Amino Acids and Peptides; 59.[1] Synthesis of Biologically Active Cyclopeptides; 9.[2] Synthesis of 16 Isomers of Dolastatin 3;[3] I. Synthesis of the 2-(1-aminoalkyl)-thiazole-4-carboxylic Acids

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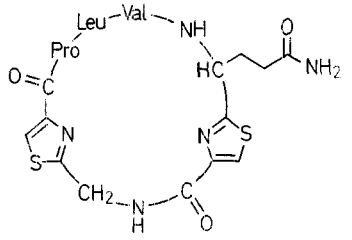
The synthesis of the optically active Dolastatin 3 building block 2-(1-amino-3-cyanopropyl)-thiazole-4-carboxylic acid is described as a prerequisite for the total synthesis of dolastatin isomers.

Numerous biologically active peptides and cyclopeptides incorporating thiazole and thiazoline nuclei have been identified in recent years as metabolic products of fungi and primitive marine animals and their structures elucidated. A number of cytotoxic cyclopeptides belonging to this class contain chiral 2-(aminoalkyl)thiazole-4 carboxylic acids of the *R*- or *S*-series as characteristic ring structural elements, derived biogenetically from cysteine peptides.

In addition to several other "cell growth inhibitory" peptides 1 mg of a substance called Dolastatin 3, with "cell growth inhibitory activity against murine P388 lymphocytic leukemia cells" was isolated by Pettit et al.[4] from *Dolabella auricularia*. This small quantity was sufficient to allow determination of the

amino acid sequence but not of the configuration. The American authors deduced the structure **1** from the fragmentation pattern in the high resolution mass spectrum, but were unable to exclude the possibility that the molecule had the reverse order of bonding as in **2**. However, the published fragment masses are not exact, and not well suited for structural assignment. As each of the structures proposed contains 4 asymmetric centres, synthesis of a total of 32 cyclopeptides with amino acid sequences corresponding to **1** and **2** would be necessary for rigorous proof of structure and configuration by comparison of the natural with a synthetic product. For an identification resting on spectroscopic data, the synthesis of 16 diastereoisomers is sufficient.



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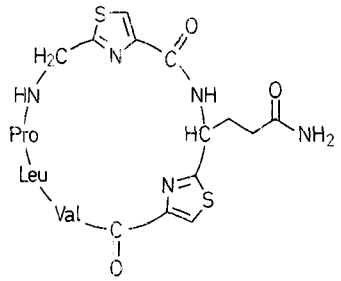

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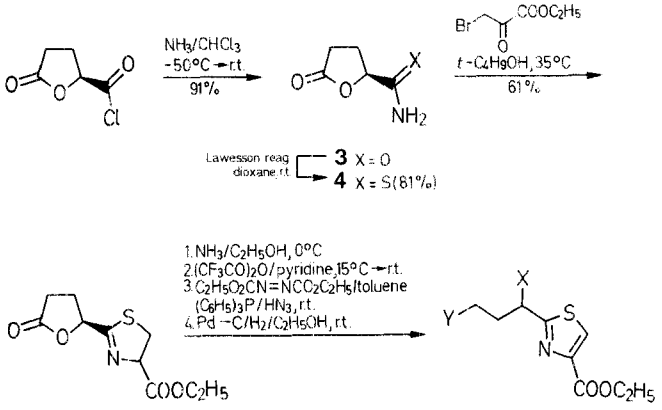
Enthusiastic reports[5] on the biological properties of this natural product induced several industrial and university research groups to undertake the synthesis of these Dolastatin isomers. As about 0.5–1 g of a compound is required for comprehensive evaluation of cancerostatic activity, a prerequisite for an economical synthesis of the diastereoisomers on this scale was a practicable construction of the optical active 2-(1-amino-3-carboxypropyl)-thiazole-4-carboxylic acid. Once we had developed two synthetic routes[1,6] to optically active 2-(aminoalkyl)-thiazole-4-carboxylic acids, and had at our disposal a ring closure method which affords virtually quantitative yields in this series of derivatives, we were in a position to prepare gram quantities of all 16 diastereoisomers of structures **1** and **2** with the *R*-thiazole compound **9** (Y = $CONH_2$). In our first publication[3] describing the successful synthesis of 16 isomers of **1** and **2**, incorporating chiral, thiazole-containing ring elements, we reported NMR-spectroscopic results showing that none of the compounds we had synthesized was identical with Dolastatin 3, and that the proposed structures must therefore be incorrect. A few months later, Hamada and Shioiri[7] arrived at the same conclusion by a completely different route. In a paper published the following year, Pettit[8] described the preparation of the racemic thiazole compound and hence of a mixture of two diastereoisomers of **2**, from which he was able to isolate 10 mg of a pure diastereoisomer.

We have previously described two synthetic routes from amino acids to chiral 2-(aminoalkyl)thiazole-4-carboxylic acids.[1,6] In the synthesis of Dolastatin 3 isomers we chose the cyano group, as a relatively inert precursor of the γ-carboxy group, because it permits facile conversion to the carboxamide group on treatment with hydrogen peroxide under mild conditions applicable to the cyclopeptide. Since they contain no primary amide groups, all the intermediates are of low polarity and easy to purify by chromatography. Because of an almost complete racemisation in the Hantzsch synthesis with optically active acylaminocarboxylic acid thioamides and bromopyruvate is described by several authors,[9–11] we chose the thiazole synthesis from optically active acyloxycarboxylic acid thioamides. Starting with (*S*)-butyrolactone-3-carboxylic acid chloride[12] the optically active thiazole compound **5** was obtained *via* the amide **3** and the thioamide **4**.

If the thiazole synthesis is carried out in ethanol, the lactone ring is opened and the reaction product contains two ester functions which are difficult to differentiate. Cleavage of the cyclic ester function can be avoided by running the reaction in *t*-butanol. The transformation of **5** into **9** *via* the amide **6** and the nitrile **7** is performed by Mitsunobu reaction[13,14] to give **8**, followed by catalytic hydrogenation (Scheme A).

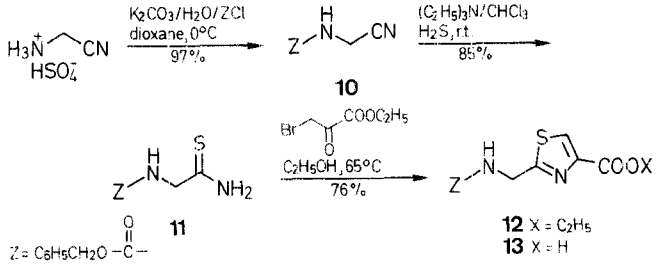


5 **(*S*)-6** (83%) **(*S*)-7** (79%) **(*R*)-8** (90%) **(*R*)-9** (80%)

	X	Y
6	OH	$CONH_2$
7	OH	CN
8	N_3	CN
9	NH_2	CN

Scheme **A**

The synthesis of the second building block 2-(aminomethyl)-thiazole-4-carboxylic acid **13** has been described.[15] In the experimental section we give detailed procedures which have proved useful for preparing larger quantities of the ester **12** starting from (*Z*)-glycinenitrile **10** (Scheme **B**).



Scheme **B**

(*S*)-γ-Butyrolactone-γ-carboxamide (3):
To a stirred solution of ammonia (81.6 g, 4.79 mol) in chloroform (2 l) at −50 °C (*S*)-γ-butyrolactone-γ-carboxylic acid chloride[12] (256 g),

2.4 mol) is added as rapidly as possible. The mixture is stirred at room temperature overnight. The precipitated ammonium chloride is filtered off, and the filtrate concentrated *in vacuo* to afford the crude amide **3**, which is recrystallized from ethyl acetate; yield: 284 g (91%); m.p. 94.5°C; $[\alpha]_D^{20}$: −6.85° (c = 2.6, ethanol).

$C_5H_7NO_3$ (129.1) calc. C 46.51 H 5.46 N 10.85
found 46.59 5.39 10.70

^{1}H-NMR (DMSO-d_6/TMS): δ = 1.73–2.78 (m, 4H); 4.85 (m. 1H); 7.53 ppm (br d, 2H).

(*S*)-γ-Butyrolactone-γ-carbothioamide (4):
A solution of the amide **3** (65.6 g, 0.508 mol) and Lawesson's reagent[16] (101 g, 0.254 mol) in absolute dioxane (480 ml) is stirred at room temperature for 48 h. After filtration and evaporation, the residue is recrystallized from chloroform to give **4**; yield: 60 g (81%); m.p. 113–115°C; R_f = 0.5 (ethyl acetate); $[\alpha]_D^{20}$: −3.85° (c = 1.32, methanol).

$C_5H_7NO_2$ (145.2) calc. C 41.36 H 4.86 N 9.65 S 22.09
found 41.33 4.79 9.57 22.24

^{1}H-NMR (DMSO-d_6/TMS): δ = 1.78–2.92 (m, 4H); 5.16 (m, 1H); 9.27 (br s, 1H); 9.78 ppm (br s, 1H).

4-Ethoxycarbonyl-2-(oxolan-2′-on-5′-yl)thiazole (5):
A mixture of the carbothioamide **4** (30 g, 0.21 mol) and ethyl bromopyruvate (53 g, 0.245 mol) in *t*-butanol (200 ml) is warmed to 35°C for 3 h. After evaporation *in vacuo* the residue is filtered through basic alumina (ethyl acetate/petroleum ether, 1:1). The filtrate is evaporated and the residue recrystallized from ethyl acetate/petroleum ether (1:1) to give the thiazole **5**; yield: 30.5 g (61%); m.p. 106–107°C; R_f = 0.38 (ethyl acetate); $[\alpha]_D^{20}$: −46.7° (c = 1.29, dichloromethane); ee > 98% (based on the optical purity of compound **7**).

$C_{10}H_{11}NO_4S$ (241.3) calc. C 49.78 H 4.60 N 5.81 S 13.29
found 49.54 4.46 5.55 13.33

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.40 (t, 3H, J = 7 Hz); 2.40–3.02 (m, 4H); 4.46 (q, 2H, J = 7 Hz); 5.85 (m, 1H); 8.28 ppm (s, 1H).

4-Ethoxylcarbonyl-2-(3′-carbamoyl-1-(*S*)-hydroxy-prop-1′-yl)thiazole (6):
Thiazole **5** (25.3 g, 0.105 mol) is added at 0°C to rapidly stirred ethanol (300 ml, saturated with ammonia). After 40 min the solvent is distilled off *in vacuo* and the oily residue is recrystallized from dichloromethane/diethyl ether to give **6**; yield: 22.3 g (83%); m.p. 127–128°C; $[\alpha]_D^{20}$: −41.2° (c = 2.05, ethanol); ee > 98% (based on the optical purity of compound **7**).

$C_{10}H_{14}N_2O_4S$ (258.3) calc. C 46.50 H 5.46 N 10.85 S 12.41
found 46.47 5.48 10.75 12.35

^{1}H-NMR (DMSO-d_6/TMS): δ = 1.32 (t, 3H, J = 7 Hz): 1.67–2.50 (m, 4H); 4.33 (q, 2H, J = 7 Hz); 4.93 (m, 1H); 6.50 (d, 1H, J = 5 Hz); 6.83 (br s, 1H); 7.37 (br s, 1H); 8.88 ppm (s, 1H).

4-Ethoxycarbonyl-2-(3′-cyano-1′-(*S*)-hydroxy-prop-1′-yl)thiazole (7):
To a stirred solution of **6** (20 g, 77.4 mmol) in absolute pyridine (65 ml) at 15°C is added over 45 min trifluoroacetic anhydrid (28 ml, 200 mmol). The mixture is allowed to come to room temperature and stirred for further 6 h. After careful addition of cold water, the mixture is extracted with chloroform (3 × 100 ml). The combined organic layer is washed successively with 2 normal hydrochloric acid (250 ml), water (100 ml), dried with magnesium sulfate and evaporated. The residue is filtered on silica gel (ethyl acetate/petroleum ether, 1:1), the filtrate evaporated and the oily residue is recrystallized from ether/hexane to give the nitrile **7**; yield: 14.7 g (79%); m.p. 72–73°C; R_f = 0.42 (ethyl acetate); $[\alpha]_D^{20}$: −28.8 (c = 1.14, dichloromethane); ee > 98%. For determination of optical purity (*S*)-**7** and (*R*)-**7** are examined by HPLC (Baker DNBPG column, isopropanol/hexane 1:9), R_t (*S*)-**7** = 14.19 min, R_t (*R*)-**7** = 14.82 min.

$C_{10}H_{12}N_2O_3S$ (240.3) calc. C 49.99 H 5.03 N 11.66 S 13.34
found 50.00 4.94 11.59 13.61

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.42 (t, 3H, J = 7 Hz); 1.87–2.80 (m, 4H); 4.40 (q, 2H, J = 7 Hz); 4.89 (d, 1H, J = 5.5 Hz); 5.23 (m, 1H); 8.15 ppm (s, 1H).

4-Ethoxycarbonyl-2-(3′-cyano-1′-(*R*)azido-prop-1′-yl)thiazole (8):
To a stirred solution of **7** (13.2 g, 55 mmol) and triphenylphosphine (15.9 g, 60.5 mmol) in toluene (225 ml) at room temperature are successively added a solution of hydrazoic acid in absolute toluene (60.5 mmol, 110 ml) and diethyl azidocarboxylate (10.5 g, 60.5 mmol) in absolute toluene (110 ml). Stirring at room temperature is continued over night and the solvent is distilled off *in vacuo*. To separate off triphenylphosphine oxide the residue is treated with ethyl acetate/petroleum ether (1:1). The filtrate is evaporated and the residue is first filtered on silica gel (ethyl acetate/petroleum ether, 1:1) next on aluminium oxide basic (ethyl acetate/petroleum ether, 3:7) to give the azide **8**; yield: 13.1 g (90%); m.p. 49–50°C; R_f = 0.57 (ethyl acetate); $[\alpha]_D^{20}$: +64.1 (c = 1.2, dichloromethane); ee > 98% (based on the optical purity of compound **9**).

$C_{10}H_{11}N_5O_2S$ (265.3) calc. C 45.27 H 4.18 N 26.40 S 12.09
found 45.34 4.24 26.25 11.90

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.43 (t, 3H, J = 7 Hz); 2.10–2.87 (m, 4H); 4.48 (q, 2H, J = 7 Hz); 5.15 (m, 1H); 8.35 ppm (s, 1H).

4-Ethoxycarbonyl-2(1′-(*R*)-amino-3′-cyano-prop-1′-yl)thiazole (9):
A solution of the azide **8** (5.94 g, 22.4 mmol) in absolute ethanol (250 ml) is hydrogenated (3 at) using palladium on charcoal (5%, 6 g) over 4 h. After filtration and evaporation the residue is passed through silica gel using ethyl acetate as solvent. The resulting amino thiazole **9** is pure enough for further reactions; yield: 4.29 g (80%); m.p. 55–56°C; $[\alpha]_D^{20}$: +8.4 (c = 0.75 chloroform); ee > 98%.

For determination of optical purity, thiazole compounds (*R*)-**9** and (*S*)-**9** are reacted with *N*-trifluoroacetylproline chloride. The resulting diastereomers are examined by gas chromatography using a Carlo Erba *FractoVap Series 4160 fitted with a HMDS/DPTMDS SE 52* (25 m × 0.2 mm).

$C_{10}H_{13}N_3O_2S$ (239.3) calc. C 50.19 H 5.48 N 17.56 S 13.40
found 50.35 5.36 17.44 13.37

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.33 (t, 3H, J = 7 Hz); 1.90 (s, 2H); 2.05–2.78 (m, 4H); 4.38 (m, 1H); 4.44 (q, 2H, J = 7 Hz); 8.19 ppm (s, 1H).

***N*-Benzyloxycarbonyl glycine nitrile (10):**
To a vigorously stirred mixture of water (400 ml) potassium hydrogen carbonate (100.72 g, 1 mol) and dioxane (200 ml) at 0°C are added at the same time simultaneously hydrogen sulfate of aminoacetonitrile[17] (50 g, 0.324 mol) in water (120 ml) and benzyl chloroformate (55.27 g, 0.324 mol) in dioxane (50 ml) over a period of 3 h. After evaporation of dioxane, the residual solution is extracted with ethyl acetate (3 × 200 ml). The organic layer is dried with magnesium sulfate and evaporated. The residue is recrystallized from petroleum ether/ethyl acetate (1:1); yield: 59.8 g (97%); R_f = 0.7 (petroleum ether/ethyl acetate, 1:1); m.p. 65–67°C.

$C_{10}H_{10}N_2O_2$ (190.2) calc. C 63.15 H 5.30 N 14.73
found 63.11 5.40 14.62

^{1}H-NMR ($CDCl_3$/TMS): δ = 3.96 (d, 2H, J = 6 Hz); 5.13 (s, 2H); 7.37 ppm (m, 6H).

***N*-Benzyloxycarbonyl-glycine-carbothioamide (11):**
To a solution of the nitrile **10** (40.1 g, 0.211 mol) and absolute triethylamine (21.34 g, 0.211 mol) in absolute chloroform (300 ml) hydrogen sulfide is passed during 20 h. *n*-Hexane (50 ml) is added and the separated product is filtered and recrystallized from ethyl acetate/chloroform/hexane (1:10:2); yield: 40.4 g (85%); R_f = 0.32 (petroleum ether/ethyl acetate, 1:1), m.p. 144–146°C.

$C_{10}H_{12}N_2O_2S$ (224.3) calc. C 53.55 H 5.39 N 12.49 S 14.30
found 53.43 5.37 12.49 14.27

^{1}H-NMR (DMSO-d_6/TMS): δ = 4.01 (d, 2H, J = 6 Hz); 5.13 (s, 2H); 7.42 (m, 6H); 8.72 (br s, 1H); 9.31 ppm (br s, 1H).

2-Benzyloxycarbonylaminomethyl-4-ethoxycarbonyl-thiazole (12):
A stirred solution of the carbothioamide **11** (20 g, 89.2 mmol) and ethyl bromopyruvate (17.4 g, 89.2 mmol) in absolute ethanol (150 ml) containing molecular sieve (3 Å) is heated for 5 h at 65°C. The solvent is distilled off, and the residue is filtered through basic alumina (petroleum ether/ethyl acetate, 6:4). The filtrate is evaporated and the residue is recrystallized from ether; yield: 21.8 g (76%); R_f = 0.29 (petroleum ether/ethyl acetate, 6:4); m.p. 67–68°C.

$C_{15}H_{16}N_2O_4S$ (320.4) calc. C 56.24 H 5.03 N 8.74 S 10.01
found 56.24 4.96 8.72 9.78

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.37 (t, 3H, J = 7 Hz); 4.41 (q, 2H, J = 7 Hz); 4.71 (d, 2H, J = 6 Hz); 5.17 (s, 2H); 6.14 (m, 1H); 7.38 (s, 5H); 8.12 ppm (s, 1H).

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