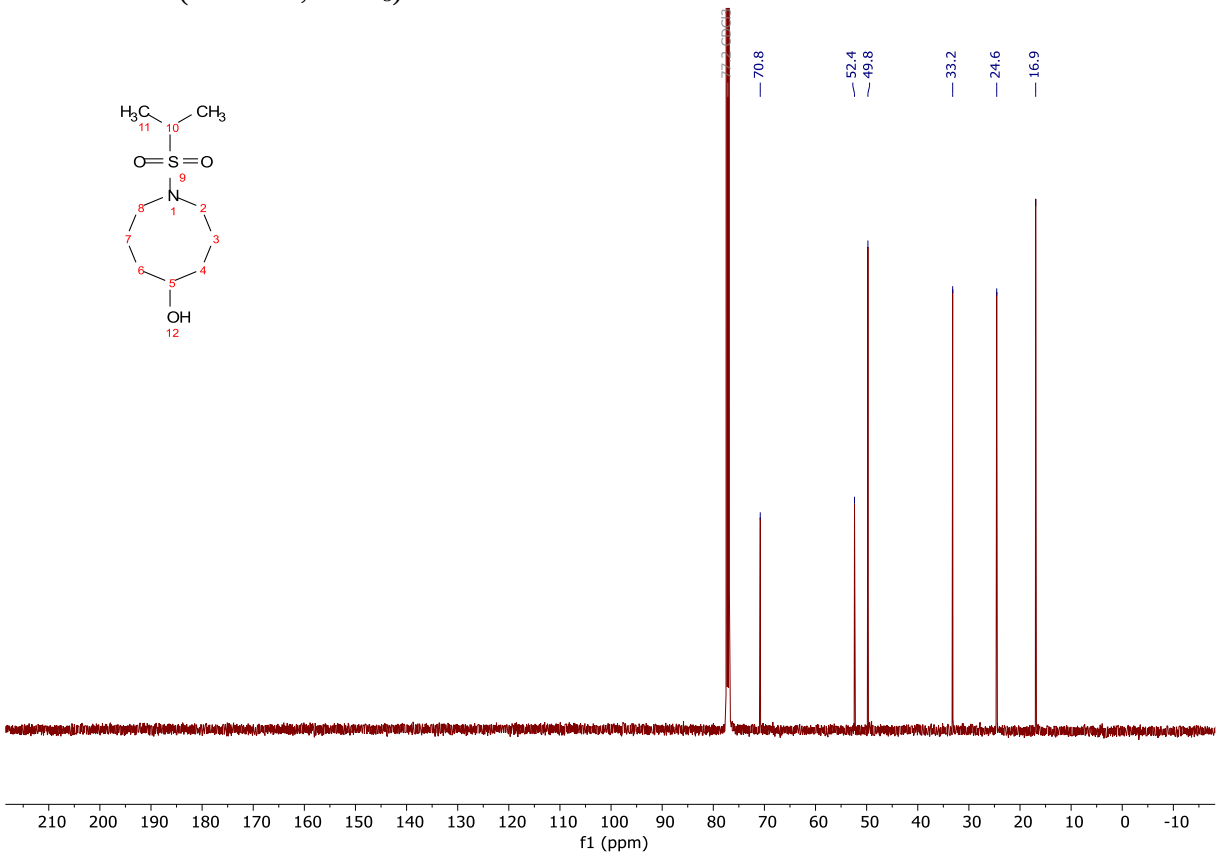
7 - ^{13}C NMR (126 MHz, $CDCl_3$)



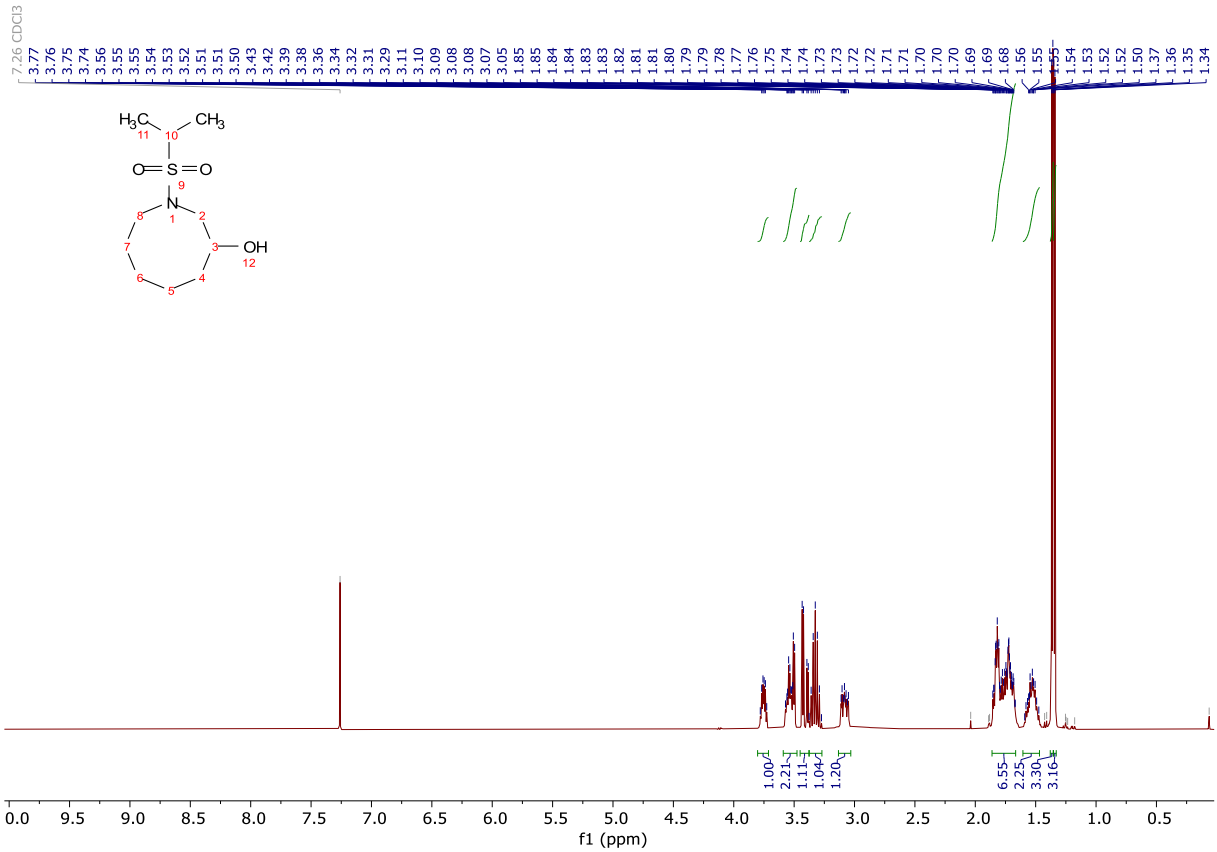
7a - ^{1}H NMR (400 MHz, $CDCl_3$)



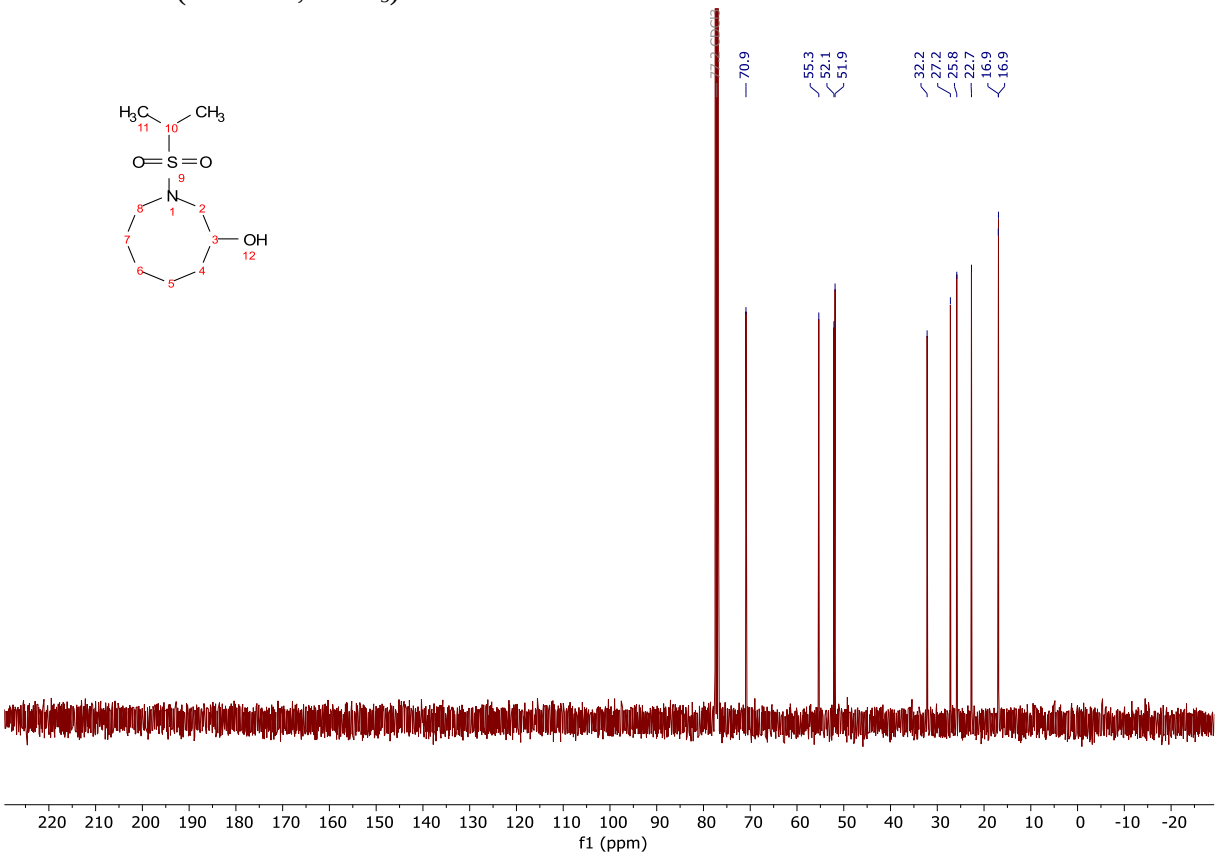
7a - ^{13}C NMR (126 MHz, $CDCl_3$)



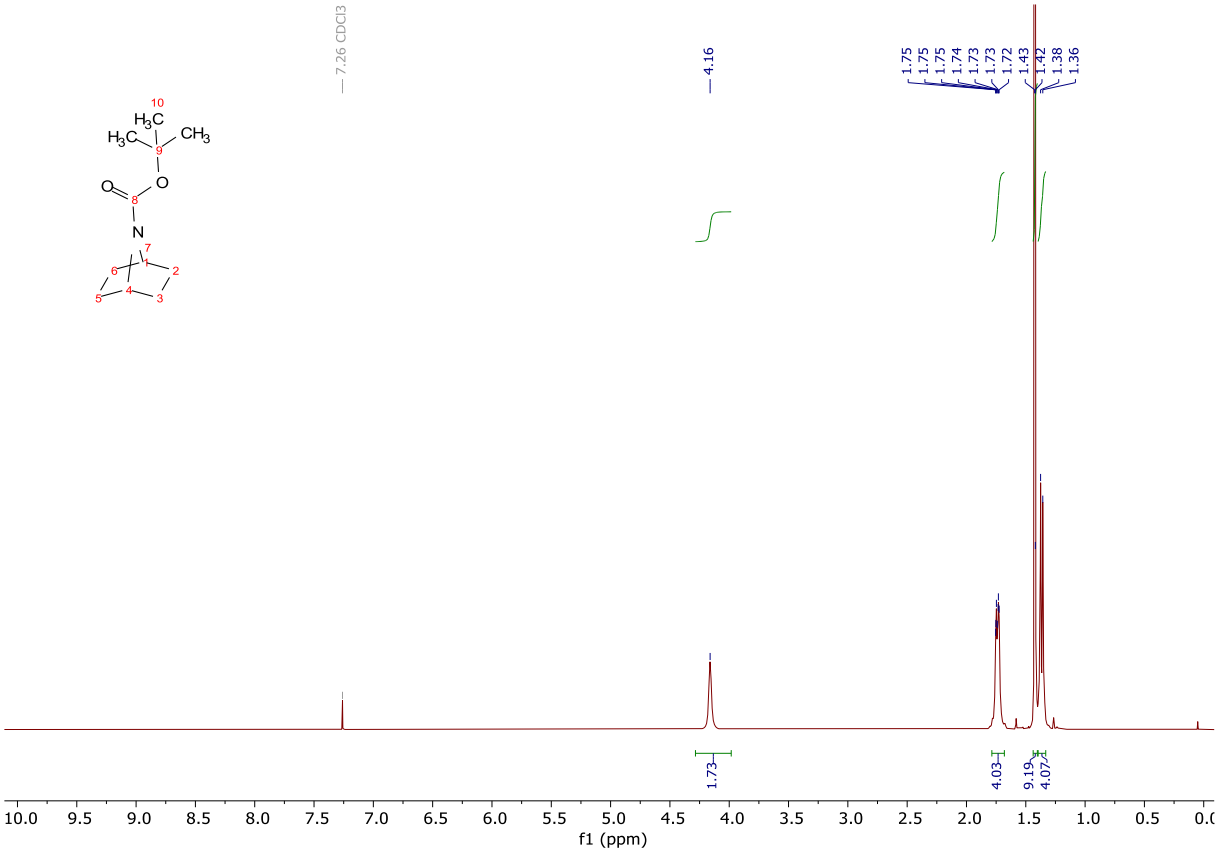
7c - ^{1}H NMR (500 MHz, $CDCl_3$)



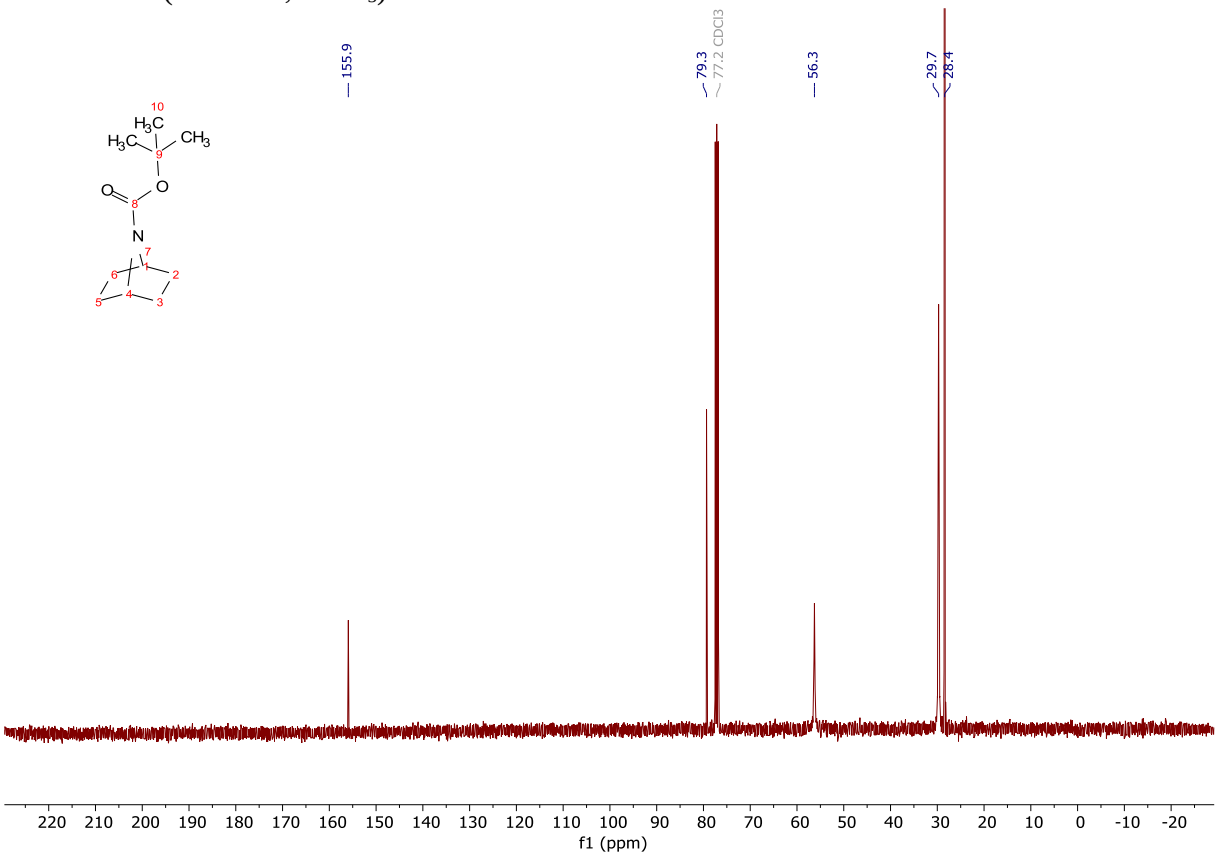
7c - ^{13}C NMR (101 MHz, $CDCl_3$)



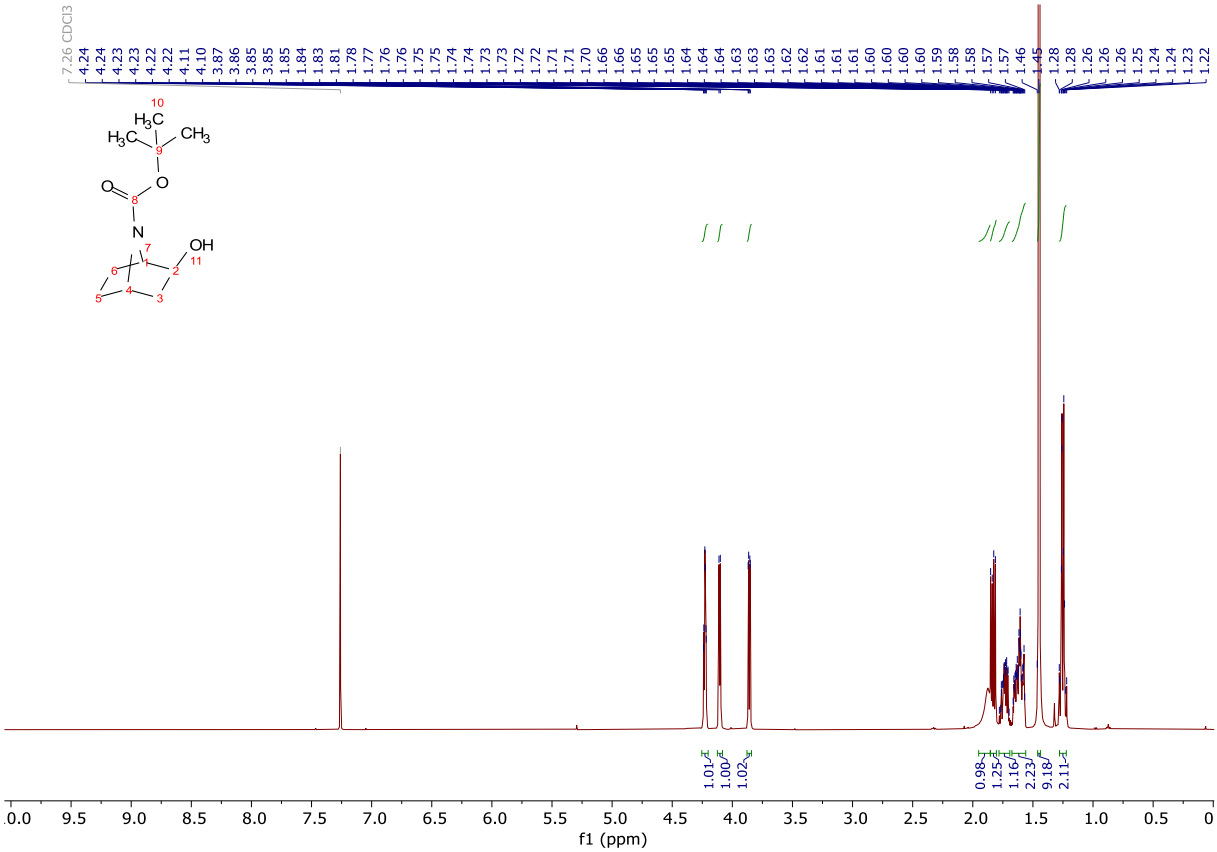
8 - ^{1}H NMR (400 MHz, $CDCl_3$)



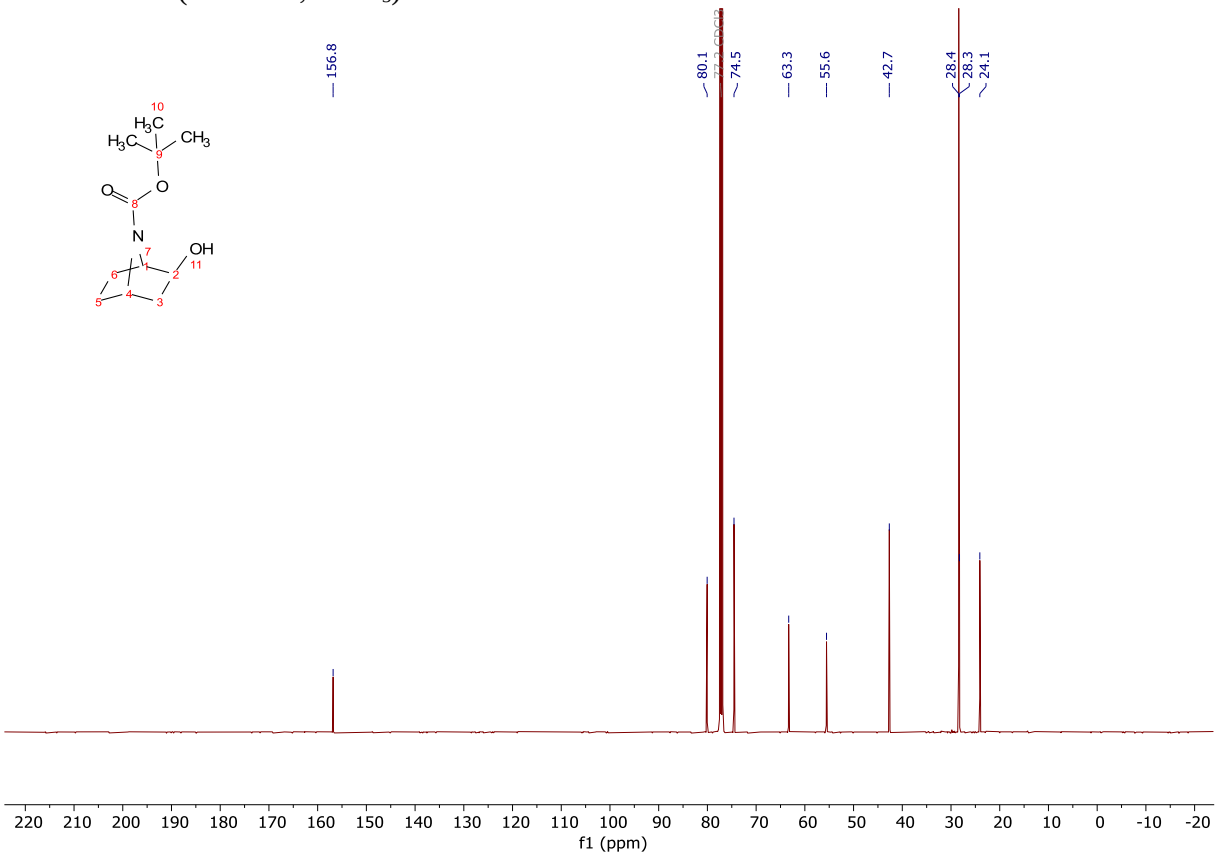
8 - ^{13}C NMR (101 MHz, $CDCl_3$)



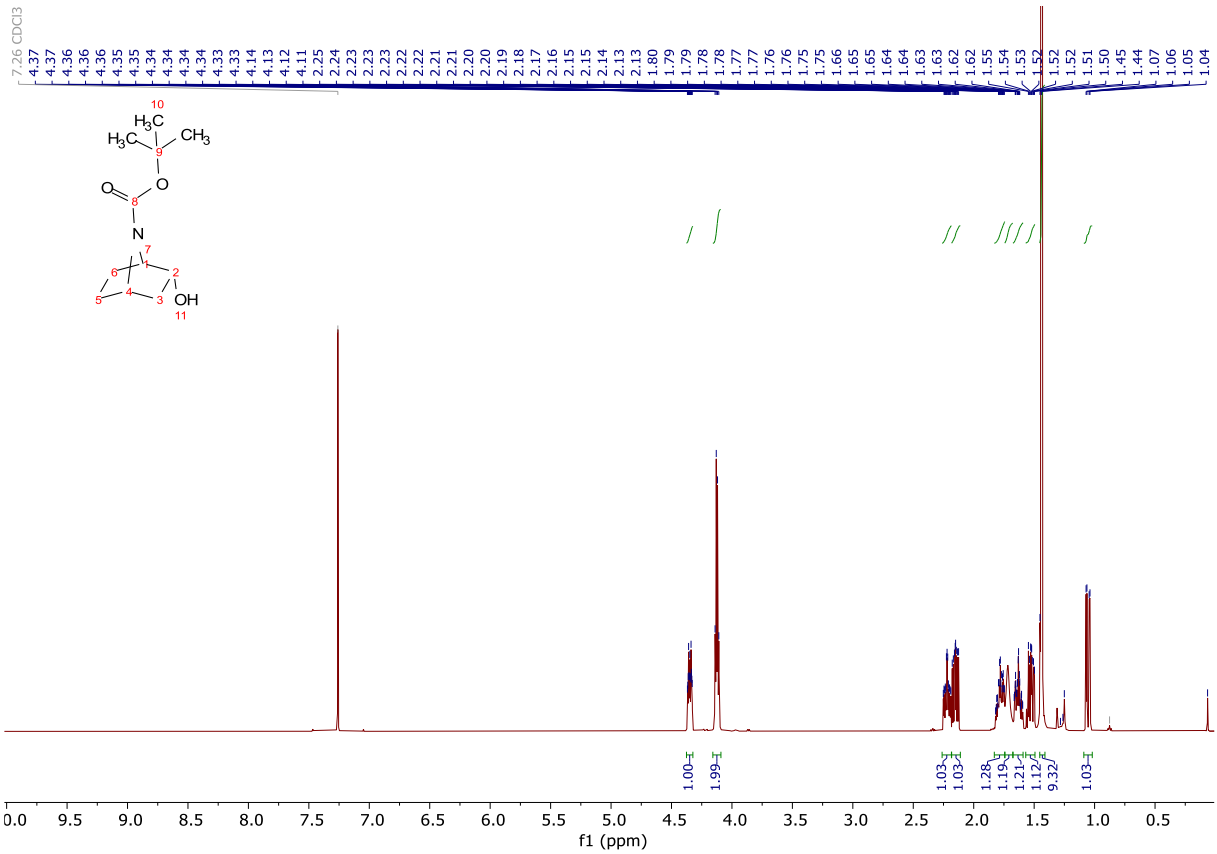
8a - ^{1}H NMR (500 MHz, $CDCl_3$)



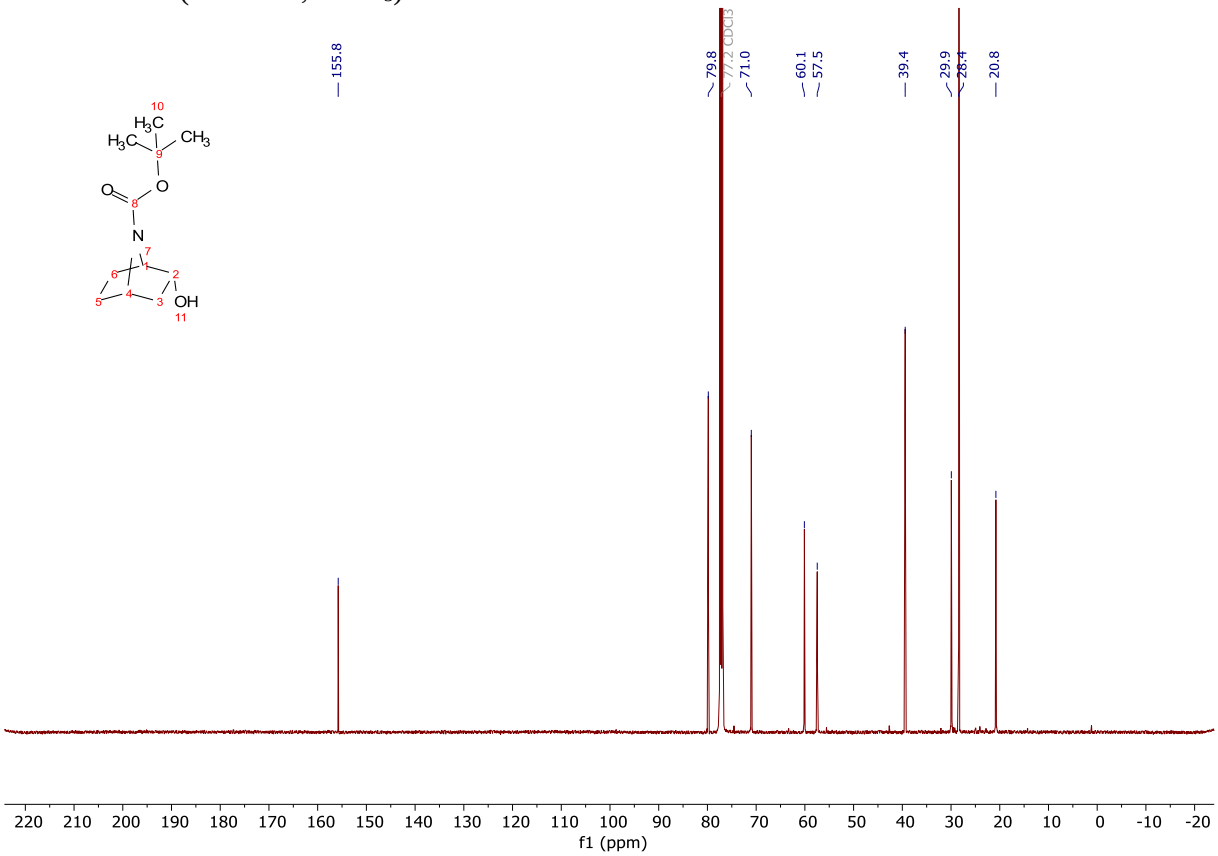
8a - ^{13}C NMR (126 MHz, $CDCl_3$)



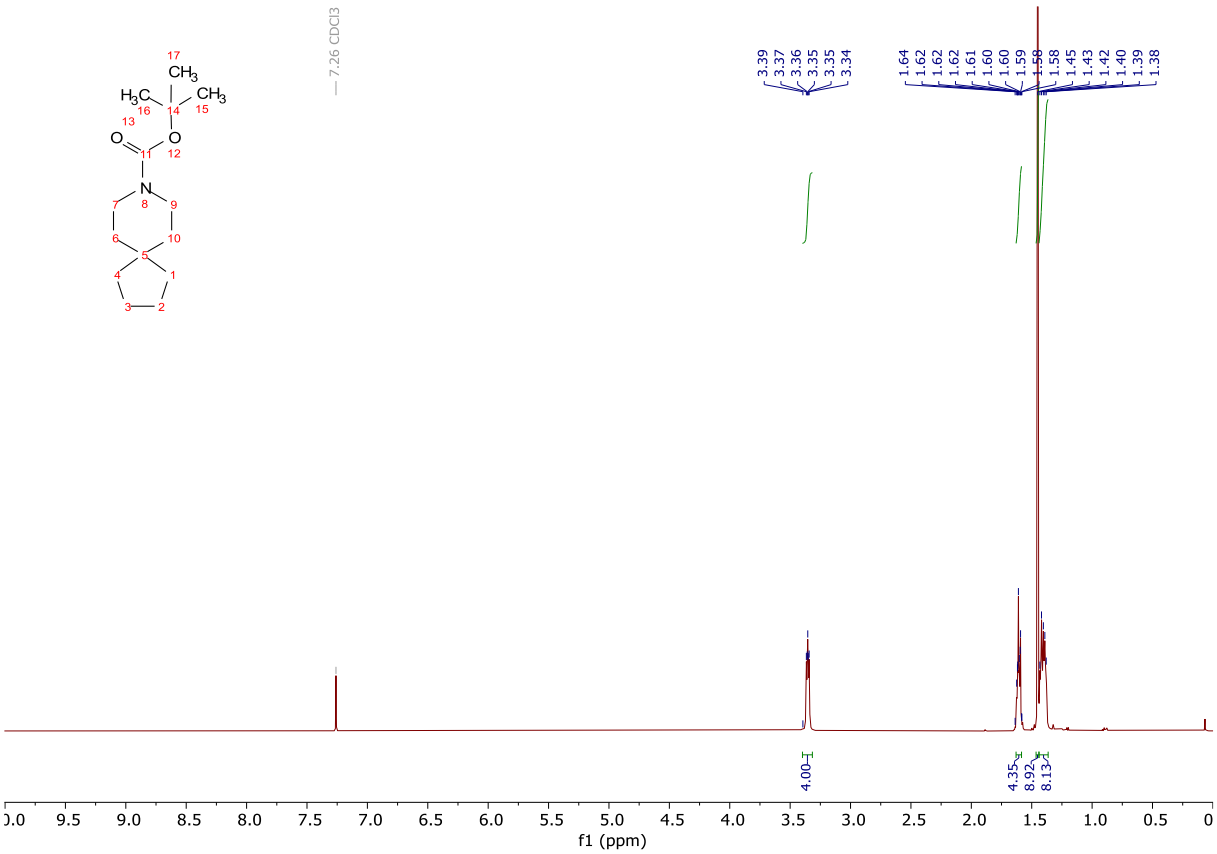
8b - ^{1}H NMR (500 MHz, $CDCl_3$)



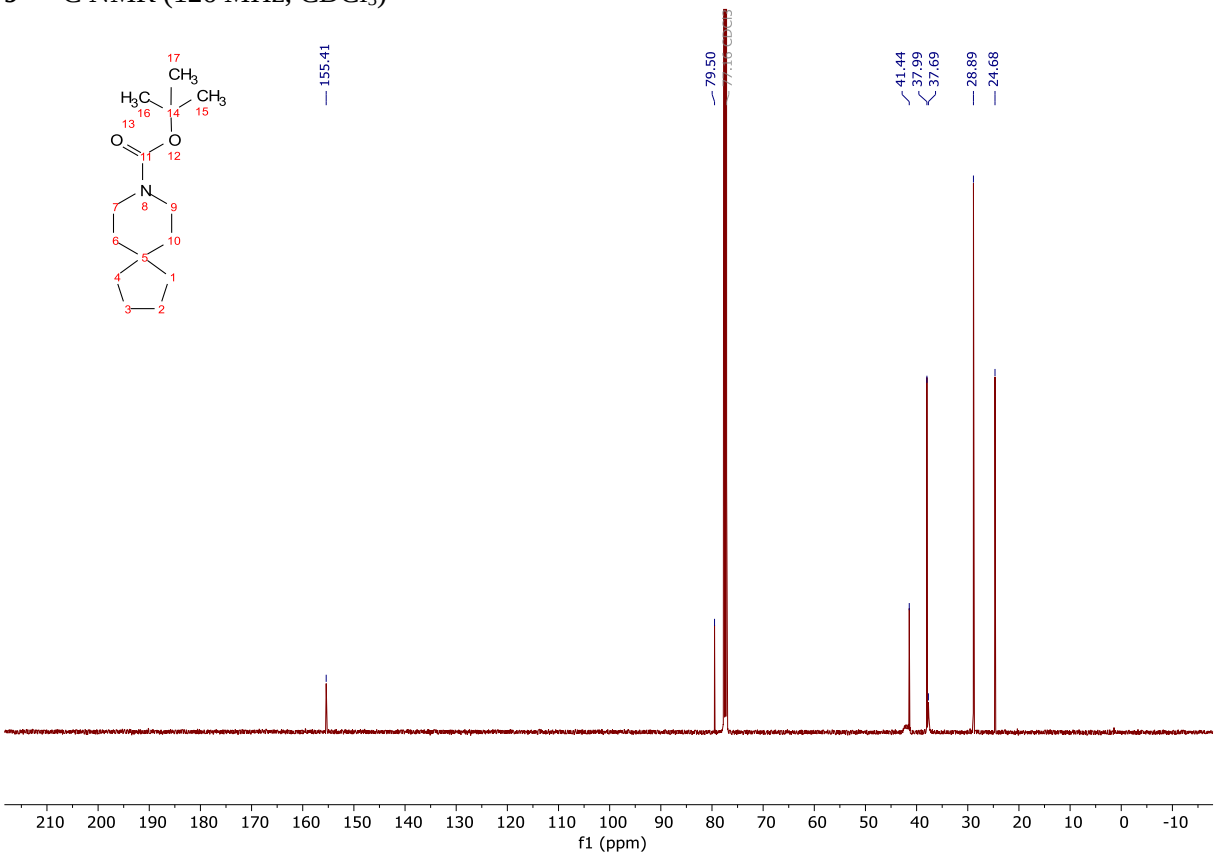
8b - ^{13}C NMR (126 MHz, $CDCl_3$)



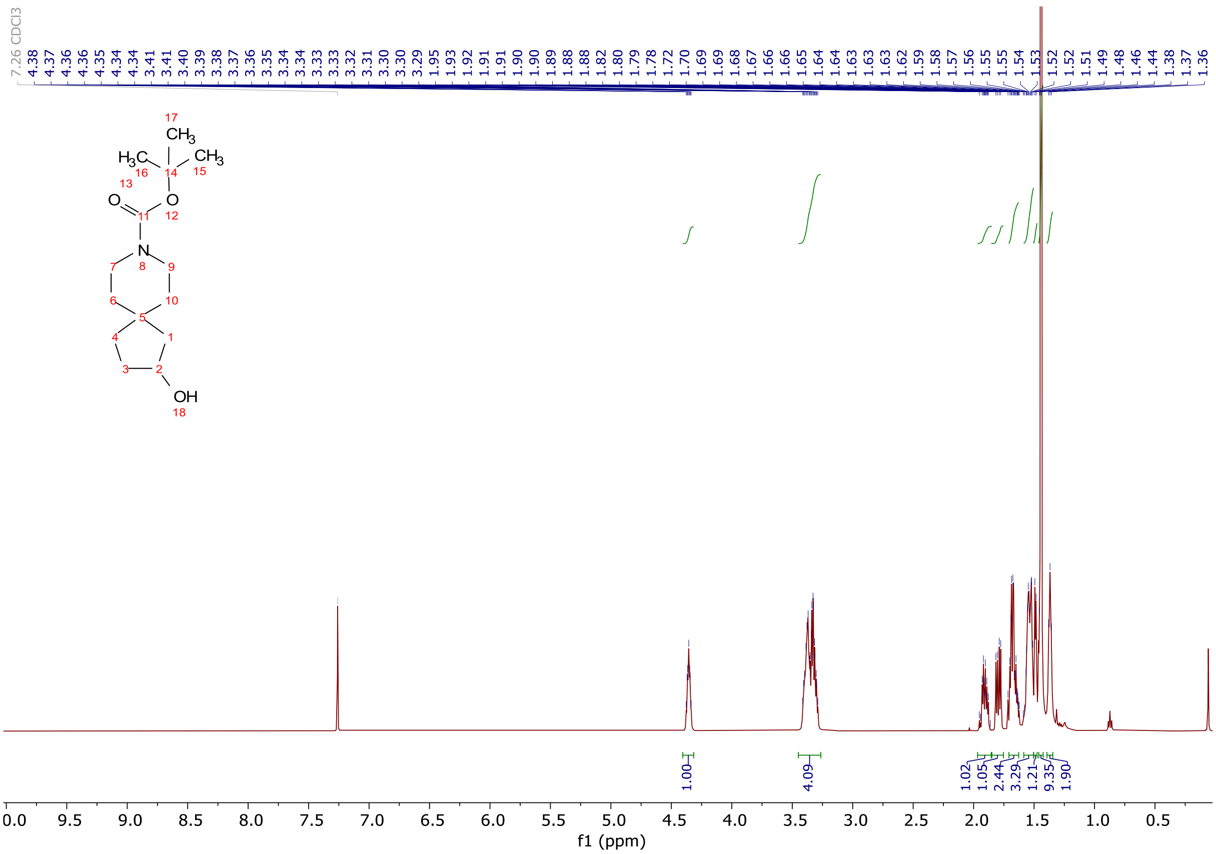
9 - ^{1}H NMR (500 MHz, $CDCl_3$)



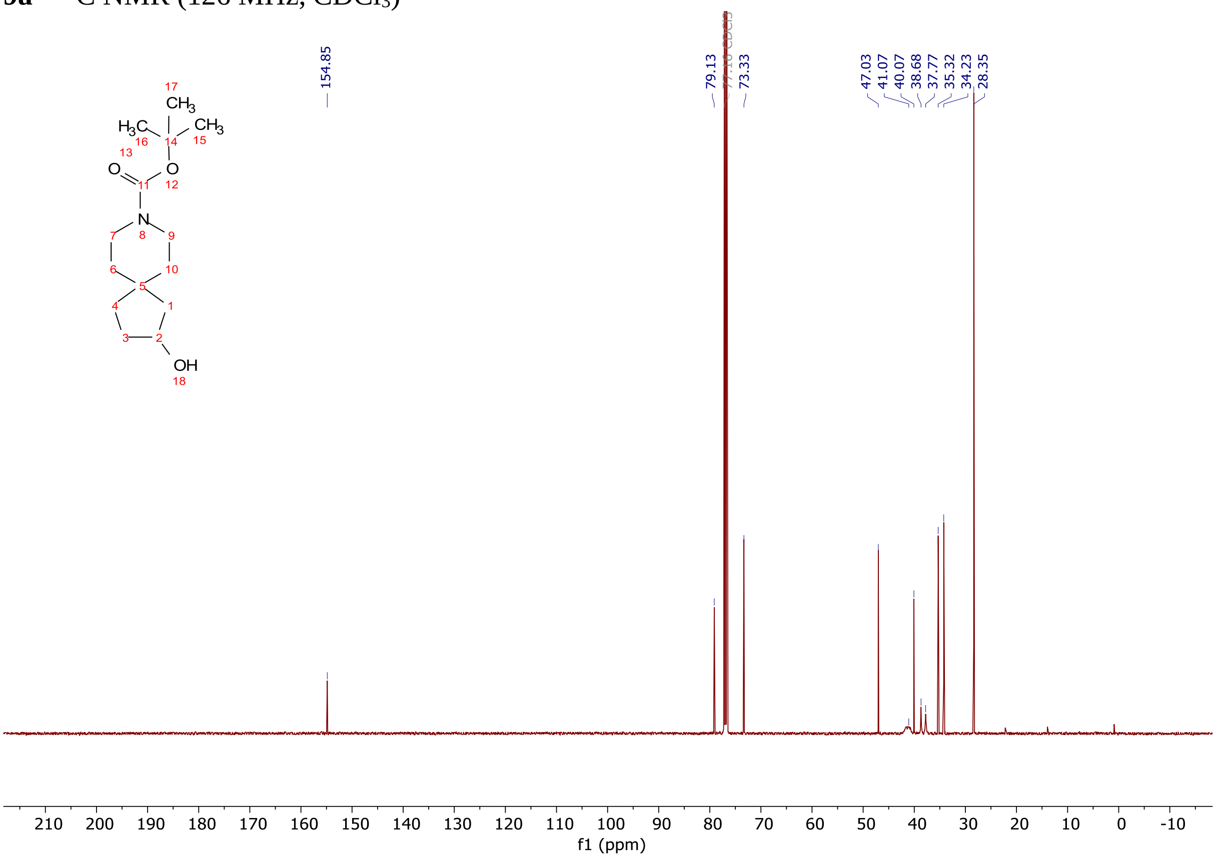
9 - ^{13}C NMR (126 MHz, $CDCl_3$)



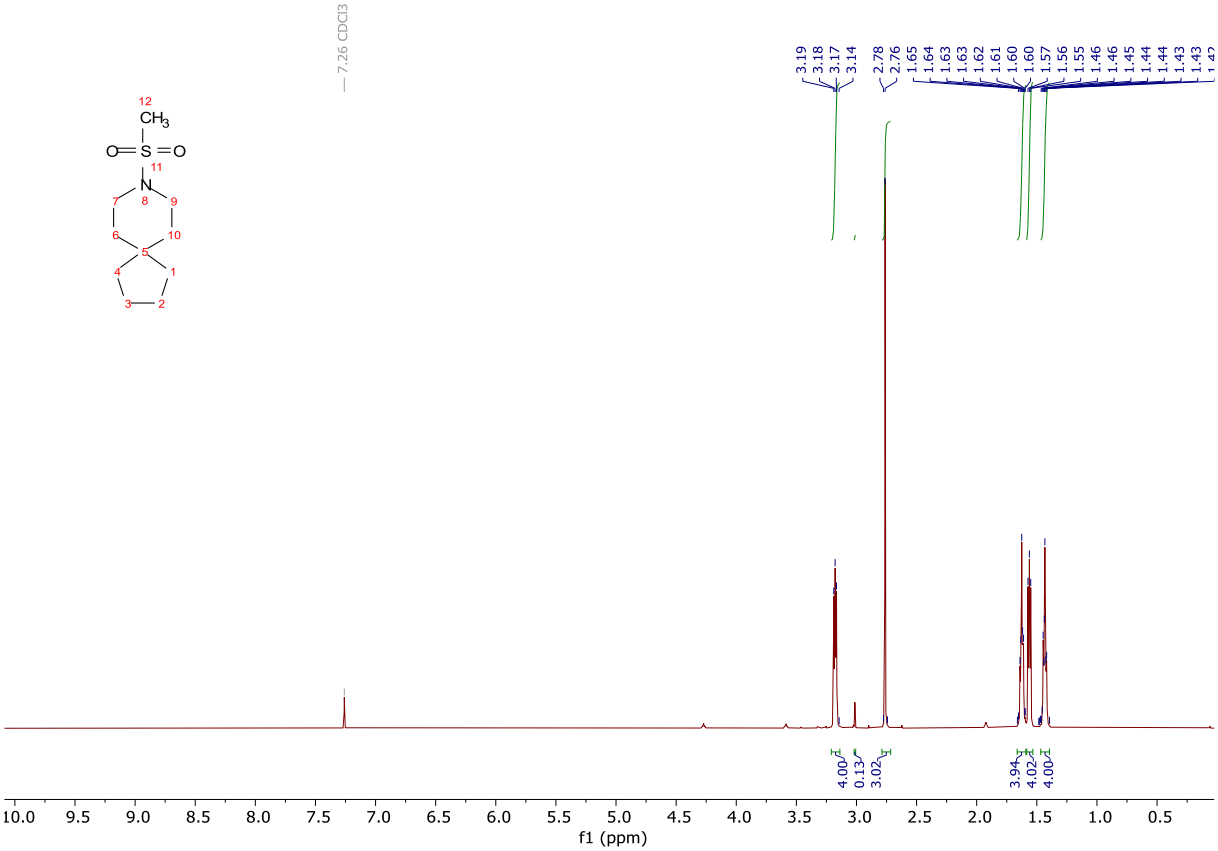
9 - ^{1}H NMR (500 MHz, $CDCl_3$)



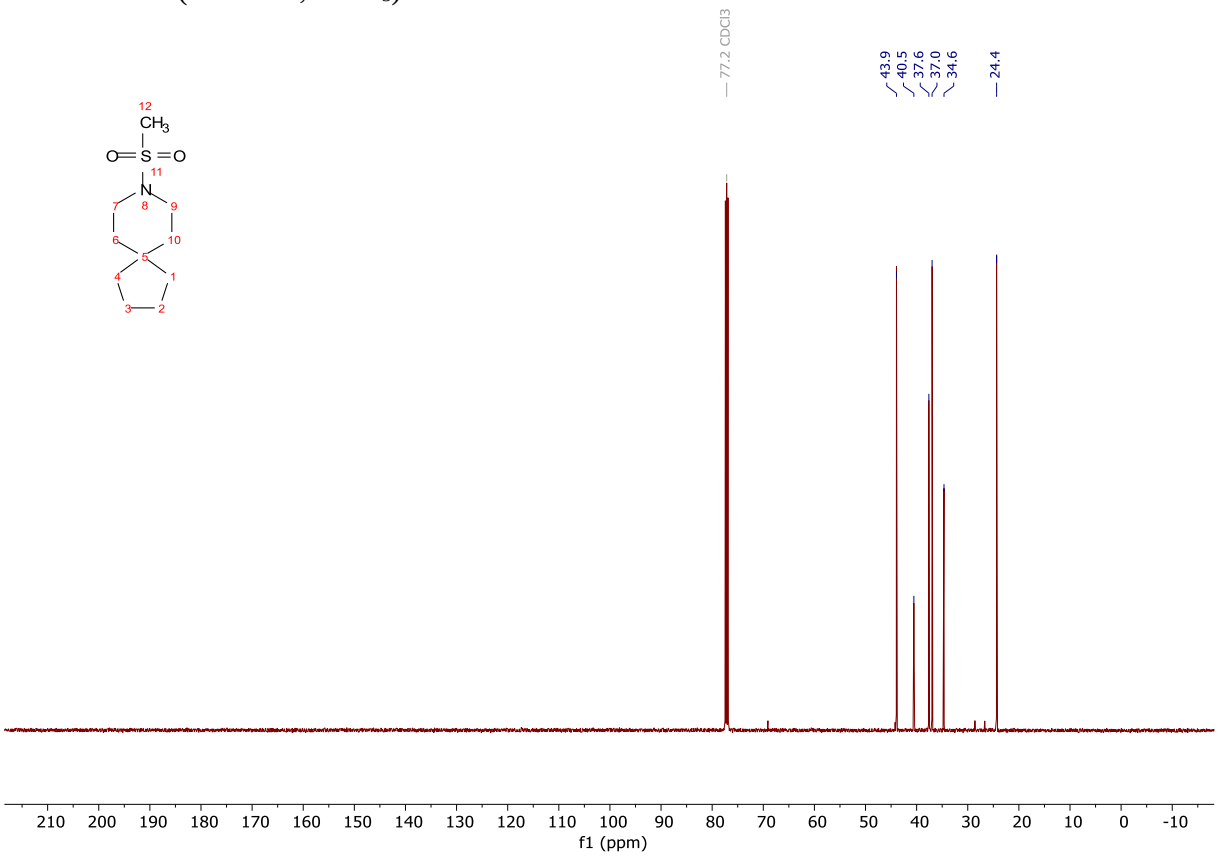
9a - ^{13}C NMR (126 MHz, $CDCl_3$)



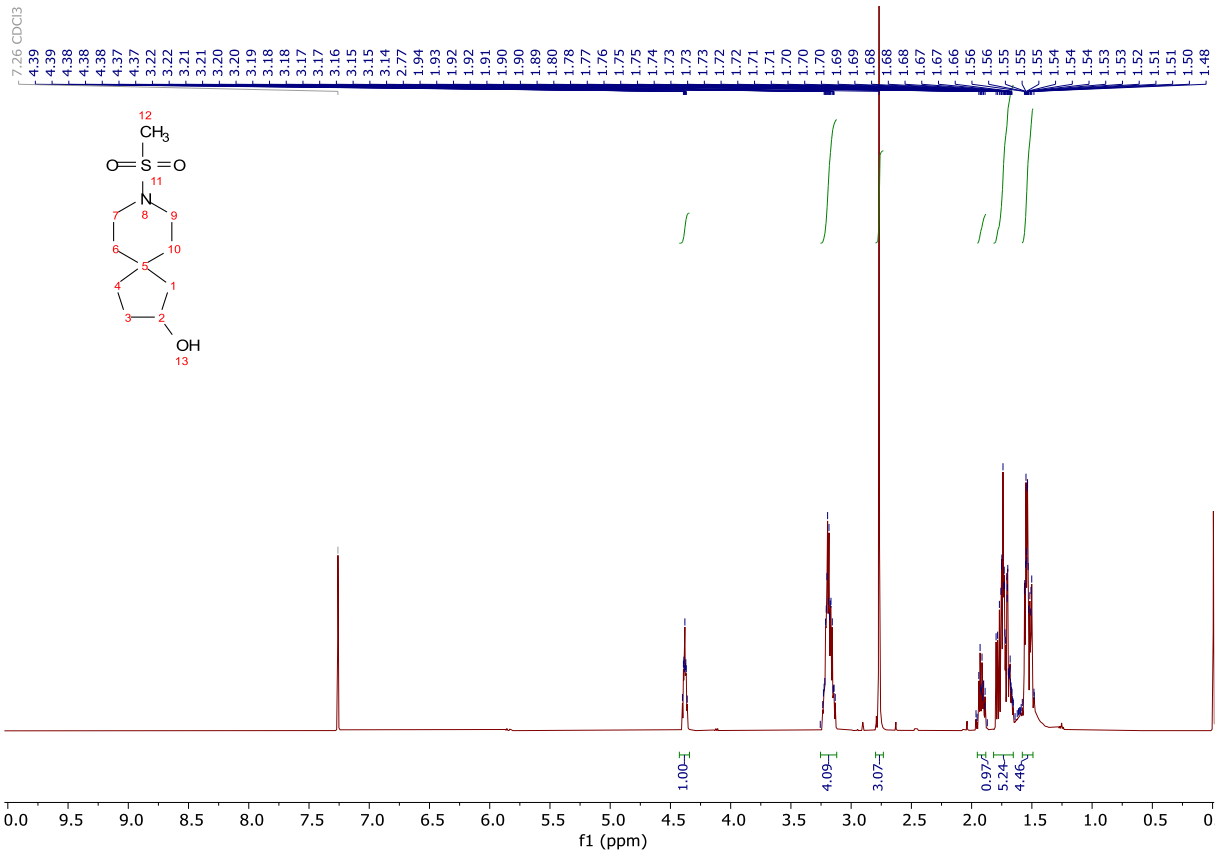
10 - ^{1}H NMR (500 MHz, $CDCl_3$)



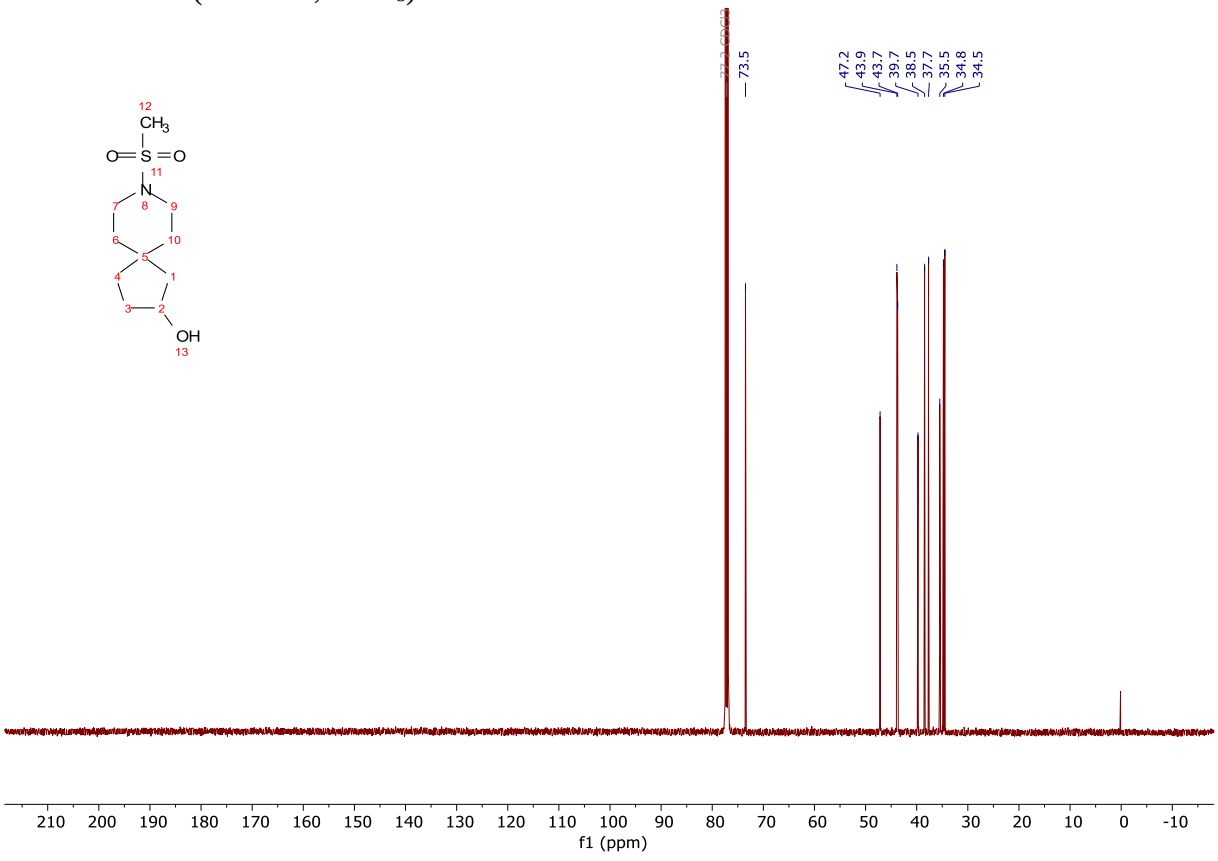
10 - ^{13}C NMR (126 MHz, $CDCl_3$)



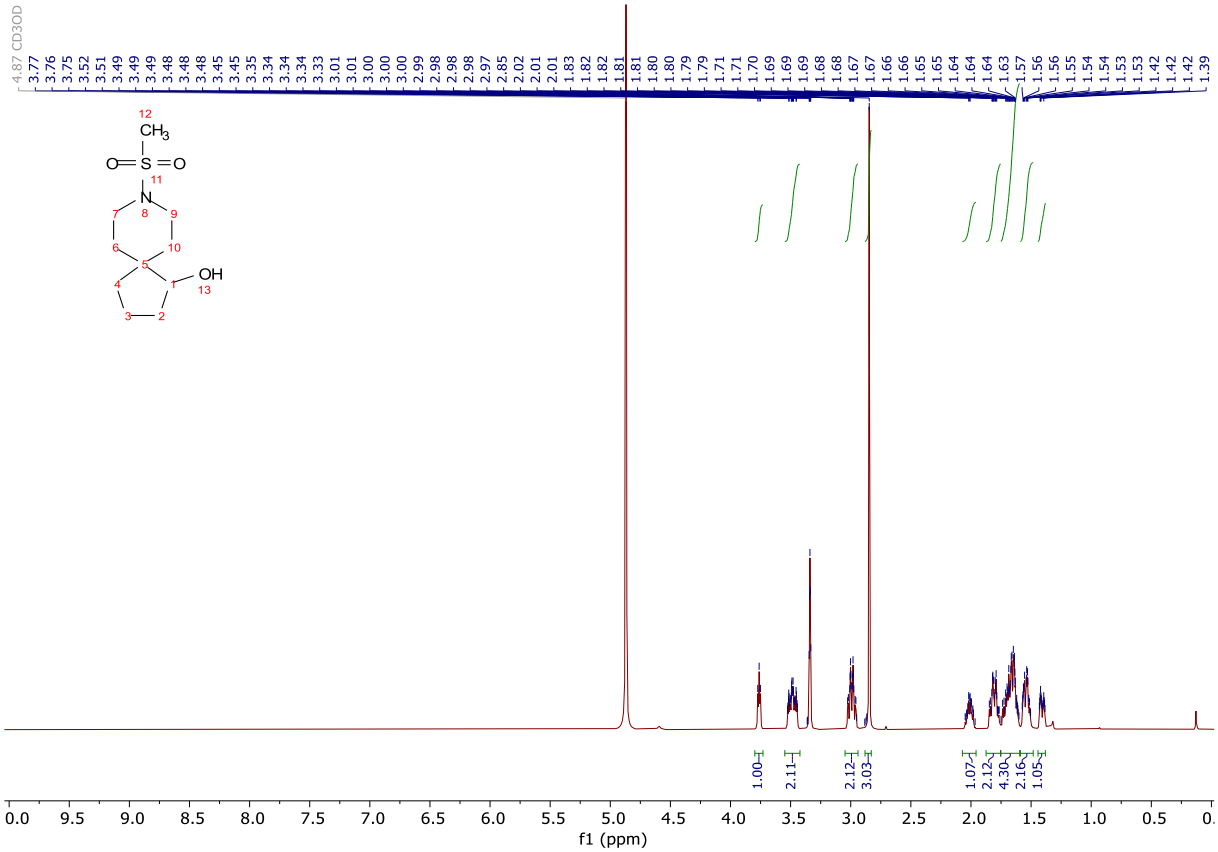
10a - ^{1}H NMR (500 MHz, $CDCl_3$)



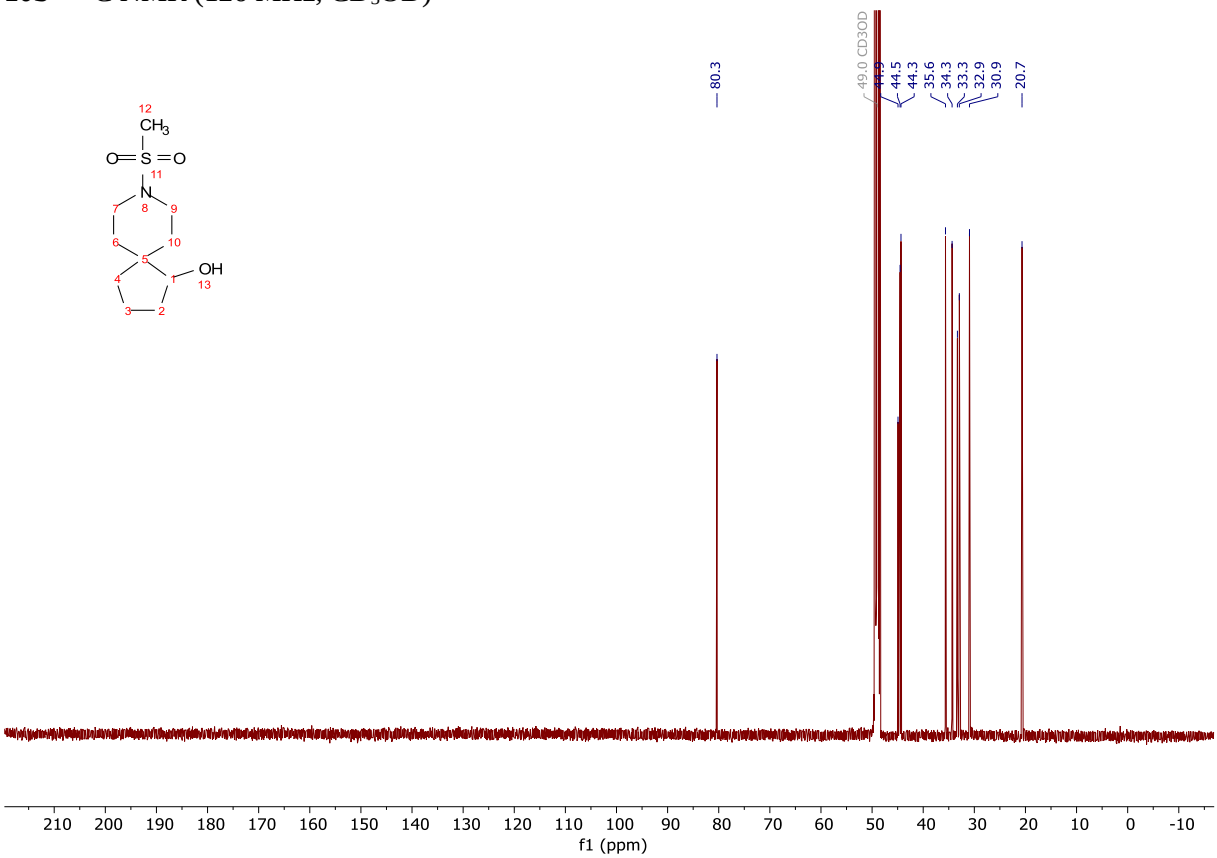
10a - ^{13}C NMR (126 MHz, $CDCl_3$)



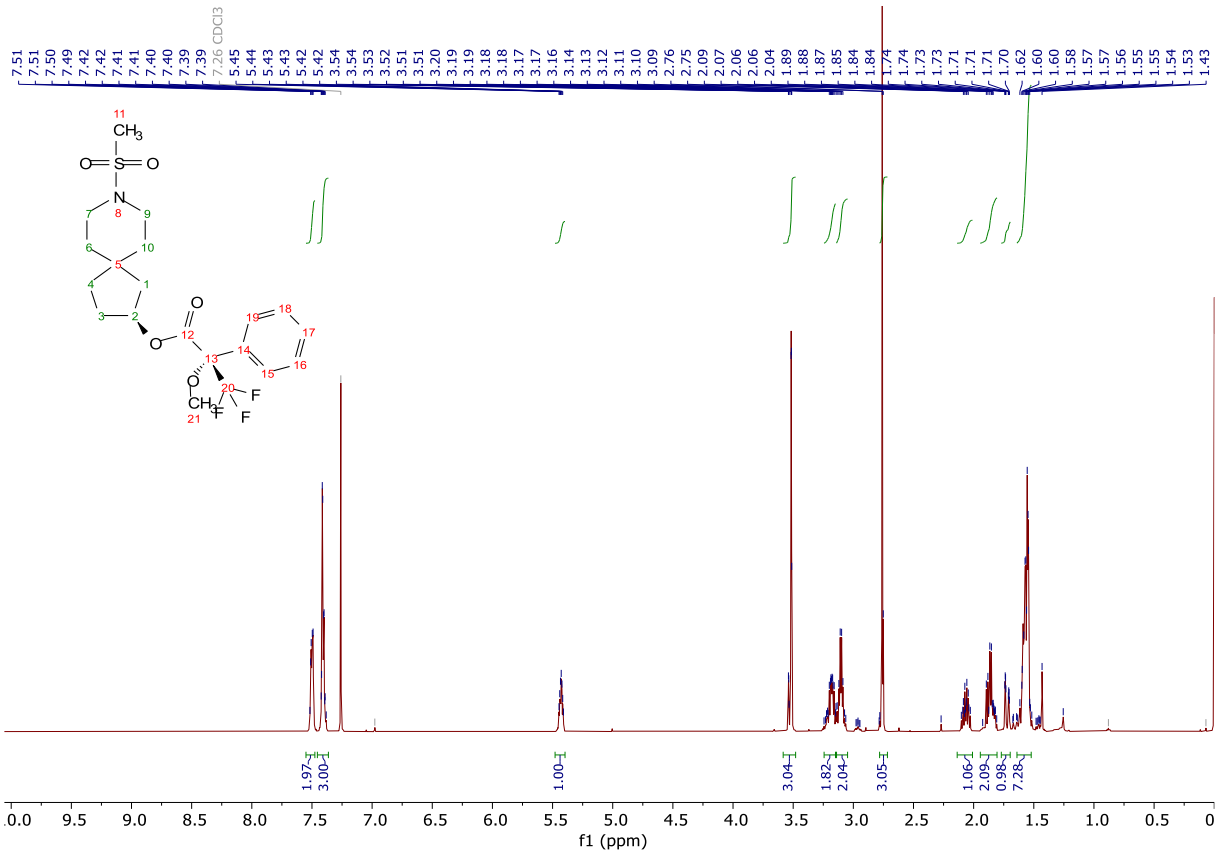
10b - ^{1}H NMR (500 MHz, CD_3OD)



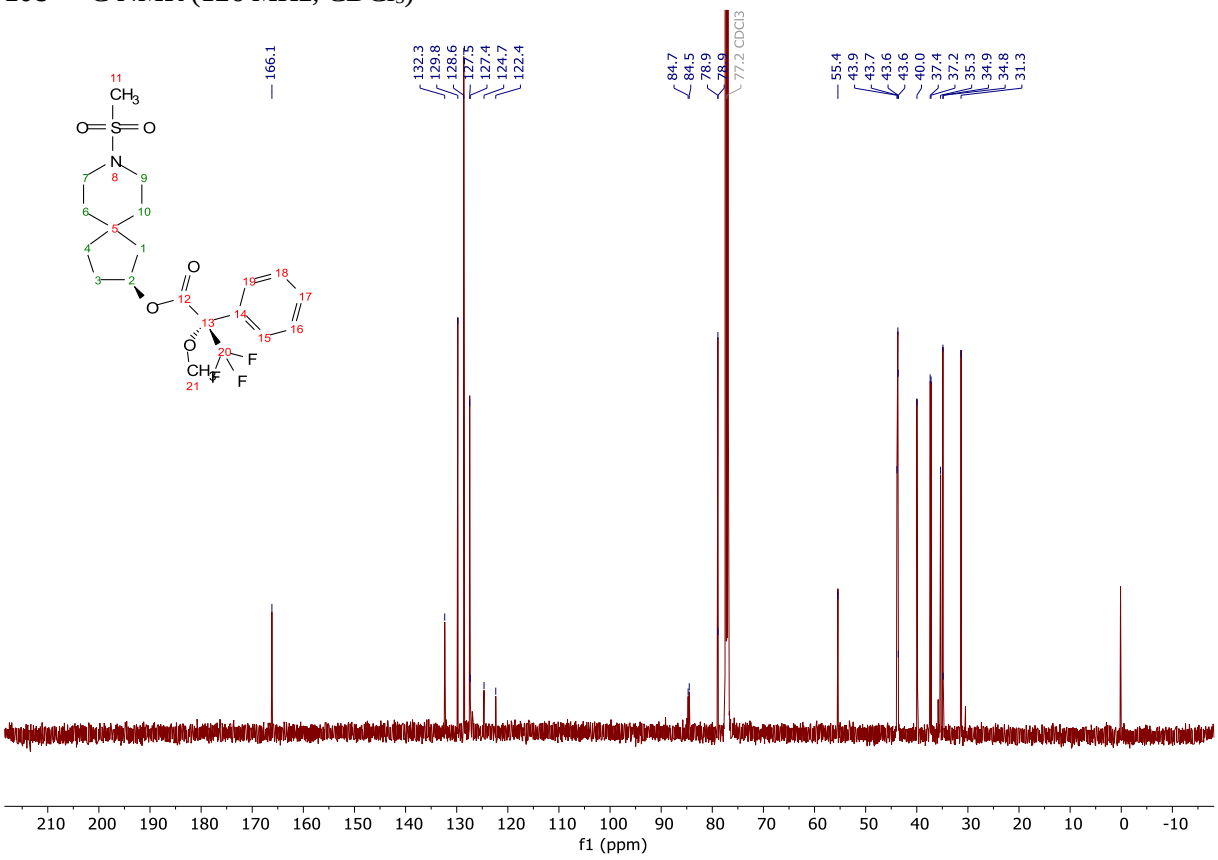
10b - ^{13}C NMR (126 MHz, CD_3OD)



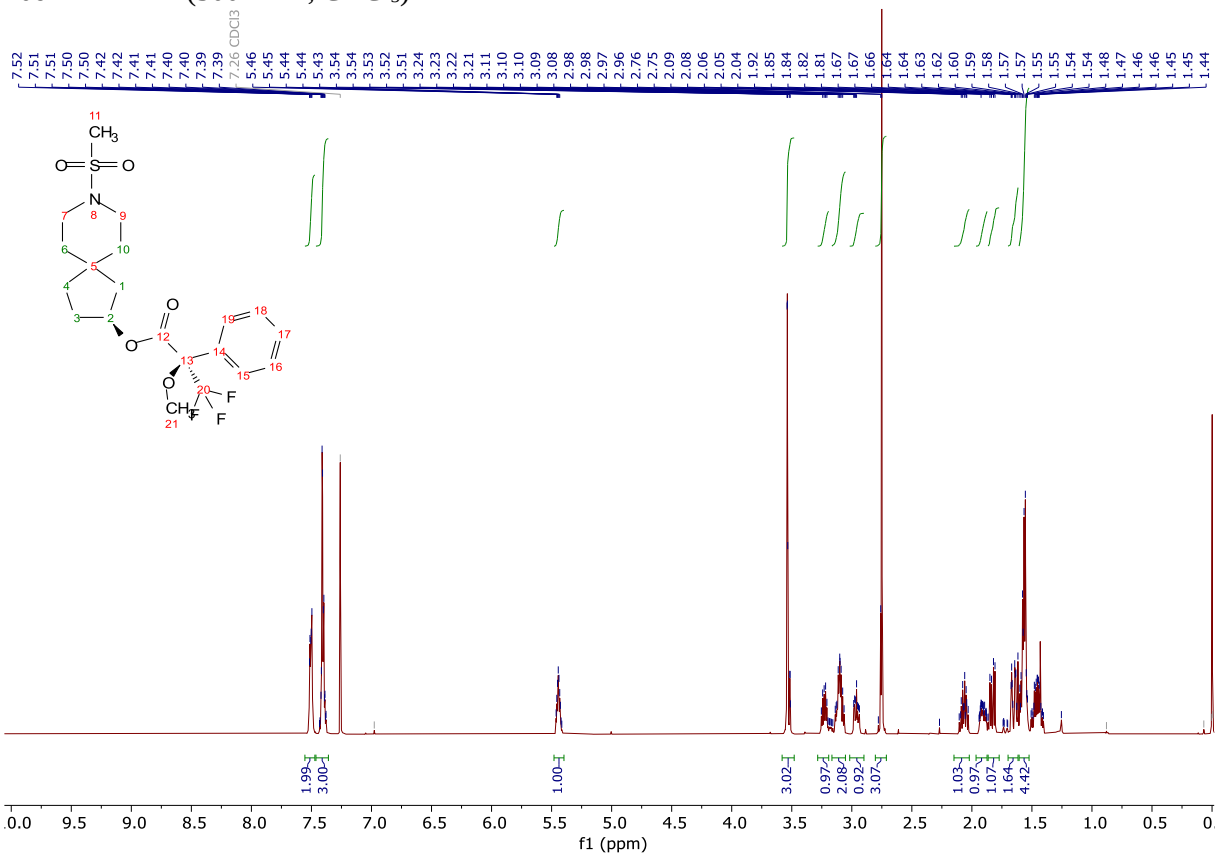
10c - ^{1}H NMR (500 MHz, $CDCl_3$)



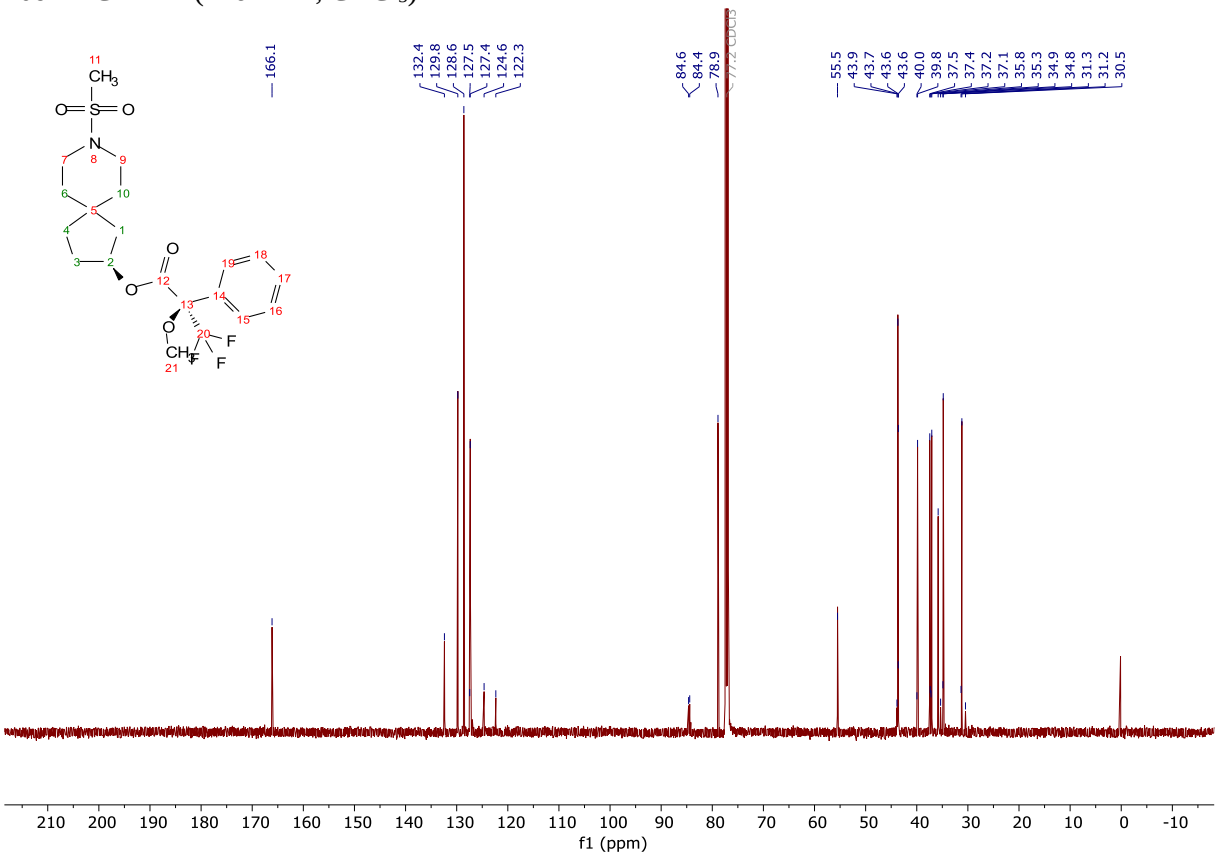
10c - ^{13}C NMR (126 MHz, $CDCl_3$)



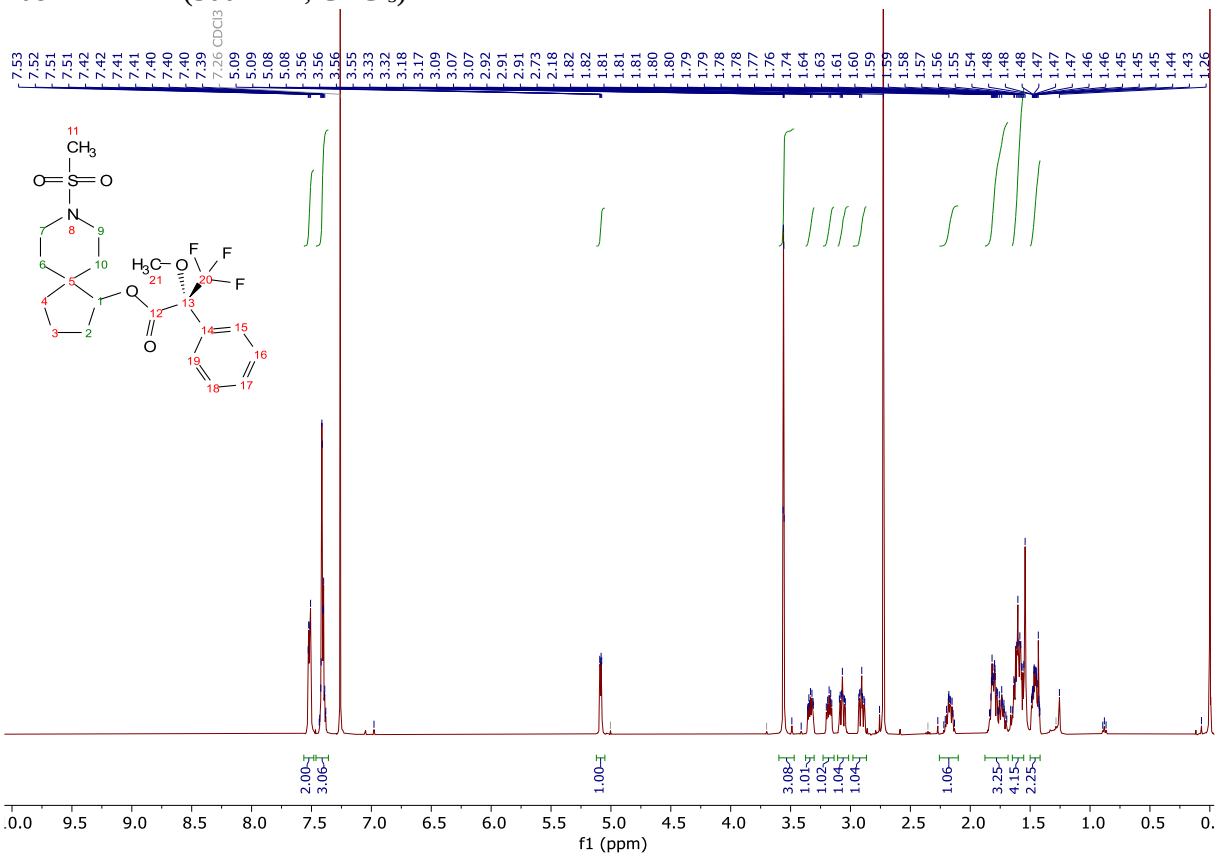
10d - ^{1}H NMR (500 MHz, $CDCl_3$)



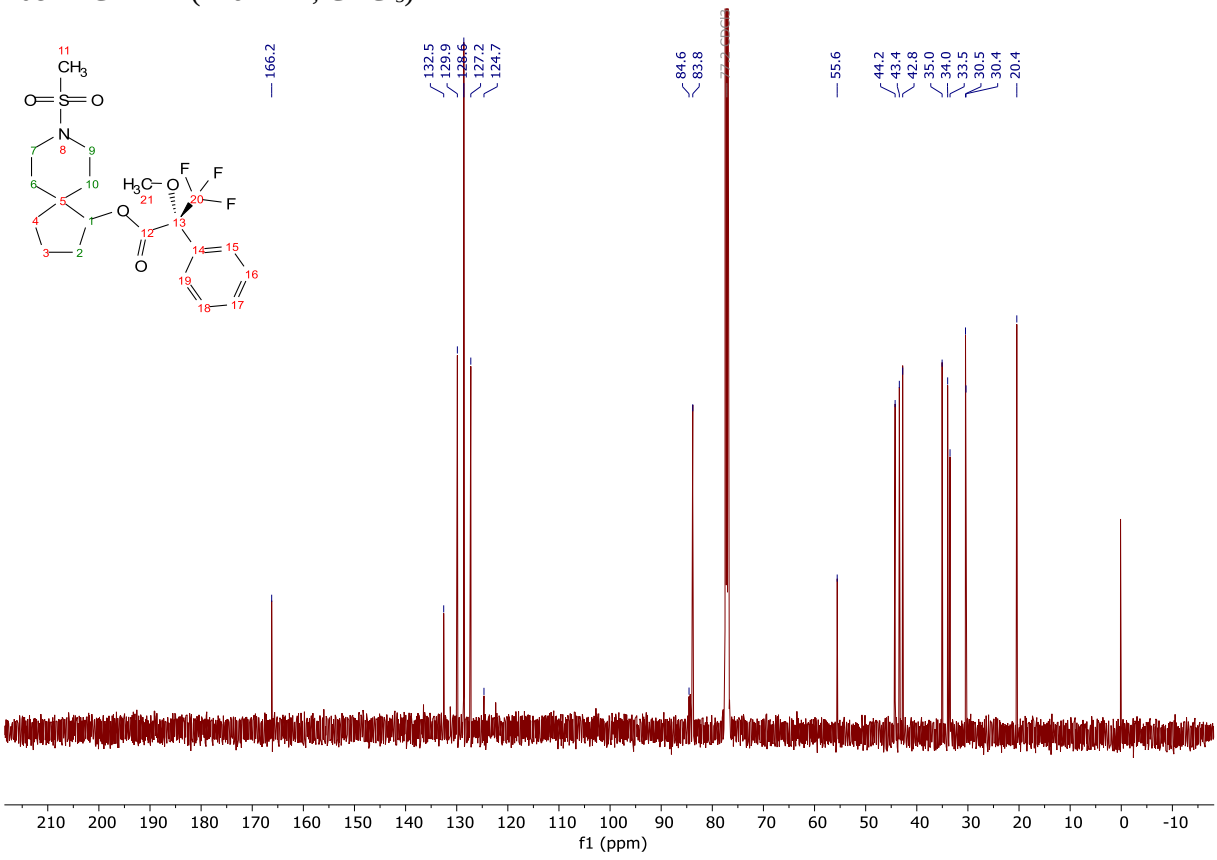
10d - ^{13}C NMR (126 MHz, $CDCl_3$)



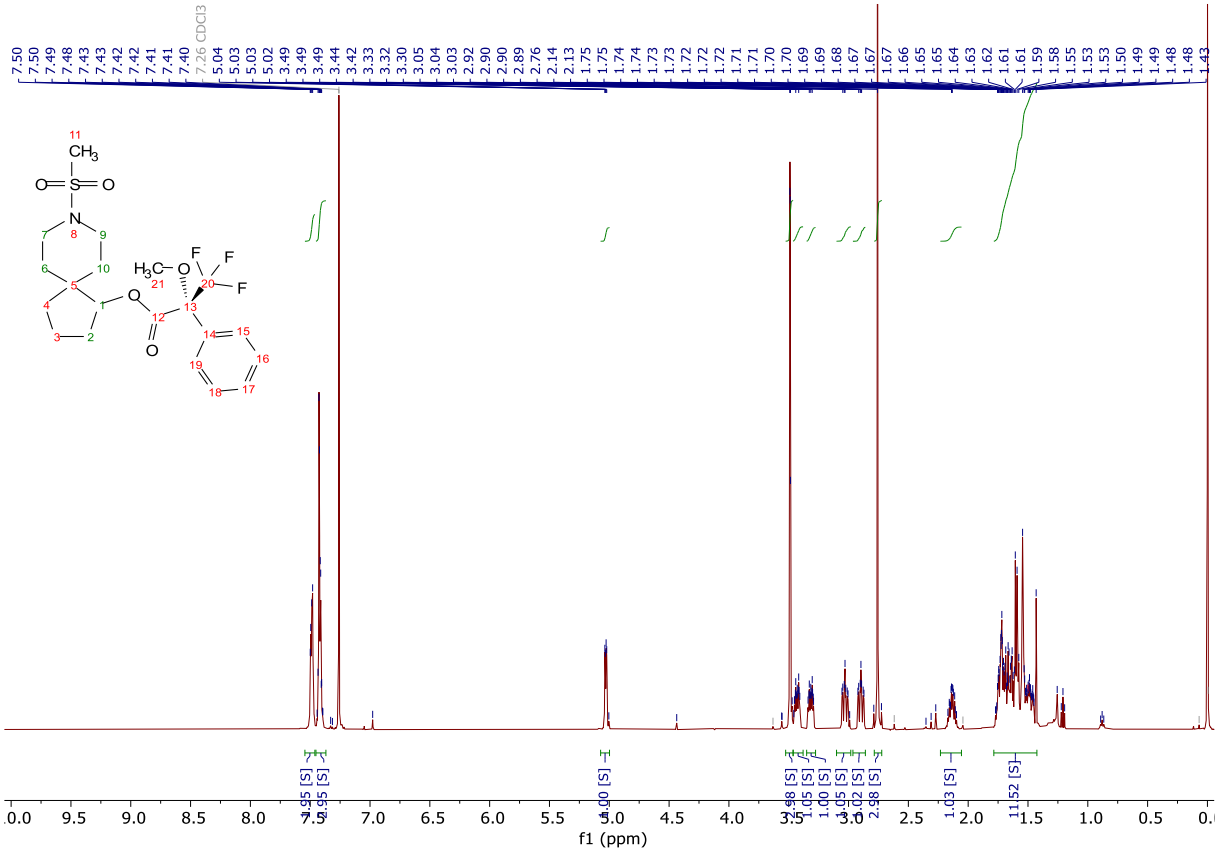
10e - ^{1}H NMR (500 MHz, $CDCl_3$)



10e - ^{13}C NMR (126 MHz, $CDCl_3$)



10f - ^{1}H NMR (500 MHz, $CDCl_3$)



10f - ^{13}C NMR (126 MHz, $CDCl_3$)