Supplementary Material

New phosphoramidites of RNA cleaving trisbenzimidazole catalysts: improved compatibility with oligonucleotide solid phase synthesis

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Removal of tosyl from trisbenzimidazole-DNA conjugates

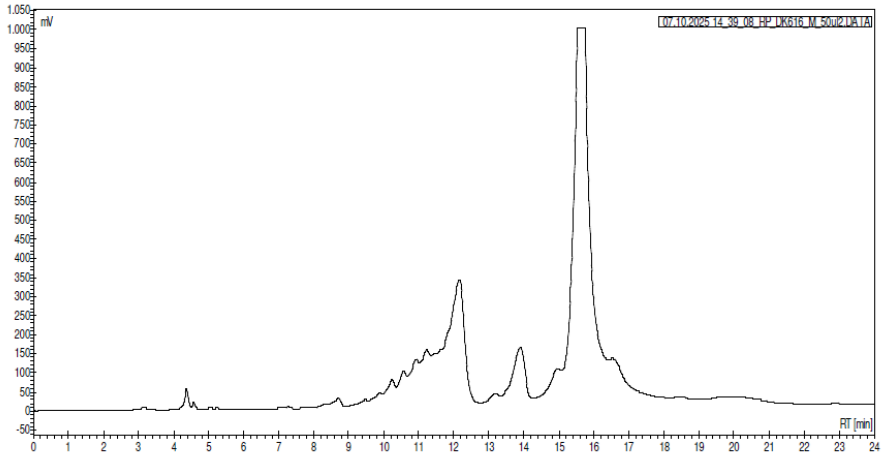


Figure S1. HPLC of crude conjugate **39**, prepared from the tosyl protected catalyst phosphoramidite **4**. Treatment with AMA at 55 °C overnight: tosyl groups are completely removed. Conjugate **39** appears at 16 min. Column: Phenomenex Jupiter 4 µm Proteo 90 Å (250 x 10 mm). Solvent A: MeOH. Solvent B: Aqueous hexafluoroisopropanol/Et_3N buffer (400 mM HFIP, 16 mM Et_3N, pH 7.9). Gradient: 5 % A and 95 % B from 0 to 2 min, 5 % to 69 % A and 95 to 31 % B from 2 to 22 min. Column temperature: 60 °C.

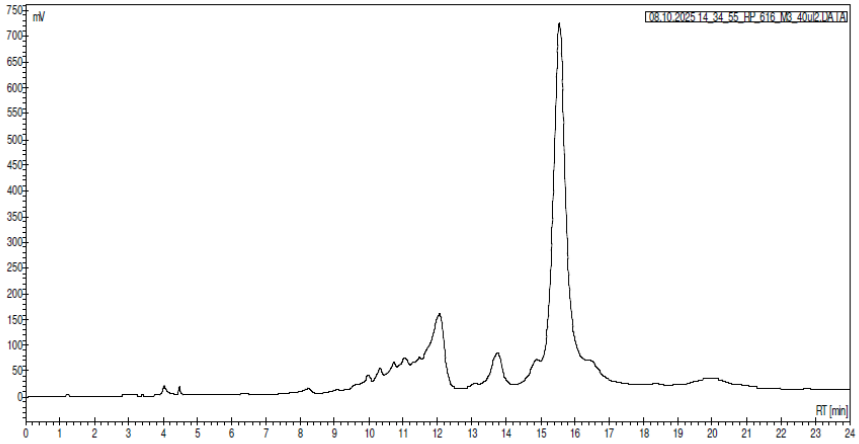


Figure S2. HPLC of crude conjugate **39** after treatment with AMA at 45 °C overnight: tosyl groups are completely removed.

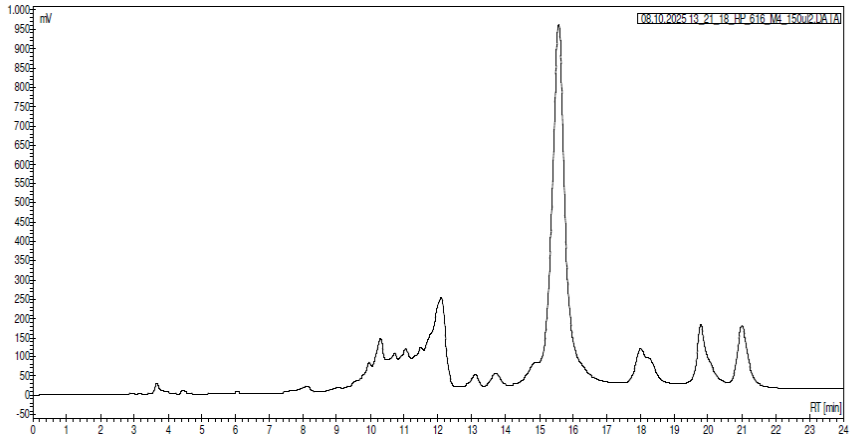


Figure S3. HPLC of crude conjugate **39** after treatment with NH_3 at 55 °C overnight: tosyl removal is incomplete. Conjugates containing one, two or three tosyl residues appear at 18, 20, and 21 min.

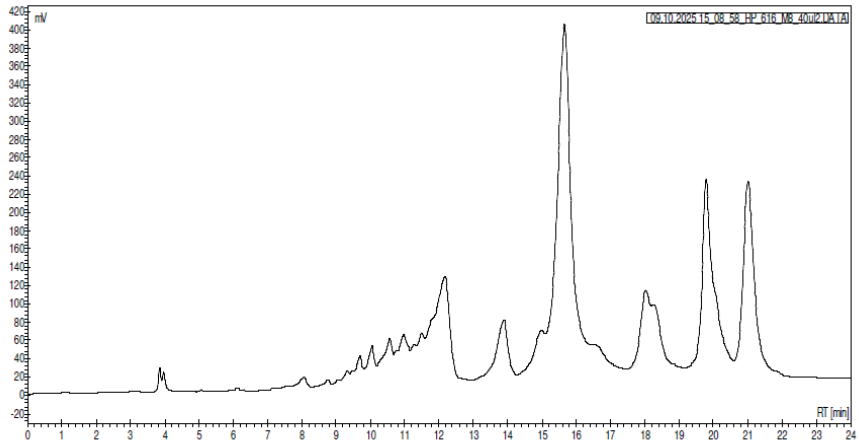


Figure S4. HPLC of crude conjugate **39** after treatment with AMA at 65 °C for 1h:

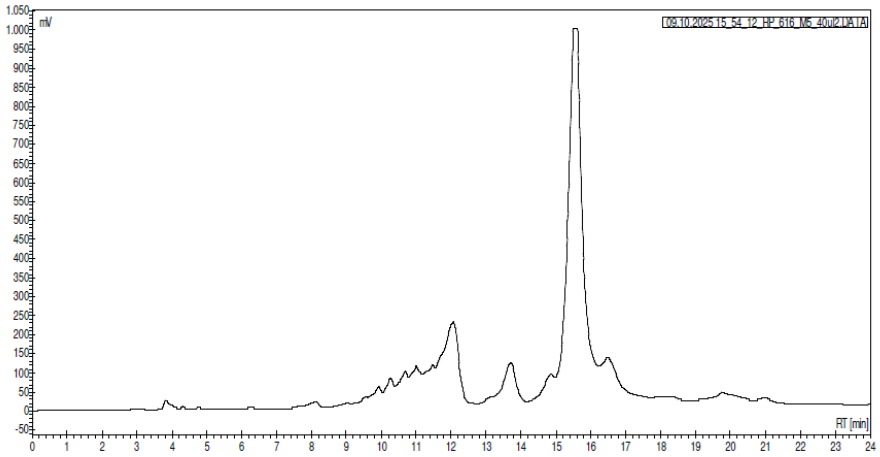


Figure S5. HPLC of crude conjugate **39** after treatment with AMA at 65 °C for 3h.

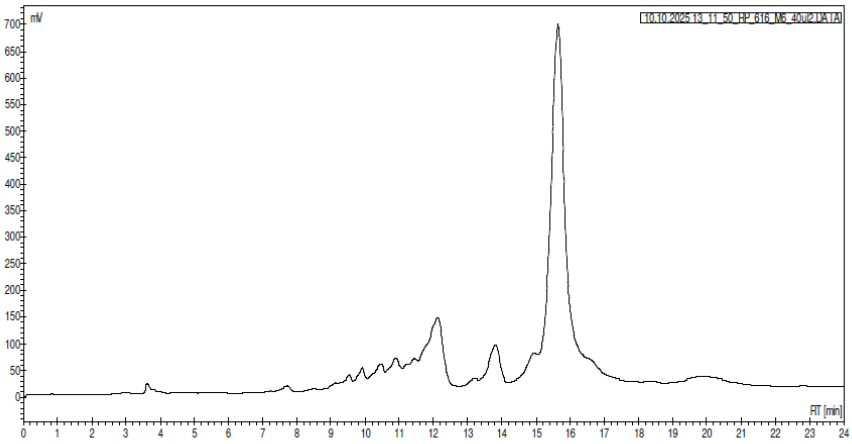


Figure S6. HPLC of crude conjugate **39** after treatment with AMA at 65 °C for 5h. Removal of tosyl is complete.

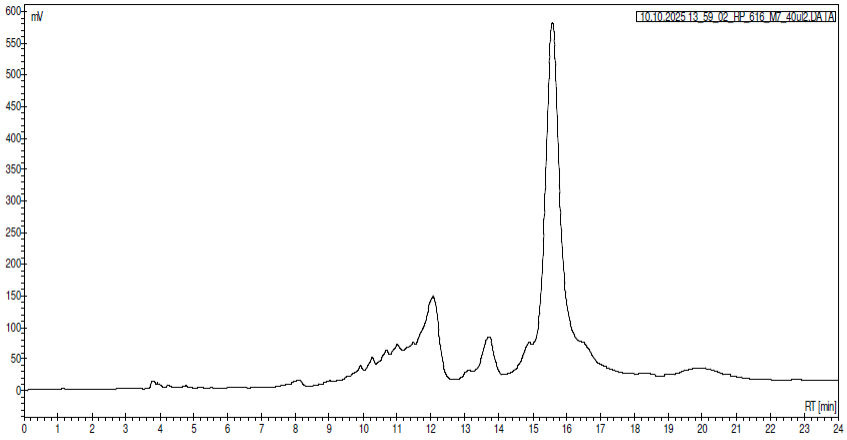


Figure S7. HPLC of crude conjugate **39** after treatment with AMA at 65 °C for 7h. No further change.

HPLC purification of conjugates 35 – 40

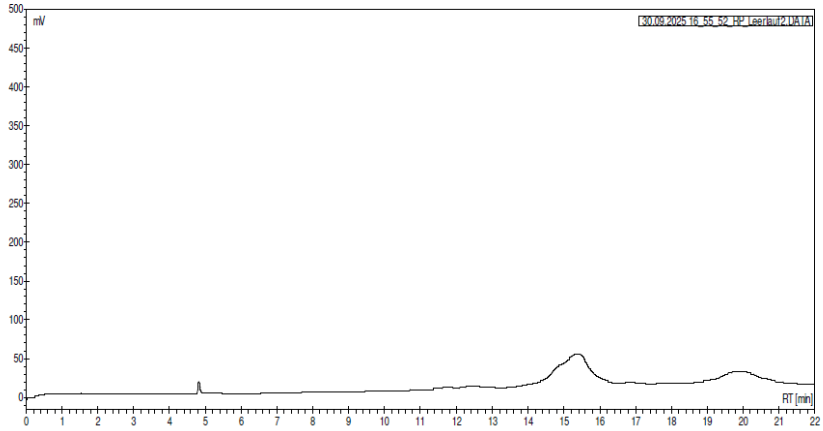


Figure S8. HPLC purification of conjugates **35** – **40**. Baseline in absence of conjugates. Column: Phenomenex Jupiter 4 µm Proteo 90 Å (250 x 10 mm). Solvent A: MeOH. Solvent B: Aqueous hexafluoroisopropanol/Et_3N buffer (400 mM HFIP, 16 mM Et_3N, pH 7.9). Gradient: 5 % A and 95 % B from 0 to 2 min, 5 % to 69 % A and 95 to 31 % B from 2 to 22 min. Column temperature: 60 °C.

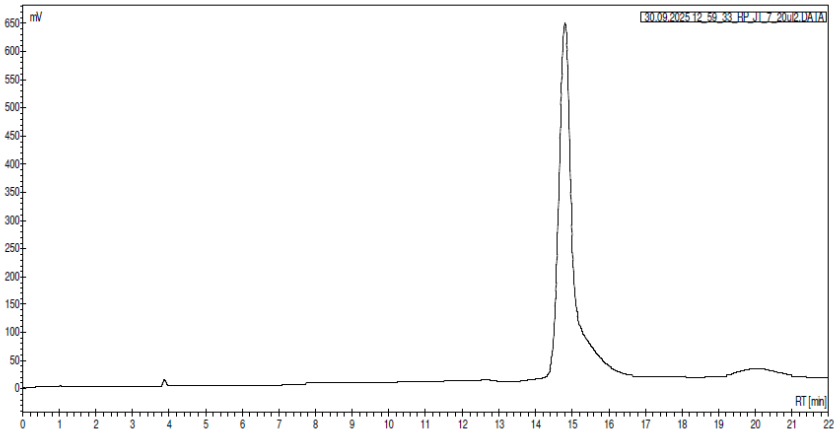


Figure S9. HPLC purification of conjugate **35**. The peak appearing around 20 min is part of the baseline.

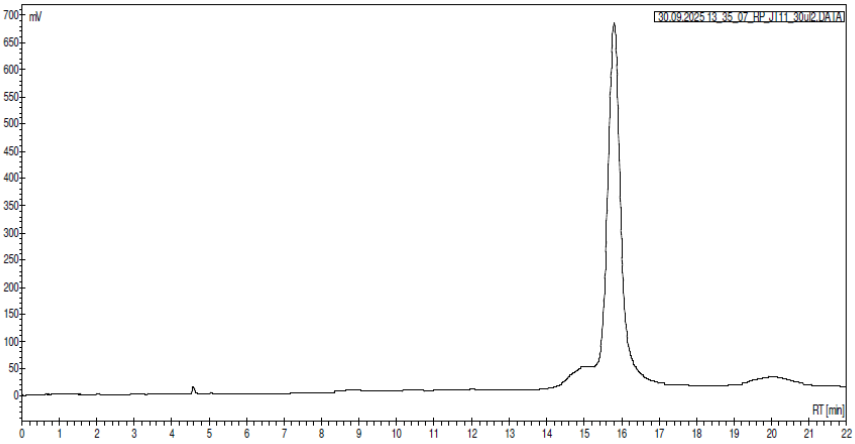


Figure S10. HPLC purification of conjugate **36**. The peaks appearing around 15 and 20 min are part of the baseline.

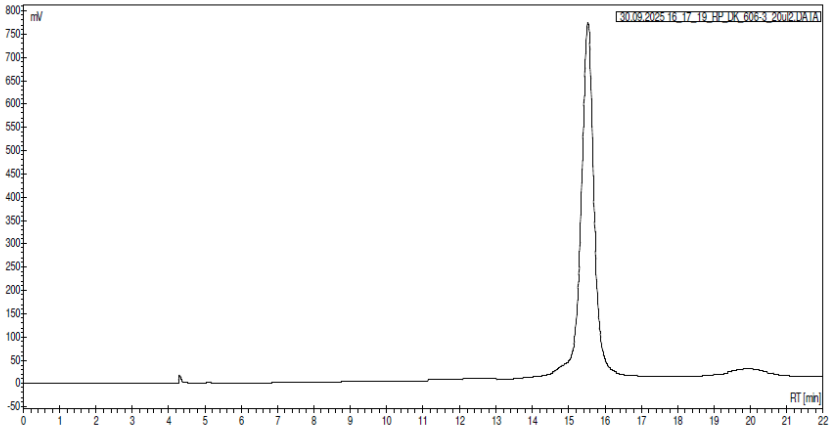


Figure S11. HPLC purification of conjugate **37**. The peak appearing around 20 min is part of the baseline.

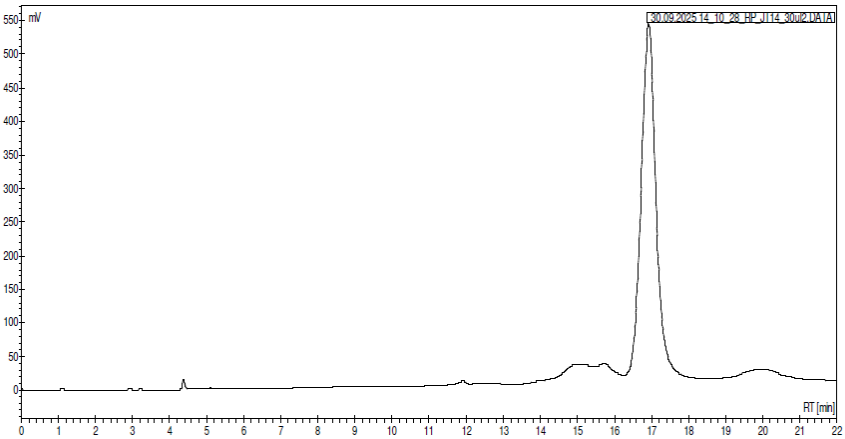


Figure S12. HPLC purification of conjugate **38**. The peaks appearing around 15 and 20 min are part of the baseline.

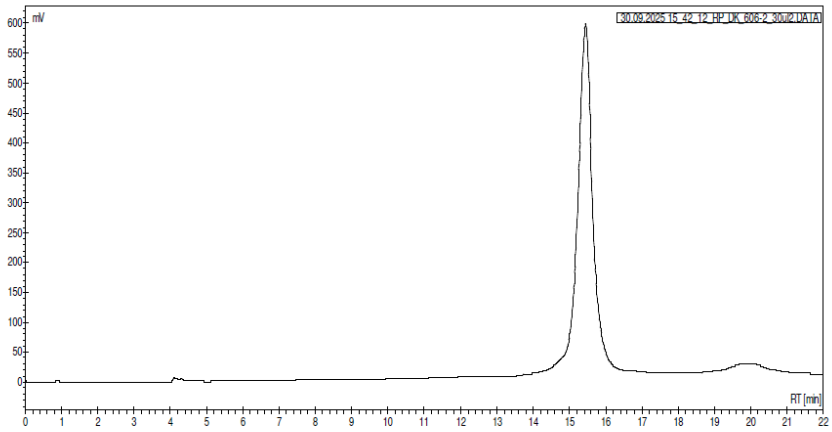


Figure S13. HPLC purification of conjugate **39**. The peak appearing around 20 min is part of the baseline.

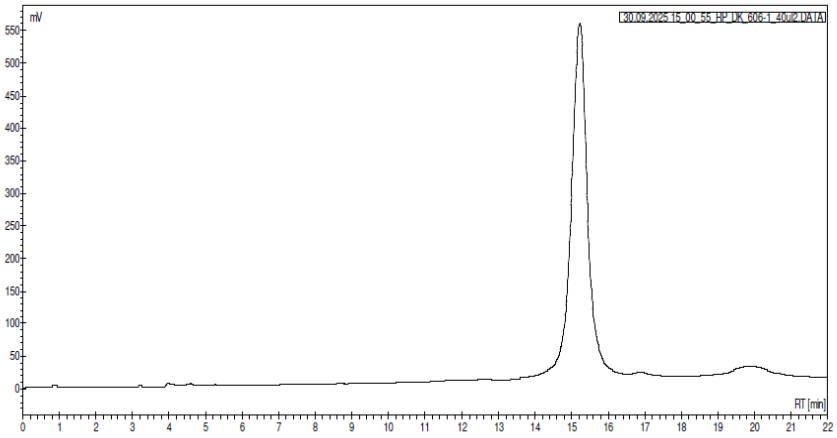


Figure S14. HPLC purification of conjugate **40**. The peak appearing around 20 min is part of the baseline.

Mass spectra of conjugates 35 – 40

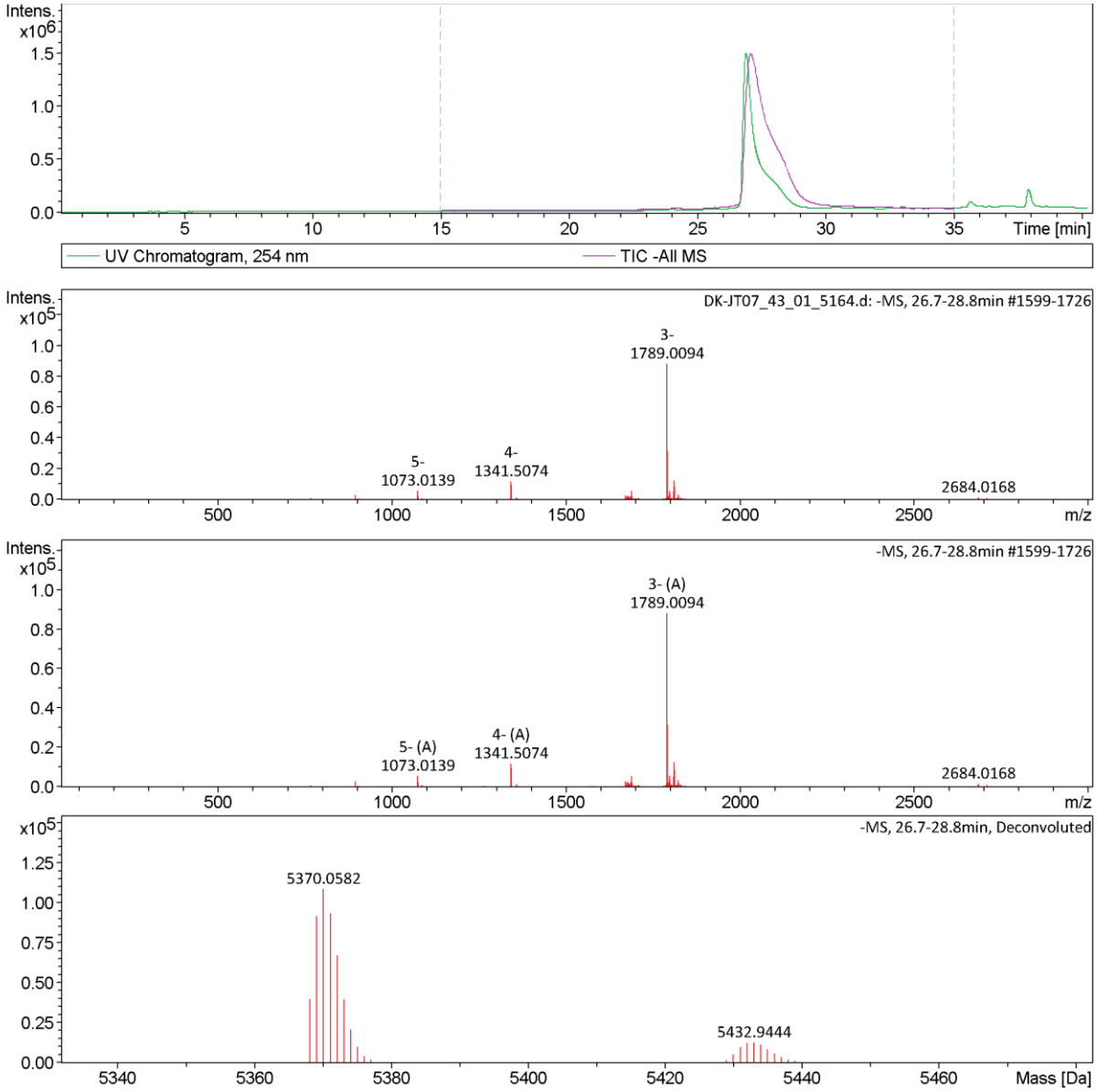


Figure S15. Conjugate **35** (C_6 linker with CC triple bond). Calculated mass for $[M\text{-}3H^+]^{3-}$: 1789.02; exact mass [M]: 5368.08.

The additional peak at 5432 mass units may point to the possibility of metal ion contaminatons. To exclude the impact of such ions on cleavage kinetics, the reactions of conjugates **37**, **38**, **39**, and **40** have been repeated in the presence of 1 mM EDTA without causing changes in rates or fragment patterns.

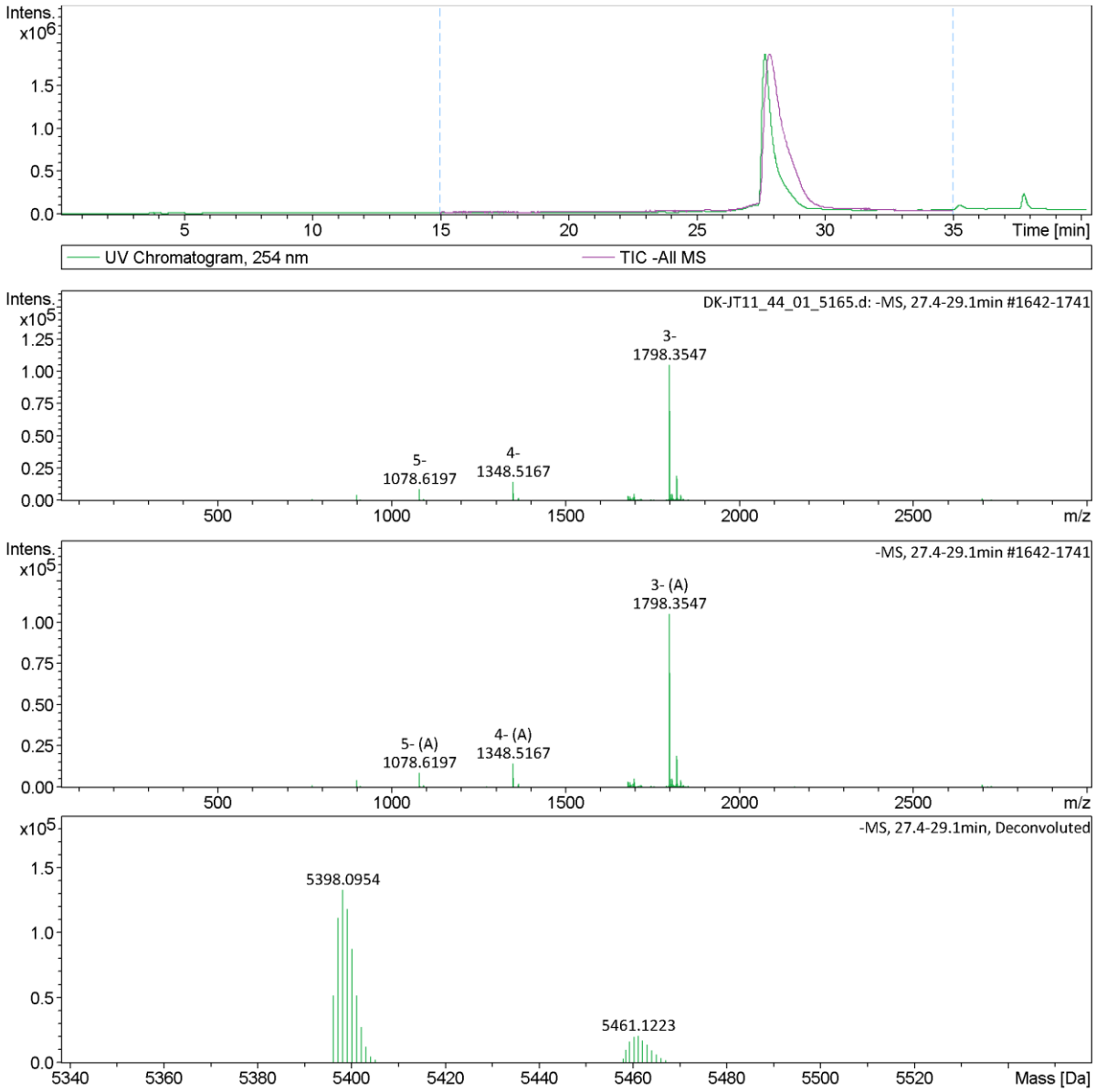


Figure S16. Conjugate **36** (C_8 linker with CC triple bond). Calculated mass for $[M\text{-}3H^+]^{3-}$: 1798.36; exact mass [M]: 5396.11.

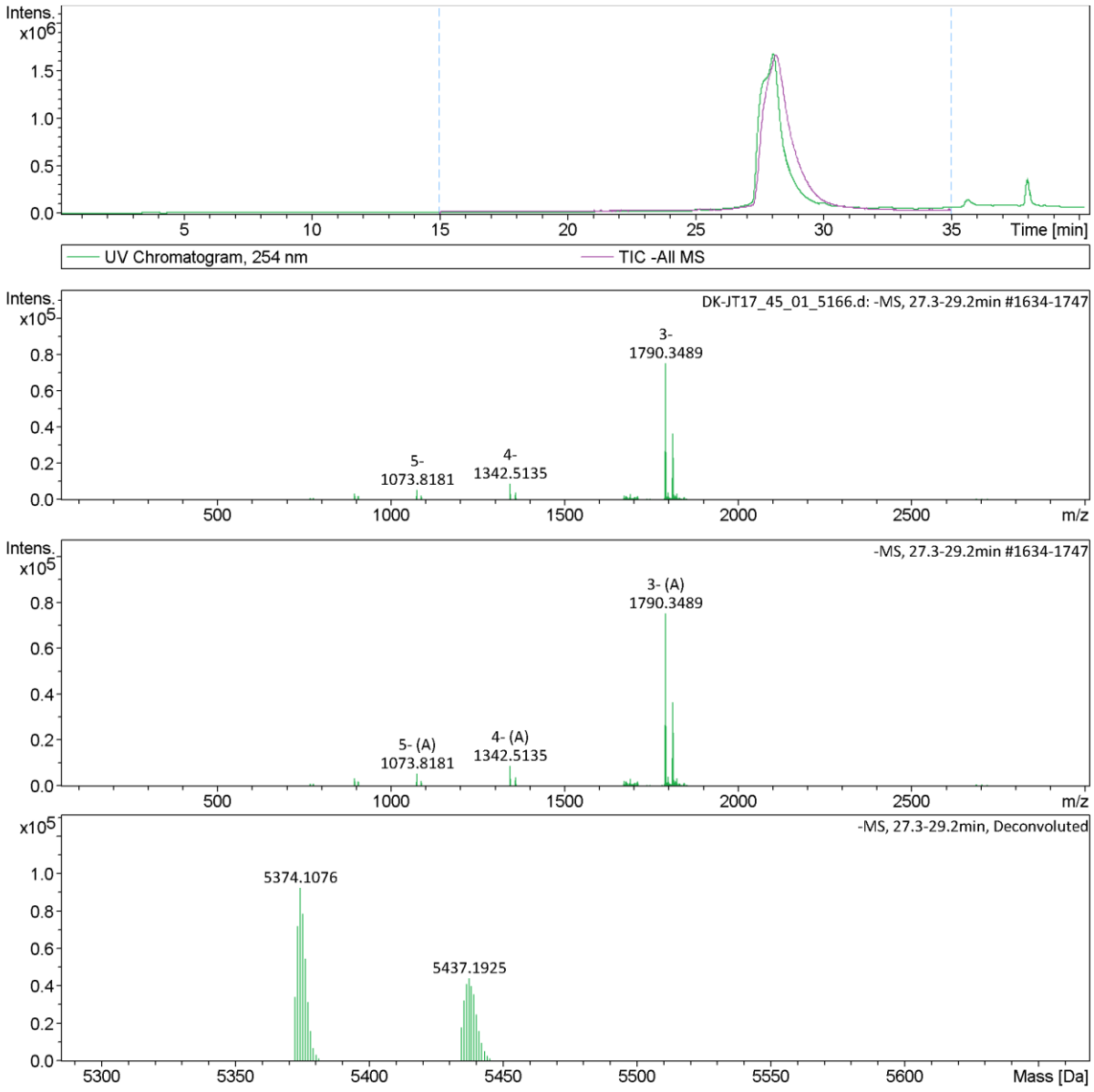


Figure S17. Conjugate **37** (saturated C_6 linker). Calculated mass for $[M\text{-}3H^+]^{3-}$: 1790.37; exact mass [M]: 5372.11

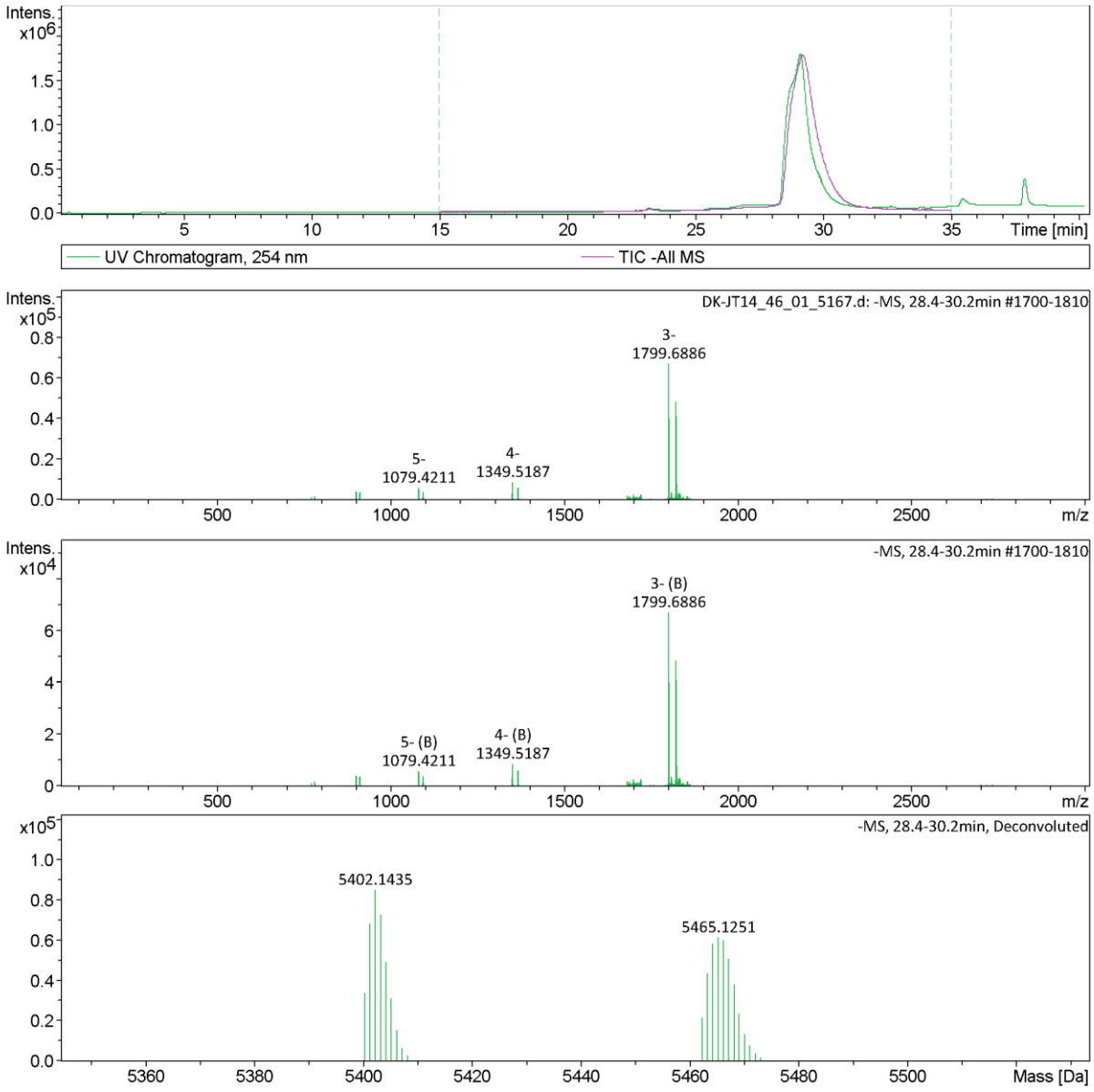


Figure S18. Conjugate **38** (saturated C_8 linker). Calculated mass for $[M\text{-}3H^+]^{3-}$: 1799.71; exact mass [M]: 5400.14.

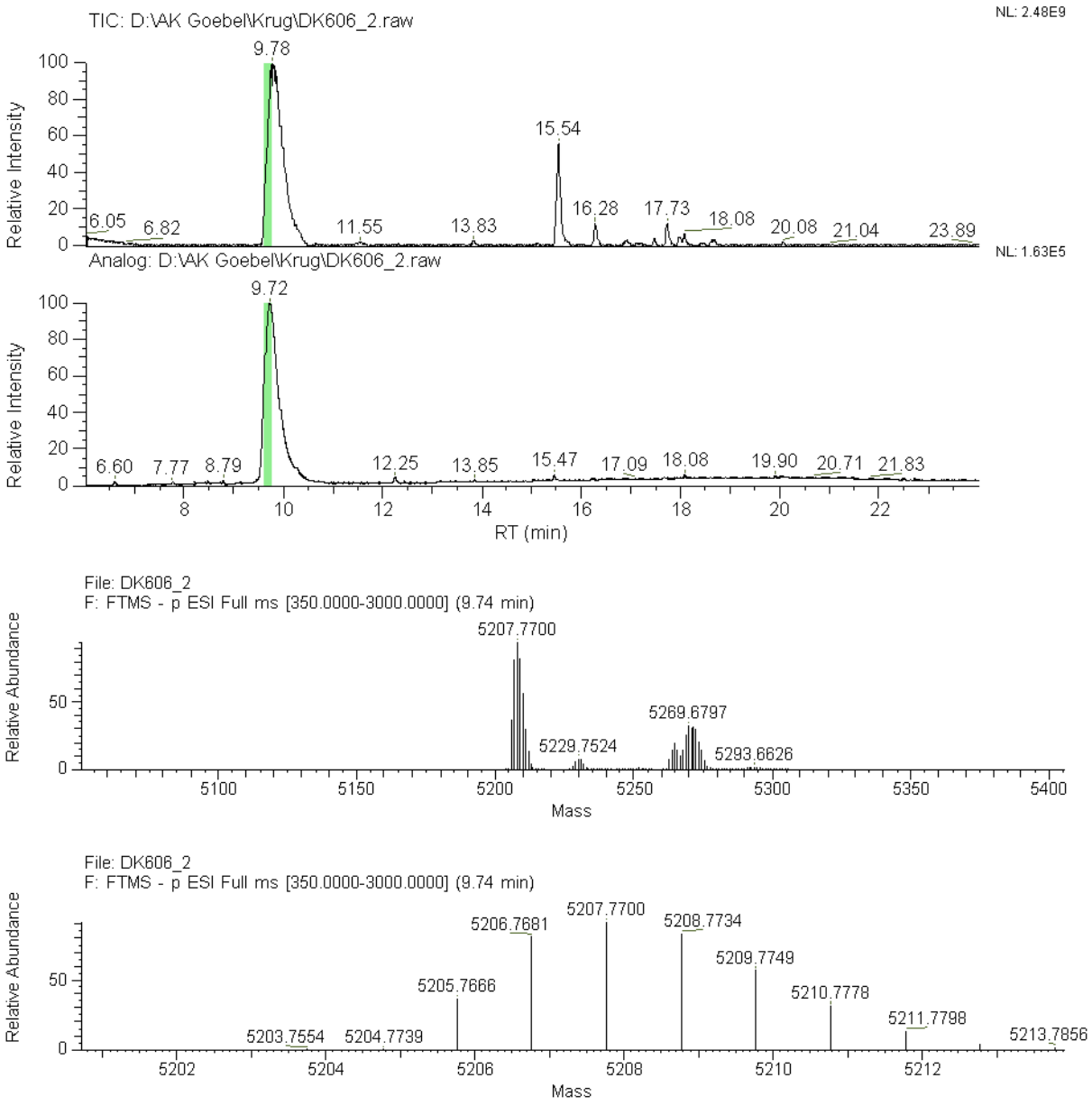


Figure S19. Conjugate **39** (saturated C_6 linker) prepared from tosyl-protected phosphoramidite **4**. Calculated exact mass [M]: 5207.11.

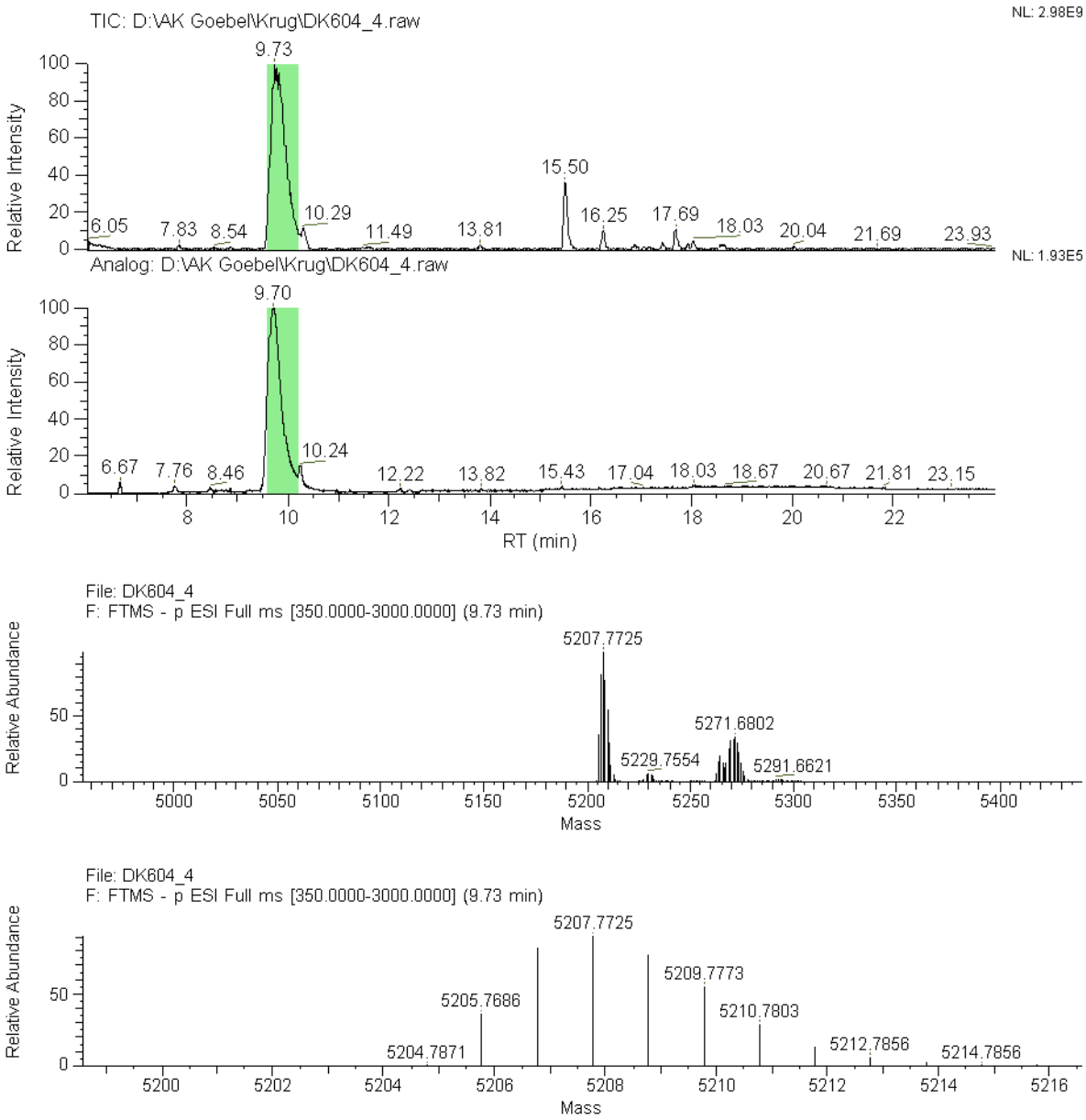


Figure S20. Conjugate **39** (saturated C_6 linker) prepared from Boc-protected phosphoramidite **6**. Calculated exact mass [M]: 5207.11.

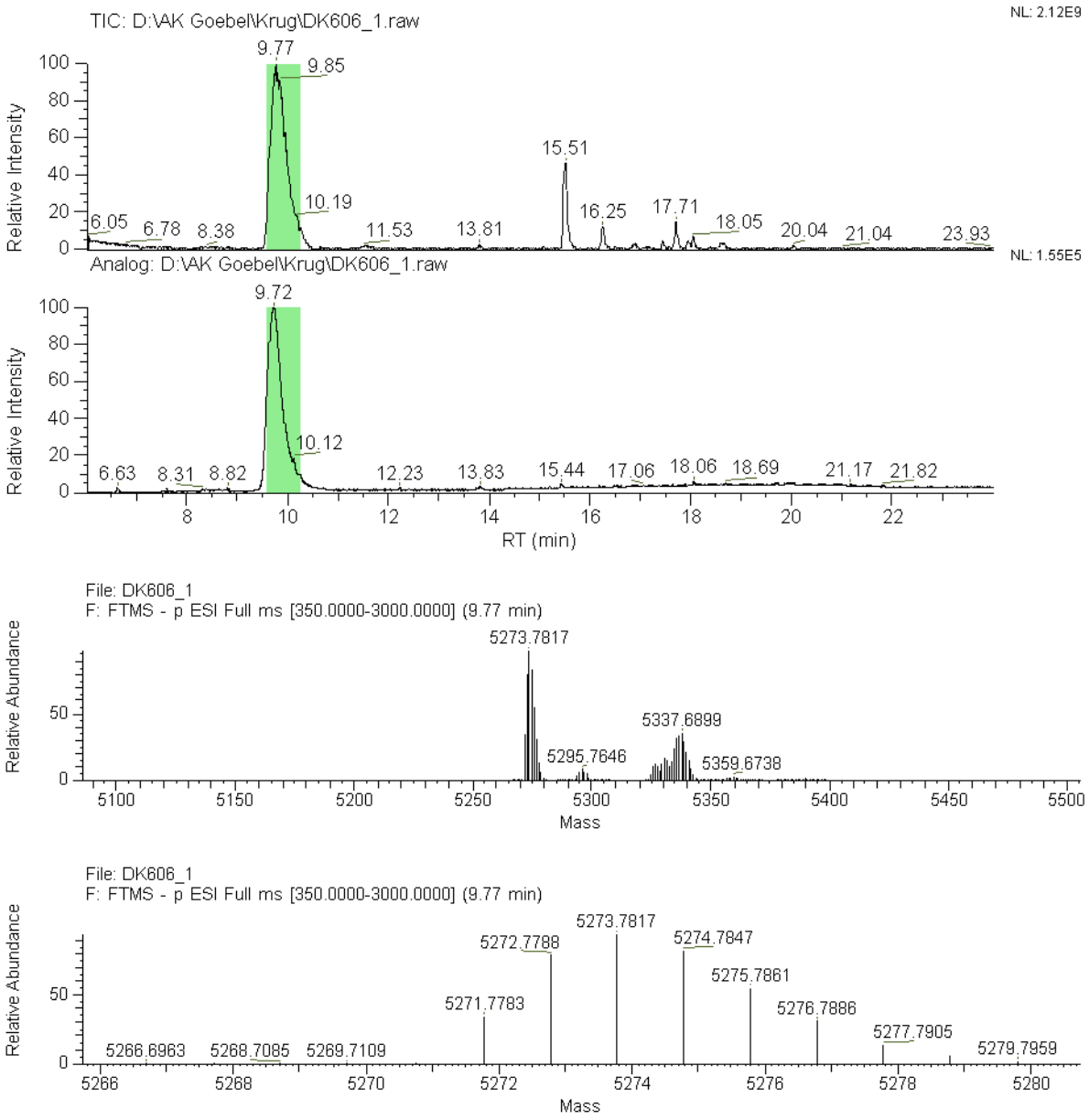


Figure S21. Conjugate **40** (saturated C_6 linker) prepared from tosyl-protected phosphoramidite **4**. Calculated exact mass [M]: 5273.12.

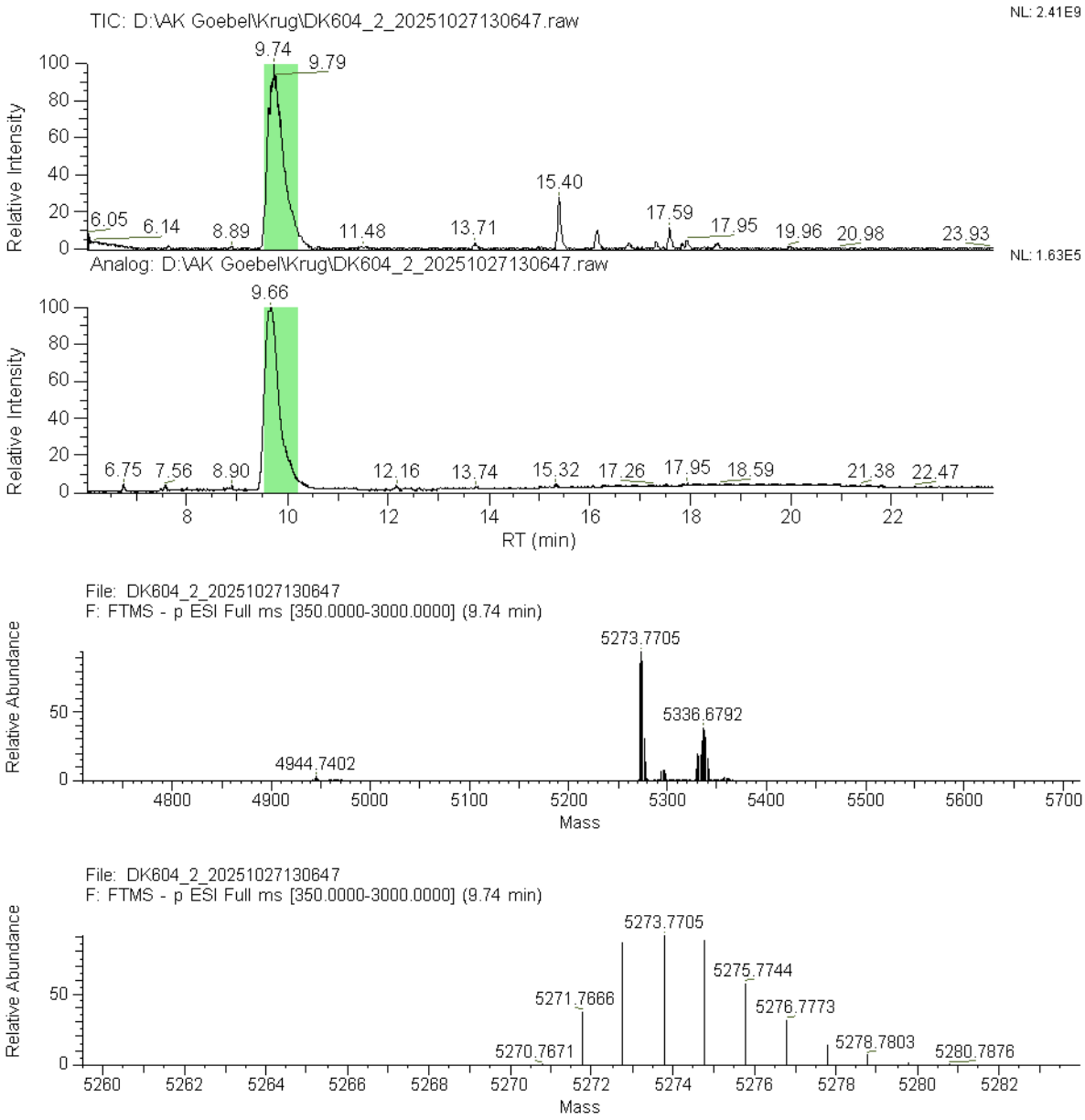


Figure S22. Conjugate **40** (saturated C_6 linker) prepared from Boc-protected phosphoramidite **6**. Calculated exact mass [M]: 5273.12.

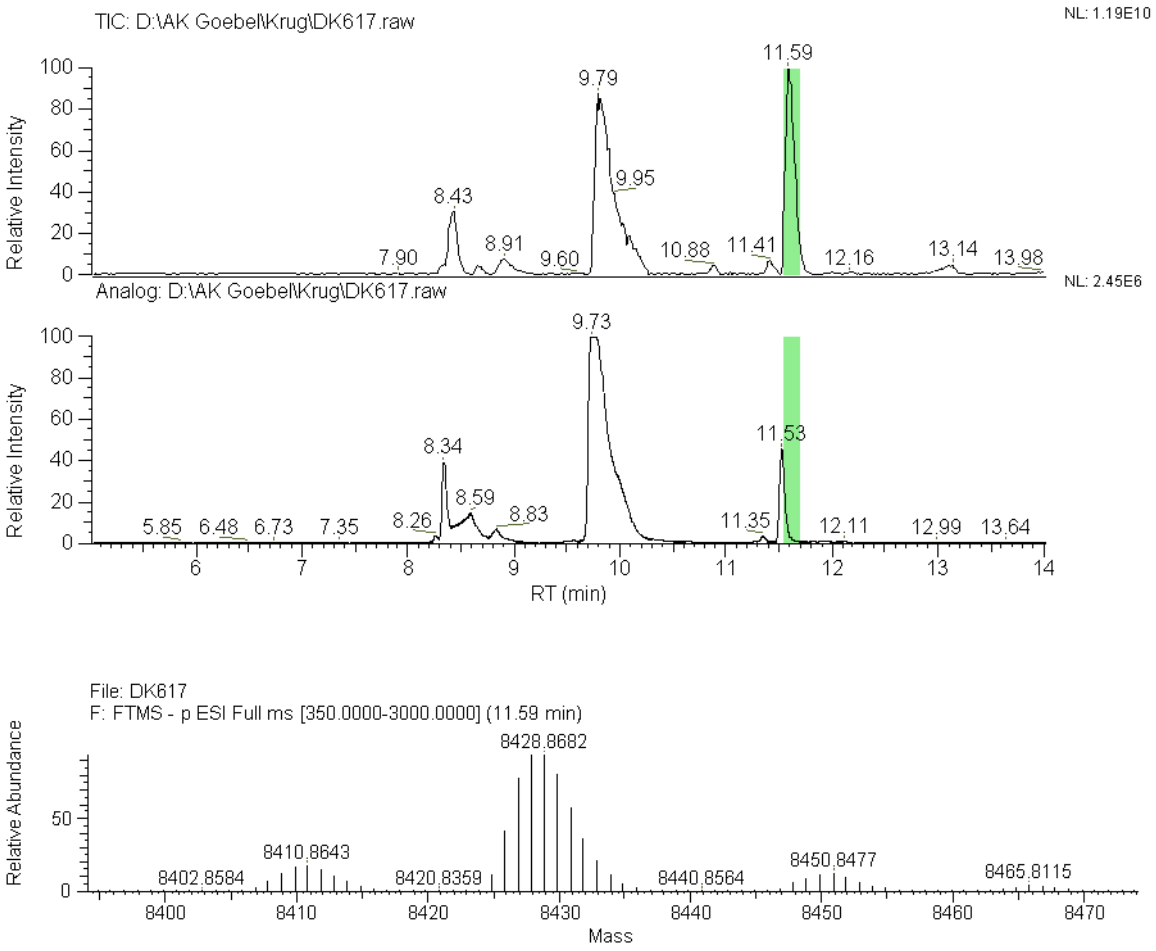


Figure S23. Analysis of the cleavage product. RNA **41** (8 µM) und conjugate **37** (68 µM, prepared from phosphoramidite **6**) were incubated for 22h at 37 °C (sodium free 50 mM Tris-HCl buffer pH 8.0). The reaction mixture was directly injected into the HPLC-MS system. The peak appearing at 11.5 min corresponds to the fragment Cy5-T_{10}-ribo$^{5'}$(CAA CAU UUA CAA CUC)$^{3'}$phosphate. The calculated exact mass [M] is 8426.4 for the 2‘ or 3‘ monophosphates and 8408.4 for the 2‘,3‘ cyclic phosphate: the monophosphates prevail in the mixture.

RNA cleavage part I. Saturation experiments

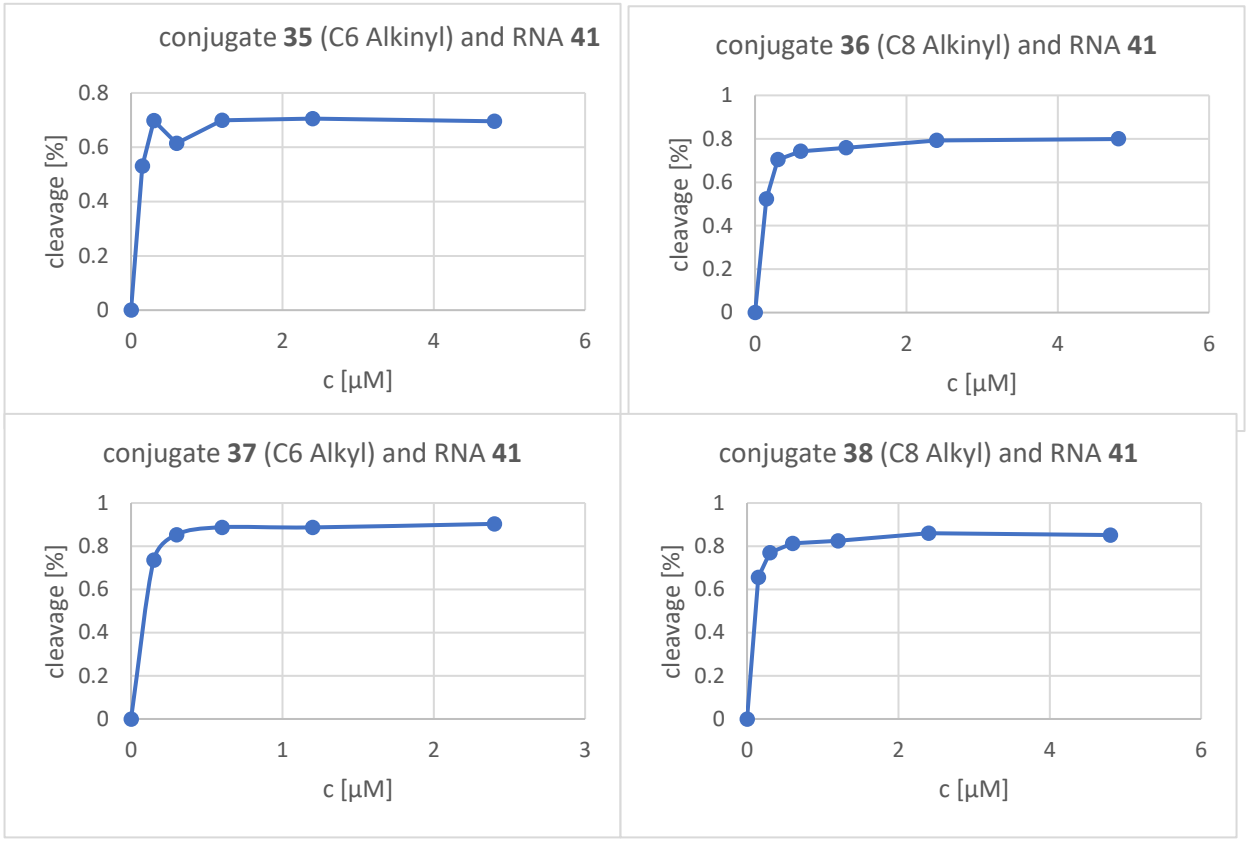


Figure S24. 150 nM RNA **41** was mixed with 150 nM – 4.8 µM of conjugates in 50 mM buffer pH 8.0 and incubated for 16 h at 37 °C. The total amount of cleavage was then determined.

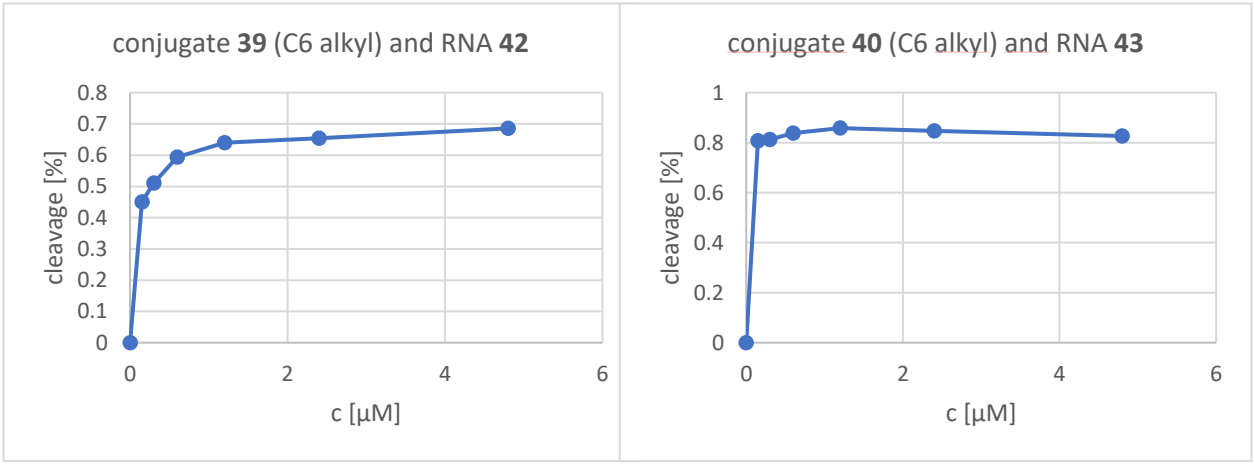


Figure S25. 150 nM RNA **42** or **43** was mixed with 150 nM – 4.8 µM of conjugates in 50 mM buffer pH 8.0 and incubated for 16 h at 37 °C. The total amount of cleavage was then determined.

RNA cleavage part II. Reaction kinetics

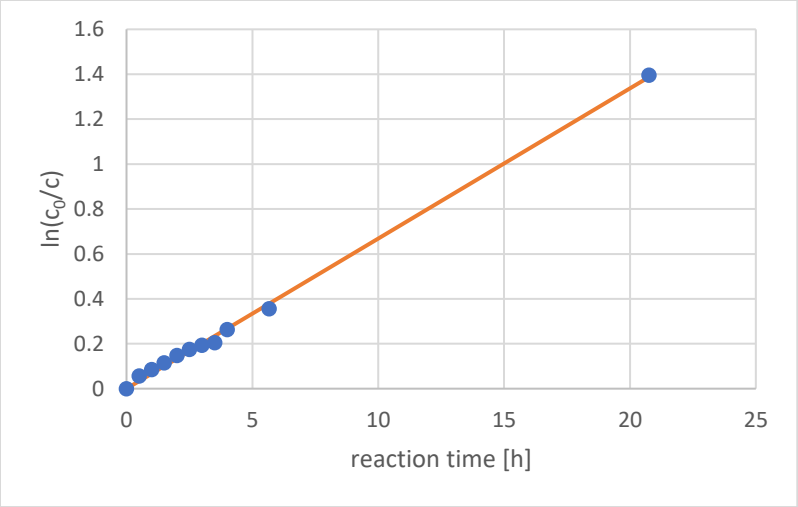


Figure S26. Cleavage kinetics of RNA **41** (150 nM) and conjugate **35** (1200 nM). Blue: data points; orange: linear fit.

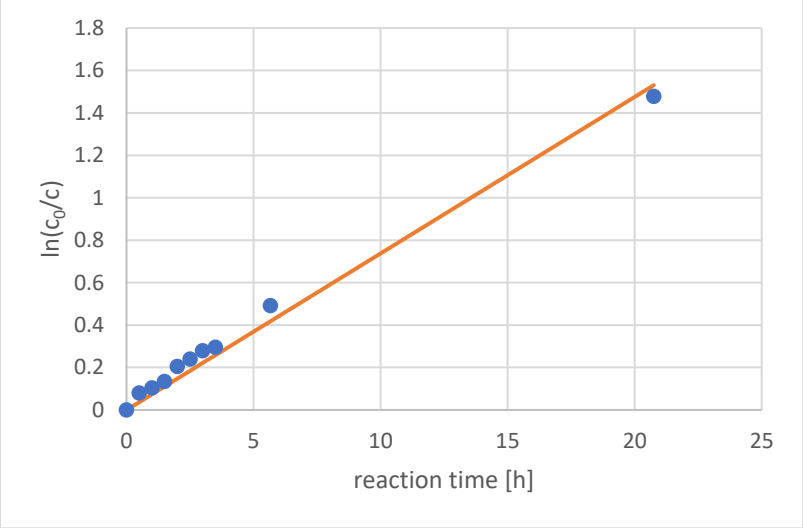


Figure S27. Cleavage kinetics of RNA **41** (150 nM) and conjugate **36** (1200 nM). Blue: data points; orange: linear fit.

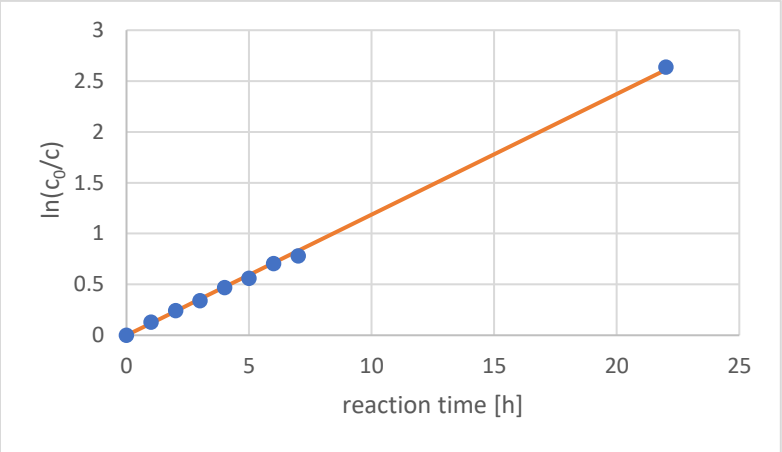


Figure S28. Cleavage kinetics of RNA **41** (150 nM) and conjugate **37** (1200 nM). Blue: data points; orange: linear fit.

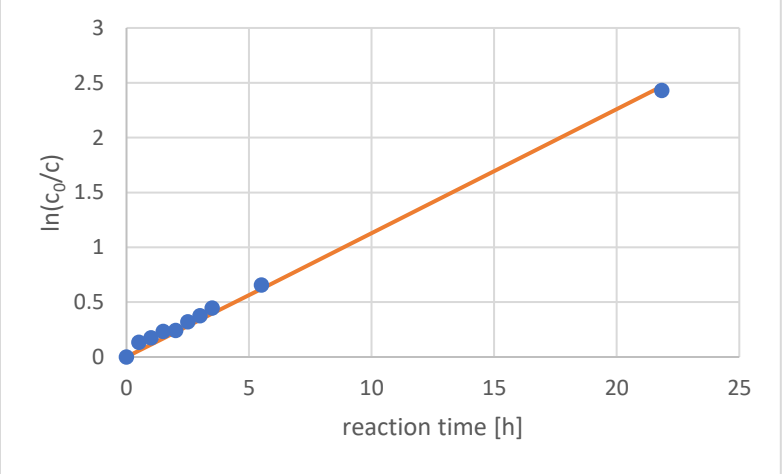


Figure S29. Cleavage kinetics of RNA **41** (150 nM) and conjugate **38** (1200 nM). Blue: data points; orange: linear fit.

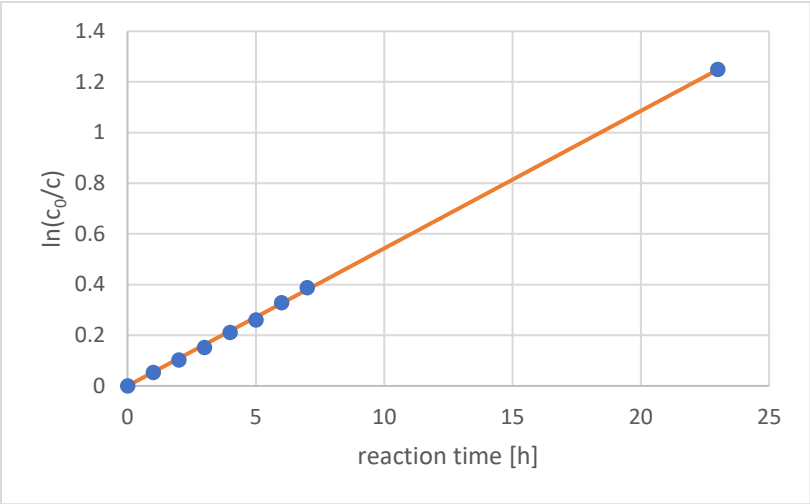


Figure S30. Cleavage kinetics of RNA **42** (150 nM) and conjugate **39** (1200 nM). Blue: data points; orange: linear fit.

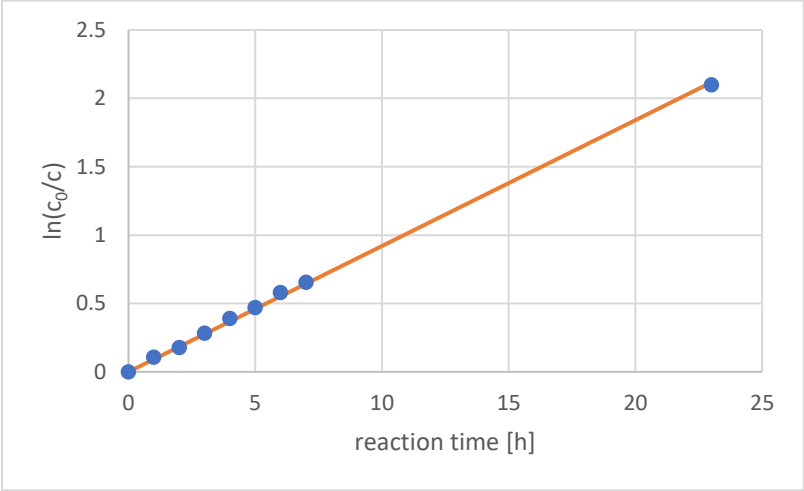
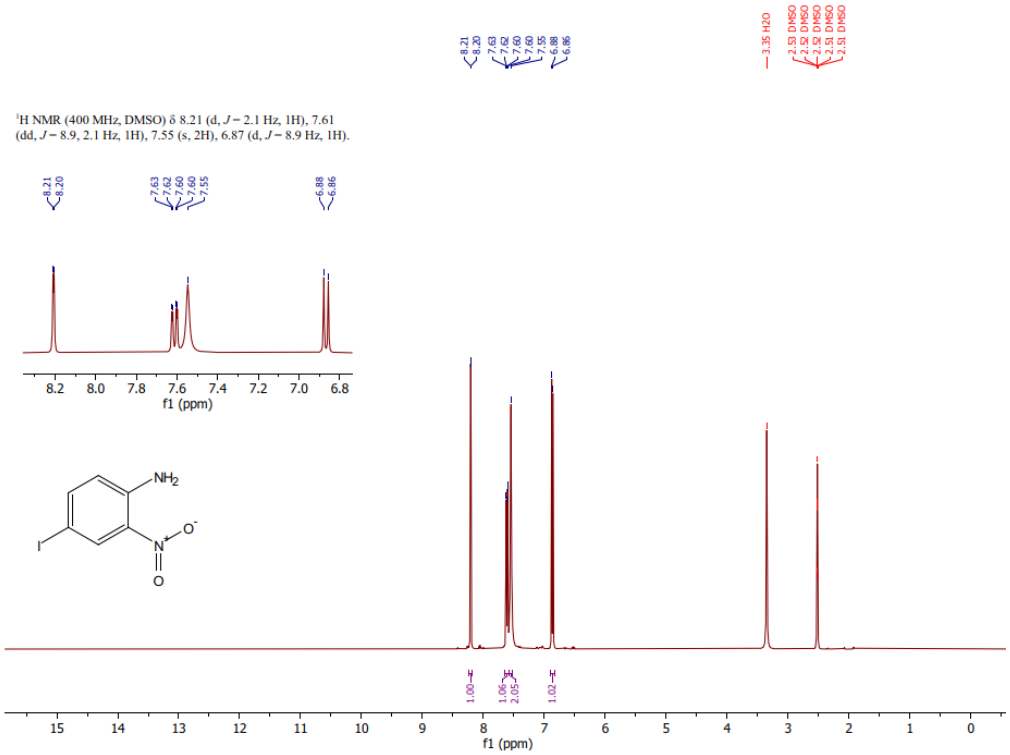
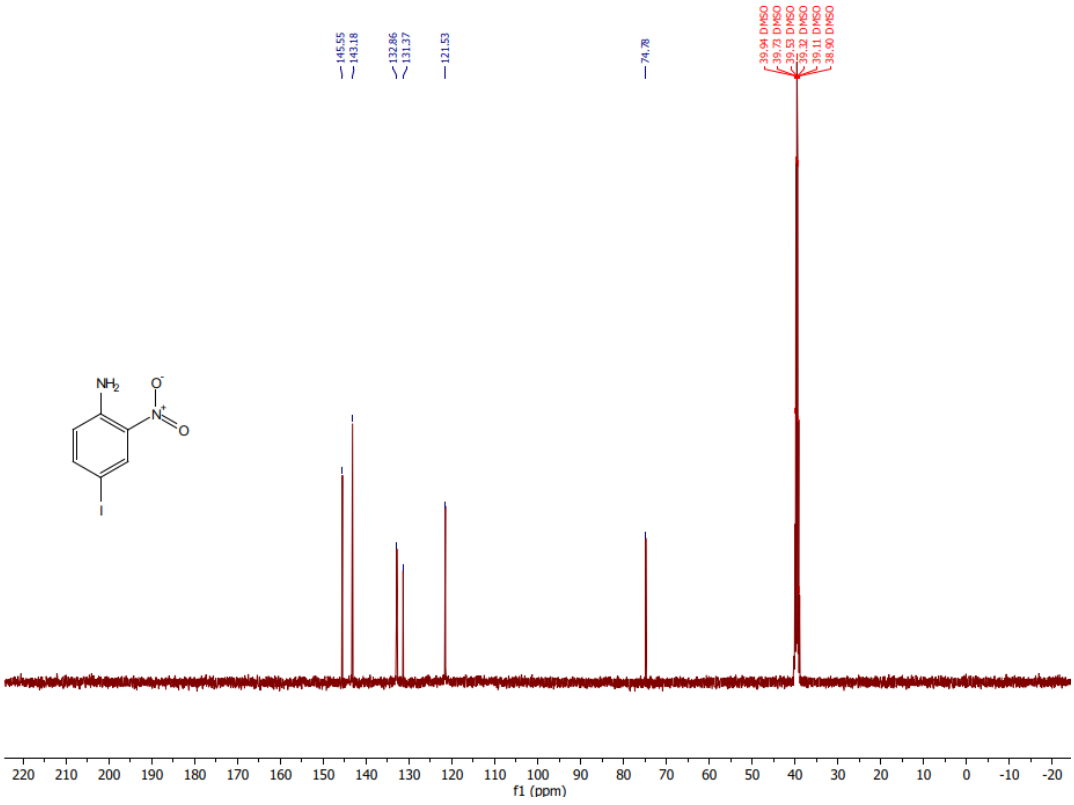


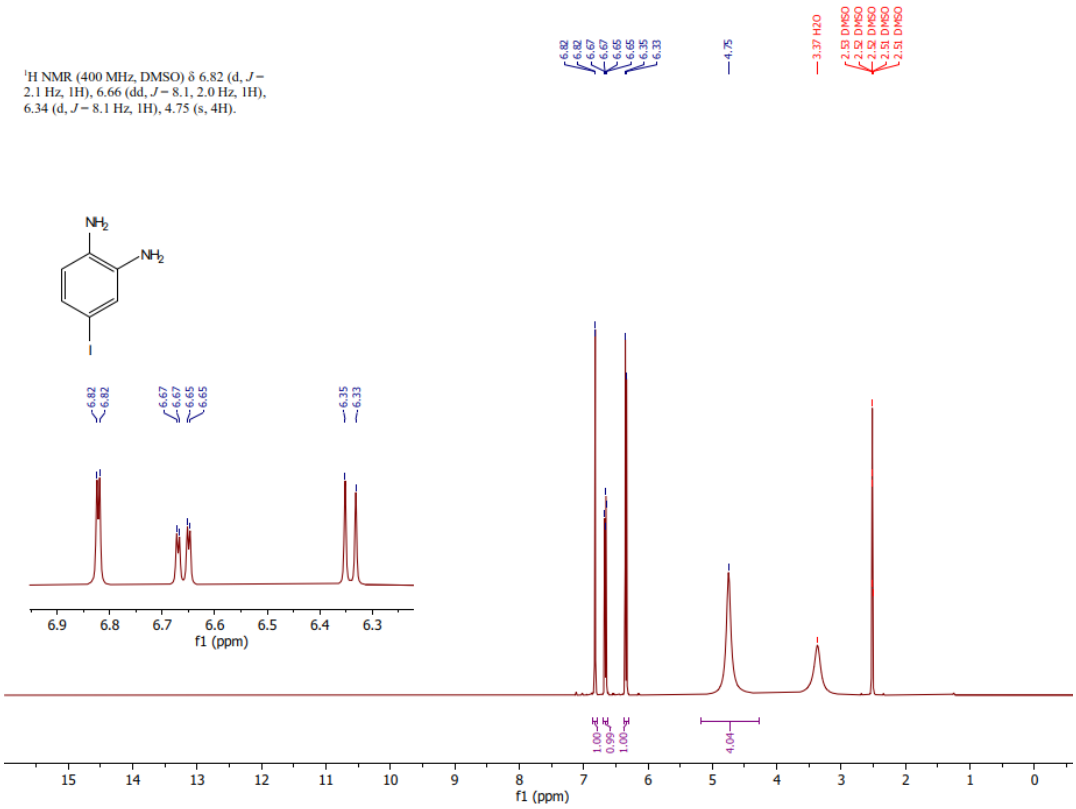
Figure S31. Cleavage kinetics of RNA **43** (150 nM) and conjugate **40** (1200 nM). Blue: data points; orange: linear fit.



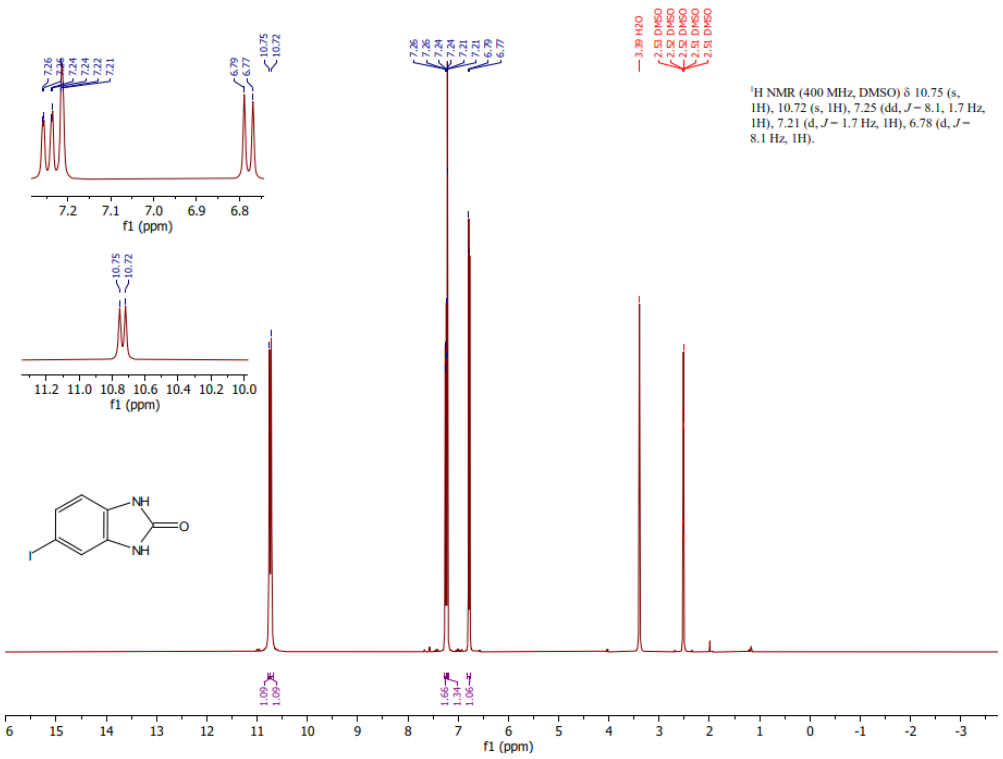
Compound **8**, ^{1}H-NMR



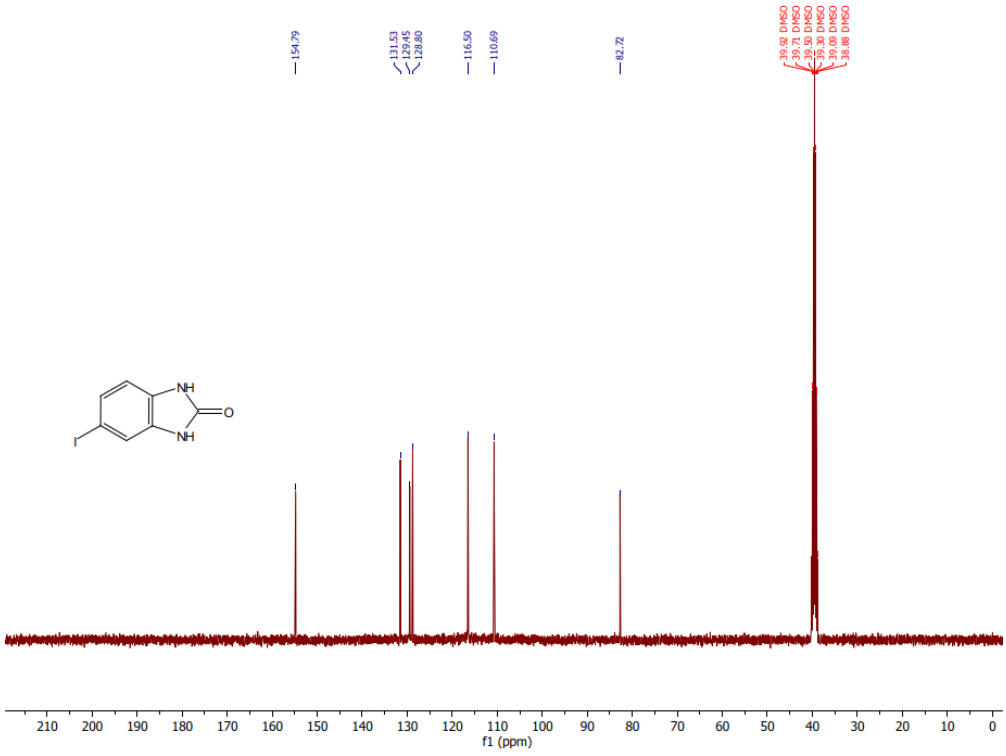
Compound **8**, ^{13}C-NMR



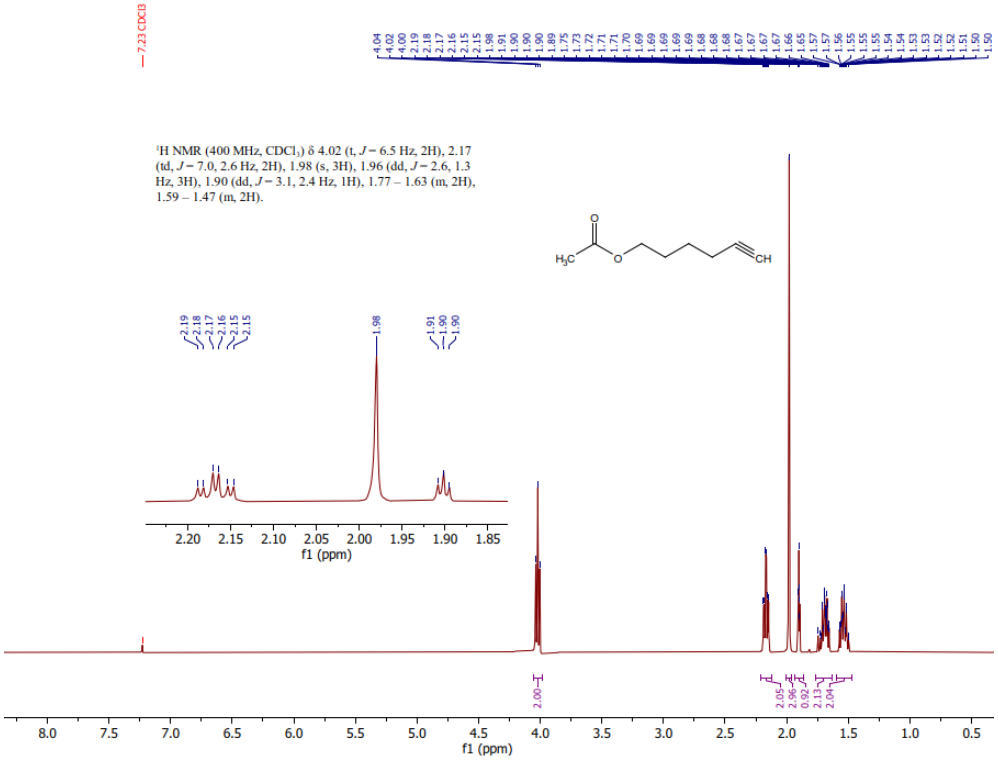
4-Iodobenzene-1,2-diamine, ^{1}H-NMR



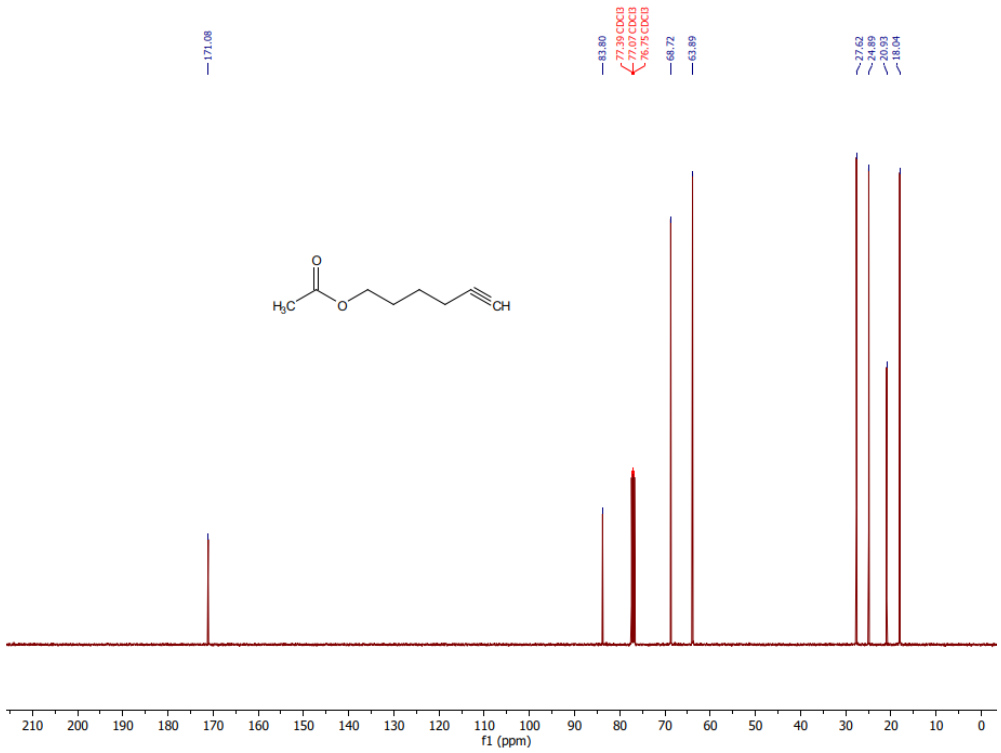
Compound **9**, ^{1}H-NMR



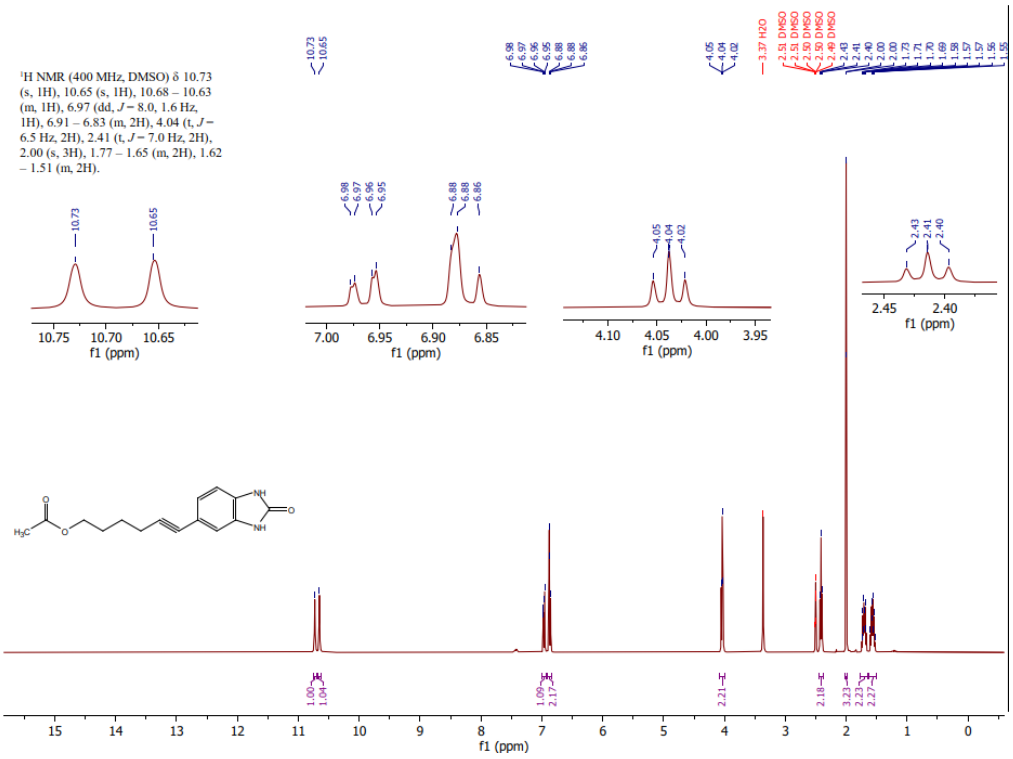
Compound **9**, ^{13}C-NMR



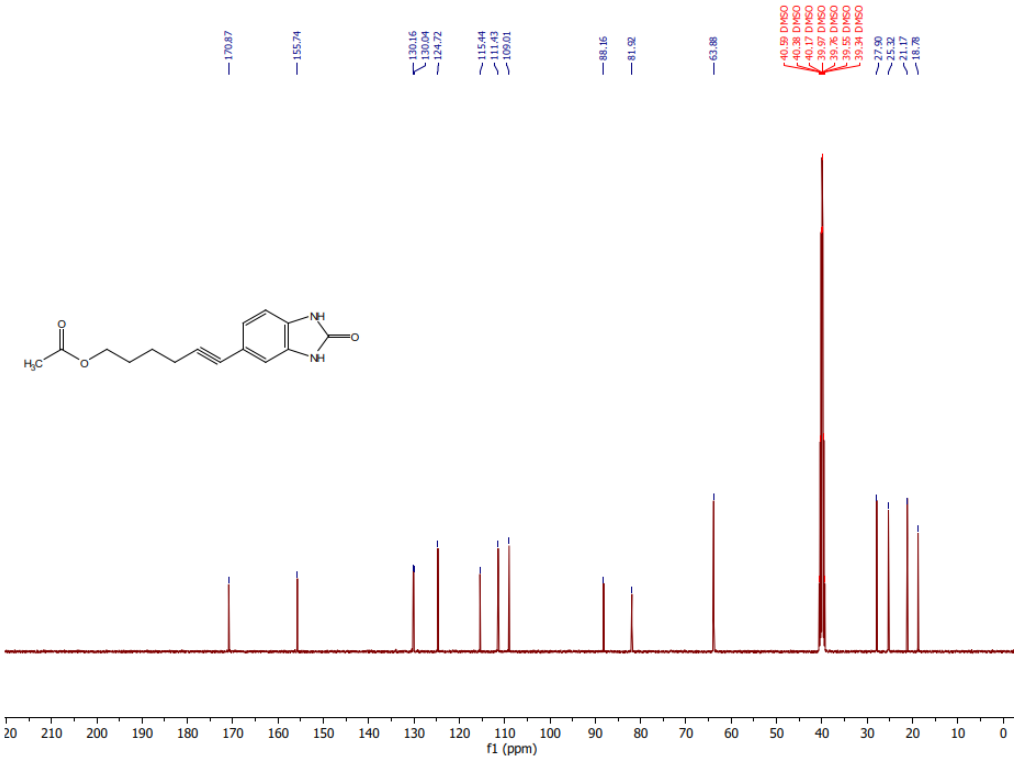
Compound **10**, ^{1}H-NMR



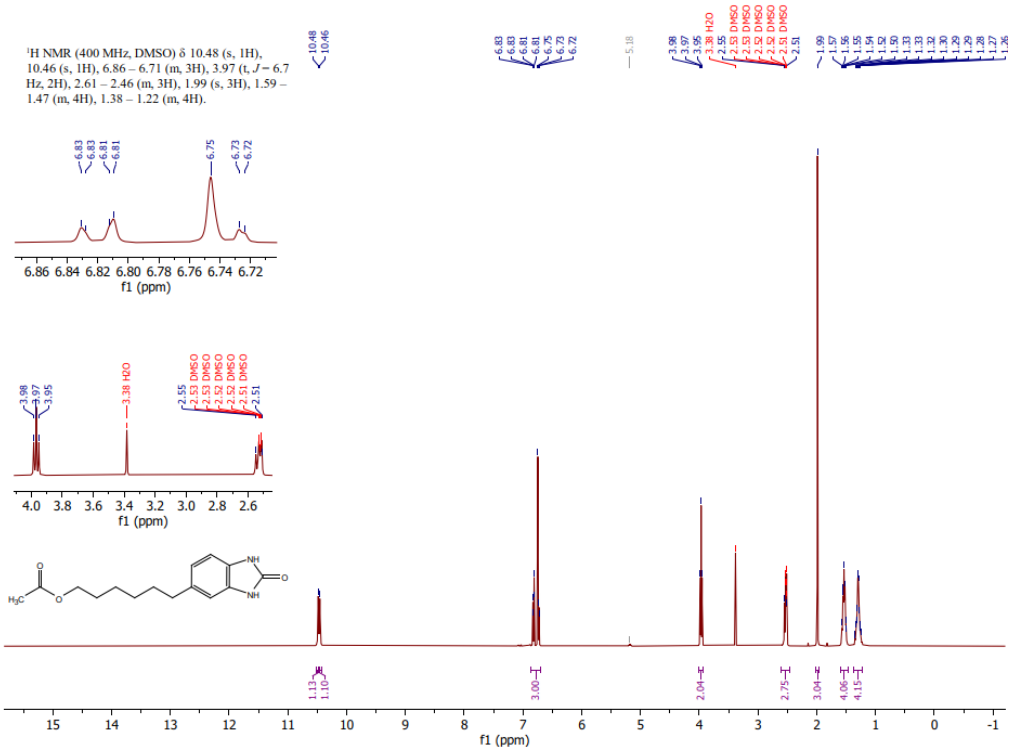
Compound **10**, ^{13}C-NMR



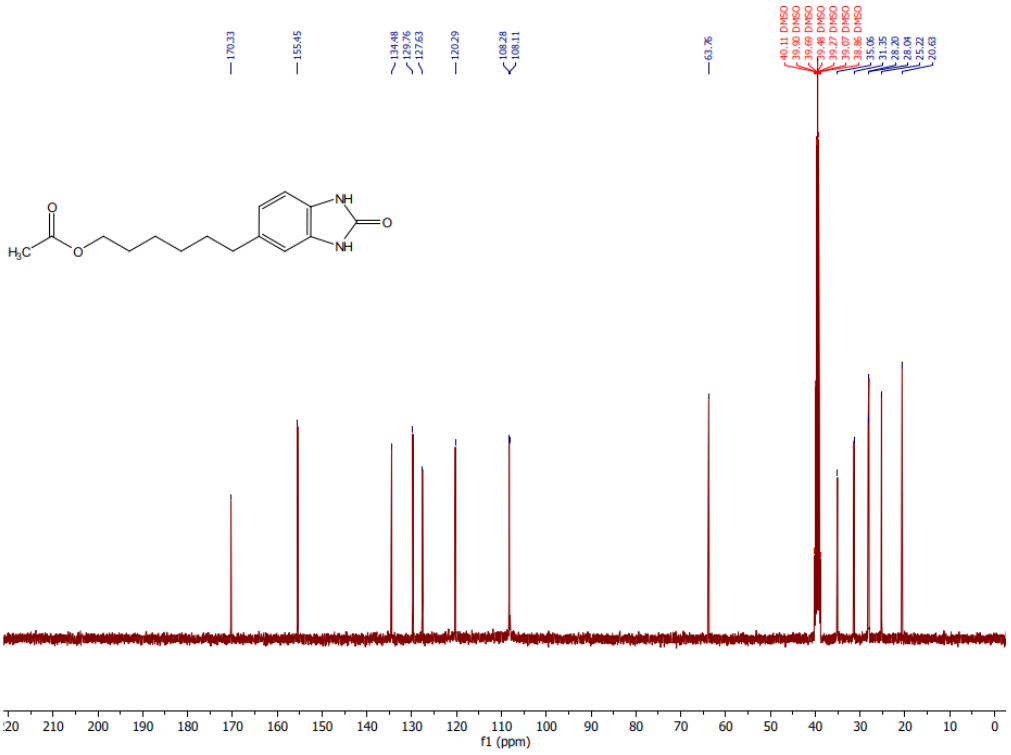
Compound **11**, ^{1}H-NMR



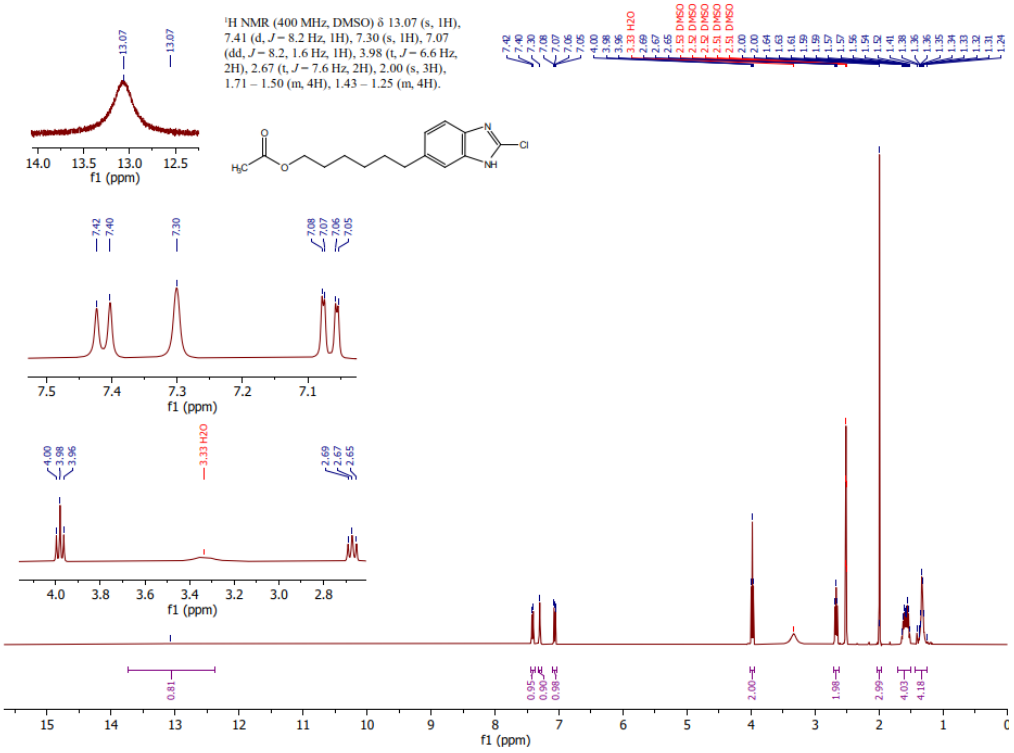
Compound **11**, ^{13}C-NMR



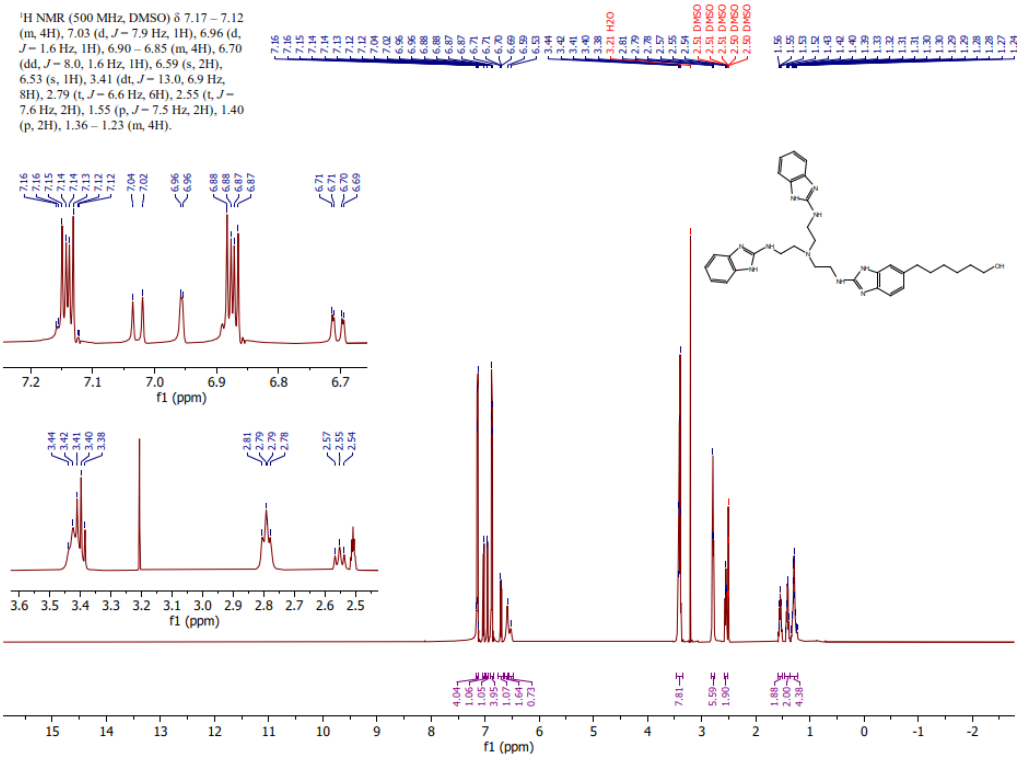
Compound **12**, ^{1}H-NMR



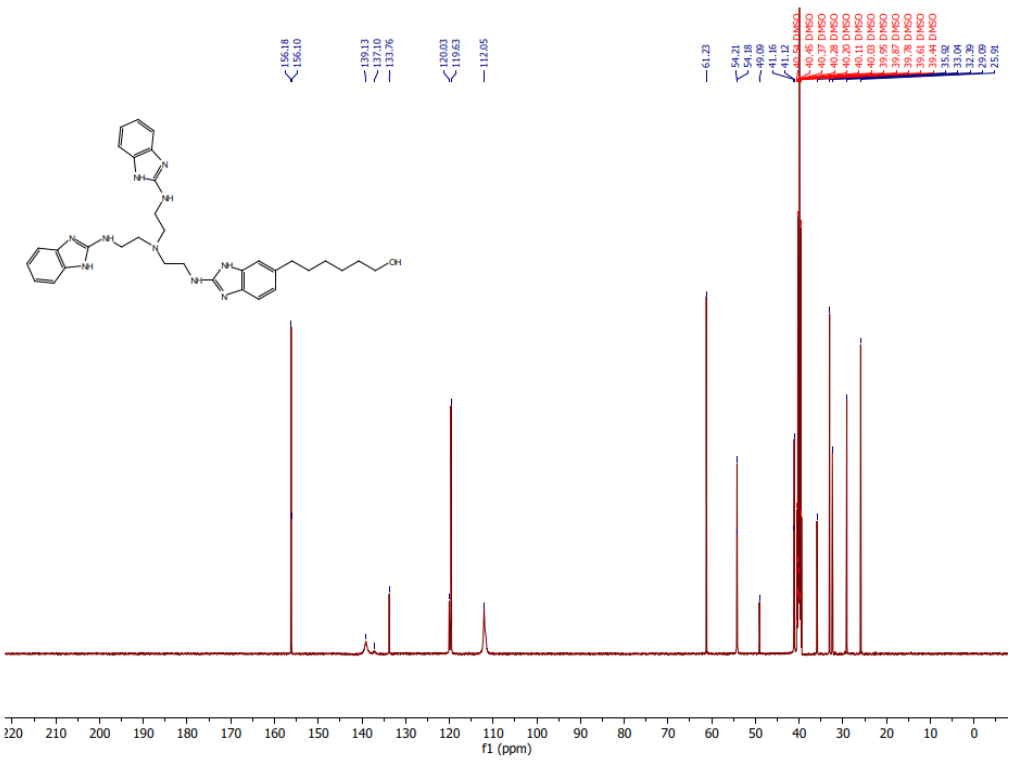
Compound **12**, ^{13}C-NMR



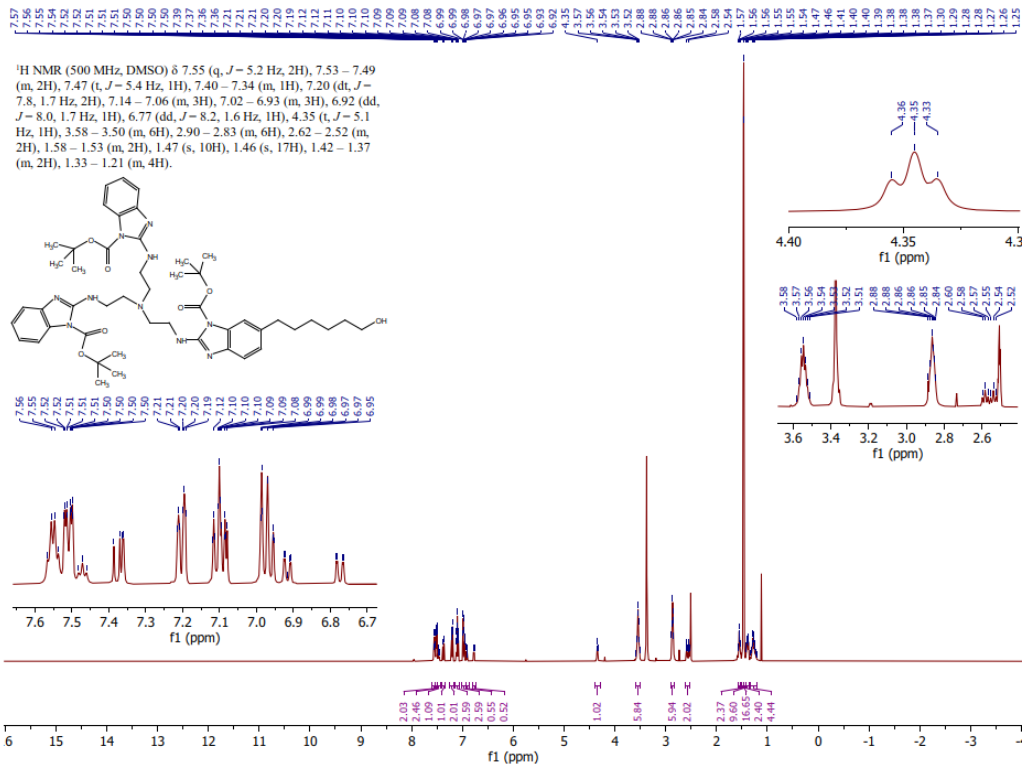
Compound **13**, ^{1}H-NMR



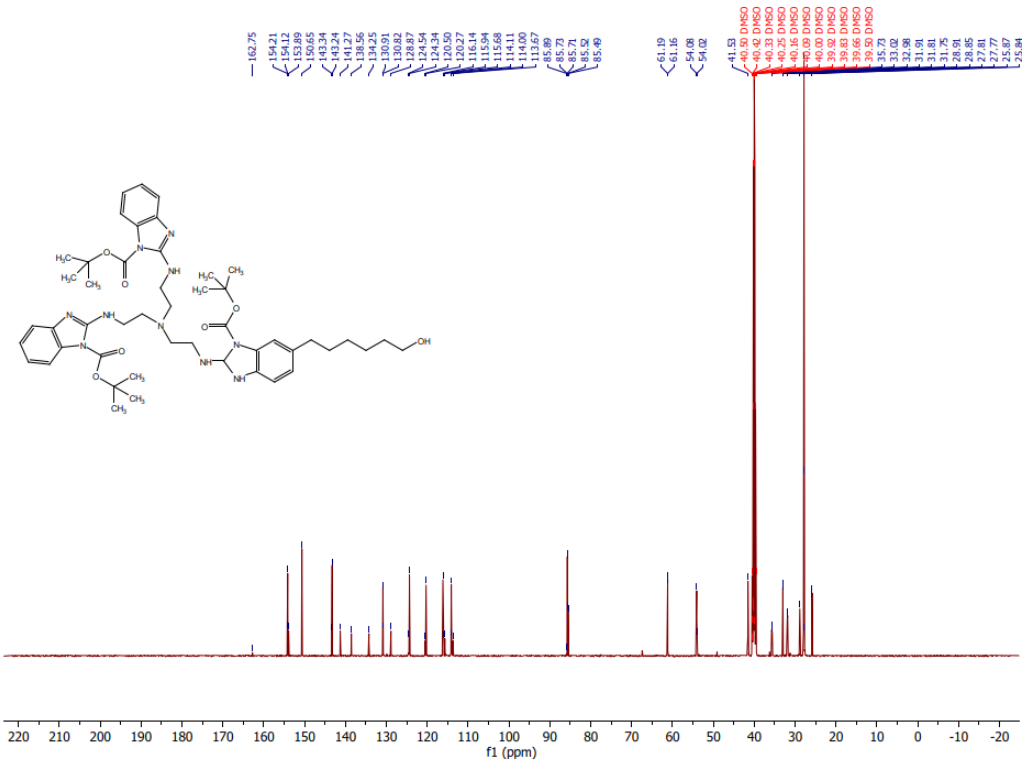
Compound **15**, ^{1}H-NMR



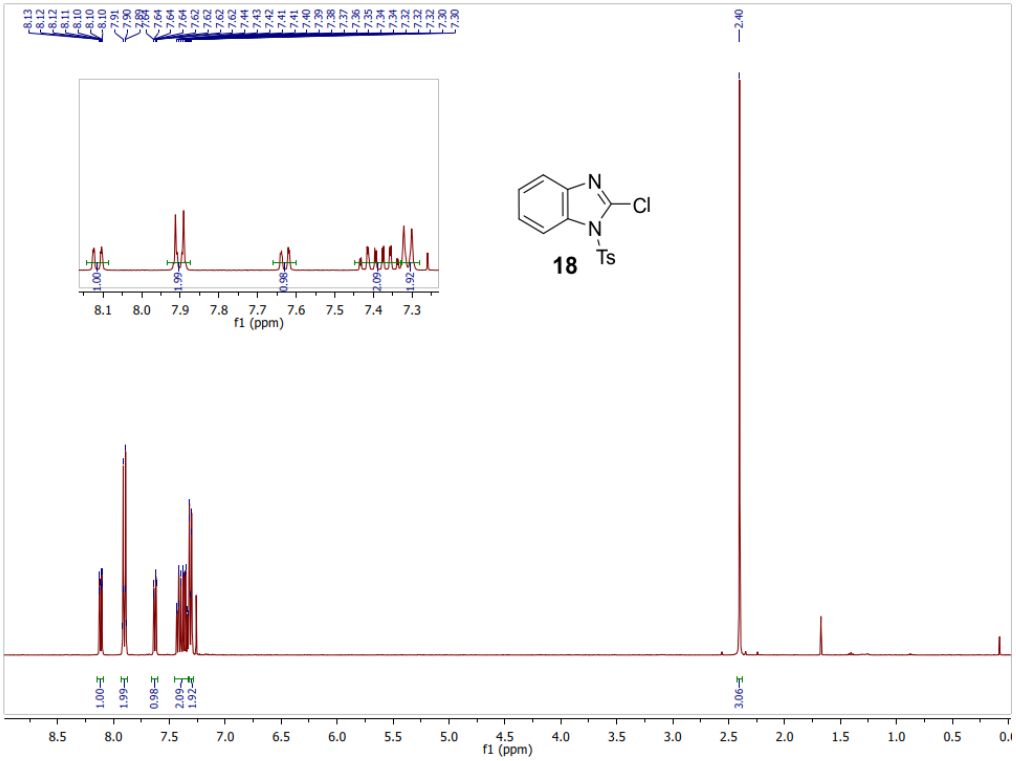
Compound **15**, ^{13}C-NMR



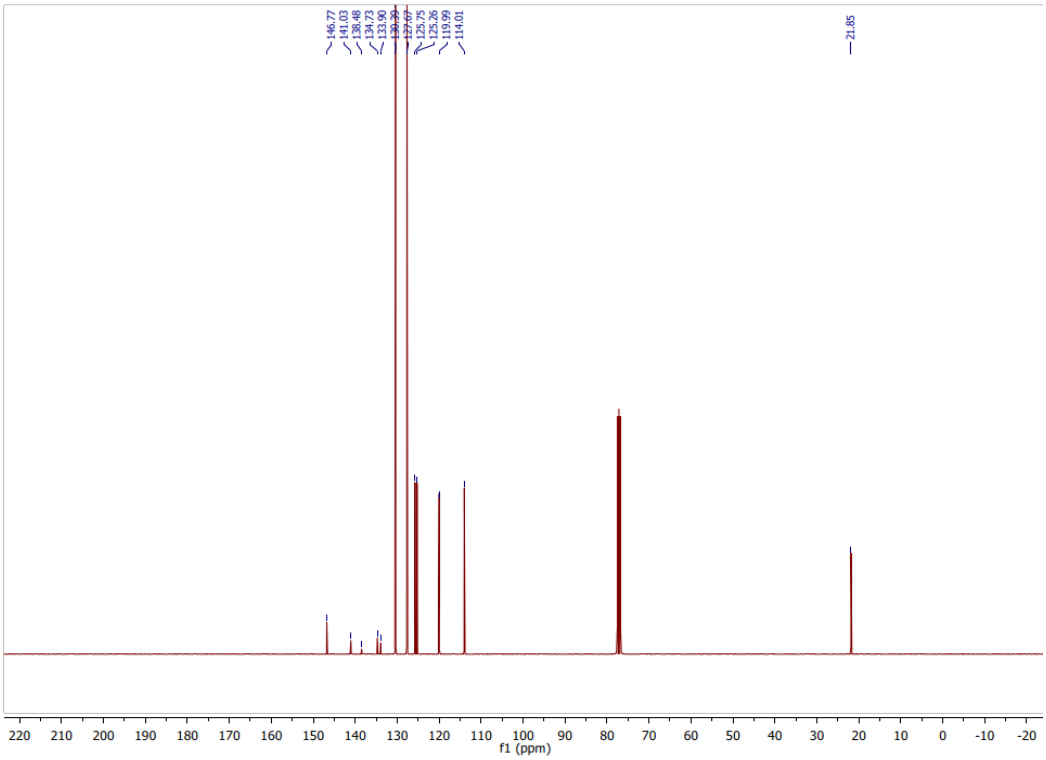
Compound **16**, prepared from benzimidazolone **12**, ^{1}H-NMR (mixture of two constitutional isomers)



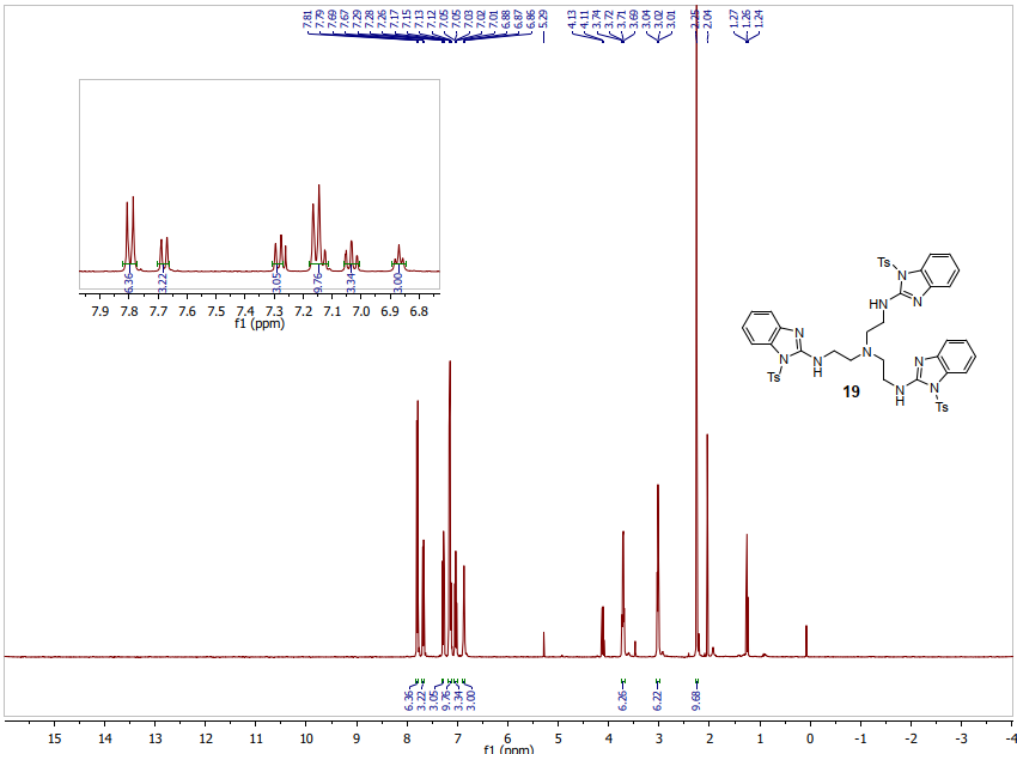
Compound **16**, prepared from benzimidazolone **12**, ^{13}C-NMR (mixture of two constitutional isomers)



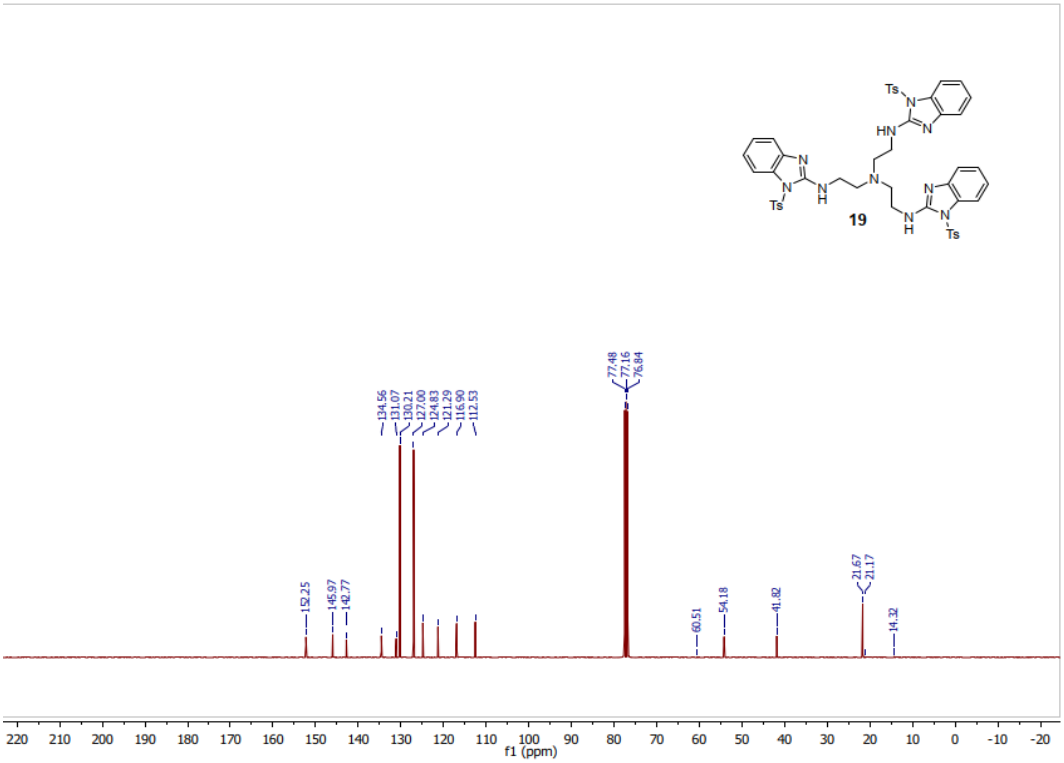
Compound **18**, ^{1}H-NMR



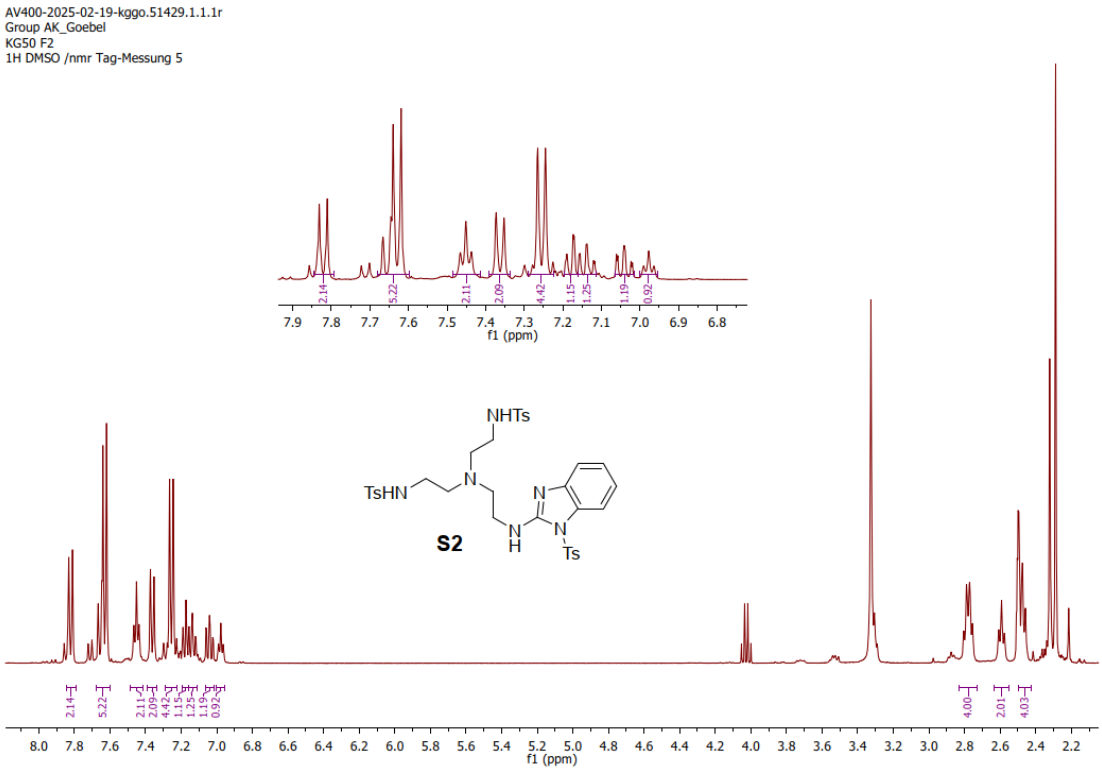
Compound **18**, ^{13}C-NMR



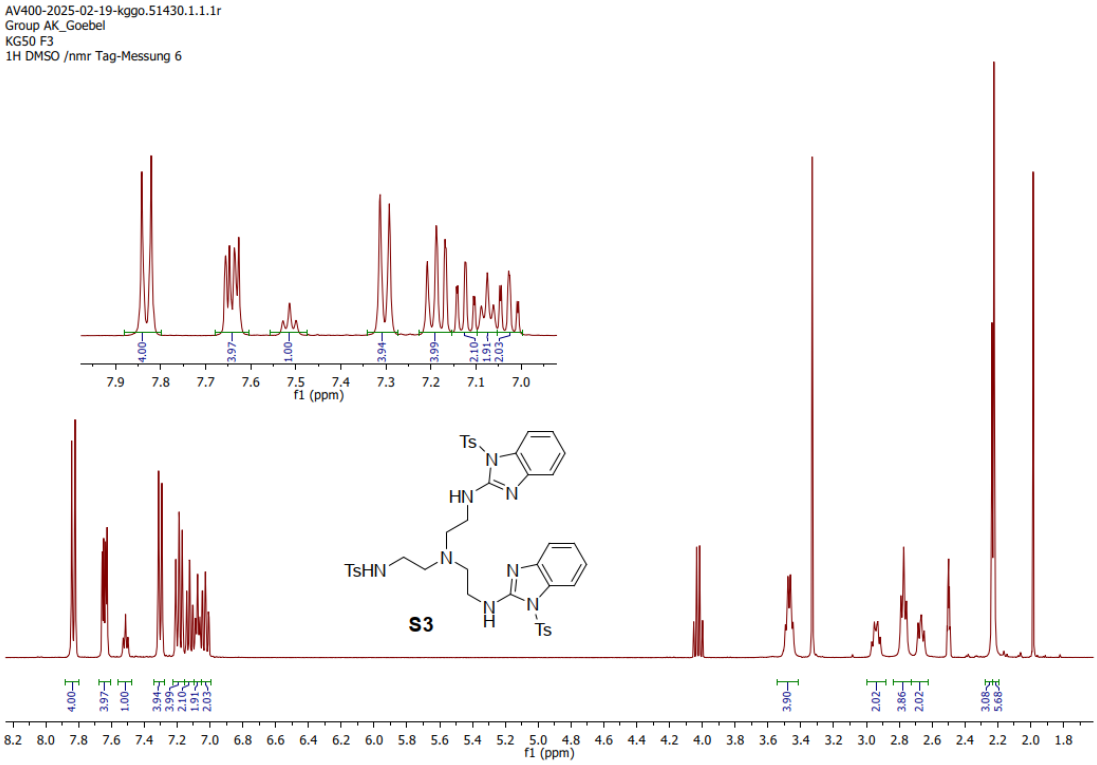
Compound **19**, ^{1}H-NMR



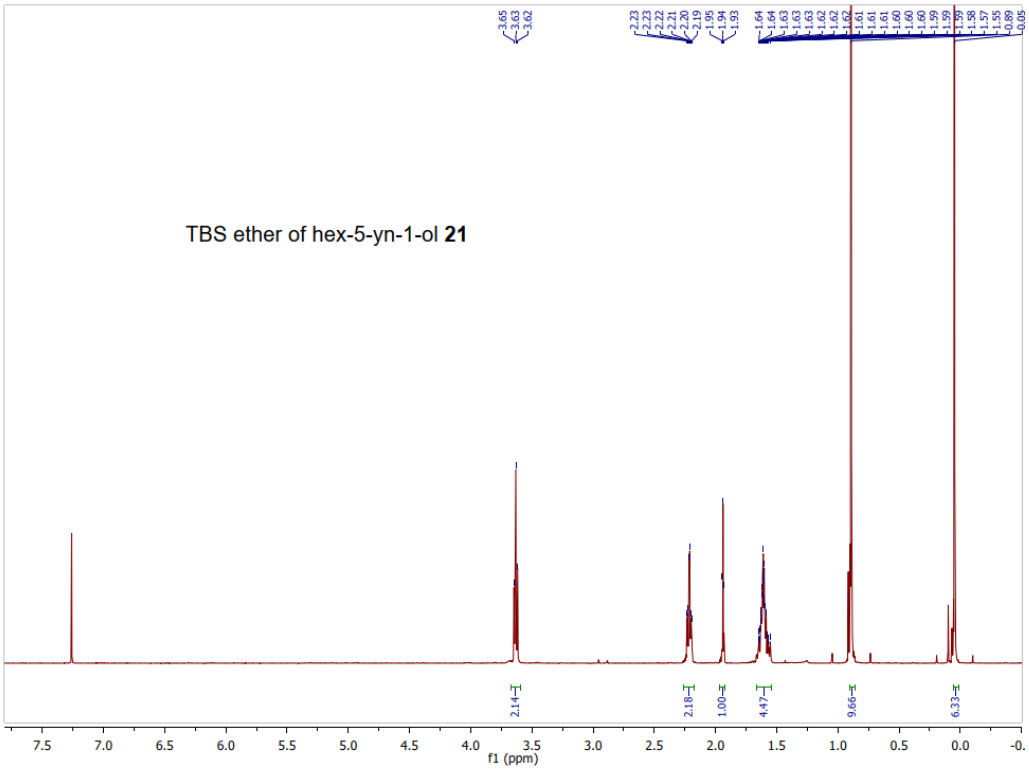
Compound **19**, ^{13}C-NMR



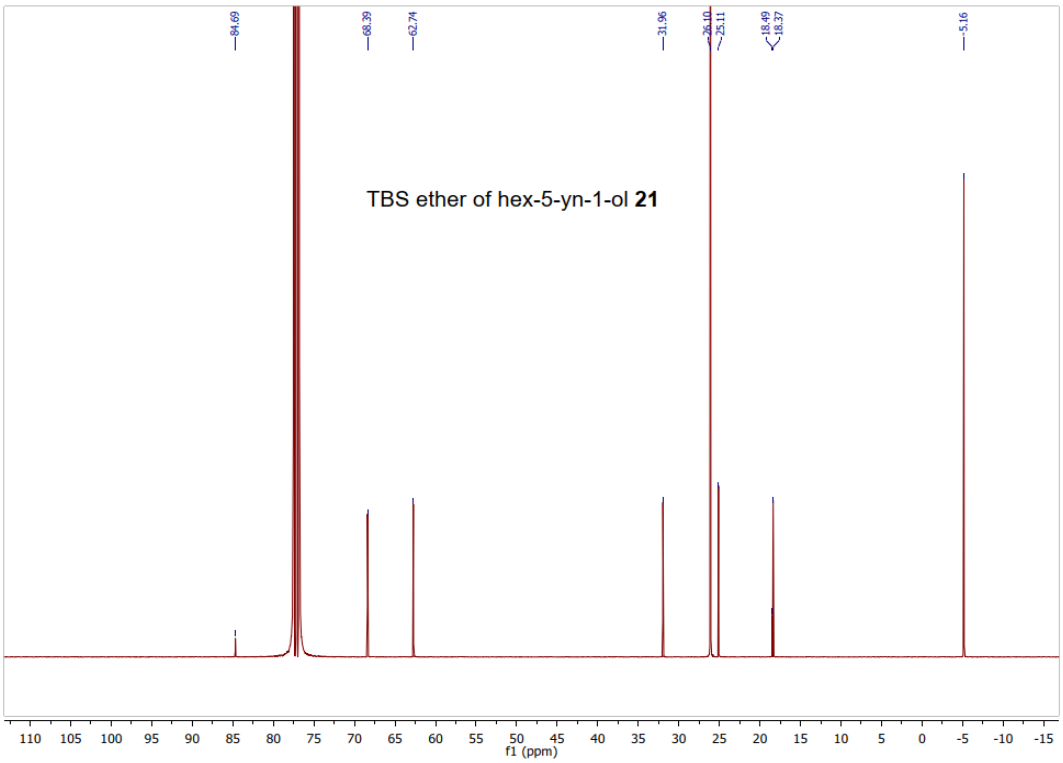
Compound **S2**, ^{1}H-NMR



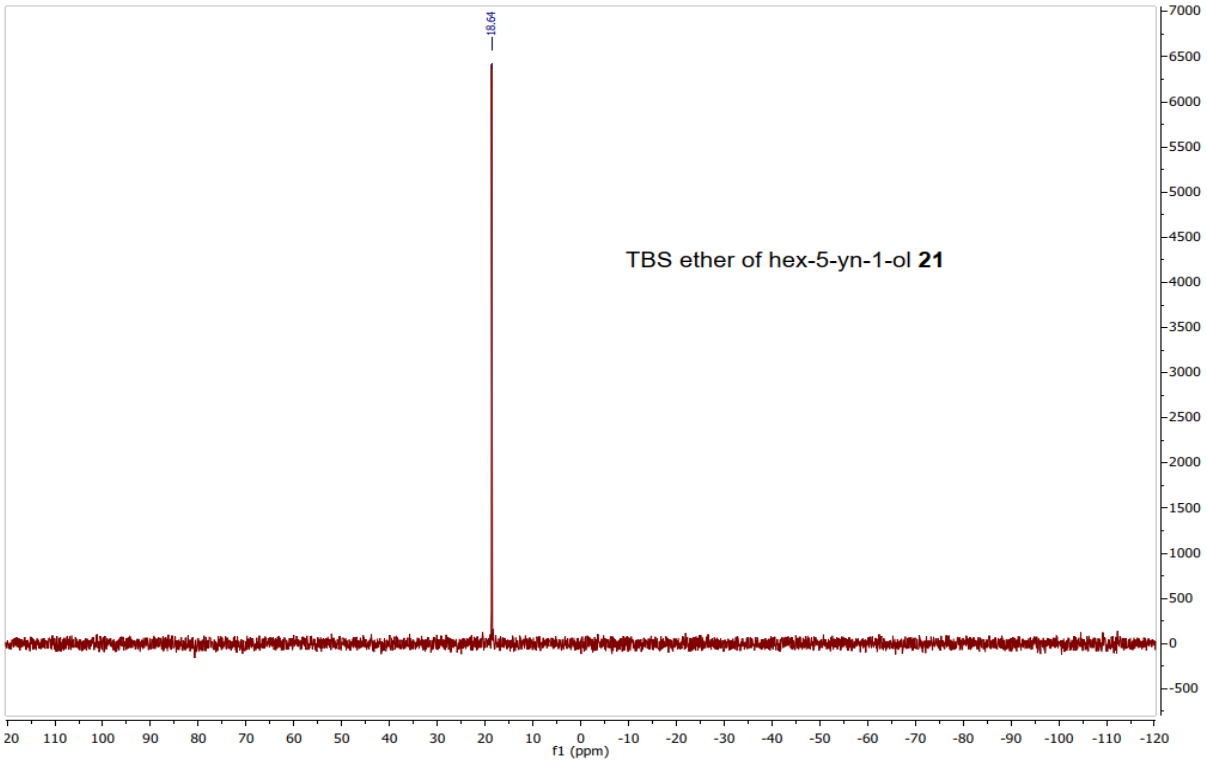
Compound **S3**, ^{1}H-NMR



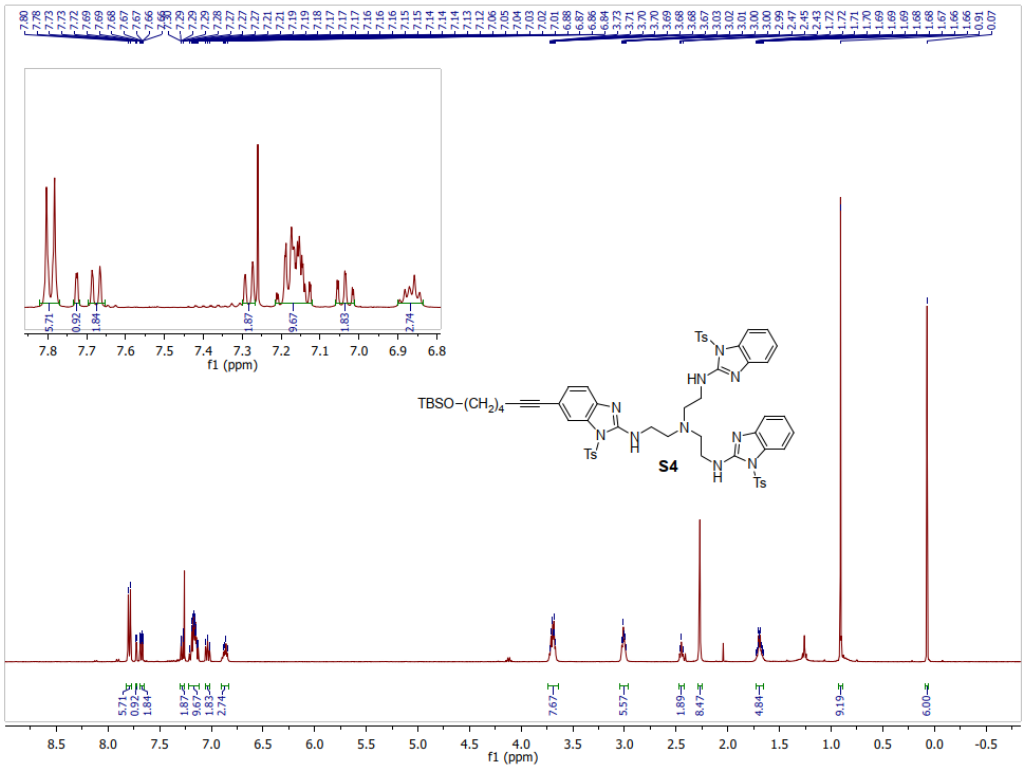
Compound **21**, ^{1}H-NMR



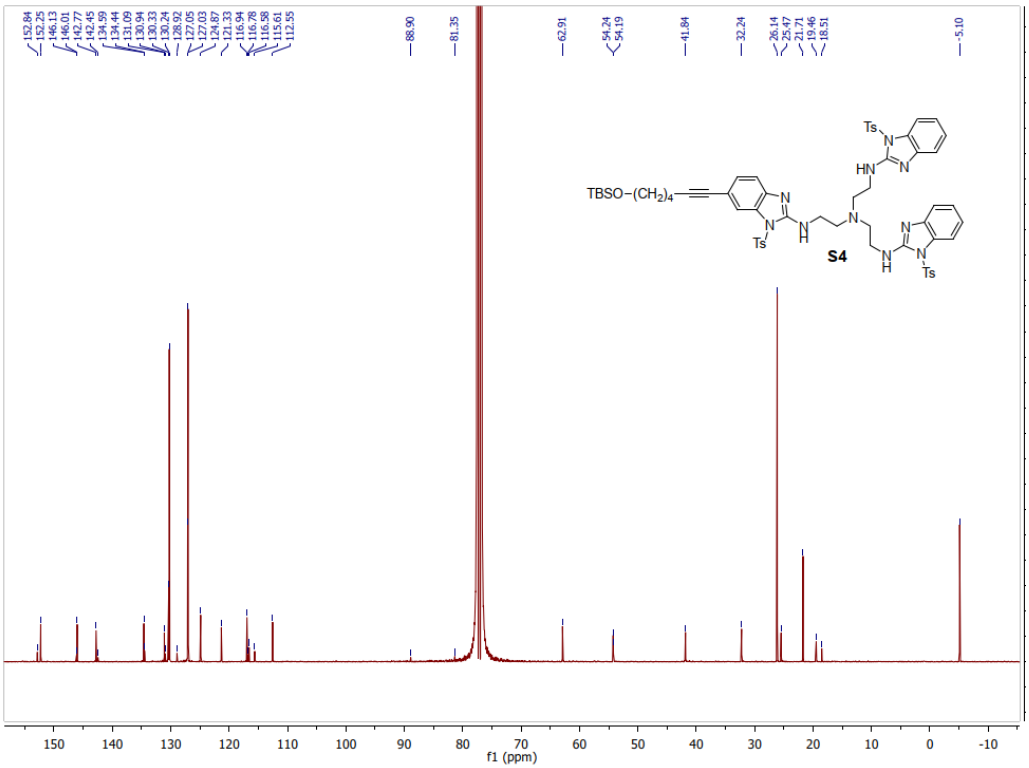
Compound **21**, ^{13}C-NMR



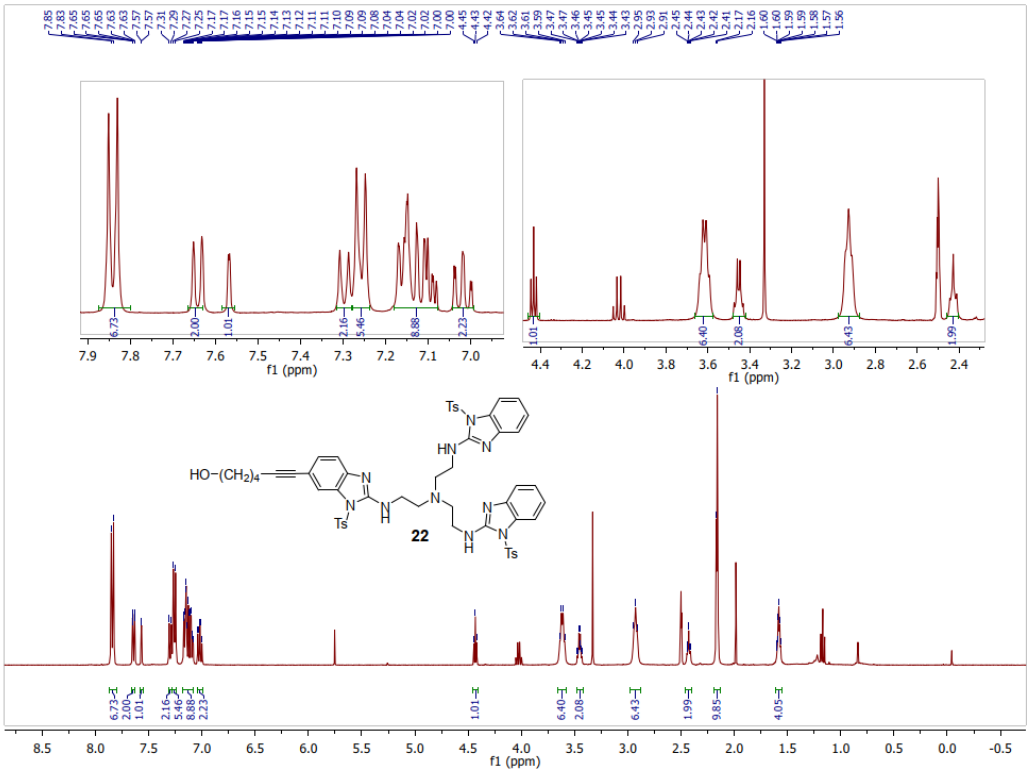
Compound **21**, ^{29}Si-NMR



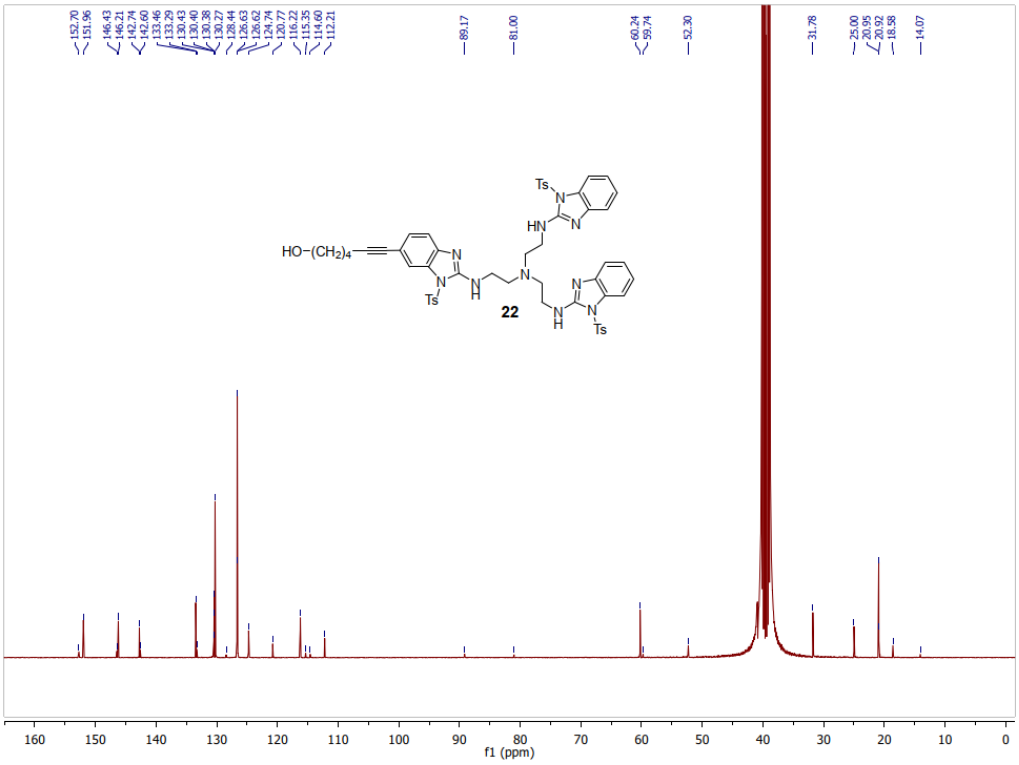
Compound **S4**, ^{1}H-NMR



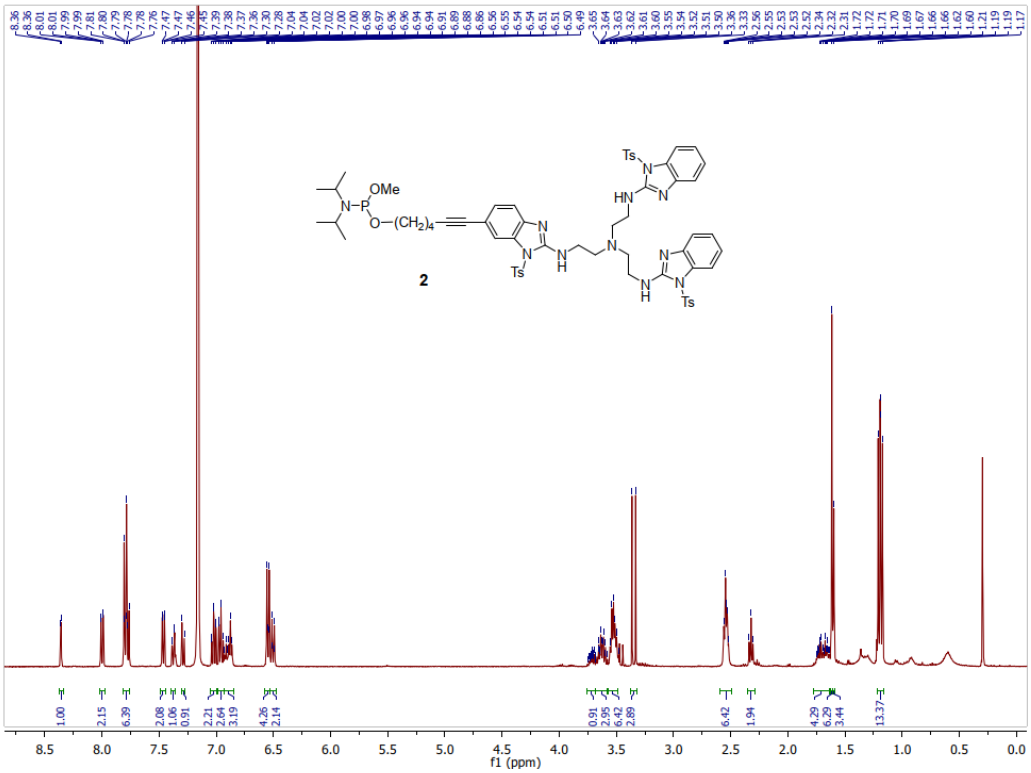
Compound **S4**, ^{13}C-NMR



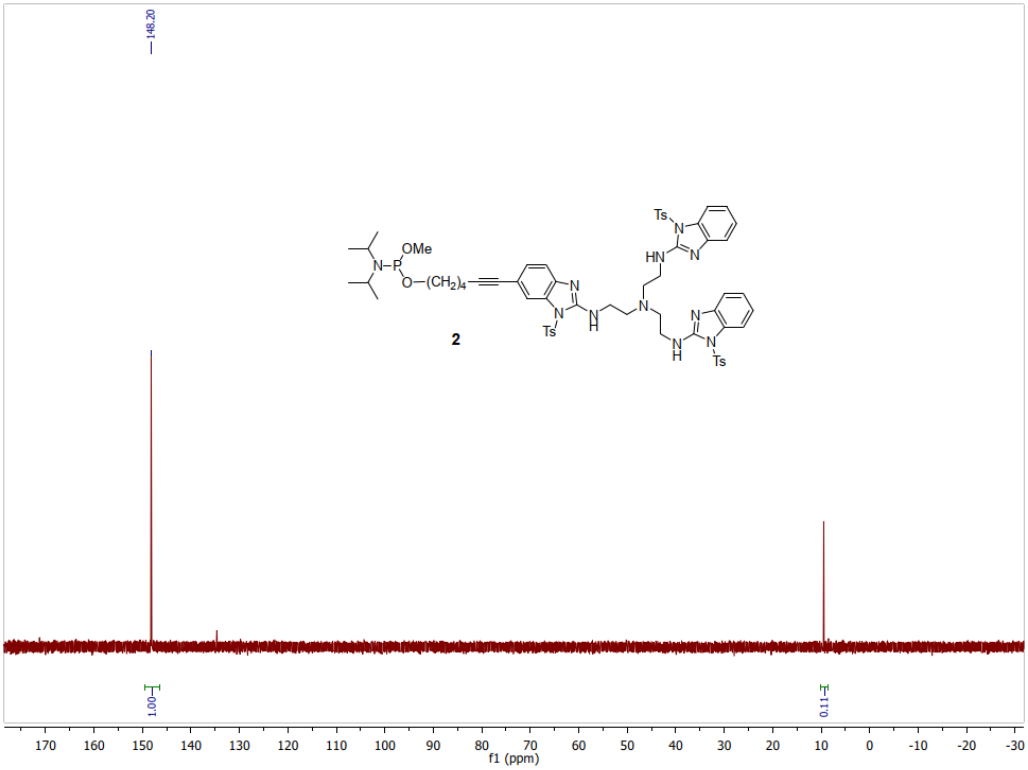
Compound **22**, ^{1}H-NMR



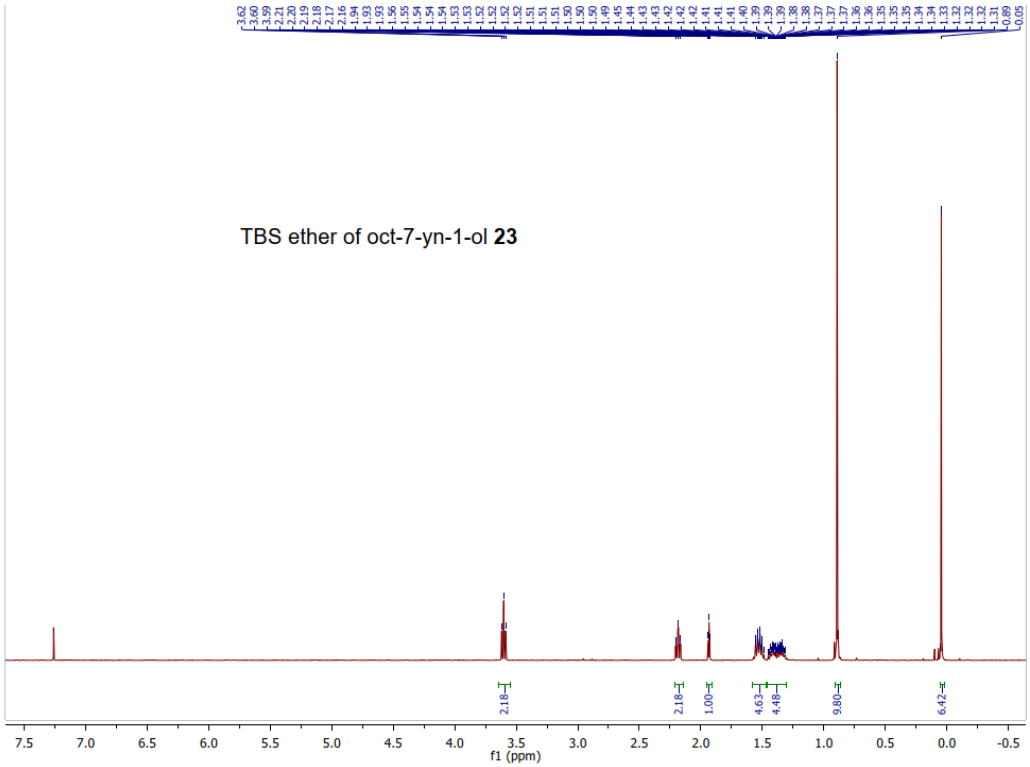
Compound **22**, ^{13}C-NMR



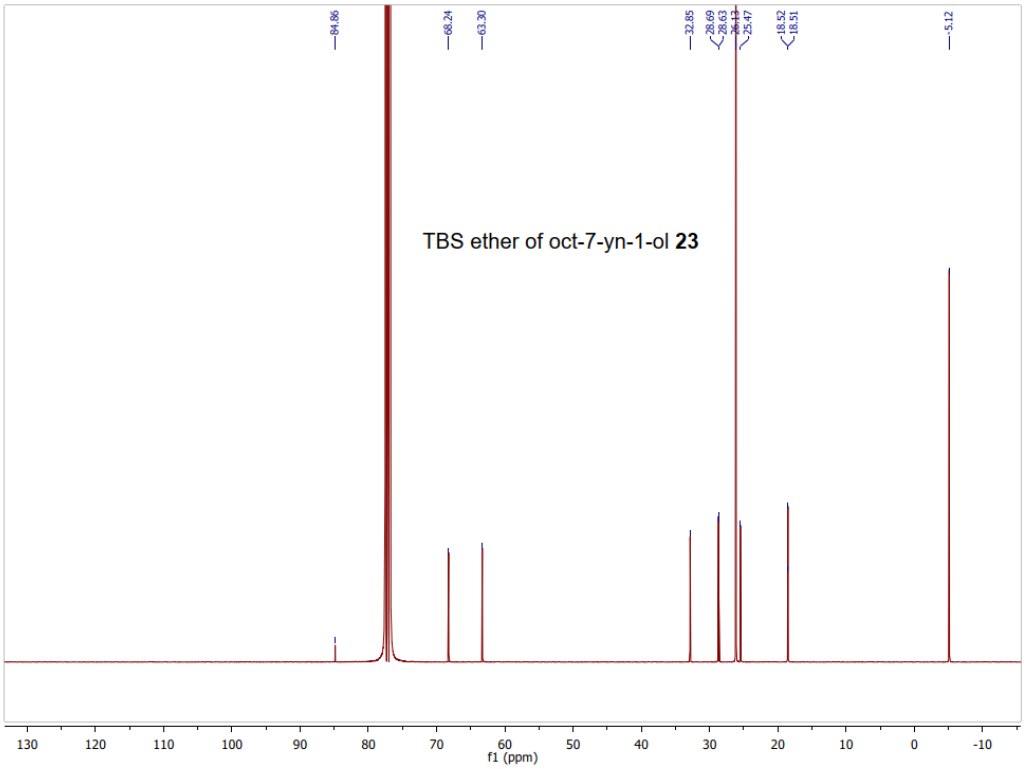
Compound **2**, ^{1}H-NMR



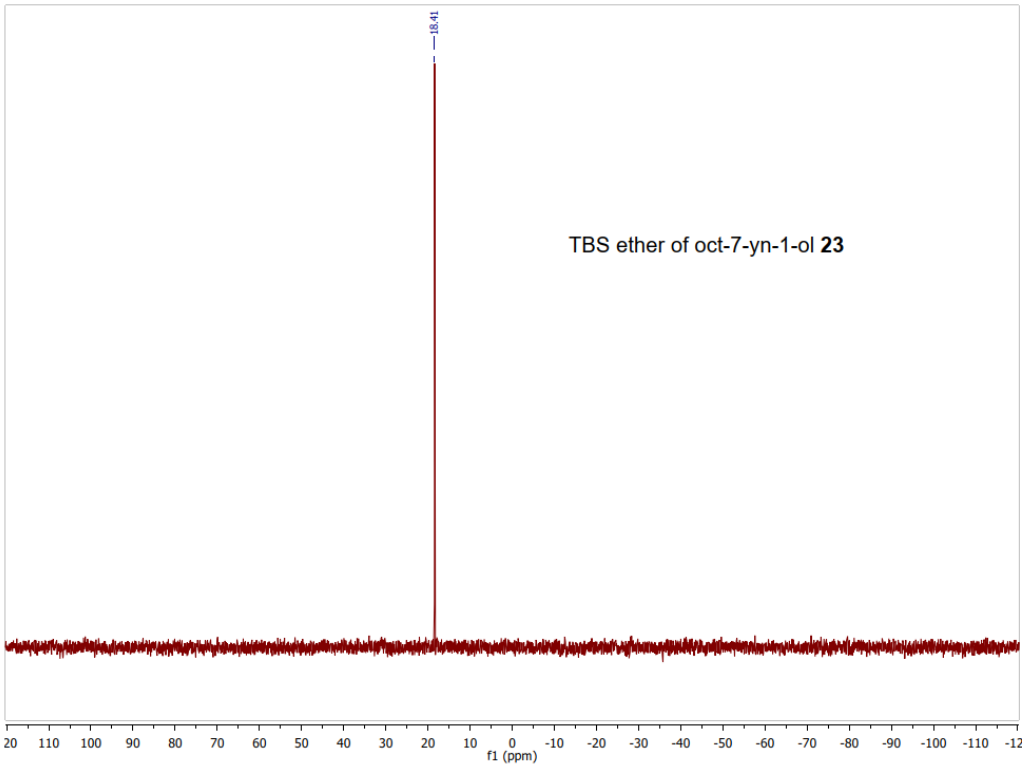
Compound **2**, ^{31}P-NMR



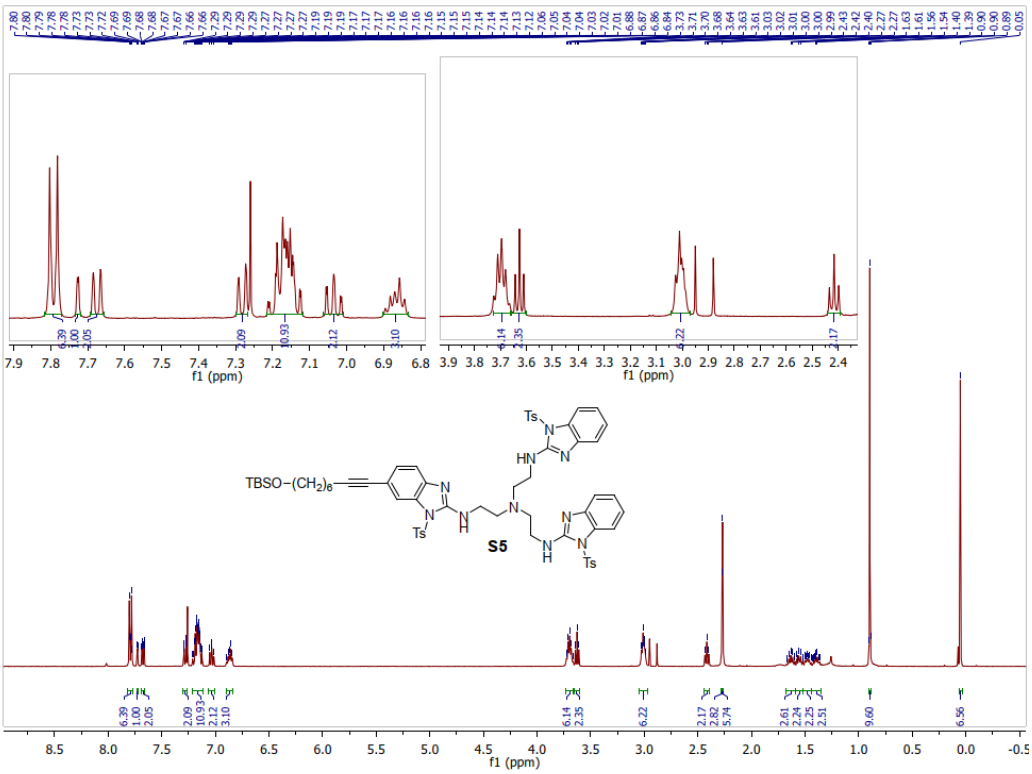
Compound **23**, ^{1}H-NMR



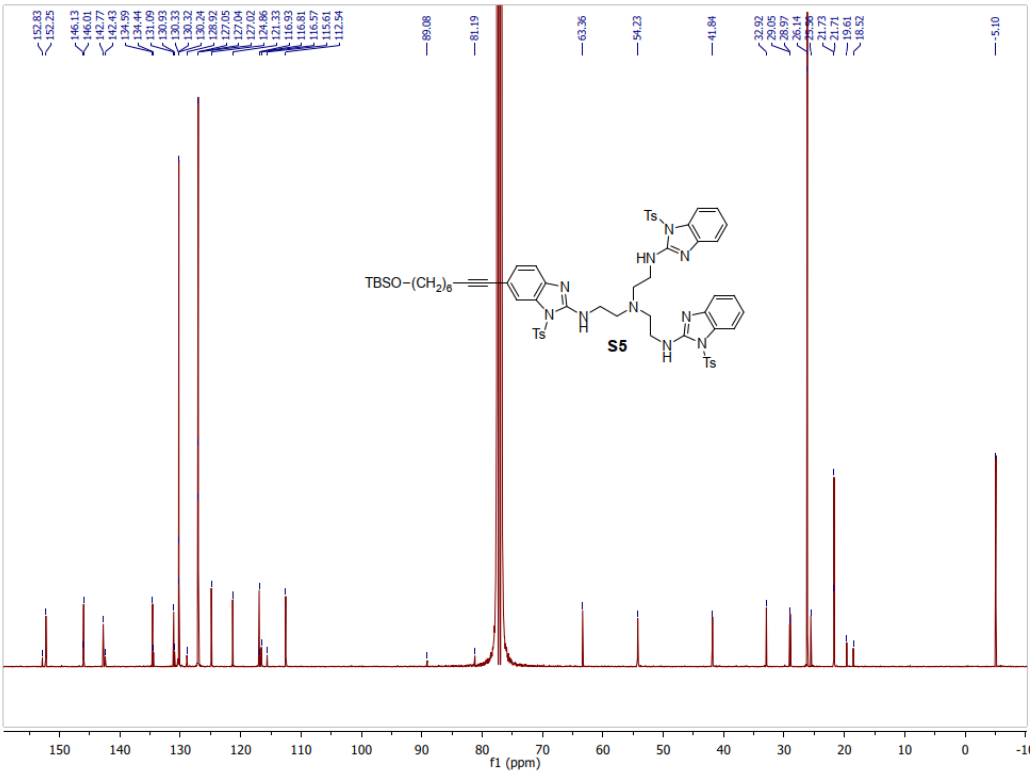
Compound **23**, ^{13}C-NMR



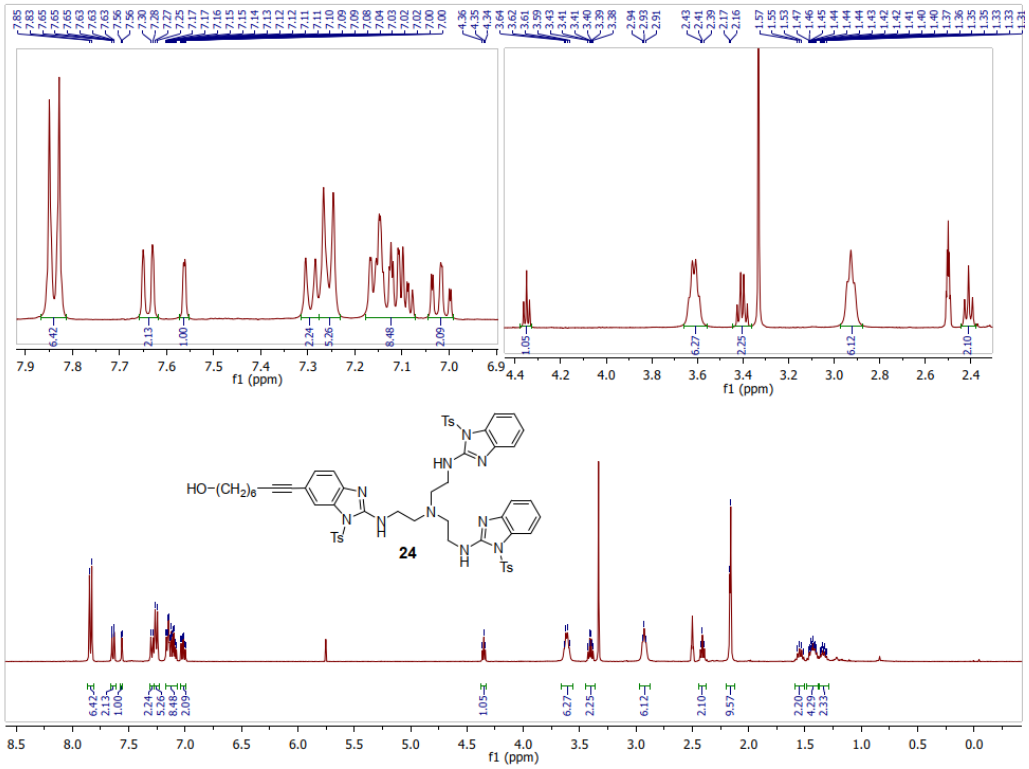
Compound **23**, ^{29}Si-NMR



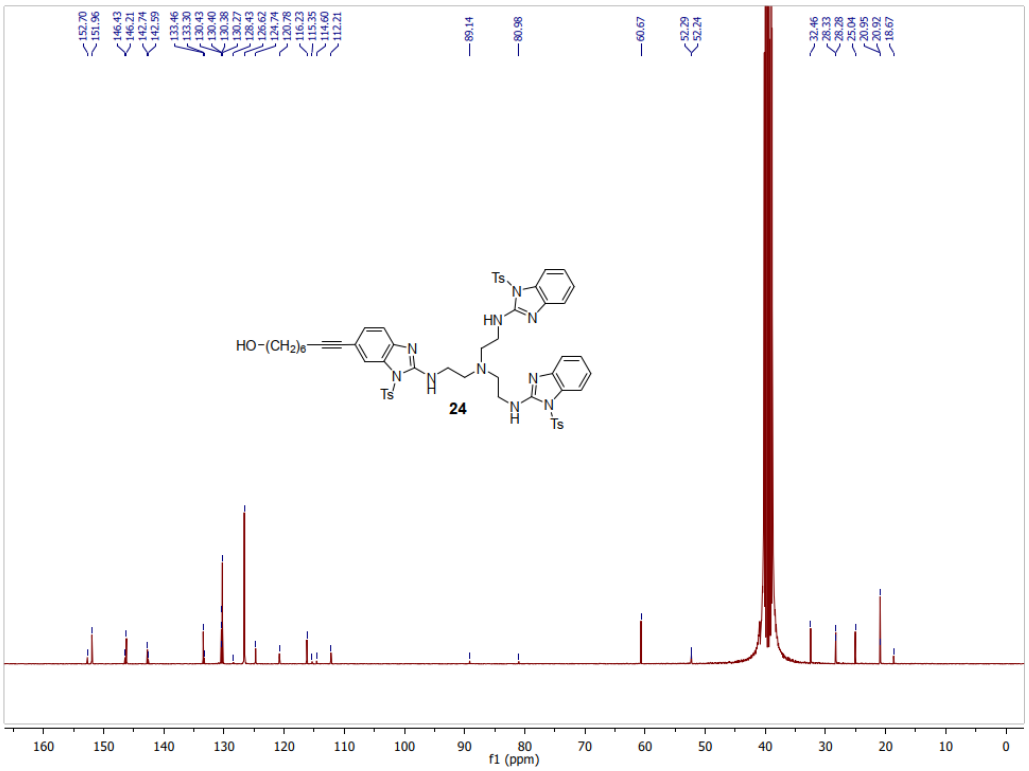
Compound **S5**, ^{1}H-NMR



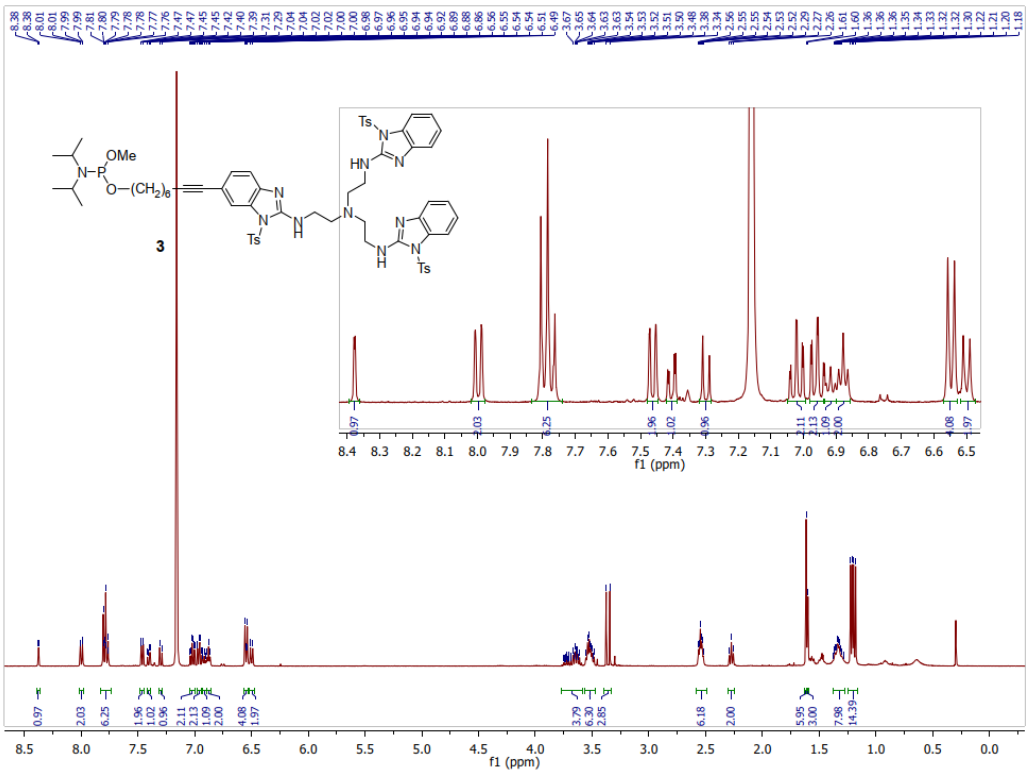
Compound **S5**, ^{13}C-NMR



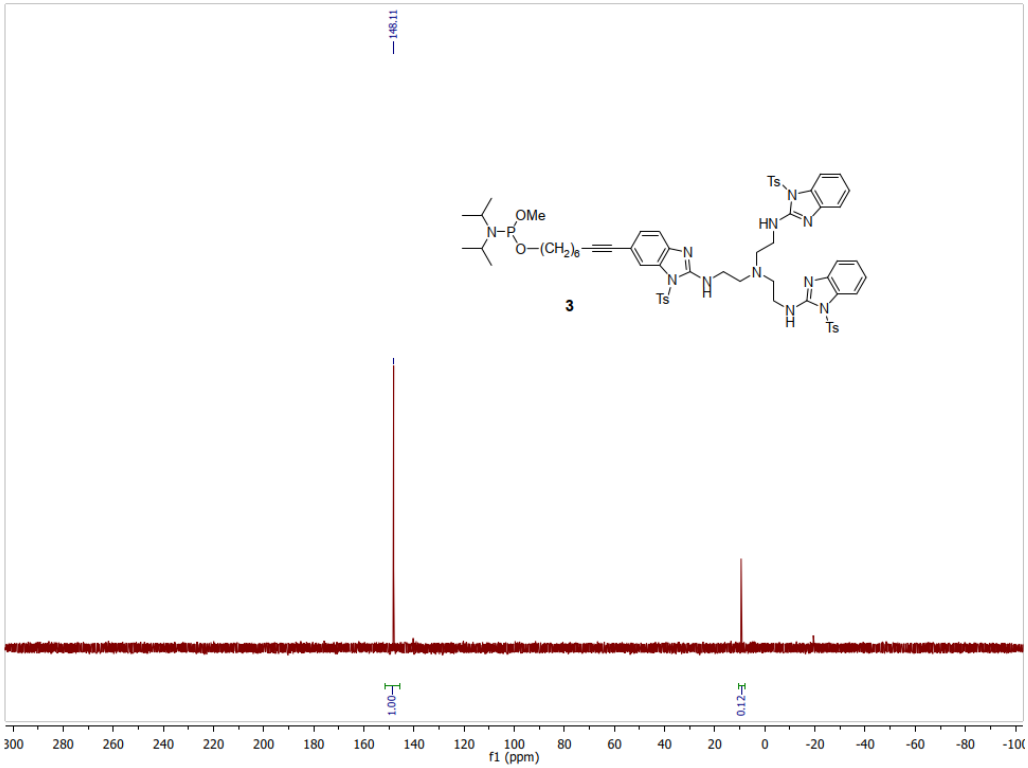
Compound **24**, ^{1}H-NMR



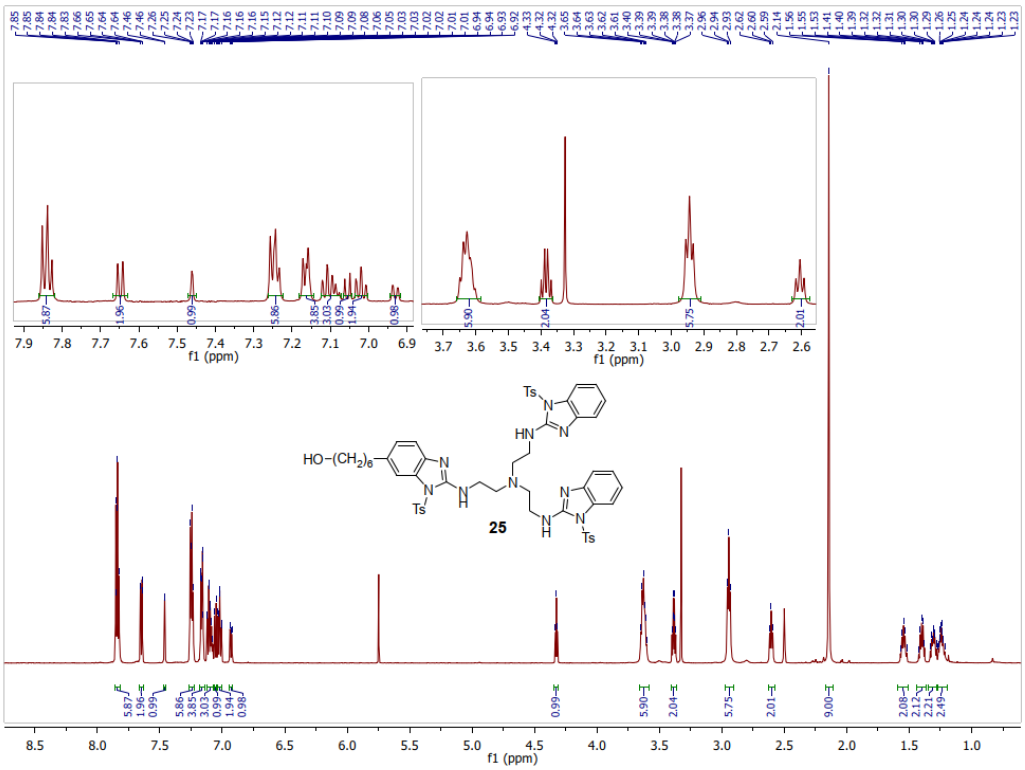
Compound **24**, ^{13}C-NMR



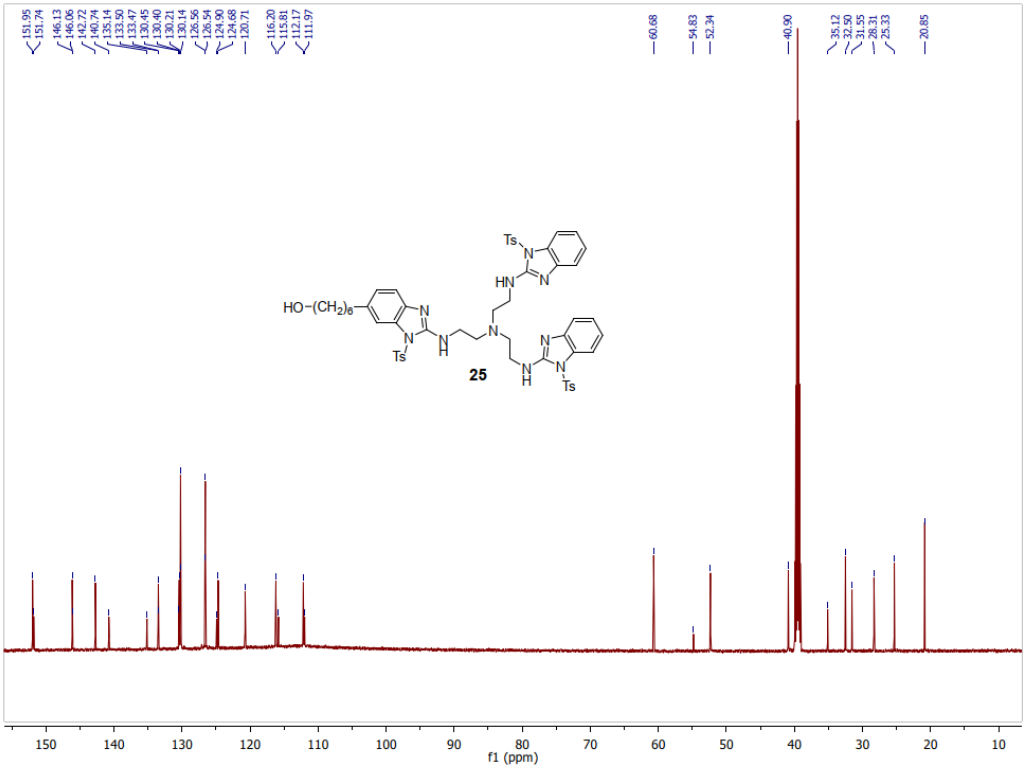
Compound **3**, ^{1}H-NMR



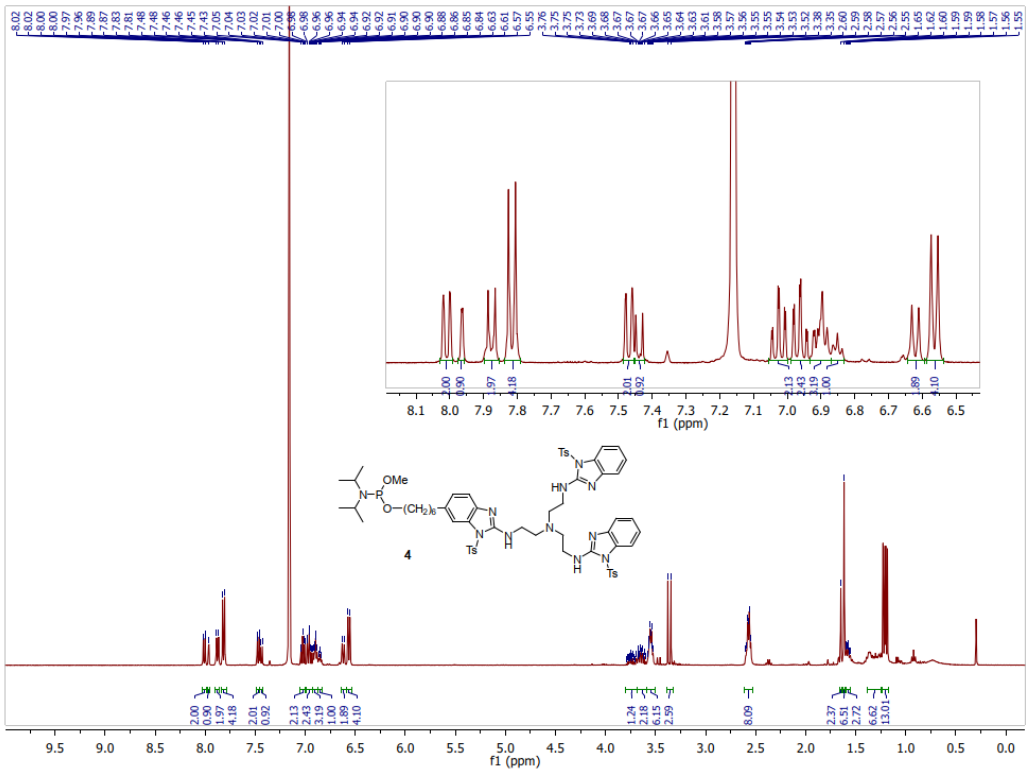
Compound **3**, ^{31}P-NMR



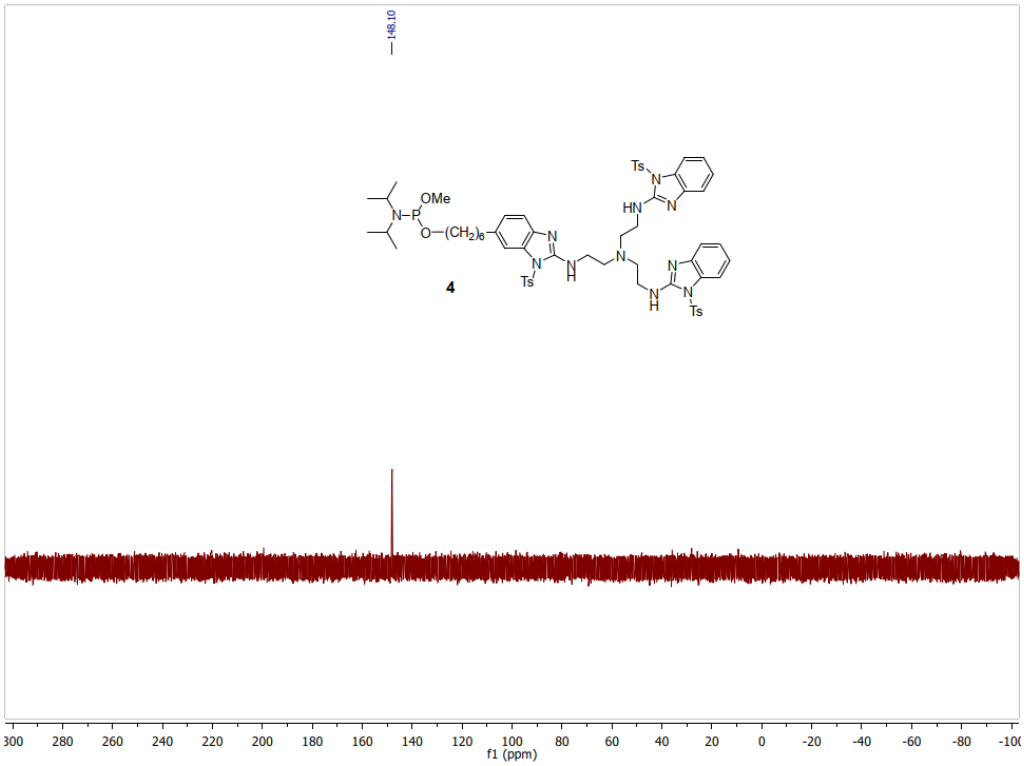
Compound **25**, ^{1}H-NMR, prepared via Suzuki reaction



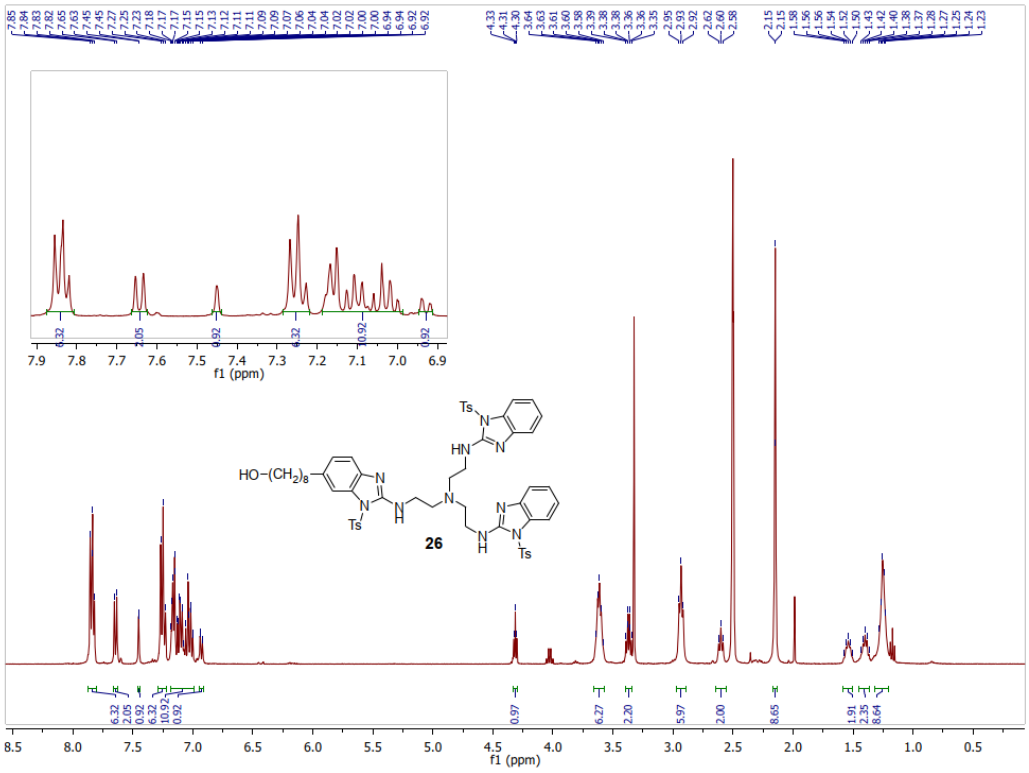
Compound **25**, ^{13}C-NMR, prepared via Suzuki reaction



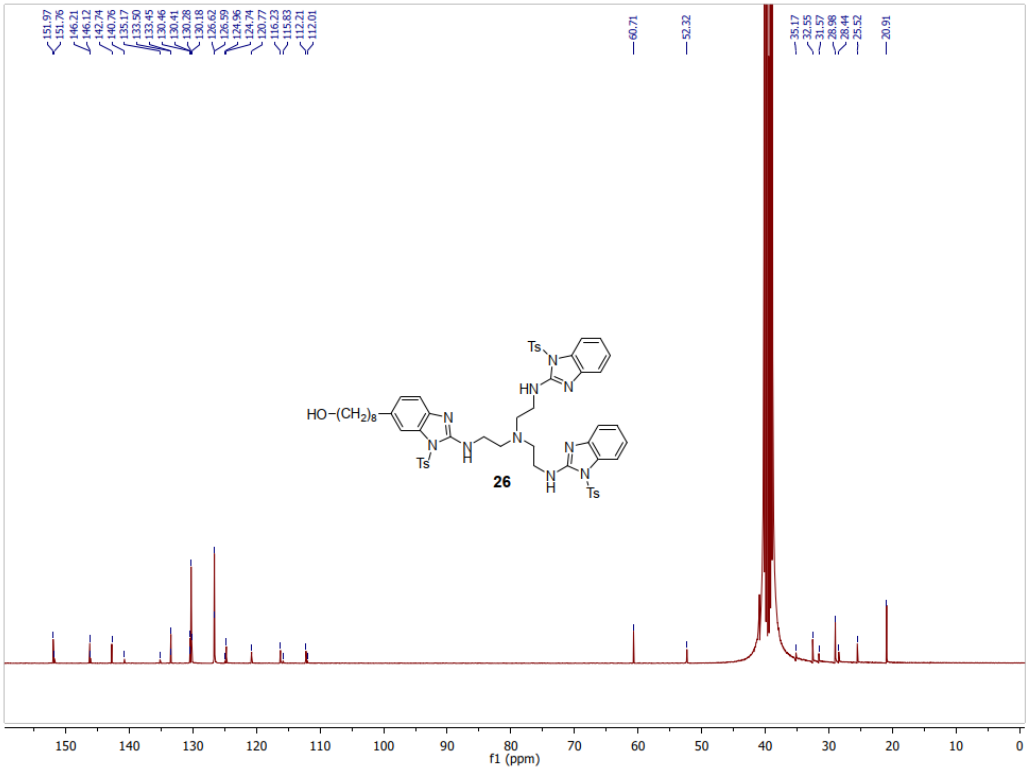
Compound **4**, ^{1}H-NMR, prepared via Suzuki reaction



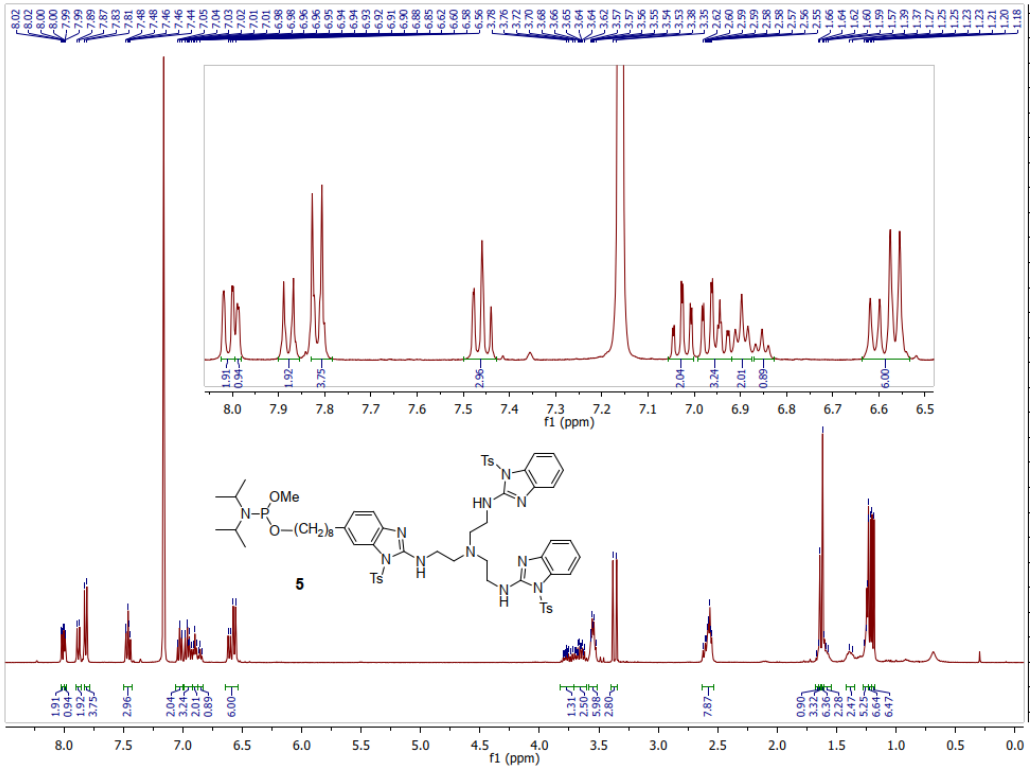
Compound **4**, ^{31}P-NMR, prepared via Suzuki reaction



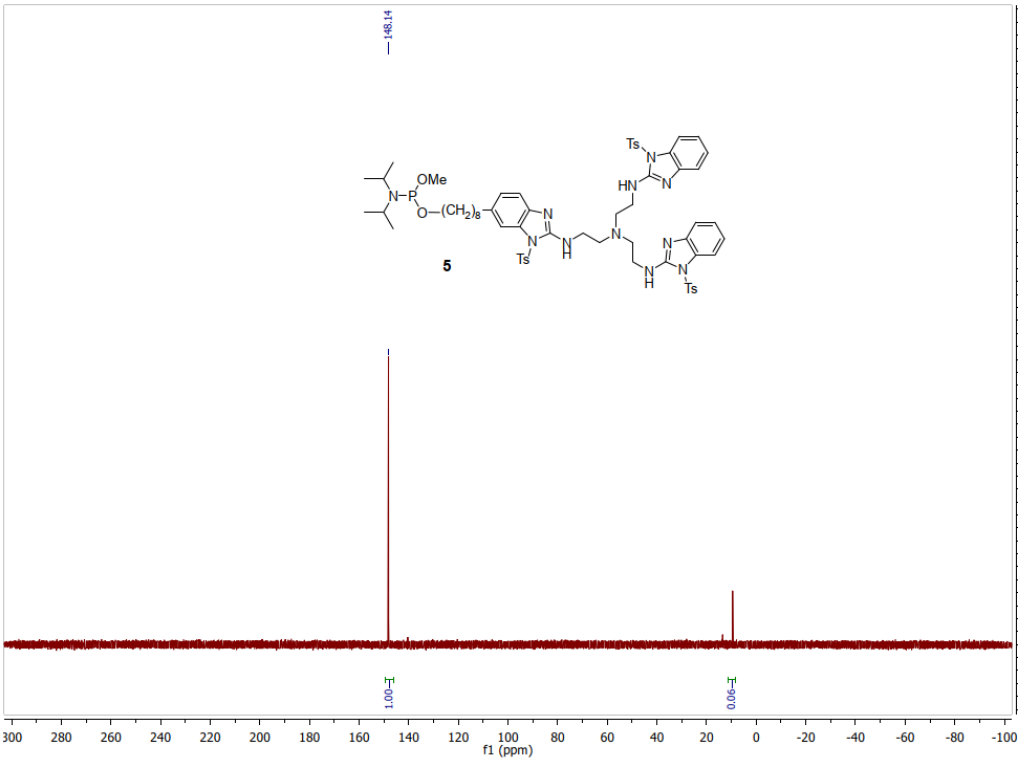
Compound **26**, ^{1}H-NMR



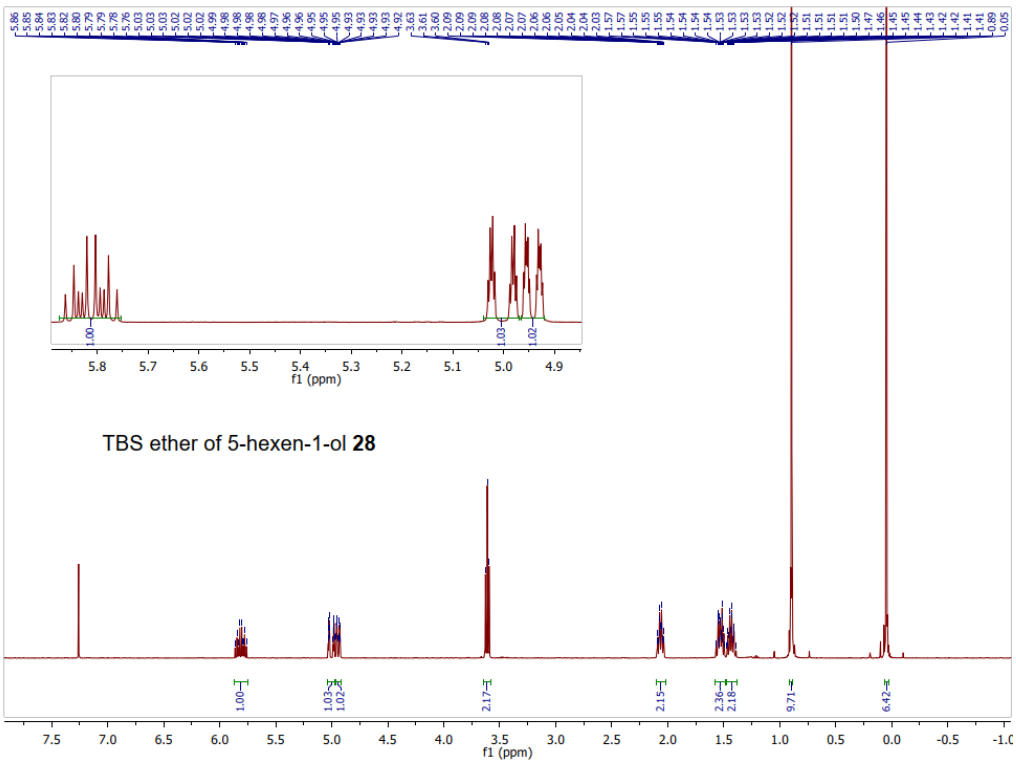
Compound **26**, ^{13}C-NMR



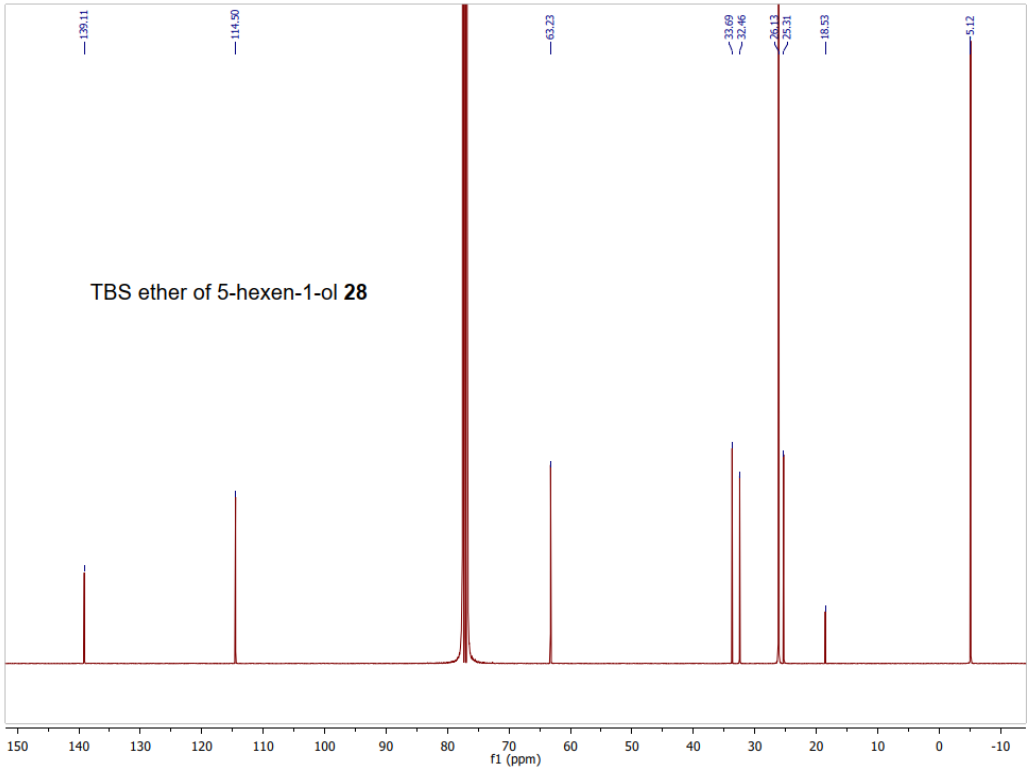
Compound **5**, ^{1}H-NMR



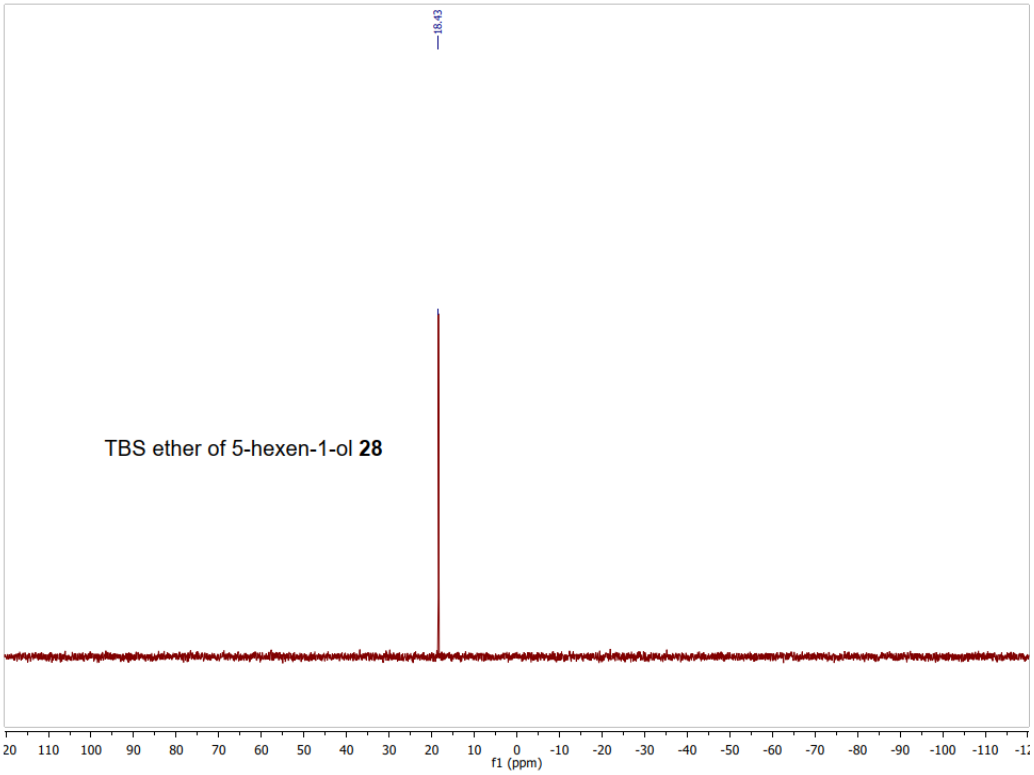
Compound **5**, ^{31}P-NMR



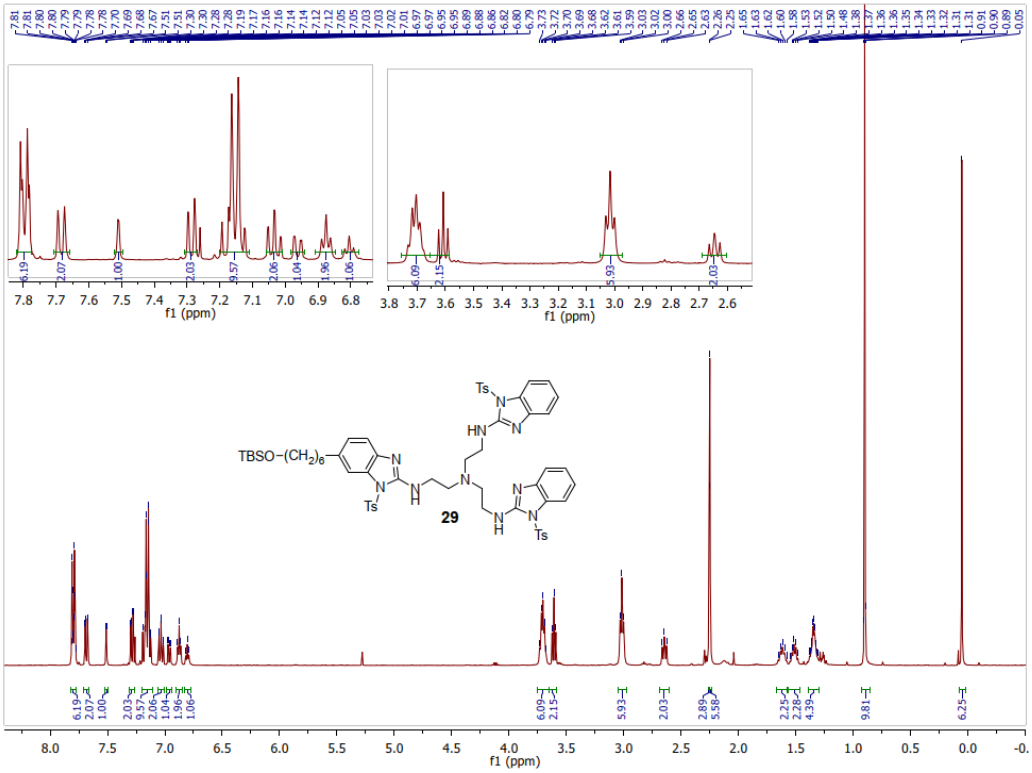
Compound **28**, ^{1}H-NMR



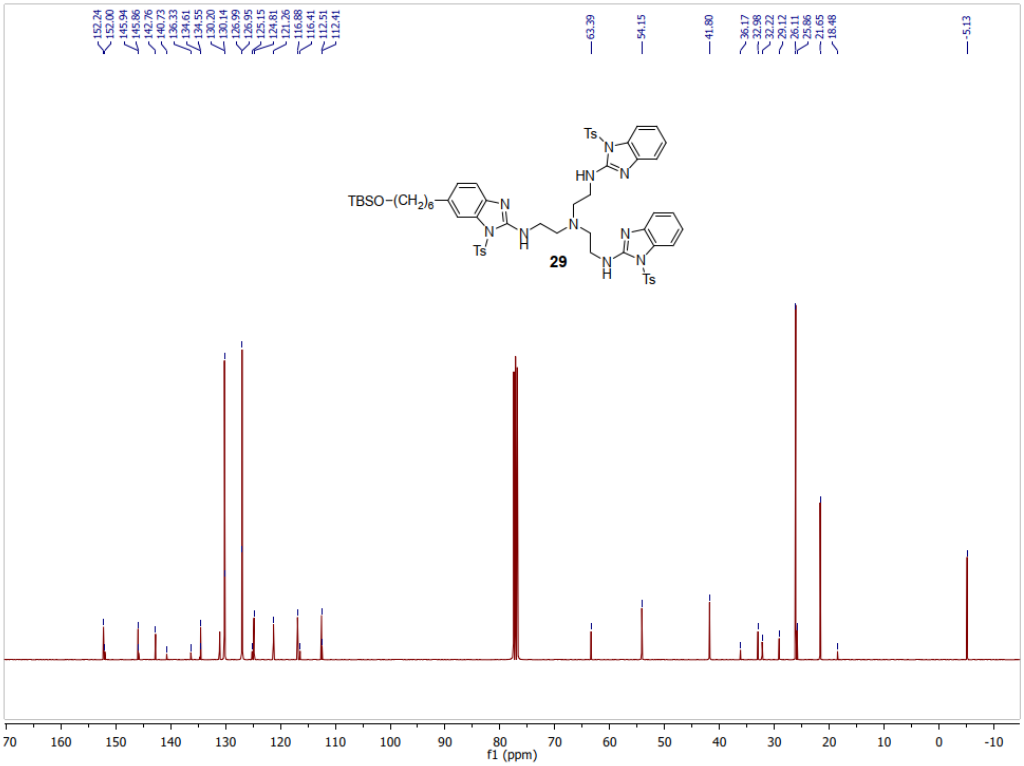
Compound **28**, ^{13}C-NMR



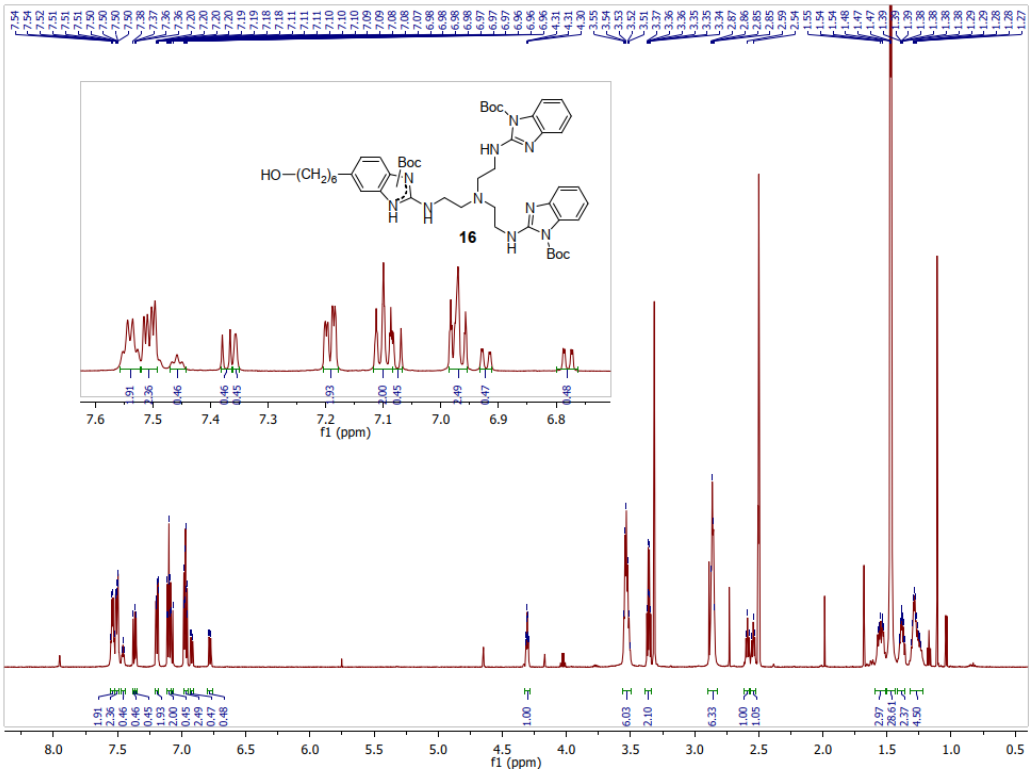
Compound **28**, ^{29}Si-NMR



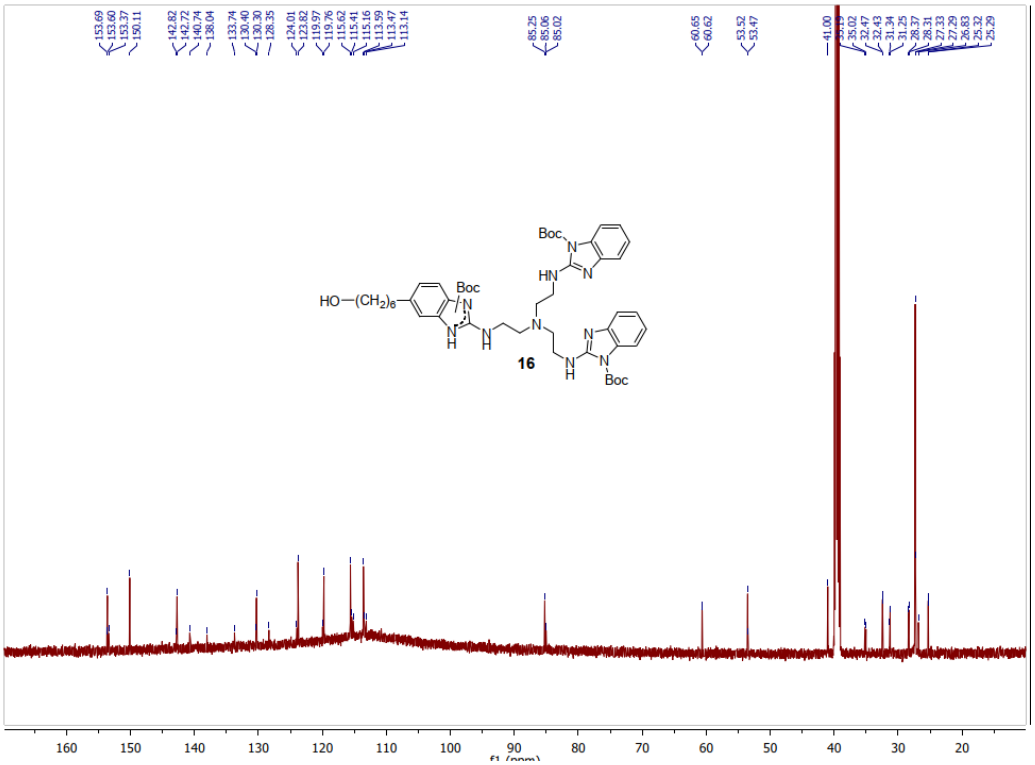
Compound **29**, ^{1}H-NMR



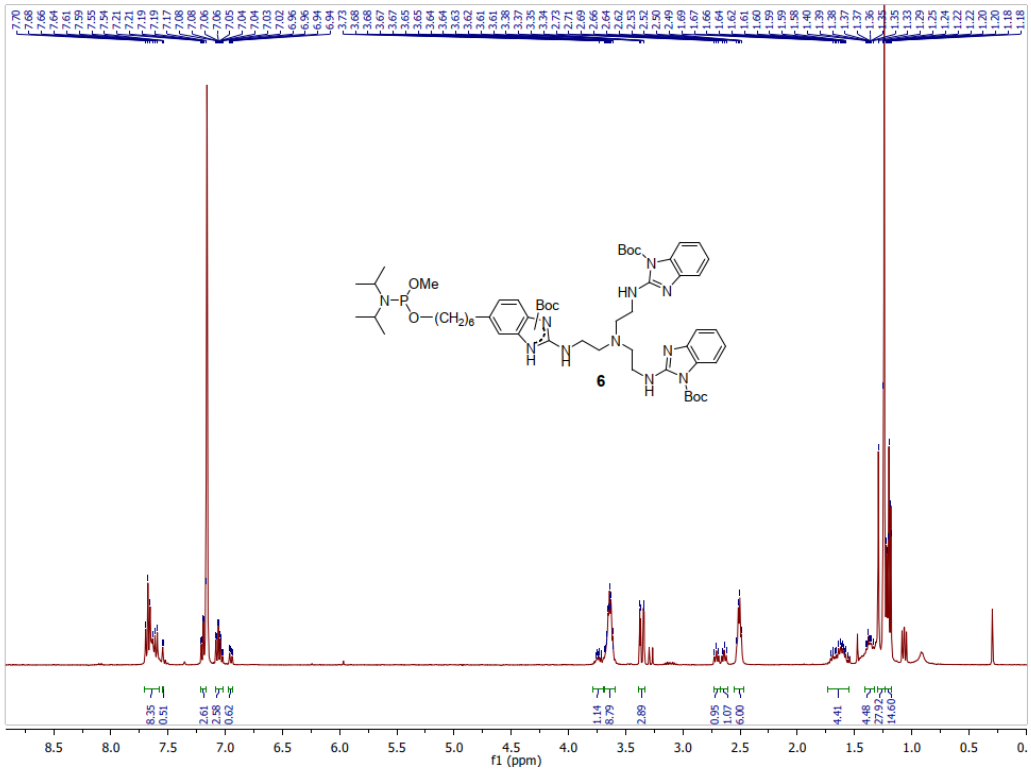
Compound **29**, ^{13}C-NMR



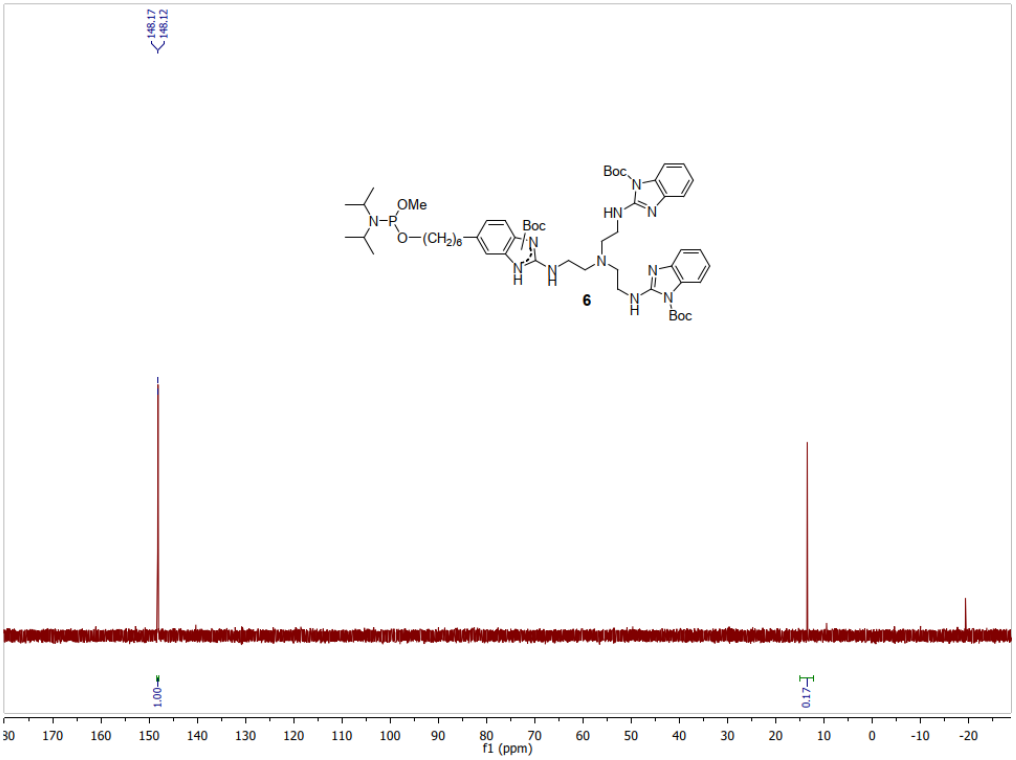
Compound **16**, prepared from trisbenzimidazole **19**, ^{1}H-NMR (mixture of two constitutional isomers)



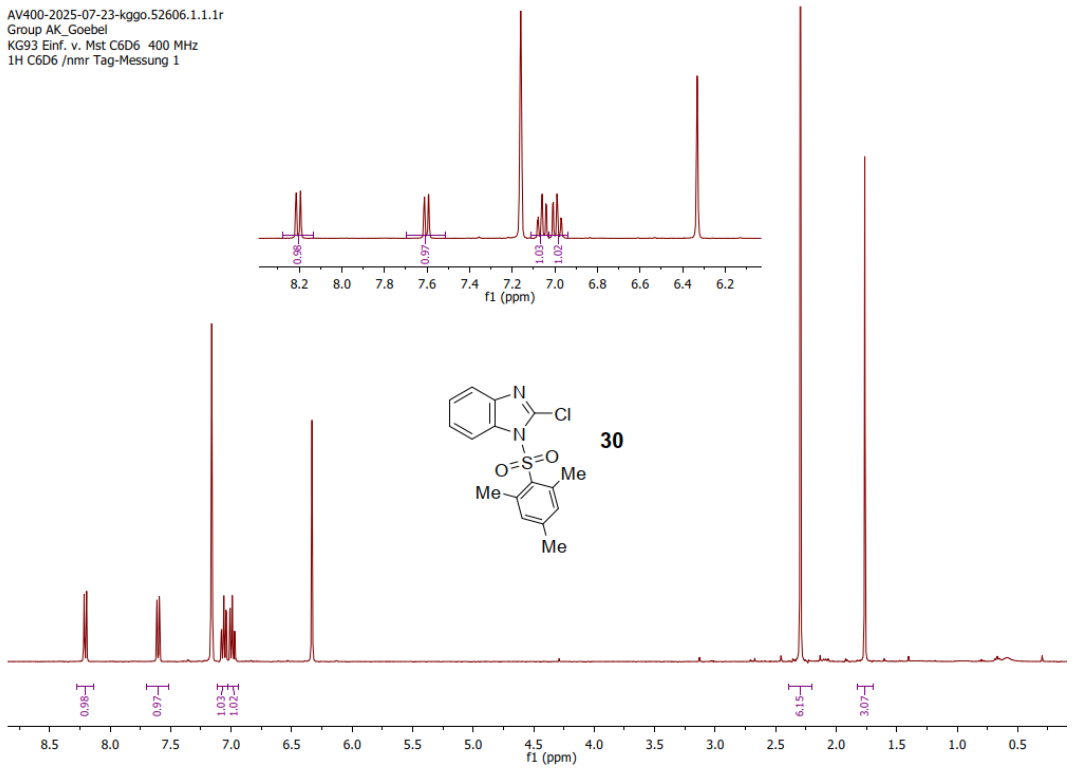
Compound **16**, prepared from trisbenzimidazole **19**, ^{13}C-NMR (mixture of two constitutional isomers)



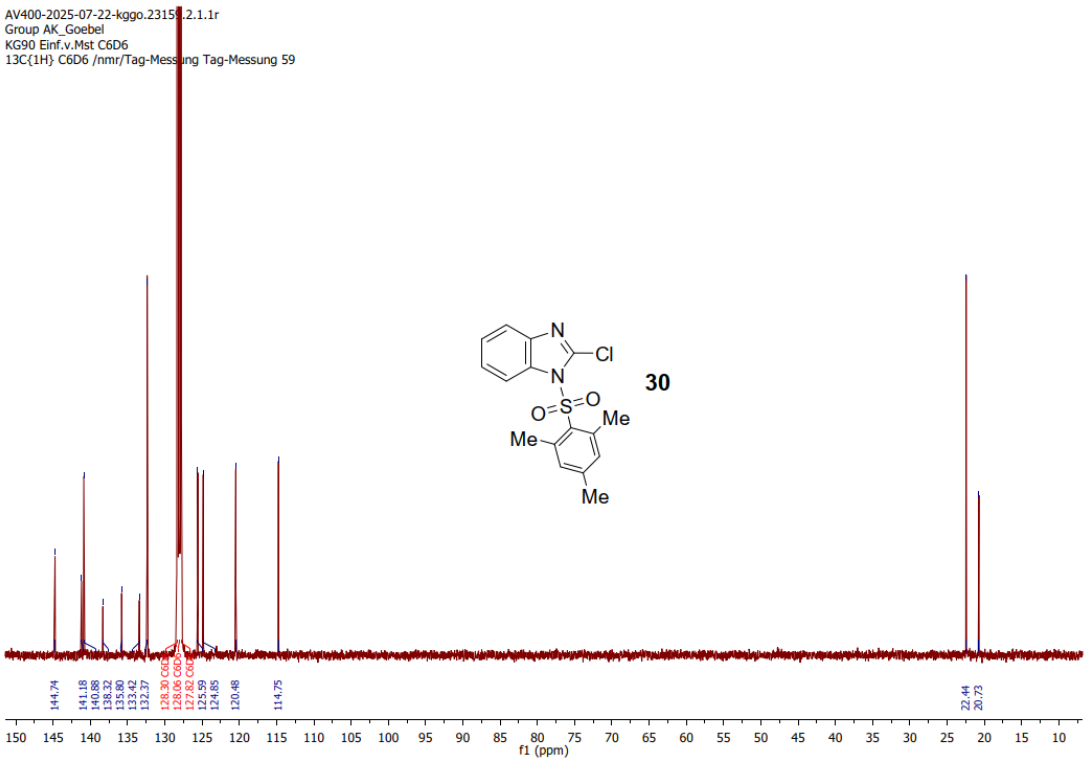
Compound **6**, ^{1}H-NMR (mixture of two constitutional isomers)



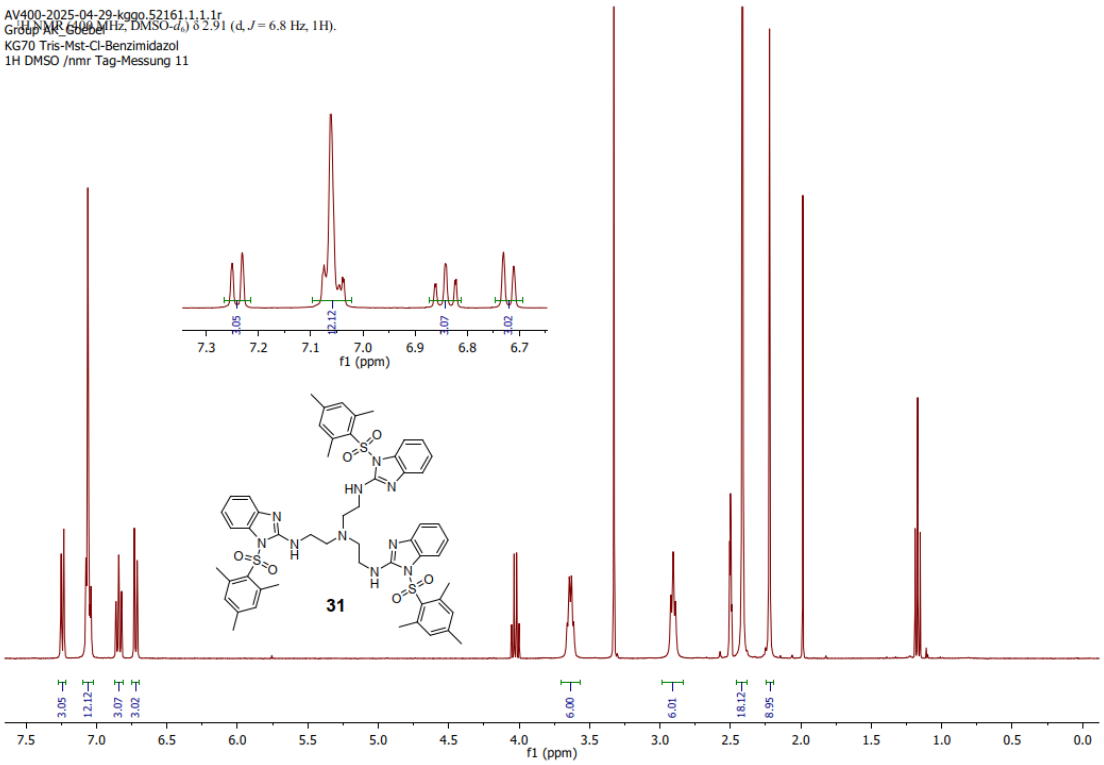
Compound **6**, ^{31}P-NMR (mixture of two constitutional isomers)



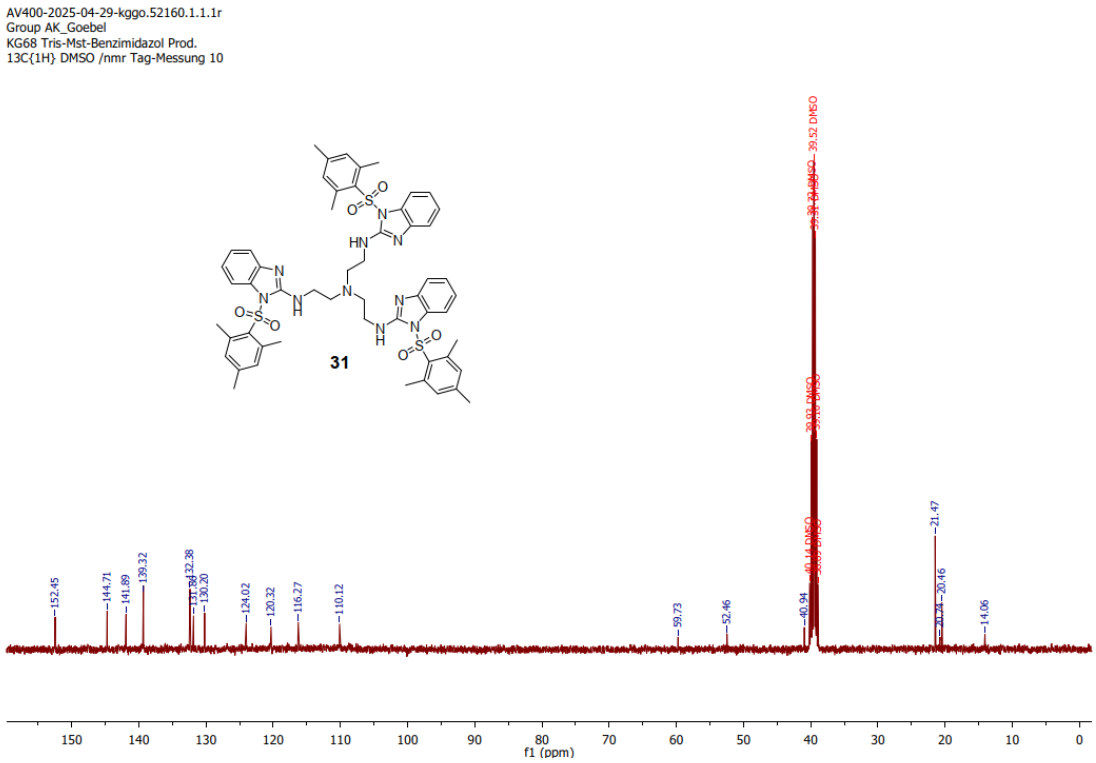
Compound **30**, ^{1}H-NMR



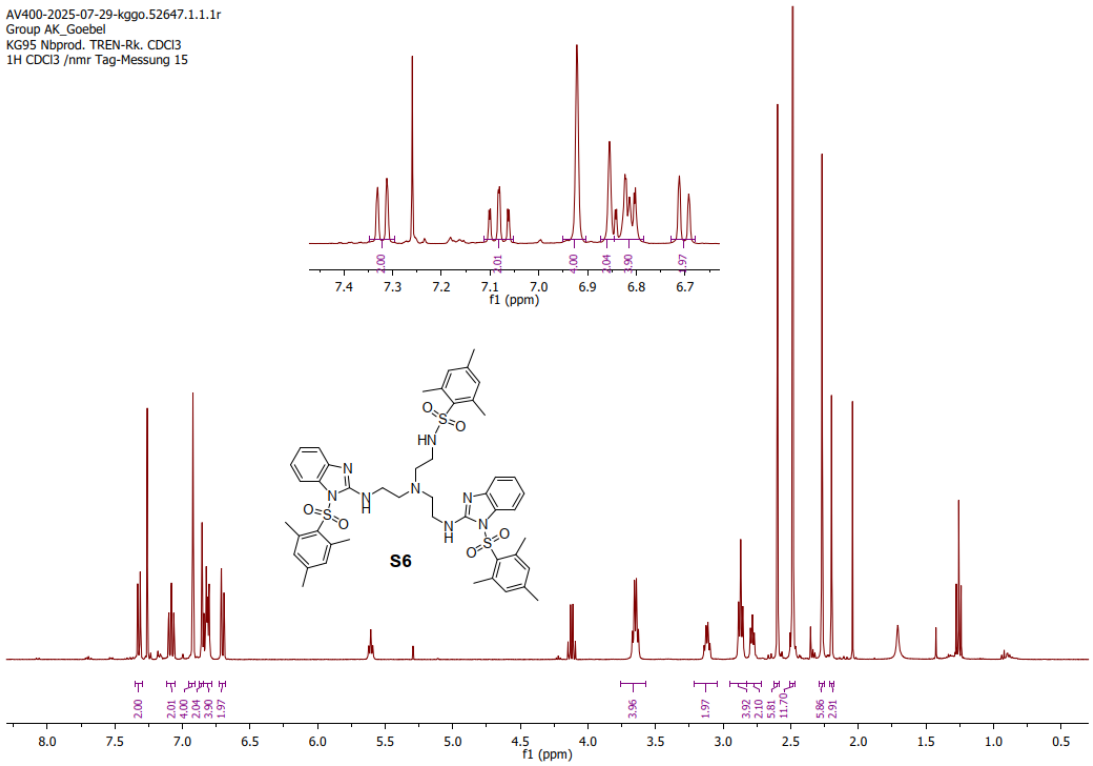
Compound **30**, ^{13}C-NMR



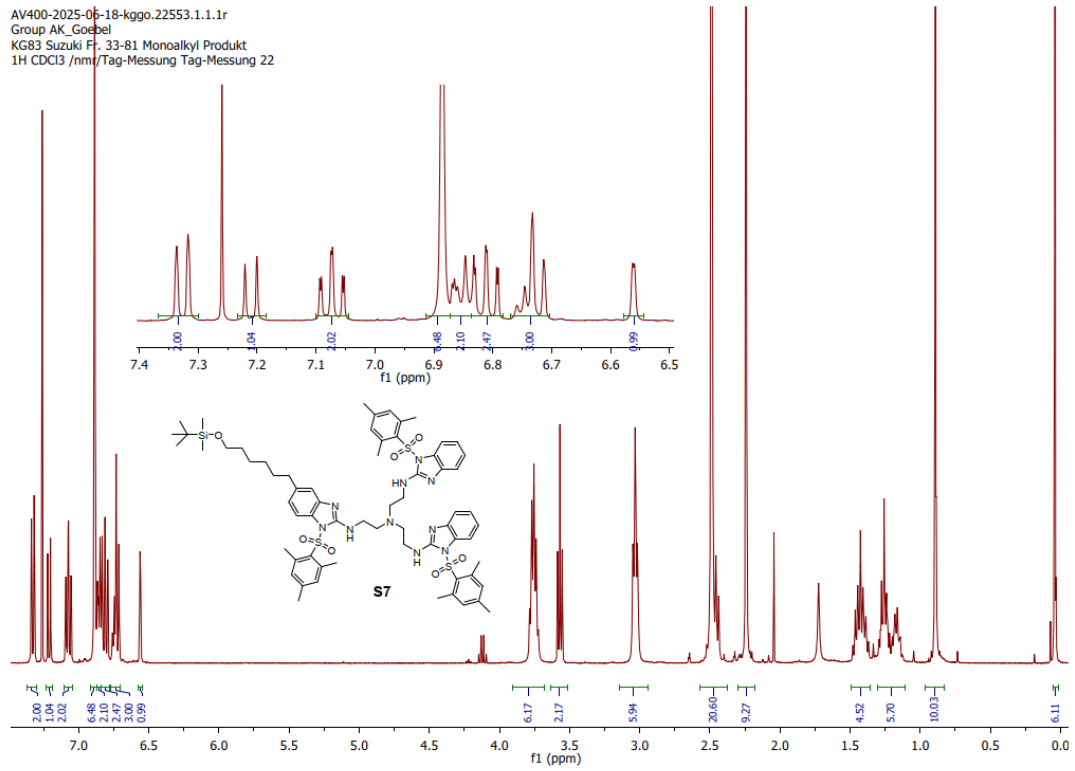
Compound **31**, ^{1}H-NMR



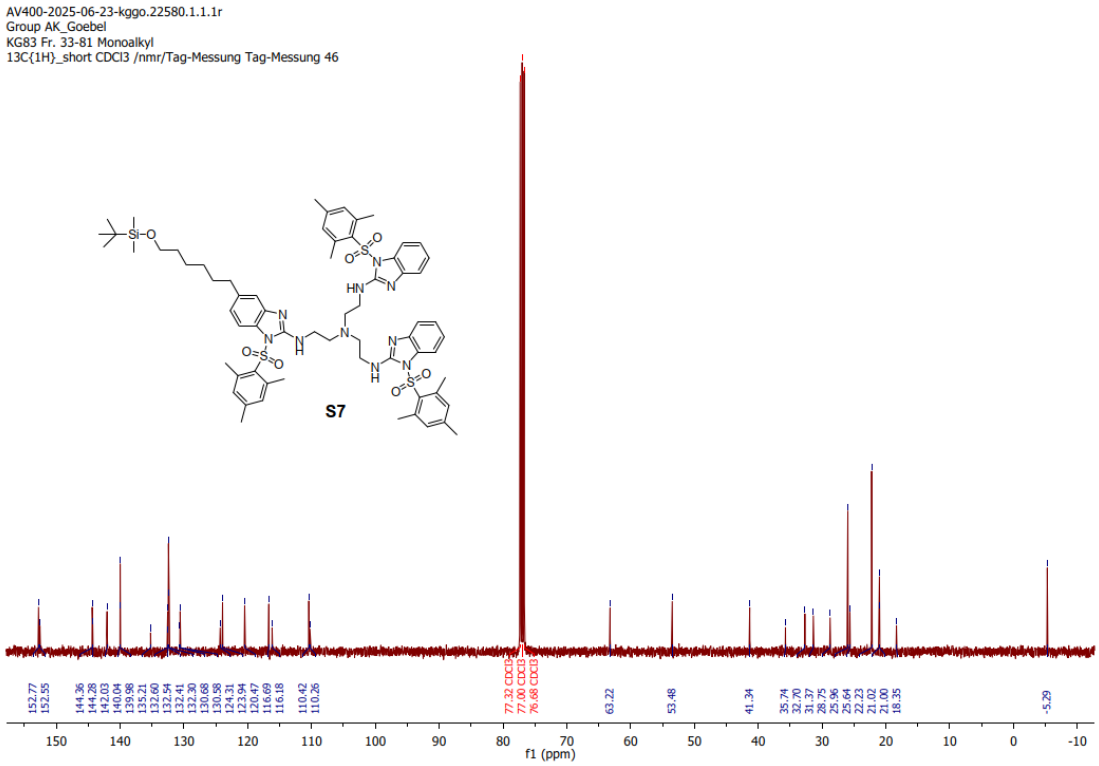
Compound **31**, ^{13}C-NMR



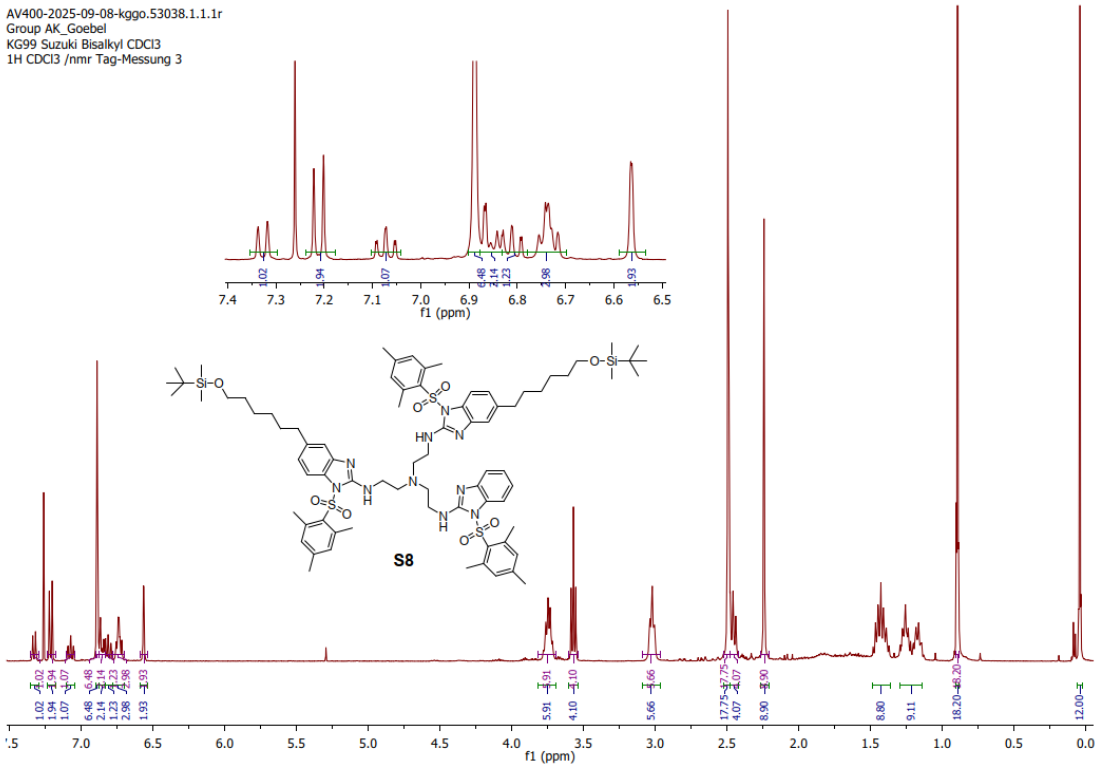
Compound **S6**, ^{1}H-NMR



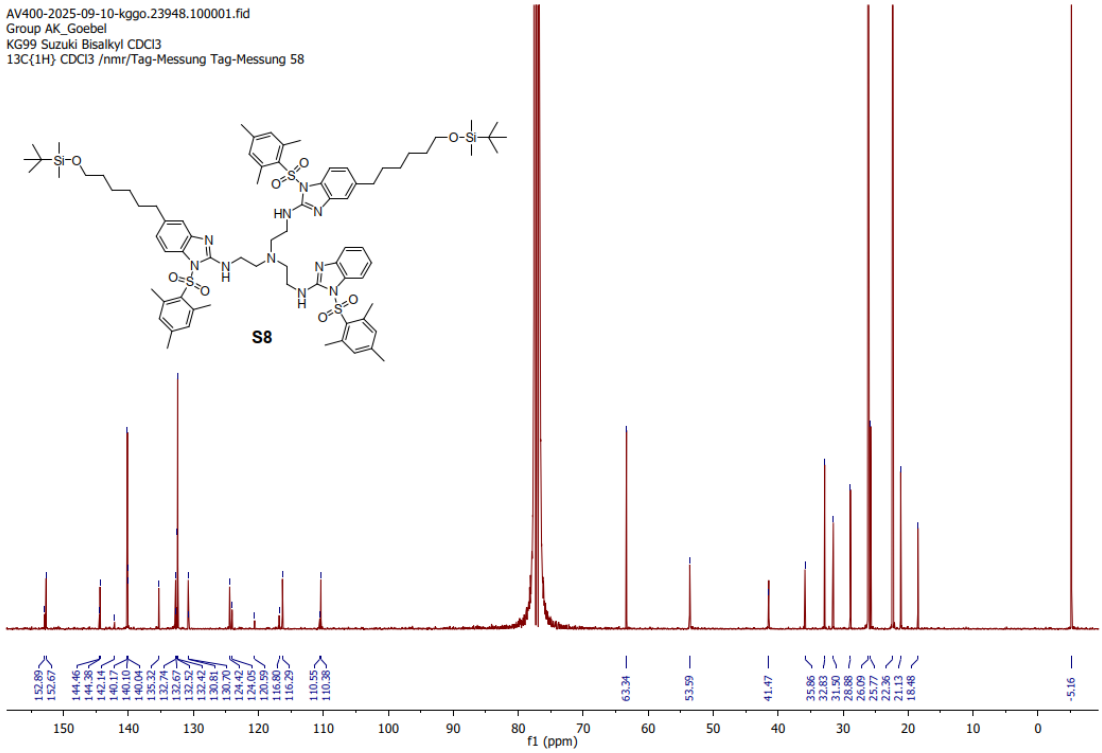
Compound **S7**, ^{1}H-NMR



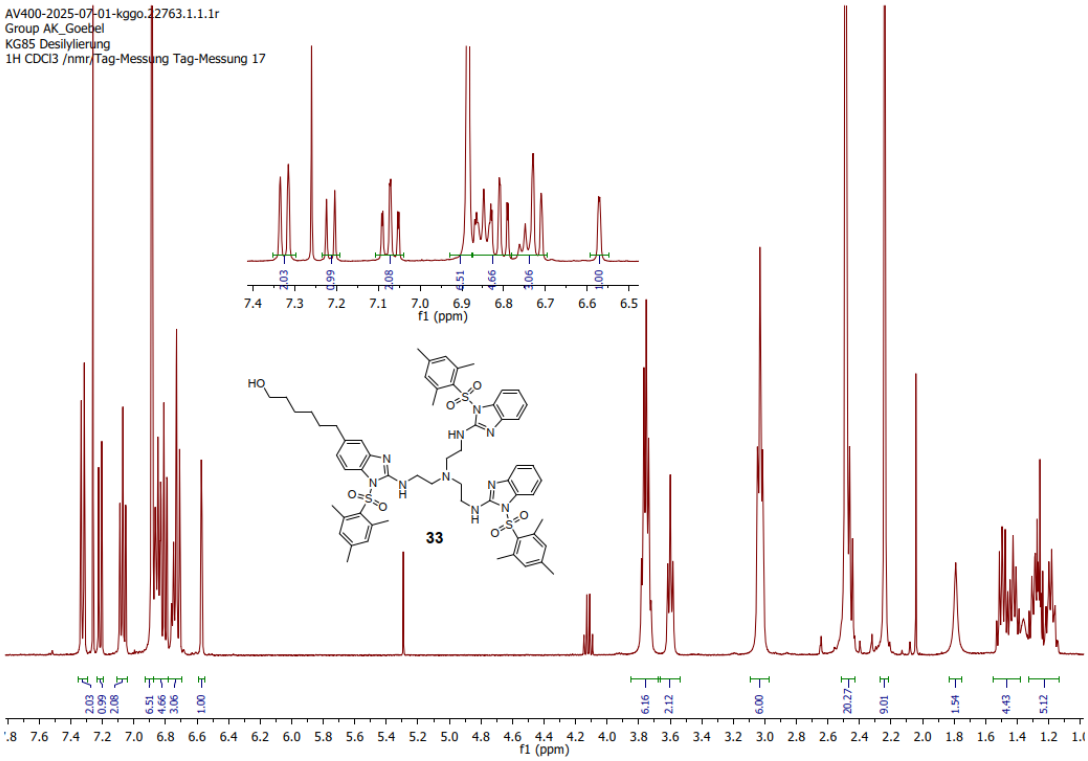
Compound **S7**, ^{13}C-NMR



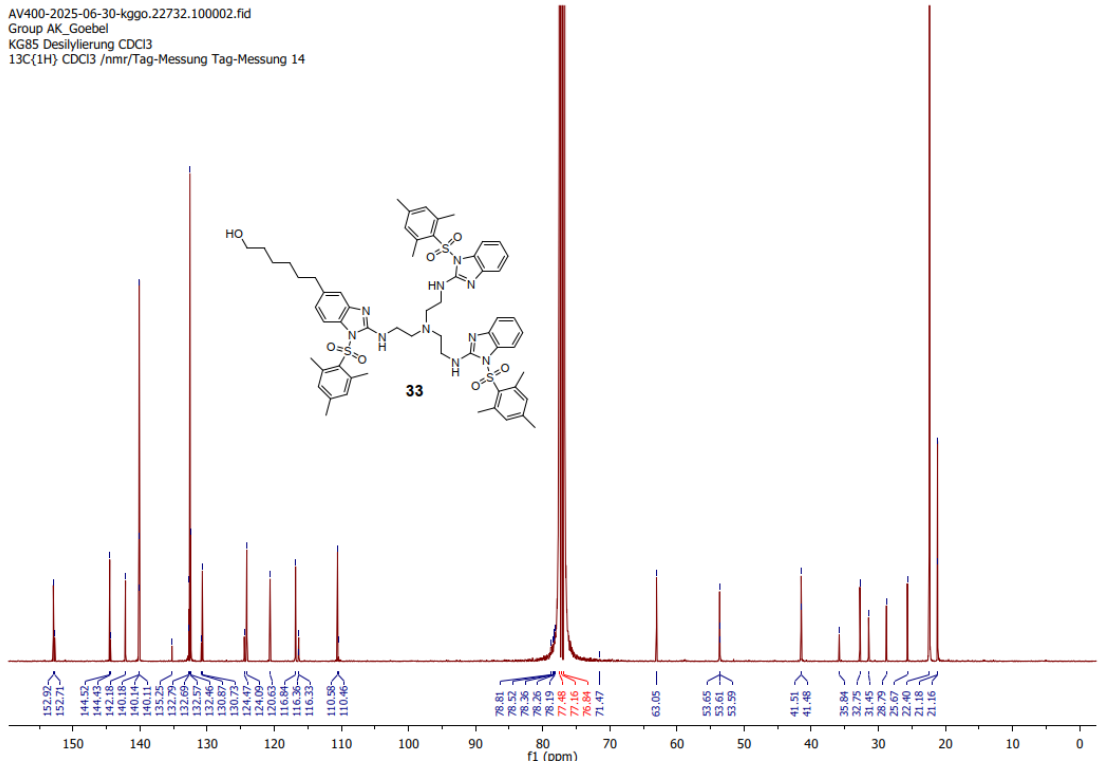
Compound **S8**, ^{1}H-NMR



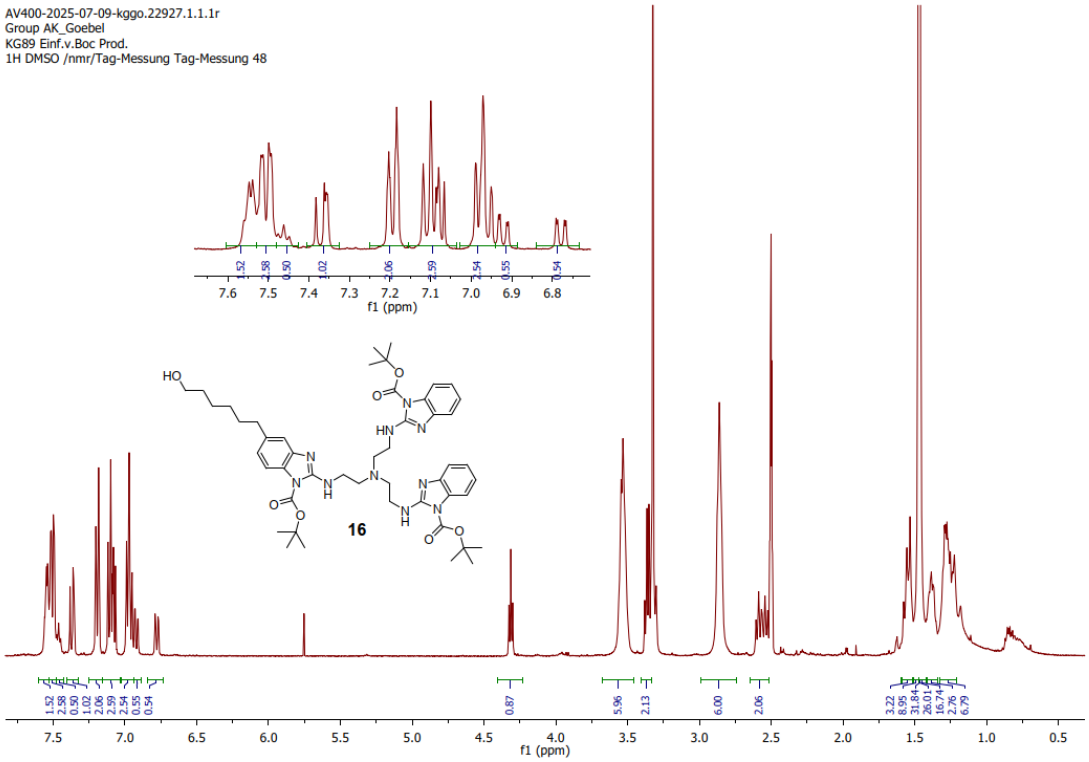
Compound **S8**, ^{13}C-NMR



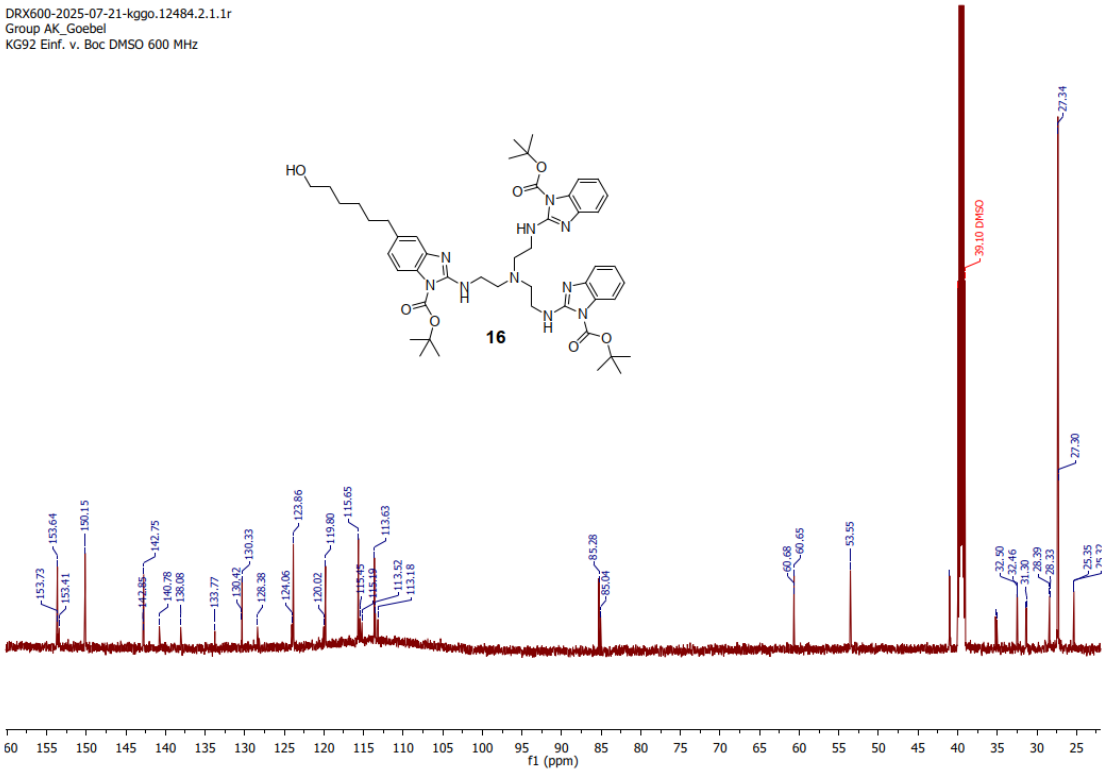
Compound **33**, ^{1}H-NMR



Compound **33**, ^{13}C-NMR



Compound **16**, prepared from trisbenzimidazole **31**, ^{1}H-NMR (mixture of two constitutional isomers)



Compound **16**, prepared from trisbenzimidazole **31**, ^{13}C-NMR (mixture of two constitutional isomers)