The Synthesis of Azatryptophane Derivatives

Jurij Svete*, Branko Stanovnik*, and Miha Tišler

Department of Chemistry, University of Ljubljana, 61000 Ljubljana, Slovenia

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Optically active and racemic isomers of aspartic acid (**1**) were transformed into the corresponding *N*-trifluoroacetyl-5-bromo-4-oxonorvaline methyl esters (**6**, R = Me) and *N*-trifluoroacetyl-3-formylalanine methyl esters **13**, which were used as starting compounds in the synthesis of β-heteroaryl substituted-α-amino acids.

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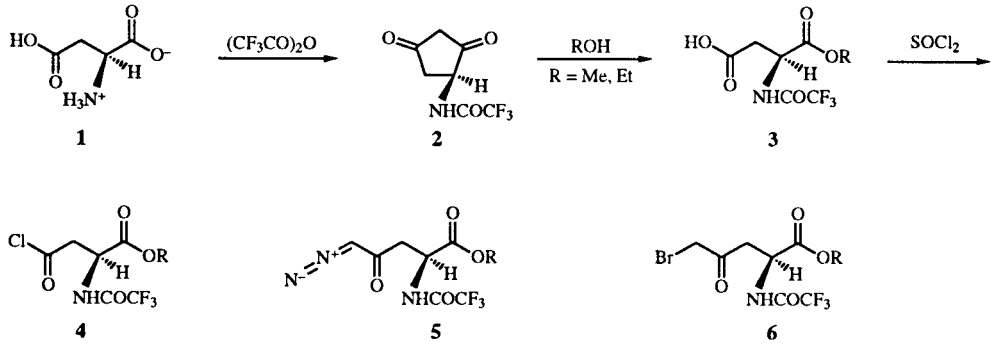
In continuation of our research in the area of heteroaryl substituted α-amino acids and heterocyclic systems containing an α-amino acid structural element [1] we report in this paper a novel synthesis of β-heteroaryl substituted α-amino acids and their derivatives. We have chosen (*R,S*), *R*, and *S* aspartic acid as the starting compounds. Our basic approach to this synthesis was the transformation of β-carboxy group of aspartic acid into a group which could be readily cyclized into a heterocyclic system. The synthesis of *N*-trifluoroacetyl-5-diazo-4-oxonorvaline ethyl ester which gives upon treating with hydrogen bromide *N*-trifluoroacetyl-5-bromo-4-oxonorvaline ethyl ester (**6**, R = Et) has been reported [2]. Later on, the same or similar norvaline derivatives have been prepared [3-5]. By this synthetic route L-aspartic acid (**1**) was transformed into L-*N*-trifluoroacetylaspartic acid anhydride (**2**) which upon treatment with an alcohol gave predominantly the α-ester of *N*-trifluoroacetylaspartic acid (**3**). The non-esterified carboxy group was transformed into an acid chloride **4** followed by the reaction with an excess of diazomethane, giving a diazo ketone **5** (Scheme 1).

Recently a similar transformation of aspartic acid into 3-(thiazol-4-yl)alanine and 3-(selenazol-4-yl)alanine derivatives using hexafluoroacetone as protective group was reported [7].

α-Halo ketones react with variety of 1,3-dinucleophiles giving heterocyclic systems as products [8]. The aim of our work was to determine whether *N*-trifluoroacetyl-5-bromo-4-oxonorvaline methyl ester (an α-haloketone) reacts with 1,3-dinucleophiles under relatively mild conditions in order to prevent the racemisation. For this purpose, 2-aminopyridine, 2-aminopyrimidine, 3-amino-6-chloropyridazine, thiobenzamide, and thiourea were chosen as 1,3-dinucleophiles. When (*S*)-*N*-trifluoroacetyl-5-bromo-4-oxonorvaline methyl ester (**6**, R = Me) was refluxed in methanol in the presence of 2-aminopyridine, 2-aminopyrimidine or 3-amino-6-chloropyridazine, (*S*)-*N*-trifluoroacetyl-3-(imidazo[1,2-*a*]pyridin-2-yl)alanine methyl ester (**7**), (*S*)-*N*-trifluoroacetyl-3-(imidazo[1,2-*a*]pyrimidin-2-yl)alanine methyl ester (**8**) and (*S*)-*N*-trifluoroacetyl-3-(6-chloroimidazo[1,2-*b*]pyridazin-2-yl)alanine methyl ester (**9**) were obtained respectively. Thiourea and thiobenzamide gave 3-thiazolyl substituted (*S*)-*N*-trifluoroacetylalanine methyl esters, **10**, **11**). The hydrolysis of **9** in 5*N* hydrochloric acid gave free 3-(6-chloroimidazo[1,2-*b*]pyridazin-2-yl)alanine (**12**) (Scheme 2).

Scheme 1



N-Trifluoroacetylaspartic acid-4-chloride-1-methyl ester (**4**, R = Me) was reduced into *N*-trifluoroacetyl-3-formylalanine methyl ester (**13**) by Rosenmund reduction [5,6]. The reduction proceeded smoothly in boiling benzene. We found this compound very interesting since it can be easily transformed into heteroarylhydrazones **14**, **15**, and **16** which were then oxidatively cyclised with bromine, according to the procedure described earlier for

Scheme 2

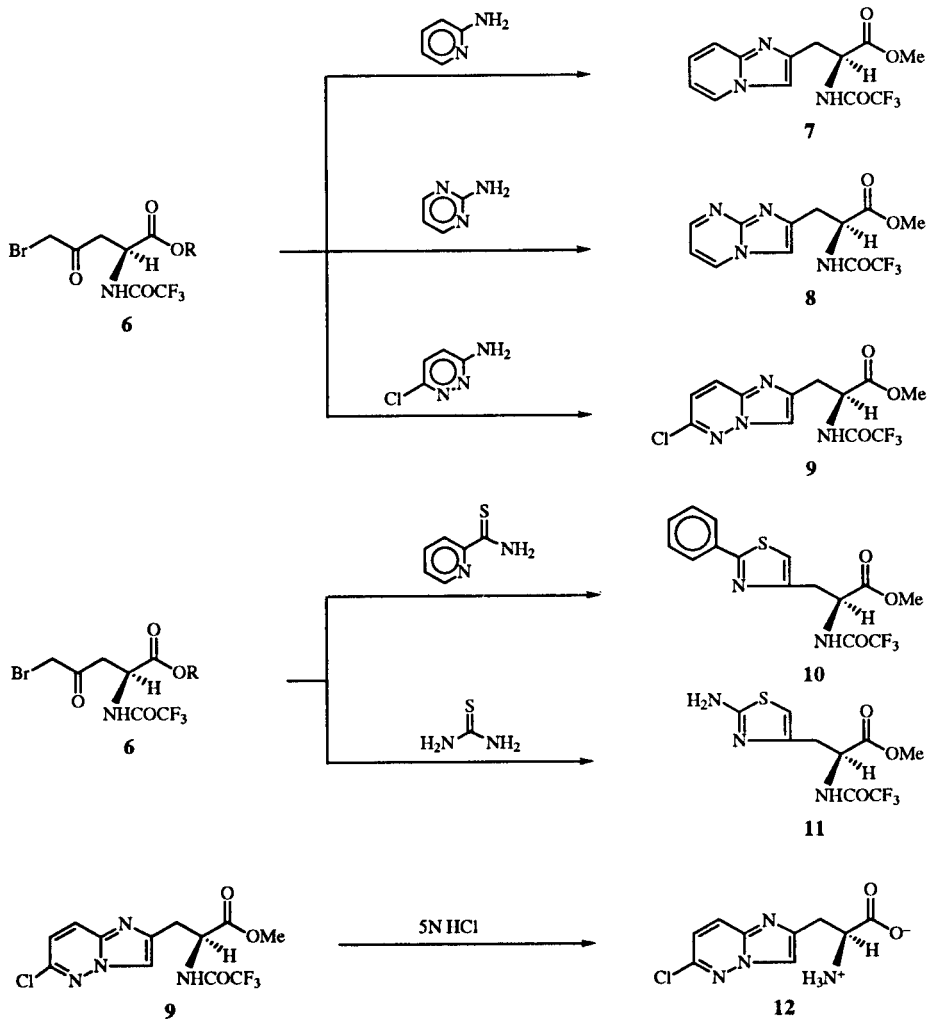


Table 1

Separation Conditions and Results [a]

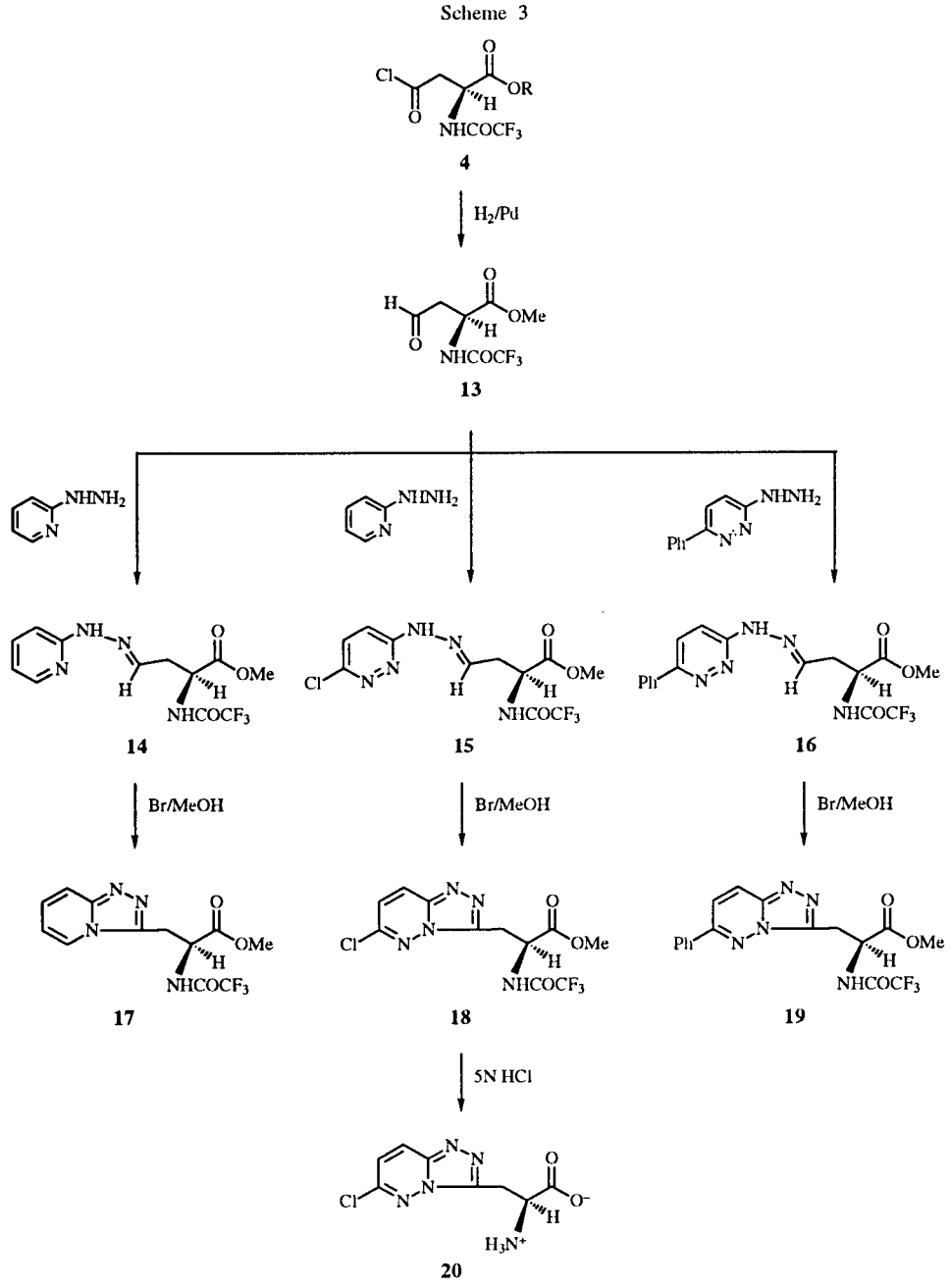
Compound	Mobile Phase	Retention Time (minutes)	Area %
9 *R,S*	A	25.865	41.17
		27.582	58.83
9 *R*	A	27.287	100.00
9 *S*	A	25.788	100.00
18 *R,S*	B	35.358	37.16
		36.741	62.84
18 *R*	B	36.694	100.00
18 *S*	B	35.634	100.00

[a] The following instrumentation and conditions apply: liquid chromatograph: Hewlett Packard Series 1050; column: Supelco LC-(*R*)-naphthyl urea, 250 x 4.6 mm; flow: 1 ml/minute; detection: uv 319 nm, BW 2 nm; sample amount: 10 µl, c = 1 mg/ml (for *R,S* isomers), c = 0.5 mg/ml (for *R* and *S* isomers); mobile phase: A (*n*-heptane 80%, ethyl acetate 20%), B (*n*-heptane 73.5%, ethyl acetate 25%, 2-propanol 1.5%). [b] The peaks of pure enantiomeric forms have a tailing shape, which explains the greater area percent amount of the enantiomeric form with larger retention time.

the synthesis of 3-alkyl- and 3-aryl substituted *s*-triazolo[4,3-*b*]pyridazine derivatives [8] into *N*-trifluoroacetyl-3-(*s*-triazoloazinyl)alanine methyl esters **17**, **18**, and **19**. For these transformations the following heteroarylhydrazines were chosen: 2-hydrazinopyridine, 3-hydrazino-6-chloropyridazine, and 3-hydrazino-6-phenylpyridazine. The hydrolysis of **18** gave free 3-(6-chloro-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine (**20**) (Scheme 3).

All three isomers of 3-(6-chloroimidazo[1,2-*b*]pyridazin-2-yl)alanine (**12**) and 3-(6-chloro-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine (**20**) have been synthesised in order to study the extent of racemisation. For the determination of optical purity of intermediate compounds we separated racemic compounds **9** and **18** by means of hplc using a chiral supported column. When (*R*) or (*S*) enantiomeric form of the compound **9** or **18** was eluted through a chiral supported column, no other enantiomeric form was detected. Supported by this observation and by comparing the melting points and optical rotations of (*R*) and (*S*) enantiomeric forms of intermediate

Scheme 3



compounds **2-6**, **9** and **18**, we assumed that there was no significant racemisation during the synthesis and isolation of products (see experimental details). However, these facts do not prove the optical purity of the compounds synthesised because the racemisation might take place to the same extent for both enantiomeric forms (Table 1, Experimental).

EXPERIMENTAL

Melting points were taken on a Büchi 535 melting point apparatus. The ^1H nmr spectra were obtained on a Varian EM360L (60 MHz) spectrometer and the ir spectra on a Perkin-Elmer 1310 spectrophotometer. The microanalyses for C, H, and N were determined on a Perkin-Elmer Analyser 2400. The optical rotations were measured on a Perkin-Elmer 241 MC Polarimeter. Chromatographic separations of enantiomers and determinations of optical purity were achieved on a Hewlett Packard Liquid Chromatograph 1050. For further details concerning chromatographic separations see Table 1.

(*S*)-*N*-Trifluoroacetylaspartic Acid-4-chloride-1-methyl Ester (**4**, R = Me).

This compound was prepared by a slightly modified procedure described in the literature [2-4]. Trifluoroacetic anhydride (80 ml) was slowly added through a reflux condenser to finely

powdered L-aspartic acid (26.6 g, 0.2 mole) while vigorous stirring at -15° and then stirred at this temperature for one hour. The reaction mixture was then allowed to warm slowly (in a period of one hour) to 30-40° when the solution first became homogenous but then (*S*)-*N*-trifluoroacetylaspartic acid anhydride (**2**) precipitated. From this solid mass volatile components (excess TFAA and TFA) were removed first by evaporation *in vacuo* at 30-40° and then by drying over sodium hydroxide in an evacuated desiccator for several hours. Dry methanol (150 ml) was added to a crude anhydride and the mixture was heated under reflux for one hour. Methanol was removed *in vacuo* to give a solid (*S*)-*N*-trifluoroacetylaspartic acid-1-methyl ester (**3**, R = Me). The crude ester was treated with 100 ml of dry toluene and 30 ml of thionyl chloride and stirred at 60-70° for one hour. The reaction mixture was then allowed to cool to room temperature and the (*S*)-*N*-trifluoroacetylaspartic acid-4-chloride-1-methyl ester (**4**, R = Me) was filtered off and washed with cold toluene. The crude product was dried overnight *in vacuo* over sodium hydroxide, and then if necessary, recrystallised from benzene, overall yield, 40.0 g (76%).

(*R*) And (*R,S*) isomers of **4** were prepared in the same manner.

Analogously the following compounds were prepared:

N-Trifluoroacetylaspartic Acid Anhydride (**2**).

S-Isomer [2-4].

This compound was prepared from the (*S*)-aspartic acid (**1**) and trifluoroacetic anhydride, mp 133-134° (from *n*-hexane/ethyl acetate), $[\alpha]_D^{22}$ = -44 0° (c = 2.0, ethyl acetate), lit [2] mp 133-134° (from acetone/ether), $[\alpha]_D^{22}$ = -22.3° (c = 0.63, absolute THF).

R-Isomer.

This compound was prepared from the (*R*)-aspartic acid (**1**) and trifluoroacetic anhydride, mp 134° (from cyclohexane/ethyl acetate), $[\alpha]_D^{23}$ = +43.5° (c = 2.42, ethyl acetate).

R,S-Isomer.

This compound was prepared from the (*R,S*)-aspartic acid (**1**) and trifluoroacetic anhydride, mp 130-132° (from *n*-hexane/ethyl acetate).

N-Trifluoroacetylaspartic Acid-4-chloride-1-methyl Ester (**4**, R = Me).

S-Isomer [2-4].

This compound was prepared from the (*S*)-isomer of **2** and methanol, following by the reaction with thionyl chloride, mp 114-115° (from *n*-heptane), $[\alpha]_D^{22}$ = -21.8° (c = 3.46, ethyl acetate), lit [4] mp 114-115° (from benzene), $[\alpha]_D^{27}$ = +62.6° (c = 1.830, chloroform).

R-Isomer.

This compound was prepared from the (*R*)-isomer of **2** and methanol, following by the reaction with thionyl chloride, mp 114-116° (from benzene), $[\alpha]_D^{22}$ = +22.2° (c = 2.98, ethyl acetate).

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **2** and methanol, following by the reaction with thionyl chloride, mp 101-103° (from *n*-heptane).

N-Trifluoroacetyl-5-diazo-4-oxonorvaline Methyl Ester (**5**, R = Me).

This compound was prepared by a slightly modified procedure described in the literature [3,4]. To an excess of cold, ethanol-free, ethereal solution of diazomethane the solution of *N*-trifluoroacetylaspartic acid-4-chloride-1-methyl ester (**4**, R = Me) (13.08 g, 0.05 mole) in dry ethyl acetate (100 ml) was slowly added at 0-5°. The mixture was left at 0° until the evolution of nitrogen have ceased (approximately 3/4 hour), and then allowed to warm at room temperature. The solution was left overnight in a good ventilated hood, and the excess diazomethane and solvent allowed to evaporate. The yield of crude product is almost quantitative.

S-Isomer.

This compound was prepared from the (*S*)-isomer of **4** (R = Me) and diazomethane, mp 105-107° (from toluene), $[\alpha]_D^{22}$ = -14.2° (c = 3.56, methanol); ir (potassium bromide): 2135 (N_2) cm^{-1}, lit [4] mp 104-105° (from chloroform/petroleum ether), $[\alpha]_D$ = -15.0° (c = 0.746, methanol); ir (Nujol): 2105 (N_2) cm^{-1}.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **4** (R = Me) and diazomethane, mp 106-107° (from toluene), $[\alpha]_D^{22}$ = +17.1° (c = 2.187, methanol); ir (potassium bromide): 2125 (N_2) cm^{-1}.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **4** (R = Me) and diazomethane, mp 95-97° (from toluene), ir (potassium bromide): 2125, 2135 (N_2) cm^{-1}. (During the crystallisation the toluene was not heated over 90°!)

N-Trifluoroacetyl-5-bromo-4-oxonorvaline Methyl Ester (**6**, R = Me).

To the suspension of *N*-trifluoroacetyl-5-diazo-4-oxonorvaline methyl ester (**5**, R = Me) (8.01 g, 0.03 mole) in 50 ml of dry ether the solution of hydrogen bromide in acetic acid (33% in acetic acid, 7 ml, *ca.* 0.04 mole) was slowly added at 0°. Stirring was continued for 15 minutes and the reaction mixture was allowed to warm to room temperature. Ethyl acetate (100 ml) was added and the solution washed first with water (100 ml) and then three times with 100 ml of aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, filtered, and the solvent removed *in vacuo*. The crude product was recrystallised from *n*-heptane.

S-Isomer.

This compound was prepared from the (*S*)-isomer of **5** (R = Me) and hydrogen bromide in 80% yield, mp 109-110° (from cyclohexane), $[\alpha]_D^{23}$ = +3.2° (c = 2.258, ethyl acetate); ^1H nmr (deuteriochloroform): δ 3.37-3.50 (2H m, CH_2CH), 3.80 (3H, s, OMe), 3.91 (2H, s, CH_2Br), 4.87 (1H, dt, $CHCOOMe$), 7.40 (1H, broad peak, NH), J_{CH-NH} = 8 Hz, J_{CH2-CH} = 4 Hz.

Anal. Calcd. for $C_8H_9BrF_3NO_4$: C, 30.02; H, 2.83; N, 4.38. Found: C, 30.28; H, 2.60; N, 4.44.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **5** (R = Me) and hydrogen bromide in 83% yield, mp 110° (from cyclohexane), $[\alpha]_D^{22}$ = -2.6° (c = 2.258, ethyl acetate): ^1H nmr (deuteriochloroform): δ 3.33-3.45 (2H, m, CH_2CH), 3.80 (3H, s,

OMe), 3.88 (2H, s, CH_2Br), 4.85 (1H, dt, C*H*COOMe), 7.33 (1H, broad peak, NH), J_{CH-NH} = 8 Hz, J_{CH-CH2} = 4 Hz.

Anal. Calcd. for $C_8H_9BrF_3NO_4$: C, 30.02; H, 2.83; N, 4.38. Found: C, 30.18; H, 2.59; N, 4.42.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **5** (R = Me) and hydrogen bromide in 91% yield, mp 94-95° (from cyclohexane); ^1H nmr (deuteriochloroform): δ 3.33-3.45 (2H, m, CH_2CH), 3.79 (3H, s, OMe), 3.89 (2H, s, CH_2Br), 4.85 (1H, dt, C*H*COOMe), 7.35 (1H, broad peak, NH), J_{CH-NH} = 8 Hz, J_{CH-CH2} = 4 Hz.

Anal. Calcd. for $C_8H_9BrF_3NO_4$: C, 30.02; H, 2.83, N, 4.38. Found: C, 30.28; H, 2.60; N, 4.44.

N-Trifluoroacetyl-3-(imidazo[1,2-*x*]azin-2-yl)alanine Methyl Esters **7**, **8**, and **9**. General Procedure.

The mixture of *N*-trifluoroacetyl-5-bromo-4-oxonorvaline methyl ester (**6**, R = Me) (1.600 g, 0.01 mole), *o*-aminoazine (0.01 mole) and methanol (50 ml) was heated under reflux for 12 hours, cooled, and the solvent evaporated *in vacuo*. Aqueous sodium hydrogen carbonate (50 ml) was added to the residue, stirred, and the mixture left at the room temperature until the oily residue crystallised (about 2-3 hours). The precipitate was filtered off and washed with water.

The following compounds were prepared in this manner:

(*S*)-*N*-Trifluoroacetyl-3-(imidazo[1,2-*a*]pyridin-2-yl)alanine Methyl Ester (**7**).

This compound was prepared from the (*S*)-isomer of **6** (R = Me) and 2-aminopyridine in 57% yield, mp 153-154° (from *n*-heptane), $[\alpha]_D^{24}$ = -41.1° (c = 2.252, ethyl acetate); ^1H nmr (deuteriochloroform): δ 3.33 and 3.37 (2H, 2d, CH_2CH), 3.69 (3H, s, OMe), 4.95 (1H, dt, C*H*COOMe), 6.82 (1H, dt, $H_{6'}$), 7.16 (1H, ddd, $H_{7'}$), 7.45 (1H, s, $H_{3'}$), 7.50 (1H, dd, $H_{8'}$), 8.12 (1H, br d, $H_{8'}$) 9.33 (1H, broad peak, NH), J_{CH-CH2} = 4.5 Hz, J_{CH-NH} = 8 0 Hz.

Anal. Calcd. for $C_{13}H_{12}F_3N_3O_3$: C, 49.53; H, 3.84; N, 13.33. Found: C, 49.75; H, 3.60; N, 13.31.

(*S*)-*N*-Trifluoroacetyl-3-(imidazo[1,2-*a*]pyrimidin-2-yl)alanine Methyl Ester (**8**).

This compound was prepared from the (*S*)-isomer of **6** (R = Me) and 2-aminopyrimidine in 16% yield, mp 172-174° dec (from diisopropyl ether), $[\alpha]_D^{22}$ = -6.6° (c = 1.756, methanol); ^1H nmr (DMSO-d_6): 3.30 (2H, d, CH_2), 3.70 (3H, s, OMe), 4.83 (1H, t, C*H*COOMe), 7.03 (1H, dd, $H_{6'}$), 7.80 (1H, s, $H_{3'}$), 8.57 (1H, dd, $H_{7'}$), 9.03 (1H, dd, $H_{5'}$), 10.04 (1H, broad peak, NH), J_{CH-CH2} = 7.0 Hz, J_{CH-NH} = 7 0 Hz, $J_{H6'H5'}$ = 6.6 Hz, $J_{H6'H7'}$ = 4 0 Hz, $J_{H7'H5'}$ = 2.1 Hz.

*Anal.*Calcd for $C_{12}H_{11}F_3N_4O_3$: C, 45.58; H, 3.51; N, 17.72. Found: C, 45.48; H, 3.43; N, 17.65.

The crude oily product **8** sometimes does not crystallise. In this case the crude product is redissolved in methanol (30 ml), 4 g of silica gel (0.035-0.070 mm) is added and the mixture is carefully evaporated. This silica gel is poured in a stabilised column filled with silica gel (0.035-0.070 mm) and the product is eluted with a mixture of chloroform and methanol (25:1). The solution of purified product is evaporated *in vacuo* and the residue crystallised from *n*-heptane.

N-Trifluoroacetyl-3-(6-chloroimidazo[1,2-*b*]pyridazin-2-yl)-alanine Methyl Ester (**9**).

S-Isomer.

This compound was prepared from the (*S*)-isomer of **6** (R = Me) and 3-amino-6-chloropyridazine in 46% yield, mp 114-116° (from methanol/water 1:2), $[\alpha]_D^{23}$ = +4.0° (c = 1.301, ethyl acetate); ^1H nmr (deuteriochloroform): δ 3.38 and 3.42 (2H, 2d, CH_2), 3.72 (3H, s, OMe), 4.99 (1H, dt, C*H*COOMe), 7.10 (1H, d, $H_{7'}$), 7.80 (s, 1H, $H_{3'}$), 7.87 (d, 1H, $H_{8'}$), 8.63 (1H, broad peak, NH), J_{CH2-CH} = 4.4 Hz, J_{CH-NH} = 8.1 Hz, $J_{H7'H8'}$ = 9.8 Hz.

Anal. Calcd. for $C_{12}H_{10}ClF_3N_4O_3$: C, 41.10; H, 2.87; N, 15.98. Found: C, 40.80; H, 2.54; N, 16. 14.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **6** (R = Me) and 3-amino-6-chloropyridazine in 74% yield, $[\alpha]_D^{22}$ = -3.95° (c = 2.531, ethyl acetate), mp 118-119° (from methanol/water 1:2); ^1H nmr (deuteriochloroform): δ 3.37 and 3.42 (2H, 2d, CH_2), 3.73 (s, 3H, OMe), 4.99 (1H, dt, C*H*COOMe), 7.08 (1H, d, $H_{7'}$), 7.79 (1H, s, $H_{3'}$), 7.87 (1H, d, $H_{8'}$), 8.63 (1H, broad peak, NH), J_{CH2-CH} = 4.4 Hz, J_{CHNH} = 8.1 Hz, $J_{H7'H8'}$ = 9.8 Hz.

Anal. Calcd. for $C_{12}H_{10}ClF_3N_4O_3$: C, 41.10; H, 2.87; N, 15.98. Found: C, 41.06; H, 2.60; N, 16.06.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **6** (R = Me) and 3-amino-6-chloropyridazine in 74% yield, mp 157-158° (from methanol/water 1:1); ^1H nmr (deuteriochloroform): δ 3.42 and 3.46 (2H, 2d, CH_2), 3.77 (3H, s, OMe), 5.03 (1H, dt, C*H*COOMe), 7.13 (1H, d, $H_{7'}$), 7.79 (1H, s, $H_{3'}$) 7.90 (1H, d, $H_{8'}$), 8.67 (1H, broad peak, NH), J_{CH2-CH} = 4.2 Hz, J_{CHNH} = 8.4 Hz, $J_{H7'H8'}$ = 10 Hz.

Anal. Calcd. for $C_{12}H_{10}ClF_3N_4O_3$: C, 41.10; H, 2.87; N, 15.98. Found: C, 40.90; H, 2.55; N, 16.01.

(*S*)-*N*-Trifluoroacetyl-3-(2-phenylthiazol-4-yl)alanine Methyl Ester (**10**).

The mixture of (*S*)-*N*-trifluoroacetyl-5-bromo-4-oxonorvaline methyl ester (**6**, R = Me) (3.200 g, 0.01 mole), thiobenzamide (1.370 g, 0.01 mole), and methanol (50 ml) was refluxed for 12 hours, cooled, and the solvent evaporated *in vacuo*. Aqueous sodium hydrogen carbonate (100 ml) was added to the residue, stirred and the mixture was left at room temperature until the oily residue crystallised (about 24 hours). The precipitate was filtered off and crystallised from *n*-hexane, yield 2.27 g (63%), mp 85-86° (from *n*-hexane), $[\alpha]_D^{24}$ = -43.0° (c = 2.527, ethyl acetate); ^1H nmr (deuteriochloroform): δ 3.37 and 3.40 (2H, 2d, CH_2), 3.73 (3H, s, OMe), 4.96 (1H, dt, C*H*COOMe), 7.09 (1H, s, $H_{5'}$), 7.43-7.63 (3H, m, Ph), 7.90-8.07 (2H, m, Ph), 8.72 (1H, broad peak, NH), J_{CH-CH2} = 4.2 Hz, J_{CHNH} = 8.0 Hz.

Anal. Calcd. for $C_{15}H_{13}F_3N_2O_3S$: C, 50.28; H, 3.66; N, 7.82. Found: C, 50.48; H, 3.30; N, 7.90.

(*S*)-*N*-Trifluoroacetyl-3-(2-aminolthiazol-4-yl)alanine Methyl Ester (**11**).

The solution of (*S*)-*N*-trifluoroaceyl-5-bromo-4-oxonorvaline methyl ester (**6**, R = Me) (1.600 g, 0.005 mole), and thiourea (0.380 g, 0.005 mole) in methanol (25 ml) was left at room temperature for 24 hours. *N*-methylmorpholine (0.55 ml) was added to the solution, left for one hour and the solvent was evaporated *in vacuo*. The residue was dissolved in 80 ml of chloroform, washed

twice with 1.5 ml of water, dried over anhydrous sodium sulphate, filtered, and chloroform evaporated *in vacuo*. Petroleum ether (30 ml) was added to the residue, stirred until the oily product crystallised, and the precipitate filtered off, yield 1.21 g (81%), mp 92-93° (from water), $[\alpha]_D^{22}$ = -51.0° (c = 1.803, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.08 (2H, d, CH_2), 3.72 (3H, s, OMe), 4.65-5.04 (3H, m, C*H*COOMe and NH_2), 6.22 (1H, s, $H_{5'}$), 8.4 (1H, broad peak, NH), J_{CH2CH} = 5.2 Hz.

Anal. Calcd. for $C_9H_{10}F_3N_3O_3S$: C, 36.37; H, 3.39; N, 14.14. Found: C, 36.07; H, 3.03; N, 14.47.

3-(6-Chloroimidazo[1,2-*b*]pyridazin-2-yl)alanine (**12**).

The mixture of *N*-trifluoroacetyl-3-(6-chloroimidazo[1,2-*b*]pyridazin-2-yl)alanine methyl ester (**9**) (1.758 g, 0.005 mole) and 5*N* hydrochloric acid (30 ml) was heated under reflux for 5 hours, cooled and volatile components evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of water, and the solution neutralised with *N*-methylmorpholine to *p*H value 7. Methanol (20 ml) was added and the mixture left overnight at room temperature. The product was filtered off and washed with methanol.

S-Isomer.

This compound was prepared from the (*S*)-isomer of **9** and 5*N* hydrochloric acid in 70% yield, mp 264-265° dec (from water), $[\alpha]_D^{24}$ = -3.85° (c = 0.78, water).

Anal. Calcd. for $C_9H_9ClN_4O_2$: C, 44.92; H, 3.75; N, 23.28. Found: C, 44.53; H, 3.57; N, 23.40.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **9** and 5*N* hydrochloric acid in 65% yield, mp 264-265° dec (from water), $[\alpha]_D^{22}$ = +2.54° (c = 0.236, water).

Anal. Calcd. for $C_9H_9ClN_4O_2$: C, 44.92; H, 3.75; N, 23.28. Found: C, 45.24; H, 3.44; N, 23.16.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **9** and 5*N* hydrochloric acid in 69% yield, mp 274-275° (from water); ms: 240.043200 (M^+, $C_9H_9ClN_4O_2$).

Anal. Calcd. for $C_9H_9ClN_4O_2$: C, 44.92; H, 3.75; N, 23.28. Found: C, 44.51; H, 3.41; N, 23.62.

N-Trifluoroacetyl-3-formylalanine Methyl Ester (**13**).

A slow stream of dry hydrogen was passed through a stirred mixture of dry benzene (100 ml), 10% Pd-C catalyst (300 mg), and *N*-trifluoroacetylaspartic acid-4-chloride-1-methyl ester (**4**, R = Me) (5.230 g, 0.02 mole) at the reflux temperature for 3-5 hours. When no more hydrogen chloride was formed, the introduction of hydrogen was ceased. The reaction mixture was filtered hot through a glass filter and the filtrate evaporated *in vacuo*. For further transformations the crude product was employed.

S-Isomer.

This compound was prepared by hydrogenating the (*S*)-isomer of **4** (R = Me) in 96% yield, mp 77-78° (from *n*-hexane), $[\alpha]_D^{22}$ = -21.7° (c = 3.15, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.21 and 3.24 (2H, 2d, CH_2), 3.79 (3H, s, OMe), 4.83 (1H, dt, C*H*COOMe), 7.37 (1H, broad peak, NH), 9.82 (1H, s, CHO), J_{CH2-CH} = 4.5 Hz, J_{CH-NH} = 8 0 Hz.

Anal. Calcd. for $C_7H_8F_3NO_4$: C, 37.02; H, 3.55; N, 6.17. Found: C, 37.37; H, 3.29; N, 6.33.

R-Isomer.

This compound was prepared by hydrogenating the (*R*)-isomer of **4** (R = Me) in 78% yield, mp 76-77° (from *n*-hexane), $[\alpha]_D^{23}$ = 21.0° (c = 2.337, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.23 and 3.26 (2H, 2d, CH_2), 3.83 (3H, s, OMe), 4.86 (1H, dt, C*H*COOMe), 7.37 (1H, broad peak, NH), 9.83 (1H, s, CHO), J_{CH2-CH} = 4.5 Hz, J_{CH-NH} = 8.0 Hz.

Anal. Calcd. for $C_7H_8F_3NO_4$: C, 37.02; H, 3.55; N, 6.17. Found: C, 36.95; H, 3.39; N, 6.24.

R,S-Isomer.

This compound was prepared by hydrogenating the (*R,S*)-isomer of **4** (R = Me) in 85% yield, mp 52-53° (from cyclohexane), nmr (deuteriochloroform): δ 3.24 and 3.27 (2H, 2d, CH_2), 3.83 (3H, s, OMe), 4.86 (1H, dt, C*H*COOMe), 7.40 (1H, broad peak, NH), 9.86 (1H, s, CHO), J_{CH2-CH} = 4.5 Hz, J_{CH-NH} = 8.0 Hz.

Anal. Calcd. for $C_7H_8F_3NO_4$: C, 37.02; H, 3.55; N, 6.17. Found: C, 37.35; H, 3.32; N, 6.26.

N-Trifluoroacetyl-3-formylalanine Methyl Ester Heteroarylhydrazones **14**, **15**, and **16**. General Procedure.

To a stirred mixture of heteroarylhydrazine (0.001 mole), methanol (3 ml) and 0.1 ml of acetic acid 227 mg (0.001 mole) of *N*-trifluoroacetyl-3-formylalanine methyl ester (**9**) were added and the solution stirred at room temperature for one hour. Then water (10 ml) was added and the reaction mixture was neutralised with sodium hydrogen carbonate. The suspension was stirred for a while, and the precipitate filtered off.

The following compounds were prepared in this manner:

(*S*)-*N*-Trifluoroacetyl-3-formylalanine Methyl Ester-(2-pyridyl)hydrazone (**14**).

This compound was prepared from the (*S*)-isomer of **13** and 2-hydrazinopyridine in 78% yield, mp 156° (from methanol/water), $[\alpha]_D^{23}$ = -38.3° (c = 2.09, DMF); ^{1}H nmr (DMSO-d_6): δ 2.67-2.90 (2H, m, CH_2), 3.70 (3H, s, OMe), 4.55-4.93 (1H, m, C*H*COOMe), 6.70 (1H, ddd, $H_{5'}$), 7.00 (1H, ddd, $H_{3'}$), 7.36 (1H, t, CH=N), 7.62 (1H, dt, $H_{4'}$), 8.10 (1H, ddd, $H_{6'}$), 9.97 (1H, broad peak, N*H*CH), 10.44 (1H, s, NH-Het), J_{CH2-CH} = 4.8 Hz.

Anal. Calcd. for $C_{12}H_{13}F_3N_4O3$: C, 45.29; H, 4.12; N, 17.60. Found: C, 45.37; H, 3.80; N, 17.79.

N-Trifluoroacetyl-3-formylalanine Methyl Ester-(6-chloro-3-pyridazinyl)hydrazone (**15**).

S-Isomer.

This compound was prepared from the (*S*)-isomer of **13** and 6-chloro-3-hydrazinopyridazine in 82% yield, mp 191-192° (from methanol), $[\alpha]_D^{22}$ = -37.8° (c = 2.02, DMF); ^{1}H nmr (DMSO-d_6): δ 2.70-2.93 (2H, m, CH_2), 3.68 (3H, s, OMe), 4.61-4.91 (1H, m, C*H*COOMe), 7.33 (1H, d, $J_{H4'H5'}$ = 9.2 Hz, $H_{5'}$), 7.44 (1H, t, CH=N), 7.65 (d, 1H, $H_{4'}$), 9.95 (1H, broad peak, N*H*CH), 11.36 (1H, s, NH-Het), J_{CH2-CH} = 5.0 Hz.

Anal. Calcd for $C_{11}H_{11}ClF_3N_5O_3$: C, 37.36; H, 3.13; N, 19.80. Found: C, 37.46; H, 2.82; N, 19.75.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **13** and 6-chloro-3-hydrazinopyridazine in 43% yield, mp 191° (from methanol), $[\alpha]_D^{23}$ = +37.4° (c = 2.46, DMF); ^{1}H nmr (DMSO-d_6): δ 2.70-2.93 (2H, m, CH_2), 3.70 (3H, s, OMe), 4.62-4.93

(1H, m, C*H*COOMe), 7.42 (1H, d, $J_{H4'H5'}$ = 9.5 Hz, $H_{5'}$), 7.50 (1H, t, CH=N), 7.70 (d, 1H, $H_{4'}$), 10.04 (1H, broad peak, N*H*CH), 11.44 (1H, s, NH-Het), J_{CH2-CH} = 4 6 Hz.

Anal. Calcd. for $C_{11}H_{11}ClF_3N_5O_3$: C, 37.36; H, 3.13; N, 19.80. Found: C, 37.27; H, 2.92; N, 20. 16.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **13** and 6-chloro-3-hydrazinopyridazine in 84% yield, mp 188-189° (from methanol); ^{1}H nmr (DMSO-d_6): δ 2.70-2.91 (2H, m, CH_2), 3.70 (3H, s, OMe), 4.64-4.90 (1H, m, C*H*COOMe), 7.37 (1H, d, $J_{H4'H5'}$ = 9.4 Hz, $H_{5'}$), 7.46 (1H, t, CH=N), 7.66 (d, 1H, $H_{4'}$), 9.98 (1H, broad peak, N*H*CH), 11.37 (1H, s, NH-Het), J_{CH2-CH} = 4.5 Hz.

Anal. Calcd. for $C_{11}H_{11}ClF_3N_5O_3$: C, 37.36; H, 3.13; N, 19.80. Found: C, 37.37; H, 2.84; N, 19.65.

(*S*)-*N*-Trifluoroacetyl-3-formylalanine Methyl Ester-(6-phenyl-3-pyridazinyl)hydrazone (**16**).

This compound was prepared from the (*S*)-isomer of **13** and 3-hydrazino-6-phenylpyridazine in 74% yield, mp 194° dec (from methanol), $[\alpha]_D^{22}$ = 37 3° (c = 2.276, DMF); ^{1}H nmr (DMSO-d_6): δ 2.76-2.95 (2H, m, CH_2), 3.71 (3H, s, OMe), 4.80 (1H, m, C*H*COOMe), 7.31-7.56 (5H, m, 3H-Ph, CH=N, and $H_{5'}$), 8.03-8.17 (2H, m, Ph), 8.10 (1H, d, $J_{H4'H5'}$ = 9 Hz, $H_{4'}$), 10.00 (1H, d, N*H*CH), 11.33 (1H, s, NH-Het), J_{NH-CH} = 8 Hz.

Anal. Calcd. for $C_{17}H_{16}F_3N_5O_3$: C, 51.56; H, 4.08; N, 17.71. Found: C, 51.31; H, 3.87; N, 18.15.

N-Trifluoroacetyl-3-(*s*-triazolo[4,3-*x*]azin-3-yl)alanine Methyl Esters **17**, **18**, and **19**. General Procedure.

To a stirred mixture of *N*-trifluoroacetyl-3-formylalanine methyl ester heteroarylhydrazone (**10**) (0.01 mole), sodium acetate (0.03 mole) and methanol (50 ml) the solution of bromine (0.51 ml, 0.01 mole) in 50 ml of methanol was added dropwise in a period of 30 minutes. The solution was stirred for one hour, methanol evaporated *in vacuo*, and 50 ml of aqueous sodium bicarbonate was added to the residue and the mixture was stirred for another hour. The precipitate was filtered off, and the filtrate extracted four times with 15 ml of chloroform. The organic phases were collected, dried over anhydrous sodium sulfate, filtered, and the filtrate evaporated *in vacuo*. The residue was triturated with 15 ml of ether, cooled in a refrigerator, and the crystals filtered off. Both precipitates were collected.

The following compounds were prepared in this manner:

(*S*)-*N*-Trifluoroacetyl-3-(*s*-triazolo[4,3-*a*]pyridin-3-yl)alanine Methyl Ester (**17**).

This compound was prepared fom the (*S*)-isomer of **14** and bromine in 81% yield, mp 115-117° (from benzene), $[\alpha]_D^{23}$ = +18.8° (c = 2.292, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.75 (2H, t, CH_2), 3.77 (3H, s, OMe), 5.29 (1H, dt, C*H*COOMe), 6.96 (1H, ddd, $H_{6'}$), 7.36 (1H, ddd, $H_{7'}$), 7.80 (1H, ddd, $H_{8'}$), 8.10 (1H, ddd, $H_{5'}$), 8.86 (1H, br d, NH), J_{CH2-CH} = 5.1 Hz, J_{NH-CH} = 8.2 Hz.

Anal. Calcd. for $C_{12}H_{11}F_3N_4O_3$: C, 45.58; H, 3.51; N, 17.72. Found: C, 45.37; H, 3.39; N, 17.73.

N-Trifluoroacetyl-3-(6-chloro-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine Methyl Ester (**18**).

S-Isomer.

This compound was prepared from the (S)-isomer of **15** and bromine in 81% yield, mp 101-102° (from diisopropyl ether), $[\alpha]_D^{23}$ = -16.0° (c = 3.287, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.77-3.91 (2H, overlapped peak, CH_2), 3.83 (3H, s, OMe), 5.26 (1H, dt, C*H*COOMe), 7.20 (1H, d, $H_{7'}$), 8.08 (1H, broad peak, NH), 8.16 (1H, d, $H_{8'}$), J_{CH2-CH} = 5.1 Hz, J_{CHNH} = 8.2 Hz, $J_{H7'H8'}$ = 9.5 Hz.

Anal. Calcd. for $C_{11}H_9ClF_3N_5O_3$: C, 37.57; H, 2.58; N, 19.91. Found: C, 37.86; H, 2.11; N, 19.90.

R-Isomer.

This compound was prepared from the (*R*)-isomer of **15** and bromine in 77% yield, mp 102° (from diisopropyl ether/ethyl acetate 7:1), $[\alpha]_D^{22}$ = +17.3° (c = 2.981, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.77-3.90 (2H, overlapped peak, CH_2), 3.80 (3H, s, OMe), 5.27 (1H, dt, C*H*COOMe), 7.21 (1H, d, $H_{7'}$), 8.11 (1H, broad peak, NH), 8.19 (1H, d, $H_{8'}$), J_{CH2-CH} = 5.1 Hz, J_{CHNH} = 8.2 Hz, $J_{H7'H8'}$ = 9.5 Hz.

Anal. Calcd. for $C_{11}H_9ClF_3N_5O_3$: C, 37.57; H, 2.58; N, 19.91. Found: C, 37.73; H, 2.28; N, 19.74.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **15** and bromine in 75% yield, mp 132° (from benzene); ^{1}H nmr (deuteriochloroform): δ 3.73-3.88 (2H, overlapped peak, CH_2), 3.80 (3H, s, OMe), 5.23 (1H, dt, C*H*COOMe), 7.16 (1H, d, $H_{7'}$), 8.06 (1H, broad peak, NH), 8.13 (1H, d, $H_{8'}$), J_{CH2-CH} = 5.1 Hz, J_{CHNH} = 8.5 Hz, $J_{H7'H8'}$ = 10 Hz.

Anal. Calcd. for $C_{11}H_9ClF_3N_5O_3$: C, 37.57; H, 2.58; N, 19.91. Found: C, 37.29; H, 2.39; N, 19.90.

(*S*)-*N*-Trifluoroacetyl-3-(6-phenyl-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine Methyl Ester (**19**).

This compound was prepared from the (*S*)-isomer of **16** and bromine in 86% yield, mp 169-170° (from benzene), $[\alpha]_D^{22}$ = -13.9° (c = 0.898, ethyl acetate); ^{1}H nmr (deuteriochloroform): δ 3.79 (3H, s, OMe), 3.79-4.02 (2H, m, CH_2), 5.32 (1H, dt, C*H*COOMe), 7.52-7.71 (4H, m, 3H-Ph and $H_{7'}$), 7.95-8.13 (3H, m, 2H-Ph and NH), 8.21 (1H, d, $H_{8'}$), J_{CH2-CH} = 5 Hz, J_{CH-NH} = 8.2 Hz, $J_{H7'H8'}$ = 9.5 Hz.

Anal. Calcd for $C_{17}H_{14}F_3N_5O_3$: C, 51.91; H, 3.59; N, 17.81. Found: C, 51.67; H, 3.26; N, 17.89.

3-(6-Chloro-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine (**20**).

The mixture of *N*-trifluoroacetyl-3-(6-chloro-*s*-triazolo[4,3-*b*]pyridazin-3-yl)alanine methyl ester (**18**) (1.758 g, 0.005 mole) and 5*N* hydrochloric acid (30 ml) was heated under reflux for 5 hours, cooled and volatile components evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of water, and the solution neutralised with *N*-methylmorpholine to *p*H value 7. Methanol (20 ml) was added and the mixture left overnight at room temperature. The product was filtered off and washed with methanol.

S-Isomer.

This compound was prepared from the (*S*)-isomer of **18** and 5*N* hydrochloric acid in 81% yield, mp 251-252° dec (from water), $[\alpha]_D^{22}$ = +14.9° (c = 0.525, water).

Anal. Calcd. for $C_8H_8ClN_5O_2$: C, 39.77; H, 3.34; N, 28.98. Found: C, 39.52; H, 2.96; N, 29.37.

R-Isomer:

This compound was prepared from the (*R*)-isomer of **18** and 5*N* hydrochloric acid in 65% yield, mp 250-251° (from ethanol/water), $[\alpha]_D^{23}$ = -16.5° (c = 0.544, water).

Anal. Calcd. for $C_8H_8ClN_5O_2$: C, 39.77; H, 3.34; N, 28.98. Found: C, 40.14; H, 3.11; N, 28.10.

R,S-Isomer.

This compound was prepared from the (*R,S*)-isomer of **18** and 5*N* hydrochloric acid in 78% yield, mp 264-266° (from water).

Anal. Calcd. for $C_8H_8ClN_5O_2$: C, 39.77; H, 3.34; N, 28.98. Found: C, 39.54; H, 3.03; N, 28.72.

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