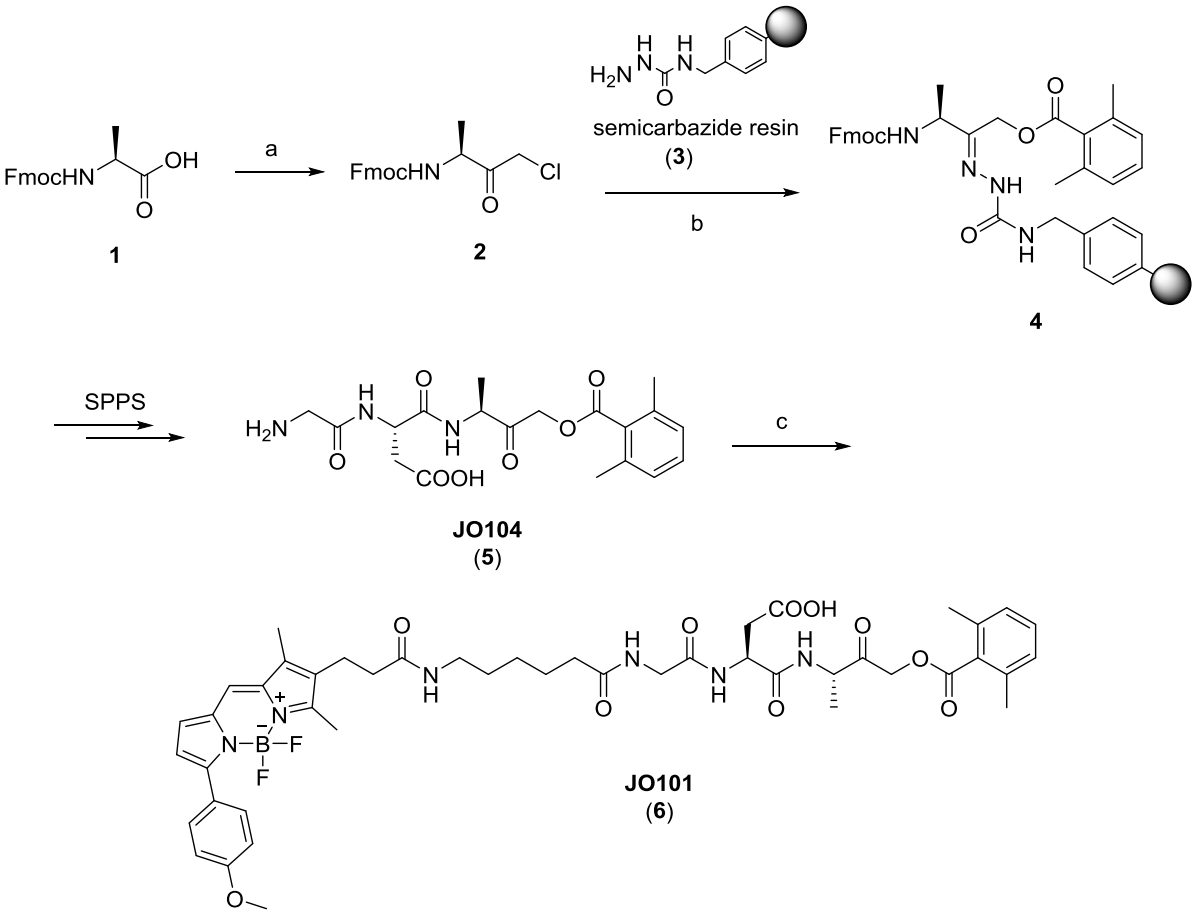
Supplemental File S1: Chemical synthesis of AOMK probes

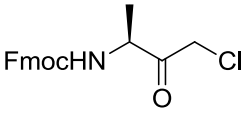
For synthesis of the AOMK probes, a solid phase-based approach was used following essentially protocols by Kato *et al*. (*Nat. Chem. Biol.* **2005**, *1*, 33-38).

Synthesis of GDA-AOMK probes



Scheme 1. Chemical synthesis of the GDA-AOMK probes **JOGDA1** (**6**) and **JOGDA2** (**5**). a) i) *iso*-butyl chloroformate (1.15 eq.), NMM (1.25 eq.), THF, -10 °C, 25 min, ii) diazomethane (4 eq.), 0 °C to rt, 3 h, iii) aq. conc. HCl/AcOH (1:1), 0 °C, 1 h; b) semicarbazide resin **3**, 2,6-dimethyl benzoic acid (3.75 eq.), KF (7.5 eq.), DMF; c) Bodipy-Ahx-NHS (0.33 eq.), DIEA (17.5 eq.), DMF, rt, 16 h.

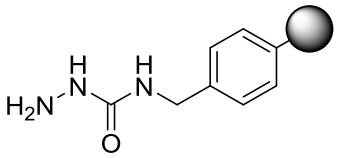
Synthesis of Fmoc-Ala-CMK (2)



A 0.2 M solution of Fmoc-Ala-OH (1.65 g, 5 mmol) in anhydrous THF was stirred in an ice/acetone bath at -10 °C. To this solution, *N*-methylmorpholine (686 µL, 6.25 mmol, 1.15 eq.) and *iso*-butyl chloroformate (752 µL, 5.75 mmol, 1.25 eq.) were sequentially added, resulting in the formation of a white precipitate. The reaction mixture was stirred for additional 25 min at -10 °C. The required diazomethane was generated *in situ* using the procedure described in the Aldrich Technical Bulletin (AL-180). This ethereal diazomethane solution (20 mmol, 4 eq.) was transferred to the stirred solution of the mixed anhydride at 0 °C and the resulting reaction mixture was allowed to warmed to room temperature over 3 h. To obtain the desired chloromethyl ketone, a solution of concentrated hydrochloric acid and acetic acid (1:1, 15 mL) was then added dropwise to the reaction mixture at 0 °C and stirred for 1 h. Ethyl acetate was added, the organic layer was separated, washed with water, brine, sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated to dryness, yielding 1.82 g (>98%) of **2** in sufficient purity for the next synthetic manipulations.

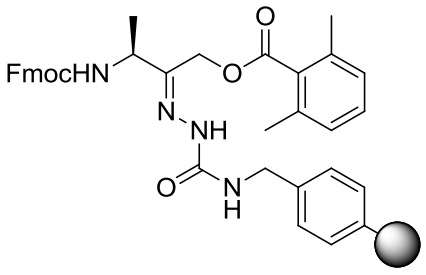
^{1}H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.57 (dd, *J* = 18.6, 14.1 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.32 (td, *J* = 7.4, 1.0 Hz, 2H), 5.38 (d, *J* = 6.5 Hz, 1H), 4.67 – 4.54 (m, 1H), 4.45 (ddd, *J* = 29.4, 10.6, 7.0 Hz, 2H), 4.22 – 4.17 (m, 2H), 1.45 – 1.27 (m, 3H); **^{13}C NMR** ($CDCl_3$) δ 201.70, 155.83, 143.78, 141.47, 127.92, 127.22, 125.15, 125.06, 120.15, 67.06, 53.61, 47.32, 46.10, 27.73, 18.84, 17.63; **LC-MS** (ESI): t_R = 9.29 min, 344.10 calcd. for $C_{19}H_{19}ClNO_3^+$ $[M+H]^+$, found 344.61.

Synthesis of semicarbazide resin 3



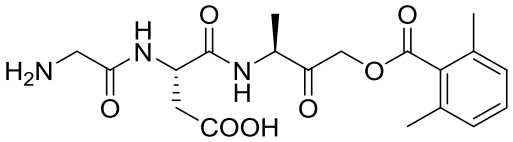
Aminomethylpolystyrene resin (0.5 g, 0.45 mmol/g) was dried under vacuum overnight in a 10 mL-polypropylene cartridge. The resin was presolvated with DMF for 30 min, the solvent was removed by filtration and a presolvation step with DCM for additional 30 min was performed. A 1 M solution of *N*, *N*'-carbonyldiimidazole (0.8 g, 4.95 mmol, 11 eq.) in DCM was added to the resin and the resin was shaken at room temperature for 3 h. The reagent was drained and the resin was washed with DCM followed by DMF. A 10 M solution of hydrazine (1.55 mL, 49.5 mmol, 110 eq.) in DMF was added to the resin, and the resin was shaken at room temperature for 1 h. The resin was washed with DMF followed by DCM, dried in vacuo, and stored until further use at 4 °C.

Synthesis of resin bound Fmoc-Ala-AOMK (4)



A 0.5 M solution of Fmoc-Ala-CMK (**2**, 0.2 g, 0.585 mmol) in DMF was added to the resin. The cartridge was tightly sealed and shaken at 50 °C for 3 h. The resin was washed with DMF. A 0.5 M solution of 2,6-dimethylbenzoic acid (170 mg, 2.2 mmol, 3.75 eq.) and potassium fluoride (128 mg, 4.4 mmol, 7.5 eq.) in DMF was added to the resin. The resulting suspension was shaken at room temperature overnight. After the solution was removed from the resin, the resin was washed with DMF followed by DCM, and dried in vacuo. The loading of the resin was determined as 0.3 mmol/g *via* the Fmoc loading assay.

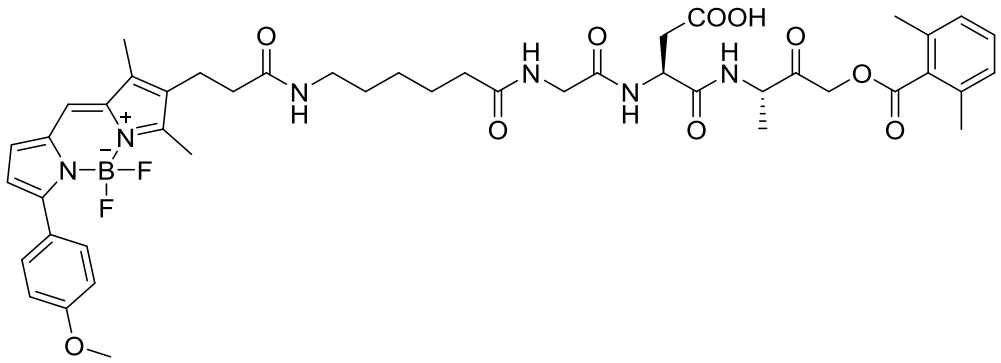
Synthesis of H-GDA-AOMK (JO104, 5)



This AOMK probe was obtained from resin **4**, using standard solid phase peptide synthesis. To this end, the following general coupling conditions were used: All amino acid couplings were performed in a syringe reactor, using commercially available Fmoc-amino acids (4 eq.), HOBt (4 eq.), HBTU (4 eq.) and DIEA (4 eq.) in DMF at room temperature with a coupling time of 2 h. High coupling rates of the different coupling steps was verified by Kaiser tests. Fmoc cleavages were performed with 20% piperidine in DMF for 15 min. After each coupling or Fmoc cleavage step, the resin was washed six times with DMF. Cleavage from the resin and simultaneous deprotection of amino acid side-chains was achieved by agitation of the resin for 2 h in a cleavage solution containing 95% TFA, 2.5% TIS and 2.5% H_2O. The cleavage-solution, containing the desired product, was collected and the resin was rinsed twice more with the cleavage solution. The combined solutions were evaporated to dryness and the crude product was purified by RP-HPLC.

LC-MS (ESI): t_R = 5.07 min, 408.17 calcd. for $C_{19}H_{26}N_3O_7^+$ $[M+H]^+$, found 408.37.

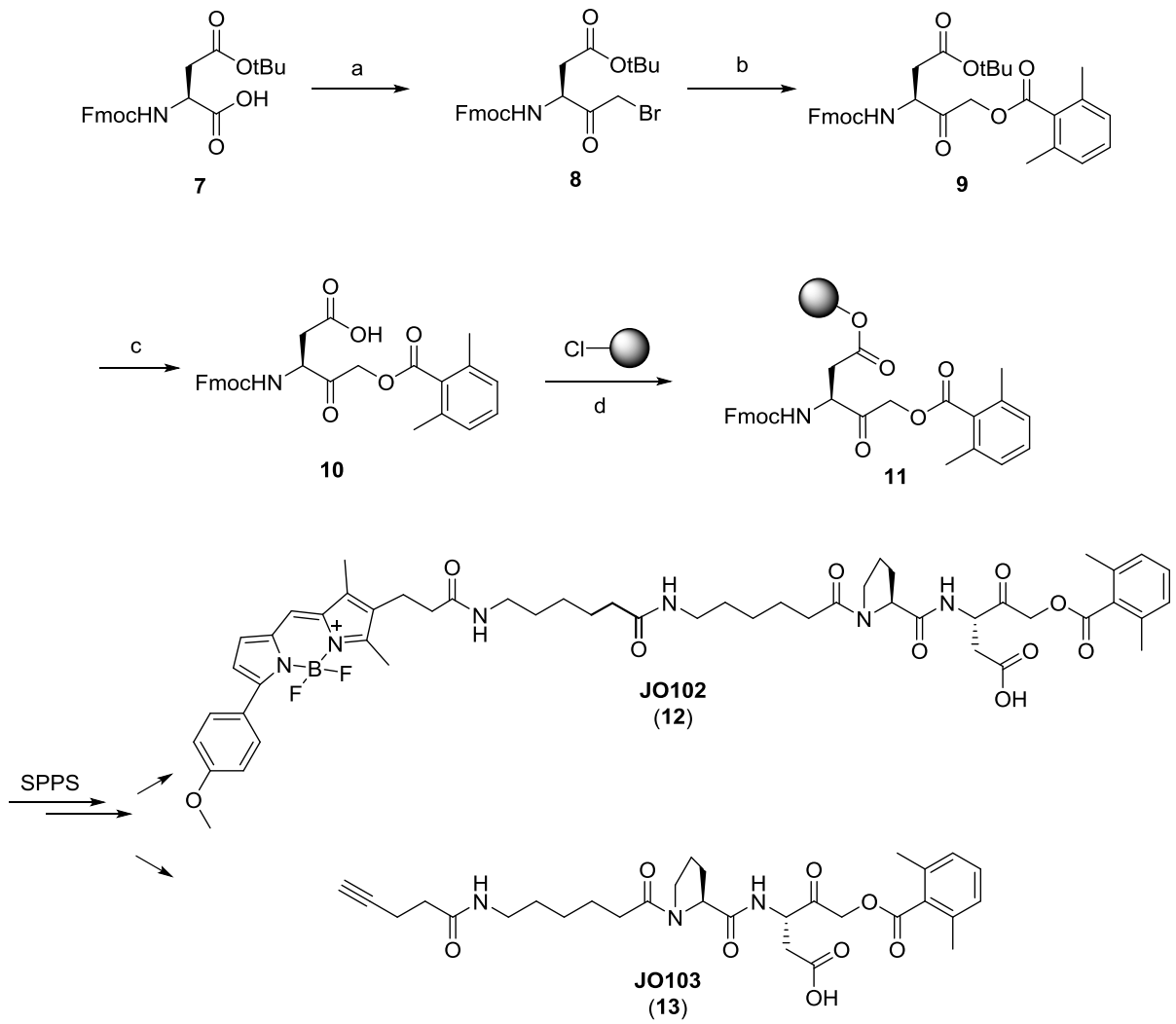
Synthesis of Bodipy-GDA-AOMK (JOGDA1, 6)



Due to the high price of commercial Bodipy-Ahx-NHS, we used this component as the limiting factor in this step. H-GDA-AOMK (**JOGDA2**, 2 mg, 4.9 µmol) was dissolved in DMF (0.5 mL) and DIEA (5 µL, 3.7 mg, 17.5 eq.) and a solution of Bodipy-Ahx-NHS (1 mg, 1.64 µmol) in DMF (0.5 mL) was added. The resulting reaction mixture was stirred for 16 h at rt. Afterwards, all volatiles were removed *via* reduced pressure and the crude product was purified by RP-HPLC, yielding 1.46 mg (1.62 µmol, 99%) of the desired product **JOGDA1** (**6**).

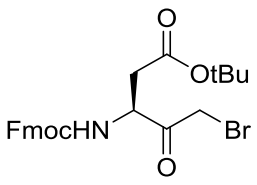
LC-MS (ESI): t_R = 8.64 min, 923.39 calcd. for $C_{46}H_{55}BF_2N_6NaO_{10}^+$ $[M+Na]^+$, found 923.45.

Synthesis of PD-AOMK probes



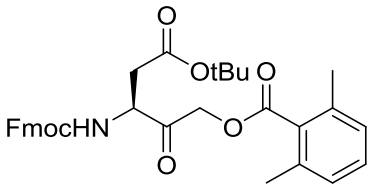
Scheme 2. Chemical synthesis of PD-AOMK probes **JOPD2** (**12**) and **JOPD2** (**13**). a) i) *iso*-butyl chloroformate (1.15 eq.), NMM (1.25 eq.), THF, -10 °C, 25 min, ii) diazomethane (4 eq.), 0 °C to rt, 3 h, iii) 30% HBr in AcOH, 0 °C, 1 h; b) 2,6-dimethyl benzoic acid (1.2 eq.), KF (3 eq.), DMF, 0 °C, overnight; c) TFA/DCM (1:4), rt, 1 h; d) 2-chloro trityl resin (0.83 eq.), DIEA (5 eq.), DCM, rt, 12 h.

Synthesis of Fmoc-Asp(O*t*Bu)-BMK (8)



A 0.2 M solution of Fmoc-Asp(*t*Bu)-OH (2 g, 5 mmol) in anhydrous THF was stirred in an ice/acetone bath at -10 °C. To this solution, *N*-methylmorpholine (686 µL, 6.25 mmol, 1.15 eq.) and *iso*-butyl chloroformate (752 µL, 5.75 mmol, 1.25 eq.) were sequentially added, resulting in the formation of a white precipitate. The reaction mixture was stirred for additional 25 min at -10 °C. The required diazomethane was generated *in situ* using the procedure described in the Aldrich Technical Bulletin (AL-180). This ethereal diazomethane solution (20 mmol, 4 eq.) was transferred to the stirred solution of the mixed anhydride at 0 °C and the resulting reaction mixture was allowed to warmed to room temperature over 3 h. To obtain the desired bromomethyl ketone, a solution of 30% HBr in acetic acid (10 mL) was then added dropwise to the reaction mixture at 0 °C and stirred for 1 h. Ethyl acetate was added, the organic layer was separated, washed with water, brine, sat. aq. $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated to dryness, yielding 2.4 g (>98%) of **8** in sufficient purity for the next synthetic manipulations.

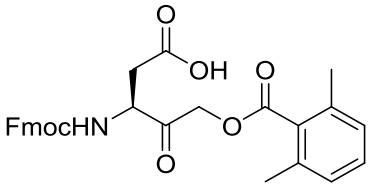
Synthesis of Fmoc-Asp(O*t*Bu)-AOMK (9)



A 0.2 M solution of **8** (2.4 g, 5 mmol) in DMF was stirred at 0 °C. To this solution, potassium fluoride (870 mg, 15 mmol, 3 eq.) and 2,6-dimethylbenzoic acid (900 mg, 6 mmol, 1.2 eq.) were added. The reaction mixture was allowed to warm to room temperature and stirred overnight. It was diluted by addition of ethyl acetate, the organic layer was separated and washed with water, brine, sat. aq. $NaHCO_3$ solution and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (ethyl acetate/cyclohexane = 1:5) to obtain 1.65 g (60%) of pure product **9** as a white solid.

TLC (ethyl acetate/cyclohexane = 1:5): R_f = 0.2; **^{1}H NMR** ($CDCl_3$) δ = 7.82 (d, *J* = 8.2 Hz, 2H), 7.63 (m, 2H), 7.41 (m, 2H), 7.33 (m, 2H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 7.64 Hz, 2H), 5.89 (d, *J* = 8.8 Hz, 1H), 5.07 (q, *J* = 16.8 Hz, 3H), 4.65 (m, 2H), 4.24 (t, *J* = 6.44 Hz, 1H), 2.97 (dd, *J* = 17.1, 4.88 Hz, 1H), 2.91 (dd, J = 17.1, 4.88 Hz, 1H), 2.40 (s, 6H), 1.45 (s, 9H); **^{13}C NMR** ($CDCl_3$) δ = 201.1, 169.0, 156.2, 143.7, 141.5, 141.5, 135.8, 132.7, 129.8, 127.9, 127.8, 125.2, 120.2, 120.2, 82.4, 67.3, 66.8, 54.9, 47.4, 36.7, 28.1, 27.1, 20.0; **LC-MS** (ESI): t_R = 11.44 min, 580.23 calcd. for $C_{33}H_{35}NNaO_7^+$ $[M+Na]^+$, found 580.11.

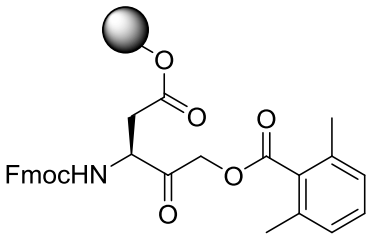
Synthesis of Fmoc-Asp-AOMK (10)



Fmoc-Asp(O*t*Bu)-AOMK (**9**, 1.65 g, 2.96 mmol) was dissolved in TFA/DCM (1:4, 15 mL) and stirred for 1 h at room temperature. The reaction mixture was diluted by addition of DCM, sufficient amounts of toluene were added and the cleavage solution was removed by co-evaporation. The product was dried *in vacuo*. The crude product **10** was used without further purification.

LC-MS (ESI): t_R = 9.70 min, 524.16 calcd. for $C_{29}H_{27}NNaO_7^+$ $[M+Na]^+$, found 524.37.

Synthesis of resin-bound Fmoc-Asp-AOMK (11)



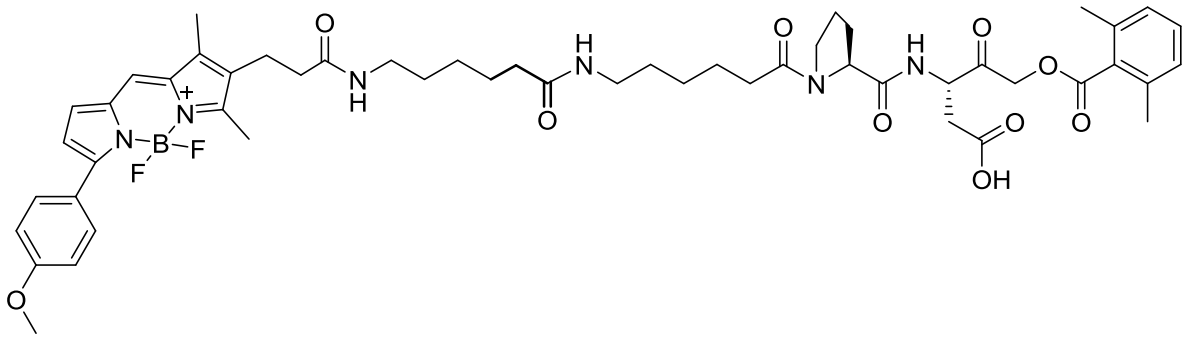
2-Chlorotrityl resin (500 mg, 0,685 mmol, maximal loading of 1.37 mmol/g) was loaded with Fmoc-Asp-AOMK (**10**, 420.8 mg, 0.84 mmol, 1.2 eq.) in the presence of DIEA (731 µL, 4.1 mmol, 6 eq.) in dry DCM (8 mL) under an argon atmosphere for 12 h at room temperature. It was washed 3x with DCM and 3x with DMF, capped for 30 min by addition of DCM/MeOH/DIEA (17 : 1 : 2 ,15 mL). The resin was washed again 5x with DMF and 5x with DCM and was subsequently dried under high vacuum. The resulting loading of the resin was determined as 0.49 mmol/g *via* the Fmoc loading assay.

General procedure for the SPPS to JOPD2 (12) and JOPD2 (13)

The AOMK probes were assembled by solid phase synthesis. To this end, the following general conditions were used: All amino acid couplings were performed in a syringe reactor, using commercially available Fmoc-amino acids (4 eq.) or 4-pentynoic acid (4 eq.), HOBt (4 eq.), HBTU (4 eq.) and DIEA (4 eq.) in DMF at room temperature with a coupling time of 2 h. For coupling of the Bodipy-Ahx moiety, commercially available Bodipy-Ahx-OSu reagent (1 eq.) was however used. High coupling rates of the different coupling steps was verified by Kaiser tests. Fmoc cleavages were performed with 20% piperidine in DMF for 15 min. After each coupling or Fmoc cleavage step, the resin was washed six times with DMF. Cleavage from the resin and simultaneous deprotection of amino acid side-chains was achieved by agitation of the resin for 2 h in a cleavage solution containing 95% TFA, 2.5% TIS and 2.5% H_2O. The cleavage-solution, containing the desired product, was

collected and the resin was rinsed twice more with the cleavage solution. The combined solutions were evaporated to dryness and the crude product was purified by RP-HPLC.

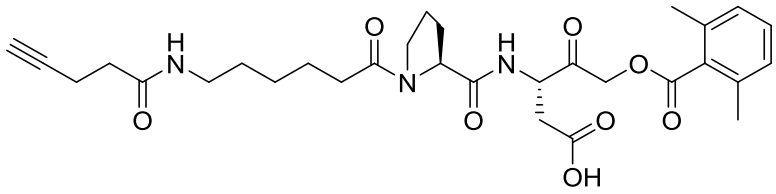
Synthesis of Bodipy-PD-AOMK (JOPD1, 12)



Following the above protocol for SPPS led to 0.82 mg of the product **JOPD1** as a yellowish solid.

LC-MS (ESI): t_R = 8.71 min, 963.48 calcd. for $C_{52}H_{65}BFN_6O_{10}^+$ $[M+H]^+$, found 963.52.

Synthesis of pent-4-ynoic-PD-AOMK (JOPD2, 13)



Following the above protocol for SPPS led 4.4 mg of the product **JOPD2** as a colorless solid.

LC-MS (ESI): t_R = 6.93 min, 570.28 calcd. for $C_{30}H_{40}N_3O_8^+$ $[M+H]^+$, found 570.24.