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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.* from Dolabella auriculata. This small quantity was sufficient to allow determination of the
Enthusiastic reports 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 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 5(Y = CONH₂). In our first publication 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 arrived at the same conclusion by a completely different route. In a paper published the following year, Pettit 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. In the synthesis of Dolastatin 3 isomers we chose the cyanogroup, 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, we chose the thiazole synthesis from optically active aroylcarboxylic acid thioamides. Starting with (S)-butyrolactone-3-carboxylic acid chloride the optically active thiazole compound 5 was obtained via the amide 3 and the thioumic 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 to give 8, followed by catalytic hydrogenation (Scheme A).

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
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<tbody>
<tr>
<td>OH</td>
<td>CONH₂</td>
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<tr>
<td>OH</td>
<td>CN</td>
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<tr>
<td>N₃</td>
<td>CN</td>
</tr>
<tr>
<td>NH₂</td>
<td>CN</td>
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Scheme A

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

![Scheme B](image_url)
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; [α]D20 = −6.85° (c = 2.6, ethanol).

C₇H₈NO₃ calc. C 46.51 H 5.46 N 10.85 (129.1) found 46.59 5.39 10.70

1H-NMR (DMSO-d₆/TMS): δ = 1.73 − 2.78 (m, 4 H); 4.85 (m, 1 H); 7.55 ppm (br d, 2 H).

(5)-β-Butyrolactone-β-carbioamide (4): A solution of the amide 3 (65.6 g, 0.508 mol) and Lawesson's reagent (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; Rf = 0.5 (ethyl acetate); [α]D20 = −3.85° (c = 1.32, methanol).

C₇H₈NO₃ calc. C 41.36 H 4.86 N 9.65 S 22.09 (145.2) found 41.33 4.79 9.57 22.24

1H-NMR (DMSO-d₆/TMS): δ = 1.78 − 2.92 (m, 4 H); 5.16 (m, 1 H); 9.27 (br s, 1 H); 9.78 ppm (br s, 1 H).

4-Ethoxy carbonyl-2-(4-oxolan-2-yl)-5-thiazole (5): A mixture of the carbioamide 4 (30 g, 0.21 mol) and ethyl bromopyruvate (53 g, 0.245 mol) in β-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; Rf = 0.38 (ethyl acetate); [α]D20 = −46.7° (c = 1.29, dichloromethane); ee > 98% (based on the optical purity of compound 7).

C₇H₆N₂O₄S calc. C 49.78 H 4.60 N 5.81 S 13.29 (241.3) found 49.54 4.46 5.55 13.33

1H-NMR (CDCl₃/TMS): δ = 1.40 (t, 3 H, J = 7 Hz); 2.40−3.02 (m, 4 H); 4.46 (q, 2 H, J = 7 Hz); 5.65 (m, 1 H); 8.28 ppm (s, 1 H).

1H-NMR (CDCl₃/TMS): δ = 1.33 (t, 3 H, J = 7 Hz); 1.90 (s, 2 H); 2.05−2.78 (m, 4 H); 4.38 (m, 1 H); 4.44 (q, 2 H, J = 7 Hz); 8.19 ppm (s, 1 H).

N-Benzoxycarbonyl glycine nitrate (10): To a vigorously stirred mixture of water (400 ml) potassium hydroxide solution (100.72 g, 1 mol) and dioxane (200 ml) at 0°C are added at the same time simultaneously hydrochloric acid of acentoarctitoxin (50 g, 0.324 mol) in water (120 ml) and benzyl chloride (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%); Rf = 0.7 (petroleum ether/ethyl acetate, 1:1); m.p. 65−67°C.

C₉H₁₂N₂O₄S calc. C 56.31 H 5.03 N 14.73 (190.2) found 56.31 4.90 14.62

N-Benzoxycarbonyl glycine-carbioamide (11): To a solution of the nitrite (10.40 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. 4-Nitrobenzene (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%); Rf = 0.32 (petroleum ether/ethyl acetate, 1:1); m.p. 144−146°C.

C₉H₁₂N₂O₄S calc. C 53.55 H 3.93 N 12.49 S 14.30 (224.3) found 53.43 3.72 12.49 14.29

1H-NMR (DMSO-d₆/TMS): δ = 4.01 (d, 2 H, J = 6 Hz); 5.13 (s, 2 H); 4.72 (m, 2 H); 8.72 (br s, 1 H); 9.31 ppm (br s, 1 H).

2-Benzoxycarbonylaminomethyl-4-ethylcarbonyl-thiazole (12): A stirred solution of the carbioamide 11 (20 g, 89.2 mmol) and ethyl bromopyruvate (17.4 g, 89.2 mmol) in absolute ethanol (150 ml) containing molecular sieves (3 Å) is heated for 5 h at 65°C. The solution 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%); Rf = 0.29 (petroleum ether/ethyl acetate, 6:4); m.p. 67−68°C.
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