Supporting Information

Recognition-Domain Focused (RDF) Chemosensors: Versatile and Efficient Reporters of Protein Kinase Activity

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TABLE OF CONTENTS **PAGE**

I. General information

Unless otherwise noted, all solvents and reagents were obtained commercially and used without further purification. N^{α}-Fmoc-protected amino acids [Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Cyc(Mmt)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(PO(OBn)OH)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Thr(PO(OBn)OH)-OH, Fmoc-Val-OH][§] were purchased from Novabiochem. Whenever anhydrous and/or degassed CH_2Cl_2 was necessary, it was distilled from calcium hydride and degassed by bubbling argon for at least 20 min. Analytical TLC was performed on silica gel 60 F_{254} precoated plates (EMD Chemicals Inc.) and visualized by UV. Flash column chromatography was performed as previously described[1] using forced flow of the indicated solvent on AdTech Flash Silica Gel (32-60 μm packing, 60 Å pore diameter, Adedge Technologies). Organic solutions were concentrated *in vacuo* by rotary evaporation at ~10 Torr (house vacuum) at 25-40 ^{o}C, then at ~0.5 Torr (vacuum pump), unless otherwise indicated. Peptides were purified *via* preparative reverse-phase HPLC employing a gradient of solvents A (H_2O with 0.1% v/v TFA) and B (CH_3CN with 0.1% v/v TFA). Compounds were characterized by ^{1}H and ^{13}C NMR and mass spectroscopy. Peptide purity was determined by analytical reverse-phase HPLC.

II. Instrumentation

NMR: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz Avance spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to $CDCl_3$ (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). Coupling constants (*J*) are reported in Hertz (Hz) and multiplicities are abbreviated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t) and multiplet (m).

HPLC: HPLC was carried out on Waters Prep LC 4000 System or Waters Delta 600 System equipped with Waters 2487 dual wavelength absorbance detectors. Columns used: C_{18} analytical

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[§] Abbreviations: Abl: Abelson kinase, ATP: adenosine triphosphate, BME: β-mercaptoethanol, Bn: benzyl, tBu: *t*-butyl, Boc: *t*-butoxycarbonyl, BSA: bovine serum albumin, BTF: β-turn focused, DIEA: diisopropylethylamine, DMF: *N*,*N*-dimethylformamide, DMSO: dimethyl sulfoxide, DTT: dithiothreitol, EDT: 1,2-ethanedithiol, EDTA: ethylenediaminetetraacetic acid, EGTA: glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid, ERK1/2: extracellular signal-regulated kinase 1/2, ESI-MS: Electrospray Ionization Mass Spectrometry, Fmoc: 9-fluorenylmethoxycarbonyl, FPR: fluorescence plate reader, HEPES: 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid, HOAt: 7-aza-1-hydroxybenzotriazole, HOBt: 1-hydroxybenzotriazole, HPLC: high performance liquid chromatography, HRMS: high resolution mass spectrometry, IRK: insulin receptor kinase, MALDI-TOF MS: Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry, MK2: mitogen-activated protein kinase-activated protein kinase-2, Mmt: 4-methoxytrityl, NMR: Nuclear Magentic Resonance, Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl, PKA: protein kinase A, PKC: protein kinase C, PyAOP: (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, PyBOP: Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate, RDF: recognition-domain focused, Sox-Br: 2-bromomethyl-8-*t*-butyldiphenylsilyloxy-5-(*N*,*N*-dimethyl)sulfonamidoquinoline, SPPS: solid-phase peptide synthesis, Src: sarcoma kinase, TFA: trifluoroacetic acid, TIS: triisopropylsilane, TLC: thin-layer chromatography, TNBS: 2,4,6-trinitrobenzene sulfonic acid, UV: Ultraviolet.

(flow rate = 1 mL/min), Beckman Ultrasphere ODS, 5 μm, 150 x 4.6 mm; C_{18} preparatory (flow rate = 15 mL/min), YMC-Pack Pro, 5 μm, 250 x 20 mm.

ESI-MS: Applied Biosystems Mariner mass spectrometer.

MALDI-TOF MS: PerSeptive Biosystems Voyager MALDI-TOF instrument.

HRMS: The Department of Chemistry Instrumentation Facility (DCIF), MIT.

UV-Vis Spectrophotometer: Shimadzu UV-2401PC.

Fluorometer: Fluoromax 3 from Jobin Yvon. Cuvette: Starna Cells (16.100F-Q-10) 100 μL sub-micro cuvette, 1 cm path length.

Fluorescence Plate Reader: HTS 7000 Bio Assay Reader from Perkin Elmer. Plate: Corning (3992) assay plate, 96-well, half area, no lid, flat bottom, non-binding surface, non-sterile, white polystyrene.

III. Fmoc-C(Sox[TBDPS])-OH synthesis

a. Fmoc-Cys-OAllyl

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