Supporting Information

Luminescent Vesicular Receptors with Phosphate-ion Binding Affinity

Benjamin Gruber, Stefan Stadlbauer, Kristina Woinaroschy and Burkhard König

Institut für Organische Chemie, Universität Regensburg, D-93040 Regensburg, Germany

Contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Synthesis and characterization of compound 3</td>
<td>S1</td>
</tr>
<tr>
<td>2. $^1$H-NMR spectra of selected compounds</td>
<td>S5</td>
</tr>
<tr>
<td>3. Hydrolytic stability of ATP under assay conditions</td>
<td>S11</td>
</tr>
<tr>
<td>3.1 NMR</td>
<td></td>
</tr>
<tr>
<td>3.2 LC-MS</td>
<td>S12</td>
</tr>
<tr>
<td>4. Indicator Displacement Assays</td>
<td>S14</td>
</tr>
<tr>
<td>4.1 CMS vs 1</td>
<td></td>
</tr>
<tr>
<td>4.2 CMS vs VR-4</td>
<td>S15</td>
</tr>
<tr>
<td>5. Raw spectra for emission changes of 2 and 3</td>
<td>S15</td>
</tr>
<tr>
<td>6. NMR experiment of 1 vs UDP</td>
<td>S16</td>
</tr>
<tr>
<td>7. Structures of DSPC and DSPC-PEG350</td>
<td>S17</td>
</tr>
</tbody>
</table>
1. Synthesis and characterization of compound 3

Synthesis of fluorescent bis-Zn(II) cyclen complex 3. (a) 5-Dimethylamino-naphthalene-1-sulfonic acid (2-amino-ethyl)-amide 17, K$_2$CO$_3$, dioxane, reflux, 72 h; (b) TFA, DCM RT 12 h, basic ion exchanger resin; (c) Zn(ClO$_4$)$_2$, H$_2$O, 65 °C.
5-Dimethylamino-naphthalene-1-sulfonic acid \{2-[4,6-bis-(1,4,7,10-tetraaza-cyclododec-1-yl)-[1,3,5]triazin-2-ylamino]-ethyl\}-1,4,7-tricarboxylic acid tri-tert-butyl ester (19)

A solution of 18\(^1\) (0.89 g, 0.84 mmol) in 25 mL of dioxane was stirred under nitrogen for 5 min, then a solution of 5-dimethylamino-naphthalene-1-sulfonic acid (2- amino-ethyl)-amide 17\(^2\) (0.62 g, 2.1 mmol) in dioxane (75 mL) was added dropwise and K\(_2\)CO\(_3\) (0.58 g, 4.2 mmol, 5 eq.) was added. The mixture was refluxed for 72 h at 140 °C under nitrogen atmosphere. After completion, all inorganic salts were filtered off, the solvent was removed under reduced pressure and the crude product was purified by column chromatography on neutral alumina (EE/PE 3:7 to 2:3). Compound 19 was obtained as a yellow solid (0.82 g, 75 %). MP: 113 °C (sublimation). \(^1\)H-NMR (400 MHz; CDCl\(_3\)): \(\delta = 1.41\) (bs, 54 H, CH\(_3\)-Boc), 2.86 (s, 6 H, CH\(_3\)N), 3.10-3.68 (m, 36 H, CH\(_2\) chain and CH\(_2\) cyclen), 4.77 (bs, 1 H, NHSO\(_2\)), 6.95 (bs, 1 H, NH-triazine), 7.13 (d, 1 H, \(^3\)J = 7.6 Hz, CH), 7.47 (dd, 1 H, \(^3\)J = 7.6, 8.7 Hz, CH), 7.48 (dd, 1 H, \(^2\)J = 7.3, 8.5 Hz, CH), 8.21 (d, 1 H, \(^3\)J = 7.3 Hz, CH), 8.36 (d, 1 H, \(^2\)J = 8.7 Hz, CH), 8.5 (d, 1 H, \(^2\)J = 8.5 Hz, CH). \(^1\)C-NMR (400 MHz; CDCl\(_3\)): \(\delta = 28.5\) (+, CH\(_3\)-Boc), 41.8 (+, CH\(_2\)NHSO\(_2\)), 43.6 (--, CH\(_2\)NH-triazine), 45.4 (+, (CH\(_3\))\(_2\)N), 50.09, 50.16, 50.25, 50.29 (--, CH\(_2\) cyclen), 79.78, 80.1, 80.3 (C\(_q\), Boc), 115.0, 123.1, 127.78, 127.82, 129.8, 129.8, 129.9, 129.9 (+, CH); 135.08 (C\(_q\), Cq-SO\(_2\)), 151.7 (C\(_q\), Cq-N-(CH\(_3\))\(_2\)), 156.2 (C\(_q\), Boc), 165.8 (C\(_q\), triazine). \(\text{IR (KBr) [cm}^{-1}\): \(\nu = 3267, 2975, 2933, 2361, 2200, 1686, 1541, 1499, 1474, 1410, 1366, 1321, 1249, 1163, 1106, 1050, 970, 946, 858, 777. \(\text{UV (CH\(_2\)Cl\(_3\)): \(\lambda_{max} (lg \epsilon) = 340\) nm (3.655). \(\text{MS (ESI, DCM/MeOH + 10 mmol/l NH}_4\text{Ac): \(m/z\) (%) = 1313.9 (100)}}\)

\(^1\) M. Subat, K. Woinaroschy, S. Anthofer, B. Malterer, B. König Inorg. Chem. 2007, 46, 4336-4356

5-Dimethylamino-naphthalene-1-sulfonic acid \{2-[4,6-bis\{1,4,7,10-tetraaza-cyclododec-1-yl\}-[1,3,5]triazin-2-ylamino]-ethyl\}-amide (20)

A solution of 19 (0.77 g, 0.59 mmol) in CH\textsubscript{2}Cl\textsubscript{2} was treated with TFA (3.6 mL, 47 mmol) and the reaction mixture was stirred for 24 h. After completion of the reaction the solvent was removed under reduced pressure, the obtained pale yellow solid (quantitative yield) was dissolved in water and passed through a column of pre-swelled (pH = 7) basic ion exchanger resin. The fractions having a basic pH were collected and the resulting aqueous solution was lyophilised. The product was obtained as a yellow solid (0.26 g, 62 %). **MP**: 92 °C.

**\textsuperscript{1}H NMR** (300 MHz; MeOD): \(\delta = 2.62\) (bs, 8 H, CH\textsubscript{2}cyclen), 2.70 (bs, 8 H, CH\textsubscript{2}cyclen), 2.87 (s, 14 H, (CH\textsubscript{3})\textsubscript{2}N and CH\textsubscript{2}cyclen), 3.00 (t, 2 H, \(^{3}J = 6.0\) Hz, CH\textsubscript{2}NH-triazine), 3.32 (t, 2 H, \(^{3}J = 6.0\) Hz, CH\textsubscript{2}NHSO\textsubscript{2}), 3.7 (bs, 8 H, CH\textsubscript{2}cyclen), 7.23 (d, 1 H, \(^{3}J = 7.6\) Hz, CH), 7.50 (dd, 1 H, \(^{3}J = 7.6, 8.6\) Hz, CH), 7.55 (dd, 1 H, \(^{3}J = 7.3, 8.5\) Hz, CH), 8.17 (d, 1 H, \(^{3}J = 7.3\) Hz, CH), 8.26 (d, 1 H, \(^{3}J = 8.6\) Hz, CH), 8.53 (d, 1 H, \(^{3}J = 8.5\) Hz, CH).

**\textsuperscript{13}C NMR** (300 MHz; MeOD): \(\delta = 41.2\) (—, CH\textsubscript{2}NHSO\textsubscript{2}), 44.5 (—, CH\textsubscript{2}NH-triazine), 45.8 (—, (CH\textsubscript{3})\textsubscript{2}N), 46.9, 48.6, 48.7, 48.9 (—, CH\textsubscript{2}cyclen), 116.28 (+, CH), 120.4 (+, CH), 124.2 (+, CH), 129.0 (+, CH), 130.1 (+, CH), 131.0 (C\textsubscript{q}), 131.1 (+, CH), 131.2 (C\textsubscript{q}), 136.9 (C\textsubscript{q}, Cq-SO\textsubscript{2}), 153.2 (C\textsubscript{q}, Cq-N-(CH\textsubscript{3})\textsubscript{2}), 167.2 (C\textsubscript{q}, Cq-triazine cyclen), 168.0 (C\textsubscript{q}, Cq-triazine NH). **IR** (KBr) [cm\textsuperscript{-1}]: \(\tilde{\nu} = 3397, 2938, 2840, 3261, 2200, 1542, 1497, 1416, 1362, 1294, 1142, 1063, 940, 792, 625, 572.**

**UV** (CH\textsubscript{2}Cl\textsubscript{2}): \(\lambda_{\text{max}} (\log \varepsilon) = 336\) nm (3.766). **MS** (ESI, TFA/AcN/H\textsubscript{2}O): \(m/z\ (%)\): 357.4 (100) \([M + 2 \text{H}^{+}]^{2+}\), 713.6 (20) [MH]\textsuperscript{+}. **HRMS** Calcd for C\textsubscript{33}H\textsubscript{56}N\textsubscript{14}O\textsubscript{2}S: 712.4331; found: 712.4421.
**Bis-Zn(II)-cyclen dansyl (3)**

Compound 20 (120 mg, 0.17 mmol) was dissolved in 1 mL of water and heated to 65 °C to give a clear yellow solution. Subsequently zinc(II)-perchlorate (64 mg, 172 µmol) dissolved in 1 ml of water was added slowly. The pH was adjusted by addition of 1 M NaOH (approx. 2 mL) to pH 7. The reaction mixture was stirred for additional 23 h at 70 °C. The solvent was removed in vacuo and the residue was redissolved in water and lyophilized. The crude product (200 mg) was recrystallized from an EtOH / H₂O (4:1) mixture as a yellow solid (89 mg, 41 %). **MP**: 180-182°C. – **¹H NMR** (300 MHz; CD₃CN): δ = 2.65-2.90 (m, 18 H, CH₂(cyclen), (CH₃)₂N), 2.94-3.15 (m, 14 H, CH₂ cyclen, CH₂NHSO₂), 3.23-3.46 (m, 6 H, CH₂ cyclen, CH₂NH-triazine), 4.24-4.44 (m, 4 H, CH₂ cyclen), 6.11 (m, 1 H, NH-triazine), 7.23 (d, 1 H, 3J = 7.4 Hz, CH), 7.53 (dd, 1 H, 3J = 7.4, 8.8 Hz, CH), 7.57 (dd, 1 H, 3J = 7.4, 8.2 Hz, CH), 8.15 (d, 1 H, 3J = 7.4 Hz, CH), 8.18 (d, 1 H, 3J = 8.8 Hz, CH), 8.51 (d, 1 H, 3J = 8.2 Hz, CH). – **¹³C NMR** (300 MHz; CD₃CN): δ = 41.9 (−, CH₂NH-triazine), 43.0 (−, CH₂NHSO₂), 44.4 (+, (CH₃)₂N), 45.3, 45.8, 46.1, 46.4 (−, CH₂ cyclen), 114.8, 118.3, 123.1, 127.9, 128.6, 128.9, 129.3, 129.9 (+, CH), 135.0 (C₉, C₉-SO₂), 151.7 (C₉, C₉-N-(CH₃)₂), 165.5 (C₉, C₉-triazine). – **IR** (KBr) [cm⁻¹]: ν = 3427, 3283, 2931, 2361, 2200, 1636, 1560, 1419, 1346, 1312, 1143, 1090, 979, 795, 627, 575. – **UV** (HEPES pH 7.4, 25 mM): λₑₓ (lg ε) = 330 nm (3.575), 227 (4.637). – **MS** (ESI(+), H₂O/MeOH + 10 mmol/L NH₄Ac): m/z (%) = 479.1 (100) [M⁺²⁺ + 2 CH₃COO]²⁺, 449.1 (82) [M⁺²⁺ – H⁺ + CH₃COO]³⁺, 420.1 (20) [M⁺²⁺ – 2 H⁺]²⁺. – C₃₃H₅₆N₁₄O₁₈SCl₄Zn₂ · EtOH (1241.52 g/mol): Calcd.: C 32.65, H 4.85, N 15.23; found: C 32.52, H 4.87, N 15.04.
2. $^1$H-NMR spectra of selected compounds

Compound 3 (300 MHz; CD$_3$CN)

![H-NMR spectrum of Compound 3](image)

Compound 6 (300 MHz; CDCl$_3$)

![H-NMR spectrum of Compound 6](image)
Compound 8 (300 MHz; CDCl₃)

Compound 9 (300 MHz; CDCl₃)
Compound 15 (300 MHz; CDCl$_3$)

Compound 16 (400 MHz; CDCl$_3$)
Compound 10 (600 MHz; CDCl₃)

Compound 11 (300 MHz; CDCl₃)
Compound 13 (400 MHz; CDCl$_3$)

Compound 14 (600 MHz; CDCl$_3$)
Compound 5 (600 MHz; CDCl$_3$ / MeOH 1:1)
3. Hydrolytic stability of ATP under assay conditions

3.1. NMR

$^{31}$P-NMR spectrum of ATP (50 mM) in the presence of VR-6 (0.15 mM [Zn$^{2+}$]) after 24 hours. $^{31}$P-NMR spectra of ATP, pyrophosphate and inorganic phosphate are included for comparison. All samples were measured in HEPES buffer (pH 7.4) with 10 % D$_2$O.
3.2. LC-MS

![Graph depicting LC-MS analysis](image-url)
4. Indicator Displacement Assays

4.1. CMS + 1

Quenching of CMS emission ($5 \times 10^{-6}$ M) in the presence of 1 in 25 mM HEPES buffer ($\lambda_{\text{ex}} = 396$ nm) and displacement by re-titration with UTP.

Color of a mixture of 80 μM CMS and 40 μM 1 upon irradiation in the presence and absence of various anions (800 μM) and phosphate anions (80 μM). Titrations were performed at 25 °C in 10 mM Hepes buffer, pH 7.4.

Relative intensity changes of 1/CMS in the presence of selected analytes and comparison to the emission response of 2 and 3.

Binding isotherms obtained by indicator displacement assay (IDA) for vesicular receptor VR-4 and various phosphate anions and anion selectivity of vesicular receptor VR-4.
4.2. CMS + VR-4

Quenching of CMS emission ($5 \times 10^{-6}$ M) in the presence of VR-4 in 25 mM HEPES buffer ($\lambda_{\text{ex}} = 396$ nm) and displacement by re-titration with inorganic phosphate.

5. Raw spectra for emission changes of 2 and 3

Lack of emission response of 2 and 3 ($5 \times 10^{-6}$ M) in 25 mM HEPES buffer (pH 7.4) in the presence of UTP ($\lambda_{\text{ex}} (2) = 330$ nm, $\lambda_{\text{ex}} (3) = 335$ nm).
6. NMR experiment of 1 vs UDP

$^1$H-NMR spectrum of UDP (25 mM) and UDP in the presence of 1 (1.0 eq). The selected region of the spectrum shows the shift and signal broadening of the imide moiety of UDP and therefore indicates a binding to the Zn(II) complex 1. All samples were measured in H$_2$O/D$_2$O (9:1) at 400 Mhz with water suppression.

$^{31}$P-NMR spectrum of UDP (25 mM) and UDP in the presence of 1 (1.0 eq). The selected region of the spectrum shows the shift of the phosphate moieties of UDP and therefore indicates a binding to the Zn(II) complex 1. All samples were measured in H$_2$O/D$_2$O (9:1) at 400 Mhz.
7. Structures of DSPC and DSPC-PEG350

![Structures of DSPC and DSPC-PEG350](image-url)