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Synthesis of the Thiazoline-based Siderophore (*S*)-Desferrithiocin

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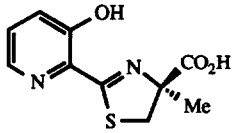
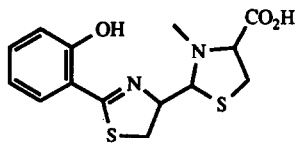
Abstract: A total synthesis of the new thiazoline-based siderophore desferrithiocin **1**, isolated from *Streptomyces antibioticus*, is described. Thus, a concise synthesis of (*S*)-2-methylcysteine hydrochloride **11** is first developed based on a modification of Seebach's "self-reproduction of chirality" protocol using the thiazolidine intermediate **8** derived from (*S*)-cysteine and pivaldehyde as a key intermediate. When a solution of (*S*)-2-methylcysteine hydrochloride is heated with 2-cyano-3-hydroxypyridine in the presence of triethylamine, (*S*)-desferrithiocin is produced in 97% yield. In a similar manner, use of (*R*)-2-methylcysteine in a cyclocondensation with 2-cyano-3-hydroxypyridine led to (*R*)-desferrithiocin, in a similar yield.

Desferrithiocin **1** is a unique and unusual naturally occurring ferric ion chelator, *i.e.* siderophore, which was first isolated in 1980 from *Streptomyces antibioticus*.[1] Whereas most siderophores use either catecholamide or hydroxamate ligands,[2] desferrithiocin binds to ferric ion through its thiazoline nitrogen centre and its phenolic and carboxylate oxygen atoms;[1,3] in this manner it is able to form strong 2:1 metal complexes with high formation constants.[4] Desferrithiocin has a similar affinity for iron as desferrioxamine, a compound which is currently used therapeutically in the treatment of disorders associated with iron overload *e.g.* thalassaemia.[5] It has therefore been suggested that desferrithiocin **1** and its analogues could have potential in iron-chelation therapy.[6]



(**1**)



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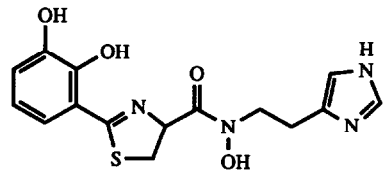
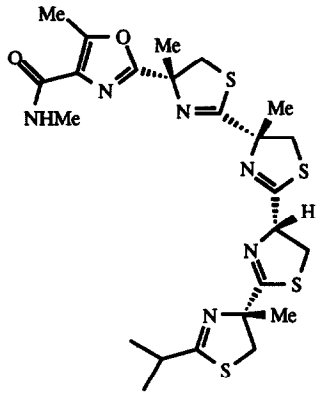


(**3**)

Thiazoline containing siderophores are somewhat rare, with pyochelin **2**[7] and anguibactin **3**[8] being the only other examples which have been fully characterised at this time. Here again desferrithiocin differs from **2** and **3** in that its biosynthesis ostensibly uses 2-methylcysteine **6**, in its (*S*)-form, as a starter unit rather than (*R*)-cysteine which presumably is used by pyochelin and anguibactin in their biosynthesis. Indeed, until 1990 desferrithiocin was the only example of a naturally occurring 4-methyl substituted Δ^2-thiazoline. Since this time however the novel family of linear fused 2,4-disubstituted poly-thiazoline/thiazole based tantazoles/mirabazoles *e.g.* tantazole A **4**[9] and thiangazole **5**[10] from blue green algae and from *Polyangium sp.* respectively, have been described. It is again significant that the biosyntheses of **4** and **5** ostensibly uses (*R*)-2-methylcysteine, rather than the (*S*)-optical antipode used by desferrithiocin.



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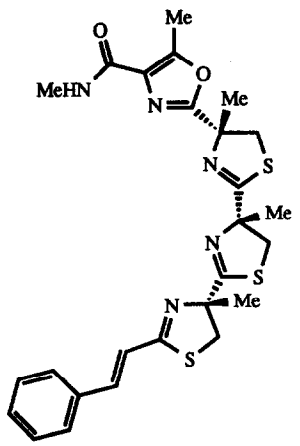
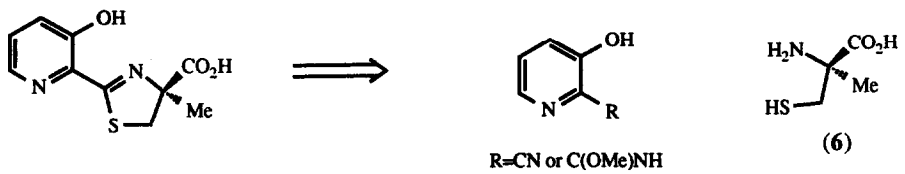


(5)

In connection with studies designed to probe and explore cellular recognition processes, and the ensuing transport phenomena, amongst siderophores and iron-complexed siderophores, we required ready access to desferrithiocin **1**, its enantiomer and some of its analogues. Hitherto no synthesis of desferrithiocin has been reported. This feature is no doubt associated with the fact that there is a dearth of suitable synthetic routes to (*S*)-2-methylcysteine **6**, a logical synthetic intermediate. In this paper we describe a facile synthesis of (*S*)-2-methylcysteine and the use of this amino acid in a concise synthesis of (-)-(*S*)-desferrithiocin, according to Scheme 1.

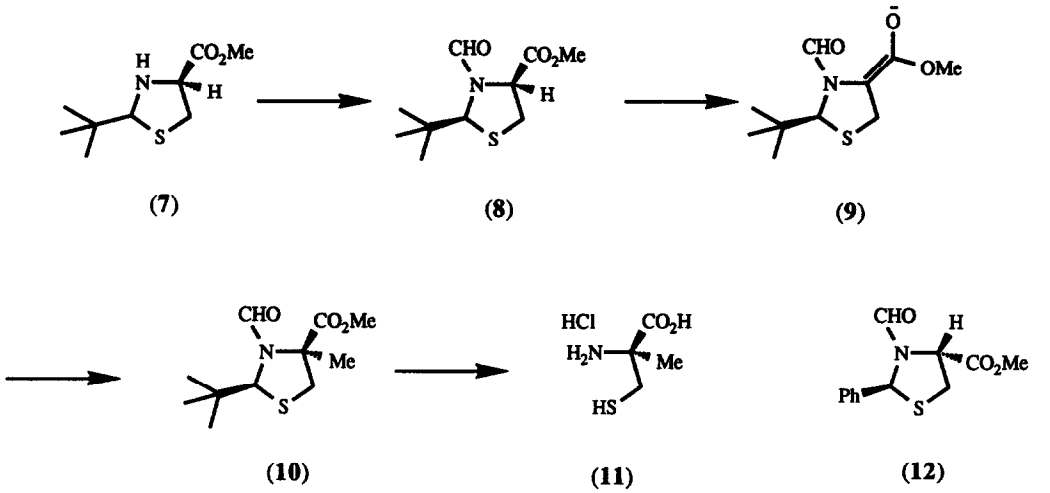


R=CN or C(OMe)NH

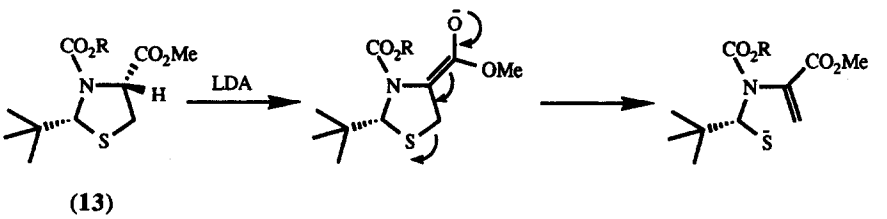
(6)

Scheme 1

We began our studies by first investigating a synthesis of (*S*)-2-methylcysteine **6**. Although Schöllkopf *et al.*[11] have applied their method based on metallation and alkylation of *bis*-lactim ethers derived from (*S*)-valine to synthesise S-benzyl and S-butyl (*S*)-2-methylcysteine esters, we were attracted to the possibility of preparing **6** using a modification of Seebach's "self-reproduction of chirality" protocol.[12] In this procedure we planned to first elaborate the thiazolidine **8** from (*S*)-cysteine methyl ester and pivaldehyde *via* **7**, and then to methylate the corresponding enolate **9** leading to the chiral C-4-methyl substituted thiazolidine **10** precursor to **11**.



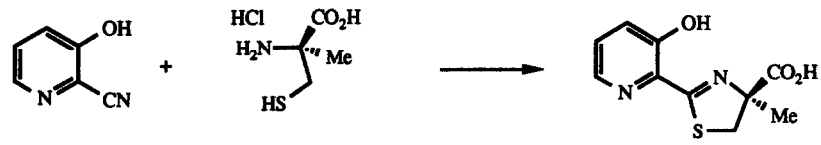
Thus, N-formylation of the thiazolidine **7** derived from (*S*)-cysteine methyl ester hydrochloride and pivaldehyde, using sodium formate in the presence of formic acid, first led to a single *syn*-diasteroisomer of the crystalline formate **8** in 87% yield. Treatment of **8** in tetrahydrofuran at -78°C with lithium diisopropylamide in the presence of DMPU, followed by quenching the resulting enolate **9** with iodomethane next produced the corresponding 4-methylthiazolidine **10** in 45% yield. In this methylation, the methyl group was delivered exclusively *anti*-to the bulky t-butyl group in (**9**); we were able to detect the co-formation of the diastereomer corresponding to **10** amongst the reaction product. A similar observation was made by us when using the C-4 (*R*)-enantiomer corresponding to **8**, where the stereochemistry of the methylation was established by X-ray crystallography of an amide derivative.[13] It is interesting that Seebach *et al.*[12] have earlier demonstrated that the N-*ester* derivative **13** undergoes facile β-elimination under similar conditions (Scheme 2), whereas the corresponding N-formyl thiazolidine **12** derived from cysteine and benzaldehyde fails to undergo C-4 alkylation following deprotonation and reaction of the resulting enolate with electrophiles.[14,15] It is clear that there are subtleties associated with the deprotonations and alkylations of substituted thiazolidines like **8**, **12** and **13** that merit further study. Having secured a practical synthesis of the methyl substituted thiazolidine **10**, all that now remained was to treat **10** with 5M hydrochloric acid which produced the hydrochloride **11** of (*S*)-2-methylcysteine as a clean white solid.



Scheme 2

Although there are a number of routes described in the literature for the synthesis of Δ^2-thiazolines,[16] our contemporaneous synthetic studies with the tantazole/mirabazole family of secondary metabolites, *viz* **4**,[17] had shown us that a practical route to such compounds is that involving the reaction between cysteine esters and

nitriles, *e.g.* **14**, or their corresponding imino ethers. The thiazoline ring in desferrithiocin proved to be no exception, and when a solution of the (*S*)-2-methylcysteine hydrochloride **11** was warmed with the pyridine nitrile **14**[18] in methanol in the presence of triethylamine, (*S*)-desferrithiocin **1** was secured in 97% yield. The synthetic desferrithiocin showed spectroscopic data which duplicated completely those data reported for the natural product; it also showed an optical rotation of $[\alpha]_D$ +29.9° (c 0.99, MeOH) consistent with the natural product, *i.e.* $[\alpha]_D$ +30.1 (c 1.01, MeOH). In a similar manner to that described for (*S*)-desferrithiocin, the (*R*)-enantiomer corresponding to **7** was elaborated to (*R*)-desferrithiocin.



(14) **(11)** **(1)**

The ready availability of desferrithiocin, its enantiomer, and some analogues, using the chemistry summarised in this paper has permitted us to carry out studies related to the chelation, metal transport and biological profiles of these novel thiazoline containing siderophores. The outcome of these studies will be published separately.

Experimental

4S-Methyl 2-tert-butyl-1,3-thiazolidine-4-carboxylate (**7**). - Triethylamine (4.8ml, 34mmol) was added dropwise over 5 min to a stirred suspension of (*S*)-cysteine methyl ester hydrochloride (5.4g, 31mmol) and trimethylacetaldehyde (3.7ml, 34mmol) in light petroleum (60ml). The resulting suspension was heated under reflux with continuous removal of water for 8h. The mixture was cooled and filtered, and the residue was then washed with ether. The filtrate was evaporated *in vacuo* to leave the thiazolidine (5.8g, 89%), as an oily 1.6:1 mixture of diastereomers; ν_{max}(film) 3316, 2954, 2868, 1743, 1477, 1436, 1365 and 1120 cm^{-1}; δ_H (270MHz; $CDCl_3$) major isomer, 4.47 (1H, s, C*H*C$(CH_3)_3$), 3.82 (1H, dd, *J* 9.9, 6.9 Hz, C*H*H), 3.79 (3H, s, CO_2CH_3), 3.26 (1H, dd, *J* 10.2, 6.9 Hz, CH*H*), 2.69 (1H, app. t, *J* ~10, C*H*CO_2CH_3), 1.08 (9H, s, C$(CH_3)_3$); minor isomer, 4.54 (1H, s, C*H*C$(CH_3)_3$), 4.15 (1H, t, *J* 5.9 Hz, C*H*CO_2CH_3), 3.76 (3H, s, CO_2CH_3), 3.16-3.00 (2H, m, CH_2), 0.99 (9H, s, C$(CH_3)_3$); δ_C (67.8MHz; $CDCl_3$) major isomer, 171.6 (CO), 81.7 (*C*HC$(CH_3)_3$), 65.2 (*C*HCO_2CH_3), 52.2 (OCH_3), 37.2 (CH_2), 33.8 (*C*$(CH_3)_3$), 26.8 (C(*C*$H_3)_3$); m/z (EI) 203 (M^+, 10%), 188 (19) 148 (63), 147 (85), 146 (100) and 144 (41).

2S,4S-Methyl 2-tert-butyl-1,3-thiazolidine-3-formyl-4-carboxylate (**8**). - Acetic anhydride (7.7ml, 81.4mmol) was added dropwise, over 1h, to a stirred solution of formic acid (40ml), (4*S*) methyl 2-tert-butyl-1,3-thiazolidine-4-carboxylate (5.5g, 27.0mmol) and sodium formate (2.0g, 30.0mmol) at 0-5°C. The solution was warmed to room temperature and then stirred overnight. The solvents were removed *in vacuo* and the residue was then carefully neutralised with $NaHCO_3$ solution and extracted with ether (3x25ml). The combined ether extracts were dried, and evaporated *in vacuo* to leave a white solid. Recrystallisation from petrol-ether gave the thiazolidine (5.4g, 87%) as white crystals, m.p. 78-80°C (8:1 mixture of conformers); $[\alpha]_D$ +128.9° (c 1.42 in $CHCl_3$); ν_{max} ($CHCl_3$) 2956, 1753, 1670 and 1364 cm^{-1}; δ_H (250MHz; $CDCl_3$) major conformer, 8.36 (1H, s, CHO), 4.90 (1H, app. t, *J* ~8.5 Hz, C*H*(CO_2CH_3)), 4.75 (1H, s, C*H*C$(CH_3)_3$), 3.75 (3H, s, CO_2CH_3), 3.30 (1H, dd, *J* 8.6, 11.5 Hz, CH_2), 3.29 (1H, dd, *J* 8.9, 11.5 Hz, CH_2), 1.04 (9H, s, C$(CH_3)_3$); δ_C (67.8MHz; $CDCl_3$) 170.0 (CO), 162.6 (CHO), 75.1 (*C*HC$(CH_3)_3$), 61.4 (*C*H(CO_2CH_3)), 52.6 (OCH_3), 38.5 (*C*$(CH_3)_3$), 32.8 (CH_2), 26.3 (C(*C*$H_3)_3$); m/z Found 231.0962; $C_{10}H_{17}NO_3S$ requires *M* 231.0929.

The corresponding 2*R*,4*R*-isomer showed $[\alpha]_D$ -130.4° (c 1.08 in $CHCl_3$) (Found: C, 52.2; H, 7.6; N, 6.2%. $C_{10}H_{17}NO_3$ requires C, 51.9; H, 7.4; N, 6.1%).

2S,4S-Methyl 2-tert-butyl-1,3-thiazolidine-3-formyl-4-methyl-4-carboxylate (**10**). - A solution of butyllithium (1.6M) in hexane (23ml, 37mmol) was added dropwise over 5 min to a stirred solution of diisopropylamine (7.4ml, 52.7mmol) in dry THF (165ml) at -78°C under a nitrogen atmosphere. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (24ml) was added in one portion, and the mixture was then stirred at -78°C for 1h. A solution of 2*S*,4*S*-methyl 2-tert-butyl-1,3-thiazolidine-3-formyl-4-carboxylate (8.12g, 35mmol) in THF (5ml) was added over 10 min, keeping the internal temperature at -90°C. The resulting solution was stirred for 0.75h at -90°C, and then iodomethane (2.6ml, 42mmol) was added dropwise over 5 min. The mixture was stirred for 2h at -90°C, and then warmed to room temperature. The solvents were evaporated *in vacuo* to leave an oily residue which was partitioned between brine (200ml) and ether (100ml). The separated aqueous layer was extracted with ether (3x300ml), and the combined ether extracts were then dried and evaporated *in vacuo* to leave an oil. The oil was purified by chromatography on silica gel, using 10% ethyl acetate-light petroleum as eluant, to give the thiazolidine (3.9g, 45%) which recrystallised from ether-light petroleum as white crystals, m.p. 51-52°C, $[\alpha]_D$ +98.5° (c 1.1 in $CHCl_3$); ν_{max} (film) 2957, 1741, 1673, 1393 and 1313 cm^{-1}; δ_H (250MHz; $CDCl_3$), (2.3:1 mixture of conformers); major conformer, 8.3 (1H, s, CHO), 4.72 (1H, s, CH), 3.78 (3H, s, OCH_3), 3.33 (1H, d, *J* 11.6 Hz, C*H*H), 2.76 (1H, d, *J* 11.6 Hz, CH*H*), 1.77 (3H, s, CH_3), 1.08 (9H, s, $C(CH_3)_3$); minor conformer, 8.43 (1H, s, CHO), 5.31 (1H, s, CH), 3.85 (3H, s, OCH_3), 3.66 (1H, d, *J* 12.4 Hz, CH*H*), 2.91 (1H, d, *J* 12.4 Hz, C*H*H), 1.82 (3H, s, CH_3), 0.98 (9H, s, $C(CH_3)_3$); δ_C (67.8MHz; $CDCl_3$) major conformer, 172.0 (CO), 161.1 (CHO), 74.3 (CH), 70.0 (CCO_2CH_3), 52.8 (OCH_3), 41.5 (CH_2), 39.4 ($C(CH_3)_3$), 26.7 ($C(CH_3)_3$), 20.6 (CH_3); minor conformer, 173.1 (s), 162.8 (d), 71.8 (d), 69.9 (s), 53.2 (q), 42.1 (t), 40.3 (s), 28.2 (q), 27.1 (q); m/z Found 245.1087; $C_{11}H_{19}NO_3S$ requires *M* 245.1086.
The corresponding 2*R*,4*R*-isomer showed $[\alpha]_D$ -100.2° (c 1.39 in $CHCl_3$) (Found: C, 53.8; H, 8.0; N, 5.9%. $C_{11}H_{19}NO_3S$ requires: C, 53.8; H, 7.8; N, 5.7%).

(S)-2-Methylcysteine hydrochloride (**11**). - 5M Hydrochloric acid (35ml) was added to 2*S*,4*S*-methyl-2-tert-butyl-1,3-thiazolidine-3-formyl-4-methyl carboxylate (2.28g, 9.3mmol) and the solution was then heated under reflux in an atmosphere of nitrogen for 3 days. The solution was washed with ethyl acetate (3x20ml), and then the aqueous layer was evaporated *in vacuo* to leave the hydrochloride salt (1.4g, 85%) as a white solid, m.p. 155-158°C (decomp.); $[\alpha]_D$ -8.07° (c 1.04 in H_2O); δ_H (270MHz; D_2O) 3.10 (1H, d, *J* 15.1 Hz, C*H*H), 2.82 (1H, d, *J* 15.1 Hz, CH*H*), 1.52 (3H, s, CH_3); δ_C (67.8MHz; D_20) 173.8 (CO), 62.3 ($C(CO_2H)$), 31.1 (CH_2), 22.1 (CH_3); m/z (FAB) 136 (MH^+-HCl, 100%).
The corresponding *R*-2-methylcysteine hydrochloride showed $[\alpha]_D$ +8.13° (c 1.58 in H_2O).

2-Cyano-3-hydroxypyridine (**14**). - The cyanopyridine was prepared (~45%) from the corresponding 2-iodopyridine using the method described by Broekman *et al.*[18] It showed m.p. 213-4°C (lit.[18] m.p. 211-2°), ν_{max} (KBr) 3424, 2238cm^{-1}; δ_H (270MHz; CD_3OD) 7.4-8.0 (1H, m, CH), 7.27-7.4 (2H, m, 2xCH) 4.95 (1H, broad, OH); δ_C (67.8MHz; CD_3OD) 159.2, 143.0, 129.8, 125.6, 122.0, 116.4; m/z Found: 120.0314; $C_6H_4N_2O$ requires *M* 120.0323.

(S)-Desferrithiocin. (**1**). - Triethylamine (116µl, 0.833mmol) was added to a solution of methyl (*S*)-2-methylcysteine hydrochloride (143mg, 0.833mmol)) and 2-cyano-3-hydroxypyridine (100mg, 0.833mmol) in methanol (5ml). The solution was heated under reflux for 24h in an atmosphere of nitrogen and then cooled to room temperature and diluted with ethyl acetate (20ml). The solution was extracted with water and the separated aqueous layer was then acidified to pH 2.0 with concentrated hydrochloric acid and again extracted with ethyl

acetate (2x15ml). Evaporation of the dried organic extracts left the siderophore (194mg, 97%) as a fawn powder. Recrystallisation from water-methanol or dichloromethane-hexane gave crystals, m.p. 130-1°C (lit.[1,6a] m.p. 90-2°C and 154°C); $[\alpha]_D$ +29.9° (c 0.99 in MeOH) (lit.[1] $[\alpha]_D$ +30.1° (c 1.01 in MeOH)); (Found: C, 49.9; H, 4.6; N, 11.7. $C_{10}H_{10}N_2O_3S$ requires C, 50.4; H, 4.2; N, 11.7%); υ_{max} (film) 3450 and 1734 cm^{-1}; δ_H (270MHz; $CDCl_3$) 11.21 (2H, broad, 2xOH), 8.22 (1H, d, *J* 3.8 Hz, CH), 7.31-7.42 (2H, m, 2xCH), 3.90 (1H, d, *J* 11.7 Hz, C*H*H), 3.27 (1H, d, *J* 11.7 Hz, CH*H*), 1.76 (3H, s, CH_3); δ_C (67.8MHz; $CDCl_3$) 177.3 (CO), 175.4 (C), 155.9 (C), 140.7 (CH), 133.4 (C), 127.7 (CH), 125.4 (CH), 83.4 (C), 39.2 (CH_2), 24.4 (CH_3); m/z Found 238.0407; $C_{10}H_{10}N_2O_3S$ requires *M* 238.0412.

(R)-Desferrithiocin was prepared using an identical procedure, and showed $[\alpha]_D$ -31.2° (c 5.0 in MeOH).

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