m, 1 H), 7.95 (br m, 1 H, 3 × NHCO), 8.07 (br s, 1 H), 8.13 (br s, 1 H, 2 × thiazole CH), 8.63 (br m, 1 H, NHCO).

Acknowledgment. The very necessary financial assistance for this research was contributed by Mary Dell Pritzlaff, the Olin Foundation (S.T. and A.W.), the Fannie E. Rippel Foundation, Eleanor W. Libby, the Waddell Foundation (Donald Ware), The Upjohn Co., the Flinn Foundation, and the Robert B. Dalton Endowment Fund. In addition, this investifation was supported by PHS Grant CA-16049-08A2, awarded by the National Cancer Institute, DHHS, the South African Council for Scientific and Industrial Research (financial support for C.W.H.), and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723).

Registry No. (*R*)-**2**, 104549-00-0; (*S*)-**2**, 104639-79-4; **4**·HCl, 96363-14-3; **5**, 104549-01-1; **6**·HBr, 104549-02-2; **6**·TFA, 104549-04-4; (*R*)-**7**, 104549-05-5; (*S*)-**7**, 104639-80-7; (*R*)-**8**, 104640-66-6; (*S*)-**8**, 104549-06-6; (*R*)-**9**·HBr, 104549-07-7; (*S*)-**9**·HBr, 104639-81-8; *N*-Boc-(gly)Thz, 71904-80-8; *N*-Boc-(gly)Thz-ONSu, 104549-09-9; (*R*)-Z-(gln)Thz-OH, 96363-15-4; (*S*)-Z-(gln)Thz-OH, 95716-10-2; (*R*)-*N*-Z-(gln)Thz-(gly)Thz-Val-Leu-Pro-OH, 104549-08-8; (*S*)-*N*-Z-(gln)Thz-(gly)Thz-Val-Leu-Pro-OH, 104639-82-9; dolastatin 3, 80387-90-2.

Synthesis of (*R*)- and (*S*)-(glu)Thz and the Corresponding Bisthiazole Dipeptide of Dolastatin 3

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Received October 23, 1984

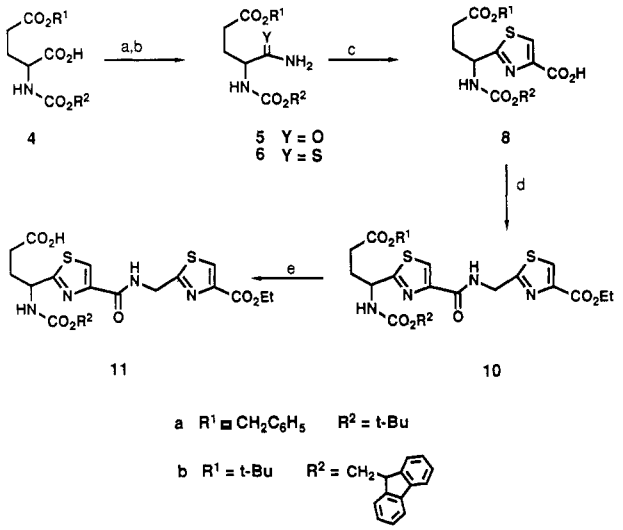
Dolastatin 3 (**1**) has been reported to be a cyclic pentapeptide containing two thiazole amino acids, (gly)Thz (**2**) and (gln)Thz (**3**). The syntheses of (*R*)- and (*S*)-(glu)Thz (**8a,b**) and (glu)Thz-(gly)Thz (**11a,b**) derivatives suitable for elaboration to dolastatin 3 are described. A key feature of the (glu)Thz syntheses is the selective thiation of a primary amide with Lawesson's reagent in the presence of ester and urethane functionalities.

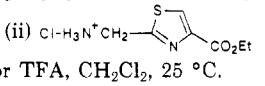
Pettit and co-workers have disclosed their work on a series of compounds from the Indian Ocean sea hare (Dolabella auricularia) which they have named dolastatin.[1] These compounds exhibit high cytotoxicity against the in vitro P388 leukemic cell line and have given good increases in life span in mice inoculated with the same cell line.[1a] Due to shortages of materials only fragmentary structures have been decipherable for most of these materials, but it is clear that they all are peptidic in nature. One of these compounds, dolastatin 3, was sufficiently characterized that a tentative bonding proposal as a cyclic pentapeptide (**1**), lacking stereochemistry, was possible.[1b]

The high activity, the unusual structure, and the structural uncertainty in this family prompted us to begin synthetic studies aimed at providing more definitive structural information.[2] Our first point of focus in developing synthetic plans for this series was the amino acid thiazoles (gly)Thz (**2**) and (gln)Thz (**3**). Several members of this class, including (gly)Thz, (ala)Thz, and (val)Thz have been described previously as constitutents of certain antibiotic and cytotoxic peptides.[3] A method has been developed for the synthesis of these simple bifunctional amino acids,[4] and we adopted this method in our preparation of (gly)Thz derivatives.

The (gln)Thz, on the other hand, is new[5] and as a result of additional functionality brings extra complexity to the

Scheme I[a]



[a] (a) (i) *i*-BuOCOCl, Et_3N or DCC; (ii) NH_3(g); (b) (p-$MeOC_6H_4PS_2)_2$, C_6H_6, 80 °C; (c) $BrCH_2COCO_2H$ (**7**); (d) (i) DCC, HOBt, DMF; (ii)  (**9**), Et_3N; (e) H_2,Pd/C or TFA, CH_2Cl_2, 25 °C.

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(2) After the completion of our work, the synthesis of the proposed dolastatin 3 structure and other isomers was reported by other workers: (a) Hamada, Y.; Kohda, K.; Shioiri, T. *Tetrahedron Lett.* **1984**, *25*, 5303. (b) Schmidt, U; Utz, R; *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 725. (c) Pettit, G. R.; Nelson, P. S.; Holzapfel, C. W. *J. Org. Chem.* **1985**, *50*, 2654.

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(4) (a) Cross, D. F. W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E. *J. Chem. Soc.* **1963**, 2143. (b) Brooks, P.; Clark, R. J.; Majhover, B.; Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1960**, 925.

(5) The workers in ref 2 generated syntheses of (glu)Thz differing substantially from ours. See ref 2a,b and Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.* **1985**, *50*, 2323.

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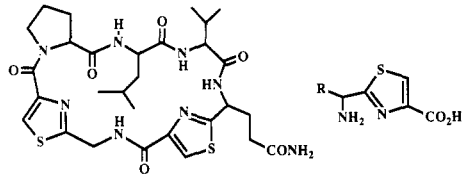
synthetic picture. This same functionality also allows extra flexibility in the intended peptide elaboration, a factor we wish to take advantage of. Thus, we decided to develop (glu)Thz derivatives suitably functionalized so that the γ-carboxylate could be used as the point of attachment for a solid phase peptide synthesis or, after conversion to bisthiazole dipeptide, transformed to the gln γ-amide and used in solution phase synthesis. With these considera-



1 dolastatin 3 2 R = H

Cyclo[Pro-Leu-Val-(glu)Thz-(gly)Thz] 3 R = $CH_2CH_2CONH_2$

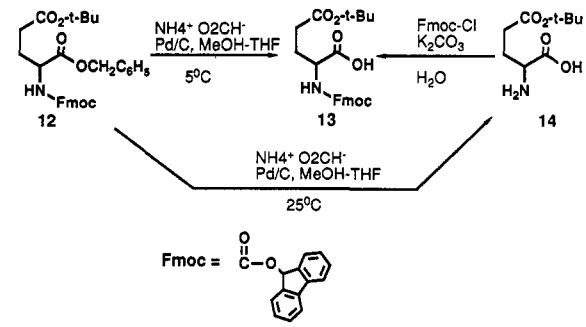
tions in mind we felt that the methodology employed in the (gly)Thz synthesis, namely, hydrothiation of a nitrile to a thioamide and subsequent conversion via a Hantzsch synthesis to a thiazole, if applied to the (gln)Thz would prove unsatisfyingly long. This point is doubly important because the chirality of (gln)Thz in dolastatin 3 is unknown and we wished to carry out the synthesis of both antipodes.

Our syntheses of two protected (glu)Thz's are shown in Scheme I. Of two commercially available L-glutamic acid derivatives with free γ-carboxylates, we first looked at the less expensive **4a**. This was converted to its amide **5** with isobutyl chloroformate or DCC followed by ammonia. The next step involved thiation of **5** with Lawesson's reagent in refluxing benzene to give the thioamide **6**.[6] This transformation is remarkable in its selectivity, giving an 80% yield of **6**, with only traces of additional thiation of the urethane and no evidence of thiation of the ester.

The next step is slightly unusual. On surveying the literature we found that thiazole-4-carboxylic acids have nearly universally been prepared by Hantzsch condensation of a thioamide with a 3-bromopyruvic ester followed by hydrolysis to the acid.[7] When we tried such reactions on **6** we were able to prepare the esters corresponding to **8a** in excellent yield but were unable to effect any selectivity in the hydrolysis. Even more discouraging was the finding that attempted hydrogenolysis of the γ-benzyl ester of **8a** under a variety of conditions occurred only in ethanol. This resulted in significant ester scrambling and diethyl ester formation.

We then turned to another approach, namely, direct condensation with bromopyruvic acid. Only a few citations[8] (in fairly remote sources) for such chemistry are presented in the literature. In the event, condensation of

Scheme II



thioamide **6a** with bromopyruvic acid (**7**) in ethanol containing added $CaCO_3$ to soak up generated HBr resulted in an excellent yield of **8a**.

To this point no mention has been made of the optical activity of any of the intermediates in this scheme. This is due to a remarkable set of circumstances. The amide **5a** is optically active but on thiation produces **6a** which shows no rotation at the sodium or mercury lines and no rotation from 250 to 350 mm in the CD. Yet we know that even though **6a** shows no rotation, it is still not racemic because in the next step it is converted to **8a** which again shows a significant rotation. This is our first encounter with a chiral molecule which displays such extremely low to nonexistent rotation over a broad range of wavelengths. Further work was not done using other methods to determine the optical purity of **6a** because of the difficulties in removal of the benzyl ester from **8a** as mentioned above or from **10a** described below.

The (glu)Thz (**8a**) and **9** were coupled by using DCC and HOBT, giving the bis-thiazole dipeptide **10a**. While some debenzylated material **11a** could be produced from **10a** by hydrogenolysis, the yields were generally quite poor. We believe that the thiazole moiety is poisoning the catalyst in these reactions because we have had difficulty in removing benzyl esters in every case when the thiazole ring is also present.

These circumstances caused us to consider the alternate commercially available L-glutamic acid **4b** as a starting material. The reactions from **4b** to **10b** proceeded in good yield analogously to the **a** series described above. Interestingly in this case the thioamide **6b** showed reasonably large rotation values. The protected (glu)Thz **8b** also showed optical activity but again at this point we had no measure of its optical purity. This was determined by chromatography on a Pirkle chiral column[9] (see Experimental Section) and found to have an ee of 60%.[10] Deprotection of **10b** with TFA in methylene chloride gave an excellent yield of the desired dolastatin 3 precursor **11b**. The acid moiety of **11b** is available for direct conversion to the γ-amide of the (gln)Thz or it may be used to anchor the peptide for further elaboration by solid phase synthesis.

Having obtained the desired precursors in the *S* series we next turned our attention to the *R* series. The *N*-α-Fmoc-D-glutamic acid γ-*tert*-butyl ester (**4b**) was prepared in a slight modification of that described in the literature (see Experimental Section) (ref 11). What is of particular interest about our modified approach is that we carried out a selective phase transfer catalytic hydrogenolysis of

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(9) Pirkle, W. H.; House, D. W.; Fina, J. M. *J. Chromatogr.* **1980**, *192*, 143.

(10) Some racemization is known to occur in the Hantzsch reaction (see ref 5). We suspect that we had less than complete racemization due to our buffering the reaction with calcium carbonate.

(11) Chang, C.; Waki, M.; Ahmed, M.; Meienhofer; Lundell, E. A.; Haug, J. D. *Int. J. Peptide Protein Res.* **1980**, *15*, 59.

the Fmoc on **12** (Scheme II) to produce **13** (ref 12, 13). The reaction could not be carried out selectively at 25 °C but was quite selective at 5 °C. At 0 °C the hydrogenolysis of even the benzyl ester slowed nearly to a stop.

To finish this series **13** was carried through the identical sequence as **4b**, producing the *R* isomers of **8b**, **10b**, and **11b**. Again analysis of (*R*)-**8b** on the Pirkle chiral column showed it to have an ee of 60%. While it would have been most desirous to achieve a synthesis with near to 100% optical purity, we did not anticipate any difficulty with the diminished optical purity obtained in this case. It was expected, and borne out in fact, that elaboration of the materials to the tri-, tetra-, and/or pentapeptide would produce diasteriomeric mixtures which would be chromatographically separable. The results of these studies and on the synthesis of several isomers of the proposed dolastatin 3 structure will be the subject of a subsequent communication.

Experimental Section

After workup solvents were dried over anhydrous magnesium sulfate. Tetrahydrofuran was dried by distillation from benzophenone ketyl. Analtech silica gel GF (0.25 mm) plates were used for TLC. Spots were visualized by UV, phosphomolybdic acid, or ninhydrin. Column chromatography was performed on silica gel (70–230 mesh) supplied by E. Merck, Darmstadt. Melting points were taken on a Kofler hot stage or a Hoover-Thomas Unimelt apparatus. Rotations were performed on a Perkin-Elmer 241 polarimeter, UV spectra on a Perkin-Elmer Lambda 3 spectrophotometer, and IR spectra on a Perkin-Elmer 197 spectrophotometer in Nujol mull. ^{1}H NMR spectra were recorded on a Varian 390 and ^{13}C NMR on a Varian CFT-20 spectrophotometer using internal tetramethylsilane as a standard. FAB mass spectra were run on a JG ZAB 2F spectrophotometer by Dr. L. Baczynskyj and EI mass spectra were run on a CEC 21-110B spectrophotometer by R. Wnuk. Elemental analyses were carried out in the Physical and Analytical Department of The Upjohn Company.

***N*-Boc-L-Glu α-Amide γ-Benzyl Ester (5a).** To a solution of *N*-Boc-L-Glu γ-benzyl ester (10 g, 29.6 mmol) and triethylamine (6.2 mL, 4.5 g, 44.5 mmol) in tetrahydrofuran (100 mL) was added isobutyl chloroformate (4.86 g, 35.6 mmol), and the resultant solution was stirred 45 min at 25 °C. Ammonia gas from the evaporation of the liquid (1.5 mL) was bubbled through the reaction over 10 min. After 45 min the reaction was diluted with ethyl acetate and extracted with 5% aqueous sodium bicarbonate, 1 N aqueous hydrochloric acid, and saturated brine. The organic layer was dried and evaporated. Chromatography of the residue over silica gel eluted with ethyl acetate–toluene mixtures gave a colorless solid (6.79 g yield): mp 122.5–124.0 °C; $[\alpha]^{25}_{578}$ −0.27° (*c* 1.1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 1.7–2.7 (m, 4 H), 4.0–4.4 (m, 1 H), 5.17 (s, 2 H), 5.3–5.6 (d, 1 H), 7.43 (s, 5 H). Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.92; H, 7.10; N, 8.11.

***N*-Fmoc-L-Glu α-Amide δ-*tert*-Butyl Ester (5b).** To a stirred mixture of *N*-Fmoc-L-Glu γ-*tert*-butyl ester (1.0 g, 2.35 mmol) and 1-hydroxybenzotriazole monohydrate (0.36 g, 2.35 mmol) in methylene chloride (25 mL) at 0 °C was added dicyclohexylcarbodiimide (0.484 g, 2.35 mmol). After 5 min the reaction was allowed to warm to 25 °C and stir an additional hour. This mixture was then treated with a −78 °C solution of ammonia (0.22 mL) in methylene chloride (1.0 mL). After another hour at 0 °C the reaction was filtered and the solid washed with methylene chloride. The combined filtrate was washed with 5% aqueous sodium bicarbonate, 1 N hydrochloric acid, and brine and then dried. After evaporation of the solvent the residue was chromatographed over 100 g of silica gel, eluting with ethyl acetate–hexane mixtures. There was obtained 0.89 g (89% yield) of product as a solid: ^{1}H NMR (acetone-d_6, methanol-d_4) δ 1.4 (s, 9 H), 1.8–2.5 (m, 4 H), 4.0–4.5 (m, 6 H), 7.2–7.6 (m, 4 H), 7.7–8.0 (m, 4 H); $[\alpha]^{25}_{578}$ −2.5° (*c* 1.0, MeOH); MS(EI), *m/e* 424 (M^+).

α-*N*-Boc-L-Glu α-Thioamide γ-Benzyl Ester (6a). Amide **5** (6.29 g, 18.7 mmol) and Lawesson's reagent (4.53 g, 11.2 mmol) were added to benzene (80 mL) and the mixture was heated at reflux under a nitrogen atmosphere for 2 h resulting in a clear yellow solution. After cooling to 25 °C and standing overnight, the solvent was evaporated. The residue was chromatographed over silica gel eluted with 30% ethyl acetate in toluene. There was obtained 5.90 g (90% yield) of solid: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 1.7–2.6 (m, 4 H), 4.4–4.8 (m, 1 H), 5.13 (s, 2 H), 5.6–6.0 (m, 1 H), 7.38 (s, 5 H); exact mass 352.1457 (calcd for $C_{17}H_{24}N_2O_4S$, 352.1473). This compound displayed zero rotation between 230 and 350 nm in the CD.

***N*-Fmoc-L-Glu α-Thioamide γ-*tert*-Butyl Ester (6b).** Amide **5b** (0.89 g, 2.1 mmol) and Lawesson's reagent (0.51 g, 1.26 mmol) were added to benzene (20 mL) and the mixture was heated under reflux for 15 min. The reaction was then cooled and evaporated under vacuum. The crude product was chromatographed over 100 g of silica gel eluted with mixtures of ethyl acetate and *n*-hexane. There was obtained 0.86 g (93% yield) of product as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9 H), 1.8–2.8 (m, 5 H), 4.0–4.8 (m, 3 H), 6.2–6.4 (d, 1 H), 7.2–7.5 (m, 4 H), 7.5–7.9 (m, 4 H), 8.2–8.5 (br, 2 H); ^{13}C NMR ($CDCl_3$) ppm 208.3, 173.04, 156.25, 143.81, 141.39, 127.81, 127.52, 127.19, 125.27, 120.04, 81.86, 67.32, 59.40, 47.23, 31.75, 30.98, 28.11; $[\alpha]^{25}_{578}$ −2.3 (*c* 1.1, MeOH); MS(EI), *m/e* 440 (M^+).

(*S*)-*N*-Boc-(glu)Thz γ-Benzyl Ester (8a). Bromopyruvic acid (1.93 g, 11.6 mmol), thioamide **6** (2.83 g, 8.03 mmol), and calcium carbonate (2.32 g, 23.2 mmol) were added to absolute ethanol (60 mL), and the mixture was stirred at 25 °C under an argon atmosphere for 265 min. The reaction was then diluted with ethyl acetate (600 mL) and filtered through diatomaceous earth. The filtrate was washed with 1 N hydrochloric acid and brine and dried. Evaporation of the solvent left a golden oil (4.32 g). This was chromatographed over silica gel eluted with toluene containing increasing concentrations of ethyl acetate, giving the product as a solid (2.75 g) on evaporation. Crystallization from ethyl acetate–*n*-hexane produced small white crystals: 2.12 g (63% yield); mp 118–120 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 1.9–2.8 (m, 4 H), 5.14 (m, 3 H), 5.3–6.3 (m, 1 H), 7.37 (s, 5 H), 8.23 (s, 1 H), 10.0 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 173.67, 172.75, 163.98, 155.60, 146.60, 135.72, 128.58, 128.28, 80.53, 66.62, 53.07–52.63, 30.57, 30.43, 28.27; $[\alpha]^{25}_{578}$ −4.9 (*c* 0.9, MeOH); UV γ_{max} 233 (ε 6450). Anal. Calcd for $C_{20}H_{24}N_2O_6S$: C, 57.13; H, 5.75; N, 6.66; S, 7.63. Found: C, 56.81; H, 5.79; N, 6.55; S, 7.32.

(*S*)-*N*-Fmoc-(glu)Thz γ-*tert*-Butyl Ester (8b). Similarly as described above, bromopyruvic acid (0.47 g, 2.81 mmol) and thioamide **6b** (0.86 g, 1.95 mmol) gave 0.70 g (71% yield) of **8b** after chromatography: ^{1}H NMR ($CDCl_3$, MeOH-d_4) δ 1.5 (s, 9 H), 1.8–2.6 (m, 5 H), 4.1–4.6 (m, 4 H), 4.9–5.2 (br, 1 H), 7.2–7.9 (m, 8 H), 8.2 (s, 1 H); ^{13}C NMR ($CDCl_3$, MeOH) ppm 173.91, 173.10, 163.30, 157.02, 147.87, 144.16, 141.75, 128.09, 127.46, 125.87, 120.29, 81.52, 67.26, 53.24, 47.64, 32.25, 30.62, 28.17; MS(EI), *m/e* 434 [M^+ − $HOC(CH_3)_3$]; $[\alpha]^{25}_{578}$ −7.8° (*c* 1.0, MeOH).

(gly)Thz Ethyl Ester Hydrochloride (9). A solution of glycinethiazole ethyl ester benzamide (47.8 g, 165 mmol) in 4.8 N ethanolic hydrogen chloride (900 mL) was heated under reflux for 17 h. At this time TLC indicated the reaction to be incomplete and titration showed that the concentration of hydrochloric acid had dropped to 3.2 N. Hydrogen chloride was bubbled in until a concentration of 4.8 N was again reached. The reaction was again heated under reflux an additional 8 h. The reaction was then evaporated under vacuum and the residue stirred with ether. The resulting solid hydrochloride salt was collected by filtration giving 18.4 g of product, mp 144–146 °C. The mother liquors were reevaporated and crystallized from ethanol–ether to give 9.6 g of product (total yield, 28 g; 76%): ^{1}H NMR ($CDCl_3$) δ 1.3–1.5 (t, 3 H), 4.3–4.6 (q, 2 H), 4.7 (s, 2 H), 8.6 (s, 1 H). Anal. Calcd for $C_7H_{11}ClN_2O_2S$: C, 37.75; H, 4.98; Cl, 15.92; N, 12.58; S, 14.40. Found: C, 37.52; H, 4.98; Cl, 16.47; N, 12.68; S, 14.58.

(*S*)-*N*-Boc-(glu)Thz-(gly)Thz γ-Benzyl Ester, Ethyl Ester (10a). Acid **8a** (2.36 g, 5.61 mmol) and 1-hydroxybenzotriazole (1.29 g, 8.45 mmol) in glyme (9 mL) were treated with *N,N'*-dicyclohexylcarbodiimide (1.30 g, 6.32 mmol) dissolved in di-

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methylformamide (4 mL). After stirring 1 h at 0 °C the above mixture was filtered and the solids were washed with glyme (2 mL). The combined filtrates were added to a solution of amine hydrochloride **9** (1.38 g, 6.17 mmol) in dimethylformamide (11 mL) which had been adjusted to pH 8 (Fisher Universal Indicator) with *N*-methylmorpholine. After being stirred 18 h at 25 °C, the reaction was treated with additional *N*-methylmorpholine (0.28 mL, 2.6 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.10 g, 0.5 mmol). Stirring was continued an additional 24 h. The reaction was then diluted with ethyl acetate (ca. 300 mL) and filtered, and the filtrate was washed with 1 N hydrochloric acid, 5% aqueous sodium bicarbonate, and saturated brine. After the organic solution was dried and evaporated, the residue was chromatographed over silica gel (200 g) eluted with 50% ethyl acetate–toluene. The product eluted containing some dicyclohexylurea. This impurity was removed by dissolving the product in ether, filtering, and evaporating. The product (2.99 g, 91% yield) was obtained as a colorless, foamy glass: ^{1}H NMR ($CDCl_3$) δ 1.24–1.67 (s + t, 12 H), 1.77–3.00 (m, 4 H), 4.38 (q, J = 7 Hz, 2 H), 4.7–5.3 (m, 5 H), 6.16–6.6 (m, 1 H), 7.39 (s, 5 H), 8.08 (d, J < 3 Hz, this proton appears as a singlet in MeOH-d_4, 1 H), 8.17 (s, 2 H), 8.5–9.1 (m, 2 H); ^{13}C NMR ($CDCl_3$, ref. to $CDCl_3$) ppm 172.29, 171.91, 168.37, 160.81, 160.50, 154.71, 148.28, 146.23, 135.25, 128.35, 127.91, 127.55, 123.42, 79.42, 65.73, 60.67, 51.46, 51.38, 40.20, 29.97, 28.33, 27.73, 13.64; $[\alpha]^{25}_{578}$ −5.5° (*c* 1.1, MeOH); UV λ_{max} (EtOH) 204 nm (ε 40 750), 237 (18 550), 300 (159); MS(FAB), *m/e* 589 (M + H$^+$).

(*S*)-*N*-Fmoc-(glu)Thz-(gly)Thz γ-*tert*-Butyl Ester, Ethyl Ester (10b). Similarly as described above, acid **8b** (0.70 g, 1.38 mmol) and amine hydrochloride **9** (0.34 g, 1.52 mmol) gave 0.72 g (72% yield) of **10b** as a foamy solid: ^{1}H NMR ($CDCl_3$) δ 1.2–1.5 (t, 3 H), 1.45 (s, 9 H), 1.7–2.5 (m, 5 H), 4.0–4.5 (m, 4 H), 4.8–5.2 (br, 3 H), 6.7–7.9 (m, 9 H), 7.95 (s, 1 H), 8.1 (s, 1 H), 8.45–8.95 (m, 1 H); ^{13}C NMR ($CDCl_3$) ppm 172.27, 168.69, 161.31, 161.17, 156.01, 149.05, 146.92, 143.77, 141.35, 128.12, 127.74, 127.09, 125.03, 124.09, 120.01, 80.92, 66.99, 61.43, 52.77, 47.27, 40.73, 31.72, 29.73, 28.09, 14.32; MS(FAB), *m/e* 677 (M + H$^+$); $[\alpha]^{25}_{578}$ −5.9° (*c* 1.0, MeOH).

(*S*)-*N*-Boc-(glu)Thz-(gly)Thz Ethyl Ester (11a). A flask was charged with ammonium formate (0.59 g, 9.4 mmol), benzyl ester **10a** (1.4 g, 2.4 mmol), 10% Pd/C (0.24 g), acetic acid (7.2 mL), and water (4.8 mL). After stirring at 25 °C for 24 h the reaction was diluted with ethyl acetate and filtered through diatomaceous earth. The filtrate was washed with brine, dried, and evaporated, leaving a solid residue (0.28 g). This was chromatographed over CC-4 silica gel (100 g) eluted with 40% toluene in ethyl acetate, giving the desired product (0.12 g, 10% yield): ^{1}H NMR ($CDCl_3$ + MeOH-d_4) δ 0.7–1.64 (m, 12 H), 1.64–2.68 (m, 4 H), 4.45 (q, 2 H), 4.74–5.23 (m, 3 H), 6.0–6.3 (m, 1 H), 8.17 (s, 1 H), 8.23 (s, 1 H), 8.48–8.78 (m, 1 H).

(*S*)-*N*-Fmoc-(glu)Thz-(gly)Thz Ethyl Ester (11b). *tert*-Butyl ester **10b** (0.10 g) was added to methylene chloride (2.5 mL) and trifluoroacetic acid (2.5 mL) and the resultant solution stirred for 30 min. The reaction was then evaporated and reevaporated with carbon tetrachloride. The crude residue was chromatographed over CC-4 silica gel (10 g) eluted with ethyl acetate. The product was obtained as a foamy solid (77 mg, 84% yield): ^{1}H NMR ($CDCl_3$) δ 1.2–1.5 (t, 3 H), 1.9–2.6 (m, 5 H), 4.0–4.7 (m, 4 H), 4.7–5.3 (m, 3 H), 6.0–6.4 (m, 1 H), 7.1–7.9 (m, 7 H), 8.1 (s, 2 H), 8.4–9.4 (m, 3 H); ^{13}C NMR ($CDCl_3$) ppm 172.10, 168.57, 161.55, 161.10, 156.21, 148.71, 146.59, 143.65, 141.41, 128.21, 127.85, 124.94, 120.07, 67.26, 61.77, 52.76, 47.25, 40.40, 30.21, 29.79, 14.22; $[\alpha]^{25}_{578}$ −5.8° (*c* 1.1, MeOH).

***N*-Fmoc-D-Glu α-Benzyl Ester and γ-Benzyl Ester.** *N*-Fmoc-D-Glu (1.4 g, 3.8 mmol) and *N,N'*-dicyclohexylcarbodiimide (0.87 g, 4.2 mmol) were dissolved in tetrahydrofuran (3 mL) and the solution was stirred for 23 h. The precipitated dicyclohexylurea was removed by filtration and the filtrate evaporated leaving the crude anhydride. This material was redissolved in tetrahydrofuran (5 mL) and the solution was treated with benzyl alcohol and potassium carbonate. After 3.5 h the reaction was filtered and the solids were washed with methylene chloride. The filtrate was diluted with additional methylene chloride, and the resultant solution washed with 1 N hydrochloric acid and saturated brine and dried. The crude residue on evaporation (7.62 g) was chromatographed over silica gel (200 g) eluted first with 40% ethyl acetate in *n*-hexane to remove neutrals and then with (1:40:60) acetic acid–ethyl acetate–*n*-hexane. The α-benzyl ester (1.21 g, 70% yield) was eluted first: ^{1}H NMR ($CDCl_3$) δ 1.7–2.6 (m, 4 H), 4.0–4.6 (m, 4 H), 5.0–5.2 (s, 2 H), 5.6–5.9 (d, 1 H), 7.1–7.8 (m, 13 H); ^{13}C NMR ($CDCl_3$) ppm 177.60, 171.75, 156.21, 143.75, 141.32, 135.15, 128.62, 128.29, 128.01, 127.74, 127.11, 125.05, 119.98, 67.41, 53.49, 47.17, 29.92, 27.29; IR (mull) cm^{-1} 3323, 1742, 1695; $[\alpha]^{25}_{578}$ +17.7° (*c* 0.84, MeOH); MS(EI), 441.1602 (M$^+$ − H_2O), calcd for $C_{27}H_{23}NO_5$ 441.1576. The γ-benzyl ester **16** (0.43 g, 25% yield) eluted second: ^{1}H NMR ($CDCl_3$) δ 1.7–2.6 (m, 4 H), 4.0–4.6 (m, 4 H), 5.0–5.1 (s, 2 H), 5.6–5.9 (d, 1 H), 7.1–7.9 (m, 13 H), 9.6–9.9 (b, 1 H); ^{13}C NMR ($CDCl_3$) ppm 172.79, 156.39, 143.76, 143.62, 141.31, 135.66, 128.56, 128.26, 127.74, 127.11, 125.02, 119.98, 67.30, 66.64, 53.46, 47.11, 30.27, 27.27; IR (mull) cm^{-1} 3314, 1728, 1690; $[\alpha]^{25}_{578}$ +6.2° (*c* 0.9, MeOH); MS(EI) on bis TMS *m/e* 497.2067 (M$^+$ − $OCHC_6H_5$), calcd for $C_{26}H_{35}NO_5Si_2$ 497.2054.

***N*-Fmoc-D-Glu α-Benzyl, γ-*tert*-Butyl Ester (12).** *N*-Fmoc-D-Glu α-benzyl ester (4.0 g, 8.71 mmol), concentrated sulfur acid (1 mL), and methylene chloride (40 mL) were added to a Parr bottle and cooled to −78 °C in a dry ice–acetone bath.[14] To this was added isobutylene (40 mL) condensed at −78 °C. The resultant mixture was attached to a Parr apparatus and shaken for 6 h at 25 °C (maximum pressure attained after 1 h was 29 psi). The reaction was then vented, diluted with methylene chloride, and extracted with 5% aqueous sodium bicarbonate. The organic layer was dried and evaporated. The crude residue was chromatographed over silica gel (400 g) eluted with 40% ethyl acetate in *n*-hexane. The product (2.46 g) was isolated as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9 H), 1.82–2.4 (m, 4 H), 3.9–4.6 (m, 4 H), 5.2 (s, 2 H), 5.6–5.8 (d, 1 H), 7.1–7.8 (m, 13 H); ^{13}C NMR 171.97, 171.89, 155.98, 143.99, 143.83, 141.39, 135.34, 128.66, 128.51, 128.34, 127.75, 127.12, 125.14, 120.00, 80.81, 67.33, 67.19, 53.75, 47.27, 31.42, 28.10, 27.58. The yield in this reaction varied from 55 to 85%. It appeared that the colder the workup and the faster the neutralization with sodium bicarbonate, the higher the yield.

***N*-Fmoc-D-Glu γ-*tert*-Butyl Ester (13).** Benzyl ester **12** (0.24 g, 0.47 mmol), ammonium formate (90 mg), 10% Pd/C (90 mg) in methanol (2.5 mL), and tetrahydrofuran (2.5 mL) were stirred at 0 °C. After 10 min at this temperature almost no reaction occurred. The reaction was warmed to 5–7 °C for 10 min which brought the reaction essentially to completion. The reaction was then diluted with ethyl acetate and filtered over diatomaceous earth. The residues were washed with ethyl acetate, and the combined filtrates were evaporated. The residue was chromatographed over silica gel (25 g) eluted with (1:40:60) acetic acid–ethyl acetate–*n*-hexane, giving the product as a colorless solid (0.20 g, 100% yield): ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9 H), 1.8–2.6 (m, 4 H), 4.1–4.7 (m, 4 H), 5.7–6.0 (d, 1 H), 7.2–7.9 (m, 8 H), 9.8–10.1 (b, 1 H); ^{13}C NMR ($CDCl_3$) ppm 175.71, 172.55, 156.42, 143.88, 143.74, 141.37, 127.77, 127.14, 125.12, 120.01, 81.20, 67.34, 53.59, 47.21, 31.62, 28.08, 27.40; $[\alpha]^{25}_{578}$ +8.4 (*c* 1.0, MeOH).

***N*-Fmoc-D-Glu γ-*tert*-Butyl Ester (13) via D-Glu γ-*tert*-Butyl Ester (14).** Benzyl ester **12** (0.24 g, 0.47 mmol), ammonium formate (90 mg), and 10% Pd/C (90 mg) were stirred in methanol (1.5 mL) at 25 °C. TLC on silica gel developed with (1:40:60) acetic acid–ethyl acetate–*n*-hexane showed in 5 min the development of three spots at R_f 0.8 (UV, 9-methylfluorene), R_f 0.45 (UV, acid **13**), and R_f 0.05 (ninhydrin, **14**). After 50 min the starting **12** and the intermediate acid **13** were completely consumed and only the R_f 0.05 material remained. The reaction was then diluted with ethyl acetate, filtered, and evaporated leaving crude **14**. This was dissolved in water (5 mL) and dioxane (10 mL) and the solution cooled to 0 °C. This was treated with sodium carbonate (0.112 g, 1.06 mmol) followed by the dropwise addition of 9-fluorenylmethyl chloroformate (0.17 g, 0.66 mmol). The reaction was stirred 2 h at 0 °C and 18 h at 25 °C and was then partitioned between water and ether. The ether layer was washed with saturated brine, dried, and evaporated. The residue was chromatographed over silica gel (25 g) eluted with (1:40:60) acetic acid–ethyl acetate–*n*-hexane. *N*-Fmoc-Glu γ-*tert*-butyl ester (**13**, 0.140 g, 70% yield) was obtained with properties identical with those obtained above.

D and *R* Series (glu)Thz. These materials were prepared as for the L- and *S* series described above.

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***N*-Fmoc-D-Glu α-Amide γ-*tert*-Butyl Ester (D-5b).** *N*-Fmoc-D-Glu γ-*tert*-butyl ester (**18**, 6.47 g produced the α-amide D-**5b**, 5.78 g, 92% yield): ^{13}C NMR ($CDCl_3$) ppm 173.78, 172.89, 156.31, 143.82, 141.37, 127.76, 127.12, 125.10, 120.01, 81.09, 67.09, 54.10, 47.26, 31.75, 28.09; $[\alpha]^{25}_{578}$ +2.5° (*c* 1.3, MeOH).

***N*-Fmoc-D-Glu α-Thioamide γ-*tert*-Butyl Ester (D-6b).** Amide D-**5b** (5.7 g) produced thioamide D-**6b** (5.52 g, 94% yield); $[\alpha]^{25}_{578}$ +2.2° (*c* 1, MeOH).

(*R*)-*N*-Fmoc-(glu)Thz γ-*tert*-Butyl Ester (D-8b). Thioamide D-**6b** (5.42 g) produced thiazole acid D-**8b** (4.93 g, 79% yield): $[\alpha]^{25}_{578}$ +9.6° (*c* 0.8, MeOH).

(*R*)-*N*-Fmoc-(glu)Thz-(gly)Thz γ-*tert*-Butyl Ester, Ethyl Ester (D-10b). Acid D-**8b** (1.0 g) produced bis-thiazole dipeptide diester D-**10b** (1.17 g, 88% yield): $[\alpha]^{25}_{578}$ +8.2° (*c* 1, MeOH).

(*R*)-*N*-Fmoc-(glu)Thz-(gly)Thz Ethyl Ester (D-11b). *tert*-Butyl ester D-**10b** (1.17 g) produced acid D-**11b** (1.07 g, 88% yield); +7.5° (*c* 1, MeOH).

Determination of Optical Purity of (*S*)-8b and (*R*)-8b. The materials were first converted to their methyl esters by treatment with ethereal diazomethane. The residues on evaporation were dissolved in methylene chloride (2 mg/mL) and 5 μL of this solution injected onto a Pirkle-type 10A column (0.46 × 25 cm, Regis Chemical Co.). The materials were eluted with 30% ethyl acetate in *n*-hexane pumped at a flow rate of 1 mL/min and monitoring the peak elution at 265 nm. Under these conditions the *S* isomer eluted in 18.3 min and the *R* isomer in 19.1 min. Peak height measurements show that the particular sample of *S* isomer run had an ee of 56%. We have subsequently learned that the extent of racemization varies in the Hantzsch condensation and can be complete at higher temperatures or in the absence of base to absorb the generated hydrobromic acid.

Registry No. **1**, 80387-90-2; **2**, 25438-22-6; **4a**, 13574-13-5; **4b**, 71989-18-9; **5a**, 18800-73-2; **5b**, 104090-92-8; D-**5b**, 104090-93-9; **6a**, 104090-94-0; **6b**, 104090-95-1; D-**6b**, 104090-96-2; **7**, 1113-59-3; **8a**, 104090-97-3; **8b**, 104090-98-4; D-**8b**, 104090-99-5; **9**, 104091-00-1; **10a**, 104091-01-2; **10b**, 104091-02-3; D-**10b**, 104091-03-4; **11a**, 104091-04-5; **11b**, 104091-05-6; D-**11b**, 104091-06-7; **12**, 104091-07-8; **13**, 104091-08-9; **14**, 45125-00-6; *N*-Fmoc-D-Glu, 104091-09-0; *N*-Fmoc-D-Glu-OCH_2Ph, 104091-10-3; *N*-Fmoc-D-Glu(OCH_2Ph), 104091-11-4; Fmoc-Cl, 28920-43-6; isobutylene, 115-11-7.

Total Synthesis of (±)-3β-Hydroxynagilactone F

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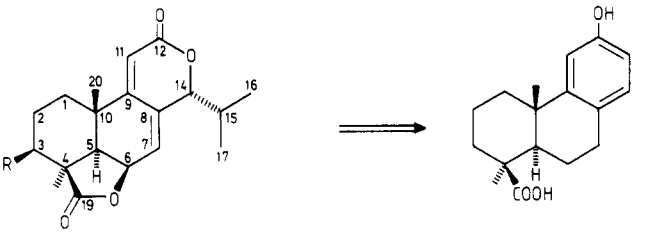
Received December 16, 1985

The total synthesis of racemic 3β-hydroxynagilactone F (**3**), a biologically active norditerpenoid dilactone isolated from *Podocarpus nagi* (Thunberg) Pilger, is described. Starting from the trans-fused bicyclic keto ester **5a**, two possible synthetic routes were explored. Annelation of the δ-lactone to **5a** gave the lactone **9** which was transformed to the 7(8),9(11)-dienolide **17a**. Ring closure of **17a** to a γ-lactone followed by demethylation afforded 1 mg of the title compound **3**. The overall yield of **3** from **5a** was 0.2%.

Norditerpenoid dilactones isolated from various species of *Podocarpus* plants have attracted considerable attention[1,2] because of their remarkable biological activity in such areas as plant growth regulation,[3] insect toxicity,[2] and antitumor activity.[2,4] Some studies have appeared which deal with the synthesis of potentially useful intermediates for this type of compound,[5] but until now only one total synthesis of a norditerpenoid dilactone has been reported. In 1982, Hayashi et al.[6] published the synthesis of nagilactone F (**1**), starting from the natural product, podocarpic acid (**2**) (Scheme I). A great number of norditerpene dilactones with interesting biological activity have structures with a functionalized ring A, and these compounds are accessible with difficulty from natural products such as podocarpic acid (**2**). Our aim was to develop a route to these ring-A functionalized dilactones, and initially 3β-hydroxynagilactone F (**3**) was chosen as the target molecule. This compound was isolated from the root bark of *Podocarpus nagi* (Thunberg) Pilger.[7] The 3β-hydroxy

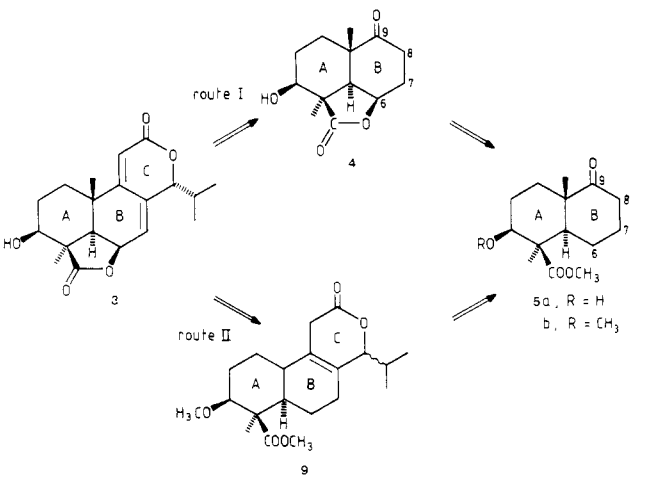
Scheme I



1, R = H
3, R = OH

2

Scheme II



4

3

5a, R = H
b, R = CH₃

9

keto ester **5a**[8] seemed a convenient starting compound for the synthesis of **3**. The two major synthetic problems in

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