Synthesis of new lophine-carbohydrate hybrids as cholinesterase inhibitors: cytotoxicity evaluation and molecular modelling

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Supplementary Information

Table S1. ADME properties computed by the QikProp tool.

Property	Description
#stars	Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. A large number of stars suggests that a molecule is less drug-like than molecules with few stars.
#rtvFG	Number of reactive functional groups that can lead to false positives in HTS assays and to decomposition, reactivity, or toxicity problems *in vivo*.
CNS	Predicted central nervous system activity on a −2 (inactive) to +2 (active) scale.
mol_MW	Molecular weight.
donor_HB	Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution over a number of molecular configurations.
acceptor_HB	Estimated number of hydrogen bonds that would be accepted by the solute to water molecules in an aqueous solution over a number of molecular configurations.
QPlogPo/w	Predicted octanol/water partition coefficient.
QPlogS	Predicted aqueous solubility, log S. S in mol dm^{-3} is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid.
CIQPlogS	Conformation-independent predicted aqueous solubility, log S.
QPlogHERG	Predicted IC_{50} value for blockage of HERG K^+ channels.
QPPCaco	Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells are a model for the gut-blood barrier. QikProppredictions are for non-activetransport.
QPlogBB	Predicted brain/blood partition coefficient for orally delivered drugs.
QPPMDCK	Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier. QikProppredictions are for non-activetransport.
#metab	Number of likely metabolic reactions.
PercentHumanOralAbsorption	Predicted human oral absorption on 0 to 100% scale. The prediction is based on a quantitative multiple linear regression model.
Ro5	Number of violations of Lipinski's rule of five (Lipinski et al., 1997). The rules are: mol_MW< 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. Compounds that satisfy these rules are considered drug-like.

Ro3	Number of violations of Jorgensen's rule of three. The three rules are: QPlogS> -5.7, QP PCaco> 22 nm/s, # Primary Metabolites < 7. Compounds with fewer (and preferably no) violations of these rules are more likely to be orally available.

Table S2. Ensemble docking results for the lofine-carboidrate derivatives against the four AChE conformations and the BChE.

Compound (linkersize)	**Carbohydrate**	**AChE**		**BChE**	**MeasuredAffinity[b]**	
		PDB	**GlideScore[a]**	**GlideScore[a]**	**AChE**	**BChE**
7a (n = 4)	Xylose	4EY7	-8.553	-7.556	n.a.	0.295
7b (n = 5)	Xylose	4EY7	-9.634	-8.283	n.a.	0.277
7c (n = 6)	Xylose	1ZGC	-9.488	-7.674	n.a.	0.708
7d (n = 7)	Xylose	1Q84	-10.493	-8.267	n.a.	0.399
7e (n = 8)	Xylose	4EY7	-9.431	-8.650	n.a.	1.300
8a (n = 6)	Ribose	2CKM	-8.674	-7.865	n.a.	0.396
8b (n = 7)	Ribose	2CKM	-8.836	-7.882	2.75	0.499
9a (n = 6)	Galactose	4EY7	-9.768	-6.958	n.a.	0.174
9b (n = 7)	Galactose	1ZGC	-11.236	-8.397	n.a.	0.619
Bis(7)-tacrine	**-**	**-12.026 (2CKM)**		**-6.434 (5K5E)**	**0.0019**	**0.0091**

[a]Given in kcal/mol. [b]Given in μM.

Table S3. ADME properties predicted by QikProp for the lophine-carbohydrate derivatives, bis(7)-tacrine and donepezil.

molecule	#stars	#rtvFG	CNS	mol_MW	donorHB	accptHB	QPlogPo/w	QPlogS	CIQPlogS	QPlogHERG	QPPCaco	QPlogBB	QPPMDCK	#metab	PHOA	Ro5	Ro3
7a	3	2	1	539,673	2	7,9	6,234	-6,952	-7,451	-8,408	933,666	-0,022	508,171	2	90,688	2	1
7e	6	2	1	595,78	2	7,9	7,354	-7,925	-8,595	-8,619	760,775	-0,401	407,269	2	95,66	2	1
8a	1	2	1	581,753	1	7,9	6,766	-5,816	-8,376	-7,223	1682,933	0,187	960,686	1	100	2	1
7d	7	2	1	581,753	2	7,9	7,276	-8,465	-8,308	-9,038	737,427	-0,392	393,776	2	94,958	2	1
9b	5	3	1	651,844	1	7,7	8,552	-8,518	-10,025	-8,054	1936,934	0,235	1118,322	1	100	2	1
8b	2	2	1	595,78	1	7,9	6,868	-5,209	-8,666	-6,607	2134,659	0,238	1242,209	1	100	2	0
9a	7	3	1	637,817	1	7,7	8,313	-8,932	-9,733	-8,622	1464,412	0,139	826,594	1	100	2	1
7c	4	2	1	567,727	2	7,9	6,656	-7,222	-8,022	-8,382	694,819	-0,301	369,243	2	90,863	2	1
7b	3	2	1	553,7	2	7,9	6,497	-7,586	-7,736	-8,718	552,946	-0,388	288,47	2	88,16	2	1
Bis(7)-tacrine	4	0	0	492,706	2	4	8,236	-9,876	-8,711	-7,493	4872,653	-0,435	2739,953	8	100	1	2
donepezil	0	0	1	379,498	0	5,5	4,427	-4,74	-4,338	-6,743	871,96	0,099	471,969	6	100	0	0
Reference	*0 – 5*	*0 – 2*	*−2 (inactive), +2 (active)*	*130.0 – 725.0*	*0.0 – 6.0*	*2.0 – 20.0*	*−2.0 – 6.5*	*−6.5 – 0.5*	*−6.5 – 0.5*	*<-5*	*<25 poor, >500 great*	*−3.0 – 1.2*	*<25 poor, >500 great*	*1 – 8*	*>80% is high <25% is poor*	*<=4*	*<=3*

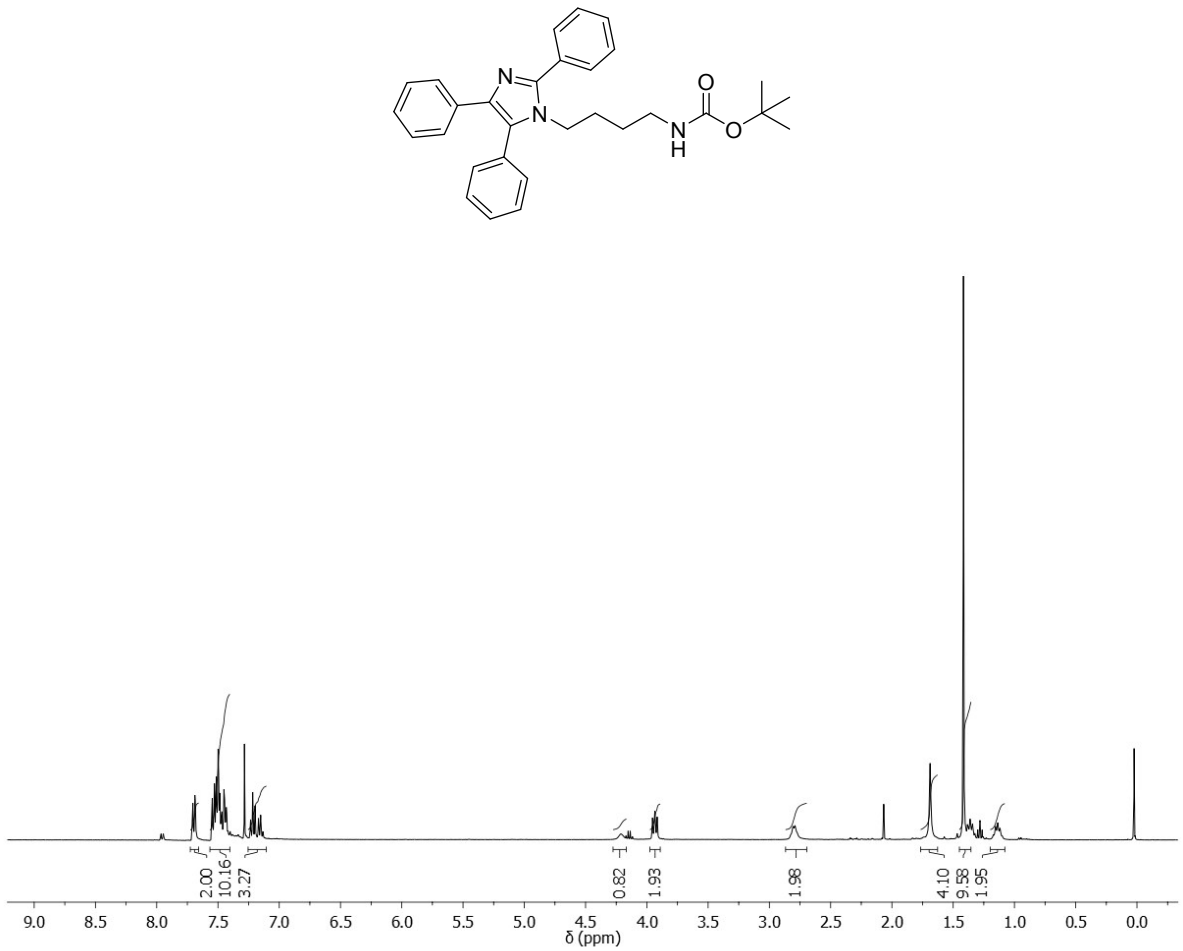


Figure S1. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **2a**.

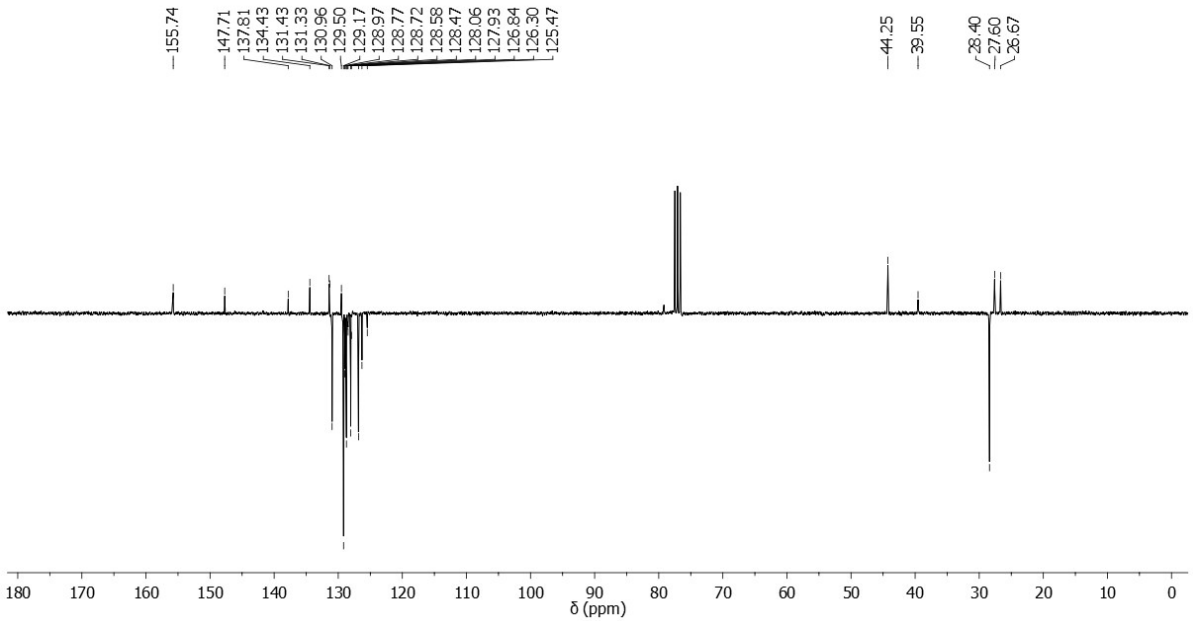


Figure S2. ^{13}C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **2a.**

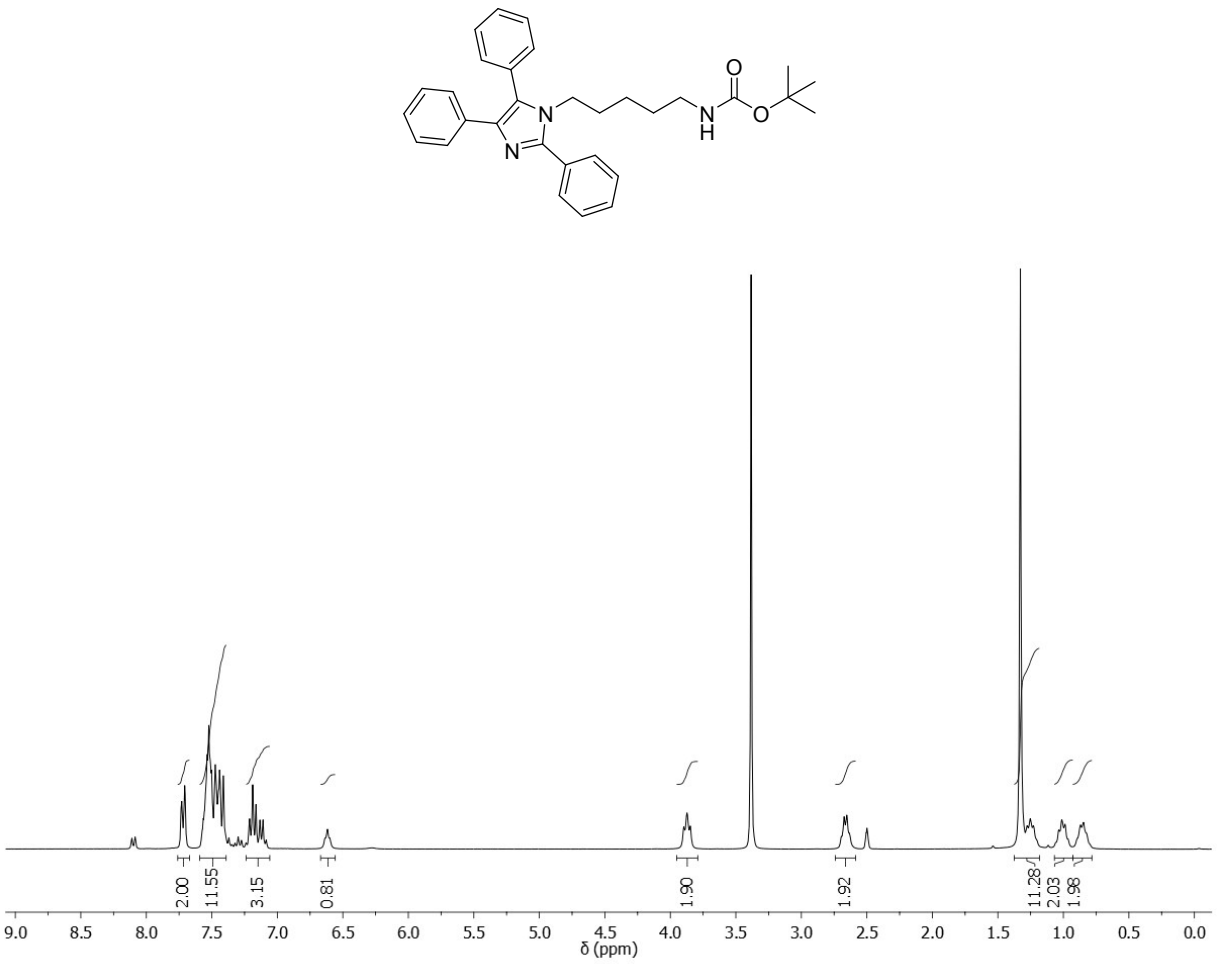


Figure S3. ^{1}H NMR Spectrum (DMSO-*d6*, 300 MHz) of compound **2b**.

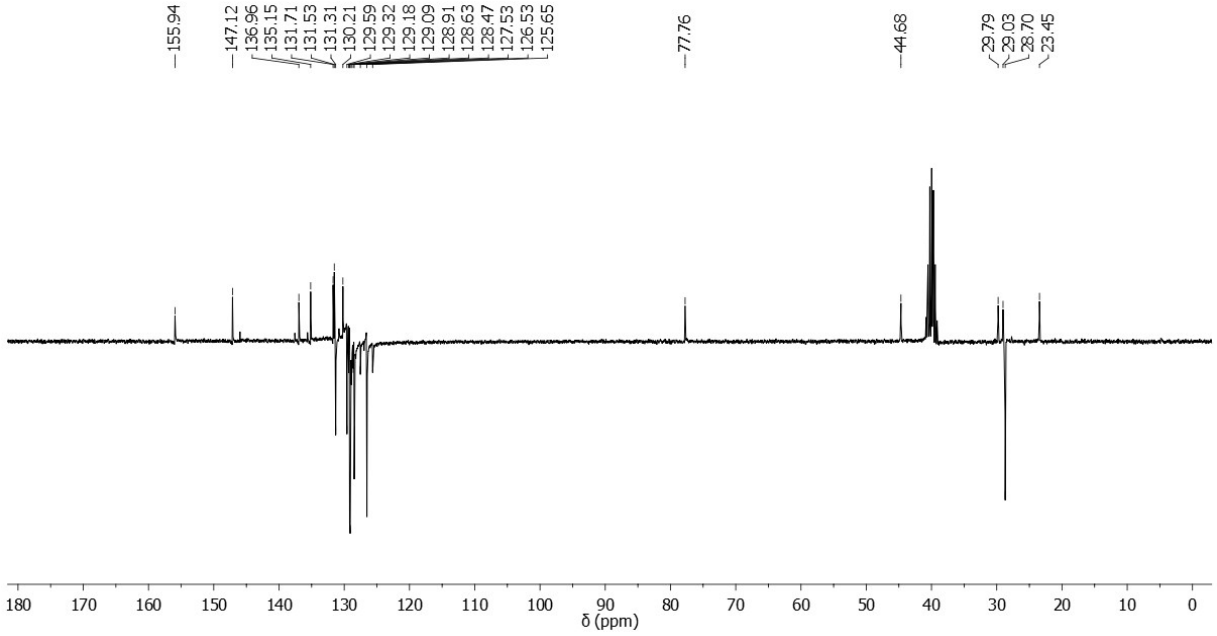


Figure S4. ^{13}C NMR Spectrum, APT (DMSO-*d6*, 75 MHz) of compound **2b**

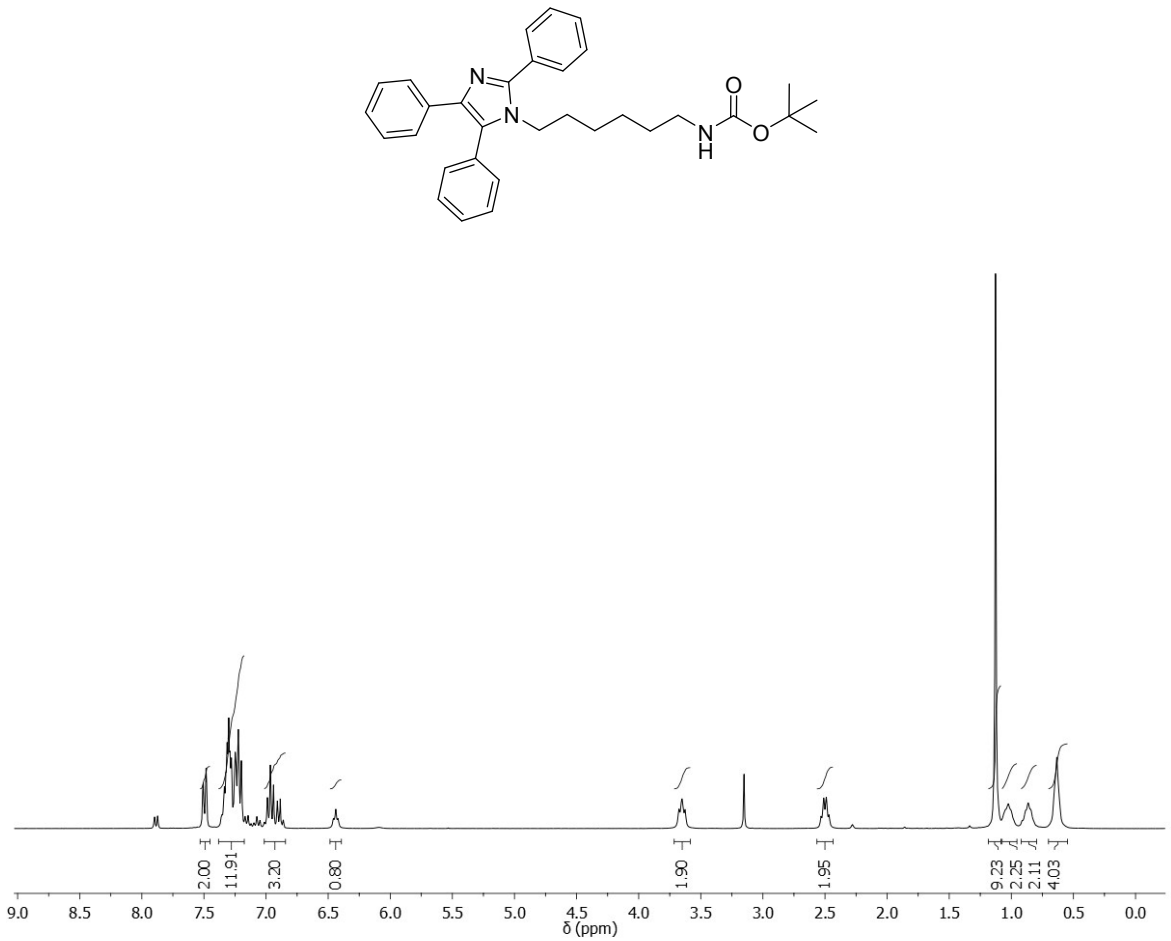


Figure S5. ^{1}H NMR Spectrum (DMSO-*d6*, 300 MHz) of compound **2c**.

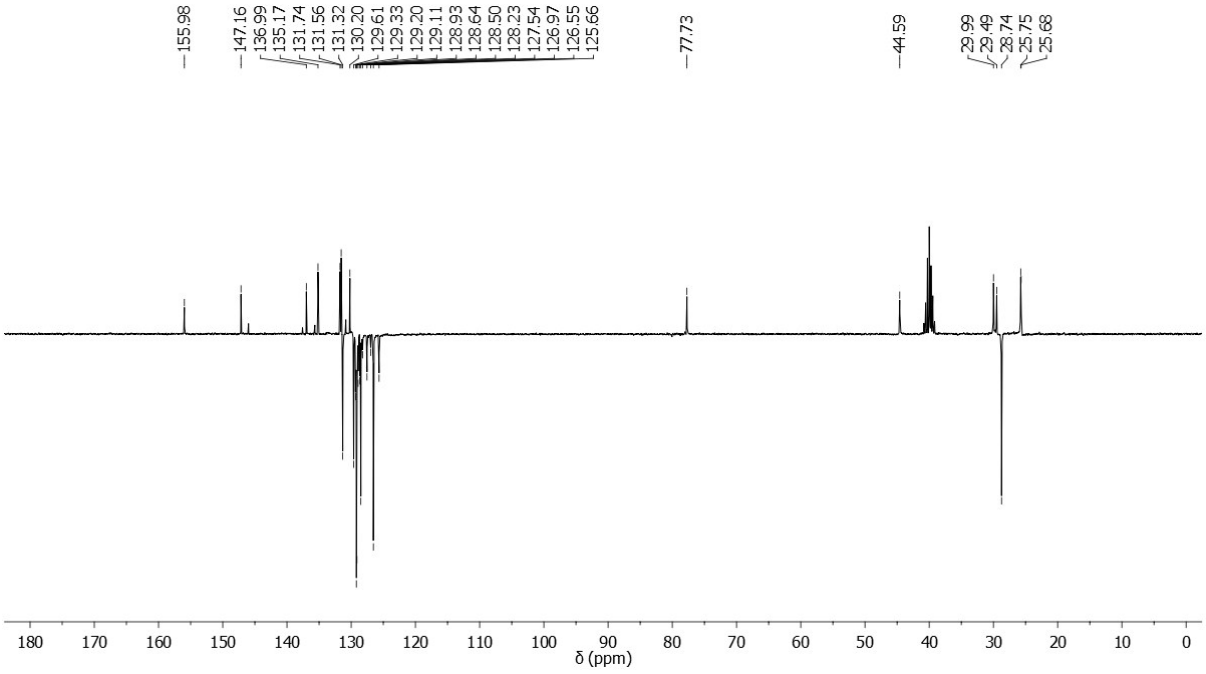
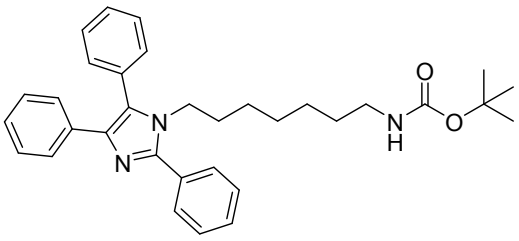


Figure S6. ^{13}C NMR Spectrum, APT (DMSO-*d6*, 75 MHz) of compound **2c.**



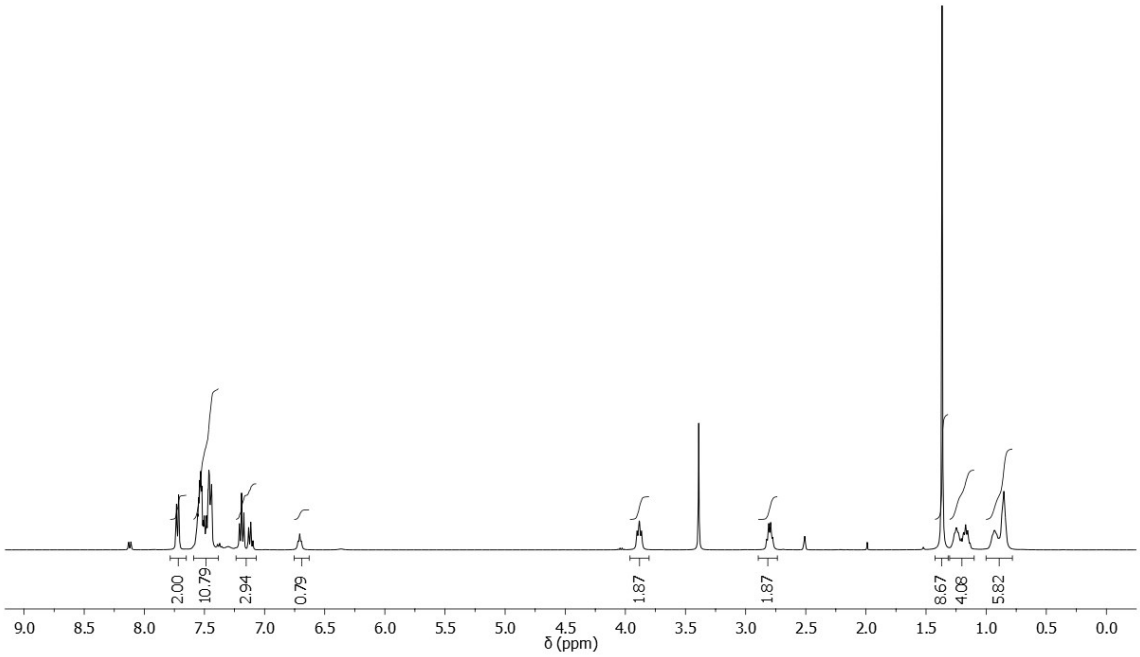


Figure S7. ^{1}H NMR Spectrum (DMSO-*d6*, 300 MHz) of compound **2d**.

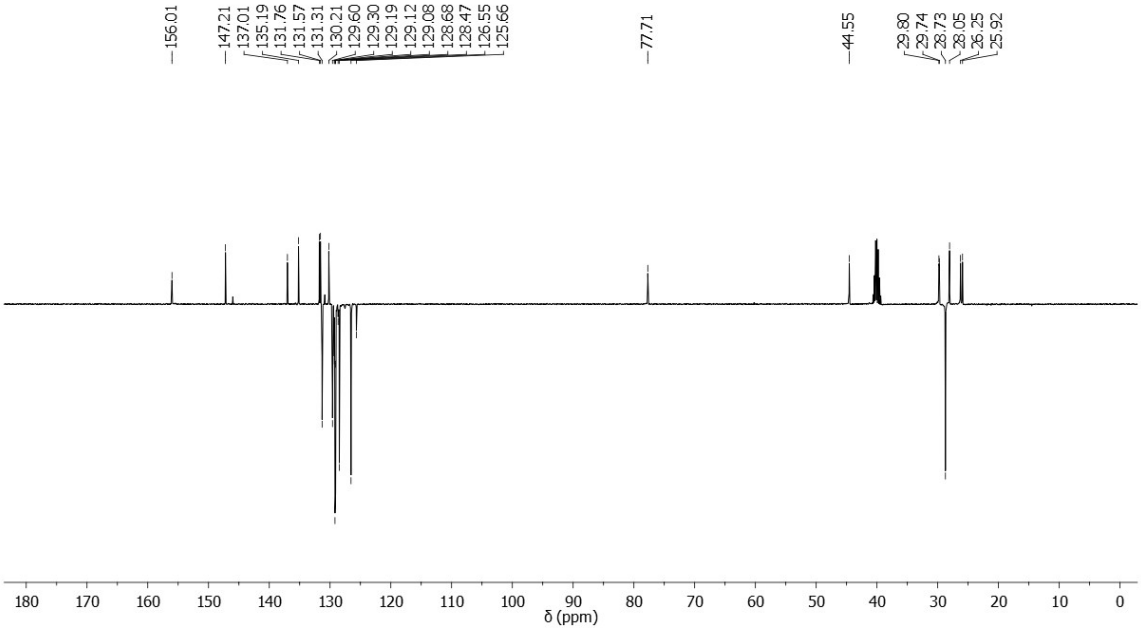


Figure S8. ^{13}C NMR Spectrum, APT (DMSO-*d6*, 75 MHz) of compound **2d.**

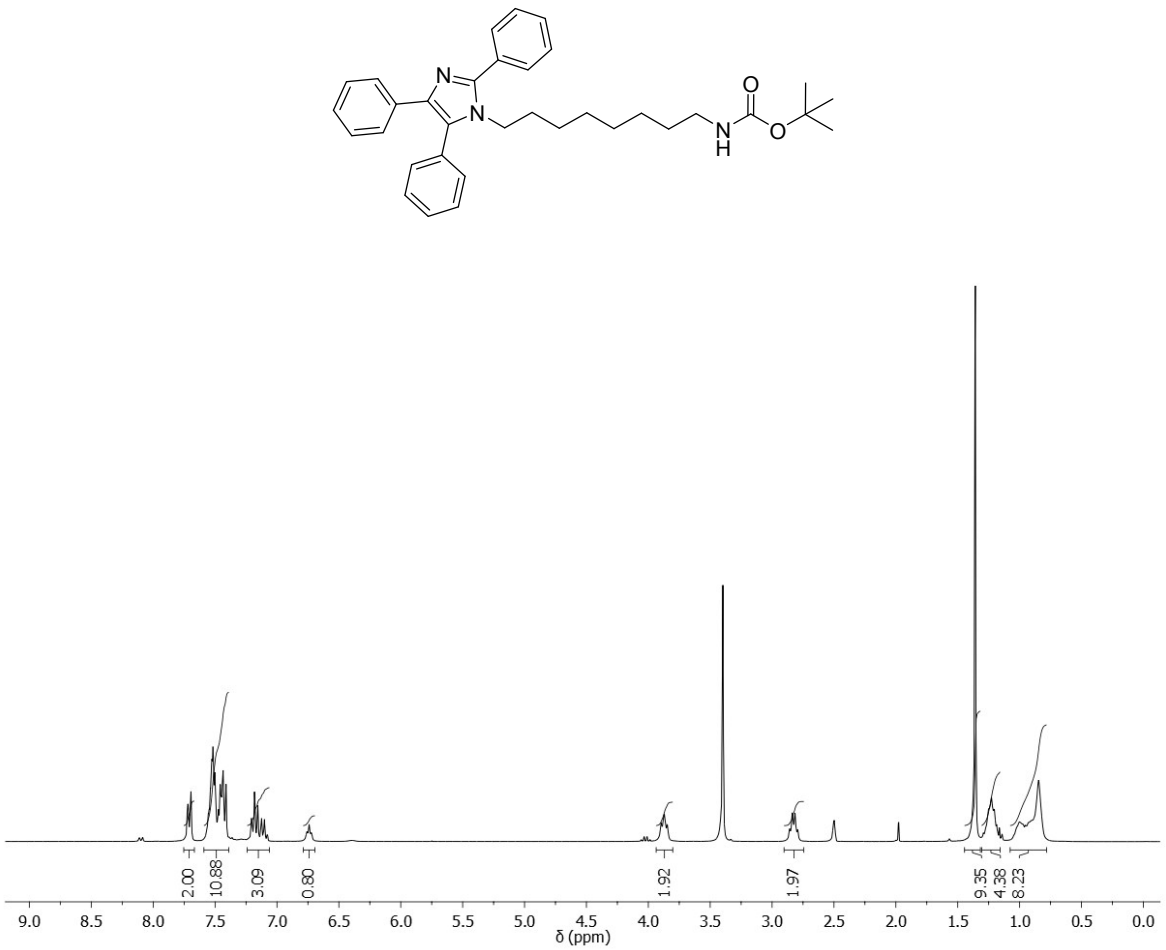


Figure S9. ^{1}H NMR Spectrum (DMSO-*d6*, 300 MHz) of compound **2e**.

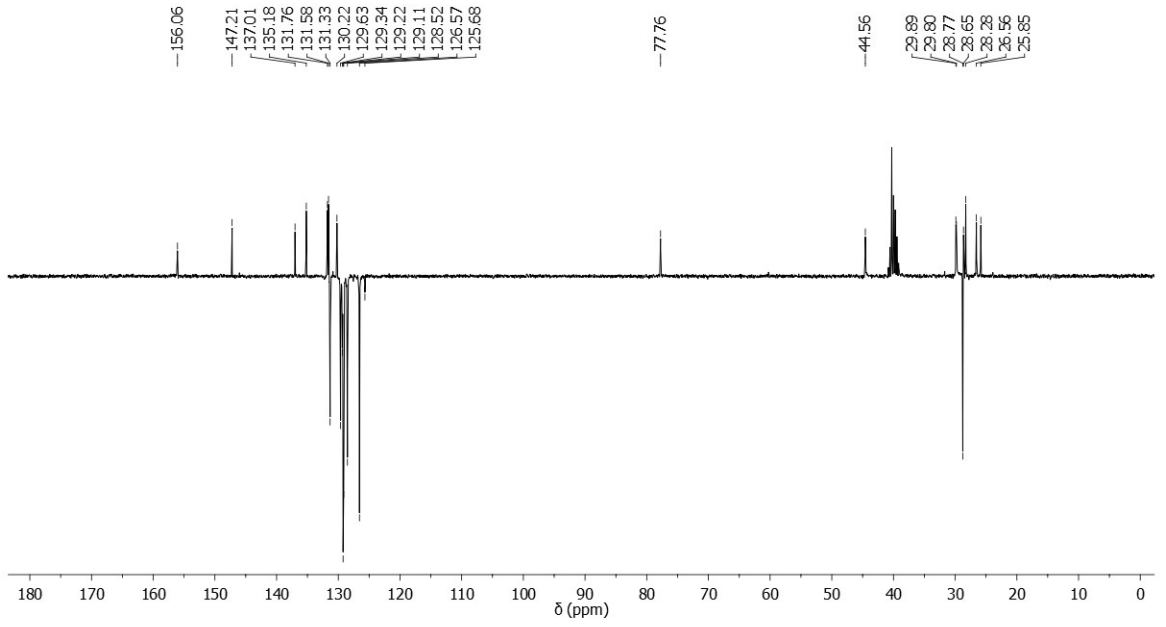


Figure S10. ^{13}C NMR Spectrum, APT (DMSO-*d6*, 75 MHz) of compound **2e.**

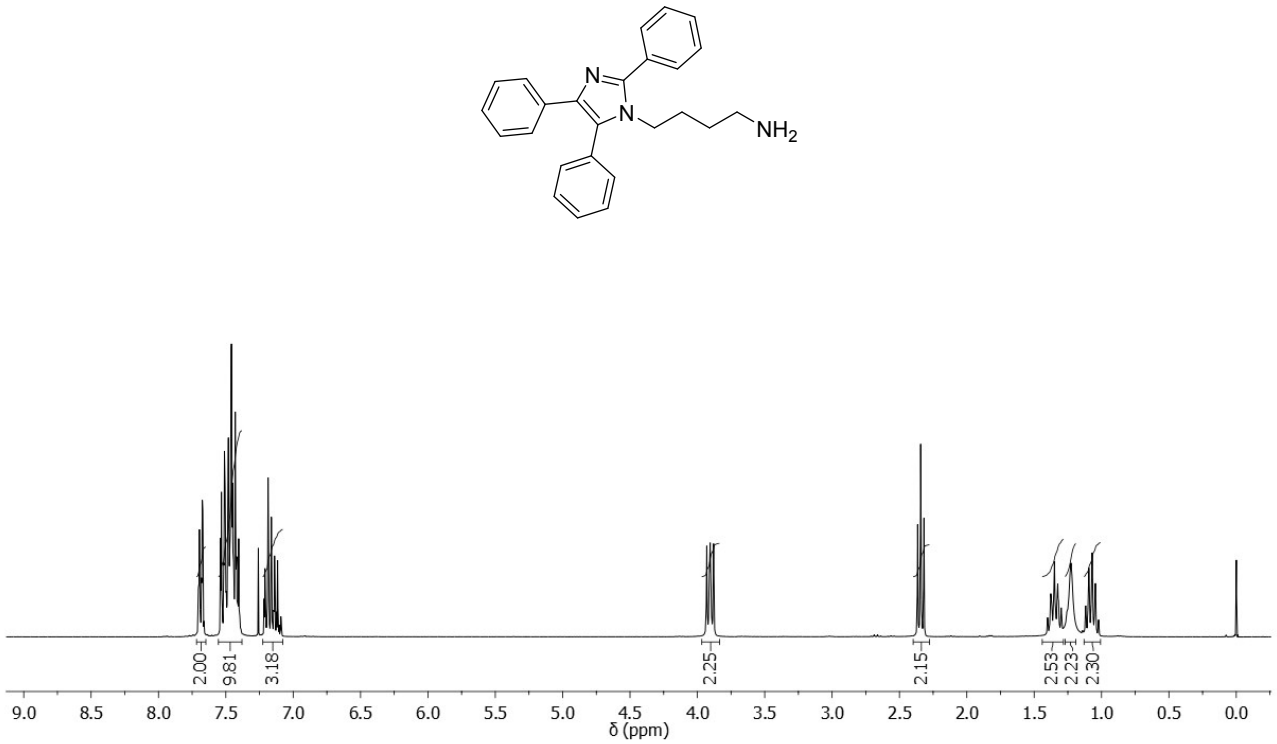


Figure S11. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **3a**.

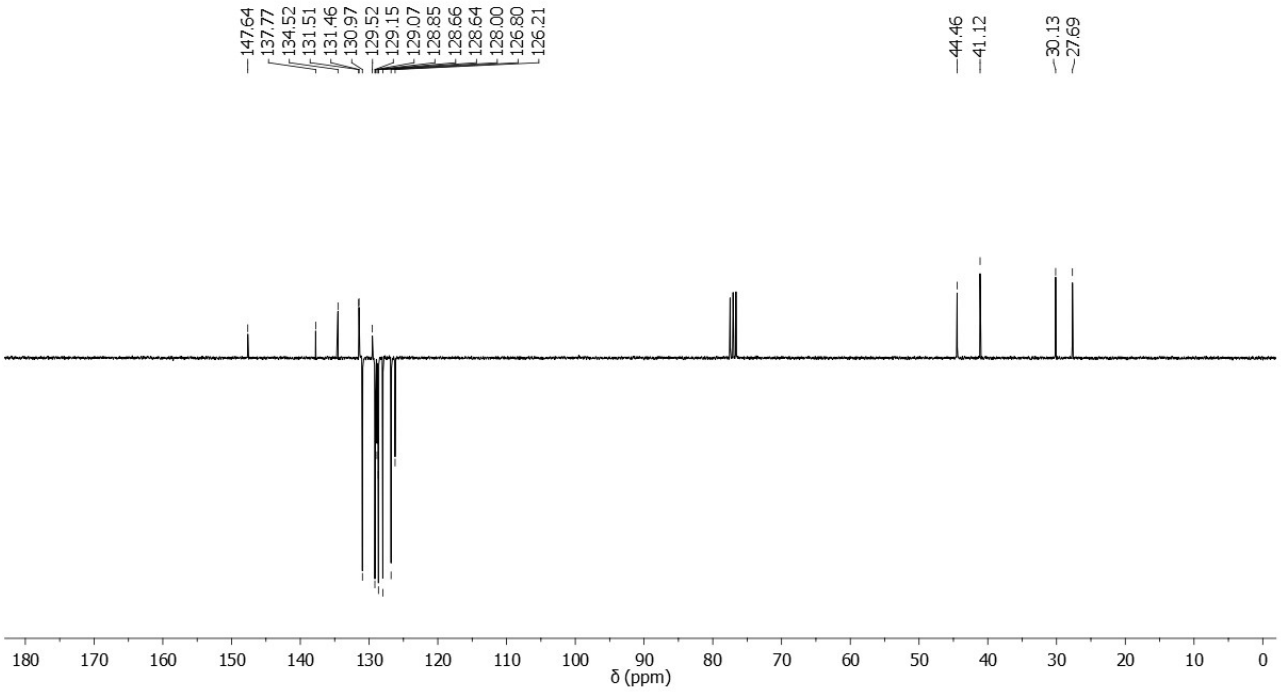


Figure S12. ^{13}C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **3a.**

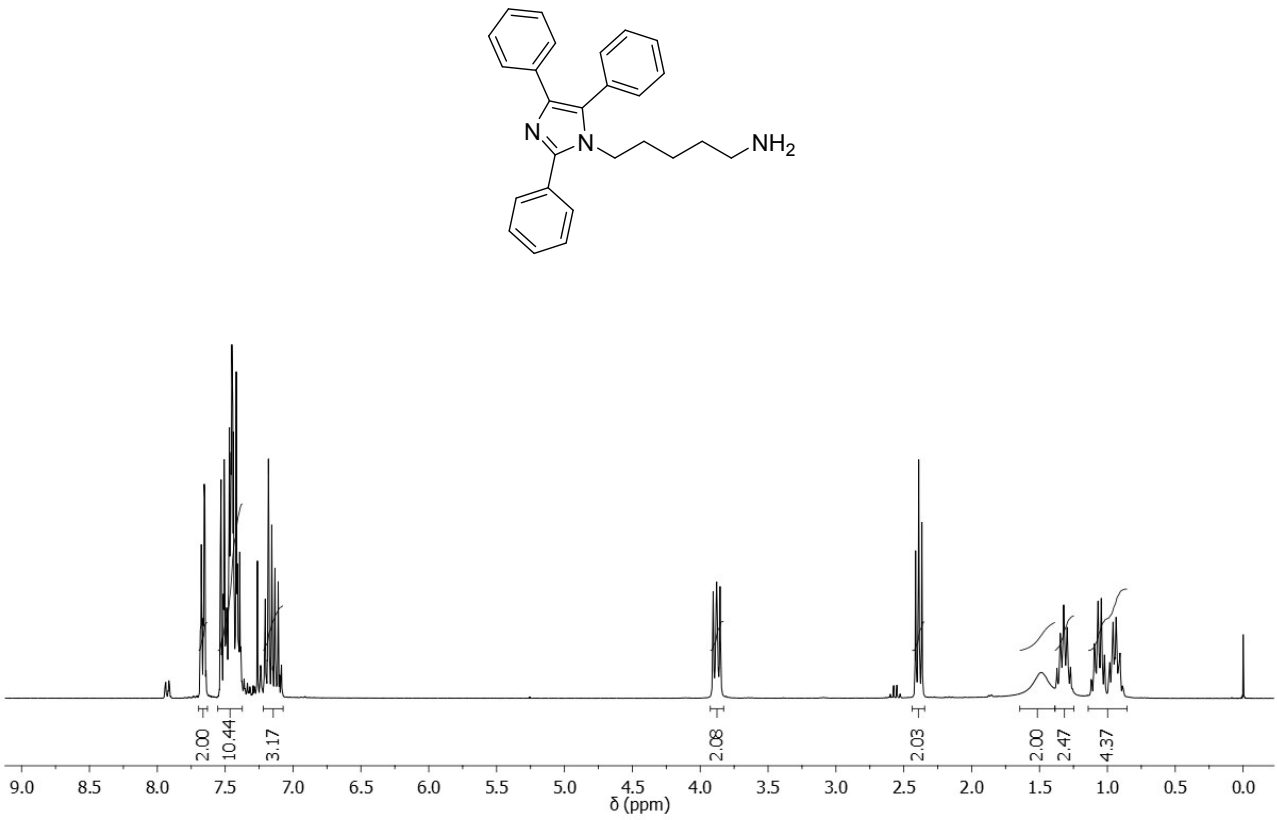


Figure S13. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **3b**.

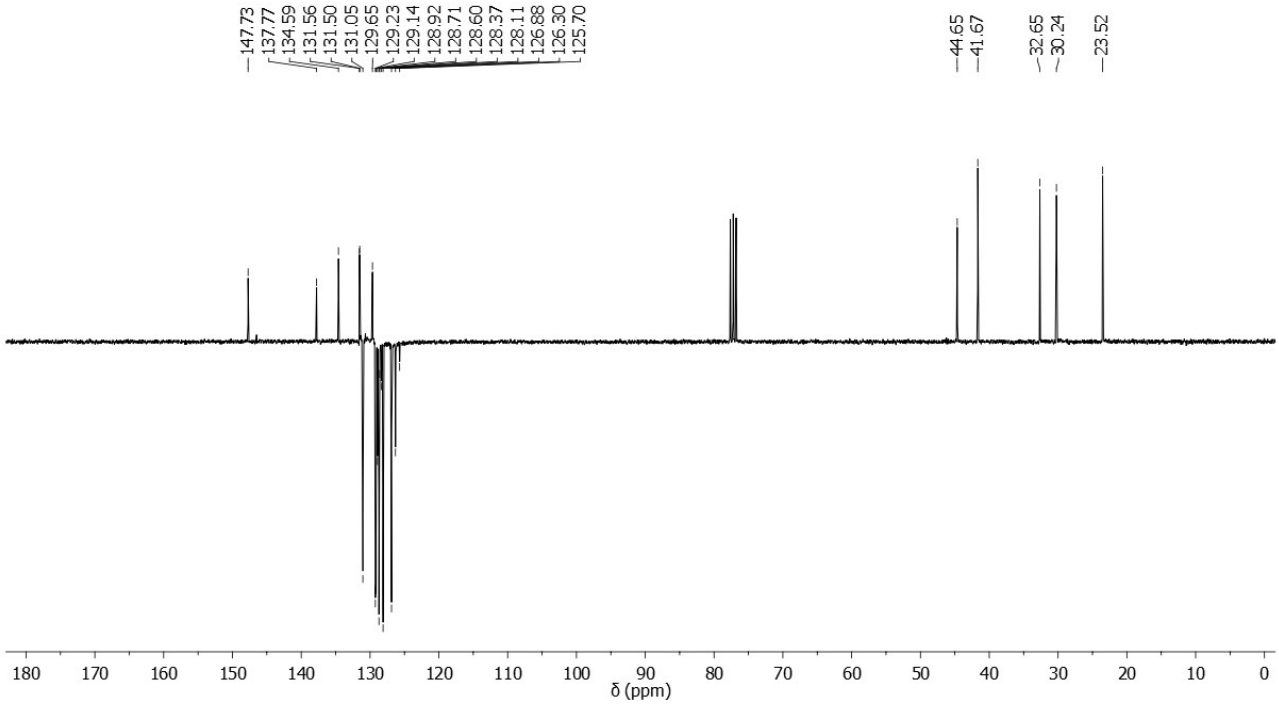
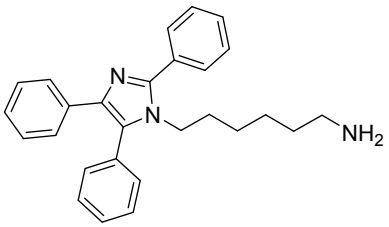


Figure S14. ^{13}C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **3b.**



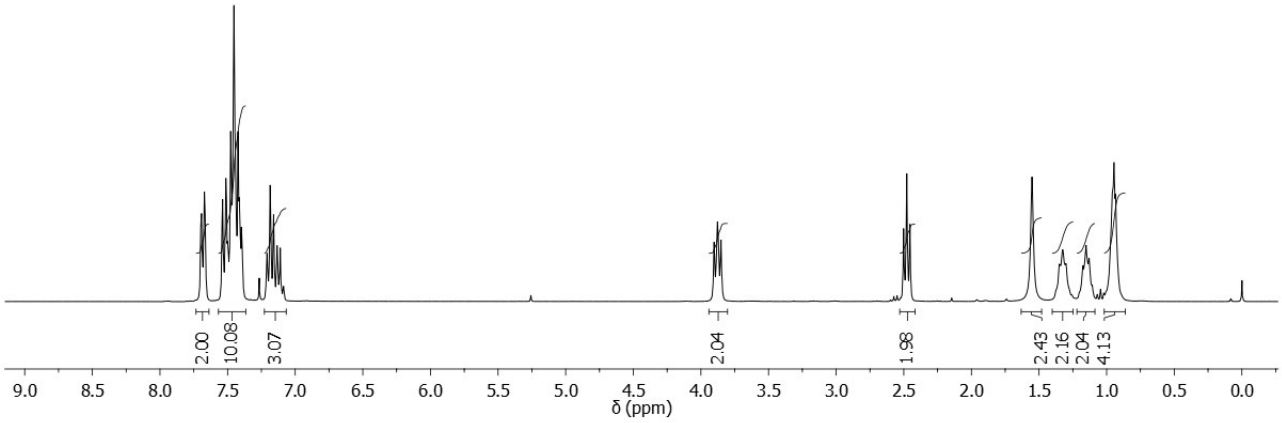


Figure S15. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **3c**.

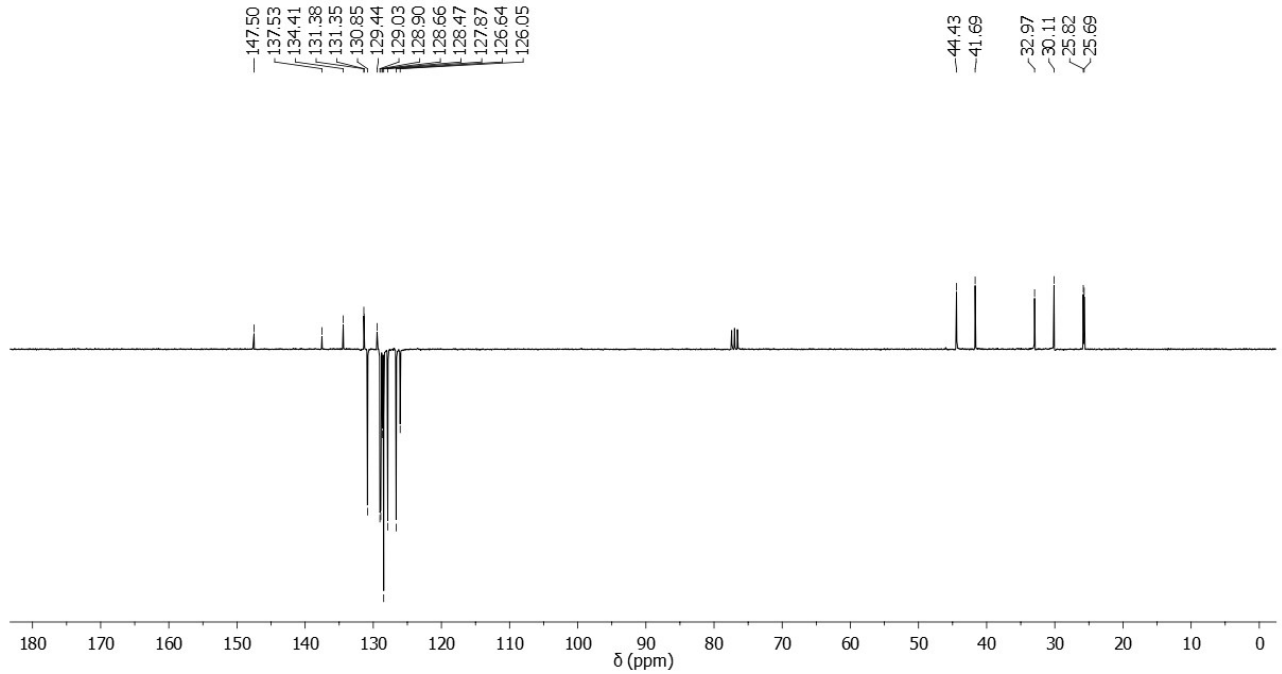


Figure S16. 13C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **3c.**

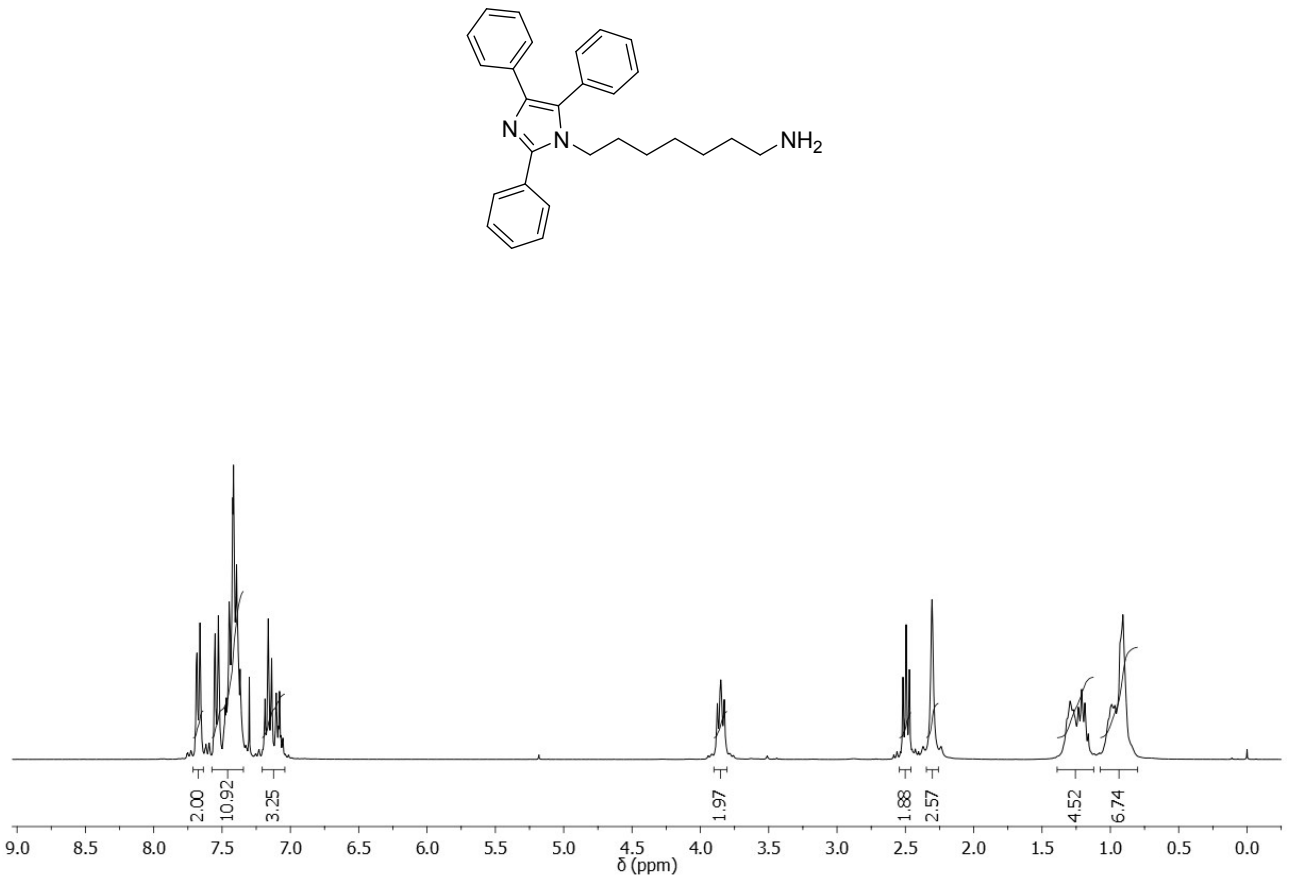


Figure S17. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **3d**.

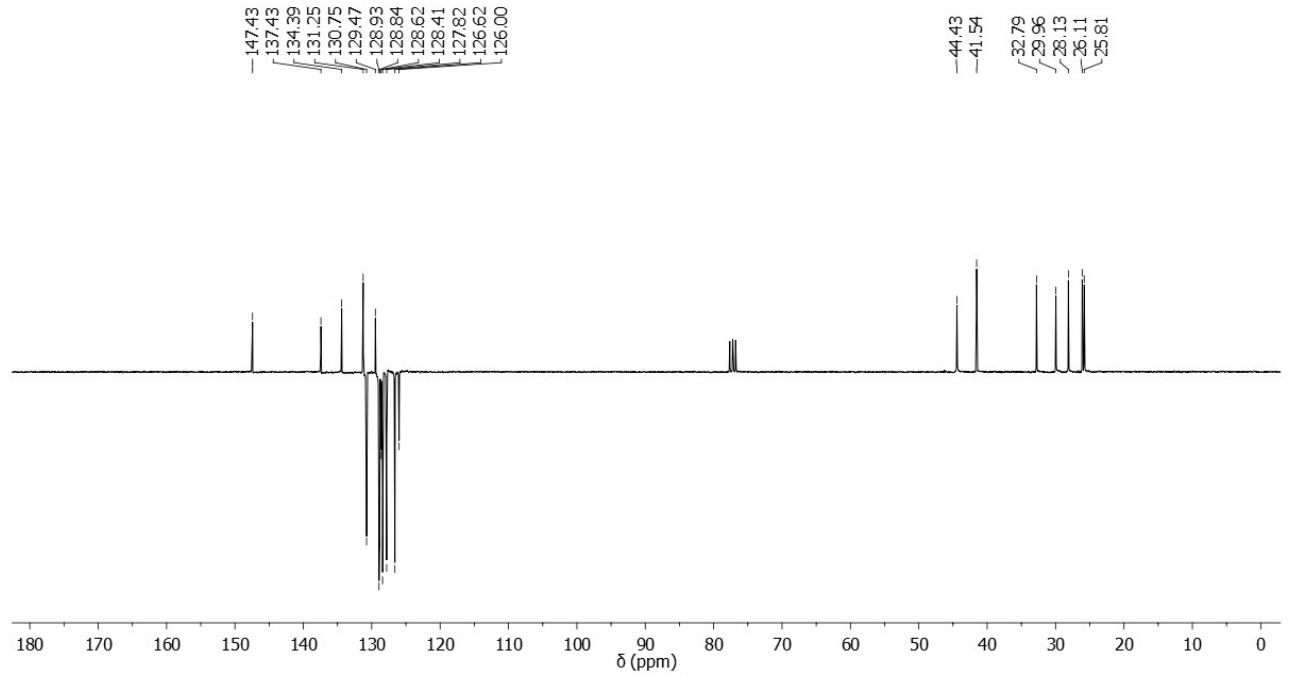


Figure S18. ^{13}C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **3d.**

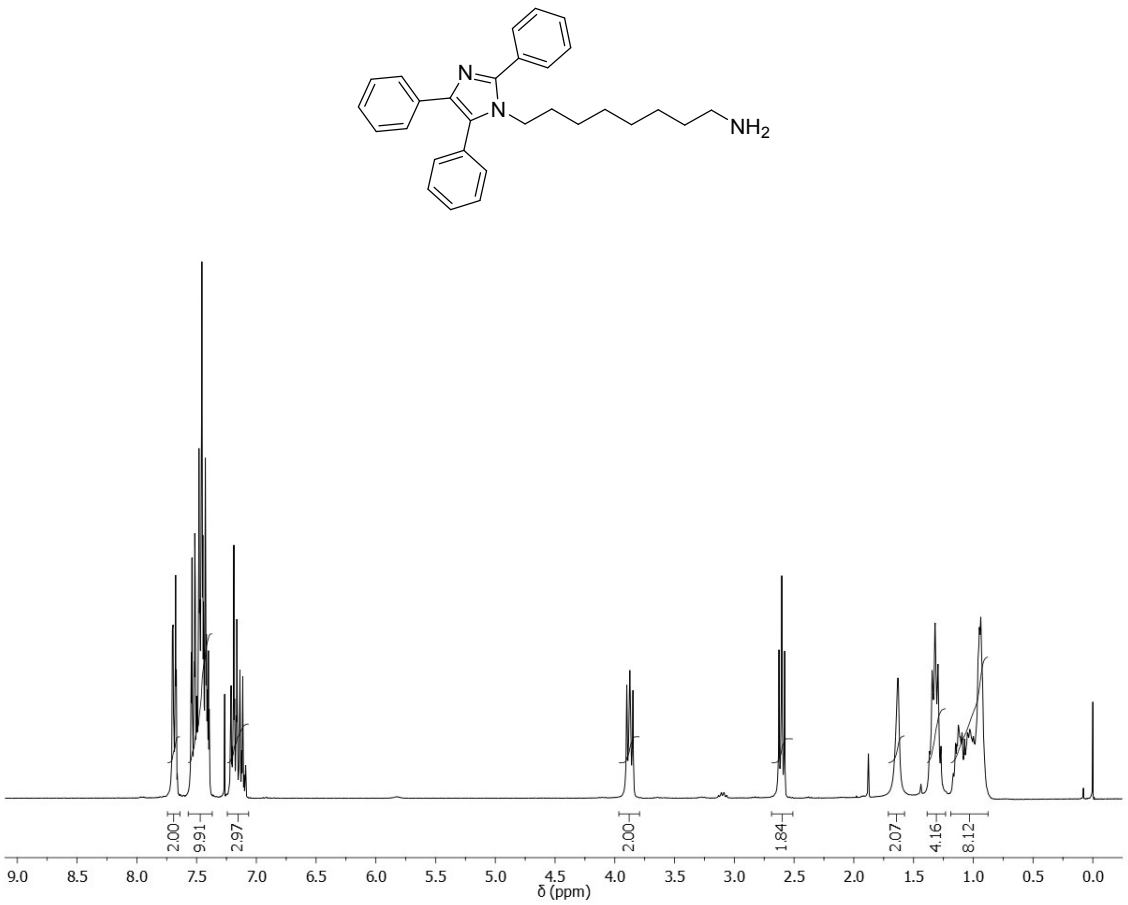


Figure S19. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **3e**.

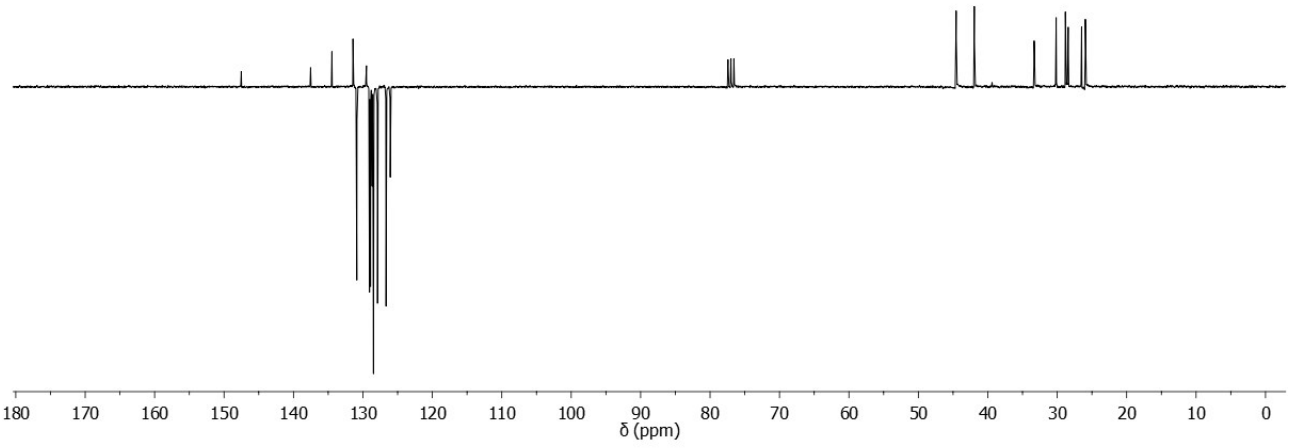


Figure S20. ^{13}C NMR Spectrum, APT ($CDCl_3$, 75 MHz) of compound **3e**.

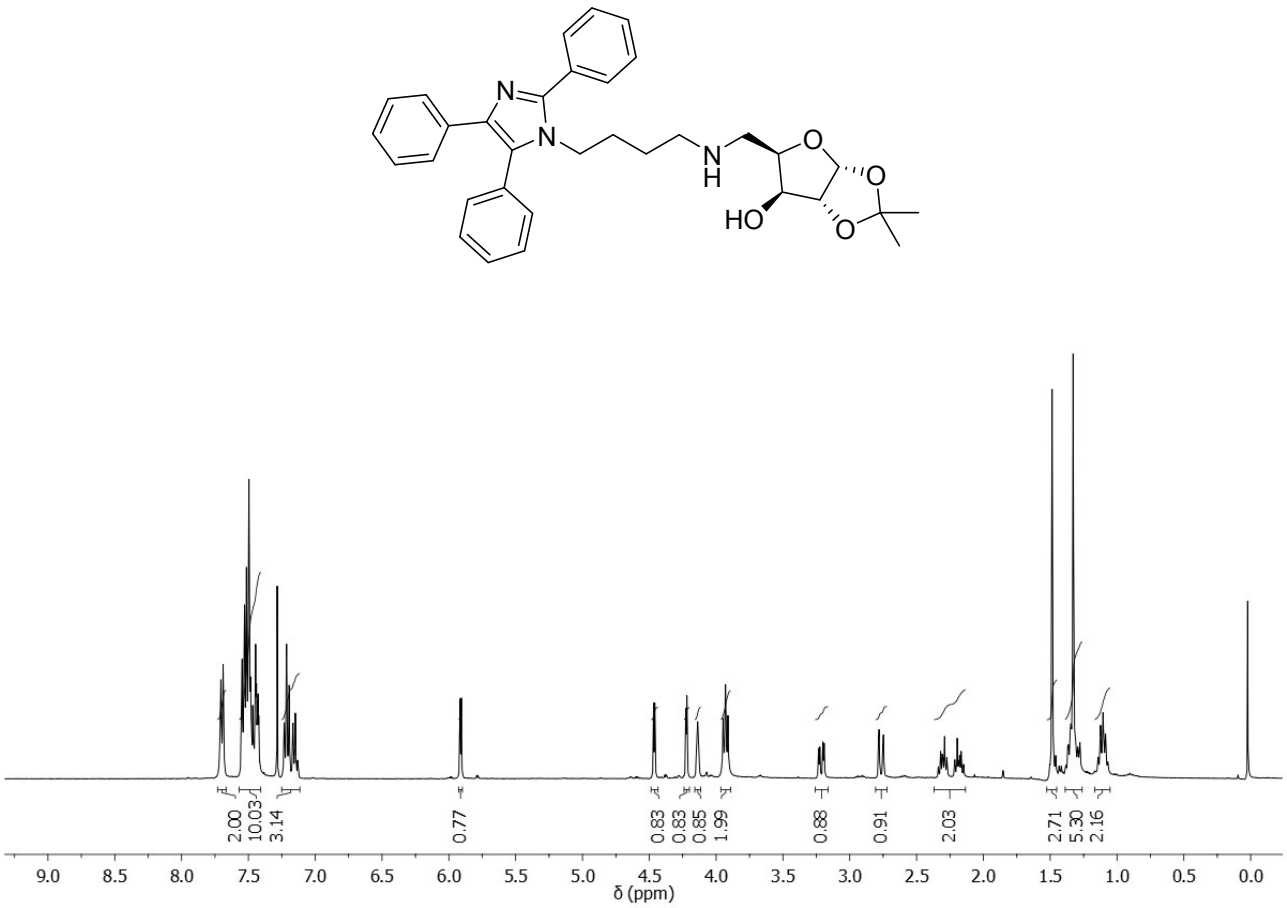


Figure S21. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **7a**.

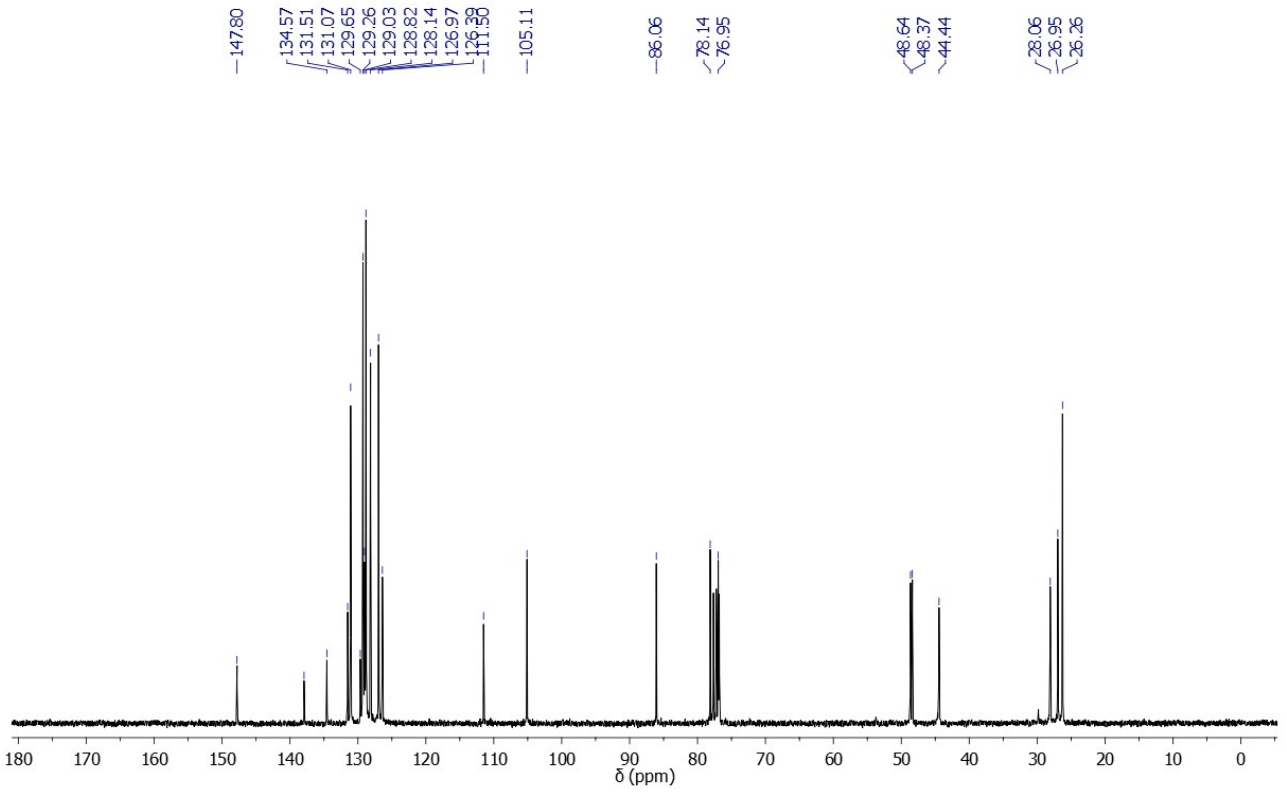


Figure S22. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **7a**.

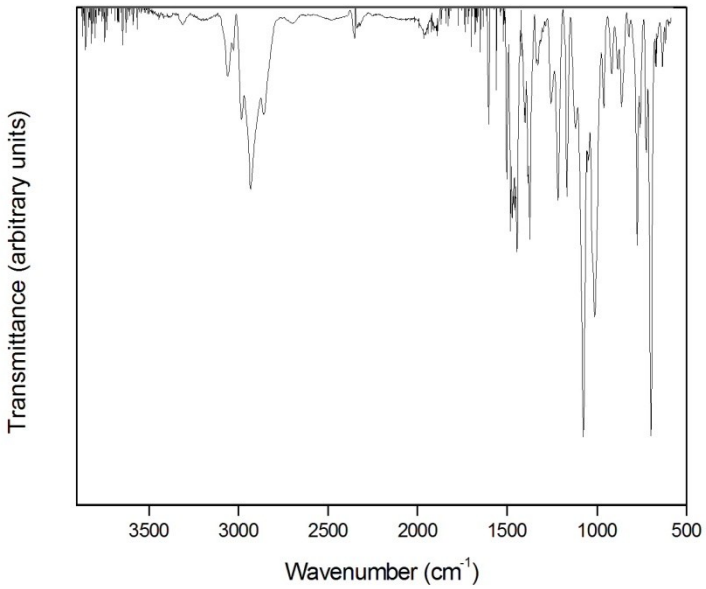


Figure S23. IR Spectrum (KBr pellet) of compound **7a**.

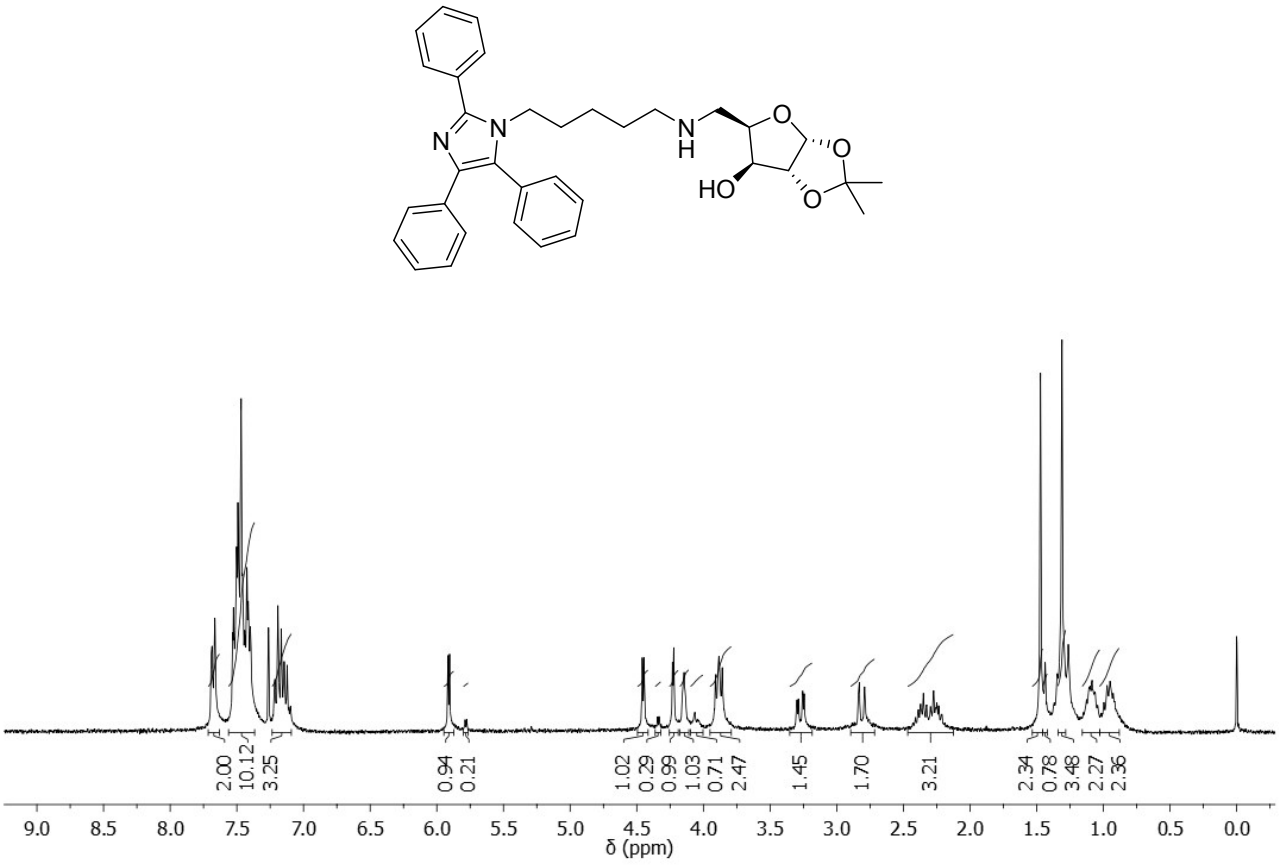


Figure S24. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **7b**.

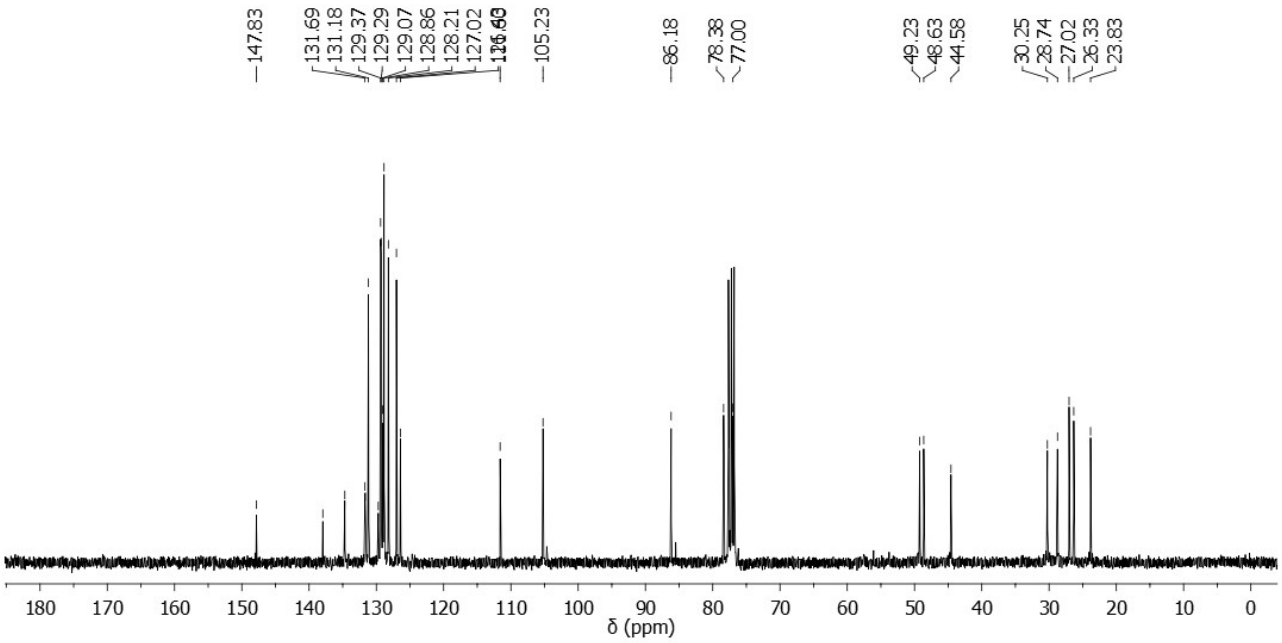


Figure S25. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **7b**.

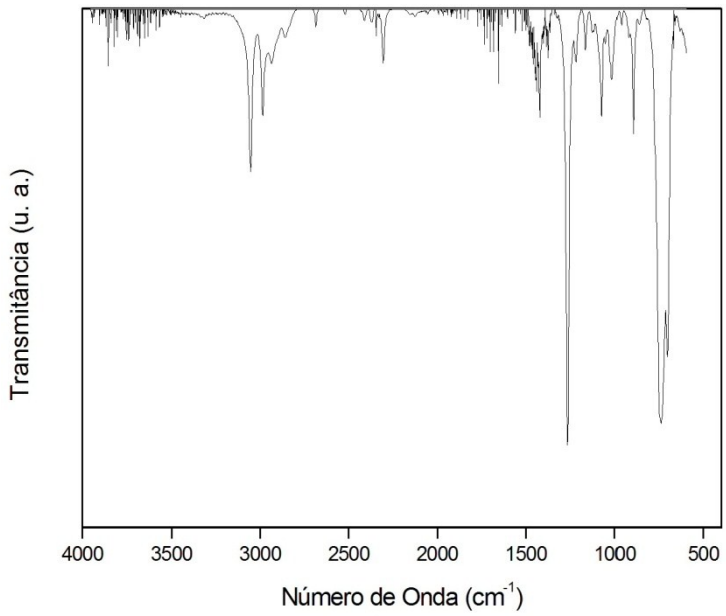


Figure S26. IR Spectrum (KBr pellet) of compound **7b**.

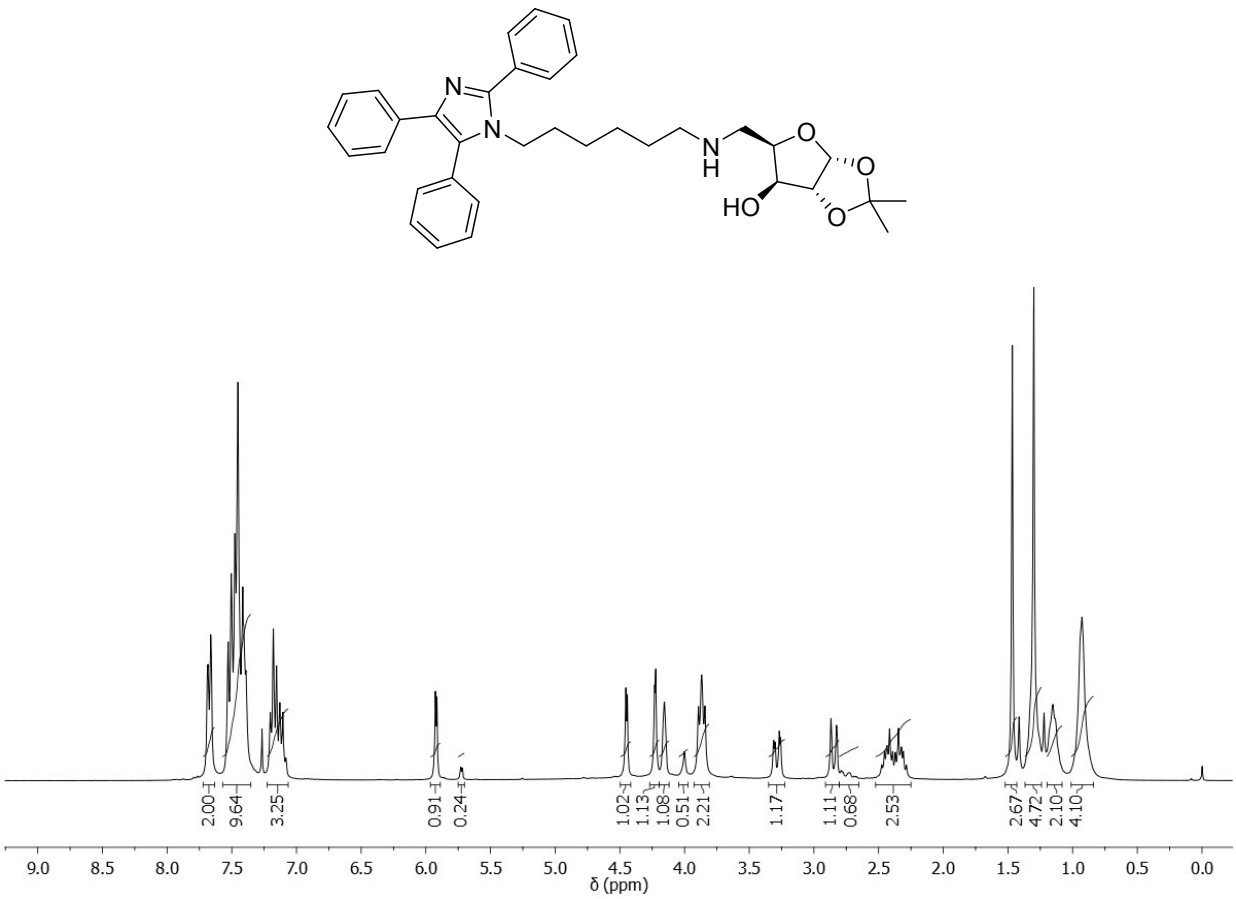


Figure S27. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **7c**.

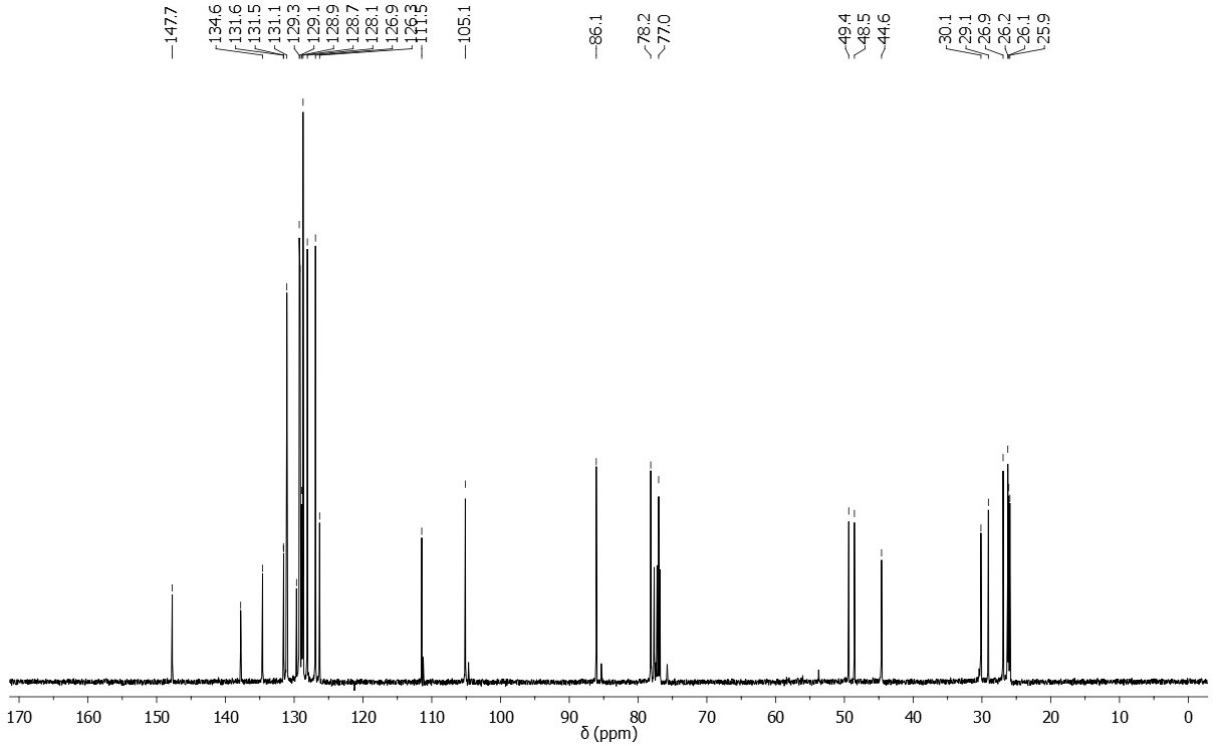


Figure S28. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **7c**.

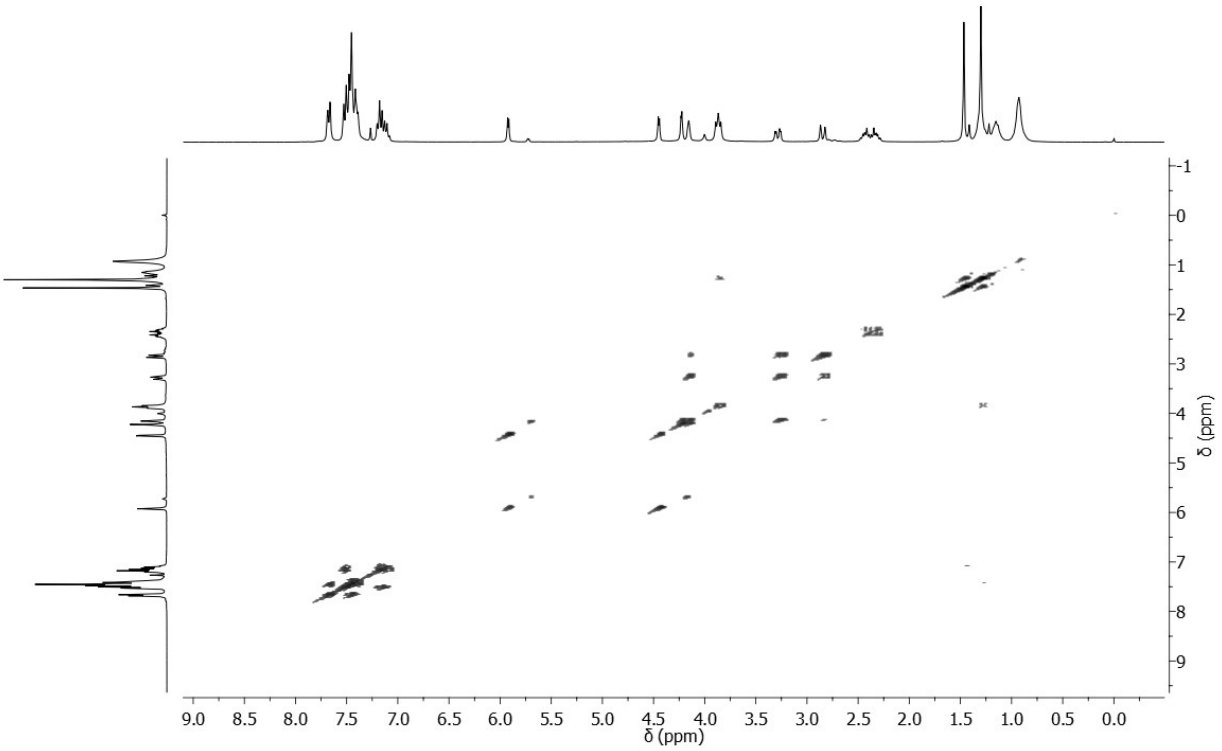


Figure S29. 2D COSY NMR Spectrum of compound **7c**.

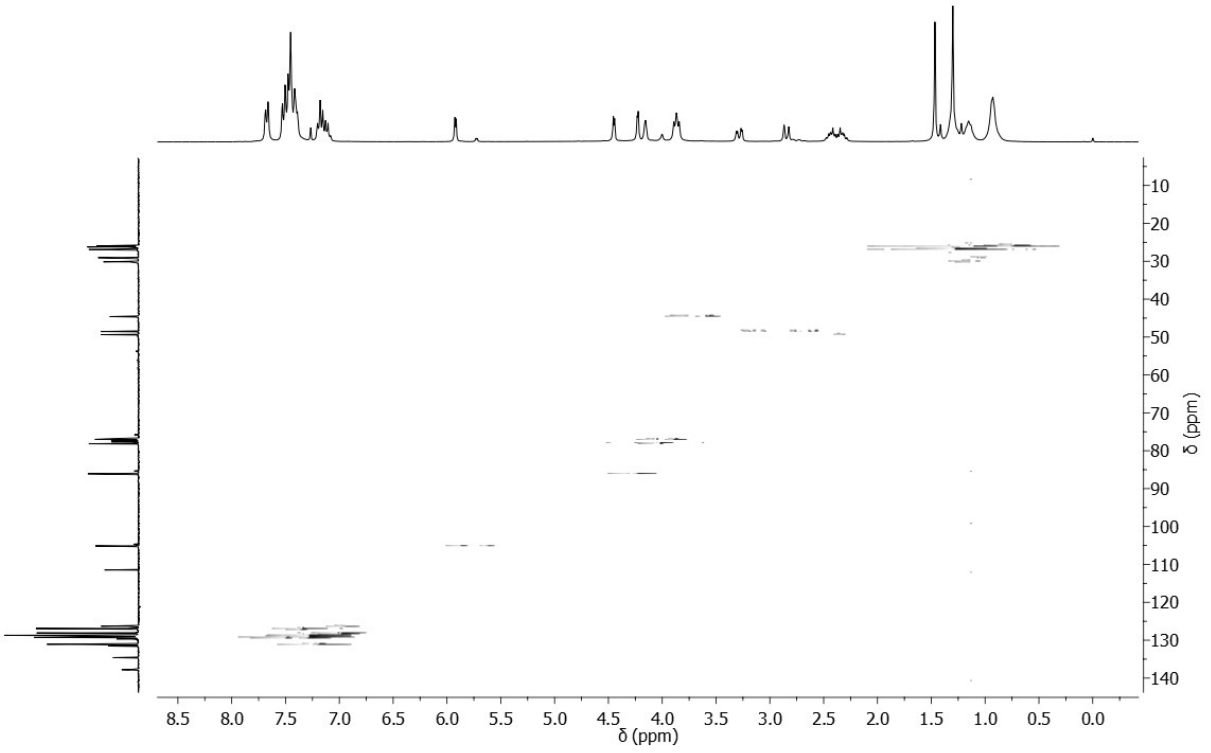


Figure S30. HMQC NMR Spectrum of compound **7c**.

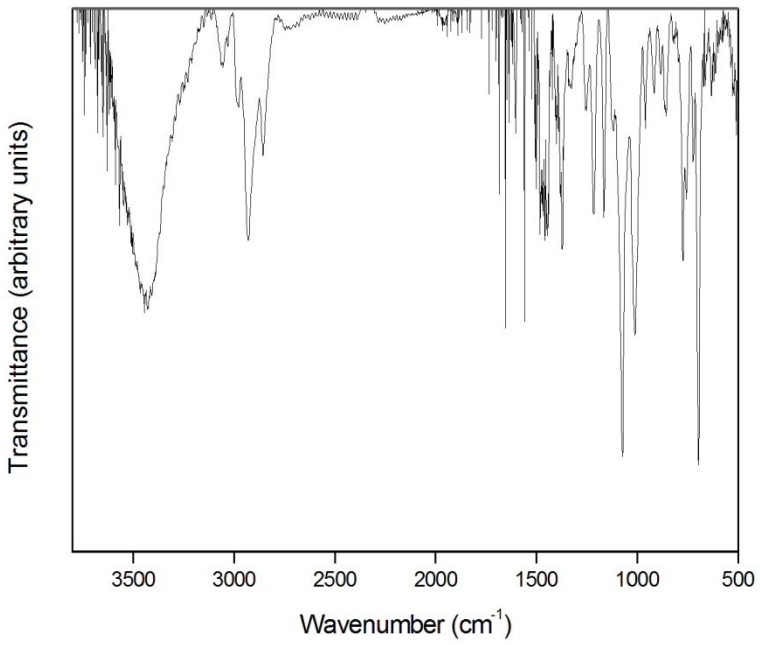


Figure S31. IR Spectrum (KBr pellet) of compound **7c**.

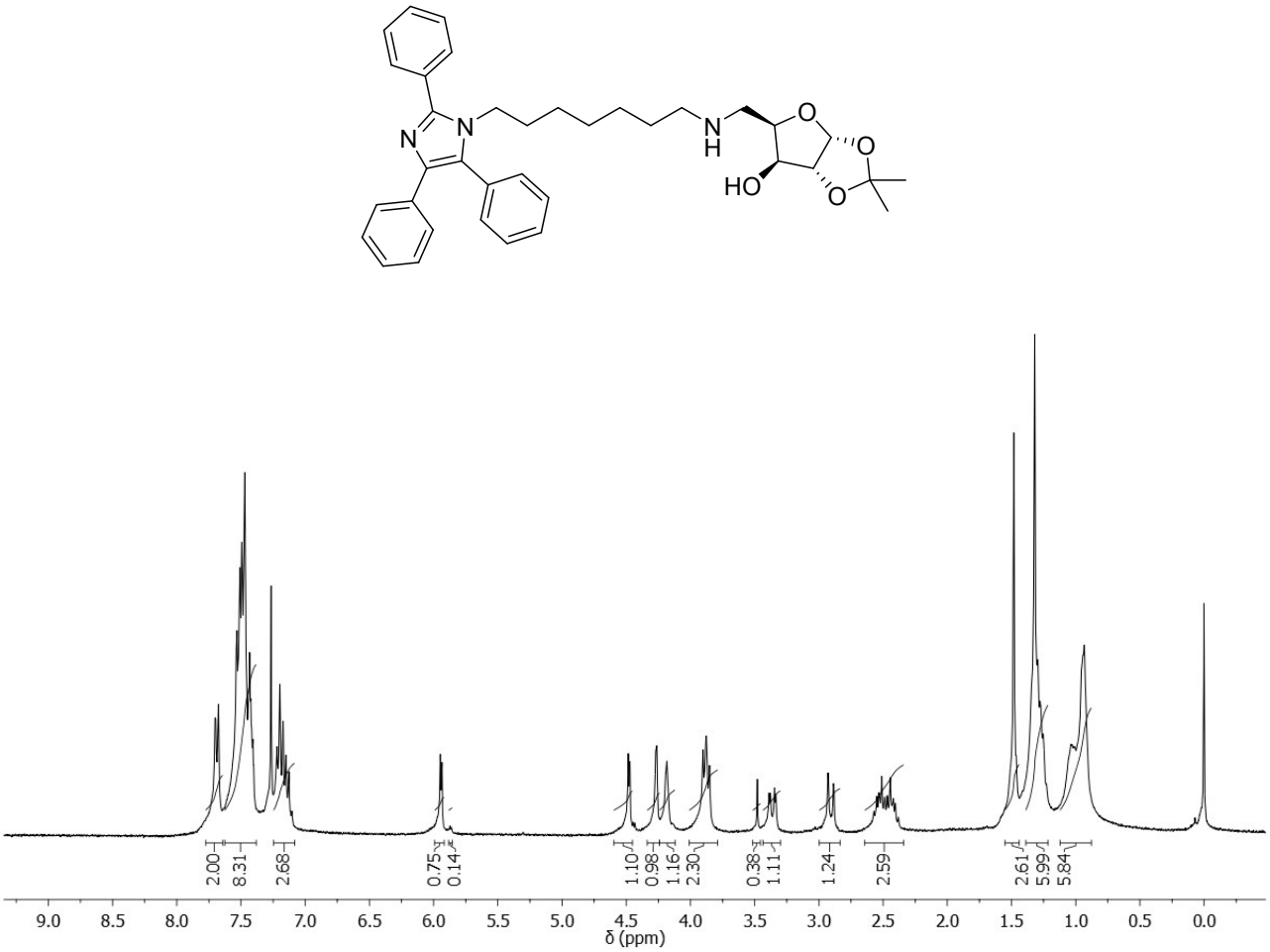


Figure S32. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **7d**.

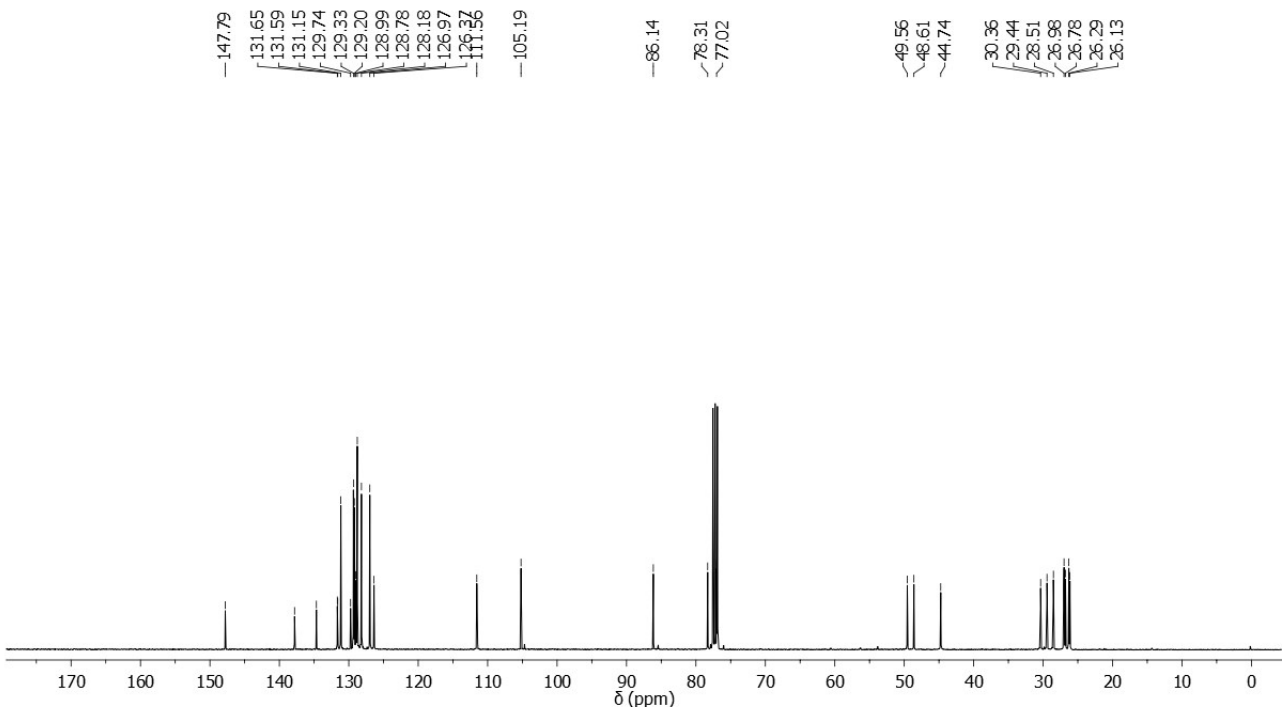


Figure S33. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **7d**.

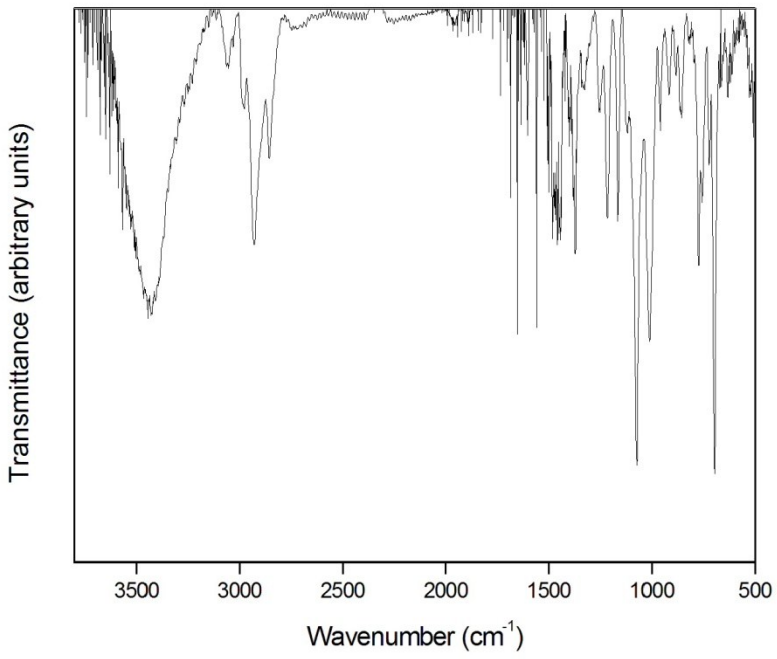


Figure S34. IR Spectrum (KBr pellet) of compound **7d**.

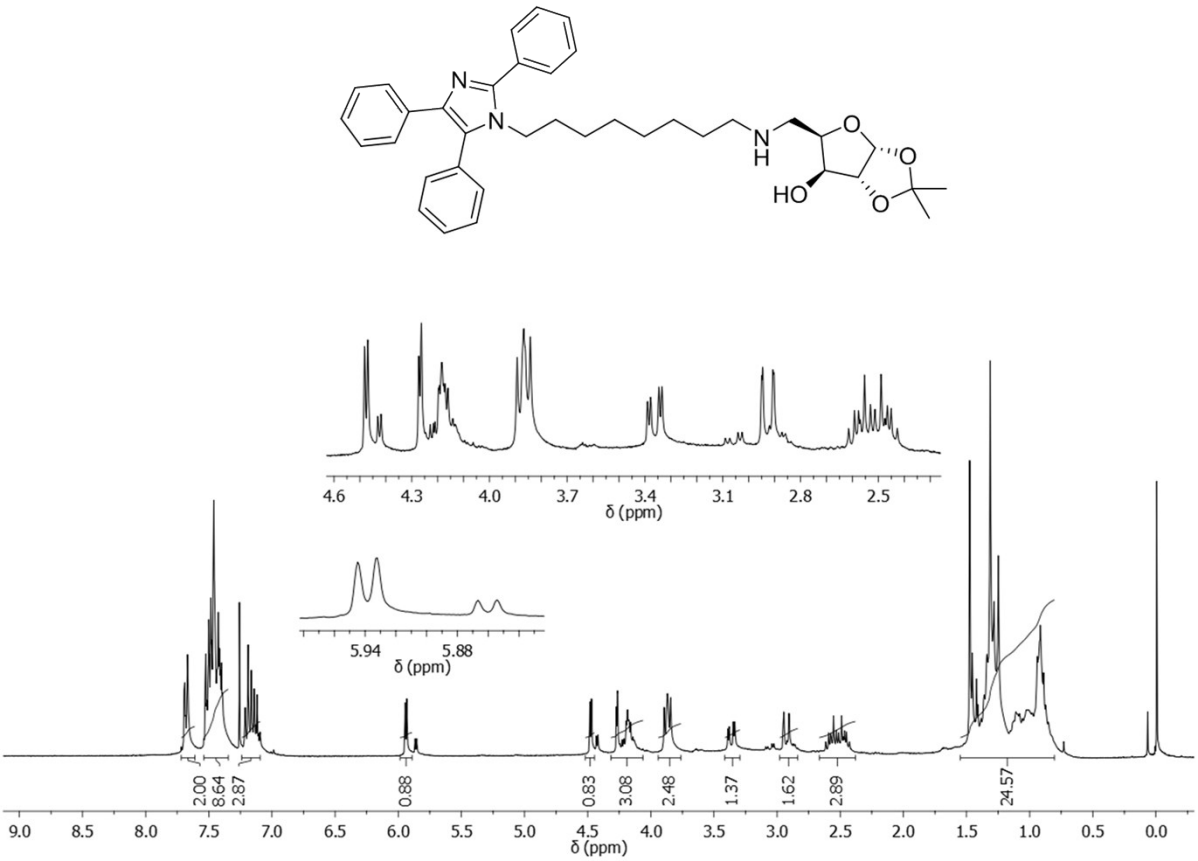


Figure S35. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **7e**.

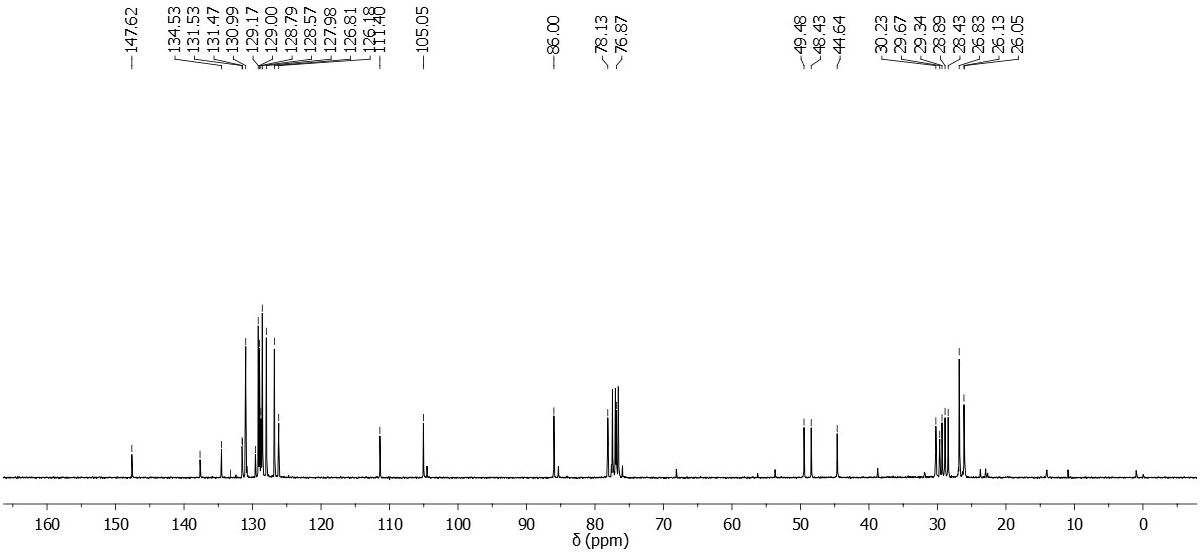


Figure S36. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **7e**.

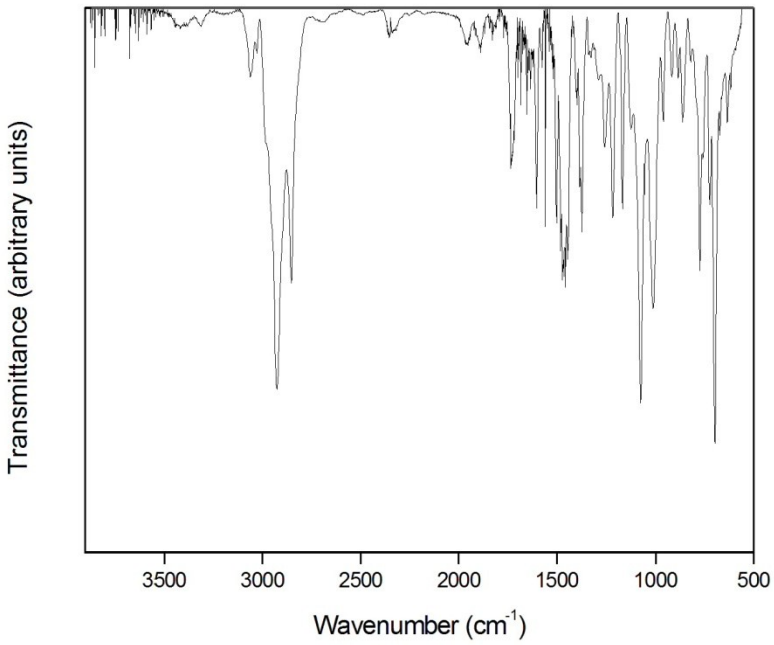


Figure S37. IR Spectrum (KBr pellet) of compound **7e**.

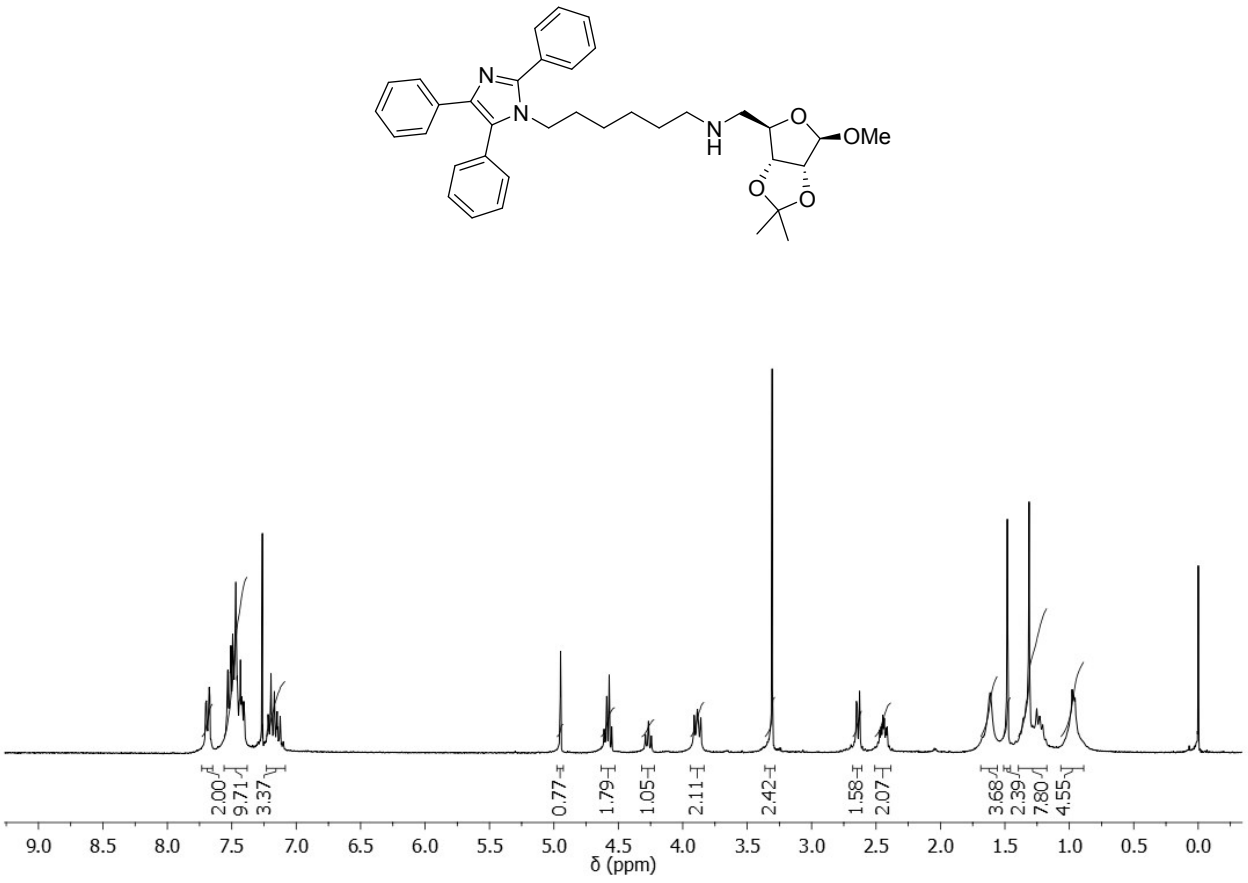


Figure S38. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **8a**.

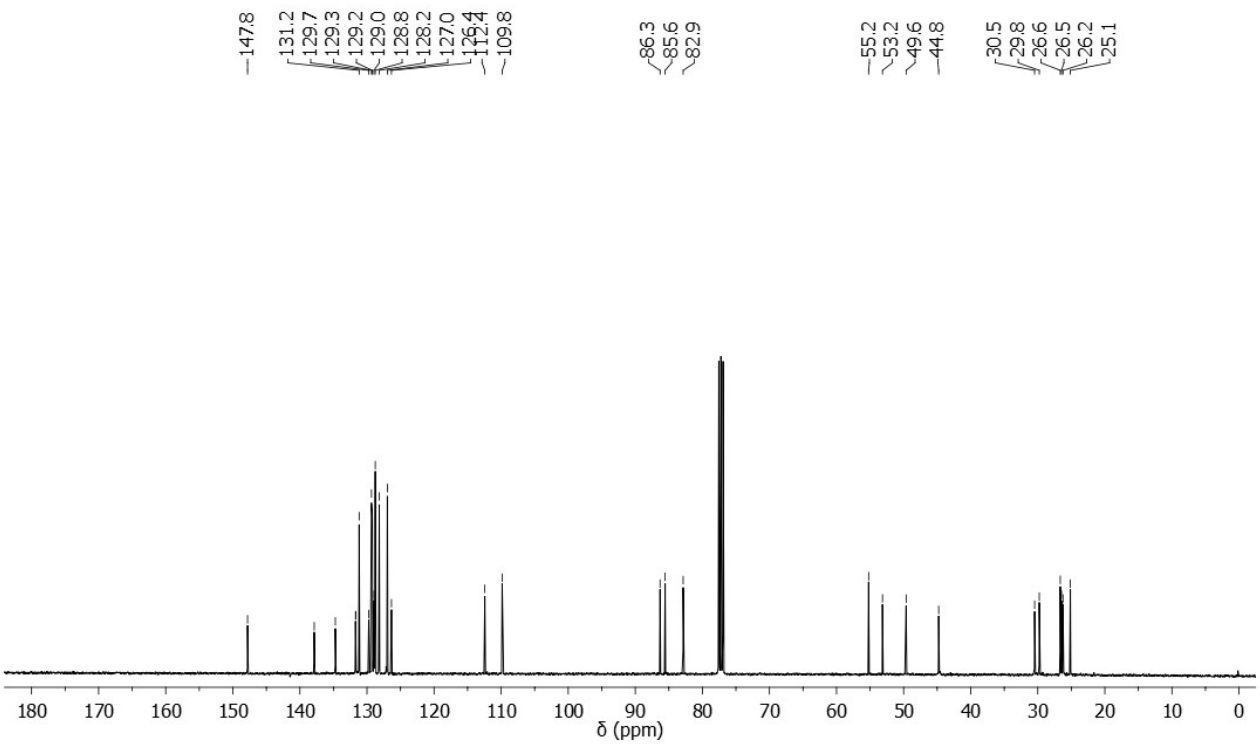


Figure S39. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **8a**.

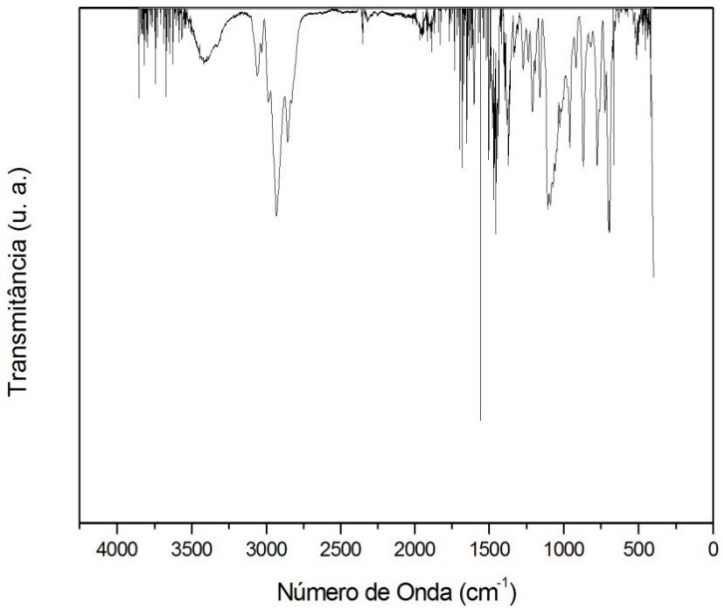
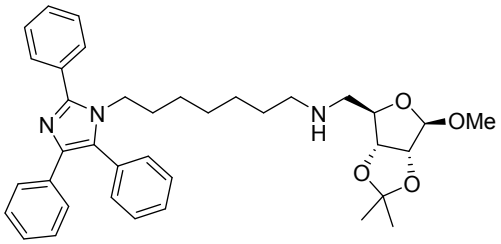


Figure S40. IR Spectrum (KBr pellet) of compound **8a**.



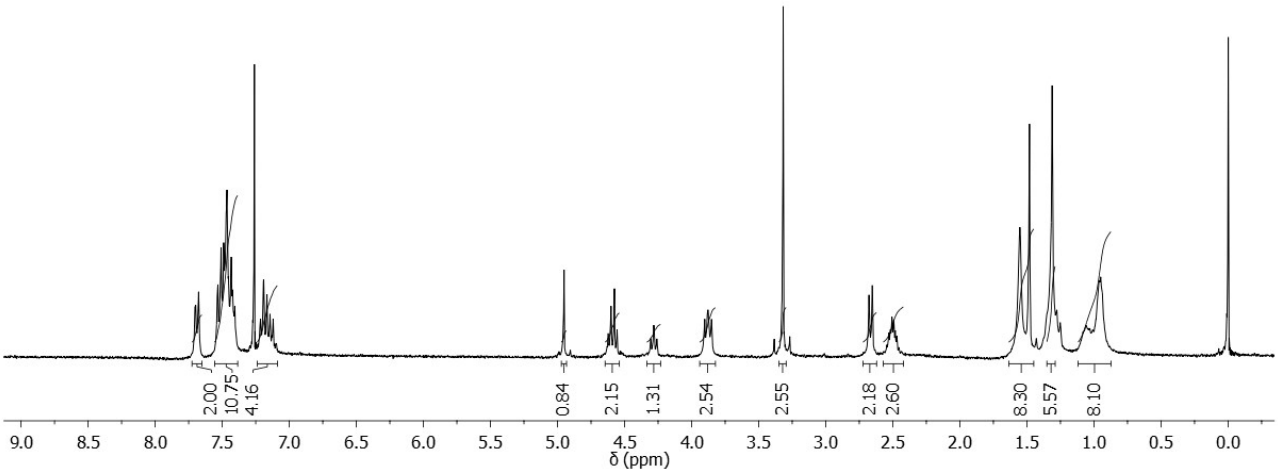


Figure S41. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **8b**.

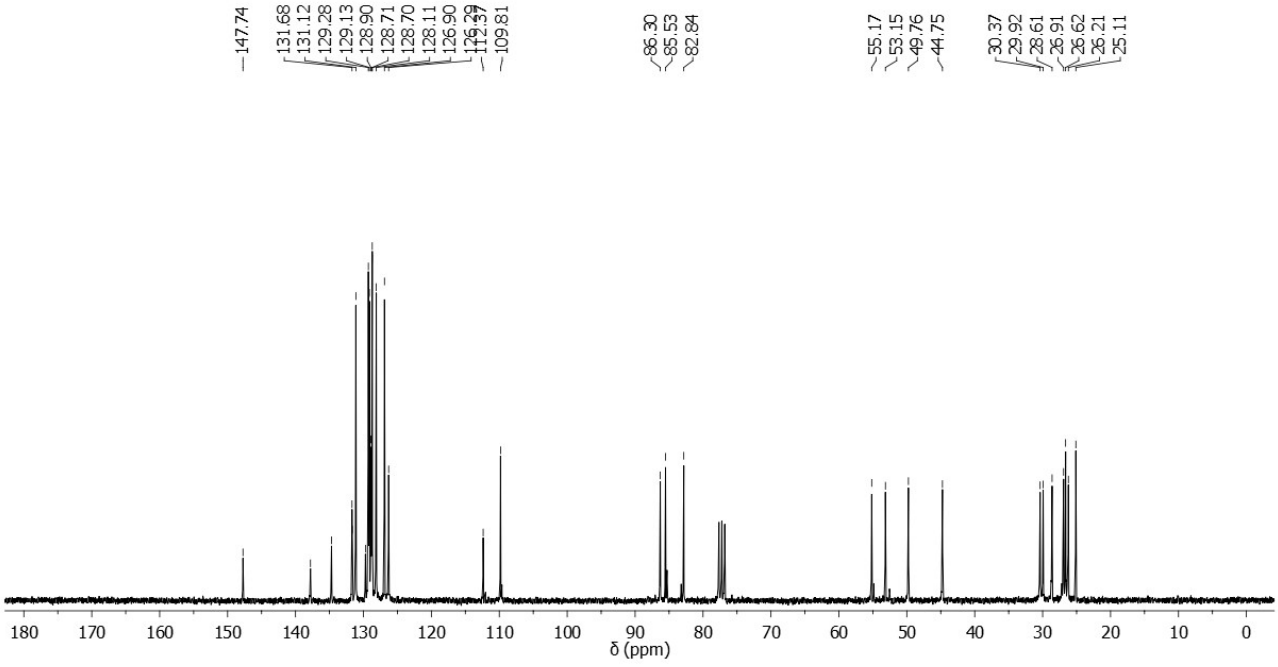


Figure S42. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **8b**.

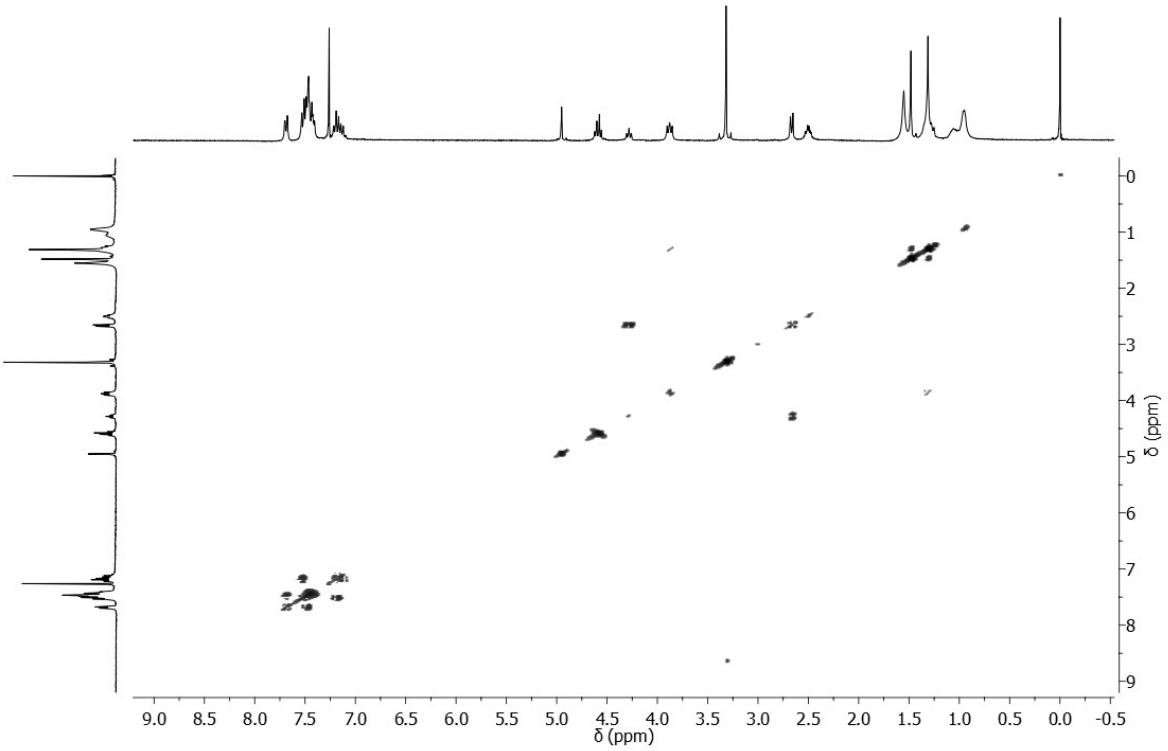


Figure S43. 2D COSY NMR Spectrum of compound **8b**.

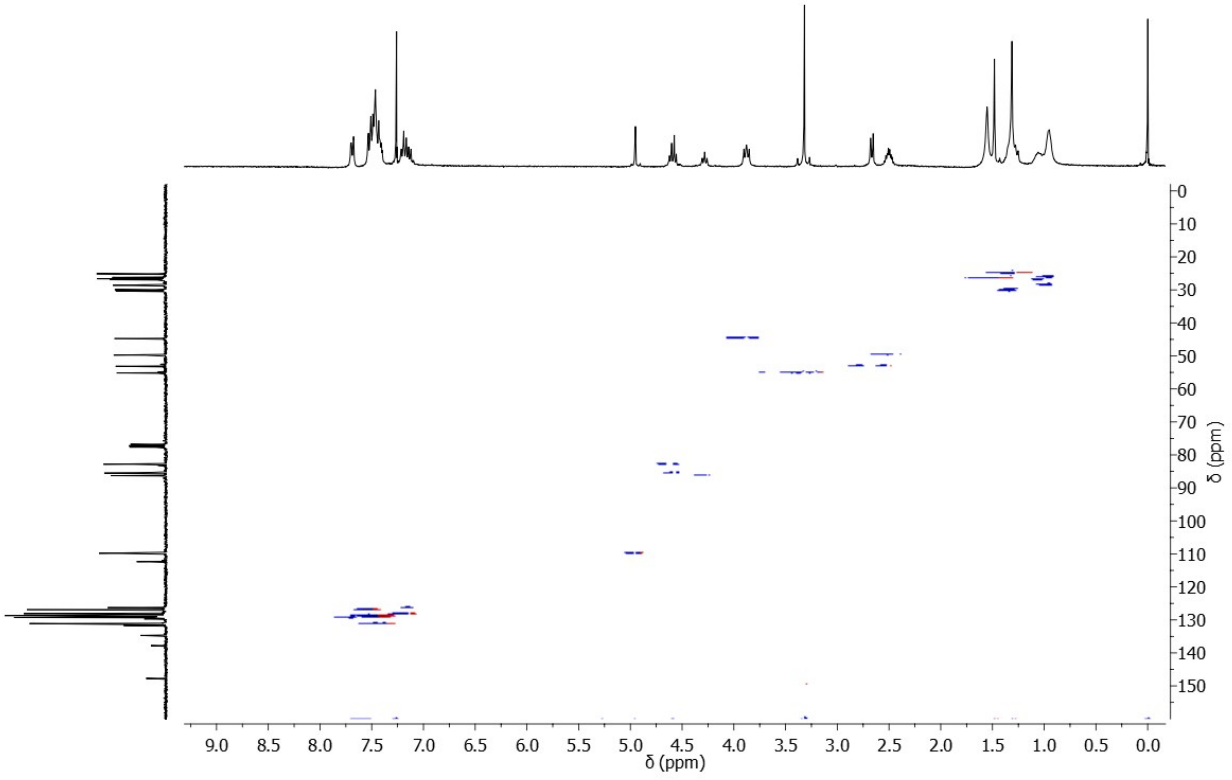


Figure S44. HMQC NMR Spectrum of compound **8b**.

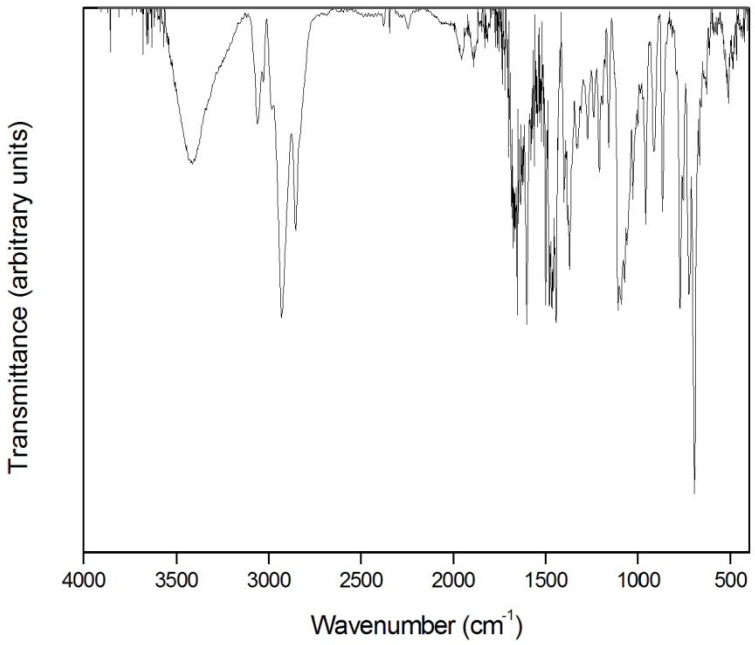
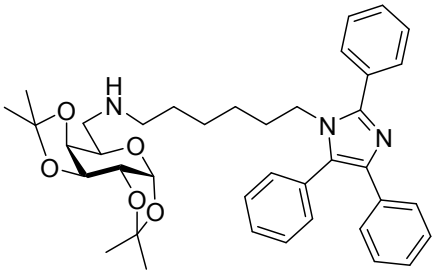


Figure S45. IR Spectrum (KBr pellet) of compound **8b**.



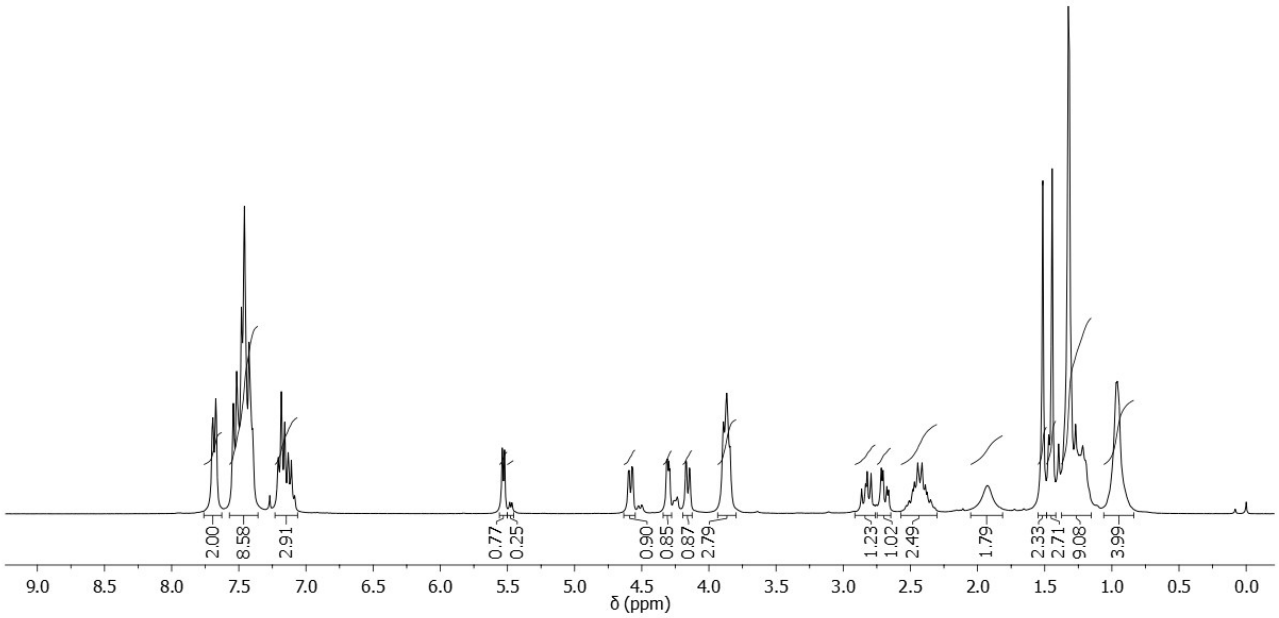


Figure S46. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **9a**.

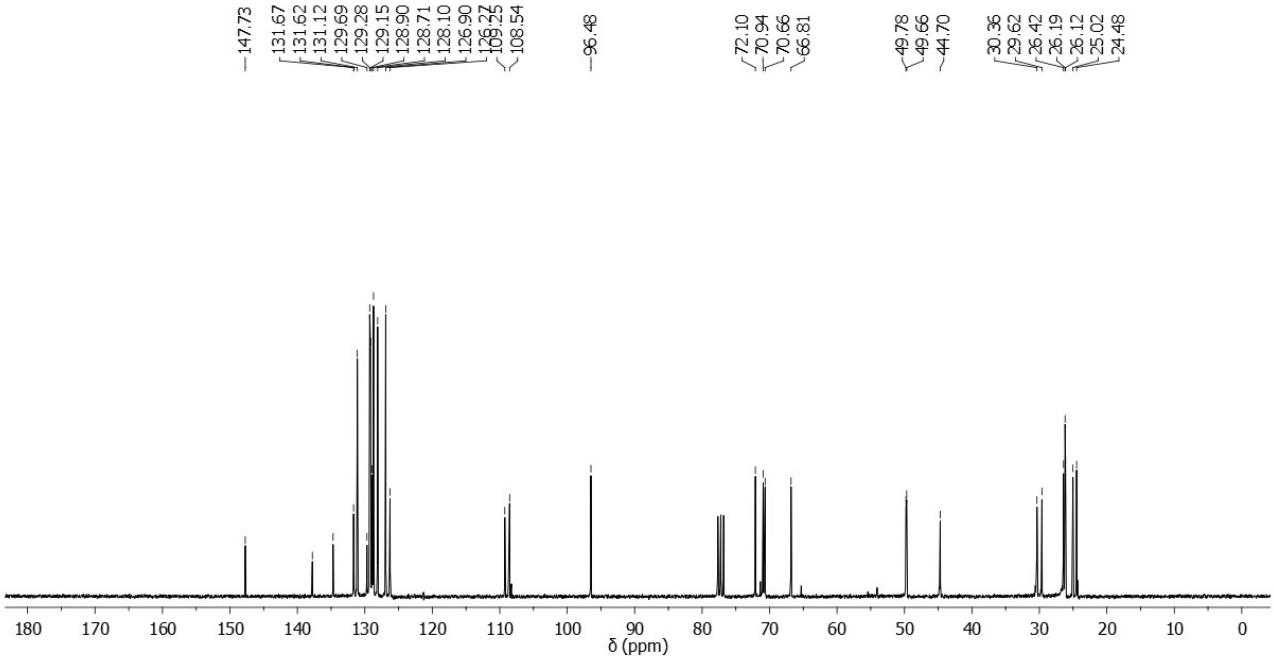


Figure S47. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **9a**.

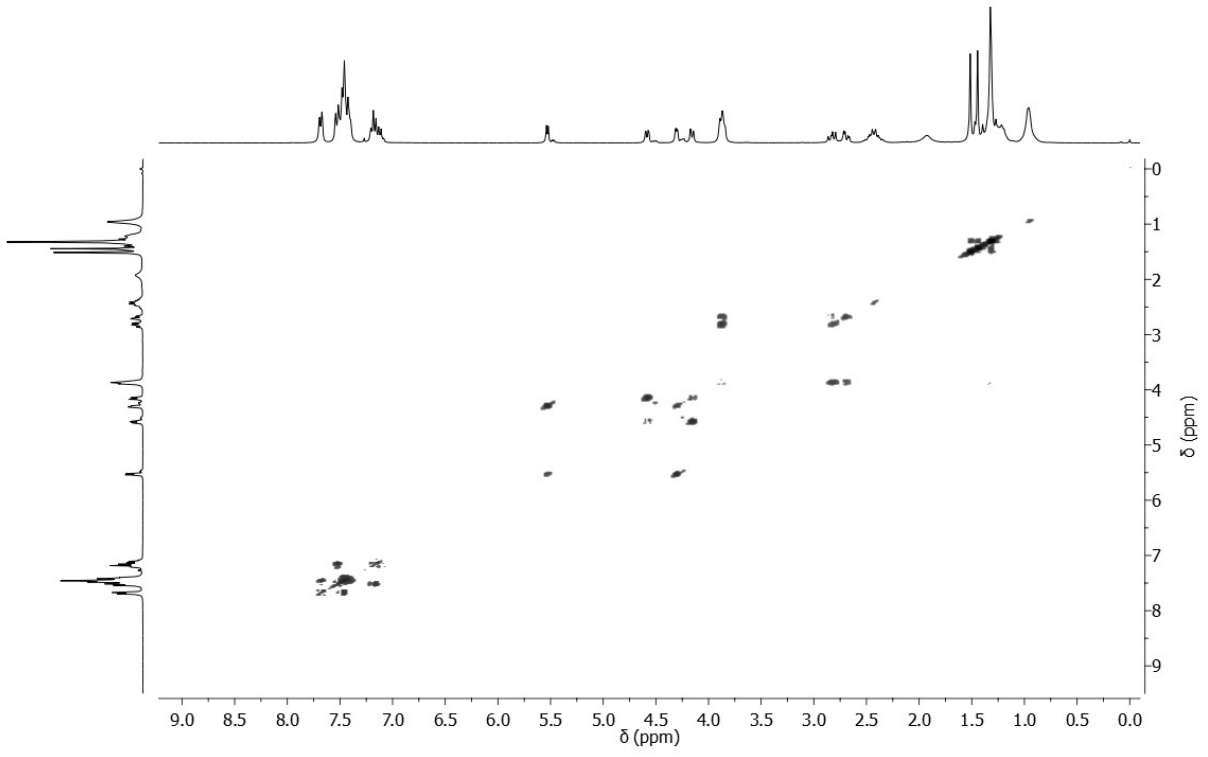


Figure S48. 2D COSY NMR Spectrum of compound **9a**.

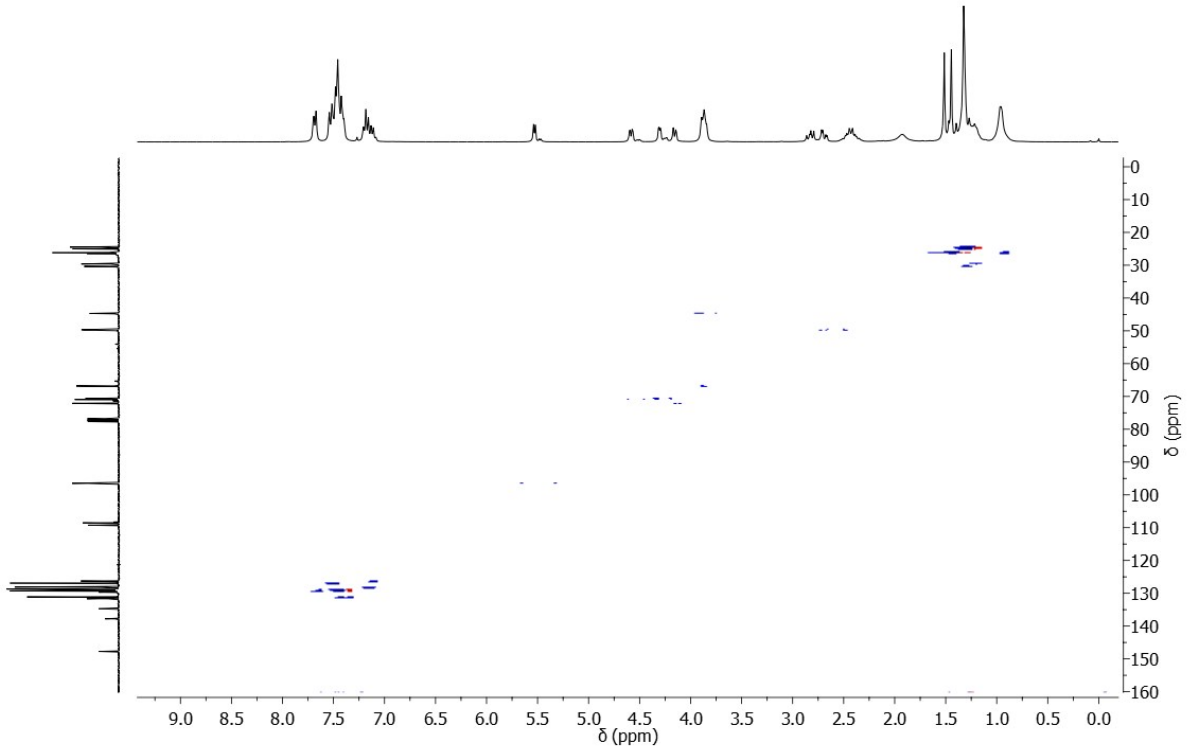


Figure S49. HMQC NMR Spectrum of compound **9a**.

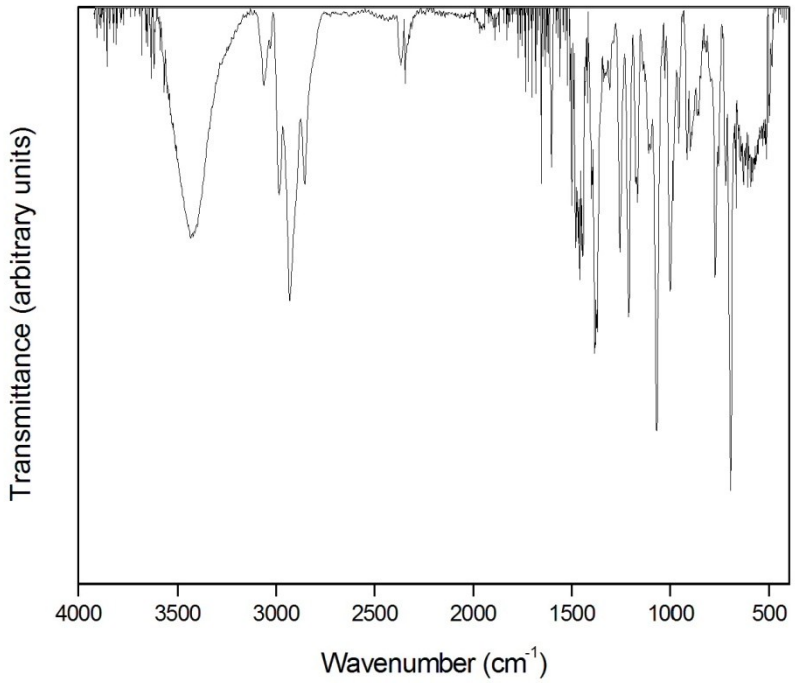


Figure S50. IR Spectrum (KBr pellet) of compound **9a**.

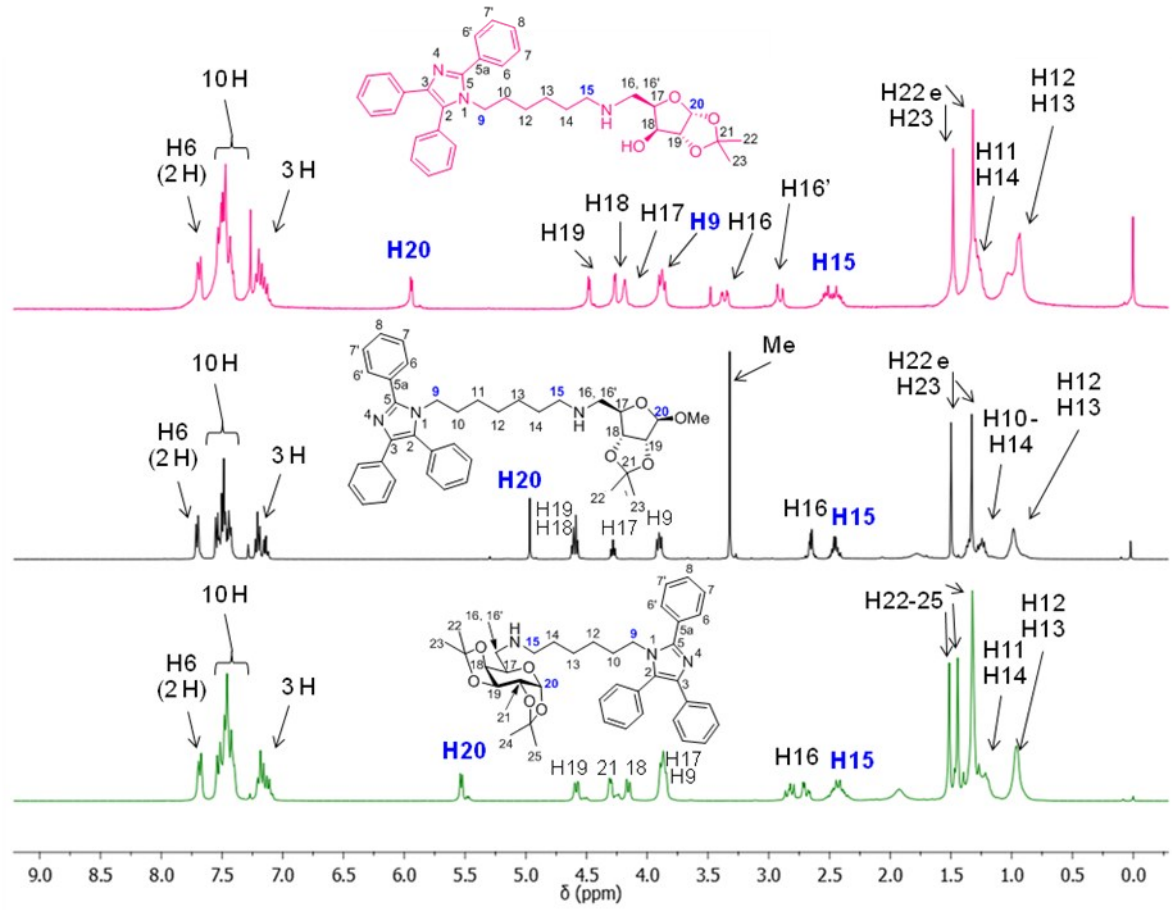


Figure S51. Comparison of ^{1}H-NMR spectra ($CDCl_3$, 300 MHz) of compounds **7c** (D-xylose), **8b** (D-ribose) and **9a** (D-galactose).

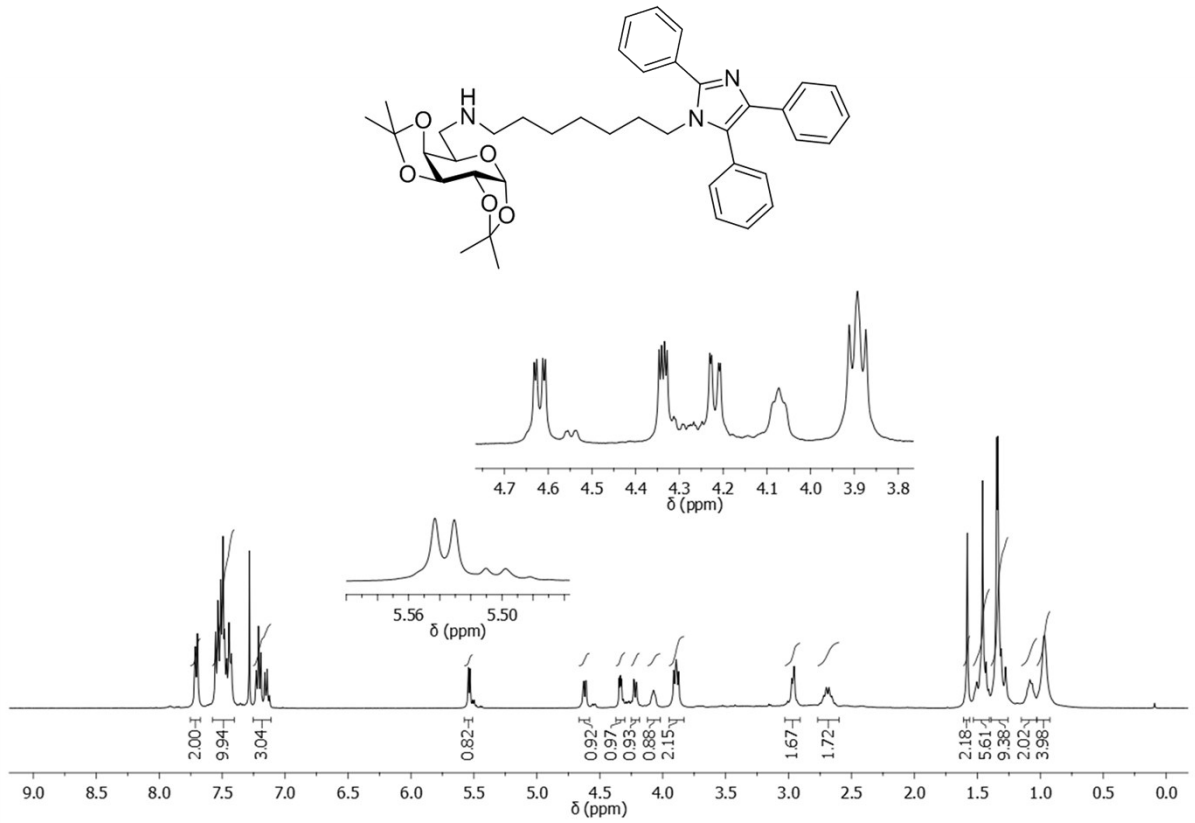


Figure S52. ^{1}H NMR Spectrum ($CDCl_3$, 300 MHz) of compound **9b**.

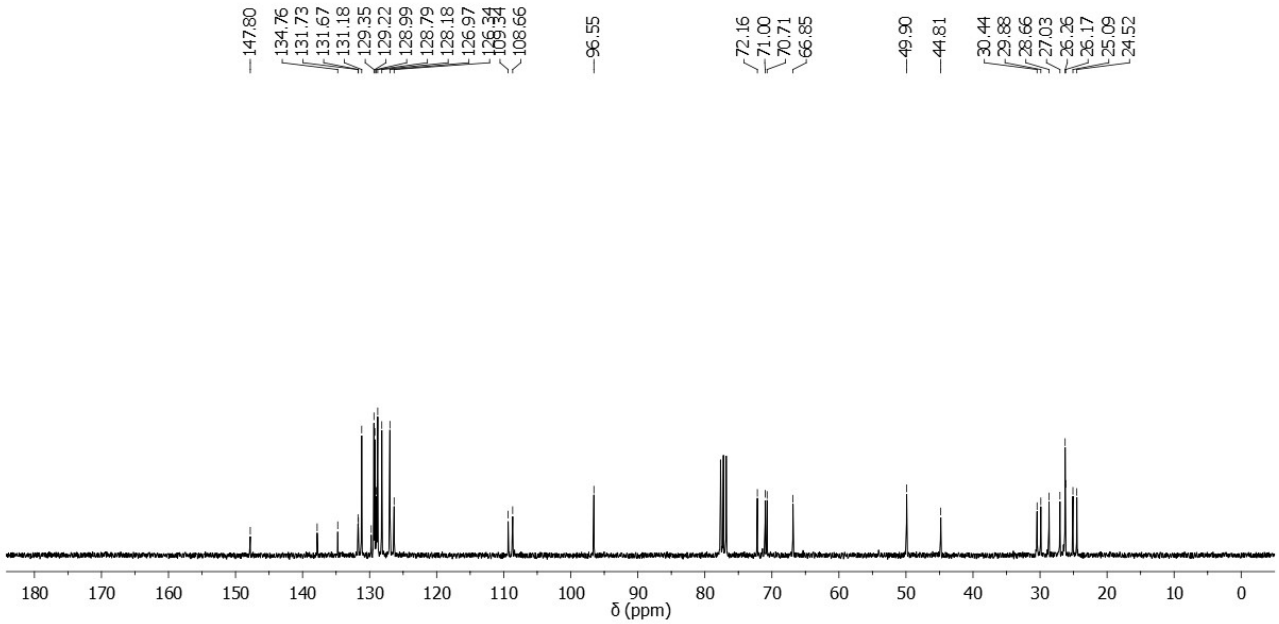


Figure S53. ^{13}C NMR Spectrum ($CDCl_3$, 75 MHz) of compound **9b**.

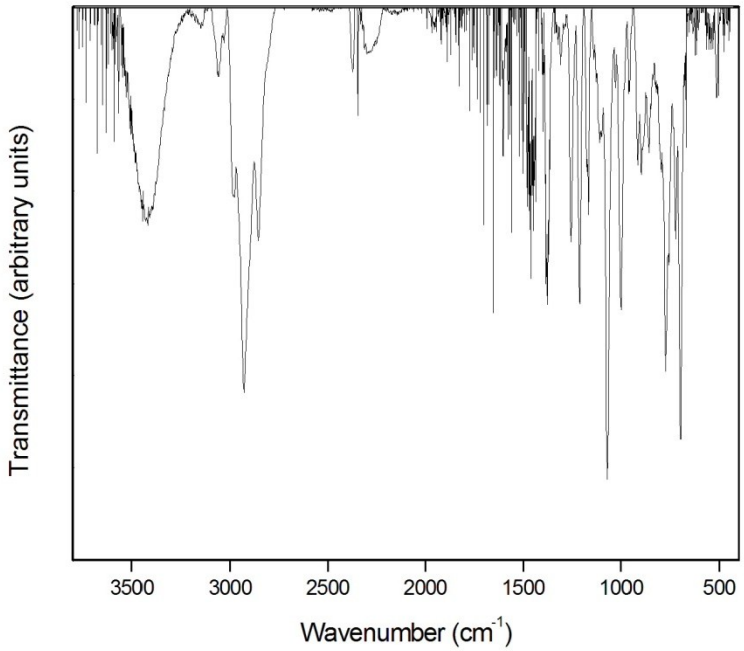


Figure S54. IR Spectrum (KBr pellet) of compound **9b**.

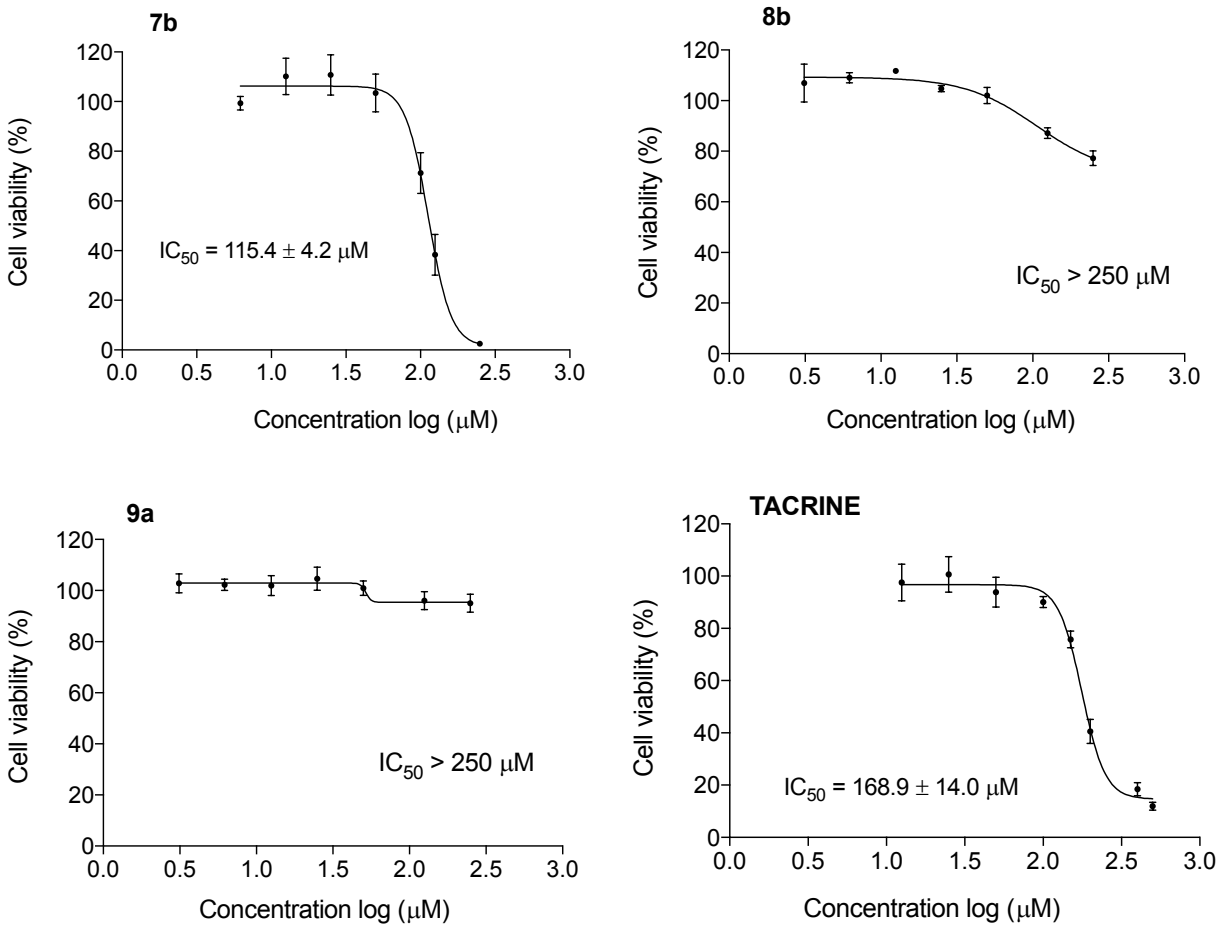


Figure S55.Cytotoxic evaluation on Vero cell line exposed to different concentrations of the compounds for 24 h. Dose-response curves of compounds **7b**, **8b**, **9a** and tacrine treatments on Vero cells (% of viable cells versus log[concentrations]).

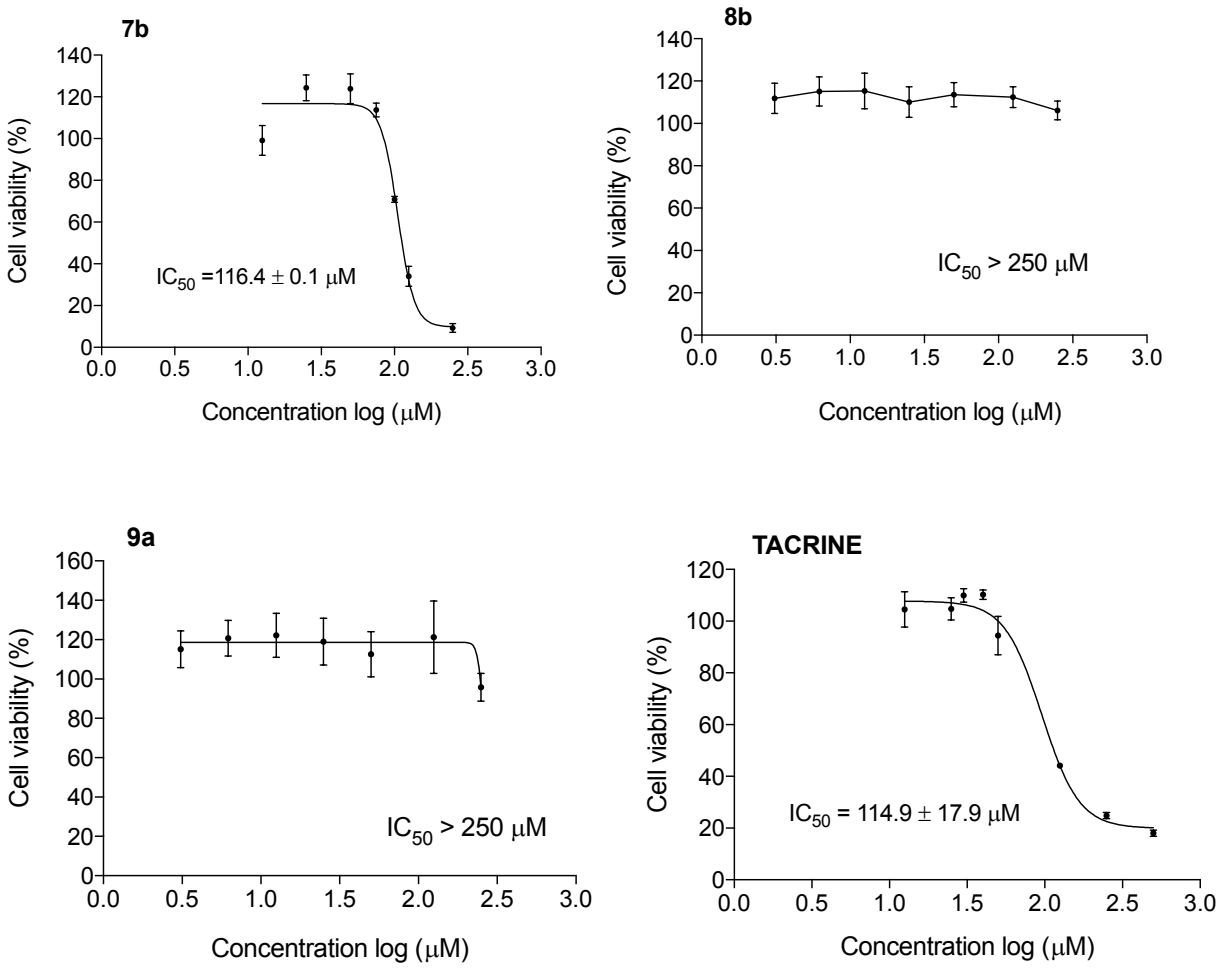


Figure S56.Cytotoxic evaluation on HepG2 cell line exposed to different concentrations of the compounds for 24 h. Dose-response curves of compounds **7b**, **8b**, **9a** and tacrine treatments on HepG2 cells (% of viable cells versus log[concentrations]).

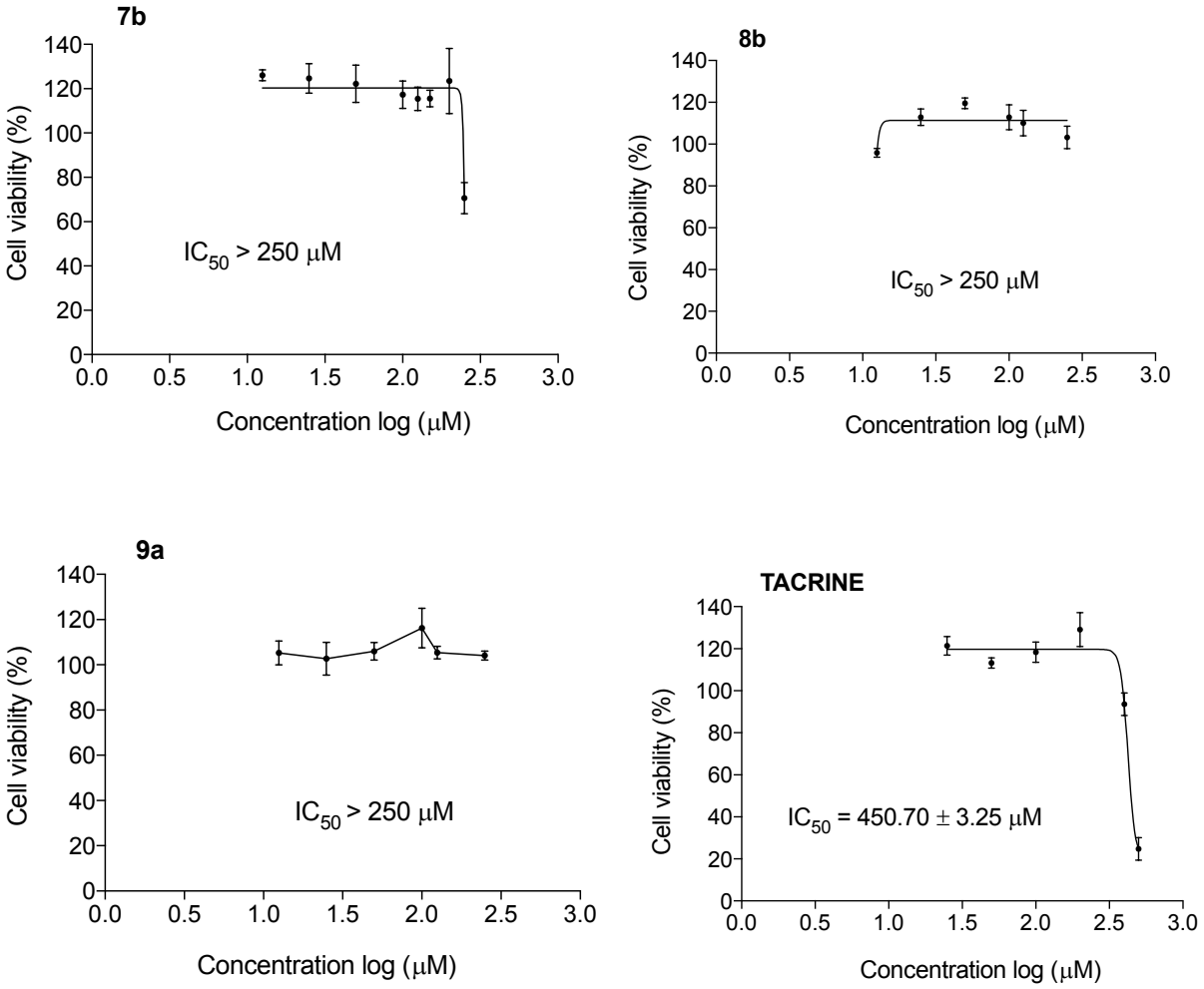
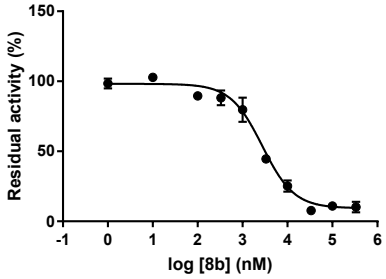
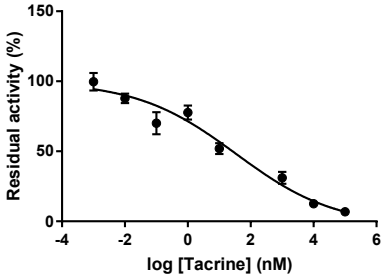


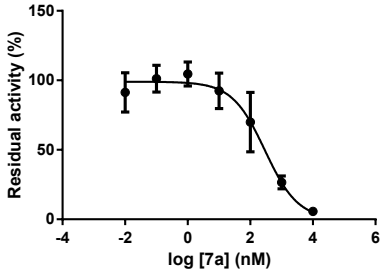
Figure S57.Cytotoxic evaluation on C6 cell line exposed to different concentrations of the compounds for 24 h. Dose-response curves of compounds **7b**, **8b**, **9a** and tacrine treatments on C6 cells (% of viable cells versus log[concentrations]).

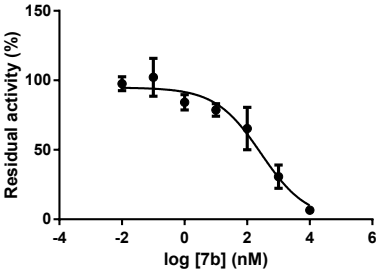
A)

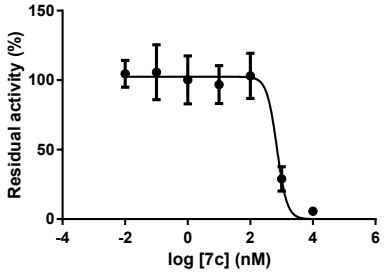

Residual activity (%)
150
100
50
0
-1
0
1
2
3
4
5
6
log [8b] (nM)

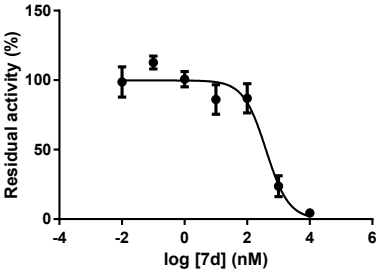

Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [Tacrine] (nM)

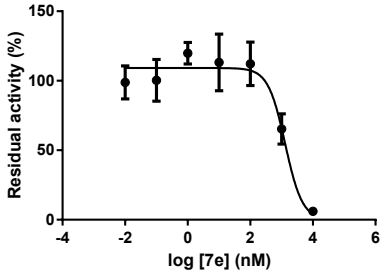
B)

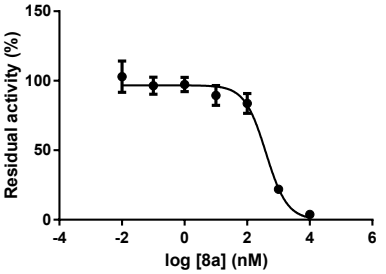

Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [7a] (nM)


Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [7b] (nM)


Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [7c] (nM)


Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [7d] (nM)


Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [7e] (nM)


Residual activity (%)
150
100
50
0
-4
-2
0
2
4
6
log [8a] (nM)

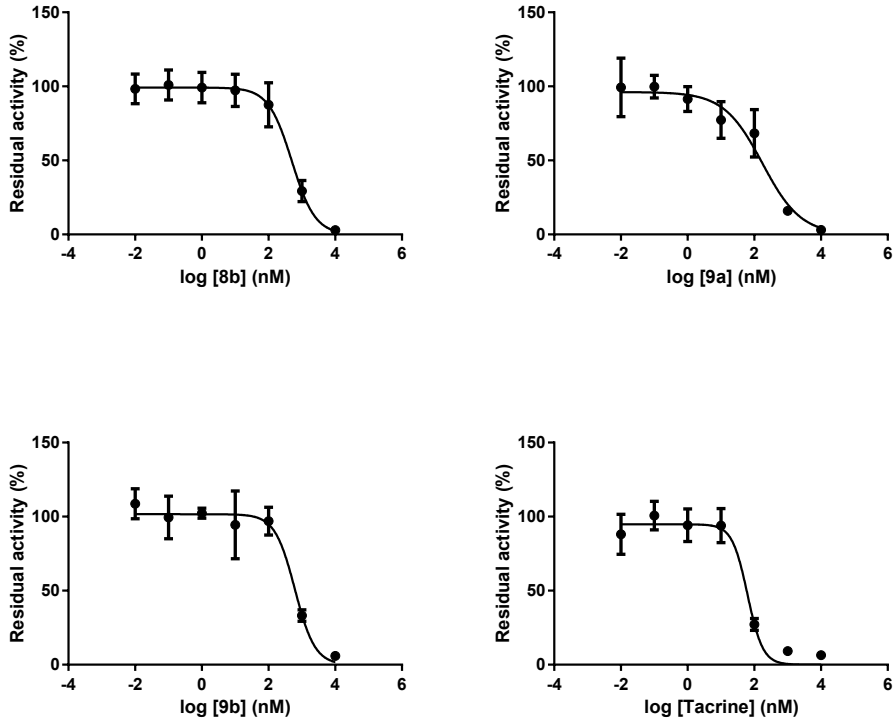


Figure S58. Dose–response curves of lophine-carbohydrate hybrids derivatives on acetylcholinesterase (A) and butyrylcholinesterase (B) activity. Enzymatic assays were was described on materials and methods. Percent values were calculated using the mean of control group and data are expressed as mean ± standard deviation of at least two independent experiments, each one in triplicate.