Supporting Information

A Highly Selective and Potent Inhibitor for PTP-MEG2 with Therapeutic Potential in Type 2 Diabetes

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Materials. Dimethylformamide (DMF), isopropanol, dichloromethane (DCM), N-methyl morpholine (NMM), and acetic acid (AcOH), disposable syringes (with a frit) were from Fisher Scientific. Diethyl ether, piperidine, trifluoroacetic acid (TFA), triisopropylsilane (TIS), tetrakis(triphenylphosphine)-palladium(0), 3-iodobenzoic acid (mIBA), and 3-bromo-4-methylbenzoic acid (BMBA), homovanillic acid (HMVA) were from Aldrich. The Rink amide resin, O-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), N-hydroxybenzotriazole (HOBt), Fmoc-Phe-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Orn(Boc)-OH, Fmoc-β-Ala-OH, Fmoc-Orn(Alloc)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Phe(4-I)-OH, Fmoc-Dpr(Boc)-OH were from Advanced ChemTech. 5-Carboxyfluorescein (5-FAM) was from Anaspec. Fmoc-F_2Pmp-OH was prepared following the literature procedures.[1-2] Compound **C1** (Figure S1) was prepared as previously reported.[3]

Instrumentation. HPLC purification was carried out on a Waters Breeze HPLC system equipped with a Waters Atlantis dC18 column (10 μm, 19 mm × 100 mm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500-MHz NMR spectrometer. Analytical HPLC analysis was carried out on a Waters Breeze HPLC system equipped with a Agilent Eclipse XDB-C18 column (5 μm, 4.6 mm × 150 mm).

General Procedure A for Rink Amide Resin Activation. Rink amide resin (Advanced ChemTech) was mixed with DCM (1 mL per 100 mg resin) and then shaken for 30 min. After activation, resin was washed three times with DMF (1 mL per 100 mg resin).

General Procedure B for the Removal of the Fmoc Group from the Rink Amide Resin. Rink amide resin was mixed with 20% piperidine in DMF (1 mL per 100 mg resin) and shaken for 30 min, and then washed with DMF (1 mL per 100 mg resin, 3 times), isopropanol (1 mL per 100 mg resin, 3 times), and DCM (1 mL per 100 mg resin, 3 times) sequentially. The removal of the Fmoc group was confirmed by the ninhydrin test.

General Procedure C for the Removal the Alloc Group from the Rink Amide Resin. The resin (200 mg) was washed with DCM (2 mL, 5 times) and shaken under N_2 overnight with a solution of tetrakis(triphenylphosphine)palladium(0) (10 mg), AcOH (0.5 mL), and NMM (0.2 mL) in DCM (10 mL). The resin was then washed with DMF (2 mL, 3 times), isopropanol (2 mL, 3 times), and DCM (2 mL, 3 times). The removal of the Alloc group was confirmed by the ninhydrin test.

General Procedure D for the Removal the Mtt Group from the Rink Amide Resin. The resin was washed with DCM (1 mL per 100 mg resin, 10 times). The resin was shaken with TFA (1% in DCM, 1 mL per 100 mg resin) for 1 min (repeat 10 times). The resin was then washed with DCM (1 mL per 100 mg resin, 3 times), DMF (1 mL per 100 mg resin, 3 times), isopropanol (1 mL per 100 mg resin, 3 times), and DCM (1 mL per 100 mg resin, 3 times). The removal of the Mtt group was confirmed by the ninhydrin test.

General Procedure E for the Coupling of Carboxylic Acids to the Rink Amide Resin. Carboxylic acids (5 equiv, 0.5 M in DMF) were first mixed with HBTU (5 equiv, 0.5 M in DMF), HOBt (5 equiv, 0.5 M in DMF), and NMM (15 equiv, 1.5 M inDMF). The mixed solution was then added to the resin and shaken for 2 h. The resin was then washed with DMF (1 mL per 100 mg resin, 3 times), isopropanol (1 mL per 100 mg resin, 3 times), and DCM (1 mL per 100 mg resin, 3 times). The completion of the coupling reaction was confirmed by the ninhydrin test.

General Procedure E for Peptide Cleavage from the Rink Amide Resin. The resin was washed with DCM (1 mL per 100 mg resin, 5 times) and subsequently shaken with a solution of 95% TFA, 2.5% TIS, and 2.5% H2O (1 mL per 100 mg resin) for 2 hours. The resin was removed by filtration, and the TFA was evaporated under vacuum. The crude peptide was obtained after trituration with diethyl ether (5 mL per 100 mg resin, 2 times).

*Synthesis of Compound **2a***. Compound **2a** was synthesized using standard Fmoc chemistry on the Rink amide resin in a disposable syringe with a frit (Scheme **S1**). Rink amide

resin (200 mg, 0.7 mmol/g loading, 0.14 mmol) was first activated with DCM (2 mL, general procedure A). Fmoc group was removed by 20% piperidine (general procedure B). The resin was the coupled with Fmoc-Lys(Alloc)-OH (general procedure E). After the deprotection of Fmoc group (general procedure B), the resin was couple with Fmoc-β-Ala-OH (general procedure E). The resin was treated with piperidine (general procedure B) and coupled with Fmoc-Orn(Boc)-OH (general procedure E). The Fmoc group was removed (general procedure B), and the resin was coupled with Fmoc-F_2Pmp-OH (general procedure E). The Fmoc group was removed (general procedure B), and resin was coupled with AcOH (general procedure E). Then, the resin was tread with Pd(0) for the removal of Alloc group (general procedure C) and coupled with 5-FAM (general procedure E). Compound **2a** was cleaved from beads (general procedure F). Crude peptide was purified by HPLC to afford **2a** (12.1 mg, 9% yield). MS (ESI): calcd for [M] 1007, found $[M+H]^+$ 1008.

*Synthesis of the Library **2b**.* The library was prepared on a Freedom EVO workstation (Tecan) with a 96 channel MCA tip block using disposable tips (Rainin). The detailed procedure is as follows: the 576 different carboxylic acids (40 mM, 10 μL) in DMF were placed in six 96-well microplates. HBTU (35 mM in DMF, 10 μL), HOBt (50 mM in DMF, 10 μL), and NMM (200 mM in DMF, 10 μL) were sequentially added to each well of the six plates and mixed for 5 min for the activation of carboxylic acids. The precursor **2a** (2 mM in DMF, 10 μL) was then added to each well. The reaction was quenched with cylcohexylamine (87 mM in DMF, 10 μL) after 1 hour. Finally, 190 μL of DMSO were added to each well to create the ready-for-screening format. The library was stored in a -20 ^oC freezer before screening.

*Synthesis of Compound **3***. Compound **3** was synthesized using standard Fmoc chemistry on the Rink amide resin (Scheme S2). The resin (200 mg, 0.7 mmol/g loading, 0.14 mmol) was first activated by DCM (General procedure A). The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Orn(Alloc)-OH (general procedure E). The Fmoc group was removed (General procedure B) and Fmoc-

F_2Pmp-OH was attached to resin (General procedure E). The Fmoc group was again removed (general procedure B) and the amine group on the F_2Pmp residue was capped by AcOH (general procedure E). The resin was treated with Pd(0) for the deprotection of Alloc group (general procedure C). 3-Iodobenzoic acid (mIBA) was attached to resin (general procedure E). Compound **3** was cleaved from beads (General procedure F). Crude peptide was purified by HPLC to afford **3** (15.1 mg, 16% yield). The assignment of proton NMR utilized additional information from COSY. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.17 (s, 1 H, mIBA-Ar**H**), 7.87 (d, J = 7.9 Hz, 1 H, mIBA-Ar**H**) 7.79 (d, J = 7.9 Hz, 1 H, mIBA-Ar**H**) 7.50 (d, J = 7.9 Hz, 2 H, F_2Pmp-Ar**H**), 7.33 (d, J = 7.9 Hz, 2 H, F_2Pmp-Ar**H**), 7.24-7.20 (m, 1 H, mIBA-Ar**H**), 4.59-4.53 (m, 1 H, F_2Pmp-C_α**H**), 4.37-4.32 (m, 1 H, Orn-C_α**H**), 3.40-3.32 (m, 2 H, Orn-C_δ**H**$_2$), 3.07-3.02 (m, 2 H, F_2Pmp-C_β**H**$_2$), 1.94 (s, 3 H, -COC**H**$_3$). 1.80-1.71 (m, 1 H, Orn-C_β**H**H'), 1.68-1.54 (m, 3 H, Orn-C_βH**H'**, Orn-C_γ**H**$_2$). ^{13}C NMR (125 MHz, CD3OD): δ = 173.32, 173.16, 141.53, 140.72, 137.78, 137.35, 131.36, 130.32, 127.59, 94.68, 56.43, 40.36, 38.63, 30.40, 26.72, 22.37. MS (ESI): calcd for [M], 680, found $[M+H]^+$ 681. HPLC purity analysis: > 95% (UV, λ = 254 nm).

*Synthesis of Compound **4a***. Compound **4a** was synthesized using standard Fmoc chemistry on the Rink amide resin in a disposable syringe with a frit (Scheme S3). Rink amide resin (200 mg, 0.7 mmol/g loading, 0.14 mmol) was first activated with DCM (2 mL, general procedure A). Fmoc group was removed by piperidine (20% solution in DMF, 2mL, general procedure B). The resin was the coupled with Fmoc-Lys(Mtt)-OH (general procedure E). After the deprotection of Fmoc group (general procedure B), the resin was couple with Fmoc-β-Ala-OH (general procedure E). The resin was treated with piperidine (general procedure B) and coupled with Fmoc-Orn(Alloc)-OH (general procedure E). The Fmoc group was removed (general procedure B), and the resin was coupled with Fmoc-F_2Pmp-OH (general procedure E). The Alloc group was removed (general procedure C), and resin was coupled with mIBA (general procedure E). The resin was treated with 1% TFA in DCM for the removal of Mtt group (general procedure D) and coupled with 5-FAM (general procedure E). The resin was treated with

piperidine to remove Fmoc group. Compound **4a** was cleaved from beads (general procedure F). Crude peptide was purified by HPLC to afford **4a** (10.4 mg, 6% yield). MS (ESI): calcd for [M] 1195, found $[M+H]^+$ 1196.

*Synthesis of the Library **4b***. The library was prepared in the same procedure as the library **2b**, except that compound **4a** (2 mM in DMF) was used as the library precursor.

*Synthesis of Compound **5***. Compound **5** was synthesized using standard Fmoc chemistry on the Rink amide resin (Scheme S4). The resin (200 mg, 0.7 mmol/g loading, 0.14 mmole) was first activated by DCM (General procedure A). The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Orn(Alloc)-OH (general procedure E). The Fmoc group was removed (General procedure B) and Fmoc-F_2Pmp-OH was attached to resin (General procedure E). The Fmoc group was again removed (general procedure B) and the resin was couple with BMBA (general procedure E). The resin was treated with Pd(0) for the deprotection of Alloc group (general procedure C). 3-Iodobenzoic acid (mIBA) was attached to resin (general procedure E). Compound **5** was cleaved from beads (General procedure F). Crude peptide was purified by HPLC to afford **5** (13.6 mg, 12% yield). The assignment of proton NMR utilized additional information from COSY. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.14 (s, 1 H, mIBA-Ar**H**), 7.98 (s, 1 H, BMBA-Ar**H**), 7.84 (d, J = 7.9 Hz, 1 H, mIBA-Ar**H**), 7.76 (d, J = 7.9 Hz, 1 H, mIBA-Ar**H**) 7.64 (d, J = 8.2 Hz, 1 H, BMBA-ArH), 7.51 (d, J = 7.9 Hz, 2 H, F_2Pmp-Ar**H**), 7.38 (d, J = 7.9 Hz, 2 H, F_2Pmp-Ar**H**) 7.31 (d, J = 8.2 Hz, 1 H, BMBA-Ar**H**), 7.21-7.17 (m, 1 H, mIBA-Ar**H**), 4.79-4.73 (m, 1 H, F_2Pmp-C_α**H**), 4.41-4.34 (m, 1 H, Orn-C_α**H**), 3.40-3.32 (m, 2 H, Orn-C_δ**H**$_2$), 3.20-3.15 (m, 2 H, F_2Pmp-C_β**H**$_2$), 2.41 (s, 3 H, BMBA-Ar-C**H**$_3$). 1.82-1.75 (m, 1 H, Orn-C_β**H**H'), 1.75-1.61 (m, 3 H, Orn-C_βH**H'**, Orn-C_γ**H**$_2$). ^{13}C NMR (125 MHz, CD_3OD): δ = 173.28, 168.65, 143.18, 141.53, 137.75, 137.39, 134.53, 132.51, 131.98, 131.36, 130.35, 127.64, 127.59, 125.70, 94.75, 57.05, 40.40, 38.44, 30.44, 26.76, 23.07. MS (ESI): calcd for [M], 834, found $[M+H]^+$ 835. HPLC purity analysis: > 95% (UV, λ = 254 nm).

*Synthesis of Compound **6a***. Compound **6a** was synthesized using standard Fmoc chemistry on the Rink amide resin in a disposable syringe with a frit (Scheme S5). Rink amide resin (200 mg, 0.7 mmol/g loading, 0.14 mmol) was first activated with DCM (2 mL, general procedure A). Fmoc group was removed by piperidine (20% solution in DMF, 2mL, general procedure B). The resin was the coupled with Fmoc-Lys(Mtt)-OH (general procedure E). After the deprotection of Fmoc group (general procedure B), the resin was couple with Fmoc-β-Ala-OH (general procedure E). The resin was treated with piperidine (general procedure B) and coupled with Fmoc-Dpr(Boc)-OH (general procedure E). The resin was treated with piperidine (general procedure B) and coupled with Fmoc-Orn(Alloc)-OH (general procedure E). The Fmoc group was removed (general procedure B), and the resin was coupled with Fmoc-F_2Pmp-OH (general procedure E). The resin was treated with piperidine (general procedure B) and coupled with BMBA (general procedure E). The Alloc group was removed (general procedure C), and resin was coupled with mIBA (general procedure E). The resin was treated with 1% TFA in DCM for the removal of Mtt group (general procedure D) and coupled with 5-FAM (general procedure E). Compound **6a** was cleaved from beads (general procedure F). Crude peptide was purified by HPLC to afford 6a (8.6 mg, 4% yield). MS (ESI): calcd for [M] 1477, found $[M+H]^+$ 1478.

*Synthesis of Library **6b***. The library was prepared in the same procedure as the library **2b**, except that compound **6a** (2 mM in DMF) was used as the library precursor.

Synthesis of Compound 7. Compound **7** was synthesized using standard Fmoc chemistry on the Rink amide resin (Scheme S6). The resin (200 mg, 0.7 mmol/g loading) was first activated by DCM (General procedure A). The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Dpr(Boc)-OH. The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Orn(Alloc)-OH. The Fmoc group was removed (General procedure B) and Fmoc-F2Pmp-OH was attached to resin (General procedure E). The Fmoc

group was again removed (general procedure B) and the amine group on the F_2Pmp residue was coupled with BMBA (general procedure E). The resin was treated with Pd(0) for the deprotection of Alloc group (general procedure C). 3-Iodobenzoic acid (mIBA) was attached to resin (general procedure E). The resin was treated with TFA (general procedure F) to give the crude peptide intermediate, which was treated with a mixture of HVA (0.5 M in DMF, 100 μL), HBTU (0.5 M in DMF, 100 μL), HOBt (0.5 M in DMF, 100 μL) and NMM (1.5 M in DMF, 100 μL) to give the crude product **7**. The crude product was purified by HPLC to afford **7** (21.5 mg, 14% yield). The assignment of proton NMR utilized additional information from COSY. ^{1}H NMR (500 MHz, CD_3OD): δ = 8.16 (s, 1 H, mIBA-Ar**H**), 7.92 (s, 1 H, BMBA-Ar**H**), 7.86 (d, *J* = 7.9 Hz, 1 H, mIBA-Ar**H**), 7.78 (d, *J* = 7.9 Hz, 1 H, mIBA-Ar**H**) 7.64 (d, *J* = 8.2 Hz, 1 H, BMBA-ArH), 7.58-7.52 (m, 3 H, BMBA-Ar**H**, F_2Pmp-Ar**H**), 7.39 (d, *J* = 7.9 Hz, 2 H, F_2Pmp-Ar**H**) 7.27 (d, *J* = 8.2 Hz, 1 H, BMBA-Ar**H**), 7.21-7.16 (m, 1 H, mIBA-Ar**H**), 6.81-6.78 (m, 1 H, HVA-Ar**H**), 6.71-6.63 (m, 2 H, HVA-Ar**H**), 4.83-4.80 (m, 1 H, F_2Pmp-C_α**H**), 4.48-4.42 (m, 1 H, Dpr-C_α**H**), 4.30-4.24 (m, 1 H, Orn-C_α**H**), 3.77 (s, 3 H, HVA-OC**H**$_3$), 3.63-3.58 (m, 1 H, Dpr-C_β**H**H'), 3.51-3.45 (m, 1 H, Dpr-C_βH**H'**), 3.41 – 3.33 (m, 5 H, Orn-C_δ**H**$_2$, F_2Pmp-C_β**H**H', HVA-C**H**$_2$-CO-), 3.16-3.09 (m, 1 H, F_2Pmp-C_βH**H'**), 2.39 (s, 3 H, BMBA-Ar-C**H**$_3$). 1.92-1.85 (m, 1 H, Orn-C_β**H**H'), 1.75-1.62 (m, 3 H, Orn-C_βH**H'**, Orn-C_γ**H**$_2$). ^{13}C NMR (125 MHz, CD3OD): δ = 175.72, 174.20, 174.15, 168.85, 168.48, 148.97, 146.60, 143.20, 141.53, 141.41, 137.67, 137.41, 134.42, 132.45, 131.95, 131.35, 130.25, 127.83, 127.63, 127.60 127.53, 125.65, 122.83, 116.30, 113.84, 101.39, 94.73, 56.73, 56.43, 55.33, 43.37, 42.17, 40.39, 37.76, 29.54, 26.96, 23.03. MS (ESI): calcd for [M], 1084, found $[M+H]^+$ 1085. HPLC purity analysis: > 95% (UV, λ = 254 nm).

*Synthesis of Compound **8***. Compound **8** was synthesized using standard Fmoc chemistry on the Rink amide resin (Scheme S7). The resin (200 mg, 0.7 mmol/g loading) was first activated by DCM (General procedure A). The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Dpr(Boc)-OH.

The Fmoc group on the resin was removed by piperidine in DMF (General procedure B). The resin was then coupled with Fmoc-Orn(Alloc)-OH. The Fmoc group was removed (General procedure B) and Fmoc-Phe-OH was attached to resin (General procedure E). The Fmoc group was again removed (general procedure B) and the amine group on the F_2Pmp residue was coupled with BMBA (general procedure E). The resin was treated with Pd(0) for the deprotection of Alloc group (general procedure C). 3-Iodobenzoic acid (mIBA) was attached to resin (general procedure E). The resin was treated with TFA (general procedure F) to give the crude peptide intermediate, which was treated with a mixture of HVA (0.5 M in DMF, 100 uL), HBTU (0.5 M in DMF, 100 uL), HOBt (0.5 M in DMF, 100 uL) and NMM (1.5 M in DMF, 100 uL) to give the crude product **8**. The crude product was purified by HPLC to afford **8** (15.8 mg, 12% yield). The assignment of proton NMR utilized additional information from COSY. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.70 (d, J = 8.1 Hz, 1 H, BMBA-N**H**), 8.60-8.55 (m, 1 H, mIBA-N**H**), 8.35 (d, J = 6.9 Hz, 1 H, Phe-N**H**), 8.19 (s, 1 H, mIBA-Ar**H**), 8.04-7.95 (m, 2 H, Orn-N**H**, BMBA-Ar**H**), 7.91-7.83 (m, 3 H, mIBA-Ar**H**, HVA-N**H**), 7.67 (d, J = 7.5 Hz, 1 H, BMBA-Ar**H**), 7.40-7.15 (m, 9 H, BMBA-Ar**H**, Phe-Ar**H**, -CON**H**$_2$, mIBA-Ar**H**), 6.77 (s, 1 H, HVA-Ar**H**), 6.63 (d, J = 7.6 Hz, 1 H, HVA-Ar**H**), 6.58 (d, J =7.6 Hz, 1 H, HVA-Ar**H**), 4.78-4.72 (m, 1 H, Phe-C$_\alpha$**H**), 4.30-4.22 (m, 2 H, Dpr-C$_\alpha$**H**, Orn-C$_\alpha$**H**), 3.70 (s, 3 H, HVA-OC**H**$_3$), 3.40-3.35 (m, 1 H, Dpr-C$_\beta$**H**H'), 3.35-3.18 (m, HVA-C**H**$_2$-CO, Dpr-C$_\beta$H**H'**, Orn-C$_\delta$**H**$_2$,Phe-C$_\beta$**H**H'), 3.04-2.96 (m, 1 H, Phe-C$_\beta$H**H'**), 2.35 (s, 3 H, BMBA-Ar-C**H**$_3$), 1.81-1.74 (m, 1 H, Orn-C$_\beta$**H**H'), 1.68-1.52 (m, 3 H, Orn-C$_\beta$H**H'**, Orn-C$_\gamma$**H**$_2$). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 171.79, 171.39, 171.25, 171.17, 164.70, 164.52, 147.13, 144.94, 140.65, 139.46, 138.30, 136.49, 135.51, 133.34, 130.75, 130.32, 129.00, 127.92, 126.62, 126.54, 126.10, 123.78, 121.26, 115.05, 113.13, 94.53, 55.37, 54.77, 53.01, 52.80, 41.74, 40.57, 36.68, 28.99, 25.46, 22.26. MS (ESI): calcd for [M], 954, found [M+H]$^+$ 955. HPLC purity analysis: > 95% (UV, λ = 254 nm).

*Screening of Library **2b**.* The library screening assay was carried out on a Tecan Genesis workstation with a 96-channel tip block with fixed tips. Before screening, the library compounds

were diluted from the DMSO stock solution into 3,3-dimethylglutarate buffer (50 mM 3,3-dimethylglutarate buffer, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15 M adjusted by addition of NaCl), resulting in a set of six daughter plates with an ~75 nM concentration of each compound in each well. In the first screen, PTP-MEG2 (2 μM in 50 mM 3,3-dimethylglutarate buffer, 50 μL, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15 M adjusted by addition of NaCl) was dispensed into each well of a 384-well plate, and then 2 μL of the fluorescein-tagged library compounds were transferred from four 96-well intermediate plates to the 384-well plate (final compound concentration ~3 nM). The fluorescence polarization values (A_1) were recorded on an Envision 2021 Multilabel Microplate Reader (Perkin-Elmer). In the second screen, 50 μL of a mixture of 2 μM PTP-MEG2 and 20 μM compound **C1** (as a competitive ligand) in 3,3-dimethylglutarate buffer (50 mM 3,3-dimethylglutarate buffer, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15 M adjusted by addition of NaCl) were dispensed into each well of another 384-well plate, followed by the addition of 2 μL of the fluorescein tagged library compounds (75 nM in 3,3-dimethylglutarate buffer, pH 7.0, containing 1 mM EDTA with an ionic strength of 0.15 M adjusted by addition of NaCl). The fluorescence polarization values (A_2) were again measured. A displacement percentage was calculated for each library compound as $(A_1 - A_2)/(A_1 - A_0) \times 100\%$, where A_1 and $A2$ are the fluorescence anisotropy values of each sample as described, and A_0 is the fluorescence anisotropy of free library compounds in 3,3-dimethylglutarate buffer. To simplify the calculation, A_0 was set to 30. The binding affinity ranking of each compound was determined on the displacement percentage: the smaller the displacement percentage, the higher the binding affinity. The best hits were selected based on affinity and are listed in Table S1.

*Screening of Library **4b***. The library was screened using the same protocol as the library **2b**, except that compound **3** (finial concentration as 10 μM) was used as the competitive ligand. The best hits were selected based on affinity and are listed in Table S2.

*Screening of Library **6b***. The library was screened using the same protocol as the library **2b**, except that compound **5** (finial concentration as 10 μM) was used as the competitive ligand. The best hits were selected based on affinity and are listed in Table S3.

References:

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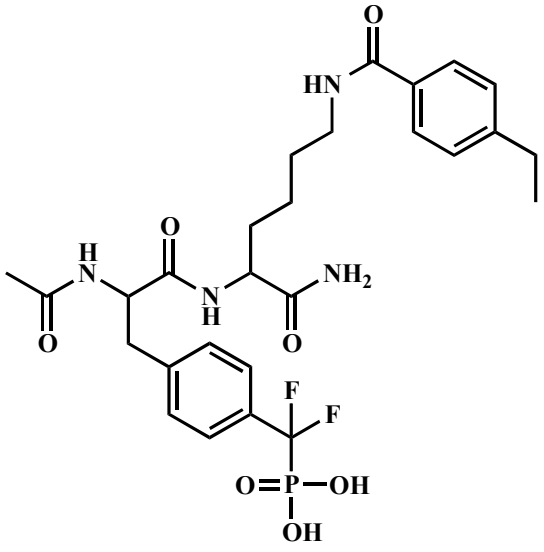
3. Zhang, S.; Chen, L.; Luo, Y.; Gunawan, A.; Lawrence, D. S.; Zhang, Z. Y. *J. Am. Chem. Soc.* **2009**, *131*, 13072-13079.



C1

Figure S1. The structure of compound **C1**.

Figure S2

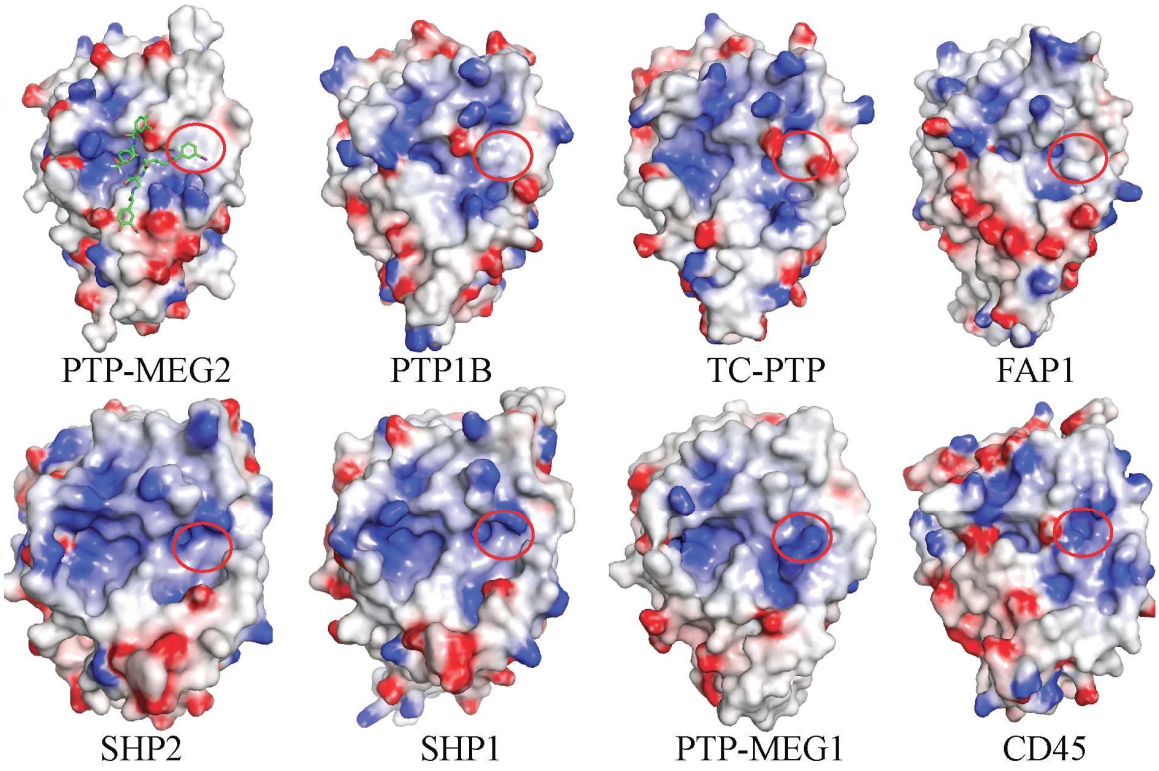


Figure S2: PTP-MEG2 has a unique binding site for the P+1 3-iodobenzoic amide moiety. Surface representations showing the calculated electrostatic potential of PTP family members from their crystal structures. The molecule of compound **7** is also shown in the PTP-MEG2 structure, the binding site for the P+1 3-iodobenzoic amide moiety is marked by a red circle; the corresponding area in other PTP members are also marked by a red circle. The figure was prepared by PyMol.

Figure S3

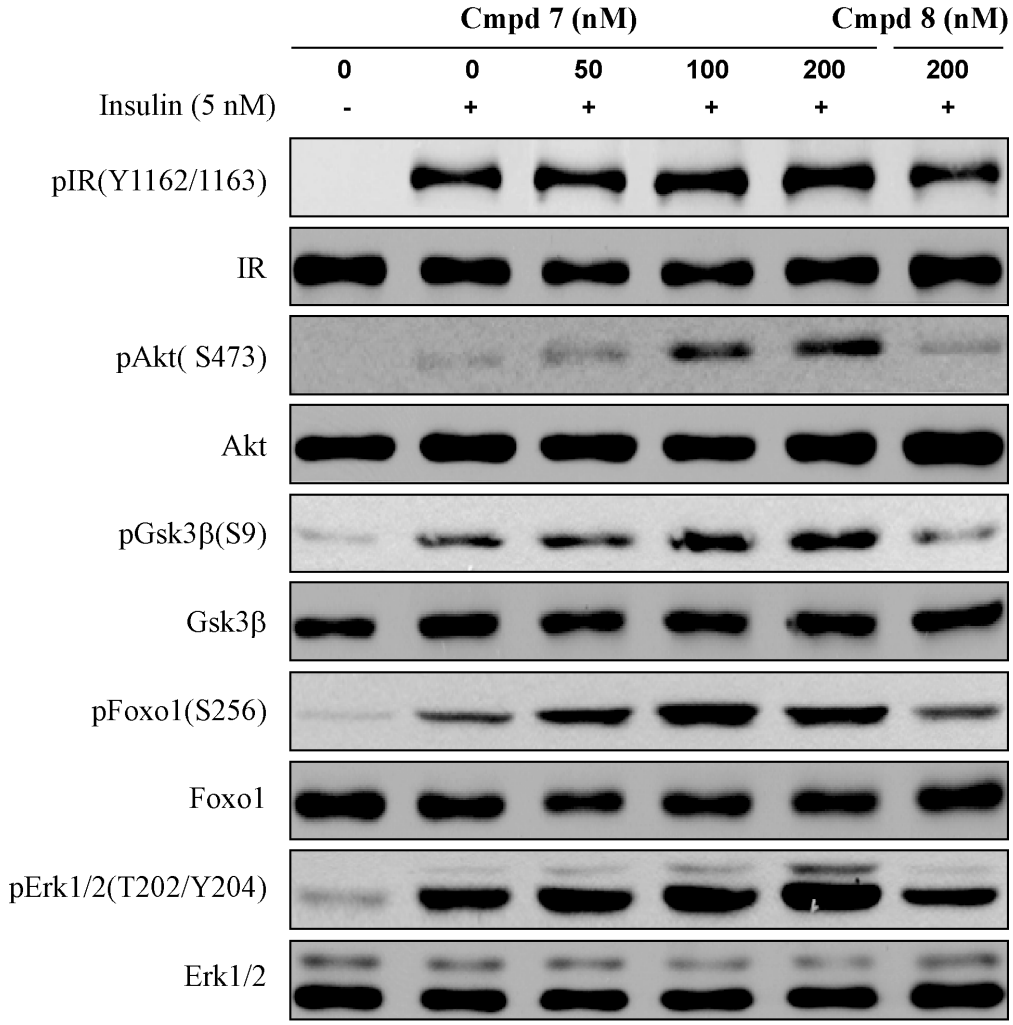


Figure S3. The prolonged insulin sensitization by Compound **7**. Mouse primary hepatocytes were cultured in DMEM medium plus 0.5% FBS overnight. Prior to insulin stimulation, the cells were preincubated with either vehicle DMSO, Compound **7**, or Compound **8** for 1 hour. After 30 minutes of stimulation by 5 nM insulin, the hepatocytes were harvested for signaling analysis using phosphor-specific or total protein antibodies.

Figure S4

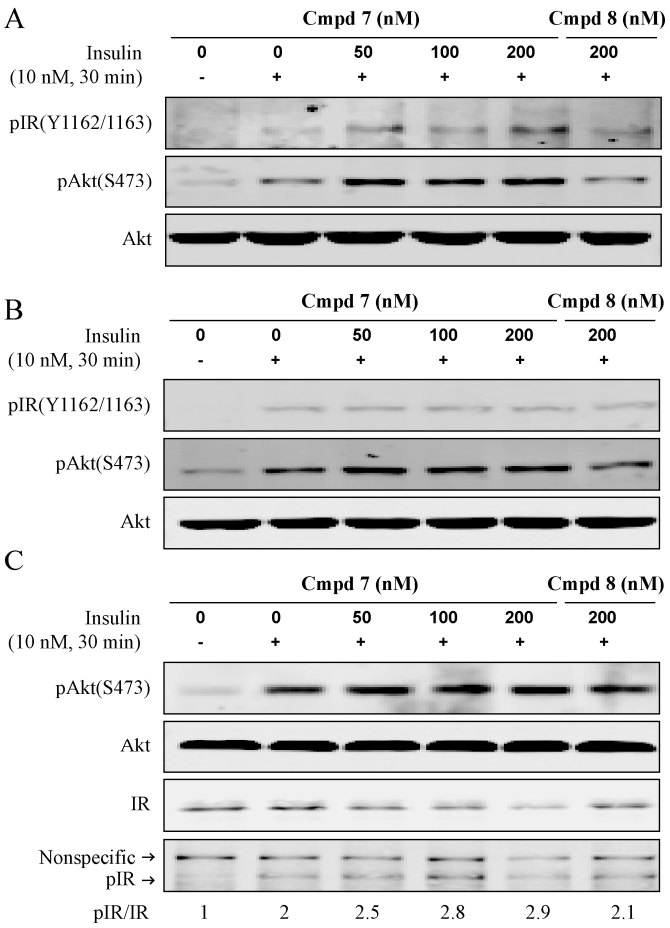


Figure S4. Compound **7**, but not Compound **8**, augmented insulin signaling in three insulin sensitive cell lines: 3T3-L1 differentiated adipocytes, HepG2 hepatocytes, and C2C12 myotubes. (A) 3T3-L1 adipocytes (10-day differentiation) were serum starved overnight, treated with Compound **7** or **8** (final DMSO 0.5%) for 1 hour before stimulation with insulin. Cell lysate (20 µg protein) was subjected to 10% SDS-PAGE and probed with phospho-Akt, phospho-IR (Y1162/1163) or Akt antibody, respectively. (B) HepG2 cells were maintained in DMEM with 10% FBS, serum starved overnight, treated with Compound **7** or **8** (final DMSO 0.5%) for 1 hour before stimulation with 10 nM insulin for 30 min. Cell lysate (20 µg protein) was subjected to 10% SDS-PAGE and probed with phospho-IR(Y1162/1163), phospho-Akt (pS473) or total Akt antibody, respectively. (C) C2C12 myotubes (7-day differentiation) were serum starved overnight, treated with Compound **7** or **8** (final DMSO 0.5%) for 1 hour before stimulation with 100 nM insulin for 30 min. Cell lysate (20 µg protein) or insulin receptor immonoprecipitation beads were subjected to 10% SDS-PAGE and probed with phospho-Akt, Akt, insulin receptor beta or phospho-tyrosine antibody (4G10), respectively. The non-specific band from insulin receptor immunoprecipitates served as a loading control for insulin receptor and phospho-insulin receptor.

Figure S5

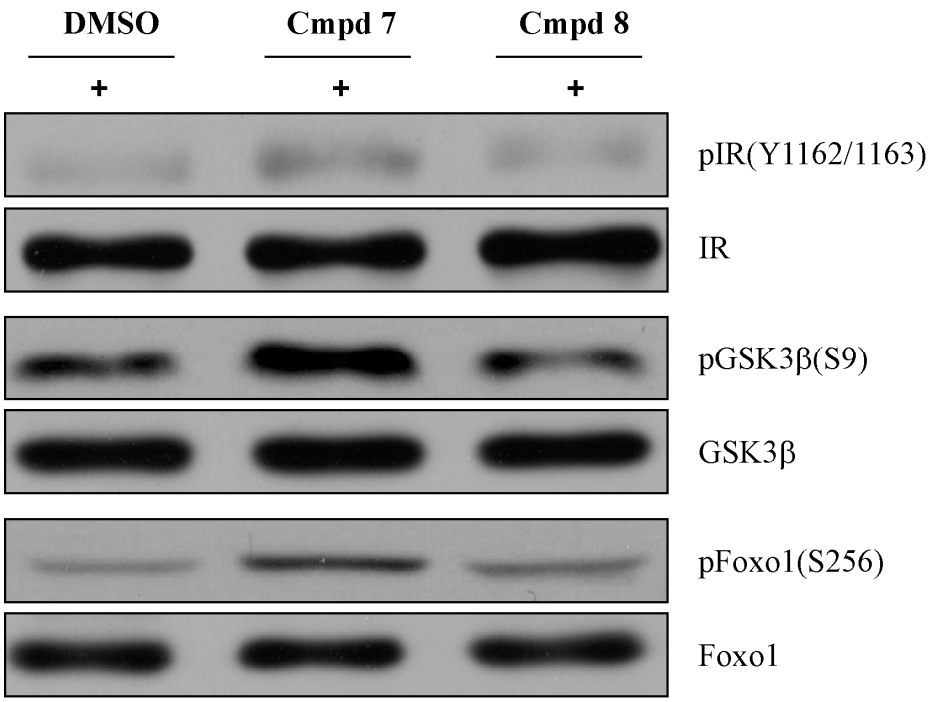
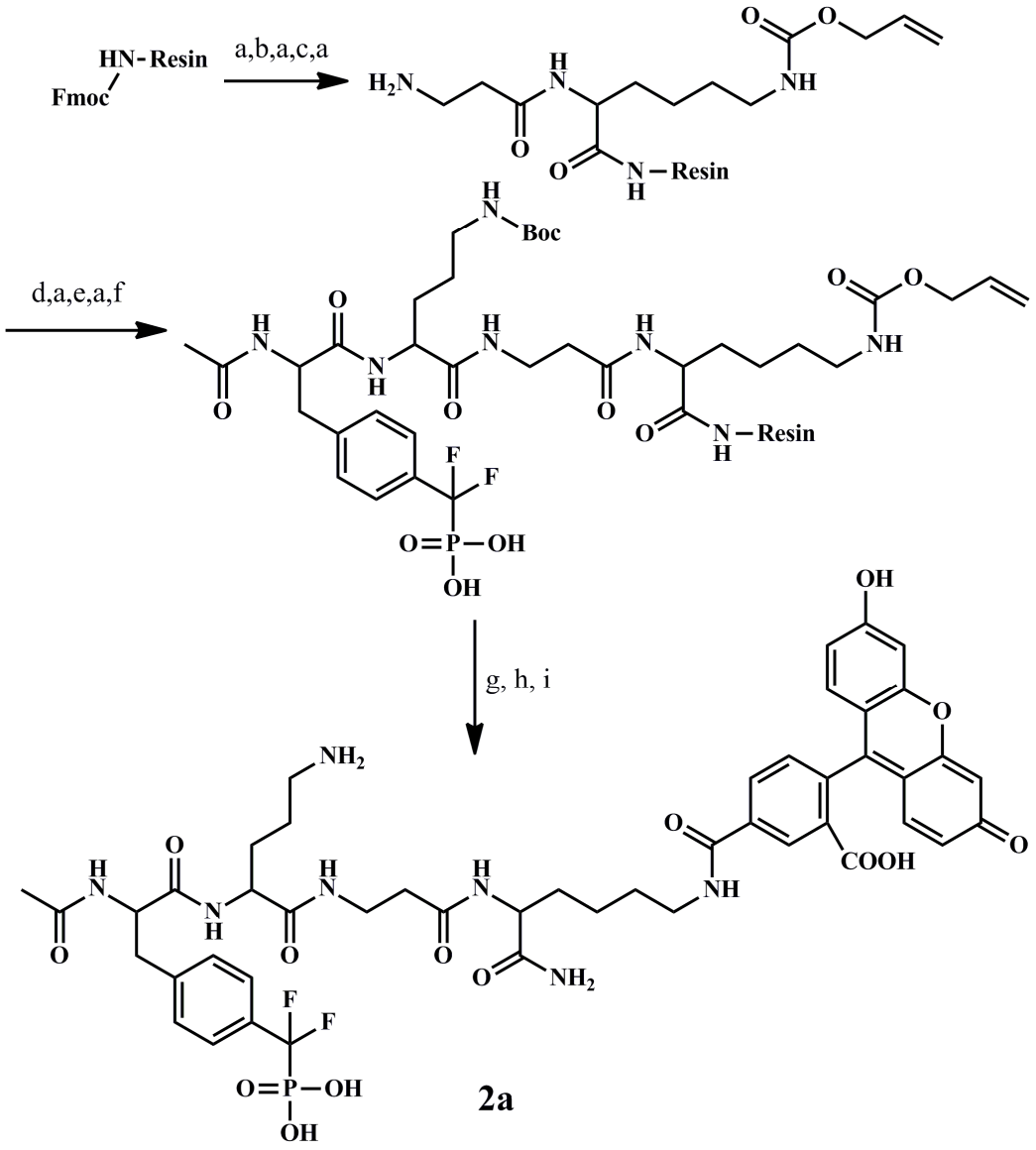


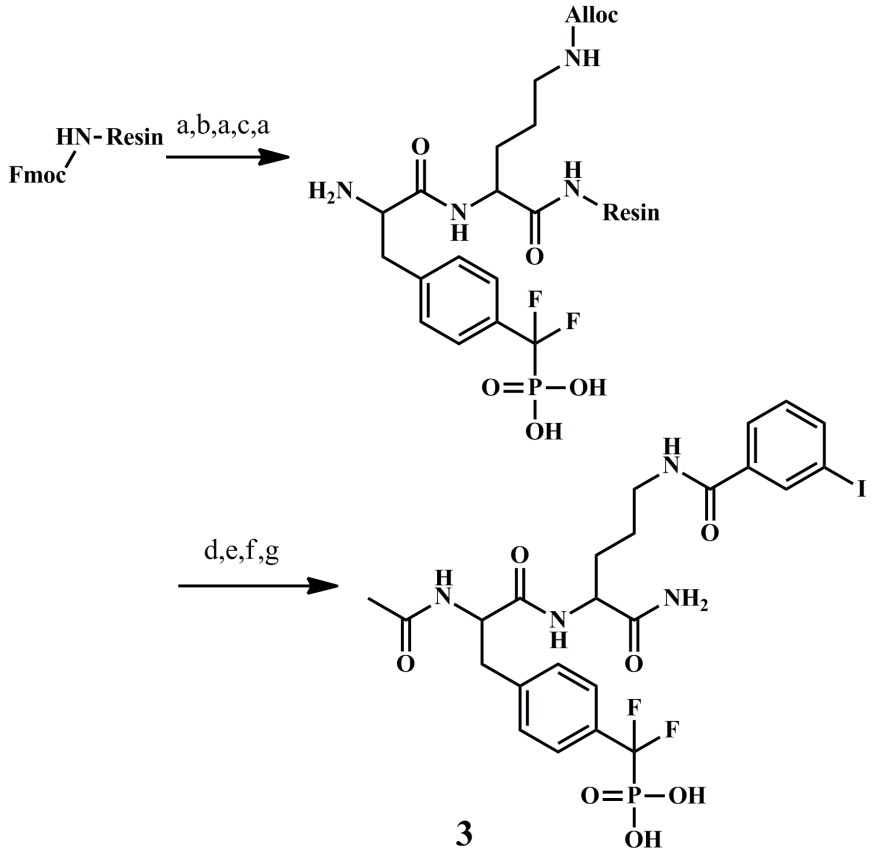
Figure S5. The insulin signaling pathway is enhanced by Compound **7** even under basal conditions. Mouse primary hepatocytes were isolated and cultured in DMEM medium with 0.5% FBS for 12 hours, and then were incubated with either vehicle DMSO, Compound **7** (100 nM), or Compound **8** (100 nM) for 1 hour. Phosphorylated IR, Gsk3β, and Foxo1 and the corresponding total proteins were analyzed by Western blots.

Scheme **S1**. Synthesis of compound **2a**.[a]



[a] (a) 30% piperidine/DMF; (b) Fmoc-Lys(Alloc)-OH/HBTU/HOBt/NMM; (c) Fmoc-β-Ala-OH/HBTU/HOBt/NMM; (d) Fmoc-Orn(Boc)-OH/HBTU/HOBt/NMM; (e) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (f) AcOH/HBTU/HOBt/NMM; (g) Pd(0)/NMM/AcOH; (h) 5-Carboxy-fluorescein/HBTU/HOBt/NMM; (i) 95% TFA/H_2O/TIS.

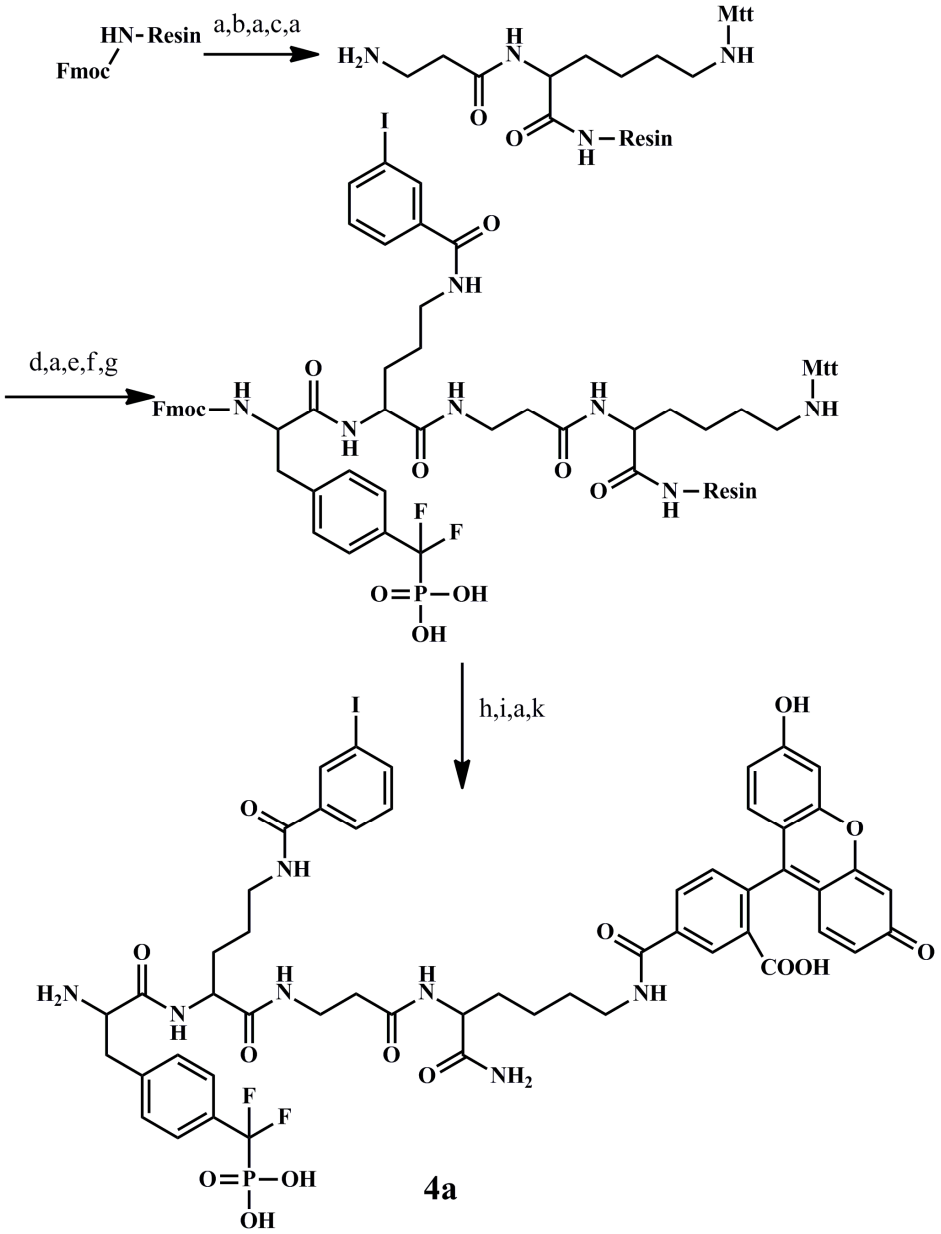
Scheme **S2**. Synthesis of compound **3**.[a]



3

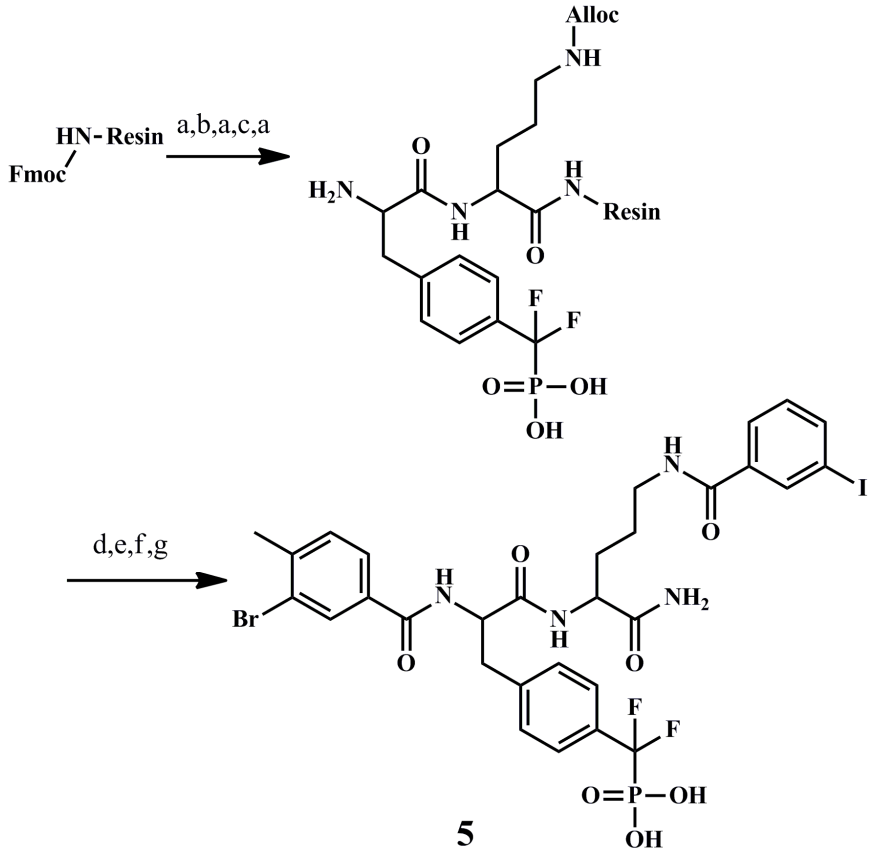
[a] (a) 30% piperidine/DMF; (b) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (c) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (d) AcOH/HBTU/HOBt/NMM; (e) Pd(0)/NMM/AcOH; (f) 3-iodobenzoic acid /HBTU/HOBt/NMM; (g) 95% TFA/H_2O/TIS.

Scheme **S3**. Synthesis of compound **4a**.[a]



[a] (a) 30% piperidine/DMF; (b) Fmoc-Lys(Mtt)-OH/HBTU/HOBt/NMM; (c) Fmoc-β-Ala-OH/HBTU/HOBt/NMM; (d) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (e) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (f) Pd(0)/NMM/AcOH; (g) 3-iodobenzoic acid/HBTU/HOBt/NMM; (h)1% TFA/TIS/DCM; (h) 5-Carboxy-fluorescein/HBTU/HOBt/NMM; (i) 95% TFA/H_2O/TIS.

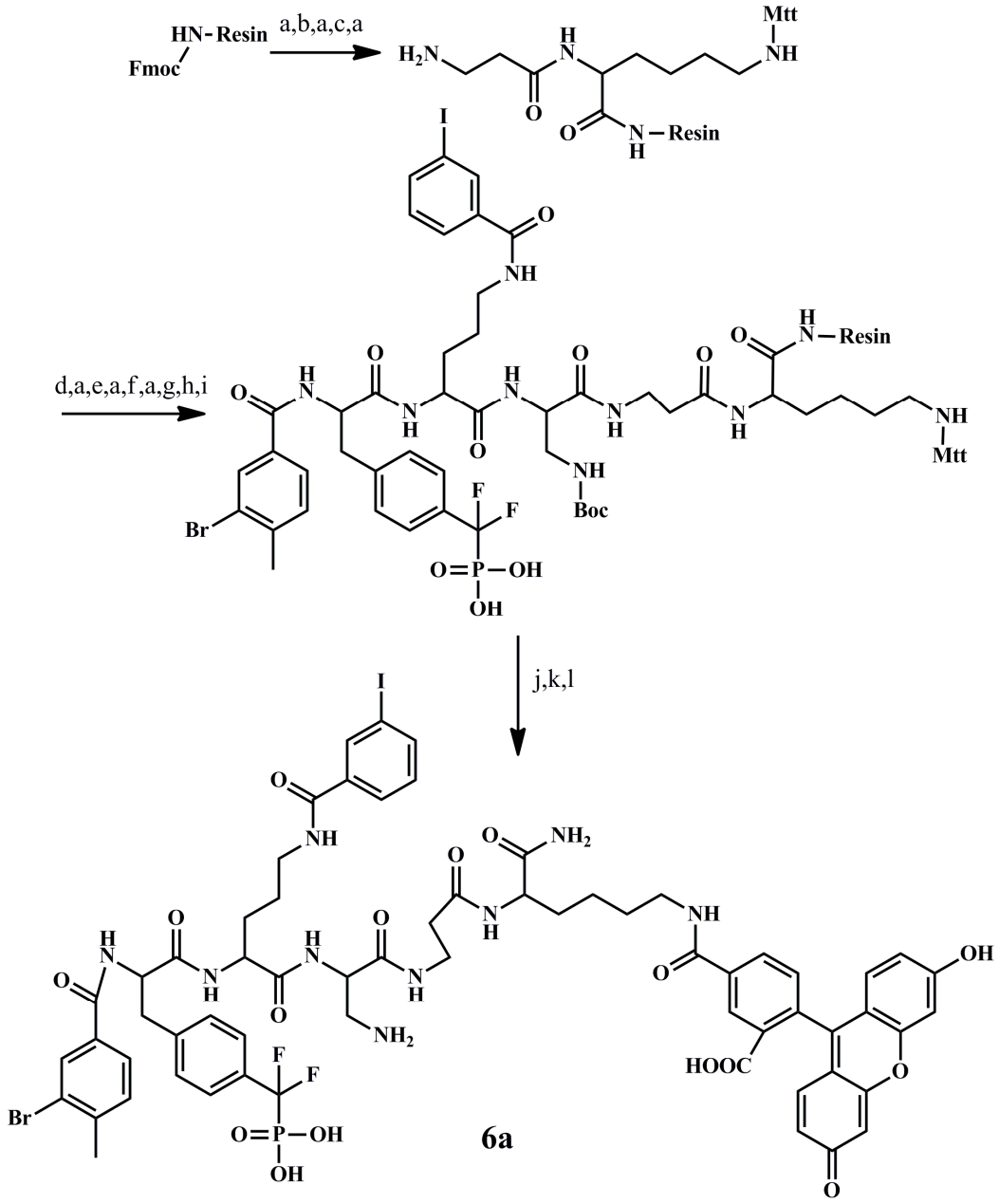
Scheme **S4**. Synthesis of compound **5**.[a]



5

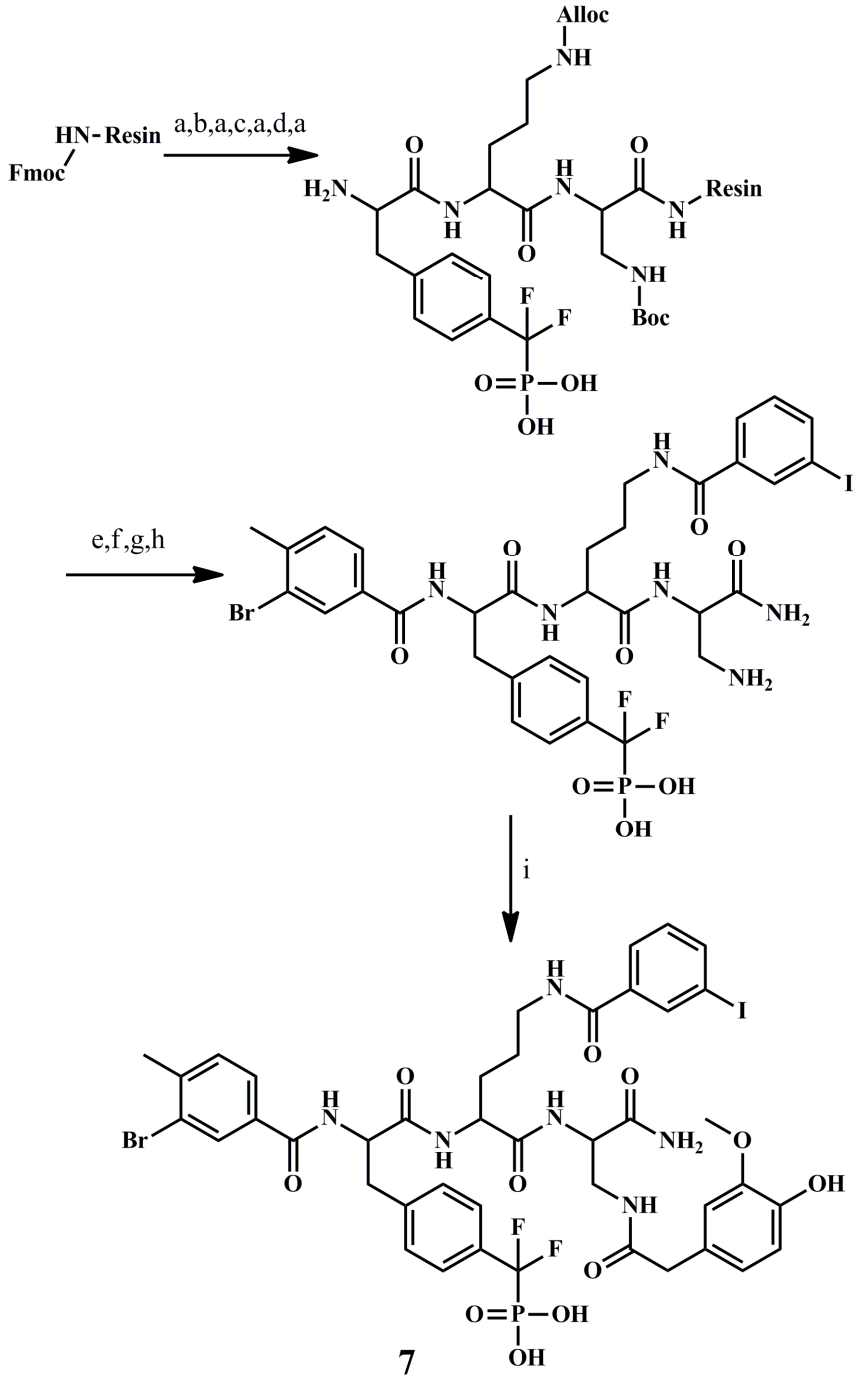
[a] (a) 30% piperidine/DMF; (b) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (c) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (d) 3-bromo-4-methylbenzoic acid/HBTU/HOBt/NMM; (e) Pd(0)/NMM/AcOH; (f) 3-iodobenzoic acid /HBTU/HOBt/NMM; (g) 95% TFA/H_2O/TIS.

Scheme **S5**. Synthesis of compound **6a**.[a]



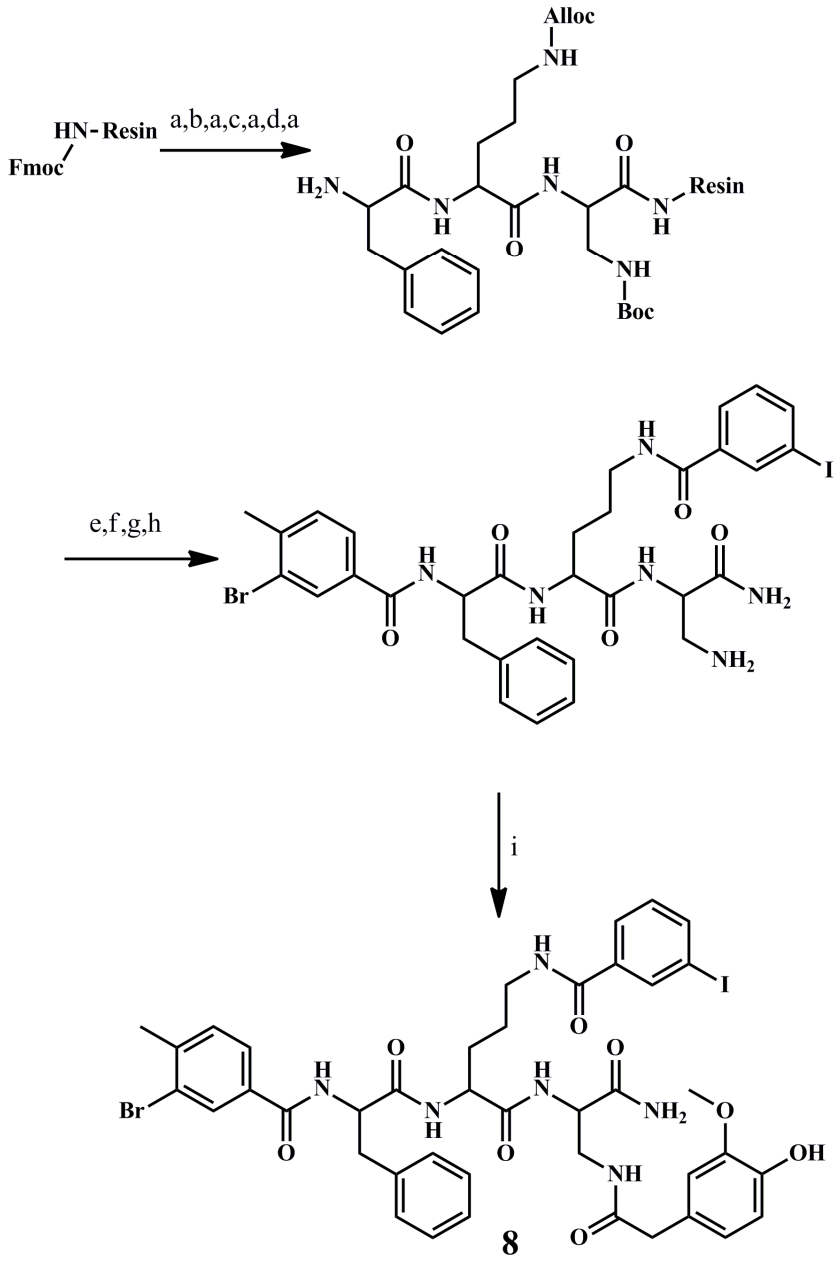
[a] (a) 30% piperidine/DMF; (b) Fmoc-Lys(Mtt)-OH/HBTU/HOBt/NMM; (c) Fmoc-β-Ala-OH/HBTU/HOBt/NMM; (d) Fmoc-Dpr(Boc)-OH/HBTU/HOBt/NMM; (e) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (f) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (g) 3-bromo-4-methylbenzoic acid/HBTU/HOBt/NMM; (h) Pd(0)/NMM/AcOH; (i) 3-iodobenzoic acid/HBTU/HOBt/NMM; (j)1% TFA/TIS/DCM; (k) 5-Carboxy-fluorescein/HBTU/HOBt/NMM; (l) 95% TFA/H_2O/TIS.

Scheme **S6**. Synthesis of compound **7**.[a]



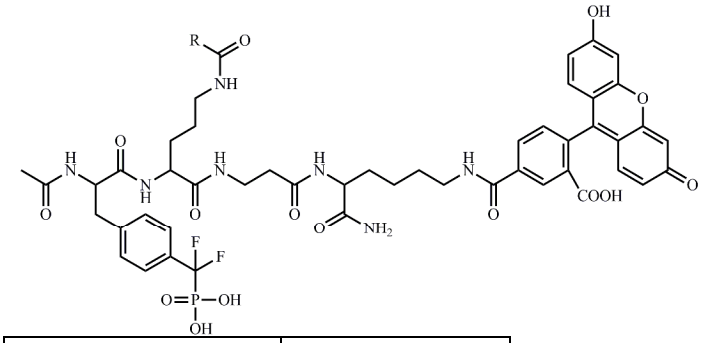
[a] (a) 30% piperidine/DMF; (b) Fmoc-Dpr(Boc)-OH/HBTU/HOBt/NMM; (c) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (d) Fmoc-F_2Pmp-OH/HBTU/HOBt/NMM; (e) 3-bromo-4-methylbenzoic acid/HBTU/HOBt/NMM; (f) Pd(0)/NMM/AcOH; (g) 3-iodobenzoic acid /HBTU/HOBt/NMM; (h) 95% TFA/H_2O/TIS; (i) homovanillic acid/HBTU/HOBt/NMM.

Scheme **S7**. Synthesis of compound **8**.[a]



[a] (a) 30% piperidine/DMF; (b) Fmoc-Dpr(Boc)-OH/HBTU/HOBt/NMM; (c) Fmoc-Orn(Alloc)-OH/HBTU/HOBt/NMM; (d) Fmoc-Phe-OH/HBTU/HOBt/NMM; (e) 3-bromo-4-methylbenzoic acid/HBTU/HOBt/NMM; (f) Pd(0)/NMM/AcOH; (g) 3-iodobenzoic acid /HBTU/HOBt/NMM; (h) 95% TFA/H_2O/TIS; (i) homovanillic acid/HBTU/HOBt/NMM.

Table **S1**. Hits from the library **2b**.



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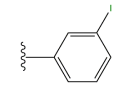
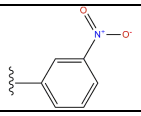
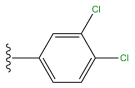
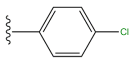
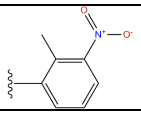
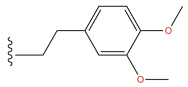
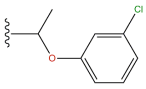
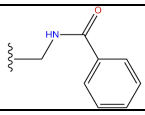
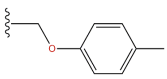
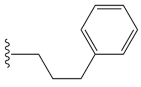
	1
	2
	3
	4
	5
	6
	7
	8
	9
	10

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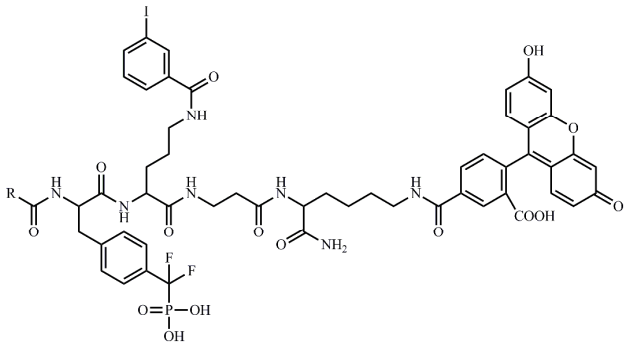

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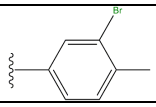
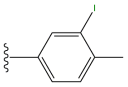
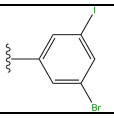
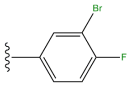
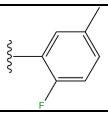
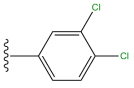
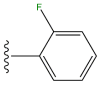
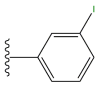
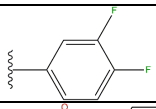
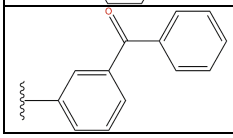
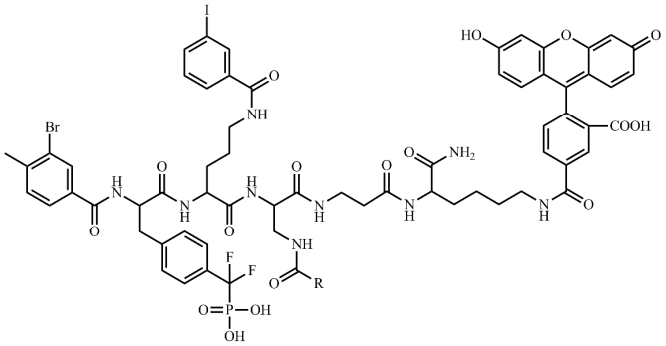
	1
	2
	3
	4
	5
	6
	7
	8
	9
	10

Table **S3**. Hits from the library **6b**.



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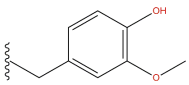
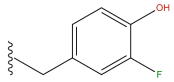
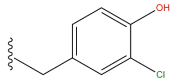
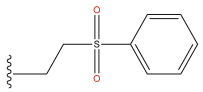
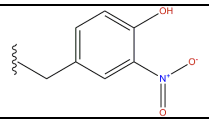
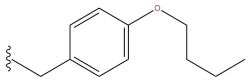
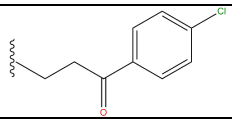
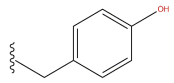
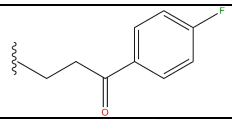
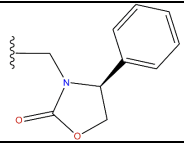
	1
	2
	3
	4
	5
	6
	7
	8
	9
	10