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SUPPORTING INFORMATION

Title: A Fluorous Capping Strategy for Fmoc-Based Automated and Manual Solid-Phase Peptide Synthesis
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Peptide Synthesis

Peptides were prepared using the *N*–Fluorenylmethoxycarbonyl (Fmoc) amino acid derivatives for Merrifield solid-phase synthesis (NovaGel Rink amide resin). *N*–*α*–Fmoc-*α*-*S*–amino acids were used with standard side chain protecting groups.

Two identical peptides were synthesized in tandem, either manually or using an Advanced Chemtech 348 Ω synthesizer. Capping at the sites of incomplete couplings was performed with **1** (or **2**) in the case of one peptide while the other was capped with Ac_2O to serve as a control.

Manual couplings were carried out using 4 eq Fmoc- amino acid, 3.6 eq HBTU, and 6 eq DIEA in DMF for 30 min. In case of a positive Kaiser test, the coupling was repeated. Deprotection was effected by 50% v/v piperidine in DMF (2 × 15 min).

Automated couplings were carried using 7.5 eq Fmoc- amino acid, 7 eq HOBt, and 7 eq DIC in NMP for 30 mins. Deprotection was effected by 50% v/v piperidine in NMP (2 × 10 mins).

Incomplete couplings were generated as described below under *Fluorous Capping* Methods. Peptides were cleaved from the resin using TFA/triisopropyl silane (TIS)/H_2O = 95:2.5:2.5 or TFA/TIS/ethanedithiol (EDT)/H_2O = 94:1:2.5:2.5 for 2-3 hrs. The crude reaction mixture after cleavage and rotary evaporation was treated with cold Et_2O. The precipitated peptides were then collected on a medium porosity frit, redissolved in 10% AcOH, lyophilized and then subjected to ESI-MS and reversed-phase HPLC analysis.

The synthesis and characterization of reagent **1** has been reported previously (*J. Am. Chem. Soc.* **2004**, *126*, 9528-9529).

Preparation and characterization of reagent 2

1-(p-Toluensulfonyl)oxy-1*H*,1*H*-perfluorodecane (S1)

In a 250 mL round bottom flask, 1*H*,1*H*-perfluorodecanol (24.88 g, 49.8 mmol) and tosyl chloride (9.53 g, 49.6 mmol) were suspended in CH_2Cl_2 (150 mL). Triethylamine (7.25 mL, 52 mmol) and 4-dimethylaminopyridine (50 mg) were added and the reaction mixture stirred for 12 hrs at 22 °C. The resulting white suspension was diluted to 350 mL with CH_2Cl_2 and extracted with H_2O (3 × 100 mL). The organic layer was evaporated and dried *in vacuo* yielding 30.94 g (95%) of 1-(p-toluensulfonyl)oxy-1*H*,1*H*-perfluorodecane as a white powder.

^1H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 2.5 (s, 3 H), 4.5 (t, 2H, J = 18 Hz, CH_2), 7.4 (d, 2H), 7.8 (d, 1H).

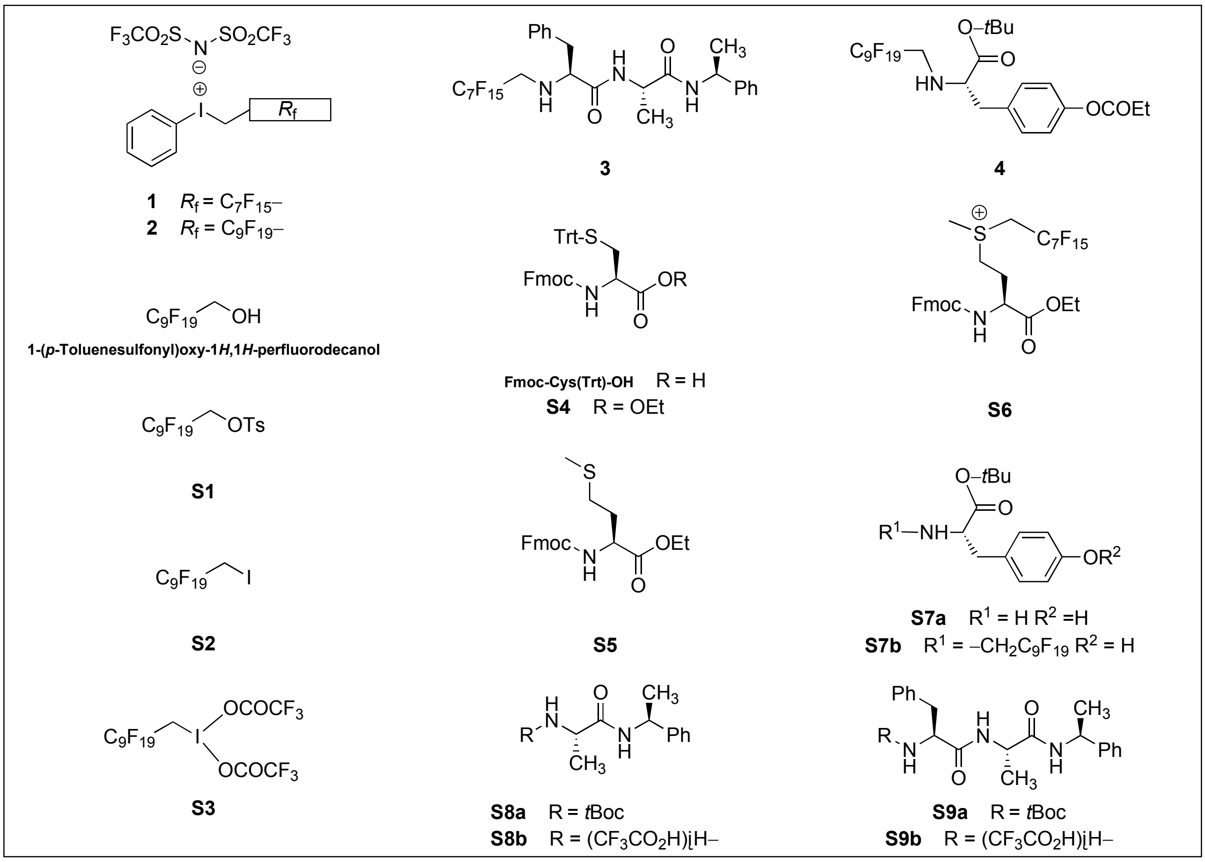

Figure S1. Structures of compounds in the manuscript main text and in the Supporting Information. Compounds mentioned only in the Supplementary Information are labeled **S1** through **S9b.**

1-Iodo-1*H*,1*H*-perfluorodecane (S2)

A solid mixture of **S1** (19.63 g, 30 mmol), KI (9.96 g, 60 mmol) and PEG 4000 (40 g) was liquefied by heating to 150 °C and stirred for 7 hours. The reaction was allowed to cool, followed by addition of H_2O (100 mL). The reaction flask was then fitted with a Dean-Stark trap and heating was resumed. The ensuing steam-distillation caused the product to collect in the bottom of the trap as a liquid that tended to solidify. After 2 hrs the product was melted by heating the trap with a heat gun. The product formed a clear lower phase which was drained into a vial where it resolidified into a white powder. The isolated yield was 9.02 g (14.78 mmol, 49%).

^{1}H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 3.6 (t, CH_2R_f).

The solid that remained in the still was collected on a glass frit and thoroughly washed with H_2O and then dried. ^{1}H NMR showed pure starting material. Recovery was 8.54 g (13.05 mmol), material balance 93%. In a similar experiment, 1-iodo-1*H*,1*H*-perfluorooctane was obtained by the same method in 73% yield. The recovered tosylate was 17%.

(Bis-trifluoroacetoxy)iodo-1*H*,1*H*-perfluorodecane (S3)

This compound was obtained as described previously for (bis-trifluoroacetoxy)iodo-1*H*,1*H*-perfluorooctane (*J. Am. Chem. Soc.* **2004**, *126*, 9528-9529). In a typical experiment, hydrogen peroxide (50% w/w, 1.4 mL, 24 mmol) was added dropwise to trifluoroacetic anhydride (TFAA) (40 mL) under magnetic stirring and cooling in an ice/salt bath. Following this, 1-iodo-1*H*,1*H*-perfluorodecane (12.29 g, 20.1 mmol) was added rapidly, the cooling bath was removed, and the reaction allowed to proceed under N_2 for 16 hrs. The volatiles were removed under vacuum yielding 15.92 g of a white powder. Fresh TFAA (10 mL) was then added, the mixture stirred for an additional 24 hrs, and the volatiles removed to yield 15.95 g (95%) of **S3** that was used directly to prepare iodonium salt **2**.

Phenyl-1*H*,1*H*-perfluorodecyl-iodonium *N*,*N*'-bis-triflimide salt (Reagent 2)

To **S3** (15.95 g) was added in one portion $(CF_3SO_2)_2NH$ (6.63 g, 23.6 mmol). The two solids were slurried in 30 mL FC-72 (perfluorohexane) and TFAA (3 mL). After 15 mins, benzene (2.25 mL, 25 mmol) was added in one portion and a clear solution resulted. The reaction mixture began to separate into two phases within 20 mins; stirring was continued under N_2 for 22 hrs at ambient temperature. Evaporation of the volatiles followed by stirring with ice/water produced a white precipitate. The precipitate was collected on a glass frit, dried in air and further *in vacuo* yielding 16.18 g (87%) of **2**, which was used directly in capping reactions. ^{1}H NMR ($CDCl_3$ /CH_3CN = 95/5, 300 MHz, relative to TMS = 0 ppm) δ 4.8 (t, 2H, J = 18 Hz, CH_2R_f), 7.6 (m, 2H), 7.8 (m, 1H), 8.1 (m, 2H).

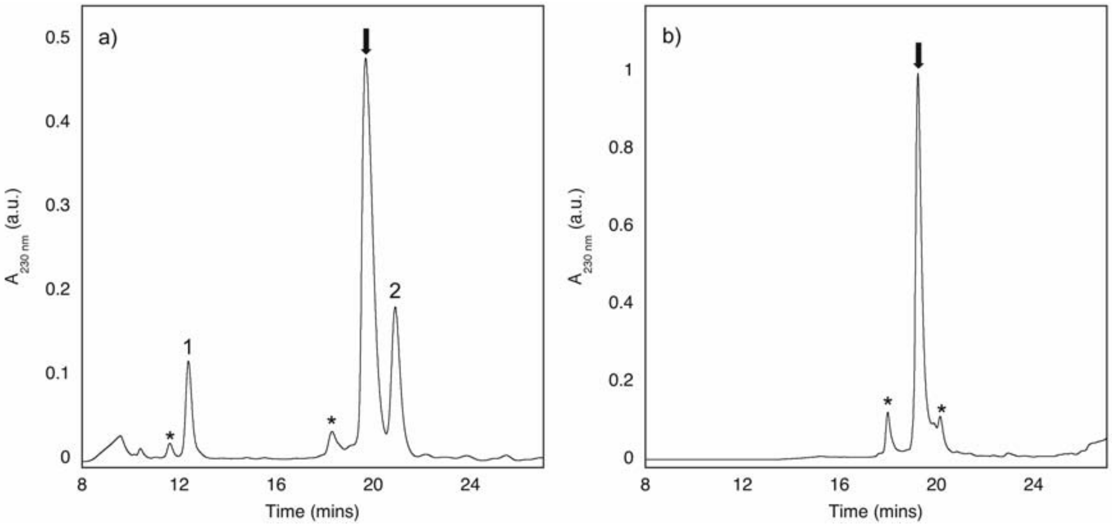

Figure S2. Reverse phase analytical HPLC of peptide **P2** synthesized using capping at selected sites marked by asterisks in **Figure 2** (main text) with Ac_2O (a) or fluorinated reagent **2** (b). The arrows indicate the full length peptide. In (a) peaks marked 1 and 2 had masses of 1088.4 and 1308.5 respectively and are the acetyl capped products (see Table 1, Supp Inf). Peaks indicated by asterisks in both (a) and (b) are unknown impurities.

Fluorous capping method using Automated Synthesizer

Two identical peptides were prepared in tandem (typically on a 0.1 mmol scale) on NovaGel Rink amide resin in individual wells of a 40-well reaction block (Advanced Chemtech 348 Ω synthesizer). At the sites of intentional incomplete couplings, the synthesizer was stopped after removal (deprotection step) of the terminal Fmoc group. The resin in the wells were transferred into independent tared vials, using CH_2Cl_2 and Et_2O. The solvents were decanted and the resin further dried *in vacuo* for 1 hr. Twenty percent by weight of each resin was then removed, the remainder returned to the synthesizer and swelled in DMF. The synthesis was resumed and the next amino acid coupling step was carried out. Following this, the 20% portions of each resin were returned to the corresponding wells. At this juncture, each well presumably had resin, 80% of it bearing the desired Fmoc-protected peptide, and 20% bearing peptide lacking the terminal Fmoc protected amino acid. The unprotected resin fractions were now capped employing the following parallel protocol.

WELL A: Fluorous capping, WELL B: Ac_2O capping

STEP 1: The resin was rinsed twice with CH_2Cl_2 to displace DMF. In WELL A, 3.5 mL of a solution of **2** (675 mg, 0.75 mmol) in CH_2Cl_2 (7 mL, containing 2% v/v DMF), and in WELL B, 3.5 mL of the acetylation mixture was added [DMF/Ac_2O/DIEA (10:0.6:0.1)]; the block was then shaken at 450 rpm for 3 mins.

STEP 2: In WELL A, 0.5 mL of a solution of collidine was added (50 µL collidine in 0.5 mL CH_2Cl_2), in WELL B, another 0.5 mL of acetylation mixture was added; the block was shaken at 450 rpm for 10 mins; and each well then washed twice with CH_2Cl_2.

To ensure complete capping, the above protocol was repeated once more. Then the usual sequence of deprotection, washing, and coupling cycles was resumed. The operations above were repeated to introduce deletions at other sites.

Fluorous capping with reagent 2: manual flow method

Two 25 mL peptide synthesis vessels fitted with a stopcock and a coarse porosity frit (CHEMGLASS Cat. No: CG-1860-11) were tared. Synthesis was again started in tandem for two identical peptides typically on a 0.1 mmol scale on NovaGel rink amide resin. At the sites of intentional incomplete couplings, after deprotection with piperidine/DMF, the resins were rinsed with CH_2Cl_2, then the loosely capped vessels were placed in a vacuum desiccator for one hour. The amount of resin in each vessel was determined by difference, then 20% was removed. One coupling step was carried out, then the 20% resin fractions previously removed were returned to the respective vessels. After rinsing and swelling with 3 × 10 mL CH_2Cl_2 shake washes, the parallel fluorous and Ac_2O cappings were conducted as follows:

VESSEL A: Fluorous capping, VESSEL B: Ac_2O capping

STEP 1: In VESSEL A, the same solution of **2** as in the case of the automated protocol was added, with mild shaking for 3 min. The fluorous-capping solution was then drained into the vial from which it was added. If the solution so drained became cloudy because of presumable entrainment of DMF by the resin, resulting in partial precipitation of the reagent, addition of 10-20 µL DMF to the vial was sufficient to redissolve **2** recovered in this manner.

Into VESSEL B, 10 mL of the acetylation mixture was added.

STEP 2: Into VESSEL A was added collidine (150 µL in 3 mL CH_2Cl_2) with mild shaking for 10 mins.

STEP 3: Fluorous capping was repeated using the recovered solution of **2** . (Recovered solutions that had been kept for several days in the refrigerator could still be used successfully). During the fluorous cappings, acetylation was allowed to continue in VESSEL B.

Finally both resins were washed with DMF and the regular synthesis was resumed.

Control experiments

Compatibility with Cys and Met residues

Model compound Fmoc-Cys(Trt)OEt (**S4**) was prepared by stirring Fmoc-Cys(Trt)OH (146 mg, 0.25 mmol), EtI (25 μL, 0.3 mmol), and $NaHCO_3$ (85 mg, 1 mmol) in DMF (1 mL) for 18 hours. TLC showed one spot (R_f = 0.8, Et_2O). Dilution with Et_2O (30 mL) and extraction with H_2O (2 × 30 mL), drying and evaporation gave 140 mg (91%).

^{1}H NMR (CD_2Cl_2, 300 MHz, relative to TMS = 0 ppm) δ 1.1 (t, 3 H, OCH_2CH_3), 4.0 (q, 2H, OCH_2CH_3), 4.2-4.3 (m, 3H, cysteine CH, CH_2), 5.1-5.2 (br, 3H, Fmoc CH, CH_2), 7.0-8.0 (m, 23 H, aromatic H).

NMR experiment: Fmoc-Cys(Trt)OEt **S4** (15 mg, 0.024 mmol) and **1** (21 mg, 0.024 mmol) were dissolved in CD_2Cl_2 (0.75 mL). After 2.5 hrs the NMR signals observed were of unchanged Fmoc-Cys(Trt)OEt . The baseline-separated signals of **1** at 4.8 (CH_2) and 8.0 (*ortho* aromatic H) were also unchanged. Small amounts of unknown products were visible after 30 hrs, but the original signals were largely unchanged.

Similarly was prepared Fmoc-Met-OEt (**S5**) in 75% yield.

^{1}H NMR (CD_2Cl_2, 300 MHz, relative to TMS = 0 ppm) δ 1.1 (t, 3 H, OCH_2CH_3), 2.0 (s, 3 H, SCH3), 4.0 (q, 2H, OCH_2CH_3), 4.1-4.3 (m, 5H, methionine CH, CH_2 , CH_2), 5.1-5.2 (br, 3H, Fmoc CH, CH_2), 7.2-7.8 (m, 8 H, Fmoc aromatic).

NMR experiment: Fmoc-Met-OEt **S5** (12 mg, 0.030 mmol) and **1** (26 mg, 0.030 mmol) were dissolved in CD_2Cl_2 (0.75 mL). After 15 min the iodonium salt signals and nearly all the S-CH_3 singlet had disappeared. New signals at 3.1 (s) and 3.5-4.0 (br, unresolved) presumably were the methyl and R_fCH_2 groups of the fluoroalkyl sulfonium salt **S6**.

Fluoroalkylation of H-Tyr-O-*t*Bu (S7a) with 2

Into a solution of **S7a** (118 mg, 0.5 mmol) and iodonium salt **2** (483 mg, 0.5 mmol) in CH_2Cl_2 (7 mL), was added a solution of 2,4,6-collidine (73 μL, 0.55 mmol) in CH_2Cl_2 (3 mL), were stirred at rt for 1 hr (by TLC the reaction seemed complete in <10 min). Dilution with CH_2Cl_2, extraction with 2 × 50 mL 0.1 M citric acid and evaporation, followed by chromatography (Et_2O:hexanes 1:1) gave 287 mg (80% yield) of **S7b** as a white powder. ^{1}H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 1.4 (s, 9 H, *t*Bu), 2.8 (m, 2H, tyrosine CH_2), 3.0-3.4 (ABX_2, m, 2H, $R_fCF_2CH_2$), 3.5 (t, 1H), 6.7 (d, 2H,), 7.0 (d, 2H).

A solution containing **S7b** (287 mg, 0.4 mmol), ethyl chloroformate (42 μL, 0.44 mmol) and triethylamine (70 μL, 0.5 mmol) in CH_2Cl_2 (5 mL) was stirred at rt for 1 hr. The mixture was diluted with CH_2Cl_2 (30 ml), extracted with 2 × 30 mL 0.1 M citric acid. The organic phase was dried and evaporated to yield 286 mg (92%) of **4**.

^{1}H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 1.4 (overlapping s, 9 H, Bu*t* and t, 3H, OCH_2CH_3), 2.8 (m, 2H, tyrosine CH_2), 3.0-3.4 (ABX_2, m, 2H, $R_fCF_2CH_2$), 3.4 (t, 1H), 4.3 (q, 2 H, OCH_2CH_3), 6.7 (d, 2H,), 7.0 (d, 2H).

Control reaction to demonstrate inertness of $C_9F_{19}CH_2NH$–peptide **to Fmoc-peptide manual coupling conditions:**

A solution of Fmoc-Ala-OH (102 mg, 0.33 mmol), HBTU (112 mg, 0.3 mmol) and DIEA (85 µL, 0.5 mmol) in DMF (2 mL) was stirred for two minutes then transferred into a flask containing **4** (64 mg, 0.083 mmol). After 3 hrs, the reaction mixture was reduced to small volume and the residue was directly filtered through silica gel using hexanes:Et_2O (2:1) to yield unchanged **4** (NMR, TLC). Recovery of **4** was quantitative.

Control reaction to demonstrate inertness of R_fCH_2NH– **to Fmoc-peptide deprotection conditions:**

Model compound **3** was prepared as follows.

A solution containing Boc-Ala-OH (946 mg, 5 mmol), HBTU (1.89 g, 5 mmol) and DIEA (1.72 mL, 10 mmol) in DMF (10 mL) was stirred for 5 minutes, then (*S*-2-methyl)benzyl amine (640 µL, 5 mmol) was added in one portion. After 3 hrs the mixture was diluted with 75 mL CH_2Cl_2, extracted with 0.1 M citric acid (100 mL) and H_2O (6 × 100 mL). Rotary evaporation afforded 1.365 g (93%) of **S8a** as a white powder that was used directly in the next step.

To **S8a** was added TFA (50% in CH_2Cl_2, 6 mL) and the solution stirred 2 hrs. Rotary evaporation and further drying *in vacuo* for 3 hrs afforded crude **S8b** as an oil.

To a solution of **S8b** in DMF (5 mL), DIEA was added dropwise until the solution was neutral. Separately, Boc-Phe-OH (1.234 g, 4.65 mmol) HBTU (1.764 g, 4.65 mmol) and DIEA (1.6 mL, 9.3 mmol) were stirred for 5 min in DMF (5 mL), then transferred into the solution of **S8b**. After 3 hrs, the mixture was diluted with 75 mL CH_2Cl_2, extracted with 0.1 M citric acid (100 mL) and H_2O (6 × 100 mL) to yield 2.23 g of crude material that was dissolved in the minimum amount of CH_2Cl_2 and stored at –20 °C overnight. The resulting precipitate was triturated with cold pentane, filtered and dried to afford 1.349 g (66%) of **S9a**.

^{1}H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 1.3-1.5 (15 H, two d, s, Me, Me, Bu*t*), 3.0-3.1 (m, 2 H, $PhCH_2$), 4.3 (1 H, br, NH), 4.4 (1 H, m, PhCH), 4.9 (1 H, br, NH), 5.0 (1 H, m, CH), 6.5 (1 H, d, CH), 6.7 (1 H, br, NH), 7.1-7.4 (10 H, Ph rings).

The deprotection of the *t*-Boc group in **S9a** (220 mg, 0.5 mmol) was accomplished by addition of TFA (50% v/v in CH_2Cl_2, 3 mL) with stirring for 1 hr. The reaction solution was then evaporated to give the crude **S9b**.

To a solution of **S9b** in CH_2Cl_2 (5 mL) was added 0.5 M $NaHCO_3$ with stirring until the aqueous phase was basic to litmus (ca. 3 mL), then 2 mL more were added, followed by iodonium salt **2** (434 mg, 0.5 mmol). The biphasic mixture was stirred rapidly at rt for 1.5

hrs, at which point TLC confirmed a complete reaction. The reaction was diluted with CH_2Cl_2 (50 mL), extracted with $NaHCO_3$ (50 mL) and 0.1 M citric acid (50 mL). The organic layer was dried and concentrated to an oil that soon solidified. Further drying *in vacuo* gave 310 mg (86%) of **3** as a white powder.

^1H NMR ($CDCl_3$, 300 MHz, relative to TMS = 0 ppm) δ 1.3 and 1.5 (9 H, two d, Me, Me), 2.8 and 3.5 (2 H, two m, $PhCH_2$), 3.2 (2 H, ABX_3, R_fCH_2), 4.5 (1 H, m, PhCH), 5.1 (1 H, m, CH), 6.6 (1 H, d, CH), 7.5 (1 H, br, NH), 7.2-7.4 (10 H, Ph rings).

The fluoroalkyl compound **3** (295 mg) was dissolved in piperidine (50% v/v in DMF, 2 mL) and stirred for 20 hrs at rt. The reaction mixture was diluted with 75 mL of Et_2O and washed with 3 × 50 mL 0.1 M citric acid. TLC analysis showed only starting material ($R_f = 0.6$ in Et_2O). The Et_2O layer was dried ($MgSO_4$) and evaporated. The material thus obtained (269 mg, 91%) was unchanged starting material by NMR analysis.

Control reaction to determine compatibility with methionine sulfoxide

The pentapeptide Ac-Phe-Ala-Met(O)-Ala-Phe-CONH-resin was prepared manually by conventional Fmoc chemistry on NovaGel Rink amide resin (0.2 mmol scale, starting resin: 0.63 mmol/g, 310 mg).

The resin was split: 240 mg was subjected to cleavage to afford 62 mg crude product, identified by ESI-MS [MW 642.28, found 643.23 (+ H^+); 665.35 (+ Na^+)]; the remainder 150 mg was submitted to fluorous capping conditions as described for Bombesin 5-14 (manual synthesis), followed by cleavage yielding 26 mg of product, identical by ESI and HPLC to the one from the untreated resin.

Table S1.

Mass Spectrometry (ESI-MS)

**All spectra were recorded in POSITIVE mode.

ESI-MS Conditions: Finnigan LTQ, Thermo Electron Corp.; solutions were ~100 nM in MeOH / 5% HCOOH unless otherwise noted, flow rate: 5 µL/min, spray voltage: 5 kV, capillary voltage: 40 V.

1) Crude peptide without centrifugation.
2) Peak (fraction) isolated by HPLC, concentrated and subjected to ESI-MS by direct infusion after redissolution.
3) Analysis carried out by LC ESI-MS [VYDAC C18 238MS5415 column – 4.6 mm × 150 mm, 5 µm – flow rate 1.0 mL/min] using linear gradients of CH_3CN and H_2O (0.03% TFA).

Peptide P1 (automated)
ACP 65-74 analog

VEAAIDYIDA Ac cap with Ac_2O			
Fragment	MW_{calc} (Da)	MW_{obsd} (Da)	Sample
H_2N–VEAAIDYIDA–$CONH_2$	1061.55	1062.45	1
Ac–NH–EAAIDYIDA–$CONH_2$	1004.49	1005.32 1027.52 (+ Na)	1
–CH_2-R_f cap with reagent **2** ($C_9F_{19}CH_2$– cap)			
Fragment			
H_2N –VEAAIDYIDA–$CONH_2$	1061.55	1062.46	1
R_fCH_2–NH–EAAIDYIDA–$CONH_2$	1444.45	1445.16	1

Peptide P2 (automated)
IGF 28-41 analog

PTGYGSSSRRAPET Ac cap with Ac_2O			
Fragment	MW_{calc} (Da)	MW_{obsd} (Da)	Sample
H_2N-PTGYGSSSRRAPET–$CONH_2$	1463.57	1464.58	1, 2
Ac-NH-GYGSSSRRAPET–$CONH_2$	1307.62	1308.54	1, 2
Ac-NH-GSSSRRAPET–$CONH_2$	1087.55	1088.42	1, 2
–CH_2-R_f cap with reagent **2** ($C_9F_{19}CH_2$– cap)			
Fragment			
H_2N-PTGYGSSSRRAPET–$CONH_2$	1463.57	1464.50	1
R_fCH_2–NH–GYGSSSRRAPET–$CONH_2$	1747.58	1748.33	1
R_fCH_2–NH–GSSSRRAPET–$CONH_2$	1527.50	1528.26	1

Peptide P3 (manual)

Bombesin 5-14 analog

GNQWAVGHLC Ac cap with Ac_2O			
Fragment	MW_{calc} (Da)	MW_{obsd} (Da)	Sample
H_2N-GNQWAVGHLC–$CONH_2$	1082.50	1083.38	1, 2, 3
Ac-NH-QWAVGHLC–$CONH_2$	953.45	954.39	1, 2, 3
Ac-NH-AVGHLC–$CONH_2$	639.21	640.37	1, 2, 3
$-CH_2$-R_f cap with reagent **2** ($C_9F_{19}CH_2$– cap)			
Fragment			
H_2N-GNQWAVGHLC–$CONH_2$	1082.50	1083.40	1
R_fCH_2–NH-QWAVGHLC–$CONH_2$	1393.42	1394.27	1
R_fCH_2–NH–AVGHLC–$CONH_2$	1079.28	1080.24	1