Stereochemical Fate of (2*S*,4*R*)- and (2*S*,4*S*)-[4-^{2}H]Methionine in Nocardicin A Biosynthesis

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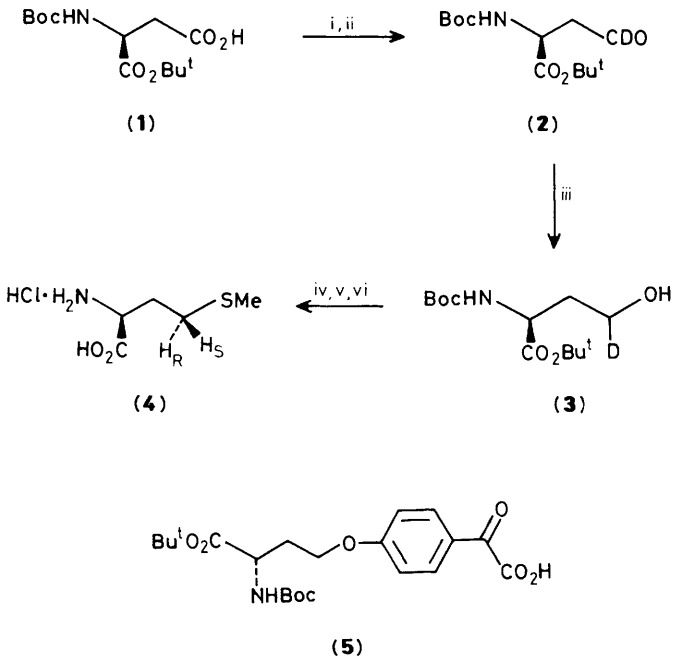
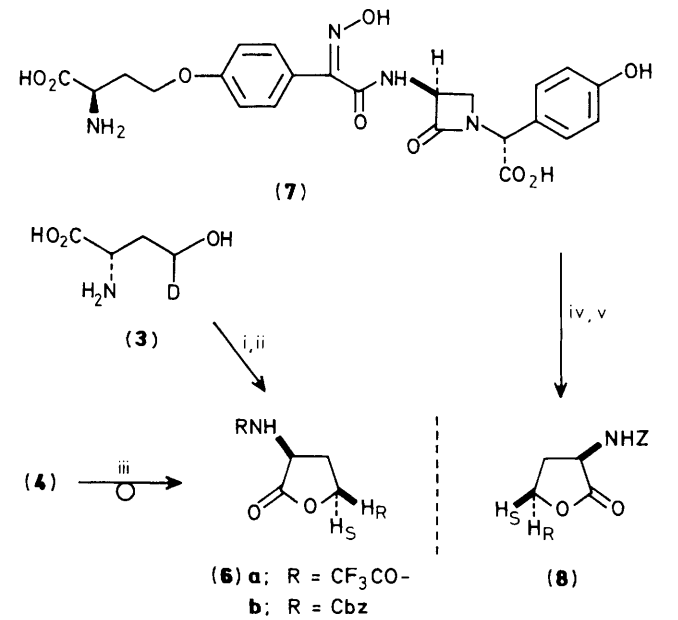
The incorporation of (2*S*,4*R*)- and (2*S*,4*S*)-[4-^{2}H]methionine into nocardicin A proceeds with inversion of configuration.

Whole-cell feeding experiments with labelled amino acids[1] in *Nocardia uniformis* subsp. *tsuyamanesis* (ATCC 21806) have established that the most direct precursors of nocardicin A (**7**) are methionine, (*p*-hydroxyphenyl)glycine, and serine for the homoseryl, aryl and β-lactam portions of the antibiotic, respectively. Similar 3-amino-3-carboxypropyl transfers from methionine have been demonstrated or appear to be active in the biosynthesis of discadenine,[2] X-base,[3] Y-base,[4] nicotianine,[5] diphthamide,[6] mugineic acid, and related compounds.[7] These group transfer reactions could be envisaged to occur in one of two ways. The first involves direct nucleophilic displacement on methionine, activated presumably as *S*-adenosylmethionine (AdoMet). Stereochemical inversion required in such a process has been demonstrated only for decarboxylated AdoMet in polyamine biosynthesis.[8] The second mechanistic possibility entails γ-elimination and replacement in a PLP-dependent process similar to that described for cystathionine γ-synthase, a reaction shown to proceed with net retention.[9]

To probe the mechanism of 3-amino-3-carboxypropyl transfer in nocardicin A biosynthesis, (2*S*,4*R*)- and (2*S*,4*S*)-[4-^{2}H]methionine (**4**) were synthesised as their hydrochloride salts as shown in Scheme 1. The deuteriated aldehyde (**2**) was obtained as a low-melting solid by mixed anhydride reduction[10] with $NaBD_4$ of the known[11] (**1**) followed by oxidation under modified Swern[12] conditions. The aldehyde contained 96% deuterium as judged by integration of its ^{1}H n.m.r. spectrum and mass spectral data. Stereospecific reduction with (*R*)- and (*S*)- Alpine borane[13] (Aldrich) yielded the protected (2*S*,4*S*)- and (2*S*,4*R*)-homoserines (**3**), respectively. Mesylation, displacement with $NaSCH_3$, and deprotection gave the labelled amino acid (**4**) in 41% and 49% overall yields for the (2*S*,4*R*)- and the (2*S*,4*S*)-diastereoisomers, respectively, from (**1**).



Scheme 1. *Reagents and conditions*: i, EtOCOCl, Et_3N, −10 °C, 30 min then $NaBD_4$, 0 °C, 4 h; ii, oxalyl chloride, DMSO, −78 °C, 15 min, then Et_3N; iii, Alpine borane; iv, Et_3N, MsCl, −5 °C, 30 min; v, $NaSCH_3$, D.M.F., 25 °C, 2 h; vi, TFA, −10 °C, 30 min, then 6 M HCl. (DMSO = dimethylsulphoxide, DMF = dimethylformamide).



Scheme 2. *Reagents and conditions*: i, 4 M HCl in dioxane, 25 °C, 2 h, then Et_3N, TFAA, −10 °C to r.t., 12 h; ii, 4 M HCl in dioxane, 25 °C, 2 h, then $NaHCO_3$, CbzCl, 25 °C, overnight; iii, ICH_2CO_2H, 40 °C, 24 h, then D.M.F., pH 3, 100 °C, 2 h then $NaHCO_3$, CbzCl; iv, 4 M HCl, PtO_2, H_2, 75 °C, 24 h, then $NaHCO_3$, CbzCl, 4 h.

The configuration at C-4 was assigned by conversion of (**3**) and (**4**) as shown in Scheme 2 to the known[14] homoserine lactone (**6**), which was N-derivatized for ease of isolation. It has been shown[14] that in L-homoserine lactone the *pro-R* hydrogen at C-4 is found downfield of its diasterotopic *pro-S* partner. Our results bear this out.† For the highly crystalline *N*-trifluoroacetyl (TFA) lactone (**6a**), in which all resonances are separated at 400 MHz, the 4-*pro-R* H was found at δ 4.50 and the 4-*pro-S* at δ 4.32. Proton and deuterium n.m.r. analyses of each monodeuteriated lactone showed completely complementary chemical shift values. The TFA derivatives also allowed calculation of the minimum enantiomeric excesses (e.e.) at C-4 from integration of resolution enhanced plots. After subtraction of the contribution from 4-$^1H_2$ molecules (4% by mass spectrometry), a 76% e.e. for (2*S*,4*S*)-(**3**) [83% when corrected for the 91.3% e.e. of (+)-pinene] and a 74% e.e. for (2*S*,4*R*)-(**3**) [90% when corrected for the 81.9% e.e. of (−)-pinene] was obtained. In general, carbobenzyloxy (Cbz) was substituted for TFA as the N-protecting group (**6b**) used for isolation from complex degradation mixtures since it was found to be more easily chromatographed. However, with the less electron-withdrawing carbamate group, the α-H overlapped the *pro-R* H at C-4 in the ^1H n.m.r. spectrum. $^2H\{^1H\}$N.m.r. spectroscopy, however, does not suffer such interference since only one deuteriated site is present above natural abundance and was used, therefore, to analyse the Cbz derivatives. In addition, confirmation that the $NaSCH_3$ displacement proceeded cleanly with one inversion in the presence of 3 equiv. of thiolate was obtained by degrading[10] the methionine obtained to the already studied homoserine lactone (**6**) (Scheme 2) and examining its n.m.r. spectrum as described above.

In separate feeding experiments, (2*S*,4*R*)- and (2*S*,4*S*)-[4-^2H]-(**4**) were administered at a concentration of 0.4 mM[1] to growing cultures of *N. uniformis*. The resulting nocardicin A (**7**) was isolated by a modification of the previously described[1] procedure and degraded[15] to homoserine lactone by hydrogenolysis as shown in Scheme 2. The stereochemical course at the homoseryl γ-carbon through this reduction/lactonization process was shown to be retention with the following experiment. The model compound (**5**) was prepared through Mitsunobu coupling of (**3**) with methyl *p*-hydroxybenzoylformate[16] (inversion[17] at C-4) followed by saponification. Under the strongly acidic conditions of the hydrogenation (4 M HCl), the amino acid protecting groups of (**5**) were rapidly lost prior to reductive elimination of labelled homoserine. The latter was stereochemically correlated through its conversion to (**6**). The homoserine lactones, therefore, obtained by degradation of the labelled samples of nocardicin A isolated above were derivatized as their D-lactones (**8**) and subjected to $^2H\{^1H\}$ n.m.r. analysis. The lactone obtained from incorporation of (2*S*,4*R*)-[4-^2H]methionine gave a single resonance at δ 4.55 while that derived from its (2*S*,4*S*)-diastereomer correspondingly gave a peak at δ 4.30. Bearing in mind that the α-carbon configuration changes from L- in the precursor (**4**) to D- in the product (**7**), the derived lactones (**8**) are antipodal to the reference substances (**6**) and, hence, the sites of deuterium labelling correspond to the 4-*pro-S* and 4-*pro-R* loci, respectively. The overall stereochemical course of the 3-amino-3-carboxypropyl transfer from methionine is, therefore, inversion. This finding parallels the steric course of polyamine biosynthesis from decarboxylated AdoMet[8] and suggests a role for AdoMet itself in nocardicin biosynthesis. This expectation has been borne out in cell-free studies where partially purified extracts of *N. uniformis* have been shown to carry out such transfers in the presence of AdoMet but not

† However, as all stereochemical correlations in this study were made to homoserine lactone, the proof of inversion in the 3-amino-3-carboxypropyl transfer reaction does not depend upon knowing the absolute configuration at C-4 in (**3**) or (**4**).

methionine and ATP.[18] These observations support the view‡ that both methyl[19] and 3-amino-3-carboxypropyl transfer reactions from AdoMet operate through complementary in-line transition state geometries to achieve S_N2 displacements from the reactive sulphonium ion cofactor.

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‡ An elimination/addition pathway proceeding with inversion cannot be strictly excluded on the basis of this stereochemical result alone.