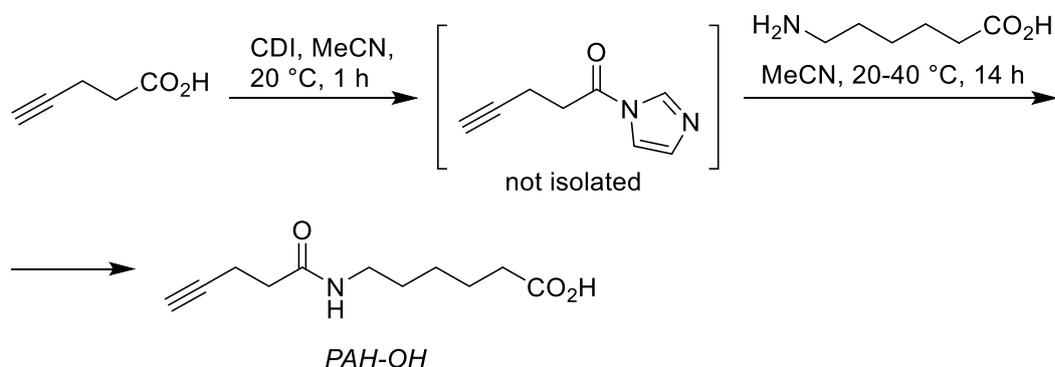


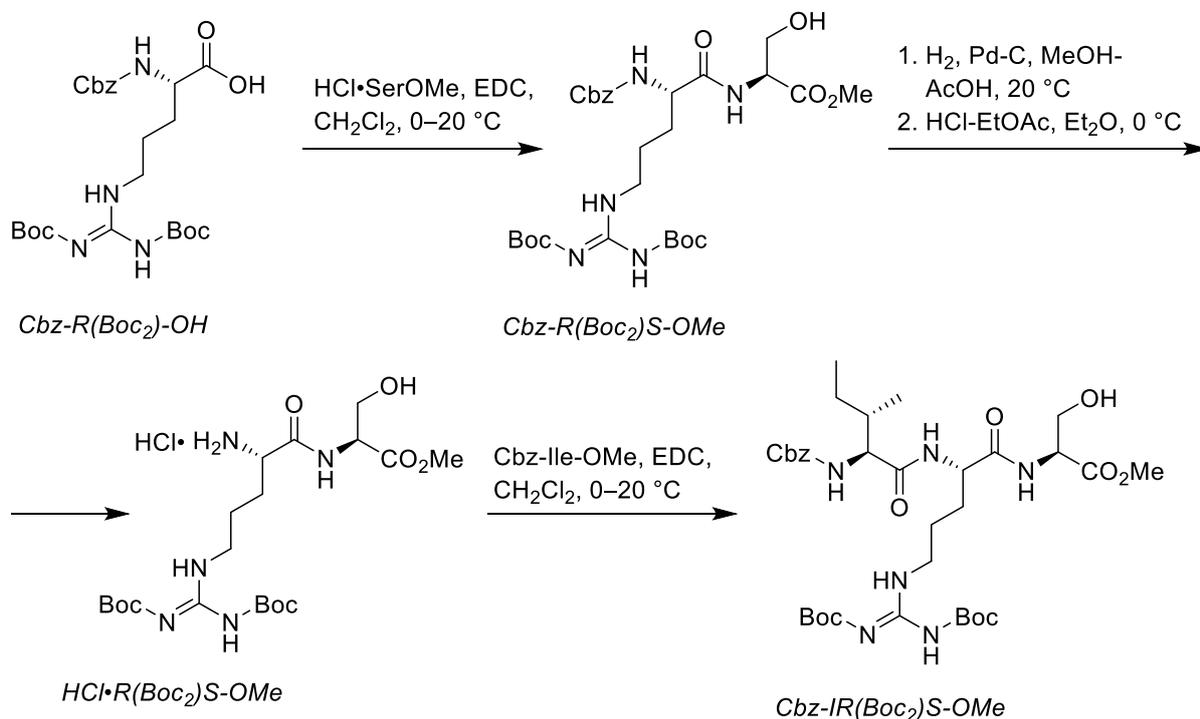
1. Synthesis of al-I and al-IRS probes

1.1. Synthesis of 6-(pent-4-ynamido)hexanoic acid (PAH-OH).



Under argon, CDI (826 mg, 5.1 mmol) was added to a stirred solution of pent-4-ynoic acid (490 mg, 5.0 mmol) in anh. MeCN (20 mL) and the mixture was stirred at room temperature for 1 h to give *in situ* the corresponding imidazolide as reactive intermediate. Then, 6-aminocaproic acid (721 mg, 5.5 mmol) was added and stirring under argon was continued for 2 h at room temperature and then for 12 h at 40 °C. Volatile components were evaporated *in vacuo*, the residue was dissolved in water (20 mL), acidified with 37% aq. HCl to pH 1, and the product was extracted with EtOAc (3×50 mL). The combined organic phase was dried over anh. Na₂SO₄, filtered, and the filtrate was evaporated *in vacuo* to give 6-(pent-4-ynamido)hexanoic acid as a yellowish oil, which solidified into a white waxy solid upon standing at room temperature. Yield: 880 mg (4.17 mmol, 83%). ¹H NMR (600 MHz, DMSO-d₆): δ *major conformer* 1.19–1.28, 1.33–1.40, and 1.44–1.51 (6H, 3m, 1:1:1), 2.18 and 2.23 (4H, 2t, 1:1, *J* = 7.4 Hz), 2.34 (2H, dt, *J* = 2.6, 7.1 Hz), 2.74 (1H, t, *J* = 2.6 Hz), 3.02 (2H, br q, *J* = 6.5 Hz), 7.84 (1H, t, *J* = 5.6 Hz), 12.04 (1H, s). ¹³C NMR (126 MHz, DMSO-d₆) δ 174.5, 170.1, 83.8, 71.3, 38.4, 34.3, 33.6, 28.9, 26.0, 24.2, 14.3. Spectral data are consistent with the literature data (Fischer-Durand et al., 2012). **Error! Bookmark not defined.**

1.2. Synthesis of methyl *N*-[*N*²-(*N*-benzyloxycarbonyl-*L*-isoleucyl)-*N*^ω,*N*^{ω'}-bis(*tert*-butoxycarbonyl)-*L*-arginyl]-*L*-serinate (Cbz-IR(Boc₂)S-OMe).



1.2.1. Synthesis of methyl *N*²-(benzyloxycarbonyl)-*N*^ω,*N*^{ω'}-bis(*tert*-butoxycarbonyl)-*L*-arginyl-*L*-serinate (Cbz-*R*(Boc₂)S-OMe). Under argon at 0 °C (ice-bath), NMM (440 μL, 4 mmol) was added to a stirred mixture of *N*²-benzyloxycarbonyl-*N*^ω,*N*^{ω'}-bis(*tert*-butoxycarbonyl)-*L*-arginine (Cbz-*R*(Boc₂)-OH) (2.034 g, 4 mmol), *L*-serine methyl ester hydrochloride (685 mg, 4.4 mmol), and anh. CH₂Cl₂ (25 mL) and the mixture was stirred at 0 °C for 10 min. Then, EDC (958 mg, 5 mmol) was added and stirring was continued for at 0 °C 1 h and then at room temperature for 12 h. CH₂Cl₂ (50 mL) was added and the combined organic phase was washed with 1 M aqueous NaHSO₄ (30 mL), saturated aq. NaHCO₃ (30 mL), and brine (30 mL). The organic phase was dried over Na₂SO₄, filtered, and the filtrate was evaporated *in vacuo*. The residue was purified by CC (column dimensions 2.5×15 cm). First, non-polar byproducts were eluted by EtOAc–hexanes 2:1 (around 6×100 mL), followed by elution of the desired dipeptide with EtOAc–hexanes 1:2 (around 5×100 mL). Fractions containing the product were combined and evaporated *in vacuo* to give Cbz-*R*(Boc₂)S-OMe. Yield: 1.442 g (2.36 mmol, 59%), white solid foam, mp 45–65 °C, [α]_D²¹ = +6.91 (c = 0.55; CH₂Cl₂). FT-IR (ATR): ν_{max} 3325, 2978, 1716, 1638, 1614, 1526, 1453, 1413, 1365, 1326, 1226, 1152, 1129, 1048, 1024, 911, 876, 807, 774, 739, 696, 611 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 1.45 and 1.48 (18H, 2s, 1:1), 1.66 (2H, p, *J* = 7.3 Hz), 1.80 (1H, dt, *J* = 14.3, 7.4

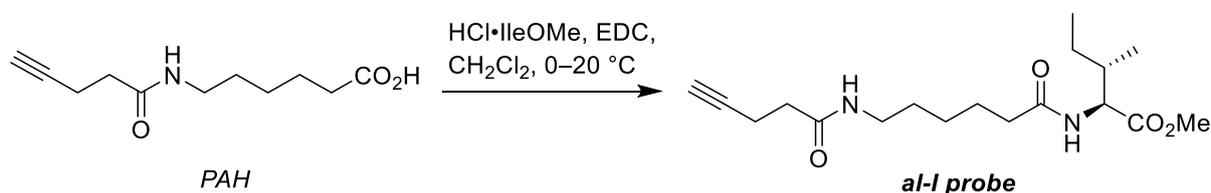
Hz), 1.89 (1H, dt, $J = 13.2, 6.7$ Hz), 3.38 (2H, dt, $J = 12.9, 6.4$ Hz), 3.76 (3H, s, OMe), 3.93 (2H, d, $J = 3.7$ Hz), 4.31 (1H, q, $J = 7.2$ Hz), 4.64 (1H, dt, $J = 7.5, 3.6$ Hz), 5.09 and 5.12 (2H, 2d, 1:1, $J = 12.2$ Hz), 6.04 (1H, d, $J = 8.1$ Hz), 7.16 (1H, d, $J = 7.8$ Hz), 7.28–7.39 (6H, m), 8.40 (1H, t, $J = 5.7$ Hz), 11.43 (1H, s). ^{13}C NMR (126 MHz, CDCl_3) δ 14.3, 25.5, 28.2, 28.3, 28.5, 40.0, 52.8, 54.8, 55.0, 62.8, 67.3, 79.8, 83.5, 128.2, 128.3, 128.3, 128.7, 136.3, 153.3, 156.5, 170.7, 172.0. m/z (HRMS): 610.3083 (MH^+). $\text{C}_{28}\text{H}_{44}\text{N}_5\text{O}_{10}$ requires m/z 610.3078.

1.2.2. Synthesis of methyl $N^\omega, N^{\omega'}$ -bis(tert-butoxycarbonyl)-L-arginyl-L-serinate hydrochloride ($\text{HCl}\cdot\text{R}(\text{Boc}_2)\text{S-OMe}$). Under argon, *Cbz-R(Boc₂)S-OMe* (982 mg, 1.61 mmol) was added to a suspension of 10% Pd-C (250 mg) in a mixture of MeOH (40 mL) and AcOH (5 mL) and the mixture was hydrogenated (3 bar of H_2 , 20 °C) for 3 h. The catalyst was removed by filtration through a fritted funnel and washed with MeOH (4×10 mL). The combined filtrate was evaporated *in vacuo* (18 mbar, 30 °C) and the residue was dissolved in anhyd. Et_2O (50 mL). While stirring under argon, the solution was cooled to 0 °C (ice-bath), 2 M HCl–EtOAc (1.5 mL, 3 mmol) was added, ice-bath was removed, and the mixture was stirred at room temperature for 5 min. The white precipitate was collected by filtration, washed with anhyd. Et_2O (4×10 mL), and dried *in vacuo* over NaOH pellets at room temperature for 12 h to give *HCl·R(Boc₂)S-OMe*. Yield: 761 mg (1.49 mmol, 92%), m.p. 152–154 °C, $[\alpha]_{\text{D}}^{20} = +6.65$ ($c = 0.77$; CH_2Cl_2). FT-IR (ATR): ν_{max} 3210, 2978, 1736, 1673, 1606, 1500, 1457, 1395, 1370, 1230, 1136, 1060, 856, 776 cm^{-1} . ^1H NMR (500 MHz, CDCl_3): δ 1.40 (1H, br s), 1.52 and 1.53 (18H, 2s, 1:1), 1.90–1.99 (2H, br m), 2.10–2.18 and 2.19–2.28 (2H, 2br m, 1:1), 3.68–3.78 (1H, m), 3.72 (3H, s), 3.80–3.88 (1H, m), 3.95 (1H, br dd, $J = 11.9, 5.8$ Hz), 4.00–4.06 (1H, br m), 4.48 (1H, br m), 4.65 (1H, br m), 8.16–8.54 (3H, br m), 9.05 (1H, d, $J = 7.6$ Hz), 9.45 (1H, br s), 11.41 (1H, br s). ^{13}C NMR (126 MHz, CDCl_3) δ 24.9, 28.0, 28.1, 28.1, 41.6, 52.7, 53.1, 55.8, 61.8, 77.4, 84.7, 152.2, 152.2, 169.4, 170.5, 174.0. m/z (HRMS): 476.2703 (MH^+). $\text{C}_{20}\text{H}_{38}\text{N}_5\text{O}_8$ requires m/z 476.2715.

1.2.3. Synthesis of methyl N -[N^2 -(N -benzyloxycarbonyl- L -isoleucyl)- $N^\omega, N^{\omega'}$ -bis(tert-butoxycarbonyl)- L -arginyl]- L -serinate ($\text{Cbz-IR}(\text{Boc}_2)\text{S-OMe}$). The reaction was carried out under argon. *HCl·R(Boc₂)S-OMe* (501 mg, 0.98 mmol) and N -Cbz- L -isoleucine (303 mg, 1.14 mmol) were dissolved in anhyd. CH_2Cl_2 (7 mL) and the mixture was stirred at 0 °C (ice-bath) for 10 min. Then, NMM (0.12 mL, 1.1 mmol) and EDC (232 mg, 1.2 mmol) were added and stirring at 0 °C was continued for 1 h. The ice-bath was removed and the mixture was stirred at room temperature for 12 h. Dichloromethane (25 mL) was added and the combined organic phase was washed with 1 M aqueous NaHSO_4 (20 mL), saturated aq. NaHCO_3 (20 mL), and

brine (20 mL). The organic phase was dried over Na₂SO₄, filtered, and the filtrate was evaporated *in vacuo* to give *Cbz-IR(Boc₂)S-OMe* as a white foam. Yield: 624 mg (0.86 mmol, 88%), [α]_D²⁰ = -10.7 (c = 0.85; CH₂Cl₂). FT-IR (ATR): ν_{max} 3305, 2966, 1719, 1639, 1617, 1525, 1413, 1328, 1225, 1049, 1025, 877, 852, 775 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ 0.83–0.95 (6H, m), 1.09–1.20 (2H, m), 1.43 and 1.48 (18H, 2s, 1:1), 1.56–1.65 (2H, m), 1.66–1.75 (1H, m), 1.77–1.92 (2H, m), 2.99–3.43 (2H, m), 3.74 (3H, s), 3.83–3.91 (1H, m), 3.91–4.08 (2H, m), 4.47–4.70 (2H, m), 4.99–5.14 (2H, m), 5.48–5.73 (1H, m), 6.69–6.96 (1H, m), 7.16 (1H, broad signal, NH), 7.27–7.38 (5H, m, Ph), 7.38–7.50 (1H, m, NH), 8.28–8.68 (1H, m, NH), 11.31–11.47 (1H, m). ¹H NMR (600 MHz, CD₃OD): δ 0.92 (3H, t, *J* = 7.5 Hz), 0.95 (3H, d, *J* = 6.8 Hz), 1.15–1.24 (2H, m), 1.46 and 1.49 (18H, 2s, 1:1), 1.62–1.79 (3H, m), 1.83 (1H, br dd, *J* = 11.7, 5.0 Hz), 1.88–1.99 (1H, m), 3.15–3.27 (1H, m), 3.36–3.52 (1H, m), 3.76 (3H, s), 3.81 (1H, dd, *J* = 11.5, 3.9 Hz), 3.89–3.97 (1H, m), 4.01 (1H, br t, *J* = 7.6 Hz), 4.42–4.66 (2H, m), 5.10 and 5.12 (2H, 2br d, 1:1, *J* = 15.3 Hz), 7.23–7.45 (5H, m). ¹³C NMR (151 MHz, CDCl₃) δ 11.5, 15.7, 25.0, 25.4, 28.2, 28.4, 28.8, 37.3, 40.3, 52.8, 53.1, 55.0, 60.2, 62.7, 67.4, 79.7, 83.4, 128.3, 128.4, 128.7, 136.2, 153.4, 156.4, 156.8, 163.4, 170.7, 171.3, 172.1. *m/z* (HRMS): 723.3920 (MH⁺). C₃₄H₅₅N₆O₁₁ requires *m/z* 723.3924.

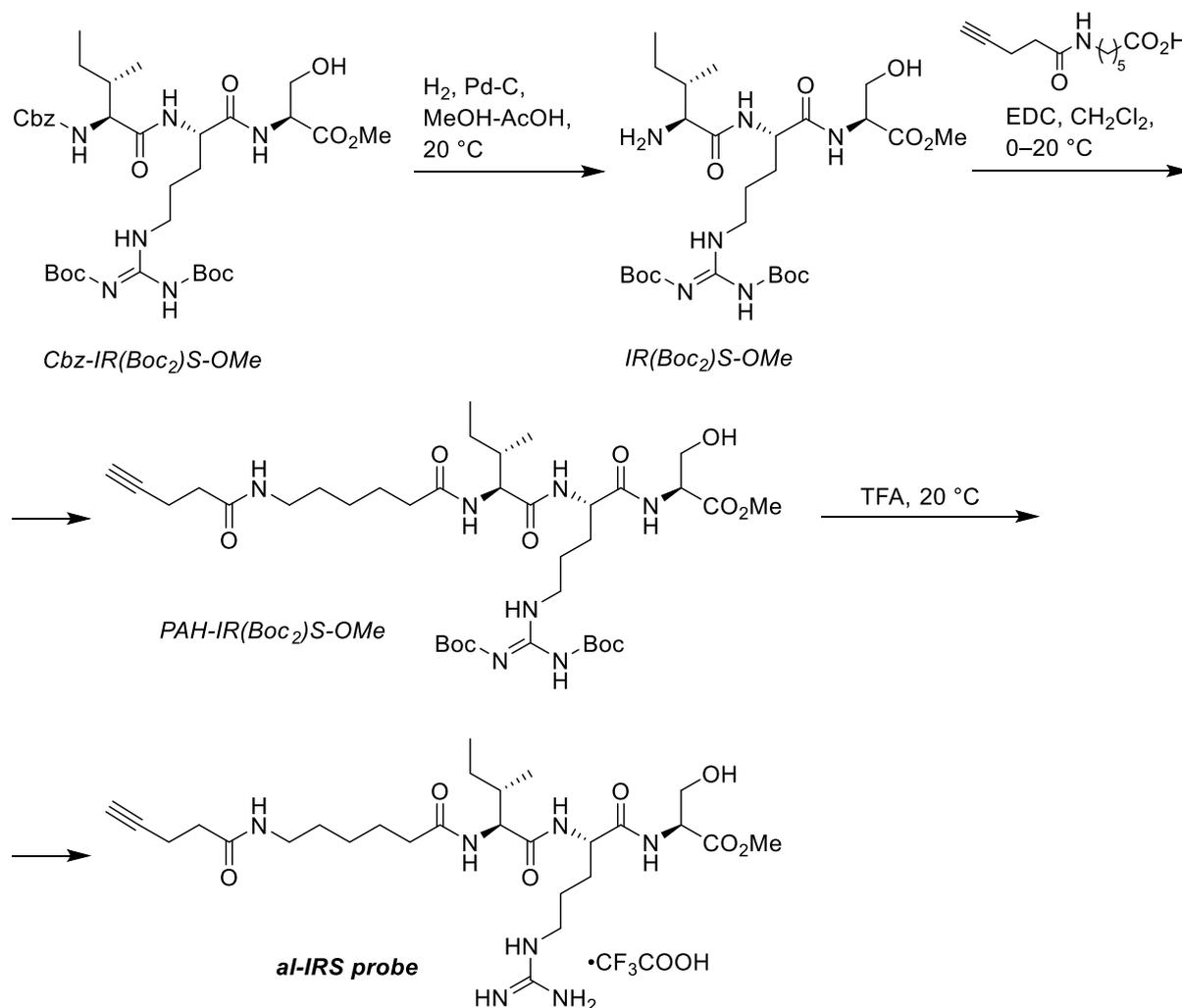
1.3. Synthesis of methyl *N*-[6-(pent-4-ynamido)hexanoyl]-*L*-isoleucinate (*al-I probe*).



The reaction was carried out under argon. 6-(Pent-4-ynamido)hexanoic acid (*PAH*) (106 mg, 0.5 mmol) and *L*-isoleucine methyl ester hydrochloride (91 mg, 0.5 mmol) were dissolved in anhyd. CH₂Cl₂ (5 mL) and the mixture was stirred at 0 °C (ice-bath) for 10 min. Then, NMM (55 μL, 0.5 mmol) and EDC (116 mg, 0.6 mmol) were added and stirring at 0 °C was continued for 1 h. The ice-bath was removed and the mixture was stirred at room temperature for 12 h. Dichloromethane (15 mL) was added and the combined organic phase was washed with 1 M aqueous NaHSO₄ (10 mL), saturated aq. NaHCO₃ (10 mL), and brine (10 mL). The organic phase was dried over Na₂SO₄, filtered, and the filtrate was evaporated *in vacuo* to give *al-I probe* as a yellowish oil. Yield: 142 mg (0.42 mmol, 84%), [α]_D²⁰ = +40.8 (c = 0.52; CH₂Cl₂). FT-IR (ATR): ν_{max} 3342, 3065, 2959, 2936, 2876, 1741, 1683, 1644, 1538, 1263, 1197, 1139, 1033, 1010, 973, 751, 706, 694 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): δ 0.89–0.95 (6H, m), 1.17 (1H, ddt, *J* = 13.6, 9.1, 7.3 Hz), 1.33–1.48 (3H, m), 1.49–1.59 (2H, m), 1.62–1.71 (2H, m),

1.82–1.93 (1H, m), 2.01 (1H, t, $J = 2.6$ Hz), 2.25 (2H, t, $J = 6.9$ Hz), 2.39 (2H, t, $J = 6.9$ Hz), 2.49–2.58 (2H, m), 3.24–3.33 (2H, m), 3.74 (3H, s), 4.60 (1H, dd, $J = 8.6, 4.9$ Hz), 5.93 (1H, br s), 6.01 (1H, br d, $J = 8.5$ Hz). m/z (HRMS): 339.2278 (MH^+). $C_{18}H_{31}N_2O_4$ requires m/z 339.2278.

1.4. Synthesis of methyl *N*-(*N*-{*N*-[6-(pent-4-ynamido)hexanoyl]-*L*-isoleucyl]-*L*-arginyl)-*L*-serinate (*al*-*IRS* probe).



1.4.1. Synthesis of *N*-(*N*²-{*N*-[6-(pent-4-ynamido)hexanoyl]-*L*-isoleucyl}-*N*^ω,*N*^{ω'}-bis(tert-butoxycarbonyl)-*L*-arginyl)-*L*-serinate (*PAH-IR*(*Boc*₂)*S*-*OMe*). Under argon, *Cbz-IR*(*Boc*₂)*S*-*OMe* (299 mg, 0.48 mmol) was added to a suspension of 10% Pd-C (70 mg) in MeOH (20 mL) and the mixture was hydrogenated (3 bar of H_2 , 20 °C) for 5 h. The catalyst was removed by filtration through a fritted funnel, washed with MeOH (2×10 mL), and the combined filtrate was evaporated *in vacuo* (18 mbar, 30 °C) to give the crude *IR*(*Boc*₂)*S*-*OMe* in quantitative yield. The crude *IR*(*Boc*₂)*S*-*OMe* was dissolved in toluene (20 mL) and the solution was

evaporated *in vacuo*. Dissolution in toluene (20 mL), followed by evaporation *in vacuo* was repeated two more times to remove MeOH. The residue was dissolved in CH₂Cl₂ (10 mL), 6-(pent-4-ynamido)hexanoic acid (126 mg, 0.6 mmol) was added, and the mixture cooled with stirring under argon to 0 °C (ice bath). Then, EDC (135 mg, 0.7 mmol) was added and the mixture was stirred at 0 °C for 1 h and then at room temperature for 12 h. The reaction mixture was diluted with CH₂Cl₂ (40 mL) and the organic phase was washed with 1 M aqueous NaHSO₄ (20 mL), saturated aq. NaHCO₃ (20 mL), and brine (20 mL). The organic phase was dried over Na₂SO₄, filtered, and the filtrate was evaporated *in vacuo* to give PAH-IR(Boc₂)S-OMe. Yield: 146 mg (0.21 mmol, 47%) of white foam, $[\alpha]_{\text{D}}^{20} = -13.4$ (c = 0.67; CH₂Cl₂). FT-IR (ATR): ν_{max} 3290, 2932, 1721, 1637, 1544, 1416, 1367, 1330, 1253, 1228, 1155, 1134, 1052, 1026, 914, 807, 776, 732, 646 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ 0.82–0.98 (6H, m), 1.12–1.22 (2H, m), 1.28–1.40 (3H, m), 1.42 (1H, s), 1.48 and 1.49 (18H, 2s, 1:1), 1.58–1.68 (4H, m), 1.69–1.78 (1H, m), 1.80–1.89 (1H, m), 1.90–1.99 (2H, m), 2.00–2.06 (1H, m), 2.17 (1H, s), 2.20–2.30 (2H, m), 2.39 (2H, br t, *J* = 7.0 Hz), 2.48–2.54 (2H, m), 3.19–3.29 (2H, m), 3.35–3.43 (1H, m), 3.76 (3H, s), 3.87 (1H, dd, *J* = 11.6, 3.0 Hz), 3.96 (1H, dd, *J* = 11.6, 3.8 Hz), 4.24 (1H, t, *J* = 7.5 Hz), 4.52 (1H, td, *J* = 7.7, 5.8 Hz), 4.61 (1H, quintet, *J* = 3.7 Hz), 6.21 (1H, t, *J* = 5.9 Hz), 6.59 (1H, d, *J* = 7.8 Hz), 7.09 (1H, d, *J* = 7.6 Hz), 7.45 (1H, d, *J* = 8.0 Hz), 8.36 (1H, t, *J* = 5.6 Hz), 11.42 (1H, s). ¹³C NMR (150 MHz, CDCl₃) δ 11.2, 15.0, 15.3, 15.6, 24.9, 25.2, 25.3, 26.2, 28.1, 28.3, 28.4, 28.6, 28.9, 35.3, 35.3, 36.0, 36.8, 39.2, 40.3, 52.6, 53.0, 55.0, 58.5, 62.6, 65.9, 69.4, 79.6, 83.2, 83.3, 153.2, 156.2, 163.3, 170.6, 171.2, 171.2, 171.9, 174.1. *m/z* (HRMS): 782.4650 (MH⁺). C₃₇H₆₄N₇O₁₁ requires *m/z* 782.4658. (Found: C, 56.72; H, 8.23; N, 12.30%. C₃₇H₆₃N₇O₁₁ requires: C, 56.83; H, 8.12; N, 12.54%).

1.4.2. Synthesis of *N*-(*N*²-[*N*-[6-(pent-4-ynamido)hexanoyl]-*L*-isoleucyl]-*L*-arginyl)-*L*-serinate (*al-IRS probe***).** PAH-IR(Boc₂)S-OMe (53 mg, 0.068 mmol) was dissolved in TFA (1 mL) and the so formed solution was left to stand at room temperature for 30 min. Volatile components were evaporated *in vacuo* (27 °C, 15 mbar) and the brownish oily residue was dried *in vacuo* (20 °C, 15 mbar) over NaOH pellets for 48 h. Yield: 46 mg (0.066 mmol, 97%) of brownish resin, $[\alpha]_{\text{D}}^{20} = -16.1$ (c = 0.267; MeOH). ¹H NMR (600 MHz, CD₃OD): δ 0.87–0.98 (6H, m), 1.17–1.27 (1H, m), 1.33–1.44 (3H, m), 1.48–1.55 (2H, m), 1.58–1.70 (4H, m), 1.71–1.77 (1H, m), 1.78–1.86 (1H, m), 1.87–1.97 (1H, m), 2.23–2.28 (2H, m), 2.34–2.40 (2H, m), 2.42–2.49 (2H, m), 3.12–3.24 (3H, m), 3.28–3.33 (2H, m, overlapped by the signal for MeOH), 3.73 (3H, s), 3.79 (1H, dd, *J* = 11.4, 3.9 Hz), 3.93 (1H, dd, *J* = 11.3, 4.4 Hz), 4.10–4.18 (1H, m), 4.45–4.53 (2H, br m). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 11.2, 15.8, 15.9, 26.1,

26.6, 27.5, 27.7, 28.1, 30.1, 36.1, 36.5, 37.5, 40.2, 42.0, 52.9, 53.8, 56.2, 59.6, 61.7, 70.4, 83.5, 115.2, 158.6, 160.8, 161.0, 169.9, 172.1, 173.6, 174.0. m/z (HRMS): 582.3599 (MH^+). $C_{27}H_{48}N_7O_7$ requires m/z 582.3610.

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