mixture of the appropriate propargylamine **22**, **23**, **24**, or **25**, 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide (1 molar equiv), and $CaCO_3$ (1 molar equiv) in DMA (2.5 mL per mmol) was stirred at room temperature for 132 h (**22**), 420 h (**23**), or 60 h (**24**, **25**) with TLC monitoring using $CHCl_3$–MeOH (10:1). The mixture was filtered and the solids washed with DMA and the combined filtrates were concentrated in vacuo. The resulting crude product, an oil, was purified by column chromatography on silica gel with $CHCl_3$–MeOH (10:1) as the eluent. Yields and analytical data of products chromatographically homogeneous in system G are given in Table II and ^1H NMR data in Table IV.

***N*-[*N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-glutamic Acid Trifluoroacetate Salt (30), *N*-[*N*-[*N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid Trifluoroacetate Salt (31), *N*-[*N*-[*N*-[*N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid Trifluoroacetate Salt (32), and *N*-[*N*-[*N*-[*N*-[4-[*N*-[(2-Amino-4-hydroxy-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-γ-glutamyl]-L-glutamic Acid Trifluoroacetate Salt (33).** A solution of **26**, **27**, **28**, or **29** in TFA (10 mL per mmol) was kept for 1 h at room temperature whereupon TLC in system H showed the absence of starting material. The solution was added dropwise to diethyl ether (100 mL per mmol). The white solid that precipitated was purified by six cycles of centrifugation–decantation–resuspension in diethyl ether. The product was dried in vacuo over KOH at 65 °C overnight. Yields, analytical data, and HPLC data are given in Table III and ^1H NMR data in Table IV.

Biochemical Evaluation. Human thymidylate synthase was partially purified from a WI-L2 cell line that overproduces TS 200-fold owing to amplification of the TS gene.[36] The enzyme preparation and assay method were as previously described for L1210 TS[33,37] except that a much purer preparation of tetrahydrofolate was used (Fluka, Neu-Ulm, West Germany) and the assay contained 0.2% bovine serum albumin. Briefly, the 0.5-mL reaction mixture contained 25 nmol of [5-^{3}H]dUMP (40 μCi/μmol), 100 nmol of (±)-L-FH_4, 1 μmol of HCHO, 5 μmol of dithiothreitol, 0.05 mL of inhibitor, and 0.2 mL of enzyme preparation. CB3717 and its polyglutamate derivatives were dissolved in 0.15 M $NaHCO_3$ and then diluted to appropriate concentrations in H_2O. The reaction was started with the addition of enzyme (diluted to the appropriate activity in 0.125 M potassium phosphate buffer, pH 7.4, containing 3 mM dithiothreitol and 0.5% bovine serum albumin—the last named from Sigma Chemical Co., Poole, Dorset, UK.) The K_i apparent's were determined by using the Goldstein equation[34] applicable to tight-binding inhibitors (zone B kinetics).[38] The data was fitted to the equation by a non-linear least squares regression.[39]

Acknowledgment. This work was supported by the Nencki Institute of Experimental Biology of the Polish Academy of Sciences, Warsaw (Grant No. CPBP 04.01), and by grants from the Cancer Research Campaign and the Medical Research Council. Miss J. Elliot of King's College kindly provided the 250-MHz NMR spectra under the auspices of the University of London Intercollegiate Research Service. We thank Kaye Long and Leticia Wolpert for their expert computer-assisted typesetting of the manuscript.

Registry No. **1**, 77766-62-2; **4**, 32677-01-3; **5**, 5891-45-2; **6**, 32719-56-5; **7**, 88063-78-9; **8**, 32719-57-6; **9**, 73167-55-2; **10**, 32816-47-0; **11**, 88443-03-2; **12**, 32719-58-7; **13**, 73167-56-3; **14**, 117559-38-3; **15**, 117559-39-4; **16**, 117559-40-7; **17**, 117559-41-8; **18**, 88050-24-2; **19**, 117559-42-9; **20**, 117559-43-0; **21**, 117559-44-1; **22**, 117581-21-2; **23**, 117559-45-2; **24**, 117559-46-3; **25**, 117559-47-4; **26**, 117559-48-5; **27**, 117559-49-6; **28**, 117559-50-9; **29**, 117559-51-0; **30**·TFA, 117559-52-1; **30** (free base), 95398-69-9; **31**·TFA, 117559-53-2; **31** (free base), 95398-70-2; **32**·TFA, 117559-54-3; **32** (free base), 95398-71-3; **33**·TFA, 117559-55-4; **33** (free base), 95398-72-4; Z-pAB-Cl, 37653-67-1; $BrCH_2{\equiv}CH$, 106-96-7; thymidylate synthase, 9031-61-2.

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Synthesis of an Analogue of Tabtoxinine as a Potential Inhibitor of D-Alanine:D-alanine Ligase (ADP Forming)

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The design and synthesis of a potential inhibitor of D-alanine:D-alanine ligase (ADP forming) (EC 6.3.2.4) are described. This enzyme, which catalyzes the second step in the biosynthesis of bacterial peptidoglycan, is believed to generate D-alanyl phosphate as an enzyme-bound intermediate. With tabtoxinine, a potent inhibitor of glutamine synthetase, as a model, β-lactams **9R** and **9S** were synthesized as potential precursors of a D-alanyl phosphate mimic.

The dipeptide D-alanyl-D-alanine is known to be an essential precursor of bacterial peptidoglycan. Its synthesis in vivo (Scheme I) involves conversion of L-alanine to D-alanine by alanine racemase (EC 5.1.1.1), followed by coupling of two molecules of D-alanine by D-alanine:D-alanine ligase (ADP forming) (EC 6.3.2.4).[1] Inhibitors of alanine racemase have been extensively studied as potential antibacterial substances.[2] Inhibitors of the ligase include cycloserine (IC_{50} = 2.5 × 10^{-4} M)[3] and D-(1-aminoethyl)phosphonic acid (IC_{50} = 9.3 × 10^{-4} M).[4] Attempts to design more effective inhibitors of the ligase have accelerated. Inhibition of the ligase by several amino phosphonic and amino phosphonamidic acids has been described.[5] The synthesis and essentially irreversible

Scheme I. Biosynthesis of D-Alanyl-D-alanine from L-Alanine by the Action of the Enzymes (1) Alanine Racemase and (2) D-Alanine:D-alanine Ligase

(1) For a recent review, see: Ghuysen, J.-M. *Topics Antiobiot. Chem.* **1980**, *5*, 9.
(2) Neuhaus, F. C.; Hammes, W. P. *Pharmacol. Ther.* **1981**, *14*, 265.
(3) Neuhaus, F. C.; Lynch, J. L. *Biochemistry* **1964**, *3*, 471–480.
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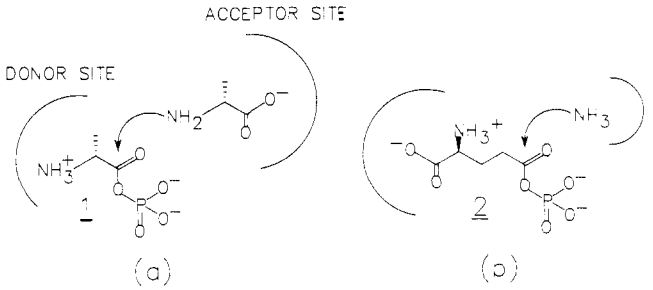
0022-2623/89/1832-0165$01.50/0

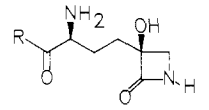
Scheme II. Proposed Amide Bond-Forming Steps for (a) D-Alanine:D-alanine Ligase (Including Conceptualized "Donor" and "Acceptor" Sites for D-Alanine) and (b) Glutamine Synthetase



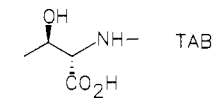
inhibition of this enzyme by phosphinic acid analogues of D-alanyl-D-alanine has also been reported.[6-8]

The ligase is known to require ATP for activity and to possess two binding sites for D-alanine; the products are ADP and the dipeptide.[9] It thus may be supposed that the carboxyl of an alanine bound in the "donor" site is activated by ATP. During or after this process, attack by the amino group of a second alanine, bound in the "acceptor" site, may occur, leading to formation of dipeptide and ADP. Although the detailed mechanism has not been determined, one possibility is the formation of D-alanyl phosphate (**1**) as an enzyme-bound intermediate (Scheme II). Strong evidence exists that a tightly bound acyl phosphate intermediate is formed during the conversion of glutamic acid to glutamine catalyzed by the enzyme glutamine synthetase.[10] Here attack by an enzyme-bound ammonia on glutamate γ-phosphate (**2**) is believed to occur (Scheme II). Thus we considered analogues that might resemble D-alanyl phosphate as potential inhibitors of D-alanine:D-alanine ligase.

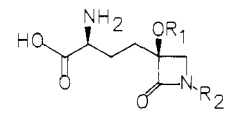
Tabtoxin (wildfire toxin) (**3**), a dipeptide toxin produced by *Pseudomonas tabaci*, is responsible for the so-called "wildfire disease" of tobacco.[11] In vivo hydrolysis of tabtoxin by aminopeptidases produces tabtoxinine (**4**), a potent and irreversible inhibitor of glutamine synthetase.[12] The resulting blockage of the photorespiratory nitrogen cycle leads to chlorosis and death of the tobacco plant. An elegant total synthesis of both tabtoxin and tabtoxinine has been achieved by Baldwin et al. using a cycloaddition route.[13] Although the mechanism of inhibition of glutamine synthetase by tabtoxinine is not known, formation of either the N- or O-phosphorylated forms, **5** or **6**, by phosphorylation of **4** in the enzyme active site, has been postulated.[11] Methionine sulfoximine (**7**) is known to be phosphorylated by glutamine synthetase to produce **8**, an exceedingly potent inhibitor of the enzyme.[14] Like methionine sulfoximine, both **5** and **6** could be viewed as analogues of glutamic acid γ-phosphate (**2**).[15]



3 R = TABTOXIN

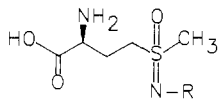


4 R = TABTOXININE



5 R_1 = -H R_2 = $-PO_3^{-2}$

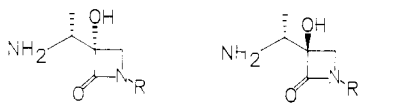
6 R_1 = $-PO_3^{-2}$ R_2 = -H



7 R = -H

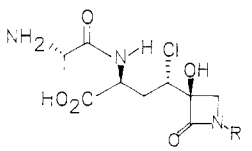
8 R = $-PO_3^{-2}$

Drawing upon the above analogy, the potential inhibition of D-alanyl:D-alanine ligase by analogues **9S** and **9R** of tabtoxinine was investigated. Here enzyme-mediated conversion to the N-phosphorylated species **10S** or **10R** might produce an analogue of D-alanyl phosphate (**1**). The configuration at the carbon atom bearing the side-chain amine in **9** was chosen as *R* to match the stereochemistry of D-alanine. Although the configuration of the hydroxyl-bearing carbon of the β-lactam ring has been determined to be *S* for tabtoxin[9] and also for the related (antibacterial) analogue **11**,[16] both *R,R* and *R,S* diastereomers of **9** (**9R** and **9S**) were studied.[17]



9S R = -H **9R** R = -H

10S R = $-PO_3^{-2}$ **10R** R = $-PO_3^{-2}$



11

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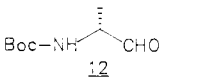
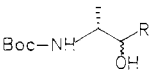
Synthesis

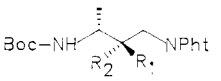
The starting material chosen for the synthesis of **9** was *N-tert*-butoxycarbonyl-D-alaninal (**12**).[18] This aldehyde was available in high yield from the protected amino ester by reduction with diisobutylaluminum hydride.[19] Due to the tendency for N-acylated α-amino aldehydes to racemize during purification on silica gel,[20] the crude aldehyde was converted immediately to cyanohydrin **13** (mixture of diastereomers). Hydrogenation of **13**, followed by treatment of the resulting amino alcohol **14** (acetate salt) with phthalic anhydride,[21] afforded crystalline alcohol **15**



12



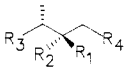
13 R = −CN
14 R = $-CH_2NH_2 \cdot HOAc$
15 R = −NPht



16 R_1, R_2 = =O
17S R_1 = −CN, R_2 = −OTMS
17R R_1 = −OTMS, R_2 = −CN

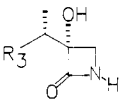
(mixture of diastereomers) in 35% overall yield (from protected amino ester). Oxidation of **15** with chromium trioxide–pyridine complex in methylene chloride afforded ketone **16**, which underwent rapid addition of trimethylsilyl cyanide in the presence of catalytic amounts of potassiusm cyanide and 18-crown-6.[22] The resulting diastereomeric cyanohydrin trimethylsilyl ethers **17S**, **17R**, although separable by MPLC on silica gel, were used as the mixture. Treatment with concentrated HCl provided amine hydrochlorides **18S**, **18R** as hygroscopic solids. These could be converted to the diallyl derivatives **19S**, **19R** by using a modification of the procedure of Ganem,[23] but difficulties in removing the allyl groups from **23S** led us instead to protect the amine as the dibenzyl derivative.[24] Thus, treatment of **18S**, **18R** with benzyl bromide and diisopropylethylamine in DMF afforded **20S**, **20R** (separated by MPLC on silica gel) in 35% overall yield (from **16**).

While the diallyl derivative **19S** was readily converted to amino ester **21S** by treatment with Amberlyst-15 acidic resin in methanol,[25] followed by removal of the phthalimido group by hydrazinolysis, the resin-promoted methanolysis of **20S** and **20R** was extremely slow. For these amides, hydrolysis with refluxing concentrated HCl, followed by reesterification to **22S** and **22R**, was superior. Cyclization of the amino esters to β-lactams **23S**, **24S**, and **24R** was brought about by the action of ethylmagnesium bromide or *tert*-butylmagnesium chloride in THF.[26]

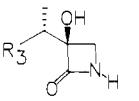


	R_1	R_2	R_3	R_4
18S	$-CONH_2$	−OH	$-NH_2$	−NPht
18R	−OH	$-CONH_2$	−NPht	$-NH_2$
19S	$-CONH_2$	−OH	$-N(CH_2CH{=}CH_2)_2$	−NPht
19R	−OH	$-CONH_2$	−NPht	$-N(CH_2CH{=}CH_2)_2$
20S	$-CONH_2$	−OH	$-N(CH_2Ph)_2$	−NPht
20R	−OH	$-CONH_2$	−NPht	$-N(CH_2Ph)_2$
21S	$-CO_2CH_3$	−OH	$-N(CH_2CH{=}CH_2)_2$	$-NH_2 \cdot HCl$
22S	$-CO_2CH_3$	−OH	$-N(CH_2Ph)_2$	$-NH_2 \cdot HCl$
22R	−OH	$-CO_2CH_3$	$-NH_2 \cdot HCl$	$-N(CH_2Ph)_2$

Lactam **23S** was identified as the *S* diastereomer by an X-ray crystal structure determination (see the Experimental Section), allowing configurational assignments for intermediates in the synthesis. Hydrogenolysis of lactams **24S** and **24R** afforded **9S** and **9R** as crystalline solids.



23S R_3 = $-N(CH_2CH{=}CH_2)_2$
24S R_3 = $-N(CH_2Ph)_2$



24R R_3 = $-N(CH_2Ph)_2$

Enzyme and Antibacterial Assays

Lactams **9S** and **9R** were assayed by using a recently described coupled alanine racemase–D-alanine:D-alanine ligase assay,[27] with alanine racemase from *Escherichia coli* (strain MB1967, Merck Sharp and Dohme Stock Culture Collection) and D-alanine:D-alanine ligase from *Streptococcus faecalis* (ATCC 8043). This assay, which was designed to detect inhibitors of either enzyme, measures the conversion of [^{14}C]-L-alanine to ^{14}C-labeled D-Ala-D-Ala. Each lactam was incubated with a mixture of the two enzymes (8 mM $MnCl_2$, 10 mM KCl) for 40 min prior to the addition of [^{14}C]-L-alanine and ATP to start the reaction (30-min duration). After termination of the reaction, the resulting mixture was analyzed for percentage conversion to labeled dipeptide versus control by descending paper chromatography. Neither **9R** nor **9S** showed inhibitory activity at a concentration of 1 mM.

Both **9R** and **9S** were inactive against *Staphylococcus aureus* and *Escherichia coli* in a qualitative antibacterial assay (see the Experimental Section).

Discussion

Both alanine racemase and D-alanine:D-alanine ligase catalyze key steps in the biosynthesis of bacterial peptidoglycan. Although inhibition of alanine racemase has been studied as a potential means of designing novel antibacterial agents, that of the ligase has not been extensively investigated. While the detailed mechanism for the ligase is not yet known, it is clear that this enzyme presents an interesting target for inhibition.

The failure of lactams **9S** and **9R**, analogues of the potent glutamine synthetase inhibitor tabtoxin, to inhibit

(17) The designation of stereochemistry for compounds in the text and in Table I refers to that of the hydroxyl-bearing carbon atom of the β-lactam ring.
(18) Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Umezawa, Y.; Morishima, H.; Saitok, S.; Takita, T.; Umezawa, H. *Tetrahedron Lett.* **1982**, *23*, 525.
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D-alanine:D-alanine ligase suggests that the mechanism of the amide-bond-forming step, or the detailed geometry of binding of the transition state for this step, may differ from that of glutamine synthetase. The N-phosphorylated lactams **10S** and **10R** derived from **9R** and **9S** (if they are indeed formed) could be viewed as conformationally restricted analogues of D-alanyl phosphate (**1**), and might not approximate the geometry of the bound acyl phosphate.[28] Attempts to design and synthesize other stable analogues of (or precursors to) D-alanyl phosphate are under investigation.

Experimental Section

^{1}H NMR spectra were recorded on a Varian XL-200 spectrometer. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (TMS) as internal standard. Coupling constants are reported in hertz. IR spectra were recorded on a Perkin-Elmer 297 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Mass spectra (MS) were taken on a Varian 731 spectrometer at 70 eV. Melting points (mp) were recorded on a Bausch and Lomb hot-stage apparatus and are uncorrected. Elemental analyses were performed by J. Gilbert and his associates, MSDRL. Thin-layer chromatography was performed on Whatman plates coated with 250 μm silica gel GF in the solvent indicated (or in the chromatography solvent indicated). Medium-pressure liquid chromatography (MPLC) was carried out with E. Merck Lobar (silica gel) columns. Amberlyst resin (Aldrich Chemical Co.) was prewashed as described previously.[25]

3(*R*)-*t*-Boc-amino-1-phthalimido-2(*RS*)-butanol (15). A solution of *N*-*t*-Boc-D-alanine methyl ester (18.5 g, 0.090 mol) in ether (400 mL) was cooled (dry ice bath) as a cooled (−78 °C) solution (1 M in hexane) of DIBAL-H (200 mL, 0.20 mol) was added over 20 min. The mixture was stirred for 15 min and then quenched by careful addition of CH_3OH (50 mL) and then a saturated solution of Rochelle salt (60 mL). The mixture was allowed to warm to room temperature and the resulting gel was diluted with Rochelle salt solution (200 mL). The aqueous layer was extracted with ether, and the combined organic portions were washed with H_2O and brine and dried ($MgSO_4$). The resulting solid aldehyde **12** (16.0 g) showed a single spot by TLC (1:1 hexanes/EtOAc), R_f = 0.60, and had an NMR spectrum ($CDCl_3$) identical with that reported for the L enantiomer.[29] The crude aldehyde was combined immediately with KCN (5.91 g, 0.90 mol), HOAc (5.4 g, 0.90 mol), and CH_3OH (200 mL), and the solution was stirred for 18 h. The solvent was removed and the residue slurried with EtOAc and filtered. The cyanohydrin **13** (*RS* mixture) remaining after evaporation showed two spots on TLC (40:1 CH_2Cl_2/CH_3OH), R_f = 0.20, 0.25. A portion of this material (200 mg) was purified on silica gel, giving a white solid: NMR ($CDCl_3$) δ 1.27, 1.30 (3 H, 2 d (diastereomeric mixture), J = 7, 7), 1.42 (9 H, s), 3.8–4.1 (1 H, m), 4.40, 4.50 (1 H, 2 d (diastereomeric mixture), J = 3, 4), 4.8–5.1 (2 H, br); IR ($CHCl_3$) 3350, 2960, 2940, 1690, 1490 cm^{-1}; MS, m/e 201 ($M^+ + 1$). A solution of the cyanohydrin **13** in HOAc (200 mL) was hydrogenated with PtO_2 (2 g) at 40 psi for 4 h. Removal of solvent afforded crude amino alcohol **14**, which showed a single major spot on TLC (100:20:3:0.5 $CHCl_3/CH_3OH/H_2O$/HOAc), R_f = 0.25. A mixture of **14**, phthalic anhydride (10.0 g, 0.067 mol), Et_3N (60 mL), and toluene (300 mL) was warmed at reflux for 6 h while H_2O was removed with a Dean–Stark head. The residue after evaporation was taken up in EtOAc (250 mL) and extracted with HCl (0.5 N), saturated aqueous $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). Evaporation of solvent and recrystallization of the resulting solid from EtOAc afforded alcohol **15** (10.6 g, 31.7 mmol; 35%), mp 140–142 °C, as a mixture of diastereomers: TLC (1:1 hexanes/EtOAc), R_f = 0.4; NMR ($CDCl_3$) δ 1.25, 1.26 (3 H, 2 d (diastereomeric mixture), J = 7, 7), 1.44, 1.46 (9 H, 2 s), 3.1–3.2 (1 H, br), 3.6–3.9 (1 H, m), 7.7–7.9 (4 H, m); IR ($CHCl_3$) 3440, 2950, 2935, 1765, 1700, 1495 cm^{-1}; MS, m/e 334 (M^+).

3(*R*)-*t*-Boc-amino-1-phthalimido-2-butanone (16). A solution of alcohol **15** (14.6 g, 43.7 mmol) in CH_2Cl_2 (150 mL) was added to a stirred suspension of CrO_3 (21.9 g) and pyridine (35.4 mL) in CH_2Cl_2 (300 mL). The mixture was stirred for 18 h and then decanted from insoluble tar. After evaporation of solvent, the residue was taken up in EtOAc (300 mL) and filtered. The filtrate was passed through silica gel (150 g) and solvent was removed, leaving a white solid. Recrystallization from hexanes/EtOAc gave ketone **16** (9.12 g, 27.5 mmol; 63%): mp 144–146 °C; NMR ($CDCl_3$) δ 1.25 (3 H, d, J = 7), 1.47 (9 H, s), 4.5–4.6 (1 H, m), 4.70 (2 H, s), 5.1–5.3 (1 H, br), 7.8–8.0 (4 H, m); IR ($CHCl_3$) 3430, 2950, 2925, 1775, 1710, 1490 cm^{-1}; MS, m/e 259 (M^+ − O-*t*-Bu); $[\alpha]^{20}_D$ = +68.3° (CH_3OH).

3(*R*)-*t*-Boc-amino-2-(phthalimidomethyl)-2-[(trimethylsilyl)oxy]butyronitrile (17S and 17R). A mixture of ketone **16** (2.42 g, 7.28 mmol), trimethylsilyl cyanide (0.97 mL, 7.28 mmol), KCN (10 mg), and 18-crown-6 (10 mg) in CH_2Cl_2 (10 mL) was stirred for 2 h. MPLC on silica gel (1:1 hexanes/EtOAc) gave the following. **17S** (2.01 g): mp 54–56 °C; TLC, R_f = 0.25; NMR ($CDCl_3$) δ 0.14 (9 H, s), 1.29 (3 H, d, J = 7), 1.38 (9 H, s), 4.02 (2 H, s), 3.9–4.1 (1 H, m), 4.70 (1 H, br d, J = 8), 7.7–7.9 (4 H, m); IR ($CHCl_3$) 3430, 2960, 1780, 1720, 1500 cm^{-1}. **17R**: TLC, R_f = 0.30; NMR ($CDCl_3$) δ 0.20 (9 H, s), 1.38 (3 H, d, J = 7), 1.42 (9 H, s), 4.0–4.1 (1 H, m), 3.93, 4.27 (2 H, AB, J_{AB} = 14), 4.70 (1 H, br d, J = 9), 7.7–7.9 (4 H, m); IR ($CHCl_3$) 3430, 2960, 1780, 1720, 1500 cm^{-1}; MS, m/e 431 (M^+); total yield 2.55 g (5.91 mmol; 81%).

3(*R*)-Amino-2-hydroxy-2-(phthalimidomethyl)butyramide (18S and 18R). A solution of nitrile **17S** (0.970 g) in concentrated HCl (25 mL) was allowed to stir at 5 °C for 18 h. Evaporation of solvent and recrystallization of the resulting solid from CH_3OH gave **18S**: mp 205–209 °C; TLC (3:1:1:1 EtOAc/BuOH/H_2O/HOAc), R_f = 0.50; NMR (D_2O) δ 1.62 (3 H, d, J = 7), 3.97 (1 H, q, J = 7), 4.09, 4.45 (2 H, AB, J_{AB} = 15), 8.0–8.1 (4 H, m). A similar treatment of nitrile **17R** (0.652 g) afforded **18R**: mp 160–162 °C; TLC, R_f = 0.50; NMR (D_2O) δ 1.50 (3 H, d, J = 7), 3.98 (1 H, d, J = 7), 4.24, 4.46 (2 H, AB, J_{AB} = 16), 8.2–8.3 (4 H, m).

3(*R*)-(Diallylamino)-2-hydroxy-2-(phthalimidomethyl)-butyramide (19S and 19R). Nitrile **17S** (0.281 g, 0.652 mmol) was treated with concentrated HCl as described above. The crude **18S** was combined with allyl bromide (0.63 mL, 7.3 mmol), i-Pr_2NEt (0.57 mL, 3.3 mmol), and DMF (3.5 mL), and the mixture was stirred for 18 h. Purification of the product on silica gel (40:1 CH_2Cl_2/CH_3OH) and recrystallization of the resulting solid (hexane/EtOAc) provided **19S** (0.298 g, 8.34 mmol; 92%): mp 136–140 °C; TLC, R_f = 0.60; NMR ($CDCl_3$) δ 1.30 (3 H, d, J = 7), 3.24 (1 H, q, J = 7), 2.73, 3.77 (4 H, ABX, J_{AB} = 14, J_{AX} = 4, J_{BX} = 8), 3.51, 4.73 (2 H, AB, J_{AB} = 13), 5.17 (2 H, d, J = 7), 5.20 (2 H, d, J = 19), 5.8–6.0 (2 H, m), 7.7–7.9 (4 H, m); MS, m/e 358 ($M^+ + 1$). Treatment of nitrile **17R** (0.089 g, 0.21 mmol) as described above provided **19R** (73% yield): mp 176–180 °C; TLC, R_f = 0.60; NMR ($CDCl_3$) δ 1.29 (3 H, d, J = 7), 2.61, 3.65 (4 H, ABX, J_{AB} = 14, J_{AX} = 4, J_{BX} = 8), 3.13 (1 H, q, J = 7), 4.19 (2 H, d, J = 2), 5.00 (2 H, d, J = 9), 5.14 (2 H, d, J = 19), 5.42 (1 H, br s), 5.6–5.8 (2 H, m), 7.06 (1 H, br s), 7.7–7.9 (4 H, m); MS, m/e 357 (M^+).

Methyl 2(*S*)-(Aminomethyl)-3(*R*)-(diallylamino)-2-hydroxybutanoate Hydrochloride (21S). A mixture of **19S** (0.913 g, 2.55 mmol), Amberlyst-15 resin (13.5 g), and CH_3OH (20 mL) was warmed at 60 °C for 7 days. The resin was rinsed with CH_3OH and then the product was eluted with 2:1 CH_3OH/Et_3N. Purification on silica gel (1:1 hexanes/EtOAc) gave phthalimido ester (0.633 g, 1.63 mmol; 63%), mp 92–94 °C (hexanes); NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7), 1.60 (s, H_2O), 2.50 (3.74 (2 H, ABX, J_{AB} = 14, J_{AX} = 4, J_{BX} = 8), 3.22 (1 H, q, J = 7), 3.84 (3 H, s), 3.95 (2 H, d, J = 2), 5.07 (2 H, d, J = 20), 5.09 (2 H, d, J = 12), 5.6–5.8 (2 H, m), 7.7–7.9 (4 H, m); MS, m/e 313 ($M^+ - CO_2CH_3$). The ester was treated with anhydrous NH_2NH_2 (75.9 mg, 2.37 mmol) in CH_3OH (4 mL). After 15 min, solvent was removed and the residue combined with HOAc (0.22 mL, 3.8 mmol) and CH_3OH (4 mL). This mixture was stirred for 24 h and then filtered to remove phthalhydrazide. The crude amino

(28) Although we did not attempt to prepare the N-phosphorylated analogues of **9S** and **9R** as potential inhibitors of the ligase, it was found that an N-sulfonated analogue of **9S** (prepared from **24S** by sulfonation, followed by hydrogenolysis of the benzyl groups) was unstable, presumably due to intramolecular or intermolecular attack of the amino group on the lactam ring.

(29) Stanfield, C. F.; Parker, J. E.; Kanellis, P. *J. Org. Chem.* **1981**, *46*, 4797.

Table I. Physical Properties of Lactams **9S**, **9R**, and Intermediates[a]

compd	mp, °C	yield, %	formula
15	140–142	35	$C_{17}H_{22}N_2O_5$
16	144–146	63	$C_{17}H_{20}N_2O_5$
17S	54–56	81[b]	$C_{21}H_{29}N_3O_5Si$
18S	205–209	90	$C_{13}H_{15}N_3O_4 \cdot HCl$[c]
18R	160–162	93	$C_{13}H_{15}N_3O_4 \cdot HCl \cdot {}^1/_2CH_3OH$
19S	136–140	92	$C_{19}H_{23}N_3O_4$
19R	176–180	73	$C_{19}H_{23}N_3O_4$
20S	oil	40[d]	$C_{27}H_{27}N_3O_4$
20R	158–159		$C_{27}H_{27}H_3O_4 \cdot {}^1/_2H_2O$
22S	glass	71	$C_{20}H_{26}N_3O_3 \cdot 2HCl$[e]
22R	glass	64	$C_{20}H_{26}N_2O_3 \cdot 2HCl$[f]
23S	115–117	54	$C_{11}H_{18}N_2O_2$
24S	149–151	53	$C_{19}H_{22}N_2O_2$
24R	145–147	81	$C_{19}H_{22}N_2O_2$
9S	112–115	81	$C_5H_{10}N_2O_2 \cdot {}^1/_4H_2O$
9R	glass	75	$C_5H_{10}N_2O_2 \cdot {}^1/_4H_2O$

[a] Unless otherwise indicated, all compounds gave satisfactory combustion analyses (C, H, N). [b] Combined yield for **17S** and **17R**. [c] Combined yield for **20S** and **20R**. [d] C: calcd, 49.77; found, 50.50. [e] C: calcd, 57.83; found, 59.33. [f] C: calcd, 57.83; found, 59.66.

ester was purified on silica gel (100:20:3:0.5 $CHCl_3/CH_3OH/H_2O$/HOAc) and then reconcentrated from CH_3OH/HCl (half-saturated), affording **21S** (0.292 g, 0.928 mmol; 78%): TLC, R_f = 0.20; NMR (CD_3OD) δ 1.53 (3 H, d, J = 7), 2.21, 2.63 (2 H, AB, J_{AB} = 12), 3.7–4.0 (2 H, m), 4.00 (3 H, s), 4.23 (1 H, q, J = 7), 5.65 (2 H, d, J = 10), 5.67 (2 H, d, J = 18), 5.9–6.2 (2 H, m).

3(*R*)-[1(*R*)-(Diallylamino)ethyl]-3-hydroxy-2-azetidinone (23S). To a suspension of amino ester **21S** (0.249 g, 0.790 mmol) in THF (2.5 mL) was added a solution (2.8 M in ether) of ethylmagnesium bromide (1.69 mL, 4.74 mmol). The mixture was stirred for 3 h, then diluted with saturated $NaHCO_3$ solution, and extracted with EtOAc. The crude product was purified on silica gel (ether), giving **23S** (90.0 mg, 0.429 mmol; 54%) as a white solid: mp 115–117 °C (CH_3OH); TLC, R_f = 0.4; NMR ($CDCl_3$) δ 1.11 (3 H, d, J = 7), 2.90, 3.30 (4 H, ABX, J_{AB} = 15, J_{AX} = 5, J_{BX} = 8), 3.0–3.2 (1 H, m), 3.11, 3.77 (2 H, AB, J_{AB} = 6), 4.21 (2 H, d, J = 10), 4.24 (2 H, d, J = 19), 5.6–5.8 (2 H, m), 5.9–6.1 (1 H, br s); MS, m/e 169 (M^+ – allyl).

X-ray Crystal Structure Analysis of 23S. Suitable crystals of **23S** ($C_{11}H_{18}N_2O_2$) for X-ray diffraction studies formed from CH_3OH with space group symmetry of $C2$ and cell constants of a = 23.764 (3) Å, b = 5.563 (1) Å, c = 9.576 (1) Å and β = 108.32 (1)° for Z = 4 and a calculated density of 1.162 g/cm³. Of the 907 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 889 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[30] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. the function $\sum w(|F_o| - |F_c|)^2$ with $w = (1/\sigma F_o)^2$ was minimized to give an unweighted residual of 0.035. No abnormally short intermolecular contacts were noted. Tables (II–IV) containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material, as is a computer-generated perspective drawing of **23S** from the final X-ray coordinates showing the relative stereochemistry (Figure 1).

3(*R*)-(Dibenzylamino)-2-hydroxy-2-(phthalimidomethyl)butyramide (20S, 20R). Ketone **16** (9.0 g, 27.1 mmol) was treated as described above with trimethylsilyl cyanide (4.6 mL, 33.9 mmol), KCN (20 mg), and 18-crown-6 (20 mg) in CH_2Cl_2 (50 mL). The crude product was taken up in concentrated HCl (300 mL) and stirred 18 h at 5 °C. Evaporation and reconcentration from CH_3OH gave a mixture of amides **18S** and **18R** as a white solid. This material was combined with benzyl bromide (29 mL, 0.244 mmol) and i-Pr_2NEt (16.6 mL, 94.8 mmol) in DMF (100 mL). The mixture was stirred for 48 h and then evaporated to dryness. A solution of the residue in EtOAc was extracted with H_2O and brine and dried ($MgSO_4$). The resulting product was purified by MPLC (ether), giving the following. **20S** (4.14 g): TLC, R_f = 0.40; NMR ($CDCl_3$) δ 1.40 (3 H, d, J = 7), 1.67 (s, H_2O), 3.21 (1 H, q, J = 7), 3.08, 4.40 (2 H, AB, J_{AB} = 12), 3.51 (4.73 (2 H, AB, J_{AB} = 14), 5.73 (1 H, s), 7.2–7.4 (12 H, m), 7.7–7.9 (4 H, m); MS (FAB), m/e 458 (M^+ + 1). **20R** (0.850 g): TLC, R_f = 0.35; NMR ($CDCl_3$) δ 1.48 (3 H, d, J = 7), 1.67 (s, H_2O), 2.89 (1 H, q, J = 7), 3.29 (2 H, d, J = 14), 3.7–3.9 (2 H, br s), 3.69, 4.27 (2 H, AB, J_{AB} = 14), 5.54 (1 H, br s), 6.07 (1 H, br s), 6.8–7.4 (10 H, br), 7.7 (4 H, s); MS (FAB), m/e 458 (M^+ + 1); total yield 4.99 g (40%).

Methyl 2-(Aminomethyl)-3(*R*)-(dibenzylamino)-2-hydroxybutanoate Hydrochloride (22S and 22R). Amide **20S** (1.95 g, 4.27 mmol) was combined with concentrated HCl (30 mL) and the mixture was warmed at reflux for 18 h. The residue after evaporation was dissolved in CH_3OH (50 mL) and the solution saturated with anhydrous HCl. After 48 h, solvent was removed, giving the product as a white foam. Purification on silica gel (100:20:3:0.5 $CHCl_3/CH_3OH/H_2O$/HOAc) and reconcentration from HCl/CH_3OH (half-saturated) afforded **22S** (1.26 g, 3.04 mmol; 71%) as a hygroscopic solid: TLC, R_f = 0.25; NMR (D_2O) δ 1.63 (3 H, d, J = 7), 3.18, 3.54 (2 H, AB, J_{AB} = 15), 3.50 (3 H, s), 3.93 (1 H, q, J = 7), 4.01, 5.07 (2 H, AB, J_{AB} = 15), 7.4–7.6 (10 H, br s). Amide **20R** (0.650 g, 1.42 mmol) was treated as described above, affording **22R** (0.379 g, 0.914 mmol; 64%) as a hygroscopic solid: TLC, R_f = 0.25; NMR (D_2O) δ 1.51 (3 H, d, J = 7), 3.07, 3.47 (2 H, AB, J_{AB} = 14), 3.68 (1 H, q, J = 7), 3.92 (3 H, s), 4.02, 4.62 (4 H, AB, J_{AB} = 13), 7.4–7.6 (10 H, br s).

3-[1(*R*)-(Dibenzylamino)ethyl]-3-hydroxy-2-azetidinone (24S and 24R). Amino ester hydrochloride **22S** (1.26 g, 3.04 mmol) was suspended in THF (10 mL) and cooled (0 °C) as a solution (2 M in THF) of *tert*-butylmagnesium chloride (9.1 mL, 18.2 mmol) was added. The mixture was stirred for 18 h and then quenched with saturated NH_4Cl solution. The aqueous layer was extracted with EtOAc, and the combined organic portions were washed with saturated NH_4Cl, H_2O, and brine and dried (Na_2SO_4). Purification of the product on silica gel (ether) gave **24S** (0.500 g, 1.61 mmol; 53%): mp 149–150 °C (hexanes/EtOAc); TLC, R_f = 0.30; NMR ($CDCl_3$) δ 1.21 (3 H, d, J = 7), 1.59 (s, H_2O), 3.25 (1 H, q, J = 7), 3.06, 3.82 (2 H, AB, J_{AB} = 5), 3.35, 3.83 (4 H, AB, J_{AB} = 12), 5.21 (1 H, br s), 5.77 (1 H, br s), 7.2–7.4 (10 H, m); IR ($CHCl_3$) 3440, 3400–3150, 2980, 2820, 2720, 1765 cm⁻¹; MS, m/e 267 (M^+ – NHCO). Similar treatment of **22R** (0.111 g, 0.268 mmol) provided **24R** (67.5 mg, 0.218 mmol; 81%): mp 145–147 °C; TLC, R_f = 0.25; NMR ($CDCl_3$) δ 1.22 (3 H, d, J = 7), 3.05 (1 H, q, J = 7), 3.03, 3.39 (2 H, AB, J_{AB} = 6), 2.87, 4.67 (4 H, AB, J_{AB} = 14), 6.14 (1 H, br s), 7.2–7.5 (10 H, br s); IR ($CHCl_3$) 3440, 3400–3150, 2980, 2720, 1760 cm⁻¹; MS, m/e 414 (M^+).

3-(1(*R*)-Aminoethyl)-3-hydroxy-2-azetidinone (9S and 9R). Lactam **24S** (41 mg, 0.132 mmol) was dissolved in CH_3OH (2 mL) and 0.1 N HCl (1 mL) and hydrogenated with 25 mg of 30% Pd(C) at 40 psi for 18 h. The product was purified on DOWEX 50W-X4 (2 g) with 0.5 N NH_4OH as eluant, giving a solid (13.9 mg, 0.107 mmol; 81%): mp 112–115 °C (CH_2Cl_2/CH_3OH); TLC (3:1:1:1 EtOAc/BuOH/H_2O/HOAc), R_f = 0.30; NMR (CD_3OD) δ 1.13 (3 H, d, J = 7), 3.12 (1 H, q, J = 7), 2.98, 3.56 (2 H, AB, J_{AB} = 6); MS, m/e 131 (M^+ + 1). Similar treatment of **24R** (53 mg, 0.17 mmol) afforded **9R** as a white solid (17 mg, 0.13 mmol; 75%): TLC, R_f = 0.30; NMR (1 N DCl in D_2O) δ 1.45 (3 H, d, J = 7), 3.38, 3.70 (2 H, AB, J_{AB} = 7), 3.83 (1 H, q, J = 7); MS, m/e 131 (M^+ + 1).

Spot Agar Assay. This is a qualitative test procedure to determine whether a compound has intrinsic antibacterial activity. The test medium for potential alanine racemase or D-alanine:D-alanine ligase inhibitors is a modification of a defined antagonist-free medium.[31,32] A single strain of *Staphylococcus aureus*

(30) The following library of crystallographic programs was used: MULTAN 80, Main, P.; et al.; University of York, York, England, 1980; ORTEP-11, Johnson, C. K.; Oak Ridge National Library, Oak Ridge, TN, 1970; SDP+v1.1, Okaya, Y.; et al.; B. A. Frenz and Associates, College Station, TX, 1984.

(31) Allen, J. G.; Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Holmes, S. W.; Lambert, R. W.; Nisbet, L. J.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, 684.

(32) Atherton, F. R.; Hall, M. J.; Hassall, C. H.; Lambert, R. W.; Ringrose, P. S. *Antimicrob. Agents Chemother.* **1979**, *15*, 677.

and *Escherichia coli* were used as test organisms. A 0.2-mL portion of overnight culture growth was diluted with 100 mL of the molten agar medium to give final concentrations approximating 2×10^6 cfu/mL. The medium for the staphylococcal culture was supplemented with saponinlyzed horse blood at a final concentration of 2% and 2,3,5-triphenyltetrazolium chloride (TTC) was added, to give a final concentration of 0.02%, to the medium for *E. coli* as an indicator for growth of the culture. Aliquots (2 mL) of the molten agar containing the microorganisms were distributed to individual wells of a tissue culture dish. After the seeded agar medium had solidified, the test was performed by adding a few crystals of **9R** of **9S** to the center of a well with a wooden applicator. D-Cycloserine was used as reference compound for inhibition of growth, and several wells were left without compound as controls for culture growth. The test dishes were incubated at 35 °C for 24–30 h, at which time they were examined for inhibition of growth. The TTC indicator in the *E. coli* culture is reduced to a red color in the presence of growth. Inhibition, therefore, is indicated if the whole well or the portion surrounding the test compound remains pale yellow. Growth or inhibition of *S. aureus* was determined visually. Neither **9R** nor **9S** showed inhibitory activity.

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Supplementary Material Available: A computer-generated drawing of **23S** derived from X-ray coordinates (Figure 1) and tables (Tables II–IV) containing the final fractional X-ray coordinates, temperature parameters, bond distances, and bond angles (4 pages). Ordering information is given on any current masthead page.

2,2-Difluoro-5-hexyne-1,4-diamine: A Potent Enzyme-Activated Inhibitor of Ornithine Decarboxylase

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2,2-Difluoro-5-hexyne-1,4-diamine was prepared in an eight-step sequence from ethyl 2,2-difluoro-4-pentenoate and tested as an inhibitor of mammalian ornithine decarboxylase. It produces a time-dependent inhibition of the enzyme in vitro which shows saturation kinetics, with $K_I = 10$ μM and $\tau_{1/2} = 2.4$ min. In rats, it produces a rapid, long-lasting, and dose-dependent decrease of ornithine decarboxylase activity in the ventral prostate, testis, and thymus. In contrast with the nonfluorinated analogue 5-hexyne-1,4-diamine (Danzin et al. *Biochem. Pharmacol.* **1983**, *32*, 941), 2,2-difluoro-5-hexyne-1,4-diamine is not a substrate of mitochondrial monoamine oxidase.

Ornithine decarboxylase (ODC, L-ornithine carboxylase, EC 4.1.1.17) is one of the rate-limiting enzymes in the biosynthesis of the polyamines spermidine and spermine.[1,2] Inhibitors of ODC show antitumoral activity[3] and are also useful in the treatment of human parasitic diseases.[3,4] Both substrate (ornithine) and product (putrescine) analogues can serve as inhibitors. In particular, α-ethynylputrescine is a potent irreversible inhibitor of ODC.[5] This compound, however, shows a mixed pharmacology owing to its in vivo oxidation to γ-ethynyl-GABA, which is an inhibitor of GABA metabolism.[6] While this oxidation, the first step of which is catalyzed by mitochondrial monoamine oxidase, can be avoided by the introduction of a methyl substituent at the δ-carbon, the consequent introduction of a second chiral center brings its own complications.[7] We sought to apply another solution to the problem of oxidation which circumvents the inconvenience of diastereomerism. Expecting that the two fluorine substituents would decrease the susceptibility of the primary amine to oxidation by monoamine oxidase,[8] we prepared 2,2-difluoro-5-hexyne-1,4-diamine (**1**). The synthesis of this compound and its behavior as an ODC inhibitor and an amine oxidase substrate are reported below.

Table I. Effects of Preincubation with Different Effectors on the Time of Half-Inactivation of ODC by **1**

additions to incubation media	time of half-inactivation of ODC, min
5 μM **1**	9 ± 1
5 μM **1** + 1 mM L-ornithine	85 ± 5
5 μM **1** + 1 mM L-2-methylornithine	>200

Chemistry

The synthesis of 2,2-difluoro-5-hexyne-1,4-diamine (**1**) is outlined in Scheme I. The starting material chosen was ethyl 2,2-difluoro-4-pentenoate (**2**), readily available in one step from allyl 2-hydroperfluoroethyl ether[9] by a modification of the literature procedure.[10,11] Ester **2** was reduced in quantitative yield ($NaBH_4$) to give alcohol **3** as a colorless, mobile oil. Reaction with triflic anhydride afforded triflate **4**, an oil which colors on standing, which was transformed into crystalline phthalimide **5** in good yield. Ozonolysis of the double bond gave aldehyde **6**, allowing the introduction of the ethynyl unit with ethynyl-

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