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Design and Synthesis of Multidentate 2-(2'-Hydroxyphenyl)-2-thiazolines for Biomedical Application†

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Abstract: A variety of multidentate ligands based on the 2-(2'-hydroxyphenyl)-2-thiazoline functionality have been synthesized by the formation of amide linkages at the carboxyl moiety in 2-(2'-hydroxyphenyl)-2-thiazoline-4-carboxylic acid **1** with a number of polyamines, using DCC and/or CDI activation methodologies.

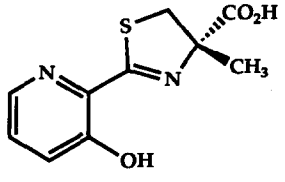


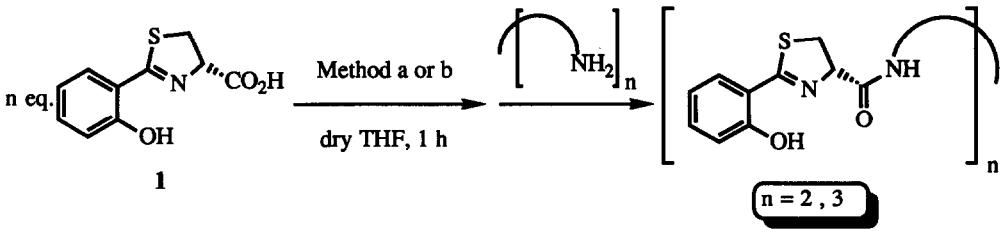
Figure 1. (S)-Desferriferrithiocin

Desferriferrithiocin [2-(3'-hydroxypyrid-2'-yl)-4-methyl-Δ^2-thiazoline-4(S)-carboxylic acid] is a chiral tridentate microbial siderophore isolated from *Streptomyces antibioticus*.[1] The tridentate mode of ligation involves the participation of the carboxylate moiety, as well as the phenolic oxygen and the thiazoline nitrogen.[2] The uniqueness of desferriferrithiocin amongst siderophores is that it is neither a catecholamide nor a hydroxamate. Nevertheless, it can sequester iron to form a 2/1 metal complex with rather high formation constants,[3] comparable to those of many hexadentate siderophores.[4]

In a continuation of our efforts to design and synthesize new and biomedically relevant multidentate ligands for complexation with group 13 metal ions -- Al, Ga, and In -- we have prepared several tripodal and bipodal multidentate ligands based on the 2-(2'-hydroxyphenyl)-2-thiazoline ligating moiety, using **1**, a synthetic analogue of desferriferrithiocin which is accessible in large quantities as a starting material.[5]

Upon close examination of desferriferrithiocin, it appeared likely that its carboxyl group could be utilized as a handle to increase its denticity. Explicitly, the carboxylate moiety could be linked to a polyamine backbone and the resulting amides, based on the chelate effect,[6] should show enhanced abilities to complex the metal, and,

† Abbreviations used: DCC, 1,3-dicyclohexylcarbodiimide; DCU, 1,3-dicyclohexylurea; CDI, 1,1'-carbonyldiimidazole; HOBT, 1-hydroxybenzotriazole; TREN, 1,1',1''-tris(aminoethyl)amine; TAME, 1,1',1''-tris(aminomethyl)ethane.



Scheme 1. Synthesis of 2-(2'-hydroxyphenyl)-2-thiazoline based polyamides. Method a: n eq. of DCC and HOBT; Method b: n eq. of CDI.

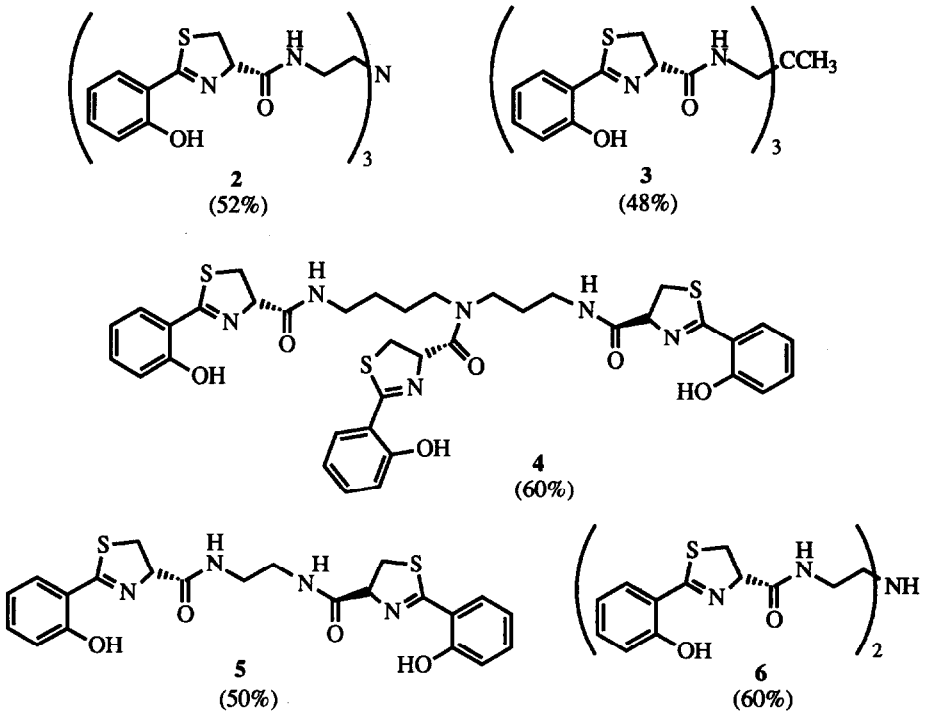


Figure 2. Multidentate 2-(2'-hydroxyphenyl)-2-thiazolines prepared according to Scheme 1.

as such, could serve as attractive models in the development of drugs for the treatment of patients suffering from iron-overload diseases.[7] Implicit in our thinking was the design feature that connecting three desferriferrithiocins into a trisamine backbone would sequester the metal ion in a hexacoordinate environment without the participation of the carboxylate moiety, while linking *two* desferriferrithiocins, might put to test the ability of the amide carbonyl groups to coordinate the metal and function (by a different mode of ligation) as hexadentate ligands. Therefore, the tripodal 2-(2'-hydroxyphenyl)-2-thiazoline ligands would form neutral complexes with trivalent metal ions of radiopharmaceutical interest such as ^{67}Ga and ^{111}In, whereas the bipodal analogues would form monocationic complexes. Given the significance of the overall charge of the metal complex in organ targetting and mobilization *in vivo*,[9] the neutral and the monocationic complexes with such trivalent metal ions would, in particular, be of great interest as potential brain and heart imaging agents respectively.

Compound **1**, an easily accessible synthetic analogue of desferriferrithiocin, was prepared by a modification of the procedure of Mathur[5a], *via* condensation of L-cysteine hydrochloride and 2-cyanophenol. The incorporation of the desferriferrithiocin analogue **1** into the backbone of various polyamines was carried out by the DCC methodology. In a typical work-up, the DCU byproduct was filtered at the end of the reaction, and the filtrate thus obtained was concentrated and chromatographed on silica gel. Into the eluant (varying amounts of EtOAc, MeOH, and/or CH_2Cl_2) was added 1% aqueous ammonium hydroxide in order to facilitate the separation of the polar polyamides by immobilizing the unreacted starting acid **1** at the base-line. In an attempt to improve the yields of **2** and **3**, CDI was used to form the activated ester; the yields of the products **2** and **3** thus obtained were considerably improved relative to those obtained by the DCC route. The yields of the polyamides **2-6**, prepared in gram quantities, ranged from good to moderate.

Comparison of selected ^1H NMR data (Table 1) reveals that the aromatic protons (H^a, $H^{a'}$, H^b, $H^{b'}$) in all compounds but **3** appear as a pair of multiplets. In the TAME based compound **3**, H^b and $H^{b'}$ separate into two multiplets, and H^c, for the first time, spreads out into a multiplet. These facts point to the greater rigidity of this tripodal compound as compared to **2** or **4**, presumably as a result of a lack of the pyramidal inversion[10] in the TAME backbone unlike the TREN and spermidine backbones in compounds **2** or **4**, respectively. Not surprisingly, in compound **4**, H^c appears as a pair of multiplets (2:1); thus indicating the non-equivalence of the three pendant arms due to the asymmetric backbone structure of spermidine.

To the best of our knowledge, the TREN based ligand **2** and the spermidine based ligand **4** are the only tripodal examples of such ligand systems. The latter could be considered as tris-thiazoline synthetic analogues of a siderophore such as vibriobactin or parabactin.[11] The tripodal hexadentate ligands **2** and **4** have been used to form metal complexes with Al, Ga, and In. All the physical data obtained so far (^1H NMR, ^{27}Al NMR, IR, FAB-MS, and EA) are consistent with neutral metal complexes in hexacoordinate environments. Importantly, these metal complexes should show enhanced stability constants (chelate effect), and, by virtue of their neutral charge, are far more lipophilic than their monoanionic desferrithiocin counterparts would be.[12]

Molecular modelling (Chem3D^{TM}) of the complexes with the bipodal ligands **5** and **6** revealed that the amide oxygen would be well within the bonding distance of a metal ion in a hexacoordinate environment;[13] hence they could act as hexadentate ligands to form monocationic complexes with the aforementioned trivalent metal ions of radiopharmaceutical interest. Such cationic metal complexes would, in particular, be of great interest as potential myocardial imaging agents. Should the binding between the amide oxygen of ligands **5** and

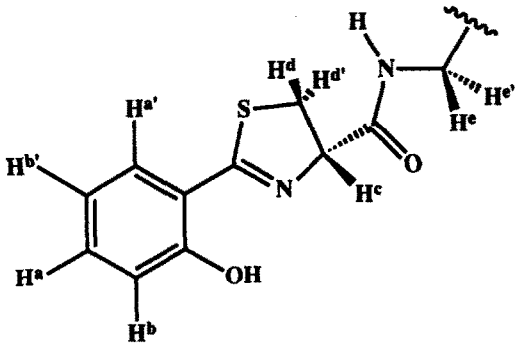


Table 1. Selected ^{1}H NMR Chemical Shifts (δ at 400 MHz in DMSO-d_6) of 2-(2'-Hydroxyphenyl)-2-thiazolines.

Entry	H^a, $H^{a'}$	H^b, $H^{b'}$	H^c	H^d, $H^{d'}$	H^e, $H^{e'}$
1	7.41-7.47 (m)	6.91-7.02 (m)	5.47 (dd) J= 12, 8 Hz	3.62-3.75 (m)	-
2	7.38-7.47 (m)	6.90-7.01 (m)	5.29 (t) J= 8 Hz	3.53-3.66 (m)	3.11-3.25 (m)
3	7.38-7.46 (m) 7.46-7.53 (m)	6.70-6.99 (m)	5.33-5.41 (m)	3.56-3.67 (m)	2.90-3.90 (m)
4	7.35-7.47 (m)	6.87-6.99 (m)	5.23-5.33 (m) 5.63-5.76 (m)	3.53-3.70 (m)	3.09-3.53 (m)
5	7.40-7.49 (m)	6.92-7.01 (m)	5.27 (t) J= 8 Hz	3.53-3.65 (m)	3.23-3.40 (m)
6	7.38-7.50 (m)	6.91-7.01 (m)	5.29 (t) J= 8 Hz	3.55-3.66 (m)	3.14-3.27 (m)

6 and the trivalent metal ion be weak or non-existent, the resulting metal complexes would present a coordinatively unsaturated metal environment. The lanthanide complexes of such ligands could hold promise as potential NMR imaging agents.

The metal complexation (with Al, Ga and In) and animal biodistribution studies (with Ga and In) of all the aforementioned compounds **1-6** are being pursued vigorously and the results will be reported in due course. In addition, the syntheses of analogous 2-(2'-hydroxyphenyl)-2-oxazoline based polyamides are currently being pursued *via* a different synthetic methodology.

EXPERIMENTAL SECTION

Materials and Methods.

All chemicals were reagent grade and were used as received: L-cysteine hydrochloride, HOBT, spermidine, CDI (Sigma), 2-cyanophenol, DCC, diethylene triamine, ethylene diamine, TREN (Aldrich). TAME was prepared according to a modified literature preparation.[14] THF was distilled from Na benzophenone ketyl under Ar prior to use. All reactions were carried out under N_2.

Instrumentation.

NMR spectra were recorded on a Bruker AC-200E (^{13}C NMR, 50 MHz), or a Bruker WH-400 (^{1}H NMR, 400 MHz). ^{1}H NMR data are reported as δ from TMS (external ref.) at 400 MHz in DMSO-d_6. Infrared spectra were recorded as KBr disks in the range 4000-400 cm^{-1} on a Perkin Elmer PE 783 spectrophotometer and were referenced to polystyrene. Mass spectra were obtained with a Kratos MS 50 (electron-impact ionization, EI), or an AEI MS 9 (fast-atom-bombardment ionization, FAB). Melting points were measured on a Mel-Temp apparatus and are uncorrected. Analyses for C, H, N, were performed in this department by Mr. Peter Borda.

2-(2'-Hydroxyphenyl)-2-thiazoline-4-carboxylic acid (1).

To a degassed solution of MeOH: 0.1 M phosphate buffer (1:1, 800 mL, pH~6) was added L-cysteine hydrochloride (30 g, 0.248 mol) and 2-cyanophenol (15 g, 0.126 mol) in one portion. The resulting mixture was stirred with heating (40 °C) for 3 days. After filtering the precipitate, MeOH was removed under reduced pressure, and the resulting solution cooled in an ice-bath. The pH of this solution was then adjusted to ~2.5 by addition of conc. ortho-phosphoric acid. The product was extracted into CH_2Cl_2, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford **1** (54%, 15.20 g), as a yellow solid, mp 116-117 °C.[15] ^{1}H NMR: 3.62-3.75 (m, 2H), 5.47 (dd, 1H, J = 12, 8 Hz), 6.91-7.02 (m, 2H), 7.41-7.47 (m, 2H), 12.46 (broad s, 1H), 13.14 (broad s, 1H).^{13}C NMR (δ at 50 MHz in DMSO-d_6): 33.52, 76.42, 115.70, 116.98, 119.43, 130.63, 133.82, 158.50, 171.39, 172.81. IR : 3430, 3220, 1715, 1620, 1590, 1400, 1255, 1220, 1060, 950, 750. MS (EI): *m/e* = 223 (M^+). Anal. Calcd for $C_{10}H_9NO_3S$: C, 53.80; H, 4.06; N, 6.27. Found: C, 53.67; H, 4.03; N, 6.35.

1,1',1''-Tris[[[2-(2'-hydroxyphenyl)-2-thiazolin-4-yl]carbonyl]-2-aminoethyl]amine (2).

A mixture of **1** (2.105 g, 9.04 mmol), and CDI (1.61 g, 9.94 mmol) in THF (150 mL) was refluxed for an hour, whereupon TREN (0.40 g, 2.47 mmol) was added and the resulting mixture was refluxed for 18 hours. The solvent was removed under reduced pressure, and the residue thus obtained was column chromatographed on silica gel (eluant: 5% MeOH in EtOAc; $R_f = 0.5$) to afford **2** (52%; 0.98 g), as a yellow solid, mp 125-127 °C. ^{1}H NMR: 2.59 (t, 6H, J = 8 Hz), 3.11-3.25 (m, 6H), 3.53-3.66 (m, 6H), 5.29 (t, 3H, J = 8 Hz), 6.90-7.01 (m, 6H), 7.38-7.47 (m, 6H), 8.15 (broad s, 3H), 12.10 (s, 3H). IR: 3280, 1660, 1620, 1590, 1485, 1250, 1220, 1030, 940, 750. Exact Mass Calcd for $C_{36}H_{39}N_7O_6S_3$: 761.2122; found: 761.2124. Anal. Calcd for $C_{36}H_{39}N_7O_6S_3.H_2O$: C, 55.44; H, 5.30; N, 12.57. Found: C, 55.37; H, 5.12; N, 12.48.

1,1',1''-Tris[[[2-(2'-hydroxyphenyl)-2-thiazolin-4-yl]carbonyl]aminomethyl]ethane (3).

A mixture of **1** (5.0 g, 22.4 mmol), and CDI (4.3 g, 26.9 mmol) in THF (300 mL) was refluxed for an hour, whereupon TAME (0.87 g, 7.4 mmol) was added and the resulting mixture was refluxed for 36 hours. The solvent was removed under reduced pressure, and the residue thus obtained column chromatographed on silica gel (eluant: 1% NH_4OH: 4% MeOH: 30% EtOAc: 65% Et_2O) to afford **3** (48%; 2.6 g), as an orange-yellow solid, mp 117-119 °C. ^{1}H NMR: 0.74 (s, 3H), 2.90-3.90 (m, 6H), 3.56-3.67 (m, 6H), 5.33-4.01 (m, 3H), 7.46-7.53 (m, 3H), 8.26-8.33 (m, 3H), 11.90 (s, 3H). IR: 3310, 1665, 1620, 1585, 1520, 1482, 1285, 1250, 1220, 1030, 950, 750. MS (FAB): m/e = 732±2 $[M+H]^+$. Anal. Calcd for $C_{35}H_{36}N_6O_6S_3$: C, 57.36; H, 4.95; N, 11.47. Found: C, 57.43; H, 5.10; N, 11.20.

1,5,10-Tris[[2-(2'-hydroxyphenyl)-2-thiazolin-4-yl]carbonyl]-1,5,10-triazadecane (4).

To a solution of **1** (2.0 g, 9 mmol) in THF (150 mL) was added HOBT (1.38 g, 9 mmol) and DCC (1.86 g, 9 mmol). The resulting mixture was stirred for an hour, whereupon 0.42 g of spermidine (2.9 mmol) was added and the reaction mixture was stirred for 18 hours. After cooling the resulting solution to 0 °C, the DCU precipitate was filtered, and the solvent removed under reduced pressure. The residue thus obtained was column chromatographed on silica gel (eluant: 1% NH_4OH: 9% MeOH: 90% EtOAc) to afford **4** (1.4 g, 60%), as a white solid, mp 84-86 °C. ^{1}H NMR: 0.81-2.01 (series of m, 10 H), 3.09-3.53 (partially obscured m, 4H), 3.53-3.70 (m, 6H), 5.23-5.33 (m, 2H), 5.63-5.76 (m, 1H), 6.87-6.99 (m, 4H), 7.35-7.47 (m, 4H), 8.21-8.35 (m, 2H), 12.10 (broad s, 3H). IR: 3310, 1660, 1620, 1590, 1520, 1485, 1290, 1250, 1220, 1030, 950, 750. MS (FAB): m/e = 761±2 $[M+H]^+$. Anal. Calcd for $C_{37}H_{40}N_6O_6S_3.0.5H_2O$: C, 57.71; H, 5.37; N,10.92. Found: C, 57.79; H, 5.25; N, 10.82.

1,2-Bis[[[2-(2'-hydroxyphenyl)-2-thiazolin-4-yl]carbonyl]amino]ethane (5).

To a solution of **1** (5 g, 22 mmol) in THF (350 mL) was added HOBT (3.43 g, 22 mmol) and DCC (4.63 g, 22 mmol). The resulting mixture was stirred for an hour, whereupon 0.64 g of ethylene diamine (11 mmol) was added and the reaction mixture was stirred for 18 hours. After cooling the resulting solution to 0 °C, the DCU precipitate was filtered, and the solvent removed under reduced pressure. The residue thus obtained was column chromatographed on silica gel (eluant: 1% NH_4OH: 4% MeOH: 95% EtOAc; $R_f = 0.41$) to afford **5** (2.60 g, 50%), as an off-white solid, mp 201-204 °C dec. ^{1}H NMR: 3.23-3.40 (partially obscured m, 4H), 3.53-3.65 (m, 4H), 5.27 (t, 2H, J = 8 Hz), 6.92-7.01 (m, 4H), 7.40-7.49 (m, 4H), 8.29 (broad s, 2H), 12.04

(s, 2H). ^{13}C NMR(δ at 50 MHz in DMSO-d_6): 33.91, 40.89, 78.05, 100.19, 115.98, 130.73, 133.66, 158.20, 169.51, 172.41. IR: 3280, 1655, 1620, 1595, 1560, 1480, 1250, 1215, 1030, 745. MS (FAB): *m/e* = 471±2 $[M+H]^+$. Anal. Calcd for $C_{22}H_{22}N_4O_4S_2$: C, 56.15; H, 4.71; N, 11.91. Found: C, 56.10; H, 4.84; N, 11.89.

N,N'-Bis[[[2-(2'-hydroxyphenyl)-2-thiazolin-4-yl]carbonyl]-2-aminoethyl]amine (6).

To a solution of **1** (5 g, 22 mmol) in THF (350 mL) was added HOBT (3.43 g, 22 mmol) and DCC (4.63 g, 22 mmol). The resulting mixture was stirred for an hour, whereupon 1.10 g of diethylene triamine (11 mmol) was added and the reaction mixture was stirred for 18 hours. After cooling the resulting solution to 0 °C, the DCU precipitate was filtered, and the solvent removed under reduced pressure. The residue thus obtained was column chromatographed on silica gel (eluant: 1% NH_4OH: 9% MeOH: 40% CH_2Cl_2: 50% EtOAc; $R_f = 0.36$) to afford **6** (2.91 g, 60%), as a yellow-orange solid, mp 48-50 °C. ^1H NMR: 2.63 (t, 4H, *J* = 6 Hz), 3.14-3.27 (m, 4H), 3.55-3.66 (m, 4H), 5.29 (t, 2H, *J* = 8 Hz), 6.91-7.01 (m, 4H), 7.38-7.50 (m, 4H), 8.40 (broad s, 2H). ^{13}C NMR (δ at 50 MHz in DMSO-d_6): 33.33, 40.47, 78.01, 100.19, 115.93, 116.95, 119.40, 137.0, 133.65, 158.21, 169.15, 172.31. IR: 3310, 1660, 1620, 1590, 1520, 1485, 1250, 1218, 1030, 945, 750. MS (FAB): *m/e* = 514±2 $[M+H]^+$. Anal. Calcd for $C_{24}H_{27}N_5O_4S_2.0.5H_2O$: C, 55.15; H, 5.40; N, 13.40. Found: C, 55.06; H, 5.34; N, 13.19.

Acknowledgment.

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7. Currently the drug of choice for the treatment of iron-overload diseases is desferrioxamine B, a hexadentate, trishydroxamate siderophore produced by large scale fermentation of a strain of *Streptomyces pilosus*; its major drawbacks are (a) relatively high cost, and (b) the required mode of administration, i.e., parenterally.[8]
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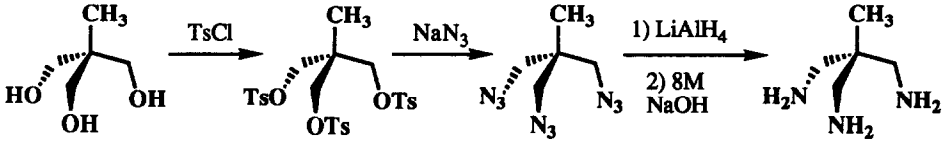
12. The lipophilicity of a radiopharmaceutical is an important feature in determining the degree of uptake and the subsequent fate of the compound. For example see Zhang, Z.; Lyster, D. M.; Webb, G. A.; Orvig, C. *Nucl. Med. Biol.* **1992**, *19*, 327.
13. Forced amide oxygen coordination to Gd^{3+} has been observed: Konings, M. S.; Dow, W. C.; Love, D. B.; Raymond, K. N.; Quay, S. C.; Rocklage, S. M. *Inorg. Chem.*, **1990**, *29*, 1488.
14. Fleischer, E. B.; Gebala, A. E.; Levey, A.; Tasker, P. A. *J. Org. Chem.*, **1971**, *36*, 3042. The sequence of reactions for preparation of TAME is as follows:



In the last step, it is crucial to use 8M NaOH, instead of 2M, to ensure good recovery of the trisamine.

15. The literature melting point for this compound is 269 °C.[5a] A careful perusal of their experimental section indicates that the authors may have isolated the sodium salt, which, according to reference 5c has a melting point of 219-220 °C.