Supporting Information

Fast, Copper-Free Click Chemistry, A Convenient Solid-Phase Approach to Oligonucleotide Conjugation

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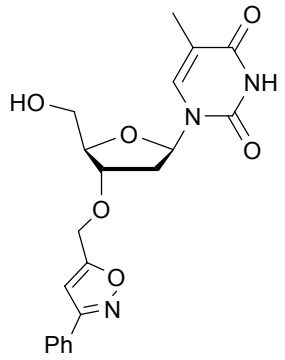
General experimental

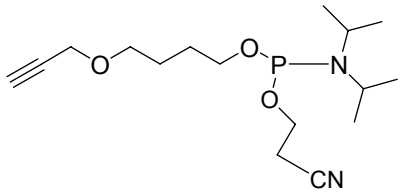
Analytical TLC was performed on precoated (250 μm) silica gel 60 F_{-254} plates from Merck. All plates were visualized by UV irradiation, and/or staining with 5% H_2SO_4 in ethanol followed by heating. Flash chromatography grade silica gel 60 (230-400 mesh) was obtained from Merck. Mass analysis was performed on an Ettan MALDI-TOF Pro from Amersham Biosciences or LASER-TOF LT3 from Scientific Analytical Instruments with 3-hydroxypicolinic acid or 2,' 4', 6'-trihydroxyacetophenone as matrix. The NMR spectra were obtained at ^{1}H (300 MHz), ^{13}C (75 MHz) and ^{31}P (121 MHz) on a Bruker instrument at 25 ºC. Chemical shifts are reported in ppm downfield from TMS as standard. HPLC was carried out using a Gilson instrument equipped with a UV detector and a Nucleosil C18 column (4.0 × 250 mm) or Phenomenex Clarity. Fluorescence spectra were recorded on a Varian Cary Eclipse instrument. All other chemical agents were purchased from Aldrich Chemical Company unless otherwise noted.



3'-O-[[3-(Phenylisoxazol-5-yl] methyl]thymidine 2

To a round bottomed flask containing benzaldehyde oxime (61 mg, 0.5 mM) and chloramine-T monohydrate (285 mg, 1.25 mM) in 4% aqueous sodium bicarbonate (3 ml) and ethanol (2 ml) was added 3'-*O*-propargylated thymidine **1**[1] (60 mg, 0.21 mM). The mixture was stirred for 12 hours at room temperature after which analysis by TLC indicated complete reaction. The product was isolated following extraction with ethyl acetate (3 x 50 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography over silica gel using dichloromethane and acetone (7:3) as eluant to give the title compound as an off-white solid in 88% yield (75 mg).

^{1}H NMR ($CDCl_3$) δ 7.81-7.78 (m, 2H), 7.46-7.44 (m, 3H), 7.38 (s, 1H), 6.59 (s, 1H), 6.13 (t, *J* = 7.2 Hz, 1H), 4.68 (d, *J* = 2.1 Hz, 2H), 4.40-4.37 (m, 1H), 4.15 (d, *J* = 2.7 Hz, 1H), 3.92 (dd, *J* = 2.7 and 11.7 Hz, 1H), 3.80 (dd, *J* = 3.0 and 11.7 Hz, 1H), 2.41-2.37 (m, 2H), 1.89 (s, 3H); ^{13}C NMR (CD_3COCD_3) δ 170.0, 163.3, 162.1, 150.5, 136.0, 130.0, 129.1, 128.9, 126.6, 109.9, 101.2, 85.0, 84.8, 80.3, 62.1, 61.7, 36.9, 11.6; HRMS (ESI) calcd for $C_{20}H_{22}N_3O_6$ 400.1509 $[M + H]^+$, found 400.1494.



4-(2-Propynyloxy)butan-1-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite, 4

4-(2-Propynyloxy)-1-butanol **3**[2] (100 mg, 0.78 mM), and benzylmercaptotetrazole (76 mg, 0.39 mM) were placed in a dried round bottomed flask under an argon atmosphere. Acetonitrile (5 ml) was added to the flask followed by diisopropylamine (56 µl, 0.39 mM) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (275 µl, 0.85 mM). The reaction mixture was stirred for 30 minutes at room temperature after which TLC analysis showed complete consumption of starting alcohol. The reaction mixture was diluted with ethyl acetate (25 ml) and washed with aqueous sodium bicarbonate (10 x 3 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give the crude alkyne phosphoramidite **4** (240 mg, 98%) which was used without purification.

^{31}P NMR (DMSO-d_6) δ 146.3.

Automated oligonucleotide synthesis

Automated oligonucleotide synthesis was performed on an Expedite 8909 DNA/RNA synthesizer on a 1.0 μmol scale using standard reagents from Link Technologies and standard coupling cycles except that double couplings of dG phosphoramidites were performed: benzylmercaptotetrazole (0.2 M in MeCN) was used as activating agent; oxidation was performed using 8:1:1 THF:pyridine:H_2O containing 0.02 M I_2, cleavage from CPG was performed with aq. methylamine at 65 °C for 30 minutes. The spin-filtered supernatant was buffered with 0.5 ml of 0.1 M TEAAc, pH 6.5, 5% (v/v) MeCN and the solution concentrated in vacuo. The crude oligonucleotides were analysed and purified using RP-HPLC (column: Phenomenex Clarity Oligo-RP 5C18); 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) eluting at 1 ml min^{-1} using a gradient consisting of: 0-5 min, 5% B; 5-35 min, 50% B; 38-48 min, 100% B; 48-58 min, 5% B. Absorbance was monitored in the range 220 – 500 nm with a diode array detector and recorded at 260 nm.

After reduction of the appropriate fractions by *ca.* 80%, the oligonucleotides were desalted using standard SepPak protocols (Waters) and concentrations determined using UV absorbance at 260 nm. Molar extinction coefficients of oligonucleotides were calculated from the nearest neighbour model.[3] The molar extinction coefficient of the mononucleotide **9c** was determined to be 13800 $M^{-1}cm^{-1}$.

Melting temperature determinations

Melting temperatures were determined using UV-spectroscopy (Cary100 Scan) measuring the absorbances at 260 nm of degassed solutions of oligonucleotides (5μM of each oligomer in 100

mM NaCl, 10 mM Na-phosphate, 1 mM Na.EDTA, pH7.0 – 500 μl) every 0.5 °C between 15 and 95 °C, using a gradient of 1 °C min^{-1} and this was repeated at least 3 times. Prior to recording the data, the solutions were heated to 80 °C and allowed to cool slowly to ambient temperature.

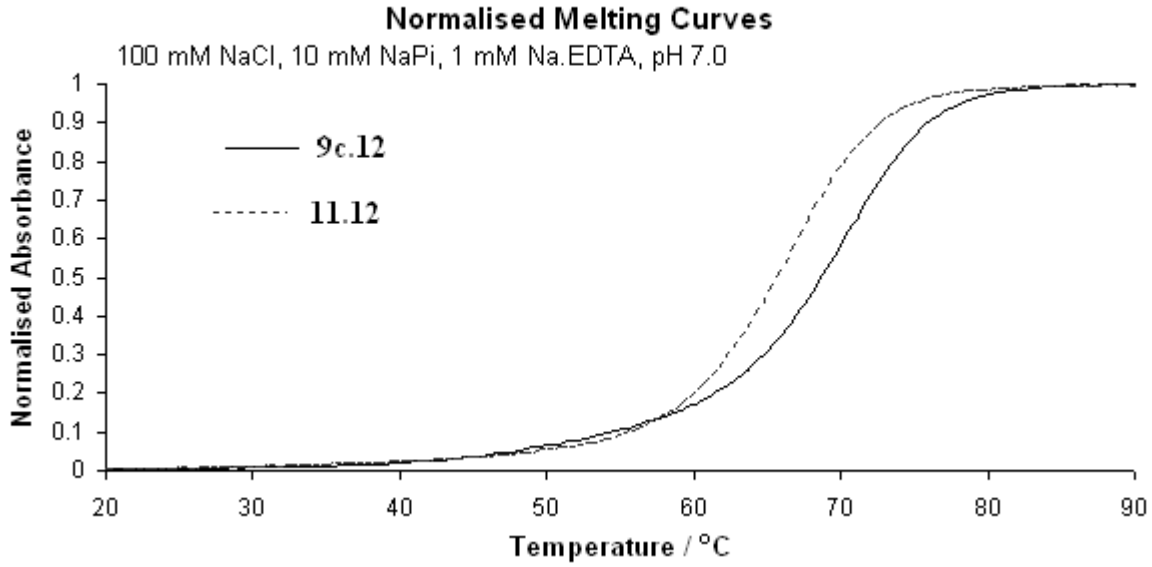


Figure 1. Hybridisation studies on **9c** with the complimentary strand **12**

DNA 11 5'-TCG CAC ACA CGC-3'

DNA 12 5'-GCG TGT GTG CGA-3'

General procedure for the phosphitylation of CPG-DNA(–OH 5'), preparation of 6a, 6b and 6c.

To manually couple the alkyne phosphoramidite **4** to the nucleotide/oligonucleotide 500 μL of **4** (100 mM in anhydrous CH_3CN) was added to the CPG-DNA (1 μmol) along with 500 μL of benzylmercaptotetrazole in CH_3CN (0.3 M). The mixture was reacted for 15 min at RT with mixing between two syringes. This reaction was repeated with a second portion of each of a new solution of phosphormamidite **4** and benzylmercaptotetrazole. The CPG was washed with CH_3CN (5 x 2 ml), oxidizer (700 μL, 0.1 M Iodine solution in THF: pyridine: water; 78:20:2) and CH_3CN (2 x 5 mL) and dried yielding CPG-DNA-alkynes **6a**, **6b** and **6c**.

General deprotection procedure

For analytical purposes a portion of the DNA was deprotected and cleaved from the CPG by incubating the CPG-DNA in 40% aqueous CH_3NH_2 (500 μL) at 65^{o}C for 30 minutes. The CH_3NH_2 was evaporated using a concentrator. The CPG was washed with H_2O (5 x 200 μL aliquots), all solutions and washings were combined to afford an aqueous solution of DNA-alkynes **7a**, **7b** and **7c**.

General method for HPLC analysis

DNA-alkynes **7a-c** were analyzed by reverse-phase HPLC with an analytical column (Nucleosil) under the following conditions; 200 μL injection loop; 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; 5-35 min, 50% B; 38-48 min, 100% B; 48-58 min, 5% B flow rate: 1.0 mL/min and detection at 260 nm.

MALDI-TOF-MS analysis of **7a** (*m/z*: 449 $[M+NH_4]^+$; found 449), **7b** (*m/z*: 3209 $[M+K]^+$; found 3211) and **7c** (*m/z*: 3806 $[M+K]^+$; found 3814)

General procedure for benzonitrile oxide click reactions on CPG-Thymidine-alkyne 6a and CPG-T_{10}-alkyne 6b

To solid supported thymidine-alkyne **6a** or CPG-DNA-alkyne **6b** (1 µM) in an eppendorf tube was added a solution of benzaldehyde oxime (48 mg) in ethanol (160 µL) followed by 4% aqueous $NaHCO_3$ (500 µL) and chloramine-T monohydrate (72 mg). The mixture was agitated at room temperature for 30 min. Following settling the supernatant liquid was removed by syringe and the CPG washed firstly with CH_3CN (5 x 300 µL) and then H_2O (5 x 300 µL). Deprotection and HPLC analysis followed by the procedures described above. MALDI-TOF-MS of **9a** (*m/z*: 573 $[M+Na]^+$; found 576) and **9b** (*m/z*: 3330 $[M+K]^+$; found 3331).

Control reaction between in situ generated benzonitrile oxide and CPG-T_{10} 5b

To solid supported thymidine **5b** (1 µM) in an eppendorf tube was added a solution of benzaldehyde oxime (48 mg) in ethanol (160 µL) followed by 4% aqueous $NaHCO_3$ (500 µL) and chloramine-T monohydrate (72 mg). The mixture was agitated at room temperature for 30 min. Following settling the supernatant liquid was removed by syringe and the CPG washed firstly with CH_3CN (5 x 300 µL) and then H_2O (5 x 300 µL). Deprotection and HPLC analysis followed by the procedures described above.

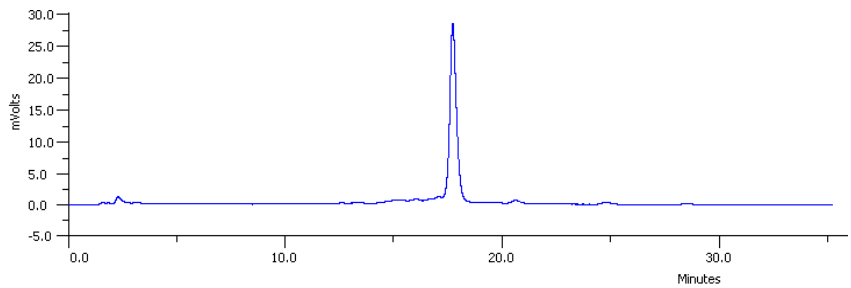


Figure 2. Reversed-phase HPLC trace of crude material obtained following deprotection and cleavage of ON from CPG-T_{10} having been exposed to the nitrile oxide alkyne click reaction conditions (UV absorbance at 260 nm vs time).

Procedure for benzonitrile oxide click reaction on CPG-heterooligonucleotide-alkyne 6c

To solid supported DNA-alkyne **6c** (0.2 μM) in an eppendorf tube was added a solution of benzaldehyde oxime (31 mg) in ethanol (330 μL) followed by 4% aqueous $NaHCO_3$ (670 μL) and chloramine-T monohydrate (114 mg). The mixture was agitated at room temperature for 10 min. Workup, deprotection and HPLC analysis followed by the procedures described above.

MALDI-TOF-MS **9c** (*m/z*: 3925 $[M+K]^+$; found 3934).

Procedure for naphthalene-1-nitrile oxide click reaction on CPG-T_{10}-alkyne 6b

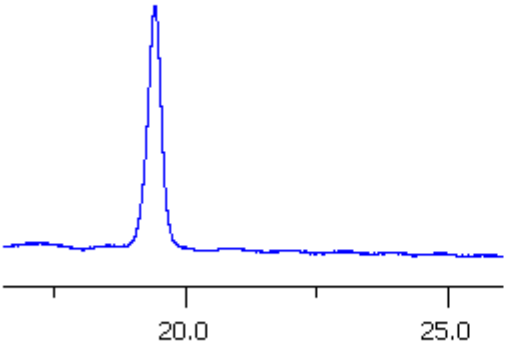
To solid supported DNA-alkyne **6b** (0.37 μM) in an eppendorf tube was added a solution of 1-naphthylaldehyde oxime (43 mg) in ethanol (500 μL) followed by 4% aqueous $NaHCO_3$ (500 μL) and chloramine-T monohydrate (114 mg). The mixture was agitated at room temperature for 12 hours. Workup, deprotection and HPLC analysis followed by the procedures described above.

MALDI-TOF-MS of **10a** (*m/z*: 3363 $[M+Na]^+$; found 3362.4).

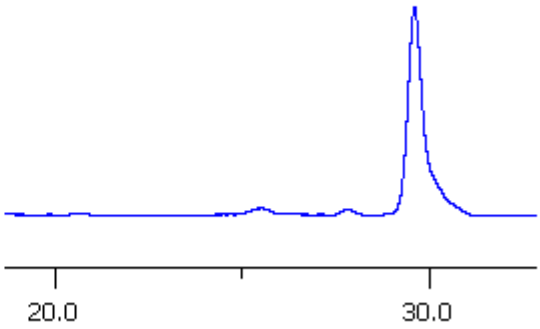
Procedure for anthracene-9-nitrile oxide click reaction on CPG-T_{10}-alkyne 6b

To solid supported DNA-alkyne **6b** (0.5 μM) in an eppendorf tube was added a solution of 9-anthraldoxime (45 mg) in ethanol (600 μL) followed by 4% aqueous $NaHCO_3$ (400 μL) and chloramine-T monohydrate (97 mg). The mixture was agitated at room temperature for 18 hours. Workup, deprotection and HPLC analysis followed by the procedures described above.

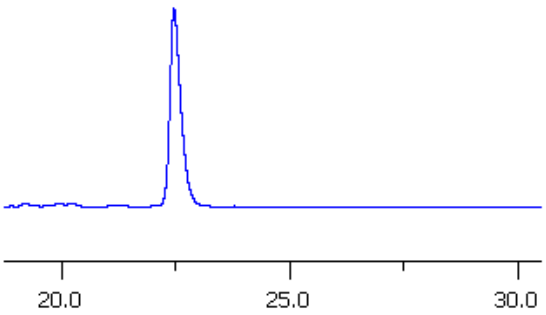
MALDI-TOF-MS of **10b** (*m/z*: 3430 $[M+K]^+$; found 3433).



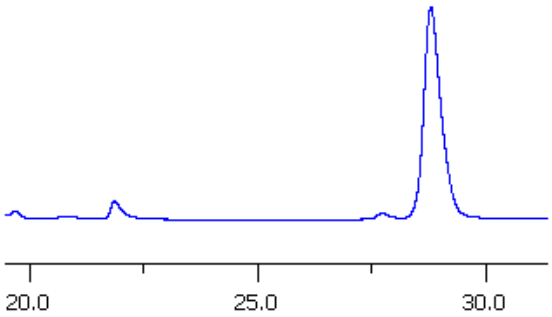
(a)



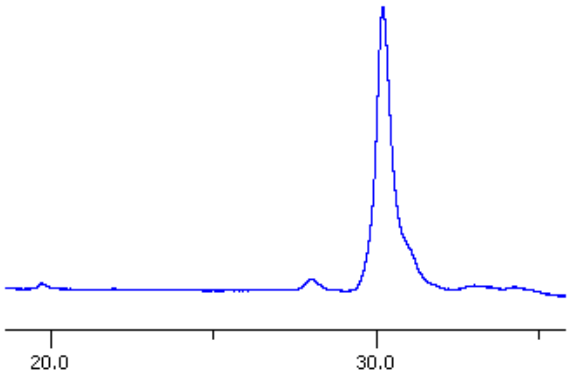
(b)



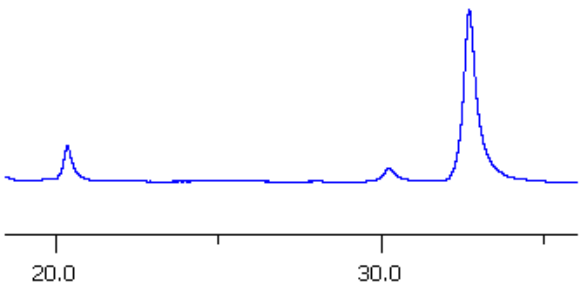
(c)



(d)



(e)



(f)

Figure 3. Reversed-phase HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time). (a) thymidine-alkyne, **7a**, (b) thymidine-isoxazole **9a**, (c) T_{10}-alkyne **7b**, (d) T_{10}-isoxazole **9b**, (e) T_{10}-isoxazole **10a**, (f) T_{10}-isoxazole **10b**.

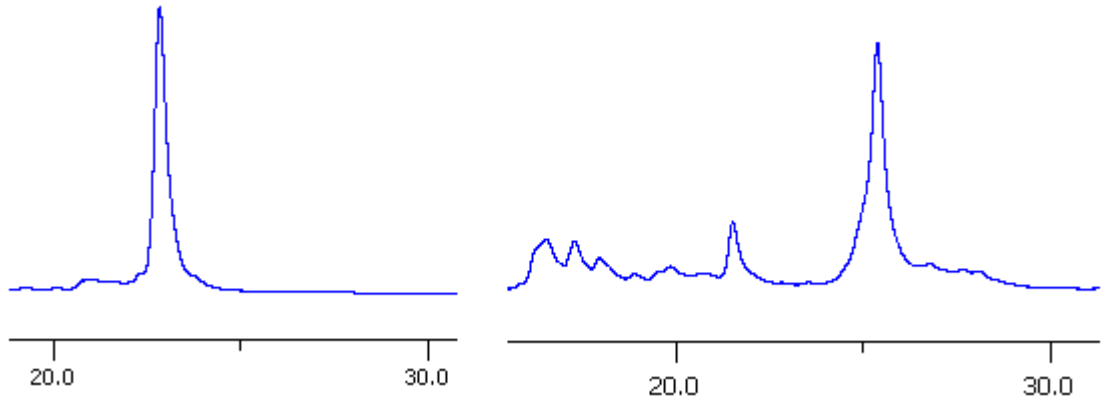
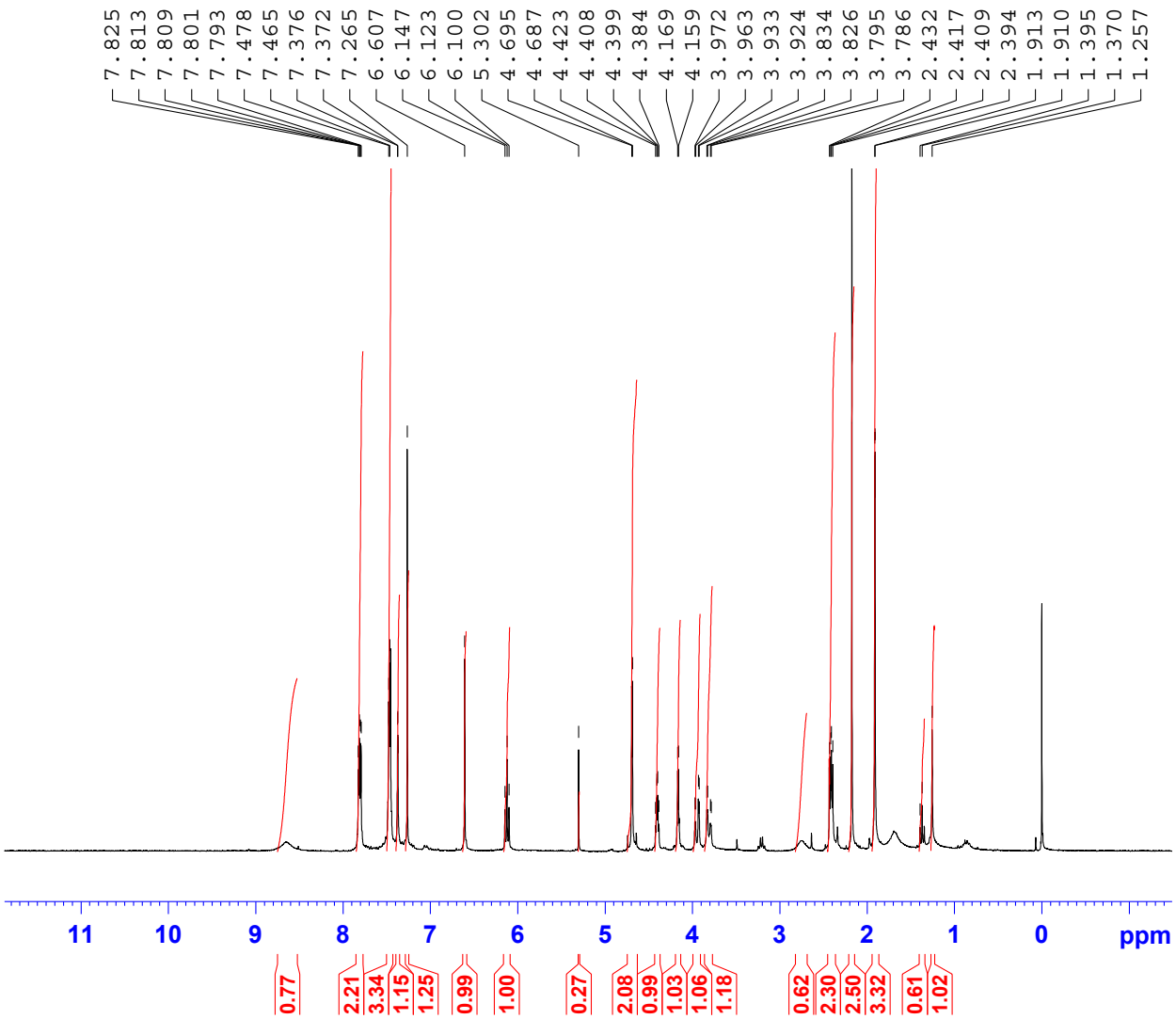
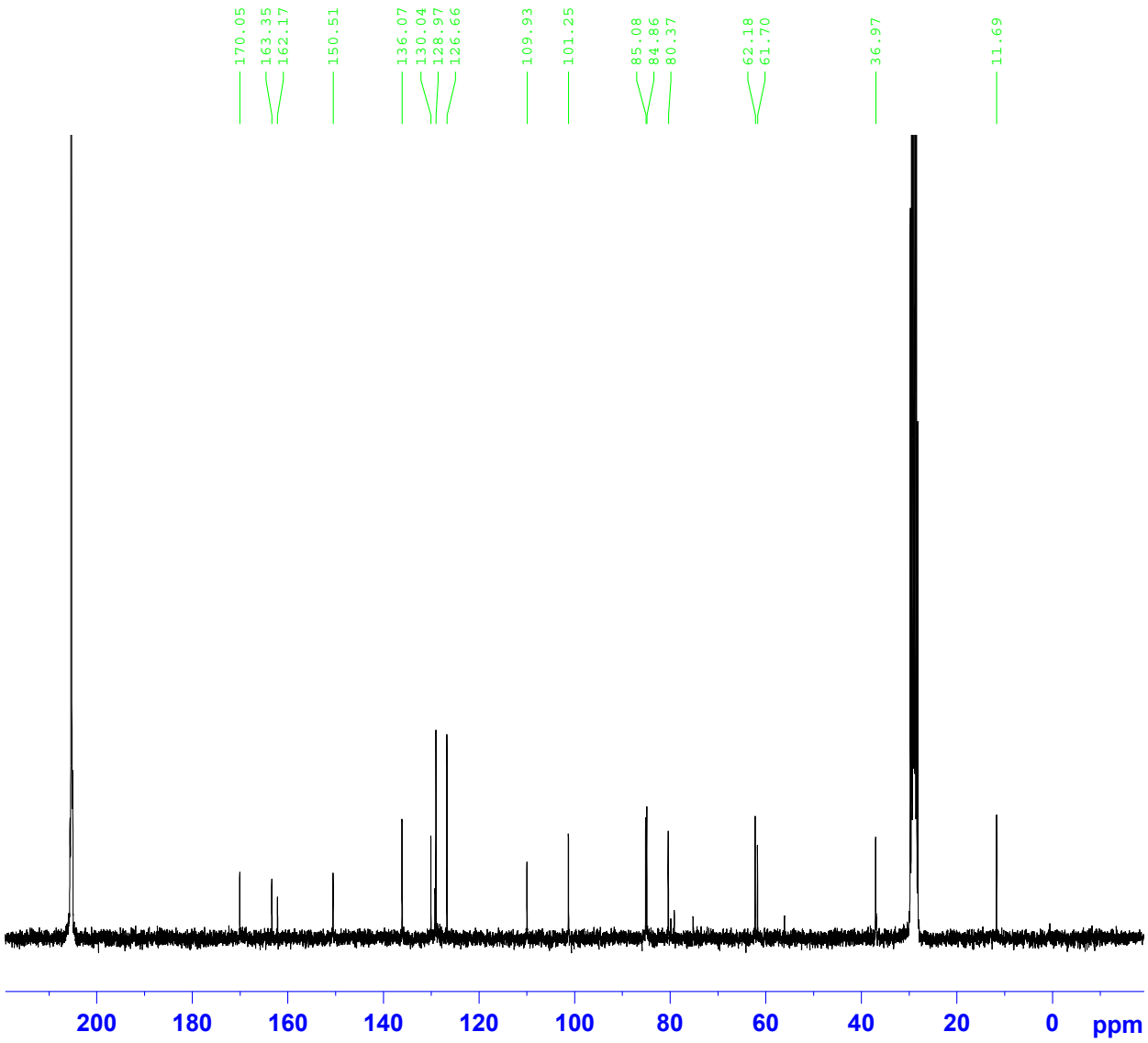


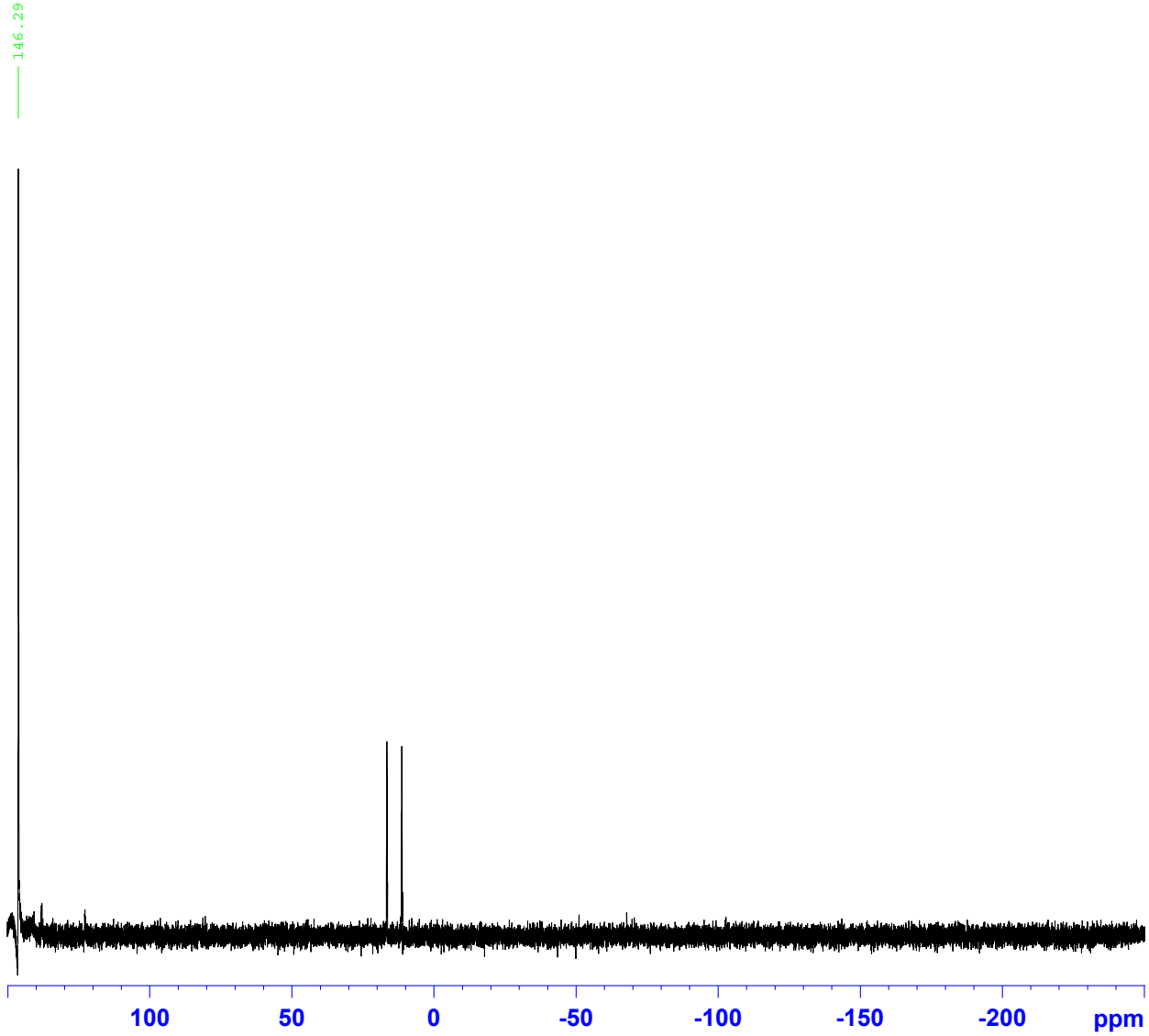
Figure 4. Reversed-phase HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time). (a) oligonucleotide-alkyne (5'-TCGCACACACGC-3') **7c**, (b) oligonucleotide-isoxazole **9c**



^{1}H NMR spectrum of **2**



^{13}C NMR spectrum of **2**



^{31}P NMR spectrum of **4**

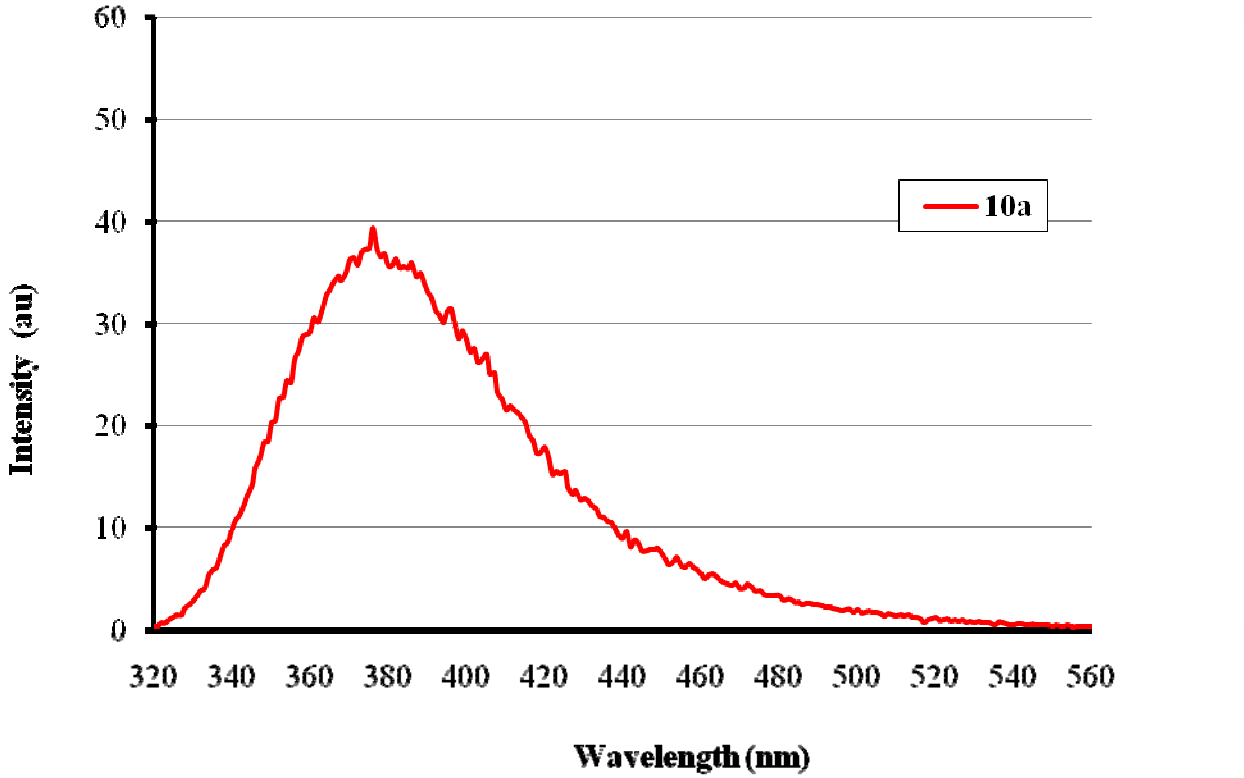


Figure 5. Fluorescense emission spectrum of **10a** (1.4 μM) in deionized water when excited at 310 nm.

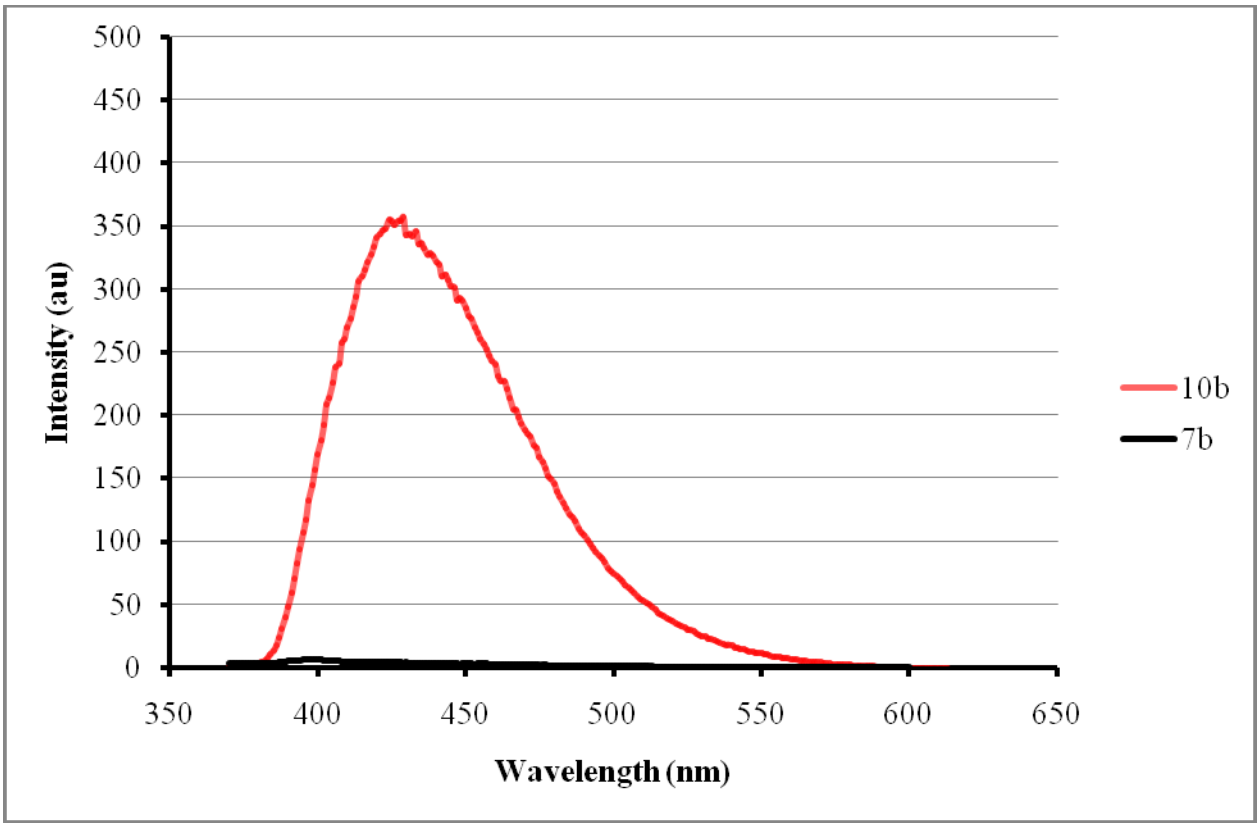
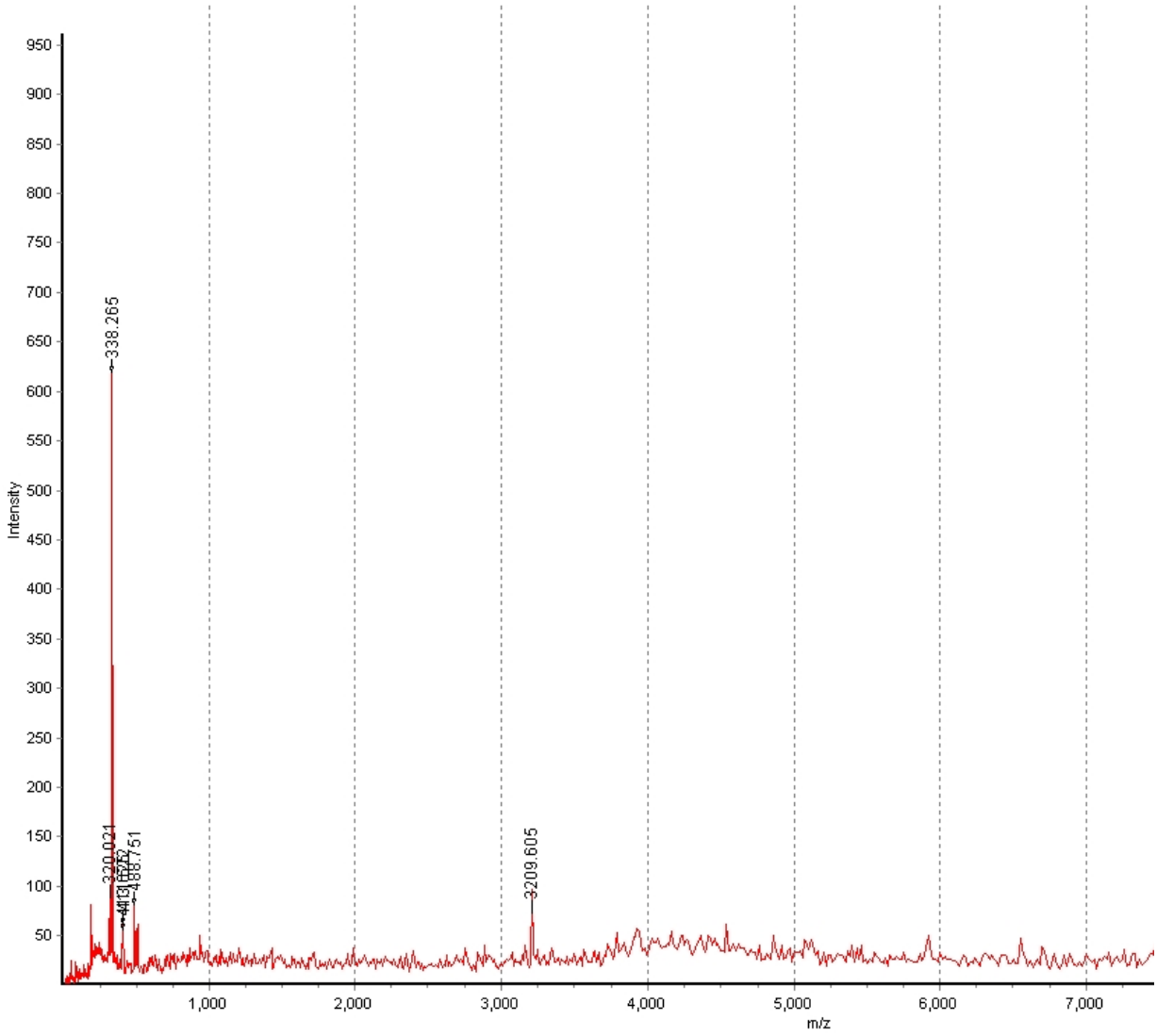
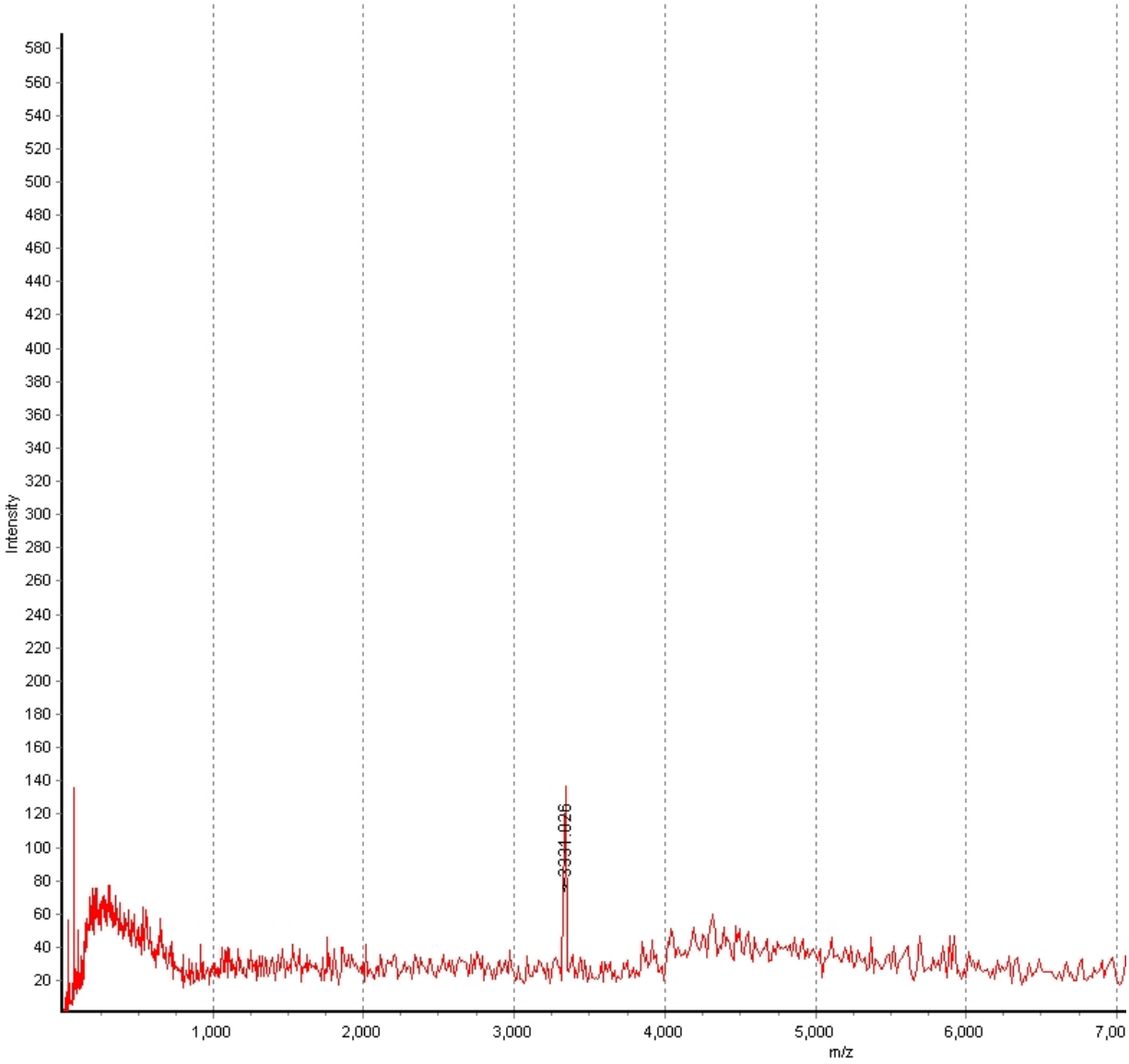


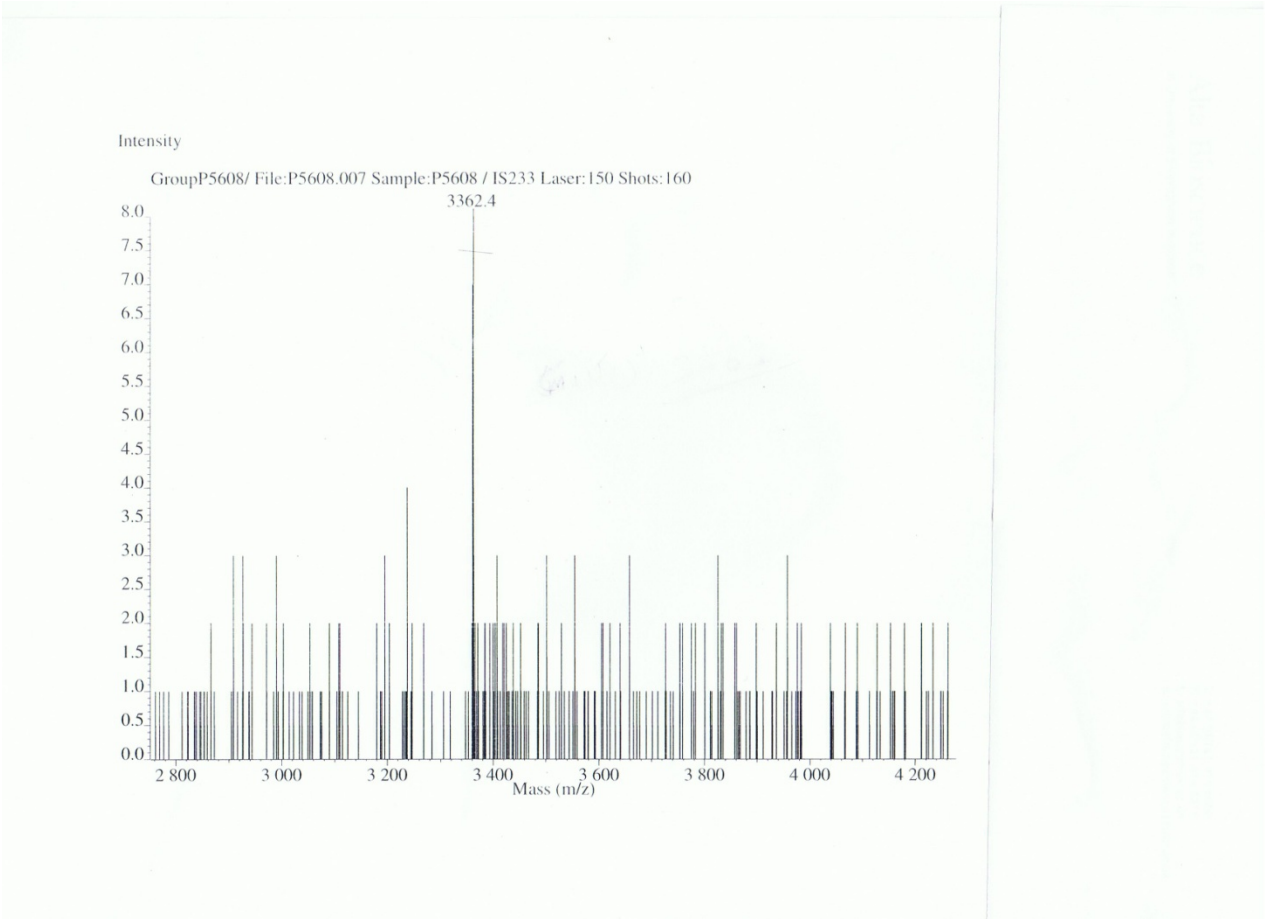
Figure 6. Fluorescense emission spectrum of **7b** and **10b** (4.9 µM) in deionized water when excited at 350 nm.



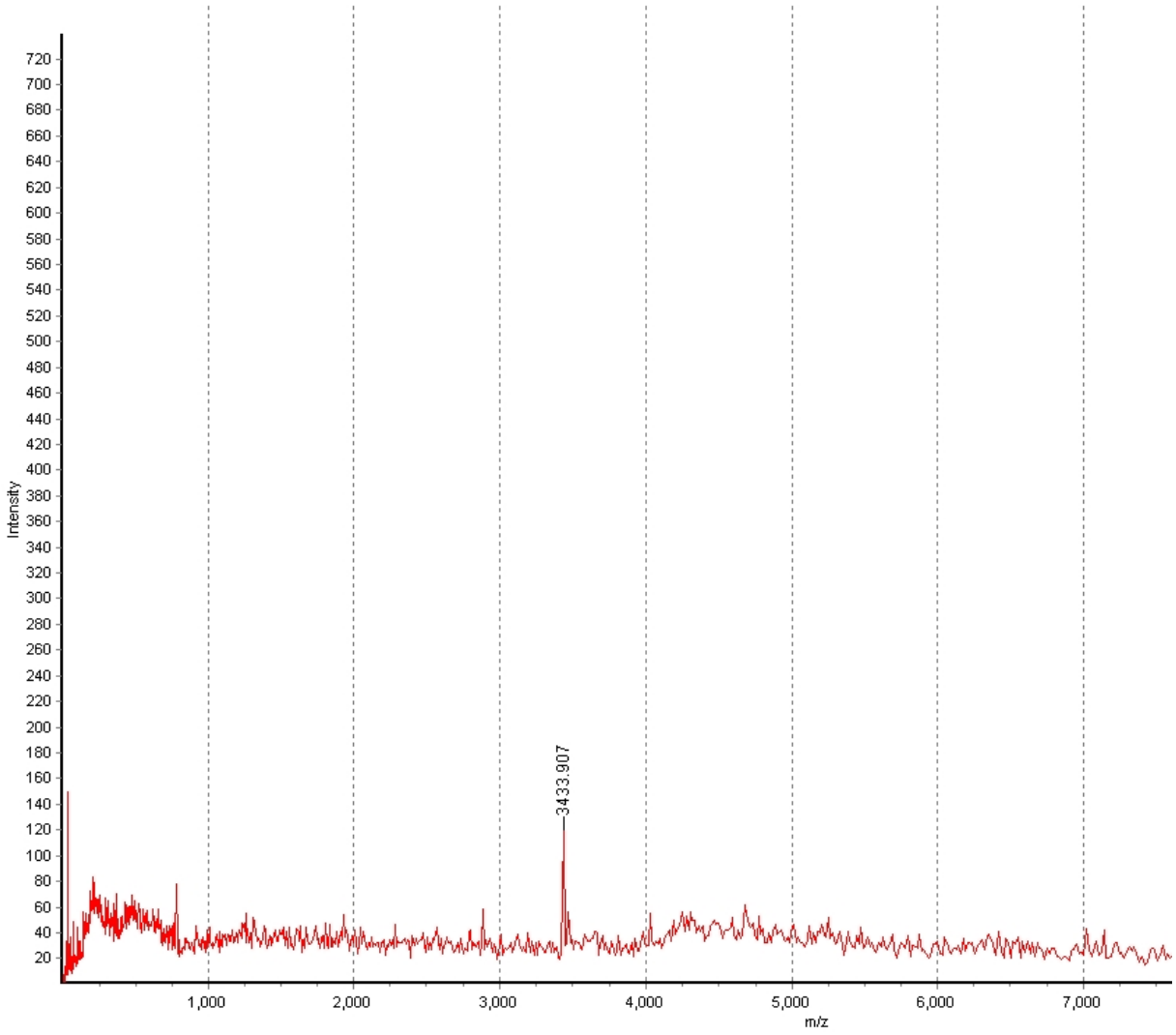
MALDI TOF MS of crude **7b**



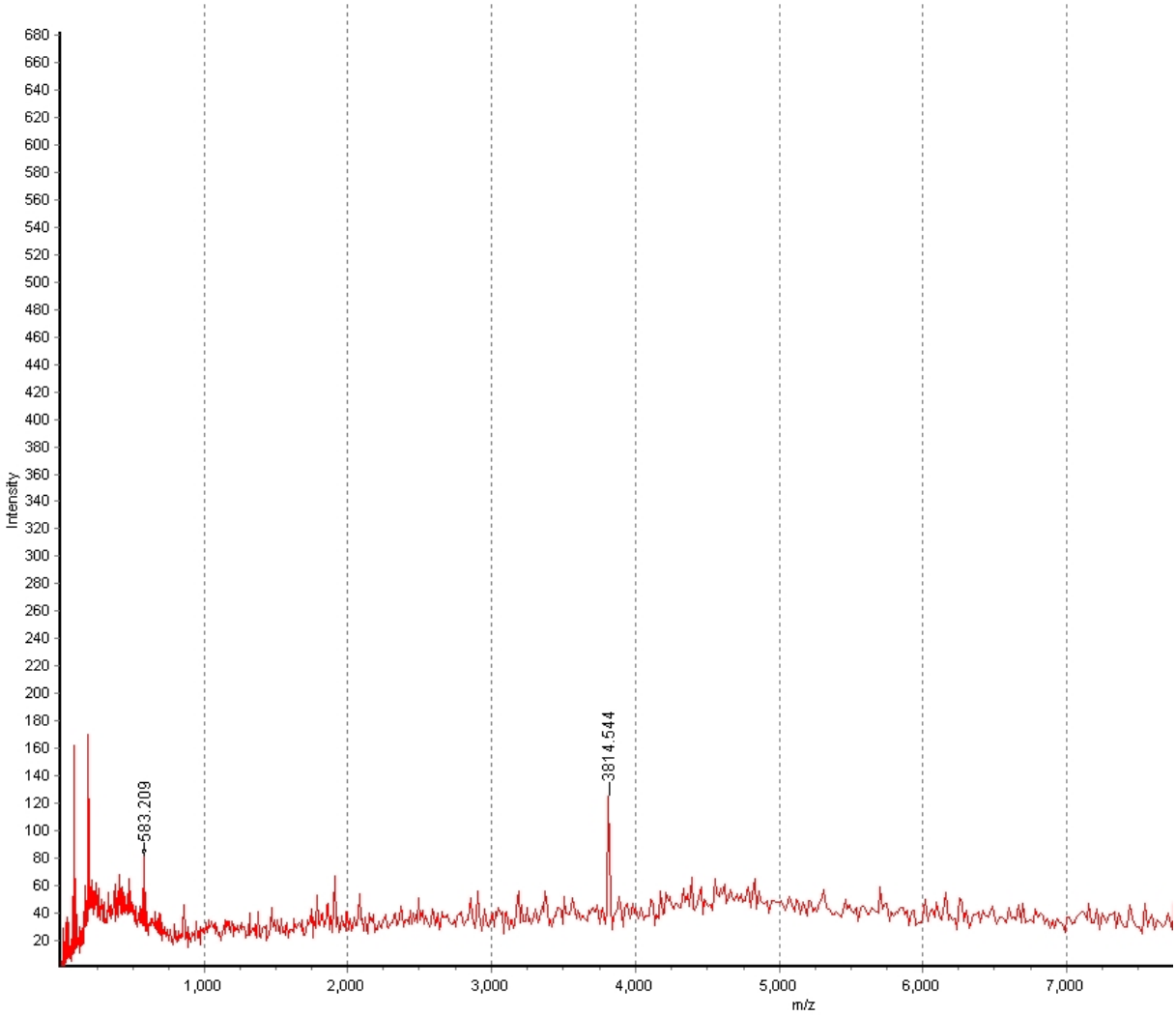
MALDI TOF MS of crude **9b**



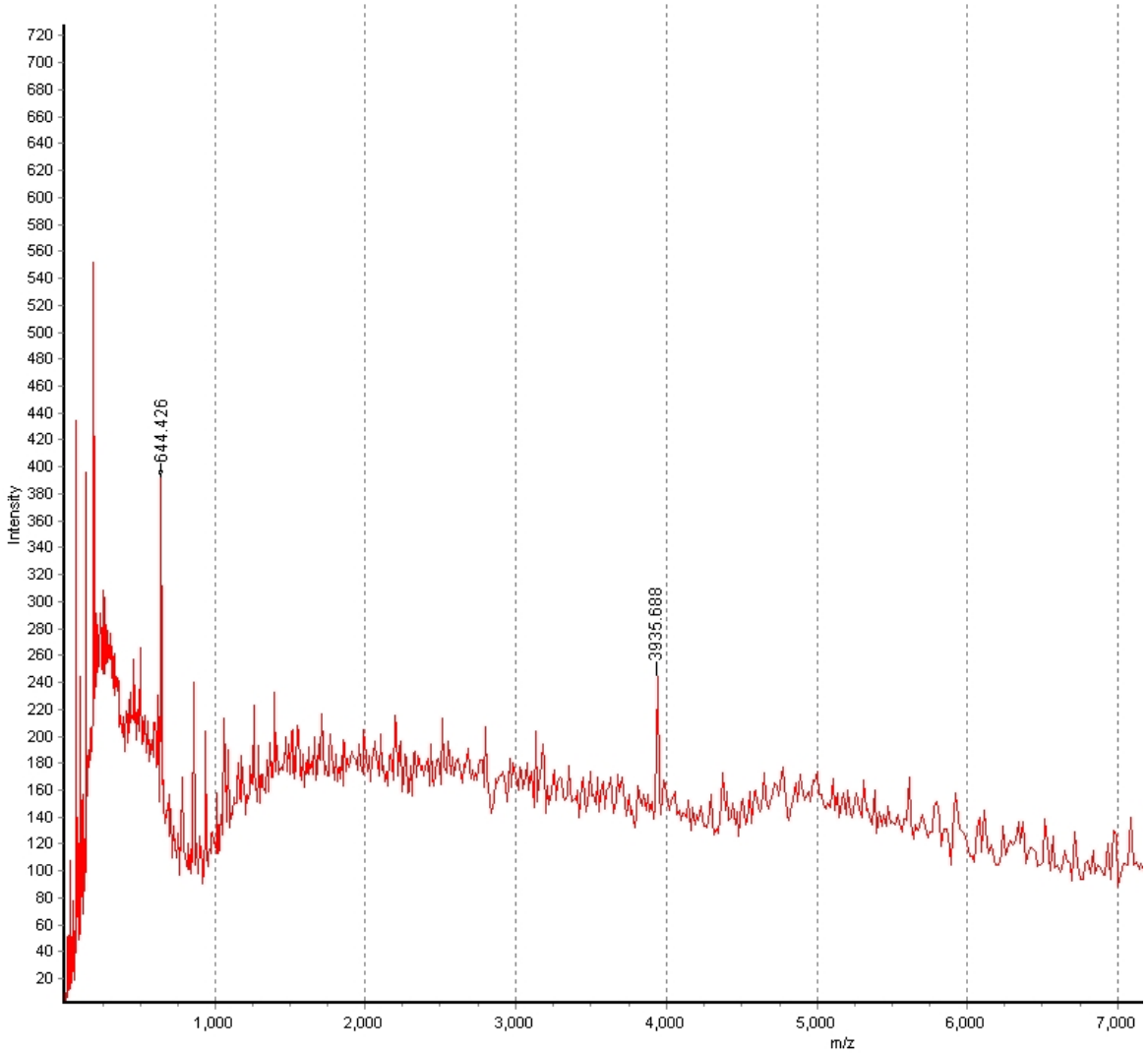
MALDI TOF MS of crude **10a**



MALDI TOF MS of crude **10b**



MALDI TOF MS of crude **7c**



MALDI TOF MS of crude **9c**

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