Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

pFe^{3+} Determination of Multidentate Ligands by a Fluorescence Assay

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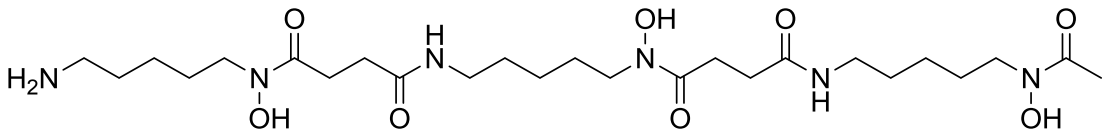
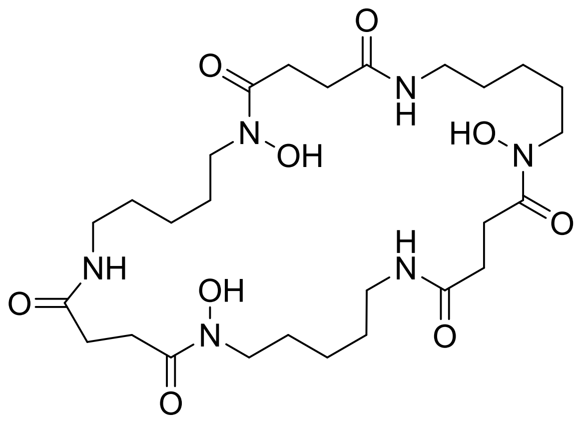
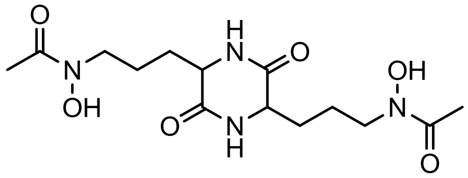
Structures of Ligands



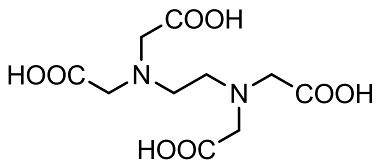
L1



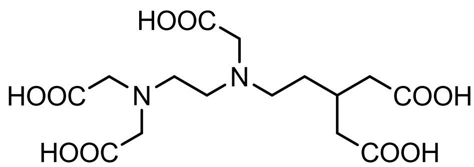
L2



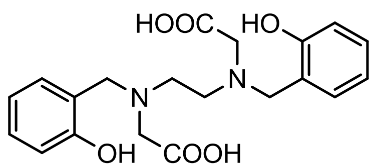
L3


COOH
HOOC
N
N
COOH
HOOC

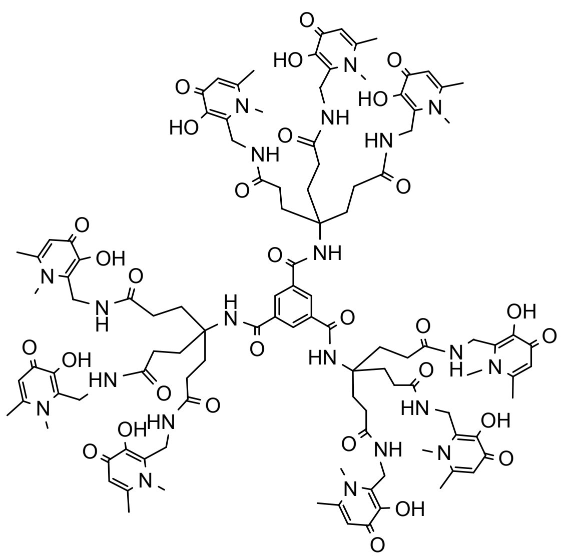
L4


HOOC
HOOC
N
N
COOH
HOOC
COOH

L5


HOOC
HO
N
N
OH
COOH

L6



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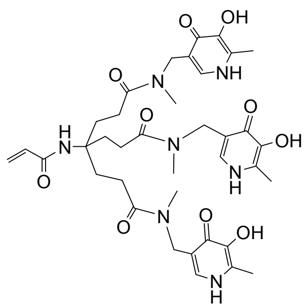
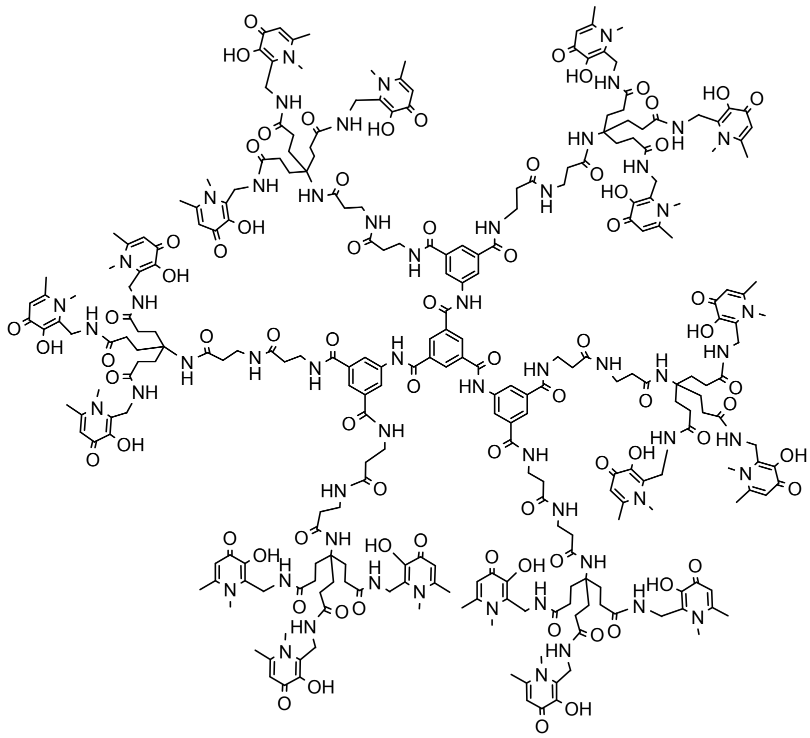
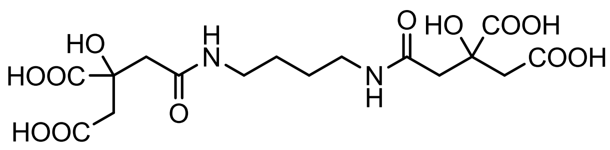


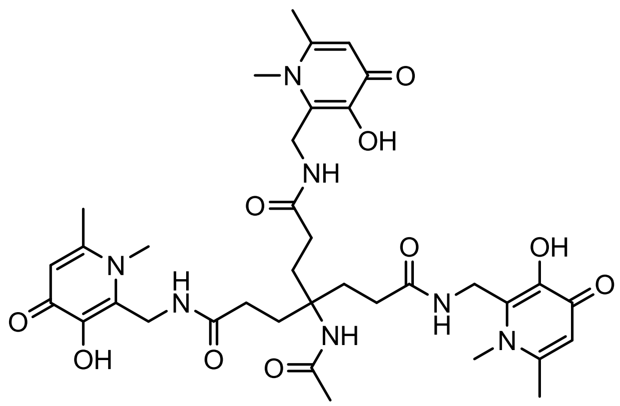
L8



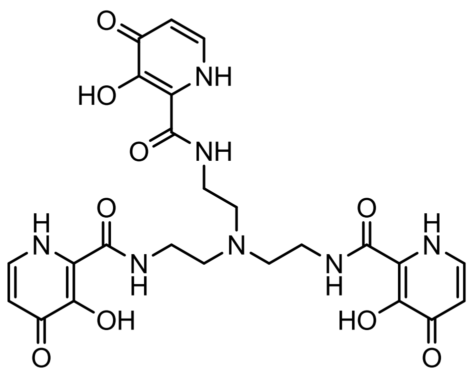
L9



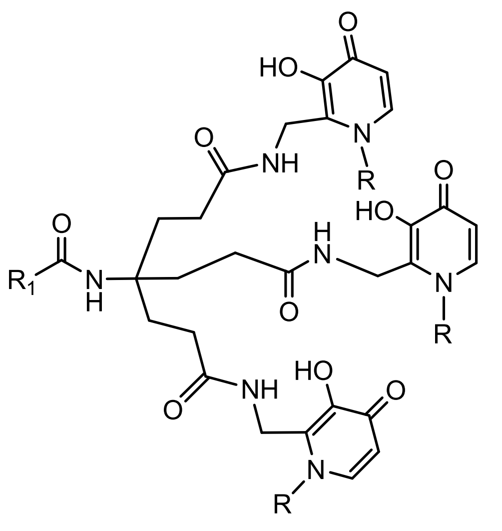
L10


O
N
OH
NH
O
N
H
OH
O
O
NH
O
NH
O
OH
O
N
H
N

L11


O
HO
NH
O
NH
H
N
O
N
H
N
OH
O
O
N
H
H
N
HO
O

L12


O
HO
N
R
O
NH
O
HO
O
R1
NH
H
N
O
H
N
R
O
NH
HO
O
N
R

L13: R_1 = CH_3, R = CH_3

L14: R_1 = n-C_3H_7， R=C_2H_5

L15: R_1 = $CH_3O(CH_2CH_2O)_2CH_2$， R_1=C_2H_5

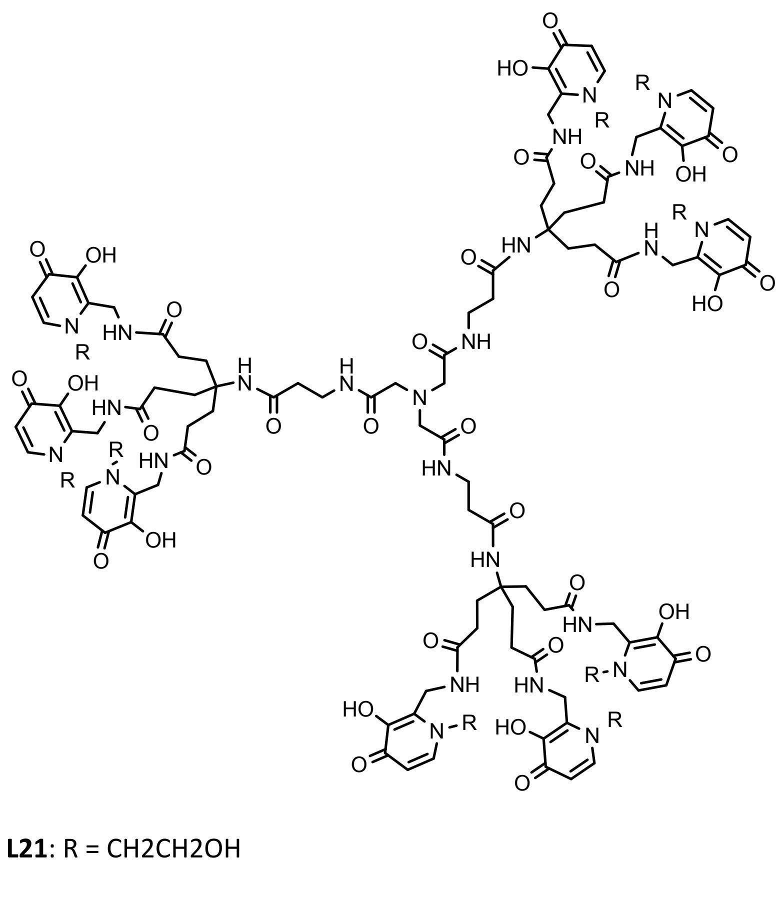
L16: R_1 = CH_3， R=n-C_4H_9

L17: R_1 = n-C_3H_7， R=n-C_4H_9

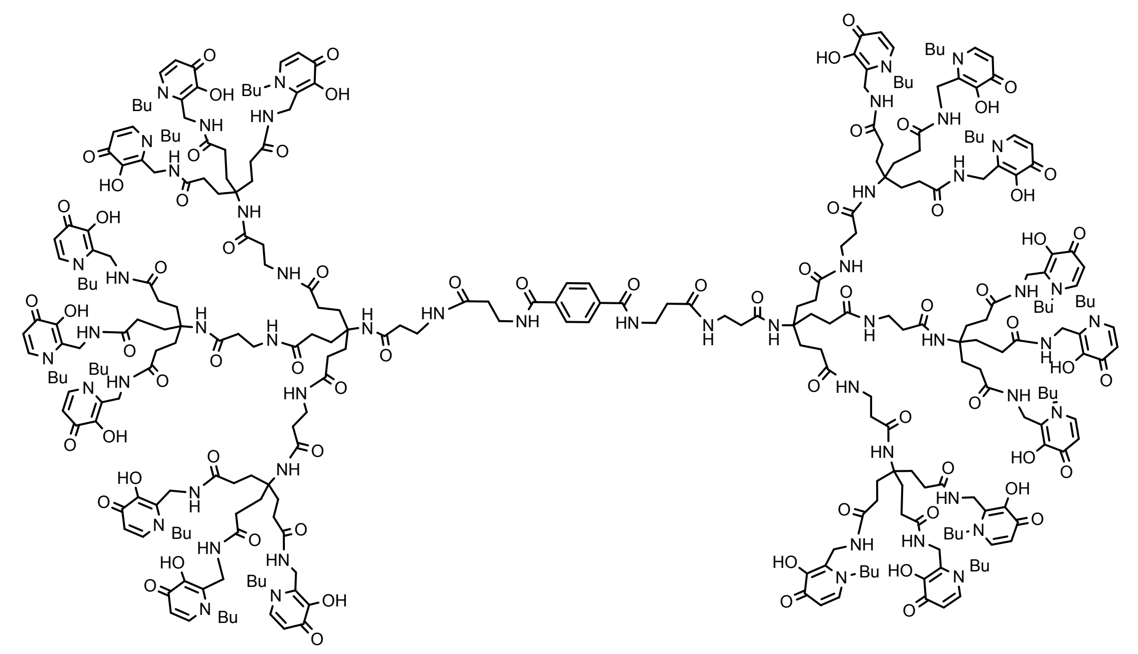
L18: R_1 = $CH_3O(CH_2CH_2O)_2CH_2$， R=n-C_4H_9

L19: R_1 = $CH_3O(CH_2CH_2O)_2CH_2$， R=n-C_6H_{13}

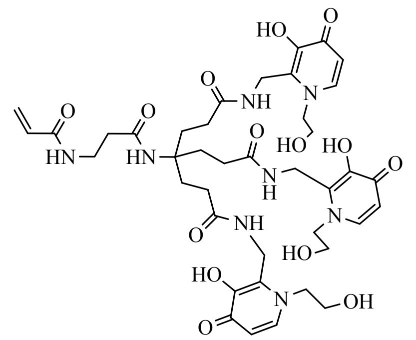
L20: R_1 = n-C_3H_7， R=CH_2CH_2OH



L21: R = CH2CH2OH

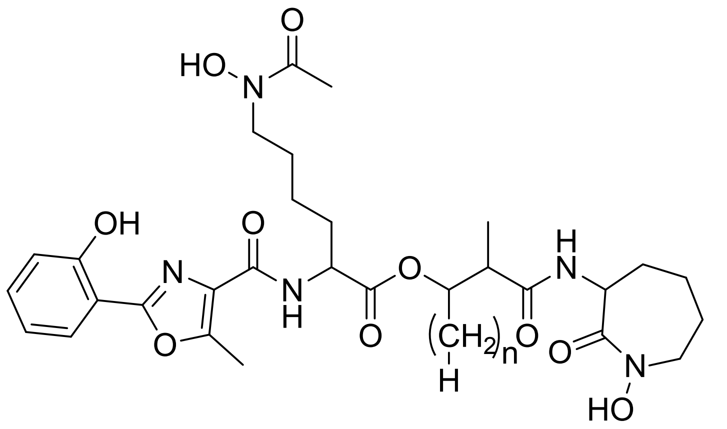


L22

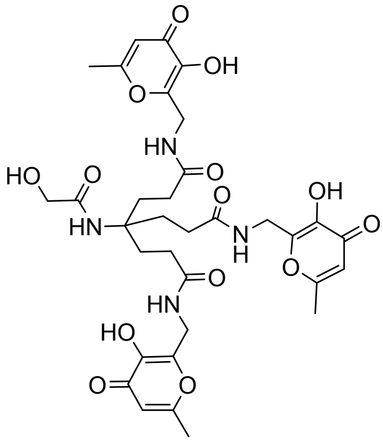


L23

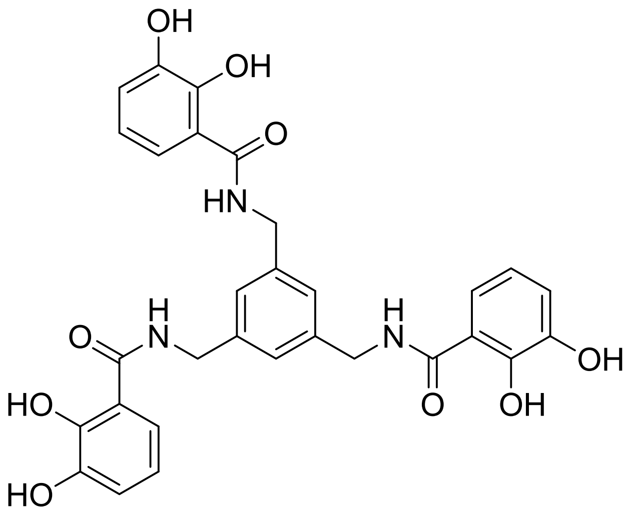
L24: Polymer of **L23** and iron binding capacity at 291 μmol/g.



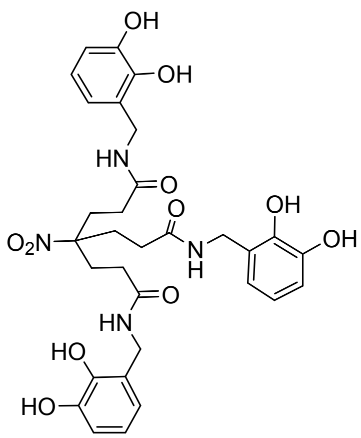
L25



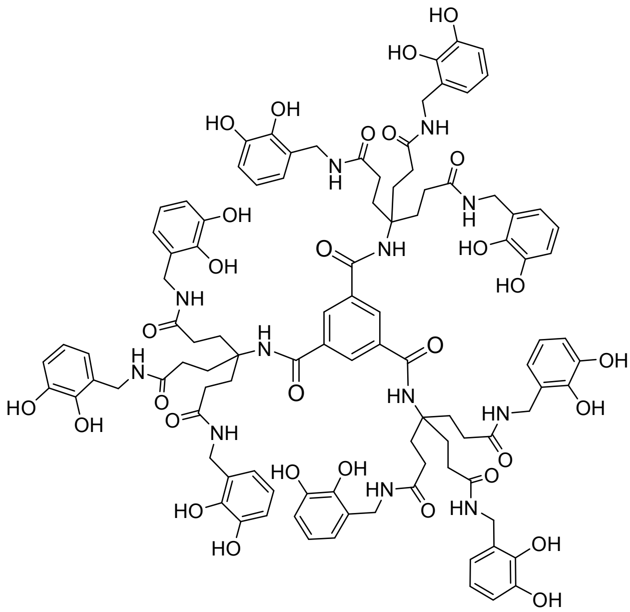
L26



L27



L28



L29

Calculation of unknown pFe value of hexadentate ligand based on the pFe value of the competing ligand and the relative fluorescence

Hexadentate ligands have simple equilibrium constants as indicated in Eq. 1 and 2. The competition between the two hexadentate ligands for iron is presented in Eq. 3.

$$Fe + L_1 \underset{}{\overset{K_1}{\rightleftharpoons}} FeL_1 \qquad \text{Eq.1}$$

$$Fe + L_2 \underset{}{\overset{K_2}{\rightleftharpoons}} FeL_2 \qquad \text{Eq.2}$$

$$FeL_1 + L_2 \underset{}{\overset{K}{\rightleftharpoons}} L_1 + FeL_2 \qquad \text{Eq.3}$$

The equilibrium constants K_1, K_2 and K can be written as follows:

$K_1 = [FeL_1] / [Fe][L_1]$

$K_2 = [FeL_2] / [Fe][L_2]$

$K = [L_1][FeL_2] / [FeL_1][L_2]$

If L_1 represents CP691 and L_2 represents DFO, then based on the exponential curve in Figure 3, at 50% relative fluorescence, the ratio of $[L_2]_{total} / [L_1]_{total} = 125$. As $[L_1]_{total} = [Fe]_{total} = 6$ μM, then $[L_2]_{total} = 750$ μM, and the point of 50% fluorescence occurs at $[L_1] = 3$ μM.

As $[L_1]_{total} = [L_1] + [FeL_1]$,

$[FeL_1]$ can be calculated by the equation $[FeL_1] = [L_1]_{total} - [L_1] = 3$ μM

As $[Fe]_{total}=[Fe]+[FeL_1]+[FeL_2]$ and the ligands are in excess and [Fe] is very low, $[FeL_2] \approx [Fe]_{total} - [FeL_1] = 3$ μM.

As $[L_2]_{total} = [L_2] + [FeL_2]$, so $[L_2] = [L_2]_{total} - [FeL_2] = 750-3 = 747$ μM.

Therefore, K = (3 μM x 3 μM)/(3 μM x 747 μM)=1 / 249

As $K = [L_1][FeL_2] / [FeL_1][L_2] = ([L_1](K_2[Fe][L_2]) / (K_1[Fe][L_1][L_2]) = K_2 / K_1$

At the condition of [L] = 10 μM, [Fe] = 1 μM and pH 7.4, [Fe] =2.5×10^{-27} μM when L = DFO (pFe^{3+} = 26.6),

Thus $K_2 = [FeL_2] / [Fe][L_2] = 1\ \mu M / (2.5 \times 10^{-27}\ \mu M \times 9\ \mu M)$

$K_1 = [FeL_1] / [Fe][L_1] = 1\ \mu M / ([Fe]_{L1} \times 9\ \mu M)$ (L_1=CP691)

So $[Fe]_{L1} = 1 / (9K_1) = 1 / (9 \times (K_2 / K)) = K / (9K_2) = (1 / 249) / (9 \times (1 / (9 \times 2.5 \times 10^{-27}\ \mu M))) = 1 \times 10^{-29}\ \mu M$

Therefore, pFe_{L1} = 29.0. In fact, the pFe_{L1} value can be calculated from any point on the exponential curve. The average value of the pFe calculated from the experimental ratio points is 28.8.