Supporting Information

Cyclic analogs of desferrioxamine E siderophore for $^{68}$Ga nuclear imaging: coordination chemistry and biological activity in *Staphylococcus aureus*

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Synthetic procedures and characterization data for the prepared compounds

The general route for the synthesis of intermediates 1 and 2 is depicted in Scheme S1.

| analog | x | y |
|--------|---|---|
| FOX 2-2 | 2 | 2 |
| FOX 2-3 | 2 | 3 |
| FOX 2-4 | 2 | 4 |
| FOX 2-5 | 2 | 5 |
| FOX 2-6 | 2 | 6 |
| FOX 3-5 | 3 | 5 |

**Scheme S1.** The general route for the synthesis of 1 and 2.

**General procedure for the synthesis of precursor for compounds 1 and 2**

To a solution of tert-butyl benzylxycarbamate (1.0 equiv.) in DMF (5 ml / 1 mmol), 50% NaH (1.1 equiv.) was added portionwise, and the reaction was stirred at RT for 30 min. After 30 min, appropriate bromoester (1.1 equiv.) was added. Reaction was heated at 70°C and its progress was monitored by LC-MS. Then poured into mixture of ice and water and the product was extracted three times by hexane. The hexane extracts were washed with brine, dried over
MgSO$_4$ and concentrated in vacuo to give the respective product as yellowish oil with a yield of 80-90%, depending on bromoester used.

Next, the oil was diluted with small amount of dioxane and 3M HCl/dioxane (10 ml / 1 g of compound) was added. Reaction progress was monitored by LC-MS. Then the reaction mixture was concentrated under reduced pressure and evaporated thrice from hexane to give the amine in a quantitative yield.

To pivaloylic anhydride of Boc-β-alanine or Boc-γ-aminobutyric acid (1.0 equiv.) in THF (tetrahydrofuran) (5 ml / 1 mmol), the solution of amine obtained above (1.0 equiv.) in THF was added along with NMM (N-methylmorpholine, 3.0 eq). Reaction was stirred at RT and its progress was monitored by LC-MS. Then the reaction mixture was diluted with EtOAc, washed twice with H$_2$O, twice with 2M HCl, twice with 5% NaHCO$_3$, brine and dried over MgSO$_4$. The residue was purified using column flash chromatography (silica gel; EtOAc/hexanes) to give the pure precursor for 1 and 2 as an oil.

Results

Precursor with $x = 2$ and $y = 2$, methyl ester: Yield: 47%; LC-MS $t_R = 4.5$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 403, [M-Boc]$^+$ = 281
Precursor with $x = 2$ and $y = 3$, ethyl ester: Yield: 58%; LC-MS $t_R = 4.7$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 431, [M-Boc]$^+$ = 309
Precursor with $x = 2$ and $y = 4$, methyl ester: Yield: 79%; LC-MS $t_R = 4.8$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 431, [M-Boc]$^+$ = 309
Precursor with $x = 2$ and $y = 5$, methyl ester: Yield: 57%; LC-MS $t_R = 4.8$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 445, [M-Boc]$^+$ = 323
Precursor with $x = 2$ and $y = 6$, ethyl ester: Yield: 78%; LC-MS $t_R = 5.2$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 473, [M-Boc]$^+$ = 351
Precursor with $x = 3$ and $y = 5$, ethyl ester: Yield: 76%; LC-MS $t_R = 5.1$ min, ESI-MS ($m/z$): [M+Na]$^+$ = 473, [M-Boc]$^+$ = 351

General procedure for the synthesis of compound 1

To a solution of precursor for 1 and 2 (1.0 equiv.) in MeOH (5 ml / 1 mmol), 1M NaOH (2.0 equiv.) was added and the reaction was stirred at RT. Reaction progress was monitored by LC-MS. Then the reaction mixture was diluted with H$_2$O, MeOH was evaporated and the residues
was acidified with 2M HCl to pH~1-2 and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over MgSO₄ and concentrated in vacuo to give the respective product.

Results:

Compound 1 with $x = 2$ and $y = 2$: Yield: quantitative; LC-MS $t_R = 4.0$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 389$, $[\text{M-Boc}]^+ = 267$, $[\text{M-H}]^- = 365$

Compound 1 with $x = 2$ and $y = 3$: Yield: quantitative; LC-MS $t_R = 4.1$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 403$, $[\text{M-H}]^- = 379$

Compound 1 with $x = 2$ and $y = 4$: Yield: quantitative; LC-MS $t_R = 4.2$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 417$, $[\text{M-H}]^- = 393$

Compound 1 with $x = 2$ and $y = 5$: Yield: quantitative; LC-MS $t_R = 4.3$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 431$, $[\text{M-Boc}]^+ = 309$, $[\text{M-H}]^- = 407$

Compound 1 with $x = 2$ and $y = 6$: Yield: quantitative; LC-MS $t_R = 4.5$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 445$, $[\text{M-Boc}]^+ = 323$, $[\text{M-H}]^- = 421$

Compound 1 with $x = 3$ and $y = 5$: Yield: quantitative; LC-MS $t_R = 4.5$ min, ESI-MS ($m/z$): $[\text{M+Na}]^+ = 445$, $[\text{M-H}]^- = 421$

General procedure for the synthesis of compound 2

The precursor for 1 and 2 was diluted with small amount of dioxane and 3M HCl/dioxane (10 ml / 1 g of compound) was added. Reaction progress was monitored by LC-MS. Then the reaction mixture was concentrated under reduced pressure and evaporated thrice from hexane to give the respective product.

Results:

Compound 2 with $x = 2$ and $y = 2$, methyl ester

Yield: quantitative

LC-MS $t_R = 2.5$ min, ESI-MS ($m/z$): $[\text{M+H}]^+ = 281$, $[\text{M+Na}]^+ = 303$

Compound 2 with $x = 2$ and $y = 3$, ethyl ester

Yield: quantitative

LC-MS $t_R = 2.8$ min, ESI-MS ($m/z$): $[\text{M-H}]^- = 309$

Compound 2 with $x = 2$ and $y = 4$, methyl ester
Yield: quantitative

LC-MS $t_R = 2.8$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 309$

Compound 2 with $x = 2$ and $y = 5$, methyl ester

Yield: quantitative

LC-MS $t_R = 3.1$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 323$

Compound 2 with $x = 2$ and $y = 6$, ethyl ester

Yield: quantitative

LC-MS $t_R = 3.3$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 351, [\text{M}+\text{Na}]^+ = 373$

Compound 2 with $x = 3$ and $y = 5$, ethyl ester

Yield: quantitative

LC-MS $t_R = 3.3$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 351, [\text{M}+\text{Na}]^+ = 373$

**General procedure for the synthesis of compound 3**

To a solution of compound 1 (1.0 equiv.) in DCM (5 ml / 1 mmol), TBTU (1.0 equiv.), TEA (3.0 equiv.) and compound 2 (1.0 equiv.) were added. Reaction was stirred at RT and its progress was monitored by LC-MS. Then the reaction mixture was diluted with EtOAc, washed twice with H$_2$O, twice with 2M HCl, twice with 5% NaHCO$_3$, brine dried over MgSO$_4$ and concentrated *in vacuo* to give the respective product. Next, the compound was diluted with small amount of dioxane and 3M HCl/dioxane (10 ml / 1 g of compound) was added. Reaction progress was monitored by LC-MS. Then the reaction mixture was concentrated under reduced pressure and evaporated thrice from hexane to give the respective product.

**Results:**

Compound 3 with $x = 2$ and $y = 2$, methyl ester

Yield: 89%

LC-MS $t_R = 3.3$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 529, [\text{M}+\text{HCOO}]^- = 573$

Compound 3 with $x = 2$ and $y = 3$, ethyl ester

Yield: 84%

LC-MS $t_R = 3.7$ min, ESI-MS ($m/z$): $[\text{M}+\text{H}]^+ = 571, [\text{M-HCOO}]^- = 615$

Compound 3 with $x = 2$ and $y = 4$, methyl ester
Yield: 92%
LC-MS $t_R = 3.6$ min, ESI-MS ($m/z$): $[M+H]^+ = 585$, $[M-Na]^+ = 607$, $[M-HCOO]^-$ = 629
Compound 3 with $x = 2$ and $y = 5$, methyl ester
Yield: 93%
LC-MS $t_R = 3.7$ min, ESI-MS ($m/z$): $[M+H]^+ = 613$, $[M-Na]^+ = 635$, $[M+HCOO]^-$ = 657
Compound 3 with $x = 2$ and $y = 6$, ethyl ester
Yield: 85%
LC-MS $t_R = 3.1$ min, ESI-MS ($m/z$): $[M+H]^+ = 655$
Compound 3 with $x = 3$ and $y = 5$, ethyl ester
Yield: 87%
LC-MS $t_R = 4.1$ min, ESI-MS ($m/z$): $[M+H]^+ = 655$, $[M+HCOO]^-$ = 699

**General procedure for the synthesis of compound 4**

To a solution of compound 1 (1.0 equiv.) in DCM (5 ml / 1 mmol), TBTU (1.0 equiv.), TEA (3.0 equiv.) and compound 3 (1.0 equiv.) were added. Reaction was stirred at RT and its progress was monitored by LC-MS. Then the reaction mixture was diluted with EtOAc, washed twice with H$_2$O, twice with 2M HCl, twice with 5% NaHCO$_3$, brine dried over MgSO$_4$ and concentrated in vacuo. The residue was purified using column flash chromatography (silica gel; EtOAc/MeOH) to give the pure compound.

To a solution of the above compound (1.0 equiv.) in MeOH (5 ml / 1 mmol), 1M NaOH (2.0 equiv.) was added and the reaction was stirred at RT. Reaction progress was monitored by LC-MS. Then the reaction mixture was diluted with H$_2$O, MeOH was evaporated and the residues was acidified with 2M HCl to pH~1-2 and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over MgSO$_4$ and concentrated in vacuo to give the respective acid.

The above acid was diluted with small amount of dioxane and 3M HCl/dioxane (10 ml / 1 g of compound) was added. Reaction progress was monitored by LC-MS. Then the reaction mixture was concentrated under reduced pressure and evaporated thrice from hexane to give the respective product.

**Results:**
Compound 4 with $x = 2$ and $y = 2$: Yield: 64%; LC-MS $t_R = 3.6$ min, ESI-MS ($m/z$): [M+H]$^+ = 763$, [M-H]$^- = 761$

Compound 4 with $x = 2$ and $y = 3$: Yield: 56%, LC-MS $t_R = 3.6$ min, ESI-MS ($m/z$): [M+H]$^+ = 806$, [M-H]$^- = 804$

Compound 4 with $x = 2$ and $y = 4$: Yield: 62%, LC-MS $t_R = 3.9$ min, ESI-MS ($m/z$): [M+H]$^+ = 847$, [M-H]$^- = 845$

Compound 4 with $x = 2$ and $y = 5$: Yield: 55%; LC-MS $t_R = 3.9$ min, ESI-MS ($m/z$): [M+H]$^+ = 889$, [M-H]$^- = 887$

Compound 4 with $x = 2$ and $y = 6$: Yield: 62%; LC-MS $t_R = 4.1$ min, ESI-MS ($m/z$): [M+H]$^+ = 932$, [M-H]$^- = 930$

Compound 4 with $x = 3$ and $y = 5$: Yield: 55%; LC-MS $t_R = 4.2$ min, ESI-MS ($m/z$): [M+H]$^+ = 932$, [M-H]$^- = 930$

**General procedure for the synthesis of compound 5**

To a solution of compound 4 (1.0 equiv.) in DCM (5 dm$^3$ / 1 mmol), EDCI (1.2 equiv.) and TEA (4.0 equiv.) were added. Reaction was stirred at RT and its progress was monitored by LC-MS. Then the reaction mixture was evaporated and the residue was diluted with EtOAc, washed twice with H$_2$O, twice with 2M HCl, twice with 5% NaHCO$_3$, brine, dried over MgSO$_4$. Analogs with benzyl groups being the precursors of the final compounds FOX 2-2, FOX 2-3 and FOX 2-4 were directly used in the next transformation. In turn, the precursors of the analogs FOX 2-5, FOX 2-6 and FOX 3-5 were purified using preparative HPLC (MeCN/H$_2$O).

**Results: compound 5 with benzyl groups:**

Compound with $x = 2$ and $y = 2$: Yield: 47%; LC-MS $t_R = 4.1$ min, ESI-MS ($m/z$): [M+H]$^+ = 745$, [M+Na]$^+ = 767$, [M-H]$^- = 743$, [M+HCOO]$^- = 789$

Compound 4 with $x = 2$ and $y = 3$: Yield: 45%; LC-MS $t_R = 4.3$ min, ESI-MS ($m/z$): [M+H]$^+ = 787$, [M+Na]$^+ = 810$, [M+HCOO]$^- = 831$

Compound 4 with $x = 2$ and $y = 4$: Yield: 55%; LC-MS $t_R = 4.5$ min, ESI-MS ($m/z$): [M+H]$^+ = 830$, [M+Na]$^+ = 852$, [M+HCOO]$^- = 873$

Compound with $x = 2$ and $y = 5$: Yield: 44%; LC-MS $t_R = 4.8$ min, ESI-MS ($m/z$): [M+H]$^+ = 872$, [M+HCOO]$^- = 916$
Compound with $x = 2$ and $y = 6$: Yield: 13%; LC-MS $t_R = 4.9$ min, ESI-MS ($m/z$): $[M+H]^+ = 914$, $[M+HCOO]^- = 958$

Compound with $x = 3$ and $y = 5$: Yield: 22%; LC-MS $t_R = 5.0$ min, ESI-MS ($m/z$): $[M+H]^+ = 914$, $[M+HCOO]^- = 958$

10% Pd on carbon catalyst was added to a solution of above compound in dioxane/H$_2$O (4/1 v/v) and the reaction was stirred under 1 atmosphere of H$_2$ (its progress was monitored by LC-MS), followed by filtration of the catalyst and evaporation of the solvent. Lyophilization from dioxane gave the pure product 5.

Results: compound 5:

Compound 5 with $x = 2$ and $y = 2$: Yield: 82%; LC-MS $t_R = 1.0$ min, ESI-MS ($m/z$): $[M+H]^+ = 475$, $[M-H]^-$ = 473

Compound 5 with $x = 2$ and $y = 3$: Yield: 88%; LC-MS $t_R = 1.7$ min, ESI-MS ($m/z$): $[M+H]^+ = 517$, $[M-H]^-$ = 515

Compound 5 with $x = 2$ and $y = 4$: Yield: 83%; LC-MS $t_R = 2.0$ min, ESI-MS ($m/z$): $[M+H]^+ = 559$, $[M-H]^-$ = 557

Compound 5 with $x = 2$ and $y = 5$: Yield: 81%; LC-MS $t_R = 2.4$ min, ESI-MS ($m/z$): $[M+H]^+ = 601$, $[M-H]^-$ = 599

Compound 5 with $x = 2$ and $y = 6$: Yield: 90%; LC-MS $t_R = 2.7$ min, ESI-MS ($m/z$): $[M+H]^+ = 643$, $[M-H]^-$ = 641

Compound 45 with $x = 3$ and $y = 5$: Yield: 70%; LC-MS $t_R = 2.8$ min, ESI-MS ($m/z$): $[M+H]^+ = 643$, $[M-H]^-$ = 641
Figure S1. NMR spectra for studied ligands: FOX 2-2(a), FOX 2-3(b), FOX 2-4(c), FOX 2-5(d), FOX 2-6(e) and FOX 3-5(f).
Table S1. Intensity maxima of the major FOX ligands and adduct ions observed by ESI-MS.

| Ligand   | Pseudomolecular ion                                    | m/z experimental | m/z simulated |
|----------|--------------------------------------------------------|------------------|--------------|
| FOX 2-2  | {[H₃L]+[H]+}⁺                                        | 475.22           | 475.21       |
|          | {[H₃L]+[Na]+}⁺                                         | 497.19           | 497.19       |
|          | {[H₃L]+[K]+}⁺                                          | 513.17           | 513.17       |
| FOX 2-3  | {[H₃L]+[H]+}⁺                                          | 517.26           | 517.26       |
|          | {[H₃L]+[Na]+}⁺                                         | 539.24           | 539.24       |
|          | {[H₃L]+[KClO₄]+[H]+}⁺                                 | 655.16           | 655.17       |
| FOX 2-4  | {[H₃L]+[H]+}⁺                                          | 559.31           | 559.31       |
|          | {[H₃L]+[H]++[KClO₄]}⁺                                  | 697.20           | 697.22       |
| FOX 2-5  | {[H₃L]+[H]+}⁺                                          | 601.35           | 601.36       |
|          | {[H₃L]+[Na]+}⁺                                         | 623.33           | 623.33       |
|          | {[H₃L]+[KClO₄]+[H]+}⁺                                 | 739.25           | 738.26       |
| FOX 3-5  | {[H₃L]+[H]+}⁺                                          | 643.41           | 643.40       |
|          | {[H₃L]+[Na]+}⁺                                         | 665.40           | 665.39       |
|          | {[H₃L]+[KClO₄]+[H]+}⁺                                 | 781.33           | 781.33       |

Conditions: [L] = 1·10⁻³ M, MeOH/H₂O: 50/50 w/w.

All peak assignments were based on the comparison between the calculated and experimental isotope patterns.

**Thermodynamic solution studies**

**ESI-MS measurements**

The ESI-MS spectra of the reaction mixture of Fe(ClO₄)₃/ligand and Ga(ClO₄)₃/ligand with a 1:1 molar ratio (Fig. 2) are characterized by the presence of a few major peaks successfully attributed to the mononuclear species (Table S2, Table S3). Experiments were performed in methanol-water system (50:50 v/v) for better solvent evaporation.
Figure S2. ESI-MS spectra for Fe(III)-FOX 2-2 (a), Ga(III)-FOX 2-2 (b), Fe(III)-FOX 2-3 (c), Ga(III)-FOX 2-3 (d), Fe(III)-FOX 2-4 (e), Ga(III)-FOX 2-4 (f), Fe(III)-FOX 2-5 (g), Ga(III)-FOX 2-5 (h), Fe(III)-FOX 3-5 (i), Ga(III)-FOX 3-5 (j), solution in metal to ligand molar ratio 1:1. The full spectra: expanded experimental (red) and simulated (black) spectra.
Table S2. Intensity maxima of the major Fe(III)-FOX complexes and adduct ions observed by ESI-MS

| Fe(III) Complexes | Pseudomolecular ion | m/z experimental | m/z simulated |
|-------------------|----------------------|------------------|--------------|
| FOX 2-2           | ([FeL] + [H]⁺)⁺      | 528.13           | 528.13       |
| FOX 2-3           | ([FeL] + [Na]⁺)⁺     | 592.15           | 592.16       |
|                   | ([FeL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 714.09        | 714.09       |
|                   | ([FeL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 957.95        | 957.97       |
| FOX 2-4           | ([FeL] + [Na]⁺)⁺     | 634.19           | 634.20       |
|                   | ([FeL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 756.13        | 756.14       |
|                   | ([FeL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 999.99        | 1000.01      |
| FOX 2-5           | ([FeL] + [Na]⁺)⁺     | 676.24           | 676.25       |
|                   | ([FeL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 798.17        | 798.19       |
| FOX 3-5           | ([FeL] + [Na]⁺)⁺     | 718.29           | 718.27       |
|                   | ([FeL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 712.26        | 734.27       |
|                   | ([FeL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 840.22        | 840.23       |

Conditions: [L] = 1·10⁻⁵ M, M:L 1:1, MeOH/H₂O: 50/50 w/w.

Table S3. Intensity maxima of the major Ga(III)-FOX complexes and adduct ions observed by ESI-MS

| Ga(III) Complexes | Pseudomolecular ion | m/z experimental | m/z simulated |
|-------------------|----------------------|------------------|--------------|
| FOX 2-2           | ([GaL] + [H]⁺)⁺      | 541.12           | 541.12       |
| FOX 2-3           | ([GaL] + [Na]⁺)⁺     | 605.14           | 605.14       |
|                   | ([GaL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 727.07        | 727.08       |
|                   | ([GaL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 927.93        | 927.96       |
| FOX 2-4           | ([GaL] + [Na]⁺)⁺     | 647.19           | 647.19       |
|                   | ([GaL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 769.12        | 769.13       |
|                   | ([GaL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 1014.97       | 1015.00      |
| FOX 2-5           | ([GaL] + [Na]⁺)⁺     | 689.23           | 689.24       |
|                   | ([GaL] + [Na]⁺ + [NaClO₄]⁻)⁺ | 811.16        | 811.18       |
|                   | ([GaL] + [Na]⁺ + 3[NaClO₄]⁻)⁺ | 1057.02       | 1057.05      |
| FOX 3-5           | ([GaL] + [Na]⁺)⁺     | 731.28           | 731.29       |
All peak assignments were based on the comparison between the calculated and experimental isotope patterns and correlate very well, with the species identified by the potentiometric and UV-Vis experiments. ESI–MS spectra collected for Fe(III)/Ga(III)-FOX analogs solutions in metal-to-ligand molar ratios of 1:1 and 1:2, confirmed the formation of monomeric complexes.

**Ligand’s protonation constants**

The acid-base properties of investigated ligands were studied by potentiometric technique. The optimized log$\beta$ values defined by equations (S1) and (S2) were calculated using SUPERQUAD\textsuperscript{1} or Hyperquad 2006\textsuperscript{2} programs (Table S4). For the sake of simplicity charges are omitted in all chemical equilibria given here.

\[
\begin{align*}
H_{n-1}L + H & \leftrightarrow H_nL & (S1) \\
K_n^H &= [H_nL]/[H_{n-1}L][H] & (S2)
\end{align*}
\]

**Table S4. Protonation (log$\beta_H$) constants for studied ligands\textsuperscript{a}**

| Assignment | Compound | FOXE$^b$ | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 |
|------------|----------|----------|----------|----------|----------|----------|----------|
| L + H$^+$ ↔ HL | 9.89 | 9.97(1) | 9.94(1) | 9.93(1) | 9.86(1) | 10.19(1) |
| HL + H$^+$ ↔ H$_2$L | 19.31 | 19.28(1) | 19.27(1) | 19.21(1) | 19.09(1) | 19.53(1) |
| H$_2$L + H$^+$ ↔ H$_3$L | 27.94 | 27.62(2) | 27.71(1) | 27.64(1) | 26.53(1) | 27.79(1) |

\textsuperscript{a}Constants determined from potentiometric titrations. Conditions: [L] = 1·10$^{-3}$ M, T = 25°C, I = 0.1 M NaClO$_4$. \textsuperscript{b}Ref \textsuperscript{3}

Novel cyclic trihydroxamate analogs possess three hydroxamate moieties. For all analogs the protonation constants are within the range for hydroxamates known from literature, such as natural trihydroxamate FOXE.\textsuperscript{3} All hydroxamate groups in FOXE are indistinguishable and because of the possible overlap of the dissociation processes, the pK values determined, cannot be ascribed unambiguously to either of the groups. Thus, to thoroughly determine the protonation processes of studied compounds, the pH-dependent NMR titrations would be
needed. However, such a detailed analysis is not necessary for the determination of stability of iron complexes and therefore was not performed.

Determination of stability constants

*Complex formation equilibria with Fe(III)*

During spectrophotometric titrations of Fe(III)–FOX systems, the color of the solutions changed from dark red in acidic pH to light yellow above pH 7. Spectral changes of LMCT bands (Fig. S3) were attributed to the formation of dihydroxamate [FeHL]^+ complex at acidic pH, and its further deprotonation and consecutive formation of trihydroxamate [FeL] complex, which persisted in solution up to pH ~ 8, where absorbance started to fade; at pH 11 we observed baseline elevation which was attributed to complex dissociation and Fe(III) hydroxide formation (data not shown). The formation of monohydroxamate [FeH₂L]^{2+} species was not observed. Fe(III)-FOX 2-5 UV-Vis spectra were however different, with only one band present during whole titration (Fig. S3 g).
Figure S3. Spectroscopic data used to calculate constants (log\(\beta\)) for studied complexes with Fe(III) ions and electronic spectra for Fe(III)-FOX 2-2 (a, b), Fe(III)-FOX 2-3 (c, d), Fe(III)-FOX 2-4 (e, f), Fe(III)-FOX 2-5 (g, h), Fe(III)-FOX 3-5 (i, j).

Conditions: \([L] = 1 \times 10^{-4} \text{ M}, \text{M:L} 1:1, T = 25^\circ\text{C}, I = 0.1 \text{ M NaClO}_4\). Experimental errors: \(\lambda_{\text{max}} = \pm 2\text{ nm}, \varepsilon = \pm 5\%\).

Stability constants gathered in Table S5 as log\(\beta[H_{\text{m}}L_n]\), defined by equations (S3) and (S4), and spectroscopic characteristics of calculated spectra given in Table S6 were determined by refinement of the spectroscopic and potentiometric data.

\[
Fe^{3+}_{(aq)} + L^{3-} \overset{K_{ML}}{\rightleftharpoons} FeL
\]  
\[\text{S3}\]

\[
K_{ML} = \frac{[FeL]}{[Fe^{3+}_{(aq)}][L^{3-}]}
\]  
\[\text{S4}\]
The uncertainties in the log$K$ values correspond to the added standard deviations in the cumulative constants.

Corresponding electronic spectra are presented in Fig. S3, while speciation plots are presented in Figure S7.

**Table S5.** Stability (log$\beta$) constants for studied complexes with Fe(III) ions$^a$

| Assignments | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 |
|-------------|---------|---------|---------|---------|---------|
| $log\beta_{[FeHL]^+}$ | 30.64 (6)$^c$ | 30.44 (5)$^c$ | 30.83 (4)$^c$ | - | 31.13$^c$ |
| $log\beta_{[FeL]}$ | 25.92 (8)$^b$ | 27.22 (2)$^b$ | - | - | 28.81 (1)$^b$ |
| $log\beta_{[FeL]}$ | 26.1$^c$ | 26.70 (9)$^c$ | 28.71 (7)$^c$ | 31.32 (8)$^c$ | 28.59 (3)$^c$ |
| $pK_{NH\text{OH}}$ | 4.72$^b$ | 3.22$^b$ | - | - | 2.32$^b$ |
| | 4.54$^c$ | 3.74$^c$ | 2.12$^c$ | - | 2.54$^c$ |

$^a$Constants determined from potentiometric$^b$ and UV-Vis$^c$ pH-dependent titrations. Conditions: [L] = 1·10$^{-3}$ M, M:L 1:1 for potentiometric assays, and 1·10$^{-4}$ M, M:L 1:1 for UV-Vis assays, $T = 25^\circ C$, $I = 0.1$ M NaClO$_4$.

Values presented in Table S5 were calculated from data collected during measurements implementing two separate methods. As we can see above, this data correlates very well and further confirms our calculations.

**Table S6.** Spectroscopic parameters for studied complexes$^a$

| Complex form | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 |
|--------------|---------|---------|---------|---------|---------|
| | $\lambda_{\text{max}}$ [nm] | $\varepsilon$ [M$^{-1}$cm$^{-1}$] | $\lambda_{\text{max}}$ [nm] | $\varepsilon$ [M$^{-1}$cm$^{-1}$] | $\lambda_{\text{max}}$ [nm] | $\varepsilon$ [M$^{-1}$cm$^{-1}$] | $\lambda_{\text{max}}$ [nm] | $\varepsilon$ [M$^{-1}$cm$^{-1}$] |
| [FeHL]$^+$ | 476 | 1620 | 478 | 1590 | 473 | 1857 | - | - |
| [FeL] | 426 | 2730 | 432 | 2140 | 430 | 2450 | 440 | 2540 | 430 | 2660 |

$^a$Conditions: [L] = 5·10$^{-5}$ M, M:L 1:1, $T = 25^\circ C$, $I = 0.1$ M NaClO$_4$. Experimental errors: $\lambda_{\text{max}} = \pm 2$ nm, $\varepsilon = \pm 5%$. 
The spectrophotometric competition experiments with EDTA

The spectrophotometric competition experiments with EDTA were performed at fixed pH 7.0, where fully formed complexes of Fe(III)-FOXE analogs were formed, characterized by strong bands at 430-440 nm. Those LMCT bands were gradually silenced after addition of fixed amount of EDTA ligand, from 0-10 molar equivalent for FOX 2-5 and 0-1.2 molar equivalent for rest of the ligands (Fig. S4).

![Graph a)](image)

![Graph b)](image)

![Graph c)](image)

![Graph d)](image)
Figure S4. UV-Vis spectra of competition reaction of Fe(III)-FOXE analog complexes vs EDTA for FOX 2-2 (a), FOX 2-3 (b), FOX 2-4 (c), FOX 2-5 (d), and FOX 3-5 (e). Conditions: [L] = 1.5·10^{-4} M, T = 25°C, I = 0.1 M NaClO₄.

The stability of ferric ion complexes with EDTA is of sufficient strength (\(\log \beta_{Fe(III)EDTA} = 10^{25.0}\)) to compete effectively with the tris-hydroxamates for ferric ion at intermediate pH values (5-8) if an excess of EDTA is used. The competition equilibrium is described by equations (S5) and (S6).

\[
Fe^{III} + EDTA \rightleftharpoons Fe^{III} EDTA + L \quad (S5)
\]

\[
K = \frac{[Fe^{III} EDTA][L]}{[Fe^{III} L][EDTA]} = \frac{\beta_{FeEDTA}^{110}}{\beta_{Fe^{III}L}^{110}} \quad (S6)
\]

The competition data were refined to obtain the overall Fe(III)-FOX binding constant \(\log \beta_{FeL}\) (Table S7) for analogs using a model involving two ligands and one metal. The protonation constants and Fe(III) formation constants for EDTA\(^4\) and the protonation constants of the analogs (The acid-base properties of investigated ligands were studied by potentiometric technique. The optimized \(\log \beta\) values defined by equations (S1) and (S2) were calculated using SUPERQUAD\(^1\) or Hyperquad 2006\(^2\) programs (Table S4). For the sake of simplicity charges are omitted in all chemical equilibria given here.

\[
H_{n-1}L + H \rightleftharpoons H_n L \quad (S1)
\]

\[
K_n^{H} = [H_n L][H_{n-1} L][H] \quad (S2)
\]

Table S4), were used as fixed parameters during data analysis.
The concentration of Fe(III)-analog complex for each derivative, [FeL], was calculated from the absorbance maximum at around 420 nm (where FeL is the only light-absorbing species) and previously determined extinction coefficients. The concentrations of other species in equation (S6) were calculated from mass balance equations using the experimental pH values:

\[
[\text{Fe}]_{\text{tot}} = \alpha_{\text{FeL}} [\text{FeL}] + \alpha_{\text{FeEDTA}} [\text{FeEDTA}] \tag{S7}^a
\]

\[
[\text{L}]_{\text{tot}} = \alpha_{\text{FeL}} [\text{FeL}] + \alpha_{\text{L}} [\text{L}] \tag{S8}^a
\]

\[
[\text{EDTA}]_{\text{tot}} = \alpha_{\text{FeEDTA}} [\text{FeEDTA}] + \alpha_{\text{EDTA}} [\text{EDTA}] \tag{S9}^a
\]

\(^a\alpha\) is the usual Ringbom’s coefficient.\(^5\)

**Table S7.** Stability (\(\log \beta_{\text{FeL}}\)) constants for studied complexes with Fe(III) ions\(^a\)

| Assignments | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 |
|-------------|---------|---------|---------|---------|---------|
| \(\log \beta_{\text{FeL}}\) | 26.57 (4) | 26.55 (3) | 27.13 (1) | 28.81 (1) | 28.38 (1) |

\(^a\)Constants determined from EDTA competition studies. Conditions: [L] = 1.5 \times 10^{-4} M, M:L ratio 1:1, pH = 7, T = 25°C, J = 0.1 M NaClO\(_4\).
Complex formation equilibria with Ga(III)

Figure S5. Spectroscopic data used to calculate (log$\beta$) constants for studied complexes with Ga(III) ions for FOX 2-2 (a), FOX 2-3 (b), FOX 2-4 (c), FOX 2-5 (d), FOX 3-5 (e) and FOXE (f).

Conditions: a), b), d) [L] = 1·10$^{-4}$ M, M:L ratio 1:1, pH = 2; c) [L] = 1·10$^{-4}$ M, M:L ratio 1:1, pH = 1.4; pH = 1.5; f) [L] = 1.5·10$^{-4}$ M, M:L ratio 1:1, pH = 2; T = 25°C, $I$ = 0.1 M NaClO$_4$. 
Table S8. Stability (logβ) constants for studied complexes with Ga(III) ions\(^a\)

| Assignments | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 | FOXE |
|-------------|---------|---------|---------|---------|---------|------|
| \(\text{log} \beta_{[GaHL]}^+\) | 28.48 (4)\(^c\) | 28.30 (4)\(^c\) | 28.84 (5)\(^c\) | - | 29.45 (1)\(^c\) | - |
| \(\text{log} \beta_{[GaL]}\) | 26.52 (5)\(^b\) | 26.02 (1)\(^b\) | 26.28 (1)\(^b\) | 29.52 (6)\(^c\) | 26.58 (4)\(^b\) | 29.79 (1) |
| \(pK_{\text{NH}_2OH}\) | 3.25 | 2.28 | 2.56 | - | 2.87 | - |

\(^a\)Constants determined from potentiometric\(^b\) and UV-Vis\(^c\) pH-dependent titrations. Conditions: [\(L\)] = 1·10\(^{-3}\) M, M:L 1:1 for potentiometric assays and [\(L\)] = 1·10\(^{-3}\) M, M:L 1:1 for competition UV-Vis titrations, and [\(L\)] = 5·10\(^{-5}\) M, M:L 1:1 for UV-Vis pH-metric titration assays; \(T = 25^\circ\text{C}, I = 0.1\) M NaClO\(_4\).

Figure S6. pH-dependent spectroscopic titration of Ga(III)-FOX 2-5 analog complex.

Conditions: [\(L\)] = 5·10\(^{-5}\) M, M:L ratio 1:1, pH range 0.1-11.9, \(T = 25^\circ\text{C}, I = 0.1\) M NaClO\(_4\).
Figure S7. Species distribution for studied complexes with Fe(III) and Ga(III) ions: a) Fe(III)-FOX 2-2, c) Fe(III)-FOX 2-3, e) Fe(III)-FOX 2-4, g) Fe(III)-FOX 2-5, h) Fe(III)-FOX 3-5, b) Ga(III)-FOX 2-2, d) Ga(III)-FOX 2-3, f) Ga(III)-FOX 2-4, h) Ga(III)-FOX 2-5, j) Ga(III)-FOX 3-5

*Plots calculated for conditions: [L] = 1·10^{-3}, [Fe(III)] = 1·10^{-3}

Complex formation equilibria with Fe(II)

a)  

\[ \begin{align*} 
\text{Fe}^{II} & \text{FOX} \ 2-2 \\
\text{Fe}^{II} & \text{FOX} \ 2-3 \\
\text{Fe}^{II} & \text{FOX} \ 2-4 \\
\text{Fe}^{II} & \text{FOX} \ 2-5 \\
\text{Fe}^{II} & \text{FOX} \ 3-5 
\end{align*} \]
Figure S8. CV voltammograms used to calculate (logβ) constants for studied complexes\textsuperscript{a} with Fe(II) ions: a) FOX 2-2, b) Fe-FOX 2-3, c) FOX 2-4, d) FOX 2-5, e) FOX 3-5

\textsuperscript{a}Conditions: [Fe(III)] = 2 \times 10^{-3} M, M:L ratio 1:1, pH = 7, T = 25°C, I = 0.1M NaClO\textsubscript{4} on glass-carbon working electrode.

For each complex the stability constant for Fe(II)-analog was calculated using eq. S10

\[ E^{0}_{\text{complex}} - E_{aq}^{0} = -59.15\log \left( \frac{\beta^{III}}{\beta^{II}} \right) \] (S10)

Where \( E_{aq}^{0} = 771 \text{ mV} \) and NHE = 222 mV.

Table S9. \( E_{1/2} \) and stability (log\( \beta \)) constants for studied complexes with Fe(II) ions\textsuperscript{a}

| Assignments | FOX 2-2 | FOX 2-3 | FOX 2-4 | FOX 2-5 | FOX 3-5 |
|-------------|---------|---------|---------|---------|---------|
| \( E^{\circ}_{\text{complex}} \) [V] | -369    | -432    | -426    | -452    | -416    |
| $\log \beta_{[\text{Fe(II)L}]}$ | 7.45 | 7.54 | 9.12 | 11.29 | 8.48 |
|-------------------------------|------|------|------|-------|------|

*aConstants determined from CV measurements. Conditions: $[L] = 1 \cdot 10^{-3}$ M, M:L ratio 1:1, pH = 7, $T = 25^\circ C$, $I = 0.1$ M NaClO₄.*

**Fig. S10.** Representative UV-HPLC chromatograms of FOX-2-5 and Fe-FOX-2-5 (UV 220 nm), radio-HPLC and radio-TLC chromatograms of $[^{68}\text{Ga}]\text{Ga-FOX 2-5}$, from top to bottom.
### Table S10. Radiochemical Purity (RCP) of $[^{68}\text{Ga}]\text{GaFOX}$ analogs as determined by SPE, HPLC and TLC

| Analog | SPE<sup>a</sup> | HPLC | TLC |
|--------|----------------|-------|------|
|        | n   | RCP  | sd  | n   | RCP  | sd  | n   | RCP  | sd  |
| FOX 2-2| 5   | 75.1 | 7.9 | 3   | 51.1 | 12.3| 5   | 13.8 | 5.4 |
| FOX 2-3| 5   | 52.5 | 6.8 | 3   | 13.4 | 4.1 | 5   | 13.8 | 5.4 |
| FOX 2-4| 5   | 94.2 | 2.3 | 5   | 83.4 | 2.4 | 5   | 15.1 | 8.8 |
| FOX 2-5| n.d.| n.d. | n.d.| 5   | 99.3 | 0.4 | 5   | 99.1 | 0.9 |
| FOX 2-6| 3   | >90<sup>b</sup> | | 4   | 90.6 | 5.9 | 4   | 90.2 | 8.0 |
| FOX 3-5| 5   | 86.5 | 24.3| 3   | 80.8 | 3.1 | 5   | n.a. | n.a. |

<sup>a</sup>RCP calculated as percentage of bound (eluted in the ethanol fraction) over total activity eluted;

<sup>b</sup>No exact calculation was made, as in one case RCP exceeded 100% due to geometry effects; n.d.: not determined (as RCP was exceeding 95% in other analysis), n.a.: not applicable due to insufficient separation.
**Fig. S11.** Growth promotion of Fe-FOX derivatives in comparison with Fe-FOX E, Fe(II)-SO₄ and no iron supply.
References

1. Gans, P.; Sabatini, A.; Vacca, A. SUPERQUAD: an improved general program for computation of formation constants from potentiometric data. *Journal of the Chemical Society-Dalton Transactions* **1985**, *(6)*, 1195-1200, DOI: 10.1039/dt9850001195.

2. Alderighi, L.; Gans, P.; Ienco, A.; Peters, D.; Sabatini, A.; Vacca, A. Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coordination Chemistry Reviews* **1999**, *(184)*, 311-318, DOI: 10.1016/s0010-8545(98)00260-4.

3. Smith, R.; Martell, A. *Hydroxamic Acids*. Springer, Boston, MA: 1989; 6, 416-420.

4. Pettit, L. D.; Powell, H. K., The IUPAC Stability Constants Database Academic Software. In Timble, York, UK: 2001; (www.acadsoft.co.uk, version 5.6).

5. Ringbom, A. Complexation in analytical chemistry : a guide for the critical selection of analytical methods based on complexation reactions. Interscience: New York, 1963.