Highly Functionalized Terpyridines as Competitive Inhibitors of AKAP–PKA Interactions**

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1. Materials and methods

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Instruments

NMR (\(^1\)H and \(^{13}\)C): 1D- and 2D-NMR spectra were recorded on a Bruker AV 300 (\(^1\)H: 300 MHz, \(^{13}\)C: 76 MHz). Chemical shifts \(\delta\) are depicted in ppm and coupling constants \(J\) in Hz. As internal standard, the measured NMR-spectra were normalized to the solvent peak used for recording the spectrum (\(d_6\)-DMSO: \(^1\)H: 2.50 ppm, \(^{13}\)C: 39.51 ppm).

The \(^{13}\)C-signals are assigned using DEPT- (distorsionless enhancement by polarization transfer) measurements; + = primary or tertiary C-atoms (positive DEPT-signal), – = secondary C-atoms (negative DEPT-signal), Cquart = quaternary C-atoms (no DEPT-signal).

LC-MS: All mass spectra were recorded on a 4000QTrap (Applied Biosystems) connected to a Shimadzu UFLC system. Ionization was done by electrospray (ESI) of an approximately 1 \(\mu\)M solution of the sample in pure MeCN, MeOH or MeCN/H\(_2\)O (1/1). All values are depicted as atomic mass units \(m/z\).

The system is equipped with a Shimadzu LC-20 system (degaser Degasys DG-2410, Autosampler SIL-20A, Controller CBM-20A) with a DAD-UV-detector (SPD-M20A). LC-MS runs were performed on an analytical Nucleodur column (100-5 C18 ec, 100 Å, 5\(\mu\)m, 50 x 4 mm, Macherey-Nagel). The flow rate was set to 1 ml/min and column temperature was set to 40°C. Injection volumes were set between 5 \(\mu\)l and 20 \(\mu\)l with an approximate sample concentration of 50 \(\mu\)M.

Analytical LC-MS runs were performed with the following gradient: water/0.1% formic acid (v/v) (solvent A) and acetonitrile/0.1% formic acid (v/v) (solvent B), 5% B to 95% B in 11 minutes. Stated retention times \(t_R\) of synthesized compounds refer to this gradient.

HRMS: High-resolution mass spectra were recorded on Ionspec QFT-7 (Varian) with a Z-spray-ESI-source (Micromass).

Melting Point Determination: Melting points of solid compounds were determined using an SMP3 machine from Bibby Sterilin Ltd.

Microwave: Microwave-assisted synthesis was performed with an InitiatorTM (Biotage). Reactions were performed in pressure-stable microwave vials with 0.2 ml-20 ml reaction volume. Microwave vials were sealed with a septum. A magnetron with 15 to 300 W generates microwave radiation with a frequency of 2.45 GHz. The temperature range of the machine can be varied from 60°C to 250°C with a heating rate of 2-5°C/min. The maximal acceptable pressure within the reaction vessel is 20 bar. During the reaction, the temperature within the vessel is kept constant by short microwave pulses. Fast cooling of the microwave vials is achieved with compressed air.

Chromatography methods

TLC: TLC plates were made off aluminum foil coated with silica gel 60 (with fluorescence indicator F254) or aluminum foil coated aluminum oxide with fluorescence indicator F254). For detection, a UV cabinet from Lamag was used (wavelengths: 254 nm and 365 nm).

Column chromatography (CC): Preparative CC was performed in glass columns with silica gel (pore size 60 Å, particle size 30-60 \(\mu\)m) from J.T. Baker as flash-chromatography with \(N_2\) overpressure. Composition of solvents used for CC is shown in volume fractions.

HPLC: The preparative HPLC system consisted of a Shimadzu LC-20 system (degasser Degasys DG-2410, Autosampler SIL-20A HT, Controller CBM-20A) with a DAD-UV-detector (SPD-M20A).

Preparative separations were performed on a Nucleodur C-18 column (100 Å, 10 \(\mu\)m 250 mm x 21 mm, Macherey-Nagel) with the flow rate set to 8.0 ml/min.

Semi-preparative separations were performed on a Nucleodur C-18 column (100 Å, 10 \(\mu\)m 250 mm x 10 mm, Macherey-Nagel) with the flow rate set to 2.0 ml/min.

Solvent A: water/0.1% acetic acid (v/v); Solvent B acetonitrile/0.1% acetic acid (v/v) (solvent B),
Chemicals

Starting materials and solvents for synthesis were purchased from Sigma-Aldrich, Fluka, Merck, J. T. Baker, Acros, and ALFA Aesar in p.a. grade. For HPLC- and LC-MS measurements, acetonitrile and methanol (HPLC grade) from J. T. Baker and purified water (Milli-Q-Plus from Millipore) were used. The anhydrous solvents DMF, THF, hexane, diethyl ether, 1,2-dichloroethane, methanol, toluene, xylene and dioxane were purchased from Sigma-Aldrich and Acros Organics. Deuterated solvents for NMR spectroscopy were purchased from Deutero GmbH.

Compound-containing extracts in organic solvents were dried over MgSO₄/Na₂SO₄ and the solvent was removed with temperature of the water bath of the rotary evaporator set to 40°C. Solvent residues were removed if necessary in high vacuum (p ≤ 10⁻³ mbar). Compounds in aqueous solutions were freeze-dried by lyophilization with an Alpha 1-2 lyophilizer (Christ).

Reactions involving moisture- or air-sensitive compounds were performed in oven-dried glass flasks under N₂-atmosphere. Anhydrous solvents were stored over molecular sieve (Fluka UOP type 3Å, 3 Å).
Chemical synthesis

$^1$H and $^{13}$C-NMR spectra of compounds are included starting on page 34

**Ethyl 3-(6-cyanopyridine-3-yl)propanoate (5)**

A 250 ml one-necked flask equipped with magnetic stir bar and condenser was charged with 5-bromo-2-cyanopyridine (2 g, 11 mmol), acrolein diethylacetal (5.08 ml, 33 mmol, d = 0.854 g/ml), n-tetrabutylammonium chloride (3.05 g, 11 mmol), tributyl-amine (5.25 ml, 22 mmol, d= 0.776 g/ml) and palladium(II) acetate (75 mg, 0.33 mmol). After addition of DMF (100 ml), the reaction mixture was heated to 120°C and stirred for 90 minutes. After the mixture had been cooled down to room temperature, it was diluted with 100 ml of 2 N hydrochloric acid. The aqueous phase was extracted three times with ethyl acetate, the combined organic phases were dried over MgSO$_4$ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : n-hexane = 1 : 2, v:v) to yield 5 (1.98 g, 88%) as yellow solid: Rf = 0.32; M. p. 36.4°C; ATR-IR (cm$^{-1}$): n = 2984, 2233, 1729, 1565, 847; $^1$H-NMR (300 MHz, d$_6$-DMSO): $\delta$ = 1.12-1.16 (t, $^3$J = 7.1 Hz, 3H, -CH$_2$-CH$_3$), 2.72-2.74 (t, $^3$J = 7.4 Hz, 2H, -CH$_2$-CO$_2$Et), 2.93-2.98 (t, $^3$J = 7.4 Hz, 2H, -CH$_2$-CH$_2$-CO$_2$Et), 4.00- 4.07 (quartet, $^3$J = 7.1 Hz, 2H, -CH$_2$-CH$_3$), 7.94-7.95 (m, 2H, H-4, H-5), 8.66 (s, 1H, H-2); $^{13}$C-NMR (75.5 MHz, d$_6$-DMSO): $\delta$ = 14.01 (-CH$_2$-CH$_3$), 27.37 (-CH$_2$-CH$_2$-CO$_2$Et), 33.70 (-CH$_2$-CO$_2$Et), 59.98 (-CH$_2$-CH$_3$), 117.60 (-CN), 128.58 (C-5), 130.34 (C-6), 137.40 (C-4), 141.07 (C-3), 151.51 (C-2), 171.72 (-CO$_2$Et); HRMS (ESI): calcd. for C$_{11}$H$_{12}$N$_2$O$_2$[H$^+$]: 205.0972, found 205.0965; HPLC-MS (ESI): tR = 5.37 min, m/z = 205.0 [M+H]$^+$. 
**Ethyl 3-[6-cyano-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-3-yl]propanoate (2)**

In an oven-dried 100 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet, rubber septum and bubbler 10 ml anhydrous THF and 2,2,6,6-tetramethylpiperidine (0.99 ml, 6.0 mmol) were added under nitrogen atmosphere. The reaction mixture was cooled down to 0°C, then 2.5 m n-butyllithium in hexane (2.35 ml, 6.0 mmol) was added dropwise into the flask via syringe and the reaction mixture was stirred for 30 minutes at 0°C. After cooling down to -75°C, ethyl 3-(6-cyanopyridine-3-yl)propanoate 5 (0.3 g, 1.5 mmol) dissolved in anhydrous THF was added drop-wise using a syringe. The reaction mixture turned deep brown and was stirred for 15 minutes at -75°C. Neat triisopropylborate (1.7 ml, 7.5 mmol, d = 0.818 g/ml) were added quickly to the reaction mixture, which was then stirred for 1 hour at -75°C. Then, pinacol (0.89 g, 7.7 mmol) dissolved in dry THF was added quickly to the reaction mixture. The solution was warmed up in a water bath (T = 40°C) and stirred for 2 hours. The reaction was quenched with water. The aqueous phase was acidified to pH 4 with 1 M hydrochloric acid and extracted three times with chloroform. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was frozen immediately and used without further purification in the next reaction step.

The product hydrolyzed to the free boronic acid under conditions used in HPLC and LC-MS runs. Analytical data represent results for the boronic acid:

**HRMS (ESI):** calcd. for C₁₁H₁₃BN₂O₄+[H⁺]: 249.1043, found 249.1032; HPLC-MS (ESI): tR = 4.18 min, m/z = 249.1 [M+H]⁺.
Ethyl 3-(5-bromo-2′-cyano-3-methyl-2,3′-bipyridin-5′-yl)-propanoate (6a)

An oven-dried 250 ml three-necked flask equipped with magnetic stir bar, condenser with bubbler, nitrogen inlet and dropping funnel was charged with 2,5-dibromo-3-methylpyridine 3a (2.2 g, 8.8 mmol), potassium phosphate (5.6 g, 26 mmol), palladium(II)acetate (0.46 g, 0.43 mmol) and triphenylphosphine (0.22 g, 1.7 mmol). After evacuation and subsequent purging with N₂ three times, 45 ml of dioxane and 5 ml of H₂O were added via the dropping funnel and the reaction mixture was stirred for 10 minutes at room temperature. After heating the mixture to 80°C, the crude ethyl-3-[6-cyano-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]propanoate 2 (6.6 g, about 8.8 mmol) of in dioxane (15 ml) was added via dropping funnel over 90 minutes. The reaction mixture was stirred for 150 minutes at 80°C, cooled down to room temperature and stirred over night. The reaction mixture was diluted with brine and extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate: n-hexane = 1 : 2) to yield 6a (2.4 g, 74%) as yellow solid: Rf = 0.27; M. p. 45.3°C; ATR-IR (cm⁻¹): n = 2978, 2229, 1714, 1367, 1107, 946; 

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.13 (t, 3J = 7.1 Hz, 3H, -CH₂-CH₃), 2.22 (s, 3H, -CH₃), 2.77 (t, 3J = 7.3 Hz, 2H, -CH₂-CO₂Et), 3.03 (t, 3J = 7.3 Hz, 2H, -CH₂-CH₂-CO₂Et), 4.03 (quartet, 3J = 7.1 Hz, 2H, -CH₂-CH₃), 8.05 (d, 4J = 1.7 Hz, 1H, H-4'), 8.20 (d, 4J = 1.6 Hz, 1H, H-4), 8.70 (d, 4J = 1.7 Hz, 1H, H-6'), 8.74 (d, 4J = 1.6 Hz, 1H, H-6);

¹³C-NMR (75.5 MHz, d₆-DMSO): δ = 14.01 (+, -CH₂-CH₃), 18.30 (+, -CH₃), 27.24 (-, -CH₂-CH₂-CO₂Et), 33.59 (-, -CH₂-CO₂Et), 60.00 (-, -CH₂-CH₃), 114.57 (Cquat, -CN), 121.58 (Cquat, C-5), 129.56 (C-2'), 134.49 (C, C-3), 137.68 (+, C-4'), 138.97 (Cquat, C-5'), 140.53 (Cquat, C-3'), 141.04 (+, C-4), 147.64 (+, C-6'), 151.01 (+, C-6), 151.55 (Cquat, C-2), 171.72 (-CO₂Et);

HRMS (ESI): calcd. for C₁₇H₁₆BrN₃O₂+[H⁺]: 374.0499, found 374.0496; HPLC-MS (ESI): tR = 7.55 min, m/z = 374.1 [M+H]+'.
Ethyl 3-(5-bromo-2’-cyano-3,4-dimethyl-2,3’-bipyridine-5’-yl)-propanoate (6b)

An oven-dried 250 ml three-necked flask equipped with a magnetic stir bar, condenser with bubbler, nitrogen inlet and dropping funnel was charged with 2,5-dibromo-3,4-dimethylpyridine 3b (2.0 g, 7.5 mmol), potassium phosphate (1M, 5.7 ml in H₂O), palladium(II)acetate (0.1 g, 0.45 mmol) and triphenylphosphine (0.5 g, 1.8 mmol). After evacuation and subsequent purging with N₂ for three times, 45 ml of dioxane and 5 ml of H₂O were added via the dropping funnel and the reaction mixture was stirred for 10 minutes at room temperature. After heating the mixture to 80°C, the crude ethyl 3-[6-cyano-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-3-yl]propanoate 2 (4.1 g, about 12.7 mmol) of in dioxane (15 ml) was added via dropping funnel over 90 minutes. The reaction mixture was stirred for 150 minutes at 80°C, cooled down to room temperature and stirred over night. The reaction mixture was diluted with brine and extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate: n-hexane = 1 : 4) to yield 6b (1.6 g, 56%) as yellow solid.

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.11-1.16 (t, 3J = 7.1, 3H, -CH₂-C₃H₃), 2.19 (s, 3H, -CH₃), 2.48 (s, 3H, -CH₃), 2.74-2.79 (t, 3J = 7.4, 2H, -CH₂-C₂H₃), 3.00-3.05 (t, 3J =7.4, 2H, -CH₂-C₂H₃), 4.00-4.07 (quartet, 3J = 7.1, 2H, -CH₂-C₃H₃), 7.98-7.99 (d, 3J = 1.9, 1H, H-4), 8.69 (s, 1H, H-11), 8.74-8.74 (d, 3J = 1.9, 1H, H-2);

¹³C-NMR (76 MHz, d₆-DMSO): δ = 14.02 (-CH₂-C₃H₃), 16.81 (-CH₃), 19.35 (-CH₃), 27.25 (-CH₂-C₂H₃), 33.56 (-CH₂-C₂H₃), 60.00 (-CH₂-C₃H₃), 116.60 (-CN), 123.79 (C-10), 129.61 (C-6), 133.18 (C-8), 137.91 (C-4), 139.76 (C-5), 140.56 (C-3), 146.71 (C-9), 148.09 (C-11), 150.92 (C-2), 151.65 (C-7), 171.74 (-CO₂Et);

HRMS (ESI): calcd. for C₁₈H₁₈BrN₃O₂+[H⁺]: 388.0655, found 388.0638; HPLC-MS (ESI): tR = 9.07 min, m/z = 388.2 [M+H]⁺.
3-(6,7-dihydro-5H-cyclopenta[\(c\)]pyridin-3-yl)-propanenitrile (9)

An oven-dried 500 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet and bubbler was charged under nitrogen atmosphere with succinonitrile 8 (10 g, 125 mmol), \(\text{Cp}^*\text{Ru(cod)Cl} \) (600 mg, 1.63 mmol) and 1,2-dichloroethane (300 ml). The reaction mixture was cooled down to 0°C and 1,6-heptadiyne 7 (7.6 g, 82.5 mmol) in 1,2-dichloroethane (50 ml) was added slowly via dropping funnel over 30 minutes. The reaction mixture was warmed up to room temperature and stirred for 20 hours. After concentration under reduced pressure the reaction mixture was chromatographed on silica gel (ethyl acetate: n-hexane = 2 : 1, v:v) to yield 9 (11.2 g, 79\%) as white solid; \(\text{Rf} = 0.39\); M. p. 68.5°C; ATR-IR (cm\(^{-1}\)): \(n = 2937, 2245, 1607, 896, 883\);

\(^1\text{H-NMR (300 MHz, }\text{d}_6\)-DMSO\): \(\delta = 2.03\) (q, \(^3J = 7.5\) Hz, 2H, H-6), 2.84-2.92 (m, 6H, -CH\(_2\)-CN, H-5, H-7), 2.99 (t, \(^3J = 7.3\) Hz, 2H, -CH\(_2\)-CH\(_2\)-CN), 7.22 (s, 1H, H-4), 8.36 (s, 1H, H-1);

\(^13\text{C-NMR (76 MHz, }\text{d}_6\)-DMSO\): \(\delta = 15.89\) (,-CH\(_2\)-CN), 25.64 (-, C-6), 29.31 (,- -CH\(_2\)-CH\(_2\)-CN), 31.95, 32.27 (-, C-5, C-7), 118.60 (C\(_{\text{quat}}\), -CN), 118.97 (+, C-4), 138.03 (C\(_{\text{quat}}\), C-7'), 144.63 (+, C-1), 153.96 (C\(_{\text{quat}}\), C-4'), 155.17 (C\(_{\text{quat}}\), C-3);

HRMS (ESI): calcd. for C\(_{11}H_{12}N_2\)+[H\(^+\)]; 173.1073, found 173.1070; HPLC-MS (ESI): \(\text{t}R = 1.13\) min, \(m/z = 173.0\) \([M+H]\)^+.
3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)propan-1-amine (10)

An oven-dried 500 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet, condenser with bubbler and dropping funnel was charged with 9 (5 g, 29 mmol) and anhydrous THF (200 ml) under nitrogen atmosphere. Neat BH$_3$*SMe$_2$ (12.6 g, 165.3 mmol) was added in one portion to the solution via dropping funnel. The reaction mixture was heated up to 60°C and stirred at this temperature over night. The solution was cooled down to room temperature, and the nitrogen inlet and dropping funnel were removed from the apparatus. 10 M hydrochloric acid was added very carefully (gas development!) over several hours until the white precipitate was completely dissolved. The reaction mixture was then heated to 70°C and stirred for 3 hours. After cooling down to room temperature, the aqueous phase was washed three times with diethyl ether. The aqueous phase was cooled down to 0°C and treated with 10 M sodium hydroxide solution to pH >11. The aqueous phase was extracted three times with chloroform. The combined organic phases were dried over a 1:1 mixture of MgSO$_4$ and K$_2$CO$_3$. Removal of the solvent under reduced pressure gave the crude amine 10 (4.4 g, 86%) as light brown oil. For the synthesis of dibenzyl amine 11a the amine 10 was used without further purification. Rf = 0.09;

$^1$H-NMR (300 MHz, d$_6$-DMSO): δ = 1.69-1.79 (m, 2H, -CH$_2$-CH$_2$-NH$_2$), 1.95-2.05 (m, 2H, H-6), 2.58 (t, $^3$J = 7.0 Hz, 2H, -CH$_2$-NH$_2$), 2.69 (t, $^3$J = 7.6 Hz, 2H, -CH$_2$-CH$_2$-CH$_2$-NH$_2$), 2.83 (t, $^3$J = 7.4 Hz, 4H, H-5, H-7), 7.11 (s, 1H, H-4), 8.30 (s, 1H, H-1);

$^{13}$C-NMR (76 MHz, d$_6$-DMSO): δ = 24.63 (-, C-6), 29.26 (-, -CH$_2$-CH$_2$-NH$_2$), 31.93, 32.30 (-, C-5, C-7), 34.59 (-, -CH$_2$-CH$_2$-CH$_2$-NH$_2$), 40.67 (-, -CH$_2$-NH$_2$), 118.58 (+, C-4), 136.90 (C$_{quat}$, C-7'), 144.39 (+, C-1), 153.52 (C$_{quat}$, C-4'), 158.78 (C$_{quat}$, C-3);

HRMS (ESI): calcd. for C$_{11}$H$_{17}$N$_2$+$[H]^+$: 177.1386, found 177.1380; HPLC-MS (ESI): tR = 1.01 min, m/z = 177.1 [$M+H]^+$.
N,N-dibenzyl-3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)-propan-1-amine (11a)

To a solution of crude amine 10 (5 g, 28.2 mmol) in acetonitrile (75 ml), benzyl bromide (6.7 ml, 56.4 mmol) and triethylamine (7.86 ml, 56.4 mmol) were added. The reaction mixture was stirred for 3 hours at 60°C or for 24 hours at room temperature. The solvent was removed under reduced pressure and the residue was partitioned between brine and chloroform. The aqueous phase was extracted twice with chloroform. The combined organic phases were dried over MgSO₄; the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : n-hexane = 3 : 7, v:v) to yield 11a (5.52 g, 55%) as yellow oil: Rf = 0.37; ATR-IR (cm⁻¹): n = 2941, 2793, 1631, 1607, 742, 697;

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.80-1.90 (m, 2H, -CH₂-CH₂-NBn₂), 1.93-2.03 (m, 2H, H-6), 2.35-2.40 (t, 3J = 7.0 Hz, 2H, -CH₂-NBn₂), 2.58-2.63 (t, 3J = 7.5 Hz, 2H, -CH₂-CH₂=CH₂-NBn₂), 2.75-2.83 (m, 4H, H-5, H-7), 3.52 (s, 4H, Bn-CH₂), 6.92 (s, 1H, H-4), 7.22-7.32 (m, 10H, Bn-H), 8.26 (s, 1H, H-1);

¹³C-NMR (76 MHz, d₆-DMSO): δ = 24.62 (–, C-6), 26.51 (–, -CH₂-CH₂-NBn₂), 29.25 (–, C-5), 31.92 (–, C-7), 34.94 (–, -CH₂-CH₂=CH₂-NBn₂), 52.12 (–, -CH₂-NBn₂), 57.48 (–, Bn-CH₂), 118.52 (+, C-4), 126.69 (+, C-d), 128.09 (+, C-c), 128.46 (+, C-b), 136.79 (Cquat, C-7'), 139.50 (Cquat, C-a), 144.36 (+, C-1), 153.35 (Cquat, C-4'), 158.72 (Cquat, C-3);

HRMS (ESI): calcd. for C₂₅H₂₈N₂⁺[H⁺]: 357.2325, found 357.2329; HPLC-MS (ESI): tR = 2.86 min, m/z = 357.2 [M+H]⁺.
An oven-dried 500 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet, bubbler and dropping funnel was charged with 2-dimethylaminoethanol (5.8 ml, 56 mmol) in anhydrous hexane under nitrogen atmosphere. The reaction mixture was cooled down to 0°C and 2.5 M nBuLi in hexane (44.8 ml, 112 mmol) was added to the solution by syringe. The mixture was stirred for 15 min at 0°C and then cooled down to -65°C. 11a (5 g, 14 mmol) in diethyl ether was added to reaction mixture by syringe. The solution turned deep red and was stirred at -65°C for 30 minutes. The mixture was cooled down to -75°C and hexachloroethane (16.5 g, 70 mmol) in anhydrous hexane (45 ml) was added in one shot by dropping funnel. The reaction mixture was stirred at -75°C for 30 minutes and quenched with water. The aqueous phase was extracted three times with chloroform. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : n-hexane = 1 : 4, v:v) to yield 4a (3.2 g, 59%) as white solid: Rf = 0.33; M. p. 48.7°C; ATR-IR (cm⁻¹): ν = 2943, 2802, 1600, 1545, 745, 692; ¹H-NMR (300 MHz, d₆-DMSO): δ = 1.88 (m, 2H, –CH₂-CH₂-NBn₂), 2.02 (q, ³J = 7.5 Hz, 2H, H-6), 2.36 (t, ²J = 6.9 Hz, 2H, –CH₂-NBn₂), 2.60 (t, ²J = 7.3 Hz, 2H, –CH₂–CH₂–CH₂–NBn₂), 2.82 (t, ²J = 7.5 Hz, 4H, H-5, H-7), 3.51 (s, 4H, Bn-CH₂), 6.93 (s, 1H, H-4), 7.22-7.32 (m, 10H, Bn-H);
¹³C-NMR (76 MHz, d₆-DMSO): δ = 23.46 (–, C-6), 26.08 (–, –CH₂-CH₂-NBn₂), 30.37 (–, C-5), 33.20 (–, C-7), 34.62 (–, –CH₂–CH₂–CH₂–NBn₂), 51.78 (–, –CH₂-NBn₂), 57.48 (–, Bn-CH₂), 118.36 (+, C-4), 126.70 (+, C-d), 128.08 (+, C-c), 128.47 (+, C-b), 135.26 (C quat, C-7'), 139.42 (C quat, C-a), 145.47 (C quat, C-1), 157.22 (C quat, C-4'), 160.33 (C quat, C-3).
HRMS (ESI): calcd. for C₂₅H₂₇N₂Cl+[H⁺]: 391.1936, found 391.1930; HPLC-MS (ESI): tR = 5.44 min, m/z = 391.2 [M+H]⁺.
N-benzyl-3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)-propan-1-amine (10BN)

The amine 10 (880 mg, 5 mmol), previously purified by column chromatography, was dissolved in anhydrous methanol (20 ml). Anhydrous magnesium sulphate (200 mg), benzaldehyde (500 µL, 5 mmol) and acetic acid (570 µL, 10 mmol) were added and reaction mixture was stirred at room temperature for 2 hours. Sodium cyanoborohydride (500 mg, 8 mmol) was added in three portions over 10 min and reaction mixture was stirred for additional 10 minutes. Reaction was quenched with brine and pH was adjusted to 9 by adding of 670 µL of 10 M NaOH. The aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : methanol : triethylamine = 8 : 2 : 0.1, v:v:v) to yield 10BN (750 mg, 57%) as white oil: Rf = 0.58;

1H-NMR (300 MHz, d₆-DMSO): δ = 1.87 (t, 3J = 7.3 Hz, 2H, -CH₂-CH₂-NHBn), 1.99-2.03 (m, 2H, H-6), 2.64-2.75 (m, 4H, CH₂-CH₂-CH₂-NHBn, -CH₂-NHBn), 2.83 (t, 3J = 7.4 Hz, 4H, H-5, H-7), 3.84 (s, 4H, Bn-CH₂), 7.11 (s, 1H, H-4), 7.22-7.36 (m, 5H, Bn-H ), 8.28 (s, 1H, H-1);

13C-NMR (76 MHz, d₆-DMSO): δ = 24.64 (–, C-6), 28.04 (–, -CH₂-CH₂-NHBn), 29.28 (–, C-5), 31.95 (–, C-7), 34.70 (–, -CH₂-CH₂-CH₂-NHBn), 47.50 (–, -CH₂-NHBn), 51.86 (–, Bn-CH₂), 118.71 (+, C-4), 127.32 (+, C-d), 128.25 (+, C-c), 128.56 (+, C-b), 137.08 (C₄quat, C-a), 137.75 (C₄quat, C-7'), 144.39 (+, C-1), 153.67 (C₄quat, C-4'), 158.34 (C₄quat, C-3);

HRMS (ESI): calcd. for C₁₈H₂₂N₂+H⁺: 267.1856, found 267.1853; HPLC-MS (ESI): tR = 1.13 min, m/z = 267.1 [M+H]+.
N-benzyl-3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)-N-(3-methoxybenzyl)-propan-1-amine (11b)

An oven-dried 15 ml microwave-flask equipped with magnetic stir bar was charged with benzyl amine 10BN (500 mg, 1.88 mmol), 3-methoxy-benzylbromide (264 µL, 1.88 mmol) and sodium hydroxide (100 mg, 2.44 mmol). Dioxane (3.5 ml) and water (3 ml) were added and reaction mixture was heated in the microwave to 100°C and stirred at this temperature for 30 minutes. After cooling down to room temperature, the reaction mixture was diluted with brine and extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : n-hexane = 1 : 1, v:v) to yield 11b (63%); Rf = 0.67;

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.87 (m, 2H, -CH₂-CH₂-NBn₂), 1.99 (q, 3J = 7.3 Hz, 2H, H-6), 2.38 (t, 3J = 7.5 Hz, 2H, CH₂-CH₂-CH₂-NBn₂), 2.62 (t, 3J = 7.0 Hz, 2H, CH₂-CH₂-CH₂-NBn₂), 2.83 (qv, 4H, H-5, H-7), 3.49, 3.52 (s, 4H, NBn-CH₂), 3.73 (s, 3H, OCH₃), 6.77-6.81 (m, 1H, H-d’), 6.89-6.91 (m, 2H, H-b’, H-f’), 6.93 (s, 1H, H-4) 7.19-7.32 (m, 6H, NBn-H, H-e’), 8.25 (s, 1H, H-1);

¹³C-NMR (76 MHz, d₆-DMSO): δ = 24.62 (-, C-6), 26.58 (-, -CH₂-CH₂-NBn₂), 29.25 (-, C-5), 31.91 (-, C-7), 34.97 (-, -CH₂-CH₂-CH₂-NBn₂), 52.24 (-, -CH₂-CH₂-NBn₂), 54.83 (+, OCH₃) 57.45, 57.41 (-, BN-CH₂), 112.06 (+, C-d’), 113.88 (+, C-b’), 118.50 (+, C-4), 120.59 (+, C-f’), 126.69 (+, C-d), 128.09 (+, C-c), 128.45 (+, C-b), 129.11 (+, C-e’), 136.80 (Cquat, C-7’), 139.48 (Cquat, C-a), 141.27 (Cquat, C-a’), 144.36 (+, C-1), 153.35 (Cquat, C-4’), 158.74 (Cquat, C-3), 159.19 (Cquat, C-e’);

HRMS (ESI): calcd. for C₂₆H₃₀N₂O+[H⁺]: 387.2431, found 387.2433; HPLC-MS (ESI): tR = 5.55 min, m/z = 387.2 [M+H]+.
N-benzyl-3-(1-chloro-6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)-N-(3-methoxybenzyl)propan-1-amine (4b)

An oven-dried 500 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet, bubbler and dropping funnel was charged with 2-dimethylaminoethanol (676 µL, 6.72 mmol) in anhydrous hexane under nitrogen atmosphere. The reaction mixture was cooled down to 0°C and 2.5 M nBuLi in hexane (5.37 ml, 13.44 mmol) was added to the solution by syringe. The mixture was stirred for 15 minutes at 0°C and then cooled down to -78°C. 11b (164 mg, 0.42 mmol) in diethyl ether was added to reaction mixture by syringe. The solution turned deep red and was stirred at -78°C for 30 minutes. The mixture warmed up to -30°C and hexa-chloroethane (500 mg, 2.1 mmol) in anhydrous hexane (45 ml) was added in one shot by dropping funnel. The reaction mixture was then stirred at 0°C for 30 minutes and quenched with saturated ammonium chloride solution. The aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate : n-hexane = 1 : 4, v:v) to yield 4b (138 g, 78%) as white oil: Rf = 0.45;

H-NMR (300 MHz, d₆-DMSO): δ = 1.84 (m, 2H, -CH₂-CH₂-NBn₂), 2.01-2.06 (m, 2H, H-6), 2.38 (t, 3J = 6.9 Hz, 2H, -CH₂-NBn₂), 2.62 (t, 3J = 7.3 Hz, 2H, -CH₂-CH₂-NBn₂), 2.86 (t, 3J = 7.5 Hz, 4H, H-5, H-7), 3.50, 3.52 (2s, 4H, Bn-CH₂), 3.74 (s, 3H, OCH₃), 6.69-6.82 (m, 1H, H-d'), 6.89-6.91 (m, 2H, H-b', H-f'), 6.96 (s, 1H, H-4), 7.19-7.32 (m, 6H, Bn-H, H-c');

C-NMR (76 MHz, d₆-DMSO): δ = 23.46 (C-6), 26.17 (-CH₂-CH₂-NBn₂), 30.38 (C-5), 32.84 (C-7), 34.30 (-CH₂-CH₂-CH₂-NBn₂), 51.91 (-CH₂-NBn₂), 54.85 (+, OCH₃) 57.45, 57.51 (+, Bn-CH₂), 112.09 (+, C-d'), 113.87 (+, C-b'), 118.35 (+, C-4), 120.61 (+, C-f'), 126.70 (+, C-d), 128.09 (+, C-c), 128.47 (+, C-b), 129.11 (+, C-e'), 135.27 (C', C-7'), 139.42 (C', C-a'), 141.21 (C', C-a), 145.46 (C', C-1), 157.26 (C', C-4'), 159.19 (C', C-3), 160.36 (C', C-c');

HPLC-MS (ESI): tR = 5.44 min, m/z = 421.9 [M+H]+.

Notice: The microwave-aided procedure for one-pot borylation and cross coupling reaction was optimized for chloride 4a and its bromide analog 4a-Br (not shown here). Considering the fact that the up-scaling of the microwave-assisted reaction on our machine was limited, the procedure was tested for chloride 4a also using standard thermal conditions.
Ethyl 3-(2'-cyano-5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-5'-yl)-propanoate (12a)

Microwave procedure: An oven-dried 20 ml microwave-flask equipped with magnetic stir bar was charged with ethyl 3-(5-bromo-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoate 6a (400 mg, 1.1 mmol), potassium acetate (320 mg, 3.2 mmol), bis(pinacolato) diboron (330 mg, 1.3 mmol) and in the Supporting Information Table S1 indicated amount of Pd(0) source and phosphine ligand and was sealed with a microwave septum. The flask was evacuated and flushed with nitrogen. After addition of 12 ml of the indicated solvent, the reaction mixture was heated in the microwave to 130°C and stirred at this temperature for 7 (DMF) or 32 (dioxane) minutes. After cooling down to room temperature, 0.75 mmol of the dihydro-5H-cyclopenta[c]pyridine halide 4a or 4a-Br in 3 ml of solvent and 1.6 mmol of base in 1 ml of H2O were added to reaction mixture via syringe. The reaction mixture was heated in the microwave to 130°C and stirred at this temperature for 30-40 minutes. After cooling down to room temperature, the reaction mixture was diluted with brine and extracted three times with ethyl acetate. The combined organic phases were dried over MgSO4 and the solvent was removed under reduced pressure. Column chromatography (EtOAc : hexane = 1 : 1 containing 1% Et3N, v:v:v) gave the product 12a as orange oil.

Supporting Table S1. Reaction conditions of 4a-Br and 4a.

|        | 4a-Br                  | 4a         |
|--------|------------------------|------------|
| Pd(0) source | 12 mg (0.05 mmol) Pd(OAc)2 | 25 mg (0.03 mmol) Pd2dba3 |
| phosphine  | 56 mg (0.21 mmol) of PPh3 | 102 mg (0.2 mmol) XPhos |
| solvent   | DMF                    | dioxane    |
| base      | 0.22 g (1.6 mmol) K2CO3 | 0.34 g (1.6 mmol) K2PO4 |
| yield of 12a | 48%                    | 54%        |

Thermal procedure: An oven-dried 100 ml three-necked flask equipped with magnetic stir bar, nitrogen inlet, rubber septum and condenser with bubbler was charged with ethyl 3-(5-bromo-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoate 6a (150 mg, 0.4 mmol), potassium acetate (120 mg, 1.2 mmol), bis(pinacolato)diboron (120 mg, 0.48 mmol), 0.01 g (0.01 mmol) tris(dibenzylidenacetone) dipalladium(0) (10 mg, 0.01 mmol) and XPhos (40 mg, 0.08 mmol). The flask was evacuated and flushed with nitrogen for at least three times. After addition of 3 ml dioxane the reaction mixture was heated to 102°C and stirred at this temperature for 1 hour. Then, N,N-dibenzyld-3-(1-chloro-6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)propan-1-amine 4a (100 mg, 0.28 mmol) in 3 ml of dioxane and potassium phosphate (130 mg, 0.6 mmol) in 0.3 ml of water were added to reaction mixture via syringe. The reaction mixture was stirred at 102°C for 3 more hours. After cooling down to room temperature, the reaction mixture was diluted with brine and extracted three times with ethyl acetate. The combined organic phases were dried over MgSO4 and the solvent was removed under reduced pressure. Column chromatography (EtOAc : hexane = 1 : 1 containing 1% Et3N) gave the product 12a in 54% yield as orange oil: Rf = 0.25;

1H-NMR (300 MHz, d6-DMSO): δ = 1.13 (t, 3J = 7.1 Hz, 3H, -CH3-CH2), 1.94-2.07 (m, 4H, -CH2-CH2-NBn2 and H-6”), 2.29 (s, 2H, -CH2), 2.43 (t, 3J = 6.9 Hz, 2H, -CH2-NBn2), 2.70-2.85 (m, 4H, -CH2-CO2Et, -CH2-CH2-CH2-NBn2), 2.88 (t, 3J = 7.4 Hz, 2H, H-5”), 3.07-3.13 (m, 4H, -CH2-CH2-CO2Et and H-7”), 3.53 (s, 4H, Bn-H), 4.03 (quartet, 3J = 7.1 Hz, 2H, -CH2-CH2), 7.03 (s, 1H, H-4”), 7.19-7.38 (m, 10H, Bn-H), 8.08 (s, 1H, H-4”), 8.15 (s, 1H, H-6”), 8.74 (s, 1H, H-6”); 13C-NMR (76 MHz, d6-DMSO): δ 14.36 (+, -CH2-CH3), 18.97 (+, -CH3), 25.26 (-, C-6”), 26.70 (-, -CH2-CH2-NBn2), 27.59 (-, -CH2-CH2-CO2Et), 31.76, 32.53 (-, C-5”, C-7”), 33.96 (-, -CH2-CO2Et), 35.22 (-, -CH2-CH2-CH2-NBn2), 52.44 (-, -
CH$_2$-NBn$_2$), 57.92 (C, -Bn-C), 60.33 (C, -CH$_2$-CH$_3$), 116.68 (C$_{quat}$, CN), 118.93 (C, C-4’’), 127.03 (+, C-d), 128.42 (+, C-c), 128.81 (+, C-b), 130.08 (C$_{quat}$, C-2’’), 131.81 (C$_{quat}$, C-3), 135.43 (C$_{quat}$, C-7b), 135.95 (C$_{quat}$, C-5), 138.17, 138.24 (+, C-4’’, C-4), 139.87 (C$_{quat}$, C-5’’), 140.17 (C$_{quat}$, C-3’’), 140.78 (C$_{quat}$, C-a), 146.67 (+, C-6), 148.99 (C$_{quat}$, C-4a), 151.14 (+, C-6’’), 152.19 (C$_{quat}$, C-2), 155.91 (C$_{quat}$, C-1’’), 159.82 (C$_{quat}$, C-3’’), 172.11 (-CO$_2$Et);

**HRMS (ESI):** calcd. for C$_{43}$H$_{43}$N$_5$O$_2$+H$^+$: 651.3490, found 650.3491; HPLC-MS (ESI): tR = 5.87 min, m/z = 649.9 [M+H]$^+$. 
Ethyl 3-(5-(3-(3-benzyl-(3-methoxbenzyl)-amino)-propyl)-6,7-dihydro-5H-cyclopaenta[c]pyridine-1-yl)-2'-cyano-3-methyl-2,3’-bipyridine-5’-yl)-propanoate (12b)

An oven-dried 15 ml microwave-flask equipped with magnetic stir bar was charged with ethyl 3-(5-bromo-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoate 6a (142 mg, 0.38 mmol), potassium acetate (56 mg, 0.57 mmol), bis-(pinacolato) diboron (116 mg, 1.3 mmol), tris(dibenzylideneacetone) dipalladium(0) (8 mg, 8.74 µmol) and XPhos (36 mg, 0.76 mmol) and dioxane (3.5 ml). The solution was stirred and bubbled with nitrogen for 30 min. The flask was sealed and reaction mixture was heated in the microwave to 130°C and stirred at this temperature for 30 minutes. After cooling down to room temperature, the reaction mixture was diluted with brine and extracted three times with ethyl acetate. The aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried over MgSO₄ and the solvent was removed under reduced pressure. The residue was chromatographed twice on silica gel first column (chloroform : n-hexane : methanol = 60 : 40 : 2, v:v:v), second column (ethyl acetate : n-hexane : methanol = 50 : 50 : 1, v:v:v) to yield 12b (130 mg, 50%) as white oil: Rf = 0.61;

1H-NMR (300 MHz, d₆-DMSO): δ = 1.14 (t, 3J = 7.1 Hz, 3H, -CH₂-C₂H₃), 1.93-2.07 (m, 4H, -CH₂-C₂H₂-NBn₂ and H-6-”), 2.29 (s, 3H, -CH₃), 2.45 (t, 3J = 6.9 Hz, 2H, -CH₂-NBn₂), 2.72-2.82 (m, 4H, -CH₂-CO₂Et, -CH₂-C₂H₂-NBn₂), 2.89 (t, 3J = 7.4 Hz, 2H, H-5’”), 3.02-3.13 (m, 4H, -CH₂-C₂H₂-CO₂Et, H-7””), 3.55, 3.54 (2s, 4H, Bn-O), 3.71 (s, 3H, OCH₃), 4.03 (quartet, 3J = 7.1 Hz, 2H, -CH₂-C₂H₃), 6.77-6.79 (m, 1H, H-d’), 6.90-6.92 (m, 2H, H-f’, H-b”), 7.04 (s, 1H, H-4’”), 7.17-7.34 (m, 6H, Bn-H, H-e’), 8.08 (d, 3J = 1.6 Hz, 1H, H-4”), 8.15 (d, 3J = 1.4 Hz, H-4), 8.74 (d, 3J = 1.6 Hz, 1H, H-6”), 8.89 (d, 3J = 1.4 Hz, 1H, H-6);

13C-NMR (76 MHz, d₆-DMSO): δ 14.02 (+, -CH₂-C₂H₃), 18.62 (+, -CH₃), 24.91 (+, C-6’”), 26.43 (+, -CH₂-C₂H₂-NBn₂), 27.25 (+, -CH₂-C₂H₂-CO₂Et), 31.54 (+, C-5’’), 32.17 (+, C-7’”), 33.62 (+, -CH₂-CO₂Et), 34.90 (+, -CH₂-C₂H₂-NBn₂), 52.23 (+, -CH₂-NBn₂), 54.82 (+, OCH₃), 57.75 (+, Bn-C), 60.00 (+, -CH₂-C₂H₃), 112.05 (+, C-d”), 113.89 (+, C-b’), 116.93 (C-quart-CN), 118.58 (+, C-4’”), 120.61 (+, C-f’), 126.70 (+, C-d), 128.09 (+, C-c), 128.46 (+, C-b), 129.11 (+, C-e’), 129.74 (C-quart-C-2’), 131.47 (C-quart-C-3), 135.09 (C-quart-C-7a), 135.61 (C-quart-C-5), 137.84, 137.91 (+, C-4”), 139.52 (C-quart-C-5”), 139.84 (C-quart-C-3’), 140.45 (C-quart-C-a), 141.31 (C-quart-C-a’), 146.34 (+, C-6), 148.66 (C-quart-C-4a), 150.45 (+, C-6”), 151.86 (C-quart-C-2), 155.59 (C-quart-C-1’”), 159.19 (C-quart-C-3’”), 159.50 (C-quart-C-c’), 172.11 (-CO₂Et);

HRMS (ESI): calcd. for C₈₇H₈₅N₇O₉[Z+H]: 680.3595, found 680.3614; HPLC-MS (ESI): tR = 6.10 min, m/z = 680.3 [M+H]⁺.
3-(2′-carbamoyl)-(5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-3-methyl-2,3′-bipyridine-5′-yl)-propanoic acid (1a)

A 50 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terpyridine 12a (431 mg, 0.66 mmol) and sodium hydroxide (266 mg, 6.6 mmol). After addition of ethanol (20 ml) and water (6 ml), the reaction mixture was refluxed overnight. After cooling down to room temperature, the reaction mixture was diluted with water and extracted three times with diethyl ether. The aqueous phase was acidified to pH 1 with 1 M HCl and extracted three times with n-butanol. The aqueous phase was then alkalized to pH 9 with saturated NaHCO₃ solution and extracted three times with n-butanol. The combined organic phases from pH 9 were dried over MgSO₄ and the solvent was removed under reduced pressure. The product was purified by preparative HPLC (gradient 20 to 90% Solvent B in 50 min). The terpyridine 1a (338 mg, 80%) was obtained as white solid after lyophilisation; m.p. 117.3°C, tR = 32.7 min; ATR-IR (cm⁻¹): n = 2922, 1722, 1671, 1197, 1086, 719, 700;

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.90-2.07 (m, 4H, -CH₂-CH₂-NBn₂ and H-6′′′), 2.10 (s, 3H, -CH₃), 2.45 (t, ³J = 7.3 Hz, 2H, -CH₂-NBn₂), 2.66 (t, ³J = 7.2 Hz, 2H, -CH₂-CH₂-CH₂-NBn₂), 2.74 (t, ³J = 7.1 Hz, 2H, -CH₂-CO₂H), 2.88 (t, ²J = 7.2 Hz, H-5′′′), 2.97 (t, 2H, ³J = 7.2 Hz, H-7′′′), 3.09 (t, 2H, ⁴J = 7.0 Hz, 2H, -CH₂-CH₂-CO₂H), 3.56 (s, 4H, HN=CH), 7.00 (s, 1H, H-4′′′), 7.18-7.37 (m, 6H, Bn-H), 7.67 (d, ³J = 1.6 Hz, 1H, H-4′′′), 7.97 (d, ³J = 1.4 Hz, H-4), 8.57 (d, ⁴J = 1.6 Hz, 1H, H-6′′′), 8.72 (d, ⁴J = 1.4 Hz, 1H, H-6);

¹³C-NMR (76 MHz, d₆-DMSO): δ = 18.86 (+, -CH₃), 24.94 (-, C-6′′′), 26.38 (-, -CH₂-CH₂-NBn₂), 27.06 (-, -CH₂-CH₂-CO₂Et), 31.57 (-, C-5′′′), 32.20 (-, C-7′′′), 34.31 (-, -CH₂-CO₂Et), 34.96 (-, -CH₂-CH₂-CH₂-NBn₂), 52.15 (-, -CH₂-NBn₂), 57.56 (-, Bn-C), 118.11 (+, C-4′′′), 126.71 (+, C-d), 128.10 (+, C-c), 128.450 (+, C-b), 130.35 (Cquat. C-3), 133.80 (Cquat. C-3′), 134.59 (Cquat. C-7a), 135.36 (Cquat. C-5), 136.12 (+, C-4), 138.42 (+, C-4′), 138.56 (Cquat. C-5′), 139.53 (Cquat. C-a), 145.16 (+, C-6), 147.27 (Cquat. C-4a), 147.76 (+, C-6′′′), 149.45 (Cquat. C-2′′′), 155.34 (Cquat. C-1′′′), 157.26 (Cquat. C-2), 159.27 (Cquat. C-3′′′), 166.88 (Cquat.-CONH₂), 173.42 (Cquat.-CO₂H);

HRMS (ESI): calcd. for C₄₀H₄₁N₅O₅⁺[H⁺]: 640.3282, found 640.3276; HPLC-MS (ESI): tR = 4.11 min, m/z = 640.3 [M+H]+.
5''-(2'-carboxyethyl)-(5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-2'-carboxylic acid) (1b)

A 50 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terpyridine 12a (60 mg, 0.09 mmol). After addition of 6 M HCl (3 ml), the reaction mixture was steered at 95°C for 90 min. The reaction mixture was concentrated in vacuum and the residue was dissolved in DMSO (1 ml) and purified by preparative HPLC (gradient 5 to 99% of Solvent B in 40 min). Terpyridinyl dicarboxilic acid 1b (43 mg, 71%) was obtained as white solid after lyophilisation; tR = 26 min;

$^1$H-NMR (300 MHz, d$_6$-DMSO): $\delta$ = 1.91-2.05 (m, 4H, -CH$_2$-CH$_2$-NBn$_2$ and H-$6''$), 2.19 (s, 3H, -CH$_3$), 2.44 (t, $^3J$ = 6.6 Hz, 2H, -CH$_2$-NBn$_2$), 2.67-2.74 (m, 4H, -CH$_2$-NBn$_2$), 2.87 (t, $^3J$ = 7.1 Hz, 2H, H-$5''$), 2.96 (t, $^3J$ = 7.1 Hz, 2H, H-$5''$), 3.07 (t, $^3J$ = 6.9 Hz, 2H, H-$5''$), 3.55 (s, 4H, Bn-H), 7.00 (s, 1H, H-$4''$), 7.21-7.34 (m, 10H, Bn-H), 7.79 (s, 1H, H-$4$), 8.02 (s, 1H, H-$4$), 8.58 (s, 1H, H-$6''$), 8.74 (s, 1H, H-$6''$);

$^{13}$C-NMR (76 MHz, d$_6$-DMSO): $\delta$ = 18.91 (+, -CH$_3$), 24.92 (-, C-$6''$), 26.35 (-, -CH$_2$-CH$_2$-NBn$_2$), 27.12 (CH$_2$-CH$_2$-CO$_2$Et), 31.51, 32.19 (-, C-$5''$, C-$7''$), 34.27 (-, -CH$_2$-CO$_2$Et), 34.93 (-, -CH$_2$-CH$_2$-CH$_2$-NBn$_2$), 52.11 (-, -CH$_2$-NBn$_2$), 57.56 (-, Bn-C), 118.26 (+, C-$4''$), 126.74 (+, C-d), 128.10 (+, -C-c), 128.51 (+, -C-b), 130.45 (C$_{quat}$, C-3), 134.27 (C$_{quat}$, C-7a), 134.75 (C$_{quat}$, C-3'), 135.39 (C$_{quat}$, C-5), 136.93, 137.87 (+, C-4', C-4), 138.30 (C$_{quat}$, C-5'), 139.44 (C$_{quat}$, C-a), 145.54 (+, C-6), 147.11 (C$_{quat}$, C-2'), 148.29 (C$_{quat}$, C-4a), 149.18 (+, C-6'), 155.41 (C$_{quat}$, C-2), 155.74 (C$_{quat}$, C-1''), 159.32 (C$_{quat}$, C-3''), 167.40 (C$_{quat}$, -CO$_2$H), 173.42 (C$_{quat}$, -CH$_2$-CO$_2$H);

HRMS (ESI): calcld. for C$_{40}$H$_{30}$N$_{4}$O$_{4}$+[H$^+$]: 641.3122, found 641.3139; HPLC-MS (ESI): tR = 3.92 min, m/z = 641.3 [M+H$^+$].
3-(5-(3-(dibenzyl(3-metoxbyenzy)amino)propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-2’-carbamoyl-3-methyl-2,3’-bipyridine-5’-yl)-propanoic acid (1c)

A 50 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terpyridine 12b (70 mg, 0.10 mmol) and sodium hydroxide (16.5 mg, 0.41 mmol). After addition of ethanol (7 ml) and water (3 ml), the reaction mixture was refluxed for 6 h. After cooling to room temperature, acetic acid was added to the reaction mixture and the pH was adjusted to 4. The reaction mixture was concentrated under vacuum; the residue was dissolved in DMSO and purified by preparative HPLC (gradient 5 to 95% acetonitrile in 60 min). Product 1c (39 mg, 57%) was obtained as white solid after lyophilisation; tR = 34.7 min;

^1H-NMR (300 MHz, d_6-DMSO): δ = 1.90-2.07 (m, 4H, -CH₂-CH₂-NBn₂ and H-6”), 2.09 (s, 3H, -CH₃), 2.45 (t, ^3J = 6.7 Hz, 2H, -CH₂-NBn₂), 2.65 (t, ^3J = 7.3 Hz, 2H, -CH₂-CH₂-CH₂-NBn₂), 2.73 (t, ^3J = 6.7 Hz, 2H, -CH₂-CO₂H), 2.87 (t, 2H, ^3J = 7.2 Hz, H-5”), 2.95 (t, 2H, ^3J = 7.2 Hz, H-7”), 3.01 (t, 2H, ^3J = 7.0 Hz, 2H, -CH₂-CH₂-H), 3.14, 3.54 (2s, 4H, Bn), 3.72 (s, 3H, OCH₃), 6.76-6.79 (m, 1H, H-d”), 6.90-6.92 (m, 2H, H-f’, H-b”), 6.99 (s, 1H, H-4”), 7.18-7.35 (m, 6H, Bn-H, H-c’), 8.08 (d, ^4J = 1.6 Hz, 1H, H-4”), 8.15 (d, ^4J = 1.4 Hz, H-4), 8.74 (d, ^4J = 1.4 Hz, H-f”, 8.89 (d, ^4J = 1.6 Hz, 1H, H-6”);

^13C-NMR (76 MHz, d_6-DMSO): δ = 18.62 (+, -CH₃), 24.95 (-, C-6”), 26.48 (-, -CH₂-CH₂-NBn₂), 27.09 (-, -CH₂-CH₂-CO₂Et), 31.59 (-, C-5”), 32.20 (-, C-7”), 34.36 (-, -CH₂-CO₂Et), 35.01 (-, -CH₂-CH₂-CH₂-NBn₂), 52.30 (-, -CH₂-NBn₂), 54.85 (+, OCH₃), 57.55, 57.61 (-, Bn-C), 112.09 (+, C-d”), 113.90 (+, C-b”), 118.10 (+, C-4”), 120.63 (+, C-f”), 126.73 (+, C-d), 128.12 (+, C-c), 128.49 (+, C-b), 129.14 (+, C-c’), 130.37 (C_quat, C-3), 133.82 (C_quat, C-3’), 134.62 (C_quat, C-7a), 135.38 (C_quat, C-5), 136.15 (+, C-4), 138.47 (C_quat, C-5’), 138.58 (+, C-4”), 139.54 (C_quat, C-a), 141.32 (C_quat, C-a’), 145.18 (+, C-6), 147.27 (C_quat, C-4a), 147.78 (+, C-6”), 149.46 (C_quat, C-2”), 155.37 (C_quat, C-1”), 157.27 (C_quat, C-2), 159.21 (C_quat, C-3”), 159.31 (C_quat, C-c”), 166.90 (C_quat,-CONH₂), 173.46 (C_quat,-CO₂H);

HRMS (ESI): calcd. for C₄₁H₄₃N₅O₁₊[H]: 670.3388, found 670.3391; HPLC-MS (ESI): tR = 4.15 min, m/z = 670.4 [M+H]^+. 

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A 10 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terephthalic acid 12b (14 mg, 0.02 mmol). After addition of 6 M HCl (1 ml), the reaction mixture was steered at 95°C for 90 min. The reaction mixture was concentrated in vacuum and the residue was dissolved in DMSO (1 ml) and purified by semipreparative HPLC (gradient 5 to 99% acetonitrile in 40 min). Terephthalic acid dicarboxylic acid 1d (7 mg, 47%) was obtained as white solid after lyophilisation; tR = 25.3 min;

\[ \text{1H-NMR (300 MHz, d6-DMSO):} \delta = 1.91-2.06 (m, 4H, -CH2-CH2-NBn2 and H-6"), 2.19 (s, 3H, -CH3), 2.44 (m, 6H, 2H, -CH2-NBn2), 2.63-2.74 (m, 4H, -CH2-CO2H, -CH2-CH2-CH2-NBn2), 2.87 (t, \( J = 7.1 \) Hz, 2H, H-5'"), 2.96 (t, \( J = 7.1 \) Hz, 2H, H-7'"), 3.07 (t, \( J = 6.9 \) Hz, 2H, -CH2-CH2-CO2H), 3.56 (s, 4H, Bn-H), 3.70 (s, 3H, OCH3), 5.76 (s, 1H, CH=O), 6.76-6.79 (m, 1H, H-d'), 6.90-6.92 (m, 2H, H-f', H-b"), 7.02 (s, 1H, H-4'"), 7.18-7.35 (m, 6H, Bn-H, H-1'"), 7.79 (s, 1H, H-4"'), 8.02 (s, H-4), 8.58 (s, 1H, H-6"'), 8.74 (s, 1H, H-6);

\[ \text{13C-NMR (76 MHz, d6-DMSO):} \delta = 18.91 (+, -CH3), 24.92 (-, C-6"'), 26.26 (-, -CH2-CH2-NBn2), 27.12 (CH2-CH2-CO2Et), 31.51, 32.19 (-, C-5"'), 34.26 (-, -CH2-CO2Et), 34.93 (-, -CH2-CH2-CH2-NBn2), 52.22 (-, -CH2-NBn2), 54.85 (-, OCH3), 57.56 (-, Bn-C), 111.00 (+, C-d"'), 112.0 (+, C-b"), 118.25 (+, C-4"'), 120.67 (+, C-f"), 126.79 (+, C-d), 128.13 (+, C-c), 128.53 (+, C-b), 129.15 (+, C-e"'), 130.45 (Cquat, C-3'), 134.27 (Cquat, C-3"'), 134.78 (Cquat, C-7a), 135.38 (Cquat, C-5'), 136.93 (+, C-4), 137.89 (Cquat, C-5"'), 138.33 (+, C-4"'), 139.54 (Cquat, C-a), 141.34 (Cquat, C-a"'), 145.54 (+, C-6), 147.05 (Cquat, C-4a), 148.30 (+, C-6"'), 149.19 (Cquat, C-2'), 155.44 (Cquat, C-1"'), 155.72 (Cquat, C-2"'), 159.20 (Cquat, C-3"'), 159.20 (Cquat, C-c"'), 167.04 (Cquat, -CONH2), 173.42 (Cquat, -CO2H);

\[ \text{HRMS (ESI):} \text{calcd. for C41H34N2O5+[H]+:} 671.3228, \text{found 671.3240; HPLC-MS (ESI):} \text{tR = 4.16 min, m/z = 671.3 [M+H]+.} \]
3-(5-(3-(dibenzy1-(3-metoxybenzyl)-amino)-propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-2’-cyano-3-methyl-2,3’-bipyridine-5’-yl)-propanoic acid (1e)

A 50 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terpyridine 12b (60 mg, 0.09 mmol) and sodium hydroxide (14 mg, 0.35 mmol). After addition of ethanol (7 ml) and water (3 ml), the reaction mixture was refluxed for 6 h. After cooling down to room temperature, the acetic acid was added to the reaction mixture and pH was adjusted to 4. The reaction mixture was concentrated in vacuum; the residue was dissolved in DMSO and purified by preparative HPLC (acetonitrile:H₂O + 0.1% CH₃COOH, gradient 5 to 99% acetonitrile in 60 min). Product 1e (19 mg, 33%) was obtained as white solid after lyophilisation; tR = 40.5 min;

¹H-NMR (300 MHz, d₆-DMSO): δ = 1.90-2.07 (m, 4H, -CH₂-CH₂-NBn₂ and H-6’’), 2.28 (s, 3H, -CH₃), 2.44 (t, J = 6.7 Hz, 2H, -CH₂-NBn₂), 2.65-2.75 (m, 4H, -CH₂-CH₂H -CH₂-CH₂-CH₂-NBn₂), 2.89 (t, 2H, J = 7.2 Hz, H-5’’), 2.95-3.05 (t, 4H, H-7’’, -CH₂-CH₂-CO₂H), 3.52, 3.54 (2s, 4H, Bn-H), 3.72 (s, 3H, OCH₃), 6.76-6.79 (m, 1H, H-d’), 6.90-6.92 (m, 2H, H-f’, H-b’), 7.03 (s, 1H, H-4’’), 7.18-7.35 (m, 6H, Bn-H, H-e’), 8.06 (d, J = 1.6 Hz, 1H, H-4’), 8.13 (d, J = 1.4 Hz, H-4), 8.73 (d, J = 1.6 Hz, 1H, H-6’’), 8.89 (d, J = 1.4 Hz, 1H, H-6);

¹³C-NMR (76 MHz, d₆-DMSO): δ = 18.65 (+, -CH₃), 24.92 (-, C-6’’), 26.44 (-, -CH₂-CH₂-NBn₂), 27.37 (-, -CH₂-CH₂-CO₂Et), 31.43 (-, C-5’’), 32.18 (-, C-7’’), 33.42 (-, -CH₂-CO₂Et), 34.92 (-, -CH₂-CH₂-CH₂-NBn₂), 52.23 (-, -CH₂-NBn₂), 54.82 (+, OCH₃), 57.56, 57.62 (-, Bn-C), 112.06 (+, C-d’), 113.89 (+, C-b’), 118.56 (+, C-4’’), 120.61 (+, C-f’), 126.71 (+, C-d), 128.09 (+, C-c), 128.47 (+, C-b), 129.11 (+, C-e’), 129.64 (C_quat C-3), 131.48 (C_quat C-3’), 135.09 (C_quat C-7a), 135.60 (C_quat C-5), 137.80 (+, C-4), 137.90 (+, C-4’), 139.51 (C_quat C-a), 139.83 (C_quat C-5’’), 140.90 - 141.30 (C_quat C-a’), 146.33(+, C-6), 148.66 (C_quat C-4a), 150.83 (+, C-6’’), 151.89 (C_quat C-2’’), 155.58 (C_quat C-1’’), 157.27 (C_quat C-2), 159.19 (C_quat C-3’’), 159.50 (C_quat C-e’), 173.34 (C_quat -CO₂H);

HRMS (ESI): calcd. for C₄₁H₄₁N₅O₅+[H⁺]: 652.3291, found 652.3282; HPLC-MS (ESI): tR = 5.28 min, m/z = 652.3 [M+H⁺].
A 10 ml one-necked flask equipped with magnetic stir bar and condenser was charged with terpyridine 12c (30 mg, 0.05 mmol) and sodium hydroxide (18 mg, 0.45 mmol). After addition of ethanol (2.25 ml) and water (0.75 ml), the reaction mixture was refluxed overnight. After cooling down to room temperature, the reaction mixture was diluted with water and extracted three times with diethyl ether. The aqueous phase was acidified to pH 1 with 1 M HCl and extracted three times with n-butanol. The aqueous phase was then alkalized to pH 9 with saturated NaHCO₃ solution and extracted three times with n-butanol. The combined organic phases from pH 9 were dried over MgSO₄ and the solvent was removed under reduced pressure. The product was purified by preparative HPLC (gradient 20 to 90% Solvent B in 50 min). The terpyridine 1f (17.9 mg, 56%) was obtained as white solid after lyophilisation; m.p. 68.5 °C.

¹H-NMR (600 MHz, d₆-DMSO): δ = 1.90-1.92 (m, 2H, -CH₂-CH₂-NBn2), 1.98-2.01 (m, 7H, -CH₃, H-6'', H-7''), 2.39-2.42 (t, 3J = 7.3 Hz, 2H, -CH₂-CO₂H), 2.49 (s, 3H, overlapped with DMSO-peak), 2.63-2.70 (m, 6H, -CH₂-NBn₂, -CH₂-CH₂-NBn2), 2.88-2.91 (t, 3J = 7.3 Hz, 2H, CH₂-CH₂-CO₂H), 2.93-2.95 (t, 3J = 7.3 Hz, 2H, H₅''), 3.52 (s, 4H, Bn-H), 7.00 (s, 1H, H-4''), 7.21-7.22 (m, 2H, Bn-H⁴), 7.28-7.32 (m, 8H, Bn-H), 7.61 (s, 1H, H-4''), 7.92 (s, 2H, -NH₂), 8.11 (s, 1H, H-5), 8.72 (s, 1H, H-5').

¹³C-NMR (76 MHz, d₆-DMSO): δ = 15.93, 24.49, 26.45, 27.04, 30.39, 32.37, 34.29, 34.92, 52.13, 57.53, 123.15, 126.67, 128.07, 128.43, 129.71, 131.01, 136.03, 136.55, 138.25, 138.70, 139.52, 144.99, 147.72, 150.81, 156.62, 157.97, 159.64, 166.87, 173.42;

HRMS (ESI): calcd. for C₄₁H₄₃N₅O₃+H⁺: 654.3439, found 654.3444; HPLC-MS (ESI): tR = 3.98 min, m/z = 654.3 [M+H⁺].
Generation of recombinant AKAP18α, D/D domain of RIIα, full-length RIIα and GSKIP

Recombinant AKAP18α was generated from the vector pGEX-4T-3 encoding a GST-tagged version of AKAP18α (GST-Δ(2-10)-AKAP18α), lacking the membrane targeting domain, i.e. palmitoylation and myristoylation sites. His-tagged versions of the D/D domain (amino acids 1-44) of RIIα and full-length RIIα were obtained from vectors pET46 and pET28, respectively. GSKIP was generated as a His-tagged version from the vector pET28. All constructs were expressed in E. coli (strain Rosetta DE3) in the presence of the respective antibiotics.

Pre-cultures from cryo stocks were grown in 50 LB medium at 25 to 37°C overnight, centrifuged and resuspended to inoculate up to four 500 ml Overnight Express™ Instant TB Medium (autoinduction medium (AIM); Novagen). Cultures were grown at least for 24 h at 20 - 37°C under constant agitation (110 rpm) in 2 l Erlenmeyer flasks. For expression of 15N-labelled His-RIIα D/D (amino acids 1-44) bacteria were grown over night in 500 ml Minimal Medium (2 x M9) with 15N-NH₄Cl in 2 l Erlenmeyer flasks at 25°C. Expression was induced with 0.5 mM IPTG at a cell density of A₆₀₀=1.0. Cells were harvested by centrifugation, washed with PBS and stored at -80°C.

For purification of tag-free RIIα D/D (amino acids 1-44) E. coli cells were thawed on ice and resuspended in extraction buffer (40 mM phosphate buffer, pH 7.5, 300 mM NaCl, 5 mM imidazole, 5 mM β-mercaptoethanol, 0.5 mM PMSF, Roche Protease Inhibitor tablet, benzonase (5 µl/100 ml). Lysates were prepared in a French press, cell debris was removed by centrifugation at 21000 rpm at 4°C for 30 min. The supernatant was cleared by filtration through a 0.45 µm filter and applied to a TALON cobalt affinity column (Clontech) in a ÄKTA system pre-equilibrated with 40 mM phosphate buffer, pH 7.5, 300 mM NaCl, 5 mM imidazole. The TALON column was then washed with equilibration buffer and eluted with 40 mM phosphate buffer, pH 7.5, 300 mM NaCl and an imidazole gradient up to 300 mM. His-RII D/D (amino acids 1-44) containing fractions were pooled. The N-terminal His-tag was removed by Enterokinase digestion (5 U/mg) at 4°C while dialysing against 20 mM phosphate buffer, pH 7.5, 150 mM NaCl. Still undigested His-RIIα D/D (amino acids 1-44) and the His-tag were removed by re-running the protein solution on a TALON column. Completely digested RIIα D/D (amino acids 1-44) was collected in the flow through. The final polishing step was a gel filtration with Superdex 75 (GE Healthcare) in 20 mM HEPES, 300 mM NaCl, pH 7. The most pure fractions were pooled, supplemented with 20% glycerol and stored at -80°C.

For purification of GST-Δ(2-10)-AKAP18α E. coli cells were lysed in 20 mM phosphate buffer, pH 7.5, 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, Roche Protease Inhibitor tablet, benzonase (5 µl/100 ml) in a French Press. The lysates were incubated in the presence of 1 % Triton X-100 for 30 min at 4°C under agitation. Affinity purification was carried out as recommended by the supplier of the pGEX-4T-3 vector (Amersham Biosciences). The sample was dialysed against 20 mM phosphate buffer, pH 7.5, 50 mM NaCl and subsequently loaded on an anion exchange column, Res Q. Protein was eluted with a gradient of up to 1 M NaCl in 20 mM Tris-HCl, pH 8. The final polishing step was by gel filtration with Superdex 75 (GE Healthcare) in 20 mM HEPES, 300 mM NaCl, pH 7. The most pure fractions were pooled, supplemented with 20% glycerol and stored at -80°C.

Purification of full-length RIIα with a C-terminal His-tag followed the protocol for purification of RIIα D/D up to the TALON cobalt affinity column (Clontech). After dialysis against 20 mM HEPES, 20 mM NaCl, pH 7, the TALON elution pool was loaded on an anion exchange column, Res Q, equilibrated with the same buffer. Protein was eluted with a gradient of up to 1 M NaCl in 20 mM HEPES, pH 7. The final polishing step was by gel filtration with Superdex 75 (GE Healthcare) in 20 mM HEPES, 300 mM NaCl, pH 7.

His-tagged GSKIP was purified by affinity chromatography using Ni-NTA with 20 mM HEPES, 300 mM NaCl, 10 mM β-Mercaptoethanol, pH 8. Eluted fractions were loaded on a MonoQ anion exchange column at 20 mM Tris-HCl, pH 7.5. Fractions containing GSKIP were eluted by a gradient of up to 1 M NaCl under the same buffer conditions. GSKIP-containing fractions were finally dialysed against 20 mM phosphate, 100 mM NaCl, pH 7.5.
Enzyme-linked immunoabsorbant assay (ELISA)

ELISAs were essentially carried out as previously described. Micro-titer plates (Corning, 384 well, high binding, polystyrene) were coated overnight at 4°C with RIIa-His (20 nM) in incubation buffer (20 µl PBS with 0.5 mM PMSF, 1 mM benzamidine, 3.2 µg/ml trypsin inhibitor I-S and 2 µg/ml aprotinin). Liquid was removed and blocking was carried out for 60 min with 95 µl of blocking buffer (PBS with 0.05% Tween 20, 0.3% skimmed milk powder). The buffer was removed and subsequently 10 µl blocking buffer, 1 µl of serial dilution of each test compound in DMSO, 10 µl of His-GSKIP (20 nM) in blocking buffer were added to the wells. The micro-titer plates were incubated for 30 min at room temperature under constant agitation. The wells were washed three times with washing buffer (PBS with 0.05% Tween 20) and incubated for 60 min with 20 µl GSKIP-antibody (custom-made affinity-purified anti-GSKIP antibody (rabbit polyclonal antiserum raised against recombinant full-length GSKIP (139 amino acid residues; Biogenes, Berlin, Germany), 1:3000)) in blocking buffer. The wells were washed as before, 20 µl horseradish peroxide-conjugated secondary antibody (PO-conjugated AffiniPure F(ab)2 fragment donkey anti-rabbit IgG (ImmunoResearch), 1:3000) in blocking buffer was added and the plates were incubated for 60 min at room temperature. The wells were washed again as before and incubated for 15 min with 20 µl tetramethylbenzidine (TMB) solution (3,3',5,5'-Tetramethylbenzidine Liquid Substrate, Supersensitive, for ELISA; Sigma). The reaction was quenched with 20 µl 1 M HCl, and the absorbance was measured at λ = 450 nm. IC₅₀ values were calculated with GraphPad Prism using nonlinear fit of log(inhibitor) vs. response – Variable slope.

Homogeneous time resolved fluorescence (HTFR) assays

The His-tagged RIIa D/D domain (see above; 5 µl of a 50 nM solution in Assay buffer (PBS containing 0.05% BSA and 0.05% Tween 20) was applied to columns 1-23 (Perkin Elmer LAS (Germany) GmbH, Rodgau-Jügesheim, Germany; Proxiplate-384 Plus, white, Cat. 6008289). Compounds 1a-f or the peptides AKAP188-L314E or AKAP188-PP (0.2 µl) were added to columns 1-22 in the indicated concentrations (Figure 2), columns 23 and 24 were incubated with DMSO, the solvent for the compounds. As a negative control, column 24 was incubated under the same conditions but in the absence of the D/D domain and AKAP18α. The samples were incubated for 1 h at room temperature. GST-Δ(2-10)-AKAP18α (see above; 5 µl of a 50 nM solution in Assay buffer) together with anti-GST antibody coupled to terbium (Donor) and anti-6-His-tag antibody coupled to XL665 (Acceptor) were added to columns 1-23 and incubated for 1 h. Final antibody dilutions were 1:200; both antibodies were purchased from CisBio (http://www.htrf.com/htrf-anti-tag-reagents-toolbox; Cisbio Bioassays BP 84175, Bagnols-sur-Ceze Cedex, France). FRET signals were recorded using a Genios Pro plate reader (Tecan Genios Pro Art.Nr.: F129035; Tecan Austria GmbH, Grödig, Austria).

Homology modeling / docking procedure

Based on functional data from previous mutagenesis and modeling studies of peptides at the D/D domain we here used the NMR structures PDB code 1L6E and 2H9R as templates for the D/D domain. The NMR structure, 2H9R contains the helical portion of AKAP79 as bound ligand and was used for the AKAP188-L314E interaction model (Supporting Information Figure S1). To further map the ligand binding area of the terpyridine scaffolds, the terpyridine scaffolds into the D/D domain, not only previous substitution studies of AKAP18 peptides were used but also a set of NMR chemical shift data of the terpyridine scaffolds. These shifts were recorded upon terpyridine scaffold binding to identify the most important functional groups of the D/D domain. The binding pocket formed by helix I and I’ is rather hydrophobic (I5, P6, L9, T10, L13, T17, L21 (yellow in Figure 4 and Supporting Information Figure S1)) and contains the possible proton-donators Q4, Q14 (blue in Figure 4 and Supporting Information Figure S1).

Docking studies of the terpyridine scaffolds were performed using the pharmacophore module in MOE (Molecular Operating Environment software). For this the proton donators of the D/D domain, derived from experimental findings (see above), were addressed by possible proton acceptors of the terpyridine scaffolds. 25-30 top-scoring poses in terms of affinity (kcal mol⁻¹ of total estimated binding energy) were automatically selected for further analysis, using the build-in ‘Affinity dG’ scoring protocol. Further energy minimization was carried out by using the MMFF94 force field.
2. Results

Model of the complex of the D/D domain of regulatory RIIα subunits of PKA with a peptide representing the RII-binding domain of AKAP18δ

Supporting Figure S1. Model of the complex of the D/D domain of RIIα and the peptide AKAP18δ-L314E based on the structures pdb-code 1L6E, and 2H9R. The peptide is shown in magenta, helices I and II of the D/D domain in green, and helices I′ and II′ in gray. Interacting residues of the D/D domain are shown as translucent surface (yellow: hydrophobic, blue: hydrophilic). The D/D domain contains several amino acids showing shifts in the NMR-spectrum upon binding of peptides or terpyridine scaffolds (see Supporting Information Figure S3).
Peptides derived from the PKA binding domain of AKAP18δ inhibit the interaction between regulatory RIIα subunits of PKA with the AKAP glycogen synthase 3β-interacting protein (GSKIP) in a concentration-dependent manner.

Supporting Table S2

| Peptide sequence | AKAP18δ-IC\textsubscript{50} [\mu M] [a] |
|------------------|---------------------------------------|
| LVRLSKRLVENAVE-  | P14                                   | 223.0 |
| LVRLSKRLVENAVEKα-| P16                                   | 9.3   |
| ELVRLSKRLVENAVEKαQ-| P18                                 | 2.0   |
| ELVRLSKRLVENAVEKAVQQ-| P20                                | 0.4   |
| DAELVRLSKRLVENAVEKAVQQY-| P23                               | 0.009 |
| PEDAEELVRLSKRLVENAVEKAVQQY-| L314E                              | 0.004 |

[a] IC\textsubscript{50} values are measured in ELISA (see Supporting Figure S1) using recombinant full-length His-tagged RIIα and His-tagged GSKIP.

Supporting Figure S2. The sequences of the indicated peptides are listed in Supporting Information Table S2. Micro-titer plates were coated with RIIα (20 nM solution; 20 µl/well) and incubated with GSKIP (10 nM solution; 20 µl/well) in the presence of the indicated PKA anchoring disruptor peptide.\textsuperscript{1-3} Binding of GSKIP to the RII subunits was detected by incubation with an anti-GSKIP and secondary peroxidase-coupled antibody, and a color reaction with 3, 3′,5,5′-Tetramethylbenzidine-solution. Absorbance was measured at λ = 450 nm in a Safire reader (Tecan; n = 8; shown are means ± S.E.). The IC\textsubscript{50} values were calculated using GraphPad Prism by nonlinear fit of log(inhibitor) vs response – Variable slope.
15N-SOFAST-HMQC experiments show an interaction of terpyridines with the D/D domain of regulatory RI α subunits of PKA

To confirm that the new compounds bind to the D/D-domain 15N-SOFAST-HMQC experiments were carried out. Prior work with the D/D domain carried out at pH 4 showed that the interaction between tightly binding PKA anchoring disruptor peptides and the D/D domain breaks the symmetry of the R subunit dimer, yielding two complete sets of resonances. The largest differences between the two sets of resonances appear at the binding site, the helix between residues T10 to Q24 and the N-terminus. We performed all NMR experiments, including a reassignment of the 1H,15N correlation spectrum, at pH 7 where the symmetry is already partly broken, most likely due to a different distribution of charges in the first helix (for example there are two peaks for G15 in the spectrum shown in Supporting Information Figure S3). We could nevertheless confirm that adding of tightly binding peptides results in two new and complete sets of resonances using peptides P16 and P18 (sequences in Supporting Information Table S2). An interaction with weaker binding peptides (P14) does not result in two sets of resonances but instead results in disappearances of a shift of the signals already present without ligand (Supporting Information Figure S3). Compounds 1a-f caused similar shifts or disappearances of signals as the peptides derived from AKAP18δ-L314E, indicating that they bind to the same site on the D/D domain (Supporting Figure S3).
Supporting Information Figure S3. $^{15}$N-SOFAST-HMQC spectra of the D/D domain of RIIα subunits of PKA recorded in the absence (black) or presence of 1a, 1b, 1f, 1d and peptides P14, P16 and P18. The spectra were recorded using 128 scans and 256 FIDs over 1.5 hours using a 600 MHz-spectrometer and a conventional 5mm RT-QXI probe. Protein and compound/peptide concentrations were 100 and 300 µM, respectively; the measurements were performed at pH 7.
Isothermal titration calorimetric measurements for determination of the $K_D$ values for the interaction of compound 1d with the D/D domain of RIIα

Supporting Information Figure S4. Isothermal titration calorimetric measurements for determination of the $K_D$ values for the interaction of compounds 1d with the D/D domain of RIIα. Measurements consisted of successive 10 µL injections of D/D domain into a less concentrated solution of compound in a MicroCal VP-ITC instrument, using 0.6 mM D/D domain and 0.03 mM 1d. The data were fitted to a one site binding model. Figure 1 shows representative results obtained with compounds 1b and 1f, which bind the D/D domain with an estimated $K_D$=148 µM and $K_D$= 31 µM, respectively. The binding is mainly driven by a large positive entropy change. 1d bound the D/D with a $K_D$ approximately similar to 1f (10-60 µM) but the measurement was of lower quality.
3. NMR spectra

Ethyl 3-(6-cyanopyridine-3-yl)propanoate (5)

Supporting Information Figure S5. $^1$H-NMR-spectrum of 5 (300 MHz, d$_6$-DMSO)

Supporting Information Figure S6. $^{13}$C-NMR spectra of 5 (76 MHz, d$_6$-DMSO)
Ethyl 3-(5-bromo-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoate (6a)

Supporting Information Figure S7. $^1$H-NMR-spectrum of 6a (300MHz, d$_6$-DMSO)

Supporting Information Figure S8. $^{13}$C-NMR spectra of 6a (76 MHz, d$_6$-DMSO)
Ethyl 3-(5-bromo-2'-cyano-3,4-dimethyl-2,3'-bipyridine-5'-yl)propanoate (6b)

Supporting Information Figure S9. $^1$H-NMR spectrum of 6b (300MHz, d$_6$-DMSO)

Supporting Information Figure S10. $^{13}$C-NMR spectrum of 6b (76 MHz, d$_6$-DMSO)
3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)propanenitrile (9)

Supporting Information Figure S11. $^1$H-NMR-spectrum of 9 (300MHz, $d_6$-DMSO)

Supporting Information Figure S12. $^{13}$C-NMR spectra of 9 (76 MHz, $d_6$-DMSO)
N-benzyl-3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)propan-1-amine (10BN)

Supporting Information Figure S13. $^1$H-NMR spectrum of 10BN (300MHz, d$_6$-DMSO)

Supporting Information Figure S14. $^{13}$C-NMR spectrum of 10BN (76 MHz, d$_6$-DMSO)
N-benzyl-3-(6,7-dihydro-5H-cyclopenta[c]pyridine-3-yl)-N-(3-m ethoxybenzyl)propan-1-amine (11b)

Supporting Information Figure S15. $^1$H-NMR-spectrum of 11b (300MHz, d$_6$-DMSO)

Supporting Information Figure S16. $^{13}$C-NMR spectra of 11b (76 MHz, d$_6$-DMSO)
Ethyl 3-(2'-cyano-5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-5'-ylpropanoate (12a)

Supporting Information Figure S17. $^1$H-NMR spectrum of 12a (300MHz, d$_6$-DMSO)

Supporting Information Figure S18. $^{13}$C-NMR spectrum of 12a (76 MHz, d$_6$-DMSO)
Ethyl 3-(5-(3-benzy|3-methox|benzyl)amino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoate (12b)

Supporting Information Figure S19. $^1$H-NMR-spectrum of 12b (300MHz, d$_6$-DMSO)

Supporting Information Figure S20. $^{13}$C-NMR spectra of 12b (76 MHz, d$_6$-DMSO)
Supporting Information Figure S21. $^{13}$C-NMR spectra of 12b (76 MHz, d$_6$-DMSO)
3-(2'-carbamoyl-(5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-5'-yl)propanoic acid (1a)

Supporting Information Figure S22. $^1$H-NMR-spectrum of 1a (300MHz, d$_6$-DMSO)

Supporting Information Figure S23. $^{13}$C-NMR spectra of 1a (76 MHz, d$_6$-DMSO)
5'-({2'}-carboxyethyl)-(5-(3-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-2'carboxylic acid) (1b)

Supporting Information Figure S24. $^1$H-NMR spectrum of 1b (300MHz, d$_6$-DMSO)

Supporting Information Figure S25. $^{13}$C-NMR spectrum of 1b (76 MHz, d$_6$-DMSO)
3-(5-(3-(dibenzyl(3-methoxybenzyl)amino)propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-2'-carbamoyl-3-methyl-2,3'-bipyridine-5'-yl)propanoic acid (1c)

Supporting Information Figure S26. $^1$H-NMR spectrum of 1c (300MHz, $d_6$-DMSO)

Supporting Information Figure S27. $^{13}$C-NMR spectrum of 1c (76 MHz, $d_6$-DMSO)
Supporting Information Figure S28. $^{13}$C-NMR spectrum of 1c (76 MHz, d$_6$-DMSO)
5'-[(2'-carboxyethyl)-(5-(3-(3-benzyl(3-methoxybenzyl)amino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3-methyl-2,3'-bipyridine-2'carboxylic acid) (1d)

Supporting Information Figure S29. $^1$H-NMR-spectrum of 1d (300MHz, d$_6$-DMSO)

Supporting Information Figure S30. $^{13}$C-NMR spectra of 1d (76 MHz, d$_6$-DMSO)
3-(5-(3-(dibenzyl(3-methoxybenzy)amino)propyl)-6,7-dihydro-5H-cyclopenta-[c]pyridine-1-yl)-2'-cyano-3-methyl-2,3'-bipyridine-5'-yl)propanoic acid (1e)

Supporting Information Figure S31. $^1$H-NMR-spectrum of 1e (300MHz, d$_6$-DMSO)

Supporting Information Figure S32. $^{13}$C-NMR spectra of 1e (76 MHz, d$_6$-DMSO)
Supporting Information Figure S33. $^{13}$C-NMR spectra of 1e (76 MHz, $d_6$-DMSO)
3-(2'-carbamoyl-(5-(3-(dibenzylamino)propyl)-6,7-dihydro-5H-cyclopenta[c]pyridine-1-yl)-3,4-dimethyl-2,3'-bipyridine-5'-yl)propanoic acid (1f)

Supporting Information Figure S34. $^1$H-NMR spectra of 1f (600MHz, d$_6$-DMSO)

Supporting Information Figure S35. $^{13}$C-NMR spectrum of 1f (76 MHz, d$_6$-DMSO)
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