Supporting Information

The first ratiometric fluorescent probe for aminopeptidase N

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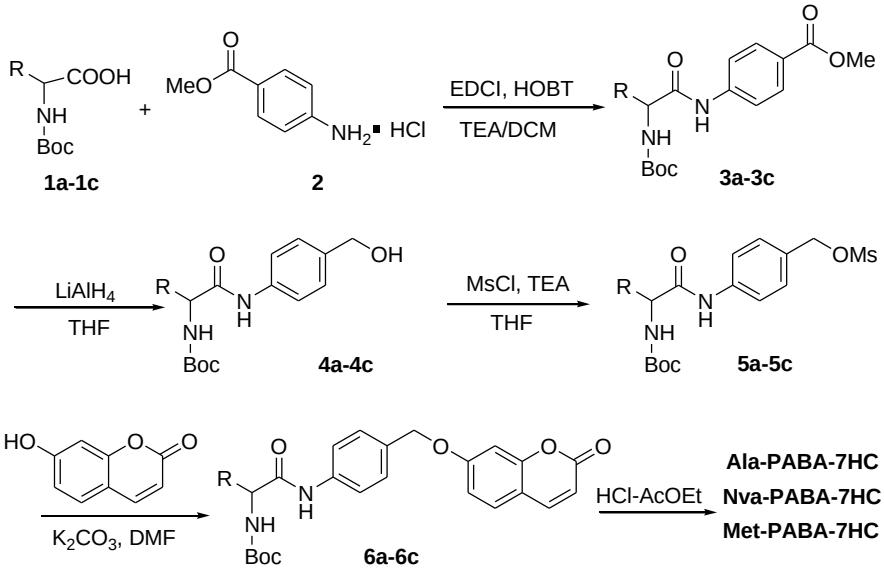
1. Materials and instruments

All reagents were purchased from Acros and Aldrich. Twice-distilled water was used throughout all experiments. ^{1}H NMR, ^{13}C NMR were recorded on a Bruker 300 NMR, 400 NMR, 600 NMR spectrometer. Mass spectra were performed by the analytical and the mass spectrometry facilities at Shandong University.

2. Synthesis



Scheme S1. Synthesis of probe **Ala-PABA-7HC, Nva-PABA-7HC, Met-PABA-7HC.**

1a-1c and **2** were synthesized according to literature procedures[1].

Methyl 4-(2-(tert-butoxycarbonyl)propanamido)benzoate (3a). To a stirred solution of Boc-Ala (**1a**) (1.0 g, 5.5 mmol) in 50 mL DCM was added HOBT (0.75 g, 5.5 mmol) and EDCI (1.05 g, 5.5 mmol) at 0°C. After 30 min, **2** (0.93 g, 5 mmol) was added to the solution rapidly and the mixture was stirred at room temperature for 10 h. Saturated citric acid was added to the mixture. The produced precipitation was filtered. The organic layer was washed by saturated citric acid (50 mL*3), saturated $NaHCO_3$ (50 mL*3), brine (50 mL*1) and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then recrystallized with AcOEt-*n*-hexane to yield a white solid, 0.94 g, yield = 85.4%, mp: 167.9-170.4°C.

Methyl 4-(2-(tert-butoxycarbonyl)pentanamido)benzoate (3b). The synthesis was analogous to **3a** with Boc-Nva (**1b**) as the starting material. The product was obtained as a white solid. Yield = 69.1%, mp: 149.8-151.0°C.

methyl 4-(2-(tert-butoxycarbonyl)-4-(methylthio)butanamido)benzoate (3c). The synthesis is analogous to **3a** with Boc-Met (**1c**) as the starting material. The product was obtained as a white solid. Yield = 68.0%, mp: 100.1-102.3°C.

***tert*-butyl 1-(4-(hydroxymethyl)phenylamino)-1-oxopropan-2-ylcarbamate (4a)**.

Compound **3a** (0.63 g, 2.0 mmol) was dissolved in THF (20 mL) and slowly added $LiAlH_4$ (0.23 g, 6 mmol) at 0℃. After stirring for 1h at room temperature, the mixture was adjusted pH to 7 with 2M HCl. After filtration, the filtrate was concentrated with a rotary evaporator. The residue was dissolved in AcOEt and washed with brine. The AcOEt solution was dried over Na_2SO_4, concentrated with a rotary evaporator and purified by combiflash to yield a white solid, 0.34 g, yield = 68.0%, mp: 140.1-142.2°C.

***tert*-butyl 1-(4-(hydroxymethyl)phenylamino)-1-oxopentan-2-ylcarbamate (4b).** The synthesis was analogous to **4a** with (**3b**) as the starting material. The product was obtained as colorless oil, yield = 59.0%.

***tert*-butyl 1-(4-(hydroxymethyl)phenylamino)-4-(methylthio)-1-oxobutan-2-yl carbamate (4c).** The synthesis was analogous to **4a** with **3c** as the starting material. The product was obtained as colorless oil, yield = 61.1%.

4-(2-(*tert*-butoxycarbonyl)propanamido)benzyl methanesulfonate (5a). To the stirred solution of **4a** (0.54 g,1.84 mmol) in anhydrous 50 mL THF was added triethanolamine (0.77 mL, 5.5 mmol) and methanesulfonyl chloride (0.43 mL, 5.5 mmol) at 0°C, the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure and redissolved in 50 mL AcOEt, then washed by 0.5 M HCl (50 mL*3), brine (50 mL*1) and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure to yield a white solid, 0.50 g, yield 72.8%, mp: 142.7-144.7°C.

4-(2-(*tert*-butoxycarbonyl)pentanamido)benzyl methanesulfonate (5b). The synthesis was analogous to **5a** with **4b** as the starting material. The product was obtained as obtained as brown oil, yield = 65.2%.

4-(2-(*tert*-butoxycarbonyl)-4-(methylthio)butanamido)benzyl methanesulfonate (5c). The synthesis was analogous to **5a** with **4c** as the starting material. The product was obtained as brown oil, yield = 80.1%.

***tert*-butyl 1-oxo-1-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenylamino)propan-2-yl carbamate (6a).** Anhydrous K_2CO_3 was added to the solution of 7-hydroxycoumarin (0.07 g, 0.45 mmol) in 10 mL DMF at room temperature, the mixture was stirred for 15 min. **5a** was added to the mixture and stirred at room temperature overnight. 50 mL AcOEt was added to the mixture, the organic solution was washed with 10% aqueous K_2CO_3 solution (50 mL*5), brine (50 mL*1) and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then recrystallized with AcOEt-*n*-hexane to yield a white solid, 0.05 g, yield = 38.0%, mp:192.3-194.4°C.

***tert*-butyl 1-oxo-1-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenylamino)pentan-2-yl carbamate (6b).** The synthesis was analogous to **6a** with **5b** as the starting material. The product was obtained as a white solid, yield = 21.5%, mp: 177.5-179.6°C.

tert-butyl 4-(methylthio)-1-oxo-1-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl amino)butan-2-ylcarbamate (6c). The synthesis is analogous to **6a** with **5c** as the starting material. The product was obtained as a white solid, yield = 20.0%, mp: 128.0-129.0°C.

2-amino-N-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl)propanamide hydrochloride (Ala-PABA-7HC). 6a (0.05 g, 0.11 mmol) in 5 mL EtOAc solution saturated with HCl gas was stirred at room temperature for 2 h. The mixture was filtered to obtain a white solid, 0.04 g, yield = 98.5%, mp: 234.2-235.7°C. ^{1}H-NMR (D_2O, 400 MHz): δ 7.82 (d, 1 H, J = 9.4 Hz), 7.40-7.46 (m, 5 H,), 6.91 (dd, 1 H, J = 8.7 Hz, 2.0 Hz,), 6.82 (s, 1 H), 5.03 (s, 2 H), 4.19(q, 1 H, J = 7.1 Hz), 1.60 (d, 3H, J = 7.1 Hz); ^{13}C-NMR (DMSO, 150 MHz): δ 168.0, 161.9, 160.7, 155.8, 144.8, 138.7, 132.0, 130.0, 129.3, 119.8, 113.5, 113.0, 112.9, 102.1, 70.1, 49.4, 17.7; HRMS (ESI) m/z calcd. for $C_{19}H_{19}N_2O_4$ ($[M + H]^+$) 339.1339; found 339.1333.

2-amino-N-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl)pentanamide hydrochloride (Nva-PABA-7HC). The synthesis was analogous to **Ala-PABA-7HC** with **6b** as the starting material. The product was obtained as a white solid, yield = 95.0%, mp: 249.1-250.0°C. ^{1}H-NMR (DMSO, 600 MHz): δ 8.00 (d, 1 H, J = 12 Hz), 7.70 (d, 2 H, J = 6Hz), 7.65 (d, 1 H, J = 6 Hz), 7.47 (d, 1H, J = 6Hz), 7.08 (s, 1 H), 7.02 (d, 2 H, J = 6 Hz), 6.30 (d, 1H, J = 12 Hz), 5.18 (s, 2 H), 4.04 (s, 1 H),1.79-1.82(m, 2 H), 1.35-1.42 (m, 2 H), 0.90(t, 3H, J = 6 Hz); ^{13}C-NMR (DMSO, 150 MHz): δ 168.1, 161.9, 160.7, 155.8, 144.8, 138.5, 132.2, 130.0, 129.2, 119.9, 113.5, 113.1, 113.0, 102.1, 70.1, 53.3, 33.7, 18.2, 14.1; HRMS (ESI) m/z calcd. for $C_{21}H_{23}N_2O_4$ ($[M + H]^+$) 367.1652; found 367.1644.

2-amino-4-(methylthio)-N-(4-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl)butanami de hydrochloride (Met-PABA-7HC). The synthesis was analogous to **Ala-PABA-7HC** with **6c** as the starting material. The product was obtained as a white solid, yield 93.9%, mp:225.7-228.2°C. ^{1}H-NMR (D_2O, 400 MHz): δ 7.89 (d, 1 H, J = 10 Hz), 7.49-7.53 (m, 5 H), 6.95-6.99 (m, 2 H), 6.28 (d, 1H, J = 10 Hz), 5.19 (s, 2 H), 4.22-4.25(m, 1 H), 2.65 (t, 2H, J = 7.2 Hz), 2.28-2.30 (m, 2H), 2.09 (s, 3H); ^{13}C-NMR (DMSO, 150 MHz): 167.5, 161.9, 160.7, 155.8, 144.8, 138.5, 132.2, 130.0, 129.3, 120.0, 113.5, 113.0, 113.0, 102.0, 70.0, 52.8, 31.4, 28.8, 15.0. HRMS (ESI) m/z calcd. for $C_{21}H_{23}N_2O_4S$ ($[M + H]^+$) 399.1373; found 399.1375.

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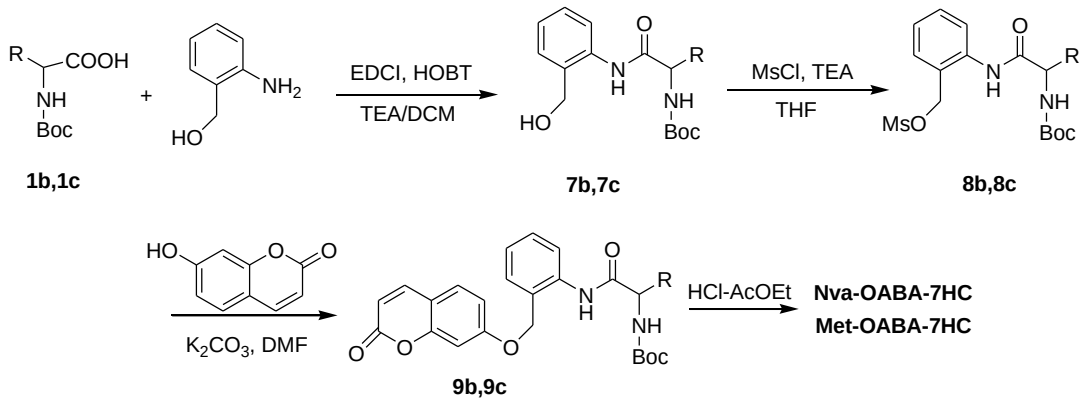


Scheme S2. Synthesis of probe **Nva-OABA-7HC, Met-OABA-7HC.**

***tert*-butyl 1-(2-(hydroxymethyl)phenylamino)-1-oxopentan-2-ylcarbamate (7b).** To a stirred solution of Boc-Nva (**1b**) (1.2 g, 5.5 mmol) in 50 mL DCM was added HOBT (0.75 g, 5.5 mmol) and EDCI (1.05 g, 5.5 mmol) at 0°C. After 30 min, 2-amino benzyl alcohol (0.62 g, 5 mmol) was added to the solution rapidly and the mixture was stirred at rt for 10 h. Saturated citric acid was added to the mixture. The produced precipitation was filtered. The organic layer was washed by saturated citric acid (50 mL*3), saturated $NaHCO_3$ (50 mL*3), brine (50 mL*1) and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then purified by combiflash to yield a white solid, 0.97 g, yield = 60.0%, mp: 110.0-112.3°C.

***tert*-butyl 1-(2-(hydroxymethyl)phenylamino)-4-(methylthio)-1-oxobutan-2-yl carbamate (7c).** The synthesis was analogous to **7b** with **1c** as the starting material. The product was obtained as a white solid, yield = 72.9%, mp:100.1-101.0°C.

2-(2-(*tert*-butoxycarbonyl)pentanamido)benzyl methanesulfonate (8b). The synthesis was analogous to **5a** with **7b** as the starting material. The product was obtained as brown oil, yield = 80.1%.

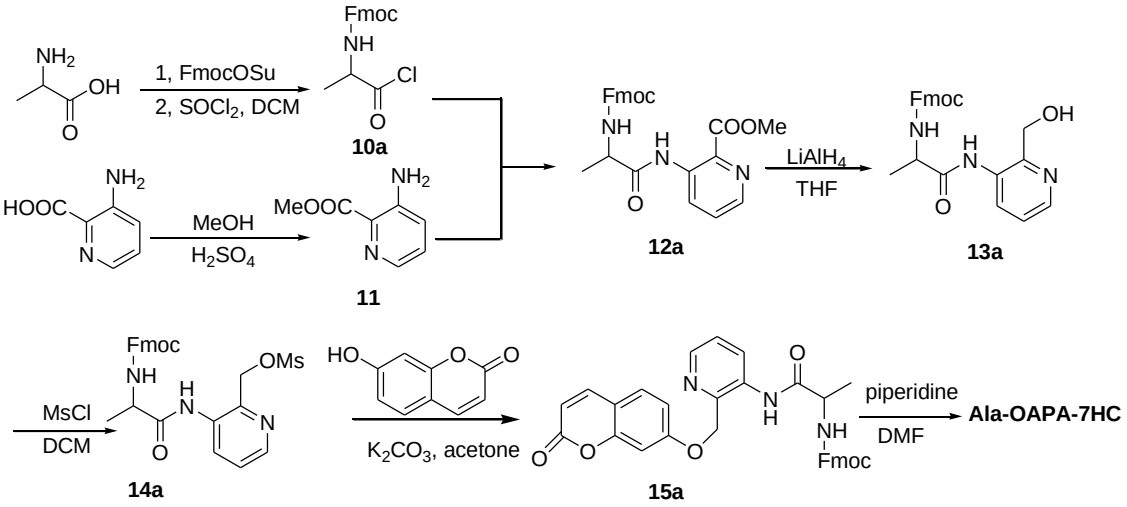
2-(2-(*tert*-butoxycarbonyl)-4-(methylthio)butanamido)benzyl methanesulfonate (8c). The synthesis was analogous to **5a** with **7c** as the starting material. The product was obtained as brown oil, yield = 65.3%.

***tert*-butyl 1-oxo-1-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenylamino)pentan-2-yl carbamate (9b).** The synthesis was analogous to **6a** with **8b** as the starting material. The product was obtained as a white solid, yield = 20.1%, mp: 158.0-160.1°C.

tert-butyl 4-(methylthio)-1-oxo-1-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl amino)butan-2-ylcarbamate (9c). The synthesis was analogous to **6a** with **8c** as the starting material. The product was obtained as a white solid, yield = 19.7%, mp: 92.1-94.3°C.

2-amino-N-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl)pentanamide hydrochloride (Nva-OABA-7HC). The synthesis was analogous to **Ala-PABA-7HC** with **9b** as the starting material. The product was obtained as a white solid, yield = 95.1%, mp:230.7-232.2°C. ^{1}H-NMR (D_2O, 300 MHz): δ 7.91 (d, 1 H, J = 9.6 Hz), 7.31-7.57 (m, 5H), 6.94-6.98 (m, 2 H), 6.28 (d, 1H, J = 9.6 Hz), 5.13 (d, 1H, J = 11.4 Hz), 5.03 (d, 1H, J = 11.4 Hz), 4.07 (t, 1H, J = 6.2 Hz), 1.61-1.74 (m, 2H), 1.18-1.31(m, 2H), 0.57 (t, 3H, J = 7.5 Hz); ^{13}C-NMR (DMSO, 150 MHz): δ 168.6, 161.8, 160.7, 155.7, 144.8, 135.2, 131.0, 130.0, 129.4, 129.2, 126.8, 126.2, 113.4, 113.1, 113.0, 101.9, 67.3, 53.0, 33.7, 18.2, 14.0. HRMS (ESI) m/z calcd. for $C_{21}H_{23}N_2O_4S$ ($[M + H]^+$) 367.1652; found 367.1644.

2-amino-4-(methylthio)-N-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)phenyl)butanamide hydrochloride (Met-OABA-7HC). The synthesis was analogous to **Ala-PABA-7HC** with **9c** as the starting material. The product was obtained as a white solid, yield = 97.3%, mp: 127.5-130.0°C. ^{1}H-NMR(D_2O, 400 MHz): δ 7.95 (d, 1 H, J = 9.5 Hz), 7.38-7.60 (m, 5 H), 6.98-7.01 (m, 2 H), 6.32(d, 1H, J = 9.6 Hz), 5.17 (d, 1 H, J = 11.0 Hz), 5.08 (d, 1 H, J = 11.0 Hz), 4.24 (t, 1H, J = 6.0 Hz), 2.48 (t, 2H, J = 7.5 Hz), 2.00-2.07 (m, 2H), 1.87 (s, 3H); ^{13}C-NMR (DMSO, 150 MHz): δ 168.1, 161.7, 160.7, 155.7, 144.8, 135.0, 131.2, 130.0, 129.2, 129.1, 126.8, 126.3, 113.4, 113.1, 113.1, 102.0, 67.2, 52.5, 31.4, 28.8, 14.8. HRMS (ESI) m/z calcd. for $C_{21}H_{23}N_2O_4S$ ($[M + H]^+$) 399.1373; found 399.1385.



Scheme S3. Synthesis of probe **Ala-OAPA-7HC.**

10a and 11 were synthesized according to literature procedures [2-3].

methyl 3-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)propanamido)picolinate (12a). To a stirred solution of **11** (2.67 g, 8.0 mmol) and Pyridine (3 mL, 36 mmol) in 50 mL dioxane was added **10a** (0.63 g, 4.0 mmol), the mixture was refluxed at 120°C for 1 hour. After cooling and concentration, the residue was redissolved in AcoEt (200 mL) and

washed with saturated $NaHCO_3$ (50 mL*3), brine (50 mL*1), dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then recrystallized with AcOEt to yield a white solid yield 0.44 g, yield = 25.0%, mp: 136.6-138.6°C

(9*H*-fluoren-9-yl)methyl 1-(2-(hydroxymethyl)pyridin-3-ylamino)-1-oxopropan-2-yl carbamate (13a) The synthesis was analogous to **4a** with **12a** as the starting material. The product was obtained as a white solid, yield = 17.9%, mp:166.8-168.7°C

(3-(2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)propanamido)pyridin-2-yl)methyl methanesulfonate (14a) The synthesis was analogous to **5a** with **13a** as the starting material. The product was obtained as a white solid, yield = 66.6%, mp:139.3-141.7°C

(9H-fluoren-9-yl)methyl 1-oxo-1-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)pyridin-3-ylamino)propan-2-ylcarbamate (15a). Anhydrous K_2CO_3 (22 mg, 0.16 mmol)was added to the solution of 7-hydroxycoumarin (0.02 g, 0.12 mmol) in 10 mL acetone at room temperature, the mixture was stirred for 30 min. **14a** (40 mg, 0.08 mmol) was added to the mixture and stirred at room temperature for 30 min. The mixture was concentrated and redissolved in 100 mL AcOEt, the organic solution was washed with 10% aqueous K_2CO_3 solution (50 mL*5), brine (50 mL*1) and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then purified by combiflash to yield a white solid, 0.03 g, yield = 66.2%, mp:150.1-151.9°C.

2-amino-N-(2-((2-oxo-2*H*-chromen-7-yloxy)methyl)pyridin-3-yl)propanamide (Ala-OAPA-7HC). The solution of **15a** (30 mg，0.053 mmol) in 8 mL 20% piperidine-DMF was stirred at room temperature for 1 h. The reation mixture was concentrated under reduced pressure at 90°C to remove piperidine. To the residue was added AcOEt (50 mL), the organic layer was collected and the water layer was extracted by AcOEt (50*3 mL). The combine organic layer was washed by water (50 mL*3), brine (50 mL*1), and dried over anhydrous $MgSO_4$ for 24 h. The solution was concentrated under reduced pressure and then purified by Pre-TLC to yield a white solid, 0.015 g, yield = 83.0%, 59.0-61.3°C. ^{1}H-NMR (DMSO, 600 MHz): δ 8.39 (d, 1 H, J = 6 Hz), 8.34 (d, 1 H, J = 6 Hz), 7.99 (d, 1 H, J = 12 Hz), 7.64 (d, 1H, J = 12 Hz), 7.44 (dd, 1 H, J = 12 Hz, 6 Hz), 7.13 (d, 1 H, J=6 Hz), 7.07(dd, 1H, J = 12 Hz, 6 Hz), 6.31 (d, 1 H, J = 12 Hz), 5.38 (s, 2 H), 3.45 (q, 1 H, J = 6 Hz), 1.24 (d, 3H, J = 6 Hz); ^{13}C-NMR (DMSO, 150 MHz): 175.8, 161.4 160.7 155.6 145.9 144.7, 144.5, 134.4 129.9, 129.6 124.6 113.4, 113.3, 113.3 102.4 70.6, 51.2, 21.4; HRMS m/z calcd. for $C_{18}H_{18}N_3O_4$ ($[M + H]^+$) 340.1292; found 340.1287.

3. Reference

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4. Cell culture and inhibition assay

4.1 IC_{50} assay on enzyme level

IC_{50} of bestatin on enzyme level against APN were determined as previously described[1] using Ala-PABA-7HC (25 μM final concentration) or *L*-Leu-*p*-nitroanilide (400 μM final concentration) as the substrates and porcine kidney aminopeptidase (BioCol GmbH) as the enzyme in a tris-HCl buffer (100 mM, pH 7.5). The hydrolysis of the substrate was monitored by following the changes in the ratios of fluorescent intensities at 450 and 390 nm (λex = 330 nm) for **Ala-PABA-7HC** or in absorbance measured at 405 nm for *L*-Leu-*p*-nitroanilide. All solutions of the inhibitor were prepared in the assay buffer. All inhibitors were preincubated with APN for 30 min at 37°C. The assay mixture, which contained the inhibitor solution (with its concentration from 0.205-210 μM), the enzyme solution (1 mIU/mL for Ala-PABA-7HC, 5 mIU/mL for *L*-Leu-*p*-nitroanilide final concentration), and the assay buffer, was adjusted to 200 μL. IC_{50} values were calculated by nonlinear regression of at least five data points using SigmaPlot (Figure S1).

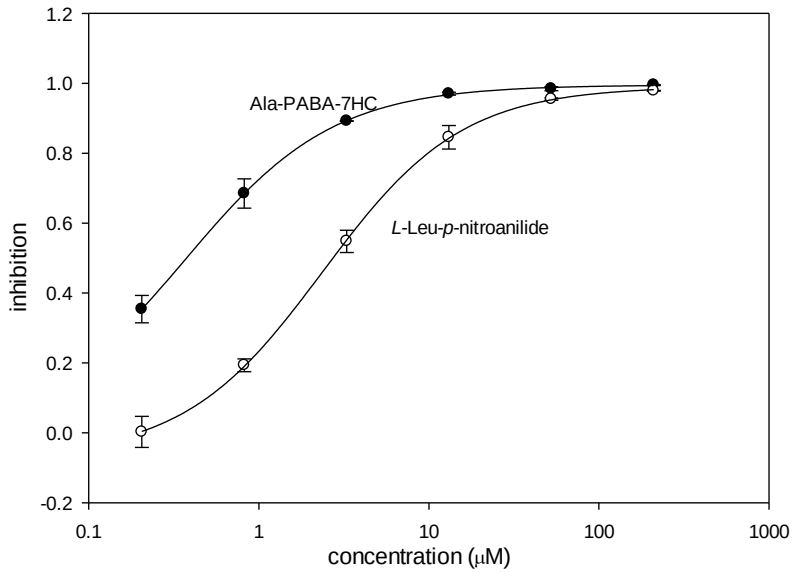


Figure S1. Inhibition of porcine kidney APN by bestatin using colorimetric (*L*-Leu-*p*-nitroanilide) and retiometric fluorescent (**Ala-PABA-7HC**) assays.

4.2 IC_{50} assay on cell level

Human ovarian clear cell carcinoma cell (ES-2) was grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum in an atmosphere of 5% CO_2, 95% air at

37 °C. IC_{50} of bestatin against APN expressed on the surface of ES-2 using Ala-PABA-7HC as substrate was determined as the method described above except using 20,000 cell to replace 1mIU enzyme, and using RPMI-1640 medium to replace the tris-HCl buffer.

IC_{50} of bestatin against APN expressed on the surface of ES-2 by using *L*-Leu-*p*-nitroanilide as substrate was similarly examined to IC_{50} assay at the enzyme level except the followings:

1) Using 150,000 cells to replace 5mIU enzyme, and using PBS (Na_2HPO_4, 10mM, PH 7.4) buffer to replace the tris-HCl buffer; 2) Cells were removed by centrifuging before measuring the absorbance at 405 nm. IC_{50} values were calculated by nonlinear regression of at least five data points using SigmaPlot (Figure S2).

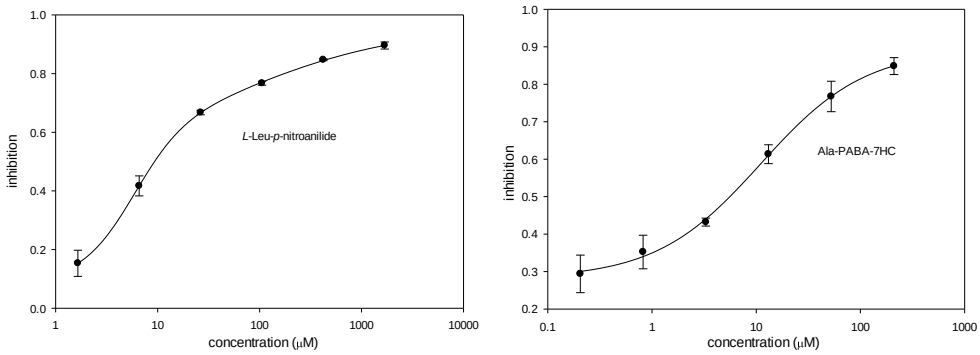


Figure S2. Inhibition of APN expressed on the surface of ES-2 cell by bestatin using colorimetric (*L*-Leu-*p*-nitroanilide, left) and retiometric fluorescent (**Ala-PABA-7HC,** right) assays.

4.3 Vmax and Km

Kinetic constants (Vmax and Km) were determined following the methods described in the literature [4]. For substrate **Ala-PABA-7HC** and *L*-Leu-AMC, an enzyme concentration of 0.005 IU/ml and [S] final between 0.0025 and 1.6 mM were used. For substrate *L*-Leu-*p*-nitroanilide, an enzyme concentration of 0.05 IU/ml and [S] final between 0.0025 and 1.6 mM were used. The assays were performed in quadruplicate. Km and Vmax were calculated by Lineweaver-Burk plot using SigmaPlot 12.

4.3 LOD

The LOD was defined as the amount of enzyme needed to generate activity that was three times the value of the blank. **LODs** were determined as the following, for substrate **Ala-PABA-7HC** and *L*-Leu-AMC, an enzyme concentration between 0.156 and 10.0 mIU/well and [S] final 22.5 μM were gentlely shaked at 37°C for 30 min. The hydrolysis

of the substrate was monitored by following the changes in the ratios of fluorescent intensities at 450 and 390 nm (λex = 330 nm) for **Ala-PABA-7HC** or the changes in fluorescent intensities at 465 nm for *L*-Leu-AMC. For *L*-Leu-*p*-nitroanilide, the enzyme concentration was between 0.156 and 10.0 mIU/well, and [S] final was 225 μM, and the shaking time was 30 min, and the hydrolysis of the substrate was monitored by following the changes in absorbance measured at 405 nm. The assays were performed in quadruplicate. LODs were calculated by linear curves fitting using SigmaPlot 12.

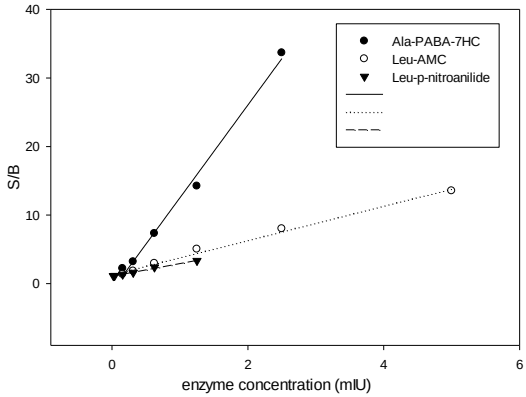


Figure S3. LOD for **Ala-PABA-7HC**, Leu-AMC and Leu-p-nitroanilide.

5. Fluorometeric Analysis

Buffer reagents were purchased from Aldrich and Acros and were used without purification. Water used for the fluorescence studies was doubly distilled and further purified with a Mill-Q filtration system. Vario skan (thermo electron corporation) was used for all fluorescent studies. Solutions of compounds **Ala-PABA-7HC, Nva-PABA-7HC, Met-PABA-7HC** were prepared in 0.1 M phosphate buffer at pH 7.4 (0.2% DMSO).

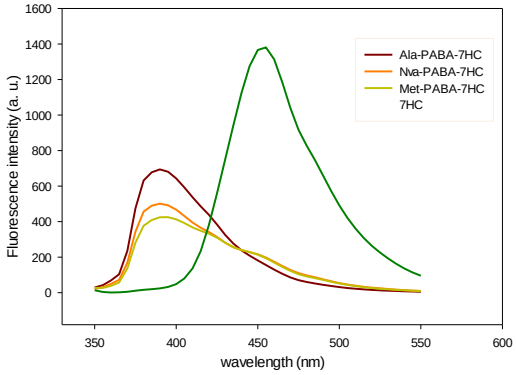
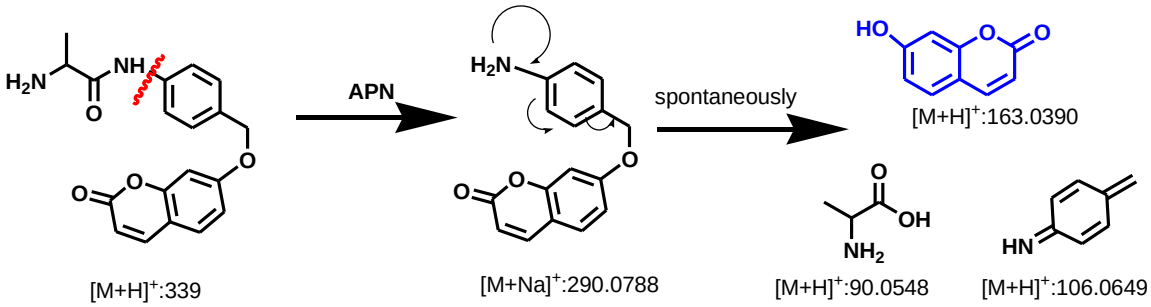


Figure S4 The emission spectrial of probes **Ala-PABA-7HC** (500 μM), **Nva-PABA-7HC** (500μM), **Met-PABA-7HC** (500 μM) and 7-hydroxy coumarin (50 μM), excitation at 330 nm.

6.Mechanism study

HRMS analysis was used for mechanism study, Figture S5 showed the structures of hydrolyzed products and their calculated m/z in Mass spectrometer. Figture S6 showed HRMS result after after adding 0.05 IU APN to the solution of **Ala-PABA-7HC** (200 μM) for 30 min. All of the hydrolyzed products had been found in the HRMS.



Figture S5. structures of hydrolyzed products and their calculated m/z in Mass spectrometer.

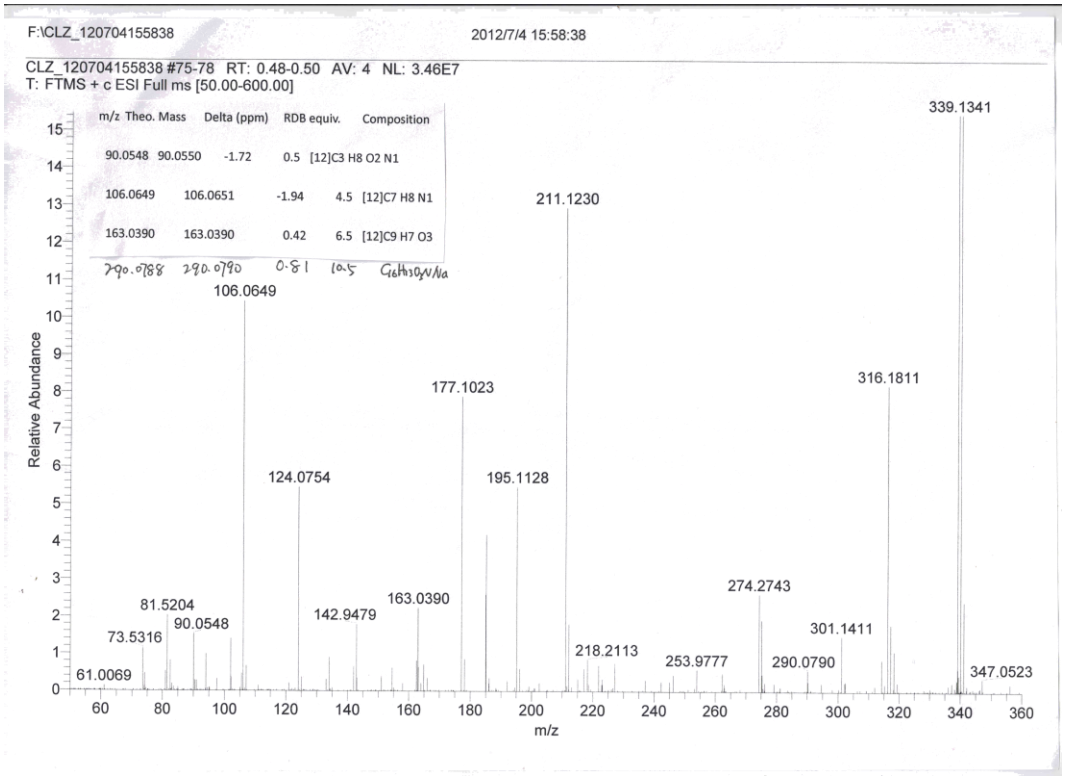


Figure S6. HRMS. 30 min after adding 0.05 IU APN to the solution of **Ala-PABA-7HC** (200 μM)

7. Stability of Ala-PABA-7HC

Stability of **Ala-PABA-7HC** in tris-HCl buffer and artificial gastric juice was tested by measurement of the time course for the generation of fluorescence (λ_{ex} = 330 nm, λ_{em} = 450 nm) in a solution of 100 μM.

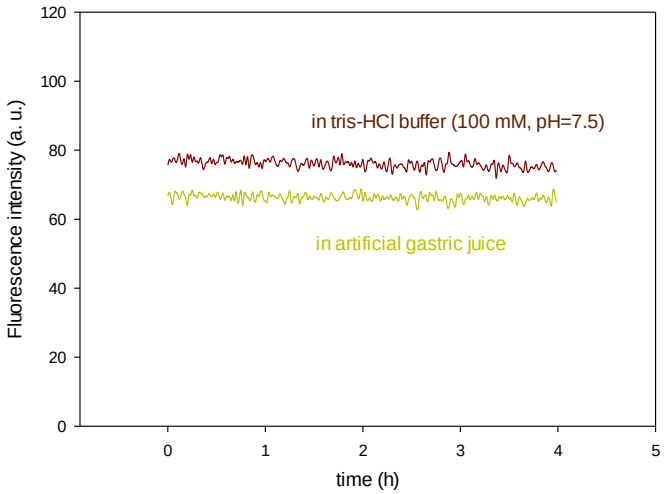


Figure S7. Time course for the generation of fluorescence (Ex = 330 nm, Em = 450 nm) of **Ala-PABA-7HC** (100 μM) in the solution of tris-HCl buffer (100 mM, pH=7.5) and artificial gastric juice.

8. ^{1}H NMR spectra

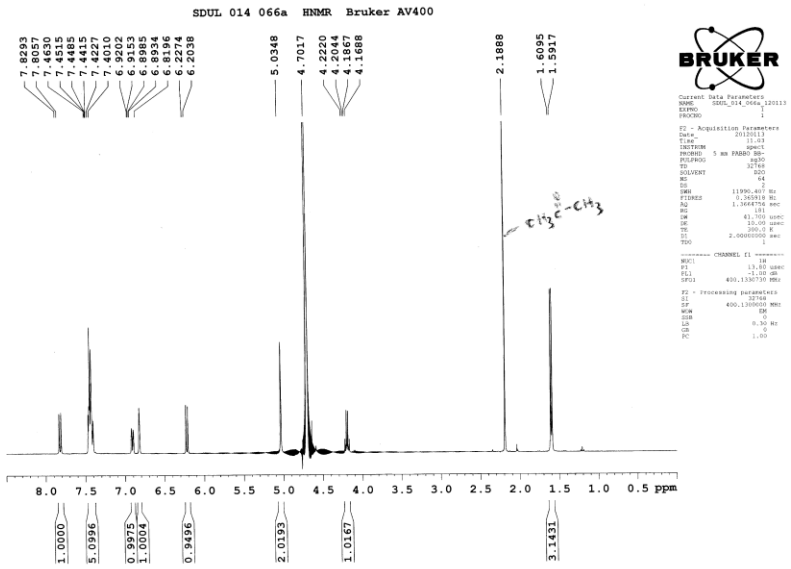


Figure S8. ^{1}H NMR (400 MHz) spectrum of Probe **Ala-PABA-7HC**.

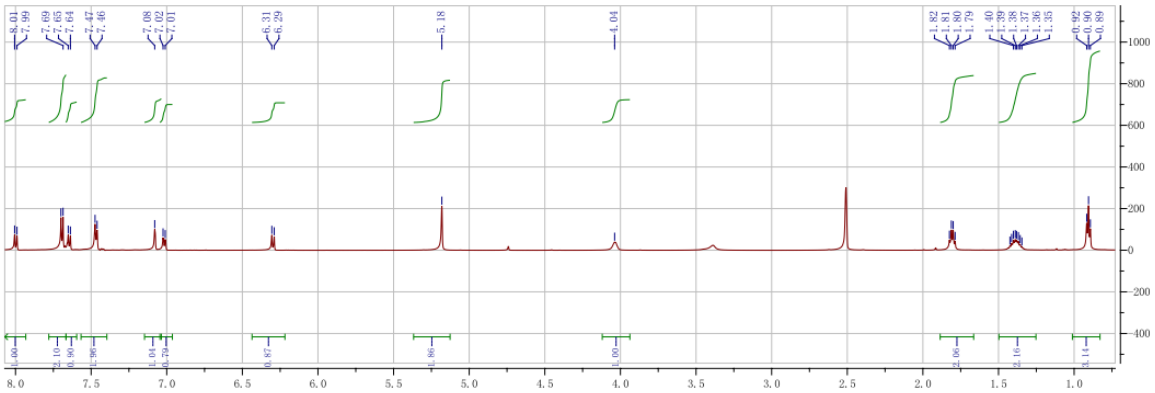


Figure S9. ^{1}H NMR (600 MHz) spectrum of Probe **Nva-PABA-7HC**.

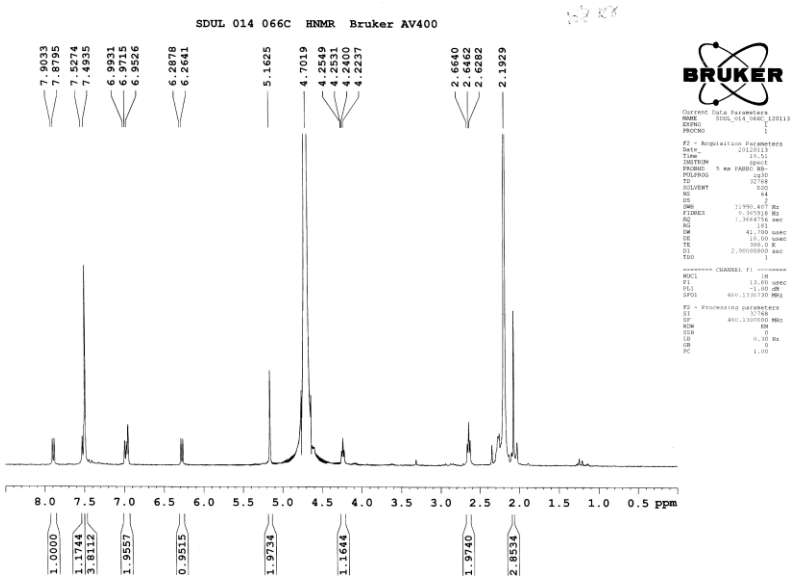


Figure S10. [1]H NMR (400 MHz) spectrum of Probe **Met-PABA-7HC**.

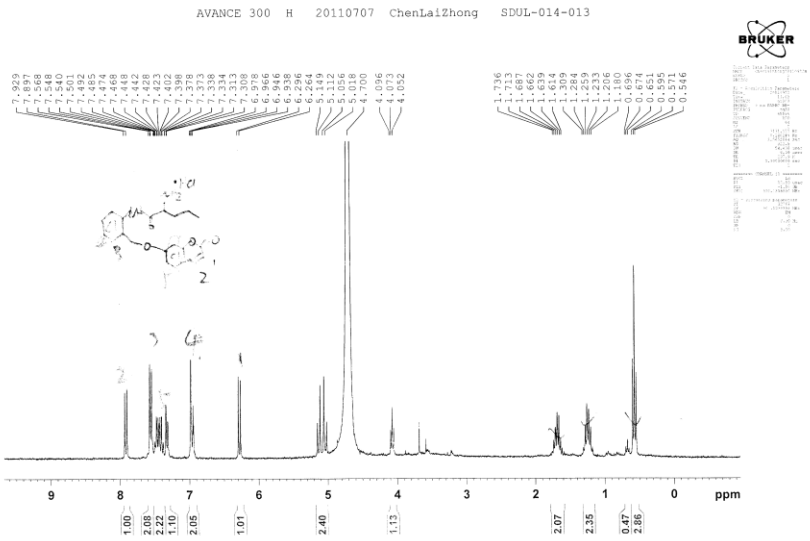


Figure S11. [1]H NMR (300 MHz) spectrum of Probe **Nva-OABA-7HC**.

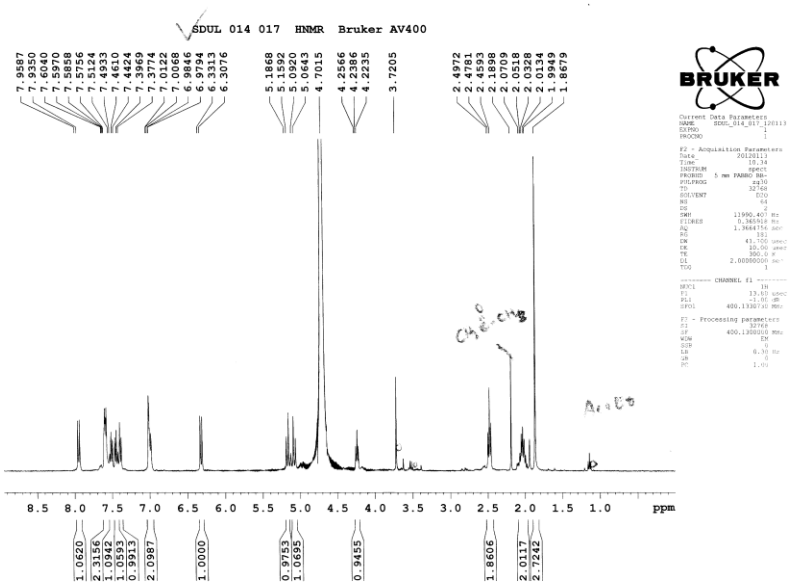


Figure S12. ^{1}H NMR (400 MHz) spectrum of Probe **Met-OABA-7HC**.

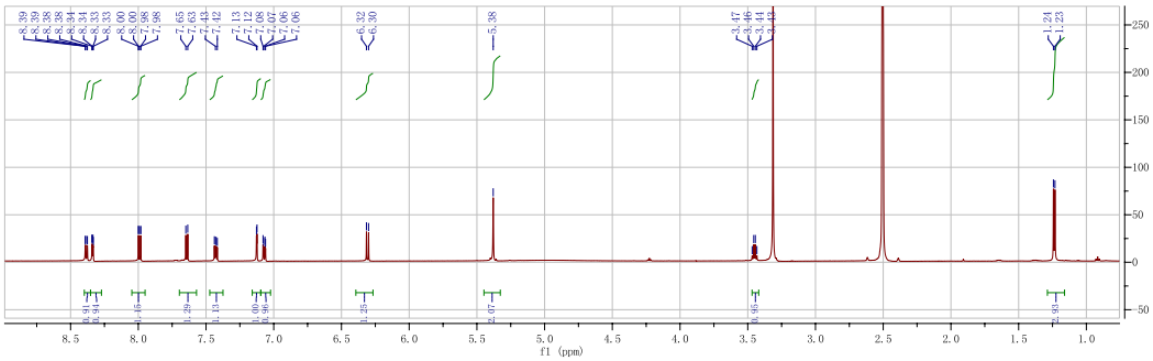


Figure S13. ^{1}H NMR (600 MHz) spectrum of Probe **Ala-OAPA-7HC**.

7. ^{13}C NMR spectra

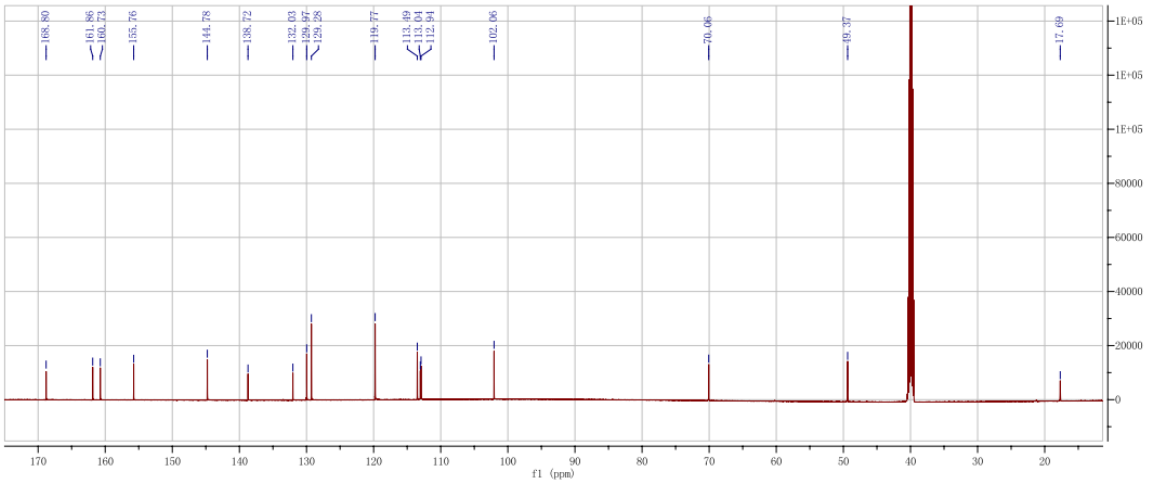


Figure S14. ^{13}C NMR (150 MHz) spectrum of Probe **Ala-PABA-7HC**.

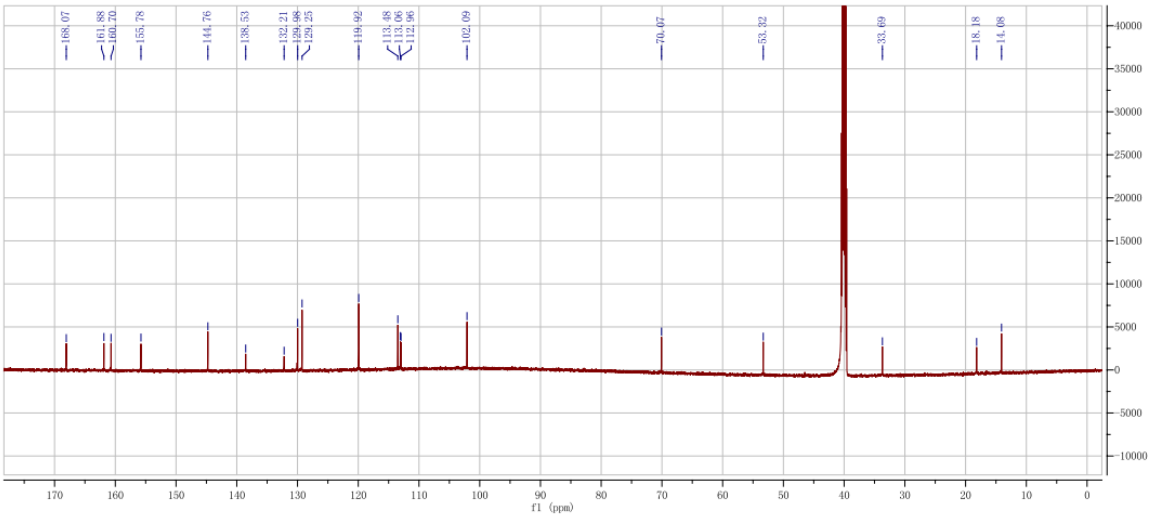


Figure S15. ^{13}C NMR (150 MHz) spectrum of Probe **Nva-PABA-7HC**.

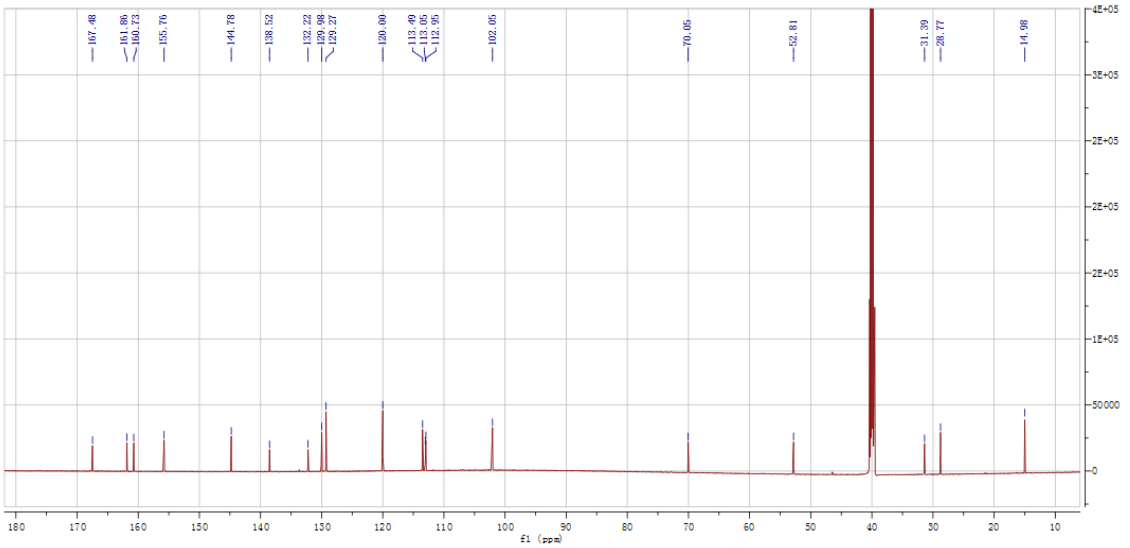


Figure S16. ^{13}C NMR (150 MHz) spectrum of Probe **Met-PABA-7HC**.

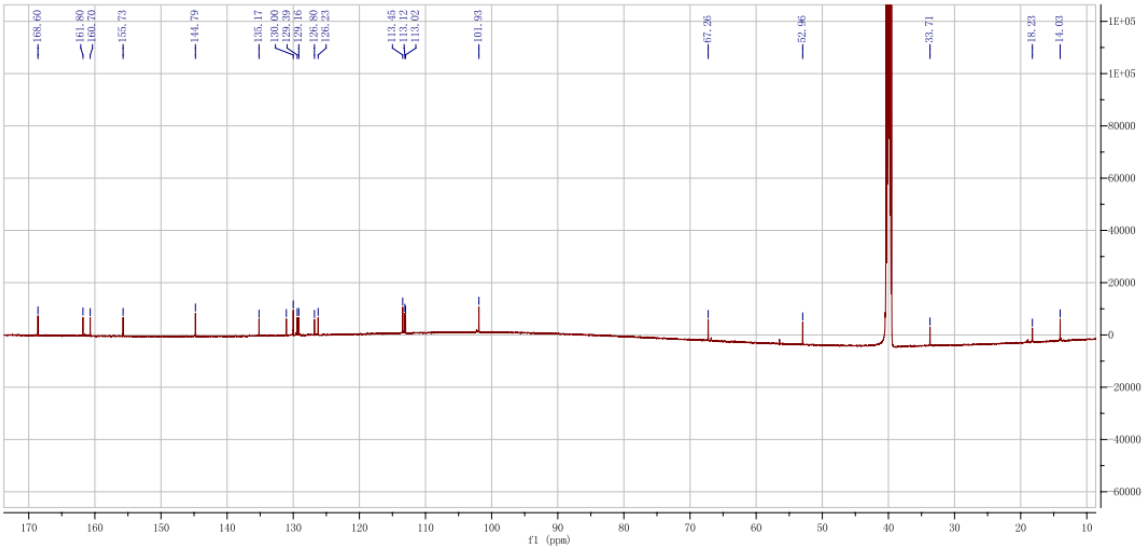


Figure S17. ^{13}C NMR (150 MHz) spectrum of Probe **Nva-OABA-7HC**.

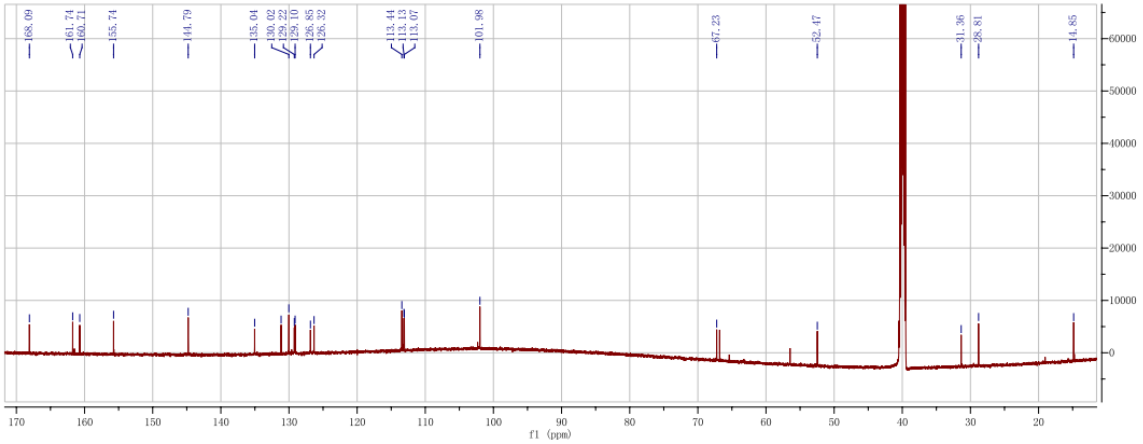


Figure S18. ^{13}C NMR (150 MHz) spectrum of Probe **Met-OABA-7HC**

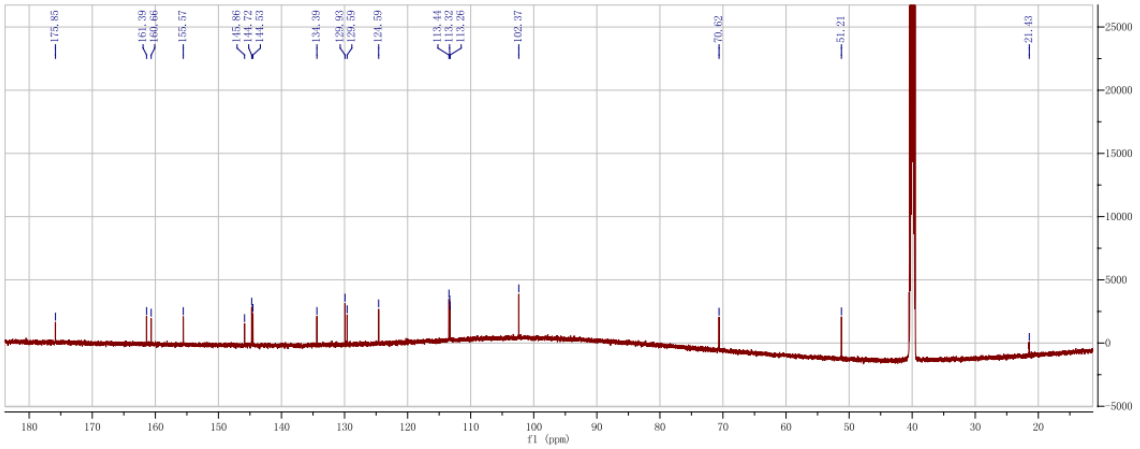
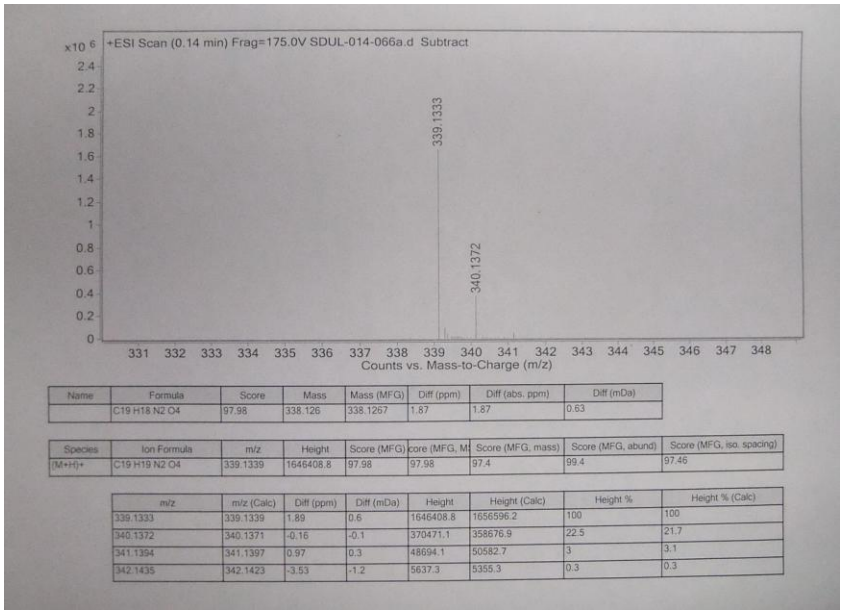


Figure S19. ^{13}C NMR (150 MHz) spectrum of Probe **Ala-OAPA-7HC**

8. HRMS

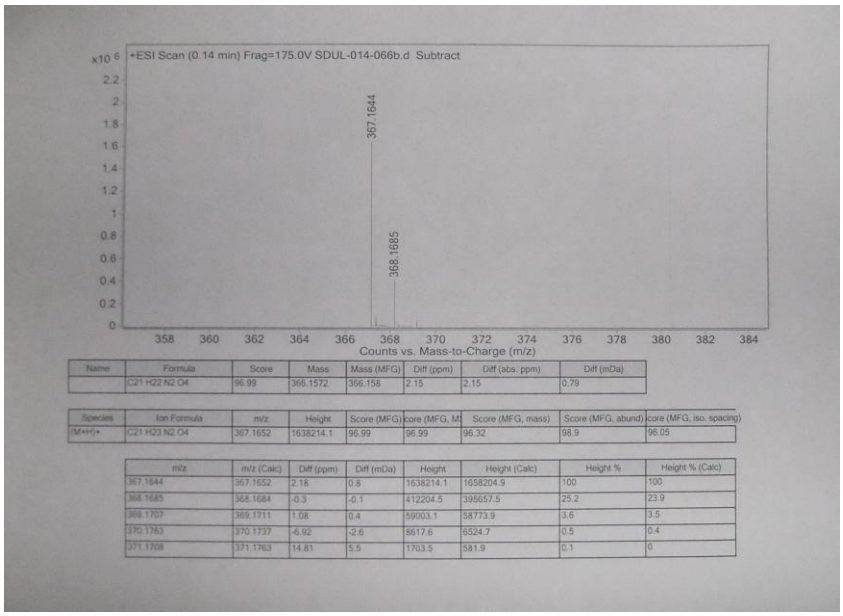


Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C19 H18 N2 O4	97.98	338.126	338.1267	1.87	1.87	0.63

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, MS	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C19 H19 N2 O4	339.1339	1646408.8	97.98	97.98	97.4	99.4	97.46

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
339.1333	339.1339	1.89	0.6	1646408.8	1656596.2	100	100
340.1372	340.1371	-0.16	-0.1	370471.1	358676.9	22.5	21.7
341.1394	341.1397	0.97	0.3	48694.1	50582.7	3	3.1
342.1435	342.1423	-3.53	-1.2	5637.3	5355.3	0.3	0.3

Figure S20. High resolution mass spectrum of Probe **Ala-PABA-7HC**.

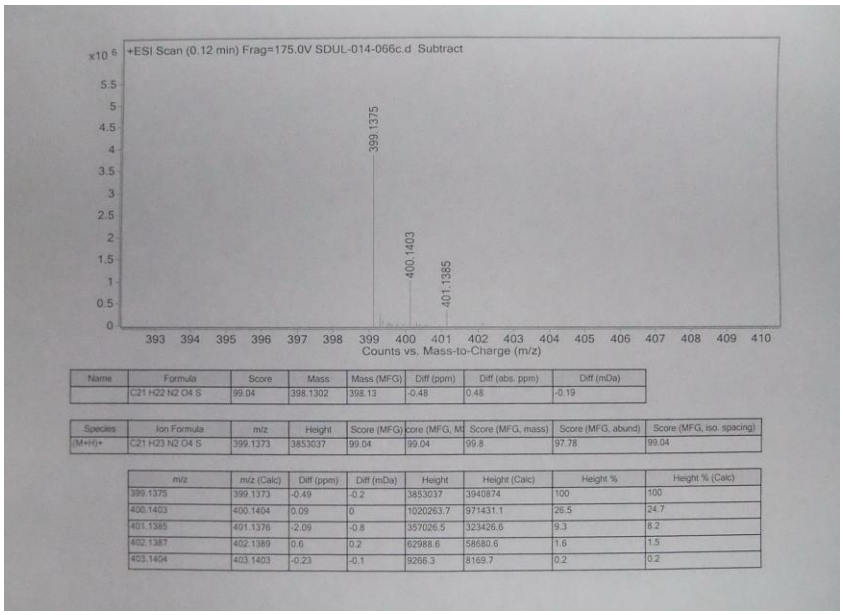


Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C21 H22 N2 O4	96.99	366.1572	366.158	2.15	2.15	0.79

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, MS	Score (MFG, mass)	Score (MFG, abund)	core (MFG, iso. spacing)
(M+H)+	C21 H23 N2 O4	367.1652	1638214.1	96.99	96.99	96.32	98.9	96.05

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
367.1644	367.1652	2.18	0.8	1638214.1	1658204.9	100	100
368.1685	368.1684	-0.3	-0.1	412204.5	395657.5	25.2	23.9
369.1707	369.1711	1.08	0.4	59003.1	58773.9	3.6	3.5
370.1763	370.1737	-6.92	-2.6	8617.6	6524.7	0.5	0.4
371.1708	371.1763	14.81	5.5	1703.5	581.9	0.1	0

Figure S21. High resolution mass spectrum of Probe **Nva-PABA-7HC**.

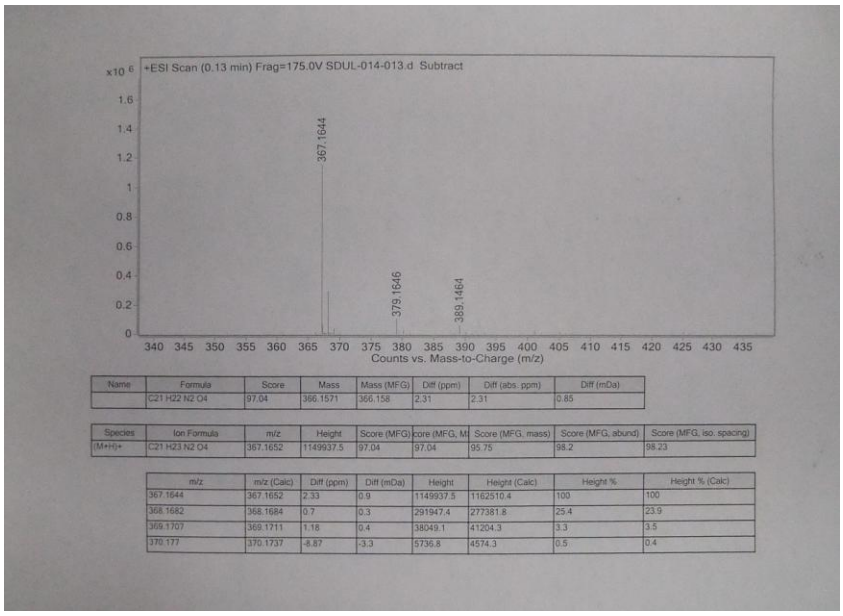


Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C21 H22 N2 O4 S	99.04	398.1302	398.13	-0.48	0.48	-0.19

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, M	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C21 H23 N2 O4 S	399.1373	3853037	99.04	99.04	99.8	97.78	99.04

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
399.1375	399.1373	-0.49	-0.2	3853037	3940874	100	100
400.1403	400.1404	0.09	0	1020263.7	971431.1	26.5	24.7
401.1385	401.1376	-2.09	-0.8	357026.5	323426.6	9.3	8.2
402.1387	402.1389	0.6	0.2	62988.6	58680.6	1.6	1.5
403.1404	403.1403	-0.23	-0.1	9266.3	8169.7	0.2	0.2

Figure S22. High resolution mass spectrum of Probe **Met-PABA-7HC**.

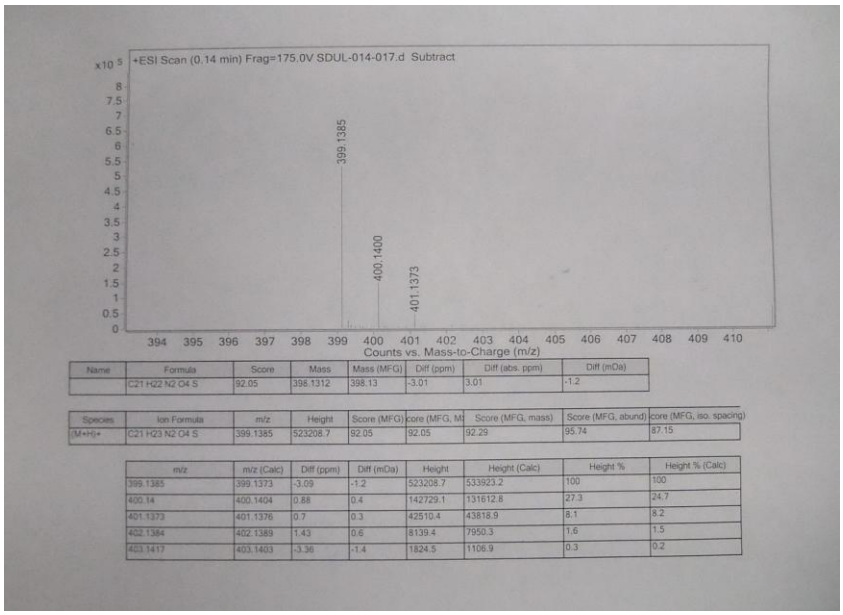


Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C21 H22 N2 O4	97.04	366.1571	366.158	2.31	2.31	0.85

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, M	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C21 H23 N2 O4	367.1652	1149937.5	97.04	97.04	95.75	98.2	98.23

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
367.1644	367.1652	2.33	0.9	1149937.5	1162510.4	100	100
368.1682	368.1684	0.7	0.3	291947.4	277381.8	25.4	23.9
369.1707	369.1711	1.18	0.4	38049.1	41204.3	3.3	3.5
370.177	370.1737	-8.87	-3.3	5736.8	4574.3	0.5	0.4

Figure S23. High resolution mass spectrum of Probe **Nva-OABA-7HC**.

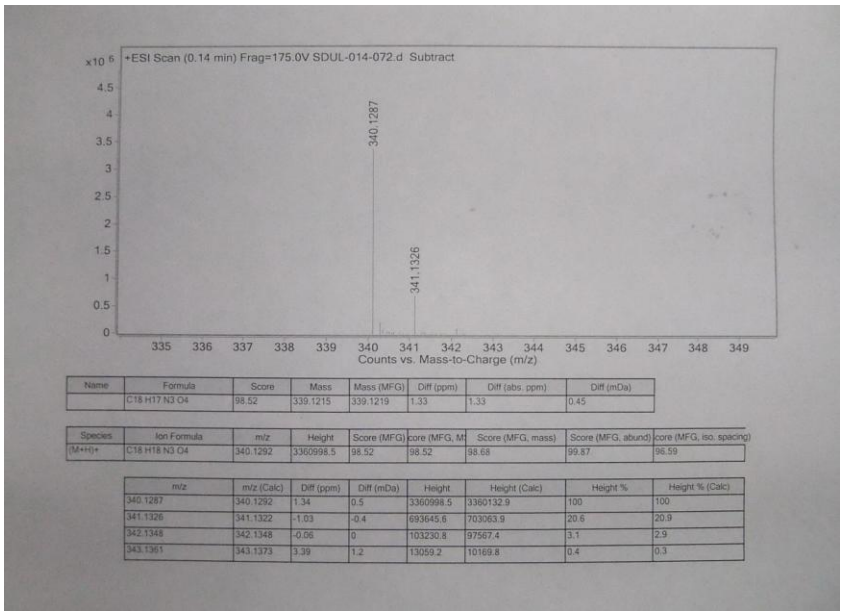


Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C21 H22 N2 O4 S	92.05	398.1312	398.13	-3.01	3.01	-1.2

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, MS	Score (MFG, mass)	Score (MFG, abund)	core (MFG, iso. spacing)
(M+H)+	C21 H23 N2 O4 S	399.1385	523208.7	92.05	92.05	92.29	95.74	87.15

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
399.1385	399.1373	-3.09	-1.2	523208.7	533923.2	100	100
400.14	400.1404	0.88	0.4	142729.1	131612.8	27.3	24.7
401.1373	401.1376	0.7	0.3	42510.4	43818.9	8.1	8.2
402.1384	402.1389	1.43	0.6	8139.4	7950.3	1.6	1.5
403.1417	403.1403	-3.36	-1.4	1824.5	1106.9	0.3	0.2

Figure S24. High resolution mass spectrum of Probe **Met-OABA-7HC**.



Name	Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
	C18 H17 N3 O4	98.52	339.1215	339.1219	1.33	1.33	0.45

Species	Ion Formula	m/z	Height	Score (MFG)	core (MFG, MS	Score (MFG, mass)	Score (MFG, abund)	core (MFG, iso. spacing)
(M+H)+	C18 H18 N3 O4	340.1292	3360998.5	98.52	98.52	98.68	99.87	96.59

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)
340.1287	340.1292	1.34	0.5	3360998.5	3360132.9	100	100
341.1326	341.1322	-1.03	-0.4	693645.6	703063.9	20.6	20.9
342.1348	342.1348	-0.06	0	103230.8	97567.4	3.1	2.9
343.1361	343.1373	3.39	1.2	13059.2	10169.8	0.4	0.3

Figure S25. High resolution mass spectrum of Probe **Ala-OAPA-7HC**.