Supporting Information

Anthracene-based receptors with a turn-on fluorescence response for nitrate

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General

All the solvents were dried according to standard procedures. Reactions were performed in oven-dried round bottom flask. Crude products were purified by column chromatography on silica gel 100-200 mesh. TLC plates were visualized by exposure to ultraviolet light and/or by exposure to acidic ethanolic
solution of ninhydrin followed by heating (<1 min) on a heat gun (~250 °C). Organic solutions were concentrated on rotary evaporator at 35–40 °C. **NMR Spectra** were measured on ASCEND 600 FT spectrometer (Bruker Corp., Billerica, MA), 600 MHz for \(^1\)H NMR and 150.9 MHz for \(^13\)C NMR. The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The solvent used is reported for each spectrum. **Mass Spectra**: Finnigan MAT TSQ 7000 (ESI). **Melting Point**: Melting Points were determined on Büchi SMP or a Lambda PhotometricsOptiMelt MPA 100. **Absorption spectra** were measured in 1 cm quartz cuvettes with Varian Cary BIO 50 UV/VIS/NIR Spectrometer. **Emission spectra** were recorded with aqueous buffered solution in 1 cm quartz cuvettes (Hellma) on a FluoroMax 4 (Horiba) with a temperature control. **pH-Measurements** were carried out on a Mettler Toledo G20 Titrator equipped with a DG115-SC pH-electrode. The electrode was calibrated with standard calibrating solutions from Mettler Toledo. The reaction vessels were kept at constant temperature 23°C. The starting compounds were purchased from TCI, Sigma-Aldrich and Acros Chemicals.

**Synthetic procedures**

9,10-anthracene dialdehyde was prepared according to the literature known procedure.[1, 2]

**Mono-Boc-tren**

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \\
\text{N} & \quad \text{NHBoc}
\end{align*}
\]

The compound was prepared according to the known procedure with small modifications.[3]

To a stirred solution of tris-(2-aminoethyl) amine (5.1 mL, 35 mmol) in dioxane (30 mL) under nitrogen a solution of di-tert-butyl-dicarbonate (1.2 mL, 5.5 mmol) in dioxane (30 mL) was added over 1 h at rt. The reaction mixture was stirred for 17 h. The solvent was removed in vacuo and the residue was dissolved in water (10 mL). The aqueous solution was extracted with dichloromethane (6x 15 mL). The organic phases were combined. The removal of the solvent in vacuo gave the product (1.247 g, 92%) as viscous oil.

**Boc-protected compound 1 (2BOC-1)**

\[
\begin{align*}
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NHBoc}
\end{align*}
\]

N,N-Bis(2-aminoethyl)-N-[2-(tert-butylcarbamoyl)ethyl]-amine (0.98 g, 4.0 mmol) was dissolved in 500 mL of CH\(_3\)CN. Under stirring, a solution of anthracene-9,10-dicarbaldehyde (0.94 g, 4.0 mmol) in CH\(_3\)CN 300 mL was added dropwise over 2 h at rt. After 20 h stirring, the solvent was removed under reduced pressure, and the crude product was diluted with 500 mL of methanol. The solution was heated to 50°C and NaBH\(_4\) was added (0.92g, 24.3 mmol). When the addition was complete, the reaction mixture was stirred at 70°C for 2 h. The solvent was then removed and the residue was dissolved in basic water (20 mL, pH=9) and extracted with CH\(_2\)Cl\(_2\) (4 x 50 mL). The collected organic phases were dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure, and the crude product was recrystallized from methanol. Product was isolated in a 44% yield (1.57 g) as a yellow solid. M.p.178-180 °C. \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ (ppm) 8.13-8.06 (m, 8H), 7.39-7.28 (m, 8H), 4.30 (s, 8H), 3.20-3.10 (m, 4H),
2.51 (t, J = 5.7 Hz, 4H), 2.44-2.30 (m, 16H), 1.42 (s, 18 H). $^{13}$C NMR (400 MHz, CDCl3): δ (ppm) 156.0, 131.8, 129.8, 125.4, 124.7, 78.9, 54.5, 53.9, 46.9, 44.9, 38.9, 28.4. HRMS (ESI-TOF): m/z: [M+H]$^+$ Calcd for C$_{54}$H$_{73}$N$_8$O$_4$: 897.5749; Found: 897.5744.

$^1$H and $^{13}$C NMR spectra of the product:
Compound 1
To a stirred solution of the boc-protected diamine 2BOC-1 (1.2 g, 1.3 mmol) in dioxane (180 mL) conc. HCl (6 mL) was added dropwise under nitrogen atmosphere at 0 °C. The reaction was continued under stirring conditions at rt for 1 h. After the solvent was removed in vacuo and the crude product was dissolved in basic water (120 mL, pH=9-10) and extracted with CH₂Cl₂ (2 x 200 mL). The collected organic phases were combined and dried over Na₂SO₄. The solvent was removed yielding compound 1 in a quantitative yield (0.93 g). M.p. 50-53 °C. ¹H NMR (400 MHz, DMSO-d₆): δ (ppm) 8.15 (m, 8H), 7.19 (m, 8H), 4.36 (s, 8H), 2.84 (m, 8H), 2.64-2.58 (m, 14H). ¹³C NMR (400 MHz, CDCl₃): δ (ppm) 131.8, 129.7, 125.4, 124.9, 66.8, 54.5, 47.7, 45.9. HRMS (ESI-TOF): m/z [M+H]⁺ calcd for C₄₄H₅₇N₈: 697.4701; found: 697.4705.

¹H and ¹³C NMR spectra of the product:
A solution of 9,10-anthracenedicarboxaldehyde (318 mg, 1.34 mmol) and diamine 1 (960 mg, 1.34 mmol) in 30 mL of DCM and 10 mL of MeOH mixture (3:1 ratio) was stirred for 20 h at rt. After 20 h of stirring, the reaction mixture was cooled to 0°C, NaBH₄ (282 mg, 7.4 mmol) was added in portions. The mixture was allowed to warm to room temperature and stirred for additional 4 h. The mixture was diluted with dichloromethane 100 mL and washed with water (4 x 50 mL). The solvent of the combined and removed under vacuum. The crude product was recrystallized from methanol: DCM (75: 25mL) giving the product in a 50% yield (602 mg) as a yellow solid M.p. 195-197 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 7.98-7.84 (m, 12H), 6.72-6.41 (m, 12H), 4.46 (s, 12H), 3.12-3.01 (m, 12H), 2.82-2.68 (m, 12H), 1.50 (brs, 6H) ppm; ¹³C NMR (400 MHz, CDCl₃): δ (ppm) 131.0, 129.4, 125.3, 124.0, 54.5, 48.1, 47.0. HRMS (ESI-TOF): m/z [M+H]+ Calcd for C₆₀H₆₇N₈: 899.5483; Found: 899.5498.

¹H and ¹³C NMR spectra of the product:
A solution of isophthalaldehyde (172 mg, 1.29 mmol) and diamine (900 mg, 1.29 mmol) in 150 mL DCM and 50 mL MeOH mixture (3:1 ratio) was stirred for 20 h at room temperature. After 20 h stirring, the reaction mixture was cooled to 0°C, NaBH₄ (265 mg, 7 mmol) was added in portions. The mixture was allowed to warm to room temperature and stirred for additional 4 h. The mixture was diluted with dichloromethane 100 mL and washed with water (4 x 50 mL). The solvent of the combined organics was removed under vacuum and the crude product was purified by silica gel column chromatography using as 95% THF/ 3% MeOH/ 2% aq. NH₃ eluent, giving the product in 48% yield as a yellow solid (496 mg). M.p. 130-132 °C. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.25-8.13 (m, 8H), 7.19-7.09 (m, 8H), 6.97 (s, 1H), 6.18-6.02 (m, 3H), 4.55 (q, J = 12.3 Hz, 8H), 2.99-2.89 (m, 4H), 2.86-2.71 (m, 8H), 2.55-2.48 (m, 4H), 2.47-2.39 (m, 8H), 2.27-2.24 (m, 4H) ppm; ¹³C NMR (400 MHz, CDCl₃): δ (ppm) 131.8, 129.8, 126.7, 126.3, 125.5, 124.6, 56.0, 48.8, 46.8, 46.4. HRMS (ESI-TOF): m/z calcd for C₅₂H₆₈N₈: 799.5170; found: 799.5172.

¹H and ¹³C NMR spectra of the product:
The titrations were carried out by sequential addition of sodium salts to the NMR tube containing the receptors followed by the measurements. The 10%DMSO–buffer mixture was used because of

\[ 1^H \text{NMR titrations} \]
solubility limitation of the receptors at 0.5 mM concentration. The following conditions were used: 0.5 mL of 0.5 mM solution of receptors in a 1:9 DMSO-$d_6$-$D_2$O (50 mM acetate buffer, pH 3.6) mixture. Sodium salts dissolved in the same $D_2$O-based buffer (0.0625 M) were added as follows (equiv): 0.25; 0.5; 0.75; 1; 1.25; 1.5; 2; 3; 5; 10; 15; 30; 45. The fitting was performed by HypNMR program.

**Figure S1.** $^1$H NMR titration of 2 with Na$_2$C$_2$O$_4$ and the fitting (black line) of experimental points (triangles) showing strong 1:1 binding ($\log K > 4$) and the second binding event.

**Figure S2.** $^1$H NMR titration of 2 with NaClO$_4$ and the fitting (black line) of experimental points (triangles) to a 1:1 binding mode. The calculated binding constant is $\log K_{11} = 2.99 \pm 0.01$
**Figure S3.** $^1$H NMR titration of 2 with NaCl and the fitting (black line) of experimental points (triangles) to a 1:1 binding mode. The calculated binding constant is $\log K_{11} = 2.99 \pm 0.01$.

**Figure S4.** $^1$H NMR titration of 2 with NaF and the fitting (black line) of experimental points (triangles) to a 1:1 binding mode. The calculated binding constant is $\log K_{11} > 4$.

**Figure S5.** $^1$H NMR titration of 2 with Na$_2$SO$_4$ and the fitting (black line) of experimental points (triangles) to a 1:1 binding mode. The calculated binding constant is $\log K_{11} > 4$.

**ITC Titrations**

The ITC experiments were evaluated by the help of the program SupraFit [C. Hübler, Institut of Organic Chemistry, TU Freiberg, Suprafit https://github.com/conradhuebler/SupraFit 27.08.2019]. The calculated results are in good agreement with those obtained by the program NanoAnalyze™ (TA Instruments).
**Fluorescence and UV-Vis titrations**

Stock solutions of receptors with concentrations of $10^{-5}$ M in a 50 mM acetate buffer (5% DMSO) were prepared for UV-Vis binding studies. The titrant (sodium salt, 0.01M) was sequentially added to a 2 mL sample of the host stock solution in the spectrometric cell and the changes in the spectral features were monitored. The total number of data points was 20-40, depending on the stoichiometry of complexation; for a presumed 1:1 complex 20 points were usually measured. The following setup parameters were used for fluorescence titration experiments: ex. 370 nm, slit 2/2, em: 380-530 nm. The resulting data was imported in HypSpec program and the data was fitted to obtain stability constants with anions. Concentration of receptors is $10^{-5}$ M.

**Figure S6.** ITC titration: heat vs. time plots for addition of Na$_2$C$_2$O$_4$ and NaClO$_4$ together with fitting graphics.

**Figure S7.** Fluorescence changes observed by the addition of nitrate to a solution of receptor 2.
**Figure S8.** UV-Vis changes and the fitting of receptor 2 titrated with sodium nitrate. The obtained binding constant is 3.10 ± 0.01. Fluorescence titration of 2 with methanol.

**Figure S9.** Job plot: stoichiometry determination of receptor 3 in the presence of fluoride anion, indicating 1:2 major binding mode. Conditions: 0.01 mM receptor and NaF concentration in 50 mM acetate buffer

**Binding constants**

**Table S1.** Binding constants of receptors 2 and 3 for anions as determined from fluorescence titrations in a 50mM acetate buffer (pH 3.6) at 23°C. The values correspond to logK for a 1:1 binding or to logβ for the 1:2 binding.

<table>
<thead>
<tr>
<th>Anions</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₂C₂O₄</td>
<td>1:1 binding 6.52±0.03</td>
<td>1:1 binding 5.02±0.01</td>
</tr>
<tr>
<td></td>
<td>1:2 binding 10.37±0.03</td>
<td>1:2 binding 8.33 ±0.01</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>4.17±0.01</td>
<td>4.70±0.01</td>
</tr>
<tr>
<td>NaBr</td>
<td>2.59±0.01</td>
<td>2.93±0.01</td>
</tr>
<tr>
<td>NaCl</td>
<td>3.27±0.05</td>
<td>2.57±0.01</td>
</tr>
<tr>
<td>NaClO₄</td>
<td>3.27±0.01</td>
<td>3.28±0.01</td>
</tr>
<tr>
<td>NaF</td>
<td>1:1 binding 5.28±0.03</td>
<td>1:2 binding 6.14±0.02</td>
</tr>
<tr>
<td></td>
<td>1:2 binding 8.90±0.02</td>
<td>1:3 binding 10.21±0.01</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>3.37±0.01</td>
<td>3.50±0.01</td>
</tr>
<tr>
<td>NaI</td>
<td>2.37±0.01</td>
<td>2.65±0.01</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>3.25±0.01</td>
<td>3.22±0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.14±0.11</td>
</tr>
<tr>
<td>NaSCN</td>
<td>2.66±0.01</td>
<td>4.5±0.05</td>
</tr>
<tr>
<td>NaBF₄</td>
<td>2.89±0.01</td>
<td>2.96±0.01</td>
</tr>
<tr>
<td>Na₂SO₃</td>
<td>5.15±0.02</td>
<td>4.70±0.02</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>2.11±0.02</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NaPF₆</td>
<td>2.58±0.02</td>
<td>2.33±0.01</td>
</tr>
</tbody>
</table>
Table S2. Binding constants of receptors 2 for different nitrate salts as determined from fluorescence titrations in a 50 mM acetate buffer (pH 3.6) at 23°C.

<table>
<thead>
<tr>
<th>Salt</th>
<th>5% DMSO pH 3.6 acetate buffer 50 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiNO₃</td>
<td>3.04±0.01</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>3.25±0.01</td>
</tr>
<tr>
<td>KNO₃</td>
<td>3.13±0.07</td>
</tr>
<tr>
<td>CsNO₃</td>
<td>3.05±0.09</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>3.06±0.01</td>
</tr>
<tr>
<td>Ba(NO₃)₂</td>
<td>3.35±0.01</td>
</tr>
</tbody>
</table>

Table S3. Absorption and fluorescence properties of compounds determined in a 50 mM acetate buffer pH 3.6. Quantum yields were measured relative to anthracene dye.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ε(max)[Lmol⁻¹ cm⁻¹]</th>
<th>λabs(max)</th>
<th>λ(em)</th>
<th>Quantum yield (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>11057</td>
<td>375</td>
<td>424</td>
<td>0.11</td>
</tr>
<tr>
<td>2+300 equiv NaNO₃</td>
<td>10480</td>
<td>375</td>
<td>424</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Fluorescence titrations of 2
The fitting curves for the titration of anion mentioned above in Table S1 were exported from HypSpec program showing fitting over the whole spectrum.
**NaCl**

Data at 426

% formation relative to L

Obs-Calc intensity (unweighted)

value

wavelength

**NaClO₄**

Data at 428

% formation relative to L

Obs-Calc intensity (unweighted)

value

wavelength
NaI

Data at 403

Intensity

% formation relative to L

Obs-Calc intensity (unweighted)

titre volume/ml

Value

Point 10 titre .014

NaNO₃

Data at 424

Intensity

% formation relative to L

Obs-Calc intensity (unweighted)

titre volume/ml

Value

Point 10 titre .014

wavelength

value
NaSCN

Data at 401

Obs-Calc intensity (unweighted)

% formation relative to L

Obs-Calc intensity (unweighted)

Point 10 titre .014

ignored

NaBF₄

Data at 424

Obs-Calc intensity (unweighted)

% formation relative to L

Obs-Calc intensity (unweighted)

Point 10 titre .014

ignored
Na₂SO₃

Data at 402

% formation relative to L

Obs-Calc intensity (unweighted)

value

titre volume/ml

NaNO₂

Data at 402

% formation relative to L

Obs-Calc intensity (unweighted)

value

titre volume/ml
Fluorescence titrations of 3

Na$_2$C$_2$O$_4$

NaPF$_6$
NaCl

Data at 402

Intensity

0 10 20 30 40 50 60 70 80 90 100
% formation relative to L

Obs-Calc intensity (unweighted)

value

0 2000 4000

titre volume/ml

Point 10 titre .014

Intensity

0 10 20 30 40 50 60 70 80 90 100
% formation relative to L

Obs-Calc intensity (unweighted)

value

-5000 0 5000

wavelength

NaClO₄

Data at 423

Intensity

2.0E5 2.2E5 2.4E5 2.6E5 2.8E5

% formation relative to L

Obs-Calc intensity (unweighted)

value

-5000 0 5000

titre volume/ml

Point 10 titre .014

Intensity

2.0E5 2.2E5 2.4E5 2.6E5 2.8E5

% formation relative to L

Obs-Calc intensity (unweighted)

value

-5000 0 5000

wavelength
NaF

Data at 424

% formation relative to L

Obs-Calc intensity (unweighted)

value

Intensity

0
10
20
30
40
50
60
70
80
90
100

titre volume/ml

Point 10 titre .014

NaH₂PO₄

Data at 407

% formation relative to L

Intensity

Obs-Calc intensity (unweighted)

value

Intensity

0
10
20
30
40
50
60
70
80
90
100

titre volume/ml

Point 10 titre .014
NaI

Data at 401

Point 10 titre .014

NaNO₃

Data at 422

Point 10 titre .014
NaSCN

Data at 402

\[
\text{Obs-Calc intensity (unweighted)}
\]

\[
\text{% formation relative to L}
\]

\[
\text{Intensity}
\]

\[
0,10,20,30,40,50,60,70,80,90,100
\]

\[
\text{Obs-Calc intensity (unweighted)}
\]

\[
\text{titre volume/ml}
\]

\[
-4000,-2000,0,2000,4000
\]

\[
\text{value}
\]

\[
0,1,2,3,4
\]

\[
\text{Point 10 titre .014}
\]

NaBF₄

Data at 422

\[
\text{Obs-Calc intensity (unweighted)}
\]

\[
\text{% formation relative to L}
\]

\[
\text{Intensity}
\]

\[
0,10,20,30,40,50,60,70,80,90,100
\]

\[
\text{Obs-Calc intensity (unweighted)}
\]

\[
\text{titre volume/ml}
\]

\[
-4000,-2000,0,2000,4000
\]

\[
\text{value}
\]

\[
0,1,2,3,4
\]

\[
\text{Point 10 titre .014}
\]
\[ \text{Na}_2\text{SO}_3 \]

**Data at 455**

- Obs-Calc intensity (unweighted)
  - Intensity
    - Value: 380 420 460 500
  - Wavelength
    - Value: -2000 0 2000

- % formation relative to L
  - Value: 0,1 0,3 0,5

- Titre volume/ml
  - Value: -10000 -5000 0 5000 10000

**Point 10 titre 0.014**

**NaPF_6**

**Data at 395**

- Obs-Calc intensity (unweighted)
  - Intensity
    - Value: 1,4E5 1,8E5 2,2E5 2,6E5 3,0E5
  - Wavelength
    - Value: -10000 0 10000

- % formation relative to L
  - Value: 1,0E5 2,0E5 3,0E5 4,0E5

**Point 10 titre 0.014**

S25
Fluorescence titrations of 4 and 5

Figure S10. Fluorescence changes induced by addition of anions up to 200 equiv to control receptor 4. The calculated binding constants are NaBF₄: logK=3.24±0.05; Na₂C₂O₄: 3.30±0.03; NaClO₄: 3.50±0.06; NaNO₃: 3.40±0.04. Conditions: receptors concentration 10⁻⁵ M, 50 mM acetate buffer solution (pH 3.6) containing 5% DMSO. Excitation at 370 nm, emission 380-600 nm, slit 2/2.

Figure S11. Fluorescence changes induced by addition of anions up to 200 equiv to control receptor 5. The calculated binding constants are NaBF₄: logK=3.14±0.02; Na₂C₂O₄: 3.82±0.02; NaClO₄: 3.42±0.03; NaNO₃: 3.28±0.01. Conditions: receptors concentration 10⁻⁵ M, 50 mM acetate buffer solution (pH 3.6) containing 5% DMSO. Excitation at 370 nm, emission 380-600 nm, slit 2/2.
**Competition experiment**

![Fluorescence intensity of receptor 2 (0.01 M) in the presence of 100 equiv of nitrate (1mM) and after addition of a competing anions in a quantity of 0, 50, 100, 200 and 300 equiv.](image)

**Figure S12.** Fluorescence intensity of receptor 2 (0.01 M) in the presence of 100 equiv of nitrate (1mM) and after addition of a competing anions in a quantity of 0, 50, 100, 200 and 300 equiv.

**Determination of pK_a values**

Protonation constants were determined based on the fluorescence measurements of receptors at different pH values. Conditions: 0.01 mM receptor, 5% of DMSO in Britton-Robinson buffers, I = 0.1 M. [5] Fitting of the obtained curves were performed with the help of HypSpec program. The last three protonation constants (pK_a) were calculated as: for receptor 2: 7.15; 5.54; 3.90 and for receptor 3: 6.48, 4.41; 3.31

![Fluorescence intensity vs. pH a) for receptor 2 and b) for receptor 3. c) and d) are the fitting of the experimental data for 2 and 3 respectively, showing the distribution of protonated forms over pH range.](image)

**Figure S13.** Fluorescence intensity vs. pH a) for receptor 2 and b) for receptor 3. c) and d) are the fitting of the experimental data for 2 and 3 respectively, showing the distribution of protonated forms over pH range.
Figure S14. Determination of pH windows for the turn-on fluorescence response of receptors a) 2 and b) 3 in the presence of NaNO₃. Conditions: 5% of DMSO in Britton-Robinson buffers, I = 0.1 M.

Potentiometric titrations for receptor 2.

All solutions were prepared with 0.1M NaClO₄ with ca. 0.001-0.003 M concentration of compounds (in a mixture 60% water and 40% MeOH). For titrations standard 0.1M solution of NaOH was used. The potentiometric titrations were carried out on a Mettler Toledo G20 Titrator equipped with a DGi 102-Mini pH-electrode. The electrode was calibrated with standard calibrating solutions from Mettler Toledo. The reaction vessel was kept at constant temperature 23°C. The value of Kw was determined from data obtained in the alkaline range of the titration, and found to be equal to 14.52 in our experimental conditions. The titration experiment was carried out as follows: in the reaction vessel was placed a solution of a compound (and calculated amount of HClO₄); after stirring the solution for 5 minutes the titrations was started. The experiment was repeated 3-5 times. For the experiment in the presence of an anion, the corresponding amount of its solution was added prior to the titrations. The obtained data was imported to the HYPEQUAD 2008 program and fitted to obtained protonation constants.

As can be seen from Figure S14, the protonation constants are different from those obtained by fluorescence measurements because of a large amount of methanol used to rich 10⁻³ M concentration of the receptor, which is required for solubilization of protonated and unprotonated receptor.

<table>
<thead>
<tr>
<th>Protonation form</th>
<th>LogK_i</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH⁺</td>
<td>8.66(5)</td>
</tr>
<tr>
<td>LH₂⁺</td>
<td>7.26(8)</td>
</tr>
<tr>
<td>LH₃⁺</td>
<td>6.11(8)</td>
</tr>
<tr>
<td>LH₄⁺</td>
<td>5.70(11)</td>
</tr>
<tr>
<td>LH₅⁺</td>
<td>5.58(12)</td>
</tr>
<tr>
<td>LH₆⁺</td>
<td>3.60(10)</td>
</tr>
</tbody>
</table>

Figure S15. Fitting graph exported from Hyperquad 2008 program and table with protonation constants as determined by fitting of the potentiometric data.

Determination of nitrate concentration in drinking water samples.

The calculated LOD value for nitrate detection without the presence of sulfate is 0.1 mM. The following drinking water samples containing various competing ions were analyzed:

Table S3. Known concentrations of ions in water samples taken for the analysis.
The exact amount of nitrate was added to the solutions to generate nitrate containing sample with concentration of nitrate below and above the maximum allowed concentrations as reported by World Health Organization: 10, 25, 50, and 100 mM.

To determine the nitrate concentration we used the calibration curves reported in the paper Figure 3e (see the article) by choosing the curve with the closest value of sulfate concentrations.

**Table S4.** Determined values of nitrate in water samples in three independent measurements.

<table>
<thead>
<tr>
<th>Concentration of nitrate in the sample/ Water sample /calibration curve used</th>
<th>Found concentration of nitrate in independent measurements 1,2 and 3</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0mM /Sample 1/ 10mM SO₄²⁻</td>
<td>11.5 mM</td>
<td>10.6 mM</td>
</tr>
<tr>
<td>25.0mM /Sample 2/ 100mM SO₄²⁻</td>
<td>25.2 mM</td>
<td>25.5 mM</td>
</tr>
<tr>
<td>50.0 mM /Sample 1/ 10 mM SO₄²⁻</td>
<td>44.7</td>
<td>49.2 mM</td>
</tr>
<tr>
<td>100.0 mM /Sample 2/100 mM SO₄²⁻</td>
<td>116.6</td>
<td>106.9 mM</td>
</tr>
</tbody>
</table>

The fluorescence measurements were carried out in the following sequence: 1) The stock solution of cryptand (4*10⁻⁵M) in acetate buffer (200mM, pH 3.6) were prepared in 25 ml volumetric flask. This solution (0.5 ml) was added to a determined sample (1.5 ml) to generate final parameters of the mixture: 50mM acetate buffer, pH 3.6, concentration of the cryptand 10⁻⁵M. After that fluorescence spectrum was recorded. Following setup parameters were used ex. 370 nm, slit 2/2, em: 380-530 nm. The experiment was repeated 3-5 times. The concentration of the nitrate was determined from the calibration curve.

**X-ray crystal structure determination.**

The crystals for free compounds 2 were grown from methanol by slow evaporation. The crystals of 3 were grown from chloroform by slow evaporation. Attempts to grow crystals of receptors in a
protonated from were successful only with receptor 2, because salts of 3 are very insoluble. Nitrate and perchlorate salts of 2 were obtained by addition of aqueous solution of the acid (6 equiv) to a methanolic solution of 2 and letting the mixtures stay for several days.

The single-crystal X-ray diffraction data for compounds 2, 3, [2H₃][ClO₄]₃ and [2H₆][NO₃]₆ were collected on the ‘Belok’ beamline of the National Research Center ‘Kurchatov Institute’ (Moscow, Russian Federation) using a Rayonix SX165 CCD detector. For each crystal, 720 frames were collected with an oscillation range of 1.0° in the φ scanning mode using two different orientations. The semi-empirical correction for absorption was applied using the Scale program [1]. The data were indexed and integrated using the utility iMOSFLM from the CCP4 software suite [2]. For details, see Table 1. The structures were solved by intrinsic phasing modification of direct methods [3] and refined by a full-matrix least-squares technique on \( F^2 \) with anisotropic displacement parameters for all non-hydrogen atoms. The crystals of 2, [2H₃][ClO₄]₃ and [2H₆][NO₃]₆ contained methanol and water solvate molecules, and the crystals of 3 – chloroform and water solvate molecules. In the case of [2H₃][ClO₄]₃ and [2H₆][NO₃]₆, all attempts to model and refine positions of the most solvate molecules were unsuccessful. Therefore, their contribution to the total scattering pattern was removed by use of the utility SQUEEZE in PLATON06 [4]. The hydrogen atoms of the NH-groups as well as the methanol and water solvate molecules were localized in the difference-Fourier map and included in the refinement within the riding model with fixed isotropic displacement parameters. The other hydrogen atoms in all compounds were placed in calculated positions and refined within the riding model with fixed isotropic displacement parameters \([U_{iso}(H) = 1.5U_{eq}(C) \text{ for the methyl groups and } 1.2U_{eq}(C) \text{ for the other groups}]\). All calculations were carried out using the SHELXTL program suite [5].

Crystallographic data for \( 2•2\frac{1}{2}\text{MeOH}•1\frac{1}{4}\text{H}_2\text{O} \), \( 3•\text{CHCl}_3•\frac{3}{4}\text{H}_2\text{O} \), [2H₃][ClO₄]₃•1½MeOH and [2H₆][NO₃]₆•3MeOH have been deposited with the Cambridge Crystallographic Data Center, CCDC 1921044, CCDC 1921045, CCDC 1921046, and CCDC 1921047, respectively. The supplementary crystallographic data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

References to the X-ray experiment


<table>
<thead>
<tr>
<th>Identification code</th>
<th>2•2½MeOH•1¼H₂O</th>
<th>3•CHCl₃•¾H₂O</th>
<th>[2H₃][ClO₄]₃•1½MeOH</th>
<th>[2H₆][NO₃]₆•3MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C₅₄H₆₇N₆O₪₂₅</td>
<td>C₆₃H₆₇Cl₃N₆O₧₂₅</td>
<td>C₅₃H₇₁Cl₃N₆O₪₃₅</td>
<td>C₅₃H₆₈N₁₄O₂₁</td>
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<tr>
<td>Formula weight</td>
<td>910.73</td>
<td>1023.08</td>
<td>1148.54</td>
<td>1273.33</td>
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<td>Temperature, K</td>
<td>100(2)</td>
<td>100(2)</td>
<td>100(2)</td>
<td>100(2)</td>
</tr>
<tr>
<td>Crystal size, mm</td>
<td>0.20×0.20×0.20</td>
<td>0.07×0.15×0.30</td>
<td>0.10×0.10×0.12</td>
<td>0.12×0.18×0.20</td>
</tr>
<tr>
<td>Wavelength, Å</td>
<td>0.96990</td>
<td>0.96990</td>
<td>0.78790</td>
<td>0.79475</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
<td>Monoclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
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<tr>
<td>----------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Space group</td>
<td>P-1</td>
<td>P2/n</td>
<td>P-1</td>
<td>P2/n</td>
</tr>
<tr>
<td>a, Å</td>
<td>13.164(3)</td>
<td>13.389(3)</td>
<td>14.575(3)</td>
<td>18.058(4)</td>
</tr>
<tr>
<td>b, Å</td>
<td>13.399(3)</td>
<td>18.842(4)</td>
<td>20.618(4)</td>
<td>16.473(3)</td>
</tr>
<tr>
<td>c, Å</td>
<td>14.852(3)</td>
<td>21.675(4)</td>
<td>21.813(4)</td>
<td>23.740(5)</td>
</tr>
<tr>
<td>α, deg.</td>
<td>85.00(3)</td>
<td>90</td>
<td>103.33(3)</td>
<td>90</td>
</tr>
<tr>
<td>β, deg.</td>
<td>89.04(3)</td>
<td>103.44(3)</td>
<td>92.24(3)</td>
<td>90.34(3)</td>
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<tr>
<td>γ, deg.</td>
<td>80.85(3)</td>
<td>90</td>
<td>109.65(3)</td>
<td>90</td>
</tr>
<tr>
<td>V, Å³</td>
<td>2576.5(10)</td>
<td>5318(2)</td>
<td>5958(3)</td>
<td>7062(3)</td>
</tr>
<tr>
<td>Z</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Density (calc.), Mg/m³  
1.174  
1.278  
1.280  
1.198  

µ, mm⁻¹  
0.154  
0.509  
0.292  
0.119  

F(000)  
985  
2170  
2428  
2704  

Theta range, deg.  
3.65 – 36.00  
3.23 – 38.44  
1.07 – 31.04  
1.68 – 31.02  

Index ranges  
-15 ≤ h ≤ 15, -17 ≤ h ≤ 17, -19 ≤ h ≤ 19, -23 ≤ h ≤ 23,  
-16 ≤ k ≤ 16, -22 ≤ k ≤ 24, -26 ≤ k ≤ 26, -20 ≤ k ≤ 21,  
-17 ≤ l ≤ 17, -24 ≤ l ≤ 27, -28 ≤ l ≤ 28, -30 ≤ l ≤ 00  

Reflections collected  
33711  
51333  
121228  
58952  

Independent reflections, Rint  
9027, 0.110  
11197, 0.116  
27557, 0.107  
15976, 0.059  

Reflections observed  
5842  
8972  
13072  
11596  

R1 / wR2 (I > 2σ(I))  
0.128 / 0.262  
0.054 / 0.137  
0.088 / 0.195  
0.064 / 0.164  

R1 / wR2 (all data)  
0.171 / 0.290  
0.068 / 0.149  
0.175 / 0.238  
0.087 / 0.180  

Goodness-of-fit on F²  
1.003  
1.110  
1.045  
1.055  

Extinction coefficient  
0.0070(12)  
0.0019(4)  
—  
0.0051(5)  

Tmin / Tmax  
0.960 / 0.960  
0.850 / 0.950  
0.950 / 0.963  
0.970 / 0.980  

Δρmax / Δρmin, eÅ⁻³  
0.569 / -0.582  
0.395 / -0.587  
1.098 / -0.852  
0.541 / -0.523  

References  