of **15h,i** (1:1). Flash chromatography on silica gel (9:1 petroleum ether/acetone) gave 0.097 g (42% yield) of **15i** as a slightly yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.20 (m, 5 H), 4.93 (s, 2 H), 4.16 (s, 1 H), 4.07 (m, 1 H), 2.55 (t, J = 6.4 Hz, 2 H), 1.74 (t, J = 6.4 Hz, 2 H), 1.31 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 175.6, 144.0, 137.5, 128.3, 126.6, 126.3, 91.8, 46.4, 38.5, 34.0, 27.1, 27.0.

Reaction of 15j with "$Cp_2Ti{=}CH_2$" Sources (results presented in Table III). In runs 1–5, **15j** was dissolved in 1 mL of PhMe, 0.1 mL of pyridine added, and the mixture stirred at the desired temperature. 1 (in 6 mL of PhMe) was added dropwise over 5 min and stirring continued for 0.5 h at temperature listed and an additional 0.25 h without temperature bath. Workup[5] gave a quantitative yield of **15k** and unreacted **15j** as a yellow oil. ^{1}H NMR integration gave percent yield of **15k**. In runs 6 and 7 THF was used in place of PhMe while in run 8 the imide was dissolved in THF and 1 in PhMe. Runs 9–11 were NMR tube experiments. Spectral data of **15k**: ^{1}H NMR ($CDCl_3$) δ 4.21 (s, 1 H), 4.12 (m, 1 H), 3.12 (s, 3 H), 2.56 (t, J = 6.4 Hz, 2 H), 1.69 (t, J = 6.4 Hz, 2 H), 1.24 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 176.3, 145.7, 90.4, 38.5, 34.5, 34.4, 27.0, 26.7.

The Titanium Enolate of 3,3-Dimethyl-1-(2,6-dimethylphenyl)-2,6-piperidinedione (16d). To a Schlenk tube charged with **3a** (0.085 g, 0.34 mmol) and **15f** (0.095 g, 0.35 mmol) was added 2 mL of PhMe via syringe. The resulting mixture was stirred 0.5 h with the formation of a bright orange precipitate. Additional PhMe was added (7 mL) to give a clear orange solution which was slowly cooled to −50 °C. Isolation of the resulting orange crystals gave 0.065 g (44% yield) of **16d**: ^{1}H NMR (C_6D_6) δ 6.96 (m, 3 H), 5.47 (s, 10 H), 3.81 (t, J = 4.5 Hz, 1 H), 2.13 (d, J = 4.5 Hz, 2 H), 2.10 (s, 6 H), 1.34 (s, 6 H), 0.58 (s, 3 H); ^{13}C NMR (C_6D_6) δ 173.8, 155.8, 137.7, 137.1, 129.6, 129.4, 113.1, 76.0, 40.6, 38.1, 34.4, 25.2. Anal. Calcd for $C_{26}H_{31}NO_2Ti$: C, 71.39; H, 7.14; N, 3.20. Found: C, 71.25;, H, 7.11;, N, 3.18.

Acknowledgment. Support of this research by the National Institutes of Health (GM-31332) is gratefully acknowledged. Helpful discussions with Professors R. E. Ireland and D. A. Straus were also greatly appreciated.

Registry No. 1, 67719-69-1; **2**, 83876-46-4; **3a**, 80122-07-2; **3b**, 75687-68-2; **4a**, 96326-46-4; **4b**, 96326-47-5; **4c**, 96326-48-6; **5b**, 96326-49-7; **5c**, 96326-50-0; **6b**, 96326-51-1; **6c**, 96326-52-2; **7a**, 96326-60-2; **7b**, 1282-51-5; **7c**, 12156-48-8; **8**, 94-41-7; **9**, 96348-34-4; **10**, 96348-35-5; **12a**, 83-25-0; **12b**, 96326-28-2; **12c**, 96326-29-3; **12d**, 1121-07-9; **12e**, 50782-57-5; **12f**, 96326-30-6; **12g**, 75619-07-7; **12h**, 96326-31-7; **12i**, 96326-32-8; **12j**, 6144-75-8; **12k**, 96326-33-9; **12l**, 96326-34-0; **12m**, 96326-35-1; **12n**, 96326-36-2; **12o**, 96326-37-3; **12p**, 96326-38-4; **13a**, 83-24-9; **13b**, 930-87-0; **13c**, 70319-57-2; **14a**, 96348-31-1; **15a**, 96326-39-5; **15b**, 96348-32-2; **15c**, 5768-13-8; **15d**, 96326-40-8; **15e**, 96326-41-9; **15f**, 96326-42-0; **15g**, 96326-43-1; **15h**, 96326-44-2; **15i**, 96348-33-3; **15j**, 1195-95-5; **15k**, 96326-45-3; **16a**, 96326-53-3; **16b**, 96326-54-4; **16c**, 96326-55-5; **16d**, 96326-56-6; **16e**, 96348-36-6; **16f**, 96326-57-7; **17**, 96326-58-8; **18**, 96326-59-9; $(PhCO)_2O$, 102-09-0; Cp_2TiCl_2, 1271-19-8; $NaO_2CC(CH_3)_3$, 1184-88-9; $(Cp_2T_1{=}O)_n$, 59487-89-7; pivalic anhydride, 1538-75-6; acetic anhydride, 108-24-7; benzaldehyde, 100-52-7; phthalic anhydride, 85-44-9; glutaric anhydride, 108-55-4; pinacolone, 75-97-8; 3,3-dimethyl-2,5-pyrrolidinedione, 3437-29-4.

Supplementary Material Available: ^{1}H NMR data for **12a,d,g,j**, **13a,b**, and **15c,j** (1 page). Ordering information is given on any current masthead page.

Synthesis of the Dolastatin Thiazole Amino Acid Component (gln)Thz[1]

Cedric W. Holzapfel and George R. Pettit*

Cancer Research Institute and Chemistry Department, Arizona State University, Tempe, Arizona 85287

Received August 8, 1984

The new thiazole amino acid (gln)Thz, found to occur as one unit of the marine sea hare cyclic pentapeptide dolastatin 3, has been synthesized from L-glutamic acid by the route **2** → **10e**. The synthesis of Z-L-isoglutamine (**4**) was improved by selective ammonolysis of anhydride **3** at −60 °C. A variety of reaction conditions were found to cause complete racemization during the Hantzsch thiazole synthesis step (**9** → **10**). Deuterium labeling experiments indicated loss of the chiral center prior to formation of the thiazole system and suggested an imine–enamine type equilibration involving intermediates A ⇌ B (Scheme II). The *N*-benzyloxycarbonyl derivative (**10d**) of (gln)Thz was partially resolved by employing brucine.

Until discovery of the marine Mollusca (sea hare)[2,3] and Urochordata (tunicate)[4] thiazole cyclic peptides such interesting amino acid structural units[5,6] were only known in *Streptomyces* antibiotics of the thiostrepton[7] and nosiheptide[8] types. Only a few natural thiazole amino acids have been prepared by synthesis.[9,10] These earlier studies were primarily concerned with Gly, Ala, and Val conversions to (gly)Thz (**1a**),[2] (ala)Thz (**1b**), and (val)Thz (**1c**) needed in part for thiostrepton structural efforts.[9] Preparatory to further structural investigations and total syntheses of the potent cell growth inhibitor (P388 lymphocytic leukemia cell line) dolastatin 3[2] and related dolastatins[3] from the sea hare *Dolabella auricularia* we began

(1) Parts 108 and 23 respectively of the series Antineoplastic Agents and Structural Biochemistry. For the preceding contributions refer to Pettit, G. R.; Numata, A.; Takemura, T.; Narula, A. S.; Ode, R. H.; Schmidt, J. M.; Cragg, G. M.; Pase, C. P. *J. Nat. Prod.*, submitted for publication. Pettit, G. R.; Cragg, G. M.; Holzapfel, C. W.; Gieschen, D. P. *Anal. Biochem.*, in preparation.

(2) Pettit, G. R.; Kamano, Y.; Brown, P.; Gust, D.; Inoue, M.; Herald, C. L. *J. Am. Chem. Soc.* **1982**, *104*, 905–907.

(3) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L.; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. J.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482–485.

(4) (a) Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688–5691. (b) Ireland, C. M.; Durso, A. R.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807–1811. (c) Biskupiak, J. E.; Ireland, C. M. *J. Org. Chem.* **1983**, *48*, 2302–2304.

(5) Puar, M. S.; Ganguly, A. K.; Afonso, A.; Brambilla, R.; Mangiaracina, P.; Sarre, O.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1981**, *103*, 5231–5233 and references cited therein.

(6) Hamamoto, Y.; Endo, M.; Nakagawa, M.; Nakanishi, T.; Mizukawa, K. *J. Chem. Soc. Chem. Commun.* **1983**, 323–324.

(7) Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983** *36*, 814–831.

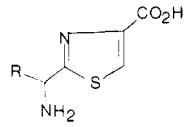
(8) Pascard, C.; Ducruix, A.; Lunel, J.; Prange, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418–6423.

(9) Cross, D. F. W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E. *J. Chem. Soc.* **1963**, 2143–2150.

(10) Brooks, P.; Clark, R. J.; Majhover, B.; Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1960**, 925–931.

0022-3263/85/1950-2323$01.50/0 © 1985 American Chemical Society

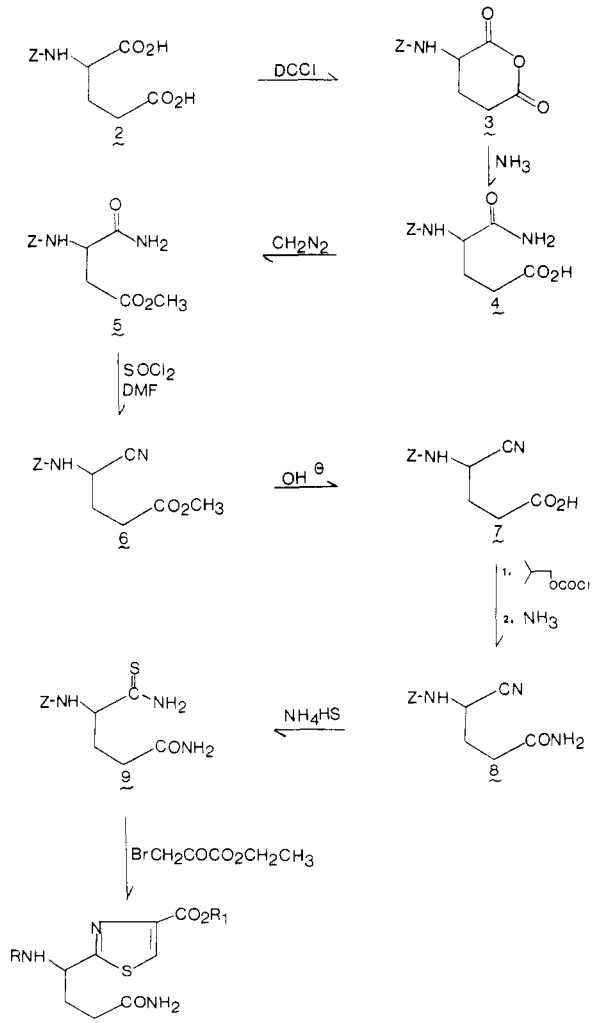
a broad synthetic study of thiazole amino acids derived from the common natural amino acids. The first objective was (gln)Thz (**1d**) the dolastatin 3 structural component that presumably arises from the biosynthetic condensation of Gln and Cys.[2]



1a, R=H
b, R=CH_3
c, R=$CH(CH_3)_2$
d, R=$CH_2CH_2CONH_2$

Synthesis of (gln)Thz (**1d**) by a Hantzsch synthesis (Scheme II), involving reaction of an appropriate optically active thioamide and ethyl bromopyruvate, was considered the route of choice. The only real uncertainty involved whether or not optical integrity could be preserved. With Z-L-Glu (**2**) as starting material the required thioamide (**9**) was prepared by the method summarized in Scheme I. In agreement with previous results[11] conversion of Z-L-Glu to anhydride **3** by reaction with acetic anhydride was found to result in partial racemization. But use of dicyclohexylcarbodiimide (DCCI) as dehydrating agent was found to provide optically pure anhydride **3** in reasonable yield (75%). For preparation of *N*-(benzyloxycarbonyl)-L-isoglutamine (**4**) the reaction[11,12] of ammonia with *N*-(benzyloxycarbonyl)-L-glutamic acid anhydride (**3**) at 0 °C was evaluated and found to furnish substantial amounts of the isomeric *N*-(benzyloxycarbonyl)-L-glutamine. After two crystallizations from water, pure isoglutamine **4** was obtained in only 25% yield. The mixed anhydride method[13] for converting Z-L-Glu directly to *N*-(benzyloxycarbonyl)-L-isoglutamine did not prove advantageous.[14] When reaction of ammonia with *N*-(benzyloxycarbonyl)-L-glutamic acid anhydride was reinvestigated, selective nucleophilic attack at the anhydride α-carbonyl group was found to be substantially improved at −60 °C. By this means pure *N*-(benzyloxycarbonyl)-L-isoglutamine (**4**) was obtained in 69% yield.

Scheme I



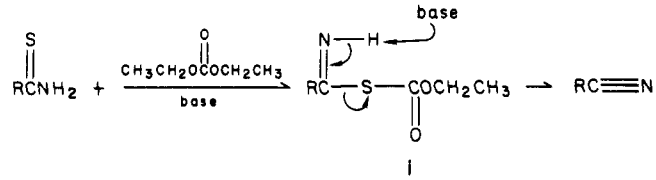
10a, R=Z, R_1=CH_2CH_3
b, R=$COCH_3$, R_1=CH_2CH_3
c, R=$COCH_3$, R_1=H
d, R=Z, R_1=H
e, R=R_1=H
f, R=$CO_2C(CH_3)_3$, R_1=H

Methyl ester **5**[15] was prepared by reaction of isoglutamine **4** with excess diazomethane. Subsequently it was found practical (~60% overall yields) to prepare ester **5** from Z-L-Glu without isolation and purification of the intermediate anhydride (**3**) or amide (**4**). For efficient conversion of amide **5** to *N*-(benzyloxycarbonyl)-L-γ-amino-γ-cyanobutyric acid methyl ester (**6**) several procedures for amide dehydration were studied, including reaction with DCCI (both in the presence and absence of acid catalysts), phosphorous oxychloride–dimethylformamide, and thionyl chloride–dimethylformamide. Best results were obtained by employing a modification of Ressler's[14,16] thionyl chloride–dimethylformamide method with *N*-methylmorpholine and controlling the exothermic reaction.

Saponification of the ester (**6**) group, preparation of a mixed carbonic anhydride, and reaction with ammonia afforded cyano amide **8**. Treatment of nitrile **8** with ethanol saturated (at 0 °C) with ammonia and hydrogen sulfide led to thioamide **9**. Because the specific rotation of thioamide **9** was strongly reduced compared to that of nitrile **8**, the product was transformed back to nitrile **8** by reaction with diethyl carbonate in the presence of *N*-methylmorpholine.[17] The reformed nitrile showed the same specific rotation as the original (**8**) and this result confirmed that formation of thioamide **9** occurred without racemization. Application of a Hantzsch synthesis for conversion of thioamide **9** Z-(gln)Thz ethyl ester (**10**) proceeded as follows.

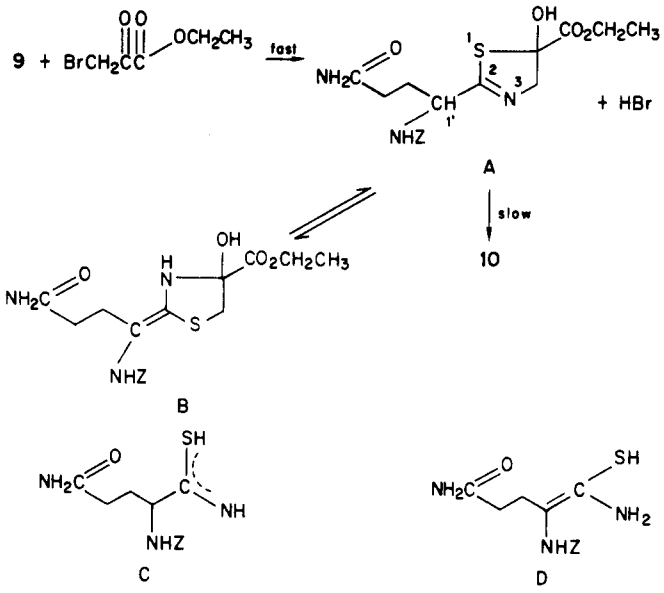
Condensation[9] of thioamide **9** with ethyl bromopyruvate in hot ethanol (several hours) furnished the optically inactive thiazole (**10a**) in excellent yield. Unfortunately, complete racemization occurred. Previously thiazole amino acids synthesized from naturally occurring amino acids were believed[9,18] to racemize at the last step of the syn-

(11) Shealy, Y. F.; Oplinger, C. E.; Montgomery, J. A. *J. Pharm. Sci.* **1968**, *57*, 757–764.
(12) Bergmann, M.; Zervas, L. *Chem. Ber.* **1932**, *65*, 1192–1201.
(13) Ressler, C. *J. Am. Chem. Soc.* **1960**, *82*, 1641–1644.
(14) Ressler, C.; Nagarajan, G. R.; Kirisawa, M.; Kashelikar, D. V. *J. Org. Chem.* **1971**, *36*, 3960–3966.
(15) Sondheimer, E.; Holley, R. W. *J. Am. Chem. Soc.* **1954**, *76*, 2467–2470.
(16) Ressler, C.; Kashelikar, D. V. *J. Am. Chem. Soc.* **1966**, *88*, 2025–2035.
(17) Presumably this mild thioamide → nitrile conversion proceeds via intermediate i.



i

Scheme II



thesis, viz., removal of an *N*-protecting group by heating with strong acid solutions of the type employed for peptide hydrolyses. The reaction leading to thiazole **10a** produced 1 mol equiv of hydrogen bromide and suggested that the racemization observed was acid catalyzed. However, repeating the condensation under conditions that would ensure removal of acid such as in dioxane (or ethanol) with anhydrous sodium carbonate (or 1 mol equiv of *N*-methylmorpholine) or prior reaction of thioamide **9** with 1 equiv of butyllithium (at −23 °C to produce the corresponding lithio salt) resulted in very little thiazole (**10a**) formation due to rapid base-catalyzed decomposition of the bromopyruvate. Eventually, it was found that the reaction time in refluxing ethanol or methanol could be reduced to 15 min. Under these conditions, reaction in deuteriomethanol (CH_3OD) furnished the racemic thiazole fully deuterated at the 1′-position (chiral center) as evidenced by disappearance of a one-proton multiplet at δ 4.95 and replacement of a doublet (N*H*CH) at δ 8.35 by a singlet in the ^1H NMR spectrum. Treatment of the undeuterated thiazole (**10a**) with refluxing deuteriomethanol containing 1–5 equiv of hydrogen bromide (to 30 min) did not result in significant deuterium incorporation. These results showed that an intermediate in the Hantzsch thiazole synthesis and not the final product was involved in the racemization step.

The slow step in the Hantzsch synthesis seems to be the dehydration of cyclic intermediate A (Scheme II).[19] From the preceding racemization results it would appear that intermediate A with a chiral center containing an enolizable proton at position 1′ enters into an imine–enamine type equilibrium (acid catalyzed) as indicated in Scheme II ($\alpha \rightleftharpoons$ B). Of course this assumes that initial enolization involving the thioamide proceeds via intermediate C rather than D. Further attempts to suppress the imine–enamine racemization step did not circumvent this problem but did lead to nice improvements in the Hantzsch synthesis reaction conditions. Specifically, it was found that thiazole **10a** could be prepared by reaction of the thioamide (**9**) with ethyl bromopyruvate under very mild conditions such as reaction in methanol at room temperature with 1 equiv of silver tetrafluoroborate or even more simply by reaction in dry dimethylformamide at room temperature. The reaction in dimethylformamide was repeated in a polarimeter tube to follow the change in specific rotation. The results proved conclusively that racemization occurred prior to isolation of the thiazole.

The *N*-protecting group of thiazole **10a** was removed by treatment with hydrogen bromide in acetic acid under conditions which did not affect the primary amide. The resulting amine hydrobromide was acetylated to yield *N*-acetyl derivative **10b**. The carboxylic acid derivative (**10c**) prepared by saponification of ester **10b** was stable to treatment with hog renal acylase **1**, a useful method[20] for the resolution of certain *N*-acyl amino acids. Instead, optically active *N*-(benzyloxycarbonyl)-(gln)Thz with $[\alpha]^{25}_D$ −6.2° (DMF) was obtained by alkaline hydrolysis of ester **10a** and acidification to provide *N*-(benzyloxycarbonyl)-DL-(gln)Thz (**10d**) followed by several crystallizations of the brucine salt, acidification, and purification of the resulting acid. The asymmetric thiazole proved to be optically stable under usual conditions of the Hantzsch synthesis, namely, heating (to 30 min) with 1 equiv of hydrogen bromide in ethanol. In view of the negative rotations exhibited by the preceding series of L-glutamic acid derivatives the product (**10d**) of partial resolution may be the L isomer.

The preceding results suggest that conversion of optically active α-amino acids into thiazoles by using the Hantzsch reaction will usually lead to complete or partial racemization of the chiral center.

Experimental Section

All solvents were redistilled and solvent extracts were dried over sodium sulfate. Ether refers to diethyl ether and petroleum ether to a mixture of hexanes. Tetrahydrofuran and dimethylformamide were distilled from lithium aluminium hydride and calcium hydride, respectively. Analtech Silica Gel GF (0.25 mm) plates were used for thin-layer chromatography (TLC) and developed with either concentrated sulfuric acid or 1% palladium chloride sprays. Ultraviolet light was also employed for visualization. Column chromatography was performed with silica gel (70–230 mesh) supplied by E. Merck (Darmstadt) or Sephadex LH-20 manufactured by Pharmacia Fine Chemicals, AB, Uppsala, Sweden.

Melting points were observed with a Kofler melting point apparatus. Potassium bromide disks spectra were obtained in methanol. Disks were employed for infrared measurements. Unless otherwise noted, deuteriochloroform was used as solvent and tetramethylsilane as an internal standard for nuclear magnetic resonance measurements. In addition to spectral results entered for *N*-Z-DL-(gln)Thz each of the other new substances gave spectral data consistent with the assigned structures.

***N*-(Benzyloxycarbonyl)-L-glutamic Acid (2).** The crude product[12,22] was recrystallized from ethyl acetate–petroleum ether to furnish *N*-Z-L-glutamic acid (19.4 g, 78%): mp 118–120 °C (lit.[12] mp 120 °C); $[\alpha]^{25}_D$ −5.9° (*c* 10, acetic acid) [lit.[12] $[\alpha]^{19}_D$ −7.1° (*c* 10, acetic acid)].

***N*-(Benzyloxycarbonyl)-L-glutamic Acid Anhydride (3).** To a solution of Z-L-glutamic acid (5.6 g, 0.02 mol)[12,22] in tetrahydrofuran (dry, 50 mL) was added dicyclohexylcarbodiimide (4.12 g, 0.02 mol) in the same solvent (10 mL). The mixture was protected from moisture (calcium chloride drying tube), left for 14 h at room temperature, and cooled to 0 °C. The precipitated urea was removed by filtration and washed with tetrahydrofuran

(18) Dean, B. M.; Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394–3400.
(19) Arakawa, K.; Miyasaka, T.; Ohtsuka, H. *Chem. Pharm. Bull.* **1972**, *20*, 1041–1046.
(20) Greenstein, J. P.; Winitz, M. In "Chemistry of the Amino Acids"; Wiley: New York, 1961; Vol. 3, p 1831. Edgar, M. T.; Pettit, G. R.; Krupa, T. S. *J. Org. Chem.* **1979**, *44*, 396–400.
(21) Pettit, G. R.; Holzapfel, C. W.; Cragg, G. M.; Herald, C. L.; Williams, P. *J. Nat. Prod.* **1983**, *46*, 917–922.
(22) Pettit, G. R.; Cragg, G. M.; Herald, C. L. In "Biosynthetic Products for Cancer Chemotherapy"; Elsevier Pub. Co.: Amsterdam; Vol. 4, in press.

(2 × 8 mL). The combined tetrahydrofuran fraction was concentrated in vacuo at 43 °C. The residue (5.15 g) was crystallized from chloroform–ether at 0 °C to give the anhydride (3.94 g, 75%): mp 93–94 °C (lit.[11] mp 94–95 °C); $[\alpha]^{25}_D$ −39.5° (*c* 10, acetic acid) [lit.[11] $[\alpha]^{25}_D$ −43° (*c* 10, acetic acid)].

***N*-(Benzyloxycarbonyl)-L-isoglutamine (4).** A stirred solution of *N*-Z-L-glutamic acid anhydride (3.5 g) in chloroform (40 mL, freshly distilled from sodium carbonate) and ether (60 mL, freshly distilled from sodium) was cooled to −60 °C (chloroform–dry ice). Ammonia gas was introduced into the stirred solution for 30 min. An exothermic reaction occurred and the internal temperature increased momentarily to −45 °C during the first 5 min. The solution was allowed to warm to room temperature, solvent was removed in vacuo at 40 °C and the residue was treated with water (37 mL). The resultant solution was filtered to remove insoluble material and acidified with 1 N hydrochloric acid to pH 3.4 at 0 °C. The precipitated gel was collected by filtration and washed with water (100 mL) and ether (50 mL). The solid was dried in vacuo and weighed 2.12 g. Analysis by TLC (6:3:93) methanol–acetic acid–ethyl acetate) indicated one major compound and a minor impurity. Recrystallization from a minimum of water afforded pure *N*-Z-L-isoglutamine (2.48 g, 69%): mp 173–175 °C (lit.[11] mp 174–175 °C); $[\alpha]^{25}_D$ −6.5° (*c* 2, methanol) [lit.[11] $[\alpha]^{25}_D$ −7.1° (*c* 2, methanol)].

***N*-(Benzyloxycarbonyl)-L-isoglutamine Methyl Ester (5). Method A.** To 1 g of finely ground *N*-(benzyloxycarbonyl)-L-isoglutamine in methanol (25 mL) was added an excess of ethereal diazomethane and the mixture held at room temperature for 30 min. Excess diazomethane was decomposed with acetic acid. The solution was filtered and solvent evaporated (in vacuo). The residue, crystallized from 1:1 methanol–water, provided *N*-Z-L-isoglutamine methyl ester (0.79 g): mp 118–120 °C (lit.[15] mp 118–120 °C); $[\alpha]^{25}_D$ −5.5° (*c* 1, methanol) [lit.[15] $[\alpha^{24}]_D$ −5.7° (*c* 1, methanol)].

Method B. The following combined procedure was found very convenient for obtaining ester **5**. A solution of *N*-Z-L-glutamic acid (22.4 g) in dry tetrahydrofuran (150 mL) was treated with dicyclohexylcarbodiimide (17.68 g) in tetrahydrofuran (25 mL). After 12 h at room temperature and at 0 °C for 2 h, precipitated dicyclohexylurea was removed by filtration. The urea was washed with chloroform (150 mL). The combined filtrate was cooled to −60 °C, stirred, and protected from moisture (calcium chloride drying tube). Ammonia (dried by passing over sodium hydroxide pellets) was bubbled into the solution for 30 min and a thick white precipitate formed. Solvent was removed in vacuo at 40 °C and the residue dissolved in water (150 mL). Insoluble material was removed by filtration and the aqueous phase acidified (3 N hydrochloric acid) to pH 2 at 0 °C. The resultant precipitate was collected by filtration, washed with water, and dried in vacuo. The solid acid (19.8 g) without any further purification was dissolved in 2:1 methanol–chloroform (150 mL) and treated with an excess of diazomethane (prepared from 20 g of nitrosomethylurea). Evaporation of solvent and crystallization of the solid residue from 1:1 methanol–water furnished *N*-Z-L-isoglutamine methyl ester (13.9 g, 60%): mp 118–120 °C; $[\alpha]^{25}_D$ −5.8° (*c* 1, methanol); MS (EI), *m/e* 294 (M^+, $C_{14}H_{18}N_2O_5$ requires M^+, 294).

***N*-(Benzyloxycarbonyl)-L-γ-amino-γ-cyanobutyric Acid (7).** A solution of Z-L-isoglutamine methyl ester (1.16 g, 3 mM) in dimethylformamide (2.5 mL) containing *N*-methylmorpholine (0.78 mL, 8.4 mM) was stirred, protected from moisture, and cooled in ice. Thionyl chloride (0.3 mL, 4.2 mM freshly distilled) was added (dropwise) over a period of 3 min and the reaction mixture temperature increased to 30 °C. The solution was kept at 0 °C for 2 h and gradually became light brown and TLC showed some unreacted starting material. Additional thionyl chloride (0.1 mL) was added and the mixture allowed to warm to room temperature. After 1 h it was poured into a mixture of crushed ice (50 g) and sodium bicarbonate (10 g). The mixture was extracted with ethyl acetate (60 mL), the organic phase washed with water (2 × 50 mL) and dried, and solvent evaporated in vacuo to give a brown oil (1.24 g). Column chromatography on silica gel (50 g) and elution with 1:1 hexane–ethyl acetate afforded nitrile **6** (0.795 g, 70%) as a colorless oil which solidified on standing. A sample recrystallized from ether–hexane melted at 50–51 °C (lit.[14] mp 51–52.5 °C).

To the crude nitrile (**6**, 0.89 g) in acetone (4.5 mL) and water (1.5 mL) was added 1 N sodium hydroxide (3 mL, 3 mM) dropwise with stirring over 15 min. The mixture was stirred for a further 30 min, diluted with water (10 mL), and extracted with ether (2 × 20 mL). The aqueous phase was acidified with dilute hydrochloric acid and extracted with chloroform (2 × 25 mL). The organic phase was washed with water (13 mL) and dried and solvent evaporated to dryness in vacuo. The residue (0.80 g) was crystallized from ethyl acetate–hexane to yield pure *N*-Z-L-γ-amino-γ-cyanobutyric acid (**7**, 0.62): mp 116–117 °C (lit.[14] mp 110.5–112 °C); $[\alpha]^{25}_D$ −48.9° (*c* 1, methanol) [lit.[14] $[\alpha]_D$ −49.7° (*c* 0.8, methanol)]; MS (EI), *m/e* 262 (M^+, $C_{13}H_{14}N_2O_4$ requires M^+, 262).

***N*-(Benzyloxycarbonyl)-L-γ-amino-γ-cyanobutyramide (8). Procedure A. From Carboxylic Acid 7.** To a solution of carboxylic acid **7** (4.93 g, 18.8 mM) in dry tetrahydrofuran (80 mL) cooled to −23 °C (with magnetic stirring) was added *N*-methylmorpholine (1.9 mL, 18.8 mM) followed by isobutylchloroformate (2.44 mL, 18.8 mM). The mixture was left at −23 °C for 25 min and ammonia was bubbled through the solution (at −23 °C) for 30 min. The mixture was allowed to warm to room temperature, precipitated salts were removed by filtration, and solvent was removed in vacuo. The residue in chloroform (300 mL) was washed with water (2 × 30 mL), dried, and concentrated in vacuo to give amide **8** as a solid (4.64 g) that exhibited only one spot on TLC (1:1 acetone–chloroform). Recrystallization from acetone–chloroform afforded a pure specimen: mp 147–148 °C; $[\alpha]^{25}_D$ −41.5 °C (*c* 1, methanol). Anal. Calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08. Found: C, 59.44; H, 5.68; N, 15.88.

Procedure B. From Thioamide 9. To a solution of thioamide **9** (0.30 g, 1 mM), prepared as described directly below, in dimethoxyethane (1 mL, freshly distilled from lithium aluminum hydride) was added diethyl carbonate (700 μL, 7 mM freshly distilled) and 200 μL (2 mM) of *N*-methylmorpholine. The solution was stirred at room temperature for 48 h, diluted with water (20 mL), and extracted with ethyl acetate (2 × 20 mL). The organic phase was washed with 2% citric acid (20 mL) and water (20 mL) and dried and solvent evaporated. The residual gum was chromatographed on a column of silica gel (30 g) in ethyl acetate to furnish nitrile **8** (40.1 mg): mp 146–147 °C; $[\alpha]^{25}_D$ −39.2° (*c* 1, methanol); identified by direct comparison (IR, NMR) with an authentic sample.

***N*-(Benzyloxycarbonyl)-L-γ-amino-γ-(thiocarbamoyl)-butyramide (9).** Absolute ethanol (120 mL) was saturated with ammonia followed by saturation with hydrogen sulfide at 0 °C. Ammonium hydrogen sulfide began to crystallize in quantity and a solution prepared from *N*-Z-L-γ-amino-γ-cyanobutyramide (**8**, 6 g) in boiling ethanol (150 mL) was cooled and added to this mixture. The resulting mixture rapidly became bright yellow and most of the ammonium hydrogen sulfide dissolved. After 5 h at room temperature, TLC analysis indicated the reaction was complete. The mixture was diluted with water (500 mL) and extracted with chloroform (2 × 250 mL). The organic phase was washed with water (100 mL) and dried and the solvent evaporated in vacuo to a gum (5.87 g). Crystallization from ethyl acetate furnished pure thioamide **9** (4.2 g): mp 120–121 °C; $[\alpha]^{25}_D$ −8° (*c* 1, ethanol); ν_{max} 3412, 3300, 3225, 1714, 1658 and 1535 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_3O_3S$: C, 52.87; H, 5.80; N, 14.22; S, 10.86. Found: C, 52.98; H, 5.67; N, 14.37; S, 10.61.

***N*-(Benzyloxycarbonyl)-DL-(gln)Thz Ethyl Ester (10a). Method A.** A solution of thioamide **9** (0.59 g, 2 mM) and ethyl bromopyruvate (276 μL, 2.2 mM) in absolute ethanol (9 mL) was heated at reflux 15 min. The solution became light yellow and TLC showed complete reaction. Solvent was removed in vacuo and the residue distributed between chloroform (50 mL) and 5% sodium bicarbonate (20 mL). The chloroform was evaporated (in vacuo) and the residue (0.75 g) was crystallized from ethyl acetate–ether to furnish pure thiazole ester **10a** (0.95 g): mp 154–155 °C; ν_{max} 3420, 3300, 1720, 1690, 1660, 1620, and 1540 cm^{-1}; ^1H NMR δ 1.43 (t, *J* = 7 Hz, 3 H, CH_3CH_2), 1.8–2.7 (m, 4 H, CH_2CH_2), 4.38 (q, *J* = 7 Hz, 2 H, CH_3CH_2O), 4.95 (m, 1 H, NHC*H*), 5.15 (s, 2 H, $C_6H_5CH_2O$), 6.9 (br, 1 H) and 7.4 (br, 1 H) ($CONH_2$), 8.35 (d, *J* = 7.5 Hz, N*HCH*), 8.52 (s, 1 H, thiazole CH); MS (EI), *m/e* 391 (M^+); MS(SP-SIMS) (sodium iodide–glycerol matrix),[21] *m/e* 392 $[M + H]^+$ and 414 $(M + Na]^+$. Anal. Calcd for $C_{18}H_{21}N_3O_5S$: C, 55.23; H, 5.41; N, 10.73; S, 8.19. Found: C,

55.45; H, 5.37; N, 10.57; S, 8.07.

When the reaction was repeated with methanol substituted for ethanol or by conducting the reaction at room temperature for 1 h in the presence of 1 mol equiv of silver tetrafluoroborate comparable yields of thiazole **10a** were realized.

Method B. To a stirred dimethylformamide (10 mL) solution of thioamide **9** (1.04 g, 3.53 mM) was added ethyl bromopyruvate (600 μL, 40% excess). The reaction mixture rapidly became yellow and TLC at 40 min showed that all starting material was consumed. The mixture was diluted with ethyl acetate (150 mL) and washed with 1% sodium bicarbonate (100 mL) and water (3 × 100 mL). The organic phase was dried, solvent evaporated, and the residue crystallized from ethyl acetate–ether to afford 1.19 g of thiazole **10a**, mp 155–156 °C.

***N*-Acetyl-DL-(gln)Thz Ethyl Ester (10b).** Thiazole **10a** (103 mg) was treated with 0.7 mL of 27% hydrogen bromide in acetic acid at 0 °C. A clear solution was obtained within 5 min and allowed to warm to room temperature. After 1 h at room temperature a considerable quantity of yellow gummy material had precipitated and TLC did not detect any starting material. Solvent was evaporated (in vacuo) and the residue treated with pyridine (0.5 mL)–acetic anhydride (0.5 mL). Acylation was complete (by TLC) in 1 h and crystalline material separated from solution. The solvent was removed in vacuo and the residue dissolved in chloroform (100 mL) was washed with 5% sodium bicarbonate (10 mL). The organic phase was dried and solvent evaporated to a white solid (84 mg) which crystallized from ethanol–ether to yield *N*-acetyl-(gln)Thz ethyl ester **10b** melting at 226–227 °C. Anal. Calcd for $C_{12}H_{17}N_3O_4S$: C, 48.15; H, 5.73; N, 14.03; S, 10.71. Found: C, 48.37; H, 5.59; N, 14.15; S, 10.53.

***N*-Z-DL-(gln)Thz (10d).** A solution prepared from thiazole ester **10a** (3.5 g, 9.33 mM), dioxane (60 mL), water (60 mL) and 1.0 N sodium hydroxide (10 mL) was allowed to react at room temperature. After 2 h, TLC showed complete saponification and the mixture was extracted with ether (200 mL). The aqueous phase was acidified with 3 N hydrochloric acid and the precipitate collected by filtration. Crystallization from ethanol–hexane yielded pure ***N*-Z-DL-(gln)Thz (10d**, 2.8 g, 86%): mp 185–186 °C; ν_{max} 3475, 3352, 3132, 1720, 1660, 1634, 1586, 1543, and 1338 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.89–2.33 (m, 4 H, CH_2CH_2), 4.90 (m, 1 H, C*H*NH), 5.07 (s, 2 H, PhCH_2O), 6.86 (br, 1 H) and 7.35 (br, 1 H) (CONH_2), 8.30 (d, J = 7 Hz, N*H*CH), 8.46 (s, 1 H, thiazole CH); ^{13}C NMR (Me_2SO-d_6) δ 29.9 (t), 31.1 (t), 53.0 (d), 65.6 (t), 127.5 (d, 2×), 127.7 (d, 2×), 128.3 (d), 128.7 (d), 136.8 (s), 147.0 (s), 155.9 (s), 162.0 (s), 173.3 (s), 174.4 (s); MS(SP-SIMS) (sodium iodide–glycerol matrix),[21] m/e 386 $[M + Na]^+$ and 364 $[M + H]^+$. Anal. Calcd for $C_{16}H_{17}N_3O_5S$: C, 52.88; H, 4.71; N, 11.56; S, 8.82. Found: C, 52.67; H, 4.50; N, 11.52; S, 8.55.

DL-(gln)Thz (1e) and *N*-(*tert*-Butyloxycarbonyl)-DL-(gln)Thz (10e). To *N*-Z-DL-(gln)Thz ethyl ester (**10a**) (1.96 g, 0.5 mM) suspended in dry methylene chloride (20 mL) at 0 °C was added 20 mL of 32% hydrogen bromide in acetic acid and the mixture was stirred at 0 °C for 4 h. A clear light yellow solution was obtained. Ether (60 mL) was added slowly and the precipitated hydrobromide (1.77 g) was collected and dried in vacuo. Without further purification the hydrobromide was dissolved in a mixture of dioxane (20 mL) and water (10 mL). The solution was neutralized with dilute sodium hydroxide solution and an additional 5 mL of 1 N sodium hydroxide was added. The solution was stirred at room temperature until hydrolysis of the ester was complete (4 h by TLC). The **DL-(gln)Thz (1e)** sodium salt was characterized by direct conversion to the Boc derivative **10f** as follows. The aqueous solution of thiazole **10e** sodium salt was cooled to 0 °C and di-*tert*-butyl pyrocarbonate (1.4 g) in dioxane (3 mL) was added. The reaction mixture was stirred at 0 °C for 24 h and acidified (to pH 2) with 3 N hydrochloric acid. The precipitate (1.64 g) was collected, dried, and crystallized from methanol–ether to give a pure specimen of ***N*-Boc-DL-(gln)Thz (10e**, 1.36 g, 83%); mp 124–126 °C; ^1H NMR (Me_2SO-d_6) δ 1.52 (s, 9 H, $(CH_3)_3C$), 4.77 (m, 1 H, C*H*NH), 8.25 (d, J = 7.5 Hz, CHN*H*), 8.49 (s, H, thiazole CH); MS (SP-SIMS) (glycerol matrix),[21] m/e 330 $[M + H]^+$. Anal. Calcd for $C_{13}H_{19}N_3O_5S$: C, 47.41; H, 5.81; N, 12.76; S, 9.73. Found: C, 47.60; H, 5.75; N, 12.70; S, 10.08.

Partial Resolution of *N*-Z-(gln)Thz (10d). The DL-thiazole carboxylic acid (**10d**, 1.08 g, 3 mM) was treated with brucine (1.18 g, 3 mM) in hot 1:1 methanol–water. The crystals which slowly separated in cooling were collected and recrystallized (three times) from 1:1 methanol–water. The crystaline salt (198 mg, mp 233–235 °C) was treated with dilute hydrochloric acid. The *N*-Z-Thz(gln) was separated and purified by crystallization from ethanol–hexane to afford 62 mg melting at 182–183 °C; $[\alpha]^{25}_D$ –6.2° (*c* 1.5, DMF). Because of the negative rotations shown by the preceding L-Glu derivatives this isomer may also have the L configuration. The product with $[\alpha]_D$ –6.2° (36 mg, 0.1 mM) was heated in a refluxing solution of hydrogen bromide (0.1 mM) in ethanol (3 mL) for 30 min. The recovered starting material (22 mg) displayed $[\alpha]^{25}_D$ –5.6° (*c* 0.5, DMF).

Acknowledgment. With thanks and appreciation we acknowledge the financial support contributed by PHS Grant No. CA-16049-07-08 awarded by the National Cancer Institute, DHHS, Mrs. Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation, Mrs. Eleanor W. Libby, the Donald Ware Waddell Foundation, the Robert B. Dalton Endowment Fund, The Upjohn Company, Mrs. Virginia L. Bayless, and Mr. Elias M. Romley. We are also pleased to thank Roger Bontems for assistance with some related experiments.

Registry No. **1e**, 96307-11-8; **2**, 1155-62-0; **3**, 4124-76-9; **4**, 6398-06-7; **5**, 51163-40-7; **6**, 31883-92-8; **7**, 31883-94-0; **8**, 96307-06-1; **9**, 96307-07-2; **10a**, 96307-08-3; **10b**, 96307-09-4; **10d**, 96391-81-0; **10e**, 96307-10-7; H-DL-(gln)Thz ethyl ester hydrobromide, 96307-12-9; *N*-Z-(gln)Thz brucine salt, 96307-13-0; *N*-Z-(gln)Thz, 95716-10-2; ethyl bromopyruvate, 70-23-5; di-*tert*-butyl pyrocarbonate, 24424-99-5; brucine, 357-57-3; dolastatin 3, 80387-90-2.

Chiral Synthesis of the Key Intermediates of (+)- and (–)-Thienamycin

Tetsuji Kametani,* Takayasu Nagahara, and Toshio Honda

Institute of Medicinal Chemistry, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan

Received October 11, 1984

Synthesis of the optically active β-keto ester **11a**, the key intermediate in the preparation of (+)-thienamycin, has been achieved. An enantioselective [3 + 2] cycloaddition of the chiral nitrone with benzyl crotonate was employed as a key reaction.

The recent discoveries of the potent antibiotics thienamycin[1] and its relatives[2] provided impetus to the design of general strategies for the synthesis of these naturally occurring carbapenem antibiotics, members of nonclassical

0022-3263/85/1950-2327$01.50/0 © 1985 American Chemical Society