Supplementary Material (ESI) for Chemical Communications

Supporting Information

Solid Phase Strain Promoted "Click" Modification of DNA *via* [3+2]-Nitrile Oxide Cyclooctyne Cycloadditons

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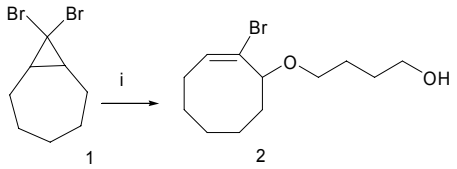
General experimental

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 with 2',4',6'-trihydroxyacetophenone as matrix or LCMS TOF from Agilent Technologies. The NMR spectra were obtained (^{1}H at 300 MHz, ^{13}C at 75 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 or a diode array detector and a Nucleosil C18 column. Oligonucleotides were purchased from Biotez Berlin Germany. All other chemical agents were purchased from Aldrich Chemical Company unless otherwise noted.

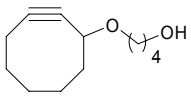
Synthesis of (*E*)-4-(2-bromocyclooct-2-enyloxy)butan-1-ol 2



i)1,4-Butanediol, silver perchlorate, toluene, pyridine, reflux 4 h,

A stirred suspension of 1,4-butanediol (10.15 g, 112.6 mmol) and silver perchlorate (2.33 g, 11.2 mmol) in toluene (2.7 mL) were added to a solution of 8,8-dibromobicyclo[5.1.0]octane **1**[1] (1.00 g, 3.7 mmol) in a mixture of toluene (2.0 mL) and pyridine (2.7 mL). The resulting mixture was heated to reflux for 4 hr in the dark after which the insoluble silver salts were removed by filtration. Brine (70 mL) was added and the aqueous layer was extracted with ether (5 x 30 mL). The combined organic extracts were washed with water (5 x 30 mL) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to yield pale yellow oil which was used without further purification (0.87 g, 89% yield).
^{1}H NMR ($CDCl_3$) δ 6.15-6.20 (dd, *J* = 11.4 and 3.9 Hz, 1H), 3.84-3.89 (m, 1H), 3.55-3.71 (m, 3H), 3.28-3.35 (m, 1H), 2.67-2.80 (m, 1H,) 2.25-2.31 (m, 2H), 1.90-2.05 (m, 4H), 1.70-1.72 (m, 5H), 1.41-1.56 (m, 1H), 1.23-1.34 (m, 1H), 0.74-0.85 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 133.1, 131.4, 85.1, 68.8, 62.8, 39.5, 36.5, 33.3, 30.2, 28.9, 26.6, 26.4; HRMS (ESI) calcd for $C_{12}H_{21}BrO_2$ 277.0798 $[M + H]^+$ found 277.0778.

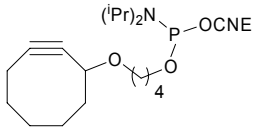
Synthesis of 4-(cyclooct-2-yn-1-yloxy)butan-1-ol 3



The bromo-cycloalkene **2** (450 mg, 1.7 mmol) was dissolved in DMF:THF (1:1, 30 mL) and NaH (300 mg, 12.5 mmol) was added. The resulting mixture was stirred at room temperature for 5 h after which it was cooled to 0 ^{0}C and neutralised by addition of 1 M HCl. The aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic extracts were washed with water (3 x 10 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by flash column chromatography (SiO_2, DCM:acetone; 95:5) to yield a colourless oil (166 mg, 52%).

^{1}H NMR ($CDCl_3$) δ 4.15-4.19 (m, 1H), 3.60-3.66 (m, 3H), 3.31-3.38 (m, 1H), 2.41 (br s, 1H), 2.07-2.32 (m, 3H), 1.78-2.00 (m, 4H,) 1.62-1.70 (m, 6H), 1.45-1.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 100.1, 92.8, 72.6, 69.3, 62.7, 42.3, 34.3, 30.2, 29.7, 26.6, 26.32, 20.7; HRMS (ESI) calcd for $C_{12}H_{20}O_2$ 197.1536 $[M + H]^+$, found 197.1546.

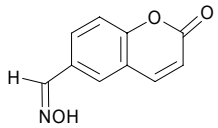
Synthesis of 2-cyanoethyl 4-(cyclooct-2-ynyloxy)butyl diisopropylphosphoramidite 4



The alcohol **3** (167 mg, 0.85 mmol), and benzylmercaptotetrazole (82 mg, 4.2 mmol) were placed in a dried round bottomed flask under an argon atmosphere. Anhydrous acetonitrile (6 mL) was added to the flask followed by diisopropylamine (65 µl, 0.42 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (327 µl, 0.935 mmol). The resulting solution was stirred for 30 minutes at room temperature after which TLC analysis (SiO_2, hexane:EtOAc; 50:50) showed complete consumption of the starting alcohol. The reaction mixture was diluted with ethyl acetate (25 mL) and washed with aqueous sodium bicarbonate (3 x 10 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to give the crude alkyne phosphoramidite which was used without purification.

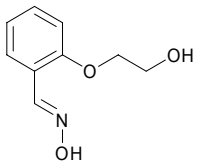
^{31}P NMR (DMSO-d_6) δ 146.3.

Synthesis of coumarin 6-carboxaldoxime 12



A mixture of the aldehyde (522 mg, 3 mmol), hydroxylamine hydrochloride (248 mg, 3.6 mmol) and sodium acetate (295 mg, 3.6 mmol) in EtOH (50 mL) and water (500 μL) was stirred at reflux for 18 h. Following solvent removal under reduced pressure inorganic salts were removed by shaking with water. Following decanting of the aqueous layer the solid residue was taken up in dioxane. The solution was dried and evaporated to yield the title compound as a white solid (340 mg, 60%). The product presented as a mixture of geometrical isomers in ~5:1 ratio and was used without further purification. m.p. 231-233 ^{0}C. ^{1}H NMR (DMSO-d_6) δ 11.90 and 11.38 (2 x s, 1H), 8.50 and 8.12 (2 x s, 1H), 8.11 (m, 1.17 H), 7.92 (m, 0.83 H), 7.50 (m, 0.83 H), 7.45 (m, 1.17H), 6.54 (d, J = 9.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 159.7, 153.8, 146.8, 144.1, 129.5, 126.3, 118.9, 116.9, 116.7; HRMS (ESI) calcd for $C_{10}H_7NO_3$ 191.0531 $[M + H]^+$, found 191.0538.

Synthesis of 2-(2-hydroxyethoxy)benzaldoxime 13



A solution of the aldehyde (1.00 g, 6 mmol) and hydroxylamine hydrochloride (1.26 g, 24 mmol) in EtOH (35 mL) was stirred at r.t. for 10 min, after which a solution of sodium acetate (1.68 g, 24 mmol) in H_2O (15 mL) was added. The mixture was heated under reflux for 40 minutes. Following solvent removal under reduced pressure the crude product, obtained as a white solid, was taken up in EtOAc (30 mL), washed with H_2O (3 x 20 mL) and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure yielded the pure product as a white solid in excellent yield (1.05 g, 96%), m.p. 98-100 ^{0}C.
^{1}H NMR ($CDCl_3$) δ 8.40 (brs, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 7.26 (s, 1H), 6.94-7.03 (m, 2H), 4.17 (t, J = 4.2 Hz, 2H), 3.98 (t, J = 4.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 156.3, 147.7, 131.1, 128.8, 121.5, 113.6, 70.6, 61.1; HRMS (ESI) calcd for $C_9H_{11}NO_3$ 204.0631 $[M + Na]^+$, found 204.0622.

General procedure for phosphitylation reactions: preparation of 6a,b

To manually couple the cyclooctyne phosphoramidite **4** to the oligonucleotide, 500 μL of a 100 mM solution of the phosphoramidite in anhydrous CH_3CN was added to the CPG-DNA (1 μmol) along with 500 μL of a 0.3 M solution of benzylmercaptotetrazole in CH_3CN. The mixture was allowed to react for 15 minutes at room temperature with mixing between two syringes. This procedure was repeated with a second portion of each of a new solution of phosphoramidite and benzylmercaptotetrazole. The CPG was washed with CH_3CN (5 x 2 ml), then exposed to oxidizer (700 μL, 0.1 M iodine solution in THF: pyridine: water; 78:20:2) for one minute. Following washing with CH_3CN (2 x 5 mL) the resin was dried yielding CPG-DNA-alkynes **6a**,**b**. Cleavage from the resin proceeded by the method described below furnishing samples of **7a**,**b** respectively.

General cleavage/deprotection procedure

For analytical purposes a portion of the DNA was deprotected and cleaved from the CPG by incubating the supported material 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 (4 x 200 μL aliquots), all solutions and washings were combined to afford an aqueous solution of the DNA products which were concentrated on a vacuum concentrator prior to HPLC analysis.

General method for HPLC analysis

The DNA conjugates were analyzed by reverse-phase HPLC with an analytical column (Nucleosil C-18) under the following conditions; 200 μL injection loop. For DNA alkynes and DNA conjugates 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, 95% B; 20-28 min, 95% B, flow rate: 1.0 mL/min, and detection at 260 nm alternatively absorbance was monitored in the range 220–500 nm with a diode array detector and recorded at 260 nm.

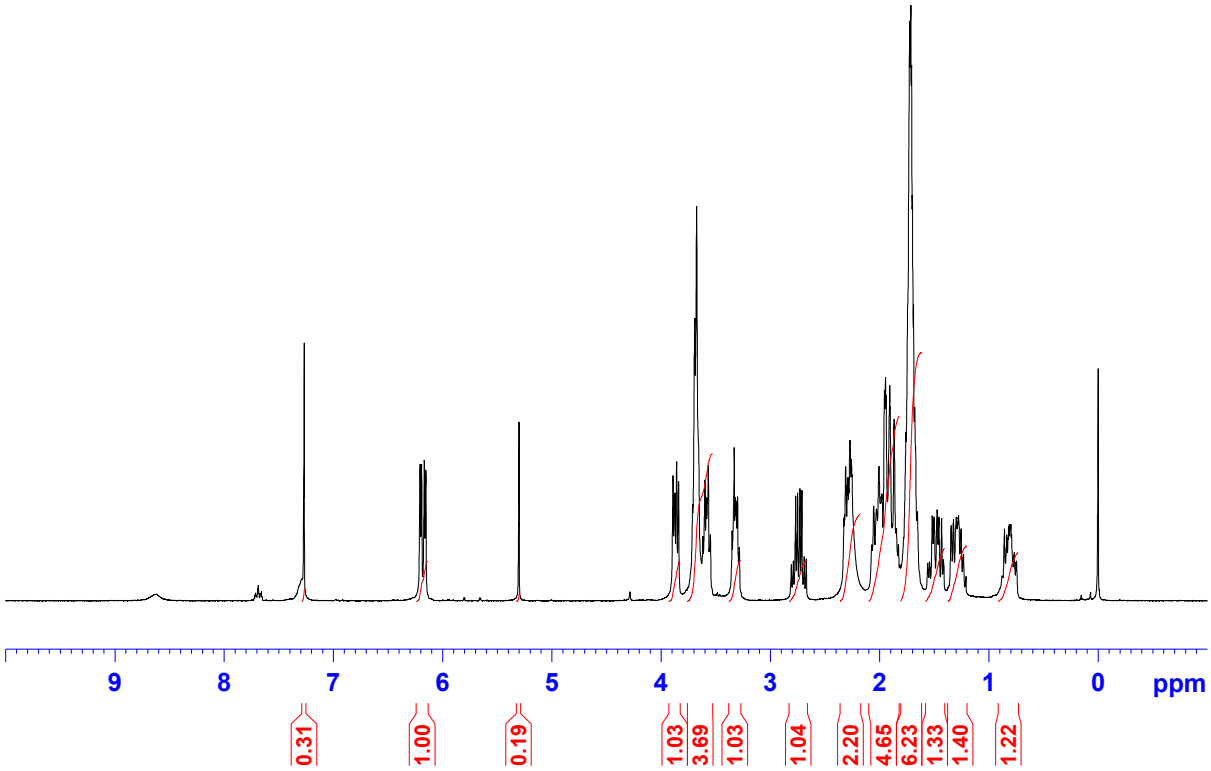
General procedure for nitrile oxide click reactions on CPG- DNA-alkynes 6a-b.

To solid supported DNA alkynes **6a** and **6b** (0.2 μmoles) in an eppendorf tube was added 50 μL ethanol and 50 μL water followed by 10 μL of a premixed solution of the oxime (3.3 μmoles) and chloramine-T monohydrate (3.3 μmoles) in 50% aqueous ethanol. The combined mixture was agitated at room temperature for 10 min. Following settling the

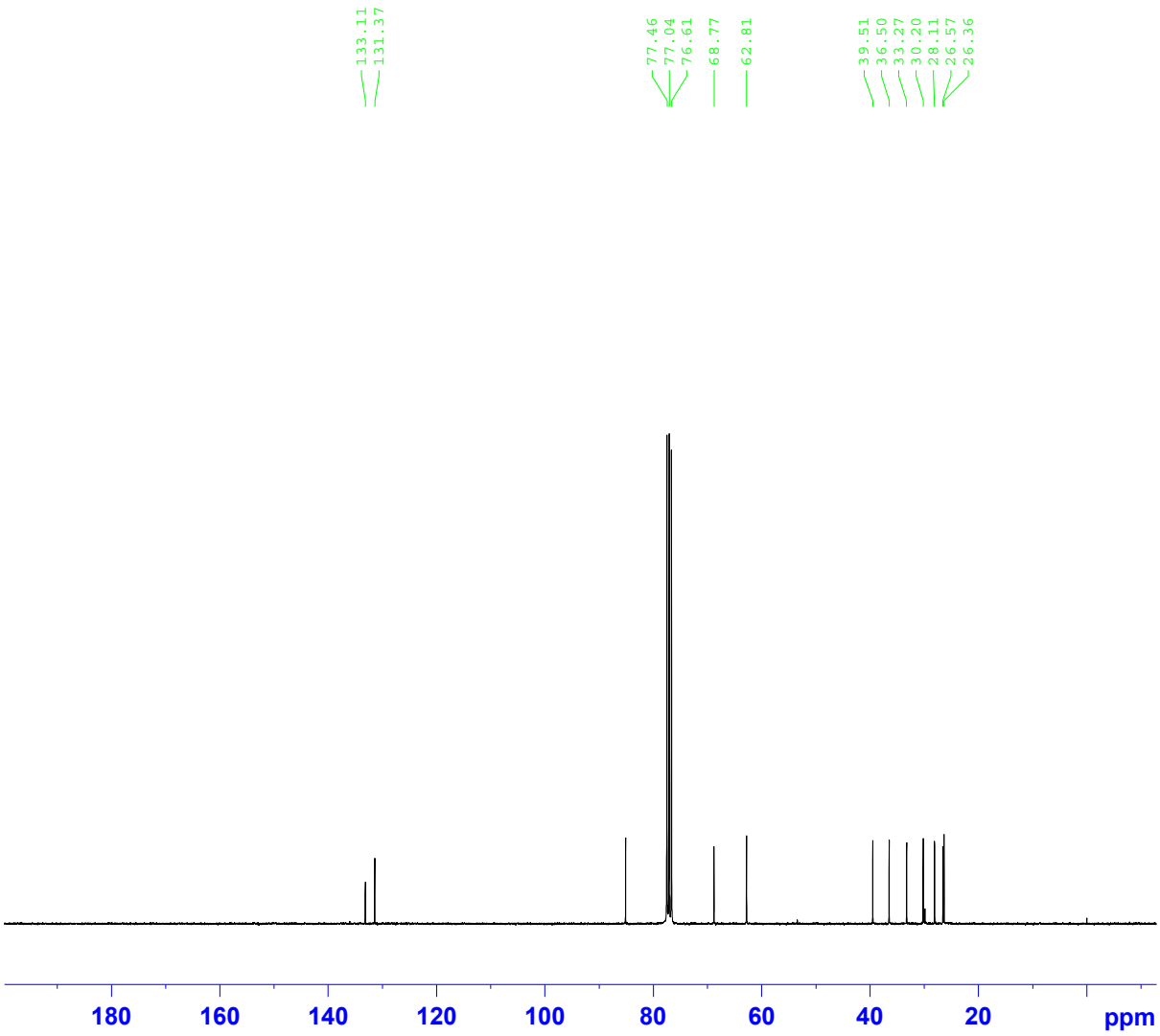
supernatant liquid was removed by syringe and the CPG washed firstly with CH_3CN (5 x 300 µL), CH_3OH (3 x 200 µL) and then H_2O (4 x 300 µL).

Reaction with pyrene oxime and coumarin 6-carboxaldoxime **12** was conducted as above with the following modifications: reaction solvent DMF (70 µL) EtOH (30 µL); solvent for premixing of oxime and chloramine-T monohydrate 10 µL of DMF;EtOH 1:1; post reaction washings DMF (5 x 300 µL), CH_3OH (3 x 200 µL) and then H_2O (4 x 300 µL).

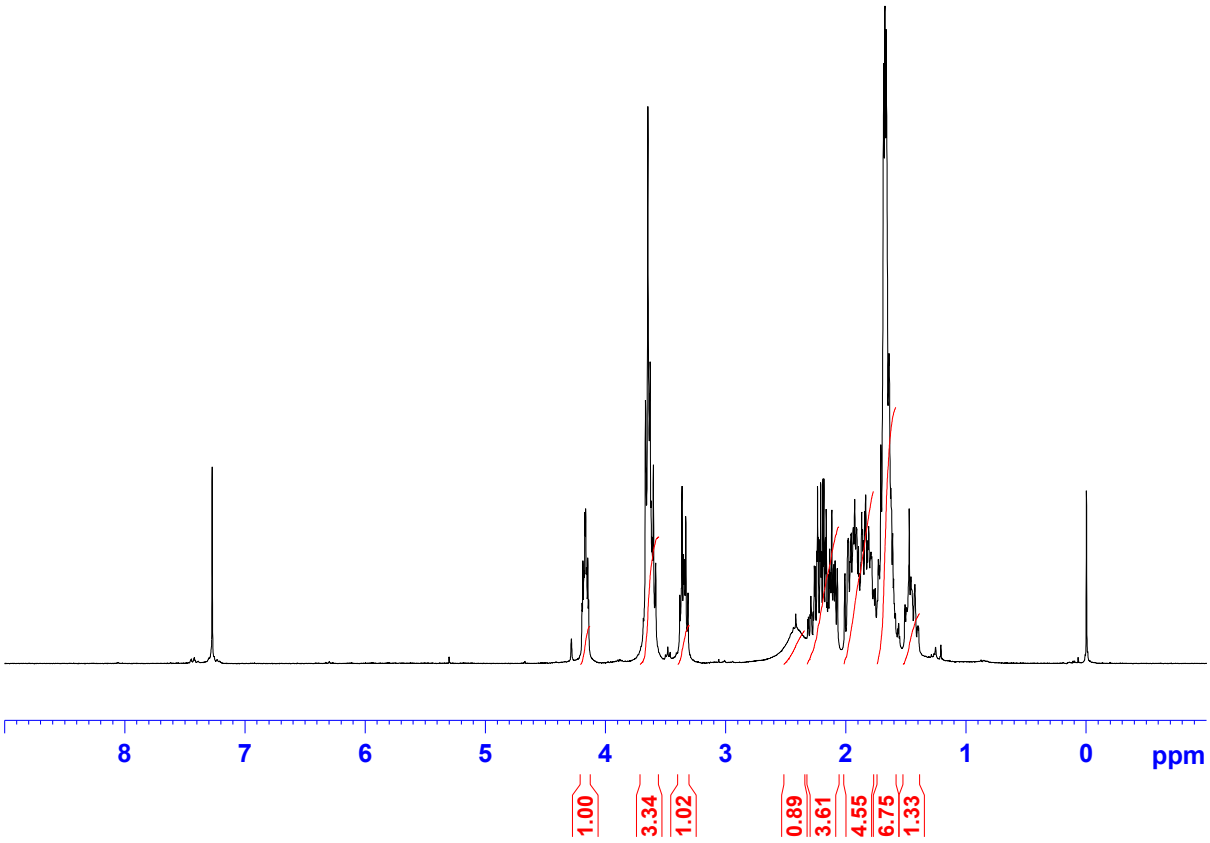
Copies of ^{1}H, ^{13}C and ^{31}P NMR spectra



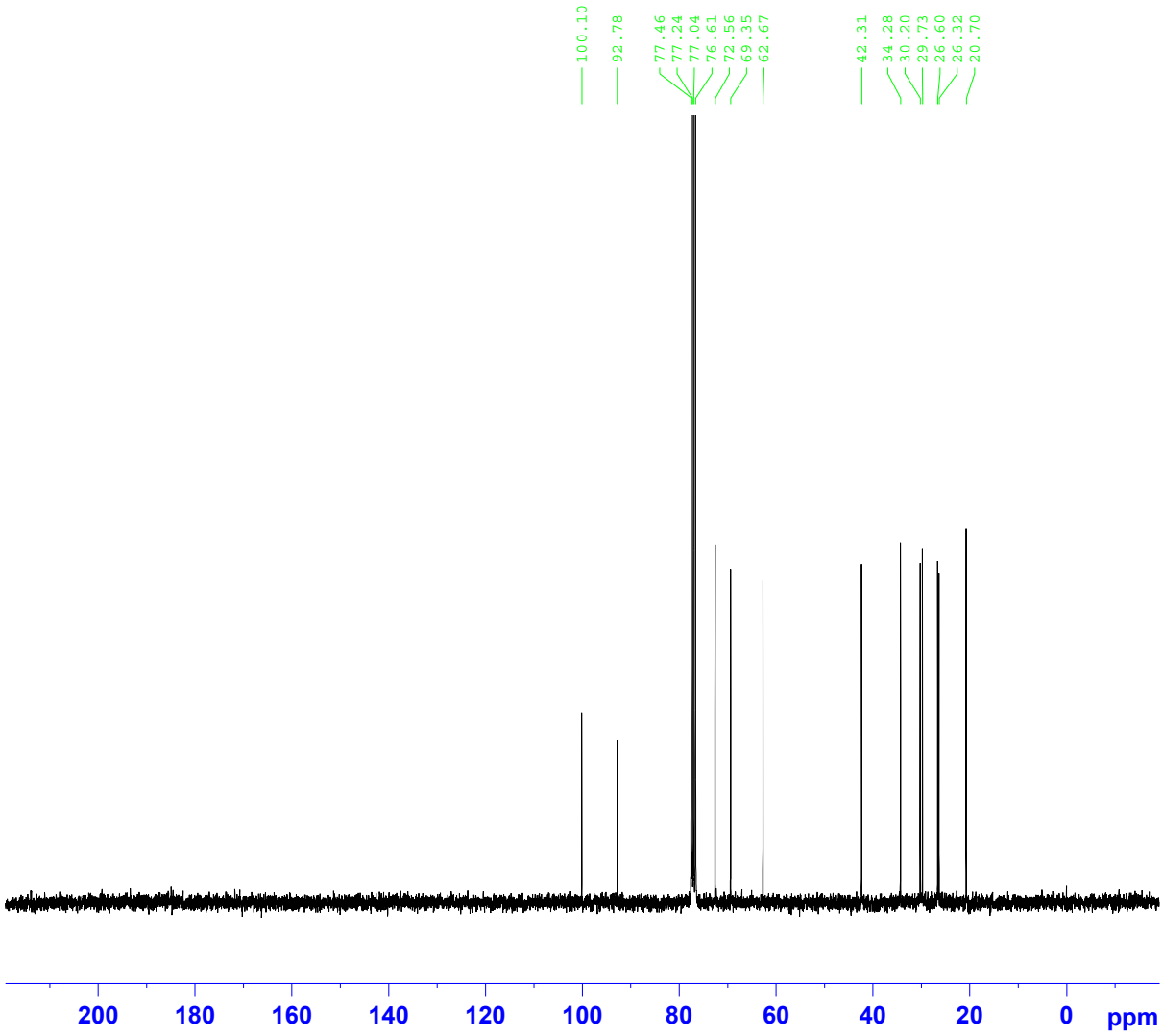
^{1}H NMR spectrum of 2



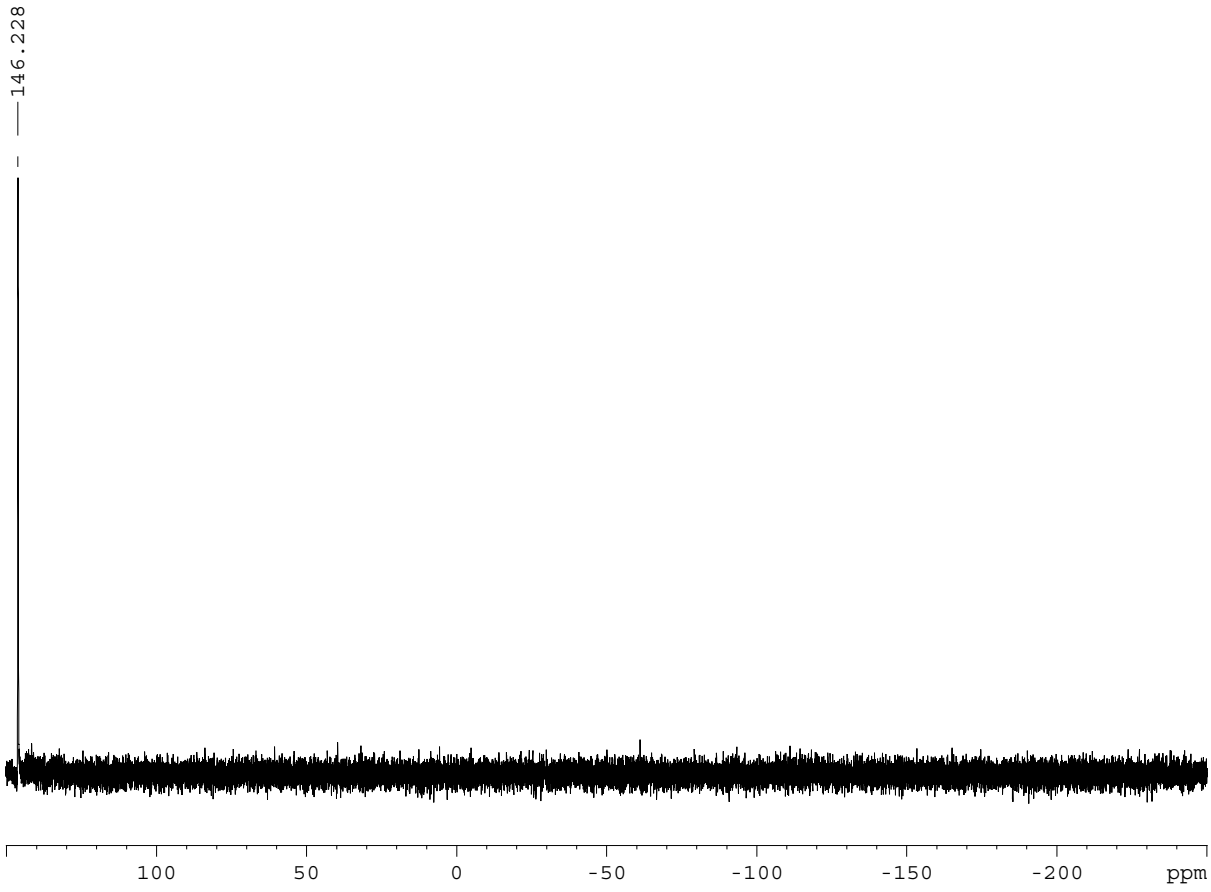
^{13}C NMR spectrum of 2



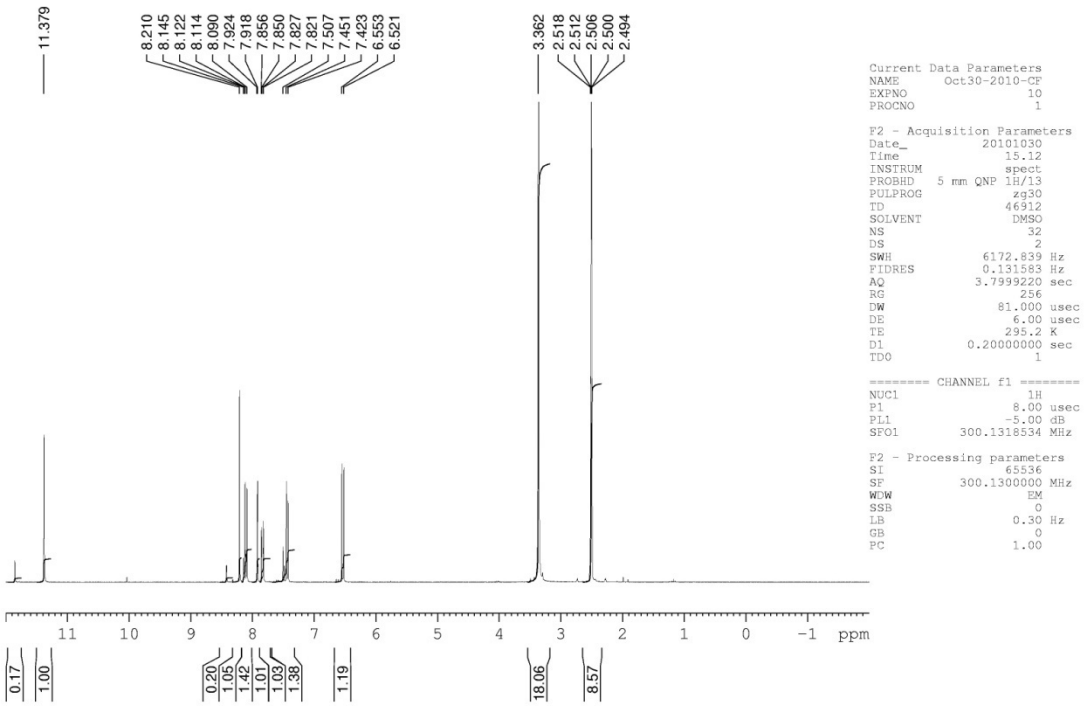
^{1}H NMR spectrum of 3



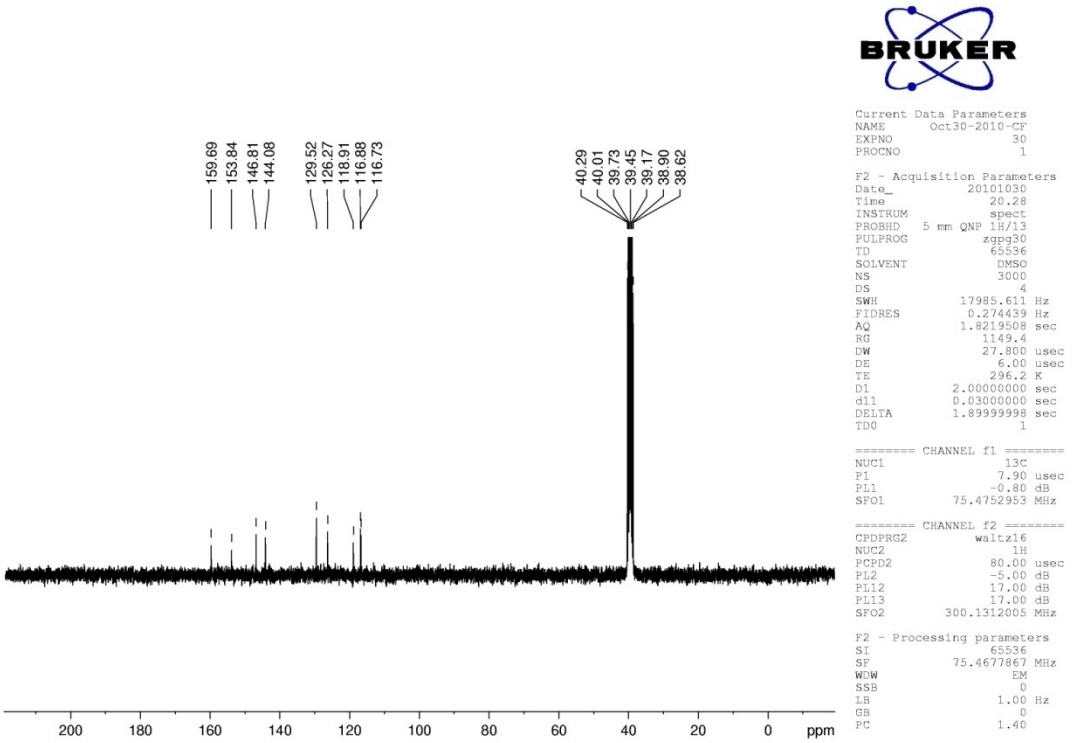
^{13}C NMR spectrum of 3



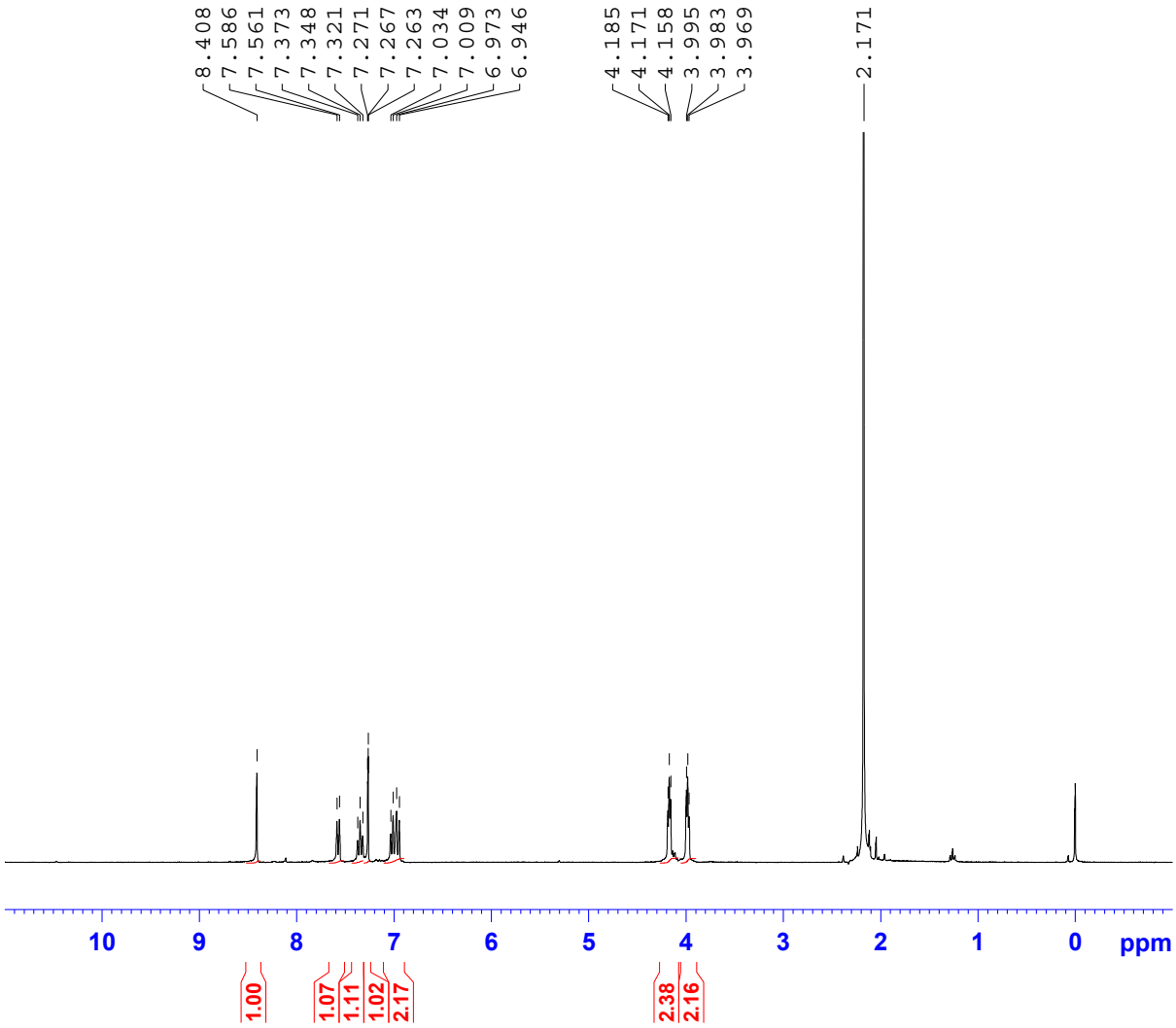
^{31}P NMR spectrum of 4



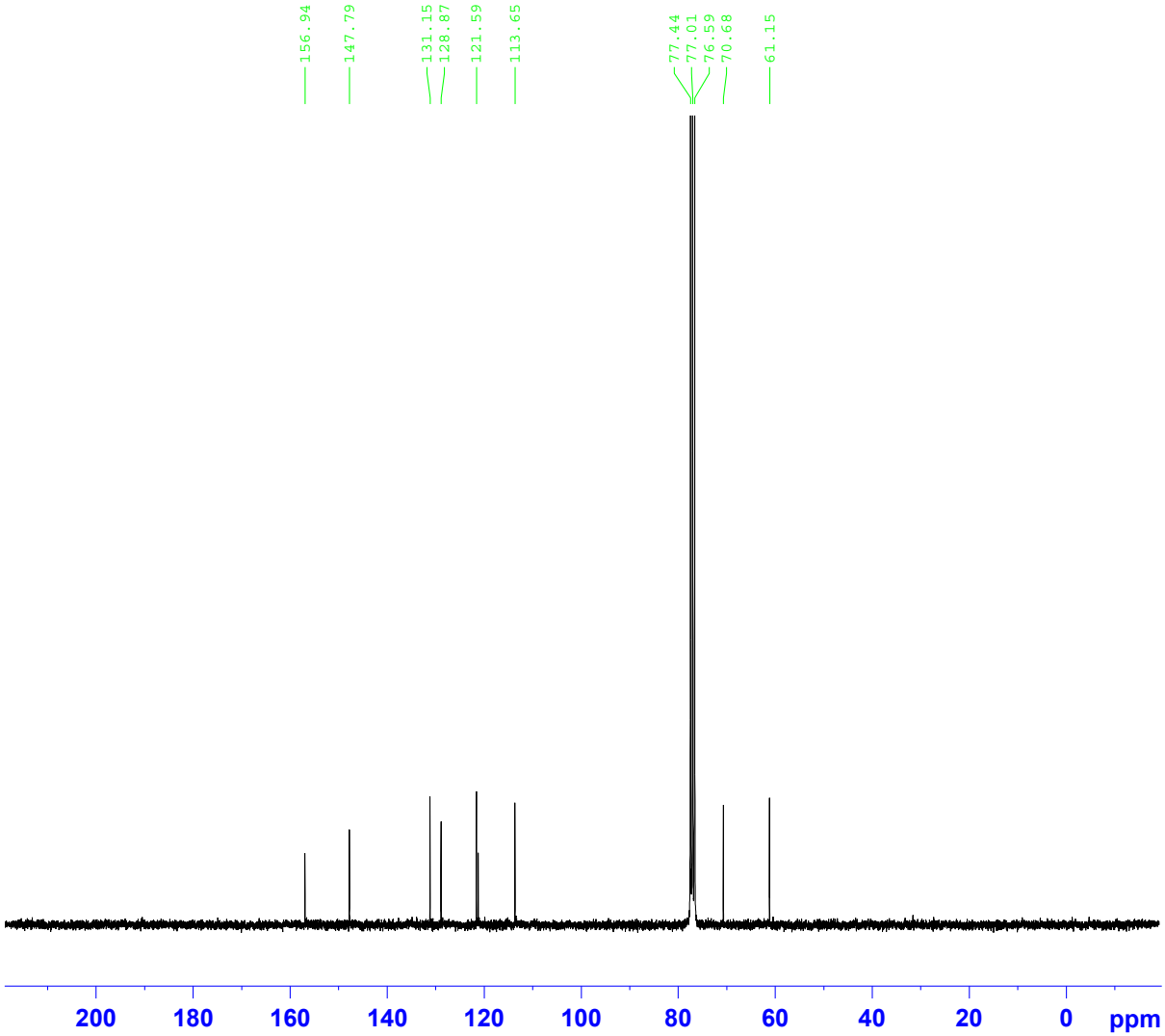
^{1}H NMR spectrum of coumarin 6-carboxaldoxime 12



^{13}C NMR spectrum of coumarin 6-carboxaldoxime 12



^{1}H NMR spectrum of 2-(hydroxyethoxy)benzaldoxime 13



^{13}C NMR spectrum of 2-(2-hydroxyethoxy)benzaldoxime 13

HPLC Traces

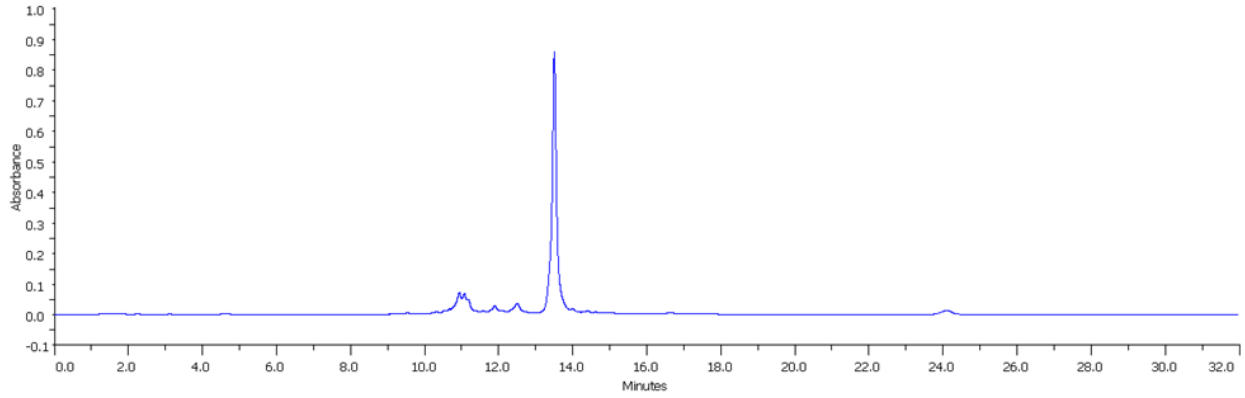


Figure 1 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **7a**

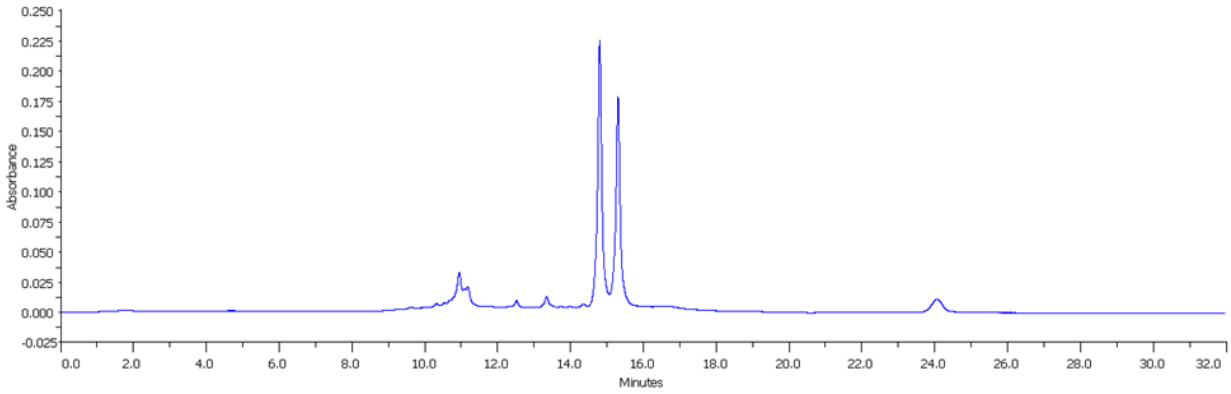


Figure 2 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10a(i)/11a(i)**

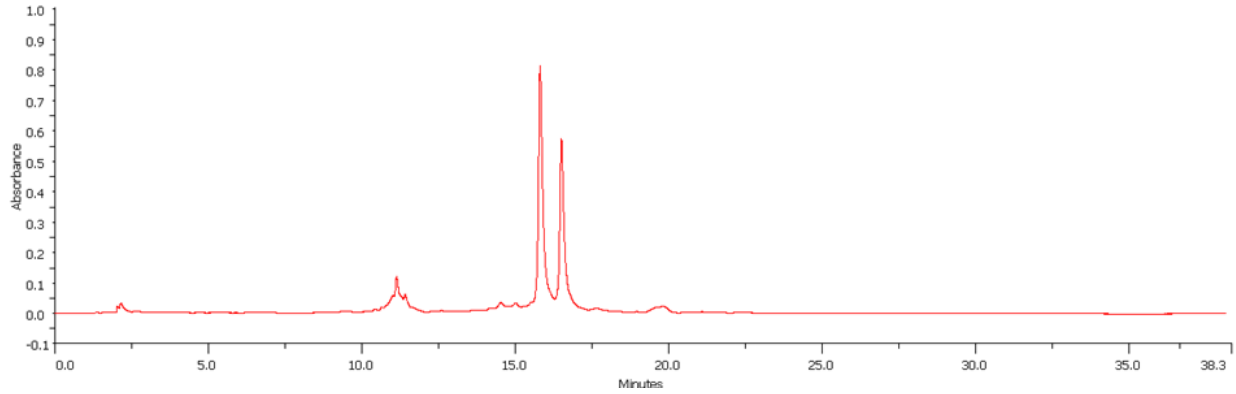


Figure 3 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10a(ii)/11a(ii)**

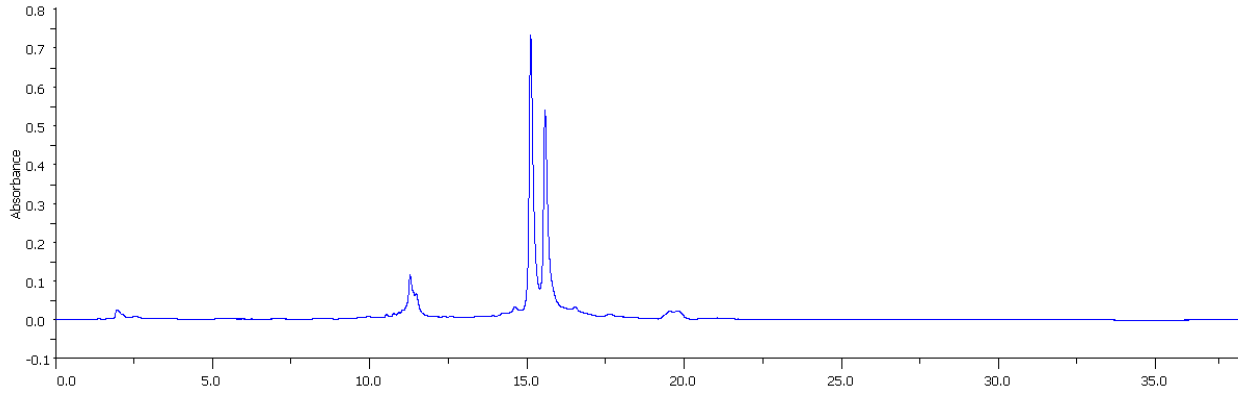


Figure 4 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10a(iii)/11a(iii)**

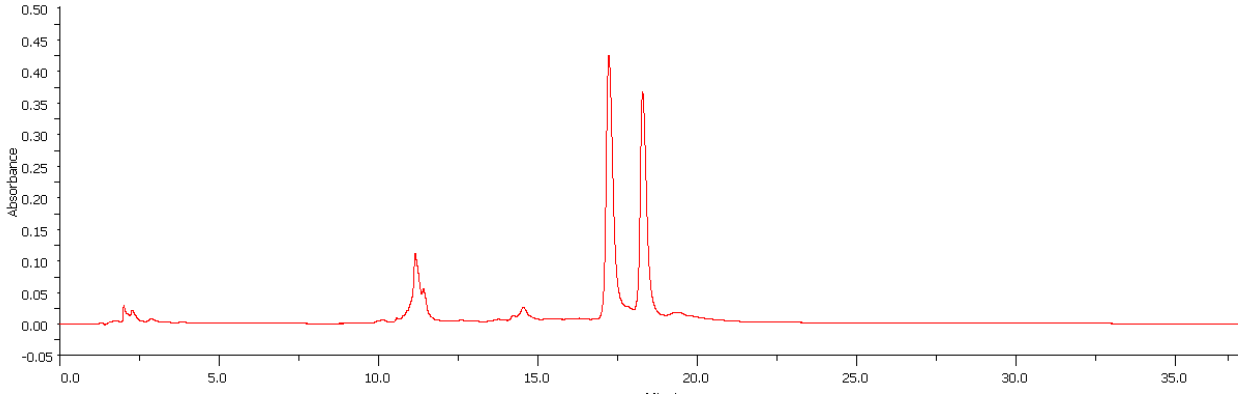


Figure 5 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10a(iv)/11a(iv)**

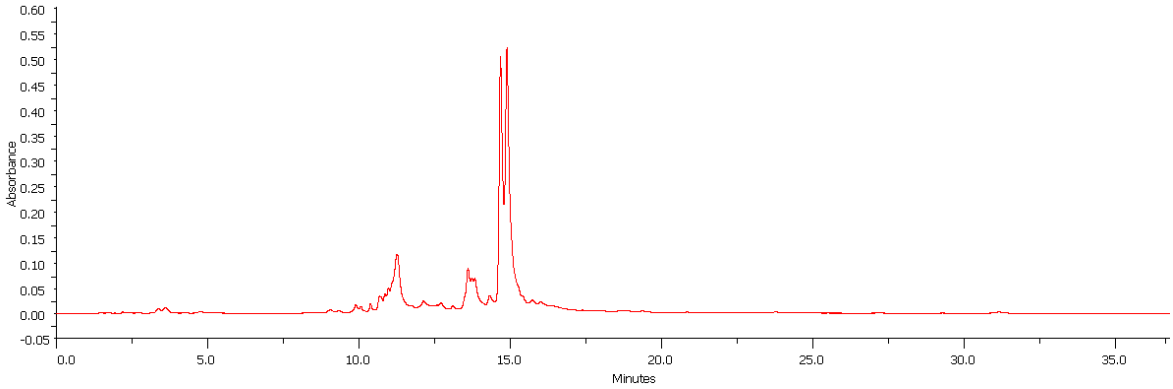


Figure 6 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10a(v)/11a(v)**

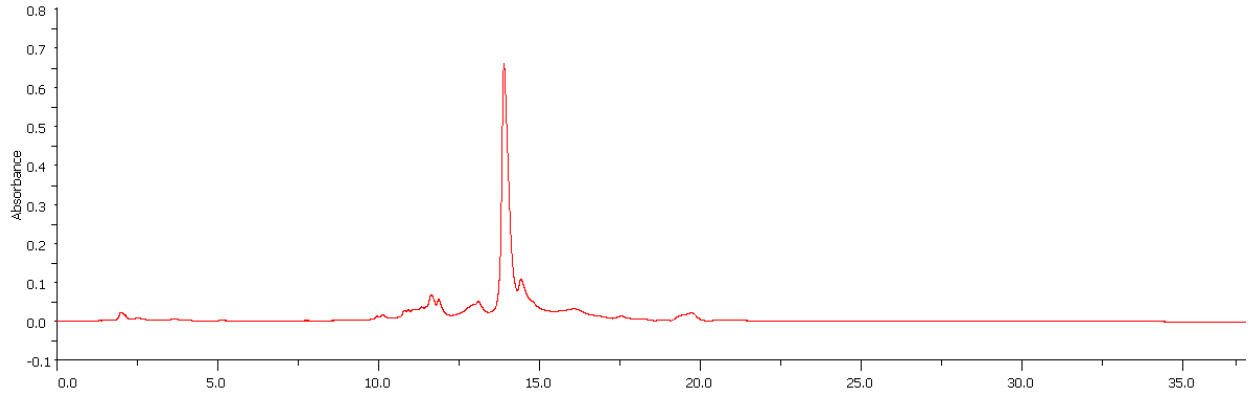


Figure 7 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **7b**

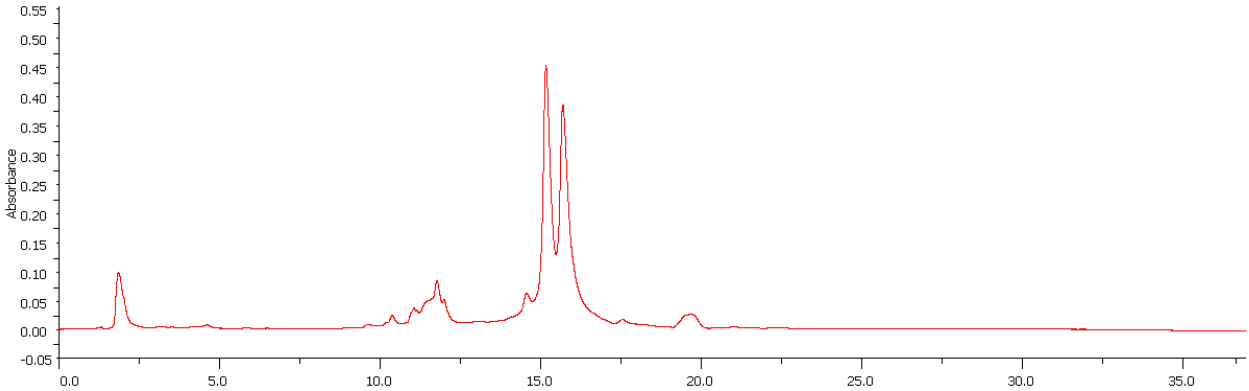


Figure 8 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10b(i)/11b(i)**

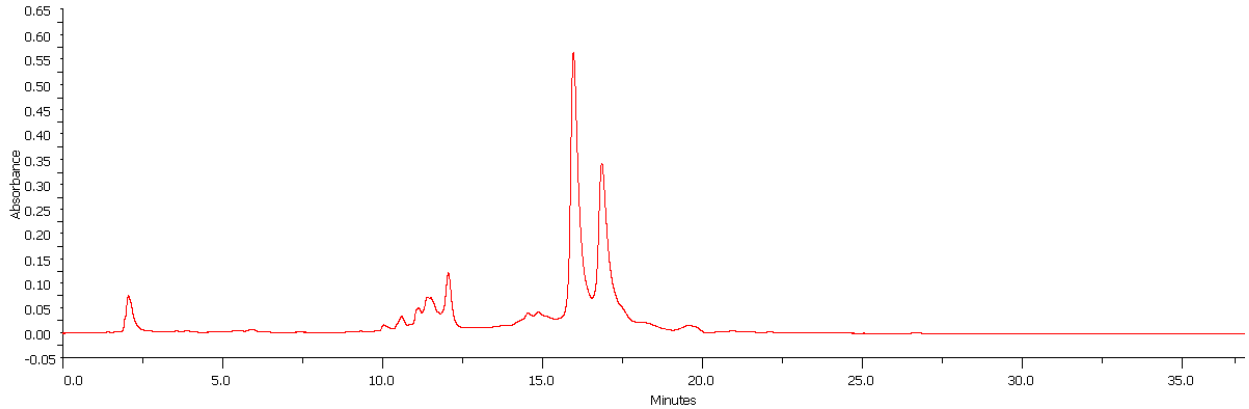


Figure 9 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10b(ii)/11b(ii)**

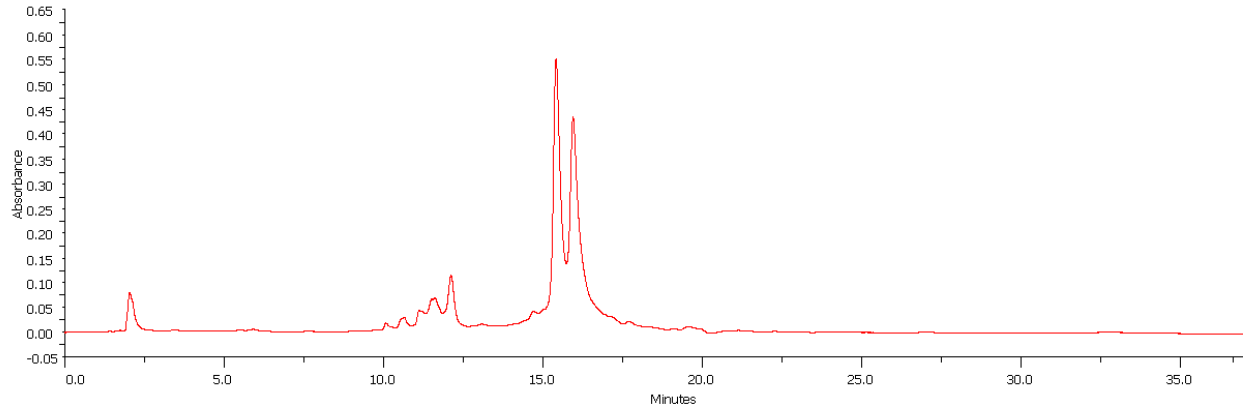


Figure 10 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10b(iii)/11b(iii)**

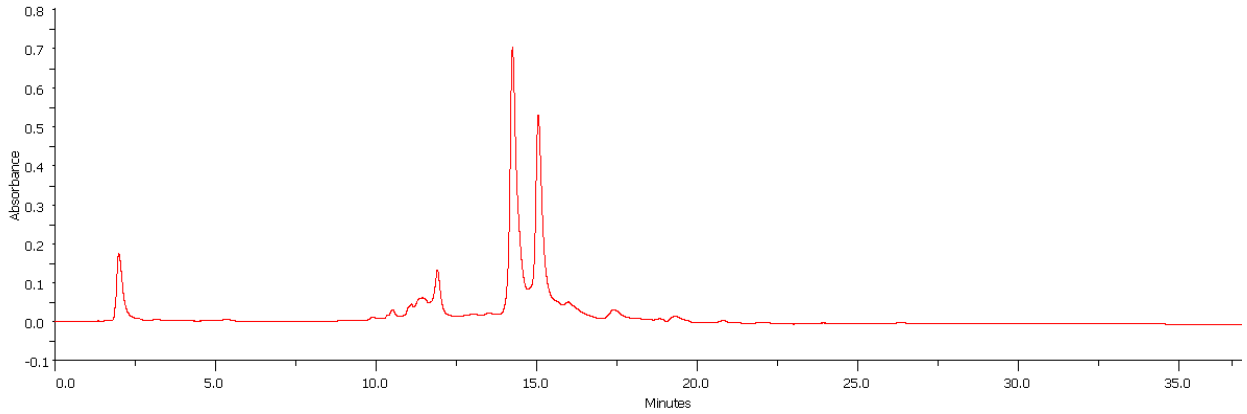
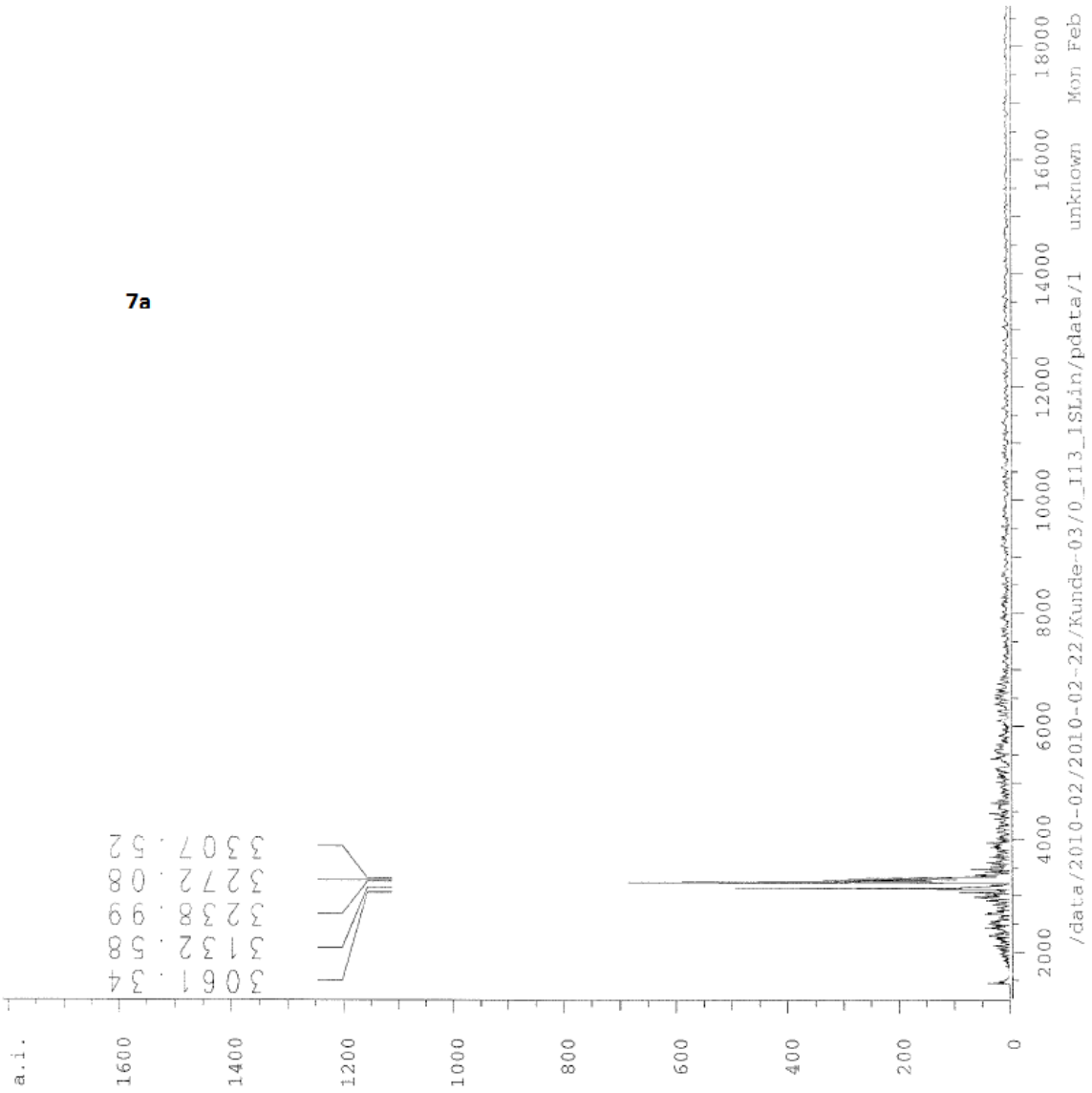


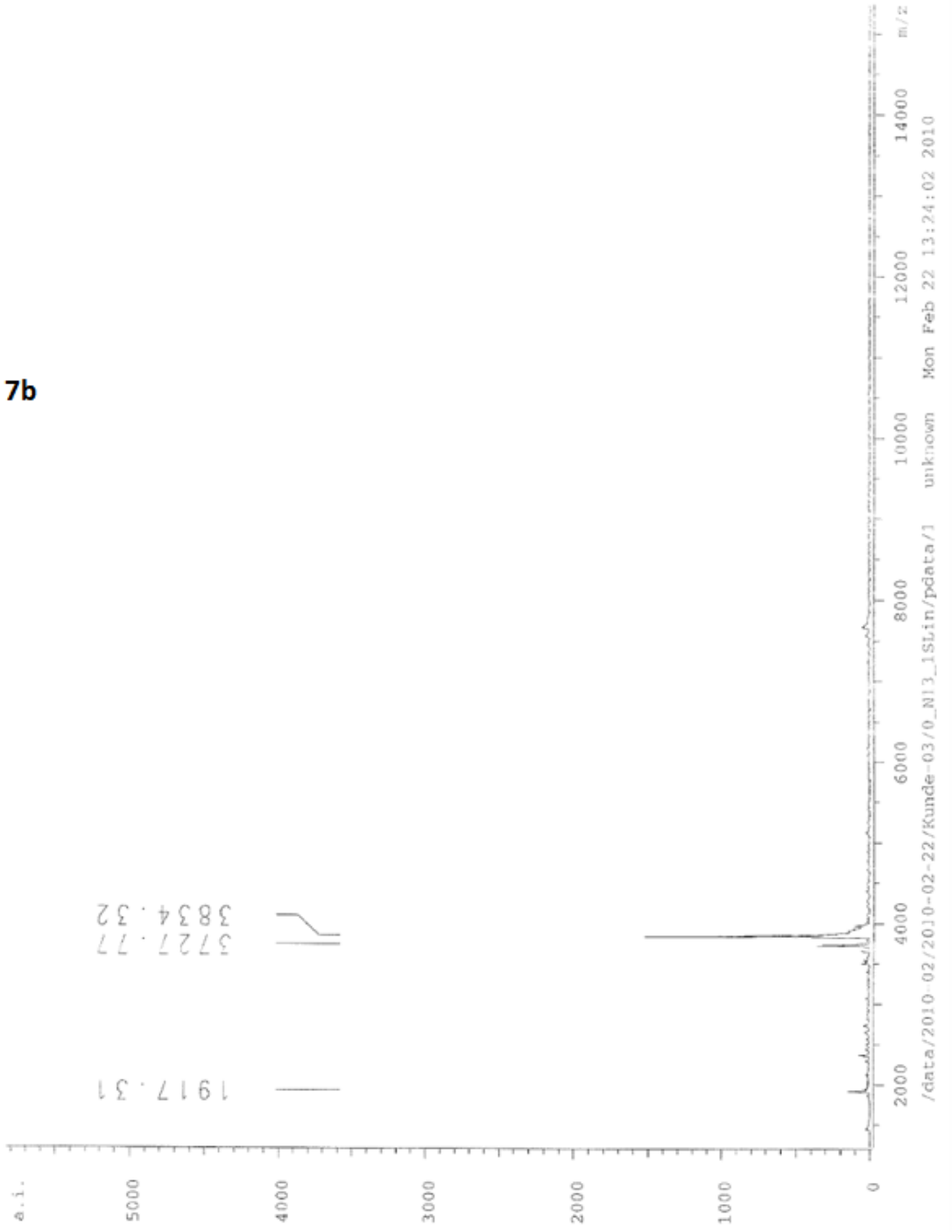
Figure 11 RP-HPLC analysis of crude reaction products (UV absorbance at 260 nm vs time) **10b(v)/11b(v)**

MALDI Analysis

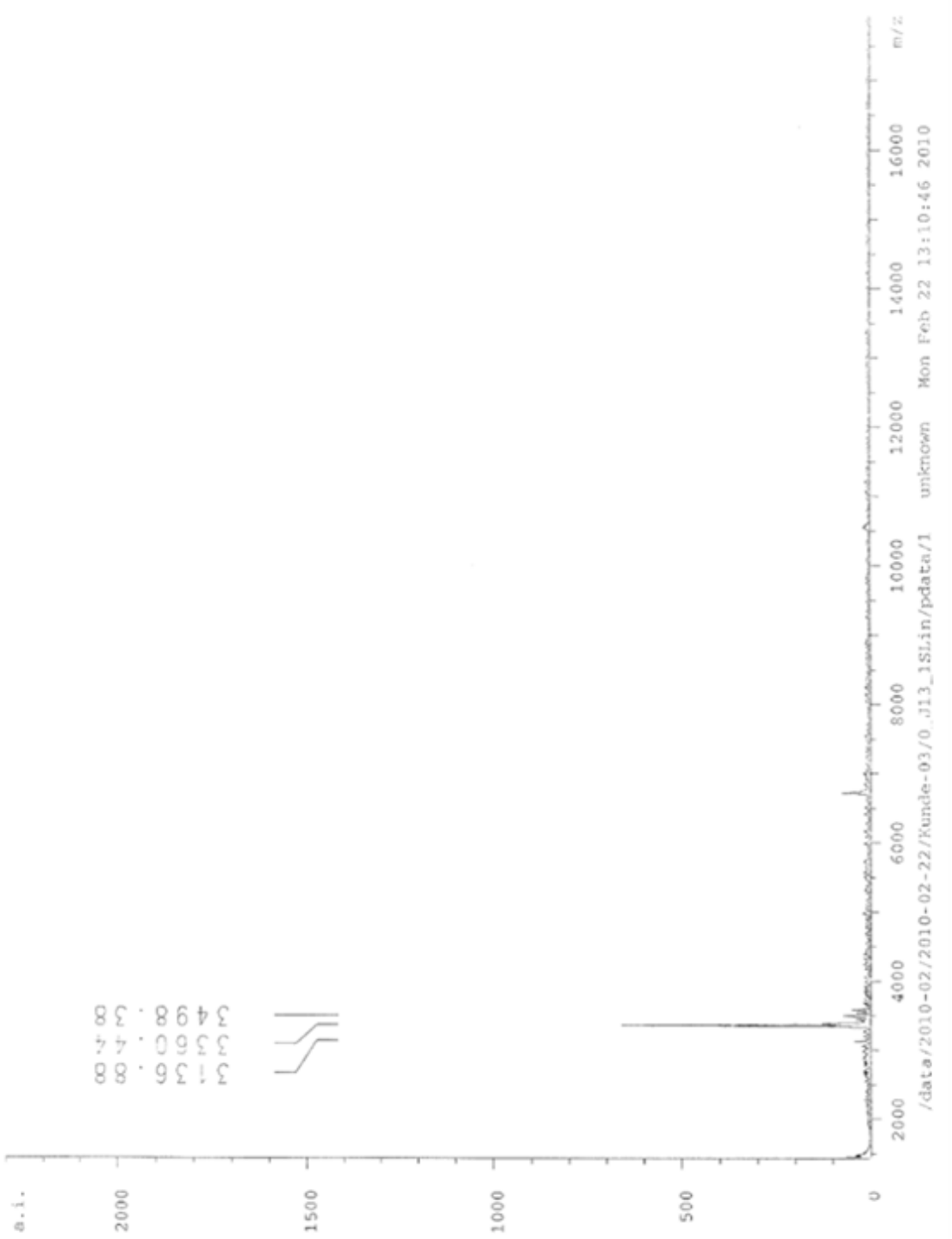
Compound no	Mass calcd.	Mass found
7a	3238	3238
7b	3833	3834
10a(i)/11a(i)	3357	3360
10a(ii)/11a(ii)	3407	3408
10a(iii)/11a(iii)	3375	3372
10a(iv)/11a(iv)	3481	3478
10a(v)/11a(v)	3445 $[M+NH_4]^+$	3443$[M+NH_4]^+$
10b(i)/11b(i)	3952	3953
10b(ii)/11b(ii)	4002	3997
10b(iii)/11b(iii)	3970	3967
10b(v)/11b(v)	4012	4006



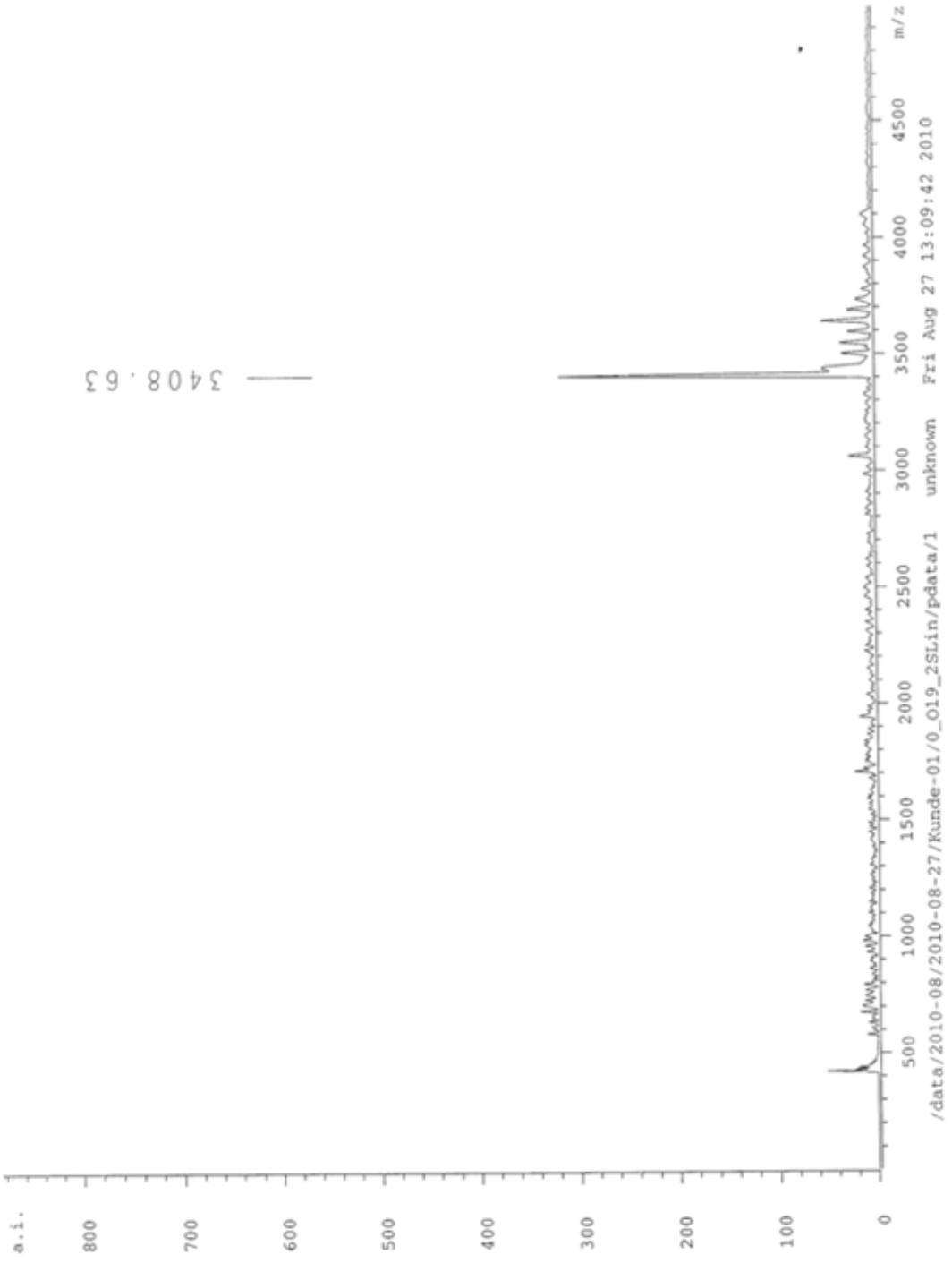
MALDI TOF MS of crude **7a**



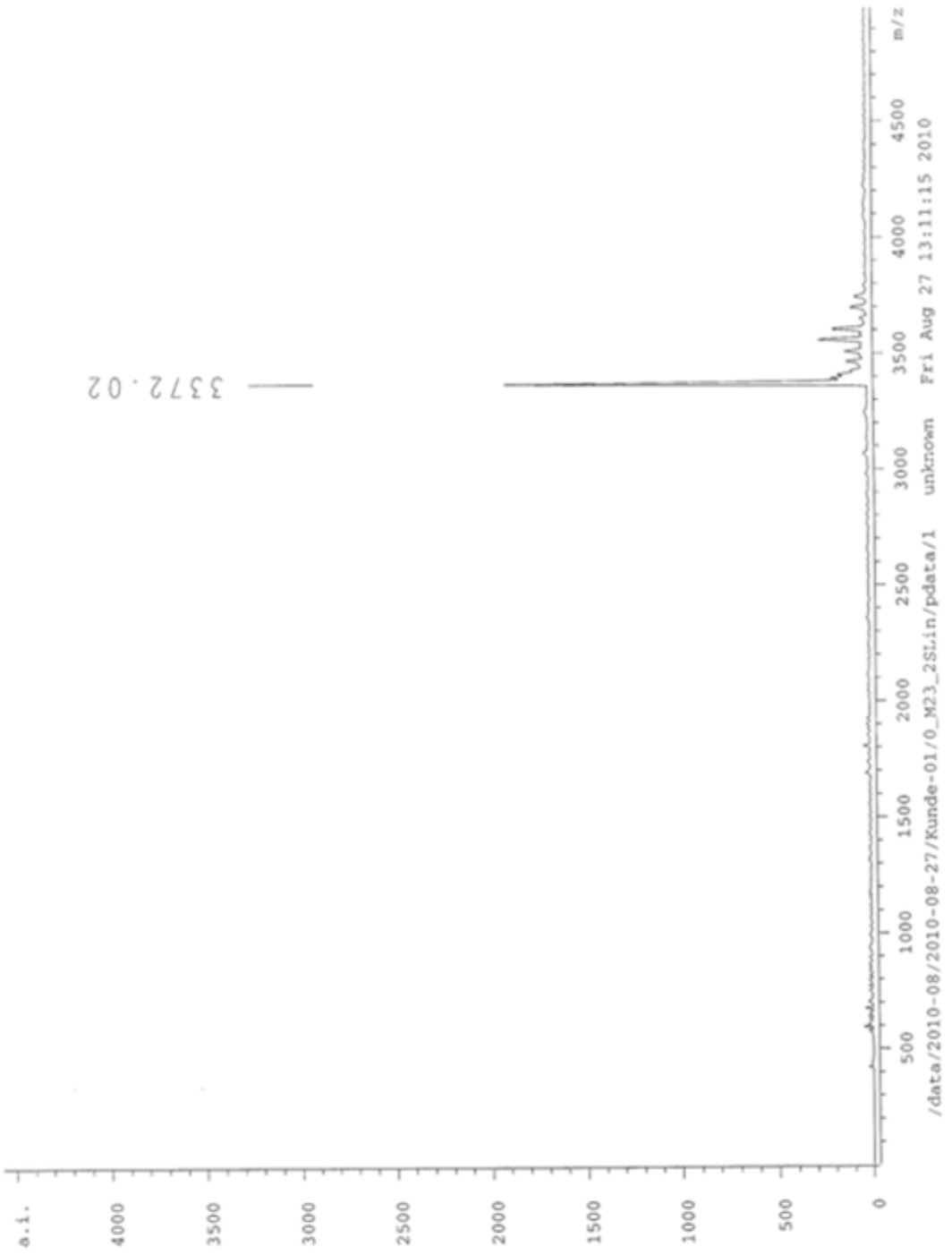
MALDI TOF MS of crude **7b**



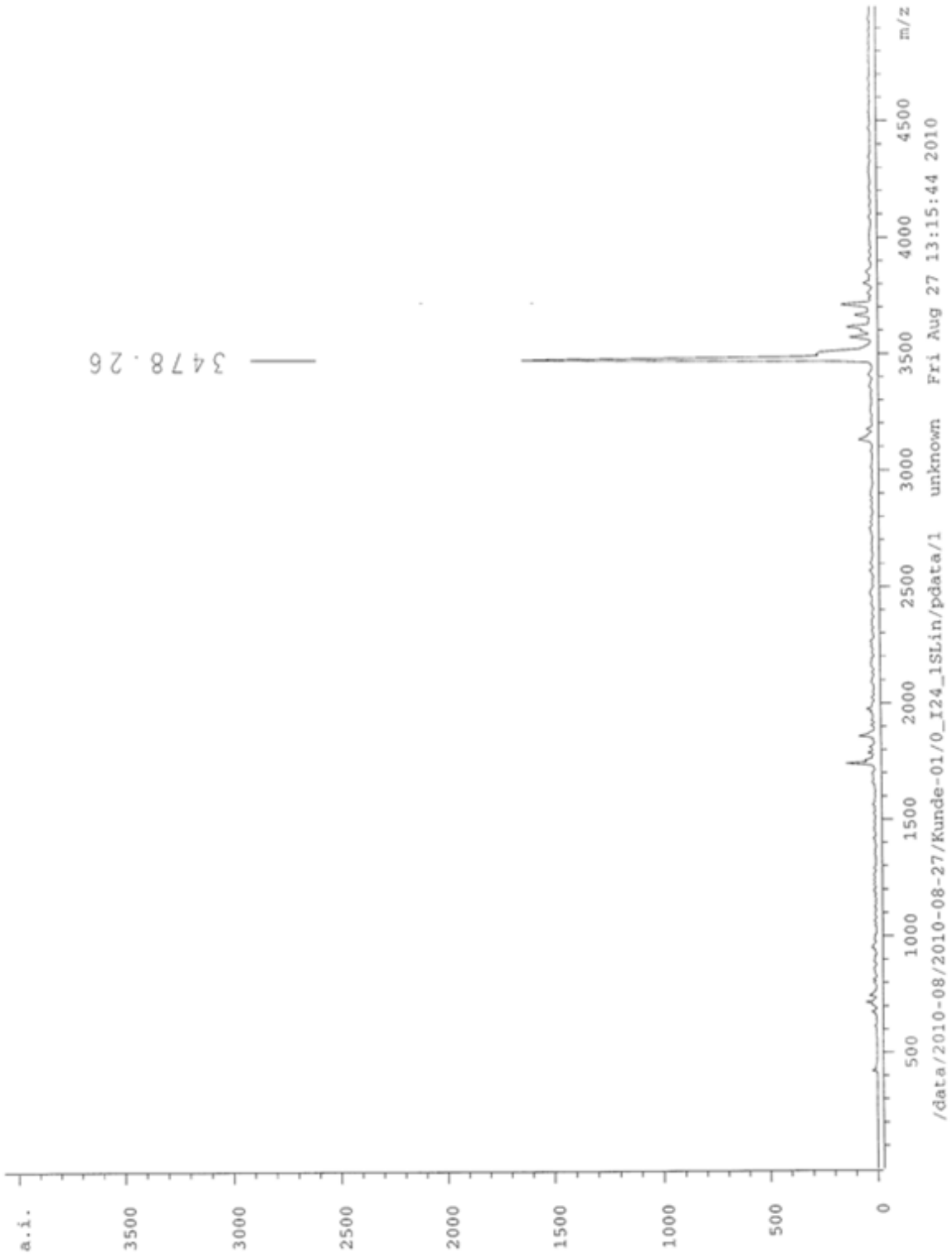
MALDI TOF MS of crude **10/11a(i)**



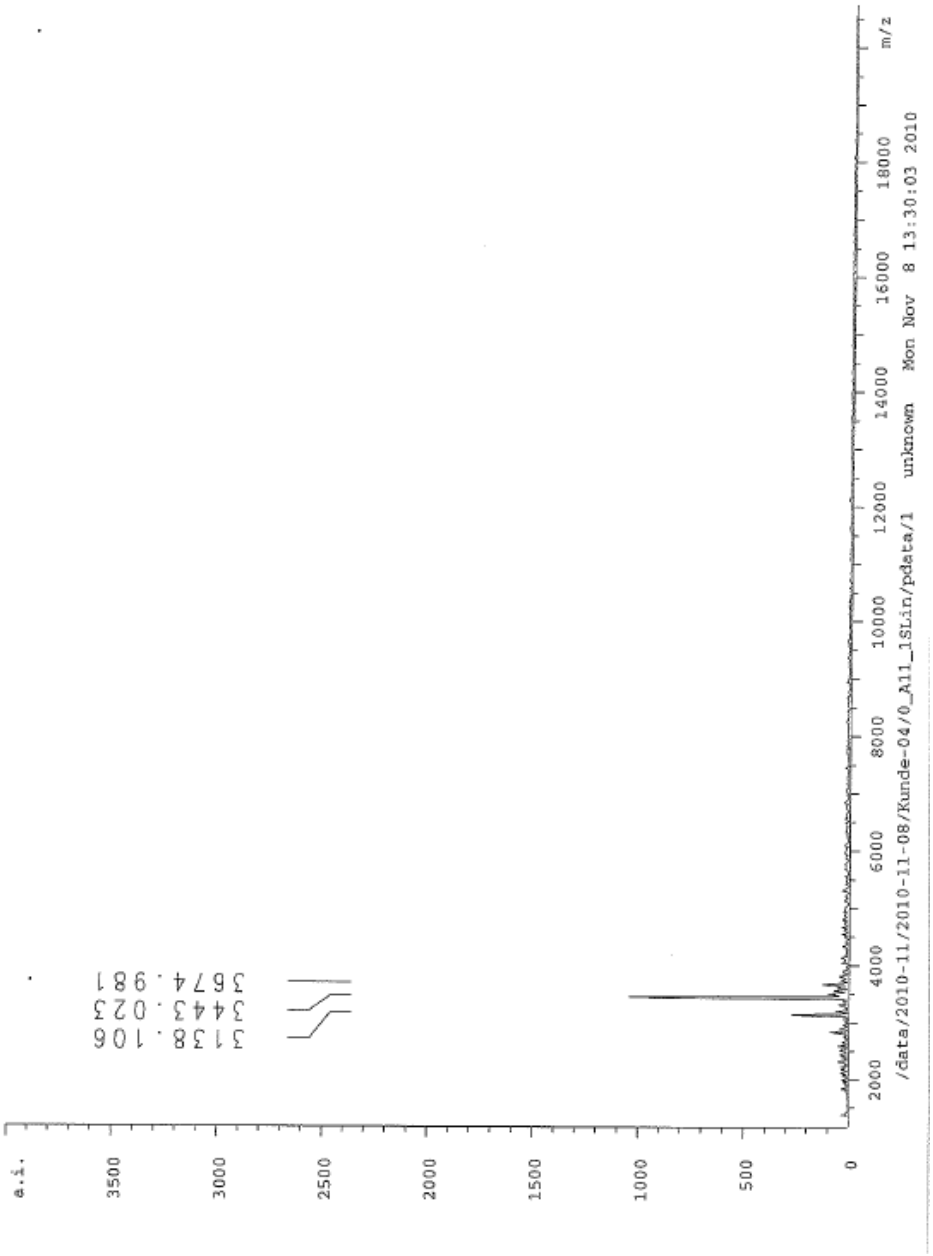
MALDI TOF MS of crude **10/11a(ii)**



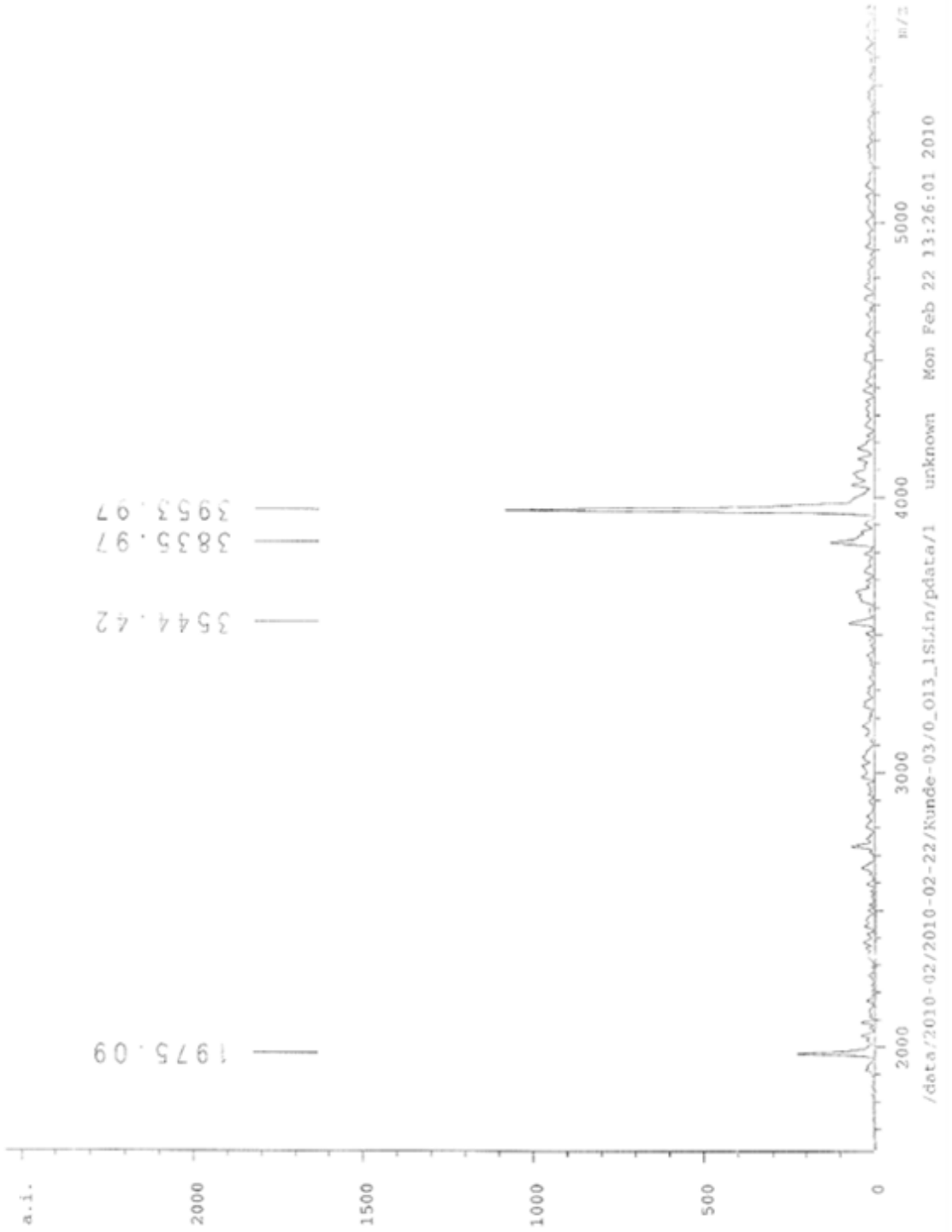
MALDI TOF MS of crude **10/11a(iii)**



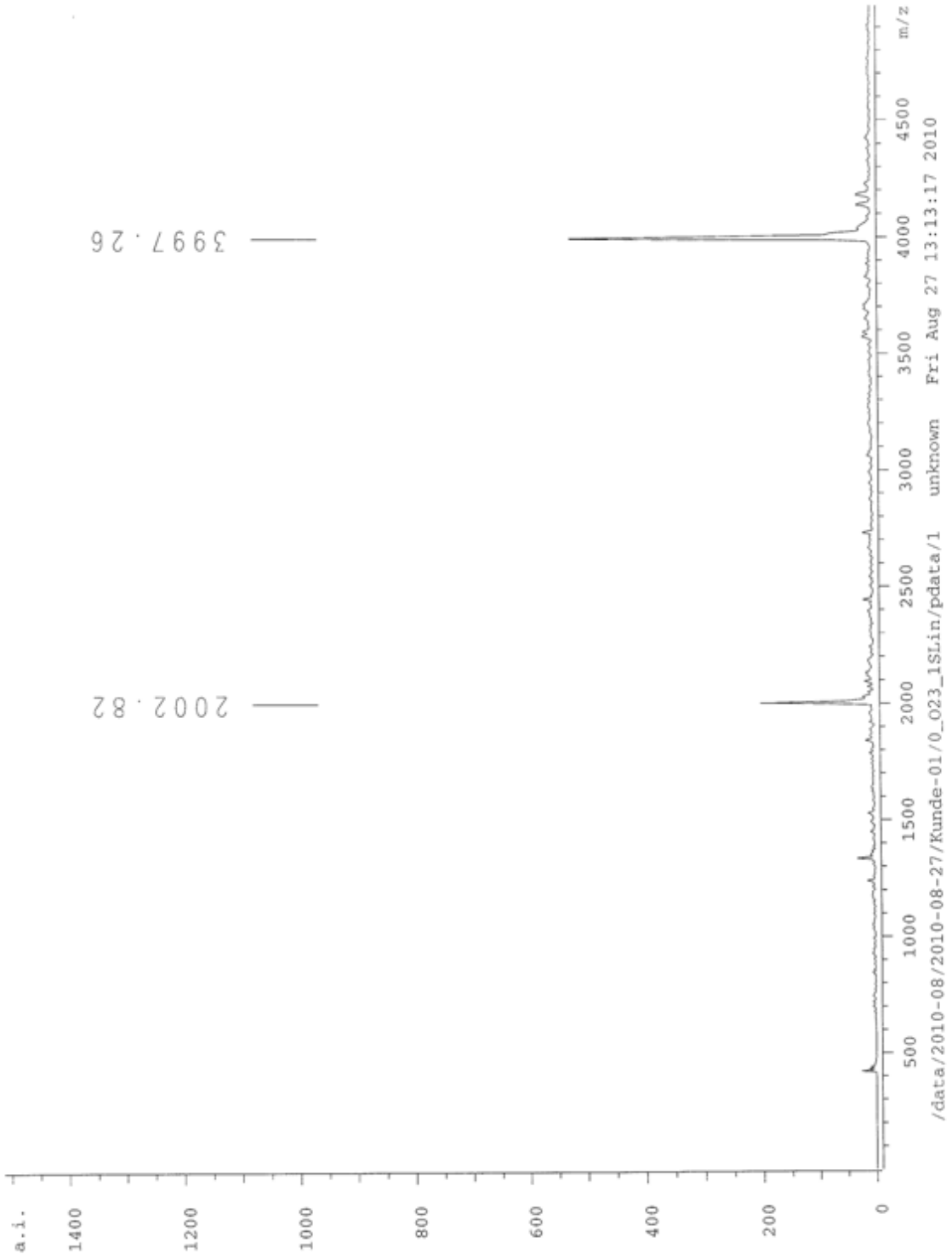
MALDI TOF MS of crude **10/11a(iv)**



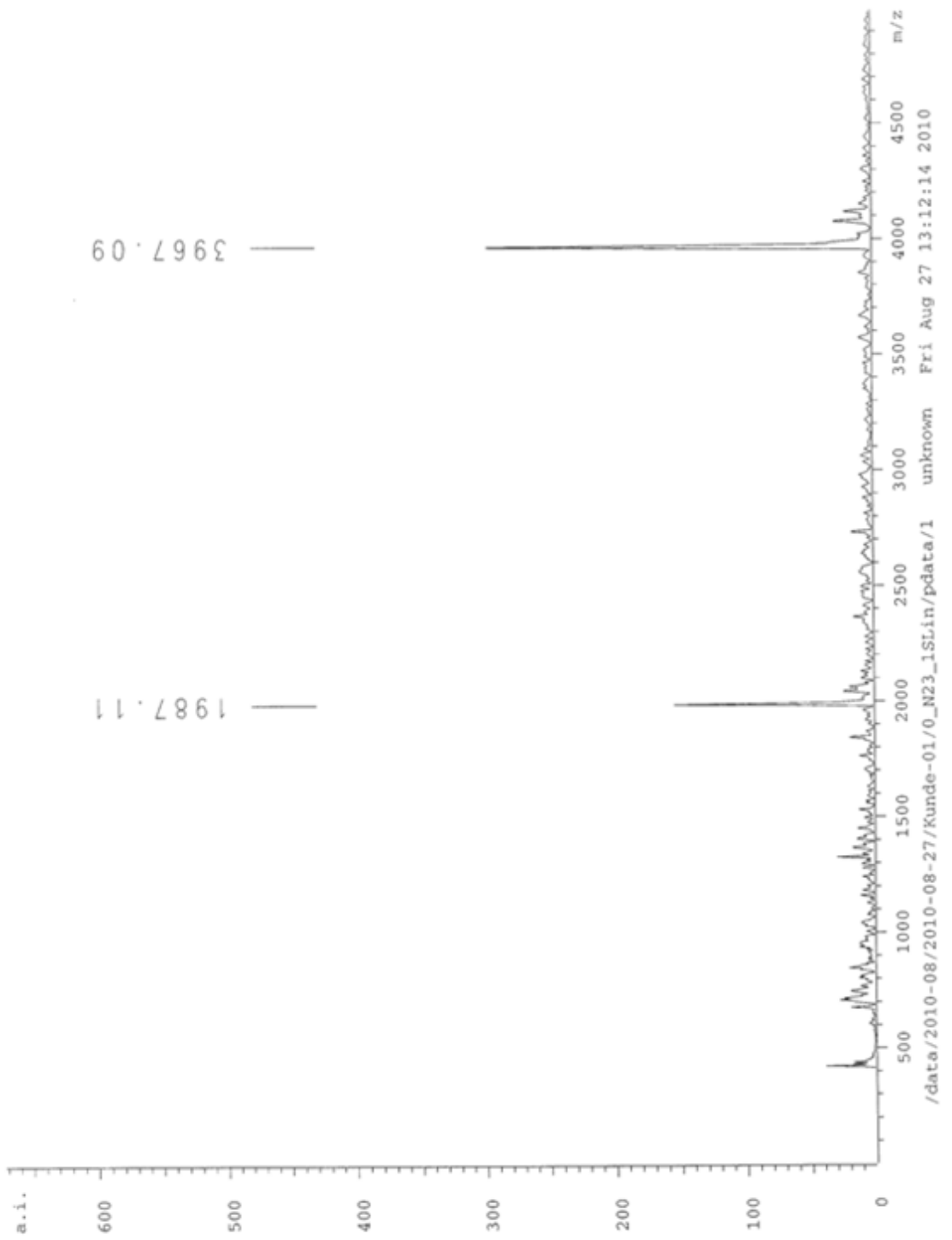
MALDI TOF MS of crude **10/11a(v)**



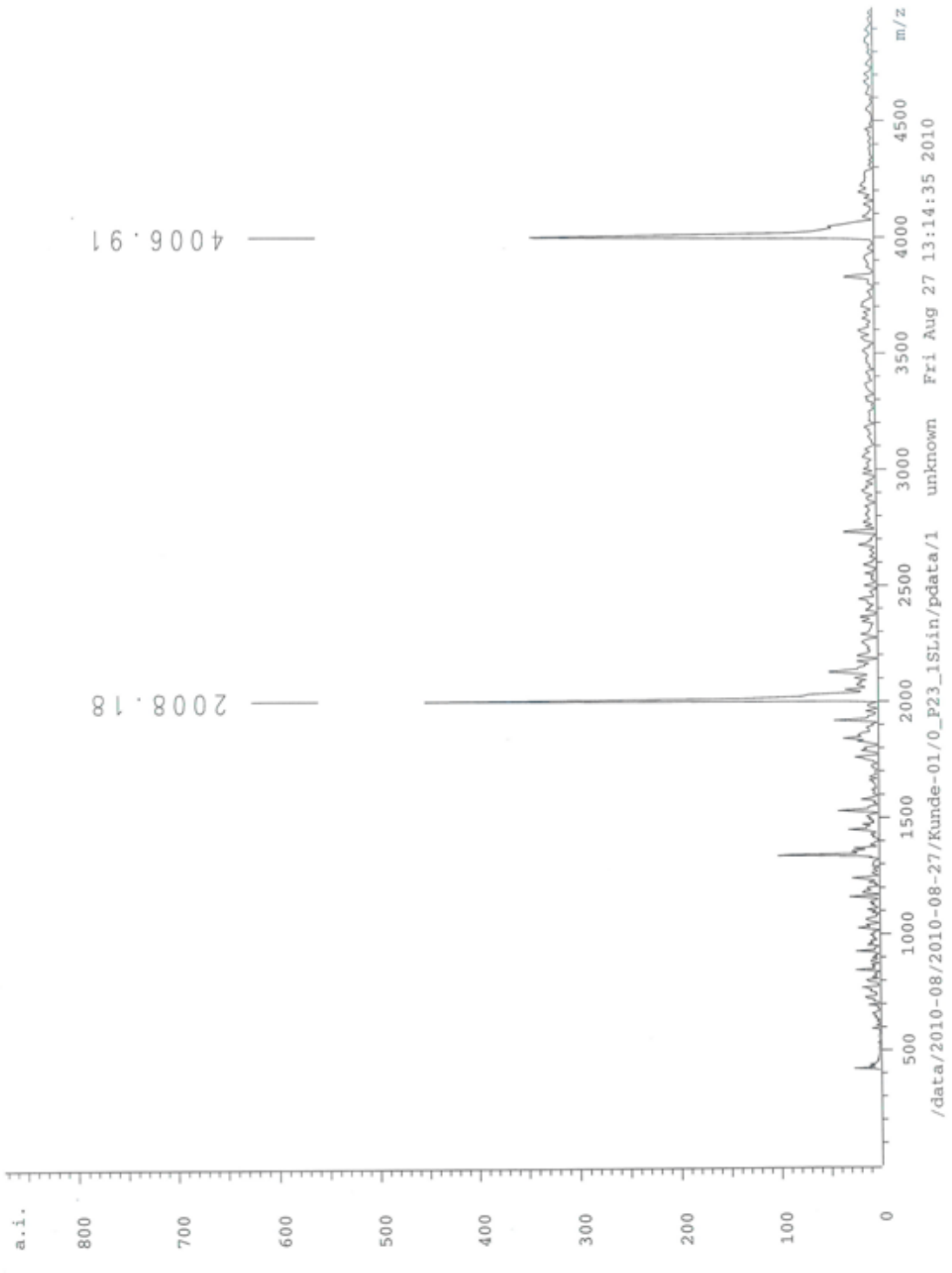
MALDI TOF MS of crude **10/11b(i)**



MALDI TOF MS of crude **10/11b(ii)**



MALDI TOF MS of crude **10/11b(iii)**



MALDI TOF MS of crude **10/11b(v)**

References

1. L Skattebol and S. Solomon, Organic Syntheses, 1973. **Coll. Vol. 5**: p. 306-310.