A Phosphinate Inhibitor of the *meso*-Diaminopimelic Acid-Adding Enzyme (MurE) of Peptidoglycan Biosynthesis

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The emergence of multi-drug-resistant bacteria has led to an increased demand for new types of antibiotics.[1] The biosynthetic pathway for bacterial cell wall (peptidoglycan) formation represents an attractive target for inhibitor design since many clinically useful antibiotics (such as penicillin and vancomycin) inhibit enzymes of the later stages of this pathway.[2] A set of ligases (MurC-F) is responsible for the synthesis of the UDP-MurNAc-pentapeptide (Figure 1) that is a common precursor to peptidoglycan biosynthesis in Gram-negative bacteria.[3] The *meso*-diaminopimelic acid-adding enzyme (MurE) is an ATP-dependent amino acid ligase that is responsible for the formation of UDP-MurNAc-L-Ala-D-Glu-*m*-Dap.[4,5] Here we report the synthesis and initial evaluation of the first effective inhibitor **1** of MurE.[6]

The mechanism of the MurE reaction is assumed to resemble that employed by many of the well-characterized ATP-dependent ligases.[7−10] An initial phosphorylation of the glutamate γ-carboxylate is followed by attack from the (*S*)-amino group of *meso*-diaminopimelic acid to form a tetrahedral intermediate (Figure 1). A subsequent loss of phosphate yields the product amide. An effective strategy for inhibiting such enzymes has been to mimic the tetrahedral intermediate with a corresponding phosphinic acid.[10b−d,11−14] In certain cases, it has been established that phosphorylation of the phosphinic acid occurs and results in slow-tight binding inhibition. Previous work in this lab demonstrated that the D-glutamate-adding enzyme (MurD) was strongly inhibited by a phosphinic acid analogue in which the MurNAc moiety was replaced by a hydrophobic hexanoate linker.[13] With this precedence in mind we designed the MurE inhibitor **1** (Scheme 1).

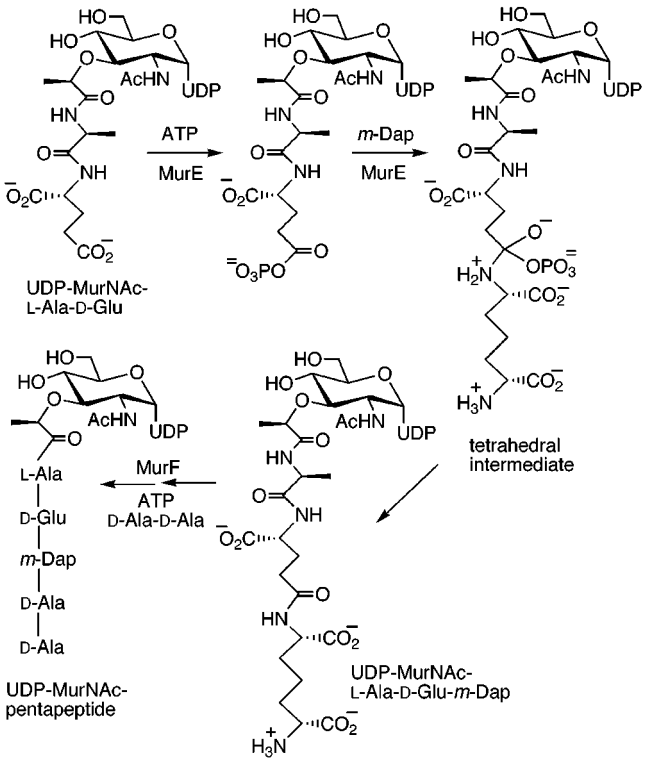

Figure 1. The reactions catalyzed by MurE and MurF and the proposed intermediates formed during the MurE reaction.

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(4) Abbreviations: UDP = uridine diphosphate; UMP = uridine monophosphate; *m*-Dap = *meso*-diaminopimelic acid; MurNAc = *N*-acetylmuramic acid; HOBt = 1-hydroxybenzotriazole; LSI-MS = liquid secondary ion mass spectrometry.

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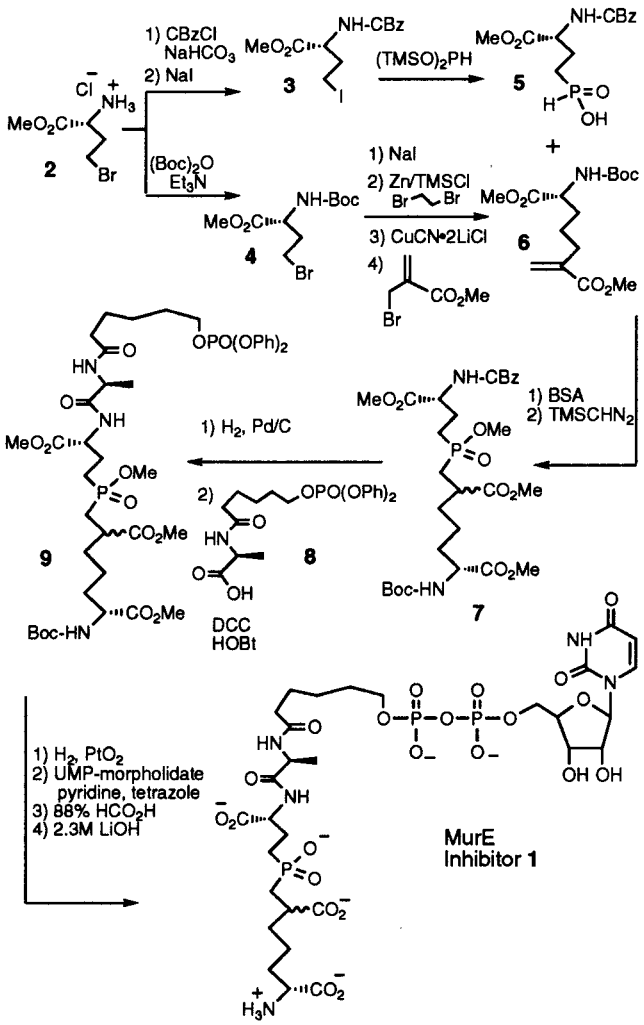
Scheme 1



The synthesis of **1** involves the preparation of a D-Glu-*m*-Dap dipeptide analogue in which a methylenephosphinic acid replaces the amide bond (compound **7** in Scheme 1). The preparation of β-carboxyphosphinic acids can readily be achieved by using conjugate addition chemistry[15] and this strategy is appropriate for use in the construction of the *m*-Dap-phosphinic acid linkage. This strategy cannot be used with simple primary phosphinic acids such as the γ-phosphinic acid analogue of glutamate, **5** (note that the linkage of the MurE product is via the γ-carboxylate of D-Glu). While routes to the racemic phosphinic acid analogue of glutamate are known,[16] we wished to prepare optically pure **5**, and since **2** could be used as a common precursor to both amino acid analogues, we chose to prepare **5** using Arbuzov-like chemistry.[17]

Compound **2** was prepared from D-aspartate in six steps,[18] protected as a benzyloxycarbonyl derivative and then converted to iodide **3** under previously reported conditions.[19] Treatment of **3** with bis(trimethylsilyl)-phosphonite under mild conditions (room temperature, CH_2Cl_2) gave the D-Glu analogue **5** in 32% yield.[17] The main side product in this reaction displayed MS and ^{1}H NMR data that were consistent with an amino acid containing a reduced propyl side chain. It may have been possible to increase this yield by carrying out the displacement at elevated temperatures in toluene, as has recently been reported by Chen and Coward.[20]

Compound **2** was also protected as a *tert*-butoxycarbonyl derivative to give **4.** Compound **4** was converted to the corresponding iodide and then directly coupled to the methyl ester of 2-(bromomethyl)acrylate using zinc/copper chemistry to give **6** in a 33% yield (both steps). Excellent precedence was available for carrying out this coupling reaction on amino acid side chains and it was shown to proceed without racemization (albeit in relatively low yields).[21] Compound **5** was silylated with *N*,*O*-(bistrimethylsilyl)acetamide and attached to compound **6** via a conjugate addition.[15,22] The resulting phosphinic acid was formed as an approximately 3:2 mixture of two diastereomers, as judged by the intensity of the ^{31}P NMR signals (CD_3OD, sodium salt; 37.79 and 37.63 ppm). The phosphinic acid was then esterified with (trimethylsilyl)-diazomethane to give **7** in an overall 50% yield (both steps).[23]

With the dipeptide analogue in hand, the next task was to attach the portion of the inhibitor designed to mimic UDP-MurNAc-L-Ala. The linker compound **8** was prepared from 5-carboxypentyl diphenyl phosphate[13] via a DCC-mediated addition of the *tert*-butyl ester of L-alanine followed by treatment with 88% formic acid. The "D-glutamate" amino group of **7** was deprotected by hydrogenation and coupled to **8** by using DCC/1-hydroxybenzotriazole to give **9** in an 86% yield. In previous work on MurD inhibitors, construction of the diphosphate linkage was performed after the complete deprotection of the phosphinic acid/peptide-linker moiety.[13,14] The phosphoramidate coupling yields were quite low, however, and in this work the coupling was performed prior to deprotection.[24] Compound **9** was hydrogenated over PtO_2 at 50 psi and the resulting phosphate was coupled to UMP-morpholidate in the presence of 1*H*-tetrazole.[25] Coupling yields as high as 65% were obtained in this fashion. Complete deprotection was achieved by treatment with 88% formic acid followed by 2.3 M lithium hydroxide to

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give the target inhibitor **1** as a mixture of two diastereomers (overall 35% yield from **9**).[26]

Compound **1** was found to be a potent inhibitor of the *E. coli* MurE. Using an HPLC based assay, the concentration of the inhibitor necessary to reduce the rate of reaction by one-half (IC_{50} value) was determined to be 1.1 ± 0.1 μM. This result supports the notion that the MurE reaction follows a mechanism similar to that of other ATP-dependent amino acid ligases and that the enzyme will bind tightly to compounds which mimic the structure of the putative tetrahedral intermediate (Figure 1). When compound **9** was fully deprotected and tested as an inhibitor, the IC_{50} was only 700 ± 50 μM, indicating that the complete UDP moiety is necessary for good inhibition and a simple phosphate will not suffice.

This work provides a starting point for the rational design of even more potent inhibitors of MurE. In studies on analogous phosphinate inhibitors of MurD it was found that the incorporation of a MurNac residue in place of the simple hydrophobic linker increased the potency of the inhibitor by almost 2 orders of magnitude.[14] The preparation of a MurNAc-containing MurE inhibitor is currently under way in our laboratories.

Experimental Section

General Procedures. Pyridine was refluxed with KOH for 24 h and then distilled from BaO under dry argon. CH_2Cl_2 was distilled under N_2 from P_2O_5. THF, Et_3N, and DMF were dried and distilled from CaH_2 under nitrogen. Hydrophilic size-exclusion chromatography was performed on Bio-Gel P-2 gel, fine (Bio-Rad Laboratories). Lipophilic size-exclusion chromatography was performed on Sephadex LH-20 (Sigma). Methyl (2*R*)-amino-4-bromobutyrate hydrochloride (**2**) and methyl (2*R*)-[(benzyloxycarbonyl)amino]-4-iodobutyrate (**3**) were prepared according to previously published procedures.[18,19]

Methyl (2*R*)-[(*tert*-Butyloxycarbonyl)amino]-4-bromobutyrate (4). Methyl (2*R*)-amino-4-bromobutyrate hydrochloride (**2**) (3.0 g, 11 mmol) was suspended in THF (25 mL) and Et_3N (2.4 g, 24 mmol). The suspension was cooled to 0 °C and a solution of di-*tert*-butyl dicarbonate (2.2 g, 10 mmol) in THF (5 mL) was added dropwise over 10 min. The mixture was allowed to warm to room temperature and stirred for 6 h and then warmed to 50 °C for a further 2 h. The solvent was removed in vacuo and the residue partitioned between Et_2O (30 mL) and H_2O (30 mL). The aqueous phase was extracted with Et_2O (2 × 30 mL), and the combined organic phases were washed with 3% HCl (50 mL), $NaHCO_3$ (50 mL, saturated), and NaCl (50 mL, saturated). Drying (Na_2SO_4) and evaporation of the solvent afforded the title compound as an oil (2.9 g, 89%) with $R_f = 0.3$ in 3% $CH_3OH/CHCl_3$: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.22 (d, 1 H, $J = 8.1$ Hz), 4.44−4.20 (m, 1 H), 3.75 (s, 3 H), 3.39 (t, 2 H, $J = 7.1$ Hz), 2.45−2.05 (m, 2 H), 1.40 (s, 9 H); +LSI-MS (thioglycerol) 240 (M(^{79}Br) + H$^+$ − C_4H_8, 100%), 242 (M(^{81}Br) + H$^+$ − C_4H_8, 98%), 196 (M(^{79}Br) + H$^+$ − C_4H_8 − CO_2, 88%), 198 (M(^{81}Br) + H$^+$ − C_4H_8 − CO_2, 80%), 296 (M(^{79}Br) + H$^+$, 40%), 298 (M(^{81}Br) + H$^+$, 38%). +LSI−HRMS calcd for $C_{10}H_{19}NO_4{}^{79}Br$: 296.0497. Found: 296.0495.

[(3*R*)-[3-(Benzyloxycarbonyl)amino]-3-carbomethoxypropyl]phosphinate (5), As an *N*,*N*-Diisopropylethylamine Salt. To a solution of ammonium phosphinate (0.20 g, 2.4 mmol) in dry CH_2Cl_2 (6 mL) was added *N*,*N*-diisopropylethylamine (0.68 g, 5.3 mmol) and trimethylsilyl chloride (0.58 g, 5.3 mmol) at 0 °C under argon. The solution was stirred at room temperature for 3 h and then cooled to 0 °C. Methyl (2*R*)-[(benzyloxycarbonyl)amino]-4-iodobutyrate (**3**) (0.67 g, 1.8 mmol) in dry CH_2Cl_2 (2 mL) was added and the reaction was stirred for 20 h under argon at room temperature. The reaction was quenched with 3% HCl and the separated organic layer washed with 3% HCl (2 × 20 mL) and water (20 mL) and then dried over Na_2SO_4. Filtration and removal of the solvent in vacuo yielded an oil. The crude product was purified by chromatography on silica gel (4% CH_3OH/CH_2Cl_2) to give compound **5** as an *N*,*N*-diisopropylethylamine salt (0.3 g, 32%) with $R_f = 0.4$ in 2% CH_3OH/CH_2Cl_2: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.26 (m, 5 H), 7.02 (d, 1 H, $J = 540$ Hz), 6.58 (br d, 1 H, $J = 8.0$ Hz), 5.02 (s, 2 H), 4.40−4.20 (m, 1 H), 3.60 (s, 3 H), 3.75−3.40 (m, 2 H), 3.02−2.80 (m, 2 H), 2.20−1.40 (m, 4 H), 1.42−1.10 (m, 15 H); ^{31}P NMR (81 MHz, $CDCl_3$) δ 33.69; −LSI-MS (thioglycerol/$CHCl_3$ matrix) 300 (M − CH_3^+, 100%), 314 (M − H$^+$, 53%). −LSI-HRMS calcd for $C_{13}H_{17}NO_6P$: 314.0794. Found: 314.0799.

Dimethyl (2*R*)-[(*tert*-Butyloxycarbonyl)amino]-6-methyleneheptanedioate (6). A solution of methyl (2*R*)-[(*tert*-butyloxycarbonyl)amino]-4-bromobutyrate, (**4**) (0.89 g, 3 mmol), and NaI (0.67 g, 4.5 mmol) in acetone (10 mL) was stirred at room temperature for 20 h. Precipitated NaBr was removed by filtration and the solution was evaporated to dryness. The residue was partitioned between Et_2O (50 mL) and H_2O (30 mL). The ethereal solution was washed with a 5% sodium thiosulfate solution (30 mL) and water (2 × 30 mL), dried over Na_2SO_4, and evaporated to give methyl (2*R*)-[(*tert*-butoxycarbonyl)amino]-4-iodobutyrate as a pale brown oil (0.96, 93%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.20 (d, 1 H, $J = 7.8$ Hz), 4.45−4.18 (m, 1 H), 3.72 (s, 3 H), 3.12 (t, 2 H, $J = 7.5$ Hz), 2.50−2.00 (m, 2 H), 1.39 (s, 9 H). This compound was used directly in the next reaction.

A suspension of zinc (1.1 g, 16.8 mmol) in dry THF (1.2 mL) and 1,2-dibromoethane (0.16 g, 0.8 mmol) was heated under argon to 60 °C for 3 min in a 50-mL two-neck flame-dried flask fitted with a septum and a condenser. After allowing the mixture to cool to room temperature, trimethylsilyl chloride (0.02 g, 0.18 mmol) was added and the mixture was vigorously stirred for 30 min. The solution was warmed to 35 °C, methyl (2*R*)-[(*tert*-butoxycarbonyl)amino]-4-iodobutyrate (0.98 g, 3.0 mmol) in dry THF (5.6 mL) was slowly added, and the mixture was stirred for 1 h until no starting material remained (as judged by TLC, 10:1 toluene/ethyl acetate). The solution was then cooled to −10 °C, and a solution prepared from CuCN (0.27 g, 3.0 mmol) and LiCl (0.25 g, 6.0 mmol) in THF (5.6 mL) was added. The solution was stirred at 0 °C for 10 min. Methyl 2-(bromomethyl)acrylate (0.55 g, 3.0 mmol) was added at −25 °C, and the flask contents were stirred at 0 °C for 3 h. The cooling bath was removed, and once the solution reached room temperature, the mixture was diluted with ethyl acetate (50 mL), washed with aqueous sodium hydrogen carbonate (25 mL, saturated) and water (2 × 25 mL), dried, and concentrated under reduced pressure to yield crude oil. Flash chromatography over silica gel (8% hexanes/ethyl ether, v/v) afforded the product **6** as an oil (0.31 g, 35%) with $R_f = 0.3$ in 1:1 hexanes/ethyl ether: ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.10 (d, 1 H, $J = 1.5$ Hz), 5.48 (d, 1 H, $J = 1.4$ Hz), 5.02 (br d, 1 H, $J = 8.3$ Hz), 4.24 (t, 1 H, $J = 6.9$ Hz), 3.70 (s, 6 H), 2.27 (t, 2 H, $J = 7.5$ Hz), 2.00−1.20 (m, 4 H), 1.20 (s, 9 H); +LSI-MS (thioglycerol matrix) 216 (M + H$^+$ − C_4H_8 − CO_2, 100%), 260 (M + H$^+$ − C_4H_8, 25%), 316 (M + H$^+$, 11%). +LSI−HRMS calcd for $C_{15}H_{26}NO_6$: 316.1760. Found: 316.1770.

Compound 7. Compound **5** (0.07 g, 0.16 mmol) was dissolved in dry CH_2Cl_2 (0.8 mL), and compound **6** (0.07 g, 0.23 mmol) and *N*,*O*-bis(trimethylsilyl)acetamide (0.1 g, 0.5 mmol) were added. The solution was stirred at room temperature for 20 h. The reaction was quenched with 1 M HCl, and the separated organic layer was washed with 1 M HCl (2 × 10 mL) and water (10 mL) and then dried over Na_2SO_4. Filtration and concentration in vacuo gave an oil. The crude product was purified by chromatography on silica gel (7% CH_3OH/CH_2Cl_2) to give the disubstituted phosphinic acid as a white foam (0.07 g, 69%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65 (br s, 1 H), 7.20 (s, 5 H), 6.02 (br s, 1 H), 5.60 (br s, 1 H), 5.18 (br s, 1 H), 4.95 (s, 2 H), 4.40−4.00 (m, 2 H), 3.70−3.30 (m, 9 H), 2.60 (br s, 1 H), 2.20−1.00 (m, 21 H); ^{31}P NMR (81 MHz, CD_3OD) δ 37.79 and 37.63; −LSI-MS (thioglycerol/$CHCl_3$ matrix) 629 (M − H$^+$, 100%), 615 (M − CH_3^+, 14%). This compound was used directly in the next reaction.

The disubstituted phosphinic acid from above (0.07 g, 0.11 mmol) was taken up in toluene/methanol (4:1 v/v, 0.5 mL) and treated with (trimethylsilyl)diazomethane ($TMSCHN_2$) until evolution of nitrogen ceased and the yellow color of diazomethane

(26) To check for possible epimerization during the final deprotection step, a sample was deprotected by using 2.3 M LiOD/D_2O. Mass spectral analysis (−LSI-MS) of the resulting compound **1** indicated that not more than 10% of the material contained deuterium and that significant epimerization had not occurred.

remained permanent. The solution was stirred for 45 min at room temperature and the excess $TMSCHN_2$ was quenched with acetic acid. The reaction mixture was evaporated to dryness and was chromatographed (ethyl acetate) to give **7** as a colorless oil (0.05 g, 71%) with $R_f = 0.2$ in ethyl acetate: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (s, 5 H), 5.80−5.60 (m, 1 H), 5.10 (s, 2 H), 5.10−5.00 (br, 1 H) 4.45−4.20 (m, 2 H), 3.80−3.50 (m, 12 H), 2.68 (br s, 1 H), 2.20−2.05 (m, 2 H) 2.00−1.50 (m, 8 H), 1.40 (s, 9 H) 1.35−1.25 (m, 2 H); ^{31}P NMR (81 MHz, $CDCl_3$) δ 53.44, 52.84; +LSI-MS (3-nitrobenzyl alcohol/$CHCl_3$ matrix) 545 ($M + H^+ - C_4H_8 - CO_2$, 100%), 645 ($M + H^+$, 13%). +LSI-HRMS calcd for $C_{29}H_{46}N_2O_{12}P$: 645.2788. Found: 645.2779.

***N*-(2-*S*-Propanoic acid)-6-[(diphenoxyphosphinyl)oxy]-hexanamide (8).** The precursor to **8**, 5-carboxypentyl diphenyl phosphate, was prepared by a slight modification of the previously reported synthesis.[13] A solution of ϵ-caprolactone (8.0 g, 70 mmol) and NaOH (3.0 g, 75 mmol) in water (2 mL) was heated to reflux for 2 h. The solvent was evaporated to yield a white solid. To a solution of the sodium 6-hydroxyhexanoate from above (0.8 g, 5 mmol) in dry pyridine (25 mL) was slowly added diphenyl phosphorochloridate (2.3 g, 8.5 mmol). The mixture was stirred at room temperature for 12 h, and the solvent was removed in vacuo. The residue was dissolved in CH_2Cl_2 and washed with 1 M HCl (2 × 30 mL) and water (30 mL) and dried over Na_2SO_4. Filtration and concentration in vacuo gave an oil. The crude product was purified by flash chromatography on silica gel (1:1 hexanes/ethyl ether) to give 5-carboxypentyl diphenyl phosphate as a colorless oil (1.5 g, 83%). The spectral characteristics were identical to those reported earlier.[13]

A solution of 5-carboxypentyl diphenyl phosphate (1.1 g, 2.9 mmol) in CH_2Cl_2/DMF (4:1 v/v, 5 mL) was prepared. To this solution were added 1-hydroxybenzotriazole monohydrate (0.44 g, 3.2 mmol) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (1.3 g, 2.9 mmol). The mixture was stirred at room temperature for 1 h during which time a white precipitate formed. A solution of *tert*-butyl L-alanine hydrochloride (0.55 g, 2.9 mmol) and dry pyridine (0.4 g, 5 mmol) in DMF (2 mL) was then added, and the mixture was allowed to stir at room temperature for 24 h. The solution was filtered and the filtrate was partitioned between water (60 mL) and CH_2Cl_2 (60 mL). The organic layer was washed with water (2 × 60 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to yield an oil. The crude product was purified by flash chromatography on silica gel (3:1 v/v, ethyl ether/hexanes) to give *tert*-butyl ester of **8** as a pale yellow oil (0.87 g) with $R_f = 0.8$ in ethyl ether: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.10 (m, 10 H), 6.03 (br d, 1 H, $J = 6.8$ Hz), 4.55−4.35 (m, 1 H), 4.21 (dt, 2 H, $J = 7.6$, 6.6 Hz), 2.12 (t, 2 H, $J = 7.6$ Hz), 1.75−1.55 (m, 4 H), 1.45 (s, 9 H), 1.34 (m, 2 H), 1.32 (d, $J = 7.1$ Hz, 3 H); ^{31}P NMR (81 MHz, $CDCl_3$) δ −12.00; −LSI-MS (glycerol/H_2O matrix) 414 ($M - C_6H_5$, 100%), 490 ($M - H^+$, 5%). −LSI-HRMS calcd for $C_{25}H_{33}NO_7P$: 490.1995. Found: 490.1985.

A solution of 88% HCO_2H (5 mL) containing the *tert*-butyl ester of **8** (0.87 g, 1.8 mmol) was stirred at room temperature for 48 h. The formic acid was completely removed in vacuo and the oil was dissolved in CH_2Cl_2 (20 mL). The organic phase was washed with water (3 × 10 mL) and dried over $MgSO_4$. Filtration and concentration in vacuo gave the acid **8** as a pale yellow oil (0.70 g, 57% in two steps). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.35−7.00 (m, 10 H), 6.55 (d, 1 H, $J = 7.2$ Hz), 4.60−4.35 (m, 1 H), 4.18 (dt, 2 H, $J = 7.2$, 7.0 Hz), 2.13 (t, 2 H, $J = 7.2$ Hz), 1.75−1.45 (m, 4 H), 1.40−1.15 (m, 5 H); ^{31}P NMR (81 MHz, $CDCl_3$) δ −12.27; +LSI-MS (thioglycerol matrix) 436 ($M + H^+$, 100%). +LSI-HRMS calcd for $C_{21}H_{27}NO_7P$: 436.1525. Found: 436.1514.

Compound 9. A solution of compound **7** (67 mg, 0.10 mmol) in methanol (3 mL) containing 10% Pd/C (46 mg) was stirred under 1 atm of hydrogen at room temperature for 10 h. The mixture was filtered, and the solvent was removed in vacuo to give the amine as colorless oil (52 mg). To a solution of the acid **8** (53 mg, 0.12 mmol) in CH_2Cl_2/DMF (4:1 v/v, 1 mL) were added 1-hydroxybenzotriazole monohydrate (19 mg, 0.14 mmol) and dicyclohexylcarbodiimide (29 mg, 0.13 mmol). The mixture was stirred at room temperature for 1 h, during which time a white precipitate formed. A solution of the amine from above (46 mg) in DMF (1 mL) was added, and the mixture was allowed to stir at room temperature for 20 h. The solution was filtered and the filtrate was partitioned between water (3 mL) and CH_2Cl_2 (3 mL). The organic layer was washed with water (2 × 3 mL) and dried over Na_2SO_4. The solvent was removed in vacuo to yield an oil. The crude product was purified on a Sephadex LH-20 column (1.5 × 100 cm in diameter/length), eluted with CH_3OH/$CHCl_3$ (4:3 v/v). The fastest moving fractions gave **9** as colorless oil (83 mg, 86%) with $R_f = 0.4$ in 20% methanol/ethyl acetate: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50−7.10 (m, 10 H), 6.12 (br, 1 H), 5.07 (br, 1 H), 4.60−4.45 (m, 2 H), 4.23 (m, 3 H), 3.80−3.60 (m, 13 H), 2.72 (br s, 1 H), 2.40−1.30 (m, 20 H), 1.42 (s, 9 H), 1.36 (d, $J = 7.0$ Hz, 3 H); ^{31}P NMR (81 MHz, $CDCl_3$) δ 55.94, 55.73, 55.49, 55.11, −12.04; +LSI-MS (thioglycerol/CH_3OH matrix) 828 ($M + H^+ - C_4H_8 - CO_2$, 100%), 928 ($M + H^+$, 22%). +LSI-HRMS calcd for $C_{42}H_{64}N_3O_{16}P_2$: 928.3762. Found: 928.3771.

Compound 1. A solution of **9** (76 mg, 82 μmol) in methanol (3 mL) containing PtO_2 monohydrate (120 mg) was stirred under 50 psi of hydrogen at room temperature for 48 h. The mixture was filtered, and the solvent was removed in vacuo to give the phosphoric acid as colorless oil (62 mg). A mixture of the acid from above (36 mg, 46 μmol) and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (70 mg, 0.1 mmol) was dried by the addition of dry pyridine (3 × 1 mL) and evaporation under reduced pressure. 1*H*-Tetrazole (15 mg, 0.2 mmol) and dry pyridine (0.8 mL) were added, and the solution was stirred under argon at room temperature for 48 h. Additional uridine 5′-monophosphomorpholidate (1 equiv) was dried as above and then added to the reaction in dry pyridine (0.5 mL). The reaction was stirred for additional 24 h, and the solvent was removed in vacuo to yield an oil. The oil was dissolved in water (10 mL) and washed with CH_2Cl_2 (2 × 10 mL). Removal of the water in vacuo yielded an oil that was purified on a Bio-Gel P-2 column (1.5 × 100 cm) eluted with 0.1 M NH_4HCO_3. The fastest moving UV active fractions were collected and concentrated under reduce pressure to give the protected **1** as a white foam (32 mg, 65%) with $R_f = 0.5$ in 2-propanol/0.1 M NH_4HCO_3 (3:1 v/v): ^{1}H NMR (400 MHz, D_2O) δ 7.91 (d, 1 H, $J = 8.1$ Hz), 5.91−5.85 (m, 2 H), 4.50−4.05 (m, 8 H), 3.89 (dt, 2 H, $J = 7.5$ Hz, $J = 6.6$ Hz), 3.80−3.60 (m, 12 H), 2.74 (br s, 1 H), 2.40−1.20 (m, 20 H), 1.38 (s, 9 H), 1.35 (d, $J = 7.2$ Hz, 3 H); ^{31}P NMR (121.5 MHz, D_2O) δ 63.02, 62.86, 62.33, 62.17, −10.50 (d, $J = 20.7$ Hz), −11.19 (d, $J = 21.0$ Hz); −LSI-MS (thioglycerol matrix) 1080 ($M - H^+$, 100%), 1066 ($M - CH_3$, 63%). +LSI-HRMS calcd for $C_{39}H_{67}N_5O_{24}P_3$: 1082.3389. Found: 1082.3406.

A solution of 88% HCO_2H (0.5 mL) containing the protected **1** (32 mg, 30 μmol) was stirred at room temperature for 3 h. The formic acid was removed in vacuo to yield the amine formate salt as a colorless oil. This was dissolved in 2.3 M LiOH (0.5 mL) and stirred at room temperature for 24 h. The solution was carefully brought to pH 9 by the addition of Dowex resin (H^+ form) and filtered. The solution was concentrated to 0.5 mL under reduced pressure and applied to a Bio-Gel P-2 column (1.5 × 19 cm) that was eluted with 0.1 M NH_4HCO_3. The fastest moving UV active fractions were collected, concentrated, and pumped under high vacuum to give **1** as a white solid (15 mg, 54% for 2-steps): ^{1}H NMR (400 MHz, D_2O) δ 7.94 (d, 1 H, $J = 8.2$ Hz), 6.00−5.92 (m, 2 H), 4.40−4.10 (m, 7 H), 3.91 (dt, 2 H, $J = 6.8$, 6.4 Hz), 3.75−3.66 (m, 1 H), 2.52 (br s, 1 H), 2.27 (t, 2 H, $J = 7.5$ Hz), 2.08−1.26 (m, 21 H); ^{31}P NMR (121.5 MHz, D_2O) δ 43.12, −10.45 (d, $J = 20.0$ Hz), −11.14 (d, $J = 20.2$ Hz); +LSI-MS (thioglycerol/CH_3OH, matrix) 926 ($M + H^+$, 100%). +LSI-HRMS calcd for $C_{30}H_{51}N_5O_{22}P_3$: 926.2239. Found: 926.2214.

Enzyme Assays. The activity of MurE was assayed at pH 8.1 in 100 mM Bis-Tris propane, containing 5 mM ATP, 75 μM UDP-MurNAc-L-Ala-D-Glu ($K_M = 76$ μM),[5] and 10 μM *meso*-DAP ($K_M = 36$ μM).[5] Commercially available [^{3}H]*meso*-DAP was used as a tracer at 10 μCi/mL final concentration. The reactions were initiated by the addition of MurE to a final concentration of 0.8 μg/mL. After 30 min, the reactions were quenched with 300 mM potassium phosphate, pH 3.5. The reactions were analyzed by HPLC cation exchange chromatography (Shimadzu) with radioflow detection (INUS) using an isocratic system of 150 mM potassium phosphate, pH 3.5. Extent of reaction as a function of inhibitor concentration was calculated from the ratio peak area of product/(peak area of substrate + peak area of product). To determine the IC_{50} value, the data were first graphically analyzed by plotting the inhibitor concentration versus the activity by using a Sigma Plot (Jandel Scientific) and fitted to

the equation $y = 1/(1 + x/b)$, where x is the inhibitor concentration, y is the relative activity, and b is the IC_{50} value. The concentrations of stock solutions of **1** were calculated from A_{262} measurements using $\epsilon = 9890\ M^{-1}\ cm^{-1}$.

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Supporting Information Available: ^{1}H NMR spectra for compounds **4**−**9** and **1** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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Additions and Corrections

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Yu Liu,* Yi-Min Zhang, Ai-Di Qi, Rong-Ti Chen, Keiko Yamamoto, Takehiko Wada, and Yoshihisa Inoue*. Molecular Recognition Study on a Supramolecular System. 10. Inclusion Complexation of Modified *β*-Cyclodextrins with Amino Acids: Enhanced Enantioselectivity for L/D-Leucine.

Page 1827. After publishing the paper, we found that mono[6-(*m*-toluidinyl)-6-deoxy]-*β*-cyclodextrin (**1**) was contaminated by the starting material, mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-cyclodextrin, probably due to the insufficient reaction period and the subsequent incomplete purification. As for the ^{1}H NMR data reported, the signals observed at *δ* 7.41−7.44 and 7.73−7.75, which had been erroneously assigned to the aromatic protons of **1**, should be assigned to those of the starting material, mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-cyclodextrin. From the integrated area of the original spectrum, we found that the "toluidinyl-CD" prepared previously contained *ca.* 40−50% of the starting material.

We therefore prepared a pure sample of compound **1** again and determined the binding constants for the enantiomeric pairs of the same amino acids. The revised synthetic procedure described below is similar to that reported earlier, except for the extended reaction period and the isolation procedure. The enantioselectivities calculated from the corrected binding constants have turned out to be considerably smaller than the previous values.

Mono[6-(*m*-toluidinyl)-6-deoxy]-*β*-cyclodextrin was prepared by the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-cyclodextrin with *m*-toluidine (10 mL) in *N*,*N*-dimethylformamide (20 mL) at 85 °C with stirring for 3 days under N_2. The reaction mixture was evaporated in vacuo at 40 °C to dryness. The residue was dissolved in water, and actone was added to the resulting solution to give a gray precipiate. After drying, the product was purified by chromatography over Sephadex to give the pure sample of **1** in 50% yield. MS: *m*/*e* 1246 (calcd for (M + Na − H) 1246). IR (KBr)/cm^{-1}: 3364, 2909, 1716, 1625, 1542, 1412, 1361, 1338, 1307, 1233, 1147, 1071, 1018, 933, 841, 749, 695, 573, 518. ^{1}H NMR (DMSO-d_6, TMS, ppm): *δ* 2.1 (s, 3H, CH_3), 3.1−3.8 (m, 42H), 4.3−4.6 (m, 6H), 4.6−4.9 (m, 7H), 5.0−5.2 (m, 1 H, NH), 5.6−5.9 (m, 14H), 6.2−6.4 (m, 3H, Ar-H), 6.8-6.9 (t, 1H, Ar-H). Anal. Calcd for $C_{49}O_{34}H_{77}N{\cdot}5H_2O$: C, 44.78; H, 6.67; N, 1.07. Found: C, 44.63; H, 6.75; N, 1.01.

We further repeated the complexation experiment with the pure host **1** prepared above to determine the complex stability constants (K_s), according to the reported procedure. The data obtained are listed in Table 1A.

Table 1A. Complex Stability Constant (K_s) and Gibbs Free Energy Change ($-\Delta G$) for the Supramolecular System Formed by 6-(*m*-Toluidinyl)-6-deoxy-*β*-cyclodextrin (1) and Some Aliphatic Amino Acids[a]

host	guest	K_s	log K_s	$-\Delta G$ (kJ/mol)	$-\Delta\Delta G$ (kJ/mol)
6-(*m*-toluidinyl)-6-deoxy-*β*-cyclodextrin	L-Ala	1197	3.08	17.3	0.4
	D-Ala	1430	3.16	17.7	
	L-Ser	843	2.93	16.4	1.3
	D-Ser	1406	3.15	17.7	
	L-Val	416	2.62	14.7	0.9
	D-Val	609	2.78	15.6	
	L-Leu	1439	3.16	17.7	−0.9
	D-Leu	1002	3.00	16.8	

[a] Measured in a phosphate buffer (pH 7.20) at room temperature (20−23 °C).

As can be seen from Table 1A, the stability constants, especially the enantioselectivities, are significantly different from the previous data. One reasonable explanation is that the compound reported previously contained a considerable amount of mono[6-*O*-(*p*-toluenesulfonyl)]-*β*-cyclodextrin, which is not fluorescent at all but yet influences the inclusion complexation. Another possible reason would be that there are some technical problems inherently in the Benesi−Hildebrand method, so the constants obtained in that way should be taken with some reservations (Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiado: Budapest (Hungary), 1982; p 199).

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Lin-Hua Zhang,* Goss S. Kauffman, Jaan A. Pesti, and Jianguo Yin. Rearrangement of N_α-Protected L-Asparagine with Iodosobenzene Diacetate. A Practical Route to *β*-Amino-L-alanine Derivatives.

Page 6919, Table 1. Compounds **3** and **4** mentioned in this table have been previously prepared by Dr. R. Pascal et al. using 1,1-diacetoxyiodobenzene in DMF (Mendre, C.; Pascal, R.; Calas, B. *Tetrahedron Lett.* **1994**, *35*, 5429−5432. Sola, R.; Saguer, P.; David, M.-L.; Pascal, R. *Chem. Commun.* **1993**, 1786−1788). We thank Dr. Pascal for bringing this to our attention.

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