SUPPLEMENTARY MATERIAL

Synthesis of side-chain modified peptides using iterative solid phase ‘click’ methodology

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1. General Experimental Information and synthetic procedures pS2

2. Copies of NMR spectra for 1-7 pS5

3. Anion titration data for 4•Zn_2; 6•Zn_2 and 7•Zn_2 pS19
General

$^1$H NMR spectra were recorded at 300 K on a Bruker Avance III 500 at a frequency of 500.13 MHz, a Bruker Avance DPX 400 at a frequency of 400.13 MHz, a Bruker Avance DPX 300 at a frequency of 300.13 MHz or a Bruker Avance DPX 200 at a frequency of 200.13 MHz and are reported as parts per million (ppm) with the residual protons in deuterated solvents as internal references. $^1$H NMR signals are reported as chemical shift values $\delta$ (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets and m = multiplet), coupling constant ($J$ Hz) and relative integral. $^{13}$C NMR were recorded using a Bruker Avance III 500 at a frequency of 125.76 MHz, a Bruker Avance DPX 400 at a frequency of 100.61 MHz or a Bruker Avance DPX 300 at a frequency of 75.47 MHz and are reported as parts per million (ppm) with CDCl$_3$ ($\delta_C$ 77.16 ppm), CD$_3$OD ($\delta_C$ 49.00 ppm) or DMSO-$d_6$ ($\delta_C$ 40.45 ppm) as an internal reference standard.

Melting points were measured using a Stanford Research Systems Optimelt melting point apparatus and are uncorrected. Optical rotations were performed at 20 °C using the indicated spectroscopic grade solvent on a Perkin Elmer model 341 polarimeter or a PO LAR 2001 polarimeter at 589 nm. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer using attenuated total reflection (ATR) of a thin film.

Low resolution mass spectra were recorded on a Thermo Finnigan LCQ Deca Ion Trap mass spectrometer and high resolution mass spectra were recorded on a 4.7 T Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR). Ionization of all samples was carried out using electrospray ionisation (ESI) or atmospheric pressure chemical ionization (APCI).

Liquid chromatography mass spectrometry (LCMS) data was obtained on a Shimadzu Separation Products: Spectra System based on a P400 Pump, a UV6000LP photodiode array detector, a Phenomenex Jupiter column (5 µm, 2.1 × 150mm) and a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI). Flow rate was maintained at 0.2 mL min$^{-1}$ with mobile phases of 0.1% formic acid in Milli-Q water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Analytical reverse phase high performance liquid chromatography (analytical
RP-HPLC) was performed on a Waters 2695 separations module with an Alliance series column heater, a Waters SunFire™ C18 column (5 µm, 2.1 × 150 mm) and a Waters 2996 photodiode array detector. The experiments were carried out at 30 °C with a flow rate maintained at 0.2 mL/min (mobile phases solvent A: 0.1% formic acid in Milli-Q water and solvent B: 0.1% formic acid in acetonitrile). Preparative RP-HPLC was performed on a Waters 600 controller with a Waters 600 pump, a 2998 photodiode array detector and Waters Empower 2 software. Separation was achieved on a XBridge™ Prep Shield C18 OBD™ (5 µm, 19 × 150 mm) column at a flow rate of 7.0 mL min⁻¹, using mobile phases of 0.05% ammonia in Milli-Q water (solvent A) and 0.05% ammonia in acetonitrile (solvent B). The collected fractions from preparative HPLC were lyophilized using a Labconco FreeZone 6 liter console freeze dry system after removal of acetonitrile.

Fluorescence spectra were recorded using a Varian Cary Eclipse Fluorescence Spectrophotometer. UV-Vis data was recorded using a Varian Cary 4000 UV-Vis Spectrophotometer. Temperature control was provided by a Varian Cary PCB 150 Water Peltier System and pH values were determined using an Activon Model 209 pH/mV meter.

**Synthesis**

**1-(2-Bromoethyl)uracil (15)**

![1-(2-Bromoethyl)uracil (15)](image)

Uracil (1.00 g, 8.9 mmol) and ClSiMe₃ (0.54 mL, 4.5 mmol) in hexamethyldisilazane (5.7 mL, 27.0 mmol) were allowed to reflux under argon for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in DMF (5 mL) followed by addition of 1,2-dibromoethane (2.3 mL). The resulting mixture was heated at 80 °C for 24 h, cooled and treated with water (80 mL). After filtration, the aqueous filtrate was then extracted with CH₂Cl₂ (3 x 200 mL). The combined organic extracts were dried over MgSO₄ and concentrated to afford the product 15 (0.60 g, 31%). ¹H NMR (300 MHz, CDCl₃): δ 8.81 (s, 1H), 7.29 (d, J = 7.8 Hz, 1H), 5.75 (d, J = 7.8 Hz, 1H), 4.16 (t, J = 5.8 Hz, 2H), 3.70 (t, J = 5.8 Hz, 2H); HRMS (ESI) calcd. for C₆H₇BrN₂O₂Na [M + Na]⁺ 240.9583, found 240.9584.
1-(2-Azidoethyl)uracil (14)

A mixture of 15 (0.60 g, 2.7 mmol), sodium azide (0.51 mg, 8.2 mmol) and tetrabutylammonium chloride (0.76 mg, 2.7 mmol) in acetone (3 mL) and water (3 mL) was allowed to stir at rt for 40 h. Extracted with ethyl acetate (5 x 20 mL), the combined organic layers were dried and evaporated to give a gum which was then purified by flash column chromatography (silica gel; 100% ethyl acetate). Compound 14 was obtained as a colourless solid (0.26 g, 52%). IR νmax/cm⁻¹ 3263, 3014, 2157, 1660, 1478, 1416, 1206, 837; \(^1\)H NMR (200 MHz, CDCl₃): δ 7.34 (d, \(J = 8.0\) Hz, 1H), 5.61 (d, \(J = 8.0\) Hz, 1H), 3.86 (t, \(J = 5.0\) Hz, 2H), 3.60 (t, \(J = 5.0\) Hz, 2H).
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TE: 300.0 K
D1: 2.26709890 sec
TD0: 1

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PL1: 4.50 dB
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SOLVENT            MeOD
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SWH           10000.000 Hz
FIDRES         0.152588 Hz
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DE                 6.50 usec
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D1           2.00000000 sec

 NASCAR                       11000.000 Hz
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APOL            0 Hz

F2 - Processing parameters
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WDW                  no
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SWH           10000.000 Hz
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DW               50.000 usec
DE                 6.50 usec
TE                300.0 K

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SFO1        500.1330008 MHz

F2 - Processing parameters
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WDW                  no
SSB      0
LB       0 Hz
GB       0
PC                 1.00

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| PROCNO  | 1 |

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| TD              | 65536 |
| SOLVENT         | MeOD |
| NS              | 25600 |
| DS              | 4 |
| SWH             | 27573.529 Hz |
| FIDRES          | 0.420739 Hz |
| AQ              | 1.1884362 sec |
| RG              | 198.77 |
| DW              | 16.133 usec |
| DE              | 6.50 usec |
| TF              | 300.0 K |
| D1              | 2.00000000 sec |
| D11             | 0.03000000 sec |

**F2 - Processing parameters**

| SI              | 65536 |
| SF              | 125.75976147 MHz |
| WDW             | EM |
| SSB             | 0 |
| LB              | 1.00 Hz |
| GR              | 1.40 |

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PROCNO: 1

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TD: 65536
SOLVENT: MeOD
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AQ: 0.152588 Hz
RG: 72.03
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DE: 6.50 usec
DI: 2.00000000 sec

F2 - Processing parameters
SI: 32768
SF: 500.1300107 MHz
WDW: EM
SSB: 0
LB: 0.30 Hz
PC: 1.00
Current Data Parameters

NAME     LLL-tripeptide-zin titration
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
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PULPROG              zg
TD                48076
SOLVENT            MeOD
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FIDRES         0.166403 Hz
AQ            3.0048001 sec
RG                  144
DW               62.500 usec
DE                10.20 usec
TE                296.4 K
D1           5.00000000 sec

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SFO1        400.1324008 MHz

F2 - Processing parameters
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SF          400.1300076 MHz
WDW                  no
SSB      0
LB       0 Hz
GB       0
PC                 1.00

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\[ \text{Diagram of chemical structures} \]
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EXPNO: 1
PROCNO: 1

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Date: 20120704
Time: 18.56
INSTRUM: spect
PROBHD: 5 mm QNP 1H/13C
PULPROG: zgpg30
TD: 65536
SOLVENT: C6D6
NS: 13056
DS: 4
SWH: 18832.393 Hz
AQ: 1.7400308 sec
RG: 1625.5
DW: 26.550 usec
DE: 6.50 usec
TE: 300.0 K
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D1: 0.03000000 sec
TD0: 17

F2 - Processing parameters
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SF: 75.4681374 MHz
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SSB: 0
LB: 3.00 Hz
PC: 1.40

--- CHANNEL f1 ---
N1: 1.3C
PCPD1: 80.00 usec
PL1: 2.50 dB
SF2: 75.4760505 MHz

--- CHANNEL f2 ---
CPDPO2: walt16
N1: 1.3H
PCPD2: 80.00 usec
PL2: 4.50 dB
PL12: 23.00 dB
SF2: 300.1322005 MHz

F2 - Processing parameters
DI: 32768
DI: 75.4681374 MHz
WM: 0
LB: 3.00 Hz
PC: 1.40
Current Data Parameters
NAME: LLO TRIPETIDE
EXPERIMENT:
PROCEDURE:

F2 - Acquisition Parameters
Data: 2012111
Time: 11:48
INSTRUMENT: spect
PROBHD: 5 mm PABBO BB-
PULPROG: zg
TD: 65536
SOLVENT: MeOD
NS: 18
DS: 0
SWH: 10000.00 Hz
FIDRES: 0.152588 Hz
AQ: 3.2788500 sec
RG: 32.64
DW: 50.00 usec
TE: 300.0 K
D1: 2.00000000 sec

-------- CHANNEL f1 --------
NUC1: 1
P1: 12.00 usec
PLW1: 15.48799992 W
SFO1: 500.1330008 MHz

F2 - Processing parameters
SI: 32768
SF: 500.1300106 MHz
WDW: no
SSB: 0
LB: 0 Hz
GB: 0
PC: 1.00
Current Data Parameters

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PROCNO                1

F2 - Acquisition Parameters
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INSTRUM           spect
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PULPROG          zgpg30
TD                65536
SOLVENT            MeOD
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AQ            1.1884362 sec
RG               198.77
DW               18.133 usec
DE                 6.50 usec
TE                300.1 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
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PCPD2                 80.00 usec
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PLW13          0.22303000 W
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PROCNO 1

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INSTRUM spect
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PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 16058
DS 4
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FIDRES 0.336591 Hz
AQ 1.4855326 sec
RG 203
DW 22.667 usec
DE 6.50 usec
TE 300.2 K
D1 2.00000000 sec
D11 0.00000000 sec

====== CHANNEL f1 ======
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SFO1 100.6223244 MHz

====== CHANNEL f2 ======
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NUC2 1H
PCPD2 90.00 usec
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PLW12 0.32877001 W
PLW13 0.26629999 W
SFO2 400.1316005 MHz

F2 - Processing parameters
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WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
**Fluorescence titration data**

**Compound 4•Zn₂**

![Graph showing changes in fluorescence intensity of 4•Zn₂](image1)

**Figure S1** Changes in fluorescence intensity of 4•Zn₂ (5 µM) upon addition of PPi (sodium salt), [PPi] = 0.5, 1.5, 3.0, 4.5, 9.6, 20.0, 40.6, 70.7, 100.8, 130.9, 191.1, 221.2, 251.3 µM in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, λₑₓ = 390 nm, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

![Graph showing changes in fluorescence intensity for 4•Zn₂ at 480 nm](image2)

**Figure S2** Changes in fluorescence intensity for of 4•Zn₂ (5 µM) at 480 nm upon addition of up to 50 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, λₑₓ = 390 nm, slit 5/5. I = normalized fluorescence intensity.
Figure S3 Changes in fluorescence intensity of 6•Zn₂ (5 µM) upon addition of PPi (sodium salt), [PPi] = 0.5, 1.0, 2.1, 2.6, 3.1, 4.2, 5.2, 6.7, 8.2, 9.6, 12.4, 15.2, 20.0, 25.2, 30.4, 40.3, 50.3 µM in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, λ<sub>ex</sub> = 390 nm, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

Figure S4 Changes in fluorescence intensity of 6•Zn₂ (5 µM) at 480 nm upon addition of up to 10 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, λ<sub>ex</sub> = 390 nm, slit 5/5. I = normalized fluorescence intensity.
Figure S5 Changes in fluorescence intensity of $7\cdot$Zn$_2$ (5 µM) upon addition of PPi (sodium salt), [PPi] = 1.0, 1.9, 2.9, 3.9, 4.8, 6.4, 8.0, 9.6, 12.4, 15.1, 19.9, 24.6, 29.4, 39.7, 50.0 µM in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, $\lambda_{ex} = 390$ nm, slit 5/5. Inset: 1:1 fitting curve at 480 nm.

Figure S6 Changes in fluorescence intensity of $7\cdot$Zn$_2$ (5 µM) at 480 nm upon addition of up to 10 equiv. of anions (sodium salts) in aqueous solutions of HEPES buffer (5 mM, 145 mM NaCl, pH 7.4) at 25 °C, $\lambda_{ex} = 390$ nm, slit 5/5. I = normalized fluorescence intensity.