Electronic Supplementary Information

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

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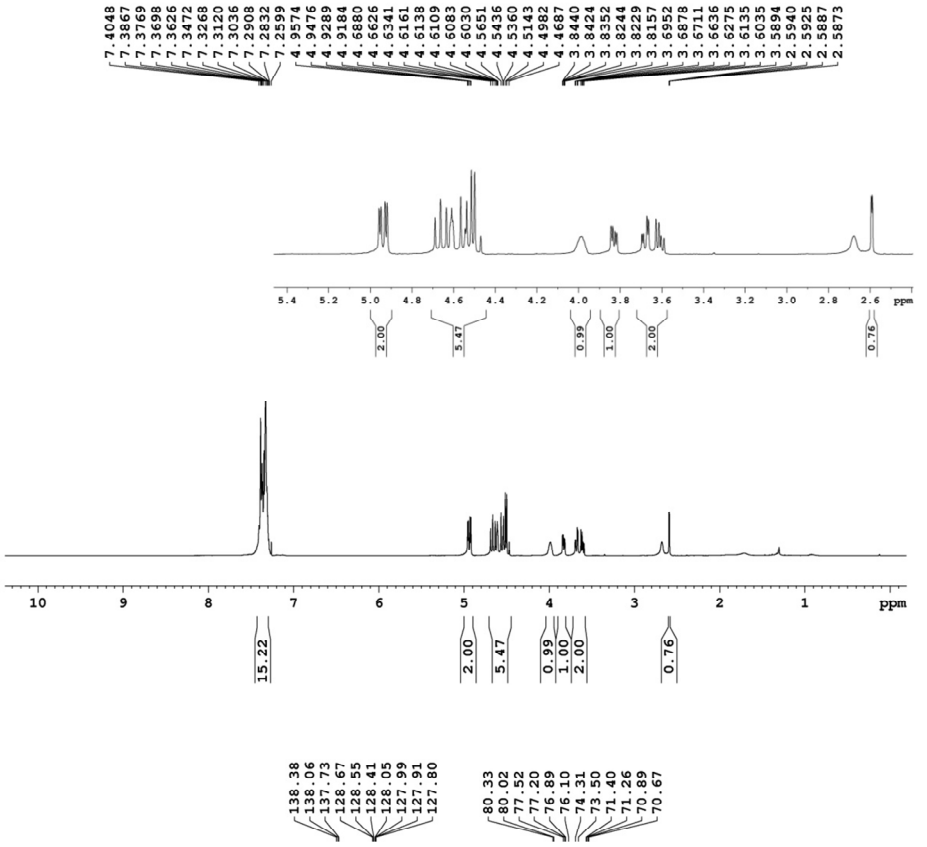
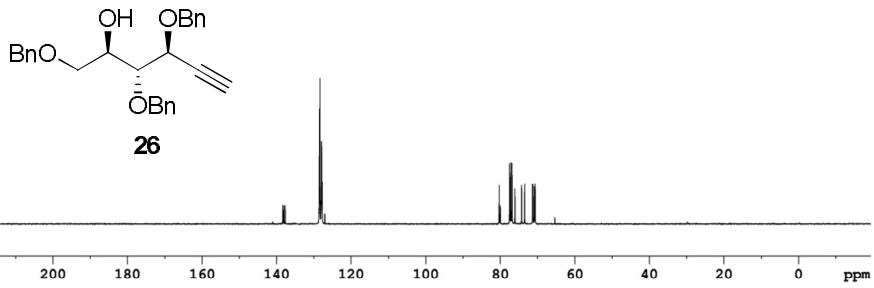
Contents **Page no.**

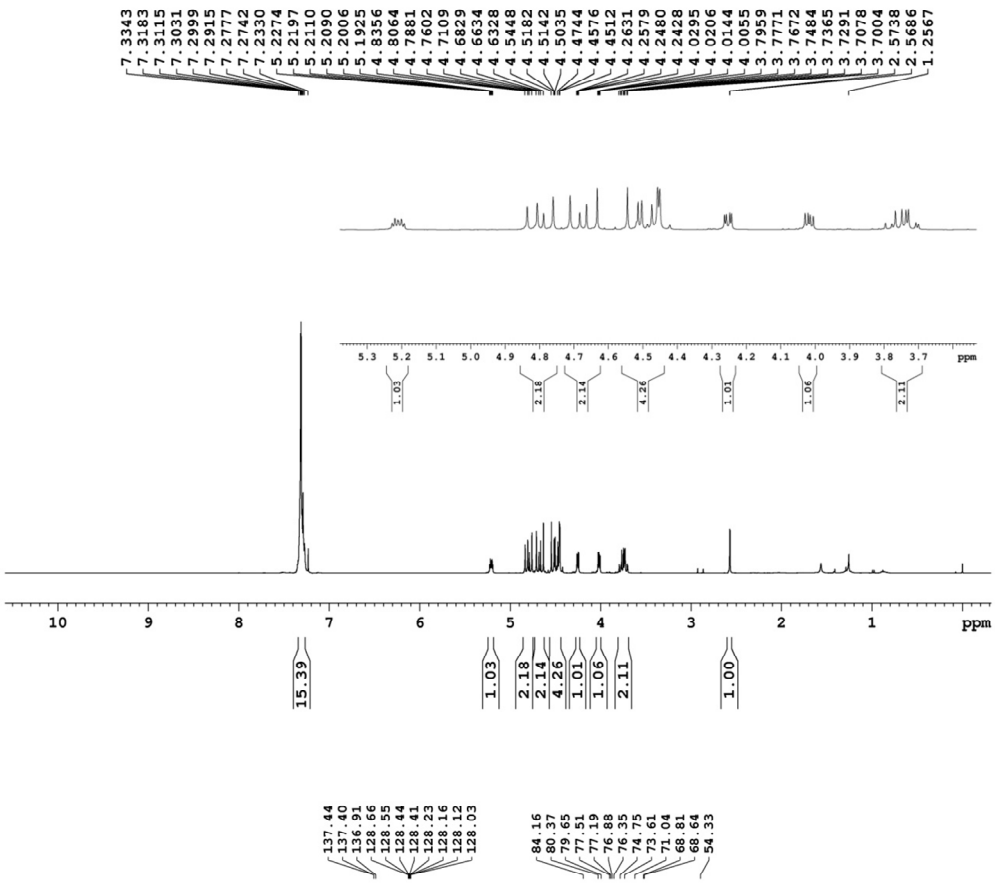
Abbreviations

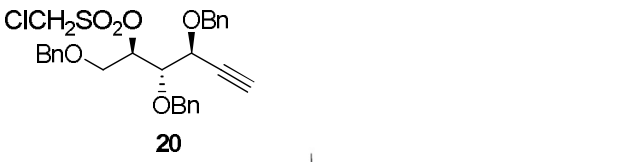
Bn	Benzyl
DCM	Dichloromethane
DMF	Dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
Et_2NH	Diethylamine
eq	Molar equivalent(s)
MeOH	Methanol
mmol	millimoles
NMR	Nuclear magnetic resonance
THF	Tetrahydrofuran
TLC	Thin Layer chromatogram



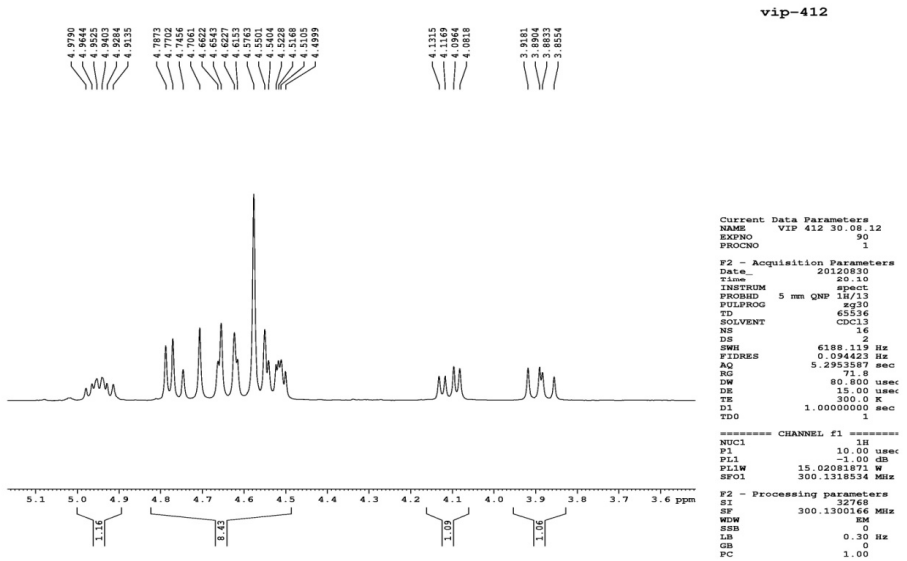


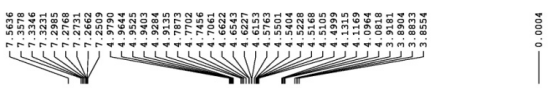
^{1}H and ^{13}C NMR Spectrum of 26

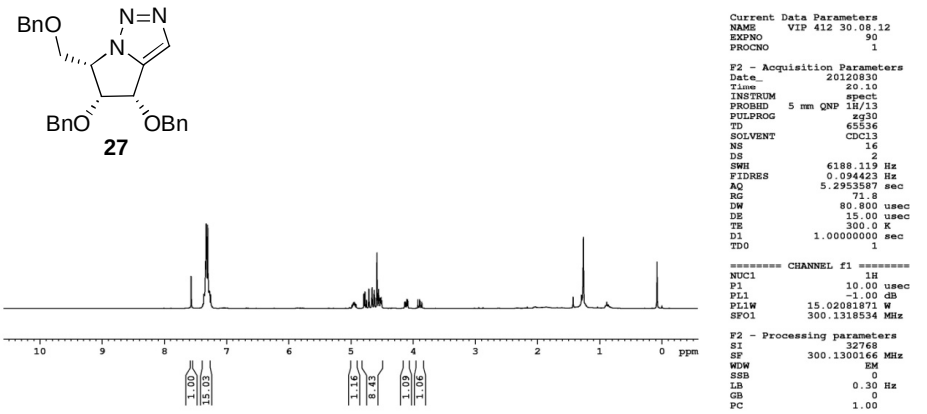




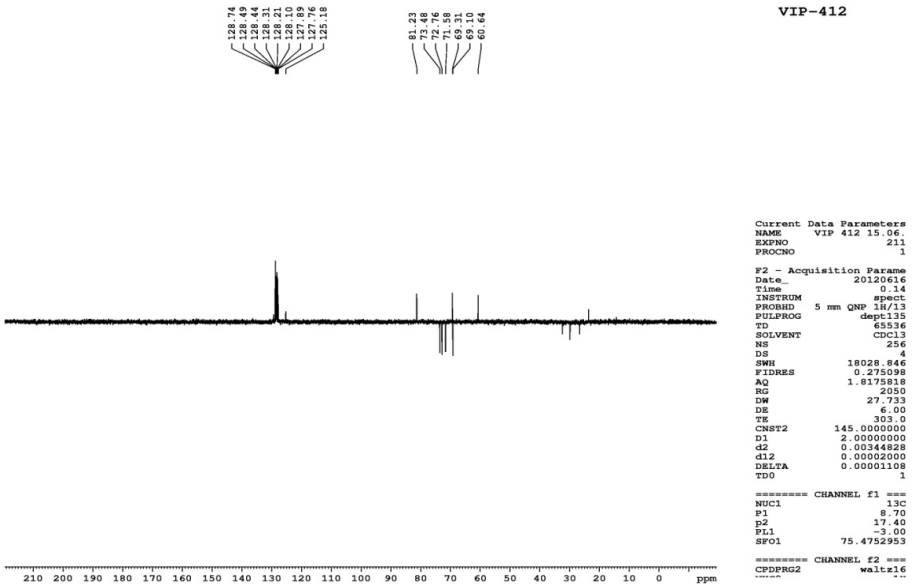
^{1}H and ^{13}C NMR Spectrum of 20

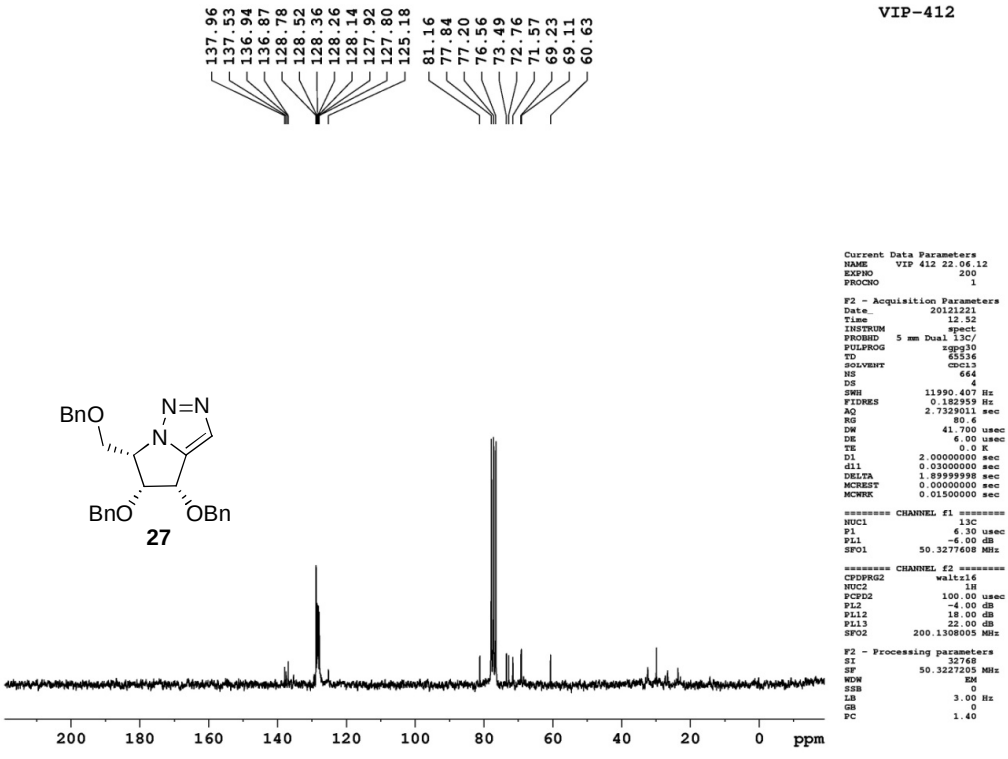




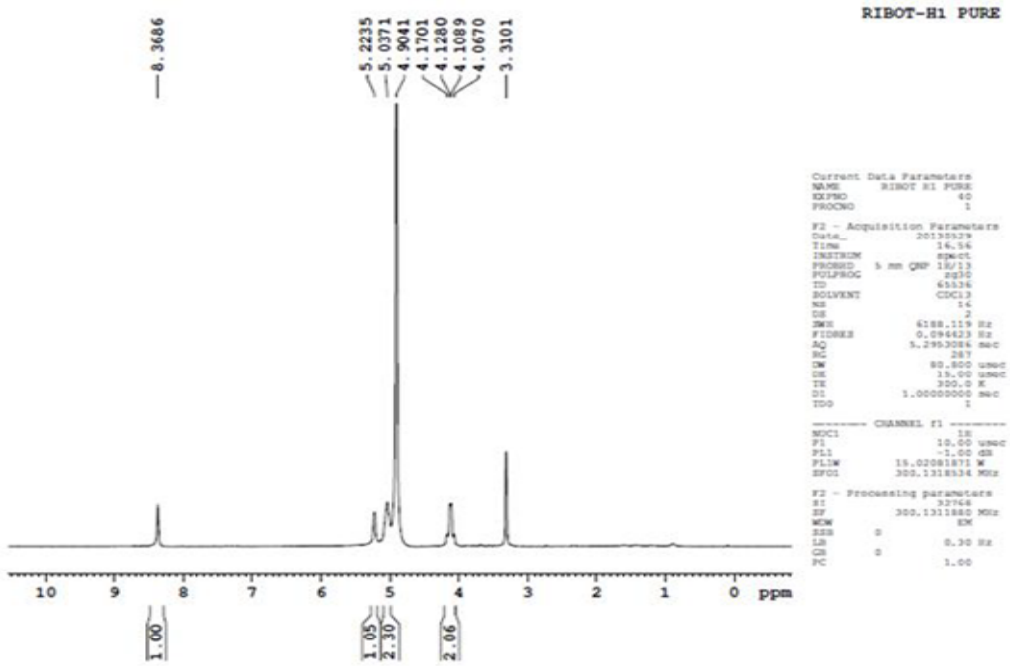


^{1}H NMR Spectrum of 27





^{13}C NMR Spectrum of 27



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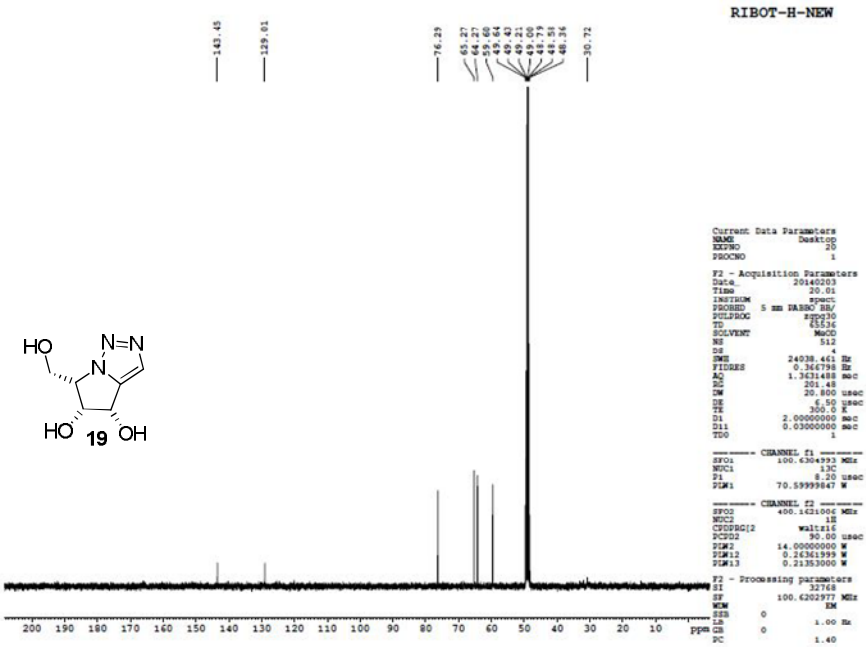
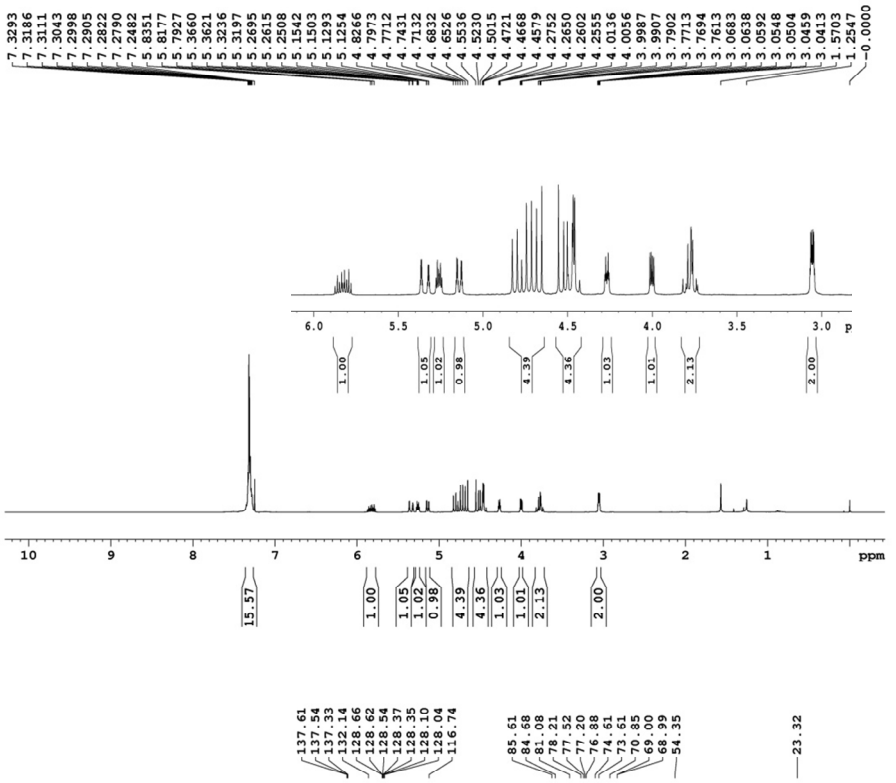
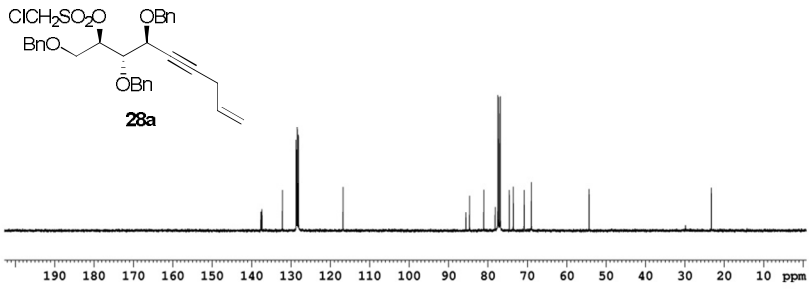
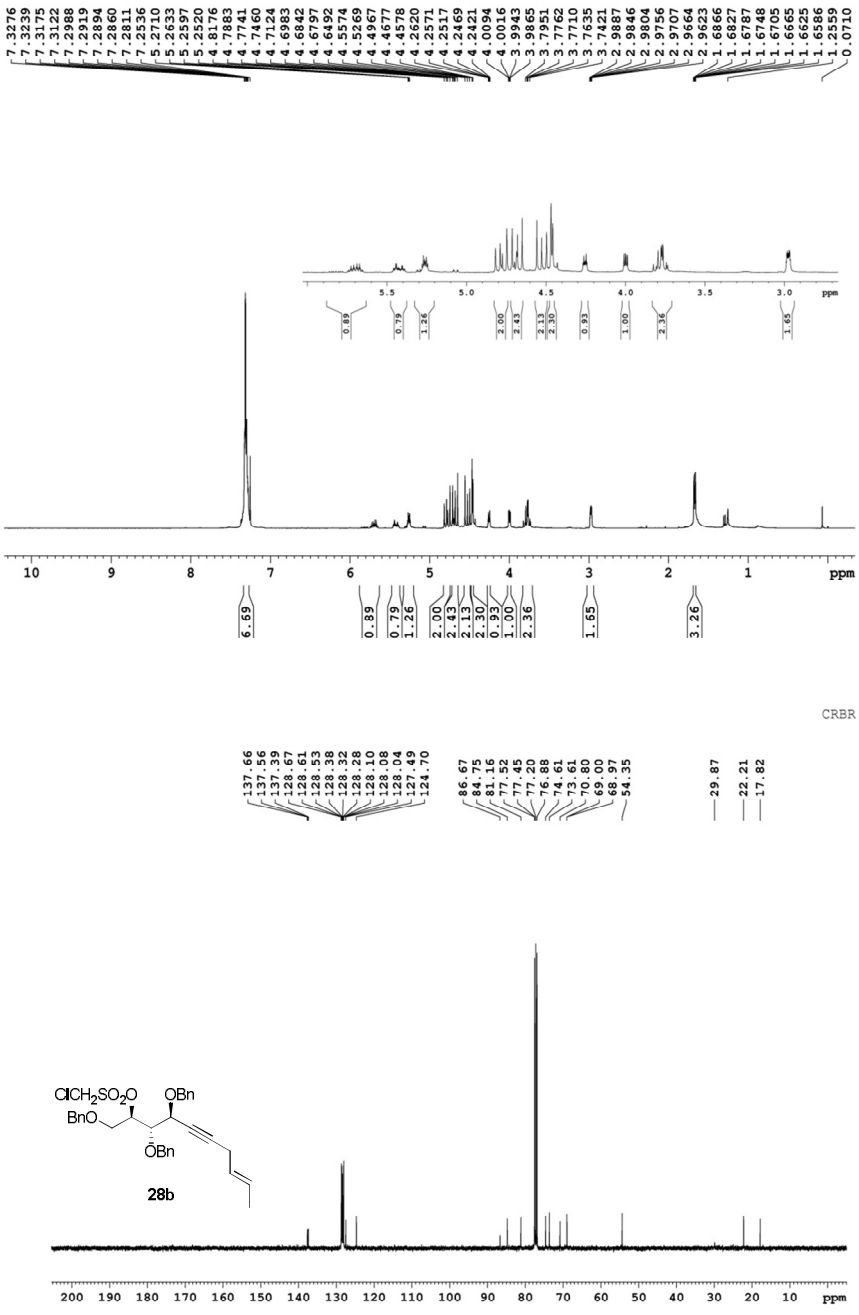


^{13}C NMR Spectrum of 19





^{1}H and ^{13}C NMR Spectrum of 28a



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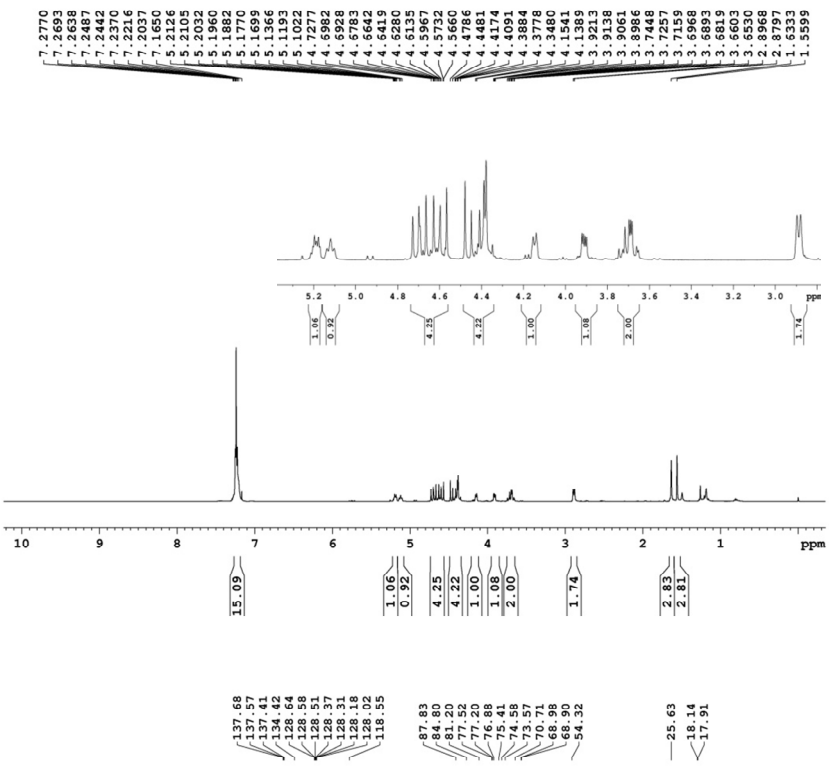

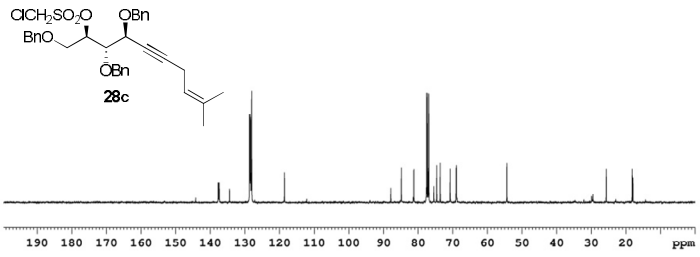

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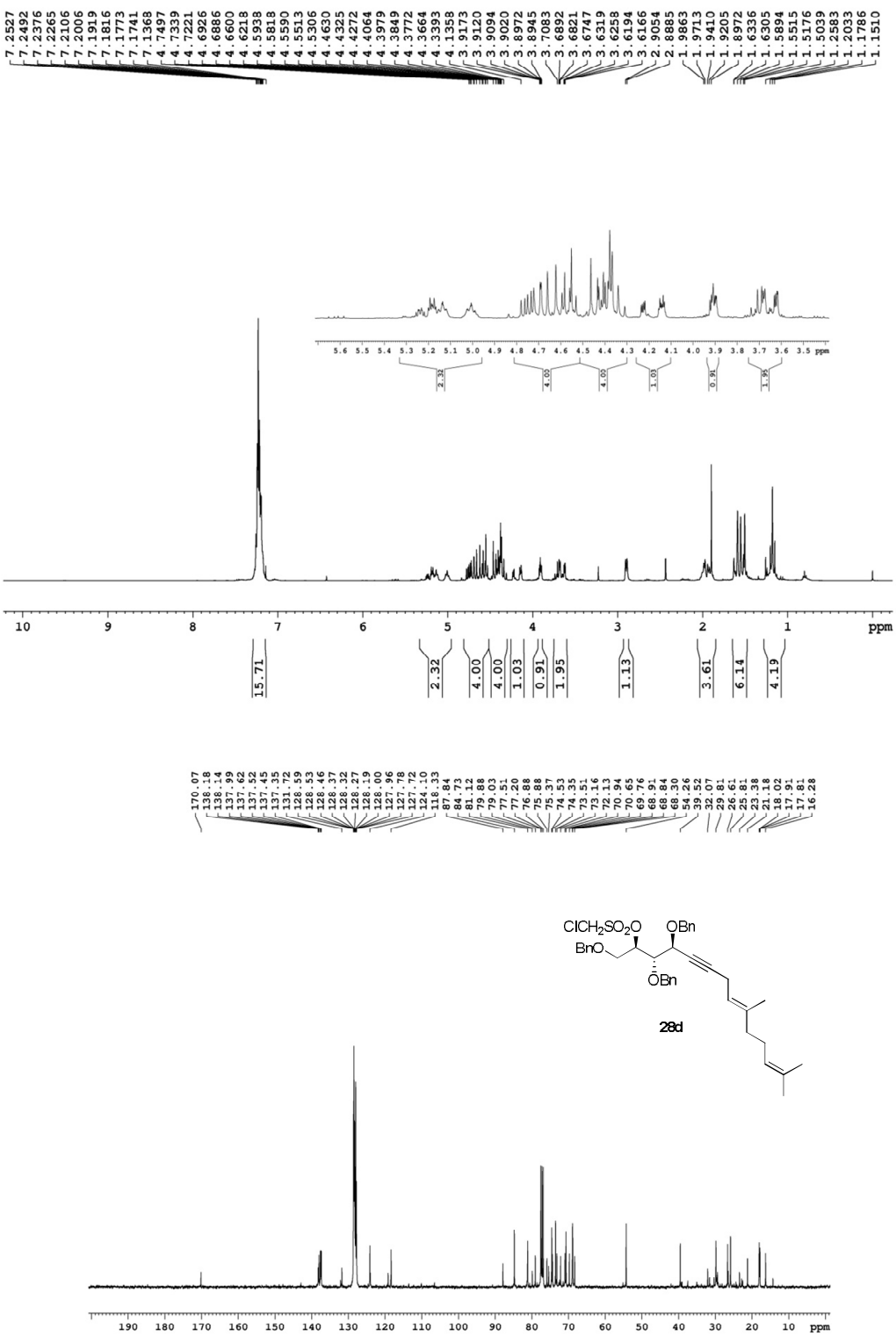
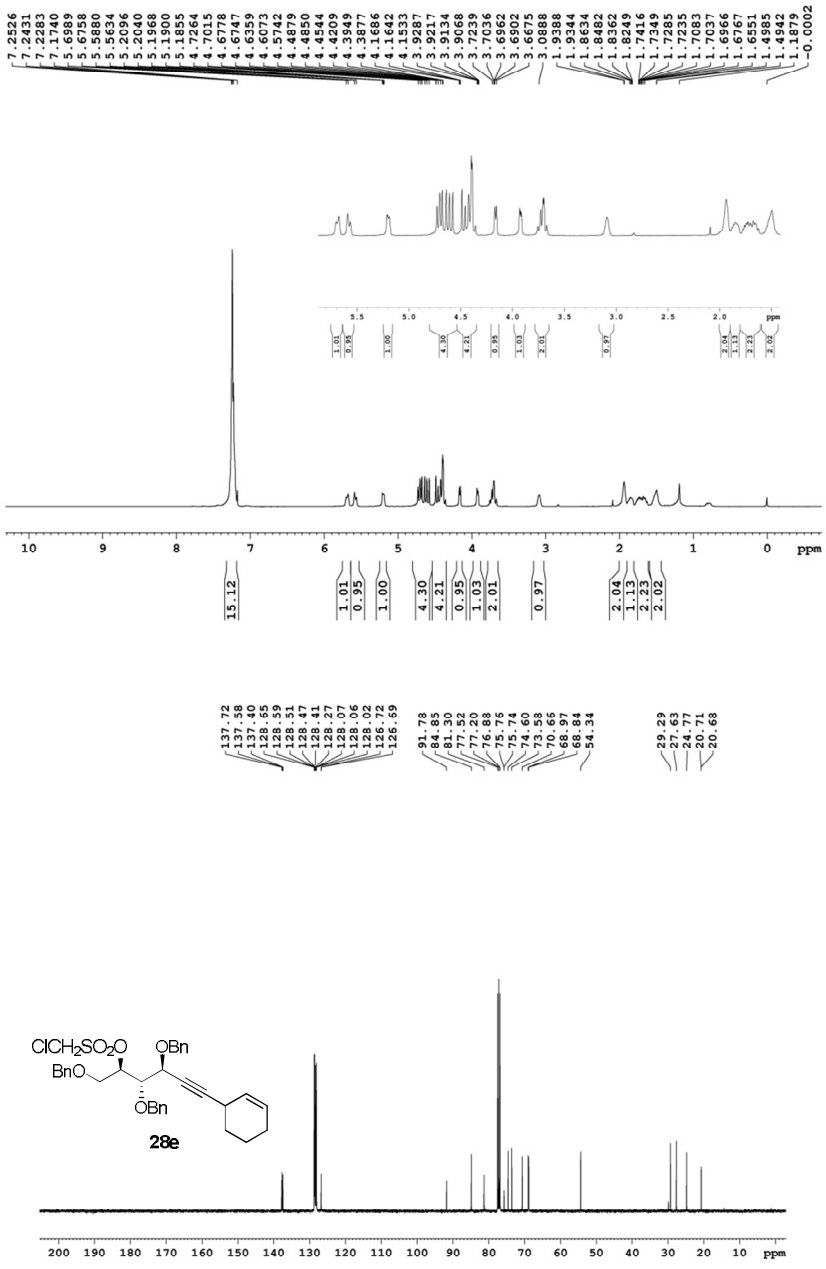
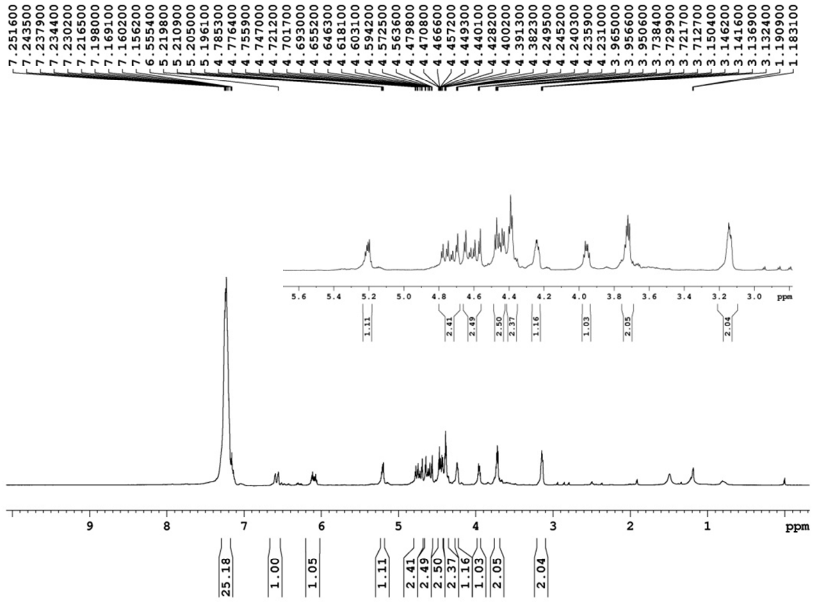
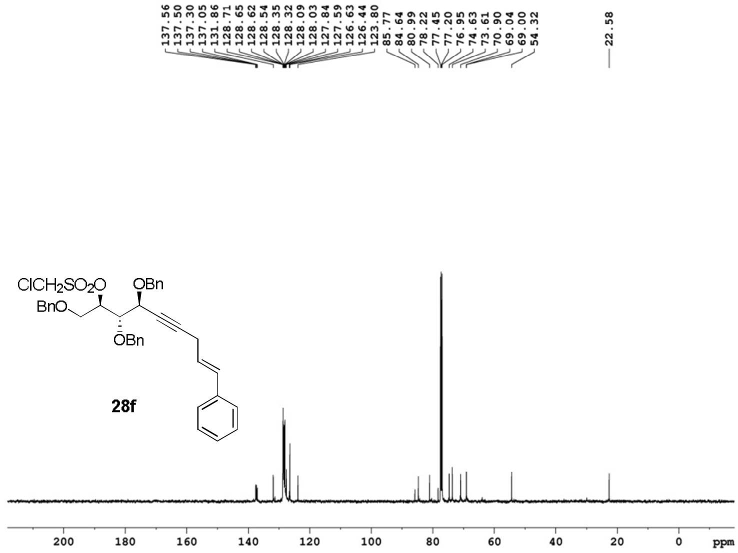


^{1}H and ^{13}C NMR Spectrum of 28d

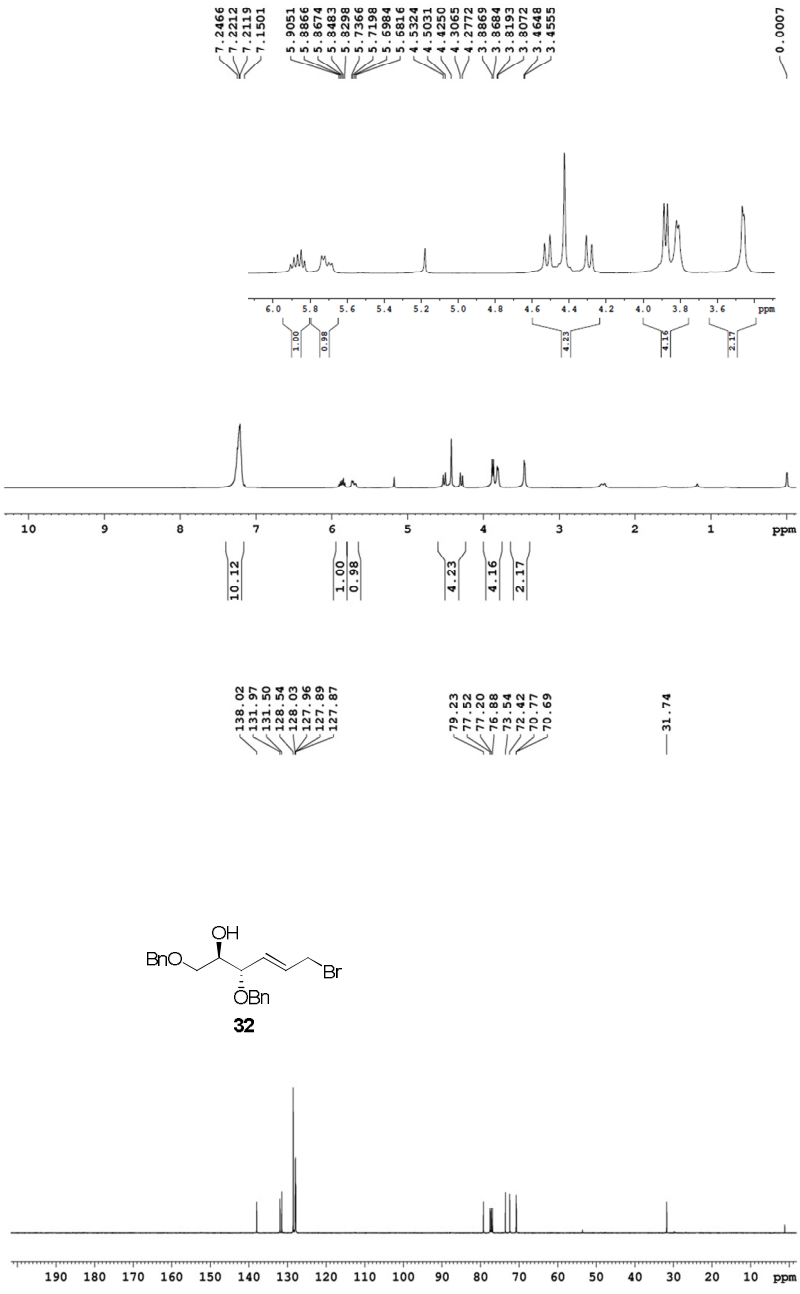


^{1}H and ^{13}C NMR Spectrum of 28e



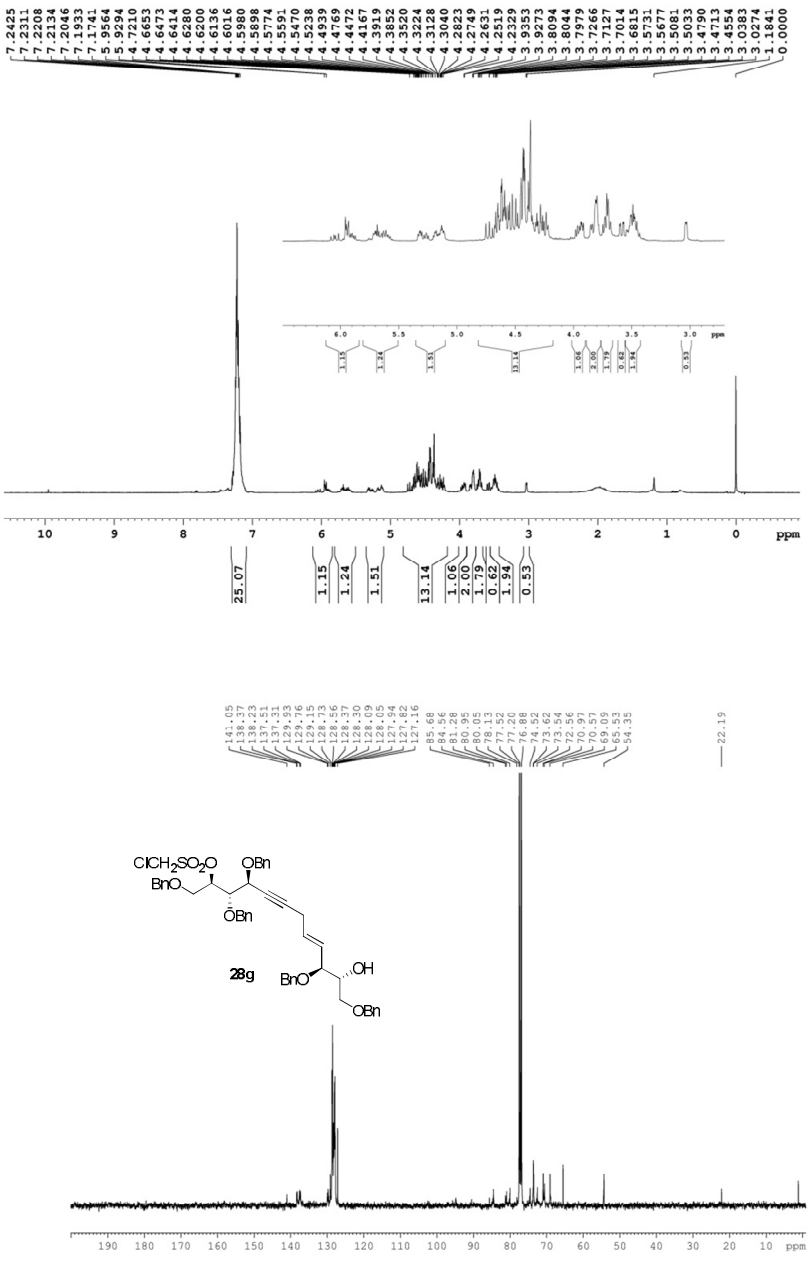


[1]H and [13]C NMR Spectrum of 28f

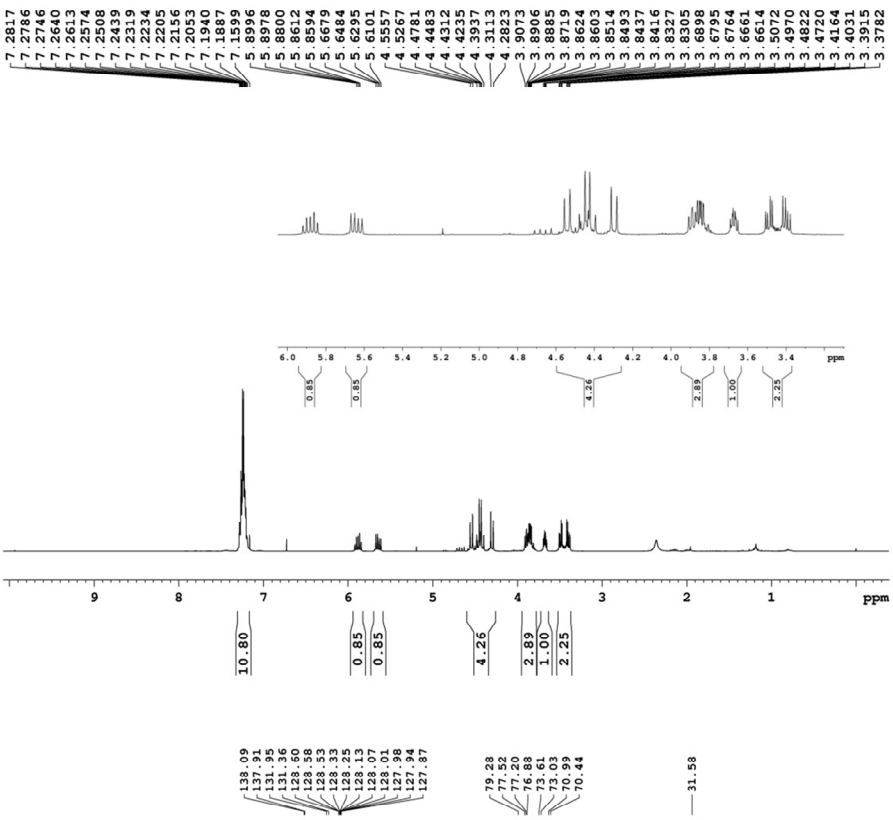


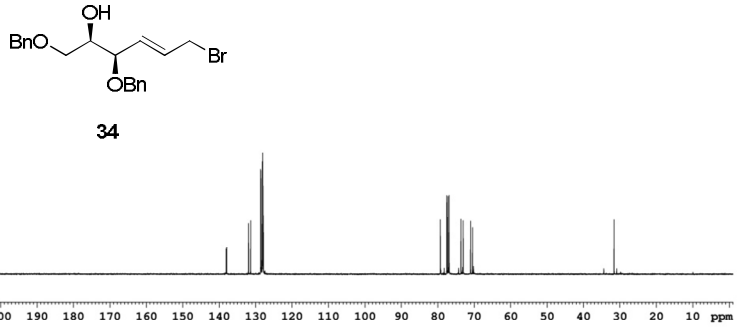
32

^{1}H and ^{13}C NMR Spectrum of 32



^{1}H and ^{13}C NMR Spectrum of 28g





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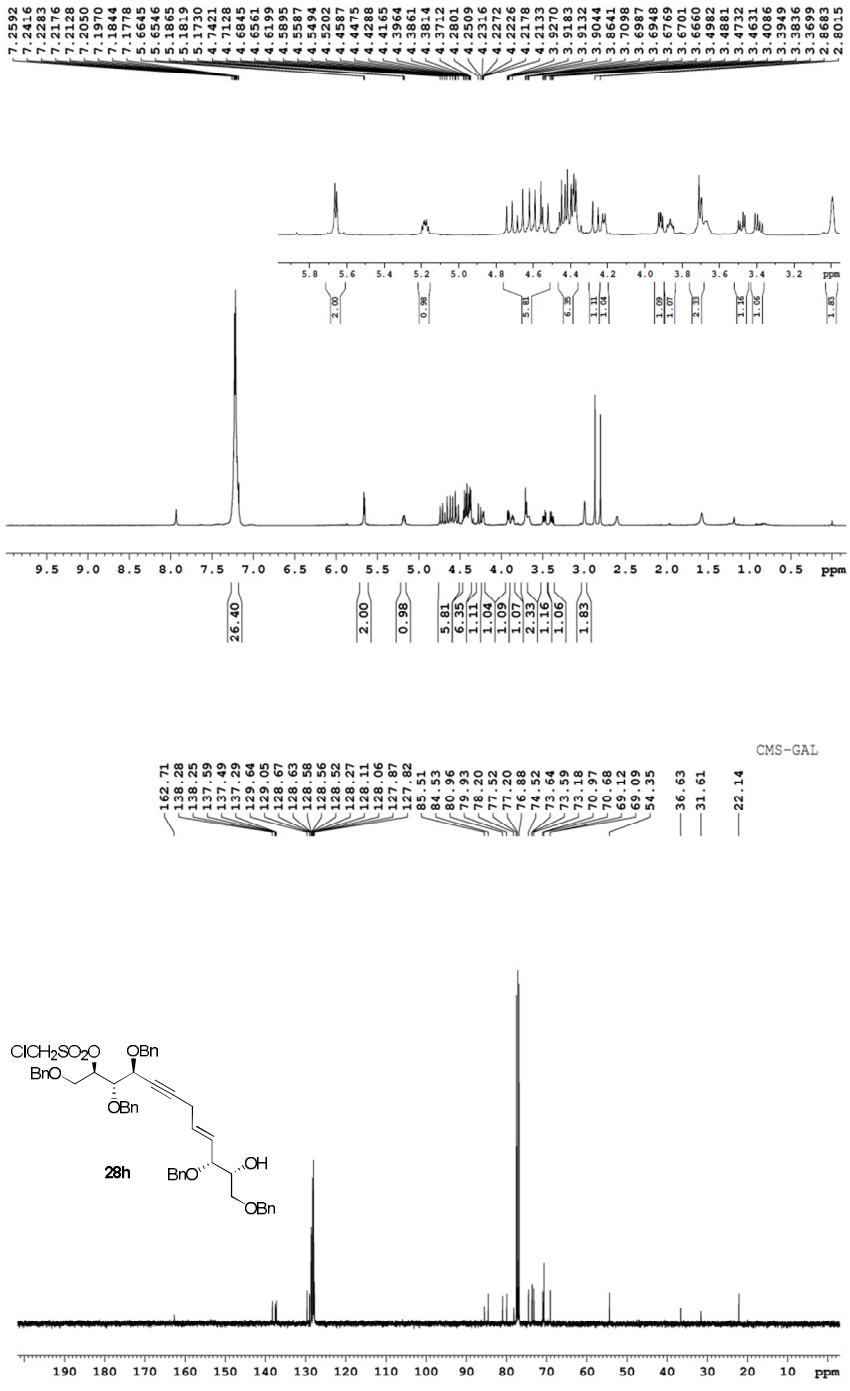
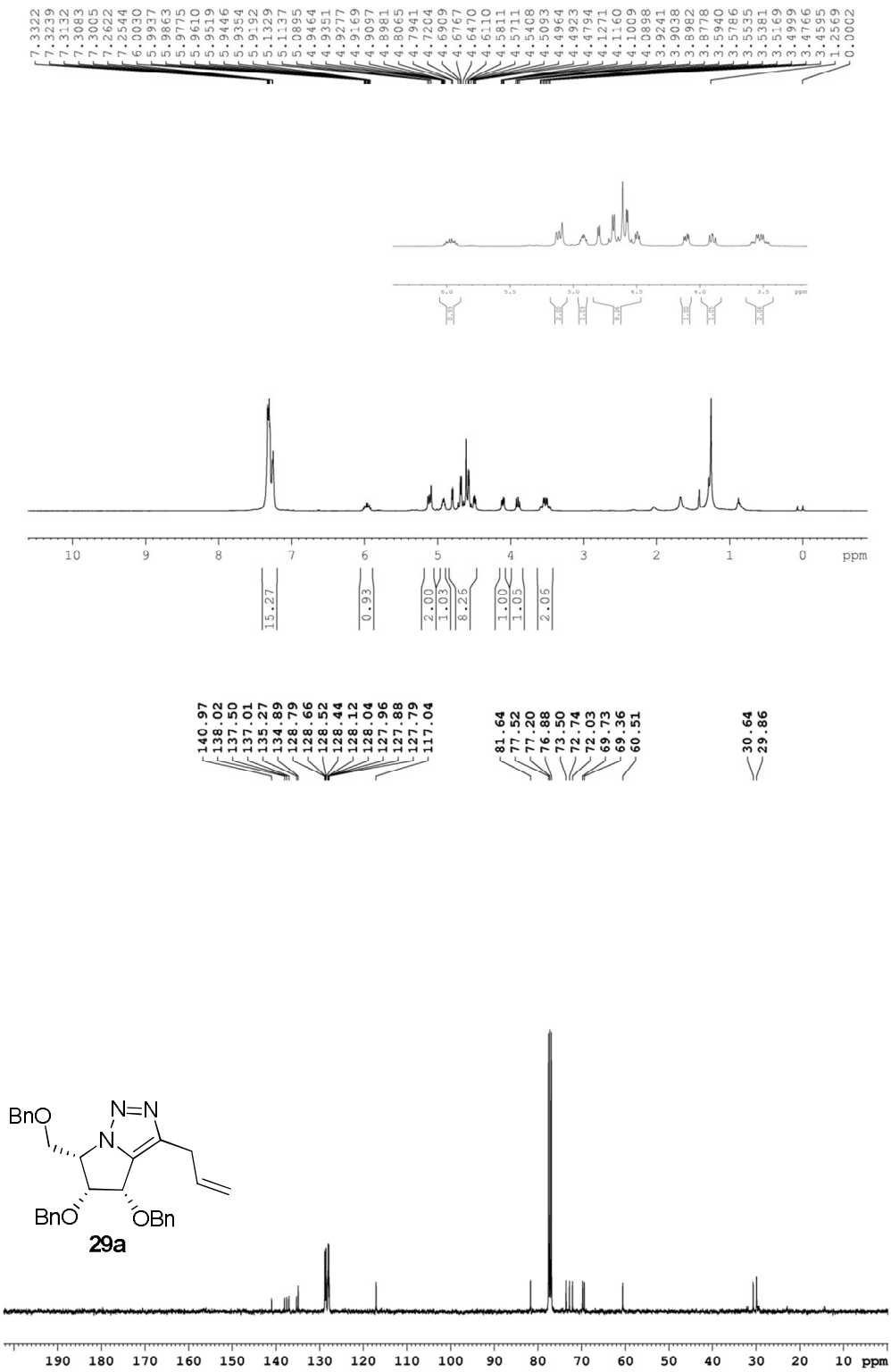
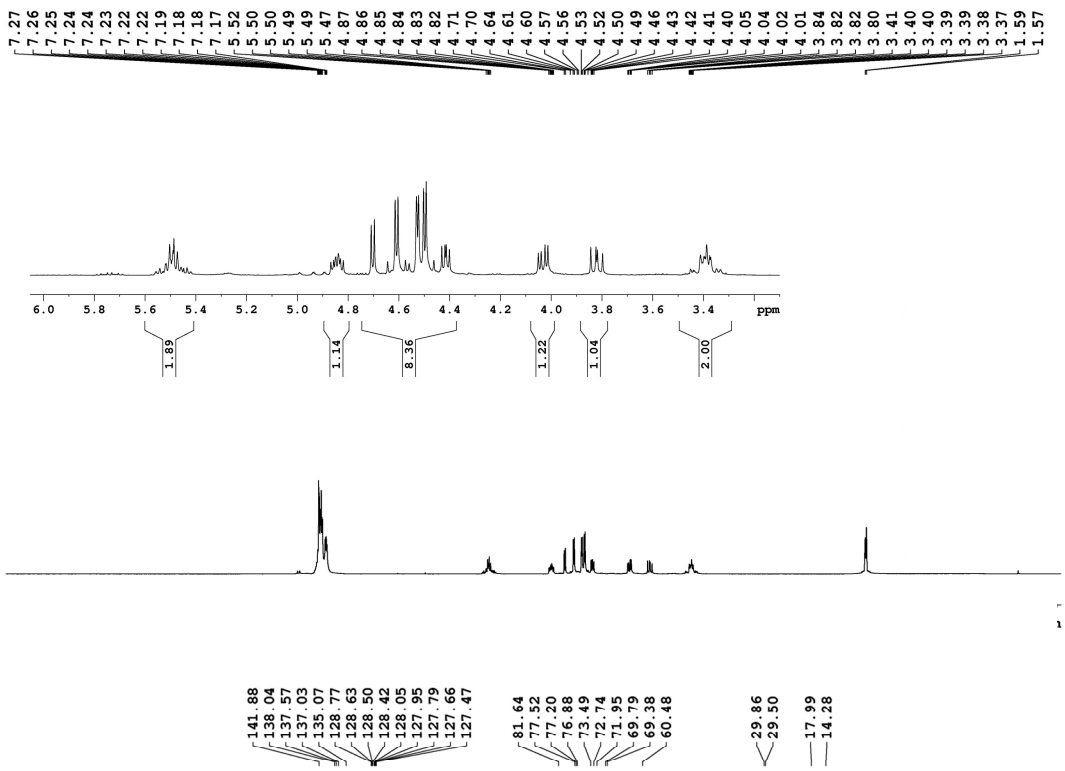
^{1}H and ^{13}C NMR Spectrum of 34

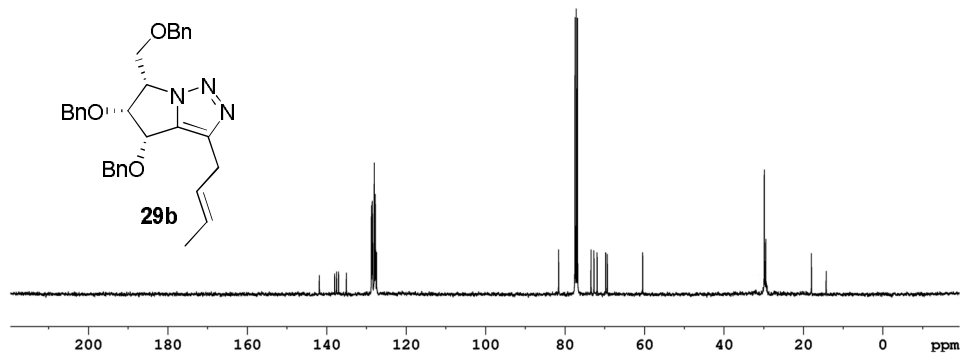


^{1}H and ^{13}C NMR Spectrum of 28h

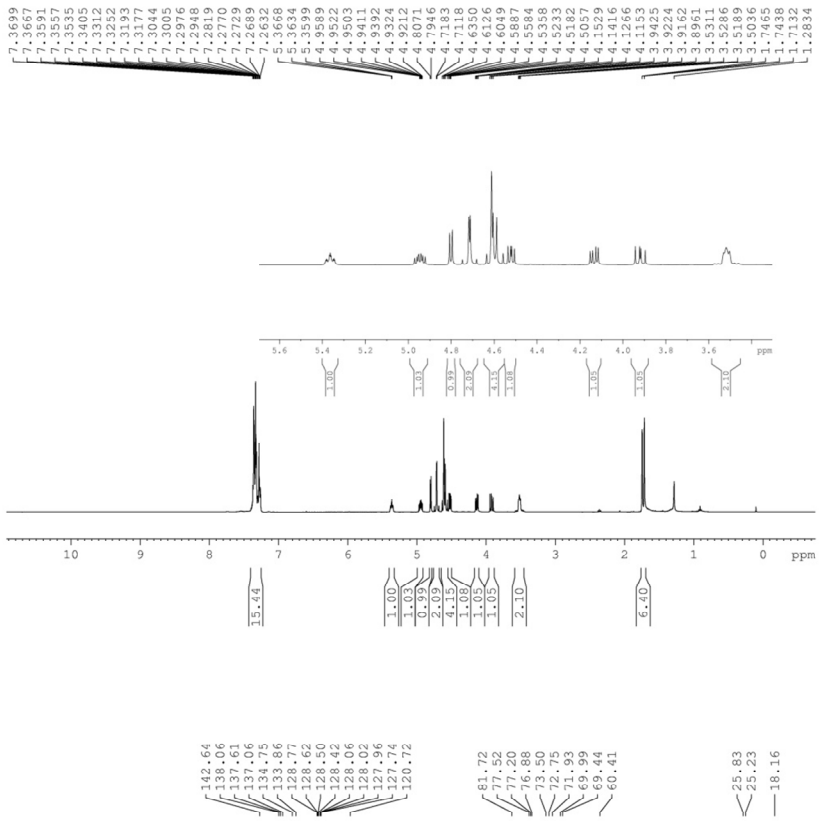


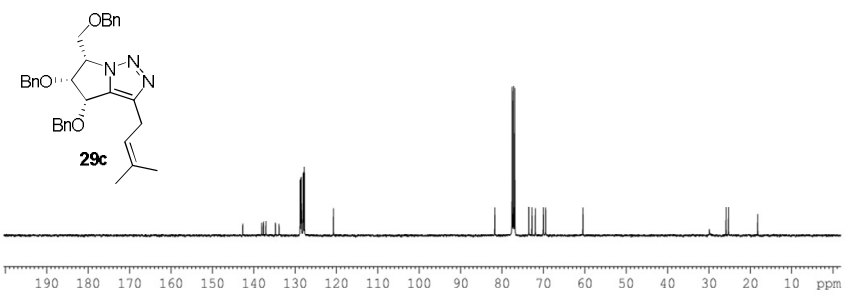
^{1}H and ^{13}C NMR Spectrum of 29a



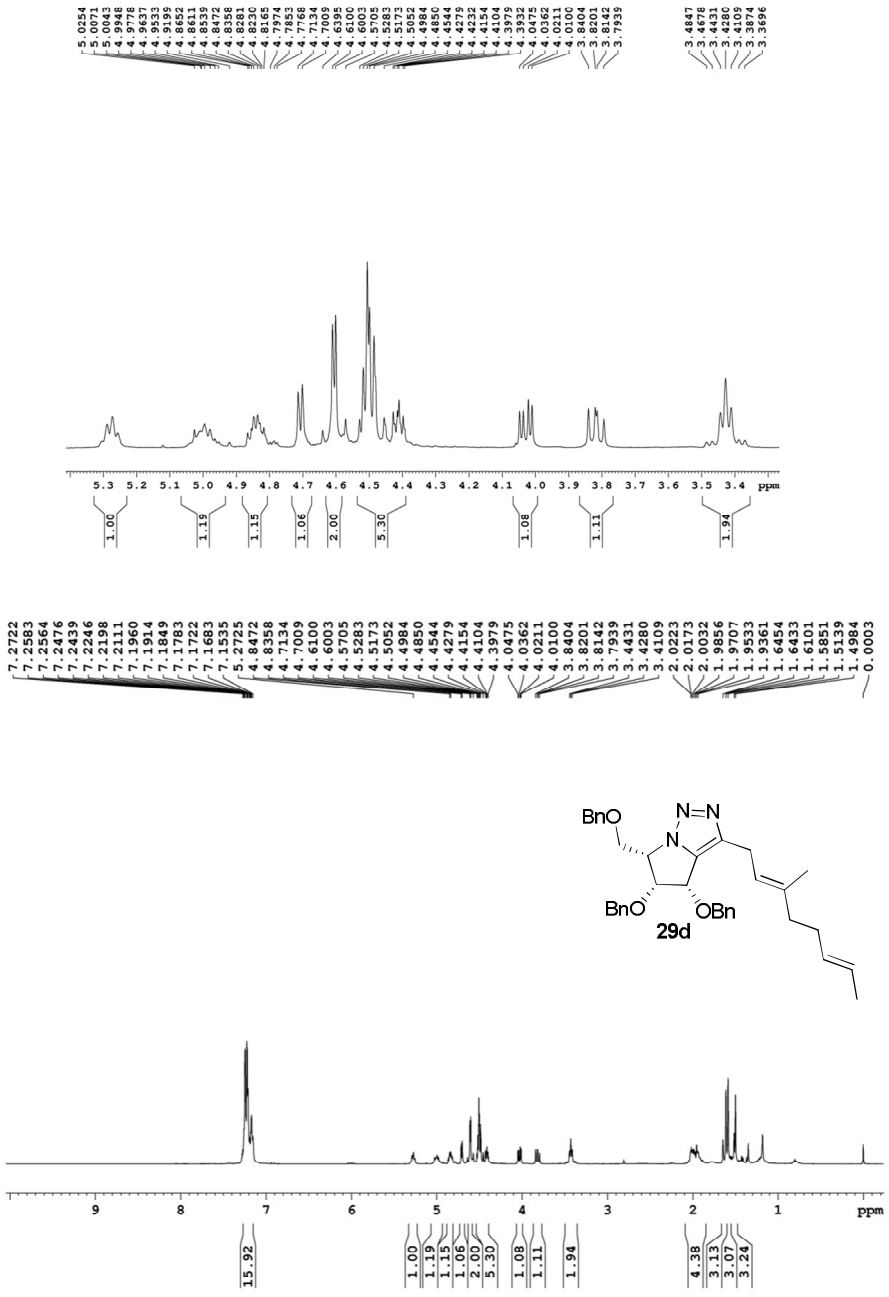


^{1}H and ^{13}C NMR Spectrum of 29b

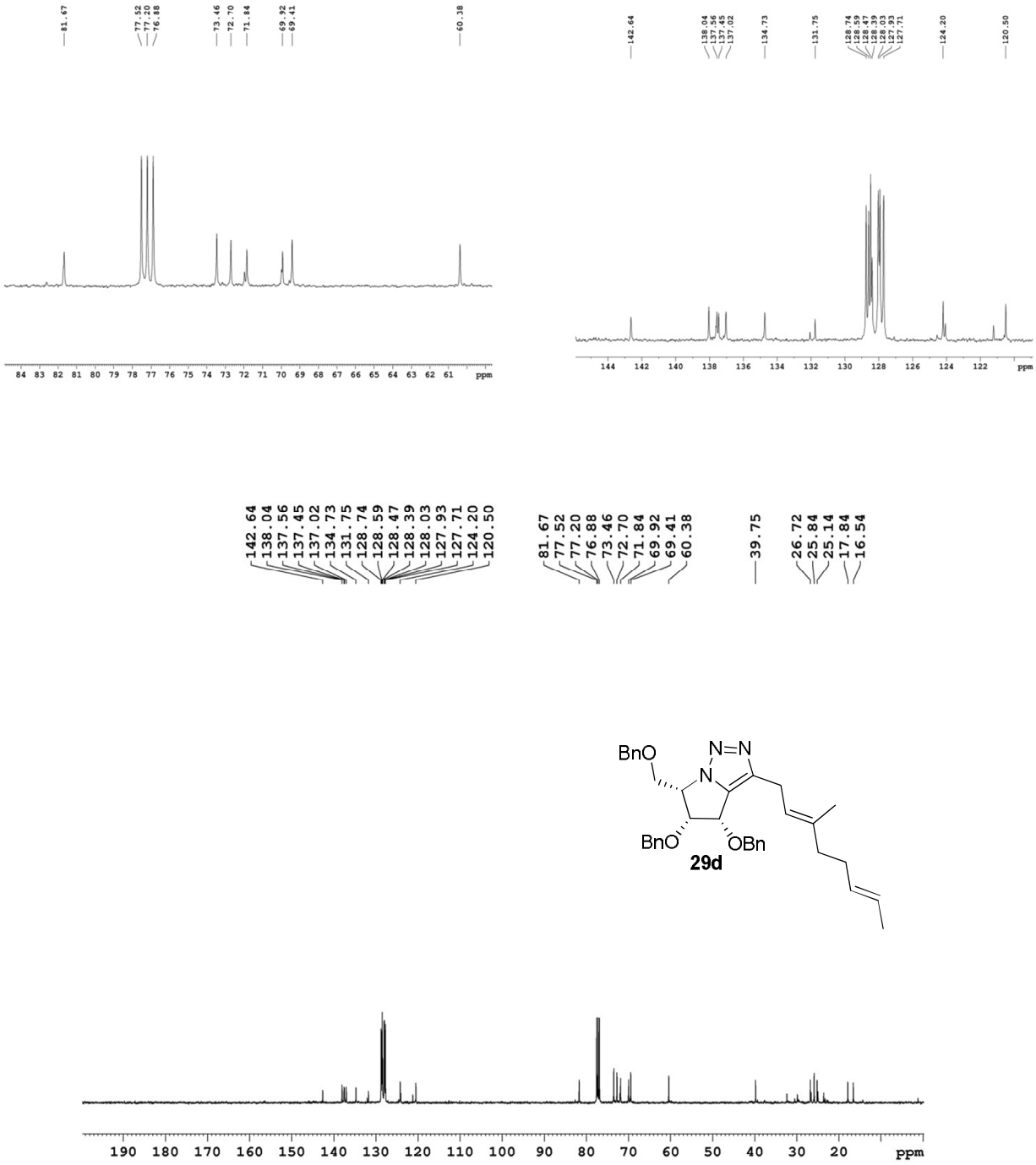




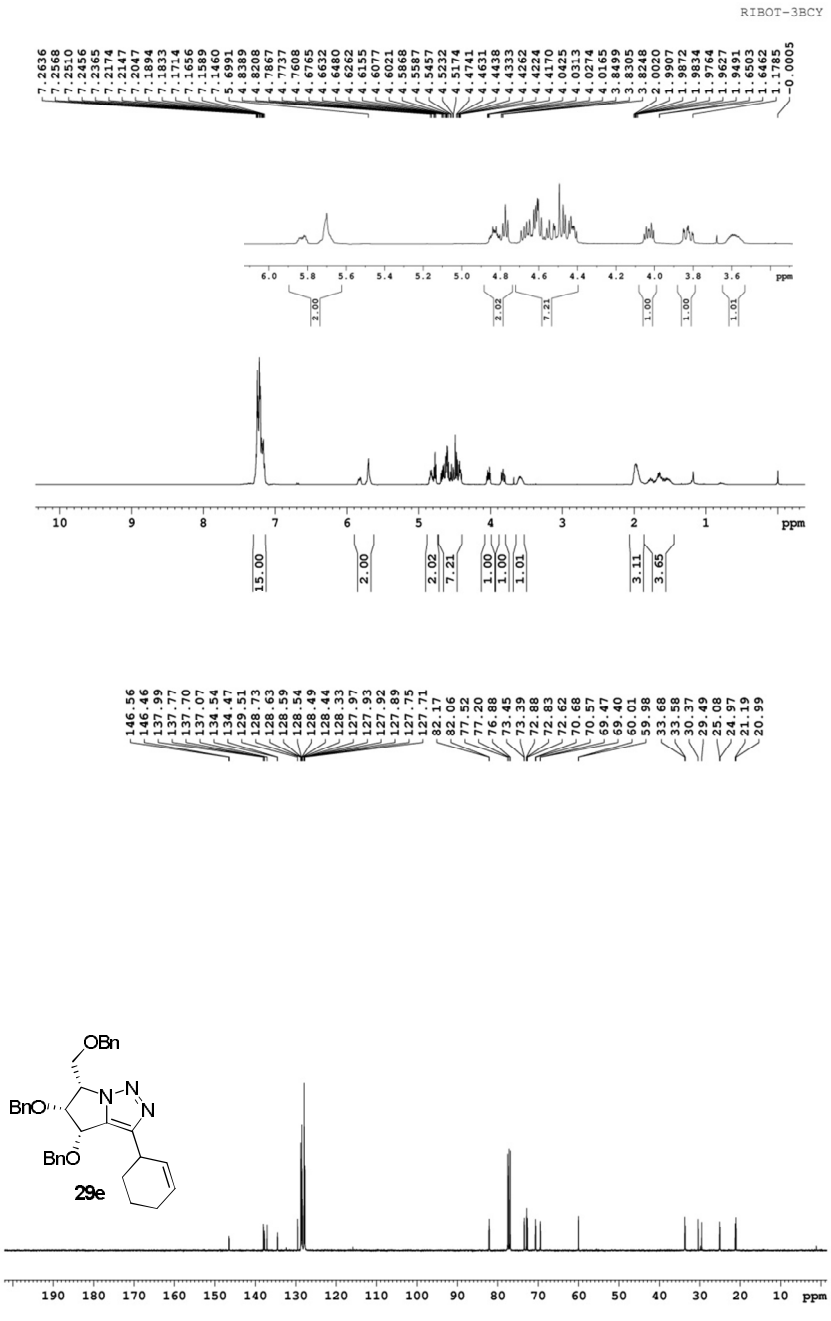
^{1}H and ^{13}C NMR Spectrum of 29c



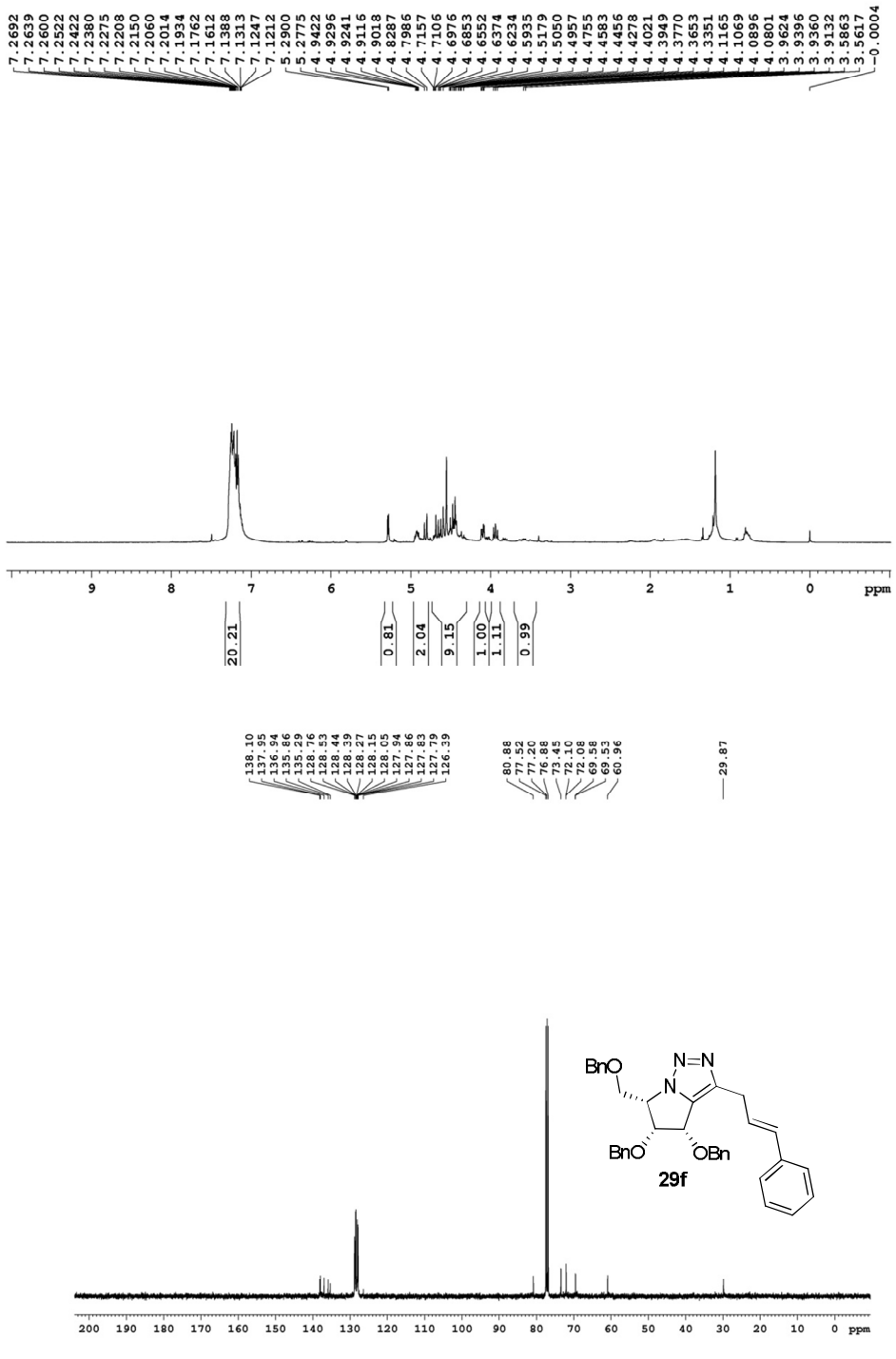
^{1}H NMR Spectrum of 29d



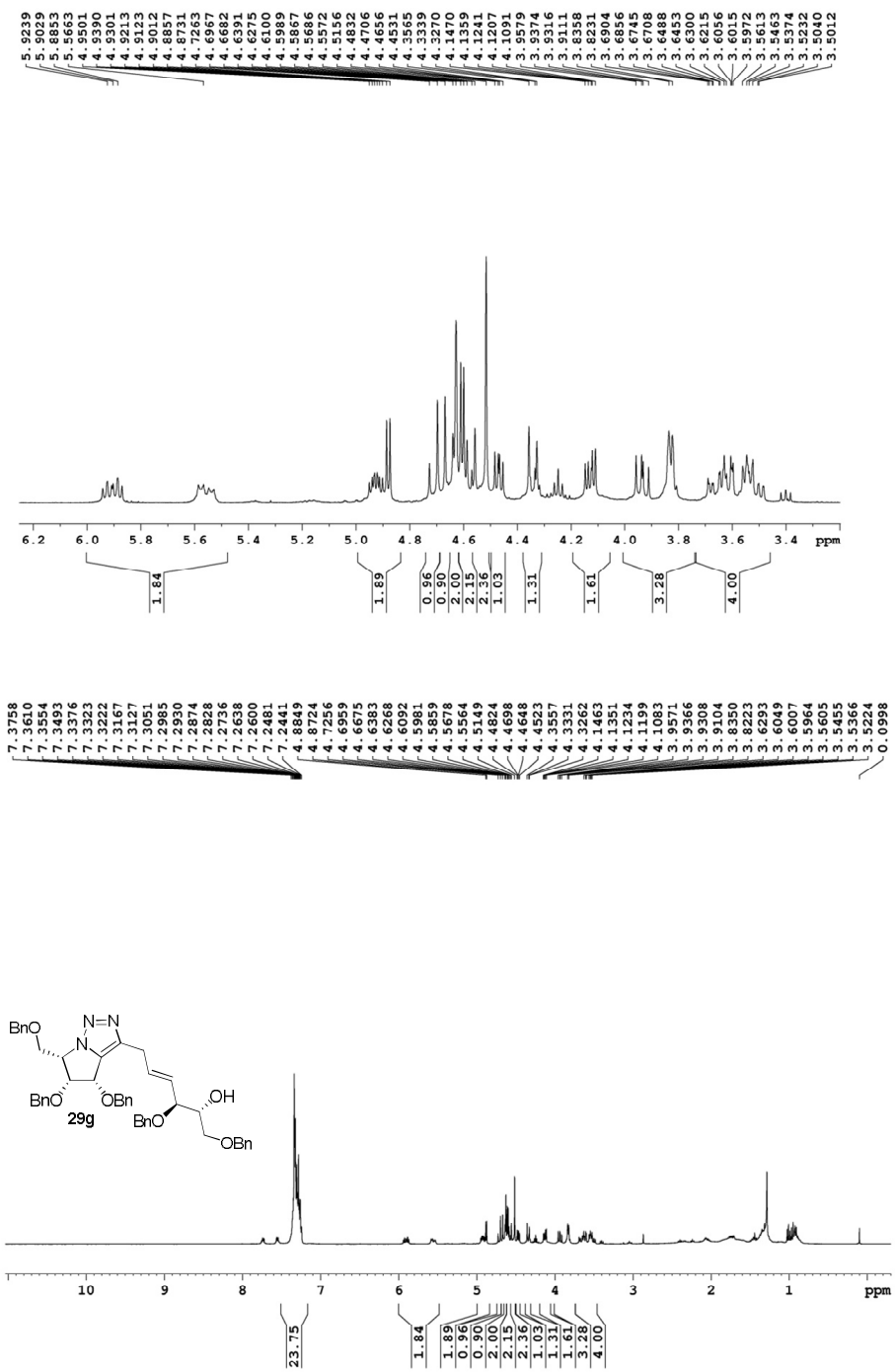
^{13}C NMR Spectrum of 29d



^{1}H and ^{13}C NMR Spectrum of 29e



^{1}H and ^{13}C NMR Spectrum of 29f



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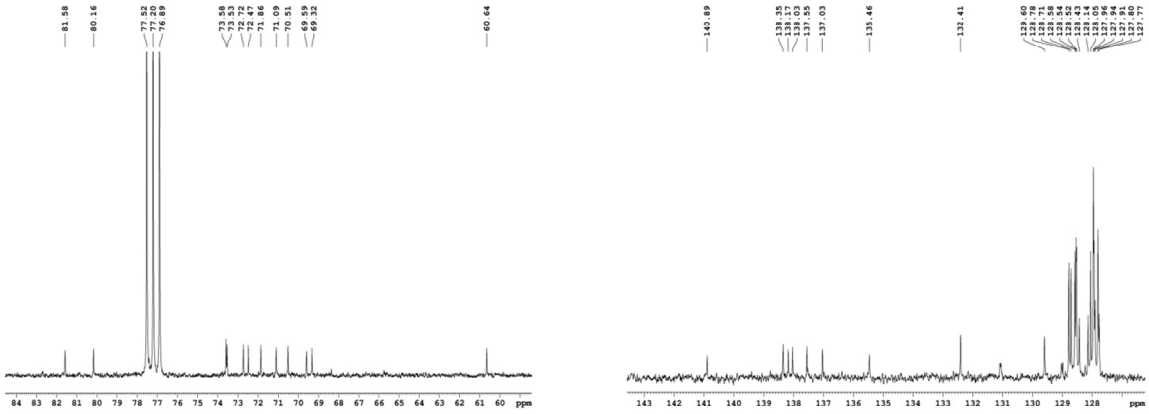

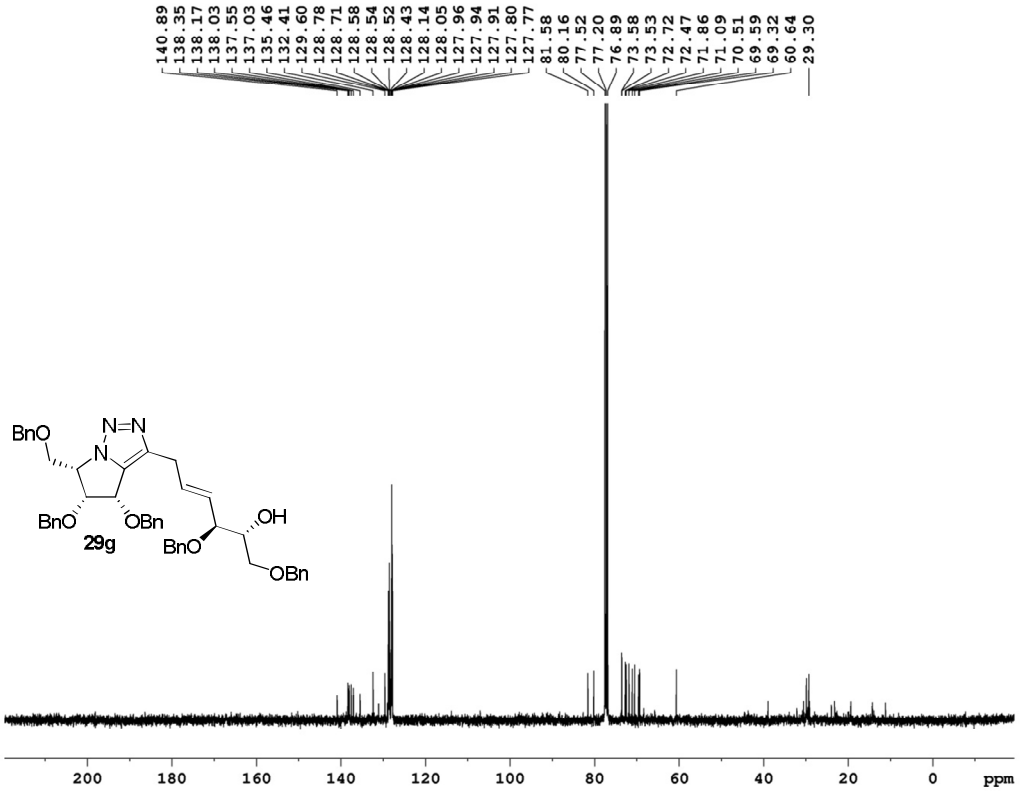

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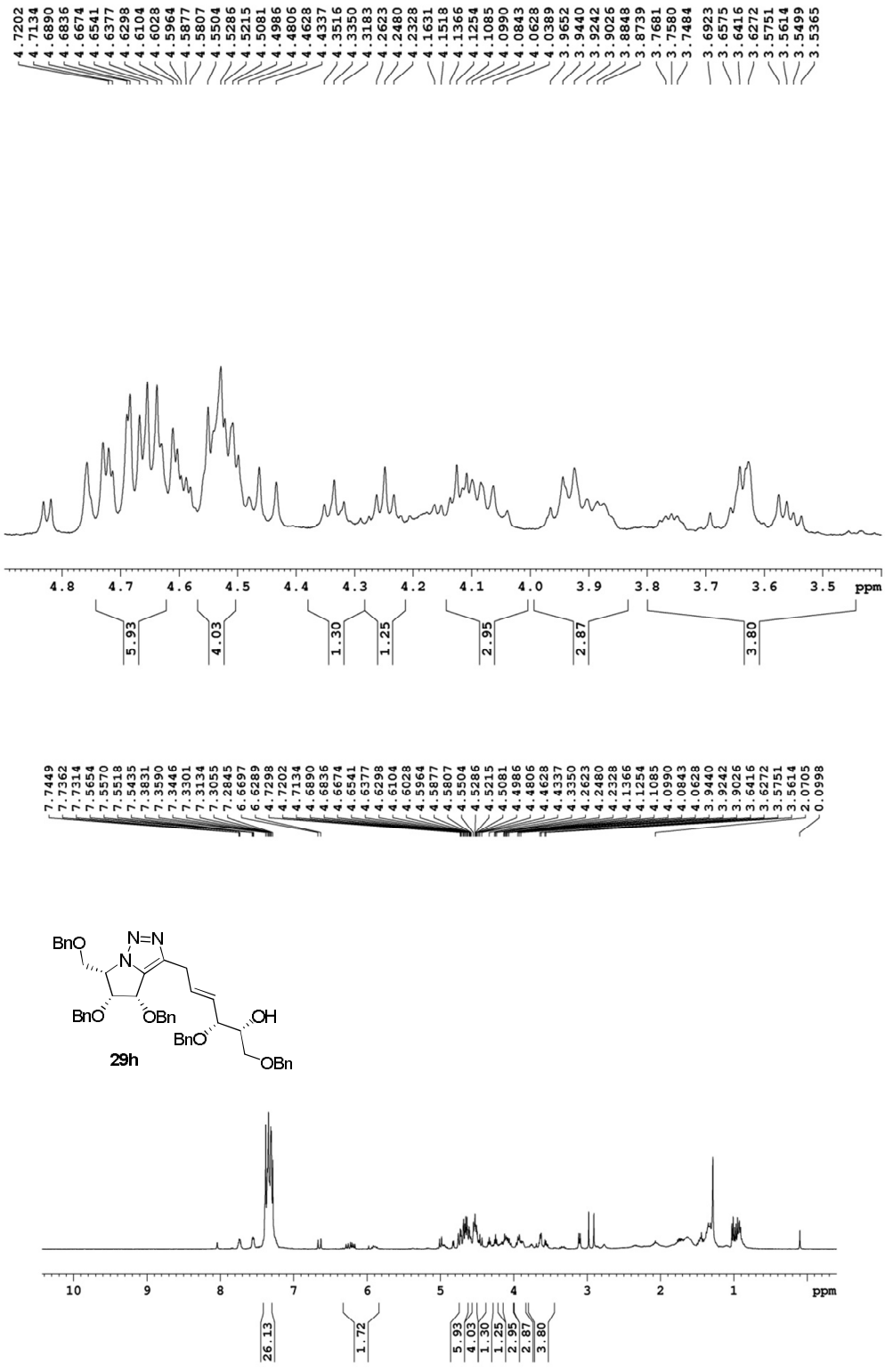
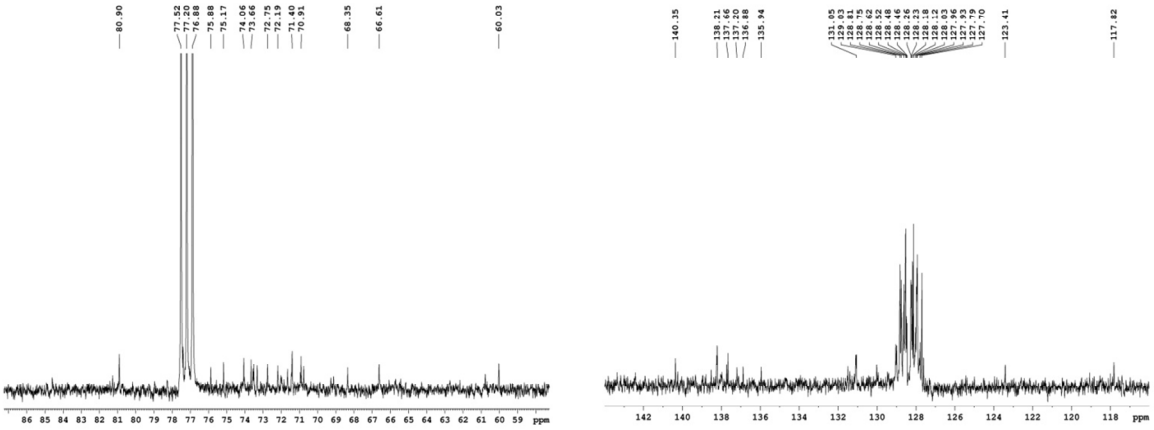
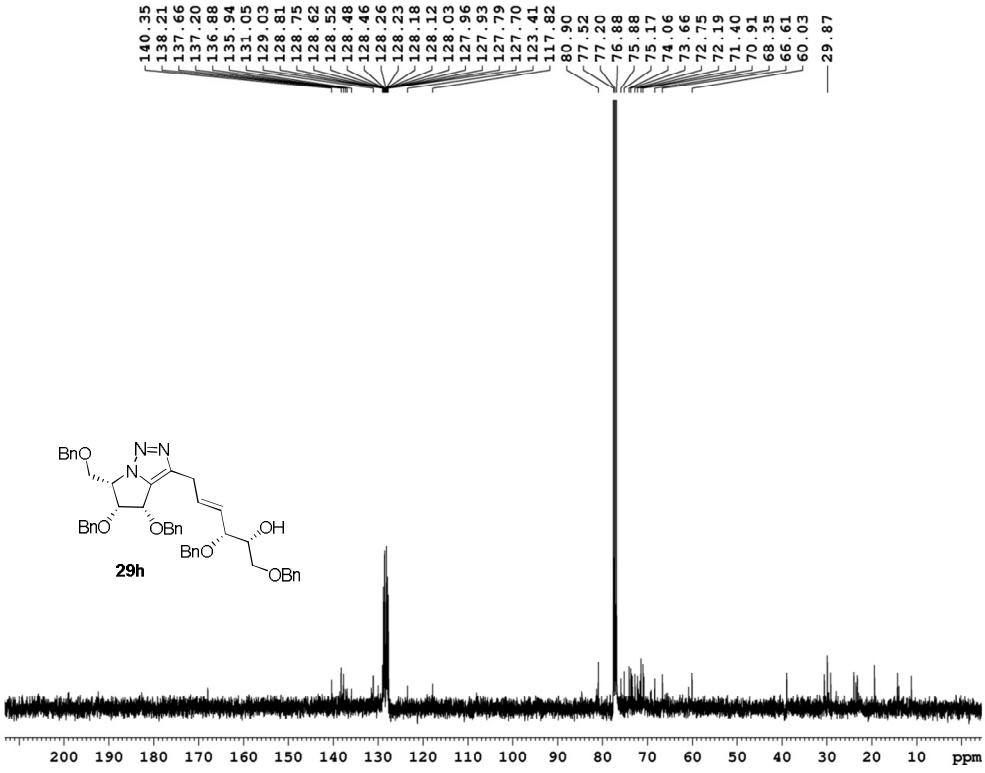
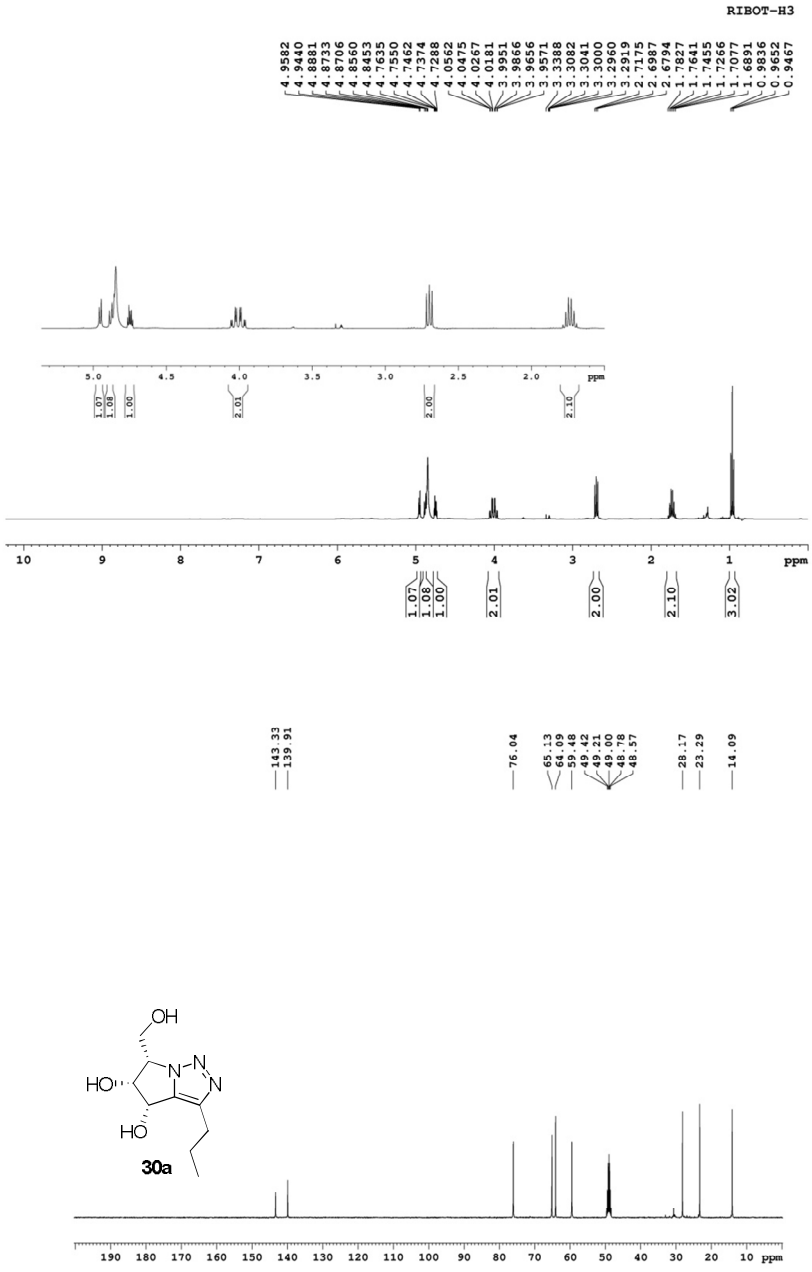


^{1}H NMR Spectrum of 29h

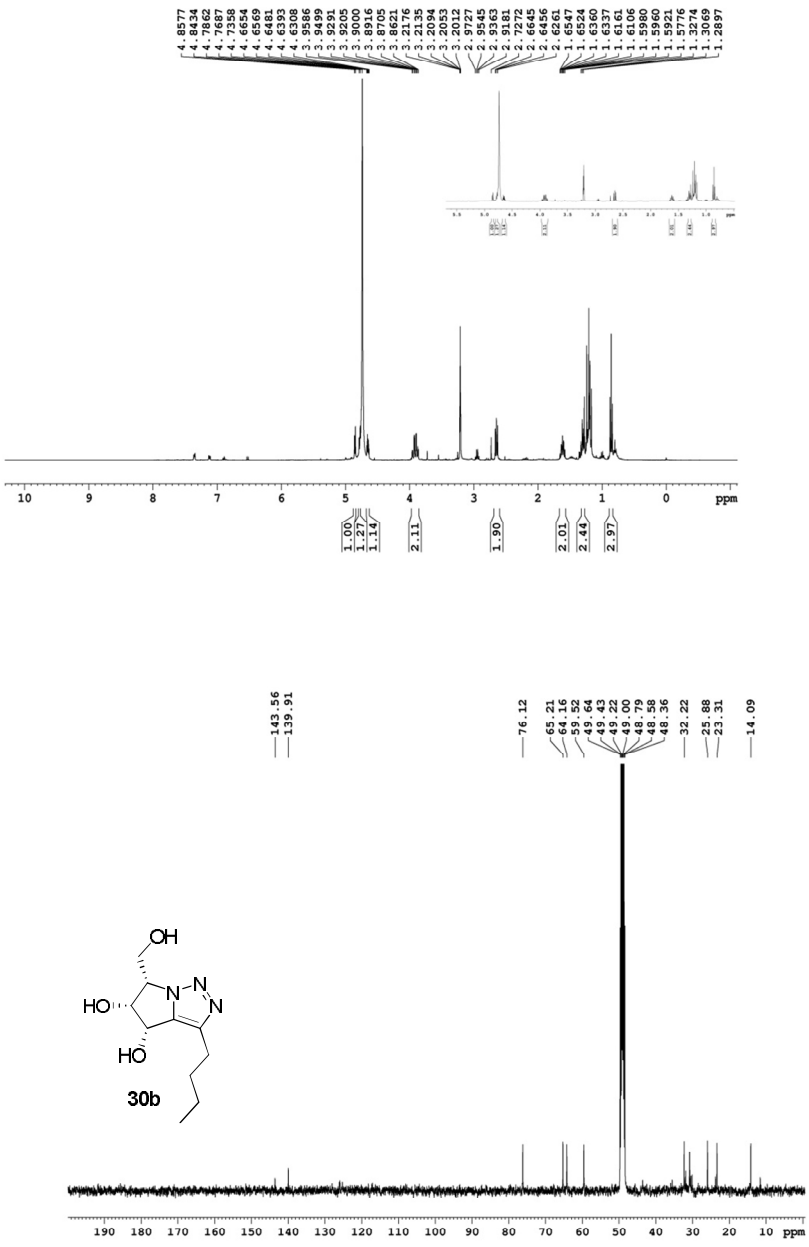




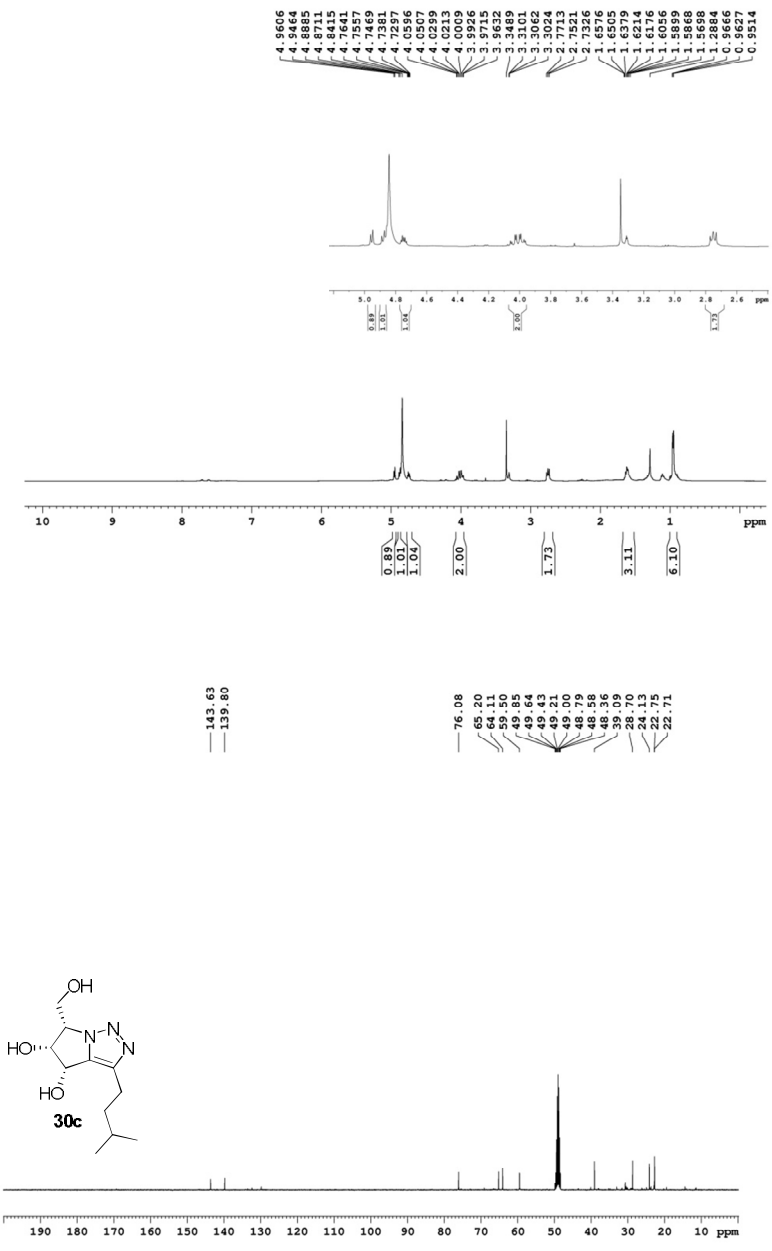
^{13}C NMR Spectrum of 29h



^{1}H and ^{13}C NMR Spectrum of 30a



^{1}H and ^{13}C NMR Spectrum of 30b



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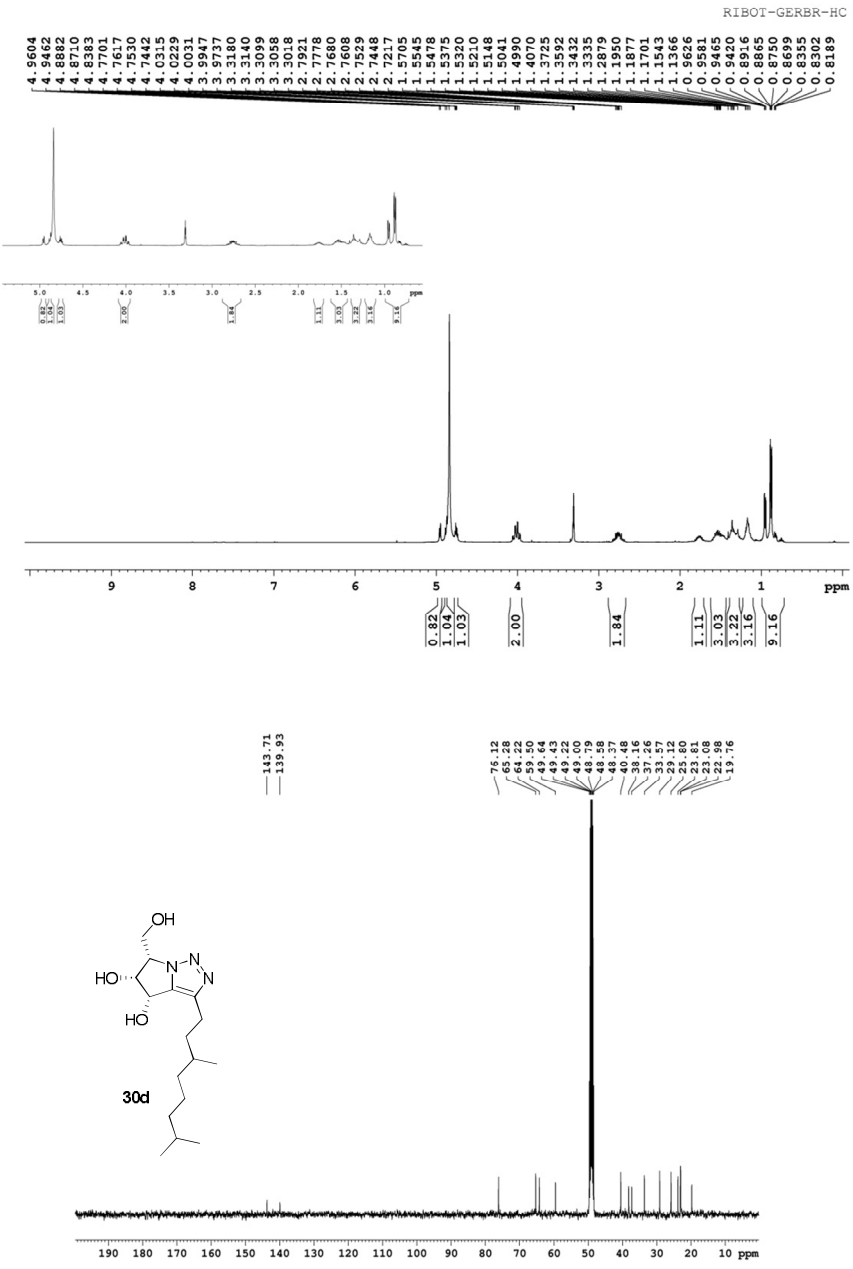

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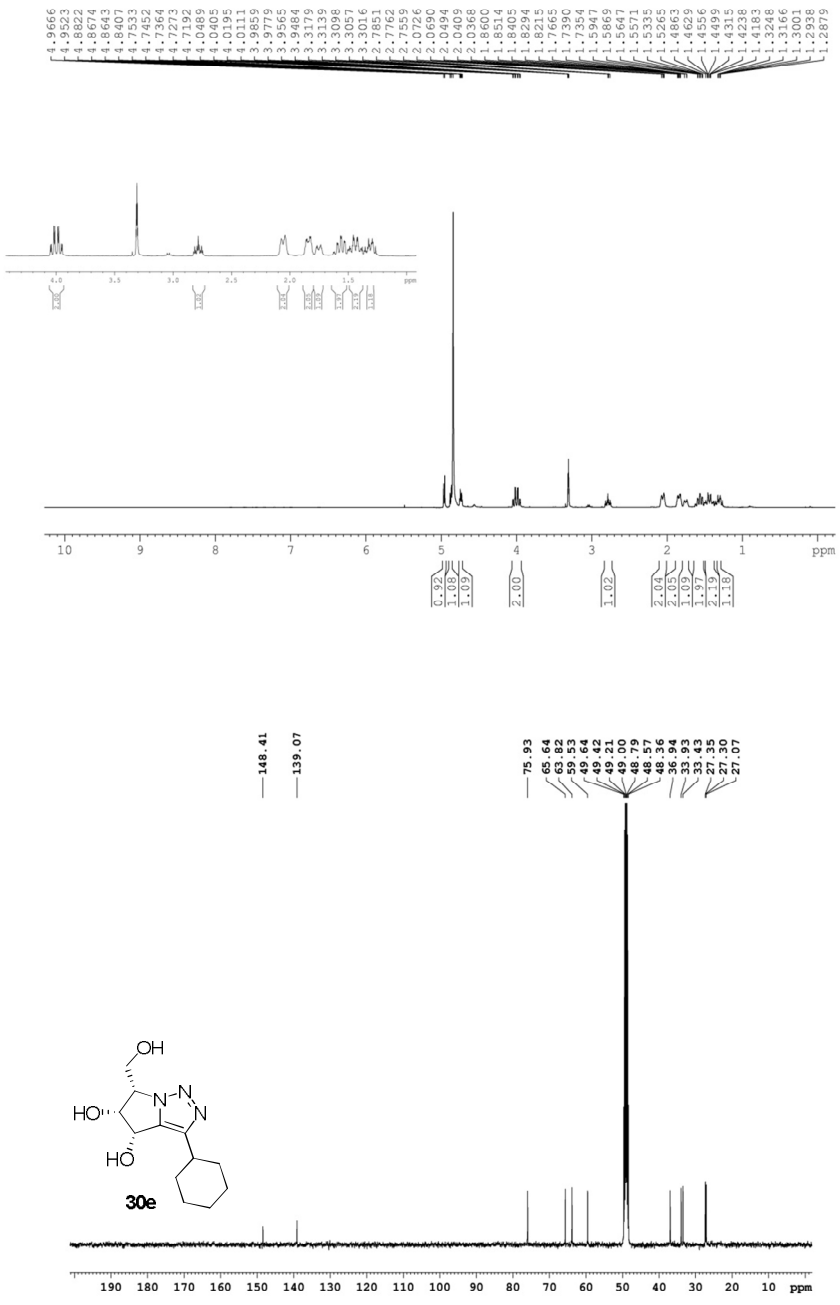

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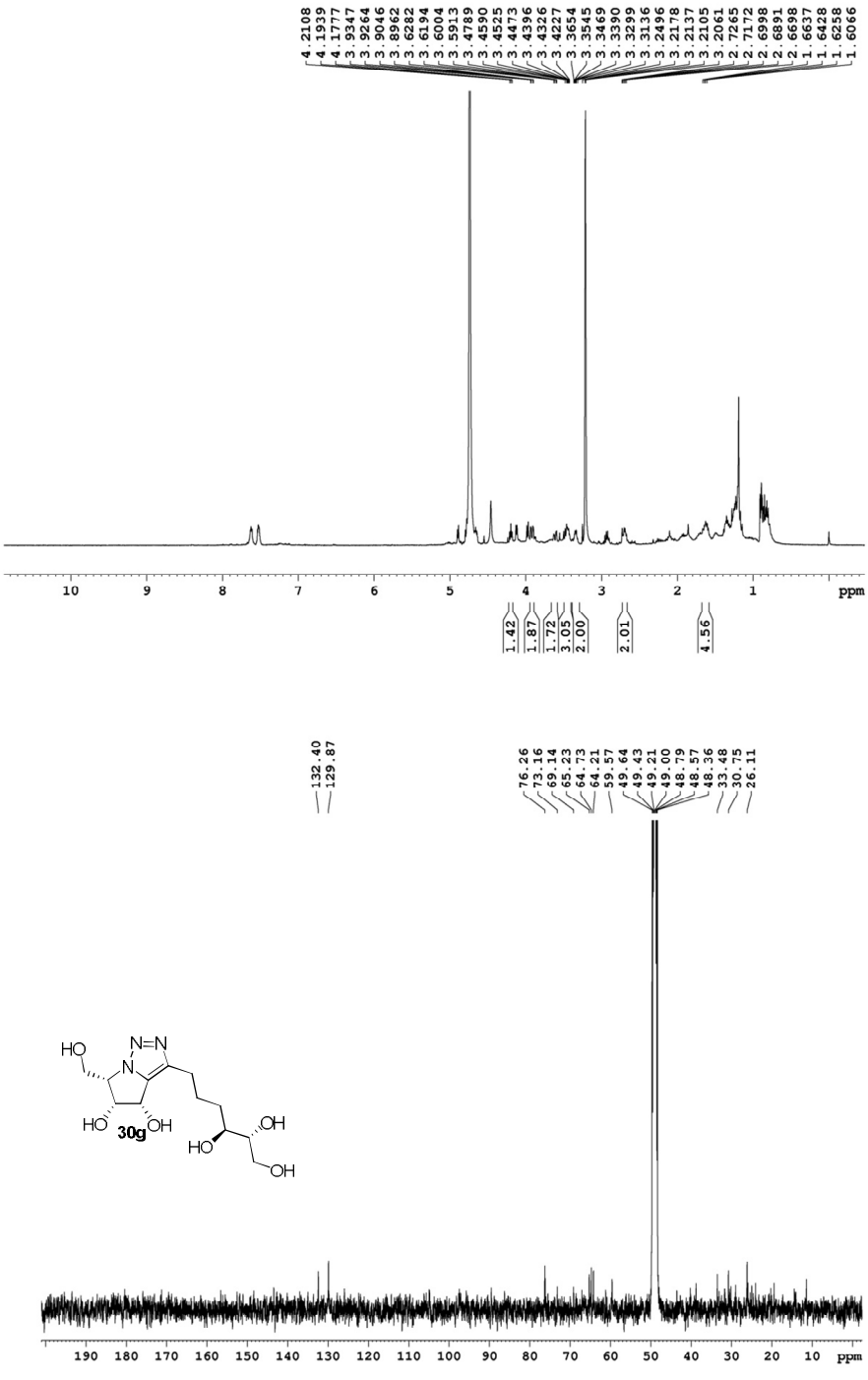

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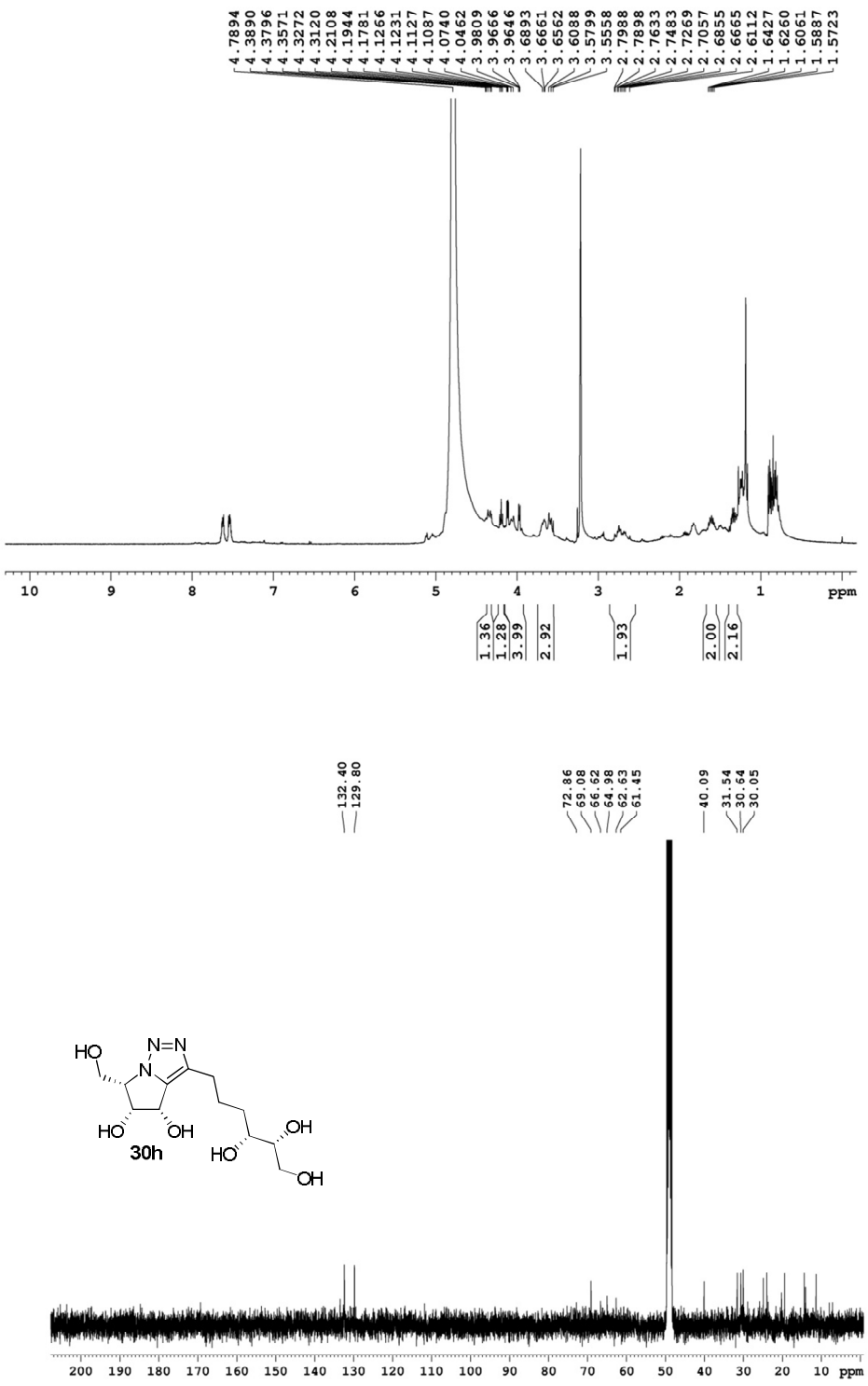

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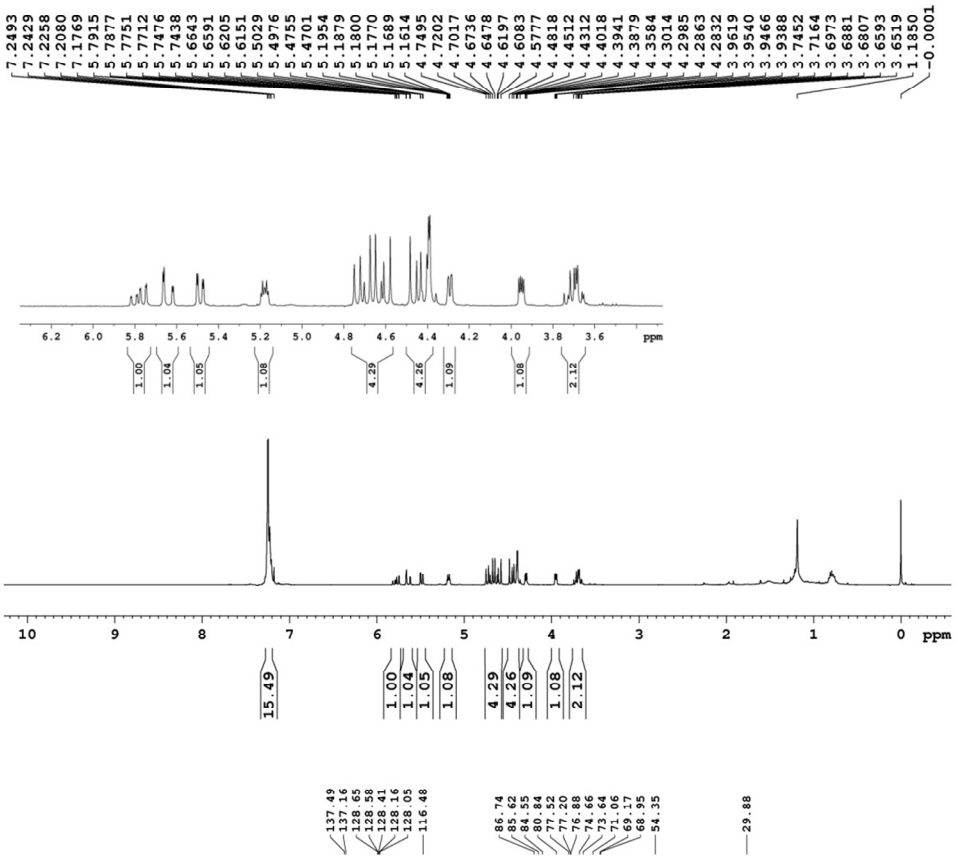
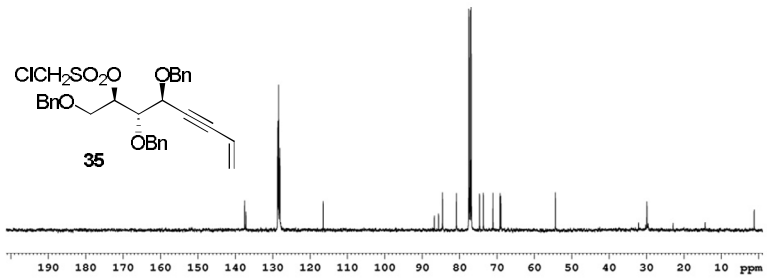
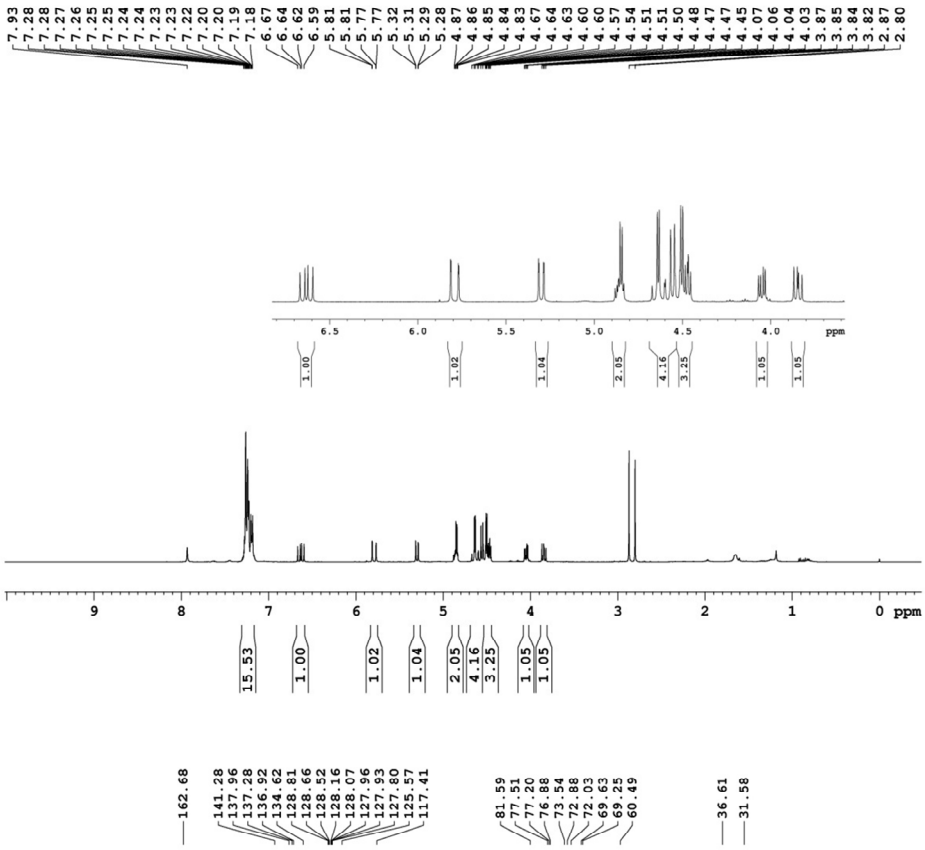
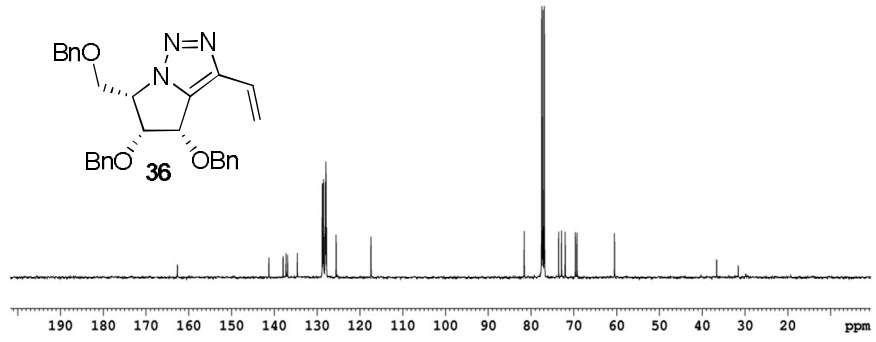


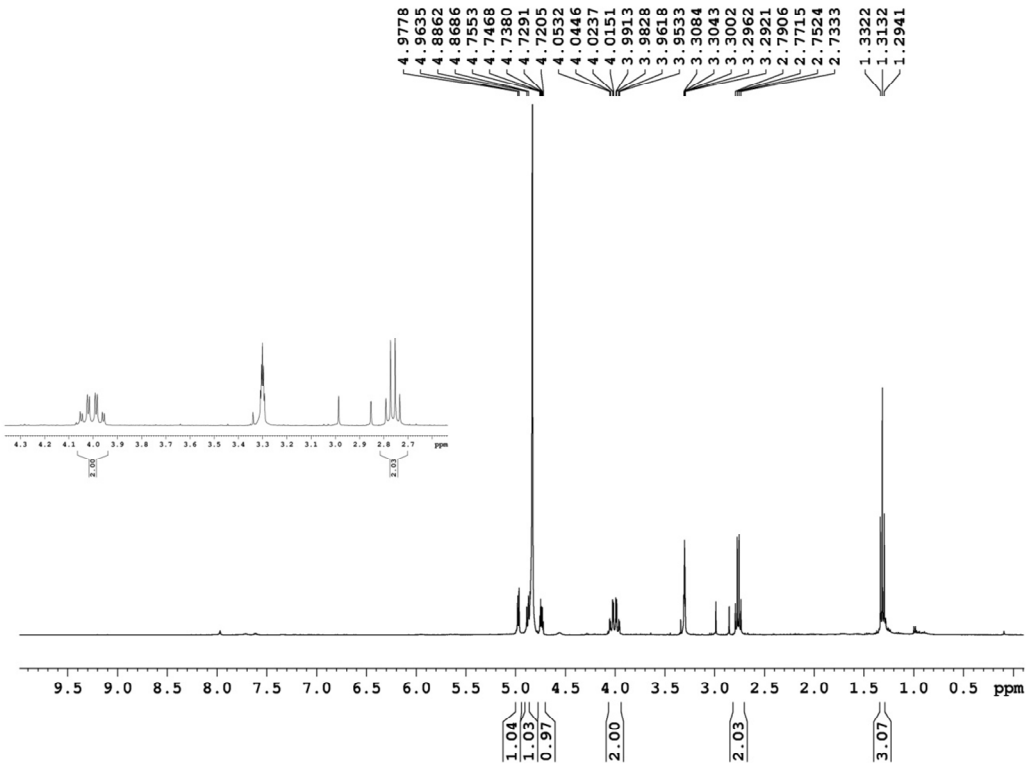


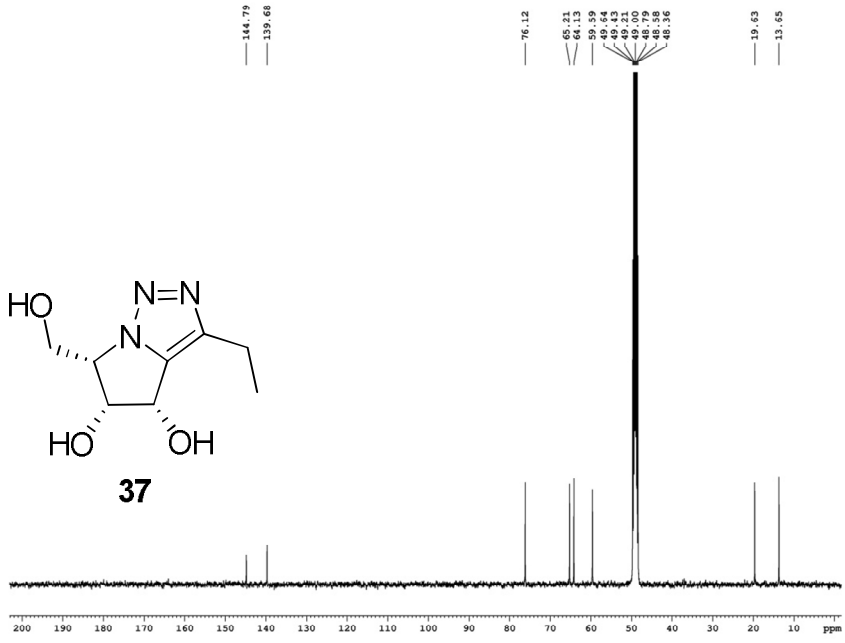
^{1}H and ^{13}C NMR Spectrum of 35





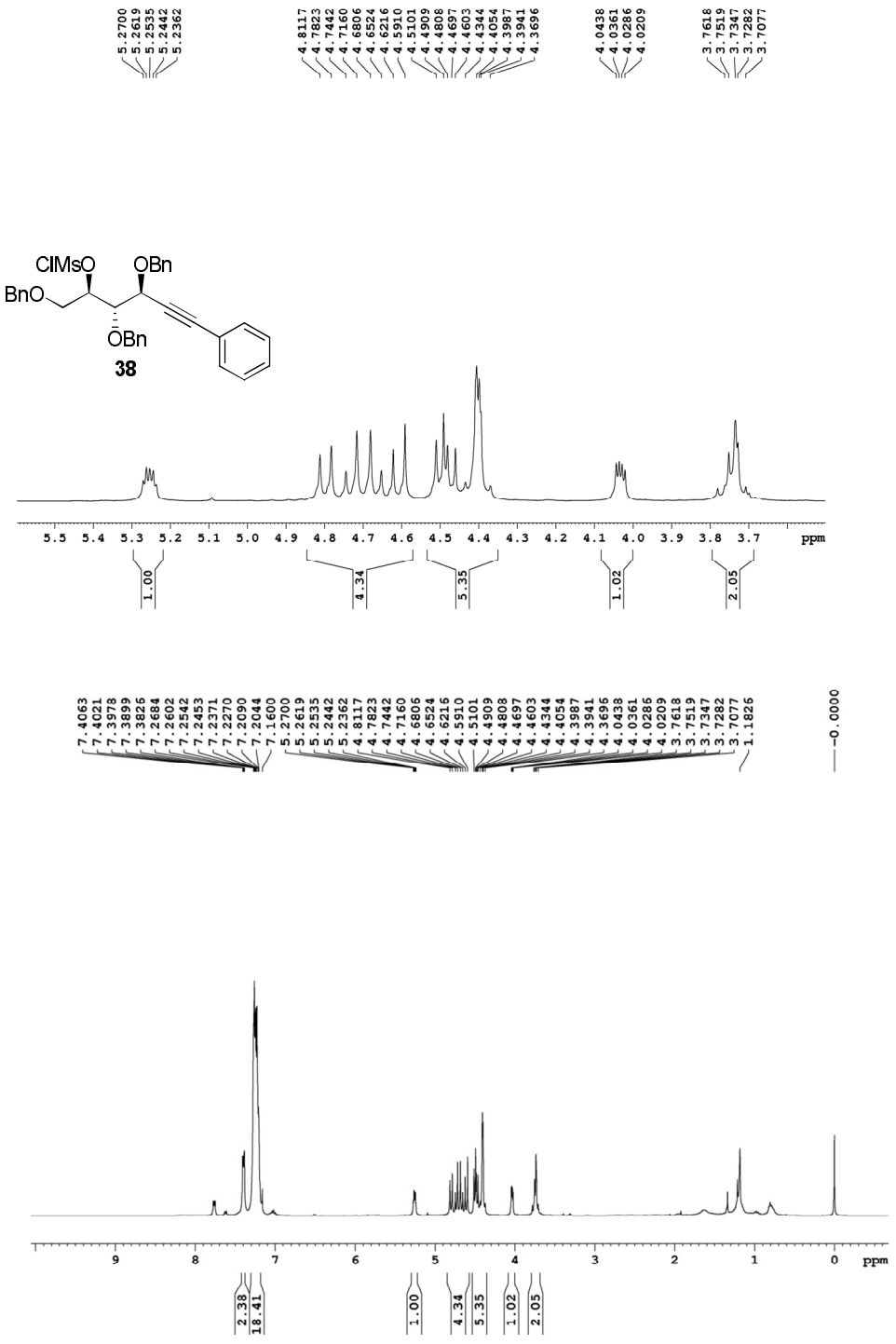
^{1}H and ^{13}C NMR Spectrum of 36



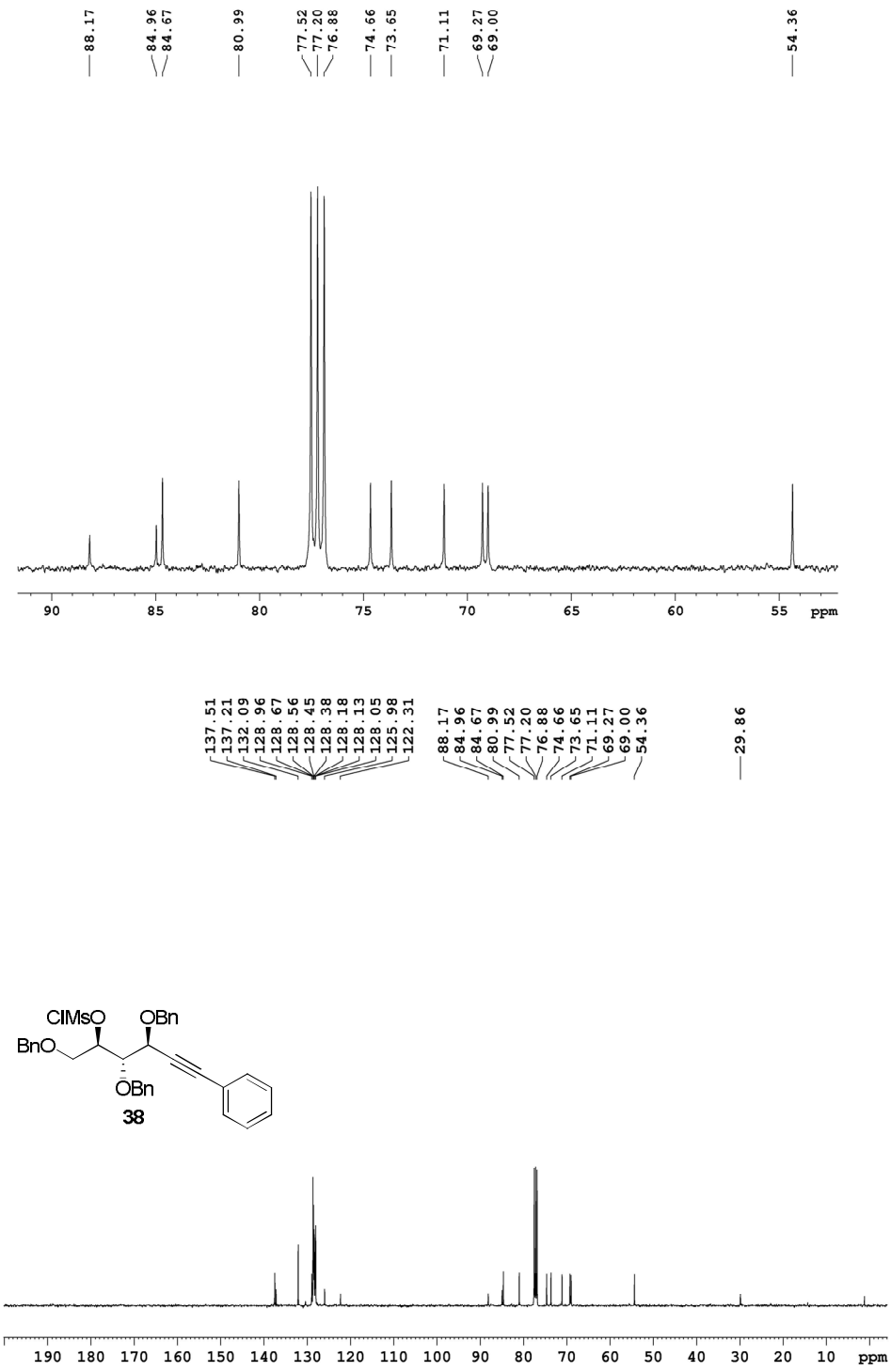


37

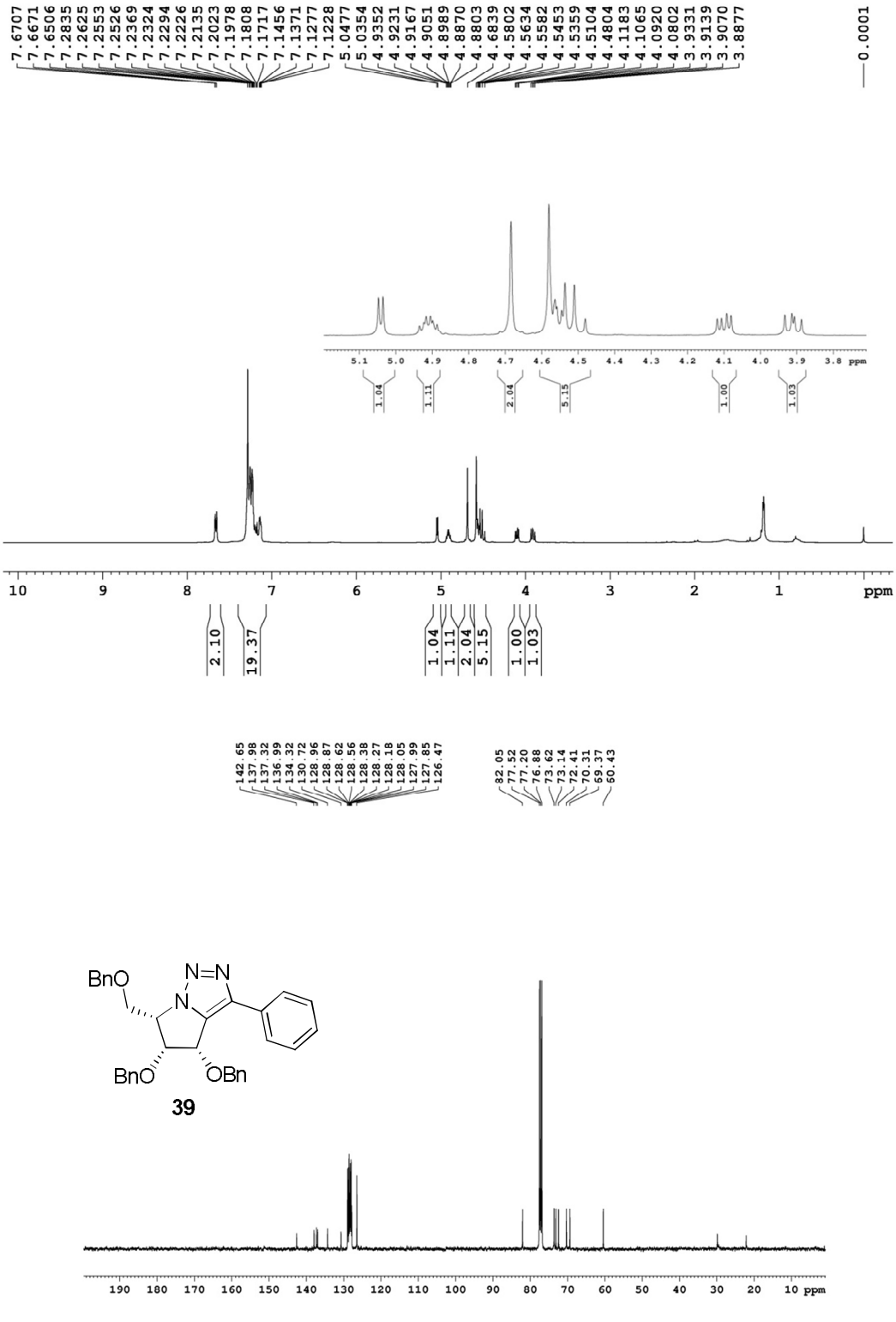
^{1}H ad ^{13}C NMR Spectrum of 37



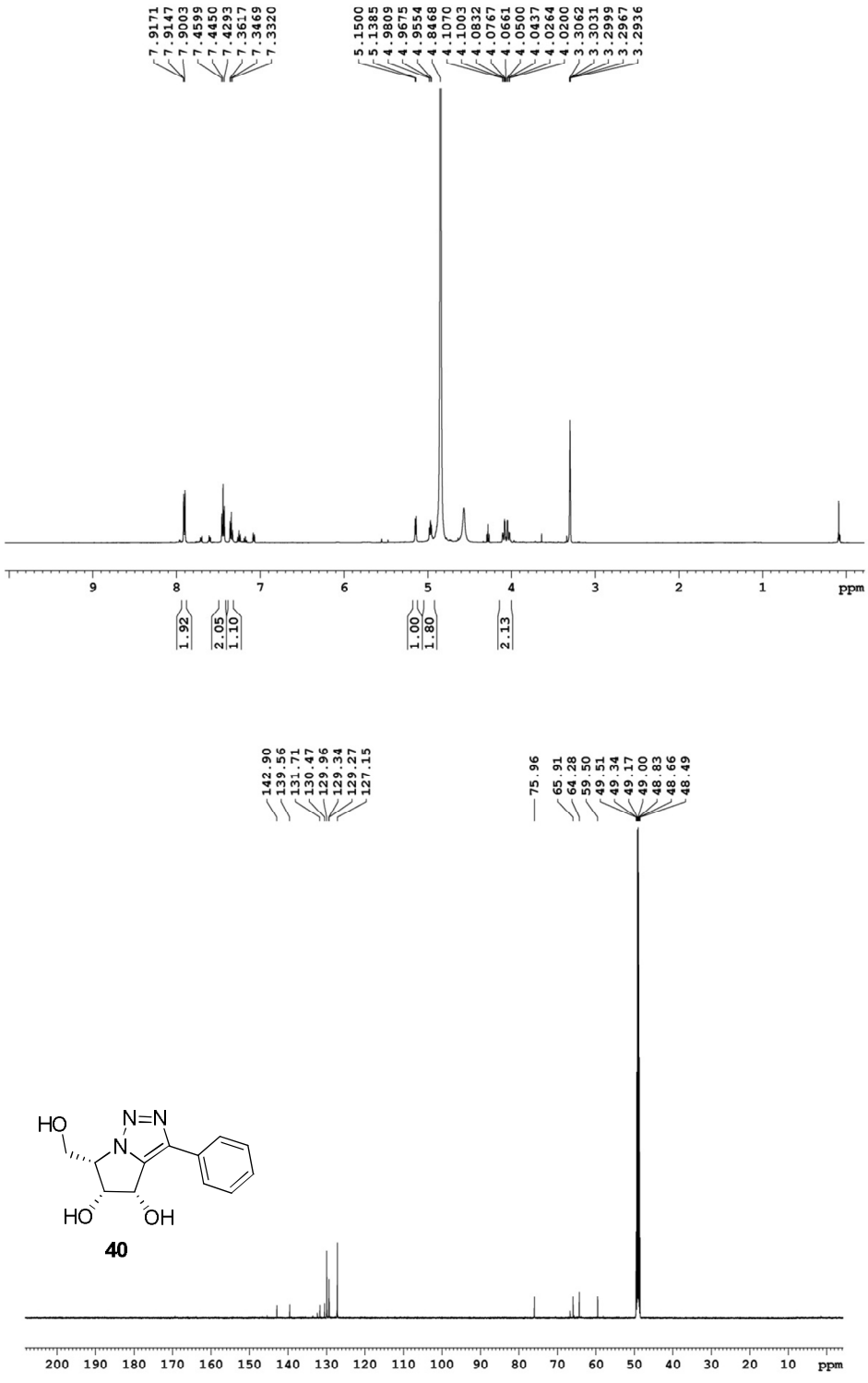
^{1}H NMR Spectrum of 38



^{13}C NMR Spectrum of 38



^{1}H and ^{13}C NMR Spectrum of 39



^{1}H and ^{13}C 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 specterophometrically. 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)	*p*-nitrophenyl *α*-D-galactopyranoside	Citrate phosphate buffer (50 mM, pH 6.5	25°C
2	*β-galactosidase (bovine liver)*	*p*-nitrophenyl *β*-D-galactosidase	Citrate phosphate buffer (50 mM, pH 4.5)	30°C
3	*α*-mannosidase (jack bean)	*p*-nitrophenyl *α*-D-mannpyranoside	Acetate buffer (50 mM, pH 4.5)	25 °C
4	*β*-glucosidase (almond)	*p*-nitrophenyl *β*-D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 5.5)	37 °C
5	*α*-glucosidase (yeast)	*p*-nitrophenyl *α*-D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
6	Glucosidase (*A. niger*)	*p*-nitrophenyl *α*-D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
7	*α*-glucosidase (rice)	*p*-nitrophenyl *α*-D-glucopyranoside	Citrate phosphate buffer (50 mM, pH 6.8)	37 °C
8	*α*-L-fucosidase (bovine kidney)	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 μM.

(% 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 (*A. niger*)	**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 μM.

(% of inhibition at 1000 μM)

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 (*A. niger*)	**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.

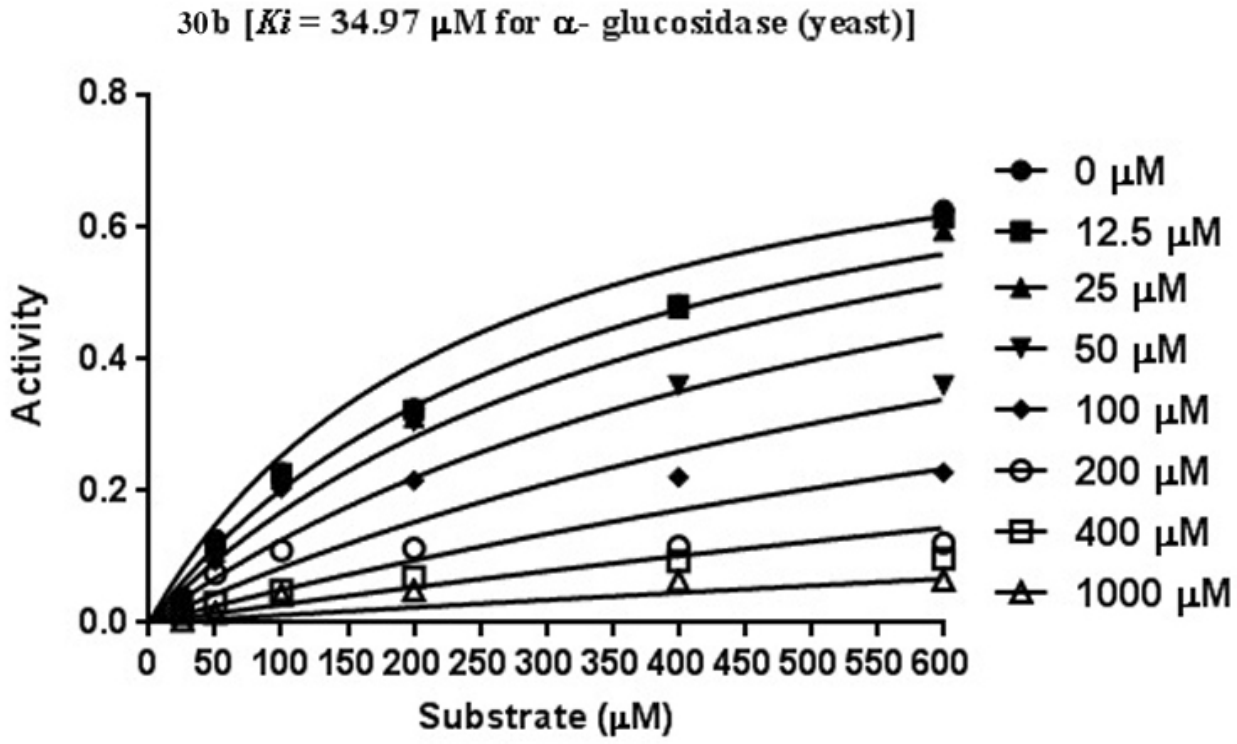


Figure 1

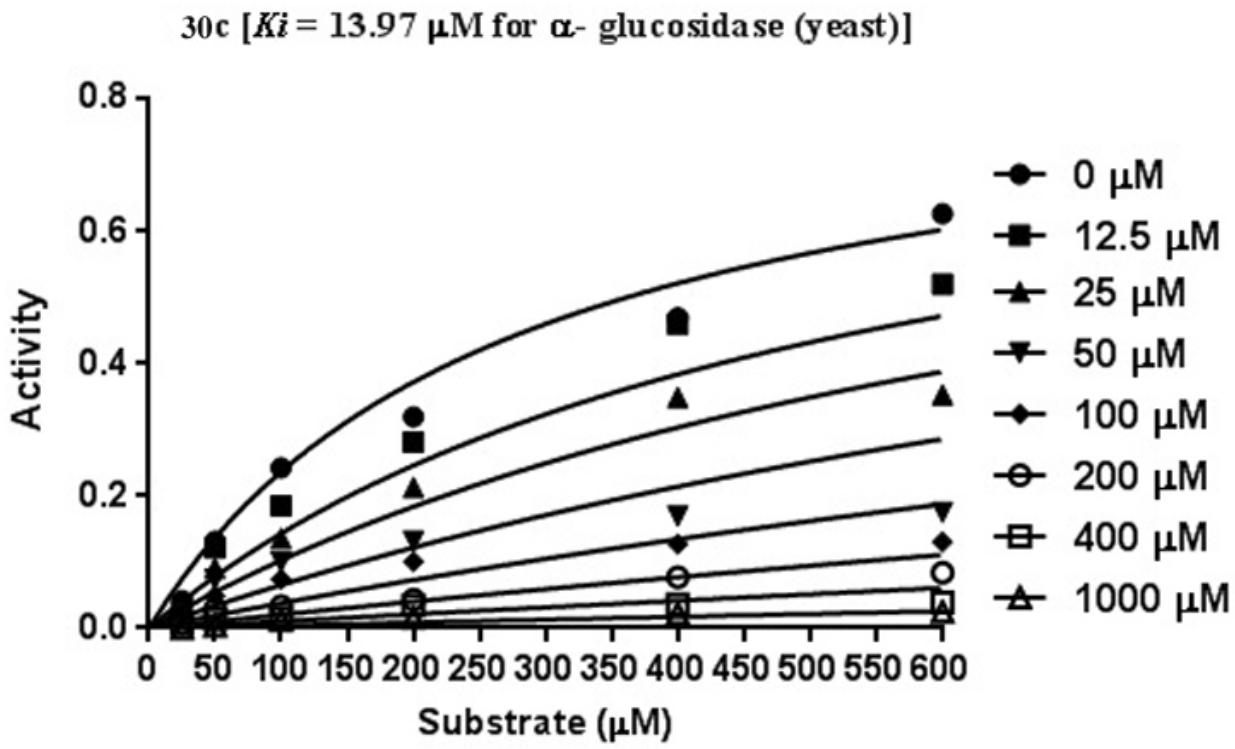


Figure 2

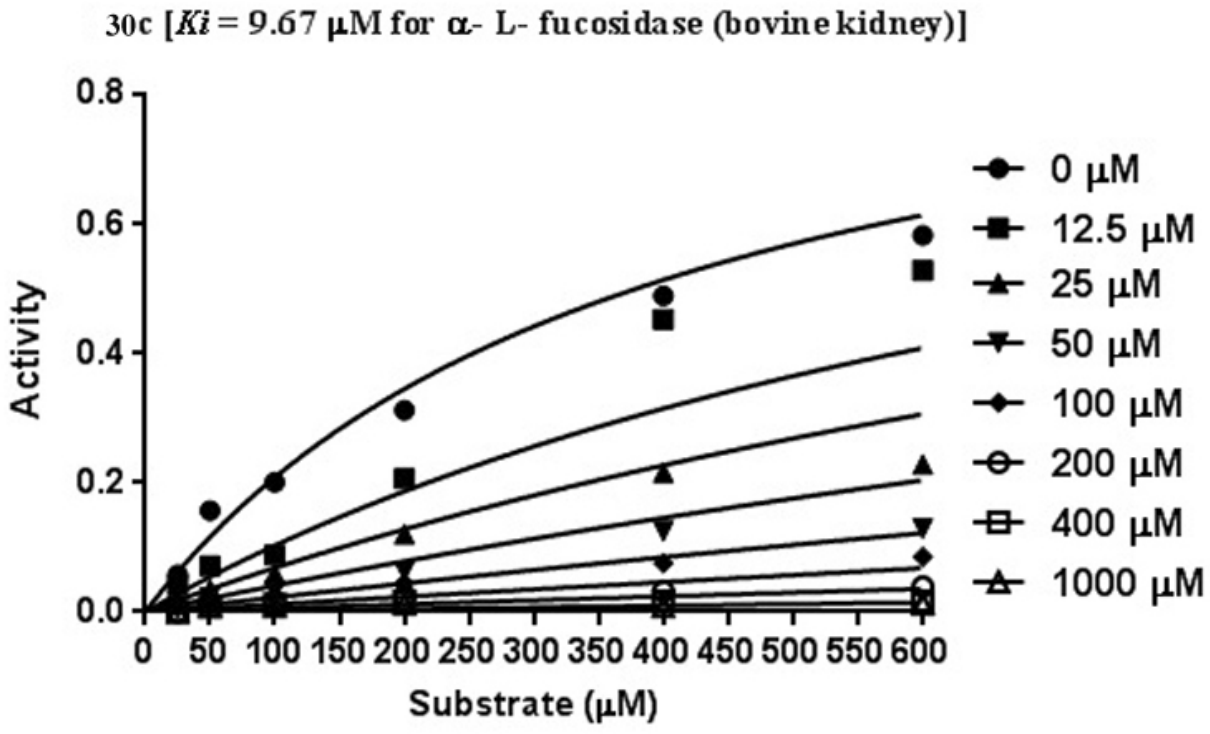


Figure 3

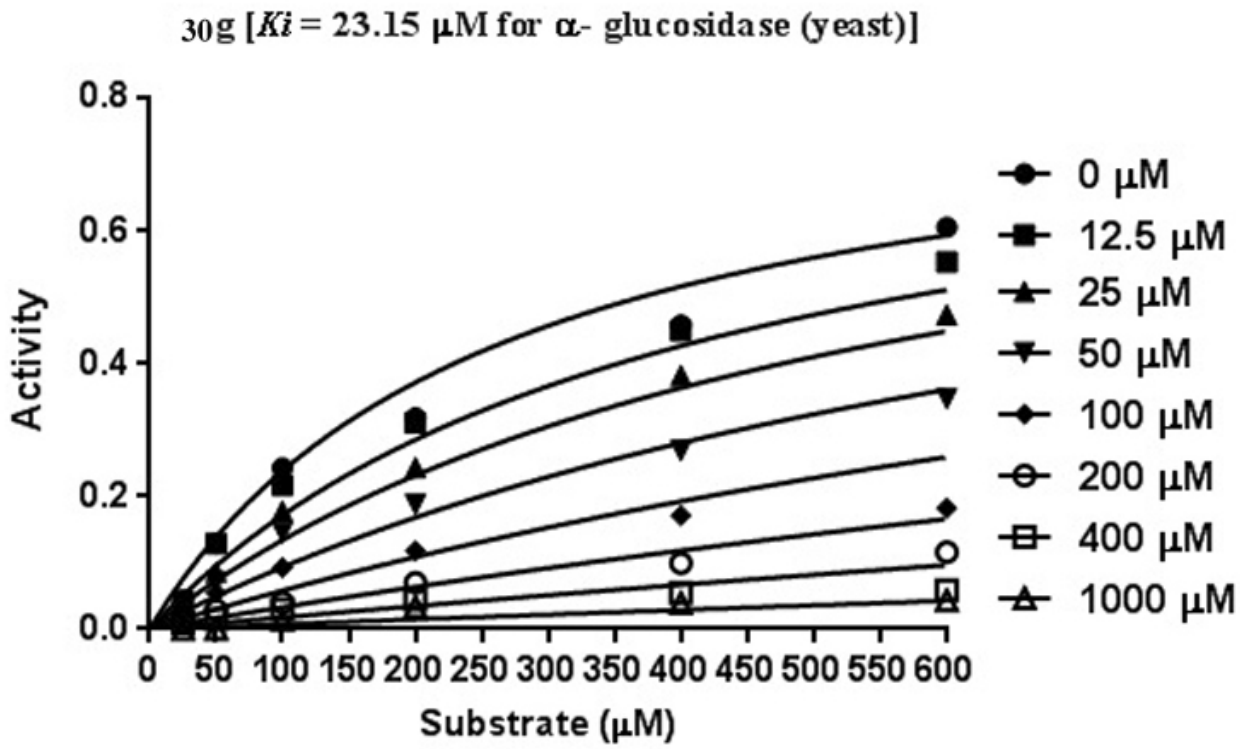


Figure 4

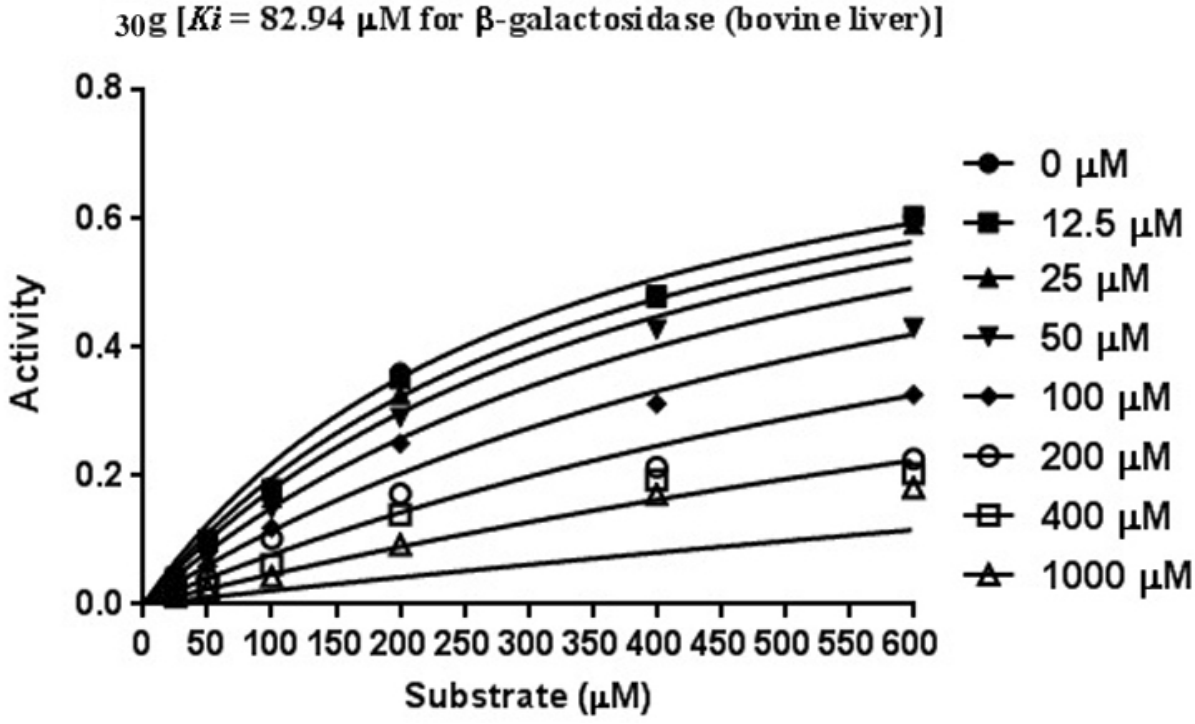


Figure 5

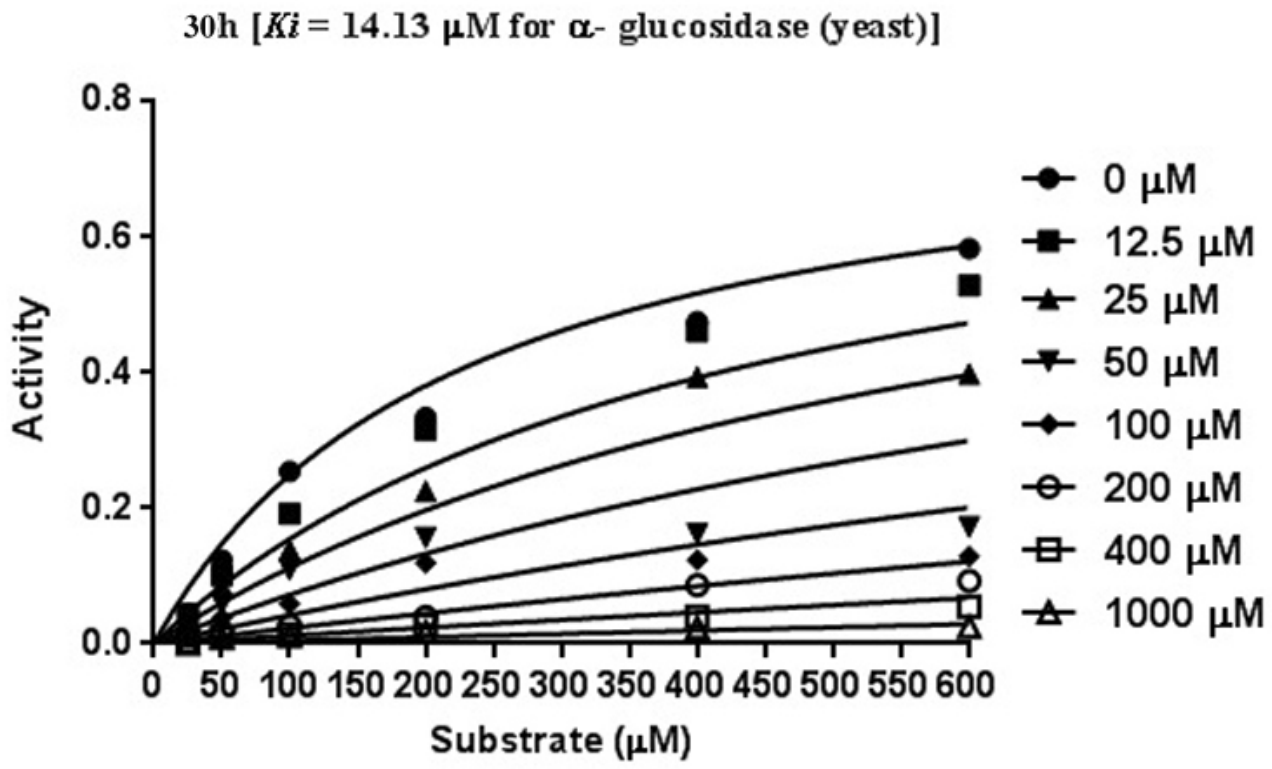


Figure 6

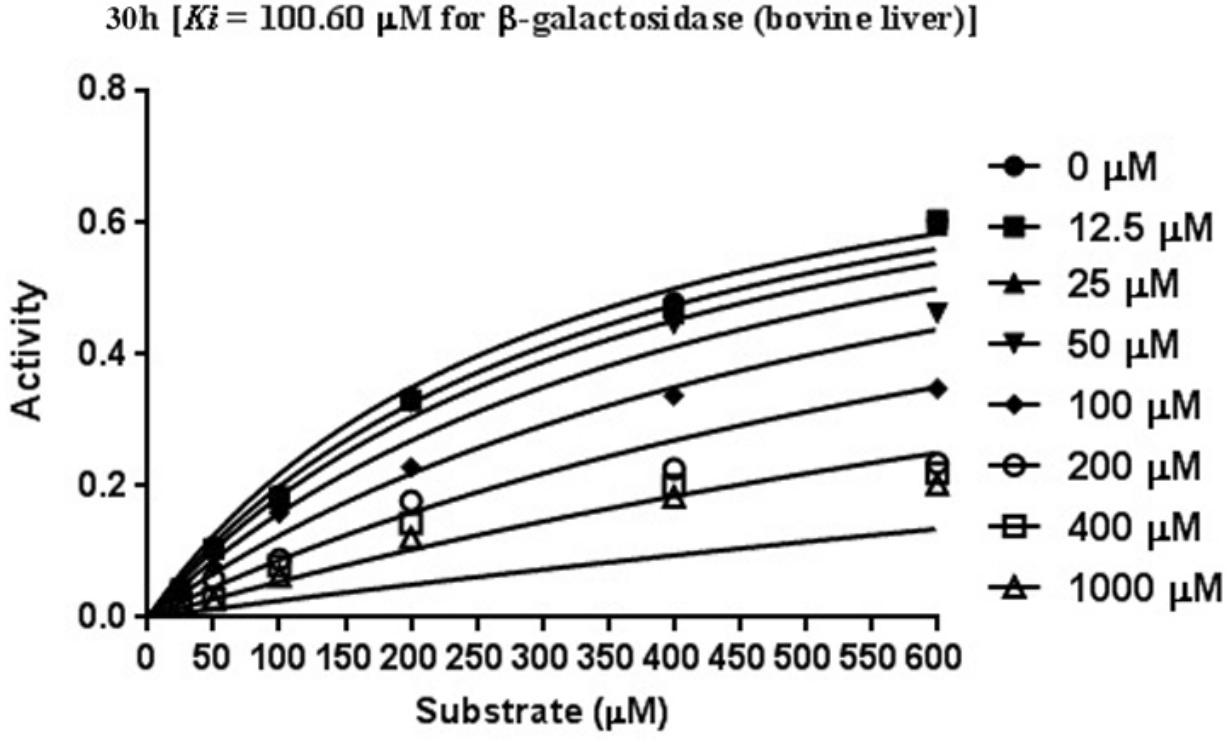


Figure 7

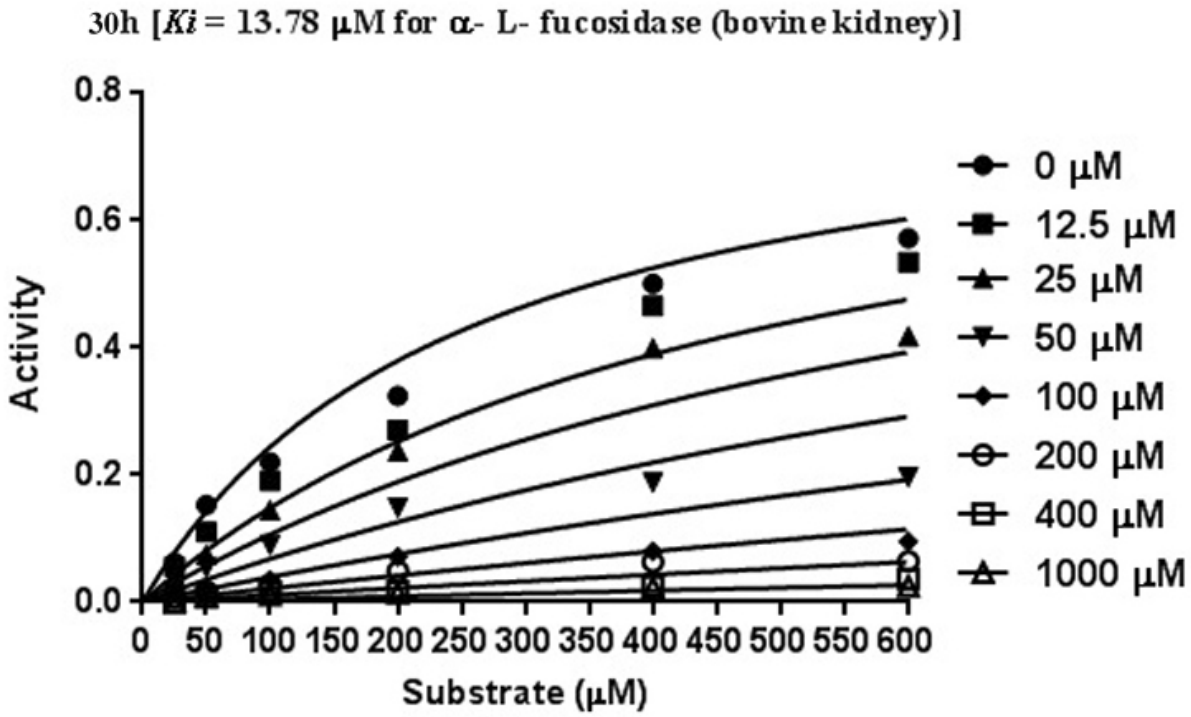


Figure 8

References

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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. Robertsonand D. W. S. Wong, *Enzyme Microb. Technol.*, 2007, **4**, 747-753.