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Synthesis and Evaluation of Inhibitors for *Escherichia coli* Glucosamine-6-phosphate Synthase

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The design, synthesis and evaluation of potential affinity labels of *Escherichia coli* glucosamine-6-phosphate synthase (glmS) are described. Among the inhibitors described, 2-amino-3-((*N*-halomethyl)amino)propanoates **1a** and **2a** and 2-amino-3-(*N*-maleimidyl)propanoate **4a** exhibited time-dependent inhibition parameters similar to those previously obtained for N^3-(4-methoxyfumaroyl)diaminopropanoate **5**, the most efficient synthetic inhibitor of glmS reported to date. From the recently elucidated mechanism of glmS inactivation by **5**, the alkylation of cysteine-1-thiol by **1a**, **2a** and **4a** seems very likely.

INTRODUCTION

Glucosamine-6P synthase is a key enzyme on the hexosamine biosynthetic pathway. The bacterial protein, encoded by the *glmS* gene (*1*), exists as an homodimer with subunits of relative molecular mass 67,000 (*1, 2*). Each subunit, which consists in a 240-amino acid amino-terminal glutamine-binding domain attached to a 40,000-Da fructose-6P binding domain (Denisot *et al.*, submitted), is responsible for the exclusively glutamine-dependent generation of ammonia (amidohydrolase) coupled to an Heyns (*3*) rearrangement between ammonia and the phosphosugar (ketose/aldose isomerase). Kinetic analyses have shown (*4*) the reaction to obey an ordered bi–bi mechanism:

fructose-6P + L-glutamine → L-glutamate + glucosamine-6P

The unique role of the amino-terminal cysteine residue in ammonia formation (*2*) confers to any glutamine-site directed compound bearing an electrophilic residue the potential ability to irreversibly inhibit the enzyme catalysis. This has been recently demonstrated with the N^3-fumaroyl-L-2,3-diaminopropionic derivatives (*5*): irreversible inhibition of *Escherichia coli* glucosamine-6P synthase by **5** stems from nucleophilic attack of the protein cysteine-1-thiol at the β position of the ester group of this diaminopropionate derivative generating a stable enzyme–inhibitor adduct. In an effort to increase the efficacy of these affinity labels, we synthesized derivatives of L-diaminopropionate bearing an electrophilic center distant of two

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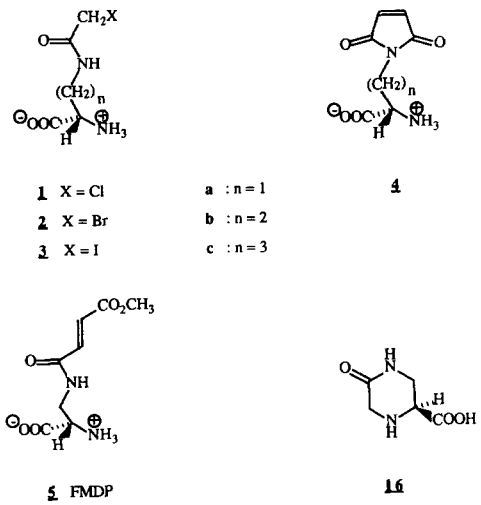


FIGURE 1

carbons from the α-nitrogen: the halo ketones **1a, 2a,** and **3a** and the maleimide **4a** (Fig. 1) fulfill these requirements. Finally to explore the topology of the glutamine active site, the corresponding homologues **1b–1c 2b–2c, 3b,** and **4b** have been obtained.

EXPERIMENTAL PROCEDURES

Synthetic Chemistry

General

Except when notified all the reagents were used as obtained commercially. Maleic anhydride was recrystallized from hot chloroform. Unless otherwise specified, reaction workups culminated in washing the organic layer (ethyl acetate) several times with brine, drying over magnesium sulfate, and evaporating the solvent under reduced pressure. Thin-layer chromatography (TLC) refers to silica gel chromatography (Merck, $60PF_{254}$) in solvent A (AcOEt/MeOH, 70/30), solvent B (AcOEt/MeOH, 50/50), solvent C (*n*-BuOH/AcOH/H_2O, 2/1/1), solvent D (*n*-BuOH/AcOH/H_2O, 3/1/1) or solvent E (*n*-PrOH/H_2O, 80/20); the amino acids were visualized with ninhydrin. Melting points were determined on a Metler FP62 apparatus. Mass spectra were recorded on a Nermag R10-10C spectrometer using chemical ionisation method and ammonia as the reactive gas; ^1H NMR spectra were recorded on a Bruker 250 spectrometer. The preparative purifications were performed either on ion-exchange resin (Bio-Rad) or reverse-phase HPLC using a radial compression cartridge (200 g of 15–20 μm C_{18} Lichroprep (Merck)).

General Procedure for the Synthesis of (S)-2-Amino-ω-((N-carbobenzoxy)amino)alkanoic Acids **7**

L-α,ω-Diaminoalkanoic acid (10 mmol) and copper(II) acetate (5 mmol) were dissolved in water (100 ml). After pH adjustment (pH ~5) with 1 N NaOH, magnesium oxide (500 mg, 12.5 mmol) was added followed by dropwise addition of benzyl chloroformate (28 mmol). The solution was magnetically stirred overnight and the blue precipitate was filtered and washed successively with ice-cold water (3 × 50 ml), methanol (50 ml), and ether (50 ml). The finely ground precipitate was suspended in water (100 ml) and treated for 15 min with H_2S; the resulting black solution was filtered through Celite and compound **7** crystallized on cooling.

(S)-2-Amino-4-((N-carbobenzoxy)amino)butanoic acid (**7b**). Yield, 53%; mp 154°C; TLC (solvent D) R_f 0.61.

(S)-2-Amino-5-((N-carbobenzoxy)amino)pentanoic acid (**7c**). Yield, 32%; mp 152°C; TLC (solvent D) R_f 0.58.

General Procedure for the Synthesis of (S)-2-((N-tert-Butoxycarbonyl)amino)-ω-((N-carbobenzoxy)amino)alkanoic Acids **8**

The Cbz compound **7** (10 mmol) dissolved in 1 : 1 dioxane–water (200 ml) containing triethylamine (20 mmol) was treated at 0°C with di-*tert*-butyl dicarbonate (5.5 mmol). After being stirred overnight at room temperature the solution was concentrated to 50 ml *in vacuo*, washed with ether (2 × 50 ml), acidified to pH 2 with citric acid and worked up as described under General.

(S)-2-((N-tert-Butoxycarbonyl)amino)-4-((N-carbobenzoxy)amino)butanoic acid (**8b**). Yield, 95%; TLC (solvent B) R_f 0.74; ^{1}H NMR (CD_3OD) δ 1.42 (s, 9), 1.92 (m, 2), 3.30 (m, 2), 4.19 (m, 1), 5.10 (s, 2), 7.32 (s, 5); MS, *m/e* 370 (MNH_4^+), 353 (MH^+).

(S)-2-((N-tert-Butoxycarbonyl)amino)-5-((N-carbobenzoxy)amino)pentanoic acid (**8c**). Yield, 80%; TLC (solvent B) R_f 0.72; ^{1}H NMR (CD_3OD) δ 1.44 (s, 9), 1.50–1.90 (m, 4), 3.13 (m, 2), 4.09 (m, 1), 5.06 (s, 2), 7.33 (s, 5); MS, *m/e* 384 (MNH_4^+), 367 (MH^+).

General Procedure for Hydrogenolytic Deprotection: Compounds **9**

The carbobenzoxy substrate (8 mmol) dissolved in methanol (50–100 ml) was heated under reflux with ammonium formate (1.6 g, 25 mmol) and 10% Pd/C (1 g); after 45 min the catalyst was removed by filtration through Celite and the solvent was removed under reduced pressure to afford the compound as a white powder.

(S)-2-((N-tert-Butoxycarbonyl)amino)-4-aminobutanoic acid (**9b**). Yield, 62%; mp 204°C; TLC (solvent D) R_f 0.58; ^{1}H NMR (D_2O) δ 1.24 (s, 9), 1.80 (m, 2), 2.87 (m, 2), 3.76 (m, 1); MS, *m/e* 219 (MH^+), 201 ($MH^+ - H_2O$).

(S)-2-((N-tert-Butoxycarbonyl)amino)-5-aminopentanoic acid (**9c**). Yield, 68%; mp 204°C; TLC (solvent E) R_f 0.54; ^{1}H NMR (D_2O) δ 1.23 (s, 9), 1.40–1.70 (m, 4), 2.81 (m, 2), 3.71 (m, 1); MS, *m/e* 233 (MH^+), 215 ($MH^+ - H_2O$).

General Method for the Synthesis of (S)-2-((N-tert-Butoxycarbonyl)amino)-ω-((N-haloacetyl)amino)alkanoic Acids **11, 12,** *and* **13**

(*S*)-2-((*N-tert*-Butoxycarbonyl)amino)-ω-aminoalkanoic acid **9** (10 mmol) was dissolved in water (10–30 ml) containing $HNaCO_3$ (1.68 g, 20 mmol). Acetyl halide (11 mol, chloride or bromide) was added dropwise at room temperature and the solution was stirred for 4 h. The aqueous solution was washed with ether (2 × 10 ml), acidified to pH 3 (citric acid), and worked up; the resulting solid was washed with dry ether and dried under vacuum.

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-chloroacetyl)amino)propanoic acid (**11a**). Yield, 87%; mp 123–124°C; TLC (solvent A) R_f 0.45; ^{1}H NMR (acetone-d_6) δ 1.46 (s, 9), 3.8 (m, 2), 4.2 (s, 2), 4.42 (m, 1), 6.38 (br s, 1), 6.75 (br s, 1), 7.82 (br s, 1); MS (DCI, NH_3), *m/e* 298 + 300 (MNH_4^+, 25:75), 281 + 283 (MH^+, 25:75), 242 + 244 (MH^+ − *t*-Bu, 25:75).

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-bromoacetyl)amino)propanoic acid (**12a**). Yield, 63%; mp 152–153°C; TLC (solvent A) R_f 0.38; ^{1}H NMR (acetone-d_6) δ 1.46 (s, 9), 3.8 (m, 2), 4.1 (s, 2), 4.5 (m, 1), 6.4 (br s, 1), 6.75 (br s, 1), 8 (br s, 1); MS (DCI, NH_3), *m/e* 342 + 344 (MNH_4^+, 50:50), 286 + 288 (MNH_4^+ − *t*-Bu, 50:50).

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-chloroacetyl)amino)butanoic acid (**11b**). Yield, 56%; oil; TLC (solvent B) R_f 0.70.

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-bromoacetyl)amino)butanoic acid (**12b**). Yield, 60%; oil; TLC (solvent B) R_f 0.70.

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-chloroacetyl)amino)pentanoic acid (**11c**). Yield, 88%; oil; TLC (solvent B) R_f 0.72.

(S)-2-((N-tert-Butoxycarbonyl)amino)-3-((N-bromoacetyl)amino)pentanoic acid (**12c**). Yield, 88%; oil; TLC (solvent B) R_f 0.72.

General Method for the Deprotection of (S)-2-((N-tert-Butoxycarbonyl)amino)-ω-((N-haloacetyl)amino)alkanoic Acids **1, 2,** *and* **3**

The Boc derivative (1 mmol) was solubilized at 0°C either in dioxane (10 ml) saturated with HCl gas (solution, 5 to 6 N) or neat trifluoroacetic acid (5 ml). After being stirred for 2 h at room temperature, the suspension was concentrated by evaporation to eliminate HCl (several additions of dioxane were necessary). The precipitate was finally washed with dry ether and dried. The resulting solid was dissolved in water and purified by ion-exchange (AG1 × 2 AcO^- form) using water as eluant.

(S)-2-Amino-3-((N-chloroacetyl)amino)propanoic acid (**1a**). Yield, 65%; mp 210°C (dec); TLC (solvent C) R_f 0.54; ^{1}H NMR (D_2O) δ 3.50 (dd, 1, J = 7, 14.8 Hz), 3.64 (dd, 1, J = 3.7, 14.8 Hz), 3.73 (dd, 1, J = 3.7, 7 Hz), 3.99 (s, 2); MS (DCI/NH_3), *m/e* 198 + 200 (MNH_4^+, 25:75), 181 + 183 (MH^+, 25:75).

(S)-2-Amino-3-((N-bromoacetyl)amino)propanoic acid (**2a**). After ion-exchange chromatography the three compounds **1a, 2a** and **16** were purified by preparative C_{18} HPLC. **2a:** mp 150°C (dec); TLC (solvent C) R_f 0.53; ^{1}H NMR (D_2O) δ 3.48 (dd, 1, J = 6.9, 14.6 Hz), 3.63 (dd, 1, J = 3.7, 14.6 Hz), 3.74 (dd, J = 3.7, 6.9 Hz), 3.78 (s, 2); MS (DCI/NH_3), *m/e* 225 + 227 (M^+, 50:50). **16:** TLC (solvent

B) R_f 0.34; ^{1}H NMR (D_2O) δ 3.46 (dd, 1, J = 10, 13.8 Hz), 3.65 (dd, 1, J = 4.7, 14.6 Hz), 3.77 (s, 2), 3.98 (dd, 1, J = 4.7, 10 Hz).

(S)-2-Amino-4-((N-chloroacetyl)amino)butanoic acid (**1b**). Yield, 10%; mp 159°C; TLC (solvent D) R_f 0.38; ^{1}H NMR (D_2O) δ 1.88 (m, 2), 3.19 (m, 2), 3.52 (m, 1), 3.92 (s, 2); MS, 195 + 197 (MH^+, 25:75), 177 + 179 ($MH^+ - H_2O$).

(S)-2-Amino-4-((N-bromoacetyl)amino)butanoic acid (**2b**). Yield, 9%; mp >300°C; TLC (solvent D) R_f 0.39; ^{1}H NMR (D_2O) δ 1.89 (m, 2), 3.18 (m, 2), 3.51 (m, 1), 3.72 (s, 2); MS, *m/e* 256 + 258 (MNH_4^+, 50:50), 239 + 241 (MH^+, 50:50).

(S)-2-Amino-5-((N-chloroacetyl)amino)pentanoic acid (**1c**). Yield, 11%; mp 163°C; TLC (solvent D) R_f 0.42; ^{1}H NMR (D_2O) δ 1.43 (m, 2), 1.69 (m, 2), 3.11 (m, 2), 3.56 (m, 1), 3.94 (s, 2); MS, *m/e* 209 + 211 (MH^+, 25:75).

(S)-2-Amino-5-((N-bromoacetyl)amino)pentanoic acid (**2c**). Yield, 9%; mp 143°C; TLC (solvent D) R_f 0.46; ^{1}H NMR (D_2O) δ 1.66 (m, 2), 1.89 (m, 2), 3.30 (m, 2), 3.77 (m, 1), 3.93 (s, 2); MS, *m/e* 253 + 255 (MH^+, 50:50).

Synthesis of (S)-2-Amino-3-((N-iodoacetyl)amino)propanoic Acid (**3a**)

9a 576 mg, 2.82 mmol and 475 mg (5.64 mmol, 2 eq) $HNaCO_3$ dissolved in water (15 ml) were treated with iodoacetic anhydride (1 g, 2.82 mmol). After 1 h stirring at room temperature, the solution was brought to pH 2 with citric acid and worked up as usual. The oily residue (1.3 g) as a mixture of **13a** with iodoacetic acid was treated for 30 min with trifluoroacetic acid (25 ml). The white solid resulting from solvent evaporation was purified by preparative HPLC (C_{18}, water as eluant) to afford 315 mg (50%) of an hygroscopic white powder. Mp ~150°C (dec); TLC (solvent C) R_f 0.54; ^{1}H NMR (D_2O) δ 3.43 (dd, 1, J = 6.6., 14.9 Hz), 3.60 (dd, 1, J = 3.6, 14.9 Hz), 3.61 (s, 2), 3.72 (dd, 1, J = 3.6, 6.6 Hz).

General Procedure for the Synthesis of (S)-2-Amino-ω-maleimidylalkanoic Acids **4**

(S)-2-((N-tert-Butoxycarbonyl)amino)-ω-((N-maleyl)amino)alkanoic acids **15**. Maleic anhydride 480 mg, 4.9 mmol was added to 4.9 mmol of (*S*)-2-((*N-tert*-butoxycarbonyl)amino)-ω-amino alkanoic acid **9** in acetic acid (10 ml). The solution was stirred for 4 h and evaporated under vacuum, and the solid residue recrystallized in acetone/pentane mixture.

15a: yield, 82%; mp 139°C; TLC (solvent E) R_f 0.47; ^{1}H NMR (D_2O) δ 1.3 (s, 9), 3.4 (m, 2), 4.15 (m, 1), 6.19 (d, 1, J = 12.2), 6.36 (d, 1, J = 12.2); MS (DCI/NH_3), *m/e* 320 (NH_4^+), 303 (MH^+) 264 ($MH_4^+ - t$Bu), 247 ($MH^+ - t$-Bu).

15b: yield, 65%; oil; TLC (solvent D) R_f 0.71; ^{1}H NMR (D_2O) δ 1.4 (s, 9), 2.1 (m, 4), 3.3 (m, 2), 4.1 (m, 1), 6.3 (AB, 2, J = 12 Hz).

15c: yield, 42%; oil; TLC (solvent D) R_f 0.71; ^{1}H NMR (D_2O) δ 1.4 (s, 9), 1.7 (m, 2), 3.2 (m, 2), 4.1 (m, 2), 6.3 (AB, 2, J = 12 Hz).

(S)-2-((N-tert-Butoxycarbonyl)amino)-ω-(N-maleimidyl)alkanoic acids **14**. Compound **15** (1 mmol) and triethylamine (2 mmol, 2 eq) in toluene (30 ml) were heated under reflux for 4 h in a Dean–Stark. The solvent was separated from the oily residue which has appeared and evaporated under vacuum. Water (20 ml) was

added and the solution was brought to pH 2 with citric acid. The title compound was isolated after the classical workup.

14a: yield, 55%; mp 96°C; TLC (solvent C) R_f 0.68; ^{1}H NMR (acetone-d_6) δ 1.4 (s, 9), 3.8 (m, 2), 4.4 (m, 1), 6.1 (br s, 1), 6.9 (s, 2), 8 (s, 1).

14b: yield 40%; oil; TLC (solvent A) R_f 0.49; ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9), 2.1 (m, 2), 3.7 (m, 2), 4.4 (m, 1), 6.7 (s, 2); MS, *m/e* 316 (MNH_4^+), 299 (MH^+).

14c: yield 61%; oil; TLC (solvent A) R_f 0.72; ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9), 1.8 (m, 4), 3.6 (m, 2), 4.3 (m, 1), 6.7 (s, 2); MS, *m/e* 330 (MNH_4^+).

(S)-2-Amino-ω-(N-maleymidyl)alkanoic acids **4**. Boc removal was performed by treatment HCl/dioxane as described above. The compound was purified by chromatography on AG1 × 2 resin (AcO^- form) eluting with water.

4a: yield, 79%; TLC (solvent C) R_f 0.33; ^{1}H NMR (D_2O) δ 3.79 (dd, 1, J = 3.6, 7.3 Hz), 3.80 (dd, 1, J = 3.6, 15 Hz), 3.88 (dd, 1, J = 7.3, 15 Hz), 6.75 (s, 2); MS, *m/e* 202 (MNH_4^+), 185 (MH^+).

4b: yield, 53%; mp > 300°C; TLC (solvent D) R_f 0.33; ^{1}H NMR (D_2O) δ 1.95 (m, 2), 3.50 (m, 3), 6.65 (s, 2); MS, *m/e* 199 (MH^+), 181 ($MH^+ - H_2O$).

4c: yield, 54%; mp >300°C; TLC (solvent D) R_f 0.38; ^{1}H NMR (D_2O) δ 1.40–1.70 (m, 4), 3.35 (m, 2), 3.53 (m, 1), 6.64 (s, 2); MS, *m/e* 230 (MNH_4^+), 213 (MH^+).

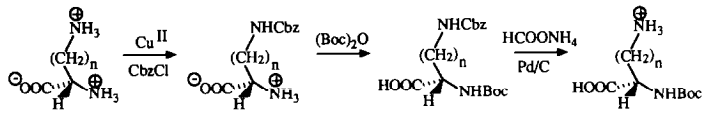
Enzymology

The enzyme purification has been previously described (*6*). Enzyme assay used glutamate dehydrogenase as the coupling enzyme in the presence of the cofactor analogue acetylpyridine adenine dinucleotide (APAD) (*2*). For time-dependent inhibition assays, enzyme was preincubated at 20°C in 100 mM potassium phosphate, 10 mM Fru-6P, pH 7.2, using enzyme concentrations ranging from 0.3 to 3 μM; the ratio enzyme/inhibitor was between 10 and 100. The kinetic parameters K_{irr} and k_{inact}, which characterized the inhibitor under study were determined from the Kitz–Wilson equation (*7*) using a double reciprocal plot of apparent first-order inactivation constant versus inhibitor concentration.

RESULTS AND DISCUSSION

Synthetic Chemistry

The synthetic route utilized in the preparation of the amides **1, 2,** and **3** is depicted in Scheme 2. The differentially protected 2,3-diaminopropanoic acid derivative **9a** was prepared from the commercially available *N*-Boc-L-serine, by a regioselective opening of the corresponding lactone with ammonia as described (*8*). The homologous N^2-protected L-2,4-diaminobutanoic and L-2,5-diaminopentanoic acids were synthesized from the corresponding free amino acid as shown in Scheme 1. The terminal amino group was first selectively protected as benzyloxycarbonyl derivative by complexation of the amino acid function with copper(II). This procedure (*9*) was specially convenient in our case since L-2,4-diaminobutanoic and L-2,5-diaminopentanoic acids (L-ornithine) are commercially available. Subsequent protection of the 2-amino group with Boc followed by hydrogenolysis afforded



6 7 8 9

a : n = 1
b : n = 2
c : n = 3

SCHEME 1

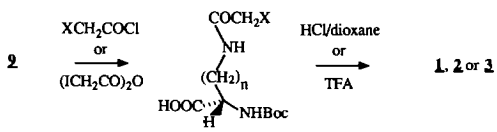
compounds **9** which were quantitatively converted into the haloacetyl derivatives using chloroacetyl chloride (compounds **11a, 11b,** and **11c**), bromoacetyl chloride (compounds **12a, 12b,** and **12c**) or iodoacetic anhydride (compound **13a**). The deprotection of compounds **12** and **13** required trifluoroacetic acid since the classical conditions HCl/dioxane promoted halogen exchange. The chloro, bromo, and iodo derivatives **1, 2,** and **3** were isolated in pure form using reverse-phase HPLC when necessary.

The three maleimide derivatives **4a, 4b,** and **4c** were obtained using the strategy described in Scheme 3. The *N*-maleoyl derivatives **15,** obtained from reaction with maleic anhydride, were heated as triethylammonium salts in toluene with continuous water removal (*10*) to afford **14** in moderate yields.

Enzymology

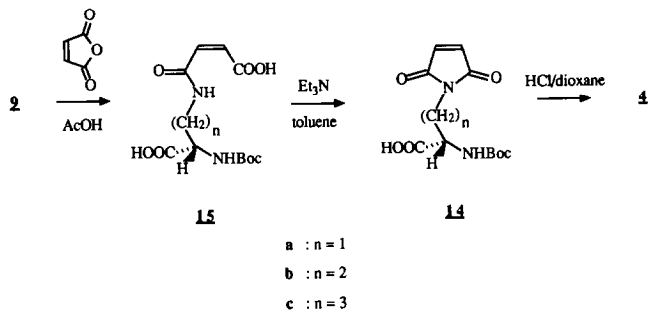
The bacterial glucosamine-6-phosphate synthase was purified from the overproducer as described (*6*) omitting the phenyl-Sepharose step; 110 mg of homogeneous enzyme was purified from 40 g of wet cells.

The compounds synthesized were first characterized by their IC_{50} that is the inhibitor concentration giving half the activity in the enzymatic assay using 10 mM Fru-6P and 1 mM glutamine. The inhibition constants were then determined for the most active compounds. All the inhibitors reported in Table 1 were found to be competitive versus glutamine. As expected, the halomethyl ketones and the maleimide derivative of diaminopropanoic acid behaved as affinity labels of the



11 X = Cl a : n = 1
12 X = Br b : n = 2
13 X = I c : n = 3

SCHEME 2



SCHEME 3

purified enzyme. The affinity of the haloacetyl derivatives, reflected by the K_i values, increased in the order Cl < Br < I meaning there is enough space available in the glutamine binding site for a bulky hydrophobic substituent. The participation of the catalytic thiol group, responsible for enzyme inactivation with FMDP **5** (**5**), seems very likely to account for enzyme inactivation; its nucleophilicity toward the halogen atom is indeed reflected by the apparent second-order rate constant k_{inact}/K_{irr} for the reaction between free enzyme and inhibitor. This value would have certainly culminated with the iodo derivative **3a** if its intrinsic reactivity had not precluded the determination of the kinetic parameters. As a matter of fact, **3a** cyclized rapidly at neutral pH to give the substituted piperazinone **16** devoid of inhibitory properties. The K_i estimate reported in Table 1 for **3a** did not take this fact into account.

N^3-Maleimidyldiaminopropionate **4a** combined the recognition moiety of diaminopropanoic acid with the reactivity of the maleimide derivatives towards SH groups (*11*). Indeed the enzyme inactivation with **4a** is roughly as efficient as with **2a** that is k_{inact}/K_{irr} = 2000 M^{-1} s^{-1}. The interesting trends exhibited by the results described here is the optimum distance between the amino acid function, anchoring

TABLE 1

Inhibition of Glucosamine-6P Synthase by Affinity Labels

Compound	IC_{50} (μM)	K_i (μM)	k_{inact} (min^{-1})	K_{irr} (μM)	k_{inact}/K_{irr} (M^{-1} s^{-1})
5	0.8	0.15	1.04	5.5	2950
1a	60	12	0.65	91	117
2a	0.6	0.1	1.92	14.7	2170
3a	0.4	0.08	[a]	[a]	[a]
1b, 2b	>1000	—		no inactivation	
1c, 2c	>1000	—		no inactivation	
4a	175	nd	9.2	77.7	2000
4b, 4c	>1000	—		no inactivation	

[a] Cyclization into **16** precluded the determination of kinetic parameters.

the compound in the active site, and the electrophilic group susceptible to react with the amino terminal cysteine of the protein. This conclusion is supported by the entire lack of inhibition by the higher homologues **1b**, **2b**, **4b**, or **4c**. The efficiency of compounds **2a** and **4a**, close to the one of the leading compound **5**, in glucosamine-6P synthase inhibition, argues in favor of our strategy in the design of affinity label inhibitors. These compounds need to be incorporated into peptide vectors to exhibit any antibacterial or antifungal activity. Despite the good anticandidal properties of peptidic FMDP conjugates (*12*), the intrinsic reactivity of the warehead toward nucleophiles, previously demonstrated by the Michael acceptor reactivity of FMDP (*5*), might preclude further applications of such antibiotics. The high reactivity of N^3-iodoacetyl-L-2,3-diaminopropionic acid **3a** described in this study might explain the poor antimicrobiol properties of the corresponding peptidic conjugates recently synthesized (*13*). The present study together with the results accumulated for the past 5 years on glucosamine-6P synthase inhibition, will be helpful in the design of inhibitors based on a somewhat different concept.

REFERENCES

1. WALKER, J. E., GAY, N. J., SARASTE, M., AND EBERLE, A. N. (1984) *Biochem. J.* **224,** 799–815.
2. BADET, B., VERMOOTE, P., HAUMONT, P. Y., LEDERER, F., AND LE GOFFIC, F. (1987) *Biochemistry* **26,** 1940–1948.
3. KORT, M. J. (1970) *in* Advances in Carbohydrate Chemistry and Biochemistry (Tipson, R. S., Ed.), Vol. 25, pp. 311–349, Academic Press, New York/London.
4. BADET, B., VERMOOTE, P., AND LE GOFFIC, F. (1988) *Biochemistry* **27,** 2282–2287.
5. KUCHARCZYK, N., DENISOT, M. A., LE GOFFIC, F., AND BADET, B. (1990) *Biochemistry* **29,** 3668–3676.
6. DUTKA-MALEN, S., MAZODIER, P., AND BADET, B. (1988) *Biochimie* **70,** 287–290.
7. KITZ, R., AND WILSON, I. B. (1962) *J. Biol. Chem.* **237,** 3245–3249.
8. KUCHARCZYK, N., BADET, B., AND LE GOFFIC, F. (1989) *Synth. Commun.* **19,** 1603–1609.
9. LEDGER, R., AND STEWART, F. H. C. (1965) *Aust. J. Chem.* **18,** 933–944.
10. RICH, D. H., GESELLCHEN, P. D., TONG, A., CHEUNG, A., AND BUCKNER, C. K. (1975) *J. Med. Chem.* **18,** 1004–1008.
11. LUNDBLAD, R. L., AND NOYES, C. M. (1988) Chemical Reagent for Protein Modification, Vol. 1, Chap. 6, CRC Press, FL.
12. ANDRUSZKIEWICZ, R., MILEWSKI, S., ZIENIAWA, T., AND BOROWSKI, E. (1990) *J. Med. Chem.* **33,** 132–135.
13. ANDRUSZKIEWICZ, R., CHMARA, H., MILEWSKI, S., ZIENIAWA, T., AND BOROWSKI, E. (1990) *J. Med. Chem.* **33,** 2755–2759.