Preparation of Some Novel *S*-Nitroso Compounds as Potential Slow-release Agents of Nitric Oxide *in vivo*

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The optimum conditions for the preparation of *N*-acetyl-*S*-nitrosopenicillamine (SNAP) **3** were determined and applied to the synthesis of the corresponding *N*-formyl compound **7**. The nitrosation of penicillamine dipeptides was investigated and bis-thionitroso compounds **13**, **18**, **26** and **27** were isolated. *S,S'*-Dinitroso dithiol **13** showed biological activity akin to that of glyceryl trinitrate in decreasing systemic arterial blood pressure in anaesthetized rats and rabbits. Concomitant inhibition of collagen- or ADP-induced platelet aggregation was observed.

Nitric oxide has been shown to be an endogenous molecule of major importance in a variety of physiological processes,[1-3] particularly in the relaxation of vascular smooth muscle and the cytotoxic action of macrophages. It has also been found in certain brain cells.

The vascular smooth muscle of blood vessels relaxes when neurotransmitters bind to endothelium cells on the inner vessel wall. These cells then release a low-molecular-mass substance, referred to as endothelium-derived relaxing factor (EDRF), which activates the enzyme guanylate cyclase. This results in the formation of cyclic guanosine monophosphate (cGMP) which induces blood-vessel dilation. EDRF has been found to be identical with nitric oxide.[4-9]

Macrophages are activated when substances which stimulate the immune system, *e.g.* γ-interferon, transmit signals to macrophage cell nuclei. These signals cause production of nitric oxide synthase which acts on L-arginine to produce nitric oxide and L-citrulline. The resulting nitric oxide destroys tumour cells by inhibiting Krebs' cycle and electron-transport activity, as well as DNA synthesis.[10,11]

Nitric oxide synthase also occurs in neurons, cells which make up 15% of the brain. Binding of glutamate to the *N*-methyl-D-aspartate (NMDA) receptor causes calcium ions to enter neurons wherein the ions bind to calmodulin, thereby activating nitric oxide synthase. The resulting nitric oxide is capable of killing adjacent neurons, possibly explaining the neurotoxic effect of glutamate. Neuronal nitric oxide production also leads to formation of cGMP in adjacent nerve cells.[12,13]

The involvement of nitric oxide in these processes has significance for the understanding and treatment of cardiovascular diseases, such as hypertension and angina, as well as for neurodegenerative disorders such as stroke, Huntington's disease and Alzheimer's disease.

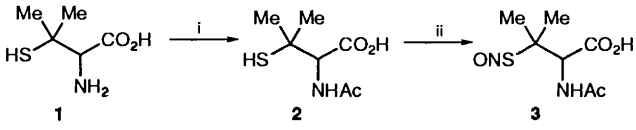
The activity of nitrovasodilators, such as sodium nitroprusside and glyceryl trinitrate, has been found to be related to the formation of *S*-nitrosothiols (thionitrites) *in vivo*.[14] It has been suggested that EDRF is more likely to be an *S*-nitrosothiol rather than free nitric oxide.[15] *N*-Acetylcysteine has been shown to potentiate the inhibition of platelet aggregation by vasodilators, a process which plays an important role in the pathogenesis of vascular disorders. This potentiation is attributed to the formation of *S*-nitrosocysteine *in vivo*. *S*-Nitrosocysteine is itself an inhibitor of platelet aggregation.[16] The stable *S*-nitrosothiol, *N*-acetyl-*S*-nitrosopenicillamine (SNAP),[17] has also been shown to inhibit platelet aggregation[18] and to have vasodilatory activity.[19] *S*-Nitrosothiol derivatives of some inhibitors of angiotensin I-converting enzyme, *e.g.* captopril, have also demonstrated vasodilatory and platelet aggregation inhibitory effects.[20] SNAP has also been shown to have a protective effect against intestinal damage induced by endotoxins.[21] *S*-Nitrosothiols would therefore appear to be important mediators in the biological activity of nitric oxide and could function as useful nitric oxide-releasing compounds.

S-Nitrosothiols can be generally prepared by the reaction of thiols with nitrosating agents.[21,22] They are mostly unstable and decompose to give disulfides and nitric oxide. The more stable examples tend to be those with bulky substituents, *e.g.* *tert*-butyl thionitrite,[23] triphenylmethyl thionitrite[24] and SNAP.[17] The functionality and unique stability of SNAP suggests that penicillamine derivatives should be good substrates for the synthesis of novel stable *S*-nitrosothiols. These should also be sufficiently water soluble to facilitate biological testing.

Penicillamine derivatives worthy of synthesis in this context would include simple α-amino derivatives, other than acetyl, with the ultimate aim of preparing the underivatized *S*-nitrosopenicillamine. Also of particular interest would be penicillamine dipeptides, as the *S,S'*-dinitrosodithiols formed from these should be capable of releasing two equivalents of nitric oxide per molecule with possible concomitant formation of a non-toxic cyclic disulfide. Our efforts in this context are detailed below.

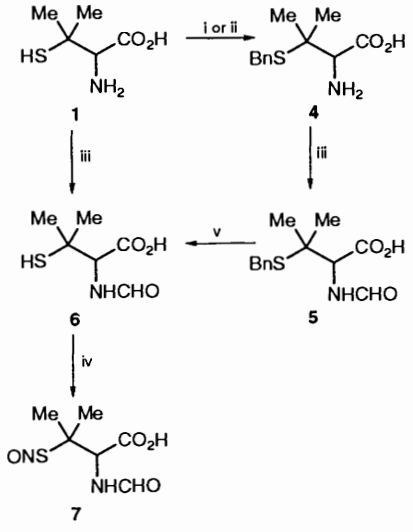
Results and Discussion

Repetition of the reported preparations of SNAP[17,25] was carried out. Penicillamine **1** was *N*-acetylated *via* a literature method, using acetic anhydride in an aqueous solution of sodium acetate and hydrobromic acid,[26] to give *N*-acetylpenicillamine **2** in low yield. Nitrosation of compound **2** with sodium nitrite in 1 mol dm^{-3} hydrochloric acid and methanol[17] gave the *S*-nitrosothiol **3**, as a green solid, in 79% yield (Scheme 1). Samples of SNAP **3** prepared in this manner could be fully characterized, giving UV, IR and NMR data as expected. Accurate mass measurements on the molecular ion could be obtained by using high-resolution FAB-MS but not by other techniques. Nitrosation of compound **2** with *tert*-butyl nitrite has also been reported[25] but this strategy was not found to be as efficient as the above method.



Scheme 1 i, Ac_2O, HBr, AcONa; ii, $NaNO_2$, 1 mol dm^{-3} HCl, MeOH, $H_2SO_{4(cat.)}$

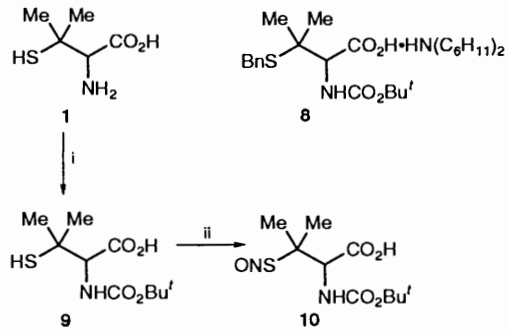
In order to determine whether the stability of SNAP would also be exhibited by other simple derivatives of penicillamine, the preparation of *N*-formylpenicillamine was undertaken. Protection of the penicillamine sulfydryl group appeared preferable prior to formylation. Attempts to protect the thiol as an acetamido sulfide were unsuccessful; however, penicillamine could be *S*-benzylated by using benzyl chloride in either liquid ammonia or in aq. sodium hydroxide (pH 8). *S*-Benzylpenicillamine **4** could be formylated using standard conditions (acetic anhydride in aq. formic acid)[27] and the resulting *N*-formyl derivative **5** was debenzylated with sodium in liquid ammonia to give *N*-formylpenicillamine **6** in 58–73% yield from penicillamine **1**. Standard formylation conditions could also be used directly on unprotected penicillamine **1** to give compound **6** in 78% yield. Nitrosation, using the conditions described above for the successful preparation of SNAP, gave the *S*-nitrosothiol **7**, as a stable green solid, which could be fully characterized, in 61% yield (Scheme 2).



Scheme 2 i, BnCl, $NH_{3(l)}$; ii, BnCl, $NaOH_{(aq.)}$ (pH 8); iii, Ac_2O, HCO_2H; iv, $NaNO_2$, 1 mol dm^{-3} HCl, MeOH, $H_2SO_{4(cat.)}$; v, Na, $NH_{3(l)}$; $NH_4Cl_{(aq)}$; H^+

Direct nitrosations of penicillamine **1** and cysteine have been reported, the products being thiiranecarboxylic acids formed *via* nitrosation of the α-amino group followed by intramolecular substitution of the resulting diazonium group by the adjacent thiol.[28] However, there is evidence to suggest that an *S*-nitrosothiol was the primary product in these reactions, followed by a slower S–N nitroso-migration step.[29] As the *S*-nitrosopenicillamine derivatives prepared above appeared to show reasonable stability to acid conditions, it was possible that *S*-nitrosopenicillamine might be isolable, as the free amino acid, from a precursor bearing an acid-labile α-amino protecting group, provided suitable conditions for the deprotection could be found. The standard *tert*-butoxycarbonyl (Boc) group was selected.

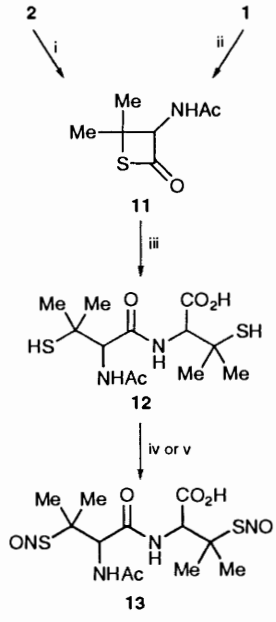
The Boc group could be introduced onto *S*-benzylpenicillamine **4** by using *S*-*tert*-butoxycarbonyl-2-mercapto-4,5-dimethylpyrimidine (Boc-S), provided that the product was isolated and purified as its dicyclohexylammonium salt **8**. Debenzylation of compound **8** could not be effected satisfactorily, however. Direct introduction of the Boc group could be carried out under standard conditions [$(Boc)_2O$, sodium hydrogen carbonate] to give α-Boc-penicillamine **9** in 88% yield. Compound **9** could be nitrosated, using sodium nitrite in 1 mol dm^{-3} hydrochloric acid and methanol, to give N^{α}-Boc-*S*-nitrosopenicillamine **10** (in 74% yield) as a stable solid which could be fully characterized (Scheme 3). Attempted



Scheme 3 i, Boc_2O, $NaHCO_{3(sat.)}$, DMF; ii, $NaNO_2$, 1 mol dm^{-3} HCl, MeOH, $H_2SO_{4(cat.)}$

removal of the Boc group from compound **10** with trifluoroacetic acid, 3 mol dm^{-3} hydrochloric acid, or anhydrous hydrogen chloride in methanol or diethyl ether resulted in rapid decomposition.

Penicillamine derivatives can be activated for peptide coupling by conversion into thietan-2-ones (propiothiolactones), further reaction of which with penicillamine gives penicillaminyl-penicillamine dipeptides.[30] The thietan-2-one **11** was prepared directly from penicillamine,[31] by reaction with acetic anhydride in pyridine, in modest yield. Reaction of compound **11** with penicillamine **1** in a chloroform–1 mol dm^{-3} aq. sodium hydroxide system gave *N*-(*N*-acetylpenicillaminyl)-penicillamine **12**, in 70% yield, as a 1:1 mixture of diastereoisomers. Nitrosation of this mixture using the sodium nitrite–hydrochloric acid–methanol conditions employed previously did not give an isolable product. However, nitrosation using sodium nitrite in acetic acid or *tert*-butyl nitrite in acetone gave the required *S*,*S*′-dinitrosodithiol **13** as a green resin in 100 or 73% yield respectively (Scheme 4). This material could be

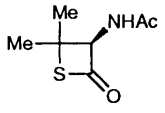


Scheme 4 i, Bu^tO_2CCl, Et_3N, $CHCl_3$; ii, Ac_2O, pyridine; iii, penicillamine **1**, $CHCl_3$, 1 mol dm^{-3} NaOH(aq); iv, $NaNO_2$, AcOH; v, Bu^tONO

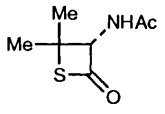
characterized by UV, IR and NMR but was found to be involatile at temperatures below which it decomposes and consequently a molecular ion mass spectral peak match could not be obtained.

Use of racemic penicillamine **1** in the reactions outlined above resulted in products **12** and **13** being obtained as mixtures of

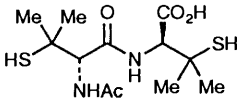
diastereoisomers. Use of homochiral D- or L-penicillamine would avoid this complication and possibly provide more stable forms of dithionitrite **13**. Thietan-2-ones **14** [≡(*R*)-**11**] and **15** [≡(*S*)-**11**] were prepared from D- and L-penicillamine, respectively. These were subsequently allowed to react to give *N*-(*N*-acetyl-D-penicillaminyl)-D-penicillamine **16** (≡D,D-**12**), and the L,L-enantiomer **17** (≡L,L-**12**), in 14 and 9% yield from the respective homochiral acids. Nitrosation of compounds **16** and **17** with sodium nitrite in acetic acid gave green resins which appeared to be *S*-nitrosothiols by UV and IR but which decomposed rapidly. Recrystallization of equimolar quantities of enantiomers **16** and **17** gave racemic *N*-(*N*-acetylpenicillaminyl)penicillamine consisting of a single diastereoisomer. Nitrosation of the racemate, using sodium nitrite in 1 mol dm^{-3} hydrochloric acid and dimethylformamide (DMF), gave the *S*,*S*′-dinitrosodithiol **18** (≡D,D-**13** + L,L-**13**) as a stable green solid in 40% yield. Compound **18** could be fully characterized.



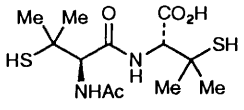
14 [≡(*R*)-**11**]



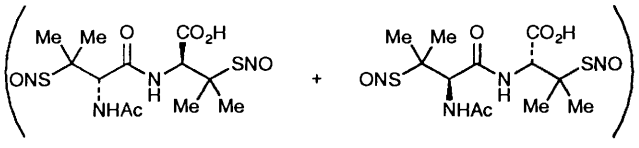
15 [≡(*S*)-**11**]



16 (≡D, D-**12**)

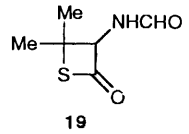


17 (≡L, L-**12**)

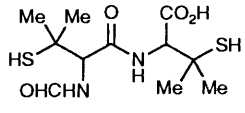


(D, D-**13**) **18** (L, L-**13**)

Synthesis of the *N*-formyl analogues of *S*-nitrosothiols **13** and **18** was also undertaken. The thietanone **19** could be prepared from *N*-formylpenicillamine **6** by dicyclohexylcarbodiimide (DCC)-induced cyclization, in 60% yield. Coupling reactions of compound **19** with penicillamine, using the chloroform–aq. sodium hydroxide conditions described above, gave the required *N*-(*N*-formylpenicillaminyl)penicillamine **20**, which could be obtained in 77% yield as 1:1 mixture of diastereoisomers following recrystallization from methanol–diethyl ether. A single pair of diastereoisomers could be isolated, in 31% yield, by repeated recrystallization from methanol. A one-step literature synthesis[30] of compound **20**, involving *in situ* preparation of the thietanone **19** by treatment of penicillamine with isobutyl chloroformate, was also carried out, but this method gave poor yields of product.

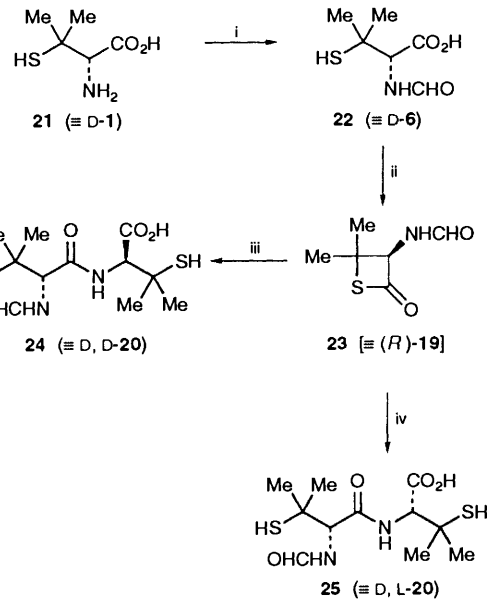


19



20

Subsequently a single isomer of each pair of diastereoisomers was prepared from D-penicillamine **21**. Formylation (to give compound **22**) and DCC cyclization of D-penicillamine **21** gave (3*R*)-3-formylamino-4,4-dimethylthietan-2-one **23** in 34% yield. Thietanone **23** was coupled with both D- and L-penicillamine to give the D,D- (**24**) and D,L- (**25**) *N*-(*N*-formylpenicillaminyl)-penicillamines, in 73 and 71% yield respectively (Scheme 5). Study of the NMR data of compounds **24** and **25** showed that

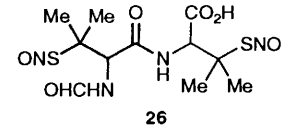


21 (≡D-**1**) **22** (≡D-**6**) **23** [≡(*R*)-**19**] **24** (≡D, D-**20**) **25** (≡D, L-**20**)

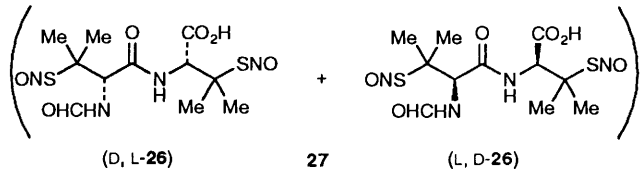
Scheme 5 i, Ac_2O, HCO_2H; ii, DCC, CH_2Cl_2; iii, D-penicillamine **21**, 1 mol dm^{-3} NaOH, $CHCl_3$; iv, L-penicillamine, 1 mol dm^{-3} NaOH, $CHCl_3$

the diastereoisomers isolated by recrystallization were the D,L and L,D pair.

Nitrosation of *N*-(*N*-formylpenicillaminyl)penicillamine, both as a mixture of diastereoisomers and as a racemic pair of enantiomers, gave stable *S*,*S*′-dinitrosodithiols, **26** and **27** respectively, as stable solids which could be fully characterized, both in 57% yield.



26



(D, L-**26**) **27** (L, D-**26**)

Biological Activity.—The *S*,*S*′-dinitrosodithiol **13** was found to induce dose-related decreases in systemic arterial blood pressure (MAP) in anaesthetized rats. The compound was less potent, by a factor of ~ 10, than sodium nitroprusside (NP) or glyceryl trinitrate (GTN) but induced a longer lasting response ($t_{\frac{1}{2}}$ ~ 3 min; NP, GTN $t_{\frac{1}{2}}$ ~ 1 min).

Compound **13** demonstrated an equal dose-related reponse on all tissues of a cascade composed of rabbit thoracic aortic strips and a final tissue of guinea pig trachea. The order of potency was: GTN > **13** > NP.

Compound **13** was found to be equipotent to GTN in inhibition of adenosine diphosphate (ADP)-induced platelet aggregation in human platelet-rich plasma, and more potent than GTN with respect to inhibition of collagen-induced aggregation in human washed platelets.

In anaesthetized rabbits, compound **13** caused a dose-related decrease in MAP and a corresponding increase in heart rate at doses between 100–300 nmol kg^{-1} min^{-1}. Concomitant inhibition of collagen- or ADP-induced platelet aggregation was exhibited at doses of 100 nmol kg^{-1} min^{-1} or above. Full inhibition was observed at 1000–3000 nmol kg^{-1} min^{-1}. An

infusion of 3000 nmol kg^{-1} min^{-1} caused a fall in blood pressure of ~ 25 mmHg. This returned to basal levels 5 min post infusion. Heart rate returned more slowly. Platelet aggregation *ex vivo* remained fully inhibited to both collagen and ADP challenge for 10 min and was still partially inhibited 20 min post infusion.

S,S'-Dinitrosodithiol **26** caused an equal dose-related reponse on a rabbit aortic strip cascade (4 tissues) but was ~ 30 times less potent than GTN or NP. Compound **26** caused an increase in cGMP levels in RFL-6 cells, the potency being ~ 0.5 that of NP.

Conclusions.—The *S*-nitroso compounds **8**, **13**, **18**, **26**/**27** based on the penicillamine core structure were synthesized and shown to be relatively stable. One compound (**13**) showed *in vitro* and *in vivo* properties consistent with release of free NO (*e.g.*, hypotensive effects in rats and rabbits) and in addition showed an interesting inhibition of platelet aggregation.

Studies on the design and synthesis of slow-release agents for NO are continuing in the Exeter laboratories.

Experimental

General.—M.p.s were determined on a capillary apparatus and are uncorrected. Optical rotations were measured using an Optical Activity AA-1000 polarimeter with a 2 dm pathlength cell, and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were carried out by Butterworth Laboratories Ltd. UV spectra were recorded on a Philips PU 8720 UV–VIS scanning spectrophotometer. IR spectra were recorded on a Perkin-Elmer 881 IR grating spectrometer. ^1H NMR spectra were recorded on a Hitachi Perkin-Elmer R-24B, a JEOL FX100, a Bruker AM250 or a Bruker AM300 spectrometer at 60, 100, 250 or 300 MHz respectively. ^{13}C NMR spectra were recorded on a Bruker AM250 or a Bruker AM300 at 63 or 76 MHz respectively. All chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (J) are quoted in Hz. High-resolution mass spectra were recorded at the SERC Mass Spectroscopy Centre, Swansea, or on a Kratos Profile HV3 mass spectrometer. Light petroleum refers to the fraction of boiling range 40–60 °C and was distilled before use, as was ethyl acetate. Dichloromethane was distilled from CaH_2 prior to use. Pyridine was distilled from KOH. Preparative column chromatography was performed using silica gel 60H (0.04–0.063 mm/230–400 mesh; Merck 9385). Compounds were dried *in vacuo* over P_2O_5. pH was determined using universal pH indicator paper. DMSO is dimethyl sulfoxide.

(±)-N-*Acetylpenicillamine*[26] **2**.—A suspension of penicillamine **1** (3.00 g, 0.020 mol), sodium acetate (2.24 g, 0.027 mol) and hydrobromic acid (1.62 cm^3 of 60% aq. solution, 0.020 mol) in water (10 cm^3) was kept for 15 min after which acetic anhydride (2.30 g, 0.023 mol) was added. The reaction mixture was stirred for 1 h and then was kept for 3 h. The resulting precipitate was collected by filtration and recrystallized from water to give the amide **2** as a crystalline solid (1.16 g, 30%), m.p. 190–192 °C (lit.,[26] 186–187 °C); ν_{max}(KBr)/cm^{-1} 3360 (NH), 2900 (CO_2H), 2530w (SH), 1700 (CO_2H) and 1620 (NHCO); δ_H(60 MHz; [$^2H_6$]DMSO) 1.46 (6 H, s, Me × 2), 2.00 (3 H, s, COMe), 2.60 (1 H, br s, SH), 4.50 (1 H, d, J 9, CH) and 8.08 (1 H, d, J 9, NH); δ_C(25 MHz; [$^2H_6$]DMSO) 26.36, 33.61, 33.96 (Me), 49.17 (CH), 65.38 (C), 173.55 and 175.19 (CO) [Found: ($M^+ + NH_4$), 209.0960. $C_7H_{13}NO_3S$ requires ($M + NH_4$), 209.0960].

(±)-N-*Acetyl*-S-*nitrosopenicillamine*[17] **3**.—A solution of sodium nitrite (606 mg, 8.78 mmol) in water (10 cm^3) was added, during 25 min, to a solution of (±)-*N*-acetylpenicillamine **2** (840 mg, 4.39 mmol) and hydrochloric acid (7 cm^3 of a 1 mol dm^{-3} solution) in methanol (10 cm^3). The resulting green solution was stirred for 15 min after which the precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the *S*-nitrosothiol **3** as a green solid with red reflections (762 mg, 79%), m.p. 152–154 °C (lit.,[17] 152–154 °C); λ_{max}(MeOH)/nm 350 (ε/dm^3 mol^{-1} cm^{-1} 500) and 595 (28); ν_{max}(KBr)/cm^{-1} 3440 (NH), 3344 (OH), 1920w (SNO), 1715 (CO_2H), 1612 (NHCO) and 1549 (NO); δ_H(100 MHz; CD_3OD) 1.95 (3 H, s, Me), 2.00 (3 H, s, Me), 2.01 (3 H, s, Me) and 5.31 (1 H, s, CH); δ_C(25 MHz; CD_3OD) 22.35, 25.98 and 27.26 (Me), 58.80 (CH), 61.25 (C), 131.35 and 173.17 (CO) [Found: ($M^+ + H$), 221.0596. Calc. for $C_7H_{12}N_2O_4S$: (M + H), 221.0596].

(±)-S-*Benzylpenicillamine* **4**.—*Method* 1. Benzyl chloride (0.890 g, 7.030 mmol) was added to a solution of (±)-penicillamine **1** (1.049 g, 7.030 mmol) in liquid ammonia (35 cm^3). The reaction mixture was stirred for 4 h after which the ammonia was allowed to evaporate off. Any remaining ammonia was removed under reduced pressure. The residual solid was suspended in water (100 cm^3) and the suspension was acidified to pH 3 by addition of acetic acid. The suspension was heated to boiling point, then was filtered, and the filtrate was allowed to cool to room temperature and was then stored at ~ 3 °C overnight. The resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the *sulfide* **4** as a crystalline solid (1.324 g, 79%), m.p. 201–202 °C; ν_{max}(KBr)/cm^{-1} 3061, 2553, 2058 (NH_3^+) and 1656 (CO_2^-); δ_H(60 MHz; CF_3CO_2D) 1.00 (3 H, s, Me), 1.50 (3 H, s, Me), 3.45 (2 H, s, CH_2) and 6.50–7.20 (5 H, m, Ph); δ_C(63 MHz; CF_3CO_2D) 31.93 and 35.24 (Me), 43.14 (CH_2), 57.00 (C), 70.04 (CH), 137.89 (ArCH), 138.03, 139.15 (ArCH × 2), 146.50 (ArC) and 179.78 (CO) [Found: ($M^+ + H$), 240.1058. $C_{12}H_{17}NO_2S$ requires (M + H), 240.1058].

Method 2. (±)-Penicillamine **1** (3.728 g, 0.025 mol) was added to 2 mol dm^{-3} aq. sodium hydroxide (10 cm^3, 0.020 mol) to give a solution with pH 9.8. Benzyl chloride (3.300 g, 0.026 mol) was added and the reaction mixture was stirred vigorously for 2.5 h. The resulting precipitates were collected by filtration, washed with water, and dried *in vacuo* to give the sulfide **4** as a powder (6.480 g, 100%), m.p. 210–212 °C; ν_{max} and δ_H as above.

(±)-S-*Benzyl*-N-*formylpenicillamine* **5**.—Acetic anhydride (1.25 cm^3) was added to a solution of (±)-*S*-benzylpenicillamine **4** (355 mg, 1.483 mmol) in formic acid (4 cm^3 of a 90% aq. solution). The reaction was stirred for 1 h at room temperature after which water (1 cm^3) was added and the solution was concentrated under reduced pressure. The resulting precipitate was recrystallized from aq. ethanol to give the *amide* **5** as a crystalline solid (349 mg, 88%), m.p. 150 °C; ν_{max}(KBr)/cm^{-1} 3311 (NH), 1697 (CO_2H) and 1639 (NHCO); δ_H(250 MHz; [$^2H_6$]DMSO) 1.32 (3 H, s, Me), 1.40 (3 H, s, Me), 3.85 (2 H, s, CH_2), 4.59 (1 H, d, J 9.5, CH), 7.12–7.38 (5 H, m, Ph), 8.11 (1 H, d, J 1, CHO) and 8.51 (1 H, dd, J 9.5 and 1, N*H*CHO); δ_C(63 MHz; [$^2H_6$]DMSO) 25.02 and 25.98 (Me), 32.21 (CH_2), 47.25 (C), 57.03 (CH), 126.76 (ArCH), 128.27, 129.04 (ArCH × 2), 137.42 (ArC), 160.96 (CHO) and 170.93 (CO) [Found: ($M^+ + H$), 268.1007. $C_{13}H_{17}NO_3S$ requires (M + H), 268.1007].

(±)-N-*Formylpenicillamine* **6**.—*From* (±)-S-*benzyl*-N-*formylpenicillamine* **5**. Sodium metal was added to a solution of (±)-*S*-benzyl-*N*-formylpenicillamine **5** (777 mg, 2.906 mmol) in liquid ammonia (12 cm^3) until a uniform blue solution was maintained for 1 min (~ 140 mg of sodium metal was required). The reaction was quenched by addition of ammonium chloride (500 mg) and the ammonia was allowed to evaporate off. The residue was dissolved in water (2.3 cm^3). The solution was acidified to pH 1 by addition of 6 mol dm^{-3} hydrochloric acid and was then kept overnight at ~ 3 °C. The resulting

precipitates were collected by filtration and washed with water to give the *thiol* **6** as a crystalline solid (430 mg, 83%), m.p. 142–145 °C; ν_{max}(KBr)/cm^{-1} 3319 (NH), 2569w (SH), 1706 (CO_2H) and 1621 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.37 (3 H, s, Me), 1.40 (3 H, s, Me), 2.85 (1 H, br s, SH) 4.46 (1 H, dd, *J* 9.5 and 1, C*H*NHCHO), 8.10 (1 H, dd, *J* 2 and 1, CHNHC*H*O) and 8.45 (1 H, br d, *J* 9.5, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 29.52 and 26.69 (Me), 44.99 (C), 59.83 (CH), 160.97 (CHO) and 170.69 (CO) [Found: (M^+ + H), 178.0538. $C_6H_{11}NO_3S$ requires (M + H), 178.0524].

From (±)-*penicillamine* **1**. Acetic anhydride (50 cm^3) was added dropwise (during *ca.* 45 min) to an ice-cooled solution of (±)-penicillamine **1** (4.496 g, 0.030 mol) in formic acid (100 cm^3 of a 90% aq. solution). The reaction mixture was stirred for 3 h at room temperature, after which the solvents were evaporated off. The residue was dissolved in methanol (200 cm^3) and water (50 cm^3) was added. The solution was concentrated under reduced pressure and the resulting suspension was acidified to pH 1 by addition of 6 mol dm^{-3} hydrochloric acid. The suspension was cooled to 0 °C for 1 h, after which the precipitate was collected by filtration and dried *in vacuo* to give the product **6** as a crystalline solid (4.142 g, 78%), m.p. 153–155 °C; ν_{max} and δ_H as above.

(±)-N-*Formyl*-S-*nitrosopenicillamine* **7**.—A solution of sodium nitrite (13 mg, 0.192 mmol) in water (0.20 cm^3) was added, over a period of 10 min, to a solution of (±)-*N*-formylpenicillamine **6** (17 mg, 0.096 mmol) in methanol (0.20 cm^3)–1 mol dm^{-3} hydrochloric acid (0.20 cm^3) containing a trace of conc. sulfuric acid (~ 1 mm^3). The reaction mixture was stirred for 20 min, after which methanol (0.20 cm^3) was added and the solution was concentrated under reduced pressure to ~ 0.20 cm^3. The resulting suspension was cooled to 0 °C for 30 min, after which the supernatant liquid was removed carefully and the remaining solid dried *in vacuo*. This gave the S-*nitrosothiol* **7** as a green crystalline solid with red reflections (12 mg, 61%), m.p. 125 °C (decomp.); λ_{max}(MeOH)/nm 342 (ε/dm^3 mol^{-1} cm^{-1} 500) and 598 (27); ν_{max}(KBr)/cm^{-1} 3356 (NH), 1936w (SNO), 1707 (CO_2H), 1622 (HNCO), 1513 (NO) and 687 (SN); δ_H(100 MHz; [$^2H_6$]DMSO) 2.00 (6 H, s, Me × 2), 5.20 (1 H, d, *J* 13, CH), 8.10 (1 H, d, *J* 1.5, CHO) and 8.84 (1 H, dd, *J* 13 and 1.5, CHN*H*CHO); δ_C(25 MHz; [$^2H_6$]DMSO) 29.17 and 30.16 (Me), 61.81 (CH), 61.99 (C), 165.01 (CHO) and 174.14 (CO_2H) [Found: (M^+ + H), 207.0440. $C_6H_{10}N_2O_4S$ requires (M + H), 207.0439].

(±)-S-*Benzyl*-N-(tert-*butoxycarbonyl*)*penicillamine Dicyclohexylammonium Salt* **8**.—A solution of Boc-S (887 mg, 3.690 mmol) in DMF (2 cm^3) was added to a solution of (±)-*S*-benzylpenicillamine **4** (738 mg, 3.082 mmol) and triethylamine (468 mg, 4.623 mmol) in water (2 cm^3). The reaction mixture was stirred at room temperature for 1 h, after which it was dissolved in water (15 cm^3), acidified, to pH 3, with dil. hydrochloric acid, and extracted with ethyl acetate (3 × 10 cm^3). The combined extracts were dried over sodium sulfate, filtered, and the solvent was evaporated. The residue was dissolved in ethanol (5 cm^3) and dicyclohexylamine (559 mg, 3.082 mmol) was added. The solvent was evaporated off and the residue was crystallized from diethyl ether–light petroleum to give a pale yellow solid, which was recrystallized from dichloromethane–light petroleum to give the *product* **8** as a pale yellow powder (970 mg, 60%), m.p. 152–153 °C (Found: C, 66.5; H, 8.9; N, 5.8; S, 6.5. $C_{29}H_{48}N_2O_4S$ requires C, 66.9; H, 9.3; N, 5.8; S, 6.2%); ν_{max}(KBr)/cm^{-1} 3299 (NH), 2590w ($R_2NH_2^+$), 1688 ($NHCO_2R$), 1616 (CO_2^-) and 1372 (CO_2^-); δ_H(250 MHz; $CDCl_3$) 1.01–2.12 (26 H, m, CH_2 × 10), 1.45 (9 H, s, Me × 3), 2.94 (2 H, tt, *J* 11.5 and 2.5, CH × 2), 3.86 (2 H, s, CH_2Ph), 4.11 (1 H, d, *J* 10, C*H*NHBoc), 5.58 (1 H, d, *J* 10, CHN*H*Boc), 7.09–7.41 (5 H, m, Ph) and 8.82 (2 H, br s, NH_2^+); δ_C(63 MHz; $CDCl_3$) 24.80 (CH_2 × 4), 25.14 (CH_2 × 2), 26.42 and 27.19 (Me), 28.48 (Me × 3), 29.03 (CH_2 × 4), 33.20 (CH_2), 49.34 (C), 52.53 (CH × 2), 63.13 (CH), 78.52 (C), 126.62 (ArCH), 128.24 and 129.20 (ArCH × 2), 138.41 (ArC), 155.75 and 174.15 (CO).

(±)-N-(tert-*Butoxycarbonyl*)*penicillamine* **9**.—A suspension of (±)-penicillamine **1** (634 mg, 4.25 mmol) and di-*tert*-butyl pyrocarbonate (1.218 g, 5.58 mmol) in DMF (10 cm^3) and saturated aq. sodium carbonate (10 cm^3) was stirred vigorously for 16 h. Water (30 cm^3) was added and the mixture was acidified, to pH 1, with 6 mol dm^{-3} hydrochloric acid. The solution was extracted with ethyl acetate (3 × 30 cm^3) and the extracts were dried over magnesium sulfate, filtered, and the solvent was evaporated off. The solid residue was triturated with light petroleum to give the *product* **9** as a powder (930 mg, 88%), m.p. 151–152 °C (Found: C, 48.1; H, 7.5; N, 5.8; S, 12.8. $C_{10}H_{19}NO_4S$ requires C, 48.2; H, 7.7; N, 5.8; S, 12.9%); ν_{max}(KBr)/cm^{-1} 3435 and 3306 (NH), 2579w (SH), 1704 and 1643 (CO); δ_H(100 MHz; [$^2H_6$]DMSO) 1.41 (15 H, s × 2, Me × 5), 2.57 (1 H, br s, SH), 4.10 (1 H, d, *J* 9, CH) and 6.41 (1 H, br d, *J* 9, NH); δ_C(25 MHz; [$^2H_6$]DMSO) 31.92 (Me × 3), 33.67 (Me × 2), 49.23 (CH), 66.84 (C × 2), 158.93 (CO) and 175.25 (CO).

(±)-N-(tert-*Butoxycarbonyl*)-S-*nitrosopenicillamine* **10**.—A solution of sodium nitrite (80 mg, 1.159 mmol) in water (1 cm^3) was added to a suspension of (±)-*N*-(*tert*-butoxycarbonyl)-penicillamine **9** (143 mg, 0.574 mmol) in methanol (1 cm^3)–1 mol dm^{-3} hydrochloric acid (1 cm^3) containing a trace quantity of sulfuric acid (~ 1 mm^3). The reaction mixture was stirred for 15 min, after which the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the S-*nitrosothiol* **10** as a green powder with red reflections (119 mg, 74%), m.p. 95–100 °C; λ_{max}(MeOH)/nm 342 (ε/dm^3 mol^{-1} cm^{-1} 628) and 595 (14); ν_{max}(KBr)/cm^{-1} 3392 (NH), 1711 (CO_2H), 1666 ($NHCO_2$), 1511 (NO) and 672 (SN); δ_H(250 MHz; [$^2H_6$]DMSO) 1.36 (9 H, s, Me × 3), 1.92 (3 H, s, Me), 2.00 (3 H, s, Me), 4.82 (1 H, d, *J* 10, CH) and 7.45 (1 H, br d, *J* 10, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 24.96 and 26.16 (CH_3), 27.98 (CH_3 × 3), 58.44 (C), 61.09 (CH), 78.65 (C) and 170.83 (CO × 2) [Found: (M^+ + H), 279.1015. $C_{10}H_{18}N_2O_5S$ requires (M + H), 279.1015].

3-*Acetamido*-4,4-*dimethylthietan*-2-*one* **11**.—*From* (±)-*penicillamine*[31] **1**. Acetic anhydride (4.023 g, 0.039 mol) was added, during 30 min, to an ice-cooled suspension of (±)-penicillamine **1** (2.462 g, 0.017 mol) in dry pyridine (10 cm^3). The reaction mixture was stirred for 14 h, after which it was dissolved in chloroform (100 cm^3), washed with dil. hydrochloric acid (3 × 50 cm^3), dried over magnesium sulfate, filtered, and evaporated. Trituration of the residue with light petroleum, followed by recrystallization from ethanol, gave the *product* **11** as a pale yellow crystalline solid (1.017 g, 35%), m.p. 128–130 °C; ν_{max}(KBr)/cm^{-1} 3254 and 3057 (NH), 1755 (SCO) and 1656 (NCO); δ_H(60 MHz; $CDCl_3$) 1.76 (3 H, s, Me), 1.97 (3 H, s, Me), 2.16 (3 H, s, Me), 5.79 (1 H, d, *J* 8, CH) and 7.45 (1 H, br d, *J* 8, NH); δ_C(63 MHz; $CDCl_3$) 22.42, 26.13 and 30.24 (CH_3), 51.19 (C), 76.41 (CH), 169.96 (NCO) and 193.35 (SCO) [Found: (M^+ + H), 174.0589. $C_7H_{11}NO_2S$ requires (M + H), 174.0589].

From (±)-N-*acetylpenicillamine* **2**.[30] Isobutyl chloroformate (149 mg, 1.091 mmol) was added to a solution of (±)-*N*-acetylpenicillamine **2** (139 mg, 0.727 mmol) in dry chloroform (5 cm^3), under nitrogen, at −10 °C. The reaction mixture was stirred at −10 °C for 15 min, and then at room temperature for 24 h, after which the solution was filtered and the solvent was evaporated

off to give a clear oil. This was subjected to silica gel chromatography, with ethyl acetate–light petroleum (2:3) as eluent, to give the product **11** as a solid (17 mg, 14%), m.p. 108–110 °C; ν_{max} and δ_H as above.

(±)-N-(N-*Acetylpenicillaminyl*)*penicillamine*[30] **12**.—A solution of 3-acetamido-4,4-dimethylthietan-2-one **11** (300 mg, 1.73 mmol) in chloroform (2 cm^3) and a solution of (±)-penicillamine **1** (259 mg, 1.74 mmol) in 1 mol dm^{-3} sodium hydroxide (2.59 cm^3) were stirred vigorously for 1.5 h. The aqueous layer was then separated and acidified, to pH 1, by addition of 0.5 mol dm^{-3} hydrochloric acid. The resulting precipitate was collected by filtration and recrystallized from methanol to give the product **12** as a crystalline solid which consisted of a 1:1 mixture of diastereoisomers (389 mg, 70%), m.p. 198–200 °C (lit.,[30] 205–206 °C); ν_{max}(KBr)/cm^{-1} 3304 and 3073 (NH), 2982 and 2939 (OH), 2560w (SH), 1723 (CO_2H) and 1638 (NHCO); δ_H(250 MHz; [$^2H_6$]DMSO) 1.34–1.42 (12 H, m, Me × 4), 1.96 (3 H, s, Ac), 2.76 (1 H, s, SH isomer a), 2.82 (1 H, s, SH isomer b), 2.92 (1 H, s, SH isomer a), 2.98 (1 H, s, SH isomer b), 4.37 (1 H, d, *J* 8, CH isomer a), 4.43 (1 H, d, *J* 9, CH isomer b), 4.47 (1 H, d, *J* 9, CH isomer a), 4.80 (1 H, d, *J* 10, CH isomer b), 7.91 (1 H, d, *J* 9, NH isomer a), 7.99 (1 H, d, *J* 10, NH isomer b), 8.13 (1 H, d, *J* 8, NH isomer a) and 8.24 (1 H, d, *J* 9, NH isomer b); δ_C(63 MHz; [$^2H_6$]DMSO) 28.34, 29.38, 29.65, 29.70 and 30.41 (CH_3), 44.76, 45.02 (C isomer a), 46.11, 46.21 (C isomer b), 60.27, 60.33 (CH isomer a), 61.57 and 61.71 (CH isomer b), 169.19 (CO), 169.32 (CO × 2), 169.42, 170.70 and 170.82 (CO) [Found: (M^+ + H), 323.1099. Calc. for $C_{12}H_{22}N_2O_4S_2$: (M + H), 323.1099].

(±)-(N-*Acetyl*-S-*nitrosopenicillaminyl*)-S-*nitrosopenicillamine* **13**.—*Method* 1. A solution of sodium nitrite (47 mg, 0.681 mmol) in water (0.75 cm^3) was added to a solution of (±)-*N*-(*N*-acetylpenicillaminyl)penicillamine **12** (104 mg, 0.322 mmol) in glacial acetic acid (7 cm^3). The reaction mixture was stirred for 15 min, after which it was dissolved in dichloromethane (20 cm^3). The solution was washed with brine (3 × 5 cm^3), dried over sodium sulfate, filtered, and evaporated to give the product **13** as a green resin, red under direct light, consisting of a 1:1 mixture of diastereoisomers (122 mg, 100%), λ_{max}(MeOH)/nm 343 (ε/dm^3 mol^{-1} cm^{-1} 714) and 597 (94); ν_{max}(CH_2Cl_2)/cm^{-1} 3688 (NH), 1758 (CO_2H), 1715 (CONH), 1680 (CONH), 1509 (NO) and 683 (SN); δ_H[250 MHz; $(CD_3)_2CO$] 1.92–2.17 (15 H, m, Me × 5), 5.39 (1 H, d, *J* 10, CH isomer a), 5.43 (1 H, d, *J* 10, CH, isomer b), 5.52 (1 H, d, *J* 10, CH isomer a), 5.57 (1 H, d, *J* 10, CH isomer b), 7.71 (1 H, d, *J* 10, NH) and 8.36 (1 H, d, *J* 10, NH); δ_C[63 MHz; $(CD_3)_2CO$] 20.47, 22.57, 25.60, 25.64, 25.84, 26.00, 26.91, 27.18, 27.25 and 27.46 (CH_3), 58.48, 58.56, 59.84 and 60.03 (C), 60.31 (CH × 2), 60.62 and 60.66 (CH), 170.07, 170.11, 170.60, 170.65, 171.09 and 172.39 (CO).

Method 2. *tert*-Butyl nitrite (1.170 g, 11.350 mmol) was added to a solution of (±)-*N*-(*N*-acetylpenicillaminyl)penicillamine **12** (216 mg, 0.670 mmol) in acetone (50 cm^3). The reaction mixture was stirred for 1 h, after which the solvent was evaporated off under reduced pressure to give the product **13** as a green resin (189 mg, 73%); δ_H as above.

(3R)-3-*Acetamido*-4,4-*dimethylthietan*-2-*one*[31] **14** [≡(R)-**11**].—Acetic anhydride (2.15 g, 21.0 mmol) was added, over a period of 30 min, at 0 °C to a suspension of D-penicillamine **21** (1.317 g, 8.83 mmol) in pyridine (8 cm^3). The reaction mixture was stirred at room temperature for 15 h, after which it was dissolved in chloroform (50 cm^3). The solution was washed with dil. hydrochloric acid (3 × 20 cm^3), dried over magnesium sulfate, filtered, and evaporated. The residue was triturated with light petroleum and the resulting solid was recrystallized from ethanol to give the product **14** as a crystalline solid (366 mg, 24%), m.p. 160–162 °C (lit.,[31] 158–160 °C); $[\alpha]_D^{25}$ −113 (*c* 0.16, $CHCl_3$); ν_{max} and δ_H as above.

N-(N-*Acetyl*-D-*penicillaminyl*)-D-*penicillamine* **16** (≡D,D-**12**).—A solution of (3*R*)-3-acetamido-4,4-dimethylthietan-2-one **14** (129 mg, 0.744 mmol) in chloroform (2 cm^3) and a solution of D-penicillamine **21** (111 mg, 0.744 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (1.11 cm^3) were mixed and stirred vigorously for 1 h at room temperature. The aqueous layer was then separated and the remaining chloroform layer washed with water (1 cm^3). The combined aqueous layers were neutralized with dil. hydrochloric acid and the water was removed by freeze-drying. The residue was recrystallized from water to give the *product* **16** as a powder (140 mg, 58%), m.p. 214–215 °C; $[\alpha]_D^{25}$ +8 (*c* 0.07, MeOH); ν_{max}(KBr)/cm^{-1} 3420, 3310 (NH), 2990 (OH), 2560w (SH), 1732 (CO_2H), 1660 (CONH) and 1635 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.37 (12 H, s × 4, Me × 4), 1.92 (3 H, s, Me), 2.53 (2 H, br m, SH × 2), 4.34 (1 H, d, *J* 8, CH), 4.74 (1 H, d, *J* 8, CH), 7.89 (1 H, d, *J* 8, NH), 8.11 (1 H, d, *J* 8, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 22.38 (CH_3), 29.39 (CH_3 × 3), 29.66 (CH_3), 44.79 and 46.21 (C), 60.36 and 61.74 (CH), and 169.26, 169.40 and 170.70 (CO) [Found: (M^+ + H), 323.1099. $C_{12}H_{22}N_2O_4S_2$ requires (M + H), 323.1099].

Nitrosation of N-(N-*Acetyl*-D-*penicillaminyl*)-D-*penicillamine* **16**.—A solution of sodium nitrite (11 mg, 0.166 mmol) in water (0.2 cm^3) was added to a solution of *N*-(*N*-acetyl-D-penicillaminyl)-D-penicillamine **16** (21 mg, 0.055 mmol) in glacial acetic acid (1 cm^3). The reaction mixture was stirred for 15 min, after which it was dissolved in dichloromethane (4 cm^3). The solution was washed with brine (3 × 2 cm^3), dried over sodium sulfate, filtered, and evaporated to give a green resin (18 mg, 81%), λ_{max}(MeOH)/nm 342 (ε/dm^3 mol^{-1} cm^{-1} 1151) and 595 (29); ν_{max}(CH_2Cl_2)/cm^{-1} 3687 (NH), 1758 (CO_2), 1713 (CONH), 1509 (NO) and 684 (SN). The material decomposed rapidly.

(3S)-3-*Acetamido*-4,4-*dimethylthietan*-2-*one* **15** [≡(S)-**11**].—Acetic anhydride (449 mg, 4.40 mmol) was added to a suspension of L-penicillamine (276 mg, 1.85 mmol) in pyridine (2 cm^3). The reaction mixture was stirred, under nitrogen, for 18 h, after which it was dissolved in dichloromethane (10 cm^3). The solution was washed with dil. hydrochloric acid (3 × 2 cm^3), dried over magnesium sulfate, filtered, and evaporated. The residue was triturated with light petroleum and the resulting solid was recrystallized from ethanol to give the *product* **15** as a crystalline solid (65 mg, 20%), m.p. 200 °C (decomp.); $[\alpha]_D^{25}$ +85 (*c* 2.6, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3253 and 3055 (NH), 1753 (SCO) and 1653 (NHCO); δ_H(100 MHz; $CDCl_3$) 1.65 (3 H, s, Me), 1.85 (3 H, s, Me), 2.03 (3 H, s, Me), 5.68 (1 H, d, *J* 8, CH) and 7.08 (1 H, br d, *J* 8, NH); δ_C(25 MHz; $CDCl_3$) 22.46, 26.20 and 30.30 (CH_3), 51.24 (C), 76.52 (CH) and 169.71 and 192.29 (CO) [Found: (M^+ + H), 174.0590. $C_7H_{11}NO_2S$ requires (M + H), 174.0590].

N-(N-*Acetyl*-L-*penicillaminyl*)-L-*penicillamine* **17** (≡L,L-**12**).—A solution of (3*S*)-3-acetamido-4,4-dimethylthietan-2-one **15** (192 mg, 1.108 mmol) in chloroform (2 cm^3) and a solution of L-pencillamine (165 mg, 1.108 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (1.65 cm^3) were stirred together vigorously for 2 h. The aqueous layer was separated and acidified, to pH 1, by addition of dil. hydrochloric acid. The resulting suspension was cooled to 0 °C for several hours after which the precipitate was collected by filtration and recrystallized from ethanol–water to give the *product* **17** as a crystalline solid (148 mg, 46%), m.p. 200–201 °C; $[\alpha]_D^{23}$ −9 (*c* 0.35, MeOH); ν_{max}(KBr)/cm^{-1} 3402 and 3315 (NH), 2932 (OH), 2555w (SH), 1730 (CO_2) and 1660 and 1625 (CONH); δ_H(250

MHz; [$^2H_6$]DMSO) 1.36 (3 H, s, Me), 1.37 (6 H, s, Me × 2), 1.39 (3 H, s, Me), 1.95 (3 H, s, Ac), 2.76 (2 H, br m, SH × 2), 4.36 (1 H, d, *J* 7, CH), 4.74 (1 H, d, *J* 7, CH), 7.93 (1 H, d, *J* 7, NH) and 8.11 (1 H, d, *J* 7, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 22.37 (CH_3), 29.38 (CH_3 × 2), 29.64 (CH_3 × 2), 44.77 and 46.21 (C), 60.34 and 61.73 (CH) and 169.28, 169.41 and 170.70 (CO) [Found: (M^+ + H), 323.1100. $C_{12}H_{22}N_2O_4S_2$ requires (M + H), 323.1099].

N-(N-*Acetylpenicillaminyl*)*penicillamine* (D,D *and* L,L *Racemate*) **16**/**17**.—Equimolar quantities of *N*-(*N*-acetyl-D-penicillaminyl)-D-penicillamine **16** (46 mg, 0.143 mmol) and *N*-(*N*-acetyl-L-penicillaminyl)-L-penicillamine **17** (46 mg, 0.143 mmol) were mixed and the mixture was recrystallized from methanol to give the racemate **16**/**17** as a crystalline solid (81 mg, 88%), m.p. 195–197 °C; ν_{max}(KBr)/cm^{-1} 3409 and 2982 (NH), 2929 (OH), 2559w (SH), 1722 (CO_2) and 1642 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.36 (3 H, s, Me), 1.37 (3 H, s, Me), 1.38 (3 H, s, Me), 1.42 (3 H, s, Me), 1.92 (3 H, s, Ac), 3.30 (2 H, br m, SH × 2), 4.36 (1 H, d, *J* 9, CH), 4.74 (1 H, d, *J* 9, CH), 7.92 (1 H, d, *J* 9, NH) and 8.11 (1 H, d, *J* 9, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 22.37 (CH_3), 29.36 (CH_3 × 2), 29.41 and 29.65 (CH_3), 44.75 and 46.23 (C), 60.31 and 61.73 (CH) and 169.26, 169.42 and 170.71 (CO) [Found: (M^+ + H), 323.1100. Calc. for $C_{12}H_{22}N_2O_4S_2$: (M + H), 323.1099].

N-(N-*Acetyl*-S-*nitrosopenicillaminyl*)-S-*nitrosopenicillamine* (D,D *and* L,L *Racemate*) **18**.—A solution of sodium nitrite (18 mg, 0.172 mmol) in water (1.0 cm^3) was added to a solution of *N*-(*N*-acetylpenicillaminyl)penicillamine (D,D and L,L racemate) **16**/**17** (22 mg, 0.068 mmol) in DMF (0.6 cm^3) and 1 mol dm^{-3} hydrochloric acid (0.6 cm^3) containing a trace of conc. sulfuric acid (~1 mm^3). The reaction mixture was stirred for 15 min, after which the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the *S*,*S*′-dinitrosodithiol **18** as a green powder with red reflections (10 mg, 40%), m.p. 130 °C (decomp.); λ_{max}(MeOH)/nm 343 (ε/dm^3 mol^{-1} cm^{-1} 1251) and 597 (22); ν_{max}/cm^{-1} (KBr) 3319 (NH), 1724 (CO_2), 1657 (CONH), 1500 (NO) and 669 (SN); δ_H(250 MHz; [$^2H_6$]DMSO) 2.36 (3 H, s, Me), 2.48 (3 H, s, Me), 2.53 (3 H, s, Me), 2.55 (3 H, s, Me), 2.57 (3 H, s, Me), 5.10 (1 H, d, *J* 10, CH), 5.38 (1 H, d, *J* 10, CH), 8.24 (1 H, d, *J* 10, NH), and 8.89 (1 H, d, *J* 10, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 27.42, 29.94, 30.53, 31.44 and 32.25 (CH_3), 63.03 (C), 63.60 and 64.94 (CH), 65.25 (C) and 174.19, 174.51 and 175.36 (CO); *m*/*z* (FAB) 381 (MH^+).

(±)-3-*Formamido*-4,4-*dimethylthietan*-2-*one* **19**.—A solution of (±)-*N*-formylpenicillamine **6** (1.223 g, 6.901 mmol) and DCC (1.424 g, 6.901 mmol) in dichloromethane (40 cm^3) was stirred at room temperature for 23 h, after which the reaction mixture was filtered and the filtrate was evaporated. The residue was subjected to silica gel chromatography, with ethyl acetate–light petroleum (2:3) as eluent, to give the *thietanone* **19** as a crystalline solid (654 mg, 60%), m.p. 82–85 °C; ν_{max}(KBr)/cm^{-1} 3324 (NH), 1746 (SCO) and 1659 (NHCO); δ_H(100 MHz; $CDCl_3$) 1.64 (3 H, s, Me), 1.82 (3 H, s, Me), 5.63 (1 H, d, *J* 8.5, CH), 7.53 (1 H, br dd, *J* 8.5 and 1.5, NH) and 8.13 (1 H, d, *J* 1.5, CHO); δ_C(25 MHz; $CDCl_3$) 26.20 and 30.24 (CH_3), 50.77 (C), 74.88 (CH), 160.64 (CHO) and 191.18 (CO) [Found: (M^+ + H), 160.0430. $C_6H_9NO_2S$ requires (M + H), 160.0432].

(±)-N-(N-*Formylpenicillaminyl*)*penicillamine* **20**.—A solution of (±)-3-formamido-4,4-dimethylthietan-2-one **19** (190 mg, 1.193 mmol) in chloroform (1.5 cm^3) and a solution of (±)-penicillamine **1** (178 mg, 1.193 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (1.78 cm^3) were stirred together vigorously for 1.5 h. The aqueous layer was then separated and acidified, to pH 1, by addition of 2 mol dm^{-3} hydrochloric acid. The resulting suspension was cooled to 0 °C for several hours, after which time the precipitate was collected by filtration and was recrystallized from methanol–diethyl ether to give the *product* **20** as a crystalline solid consisting of a (1:1) mixture of diastereoisomers (284 mg, 77%), m.p. 158–164 °C; ν_{max}(KBr)/cm^{-1} 3286 (NH, OH), 2560w (SH), 1725 (CO_2), 1685 (CONH) and 1637 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.26–1.49 (12 H, m, Me × 4), 2.75 (1 H, br s, SH), 2.79 (1 H, br s, SH), 4.36 (1 H, d, *J* 9.5, CH isomer a), 4.41 (1 H, d, *J* 9.5, CH isomer b), 4.80 (1 H, d, *J* 9.5, CH isomer a), 4.89 (1 H, d, *J* 9.5, CH isomer b), 8.07 (1 H, m, CHO), 8.21–8.42 (1 H, m, NH) and 12.60 (1 H, br s, CO_2H); δ_C(63 MHz; [$^2H_6$]DMSO) 28.41, 29.21 and 29.36 (CH_3), 29.55 (CH_3 × 2), 29.64, 29.82 and 30.48 (CH_3), 44.75, 44.94, 46.10 and 46.22 (C), 58.54 and 58.65 (CH), 61.75 (CH × 2), 160.78 and 160.97 (CHO) and 168.97, 169.06, 170.63 and 170.76 (CO) [Found: (M^+ + H), 309.0924. $C_{11}H_{20}N_2O_4S_2$ requires (M + H), 309.0943].

N-(N-*Formylpenicillaminyl*)*penicillamine* **20** (*Single Diastereoisomer*).—A solution of (±)-3-formamido-4,4-dimethylthietan-2-one **19** (654 mg, 4.108 mmol) in chloroform (6 cm^3) and a solution of (±)-penicillamine **1** (597 mg, 4.002 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (6 cm^3) were stirred together vigorously for 2 h. The aqueous layer was separated and acidified, to pH 1, by addition of 1 mol dm^{-3} hydrochloric acid. The resulting precipitate was collected by filtration and was twice recrystallized from methanol to give the product **20** as a crystalline solid consisting predominantly of a *single diastereoisomer* (387 mg, 31%), m.p. 162–166 °C; ν_{max}(KBr)/cm^{-1} 3440 and 3280 (NH, OH), 2500w (SH), 1726 (CO_2), 1686 (CONH) and 1639 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.21–1.55 (12 H, m, Me × 4), 2.70–2.82 (2 H, br m, SH × 2), 4.42 (1 H, d, *J* 10, CH), 4.89 (1 H, d, *J* 10, CH), 8.08 (1 H, d, *J* 1, CHO), 8.34 (1 H, dd, *J* 10 and 1, NH) and 12.70 (1 H, br s, CO_2H); δ_C(63 MHz; [$^2H_6$]DMSO) 28.39, 29.54, 29.81 and 30.48 (CH_3), 44.93 and 46.10 (C), 58.49 and 61.73 (CH), 160.70 (CHO), 166.98 (CO) and 170.76 (CO) [Found: (M^+ + H), 309.0940. $C_{11}H_{20}N_2O_4S_2$ requires (M + H), 309.0943].

N-*Formyl*-D-*penicillamine* **22** (≡D-**6**).—Acetic anhydride (7.7 cm^3) was added to an ice–water-cooled solution of D-penicillamine **21** (1.601 g, 0.011 mol) in formic acid (23 cm^3 of 98% aq. solution) such that the reaction temperature was kept below 10 °C. The reaction mixture was then stirred at room temperature for 1.5 h, after which water (10 cm^3) was added and the solution was concentrated under reduced pressure. Extra water (5 cm^3) was added to the concentrated solution, which was then further concentrated to ~3 cm^3, after which the resulting suspension was kept at ~3 °C overnight. The resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the *amide* **22** as a crystalline solid (1.22 g, 63%), m.p. 143–146 °C (Found: C, 40.8; H, 6.1; N, 7.9; S, 17.4. $C_6H_{11}NO_3S$ requires C, 40.7; H, 6.3; N, 7.9; S, 18.1%); $[\alpha]_D^{29}$ +6.3 (*c* 0.285, MeOH); ν_{max}(KBr)/cm^{-1} 3295 (NH, OH), 2503w (SH), 1699 (CO_2) and 1614 (CONH); δ_H(300 MHz; [$^2H_6$]DMSO) 1.37 (3 H, s, Me), 1.42 (3 H, s, Me), 2.84 (1 H, br s, SH), 4.46 (1 H, d, *J* 10, CH), 8.11 (1 H, d, *J* 1.5, CHO), 8.45 (1 H, dd, *J* 10 and 1.5, NH) and 12.84 (1 H, br s, CO_2H); δ_C(75 MHz; [$^2H_6$]DMSO) 29.58 and 29.75 (CH_3), 45.06 (C), 59.88 (CH), 161.05 (CHO) and 170.79 (CO).

(3R)-3-*Formamido*-4,4-*dimethylthietan*-2-*one* **23** [≡(*R*)-**19**].—A suspension of *N*-formyl-D-penicillamine **22** (342 mg, 1.930 mmol) and DCC (398 mg, 1.930 mmol) in dry dichloromethane (40 cm^3) was stirred for 20 h under nitrogen. The reaction mixture was then filtered and the filtrate was evaporated. The residue was suspended in ethyl acetate–diethyl

ether (1:2; 50 cm^3), the mixture was filtered, and the filtrate was evaporated. The resulting oil was subjected to silica gel chromatography, with ethyl acetate–light petroleum (1:1) as eluent, to give the thietanone **23** as a crystalline solid (165 mg, 54%), m.p. 63–65 °C; $[\alpha]_D^{28}$ −83.4 (*c* 1.65, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3282 (NH), 1747 (SCO) and 1666 (CONH); δ_H(300 MHz; $CDCl_3$) 1.64 (3 H, s, Me), 1.83 (3 H, s, Me), 5.70 (1 H, d, *J* 8, CH), 7.26 (1 H, br d, *J* 8, NH) and 8.16 (1 H, s, CHO); δ_C(75 MHz; $CDCl_3$) 26.23 and 30.23 (CH_3), 50.83 (C), 74.77 (CH), 160.60 (CHO) and 191.41 (CO) [Found: (M^+ + NH_4), 177.0698. $C_6H_9NO_2S$ requires (M + NH_4), 177.0698].

N-(N-*Formyl*-D-*penicillaminyl*)-D-*penicillamine* **24** (≡D,D-**20**).—A solution of (3*R*)-3-formamido-4,4-dimethylthietan-2-one **23** (74 mg, 0.46 mmol) in chloroform (1.5 cm^3) and a solution of D-penicillamine **21** (69 mg, 0.46 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (0.69 cm^3) were stirred together vigorously for 2.5 h. The aqueous layer was separated and acidified, to pH 1, with 2 mol dm^{-3} hydrochloric acid. The resulting suspension was kept overnight at ~0 °C, after which the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the product **24** as a crystalline solid (103 mg, 73%), m.p. 95–100 °C: $[\alpha]_D^{29}$ +24.7 (*c* 0.46, MeOH); ν_{max}(KBr)/cm^{-1} 3315 (NH, OH), 2563w (SH), 1721 (CO_2) and 1643 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.34 (3 H, s, Me), 1.37 (3 H, s, Me), 1.39 (3 H, s, Me), 1.40 (3 H, s, Me), 2.74 (1 H, s, SH), 2.99 (1 H, s, SH), 4.37 (1 H, d, *J* 9, CH), 4.82 (1 H, d, *J* 9, CH), 8.08 (1 H, d, *J* 1, CHO), 8.26 (1 H, d, *J* 9, NH) and 8.32 (1 H, dd, *J* 9 and 1, N*H*CHO).

N-(N-*Formyl*-D-*penicillaminyl*)-L-*penicillamine* **25** (≡D,L-**20**).—A solution of (3*R*)-3-formamido-4,4-dimethylthietan-2-one **23** (57 mg, 0.36 mmol) in chloroform (0.5 cm^3) and a solution of L-penicillamine (54 mg, 0.36 mmol) in 1 mol dm^{-3} aq. sodium hydroxide (0.54 cm^3) were mixed and stirred vigorously for 1 h. The aqueous layer was then separated and acidified, to pH 1, with 1 mol dm^{-3} hydrochloric acid. The resulting suspension was kept at ~0 °C overnight, after which the precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the product **25** as a powder (79 mg, 71%), m.p. 116–122 °C; ν_{max}(KBr)/cm^{-1} 3318 (NH, OH), 2562w (SH), 1721 (CO_2) and 1641 (CONH); δ_H(250 MHz; [$^2H_6$]DMSO) 1.24 (3 H, s, Me), 1.29–1.39 (9 H, m, Me × 3), 2.43 (1 H, br s, SH), 2.71 (1 H, br s, SH), 4.36 (1 H, d, *J* 10, CH), 4.80 (1 H, d, *J* 10, CH), 8.00 (1 H, d, *J* 1, CHO), 8.28 (1 H, dd, *J* 10 and 1, N*H*CHO) and 8.32 (1 H, d, *J* 10, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 28.42, 29.56, 29.82 and 30.47 (CH_3), 44.95 and 46.10 (C), 58.54 and 61.75 (CH), 160.78 (CHO) and 168.98 and 170.76 (CO).

(±)-N-(N-*Formyl*-S-*nitrosopenicillaminyl*)-S-*nitrosopenicillamine* **26**.—A solution of sodium nitrite (157 mg, 2.27 mmol) in water (2 cm^3) was added to a solution of (±)-*N*-(*N*-formylpenicillaminyl)penicillamine **20** (175 mg, 0.57 mmol) in a mixture of DMF (2.5 cm^3) and 1 mol dm^{-3} hydrochloric acid (2.5 cm^3) containing a trace of conc. sulfuric acid (1 mm^3). The reaction mixture was stirred for 15 min, after which the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the S,S′-*dinitrosodithiol* **26** as a green powder, with red reflections, consisting of a 1:4 mixture of diastereoisomers (with the D,D–L,L diastereoisomeric pair predominant) (120 mg, 57%), m.p. 127 °C (decomp.); λ_{max}(MeOH)/nm 341 (ε/dm^3 mol^{-1} cm^{-1} 897) and 596 (18); ν_{max}(KBr)/cm^{-1} 3308 (NH, OH), 1724 (CO_2), 1653 (CONH), 1625 (CONH), 1512 (NO) and 658 (SN); δ_H(250 MHz; [$^2H_6$]DMSO) 1.75–2.06 (12 H, m, Me × 4), 5.09 (1 H, d, *J* 9, CH major isomer), 5.16 (1 H, d, *J* 9, CH minor isomer), 5.42 (1 H, d, *J* 10, CH major isomer), 5.50 (1 H, d, *J* 10, CH minor isomer), 7.89 (1 H, d, *J* 1, CHO), 8.58 (1 H, dd, *J* 10 and 1, N*H*CHO major isomer), 8.84 (1 H, dd, *J* 10 and 1, N*H*CHO minor isomer), 9.00 (1 H, d, *J* 9, NH major isomer) and 9.18 (1 H, d, *J* 9, NH minor isomer); δ_C(63 MHz; [$^2H_6$]DMSO) (major isomer only) 29.85, 30.49, 31.44 and 32.06 (CH_3), 62.35 (CH), 63.07 (C), 64.90 (CH), 64.89 (C), 166.01 (CHO), 173.61 (CO) and 175.30 (CO) [Found: (M^+ + H), 367.0746. $C_{11}H_{18}N_4O_6S_2$ requires (M + H), 367.0746].

N-(N-*Formyl*-S-*nitrosopenicillaminyl*)-S-*nitrosopenicillamine* (L,D *and* D,L *Racemate*) **27**.—A solution of sodium nitrite (53 mg, 0.77 mmol) in water (0.4 cm^3) was added to a solution of *N*-(*N*-formylpenicillaminyl)penicillamine (L,D and D,L racemate) **20** (59 mg, 0.19 mmol) in a mixture of DMF (0.65 cm^3) and 1 mol dm^{-3} hydrochloric acid (0.65 cm^3) containing a trace of conc. sulfuric acid (~5 mm^3). The reaction mixture was stirred for 10 min, after which the resulting precipitate was collected by filtration, washed with water, and dried *in vacuo* to give the *S*,*S*′-dinitrosodithiol **27** as a green powder with red reflections (40 mg, 57%), m.p. 125 °C (decomp.); λ_{max}(MeOH)/nm 344 (ε/dm^3 mol^{-1} cm^{-1} 912) and 590 (22); ν_{max}(KBr)/cm^{-1} 3275 (NH, OH), 1924w (SNO), 1735 (CO_2), 1646 (CONH), 1499 (NO) and 661 (SN); δ_H(250 MHz; [$^2H_6$]DMSO) 1.88 (3 H, s, Me), 1.93 (6 H, s, Me × 2), 2.03 (3 H, s, Me), 5.22 (1 H, d, *J* 10, CH), 5.55 (1 H, d, *J* 10, CH), 7.99 (1 H, d, *J* 1, CHO), 8.66 (1 H, dd, *J* 10 and 1, N*H*CHO) and 9.26 (1 H, d, *J* 10, NH); δ_C(63 MHz; [$^2H_6$]DMSO) 24.82, 26.63, 29.21 and 30.14 (CH_3), 56.15 (CH), 57.86 and 59.48 (C), 59.55 (CH), 160.75 (CHO), 166.45 and 170.21 (CO); *m*/*z* (FAB) 367 (MH^+).

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