

Electronic Supplementary Information

Aglycone Mimics for Tuning of Glycosidase Inhibition: Design, Synthesis and Biological Evaluation of Bicyclic Pyrrolidotriazole Iminosugars.

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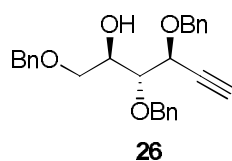
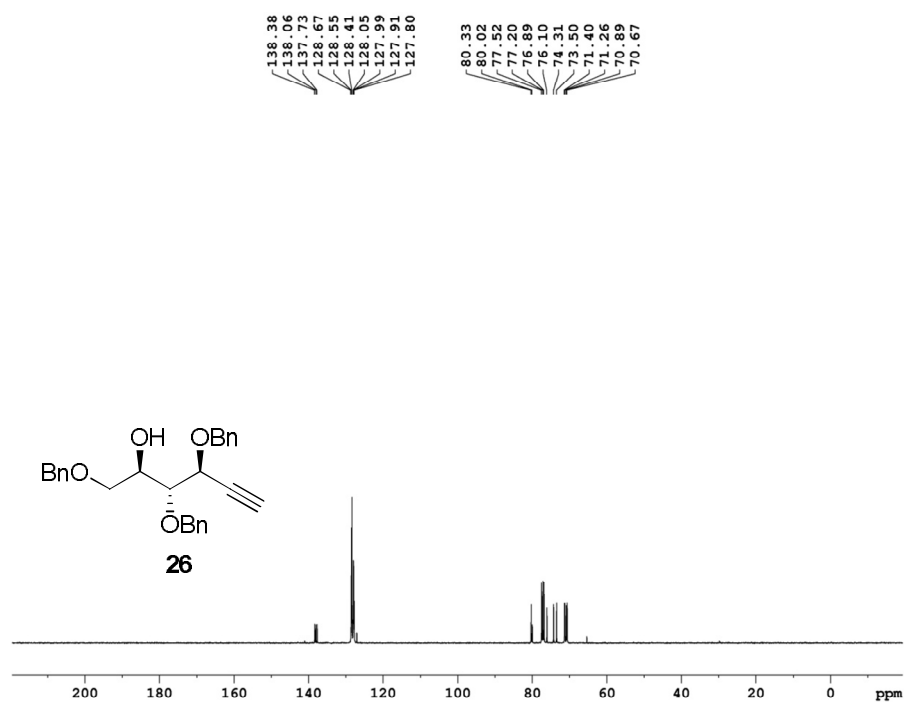
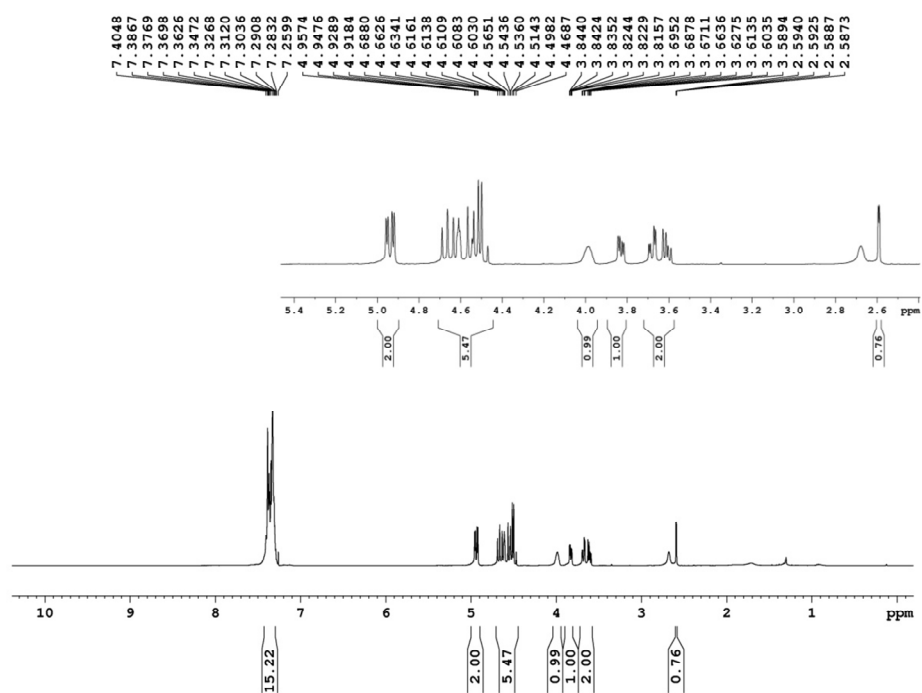
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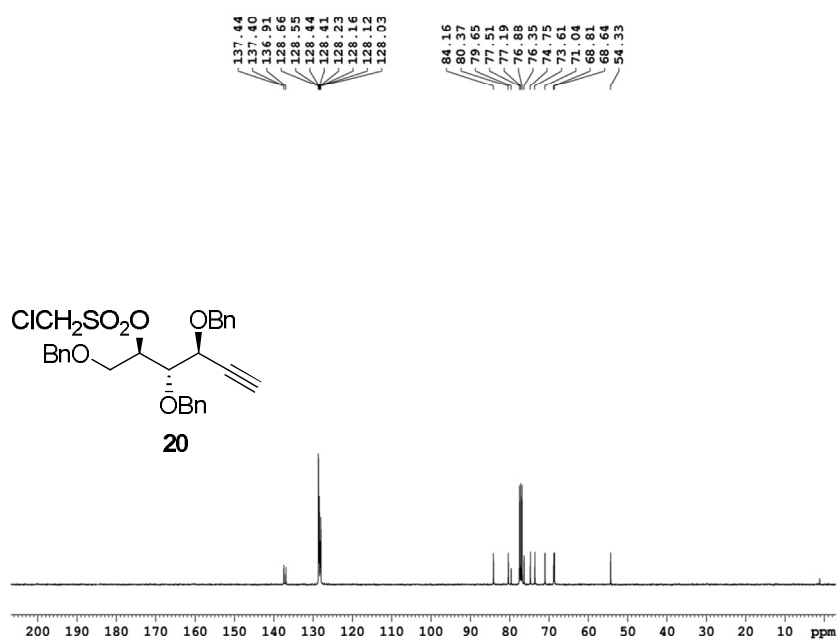
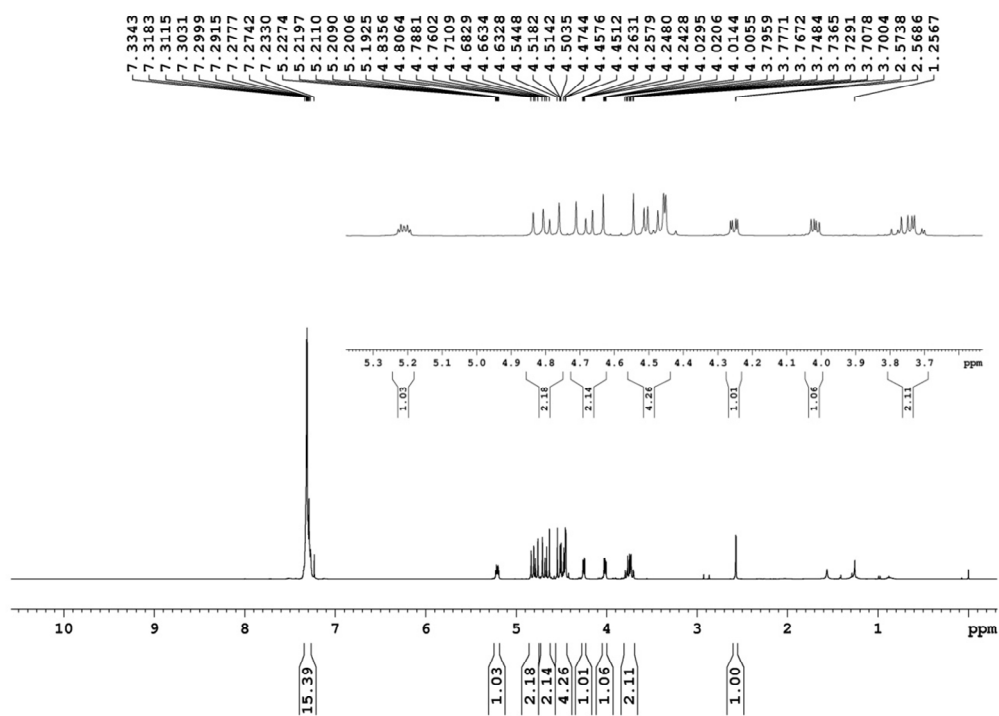
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Abbreviations

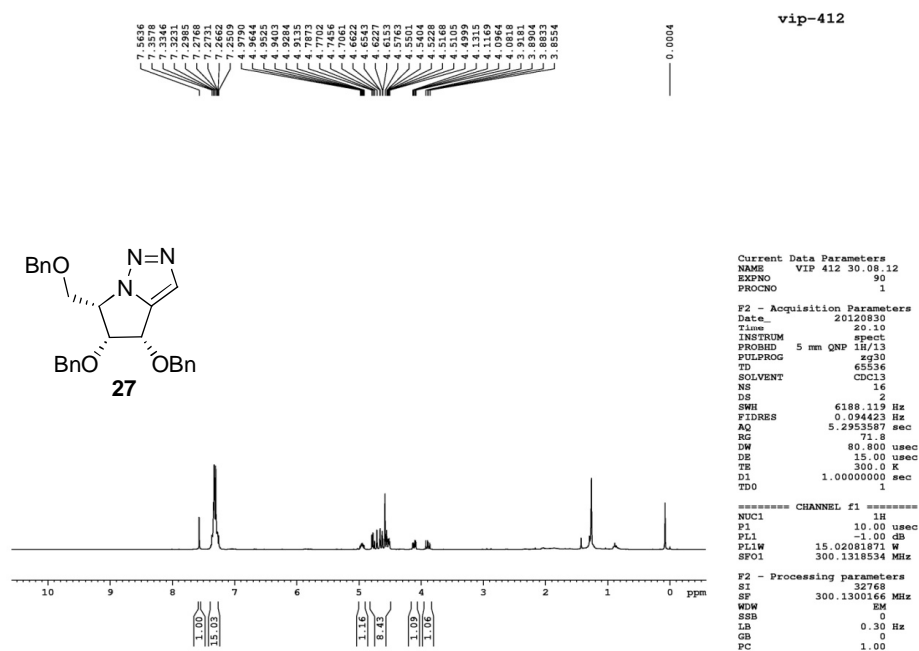
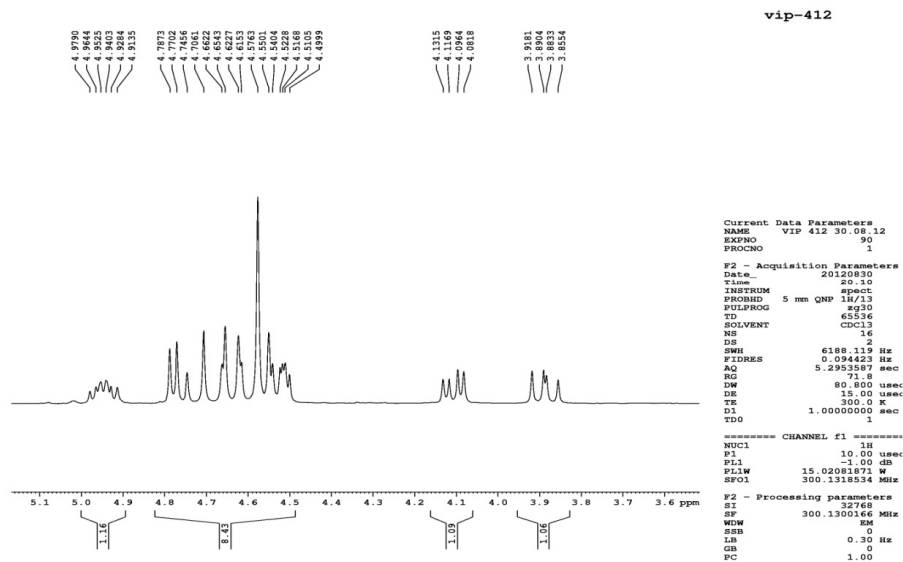
Bn	Benzyl
DCM	Dichloromethane
DMF	Dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
Et ₂ NH	Diethylamine
eq	Molar equivalent(s)
MeOH	Methanol
mmol	millimoles
NMR	Nuclear magnetic resonance
THF	Tetrahydrofuran
TLC	Thin Layer chromatogram



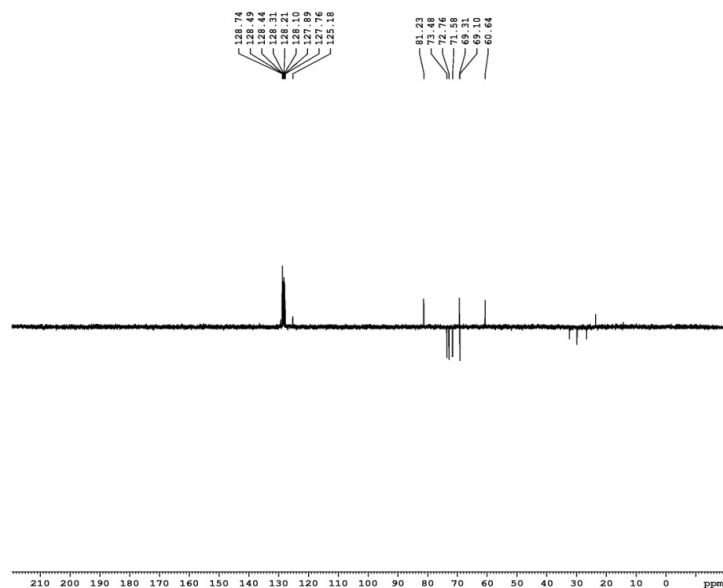
¹H and ¹³C NMR Spectrum of 26



¹H and ¹³C NMR Spectrum of 20



¹H NMR Spectrum of 27



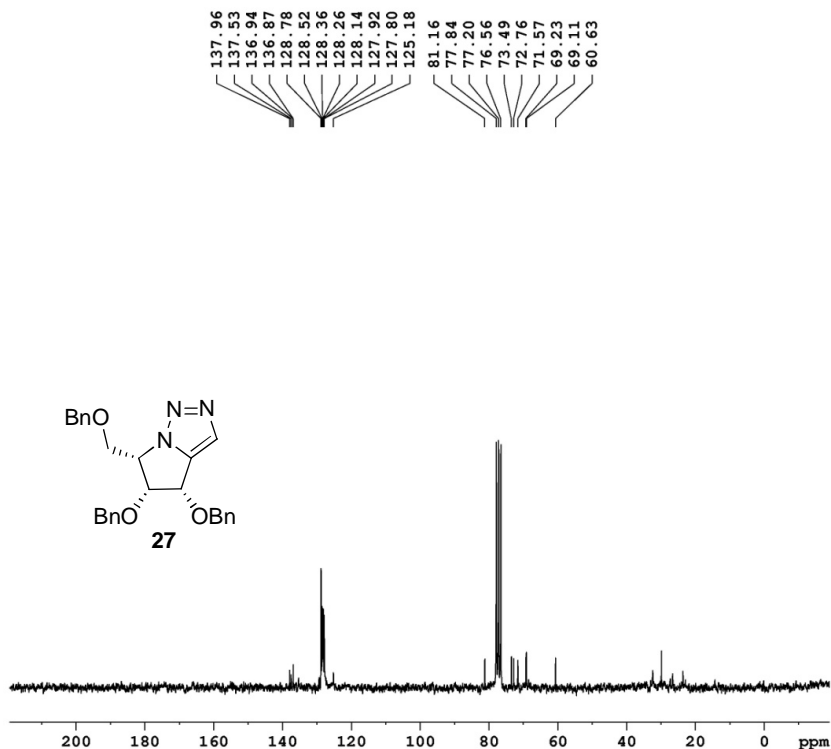
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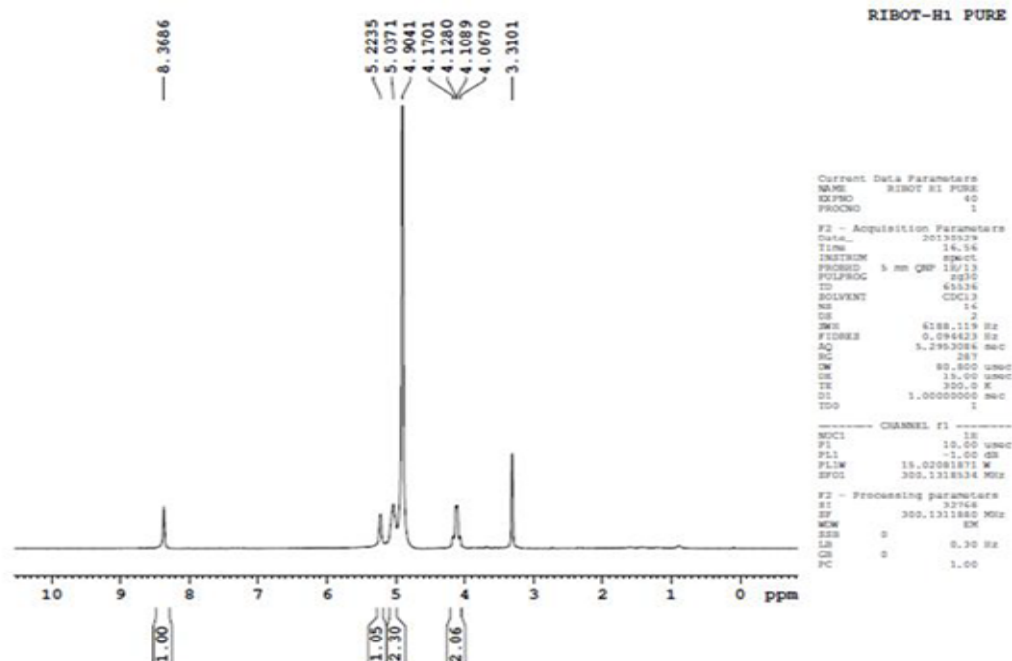
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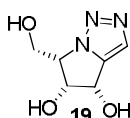
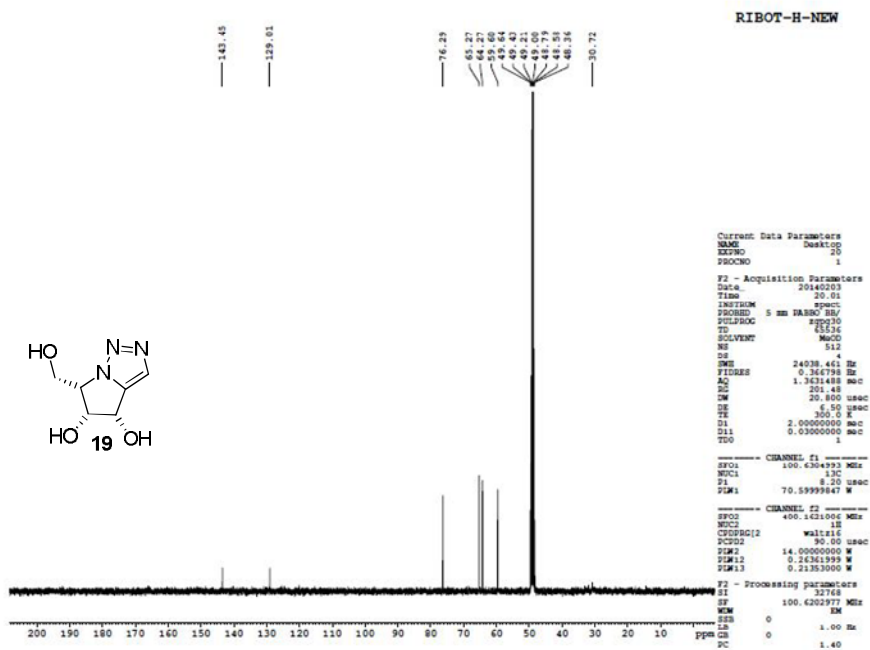
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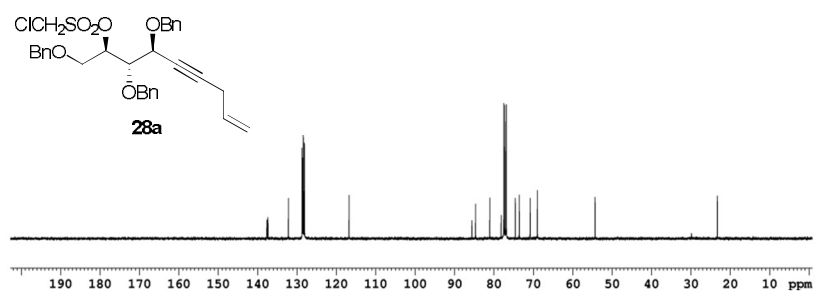
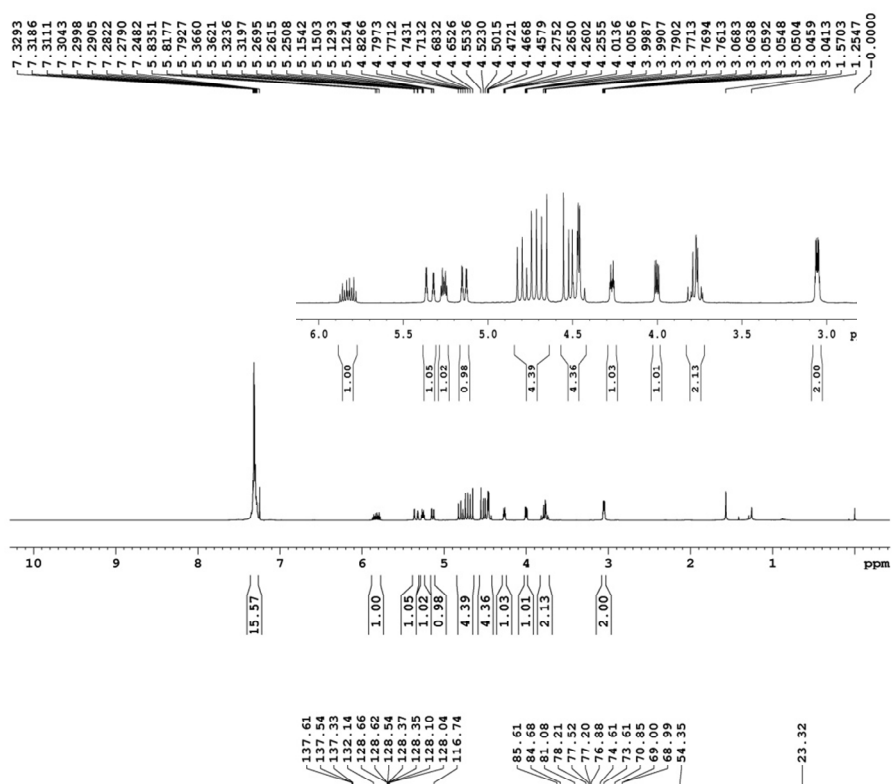
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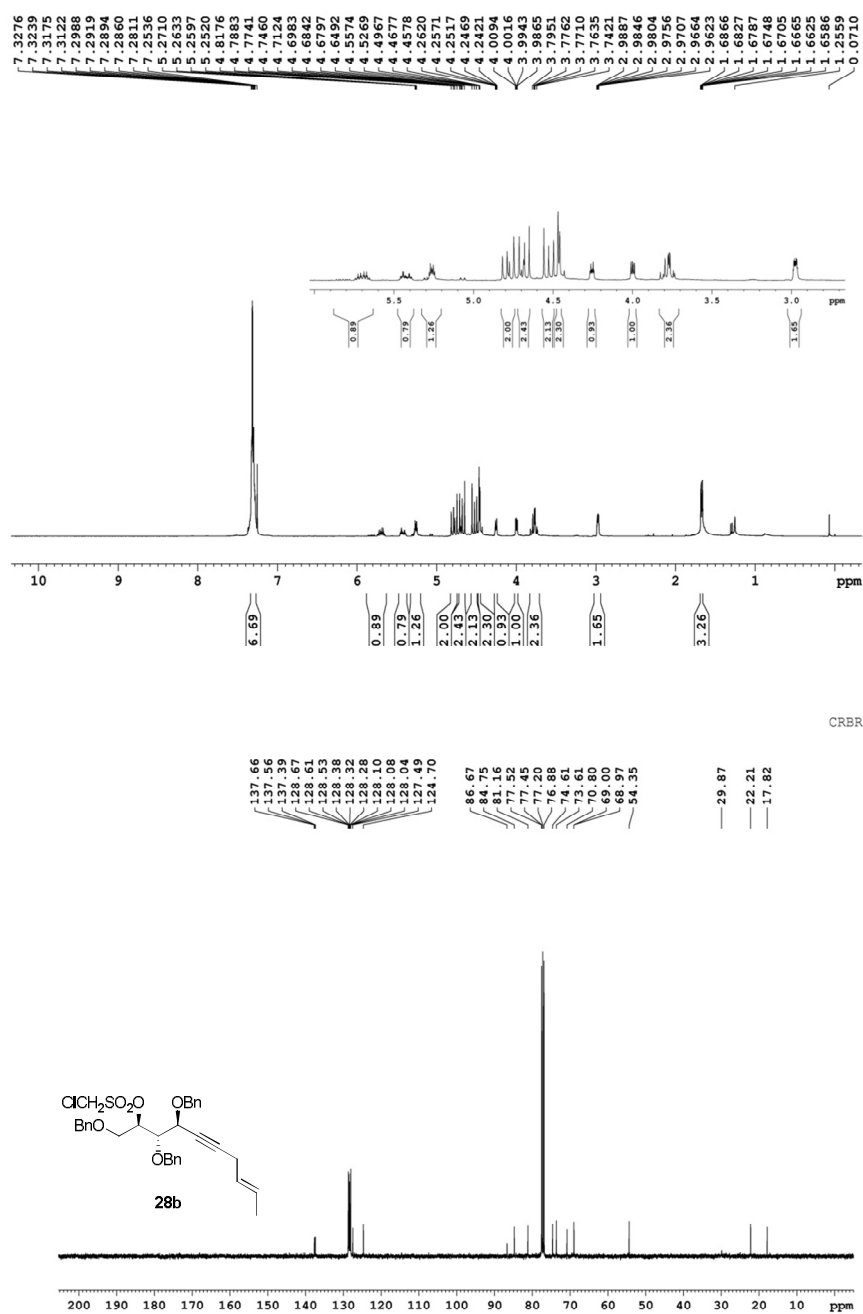
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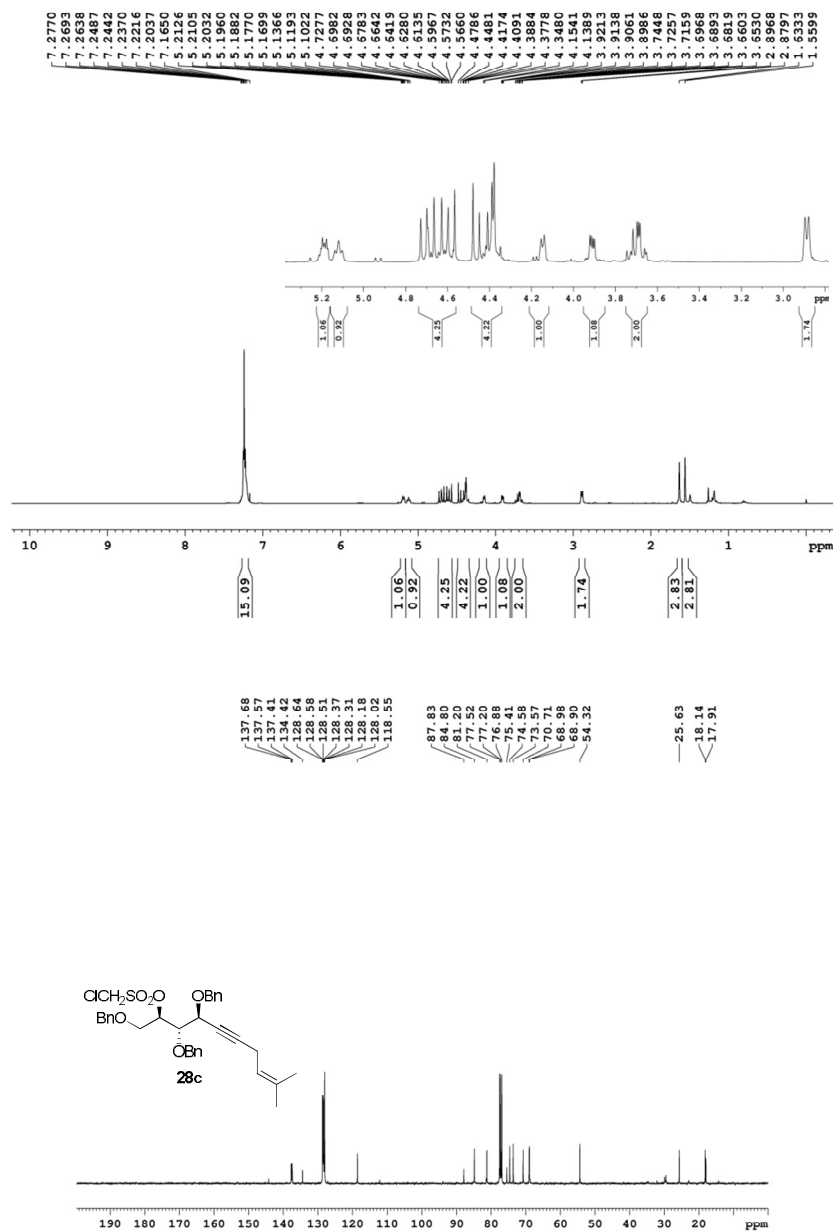
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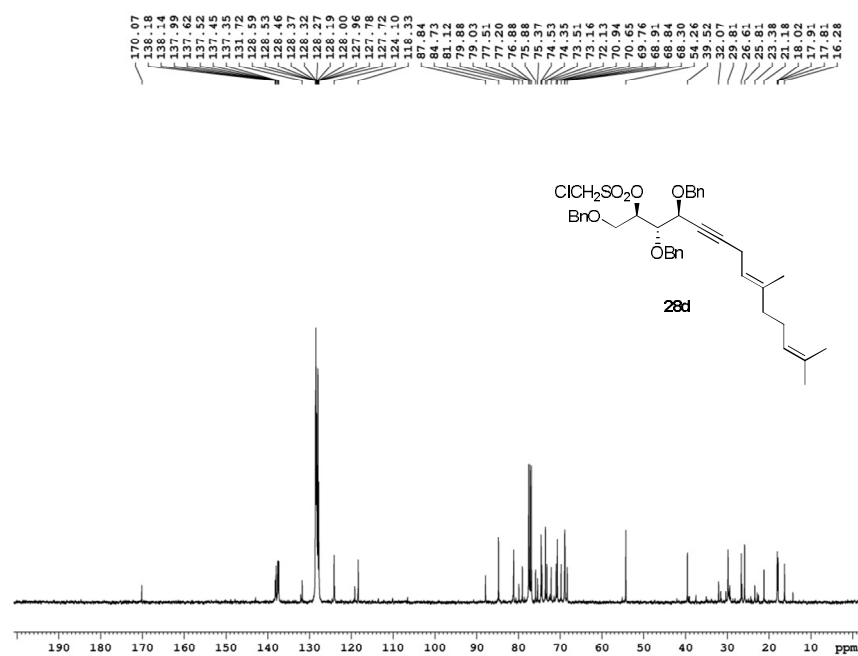
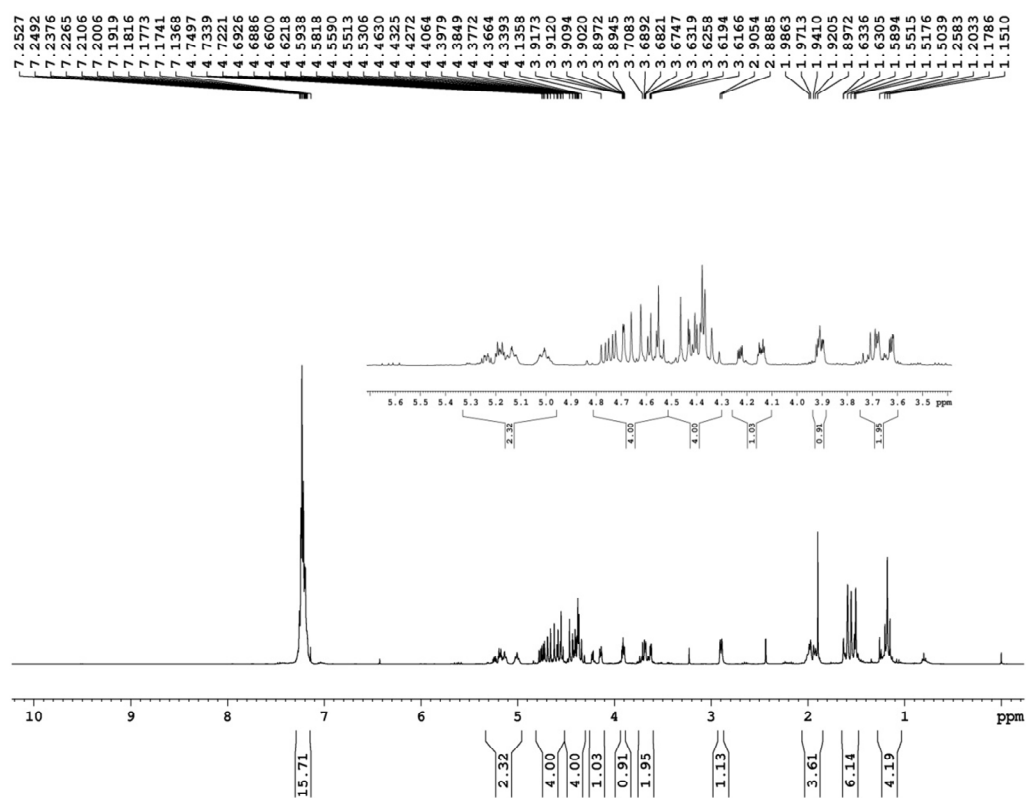
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¹H and ¹³C NMR Spectrum of 28b

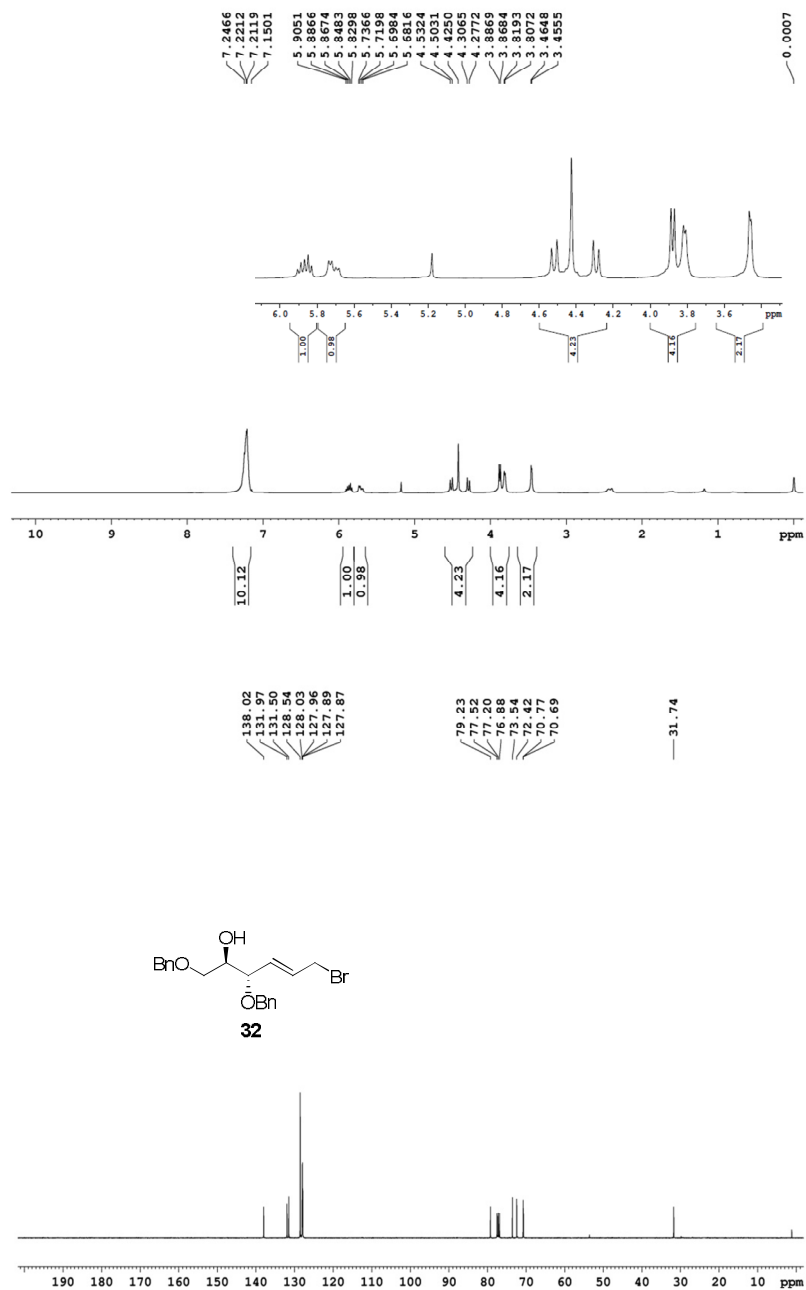


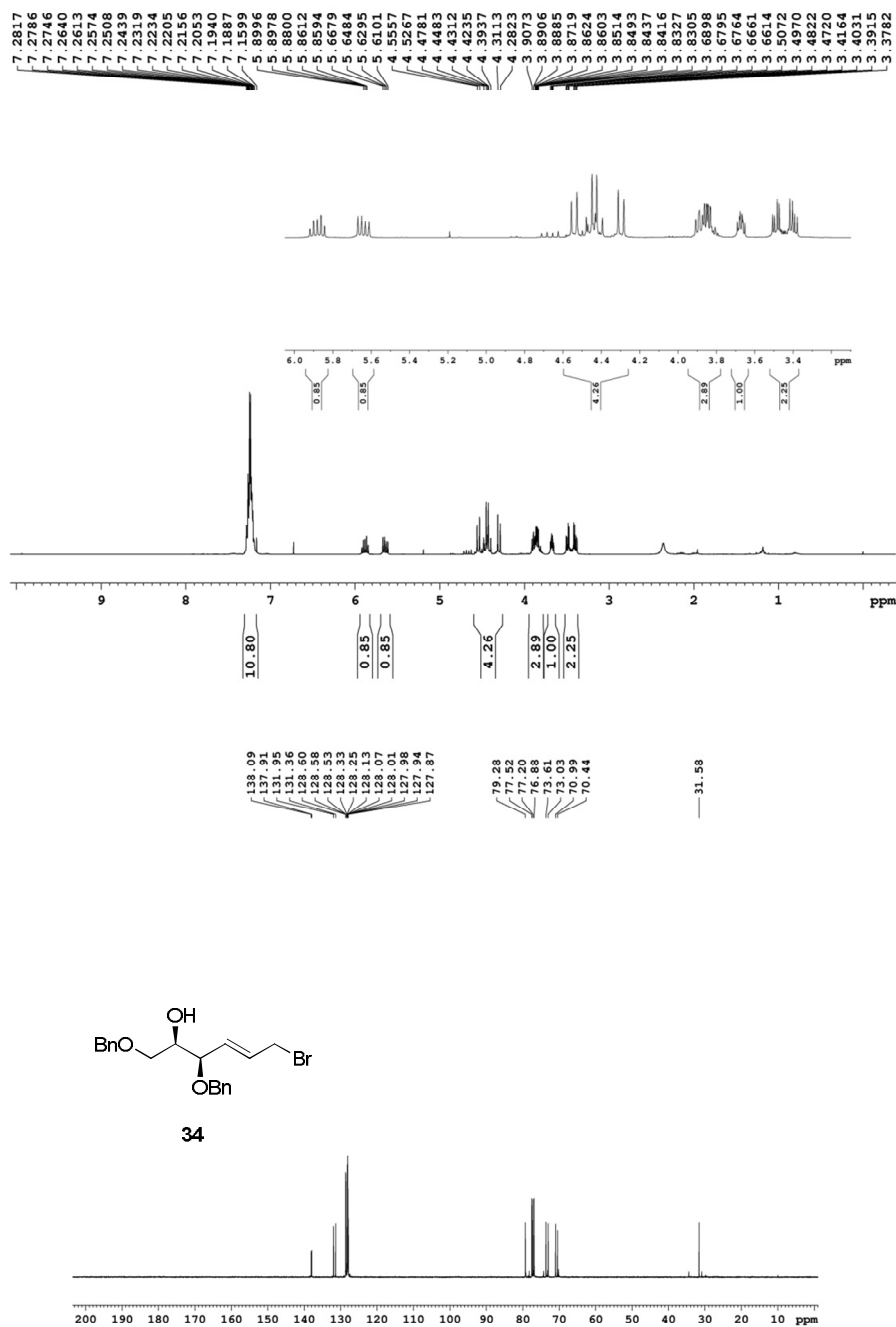
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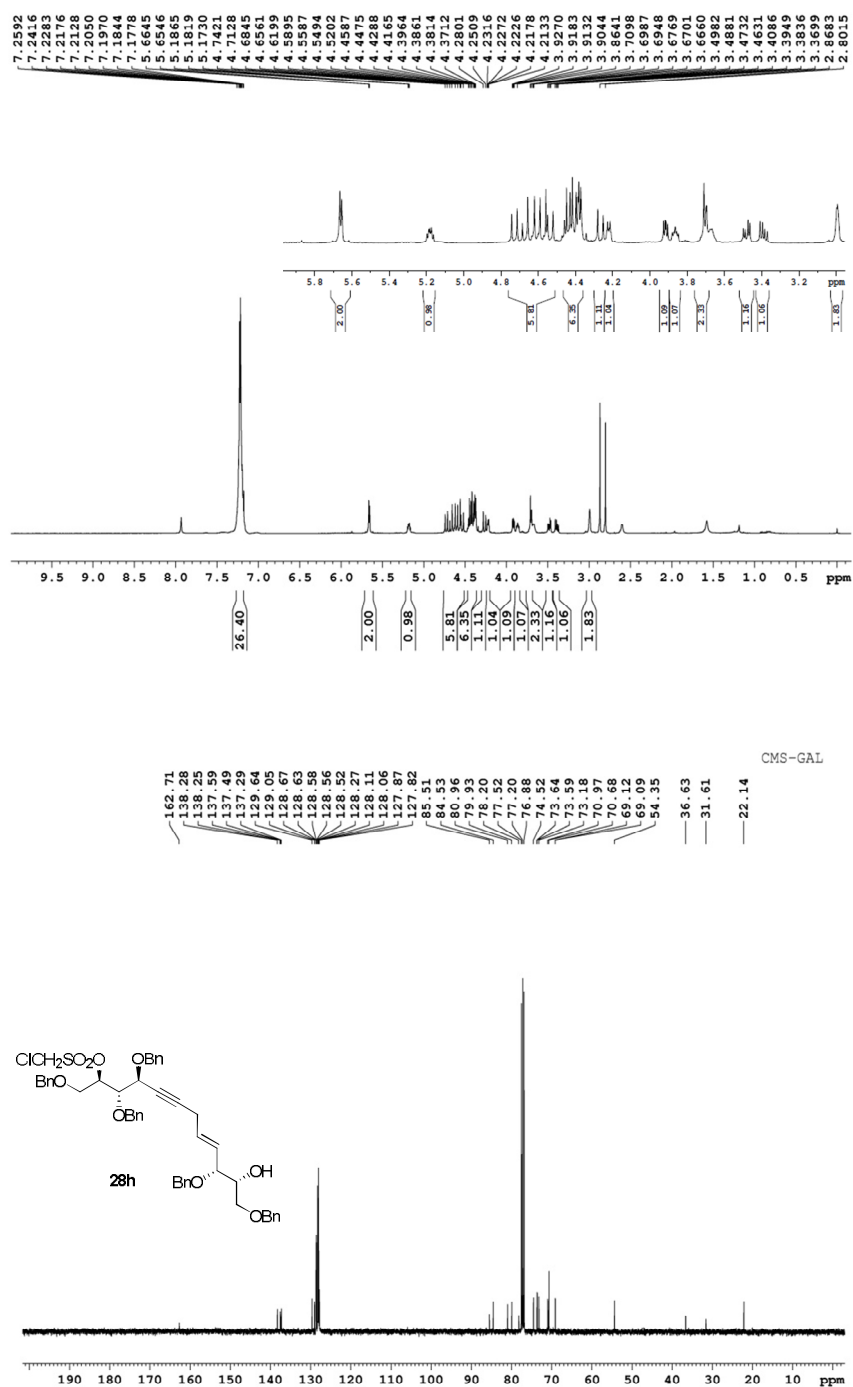
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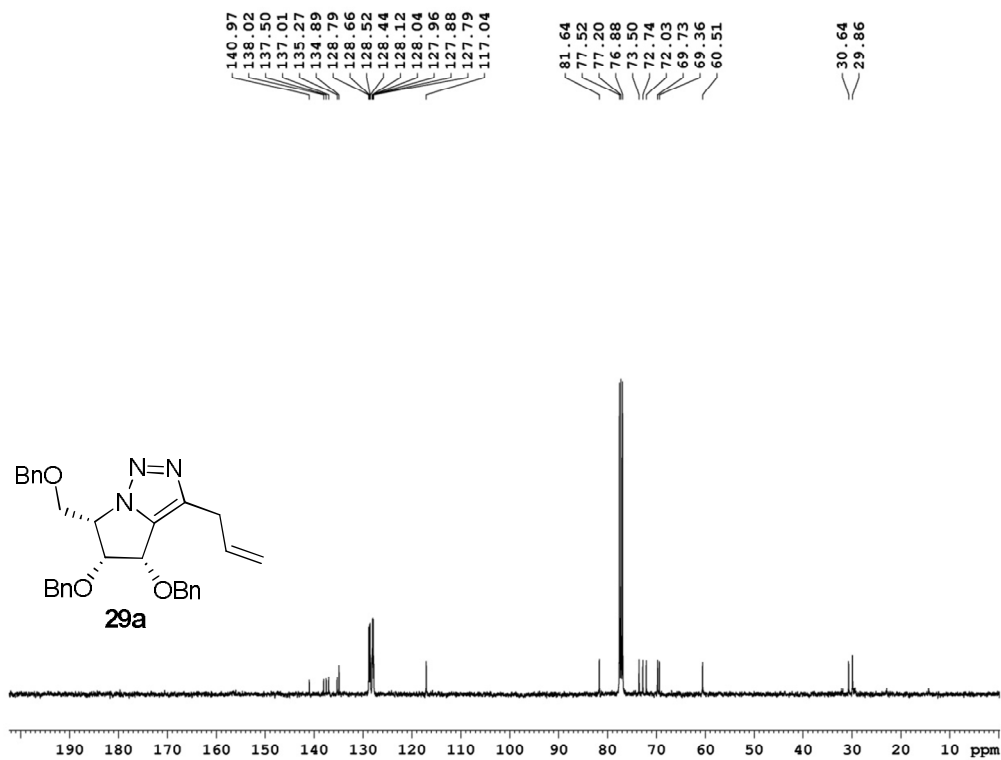
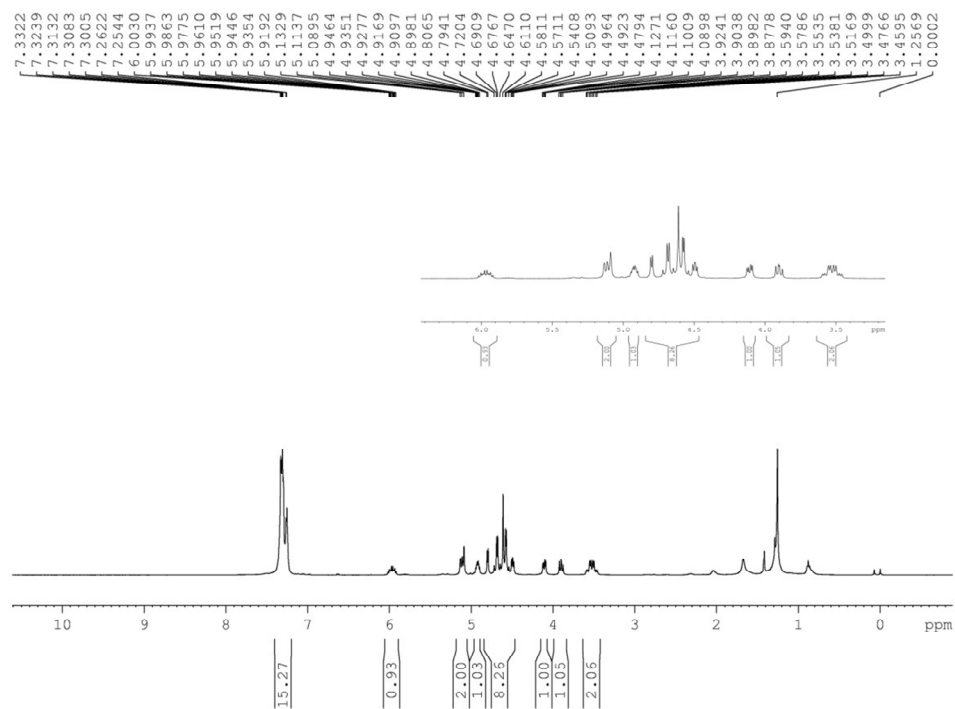




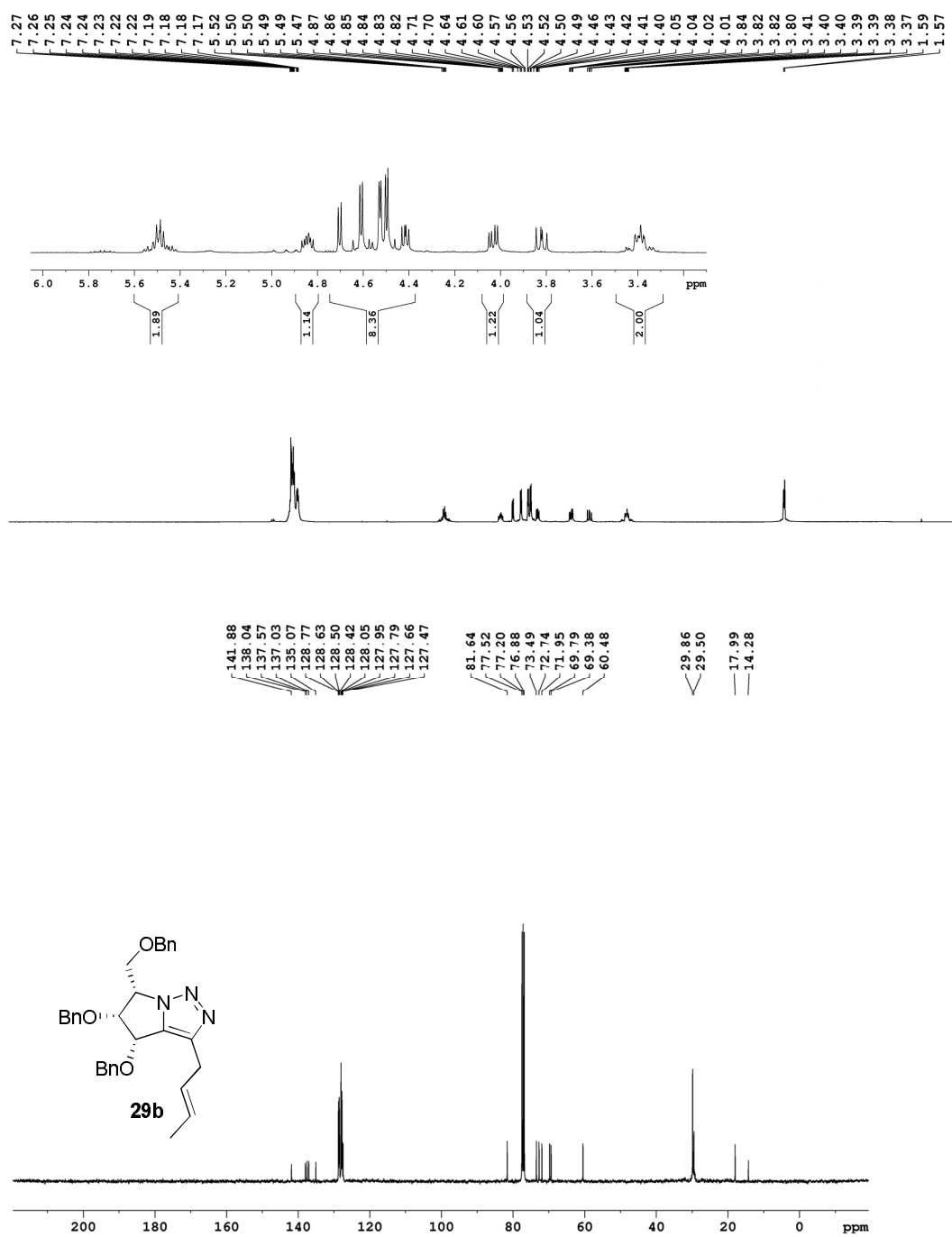
¹H and ¹³C NMR Spectrum of 34



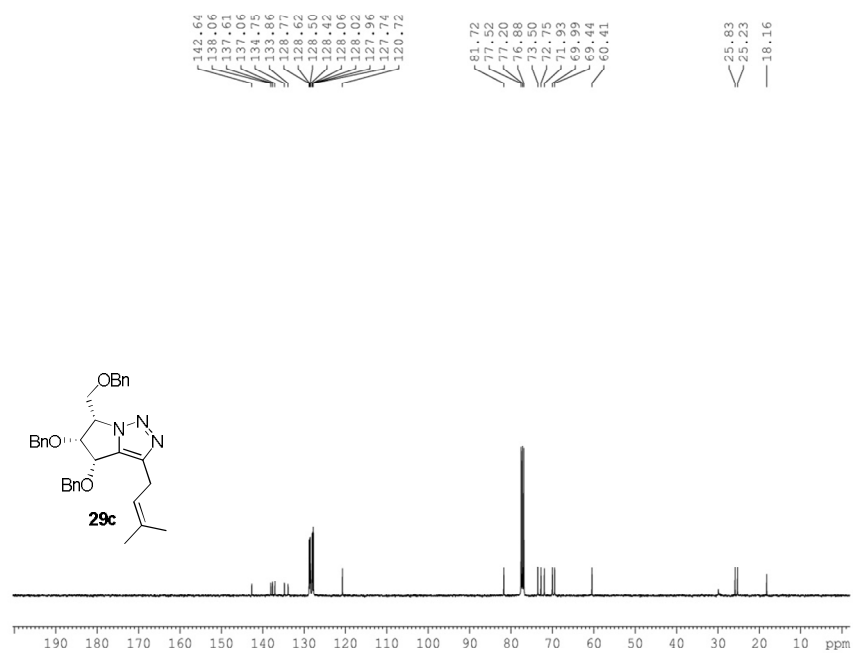
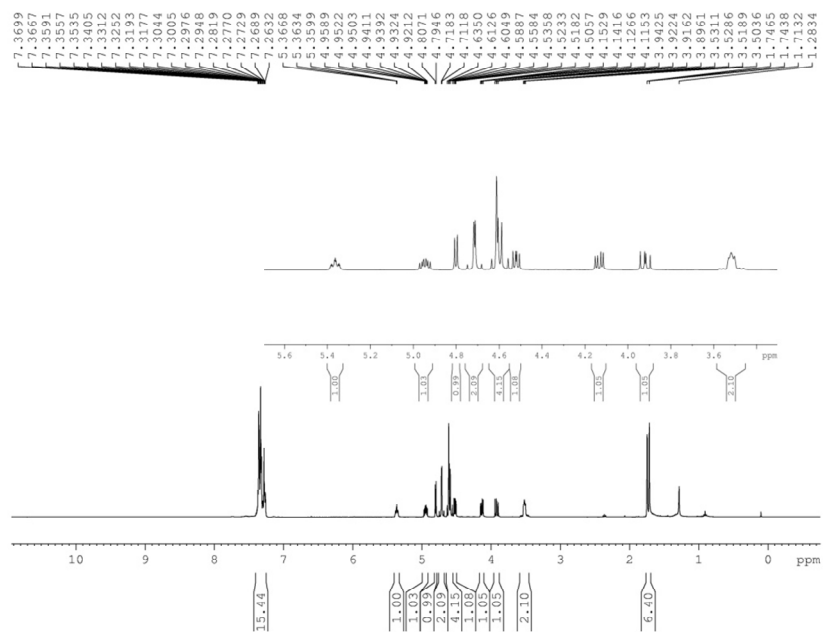
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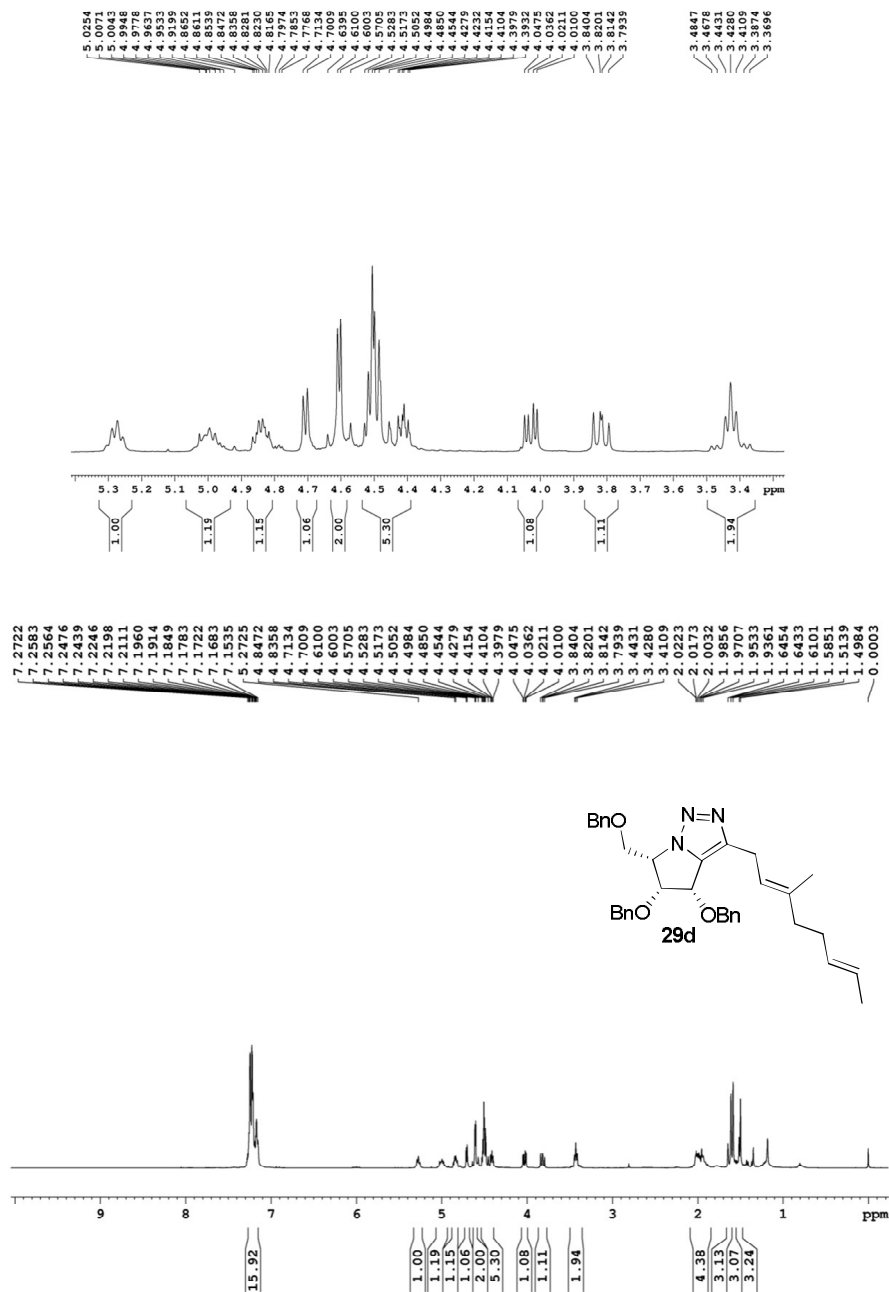
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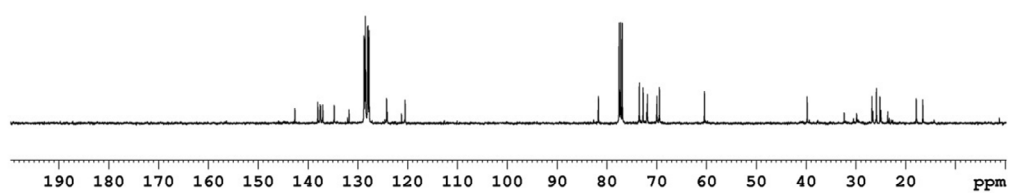
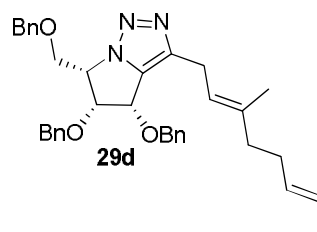
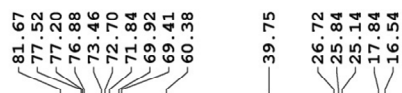
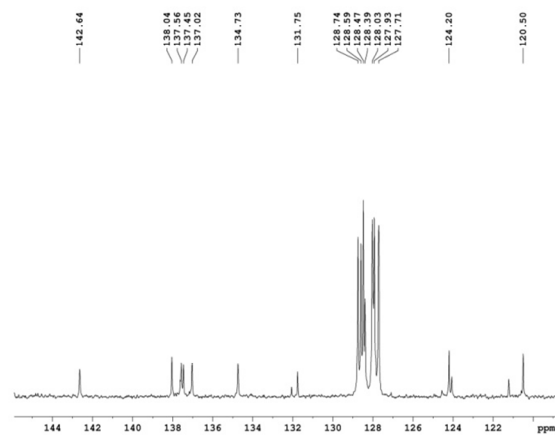
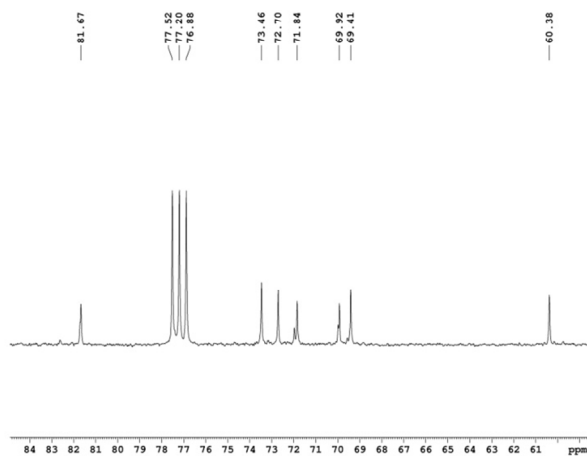


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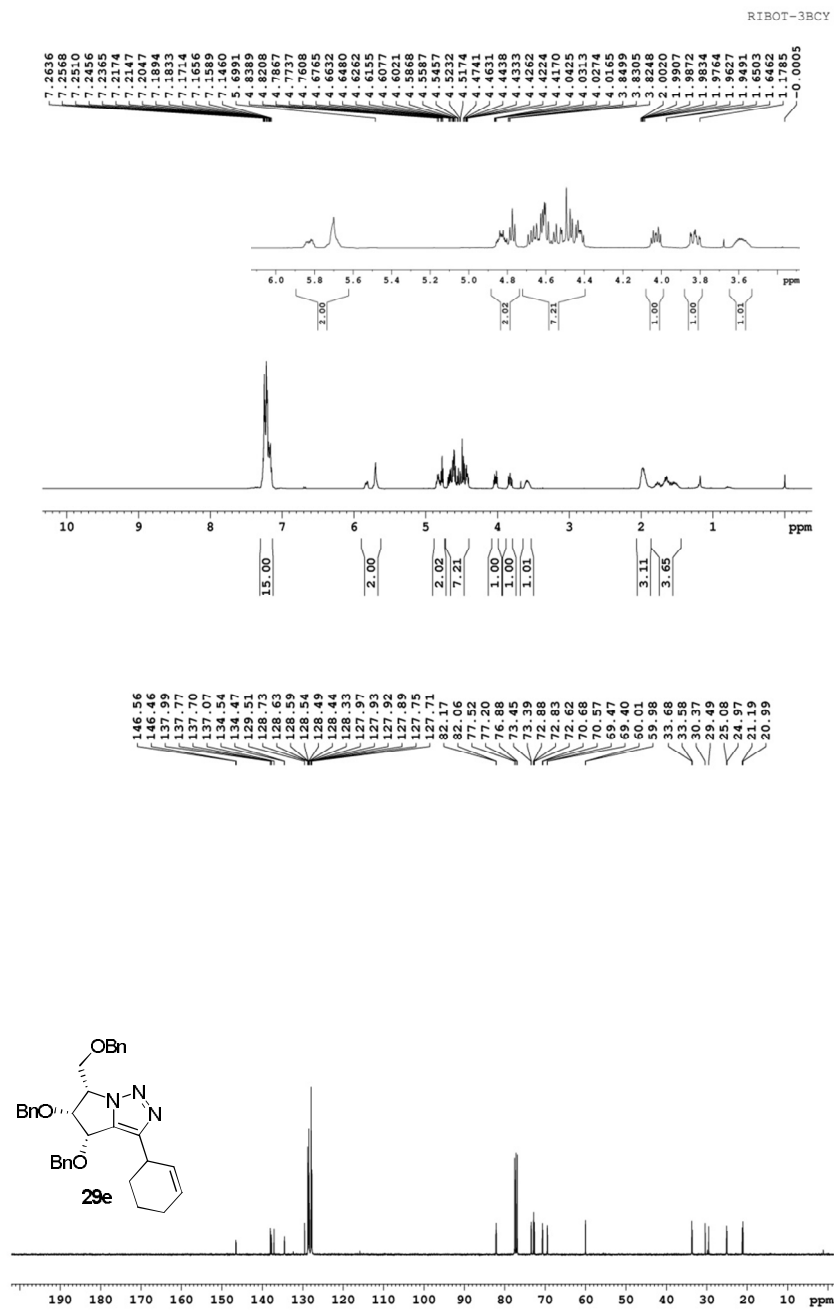


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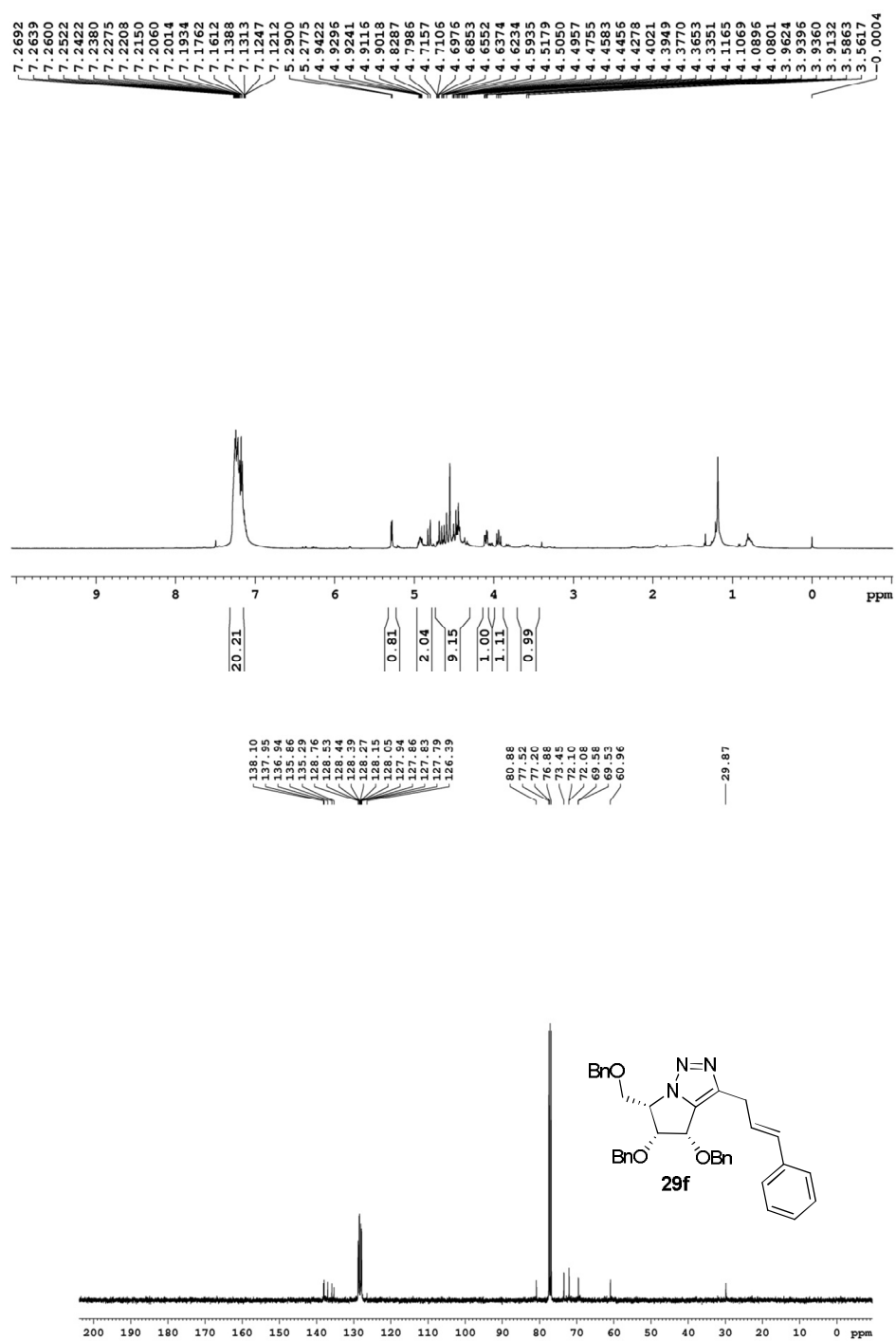




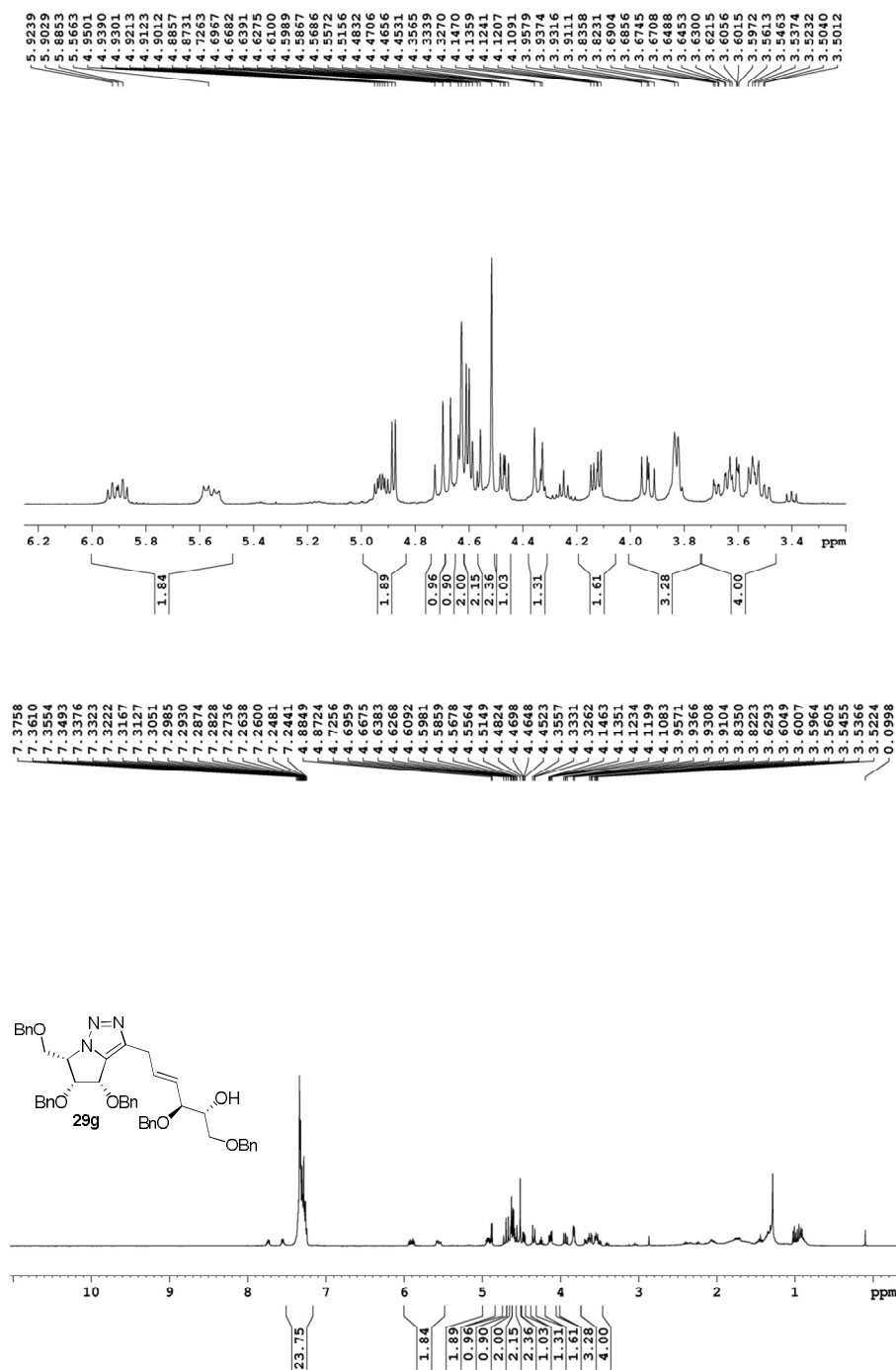
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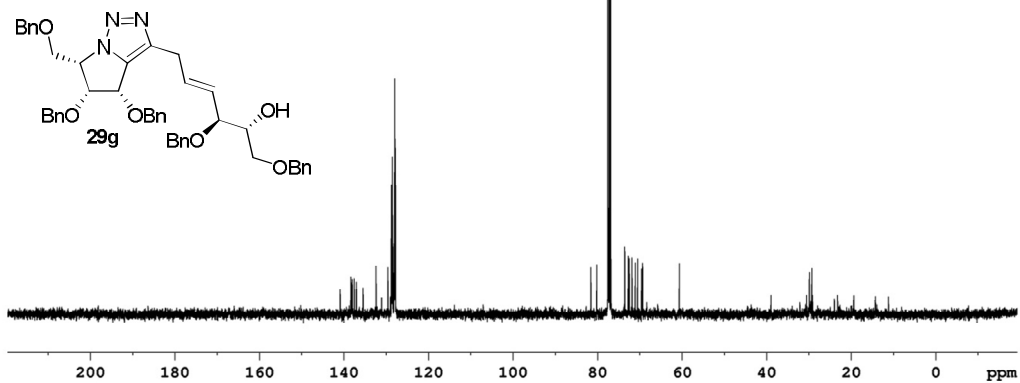
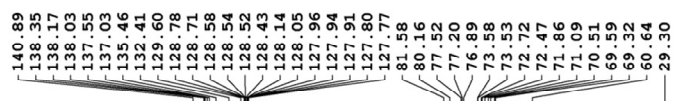
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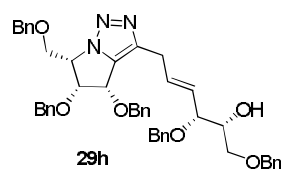
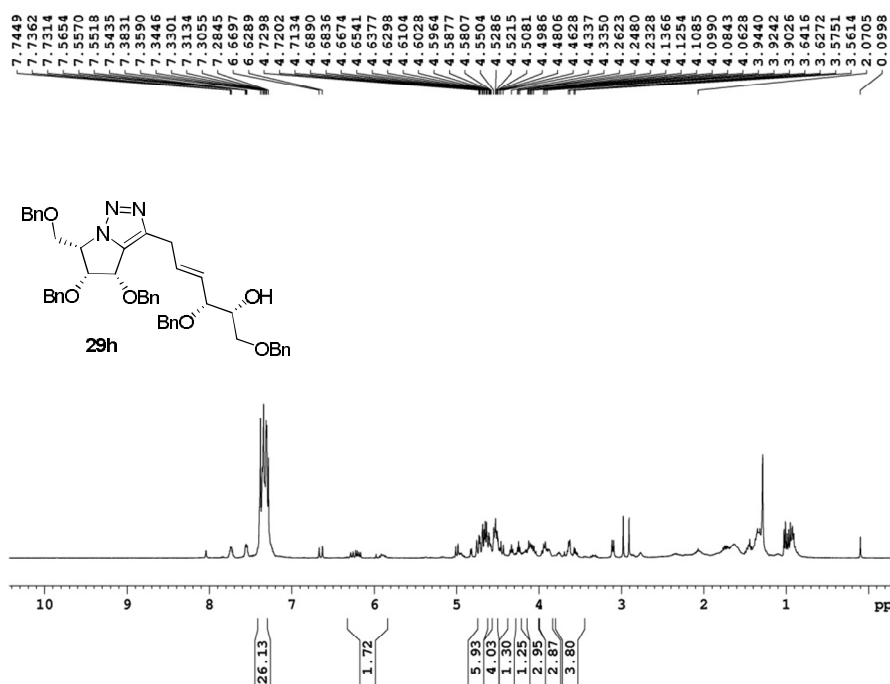
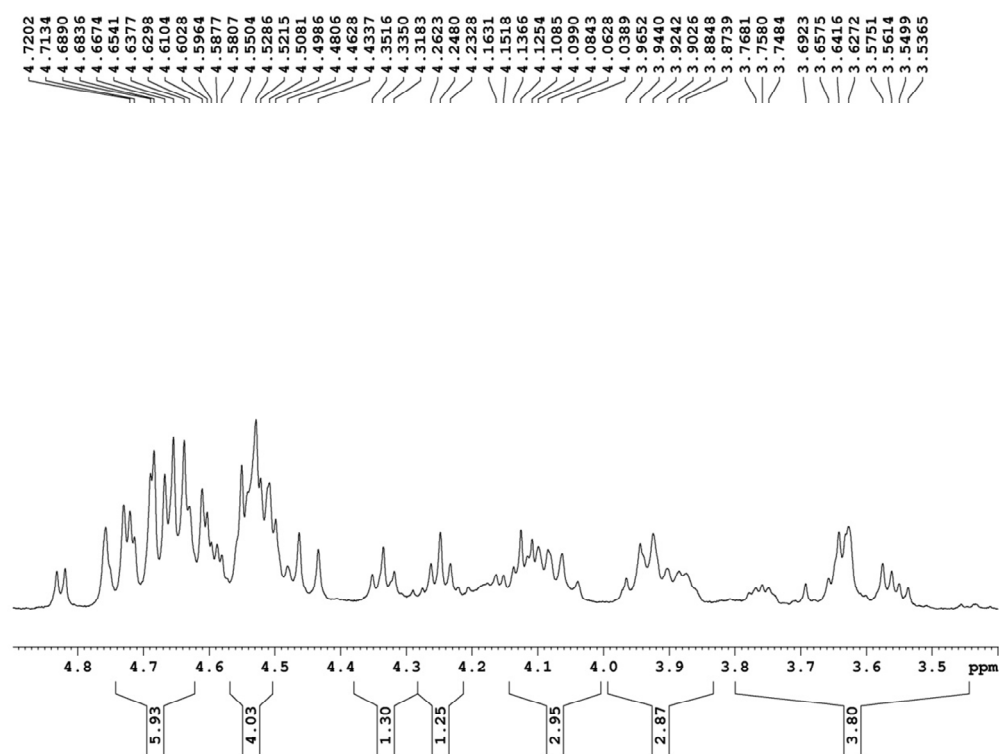


¹H and ¹³C NMR Spectrum of 29f

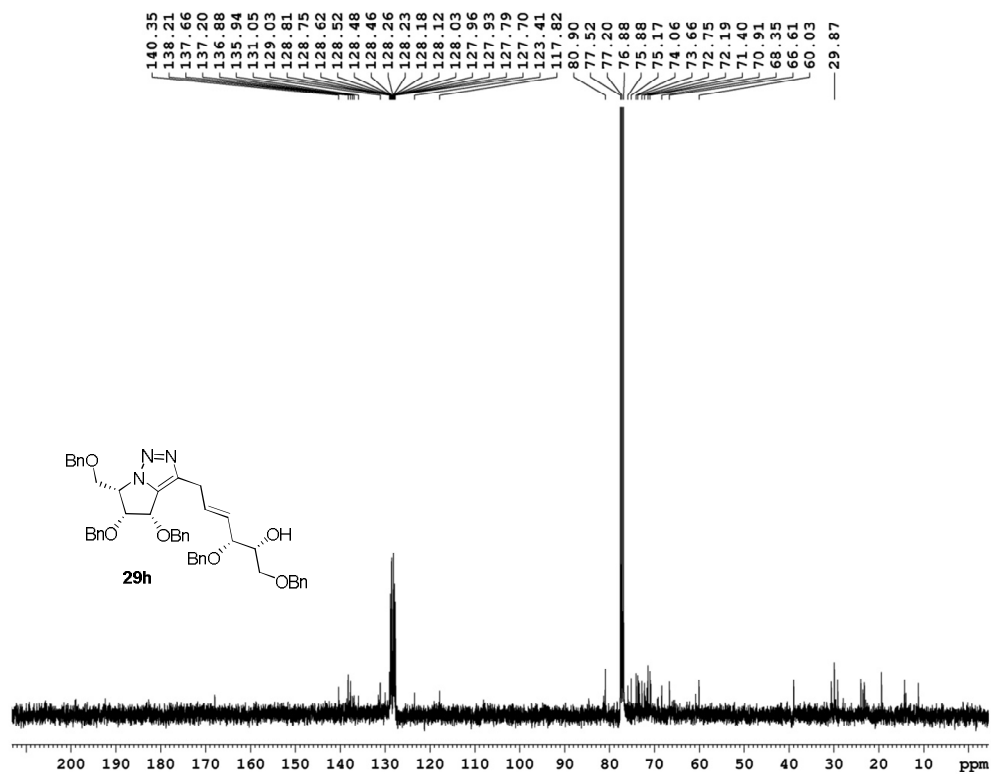
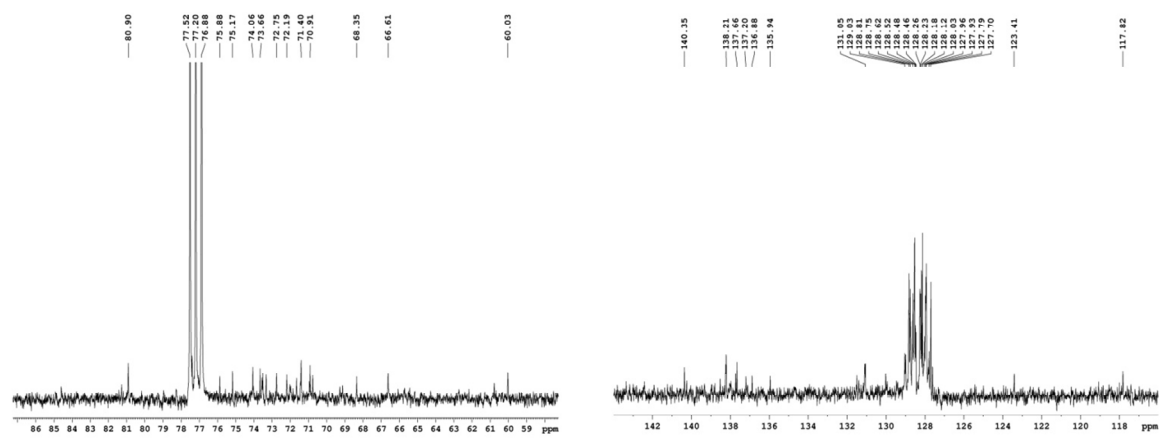


¹H NMR Spectrum of 29g

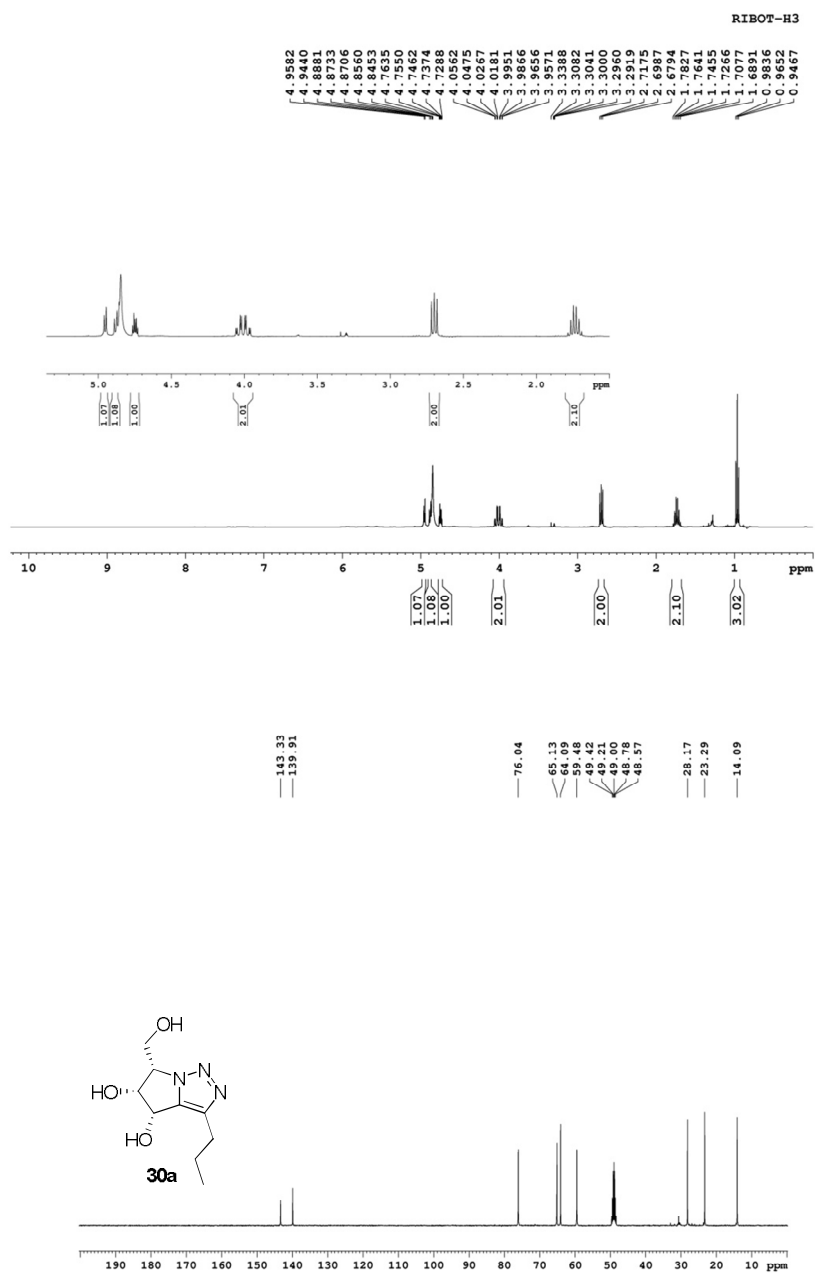




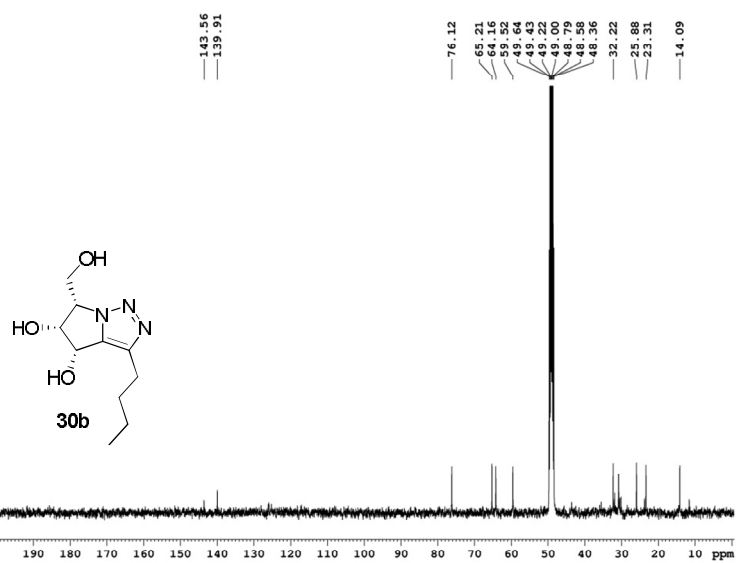
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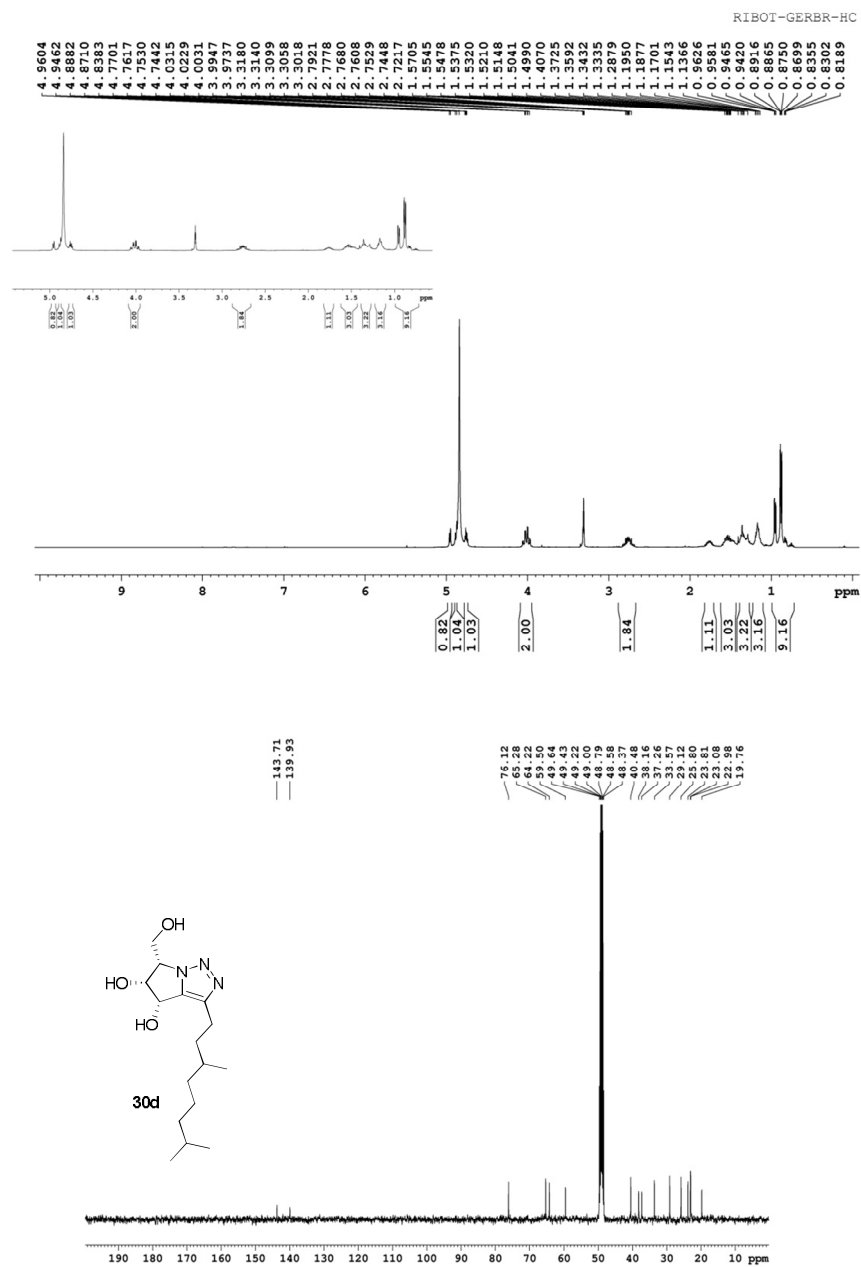


^{13}C NMR Spectrum of 29h

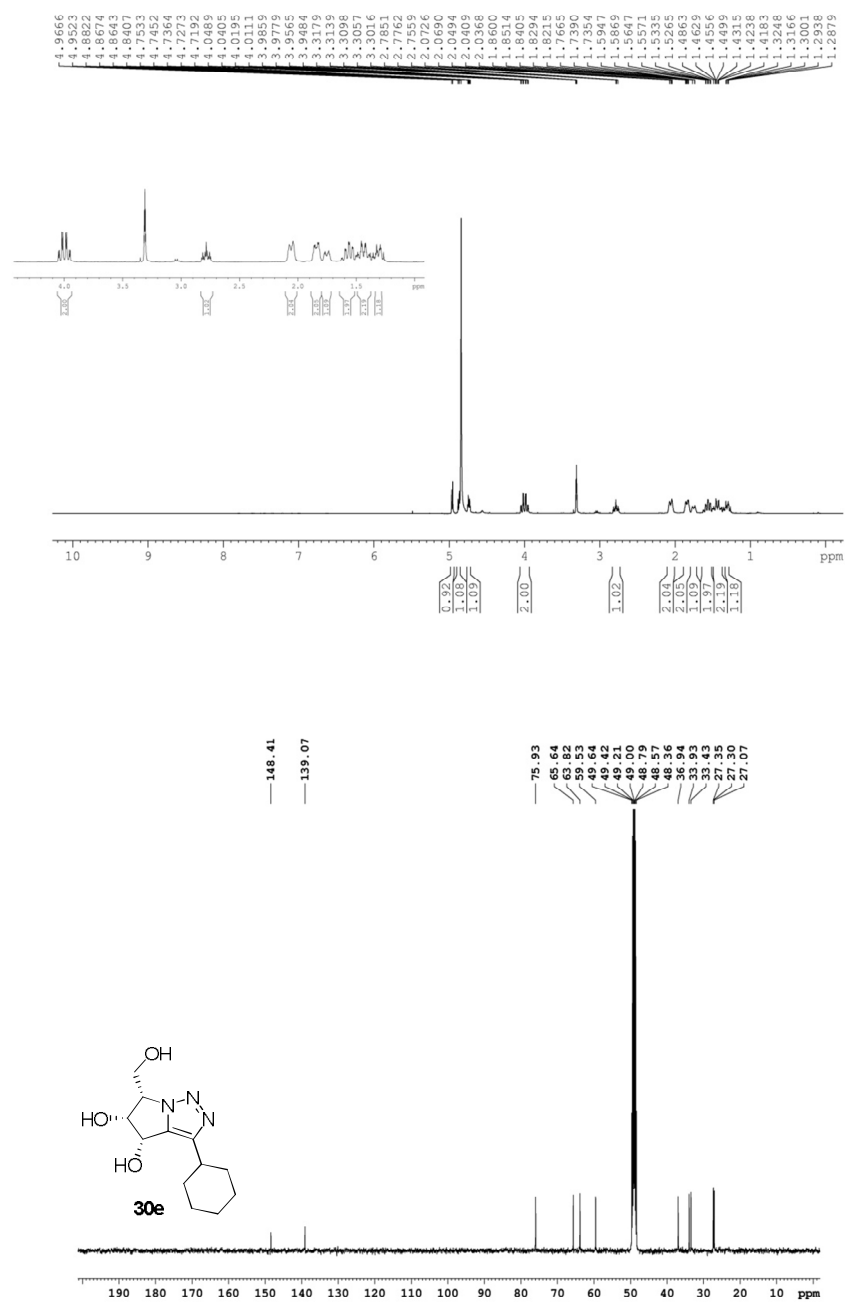


¹H and ¹³C NMR Spectrum of 30a

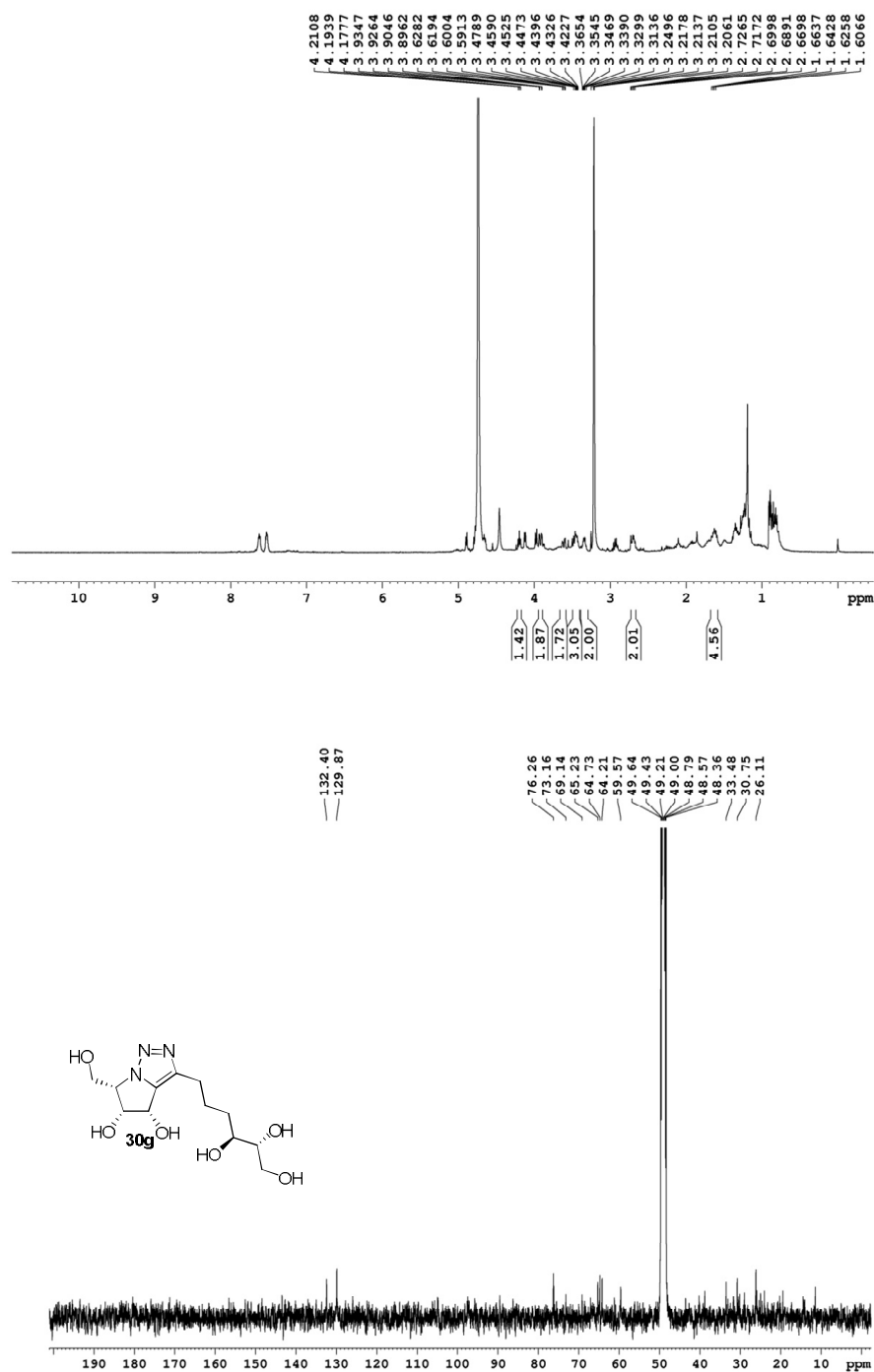




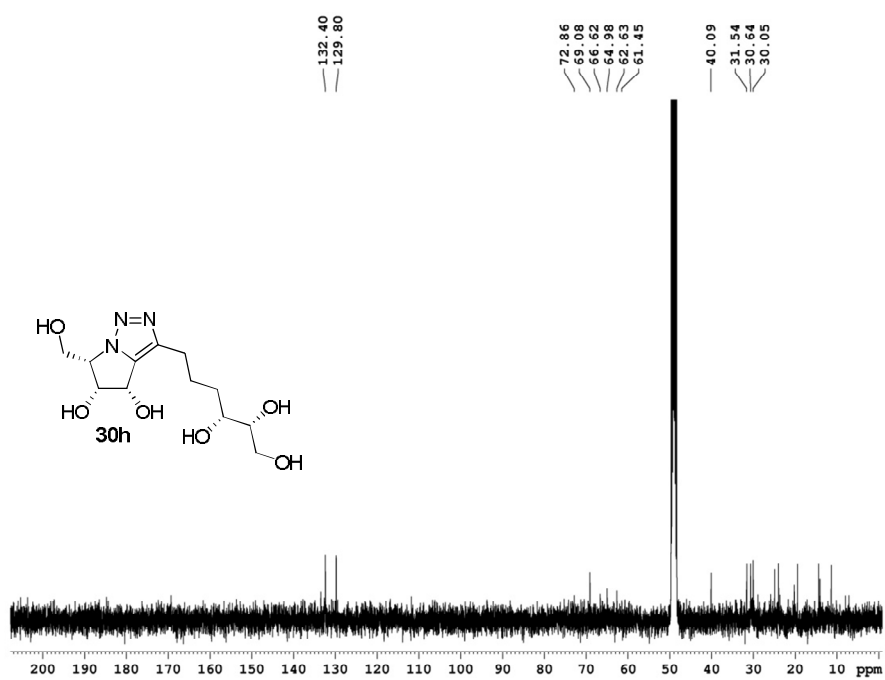
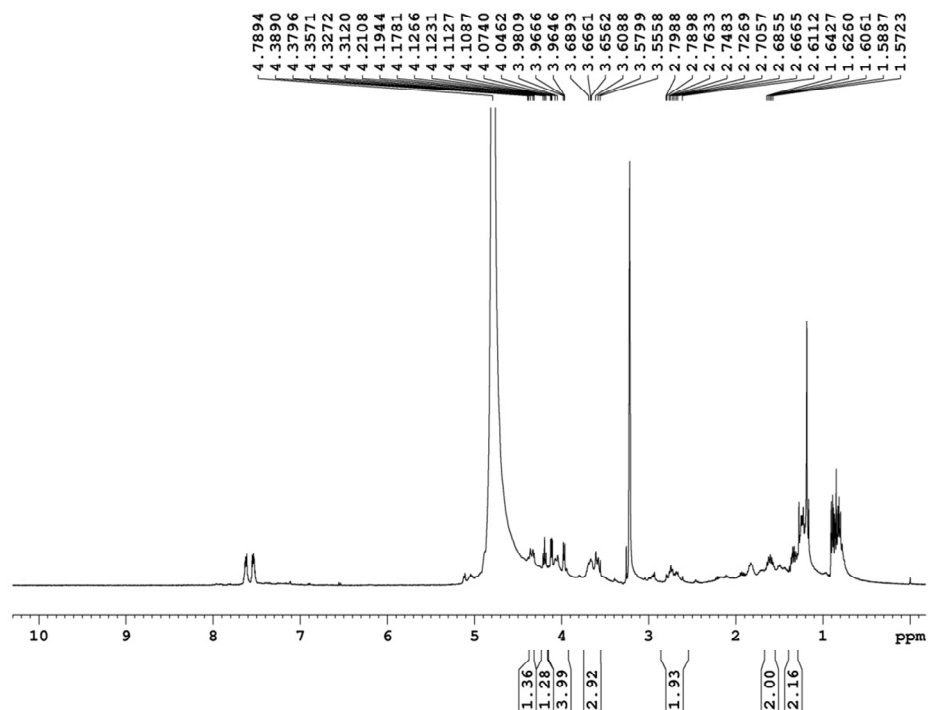
¹H and ¹³C NMR Spectrum of 30d



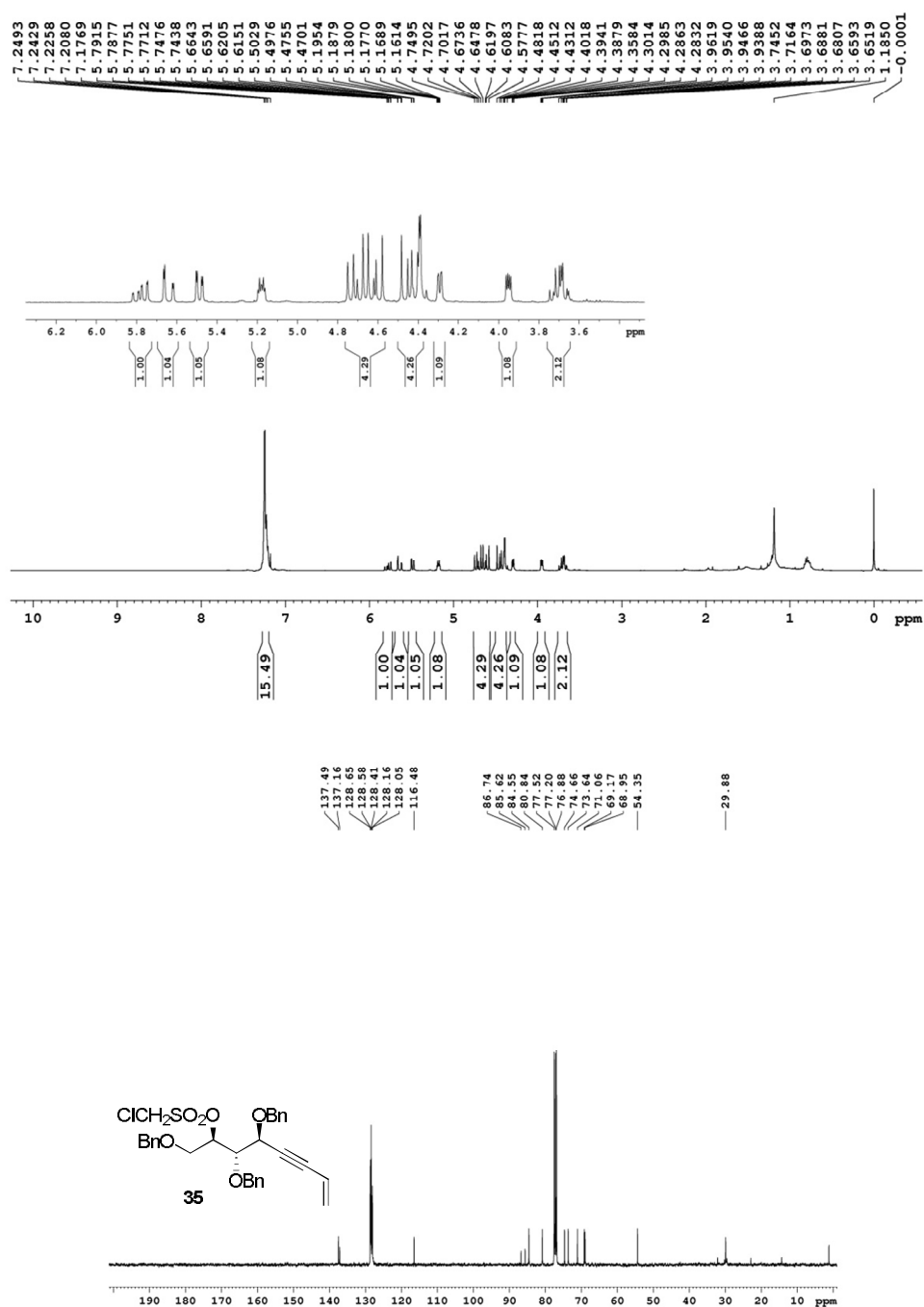
¹H and ¹³C NMR Spectrum of 30e



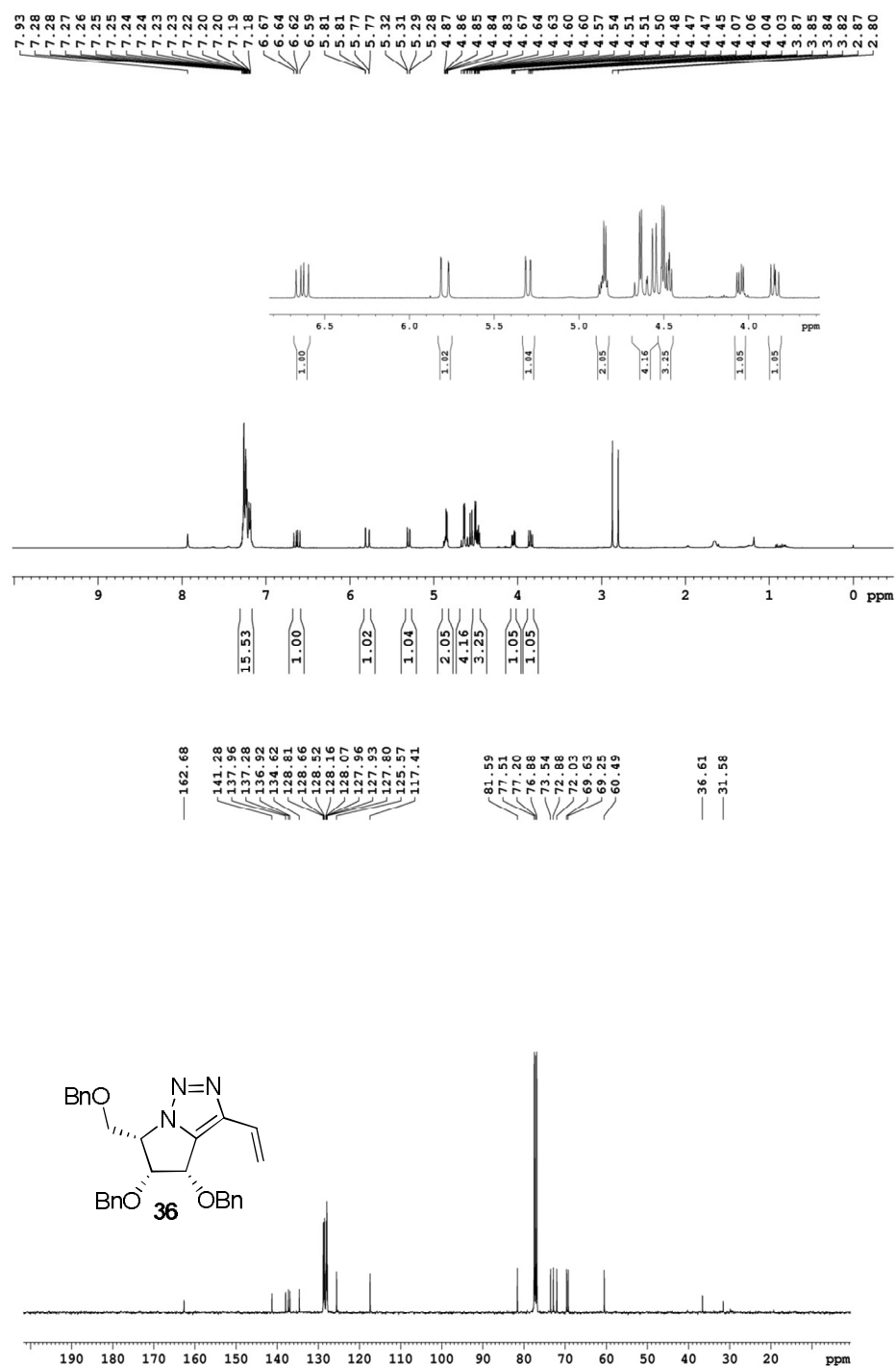
¹H and ¹³C NMR Spectrum of 30g



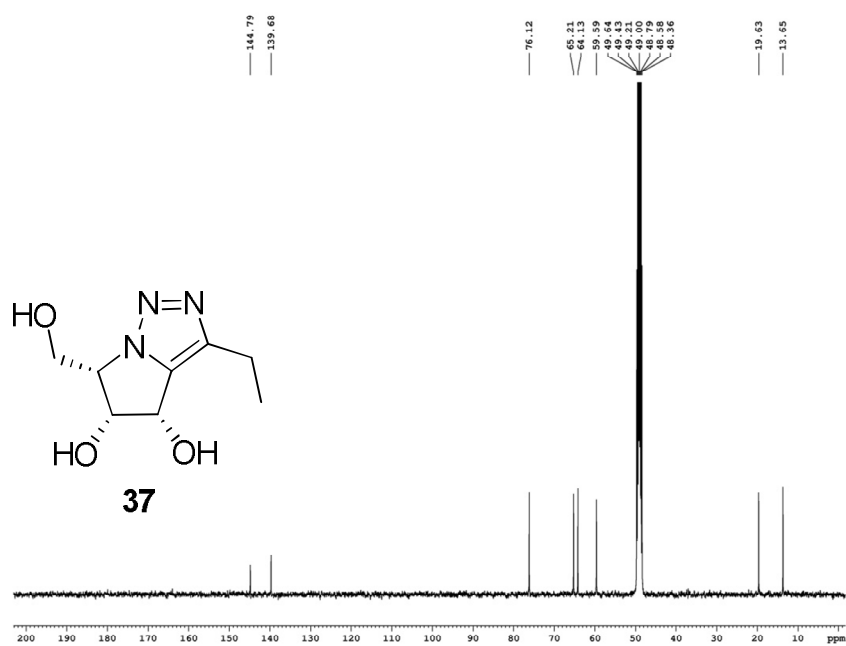
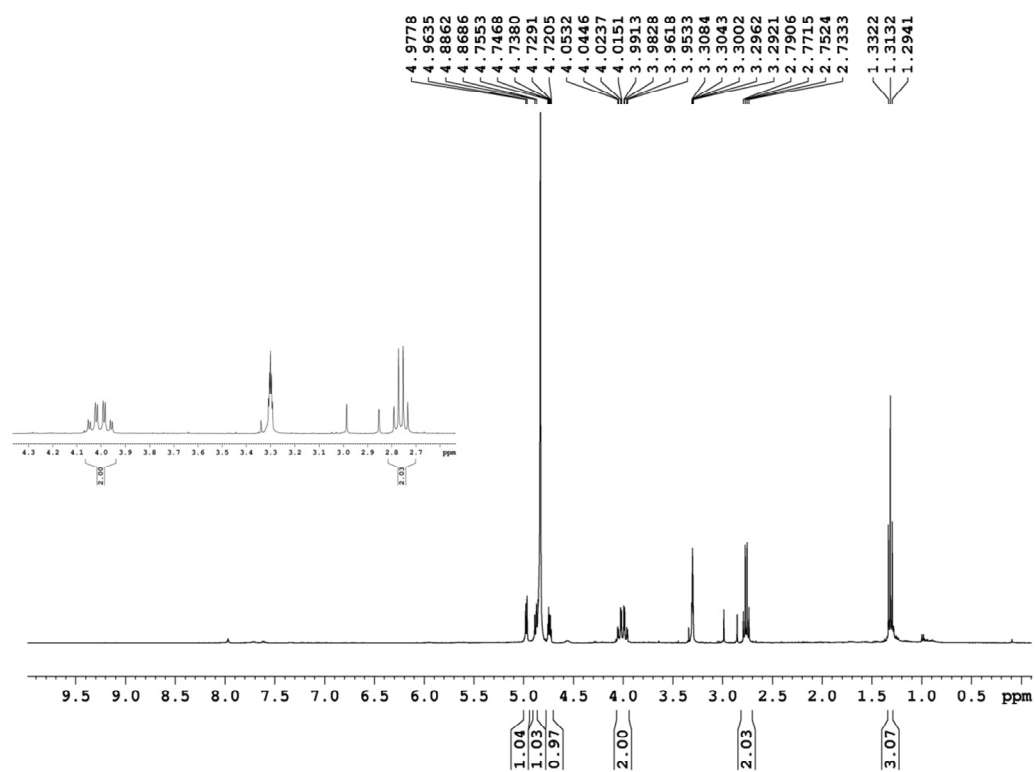
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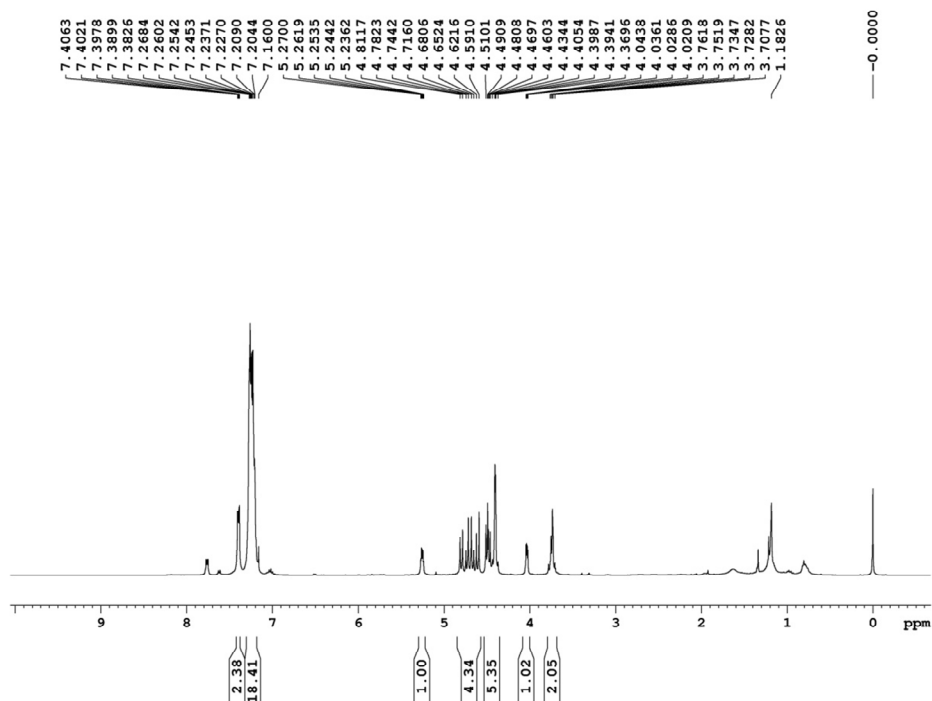
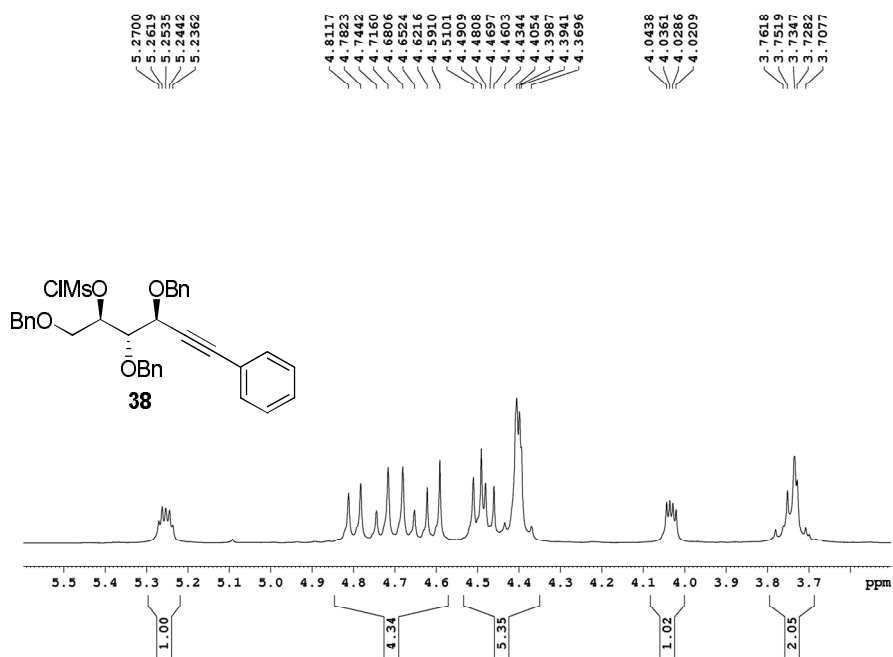
¹H and ¹³C NMR Spectrum of 35



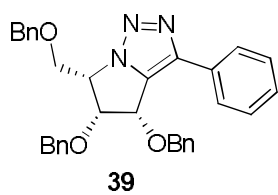
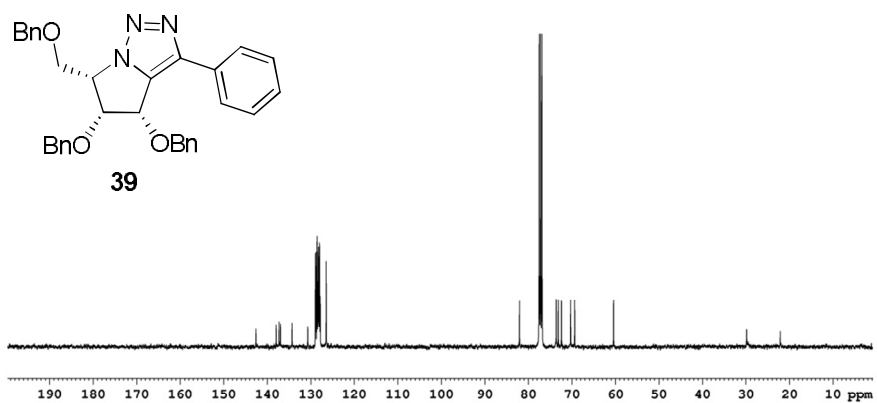
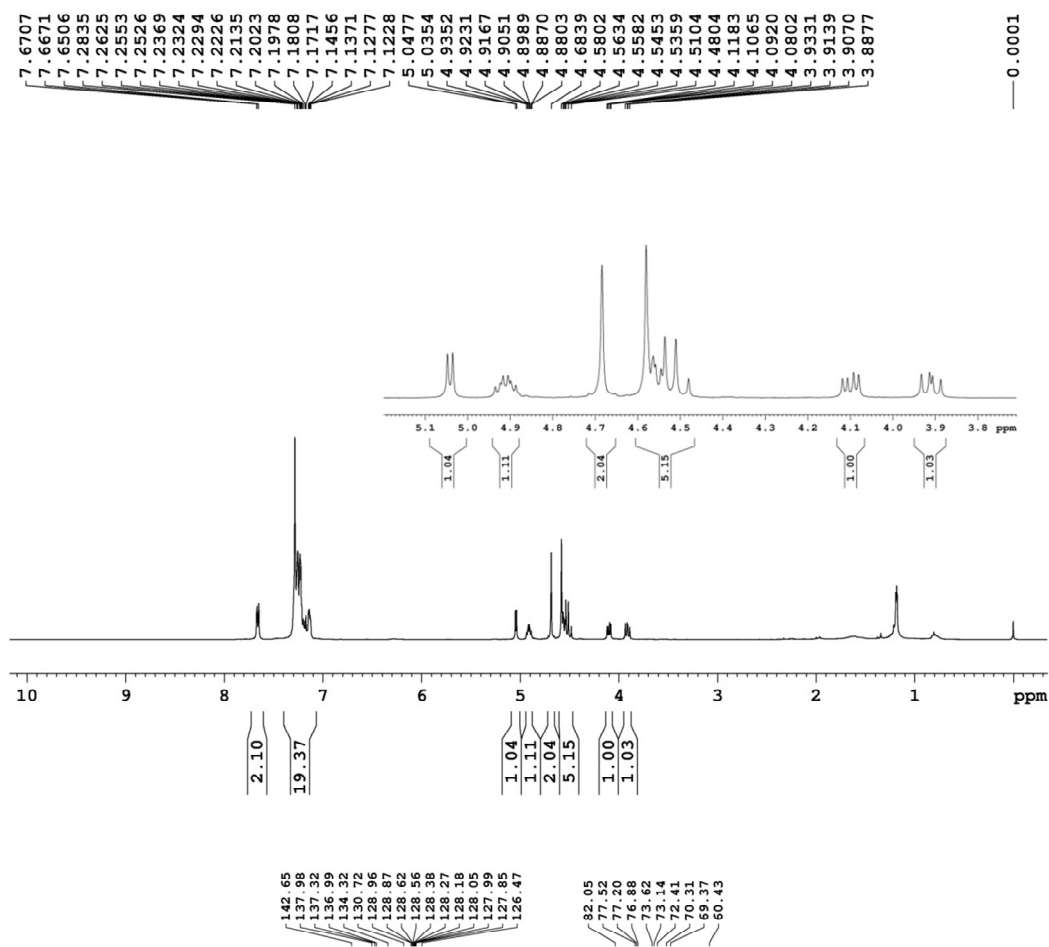
¹H and ¹³C NMR Spectrum of 36



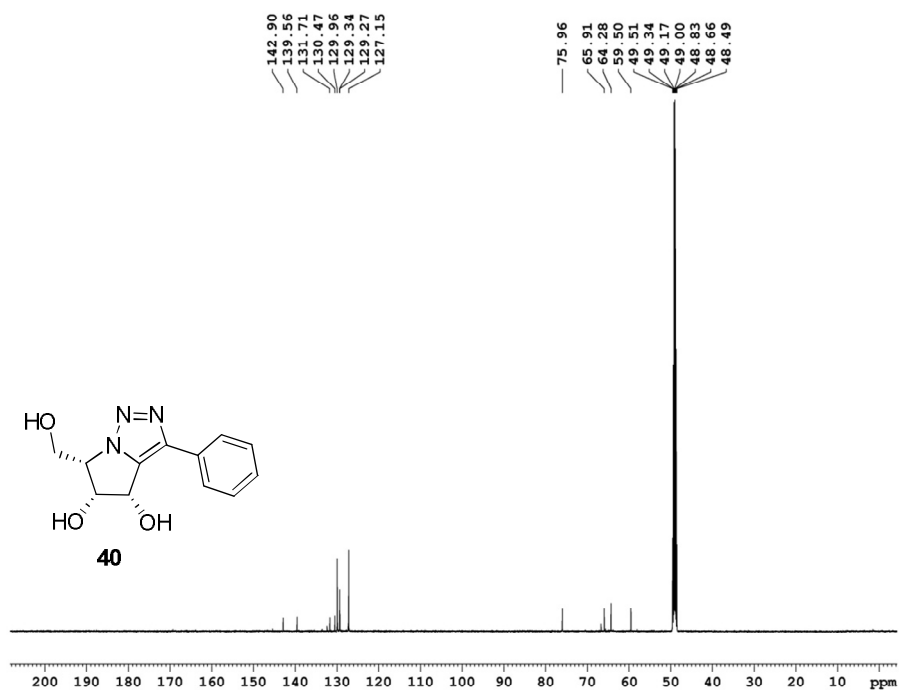
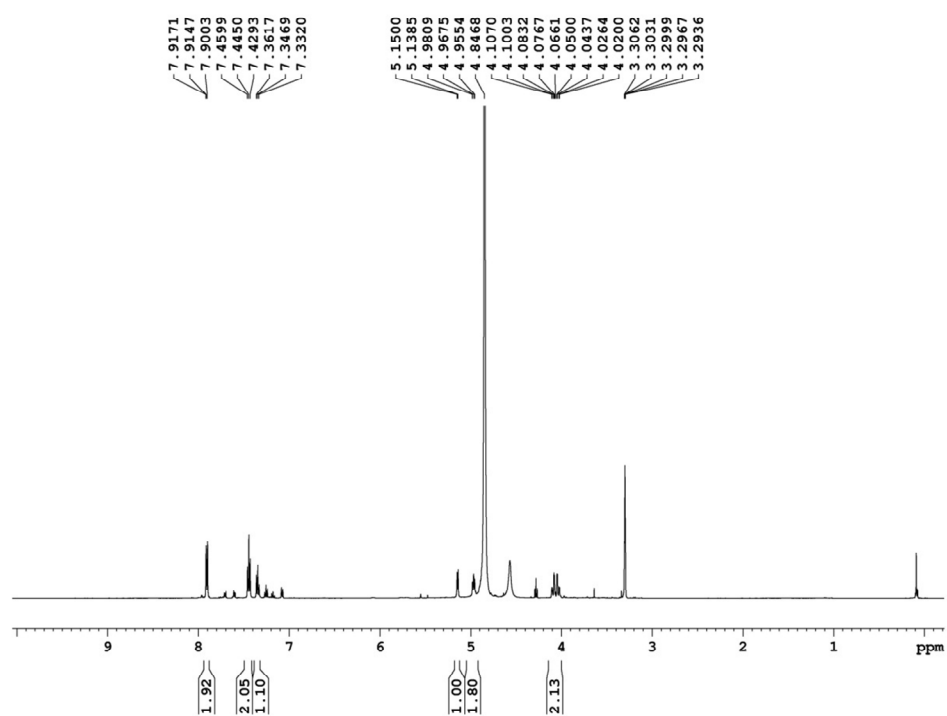
¹H and ¹³C NMR Spectrum of **37**



^1H NMR Spectrum of 38



¹H and ¹³C NMR Spectrum of 39



¹H and ¹³C NMR Spectrum of 40

Glycosidase Inhibition

General Methods are mentioned in the manuscript text. Inhibition potencies of compounds were determined according to Gunter and Stefan (1986), Li *et al* (2011) by minute modifications, measuring the residual hydrolytic activities of glycosidase of the corresponding *p*-nitrophenyl glycosides in presences of compounds spectrophotometrically. Michaelis-Menten plot of Activity versus Substrate concentration for inhibition and K_i were determined by nonlinear regression using data to a competitive inhibition model using Graph Pad Prism (version 6.01 for Windows, Graph Pad Software, San Diego California (USA)).¹⁻³

Table S1: List of enzymes, respective substrates, buffers and optimum incubation temperatures.

S.No	Enzyme	Substrate	Buffer	Incubation temperature.
1	α -galactosidase (green coffee bean)	<i>p</i> -nitrophenyl α -D-galactopyranoside	Citrate phosphate buffer (50 mM, pH 6.5)	25°C
2	β -galactosidase (<i>bovine liver</i>)	<i>p</i> -nitrophenyl β -D-galactosidase	Citrate phosphate buffer (50 mM, pH 4.5)	30°C
3	α -mannosidase (jack bean)	<i>p</i> -nitrophenyl α -D-mannopyranoside	Acetate buffer (50 mM, pH 4.5)	25 °C
4	β -glucosidase (almond)	<i>p</i> -nitrophenyl β -D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 5.5)	37 °C
5	α -glucosidase (yeast)	<i>p</i> -nitrophenyl α -D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
6	Glucosidase (<i>A. niger</i>)	<i>p</i> -nitrophenyl α -D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
7	α -glucosidase (rice)	<i>p</i> -nitrophenyl α -D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
8	α -L-fucosidase (<i>bovine kidney</i>)	4-nitrophenyl α -L-fucopyranoside	Citrate phosphate buffer (50 mM, pH 5)	30 °C

Table S2: % of inhibition of glycosidases by 30a-e, 30g, 30h and 37 at 400 μ M.

(% of inhibition at 400 μM)								
Enzyme	30a	30e	30b	30c	30d	30h	30g	37
α -galactosidase (green coffee bean)	3.74	1.7	0.34	0.34	0	1.7	2.89	3.23
β -galactosidase (bovine liver)	4.93	3.15	0.16	1.49	1.49	64.59	63.74	5.47
α -mannosidase (jack bean)	1.4	1.4	0.9	0.7	2.83	0	0	3.3
β -glucosidase (almond)	2.7	1.72	0	0.57	0.95	0	0	2.86
α -glucosidase (yeast)	4.79	5.43	84.51	93.73	4.15	90.27	90.63	3.83
Glucosidase (<i>A. niger</i>)	5.93	5.42	1.01	0.84	2.54	0	0.84	0.84
α -glucosidase (rice)	2.94	4.85	1.47	1.17	1.17	0.29	1.47	3.52
α -L-fucosidase (bovine kidney)	3.26	3.95	2.92	97.12	3.09	93.63	2.40	0.34

Table S3: % of inhibition of glycosidases by 30a-e, 30g, 30h and 37 at 1000 μ M.

(% of inhibition at 1000 μ M)								
Enzyme	30a	30e	30b	30c	30d	30h	30g	37
α -galactosidase (green coffee bean)	4.93	3.74	2.04	1.36	1.36	3.06	2.04	4.76
β -galactosidase (bovine liver)	6.13	6.30	2.48	2.82	3.48	69.94	66.49	6.80
α -mannosidase (jack bean)	3.70	3.70	2.10	3.70	6.14	0.70	0.70	5.66
β -glucosidase (almond)	4.78	4.78	0.57	0.57	2.48	0	0	4.78
α -glucosidase (yeast)	7.66	7.34	89.62	95.97	4.58	92.83	95.68	4.47
Glucosidase (<i>A. niger</i>)	7.11	7.11	3.55	1.69	5.08	0	1.69	2.03
α -glucosidase (rice)	5.58	6.61	2.20	2.94	4.41	1.76	2.94	6.81
α -L-fucosidase (bovine kidney)	4.29	4.46	4.41	97.84	5.15	95.59	2.92	2.92

Figures 1-8 represent Michaelis-Menten plot of Activity versus Substrate concentration for inhibition of various enzymes (see methods) by compounds 30b, 30c, 30 g and 30h. The K_i were determined by nonlinear regression using Graph Pad Prism (version 6.01 for Windows, Graph Pad Software, San Diego California USA). These curves show that the inhibition is competitive. Activity represented absorbance of liberated *p*-nitrophenol measured at 405 nm.

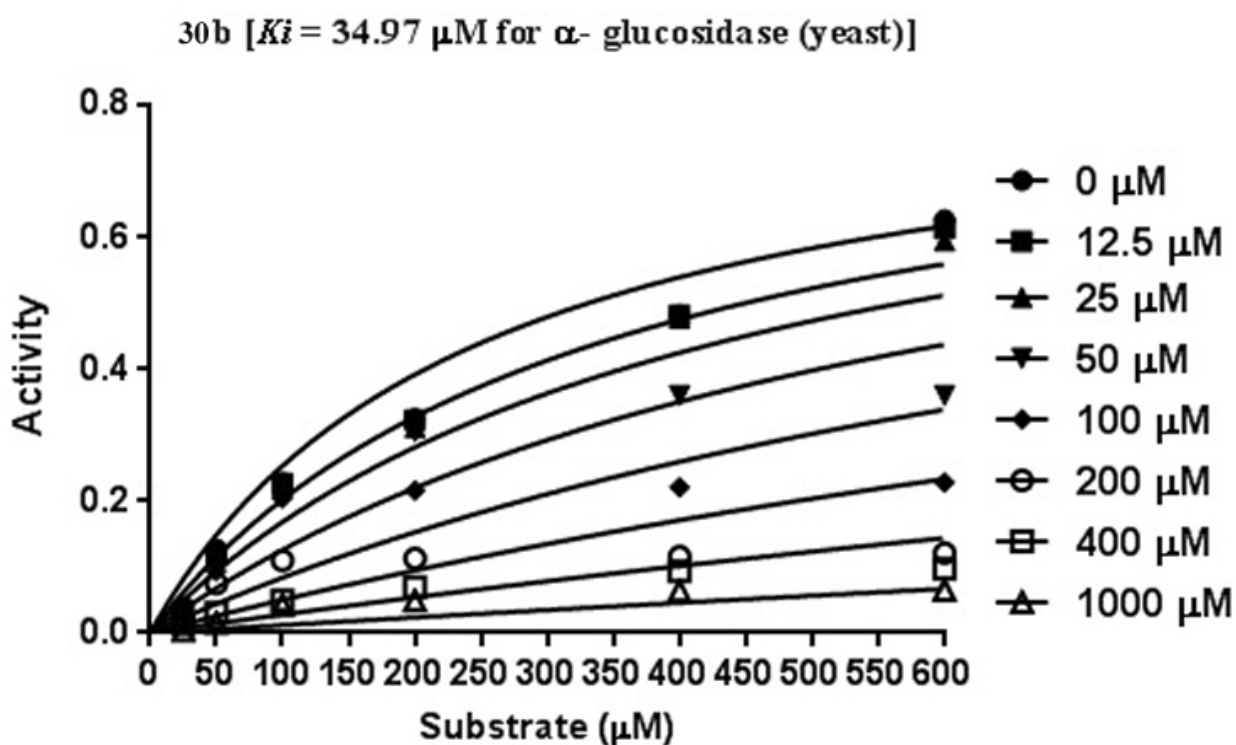


Figure 1

30c [$K_i = 13.97 \mu\text{M}$ for α - glucosidase (yeast)]

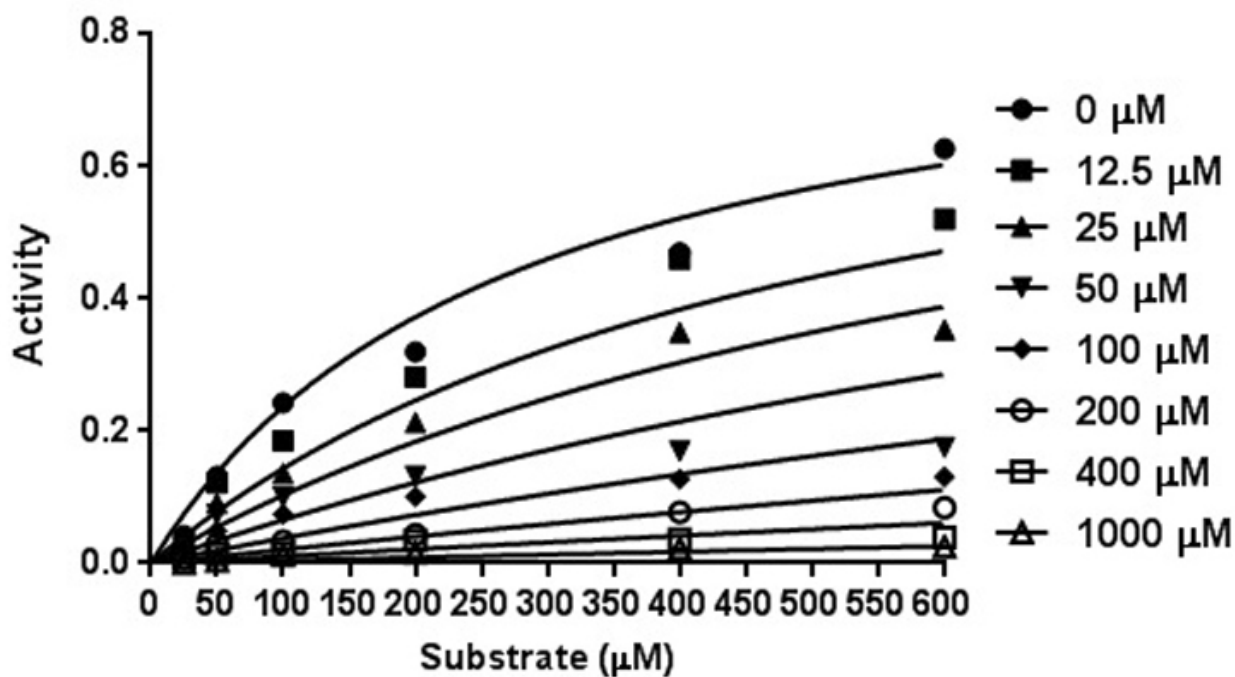


Figure 2

30c [$K_i = 9.67 \mu\text{M}$ for α - L- fucosidase (bovine kidney)]

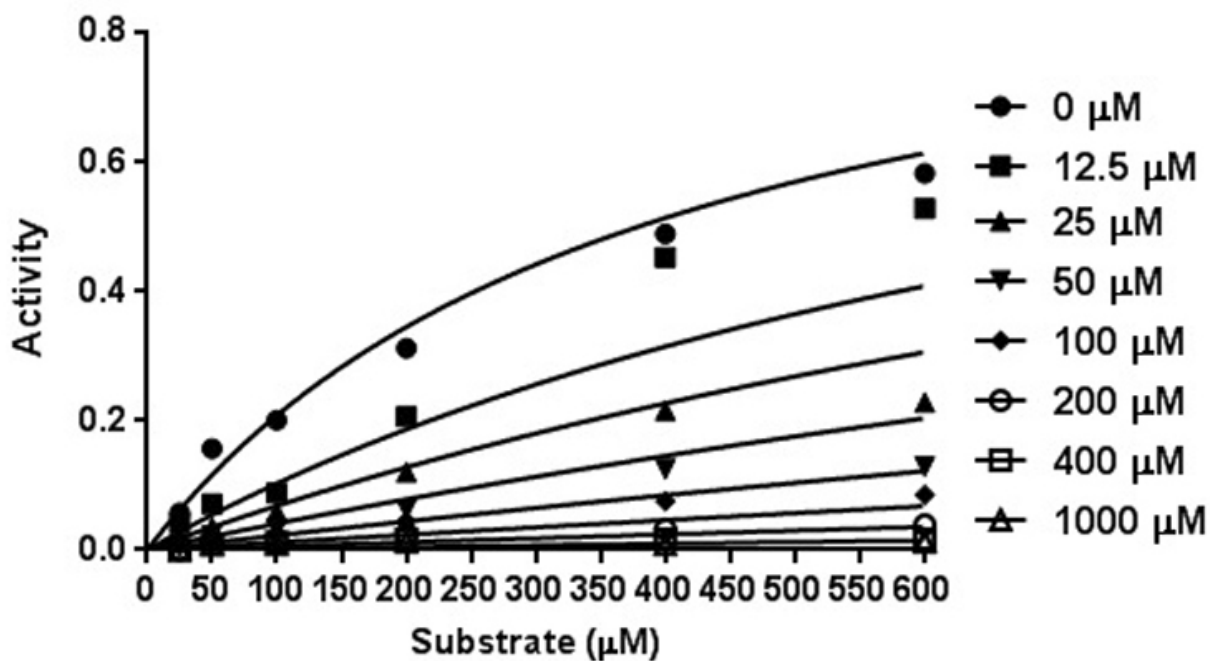


Figure 3

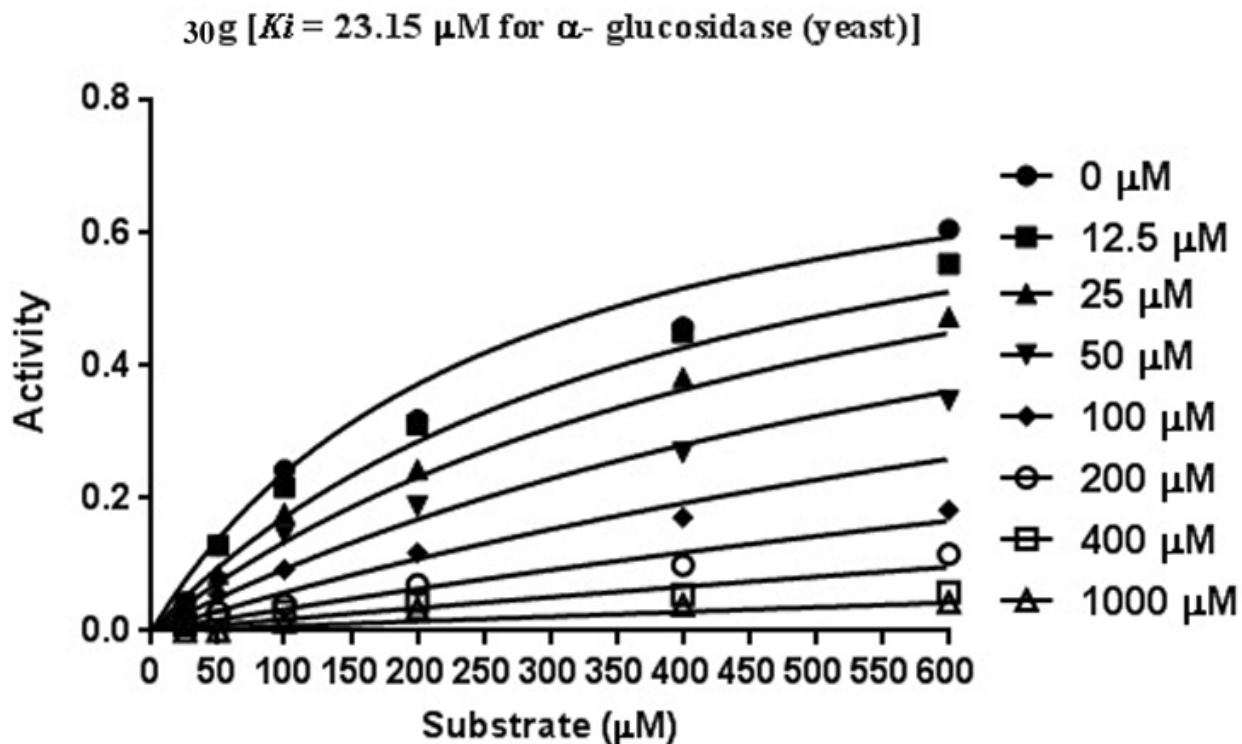


Figure 4

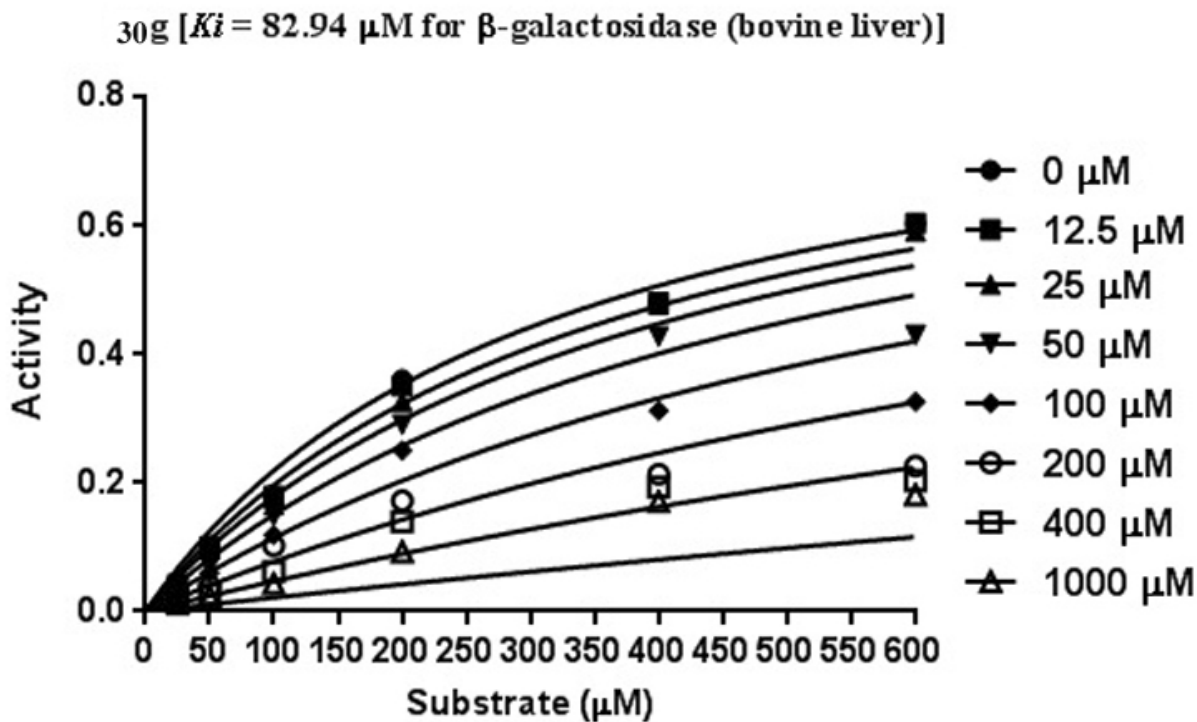


Figure 5

30h [$K_i = 14.13 \mu\text{M}$ for α -glucosidase (yeast)]

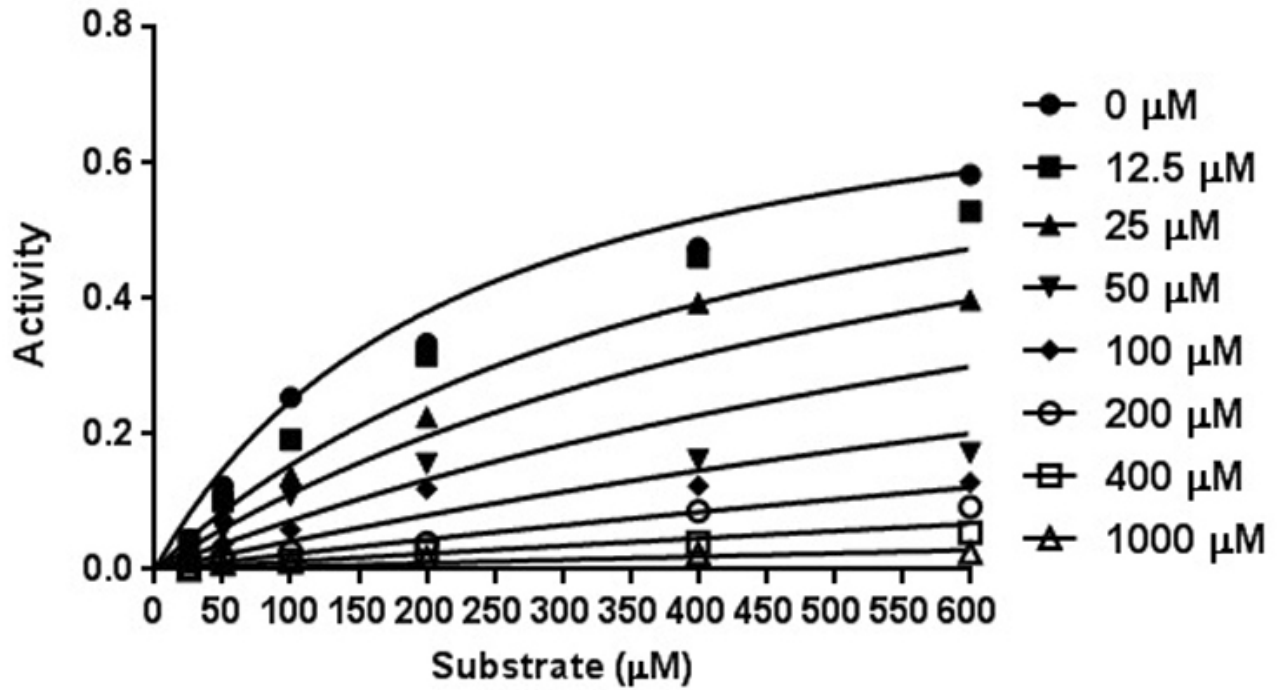


Figure 6

30h [$K_i = 100.60 \mu\text{M}$ for β -galactosidase (bovine liver)]

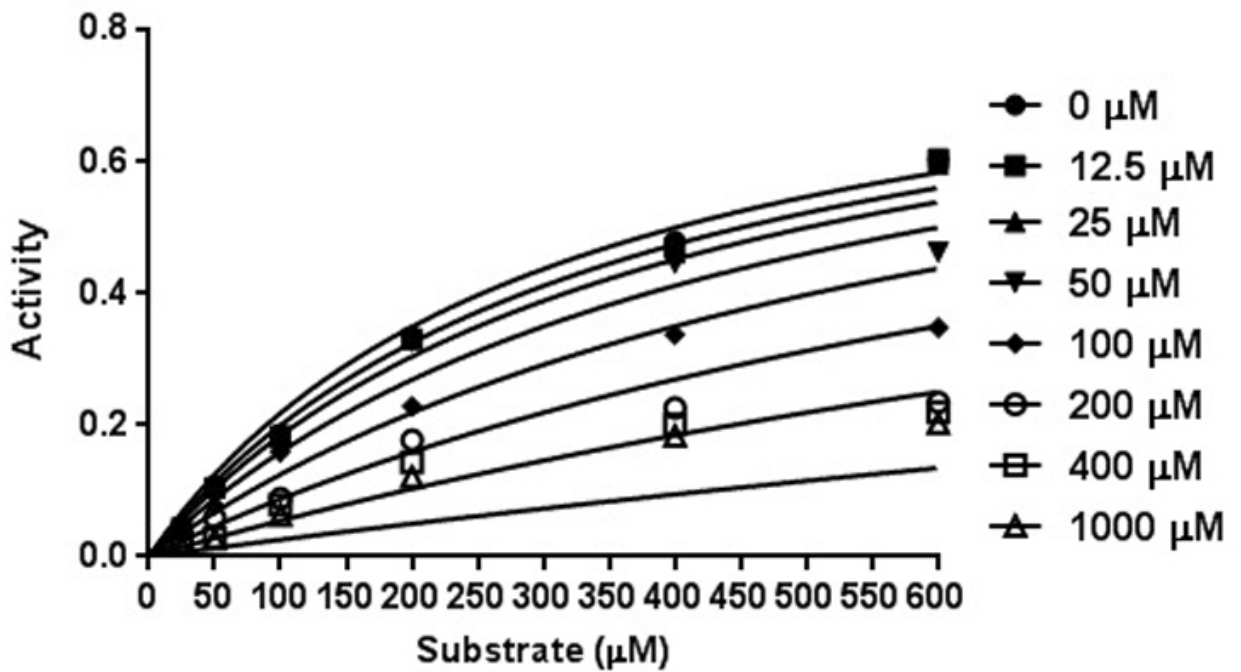


Figure 7

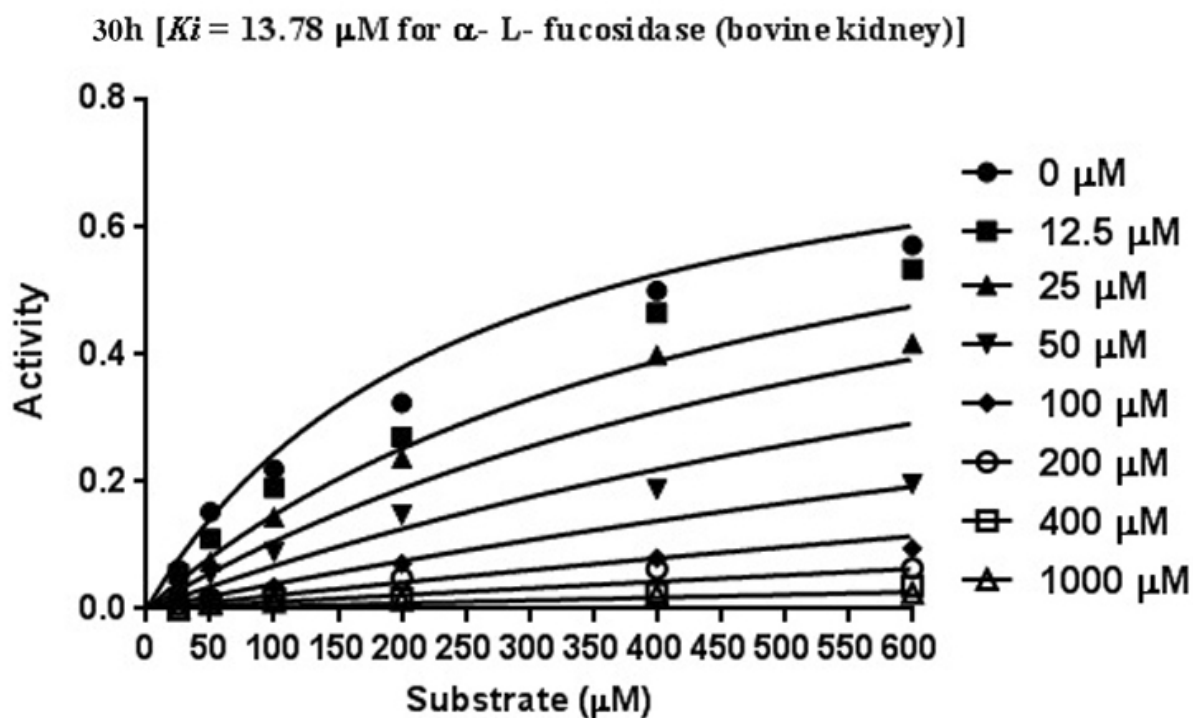


Figure 8

References

1. L. Gunter and P. Stefan, *Carbohydr. Res.*, 1986, **155**, 119-129.
2. Y. X. Li, M. H. Huang, Y. Yamashita, A. Kato, Y. M. Jia, W. B. Wang, G. W. J. Fleet, R. J. Nash and C. Yu, *Org. Biomol. Chem.*, 2011, **9**, 3405-3414.
3. K. Wagschal, D. Franqui-Espiet, C. C Lee, R. E. Kibblewhite-Accinelli, G. H. Robertson and D. W. S. Wong, *Enzyme Microb. Technol.*, 2007, **4**, 747-753.