^{1}H NMR (300 MHz, $CDCl_3$) δ 2.47 (3 H, m), 3.51 (2 H, d, J = 5.3), 3.92–4.04 (3 H, m), 4.19 (2 H, m), 5.15–5.45 (2 H, m), 7.34 (2 H, d, J = 8.2), 7.80 (2 H, d, J = 8.2). The ee of compound (−)-**15b** was determined to be 90% by comparing the optical rotation of the acetylated product with (+)-**16b**.

Preparation of (+)-3-(Allyloxy)-1,2-epoxypropane (18) from (−)-15b. To a solution of (−)-**15b** (294 mg, 1.03 mmol) in dry THF (6 mL) was added sodium hydride (72 mg) at 0 °C over a period of 10 min, and the mixture was stirred at room temperature for 30 min. Ice water was added, and the mixture was extracted with ethyl acetate. After removing the solvent under reduced pressure, the product was purified by column chromatography (ethyl acetate–n-hexane = 1:4 v/v) on silica gel to give 3-(allyloxy)-1,2-epoxypropane (**18**) (110 mg, 94% yield): $[\alpha]^{23}_D$ = +1.97° (c = 3.78, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.66 (1 H, m), 2.85 (1 H, m), 3.17 (1 H, m), 3.44 (1 H, dd, J = 3, 11.4 Hz), 4.01–4.11 (2 H, m), 5.12–5.30 (2 H, m), 5.80–6.01 (1 H, m). Anal. Calcd for $C_6H_{10}O_2$: C, 63.16; H, 8.77. Found: C, 63.20; H, 8.18. On the basis of the optical purity of the starting material, the epoxide should have 90% ee.

Preparation of (−)-3-(Allyloxy)-1,2-epoxypropane (19) from (+)-16b. To a solution of (+)-**16b** (370 mg, 1.16 mmol) in 5 mL of methanol was added KOH (78 mg, 1.39 mmol) with stirring at room temperature. After 1 h, water was added, the solution was extracted with ethyl acetate, and the solvent was removed under reduced pressure. The product was purified by column chromatography (ethyl acetate–n-hexane = 1:4 v/v) on silica gel to yield 3-(allyloxy)-1,2-epoxypropane (**19**) (115 mg, 87% yield): $[\alpha]^{23}_D$ = −2.08° (c = 2.31, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65 (1 H, m), 2.85 (1 H, m), 3.16 (1 H, m), 3.44 (1 H, dd, J = 3, 11.4) 4.01–4.12 (2 H, m), 5.15–5.38 (2 H, m), 5.85–6.00 (1 H, m). Anal. Calcd for $C_6H_{10}O_2$: C, 63.16; H, 8.77. Found: C, 63.20; H, 8.66. On the basis of the optical purity of the starting material, the epoxide should have 94% ee.

Absolute Stereochemistry of 18 and That of 15b, 16b, and 19. Authentic (2S)-(+)-glycidol tosylate was converted to (2R)-(+)-3-(allyloxy)-1,2-epoxypropane via reaction with allyl alcohol. The product had $[\alpha]^{23}_D$ = +2.1° (c = 2.4, $CHCl_3$) and had the same optical rotation as **18**. Therefore, compound **18** was established to have R configuration and **15b**, **16b**, and **19** should be R, S, and S as indicated.

D-Glyceraldehyde 3-Phosphate. To a solution of water containing 24.4 mmol of Na_2HPO_4 and 1.2 mmol of Na_3PO_4 (250 mL) was added to compound **4** (12.2 mmol).[9] The solution was heated to 75 °C for 30 h. After cooling, the solution was adjusted to pH 1.1 with 1 N HCl and incubated at 37 °C for 24 h. Enzymatic analysis[17] indicated that the solution contained 7.3 mmol (60% yield) of D-glyceraldehyde 3-phosphate. The solution was adjusted to pH 3.5 with 1 N NaOH and stored in the refrigerator. No further purification was attempted. This preparation has been used in the synthesis of 2-deoxyribose-5-phosphate using 2-deoxyribose-5-phosphate aldolase as catalyst[18] (eq 9).

Acknowledgment. This research was supported by the NIH (GM 94154-01).

Supplementary Material Available: ^{13}C NMR spectra for compounds **7** and **9** (2 pages). Ordering information is given on any current masthead page.

(17) Bergmeyer, H. U. *Methods of Enzymatic Analysis*; Verlag Chemie: New York, 1974.
(18) Barbas, C. F., III; Wang, Y.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **1990**, *112*, 2013.

Preparative Separation of the Diastereoisomers of Dioxindolyl-L-alanine and Assignment of Stereochemistry at C-3[1]

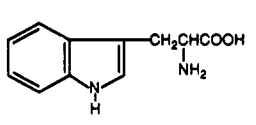
Rita B. Labroo and Louis A. Cohen*

Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

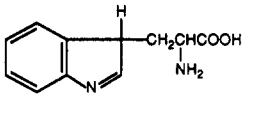
Received March 14, 1990

The diastereoisomers of dioxindolyl-L-alanine are obtained in a 1:1 ratio by air oxidation of oxindolyl-L-alanine. Preparative separation of the diastereoisomers is achieved by reverse-phase HPLC. Stereochemistry of the hydroxyl group at C-3 is assigned by transformation to a related pair of diastereoisomers of known stereochemistry.

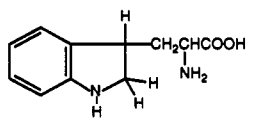
The biosynthesis of tryptophan by tryptophan synthase (TSase), as well as its degradation by tryptophanase (TPase), are considered to involve an enzyme-bound intermediate—the indolenine tautomer (**2**) of L-tryptophan (**1**). Two analogues of **2**, which contain an sp^3 carbon at C-3 (**3** and **4**), were found to be significant inhibitors of both enzymes while analogues of **1** were not inhibitory.[2] The diastereoisomers of **3** were separated by HPLC and were given stereochemical designations on the basis of literature data.[3] Unexpectedly, the αS,3S isomer was found to inhibit only TSase, while the αS,3R isomer inhibited only TPase. Thus, a pair of enzymes, which catalyze essentially the same reaction in opposite directions, are found to have mirror-image stereochemical requirements for the heterocyclic portion of the substrate or the product. Although oxindolyl-L-alanine (**4**) also has a chiral center at C-3, no effort had been made in our earlier work to extend the mirror-image test, since it was already known that H-3 in **4** undergoes facile isotope exchange in mildly acidic D_2O.[4] Furthermore, chiral 3-hydroxyoxindole had been found to racemize rapidly in mild base.[5] We have



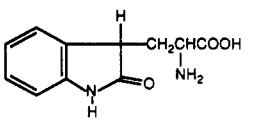
1



2



3



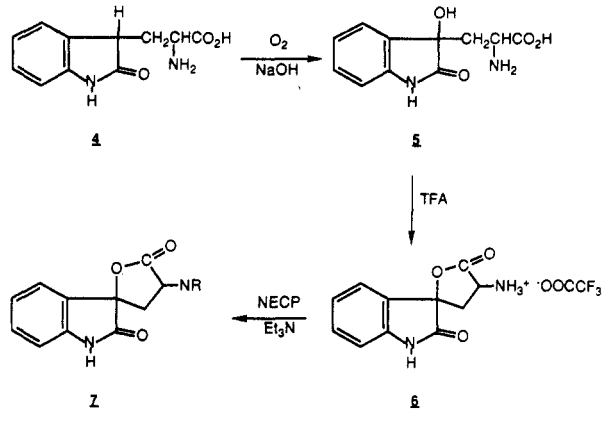
4

(1) Taken in part from the Ph.D. Dissertation (1990) of Rita B. Labroo, George Washington University, Washington, D. C.

(2) Phillips, R. S.; Miles, E. W.; Cohen, L. A. *Biochemistry* **1984**, *23*, 6228.
(3) Rebek, J., Jr.; Tai, D. F.; Shue, Y. K. *J. Am. Chem. Soc.* **1984**, *106*, 1813.
(4) Ohno, M.; Spande, T. F.; Witkop, B. *J. Org. Chem.* **1974**, *39*, 2635.
(5) (a) McKenzie, A.; Stewart, P. A. *J. Chem. Soc.* **1935**, 104. (b) At neutral pH, 3-(hydroxymethyl)oxindole dehydrates to 3-methyleneoxindole, presumably through a C-3 carbanion. Tuli, V.; Moyed, H. S. *Plant Physiol.* **1967**, *42*, 425.

0022-3263/90/1955-4901$02.50/0

Scheme I



TFA = Trifluoroacetic acid; NECP = N-(Ethoxycarbonyl)phthalimide; R = Phthaloyl

now been able to separate the diastereoisomers of **4** by careful HPLC; unfortunately, epimerization at C-3 appears to be complete in 2–3 h, even at pH 3–4. Thus, any attempt to evaluate the inhibitory activities of the separated diastereoisomers of **4** would be fruitless.

An alternative approach would make use of chirally stable 3-substituted analogues of **4**, such as dioxindolyl-L-alanine (**5**). Although we had no basis to expect that any substituent at C-3 larger than hydrogen would be acceptable to the enzymes, the ease of synthesis of **5** seemed to justify the investigation. However, the enzyme studies required not only moderate quantities of both pure diastereoisomers of L-**5**, but a knowledge of stereochemistry at both chiral centers. Early approaches to DL-**5** involved total synthesis,[6] or oxidation of DL-**4** with iodine in alkaline media.[6,7] The expected diastereoisomeric pairs of enantiomers were isolated by the first method, but only one pair was isolated by fractional crystallization from water following iodine oxidation. Interestingly, both DL pairs are obtained by fractional crystallization from water following air oxidation of an alkaline solution of DL-**4**.[7] Iodine oxidation was also performed with L-**4**;[8] analytical TLC suggested that both diastereoisomers had been formed, but neither was isolated.

In our hands, air oxidation of an alkaline solution of L-**4** proceeded very cleanly to give only the diastereoisomers of L-**5** (Scheme I). According to analytical HPLC and the intensities of the doubled ^{1}H NMR signals, the products are formed in a 1:1 ratio.[9] Although fractional crystallization from water had led to the separation and recovery of both DL pairs, the diastereoisomers of L-**5** could not be separated by fractional crystallization from water. Extensive exploration of HPLC systems finally provided a method for the recovery of both isomers in pure form. The same procedure was used to synthesize and separate the diastereoisomers of D-**5**, starting from D-tryptophan.

The enantiomeric purity of L-**4** was demonstrated by comparison with D-**4** on a chiral TLC plate, the R_f values providing a clear differentiation. Similarly, the diastereoisomers of L-**5** were compared with those of D-**5**. Although one of the isomers of L-**5** was distinguishable from its D enantiomer, the D and L enantiomers of the other isomer could not be differentiated. All four isomers of **5** were then allowed to react with the chiral reagent, 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose isothiocyanate (GITC).[10] Analysis by HPLC showed each derivative to consist of a single peak with a different retention time, thus establishing the enantiomeric purity of each isomer.

Table I. Assignment of Stereochemistry to Diastereoisomers of *N*-Phthaloyldioxindolyl-L-alanine Spirolactone (7) and Dioxindolyl-L-alanine (5)

isomer	mp, °C	$[\alpha]_D$, deg	R_f[b]	t_R,[b]	ref
L-**7A**	277–280	−147 (23 °C)[a]	0.62	35.4	*c*
($\alpha S,3S$)	275–277	−133 (25 °C)[a]			11
	271–273	−163 (20 °C)[a]			16
	265–268				13
L-**7B**	259–262	−204 (23 °C)[a]	0.53	32.8	*c*
($\alpha S,3R$)	279–281	−206 (25 °C)[a]			11
	261–263	−220 (20 °C)[a]			16
L-**5A**	235–240	−20.4 (22 °C)[d]	0.59	10.0	*c*
($\alpha S,3S$)					
L-**5B**	161–171	+42.8 (22 °C)[d]	0.55	13.2	*c*
($\alpha S,3R$)					

[a] In acetone. [b] See Experimental Section for details. [c] Present work. [d] In 0.01 N NaOH.

In earlier work, the stereochemistry of the *N*-phthaloyl derivatives (**7**) of the spirolactones related to L-**5** had been assigned[11] by comparison with the tryptophan-based alkaloid, nortryptoquivaline, whose stereochemistry had been determined by X-ray analysis.[12] In independent studies, the same assignments had been made by analysis of ^{1}H NMR coupling constants.[13] Thus, conversion of each isomer of L-**5** to that of L-**7** would permit the assignment of stereochemistry to the C-3 chiral center in **5**. Each isomer of **5** was found to undergo rapid cyclization to the corresponding aminolactone salt (**6**) in neat trifluoroacetic acid. Although we were hopeful that the acid-catalyzed cyclization would follow the normal esterification mechanism, the results of related studies[14] required that we consider the possibility of carbocation formation at C-3. Since each diastereoisomer of **6** hydrolyzed back in water to form *only* the original isomer of **5** (RPTLC and HPLC analysis), we were confident that no loss of chiral integrity had occurred at either the C-3 or the α-chiral center during cyclization of **5** to amino lactone with trifluoroacetic acid.

The diastereoisomers of L-**6** were then allowed to react with *N*-(ethoxycarbonyl)phthalimide[15] (Scheme I) to form the corresponding diastereoisomers of L-**7**. Comparison of the optical rotations of L-**7A** and L-**7B** with the reported values[11,16] (Table I) permits assignment of stereochemistry to the diastereoisomers of L-**5**. Thus L-**5A** is $\alpha S,3S$ and L-**5B** is $\alpha S,3R$.[17] Isomer L-**7B** is unstable on fusion, being converted largely to L-**7A** or to D-**7B**; L-**7A**, on the other hand, is much more stable under the same conditions. The conversion of L-**7B** to D-**7A** in the presence of strong base (epimerization at the α-carbon) had been described pre-

(6) Julian, P. L.; Dailey, E. E.; Printy, H. C.; Cohen, H. L.; Hamashige, S. *J. Am. Chem. Soc.* **1956**, *78*, 3503.
(7) Savige, W. E. *Aust. J. Chem.* **1975**, *28*, 2275.
(8) Savige, W. E. *Aust. J. Chem.* **1971**, *24*, 1285.
(9) Iodine oxidation, on the other hand, gives a 2:1 ratio of isomers. This apparent induction of chiral preference should involve the α-chiral center, probably through a cyclic intermediate or transition state; the phenomenon will be discussed further in a subsequent paper.
(10) Nimura, N.; Ogura, H.; Kinoshita, T. *J. Chromatogr.* **1980**, *202*, 375.
(11) Büchi, G.; DeShong, P. R.; Katsumura, S.; Sugimura, Y. *J. Am. Chem. Soc.* **1979**, *101*, 5084.
(12) Springer, J. P. *Tetrahedron Lett.* **1979**, 339.
(13) Nagasaka, T.; Ohki, S. *Chem. Pharm. Bull.* **1971**, *19*, 603.
(14) Labroo, R. B.; Labroo, V. M.; Cohen, L. A., manuscript in preparation.
(15) McArthur, C. R.; Worster, P. M.; Okon, A. U. *Synth. Commun.* **1983**, *13*, 311.
(16) Ohnuma, T.; Kasuga, H.; Kimura, Y.; Ban, Y. *Heterocycles* **1982**, *17*, 377.
(17) The designations A and B were chosen arbitrarily on the basis of R_f values. These designations, however, acquire a new significance when stereochemistry has been assigned. For the sake of consistency, therefore, the enantiomer of L-**5A** ($\alpha S,3S$) is designated D-**5B** ($\alpha R,3R$) and that of L-**5B** ($\alpha S,3R$) is designated D-**5A** ($\alpha R,3S$).

viously.[11] Isomer L-**7B** was also found to be unstable in hot aqueous acid, with isomerization at the α carbon being assumed.[13] Our studies indicate that both the thermal and the acid-catalyzed epimerizations involve C-3 and not the α-carbon.[14]

Each diastereoisomer of L-**5** was found to inhibit only one enzyme, in the same order as observed for the diastereoisomers of **3**.[18]

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR chemical shifts are reported relative to internal TMS or H_2O (δ 4.78) as reference. Chemical ionization mass spectra were obtained with ammonia as the reagent gas.

Normal thin-layer (TLC) or reverse-phase (RPTLC) chromatography was performed using silica plates (Analtech, Inc.). Chiralplate was obtained from Alltech Assoc. Amino acids were detected by use of 0.2% ninhydrin in methanol. The following reverse-phase (RPHPLC) columns were used for high-performance liquid chromatography: analytical, Beckman Ultrasphere (5 μ, C_{18}, 4.6 mm × 25 cm); semipreparative, Beckman Ultrasphere (5 μ, C_{18}, 10 mm × 25 cm); preparative, Rainin Dynamax (8 μ, C_{18}, 21.4 mm × 25 cm). Solvent flow rates were 1, 4, and 9 mL/min, respectively. HPLC-grade solvents were used for HPLC: solvent A, 0.05% trifluoroacetic acid (TFA) in H_2O (v/v); solvent B, 0.05% TFA in CH_3CN–H_2O 7:3 (v/v).

Oxindolyl-L-alanine (4). The following procedure is a modification of that described by Savige and Fontana.[19] Dimethyl sulfoxide (DMSO, 9 mL, 125 mmol) was added slowly to 50 mL of 12 N HCl at ambient temperature. Phenol (1 g, 10 mmol) was added, followed by a suspension of L-tryptophan (10 g, 50 mmol) in 300 mL of glacial acetic acid. The mixture was stirred at ambient temperature for 5 h, at which point all the tryptophan had dissolved and the slightly viscous solution had become light pink. Acetic acid and DMSO were removed in vacuo (bath temperature 50 °C). The dark, syrupy residue was dissolved in 15 mL of glacial acetic acid, and the solution was refrigerated overnight. A rose-colored solid had crystallized, which was collected by filtration, washed with diethyl ether, and dried under vacuum to give 7.7 g (70%) of **4**, mp 240–242 °C dec (lit.[19] mp 260 °C dec). This material showed one spot by TLC (1-butanol–EtOAc–AcOH–H_2O, 1:1:1:1 v/v); L-tryptophan gives a light purple color with ninhydrin (R_f 0.70) and **4** gives a dark pink color (R_f 0.68). According to RPHPLC (see below), the product contains ca. 3% of **5**; since **4** was prepared as the starting material for **5**, removal of the trace of impurity seemed unnecessary: UV (H_2O) λ_{max} 250.4 nm; ^{1}H NMR (D_2O) δ 2.15–2.63 (m, 2 H, β-CH_2), 4.01–4.22 (m, 1 H, α-CH), 7.0–7.5 (m, 4 H, aryl H's); MS (CI, NH_3) m/z (%) 221 (100) $(M + 1)^+$.

The enantiomeric purity of L-**4** was demonstrated by TLC comparison with D-**4** on Chiralplate, using MeOH–H_2O–MeCN, 1:1:3 (v/v), as the developing solvent; L-**4** moved slightly faster, with an R_f difference of 0.04. D-Tryptophan was used to prepare D-**4**, by the same DMSO–HCl procedure.

The diastereoisomers of L-**4** showed t_R values of 13.16 and 13.68 min (relative peak areas of 52 and 48, respectively) on an analytical RPHPLC column, using solvents A and B with a 0–60% gradient of B over 35 min. The diastereoisomers were carefully collected in separate fractions; after 1–2 h, each fraction was separately reapplied to the column and showed the original pair of peaks with equivalent areas.

Dioxindolyl-L-alanine (5A, 5B). Oxindolyl-L-alanine (**4**, 1.1 g, 5 mmol) was dissolved in 100 mL of 0.1 N NaOH, and the solution was aerated for 12 h at ambient temperature (25 °C). Conversion was complete, with the formation of two products, both more polar than **4** on RPTLC (water) and showing spots of almost equal intensity (ninhydrin). The reaction mixture was adjusted to pH 4 with acetic acid and refrigerated overnight, but no solid separated. The solvent was removed in vacuo, with precipitation occurring during concentration. The precipitate was collected and dried by suction to give a pale yellow cake. The solid showed two closely associated spots by RPTLC (5 × 20 cm, water), both of which turned pinkish-purple with ninhydrin. The solid was identified as **5** from its mass spectrum and was found to be a mixture of diastereoisomers from its ^{1}H NMR spectrum. According to RPHPLC analysis, isomers **5A** and **5B** were present in equal amount and had identical UV spectra with λ_{max} at 255 nm, characteristic of the dioxindole chromophore.

Analytical separation of the reaction mixture was performed on an analytical RPHPLC column with solvent A as the eluting solvent. An attempt to separate the diastereoisomers of L-**5** by fractional crystallization from water proved unsuccessful. Some solid was formed on refrigeration, but this solid was again found to be a mixture of isomers by RPTLC and ^{1}H NMR. A semipreparative separation of the isomers was achieved by RPHPLC with solvent A. Larger scale separation was performed by applying portions of crude product (ca. 15 mg/0.5 mL of water) to a Rainin Dynamax preparative column, which had been equilibrated with solvent A, and eluting with solvent A at a flow rate of 9 mL/min. Fractions were carefully collected by monitoring changes in absorbance at 280 and 330 nm, with a sensitivity of 2 AUFS. Fractions corresponding to each isomer were pooled and evaporated in vacuo. Purity of each isomer was confirmed by RPTLC and analytical RPHPLC. Each isomer was crystallized from water, L-**5A** as a white powder and L-**5B** as a white, crystalline solid.

L-**5A**: mp 235–240 °C; UV (H_2O) λ_{max} 255 nm; ^{1}H NMR (D_2O) δ 2.27–2.32 and 2.47–2.54 (m, 2 H, β-CH_2, $J_{\alpha\beta1}$ = 3.7 Hz,[20] $J_{\alpha\beta2}$ = 9.5 Hz, $J_{\beta1\beta2}$ = 15.4 Hz), 4.20–4.24 (dd, 1 H, α-CH, $J_{\alpha\beta1}$ = 3.7 Hz, $J_{\alpha\beta2}$ = 9.6 Hz), 7.10–7.55 (m, 4 H, aryl H's); MS (CI, NH_3) m/z (%) 237 (53) $(M + 1)^+$, 219 (50) $(M + 1 - H_2O)^+$, 175 (40) $(M + 1 - H_2O - CO_2)^+$; $[\alpha]^{22}_D$ −20.4° (c 0.62, 0.01 N NaOH); t_R 9.98 min; R_f 0.59 (RP silica, 5 × 20 cm, water); ninhydrin color, pinkish purple.

L-**5B**: mp 168–171 °C; UV (H_2O) λ_{max} 255 nm; ^{1}H NMR (D_2O) δ 2.31–2.52 (m, 2 H, β-CH_2, $J_{\alpha\beta1}$ = 9.8 Hz, $J_{\alpha\beta2}$ = 3.6 Hz, $J_{\beta1\beta2}$ = 15.6 Hz), 4.29–4.34 (dd, 1 H, α-CH, $J_{\alpha\beta1}$ = 3.6 Hz, $J_{\alpha\beta2}$ = 9.7 Hz), 7.0–7.47 (m, 4 H, aryl H's); MS (CI, NH_3) m/z (%) 237 (10) $(M + 1)^+$, 219 (73) $(M + 1 - H_2O)^+$, 175 (100) $(M + 1 - H_2O - CO_2)^+$; $[\alpha]^{22}_D$ +42.8° (c 0.50, 0.01 N NaOH); t_R 13.23 min; R_f 0.55 (RP silica, 5 × 20 cm, water); ninhydrin color, pinkish purple.

The corresponding diastereoisomers of D-**5** were prepared by air oxidation of D-**4** and were separated as described above for the L series.

Enantiomeric Purity of L-5A and L-5B. The enantiomeric purity of the diastereoisomers was initially checked on Chiralplate (MeOH–H_2O–MeCN, 1:1:3 v/v) against the corresponding diastereoisomers of D-**5**, and the following R_f values were obtained: L-**5A**, 0.68; L-**5B**, 0.66; D-**5A**, 0.65; D-**5B**, 0.59. While L-**5A** and its enantiomer, D-**5B**, were readily distinguishable, the other enantiomeric pair could not be differentiated on the plate.

All four isomers were then reacted with the chiral reagent, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). Each isomer (0.1 mg) was dissolved in 50% aqueous acetonitrile containing 0.4% (w/v) triethylamine to give a final volume of 0.2 mL. To 50 μL of this stock solution was added 50 μL of 0.2% (w/v) of GITC in acetonitrile. The reaction mixture was stirred at ambient temperature for 45 min. Analysis was performed by analytical RPHPLC with solvents A and B, using a gradient of 20% B to 100% B in 40 min. Each isomer gave a single derivative peak with the following t_R values: L-**5A**, 13.71 min; L-**5B**, 14.03 min; D-**5A**, 14.63 min; D-**5B**, 14.30 min. Thus, all four isomers can be differentiated by RPHPLC analysis of their GITC derivatives.

***N*-Phthaloyldioxindolyl-L-alanine Spirolactones (7) from 5.** Dioxindolyl-L-alanine (L-**5A**, 472 mg, 2 mmol) was dissolved in trifluoroacetic acid (15 mL), and the solution was stirred at ambient temperature. After 2 h, TLC showed the formation of spirolactone **6** to be essentially complete (silica; $CHCl_3$–MeOH–AcOH, 7:2.5:0.5 v/v). Trifluoroacetic acid was removed under nitrogen, and the major product was separated by column chromatography (silica gel, 10% MeOH in $CHCl_3$). The fractions containing the major product were pooled, and the solvent was removed completely in high vacuum to give a light yellow solid

(18) Labroo, R. B.; Labroo, V. M.; Cohen, L. A., to be submitted elsewhere.

(19) Savige, W. E.; Fontana, A. *Int. J. Peptide Protein Res.* **1980**, *15*, 285.

(20) The β-CH_2 protons are arbitrarily identified as $\beta1$ and $\beta2$.

(592 mg, 85% yield). The solid was identified as 6 (trifluoroacetate salt) by mass spectrometry: MS (CI, NH_3) m/z (%) 219 (100) $(M + 1)^+$.

An aqueous solution of a small sample of L-6A was left at ambient temperature for 3-4 h and was then analyzed by RPHPLC, which showed L-5A as the only hydrolysis product.

The amino lactone salt (531 mg, 1.6 mmol) was dissolved in anhydrous DMSO (17 mL). *N*-(Ethoxycarbonyl)phthalimide (363 mg, 1.66 mmol) was added, followed by triethylamine (0.23 mL, 1.66 mmol), and the reaction mixture was stirred at ambient temperature. After 30 h, the reaction mixture was diluted with water (10 mL) and extracted with ethyl acetate (3 × 150 mL). The organic layer was washed with water (4 × 100 mL) and with saturated brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The major band was separated on a preparative silica plate (EtOAc–hexanes, 6:4 v/v) and the product (L-7A) crystallized from acetone as a white solid (100 mg, 18% yield): mp 277–280 °C (lit. mp, Table I); TLC showed that the compound was essentially unaffected by brief fusion; ^1H NMR (CD_3CN) δ 2.88–3.02 (m, 2 H, β-CH_2, $J_{\alpha\beta1}$ = 11.1 Hz, $J_{\alpha\beta2}$ = 9.8 Hz, $J_{\beta1\beta2}$ = 13.4 Hz), 5.56–5.63 (dd, 1 H, α-CH, $J_{\alpha\beta1}$ = 11.0 Hz, $J_{\alpha\beta2}$ = 10.0 Hz), 6.97–7.55 (m, 4 H, indolic aryl H's), and 7.83–7.93 (m, 4 H, phthaloylic aryl H's); MS (CI, NH_3) m/z (%) 349 (20) $(M + 1)^+$, 366 (60) $(M + 18)^+$; $[\alpha]^{22}_D$ −147° (*c* 0.97, acetone). Anal. Calcd for $C_{19}H_{12}N_2O_5$: C, 65.52; H, 3.45; N, 8.04. Found: C, 65.76; H, 3.54; N, 7.88.

Similarly, L-5B was converted into the amino lactone L-6B in trifluoroacetic acid. RPHPLC showed that only L-5B was formed on hydrolysis of L-6B and that stereochemistry had not been altered by lactonization. The amino lactone was then converted into L-7B as described for L-7A: mp 259–262 °C (lit. mp, Table I); on melting, L-7B showed major conversion to L-7A or D-7B by TLC; ^1H NMR (CD_3CN) δ 2.68–2.77 and 3.06–3.14 (m, 2 H, β-CH_2, $J_{\alpha\beta1}$ = 9.4 Hz, $J_{\alpha\beta2}$ = 12.8 Hz, $J_{\beta1\beta2}$ = 12.7 Hz), 5.61–5.68 (dd, 1 H, α-CH, $J_{\alpha\beta1}$ = 9.5 Hz, $J_{\alpha\beta2}$ = 12.2 Hz), 6.97–7.61 (m, 4 H, indolic aryl H's), and 7.83–7.92 (m, 4 H, phthaloylic aryl H's); MS (CI, NH_3) m/z (%) 349 (100) $(M + 1)^+$, 366 (50) $(M + 18)^+$, 305 (35) $(M + 1 - CO_2)^+$; $[\alpha]^{22}_D$ −204° (*c* 0.35, acetone). Anal. Calcd for $C_{19}H_{12}N_2O_5$: C, 65.52; H, 3.45; N, 8.04. Found: C, 65.28; H, 3.50; N, 7.95.

Acknowledgment. Mass and NMR spectra were provided by the Laboratory of Analytical Chemistry of this Institute. We are indebted to Dr. Virender M. Labroo of this Laboratory and Prof. Michael M. King of George Washington University for valuable advice and discussion.

Synthesis of Compounds Designed To Inhibit Bacterial Cell Wall Transglycosylation

Scott J. Hecker,* Martha L. Minich, and Karen Lackey

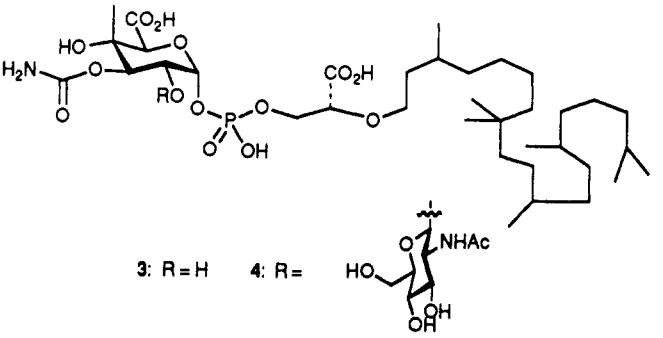
Pfizer Central Research, Groton, Connecticut 06340

Received March 6, 1990

Methods for preparation of compounds designed to inhibit the transglycosylation step in bacterial cell wall biosynthesis are described. Two hybrid structures (**5** and **31**) are synthesized, which combine features of the transglycosylase substrate with those of the natural product moenomycin, a known transglycosylation inhibitor. The compounds are synthesized by a convergent route involving the coupling as a phosphate diester of a protected sugar portion with a glycerate–lipid synthon. Details of the syntheses of the sugar and glycerate precursors are discussed.

The transglycosylation step in bacterial cell wall biosynthesis, responsible for the construction of the polyglycan chains of peptidoglycan (Figure 1), has received little attention from medicinal chemists in the search for new antibacterial agents. Only one class of inhibitors of this biosynthetic step, the phosphoglycolipids, has been discovered; the best known member of this class is moenomycin A (**2**)[1] (Figure 2).

Moenomycin A, first reported in 1965, is a potent but fairly narrow-spectrum antibiotic that has been extensively used as a growth promotant by Hoechst under the trade name Flavomycin.[1] Comparison of the structure of this antibiotic with that of the transglycosylase substrate (**1**) invites the hypothesis that much of the structure of moenomycin might not be necessary for biological activity. In fact, recent degradative studies[2] by Welzel et al. have established that this is indeed the case: Successive removal of the sugar components has shown that monosaccharide **3** and disaccharide **4** retain much of the activity of moenomycin.



3: R = H **4**: R =

The demonstration that compounds **3** and **4** retain biological activity suggests that other small, synthetically accessible molecules could inhibit the transglycosylation step.[3] We have designed a set of potential transglycosylase inhibitors, exemplified by structures **5** and **6**, which contain features of both moenomycin A and the transglycosylase substrate. In designing these targets, we have chosen to retain most of the features of **1**, while replacing the diphosphate group of **1** with the phosphoglycerate group of

(1) Huber, G. Moenomycin and Related Phosphorus-Containing Antibiotics. In *Antibiotics*; Hahn, F. E., Ed.; Springer-Verlag: New York, 1979; Vol. 5, Part 1, p 135.

(2) (a) Welzel, P.; Kunisch, F.; Kruggel, F.; Stein, H.; Ponty, A.; Duddeck, H. *Carbohydr. Res.* **1984**, *126*, C1. (b) Welzel, P.; Kunisch, F.; Kruggel, F.; Stein, H.; Scherkenbeck, J.; Hiltmann, A.; Duddeck, H.; Muller, D.; Maggio, J. E.; Fehlhaber, H.-W.; Seibert, G.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1987**, *43*, 585.

(3) At the time we initiated this work, only one example of the synthesis of a structural analogue of moenomycin had appeared in the literature: Schubert, T.; Welzel, P. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 137. More recently, the Welzel group has reported synthesis of two disaccharide analogues of moenomycin: Hohgardt, H.; Dietrich, W.; Kuhne, H.; Muller, D.; Grzelak, D.; Welzel, P. *Tetrahedron* **1988**, *44*, 5771.

0022-3263/90/1955-4904$02.50/0