Synthesis and mode of action of 1-substituted *trans*-cyclopropane 1,2-dicarboxylic acids: inhibitors of the methylaspartase reaction

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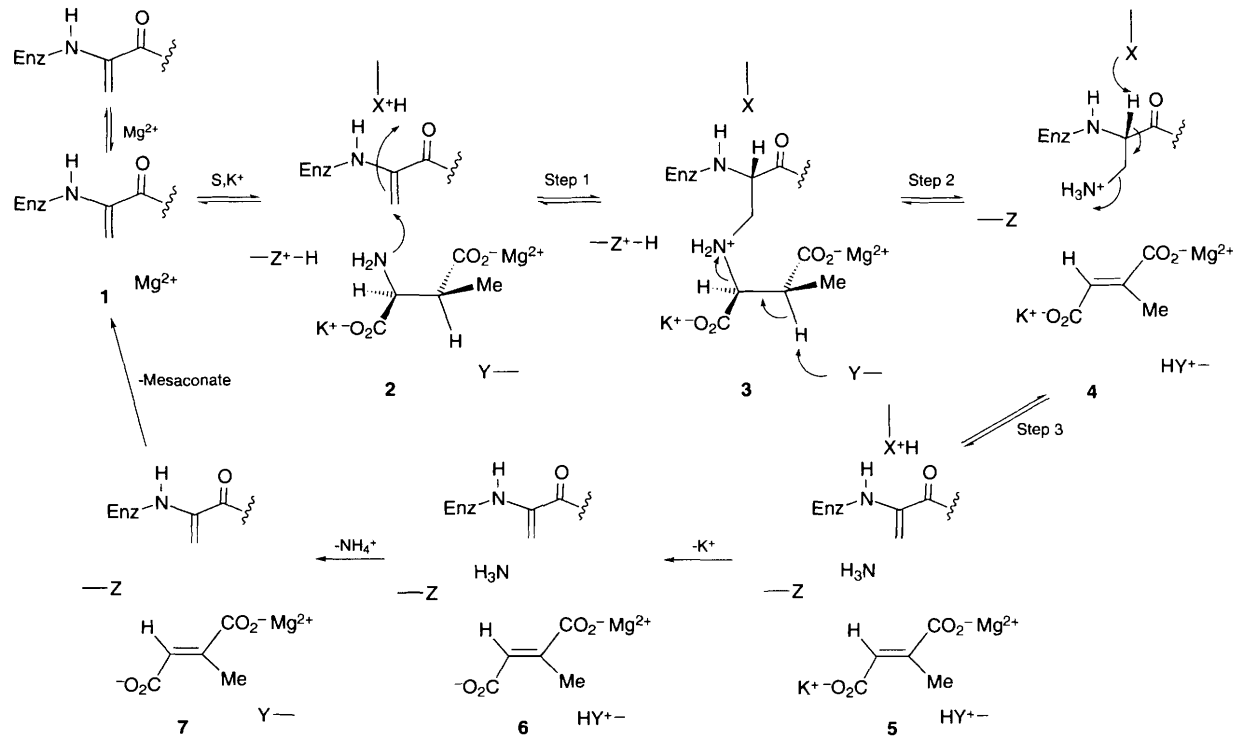
A range of 1-substituted cyclopropane 1,2-dicarboxylic acids are synthesised using short efficient routes and are found to be good to potent inhibitors of 3-methylaspartase; the crystallographically determined absolute stereochemistry and the mode of action of the most potent inhibitor, (1*S*,2*S*)-1-methylcyclopropane 1,2-dicarboxylic acid, is consistent with it acting as a transition state analogue for the central substrate deamination reaction catalysed by the enzyme.

Methylaspartase[1] (EC 4.3.1.2) catalyses the deamination of (2*S*,3*S*)-3-methylaspartic acid *via* a complex mechanism involving one Mg^{2+} ion, one K^+ ion and the intermediacy of a covalent enzyme-substrate adduct formed from a dehydroalanine residue,[2] Scheme 1. After the formation of a Mg^{2+}-enzyme–substrate complex **2** it is believed that the K^+ ion adds and the *N*-atom of the substrate attacks the dehydroalanine residue at C-3 to give the conjugate addition product **3**.[3] This covalent enzyme-substrate adduct **3** then undergoes elimination to give a covalent ammonia-enzyme adduct **4** *via* an apparently concerted process which displays a primary deuterium[4] and nitrogen isotope effect,[5] but which is not rate determining for the overall reaction.[6] In subsequent steps it is believed that the amino-enzyme **4** is deaminated to give the product complex **5** and that K^+ ion, and then ammonium ion, is released from the enzyme, prior to the release of mesaconic acid which generates the free Mg^{2+}-enzyme **1**.

In order to gain further information on the mechanism and to support crystallographic studies of the enzyme, potent reversible inhibitors were sought. Such compounds had not been described and, therefore, we set out to design transition state (TS) analogues. The transition states for the formation (Scheme 1, step 1) and break-down (step 3) of the covalent substrate **3** or covalent product **4** complexes are difficult to mimic because a large part of the TS structure involves protein–protein interactions. Therefore, attention was focussed on a TS analogue for the central substrate deamination step (step 2). In the flanking catalytically active complexes, **3** and **4** in Scheme 1, the enzyme should exist in a closed conformation with both metal ions bound and, in broad terms, the ground states should differ only in the C-3 dehydroalanine *N*-substrate bond length or distance and the hybridisation state at C-3 of the substrate. Substituted *trans*-cyclopropane 1,2-dicarboxylic acids, for example structure **8a**, accurately mimic the expected TS structure for the substrate and it was hoped that such analogues might allow the simultaneous occupation of the cleft of the enzyme by ammonia, Fig. 1.

The alkali-metal hydride catalysed formation of 1-substituted cyclopropane-1,2-dicarboxylate esters, from 2-dihalogenoethanoate esters (**9**, X = Y = Halogen) and acrylic ester **10** is



Scheme 1

well established and the *cis*-isomer **11** is the predominant product when the reaction is performed in non-polar media.[7] This preference is believed to arise because the metal ion can chelate to both of the ester groups of the initial conjugate addition product prior to the formation of the cyclopropane ring, Scheme 2. Since we required the *trans*-dioic acids to test as inhibitors, and expected that the *trans*-diesters **12** would predominate in the absence of the metal ion chelating effect, the ratio of *cis*- to *trans*-products formed in different solvents was examined. In toluene, the ratio of products [**11** to **12**] formed respectively from the reaction of methyl 2-chloropropanoate (**9**, X = Me, Y = Cl), methyl 2-dichloroethanoate (**9**, X = Y = Cl) and methyl 2-dibromoethanoate (**9**, X = Y Br) with methyl acrylate **10** was *ca.* 10 : 1. However, in DMF the ratio increased in favour of the *trans*- isomer to 0.73 : 1, for the 1-bromo derivatives (**11** and **12**, X = Br) to 1 : 3 for the 1-chloro derivatives (**11** and **12**, X = Cl) and to 1 : 5 for the 1-methyl derivatives (**11** and **12**, X = Me) in the best case, Scheme 2. Isolated yields of the *trans*- isomer were in the range 42–65%. Placing the group which is retained in the cyclopropane product on the 2-position of the acrylate ester (**14**, X = Cl or Br), such that the anion of a monohalogenoethanoate ester (**13**, X = Cl or Br) serves as the nucleophile for the conjugate addition, was expected to reduce the steric repulsion in the TS compared to the situation in which the anion is derived from a dihalogenoethanoate ester **9**. Accordingly, the 2-halogenoacrylates (**14**, X = Cl or Br) were treated with 2-chloro- and 2-bromo-ethanoate methyl ester, respectively, in the presence of NaH or KH, Scheme 3. Under optimised conditions reaction times were reduced to 6–12 h and the yields of the esters (**12**, X = Cl and X = Br) were increased to 80 and 83% respectively. The required dioic acids (**8a**, X = H, Me, Et, Cl and Br) were obtained in excellent yield through acid catalysed ester hydrolysis and each compound and synthetic intermediate displayed the expected spectral and analytical properties.†

When tested for biological activity, the racemic *trans*-cyclopropane 1,2-dioic acids (**8a**, X = H, Cl and Br) served as weak competitive inhibitors for the enzyme with K_i values of 7.1, 2.7 and 2 mmol dm^{-3}, respectively. The racemic *cis*-1-methylcyclopropane 1,2-dioic acid was a very poor inhibitor (K_i = 6 mmol dm^{-3}) but the corresponding *trans*-compound (**8a**, X = Me) served as a good competitive inhibitor, K_i = 87 μmol dm^{-3}, Table 1. Note that K_m for (2*S*,3*S*)-3-methylaspartic acid is 2.3 mmol dm^{-3} and that the value of K_i for the competitive product inhibitor, mesaconic acid, is 3 mmol dm^{-3}.[6] Replacement of the methyl group in the best inhibitor by an ethyl group (**8a**, X = Et) reduced the efficacy of the inhibitor by 30-fold (Table 1) in accord with the previous finding that K_m for (2*S*,3*S*)-3-ethylaspartic acid is large, 17.1 mmol dm^{-3}.[4] Thus it appeared that the series of inhibitors displayed the

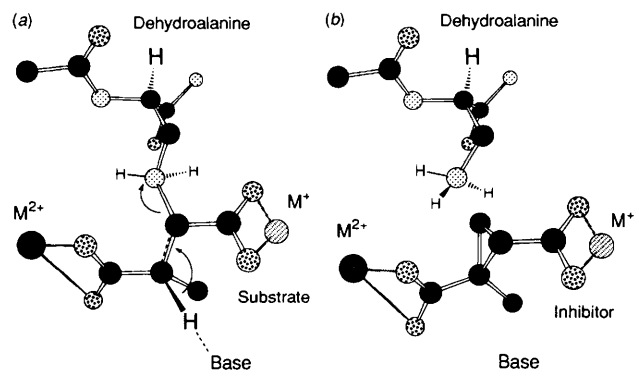
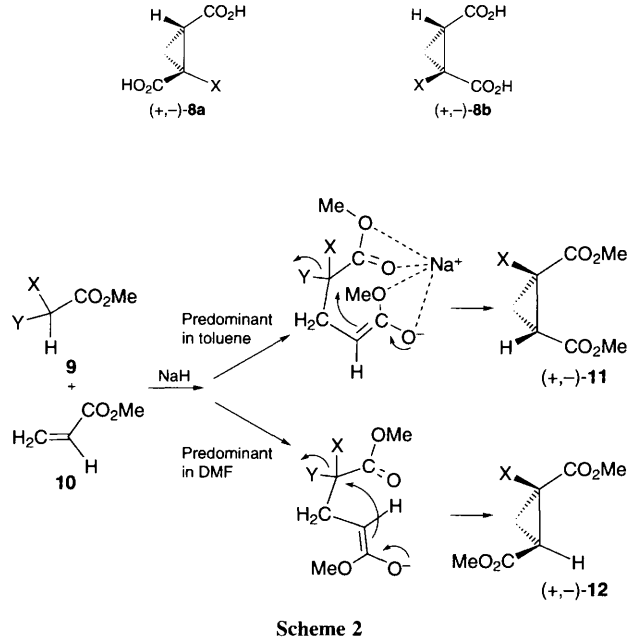
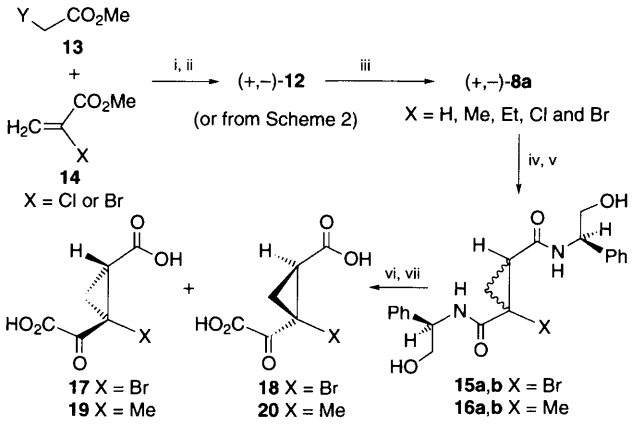


Fig. 1 (*a*) Expected transition state for C–N bond cleavage in the substrate. (*b*) Possible interaction of (1*S*,2*S*)-1-methyl cyclopropane-1,2-dioic acid **19** with the enzyme.



Scheme 2



Scheme 3 *Reagents and conditions*: i, KH, DMF 25 °C, 6 h, 80–83%; ii, column chromatrography on silica; iii, 6 mol dm^{-3} HCl, reflux, 2–4 h, 90–95%; iv, $SOCl_2$, reflux, 45 min.; v, (+)-(*R*)-2-phenylglycinol, Et_3N, 25 °C, 6–12 h, 85–90%; vi, column chromatography on silica; vii, 3 mol dm^{-3} HCl, rerflux, 12 h, 80–90%

Table 1 Properties of methylaspartase inhibitors[a]

Stereo-chemistry	X	Comp	K_i[b] mmol dm^{-3}	Mode of inhibition
(+,–)-*trans*-	H	**8a**	7.100	competitive
(+,–)-*cis*-	Me	**8b**	6.000	competitive
(+,–)-*trans*-	Me	**8a**	0.087	competitive
(+,–)-*trans*-	Et	**8a**	2.530	competitive
(+,–)-*trans*-	Cl	**8a**	2.700	competitive
(+,–)-*trans*-	Br	**8a**	2.000	n.d.,[c] IC_{50}
(1*R*,2*R*)-*trans*-	Br	**17**	0.630	mixed
(1*S*,2*S*)-*trans*-	Br	**18**	> 5.000	n.d.,[c] IC_{50}
(1*S*,2*S*)-*trans*-	Me	**19**	0.020	competitive
(1*R*,2*R*)-*trans*-	Me	**20**	0.175	competitive
(1*S*,2*S*)-*trans*-	Me	**19**	0.009	competitive

[a] Reaction conditions were as standard except for the last entry, which also included 10 mmol dm^{-3} ammonium chloride. Reaction rates were determined in triplicate at pH 9.0 and at 30 °C under standard assay conditions[4] for several different substrate and inhibitor concentrations and data was fitted using non-linear regression analysis. [b] Errors in K_i values are within ±5% of stated figure. [c] n.d. indicates that the mode of inhibition was not determined and the quoted IC_{50} value was determined at a substrate concentration of 2.3 mmol dm^{-3} [K_m for the substrate (2*S*,3*S*)-3-methylaspartic acid].

properties that might be expected if they were serving as TS analogues for the central substrate deamination step, Scheme 1, step 2.

In order to test the hypothesis further, the racemic 1-bromo- and 1-methyl-*trans*-dioic acids (**8a**, X = Br and Me) were resolved by separating the diastereoisomeric *bis*-amide derivatives of (+)-(2*R*)-phenylglycinol[8] (**15a** and **15b**) and (**16a** and **16b**) by flash column chromatography on silica, Scheme 3. The separated diastereoisomers were each hydrolysed to furnish the required enantiomers of the 1-bromo and 1-methyl cyclopropane dicarboxylic acids in excellent yield. To determine the absolute configuration of each enantiomer, the most polar diastereoisomers of each of the *bis*-amides **15b** and **16b** was subjected to X-ray crystallographic analysis using the known (2*R*)-stereochemistry of the phenylglycinol moiety for reference in each case.‡ These analyses revealed that the samples were derived from (1*S*,2*S*)-1-bromo cyclopropane-1,2-dicarboxylic acid **18** and (1*R*,2*R*)-1-methyl cyclopropane-1,2-dicarboxylic acid **20** respectively, Figs 2 and 3.

When tested for activity the (1*S*,2*S*)-1-methylcyclopropane **19** {$[\alpha]_D$ +191.4 (*c* 1.0, MeOH)} was a potent inhibitor (K_i = 20 μmol dm^{-3}) and was nine times more effective than the (1*R*,2*R*)-antipode {$[\alpha]_D$−191.4 (*c* 1.0, MeOH)} whereas the (1*R*,2*R*)-1-bromo cyclopropane **17** {$[\alpha]_D$ +91.5 (*c* 1.0, MeOH)} displayed a K_i value of 630 μmol dm^{-3} and was at least nine times more active than the (1*S*,2*S*)-antipode {$[\alpha]_D$ −91.4 (*c* 1.0, MeOH)}. Note that the spatial arrangement of similar groups is identical for the (1*S*,2*S*)-methylcyclopropane **19** and the (1*R*,2*R*)-bromocyclopropane **17** but that there are priority assignment changes. From these results it is evident that each of the inhibitors prefer to bind with the 1-substituent in the pocket for the methyl group of the substrate. Thus each carboxylate group can bind to the enzyme and metal ion cofactors as for the substrate with the cyclopropane methylene moiety disposed on the same face of the pseudo-plane described by C-1′–C-1–C-2–C-2′ as the dehydroalanine residue, Fig. 1. This is in keeping with the observation that there is enough space to accommodate hydrazine or methylamine in place and that such species support catalysis for the reverse reaction.[9]

Given that compound **19** can only bind to the enzyme as a partial substrate analogue in the absence of ammonia because the compound lacks an amino group and ammonium ion is released before the dioic acid in the forward reaction direction, Scheme 1, it was of interest to examine the inhibitory properties of the compound in the presence of ammonia. Accordingly a new set of kinetic experiments were performed in the presence of 10 mmol dm^{-3} ammonium chloride (*ca.* 0.2 times K_m for the species behaving as a substrate in the reverse reaction direction). Under these conditions compound **19** behaved as a highly potent competitive inhibitor and displayed a reduced K_i value of 9 μmol dm^{-3} (Table 1, last entry). The result indicates that ammonia and the cyclopropane dioic acid can simultaneously bind to the enzyme and together mimic the TS structure for the central deamination process. This result is at odds with the notion that the dehydroalanine residue of the related enzyme, phenylalanine ammonia-lyase, serves as a Friedel-Crafts donor rather than an amino group acceptor as has been recently suggested.[10] As yet there is no X-ray crystallographic information available for any enzyme which operates *via* the intermediacy of a catalytic dehydroalanine residue. However, compound **19**, a potent inhibitor of methylasapartase which allows the simultaneous occupation of the active-site by ammonia, is ideally suited for gaining such information.

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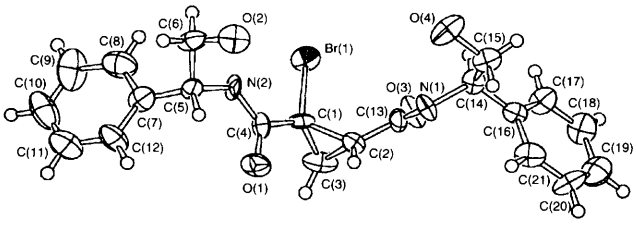
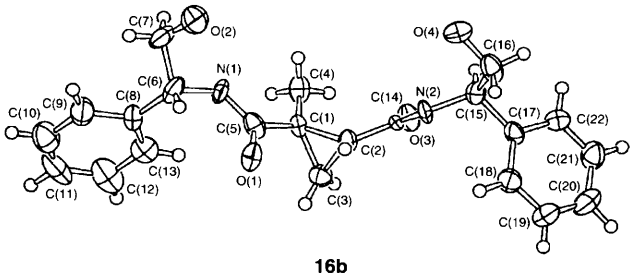


15b

Fig. 2



16b

Fig. 3

Footnotes

† All compounds were fully characterised and gave the expected spectral and analytic data.

‡ *Crystal data* for compound **15b**; $C_{21}H_{23}N_2O_4Br$, M = 447.33, orthorhombic, space group $P2_12_12_1$, a = 10.652(7), b = 33.479(7), c = 5.513(7) Å, V = 1966(2) Å^3, Z = 4, D_c = 1.511 g cm^{-3}, T = 293 K. 2081 unique reflections collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 1283 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 7.3%, $R_w(F)$ = 7.2% for 253 variables. For **16b**; $C_{22}H_{26}N_2O_4$, M = 382.46, orthorhombic, space group $P2_12_12_1$, a = 11.759(9), b = 31.84(2), c = 5.225(2) Å, V = 1956(1) Å^3, Z = 4, D_c = 1.299 g cm^{-3}, T = 293 K. 1554 unique reflections collected on a Rigaku AFC7S diffractometer employing Mo-Kα radiation (λ = 0.71069 Å) of which 842 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 5.5%, $R_w(F)$ = 4.5% for 253 variables. Both structures were solved using SIR92 and were refined using TEXSAN.[11] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

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