Supporting Information

Design and Synthesis of a Clickable Cell-Permeable Pseudopeptide Pin1 Inhibitor with Antiproliferative Effects on Human Multiple Myeloma Cell Line

Lorenzo Meneghelli,[a] Stephanie Davidson,[b] Anthony Gineste,[a] Lamia El Guermah,[c] Sabrina Kellouche-Gaillard,[c] Franck Carreiras,[c] Ludovic Carlier,[b] Simon Nadal,[a] Maud Larregola,[a,b] Julien Pytkowicz,[a] Chiara Zanato.[a]*

[a]*BioCIS UMR 8076 CY Cergy Paris Université, CNRS, 95000 Cergy Pontoise, France.*
[b]*LBM, Sorbonne Université, ENS, PSL University, CNRS, 75005 Paris, France.*
[c]*ERRMECe, MECuP, I-MAT, CY Cergy Paris Université, Cergy, 95000 France.*
**chiara.zanato@cyu.fr*

Table of contents

1 MATERIAL AND METHODS

Chemical Synthesis

All reactions were carried out in oven- or flame-dried glassware under argon atmosphere, unless stated otherwise. All commercially available reagents were used as received. Dry solvents were obtained from commercial sources and used without further purification. Reactions requiring heating were performed using an oil bath. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC), unless otherwise noted. TLCs were performed on Merck silica gel glass plates (60 PF254). Visualization was accomplished by UV light (254 nm) or staining with $KMnO_4$ solution or a solution of phosphomolybdic acid in ethanol. Flash chromatography was performed manually using silica gel (60 Å, particle size 40−63µm) purchased from Merck. Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise.

NMR

^{1}H (400.13 MHz), ^{13}C (100.58 MHz) and ^{31}P (161.98 MHz) NMR spectra were recorded on a BRUKER AVANCE NEO 400 MHz. NMR experiments for the affinity measures (titrations of Pin1 with a ligand) were recorded and on a BRUKER 500 MHz equipped with a triple resonance, z-axis pulsed-5 field-gradient cryogenic probe head optimized for ^{1}H detection. For ^{1}H NMR spectra the solvent resonance was employed as internal standard (CDCl3 δ = 7.26 ppm, CD_3OD δ = 3.31 ppm, $(CD_3)_2CO$ δ = 2.05 ppm, $(CD_3)_2SO$ δ = 2.50 ppm). ^{13}C NMR spectra were recorded with complete proton decoupling, and the solvent resonance was employed as internal standard (CDCl3 δ = 77.16 ppm, CD_3OD δ = 49.00 ppm, $C(CD_3)_2CO$ δ = 29.84 et 206.26 ppm, $(CD_3)_2SO$ δ = 39.52 ppm). ^{31}P NMR spectra were recorded with complete proton decoupling. Chemical shifts (δ) are reported in parts per million (ppm), coupling constants (*J*) are given in Hertz (Hz), and multiplicity is described using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal; or combinations thereof.

High-Resolution Mass Spectra (HRMS)

High-resolution mass analyses (HRMS) were performed using a QTOF 6530 from Agilent Technologies (source ESI, resolution 12000).

UPLC-MS

UPLC-MS analyses were performed on a Waters Acquity UPLC apparatus equipped with a Luna Omega PS-C18 Column (1.5 µM, 2.1 x 50 mm) coupled to a single quadrupole ESI-MS (Micromass ZQ).

HPLC

Reverse-phase HPLC analysis were performed on an analytical Agilent 1200 HPLC system equipped with a DAD detector. HPLC conditions for analytical analyses were as follows: Zorbax Eclipse XDB-C18 column, 5µm, 100 Å, 150 x 4.6 mm, injected volume 10 µL, flow rate 1 mL/min. Reverse-phase HPLC preparative purification was performed using an Agilent 1260 Infinity II HPLC system equipped with a DAD detector. HPLC conditions for preparative purification were as follows: Luna Omega PS-C18 column, 5µm, 100 Å, 250 x 10 mm, flow rate 4 mL/min.

Antibodies

Pin1 Antibody (G-8) / sc-46660 produced in mice and marketed by Santa Cruz Biotechnology and Alexa Fluor 488 Goat Anti-Mouse, A11029, marked by Life technologies were used for the immunofluorescence. Excitation and emission wavelengths: 499/520 nm.

Cells and Cells Culture Media

Two ovarian adenocarcinoma cell lines, SKOV3 and IGROV1, were used for the cell permeability assay. SKOV3, derived from serous ovarian adenocarcinoma, is characterized by a high migratory capacity, while IGROV1 serves as a model for chemoresistant ovarian adenocarcinoma. SKOV3 cells exhibit Pin1 localization in both the cytosol and nucleus, whereas in IGROV1 cells, Pin1 is predominantly cytoplasmic. A steroid-resistant multiple myeloma cell line, MM.1R, was used for the viability assay. The MM1.R cell line was purchased from the American Tissue Culture Collection (ATCC #CRL-2975, RRID: CVCL_8794) and cultures were started from a copy one passage away from the certified source. The human ovarian adenocarcinoma cell lines SKOV3 was purchased from the American Type Culture Collection, Manassas, USA

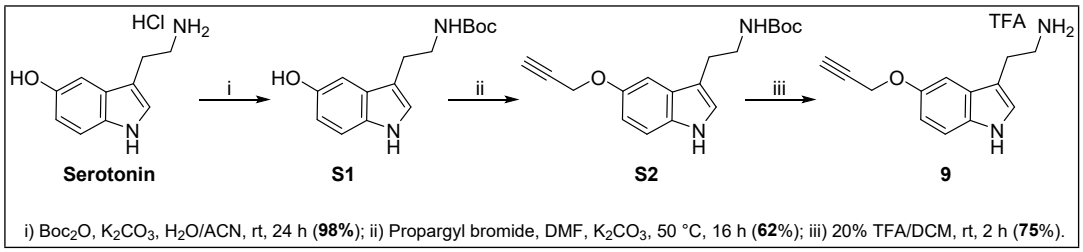
(ATCC HTB-79). IGROV1 est une lignée cellulaire d'un patient diagnostiqué d'un cancer ovarien de stade III, et a été généreusement fournie par le Dr. J. Bénard [BÉNARD, J. et al. *Characterization of a human ovarian adenocarcinoma line, IGROV1, in tissue culture and in nude mice*, *Cancer Res* 45, 4970-4979. ISSN : 0008-5472 (oct. 1985)].The cell medium used for the cell permeability and viability assays with SKOV3, IGROV1, and MM.1R cell lines was RPMI 1640 (Gibco, ATCC modification) with 10% fetal bovine serum (FBS, Gibco). Ham's F-12 medium, supplemented with 10% FBS, was used for the stability assay. Cells were cultured according to manufacturer's instruction, with a passaging at 85-90 % confluence for MM1.R.

Statistical Analysis

The results of the cell viability assay were statistically analyzed by calculating the standard deviation (SD). Data are expressed as the mean ± SD, and the values reported are from experiments performed in triplicate (technical replicates from the same cell batch). The different values were compared and analyzed using one-way ANOVA with a Tukey post-test. A p-value < 0.05 was considered statistically significant. Graphs were prepared using GraphPad Prism (version 8.0.2 for Windows, San Diego, CA, USA). In the graph, values are expressed as normalised viability compared with the control with cells and cell media at time zero.

2 SYNTHETIC PROCEDURES

2.1 Synthesis of 2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine (9)



Scheme S1: Synthesis of 2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine (**9**).

***Tert*-butyl (2-(5-hydroxy-1H-indol-3-yl)ethyl)carbamate (S1)**

Serotonin hydrochloride salt (2.0 g, 9.4 mmol, 1 eq) and potassium carbonate (2.6 g, 18.9 mmol, 2 eq) were solubilised in H_2O/CH_3CN (2:1, 50 mL) at room temperature. Di-*tert*-butyl dicarbonate (2.4 mL, 10.3 mmol, 1.1 eq) was added and the resulting yellow solution was stirred at room temperature for 24 h. The reaction mixture was extracted with EtOAc (3 x 80 mL). The organic layers were washed with H_2O (100 mL), brine (100 mL) dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product **S1** (2.5 g, 9.2 mmol, 98%) was directly submitted to the next step without any further purification. (Characterisation data in accordance with those reported in literature: J. Jose *et al ACS Med. Chem. Lett.* 2017, *8*, 1072–1076). $\mathbf{R_f}$: 0.35 (CyHex/AcOEt 70:30). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 8.00 (bs, 1 H, N*H* Indole), 7.20 (d, *J* = 8.6 Hz, 1 H, H arom), 7.01 (d, *J* = 2.2 Hz, 1 H, H arom), 6.97 (bs, 1 H, N*H*Boc), 6.80 (dd, *J* = 8.6, 2.2 Hz, 1 H, H arom), 4.69 (bs, 1 H, O*H*), 3.50-3.30 (m, 2H, C$H_2$$CH_2$NH), 2.86 (t *J* = 6.5 Hz, 2 H, CH_2CH_2NH), 1.44 (s, 6 H, C(CH_3)$_2$).

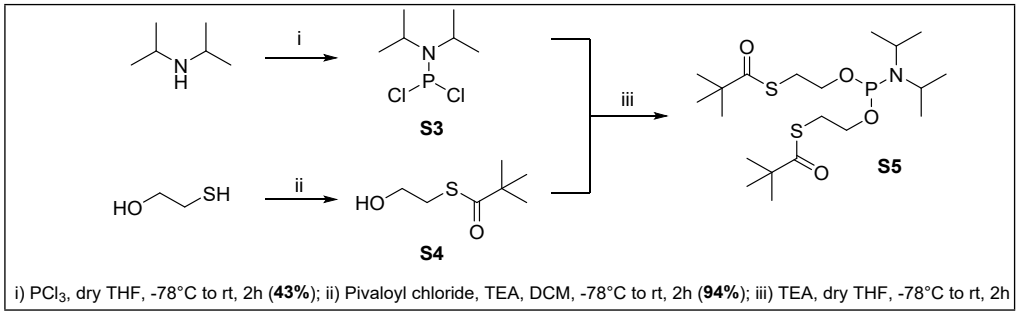
***Tert*-butyl (2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethyl)carbamate (S2)**

Boc protected serotonine **S1** (2.5 g, 9.2 mmol, 1 eq) and potassium carbonate (3.8 g, 27.6 mmol, 3 eq) was dissolved in DMF (50 mL). Propargyl bromide (1.23 mL, 13.8 mmol, 1.5 eq) was added and the resulting solution was stirred at 50°C for 16 h. The reaction mixture was cooled to room temperature, diluted with water (50 mL) and extracted with EtOAc (150 mL). The organic layers were washed with H_2O (3 x 100 mL), brine (100 mL) dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CyHex/AcOEt step gradient from 0% AcOEt to 60%) to give the alkylated compound **S2** (1.8 g, 5.70 mmol, 62%) as a pale yellow solid. $\mathbf{R_f}$: 0.50 (CyHex/AcOEt 30:70). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 8.1 (bs, 1 H, N*H* Indole), 7.27 (d, *J* = 8.8 Hz, 1 H, H arom), 7.14 (d, *J* = 1.9 Hz, 1 H, H arom), 7.01 (s, 1 H, H arom), 6.93 (dd, *J* = 8.8, 2.4 Hz, 1 H, H arom), 4.74 (d, *J* = 2.4 Hz, 2H, CH_2O), 4.64 (bs, 1 H, N*H*Boc), 3.53-3.35 (m, 2H, C$H_2$$CH_2$NHBoc), 2.91 (t, *J* = 6.6 Hz, 2 H, CH_2CH_2NHBoc), 2.51 (t, *J* = Hz, 6 H, CC*H*), 1.44 (s, 6 H, C(CH_3)$_2$). **^{13}C NMR** (100.5 MHz, $CDCl_3$) δ: 156.1 (C, CO), 152.0 (C, CO), 132.2 (C arom), 127.8 (C arom), 123.2 (CH arom), 113.2 (CH arom), 113.1 (C *arom*), 112.1 (CH arom), 103.0 (CH arom), 79.3 (C, *C*CH), 77.4 (C, *C*$(CH_3)_2$).75.3 (CH, C*CH*), 57.1 (CH_2, *CH_2*O), 40.8 (CH_2, *CH_2*CH_2NHBoc), 28.6 (3x CH_3, C(CH_3)$_2$), 25.9 (CH_2, CH_2*CH_2*NHBoc).

2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-aminium (TFA salt) (9)

The Boc protected serotonine derivative **S2** (1.8 g, 5.70 mmol, 1 eq) was dissolved in TFA/DMC (20% TFA in DCM, 25 mL) and stirred for 2 h. The solvent was evaporated under reduced pressure to give compound **9** (1.4 g, 4.27 mmol, 75%,) as pale-yellow solid. The crude product was directly submitted to the next step without any further purification. $\mathbf{R_f}$: 0.12 (DCM/MeOH 80:20). **^{1}H NMR** (400 MHz, CD_3OD) δ: 7.28 (d, *J* = 8.8 Hz, 1 H, H arom), 7.16 (s, 1 H, H arom), 7.15 (d, *J* = 2.3 Hz, 1 H, H arom), 6.85 (dd, *J* = 8.8, 2.4 Hz, 1 H, H arom), 4.74 (d, *J* = 2.4 Hz, 2H, CH_2O), 3.21 (t, *J* = 7.3 Hz, 2 H, C$H_2$$CH_2$$NH_2$), 3.08 (t, *J* = 7.3 Hz, 2 H, C$H_2$$CH_2$$NH_2$), 2.92 (t, *J* = 2.4 Hz, 1 H, CC*H*). **^{13}C NMR** (100.5 MHz, CD_3OD) δ: 151.8 (C arom), 132.7 (C arom), 127.0 (C arom), 123.8 (CH arom), 112.2 (CH arom), 111.8 (CH arom), 108.8 (C arom), 101.9 (CH arom), 79.1 (C, *C*CH), 74.8 (CH, C*CH*), 56.3 (CH_2, *CH_2*O), 39.8 (CH_2, *CH_2*$CH_2$$NH_2$), 23.3 ($CH_2$, CH_2*CH_2*NH_2). **HRMS** calcd for $C_{13}H_{14}N_2O$ $[M + H]^+$ *m/z* = 215.1179, found *m/z* = 215.1175.

2.2 Synthesis of SATE phosphoramidite (S5)



i) PCl_3, dry THF, -78°C to rt, 2h (**43%**); ii) Pivaloyl chloride, TEA, DCM, -78°C to rt, 2h (**94%**); iii) TEA, dry THF, -78°C to rt, 2h

Scheme S2: Synthesis of SATE phosphoramidite (**S5**).

1,1-dichloro-N,N-diisopropylphosphanamine (S3)

A solution of diisopropylamine (5.25 mL, 37.4 mmol, 2.3 eq) in THF (15 mL) was added dropwise into a vigorously stirred solution of PCl_3 (1.42 mL, 16.3 mmol, 1 eq) in THF (15 mL) at -78°C, under Argon atmosphere. The white suspension was stirred at room temperature for 2 h, then the hydrochloride salt was filtered off and washed with THF (15 mL). The filtrate was concentrated under reduced pressure and then distilled under vacuum (b.p. 79-80°C, 5 mbar). **S3** was obtained in a form of crude colourless oil (2.78 g, 13.8 mmol, m.p. 4°C). Yield: 42%. (Characterisation data in accordance with those reported in literature: G. F. Ruda *et al ChemMedChem* 2007, *2*, 1169-1180). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 3.98 (bs, 2 H, 2 x C*H*$(CH_3)_2$), 1.33 (s, 12 H, CH(C*H*$_3$)$_2$). **^{31}P NMR** (161.98 MHz, $CDCl_3$) δ: 169.6 (s, P).

S-(2-hydroxyethyl) 2,2-dimethylpropanethioate (S4)

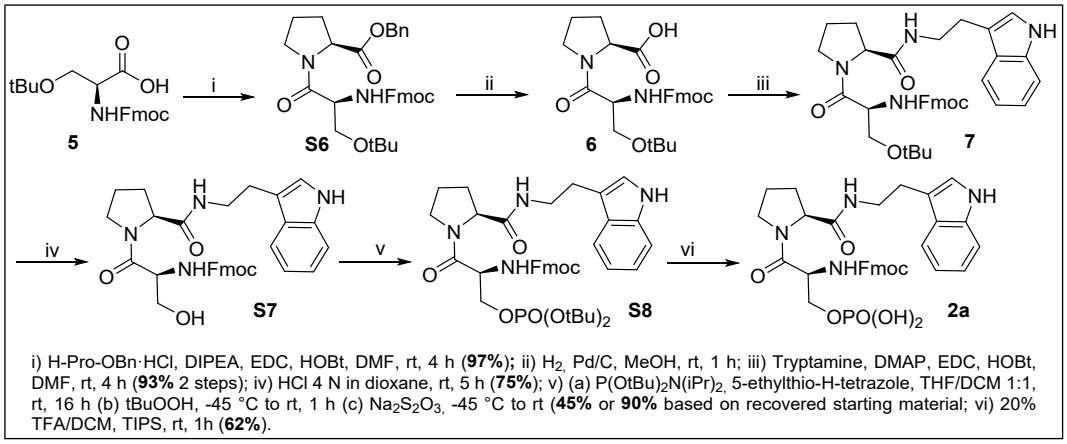
Pivaloyl chloride (1.6 mL, 13 mmol, 1 eq) was added to a stirred solution of 2-mercaptoethanol (1.4 mL, 13 mmol, 1 eq) and triethylamine (1.8 mL, 13 mmol, 1 eq) in DCM (20 mL), cooled at -78°C. The mixture was stirred at -78°C for 1 h, then allowed to warm to room temperature and stirred further for 1 h. Water (20 mL) was added, the organic layer was separated, and the aqueous phase extracted with DCM (2 x 20 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CyHex/AcOEt step gradient from 0% AcOEt to 25% AcOEt) to give compound **S4** (2.0 g, 12.2 mmol, 94%) as a colorless oil. (Characterisation data in accordance with those reported in literature: G. F. Ruda *et al ChemMedChem* 2007, *2*, 1169-1180). $\mathbf{R_f}$: 0.43 (CyHex/AcOEt 70:30). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 3.75 (d, *J* = 6.0 Hz, 2 H, C*H*$_2$O), 3.06 (d, *J* = 6.0 Hz, 2 H, C*H*$_2$S), 1.24 (s, 9 H, C(C*H*$_3$)$_3$). **^{13}C NMR** (100.5 MHz, $CDCl_3$) δ: 207.5 (C, CO), 62.1 (CH_2, CH_2O), 46.7 (C, *t*Bu), 31.7 (CH_2, CH_2S), 27.5 (3 x CH_3, *t*Bu).

SATE Phosphoramidite (S5)

A solution of *S*-pivaloyl thioethanol **S4** (517 mg, 3.2 mmol, 2 eq) and triethylamine (0.97 mL, 7.0 mmol, 4.4 eq) in THF (8 mL) was added dropwise to a solution of crude diisopropylamino dichloro phosphine **S3** (322 mg, 1.6 mmol, 1 eq) in THF (8 mL) at -78°C. The white suspension was stirred for 2 h at room temperature then the precipitate (triethylamine hydrochloride salt) was filtered off. The filtrate was concentrated under reduced pressure and hexane (5 mL) was added to the crude yellow syrup. The white precipitate formed was filtered off and the filtrate (**S5**, pale yellow oil) was directly submitted to the next step without any further purification. (Characterisation data in accordance with those reported in literature: G. F. Ruda *et al ChemMedChem* 2007, *2*, 1169-1180). **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 3.80-3.50 (m, 6 H, 2 x C*H*$_2$O + 2 x C*H*$(CH_3)_2$), 3.12-3.04 (m, 4 H, 2 x C*H*$_2$S), 1.23 (s, 18 H, 2 x C(C*H*$_3$)$_3$), 1.17 (s, 6 H, CH(C*H*$_3$)$_2$), 1.16 (s, 6 H, CH(C*H*$_3$)$_2$). **^{31}P NMR** (161.98 MHz, $CDCl_3$) δ: 147.1 (s, P).

2.3 Synthesis of Reduced Amide Series Pin1 Ligands (2a-2b)

2.3.1 Synthesis of 2a



i) H-Pro-OBn·HCl, DIPEA, EDC, HOBt, DMF, rt, 4 h (**97%**); ii) H_2, Pd/C, MeOH, rt, 1 h; iii) Tryptamine, DMAP, EDC, HOBt, DMF, rt, 4 h (**93%** 2 steps); iv) HCl 4 N in dioxane, rt, 5 h (**75%**); v) (a) $P(OtBu)_2N(iPr)_2$, 5-ethylthio-H-tetrazole, THF/DCM 1:1, rt, 16 h (b) tBuOOH, -45 °C to rt, 1 h (c) $Na_2S_2O_3$, -45 °C to rt (**45%** or **90%** based on recovered starting material; vi) 20% TFA/DCM, TIPS, rt, 1h (**62%**).

Scheme S3: Synthesis of **2a**.

Fmoc-L-Ser(O*t*Bu)-L-Pro-OBn (S6)

A solution of DIPEA (2.66 mL, 15.65 mmol, 1.5 eq) and HCl·H–Pro–OBn (2.77 g, 11.47 mmol, 1.1 eq) in DMF (16 mL) was added to a solution of Fmoc–Ser(O*t*Bu)–OH (4.00 g, 10.43 mmol, 1.0 eq), EDC (2.40 g, 12.52 mmol, 1.2 eq), and HOBt (1.92 g, 12.52 mmol, 1.2 eq) in DMF (32 mL). The mixture was stirred at rt for 16 h, then it was diluted with AcOEt (200 mL), and washed with an aqueous solution of HCl (1M 100 mL) and an aqueous solution of $NaHCO_3$ (5% w/w, 80 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CyHex/AcOEt step gradient from 0% AcOEt to 50%) to give peptide **S6** (5.77 g, 10.12 mmol, 97%, *trans-cis* rotamers mixture) as a white solid. $\mathbf{R_f}$: 0.32 (CyHex/AcOEt 30:70). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers 60:40, *minor rotamer when visible): δ : 7.67 (d, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.50 (d, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.35-7.16 (m, 9 H, 4 H x Fmoc arom. and 5 H x Bn arom.), 5.61 (m, 1 H, NH Fmoc), 5.08 (s, 2 H, Bn CH_2), 5.02-4.98* (m, 0.4 H, Hα Pro*), 4.59 (dd, *J* = 7.2, 14.3 Hz, 0.6 H, Hα Ser), 4.52-4.41* (m, 0.6 H x Hα Pro + 0.4 H x Hα Ser*), 4.29-4.20 (m, 2 H, Fmoc CH_2), 4.17-4.07 (m 1 H, Fmoc CH), 3.79-3.88 (m, 0.6 H, Hδ Pro-Hb), 3.65-3.52* (m, 0.6 H x Hδ Pro-Ha + 0.4 H x Hδ Pro-Hb*), 3.39-3.49* (m, 1.2 H x Hβ Ser + 0.4 H, Hδ Pro-Ha*), 3.33-3.25* (dd, 0.4 H J = 8.2, 10.2 Hz, Hβ Ser*), 2.19-1.98 (m, 1 H, Hβ Pro-Ha), 1.98-1.79 (m, 3 H, 2 x Hγ Pro + 1 x Hβ Pro-Ha), 1.08 (s, 5.4 H, *t*Bu), 1.04 (s, 3.6 H, *t*Bu*). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 60:40, *minor rotamer when visible) δ : 171.9* (C, CO Pro*) 171.6 (C, CO Ser), 170.4* (C, CO Pro*), 169.8 (C, CO Ser), 156.0 (C, CO Fmoc), 155.4* (C, CO Fmoc*), 144.0 (2 C, Fmoc arom.), 143.9* (2 C, Fmoc arom.*), 141.3 (2 C, Fmoc arom.), 135.8 (C, Bn arom.), 135.4* (C, Bn arom.*), 128.6 (2 CH, Bn arom.), 128.3 (CH, Bn arom.), 128.1, (2 CH, Bn arom.), 127.8 (2 CH, Fmoc arom.), 127.7 (2 CH, Fmoc arom.), 127.1 (2 CH, Fmoc arom.), 120.0 (2 CH, Fmoc arom.), 73.6 (C, *t*Bu), 67.5* (CH_2, Bn*), 67.2 (CH_2, Bn), 67.1* (CH_2, Fmoc*), 66.8 (CH_2, Fmoc), 64.0* (CH_2, Cβ Ser*), 62.9 (CH_2, Cβ Ser), 59.3* (CH, Cα Pro*), 59.2 (CH, Cα Pro), 53.0 (CH, Cα Ser), 52.6* (CH, Cα Ser*), 47.4 (CH_2, Cδ Pro), 47.2 (CH, Fmoc), 46.4* (CH_2, Cδ Pro*), 30.8* (CH_2, Cβ Pro*), 29.2 (CH_2, Cβ Pro), 27.4* (3 CH_3, *t*Bu*), 27.3 (3 CH_3, O*t*Bu), 24.9 (CH_2, Cγ Pro), 22.6* (CH_2, Cγ Pro*). **HRMS** calcd for $C_{35}H_{38}N_2O_6$ $[M + H]^+$ m/z = 571.2808, found m/z = 571.2808.

Fmoc-L-Ser(O*t*Bu)-L-Pro-OH (6)

Compound **S6** (5.50 g, 9.65 mmol, 1 eq) was dissolved in MeOH (165 mL), and 10% Pd/C (10% w/w, 550 mg) was added. The reaction was stirred at rt under 1 atm of H_2 for 1 h. The mixture was filtered through a short pad of Celite, washed with AcOEt (200 mL), and the organic solvents were evaporated under reduced pressure. The crude carboxylic acid **6** (4.63 g, 9.65 mmol, quantitative, *trans-cis* rotamers mixture),was obtained as a white solid and it was directly used for the next step without further purification. $\mathbf{R_f}$: 0.25 (DCM/MeOH 90:10). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ : 10.17 (bs 1 H, COOH), 7.75 (d, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.59 (d, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.39 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.30 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 5.94 (d, *J* = 8.4 Hz, NH Fmoc), 4.72 (dd, *J* = 8.2, 14.1 Hz, 1 H, Hα Ser), 4.60 (dd, *J* = 3.3, 7.9 Hz, 1 H, Hα Pro), 4.35 (d, *J* = 7.3 Hz, 2 H, Fmoc CH_2), 4.21 (d, *J* = 6.8 Hz, 1 H, Fmoc CH), 4.00-3.86 (m, 1 H, Hδ Pro-Hb), 3.72-3.65 (m, 1 H, Hβ Ser-Hb), 3.65-3.57 (m, 1 H, Hδ Pro-Ha), 3.57-3.48 (m, 1 H, Hβ Ser-Ha), 2.22-2.09 (m, 1 H, Hβ Pro-Ha), 2.09-1.84 (m, 3 H, 2 x Hγ Pro + 1 x Hβ Pro-Hb), 1.14 (s, 6 H, *t*Bu). **^{13}C**

NMR (100.5 MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ : 173.9 (C, COOH Pro), 171.6 (C, CO Ser), 156.0 (C, CO Fmoc), 143.9 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 141.3 (2 C, Fmoc), 127.8 (2 CH, Fmoc arom.), 127.1 (2 CH, Fmoc arom.), 125.3 (2 CH, Fmoc arom.), 120.0 (2 CH, Fmoc arom.), 73.8 (C, *t*Bu), 67.3 (CH_2, Fmoc), 62.8 (CH_2, Cβ Ser), 59.6 (CH, Cα Pro), 52.6 (CH, Cα Ser), 47.8 (CH_2, Cδ Pro), 47.1 (CH, Fmoc), 28.4 (CH_2, Cβ Pro), 27.2 (3 CH_3, *t*Bu), 24.8 (CH_2, Cγ Pro). **HRMS** calcd for $C_{27}H_{33}N_2O_6$ $[M + H]^+$ m/z = 481.2339, found m/z = 481.2337.

Fmoc-L-Ser(O*t*Bu)-L-Pro-Tryptamine (7)

The crude carboxylic acid **S6** (4.63 g, 9.65 mmol, 1 eq) and tryptamine (1.86 g, 11.58 mmol, 1.2 eq) were dissolved in DMF (150 mL) then HOBt (1.77 g, 11.58 mmol, 1.2 eq) DMAP (14.1 mg, 0.12 mmol, 0.12 eq), and EDC (2.22 g, 11.58 mmol, 1.2 eq) were added. The mixture was stirred at rt for 16 h, diluted with AcOEt (200 mL), washed with water (3 x 100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$. After filtration and evaporation under reduced pressure, the residue was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%) to give peptide **7** (5.58 g, 8.97 mmol, 93% in 2 steps, *trans-cis* rotamers mixture) as a pale-yellow solid. $\mathbf{R_f}$: 0.25 (DCM/MeOH 98:2). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ : 8.51 (bs, 1 H, NH Indole Trypt), 7.77 (d, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.62-7.53 (m, 3 H, 2 H Fmoc arom. + 1 H Trypt arom.), 7.41 (t, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.28-7.37 (m, 3 H, 2 H Fmoc arom. + 1 H Trypt arom.), 7.16 (t, *J* = 7.6 Hz, 1 H Trypt arom.), 7.09 (t, *J* = 7.6 Hz, 1 H Trypt arom.), 6.98 (bs, 1 H, NH Pro), 7.04 (s, 1 H Trypt arom.), 5.91 (d, 1 H, *J* = 7.1 Hz, NH Fmoc), 4.69 (m, 2 H, Hα Pro + Hα Ser), 4.38 (d, 2 H, *J* = 7.5 Hz, CH_2 Fmoc), 4.21 (t, 1 H, *J* = 6.9 Hz, CH Fmoc), 3.90-3.80 (m, 1 H, Hδ Pro-Hb), 3.68-3.62 (m, 2 H, Hβ Ser-Hb + Trypt CH_2-Hb), 3.56 (dd, 1 H *J* = 7.6, 16.4 Hz, Hδ Pro-Ha), 3.46-3.35 (m, 1 H, Trypt CH_2-Ha), 3.33 (m, 1 H, Hβ Ser-Ha), 2.97 (t, 2 H *J* = 6.9 Hz, Trypt $CH_2$1), 2.31-2.22 (m, 1 H, Hβ Pro-Hb), 2.07-1.92 (m, 1 H, Hβ Pro-Ha), 1.90 (m, 2 H, Hγ Pro), 1.05 (s, 9 H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ : 171.2 (C, CO Pro). 170.6 (C, CO Ser), 155.9 (C, CO Fmoc), 143.9 (2 C, Fmoc arom.), 143.7 (C, Fmoc arom.), 141.3 (2 C, Fmoc arom.), 136.4 (C, Trypt arom.), 127.8 (2 CH, Fmoc arom.), 127.4 (CH, Trypt arom.), 127.1 (2 CH, Fmoc arom.), 125.2 (2 CH, Fmoc arom.), 122.7 (CH, Trypt arom.), 121.9 (CH, Trypt arom.), 120.1 (2 CH, Fmoc arom.), 119.2 (CH, Trypt arom.), 118.6 (CH, Trypt arom.), 112.6 (C, Trypt arom.), 111.3 (C, Trypt arom.), 74.3 (C, *t*Bu), 67.2 (CH_2, Fmoc), 61.1 (CH_2, Cβ Ser), 60.6 (CH, Cα Pro), 52.4 (CH, Cα Ser), 47.9 (CH_2, Cδ Pro), 47.1 (CH, Fmoc), 40.3 (CH_2, Trypt $CH_2$2), 28.7 (CH_2, Cβ Pro), 27.3 (3 CH_3, *t*Bu), 25.4 (CH_2, Trypt $CH_2$1), 24.6 (CH_2, Cγ Pro). **HRMS** calcd for $C_{38}H_{42}N_4O_5$ $[M + H]^+$ m/z = 623.3233, found m/z = 623.3221.

Fmoc-L-Ser(OH)-L-Pro-Tryptamine (S7)

To a solution of protected compound **7** (1.00 g, 1.61 mmol, 1 eq) a solution of 4 N HCl in dioxane (40 mL) was added. The reaction stirred at rt for 5 h until the disappearance of the starting material in TLC. The solvent was removed under reduced pressure and the crude alcohol was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 15%) to give peptide **S7** (684 mg, 1.21 mmol, 75%, *trans-cis* rotamers mixture) as a pale-yellow solid. $\mathbf{R_f}$: 0.35 (DCM/MeOH 90:10). **^{1}H NMR** (400 MHz, Acetone-d6) (2 rotamers 80:20, major rotamer reported) δ : 10.04 (bs, 1 H, NH Indole Trypt), 7.85 (d, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.71 (dd, *J* = 3.2, 7.3 Hz, 2 H, Fmoc arom.), 7.59 (d, *J* = 7.7 Hz, 1 H, Trypt arom.), 7.50 (bs, 1 H, NH Trypt), 7.41 (t, J = 7.3 Hz, 2 H, Fmoc arom.), 7.37 (d, *J* = 8.4 Hz, 1 H, Trypt arom.), 7.32 (t, *J* = 7.3 Hz, 2 H, Fmoc arom.), 7.19 (d, *J* = 1.9 Hz, 1 H, Trypt arom.), 7.09 (t, *J* = 7.5 Hz, 1 H, Trypt arom.), 7.00 (t, *J* = 7.5 Hz, 1 H, Trypt arom.), 6.61 (d, *J* = 7.8 Hz, 1 H, NH Ser), 4.67 (dd, *J* = 5.4, 13.2 Hz, 1 H, Hα Ser), 4.53 (dd, *J* = 3.9, 8.3 Hz, 1 H, Hα Pro), 4.50-4.27 (m, 2 H, CH_2 Fmoc), 4.22 (t, *J* = 7.1 Hz, 1 H, CH Fmoc), 3.91-3.86 (m, 1 H, Hβ Ser-Hb), 3.86-3.79 (m, 1 H, Hδ Pro-Hb), 3.79-3.62 (m, 2 H, Hβ Ser-Hb + Hδ Pro-Hb), 3.56-3.40 (m, 2 H, Trypt $CH_2$2), 3.07-2.84 (m, 2 H, Trypt $CH_2$1), 2.13-2.01 (m, 1 H, Hβ Pro-Hb), 2.01-1.77 (m, 3 H, Hβ Pro-Ha + Hγ Pro). **^{13}C NMR** (400 MHz, Acetone-d6) (2 rotamers 80:20, major rotamer reported) δ : 171.7 (C, CO Pro), 170.2 (C, CO Ser), 156.9 (C, CO Fmoc), 144.2 (2 C, Fmoc arom.), 141.2 (2 C, Fmoc arom.), 136.8 (2 C, Trypt arom.), 127.7 (2 CH, Fmoc arom.), 127.2 (C, Trypt arom.), 125.4 (2 CH, Fmoc arom.), 122.9 (2 CH, Fmoc arom.), 122.7 (CH, Trypt arom.), 121.2 (CH, Trypt arom.), 120.0 (2 CH, Fmoc arom.), 118.6 (CH, Trypt arom.), 118.5 (CH, Trypt arom.), 112.7 (C, Trypt arom.), 11.4 (CH, Trypt arom.), 66.5 (CH_2, Fmoc), 63.4 (CH_2, Cβ Ser), 60.5 (CH, Cα Pro), 54.2 (CH, Cα Ser), 47.4 (CH_2, Cδ Pro), 47.1 (CH, Fmoc), 39.9 (CH_2, Trypt $CH_2$2), 25.2 (CH_2, Trypt $CH_2$1), 24.5 (CH_2, Cγ Pro). **HRMS** calcd for $C_{33}H_{36}N_4O_5$ $[M + H]^+$ m/z = 567.2608, found m/z = 567.2603.

Fmoc-L-Ser[PO(O*t*Bu)$_2$]-L-Pro-Tryptamine (S8)

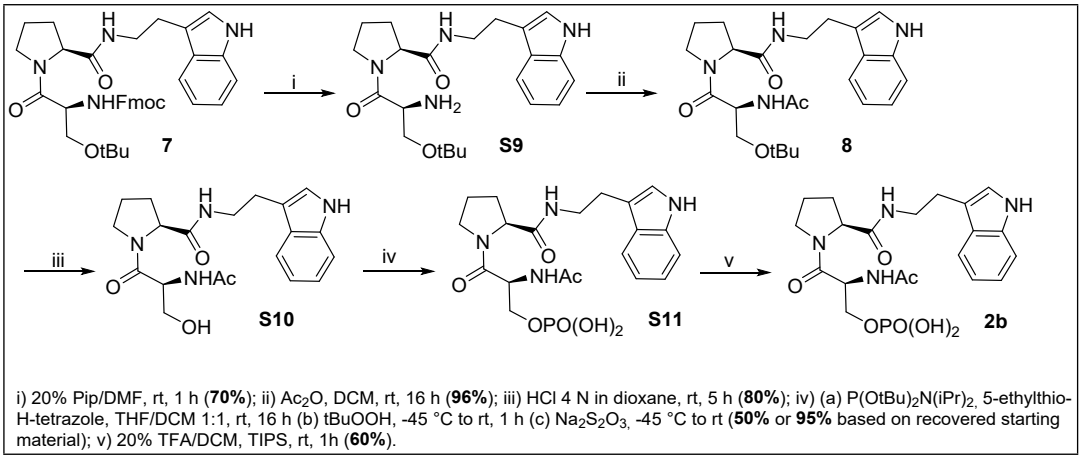
To a solution of **S7** (400 mg, 0.71 mmol, 1 eq) in DCM/THF (1:1, 20 mL) was added 5-ethylthio–H–tetrazole (184 mg, 1.41 mmol, 2 eq) and the mixture was stirred for 30 min at rt. Di*tert*-butyl-diisopropyl-phosphoramidite (0.44 mL, 1.41 mmol, 2 eq) was added and the mixture was stirred at rt for 15 h. The mixture was cooled to -45 °C, and a solution of *ter*buthyl-hydroxyperoxide (6M in decane, 0.47 mL, 2.84 mmol, 4 eq) was added dropwise. The mixture was stirred at -45 °C for 1 h and at rt for 30 minutes, then it was cooled down to -45 °C and quenched with a saturated aq solution of

$Na_2S_2O_3$ (40 mL). The layers were separeted in a separatory funnel and the acqueus phase was extracted with DCM (40 mL x 2). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was rapidly filtered on a short pad of silica gel (100% AcOEt) to give compound **S8** as a pale-yellow oil (45% yield or 90% yield based on recovered starting material). Since the purification on silica showed to be tricky (partially deprotection of one of the *t*Bu group occurs in parallel with the co-elution of the reduced phosphoyramidite) the product was used crude for the next step without further purification. $\mathbf{R_f}$: 0.64 (AcOEt/MeOH 95:5). **^{31}P NMR** (162.0 MHz, Acetone-d6) (2 rotamers 80:20, * minor rotamer) δ : -9.14, -9.68* (s, P(O)O*t*Bu_2).

Fmoc-L-Ser[OPO(OH)$_2$]-L-Pro-Tryptamine (2a)

To a solution of protected compound **S8** (242 mg, 0.32 mmol, 1 eq) 10 mL of a solution of 20% TFA in DCM, , few drops of TIPS and few drops of H_2O were added. The reaction stirred at rt for 1 h, the solvent was removed under reduced pressure and the residue was purified by semi-preparative HPLC (Luna Omega column C18, 250 x 4.5 mmm, 5µm, 100Å; Eluent: solvent A 0.1% TFA/H2O, solvent B 0.1% TFA/CH_3CN; Method: 4 mL/min, from 40% A to 90% A in 20 min then 100% A until 30 min; RT : 19.2 min) to provide **2a** as a white solid after lyophilization (128 mg, 0.20 mmol, 62% after HPLC purification). **^{1}H NMR** (400 MHz, MeOD-d4) (2 rotamers 82:18, *minor rotamer when visible) δ : 7.9 (d, *J* = 7.6 Hz, 2 H, Fmoc arom.), 7.65 (dd, *J* = 7.4 Hz, 2 H, Fmoc arom.), 7.58 (d, *J* = 7.9 Hz, 1 H, Trypt arom.), 7.38 (t, *J* = 7.5 Hz, 2 H, Fmoc arom.), 7.3 (d, *J* = 8.0 Hz, 1 H, Trypt arom.), 7.30 (t, *J* = 8.1 Hz, 2 H, Fmoc arom.), 7.09 (s, 1 H, Trypt arom.), 7.07 (t, *J* = 7.9 Hz, 1 H, Trypt arom.), 6.99 (t, *J* = 7.4 Hz, 1 H, Trypt arom.), 6.99* (t, *J* = 7.4 Hz, 0.2 H, Trypt arom.*), 4.75 (t, *J* = 6.3 Hz, 1 H, Hα Ser), 4.72-4.63* (m, 0.2 H, Hα Pro*), 4.51* (t, *J* = 7.2 Hz, 0.2 H, Hα Ser*), 4.41-4.32 (m, 3 H, Hα Pro + CH_2 Fmoc), 4.30-4.09 (m, 3 H, Hβ Ser + CH Fmoc), 3.77-3.64 (m, 2 H, Hδ Pro), 3.52-3.42 (m, 2 H, Trypt $CH_2$2), 2.95 (t, *J* = 6.1 Hz, Trypt $CH_2$2), 2.18-2.06 (m, 1 H, Hβ Pro-Hb), 1.97-1.87- (m, 2 H, Hγ Pro), 1.87-1.75 (m, 1 H, Hβ Pro-Ha). **^{13}C NMR** (100.5 MHz, MeOD-d4) (2 rotamers 82:18, major rotamer reported) δ : 174.3 (C, CO Pro), 170.5 (C, CO Ser), 158.3 (C, CO Fmoc), 145.2 (2 C, Fmoc arom.), 142.6 (2 C, Fmoc arom.), 138.1 (2 C, Trypt arom.), 128.8 (2 CH, Fmoc arom.), 128.2 (2 CH, Fmoc arom.), 126.2 (2 CH, Fmoc arom.), 123.6 (CH, Trypt arom.), 122.3 (CH, Trypt arom.), 120.9 (2 CH, Fmoc arom.), 119.6 (CH, Trypt arom.), 119.3 (CH, Trypt arom.), 113.1 (C, Trypt arom.), 112.2 (CH, Trypt arom.), 68.1 (CH_2, Fmoc), 66.2 (d, $J_{C\text{-}P}$ = 5.0 Hz, CH*2*, Cβ Ser), 62.2 (CH, Cα Pro), 54.5 ($J_{C\text{-}P}$ = 7.4 Hz, CH, Cα Ser), 47.7(CH_2, Cδ Pro, under the signal of the solvent, visible at HSQC), 47.3 (CH Fmoc, under the signal of the solvent, visible at HSQC), 41.4 (CH_2, Trypt $CH_2$2), 30.8. (CH_2, Cβ Pro), 26.1 (CH_2, Trypt $CH_2$1), 25.8 (CH_2, Cγ Pro). **^{31}P NMR** (162.0 MHz, MeOD-d4) (2 rotamers 82:18, *minor rotamer): δ : -0.16, -0.61* (s, P(O)OH_2). **^{1}H-NOESY** (400 MHz, MeOD-d4): the major rotamer is in a *trans* configuration (coupling between Hα Ser at 4.75 ppm and Hδ Pro at 3.73 ppm). **HRMS** calcd for $C_{33}H_{36}N_4O_8P$ $[M + H]^+$ m/z = : 647.2271, found m/z = 647.2264. **UPLC-MS**: Eluent - solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/CH_3CN; Method: 0.5 mL/min, from 10% B to 90% B in 3.5 min then 100% B until 5 min; RT : 2.42 min. **MS** calcd for $C_{33}H_{36}N_4O_8P$ [M + H]+ m/z = 647.22, found m/z = 647.31.

2.3.2 Synthesis of 2b



i) 20% Pip/DMF, rt, 1 h (**70%**); ii) Ac_2O, DCM, rt, 16 h (**96%**); iii) HCl 4 N in dioxane, rt, 5 h (**80%**); iv) (a) $P(OtBu)_2N(iPr)_2$, 5-ethylthio-H-tetrazole, THF/DCM 1:1, rt, 16 h (b) tBuOOH, -45 °C to rt, 1 h (c) $Na_2S_2O_3$, -45 °C to rt (**50%** or **95%** based on recovered starting material); v) 20% TFA/DCM, TIPS, rt, 1h (**60%**).

Scheme S4: Synthesis of **2b**.

L-Ser(O*t*Bu)-L-Pro-Tryptamine (S9)

To compound **7** (1.00 g, 1.61 mmol, 1 eq) a solution of 20% piperidine in DMF (10 mL) was added. The resulting mixture was stirred for 1 h at rt. AcOEt was added (100 mL) and the organic layer was washed with H_2O (3 x 50 mL) and brine (1 x 50 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 20%) to give peptide **S9** (451 mg, 1.13 mmol, 70%, *trans-cis* rotamers mixture) as a colorless oil. $\mathbf{R_f}$: 0.10 (DCM/MeOH 90:10). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers 70:30, *minor rotamer when visible) δ:9.16* (bs, 0.3 H, NH Indole Trypt*), 8.06 (bs, 0.7 H, NH Indole Trypt), 7.55 (d, *J* = 8.0 Hz, 1 H, Trypt arom.), 7.32 (d, *J* = 8.0 Hz, 1 H, Trypt arom.) 7.13 (t, *J* = 7.0 Hz, 1 H, Trypt arom.), 7.04 (t, *J* = 7.2 Hz, 1 H, Trypt arom.), 7.02 (m, 2 H, 1 H, Trypt arom. + NH Pro), 4.68* (d, *J* = 8.0 Hz, 1 H, Hα Pro*), 4.58 (d, *J* = 6.1 Hz, 1 H, Hα Pro), 3.76-3.67 (m, 2 Hα Ser + Hδ Pro-Hb), 3.61 (ddd, *J* = 6.7, 13.5, 20.0 Hz, 1 H, Trypt $CH_2$2-Hb), 3.49-3.32 (m, 3 H, Hβ Ser + Trypt $CH_2$2-Ha), 3.27 (t, *J* = 7.1 Hz, 1 H, Hδ Pro-Ha), 2.92 (t, *J* = 7.1 Hz, 2 H, Trypt $CH_2$2), 2.29-2.16 (m, 1 H, Hβ Pro-Hb), 2.02-1.80 (m, 3 H, Hβ Pro-Ha + Hγ Pro), 1.08* (s, 2.7 H, *t*Bu*), 1.04 (s, 6.3 H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 70:30, *minor rotamer when visible) δ: 174.0* (C, CO Pro*), 173.6 (C, CO Pro), 171.9* (C, CO Ser*), 171.5* (C, CO Ser*), 136.4* (C, Trypt arom.*), 136.3 (C, Trypt arom.), 127.4 (C, Trypt arom.), 127.3* (C, Trypt arom.*), 122.6 (CH, Trypt arom.), 122.5* (CH, Trypt arom.*), 122.1* (CH, Trypt arom.*), 121.7 (CH, Trypt arom.), 119.3* (CH, Trypt arom.*), 119.1 (CH, Trypt arom.),118.6 (CH, Trypt arom.), 112.5 (C, Trypt arom.), 112.3* (C, Trypt arom.*), 111.6* (CH, Trypt arom.*), 111.3 (CH, Trypt arom.), 73.7 (C, *t*Bu), 73.3* (C, *t*Bu*), 66.0 (CH_2, Cβ Ser), 65.8* (CH_2, Cβ Ser*), 60.9* (CH, Cα Pro*), 60.5 (CH, Cα Pro), 53.0 (CH, Cα Ser), 52.6* (CH, Cα Ser*), 47.4 (CH_2, Cδ Pro), 46.6* (CH_2, Cδ Pro*), 40.2 (CH_2, Trypt $CH_2$2), 31.2* (CH_2, Cβ Pro*), 28.4 (CH_2, Cβ Pro), 27.4 (3 CH_3, *t*Bu), 27.2* (3 CH_3, *t*Bu*), 25.4 (CH_2, Trypt $CH_2$1), 24.6 (CH_2, Cγ Pro), 22.4* (CH_2, Cγ Pro*). **HRMS** calcd for $C_{22}H_{33}N_4O_3$ $[M + H]^+$ m/z = 401.2553, found m/z = 401.2535.

Ac-L-Ser(O*t*Bu)-L-Pro-Tryptamine (8)

To a solution of unprotected amine **S9** (400 mg, 1.0 mmol, 1 eq) in DCM (30 mL) acetic anydride (0.10 mL, 1.1 mmol, 1.1 eq) was added. The solution stirred at rt for 16 h. AcOEt was added (60 mL) and the organic layer was washed with H_2O (100 mL) and brine (100 mL). The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 20%) to give the acetylated peptide **8** (425 mg, 0.96 mmol, 96%, *trans-cis* rotamers mixture) as a colorless oil. $\mathbf{R_f}$: 0.12 (DCM/MeOH 90:10). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers 70:30, *minor rotamer when visible) δ : 8.66 (bs, 0.7 H, NH Indole Trypt). 8.61* (bs, 0.3 H, NH Indole Trypt*), 7.55 (d, *J* = 7.8 Hz, 1 H, Trypt arom.), 7.33 (d, *J* = 8.0 Hz, 1 H, Trypt arom.), 7.14 (t, *J* = 7.1 Hz, 1 H, Trypt arom.), 7.06 (t, *J* = 7.6 Hz, 1 H, Trypt arom.), 7.02 (s, 1 H, Trypt arom.), 6.92 (t, *J* = 5.8 Hz, 1 H, NH Pro), 6.76 (d, *J* = 7.6 Hz, 0.7 H, NH Ac), 6.46* (d, *J* = 5.6 Hz, 0.3 H, NH Ac*), 4.87 (dd, *J* = 8.3, 13.5 Hz, 0.7 H, Hα Ser), 4.79* (d, *J* = 8.1 Hz, 0.3 H, Hα Ser*), 4.61 (dd, *J* = 2.5, 8.3 Hz, 0.7 H, Hα Pro), 4.44-4.36* (m, 0.3 H, Hα Pro*), 3.86-3.74 (m, 1 H, Hδ Pro-Hb), 3.63 (dd, *J* = 5.9, 8.0 Hz, 1 H, Hβ Ser-Hb), 3.59-3.47 (m, 2 H, Hδ Pro-Ha + Trypt $CH_2$2-Hb), 3.44-3.23 (m, 1 H, Trypt $CH_2$2-Ha), 3.30 (t, *J* = 8.9 Hz, 1 H, Hβ Ser-Ha), 2.92 (t, *J* = 7.1 Hz, 2 H, Trypt $CH_2$1), 2.28-2.14 (m, 1 H, Hβ Pro-Hb), 2.06-1.93 (m, 1 H, Hβ Pro-Ha), 1.98 (s, 2.1 H, Ac), 1.88* (s, 0.9 H, Ac*), 1.94-1.84 (m, 2 H, Hγ Pro), 1.10* (s, 2.7 H, *t*Bu*), 1.06 (s, 6.3 H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 7:3, *minor rotamer where visible) δ: 171.3 (C, CO Pro), 171.0* (C, CO Pro*), 170.7 (C, CO Ser), 170.6* (C, CO Ser), 170.1 (C, CO Ac), 136.4 (C, Trypt arom.), 127.6* (C, Trypt arom.*), 127.4 (C, Trypt arom.), 122.6 (CH, Trypt arom.), 122.3* (CH, Trypt arom.*), 121.8 (CH, Trypt arom.), 119.2 (CH, Trypt arom.), 118.8* (CH, Trypt arom.*), 118.6 (CH, Trypt arom.), 112.9 (C, Trypt arom.), 112.6* (C, Trypt arom.*), 111.3 (CH, Trypt arom.), 111.2* (CH, Trypt arom.*), 74.3 (C, *t*Bu Ser), 73.9* (C, *t*Bu Ser*), 62.9 (CH_2, Cβ Ser), 62.3* (CH_2, Cβ Ser*), 61.1* (CH, Cα Pro*), 60.7 (CH, Cα Pro), 52.3* (CH, Cα Ser*), 51.0 (CH, Cα Ser), 47.9 (CH_2, Cδ Pro), 46.9* (CH_2, Cδ Pro*), 40.3 (CH_2 Trypt CH22), 28.8 (CH_2, Cβ Pro), 27.2 (3 CH_3, *t*Bu), 25.4 (Trypt $CH_2$1), 24.9* (CH_2, Cγ Pro*), 24.5 (CH_2, Cγ Pro), 23.1 (CH_3, Ac), 22.8* (CH_3, Ac*). **HRMS** calcd for $C_{24}H_{35}N_4O_4$ $[M + H]^+$ m/z = 443.2658, found m/z = 443.2653.

Ac-L-Ser(OH)-L-Pro-Tryptamine (S10)

To a solution of protected compound **8** (400 mg, 1.61 mmol, 1 eq) a solution of 4 N HCl in dioxane (40 mL) was added. The reaction stirred at rt for 5 h until the disappearance of the starting material in TLC. The solvent was removed under reduced pressure and the crude alcohol was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 15%) to give peptide **8** (497 mg, 1.26 mmol, 78%, *trans-cis* rotamers mixture) as a white solid. $\mathbf{R_f}$: 0.15 (DCM/MeOH 90:10). **^{1}H NMR** (400 MHz, MeOD) (2 rotamers 80:20, *minor rotamer when visible) δ: 7.90 (t, *J* = 4.9 Hz, 1H, NH), 7.55 (d, *J* = 7.7 Hz, 1 H, Trypt arom.), 7.32 (d, *J* = 8.1 Hz, 1 H, 1 H, Trypt arom.), 7.09 (t, *J* = 7.3 Hz, 1 H, Trypt arom.), 7.06 (s, 1 H, Trypt arom.), 6.99 (t, *J* = 7.4 Hz, 1 H, 1 H, Trypt arom.), 4.74 (t, *J* = 6.9 Hz, 0.7 H, Hα Ser), 4.43 (dd, *J* = 3.4, -8.4 Hz, 0.8 H, Hα Pro), 4.32* (t, *J* = 7.0 Hz, 0.2 H, Hα Ser*), 4.09* (q, *J* = 7.1 Hz, 0.2 H, Hα Pro*), 3.85-3.65 (m, 4

H, Hδ Pro + Hβ Ser), 3.59-3.37 (m, 2 H, Trypt $CH_2$2), 2.92 (t, *J* = 7.2 Hz, 2 H Trypt $CH_2$1), 2.15-2.02 (m, 1 H, Hβ Pro-Hb), 1.97 (s, 2.4 H, Ac), 1.89* (s, 0.6 H, Ac*), 1.89-1.74 (m, 3 H, Hβ Pro-Ha + Hγ Pro). **^{13}C NMR** (400 MHz, MeOD) (2 rotamers 80:20, *minor rotamer when visible) δ: 174.1* (C, CO Pro*), 174.0 (C, CO Pro), 173.3 (C, CO Ser), 172.0 (C, CO OAc), 138.1 (C, Trypt arom.), 129.0* (C, Trypt arom.*), 128.8 (C, Trypt arom.), 123.6 (CH, Trypt arom.), 122.3 (CH, Trypt arom.), 119.6 (CH, Trypt arom.) 119.2 (CH, Trypt arom.), 113.2* (C, Trypt arom.*), 113.0 (C, Trypt arom.), 112.2 (CH, Trypt arom.), 63.1* (CH_2, Cβ Ser*), 61.9* (CH, Cα Pro*), 61.8 (CH, Cα Pro), 54.8* (CH, Cα Ser*), 54.5 (CH, Cα Ser), 48.8 (CH_2, Cδ Pro, under the signal of the solvent, visible at HSQC), 41.4* (Trypt $CH_2$2*) 41.3 (Trypt $CH_2$2), 30.6 (CH_2, Cβ Pro), 25.9 (Trypt $CH_2$1), 25.6* (CH_2, Cγ Pro*), 25.4 (CH_2, Cγ Pro), 23.0* (CH_3, Ac*), 22.2 (CH_3, Ac). **HRMS** calcd for $C_{20}H_{27}N_4O_4$ [M + H]$^+$ m/z = 387.2032, found m/z = 387.2026.

Ac-L-Ser[PO(O*t*Bu)$_2$]-L-Pro-Tryptamine (S11)

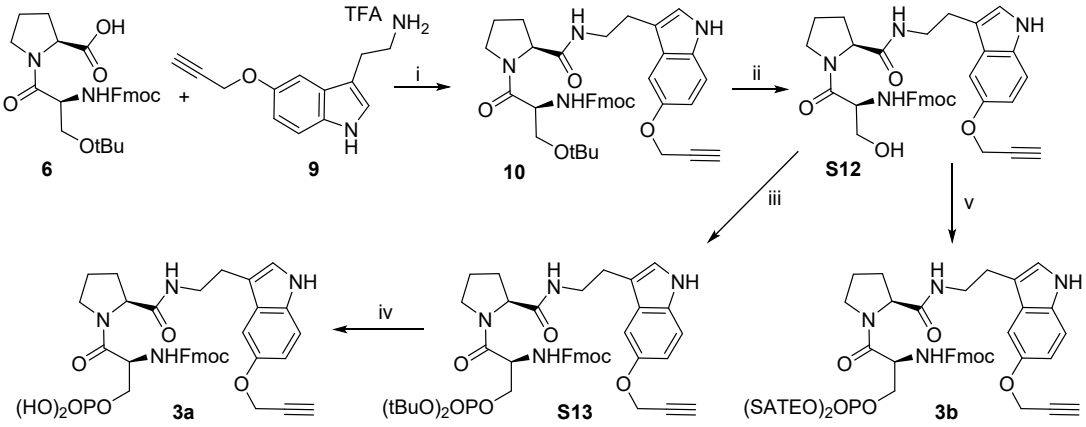
To a solution of **S10** (300 mg, 0.78 mmol, 1 eq) in DCM/THF (1:1, 20 mL) was added 5-ethylthio–H–tetrazole (203 mg, 1.56 mmol, 2 eq) and the mixture was stirred for 30 min at rt. Di*tert*-butyl-diisopropyl-phosphoramidite (0.49 mL, 1.56 mmol, 2 eq) was added and the mixture was stirred at rt for 15 h. The mixture was cooled to -45 °C, and a solution of terbuthyl-hydroxyperoxide (6M in decane, 0.52 mL, 3.12 mmol, 4 eq) was added dropwise. The mixture was stirred at -45 °C for 1 h and at rt for 30 minutes, then it was cooled down to -45 °C and quenched with asaturated aq solution of $Na_2S_2O_3$ (40 mL). The layers were separeted in a separatory funnel and the acqueus phase was extracted with DCM (40 mL x 2). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was rapidly filtered on a short pad of silica gel (100% AcOEt) to give compound **S11** as a pale-yellow oil (50% yield or 95% yield based on recovered starting material). Since the purification on silica showed to be tricky (partially deprotection of one of the *t*Bu group occurs in parallel with the co-elution of the reduced phosphoyramidite) the product was used crude for the next step without further purification. **R_f** : 0.20 (DCM/MeOH 90:10). **^{31}P NMR** (162.0 MHz, $CDCl_3$) (2 rotamers 80:20, *minor rotamer) δ : -10.43, -11.18* (s, P(O)O*t*Bu_2).

Ac-L-Ser[OPO(OH)$_2$]-L-Pro-Tryptamine (2b)

To a solution of protected compound **S11** (181 mg, 0.39 mmol, 1 eq) 10 mL of a solution of 20% TFA in DCM, few drops of TIPS and few drops of H_2O were added. The reaction stirred at rt for 1 hour, the solvent was removed under reduced pressure and the residue was purified by semi-preparative HPLC. Eluent: solvent A 0.1% TFA/H2O, solvent B 0.1% TFA/CH_3CN; Method: 4 mL/min, from 10% B to 70% B in 17 min then 100% B until 25 min; RT : 7.68 min) to provide **2b** as a white solid after lyophilization (128 mg, 0.23 mmol, 60% after HPLC purification). **^{1}H NMR** (400 MHz, MeOD-d4) (2 rotamers 83:17, *minor rotamer when visible) δ : 7.58 (d, *J* = 7.7 Hz, 1 H, Trypt arom.), 7.32 (d, *J* = 8.1 Hz, 1 H, Trypt arom.), 7.09 (s, 1 H, Trypt arom.), 7.07 (t, *J* = 7.7 Hz, 1 H, Trypt arom.), 6.99 (t, *J* = 7.4 Hz, 1 H, Trypt arom.), 4.93 (0.83 H, Hα Ser, under the signal of the solvent, visible at HSQC), 4.64* (dd, *J* = 6.4, 3.6 Hz, 0.17 H, Hα Pro*), 4.52* (t, *J* = 7.1 Hz, 0.17 H, Hα Ser*), 4.36 (dd, *J* = 8.3, 5.0 Hz, 0.83 H, Hα Pro), 4.23 (dt, *J* = 13.5, 6.8 Hz, 0.83 H, Hβ Ser-Hb), 4.16 (dt, *J* = 13.5, 7.7 Hz, 0.83 H, Hβ Ser-Hb), 4.00* (t, *J* = 7.0 Hz, 0.34 H, Hβ Ser*), 3.85-3.77 (m, 0.83 H, Hδ Pro-Hb), 3.77-3.70 (m, 0.83 H, Hδ Pro-Ha), 3.57-3.50* (m, 0.34 H, Hδ Pro*), 3.50-3.42 (m, 2 H, Trypt $CH_2$2), 3.07-2.99* (m, 0.34 H, Trypt $CH_2$1*), 2.95 (td, *J* = 7.2, 2.3 Hz, 1.66 H, Trypt $CH_2$1), 2.19-2.07 (m, 1 H, Hβ Pro-Hb), 1.99 (s, 2.49 H, Ac), 1.93 (dd, *J* = 13.3, 6.5 Hz, 2 H, Hγ Pro), *1.89 (s, 0.51 H, Ac), 1.82 (td, *J* = 11.8, 5.7 Hz, 1 H, Hβ Pro-Ha). **^{13}C NMR** (100.5 MHz, MeOD-d4) (2 rotamers 83:17, * minor rotamer when visible) δ : 174.3 (C, CO Pro), 173.5* (C, CO Pro*), 173.3 (C, CO Ser), 172.9* (C, CO Ser), 170.6* (C, CO Ac*), 170.1 (C, CO Ac), 138.1 (C, Trypt arom.), *129.0 (C, Trypt arom.*), 128.8 (C, Trypt arom.), 123.6 (CH, Trypt arom.), 122.3* (CH, Trypt arom.*), 122.2 (CH, Trypt arom.), 119.6* (CH, Trypt arom.*), 119.5 (CH, Trypt arom.), 119.3 (CH, Trypt arom.), 119.*2 (CH, Trypt arom.*), 113.2* (C, Trypt arom.*), 113.1 (C, Trypt arom.), 112.2* (CH, Trypt arom.), 112.1 (CH, Trypt arom.), 66.4* (d, $J_{C\text{-}P}$ = 5.0 Hz, CH_2, Cβ Ser*), 66.1 (d, $J_{C\text{-}P}$ = 5.0 Hz, CH_2, Cβ Ser), 62.1 (CH, Cα Pro), 53.1 (d, $J_{C\text{-}P}$ = 7.2 Hz, CH, Cα Ser), 52.7* (d, $J_{C\text{-}P}$ = 8.7 Hz, CH, Cα Ser*), 48.9 (CH, Cδ Pro under the signal of the solvent, visible at HSQC), 48.1* (CH, Cδ Pro* under the signal of the solvent, visible at HSQC), 41.8* (CH_2, Trypt $CH_2$2*), 41.4 (CH_2, Trypt $CH_2$2), 32.5* (CH_2, Cβ Pro*), 30.8 (CH_2, Cβ Pro), 26.1 (CH_2, Trypt $CH_2$1), 25.8 (CH_2, Cγ Pro), 25.6* (CH_2, Trypt $CH_2$1*), 23.1* (CH_2, Cγ Pro*), 22.3 (3 CH_3, Ac), 22.2* (3 CH_3, Ac*). **^{31}P NMR**(162.0 MHz, MeOD-d4) (2 rotamers 82:18, *minor rotamer): δ : -0.70, -0.23* (s, P(O)OH_2). **^{1}H-NOESY**(400 MHz, MeOD-d4): The major rotamer (83%) is in a *trans* configuration (coupling between Hα Ser at 4.93 ppm and Hδ Pro at 3.75-3.79 ppm). The minor rotamer (17%) is in a *cis* configuration (coupling between Hα Ser*at 4.52 ppm and Hα Pro* at 4.6 ppm). **HRMS** calcd for $C_{20}H_{28}N_4O_7P$ [M + H]$^+$ m/z = 467.1696, found m/z = 467.1688. **UPLC-MS**: Eluent – solvent A 0.1% TFA/H2O, solvent B 0.1% TFA/CH_3CN; Method: 0.5 mL/min, from 10% B to 90% B in 3.5 min then 100% B until 5 min; RT : 1.46 min. **MS** calcd for $C_{33}H_{36}N_4O_8P$ [M + H]$^+$ m/z = : 467.17, found m/z = 467.25.

2.4 Synthesis of *C*-Terminal Alkyne Pin1 Ligands (3a-3d)

2.4.1 Synthesis of 3a and 3b



i) DMAP, EDC, HOBt, DMF, rt, 4 h (**80%**); ii) HCl 4N in dioxane, rt 5 h (**76%**); iii) $P(OtBu)_2N(iPr)_2$, ETT then tBuOOH (**79%** or **90%** based on recovered starting material); iv) 20% TFA/DCM, TIPS, H_2O, rt, 1h (**62%**); v) (a) $P(OSATE)_2N(iPr)_2$, 5-ethylthio-H-tetrazole THF/DCM 1:1, rt, 16 h, (b) *t*BuOOH, -45 °C to rt, 1h (c) $Na_2S_2O_3$, -45 °C to rt (**80%** or **90%** based on recovered starting material).

Scheme S5: Synthesis of **3a** and **3b**.

Fmoc-L-Ser(OtBu)-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (10)

The crude carboxylic acid **6** (3.36 g, 6.99 mmol, 1.2 eq) and the alkylated serotonine **9** (1.9 g, 5.83 mmol, 1.0 eq) were dissolved in DMF (50 mL) then HOBt (1.07 g, 6.99 mmol, 1.2 eq) DIPEA (0.99 mL, 5.83 mmol, 1.2 eq), and EDC (1.34 g, 6.99 mmol, 1.2 eq) were added. The mixture was stirred at rt for 16 h, diluted with AcOEt (200 mL), washed with water (3 x 100 mL), and brine (100 mL). The organic layer was dried over $MgSO_4$. After filtration and evaporation under reduced pressure, the residue was purified by flash chromatography on silica gel (cyHex/AcOEt step gradient from 10% AcOEt to 50%) to give the pseudopeptide **10** (3.16 g, 4.66 mmol, 80% in 2 steps, *trans-cis* rotamers mixture) as a pale-yellow solid. $\mathbf{R_f}$: 0.38 (cyHex/AcOEt 50:50). **^{1}H NMR** (400. MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ: 8.47 (bs, 1H, N*H* Indole), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.58 (d, *J* = 7.4 Hz, 2H, Fmoc arom), 7.40 (t, *J* = 7.5 Hz, 2H, Fmoc arom), 7.31 (t, *J* = 7.4 Hz, 2H, Fmoc arom), 7.24 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.11 (d, *J* = 2.4 Hz, 1H, Tryp arom), 7.03 (s, 1H, Tryp arom), 6.95 (t, *J* = 5.7 Hz, 1H, N*H* Tryp), 6.88 (dd, *J* = 8.7, 2.4 Hz, 1H, Tryp arom), 5.91 (d, *J* = 8.1 Hz, 1H, N*H* Ser), 4.71 (d, *J* = 2.4 Hz, 2H, CH_2O), 4.71-4.59 (m, 2H, H^α Pro + H^α Ser), 4.41-4.37 (m, 2H, CH_2 Fmoc), 4.20 (t, *J* = 7.0 Hz, 1H, C*H* Fmoc), 3.88-3.78 (m, 1H, H^δ Pro-Ha), 3.72-3.60 (m, 2H, H^β Ser-Ha + CH_2CH_2NH-Ha), 3.60-3.46 (m, 1H, H^δ Pro-Hb), 3.40-3.31 (m, 1H, CH_2CH_2NH-Hb), 3.28 (t, *J* = 8.9 Hz, 1H, H^β Ser-Hb), 2.99-2.83 (m, 2H, CH_2CH_2NH), 2.50 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.32-2.20 (m, 1H, H^β Pro-Ha), 2.04-1.81 (m, 3H, H^β Pro-Hb + 2H x H^γ Pro), 1.0 (s, 9 H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 80:20, major rotamer reported) δ: 171.2 (C, CO Pro). 170.6 (C, CO Ser), 155.9 (C, CO Fmoc), 151.8 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 143.7 (C, Fmoc arom.), 141.3 (C, Fmoc arom.), 132.1 (C, Tryp arom.), 127.8 (2 x CH, Fmoc arom.), 127.7 (C, Tryp arom.), 127.1 (2 x CH, Fmoc arom.), 125.1 (2 x CH, Fmoc arom.), 123.9 (CH, Tryp arom.), 120.1 (2 x CH, Fmoc arom.), 112.7 (CH, Tryp arom.), 112.5 (C, Tryp arom.), 112.0 (CH, Tryp arom.), 102.8 (1CH, Tryp arom.), 79.4 (C, *C*CH), 75.2 (CH, C*C*H), 74.3 (C, *t*Bu), 67.2 (CH_2, Fmoc), 63.3 (CH_2, C^β Ser), 60.6 (CH, C^α Pro), 57.1 (CH_2, *C*H_2O), 52.4 (CH, C^α Ser), 47.9 (CH_2, C^δ Pro), 47.1 (CH, Fmoc), 40.1 (CH_2, CH_2CH_2NH), 28.7 (CH_2, C^β Pro), 27.2 (3 x CH_3, *t*Bu), 25.4 (CH_2, *CH*$_2$CH$_2$NH), 24.5 (CH_2, C^γ Pro). **HRMS** calcd. for $C_{40}H_{45}N_4O_6$ $[M + H]^+$ *m/z* = 677.3339, found *m/z* = 677.3343.

Fmoc-L-Ser(OH)-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (S12)

To a solution of protected compound **10** (720 mg, 1.06 mmol, 1 eq) a solution of 4 N HCl in dioxane (40 mL) was added. The reaction stirred at rt for 5 h until the disappearance of the starting material in TLC. The solvent was removed under reduced pressure and the crude alcohol was purified by flash chromatography on silica gel (cyHex/AcOEt step gradient from 10% AcOEt to 60%) to give peptide **S12** (502.7 mg, 0.81 mmol, 76%, *trans-cis* rotamers mixture) as a pale pink solid. $\mathbf{R_f}$: 0.34 (cyHex/AcOEt 50:50). **^{1}H NMR** (400.0 MHz, $CDCl_3$) (2 rotamers 90:10, major rotamer reported) δ: 8.29 (bs, 1H, N*H* Indole), 7.66 (d, *J* = 7.3 Hz, 2H, Fmoc arom), 7.47 (d, *J* = 7.4 Hz, 2H, Fmoc arom), 7.30 (t, *J* = 7.3 Hz, 2H, Fmoc arom), 7.19 (t, *J* = 7.4 Hz, 2H, Fmoc arom), 7.12 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.01 (s, 1H, Tryp arom), 6.86 (s, 1H, Tryp

arom), 6.80 (dd, *J* = 8.8, 2.1 Hz ,1H, Tryp arom), 6.6 (bs, 1H, N*H* Tryp), 5.91 (d, *J* = 7.8 Hz, 1H, N*H* Ser), 4.62 (d, *J* = 1.9, 2H, Hz, CH_2O), 4.58-4.49 (m, 1H, H$^{\alpha}$ Ser), 4.37 (t, *J* = 6.0 Hz, 1H, H$^{\alpha}$ Pro), 4.28 (t, *J* = 6.2 Hz, 2H, CH_2 Fmoc), 4.09 (t, *J* = 6.8 Hz, 1H, C*H* Fmoc), 3.73-3.63 (m, 1H, H$^{\beta}$ Ser-Ha), 3.63-3.50 (m, 1H, H$^{\delta}$ Pro-Ha), 3.50-3.29 (m, 4H, H$^{\beta}$ Ser-Hb + H$^{\delta}$ Pro-Hb + 2H x CH_2CH_2NH), 2.75 (t, *J* = 6.3 Hz, 2H, CH_2CH_2NH), 2.44 (t, *J* = 2.1 Hz, 1H, CC*H*), 1.95-1.82 (m, 2H, H$^{\beta}$ Pro), 1.82-1.64 (m, 2H, H$^{\gamma}$ Pro). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 90:10, major rotamer reported) δ: 171.7 (C, CO Pro). 170.3 (C, CO Ser), 156.0 (C, CO Fmoc), 151.8 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 143.7 (C, Fmoc arom.), 141.3 (C, Fmoc arom.), 132.0 (C, Tryp arom.), 127.8 (2 x CH, Fmoc arom.), 127.6 (C, Tryp arom.), 127.1 (2 x CH, Fmoc arom.), 125.1 (CH, Fmoc arom.), 125.0 (CH, Fmoc arom.), 123.6 (CH, Tryp arom.), 120.1 (2 x CH, Fmoc arom.), 112.7 (CH, Tryp arom.), 112.4 (C, Tryp arom.), 112.1 (CH, Tryp arom.), 102.9 (CH, Tryp arom.), 79.3 (C, *C*CH), 75.4 (CH, C*C*H), 67.1 (CH_2, Fmoc), 63.8 (CH_2, C$^{\beta}$ Ser), 60.6 (CH, C$^{\alpha}$ Pro), 60.0 (CH_2, *C*H_2O), 53.6 (CH, C$^{\alpha}$ Ser), 47.7 (CH_2, C$^{\delta}$ Pro), 47.1 (CH, Fmoc), 39.7 (CH_2, CH_2CH_2NH), 29.2 (CH_2, C$^{\beta}$ Pro), 24.9 (CH_2, CH_2CH_2NH), 24.7 (CH_2, C$^{\gamma}$ Pro). **HRMS** calcd. for $C_{36}H_{37}N_4O_6$ [M + H]$^+$ *m/z* = 621.2713, found *m/z* = 621.2717.

Fmoc-L-Ser[OPO(OtBu)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (S13)

To a solution of **S12** (150 mg, 0.27 mmol, 1.0 eq) in DCM/THF (1:1, 12 mL) was added ETT (70.3 mg, 0.54 mmol, 2.0 eq) and the mixture was stirred for 30 min at rt. *i*Pr$_2$N-P(O*t*Bu)$_2$ (0.17 mL, 0.54 mmol, 2.0 eq) was added and the mixture was stirred at rt for 16 h. The mixture was cooled to -45°C, and a solution of *t*BuOOH (6M in decane, 0.25 mL, 1.08 mmol, 4 eq) was added dropwise. The mixture was stirred at -45°C for 1 h and at rt for 30 minutes, then it was cooled down to -45°C and quenched with saturated aq solution of $Na_2S_2O_3$ (20 mL). The layers were separated in a separatory funnel and the aqueous phase was extracted with DCM (40 mL x 2). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was rapidly columned on a short pad of silica gel (100% AcOEt) to give compound **S13** as a pale-yellow oil (79% yield or 90% yield based on recovered starting material). Since the purification on silica showed to be tricky (partially deprotection of one of the *t*Bu group occurs in parallel with the co-elution of the reduced phosphoyramidite) the product was used crude for the next step without further purification. **^{31}P NMR** (162 MHz, $CDCl_3$) (2 rotamers 90:10, *minor rotamer) δ: –9.55, –10.08* (s, PO(O*t*Bu)$_2$).

Fmoc-L-Ser[OPO(OH)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (3a)

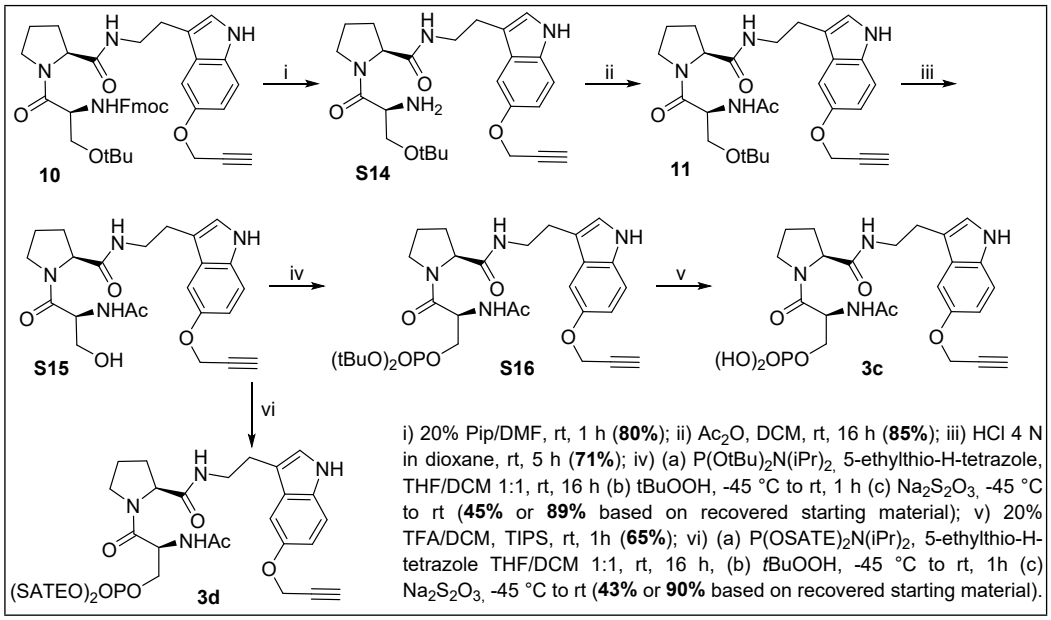
To a solution of protected compound **S13** (30 mg, 0.0395 mmol, 1 eq) 60 mL of a solution of 20% TFA in DCM, few drops of TIPS and few drops of H_2O were added. The reaction stirred at rt for 1 hour, the solvent was removed under reduced pressure and the residue was purified by semi-preparative HPLC. Eluent: solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/CH_3CN; Method: 4 mL/min, from 10% B to 65% B in 22 min then 100% B until 25 min; RT : 19.7 min) to provide **3a** as a white solid after lyophilization (17.4 mg, 0.0248 mmol, 62% after HPLC purification). **^{1}H NMR** (400 MHz, MeOD-*d$_4$*) (2 rotamers, major rotamer reported) δ: 7.79 (d, *J* = 7.5 Hz, 2H, Fmoc arom.), 7.65 (d, *J* = 7.4 Hz, 2H, Fmoc arom.), 7.39 (t, *J* = 7.4 Hz, 2H, Fmoc arom.), 7.31 (t, *J* = 7.4 Hz, 2H, Fmoc arom.), 7.23 (d, *J* = 8.8 Hz ,1H, Tryp arom.), 7.17 (d, *J* = 2.3 Hz, 1H, Tryp arom.), 7.08 (s, 1H, Tryp arom.), 6.88 (dd, *J* = 8.8, 2.4 Hz, 1H, Tryp arom.), 4.79-4.72 (m, 1H, H$^{\alpha}$ Ser), 4.72 (d, *J* = 2.4 Hz, 2H, C*H*$_2$O), 4.42-4.30 (m, 3H, H$^{\alpha}$ Pro + 2H x C*H*$_2$ Fmoc), 4.25-4.12 (m, 3H, C*H* Fmoc + 2H x H$^{\beta}$ Ser), 3.76-3.69 (m, 2H, H$^{\delta}$ Pro), 3.54-3.42 (m, 2H, CH_2CH_2NH), 3.29-2.88 (m, 3H, 2H x CH_2CH_2NH + CC*H*), 2.20-2.06 (m, 1H, H$^{\beta}$ Pro-Ha), 1.96-1.87 (m, 2H, H$^{\gamma}$ Pro), 1.87-1.79 (m, 1H, H$^{\beta}$ Pro-Hb). **^{13}C NMR** (100.5 MHz, MeOD-*d$_4$*) (2 rotamers, major rotamer reported) δ: 173.0 (C, CO Pro). 169.1 (C, CO Ser), 156.9 (C, CO Fmoc), 151.6 (C, Fmoc arom.), 143.8 (C, Fmoc arom.), 141.2 (C, Fmoc arom.), 132.5 (2 x C, Fmoc arom.), 127.6 (C, Tryp arom.), 127.4 (2 x CH, Fmoc arom.), 126.8 (2 x CH, Fmoc arom.), 124.8 (2 x CH, Fmoc arom.), 123.3 (CH, Tryp arom.), 119.5 (2 x CH, Fmoc arom.), 111.8 (CH, Tryp arom.), 111.6 (C, Tryp arom.), 111.4 (CH, Tryp arom.), 102.3 (CH, Tryp arom.), 79.3 (C, *C*CH), 74.8 (CH, C*C*H), 66.7 (CH_2, Fmoc), 64.8 (d, $J_{C\text{-}P}$ =5.4 Hz, CH_2, C$^{\beta}$ Ser), 60.8 (CH, C$^{\alpha}$ Pro), 56.4 (CH_2, *CH*$_2$O), 53.2 (d, $J_{C\text{-}P}$ = 7.3 Hz, CH, C$^{\alpha}$ Ser), 47.6 (CH_2, C$^{\delta}$ Pro - under the signal of the solvent), 47.6 (CH, Fmoc - under the signal of the solvent), 39.9 (CH_2, CH_2CH_2NH), 29.4 (CH_2, C$^{\beta}$ Pro), 24.7 (CH_2, CH_2CH_2NH), 24.4 (CH_2, C$^{\gamma}$ Pro). **^{31}P NMR** (162.0 MHz, MeOD-d4) (2 rotamers 80:20, *minor rotamer) δ: –0.19, –0.58* (s, PO(OH)$_2$). **HRMS** calcd. for $C_{36}H_{37}N_4O_9P$ [M + H]$^+$ *m/z* = 701.2371, found *m/z* = 701.2374.

Fmoc-L-Ser[OPO(OSATE)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (3b)

To a solution of **S12** (143 mg, 0.23 mmol, 1 eq) in DCM/THF (1:1, 12 mL), ETT (60 mg, 0.46 mmol, 2 eq) was added and the mixture was stirred for 30 min at rt. Phosphoramidite **S5** (209 mg, 0.46 mmol, 2 eq) was added and the mixture was stirred at rt for 16 h. The mixture was cooled to -45°C, and a solution of *t*BuOOH (6M in decane, 0.23 mL, 0.92 mmol, 4 eq) was added dropwise. The mixture was stirred at -45°C for 1 h and at rt for 30 minutes, then it was cooled down to -45°C and quenched with a saturated aq. solution of $Na_2S_2O_3$ (20 mL). The layers were separated in a separatory funnel and the aqueous phase was extracted with DCM (40 mL x 2). The combined organic layers were washed with brine (40 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash

chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%) to give pseudopeptide **3b** (182 mg, 0.184 mmol, 80% or 90% based on recovered starting material, *trans-cis* rotamers mixture) as a pale, yellow oil. $\mathbf{R_f}$: 0.43 (DCM/MeOH 95:5). **^{1}H NMR** (400.0 MHz, $CDCl_3$) (rotamers, major rotamer reported) δ: 8.74 (bs, 1H, N*H* Indole), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.59 (dd, *J* = 7.3, 3.8 Hz, 2H, Fmoc arom), 7.40 (t, *J* = 7.4 Hz, 2H, Fmoc arom), 7.30 (t, *J* = 7.4 Hz, 2H, Fmoc arom), 7.26 (d, *J* = 8.7 Hz ,1H, Tryp arom), 7.16 (d, *J* = 2.2 Hz, 1H, Tryp arom), 7.06 (d, *J* = 1.8 Hz, 1H, Tryp arom), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H, Tryp arom), 6.84 (t, *J* = 5.4 Hz, 1H, N*H* Tryp), 6.89 (d, *J* = 8.3 Hz, 1H, N*H* Ser), 4.74 (d, *J* = 7.1 Hz, 2H, CH_2O), 4.73-4.69 (m, 1H, H$^{\alpha}$ Ser), 4.56 (d, *J* = 5.8 Hz, 1H, H$^{\alpha}$ Pro), 4.37 (dd, *J* = 7.1, 4.7 Hz, 2H, CH_2 Fmoc), 4.21 (t, *J* = 7.1 Hz, 1H, C*H* Fmoc), 4.15-4.02 (m, 6H, 4H x OCH_2CH$_2$S + 2H x H$^{\beta}$ Ser), 3.69-3.57 (m, 1H, CH$_2$CH_2NH-Ha), 3.57-3.42 (m, 3H, CH$_2$CH_2NH-Hb + 2H x H$^{\delta}$ Pro), 3.18-3.05 (m, 4H, OCH$_2$CH_2S), 3.01-2.86 (m, 2H, CH_2CH$_2$NH), 2.52 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.33-2.56 (m, 1H, H$^{\beta}$ Pro-Ha), 1.96-1.86 (m, 2H, H$^{\gamma}$ Pro), 1.80-1.74 (m, 1H, H$^{\beta}$ Pro-Hb), 1.21 (s, 9H, *t*Bu), 1.20 (s, 9H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (rotamers, major rotamer reported) δ: 205.8 (C, CO SATE), 205.9 (C, CO SATE), 170.5 (C, CO Pro). 168.4 (C, CO Ser), 155.8 (C, CO Fmoc), 151.8 (C, Fmoc arom), 143.7 (C, Fmoc arom), 143.6 (C, Fmoc arom), 141.3 (2 x C, Fmoc arom), 132.1 (C, Tryp arom), 127.8 (2 x CH, Fmoc arom), 127.1 (2 x CH, Fmoc arom), 125.2 (2 x CH, Fmoc arom.), 123.3 (CH, Tryp arom.), 120.1 (2 x CH, Fmoc arom), 112.7 (CH, Tryp arom), 112.6 (C, Tryp arom), 112.1 (CH, Tryp arom), 102.9 (CH, Tryp arom), 79.4 (C, *C*CH), 75.2 (CH, C*C*H), 67.4 (CH$_2$, Fmoc), 66.6 (d, $J_{C\text{-}P}$ =5.9 Hz, 2 x CH$_2$, OCH_2CH$_2$S), 61.9 (d, $J_{C\text{-}P}$ =5.5 Hz, CH$_2$, C$^{\beta}$ Ser), 61.0 (CH, C$^{\alpha}$ Pro), 56.9 (CH$_2$, *C*H$_2$O), 52.7 (d, $J_{C\text{-}P}$ =6.5 Hz, CH, C$^{\alpha}$ Ser), 47.6 (CH$_2$, C$^{\delta}$ Pro), 47.0 (CH, Fmoc), 46.6 (2 x C, *t*Bu), 39.7 (CH$_2$, CH$_2$*C*H$_2$NH), 28.5 (d, $J_{C\text{-}P}$ =3.9 Hz, CH$_2$, OCH$_2$*C*H$_2$S), 28.5 (d, $J_{C\text{-}P}$ = 3.9 Hz, CH$_2$, OCH$_2$*C*H$_2$S), 28.3 (CH$_2$, C$^{\beta}$ Pro), 27.3 (6 x CH$_3$, *t*Bu), 24.9 (CH$_2$, *C*H$_2$CH$_2$NH), 24.8 (CH$_2$, C$^{\gamma}$ Pro). **^{31}P NMR** (162.0 MHz, $CDCl_3$) (2 rotamers 90:10, *minor rotamer) δ: –1.44, –1.48* (s, PO(OSATE)$_2$). **HRMS** calcd. for $C_{50}H_{62}N_4O_{11}PS_2$ $[M + H]^+$ *m/z* = 989.3594, found *m/z* =989.3572.

2.4.2 Synthesis of 3c and 3d



Scheme S6: Synthesis of **3c** and **3d**.

L-Ser(OtBu)-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (S14)

Compound **10** (3.67 g, 5.42 mmol, 1 eq) was dissolved in a solution of 20% piperidine in DMF (50 mL). The resulting mixture was stirred for 1 h at rt. AcOEt was added (200 mL) and the organic layer was washed with H_2O (3 x 100 mL) and brine (1 x 100 mL). The organic extracts were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 10%) to give peptide **S14** (1.97 g, 4.33 mmol, 80%, *trans-cis* rotamers mixture) as a pale, yellow oil. $\mathbf{R_f}$: 0.15 (DCM/MeOH 95:5). **^{1}H NMR** (400.0 MHz, $CDCl_3$) (2 rotamers 75:25, major rotamer reported) δ: 9.14 (bs, 1 H, N*H* Indole), 7.19 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.05 (d, *J* = 1.9 Hz, 1H, Tryp arom), 7.00-6.92 (m, 2H, Tryp arom + N*H* Tryp), 6.81 (dd, *J* = 8.8, 2.2 Hz, 1H, Tryp arom), 4.65 (d, *J* = 2.2 Hz, 2H, CH_2O), 4.52 (d, *J* = 2.2 Hz, 1H, H$^{\alpha}$ Pro), 3.73-3.63 (m, 2H, H$^{\alpha}$ Ser + H$^{\delta}$ Pro-Ha), 3.62-3.51 (m, 1H, CH$_2$CH_2NH-Ha), 3.44-3.27 (m, 3H, H$^{\delta}$ Pro-Hb + CH$_2$CH_2NH-Hb + H$^{\beta}$ Ser-Ha), 3.24 (t, *J* = 8.3 Hz, 1H, H$^{\beta}$ Ser-Hb), 2.84 (t, *J* = 7.0 Hz, 2H, CH_2CH$_2$NH), 2.49 (t, *J* = 2.3 Hz, 1H, CC*H*), 2.20-2.10 (m, 1H, H$^{\beta}$ Pro-Ha), 1.96-1.80 (m, 5H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro + 2H x NH$_2$), 1.0 (s, 9H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 75:25, major rotamer

reported) δ: 173.5 (C, CO Pro). 171.5 (C, CO Ser), 151.6 (C, Tryp arom), 132.2 (C, Tryp arom), 127.6 (C, Tryp arom), 123.8 (CH, Tryp arom), 112.5 (CH, Tryp arom), 112.2 (C, Tryp arom), 112.1 (CH, Tryp arom), 102.7 (CH, Tryp arom), 79.3 (C, *C*CH), 75.2 (CH, C*C*H), 73.7 (C, *t*Bu), 65.9 (CH_2, C^{β} Ser), 60.5 (CH, C^{α} Pro), 57.0 (CH_2, *C*H_2O), 52.9 (CH, C^{α} Ser), 47.4 (CH_2, C^{δ} Pro), 40.1 (CH_2, CH_2*C*H_2NH), 28.6 (CH_2, C^{β} Pro), 27.3 (3 x CH_3, *t*Bu), 25.4 (CH_2, *C*$H_2$$CH_2$NH), 24.6 ($CH_2$, C^{γ} Pro). **HRMS** calcd. for $C_{25}H_{35}N_4O_4$ $[M + H]^+$ *m/z* = 455.2658, found *m/z* = 455.2643.

Ac-Ser(OtBu)-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (11)

To a solution of unprotected amine **S14** (1.43 g, 3.15 mmol, 1 eq) in DCM (85 mL), acetic anhydride (0.33 mL, 3.47 mmol, 1.1 eq) was added. The solution was stirred at rt for 16 h. AcOEt was added (200 mL) and the organic layer was washed with H_2O (100 mL) and brine (100 mL). The organic extracts were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 10%) to give the acetylated peptide **11** (1.33 mg, 2.68 mmol, 85%, *trans-cis* rotamers mixture) as a colourless oil. $\mathbf{R_f}$: 0.17 (DCM/MeOH 95:5). **^{1}H NMR** (400.0 MHz, $CDCl_3$) (2 rotamers 75:25, major rotamer reported) δ: 8.32 (bs, 1H, N*H* Indole), 7.25 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.11 (d, *J* = 2.3 Hz, 1H, Tryp arom), 7.04 (d, *J* = 2.1 Hz, 1H, Tryp arom), 6.89 (dd, *J* = 8.8, 2.3 Hz, 2H, Tryp arom + N*H* Tryp), 6.56 (d, *J* = 7.7 Hz, 1H, N*H* Ser), 4.86 (td, *J* = 8.4, 5.5 Hz, 1H, H^{α} Ser), 4.73 (d, *J* = 2.3 Hz, 2H, C*H*$_2$O), 4.63 (dd, *J* = 8.3, 2.6 Hz, 1H, H^{α} Pro), 3.89-3.81 (m, 1H, H^{δ} Pro-Ha), 3.71-3.53 (m, 3H, H^{δ} Pro-Hb + CH_2C*H*$_2$NH-Ha + H^{β} Ser-Ha), 3.41-3.29 (m, 1H, CH_2C*H*$_2$NH-Hb), 3.24 (t, *J* = 8.8 Hz, 1H, H^{β} Ser-Hb), 2.90 (t, *J* = 7.0 Hz, 2H, C*H*$_2$$CH_2$NH), 2.51 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.30-2.21 (m, 1H, H^{β} Pro-Ha), 2.02-1.88 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.99 (s, 3H, Ac), 1.0 (s, 9H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 75:25, major rotamer reported) δ: 171.2 (C, CO Pro). 170.6 (C, CO Ser), 169.9 (CO, Ac), 151.8 (C, Tryp arom), 132.1 (C, Tryp arom), 127.7 (C, Tryp arom), 123.5 (C, Tryp arom), 112.7 (CH, Tryp arom), 111.9 (CH, Tryp arom), 102.8 (CH, Tryp arom), 79.3 (C, *C*CH), 75.1 (CH, C*C*H), 74.3 (C, *t*Bu), 62.9 (CH_2, C^{β} Ser), 60.6 (CH, C^{α} Pro), 57.0 (CH_2, *C*H_2O), 52.9 (CH, C^{α} Ser), 47.9 (CH_2, C^{δ} Pro), 40.1 (CH_2, CH_2*C*H_2NH), 28.7 (CH_2, C^{β} Pro), 27.2 (3 CH_3, *t*Bu), 25.4 (CH_2, *C*$H_2$$CH_2$NH), 24.5 ($CH_2$, C^{γ} Pro), 23.1 (CH_3, Ac). **HRMS** calcd. for $C_{27}H_{37}N_4O_5$ $[M + H]^+$ *m/z* = 497.2764, found *m/z* = 497.2747.

Ac-L-Ser(OH)-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (S15)

To a solution of protected compound **11** (160 mg, 0.32 mmol, 1 eq) a solution of 4 N HCl in dioxane (16 mL) was added. The reaction stirred at rt for 5 h until the disappearance of the starting material in TLC. The solvent was removed under reduced pressure and the crude alcohol was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 10%) to give peptide **S15** (100 mg, 0.22 mmol, 71%, *trans-cis* rotamers mixture) as a pale-yellow solid. $\mathbf{R_f}$: 0.15 (DCM/MeOH 90:10). **^{1}H NMR** (400.0 MHz, CD_3OD) (2 rotamers 85:15, major rotamer reported) δ: 7.81 (bs, 1H, N*H* Indole), 7.13 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.04 (d, *J* = 2.3 Hz, 1H, Tryp arom), 6.96 (s, 1H, Tryp arom), 6.71 (dd, *J* = 8.8, 2.4 Hz, 1H, Tryp arom), 4.70-4.63 (m, 1H, H^{α} Ser), 4.62 (d, *J* = 2.4 Hz, 2H, C*H*$_2$O), 4.33 (dd, *J* = 8.3, 3.8 Hz, 1H, H^{α} Pro), 3.72-3.27 (m, 6H, 2H x H^{β} Ser + 2H x H^{δ} Pro + 2H x CH_2C*H*$_2$NH), 2.83-2.75 (m, 3H, 2H x C*H*$_2$$CH_2$NH + CC*H*), 2.06-1.95 (m, 1H, H^{β} Pro-Ha), 1.87 (s, 3H, Ac), 1.82-1.65 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 85:15, major rotamer reported) δ: 171.6 (C, CO Pro). 170.5 (C, CO Ser), 170.2 (CO, Ac), 151.8 (C, Tryp arom), 132.1 (C, Tryp arom), 127.6 (C, Tryp arom), 123.5 (C, Tryp arom), 112.8 (CH, Tryp arom), 112.5 (C, Tryp arom), 112.1(CH, Tryp arom), 102.9 (CH, Tryp arom), 79.3 (C, *C*CH), 75.3 (CH, C*C*H), 63.5 (CH_2, C^{β} Ser), 60.7 (CH, C^{α} Pro), 57.0 (CH_2, *C*H_2O), 52.4 (CH, C^{α} Ser), 47.7 (CH_2, C^{δ} Pro), 39.8 (CH_2, CH_2*C*H_2NH), 29.1 (CH_2, C^{β} Pro), 24.9 (CH_2, *C*$H_2$$CH_2$NH), 24.7 ($CH_2$, C^{γ} Pro), 23.0 (CH_3, Ac). **HRMS** calcd. for $C_{23}H_{29}N_4O_5$ $[M + H]^+$ *m/z* = 441.2138, found *m/z* = 441.2141.

Ac-L-Ser[OPO(OtBu)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (S16)

To a solution of **S15** (136 mg, 0.31 mmol, 1.0 eq) in DCM/THF (1:1, 12 mL) was added ETT (81.0 mg, 0.62 mmol, 2.0 eq) and the mixture was stirred for 30 min at rt. *i*Pr_2N-P(O*t*Bu)$_2$ (0.195 mL, 0.62 mmol, 2.0 eq) was added and the mixture was stirred at rt for 16 h. The mixture was cooled to -45°C, and a solution of *t*BuOOH (6M in decane, 0.25 mL, 1.08 mmol, 4 eq) was added dropwise. The mixture was stirred at -45°C for 1 h and at rt for 30 minutes, then it was cooled down to -45°C and quenched with a saturated aq solution of $Na_2S_2O_3$ (20 mL). The layers were separated in a separatory funnel and the aqueous phase was extracted with DCM (40 mL x 2). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was rapidly columned on a short pad of silica gel (100% AcOEt) to give compound **S16** as a pale-yellow oil (45% yield or 89% yield based on recovered starting material). Since the purification on silica showed to be tricky (partially deprotection of one of the *t*Bu group occurs in parallel with the co-elution of the reduced phosphoramidite) the product was used crude for the next step without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans/cis* ratio 82:18, *minor rotamer) δ: 9.01 (s, 1H, N*H* Indole), 7.31 – 7.22 (m, 1H, Tryp arom), 7.21 – 7.11 (m, 1H, Tryp arom), 7.07 – 6.97 (m, 1H, Tryp arom), 6.91 – 6.81 (m, 2H, Tryp arom + N*H* Tryp), 6.70 (d, *J* = 7.7 Hz, 1H, N*H* Ser), 4.92 (q, *J* = 6.4 Hz, 1H, H^{α} Ser), 4.72 (d, *J* = 2.4 Hz, 2H, C*H*$_2$O), 4.62 – 4.42 (m, 1H, H^{α} Pro), 4.16 – 3.90 (m, 2H, H^{β} Ser), 3.69 – 3.42 (m, 4H, 2H x H^{δ} Pro + 2H x CH_2C*H*$_2$NH),

3.03 – 2.83 (m, 2H, CH_2CH$_2$NH), 2.51 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.26 – 2.18 (m, 1H, H$^{\beta}$ Pro-Ha), 2.04* (s, 0.60H, Ac*), 1.99 (s, 2.40H, Ac), 1.96 – 1.85 (m, 3H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro), 1.49 (s, 9H, *t*Bu 1), 1.47 (s, 9H, *t*Bu 2). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans/cis* ratio 82:18, *minor rotamer) δ: 171.32* (C, CO Ac*), 170.78 (C, CO Ac), 170.45* (C, CO Ser*), 170.08 (C, CO Ser), 169.13 (C, CO Pro), 168.10* (C, CO Pro*), 151.86* (C, Tryp*), 151.80 (C, Tryp), 132.24 (C, Tryp), 132.21* (C, Tryp*), 127.85 (C, Tryp), 127.73* (C, Tryp*), 123.43 (CH, Tryp), 123.37* (CH, Tryp*), 112.66 (CH, Tryp), 112.63 (C, Tryp), 112.14 (CH, Tryp), 103.00 (CH, Tryp), 102.80* (CH, Tryp*), 83.83 (d, *J* = 3.6 Hz, C, *t*Bu-1), 83.75 (d, *J* = 3.6 Hz, C, *t*Bu-1), 79.45 (C, *C*CH), 79.41* (C, *C*CH*), 75.31* (CH, C*CH**), 75.22 (CH, C*CH*), 65.57 (d, *J* = 5.8 Hz, CH_2, C$^{\beta}$ Ser), 65.33* (d, *J* = 6.1 Hz, CH_2, C$^{\beta}$ Ser*), 60.99 (CH, C$^{\alpha}$ Pro), 60.87* (CH, C$^{\alpha}$ Pro*), 57.11 (CH_2, *C*H_2O), 57.03* (CH_2, *C*H_2O*), 52.26* (d, *J* = 6.7 Hz, CH, C$^{\alpha}$ Ser*), 51.18 (d, *J* = 7.1 Hz, CH, C$^{\alpha}$ Ser), 47.76 (CH_2, C$^{\delta}$ Pro), 47.23* (CH_2, C$^{\delta}$ Pro*), 39.88* (CH_2, CH$_2$*C*H_2NH*), 39.77 (CH_2, CH$_2$*C*H_2NH), 31.71* (CH_2, C$^{\beta}$ Pro*), 29.93 (d, *J* = 4.3 Hz, 6 x CH_3, *t*Bu), 28.34 (CH_2, C$^{\beta}$ Pro), 24.90 (CH_2, *C*H_2CH$_2$NH), 24.78 (CH_2, C$^{\gamma}$ Pro), 24.73* (CH_2, C$^{\gamma}$ Pro*), 23.12 (CH_3, Ac), 21.18* (CH_3, Ac*). **^{31}P NMR** (162 MHz, $CDCl_3$) (2 rotamers *trans/cis* ratio 82:18, *minor rotamer) δ: –9.48, –9.72* (s, PO(O*t*Bu)$_2$).

Ac-L-Ser[OPO(OH)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (3c)

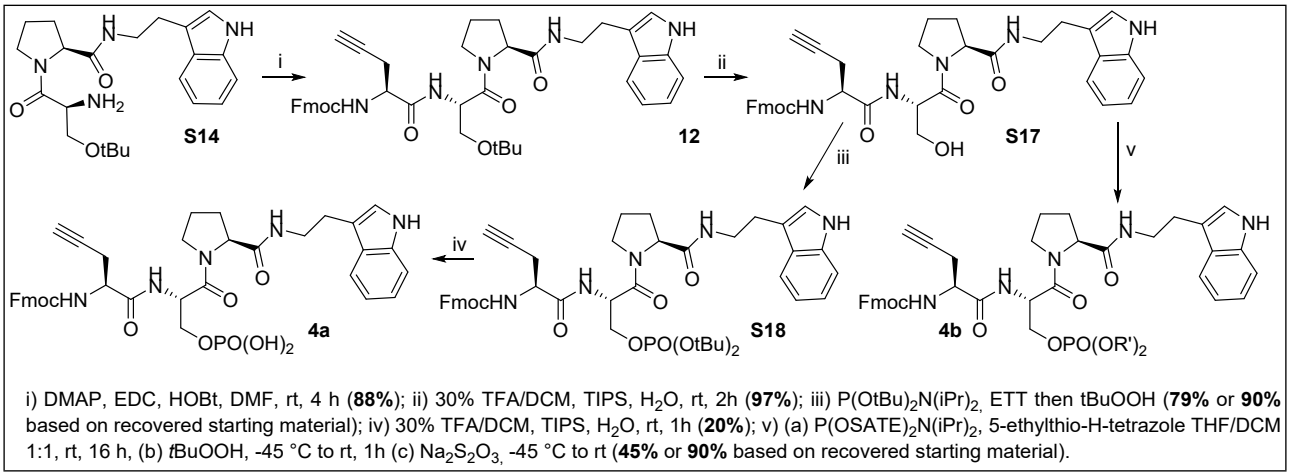
To a solution of protected compound **S13** (91.46 mg, 0.144 mmol) 6 mL of a solution of 20% TFA in DCM, few drops of TIPS and few drops of H_2O were added. The reaction stirred at rt for 1 hour, the solvent was removed under reduced pressure and the residue was purified by semi-preparative HPLC (Eluent: solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: from 10% to 50% B in 15 min; RT: 11.7 min) to provide **3c** as a white solid after lyophilisation (48.8 mg, 0.094 mmol, 65% after HPLC purification). **^{1}H NMR** (400.0 MHz, MeOD-d4) (2 rotamers *trans/cis* ratio 87:13, major rotamer reported) δ: 7.23 (d, *J* = 8.8 Hz, 1H, Tryp arom.), 7.17 (d, *J* = 2.2 Hz, 1H, Tryp arom.), 7.08 (s, 1H, Tryp arom.), 6.80 (dd, *J* = 8.8, 2.2 Hz, 1H, Tryp arom.), 4.95 (t, *J* = 6.4 Hz, 1H, H$^{\alpha}$ Ser), 4.73 (d, *J* = 2.4 Hz, 2H, C*H*$_2$O), 4.36 (dd, *J* = 8.4, 5.0 Hz, 1H, H$^{\alpha}$ Pro), 4.26-4.12 (m, 2H, H$^{\beta}$ Ser), 3.84-3.72 (m, 2H, H$^{\delta}$ Pro), 3.52-3.43 (m, 2H, CH$_2$*C*H_2NH), 2.94-2.88 (m, 3H, CC*H* + C*H*$_2$CH$_2$NH), 2.17-2.07 (m, 1H, H$^{\beta}$ Pro-Ha), 1.99 (s, 3H, Ac), 1.98-1.90 (m, 2H, H$^{\gamma}$ Pro), 1.86, 1.79 (m, 1H, H$^{\beta}$ Pro-Hb). **^{13}C NMR** (100.5 MHz, MeOD-d4) (2 rotamers *trans/cis* ratio 87:13, major rotamer reported) δ: 172.9 (C, CO Pro). 171.9 (C, CO Ser), 168.7 (C, CO Ac), 151.6 (C, Tryp arom.), 132.5 (C, Tryp arom.), 127.6 (C, Tryp arom.), 123.3 (CH, Tryp arom.), 111.8 (C, Tryp arom.), 111.6 (CH, Tryp arom.), 111.4 (CH, Tryp arom.), 102.2 (CH, Tryp arom.), 79.3 (C, *C*CH), 74.8 (CH, C*CH*), 66.7 (d, $J_{C\text{-}P}$ =5.1 Hz, CH_2, C$^{\beta}$ Ser), 60.8 (CH, C$^{\alpha}$ Pro), 56.4 (CH_2, *C*H_2O), 51.8 (d, $J_{C\text{-}P}$ = 7.2 Hz, CH, C$^{\alpha}$ Ser), 47.5 (CH_2, C$^{\delta}$ Pro), 39.9 (CH_2, CH$_2$*C*H_2NH), 29.4 (CH_2, C$^{\beta}$ Pro), 24.7 (CH_2, *C*H_2CH$_2$NH), 24.4 (CH_2, C$^{\gamma}$ Pro), 20.9 (CH_3, Ac). **^{31}P NMR** (162.0 MHz, $CDCl_3$) (2 rotamers *trans/cis* ratio 87:13, *minor rotamer) δ: –0.22, –0.63* (s, PO(OH)$_2$). **HRMS** calcd. for $C_{23}H_{29}N_4O_8P$ [M + H]$^+$ *m/z* = 521.1796, found *m/z* = 521.1805.

Ac-L-Ser[OPO(OSATE)$_2$]-L-Pro-[2-(5-(prop-2-yn-1-yloxy)-1H-indol-3-yl)ethan-1-amine] (3d)

To a solution of **S15** (134 mg, 0.31 mmol, 1 eq) in DCM/THF (1:1, 12 mL) ETT (79 mg, 0.61 mmol, 2 eq) was added and the mixture was stirred for 30 min at rt. Phosphoramidite **S5** (277 mg, 0.61 mmol, 2 eq) was added and the mixture was stirred at rt for 24 h. The mixture was cooled to -45°C, and a solution of *t*BuOOH (6M in decane, 0.20 mL, 1.22 mmol, 4 eq) was added dropwise. The mixture was stirred at -45°C for 1 h and at rt for 30 minutes, then it was cooled down to -45 °C and quenched with a saturated aq. solution of $Na_2S_2O_3$ (20 mL). The layers were separated in a separatory funnel and the aqueous phase was extracted with DCM (60 mL x 2). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 10%) to give the phosphorylated pseudopeptide **3d** (108 mg, 0.13 mmol, 43% yield or 90% yield based on recovered starting material, *trans-cis* rotamers mixture) as a pale, yellow oil. **R$_f$**: 0.25 (DCM/MeOH 95:5). **^{1}H NMR** (400.0 MHz, $CDCl_3$) (2 rotamers 88:12, major rotamer reported) δ: 8.65 (bs, 1H, N*H* Indole), 7.26 (d, *J* = 8.8 Hz, 1H, Tryp arom), 7.15 (d, *J* = 2.3 Hz, 1H, Tryp arom), 7.04 (d, *J* = 2.1 Hz, 1H, Tryp arom), 6.96 (t, *J* = 5.8 Hz, 1H, N*H* Tryp), 6.89 (dd, *J* = 8.8, 2.4 Hz, 1H, Tryp arom), 6.88 (bs, 1H, N*H* Ser), 4.96 (dt, *J* = 8.2, 4.8 Hz, 1H, H$^{\alpha}$ Ser), 4.73 (d, *J* = 2.3 Hz, 2H, C*H*$_2$O), 4.55-4.49 (m, 1H, H$^{\alpha}$ Pro), 4.23-4.00 (m, 6H, 4H x OC*H*$_2$CH$_2$S + 2H x H$^{\beta}$ Ser), 3.67-3.40 (m, 4H, 2H x H$^{\delta}$ Pro + 2H x CH$_2$C*H*$_2$NH), 3.17-3.04 (m, 4H, OCH$_2$C*H*$_2$S), 2.96-2.86 (m, 2H, C*H*$_2$CH$_2$NH), 2.52 (t, *J* = 2.4 Hz, 1H, CC*H*), 2.26-2.19 (m, 1H, H$^{\beta}$ Pro-Ha), 2.05 (s, 3H, Ac), 1.96-1.86 (m, 3H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro), 1.21 (s, 18H, *t*Bu). **^{13}C NMR** (100.5 MHz, $CDCl_3$) (2 rotamers 88:12, major rotamer reported) δ: 206.1 (C, CO SATE), 205.9 (C, CO SATE), 170.7 (C, CO Pro). 170.2 (C, CO Ser), 168.4 (C, CO Ac), 151.8 (C, Tryp arom), 132.1 (C, Tryp arom), 127.9 (C, Tryp arom), 123.3 (CH, Tryp arom), 112.7 (C, Tryp arom), 112.6 (CH, Tryp arom), 112.0 (CH, Tryp arom), 102.9 (CH, Tryp arom), 79.4 (C, *C*CH), 75.2 (CH, C*CH*), 66.6 (d, $J_{C\text{-}P}$ = 5.9 Hz, CH_2, OC*H*$_2$CH$_2$S), 66.5 (d, $J_{C\text{-}P}$ =5.9 Hz, CH_2, OC*H*$_2$CH$_2$S), 66.4 (d, $J_{C\text{-}P}$ = 5.3 Hz, CH_2, C$^{\beta}$ Ser), 61.2 (CH, C$^{\alpha}$ Pro), 57.0(CH_2, *C*H_2O), 51.1 (d, $J_{C\text{-}P}$ = 5.9 Hz, CH, C$^{\alpha}$ Ser), 47.6 (CH_2, CH$_2$*C*H_2NH), 46.6 (2 x C, *t*Bu), 39.8 (CH_2, C$^{\delta}$ Pro), 28.5 (CH_2, C$^{\beta}$ Pro), 28.4 (d, $J_{C\text{-}P}$ = 7.9 Hz, CH_2, OCH$_2$*C*H_2S), 28.2 (d, $J_{C\text{-}P}$ = 7.1 Hz, CH_2, OCH$_2$*C*H_2S), 27.3 (6 x CH_3, *t*Bu), 24.9 (CH_2, *C*H_2CH$_2$NH), 24.7 (CH_2, C$^{\gamma}$ Pro), 23.0 (CH_3, Ac). **^{31}P NMR** (162.0 MHz, $CDCl_3$) (2 rotamers 88:12, *minor rotamer) δ: –1.41, –1.62* (s, PO(OSATE)$_2$). **HRMS** calcd. for $C_{37}H_{54}N_4O_{10}PS_2$ [M + H]$^+$ *m/z* = 809.3019, found *m/z* = 809.3018.

2.5 Synthesis of *N* -Terminal Alkyne Series Pin1 Ligands

2.5.1 Synthesis of 4a and 4b



i) DMAP, EDC, HOBt, DMF, rt, 4 h (**88%**); ii) 30% TFA/DCM, TIPS, H_2O, rt, 2h (**97%**); iii) $P(OtBu)_2N(iPr)_2$, ETT then tBuOOH (**79%** or **90%** based on recovered starting material); iv) 30% TFA/DCM, TIPS, H_2O, rt, 1h (**20%**); v) (a) $P(OSATE)_2N(iPr)_2$, 5-ethylthio-H-tetrazole THF/DCM 1:1, rt, 16 h, (b) *t*BuOOH, -45 °C to rt, 1h (c) $Na_2S_2O_3$, -45 °C to rt (**45%** or **90%** based on recovered starting material).

Scheme S7: Synthesis of **4a** and **4b**.

Fmoc-L-Pra-L-Ser(OtBu)-L-Pro-Tryptamine (12)

A solution of DMAP (4.1 mg, 0.34 mmol, 0.12 eq) and the amine **S14** (1.39 g, 2.80 mmol, 1.0 eq) in DMF (20 mL) was added to a solution of the carboxylic acid (1.13 g, 3.35 mmol, 1.2 eq), EDC (636 mg, 3.35 mmol, 1.2 eq), and HOBt (512 mg, 3.35 mmol, 1.2 eq) in DMF (30 mL). The mixture was stirred at rt for 2 h, then it was diluted with AcOEt (100 mL) and washed with water (3 x 100 mL) and brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. the residue was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 2%) to give peptide **12** (1.61 g, 2.24 mmol, 88%, *trans-cis* rotamers mixture) as a pale yellow solid. $\mathbf{R_f}$**:** 0.55 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, major rotamer only) δ: 8.33 (s, 1H, N*H* Indole), 7.77 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.64 – 7.52 (m, 3H, 2H x Fmoc arom + Tryp arom), 7.47 – 7.37 (m, 3H, 2H x Fmoc arom + N*H* Ser), 7.37 – 7.26 (m, 3H, 2H x Fmoc arom + Tryp arom), 7.11 (t, *J* = 7.4 Hz, 1H, Tryp arom), 7.04 (t, *J* = 5.4 Hz, 3H, 2H x Tryp arom + N*H* Tryp), 5.60 (d, *J* = 7.9 Hz, 1H, N*H* Pra), 4.94 – 4.84 (m, 1H, H^{α} Ser), 4.58 (d, *J* = 7.0 Hz, 1H, H^{α} Pro), 4.39 (d, *J* = 10.1 Hz, 2H, H^{α} Pra + CH_2 Fmoc-Ha), 4.32 – 4.25 (m, 1H, CH_2 Fmoc-Hb), 4.22 (q, *J* = 6.3 Hz, 1H, C*H* Fmoc), 3.94 – 3.79 (m, 1H, H^{δ} Pro-Ha), 3.70 – 3.54 (m, 3H, H^{δ} Pro-Hb + H^{β} Ser-Ha + CH_2CH_2NH-Ha), 3.45 – 3.32 (m, 1H, CH_2CH_2NH-Hb), 3.29 (t, *J* = 8.5 Hz, 1H, H^{β} Ser-Hb), 3.03 – 2.87 (m, 2H, CH_2CH_2NH), 2.76 – 2.50 (m, 2H, H^{β} Pra), 2.23 (s, 1H, H^{β} Pro-Ha), 2.12 – 2.05 (m, 1H, CC*H*), 2.07 – 1.78 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.12* (s, 1H, *t*Bu*), 1.02 (s, 8H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, major rotamer only) δ: 171.16 (C, CO), 169.81 (C, CO), 169.50 (C, CO), 155.99 (C, CO), 143.70 (C, Arom), 141.42 (C, Arom), 136.40 (C, Arom), 127.95 (2 x CH, Fmoc arom), 127.43 (2 x CH, Fmoc arom), 127.25 (C, Arom), 125.23 (2 x CH, Fmoc arom), 122.87 (CH, Tryp arom), 121.97 (CH, Tryp arom), 120.20 (2 x CH, Fmoc arom), 119.30 (CH, Tryp arom), 118.60 (CH, Tryp arom), 112.80 (C, Arom), 111.31 (CH, Tryp arom), 78.97 (C, *C*CH), 74.52 (C, *t*Bu), 72.12 (CH, C*CH*), 67.54 (CH_2, Fmoc), 62.71 (CH_2, C^{β} Ser), 60.64 (CH, C^{α} Pro), 53.14 (CH, C^{α} Pra), 51.53 (CH, C^{α} Ser), 48.12 (CH_2, C^{δ} Pro), 47.10 (CH, Fmoc), 40.14 (CH_2, CH_2CH_2NH), 28.91 (CH_2, C^{β} Pro), 27.39 (3 CH_3, *t*Bu), 25.31 (CH_2, CH_2CH_2NH), 24.71 (CH_2, C^{γ} Pro), 23.12 (CH_2, C^{β} Pra). **HRMS** calcd. for $C_{42}H_{47}N_5O_6$ $[M + H]^+$ *m/z* = 718.3599 and $[M + Na]^+$ *m/z* = 740.3419, found *m/z* = 718.3592 and *m/z* = 740.3411.

Fmoc-L-Pra-L-Ser(OH)-L-Pro-Tryptamine (S17)

*t*Bu-protected compound **12** (50 mg, 0.07 mmol) was dissolved in 30% TFA/DCM (62.5 mL) with 250 µL of H_2O and 250 µL TIPS. The reactive mixture was stirred for 2 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 10%). Alcohol **S17** (45 mg, 0.068 mmol, 97%, *trans-cis* rotamers mixture) was obtained as a yellow solid. $\mathbf{R_f}$**:** 0.35 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, major rotamer reported) δ: 8.62 (s, 1H, N*H* Indole), 8.20 (d, *J* = 8.2 Hz, 1H, N*H* Ser), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.53 (d, *J* = 5.4 Hz, 2H, Fmoc arom), 7.48 (d, *J* = 7.9 Hz, 1H, Tryp arom), 7.39 (t, *J* = 7.5 Hz, 2H, Fmoc arom), 7.33 – 7.23 (m, 3H, 2H x Fmoc arom + Tryp arom), 7.16 – 7.04 (m, 2H, Tryp arom + N*H* Tryp), 7.04 – 6.92 (m, 2H, Tryp arom), 5.92 (d, *J* = 8.6 Hz, 1H, N*H* Pra), 5.01 (d, *J* = 7.3 Hz, 1H, H^{α} Ser), 4.73 – 4.63 (m, 1H, H^{α} Pra), 4.59 – 4.51 (m, 1H, H^{α} Pro), 4.23

(dd, *J* = 10.1, 7.5 Hz, 1H, CH_2 Fmoc-Ha), 4.10 (t, *J* = 7.3 Hz, 1H, C*H* Fmoc), 4.00 (dd, *J* = 10.1, 7.0 Hz, 1H, CH_2 Fmoc-Hb), 3.90 – 3.82 (m, 1H, H$^{\beta}$ Ser-Ha), 3.81 – 3.51 (m, 4H, H$^{\beta}$ Ser-Hb + 2H x H$^{\delta}$ Ser + CH_2CH_2NH-Ha), 3.51 – 3.38 (m, 1H, CH_2CH_2NH-Hb), 2.85 (t, *J* = 6.8 Hz, 2H, CH_2CH_2NH), 2.60 – 2.54 (m, 2H, H$^{\beta}$ Pra), 2.10 – 2.00 (m, 2H, H$^{\beta}$ Pro-Ha + CC*H*), 2.00 – 1.78 (m, 3H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, major rotamer reported) δ: 172.38 (C, CO), 169.89 (C, CO), 169.79 (C, CO), 156.15 (C, CO), 143.90 (C, Arom), 143.62 (C, Arom), 141.31 (C, Arom), 136.42 (C, Arom), 127.92 (2 x CH, Fmoc arom), 127.22 (2 x CH, Fmoc arom), 125.28 (2 x CH, Fmoc arom), 122.66 (CH, Tryp arom), 121.94 (CH, Tryp arom), 120.13 (2 x CH, Fmoc arom), 119.22 (CH, Tryp arom), 118.46 (CH, Tryp arom), 112.45 (C, Arom), 111.45 (CH, Tryp arom), 79.03 (C, *C*CH), 71.77 (CH, C*CH*), 67.48 (CH_2, Fmoc), 63.60 (CH_2, C$^{\beta}$ Ser), 60.77 (CH, C$^{\alpha}$ Pro), 52.95 (CH, C$^{\alpha}$ Ser), 52.74 (CH, C$^{\alpha}$ Pra), 48.13 (CH_2, C$^{\delta}$ Pro), 46.94 (CH, Fmoc), 39.99 (CH_2, CH_2CH_2NH), 29.59 (CH_2, C$^{\beta}$ Pro), 25.02 (CH_2, CH_2CH_2NH), 24.97 (CH_2, C$^{\gamma}$ Pro), 24.08 (CH_2, C$^{\beta}$ Pra). **HRMS** calcd. for $C_{38}H_{39}N_5O_6$ [M + H]$^+$ *m/z* = 662.2973 and [M + Na]$^+$ *m/z* = 684.2793, found *m/z* = 662.2976 and *m/z* = 684.2795.

Fmoc-L-Pra-L-Ser[OPO(OtBu)$_2$]-L-Pro-Tryptamine (S18)

Compound **S17** (30 mg, 0.045 mmol, 1.0 eq) and ETT (24 mg, 0.18 mmol, 4 eq) were first dissolved in dry THF (3 mL) before adding the (*i*Pr$_2$N)P(O*t*Bu)$_2$ (30 µL, 0.09 mmol, 2 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN-liquid N_2 bath) and 5-6 M *t*BuOOH in decane (40 µL, 0.18 mmol, 4 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (3 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (15 mL) and washed with water (2 x 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 2%). Phosphorylated compound **S18** (30 mg, 0.036 mmol, 79% yield or 90% yiled based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. **R$_f$**: 0.70 (DCM/MeOH 9:1). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ: 8.88 (s, 1H, N*H* Indole), 7.76 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.64 – 7.56 (m, 3H, 2H x Fmoc arom + Tryp arom), 7.44 – 7.36 (m, 3H, 2H x Fmoc arom + N*H* Ser), 7.36 – 7.27 (m, 3H, 2H x Fmoc arom + Tryp arom), 7.15 (t, *J* = 7.6 Hz, 1H, Tryp arom), 7.11 – 6.98 (m, 2H, Tryp arom), 6.89 (bs, 1H, N*H* Tryp), 5.87 (d, *J* = 8.3 Hz, 1H, N*H* Pra), 5.01 – 4.85 (m, 1H, H$^{\alpha}$ Ser), 4.59 – 4.26 (m, 4H, H$^{\alpha}$ Pro + H$^{\alpha}$ Pra + 2H x CH_2 Fmoc), 4.21 (t, *J* = 7.1 Hz, 1H, C*H* Fmoc), 4.13 – 4.01 (m, 2H, H$^{\beta}$ Ser), 3.69 – 3.41 (m, 4H, 2x CH_2CH_2NH + 2H x H$^{\delta}$ Pro), 3.06 – 2.88 (m, 2H, CH_2CH_2NH), 2.78 – 2.62 (m, 2H, H$^{\beta}$ Pra), 2.38 – 2.17 (m, 1H, H$^{\beta}$ Pro-Ha), 2.06 (s, 1H, CC*H*), 1.99 – 1.84 (m, 3H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro), 1.48 (s, 12H, *t*Bu), 1.47* (s, 6H, *t*Bu*). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ: 170.69 (C, CO), 169.68(C, CO), 168.28 (C, CO), 155.99(C, CO), 143.87 (C, C arom), 141.38 (C, C arom), 136.45 (C, C arom), 127.89 (2 x CH, Fmoc arom), 127.61 (C, C arom), 127.22 (2 x CH, Fmoc arom), 125.24 (2 x CH, Fmoc arom), 122.46 (CH, Tryp arom), 121.87 (CH, Tryp arom), 120.14 (2 x CH, Fmoc arom), 119.12 (CH, Tryp arom), 118.82 (CH, Tryp arom), 112.79 (C, C arom), 111.42 (CH, Tryp arom), 83.80 (d, $J_{C\text{-}P}$ = 7.9 Hz, 2 x C, *t*Bu), 79.12 (C, *C*CH), 72.07 (CH, C*CH*), 67.44 (CH_2, Fmoc), 65.37 (d, $J_{C\text{-}P}$ = 5.9 Hz, CH_2, C$^{\beta}$ Ser), 61.00 (CH, C$^{\alpha}$ Pro), 53.28 (CH, C$^{\alpha}$ Pra), 51.46 (d, $J_{C\text{-}P}$ = 6.7 Hz, CH, C$^{\alpha}$ Ser), 47.68 (CH_2, C$^{\delta}$ Pro), 47.14 (CH, Fmoc), 39.92 (CH_2, CH_2CH_2NH), 29.95 (d, $J_{C\text{-}P}$ = 4.3 Hz, 6 x CH_3, *t*Bu), 28.37 (CH_2, C$^{\beta}$ Pro), 24.93 (CH_2, CH_2CH_2NH), 24.80 (CH_2, C$^{\gamma}$ Pro), 22.99 (CH_2, C$^{\beta}$ Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer) δ: –9.35, –9.69* (s, PO(O*t*Bu)$_2$). **UPLC-MS**: Eluent – solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: 0.5 mL/min, from 10% to 90% B in 3.5 min then 100% B until 5 min; RT: 2.86 min. MS calcd. for $C_{46}H_{56}N_5O_9P$ [M + H]$^+$ *m/z* = : 854.39, found *m/z* = 854.58.

Fmoc-L-Pra-L-Ser[OPO(OH)$_2$]-L-Pro-Tryptamine (4a)

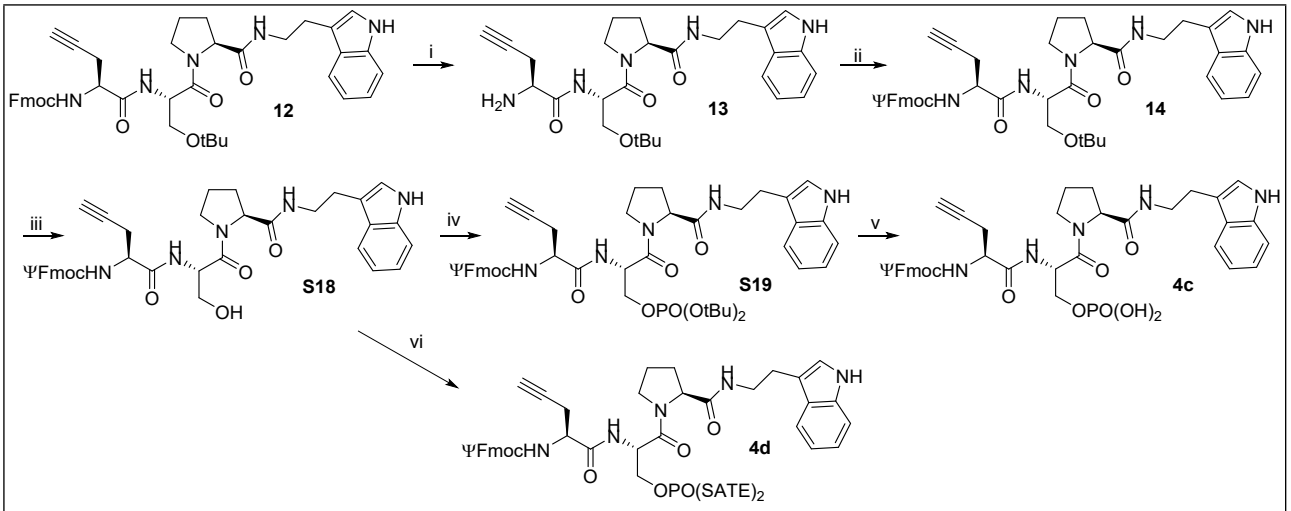
*t*Bu-protected compound **S18** (35 mg, 0.041 mmol) was dissolved in 30% TFA/DCM (62.5 mL) with 10 µL of H_2O and 10 µL TIPS. The reactive mixture was stirred for 1 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude was purified by semi-preparative HPLC (Eluent: solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: from 5% to 100% B over 20 min. RT: 14.9 min). Phosphorylated compound **4a** (6 mg, 0.008 mmol, 20%, *trans-cis* rotamers mixture) was obtained as a whitish solid after lyophilisation. **^{1}H NMR** (400 MHz, MeOD) (2 rotamers *trans-cis* ratio 80:20, major rotamer reported) δ: 7.79 (d, *J* = 7.5 Hz, 2H, Fmoc arom), 7.71 – 7.64 (m, 2H, Fmoc arom), 7.58 (d, *J* = 8.0 Hz, 1H, Tryp arom), 7.39 (t, *J* = 6.9 Hz, 2H, Fmoc arom), 7.31 (t, *J* = 6.1 Hz, 3H, 2H x Fmoc arom + Tryp arom), 7.11 – 7.03 (m, 2H, Tryp arom), 7.03 – 6.95 (m, 1H, Tryp arom), 4.97 (t, *J* = 6.3 Hz, 1H, H$^{\alpha}$ Ser), 4.45 – 4.28 (m, 4H, H$^{\alpha}$ Pro + H$^{\alpha}$ Pra + 2H x CH_2 Fmoc), 4.29 – 4.14 (m, 3H, 2H x H$^{\beta}$ Ser + C*H* Fmoc), 3.84 – 3.66 (m, 2H, H$^{\delta}$ Pro), 3.55 – 3.41 (m, 2H, CH_2CH_2NH), 2.95 (t, *J* = 7.1 Hz, 2H, CH_2CH_2NH), 2.78 – 2.52 (m, 2H, H$^{\beta}$ Pra), 2.38 (s, 1H, CC*H*), 2.18 – 1.97 (m, 1H, H$^{\beta}$ Pro-Ha), 1.96 – 1.77 (m, 3H, H$^{\beta}$ Pro-Hb + 2H x H$^{\gamma}$ Pro). **^{13}C NMR** (101 MHz, MeOD) (2 rotamers *trans-cis* ratio 80:20, major rotamer reported) δ: 174.27 (C, CO), 172.71 (C, CO), 169.67 (C, CO), 158.36 (C, CO), 145.27 (C, Arom), 145.16 (C, Arom), 142.58 (C, Arom), 138.12 (C, Arom), 128.82 (2 x CH, Fmoc arom), 128.22 (2 x CH, Fmoc arom), 126.30 (2 x CH, Fmoc arom), 123.61 (CH, Tryp arom), 122.27 (CH, Tryp arom), 120.93 (2 x

CH, Fmoc arom), 119.56 (CH, Tryp arom), 119.36 (CH, Tryp arom), 113.09 (C, Arom), 112.18 (CH, Tryp arom), 80.33 (C, *C*CH), 72.36 (CH, C*CH*), 68.26 (CH_2, Fmoc), 65.93 (CH_2, C^{β} Ser), 62.22 (CH, C^{α} Pro), 55.26 (CH, C^{α} Pra), 52.97 (CH, C^{α} Ser), 48.83 (CH_2, C^{δ} Pro), 48.38 (CH, Fmoc), 41.41 (CH_2, CH_2*CH*$_2$NH), 30.83 (CH_2, C^{β} Pro), 26.05 (CH_2, *CH*$_2$$CH_2$NH), 25.78 ($CH_2$, C^{γ} Pro), 22.90 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, MeOD) (2 rotamers *trans-cis* ratio 80:20, *minor rotamer) δ: –0.18, –0.67* (s, $PO(OH)_2$). **UPLC-MS**: Eluent – solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: 0.5 mL/min, from 10% to 90% B in 3.5 min then 100% B until 5 min; RT: 2.38 min. MS calcd. for $C_{38}H_{40}N_5O_9P$ $[M + H]^+$ *m/z* = : 742.26, found *m/z* = 742.63.

Fmoc-L-Pra-L-Ser[OPO(OSATE)$_2$]-L-Pro-Tryptamine (4b)

Compound **S17** (240 mg, 0.363 mmol, 1.0 eq) and ETT (190 mg, 1.45 mmol, 4.0 eq) were first dissolved in dry THF (18 mL) before adding the phosphoramidite **S5** (330 mg, 0.726 mmol, 2.0 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN- liquid N_2 bath) and 5-6 M *t*BuOOH in decane (290 µL, 1.45 mmol, 4 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (20 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (30 mL) and washed with water (2 x 60 mL) and brine (60 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 3%). Phosphorylated compound **4b** (172 mg, 0.163 mmol, 45% yield or 90% based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a yellow solid. R_f: 0.40 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, major rotamer reported) δ: 8.63 (s, 1H, N*H* Indole), 7.76 (d, *J* = 7.6 Hz, 2H, Fmoc arom), 7.60 (d, *J* = 8.0 Hz, 3H, 2H x Fmoc arom + Tryp arom), 7.44 – 7.28 (m, 6H, 4H x Fmoc arom + Tryp arom + N*H* Ser), 7.17 (t, *J* = 7.0 Hz, 1H, Tryp arom), 7.12 – 7.06 (m, 2H, Tryp arom), 6.86 (s, 1H, N*H* Tryp), 5.99 (d, *J* = 8.3 Hz, 1H, N*H* Pra), 4.95 – 4.81 (m, 1H, H^{α} Ser), 4.58 – 4.31 (m, 4H, H^{α} Pro + H^{α} Pra + 2H x C*H*$_2$ Fmoc), 4.25 (t, *J* = 7.3 Hz, 1H, C*H* Fmoc), 4.18 – 3.96 (m, 6H, 2H x H^{β} Ser + 4H x OC*H*$_2$$CH_2$S), 3.71 – 3.36 (m, 4H, 2H x CH_2C*H*$_2$NH + 2H x H^{δ} Pro), 3.21 – 2.88 (m, 6H, 4H x OCH_2C*H*$_2$S + 2H x C*H*$_2$$CH_2$NH), 2.85 – 2.62 (m, 2H, H^{β} Pra), 2.33 – 2.20 (m, 1H, H^{β} Pro-Ha), 2.09 (s, 1H, CC*H*), 1.99 – 1.84 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.22 (s, 18H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, major rotamer reported) δ: 206.13 (2 x C, CO SATE), 170.57 (C, CO), 169.89 (C, CO), 167.66 (C, CO), 156.20 (C, CO), 143.92 (C, Arom), 143.80 (C, Arom), 141.41 (C, Arom), 136.44 (C, Arom), 127.92 (2 x CH, Fmoc arom), 127.69 (C, Arom), 127.26 (2 x CH, Fmoc arom), 125.29 (2 x CH, Fmoc arom), 122.45 (CH, Tryp arom), 122.02 (CH, Tryp arom), 120.17 (2 x CH, Fmoc arom), 119.31 (CH, Tryp arom), 118.84 (CH, Tryp arom), 113.00 (C, Arom), 111.43 (CH, Tryp arom), 79.20 (C, *C*CH), 72.05 (CH, C*CH*), 67.56 (CH_2, Fmoc), 66.69 (d, $J_{C\text{-}P}$ = 3.6 Hz, CH_2, O*CH*$_2$$CH_2$S-1), 66.63 (d, $J_{C\text{-}P}$ = 3.8 Hz, CH_2, O*CH*$_2$$CH_2$S-2), 66.20 (d, $J_{C\text{-}P}$ = 5.2 Hz, CH_2, C^{β} Ser), 61.21 (CH, C^{α} Pro), 53.47 (CH, C^{α} Pra), 51.59 (d, $J_{C\text{-}P}$ = 4.3 Hz, CH, C^{α} Ser), 47.63 (CH_2, C^{δ} Pro), 47.19 (CH, Fmoc), 46.73 (d, $J_{C\text{-}P}$ = 4.4 Hz, 2 x C, *t*Bu), 39.97 (CH_2, CH_2*CH*$_2$NH), 28.47 (d, *J* = 7.6 Hz, CH_2, OCH_2*CH*$_2$S-1), 28.37 (d, *J* = 6.5 Hz, CH_2, OCH_2*CH*$_2$S-2), 28.26 (CH_2, C^{β} Pro), 27.45 (d, *J* = 2.2 Hz, 6 x CH_3, *t*Bu), 24.92 (2 x CH_2, *CH*$_2$$CH_2$NH + C^{γ} Pro), 22.73 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) (major rotamer reported) δ: –1.25 (s, $PO(OSATE)_2$). **HRMS** calcd. for $C_{52}H_{64}N_5O_{11}PS_2$ $[M + Na]^+$ *m/z* = 1052.3674, found *m/z* = 1052.3683.

2.5.2 Synthesis of 4c and 4d



i) 20% DEA/DCM, rt, 2 h (**95%**); ii) 2-(9H-fluoren-9-yl)acetic acid, DMAP, EDC, HOBt, DMF, rt, 4 h (**85%**); iii) 30% TFA/DCM, TIPS, H_2O, rt, 2h (**80%**); iv) (a) $P(OtBu)_2N(iPr)_2$, 5-ethylthio-H-tetrazole, THF, rt, 16 h (b) tBuOOH, -45 °C to rt, 1 h (c) $Na_2S_2O_3$, -45 °C to rt (**16%** or **80%** based on recovered starting material); 20% TFA/DCM, TIPS, H_2O rt, 1h (**18%**); (a) $P(OSATE)_2N(iPr)_2$, 5-ethylthio-H-tetrazole THF/DCM 1:1, rt, 16 h, (b) tBuOOH, -45 °C to rt, 1h (c) $Na_2S_2O_3$, -45 °C to rt (**20%** or **80%** based on recovered starting material)

Scheme S8: Synthesis of **4c** and **4d**.

L-Pra-L-Ser(OtBu)-L-Pro-Tryptamine (13)

Fmoc-protected compound **12** (732 mg, 1.02 mmol) was dissolved in 20% DEA/DCM (10 mL) solution and stirred for 2h at rt. At completion of the reaction, the solvents were co-evaporated with $CHCl_3$ under reduced pressure. The crude product was purified by flash chromatography on silica gel (AcOEt/MeOH step gradient from 0% MeOH to 20%). Amine **13** (477 mg, 0.96 mmol, 95%, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. $\mathbf{R_f}$*:* 0.22 (AcOEt/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, *minor rotamer when visible) δ: 8.25 (s, 1H, N*H* Indole), 8.03 (d, *J* = 8.0 Hz, 0.80H, N*H* Ser), 7.79* (d, *J* = 5.2 Hz, 0.20H, N*H* Ser*), 7.64* (d, *J* = 7.9 Hz, 0.20H, Tryp arom*), 7.58 (d, *J* = 8.9 Hz, 0.80H, Tryp arom), 7.34 (d, *J* = 8.0 Hz, 1H, Tryp arom), 7.16 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, Tryp arom), 7.13 – 7.04 (m, 2H, Tryp arom), 6.96 (t, *J* = 6.1 Hz, 1H, N*H* Tryp), 4.87 – 4.76 (m, 1H, 0.80H x H$^\alpha$ Ser + 0.20H x H$^\alpha$ Pro*), 4.63 (dd, *J* = 8.0, 2.7 Hz, 0.80H, H$^\alpha$ Pro), 4.36* (dt, *J* = 8.8, 5.8 Hz, 0.20H, H$^\alpha$ Ser*), 3.83 (dt, *J* = 10.5, 5.4 Hz, 1H, H$^\delta$ Pro-Ha), 3.70 – 3.43 (m, 4H, H$^\beta$ Ser-Ha + H$^\delta$ Pro-Hb + CH_2CH_2NH-Ha + H$^\alpha$ Pra), 3.43 – 3.29 (m, 2H, H$^\beta$ Ser-Hb + CH_2CH_2NH-Hb), 3.04 – 2.91 (m, 2H, CH_2CH_2NH), 2.72 (ddd, *J* = 16.9, 4.5, 2.7 Hz, 1H, H$^\beta$ Pra-Ha), 2.60 (ddd, *J* = 16.8, 7.4, 2.6 Hz, 1H, H$^\beta$ Pra-Ha), 2.45* (dd, *J* = 12.5, 6.3 Hz, 0.20H, H$^\beta$ Pro-Ha*), 2.34 – 2.26 (m, 0.80H, H$^\beta$ Pro-Ha), 2.07 (t, *J* = 2.6 Hz, 1H, CC*H*), 2.05 – 1.89 (m, 3H, H$^\beta$ Pro-Hb + 2H x H$^\gamma$ Pro), 1.14* (s, 2H, *t*Bu*), 1.05 (s, 7H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 80:20, *minor rotamer when visible) δ: 173.24* (C, CO*), 172.67 (C, CO), 171.14 (C, CO), 171.05* (C, CO*), 170.56 (C, CO), 170.29* (C, CO*), 136.39 (C, Tryp arom), 127.67* (C, Tryp arom*), 127.53 (C, Tryp arom), 122.57 (CH, Tryp arom), 122.05* (CH, Tryp arom*), 122.02 (CH, Tryp arom), 119.38 (CH, Tryp arom), 119.00* (CH, Tryp arom*), 118.77 (CH, Tryp arom), 113.36* (C, Tryp arom*), 113.02 (C, Tryp arom), 111.27 (CH, Tryp arom), 111.20* (CH, Tryp arom*), 80.27 (C, *C*CH), 74.24 (C, *t*Bu), 74.05* (C, *t*Bu*), 71.51 (CH, C*CH*), 63.00 (CH_2, C$^\beta$ Ser), 62.36* (CH_2, C$^\beta$ Ser*), 61.22* (CH, C$^\alpha$ Pro*), 60.62 (CH, C$^\alpha$ Pro), 53.66 (CH, C$^\alpha$ Pra), 53.33* (CH, C$^\alpha$ Pra*), 52.41* (CH, C$^\alpha$ Ser*), 50.92 (CH, C$^\alpha$ Ser), 47.94 (CH_2, C$^\delta$ Pro), 46.90* (CH_2, C$^\delta$ Pro*), 40.30 (CH_2, CH_2CH_2NH), 31.26* (CH_2, C$^\beta$ Pro*), 28.52 (CH_2, C$^\beta$ Pro), 27.41* (3 x CH_3, *t*Bu*), 27.32 (3 x CH_3, *t*Bu), 25.50 (CH_2, CH_2CH_2NH), 25.26 (CH_2, C$^\beta$ Pra), 24.99* (CH_2, CH_2CH_2NH*), 24.70 (CH_2, C$^\gamma$ Pro), 22.30* (CH_2, C$^\gamma$ Pro). **HRMS** calcd. for $C_{27}H_{37}N_5O_4$ $[M + H]^+$ *m/z* = 496.2924, found *m/z* = 496.2918.

ΨFmoc-L-Pra-L-Ser(OtBu)-L-Pro-Tryptamine (14)

A solution of DMAP (0.6 mg, 0.05 mmol, 0.12 eq) and the amine **13** (200 mg, 0.40 mmol, 1.0 eq) in DMF (10 mL) was added to a solution of 9H-Fluoren-9-yl-acetic acid (100 mg, 0.44 mmol, 1.1 eq), EDC (113 mg, 0.6 mmol, 1.2 eq), and HOBt (92 mg, 0.6 mmol, 1.2 eq) in DMF (15 mL). The mixture was stirred at rt for 2 h, then it was diluted with AcOEt (50 mL) and washed with water (3 x 50mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 2%). ψFmoc-protected peptide **14** (235 mg, 0.335 mmol, 85%, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. $\mathbf{R_f}$*:* 0.40 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, *minor rotamer when visible) δ: 8.77 (s, 1H, N*H* Indole), 8.14 (s, 1H, N*H* Ser), 7.78 – 7.69 (m, 2H, ΨFmoc arom), 7.61 – 7.55 (m, 1H, Tryp arom), 7.51 – 7.19 (m, 8H, 6H x ΨFmoc arom + N*H* Tryp + Tryp arom), 7.15 – 6.98 (m, 3H, Tryp arom), 6.25 (d, *J* = 6.5 Hz, 0.90H, N*H* Pra), 6.08* (s, 0.10H, N*H* Pra*), 5.19 – 5.11 (m, 0.90H, H$^\alpha$ Ser), 4.83* (d, *J* = 8.1 Hz, 0.10H, H$^\alpha$ Pro*), 4.66 (q, *J* = 5.6 Hz, 1H, H$^\alpha$ Pra), 4.54 (dd, *J* = 7.5, 3.3 Hz, 0.90H, H$^\alpha$ Pro), 4.29 (dd, *J* = 8.8, 5.7 Hz, 1H, C*H* ΨFmoc), 3.96 – 3.86 (m, 1H, H$^\delta$ Pro-Ha), 3.83 – 3.69 (m, 2H, H$^\delta$ Pro-Hb + H$^\beta$ Ser-Ha), 3.67 – 3.44 (m, 3H, H$^\beta$ Ser-Hb + 2H x CH_2CH_2NH), 3.11 – 2.91 (m, 2H, CH_2CH_2NH), 2.65 (ddd, *J* = 17.0, 5.3, 2.6 Hz, 1H, H$^\beta$ Pra-Ha), 2.53 – 2.43 (m, 1H, H$^\beta$ Pra-Hb), 2.40 – 2.29 (m, 1H, CH_2 ΨFmoc-Ha), 2.21 – 1.87 (m, 6H, CH_2 ΨFmoc-Hb + CC*H* + 2H x H$^\beta$ Pro + 2H x H$^\gamma$ Pro), 1.12 (s, 9H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, *minor rotamer when visible) δ: 171.81 (C, CO), 171.48 (C, CO), 169.81 (C, CO), 169.62 (C, CO), 146.27 (2 x C, Arom), 140.81 (C, Arom), 140.71 (C, Arom), 136.46 (C, Arom), 127.56 (2 x CH, ΨFmoc arom), 127.29 (2 x CH, ΨFmoc arom), 124.83 (C, Arom), 124.39 (2 x CH, ΨFmoc arom), 123.19 (CH, Tryp arom), 121.85 (CH, Tryp arom), 120.00 (2 x CH, ΨFmoc arom), 119.20 (CH, Tryp arom), 118.49 (CH, Tryp arom), 112.56 (C, Arom), 111.51 (CH, Tryp arom), 78.84 (C, *C*CH), 74.92 (C, *t*Bu), 71.74 (CH, C*CH*), 62.72 (CH_2, C$^\beta$ Ser), 60.54 (CH, C$^\alpha$ Pro), 51.59 (CH, C$^\alpha$ Ser), 51.45 (CH, C$^\alpha$ Pra), 48.48 (CH_2, C$^\delta$ Pro), 43.84 (CH, ΨFmoc), 40.07 (CH_2, CH_2CH_2NH), 39.96 (CH_2, ΨFmoc), 29.67 (CH_2, C$^\beta$ Pro), 27.37 (3 x CH_3, *t*Bu), 24.83 (CH_2, CH_2CH_2NH), 24.76 (CH_2, C$^\beta$ Pra), 23.28 (CH_2, C$^\gamma$ Pro). **HRMS** calcd. for $C_{42}H_{47}N_5O_5$ $[M + H]^+$ *m/z* = 702.3656, found *m/z* = 702.3650.

ΨFmoc-L-Pra-L-Ser(OH)-L-Pro-Tryptamine (S18)

*t*Bu-protected compound **14** (141 mg, 0.20 mmol) was dissolved in 30% TFA/DCM (12 mL) with 50 µL of H_2O and 50 µL TIPS. The reactive mixture was stirred for 2 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). The alcohol **S18** (95 mg, 0.147 mmol, 80%, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. $\mathbf{R_f}$**:** 0.30 (DCM/MeOH 9:1). **^{1}H NMR** (400 MHz, MeOD) (2 rotamers *trans-cis* ratio 78:22, *minor rotamer when visible) δ: 7.77 (d, *J* = 7.5 Hz, 2H, ΨFmoc arom), 7.61 – 7.50 (m, 3H, 2H x ΨFmoc arom + Tryp arom), 7.46 – 7.20 (m, 5H, 4H x ΨFmoc arom + Tryp arom), 7.11 – 7.05 (m, 2H, Tryp arom), 7.02 – 6.94 (m, 1H, Tryp arom), 4.86 – 4.78 (m, 0.78H, H$^\alpha$ Ser), 4.68 (dd, *J* = 8.3, 5.6 Hz, 1H, H$^\alpha$ Pra), 4.53* (dd, *J* = 8.1, 5.8 Hz, 0.22H, H$^\alpha$

Ser*), 4.48 – 4.33 (m, 2H, H^{α} Pro + C*H* ΨFmoc), 3.86 (dd, *J* = 10.7, 6.1 Hz, 1H, H^{β} Ser-Ha), 3.82 – 3.69 (m, 3H, H^{β} Ser-Hb + 2H x H^{δ} Pro), 3.60 – 3.40 (m, 2H, CH_2CH_2NH), 3.02 – 2.87 (m, 2H, CH_2CH_2NH), 2.78 – 2.53 (m, 4H, 2H x H^{β} Pra + 2H x CH_2 ΨFmoc), 2.40 (t, *J* = 2.6 Hz, 0.78H, CC*H*), 2.37* (t, *J* = 2.6 Hz, 0.22H, CC*H**), 2.19 – 2.04 (m, 1H, H^{β} Pro-Ha), 1.95 – 1.77 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro). **^{13}C NMR** (101 MHz, MeOD) (2 rotamers *trans-cis* ratio 78:22, *minor rotamer when visible) δ 174.63 (C, CO), 174.06 (C, CO), 172.32 (C, CO), 171.46 (C, CO), 147.76 (2 x C, Arom), 141.95 (2 x C, Arom), 138.13 (C, Arom), 128.80 (C, Arom), 128.48 (2 x CH, ΨFmoc arom), 128.30 (2 x CH, ΨFmoc arom), 125.72 (2 x CH, ΨFmoc arom), 123.58 (CH, Tryp arom), 122.31 (CH, Tryp arom), 120.83 (2 x CH, ΨFmoc arom), 119.59 (CH, Tryp arom), 119.27 (CH, Tryp arom), 113.04 (C, Arom), 112.22 (CH, Tryp arom), 80.43 (C, *C*CH), 72.40 (CH, C*CH*), 63.26 (CH_2, C^{β} Ser), 62.01 (CH, C^{α} Pro), 54.82* (CH, C^{α} Ser*), 54.39 (CH, C^{α} Ser), 53.87 (CH, C^{α} Pra), 48.76 (under the solvent signal, CH_2, C^{δ} Pro), 45.09 (CH, ΨFmoc), 41.32 (CH_2, CH_2CH_2NH), 41.10 (CH_2, ΨFmoc), 30.64 (CH_2, C^{β} Pro), 25.99 (CH_2, CH_2CH_2NH), 25.48 (CH_2, C^{γ} Pro), 22.50 (CH_2, C^{β} Pra). **HRMS** calcd. for $C_{38}H_{39}N_5O_5$ $[M + H]^+$ *m/z* = 646.3029, found *m/z* = 646.3024.

ΨFmoc-L-Pra-L-Ser[OPO(OtBu)$_2$]-L-Pro-Tryptamine (S19)

Compound **S18** (125 mg, 0.195 mmol, 1.0 eq) and ETT (100 mg, 0.776 mmol, 4 eq) were first dissolved in dry THF (10 mL) before adding the (*i*Pr$_2$N)P(O*t*Bu)$_2$ (123 µL, 0.388 mmol, 2 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN-liquid N_2 bath) and 5-6 M *t*BuOOH in decane (160 µL, 0.776 mmol, 4 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (10 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (25 mL) and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Phosphorylated compound **S19** (26 mg, 0.126 mmol, 16% yield or 80% based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ 8.83 (s, 1H, N*H* Indole), 7.74 (d, *J* = 7.2 Hz, 2H, ΨFmoc arom), 7.61 (d, *J* = 7.8 Hz, 1H, Tryp arom), 7.58 – 7.42 (m, 3H, 2H x ΨFmoc arom + N*H* Ser), 7.41 – 7.22 (m, 5H, 4H x ΨFmoc arom + Tryp arom), 7.22 – 6.96 (m, 3H, Tryp arom), 6.92 (t, *J* = 5.7 Hz, 1H, N*H* Tryp), 6.60 (d, *J* = 7.6 Hz, 1H, N*H* Pra), 4.98 – 4.89 (m, 1H, H^{α} Ser), 4.81 – 4.64 (m, 1H, H^{α} Pra), 4.58 – 4.44 (m, 2H, H^{α} Pro + C*H* ΨFmoc), 4.16 – 3.98 (m, 2H, H^{β} Ser), 3.70 – 3.42 (m, 4H, 2H x H^{δ} Pro + 2H x CH_2CH_2NH), 3.06 – 2.89 (m, 2H, CH_2CH_2NH), 2.85 – 2.54 (m, 4H, 2H x H^{β} Pra + 2H x CH_2 ΨFmoc), 2.36 – 2.15 (m, 1H, H^{β} Pro-Ha), 2.09 – 1.84 (m, 4H, CC*H* + H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.44 (d, $J_{P\text{-}H}$ = 8.2 Hz, 18H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ 171.71 (C, CO), 170.80 (C, CO), 169.63 (C, CO), 168.28 (C, CO), 146.43 (C, Arom), 146.38 (C, Arom), 140.84 (2 x C, Arom), 136.52 (C, Arom), 129.62 (C, Arom), 127.60 (2 x CH, ΨFmoc arom), 127.44 (2 x CH, ΨFmoc arom), 124.79 (C, Arom), 124.52 (2 x CH, ΨFmoc arom), 122.57 (CH, Tryp arom), 121.92 (CH, Tryp arom), 120.05 (2 x CH, ΨFmoc arom), 119.19 (CH, Tryp arom), 118.83 (CH, Tryp arom), 112.83 (C, Arom), 111.46 (CH, Tryp arom), 83.90 (d, $J_{C\text{-}P}$ = 4.3 Hz, C, *t*Bu-1), 83.82 (d, $J_{C\text{-}P}$ = 4.4 Hz, C, *t*Bu-2), 79.25 (C, *C*CH), 71.95 (CH, C*CH*), 65.50 (d, $J_{C\text{-}P}$ = 5.7 Hz, CH_2, C^{β} Ser), 60.99 (CH, C^{α} Pro), 51.73 (CH, C^{α} Pra), 51.58 (d, $J_{C\text{-}P}$ = 6.1 Hz, CH, C^{α} Ser), 47.76 (CH_2, C^{δ} Pro), 43.80 (CH, ΨFmoc), 40.53 (CH_2, ΨFmoc), 39.94 (CH_2, CH_2CH_2NH), 29.98 (d, $J_{C\text{-}P}$ = 4.5 Hz, 6 x CH_3, *t*Bu), 28.44 (CH_2, C^{β} Pro), 24.96 (CH_2, CH_2CH_2NH), 24.87 (CH_2, C^{γ} Pro), 22.48 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer) δ: –9.43, –9.75* (s, PO(O*t*Bu)$_2$).

ΨFmoc-L-Pra-L-Ser[OPO(OH)$_2$]-L-Pro-Tryptamine (4c)

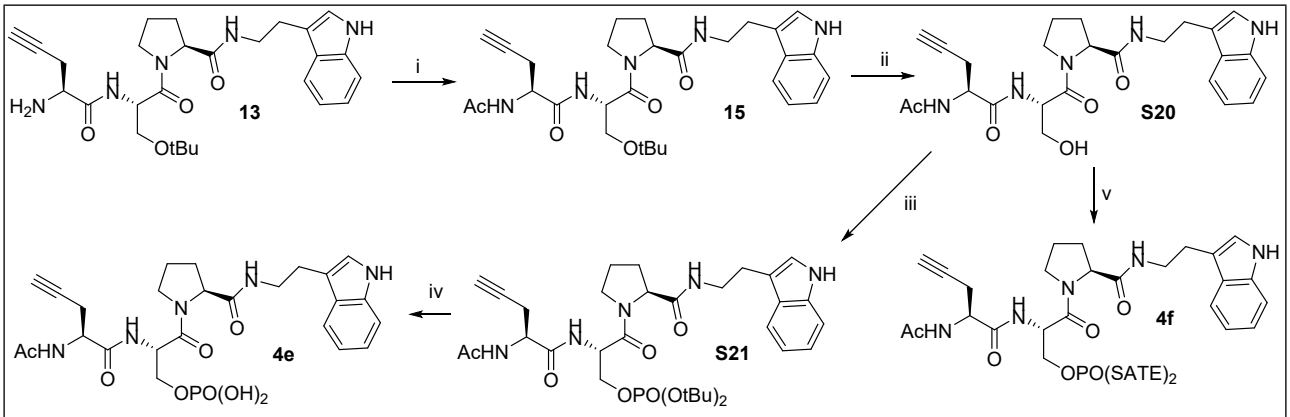
*t*Bu-protected compound (26 mg, 0.126 mmol) was dissolved in 30% TFA/DCM (2 mL) with 5 µL of H_2O and 5 µL TIPS. The reactive mixture was stirred for 1 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude was purified by semi-preparative HPLC (Eluent: solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: from 30% to 70% B over 20 min, then 100% B for 4 min. RT: 11.2 min). Phosphorylated compound L11 (5 mg, 0.007 mmol, 18%, *trans-cis* rotamers mixture) was obtained as a whitish solid after lyophilisation. RT: 7.17 min (analytical HPLC – A: 0.1% TFA/H_2O, B: 0.1% TFA/ACN; from 30% to 70% B over 10 min, then 100% B for 2 min.) **^{1}H NMR** (400 MHz, MeOD) (2 rotamers *trans-cis* ratio 83:17, *minor rotamer when visible) δ: 7.81 – 7.72 (m, 2H, ΨFmoc arom), 7.63 – 7.51 (m, 3H, 2H x ΨFmoc arom + Tryp arom), 7.40 – 7.23 (m, 5H, 4H x ΨFmoc arom + Tryp arom), 7.12 – 7.03 (m, 2H, Tryp arom), 7.00 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H, Tryp arom), 5.00 (t, *J* = 6.2 Hz, 1H, H^{α} Ser), 4.78 – 4.64 (m, 1H, H^{α} Pra), 4.44 – 4.33 (m, 2H, H^{α} Pro + C*H* ΨFmoc), 4.33 – 4.17 (m, 2H, H^{β} Ser), 3.87 – 3.70 (m, 2H, H^{δ} Pro), 3.59 – 3.46 (m, 2H, CH_2CH_2NH), 3.05 – 2.89 (m, 2H, CH_2CH_2NH), 2.81 – 2.54 (m, 4H, 2H x H^{β} Pra + 2H x CH_2 ΨFmoc), 2.41 (t, *J* = 2.6 Hz, 0.83H, CC*H*), 2.37* (t, *J* = 2.5 Hz, 0.17H, CC*H**), 2.19 – 2.06 (m, 1H, H^{β} Pro-Ha), 2.01 – 1.88 (m, 2H, H^{γ} Pro), 1.88 – 1.78 (m, 1H, H^{β} Pro-Hb). **^{13}C NMR** (101 MHz, MeOD) (2 rotamers *trans-cis* ratio 83:17, *minor rotamer when visible) δ: 174.70 (C, CO), 174.28 (C, CO), 172.30 (C, CO), 169.63 (C, CO), 147.76 (2 x C, Arom), 141.97 (2 x C, Arom), 138.14 (C, Arom), 128.84 (C, Arom), 128.49 (2 x CH, ΨFmoc arom), 128.36 (CH, ΨFmoc arom), 128.22 (CH, ΨFmoc arom), 125.77 (CH, ΨFmoc arom), 125.70 (CH, ΨFmoc arom), 123.62 (CH, Tryp arom), 122.28 (CH, Tryp arom),

120.82 (2 x CH, ΨFmoc arom), 119.58 (CH, Tryp arom), 119.36 (CH, Tryp arom), 113.12 (C, Arom), 112.19 (CH, Tryp arom), 80.40 (C, *C*CH), 72.45 (CH, C*CH*), 66.06 (d, $J_{C\text{-}P}$ = 4.5 Hz, CH_2, C^{β} Ser), 62.25 (CH, C^{α} Pro), 53.94 (CH, C^{α} Pra), 53.03 (d, $J_{C\text{-}P}$ = 7.5 Hz, CH, C^{α} Ser), 49.50 (under the solvent signal, CH_2, C^{δ} Pro), 45.06 (CH, ΨFmoc), 41.43 (CH_2, CH_2CH_2NH), 41.10 (CH_2, ΨFmoc), 30.84 (CH_2, C^{β} Pro), 26.06 (CH_2, CH_2CH_2NH), 25.79 (CH_2, C^{γ} Pro), 22.51 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, MeOD) (2 rotamers *trans-cis* ratio 83:17, *minor rotamer) δ: –0.21, –0.74* (s, $PO(OH)_2$). **HRMS** calcd. for $C_{38}H_{40}N_5O_8P$ $[M + H]^+$ *m/z* = 726.2687, found *m/z* = 726.2683.

ΨFmoc-L-Pra-L-Ser[OPO(OSATE)$_2$]-L-Pro-Tryptamine (4d)

Compound **S19** (105 mg, 0.163 mmol, 1.0 eq) was first dissolved in dry THF (8 mL) and 1*H*-tetrazole 0.45 M in ACN (1.45 mL, 0.65 mmol, 4.0 eq) was added, followed by the phosphoramidite **S5** (148 mg, 0.326 mmol, 2.0 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN-liquid N_2 bath) and 5-6 M *t*BuOOH in decane (130 µL, 0.65 mmol, 4.0 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (8 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (20 mL) and washed with water (2 x 40 mL) and brine (40 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Phosphorylated compound **4d** (31 mg, 0.0306 mmol, 20% yield or 80% based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. **R_f:** 0.35 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ: 8.82 (s, 1H, N*H* Indole), 7.74 (d, *J* = 7.5 Hz, 2H, ΨFmoc arom), 7.59 (dd, *J* = 7.9, 3.5 Hz, 2H, N*H* Ser + Tryp arom), 7.50 (dd, *J* = 7.6, 3.7 Hz, 2H, ΨFmoc arom), 7.40 – 7.22 (m, 5H, 4H x ΨFmoc arom + Tryp arom), 7.19 – 7.11 (m, 1H, Tryp arom), 7.11 – 7.01 (m, 2H, Tryp arom), 6.94 (t, *J* = 5.8 Hz, 1H, N*H* Tryp), 6.76 (d, *J* = 7.8 Hz, 1H, N*H* Pra), 4.99 – 4.90 (m, 1H, H^{α} Ser), 4.88 – 4.79 (m, 1H, H^{α} Pra), 4.55 – 4.42 (m, 2H, H^{α} Pro + C*H* ΨFmoc), 4.21 – 3.93 (m, 6H, 2H x H^{β} Ser + 4H x OCH_2CH_2S), 3.71 – 3.39 (m, 4H, 2H x H^{δ} Pro + 2H x CH_2CH_2NH), 3.14 – 2.87 (m, 6H, 2H x CH_2CH_2NH + 4H x OCH_2CH_2S), 2.79 (dd, *J* = 15.0, 6.4 Hz, 1H, CH_2 ΨFmoc-Ha), 2.73 – 2.54 (m, 3H, 2H x H^{β} Pra + CH_2 ΨFmoc-Hb), 2.25 – 2.18 (m, 1H, H^{β} Pro-Ha), 2.07 – 1.87 (m, 4H, CC*H* + H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.18 (s, 18H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ: 206.34 (C, CO SATE), 206.12 (C, CO SATE), 171.99 (C, CO), 170.80 (C, CO), 169.81 (C, CO), 167.53 (C, CO), 146.43 (C, Arom), 146.39 (C, Arom), 140.80 (2 x C, Arom), 136.46 (C, Arom), 127.59 (C, Arom), 127.56 (CH, ΨFmoc arom), 127.52 (CH, ΨFmoc arom), 127.43 (CH, ΨFmoc arom), 127.26 (CH, ΨFmoc arom), 124.78 (CH, ΨFmoc arom), 124.54 (CH, ΨFmoc arom), 122.66 (CH, Tryp arom), 121.93 (CH, Tryp arom), 120.05 (CH, ΨFmoc arom), 120.02(CH, ΨFmoc arom), 119.22 (CH, Tryp arom), 118.71 (CH, Tryp arom), 112.77 (C, Arom), 111.47 (CH, Tryp arom), 79.26 (C, *C*CH), 71.85 (CH, C*CH*), 66.64 (d, $J_{C\text{-}P}$ = 2.9 Hz, CH_2, OCH_2CH_2S-1), 66.58 (d, $J_{C\text{-}P}$ = 2.5 Hz, CH_2, OCH_2CH_2S-2), 66.37 (d, $J_{C\text{-}P}$ = 5.7 Hz, CH_2, C^{β} Ser), 61.06 (CH, C^{α} Pro), 51.74 (CH, C^{α} Pra), 51.60 (d, $J_{C\text{-}P}$ = 5.5 Hz, CH, C^{α} Ser), 47.70 (CH_2, C^{δ} Pro), 46.65 (d, *J* = 3.7 Hz, 2 x C, *t*Bu), 43.78 (CH, ΨFmoc), 40.42 (CH_2, ΨFmoc), 39.94 (CH_2, CH_2CH_2NH), 28.45 (d, $J_{C\text{-}P}$ = 2.8 Hz, CH_2, OCH_2CH_2S-1), 28.38 (d, $J_{C\text{-}P}$ = 2.9 Hz, CH_2, OCH_2CH_2S-2), 28.32 (CH_2, C^{β} Pro), 27.38 (6 x CH_3, *t*Bu), 24.91 (2 x CH_2, CH_2CH_2NH + C^{γ} Pro), 22.31 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) δ: –1.38 (s, $PO(OSATE)_2$). **HRMS** calcd. for $C_{52}H_{64}N_5O_{10}PS_2$ $[M + H]^+$ *m/z* = 1014.3905, found *m/z* = 1014.3903.

2.5.3 Synthesis of 4e and 4f



i) Ac_2O, DCM, rt, 16 h (**98%**); vi) 30% TFA/DCM, TIPS, H_2O, rt, 2h (**98%**); ii) (a) $P(OtBu)_2N(iPr)_2$, 5-ethylthio-H-tetrazole, THF, rt, 16 h (b) tBuOOH, -45 °C to rt, 1 h (c) $Na_2S_2O_3$, -45 °C to rt (**50%** or **89%** based on recovered starting material); iii) 30% TFA/DCM, TIPS, H_2O, rt, 2h (**30%**); iv) $P(OSATE)_2N(iPr)_2$, 5-ethylthio-H-tetrazole THF/DCM 1:1, rt, 16 h, (b) tBuOOH, -45 °C to rt, 1h (c) $Na_2S_2O_3$, -45 °C to rt (**35%** or **81%** based on recovered starting material).

Scheme S9: Synthesis of **4e** and **4f**.

Ac-L-Pra-L-Ser(OtBu)-L-Pro-Tryptamine (15)

Amine **13** (330 mg, 0.46 mmol) was dissolved in DCM (10 mL) and an excess of Ac_2O (200 µL) was added. The mixture was stirred at rt overnight and the solvents were evaporated under reduced pressure. The crude compound was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Peptide **15** (240 mg, 0.45 mmol, 98%, *trans-cis* rotamers mixture) was obtained as a yellow solid. $\mathbf{R_f}$: 0.50 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, *minor rotamer when visible) δ: 8.91 (s, 1H, N*H* Indole), 8.20 (s, 1H, N*H* Ser), 7.51 (d, *J* = 7.8 Hz, 1H, Tryp arom), 7.42 (bs, 1H, N*H* Tryp), 7.33 (d, *J* = 8.1 Hz, 1H, Tryp arom), 7.14 (t, *J* = 7.6 Hz, 1H, Tryp arom), 7.07 – 6.99 (m, 2H, Tryp arom), 6.20* (d, *J* = 7.8 Hz, 0.10H, N*H* Pra*), 6.08 (d, *J* = 6.7 Hz, 0.90H, N*H* Pra), 5.21 (q, *J* = 8.5 Hz, 0.90H, H^α Ser), 4.82* (d, *J* = 8.1 Hz, 0.10H, H^α Pro*), 4.62 – 4.54* (m, 0.10H, H^α Pra*), 4.47 – 4.34 (m, 1.90H, 0.90 x H^α Pro +0.90 x H^α Pra + 0.10 x H^α Ser*), 3.97 – 3.81 (m, 2H, H^δ Pro), 3.80 – 3.60 (m, 3H, 2H x H^β Ser + CH_2CH_2NH-Ha), 3.47 – 3.34 (m, 1H, CH_2CH_2NH-Hb), 3.09 – 2.87 (m, 2H, CH_2CH_2NH), 2.60 – 2.48 (m, 1H, H^β Pra-Ha), 2.34 – 2.24 (m, 1H, H^β Pra-Hb), 2.22 – 2.03 (m, 3H, 2H x H^β Pro + H^γ Pro-Ha), 1.95 – 1.84 (m, 2H, H^γ Pro-Hb + CC*H*), 1.51 (s, 3H, Ac), 1.17 (s, 9H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, *minor rotamer when visible) δ: 171.42 (C, CO), 170.55 (C, CO), 169.72 (C, CO), 169.62, (C, CO), 136.44 (C, Tryp arom), 127.27 (C, Tryp arom), 123.06 (CH, Tryp arom), 121.73 (CH, Tryp arom), 119.04 (CH, Tryp arom), 118.48 (CH, Tryp arom), 112.37 (C, Tryp arom), 111.53 (CH, Tryp arom), 78.79 (C, *C*CH), 75.13 (C, *t*Bu), 71.24 (CH, C*CH*), 62.67 (CH_2, C^β Ser), 60.39 (CH, C^α Pro), 51.54 (CH, C^α Ser), 51.02 (CH, C^α Pra), 48.62 (CH_2, C^δ Pro), 39.55 (CH_2, CH_2CH_2NH), 29.88 (CH_2, C^β Pro), 27.46 (C, *t*Bu), 24.80 (CH_2, C^γ Pro), 24.47 (CH_2, CH_2CH_2NH), 23.44 (CH_2, C^β Pra), 22.72 (CH_3, Ac). **HRMS** calcd. for $C_{29}H_{39}N_5O_5$ $[M + H]^+$ *m/z* = 538.3024 and $[M + Na]^+$ *m/z* = 560.2843, found *m/z* = 538.3046 and *m/z* = 560.2866.

Ac-L-Pra-L-Ser(OH)-L-Pro-Tryptamine (S20)

*t*Bu-protected compound **15** (60 mg, 0.11 mmol) was dissolved in 30% TFA/DCM (6 mL) with 20 µL of H_2O and 20 µL TIPS. The reactive mixture was stirred for 2 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Alcohol **S20** (53 mg, 0.11 mmol, 98%, *trans-cis* rotamers mixture) was obtained as a yellow solid. $\mathbf{R_f}$: 0.58 (DCM/MeOH 95:5). **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, major rotamer reported) δ: 9.08 (s, 1H, N*H* Indole), 8.38 (d, *J* = 8.4 Hz, 1H, N*H* Ser), 7.45 (d, *J* = 7.7 Hz, 2H, Tryp arom + N*H* Tryp), 7.34 (d, *J* = 8.1 Hz, 1H, Tryp arom), 7.13 (t, *J* = 7.6 Hz, 1H, Tryp arom), 7.03 (t, *J* = 7.5 Hz, 1H, Tryp arom), 6.95 (s, 1H, Tryp arom), 6.38 (d, *J* = 7.6 Hz, 1H, N*H* Pra), 5.17 – 5.07 (m, 1H, H^α Ser), 4.85 (bs, 1H, O*H*), 4.77 (q, *J* = 5.6 Hz, 1H, H^α Pra), 4.63 (dd, *J* = 8.2, 5.3 Hz, 1H, H^α Pro), 3.95 – 3.80 (m, 3H, 2H x H^β Ser + H^δ Pro-Ha), 3.77 – 3.67 (m, 1H, H^δ Pro-Hb), 3.68 – 3.55 (m, 1H, CH_2CH_2NH-Ha), 3.38 – 3.26 (m, 1H, CH_2CH_2NH-Hb), 2.86 – 2.65 (m, 2H, CH_2CH_2NH), 2.59 – 2.37 (m, 2H, H^β Pra), 2.23 – 1.83 (m, 5H, 2H x H^β Pro + 2H x H^γ Pro + CC*H*), 1.56 (s, 3H, Ac). **^{13}C NMR (**101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 90:10, major rotamer reported) δ: 172.72 (C, CO), 170.75 (C, CO), 169.82 (C, CO), 169.59 (C, CO), 136.51 (C, Tryp arom), 127.38 (C, Tryp arom), 122.45 (CH, Tryp arom), 121.80 (CH, Tryp arom), 119.03 (CH, Tryp arom), 118.44 (CH, Tryp arom), 112.22 (C, Tryp arom), 111.50 (CH, Tryp arom), 78.97 (C, *C*CH), 71.39 (CH, C*CH*), 63.51 (CH_2, C^β Ser), 60.51 (CH, C^α Pro), 53.12 (CH, C^α Ser), 51.04 (CH, C^α Pra), 48.38 (CH_2, C^δ Pro), 39.53 (CH_2, CH_2CH_2NH), 29.96 (CH_2, C^β Pro), 25.09 (CH_2, C^γ Pro), 24.32 (CH_2, CH_2CH_2NH), 23.83 (CH_2, C^β Pra), 22.82 (CH_3, Ac). **HRMS** calcd. for $C_{25}H_{31}N_5O_5$ $[M + H]^+$ *m/z* = 482.2398 and $[M + Na]^+$ *m/z* = 504.2217, found *m/z* = 482.2412 and *m/z* = 504.2231.

Ac-L-Pra-L-Ser[OPO(OtBu)$_2$]-L-Pro-Tryptamine (S21)

Compound **S20** (125 mg, 0.26 mmol, 1.0 eq) and ETT (135 mg, 1.04 mmol, 4 eq) were first dissolved in dry THF (13 mL) before adding the (iPr$_2$N)P(OtBu)$_2$ (164 µL, 0.52 mmol, 2 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN-liquid N_2 bath) and 5-6 M *t*BuOOH in decane (208 µL, 1.04 mmol, 4 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (13 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (25 mL) and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Phosphorylated compound **S21** (85 mg, 0.126 mmol, 50% yield or 89% based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer when visible) δ: 8.80 (s, 1H, N*H* Indole), 7.60 (d, *J* = 7.9 Hz, 1H, Tryp arom), 7.44 (d, *J* = 7.6 Hz, 1H, N*H* Ser), 7.37 (d, *J* = 8.1 Hz, 1H, Tryp arom), 7.17 (t, *J* = 7.0 Hz, 1H, Tryp arom), 7.13 – 6.98 (m, 2H, Tryp arom), 6.89 (t, *J* = 5.7 Hz, 1H, N*H* Tryp), 6.63 (d, *J* = 7.8 Hz, 1H, N*H* Pra), 4.92 (q, *J* = 6.0 Hz, 1H, H^α Ser), 4.72 – 4.61 (m, 1H, H^α Pra), 4.56 – 4.44 (m, 1H, H^α Pro), 4.18 – 3.94 (m, 2H, H^β Ser), 3.74 – 3.41 (m, 4H, 2H x H^δ Pro + 2H x CH_2CH_2NH), 3.06 – 2.88 (m, 2H, CH_2CH_2NH), 2.78 – 2.57 (m, 2H, H^β Pra), 2.40 – 1.84 (m, 8H, 2H x H^β Pro + 2H x H^γ Pro + CC*H* + 3H x Ac), 1.48 (s, 11H, *t*Bu), 1.47* (s, 7H, *t*Bu*). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, major rotamer reported) δ: 170.82 (C, CO), 170.53 (C, CO), 169.76 (C, CO), 168.21 (C, CO), 136.50 (C, Tryp arom), 127.60 (C, Tryp arom), 122.60 (CH, Tryp arom), 121.95 (CH, Tryp arom), 119.21 (CH, Tryp arom), 118.83

(CH, Tryp arom), 112.76 (C, Tryp arom), 111.45 (CH, Tryp arom), 83.98 (d, $J_{C\text{-}P}$ = 4.4 Hz, C, *t*Bu-1), 83.90 (d, $J_{C\text{-}P}$ = 4.3 Hz, C, *t*Bu-2), 79.30 (C, *C*CH), 71.81 (CH, C*CH*), 65.45 (d, $J_{C\text{-}P}$ = 6.1 Hz, CH_2, C^{β} Ser), 60.96 (CH, C^{α} Pro), 51.60 (d, $J_{C\text{-}P}$ = 6.7 Hz, CH, C^{α} Ser), 51.47 (CH, C^{α} Pra), 47.76 (CH_2, C^{δ} Pro), 39.91 (CH_2, CH_2*CH*$_2$NH), 29.98 (d, $J_{C\text{-}P}$ = 4.3 Hz, 6 x CH_3, *t*Bu), 28.39 (CH_2, C^{β} Pro), 24.91 (2 x CH_2, *CH*$_2$$CH_2$NH + C^{γ} Pro), 23.25 (CH_3, Ac), 22.45 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer) δ: –9.37, –9.65* (s, PO(O*t*Bu)$_2$). **HRMS** calcd. for $C_{33}H_{48}N_5O_8P$ $[M + Na]^+$ *m/z* = 696.3133, found *m/z* = 696.3146.

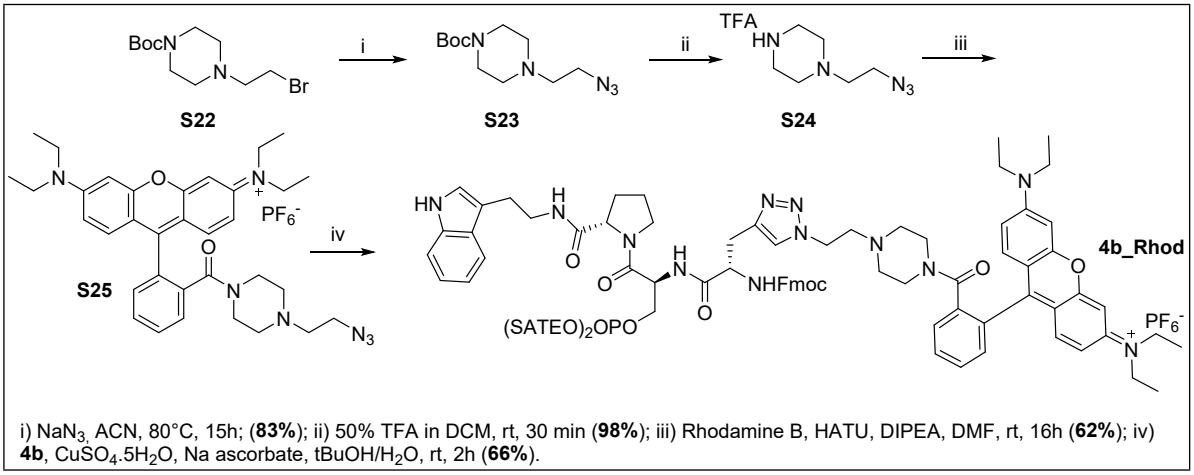
Ac-L-Pra-L-Ser[OPO(OH)$_2$]-L-Pro-Tryptamine (4e)

*t*Bu-protected compound **S21** (85 mg, 0.126 mmol) was dissolved in 30% TFA/DCM (6 mL) with 20 µL of H_2O and 20 µL TIPS. The reactive mixture was stirred for 2 h at rt until disappearance of the starting material on the TLC. Then, the solvent was evaporated under reduced pressure. The crude was purified by semi-preparative HPLC (Eluent: solvent A 0.1% TFA/H_2O, solvent B 0.1% TFA/ACN; Method: from 5% to 100% B over 20 min. RT: 9.92 min). Phosphorylated compound **4e** (21 mg, 0.0374 mmol, 30%, *trans-cis* rotamers mixture) was obtained as a whitish solid after lyophilisation. RT: 5.20 min (analytical HPLC – A: 0.1% TFA/H_2O, B: 0.1% TFA/ACN; from 10% to 100% B over 10 min) **^{1}H NMR** (400 MHz, MeOD) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer when visible) δ: 7.58 (d, *J* = 7.9 Hz, 1H, Tryp arom), 7.32 (d, *J* = 8.0 Hz, 1H, Tryp arom), 7.11 – 7.03 (m, 2H, Tryp arom), 7.04 – 6.95 (m, 1H, Tryp arom), 4.98 (d, *J* = 6.5 Hz, 0.85H, H^{α} Ser), 4.71 – 4.63* (m, 0.30H, H^{α} Ser* + H^{α} Pro*), 4.51 (dd, *J* = 8.0, 5.7 Hz, 1H, H^{α} Pra), 4.34 (dd, *J* = 8.4, 4.9 Hz, 0.85H, H^{α} Pro), 4.29 – 4.13 (m, 1.70H, H^{β} Ser), 4.08 – 4.01* (m, 0.30H, H^{β} Ser*), 3.83 – 3.66 (m, 2H, H^{δ} Pro), 3.57 – 3.41 (m, 2H, CH_2C*H*$_2$NH), 2.95 (t, *J* = 7.9 Hz, 2H, C*H*$_2$$CH_2$NH), 2.74 – 2.50 (m, 2H, H^{β} Pra), 2.39 (t, *J* = 2.6 Hz, 0.85H, CC*H*), 2.35* (t, *J* = 2.7 Hz, 0.15H, CC*H**), 2.20 – 2.05 (m, 1H, H^{β} Pro-Ha), 2.01 (s, 2.55H, Ac), 1.99* (s, 0.45H, Ac*), 1.97 – 1.87 (m, 2H, H^{γ} Pro), 1.86 – 1.76 (m, 1H, H^{β} Pro-Hb). **^{13}C NMR** (101 MHz, MeOD) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer when visible) δ: 174.28 (C, CO), 173.56 (C, CO), 172.38 (C, CO), 169.56 (C, CO), 138.12 (C, Tryp arom), 128.81 (C, Tryp arom), 123.62 (CH, Tryp arom), 122.27 (CH, Tryp arom), 119.56 (CH, Tryp arom), 119.34 (CH, Tryp arom), 113.07 (C, Tryp arom), 112.19 (CH, Tryp arom), 80.24 (C, *C*CH), 72.32 (CH, C*CH*), 65.99 (d, $J_{C\text{-}P}$ = 4.9 Hz, CH_2, C^{β} Ser), 62.19 (CH, C^{α} Pro), 53.69 (CH, C^{α} Pra), 52.94 (d, $J_{C\text{-}P}$ = 7.4 Hz, CH, C^{α} Ser), 48.88 (CH_2, C^{δ} Pro), 41.41 (CH_2, CH_2*CH*$_2$NH), 30.85 (CH_2, C^{β} Pro), 26.05 (CH_2, *CH*$_2$$CH_2$NH), 25.77 ($CH_2$, C^{γ} Pro), 22.49 (CH_2, C^{β} Pra), 22.40 (CH_3, Ac). ^{31}P NMR (162 MHz, MeOD) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer) δ: –0.16, –0.73 (s, PO(OH)$_2$). **HRMS** calcd. for $C_{25}H_{32}N_5O_8P$ $[M + H]^+$ *m/z* = 562.2061, found *m/z* = 562.2061.

Ac-L-Pra-L-Ser[OPO(OSATE)$_2$]-L-Pro-Tryptamine (4f)

Compound **S20** (125 mg, 0.26 mmol, 1.0 eq) and ETT (135 mg, 1.04 mmol, 4 eq) were first dissolved in dry THF (13 mL) before adding the phosphoramidite **S5** (236 mg, 0.52 mmol, 2 eq). The mixture was stirred at rt overnight. The mixture was then cooled down to –40°C (ACN-liquid N_2 bath) and 5-6 M *t*BuOOH in decane (208 µL, 1.04 mmol, 4 eq) was added. The reaction was allowed to reach rt and stirred for 1h. The mixture was once again cooled down to –40°C and a saturated solution of $Na_2S_2O_3$ (13 mL) was added. The reaction was allowed to reach rt and stirred for 1h. It was diluted with AcOEt (25 mL) and washed with water (2 x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Phosphorylated compound **4f** (77 mg, 0.091 mmol, 35% yield or 81% based on recovered starting material, *trans-cis* rotamers mixture) was obtained as a pale-yellow solid. **^{1}H NMR** (400 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer when visible) δ: 8.82 (s, 0.85H, N*H* Indole), 8.70* (s, 0.15H, N*H* Indole*), 7.64 – 7.55 (m, 2H, N*H* Ser + Tryp arom), 7.37 (d, *J* = 8.0 Hz, 1H, Tryp arom), 7.21 – 7.07 (m, 1H, Tryp arom), 7.12 – 7.02 (m, 2H, Tryp arom), 6.96 (t, *J* = 5.7 Hz, 1H, N*H* Tryp), 6.80 (d, *J* = 7.9 Hz, 1H, N*H* Pra), 4.93 (td, *J* = 6.8, 4.2 Hz, 1H, H^{α} Ser), 4.77 – 4.68 (m, 1H, H^{α} Pra), 4.53 – 4.41 (m, 1H, H^{α} Pro), 4.21 – 3.96 (m, 6H, 2H x H^{β} Ser + 4H x OC*H*$_2$$CH_2$S), 3.73 – 3.39 (m, 4H, 2H x H^{δ} Pro + 2H x CH_2C*H*$_2$NH), 3.17 – 3.03 (m, 4H, OCH_2C*H*$_2$S), 3.03 – 2.88 (m, 2H, C*H*$_2$$CH_2$NH), 2.76 – 2.59 (m, 2H, H^{β} Pra), 2.20 (s, 1H, H^{β} Pro-Ha), 2.09 – 1.88 (m, 7H, H^{β} Pro-Hb + CC*H* + 3H x Ac + 2H x H^{γ} Pro), 1.23 (s, 18H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) (2 rotamers *trans-cis* ratio 85:15, *minor rotamer when visible) δ: 206.44 (C, CO SATE), 206.22 (C, CO SATE), 170.91 (2 x C, CO), 169.97 (C, CO), 167.55 (C, CO), 136.48 (C, Tryp arom), 127.59 (C, Tryp arom), 122.72 (CH, Tryp arom), 121.95 (CH, Tryp arom), 119.24 (CH, Tryp arom), 118.72 (CH, Tryp arom), 112.70 (C, Tryp arom), 111.48 (CH, Tryp arom), 79.28 (C, *C*CH), 71.72 (CH, C*CH*), 66.67 (d, $J_{C\text{-}P}$ = 5.9 Hz, 2 x CH_2, O*CH*$_2$$CH_2$S), 66.35 (d, $J_{C\text{-}P}$ = 5.2 Hz, CH_2, C^{β} Ser), 61.08 (CH, C^{α} Pro), 51.62 (d, $J_{C\text{-}P}$ = 5.1 Hz, C^{α} Ser), 51.54 (CH, C^{α} Pra), 47.74 (CH_2, C^{δ} Pro), 46.72 (d, $J_{C\text{-}P}$ = 4.3 Hz, 2 x C, *t*Bu), 39.97 (CH_2, CH_2*CH*$_2$NH), 28.46 (d, $J_{C\text{-}P}$ = 7.9 Hz, 2 x CH_2, OCH_2*CH*$_2$S), 28.35 (CH_2, C^{β} Pro), 27.44 (d, $J_{C\text{-}P}$ = 1.7 Hz, 6 x CH_3, *t*Bu), 24.95 (CH_2, *CH*$_2$$CH_2$NH), 24.90 ($CH_2$, C^{γ} Pro), 23.20 (CH_3, Ac), 22.27 (CH_2, C^{β} Pra). **^{31}P NMR** (162 MHz, $CDCl_3$) δ: –1.42 (s, PO(OSATE)$_2$). **HRMS** calcd. for $C_{39}H_{56}N_5O_{10}PS_2$ $[M + H]^+$ *m/z* = 850.3279 and $[M + Na]^+$ *m/z* = 872.3098, found *m/z* = 850.3277 and *m/z* = 872.3103.

2.6 Synthesis of 4b-Rhod



i) NaN_3, ACN, 80°C, 15h; (**83%**); ii) 50% TFA in DCM, rt, 30 min (**98%**); iii) Rhodamine B, HATU, DIPEA, DMF, rt, 16h (**62%**); iv) **4b**, $CuSO_4.5H_2O$, Na ascorbate, $tBuOH/H_2O$, rt, 2h (**66%**).

Scheme S10: Synthesis of **4b-Rhod**.

***Tert*-butyl 4-(2-azidoethyl)piperazine-1-carboxylate (S23)**

Piperazine derivative **S22** (300 mg, 1.02 mmol, 1.0 eq) was dissolved in ACN (5 mL), then NaN_3 (186 mg, 2.86 mmol, 2.8 eq) was added and the solution was stirred at 82°C for 15 h. The solution was diluted with AcOEt (10 mL) and washed with water (2 x 20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude orange oil **S23** (216 mg, 0.846 mmol, 83%) was used in the next step without further purification. (Characterisation data in accordance with those reported in literature: Tahtaoui C., *et al. J. Med. Chem.* **2004**, *47*, 4300). $\mathbf{R_f}$**:** 0.30 (cyHex/AcOEt 7:3) **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 3.44 (t, *J* = 5.0 Hz, 4H, CH_2 piperazine), 3.35 (t, *J* = 6.0 Hz, 2H, $NCH_2CH_2N_3$), 2.60 (dd, *J* = 6.5, 5.5 Hz, 2H, $NCH_2CH_2N_3$), 2.44 (t, *J* = 5.0 Hz, 4H, CH_2 piperazine), 1.46 (s, 9H, *t*Bu).

1-(2-azidoethyl)piperazine, TFA salt (S24)

Boc protected compound **S23** (216 mg, 0.846 mmol) was dissolved in 1:1 TFA/DCM solution (10 mL) and stirred at rt for 30 min. The solvents were evaporated under reduced pressure and the crude whitish solid **S24** (228 mg, 0.83 mmol, 98%) was used in the next step without further purification. (Characterisation data in accordance with those reported in literature: Tahtaoui C., *et al. J. Med. Chem.* **2004**, *47*, 4300). **^{1}H NMR** (400 MHz, Acetone-d_6) δ 3.86 – 3.77 (m, 2H, $NCH_2CH_2N_3$), 3.73 – 3.66 (m, 4H, CH_2 piperazine), 3.65 – 3.51 (m, 4H, CH_2 piperazine), 3.37 – 3.23 (m, 2H, $NCH_2CH_2N_3$).

1-(2-azidoethyl)piperazine-Rhodamine, PF_6 salt (S25)

Rhodamine B (50 mg, 0.113 mmol, 1 eq), amine **S24** (45.8 mg, 0.170 mmol, 1.5 eq), HATU (76 mg, 0.2 mmol, 1.5 eq) and DIPEA (0.05 mL, 0.33 mmol, 2.9 eq) were dissolved in DMF (3 mL) The mixture was stirred at rt for 2 h, then it was diluted with AcOEt (5 mL) and washed with water (3 x 5 mL) and brine (5 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude deep-purple solid **112** (50 mg, 0.07 mmol, 62%) was used in the next step without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 7.73 – 7.61 (m, 2H, Rhod arom), 7.56 (dd, *J* = 7.1, 1.9 Hz, 1H, Rhod arom), 7.32 (dd, *J* = 7.4, 1.6 Hz, 1H, Rhod arom), 7.23 (d, *J* = 9.5 Hz, 2H, Rhod arom), 6.95 (dd, *J* = 9.6, 2.4 Hz, 2H, Rhod arom), 6.75 (d, *J* = 2.4 Hz, 2H, Rhod arom), 3.70 – 3.51 (m, 8H, $N(CH_2CH_3)_2$), 3.51 – 3.36 (m, 6H, 4H x CH_2 piperazine + 2H x $NCH_2CH_2N_3$), 2.64 (t, *J* = 5.8 Hz, 2H, $NCH_2CH_2N_3$), 2.56 – 2.42 (m, 4H, CH_2 piperazine), 1.32 (t, *J* = 7.1 Hz, 12H, $N(CH_2CH_3)_2$). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 167.56 (C, CO), 157.87 (2 x C, Rhod arom), 156.21 (C, Rhod arom), 155.77 (2 x C, Rhod arom), 135.33 (C, Rhod arom), 132.28 (2 x CH, Rhod arom), 130.78 (C, Rhod arom), 130.33 (CH, Rhod arom), 130.27 (CH, Rhod arom), 130.08 (CH, Rhod arom), 127.82 (CH, Rhod arom), 114.30 (2 x CH, Rhod arom), 113.89 (2 x C, Rhod arom), 96.31 (2 x CH, Rhod arom), 56.82 (CH_2, $NCH_2CH_2N_3$), 52.77 (2 x CH_2, CH_2 piperazine), 47.69 (CH_2, $NCH_2CH_2N_3$), 47.20 (CH_2, CH_2 piperazine), 46.18 (4 CH_2, $N(CH_2CH_3)_2$), 41.35 (CH_2, CH_2 piperazine), 12.70 (4 CH_3, $N(CH_2CH_3)_2$). **HRMS** calcd. for $C_{34}H_{42}N_7O_2^+$ $[M]^+$ *m/z* = 580.3395, found $[M]^+$ *m/z* = 580.3384.

4b-Rhod

The alkyne **4b** (30 mg, 0.03 mmol) and the azide **S25** (17 mg, 0.03 mmol) were dissolved in 2:1 *t*BuOH/H_2O solution (2 mL). The mixture was sonicated for few seconds to allow complete dissolution. $CuSO_4 \cdot 5H_2O$ (0.012 mmol) and Na ascorbate (0.012 mmol) were separately dissolved in water (around 0.3 mL for each salt) and sonicated to allow complete dissolution. They were added sequentially ($CuSO_4 \cdot 5H_2O$ first, followed by Na ascorbate) to the reactive mixture which was then stirred at rt for 2h. After being diluted with DCM (5 mL) and washed with water (2 x 5 mL) and brine (5 mL), the organic layers were collected and dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash chromatography on silica gel (DCM/MeOH step gradient from 0% MeOH to 5%). Fluorescent probe **4a-Rhod** (32 mg, 0.0182 mmol, 66%) was obtained as a deep-purple solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ: 8.99 (s, 1H, N*H* Indole), 7.73 (d, *J* = 7.7 Hz, 2H, Fmoc arom), 7.66 – 7.44 (m, 6H, 2H x Fmoc arom + 3H x Rhod arom + CH Tryp), 7.43 – 7.24 (m, 7H, 4H x Fmoc arom + CH Tryp + Rhod arom + N*H* Ser), 7.20 (d, *J* = 9.4 Hz, 2H, Rhod arom), 7.16 – 6.92 (m, 6H, 2H x Rhod arom + C*H* Triazole + 3H x CH Tryp), 6.88 (s, 1H, N*H* Tryp), 6.69 – 6.64 (m, 2H, Rhod arom), 6.16 (s, 1H, N*H* Pra), 4.87 (d, *J* = 7.0 Hz, 1H, H^{α} Ser), 4.58 (d, *J* = 6.4 Hz, 1H, H^{α} Pra), 4.48 (s, 1H, H^{α} Pro), 4.39 (bs, 2H, NC*H*$_2$CH$_2$Triazole), 4.28 (d, *J* = 6.8 Hz, 2H, C*H*$_2$ Fmoc), 4.20 (d, *J* = 7.3 Hz, 1H, C*H* Fmoc), 4.13 – 3.85 (m, 6H, 2H x H^{β} Ser + 4H x OC*H*$_2$CH$_2$S), 3.70 – 3.45 (m, 12H, 8H x N(C*H*$_2$CH$_3$)$_2$ + 2H x H^{δ} Pro + 2H x CH$_2$C*H*$_2$NH), 3.45 – 3.29 (m, 4H, C*H*$_2$ piperazine), 3.29 – 3.17 (m, 2H, H^{β} Pra), 3.18 – 2.85 (m, 6H, 4H x OCH$_2$C*H*$_2$S + 2H x C*H*$_2$CH$_2$NH), 2.74 (bs, 2H, NCH$_2$C*H*$_2$Triazole), 2.55 – 2.10 (m, 5H, 4H x C*H*$_2$ piperazine + H^{β} Pro-Ha), 2.08 – 1.83 (m, 3H, H^{β} Pro-Hb + 2H x H^{γ} Pro), 1.33 – 1.23 (m, 12H, N(CH$_2$C*H*$_3$)$_2$), 1.19 (s, 18H, *t*Bu). **^{13}C NMR** (101 MHz, $CDCl_3$) δ: 205.98 (2 x C, CO SATE), 171.06 (C, CO), 168.11 (C, CO), 167.36 (C, CO), 157.79 (C, CO), 156.34 (C, CO), 155.72 (C, CO), 144.01 (C, Arom), 141.28 (C, Arom), 136.51 (C, Arom), 135.65 (C, Arom), 132.25 (2 x CH, Rhod arom), 130.92 (C, Arom), 130.13 (CH, Rhod arom), 129.83 (2 x CH, Rhod arom), 127.78 (3 x CH, 2 x Fmoc arom + Rhod arom), 127.51 (C, Arom), 127.31 (2 x CH, Fmoc arom), 125.53 (2 x CH, Fmoc arom), 123.33 (CH, *CH* Triazole), 121.66 (CH, Tryp arom), 119.97 (2 x CH, Fmoc arom), 118.99 (CH, Tryp arom), 118.56 (CH, Tryp arom), 117.16 (C, Arom), 114.40 (2 x CH, Rhod arom), 113.90 (CH, Tryp arom), 112.38 (C, Arom), 111.59 (CH, Tryp arom), 96.11 (2 x CH, Rhod arom), 67.47 (CH$_2$, Fmoc), 66.67 (3 x CH$_2$, 2 x OC*H*$_2$CH$_2$S + C^{β} Ser), 61.18 (CH, C^{α} Pro), 57.20 (CH$_2$, NCH$_2$C*H*$_2$Triazole), 54.71 (CH, C^{α} Pra), 53.17 (CH$_2$, *CH*$_2$ piperazine), 52.46 (CH$_2$, *CH*$_2$ piperazine), 51.44 (CH, C^{α} Ser), 47.72 (2 x CH$_2$, *CH*$_2$ piperazine + C^{δ} Pro), 47.46 (CH$_2$, NC*H*$_2$CH$_2$Triazole), 47.12 (CH, Fmoc), 46.61 (2 x C, *t*Bu), 46.17 (4 x CH$_2$, N(*CH*$_2$CH$_3$)$_2$), 41.86 (CH$_2$, *CH*$_2$ piperazine), 39.81 (CH$_2$, CH$_2$*CH*$_2$NH), 28.57 (CH$_2$, C^{β} Pra), 28.49 (3 x CH$_2$, 2 x OCH$_2$*CH*$_2$S + C^{β} Pro), 27.41 (6 x CH$_3$, *t*Bu), 24.89 (2 x CH$_2$, C^{γ} Pro + *CH*$_2$CH$_2$NH), 12.71 (4 x CH$_3$, N(CH$_2$*CH*$_3$)$_2$). **^{31}P NMR** (162 MHz, $CDCl_3$) δ: –1.74 (s, PO(OSATE)$_2$), –134.97 – (–154.07) (m, PF_6^-). **HRMS** calcd. for $C_{86}H_{106}N_{12}O_{13}PS_2^+$ $[M]^+$ m/z = 1609.7176 and $[M + H]^{2+}$ *m/z* = 805.3625, found $[M]^+$ *m/z* = 1609.7156 and $[M + H]^{2+}$ *m/z* = 805.8629.

3 NMR AFFINITY ASSAY (CSPs)

3.1 Isotopically Enriched Pin1 Expression

3.1.1 Pin1 Expression

To carry out the structural and dynamical investigations on Pin1, ^{15}N-labelled protein samples were prepared using the Marley protocol (Marley, J.; Lu, M.; Bracken, C. A *J. Biomol. NMR* **2001**, *20*, 71.)
First, 50 mL of sterilised LB media is inoculated with 400 µL glycerol stock of transformed BL21 (DE3) cells containing the gene for Pin1 enzyme. This preculture is incubated overnight at 37°C, shaking at 220 rpm to reach an OD_{600} (Optical Density at 600 nm) of 4-8. 4 L of sterilised LB media is split into 500 mL aliquots in 2 L flasks and each aliquot is inoculated with the preculture to reach an OD_{600} of 0.05. 100 µg/mL ampicillin and 30 µg/mL chloramphenicol are also added to each 500 mL aliquot. The cultures are incubated at 37°C shaking at 220 rpm until an OD_{600} of 0.6-0.8 is reached. At this stage, if isotopic labelling is desired, the cultures are pooled and centrifuged at 3000 x g for 20 minutes at 20°C. The cell pellet is then resuspended in 1 L of sterilised M9 media and incubated at 37°C, shaking at 220 rpm, for 30 minutes. Carbon and nitrogen sources are then provided via 1g $^{15}NH_4Cl$ or $^{14}NH_4Cl$ and 4 g $^{12}C_6H_{12}O_6$ or $^{13}C_6H_{12}O_6$ (according to the desired labelling) and the culture is again incubated at 37°C for 30 minutes. Protein expression can then be induced with the addition of 0.5 mM IPTG. The cultures are incubated with IPTG for 4 hours at 37°C, shaking at 220 rpm. They are then pooled and centrifuged at 6000 x g for 20 minutes at 4°C. The cell pellet is resuspended in 80 mL of the appropriate binding buffer and stored at –80°C until purification.

3.1.2 Isolation of the Cell Free Extract

A frozen pellet of *E. coli* cells containing the overexpressed protein in 80 mL of Binding Buffer (25 mM NaH_2PO_4, 500 mM NaCl, 30 mM imidazole, 1 mM DTT, pH 7.8) is thawed at room temperature and then transferred to ice. The suspended cells are separated into two aliquots for lysis. To each 40 mL of suspended cells, 0.1% Triton X-100 is added along with the following cocktail of antiproteases: 0.1 mM PMSF, 1 µg/mL leupeptin and 1 µg/mL pepstatin. The two aliquots of cells are then lysed on ice using a sonicator whereby 20 cycles of 15 s pulses at an amplitude of 40% are applied, with a 59 s break between bursts. To isolate the CFE, the lysate is centrifuged at 30,000 x g for 25 minutes at 4°C. The soluble fraction is filtered using a 0.45 µm membrane prior to purification.

3.1.3 Pin1 Purification

Ni-NTA affinity chromatography is performed as the first step in the purification of Pin1. For this, an ÄKTA purifier machine, equipped with a 5 mL Ni-NTA His-trap column, is run using the UNICORN program. The column is first equilibrated in 5 column volumes (CV) of Pin1 Binding Buffer at a flow rate of 1 mL/min. The filtered CFE is then injected into the column at 1 mL/min and the flow-through (FT) collected as 45 mL fractions. The column is subsequently washed with 10 CV of binding buffer to remove weakly interacting proteins. Finally, a gradient from 0 to 80 % Pin1 Elution Buffer (25 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, 1 mM DTT, pH 7.8) is applied over 40 minutes and 3 mL fractions are collected as the His-tagged Pin1 is eluted from the column. The fractions containing Pin1 (identified by SDS-PAGE and chromatogram analysis) are pooled and incubated overnight with 0.15 mg of PreScission enzyme. After His-tag cleavage, the eluate is diluted 3-fold in Binding buffer to decrease imidazole concentration before re-injection into the Ni-NTA column. The FT (containing fully cleaved Pin1) is collected as 3 mL fractions and pooled. A pressure-based concentration method is then used to obtain a 5 mL sample of Pin1 using an Amicon stirred ultrafiltration cell equipped with a 3 kDa ultrafiltration membrane. The final step in Pin1 purification relies on size exclusion chromatography (gel filtration). A HiLoad Superdex 75 pg column is equilibrated with NMR buffer (3 0mM Tris, 50 mM NaCl, 2 mM DTT, pH 7) aT flow of 1 mL/min. The sample is injected into the column as 2.5 mL aliquots and emerges from the column after washing with ≈70 mL of buffer. The fractions containing pure Pin1 (identified by SDS-PAGE and chromatogram analysis) are pooled and concentrated using a stirred ultrafiltration cell prior to storage as aliquots at –80°C.

3.2 Assignment of Pin1 amide backbone resonances

The amide backbone resonances of isotopically enriched Pin1 were assigned through a series of 3D triple resonance experiments (HN(Cα)CO, HNCO, CβCα(CO)NH and CβCαNH), recorded at 298K on a Pin1 sample in Tris buffer (pH 7) (Figure S1). This and subsequent experiments were carried out on a Bruker 500 MHz spectrometer equipped with a 5-mm TCI cryoprobe. Unbound Pin1 structure in solution was confirmed by comparing the acquired spectra to previously reported ones (Jacobs *et al*. *J. Biomol. NMR* **2002**, *23*, 163; A. Born *et al*. *Biomol. NMR Assign.* **2019**, *13*, 85; BMRB entries 5305, 11557, and 27579) and taken as reference for future CSP experiments. It was not possible to assign all the residues, specifically: residues Gly39–Gly45 within the disordered linker region, likely due to the rapid exchange of amide protons

with water under these experimental conditions; and the amide resonances for Arg17, Ser18, and Ser19 in Loop 1 of the WW domain, as well as Ser114 in the PPIase domain. In Tris buffer, Arg68, Arg69 and Ser71, belonging to the phosphate-binding site of the PPIase domain, were also lost.

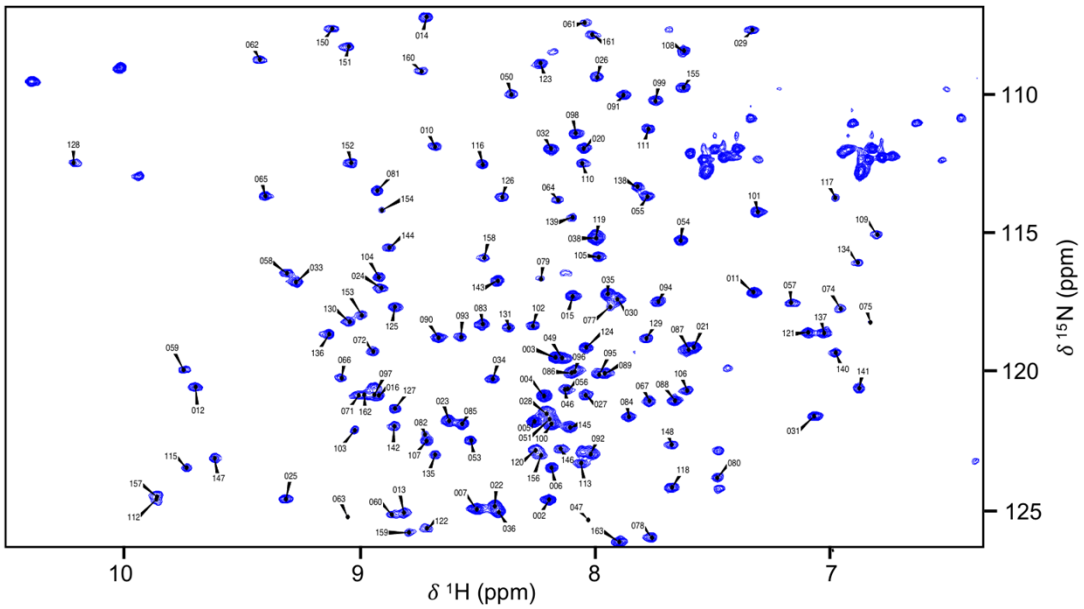
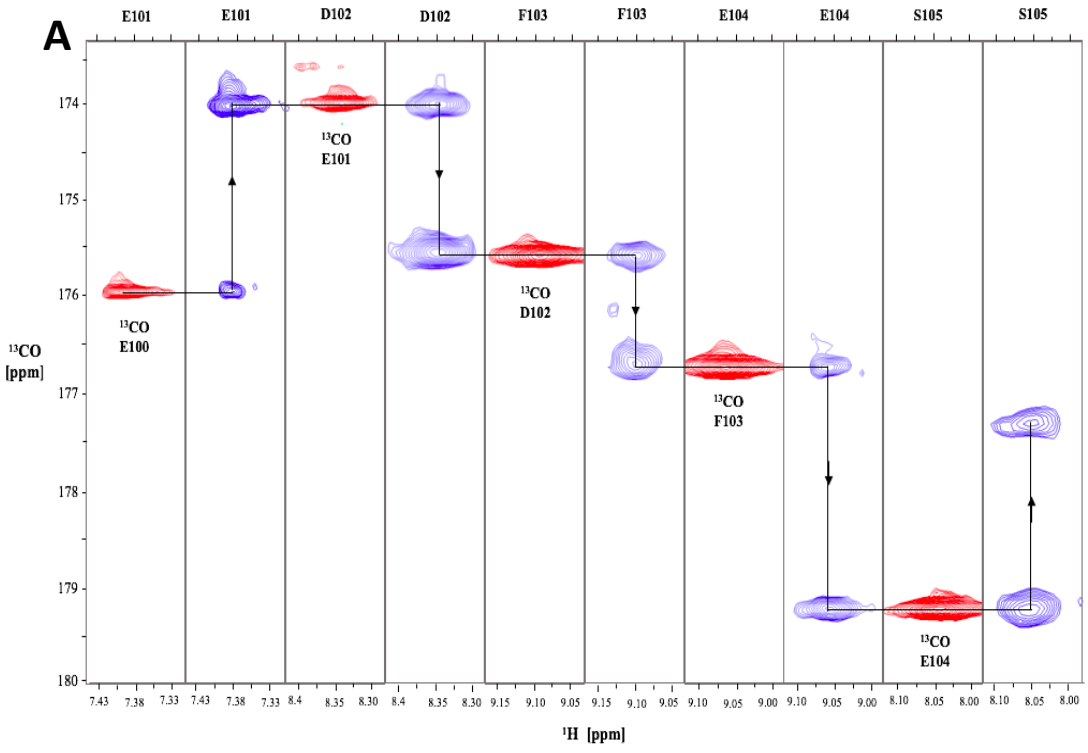


Figure S1: Assigned ^{1}H,^{15}N-HSQC spectrum of unbound Pin1. 500µL of sample containing 150µM of Pin1 in a pH 7.0 buffer solution (30mM Tris, 50mM NaCl, 2mM DTT) and 10% D_2O were analysed using a 500 MHz NMR at 298K.

More in deail, to assign H_N, N_H, CO, Cα, and Cβ resonances, five type of NH-based 3D experiments were recorded: HNCA, HN(CA)CO, HNCO, CBCA(CO)NH, and CBCANH. The combined analysis of these 3D experiments allowed to identify 147 spin systems with CO, Cα, and Cβ chemical shift values for residues *i* and *i-1* of each NH group. Sequential assignment was performed from comparison between *i* and *i-1* chemical shifts. Characteristic values of Cα chemical shift for the glycine ($\delta_{C\alpha} \approx 45$ ppm) or Cβ for alanine, serine and threonine ($\delta_{C\beta_{Thr}} \approx 68$ ppm, $\delta_{C\beta_{Ser}} \approx 63$ ppm, $\delta_{C\beta_{Ala}} \approx 19$ ppm) were used as starting points for the sequential assignment. In Figure S2 is shown an example of sequential connectivities for residues **E101** to **S105** in the 3D spectra (Figure S2).



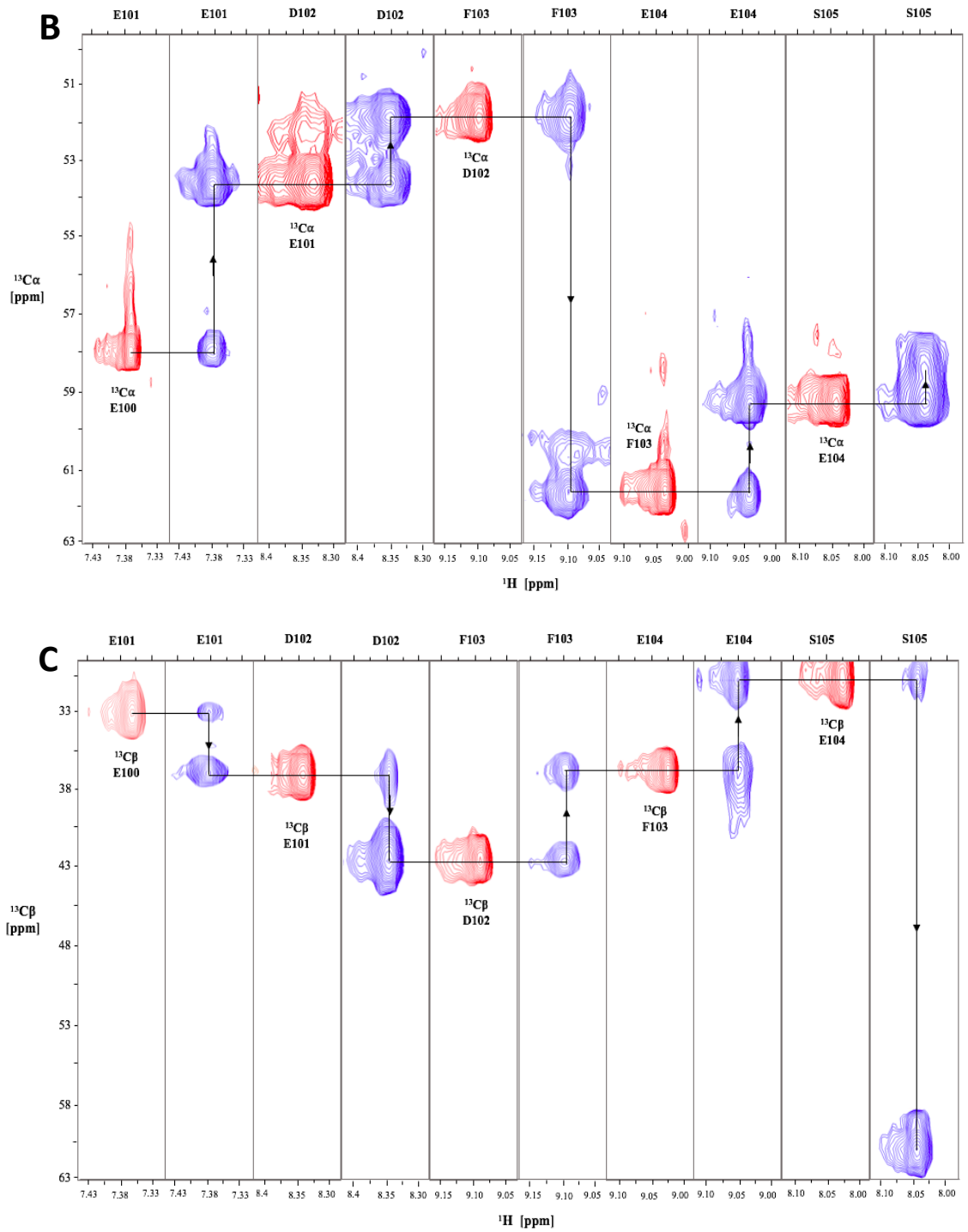


Figure S2: Example of NMR assignment of CO, Cα and Cβ nuclei of FL (Full Length) Pin1. Sequential connectivities are represented by arrows. (**A**) HNCO experiment (blue), (**B**) HN(CA)CO experiment (red) and (**C**) CBCA(CO)NH (blue) and CBCANH (red).

^{15}N-labeled samples of the isolated WW (Met1-Ser38) and PPIase (Gly50-Glu163) domains were sufficient to assign their amide resonances, from comparison of their ^{1}H-^{15}N HSQC spectra with that of FL (Full length) Pin1. No additional peaks were observed on the spectra of the isolated domains. Indeed, the NH cross-peaks that were absent in the HSQC spectrum of FL Pin1 (Arg17, Ser18, and Ser19 of WW, and Arg68, Arg69, and Ser71 of PPIase) were also absent in the spectra of the isolated domains. The assignment of both isolated domains was confirmed by previously published NMR studies of Pin1 isolated domains (BMRB entries 5248 and 11559).

3.3 Affinity Costants Evaluation by CSPs

3.3.1 NMR Titration

An NMR sample containing 150 µM ^{15}N-labelled Pin1 and 10% D_2O is prepared in Pin1 NMR buffer to a final volume of 500 µL in a 5 mm NMR tube. A reference HSQC spectrum is recorded on this sample using a 500 MHz spectrometer. Ligand stock solutions are prepared at 3 mM in Pin1 NMR buffer and the pH adjusted to 7 before the titration. The concentration of the ligand stock solution is confirmed prior to the titration by integrating isolated resonances of assigned protons in 1D ^{1}H NMR spectra and comparing the calculated volume to that of a calibration peak corresponding to a known concentration of DSS. To do this, an NMR sample is prepared containing 100 µL of the 3 mM ligand stock, 10 % D_2O, and 1 mM DSS and subsequently a 1D ^{1}H NMR spectrum is recorded using a 500 MHz spectrometer. The DSS signal is used as a reference for relative quantification of the ligand stock solution by integrating known proton peaks in ligand spectra and comparing to the volume of the DSS peak, calibrated at 1 mM. Ligand is titrated into the NMR sample to reach up to 8 molar equivalents of Pin1. The volumes added at each titration point are calculated as a molar ratio of Pin1. Typically, the following series of molar equivalents are titrated into the sample: 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0. ^{1}H-^{15}N HSQC experiments are recorded on the sample at each titration point and compared to a reference spectrum of apo-Pin1 using a 500 MHz spectrometer.

3.3.2 K_D Evaluation

The K_D were evaluated following the previously reported method (Williamson, *Prog Nucl Magn Reson Spectrosc* **2013**, 1).

Ligand	K_DWW (µM)	K_DPPIase (µM)
1b	>1000	15 ± 4
2a	26 ± 6	438 ± 80
2b	32 ± 6	>1000
3a	Not determined (Ligand not soluble in water)	Not determined (Ligand not soluble in water)
3c	439 ± 62	705 ± 302
4a	44 ± 6	88 ± 16
4c	66 ± 12	80 ± 32
4e	285 ± 32	598 ± 193

Table S1: K_D value of the synthesised ligands fro both the Pin1 domains.

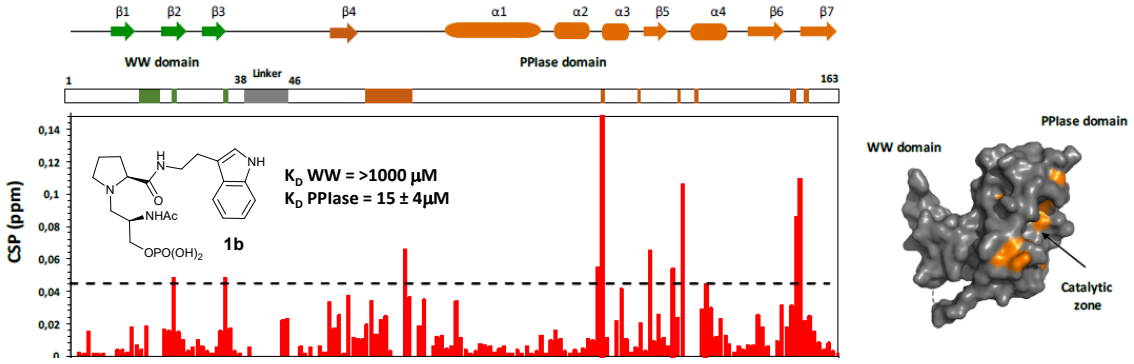


Figure S3: Analysis of the interaction of Pin1 with **1b**. CSPs of Pin1 induced by the presence of 8 molar equivalents of **1b** with the surface representation of the most significant CSPs in orange (right). The black dashed line represents the threshold CSP value (m + σ). The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

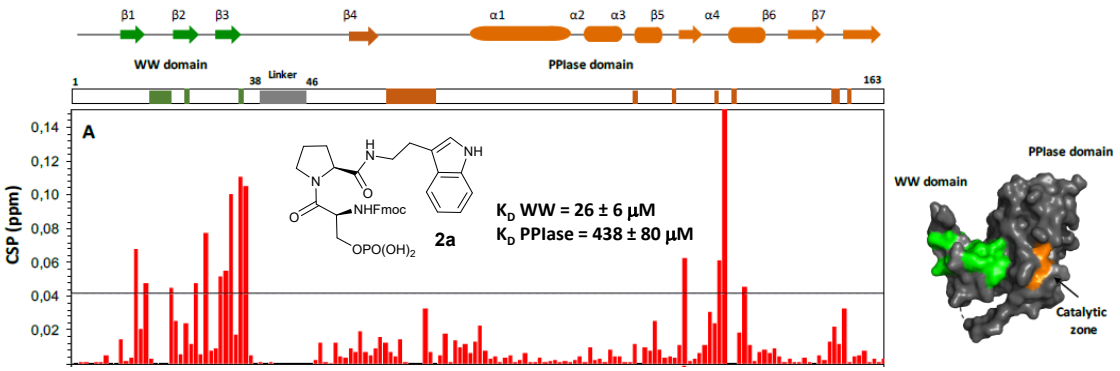


Figure S4: Analysis of the interaction of Pin1 with **2a**. CSPs of Pin1 induced by the presence of 8 molar equivalents of **2a** with the surface representation of the most significant CSPs in orange and green (right). The black dashed line represents the threshold CSP value (m + σ). The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

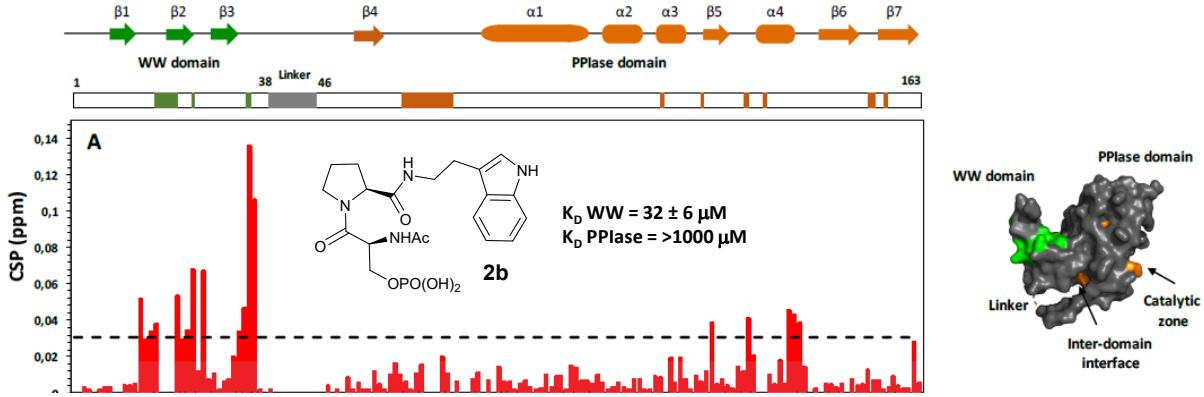


Figure S5: Analysis of the interaction of Pin1 with **2b**. CSPs of Pin1 induced by the presence of 8 molar equivalents of **2b** with the surface representation of the most significant CSPs in orange and green (right). The black dashed line represents the threshold CSP value (m + σ). The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

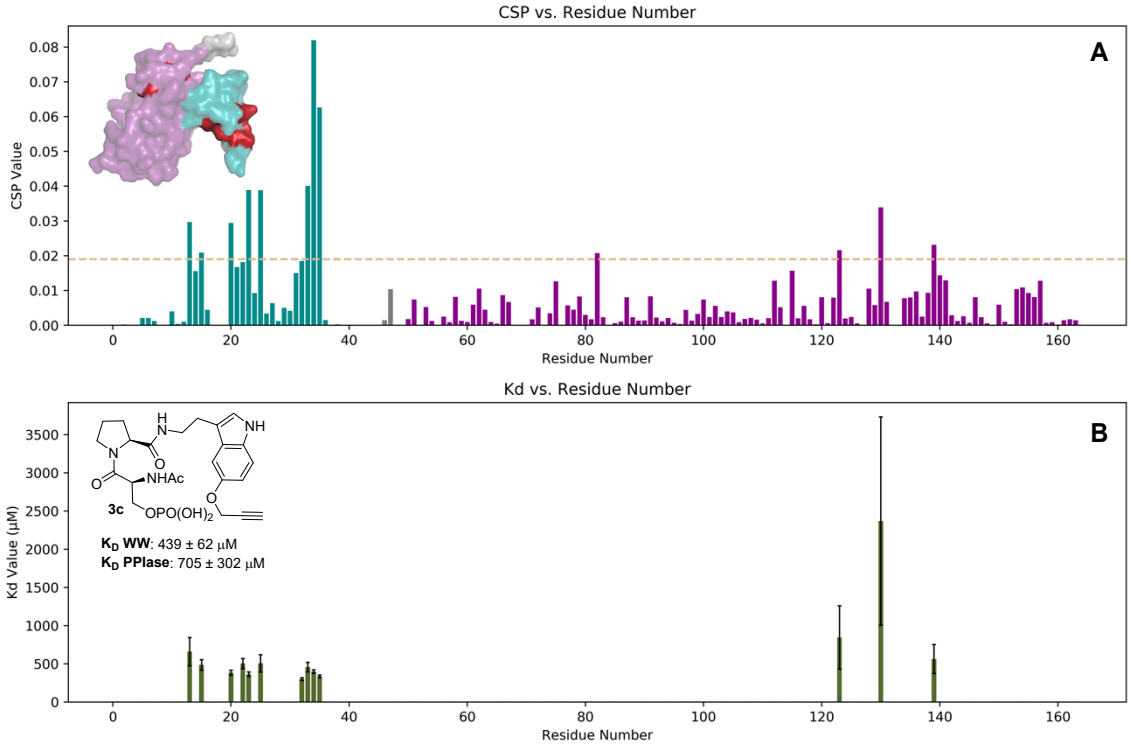


Figure S6: Analysis of the interaction of Pin1 with **3c**. **A.** CSPs of Pin1 induced by the presence of 8 molar equivalents of **3c** with the surface representation of the most significant CSPs in red (right). The orange dashed line represents the threshold CSP value (m + σ). **B.** The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

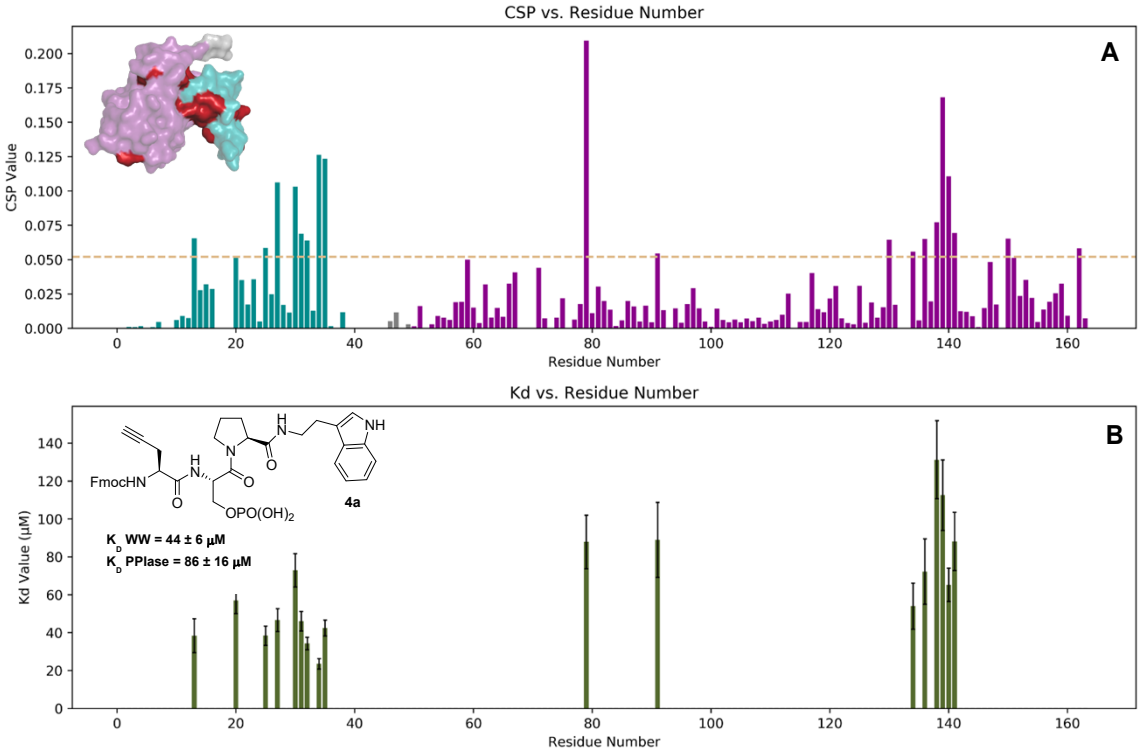


Figure S7: Analysis of the interaction of Pin1 with **4a**. **A.** CSPs of Pin1 induced by the presence of 4 molar equivalents of **4a** with the surface representation of the most significant CSPs in red (right). The orange dashed line represents the threshold CSP value (m + σ). **B.** The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

The binding analysis of **4a** to Pin1 revealed significant CSPs in both the WW (K_D: 44 ± 6 µM) and PPIase (K_D: 88 ± 16 µM) domains. Key residues in the WW domain, including Gln33, Trp34, and Glu35, which coordinate the proline group of the pSer-Pro motif, were notably affected. Additionally, residues at the WW interdomain interface (His27-Ser32) and in the PPIase domain, such as Phe134 in the proline-binding pocket and Ser138-Leu141 in the α4-helix at the interdomain interface, showed significant perturbations. However, the lack of CSPs in the PPIase phosphate-binding site (residues 63-72) suggests that the phosphate group of **4a** does not interact with this domain. The most significant CSPs in the PPIase domain correspond to the α4-helix, with K_D values similar to those in the WW domain, indicating an allosteric effect from **4a** binding to the WW domain.

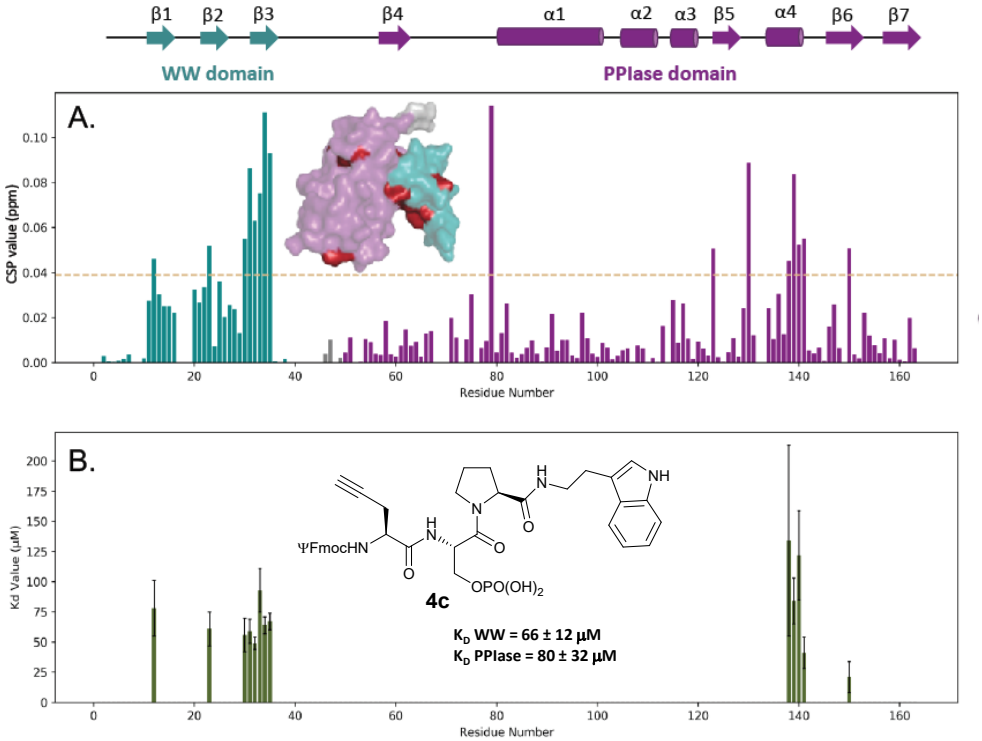


Figure S8: Analysis of the interaction of Pin1 with **4c**. **A.** CSPs of Pin1 induced by the presence of 6 molar equivalents of **4c** with the surface representation of the most significant CSPs in red (right). The orange dashed line represents the threshold CSP value (m + σ). **B.** The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

NMR titration of ^{15}N-labeled Pin1 with **4c** revealed significant CSPs in both the WW (K_D: 66 ± 12 µM) and PPIase domains (K_D: 80 ± 32 µM) similar to its parent Fmoc protected compound **4a**. As for **4a** the in the WW domain, key residues in the proline-binding pocket (Tyr23, Gln33, Trp34, Glu35) exhibited notable CSPs. In the PPIase domain, significant perturbations were observed in the proline-binding pocket (Gly123, Met130) and the α4-helix (Ser138, Phe139, Ala140, Leu141). However, the CSPs profile of the PPIase domain suggests non-specific binding, as KD values for Gly123 and Met130 likely exceed 1 mM, indicating weak or non-specific interactions. In contrast, the KD values for residues Ser138-Leu141 align with those of the WW domain, implying an allosteric effect from WW domain binding.

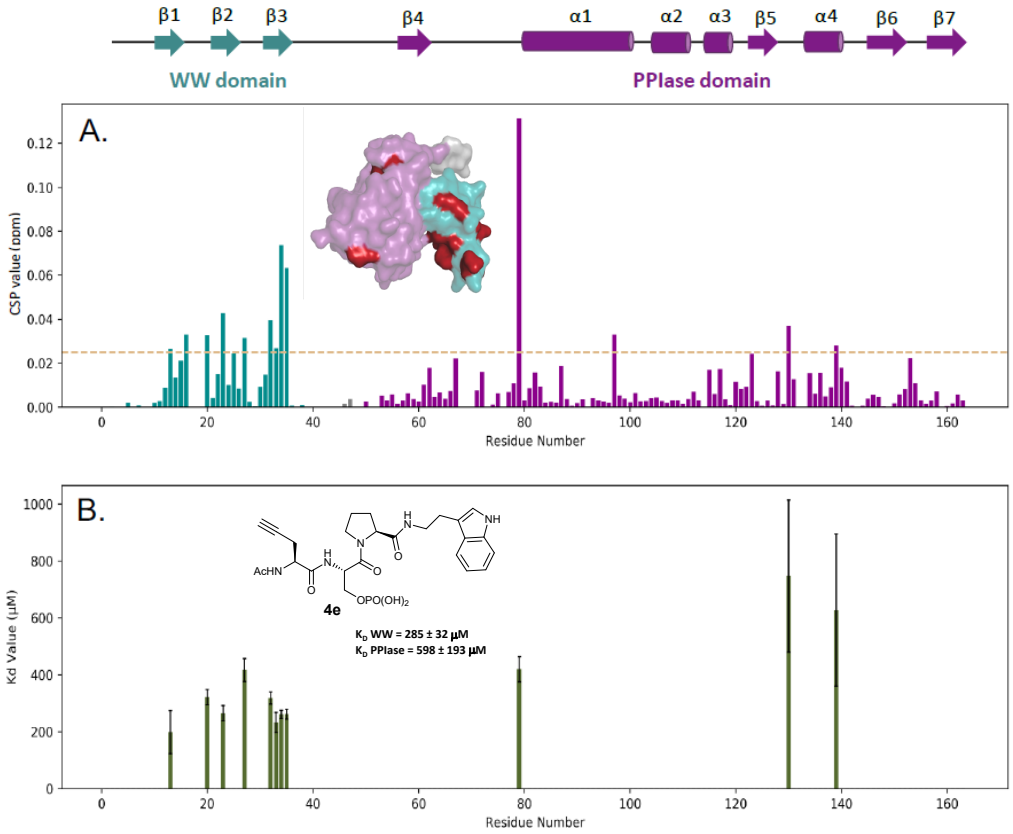


Figure S9: Analysis of the interaction of Pin1 with **4e**. **A.** CSPs of Pin1 induced by the presence of 4 molar equivalents of **4e** with the surface representation of the most significant CSPs in red (right). The orange dashed line represents the threshold CSP value (m + σ). **B.** The K_D values of residues displaying the most significant CSPs were used to calculate an average K_D for each domain.

4 STABILITY ASSAY

A 1 mM stock solution of the **4b-Rhod** was prepared in DMSO.
For the **Calibration Curve** a 100µL aliquot of 1mM stock solution is diluted in 900µL of distilled H_2O. From the obtained 100 µM solution, seven further dilutions were prepared at 50, 30, 15, 10, 5, 2.5, 1 µM. Each dilution was injected in the HPLC and the absorbance at 214 and 254 nm was registered. The analysis was done in triplicates. Two calibration curves absorbance vs. concentration (one for each wavelength) were plotted.
For the **Stability Test**, a 100µL aliquot of stock solution is diluted in 900µL of cellular medium. This step is done in triplicate. Two different cellular media are tested: RPMI 1640 medium + 10% FBS Ham's F-12 (F12) medium + 10% FBS. The samples were incubated at 37°C for 72h. 100µL aliquots were collected at 2h, 4h, 6h, 8h, 24h, 30h, 48h and 54h, diluted in cold MeOH (300µL) and centrifuged. The supernatant was later injected into HPLC to estimate concentration changes.

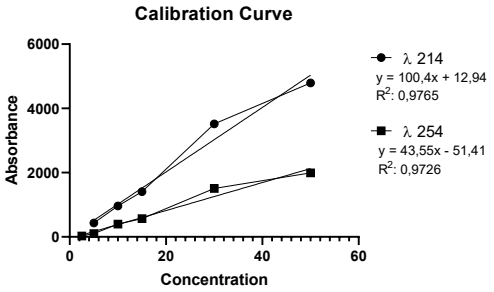


Figure S10: Calibration Curve Abs (at λ 214 and λ 254 nm) vs Concentration (micromolar) for compound **4b-Rhod**.

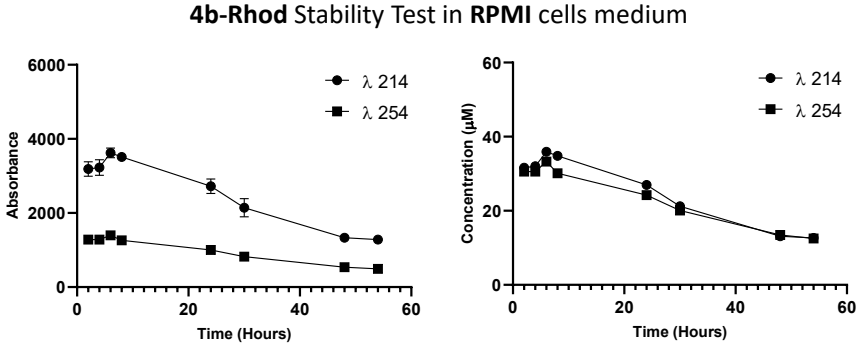


Figure S11. **On the left**: Graph Absorbance vs Time (hours) at λ 214 and λ 254 nm for compound **4b-Rhod** in RPMI cell medium. **On the right**: Graph Concentration vs Time (hours) at λ 214 and λ 254 nm for compound **4b-Rhod** in RPMI cell medium.

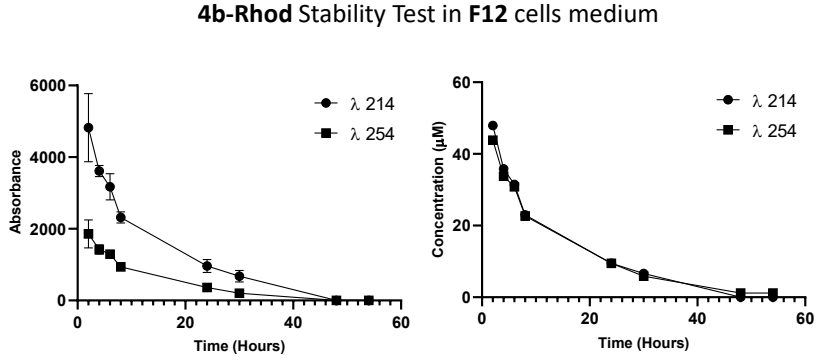


Figure S12. **On the left**: Graph Absorbance vs Time (hours) at λ 214 and λ 254 nm for compound **4b-Rhod** in F12 cell medium. **On the right**: Graph Concentration vs Time (hours) at λ 214 and λ 254 nm for compound **4b-Rhod** in F12I cell medium.

5 CELL PERMEABILITY ASSAY

5.1 Immunofluorescence

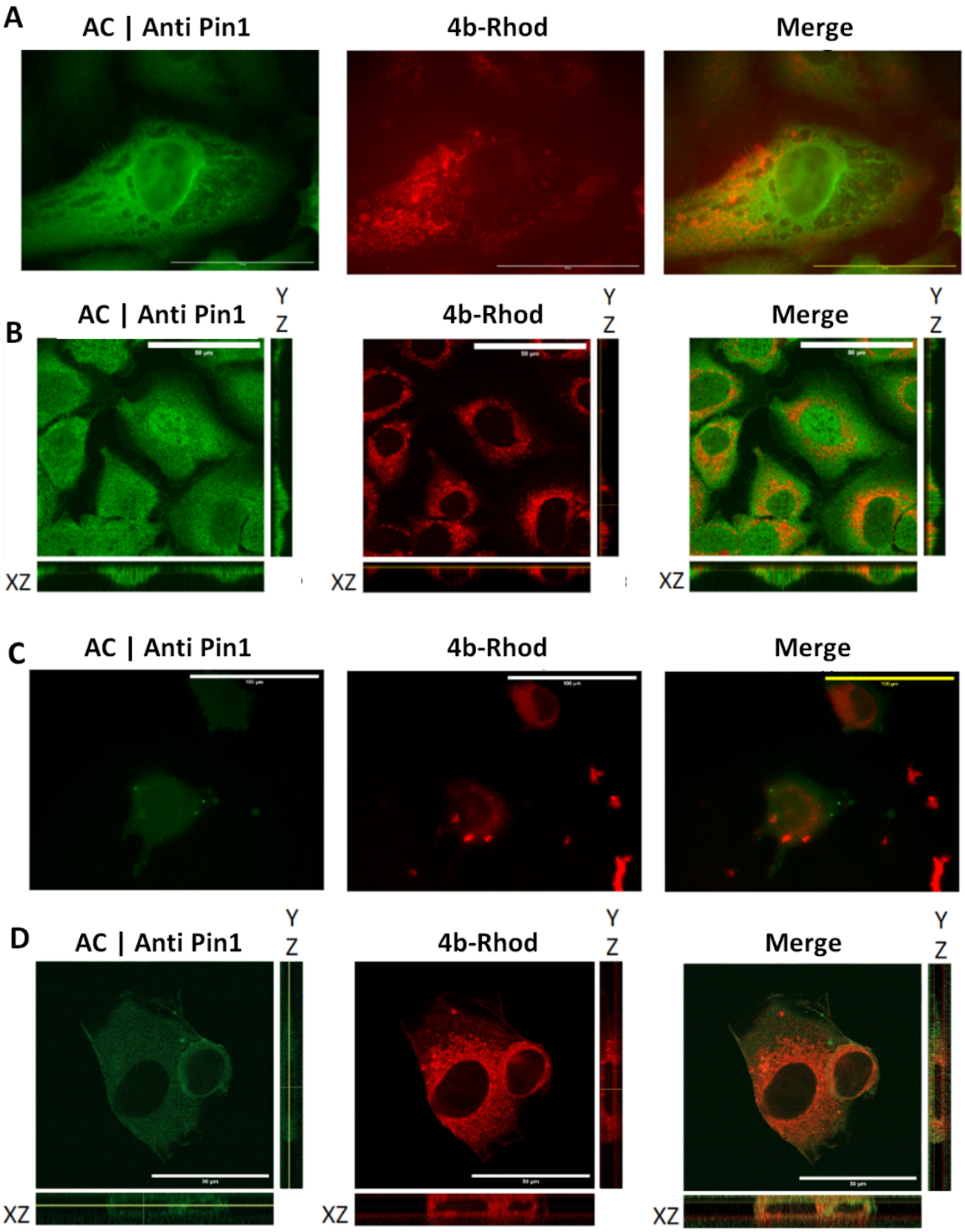


Figure S13: IF imaging on SKOV3 treated with the fluorescent probe **4b-Rhod**. **A**) epifluorescence analysis on SKOV3; **B**) Confocal microscopy analysis on SKOV3. **C**) epifluorescence analysis on IGROV1; **D**) Confocal microscopy analysis on IGROV1. (Scale: 50 µm)

A solution of 30,000 cells/mL (SKOV3 or IGROV1) is prepared in RPMI 1640 + 10% FBS cell media (Gibco). The cells are seeded in p4 plates (30,000 cells per well). In the relevant wells, a glass coverslip was placed beforehand. After 24 hours, the cells are incubated with a solution of 5 µM **4b-Rhod** in cell medium. After 24 hours of incubation, the medium is removed, and the cells are gently rinsed with PBS. Fixation with 4% PFA is carried out for 10 minutes (500 µL per coverslip). Permeabilization is performed with 500 µL of PBS-Triton 0.1% in each well, incubating for 4 minutes at 4°C. The PBS-Triton is removed and replaced with 500 µL of PBS-BSA 0.5% in each well for a 20-minute incubation. The primary anti-Pin1 antibody (Santa Cruz Biotechnology # sc-46660, RRID: AB_628132) is prepared by diluting it at 1/50 in PBS-BSA. On an annotated parafilm, 45 µL of the antibody is placed, and the coverslip is placed on top with the cells facing the antibody. This is followed by incubation in a humid chamber for 2 hours at room temperature. The coverslips are retrieved and placed back in their respective wells with the cells facing up. Three rinses with PBS-BSA are performed. The secondary antibody Alexa Fluor 488 conjugate (A11029) is prepared by diluting it at 1/400. The same incubation as for the primary antibody is conducted for 1 hour. Three PBS-BSA rinses are performed afterward. Then, 7 µL of Prolong Gold is placed on the slide, and the coverslip is positioned on the drop (cells facing the drop). The mounting is left to dry overnight.

6 CELL VIABILITY ASSAY

6.1 General Informations

4b was solubilised in sterile 100% DMSO at 10mM concentration under sterile condition in a class II biological safety cabinet. The dilutions were done in culture cell medium supplemented with 10% FBS. The final concentrations were 10 µM, 30 µM, 50 µM0. The % of DMSO in the final **4b** solutions was not exceeding 0.5%. Controls: cell media, a solution of cell media with 0.5% of DMSO and a solution of cell media whitout cells.

4b was solubilised in 100% DMSO at 10mM concentration. The dilutions were done in culture cell medium. The final concentrations were 10 µM, 25 µM, 50 µM, 75 µM, 100 µM, and 150 µM. The % of DMSO in the final **4b** solutions not exceeding the 1.5%. Controls: cell media, a solution of cell media with 2% of DMSO and a solution of cell media whitout cells.

6.2 MTT Assay of 4b

A solution of 6.2 × 10^5 MM1.R cells/mL was prepared in RPMI 1640 + 10% FBS cell media. The Invitrogen™ Countess™ 3 Automated Cell Counter was used to count live and dead cells following a 1:1 dilution (10 µL + 10 µL) with Trypan Blue (Invitrogen). The cell suspension was homogenized by gently pipetting 20 times with a 5 mL pipette.

For the viability assay at 24 hours, a volume of 190 µL, containing 4,000 cells, was seeded into a 96-well plate (5 × 3 wells in total). Cells for the calibration curve were also seeded: 190 µL containing 10,000, 8,000, 5,000, 4,000, 2,500, 2,000, and 1,000 cells were added to the same 96-well plate (7 × 3 wells in total). Seeding was performed according to the experimental plan, with three replicates per condition. An additional 1 × 3 wells were filled with 190 µL of cell media without cells as a control. Plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hours to allow for adequate cell adhesion.

After 24 hours, 20 µL of a solution of **4b** at the appropriate concentrations (10 µM, 30 µM, and 50 µM) was added to the designated wells (4 × 3 wells). For the calibration curve, 20 µL of cell media was added to each well (7 × 3 wells). For the controls, 20 µL of cell media was added to the wells without cells (1 × 3 wells). Additionally, 20 µL of cell media was added to the control with cells but without compound (1 × 3 wells), and 20 µL of a solution of DMSO in cell media was added to a separate 1 × 3 wells, yielding a final DMSO concentration of 0.5% in cell media.

After 24 hours of incubation, the medium was removed, and cells were washed with PBS. PBS was then removed, and 200 µL of a solution of yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in PBS was added to each well. Cells were incubated at 37°C with 5% CO_2 for 3 hours. Following incubation, the media was removed, and 200 µL of DMSO was added to each well. Cells were incubated at 37°C with 5% CO_2 for an additional hour, after which absorbance at 570 nm was measured using a plate reader. The same procedure was repeated for another 96-well palte with 48 hours of incubation.

For IC_{50} calculation, the protocol was followed as described, with **4b** concentrations adjusted to 10 µM, 25 µM, 50 µM, 75 µM, 100 µM, and 150 µM. Controls remained the same, and the DMSO concentration was adjusted to 1.5%. Cells were incubated for 24 hours.

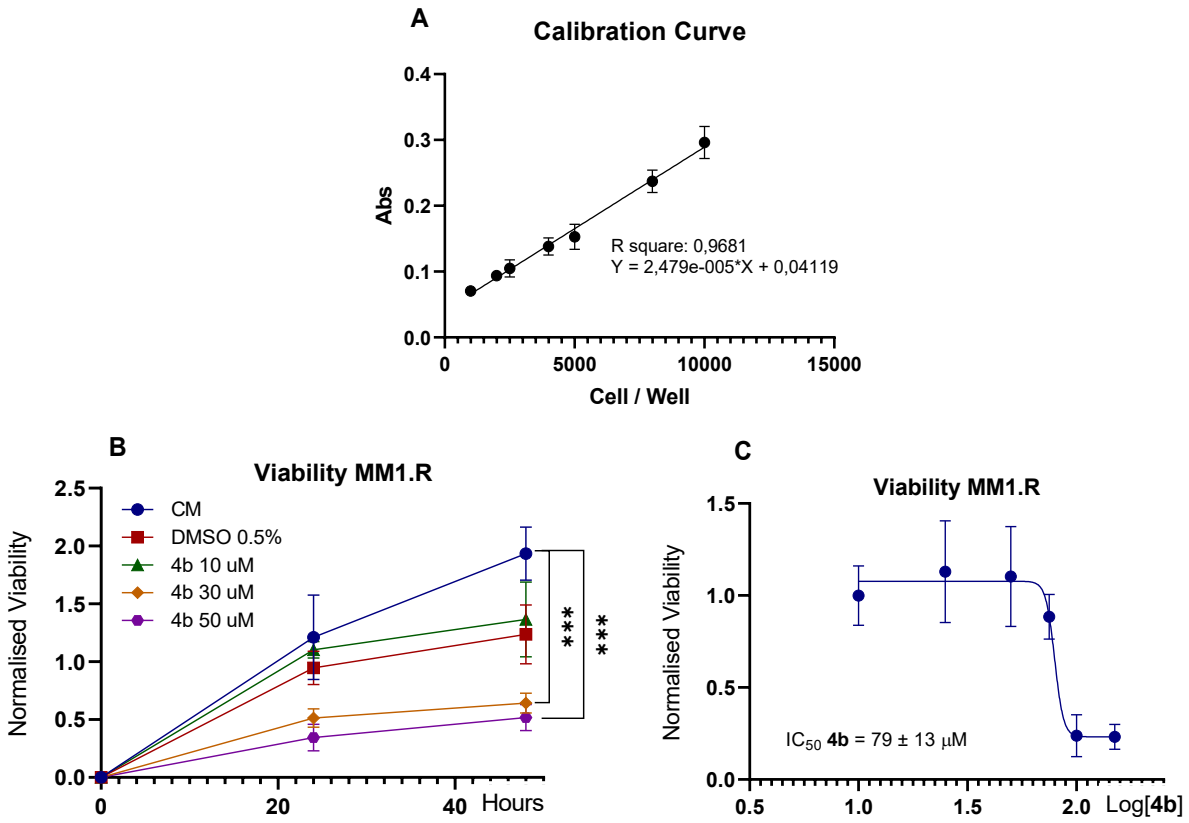


Figure S14. A: Calibration Curve for the Cell Viability Assay (MTT). Absorbance vs Number of MM.1R cells for the cells incubated with compound **4b**. **B** and **C:** Cell viability assay (MTT) of MM.1R cell line after 24h incubation with **4b**. Values are expressed as normalised viability compared with Ctrl cells in Cell Media (CM) at Day 0. The data are expressed as means ± SEM; n = 3; *** p < 0,0002 (one-way ANOVA test).

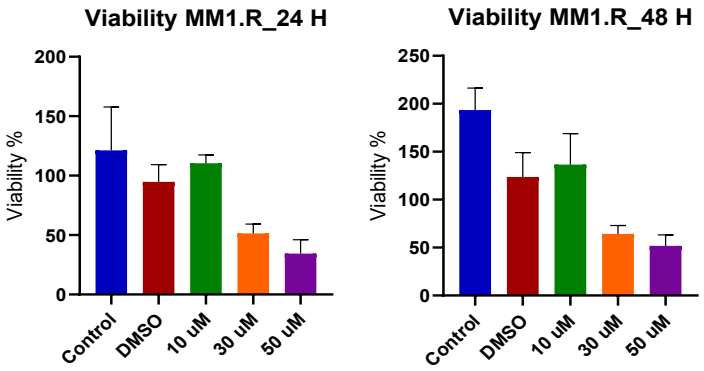


Figure S15: Cell viability assay (MTT) of MM.1R cell line after 24h (left) and 48h (right) of incubation with **4b** (istogram representation). Values are expressed as normalised viability compared with Ctrl cells in Cell Media (CM) at Day 0. The data are expressed as means ± SEM; n = 3; *** p < 0,0002 (one-way ANOVA test).

Ordinary one-way ANOVA of Viability Assay 4000 cells after incubation with 4b for 48H

Tukey's multiple comparisons test	Mean Diff	95,00% CI of diff	Significant?	**Summary**	Adjusted P Value
Control vs. DMSO	26,41	-24,43 to 77,24	No	**ns**	0,4699
Control vs. 10 uM	10,85	-39,99 to 61,68	No	**ns**	0,9513
Control vs. 30 uM	69,79	18,96 to 120,6	Yes	**	0,0076
Control vs. 50 uM	86,77	35,94 to 137,6	Yes	**	0,0016

DMSO vs. 10 uM	-15,56	-66,40 to 35,27	No	**ns**	0,8463
DMSO vs. 30 uM	43,39	-7,449 to 94,22	No	**ns**	0,105
DMSO vs. 50 uM	60,36	9,528 to 111,2	Yes	*	0,0192
10 uM vs. 30 uM	58,95	8,114 to 109,8	Yes	*	0,0222
10 uM vs. 50 uM	75,93	25,09 to 126,8	Yes	**	0,0043
30 uM vs. 50 uM	16,98	-33,86 to 67,81	No	**ns**	0,8034

Table S2: Ordinary one-way ANOVA of Viability Assay 4000 cells 24H**.** For Graph Prism 8.0.2, ns: p > 0.1234; *: p < 0.0332; **: p < 0.0021; ***: p < 0.0002; ****: p < 0.0001.

Ordinary one-way ANOVA of Viability Assay 4000 cells after incubation with 4b for 48H

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff	Significant?	**Summary**	Adjusted P Value
Control vs. DMSO	69,75	10,51 to 129,0	Yes	*	0,0202
Control vs. 10 uM	56,88	-2,366 to 116,1	No	**ns**	0,0613
Control vs. 30 uM	129	69,78 to 188,3	Yes	***	0,0002
Control vs. 50 uM	141,6	82,35 to 200,8	Yes	***	0,0001
DMSO vs. 10 uM	-12,87	-72,12 to 46,37	No	**ns**	0,9482
DMSO vs. 30 uM	59,27	0,02896 to 118,5	Yes	*	0,0499
DMSO vs. 50 uM	71,85	12,60 to 131,1	Yes	*	0,0169
10 uM vs. 30 uM	72,14	12,90 to 131,4	Yes	*	0,0165
10 uM vs. 50 uM	84,72	25,47 to 144,0	Yes	**	0,0058
30 uM vs. 50 uM	12,57	-46,67 to 71,82	No	**ns**	0,9522

Table S3: Ordinary one-way ANOVA of Viability Assay 4000 cells 48H**.** For Graph Prism 8.0.2, ns: p > 0.1234; *: p < 0.0332; **: p < 0.0021; ***: p < 0.0002; ****: p < 0.0001.

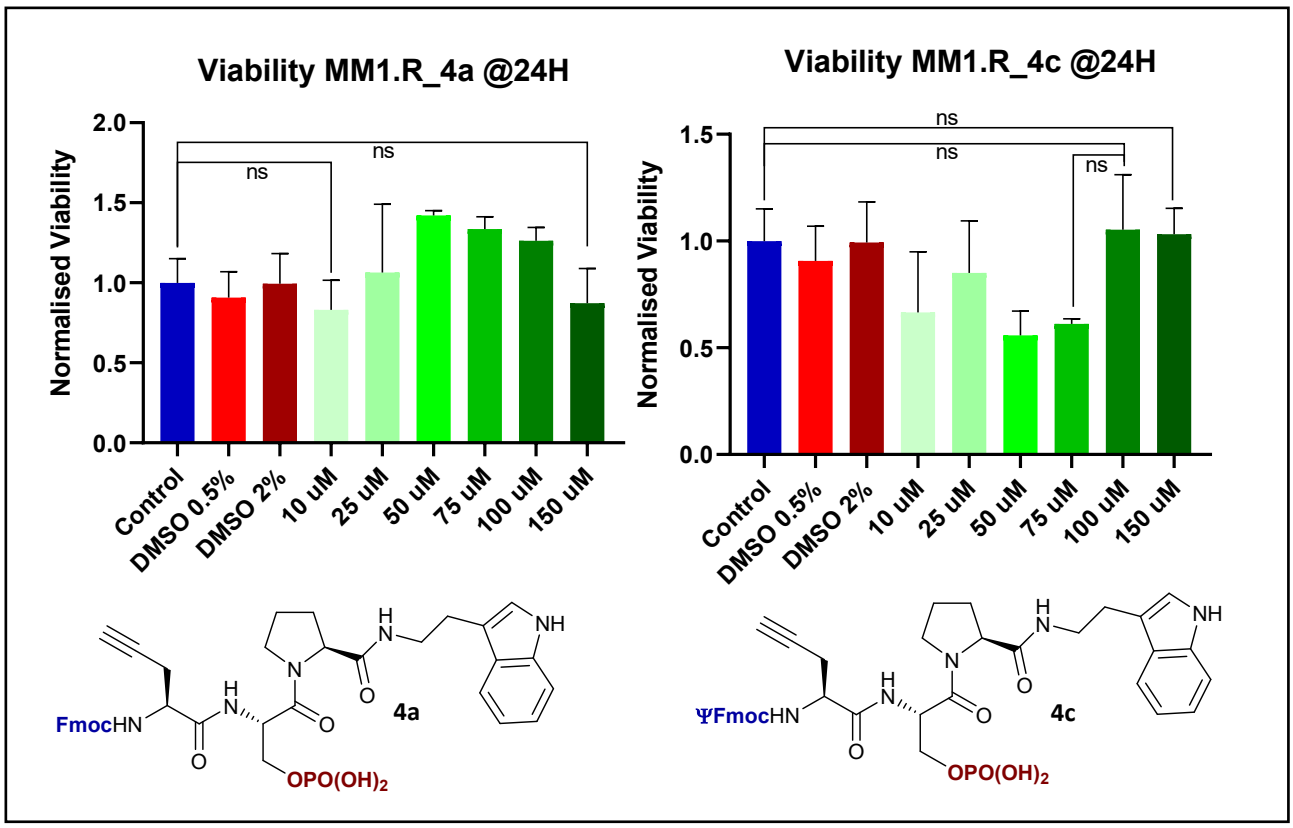
6.3 MTT Assay of 4a, 4c and 4b at 24H

A solution of 1.27 × 10^6 MM1.R cells/mL was prepared in RPMI 1640 + 10% FBS cell media. The Invitrogen™ Countess™ 3 Automated Cell Counter was used to count live and dead cells following a 1:1 dilution (10 µL + 10 µL) with Trypan Blue (Invitrogen). The cell suspension was homogenized by gently pipetting 20 times with a 5 mL pipette.

For the viability assay at 24 hours, a volume of 190 µL, containing 4,000 cells, was seeded into a 96-well plate (5 × 3 wells in total). Cells for the calibration curve were also seeded: 190 µL containing 10,000, 8,000, 5,000, 4,000, 2,500, 2,000, and 1,000 cells were added to the same 96-well plate (7 × 3 wells in total). Seeding was performed according to the experimental plan, with three replicates per condition. An additional 1 × 3 wells were filled with 190 µL of cell media without cells as a control. Plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hours to allow for adequate cell adhesion.

After 24 hours, 20 µL of a solution of each **compound** (**4a** / **4c** / **4b**) at the appropriate concentrations (10 µM, 25 µM, 50 µM, 75 µM, 100 µM, 150 µM) was added to the designated wells (6 × 3 wells for each compounds). For the calibration curve, 20 µL of cell media was added to each well (7 × 3 wells). For the controls, 20 µL of cell media was added to the wells without cells (1 × 3 wells). Additionally, 20 µL of cell media was added to the control with cells but without compound (1 × 3 wells). 20 µL of a solution of DMSO in cell media was added to a separate 1 × 3 wells, yielding a final DMSO concentration of 0.5% in cell media. 20 µL of a solution of DMSO in cell media was added to a separate 1 × 3 wells, yielding a final DMSO concentration of 2% in cell media

After 24 hours of incubation, the medium was removed, and cells were washed with PBS. PBS was then removed, and 200 µL of a solution of yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] in PBS was added to each well. Cells were incubated at 37°C with 5% CO_2 for 3 hours. Following incubation, the media was removed, and 200 µL of DMSO was added to each well. Cells were incubated at 37°C with 5% CO_2 for an additional hour, after which absorbance at 570 nm was measured using a plate reader.



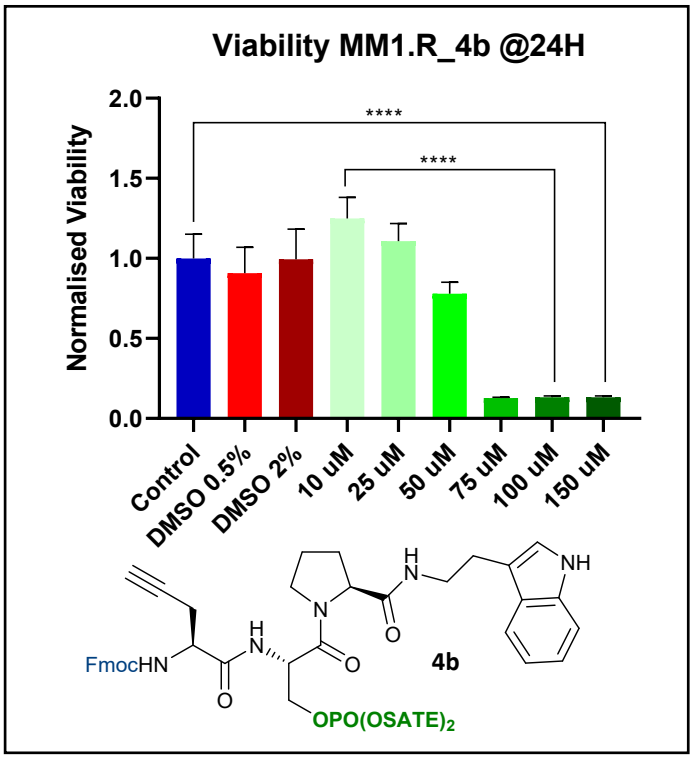


Figure S16: Cell viability assay (MTT) of MM.1R cell line after 24h of incubation with **4a, 4b** and **4c** (istogram representation). Values are expressed as normalised viability compared with Ctrl cells in Cell Media (CM) at 24h. The data are expressed as means ± SEM; n = 3; ns: $p > 0.1234$; *** $p < 0,0002$; ****: $p < 0.0001$. (one-way ANOVA test). No significant effects on cells after incubation of **4a** and **4c** (unprotected compounds). Significant effect on cells after incubation with **4b** (Pro-Drug, SATE protected compound) were mesured.

Ordinary one-way ANOVA of Viability Assay 5000 cells after incubation with 4a for 24H

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	**Summary**	Adjusted P Value
Control vs. DMSO 0.5%	-0,2811	-0,8425 to 0,2803	No	**ns**	0,7085
Control vs. DMSO 2%	-0,3679	-0,9294 to 0,1935	No	**ns**	0,3925
Control vs. 10 uM	-0,2054	-0,7669 to 0,3560	No	**ns**	0,9242
Control vs. 25 uM	-0,4388	-1,000 to 0,1226	No	**ns**	0,2018
Control vs. 50 uM	-0,7947	-1,356 to -0,2333	Yes	**	0,0025
Control vs. 75 uM	-0,7100	-1,271 to -0,1485	Yes	**	0,0076
Control vs. 100 uM	-0,6370	-1,198 to -0,07556	Yes	*	0,0195
Control vs. 150 uM	-0,2475	-0,8090 to 0,3139	No	**ns**	0,8207
DMSO 0.5% vs. DMSO 2%	-0,08683	-0,6483 to 0,4746	No	**ns**	0,9997
DMSO 0.5% vs. 10 uM	0,07567	-0,4858 to 0,6371	No	**ns**	0,9999
DMSO 0.5% vs. 25 uM	-0,1577	-0,7191 to 0,4037	No	**ns**	0,9827
DMSO 0.5% vs. 50 uM	-0,5136	-1,075 to 0,04784	No	**ns**	0,0885
DMSO 0.5% vs. 75 uM	-0,4289	-0,9903 to 0,1326	No	**ns**	0,2232
DMSO 0.5% vs. 100 uM	-0,3559	-0,9173 to 0,2055	No	**ns**	0,4331
DMSO 0.5% vs. 150 uM	0,03357	-0,5279 to 0,5950	No	**ns**	>0,9999
DMSO 2% vs. 10 uM	0,1625	-0,3989 to 0,7239	No	**ns**	0,9793
DMSO 2% vs. 25 uM	-0,07087	-0,6323 to 0,4906	No	**ns**	>0,9999
DMSO 2% vs. 50 uM	-0,4268	-0,9882 to 0,1347	No	**ns**	0,2280
DMSO 2% vs. 75 uM	-0,3420	-0,9035 to 0,2194	No	**ns**	0,4821
DMSO 2% vs. 100 uM	-0,2691	-0,8305 to 0,2924	No	**ns**	0,7509
DMSO 2% vs. 150 uM	0,1204	-0,4410 to 0,6818	No	**ns**	0,9970
10 uM vs. 25 uM	-0,2334	-0,7948 to 0,3281	No	**ns**	0,8609
10 uM vs. 50 uM	-0,5893	-1,151 to -0,02783	Yes	*	0,0355
10 uM vs. 75 uM	-0,5045	-1,066 to 0,05690	No	**ns**	0,0983
10 uM vs. 100 uM	-0,4316	-0,9930 to 0,1299	No	**ns**	0,2172
10 uM vs. 150 uM	-0,04210	-0,6035 to 0,5193	No	**ns**	>0,9999
25 uM vs. 50 uM	-0,3559	-0,9173 to 0,2055	No	**ns**	0,4331
25 uM vs. 75 uM	-0,2712	-0,8326 to 0,2903	No	**ns**	0,7437
25 uM vs. 100 uM	-0,1982	-0,7596 to 0,3632	No	**ns**	0,9370
25 uM vs. 150 uM	0,1913	-0,3702 to 0,7527	No	**ns**	0,9478
50 uM vs. 75 uM	0,08473	-0,4767 to 0,6462	No	**ns**	0,9998
50 uM vs. 100 uM	0,1577	-0,4037 to 0,7191	No	**ns**	0,9827
50 uM vs. 150 uM	0,5472	-0,01427 to 1,109	No	**ns**	0,0594
75 uM vs. 100 uM	0,07297	-0,4885 to 0,6344	No	**ns**	>0,9999
75 uM vs. 150 uM	0,4624	-0,09900 to 1,024	No	**ns**	0,1572
100 uM vs. 150 uM	0,3895	-0,1720 to 0,9509	No	**ns**	0,3254

Table S4: Ordinary one-way ANOVA of Viability Assay 5000 cells incubated with **4a** for 24H**.** For Graph Prism 8.0.2, ns: $p > 0.1234$; *: $p < 0.0332$; **: $p < 0.0021$; ***: $p < 0.0002$; ****: $p < 0.0001$.

Ordinary one-way ANOVA of Viability Assay 5000 cells after incubation with 4c for 24H

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	**Summary**	Adjusted P Value
Control vs. DMSO 0.5%	-0,2811	-0,8088 to 0,2466	No	**ns**	0,6422
Control vs. DMSO 2%	-0,3679	-0,8956 to 0,1597	No	**ns**	0,3196
Control vs. 10 uM	-0,04030	-0,5680 to 0,4874	No	**ns**	>0,9999
Control vs. 25 uM	-0,2246	-0,7523 to 0,3030	No	**ns**	0,8455
Control vs. 50 uM	0,06787	-0,4598 to 0,5955	No	**ns**	>0,9999
Control vs. 75 uM	0,01407	-0,5136 to 0,5417	No	**ns**	>0,9999
Control vs. 100 uM	-0,4276	-0,9553 to 0,1001	No	**ns**	0,1703
Control vs. 150 uM	-0,4058	-0,9334 to 0,1219	No	**ns**	0,2169
DMSO 0.5% vs. DMSO 2%	-0,08683	-0,6145 to 0,4408	No	**ns**	0,9995
DMSO 0.5% vs. 10 uM	0,2408	-0,2869 to 0,7685	No	**ns**	0,7937
DMSO 0.5% vs. 25 uM	0,05647	-0,4712 to 0,5841	No	**ns**	>0,9999
DMSO 0.5% vs. 50 uM	0,3490	-0,1787 to 0,8766	No	**ns**	0,3816
DMSO 0.5% vs. 75 uM	0,2952	-0,2325 to 0,8228	No	**ns**	0,5859
DMSO 0.5% vs. 100 uM	-0,1465	-0,6742 to 0,3812	No	**ns**	0,9839
DMSO 0.5% vs. 150 uM	-0,1247	-0,6523 to 0,4030	No	**ns**	0,9942
DMSO 2% vs. 10 uM	0,3276	-0,2000 to 0,8553	No	**ns**	0,4586
DMSO 2% vs. 25 uM	0,1433	-0,3844 to 0,6710	No	**ns**	0,9860
DMSO 2% vs. 50 uM	0,4358	-0,09187 to 0,9635	No	**ns**	0,1551
DMSO 2% vs. 75 uM	0,3820	-0,1457 to 0,9097	No	**ns**	0,2780
DMSO 2% vs. 100 uM	-0,05967	-0,5873 to 0,4680	No	**ns**	>0,9999
DMSO 2% vs. 150 uM	-0,03783	-0,5655 to 0,4898	No	**ns**	>0,9999
10 uM vs. 25 uM	-0,1843	-0,7120 to 0,3433	No	**ns**	0,9404
10 uM vs. 50 uM	0,1082	-0,4195 to 0,6358	No	**ns**	0,9978
10 uM vs. 75 uM	0,05437	-0,4733 to 0,5820	No	**ns**	>0,9999
10 uM vs. 100 uM	-0,3873	-0,9150 to 0,1404	No	**ns**	0,2634
10 uM vs. 150 uM	-0,3655	-0,8931 to 0,1622	No	**ns**	0,3272
25 uM vs. 50 uM	0,2925	-0,2352 to 0,8202	No	**ns**	0,5966
25 uM vs. 75 uM	0,2387	-0,2890 to 0,7664	No	**ns**	0,8008
25 uM vs. 100 uM	-0,2030	-0,7306 to 0,3247	No	**ns**	0,9030
25 uM vs. 150 uM	-0,1811	-0,7088 to 0,3465	No	**ns**	0,9456
50 uM vs. 75 uM	-0,05380	-0,5815 to 0,4739	No	**ns**	>0,9999
50 uM vs. 100 uM	-0,4955	-1,023 to 0,03220	No	**ns**	0,0755
50 uM vs. 150 uM	-0,4736	-1,001 to 0,05403	No	**ns**	0,0989
75 uM vs. 100 uM	-0,4417	-0,9693 to 0,08600	No	**ns**	0,1449
75 uM vs. 150 uM	-0,4198	-0,9475 to 0,1078	No	**ns**	0,1859
100 uM vs. 150 uM	0,02183	-0,5058 to 0,5495	No	**ns**	>0,9999

Table S5: Ordinary one-way ANOVA of Viability Assay 5000 cells incubated with **4c** for 24H**.** For Graph Prism 8.0.2, ns: $p > 0.1234$; *: $p < 0.0332$; **: $p < 0.0021$; ***: $p < 0.0002$; ****: $p < 0.0001$.

Ordinary one-way ANOVA of Viability Assay 5000 cells after incubation with 4b for 24H

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?	**Summary**	Adjusted P Value
Control vs. DMSO 0.5%	-0,2811	-0,5914 to 0,02922	No	**ns**	0,0938
Control vs. DMSO 2%	-0,3679	-0,6782 to -0,05762	Yes	*	0,0135
Control vs. 10 uM	-0,6237	-0,9340 to -0,3134	Yes	****	<0,0001
Control vs. 25 uM	-0,4815	-0,7918 to -0,1712	Yes	***	0,0010
Control vs. 50 uM	-0,1532	-0,4635 to 0,1571	No	**ns**	0,7223
Control vs. 75 uM	0,4983	0,1880 to 0,8086	Yes	***	0,0006
Control vs. 100 uM	0,4924	0,1821 to 0,8027	Yes	***	0,0007
Control vs. 150 uM	0,4924	0,1821 to 0,8027	Yes	***	0,0007
DMSO 0.5% vs. DMSO 2%	-0,08683	-0,3971 to 0,2235	No	**ns**	0,9831
DMSO 0.5% vs. 10 uM	-0,3426	-0,6529 to -0,03225	Yes	*	0,0242
DMSO 0.5% vs. 25 uM	-0,2004	-0,5107 to 0,1099	No	**ns**	0,4106
DMSO 0.5% vs. 50 uM	0,1279	-0,1824 to 0,4382	No	**ns**	0,8662
DMSO 0.5% vs. 75 uM	0,7794	0,4691 to 1,090	Yes	****	<0,0001
DMSO 0.5% vs. 100 uM	0,7735	0,4632 to 1,084	Yes	****	<0,0001
DMSO 0.5% vs. 150 uM	0,7735	0,4632 to 1,084	Yes	****	<0,0001
DMSO 2% vs. 10 uM	-0,2557	-0,5660 to 0,05458	No	**ns**	0,1568
DMSO 2% vs. 25 uM	-0,1135	-0,4238 to 0,1968	No	**ns**	0,9243
DMSO 2% vs. 50 uM	0,2147	-0,09562 to 0,5250	No	**ns**	0,3285
DMSO 2% vs. 75 uM	0,8662	0,5559 to 1,177	Yes	****	<0,0001
DMSO 2% vs. 100 uM	0,8604	0,5501 to 1,171	Yes	****	<0,0001
DMSO 2% vs. 150 uM	0,8604	0,5501 to 1,171	Yes	****	<0,0001
10 uM vs. 25 uM	0,1422	-0,1681 to 0,4525	No	**ns**	0,7903
10 uM vs. 50 uM	0,4704	0,1601 to 0,7807	Yes	**	0,0012
10 uM vs. 75 uM	1,122	0,8117 to 1,432	Yes	****	<0,0001
10 uM vs. 100 uM	1,116	0,8058 to 1,426	Yes	****	<0,0001
10 uM vs. 150 uM	1,116	0,8058 to 1,426	Yes	****	<0,0001
25 uM vs. 50 uM	0,3282	0,01792 to 0,6385	Yes	*	0,0335
25 uM vs. 75 uM	0,9798	0,6695 to 1,290	Yes	****	<0,0001
25 uM vs. 100 uM	0,9739	0,6636 to 1,284	Yes	****	<0,0001
25 uM vs. 150 uM	0,9739	0,6636 to 1,284	Yes	****	<0,0001
50 uM vs. 75 uM	0,6515	0,3412 to 0,9618	Yes	****	<0,0001
50 uM vs. 100 uM	0,6457	0,3354 to 0,9560	Yes	****	<0,0001
50 uM vs. 150 uM	0,6457	0,3354 to 0,9560	Yes	****	<0,0001
75 uM vs. 100 uM	-0,005867	-0,3162 to 0,3044	No	**ns**	>0,9999
75 uM vs. 150 uM	-0,005867	-0,3162 to 0,3044	No	**ns**	>0,9999
100 uM vs. 150 uM	0,000	-0,3103 to 0,3103	No	**ns**	>0,9999

Table S6: Ordinary one-way ANOVA of Viability Assay 5000 cells incubated with **4b** for 24H**.** For Graph Prism 8.0.2, ns: $p > 0.1234$; *: $p < 0.0332$; **: $p < 0.0021$; ***: $p < 0.0002$; ****: $p < 0.0001$.

7 Pin1 INHIBITION ASSAY

The inhibitory activity of **4a** (unprotected Pin1 ligand) and **4b** (SATE-protected Pin1 ligand) was evaluated using the in vitro **SensoLyte® Green Pin1 Assay Kit** (AS-72240; AnaSpec, Fremont, CA, USA). This assay utilises a fluorogenic substrate that is pretreated to adopt the *cis* conformation. Pin1 catalyses the conversion of the substrate into its *trans* form, which is then readily cleaved, generating a fluorescent signal that can be monitored at an excitation/emission wavelength of 490/520 nm. The fluorescence intensity increase is directly proportional to Pin1 activity. Fluorescence measurements were recorded using a Jasco FP-8350 Spectrofluorometer equipped with a thermostated 96-well plate reader. The experiment followed the protocol provided in the assay kit instructions (https://www.anaspec.com/assets/2e1f981b-c942-41a2-9c63-f944682629cb/tds-en-as-72240-sensolyte-green-pin1-activity-assay-kit-fluorimetric.pdf)).

The assay was conducted in a black flat-bottom 96-well plate with a non-binding surface. Compounds **4a** and **4b** were tested at final well concentrations of 10 µM (in triplicate, 0.1% DMSO) and 100 µM (in triplicate, 1% DMSO). **Tannic acid**, provided in the Kit, served as a positive control at a final concentration of 1 mM (in duplicate). **Pin1 in buffer** acted as a negative control (in duplicate). A 1% DMSO solution was used as a blank (in duplicate). Additionally, solutions of **4a** and **4b in buffer without Pin1** were tested at 10 µM (in triplicate, 0.1% DMSO) and 100 µM (in triplicate, 0.1% DMSO) as blanks. Buffer alone was also included as a blank (in triplicate).

All samples, controls, and blanks were incubated at room temperature for one hour before adding 50 µL of the fluorogenic substrate to each well. After a further 30-minute incubation, 30 µL of developer was added to initiate the reaction. Fluorescence was measured at Ex/Em = 490/520 nm every five minutes, from time 0 to 120 minutes.

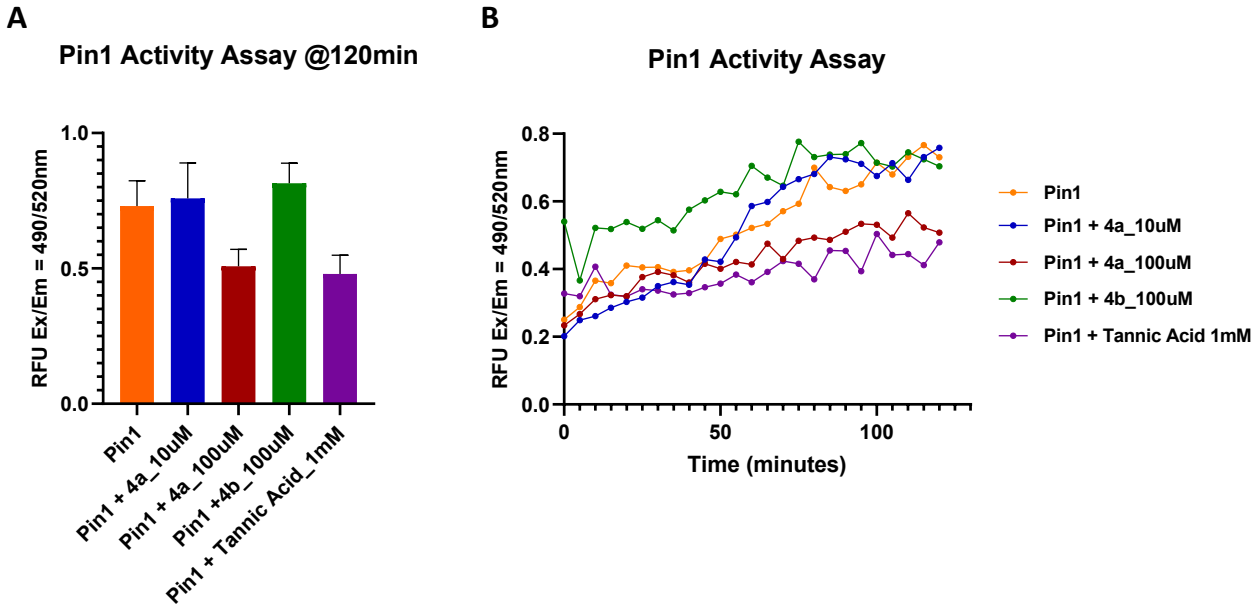


Figure S17: SensoLyte® Green Pin1 Inhibition. All fluorescent readings are expressed in Relative Fluorescence Units (RFU). **A**: RFU at 120 minutes. At low concentration (10 µM) **4a** (unprotected Pin1 ligand) does not significantely inhibit Pin1 activity. At high concentration (100 µM) **4a** (unprotected Pin1 ligand) inhibit Pin1 activity. Tannic Acid, the positive control, also inhibit Pin1 activity. **4b** (SATE-protected Pin1 ligand) does not inhibit Pin1 since the SATE group do not permit the binding. **B**:RFU from 0 to 120 minutes. For both A and B, the data are expressed as means ± SEM; n = 2 or 3.