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Supporting Information

Harnessing Natural-Product-Inspired Combinatorial Chemistry and Computation-Guided Synthesis to Develop *N*-Glycan Modulators as Anticancer Agents

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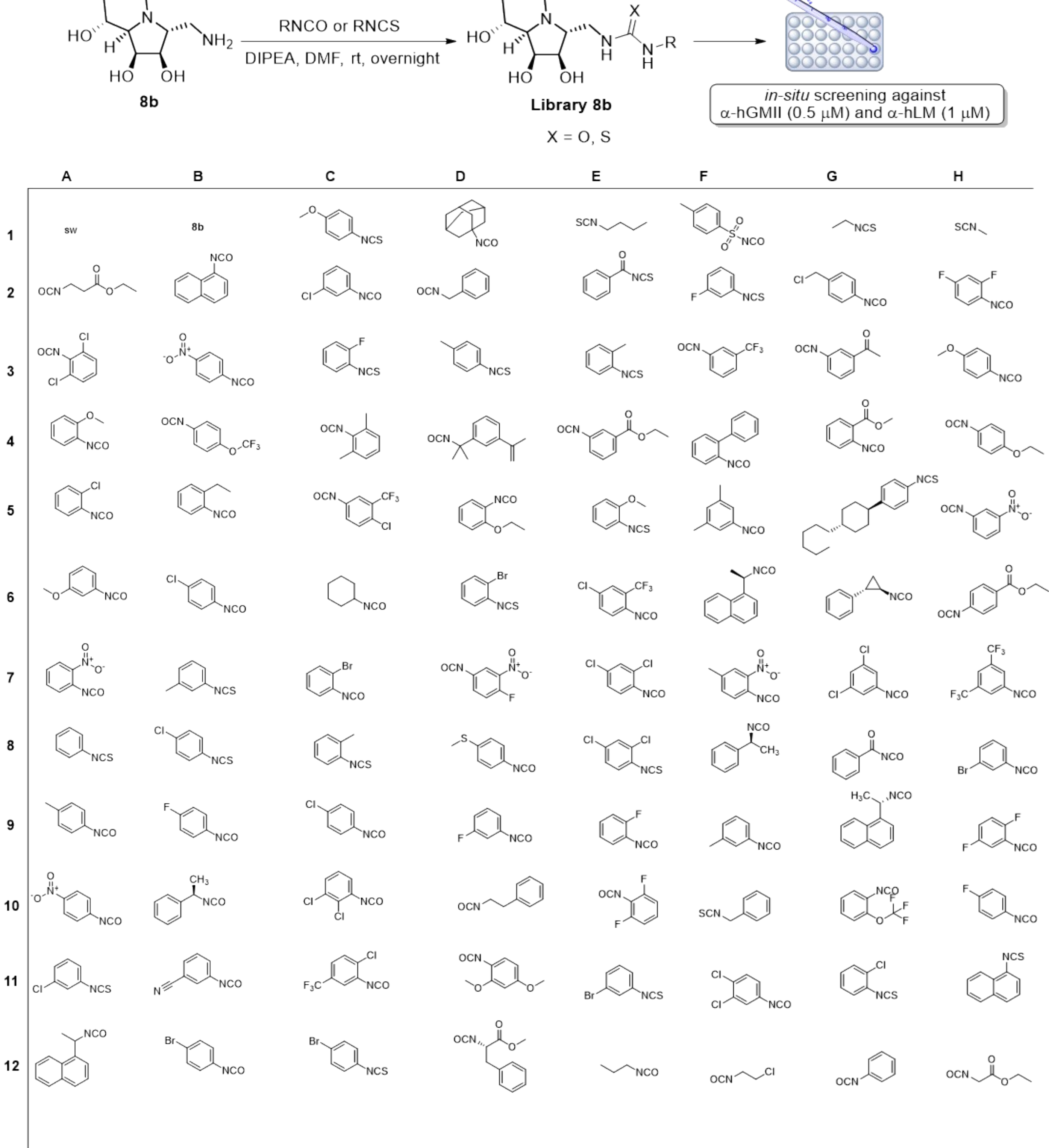
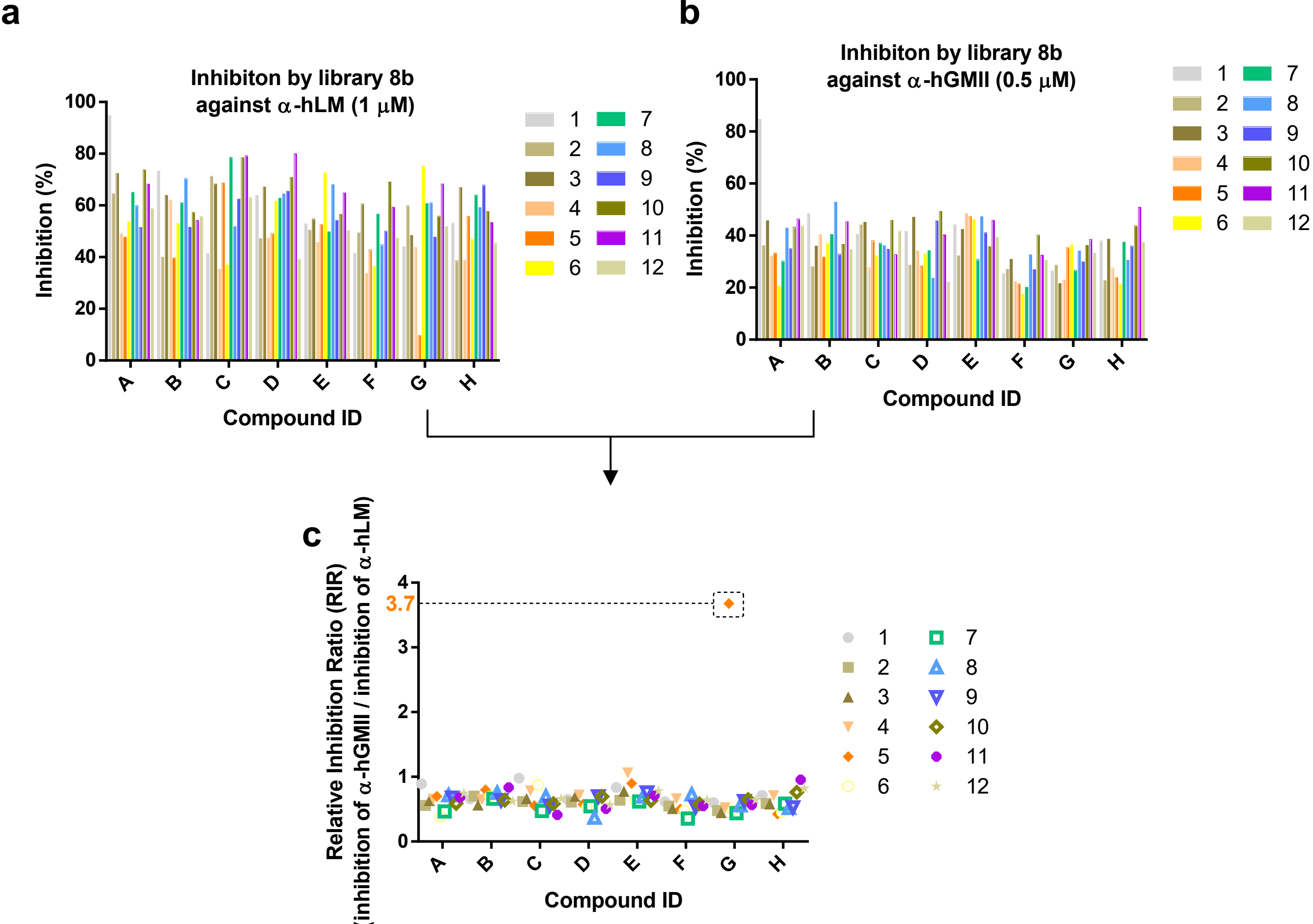
Abbreviation

Vmax: reaction rate when the enzyme is fully saturated by substrate; K_m: concentration of substrate which permits the enzyme to achieve half V_{max}; k_{cat}: turnover number; α-hGMII: human Golgi α-mannosidase II; α-hLM: human lysosomal α-mannosidase; IC_{50}: half-maximal inhibitory concentration; K_i: inhibition constant; H_2O: water; DMSO: dimethyl sulfoxide; 4MU-α-Man: 4-methylumbelliferyl-α-D-mannopyranoside; DMF: dimethylformamide; DIPEA: diisopropylamine; TLC: thin layer chromatography; MS: Mass; UPLC: ultra-performance liquid chromatography; 2AB: 2-Aminobenzamide; Man: mannose; GlcNAc: N-acetylglucosamine; Gal: galactose; $NaBH_3CN$: sodium cyanoborohydride; HILIC: hydrophilic interaction liquid chromatography; LNnT-2AB: Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-2AB; GlcNAcMan5-2AB: $GlcNAcMan_5GlcNAc_2$-2AB; Man5-2AB: $Man_5GlcNAc_2$-2AB; MALDI-TOF-MS: matrix assisted laser desorption ionization/time of flight MS; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NaCl: sodium chloride; $MnCl_2$: manganese (II) chloride; UDP-GlcNAc: Uridine diphosphate N-acetylglucosamine; GnT-1: 2-β-N-acetylglucosaminyltransferase; NA2-2AB: $(Gal\text{-}GlcNAc)_2Man_3(GlcNAc)_2$-2AB; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; CO_2: carbon dioxide; PBS: phosphate buffered saline; PGC: porous graphite carbon; FA: formic acid; LC-MS: liquid chromatography–mass spectrometry; ESI: electrospray ionization; Tris: tris(hydroxymethyl)aminomethane; HCl: hydrogen chloride; DTT: dithiothreitol; IAA iodoacetamide; Peptide-N-Glycosidase F: PNGase F; FITC: fluorescein; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PFA: paraformaldehyde; ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: blood urea nitrogen, CRE: creatinine; TBIL: total bilirubin.

Supplementary Figure 1. Attempted synthesis of **8a** from **4a** using S_N2 type cyclization.



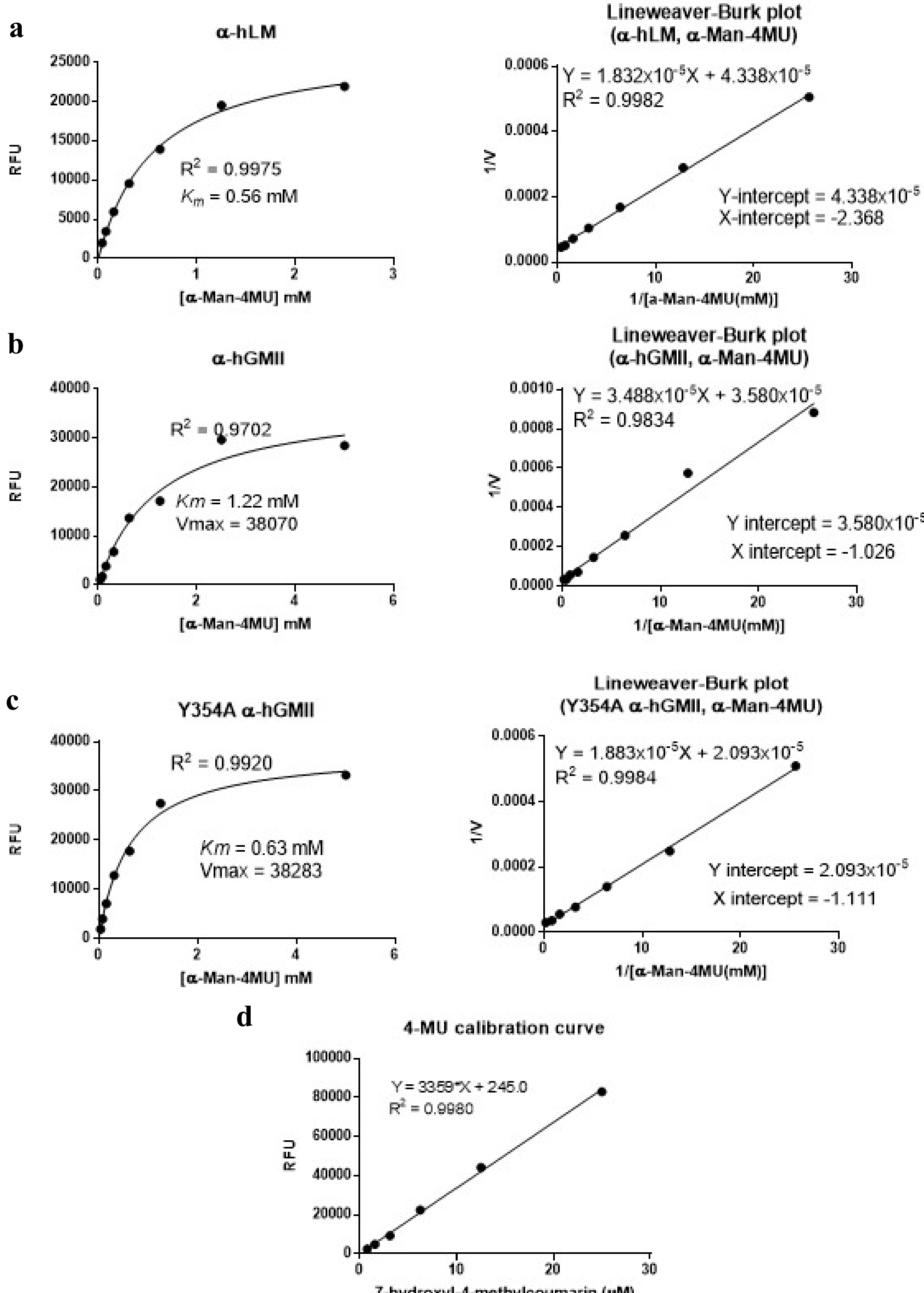
Supplementary Figure 2. Preparation of library 8b (L-8b) for *in-situ* screening and iso(thio)cyanate libraries for diversification. (sw = swainsonine)



Supplementary Figure 3. Inhibition by **L-8b** of (**a**) α-hLM (1 μM) and (**b**) α-hGMII (0.5 μM). (**c**) Screening results by comparing inhibition ratio of each compound in library **8b** and molecule 5G (**8b-1**) was found with highest inhibition ratio. Relative inhibition ratio in Supplementary Fig 3c = inhibition percentage of **L-8b** against α-hGMII in Supplementary Fig. 3b / inhibition percentage of **L-8b** against α-hLM in Supplementary Fig. 3a).

Enzyme kinetic study

The K_m and k_{cat} of α-Man-4MU toward α-hLM is about 0.56 mM and 3.41×10^{-3} (1/s), respectively (Supplementary Fig. 4a). The K_m and k_{cat} of α-Man-4MU toward α-hGMII is about 1.22 mM and 2.85×10^{-5} (1/s), respectively (Supplementary Fig. 4b). The K_m and k_{cat} of α-Man-4MU toward Y354A α-hGMII is about 0.63 mM and 1×10^{-4} (1/s), respectively (Supplementary Fig. 4c).



Supplementary Figure 4. Enzyme kinetic studies of (a) α-hLM, (b) α-hGMII and (c) Y354A α-hGMII using 4-Methylumbelliferyl α-D-mannopyranoside (α-Man-4MU) as the substrate. (d) 4MU calibration curve.

Inhibition constant (K_i) of tested compounds.

The compounds sw, **8b, 8b-1, 8b-2, 8b-3,** and **8b-4** were used as inhibitors and their corresponding K_i values were measured against α-hLM, α-hGMII, or Y354A α-hGMII.

Supplementary Figure 5. Lineweaver–Burk plot for K_i determination of inhibitor sw, **8b**, **8b-1**, **8b-2**, **8b-3** and **8b-4** against (**a**) α-hLM (**b**) α-hGMII and (c) Y354A α-hGMII.

Study of sequence similarity (homology) and 3D molecular structures of α-hLM and α-hGMII

Up to date, no structure of human lysosomal α-mannosidase (α-hLM) and human Golgi α-mannosidase II (α-hGMII) is available. In contrast, the crystal structures of bovine lysosomal α-mannosidase (α-bLM) (PDB coded 1O7D) and drosophila Golgi α-mannosidase II (α-dGMII) (PDB coded 3DDF) are accessible. Thus, the accessible crystal structure as the 3D template can be applied to build the target structure of the desired enzyme if two proteins possess more than 30% identical residues over their entire sequence lengths. In this study. the conventional homology approach (BLAST) was performed, and the results showed that (i) the sequence of α-hGMII is approximately 40% homologous to the sequence of α-dGMII (Supplementary Fig. 6a); (ii) the sequence of α-hLM is approximately 80% homologous to the sequence of α-bLM (Supplementary Fig. 6b). In addition, in the 3D molecular structures, both α-dGMII and α-hGMII are structurally identical in the region having radius of 6 Å around the central Zn^{2+} (Supplementary Fig. 7a), so are α-bLM and α-hLM (Supplementary Fig. 7b). Therefore, both α-dGMII and α-bLM, which display high sequence identity with their corresponding human enzymes, are qualified as template models.

a

Score	Expect	Method	Identities	Positives	Gaps
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b

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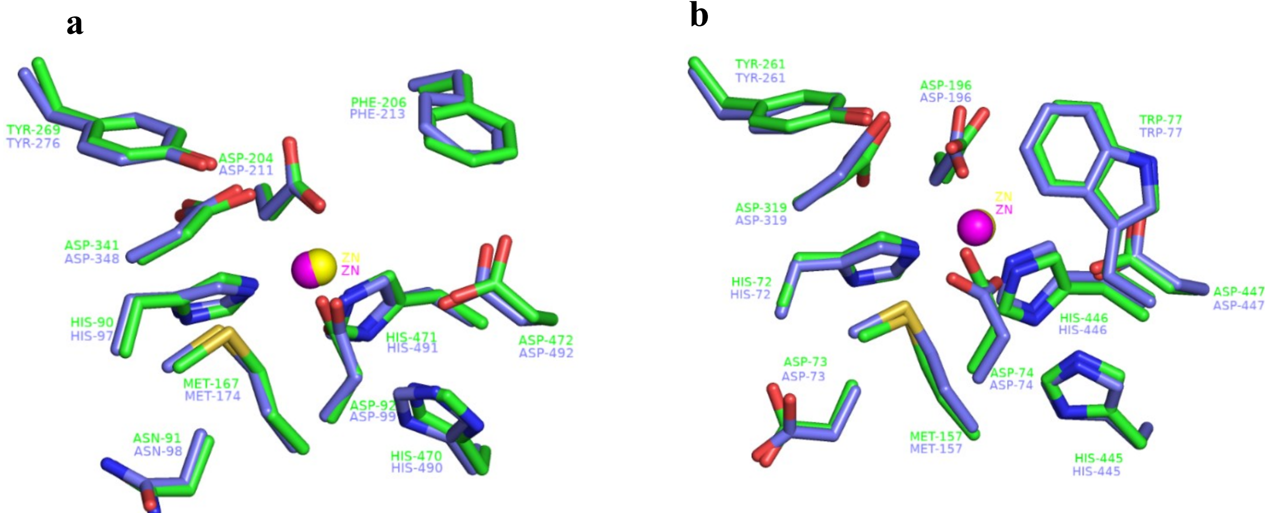
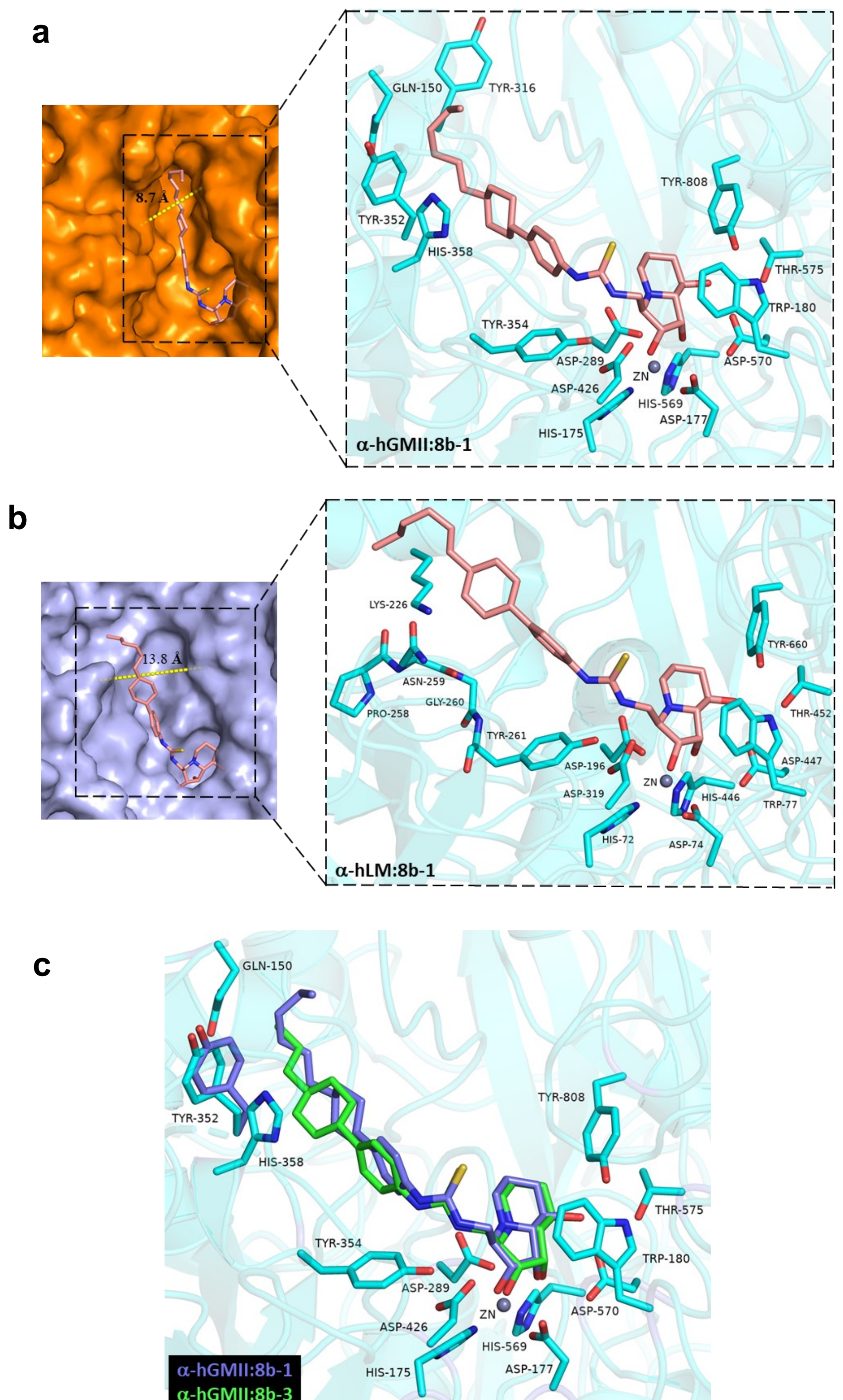
```

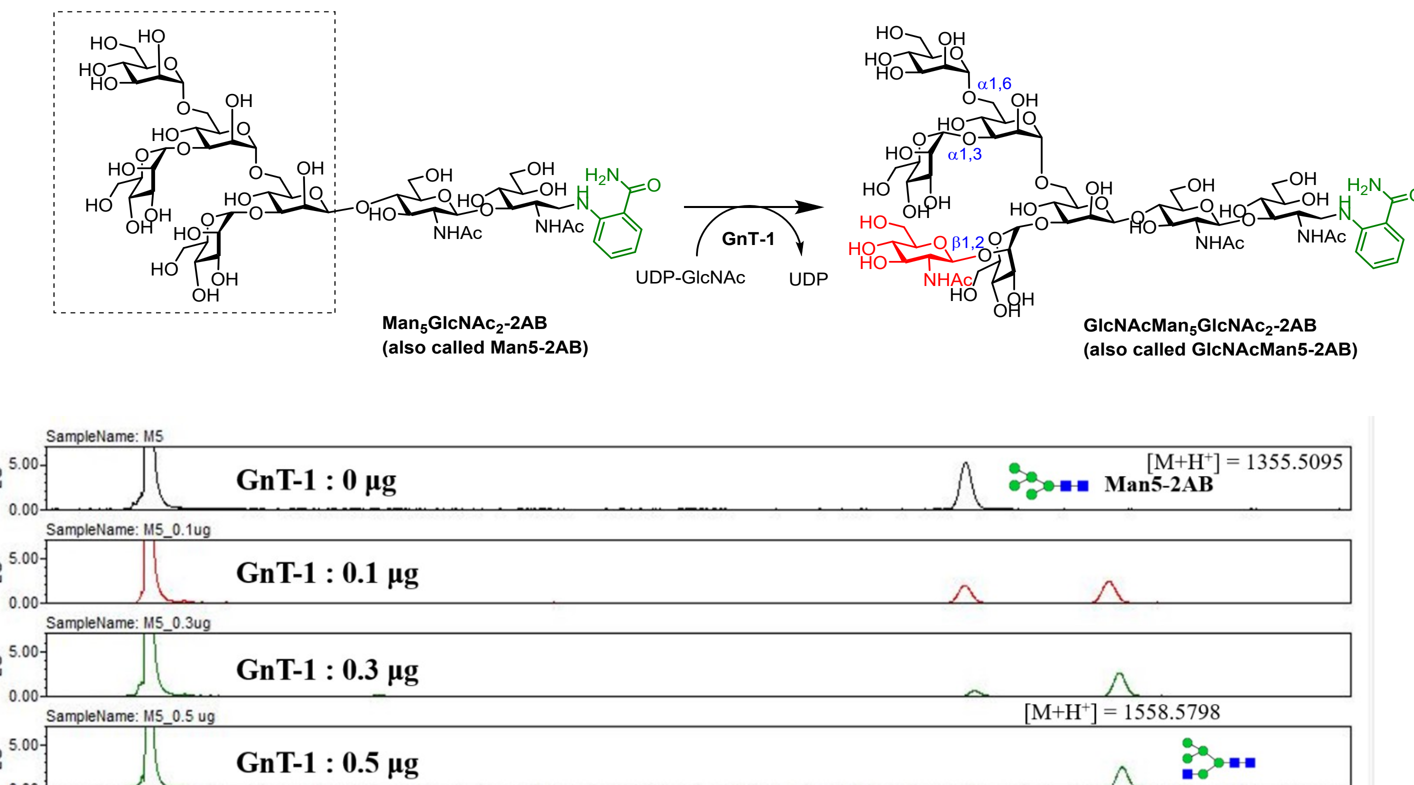
Supplementary Figure 6. Alignment of protein sequence of (**a**) drosophila Golgi α-mannosidase II (Query) and human Golgi α-mannosidase II (Sbjct) and (**b**) bovine lysosomal α-mannosidase (Query) and human lysosomal α-mannosidase (Sbjct) using BLAST.



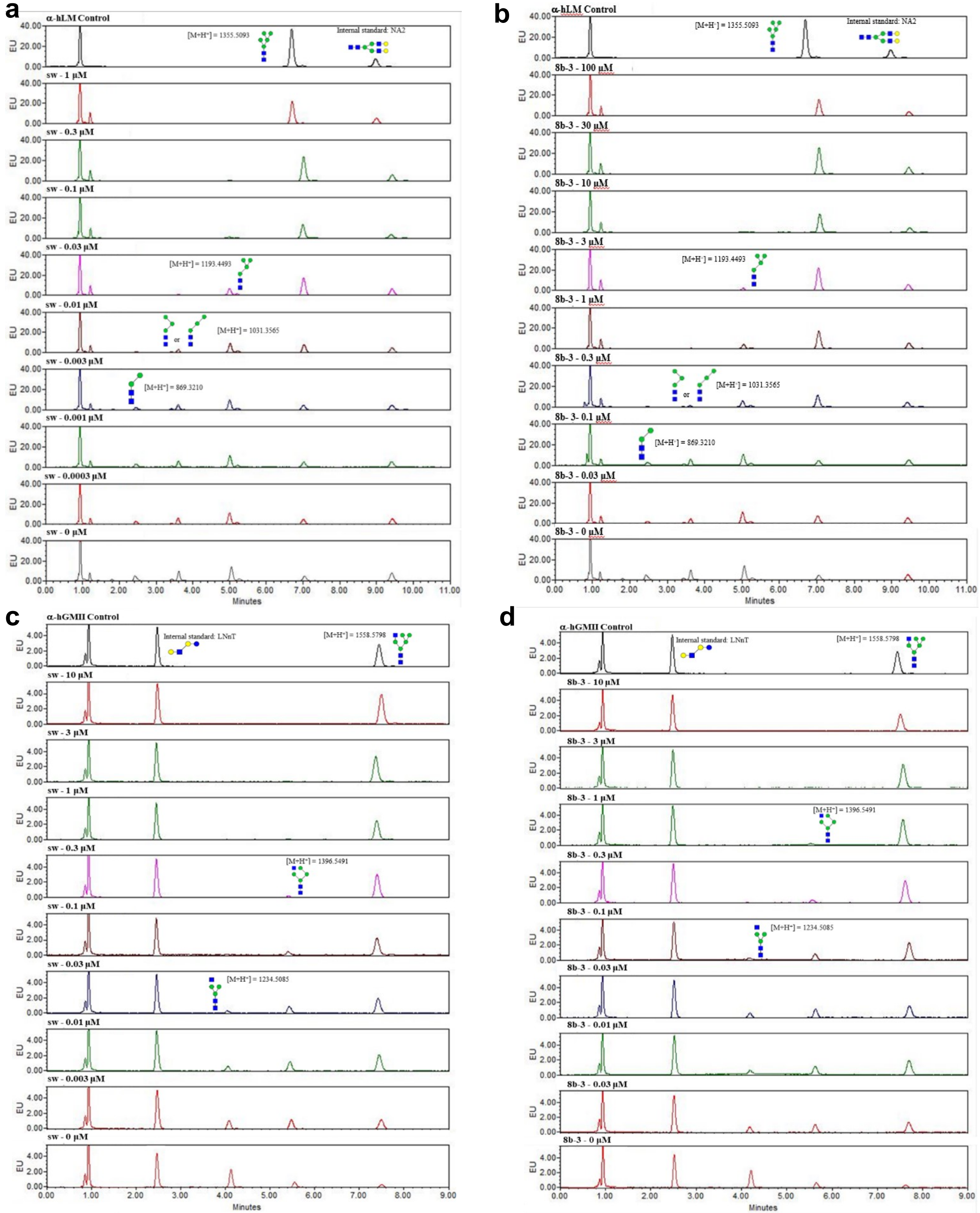
Supplementary Figure 7. Superimposed structures of (**a**) α-dGMII (3DDF) and homology model of α-hGMII and (**b**) α-bLM (1O7D) and homology model of α-hLM in an area of 6 Å around the central Zn^{2+}. Carbon atoms of 3DDF and 1O7D are colored in green. Carbon atoms of α-hGMII and α-hLM are colored in magentas



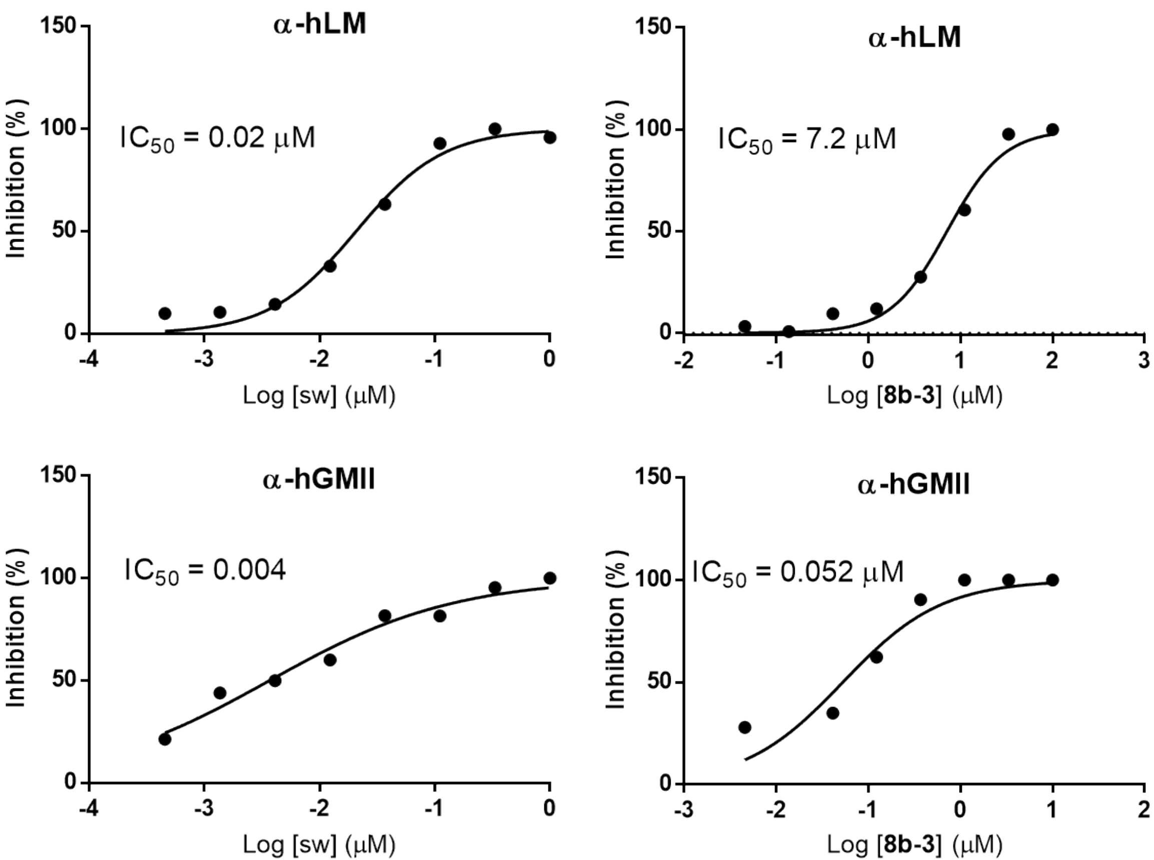
Supplementary Figure 8. Molecular modeling of the complex between (**a**) α-hGMII and **8b-1** and (**b**) α-hLM and **8b-1**. (**c**) The binding of **8b-1** (*violet*) is compared with **8b-3** (*yellow*) to α-hGMII.



Supplementary Figure 9. Enzymatic synthesis of GlcNAcMan$_5$GlcNAc$_2$-2AB (also called GlcNAcMan5-2AB) using GnT-1.



Supplementary Figure 10. Monitoring of glycan-based enzymatic assay by UPLC using LNnT (for α-hGMII) and NA2 (for α-hLM) as internal standards. The α-hLM and the glycan substrate ($Man_5GlcNAc_2$-2AB) were treated with (**a**) sw (1 to 0.0003 μM) and (**b**) **8b-3** (100 to 0.03 μM) for 24 h at 37°C. The α-hGMII and the glycan substrate ($GlcNAcMan_5GlcNAc_2$-2AB) were treated with (**c**) sw (10 to 0.003 μM) and (**d**) **8b-3** (10 to 0.03 μM) in a dose-dependent manner for 24 h at 37°C.



Supplementary Figure 11. The IC_{50} plots of sw and **8b-3** versus α-hLM and α-hGMII in UPLC assay.

Supplementary Table 1. Molecular structure of proposed compounds for computational modeling and their binding energy calculation for α-hGMII binding sites. (Compound **S8**, **S9** and **S13** were synthesized in this work)

Proposed Compound	R	MMGBSA ΔG (kcal/mol)
S4		-91.2
S5		-79.9
S6		-95.1
S7		-98.4
S8 (8b-3)		-108.4
S9 (8b-1)		-91.3
S10		-93.0
S11		-97.5
S12		-100.0
S13 (8b-4)		-94.8

Nine molecules (**S4-S13**) were designed and their α-hGMII binding energies were calculated. It was found that (i) cyclohexyl and, to a lesser extent, cyclopentyl rings are favored; (ii) hydrophobic substituents should be located at the *para* and not *meta* position; (iii) the *n*-propyl lipid chain is better than the *n*-hexyl; and (iv)

the thiourea linkage is better than the amide linkage. Thus, **8b-3** (**S8**) incorporating a 4-propylcyclohexyl moiety was selected for investigations and its molecular models with α-hGMII and α-hLM were built (Fig. 3c and 3d).

Supplementary Table 2. Analysis of high mannose glycan accumulated in 08C0015 treated with sw or **8b-3**.

$[M+H]^+$ m/z	Compositions	Proposed N-glycan structure	Relative intensity (%) in 08C0015						
			DMSO	**8b-3 (μM)**			**sw (μM)**		
				5	10	20	5	10	20
546.2	H2N		2.65	7.13	6.34	7.49	9.05	11.31	10.95
708.2	H3N		6.69	28.09	29.68	30.75	38.68	42.28	46.69
870.3	H4N		4.33	6.17	5.86	6.19	5.96	6.30	7.34
1032.3	H5N		3.77	10.13	9.89	10.85	10.17	10.19	12.11
1194.4	H6N		1.92	5.71	6.16	8.25	7.93	7.98	9.78
1356.4	H7N		1.20	3.15	3.12	4.54	4.38	4.37	5.81
1518.5	H8N	2x	0.48	1.00	1.02	2.09	2.91	3.12	4.93
1680.5	H9N		0.24	0.36	0.37	0.77	1.17	1.35	2.35
Total			21.23	61.79	62.48	70.96	80.29	86.94	100

Supplementary Table 3. *N*-glycosylation analysis of HepG2 treated with **8b-3**.

$[M+H]^+$ m/z	Compositions	Proposed N-glycan structure	Relative abundance (%) in HepG2						
			8b-3 (μM)						
			DMSO	0	0.1	0.3	1	3	10
1235.4	H5N2		1.40	1.97	1.01	2.07	1.99	1.14	3.64
1397.4	H6N2		2.00	2.71	1.74	4.17	3.74	2.39	4.68
1559.5	H7N2		2.38	3.28	1.96	4.82	5.02	3.27	7.97
1721.5	H8N2		3.11	6.38	3.48	5.40	4.77	2.94	6.84
1729.6	H5N3S1		0	0.71	0.90	0.64	1.03	0.74	0
1875.6	H5N3S1F1		0	0	0	0	0.71	1.36	0.79
1883.6	H9N2		1.45	2.55	1.73	2.36	2.61	1.27	2.53
1891.6	H6N3S1		1.85	1.44	2.97	1.95	4.13	8.42	10.07
1932.6	H5N4S1		1.73	1.74	1.10	1.35	0.326	0	0
2037.7	H6N3S1F1		0	0	0	0	1.50	6.51	11.28
2078.7	H5N4S1F1		4.58	3.86	3.75	3.96	2.54	1.00	0
2223.7	H5N4S2		12.93	12.23	11.70	11.79	9.65	7.18	3.05
2240.8	H6N4S1F1		0	0	0	0	0	0.96	0.57
2297.8	H6N5S1		0.90	0.74	0.64	0.67	0	0	0
2369.8	H5N4S2F1		24.48	20.44	25.48	22.76	23.39	18.95	6.79

2385.8	H6N4S2		1.92	0	0	0	0.30	1.06	0.93
2402.8	H7N4S1F1		0	0	0	0	0	2.56	5.54
2443.8	H6N5S1F1		1.92	1.73	1.72	1.62	0.98	0	0
2531.8	H6N4S2F1		0	0	0	0	1.28	5.39	5.33
2547.8	H7N4S2		0	0	0	0	0	0	0.59
2572.9	H5N5S2F1		1.06	0.85	1.12	0.88	1.01	0.81	0
2588.9	H6N5S2		1.96	1.73	1.82	1.69	1.43	1.08	0
2589.9	H6N5S1F2		1.07	1.04	0.96	0.81	0.58	0	0
2605.9	H7N5S1F1		0.68	0.53	0.66	0.31	0	0	0
2662.9	H7N6S1		0.81	0.76	0.76	0.68	0	0	0
2693.9	H7N4S2F1		0	0	0	0	0.67	6.51	16.35
2734.9	H6N5S2F1		5.62	4.69	5.47	5.05	4.63	2.97	0.59
2751.9	H7N5S1F2		2.05	1.65	1.92	1.65	1.53	0.98	0
2809.0	H7N6S1F1		2.39	2.59	2.84	2.50	1.74	0.83	0
2880.0	H6N5S3		1.87	3.12	2.29	2.30	1.98	3.13	4.70

2954.0	H7N6S2		0.89	0.69	0.80	0.78	0.57	0	0
3026.0	H6N5S3F1		4.06	4.37	4.01	3.90	4.09	3.30	1.40
3100.1	H7N6S2F1		3.57	3.01	3.80	3.42	3.40	2.28	0
3171.10	H6N5S4		0	1.16	0.34	0.32	0.25	0.64	0
3172.1	H6N5S3F2		3.87	5.46	4.69	4.20	5.02	5.77	3.20
3391.2	H7N6S3F1		1.37	1.31	1.48	1.21	1.36	1.05	0
3465.2	H8N7S2F1		0.54	0	0.61	0.31	0	0	0
3537.2	H7N6S3F2		1.95	2.44	2.26	1.90	2.36	2.08	0.25
3828.3	H7N6S4F2		0.76	0.85	1.05	0.77	1.01	0.88	0

Supplementary Table 4. *N*-glycosylation analysis of Huh7 treated with 8b-3

$[M+H]^+$ m/z	Compositions	Proposed *N*-glycan structure	Relative abundance (%) in Huh7						
			8b-3 (μM)						
			DMSO	0	0.1	0.3	1	3	10
1235.4	H5N2		2.27	2.72	2.31	1.20	3.21	2.88	1.76
1397.4	H6N2		3.67	4.05	3.78	1.94	4.93	4.54	2.24
1559.5	H7N2		4.59	3.40	3.77	2.19	4.91	4.93	2.90
1721.5	H8N2		4.52	6.98	4.29	3.40	5.43	4.95	4.00
1729.6	H5N3S1		1.41	1.22	1.41	1.57	1.25	0.73	0.26
1875.6	H5N3S1F1		0	0	1.07	2.31	3.05	2.47	1.56
1883.6	H9N2		1.17	3.88	1.52	1.77	2.71	1.60	1.75
1891.6	H6N3S1		1.92	2.24	4.10	7.38	7.58	3.35	8.03
1916.6	H4N4S1F1		0	0	0	0	0	0	0.76
1932.6	H5N4S1		2.99	2.68	1.45	1.07	0.27	0	0.70
2037.7	H6N3S1F1		0	0	0.99	3.69	7.75	9.94	11.34
2078.7	H5N4S1F1		4.46	4.38	3.42	2.92	1.79	1.09	1.11
2135.7	H5N5S1	Or	0	0	0	0	0.30	0.48	1.29
2223.7	H5N4S2		6.97	8.62	5.80	4.76	3.06	3.04	4.11

2240.8	H6N4S1F1		0	0	0	0.77	0.62	1.21	1.71
2281.8	H5N5S1F1	Or	0	0.54	0.26	0	0	0	0
2297.8	H6N5S1		0.65	0.92	0.61	0.53	0	0	0
2369.8	H5N4S2F1		16.2	14.9	13.46	12.90	7.76	4.91	2.77
2385.8	H6N4S2		1.19	0.90	1.28	1.73	1.76	1.68	1.18
2402.8	H7N4S1F1		0	0	0	0	1.01	2.27	4.37
2443.8	H6N5S1F1		1.71	2.08	1.82	1.76	0.47	0	0
2531.8	H6N4S2F1		0	0	0.72	2.96	5.78	8.63	6.52
2547.8	H7N4S2		0	0	0	0.76	1.48	2.74	3.56
2572.9	H5N5S2F1		1.45	1.51	1.38	1.17	0	0	0
2588.9	H6N5S2		2.74	2.20	2.07	1.73	1.08	0.82	1.05
2662.9	H7N6S1		0.93	0.63	0.63	0.27	0	0	0
2693.9	H7N4S2F1		0	0	0	1.38	6.20	14.98	18.44
2734.9	H6N5S2F1		3.52	4.35	4.78	2.82	2.15	1.30	0.58
2809.0	H7N6S1F1		3.24	0	2.31	2.03	1.22	0.79	0.79

2880.0	H6N5S3		4.58	3.67	2.69	2.75	2.13	2.50	3.23
2897.0	H7N5S2F1		0	0	0	0	0.84	1.8	2.37
2938.0	H6N6S2F1		0.65	0.58	0.61	0.55	0	0	0
2954.0	H7N6S2		1.16	0.97	0.79	0.60	0	0	0
3026.0	H6N5S3F1		7.28	7.03	8.08	7.61	3.71	4.44	3.09
3059.0	H8N5S2F1		0	0	0	0	0	0.30	0.73
3100.1	H7N6S2F1		4.53	3.89	4.17	3.61	2.32	1.48	0.97
3174.1	H8N7S1F1		0	0	0.52	0	0	0	0
3229.1	H6N6S3F1		1.36	1.18	1.19	1.19	0.38	0.31	0
3245.1	H7N6S3		1.05	0.89	0.78	0.76	0	0	0
3391.2	H7N6S3F1		3.92	2.87	3.56	3.50	2.15	1.52	0.99
3465.2	H8N7S2F1		1.07	0.85	1.06	1.02	0.62	0	0
3682.2	H7N6S4F1		1.3	1.09	1.93	2.06	1.84	1.09	0.26
3756.3	H8N7S3F1		1.19	0.62	1.35	1.11	0.87	0.55	0
4047.4	H8N7S4F1		0.60	0.50	1.01	1.11	0.76	0	0

4121.4	H9N8S3F1		0.53	0	0.71	0.74	0.25	0	0
4412.5	H9N8S4F1		0	0.52	1.00	0.97	0.69	0	0

Supplementary Table 5. Serum biochemistry test results

	ALT (U/L)	AST (U/L)	BUN (mg/dl)	CRE (mg/dl)	TBIL (mg/dl)
Naïve (n=6)	117.60±95.17	200.16±114.04	20.06±6.01	0.4±0.07	0.98±0.98
8b-3 (n=7)	101.57±70.63	223.42±157.00	22.37±4.41	0.33±0.05	0.55±0.18
Sorafenib (n=6)	61.33±54.45	135.33±90.76	23±2.53	0.35±0.19	0.73±0.32

Note: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BUN, Blood Urea Nitrogen; CRE, Creatinine; TBIL, Total Bilirubin.

Supplementary Table 6. Analysis of high mannose glycan accumulated in serum from mice in control group and 8b-3 treated group

$[M+H]^+$ m/z	Compositions	Proposed *N*-glycan structure	Relative intensity (%)	
			Control	**8b-3**
546.2	H2N		2.45	1.20
708.2	H3N		25.63	29.51
870.3	H4N		41.06	42.92
1032.3	H5N		20.66	20.10
1194.4	H6N		7.16	6.25
1356.4	H7N		0	0
1518.5	H8N		0	0
1680.5	H9N		0	0
Total			96.99	100

Supplementary Methods

Determination of kinetic parameters

For determination of V_{max} and K_m, different substrate concentrations were used in the range of 5 to 0.039 mM for human Golgi α-mannosidase II (α-hGMII) in phosphate buffer (0.1 M sodium phosphate dibasic, pH 7.0) and 2.5 to 0.039 mM for human lysosomal α-mannosidase (α-hLM) in citric acid-phosphate buffer (0.1 M sodium phosphate monobasic monohydrate, 0.5 mM citric acid monohydrate, pH 4.6). The assay was carried out at 37 °C for 1 h (α-hLM) or 2 h (α-hGMII). Stop solution (0.5 M potassium carbonate, pH 10.8) was then added to the reactions and the generation of fluorescence was determined at 355 nm excitation and 460 nm emission (SpectraMax M5, Molecular Devices). The K_m and V_{max} were determined directly from the hyperbolic curve fitting of the Michaelis-Menten equation generated using GraphPad Prism 6.0. The value of k_{cat} was determined by the formula V_{max}/E_t, where E_t is the total enzyme concentration in μg/ml. The assays were performed in 384-well black-bottom microtiter plates.

4-MU-based enzyme inhibition study

The tested compounds (20 mM stock in H_2O or DMSO) were diluted with assay buffer to a range of final concentration and incubated with wide-type α-hGMII or Y354A α-hGMII in phosphate buffer (0.1 M sodium phosphate dibasic, pH 7.0) or α-hLM in citric acid-phosphate buffer (0.1 M sodium phosphate monobasic monohydrate, 0.5 mM citric acid monohydrate, pH 4.6) at rt for 10 min prior to the addition of 4-methylumbelliferyl-α-D-mannopyranoside (4MU-α-Man, Carbosynth, 500 mM stock in DMSO) as the substrate (5 mM for wild-type α-hGMII; 2.5 mM for α-hLM and Y354A α-hGMII). The assay was carried out at 37 °C for 1 h (α-hLM) or 2 h (α-hGMII). Stop solution (0.5 M potassium carbonate, pH 10.8) was then added to the reactions and the generation of fluorescence was determined at 355 nm excitation and 460 nm emission (SpectraMax M5, Molecular Devices). The IC_{50} was calculated as relative enzyme activity to control using GraphPad Prism 6.0. Competitive K_i values were determined using four fixed concentration of substrate (4MU-α-Man) and four fixed inhibitor concentration in the assay buffer. The K_i value were calculated via nonlinear regression using GraphPad Prism 6.0. The assays were performed in 384-well black-bottom microtiter plates.

General procedure for preparation of (thio)urea libraries and *in-situ* screening

Scaffold **8b** (0.1 M in H_2O, 4 μL) were added to a 96-well reaction plate. After lyophilization, DMF (8 μL), DIPEA (0.2 M in DMF, 4 μL), and various iso(thio)cyanate (0.2 M in DMF, 4 μL) were added to the reaction well. The mixture was sonicated and shaken for 12 h. Reactions were monitored by TLC and MS. Assay buffer (phosphate buffer or citric acid-phosphate buffer) was then added to the reaction and sonicated for another 6 h to quench unreacted isocyanate. The resulting crude products were directly screened in the enzyme-based assay without further purification. Neither quenched iso(thio)cyanate reagents nor DIPEA and DMSO showed any significant inhibition. Across the total set of preliminary (thio)urea-indolizidine library screening (1 μM for α-hLM and 0.5 μM for α-hGMII), a potentially α-hGMII-selective compound **8b-1** (5G) was found (approximate 60% inhibition against α-hGMII at 0.5 μM) and excitingly display less than 10% inhibition against α-hLM at 1 μM. The compounds with the best selectivity were selected for re-synthesis and further testing.

Molecular docking studies

Molecular docking studies were performed using the Glide module of the Schrodinger Suite (Schrödinger, LLC, New York). The three-dimensional (3D) structures of the α-hGMII and α-hLM were created by homology modeling on Swiss-Model,[1] using the crystallized structures of Drosophila α-GMII (PDB coded 3DDF)[2] and Bovine α-LM (PDB coded 1O7D)[3] as molecular templates, respectively. Both of the 3D structures were refined by Prime with Protein Preparation Wizard (Schrodinger) before molecular docking. The compound structures were prepared by using LigPrep module (Schrodinger) before being input to Glide. The OPLS_2005 force field was applied in all procedures. Briefly, a docking grid (size of $46 \times 46 \times 46$ centered at the benzene moiety of (thio)urea and Vander Waal radius scale of 0.90 Å with a partial atomic charge of 0.25) was created in the cleft of the target protein, including the swainsonine (sw) binding site with considering the size of the docking compounds. The extra precision (XP) mode was applied and root mean square deviation (RMSD) from sw was checked to validate the docking pose of each compound.

MM-GBSA analysis

The MM-GBSA method in Prime module of Schrödinger was applied to calculate the optimal binding free energies, using the following equation: MMGBSA_ΔG= PrimeEnergy (Optimized Complex) – PrimeEnergy (Optimized Free Ligand) – PrimeEnergy (Optimized Free Receptor). Briefly, the VGSB2 solvent model[4] was applied with OPLS_2005 force field inclusive of an implicit solvent model in addition to physics-based corrections for hydrogen bonding, hydrophobic interactions, π-π interactions and self-contact interactions.

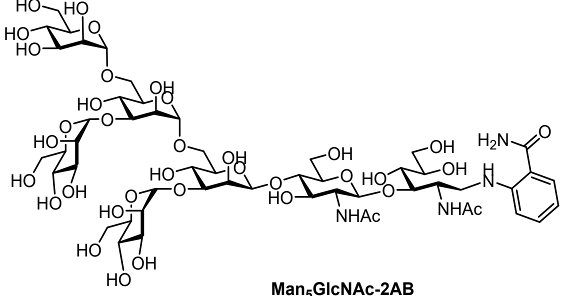
Glycan-based enzyme assay

General UPLC condition of glycan-based analysis

The glycan mixture was analyzed by Waters Acquity UPLC equipped with Acquity UPLC Glycan BEH Amide column (2.1 mm x 150 mm, 1.7 μm) with the flow rate of 0.4 ml/min.

After injection of 10 μL of samples in 30% ammonium formate buffer (0.1 M, pH 4.5) over 2 min, the linear gradient of 80% acetonitrile was applied in the following 20 min. For further analyses, the column was re-equilibrated using initial condition for 5 mins. The fluorescence of 2-Aminobenzamide (2AB) was detected at 330 nm excitation and 420 nm emission.

Preparation of $Man_5GlcNAc_2$-2AB



A mixture of 1 mg Glyko® oligomannose 5 ($Man_5GlcNAc_2$) in a solution of acetic acid (15 μL) and DMSO (35 μL) was treated with 2-aminobenzamide (2.5 mg) and $NaBH_3CN$ (3 mg) at 65°C for 3 h. The reaction mixtures were purified using an HILIC cartridge, then lyophilized. For quantification, **$Man_5GlcNAc_2$-2AB** was re-dissolved in water to give the final concentration of 50 pmol/μL. The concentration was calculated by UPLC analysis and the ratio of peak areas to the internal standard, Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-2AB LNnT (8 pmol/μL).

$Man_5GlcNAc_2$-2AB: MALDI-TOF-MS calcd for $[C_{53}H_{86}N_4O_{36}+H]^+$ 1355.5095, found 1355.5093.

Enzymatic synthesis of $GlcNAcMan_5GlcNAc_2$-2AB

A mixture of **$Man_5GlcNAc_2$-2AB** solution (10 μM, 25 μL) in the reaction buffer (20 mM HEPES, 150 mM NaCl and 20 mM $MnCl_2$, pH 7.5) composed of UDP-GlcNAc (1 mM, 75 μL) was treated with the enzyme, 0.5 μg/μL of alpha-1,3-mannosyl-glycoprotein 2-beta-*N*-acetylglucosaminyltransferase (GnT-1, 25 μL) and incubated at 37°C for 24 h. The reaction was monitored by UPLC, and the reaction mixture purified using an HILIC cartridge, then lyophilized overnight. **$GlcNAcMan_5GlcNAc_2$-2AB** was re-dissolved in water to give the final concentration of 50 pmol/μL. The yield was 60 % calculated by the ratio of peak areas to the internal standard, Gal-β-1,4-GlcNAc-β-1,3-Gal-β-1,4-Glc-2AB (LNnT-2AB) (8 pmol/μL).

$GlcNAcMan_5GlcNAc_2$-2AB: MALDI-TOF-MS calcd for $[C_{61}H_{99}N_5O_{41}+Na]^+$ 1580.5708, found 1580.5692.

MALDI-TOF mass analysis of $GlcNAcMan_5GlcNAc_2$-2AB

1 μL of sample was mixed with 1 μl matrix solution consisting of 2, 5-dihydroxybenzoic acid (50 nmol/μL in 50% acetonitrile). Then 1 μl of the resulting mixture was spotted onto the MALDI stainless steel sample plate and allowed to air dry at room temperature. Measurements were performed on an Ultraflex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). Mass spectra were obtained in the range of mass to charge ratio (*m*/*z*) from 10,000 to 30,000 with linear mode.

Glycan-based enzyme inhibitory assay of 8b-3 against α-hLM and α-hGMII

The corresponding enzyme (0.05 μg/μL for α-hLM; 5 ng/μL for α-hGMII) was added to the reaction mixtures (20 μL) of 2.5 μM glycan substrate (**Man5-2AB** for α-hLM; **GlcNAcMan5-2AB** for α-hGMII), reaction buffer (0.1 M sodium phosphate, 0.05 M citric acid, pH 4.6 buffer for α-hLM; 0.1 M potassium phosphate, pH 7.0 buffer for α-hGMII), internal standard (NA2-2AB for α-hLM; LNnT-2AB for α-hGMII), inhibitors for inhibition selectivity [sw (0.1 μM-0.0003 μM for α-hLM and α-hGMII) and **8b-3** (100 μM-0.03 μM for α-hLM; 10 μM-0.003 μM for α-hGMII)]. After incubation at 37°C for 24 h, the reactions were terminated by C4-tip (Merck Co. ZTC04S008) to remove the enzyme and mixed with 20 μL acetonitrile. After centrifugation (12000 rpm) of samples for 10 minutes, the supernatants were analyzed by UPLC. Enzyme

inhibition including IC_{50} and K_i was calculated as a relative ratio of peak areas of **Man5-2AB** or **GlcNAcMan5-2AB** to that of internal standard.

Cell culture and drugs

Human hepatocellular carcinoma cell lines HepG2 and Huh7 were obtained from ATCC. Normal human fibroblast 08C0015 was purchased from BCRC. Human primary hepatocytes (Caucasian male, aged 34) were procured from ThermoFisher Scientific (Cat. # HMCPMS, Lot # HU1962). Huh7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone SH3002202) supplemented with 10% fetal bovine serum (FBS, Gibco 1887826) at 37 ^oC in a humidified atmosphere of 5% CO_2. HepG2 cells were maintained in antibiotic-free Minimum Essential Medium (MEM, Gibco 41500-34) supplemented with 10% fetal bovine serum (FBS, Gibco 1887826) and 1.5 g/L sodium bicarbonate (Sigma Aldrich S5761). 08C0015 cells were maintained in Dulbecco's Modified Eagle medium (DMEM, Gibco 11965-092) supplemented with 1% non-essential amino acid (NEAA, Gibco 11140-050), 1% L-glutamine (Gibco A29168-01), 1% sodium pyruvate (Gibco 11360-070) and 10% fetal bovine serum (FBS, Gibco 1887826). Human primary hepatocytes were stored at vapor phase of liquid nitrogen. Post thawing, cell viability was found to be 92%. Cells were cultured in Williams E medium (Thermo WEM, A1217601), + 5% FBS + 4 μg/mL human recombinant insulin (Gibco, 2244453) + 2 mM GlutaMax (Thermo, 35050061) + 1× antibiotic + HEPES (Thermo, 15630080). Multi well plates were coated and maintained at 37°C prior to assay initiation. 6.2×10^4 cells per well were seeded in a 96-well plate and incubated overnight at 37°C with 5% CO_2.

Swainsonine (Toronto Research Chemicals, S885000), sorafenib (Cayman, 10009644) and **8b-3** were dissolved in DMSO at a concentration of 100 mM for storage and was formulated at various concentrations for *in vitro* studies.

Cell based selectivity assay (analysis of free $Man_{2\text{-}9}GlcNAc$ in cell)

Cell culture

Human normal fibroblasts (08C0015) were seeded in 10 cm culture plates at a density of 4×10^5 cells/plate. After 24 h of attachment, cells were treated for 24 h with different concentrations of compound sw and **8b-3** at various concentration.

Isolation of $Man_{2\text{-}9}GlcNAc$

After treatment, sw- or **8b-3** treated cells were washed three times with PBS and harvested into centrifuge tubes (one tube per plate) by trypsin treatment. The cell pellets were washed three times with PBS (1 mL) and resuspended in deionized water (200 μL). The cells in water suspension were disrupted for 1 min at 460 r/min in BEAD CRUSHER (TAITEC). The supernatant obtained after centrifugation at 15000 g for 10 min was applied to Amicon Ultra-0.5 Centrifugal Filter Unit (3KDa cutoff, Merck, UFC5003) and centrifuge at 15000 g for 15 min to remove molecules with high molecular weight. The filtrate was collected and the released oligomannoses were purified with porous graphitic carbon (PGC, Supelco 57127-U) spin column. Briefly, the PGC spin column was washed with 400 μL of 80% acetonitrile (acetonitrile) with 0.1% formic acid (FA) three times and conditioned with 400 μL of 0.1% FA three times before use. The released *N*-glycan mixture was then loaded onto the PGC spin column, and the column was washed with 400 μL of 0.1% FA three times. Finally, *N*-glycans were eluted with 250 μL of 50% acetonitrile with 100 mM of ammonium bicarbonate two times. The eluents were combined, and dried using SpeedVac and analyzed using LC-MS.

LC-MS

High resolution and high mass accuracy LC-MS experiments were performed on a LTQFT Ultra (Linear quadrupole ion trap Fourier transform ion cyclotron resonance) mass spectrometer (Thermo Electron, San Jose, CA) equipped with a standard ESI source, an Agilent 1100 Series binary high-performance liquid chromatography pump (Agilent Technologies, Palo Alto, CA), and a Famos autosampler (LC Packings, San Francisco, CA). The sample was injected (5 μl) at 75 μl/min flow rate on BEH Amide Column (1 mm I.D. x 100 mm, 1.7 μm, 130 Å, Waters) Chromatographic separation was using 0.1% formic acid in water as mobile phase A and 0.1% formic acid in 80% acetonitrile as mobile phase B operated at 75 μl/min flow rate. The gradient employed was 90% buffer B at 3 min to 5% buffer B at 40 min. MS conditions: mass range m/z 500-2000, resolution 50,000 at m/z 400. Electrospray voltage was maintained at 4.0 kV and capillary temperature was set at 275 ^{o}C.

N-Glycan profiling assay

Cell culture

Cells were seeded in 10 cm culture plates at a density of 10^6 cells/plate for Huh-7 and 7×10^5 cells/ plate for HepG2. After 24 h of attachment, cells were treated for 72 h with different concentrations of compound **8b-3**.

Cell Lysis

For cellular (glyco) protein extraction, the **8b-3**-treated cells were washed three times with 1 mL of PBS. After washing, 2 mL of trypsin was added onto a 10 cm tissue culture dish of cells, and the cells were harvested and transferred to a 15 mL centrifuge tube. When necessary, 10 μL of the cell suspension was removed for counting, to determine the total number of cells used. The remaining cell suspension was centrifuged at 1000 rpm for 5 min. After centrifugation, the pellet was washed three times with 1 mL of PBS. The cell pellet was transferred to 1.5 mL microcentrifuge tubes for centrifugation at 1000 rpm for 5 min. After removing PBS, for each 60 mg of cell pellet was resuspended with 150 μl of 1x RIPA buffer (10x stock, Merck Millipore, No. 20-188) and incubated on ice for 30 min. The lysate was centrifuged at 16,000 g for 15 min, and the supernatant was collected. The protein concentration of the supernatant was measured by BCA protein assay.

FANGS method for *N*-glycan release

The *N*-glycans analysis was performed as described previously.[5] The supernatant collected after cell lysis was treated with urea solution (8 M in 100 mM Tris/HCl pH 8.5) in a ratio of 4:1 urea buffer to sample solution by volume. An aliquot of this mixture (200-300 μL) was transferred to an ultrafiltration device (Amicon Ultra-0.5, Ultracel-30 membrane, nominal mass cutoff 30 kDa, Millipore) and centrifuged at 14,000g for 15 min, if necessary, repeating this step until all of the liquid had passed through the ultrafiltration device. The sample retained above the filter membrane was washed by the addition of 200 μL of the urea solution and centrifugation at 14 000g for 15 min. Freshly prepared dithiothreitol (DTT) in urea buffer (25 mM, 100 μL) was added to the ultrafiltration device and mixed at 600 rpm in thermomixer for 1 min and incubated at room temperature for 10 min. Removing DTT solution by centrifuging at 14,000 g for 10 min. Freshly prepared iodoacetamide (IAA) in urea solution (50 mM, 100 μL) was added to the ultrafiltration device and mixed at 600 rpm in thermomixer for 1 min and incubated at room temperature for 20 min before centrifuging at 14,000 g for 10 min. The sample retained above the filter membrane was washed four times by the addition of 200

μL of 50 mM ammonium bicarbonate (pH 7.5 to 8) and centrifugation at 14,000g for 10 min. The ultrafiltration device was transferred to a new collection tube, and 100 μL of 50 mM ammonium bicarbonate solution was added to, followed by 500 U (1 μL of 5×10^5 U/mL solution in 5 mM potassium phosphate, pH 7.5) of Peptide -N-Glycosidase F (PNGase F, New England Biolabs P0704L). The ultrafiltration device was sealed with Parafilm and incubated at 37 °C for 16 h. After incubation, the device was centrifuged for 10 min at 14,000g and washed twice with 200 μL of 50 mM ammonium bicarbonate followed by centrifugation for 10 min at 15 000g. The released *N*-glycans in solution were retrieved from the collection tube. Released *N*-glycans were purified with PGC spin column. Briefly, the PGC spin column was washed with 400 μL of 80% acetonitrile (acetonitrile) with 0.1% formic acid (FA) three times and conditioned with 400 μL of 0.1% FA three times before use. The released *N*-glycan mixture was then loaded onto the PGC spin column, and the column was washed with 400 μL of 0.1% FA three times. Finally, *N*-glycans were eluted with 250 μL of 50% acetonitrile with 100 mM of ammonium formate two times. The eluents were combined, dried using SpeedVac and the released *N*-glycans were analyzed by LC-MS mentioned above. The glycan search was using Mulitglycan v1.2.3 with default database. MS tol was 15 ppm and 6 ppm for isotope. Peak area was manual data processing by Xcalibur Qual Browser.

Fluorescent imaging

Cells were seeded in 24-well culture plates at a density of 10^5 cells/well for Huh-7 and 1.5×10^5 cells/ plate for HepG2. After 24 h of attachment, cells were treated for 48 h with 10 μM of compound **8b-3**. After treatment, cells were washed three times with PBS, fixed with 4% formaldehyde in PBS for 10 minutes at room temperature and blocked with PBS containing 2% BSA for 1 hour at room temperature. Cells were stained with 20 μg/mL Alexa Fluor 555-conjugated recombinant galectin-1 and 20 μg/mL FITC-conjugated ConA, staining for 1 hour at 4°C. Images were acquired and analyzed.

MTT cell proliferation assay

Cells were seeded in 96-well plates at a density of 4×10^3 cells/well for Huh7 and 10^4 cells/well for HepG2. After 24 h of attachment, cells were treated for 72 h with different concentrations of compound. After treatment, the effect of compound on cell viability was examined by the MTT assay following the

manufacturer's instructions. Briefly, a medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, M5655) at a final concentration of 0.5 mg/mL to cells which were twice washed with PBS before MTT treatment, then incubated for 1 h at 37 ^{o}C in a humidified incubator containing 5% CO_2 in air. Then, the medium was replaced with 100 μL of DMSO for 30 min at room temperature, and the 96-well plate was read by microplate spectrometer reader (BIO-RAD, Eenchmark plus^{TM}) absorbance at 565 nm to obtain the absorbance density values. The IC_{50} values of death cell lines were calculated accordingly using GraphPad Prism 6.0 software.

Transwell cell migration assay

The cells to be analyzed (5×10^4 cells/well) were seeded in the upper chambers (pore size, 8 μm, Millipore) of the 24-well plate in 400 μL serum-free medium. FBS (10%) was used as the chemoattractant in the lower chamber, and the plate was incubated under standard conditions. After incubation for 18 hours, the media in the upper chambers were aspirated and washed with PBS twice. Both non-migrated and migrated cells were fixed with 4% Paraformaldehyde (PFA, Merck, 104003) for 30 minutes at room temperature and stained with 2% crystal violet solution. The non-invading cells were removed from the upper surface of the insert membrane with a cotton swab. The images were taken using an imaging reader (Cytation 5, BioTek, VT, U.S.A.) and analyzed using NIH ImageJ software.

Transwell cell invasion assay

A sample of 5×10^4 cells/well of the indicated cancer cells was resuspended in 300 μL serum-free medium and placed into the top chamber of an insert (pore size, 8 μm, Millipore) pre-coated with 100 μg/ml Matrigel (Corning, 394230). The lower chamber of the well was filled with 600 μL complete medium. After 24 hours of incubation, the media in the upper chambers were aspirated and washed with PBS twice. Both non-migrated and migrated cells were fixed with 4% PFA for 1 hour at room temperature, permeabilized cells by 100% methanol (Merck, 106007) for 20 min at room temperature. stained with 2% crystal violet solution (Sigma, HT90132) for 20 minutes. The non-invading cells were removed from the upper surface of the insert membrane with a cotton swab. The images were taken using an imaging reader (Cytation 5, BioTek, VT, U.S.A.) and analyzed using NIH ImageJ software.

Mice xenograft models of HCC

All animal operations were in accord with institutional animal use and care regulations approved by the Academia Sinica SPF animal facility. HCC xenografts were established in 5-6 week-old male NOD-SCID mice (purchased from BioLASCO Taiwan Co., Ltd) by subcutaneous inoculation of about 5× 10^6 Huh7 cells suspended in 50 μL PBS +50 μL Matrigel (Corning, 394230) in the left and right flanks of each mouse. After one week of inoculation, tumors were palpable and the mice were assigned randomly to one of four treatment groups. The vehicle solution contained Kolliphor® EL (Sigma Aldrich, C5135), 99.9% ethanol (Merck 100983) and water in a ratio of 1:1:6. Mice received i.p (intraperitoneal) injection of vehicle, **8b-3** (30 mg/kg) or sorafenib (30 mg/kg) twice a week for 25 days. The tumor volume was assessed by the formula of a rational ellipsoid: [Volume = (shorter axis) m_1^2 x (longer axis) m_2 x 0.5236], and the tumor volume was measured twice a week. All the mice were euthanized at 27 days after the treatments started. Tumor tissues were isolated, photographed and then weighed.

Serum biochemistry assay

All blood specimens were collected in tubes and were left at 25 ^{o}C for 30 min to clot and centrifuged for 10 min at 1200 g. The concentrations of the following biochemical serum analytes collected were measured with FUJI DRI-CHEM 500i analyzer (FUJIFILM Corp., Tokyo, Japan) using the manufacturer's reagents and according to the manufacturer's instructions: Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Blood Urea Nitrogen (BUN), Creatinine (CRE) and Total Bilirubin (TBIL)

Isolation of free Oligomannose ($Man_{2\text{-}9}GlcNAc$) from serum

50 μL of serum was applied to Amicon Ultra-0.5 Centrifugal Filter Unit (3KDa cutoff, Merck, UFC5003) and centrifuge at 15000 g for 15 min to remove molecules with high molecular weight. The filtrate was collected and the released oligomannoses were purified with PGC spin column. Briefly, the PGC spin column was washed with 400 μL of 80% acetonitrile (acetonitrile) with 0.1% formic acid (FA) three times and conditioned with 400 μL of 0.1% FA three times before use. The released *N*-glycan mixture was then loaded onto the PGC spin column, and the column was washed with 400 μL of 0.1% FA three times. Finally, *N*-glycans were eluted with 250 μL of 50% acetonitrile with 100 mM of ammonium formate two times. The

eluents were combined, dried using SpeedVac and the oligomannoses ($Man_{2-9}GlcNAc$) were analyzed by LC-MS mentioned above

Statistical analysis

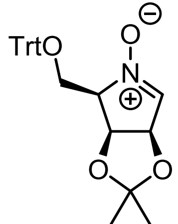
All statistical analysis was conducted using a student's t-test with GraphPad Prism 6 (GraphPad Software, Inc. La Jolla, CA). Data were presented as mean±standard deviations. p values < 0.05 were deemed statistically significant.

Experimental data

General

All chemicals were obtained from commercial suppliers and used without further purification. Reactions were magnetically stirred and monitored by thin-layer chromatography on silica gel. Column chromatography was performed using silica gel (Merck Kieselgel Si60 (40–63 μm)). Thin layer chromatography (TLC) was performed on glass plates coated to a thickness of 1 mm with Merck Kieselgel 60F254. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a Bruker AM-600 NMR spectrometer using $CDCl_3$, CD_3OD, or D_2O as solvent. Chemical shifts are given in parts per million (ppm) and J (coupling constant) values were estimated in Hertz (Hz). The following notation is used: br – broad, s – singlet, d – doublet, t – triplet, q – quartet, quin – quintet, m – multiplet, dd – doublet of doublets, ddd – doublet of doublets of doublets, dt – doublet of triplets, td – triplet of doublets. High resolution ESI mass spectra were recorded on a Bruker Daltonics spectrometer. Concentration refers to rotary evaporation.

Preparation of compound 1



Nitrone **1** was prepared in 35% yield as a white solid over seven steps from L-ribose as previously described.[6,7]

1: TLC (EtOAc), R_f= 0.25; [α]D25 = -22.4° (c = 0.2 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.29, (s,

3H), 1.40 (s, 3H), 3.69 (t, 1H, J = 9.2 Hz), 3.96 (dd, 1H, J = 9.0, 4.4 Hz), 4.13-4.16 (m, 1H), 4.97-4.99 (t, 1H, J = 5.7 Hz), 5.24-5.25 (m, 1H), 6.83 (t, 1H, J = 1.9 Hz), 7.25-7.52 (m, 15H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 26.1, 27.1, 58.8, 74.1, 75.0, 77.8, 87.3, 112.0, 127.0, 127.7, 128.8, 132.4, 143.6. HRMS (m/z): $[Na]^+$ calcd. for $C_{27}H_{27}NO_4$, 452.1832; found, 452.1832.

Preparation of compound 2

TrtO OH N CN O O

A mixture of **1** (4.9 g, 11.5 mmol) in dried methanol was treated with trimethylsilyl cyanide (2.86 mL, 22.9 mmol) at rt. The reaction mixture was heated to 50 °C. After 2 h, the mixture was concentrated and then purified by column chromatography (*n*-hexane / EtOAc = 4:1, silica gel) to give **2** as a white foam (4.7 g, 10.4 mmol, 90%).

2: TLC (*n*-hexane / EtOAc, v/v 2:1), R_f = 0.25; [α]D25 = -83.1° (c = 0.1 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.27 (s, 3H), 1.28 (s, 3H), 3.21-3.24 (m, 1H), 3.43-3.45 (m, 1H), 3.55-3.58 (m, 1H), 4.28 (s, 1H), 4.79-4.80 (m, 1H), 4.82-4.84 (m, 1H), 5.17 (s, 1H), 7.23-7.47 (m, 15H) ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.0, 25.5, 61.1, 62.4, 67.9, 77.0, 78.8, 87.2, 112.5, 114.8, 127.1, 127.8, 128.7, 143.6. HRMS (m/z): $[Na]^+$ calcd. for $C_{28}H_{28}N_2O_4$, 479.1941; found, 479.1946.

Preparation of compound S1

HO Boc N NHBoc O O

Di-*tert*-butyl dicarbonate (15 mL, 98.5 mmol) and Raney nickel (5 g) were added to a solution of compound **2** (9 g, 19.7 mmol) in MeOH (65 mL) and the mixture stirred under a hydrogen atmosphere at room temperature. After 8 h, the reaction mixture was filtered through Celite, and the filtrate was concentrated and extracted with EtOAc and water. The combined organic extracts were dried ($MgSO_4$) and then concentrated

to give fully protected iminosugar residue as colorless oil, which was directly used in next step without further purification.

HCOOH (100 mL) was added to an ice-cold solution of fully protected iminosugar residue in dry ethyl ether (120 mL) and the mixture stirred. After 1 h, the ice bath was removed and stirring continued for another 30 min. Then, the reaction mixture was diluted with EtOAc and the reaction was carefully quenched by dilution with $NaHCO_{3(s)}$ until effervescence ceased. The organic layer was washed with water and brine, dried ($MgSO_4$), concentrated, and purified by column chromatography (*n*-hexane / EtOAc = 5:1, silica gel) to give **S1** (4.5g, 11.0 mmol, 56% over 2 steps) as a white foam.

S1: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f= 0.7; [α]D25 = -50.8° (*c* = 0.2 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, 278K): δ 1.24, (s, 3H), 1.37 (s, 3H), 1.39 (s, 9H), 1.44 (s, 9H), 3.19 (m, 1H), 3.25 (m, 1H), 3.76-3.79 (m, 2H), 3.83-3.87 (m, 1H), 3.95 (m, 1H), 4.52-4.53 (m, 1H), 4.67-4.68 (m, 1H), 4.93 (br, 1H), 5.13-5.15 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$, 278K): δ 24.5, 25.9, 28.2, 28.2, 40.7, 61.9, 64.2, 64.8, 79.7, 79.9, 80.5, 81.1, 111.3, 155.3, 155.9. HRMS (m/z): $[Na]^+$ calcd. for $C_{19}H_{34}N_2O_7$, 425.2258; found, 425.2255.

Preparation of compound 3

Dess-Martin periodinane (10.7 g, 25.9 mmol) was added to a solution of **S1** (7.25 g, 17.3 mmol) in CH_2Cl_2 (60 mL) and the mixture stirred for 1 h at rt. The reaction solution was filtered through a Celite pad, then diluted with $NaHCO_{3(aq)}$ and then washed with $NaS_2O_{3(aq)}$ and CH_2Cl_2. The organic layer was concentrated to give aldehyde residue, which was purified by column chromatography (*n*-hexane / EtOAc 4:1, silica gel) to give **3** as a white foam (7.16 g, 17.1 mmol, 99%).

3: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f= 0.8; [α]D25 = -91.2° (*c* = 0.2 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, 278K): δ 1.19 (s, 3H), 1.32-1.40 (m, 21H), 3.18-3.28 (m, 1H), 3.31-3.37 (m, 1H), 4.01-4.18 (m, 2H), 4.70-4.74 (m, 1H), 4.83-4.92 (m, 1H), 5.07-5.13 (m, 1H), 9.20-9.25 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$, 278K): δ 23.9, 25.5, 25.6, 28.0, 28.1, 28.2, 40.2, 41.1, 63.3, 63.5, 67.5, 67.6, 79.6, 79.7, 79.9, 80.5, 81.6, 81.7,

82.0, 82.6, 112.2, 112.3, 153.4, 154.2, 156.1, 156.3, 197.9, 198.0. HRMS (m/z): $[Na]^+$ calcd. for $C_{19}H_{32}N_2O_7$, 423.2102; found, 423.2102.

Preparation of compound 4a

Allylmagnesium chloride (1.7 M in THF, 4.86 mL, 8.25 mmol) was added to a solution of compound **3** (1.1 g, 2.8 mmol) in THF (30 mL) at -78 °C and stirred for 1 h. The reaction was quenched with sat. $NH_4Cl_{(aq)}$ and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (*n*-hexane / EtOAc = 5:1, silica gel) to give **4a** as a white foam (788 mg, 1.8 mmol, 64%)

4a: TLC (*n*-hexane / EtOAc, v/v 2:1), R_f = 0.6. [α]D25 = -8.7° (*c* = 0.1 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, 278K): δ 1.26 (s, 3H), 1.39 (s, 3H), 1.41-1.48 (m, 18H), 2.33-2.38 (m, 1H), 2.61-2.63 (m, 1H), 3.09-3.12 (m, 1H), 3.18-3.23 (m, 1H), 3.41-3.42 (m, 1H), 4.04-4.06 (m, 1H), 4.20-4.23 (m, 1H), 4.36-4.37 (m, 1H), 4.59 (m, 1H), 5.07-5.09 (m, 1H), 5.12-5.14 (m, 1H), 5.99-6.05 (m, 1H), 6.18 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$, 278K): δ 24.9, 26.6, 28.3, 28.3, 37.8, 39.9, 65.3, 67.4, 67.8, 79.6, 79.7, 80.0, 81.3, 111.4, 116.9, 135.1, 155.2, 155.9. HRMS (m/z): $[Na]^+$ calcd. for $C_{22}H_{38}N_2O_7$, 465.2571; found, 465.2568.

Preparation of compound 5a

Borane-THF complex (4 mL of a 1 M solution in THF, 2.3 mmol) was added to a solution of **4a** (442 mg, 0.58 mmol) in THF (3.6 mL) at 0 °C for 1 h. 3N $NaOH_{(aq)}$ (0.5 mL) and $H_2O_{2\,(aq)}$ (0.5 mL) were then added to the reaction mixture at 0 °C. The reaction mixture was again stirred for 1 h. The reaction mixture was concentrated, and the residue was extracted with EtOAc and H_2O. The combined organic layers were dried

($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (*n*-hexane / EtOAc = 3:1, silica gel) to give **5a** (346 mg, 0.75 mmol, 75%) as a colorless oil.

5a: R_f= 0.4 (*n*-hexane / EtOAc 1:1); [α]D25 = -21.2° (*c* = 0.1 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.25 (s, 3H), 1.38 (s, 3H), 1.41 (s, 9H), 1.45 (s, 9H), 1.55-1.61 (m, 1H), 1.77-1.80 (m, 2H), 1.95-2.00 (m, 1H), 3.13-3.14 (m ,1H), 3.19 (m, 1H), 3.43-3.46 (m, 2H), 3.61-3.69 (m, 2H), 4.07 (br, 1H), 6.46 (br, 1H), 4.36 (d, 1H, *J* = 5.4 Hz), 4.58 (t, 1H, *J* = 4.8 Hz), 4.81 (d, 1H, *J* = 10.9 Hz), 4.86 (t, 1H, 6.5 Hz), 7.26-7.39 (m, 5H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.9, 26.5, 28.3, 29.0, 30.4, 39.9, 62.7, 65.4, 65.8, 67.7, 68.4, 79.5, 79.8, 80.5, 81.5, 111.5, 155.4, 155.9. HRMS (m/z): $[H]^+$ calcd. for $C_{22}H_{40}N_2O_8$, 461.5690; found, 461.5688.

Preparation of compound 6a

3-Butenylmagnesium bromide (4.5 mL, 2.25 mmol, 0.5 M in THF) was added to a solution of **3** (300 mg, 0.75 mmol) in THF (7.5 mL) at -78 °C, with stirring. After 1 h, the reaction was quenched with sat. $NH_4Cl_{(aq)}$ and extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (*n*-hexane / EtOAc = 7:1, silica gel) to give **6a** (267 mg, 0.59 mmol, 78%) as a white foam.

6a: TLC (*n*-hexane / EtOAc, v/v 2:1), R_f= 0.6. ; [α]D25 = -43.8° (*c* = 0.1 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.24 (s, 3H), 1.37 (s, 3H), 1.40 (s, 9H), 1.44 (s, 9H), 1.49-1.54 (m, 1H), 1.88 (br, 1H), 2.19 (br, 1H), 2.37 (m, 1H), 3.09-3.12 (m, 1H), 3.18-3.22 (m, 1H), 3.38-3.39 (m, 1H), 4.04-4.06 (m, 1H), 4.09-4.12 (m, 1H), 4.33-4.34 (m, 1H), 4.55 (br, 1H), 4.81 (br, 1H), 4.93 (d, 1H, *J* = 10.1 Hz), 5.04 (d, 1H, *J* = 17.1 Hz), 5.85 (m, 1H), 5.99 (br, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.0, 26.6, 28.3, 29.7, 32.4, 39.9, 65.4, 67.3, 68.8, 79.5, 79.8, 80.3, 81.3, 111.4, 114.3, 138.9, 155.2, 155.9. HRMS (m/z): $[H]^+$ calcd. for $C_{23}H_{41}N_2O_7$, 459.2908; found, 459.2911.

Preparation of compound 7a

Zinc bromide (1.2 g, 5.5 mmol) was added to a stirring solution of **6a** (500 mg, 1.1 mmol) in CH_2Cl_2 (4 mL). The resulting solution was stirred at rt for 8 h. After the reaction was complete, the mixture was filter, concentrated, and purified with column chromatography (MeOH / CH_2Cl_2 = 1:30, silica gel) to give a diamine crude.

The diamine crude in THF (3.6 mL) was treated with benzyl chloroformate (0.6 mL, 4.4 mmol) and conc. $NaHCO_{3\ (aq)}$ (3.6 mL) at rt and stirred for 1h (*n*-hexane / EtOAc, v/v 1:2, R_f = 0.6). After the reaction was complete, the reaction mixture was extracted with EtOAc. The organic layer was separated, and the water layer was extracted with EtOAc three times. The combined organic layer was dried ($MgSO_4$). The solvent was removed, and the product was purified by column chromatography (*n*-hexane / EtOAc = 5:1, silica gel) to give **7a** as a yellow oil (393 mg, 0.76 mmol, 69% over 2 steps).

7a: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f = 0.3. ; [α]D25 = -18.5° (*c* = 0.16 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.29 (s, 3H), 1.38 (s, 3H), , 1.59 (br, 1H), 1.93 (br, 1H), 2.26 (br, 1H), 2.42 (br, 1H), 3.22 (br, 1H), 3.35 (br, 1H), 3.51 (br, 1H), 4.20 (br, 1H), 4.25 (br, 1H), 4.45 (br, 1H), 4.60 (br, 1H), 4.99-5.15 (m, 7H), 5.78 (br, 1H), 5.90 (br, 1H), 7.29-7.34 (m, 10H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.8, 26.5, 29.7, 32.3, 40.4, 65.2, 67.1, 67.3, 67.6, 69.4, 79.4, 80.1, 111.6, 114.5, 127.9, 128.3, 128.4, 128.6, 128.7, 135.9, 136.1, 138.7, 155.5, 156.6. HRMS (m/z): $[H]^+$ calcd. for $C_{29}H_{36}N_2O_7$, 525.2595; found, 525.2599.

Preparation of compound S14

2,6-lutidine (0.11 mL, 0.96 mmol), OsO_4 (1% in H_2O, 0.05 mmol), and $NaIO_4$ (410 mg, 1.9 mmol) were added to a solution of compound **7a** (250 mg, 0.48 mmol) in 1,4-dioxane-water (3:1, 8 mL). The reaction was stirred at rt for 5 h. After the reaction was complete, H_2O and CH_2Cl_2 were added. The organic layer was

separated, and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was dried ($MgSO_4$). The solvent was removed, and the product was purified by column chromatography (*n*-hexane /EtOAc = 3:1, silica gel) to give aldehyde (*n*-hexane / EtOAc, v/v 1:1, R_f= 0.1) as a white foam.

The aldehyde in MeOH (5 mL) was added Pd/C (20 mg). The flask was evacuated, filled with H_2, and stirred for 8 h at rt. The mixture was then filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography (MeOH / CH_2Cl_2 = 1:7, silica gel) to give **S14** as a yellow oil (103 mg, 0.43 mmol, 89% over 2 steps).

S14: TLC ($CHCl_3$ / MeOH / H_2O / NH_4OH, v/v 88:58:12.5:1), R_f= 0.5. ^1H NMR (600 MHz, MeOD): δ 1.32 (s, 3H), 1.51 (m, 4H), 1.59-1.61 (m, 1H), 1.85-1.93 (m, 2H), 2.63 (br, 1H), 2.83-2.86 (m, 1H), 2.92-2.95 (m, 2H), 3.15-3.18 (m, 1H), 3.34-3.37 (m, 1H), 4.17-4.18 (m, 1H), 4.55-4.56 (m, 1H), 4.89-4.90 (m, 1H). ^{13}C NMR (150 MHz, MeOD): δ 22.8, 23.5, 26.2, 31.2, 37.3, 50.2, 64.0, 68.3, 68.4, 83.6, 83.8, 113.4. HRMS (m/z): $[H]^+$ calcd. for $C_{12}H_{22}N_2O_3$, 243.1703; found, 243.1702.

Preparation of compound 8a

6N HCl (0.5 mL) was added to a solution of **S2** (33.6 mg, 0.14 mmol) in MeOH (0.5 mL) and stirred for 8 h. The reaction mixture was concentrated and purified by column chromatography (*i*-PrOH / NH_4OH = 9:1, silica gel) to give **8a** as a yellow foam (25.4 mg, 90%).

8a: TLC (*i*-PrOH / NH_4OH, v/v 3:1), R_f= 0.5. ; [α]D25 = 3.84° (*c* = 0.1 in H_2O); ^1H NMR (600 MHz, D_2O): δ 1.26-1.29 (m, 1H), 1.54-1.59 (m, 1H), 1.79-1.84 (m, 2H), 2.58-2.65 (m, 2H), 2.87-2.91 (m, 2H), 2.96-2.98 (m, 1H), 3.03-3.06 (m, 1H), 3.93-3.95 (m, 1H), 4.00-4.01 (m, 1H), 4.17-4.19 (m, 1H). ^{13}C NMR (150 MHz, D_2O): δ 15.6, 29.6, 40.9, 45.8, 62.9, 64.8, 68.1, 70.9, 73.8. HRMS (m/z): $[H]^+$ calcd. for $C_9H_{18}N_2O_3$, 203.1390; found, 203.1389.

Preparation of compound 6b

Trifluoromethanesulfonic anhydride (0.3 mL, 1.8 mmol) was added dropwise to a solution of compound **6a** (420 mg, 0.9 mmol) and pyridine (0.37 mL, 4.6 mmol) in CH_2Cl_2 (9.2 mL) at 0 °C. The reaction was warmed to rt and stirred for 8 h. After the reaction was complete, water and CH_2Cl_2 were added. The organic layer was separated, and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with 1N HCl to remove pyridine. The organic layer was then dried ($MgSO_4$). The solvent was removed, and the product was purified by column chromatography (*n*-hexane /EtOAc = 2:1, silica gel) to give cyclic carbamate **6b** as a yellow foam (247 mg, 0.65 mmol, 73%).

6b: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f= 0.5. ; [α]D25 = 2.86° (*c* = 0.1 in CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.26 (s, 3H), 1.40 (s, 9H), 1.42 (s, 3H), 1.90-1.96 (m, 1H), 2.16-2.21 (m, 1H), 2.26-2.32 (m, 2H), 3.04-3.09 (m, 1H), 3.26-3.31 (m, 1H), 3.82-3.84 (m, 1H), 3.93-3.96 (m, 1H), 4.53-4.55 (m, 1H), 4.58-4.60 (m, 1H), 4.63-4.67 (m, 1H), 4.84 (m, 1H), 5.01 (d, 1H, *J* = 10.2 Hz), 5.07 (d, 1H, *J* = 17.2 Hz), 5.78-5.84 (m, 1H) . ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.8, 26.7, 28.4, 30.4, 41.1, 63.8, 65.0, 75.9, 76.8, 79.7, 80.4, 84.8, 113.2, 115.9, 137.0, 156.2, 161.3. HRMS (m/z): $[Na]^+$ calcd. for $C_{19}H_{30}N_2O_6$, 405.1996; found, 405.1999.

Preparation of compound 7b

The solution of LiOH•H_2O (545 mg, 13 mmol) in water (4 mL) was added to a solution of **6b** (247 mg, 0.64 mmol) in EtOH (4 mL). The mixture was stirred over 20 h at 90 °C. After the reaction was complete, the reaction mixture was concentrated, and the product was purified by column chromatography (*n*-hexane / EtOAc = 2:1, silica gel) to give amino alcohol as a white foam (167 mg, 0.47 mmol, 73 %).

The amino alcohol (167.1 mg, 0.47 mmol) in THF (5 mL) and conc. $NaHCO_{3\ (aq)}$ (5 mL) was treated with benzyl chloroformate (0.14 mL, 0.94 mmol) at 0 °C. The reaction was warmed to rt and stirred for 1 h. After

the reaction was complete, the reaction mixture was extracted with EtOAc. The organic layer was separated, and the water layer was extracted with EtOAc three times. The combined organic layer was dried ($MgSO_4$). The solvent was removed, and the product was purified by column chromatography (*n*-hexane / EtOAc = 7:1, silica gel) to give **7b** (218 mg, 95%) as a white solid.

7b: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f = 0.3. [α]D25 = -35.8° (*c* = 0.1 in CH_2Cl_2);^{1}H NMR (600 MHz, $CDCl_3$): δ 1.28 (s, 3H), 1.42 (s, 9H), 1.46 (br, 4H), 1.72 (br, 1H), 2.13 (br, 1H), 2.37 (br, 1H), 3.28 (br, 1H), 3.92 (br, 1H), 4.03 (br, 1H), 4.15 (br, 1H), 4.29 (br, 1H), 4.63 (br, 1H), 4.78-4.80 (m, 1H), 4.91-4.93 (m, 1H), 4.99-5.02 (m, 1H), 5.09-5.11 (m, 2H), 5.18 (m, 1H), 5.82 (b, 1H), 7.30-7.35 (m, 5H). ^{13}C NMR (150 MHz, $CDCl_3$, 278K): δ 23.9, 25.5, 28.3, 30.9, 32.8, 41.1, 64.3, 67.4, 69.9, 79.7, 79.9, 80.9, 111.5, 114.4, 128.2, 128.3, 128.6, 135.9, 138.8, 156.1. HRMS (m/z): $[H]^+$ calcd. for $C_{26}H_{38}N_2O_7$, 405.1996; found, 405.1999.

Preparation of compound S15

HO H N NHBoc O O

Following the above general method for **S14**, compound **7b** (50 mg, 0.1 mmol) gave **S15** (30.6 mg, 90% over 2 steps) as a white foam.

S15: TLC (EtOAc), R_f= 0.4. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.43 (s, 9H), 1.51 (s, 3H), 1.57-1.62 (m, 1H), 2.03-2.05 (m, 1H), 2.45-2.49 (m, 3H), 2.61-2.64 (m, 1H), 2.86-2.88 (m, 1H), 3.02-3.03 (m, 1H), 3.22-3.24 (m, 1H), 3.35-3.37 (m, 1H), 3.72-3.76 (m, 1H), 4.49-4.50 (m, 1H), 4.68 (br, 1H), 4.75-4.77 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 22.1, 24.6, 25.8, 28.3, 32.2, 37.3, 45.5, 65.1, 66.8, 68.5, 79.3, 79.6, 81.9, 112.6, 156.0. HRMS (m/z): $[H]^+$ calcd. for $C_{17}H_{30}N_2O_5$, 343.2227; found, 343.2258.

Preparation of compound 8b

HO H N NH_2 HO OH

Following the above general method for **8a**, compound **S3** (69 mg, 0.2 mmol) gave **8b** (40 mg, 100%) as a

yellow foam.

8b: TLC (*i*-PrOH / NH_4OH, v/v 3:1) R_f= 0.5. [α]D25 = -22.6° (c = 0.2 in H_2O); ^1H NMR (600 MHz, D_2O): δ 1.34-1.40 (m, 1H), 1.62-1.68 (m, 1H), 1.84-1.88 (m, 1H), 2.03-2.08 (m, 1H), 2.83-2.86 (m, 1H), 3.05-3.06 (m, 1H), 3.13-3.15 (m, 1H), 3.24-3.28 (m, 1H), 3.45-3.53 (m, 2H), 3.96-4.00 (m, 1H), 4.22-4.24 (m, 1H), 4.31-4.32 (m, 1H). ^{13}C NMR (150 MHz, D_2O): δ 20.4, 29.8, 38.1, 47.1, 63.9, 64.2, 68.8, 69.0, 74.3. HRMS (m/z): $[H]^+$ calcd. for $C_9H_{18}N_2O_3$, 203.1390; found, 203.1386.

Preparation of compound 9a

Following the above general method for **7a**, compound **4a** (163 mg, 0.37 mmol) gave **9a** (144 mg, 0.28 mmol, 76% over 2 steps) as a colorless oil.

9a: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f = 0.6. [α]D25 = -7.6° (c = 0.1 in CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ 1.26 (s, 3H), 1.35 (s, 3H), 2.37 (br, 1H), 2.62 (br, 1H), 3.17 (br, 1H), 3.30 (br, 1H), 3.51 (br, 1H), 4.22-4.26 (m, 1H), 4.42 (br, 1H), 4.59 (br, 1H), 5.01-5.16 (m, 8H), 5.95 (br, 1H), 6.03 (br, 1H), 7.28-7.34 (m, 10H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.8, 26.5, 37.8, 40.6, 65.2, 67.0, 67.6, 68.5, 79.6, 79.8, 111.6, 116.9, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 135.0, 136.0, 136.2, 155.6, 1560.6. HRMS (m/z): $[H]^+$ calcd. for $C_{28}H_{34}N_2O_7$, 511.2439; found, 511.2450.

Preparation of compound S16

Following the above general method for **S14**, compound **9a** (144 mg, 0.28 mmol) gave **S16** (57 mg, 0.25 mmol, 88% over 2 steps) as a yellow foam.

S16: TLC ($CHCl_3$ / MeOH / H_2O / NH_4OH, v/v 88:58:12.5:1), R_f= 0.2. ^1H NMR (600 MHz, MeOD): δ

1.32 (s, 3H), 1.52 (s, 1H), 2.08-2.12 (m, 2H), 2.59-2.62 (m, 1H), 2.69-2.72 (m, 1H), 3.12-3.18 (m, 3H), 3.46-3.48 (m, 1H), 4.57-4.63 (m, 2H), 4.79-4.80 (m, 1H). ^{13}C NMR (150 MHz, MeOD): δ 22.7, 25.7, 34.4, 42.2, 52.2, 67.8, 72.5, 73.0, 82.4, 86.1, 112.8. HRMS (m/z): $[H]^+$ calcd. for $C_{11}H_{20}N_2O_3$, 229.1547; found, 229.1550.

Preparation of compound 10a

Following the above general method for **8a**, compound **S4** (57 mg, 0.25 mmol) gave **10a** (28 mg, 95%) as a yellow foam.

10a: TLC (*i*-PrOH / NH_4OH = 2:1) R_f = 0.3. [α]D25 = 22.9° (*c* = 0.1 in H_2O); ^{1}H NMR (600 MHz, D_2O): δ 1.97-2.03 (m, 1H), 2.06-2.11 (m, 1H), 2.88-2.93 (m, 1H), 3.14-3.25 (m, 4H), 3.54-3.55 (m, 1H), 4.01-4.03 (m, 1H), 4.41-4.43 (m, 1H), 4.60-4.63 (m, 1H). ^{13}C NMR (150 MHz, D_2O): δ 35.1, 41.4, 51.3, 65.8, 66.2, 72.0, 73.0, 76.5. HRMS (m/z): $[H]^+$ calcd. for $C_8H_{16}N_2O_3$, 189.1234; found, 189.1233.

Preparation of compound 9b

Following the above general method for **6b**, compound **4a** (500 mg, 1.13 mmol) gave **9b** (318 mg, 0.85 mmol, 75%) as a yellow solid.

9b: TLC (*n*-hexane / EtOAc, v/v 1:1), R_f = 0.3. [α]D25 = 7.1° (*c* = 0.15 in CH_2Cl_2); ^{1}H H NMR (600 MHz, $CDCl_3$): δ 1.25 (s, 3H), 1.40 (s, 9H), 1.42 (s, 3H), 2.74-2.79 (m, 1H), 2.82-2.87 (m, 1H), 3.04-3.08 (m, 1H), 3.24-3.29 (m, 1H), 3.83-3.85 (m, 1H), 3.93-3.96 (m, 1H), 4.52-4.53 (m, 1H), 4.60-4.62 (m, 1H), 4.69-4.72 (m, 1H), 4.88 (br, 1H), 5.14 (d, 1H, *J* = 10.1 Hz), 5.22 (d, 1H, *J* = 17.2 Hz), 5.77-5.84 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.7, 26.6, 28.3, 33.6, 41.1, 63.5, 64.9, 75.5, 79.7, 80.3, 84.7, 113.1, 118.6, 132.5, 156.2, 161.2. HRMS (m/z): $[Na]^+$ calcd. for $C_{18}H_{28}N_2O_6$, 391.1840; found, 391.1843.

Preparation of compound S17

Following the above general method for **7b**, compound **9b** (318 mg, 0.86 mmol) gave **S17** (305 mg, 73% over 2 steps) as a white solid.

S17: TLC (*n*-hexane / EtOAc, v/v 1:1), $R_f = 0.5$. [α]D25 = -34.3° ($c = 0.1$ in CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$): δ 1.29 (s, 3H), 1.41 (s, 9H), 1.48 (s, 3H), 2.24 (br, 1H), 2.44 (br, 1H), 3.28 (br, 1H), 3.91 (br, 1H), 4.02 (br, 1H), 4.28 (br, 1H), 4.33 (br, 1H), 4.62 (br, 2H), 4.81 (m, 1H), 5.02-5.18 (m, 6H), 5.92 (br, 1H), 7.31-7.36 (m, 5H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 23.9, 25.7, 28.3, 38.3, 41.1, 64.4, 66.5, 67.6, 69.7, 79.7, 79.9, 80.8, 111.6, 116.2, 128.2, 128.3, 128.6, 135.9, 155.9, 156.1. HRMS (m/z): $[H]^+$ calcd. for $C_{25}H_{36}N_2O_7$, 477.2595, found 477.2604.

Preparation of compound S18

Following the above general method for **S14**, compound **S17** (27.3 mg, 0.06 mmol) gave **S18** (16.4 mg, 83% over 2 steps) as a white foam.

S18: TLC (CH_2Cl_2 / MeOH, v/v 20:1), $R_f = 0.1$. ^1H NMR (600 MHz, $CDCl_3$): δ 1.27 (s, 3H), 1.43 (s, 9H), 1.45 (s, 3H), 1.85-1.88 (m, 1H), 2.31-2.33 (m, 1H), 3.00-3.03 (m, 1H), 3.05-3.08 (m, 1H), 3.14 (br, 1H), 3.21-3.23 (m, 1H), 3.31 (br, 1H), 3.54-3.61 (m, 2H), 4.52 (br, 1H), 4.62-4.65 (m, 1H), 4.68-4.70 (m, 1H), 5.21 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.0, 26.7, 28.3, 35.8, 41.9, 53.4, 69.5, 72.0, 74.6, 79.4, 80.5, 85.2, 112.4, 156.1. HRMS (m/z): $[H]^+$ calcd. for $C_{16}H_{28}N_2O_5$, 329.2071; found, 329.2079.

Preparation of compound 10b

Following the above general method for **8a**, compound **S6** (16.4 mg, 0.05 mmol) gave **10b** (8.8 mg, 0.047 mmol, 94%) as a yellow foam.

10b: TLC (*i*-PrOH / NH_4OH, v/v 3:1) R_f= 0.2. [α]D25 = 3.84° (*c* = 0.1 in H_2O); ^1H NMR (600 MHz, D_2O): δ 1.74-1.79 (m, 1H), 2.08-2.13 (m, 1H), 2.65-2.69 (m, 1H), 2.86-2.90 (m, 1H), 3.07-3.15 (m, 3H), 3.30-3.32 (m, 1H), 3.92-3.94 (m, 1H), 4.11-4.13 (m, 1H), 4.48-4.51 (m, 1H). ^{13}C NMR (150 MHz, D_2O): δ 34.3, 41.3, 52.0, 64.4, 70.1, 70.3, 72.5, 76.1. HRMS (m/z): $[H]^+$ calcd. for $C_8H_{16}N_2O_3$, 189.1234, found 189.1246.

Preparation of compound 8b-1

1-(trans-4-hexylcyclohexyl)-4-isothiocyanatobenzene (16.6 mg, 0.055 mmol) was added to a mixture of **8b** (10 mg, 0.05 mmol) and Et_3N (14 μL, 0.1 mmol) in DMSO (0.5 mL) and stirred for 1 h. The reaction extracted with EtOAc and H_2O. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2 / MeOH = 20:1, silica gel) to give **8b-1** (7 mg, 0.012 mmol, 60%) as a white solid.

8b-1: TLC (CH_2Cl_2 / MeOH, v/v 10:1), R_f= 0.3. [α]D25 = 7.0° (*c* = 0.1 in MeOH); ^1H NMR (600 MHz, MeOD): δ 0.94 (t, 3H, *J* = 7.0 Hz), 1.07-1.14 (m, 2H), 1.24-1.37 (m, 8H), 1.47-1.54 (m, 2H), 1.57-1.65 (m, 2H), 1.88-1.92 (m, 4H), 1.99-2.02 (m, 1H), 2.48-2.53 (m, 2H), 2.61 (br, 1H), 2.92-2.94 (m, 1H), 3.24-3.27 (m, 1H), 3.49-3.52 (m, 1H), 3.90 (br, 1H), 3.95 (br, 1H), 4.01-4.03 (m, 1H), 4.12-4.14 (m, 1H), 4.62 (br, 1H), 7.23-7.28 (m, 4H). ^{13}C NMR (150 MHz, MeOD): δ 13.0, 22.3, 26.6, 29.3, 29.4, 31.7, 32.1, 33.3, 34.1, 37.2, 43.7, 44.2, 45.1, 48.1, 65.7, 68.1, 70.7, 79.3, 124.6, 127.4. HRMS (m/z): $[H]^+$ calcd. for $C_{28}H_{45}N_3O_3S$, 504.3254; found, 504.3249.

Preparation of compound 8b-2

Isothiocyanatobenzene (7.4 mg, 0.055 mmol) was added to a mixture of **8b** (10 mg, 0.05 mmol) and Et_3N (14 μL, 0.1 mmol) in DMSO (0.5 mL) and stirred for 1 h. After the reaction was complete, the reaction was extracted with EtOAc and H_2O. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2 / MeOH = 20:1, silica gel) to give **8b-2** (8.4 mg, 0.025 mmol, 50%) as a white solid.

8b-2: TLC (CH_2Cl_2 / MeOH, v/v 10:1), R_f = 0.3. [α]D25 = -5.2° (*c* = 0.1 in MeOH); ^{1}H NMR (600 MHz, MeOD): δ 1.26-1.32 (m, 1H), 1.60-1.69 (m, 2H), 2.00-2.04 (m, 1H), 2.58-2.61 (m, 1H), 2.68 (br, 1H), 2.96-2.99 (m, 1H), 3.29 (br, 1H), 3.54 (dd, 1H, *J* = 13.5, 7.5 Hz), 3.93-3.99 (m, 2H), 4.04 (dd, 1H, *J* = 5.7, 3.9 Hz), 4.16 (t, 1H, *J* = 5.7 Hz), 7.23 (t, 1H, *J* = 7.1 Hz), 7.36-7.41 (m, 4H). ^{13}C NMR (150 MHz, MeOD): δ 21.2, 29.3, 39.0, 43.7, 45.4, 65.6, 68.0, 70.6, 73.9, 124.3, 125.6, 129.0, 137.9, 181.0. HRMS (m/z): $[H]^+$ calcd. for $C_{16}H_{23}N_3O_3S$, 338.1533; found, 338.1540.

Preparation of compound 8b-3

1-(trans-4-propylcyclohexyl)-4-isothiocyanatobenzene (14 mg, 0.055 mmol) was added to a solution of **8b** (10 mg, 0.05 mmol) and Et_3N (14 μL, 0.1 mmol) in DMSO (0.5 mL) and stirred for 1 h. After the reaction was complete, the reaction was extracted with EtOAc and H_2O. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2 / MeOH = 20:1, silica gel) to give **8b-3** (12 mg, 0.03 mmol, 52%) as a white solid.

8b-3: TLC (CH_2Cl_2 / MeOH, v/v 10:1), R_f = 0.3. [α]D25 = 3.6° (*c* = 0.1 in MeOH); ^{1}H NMR (600 MHz, MeOD): δ 0.94 (t, 3H, *J* = 7.2 Hz), 1.07-1.14 (m, 2H), 1.24-1.42 (m, 12H), 1.47-1.54 (m, 2H), 1.61-1.68 (m, 2H), 1.88-1.91 (m, 4H), 2.00-2.03 (m, 1H), 2.49-2.55 (m, 2H), 2.63 (br, 1H), 2.94-2.96 (m, 1H), 3.26-3.27 (m, 1H), 3.50-3.53 (m, 1H), 3.92-3.96 (m, 2H), 4.01-4.03 (m, 1H), 4.12-4.14 (m, 1H), 7.23-7.28 (m, 4H). ^{13}C

NMR (150 MHz, MeOD): δ 13.3, 19.7, 21.0, 29.3, 31.8, 33.3, 34.2, 36.9, 39.5, 43.7, 44.2, 45.6, 48.4, 60.8, 65.5, 66.3, 68.0, 70.7, 72.1, 73.8, 124.7, 127.4, 146.0. HRMS (m/z): $[H]^+$ calcd. for $C_{25}H_{39}N_3O_3S$, 462.2785; found, 462.2800.

Preparation of compound 8b-4

2,5-dioxopyrrolidin-1-yl 4-((1s,4r)-4-propylcyclohexyl) benzoate (52 mg, 0.15 mmol) was added to a solution of **8b** (10 mg, 0.05 mmol) and Et_3N (14 μL, 0.1 mmol) in DMSO (0.5 mL) and stirred for 8 h. After the reaction was complete, the reaction was extracted with EtOAc and H_2O. The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The residue was purified by column chromatography (CH_2Cl_2 / MeOH = 20:1, silica gel) to give **8b-4** (12.4 mg, 0.028 mmol, 58%) as a white solid.

8b-4: TLC (CH_2Cl_2 / MeOH, v/v 10:1), R_f= 0.3. [α]D25 = -9.5° (c = 0.1 in MeOH); ^{1}H NMR (600 MHz, MeOD): δ 0.94 (t, 3H, J = 7.1 Hz), 1.08-1.14 (m, 2H), 1.19-1.42 (m, 10H), 1.49-1.55 (m, 2H), 1.62-1.64 (m, 2H), 1.90 (t, 4H, J = 11.9 Hz), 2.02-2.04 (m, 1H), 2.54-2.64 (m, 3H), 2.98-3.00 (m, 1H), 3.28-3.29 (m, 1H), 3.35-3.38 (m, 1H), 3.51 (q, 1H, J = 6.9 Hz), 3.67-3.70 (m, 1H), 3.87-3.91 (m, 1H), 4.03-4.04 (m, 1H), 4.22-4.23 (m, 1H), 7.33 (d, 2H, J = 7.3 Hz), 7.75 (d, 2H, J = 7.3 Hz). ^{13}C NMR (150 MHz, MeOD): δ 13.3, 14.0, 19.6, 21.9, 32.2, 33.2, 33.9, 36.8, 38.7, 39.5, 44.5, 45.5, 65.6, 65.8, 66.4, 68.4, 70.3, 74.3, 126.6, 127.0, 131.7, 151.7, 169.0. HRMS (m/z): $[H]^+$ calcd. for $C_{25}H_{38}N_2O_4$, 431.2904; found, 431.2906.

X-Ray ellipsoid plots of 4a.

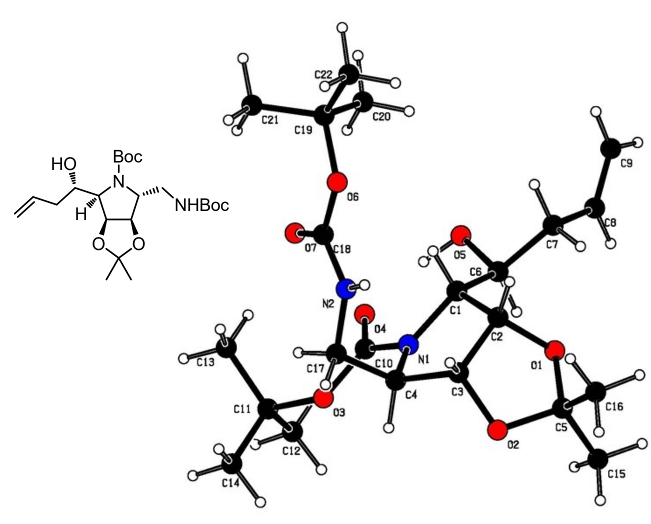


Table 1. Crystal data and structure refinement for**4a**.

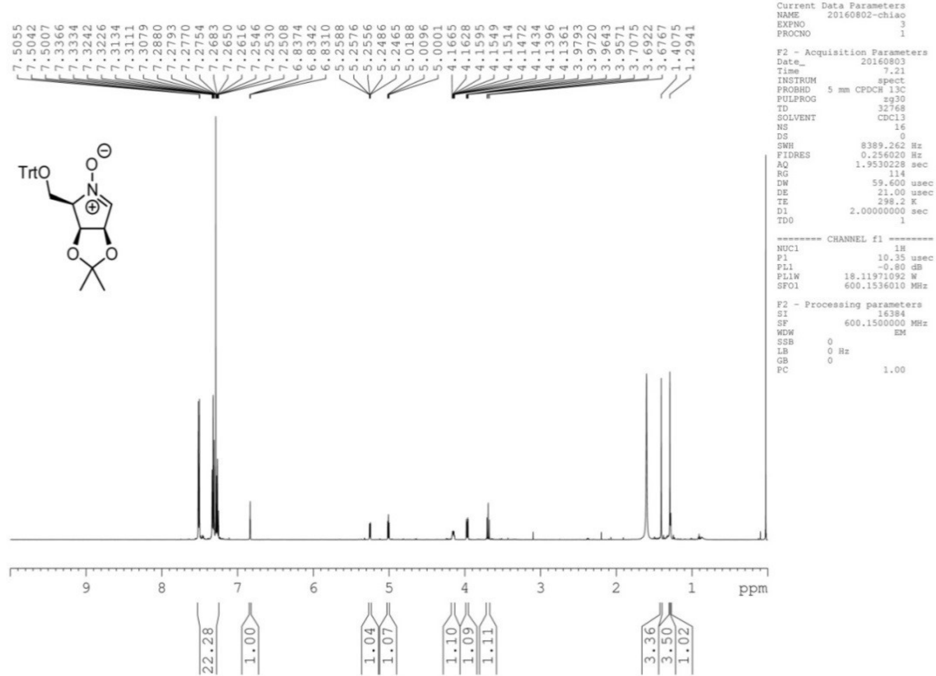
Identification code	i17332	
Empirical formula	C22 H38 N2 O7	
Formula weight	442.54	
Temperature	100.0(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 9.6080(3) Å	a= 90°.
	b = 13.2461(5) Å	b= 90°.
	c = 20.0321(6) Å	g = 90°.
Volume	2549.46(15) $Å^3$	
Z	4	
Density (calculated)	1.153 Mg/m^3	
Absorption coefficient	0.085 mm^{-1}	
F(000)	960	
Crystal size	0.528 x 0.482 x 0.402 mm^3	
Theta range for data collection	1.843 to 27.141°.	
Index ranges	-12<=h<=12, -17<=k<=17, -25<=l<=25	
Reflections collected	47973	
Independent reflections	5632 [R(int) = 0.0599]	
Completeness to theta = 25.242°	100.0 %	
Absorption correction	Numerical	
Max. and min. transmission	1 and 0.9259	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5632 / 0 / 296	

Goodness-of-fit on F^2	1.024
Final R indices [I>2sigma(I)]	R1 = 0.0345, wR2 = 0.0797
R indices (all data)	R1 = 0.0391, wR2 = 0.0824
Absolute structure parameter	-0.3(3)
Extinction coefficient	n/a
Largest diff. peak and hole	0.169 and -0.195 e.$Å^{-3}$

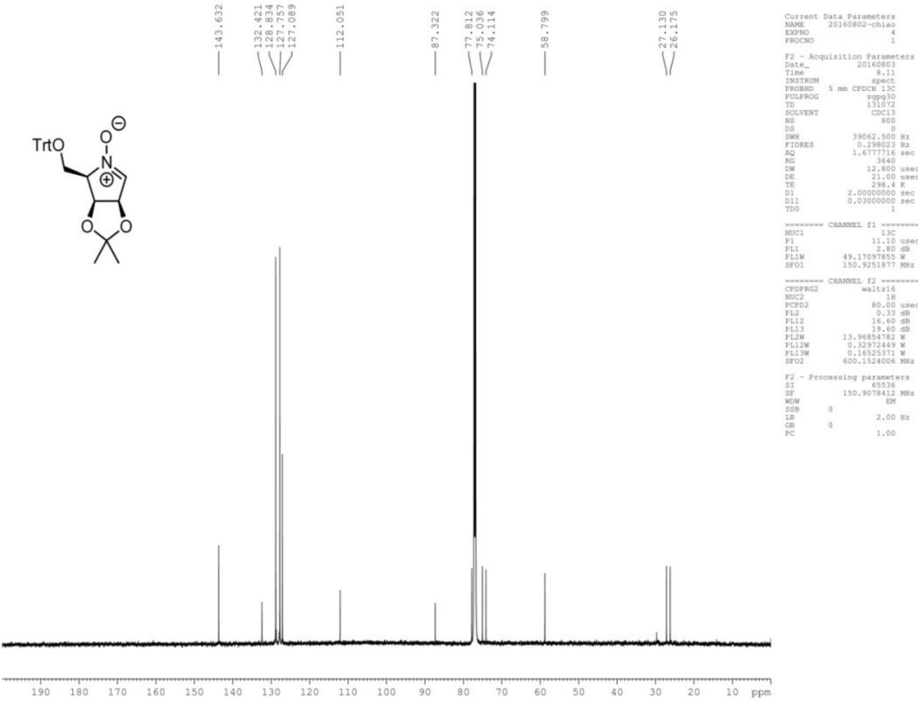
Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for i17332. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
O(1)	4495(2)	7425(1)	8930(1)	31(1)
O(2)	2339(2)	7960(1)	8716(1)	26(1)
O(3)	1639(1)	5543(1)	7162(1)	22(1)
O(4)	3749(1)	4810(1)	7226(1)	27(1)
O(5)	6133(1)	5150(1)	7731(1)	24(1)
O(6)	5387(2)	8377(1)	6070(1)	30(1)
O(7)	4260(2)	6867(1)	6040(1)	29(1)
N(1)	3340(2)	6399(1)	7635(1)	18(1)
N(2)	3482(2)	8188(1)	6655(1)	22(1)
C(1)	4782(2)	6709(1)	7813(1)	17(1)
C(2)	4527(2)	7662(1)	8232(1)	21(1)
C(3)	3032(2)	8022(1)	8088(1)	19(1)
C(4)	2395(2)	7274(1)	7605(1)	18(1)
C(5)	3386(2)	7990(2)	9213(1)	29(1)
C(6)	5687(2)	5925(1)	8172(1)	19(1)
C(7)	7030(2)	6396(2)	8431(1)	27(1)
C(8)	7891(2)	5640(2)	8800(1)	30(1)
C(9)	9210(3)	5446(2)	8696(1)	46(1)
C(10)	2981(2)	5531(1)	7328(1)	20(1)
C(11)	1019(2)	4719(1)	6768(1)	26(1)
C(12)	990(2)	3762(2)	7181(1)	37(1)
C(13)	1794(2)	4596(2)	6116(1)	37(1)
C(14)	-442(2)	5112(2)	6652(1)	37(1)
C(15)	2860(3)	7442(2)	9820(1)	45(1)
C(16)	3841(3)	9065(2)	9358(1)	35(1)
C(17)	2237(2)	7704(1)	6899(1)	21(1)
C(18)	4368(2)	7724(1)	6235(1)	22(1)
C(19)	6580(2)	8054(2)	5672(1)	34(1)

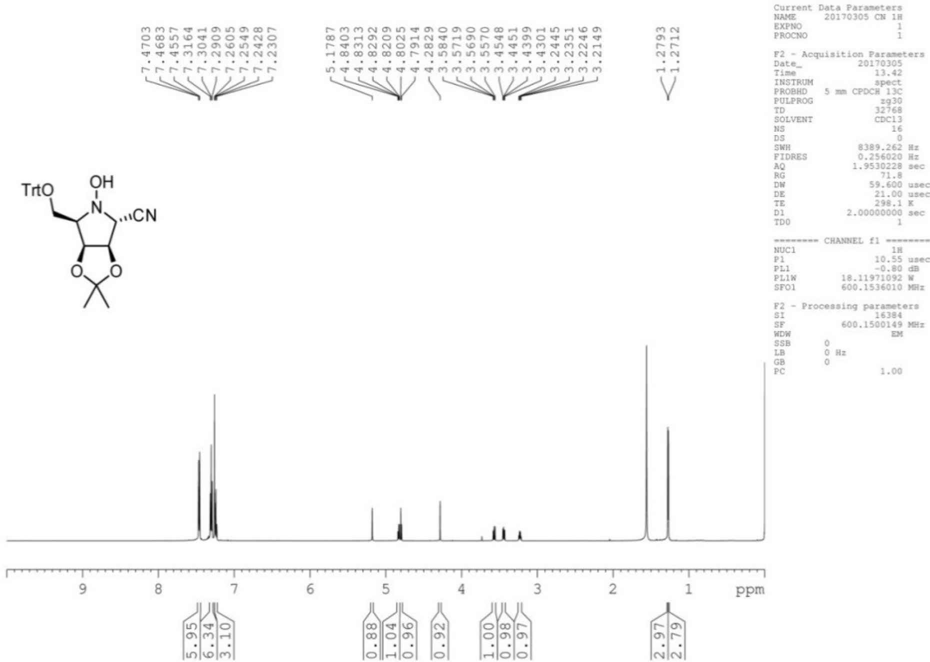
C(20)	7355(3)	7231(2)	6040(2)	57(1)
C(21)	6115(3)	7734(2)	4985(1)	51(1)
C(22)	7437(3)	9014(2)	5634(1)	51(1)



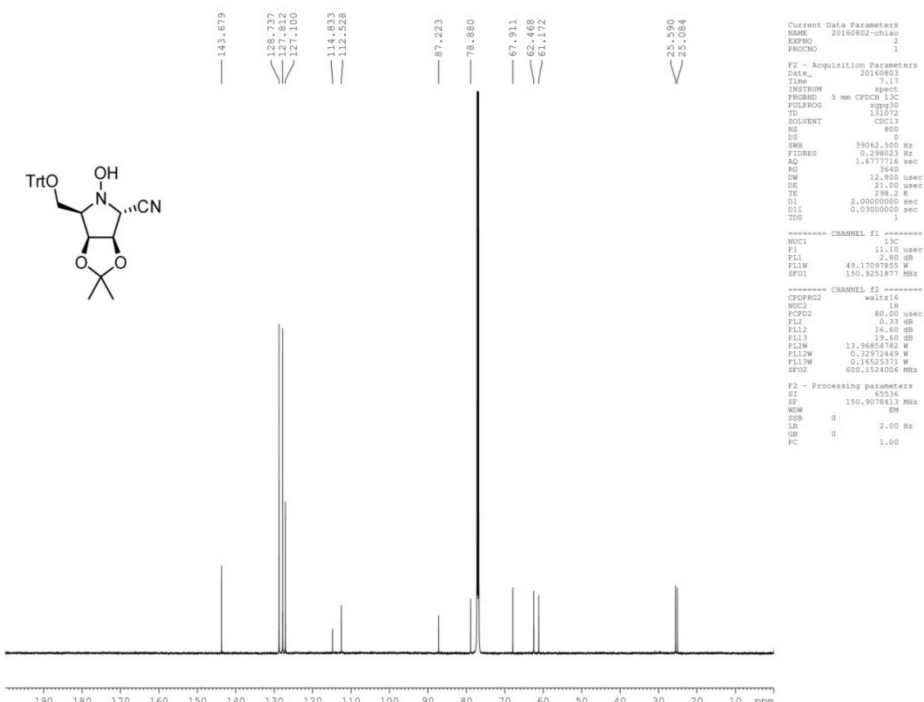
^{1}H NMR Spectra of compound **1** (600 MHz, $CDCl_3$)



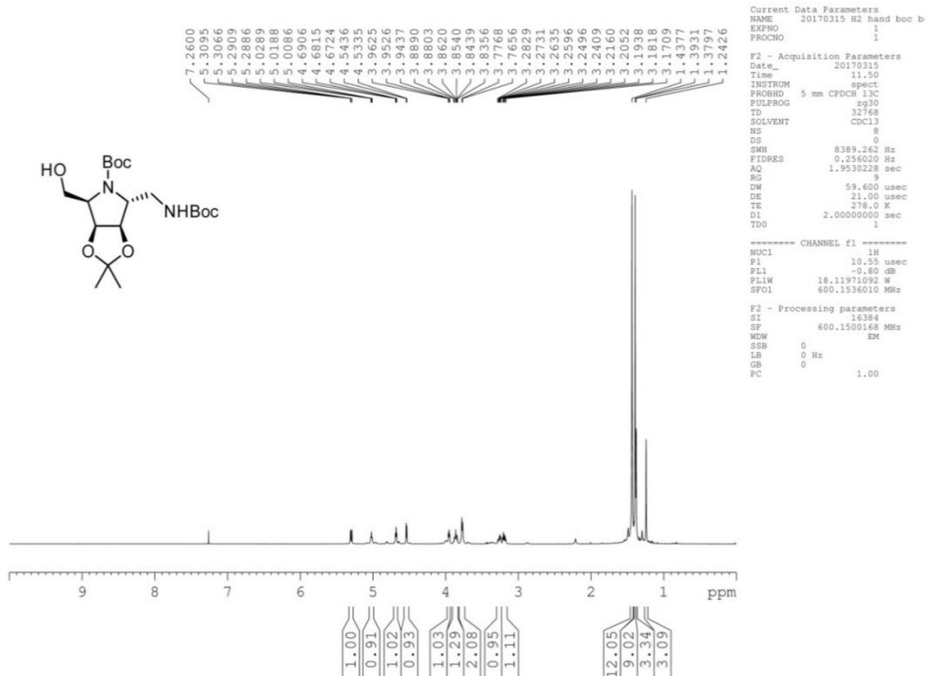
^{13}C NMR Spectra of compound **1** (150 MHz, $CDCl_3$)



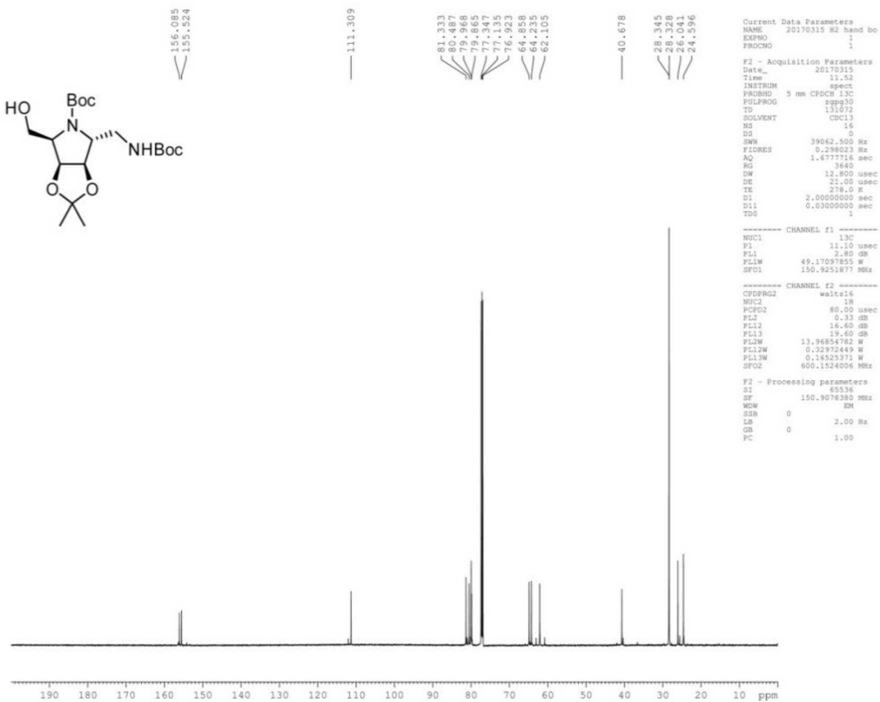
^1H NMR Spectra of compound **2** (600 MHz, $CDCl_3$)



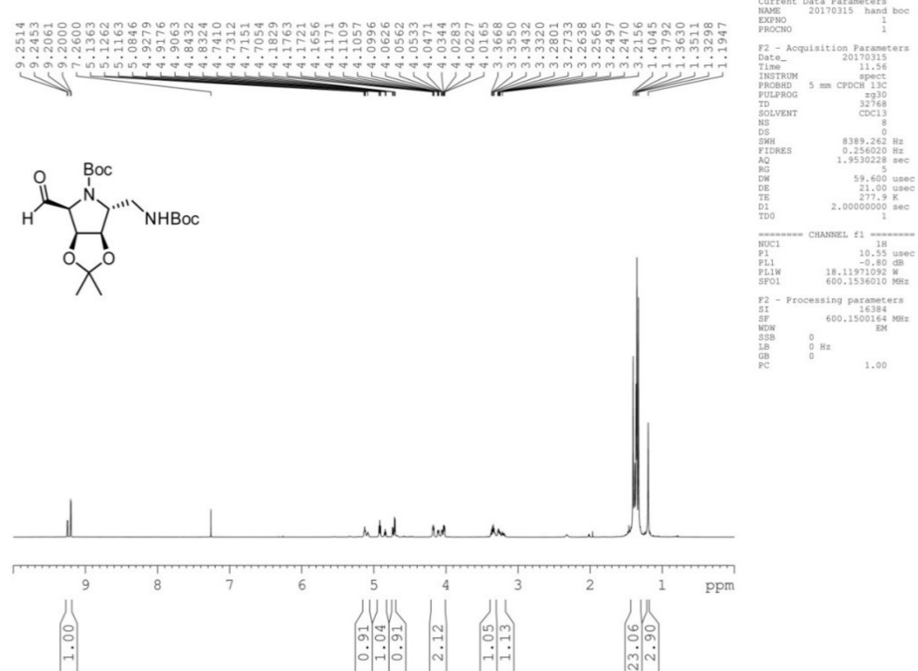
^{13}C NMR Spectra of compound **2** (150 MHz, $CDCl_3$)



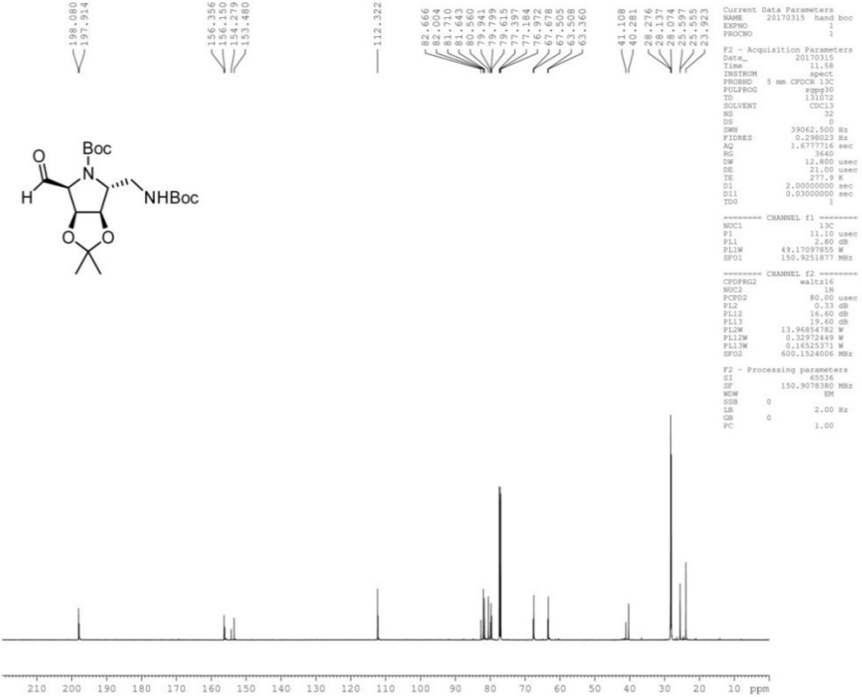
^{1}H NMR Spectra of compound **S1** (600 MHz, $CDCl_3$)



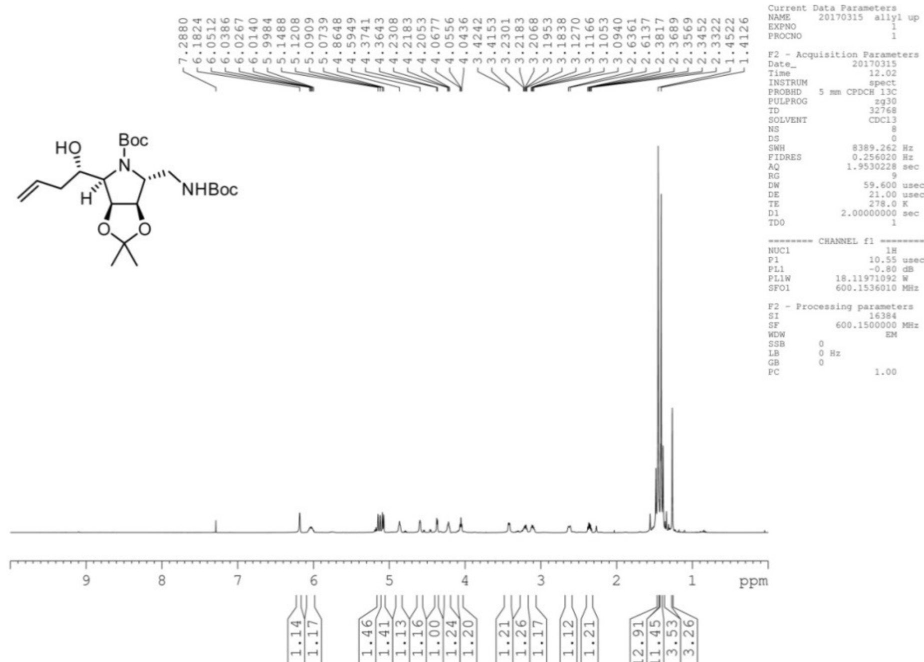
^{13}C NMR Spectra of compound **S1** (150 MHz, $CDCl_3$)



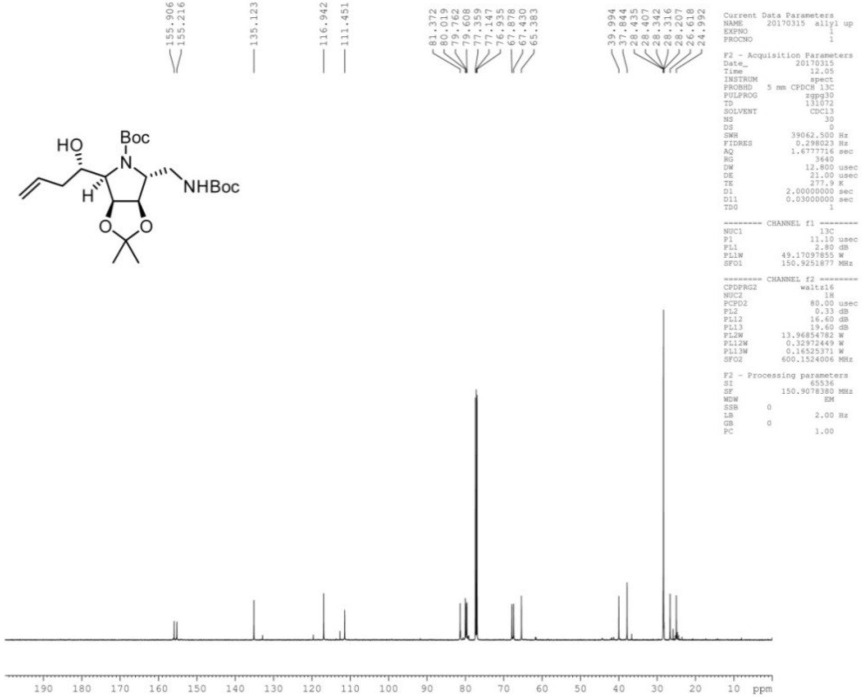
^{1}H NMR Spectra of compound **3** (600 MHz, $CDCl_3$)



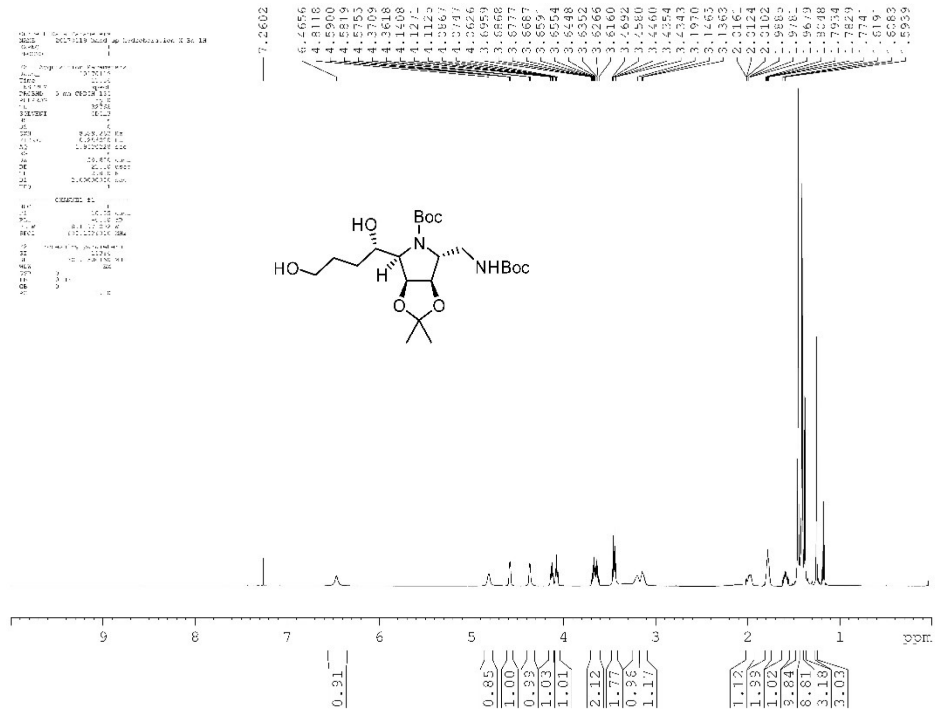
^{13}C NMR Spectra of compound **3** (150 MHz, $CDCl_3$)



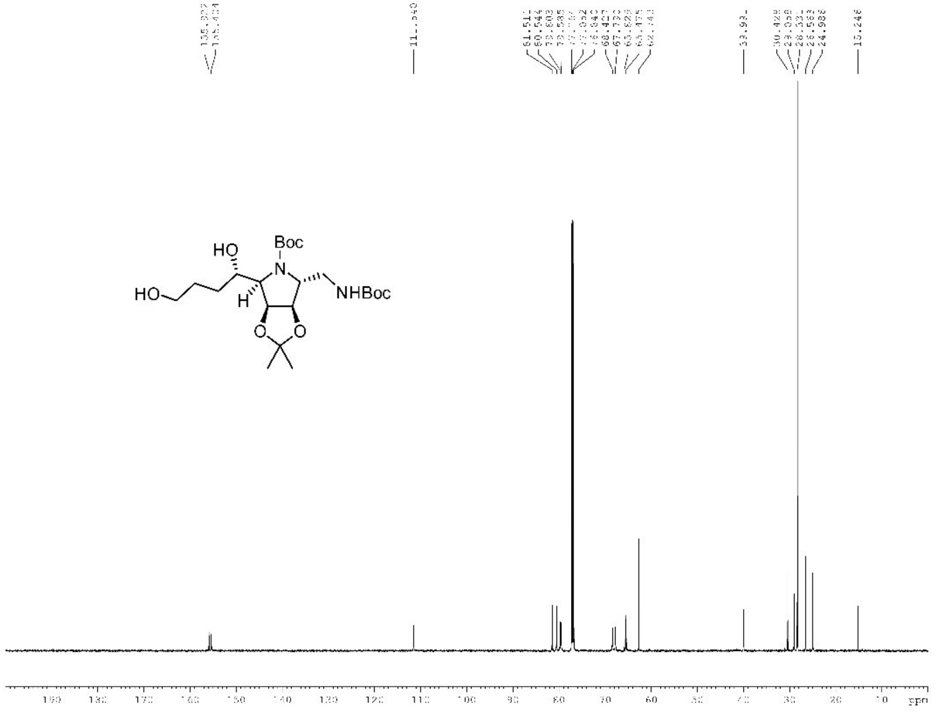
^{1}H NMR Spectra of compound **4a** (600 MHz, $CDCl_3$)



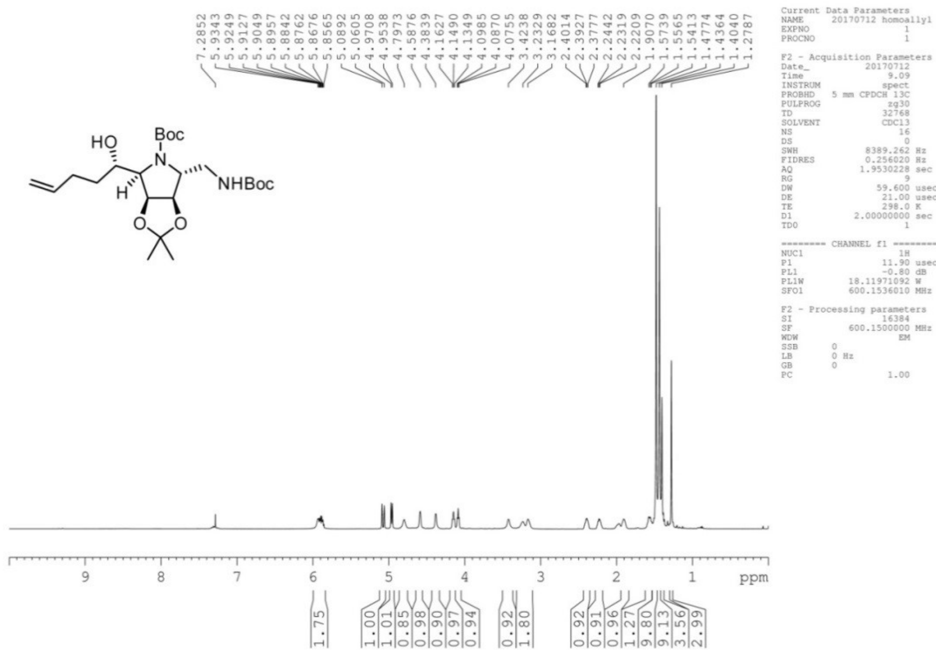
^{13}C NMR Spectra of compound **4a** (150 MHz, $CDCl_3$)



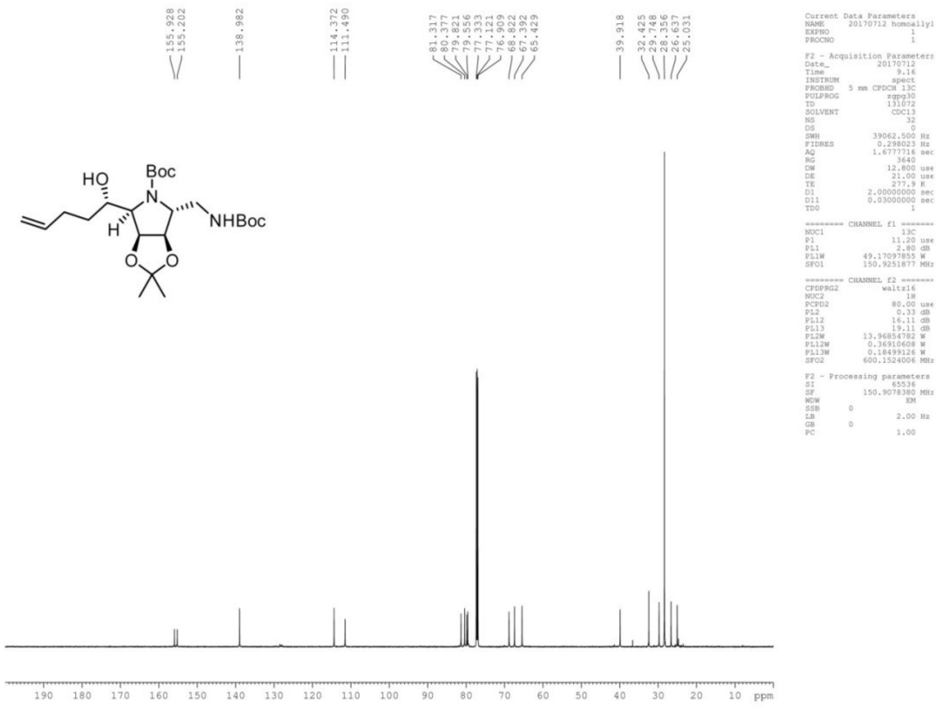
^1H NMR Spectra of compound **5a** (600 MHz, $CDCl_3$)



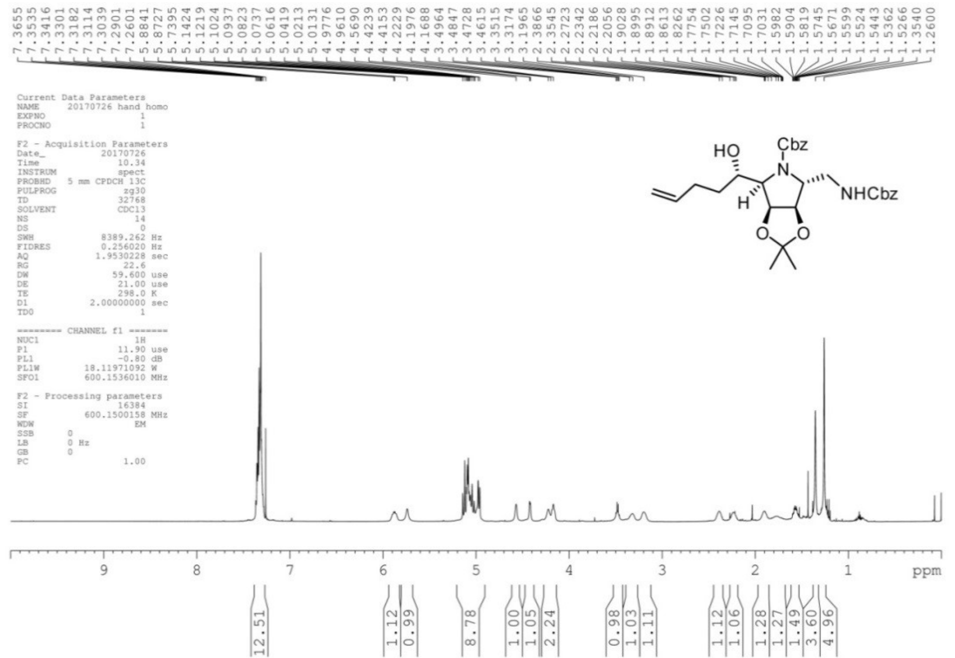
^{13}C NMR Spectra of compound **5a** (150 MHz, $CDCl_3$)



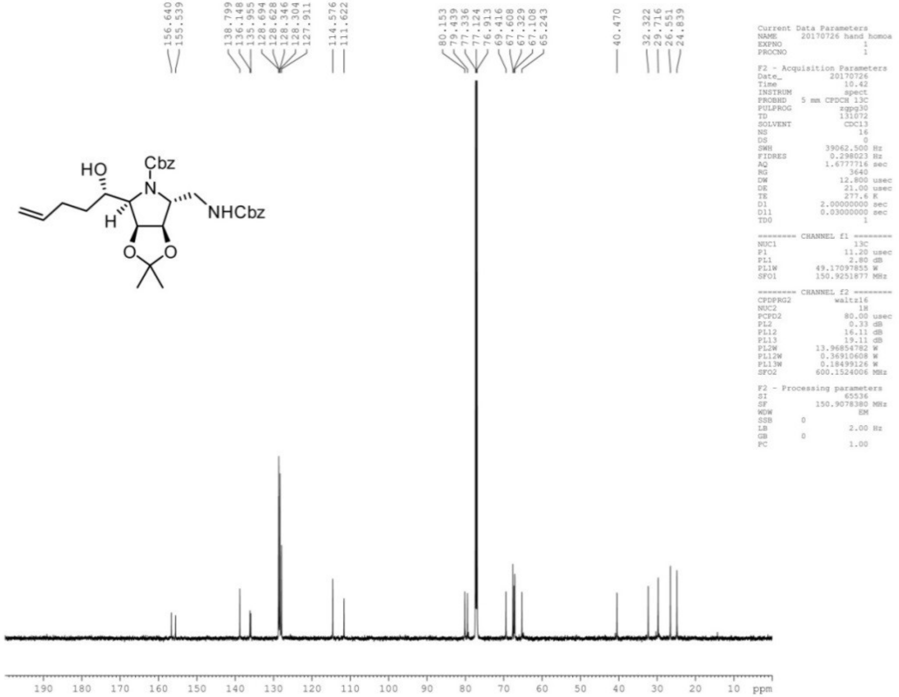
^1H NMR Spectra of compound **6a** (600 MHz, $CDCl_3$)



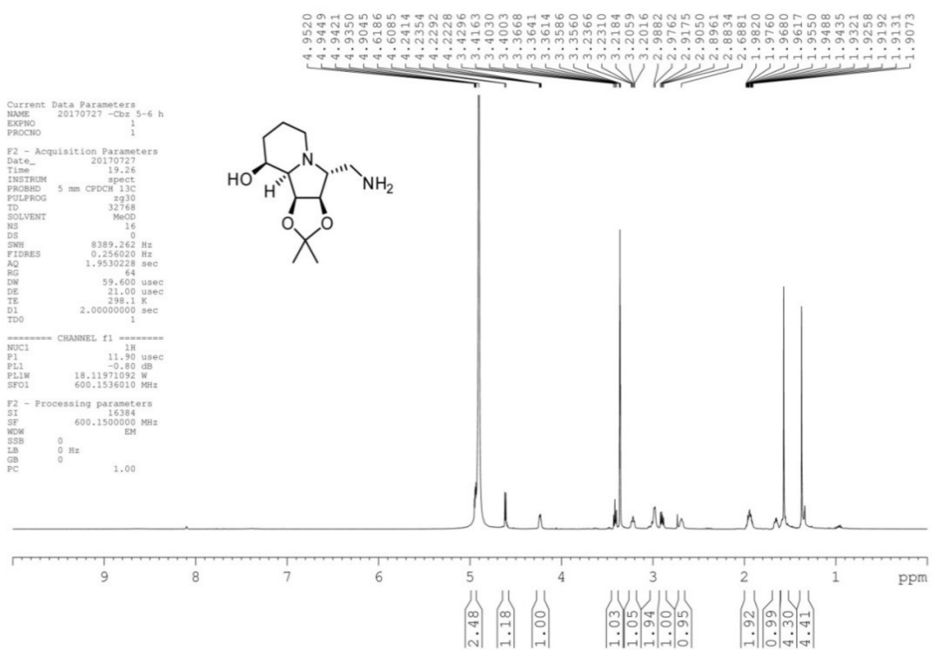
^{13}C NMR Spectra of compound **6a** (150 MHz, $CDCl_3$)



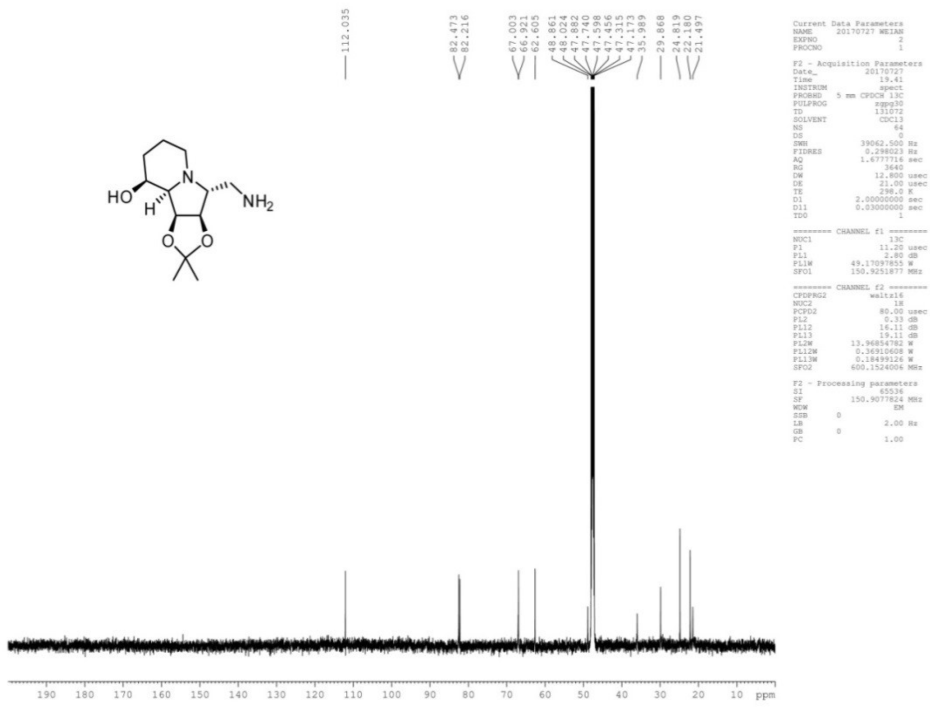
^{1}H NMR Spectra of compound **7a** (600 MHz, $CDCl_3$)



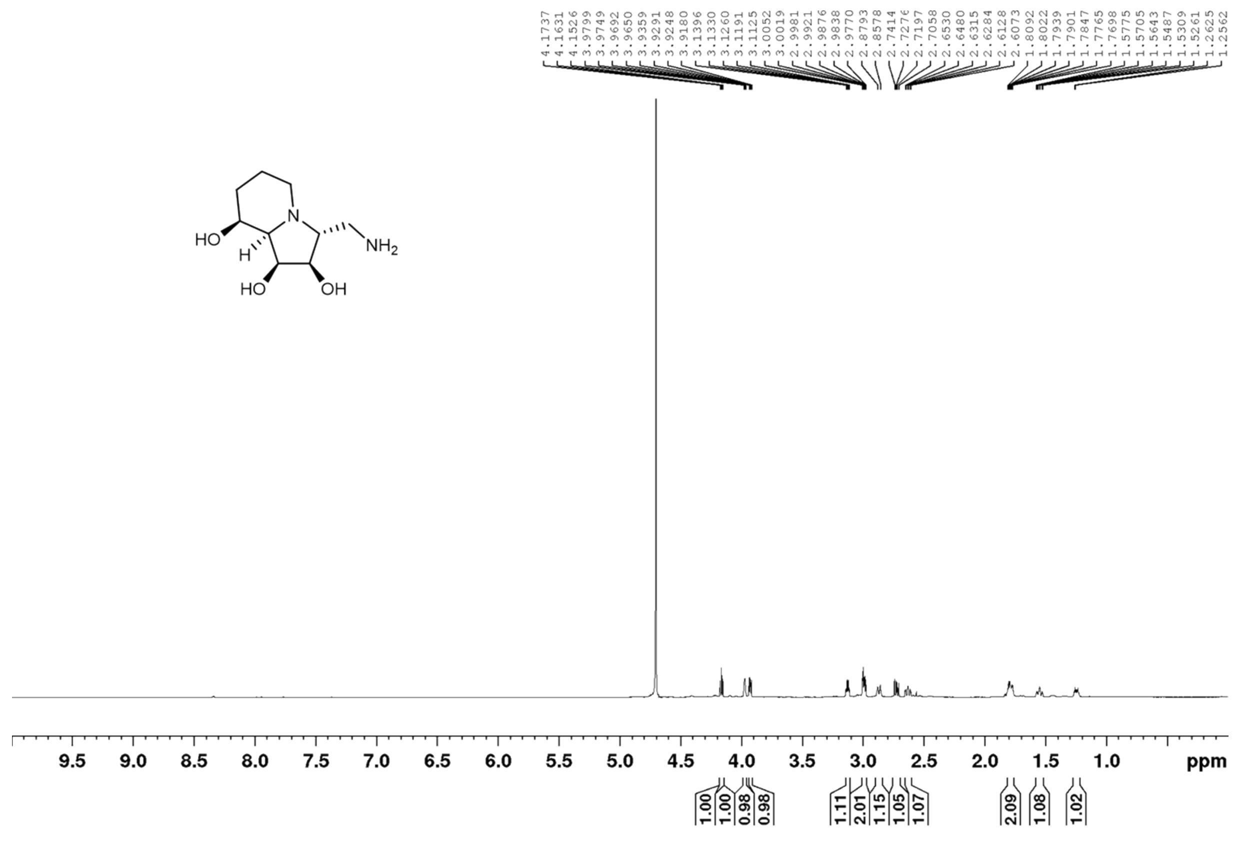
^{13}C NMR Spectra of compound **7a** (150 MHz, $CDCl_3$)



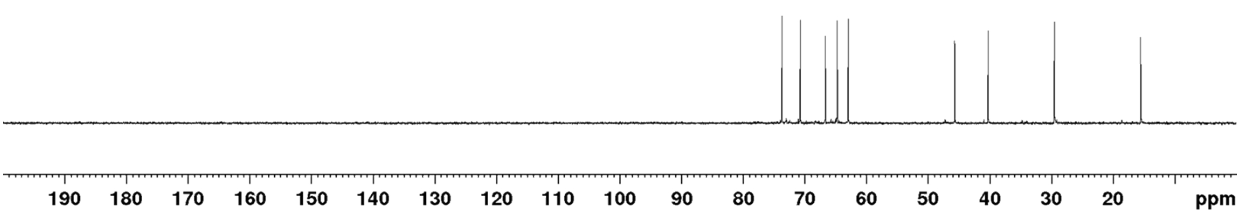
^{1}H NMR Spectra of compound **S2** (600 MHz, MeOD)



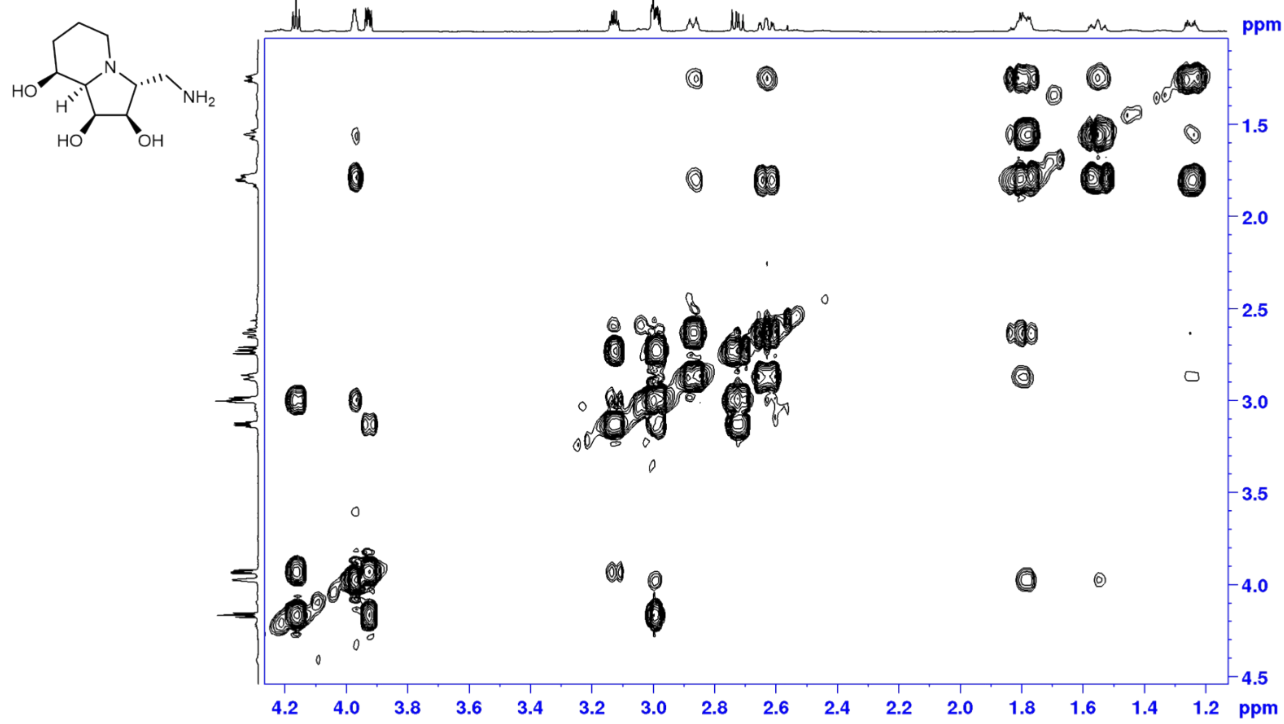
^{13}C NMR Spectra of compound **S2** (150 MHz, MeOD)



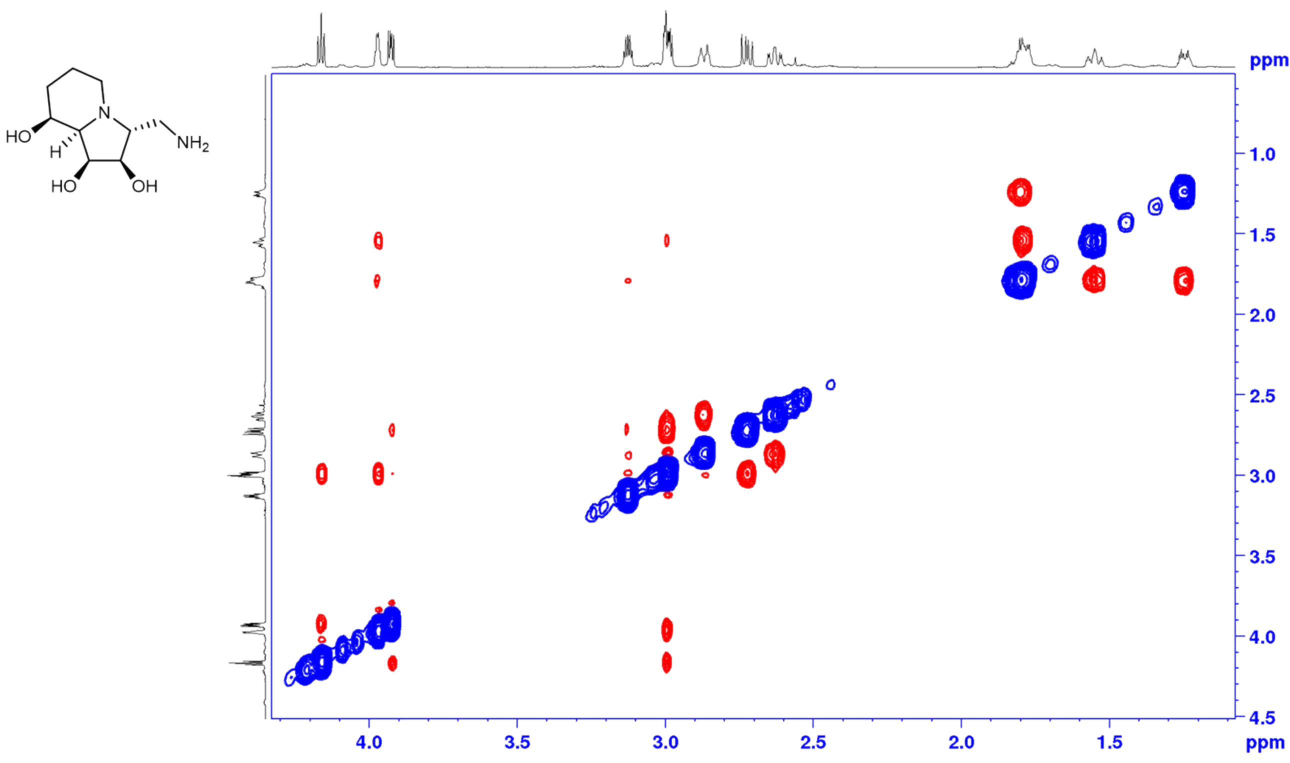
^{1}H NMR Spectra of compound **8a** (600 MHz, D_2O)



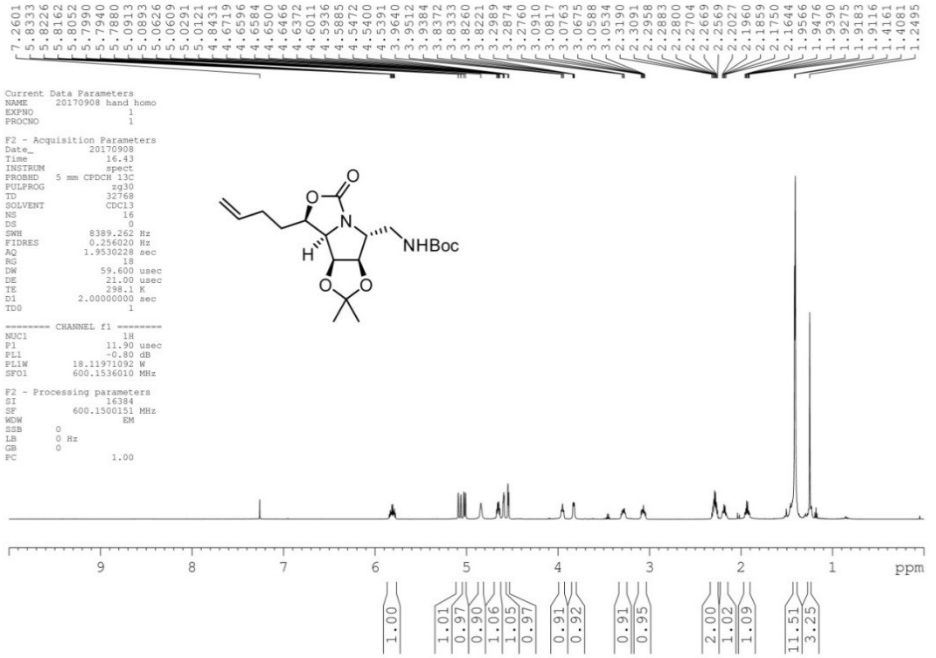
^{13}C NMR Spectra of compound **8a** (150 MHz, D_2O)



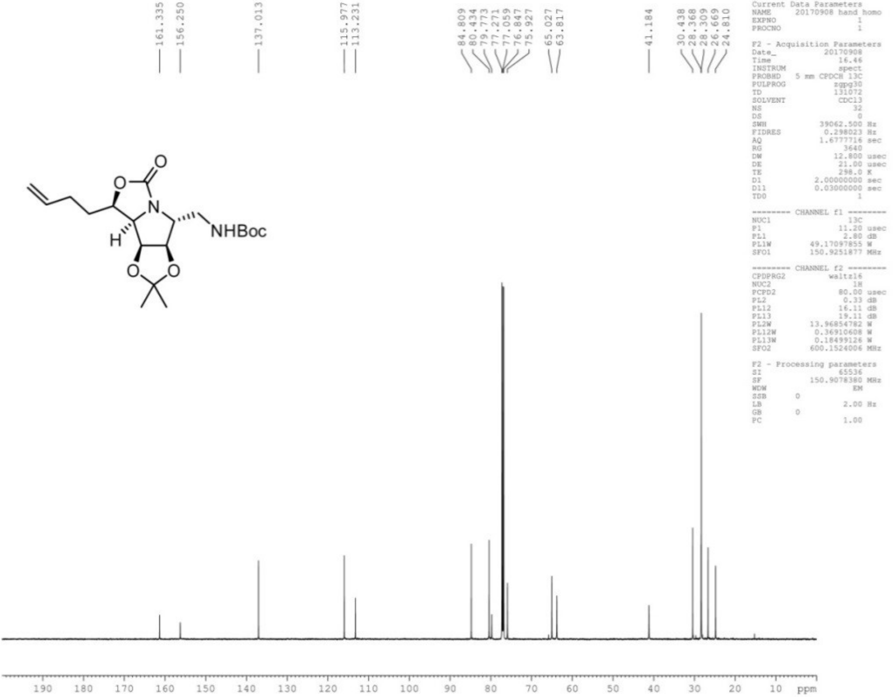
COSY Spectra of compound**8a** (600 MHz, D_2O)



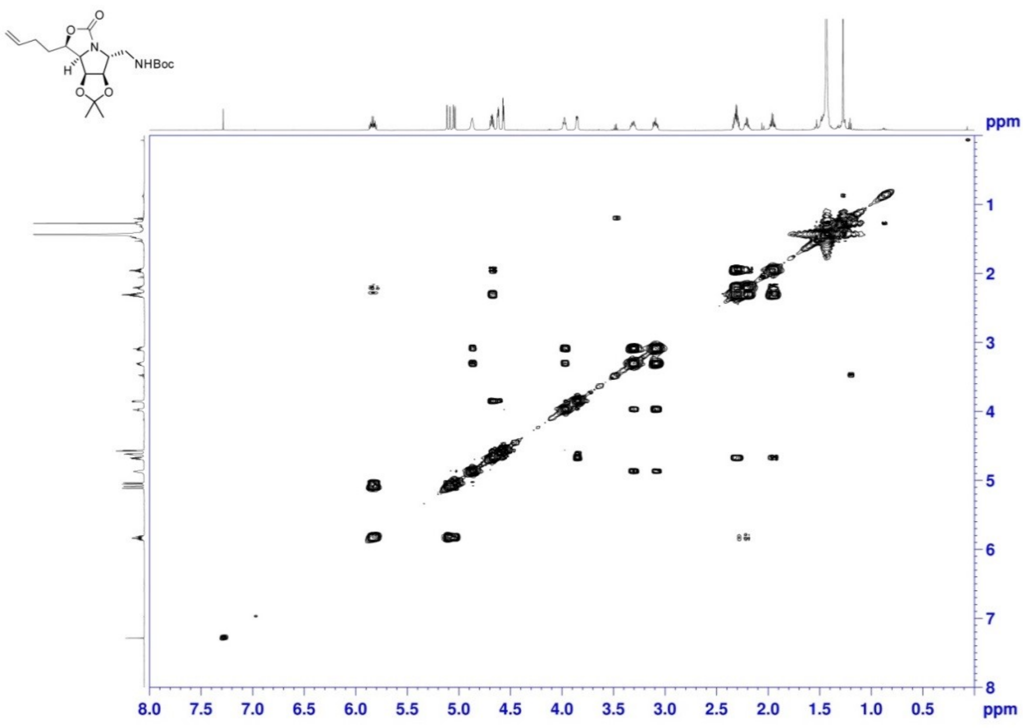
NOESY Spectra of compound **8a** (600 MHz, D_2O)



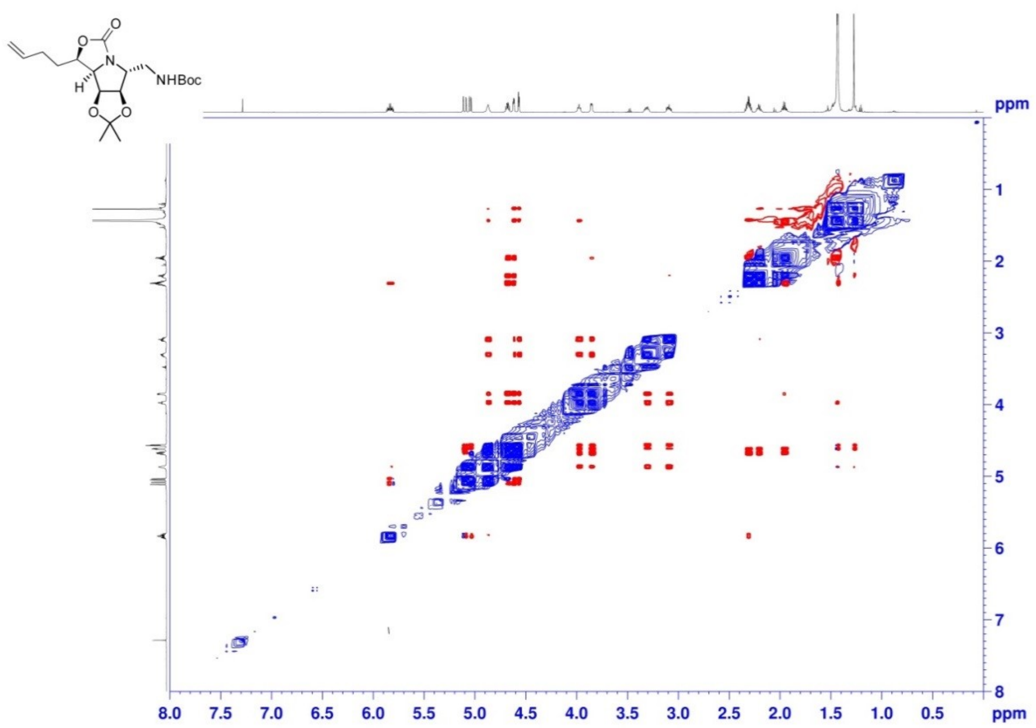
^{1}H NMR Spectra of compound **6b** (600 MHz, $CDCl_3$)



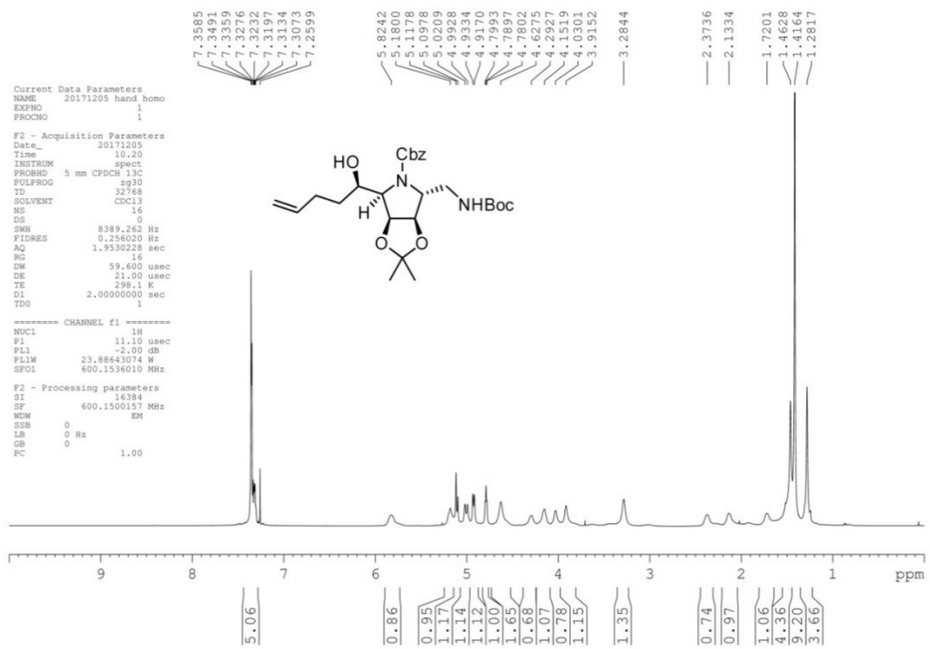
^{13}C NMR Spectra of compound **6b** (150 MHz, $CDCl_3$)



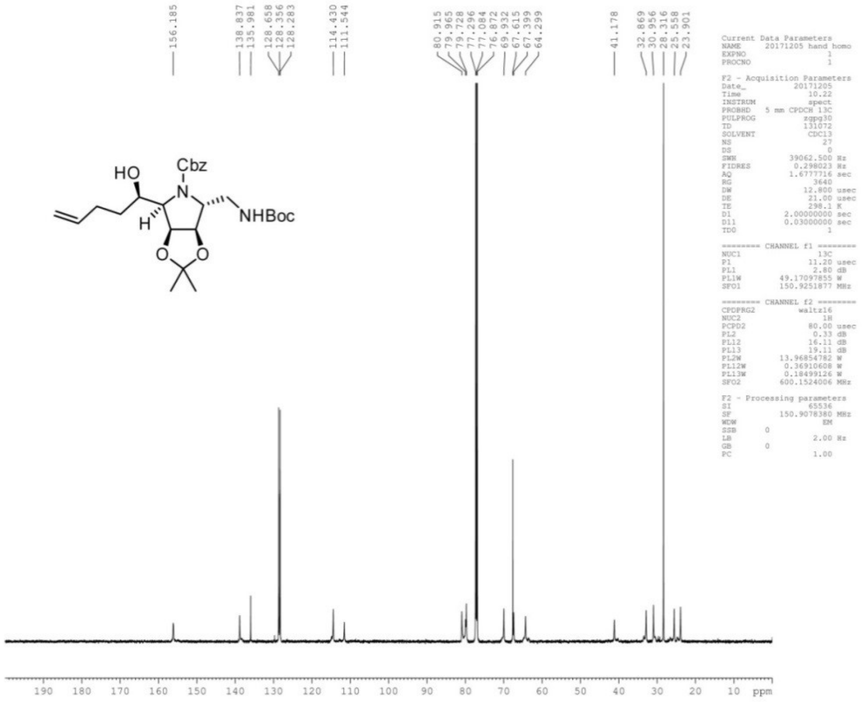
COSY Spectra of compound **6b** (600 MHz, $CDCl_3$)



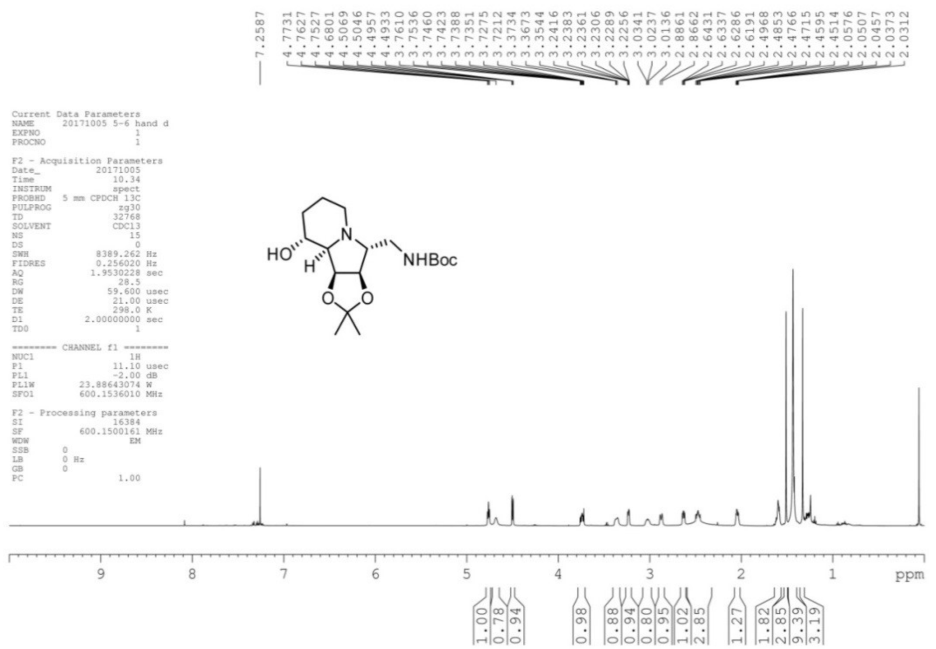
NOESY Spectra of compound **6b** (600 MHz, $CDCl_3$)



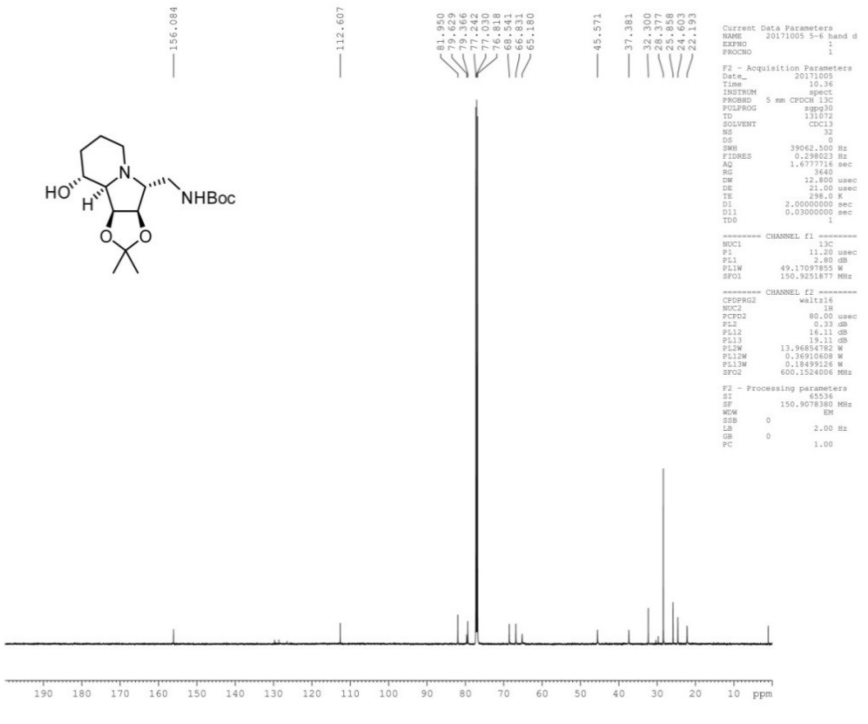
^{1}H NMR Spectra of compound **7b** (600 MHz, $CDCl_3$)



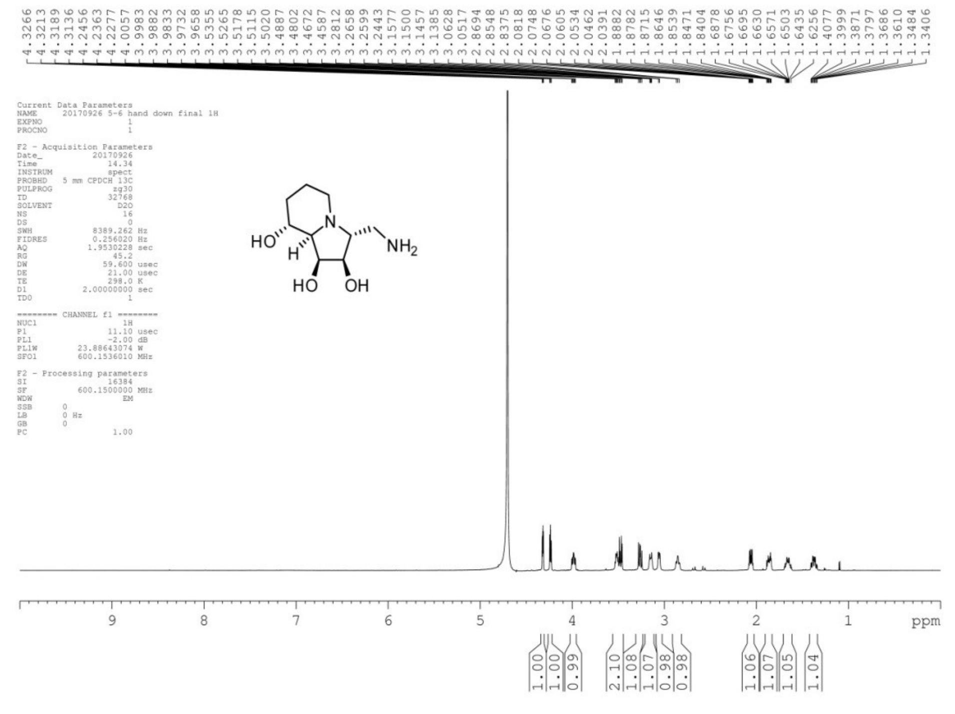
^{13}C NMR Spectra of compound**7b** (150 MHz, $CDCl_3$)



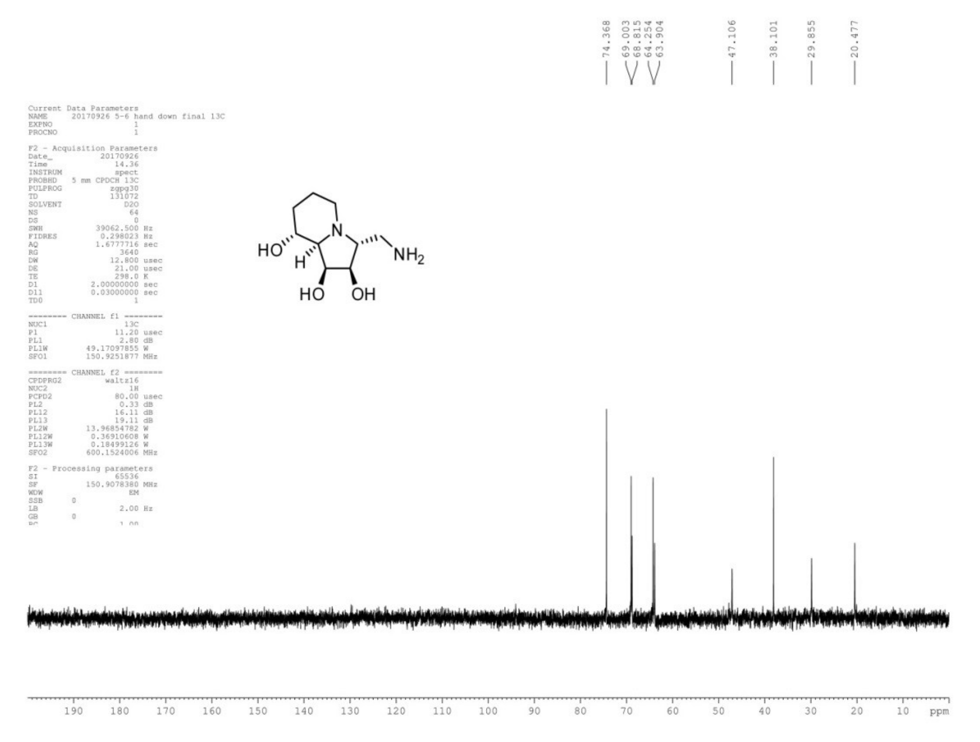
^{1}H NMR Spectra of compound **S3** (600 MHz, $CDCl_3$)



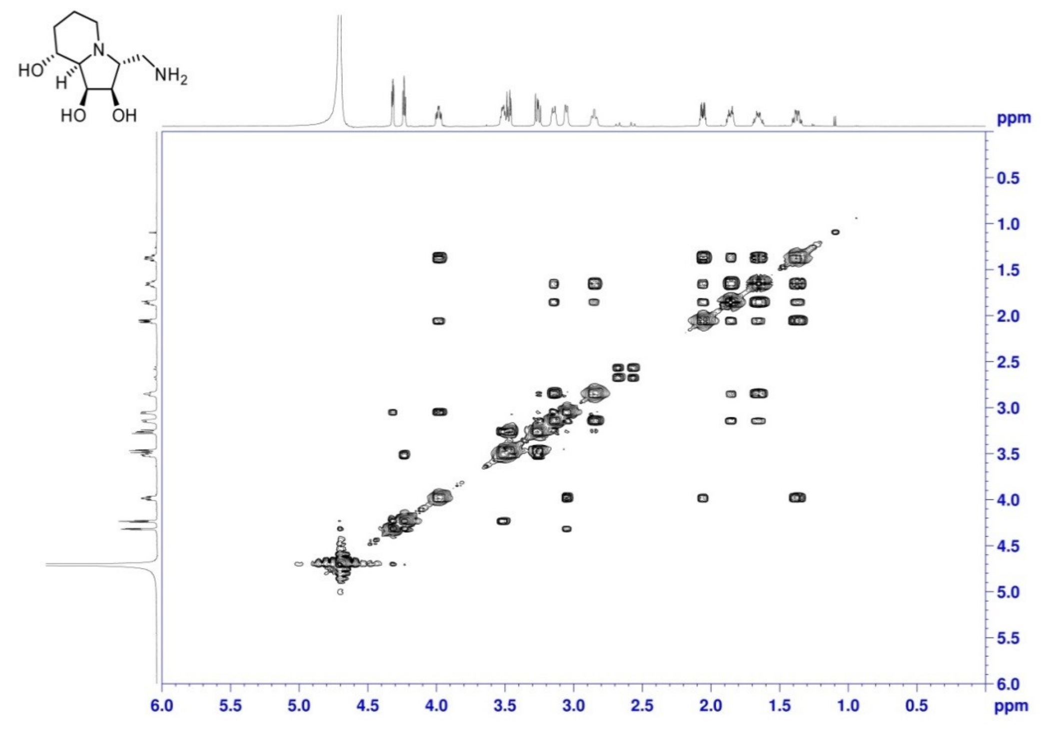
^{13}C NMR Spectra of compound **S3** (150 MHz, $CDCl_3$)



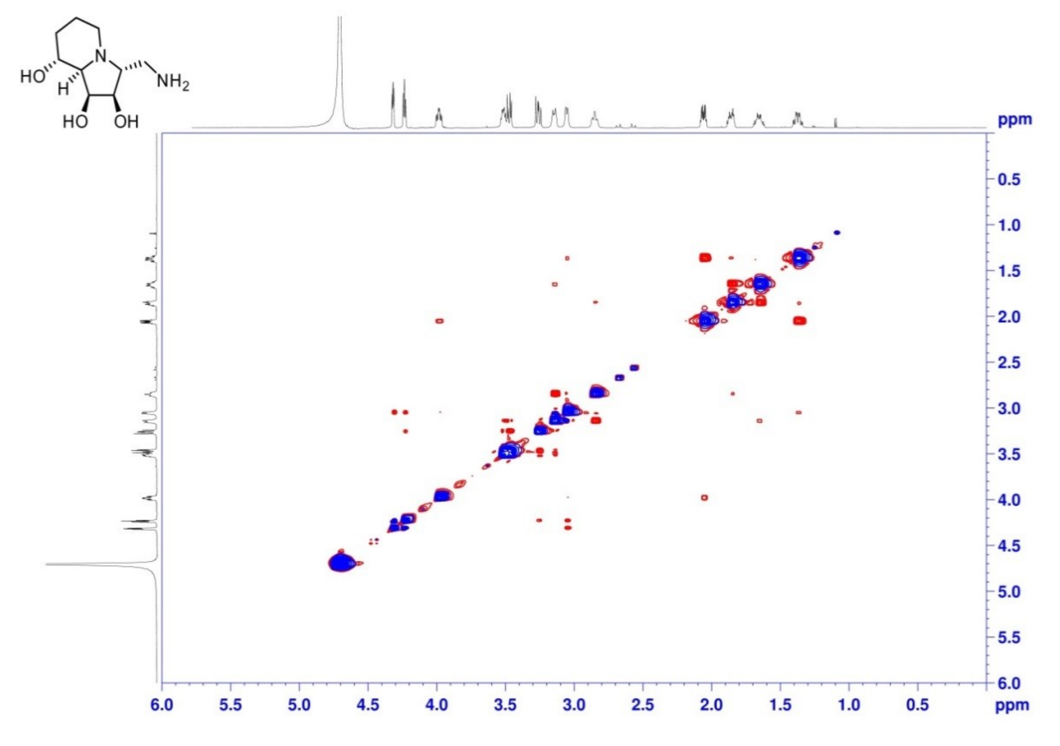
^1H NMR Spectra of compound **8b** (600 MHz, D_2O)



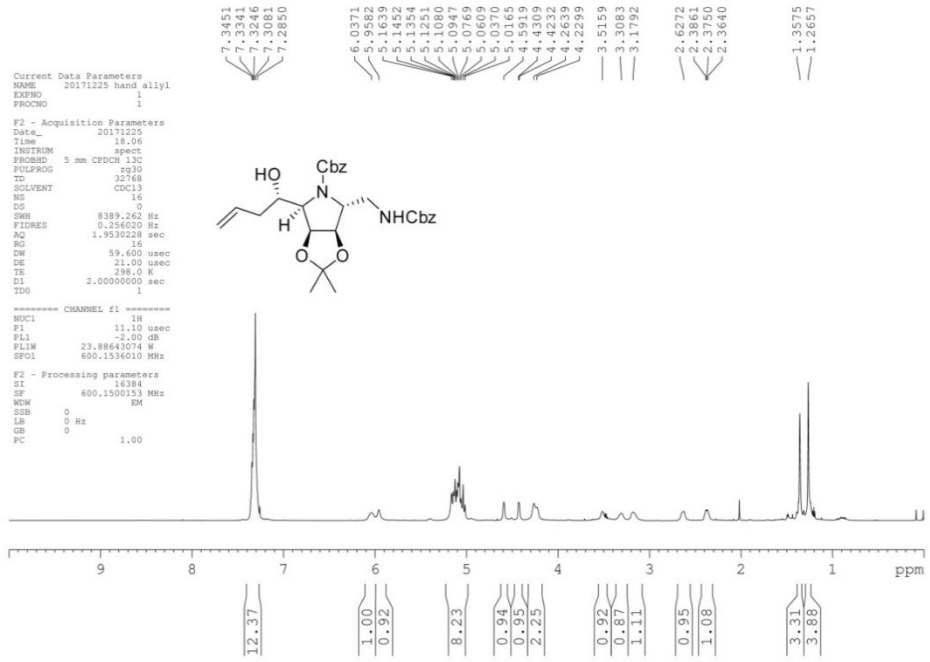
^{13}C NMR Spectra of compound **8b** (150 MHz,D_2O)



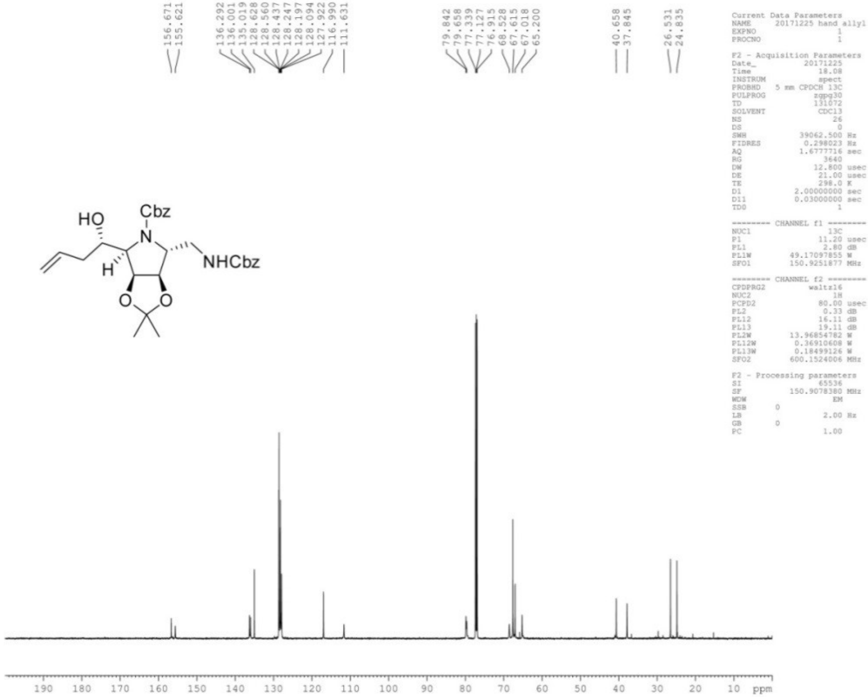
COSY Spectra of compound **8b** (600 MHz, D_2O)



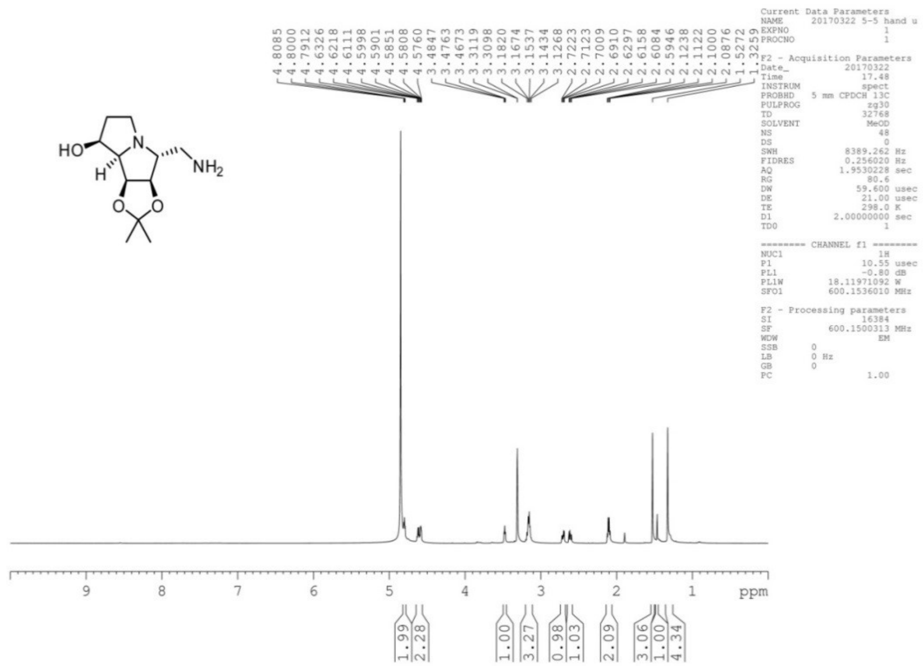
NOESY Spectra of compound **8b** (600 MHz, D_2O)



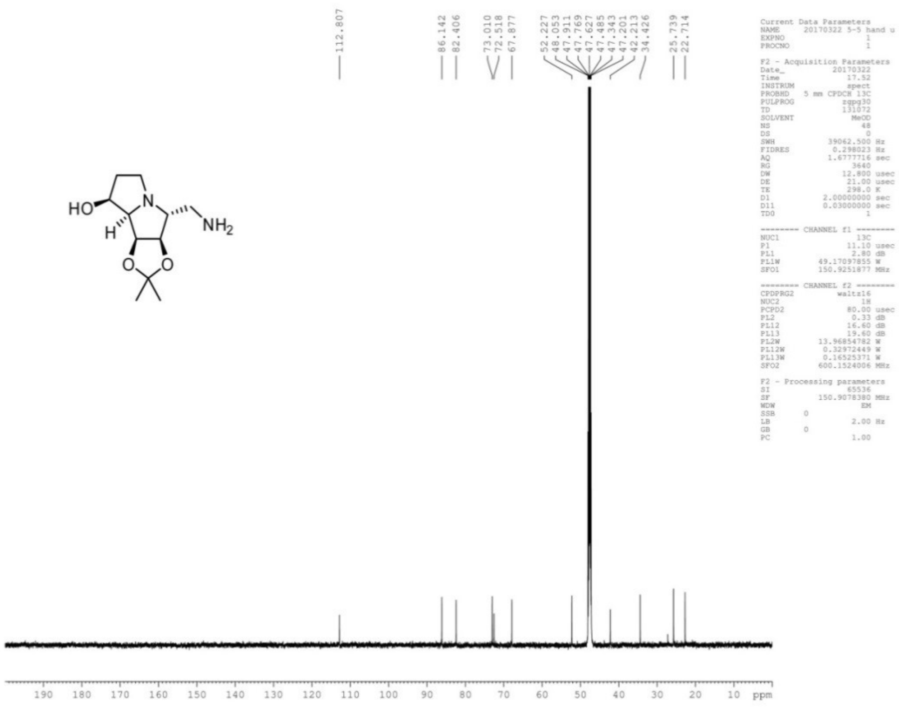
^{1}H NMR Spectra of compound **9a** (600 MHz, $CDCl_3$)



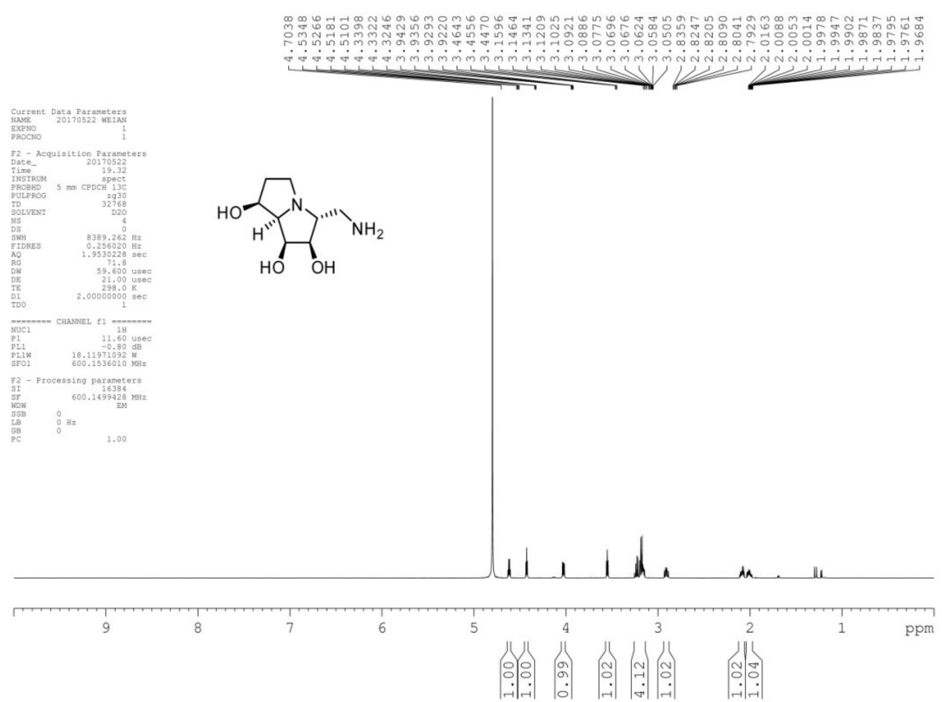
^{13}C NMR Spectra of compound **9a** (150 MHz, $CDCl_3$)



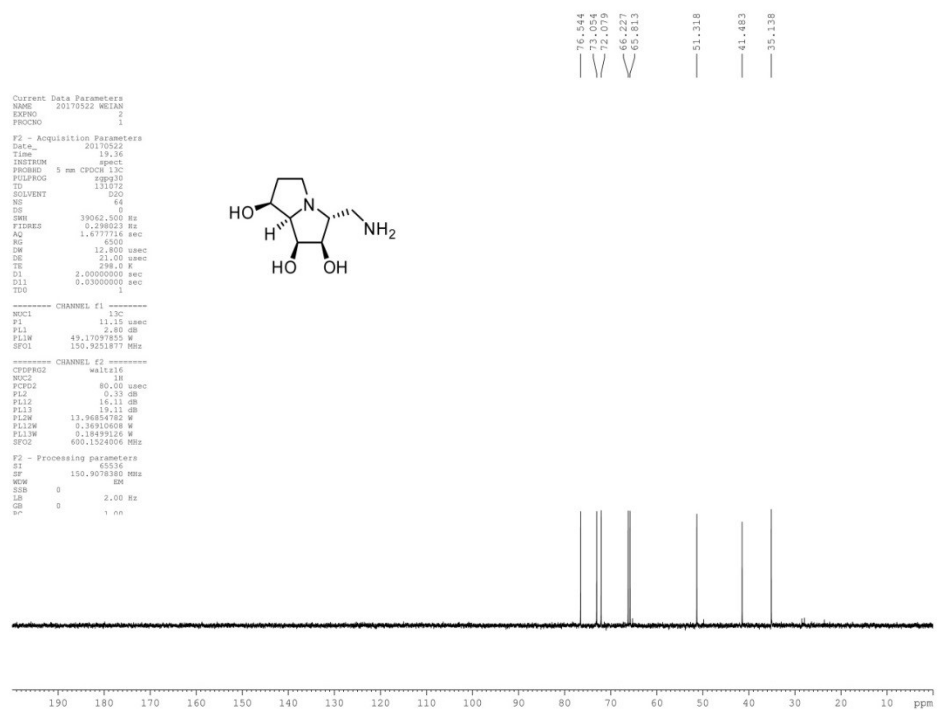
^{1}H NMR Spectra of compound **S4** (600 MHz, MeOD)



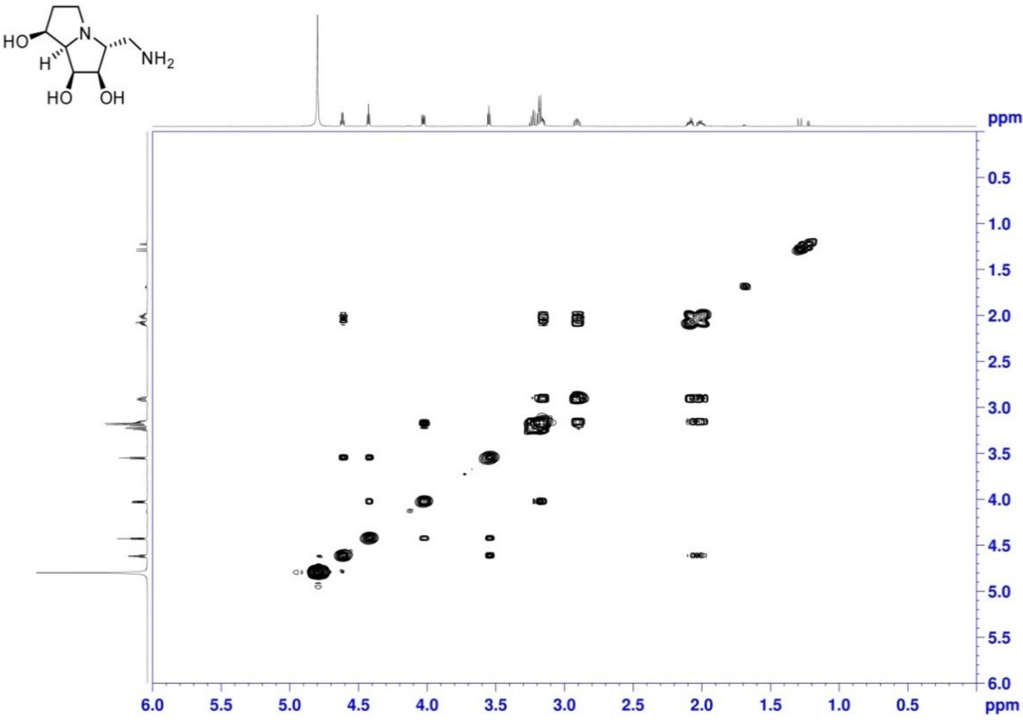
^{13}C NMR Spectra of compound **S4** (150 MHz, MeOD)



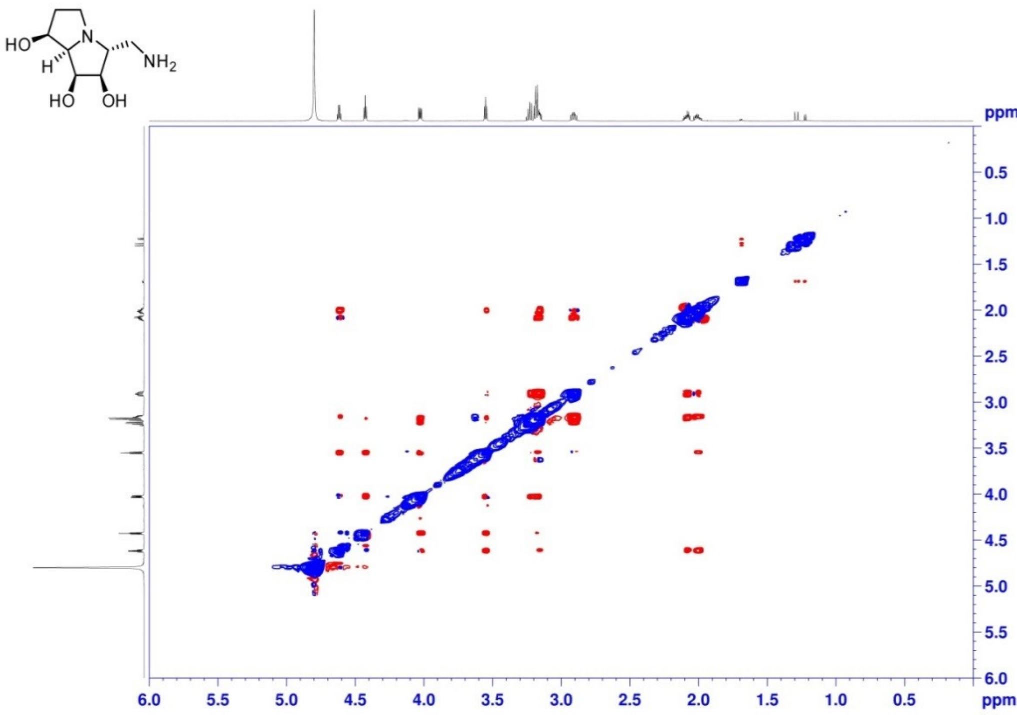
^{1}H NMR Spectra of compound **10a** (600 MHz, D_2O)



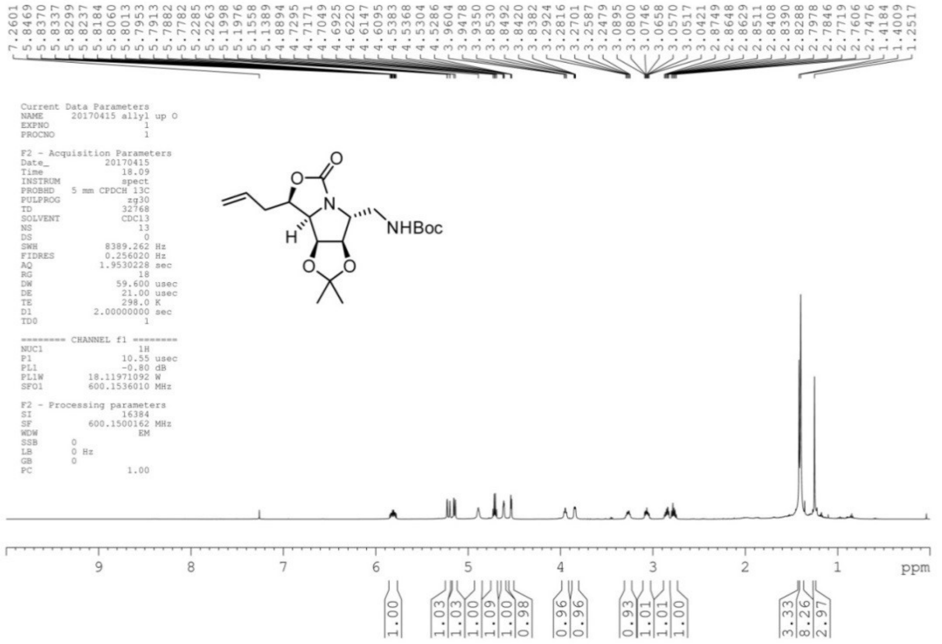
^{13}C NMR Spectra of compound **10a** (150 MHz, D_2O)



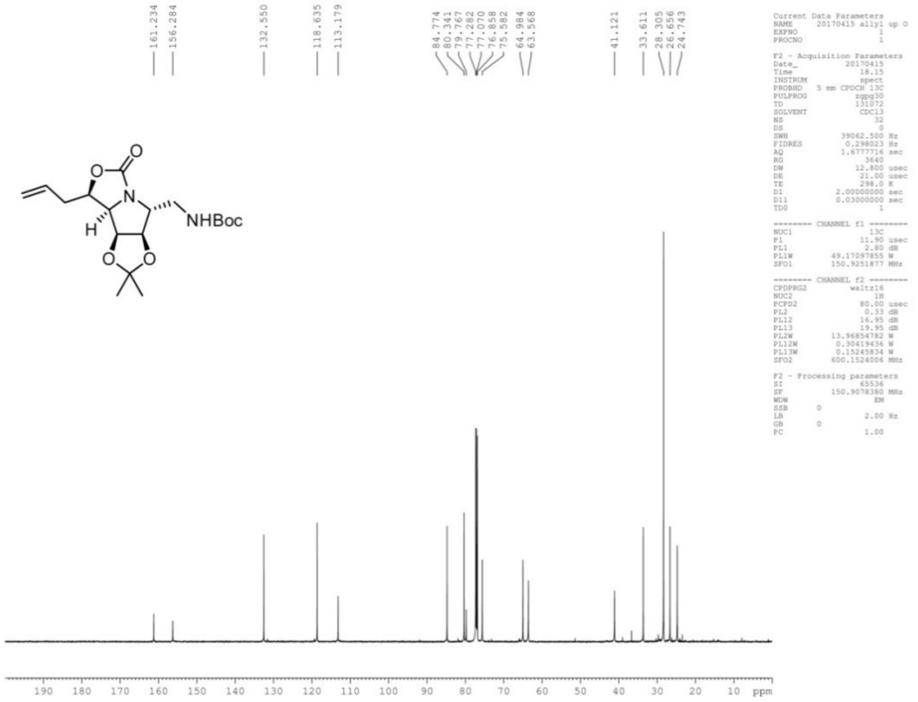
COSY Spectra of compound **10a** (600 MHz, D_2O)



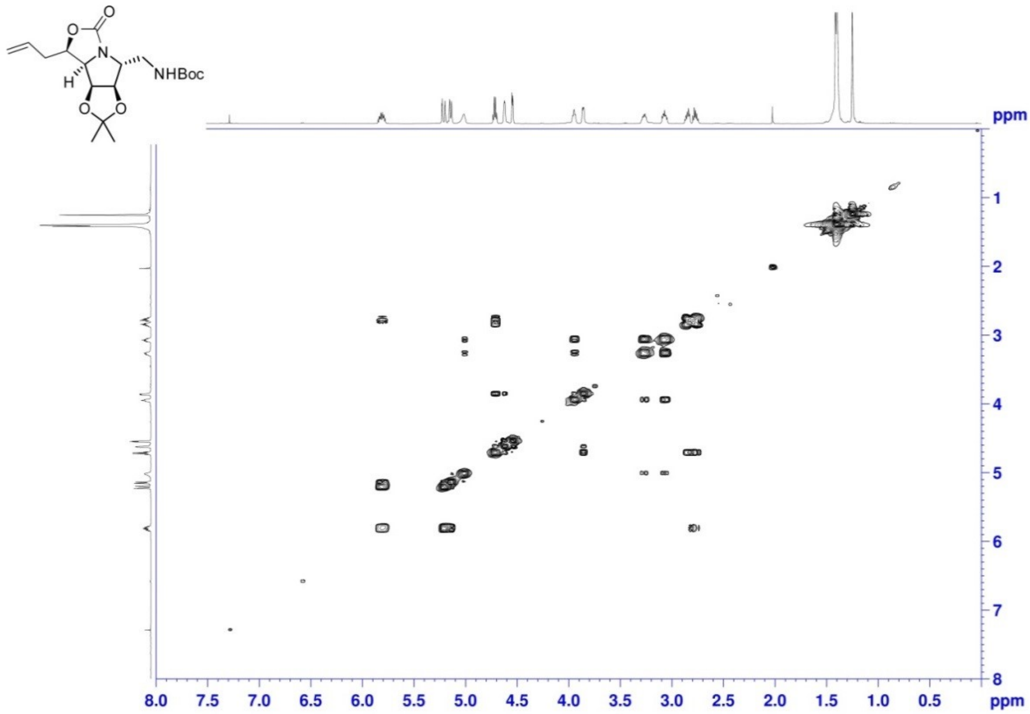
NOESY Spectra of compound **10a** (600 MHz, D_2O)



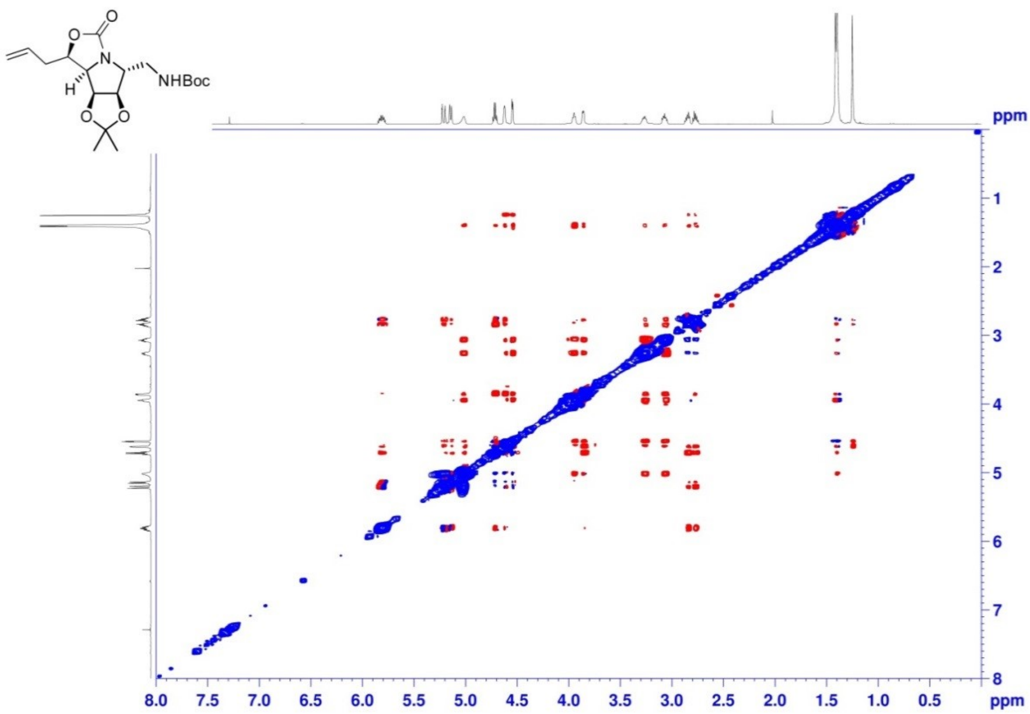
^{1}H NMR Spectra of compound **9b** (600 MHz, $CDCl_3$)



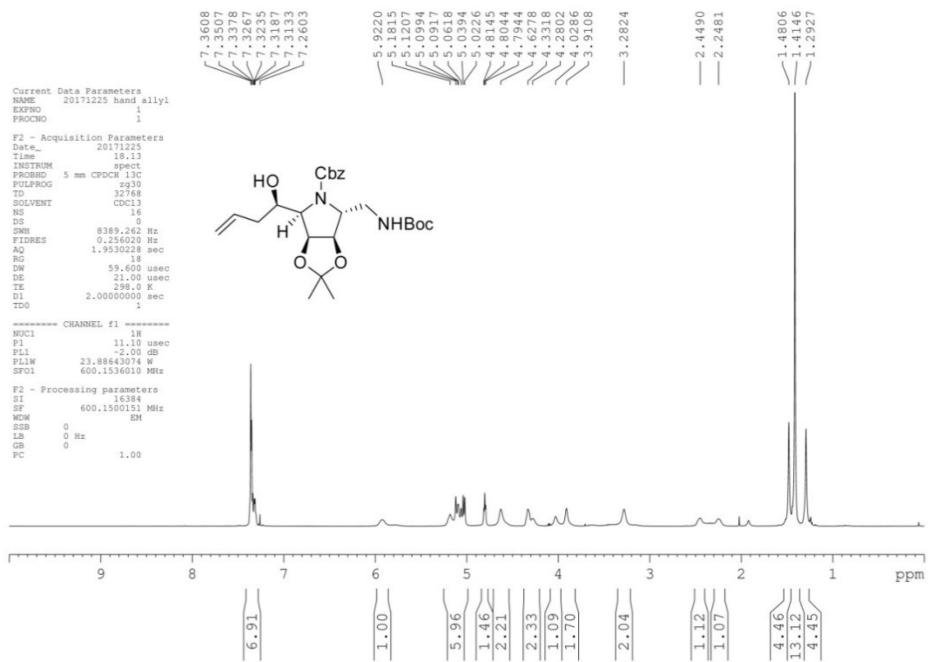
^{13}C NMR Spectra of compound **9b** (150 MHz, $CDCl_3$)



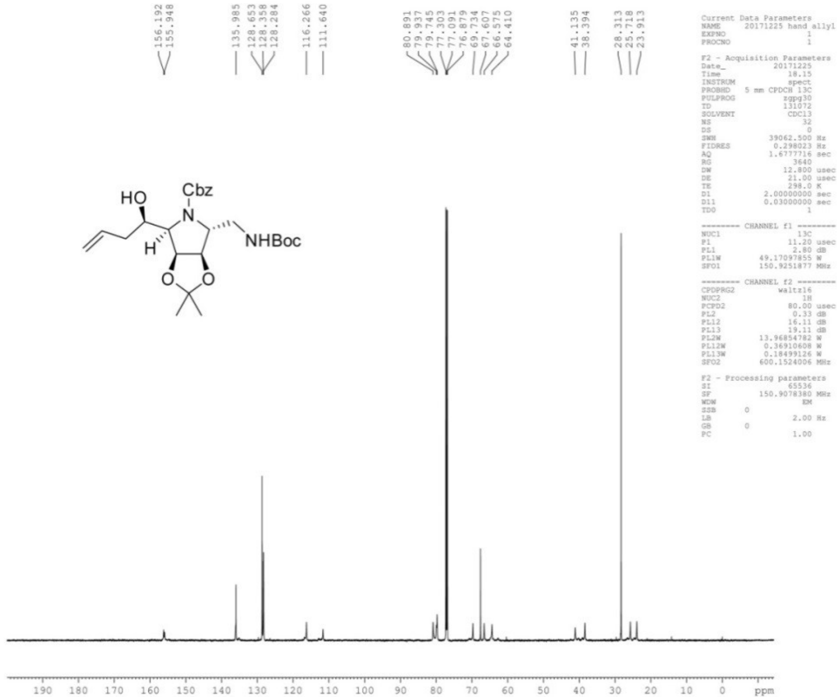
COSY Spectra of compound **9b** (600 MHz, $CDCl_3$)



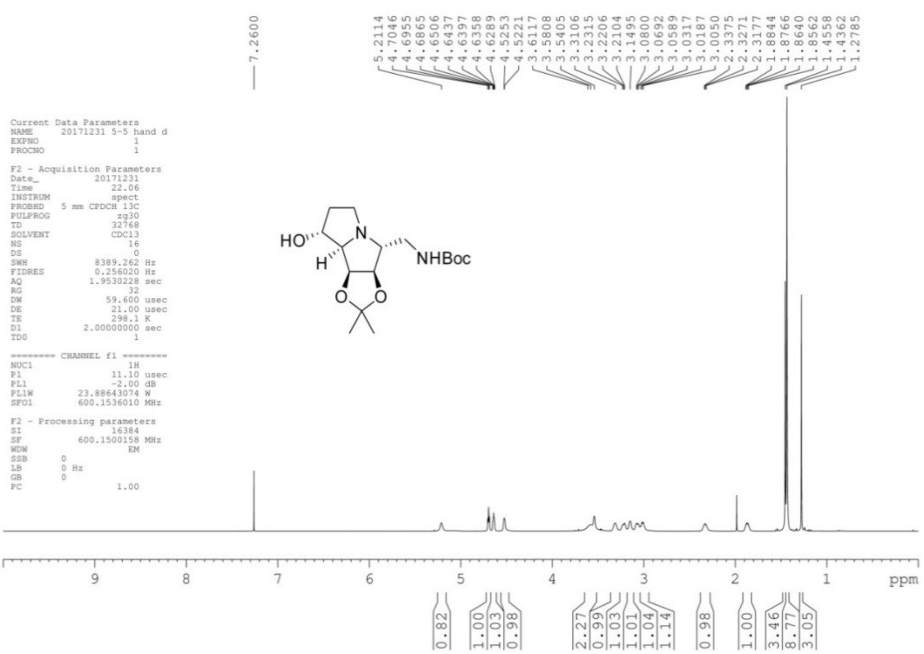
NOESY Spectra of compound **9b** (600 MHz, $CDCl_3$)



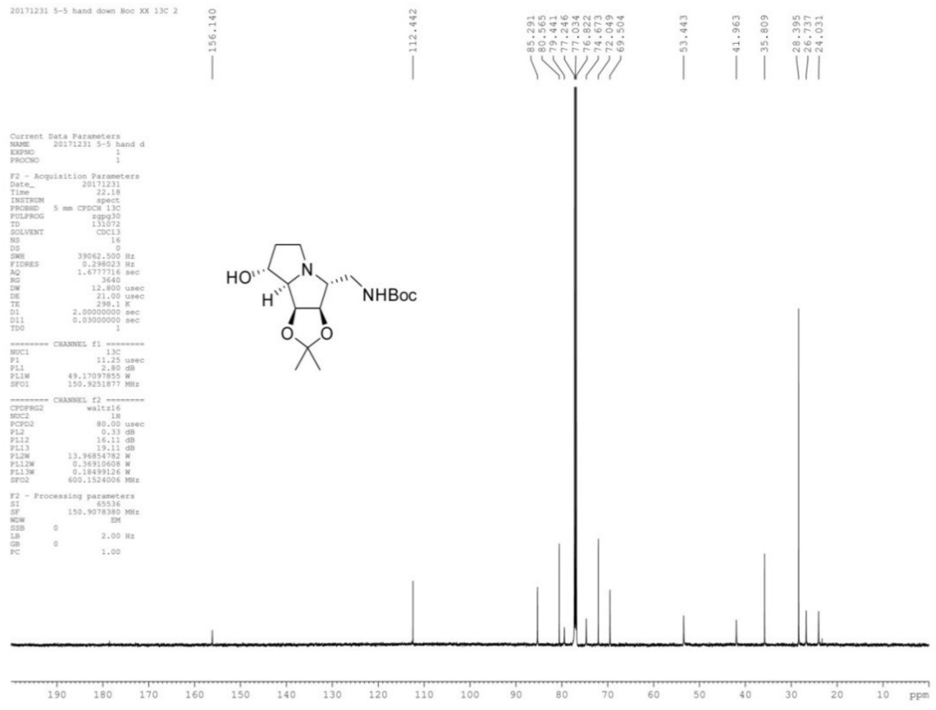
^{1}H NMR Spectra of compound **S14** (600 MHz, $CDCl_3$)



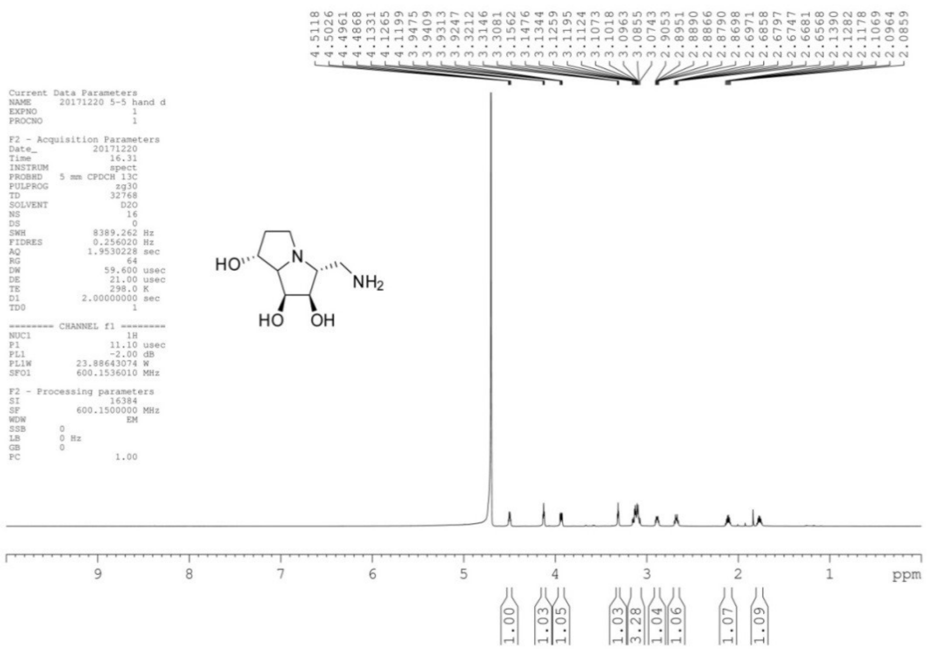
^{13}C NMR Spectra of compound **S14** (150 MHz, $CDCl_3$)



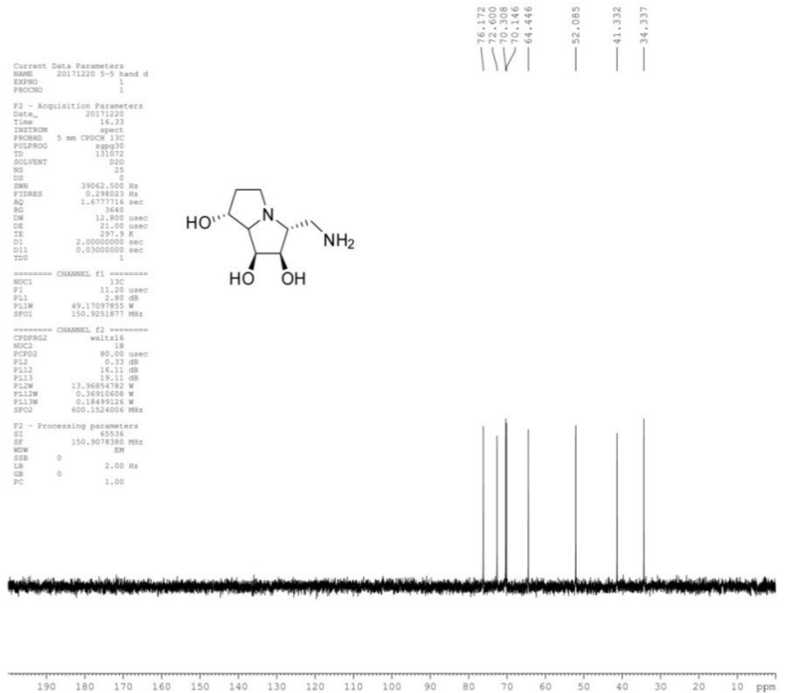
^{1}H NMR Spectra of compound **S6** (600 MHz, $CDCl_3$)



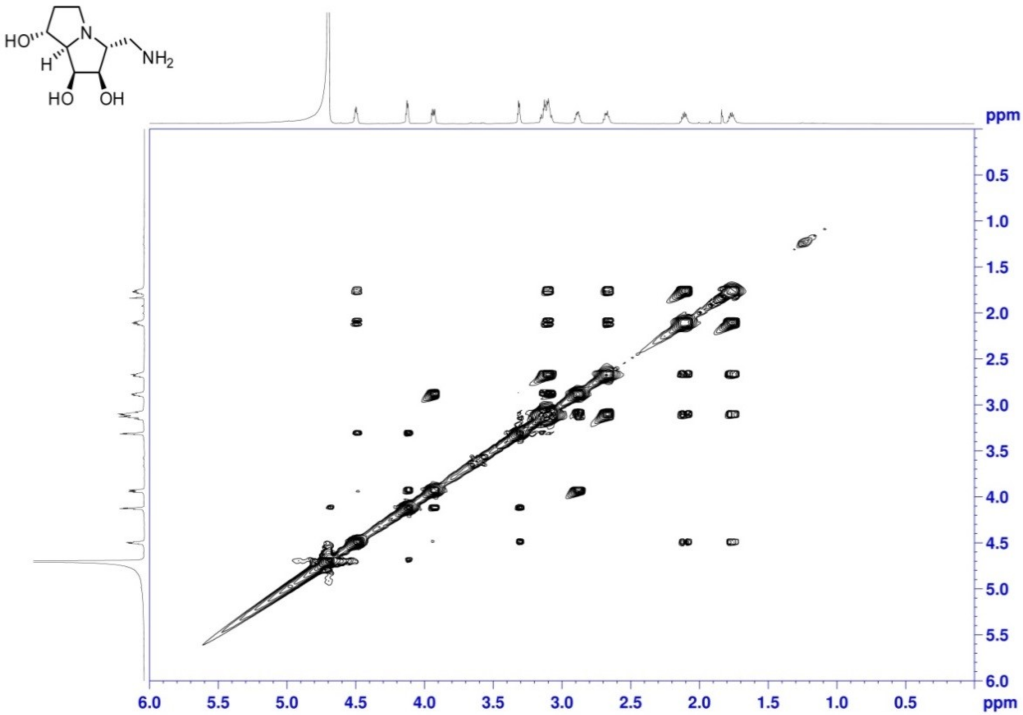
^{13}C NMR Spectra of compound **S6** (150 MHz, $CDCl_3$)



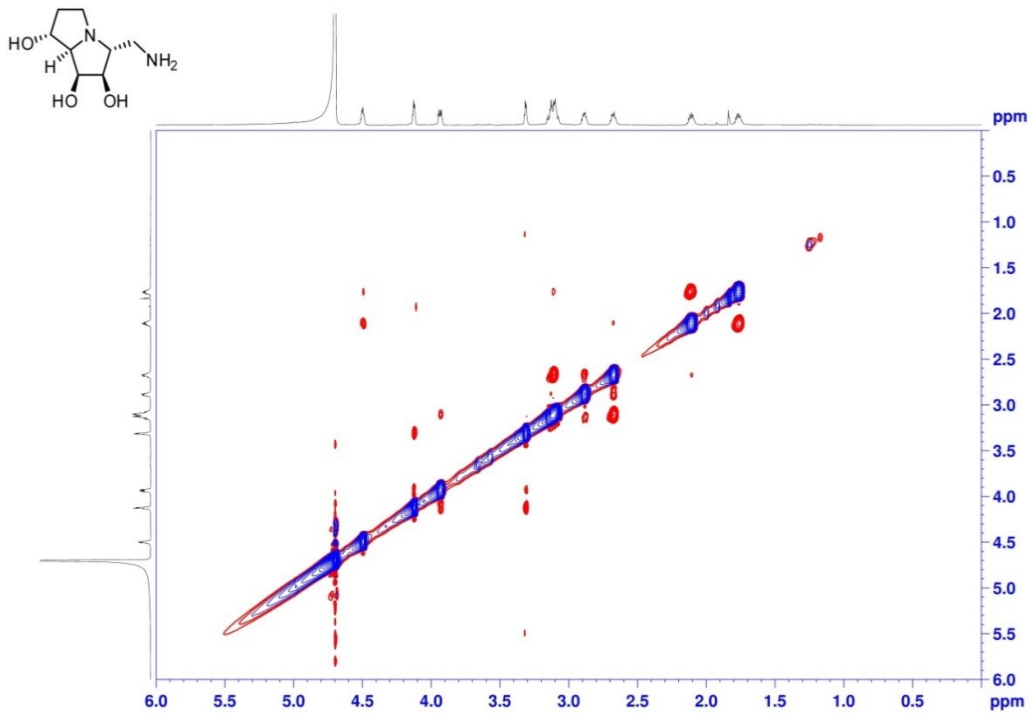
^1H NMR Spectra of compound **10b** (600 MHz, D_2O)



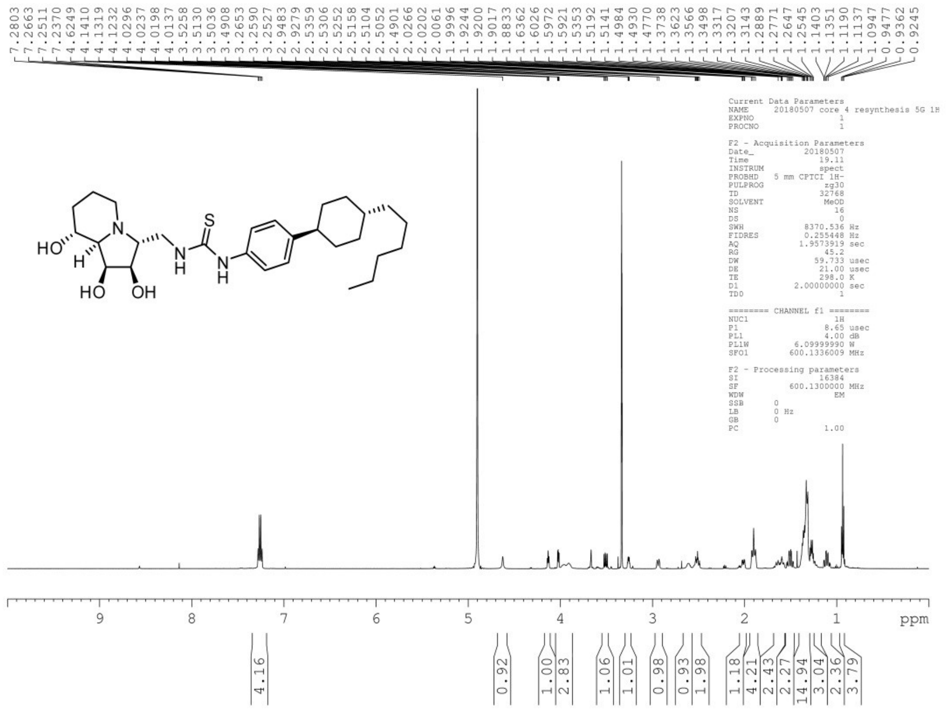
^{13}C NMR Spectra of compound **10b** (150 MHz, D_2O)



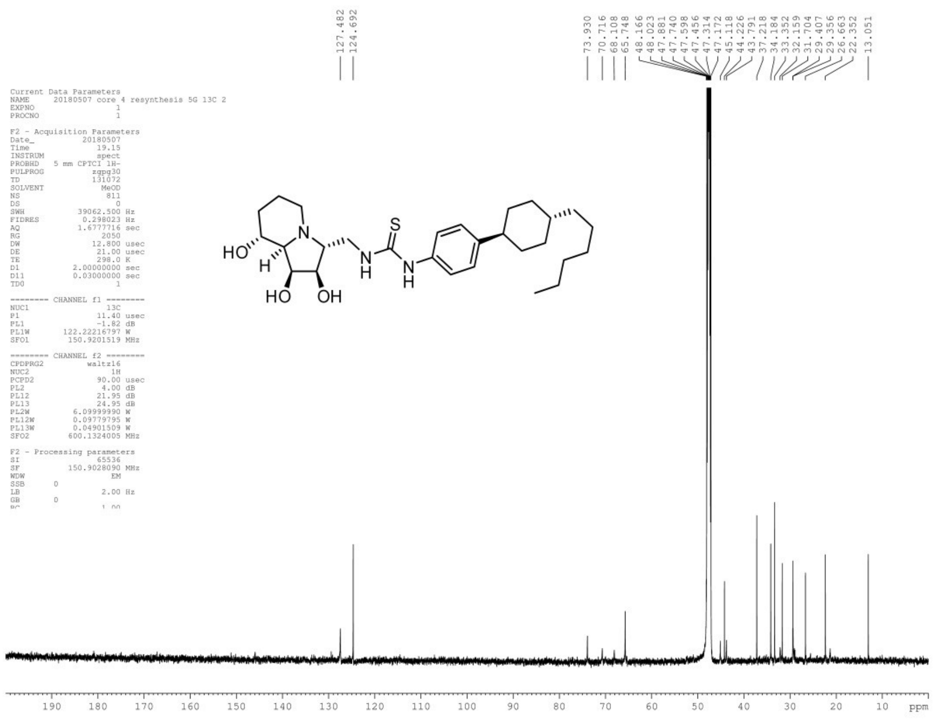
COSY Spectra of compound **10b** (600 MHz, D_2O)



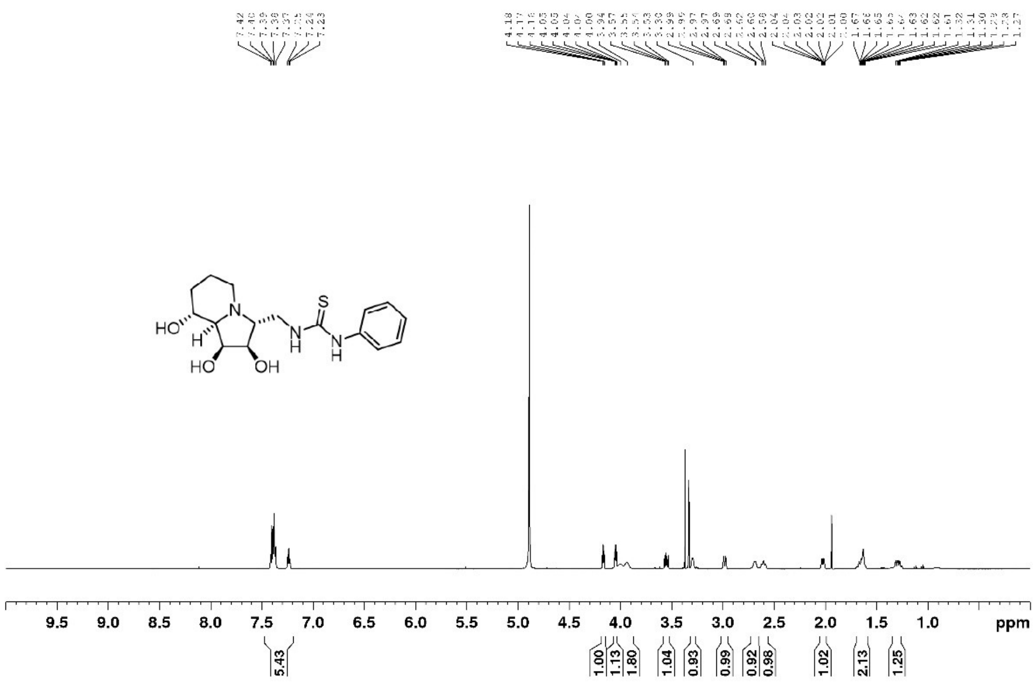
NOESY Spectra of compound **10b** (600 MHz, D_2O)



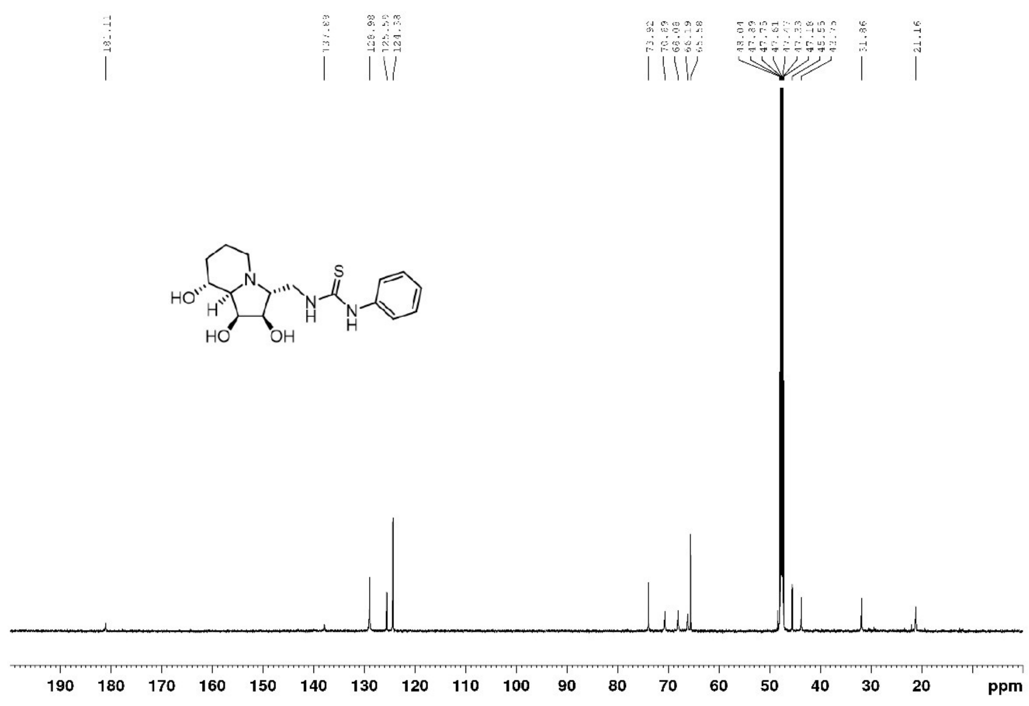
^{1}H NMR Spectra of compound **8b-1** (600 MHz, MeOD)



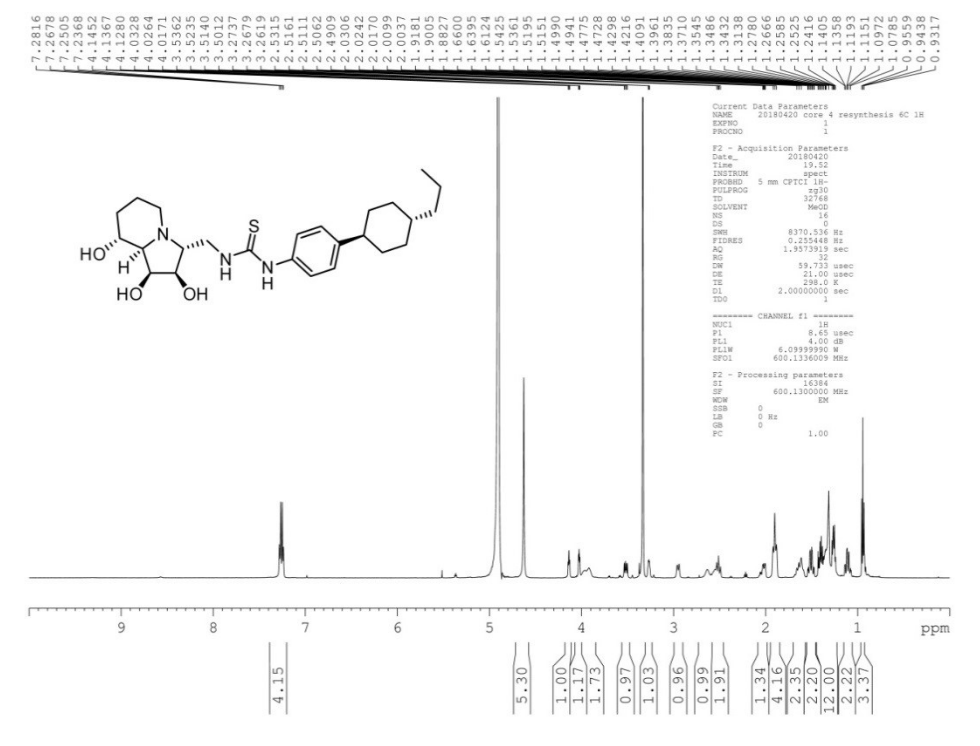
^{13}C NMR Spectra of compound **8b-1** (150 MHz, MeOD)



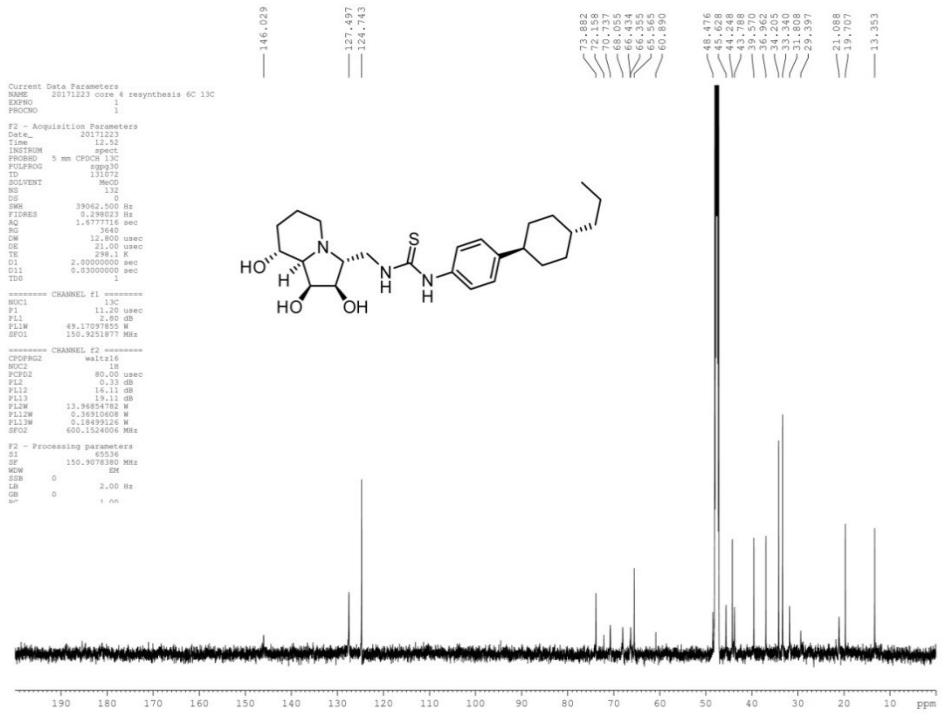
^{1}H NMR Spectra of compound **8b-2** (600 MHz, MeOD)



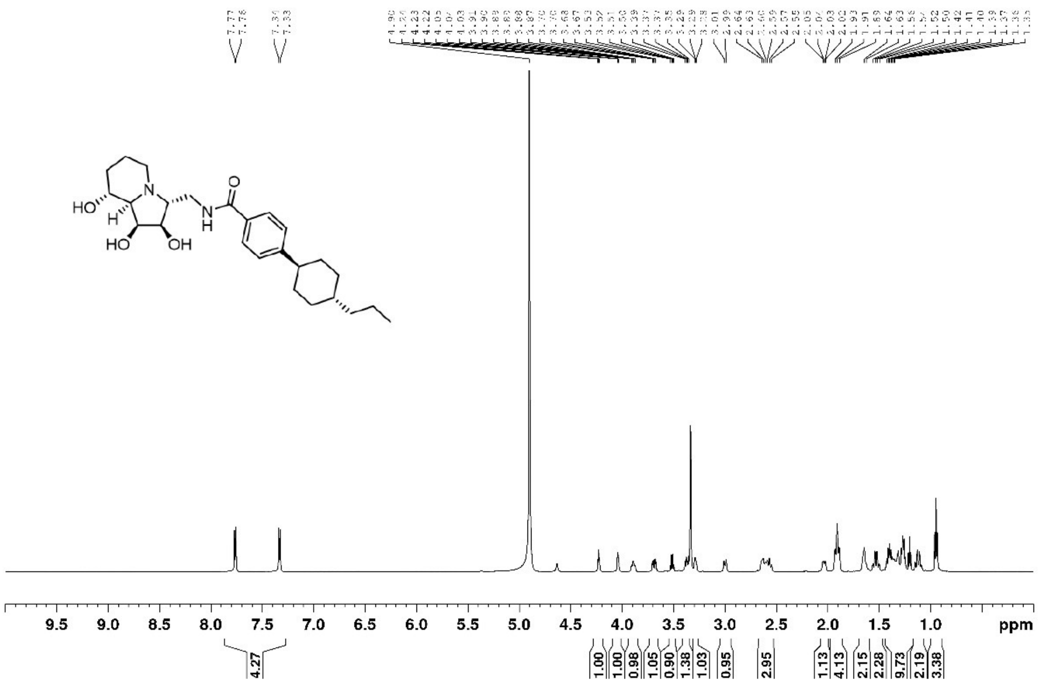
^{13}C NMR Spectra of compound **8b-2** (150 MHz, MeOD)



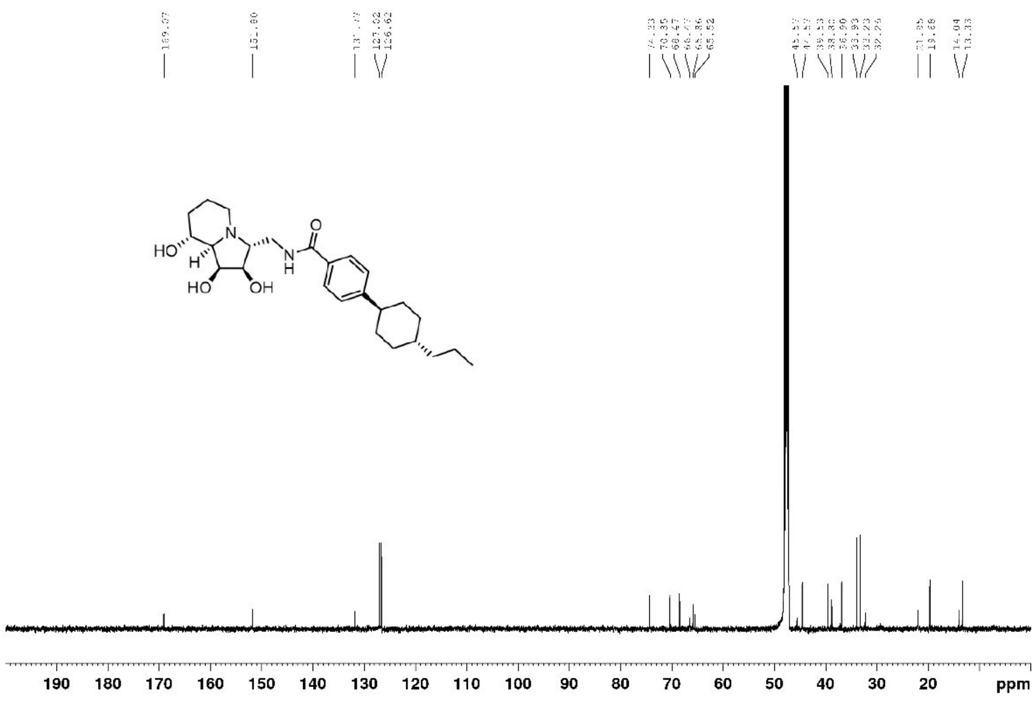
^{1}H NMR Spectra of compound **8b-3** (600 MHz, MeOD)



^{13}C NMR Spectra of compound **8b-3** (150 MHz, MeOD)



^{1}H NMR Spectra of compound **8b-4** (600 MHz, MeOD)



^{13}C NMR Spectra of compound**8b-4** (150 MHz, MeOD)

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