Electronic Supplementary Material (ESI) for Chemical Communications

A fluorogenic probe for recognizing 5-hydroxylysine inspired from serine/threonine ligation

Chun Ling Tung,[+] Hiu Yung Lam,[+] Jianchao Xu, and Xuechen Li*

Department of Chemistry, The University of Hong Kong, Hong Kong

Materials and Methods

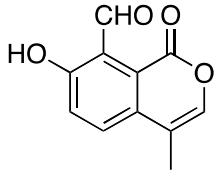
All commercial materials (Aldrich, Fluka and GL Biochem) were used without further purification. All solvents were reagent grade or HPLC grade (RCI or DUKSAN). Dry dichloromethane (CH_2Cl_2) was distilled from calcium hydride (CaH_2). HPLC separations were performed with a Waters HPLC system equipped with a photodiode array detector (Waters 2996) using a Vydac 218TP^{TM} C18 column (5 μm, 4.6 x 250 mm) at a flow rate of 0.6 mL/min for analytical HPLC and Vydac 218TP^{TM} column (10 μm, 22 x 250 mm) at a flow rate of 10 mL/min for preparative HPLC. All separations involved a mobile phase of 0.05% TFA (*v/v*) in acetonitrile (solvent A)/0.05% TFA (*v/v*) in water (Solvent B). Low-resolution mass spectral analyses were performed with a Waters 3100 mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 300 FT-NMR Spectrometer at 300 Hz for ^{1}H NMR and 75.47 MHz for ^{13}C NMR or Bruker Avance DRX 400 FT-NMR spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Fluorescence was recorded on HITACHI F-7000 Fluorescence Spectrophotometer.

General procedure of solid-phase peptide synthesis of compound 12, 13, 14 and 15 according to Fmoc-strategy

Synthesis was performed manually on 2-chlorotrityl chloride Resin (resin loading: 0.4 mmol/g). Peptides were synthesized under standard Fmoc/*t*Bu protocols. The deblock mixture was a mixture of 20/80 (*v/v*) of piperidine/DMF. The following Fmoc amino acids were employed: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Hyl(Boc)(Psi(Me, Me)pro)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Tyr(*t*Bu)-OH. Upon completion of synthesis, the peptide resin was subjected to a cleavage cocktail of TFA/TIPS/H_2O (95:2.5:2.5, *v/v/v*) and stirred at room temperature for 2 hours. The resin was filtered

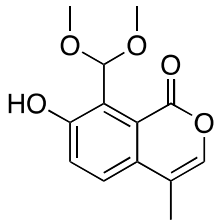
and the combined filtrates were blown off under a stream of condensed air. The crude product was triturated with cold diethyl ether to give a white suspension, which was centrifuged and the ether subsequently decanted. The remaining solid was purified by reverse phase preparative HPLC followed by concentration at reduced pressure and lyophilization to give a white powder (35% to 67%, base on resin loading).

Compound **7:**



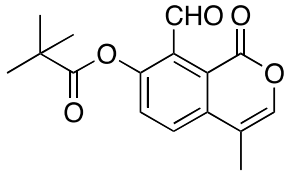
Compound **7** was prepared by typical Duff reaction. A mixture of 4 – methyl – umbelliferone (2.0 g, 11.36 mmol) and hexamine (4.0 g, 28.53 mmol) in 40 mL TFA was heated at 110 ^{o}C for 12 hours. 60 mL 10% HCl was then added and the mixture was heated at 110 ^{o}C for another 2 hours. The mixture was diluted with AcOEt (250 mL), washed with saturated $NaHCO_3$ solution (100 mL x 3) and brine (100mL x 1). The organic phase was dried over Na_2SO_4 and concentrated under *vacuo*. The mixture was purified by flash column chromatography on silica gel (hexane/AcOEt, 3:2) to give compound **11a** (320 mg, 25%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.20 (1H, broad s, O**H**), 10.61 (1H, s, C**H**O), 7.73 (1H, d, J = 9.0 Hz, Ar **H**), 6.90 (1H, d, J = 9.0 Hz, Ar **H**), 6.20 (1H, s, C=C**H**), 2.43 (3H, s, C**H**$_3$) ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.4, 165.2, 159.2, 156.1, 152.6, 132.9, 114.3, 112.1, 111.9, 108.6, 18.9 ESI MS calcd. For $C_{11}H_9O_4$ [M^+] 205.18 found 205.55

Compound **8**:



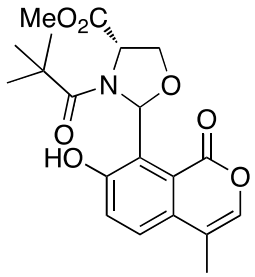
Compound **7** (245 mg, 1.20 mmol) was dissolved in 25 mL of anhydrous MeOH. $LiBF_4$ (1 mg, 0.01 mmol) and trimethylorthoformate (637 mg, 6.00 mmol) were added. The reaction mixture was heated under reflux for 4 h. The reaction mixture was diluted with AcOEt (250 mL), washed with saturated $NaHCO_3$ solution (100 mL x 3) and brine (100mL x 1). The organic phase was dried over Na_2SO_4 and concentrated under *vacuo* to give compound **8** without further purification (180 mg, 60%)

Compound **10**:



To a solution of **8** (110 mg, 0.44 mmol) and trimethylacetic acid (224 mg, 2.20 mmol) in 5 mL anhydrous CH_2Cl_2, EDCI (543 mg, 2.86 mmol) and DMAP (5 mg, 0.04 mmol) were added. The mixture was stirred at room temperature for 12 hours. The mixture was diluted with AcOEt (25 mL), washed with saturated $NaHCO_3$ solution (10 mL x 3) and brine (10 mL x 1). The organic phase was dried over Na_2SO_4 and concentrated under *vacuo*. The mixture was purified by flash column chromatography on silica gel (hexane/AcOEt, 2:1) to give compound **9** (142 mg, 97%). Compound **9** was treated with 5 mL of 95% TFA for 30 min. The solvent was blown off under a stream of condensed air. The crude product was purified by flash column chromatography on silica gel (hexane/AcOEt, 2:1) to give compound **10** (86 mg, 70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.64 (1H, s, C**H**O), 7.80 (1H, d, J = 8.6 Hz, Ar **H**), 7.02 (1H, d, J = 8.6 Hz, Ar **H**), 6.35 (1H, s, C=C**H**), 2.47 (3H, s, C**H$_3$**), 1.41 (9H, s, C(C**H$_3$**)$_3$) ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.1, 176.2, 158.7, 155.9, 152.6, 151.7, 130.1, 119.6, 118.3, 116.7, 114.8, 39.3, 27.1, 19.0 ESI MS calcd. For $C_{16}H_{16}O_5$ [M^+] 289.30 found 289.52

Compound **11**:



Compound **10** (9 mg, 0.03 mmol) and HCl · NH_2 – Ser – OMe (6 mg, 0.04 mmol) was dissolved in 6 mL Pyr/HOAc (1:1, mol/mol). The reaction mixture was stirred at room temperature. A 25 μL aquilot reaction solution at different time points during the reaction process was diluted with Pyr/HOAc (1:1, mol/ mol) into 2 mL and subjected to Fluorescence spectrometer. The fluorescence intensity was recorded at λ_{ex} = 320 nm. Upon completion of the reaction, the mixture was diluted with AcOEt (25 mL), washed with 1N HCl (10 mL x 3) and brine (10 mL x 1). The organic phase was dried

over Na_2SO_4 and concentrated under *vacuo*. The mixture was purified by flash column chromatography on neutral Aluminum oxide (CH_2Cl_2/ MeOH, 20:1) to give compound **14** (8 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (1H, d, J = 9.7 Hz, Ar **H**), 6.90 (1H, s, C**H** of oxazolidine), 6.87 (1H, d, J = 8.8 Hz, Ar **H**), 6.11 (1H, s, C=C**H**), 5.17 (2H, m, β**H** – Ser), 4.34 (1H, m, α**H** – Ser), 3.85 (3H, s, OC**H**$_3$), 2.38 (3H, s, C=C(C**H**$_3$)), 1.17 (9H, s, C(C**H**$_3$)$_3$) ESI MS calcd. For $C_{20}H_{24}NO_7$ [M^+] 390.40 found 390.14

General procedure of peptide reacting with probe 10:

Peptide was dissolved in Pyr/HOAc (1:1, mol/ mol) at a concentration of 5 mM. Compound **10** (1.2 equiv.) was added. The reaction mixture was stirred at room temperature. A 20 μL aquilot reaction solution at different time points during the reaction process was diluted with water into 2 mL and subjected to Fluorescence spectrometer. The fluorescence intensity was recorded at λ_{ex} = 320 nm.

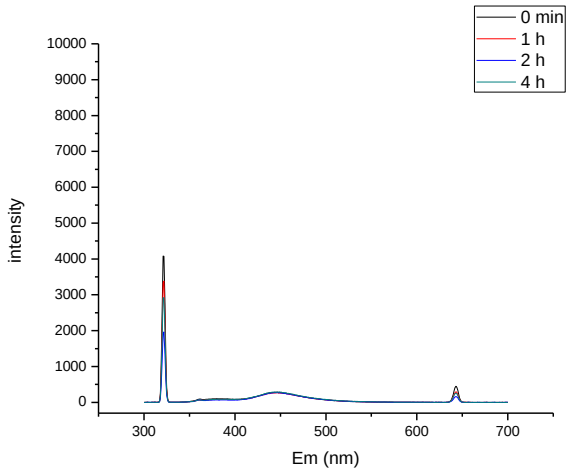


Fig. S1 Fluorescence spectrum of peptide **13** reacting with probe **10**

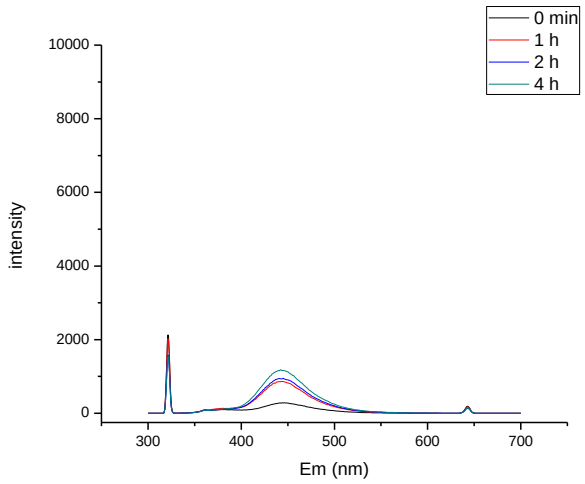


Fig. S2 Fluorescence spectrum of peptide **14** reacting with probe **10**

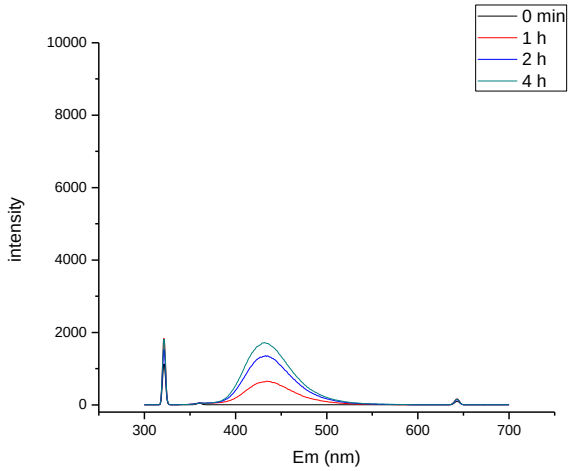


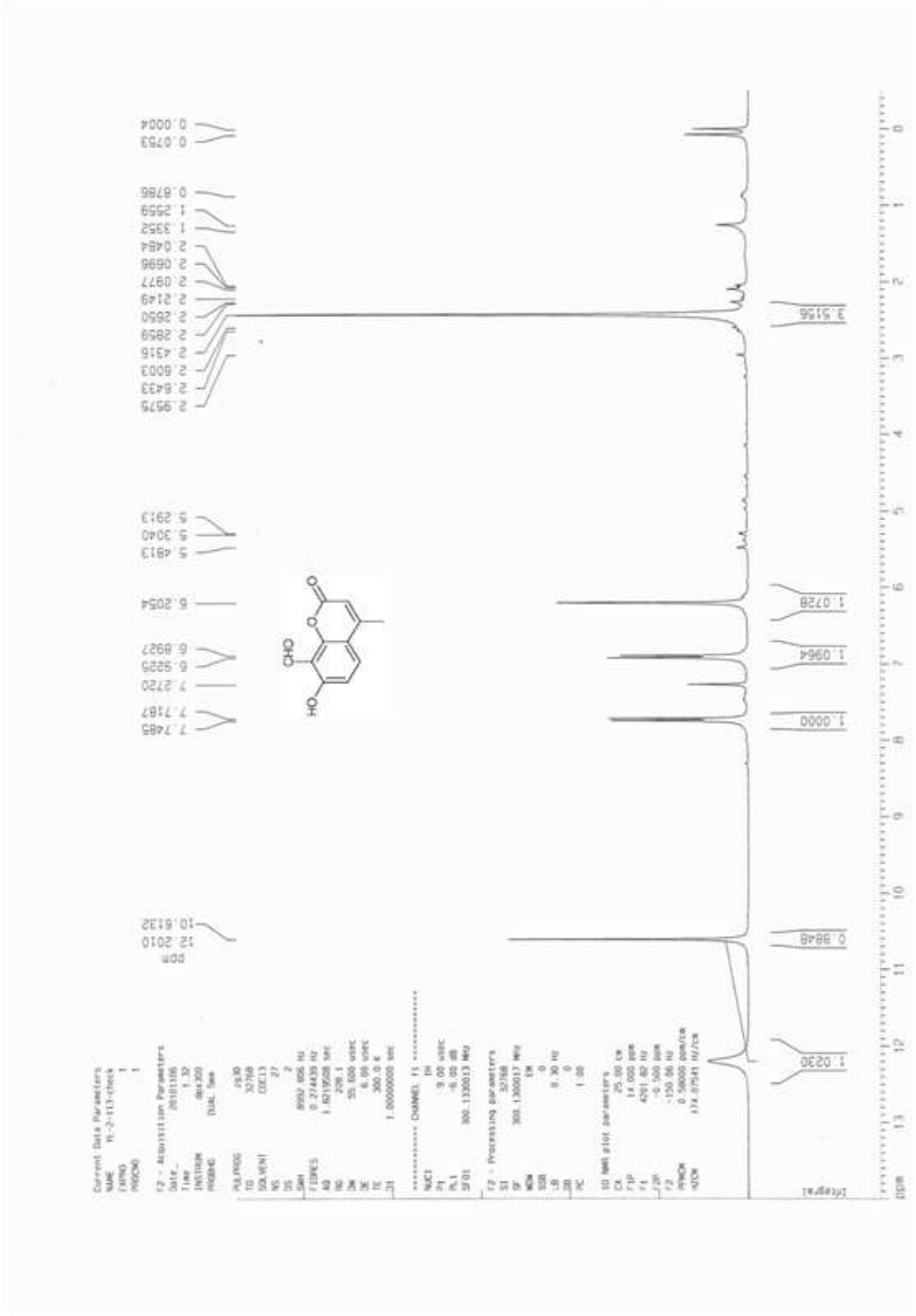
Fig. S3 Fluorescence spectrum of peptide **15** reacting with probe **10**

General procedure of fluorescence detection limit of peptide reacting with probe 10:

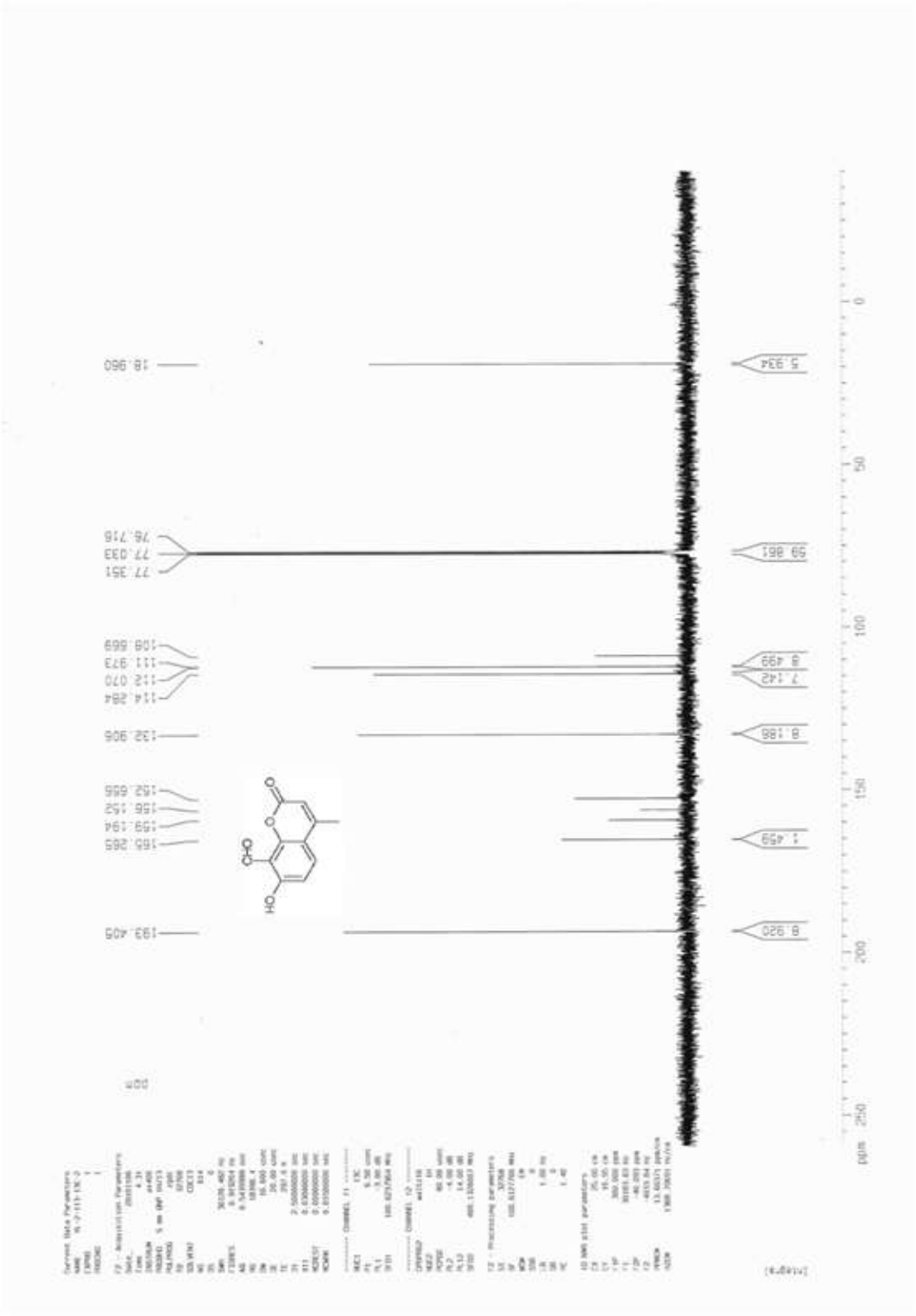
Stock solutions of peptide at concentration of 0.001 mM, 0.01 mM, 0.1 mM, 1 mM and 5 mM were prepared by dissolving in Pyr/HOAc (1:1, mol/mol). Compound **10** was added to the peptide stock solutions at a concentration of 5 mM. The reaction mixture was stirred at room temperature for 2 hours. A 20 µL aquilot reaction solution was diluted with water into 2 mL and subjected to Fluorescence spectrometer. The fluorescence intensity was recorded at λ_{ex} = 320 nm.

^{1}H and ^{13}C NMR spectra

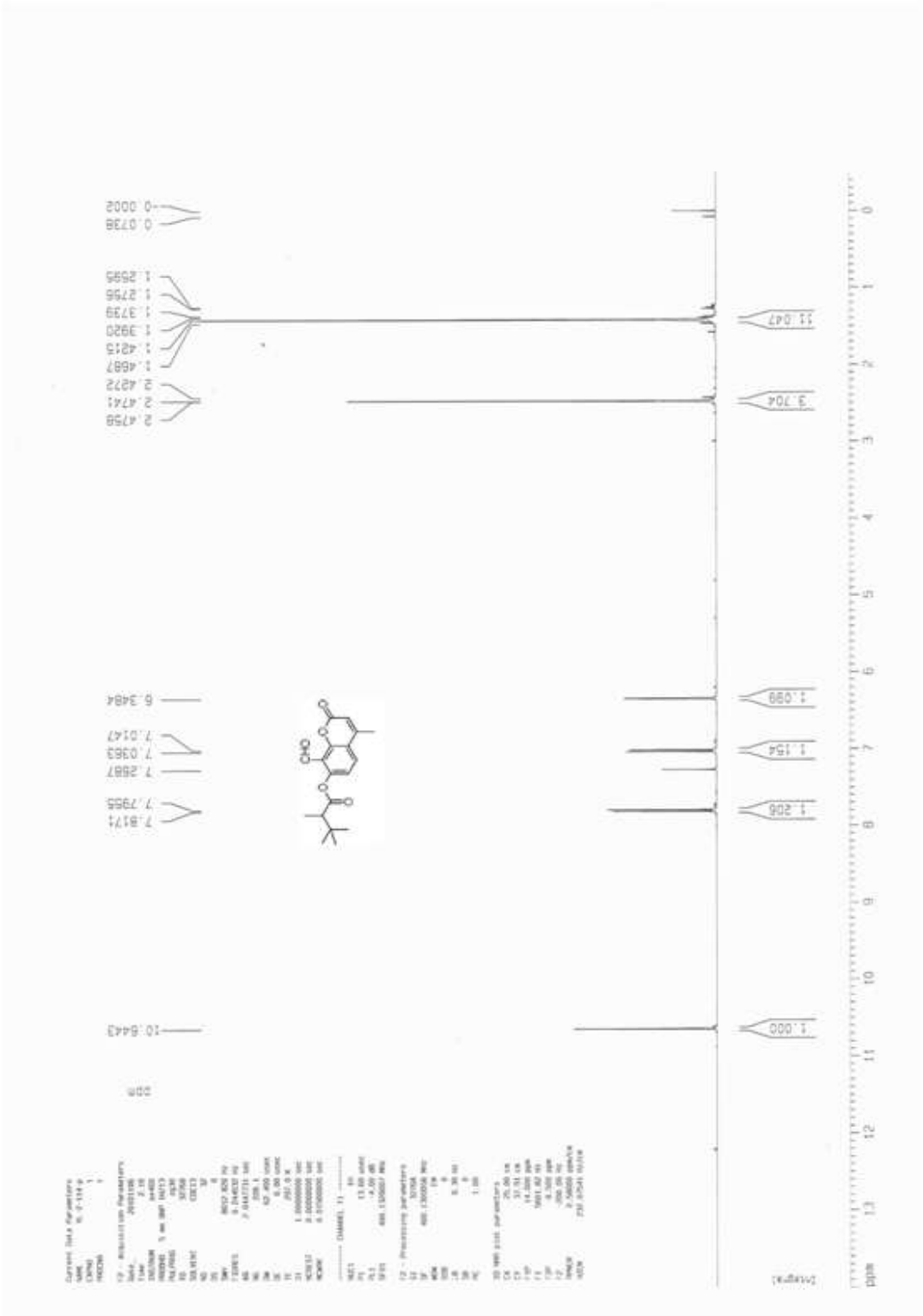
^{1}H NMR spectrum for compound **7**



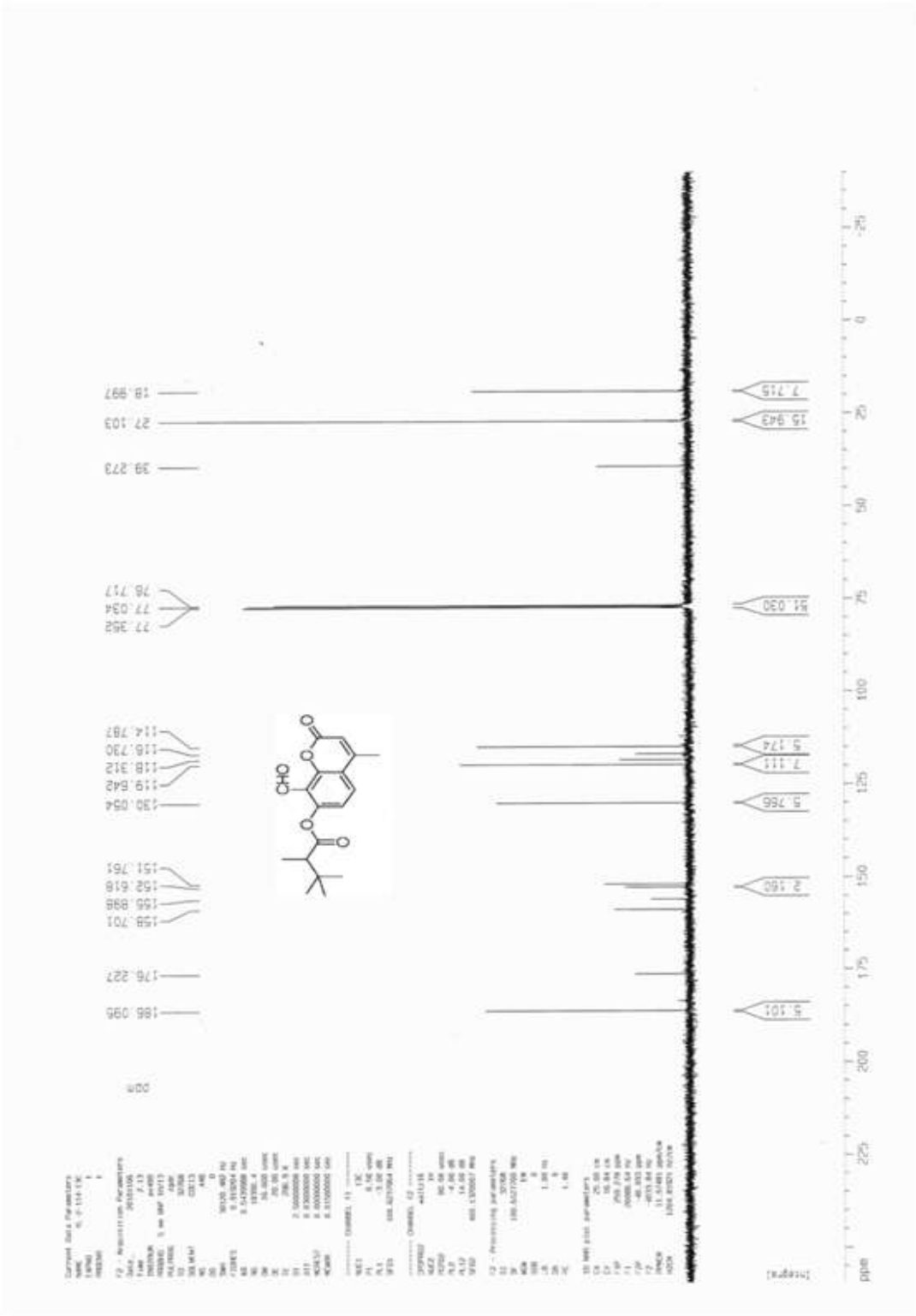
^{13}C NMR spectrum for compound **7**



^{1}H NMR spectrum for compound **10**



^{13}C NMR spectrum for compound **10**



^{1}H NMR spectrum for compound **11**