Electronic Supplementary Information

5-Nitroindole oligonucleotides with alkynyl side chains: universal base pairing, triple bond hydration and properties of pyrene "click" adducts

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General methods and materials

All chemicals and solvents were of laboratory grade as obtained from commercial suppliers and were used without further purification. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F254 (0.2 mm). Flash column chromatography (FC): silica gel 60 (40−60 μM, for flash chromatography) at 0.4 bar. UV spectra: λ_{max} (ε) in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. NMR spectra were measured at 300.15 MHz for ^1H, 75.48 MHz for ^{13}C and 121.52 MHz for ^{31}P. The *J* values are given in Hz; δ values are given in ppm relative to Me_4Si as internal standard. For NMR spectra recorded in DMSO-d_6, the chemical shift of the solvent peak was set to 2.50 ppm for ^1H NMR and 39.50 ppm for ^{13}C NMR. The ^{13}C NMR signals were assigned on the basis of DEPT-135 and ^1H-^{13}C gated-decoupled NMR spectra (Table S1†; for coupling constants see Table S2†). Reversed-phase HPLC was carried out on a 4 × 250 mm RP-18 (10 μM) LiChrospher 100 column with a HPLC pump connected with a variable wavelength monitor, a controller, and an integrator. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer. Molecular masses of oligonucleotides were determined by MALDI-TOF mass spectrometry in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix (Table S3†). Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany). T_m values were determined from the melting curves using the software MELTWIN, version 3.0 (J. A. McDowell, 1996).

Synthesis, purification, and characterization of oligonucleotides

The oligonucleotides were synthesized on an automated DNA synthesizer on a 1 μmol scale employing standard phosphoramidites as well as the phosphoramidites **10d**, **10e**, and **10f**. The coupling yields were always higher than 95%. After cleavage from the solid support, the oligonucleotides were deprotected in concentrated aqueous ammonia solution for 16 h at 55 °C. The purification of the “trityl-on” oligonucleotides was carried out on reversed-phase

HPLC using the following gradient system at 260 nm: (A) MeCN; (B) 0.1 M (Et_3NH)OAc (pH 7.0):MeCN, 95:5; gradient I: 0-3 min 10-15% A in B, 3-15 min 15−50% A in B, flow rate 0.8 mL/min. The purified "trityl-on" oligonucleotides were treated with 2.5% $CHCl_2COOH/CH_2Cl_2$ for 2 min at 0 °C to remove the 4,4′-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC with gradient II: 0-20 min 0-20% A in B, 20-25 min, 20% A in B, flow rate 0.8 mL/min (ODNs-**15**, **17**, **19**) or 0-20 min 0-30% A in B, 20-25 min, 30% A in B, flow rate 0.8 mL/min (ODN-**28**). The oligonucleotides were desalted on short column (RP-18) using water for elution of salt, while the oligonucleotides were eluted with H_2O/MeOH (2/3). The oligonucleotides were lyophilized on a Speed-Vac evaporator to yield colorless or yellow solids which were frozen at -24 °C. Extinction coefficients ε_{260} (H_2O) of nucleosides are dA, 15 400; dG, 11 700; dT, 8800; dC, 7300; **4d**, 16 600 (MeOH); **4e**, 12 700 (MeOH); **4f**, 19 800 (MeOH).

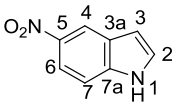
Fluorescence measurements performed on nucleoside and oligonucleotide pyrene conjugates

Fluorescence spectra of all nucleoside pyrene conjugates (**12**, **13**, **31**, **32** and **33**) were measured in methanol (concentrations 10 µM; for solubility reasons all nucleoside 'click' conjugates were first dissolved in 1% of DMSO and then diluted with methanol, 99%). Fluorescence spectra of ss oligonucleotide pyrene conjugates and their duplexes were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate buffer (pH 7.0). The measurements were performed with identical concentrations, i.e., 2 µM for ss oligonucleotides and 2 µM + 2 µM for ds oligonucleotides. Excitation wavelength of 340 nm was used.

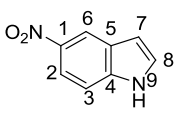
Table S1 ^{13}C-NMR chemical shifts of indole derivatives[a]

	C(8)[b] C(2)[c]	C(7) C(3)	C(5) C(3a)	C(6) C(4)	C(1) C(5)	C(2) C(6)	C(3) C(7)	C(4) C(7a)	C(1')	C(2')	C(3')	C(4')	C(5')	C≡C/ NCH_2	CH_3/ CH_2	C=O
5[1]	129.2	103.8	127.0	117.2	140.7	116.3	111.7	139.1	-	-	-	-	-	-	-	-
6	133.9	58.8	129.1	117.5	141.4	116.8	112.7	139.4	-	-	-	-	-	-	-	-
8	132.9	61.3	130.1	117.9	142.0	117.1	111.9	139.0	85.3	36.6	74.7	81.5	64.0	-	-	165.5, 165.3
4b	133.4	60.4	129.9	117.8	141.8	117.0	111.7	138.9	85.2	-[d]	70.5	87.6	61.5	-	-	-
4c	133.5	97.1	128.1	118.1	141.9	115.4	112.0	137.7	85.3	-[d]	70.5	87.7	61.4	97.5 99.5/-	0.7/-	-
4d	133.4	98.9	128.3	118.0	142.0	115.5	112.0	137.7	85.3	-[d]	70.5	87.7	61.4	75.6 83.9/-	-	-
4e	131.8	100.4	128.3	117.8	141.7	115.5	111.7	137.8	85.1	-[d]	70.5	87.6	61.5	71.3 72.0 84.3 93.2/-	-/17.3, 18.4, 27.2, 27.4	-
4f	133.1	93.3	128.7	118.1	142.0	115.2	112.1	137.8	85.3	-[d]	70.4	87.7	61.4	98.8 99.7/-	10.7/-	-
9b	133.2	60.4	130.2	117.8	141.9	117.1	112.2	138.9	85.2	-[d]	70.2	85.6	63.6	-	-	-
9d	133.2	98.8	128.5	118.0	142.0	115.5	112.5	137.9	85.4	-[d]	69.9	85.6	63.5	75.6 83.9/-	-	-
9e	131.8	100.4	128.5	117.8	141.8	115.5	112.2	137.9	85.0	-[d]	70.0	85.6	63.6	71.3 72.0 84.3 93.2/-	-/17.3, 18.4, 27.2, 27.4,	-
9f	132.7	93.3	128.7	118.0	141.9	115.1	112.4	137.8	85.2	-[d]	70.0	85.6	63.4	98.6 99.5/-	10.6/-	-
12	131.1[f]	109.2	128.5[f]	117.7	141.4	117.4	111.3	138.9	84.7	-[d]	70.5	87.4	61.6	-/51.1	-/-	-
13	131.8[f]	100.3	128.2[f]	117.7	141.5	115.4	111.6	137.8	85.0	-[d]	70.4	87.5	61.4	93.3, 71.8/ 50.6	-/18.5, 24.3, 27.7, 28.1	-
16	136.8	-[e]	125.1	118.1	142.8	117.5	111.9	138.8	85.4	-[d]	69.9	87.5	60.9	-	27.1/-	192.7

[a] Measured in DMSO-d_6 at 298 K. [b] Purine numbering. [c] Systematic numbering. [d] Superimposed by DMSO-d_6. [e] Not detected. [f] Tentative.



systematic numbering



purine numbering

Table S2 Selected ^{1}H-^{13}C coupling constants (Hz) of indole nucleosides[a,b]

		6	**8**	**4b**	**4c**	**4d**	**4e**	**4f**	**9b**	**9d**	**9e**	**9f**	**12**	**13**	**16**
C(1')	^{1}J(C(1'), H-C(1'))	-	166	164	165	165	172	164	167	165	166	166	164	163	168
C(3')	^{1}J(C(3'), H-C(3'))	-	158	149	148	149	149	149	146	147	148	150	150	148	148
C(4')	^{1}J(C(4'), H-C(4'))	-	152	147	147	147	146	147	148	149	147	146	147	148	149
C(5')	^{1}J(C(5'), H-C(5'))	-	151	140	139	140	139	139	143	143	143	142	141	139	140
C(2)	^{1}J(C(2), H-C(2))	191	193	193	195	191	191	191	193	191	192	191	-	-	190
C(2)	^{3}J(C(2), H-C(1'))	-	4.2	4.5	4.2	2.4	4.0	4.1	4.0	-	-	4.0	-	-	3.8
C(4)	^{1}J(C(4), H-C(4))	168	171	168	169	169	169	169	169	170	169	169	166	169	169
C(4)	^{3}J(C(4), H-C(6))	4.4	4.5	4.6	4.2	4.8	4.7	-	4.8	4.4	4.5	-	4.6	4.3	4.6
C(6)	^{1}J(C(6), H-C(6))	167	167	170	168	167	167	169	169	168	168	173	166	171	171
C(6)	^{3}J(C(6), H-C(4) or ^{2}J(C(6), H-C(7))	4.7	4.4	4.2	4.6	4.4	4.9	4.8	4.6	4.8	4.3	4.3	4.9	4.3	4.6
C(7)	^{1}J(C(7), H-C(7))	167	169	169	169	169	169	167	169	169	169	168	169	169	169.3
C≡C	^{1}J(C≡C, C≡C-H)	-	-	-	-	253	248	-	-	248	253	-	-	-	-

[a] Measured in DMSO-d_6 at 298 K. [b] Systematic numbering.

Table S3 Molecular masses of oligonucleotides measured by MALDI-TOF mass spectrometry[a]

Oligonucleotides	Molecular Weight	
	Calc.	**Found**
5’-d(TAG GTC A**16**T ACT) (**15**)	3713.5	3713.9
5’-d(TAG GTC A**4d**T ACT) (**17**)	3695.5	3695.7
5’-d(TAG GTC A**12**T ACT) (**18**)	3952.7	3951.3
5’-d(TAG GTC A**4e**T ACT) (**19**)	3775.6	3774.6
5’-d(TAG GTC A**13**T ACT) (**20**)	4032.9	4031.9
5’-d(TAG GTC A**4f**T ACT) (**28**)	3851.8	3853.9
5’-d(AGT A**T**T GAC CTA) (**21**)	3644.4	3645.5
5’-d(AGT A**C**T GAC CTA) (**22**)	3629.4	3628.3
5’-d(AGT A**A**T GAC CTA) (**23**)	3653.4	3654.0
5’-d(AGT A**G**T GAC CTA) (**24**)	3669.4	3669.3

[a] Measured in the positive linear mode.

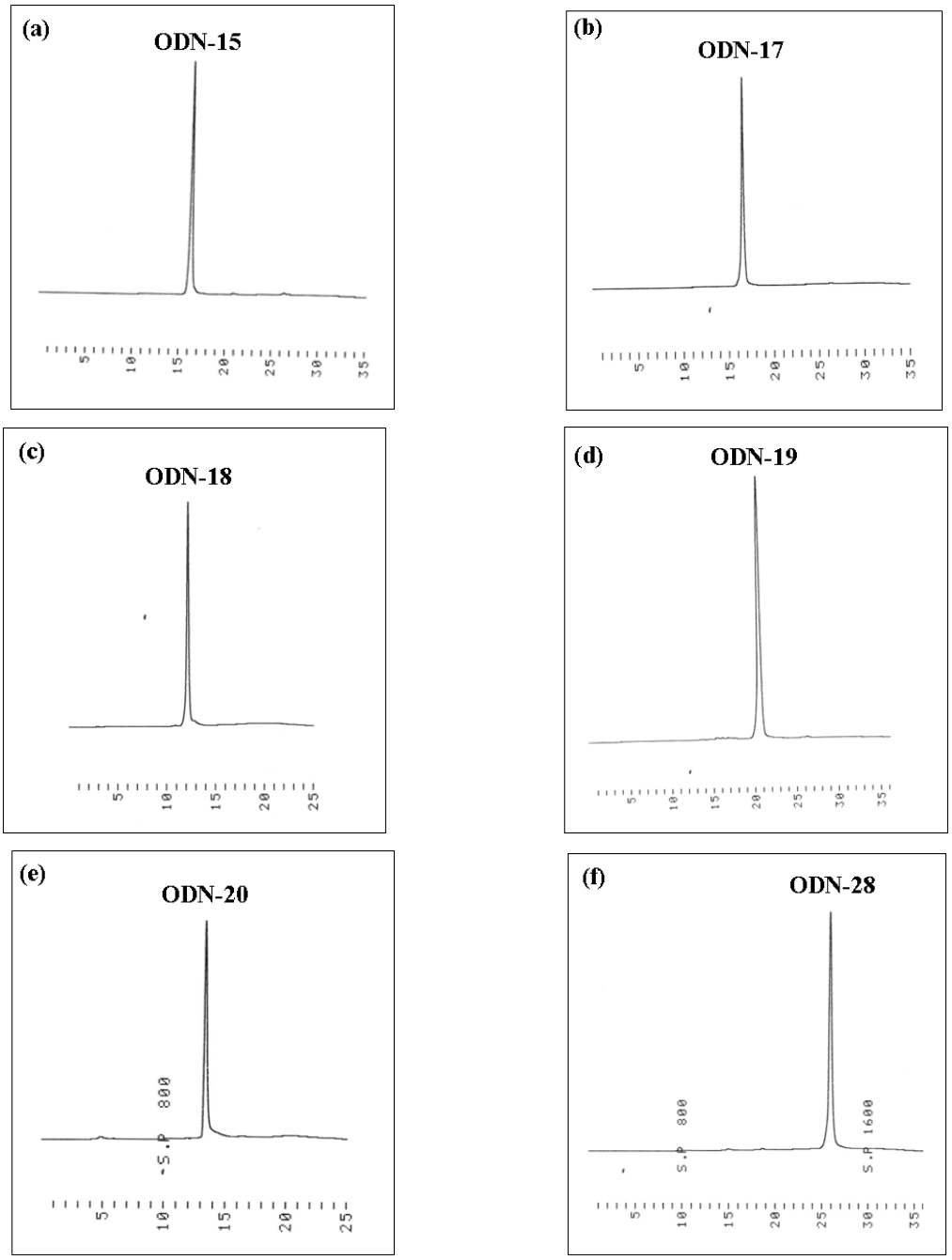
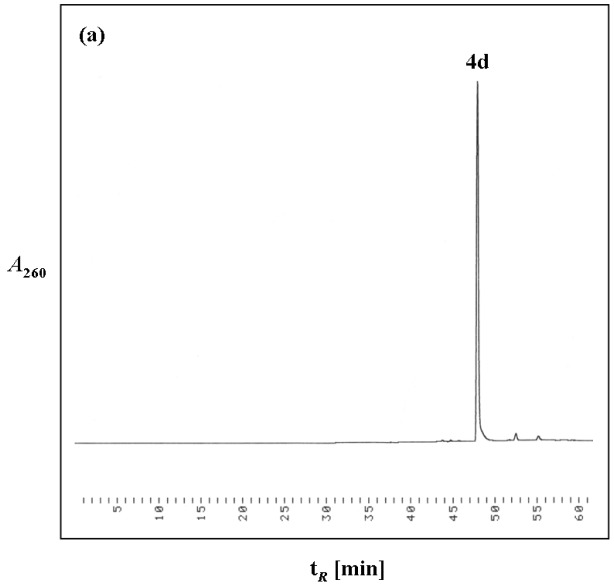
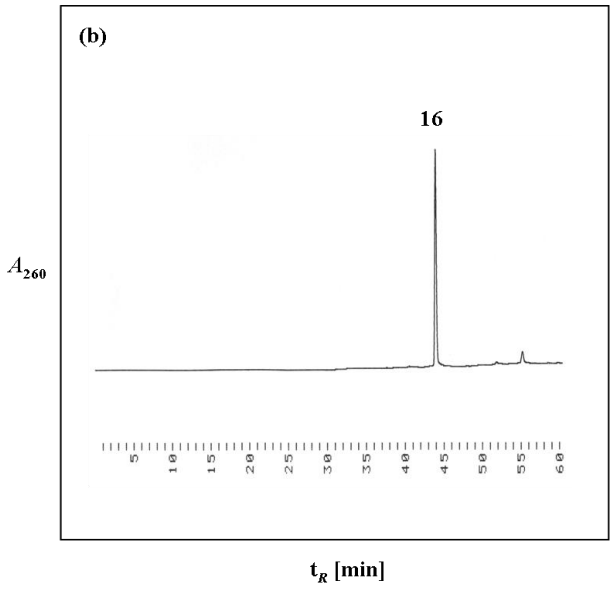
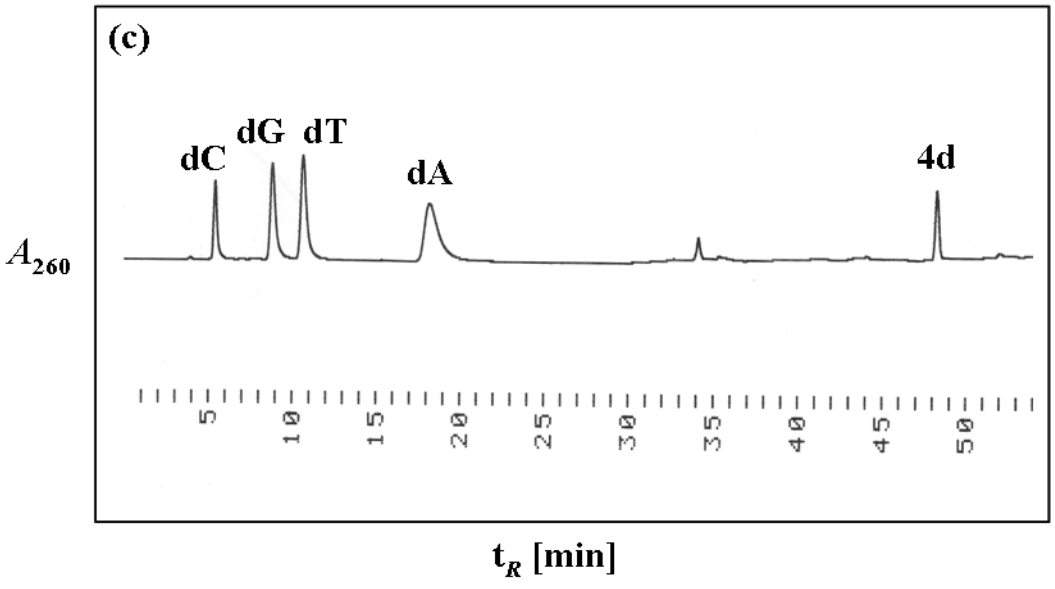


Fig. S1 Reversed-phase HPLC elution profiles of purified oligonucleotides monitored at 260 nm: (a) ODN-**15**; (b) ODN-**17**; (c) ODN-**18**; (d) ODN-**19**; (e) ODN-**20**; (f) ODN-**28**. For elution the following solvent system was used: MeCN (A) and 0.1 M (Et_3NH)OAc:MeCN, 95:5 (pH 7.0). Gradient I (for ODN-**18** and **20**), 0-3 min 10-15% A in B, 3-15 min 15-50% A in B, flow rate 0.8 mL/min. Gradient II (for ODN-**15**, **17** and **19**), 0-20 min 0-20% A in B, 20−25 min 20% A in B, flow rate 0.8 mL/min. Gradient III (for ODN-**28**), 0-20 min 0-30% A in B, 20-25 min 30% A in B, flow rate 0.8 mL/min. X-axis corresponds to retention time [min] and y-axis corresponds to absorbance.


(a)
4d
A_{260}
5 10 15 20 25 30 35 40 45 50 55 60
t_R [min]


(b)
16
A_{260}
5 10 15 20 25 30 35 40 45 50 55 60
t_R [min]


(c)
dC
dG
dT
dA
4d
A_{260}
5 10 15 20 25 30 35 40 45 50
t_R [min]

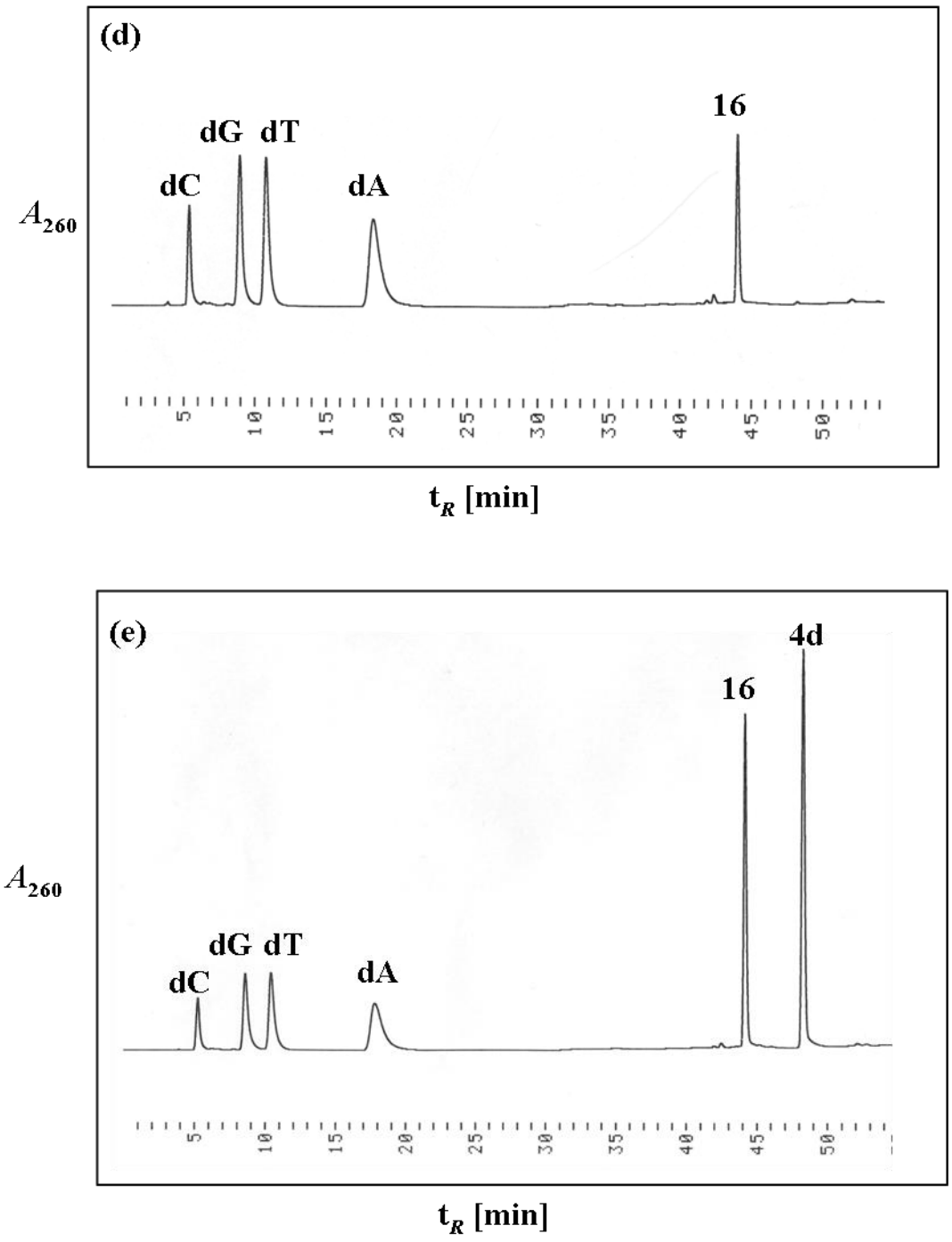


Fig. S2 Reversed-phase HPLC elution profiles of (a) purified ethynyl nucleoside **4d**; (b) purified acetyl nucleoside **16**; (c) enzymatic hydrolysis product of ODN-**15**; (d) enzymatic hydrolysis product of ODN-**17**; (e) artificial mixture of enzymatic hydrolysis product of ODN-**15** + nucleoside **4d** + nucleoside **16**. For elution the following solvent system was used: gradient III: 20 min 100% B, 20−50 min 0−60% A in B; 50-55 min 60% A in B; flow rate 0.7 mL/min (A, MeCN; B 0.1 M (Et_3NH)OAc (pH 7.0):MeCN, 95:5).

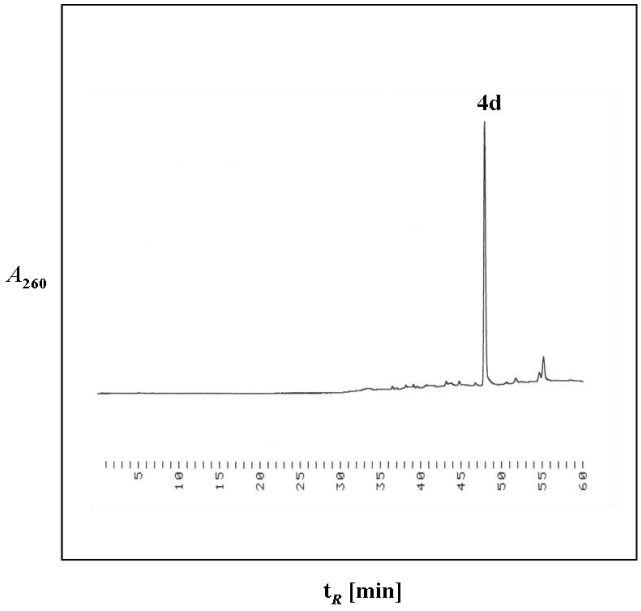


Fig. S3 Reversed-phase HPLC elution profile of the crude reaction mixture of ethynyl nucleoside **4d** treated with concentrated aqueous ammonia at elevated temperature (55 °C for 16 h), monitored at 260 nm. For elution the following solvent system was used: gradient III: 0-20 min 100% B, 20−50 min 0−60% A in B; 50-55 min 60% A in B; flow rate 0.7 mL/min (A, MeCN; B 0.1 M (Et_3NH)OAc (pH 7.0):MeCN, 95:5).

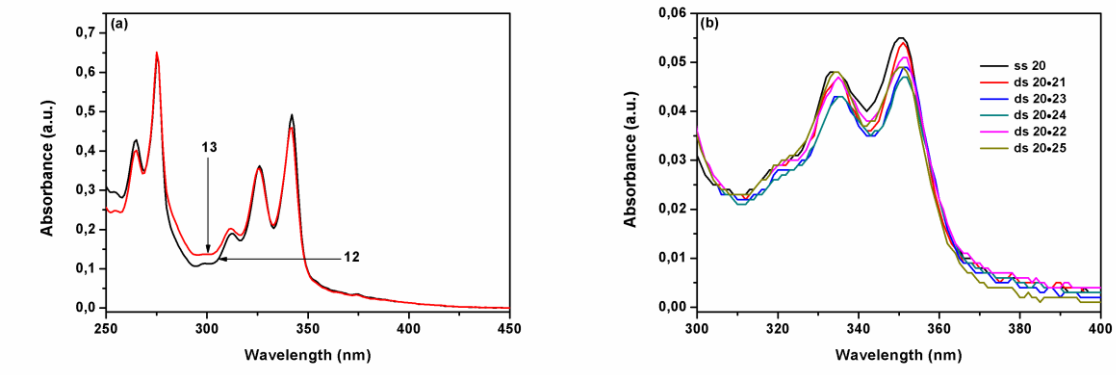


Fig. S4 (a) UV-vis spectra of pyrene conjugates **12** and **13** (10 µM); the measurements were performed in methanol containing 1% DMSO. (b) UV-vis spectra of 2 µM ss ODN-**20** and corresponding duplexes (2 µM of each strand), the measurements were performed in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate buffer (pH 7.0).

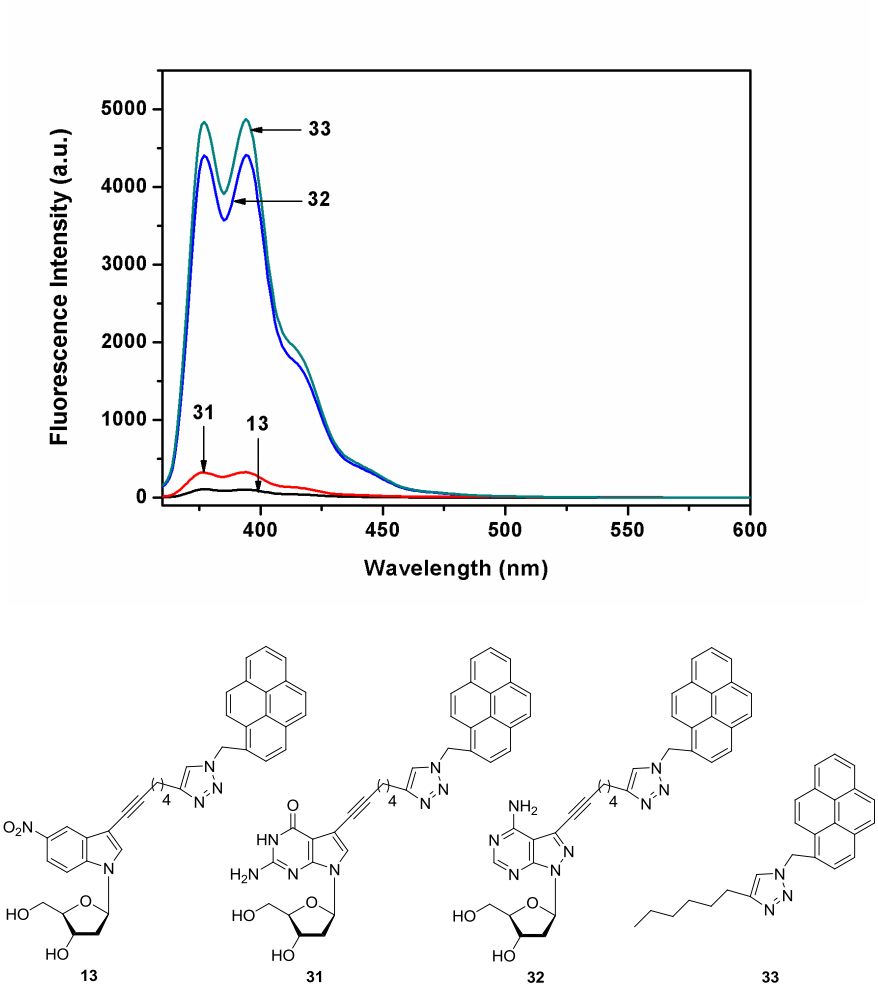


Fig. S5 Fluorescence spectra of pyrene conjugates **13**, **31**-**33** (10 µM) in methanol. Excitation wavelength 340 nm.

References

1 (a) M. S. Morales-Ríos, R. E. del Río and P. Joseph-Nathan, *Magn. Res. Chem.*, 1989, **27**, 1039-1047; (b) M. S. Morales-Ríos, J. Espiñeira and P. Joseph-Nathan, *Magn. Res. Chem.*, 1987, **25**, 377-395; (c) M. S. Morales-Ríos, P. Joseph-Nathan, B. Wrackmeyer and E. Kupče, *Magn. Res. Chem.*, 1993, **31**, 238-240; (d) G. Burton, A. A. Ghini and E. G. Gros, *Magn. Res. Chem.*, 1986, **24**, 829-831.

Fig. S6. ^{1}H NMR spectrum of compound 6.

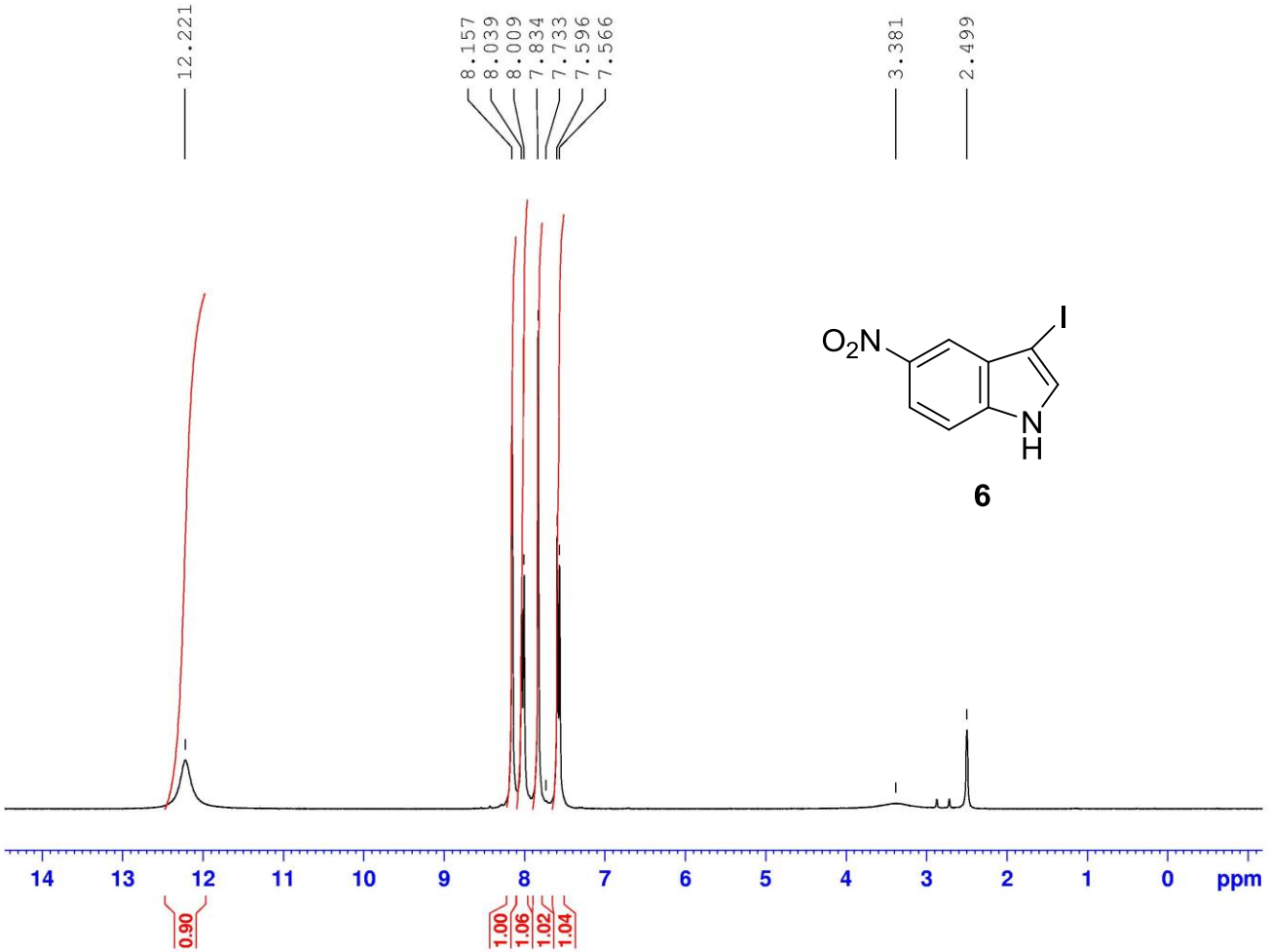


Fig. S7. ^{13}C NMR spectrum of compound 6.

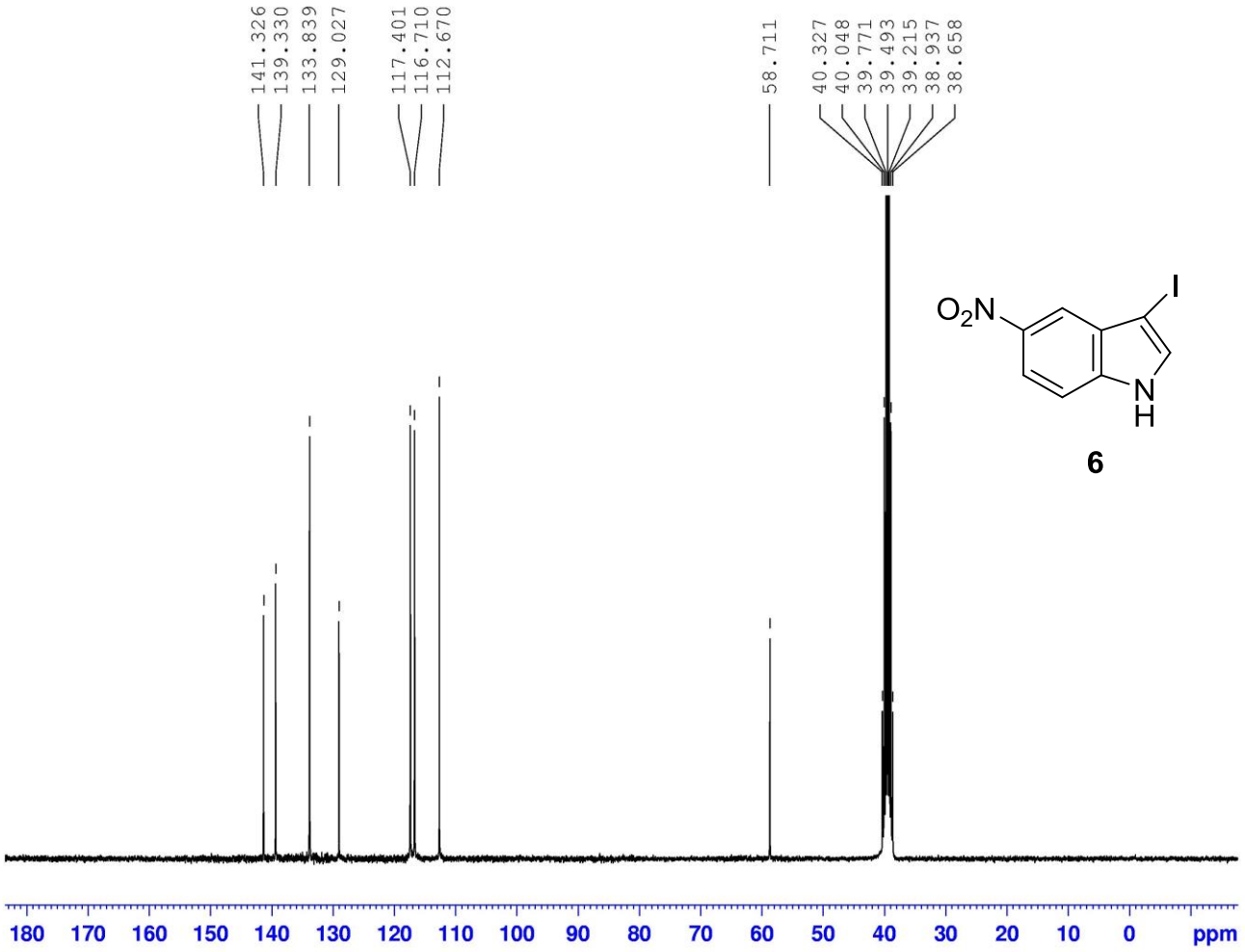


Fig. S8. DEPT-135 spectrum of compound 6.

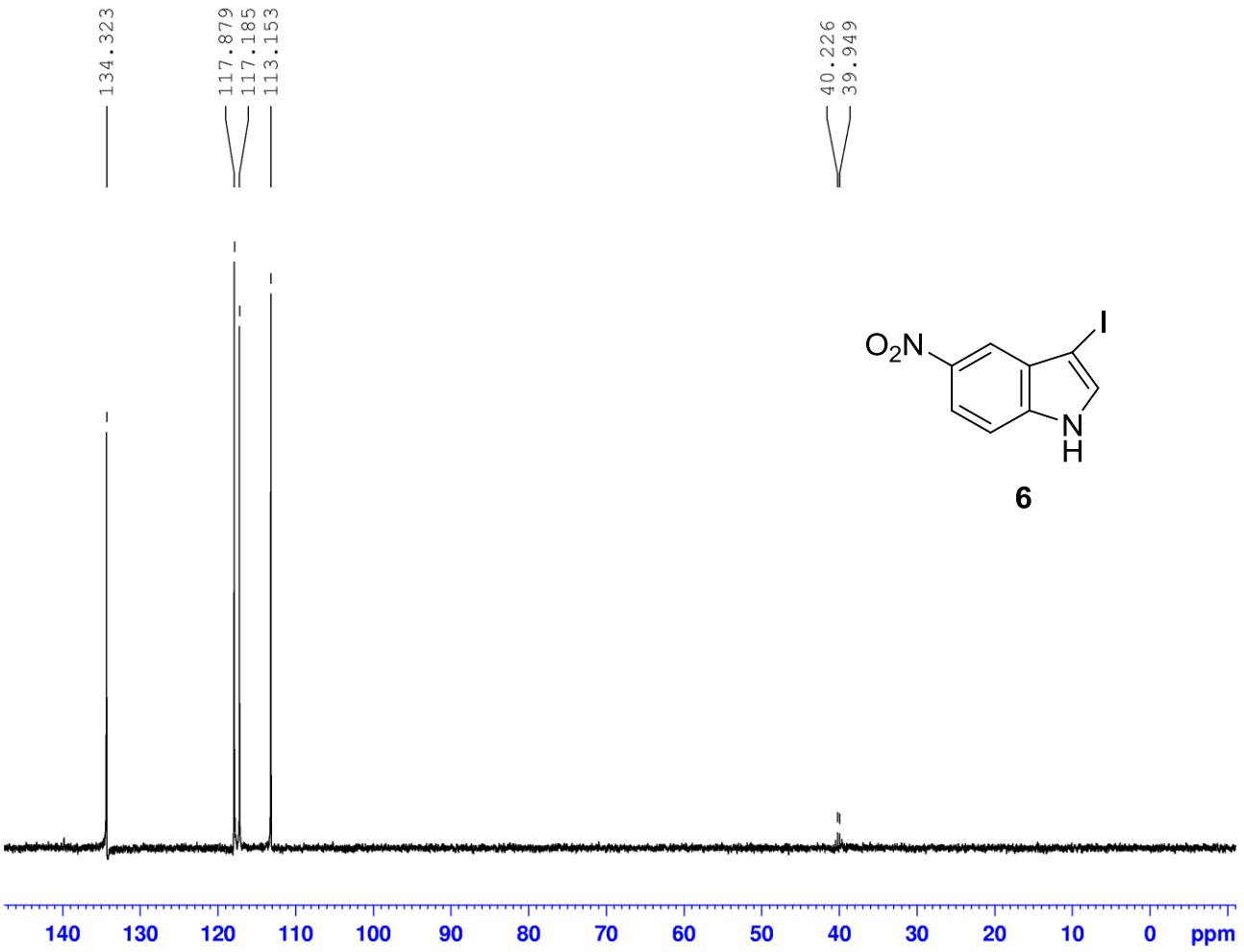


Fig. S9. ^{1}H-^{13}C gated-decoupled spectrum of compound 6.

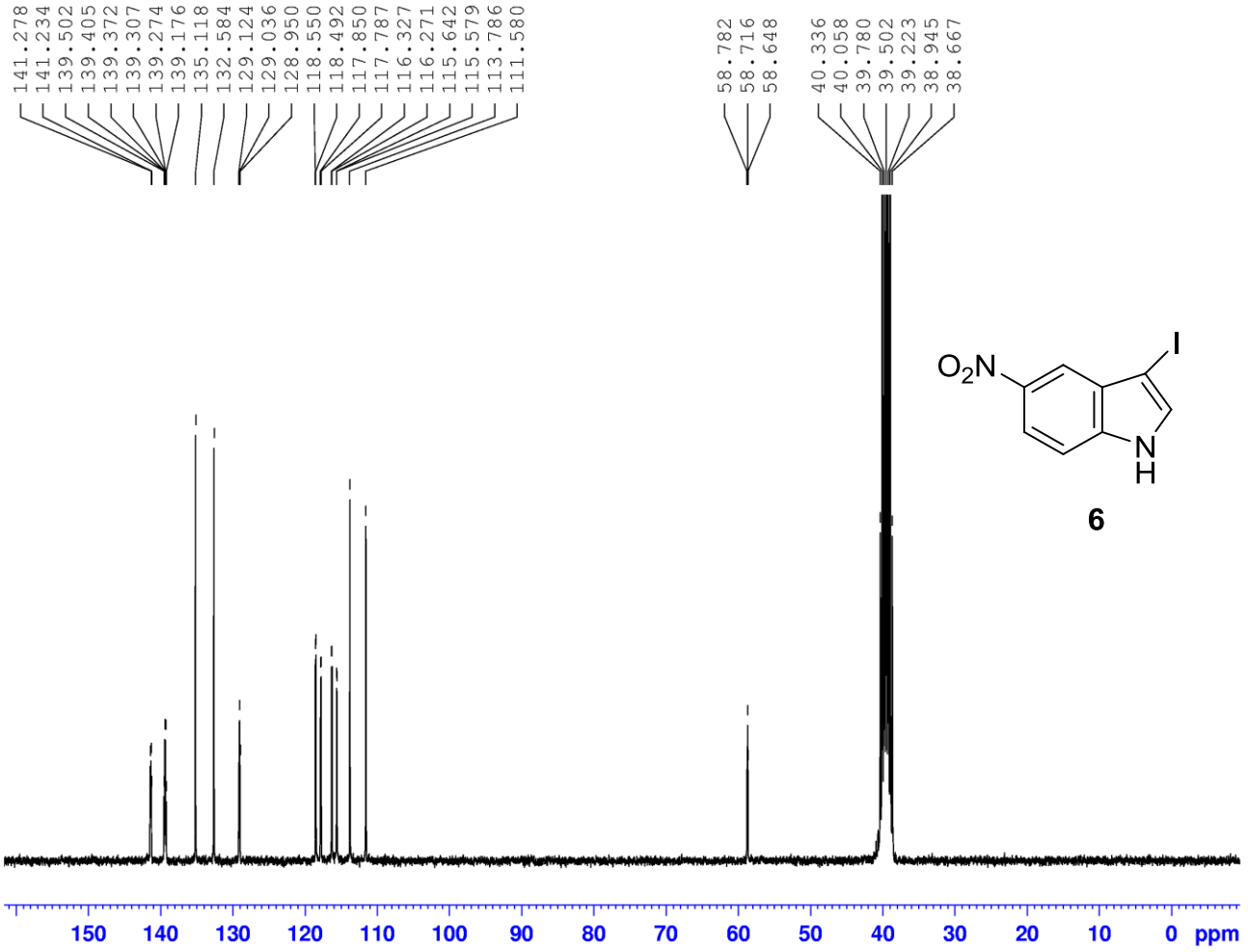


Fig. S10. ^{1}H NMR spectrum of compound 8.

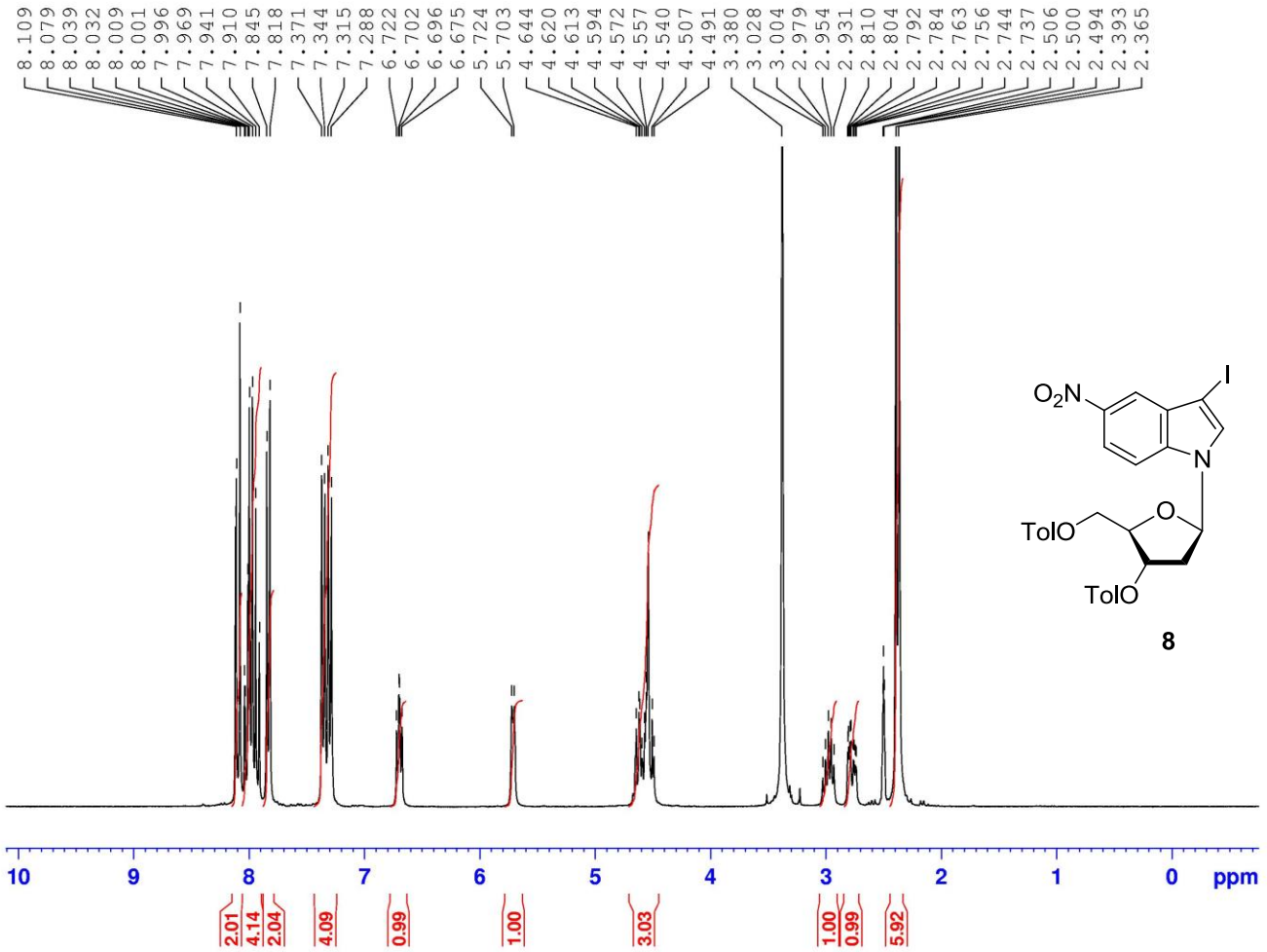


Fig. S11. ^{13}C NMR spectrum of compound 8.

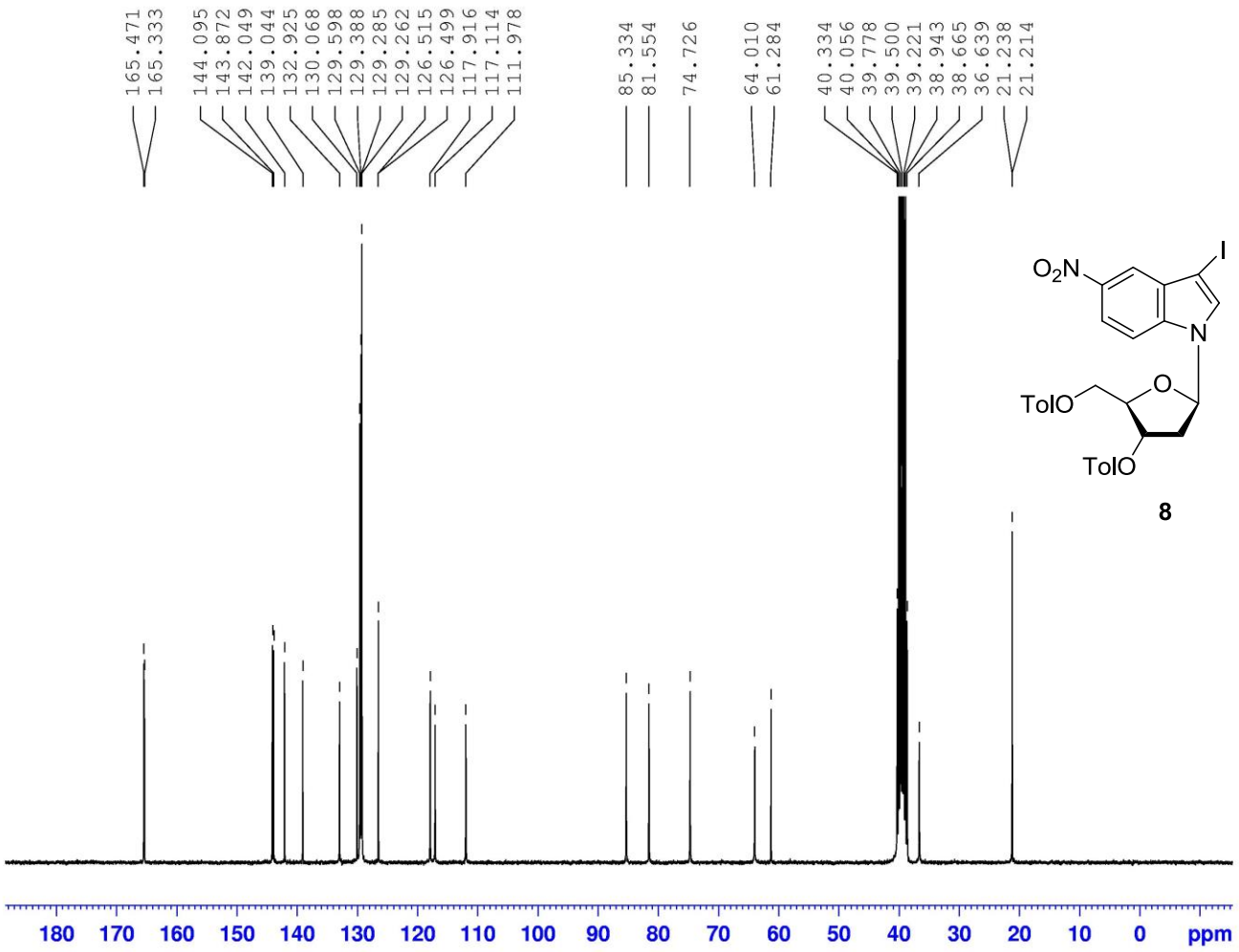


Fig. S12. DEPT-135 spectrum of compound 8.

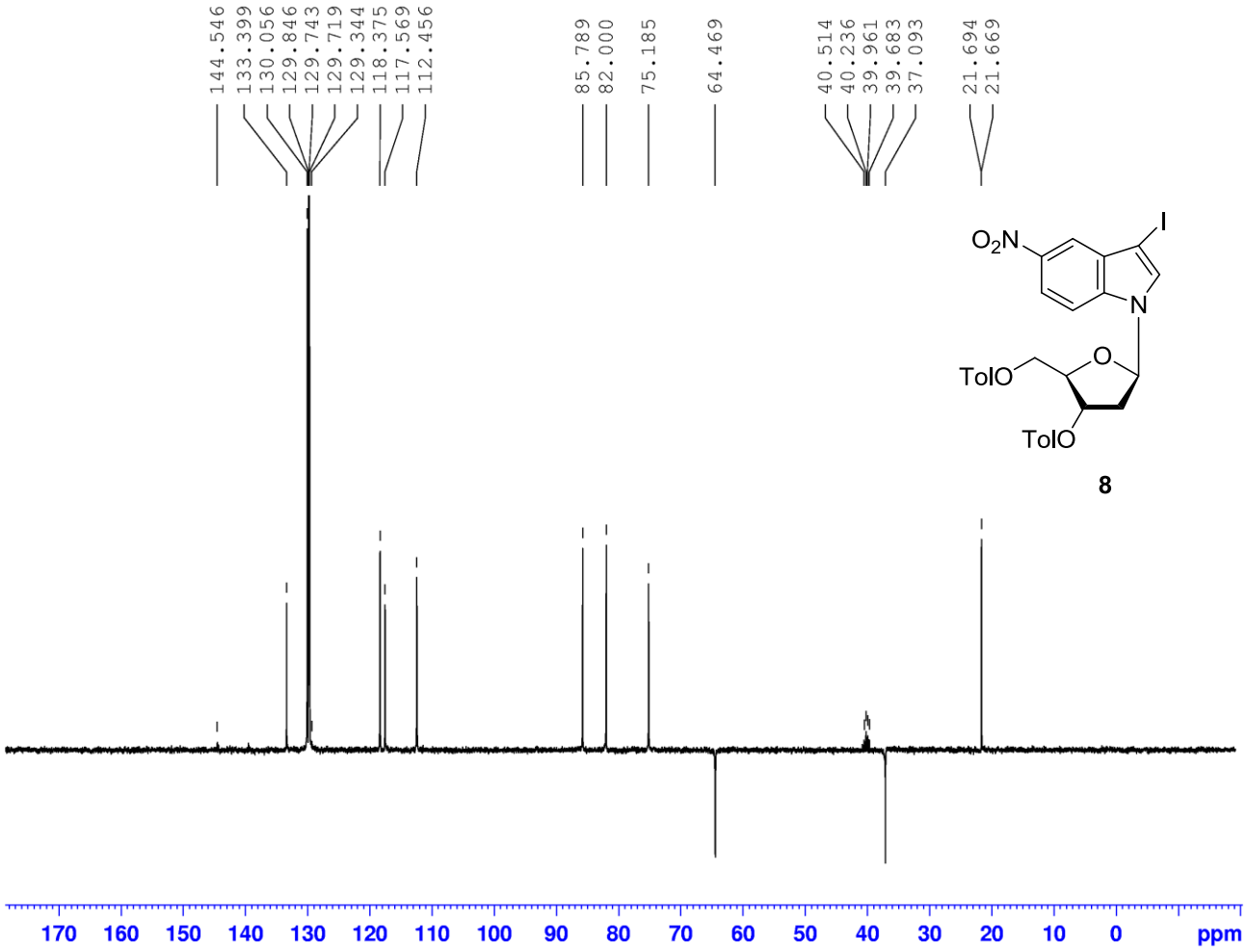


Fig. S13. ^{1}H-^{13}C gated-decoupled spectrum of compound 8.

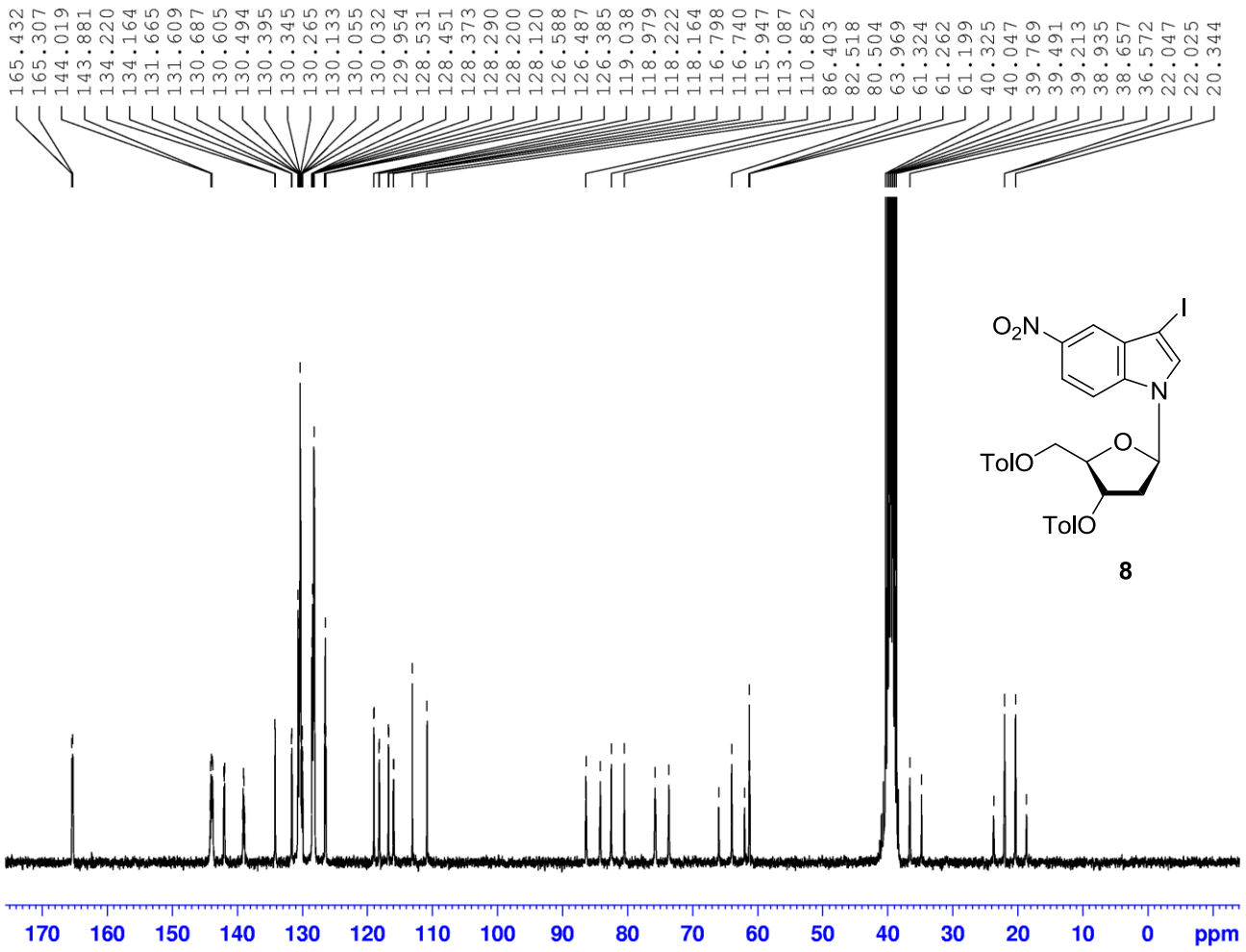


Fig. S14. ^{1}H NMR spectrum of compound 4b.

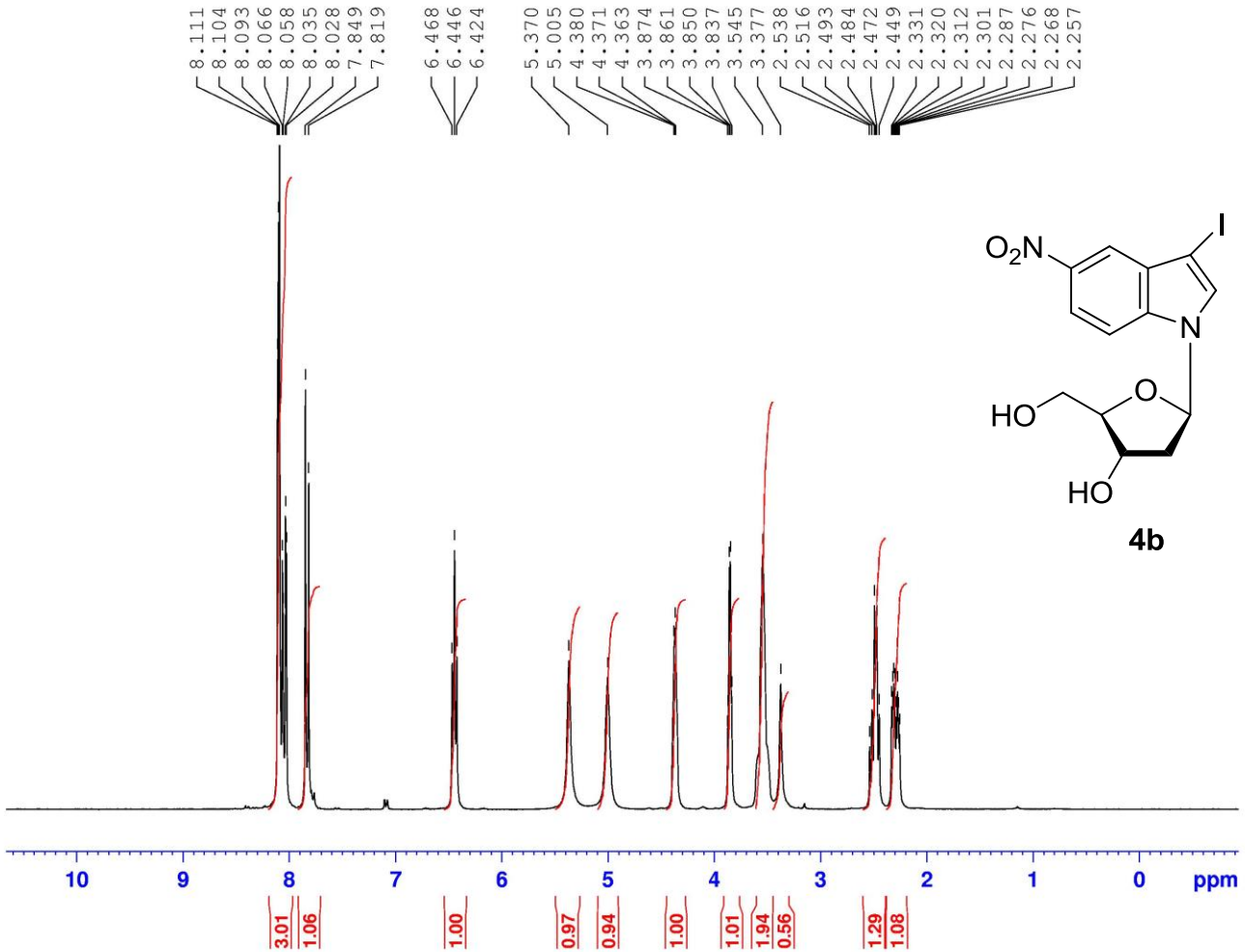


Fig. S15. ^{13}C NMR spectrum of compound 4b.

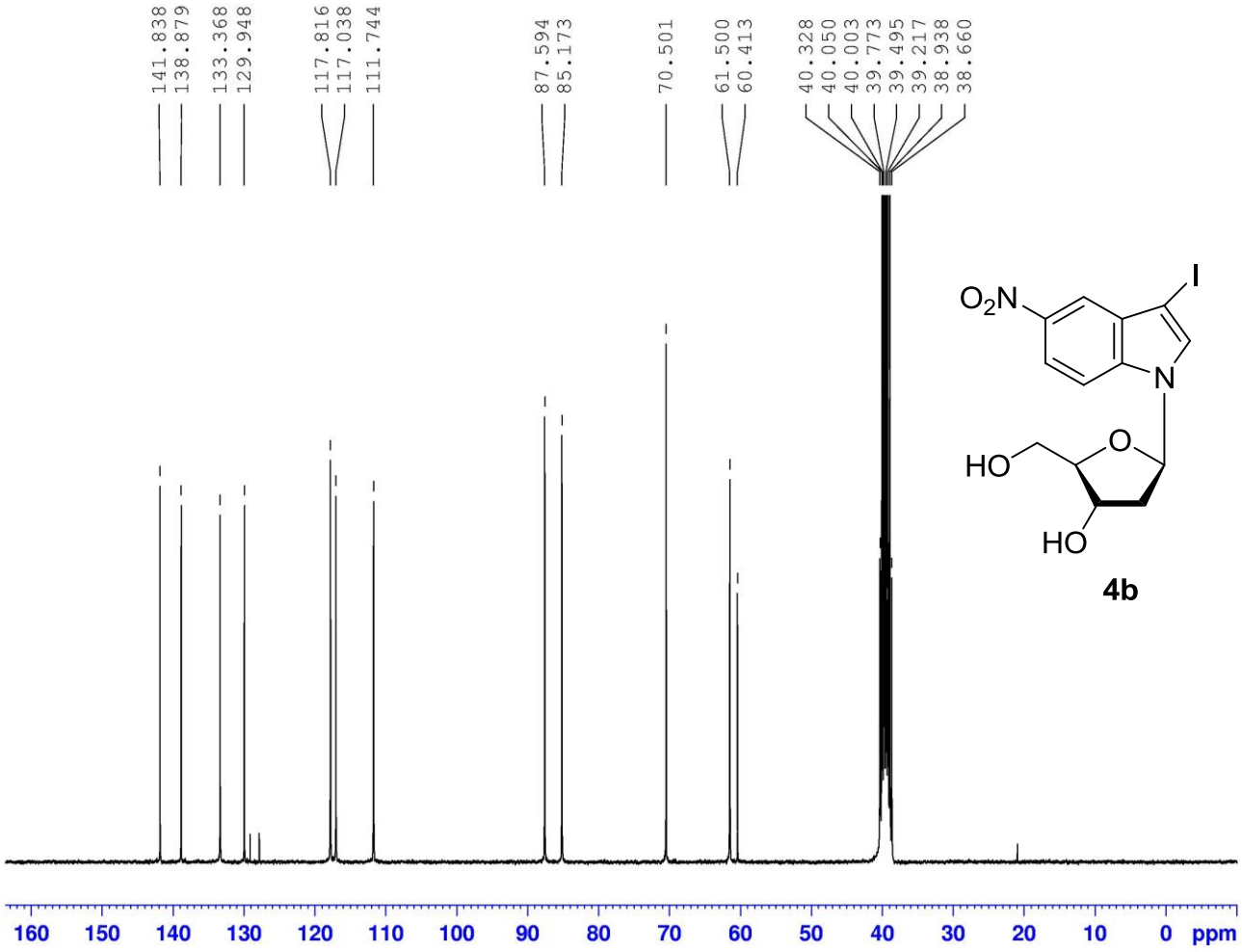


Fig. S16. DEPT-135 spectrum of compound 4b.

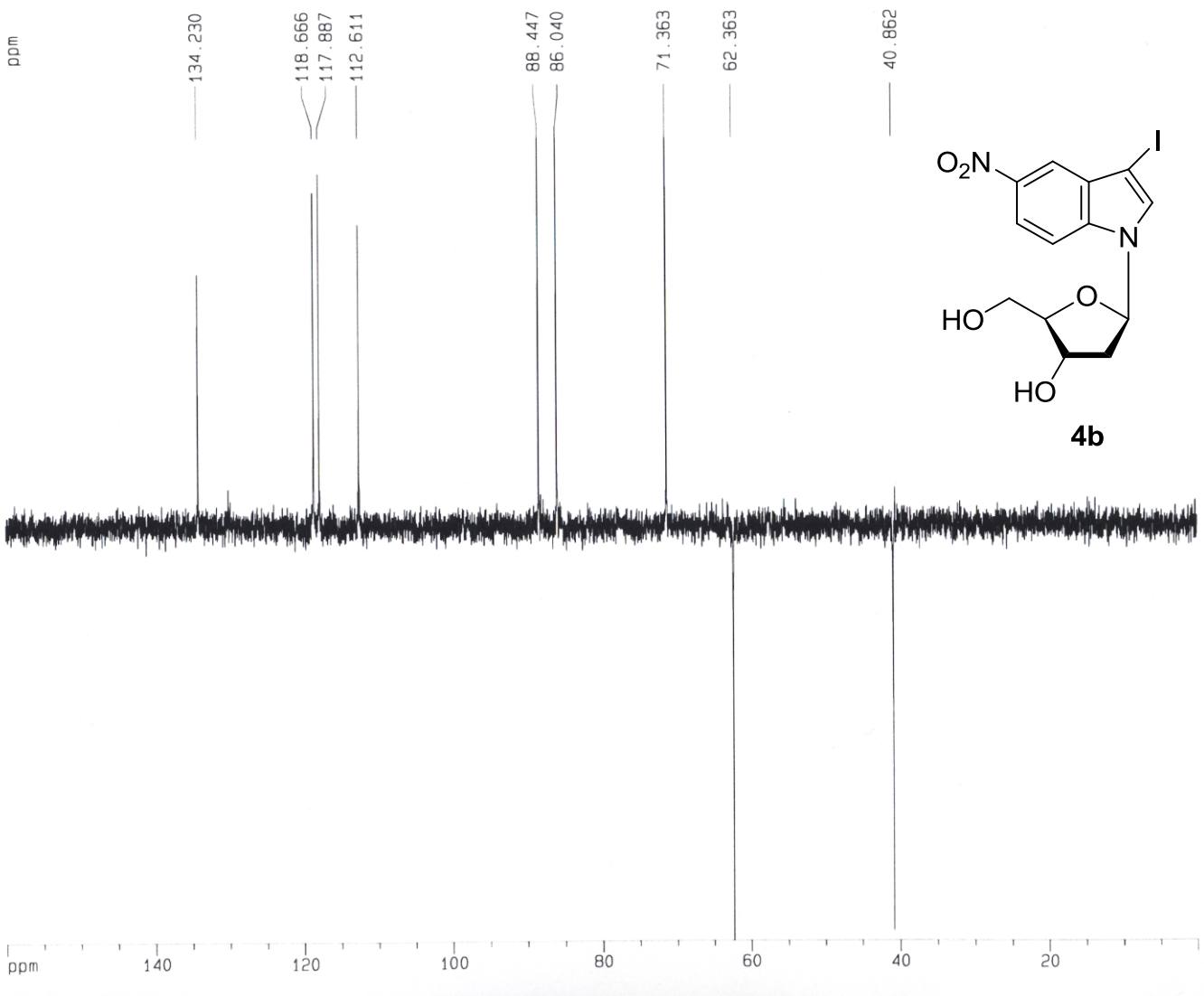


Fig. S17. ^{1}H-^{13}C gated-decoupled spectrum of compound 4b.

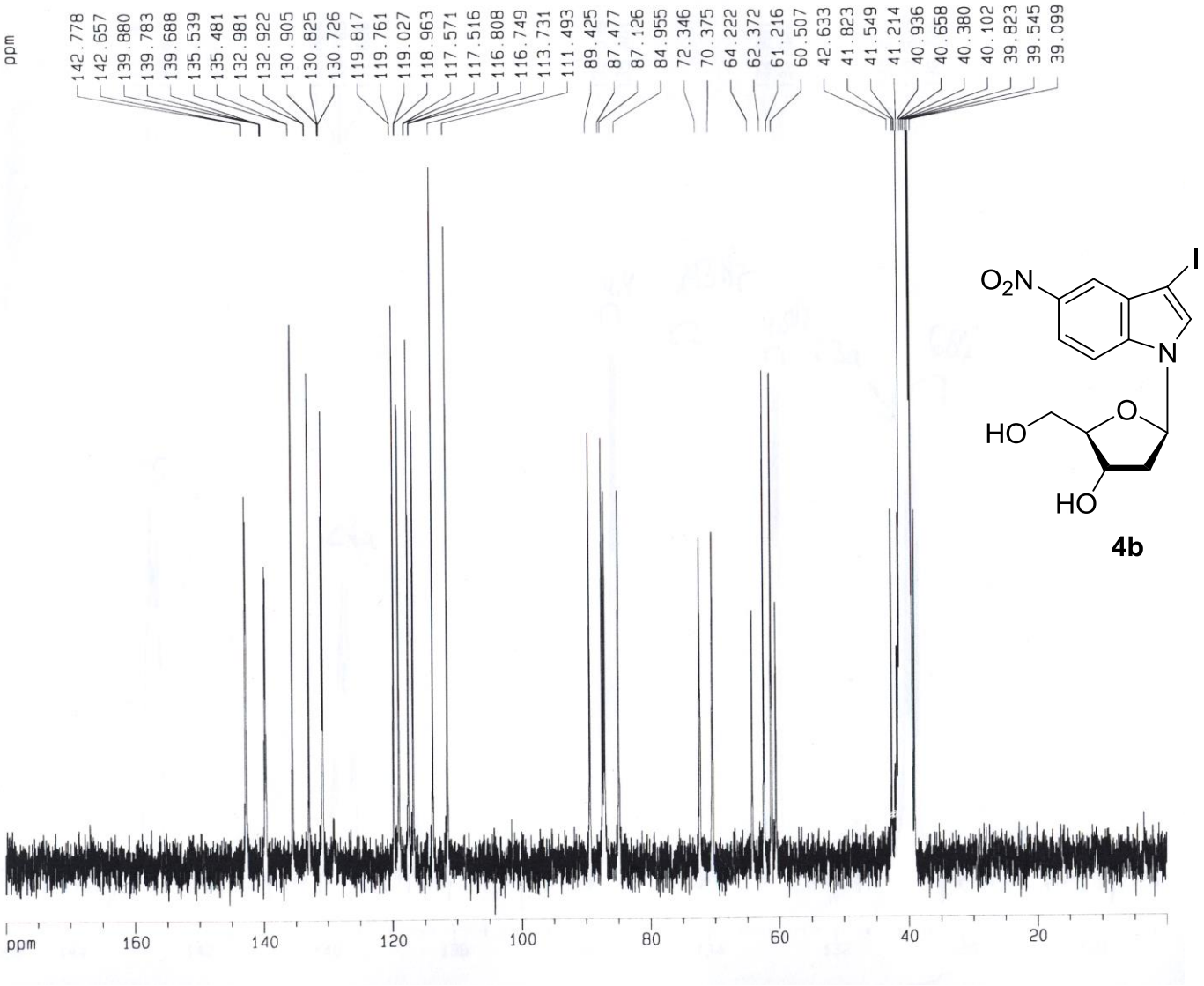


Fig. S18. ^{1}H NMR spectrum of compound 9b.

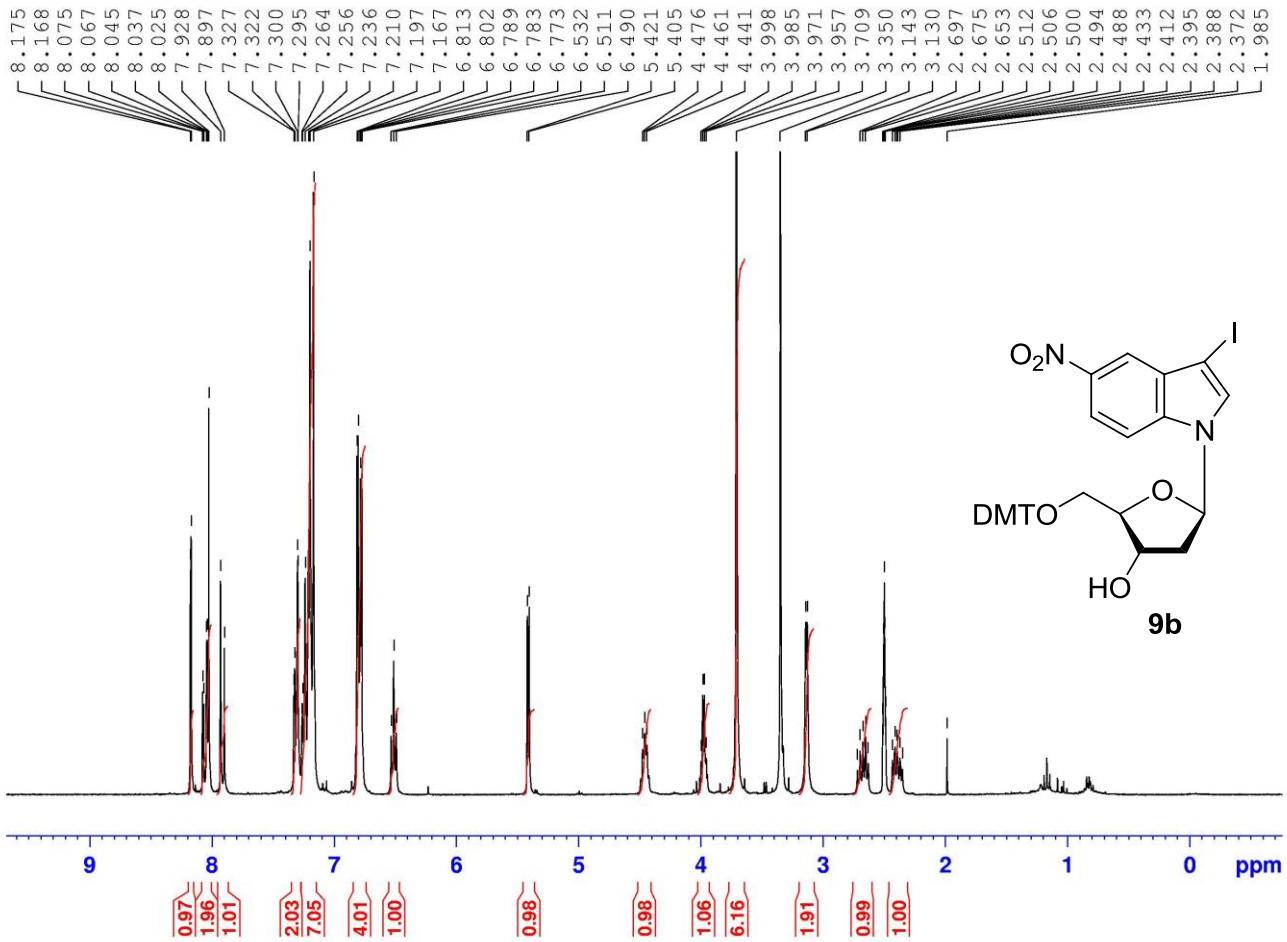


Fig. S19. ^{13}C NMR spectrum of compound 9b.

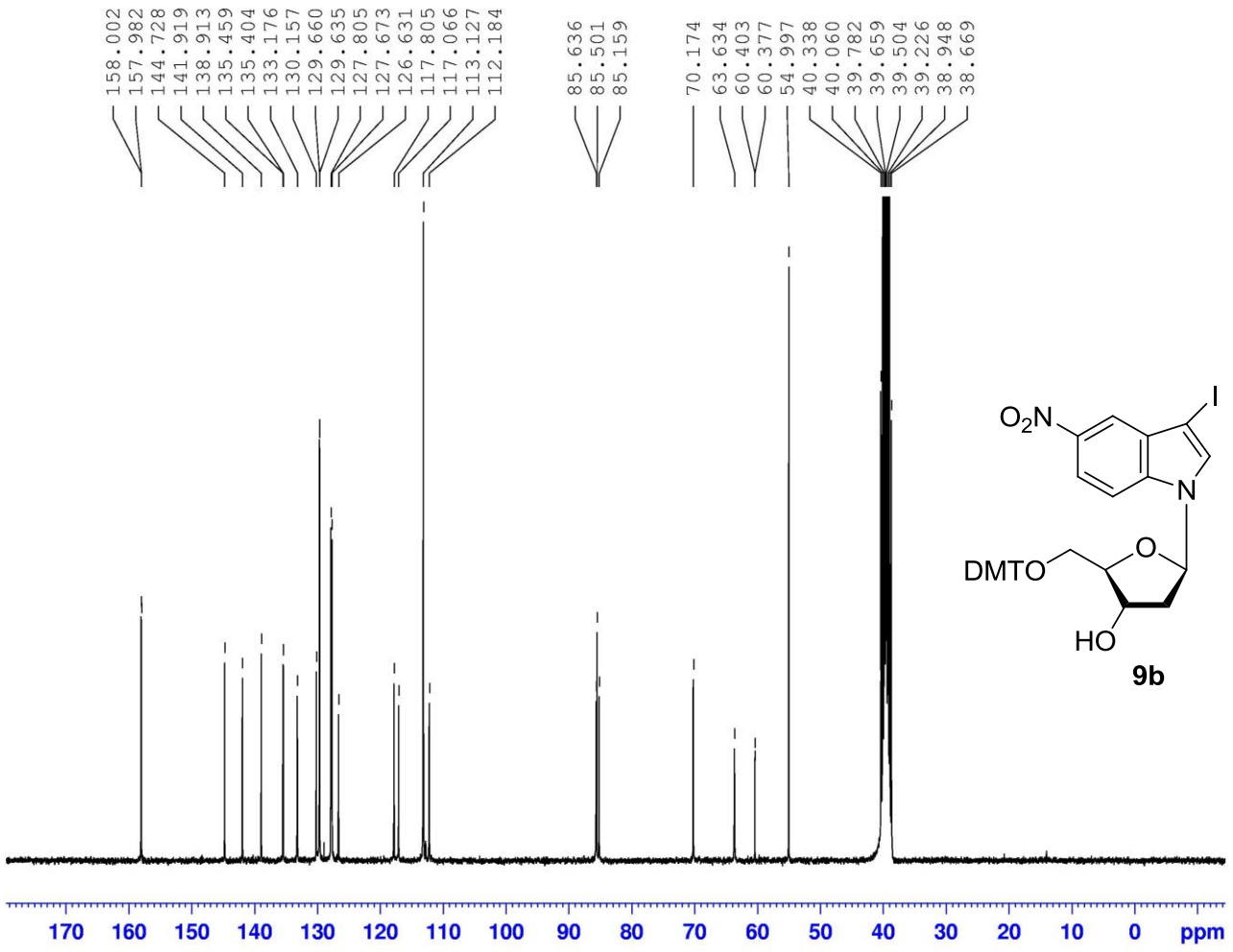


Fig. S20. DEPT-135 spectrum of compound 9b.

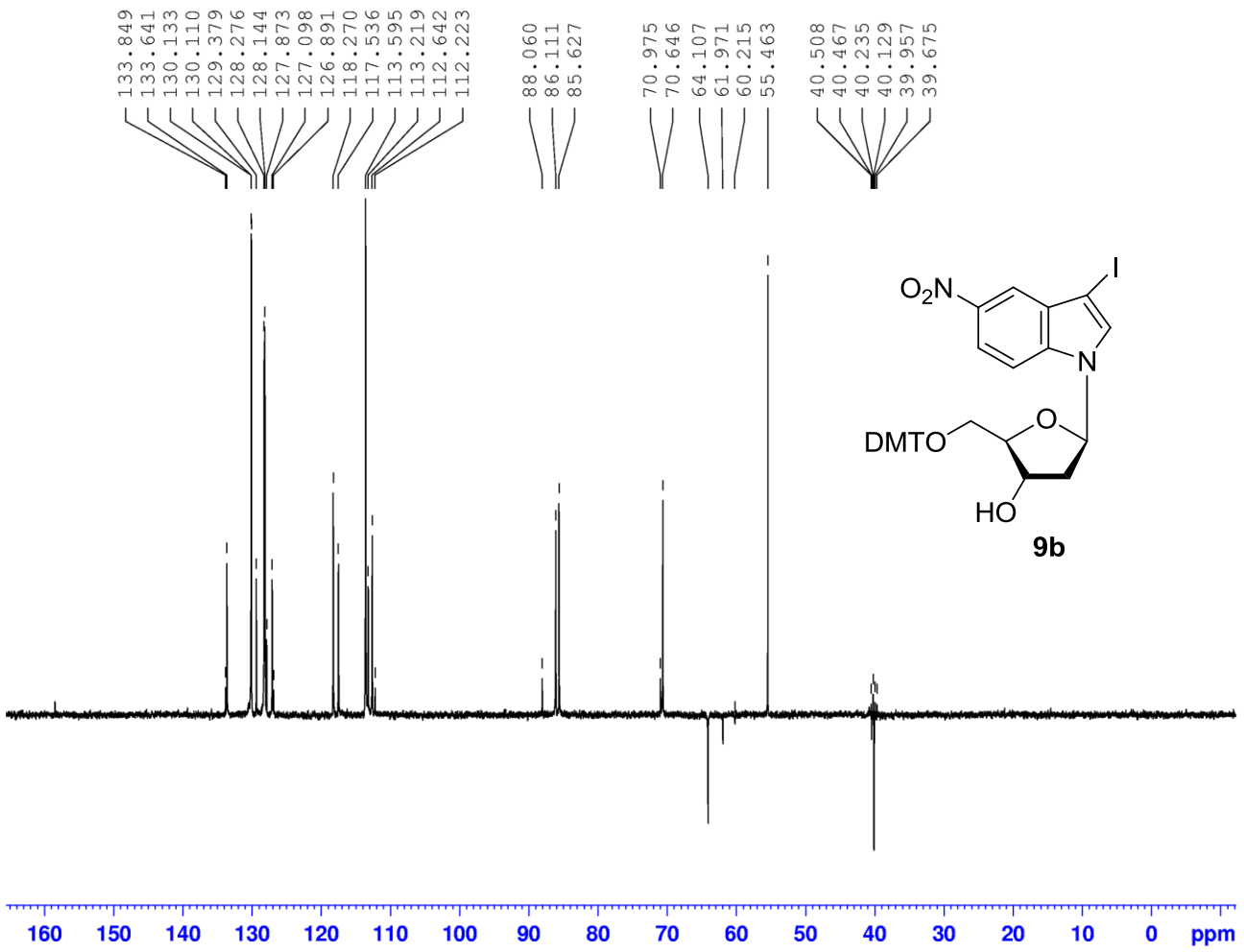


Fig. S21. ^{1}H-^{13}C gated-decoupled spectrum of compound 9b.

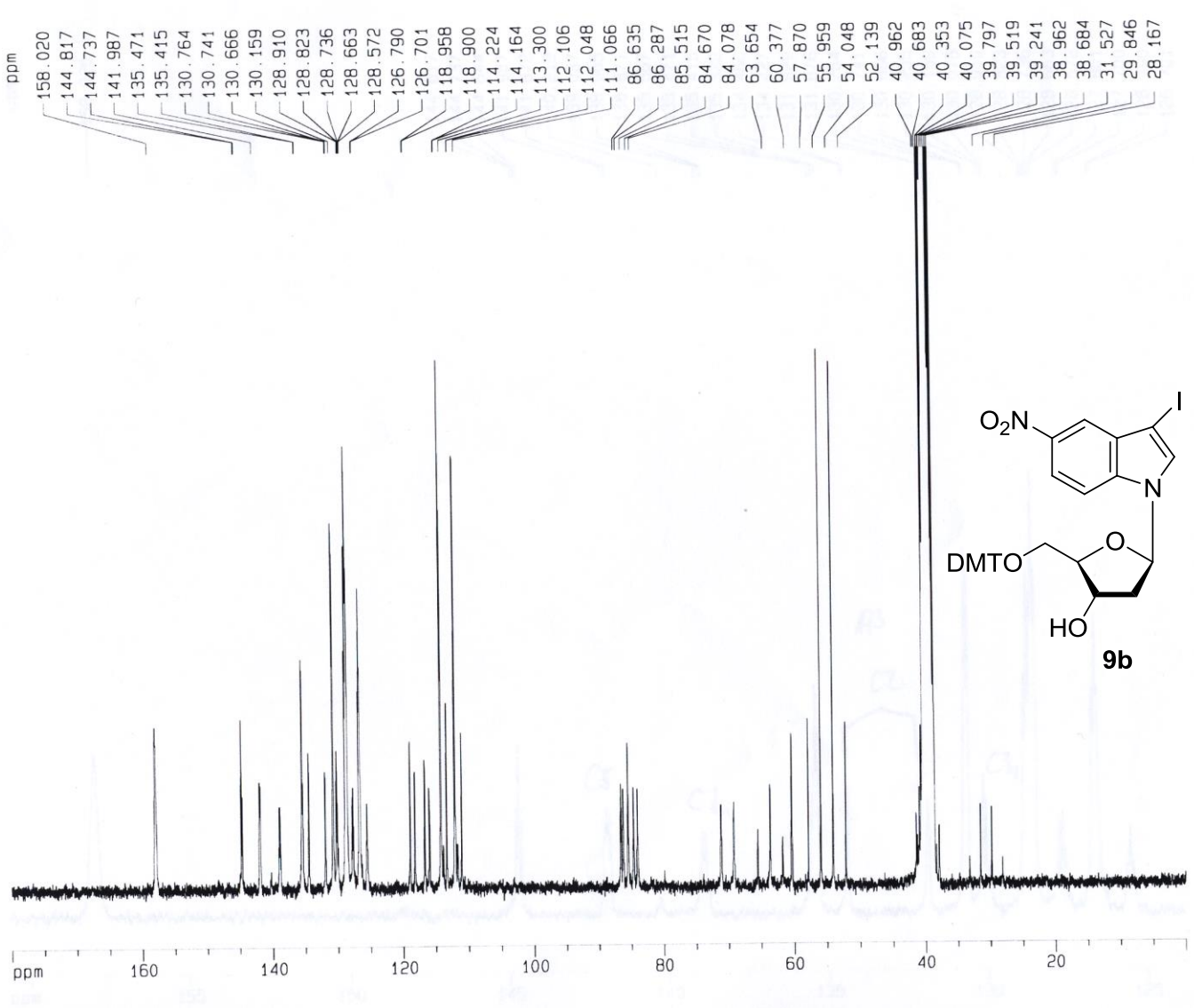


Fig. S22. ^{1}H NMR spectrum of compound 10b.

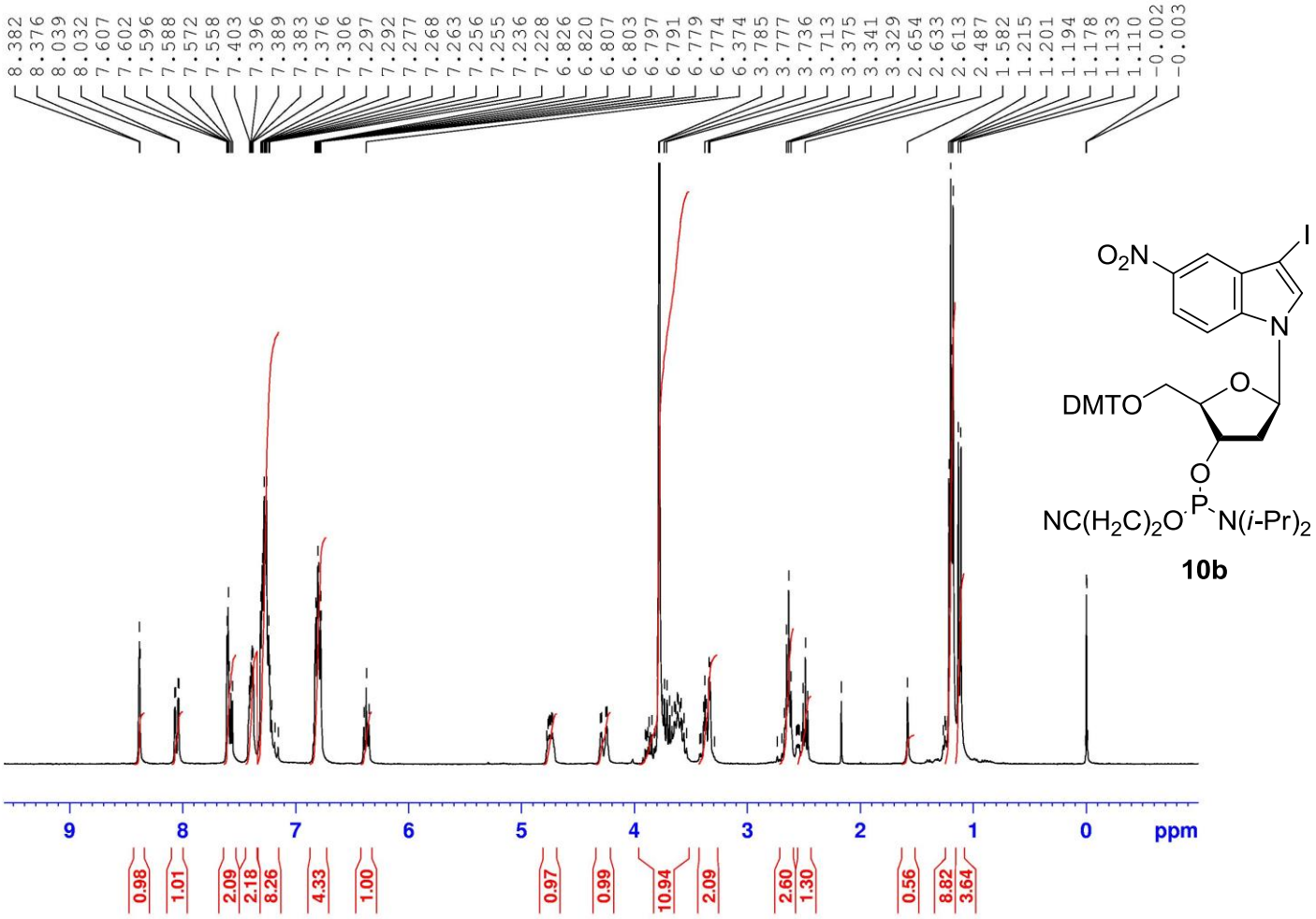


Fig. S23. ^{13}C NMR spectrum of compound 10b.

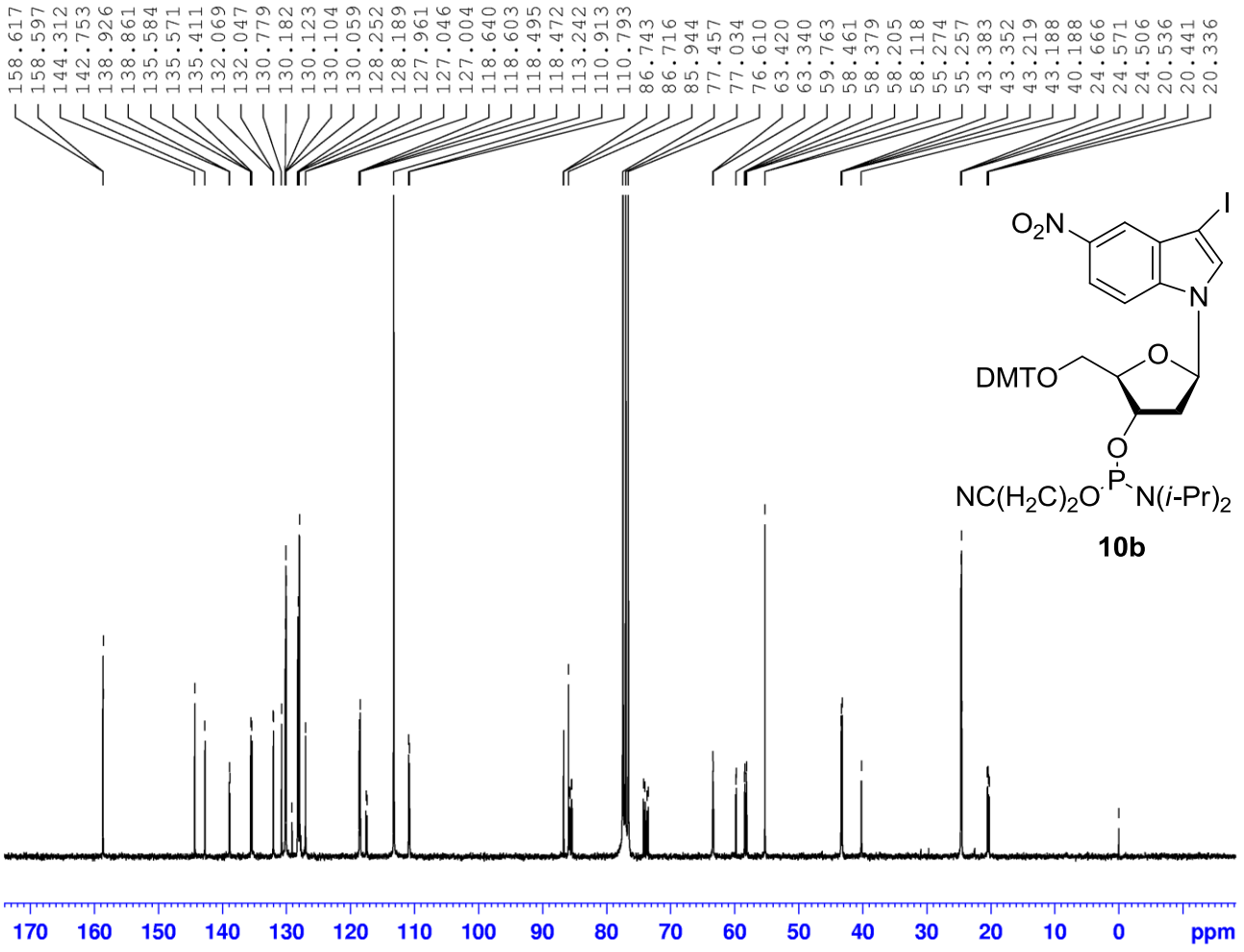


Fig. S24. ^{31}P NMR spectrum of compound 10b.

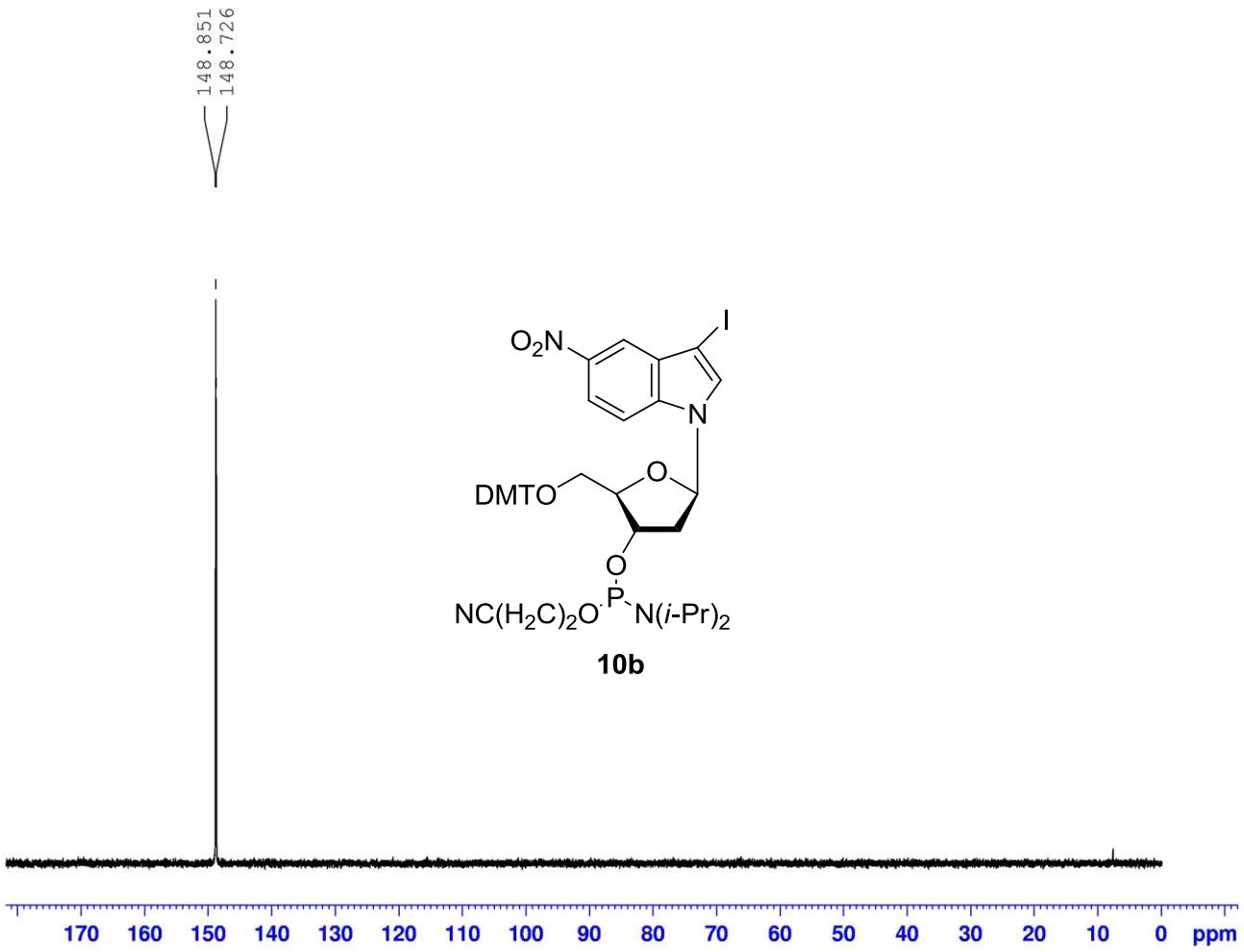


Fig. S25. ^{1}H NMR spectrum of compound 4c.

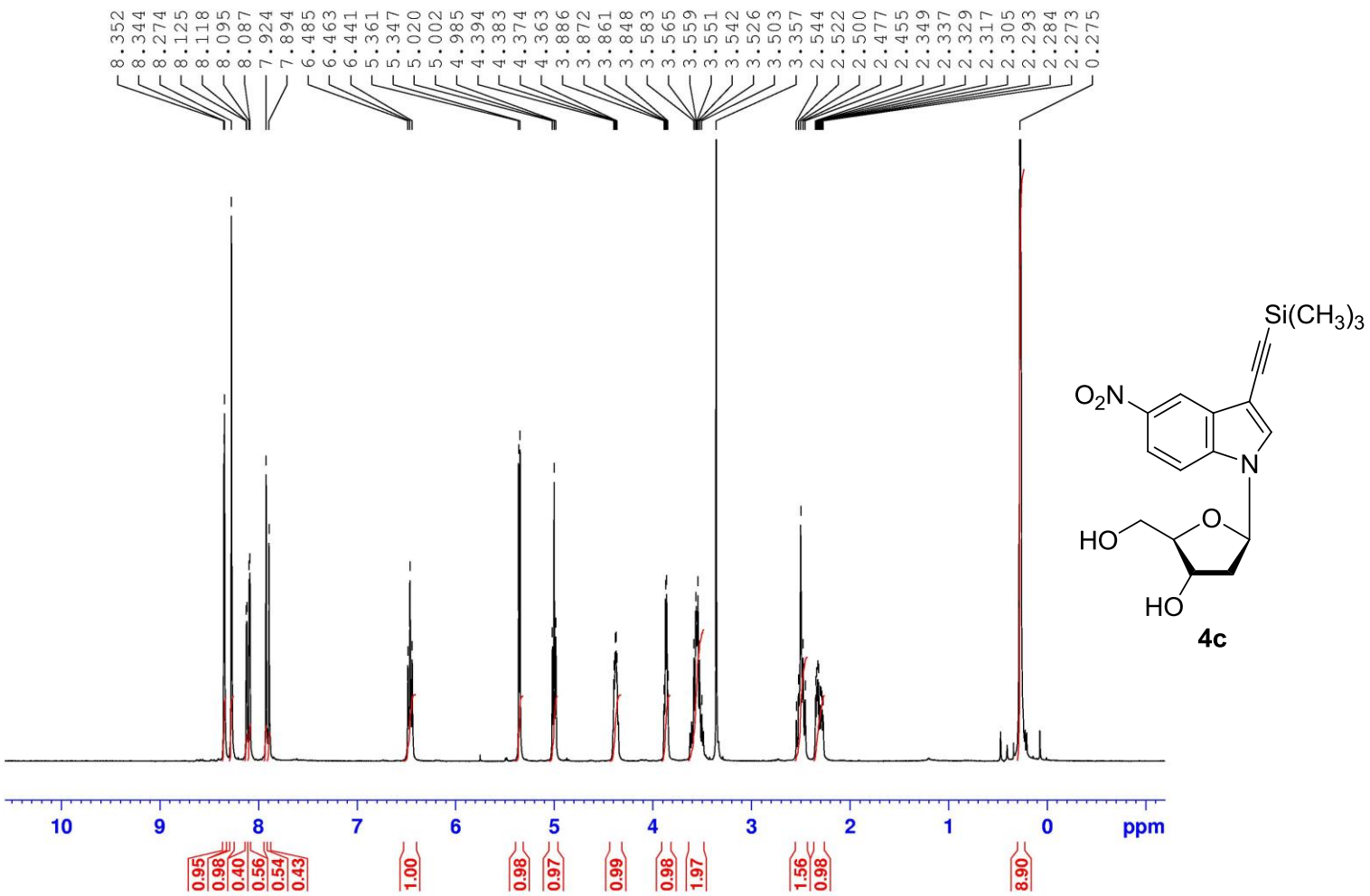


Fig. S26. ^{13}C NMR spectrum of compound 4c.

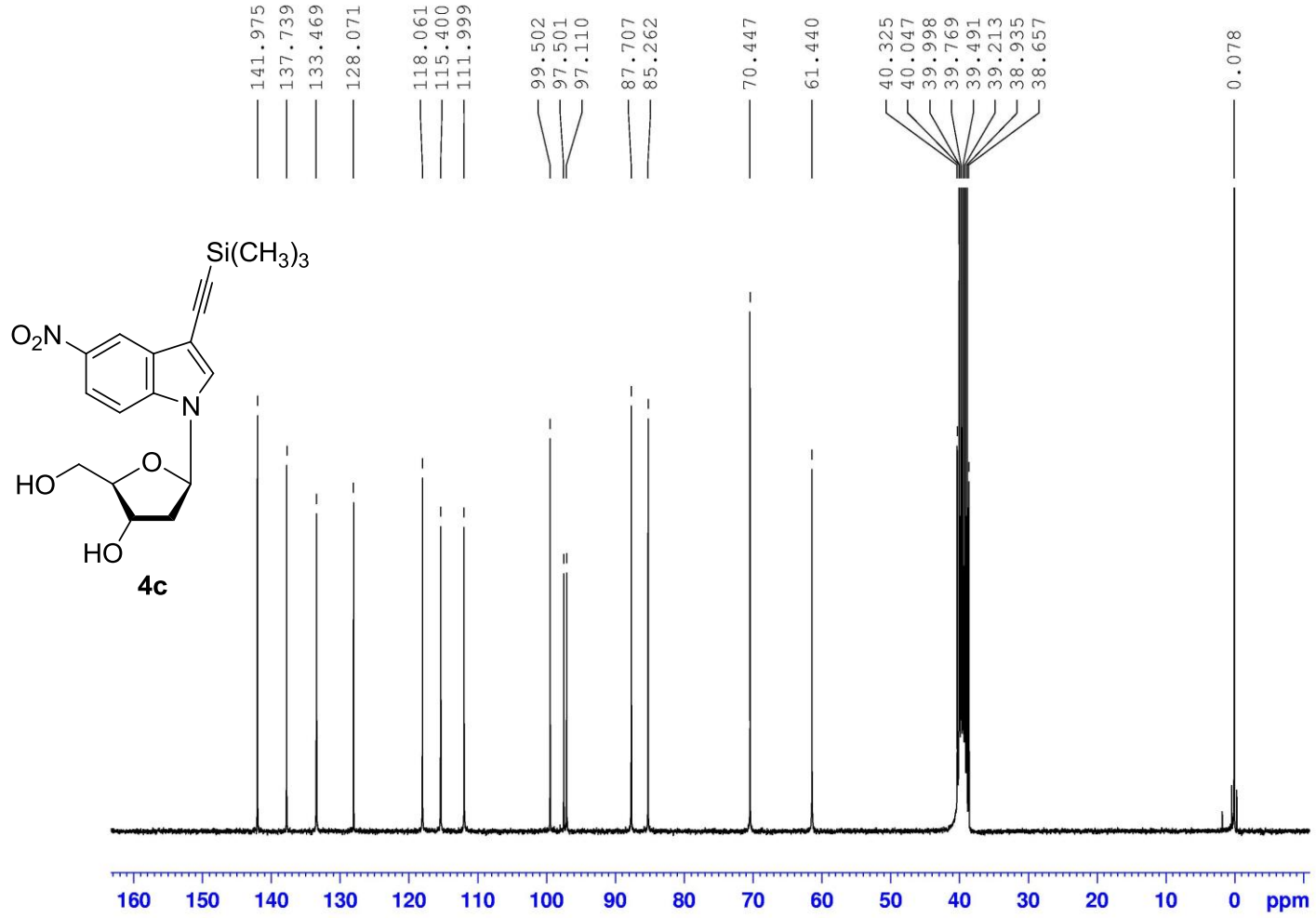


Fig. S27. DEPT-135 spectrum of compound 4c.

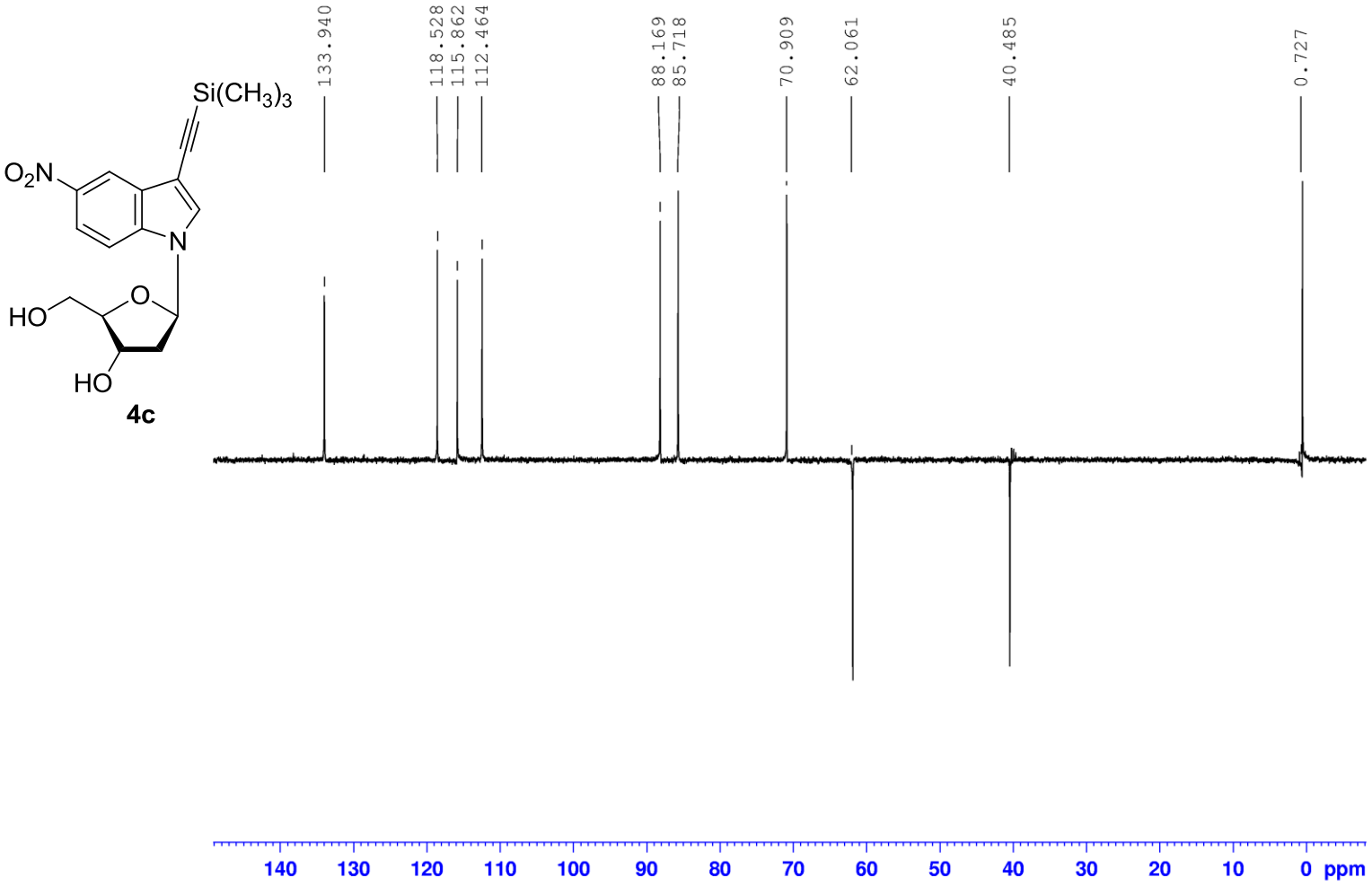


Fig. S28. ^{1}H-^{13}C gated-decoupled spectrum of compound 4c.

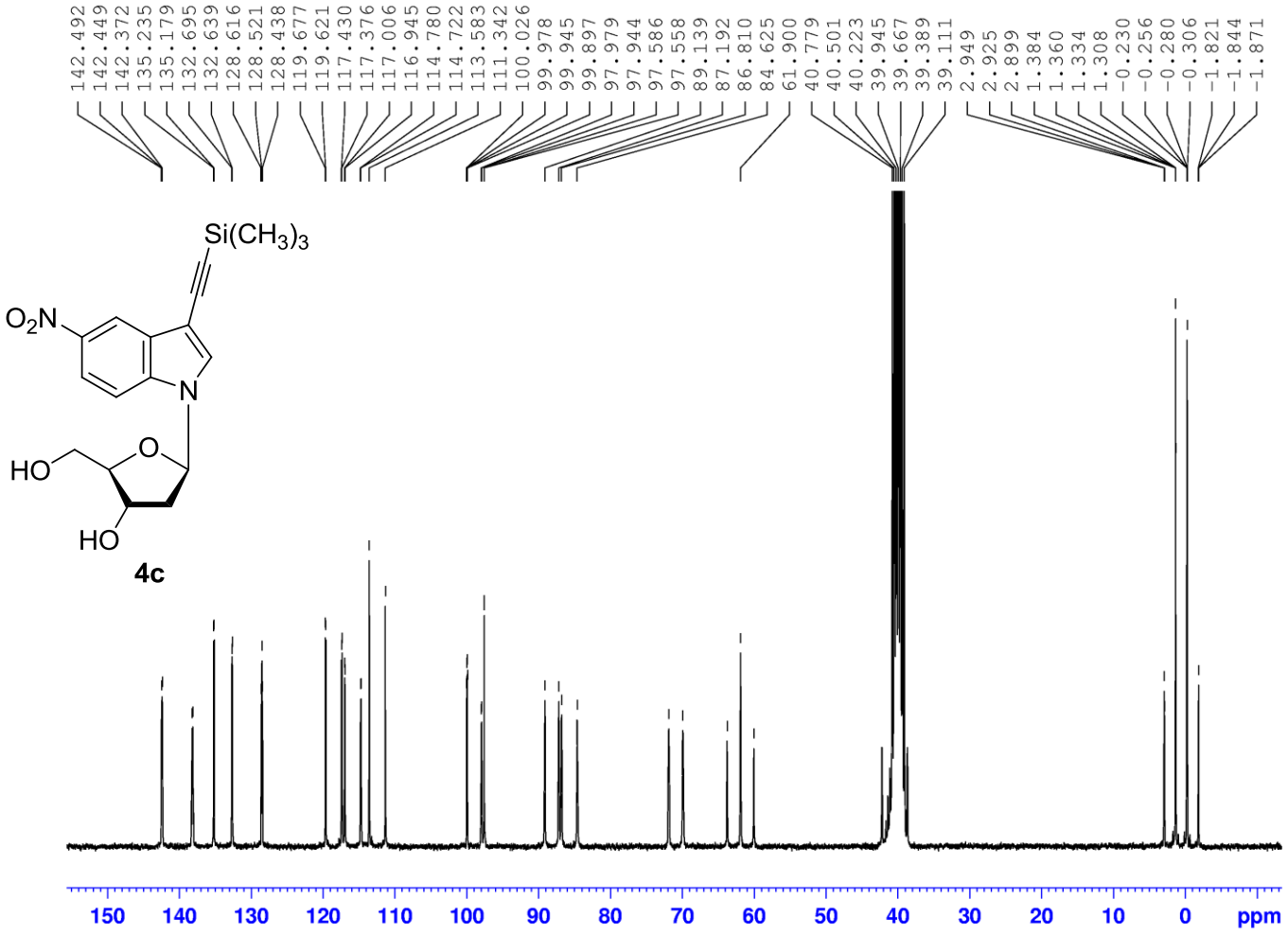


Fig. S29. ^{1}H NMR spectrum of compound 4d.

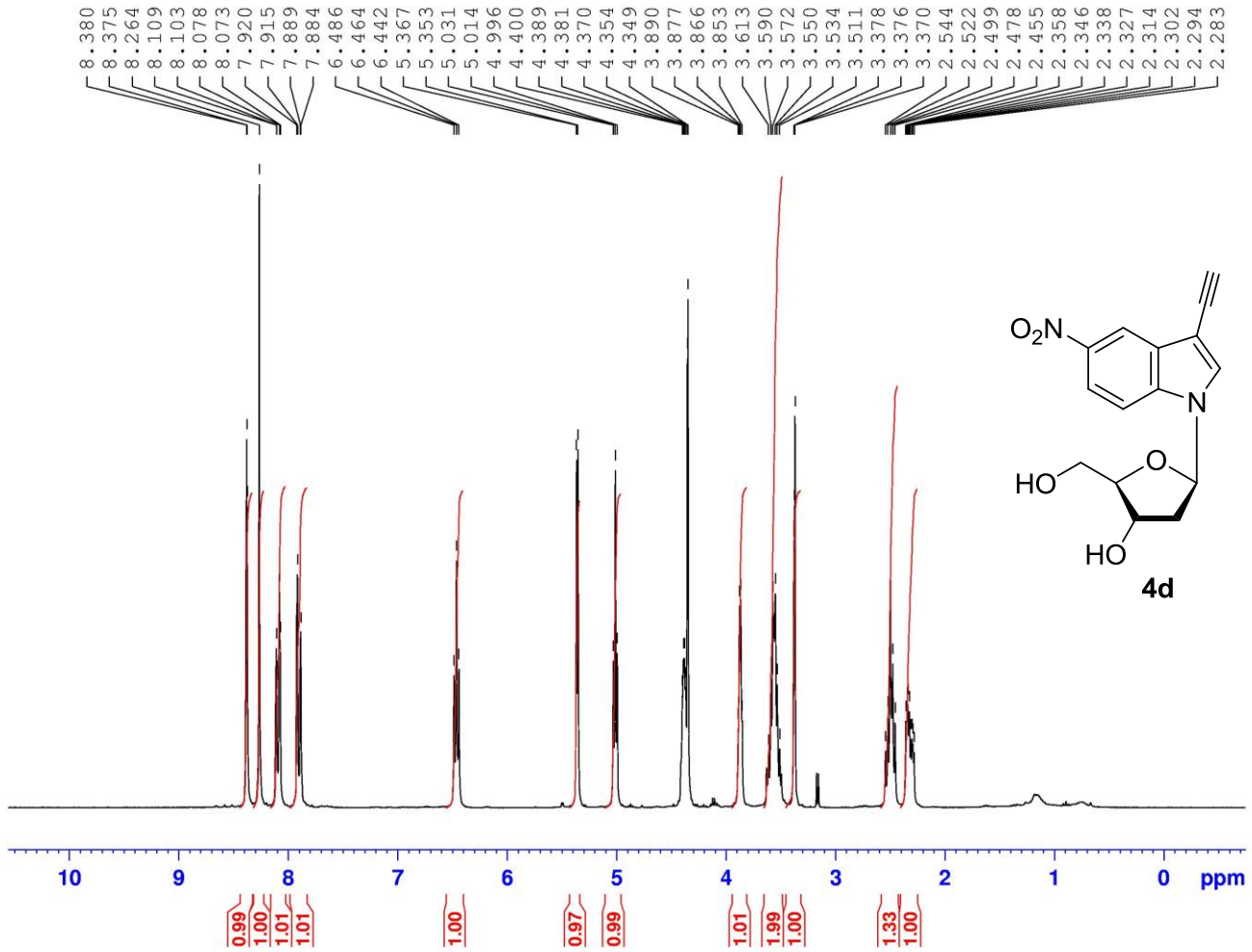


Fig. S30. ^{13}C NMR spectrum of compound 4d.

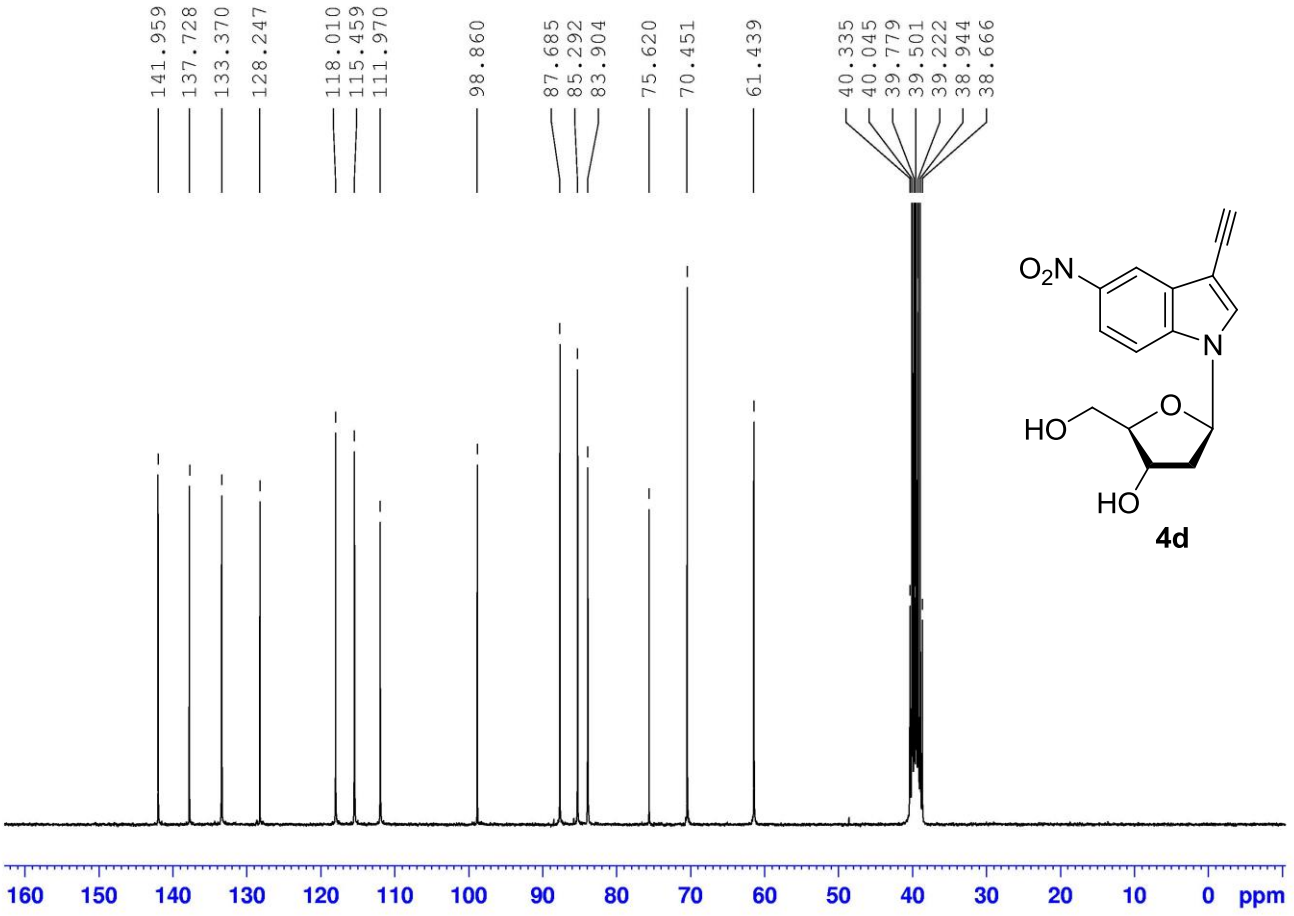


Fig. S31. DEPT-135 spectrum of compound 4d.

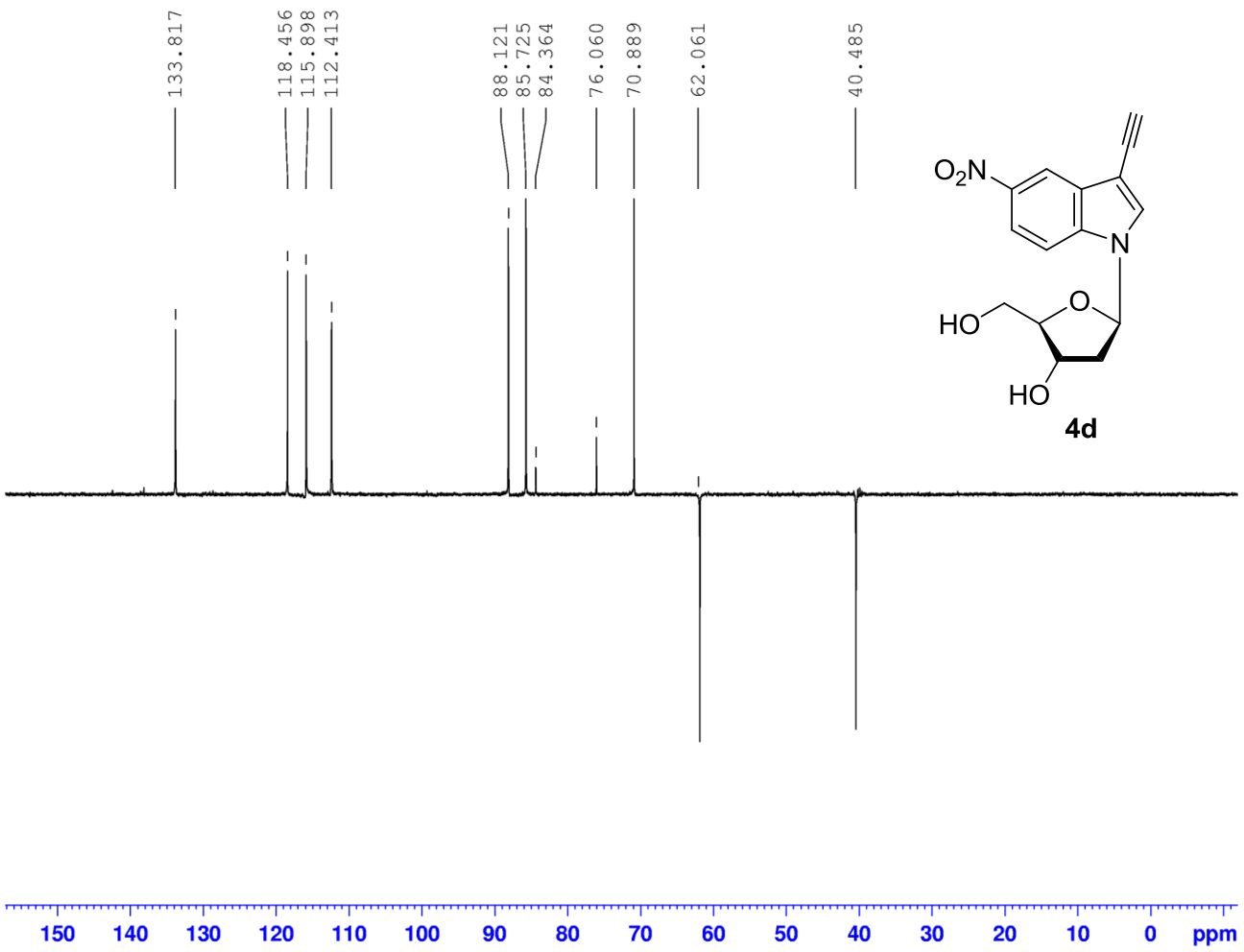


Fig. S32. ^{1}H-^{13}C gated-decoupled spectrum of compound 4d.

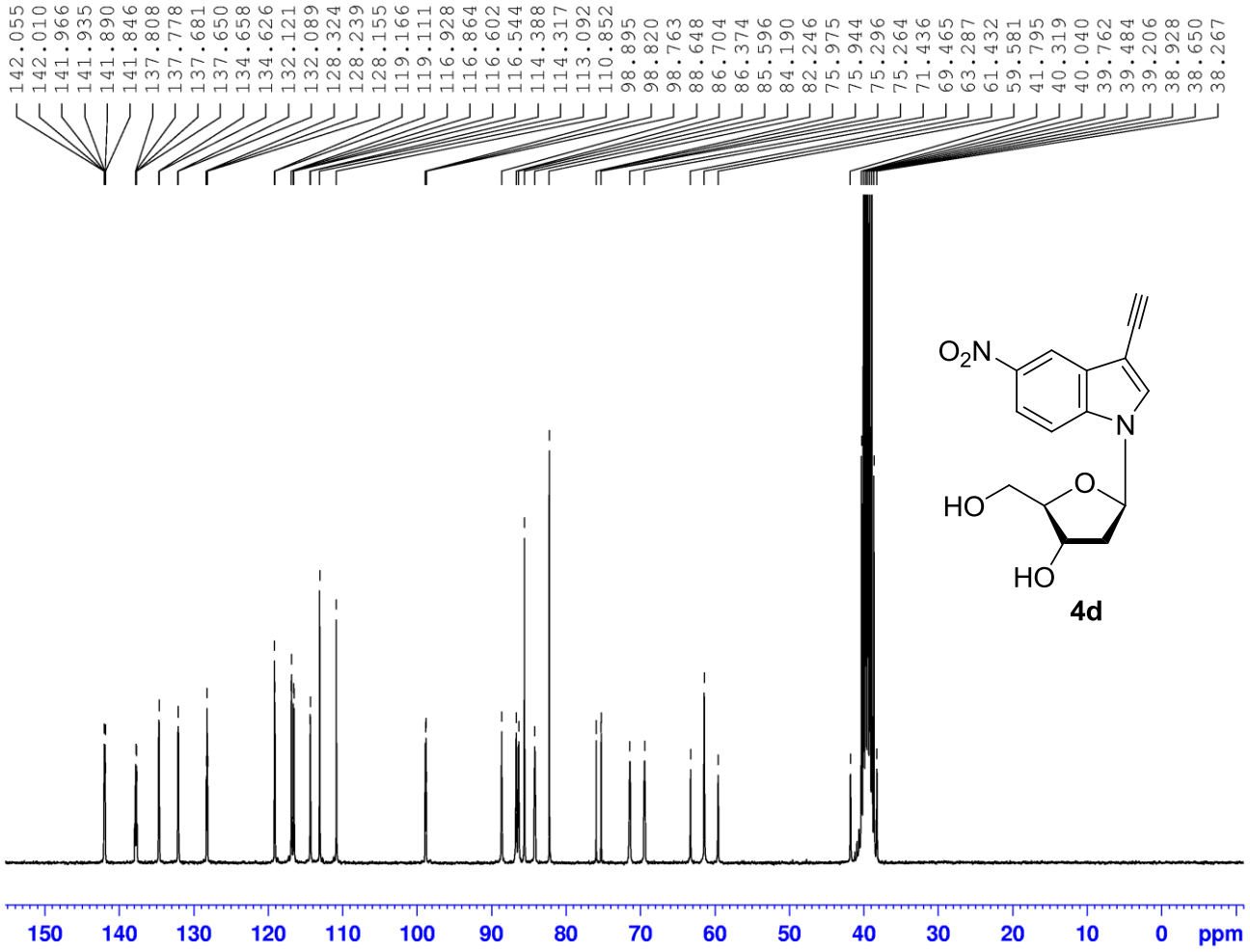


Fig. S33. ^{1}H NMR spectrum of compound 9d.

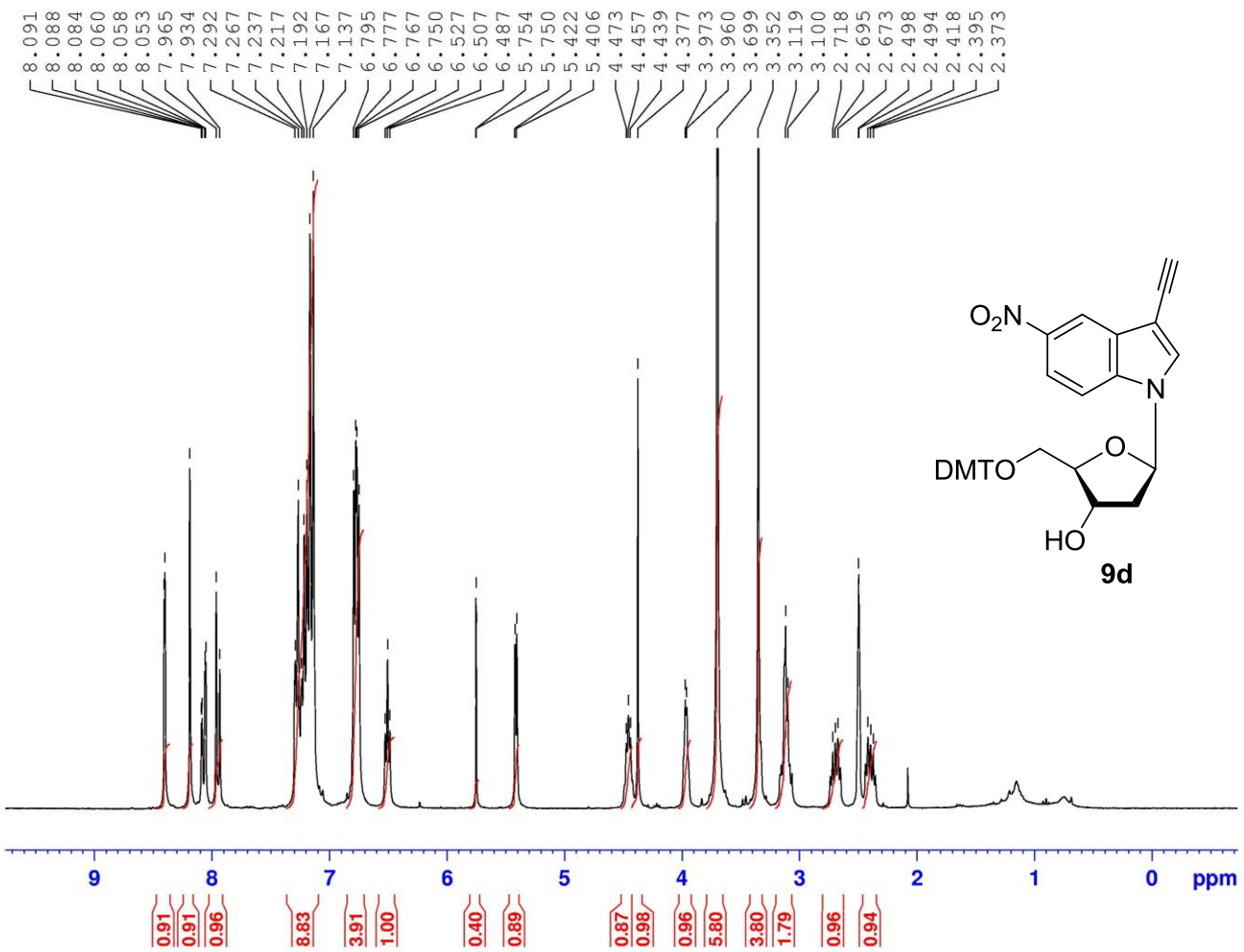


Fig. S34. ^{13}C NMR spectrum of compound 9d.

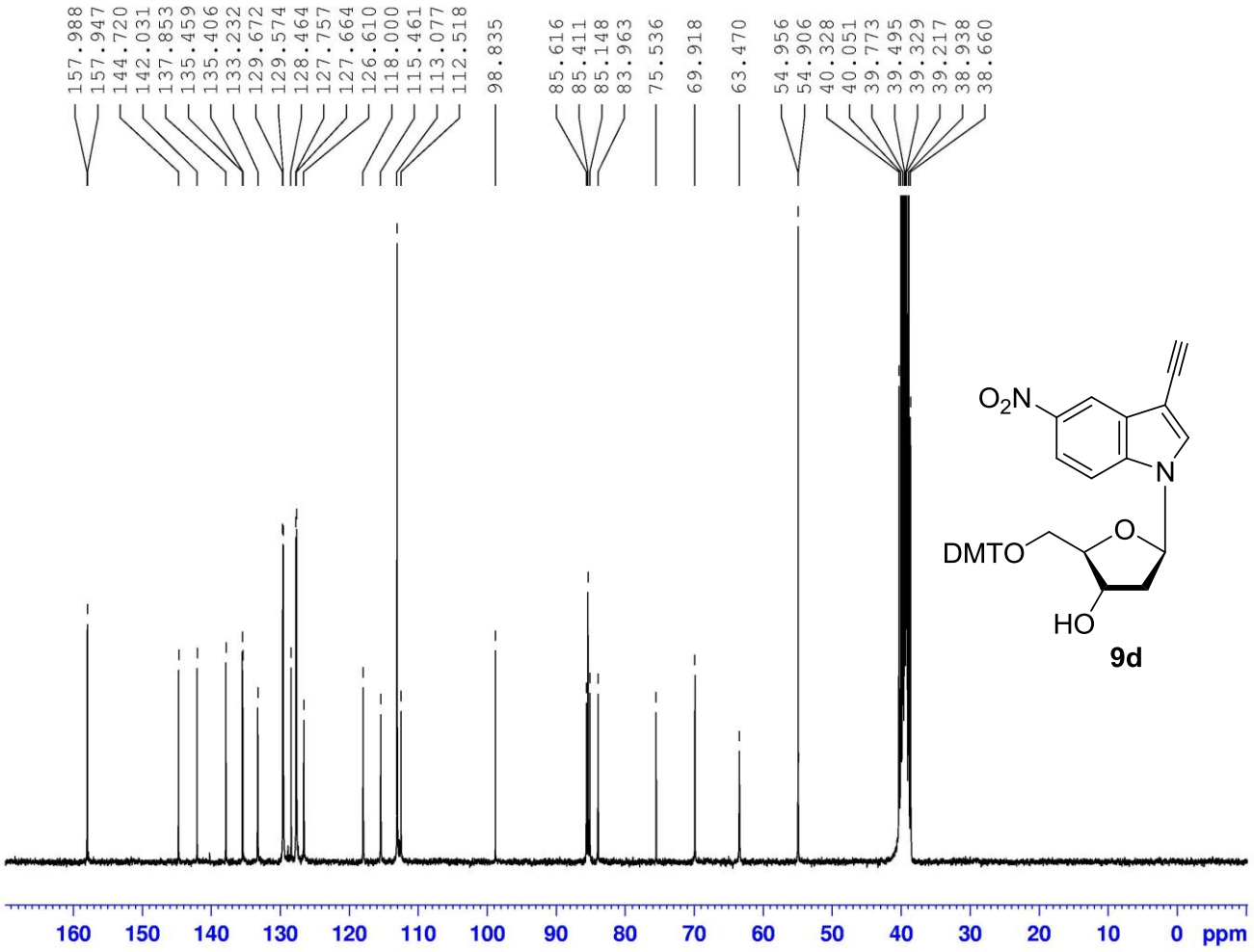


Fig. S35. DEPT-135 spectrum of compound 9d.

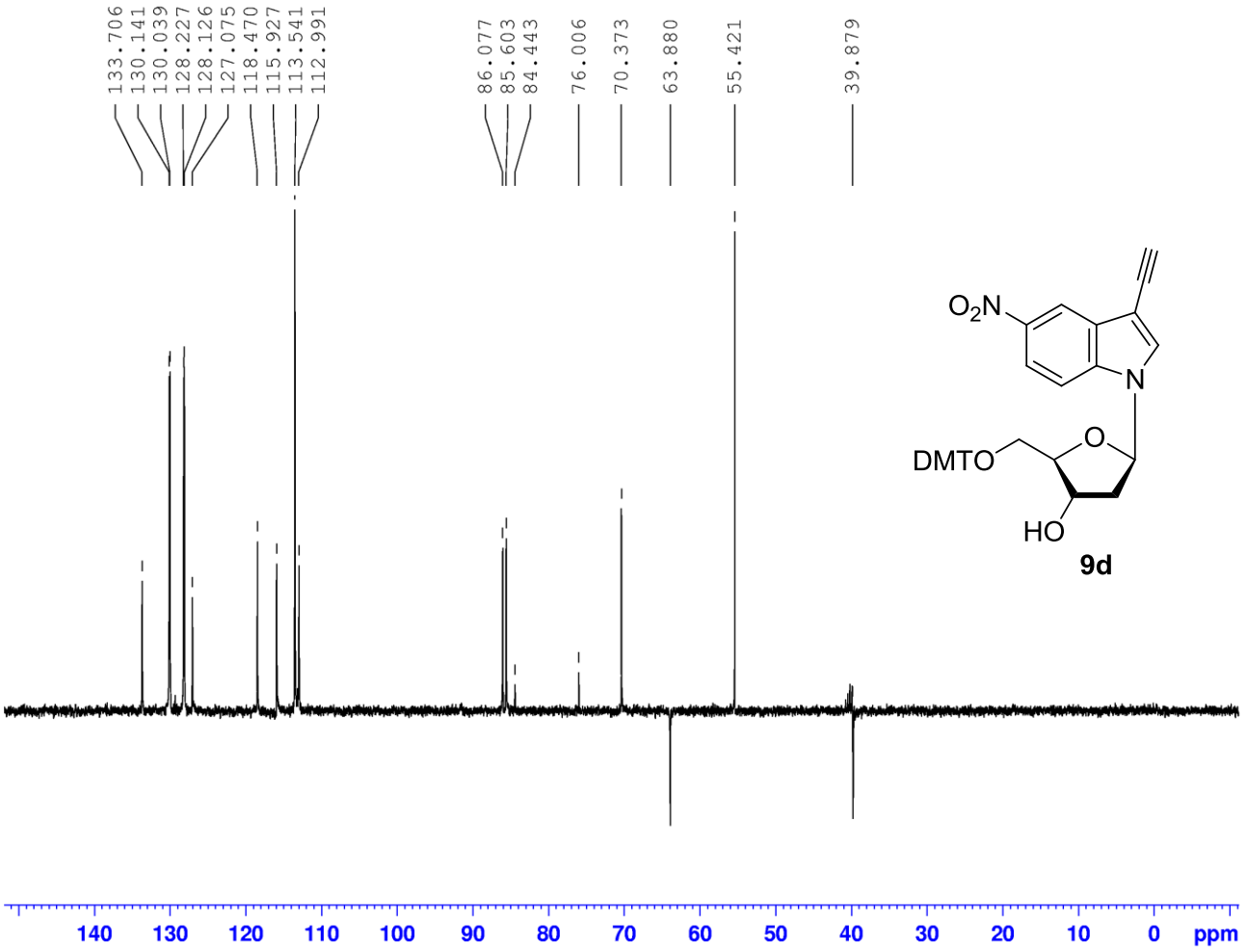


Fig. S36. ^{1}H-^{13}C gated-decoupled spectrum of compound 9d.

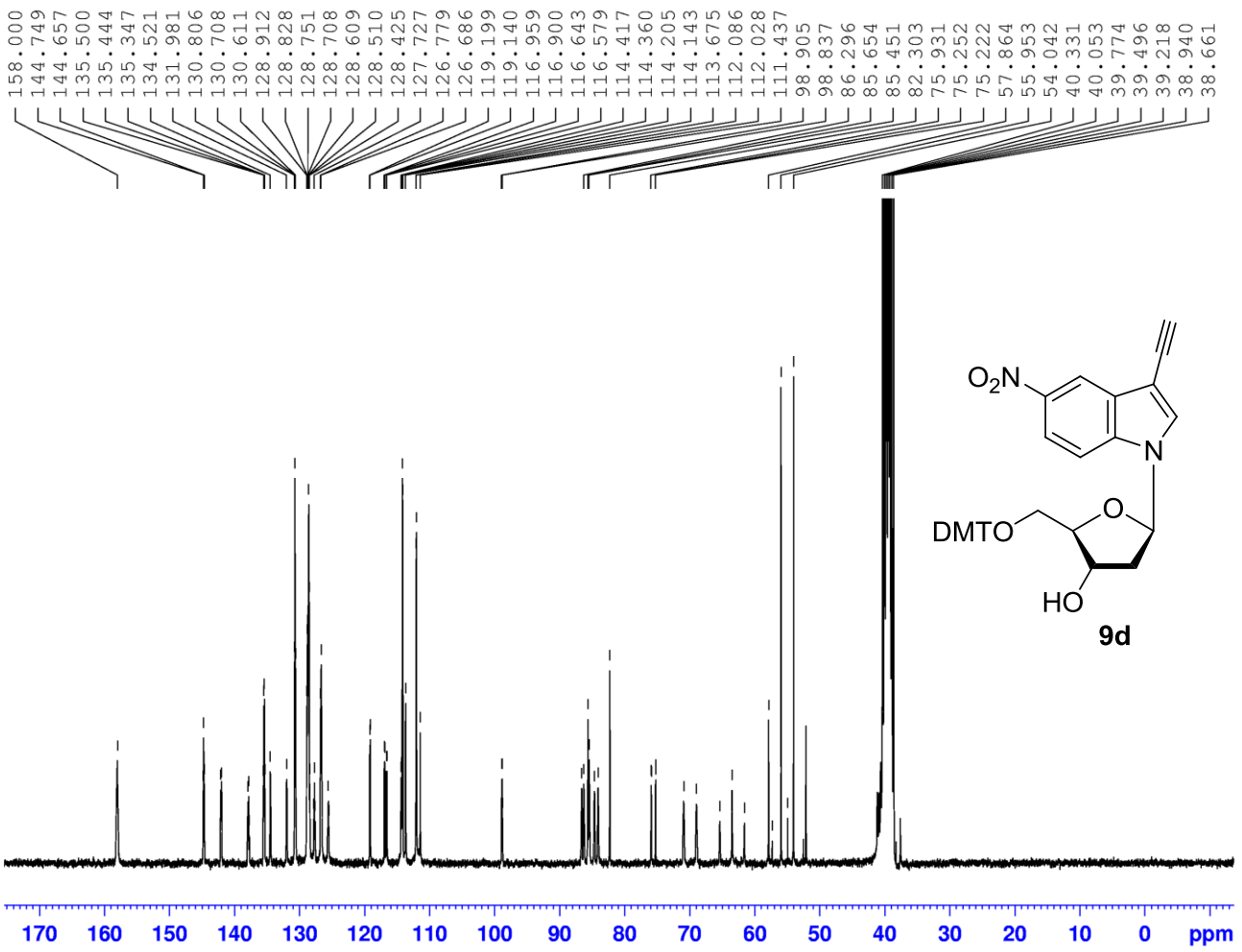


Fig. S37. ^{1}H NMR spectrum of compound 10d.

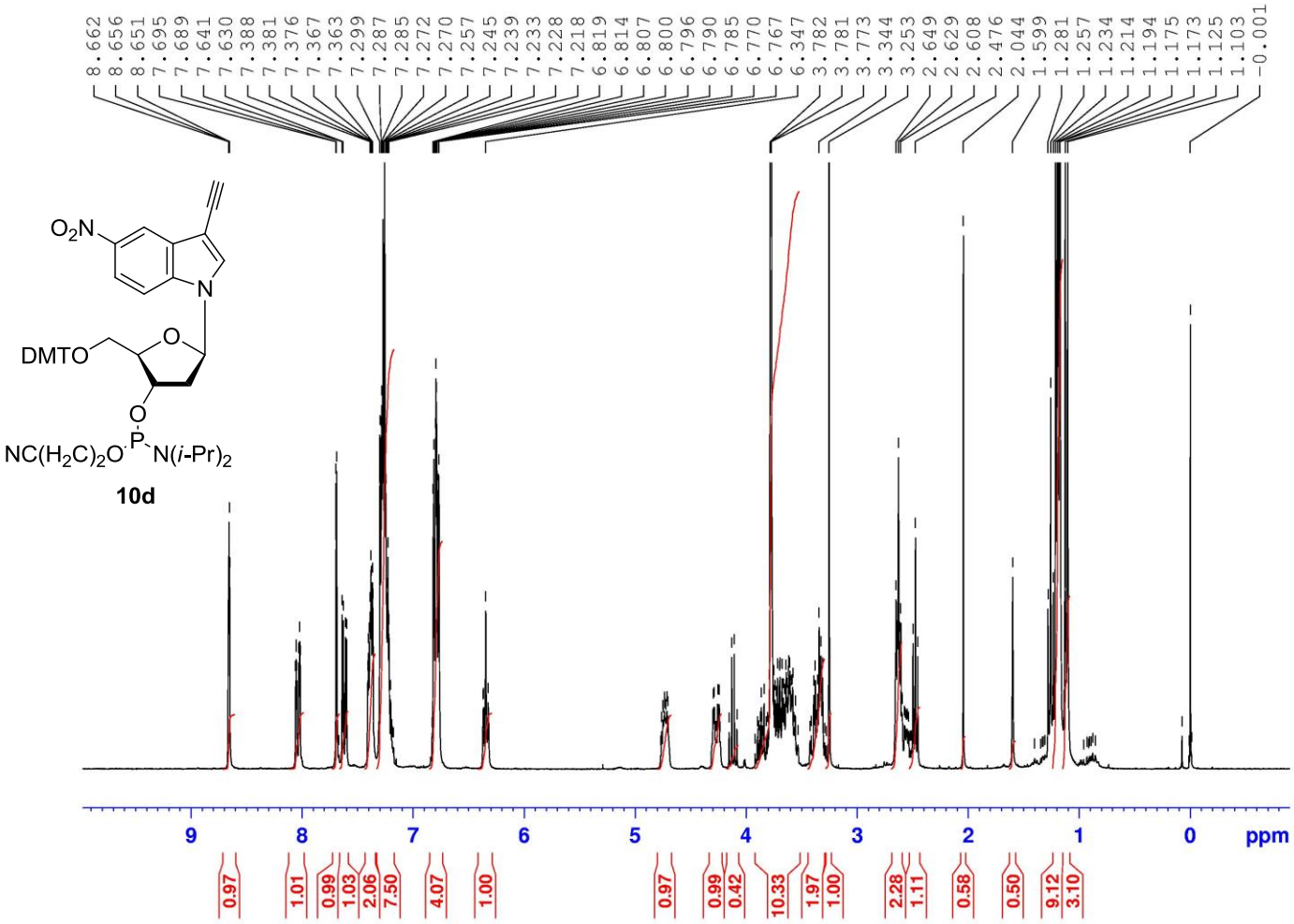


Fig. S38. ^{13}C NMR spectrum of compound 10d.

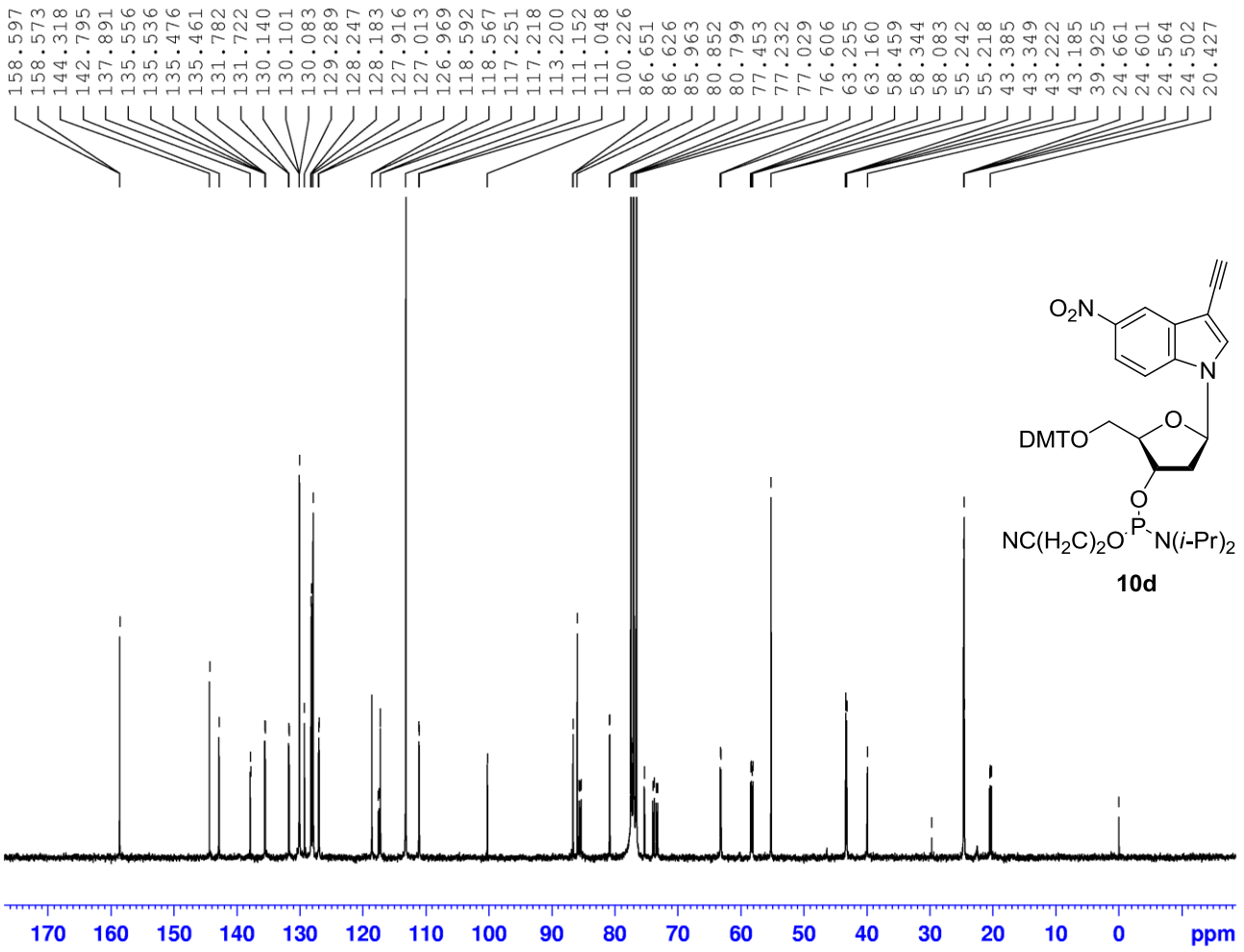


Fig. S39. ^{31}P NMR spectrum of compound 10d.

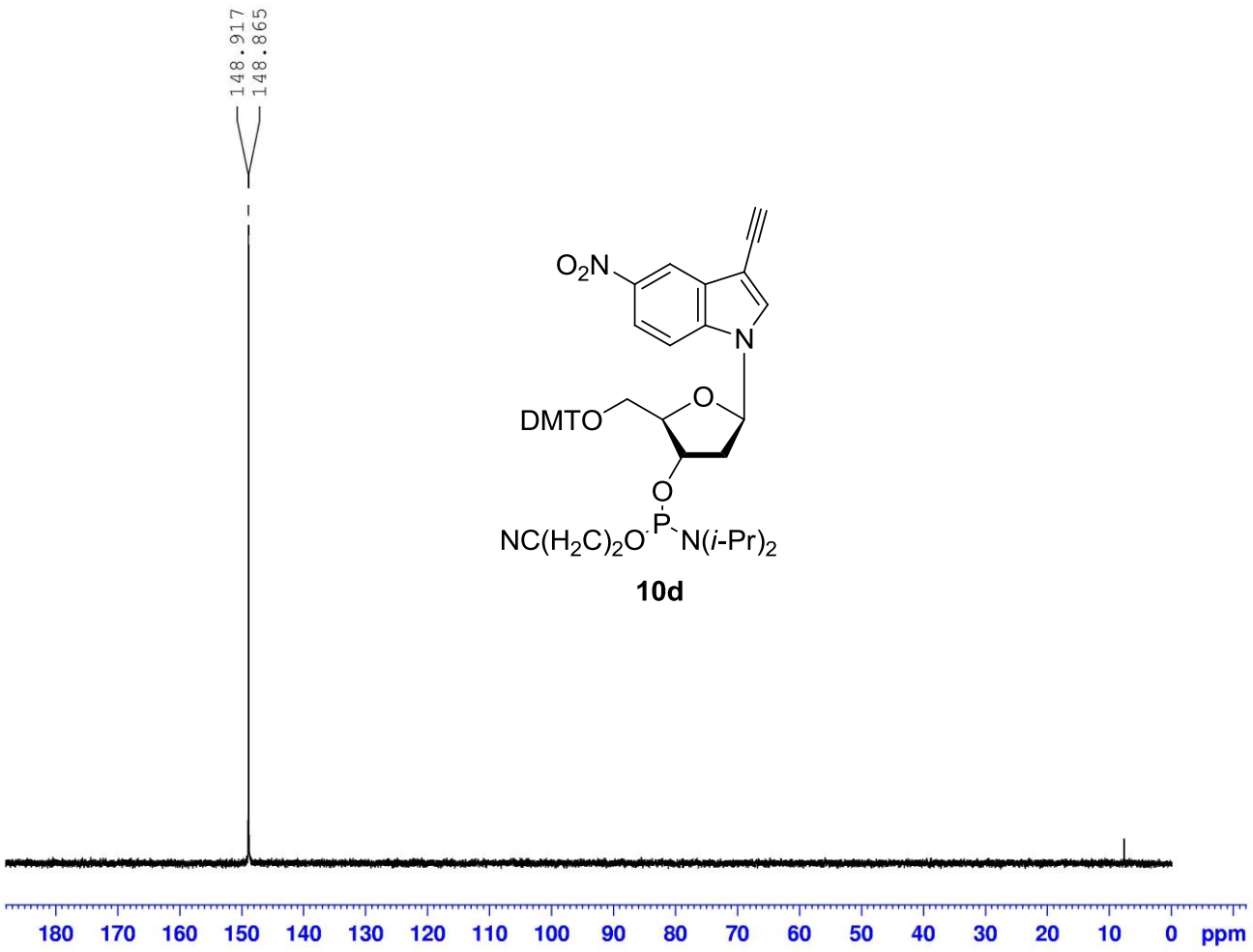


Fig. S40. ^{1}H NMR spectrum of compound 4e.

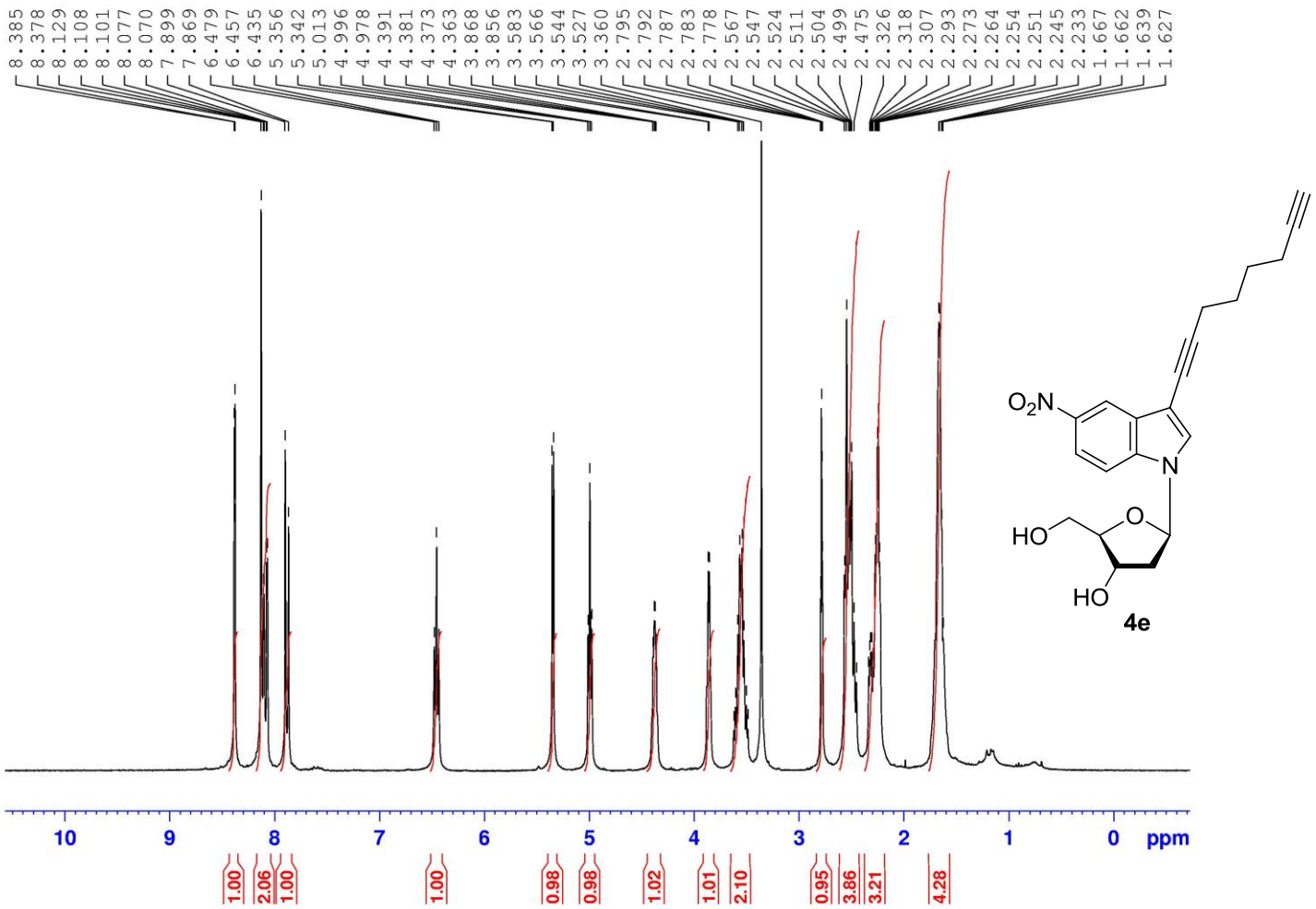


Fig. S41. ^{13}C NMR spectrum of compound 4e.

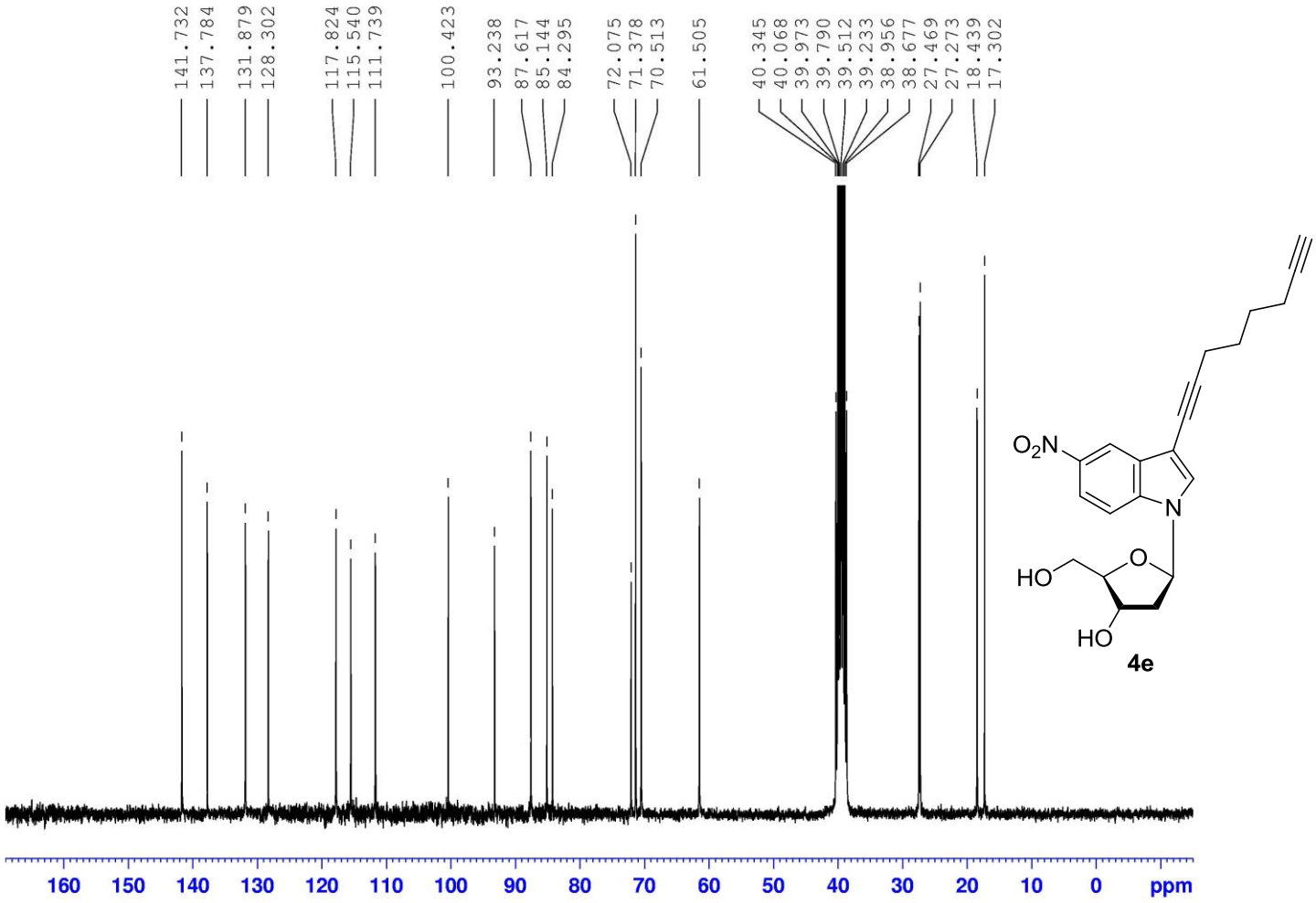


Fig. S42. DEPT-135 spectrum of compound 4e.

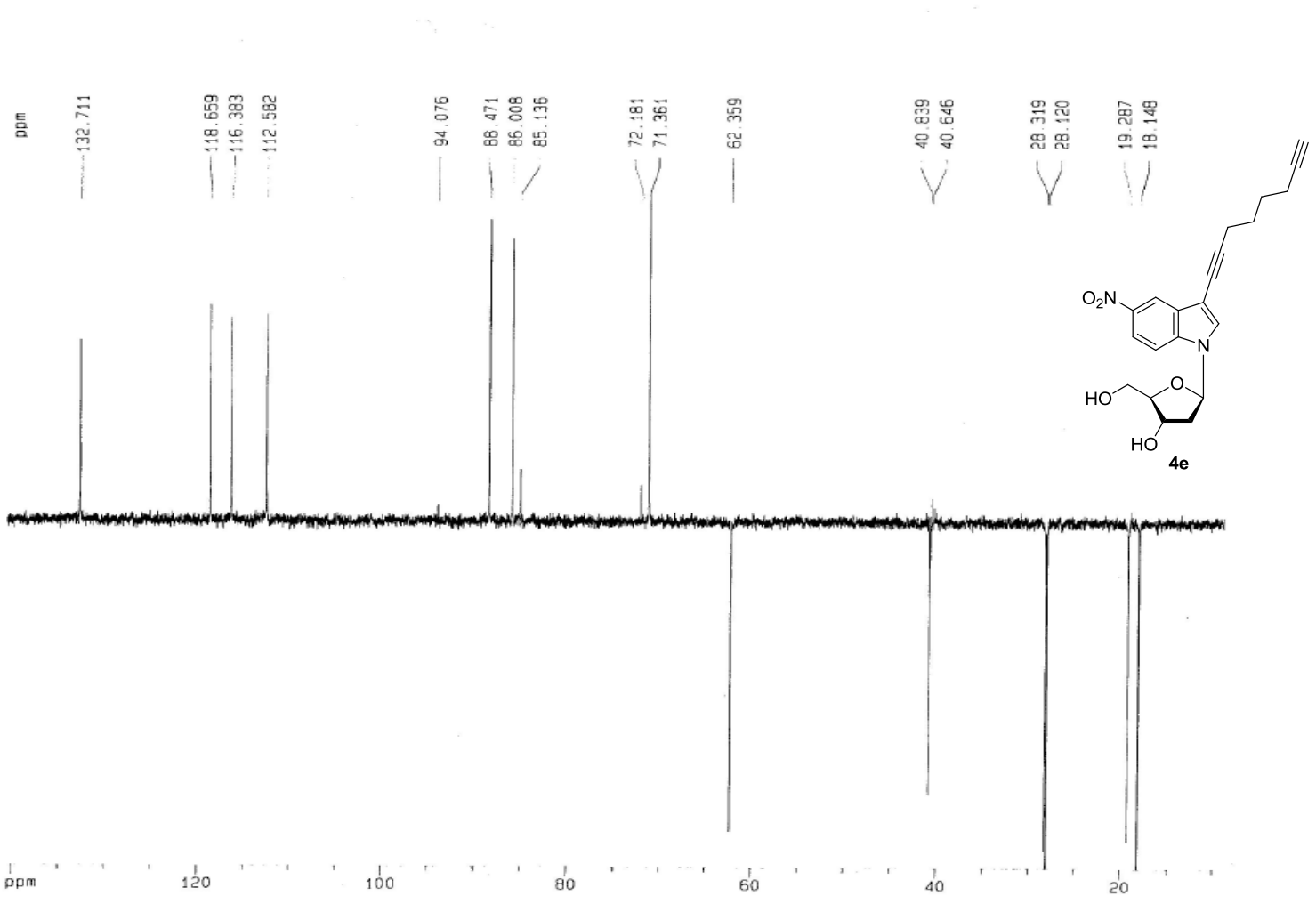


Fig. S43. ^{1}H-^{13}C gated-decoupled spectrum of compound 4e.

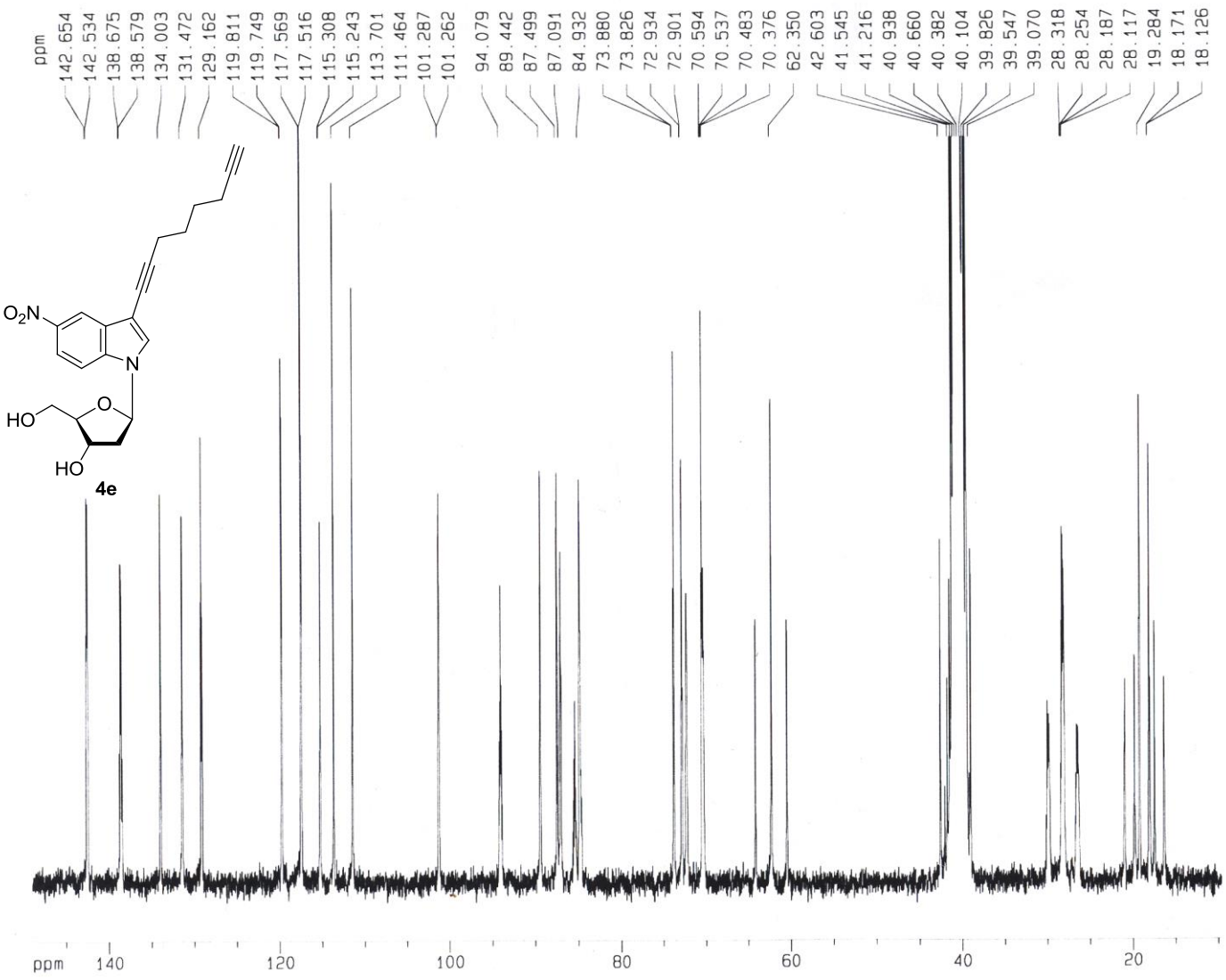


Fig. S44. ^{1}H NMR spectrum of compound 9e.

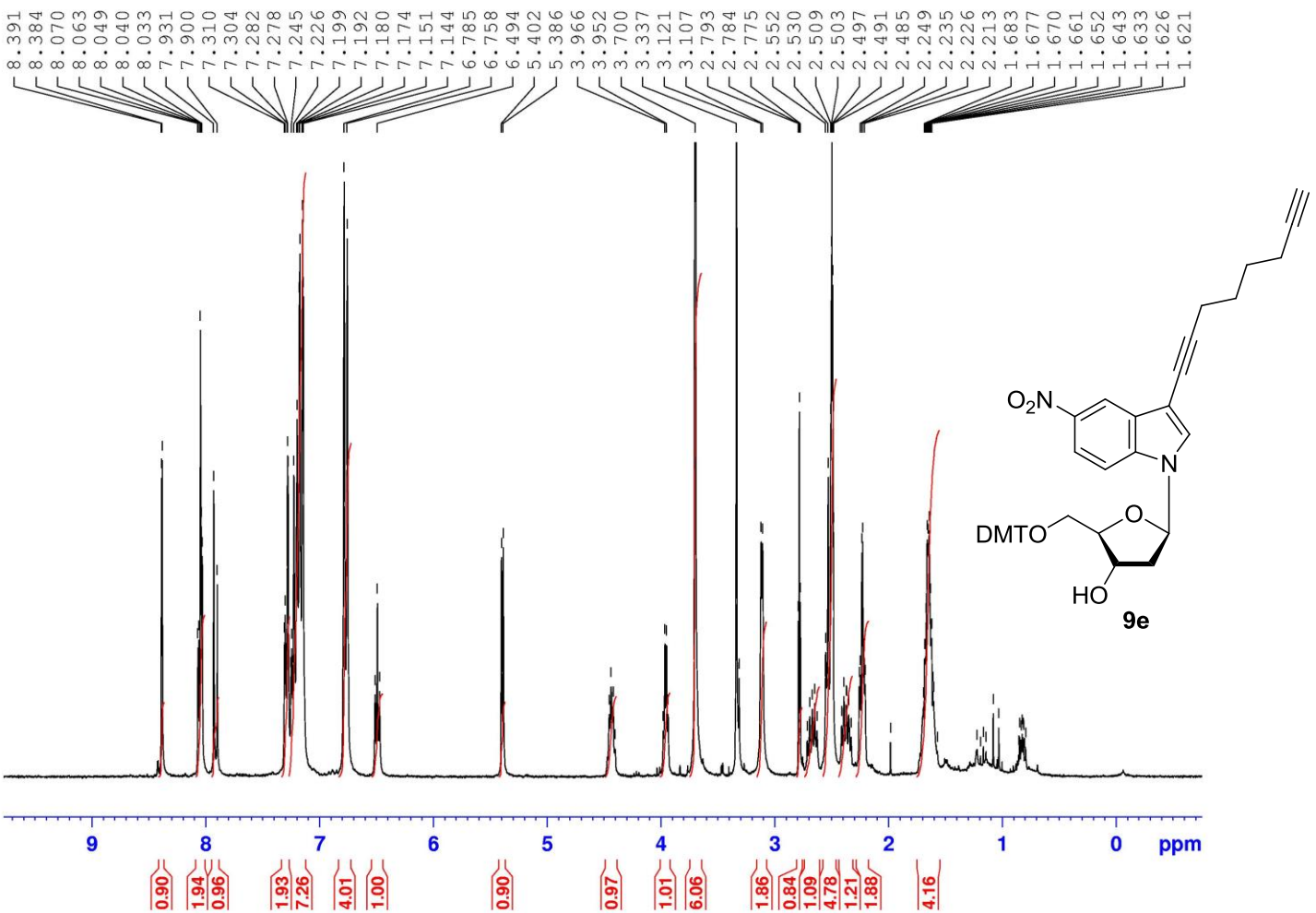


Fig. S45. ^{13}C NMR spectrum of compound 9e.

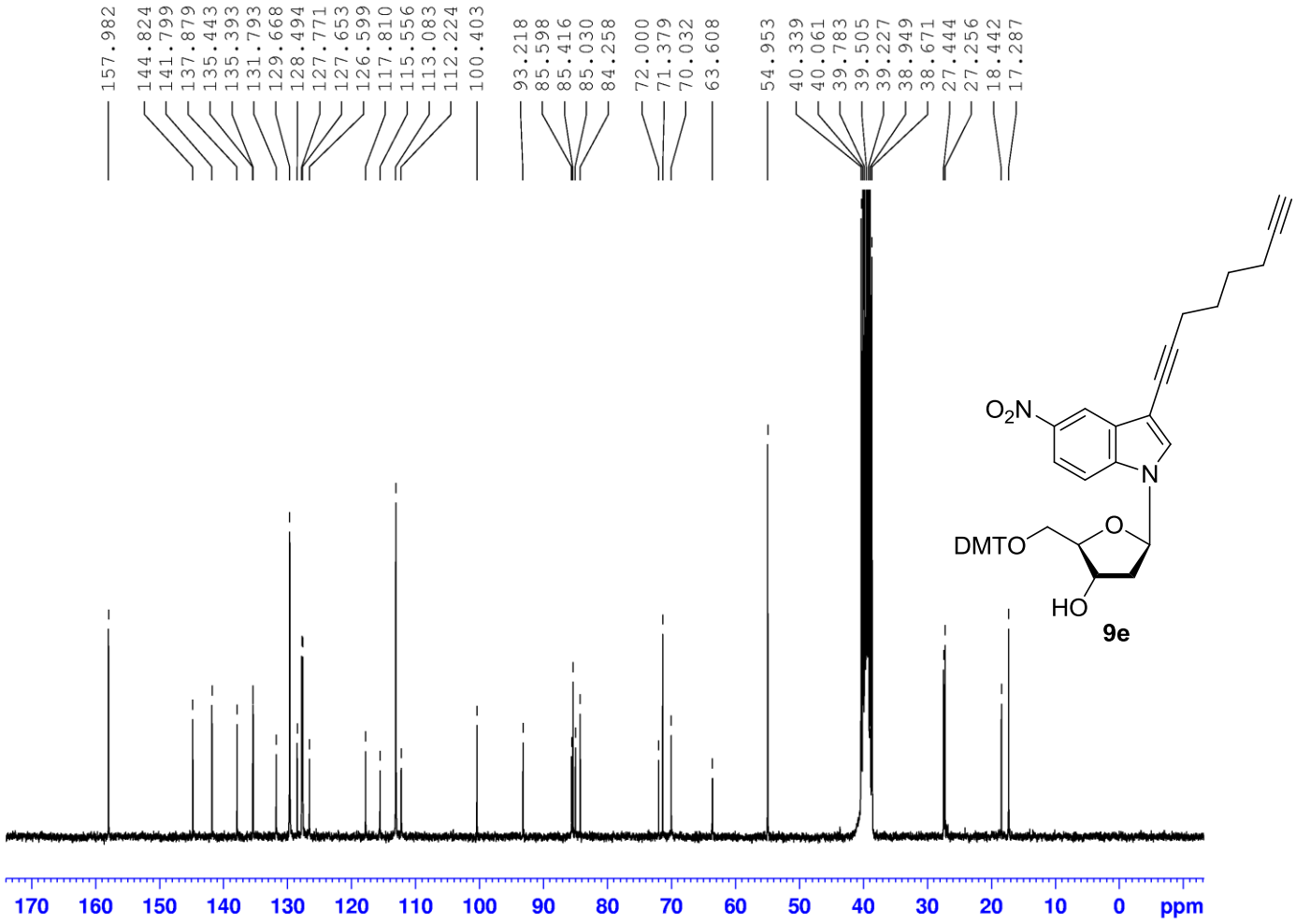


Fig. S46. DEPT-135 spectrum of compound 9e.

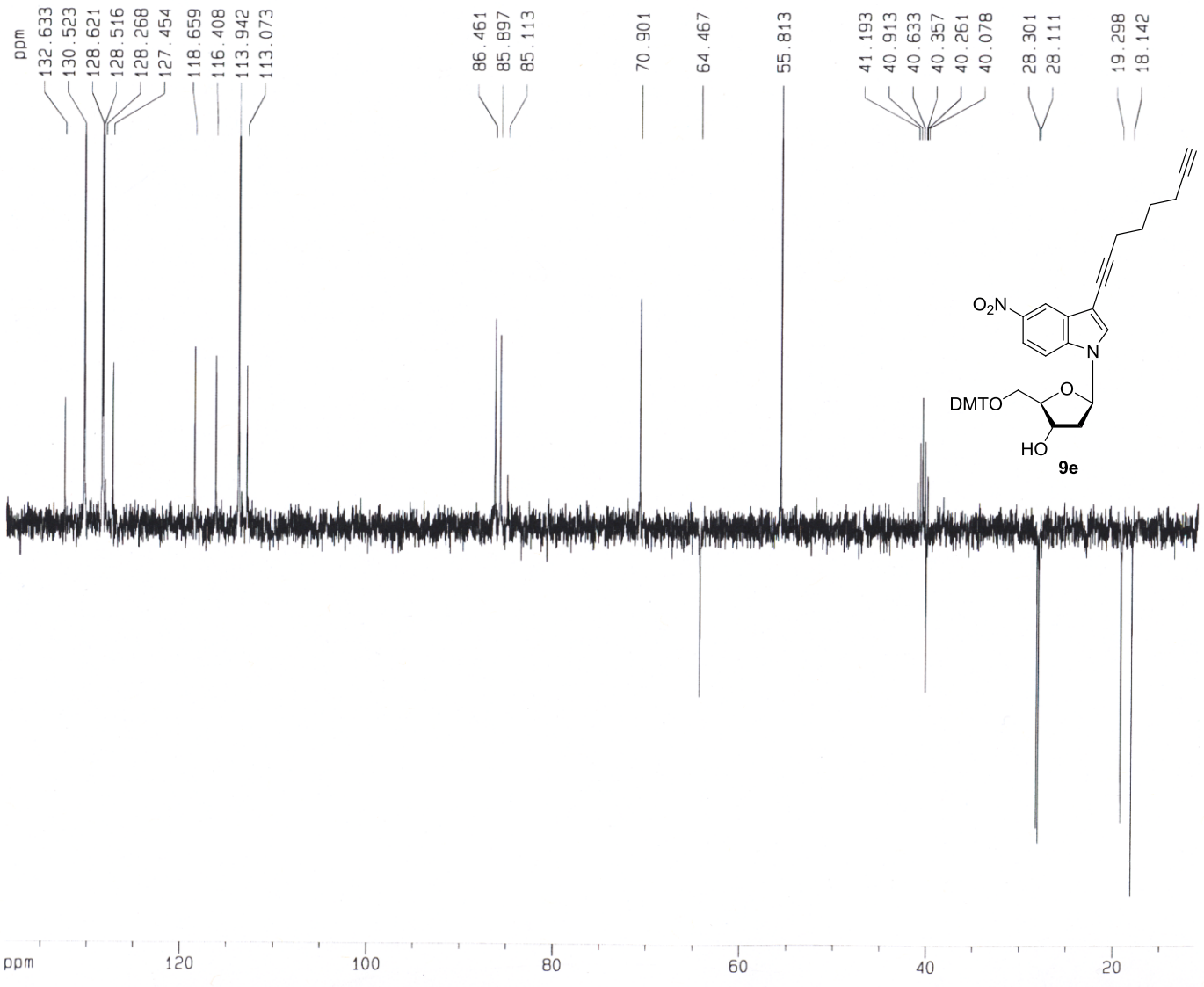


Fig. S47. ^{1}H-^{13}C gated-decoupled spectrum of compound 9e.

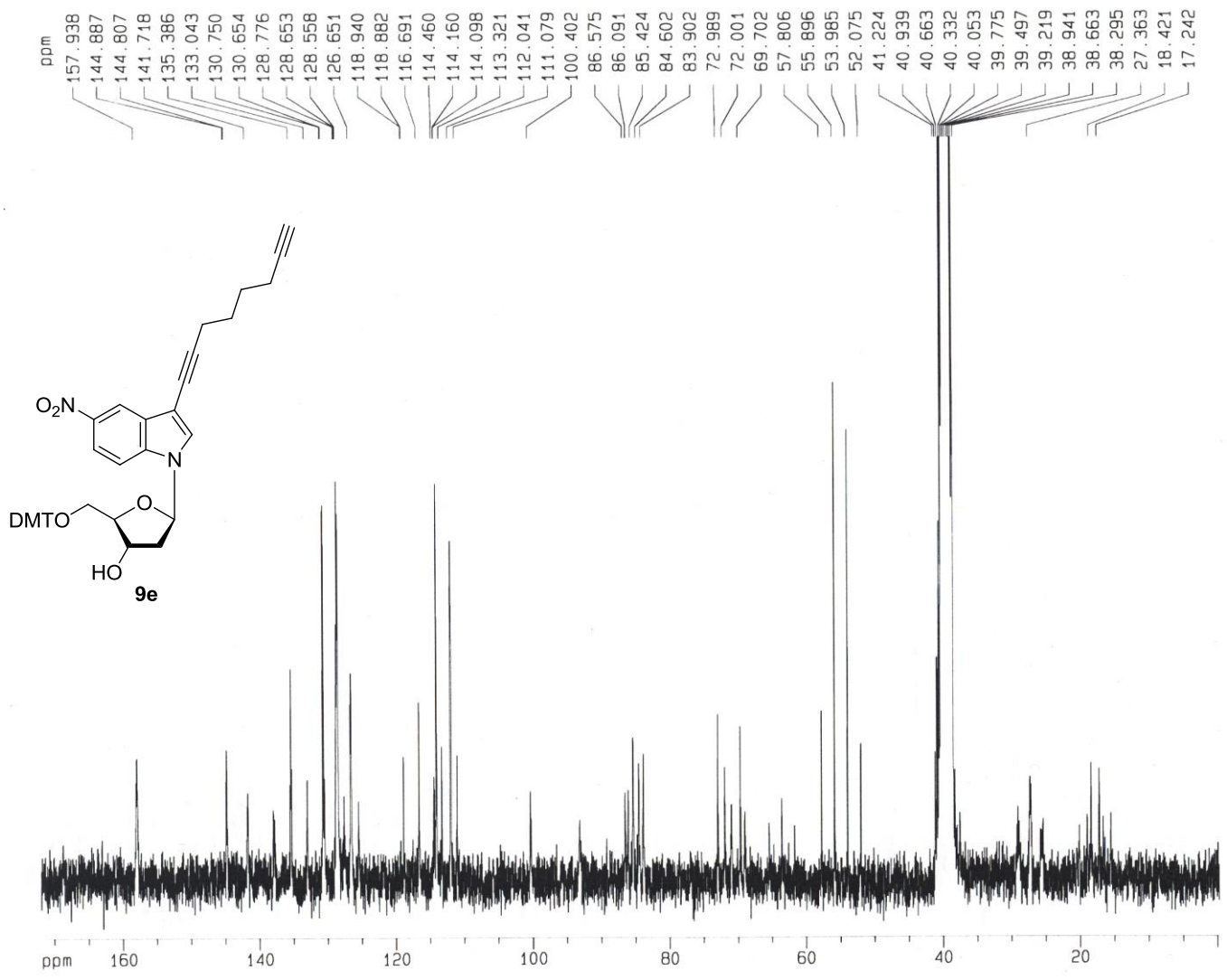


Fig. S48. ^{1}H NMR spectrum of compound 10e.

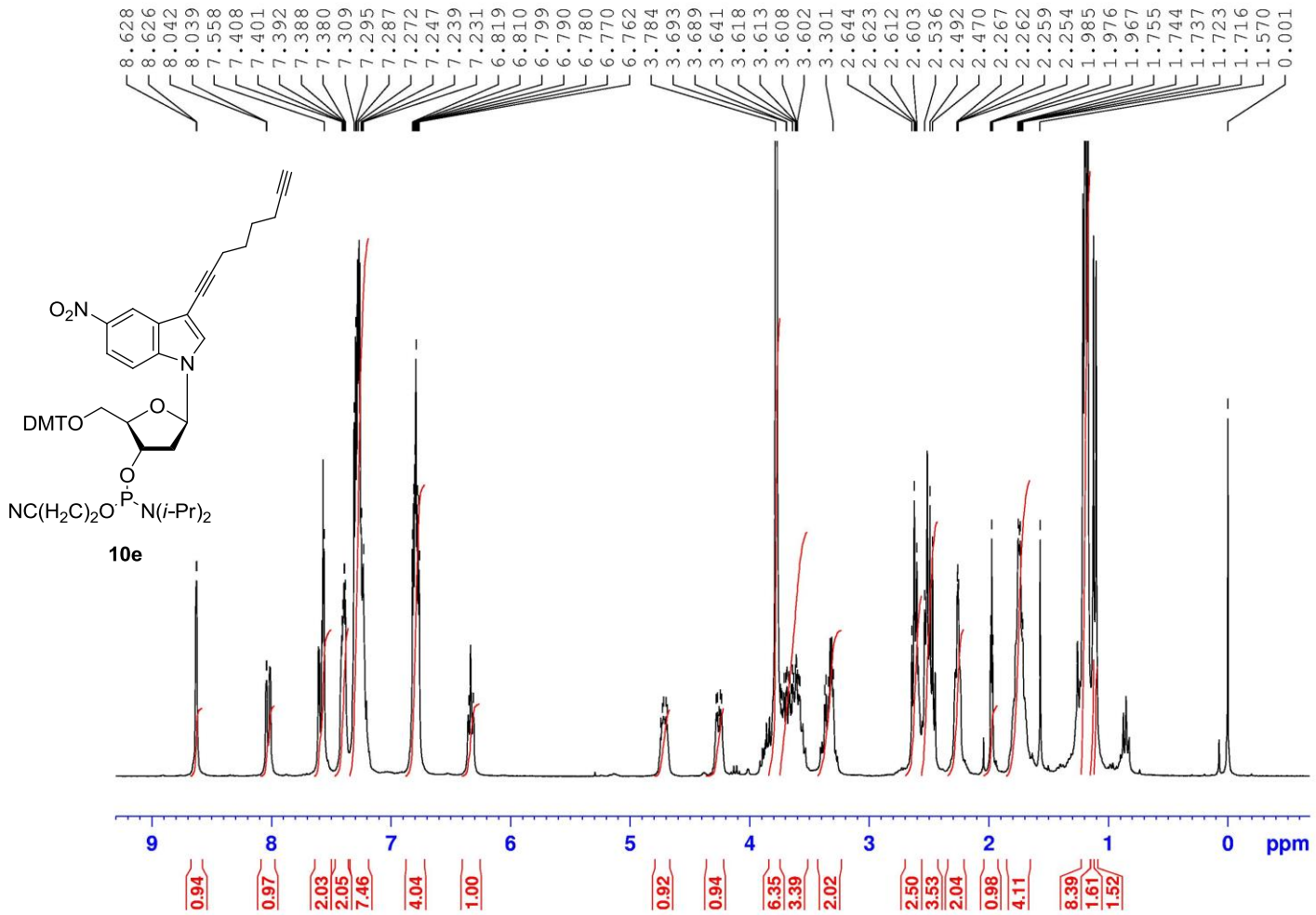


Fig. S49. ^{13}C NMR spectrum of compound 10e.

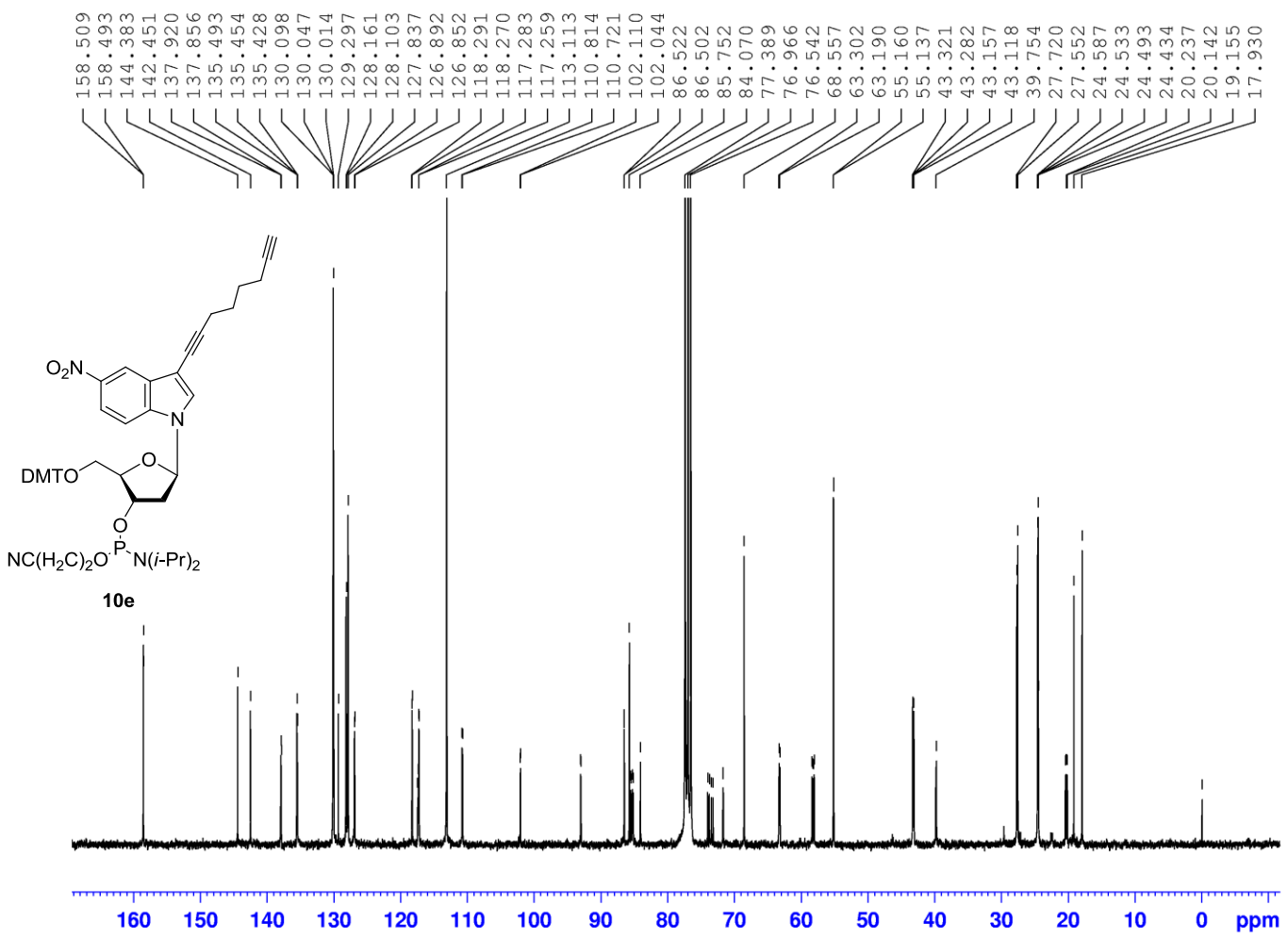


Fig. S50. ^{31}P NMR spectrum of compound 10e.

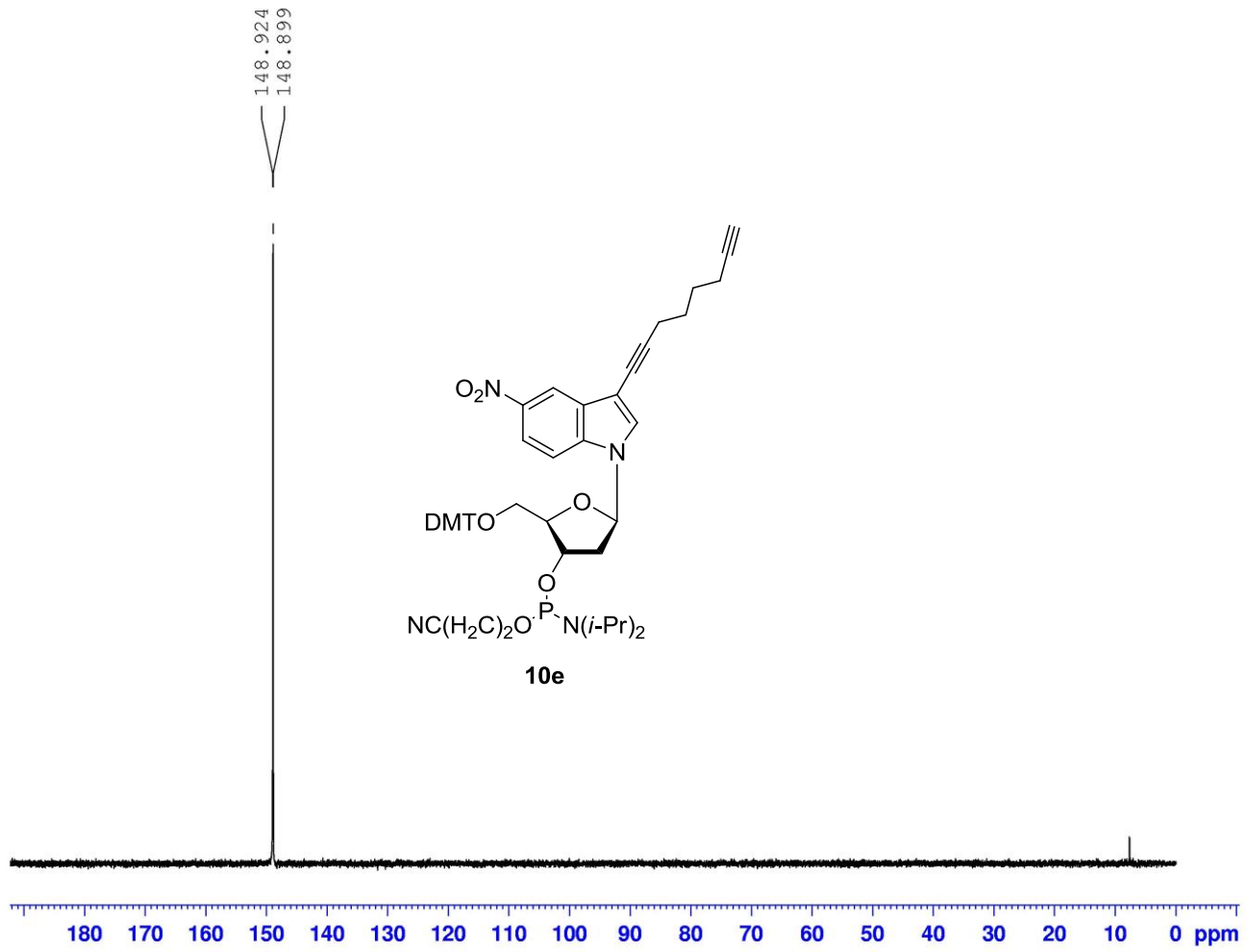


Fig. S51. ^{1}H NMR spectrum of compound 4f.

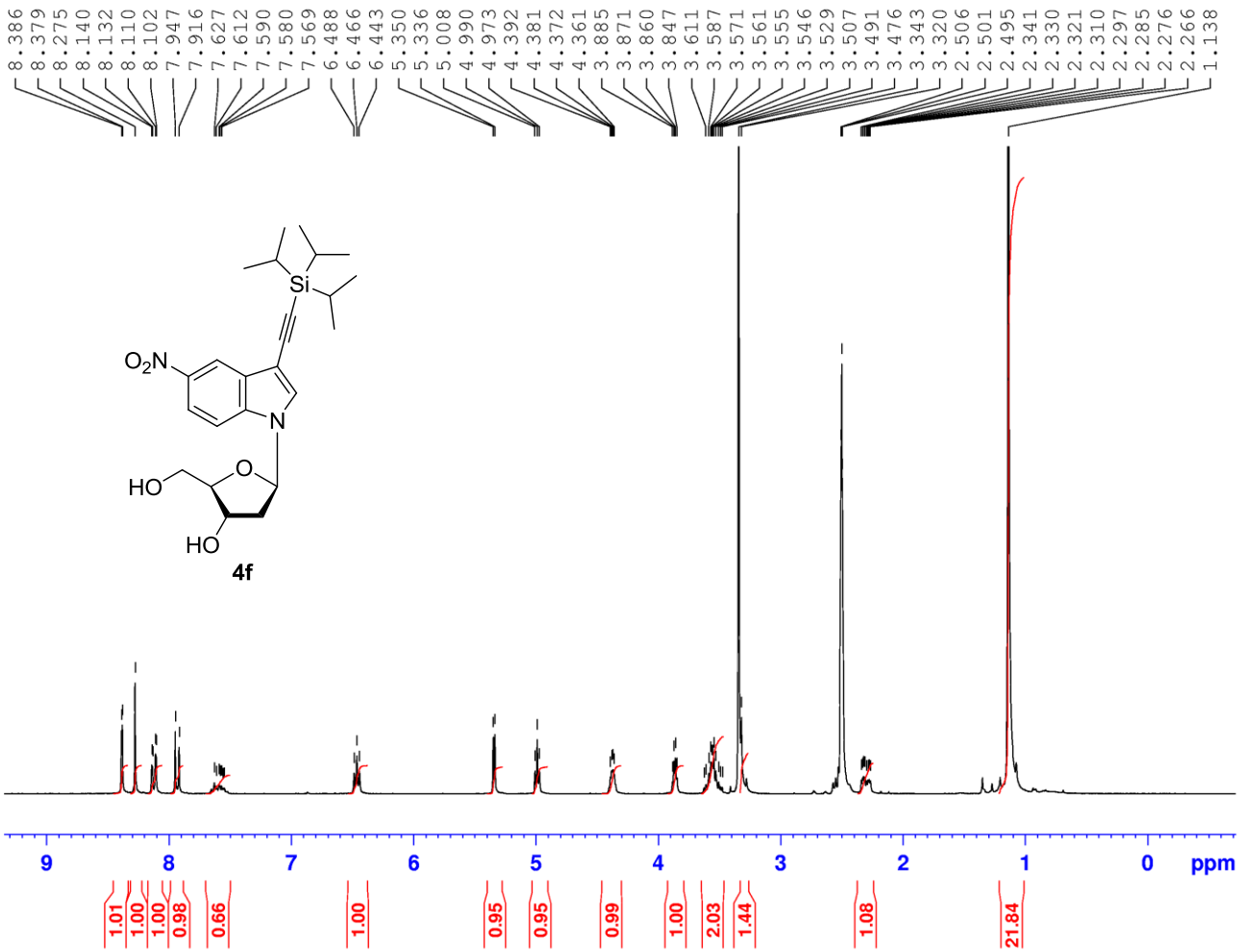


Fig. S52. ^{13}C NMR spectrum of compound 4f.

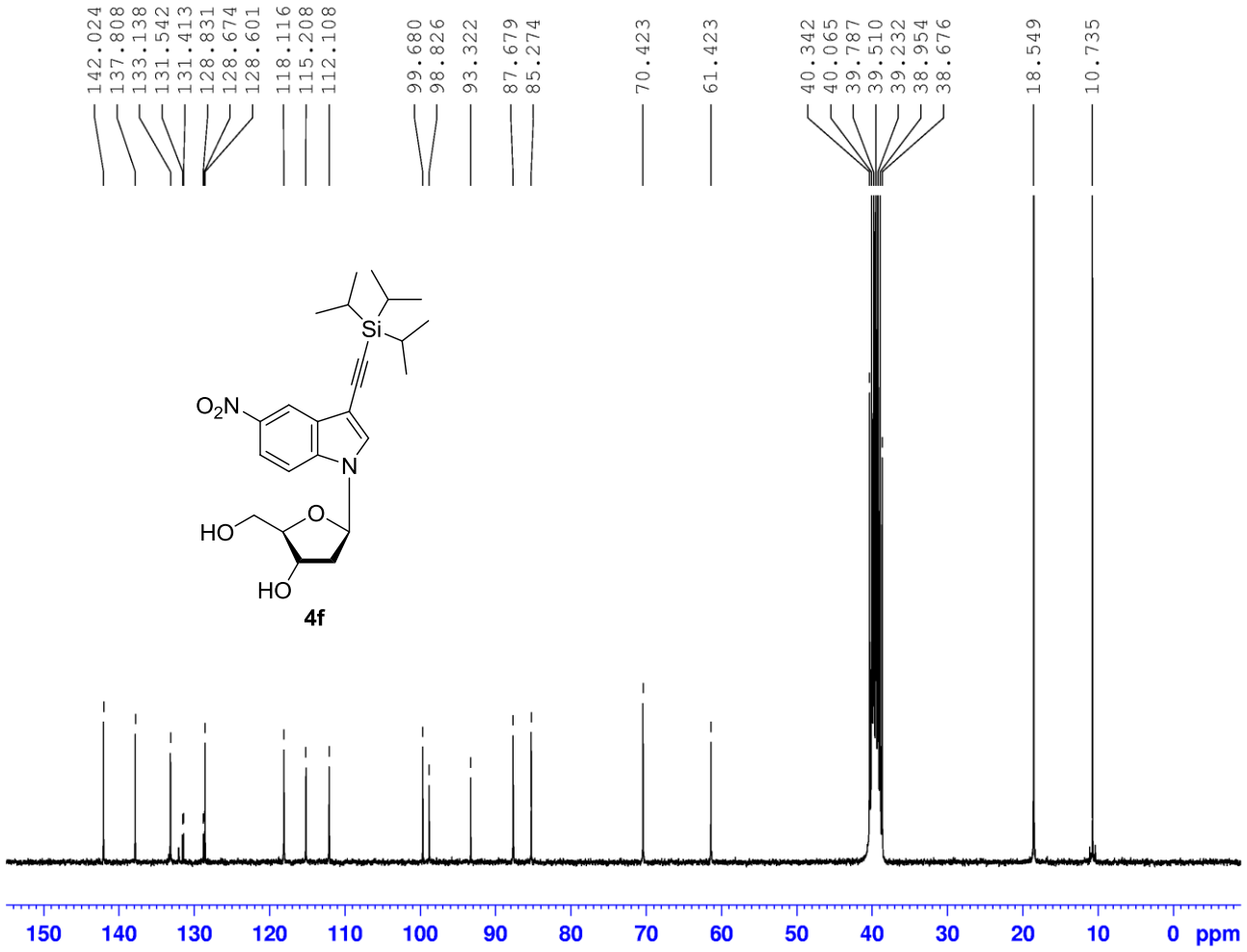


Fig. S53. DEPT-135 spectrum of compound 4f.

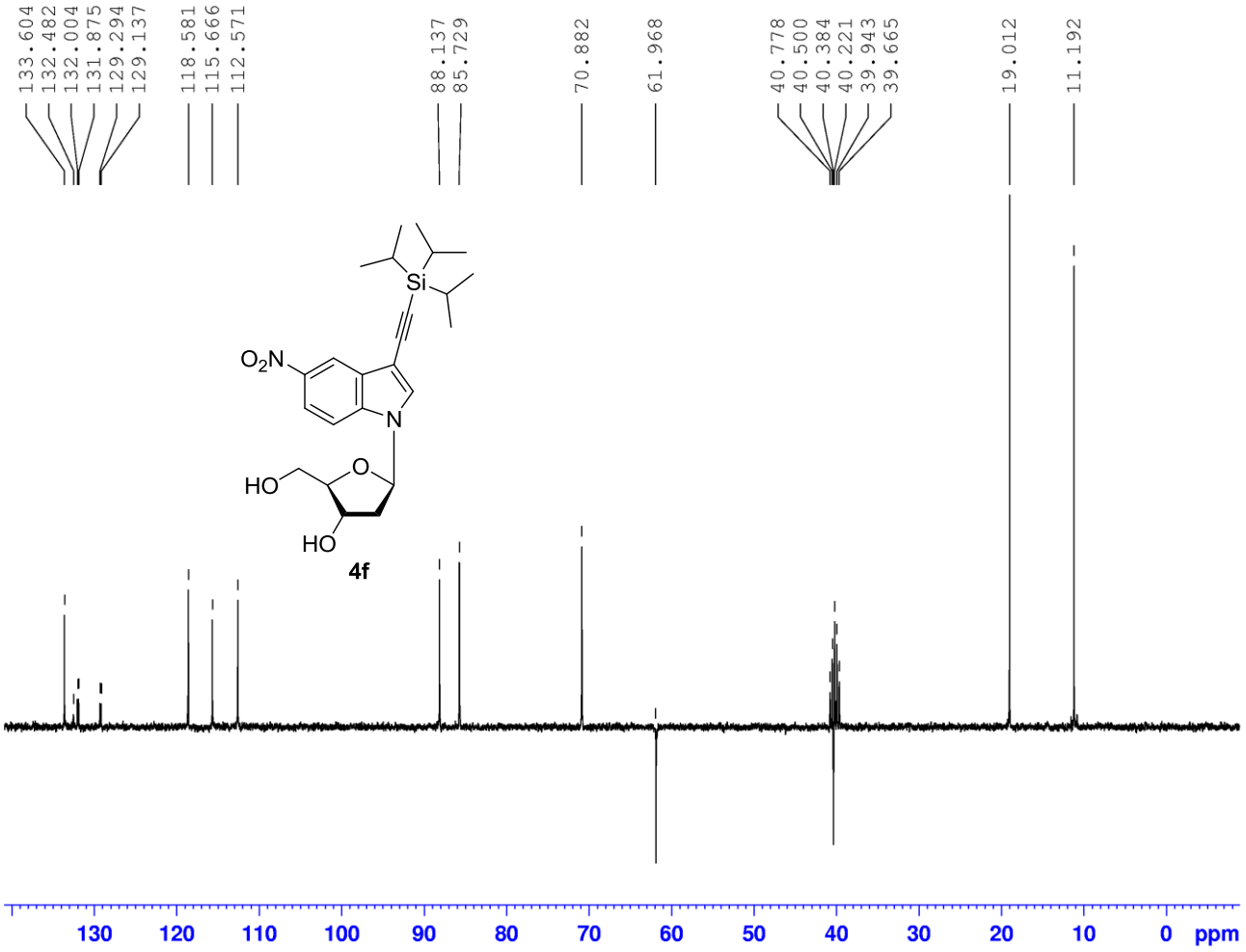


Fig. S54. ^{1}H-^{13}C gated-decoupled spectrum of compound 4f.

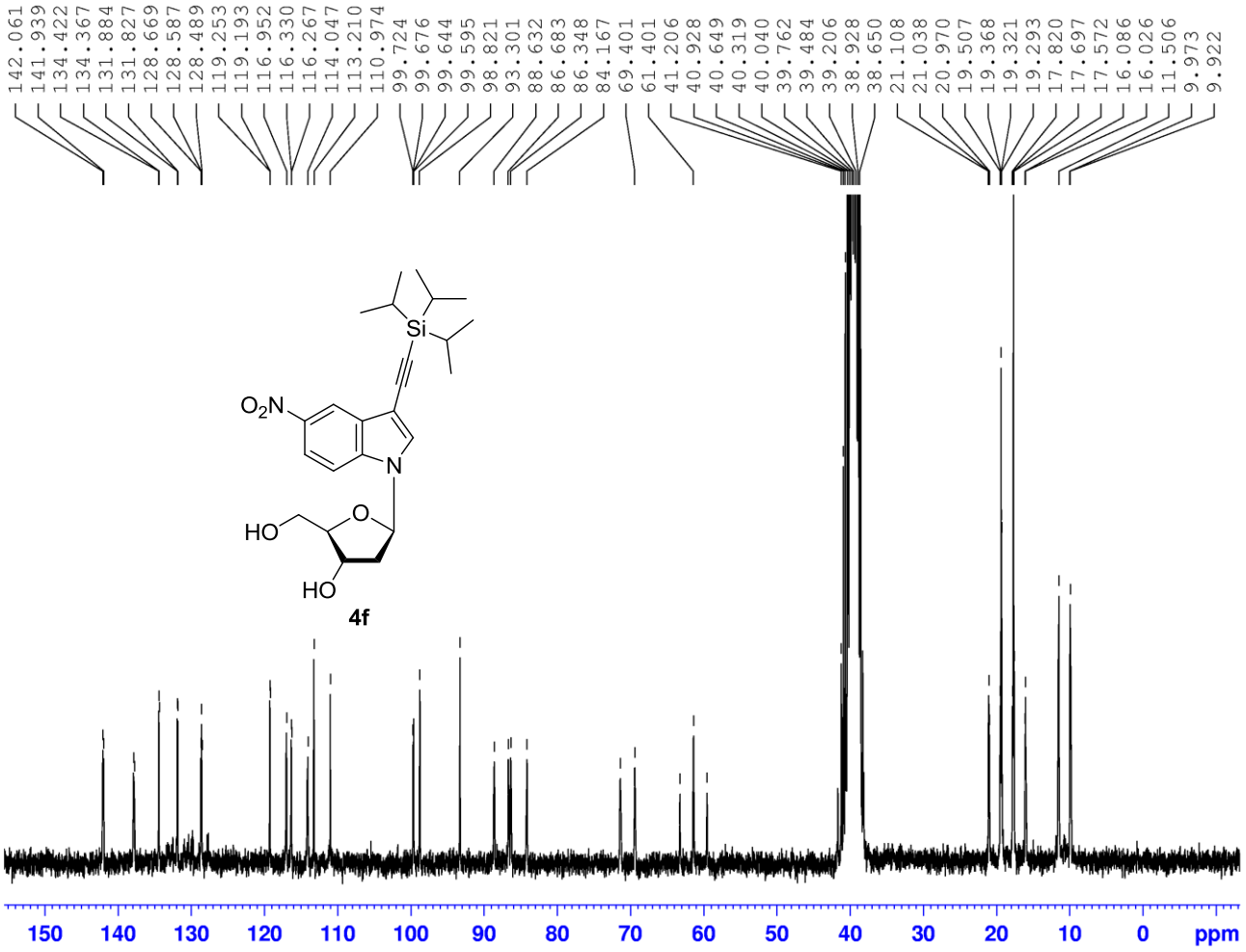


Fig. S55. ^{1}H NMR spectrum of compound 9f.

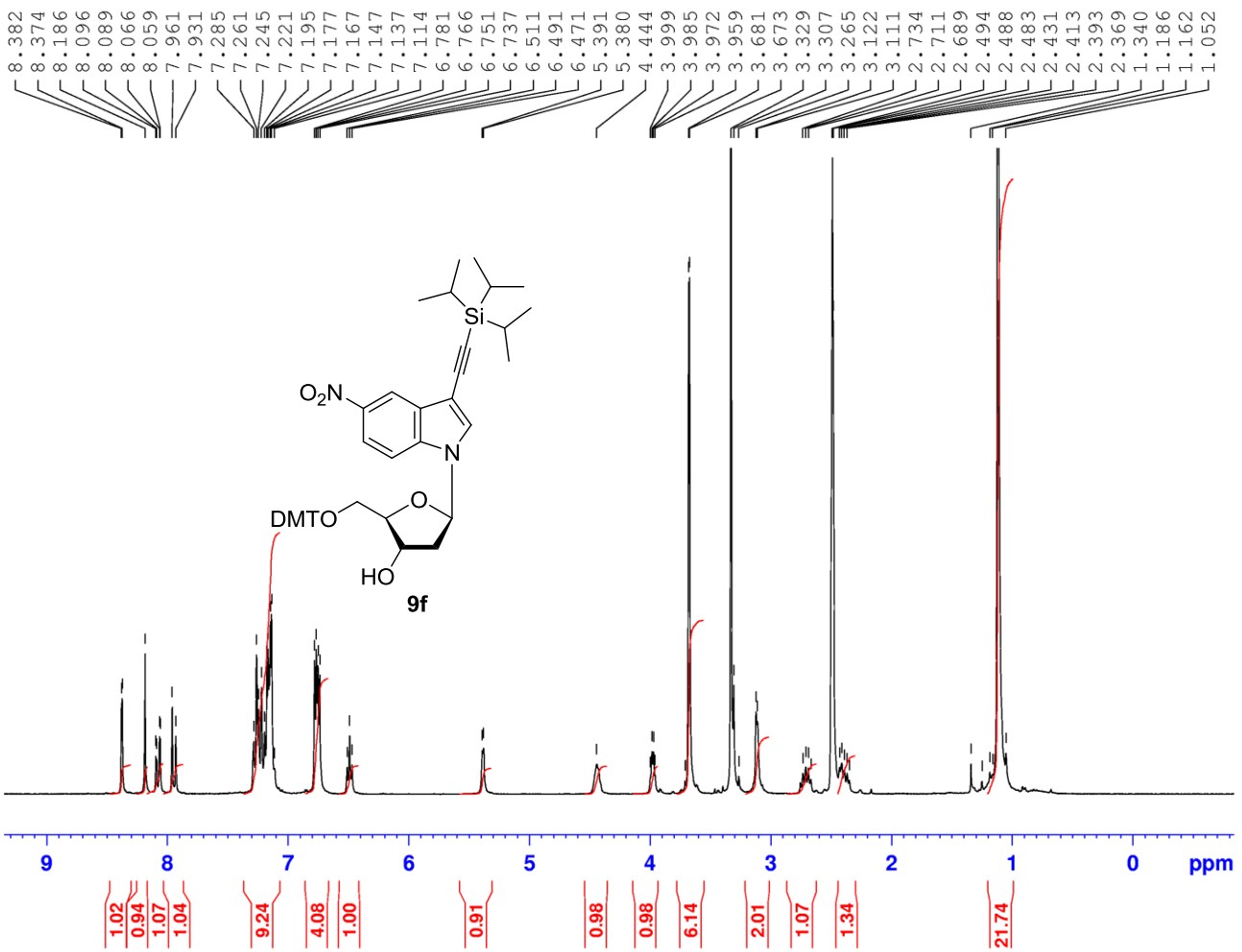


Fig. S56. ^{13}C NMR spectrum of compound 9f.

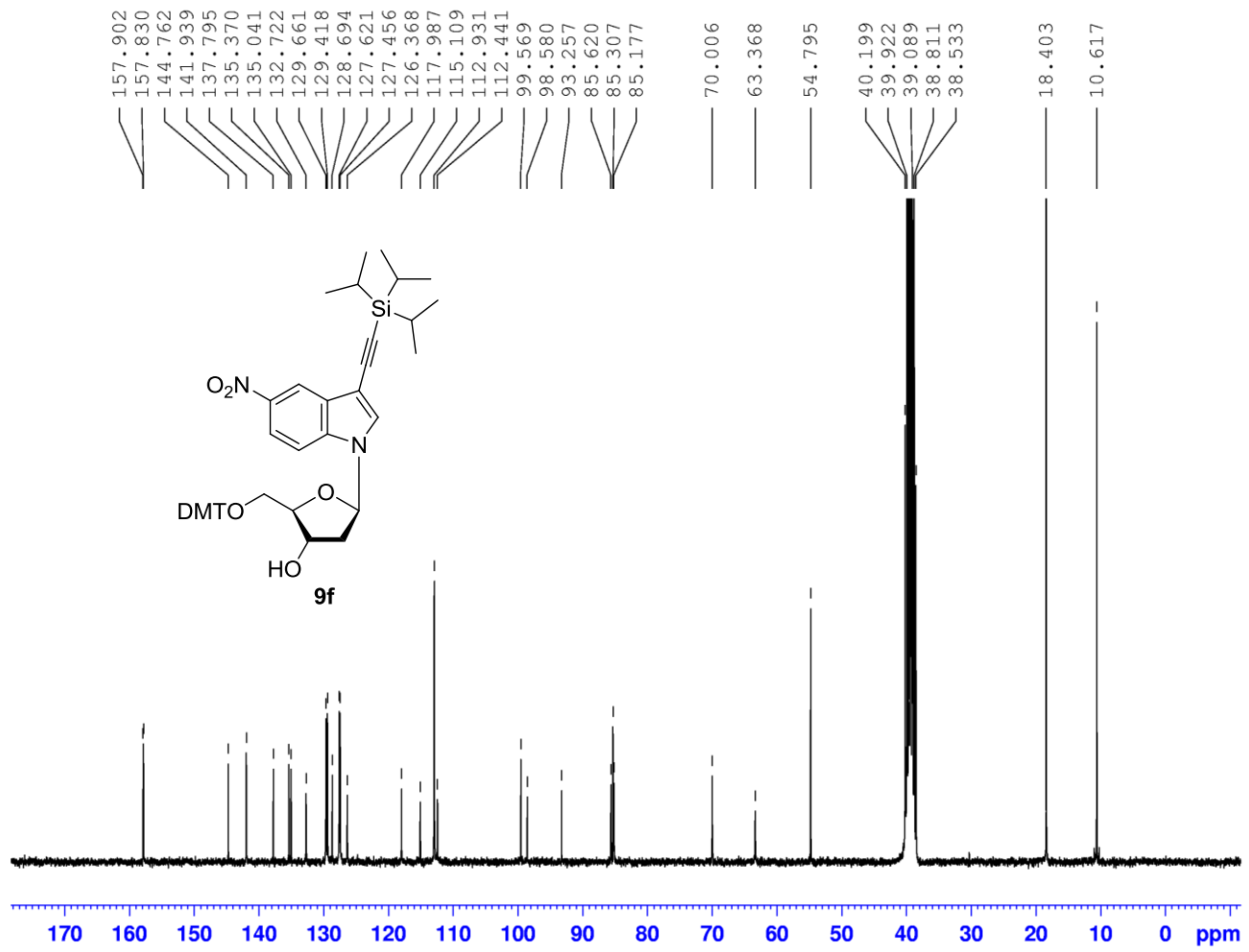


Fig. S57. DEPT-135 spectrum of compound 9f.

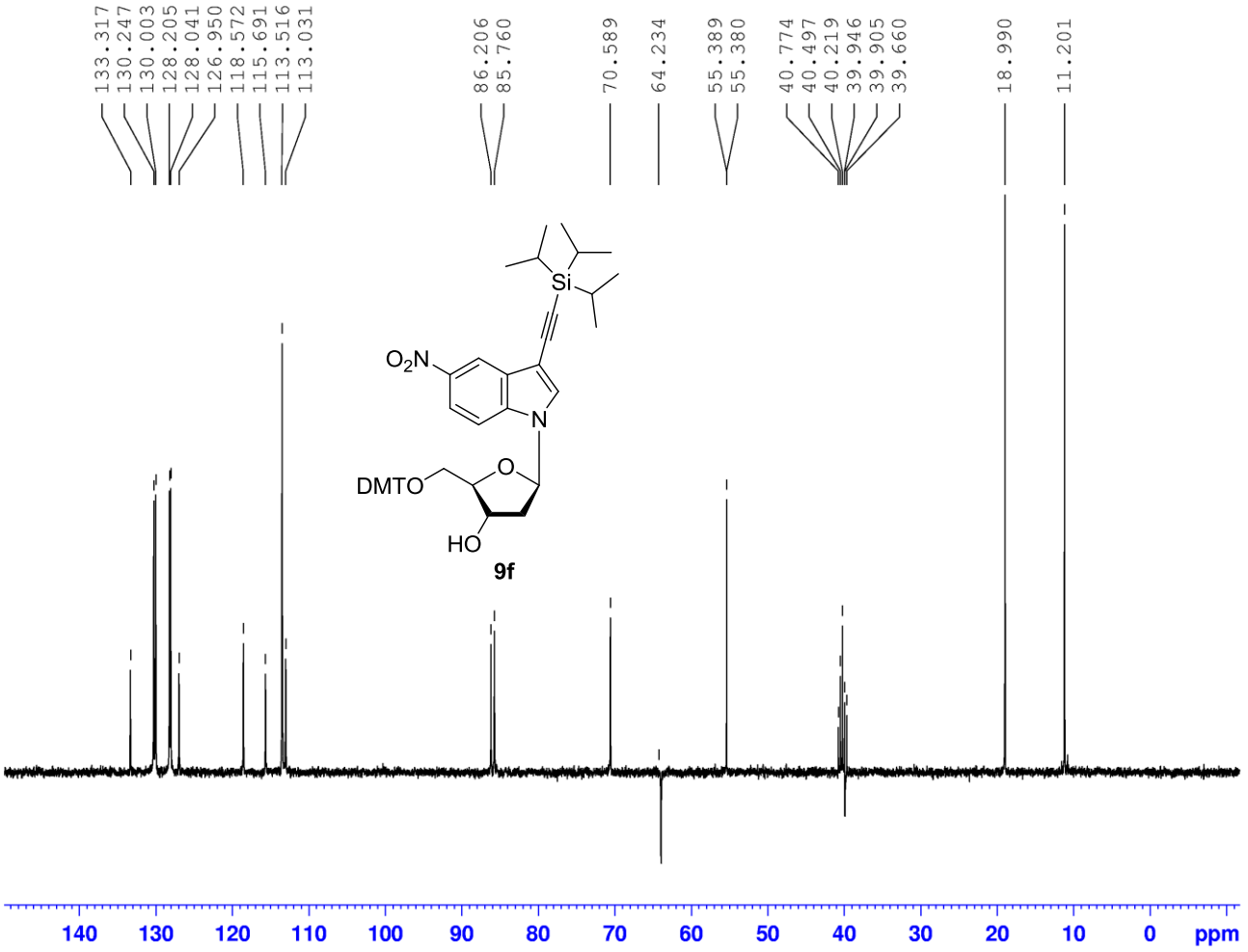


Fig. S58. ^{1}H-^{13}C gated-decoupled spectrum of compound 9f.

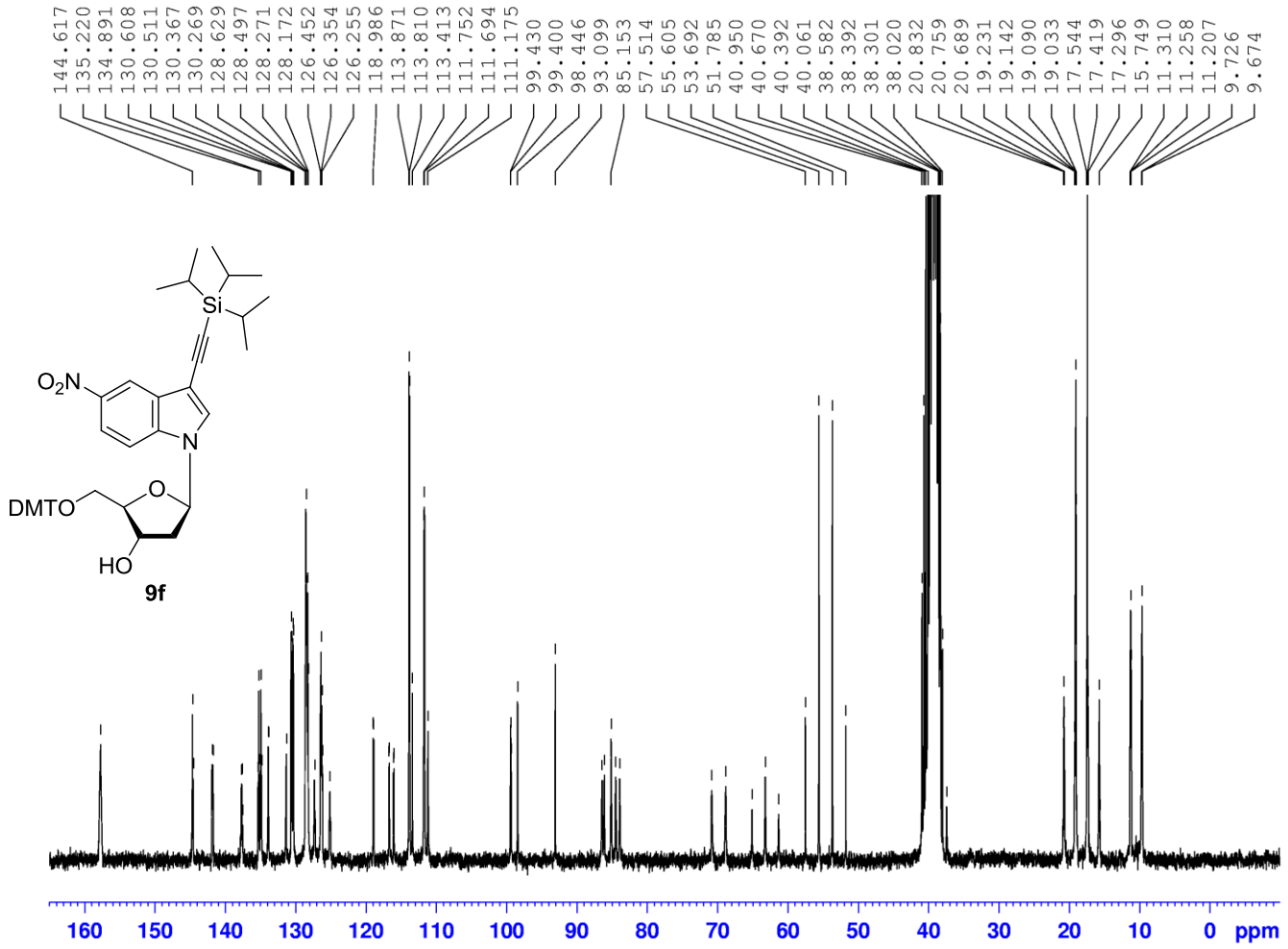


Fig. S59. ^{1}H NMR spectrum of compound 10f.

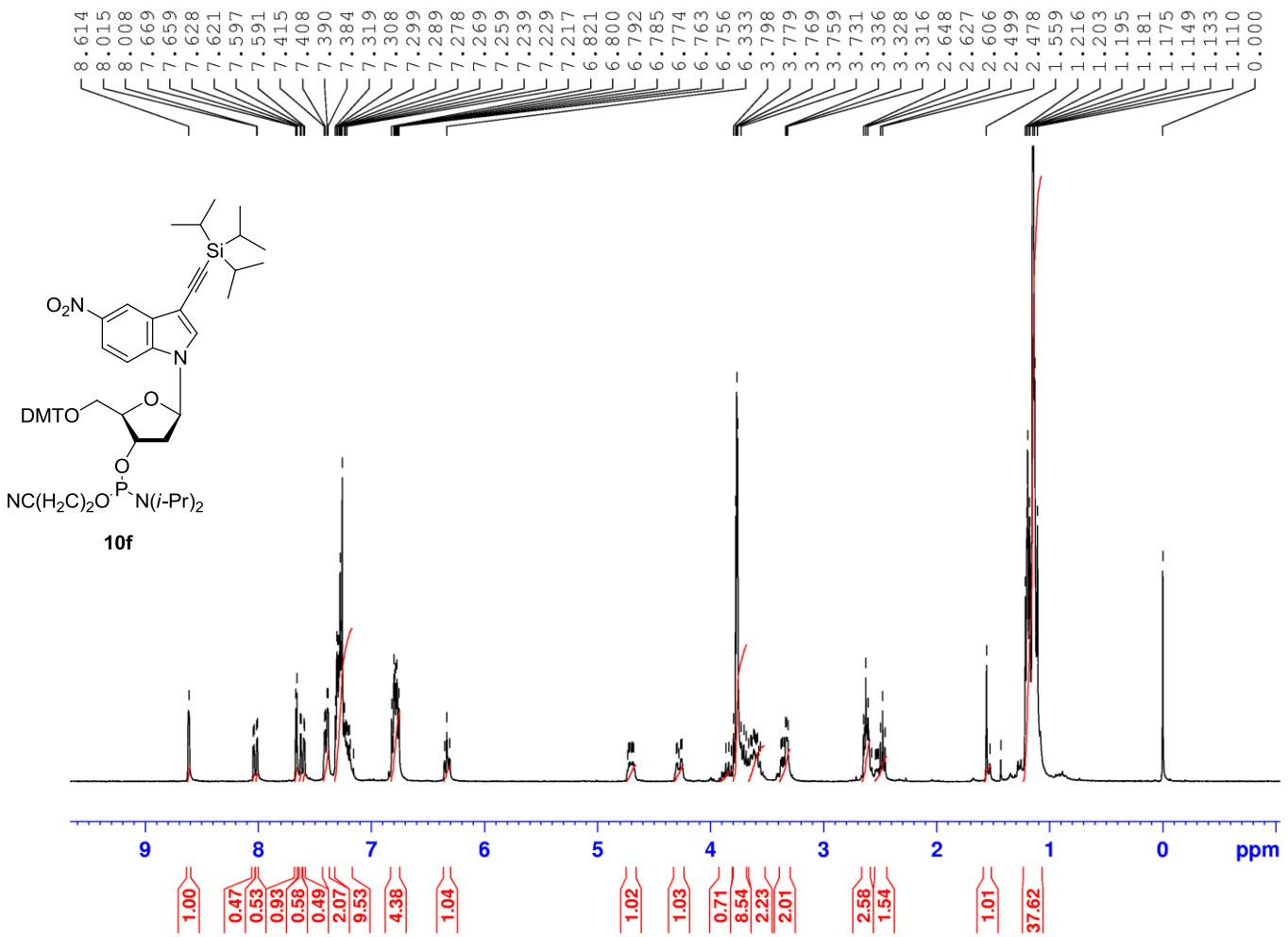


Fig. S60. ^{13}C NMR spectrum of compound 10f.

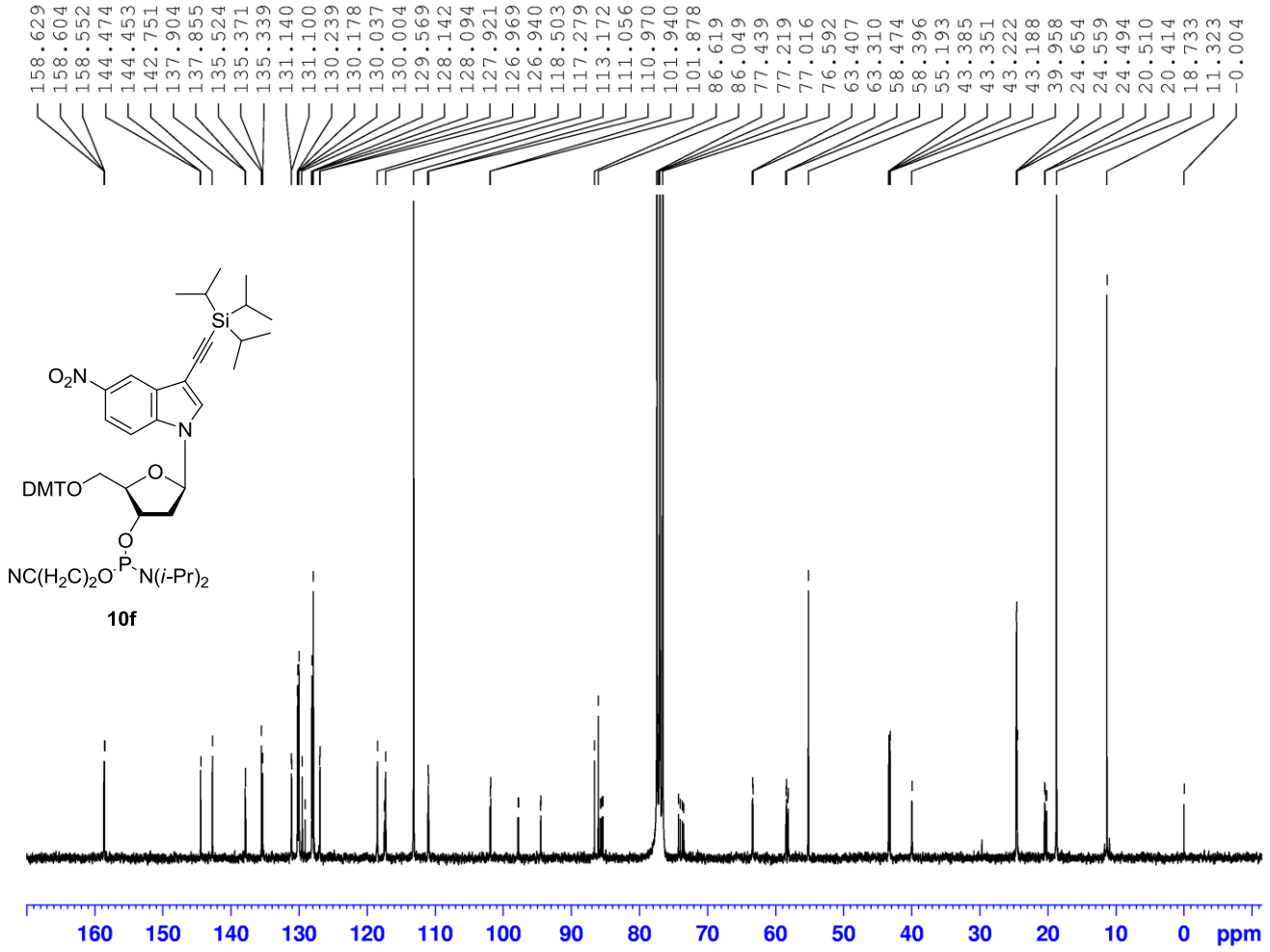


Fig. S61. ^{31}P NMR spectrum of compound 10f.

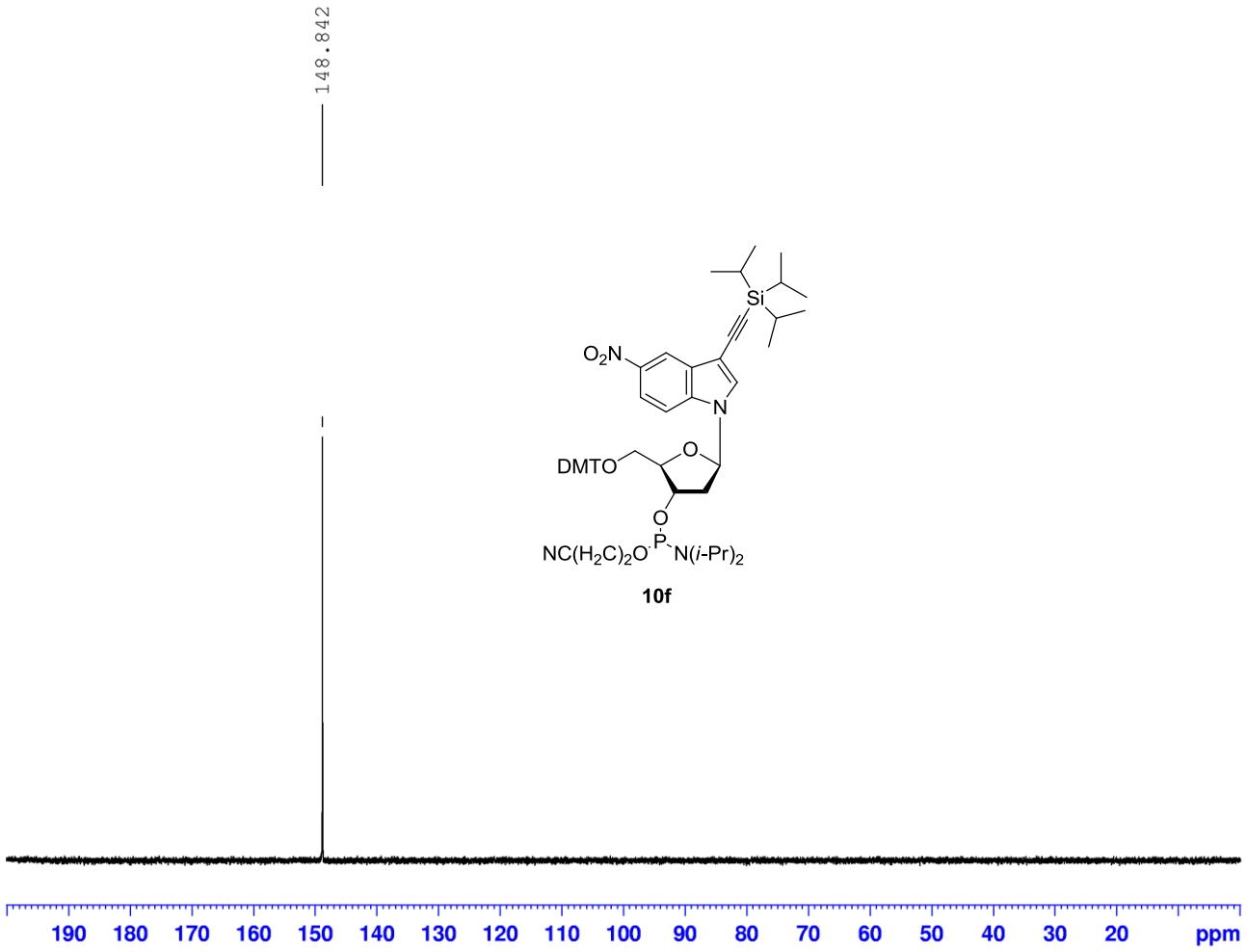


Fig. S62. ^{1}H NMR spectrum of compound 12.

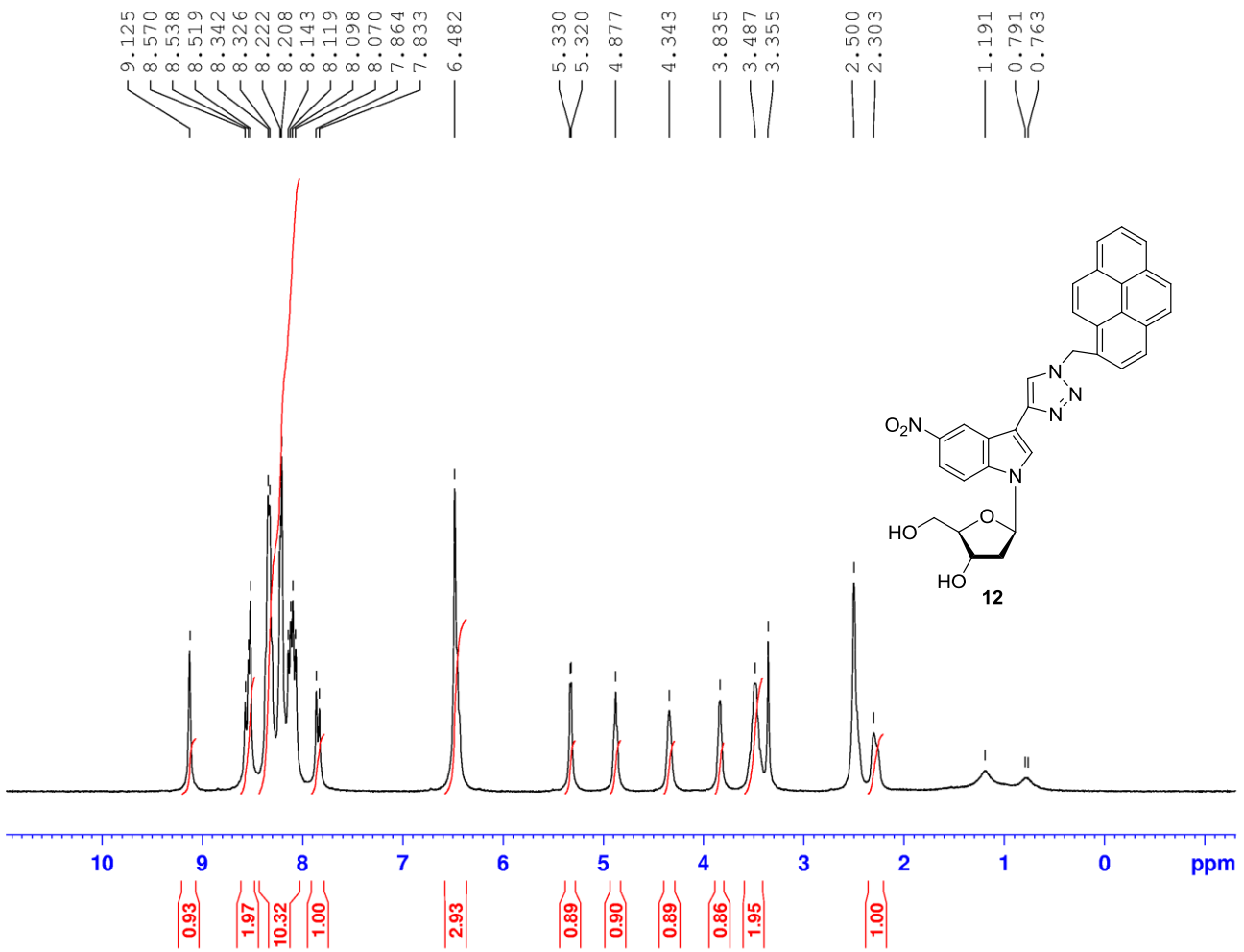


Fig. S63. ^{13}C NMR spectrum of compound 12.

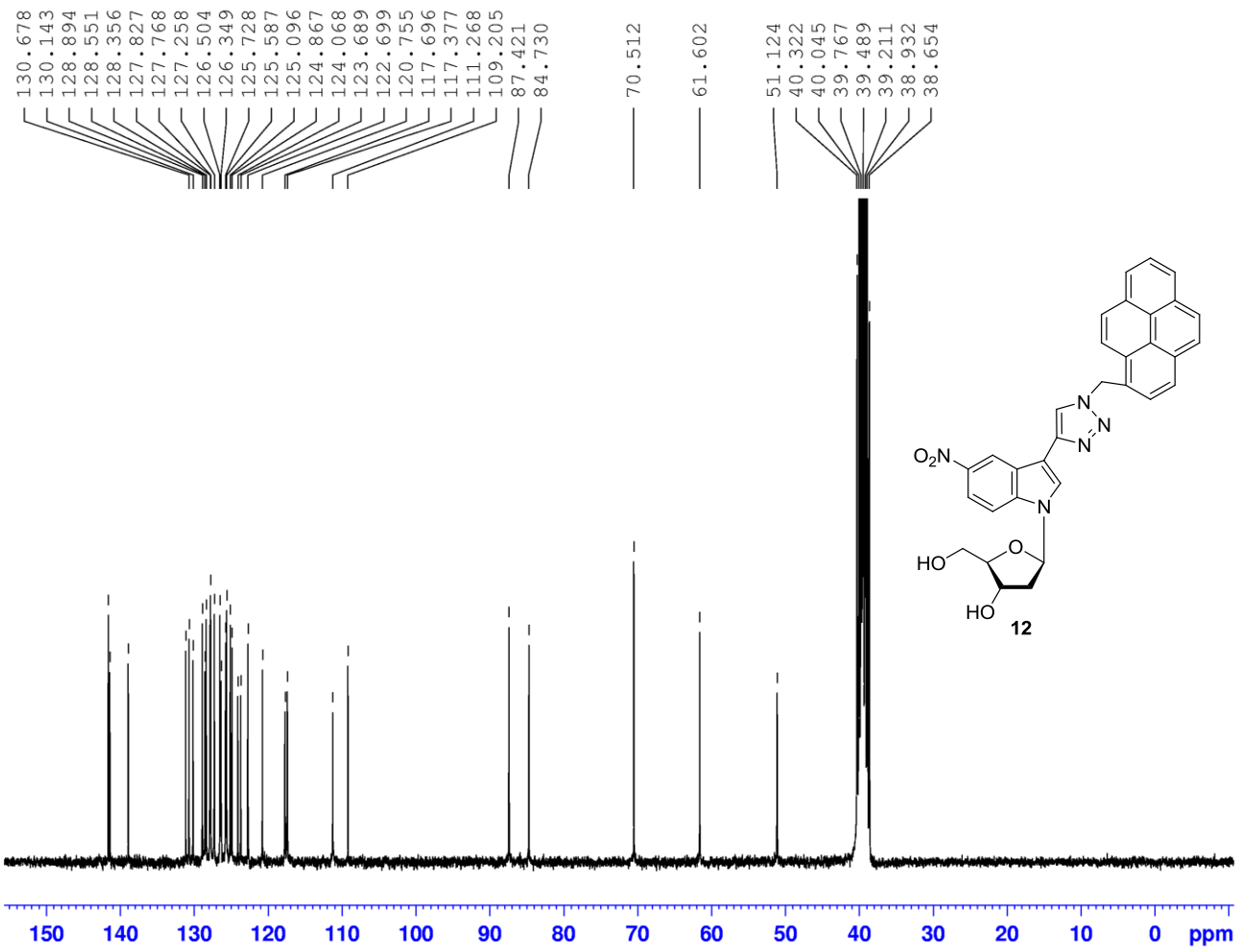


Fig. S64. DEPT-135 spectrum of compound 12.

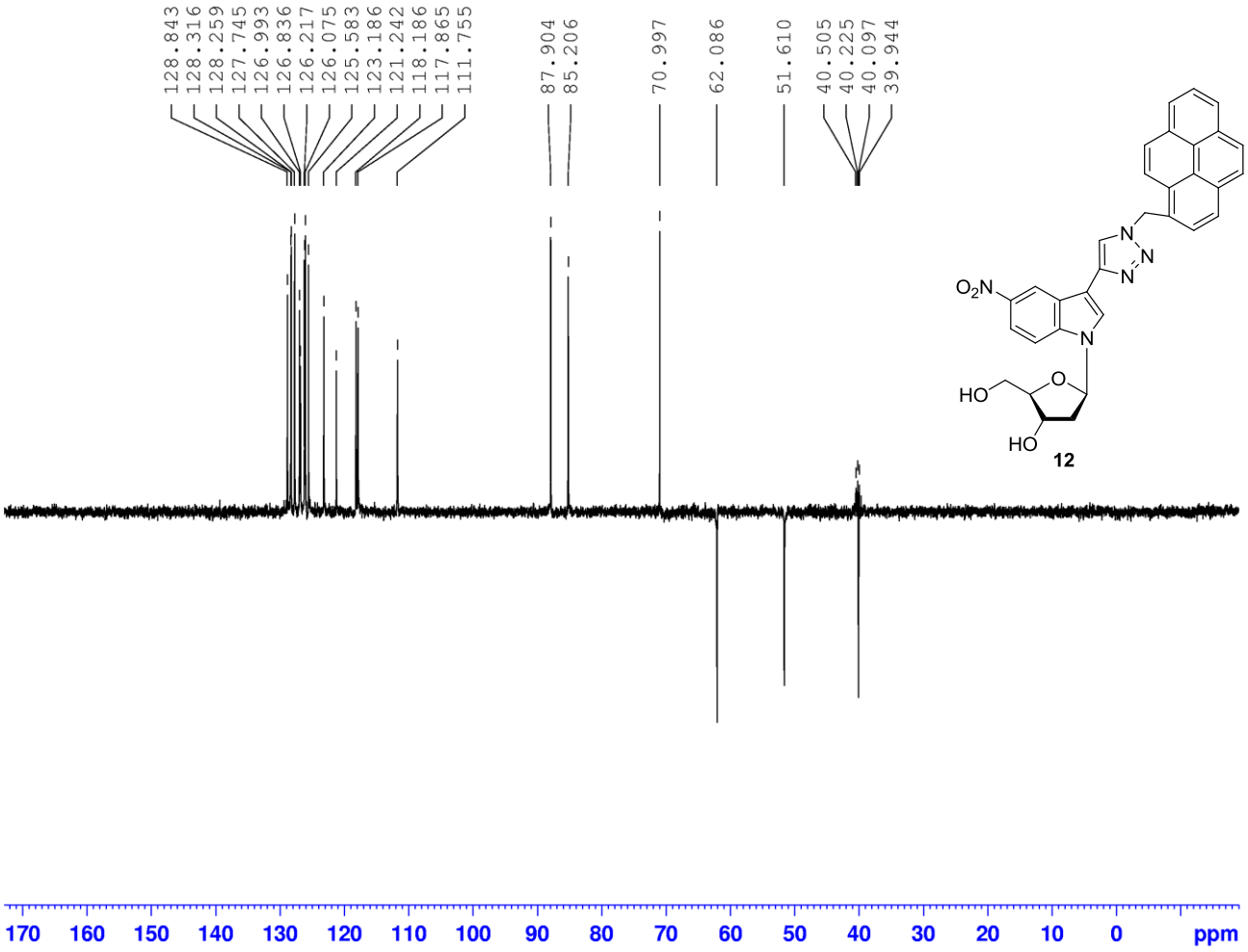


Fig. S65. ^{1}H-^{13}C gated-decoupled spectrum of compound 12.

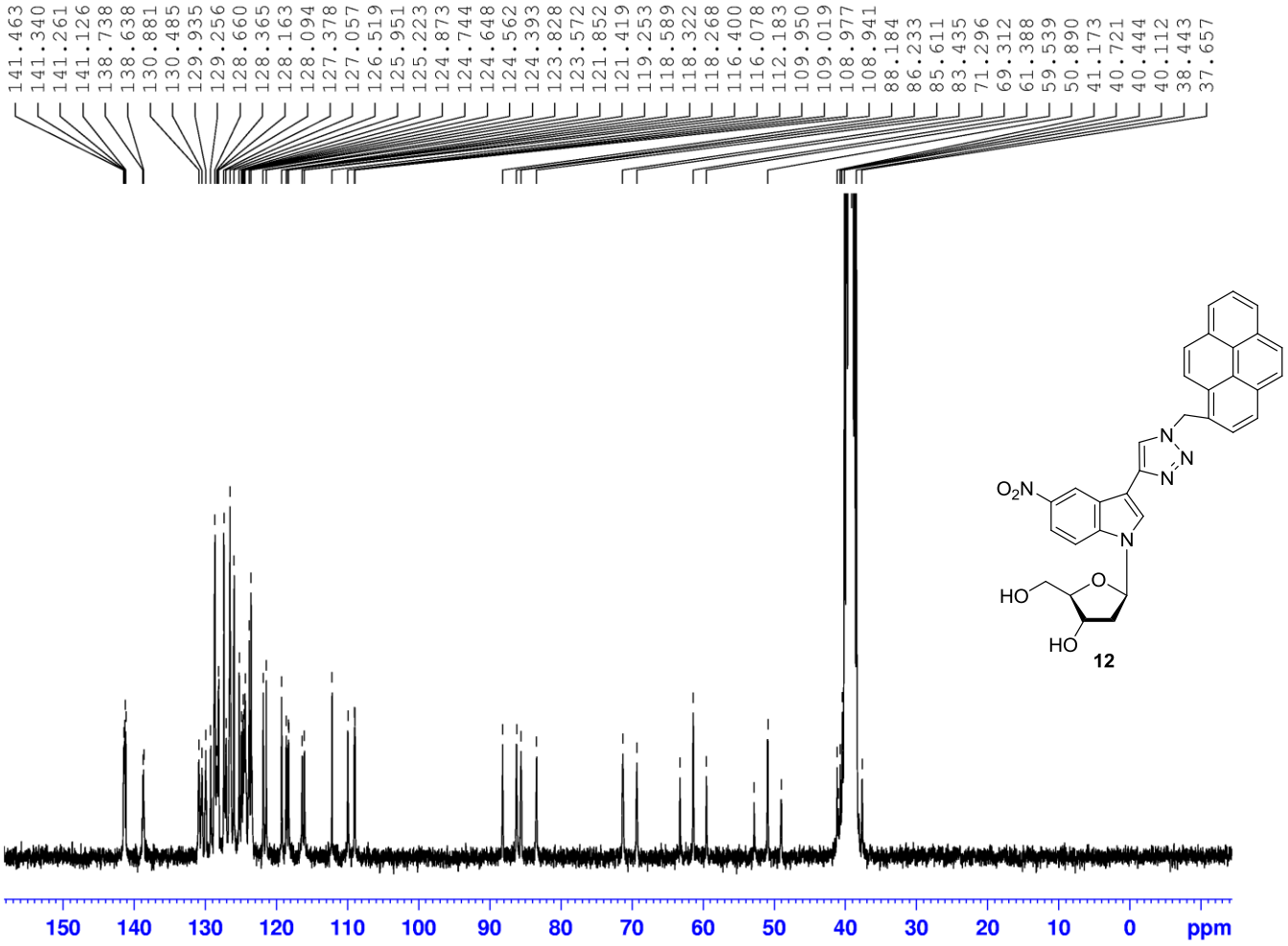


Fig. S66. ^{1}H NMR spectrum of compound 13.

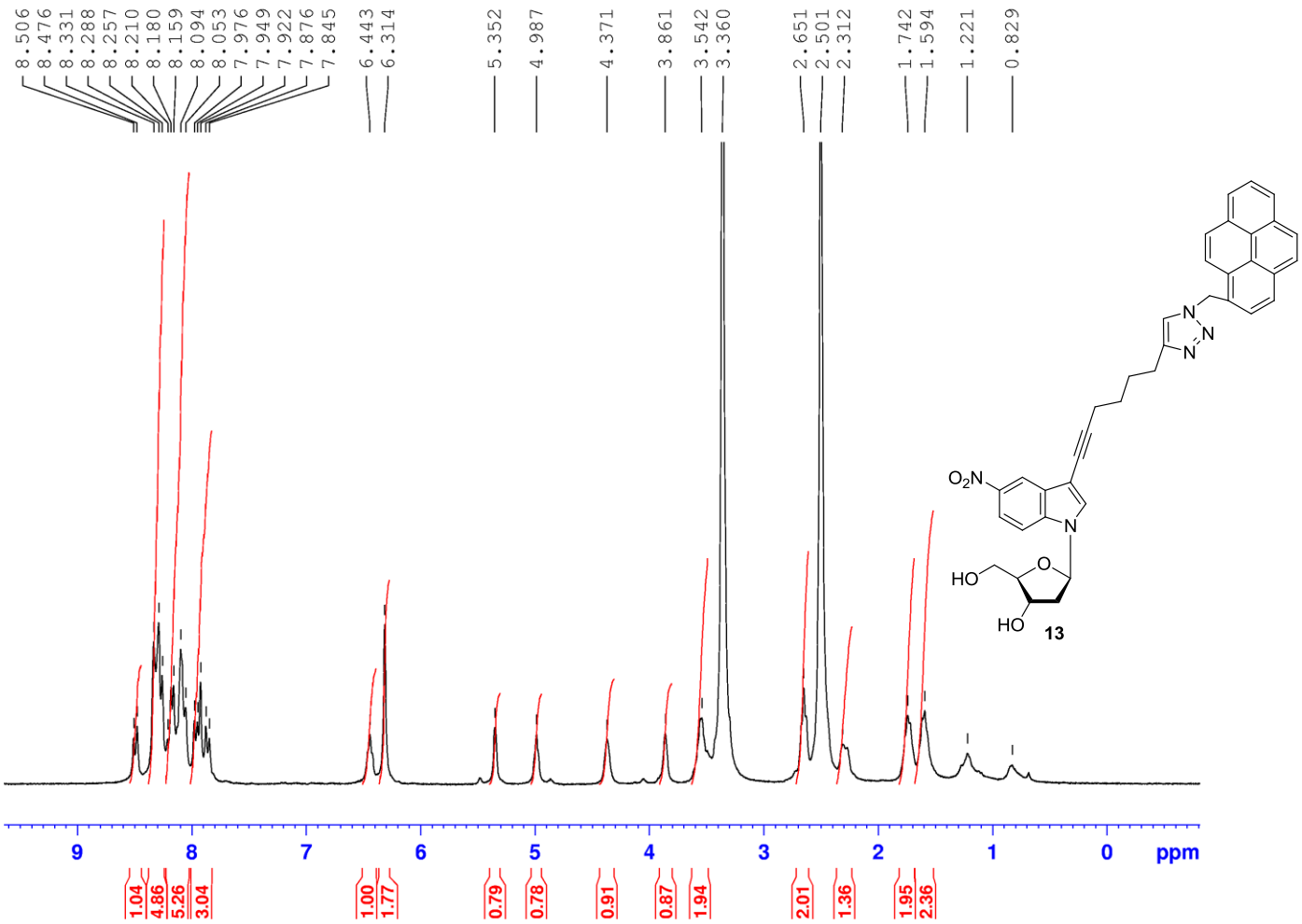


Fig. S67. ^{13}C NMR spectrum of compound 13.

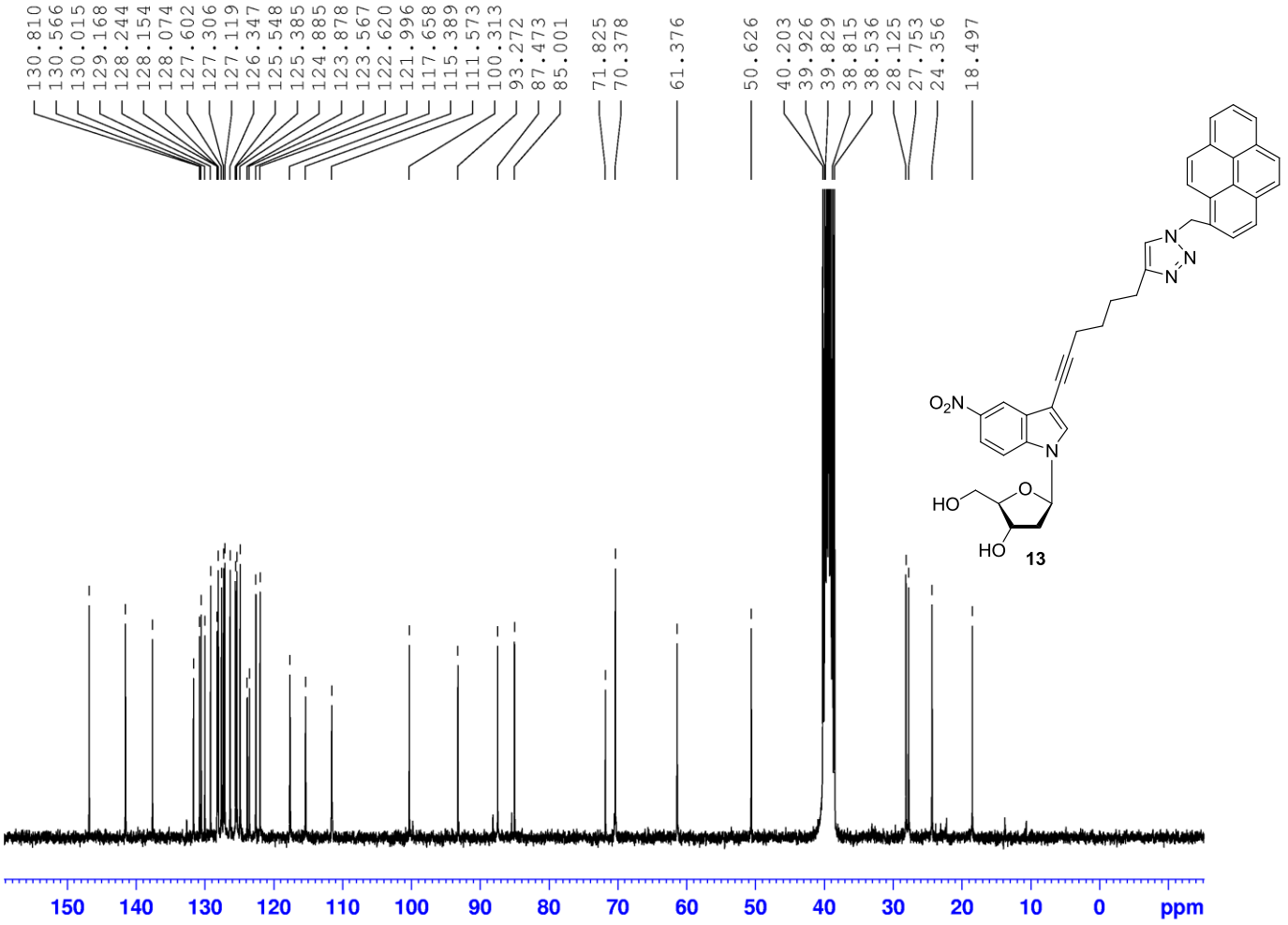


Fig. S68. DEPT-135 spectrum of compound 13.

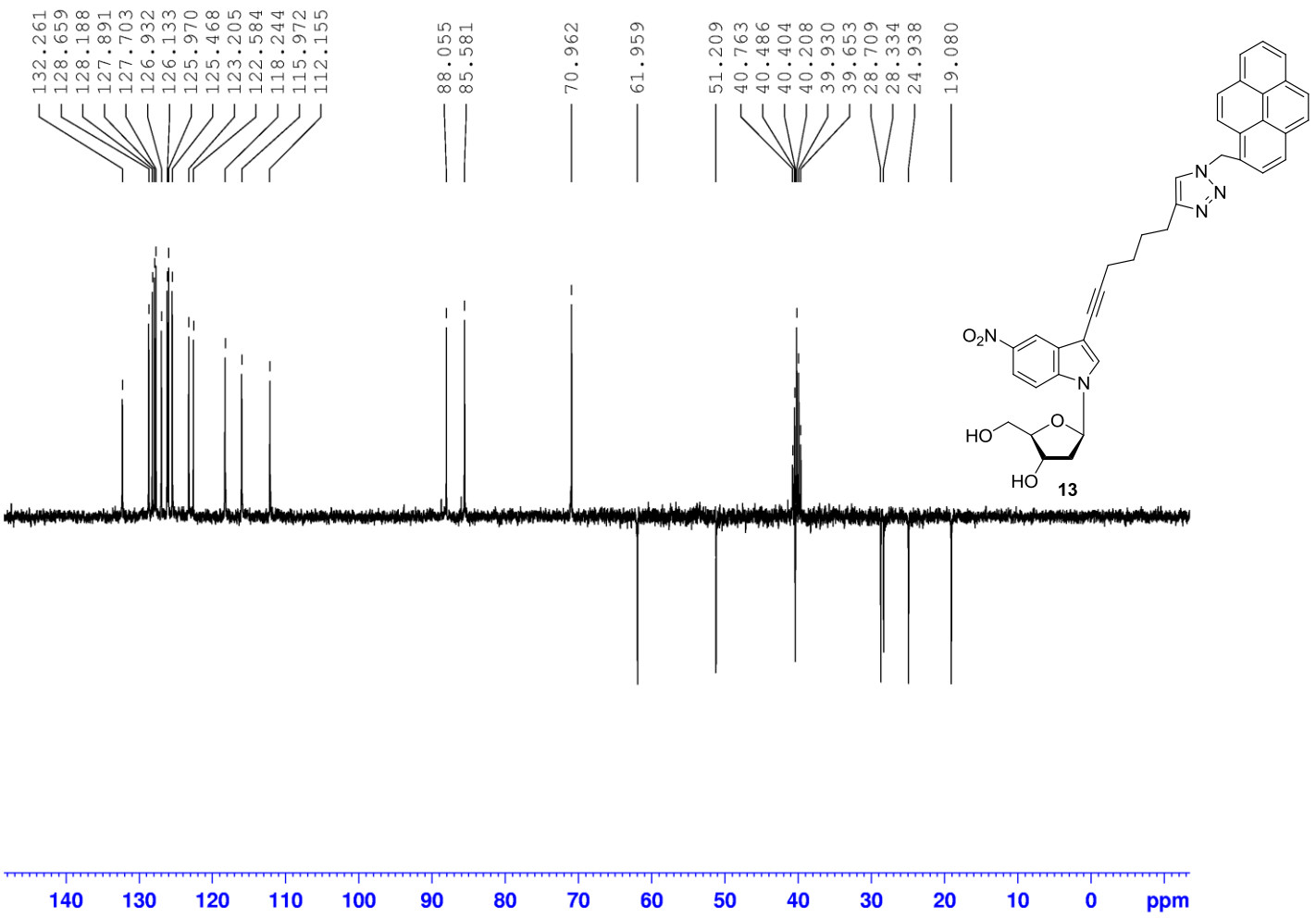


Fig. S69. ^{1}H-^{13}C gated-decoupled spectrum of compound 13.

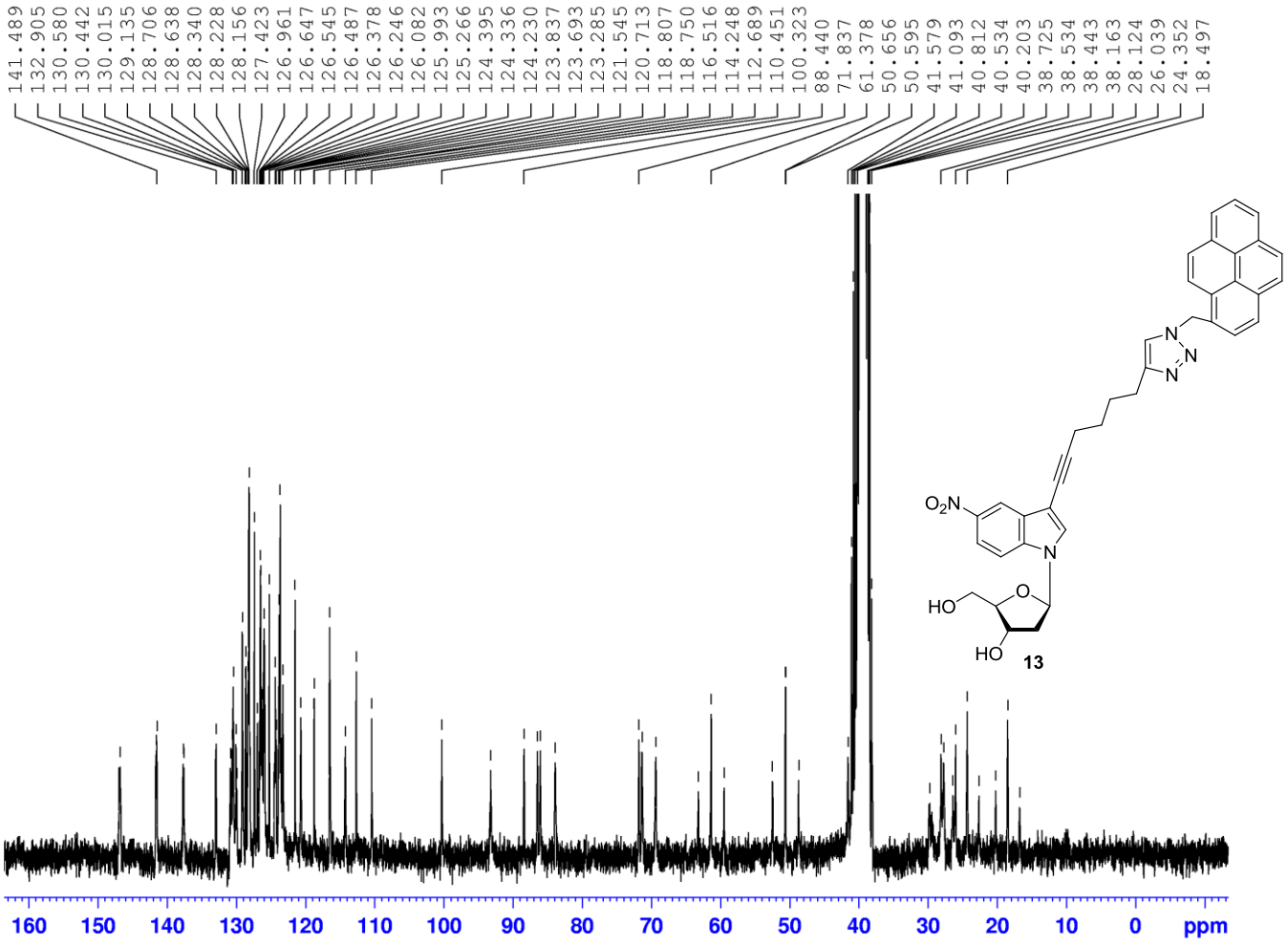


Fig. S70. ^{1}H NMR spectrum of compound 16.

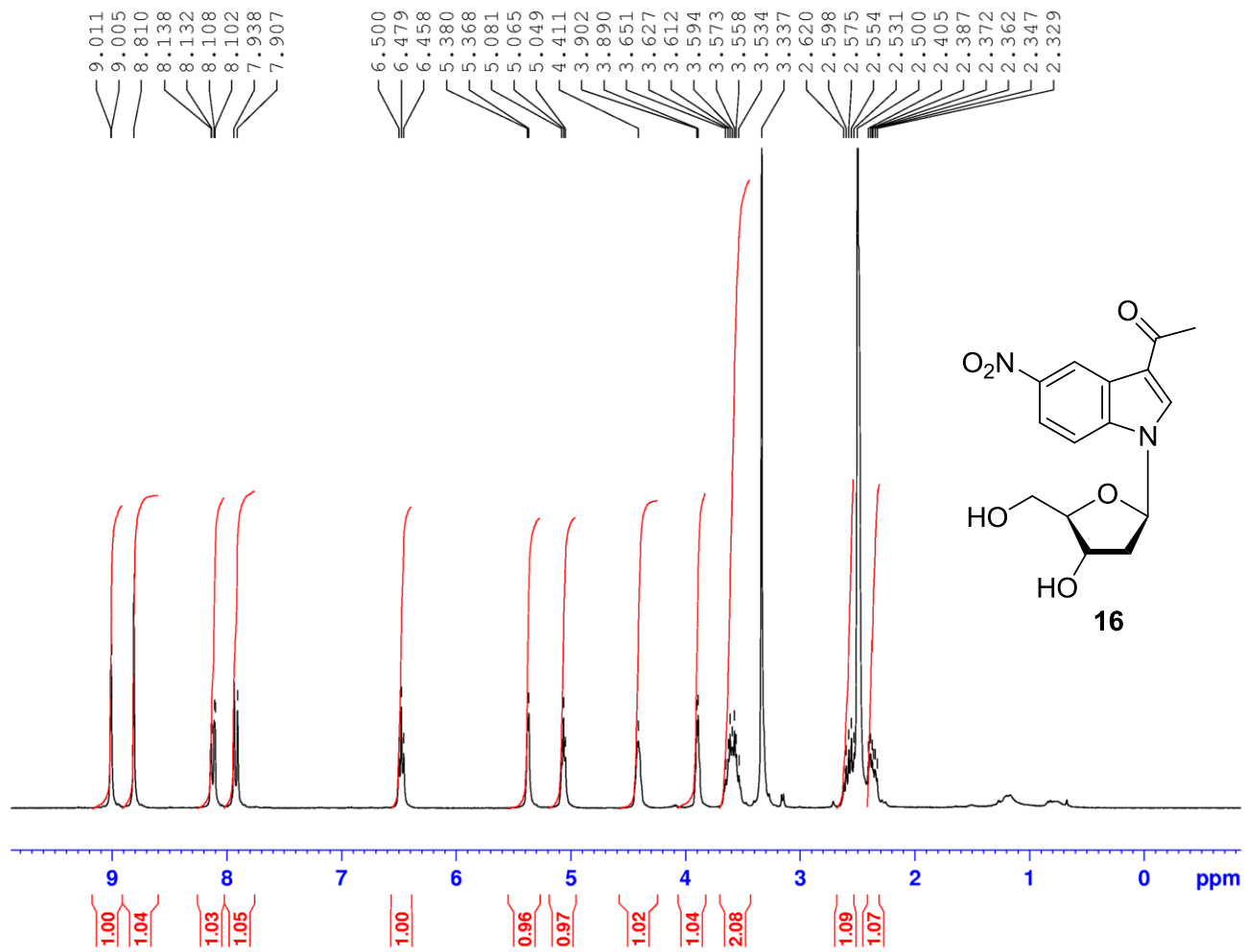


Fig. S71. ^{13}C NMR spectrum of compound 16.

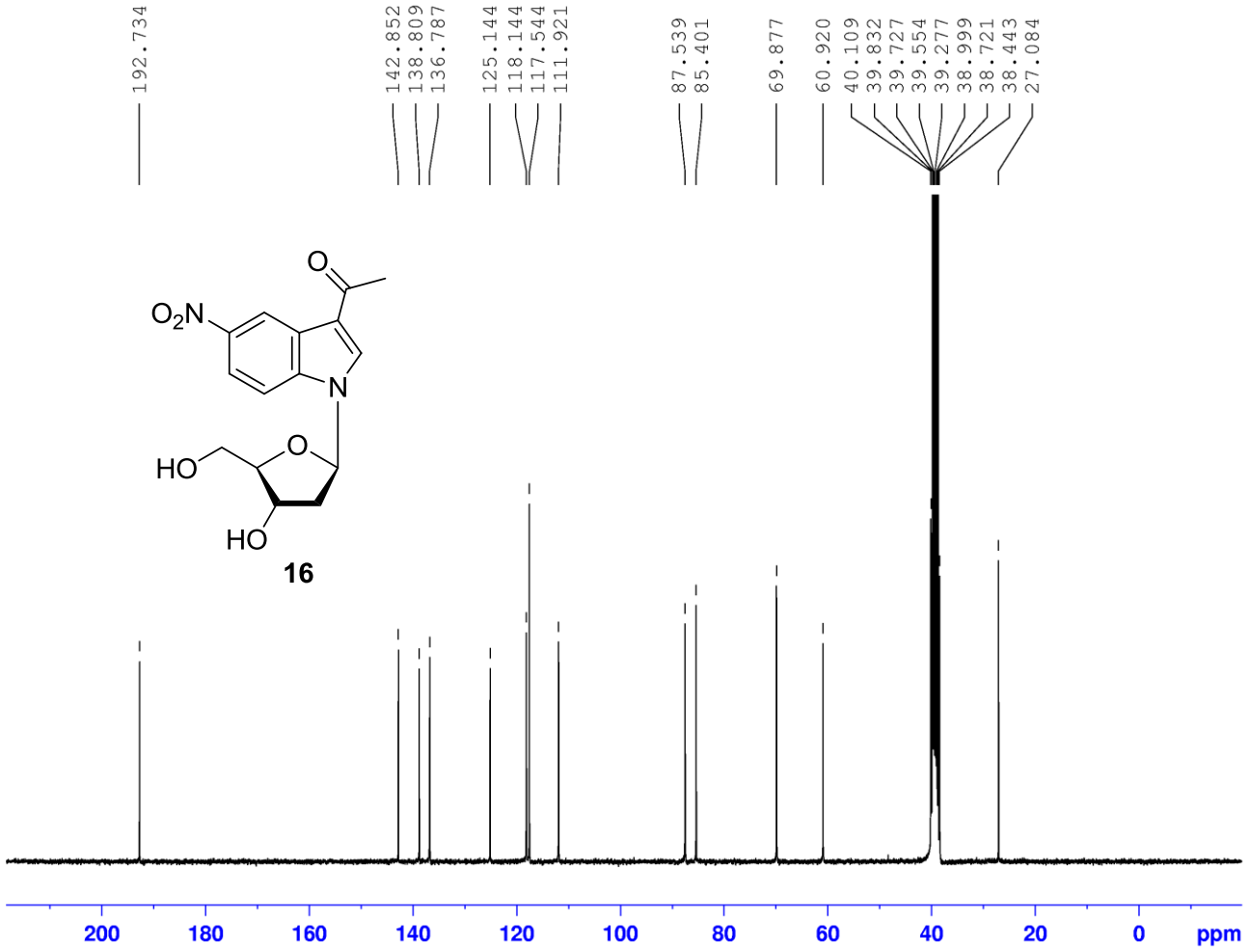


Fig. S72. DEPT-135 spectrum of compound 16.

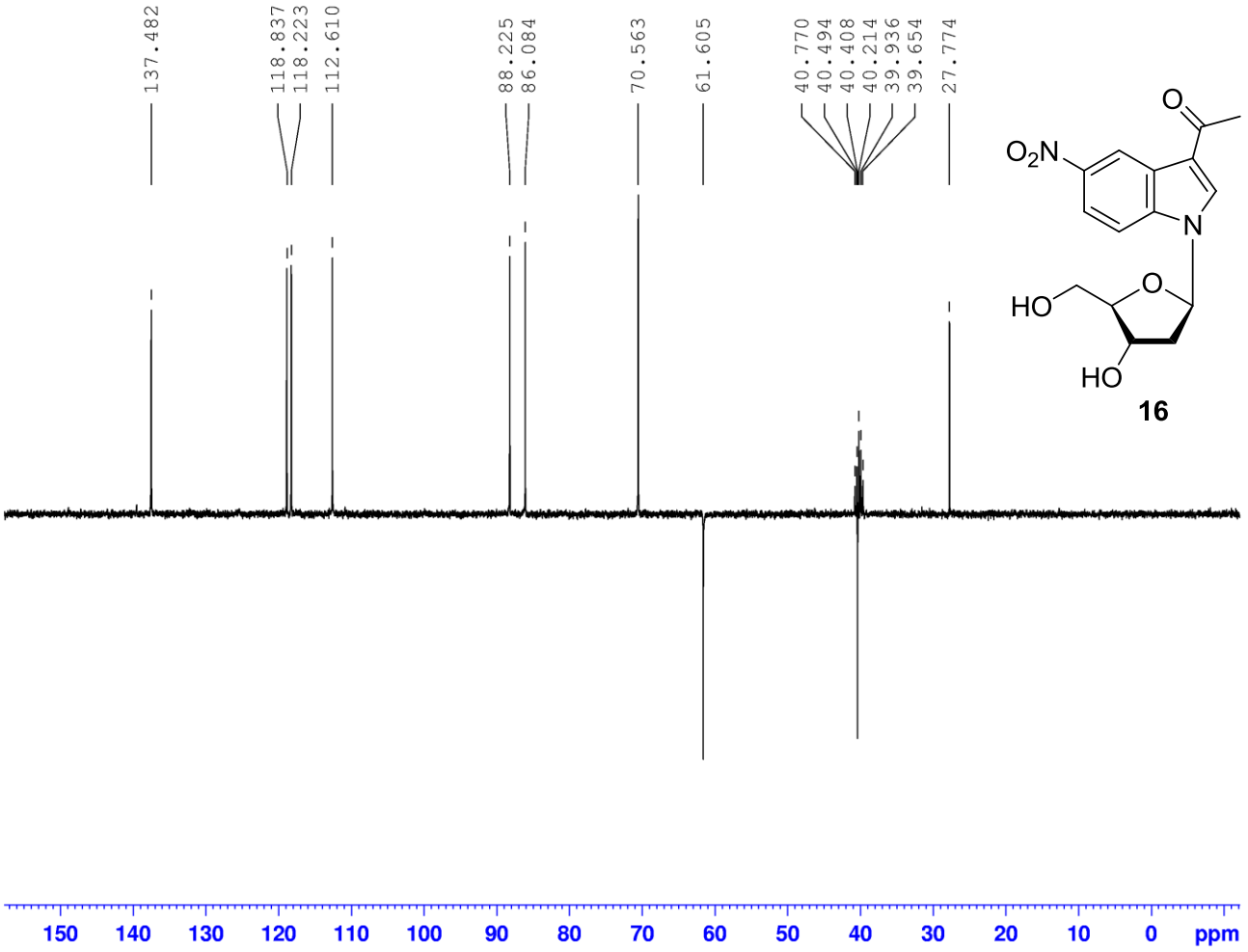


Fig. S73. ^{1}H-^{13}C gated-decoupled spectrum of compound 16.