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Synthesis and Configurational Assignment of an Unusual Bicylic Amino Acid

Saleem Farooq,*[a] William E. Swain, Jr.,[b] Richard Daeppen [c] and Grety Rihs [d]

[a] Agricultural Division, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland; [b] Agricultural Division, CIBA-GEIGY Corp., Greensboro (N.C.), USA; [c] Central Analytical Department, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland; [d] Central Physics Department, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland

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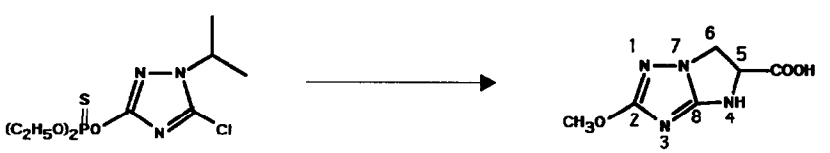
Abstract: A synthesis leading to the racemic bicyclic amino acid **1** is described. Similarly, the two enantiomers of **1** have been synthesised and their configuration determined.

Introduction

The structure **1**, shown in Fig. 1, of unknown chirality has been proposed for the major metabolite of the insecticide isazofos in corn grain.[1] Compound **1**, 5,6-dihydro-2-methoxy-4H-imidazo[1,2-b][1,2,4] triazole-5-carboxylic acid, is an example of an unusual bicyclic amino acid conjugate.



isazofos **1**

Fig. 1. Major metabolite **1** of isazofos in corn grain

For regulatory registration of isazofos it was both necessary (a) to prove the validity of the proposed structure and (b) to provide relevant documentation (e.g. toxicological data) for the metabolite **1**. This required that a synthetic route be developed to provide an appreciable quantity of **1**. We describe here the synthesis of the racemate and the two enantiomers of the bicyclic amino acid **1**.

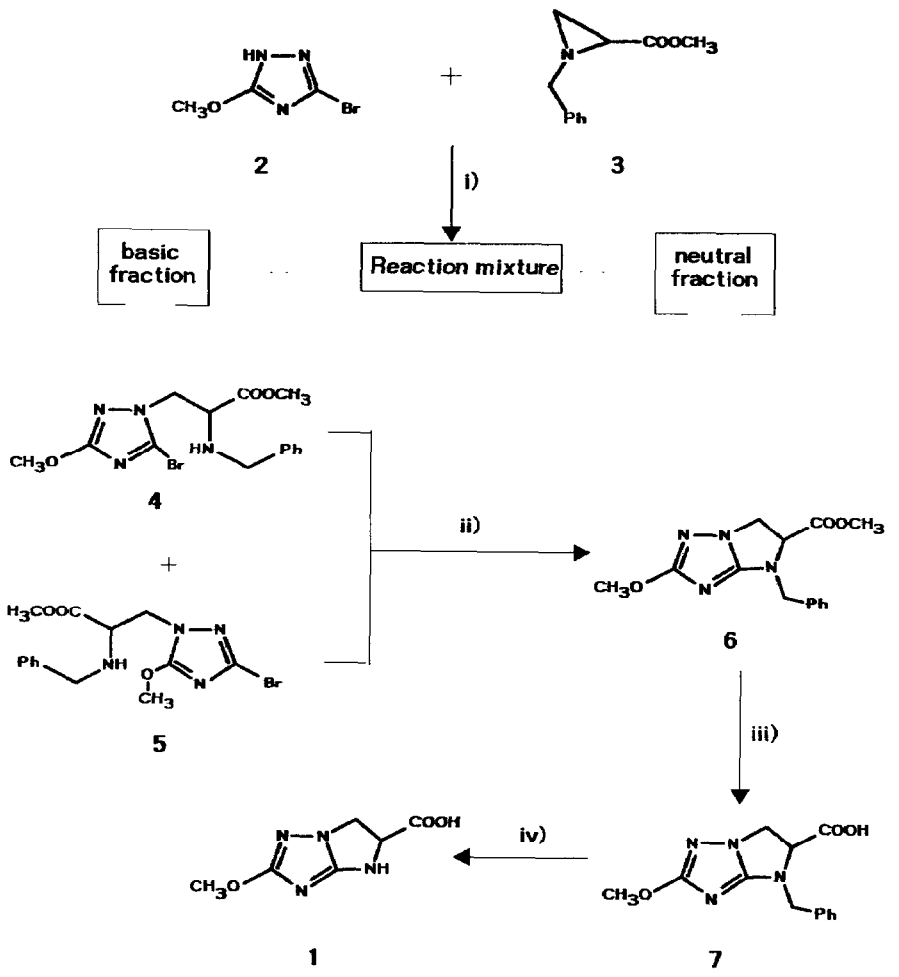
Results and Discussion

Synthesis of racemic 5,6-dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5-carboxylic acid (1)

Fig. 2 outlines the successful synthetic route to the desired amino acid **1**. The key step of the synthesis is the alkylation of the known 3-bromo-5-methoxy-1H-1,2,4-triazole **2**[3] with the nitrogen protected methyl aziridine-2-carboxylate **3**[4] using boron trifluoride diethylether complex as a Lewis acid[5]. Under the reaction conditions used this leads to a mixture consisting of the desired alkylated product **4** and the isomer **5** in a ratio of ca. 2 : 1. All attempts at separating this mixture by column chromatography failed, however the products **4** and **5** were identifiable in the chromatographed mixture by ^{13}C-NMR, see Experimental Procedures. Surprisingly, in the same reaction mixture small quantities (~ 3%) of the cyclised neutral product **6** were also isolated. Treatment of the unseparated mixture **4** and **5**[6] with HCl in methanol afforded the bicyclic intermediate **6**. This was readily hydrolysed to the acid **7** which subsequently underwent smooth hydrogenolysis, to remove the benzyl protecting group, with10% Pd / C as catalyst, to provide the desired product **1**. Elemental analysis and the spectral data of this strongly acidic (pK = 2.8), water soluble compound were in full accord with the structure **1**. The synthesised racemate **1** was compared to the natural metabolite and found to be identical in its mass spectral and two dimensional tlc behaviour[7].

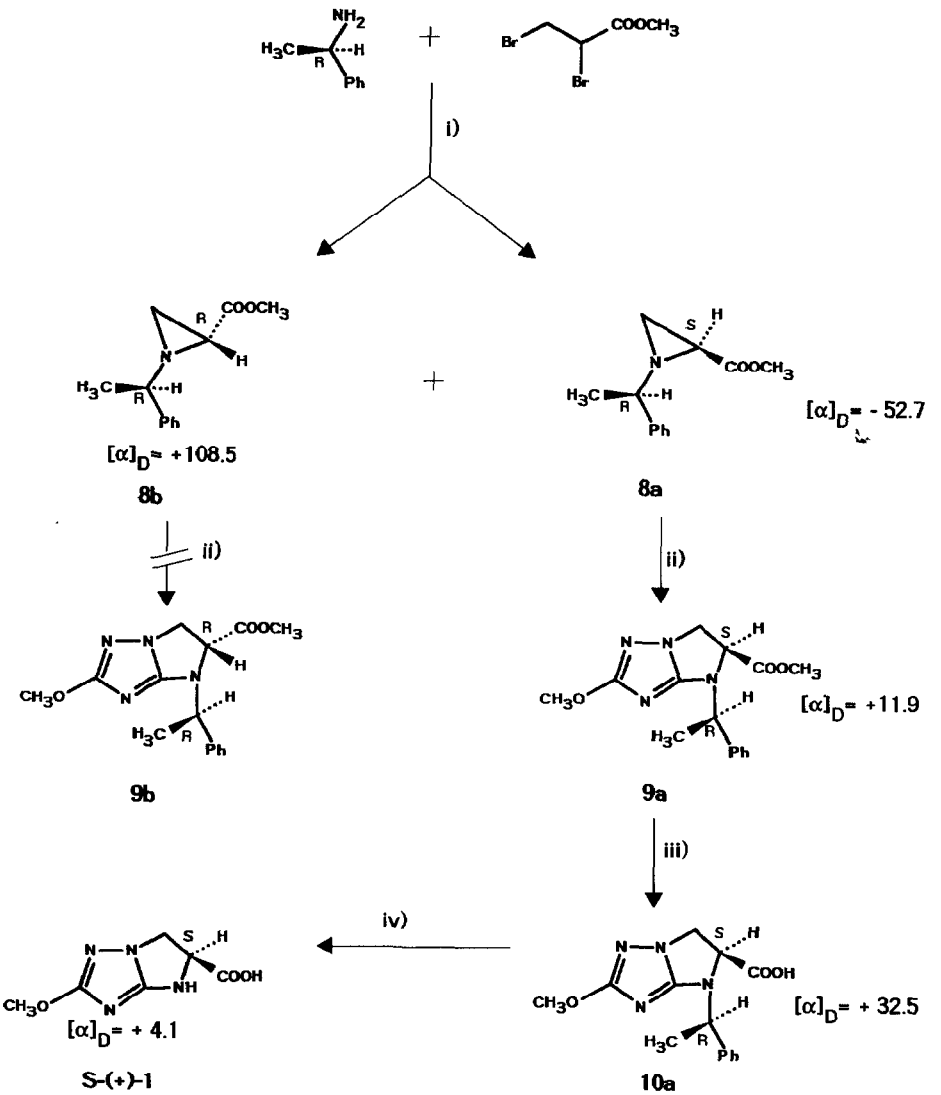
Synthesis of the enantiomers S-(+)-1 and R-(-)-1

The successful synthesis of racemic **1** prompted us to investigate a similar strategy employing a chiral aziridine, in order to obtain both enantiomers of **1**. For the preparation of chiral aziridine-2-carboxylates the chiral auxiliary 1-phenylethylamine was utilized. Hence, methyl 2,3-dibromo-propionate and (R)-1-phenylethylamine gave the two diastereoisomers **8a** and **8b**, see Fig. 3, which were conveniently separated by column chromatography on silica gel. Alkylation of the triazole **2** with the aziridine **8a**, using boron trifluoride diethylether complex as catalyst, resulted in a reaction mixture from which the neutral cyclised product **9a** was isolated in a yield of 13%. Treatment of the basic products from this same reaction mixture with HCl in methanol afforded further **9a** (7%). Hydrolysis of **9a** with KOH in aqueous methanol gave the crystalline acid **10a**. An X-ray analysis of **10a** was performed, Fig. 4, to establish the configuration at the chiral center C(5) bearing the carboxyl-group. Knowing the chirality at the benzyl-carbon, **10a** was shown to possess the S-configuration at C(5). Hydrogenolysis of **10a** to remove the chiral auxiliary group gave S-(+)-1 with an enantiomeric excess of ≥ 96% as evidenced by HPLC of the product derivatised with S-(-)-1-(naphthyl)-ethylamine, see Experimental Procedures.



i) $BF_3.OEt_2$, THF / 60°C, 3 h; ii) HCl, CH_3OH / rfl., 8 h;
iii) KOH, CH_3OH - H_2O / rt, 0.5 h; iv) H_2, 10% Pd/C, dioxane / 35°C, 5 bar, 34 h.

Fig. 2. Synthesis route to racemic 1



i) NEt_3, toluene / 90°C, 3 h; ii) 2, $BF_3.OEt_2$, THF / 60°C, 3 h;
iii) KOH, CH_3OH - H_2O / rt, 1 h; iv) H_2, 10% Pd/C, dioxane / 35°C, 5 bar, 13 h.

Fig. 3. Synthesis route to S-(+)-1

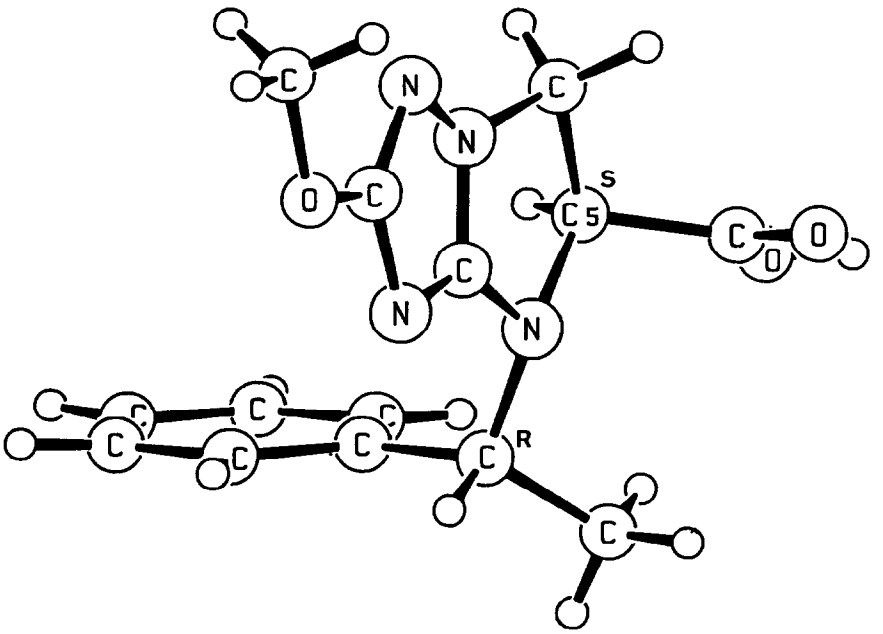


Fig. 4. The X-ray structure of **10a** showing the S-configuration at C(5)

Having established the chirality at C(5) in **10a**, it was then possible retrospectively to assign the S-chirality at C(5) in **9a**, S-chirality at C(2) in **8a** and R-chirality at C(2) in **8b**. Interestingly, an attempt to obtain the enantiomer R-(-)-**1** starting from the diastereomeric aziridine-2-carboxylate **8b**, as detailed for the synthesis of S-(+)-**1**, gave no cyclised product **9b** as evidenced by tlc. It is assumed that the possible transition state **9b≠** required in the cyclisation step, is energetically unfavourable owing to the steric interaction between the CH_3- and the $COOCH_3$-group, therefore hindering the approach of the nitrogen-nucleophile to the electrophilic C(5)-atom of the triazole ring as depicted in Fig. 5.

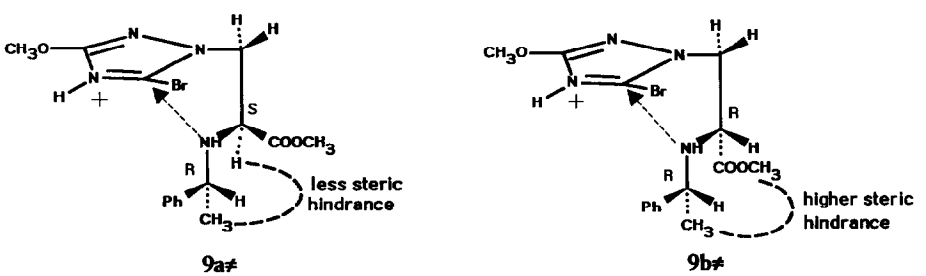


Fig. 5. Possible transition states **9a≠** and **9b≠** required for cyclisation to **9a** and **9b** respectively

The desired R-(-)-1 was, however, obtained by preparing the diastereomeric aziridine-2-carboxylate **8c** (enantiomer of **8a**) from (S)-1-phenylethylamine and carrying out the synthesis of R-(-)-1 from **8c** as described for the conversion of S-(+)-1 from **8a**. The synthesised R-(-)-1 had an enantiomeric excess of ≥ 96% as shown by HPLC, see Experimental Procedures.

Finally, the natural metabolite, derivatised with S-(-)-1-(1-naphthyl)-ethylamine, was analysed by HPLC and found to contain 83% of S-(+)-1 and 17% of R-(-)-1, Fig. 6.

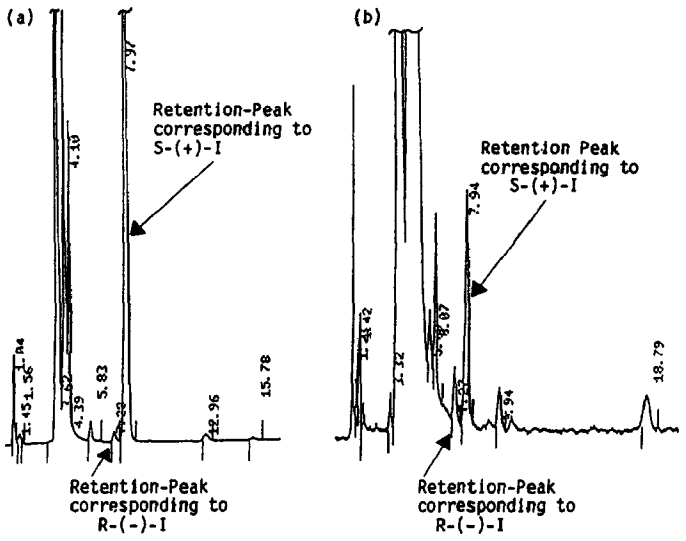


Fig. 6. HPLC of (a) S-(+)-1 derivatised with S-(-)-1-(1-naphthyl)-ethylamine and of (b) the natural metabolite derivatised with S-(-)-1-(1-naphthyl)-ethylamine

Conclusion

A facile synthesis for the proposed structure **1** of the major metabolite of the insecticide isazofos in corn grain was achieved leading to the racemate as well as to the two enantiomers. The natural bicyclic amino acid conjugate was found to contain 83% of S-(+)-1 and 17% of R-(-)-1.

Experimental Procedures

NMR spectra were recorded on a Bruker AM 300 or a Varian XL 300 spectrometer using TMS as an internal standard. Mass spectra were recorded on a Finnigan MAT 212/SS300 instrument. Optical rotations were measured on a Perkin Elmer Polarimeter 241. The melting points were recorded on a Dr. Tottoli melting point apparatus and are uncorrected.

Synthesis of racemic 5,6-dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5-carboxylic acid (1)

3-Bromo-5-methoxy-1H-1,2,4-triazole (2)

2 was prepared from 3-methoxy-1,2,4-triazole[2] (mp 96° - 98°C, Lit.[2] : 98° - 99°C) as described in the literature[3] : mp 125° - 127°C (Lit.[3] : mp 125° - 126°C).

Methyl 1-benzyl-aziridine-2-carboxylate (3)

The preparation of 3 was carried out as described in the literature[4] : bp 95° - 97°C (0.3 mm) (Lit.[4] : bp 96° - 98°C (0.2 mm)).

Methyl 2-benzylamino-3-(3-methoxy-5-bromo-1,2,4-triazol-1-yl)-propionate (4) and methyl 2-benzylamino-3-(3-bromo-5-methoxy-1,2,4-triazol-1-yl)-propionate (5)

To a solution of 2 (26.7 g, 0.15 mol) and 3 (28.7 g, 0.15 mol in dry tetrahydrofuran (150 ml) was slowly added boron trifluoride diethylether complex (5 ml) at room temperature. The reaction mixture was stirred for 3 h at 60°C and then concentrated at reduced pressure. Water (250 ml) was added, followed by solid $NaHCO_3$ until the solution was alkaline. The alkaline solution was washed twice with 300 ml-portions of ethyl acetate; the combined ethyl acetate extracts were washed 4 times with 200 ml-portions of water; these water extracts were discarded. The ethyl acetate layer was extracted twice with 100 ml-portions of 10% aqueous H_2SO_4, then twice with a saturated brine solution, dried (Na_2SO_4) and concentrated to give the neutral fraction:~ 25 g.

To the acidic (H_2SO_4) water extract was added a 30% NaOH solution until alkaline. This was then extracted twice with 100 ml-portions of ethyl acetate. The combined ethyl acetate extracts were washed twice with 100 ml-portions of saturated brine solution, dried (Na_2SO_4) and concentrated in vacuo to give the basic fraction: ~ 30 g.

(a) The basic fraction was chromatographed (flash chromatography) on silica gel (800 g) using ethyl acetate : n-hexane = 2:1 as eluant to yield 20 g (36%) of a mixture of 4 and 5 (ca. 2:1). The NMR-spectra showed the following characteristic peaks :

^{1}H-NMR ($CDCl_3$) : for 4 peaks at 3.75 (s, CH_3O), 3.90 (s, CH_3OOC) and
for 5 peaks at 3.73 (s, CH_3O), 4.07 (s, CH_3OOC) ppm.

^{13}C-NMR ($CDCl_3$) : for 4 peaks at 50.8 (td, heteroaryl-N-CH_2), 168.3 (q, C(3)) and
for 5 peaks at 48.1 (td, heteroaryl-N-CH2), 159.8 (m, C(5)) ppm.

(b) The neutral fraction was chromatographed (flash chromatography) on silica gel (800 g) using ethyl acetate : n-hexane = 2:1 as eluant to give 1.3 g (2.3%) of **6**. This material was identical (^{1}H-NMR) to **6** obtained in the next step.

Methyl 5,6-dihydro-2-methoxy-4-benzyl-imidazo [1,2-b][1,2,4] triazole-5-carboxylate (6)

A solution of a ca. 2:1 mixture of **4** and **5** (17.5 g, 47.4 mmol) in methanol (70 ml) was added to a solution of HCl in methanol (2.5 N, 10 ml) and heated under reflux for 8 h. The reaction mixture was concentrated in vacuo. The crude product was dissolved in ethyl acetate (250 ml), washed twice with 100 ml-portions of water, dried (Na_2SO_4) and concentrated at reduced pressure to yield 11 g of an oily product. Flash chromatography on silica gel (800 g) with ethyl acetate as eluant gave **6** (3.4 g (35%)), n_D(20°C)=1.5408, ^{1}H-NMR($CDCl_3$): δ = 3.78 (s, 3H, CH_3O), 3.93 (s, 3H, CH_3OOC), 4.04 (dd, 1H, J_1 = 6Hz, J_2 = 9 Hz, H_A-C(6)), 4.17 (dd, 1H, J_1=J_2= 9 Hz, H-C(5)), 4.35 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 4.43 (d, 1H, J = 15 Hz, H_AC-Ph), 4.80 (d, 1H, J = 15 Hz, H_BC-Ph), 7.2 - 7.4 (m, 5H, C_6H_5) ppm, MS : m/e = 288 (M^+, 12), 229 (M^+ - $COOCH_3$, 22), 91 ($C_6H_5CH_2^+$, 100).

5,6-Dihydro-2-methoxy-4-benzyl-imidazo [1,2-b][1,2,4] triazole-5-carboxylic acid (7)

To a solution of **6** (4.6 g, 16 mmol) dissolved in methanol (50 ml) was slowly added a solution of KOH (85%, 1.3 g, 20 mmol) in water (10 ml). The reaction mixture was stirred at room temperature for 30 minutes and then concentrated in vacuo. The residue was dissolved in water (100 ml) and extracted once with ethyl acetate (100 ml); this organic phase was discarded. The water extract was acidified with conc. sulphuric acid and extracted twice with 100 ml-portions of ethyl acetate; the combined ethyl acetate extracts were washed once with saturated brine solution (100 ml), dried (Na_2SO_4) and evaporated at reduced pressure. The solid residue was suspended in hexane, filtered and dried to yield **7** (3.6 g (82%)), mp 158° - 160°C, ^{1}H-NMR (DMSO-d_6) : δ = 3.78 (s, 3H, CH_3O), 3.97 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_A-C(6)), 4.27 (dd, 1H, J_1=J_2= 9 Hz, H-C(5)), 4.36 (d, 1H, J = 15 Hz, H_AC-Ph), 4.46 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 4.61 (d, 1H, J = 15 Hz, H_BC-Ph), 7.2 - 7.4 (m, 5H, C_6H_5), 13.50 (bs, 1H, COOH) ppm, ^{13}C-NMR (DMSO-d_6) : δ = 47.8 (td, J_1 = 150 Hz, J_2 = 7 Hz, C(6)), 49.6 (tm, J = 140 Hz, CH_2-Ph), 55.8 (q, J = 146 Hz, CH_3O), 62.2 (dm, J = 147 Hz, C(5)), phenyl carbons at 127.7, 128.3 & 135.7, 162.8 (m, C(8)), 170.1 (q, J = 4 Hz, C(2)), 170.5 (m, COOH) ppm, MS : m/e = 274 (M^+, 7), 229 (M^+ - COOH, 16), 91 ($C_6H_5CH_2^+$, 100), pK = 2.6.

5,6-Dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5-carboxylic acid (1)

7 (3.4 g, 12.4 mmol), 10% Pd/C (6.8 g) in dioxane (50 ml) were hydrogenated at 35°C/5 bar for a period of 34 h. The reaction was monitored by tlc (silica gel, pyridine : n-propanol : water : acetic acid = 10 : 35 : 12 : 3). After completion of the reaction the reaction mixture was filtered through a filter-paper and evaporated to dryness. The solid residue was dissolved in water (10 ml) and extracted once with ethyl acetate (30 ml). The water extract was separated and evaporated in vacuo to yield a glassy residue which was suspended in tetrahydrofuran, filtered and dried to afford racemic 1 (1.4 g (61.4%)), mp 176° - 177°C, Anal. calcd. for $C_6H_8N_4O_3$: C, 39.1; H, 4.4; N, 30.4; found : C, 39.4; H, 4.6; N, 29.8; ^{1}H-NMR (DMSO-d_6) : δ = 3.75 (s, 3H, CH_3O), 4.02 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_A-C(6)), 4.18 (dd, 1H, J_1=J_2= 9 Hz, H-C(5)), 4.75 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 7.34 (bs, 1H, NH), 13.32 (bs, 1H, HOOC) ppm, ^{13}C-NMR (DMSO-d_6) : δ = 48.2 (t, J = 150 Hz, C(6)), 55.8 (q, J = 146 Hz, CH_3O), 59.4 (dt, J_1 = 148 Hz, J_2 = 5 Hz, C(5)), 162.9 (dt, C(8)), 170.2 (q, J = 4 Hz, C(2)), 172.2 (m, COOH) ppm, MS : m/e = 184 (M^+, 85), 139 (M^+ - COOH, 78), 56 (100), pK = 2.8.

Synthesis of 5,6-dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5S-carboxylic acid (S-(+)-1)

Methyl 1-(1R-phenylethyl)-aziridine-2S-carboxylate (8a) and methyl 1-(1R-phenylethyl)-aziridine-2R-carboxylate (8b)

A solution containing (R)-1-phenylethylamine (36.3 g, 0.3 mol), triethylamine (60.6 g, 0.6 mol) in toluene (300 ml) was added, during a period of ca. 1 h, to a stirred solution of methyl 2,3-dibromo-propionate (73.8 g, 0.3 mol) in toluene (190 ml) at room temperature.The reaction mixture was stirred at 90°C for 3 h, cooled and washed twice with each 200 ml-portions of water, twice with each 200 ml-portions of a saturated brine solution, dried (Na_2SO_4) and concentrated under reduced pressure. The residual liquid was distilled (bp 80° - 82°C (0.15 mm)) to afford a mixture of **8a** and **8b** (51.5 g (83.7%)). This diastereoisomeric mixture was separated by flash chromatography (silica gel, ethyl acetate : hexane = 1:1) to afford two oily fractions. The first fraction (22.4 g) and the second fraction (21.7 g) were retrospectively (see Discussion) assigned the structures **8b** and **8a**.

(**8b**) : rotation $[\alpha]_D$ = + 108.5 ± 0.5 (2% in $CHCl_3$), ^{1}H-NMR ($CDCl_3$) : δ = 1.50 (d, 3H, J = 7, CH_3-C), 1.62 (dd, 1H, J_1 = 1 Hz, J_2 = 6 Hz, H_A-C(3)), 2.14 (dd, 1H, J1= J_2= 3 Hz, H_B-C(3)), 2.22 (dd, 1H, J_1 = 3 Hz, J_2 = 6 Hz, H-C(2)), 2.55 (q, 1H, J = 7 Hz, HC-Ph), 3.77 (s, 3H, CH_3OOC), 7.2 - 7.4 (m, 5H, C_6H_5) ppm.

(8a) : rotation $[\alpha]_D$ = - 52.7 ± 0.4 (3% in $CHCl_3$), ^{1}H-(NMR) : δ = 1.47 (d, 3H, J = 7 Hz, CH_3-C), 1.79 (dd, 1H, J_1 = 1 Hz, J_2 = 6 Hz, H_A-C(3)), 2.08 (dd, 1H, J_1 = 3 Hz, J_2 = 6 Hz, H-C(2)), 2.35 (dd, 1H, J_1 = 1 Hz, J_2 = 3 Hz, H_B-C(3)), 2.57 (q, 1H, J = 7 Hz, HC-Ph), 3.68 (s, 3H, CH_3OOC), 7.2 - 7.4 (m, 5H, C_6H_5) ppm.

Methyl 5,6-dihydro-2-methoxy-4-(1R-phenylethyl)-imidazo [1,2-b][1,2,4] triazole-5S-carboxylate (9a)

To a solution of **2** (14.2 g, 79.7 mmol) and **8a** (16.3 g, 79.5 mmol) in tetrahydrofuran (80 ml) was added, at room temperature, boron trifluoride diethylether complex (4 ml). The reaction mixture was stirred for 3 h at 60°C and then concentrated in vacuo; water (150 ml) was added followed by solid $NaHCO_3$ until the solution was alkaline. The alkaline solution was washed twice with 200 ml-portions of ethyl acetate; the combined ethyl acetate extracts were washed twice with 80 ml-portions of 10% aqueous H_2SO_4, then twice with a saturated brine solution, dried (Na_2SO_4) and concentrated to yield 6 g of an oily residue. Flash chromatography on silica gel (800 g) using ethyl acetate as eluant afforded **9a** as an oil (3.1 g (12.9%)), rotation $[\alpha]_D$ = + 11.9 ± 1.3 (0.8% in $CHCl_3$), ^{1}H-NMR ($CDCl_3$) : δ = 1.68 (d, 1H, J = 6 Hz, CH_3-C), 3.69 (s, 3H, CH_3O), 3.93 (s, 3H, CH_3OOC), 3.95 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_A-C(6)), 4.13 (dd, 1H, J_1=J_2 = 9 Hz, H-C(5)), 3.37 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 4.93 (q, J = 6 Hz, HC-Ph), 7.2 - 7.4 (m, 5H, C_6H_5) ppm.

Reacting **2** and the aziridine **8b** under the identical reaction conditions as described above did not give the desired product **9b**, as evidenced by tlc (see Discussion).

5,6-Dihydro-2-methoxy-4-(1R-phenylethyl)-imidazo [1,2-b][1,2,4] triazole-5S-carboxylic acid (10a)

A solution of KOH (85%, 1.3 g, 20 mmol) in water (2 ml) was added to a solution of **9a** (2.9 g, 9.6 mmol) in methanol (50 ml). The reaction mixture was stirred at room temperature for 1 h. Work-up, as described for the preparation of the acid **7**, gave a crude product which was recrystallised from ethyl acetate to yield **10a** (1.8 g (66.7%)), mp 125° - 126°C, rotation $[\alpha]_D$ = 32.5 ± 0.5 (2% in $CHCl_3$), ^{1}H-NMR (DMSO-d_6) : δ = 1.53 (d, 3H, J = 6 Hz, CH_3-C), 3.74 (s, 3H, CH_3O), 3.92 (dd, 1H, J_1 = 9 Hz, J_2 = 6 Hz, H_A-C(6)), 4.28 (dd, 1H, J_1=J_2 = 9 Hz, H-C(5)), 4.67 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 4.72 (q, 1H, J = 5 Hz, HC-Ph), 7.2 - 7.5 (m, 5H, C_6H_5), 13.40 (bs, 1H, COOH) ppm.

5,6-Dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5S-carboxylic acid(S-(+)-1)

10a (1.2 g, 4.2 mmol), 10% Pd / C (2.4 g) in dioxane (120 ml) were hydrogenated at 35°C and 5 bar for a period of 13 h. Work-up and purification as described for the preparation of racemic **1** yielded **S-(+)-1** (0.4 g (52.6%)), mp 182° - 183°C, rotation $[\alpha]_D$ = 4.1 ± 0.5 (2% in H_20), Anal. calcd. for $C_6H_8N_4O_3$: C, 39.1; H, 4.4; N, 30.4; found : C, 39.5; H, 4.5; N, 30.5; ^{1}H-NMR (DMSO-d_6) : identical to the spectrum of the racemate **1**. The enantiomeric excess as determined by HPLC was ≥ 96%, *vide infra.*

Synthesis of 5.6-dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5R-carbox-ylic acid (R-(-)-1)

Methyl 1-(1S-phenylethyl)-aziridine-2R-carboxylate (8c) and methyl 1-(1S-phenylethyl)-aziridine-2S-carboxylate (8d)

Starting with (S)-1-phenylethylamine, as described for the preparation of **8a** and **8b**, the compounds **8c** and **8d** were prepared and separated by flash chromatography (silica gel, ethyl acetate : hexane = 1 : 1) to provide two oily fractions. The first fraction and the second fraction were retrospectively (see Discussion) assigned the structures **8d** and **8c**.

(**8d**) : rotation $[\alpha]_D$ = - 100.5 ± 0.5 (2% in $CHCl_3$), ^{1}H-NMR ($CDCl_3$) : identical to that of **8b**.

(**8c**) : rotation $[\alpha]_D$ = + 50.6 ± 0.5 (2% in $CHCl_3$), ^{1}H-NMR ($CDCl_3$) : identical to that of **8a**.

Methyl 5,6-dihydro-2-methoxy-4-(1S-phenylethyl)-imidazo [1,2-b][1,2,4] triazole-5R-carboxylate (9c)

To a solution of **2** (21.7 g, 0.1 mol) and **8c** (17.8 g, 0.1 mol) in tetrahydrofuran (100 ml) was added boron trifluoride diethylether complex (5 ml) at room temperature. The reaction mixture was stirred for 3 h at 60°C. Work-up as described for **9a** afforded an oily residue which was chromatographed (flash chromatography) on silica gel (800 g) using ethyl acetate as eluant to yield **9c** as an oil (6.3 g (20.9%)), rotation $[\alpha]_D$ = - 12.0 ± 0.7 (1.3% in $CHCl_3$), ^{1}H-NMR ($CDCl_3$) : identical to the spectrum of **9a**.

5,6-Dihydro-2-methoxy-4-(1S-phenylethyl)-imidazo [1,2-b][1,2,4] triazole-5R-carboxylic acid (10c)

To a solution of **9c** (7.6 g, 25.2 mmol) in methanol (80 ml) was added KOH (85%, 2,7 g, 40 mmol) dissolved in water (2 ml). The reaction mixture was stirred at room temperature for 1 h. Work up as described for the preparation of **10a**, followed by recrystallisation of the crude product from ethyl acetate yielded **10c** (5.1 g (70.8%)), mp 122° - 123°C, rotation $[\alpha]_D$ = - 32.5 ± 0.4 (2% in $CHCl_3$), ^{1}H-NMR

($CDCl_3$) : δ = 1.67 (s, 3H, J =6 Hz, CH_3-C), 3.88 (s, 3H, CH_3O), 4.02 (dd, 1H, J_1 = 6Hz, J_2 = 9 Hz, H_A-C(6)), 4.15 (dd, 1H, J_1=J_2 = 9 Hz, H-C(5)), 4.38 (dd, 1H, J_1 = 6 Hz, J_2 = 9 Hz, H_B-C(6)), 5.08 (q, 1H, J = 5 Hz, HC-Ph), 7.2 - 7.4 (m, 5H, C_6H_5), 12.80 (bs, 1H, COOH) ppm.

5,6-Dihydro-2-methoxy-4H-imidazo [1,2-b][1,2,4] triazole-5R-carboxylic acid (R-(-)-1)

10c (3.8 g, 13.1 mmol) was hydrogenated over 10% Pd / C (7.6 g) in dioxane (190 ml) at 35°C / 5 bar during 14.5 h. Work-up and purification as described for S-(+)-1 gave R-(-)-1 (1.6 g (66.4%)), mp 179° - 180°C, rotation $[\alpha]_D$ = - 3.1 ± 1.0 (1% in H_2O), Anal. calcd. for $C_6H_8N_4O_3$: C, 39.1; H, 4.4; N, 30.4; found : C, 39.6; H, 4.5; N, 30.3; ^{1}H-NMR (DMSO-d_6) : identical to the spectrum of the racemate **1**. The enantiomeric excess as determined by HPLC was ≥ 96%, *vide infra*.

X-ray structure analysis of the intermediate 10a

A Philips PW1100 automatic diffractometer was used for data collection with CuKα radiation and graphite monochromator. The intensities of 1551 independent reflections with θ < 67° were measured, of which 1485 were classified as observed with I > 2σ(I). The structure was solved by direct methods and refined by fullmatrix least squares calculations with anisotropic (fixed for hydrogen atoms) thermal parameters to a final value of 0.069.

Crystal data: $C_{14}H_{16}N_4O_3$, orthorhombic, space group $P2_12_12_1$, a = 6.237 (1) Å,b = 13.337 (2) Å, c = 17.523 (2) Å, z = 4.

Fig. 4 shows the structure of **10a**, establishing the S-configuration at the chiral center C(5) bearing the carboxyl group.

HPLC determination of the enantiomeric purity of S-(+)-1 and R-(-)-1

To ca. 2 mg of racemic **1**, dissolved in dichloromethane, was added ca. 5 mg of 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and the reaction mixture placed in an ultrasonic bath. After 20 minutes 2 µl of S-(-)-1-(1-naphthyl)-ethylamine was added and the reaction mixture allowed to stand in the ultrasonic bath for 1 h. The dichloromethane was evaporated in a stream of nitrogen and the residue analysed on a Spectra-Physics LC-Instrument with a Perkin Elmer LC 235 UV diode array detector using Nucleosil C_{18} 250 x 4.6 mm column packed with 5 µm particles as the stationary phase, and water / acetonitrile 62 : 38, pH adjusted to 4.0 with phosphoric acid as the mobile phase at a flow rate of 2 ml/min. The injection volume was 20 µl and the detection wavelength was set at 220 nm. The chromatogramm showed two distinct peaks corresponding to the two enantiomers of **1**. Similarly S-(+)-1 and R-(-)-1 were derivatised with S-(-)-1-(1-naphthyl)-ethylamine and chromatographed using the above

conditions. Derivatised R-(-)-1 eluted before the derivatised S-(+)-1 and the retention peaks corresponded to the two retention peaks observed with the derivatised racemic 1. The enantiomeric excess of the two enantiomers S-(+)-1 and R-(-)-1 was determined by integration of the corresponding retention peaks and found to be ≥ 96%.

HPLC determination of the absolute configuration of the natural metabolite

0.7 µg of the natural metabolite were derivatised with S-(-)-1-(1-naphthyl)-ethylamine and analysed by HPLC using the conditions described in the previous section. Peak assignment based on retention time and UV spectrum showed the analysed natural metabolite to consist of 83% of the S-(+)-1 isomer and 17% of the R-(-)-1 isomer, see Fig 6.

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References and Notes

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