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DEPSIPEPTIDE ANALOGS OF THE ANTITUMOR DRUG DISTAMYCIN CONTAINING THIAZOLE AMINO ACIDS RESIDUES

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ABSTRACT

Three compounds structurally related to the natural antiviral antitumor drugs netropsin and distamycin have been synthetized. They have been designed starting from 2-((aminomethyl)-thiazole-4 carboxylic acid, gly (Thz), a key element in the structure of highly cytotoxic natural peptides. In the structure of the three new compounds, this unit replaces N-methyl pyrrole carboxylic acid which seems to play a crucial role in DNA base sequence recognition by the parent natural agents.

Distamycin-A (Dst) (Fig. 1), a compound possessing antibacterial, antiviral and antitumor activities, has attracted considerable attention of many research groups (for a review see ref. 1) since its isolation[2] and its total synthesis[3]. Physico-chemical studies indicates that Dst, like the other pyrrole amidine antibiotic netropsin (Nt) (Fig. 1), is able to block the template function of DNA by binding to specific nucleotide sequences in the minor groove of double strand DNA[1]. The molecular recognition site was found to be a AT rich segment as revealed by foot-printing or DNA-affinity cleaving [4-6] techniques. In order to understand the conformational and chemical basis of DNA binding and to delineate the role of the heterocyclic moiety in the base specificity, structural modifications of the parent molecules Dst and Nt, have been rationally carried out. Peptide analogs of Dst and Nt synthesised hitherto can be regrouped in three classes. First, those on which the pyrrole ring is replaced by phenyl[7], pyridine[8,9], thiophene[9], imidazole[10,11] groups. In the second class, the pyrrole carboxamide unit and the side chains (formyl and amidine) were present but the number of pyrrole units is different[12] or the linking chains between the heterocyclic rings is more extended[13]. In the last class, the modifications are centered on the side chain without modification of the two (Nt) or three (Dst) pyrrole units[14].

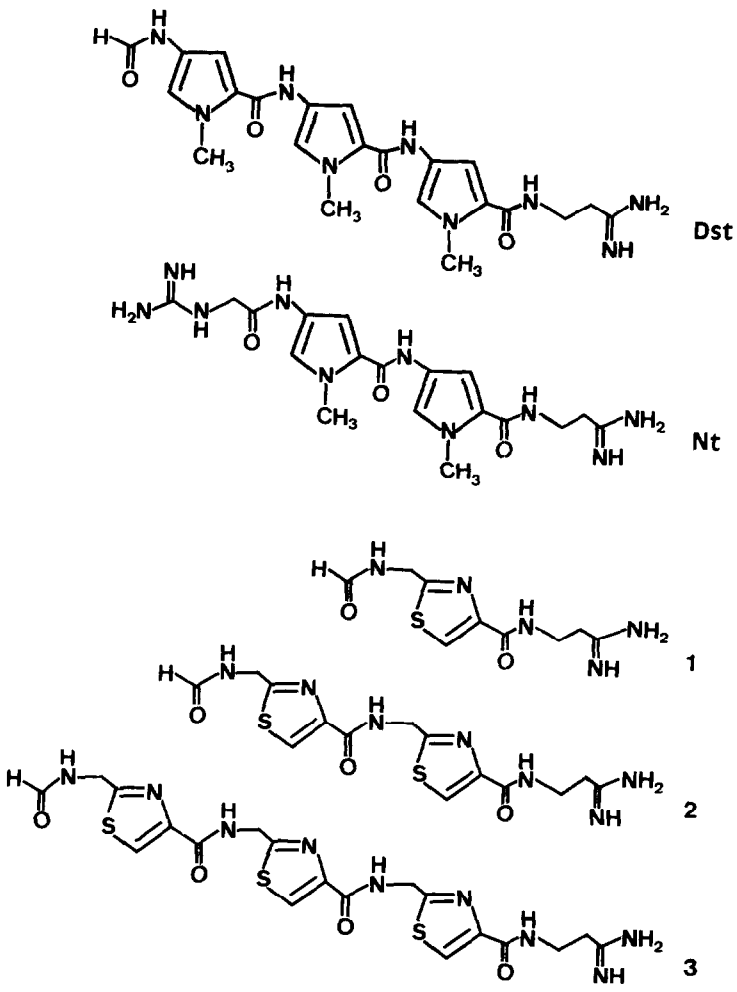


Figure 1. Structure of the oligopeptide antibiotics, Distamycin A (Dst), Netropsin (Nt) and related synthetic compound formyl-gly(Thz)-aminopropionamidine (1), formyl-gly(Thz)$_2$-aminopropionamidine (2), formyl-gly(Thz)$_3$-aminopropionamidine (3).

Taking into account these previous works, rational conformational structure-DNA binding relationships have been developed. In order to design non-intercalative DNA-binding compounds with a modified DNA specificity and enhanced biological activities compared to Dst, we report here the synthesis of three new compounds related to the Dst antibiotic (Fig. 1). The distinctive features of these three compounds is the introduction of 2-(aminomethyl) thiazole-4-carboxylic acid unit in place of the N-methyl-pyrrole unit of Dst (Fig. 1). The peptides, reported in this paper, contain the common synthon 2-aminomethylthiazole carboxylic acid. This ring has several advantages :

i) First the heterocyclic N atom is a hydrogen accepting site for the NH_2 of guanine as it has been demonstrated in similar cases[10,11].

ii) The additional methylene group undoubtedly disrupts the extensive π-electron delocalization which occurs in the parent drug structure and which has been regarded as responsible for the stabilization of the drug-DNA complex[13]. However, since the base sequence information can be read out by Van der Waals contacts[15], the supplementary CH_2 group is able to induce a modified binding specificity to DNA sequence.

iii) Are also present in the structure of the new models the formyl and amidine groups able to form electrostatic bonds with DNA phosphates and the amide bonds necessary for a relative rigid conformation and the establishment of hydrogen bonds with a heteroatom of purines and pyrimidines as found for Dst.

iv) The 2-(aminoalkyl)-thiazole-4 carboxylic acid seems to be important for the biological activity of highly cytotoxic cyclopeptides isolated from marine animals (dolastatin [16], ulicyclamide, ulithiacyclamide [17] and patellamides [18-21] from tunicates) and the thiazole ring seems to play a crucial role in the action of other naturally occurring peptides of pharmacological interest such as thiostrepton [22,23], botromycin [24], dysidenin [25] or isodysidenin [26].

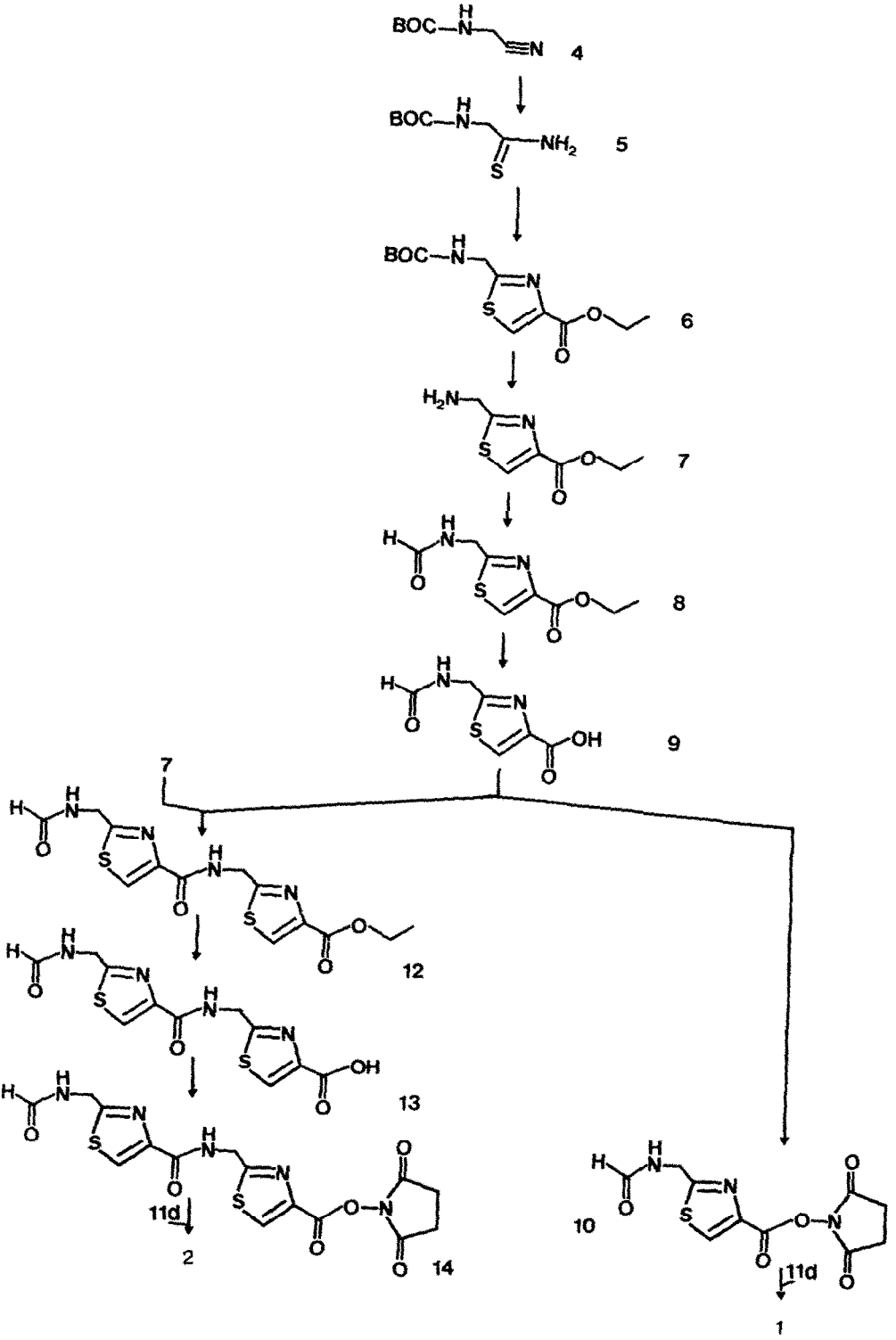
Synthetic strategy

The three new compounds formyl-gly(Thz)-aminopropionamidine[27] 1, formyl gly(Thz)$_2$-aminopropionamidine 2 and formyl-gly(Thz)$_3$-aminopropionamidine 3 have been synthesized according to schemes I and III.

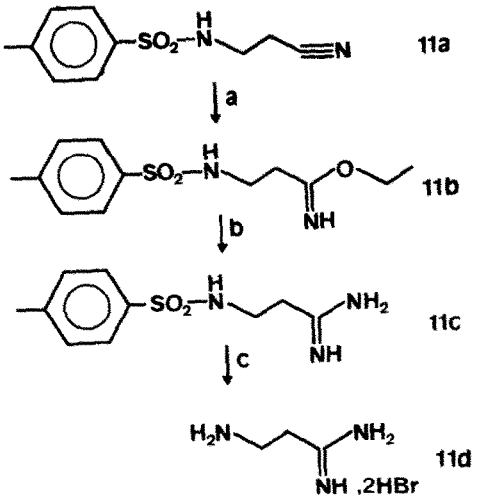
Reaction of aminoacetonitrile hydrochloride with di-t-butyldicarbonate anhydride ((BOC)$_2$0) in the presence of triethylamine afforded the BOC protected aminoacetonitrile 4 in high yield. Thionation of the nitrile 4 was accomplished with H_2S under pressure. Ethyl BOC-aminomethylthiazole carboxylate was then prepared from the thioamide 5 and ethyl bromopyruvate by an improvement of the classical Hantzsch condensation using a procedure which has previously been exploited by our group [28,29]. The resulting unstable air sensitive ester 6 was immediately converted into the stable amine 7 : cleavage of the BOC group with dry hydrogen bromide, in acetic acid, gave the desired synthon 7 in 95 % yield. N-formylation of 7 was carried out with formic acid by a classical procedure using dicyclohexylcarbodiimide (DCC) and 1H-hydroxy-1,2,3-benzotriazole as coupling agent. This procedure provided the N-formyl product 8 in 63 % yield. This approach was preferred to that which uses other reagents like formic anhydride [30] or formamide and ethyl formate [31]. Such formyl derivatives 8, 12 and 20 were more hydrophilic than their corresponding BOC protected amines and extreme precautions must be taken with all the washing procedures with aqueous media. For this reason, we decided for the Thz-Dst derivative 3 to introduce the formyl side chain in the last step of the synthetic strategy, just before the amidine moiety.

Alkaline hydrolysis of 8 afforded the formyl-gly(Thz) carboxylic acid 9, after acidification, in a 98 % yield. Coupling of the primary amine 7 with 9 in the presence of DCC-HOBt, gave the amide 12 in excellent yield and saponification of 12 with sodium hydroxide afforded the desired acid 13. With the formyl-gly(Thz) carboxylic acid 9 and the formyl-gly(Thz)$_2$ carboxylic acid 13, we now turned to the attachment of the aminopropionamidine end group. The literature[32] method of coupling, first with aminopropionitrile followed by addition of ammonia to the nitrile group via an intermediate iminoester, was in this case unsatisfactory because of the very low yield caused by the degradation of the thiazole system. Therefore an alternative procedure was examined. The direct coupling of β-aminopropionamidine dihydrobromide[33] (scheme II) with succinimidyl esters of 9 and 13 suggested an alternative method[30]. Best results were obtained by using one equivalent of the succinimidyl esters, 10, 14 (isolated as pure compound by recrystallisation from 2-propanol), 1.2 equiv. of 11d and 1.2 equiv of $NaHCO_3$. The two compounds 1 and 2 were finally obtained as pure white needles by two successive recrystallisations from 2-propanol. The method of purification used here avoided chromatography which is not suitable for such polar products because of contamination of the final compounds with inorganic salts eluted from the adsorbent.

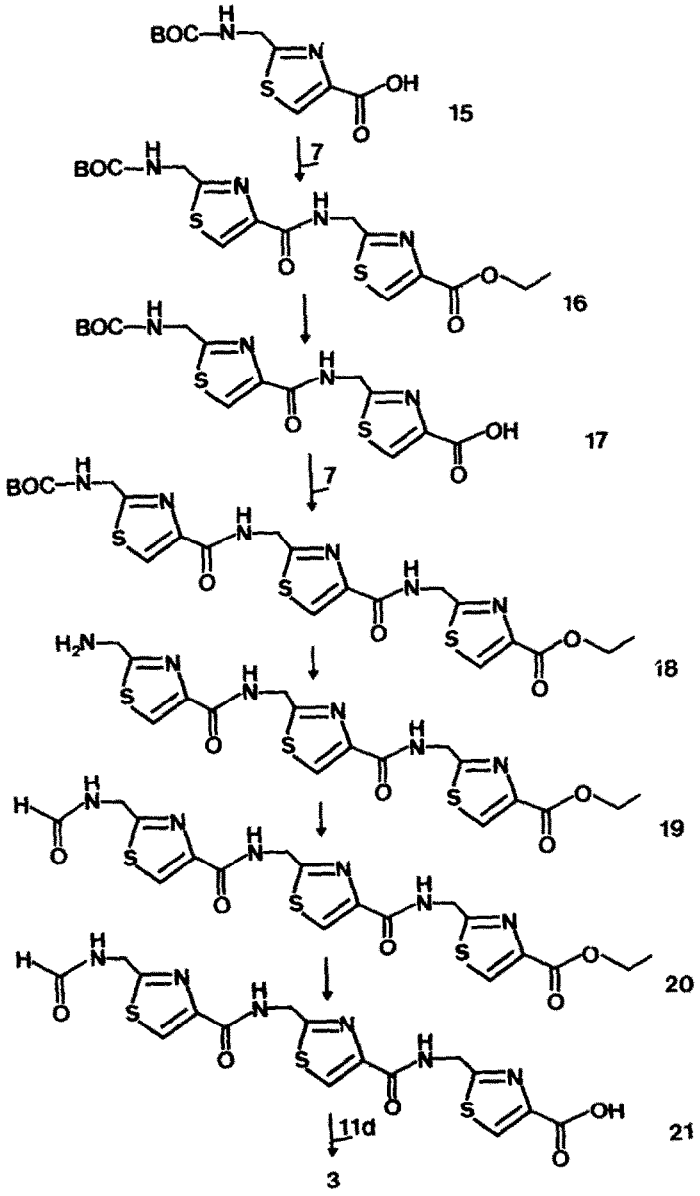
The synthesis of 3 was carried out as depicted in scheme III. This thiazole-containing Dst analogue was prepared using the BOC-protected aminoacid 15 as starting material. Two different ways could be envisaged in the synthesis of 15. First saponification of the easily obtained synthon 6 with sodium hydroxide seemed to be the more appropriate procedure. However by this method, the yield of purified 15 did not exceed 10 % and this experiment was not easily reproducible. In an alternative method, the acid 15 was obtained in a high yield by direct condensation of thioamide 5 with bromopyruvic acid[34]. This reaction, conducted in ethyl acetate, needed one equivalent of triethylamine to neutralize HBr formed in situ which could cleave the BOC group. Careful examination of the crude reaction mixture revealed the presence of unreacted 5 ($\simeq$ 1 %), and pure compound 15 was obtained by flash chromatography[35].



Scheme I



Scheme II[a] Reaction conditions : (a) HCl in dry EtOH, (b) dry NH_3 in ETOH, (c) phenol, HBr and acetic acid.



Scheme III

The condensation of 15 with amine 7 using DCC-HOBt furnished the protected dimer in a reasonable yield. After alkaline hydrolysis of the ester group, this procedure was repeated to give protected trimer 18. The BOC protecting group in 18 was conveniently removed, according to a standard procedure, with trifluoroacetic acid at room temperature. The N-formylated compound 20 was obtained from the amine 19, using the method of formylation described above for the conversion of 7 to 8.

The final incorporation of the amidine side chain in the molecule constituted a crucial step in the synthesis of 3. When the succinimidyl active ester of 21 (prepared as described for 10) was isolated, the yield was very poor and degradation of this active ester occurred readily. When DCC was used without N-hydroxysuccinimide as coupling agent, only minor amounts of 3 were produced and it was severely contaminated with several other substances. This obstacle was finally overcome by using N-hydroxysuccinimide in presence of DCC without isolation of the intermediate active ester.

Thus, the yield in the last step was unsatisfactory and was due to the difficulty in eliminating the unreacted amidine 11d without chromatographic methods unsuitable for such polar compounds. Generally two or three successive recrystallisations are necessary to obtain highly pure final products 1, 2, and 3.

Experimental section

General

- Melting points were determined in capillary tubes and are uncorrected. The IR spectra were obtained on a Perkin-Elmer 177 spectrophotometer, using KBr pellets. ^{1}H-NMR spectra were recorded on a Brucker WP 80 SY or on a Brucker AM 400 WB spectrophometers. Chemical shifts are reported in ppm from tetramethylsilane as an internal standard and are given in δ units. EI mass spectra were recorded on a Ribermag R10.10 (combined with Riber 400 data system) mass spectrophotometer at 70 eV by using direct insertion. FAB mass spectra were determined on a Kratos MS-50 RF mass spectrometer arranged in an EBE geometry. The sample was bombarded using a beam of xenon with a kinetic energy of 7 keV. The mass spectrometer was operated at 8 KV accelerating voltage with a mass resolution of 3000. Thin layer chromatography (TLC) was carried out using silica gel 60F-254 Merck (0,25 mm thick) precoated UV-sensitive plates, generally in solvent system A ($CHCl_3$-MeOH, 80 : 20 (v/v) in a satured NH_3 atmosphere). Spots were visualized by inspection under U.V. light at 254 nm and after exposure to vaporized I_2 and/or ninhydrin. Kieselgel 60 (230-400 mesh) of Merck was used for flash-chromatography according to the procedure of Still[35].

t-Butoxyaminoacetonitrile (4). To a solution of 9.25 g (0.1 mol) of 2-aminoacetonitrile hydrochloride (Aldrich) in CH_2Cl_2 (300 mL), TEA (27.7 mL, 0.2 mol) and di-t-butyldicarbonate (21.82 g, 0.1 mol) in CH_2Cl_2 (100 mL) were added. The stirred mixture was refluxed for 12 hours. Triethylamine salt was extracted twice by 30 mL of water, and the organic phase was evaporated in vacuo to yield 14.3 g (92 % yield) of an oil which solidified by trituration in petroleum ether, mp<35°C ; Rf(A) : 0.8 ; IR 3380 (NH), 2220 (C N), 1700 (O-CO) cm^{-1} ; ^{1}H-NMR ($CDCl_3$) δ 1.5 (s,9H, $(CH_3)_3$), 4.1 (d, 2H, J=6Hz, CH_2), 6.1 (m, 1H, NH). Anal. Calcd for $C_7H_{12}N_2O_2$: C,53.83 ; H, 7.69 ; N, 17.94. Found : C, 53.61 ; H, 7.65 ; N, 17.80.

t-Butoxyaminothioacetamide (5). A solution of 4 (14 g, 89.7 mmol) in dimethylformamide (50 mL) and diethylamine (20 mL) was poured in a stainless steel bomb and allowed to react with hydrogen sulfide under 2 atmospheres pressure for 12 hours at 20°C. The solvent was evaporated and the residual solid recrystallized twice from water, giving 14.45 g (85 % yield) of white crystals : mp:125°C ; Rf(A):0.8 ; IR 3420 (NH_2 thioamide), 3300 (NH-BOC) 1685 (O-CO), 1610 (C=S) cm^{-1} ; ^{1}H-NMR ($Me_2SO.d_6$) δ 1.4 (s, 9H, $(CH_3)_3$), 3.8 (d, 2H, J = 0.55Hz, CH_2), 6.9 (m, 1H, NH), 8.9 (m, 1H, NH_2-C=S), 9.6 (m, 1H, NH_2-C=S) ; MS, m/e (rel intensity) 190 (17, M^+). Anal. Calcd for $C_7H_{14}N_2O_2S$: C, 44.21 ; H, 7.36 ; N, 14.73. Found : C, 44.08 ; H, 7.47 ; N, 14.57.

Ethyl 2-(t-butoxyaminomethyl)-thiazole-4-carboxylate (6). A mixture of 5 g (26.3 mmol) of 5 and 3.29 mL (26.3 mmol) of ethyl bromopyruvate (Aldrich) in 250 mL of dry ether was stirred at room temperature for 2 h. Filtration of the precipitate afforded 6.96 g (92.5 % yield) of white unstable crystalline 6 immediately used for preparation of 7 and 15. mp:133°C ; Rf(A):0.68 ; IR 1760 ($COOCH_2$), 1705 (OCO) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.3 (t, 3H, CH_3-CH_2), 1.4 (s,9H, $(CH_3)_3$), 4.3 (q, 2H, CH_2-CH_3), 4.4 (d, 2H, CH_2-NH), 6.0 (m, 1H,$NH-CH_2$) 8.35 (s, 1H, CH), MS, m/e (rel intensity) 286 (24, M^+).

Ethyl 2-(aminomethyl) thiazole -4-carboxylate hydrobromide (7). A solution of 6.8 g (23.8 mmol) of 6 in 200 mL of glacial acetic acid was treated with dry HBr gas. After saturation, the reaction mixture was set aside at room temperature for 2 h after which time a solid precipitated. The white solid (5.97 g, 94 % yield) was collected, washed with large quantities of diethyl ether and dried under reduced pressure. An authentic specimen was recrystallised from absolute ethanol : mp:190°C, Rf(A):0.40 ; IR 3000 (NH_3), 1740 ($COOCH_2$) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.3 (t, 3H, J=6.4Hz, CH_3-CH_2), 4.3 (q, 2H,J=6.4Hz,CH_2-CH_3), 4.5 (m, 2H, CH_2-C), 7.35 (m, 1H, NH_3-CH_2), 8.6 (s, 1H, CH). MS, m/e (rel intensity) 186 (12, M^+), 158 (9.9), 112 (46), 82 (100). Anal. Calcd for $C_7H_{11}N_2O_2SBr$: C, 31.46 ; H, 4.12 ; N, 10.48. Found : C, 31.39 ; H, 4.20 ; N, 10.32.

Ethyl 2-(formamidomethyl)thiazole-4-carboxylate (8). Formic acid (0.36 mL, 9.36 mmol) in cold dry CH_2Cl_2 was stirred with dicyclohexylcarbodiimide (2.12 g, 10.3 mmol) and 1H-hydroxy-1,2,3-benzotriazole (1.58 g, 10.3 mmol) for 1 h ; a cold solution of 7 (2.5 g, 9.36 mmol) and TEA (1.3 mL, 9.36 mmol) in CH_2Cl_2 (30 mL) was added. Stirring was continued for 2 h at 0°C and 15 h at 20 °C. The dicyclohexylurea was discarded by precipitation with acetone and the CH_2Cl_2 solution was concentrated and washed with a minimum volume (5 mL) of 1N HCl and 1M $NaHCO_3$. After drying over Na_2SO_4, the solvent was removed in vacuo and compound 8 was obtained in 1.26 g (63 % yield) as a white pure powder as ascertained by TLC. mp:84-86°C, Rf(A):0.68 ; IR 1735 (COOEt), 1685 (CHO) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.3 (t, 3H, CH_3-CH_2), 4.3 (q, 2H, CH_2-CH_3), 4.6 (d, 2H, CH_2-NH), 8.15 (s, 1H, CHO), 8.35 (s, 1H, CH), 8.83 (m, 1H, NH) ; MS,m/e (rel intensity) 214 (71.3, M^+), 185 (18.3), 168 (27.8), 139 (61.0), 112 (100). Anal. Calcd. for $C_8H_{10}N_2O_3S$: C, 44.86 ; H, 4.67 ; N, 13.08 ; Found. C, 44.55 ; H, 4.77 ; N, 12.85.

2-(Formamidomethyl) thiazole-4-carboxylic acid (9). A solution of 1.2 g (5.6 mmol) of 8 in MeOH and 0.9 g (22.4 mmol) of sodium hydroxide in water (2 mL) was stirred at room temperature. The progress of the reaction was monitored by TLC and was thereby judged to be complete after 20 h . The resulting solution was cautiously acidified to pH 3.0 with a few drops of dilute HCl. Evaporation of the solvent, trituration in absolute ethanol, elimination of sodium chloride by filtration, and evaporation of ethanol, yielded 1.02 g of a white solid suitable for further synthesis (98% yield). An analytical pure sample was obtained by recrystallisation from 95 % ethanol. mp:246°C, Rf(A):0 ; IR 1730 (COOH), 1710 (CHO) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 4.0 (m, 1H, COOH) ; 4.65 (d, 2H, CH_2), 8.2 (s, 1H, CHO), 8.35 (s, 1H, CH), 8.7 (m,1H, NH) ; MS, m/e (rel intensity) 186 (30.2, M^+), 168 (77.7), 157 (24.8), 139 (100). Anal. Calcd for $C_6H_6N_2O_3S$: C, 38.71 ; H, 3.22 ; N, 15.05 ; Found : C, 38.69 ; H, 3.28 ; N, 15.16.

Succinimidyl 2-(formamidomethyl) thiazole-4-carboxylate (10). A mixture of 9 (0.5 g, 2.69 mmol) and N-hydroxysuccinimide (0.34 g, 2.95 mmol) dissolved in dry DMF (20 mL), was cooled in an ice bath. To this well-stirred solution was added solid dicyclohexylcarbodiimide (0.61 g, 2.95 mmol) and stirring was continued for an additional 1h at 0°C and then at 20°C overnight. After evaporation, the dicyclohexylurea formed was collected and washed with small portions of cold acetone. The combined yellowish filtrate and washings were evaporated to dryness under reduced pressure (temperature below 40°C). This semi-solid residue was purified by dissolution in a small quantity of acetone, and precipitation with cold ether. This material, obtained as a white powder, was finally recrystallized from 2-propanol to give a white microcrystalline solid. (0.29 g, 38 % yield) ; mp:144-146°C ; Rf(A):0.56 ; IR 1800; 1745 (COON), 1680 (CHO) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ

3.15 (m, 4H, $2CH_2CO$), 4.7 (d, 2H, CH_2NH), 8.2 (s, 1H, CHO), 8.7 (m, 1H, NH), 8.8 (s, 1H, CH). Anal. Calcd for $C_{10}H_9N_3O_5S$: C, 42.40 ; H, 3.18 ; N, 14.84 ; Found : C, 42.45; H, 3.27 ; N, 14.71.

3-Aminopropionamidine dihydrobromide (11d). The title compound was prepared as previously described by Hilgetag et al[33]. Only the physico-chemical characteristics of intermediate and final products are reported here.

3-(p-Toluenesulfonylamino)-propionitrile (11a). mp>250°C ; Rf(A) : 0.68 ; IR 2240 (C N), 1340 (SO_2) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 2.4 (s, 3H, CH_3), 2.6 (m, 2H, CH_2-NH),, 3.0 (t, 2H, CH_2-C N), 6.8 (m, 1H, $NHSO_2$), 7.5 (m, 4H, CH) ; MS, m/e (rel intensity) 224 (8.2 M^+), 184 (20.5), 155 (42.0), 91 (100).

Ethyl 3-(p-toluenesulfonylamino)-propionimidate hydrochloride (11b). mp:126°C ; Rf(A):0.93 ; IR 1340 (SO_2), 1730 (CNHOEt) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.15 (t, 3H, CH_3-CH_2), 2.4 (s, 3H, CH_3-C), 3.0 (m, 4H, CH_2-CH_2), 4.0 (q, 2H, CH_2-CH_3), 6.7 (m, 1H, NH-SO_2), 7.6 (m, 4H, CH), 7.95 (m, 1H, HN = C,) ; MS, m/e (rel intensity) 271 (0.2 M^+), 226 (11.8), 184 (35.6), 155 (89.5), 116 (94.4), 91 (100).

3-(p-Toluenesulfonylamino)-propionamidine (11c). Rf(A):0, Rf (H_2O/MeOH /HCOOH, 9/3/0.1, v:v):0.57 ; IR 1680 (amidine), 1335 (SO_2) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 2.4 (s,3H,CH_3), 3.0 (m, 2H, CH_2), 3.4 (m, 2H, CH_2), 6.5 (m, 1H, $NHSO_2$), 6.6 (m, NH), 7.5 (m, 4H, CH). No molecular ion peak was present in the 70-eV mass spectrum ; same fragmentation as observed for 11b.

3-Aminopropionamidine dihydrobromide (11d). mp:159°C ; Rf(A):0, Rf(H_2O/MeOH/ HCOOH, 9/3/0.1, v:v):0.72 ; IR 3340 (NH_2-C), 1690 (amidine) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 2.9 (m, 2H, CH_2), 3.2 (m, 2H, CH_2), 8.5 (m, NH) ; no molecular ion peak was present in the 70-eV mass spectrum (parent peak, m/e 80). Anal. Calcd for $C_3H_{11}N_3Br_2$: C, 14.45 ; H 4.41 ; N, 16.86. Found : C, 14.39 ; H, 4.48 ; N, 16.64.

[2-Formamidomethyl)thiazole-4carboxamido]propionamidine hydrobromide (1). To a well stirred solution of β-aminopropionamidine 11d (210 mg, 0.84 mmol) and $NaHCO_3$ (70.6 mg, 0.84 mmol) in dioxane - water 1 : 2 (v:v, 15 mL) was added a fresh solution of 10 (200 mg, 0.7 mmol) in dioxane (5mL). The solution was stirred for 48 h at room temperature and then evaporated ; after filtration of the solution obtained by dissolution in water (4 mL), the lyophilizated material was dissolved in hot absolute ethanol to remove NaBr and the starting amidine 11d. The alcoholic filtrate was evaporated to dryness and the residual powder recrystallised twice in 1-butanol giving 53 mg (15 % yield) of hygroscopic compound 1 pure as indicated by usual analytical criteria . Rf(A):0, Rf(H_2O/MeOH/HCOOH, 9/3/0.1, v:v):0.62 ; ^{1}H-NMR (Me_2SO-d_6) δ 2.85 (m, 2H, CH_2), 3.8 (m,2H, CH_2),4.8 (d, 2H, CH_2 - NH), 8.25 (s, 1H, CHO), 8.35 (s, 1H, CH), 8.7 (m, 1H, NH). Non analysable by MS (EI). Anal. Calcd. for $C_9H_{14}N_5O_2SBr$: C, 32.14 ; H, 4.17 ; N, 20.83. Found : C, 32.26 ; H, 4.19 ; N, 20.74

Ethyl2-[2'-(formamidomethyl)thiazole - 4'-carboxamidomethyl]thiazole-4-carboxylate (12). A solution of compound 9 (1.5 g, 8.06 mmol) in anhydrous CH_2Cl_2 (100 mL) was cooled to 0°C and dicyclohexylcarbodiimide (1.82 g, 8.87 mmol) and 1H-hydroxy-1,2,3-benzotriazole hydrate (1.35 g, 8.87 mmol) in 10 mL of CH_2Cl_2 were added. After 1 h 30, a solution of 7 (2.15 g, 8.06 mmol) in 30 mL CH_2Cl_2 and TEA (1.12 mL, 8.06 mmol) cooled to 0°C was added. The mixture was stirred at 0°C for 2 h and allowed to rise to ambient temperature, stirring was continued for 10 h. The precipitated dicyclohexylurea was collected and the CH_2Cl_2 solution was washed successively with 30 mL of 1N HCl, , H_2O and 1M $NaHCO_3$. After drying over Na_2SO_4 the solvent was removed in vacuo. The remaining dicyclohexylurea was discarded by classically precipitation with acetone. The resulting residue obtained after evaporation of the solvent was thoroughly triturated with diethylether. The yield of crude, chromatographically pure product obtained as a white powder was 3.24 g (68 % yield). mp:134-135°C, Rf(A):0.78, IR 1720 (COOEt), 1665 (CHO), 1635 (CONH)cm^{-1}, ^{1}H-NMR (Me_2SO-d_6) δ 1.3 (t, 3H, CH_3CH_2),

4.3 (q, 2H, CH_2CH_3), 4.6 (d, 2H, CH_2), 4.8 (d, 2H, CH_2), 8.2 (s, 1H, CHO), 8.35 (2s, 2H, 2CH), 8.7 (m, 1H, NH,), 9.05 (m, 1H, NH). MS-FAB 355 (75, M^++1). Anal. Calcd for $C_{13}H_{14}N_4O_4S_2$: C, 44.07 ; H, 3.95 ; N, 15.82 ; Found : C, 44.35 ; H, 4.08 ; N, 15.74.

2-[2'-(Formamidomethyl)thiazole-4'-carboxamidomethyl]thiazole-4-carboxylic acid (13). This compound was prepared using the same conditions as described for 9. Recrystallisation from absolute ethanol provided 1.64 g of 13 (94 % yield). mp:128°C, Rf(A):0, IR 1730 (COOH), 1710 (CHO), 1640 (CONH) ; ^{1}H-NMR (Me_2SO-d_6) δ 4.7 (d, 2H, CH_2), 4.8 (d, 2H, CH_2), 8.2 (s, 1H, CH), 8.3 (s, 1H, CH), 8.35 (s, 1H, CHO), 8.8 (m, 1H, NH), 9.0 (m, 1H, NH). MS, m/e (rel intensity) 326 (0.3, M^+), 320 (1.1), 305 (5.4) 223 (10.2), 141 (21.6), 113 (39.9), 98 (100). Anal. Calcd. for $C_{11}H_{10}N_4O_4S_2$: C, 40.49 ; H, 3.07 ; N, 17.18 ; Found : C,40.41 ; H, 3.15 ; N, 16.97.

Succinimidyl 2-[2'-formamidomethyl)thiazole-4'-carboxamidomethyl]thiazole-4-carboxylate (14). This succinimidyl ester was prepared according to the previously established procedure for 10. 0.26 g of needles from isopropanol. (40 % yield) ; Rf(A) : 0.45 ; IR 1745 (COON), 1680 (CHO), 1660 (CONH) ; ^{1}H-NMR (Me_2SO-d_6) δ 3.20 (m, 4H, $2CH_2CO$), 4.75 (d, 2H, CH_2), 4.8 (d, 2H, CH_2), 8.2 (s, 1H, CH), 8.3 (s, 1H, CH), 8.35 (s, 1H, CHO), 8.8 (m, 1H, NH), 9.1 (m, 1H, CH). Anal. Calcd. for $C_{15}H_{13}N_5O_6S_2$: C, 42.55 ; H, 3.07 ; N,16.55. Found : C, 42.66; H, 3.02 ; N, 16.40.

[2[2'-(Formamidomethyl)thiazole-4'-carboxamidomethyl]thiazole-4-carboxamido]propionamidine hydrobromide (2). 38 mg (17% yield) of amidine 2 were obtained according to the procedure described 1. mp:76°C ; Rf(A):0, Rf(H_2O/MeOH/HCOOH, 9/3/0.1, v:v):0.58 ; ^{1}H-NMR (Me_2SO-d_6) δ 2.5 (m, 2H, CH_2-CH_2) ; 3.6 (m, 2H, CH_2-CH_2) ; 4.7 (d, 2H, CH_2NH), 4.85 (d, 2H, CH_2-NH), 8.1 (s, 1H, CHO), 8.2 (s, 1H, CH), 8.25 (s, 1H, CH), 8.9 (m, 1H, NH), 9.1 (m, 1H, NH). FAB-MS 396 (M^++1). Anal. Calcd. for $C_{14}H_{18}N_7O_3S_2Br$: C, 35.29 ; H, 3.78 ; N, 20.59. Found C, 35.03; H, 3.71 ; N, 20.46.

2-(t-Butoxyaminomethyl)thiazole-4-carboxylic acid (15). (a) saponification of ester 6. 6 (1 g, 3.5 mmol) was dissolved in 50 mL of MeOH and a sodium hydroxide solution (0.15 g, 3.85 mmol in a minimum of water) was added. The mixture was stirred at room temperature for 30 min and then evaporated. The resulting residue was rapidly acidified with 30 mL of 1N HCl ; trituration of the gummy product gave 80 mg of the desired acid 15 in a poor yield (less than 10 %) but as a white powder essentially pure by TLC.

(b) Cyclisation using bromopyruvic acid. In an alternative procedure, 2.64 g of bromopyruvic acid (15.8 mmol, Aldrich) was added to a solution of 3 g of 5 (15.8 mmol) and 2.4 mL of TEA (17.36 mmol) in 100 mL of ethyl acetate. The mixture was stirred for 2 h at ambient temperature, the precipitate was collected and the solvent evaporated in vacuo. Trituration of a gummy product with ether gave a crude yellow crystalline substance which was collected : 2.89 g (71 % yield). Remaining traces amounts of 5 could be removed by flash chromatography (ethyl acetate : acetone (1 : 1 by volume) as eluent, followed by MeOH) to collect 15. An analytical sample could be obtained by dissolution of the chromatographed material in a minimum volume of acetone followed by addition of sufficient cold dry ether under rapid stirring. The precipitated white powder was then collected and dried. mp:184°C ; Rf(A):0.1 ; IR 1780 (COOH), 1710 (OCONH) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.45 (s, 9H, $(CH_3)_3$), 4.4 (m, 2H, CH_2), 7.1 (m, 1H, COOH), 8.35 (s. 1H, CH). MS, m/e (rel intensity) 258 (3.3, M^+), 241 (2.6), 226 (1.8), 214 (1.9), 202 (14.5), 198 (1.5), 185 (9.4), 158 (20.4) 100 (100). Anal. Calcd. for $C_{10}H_{14}N_2O_4S$: C, 46.51 ; H, 5.42 ; N, 10.85. Found : C, 46.62 ; H, 5.55 ; N, 10.79.

Ethyl 2-[2'-(t-butoxyaminomethyl)thiazole-4'- carboxamidomethyl]thiazole-4-carboxylate (16). Fresh, thoroughly dried acid 15 (2.6 g, 11.2 mmol) was coupled to the amine 7 (3 g, 11.2 mmol) using dicyclohexylcarbodiimide (2.5 g, 12.4 mmol), 1H-hydroxy-1,2,3-benzotriazole (1.9 g, 12.4 mmol) and TEA (1.55 mL, 11.2 mmol) in a mixture of CH_2Cl_2/DMF (9/1, v:v) according to the procedure described for 12. White powder, 3.24 g ; 68 % yield ; mp:118°C ; Rf(A):0.85 ; IR 1720 (COOEt), 1685 (OCONH), 1660 (CONH) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.25 (t, 3H, CH_3-CH_2), 1.4 (s, 9H, $(CH_3)_3$), 4.25 (q, 2H,

CH_2-CH_3), 4.45 (d, 2H, CH_2), 4.75 (d, 2H, CH_2), 7.7 (m, 1H, NH-BOC), 8.2 (s, 1H, CH), 8.35 (s, 1H, CH), 9.15 (m, 1H, NH-CO). Anal. Calcd for $C_{17}H_{22}N_4O_5S_2$: C, 47.88 ; H, 5.16 ; N, 13.14. Found : C, 48.01 ; H, 5.07; N, 13.02.

2-[2'(t-Butoxyaminomethyl) thiazole-4'- carboxamidomethyl]thiazole-4-carboxylic acid (17). The crude ethyl ester 16 (3 g, 7.04 mmol) was totally converted after 35 h to the corresponding acid 17, according to the method of preparation for 9. The material used for the further reaction was conveniently recovered after the following purification into a sinter glass charged with silica gel. The deposited mixture was first eluted with $CHCl_3$ to remove impurities, then with MeOH which afforded the neat 17 (2. 13 g, 76 % yield). mp:173-175°C, Rf(A):0 ; IR 1780 (COOH), 1680 (OCONH), 1665 (CONH)cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.4 (s, 9H, $(CH_3)_3$), 4.45 (d, 2H, CH_2), 4.75 (d, 2H, CH_2), 7.68 (m, 1H, NH-BOC), 8.2 (s, 1H, CH), 8.3 (s, 1H, CH), 9.4 (m, 1H, NH-CO). Anal. Calcd for $C_{15}H_{18}N_4O_5S_2$: C, 45.22 ; H, 4.52 : N, 14.07. Found : C, 45.34 ; H, 4.55 ; N, 13.93.

Ethyl 2-[2'-[[2"-(t-butoxyaminomethyl)thiazole]-4"carboxamidomethyl]thiazole-4'-carboxamidomethyl]-thiazole-4-carboxylate (18). The procedure adopted for the preparation of the tristhiazole 18 is strictly identical to that described for 12. 64 % yield, mp:123°C, Rf(A):0.85 ; IR 1720 (COOEt), 1690 (O-CO), 1665 (CONH) cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.4 (s, 9H, $(CH_3)_3$), 4.45 (d, 2H, CH_2), 4.7 (d, 2H, CH_2), 8.25 (s, 1H, CH), 8.3 (s, 1H, CH), 9.15 (m, 2H, 2NH) ; MS-FAB 567 (55, M^++1). Anal. Calcd. for $C_{22}H_{26}N_6O_6S_3$: C, 46.64 ; H, 4.59 ; N, 14.84 ; Found : C, 46.50 ; H,4.71 ; N, 14.69.

Ethyl 2-[2'-[[2"-(aminomethyl)thiazole]-4"carboxamidomethyl]thiazole-4'-carbo xamidomethyl]thiazole--4-carboxylate (19). The t-BOC protected amine 18 (1 g, 1.77 mmol) was deprotected with pure TFA (10mL) to give the corresponding free amine 19 in a good yield (>85%). After 1 h stirring, the excess of TFA was evaporated and the residue was diluted with absolute ethanol (30 mL) before evaporation of the solvent. This procedure was repeated three times and resulted in the complete elimination of TFA. A thorough drying is necessary to eliminate the retained ethanol and the resulting 19 is quite suitable for the next reaction. Hygroscopic powder ; 0.95 g ; 93 % yield ; no distinct melting point ; Rf(A):0.6 ; IR 1720 (NH_3^+ TFA^-)cm^{-1} ; ^{1}H-NMR (Me_2SO-d_6) δ 1.2 (t, 3H, CH_3-CH_2), 4.25 (q, 2H, CH_2-CH_3), 4.8 (m, 6H, $3CH_2$), 8.15 (s, 1H, CH), 8.3 (s, 1H, CH), 8.4 (s, 1H, CH), 9 (m, 3H, NH_3^+ TFA^-). MS - FAB 467 (80, M^++1).

Ethyl 2-[2'-[[2"-(formamidomethyl)thiazole]-4"carboxamidomethyl]thiazole-4'-carboxamidomethyl]thiazole-4-carboxylate (20). The formamido derivative 20 is prepared according to the procedure adopted for compound 8. Extreme care should be observed during the washing procedure due to the extreme solubility of 20 in the aqueous media. The crude residue was purified by flash chromatography (acetone : $CHCl_3$ (7 : 3)). 54 % yield, Rf(A):0.64 ; IR 1720 (COOEt), 1670 (CHO), 1660 (CONH) cm^{-1}, ^{1}H-NMR ($CDCl_3$) 1.35 (t, 3H, CH_3-CH_2), 4.35 (q, 2H, CH_2-CH_3), 4.8 (m, 6H, $3CH_2$), 7.5 (m, 1H, NH),8.0 (s, 1H, CH) ; 8.05 (s, 1H, CH), 8.1 (s, 1H, CH), 8.3 (s, 1H, CHO), 8.4 (m, 2H, 2NH) ; MS-FAB 495 (15, M^++1). Anal. Calcd for $C_{18}H_{18}O_5N_6S_3$: C, 4 3.72 ; H, 3.64 ; N,17.00. Found : C, 43.57 ; H, 3.63 ; N, 16.81.

2-[2'-[[2"-(Formamidomethyl)thiazole]-4"carboxamidomethyl]thiazole-4'-carboxamidomethyl]thiazole--4-carboxylic acid (21). The crude ethyl ester 20 was totally converted to the corresponding acid within 30 h, according to the method of preparation of 17. 69 % yield ; Rf(A):0 ; IR 1780 (COOH), 1680 (CHO), 1660 (CONH) cm^{-1} ; ^{1}H-NMR ($CDCl_3$) δ 4.8 (m, 6H, $3CH_2$), 7.5 (m, 1H NH), 8.0, 8.05, 8.10 (3s, 3H, 3CH), 8.3 (s, 1H, CHO), 8.4 (m, 2H, 2NH) ; MS-FAB 467 (45, M^++1). Anal. Calcd. for $C_{16}H_{14}O_5N_6S_3$: C, 41.20 ; H, 3.00 N, 18.03 ; Found : C, 41.16 ; H, 2.87 ; N, 17.91.

[2-[2'-[[2"-(Formamidomethyl)thiazole]-4"carboxamidomethyl]thiazole-4'-carboxamidomethyl]thiazole-4 carboxamido]propionamidine hydrobromide (3). The amidine 3 was obtained by initial in situ preparation of the corresponding succinimidyl ester and then condensation with the amino derivative 11d.

The procedures referred respectively to the preparation of structures 10 and 1. The final product is recrystallized from absolute ethanol. 15 mg ; 11 % yield, mp:188°C, Rf(A):0, Rf(H_2O/MeOH/HCOOH, 9/3/0.1, v:v):0.4 ; ^{1}H-NMR (400MHz) (D_2O) δ 3.5 (m, 2H, CH_2-C), 3.6 (m, 2H, $NHCH_2$), 4.9 (m, 6H, CH_2-NH), 8.09 (s, 1H, CH), 8.15 (s, 1H, CH), 8.155 (s, 1H, CH), 8.12 (s, 1H, CHO). FAB-MS 536 (23, M^+ - Br) Anal. Calcd for $C_{19}H_{22}N_9O_4S_3$ Br : C, 37.01 ; H, 3.57 ; N, 20.45 ; Found : C, 37.19 ; H, 3.60 ; N, 20.36.

REFERENCES

1- C. Zimmer, and U. Wahnert, Prog. Biophys. Mol. Biol., 1986, 47, 31.
2- F. Arcamone, F. Bizioli, G. Canevazzi, and A. Grein, German Pat. 1958, 1, 027,667.
3- S. Penco, S. Redaelli, and F. Arcamone, Gazz. Chim. Ital., 1967, 97, 1110.
4- B. Ward, R. Rehfuss, and J.C. Dabrowiak, J. Biomol. Struct. Dyn., 1987, 4, 685.
5- M.W. Van Dyke, and P.B. Dervan, Nucl. Acids Res., 1983, 11, 5555.
6- J.S. Taylor, P.G. Schultz, and P.B. Dervan, Tetrahedron, 1984, 40, 457.
7- D. Dasgupta, M. Rajagopalan, and V. Sasisekharan, Biochem. Biophys Res. Comm. 1986, 140, 626.
8- W.S. Wade, and P.B. Dervan, J. Am. Chem. Soc., 1987, 109, 1574.
9- D.H. Jones, and R.H. Wooldridge, J. Chem. Soc., 1968, 550.
10- J.W. Lown, K. Krowicki, U.G. Bhat, A. Skorobogaty, B. Ward, and J.C. Dabrowiak, Biochemistry, 1986, 25, 7408.
11- K. Kissinger, K. Krowicki, J.C. Dabrowiak, and J.W. Lown, Biochemistry, 1987, 26, 5590.
12- R.S. Youngquist, and P.B Dervan, Proc. Natl. Acad. Sci. USA, 1985, 82, 2565.
13- D. Dasgupta, P. Parrack, and V. Sasisekharan, Biochemistry, 1987, 26, 6381.
14- J.W. Lown, personal communication.
15- M.L. Kopka, C. Yoon, D. Goodsell, P. Pjura, and R.E. Dickerson, Proc. Natl. Acad. Sci. USA, 1985, 82, 1376.
16- G.R. Pettit, Y. Kamano, P. Brown, D. Gust, M. Inoue, and C.L. Herald, J. Am. Chem. Soc., 1982, 104, 905.
17- C.M. Ireland, and P.J. Sheuer, J. Am. Chem. Soc., 1980, 102, 5688.
18- C.M. Ireland, A.R. Durso, Jr. R.A. Newman, and M.P. Hacker, J. Org. Chem. 1982, 47, 1807.
19- J.E. Biskupiak, and C.M. Ireland, J. Org. Chem. 1983, 48, 2302.
20- Y. Hamamoto, M. Endo, M. Nakagawa, T. Nakanishi, and K. Mizukawa, J. Chem. Soc., Chem. Commun., 1983, 323.
21- J.M. Wasylyk, J.E. Biskupiak, C.E. Costello, and C.M. Ireland, J. Org. Chem., 1983, 48, 4445.
22- M. Bodanszky, J. Fried, J.T. Sheehan, N.J. Williams, J. Alicino, Cohen, A.I. B.T. Keller, and C.A. Birkhimer, J. Am. Chem. Soc., 1964, 86, 2478.
23- B. Anderson, D.C. Hodgkin, and M.A. Viswamitra, Nature (London), 1970, 225, 233.
24- J.M. Waisvicz, M.G. van der Hoeven, and B. te Nijenhuis, J. Am. Chem. Soc., 1957, 79, 4524.
25- R. Kazlauskas, R.O. Lidgard, R.J. Walls, and W. Vetter, Tetrahedron Lett., 1977, 3183.
26- C. Charles, J.C. Brackman, D. Daloze, B. Tursch, and R. Karlsson, Tetrahedron Lett., 1978, 1519.
27- The abbreviation gly(Thz) for 2-(1-aminomethyl)thiazole-4-carboxylic acid corresponds to the Pettit's proposal[16].
28- R. Houssin, M. Lohez, J.L. Bernier, and J.P. Hénichart, J. Org. Chem., 1985, 50, 2787.
29- J.L. Bernier, R. Houssin, and J.P. Hénichart, Tetrahedron, 1986, 42, 2695.
30- L. Grehn, and U. Ragnarsson, J. Org. Chem., 1981, 46, 3492.
31- F. Arcamone, V. Nicolella, S. Penco, and S. Redaelli, Gazz. Chim. Ital., 1969, 99, 632.
32- J.W. Lown, and K. Krowicki, J. Org. Chem., 1985, 50, 3774.
33- G. Hilgetag, H. Paul, J. Gunther, and M. Witt, Chem. Ber., 1964, 97, 704.
34- R.C. Kelly, I. Gebhard, and N. Wicnienski, J. Org. Chem., 1986, 51, 4590.
35- W.C. Still, M. Kahn, and A. Mitra, J. Org. Chem., 1978, 43, 2923.