# Remote Control of Bipyridine-Metal Coordination within a Peptide Dendrimer

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# **Supporting Information**

#### **General methods**

All reagents were commercially available and used without further purification. Melting points were measured with Büchi Melting Point B-545 unit and are uncorrected. Analytical HPLC was carried out with HPLC-grade acetonitrile and miliQ deionized water on Waters 600 system with Waters 996 photodiode array detector (Column: Waters Atlantis dC18 5 µm, 100 x 4.6 mm) for analytical HPLC or Waters Delta Prep 4000 Preparative Chromatography System with Waters 486 tunable absorbance Detector (Column: Waters PrepPak Cartidge with Delta-Pak C18, 15 µm, 300 Å pore size) for preparative HPLC. For dendrimer purification and identification solvent A is 0.1% TFA in H<sub>2</sub>O and solvent D is 0.1% TFA in H<sub>2</sub>O/CH<sub>3</sub>CN 40/60. All solutions prepared were deoxygenated by bubbling nitrogen for 20 min. For LC-MS analyses, mixtures of the complexes were separated on a Waters Atlantis dC<sub>18</sub> (2.1 x 100 mm, 3 µm) column in a Perkin Elmer Series 200 HPLC system equipped with an Agilent 1100 variable wavelength detector with a 5 µl micro-flowcell (Agilent Technologies, Basel, Switzerland). The wavelength was set to 541 nm. 10  $\mu$ l of sample solution was injected for each analysis. Solvent A was 0.5% formic acid in water, solvent B was 0.5% formic acid in acetonitrile. All solvents were degassed by an Erma model erc-3315 on-line degasser (Erma Inc., Tokyo, Japan). Separations were performed by applying a gradient from 10% to 50% solvent B within 15 min at a flow rate of 200 µl/min. For mass spectral analyses, the column effluent was coupled to the electrospray ion source of an Applied Biosystems API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Rotkreuz, Switzerland) without splitting. A potential of +5.5 kV was applied to the spray capillary and nitrogen was used as the curtain gas. Data were acquired by scanning quadrupole  $Q_1$  over the m/z range from 300 to 1800. Spectroscopic measurements were carried out on a Carey 100 Bio UV-Visible Spectrophotometer at 25°C using gastight cuvettes (d = 1.0 cm).

#### 2,2'-Bipyridine-5,5'-dicarboxylic acid 3<sup>1</sup>

5,5'-Dimethyl-2,2'-bipyridine **2** (5.00 g, 27.1 mmol) and KMnO<sub>4</sub> (27.50 g, 174.1 mmol) were added to 300 mL water. The mixture was stirred at 90°C until the purple color of the permanganate disappeared (after ~8 hours). It was then cooled to rt and the brown precipitate removed by filtration. Remaining dimethyl bipyridine was removed by extraction with diethylether (3 ×). The pH of the aqueous solution was set to 2 by adding 1 M HCl, the white precipitate formed was filtered off and dried under high vacuum to give **3** (5.45 g, 22.3 mmol, 82%). M.p.: >410°C [lit > 360°C]. <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$  = 13.59 (s, 2H), 9.22 (dd, *J* = 2.3, 0.9, 2H), 8.60 (dd, *J* = 8.3, 0.8, 2H), 8.47 (dd, *J* = 8.1, 2.1, 2H). <sup>13</sup>C NMR (400 MHz, D<sub>2</sub>O)  $\delta$  = 173.23, 156.60, 150.02, 138.98, 132.82, 122.53. -ESI-MS: C<sub>12</sub>H<sub>7</sub>N<sub>2</sub>O<sub>4</sub><sup>-</sup> found/calc. 243.0/243.0.

#### 5,5´-Bis(ethoxycarbonyl)-2,2´-bipyridine dimethyl-2,2´-bipyridine 4

1.50 g of **3** were added to 28 ml of EtOH and 3.9 ml of sulphuric acid (conc.). The mixture was stirred overnight at reflux yielding a clear yellowish solution. The solution was poured into 100 mL of cold (0°C) water. The precipitate was filtered and washed with water. After recrystallization from ethanol, **4** was obtained as thin, colorless needles (1.14 g, 3.80 mmol, 62%). M.p.: 149-150°C [lit<sup>2</sup> 148-149°C]. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 9.23 (d, *J* = 2.0, 2H), 8.52 (d, *J* = 8.2, 2H), 8.38 (dd, *J* = 8.3, 2.0, 2H), 4.38 (q, *J* = 7.2, 4H), 1.37 (t, *J* = 7.2, 6H). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 165.49, 158.45, 150.90, 138.62, 127.06, 121.79, 61.96, 14.68. +ESI-MS: C<sub>16</sub>H<sub>17</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup> found/calc. 301.0/301.1.

#### 5'-(Ethoxycarbonyl)-2,2'-bipyridine-5-carboxylic acid 5

The diester **4** (4.22 g, 14.0 mmol) was added to 24 ml of DCM and 120 ml of *tert*butanol. The mixture was stirred at 40°C. KOH (0.788 mg, 14.0 mmol) was slowly added in 6 mL water. After 6 hours the solvents were removed and the white precipitate dried under high vacuum. The white solid was then dissolved in water/DCM and extracted with DCM (3 ×). By adding 1 M HCl the pH of the aqueous phase was set to 5. The white solid **5** was collected via filtration and dried under high vacuum (2.92 g, 10.7 mmol, 76%). M.p.: 269-270°C [lit<sup>3</sup> 267-268°C]. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta = 13.55$  (s, 1H), 9.19 (d, J = 1.5, 2H), 8.56 (d, J = 7.8, 1H), 8.55 (d, J = 7.3, 1H), 8.46-8.43 (m, 2H), 4.38 (q, J = 7.0, 2H), 1.37 (t, J = 7.0, 3H). <sup>13</sup>C NMR (400 MHz, DMSO)  $\delta = 166.32$ , 164.79, 157.92, 157.50, 150.65, 150.38, 138.80, 138.60, 127.56, 126.61, 121.50, 61.68, 14.44. +ESI-MS: C<sub>14</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub><sup>+</sup> found/calc. 273.1/273.1.

#### 5'-(Azidocarbonyl)-2,2'-bipyridine-5-carboxylic acid ethyl ester 6<sup>4</sup>

Trietylamine (125 µL) and mono carboxylic acid **5** (244 mg, 0.897 mmol) were added to 4 ml of dry DMF and the mixture was stirred in an ice bath. Diphenylphosphoryl azide (DPPA) (194 mL, 0.897 mmol) in 1 mL of dry DMF was then added during one hour. The ice bath was removed and after 3 hours the reaction mixture was poured into a cooled (0°C) mixture of diethylether and water. After extraction with diethylether (3 ×) the combined organic phases were washed with sat. NaHCO<sub>3</sub> solution (3 ×), water (3 ×), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to yield a slightly yellowish solid. The crude product was then washed with diethylether and dried under high vacuum to yield **6** as a white solid (222 mg, 0.747 mmol, 83%). M.p.: 128-130° C [lit<sup>5</sup> 134-135°C] <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  = 9.21 (dd, *J* = 9.6, 1.4, 2H), 8.54 (dd, *J* = 7.2, 0.5, 1H), 8.51 (dd, *J* = 8.3, 0.6, 1H), 8.37 (dd, *J* = 8.4, 2.2, 1H), 8.34 (dd, J = 8.4, 2.3, 1H), 4.38 (q, J = 7.0, 2H), 1.37 (t, J = 7.2, 2H). <sup>13</sup>C NMR (400 MHz, CDCl<sub>3</sub>)  $\delta = 171.49, 165.45, 159.84, 158.21, 151.01, 150.75, 138.57, 138.30, 127.25, 127.16, 121.92, 121.89, 61.99, 14.68. +ESI-MS:C<sub>14</sub>H<sub>12</sub>N<sub>5</sub>O<sub>3</sub><sup>+</sup> found/calc. 298.0/298.1.$ 

#### 5'-Amino-2,2'-bipyridine-5-carboxylic acid 7

Compound **6** (100 mg, 0.337 mmol) was added to 3 mL of toluene and refluxed for 1 hour. The solution was then allowed to cool to rt, 1.5 mL of 6 M HCl were added and the resulting mixture refluxed for 6 hours. The solvents were evaporated yielding a yellow solid which was added to water (20 mL) and freeze dried to obtain the hydrochloride salt of **7** as a yellow solid (78.1 mg, 0.310 mmol, 92%). M.p.: 291-293°C [lit<sup>5</sup> 286-290°C]. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  = 9.09 (d, *J* = 1.5, 1H), 8.37 (dd, *J* = 8.4, 2.2, 1H), 8.31 (d, *J* = 4.8, 1H), 8.29 (d, *J* = 4.3, 1H), 8.09 (d, *J* = 2.6, 1H), 7.33 (dd, *J* = 8.7, 2.6, 1H). <sup>13</sup>C NMR (400 MHz, DMSO)  $\delta$  = 166.28, 155.57, 150.13, 147.83, 138.91, 138.07, 132.55, 125.89, 124.22, 123.46, 119.53. +ESI-MS: C<sub>11</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub><sup>+</sup> found/calc. 216.1/216.1.

#### N-(9-fluorenylmethyloxycarbonyl)-5´-amino-2,2´-bipyridine-5-carboxylic acid 1

The bpy-amino acid **7** (200 mg, 0.795 mmol) and NaHCO<sub>3</sub> (200 mg, 2.38 mmol) were added to 15 mL of water. The mixture was stirred in an ice bath and FmocCl (308 mg, 1.19 mmol) in 15 mL of dioxane was added during 30 min. Additional NaHCO<sub>3</sub> (100 mg, 1.19 mmol) was added. After 4 hours the pH of the mixture was set to 1 by adding 1 M HCl and the yellow precipitate was filtered off. It was then washed with water, MeOH and DCM and dried under high vacuum to yield **1** as a yellow solid (246 mg, 0.552 mmol, 69%). M.p.:  $153-155^{\circ}$ C <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta = 13.39$  (s, 1H), 10.18 (s, 1H), 9.12 (dd, J = 2.0, 0.8, 1H), 8.77 (s, 1H), 8.47 – 8.35 (m, 3H), 8.06 (s, 1H), 7.92 (d, J = 7.3, 2H), 7.77 (d, J = 7.4, 2H), 7.44 (t, J = 7.3, 2H), 7.37 (td, J = 7.4, 1.2, 2H), 4.58 (d, J = 6.2, 2H), 4.35 (t, J = 6.4, 1H). <sup>13</sup>C NMR (400 MHz, DMSO)  $\delta = 166.54, 158.63, 153.80, 150.48, 148.60, 144.03, 141.21, 140.02, 138.44, 137.31, 128.09, 127.52, 126.15, 125.98, 125.44, 121.92, 120.58, 119.91, 66.35, 46.94. HR +ESI-MS: C<sub>26</sub>H<sub>20</sub>N<sub>3</sub>O<sub>4</sub> found/calc. 438.1465/438.1453$ 

#### Synthesis of peptide dendrimer library<sup>6</sup>

NovasynTG<sup>®</sup> resin (Tentagel) (loading 0.23 mmol g<sup>-1</sup>) in four polypropylene syringes (100 mg, 0.023 mmol per syringe) was swollen in DCM for 15 min. After removal of the DCM the first amino acid was coupled. Stirring of the reaction mixture at any given step (coupling/deprotection/cleavage) was performed by attaching the closed syringe to a rotating axis.

#### Coupling of the Fmoc-protected amino acids

Fmoc-protected amino acid (3 eq.), PyBOP ((Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate) (3 eq.) and DIEA (diisopropyl-ethylamine) (5 eq.) in 5 mL of DMF were added to the resin and the reaction mixture was stirred for 30 min for the first 2 amino acids (for the amino acids after 1 branching unit 60 min, after 2 branching units 90 min and 3 branching units 120 min; for branching units twice the reaction time was used). The resin was then washed (3 × each) with DMF, MeOH and DCM. The effectiveness of the coupling was monitored by TNBS (trinitrobenzenesulfonic acid) test.<sup>7</sup>

#### Resin mixing and splitting

The resin was suspended in DMF/DCM (2/1, v/v), and mixed via nitrogen bubbling for 15 min and then distributed in four or eight (for  $A^3$  and  $A^4$ ) equal portions.

Cleavage of the Fmoc protecting group

The Fmoc protecting group was removed with 5 mL of a solution of piperidine/DMF (1/4, v/v) for 10 min. After filtration the procedure was repeated and the resin washed with DMF, MeOH and DCM (3 × each).

#### Acylation of bpy

To a solution of Fmoc-protected amino acid (12 eq.) in dry DCM (a few drops of DMF may be required for solubility) was added  $N,N^{\circ}$ -disiopropylcarbodiimide (DIC, 12 eq.) under nitrogen. The reaction mixture was stirred for 30 min at 0°C. The mixture was then directly added to the deprotected resin and stirred overnight. The resin was washed (3 × each) with DMF, MeOH and DCM.

Side chain deprotection

Deprotection was carried out with TFA/H<sub>2</sub>O/TIS (95/2.5/2.5 v/v/v) for 4 hours. After removing the solution the resin was washed with methanol giving a strong violet staining of the beads. The color could be removed by washing the beads 3x for 10 min with 2M HCl before methanol washing.

# **On-bead assay for Fe<sup>2+</sup>-binding**

50 mg of dry library resin were swollen in buffer (MES buffer pH 6.0, 20 mM) for 1 hour. The buffer was removed by filtration and 3 mL of a 30  $\mu$ M solution of Fe(SO<sub>4</sub>) · 7H<sub>2</sub>O was added and the resin was stirred for 5 min. The solution was then removed by filtration and washed with water (3 ×). The resin was suspended in H<sub>2</sub>O, transferred to a silica gel plate and the beads were observed under a microscope. Single red colored beads were transferred to amino acid analysis vials for sequence determination.

#### **UV-Vis titrations**

1000  $\mu$ L of a ca. 20 mM buffered solution (HEPES buffer, pH 6.5) of dendrimer was prepared in a gas tight cuvette at rt. The titration was carried out by adding portions of a stock solution of Fe<sup>2+</sup> or Cu<sup>2+</sup> (CuSO<sub>4</sub>·5H<sub>2</sub>O) (5.0 mM) with a gas tight syringe and waiting until no further change of the spectrum took place (equilibration time: ~1h for Fe<sup>2+</sup>-titrations; seconds for Cu<sup>2+</sup>-titrations). Data points were collected at 541 nm for Fe<sup>2+</sup>-titrations and at 335 nm for Cu<sup>2+</sup>-titrations and fitted with Boltzmann-fit.

# **Formation of dendrimer-Fe<sup>2+</sup>-complexes**

Solutions of dendrimers were prepared in gradient grade HPLC water at concentrations of 1 mM each. The solution containing the metal ion was prepared by dissolving  $Fe(SO_4)_2(NH_4)_2 \cdot 6H_2O$  in gradient grade water, resulting in a concentration of 1 mM. For complex formation, the solutions were combined in the desired molar ratios, mixed thoroughly, and kept at room temperature for 1 h prior to LC/MS analysis.

#### Synthesis, purification and characterization of peptide dendrimers

The dendrimers were synthesized on Rink amide resin using the same coupling conditions as described for the library synthesis. After TFA cleavage, the solution was filtered and the dendrimers precipitated with MTBE. After the MTBE was removed, the dendrimers were dissolved in 3-5 ml water/acetonitrile and purified by RP-HPLC. Yields were calculated for the TFA salts of the dendrimers.

# (AcLysAla)<sub>4</sub>(DapGluPro)<sub>2</sub>DapGlyBpyGlyLeuNH<sub>2</sub> G1

From Novasyn TGR<sup>®</sup> (200 mg, 0.23 mmol/g), **G1** was obtained as foamy colorless solid after preparative RP-HPLC (15.4 mg, 5.99  $\mu$ mol, 13%). RP-HPLC: t<sub>R</sub> = 5.5 min (A/D 80/20 to 60/40 in 10 min,  $\lambda$  = 214 nm); +ESI-MS: C<sub>94</sub>H<sub>149</sub>N<sub>29</sub>O<sub>27</sub><sup>+</sup> found/calc. 2117/2116.

# (AcLysSer)<sub>4</sub>(DapTyrAsp)<sub>2</sub>DapGlyBpyGlyLeuNH<sub>2</sub> G2

From Novasyn TGR<sup>®</sup> (200 mg, 0.23 mmol/g), **G2** was obtained as foamy colorless solid after preparative RP-HPLC (40.7 mg, 14.3 µmol, 31%). RP-HPLC:  $t_R = 4.9$  min (A/D 80/20 to 60/40 in 10 min,  $\lambda = 214$  nm); +ESI-MS:  $C_{100}H_{149}N_{29}O_{33}^+$  found/calc. 2285/2284.

#### (AcGluThr)<sub>4</sub>(DapGluVal)<sub>2</sub>DapBypBlaAspNH<sub>2</sub> E1

From Novasyn TGR<sup>®</sup> (200 mg, 0.25 mmol/g), **E1** was obtained as foamy colorless solid after preparative RP-HPLC (33.9 mg, 13.9  $\mu$ mol, 28%). RP-HPLC: t<sub>R</sub> = 5.4 min (A/D 80/20 to 60/40 in 10 min,  $\lambda$  = 214 nm); +ESI-MS: C<sub>91</sub>H<sub>134</sub>N<sub>24</sub>O<sub>40</sub><sup>+</sup> found/calc. 2203/2203.

#### (AcArgLys)<sub>4</sub>(DapHisVal)<sub>2</sub>DapBpyAmbTyrNH<sub>2</sub> E2

From Novasyn TGR<sup>®</sup> (200 mg, 0.25 mmol/g), **E2** was obtained as foamy colorless solid after preparative RP-HPLC (21.7 mg, 5.54 µmol, 11%). RP-HPLC:  $t_R = 10.7$  min (A/D 90/10 to 70/30 in 15 min,  $\lambda = 214$  nm); +ESI-MS:  $C_{115}H_{180}N_{44}O_{23}^+$  found/calc. 2547/2545.

#### (AcArgLys)<sub>4</sub>(DapPheLys)<sub>2</sub>DapBpyBlaTyrNH<sub>2</sub> E3

From Novasyn TGR<sup>®</sup> (200 mg, 0.25 mmol/g), **E3** was obtained as foamy colorless solid after preparative RP-HPLC (52.8 mg, 13.4  $\mu$ mol, 27%). RP-HPLC: t<sub>R</sub> = 10.2 min (A/D 90/10 to 70/30 in 10 min,  $\lambda$  = 214 nm); +ESI-MS: C<sub>118</sub>H<sub>188</sub>N<sub>42</sub>O<sub>23</sub><sup>+</sup> found/calc. 2563/2561.

Hit	$A^6$	$A^5$	$A^4$	$A^3$	$A^2$	$A^1$
1	Glu	Thr	Glu	Val	Bla	Asp
2	Glu	Thr	Phe	Asp	Phe	Leu
3	Glu	Thr	Glu	Tyr	Phe	Asp
4	Glu	Val	Glu	Asp	Amb	Tyr
5	Glu	Thr	Glu	Tyr	Amb	Asp
6	Glu	Val	Glu	Leu	Amb	Asp
7	Arg	Val	Phe	Leu	Bla	Leu
8	Ser	Val	Arg	Leu	Phe	Tyr
9	Arg	Val	Bla	Thr	Phe	Leu

**Table S1** Amino acid sequences from library **E**, determined by amino acid analysis. Sequences 1-6 are positive hits, 7-9 are negative hits



**Fig. S1** Job-plot of the Fe<sup>2+</sup>-complex of **G1**. X is the molar ratio of **G1** (conc.(**G1**)/conc.(**G1**)+conc.(Fe<sup>2+</sup>)). The data points were fitted using a polynomial function. Maximum absorption was observed for X = 0.758 indicating a 3 to 1 complex (**G1**/Fe<sup>2+</sup>).



**Fig. S2** a) UV-Vis titration of dendrimer **E1** (conc. = 90.0  $\mu$ M) with Fe<sup>2+</sup> (full spectrum). Insert top right: molar ratio plot at 541 nm indicating a 3 to 1 ratio of dendrimer **E1** relative to Fe<sup>2+</sup> and binding constant K<sub>a</sub> of 3.1x10<sup>14</sup> M<sup>-3</sup>; pH 6.5 (HEPES buffer 20 mM), 25°C. Data fitted with Boltzmann-fit. b) Section of spectrum a) from 400 nm to 700 nm.



**Fig. S3** Left: chromatogram of  $Fe(E1)_3$  complex after semi-preparative RP-HPLC purification (gradient: 0.1% TFA in water/0.1% TFA in 60% acetonitrile-water 80/20 to 60/40 in 10 min, detection at 214 nm). Right: Deconvoluted electrospray mass spectrum, showing the peaks of the complex  $Fe(E1)_3$  (6665.88 Da) and the free dendrimer E1 (2203.75 Da). The presence of the free dendrimer in the spectrum of the purified complex is due to partial decomplexation occurring upon electrospray ionization.



Fig. S4 a) LC-MS analysis of a mixture of dendrimers G2 and E1 in the presence of  $\text{Fe}^{2+}$  (Entry 4, Table 2); absorption at 541 nm;  $\text{Fe}(G2)_2\text{E1}$ : peaks 2-4, 6829 Da and  $\text{Fe}G2(\text{E1})_2$ : peak 5, 6747 Da ;  $\text{Fe}(G2)_3$ : peak 1, 6911 Da;  $\text{Fe}(\text{E1})_3$ : peak 6. b) Total ion current chromatogram (m/z 400-1800).  $\text{Fe}(G2)_2\text{E1}$ : peaks 2-4, 6829 Da and  $\text{Fe}G2(\text{E1})_2$ : peak 5, 6747 Da.





# 400 MHz $^{13}\text{C}$ NMR in D2O









400 MHz <sup>13</sup>C NMR in CDCl<sub>3</sub>









400 MHz <sup>13</sup>C NMR in d<sub>6</sub>-DMSO







400 MHz <sup>13</sup>C NMR in CDCl<sub>3</sub>









400 MHz <sup>13</sup>C NMR in d<sub>6</sub>-DMSO







400 MHz <sup>13</sup>C NMR in d<sub>6</sub>-DMSO



# **Peptide Dendrimer G1**



Mass spectrum, +ESI-MS: A M+, C M+Na

Analytical RP-HPLC chromatogram



# **Peptide Dendrimer G2**



Mass spectrum, +ESI-MS: A M+K+Na, B M+

Analytical RP-HPLC chromatogram



# **Peptide Dendrimer E1**



Mass spectrum, +ESI-MS: A M+, B M+Na, C M+K

Analytical RP-HPLC chromatogram



# **Peptide Dendrimer E2**





Analytical RP-HPLC chromatogram



# **Peptide Dendrimer E3**

# Mass spectrum, +ESI-MS



Analytical RP-HPLC chromatogram



# MS-spectra from LC-MS analysis of a mixture of dendrimers G2 and E1 in the presence of ${\rm Fe}^{2+}$

# Peak 1



#### Peaks 2+3



#### Peak 4



# Peak 5



# MS-spectra from LC-MS analysis of a mixture of dendrimers E1 and E2 in the presence of ${\rm Fe}^{2+}$

#### Peak 1



#### Peak 2



#### Peak 3



#### Peak 4



#### Peak 5



#### Peak 6



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