Supporting Information

Oligo switches: photoresponsive oligonucleotide conjugates by solid-supported click chemistry

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General Experimental

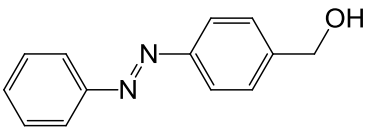
Standard reagents were supplied by either Apollo Scientific, TCI Europe, or Sigma-Aldrich and were used as received without further purification. Anhydrous or reagent grade solvents were used; the latter were dried before use according to standard procedures. 18.2 MΩ water was used for HPLC. Analytical TLC was performed on pre-coated (250 μm) silica gel 60 F-254 plates from Merck. All plates were visualised by UV irradiation. Flash chromatography was performed using silica gel 32–63 μm, 60 Å. Electrospray (ESI) mass spectra were collected on an Agilent Technologies 6410 Time of Flight LC/MS. NMR spectra were obtained with a Bruker instrument at 25 °C (^{1}H at 300 MHz; ^{13}C at 75 MHz). Chemical shifts are reported in ppm downfield from TMS as standard. NMR spectra are recorded in $CDCl_3$ unless otherwise stated. Melting point analyses were carried out using a Stewart Scientific SMP 1 melting point apparatus.

All materials used in DNA synthesis were supplied by either Link Technologies or ChemGenes. Oligonucleotide ON synthesis was carried out on an Expedite DNA synthesiser on a 1 μmol scale using standard DNA phosphoramidites. BMT (0.2 M) was used as activating agent. Oxidation was performed using 8:1:1 THF:pyridine:H_2O containing 20 mM I_2. Deprotection/cleavage of both native (trityl-on) and modified (trityl-off) DNA was achieved following incubation in 40% (w/w) aqueous CH_3NH_2 (500 μL) at 65 ^{o}C for 30 minutes. Upon cooling, the aqueous CH_3NH_2 solution was decanted and reduced in a vacuum concentrator. The concentrate was combined with the CPG washings (3 x 150 μL H_2O), and desalted using Waters Sep-Pak® C18 cartridges. Mass spectral data was obtained by MALDI-TOF MS recorded by Metabion, Germany, unless otherwise stated. UV-Visible data were obtained with a VARIAN CARY 100 Spectrometer after purification. ON samples were analysed and, as required, purified by RP-HPLC.

Conditions A: Column: Phenomenex C8 (4.6 x 250 mm). Buffer A: 0.1 M TEAAc, pH 6.5, 5% (v/v) MeCN, buffer B: 0.1 M TEAAc, pH 6.5, 65% (v/v) MeCN. Gradient: 0-4.3 min, 5% B; 4.3-16.6 min, 5→100% B. Flow rate: 1.0 mL/min. Detection at 260 nm with a diode array detector.

Conditions B: Column: Hichrom KR100-5C18 (4.6 x 250 mm). Buffer A: 0.1 M TEAAc, pH 6.5, 5% (v/v) MeCN, buffer B: 0.1 M TEAAc, pH 6.5, 65% (v/v) MeCN. Gradient: 0-5 min, 5% B; 5-20 min, 5→100% B. Flow rate: 1.0 mL/min. Detection at 260 nm with a diode array detector.

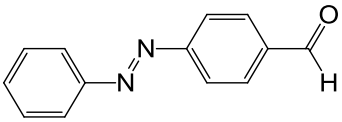
4-(Phenylazo)phenylmethanol (1a)



To a stirred solution of 4-(phenylazo)benzoic acid (1.00 g, 4.42 mmol) in anhydrous THF (25 mL) under argon, was added lithium aluminium hydride (LAH) (210 mg, 5.53 mmol) in one portion at 0 ^{o}C, and the resulting mixture was stirred at rt for 30 min. Residual LAH was then quenched with 3 % (w/v) KOH. The resulting solution was diluted with EtOAc (30 mL) and washed with brine (1 x 10 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to yield the product as an orange solid (619 mg, 66%).

m.p. = 140-142 ^{o}C (lit. value = 142 ^{o}C).[1] ^{1}H NMR data corresponded to that reported in the literature; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0 Hz, 4H), 7.52-7.45 (m, 5H), 4.76 (s, 2H).[2]

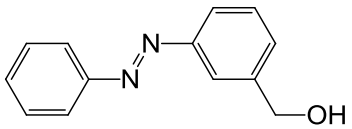
4-(Phenylazo)benzaldehyde (2a)



To a stirred solution of 4-(phenylazo)phenylmethanol (**1a**) (300 mg, 1.41 mmol) in anhydrous DCM (10 mL) at ambient temperature, was added solid Dess Martin periodinane reagent (DMP) (730 mg, 1.72 mmol) in one portion. The resulting solution was stirred at rt for 1 h. The solvent was then removed under reduced pressure and the resulting orange solid was dissolved in EtOAc (30 mL). This solution was washed with saturated $NaHCO_3$ (10 mL), brine (10 mL) and water (10 mL). The organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure, yielding the pure product as an orange solid (289 mg, 97%).

^{1}H NMR δ 10.08 (s, 1H, CHO), 8.01 (br s, 4H, ArH), 7.96-7.93 (m, 2H, ArH), 7.53-7.51 (m, 3H, ArH); ^{13}C NMR δ 191.6 (CHO), 155.9, 152.6, 137.5 (3 x ArC), 132.0 (ArCH), 130.7 (2 x ArCH), 129.2 (2 x ArCH), 123.3 (2 x ArCH), 123.3 (2 x ArCH); HRMS (ESI) calcd for $C_{13}H_{11}N_2O$ 210.0793 $[M + H]^+$, found 210.0880. m.p. = 118-119 ^{o}C

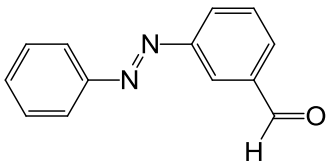
3-(Phenylazo)phenylmethanol (1b)



meta-Aminobenzyl alcohol (500 mg, 4.06 mmol) was added in one portion to a solution of nitrosobenzene (652 mg, 6.09 mmol) in CH_3COOH (5 mL) at 0 ^oC. The resulting mixture was stirred at this temperature for 1 h, after which it was warmed to rt and stirred for another 3 hr. EtOAc was added and the organic layer was washed with aqueous $NaHCO_3$ (3 x 20 mL) to remove the CH_3COOH. The organic layer was then dried over anhydrous $MgSO_4$ and concentrated under reduced pressure, yielding an orange solid as the crude product. This was then purified by flash column chromatography (*n*-hexane:EtOAc, 6:4), giving the product as an orange oil (707 mg, 82%).

^1H NMR δ 7.93-7.90 (m, 3H, ArH), 7.85 (dt, *J* = 6.8, 1.9 Hz, 1H, ArH), 7.55-7.45 (m, 5H, ArH), 4.79 (s, 1H, CH_2); ^{13}C NMR δ 152.9, 152.6, 142.1 (3 x ArC), 131.1, 129.4, 129.3, 129.1, 122.9, 122.6, 120.6 (9 x ArCH), 64.9 (CH_2); HRMS (ESI) calcd for $C_{13}H_{13}N_2O$ 213.1022 $[M + H]^+$, found 213.1030. m.p. = 35-36 ^oC

3-(Phenylazo)benzaldehyde (2b)



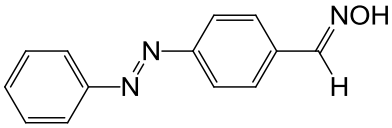
To a stirred solution of 3-(phenylazo)phenylmethanol (**1b**) (300 mg, 1.41 mmol), in anhydrous DCM (10 mL) at ambient temperature, was added solid DMP (1.20 g, 2.82 mmol) in one portion. The resulting solution was stirred at rt for 3 h. The solvent was then removed under reduced pressure and the resulting orange solid was dissolved in EtOAc (30 mL). This solution was washed with saturated $NaHCO_3$ (10 mL), brine (10 mL) and water (10 mL). The organic layer was then dried over anhydrous sodium sulphate and concentrated under reduced pressure, yielding the pure product as an orange solid (210 mg, 71%).

^1H NMR δ 10.04 (s, 1H, CHO), 8.32 (t, *J* = 1.8 Hz, 1H ArH), 8.10 (dt, *J* = 7.9, 1.3 Hz, 1H, ArH), 7.93-7.89 (m, 3H, ArH), 7.58 (t, *J* = 7.9 Hz, ArH); ^{13}C NMR δ 191.6 (CHO), 152.8, 152.3, 137.3 (3 x ArC), 131.7, 131.1, 129.8, 129.2, 128.7, 123.8, 123.2 (9 x ArCH); HRMS (ESI) calcd for $C_{13}H_{11}N_2O$ 211.0866 $[M + H]^+$, found 211.0868. m.p. = 53-56 ^oC

General procedure for the synthesis of 3- (3a) or 4-(phenylazo)benzaldehyde oximes (3b)

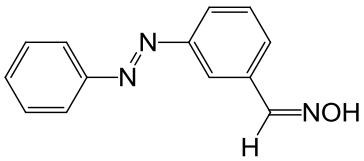
To a solution of the aldehyde (350 mg, 1.66 mmol) in EtOH (10 mL) at ambient temperature, was added hydroxylamine hydrochloride (173 mg, 2.49 mmol) and pyridine (2.49 mmol, 0.201 mL). The resulting solution was heated for 1 h under microwave irradiation (T = 125 ^{o}C, P_{max} = 300 W). Water (5 mL) was then added and the resulting orange precipitate was filtered off to yield pure product in excellent yield.

4-(Phenylazo)benzaldehyde oxime (3a)



Yield: (351 mg, 94%). ^{1}H NMR δ 8.20 (s, 1H, CHNOH), 7.96-7.92 (m, 4H, ArH), 7.74 (d, J = 8.5 Hz, 2H, ArH), 7.60 (br s, 1H, NOH), 7.56-7.49 (m, 3H, ArH); ^{13}C NMR δ 153.3, 152.6 (2 x ArC), 149.7 (CHNOH), 134.4 (ArC), 131.4, 129.2, 127.8, 123.3, 123.0 (9 x ArCH); HRMS (ESI) calcd for $C_{13}H_{12}N_3O$ 226.0975 $[M + H]^+$, found 226.0986. m.p. = 151-152 ^{o}C

3-(Phenylazo)benzaldehyde oxime (3b)



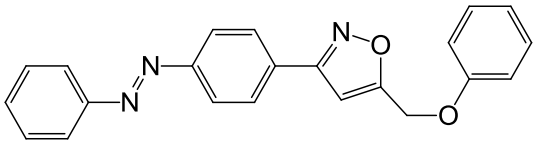
Yield: 329 mg, 88%. ^{1}H NMR δ 8.37 (br s, 1H, NOH), 8.26 (s, 1H, CHNOH), 8.13 (t, *J* = 1.7 Hz, 1H, ArH), 7.96-7.91 (m, 3H, ArH), 7.71 (d, *J* = 7.7 Hz, 1H), 7.56-7.48 (m, 4H, ArH); ^{13}C NMR δ 152.9 (ArC), 152.5 (ArC), 149.8 (CHNOH), 133.1 (ArC), 131.4, 129.6, 129.2, 129.0, 124.4, 123.0, 121.6 (10x ArCH); HRMS (ESI) calcd for $C_{13}H_{12}N_3O$ 226.0975 $[M + H]^+$, found 226.0979. m.p. = 101-103 ^{o}C

General procedure for the solution-phase synthesis of the isoxazole cycloadducts 5a and 5b

To a solution of oxime **3a** (150 mg, 0.66 mmol) in EtOH (3 mL) at ambient temperature, was added Ch-T (152 mg, 0.66 mmol)and subsequently, H_2O (1 mL). The resulting solution was stirred for 10 min at rt. Phenyl propargyl ether[3] (29 mg, 0.222 mmol) was then added followed by further aliquots of EtOH (1.5 mL) and H_2O (0.5 mL). The reaction mixture was heated at 40 ^{o}C for 1 h, diluted with H_2O (30 mL) and extracted with DCM (3 x 10 mL). Following drying of the combined organic extracts over anhydrous magnesium sulphate, the solvent was filtered and removed under reduced pressure. The crude product was purified by

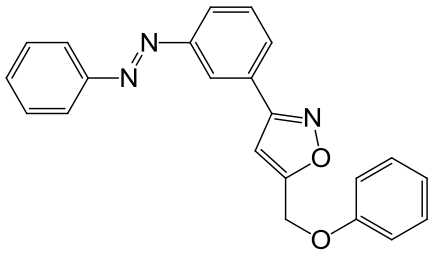
flash column chromatography (*n*-hexane:EtOAc, 4:1) yielding the pure product as an orange solid.

5-(Phenoxymethyl)-3-(4-(phenylazo)phenyl)isoxazole (5a)



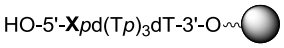
Yield: 209 mg, 89%, ^{1}H NMR δ 8.04-7.93 (m, 6H, ArH), 7.60-7.50 (m, 3H, ArH), 7.34 (t, *J* = 8.1 Hz, 2H, ArH), 7.06-7.00 (m, 3H, ArH), 6.72 (s, 1H, isox-H), 5.24 (s, 2H, CH_2); ^{13}C NMR δ 169.0, 161.9, 157.8, 153.5, 152.6 (5 x ArC), 131.4 (ArCH), 131.0 (ArC), 129.7, 129.2, 127.7, 123.4, 123.0, 122.0, 114.8 (13 x ArCH), 101.4 (isox-CH), 61.5 (CH_2); HRMS (ESI) calcd for $C_{22}H_{18}N_3O_2$ 356.1394 $[M + H]^+$, found 356.1393. m.p. = 121-122 ^{o}C

5-(Phenoxymethyl)-3-(3-(phenylazo)phenyl)isoxazole (5b)

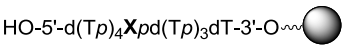


Yield: 109 mg, 46%.^{1}H NMR δ 8.32 (t, *J* = 2.1 Hz, 1H, ArH) 8.01-7.93 (m, 4H, ArH), 7.61 (t, *J* = 7.8 Hz, 1H, ArH), 7.57-7.49 (m, 3H), 7.36-7.30 (m, 2H, ArH), 7.05-6.99 (m, 3H, ArH), 6.75 (s, 1H, isox-H), 5.24 (s, 2H, CH_2); ^{13}C NMR δ 168.9, 162.1, 157.8, 153.0, 152.5 (5 x ArC), 131.4 (ArCH), 129.8 (ArC), 129.8, 129.7, 129.2, 129.0, 124.7, 123.0, 122.0, 121.1, 114.8 (13 x ArCH), 101.5 (isox-CH), 61.5 (CH_2); HRMS (ESI) calcd for $C_{22}H_{18}N_3O_2$ 356.1394 $[M + H]^+$, found 356.1391. m.p. = 105-107 ^{o}C

Synthesis of 2′-*O*-propargyl derivatised supported ON's 13 and 16



13

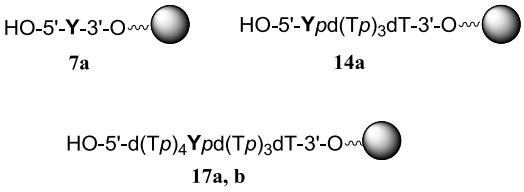


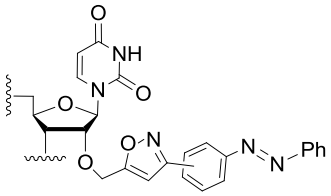
16

Separate solutions of the phosphoramidite **12** (500 µL, 100 mM in anhydrous CH_3CN), and BMT (500 µL, 0.3 mM in anhydrous CH_3CN), both in 1 mL syringes were attached to either end of a DNA synthesis column containing the CPG-T_4 **11** (1 µmol). The mixture was reacted for 15 min at rt with mixing between the two syringes. This reaction was repeated with a second portion each of a new solution of the phosphoramidite and BMT. The column was then re-installed on the DNA synthesiser where standard washing, capping, oxidation and deblocking programmes were carried out. Where the ON **13** was required, the column

was removed from the synthesiser at this point. Where the ODN **16** was required, the column was left installed on the synthesiser and the required phosphoramidite couplings were executed using the standard procedure. Deprotection/cleavage of the resin-bound ON's was followed by RP-HPLC analysis under conditions A.

Synthesis of the AB-ODN conjugates 7a (from 6), 14a (from 13) and 17a, b (from 16)





Y **a.** *para*-AB
b. *meta*-AB

To a solution of the appropriate AB-oxime, **3a** or **3b** (30 µL, 250 mM in EtOH) was added a solution of Ch-T (10 µL, 750 mM in 1:1 (v:v) EtOH:H_2O). The resulting suspension was agitated at rt for 10 minutes to facilitate formation of the nitrile oxides **4a** or **4b**. Subsequently 20 µL (3.6 µmol) of the nitrile oxide solution was added to the CPG-2'-*O*-propargylated nucleic acid material (0.12 µmol). The mixture was agitated at rt for 24 h, after which the excess reactants were removed following washing with DMSO (10 x 200 µL) and CH_3CN (2 x 200 µL). Deprotection/cleavage of the resin-bound ON's was followed by analysis and, where necessary, purification by RP-HPLC under conditions A.

Table 1. Mass data for AB- and alkyne-modified ODN conjugates

Sequence	Compound	Mass calculated	Mass found
HO-5'-**Y**-3'-OH	**8a**	528.1490 [M + Na^+]	528.1463 [M + Na^+][a]
HO-5'-d(GpCpA)p**Y**-3'-OH	**10a**	1437	1437
HO-5'-**X**pd(Tp)$_3$dT-3'-OH	**(13)**[b]	1499	1501
HO-5'-**Y**pd(Tp)$_3$dT-3'-OH	**15a**	1722	1723
HO-5'-d(Tp)$_4$**X**pd(Tp)$_3$dT-3'-OH	**(16)**[b]	2716	2714
HO-5'-d(Tp)$_4$**Y**pd(Tp)$_3$dT-3'-OH	**18a**	2938	2937
HO-5'-d(Tp)$_4$**Y**pd(Tp)$_3$dT-3'-OH	**18b**	2938	2936

[a] LC/MS-ESI-TOF mass data [b] compound obtained from **13/16** following cleavage from the resin and deprotection

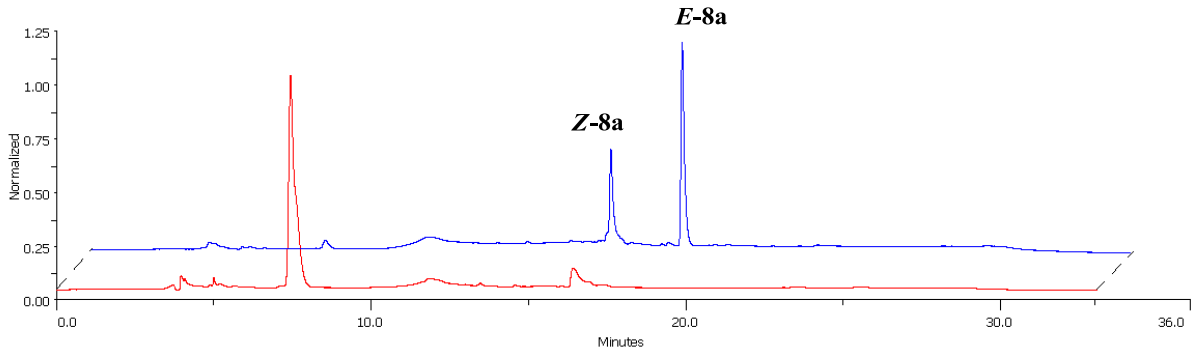


Figure 1. HPLC traces of the crude AB-labelled nucleoside **8a** (blue) and the starting material reference, 2′-*O*-propargyl uridine (red)

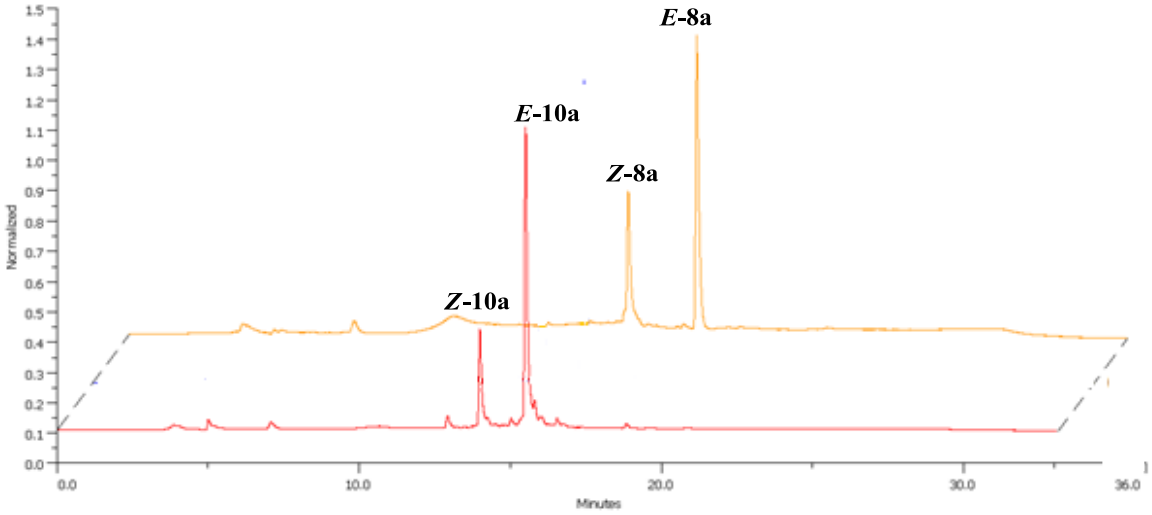


Figure 2. HPLC traces of crude AB-labelled nucleosides/ODN's; starting material reference **8a** (orange) and tetramer **10a** (red)

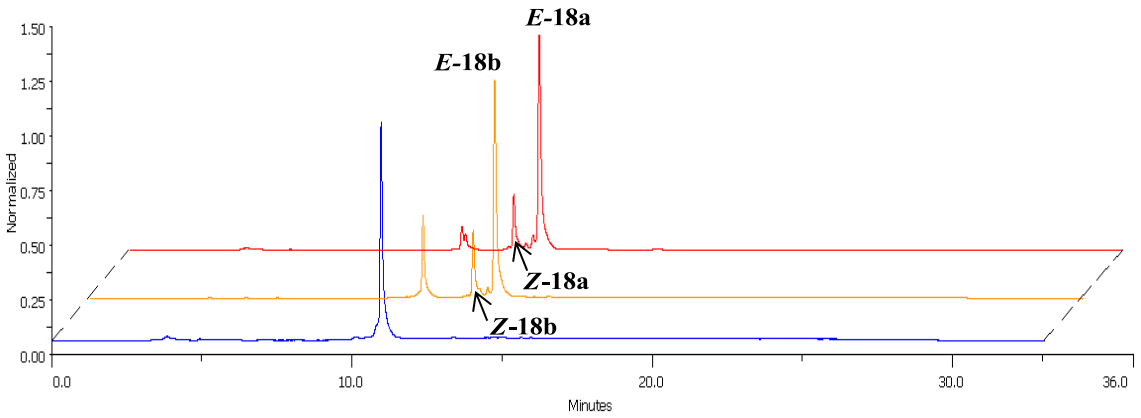


Figure 3. HPLC traces of crude ODN reaction products bearing an AB photoswitch at an internal position; starting material reference HO-5′-d(Tp)$_4$**X**pd(Tp)$_3$dT-3’OH (obtained from **16**, blue), *meta*-disubstituted cycloadduct **18b** (orange), and *para*-disubstituted cycloadduct **18a** (red)

Photochemical characterisation of the AB-ODN conjugates 18a and 18b

i. Photo-switching of AB-ON conjugates 18a and 18b

A medium pressure Hg-Arc lamp (100 W, Engelhard Hanovia of Canada Ltd.) was used to effect photo-isomerisation of the AB moieties. A band-pass filter was used for irradiation at 366 nm (4.13 W) and a cut-off filter was used for irradiation >400 nm (435 nm, 3.36 W) in combination with a water filter (1 cm) to prevent warming of the samples.

ii. UV analysis of E → Z photoswitching following irradiation at 366 nm

UV spectra of solutions of the AB-ON conjugates **18a** and **18b** (15 μM in Milli-Q H_2O) were measured following irradiation at 366nm for 2, 4, 6, 8, 10 and 600 seconds. The loss of UV-absorption arising from the *E*-AB π-π* transition at 320-340 nm was used to monitor the degree of isomerisation.

iii Determination of the mole fraction of E-18a and E-18b (χ_E) in the photostationary state using RP-HPLC

Solutions of the AB-ON conjugates **18a** and **18b** (15 μM in Milli-Q H_2O) were analysed by RP-HPLC under conditions B following irradiation at 366 nm for 10 min and at >400 nm for 2 min. The χ_E values for **18a** and **18b** in both photostationary states were quantified from the relative peak areas of *E*-**18a** and *Z*-**18a**, and *E*-**18b** and *Z*-**18b**.

Thermal stability studies of *Z*-18a and *Z*-18b

Following irradiation to the *Z*-form at 366 nm for 10 min, UV spectra of the AB-ON conjugates **18a** and **18b** (10 μM in buffer: 10 mM pH 7.0 $Na_xH_xPO_4$, 100 mM NaCl, 0.5 mM EDTA) were measured at regular intervals up to a period of 10 h. This procedure was carried out at the following temperatures; 60, 65, 70, 75, 80, 85 °C. The normalised absorbance values $[(A_t-A_0)/A_\infty-A_0)]$ at 351 nm were plotted against time, and by curve-fitting using the GraphPad Prism 5 software package, the first order rate constants (*k*) at each temperature were obtained. Arrhenius and Eyring plots were generated from these data, and from these plots a range of kinetic constants were obtained for the *Z* → *E* isomerisation process of both **18a** and **18b**.

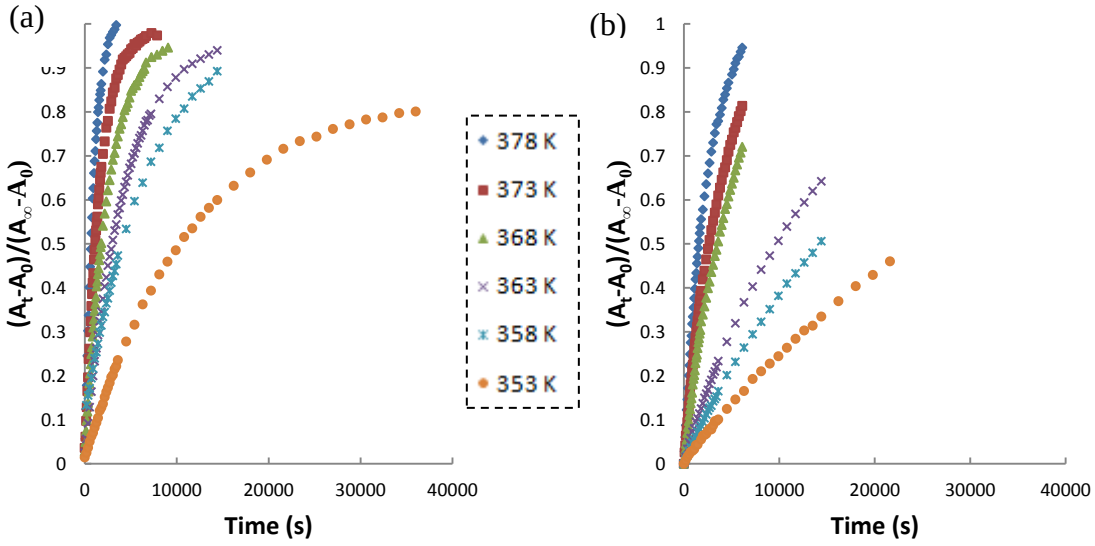


Figure 4. Plots of the normalised absorbance at 351 nm against time for the AB-modified ODN's **18a** (a) and **18b** (b)

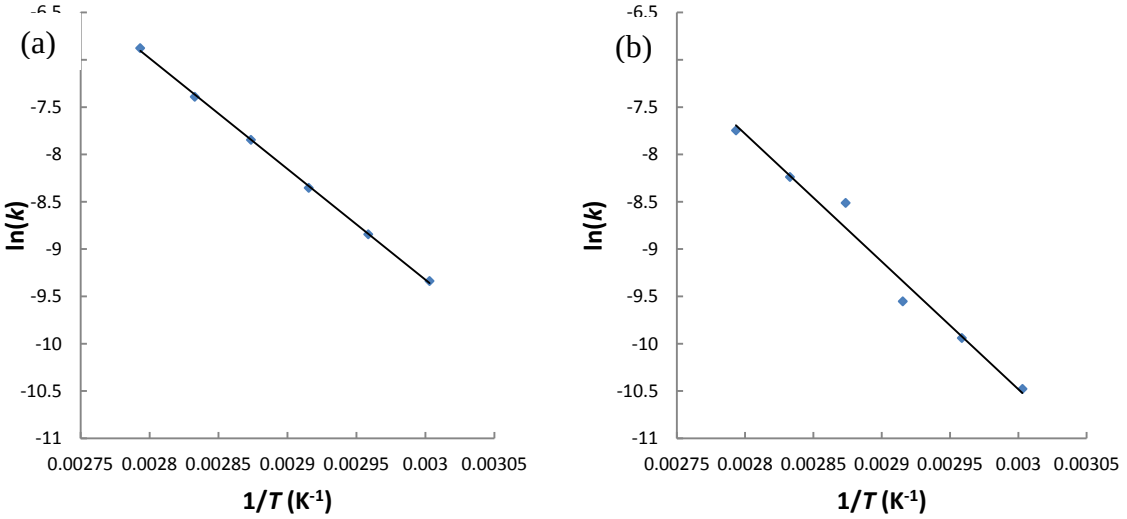


Figure 5. Arrhenius plots for the AB-modified ODN's **18a** (a) & **18b** (b)

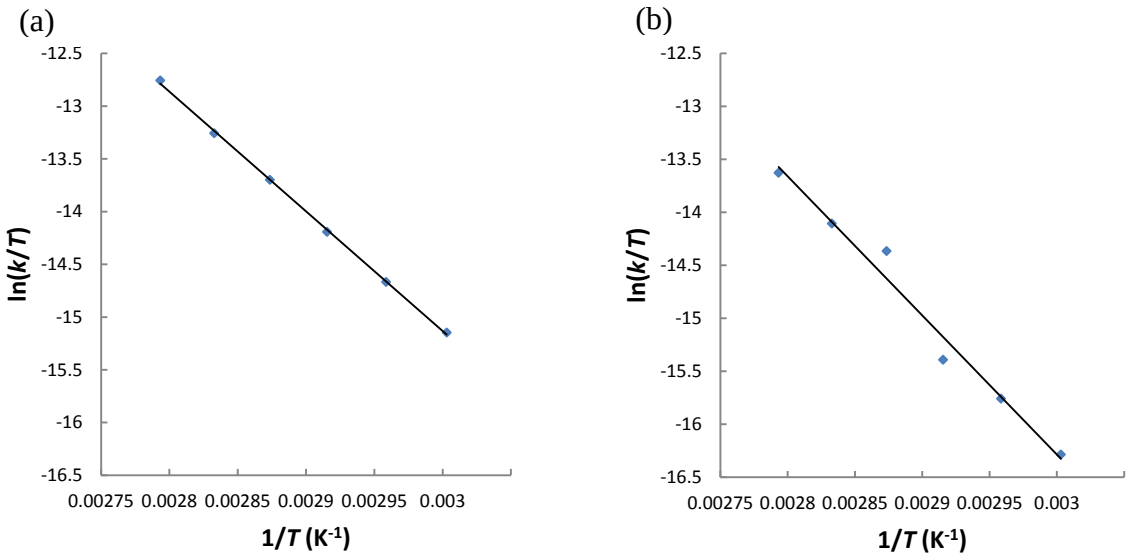


Figure 6. Eyring plots for the AB-modified ODN's **18a** (a) & **18b** (b)

Table 2. Calculated kinetic constants describing the thermal $Z \rightarrow E$ of isomerisation of **18a, b**

Compound	E_{Act} (kJ·mol^{-1})	A (s^{-1})	$\Delta H^{\ddagger}$ (kJ·mol^{-1})	$\Delta S^{\ddagger}$ (J·mol^{-1}K^{-1})	$t_{1/2}$ at 37 °C (h)
18a	97.25	1.549 x 10^{11}	94.37	-40.22	30.30
18b	112.18	1.065 x 10^{13}	109.22	-5.30	144

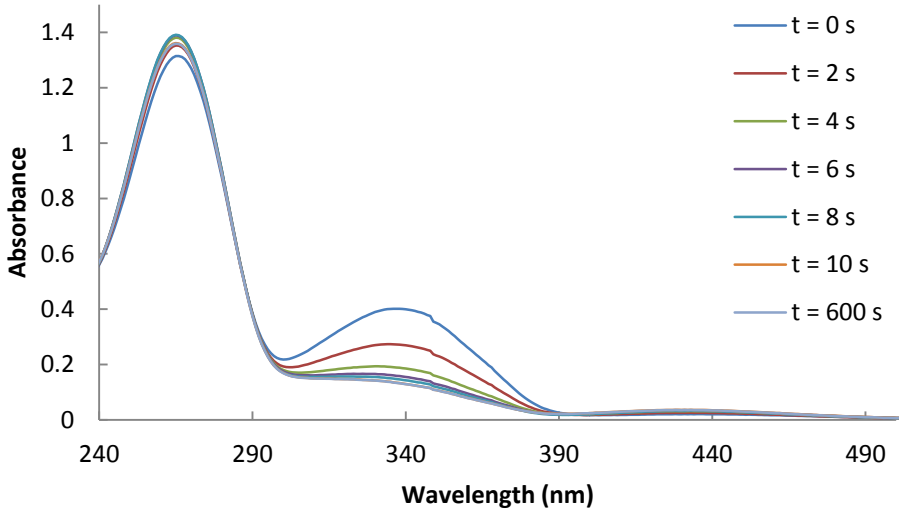


Figure 7. Overlaid UV/Vis spectra of **18a** upon irradiation at 366 nm for 0, 2, 4, 6, 8, 10 and 600 s

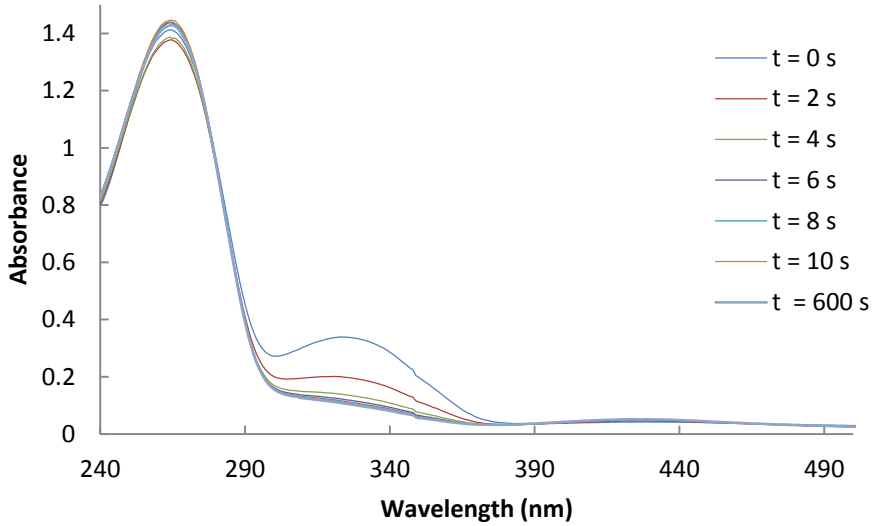


Figure 8. Overlaid UV/Vis spectra of **18b** upon irradiation at 366 nm for 0, 2, 4, 6, 8, 10 and 600 s

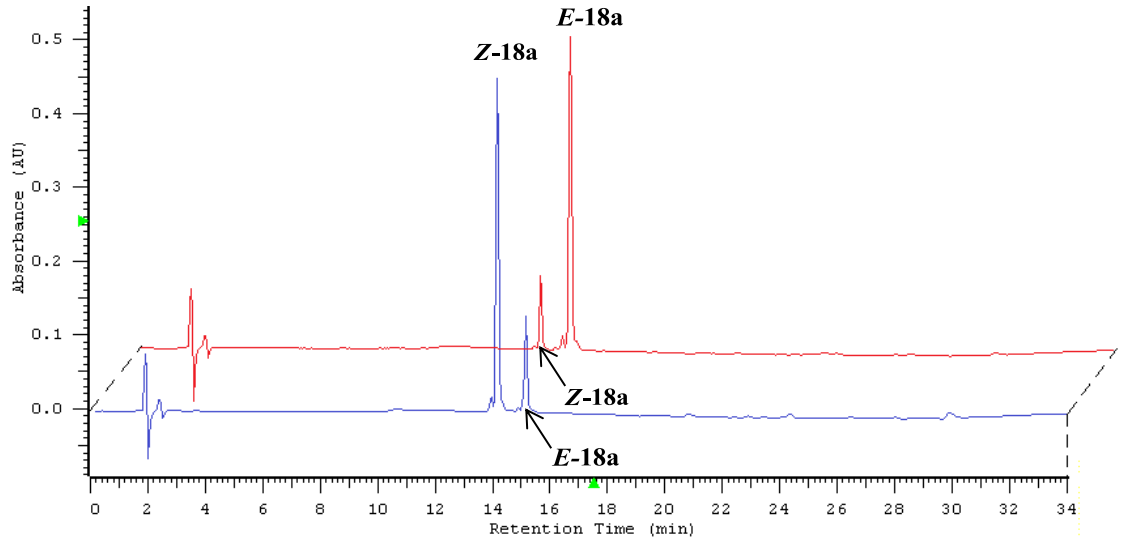


Figure 9. Overlaid HPLC traces of **18a** in the *Irr* (blue) and *DA* (red) photostationary states

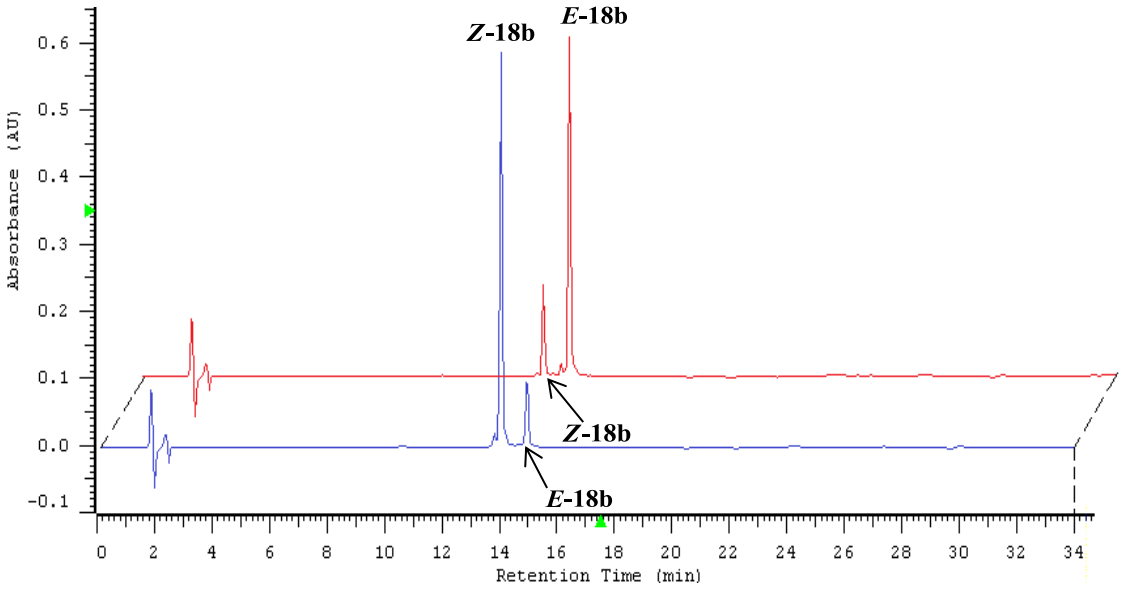


Figure 10. Overlaid HPLC traces of **18b** in the *Irr* (blue) and *DA* (red) photostationary states

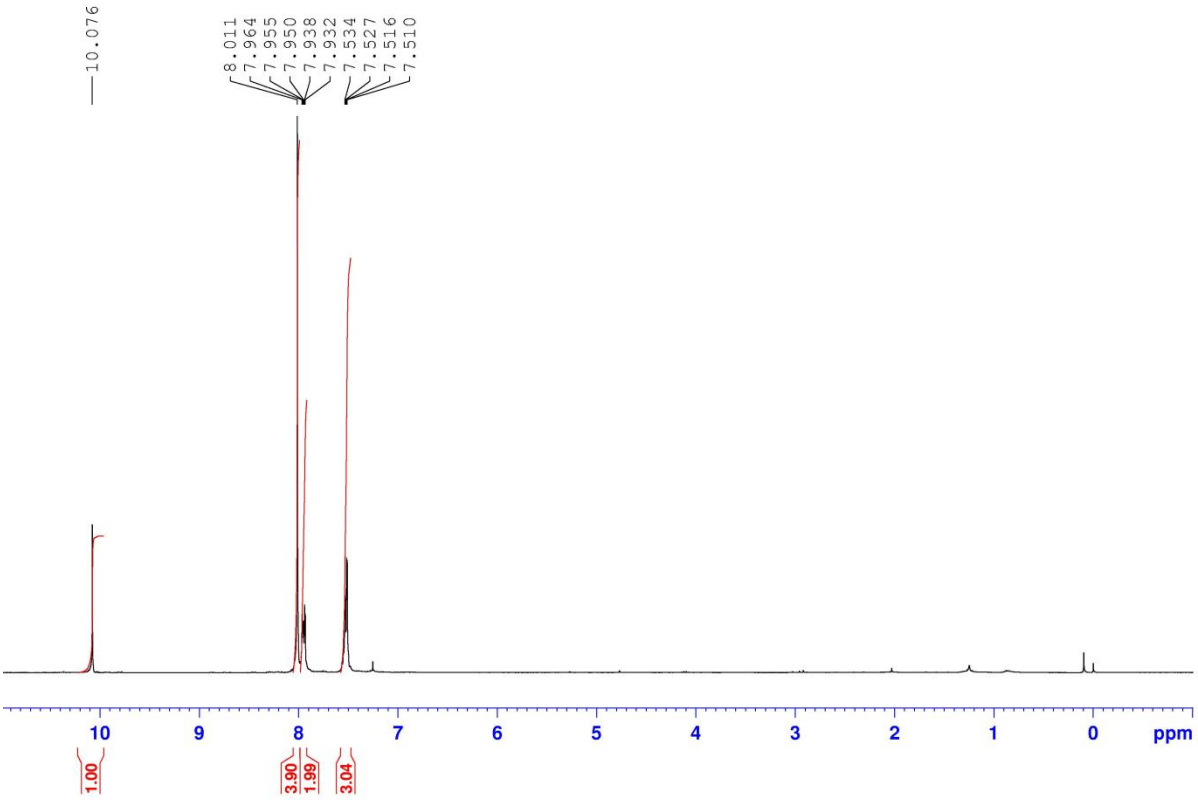


Figure 11. ^{1}H NMR spectrum of **2a**

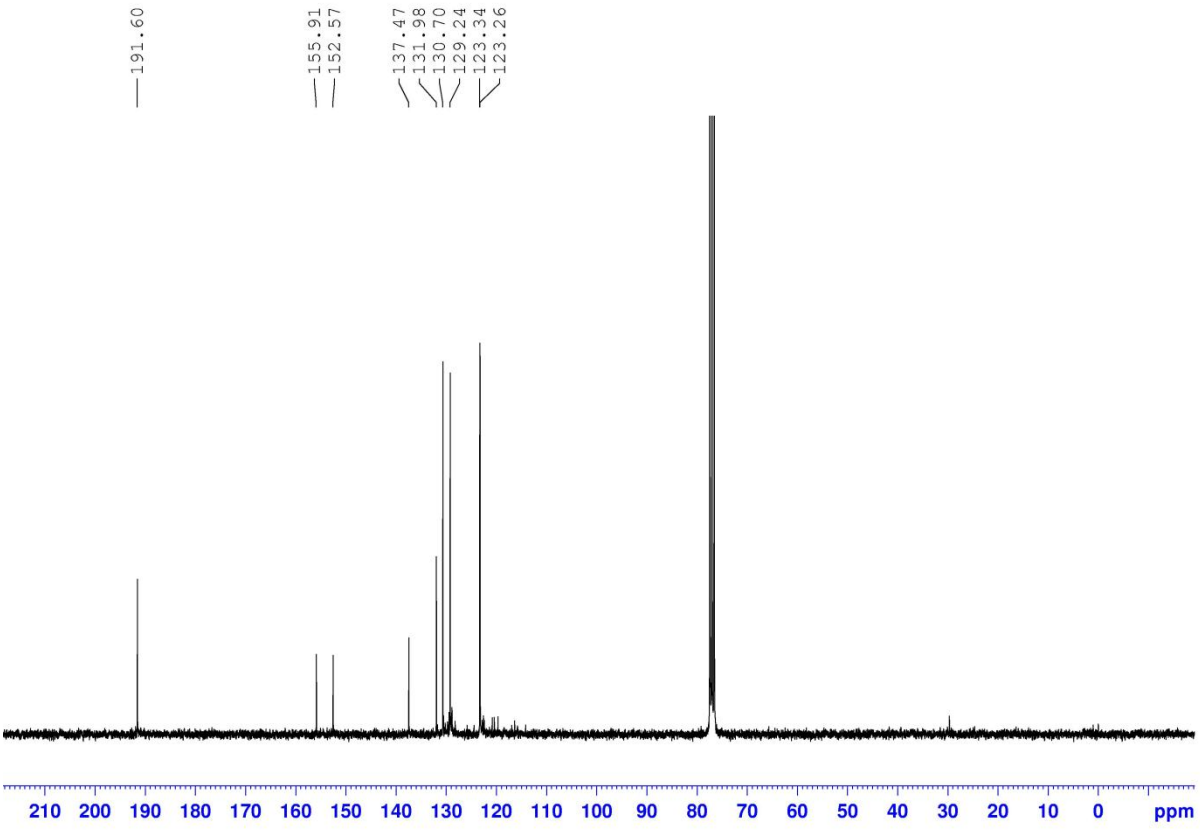


Figure 12. ^{13}C NMR spectrum of **2a**

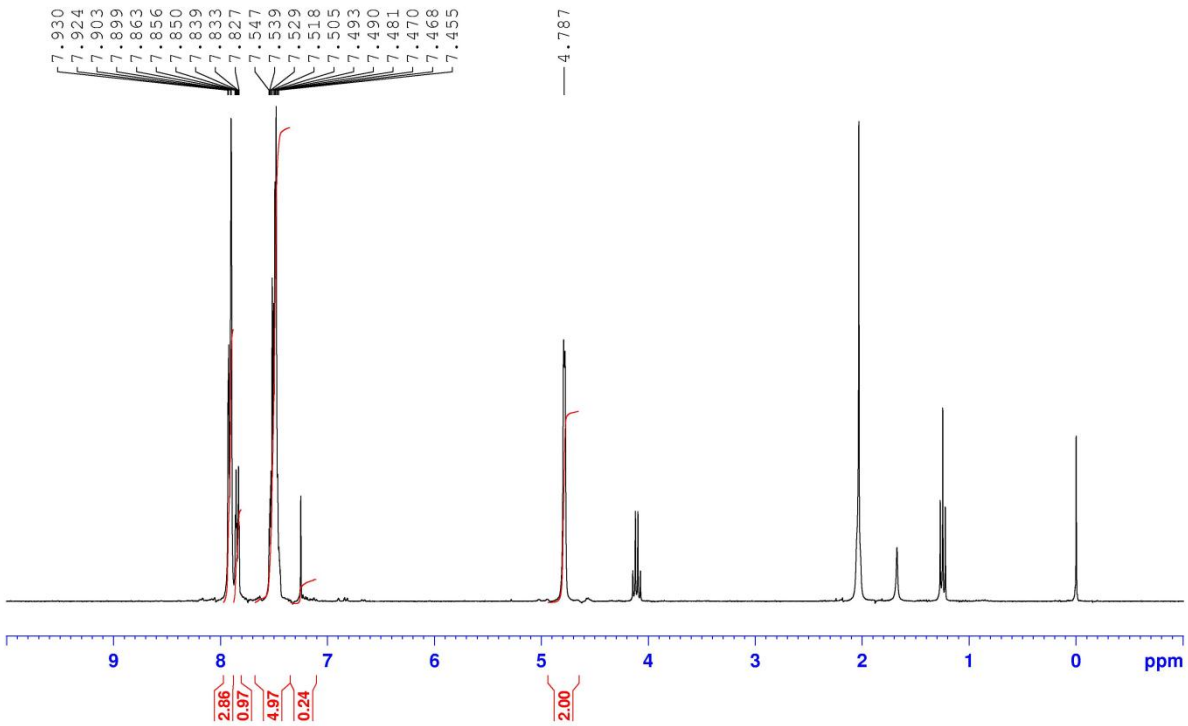


Figure 13. ^{1}H NMR spectrum of **1b**

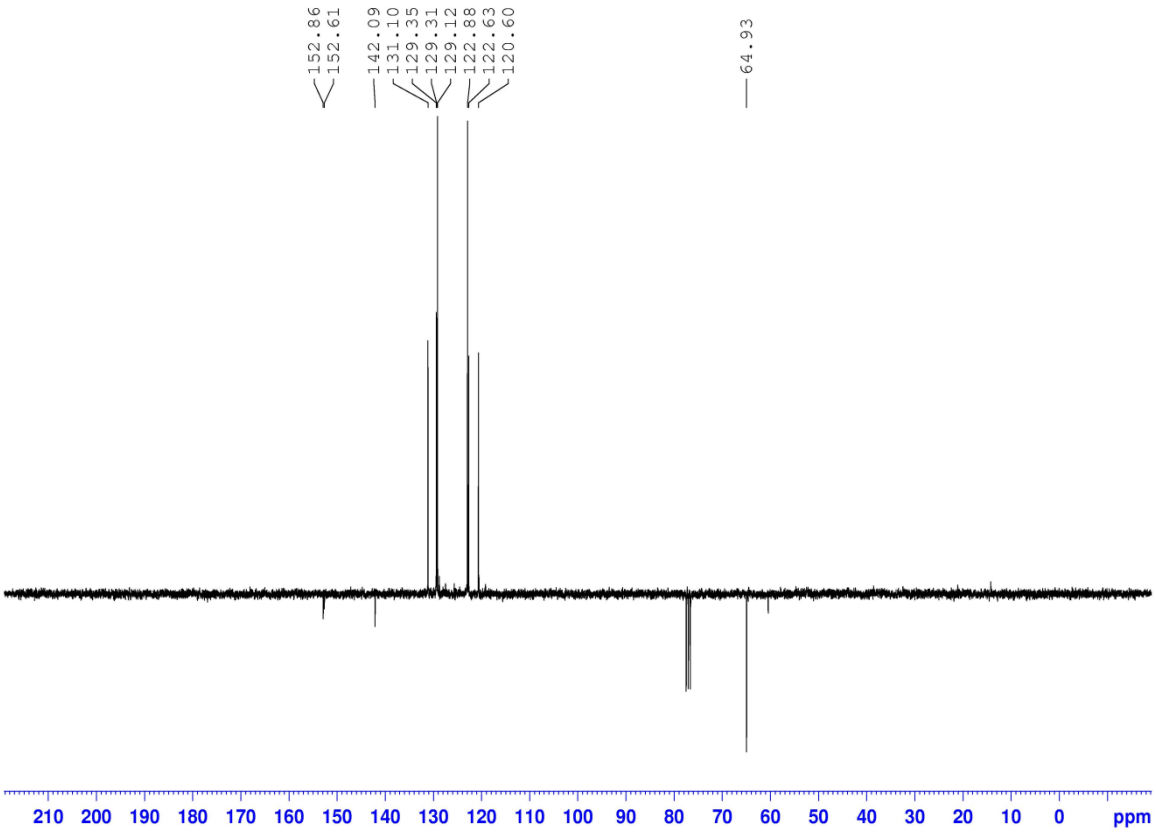


Figure 14. DEPTQ NMR spectrum of **1b**

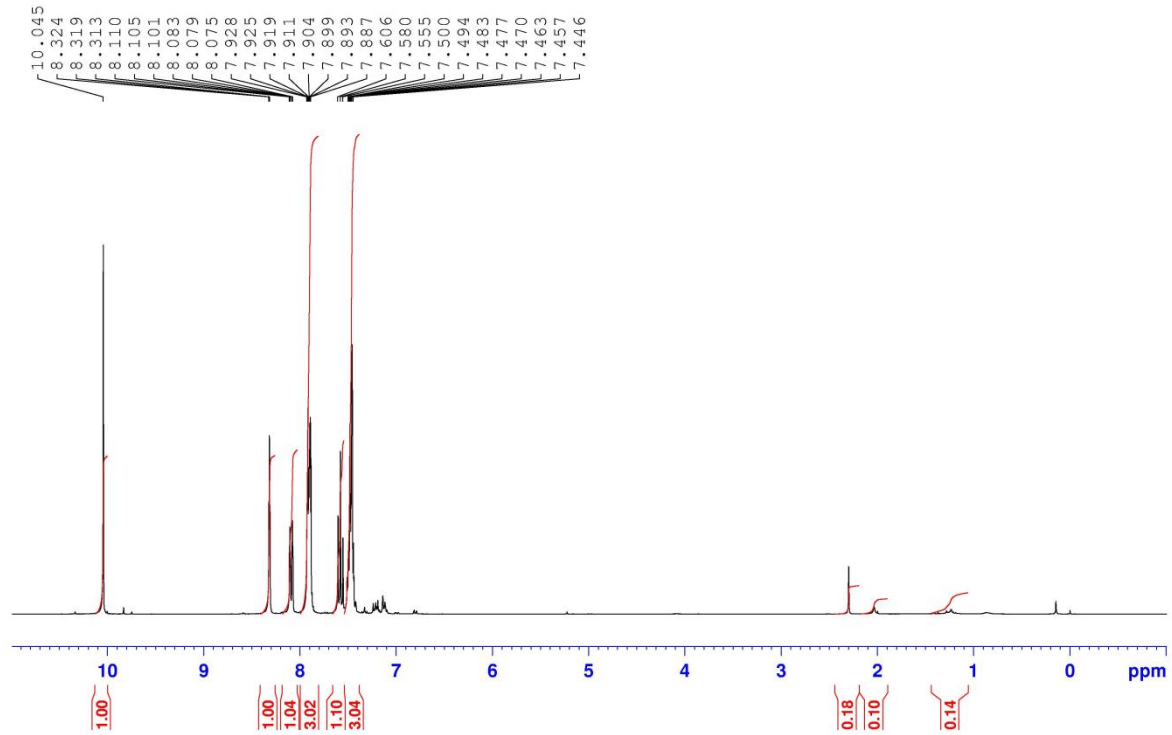


Figure 15. ^{1}H NMR spectrum of **2b**

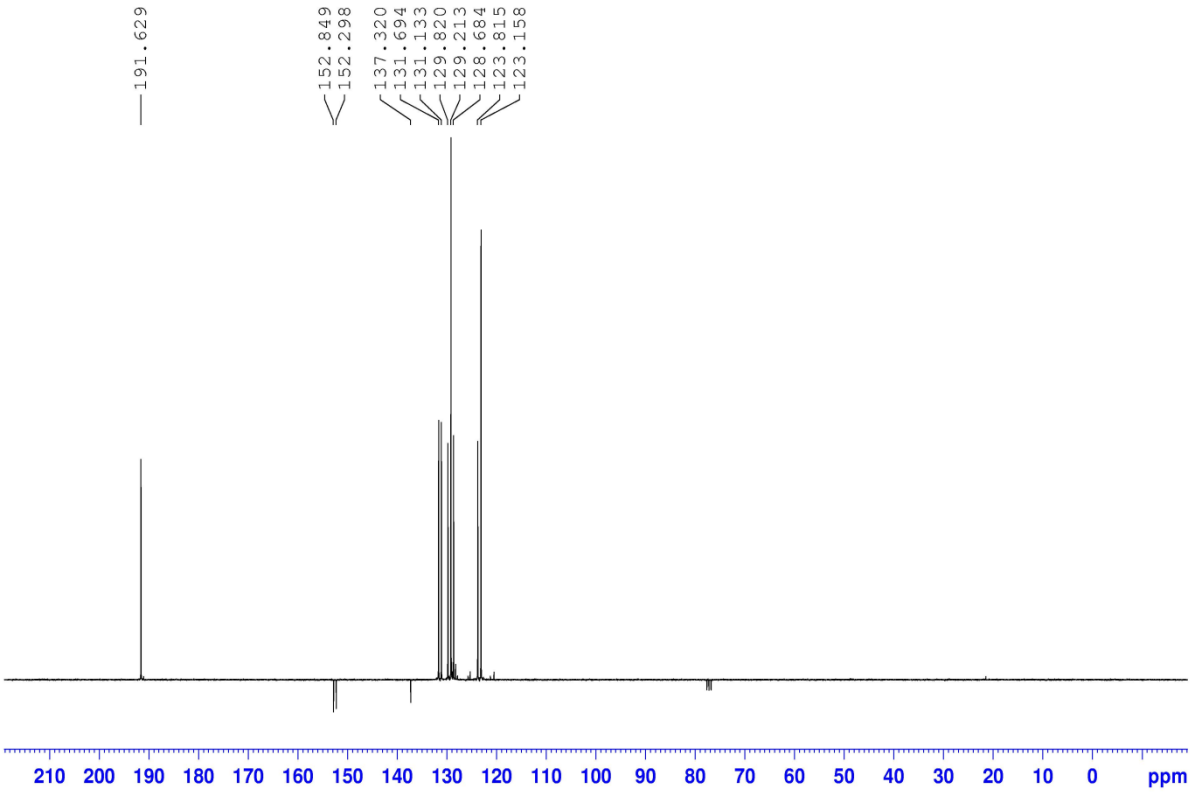


Figure 16. DEPTQ NMR spectrum of **2b**

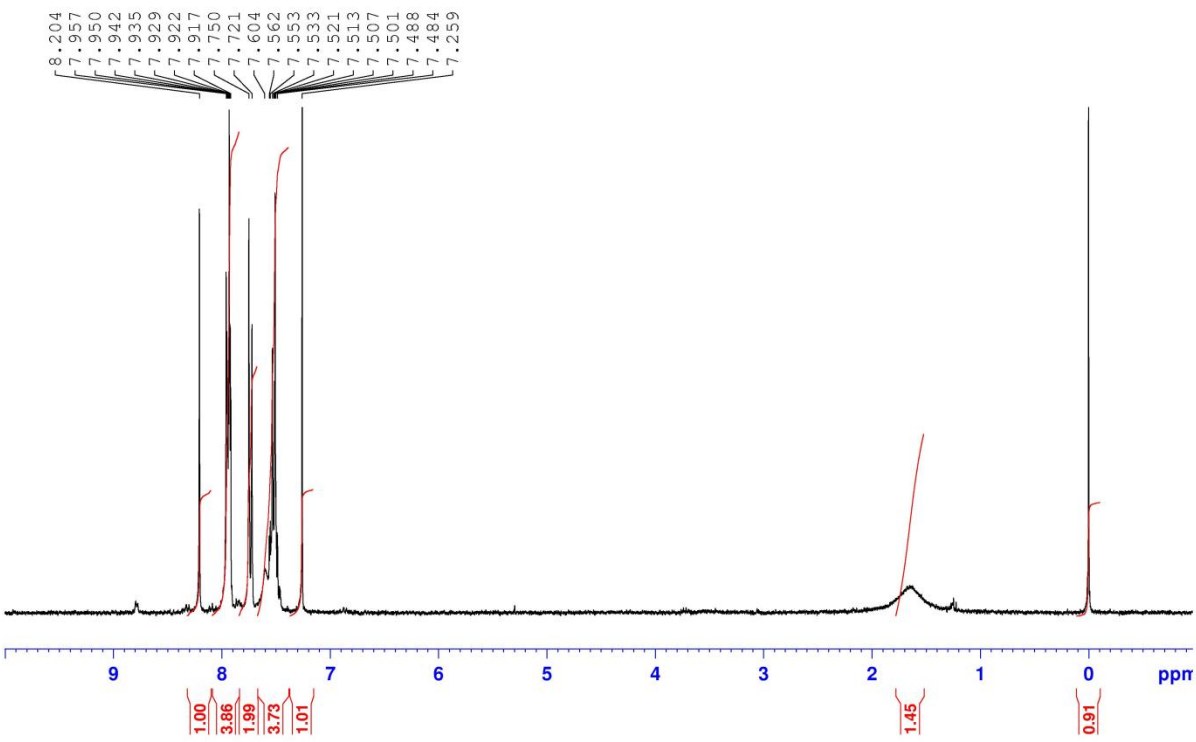


Figure 17. ^{1}H NMR spectrum of **3a**

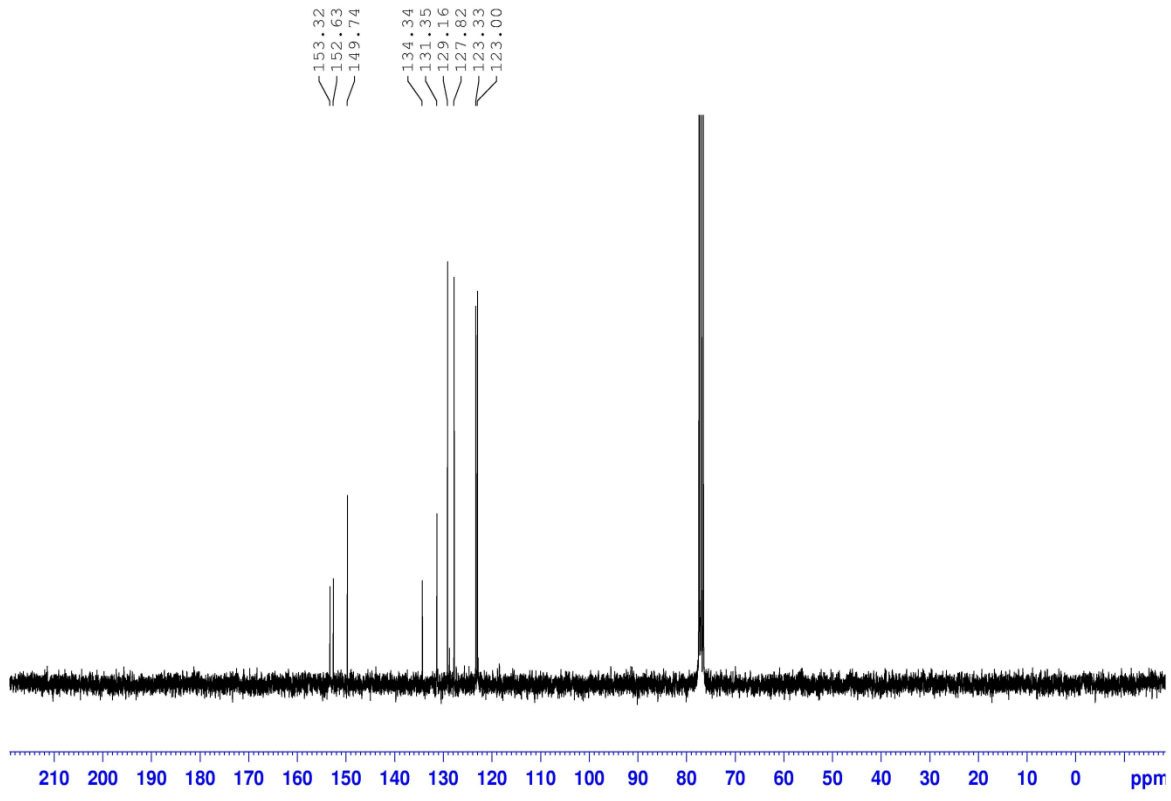


Figure 18. ^{13}C NMR spectrum of **3a**

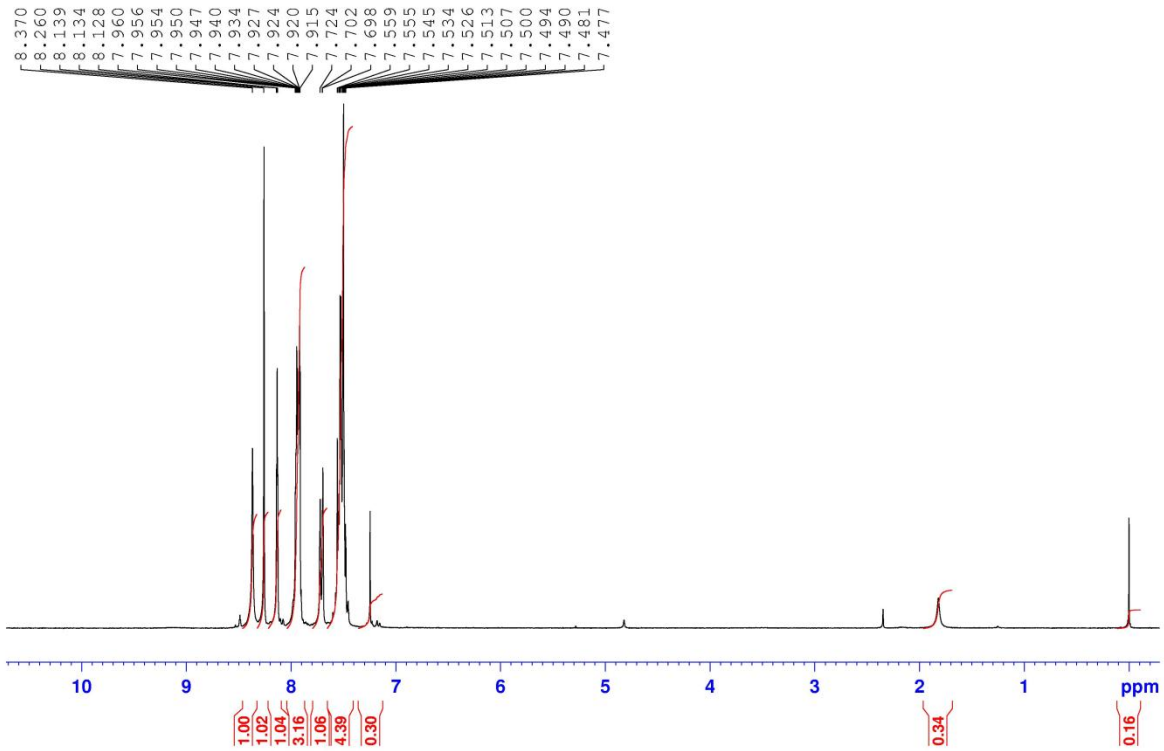


Figure 19. ^{1}H NMR spectrum of **3b**

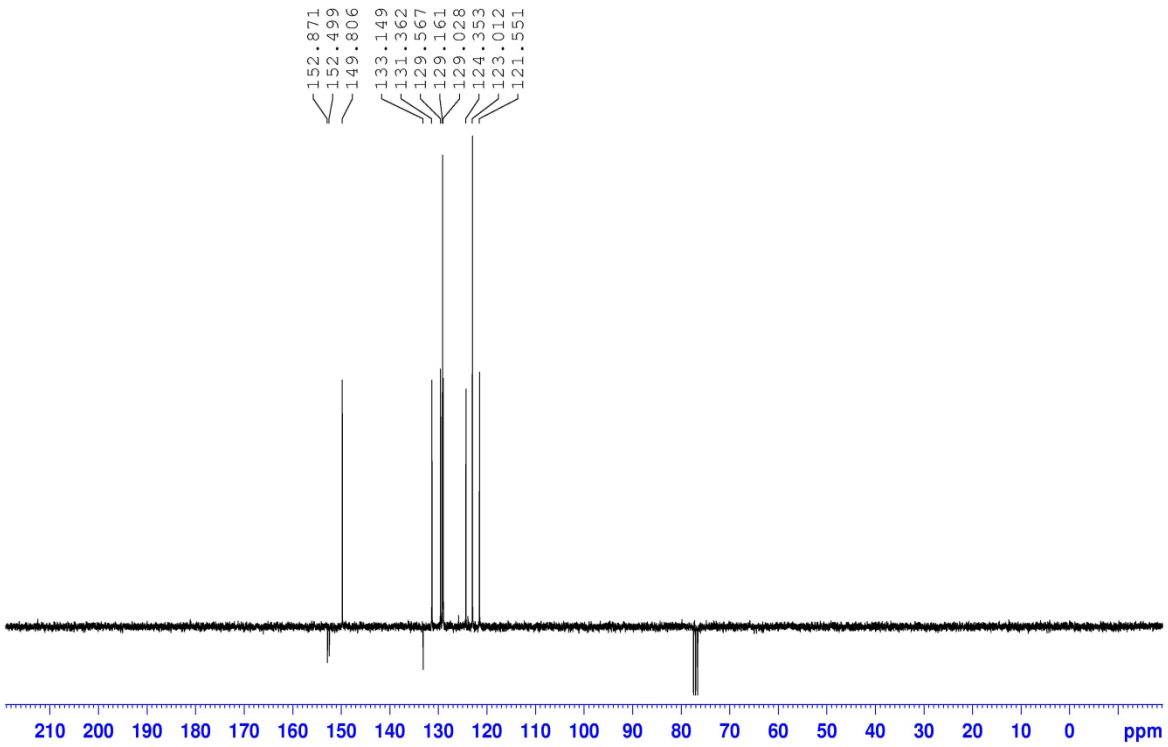


Figure 20. DEPTQ NMR spectrum of **3b**

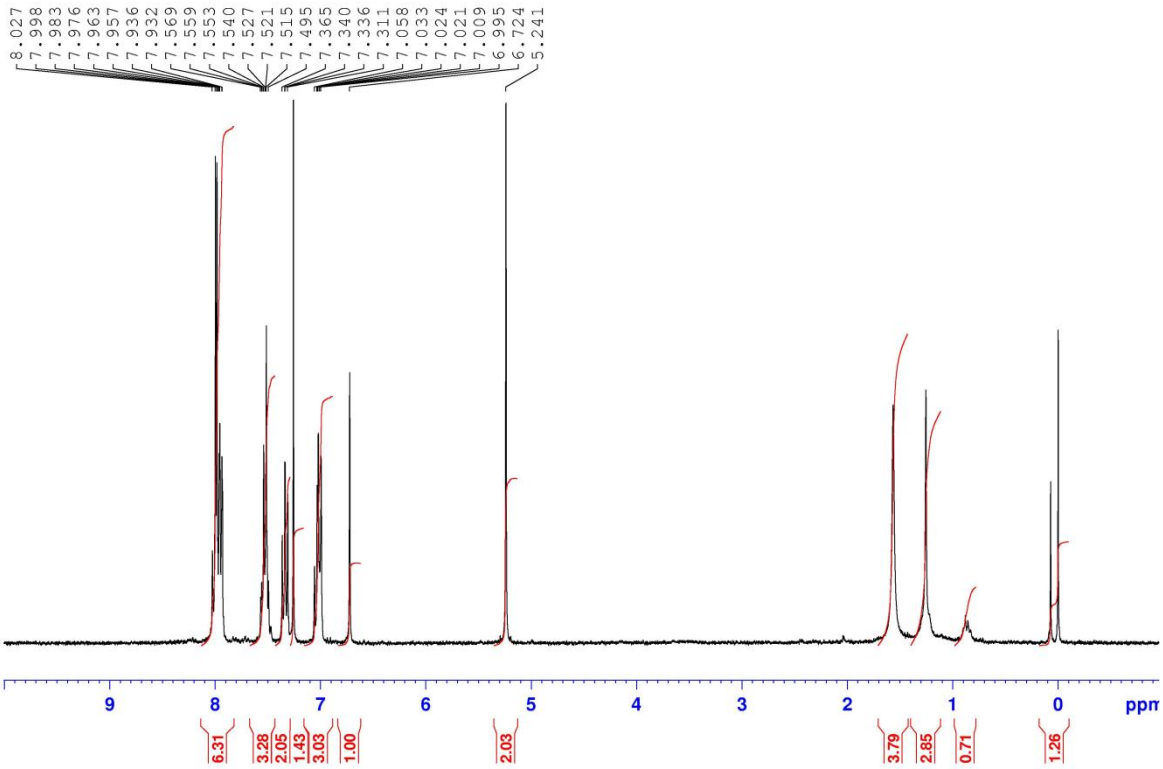


Figure 21. ^{1}H NMR spectrum of **5a**

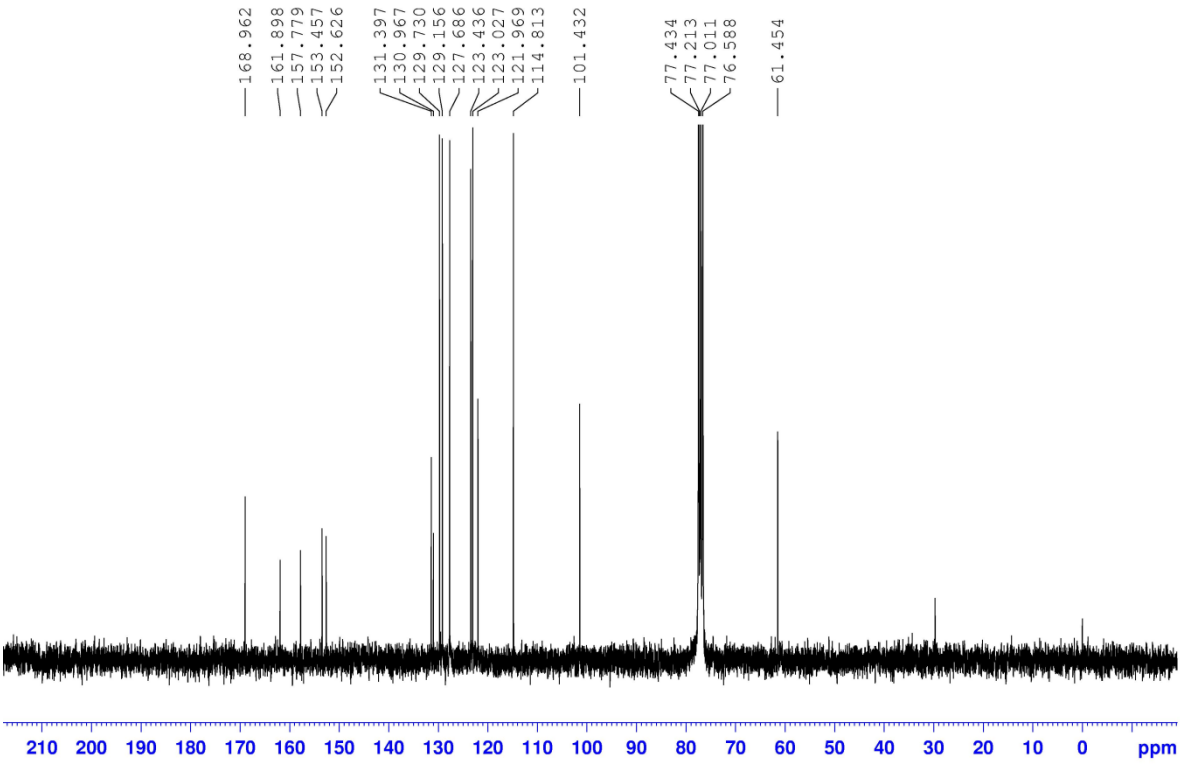


Figure 22. ^{13}C NMR spectrum of **5a**

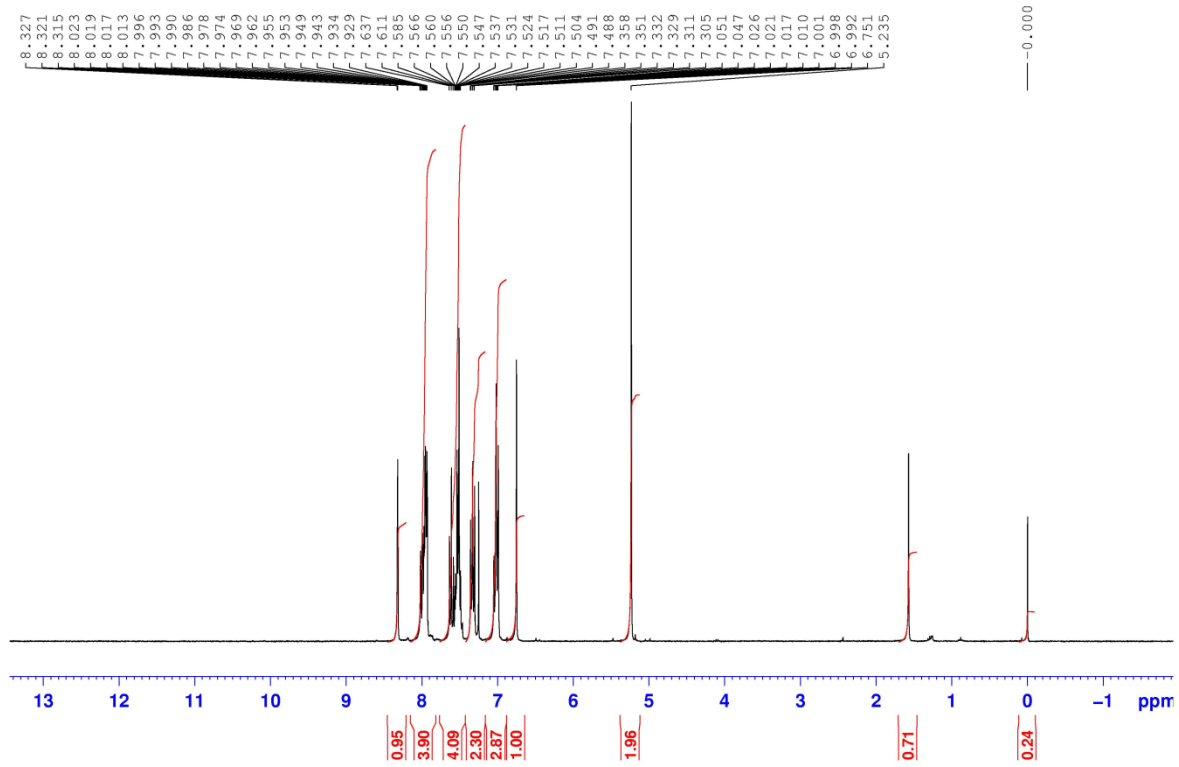


Figure 23. ^{1}H NMR spectrum of **5b**

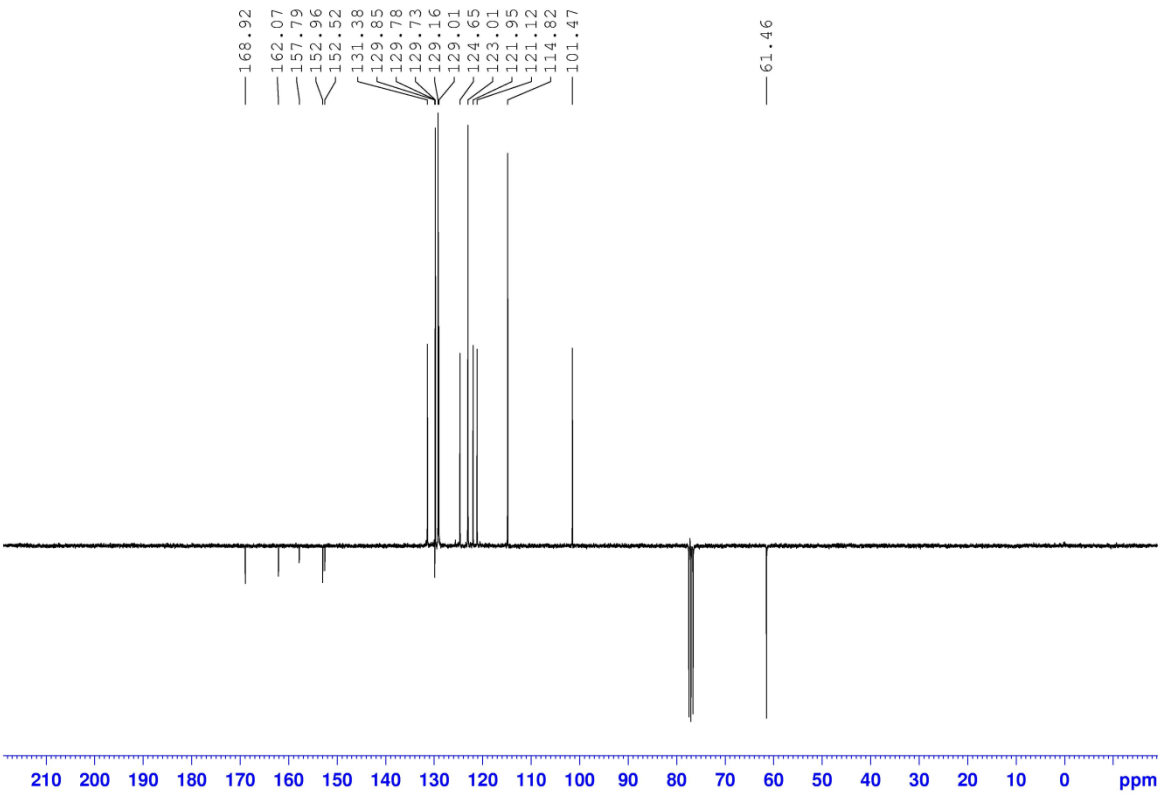


Figure 18. DEPTQ NMR spectrum of **5b**

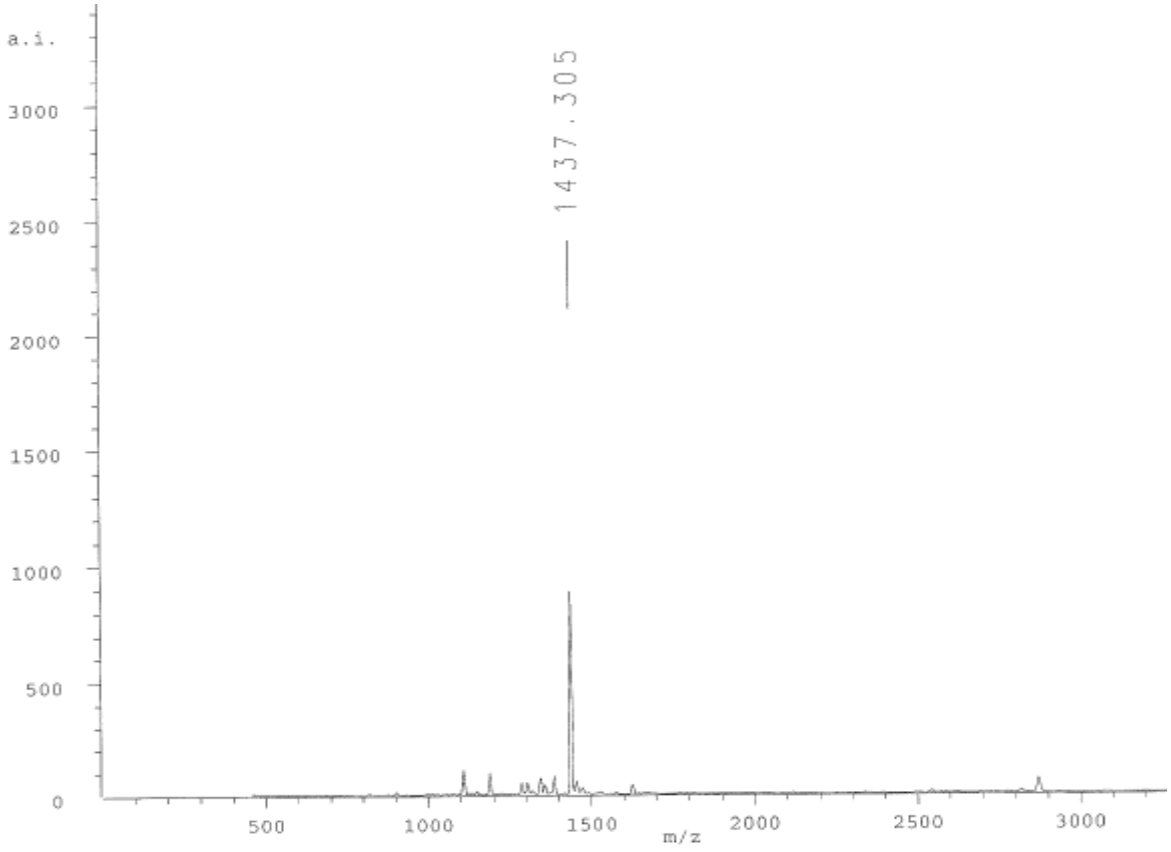


Figure 25. MALDI-TOF MS spectrum of **10a**

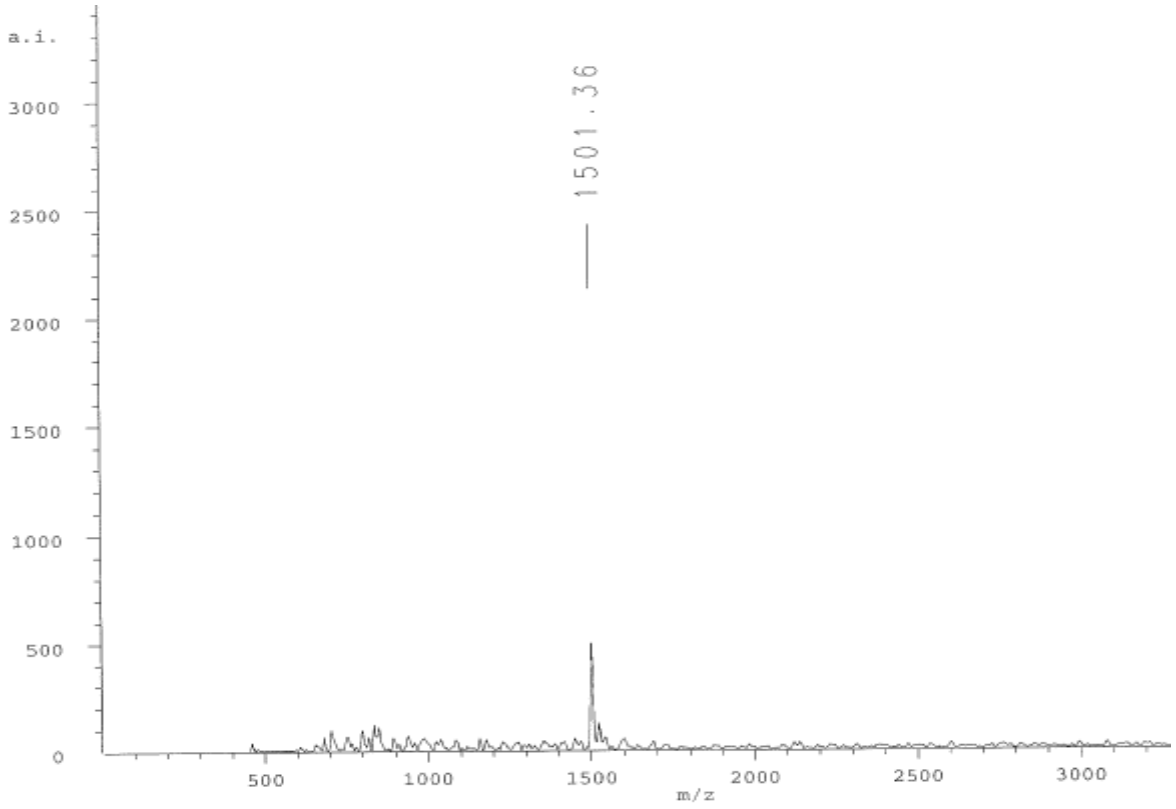


Figure 26. MALDI-TOF MS spectrum of HO-5′-**X**pd(Tp)$_3$dT-OH obtained from **13** following deprotection and cleavage from the resin

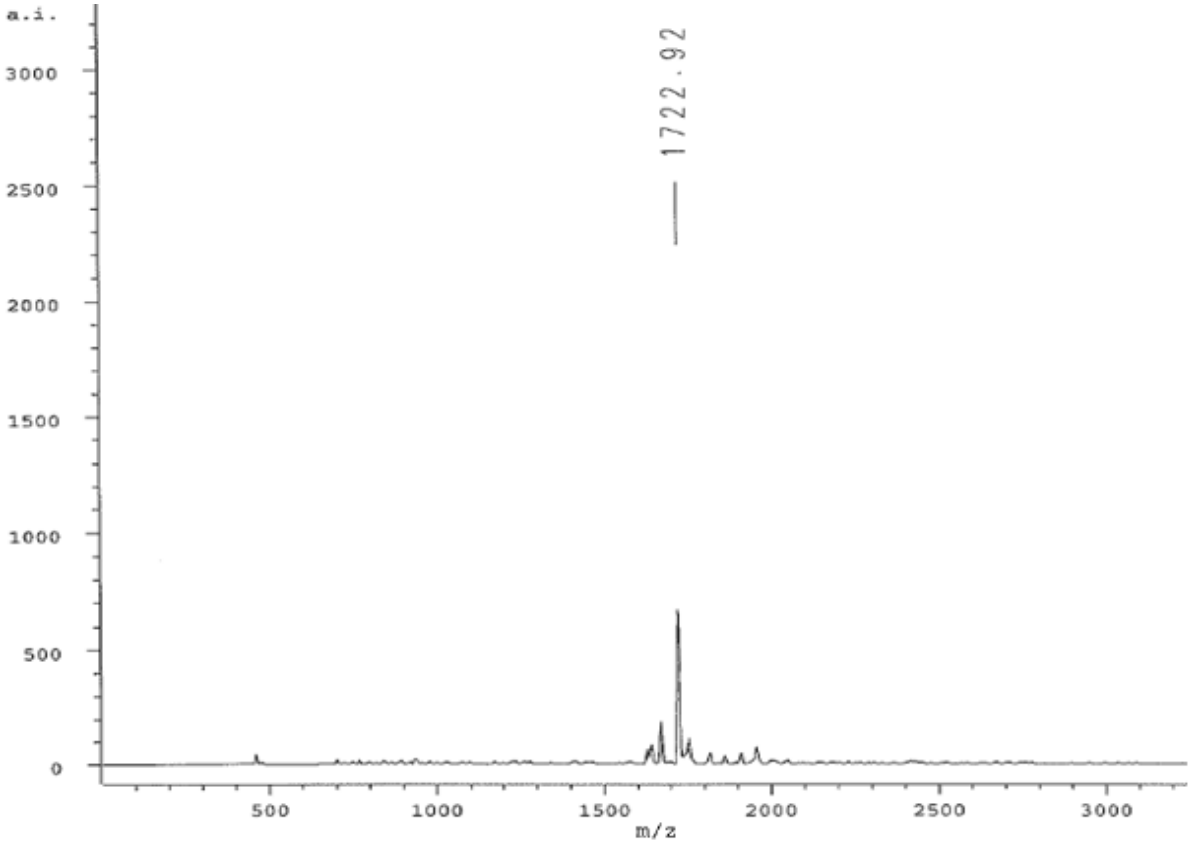


Figure 27. MALDI-TOF MS spectrum of **15a**

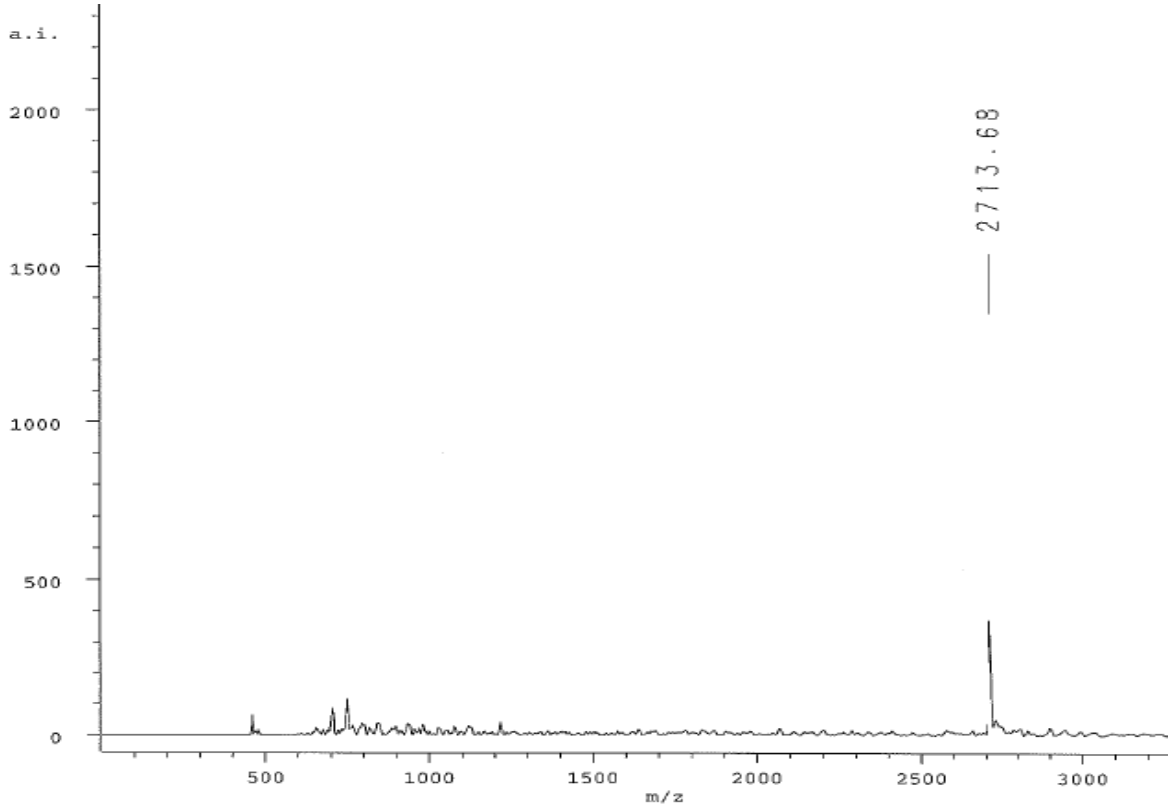


Figure 28. MALDI-TOF MS spectrum of HO-5′-d(Tp)$_4$**X**pd(Tp)$_3$dT-OH obtained from **16** following deprotection and cleavage from the resin

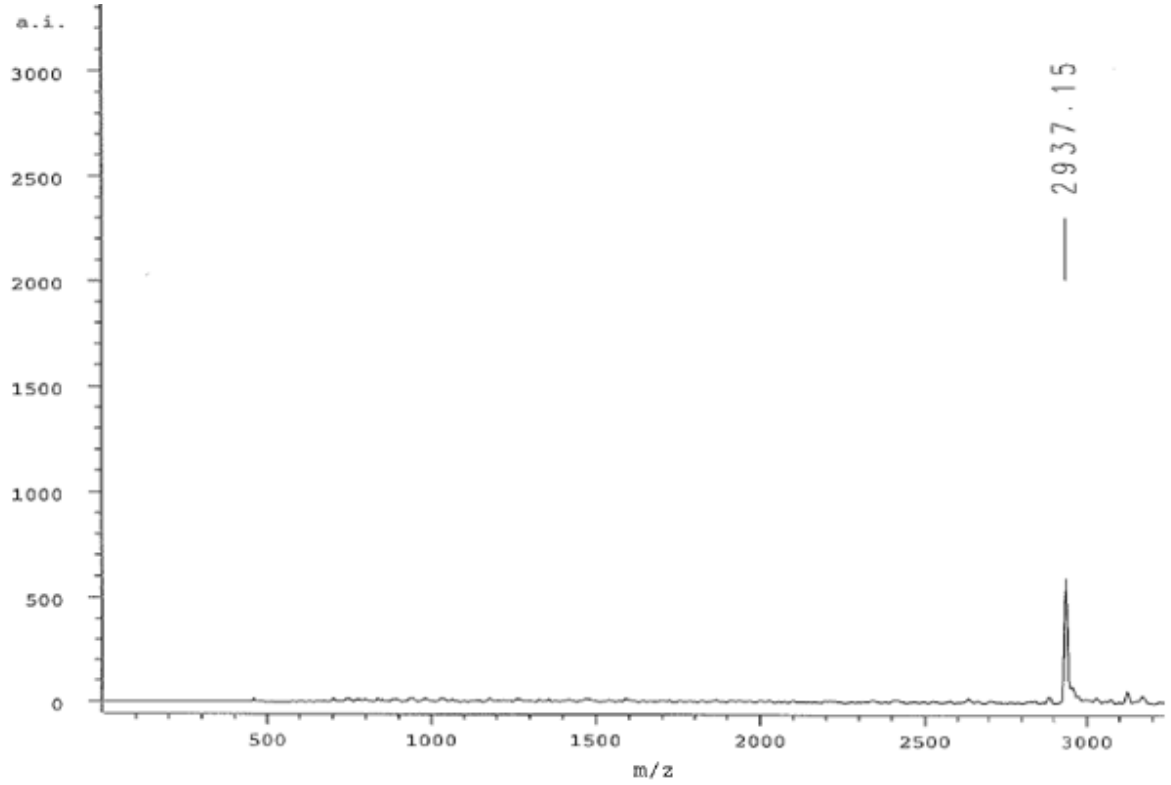


Figure 29. MALDI-TOF MS spectrum of **18a**

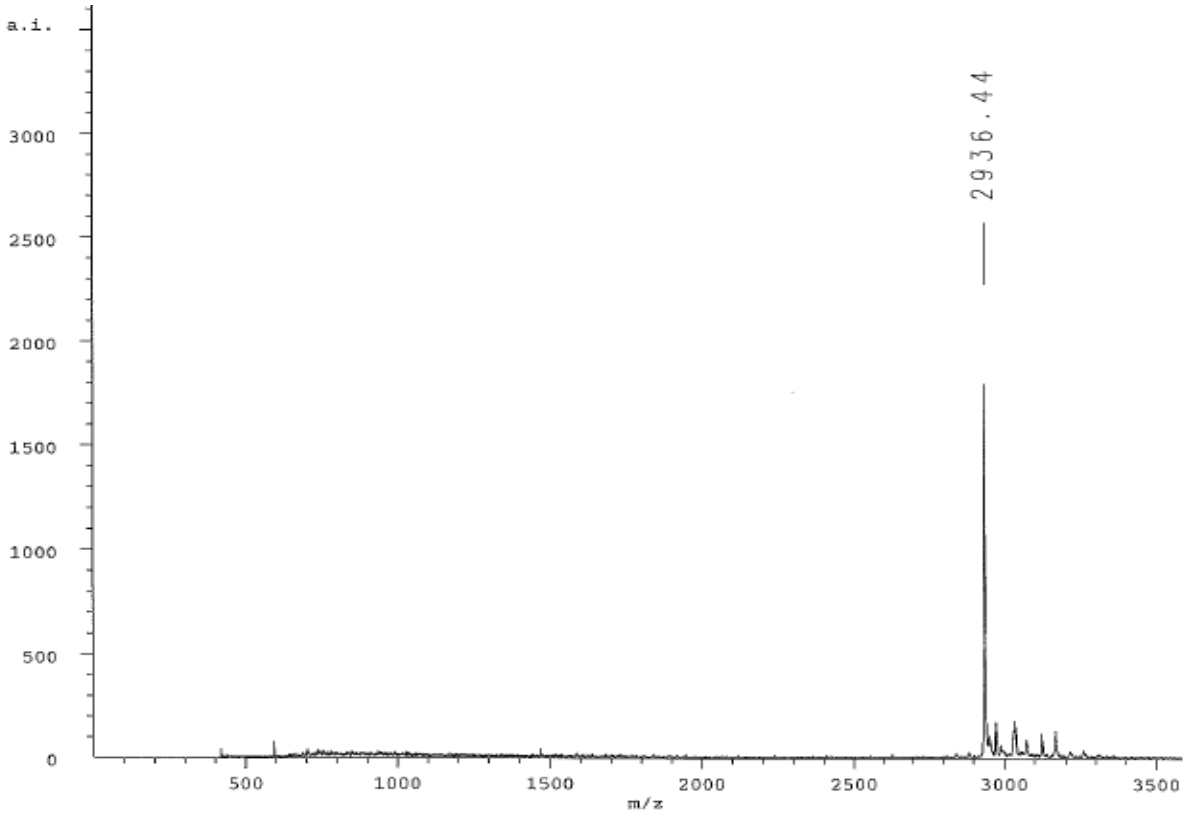


Figure 30. MALDI-TOF MS spectrum of **18b**

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2. Y. Kim, M. Koh, D. K. Kim, H. S. Choi and S. B. Park, *Journal of Combinatorial Chemistry*, 2009, **11**, 928-937.
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