Biological Studies. Organisms were obtained either from the American Type Culture Collection (ATCC) or from the Pasteur Institut Collection. *E. coli* W7 Lys, DAP mutant was kindly provided by Dr. J. Van Heijenoort, Institut de Biochimie, Orsay, France. The growth inhibition experiments were performed in the Davis and Mingioli's liquid medium.[29] The bacterial growth was followed by spectrophotometry at 660 nm, and the inhibition percentages were calculated after 6 h at 37 °C. The inoculum was 0.2 mL of 18-h liquid medium culture of the test organism. The inoculum was added to 4.8 mL of the liquid medium, and 0.1 mL of a solution of the compound was added. The MIC values were determined on the agar Davis and Mingioli's solid medium[29] eventually supplemented by amino acids[30] and were the lowest concentration that resulted in complete inhibition of growth. Antagonism studies were conducted either by a disk diffusion method on the solid medium or by supplementing the liquid medium with the amino acid. K_m and K_i values are the arithmetic means of three determinations.

E. coli ATCC 9637 *meso*-DAP decarboxylase was isolated and purified as described by White and Kelly.[21] Its specific activity was 3 μm/mg. Inhibition of *meso*-DAP decarboxylase was measured following the procedure described by Beaven et al.[31] with [^{14}C]-*meso*-DAP (CEA).

Acknowledgment. We are grateful to Drs. M. Langlois, Ph. Dostert, and A. R. Schoofs for valuable discussions and to A. Lalande, E. Caminade, and C. Meriaux for technical assistance.

Registry No. **2**, 100910-16-5; **2a**, 35047-24-6; **2b**, 100910-23-4; **2c**, 100910-24-5; **2d**, 100910-25-6; **2e**·HCl, 100910-26-7; **3**·2HCl, 100910-17-6; **3a**, 37944-29-9; **3b**, 100910-27-8; **3c**, 100910-28-9; **3d**, 100910-29-0; **3e**·HCl, 100910-30-3; **4**·HCl, 100910-18-7; **4** (free base), 100910-60-9; (DD + LL)-**4**, 100910-31-4; (-)-**4**·2HCl, 100910-32-5; (-)-**4** (free base), 100928-36-7; (+)-**4**·2HCl, 100910-33-6; (+)-**4** (free base), 100910-61-0; *meso*-**4**, 100910-34-7; **4b**, 100910-35-8; **4c**, 100938-75-8; **4d**, 100910-36-9; **4e**, 100910-37-0; **4f**, 100910-38-1; **4g**, 100910-39-2; **4h**, 100910-40-5; **4i**, 100910-41-6; **4j**, 100910-42-7; **4k**, 100910-43-8; **4l**, 100910-44-9; **4m**·2HCl, 100910-45-0; **4n**·2HCl, 100910-46-1; **5**, 100910-19-8; **5a**, 100910-47-2; **5b**, 100910-48-3; **5c**, 100928-35-6; **5d**, 100910-49-4; **5e**, 100910-50-7; **6**·2HCl, 100910-20-1; **6** (free base), 100910-62-1; **6a**, 100056-08-4; **6b**, 100910-51-8; **6c**, 100910-52-9; **6d**, 100910-53-0; **6e**, 100910-54-1; **7**, 100910-21-2; **7a**, 100910-55-2; **7b**, 100910-56-3; **7c**, 100910-57-4; **7d**, 100910-58-5; **7e**, 100910-59-6; **7f**, 100910-63-2; **8**, 98998-80-2; **9**·2HCl, 100910-22-3; **9** (free base), 52017-67-1; MesCl, 124-63-0; $(BOC)_2O$, 24424-99-5; OHCCOOEt, 924-44-7; lysine, 56-87-1; *meso*-DAP decarboxylase, 9024-75-3.

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Isozyme-Specific Enzyme Inhibitors. 11.[1] L-Homocysteine-ATP S-C5′ Covalent Adducts as Inhibitors of Rat Methionine Adenosyltransferases

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The title compounds (**14a,b**) were 5′ epimers of a derivative of a phosphonate isostere of ATP in which the CH_2OP^{α} system of ATP was replaced by $CH(R)CH_2P^{\alpha}$ [R = L-$S(CH_2)_2CH(NH_2)CO_2H$]. They resisted synthesis via attempted S-alkylation of the corresponding epimeric 5′-mercapto derivatives. A practicable route to **14a,b** commenced with Michael condensation of L-homocysteine with the diphenyl ester of the 5′,6′-vinyl phosphonate analogue of 2′,3′-*O*-isopropylideneadenosine 5′-phosphate. The resulting epimeric 5′ thioethers were separated by reverse-phase HPLC. The two phenyl groups were replaced by benzyl groups, after which the α-amino acid residue was protected as an *N*-Boc methyl ester. Both benzyl groups were removed by hydrogenolysis, and the resulting phosphonic acid was converted into its pyrophosphoryl derivative. Blocking groups were then removed under conditions that furnished **14a** and **14b** without racemization of their L-amino acid residues. Also synthesized were the P^{β}-NH-P^{γ} imido analogue (**15a**) of **14a** and the sulfoxide derivative (**16a**) of **14a**. The structures of **14a** and **16a** were verified by FAB mass spectra, which revealed the protonated molecular ions of their sodium salts. All adducts appeared to function as dual substrate site inhibitors (competitive to ATP and to methionine) of the rat normal tissue (MAT-2) form of methionine adenosyltransferase (MAT); **14a** and **15a** [$K_M(ATP)/K_i$ = 4 and 9, respectively] were the most effective. Adduct **15a** was the most effective inhibitor [$K_M(ATP)/K_i$ = 13] of the MAT-T form from rat hepatoma tissue; the kinetic data indicated dual-site inhibition by **15a** with apparently complete coverage of the ATP site and incomplete coverage of the methionine site. The inhibition properties of the adducts indicated little preference in the order in which the two MAT forms bound ATP and methionine.

Isozyme-specific enzyme inhibitors are, as discussed previously,[2] of interest as potential progenitors of new antineoplastic agents. In previous work in this series, we have studied several approaches to the design of such inhibitors, using for that purpose enzymes of the most common type, i.e., ones that catalyze reactions involving two substrates and that accordingly possess two substrate binding sites per catalytic unit. These studies have shown that two-substrate adducts linked covalently via the atoms that become bonded in the enzyme-catalyzed reaction are capable of acting as potent isozyme-selective inhibitors, either in their unsubstituted form, as was observed in the case of mammalian thymidine kinases,[3] or after the at-

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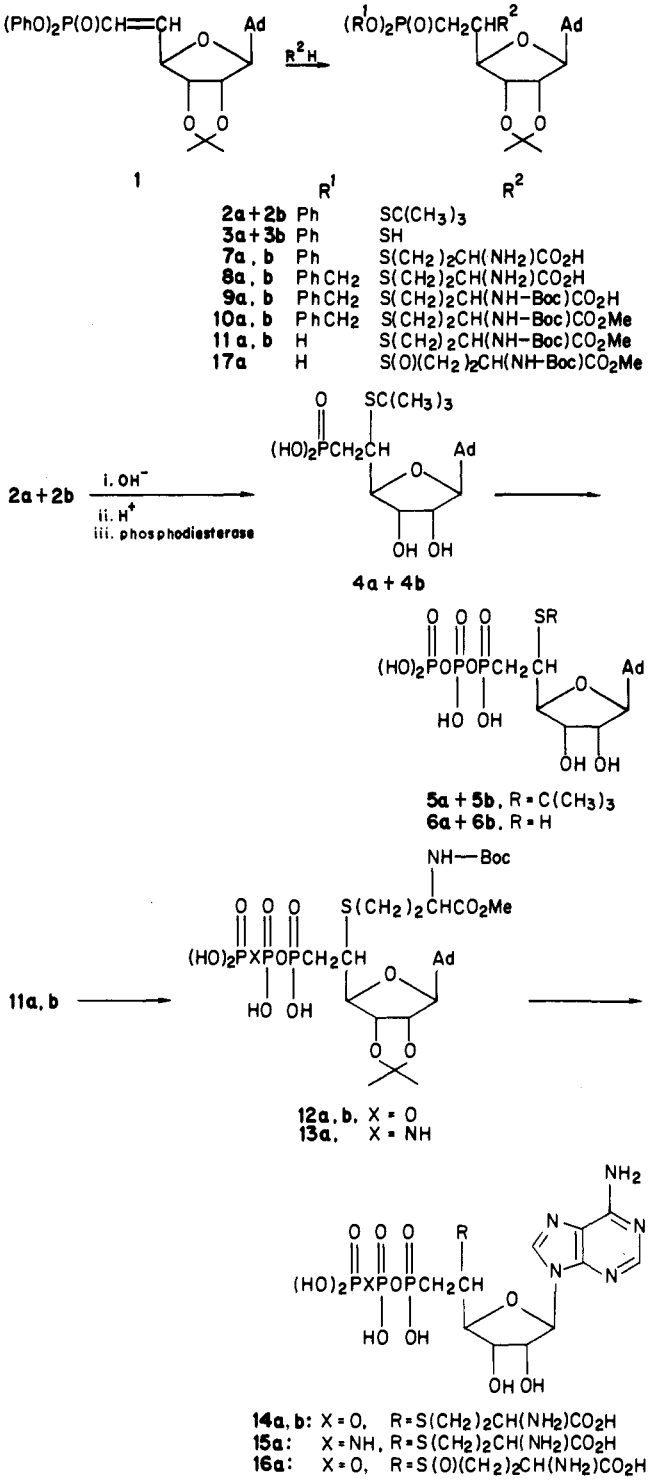
tachment of one or more substrate substituents known to produce isozyme-selective inhibition, as was observed with mammalian adenylate kinases.[4,5] Kinetic analysis indicated that the enzyme-bound adducts, in producing these effects, could be interacting simultaneously with both substrate sites. With mammalian hexokinases on the other hand, two-substrate adducts of this type acted instead as weak inhibitors.[3] This was thought to result from the sequential manner in which the substrates adsorb to hexokinases, together with structural requirements for adsorption of the first substrate that may not have been satisfied in the two-substrate adducts. The present work extends studies with the above type of two-substrate adduct to rat tissue variants of a fourth enzyme, ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) (MAT), which catalyzes attack of the sulfur of methionine on C5′ of adenosine 5′-triphosphate (ATP) to form *S*-adenosylmethionine. Evidence, summarized previously,[1] indicates that a variant of MAT found in rat hepatoma tissue is of interest as a potential target in cancer chemotherapy.

We describe here the synthesis of the two 5′ epimers (**14a,b**) of an adduct in which the sulfur of L-homocysteine is bonded to C5′ of an isostere of ATP in which a C5′-CH_2-P^{α} system replaces C5′-O-P^{α}. That **14a,b** might be able to function as dual-site inhibitors was suggested by evidence that replacement of O5′ in ATP by CH_2 or introduction of a small alkyl (*n*-propyl) group at C5′ of ATP are both compatible with good affinity for the ATP binding sites of two forms of MAT that are present in rat normal and tumor tissue, respectively.[1] The P^{β}-NH-P^{γ} isostere **15a** of adduct **14a** was also synthesized in the present work because the P^{β}-NH-P^{γ} isostere of ATP is a potent inhibitor of MAT-T.[1] In addition, the sulfoxide **16a** (as a mixture of its two stereoisomers) was synthesized to provide electronic mimicry of C5′···$S^{\delta+}(R^1)R^2$ partial bonds in transitional structures leading to enzyme-catalyzed formation of the C5′-$S^+(R^1)R^2$ system of *S*-adenosylmethionine. In this study, **14a**, **14b**, **15a**, and **16a** were analyzed kinetically as potential dual-site inhibitors of MAT-T, a form of MAT that occurs in several transplatable rat hepatomas,[6,7] and of MAT-2, a form that predominates in most normal rat tissues.[8,9]

Chemical Syntheses. Two approaches to the synthesis of **14a,b** were evaluated. Both utilized as starting material the readily accessible 2′,3′-*O*-isopropylidene derivative of the diphenyl ester of the 5′,6′-vinyl phosphonate analogue of adenosine 5′-phosphate (**1**),[10] and both were based on the known β-addition of nucleophiles to α,β-unsaturated phosphonates.[11,12] The first approach aimed to obtain **14a,b** via alkylation of the 5′-mercapto ATP analogues **6a,b** with either 4-(bromomethyl)-[13] or 4-(iodomethyl)-L-alanine[14] and to secure **6a,b** by treatment of their *S*-*tert*-butyl derivatives **5a,b** by a mild procedure (7 equiv of mercuric acetate at pH 4)[15] that quantitatively removes *S*-*tert*-butyl groups from thioethers and was found by us not to decompose ATP. (ATP was much less stable at pH 5 under these conditions, however.) Treatment of the vinyl phosphonate **1** with a large excess of *tert*-butyl mercaptan in *N,N*-dimethylformamide (DMF) in the presence of 0.5 mol equiv of NaOMe gave, after silica gel column chromatography, 65% yield of a mixture (in 2:3 ratio by HPLC

Scheme I[a]



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and ^{1}H NMR) of the 5′ epimeric β-(*tert*-butylthio)alkyl phosphonates **2a** and **2b** (Scheme I). That removal of the *tert*-butyl group was feasible in the present series of compounds was confirmed by treatment of **2a** and **2b** for 24 h with 7 equiv of $Hg(OAc)_2$ in DMF–H_2O at pH 4, when the corresponding 5′-mercapto derivatives **3a** and **3b** were obtained in good yield. The mixture of **2a** and **2b** was treated in succession with NaOH in THF–H_2O to remove one phenyl group, with trifluoroacetic acid to remove the isopropylidene group, and with phosphodiesterase to remove the second phenyl group, following which the mixed 5′ epimers of 5′-(*tert*-butylthio)-5′-deoxy-5′-[(dihydroxyphosphinyl)methyl]adenosine (**4a** and **4b**) could be isolated in homogeneous form in 80% overall yield. Modifications described previously[5] of the Hoard–Ott method for preparing 2′-deoxynucleoside 5′-triphosphates[16] were then used to convert the **4a–4b** mixture to a mixture of the corresponding ATP analogues **5a** and **5b**; these were purified by anion-exchange chromatography over DEAE-cellulose and isolated in 53% yield as triethylammonium salts that were homogeneous by HPLC analysis. The *tert*-butyl groups were removed with mercuric acetate, using conditions under which ATP, as a model triphosphate, had been found to be stable. This gave quantitative conversion to a mixture of the free thiols **6a** and **6b**, which were shown by HPLC analysis to be homogeneous and present in the same 2:3 ratio as **2a** and **2b**. The compounds were stable for more than 18 h at 4 °C under an air atmosphere in aqueous solution at pH 5. The sulfhydryl group content of the **6a–6b** mixture was 70% of theoretical as measured by the 5,5′-dithiobis(2-nitrobenzoic acid) procedure.[17] The low value possibly resulted from a slow rate of mixed disulfide formation due to steric hindrance. The **6a–6b** mixture was treated at room temperature under argon with 5 equiv of 4-(iodomethyl)-L-alanine, either in water at pH 9 or in DMF containing 4 equiv of Bu_3N or Et_3N. Analysis by HPLC, TLC, and paper electrophoresis showed major conversion of **6a,b** to less polar products of undetermined structure and furnished no evidence for formation of compounds with the properties of **14a,b**. A similar result was obtained upon treatment of **6a,b** in DMF with the methyl ester of 4-(iodomethyl)-L-alanine. Since certain bromoalkanes are known to oxidize thiols to disulfides less readily than the corresponding iodoalkanes,[18] **6a,b** were treated with 5 equiv of 4-(bromomethyl)-L-alanine in DMF containing 4 equiv of Et_3N. After 3 days at 45 °C no **14a,b** was detected, though portion of each reactant was still present. With liquid ammonia as solvent, no **14a,b** formed even at room temperature. The thiols **3a,b** likewise formed no detectable amounts of the thioethers **7a,b** upon treatment with 5 equiv of 4-(bromomethyl)- or 4-(iodomethyl)-L-alanine in DMF containing 4 equiv of Et_3N. In virtually all the foregoing attempted S-alkylations, the nucleoside 5′-thiols become extensively converted to less polar products of unelucidated structure that formed at similar rates in the absence of a haloalkane. These competing reactions mitigated against the possibility of achieving S-alkylation by the use of more forcing conditions. The difficulty in S-alkylation of **6a,b** or **3a,b** suggests steric shielding of the 5′-thiol groups from the haloalkanes.

Adducts **14a,b** were subsequently obtained via a second approach that commenced with reaction of a 3-fold excess of disodium L-homocysteinate with the vinyl derivative **1** in aqueous tetrahydrofuran at room temperature. The choice of pH was important. Thus at pH 8.0 the reaction was only 10% complete after 16 h whereas at pH 8.5 it was complete within 16 h. At pH 9.0 the reaction was more rapid, but the yield of **7a,b** was significantly less because **7a,b** underwent competing hydrolysis to the corresponding monophenyl esters which were unsuitable for the next stage of the sequence, while portion of **1** was concomitantly hydrolyzed to a monophenyl ester that did not undergo the addition reaction. Reverse-phase preparative HPLC yielded 38% of the more polar 5′ epimer **7a** and 41% of the less polar epimer **7b** when the reaction was carried out at pH 8.5.

The phenyl groups of **7a** and **7b** were replaced by benzyl groups in quantitative yield by the action of 4 equiv of sodium benzyl oxide in benzyl alcohol for 0.5 h at 22 °C. HPLC analysis indicated that epimerization at C5′ had not occurred. The isolated dibenzyl esters **8a,b** could be converted directly and quantitatively to their homogeneous *N*-Boc derivatives **9a,b** with di-*tert*-butyl pyrocarbonate in dry DMF containing 1 equiv of NEt_3. In view of the low reactivity of diazomethane for the adenine ring system under neutral conditions,[19,20] the carboxyl groups of **9a,b** could conveniently be methylated with an excess of diazomethane in tetrahydrofuran. Silica gel flash chromatography then yielded the methyl esters **10a** and **10b** in 69% and 74% yields overall from **7a** and **7b**, respectively.

The benzyl esters **10a** and **10b** were hydrogenolyzed in acetic acid in the presence of palladium black. A 2:1 ratio of palladium to **10a** or **10b** was required to compensate for the catalyst poisoning commonly encountered with thioethers.[21] The resulting phosphonic acids **11a** and **11b** were isolated in 96% yield and were converted, via their phosphoroimidazolidates, to their pyrophosphoryl derivatives **12a** and **12b** by using an established procedure[22] for the conversion of nucleoside 5′-monophosphates to nucleoside 5′-triphosphates. Treatment of **12a,b** with aqueous 90% trifluoroacetic acid quantitatively removed the isopropylidene and butyloxycarbonyl groups. The carbomethoxy group was next hydrolyzed during 6 h at 22 °C in a sodium carbonate buffer initially at pH 10.4. No significant racemization of the L-homocysteine residue was expected during this operation because the methyl esters of several dipeptides have been reported to undergo less than 0.1% racemization when treated for 3 h at 22 °C with 1.1 molar equiv of 1 N sodium hydroxide.[23] Furthermore, we found no evidence for racemization when *S*-benzylhomocysteine methyl ester was saponified under the conditions used in the present work.[24] After purification by anion-exchange chromatography, the desired adducts **14a,b** were isolated in 34% and 26% yields, respectively, from **11a,b** as tetrasodium salts that appeared homogeneous when analyzed by paper chromatographic, paper electrophoretic, and HPLC systems (see Table I). Adducts **14a,b** were further characterized by elemental analysis, UV extinction coefficient, ^{1}H and ^{31}P NMR spectra, and, in the case of **14a**, a fast atom bombardment (FAB) mass spectrum.

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Table I. Paper Chromatographic, Paper Electrophoretic, and HPLC Properties of Adenine Nucleotide Derivatives

	R_f			electrophoresis, rel mobility			
compd	syst B	syst C	syst D	pH 3.5	pH 6.8	pH 7.5	HPLC retn time, min (system)
AMP	0.1					1.00	
ATP		0.24	0.34	1.00	1.00		4 (H) and 22.4 (M)
1							7 (F)[a]
2a + 2b							20 and 21 (F)[b]
3a + 3b							8 and 11 (F)
4a + 4b	0.31					0.99	12 (G)
5a + 5b		0.60	0.77				20.5 and 25.5 (H)
6a + 6b							3.8 and 4.2 (H)
7a							13 (J)[c]
7b							16 (J)[c]
8a							20 (K)
8b							22 (K)
9a							12.4 (K)
9b							12.4 (K)
11a						0.67	22.8 (L)
11b						0.67	23.7 (L)
14a		0.23	0.32	0.85	0.84		19.8 (M)
14b		0.24	0.31	0.85	0.84		20.8 (M)
15a		0.28	0.27		0.81		
16a		0.21	0.29	0.85	0.84		18.3, 20.5 (M)
17a						0.75	19.8, 20.8 (L)
19a							8.5, 11.5 (M)

[a] 19 min (E) and 26 min (J). [b] 50 min and 54 min (E). [c] 20 min (K).

For synthesis of **15a**, which is the P^{β},P^{γ}-imido isostere of **14a**, **11a** was converted to its phosphoroimidazolidate, and, in modifications of a described procedure[16] for nucleoside 5′-triphosphate synthesis, this intermediate was freed of imidazole and added to a solution in DMF of 10 molar equiv of tri-*n*-butylammonium imidodiphosphate. Formation of the ATP derivative **13a** was complete after 4 h at room temperature. Separation of **13a** from the excess of imidodiphosphate by the usual procedure of anion-exchange chromatography on DEAE-cellulose was not feasible at the mildly alkaline pH values at which the P–N bond of **13a** was stable. However, **13a** could be completely separated from the relatively polar imidodiphosphate by adsorption from aqueous solution onto a column of C_{18} silica and subsequent desorption with aqueous methanol. Removal of blocking groups from **13a** by the methods used for the **12a** → **14a** conversion gave the homogeneous tetrasodium salt of **15a** (43% yield from **11a**) with the expected UV, ^{1}H NMR, and ^{31}P NMR spectral properties.

An aqueous solution of the tetrasodium salt of **14a** was treated for 4 days at 4 °C with an excess of H_2O_2, when HPLC and spectrophotometric analyses indicated that quantitative conversion to a 3:2 mixture of two new compounds had occurred. NMR spectra indicated that these were the two diastereomers of the sulfoxide **16a**. Thus the ^{1}H NMR spectrum of the mixed products showed H1′ as a pair of doublets in a 3:2 ratio, and the protons on the methylene and methine groups adjacent to sulfur exhibited downfield shifts of 0.47 and 0.29 ppm, respectively, relative to the same protons of the thioether **14a**, consistent with a sulfoxide structure. In addition, the P^{β} and P^{γ} resonances in the ^{31}P NMR spectrum of **16a** were almost identical in chemical shift with the same resonances in **14a**, whereas P^{α} of **16a** was deshielded by 1.7 ppm, in agreement with the enhanced electron withdrawal from P that would result from conversion of a β-thioether-substituted phosphonate to a β-sulfoxide-substituted phosphonate. A peroxyphosphate structure for **16a** is excluded by its ^{31}P NMR spectrum in comparison with that of P^{β},P^{γ}-peroxy-ATP,[25] and an adenosine N^1-oxide structure[26] is excluded by the UV spectrum of **16a**. The FAB mass spectrum of **16a** revealed the protonated molecular ions of the tetra-, tri-, di-, and monosodium salts, thus providing direct evidence for introduction into the tetrasodium salt of **14a** of a single covalently attached oxygen atom.

Synthesis of **16a** was also attempted by a route that commenced with treatment of **11a** with 1 equiv of H_2O_2 in aqueous acetic acid to give the two diastereomeric sulfoxide derivatives **17a** in quantitative yield. The mixed **17a** diastereomers were transformed to their pyrophosphoryl derivatives **18a** in 65% yield by the procedures used to convert **11a** to **13a**. The interesting observation was made, however, that application of the acidic and basic treatments necessary to remove the blocking groups from **18a** did not yield the mixed diastereomers of **16a** but gave products that appeared to be the diastereomers of the β-sulfoxide-substituted monophosphonate **19a**. Paper electrophoretic analysis indicated that the loss of inorganic pyrophosphate had taken place during the initial acidic treatment. HPLC analysis is showed, by co-injection of samples, that material indistinguishable from **19a**, and comprised of two presumed diastereomers, was formed rapidly and quantitatively from **16a** under the acidic conditions used to remove isopropylidene groups in the present work and also was formed from **17a** under the acidic and basic deblocking conditions used to convert **12a,b** into **14a,b**. It seems likely that loss of pyrophosphate from **16a** and **18a** occurs by activation of P^{α} by protonation of its ionizable oxygen and intramolecular attack on P^{α} by sulfoxide oxygen, followed by attack of a water oxygen on the resulting sulfoxonium sulfur to regenerate a sulfoxide. Thus the capacity of sulfoxide oxygen to form a cyclic sulfoxonium salt by intramolecular attack on an incipient cationic center has been directly demonstrated, and evidence has been presented for subsequent attack by water on the sulfoxonium sulfur to produce a sulfoxide of opposite chirality.[27]

Enzyme Studies. The MAT-2 form of methionine adenosyltransferase was extracted from rat kidney and the MAT-T form from rat Novikoff hepatoma cells by iden-

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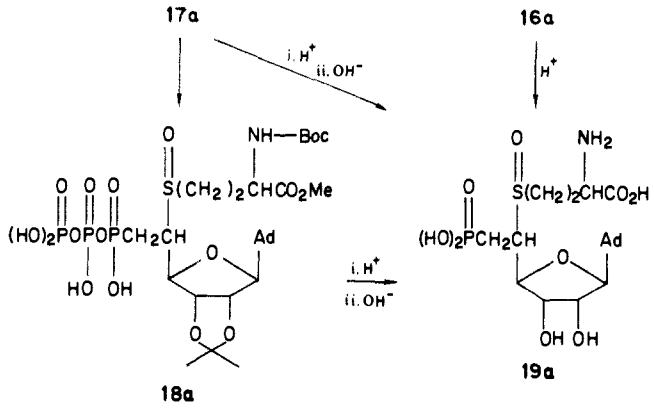

tical procedures. The crude extracts were partially purified under identical conditions by DEAE-cellulose chromatography, which sufficed to yield preparations of sufficient purity for kinetic studies of the MAT-catalyzed reaction. These studies were carried out under the same conditions with the two MAT forms, using levels of the essential cations K^+ and Mg^{2+} and a pH value known to optimize the activity for both forms.[6,28,29] Inhibition constants of **14a**, **14b**, **15a**, and **16a** were determined with use of fixed levels of methionine (for variable MgATP) or MgATP (for variable methionine) that gave near-maximal reaction velocities yet were not inhibitory. ATP and ATP derivatives were added to reaction mixtures as 1:1 complexes with Mg^{2+} in order to minimize variations in the level of uncomplexed Mg^{2+}.

Inhibition constants of the adducts **14a**, **14b**, **15a**, and **16a** are given in Table II. In the case of MAT-2, all adducts produced competitive inhibition with respect to both methionine and MgATP, suggesting that they may be able to cover both substrate binding sites. The inhibition constants obtained with variable MgATP were similar in magnitude to those with variable methionine, indicating that MAT-2 could have no marked preference for the order in which it binds MgATP and methionine under the conditions of the study and that the observed inhibition constants are a measure of the relative abilities of the various adducts to bind reversibly to an enzyme species that is not complexed with either of the two substrates. In the cases of **14a** and **15a**, the inhibition constants with variable methionine were slightly but significantly higher than with variable MgATP. If it is assumed that these adducts have higher affinity for uncomplexed MAT than for the MAT–MgATP complex, this finding may signify that MAT–MgATP is formed slightly more frequently than MAT–methionine in the initial step of the catalytic cycle. Studies of the kinetic mechanism of rat MAT-2 by the usual initial-velocity and product-inhibition approaches are apparently not yet reported. However, evidence from these approaches suggests that MgATP binds prior to methionine to a MAT, obtained from human lymphocytes, which resembles rat MAT-2.[30] In contrast, *Escherichia coli* MAT is reported to exhibit no preferential order of binding to MgATP and methionine.[31]

The inhibition constants of **14a** and **14b** with MAT-2 determined with variable MgATP levels indicate a 4.6-fold stereochemical preference for binding of **14a** to the enzyme. The inhibition constant of 5′(*S*)-propyl-ATP was found earlier to be 5.6 times higher than that of 5′(*R*)-propyl-ATP (Table II), suggesting that **14a** (and hence **15a** and **16a**) may have the 5′(*R*) configuration. Adduct **14a** is seen to be an effective inhibitor of MAT-2. Replacement of the L-homocysteine residue of **14a** by H, to give the $PCH_2C(5')$ isostere of ATP, approximately doubled K_i (Table II) to indicate that the homocysteine moiety of **14a** makes at least a small contribution of the affinity of **14a** for uncomplexed MAT-2. Conceivably, the homocysteine portion of **14a** may reduce the affinity of the phosphono-ATP portion for MAT-2, in which event the contribution of the homocyteine portion to **14a** affinity would be larger than indicated by the present K_i values.

The P^{β},P^{γ}-imido isostere **15a** of **14a** appears from its inhibition constants (Table II) to bind to the free enzyme species of MAT-2 more than twice as effectively as **14a** itself. In the case of MAT of *E. coli*, evidence indicates that the Mg of MgATP contributes to binding of MgATP to its substrate site.[32] In addition, determination of stability constants has shown that P^{β},P^{γ}-imido-ATP at pH 8.5 chelates Mg^{2+} 2.5-fold more avidly than ATP,[33] a property that could account for the enhanced binding of **15a** at pH 8.2 in the present studies if it is assumed that MgATP binds to MAT-2 and MAT-T partly via its coordinated Mg. In accord with this view, the inhibition constant of **15a** with MAT-T was almost 3 times less than that of **14a** (Table II).

Adduct **16a**, which comprised a 2:3 mixture of its two sulfoxide stereoisomers, inhibited MAT-2 less effectively than the corresponding thioether **14a**, even were it assumed that the minor isomer of **16a** was solely responsible for the observed inhibition. The decreased inhibitory potency might be associated with the greater polarity of sulfoxide oxygen compared to the methyl of methionine or of *S*-adenosylmethionine, or with unfavorable steric effects arising from introduction of the sulfoxide oxygen.

The adducts **14a**, **14b**, **15a**, and **16a** were competitive inhibitors of MAT-T with respect to MgATP (Table II). The K_i value of each adduct appeared to be slightly less than with MAT-2, except for **16a**, which inhibited MAT-T about twice as effectively as MAT-2. As with MAT-2, conversion of **14a** to the sulfoxide **16a** did not enhance inhibitory potency. The ratio of the inhibition constants of **14a** and **14b** with MAT-T was similar to that shown by 5′(*R*)- and 5′(*S*)-propyl-ATP, as was noted in the case of MAT-2. The inhibition constant of the $PCH_2C(5')$ isostere of ATP was respectively 2- and 10-fold higher than those of **14b** and **14a**, indicating that the homocysteine moieties of **14a** and **14b** probably contribute to their affinity for MAT-T. This, together with the competitive nature of their inhibition with respect to MgATP, suggests that **14a** and **14b** are able to bind in concerted fashion to the ATP site and to an area adjacent to or overlapping the methionine site.

In the presence of variable methionine levels, **15a** inhibited MAT-T noncompetitively. The inhibition constant was significantly less than with variable MgATP. On the assumption that **15a** binds more strongly to free enzyme than to the enzyme–MgATP, enzyme–methionine, or enzyme–MgATP–methionine complexes, the data suggest that MAT-T is exhibiting no preference, under the conditions of these studies, for the order in which it complexes with MgATP and methionine and that the noncompetitive inhibition by **15a** with respect to methionine indicates reversible binding of **15a**, presumably via its ATP moiety, to the enzyme–methionine complex and/or reversible

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Table II. Inhibition Constants of Adenine Nucleotide Derivatives with Kidney (MAT-2) and Novikoff Hepatoma (MAT-T) Forms of Rat Methionine Adenosyltransferase[a]

	MAT-2			MAT-T		
	K_i, μM (type of inhibn)[b]			K_i, μM (type of inhibn)		
compd	ATP varied	Met varied	K_M,[c] μM	ATP varied	Met varied	K_M, μM
Met			5			15
MgATP			140			140
14a	39 (C)	62 (C)		32 (C)		
14b	180 (C)	200 (C)		148 (C)		
15a	16 (C)	26 (C)		11 (C)	38 (NC)	
16a	140 (C)			81 (C)		
phosphono-ATP[d]	75 (C)[e]			310 (C)[e]		
5′(*R*)-Pr-ATP	48 (C)[e]			37 (C)[e]		
5′(*S*)-PR-ATP	270 (C)[e]			140 (C)[e]		

[a] When methionine (Met) was the variable substrate (S), [MgATP] was 2 mM with both MAT forms; with variable MgATP, [Met] was 60 μM with MAT-2 and 120 μM with MAT-T. For other conditions, see Experimental Section. [b] C = competitive; NC = simple noncompetitive (the inhibitor reduces V_{max} and does not change K_M). [c] K_M = Michaelis constant ([S] for 0.5 V_{max}). [d] The ATP analogue in which $PCH_2C(5')$ replaces $POC(5')$. [e] Data from ref 1.

binding of methionine to the enzyme–**15a** complex. The lesser tendency of MAT-2 to form an enzyme–**15a**–methionine complex could signify more effective obstruction of its methionine site by **15a** and is ascribable to structural differences between the two MAT forms. These differences are manifested also by the differing Michaelis constants of methionine with these forms and by differential inhibitory potencies toward them that are shown by a variety of monosubstituted ATP derivatives and by some ATP analogues.[1]

In summary, our study has demonstrated a practicable synthetic route to L-homocysteine–ATP adducts with S–C5′ linkages and has revealed that several of these act as effective dual-site inhibitors of two forms of rat L-methionine adenosyltransferase. One of these forms predominates in rat hepatoma tissue and is a potential target in cancer chemotherapy. On this basis we are currently studying other potential inhibitors of this form.

Experimental Section

Chemical Synthesis. General Procedures. *N,N*-Dimethylformamide (DMF) and hexamethylphosphoric triamide (HMPA) were distilled from CaH_2 and stored over molecular sieves. Thin-layer chromatograms were obtained on Baker-flex silica gel 1B2-F from J. T. Baker Chemical Co. in (A) $CHCl_3$–MeOH (100:6). Preparative layer chromatography was conducted with 2-mm layers of silica gel on glass from EM Laboratories. Paper chromatography was carried out by the ascending technique on Whatman No. 1 paper in (B) 2-propanol–NH_4OH–water (7:1:2); (C) isobutyric acid–NH_4OH–water (66:1:33), and (D) 1-propanol–NH_4OH–water (55:10:35). Electrophoresis was carried out on Whatman No. 1 paper at pH 3.5 (0.05 M citrate), pH 6.8 (0.05 M citrate), pH 7.5 (0.05 M $Et_3NH{\cdot}HCO_3$), and pH 9.0 (0.05 M $Na_2B_4O_7$). UV spectra were obtained on Cary Model 15 and Varian Model 635 spectrophotometers. ^1H NMR spectra were obtained on Perkin-Elmer 24B and Nicolet NT 300 WB spectrometers. ^{31}P NMR spectra were obtained on a Nicolet NT 300 WB spectrometer. Chemical shifts are given as parts per million downfield from $SiMe_4$ or 85% H_3PO_4. The sign of ^{31}P shifts are in accord with the 1976 IUPAC convention.[34] Analytical HPLC was performed on a Waters Model 204 chromatograph equipped with a dual solvent-delivery system (Model M-6000A) and a Model 660 programmer. Compounds were analyzed with a Waters RCM-100 unit using a 10-μM particle size Bondapak C_{18} adsorbant in (E) a 10-min linear gradient of 30–60% MeOH in H_2O at a 3 mL/min flow rate, (F) MeOH–H_2O (7:3) at 3 mL/min, (G) a 10-min linear gradient of 10–45% MeOH in 0.1 M KH_2PO_4–0.025 M $(n\text{-Bu})_4NHSO_4$ (pH 5) at 3 mL/min, (H) 25% MeOH in 0.1 M KH_2PO_4–0.0025 M $(n\text{-Bu})_4NHSO_4$ (pH 5) at 2 mL/min, (J) MeOH–H_2O (6:4) at 2 mL/min, (K) a 20-min linear gradient of 40–75% MeOH in H_2O at 2 mL/min, (L) a 20-min linear gradient of 0–30% MeOH in 0.01 M NH_4OAc (pH 7.4) at 2 mL/min, (M) a 20-min linear gradient of 0–30% methanol in 0.1 M KH_2PO_4–0.025 M $(n\text{-Bu})_4NHSO_4$ (pH 5) at 2 mL/min. Preparative HPLC was performed at 100 mL/min on a Waters Prep LC/System 500A equipped with two Prep Pak-500/C_{18} cartridges. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, TN, and were within ±0.4% of theoretical values unless stated otherwise. All compounds were dried at 25 °C over P_2O_5 except the **2a,b** epimeric mixture, which was dried at 50 °C for 2 days. Fast atom bombardment mass spectra were obtained on a Kratos MS-50 double-focusing mass spectrometer, equipped with a Kratos DS-55 data system. Samples were deposited in aqueous solution in a matrix of thioglycerol on a copper probe tip.

Stability of ATP in Buffers Containing Mercuric Acetate. A solution of ATP (60 mg of disodium salt; 0.096 mmol) and $Hg(OAc)_2$ (223 mg, 7.2 equiv) in water or in 0.5 M sodium acetate, pH 4 or pH 5 (1 mL), was stored in the dark at 22 °C. After 24 h, H_2S was passed through the reaction mixture for 5 min and the precipitate was removed by centrifugation. HPLC analysis of the supernate showed that the AMP–ADP–ATP ratio was 1:2:1 in the aqueous reaction mixture and 1:1.2:1 in the pH 5 reaction mixture, whereas in the pH 4 reaction mixture only ATP was detected.

5′-(*tert*-Butylthio)-5′-deoxy-5′-[(diphenoxyphosphinyl)methyl]-2′,3′-*O*-isopropylideneadenosine (Mixture of 5′ Epimers, 2a + 2b). A solution of NaOMe (3.5 mmol) in MeOH was evaporated to dryness under reduced pressure and the residue was stirred with *tert*-butyl mercaptan (60 mL) at 22–24 °C until solid dissolved (20 min). A solution of 1 (4 g, 7.46 mmol) in dry DMF (60 mL) was added, and the solution was stirred at room temperature until the reaction was complete (30 min) as indicated by HPLC (solvent G) and TLC (solvent A). CO_2 was passed through the reaction mixture until $NaHCO_3$ formation was complete. *tert*-Butyl mercaptan was removed under reduced pressure, finally by coevaporation with acetone. DMF was then removed in vacuo and the residue was evaporated with acetone and finally with $CHCl_3$. A solution of the residue in $CHCl_3$ was applied to a column (5 × 42 cm) of silica gel 60H (300 g). Elution with $CHCl_3$–CH_3CN (2:1) gave a mixture of the 5′(*S*) and 5′(*R*) isomers **2a** and **2b** (3 g) in a ratio of 2:3 as indicated by HPLC and NMR. Upon removal of solvent in vacuo at 50 °C for 2–3 h, the **2a–2b** mixture formed a white solid that showed only two components on HPLC (Table I): ^1H NMR (60 MHz, $CDCl_3$) δ 1.4 (9 H, s, $SCMe_3$), 1.4 and 1.6 (3 H, s, CMe_2), 2.6 (2 H, m, H-6′), 3.6 (1 H, m, H-5′), 4.7 (1 H, m, H-4′), 5.2 (2 H, m H-2′ and H-3′), 6.15 (1 H, d, H-1′), 6.5 (2 H, s NH_2-6), 7.2 (10 H, s, phenyl), 8.0 and 8.1 (total 1 H, s, H-8), 8.25 and 8.3 (total 1 H, s H-2); UV λ_{max} (MeOH) 261 nm (ε 14 800). Anal. ($C_{30}H_{36}N_5O_6PS$) C, H, N, P, S.

5′-Deoxy-5′-[(diphenoxyphosphinyl)methyl]-2′,3′-*O*-isopropylidene-5′-mercaptoadenosine (Mixture of 5′ Epimers, 3a + 3b). Mercuric acetate (640 mg, 2 mmol) was added to a solution of **2a + 2b** (180 mg, 0.29 mmol) in DMF–H_2O (3:1) (8 mL) followed by acetic acid (90 μL) to dissolve the suspension. The solution was stirred at 22 °C in the dark and the reaction was followed by HPLC. After 0.5 h a precipitate started to form.

(34) *Pure Appl. Chem.* **1976**, *45*, 217.

The reaction was complete after 24 h as judged by HPLC. H_2S was passed into the mixture until precipitation of HgS was complete. The solid was filtered onto Celite and washed with acetonitrile. The combined filtrate and washings were evaporated, and residual acetic acid was removed by coevaporation with toluene. The residue was chromatographed on two preparative TLC plates with $CHCl_3$–CH_3CN (1:1.5) to give **3a + 3b** (100 mg), λ_{max} (MeOH) 261 nm, as a gum that showed only two UV-absorbing components on HPLC (Table I): ^1H NMR (60 MHz, $CDCl_3$) δ 1.4 and 1.6 (3 H, s CMe_2), 2.6 (2 H, m, H-6′), 3.7 (2 H, m, H-5′ and SH-5′), 4.3 (1 H, m, H-4′), 4.9–5.1 (2 H, m, H-2′ and H-3′), 6.0 (1 H, d, H-1′), 6.3 (2 H, s, NH_2-6), 7.2 (10 H, m, phenyl), 7.8, 7.9 (total 1 H, s, H-8), 8.2 (1 H, s, H-2).

5′-(*tert*-Butylthio)-5′-deoxy-5′-[(dihydroxyphosphinyl)methyl]adenosine (Mixture of 5′ Epimers, 4a + 4b). NaOH (2 N, 11 mL) was added to a solution of **2a + 2b** (2.5 g, 4. mmol) in THF (40 mL), and the turbid mixture was stirred at 22 °C for 4 h. Volatiles were removed in vacuo. A solution of the residue in aqueous 90% trifluoroacetic acid (70 mL) was stored at 22 °C for 10 min. Solvent was immediately removed in vacuo and toluene–ethanol (3:1) was evaporated twice from the residue. This was dissolved in cold water and the solution was neutralized with diluted NH_4OH. The principal UV-absorbing component was the monophenyl ester of **4a + 4b**, R_f 0.65 in system B, HPLC retention times 39 and 45 min in G. After removal of volatiles in vacuo, EtOH was evaporated from the residue. A solution of the residue in 0.1 M Tris-HCl buffer containing 0.2 M magnesium acetate (200 mL) was brought to pH 8.8 with 2 N NaOH. Snake venom phosphodiesterase (800 mg, Sigma Chemical Co., type IV) from *Crotalus atrox* was added, and the solution was stored at 37 °C. After 24 h, more phosphodiesterase (800 mg) was added and incubation was continued until the reaction was complete (24 h). The solution was maintained at 100 °C for 3 min after which precipitated protein was removed by centrifugation and washed twice with water. The combined centrifugate and washings were diluted to 550 mL with water. The solution was brought to pH 3.5 with acetic acid and stirred for 1 h at 22 °C with partially inactivated charcoal[35] (Sigma, acid washed, 20 g) and Celite (20 g). The charcoal was collected on a bed of Celite, then washed with water and extracted with H_2O–EtOH (1:1, 1L) containing 3 mL of NH_4OH. The extracts were concentrated and applied to a DEAE (HCO_3^-) cellulose column (4 × 30 cm). Elution with a linear gradient of 0.0–0.15 M aqueous $Et_3NH{\cdot}HCO_3$ (4 L) gave the triethylammonium salt of **4a + 4b**, which was dissolved in sufficient MeOH so that on addition of 1 M NaI in MeOH (2.5 equiv) no precipitate formed. The solution was evaporated to a minimum volume and diluted with acetone (20 volumes) to precipitate the disodium salt. The precipitate was washed with acetone and dried in vacuo over P_2O_5 to give the disodium salt of **4a + 4b** (1.48 g, 80%), which contained two isomers as shown by NMR and was homogeneous in the systems of Table I: ^1H NMR (60 MHz, D_2O) δ 1.0 and 1.2 (total 9 H, s, $SCMe_3$), 2.0 (2 H, m, H-6′), 3.1 (1 H, m, H-5′), 4.0–4.9 (3 H, m, masked by H_2O, H-2′, H-3′, H-4′), 5.85 (1 H, d, J = 4 Hz, H-1′), 7.9, 8.1, and 8.2 (total 2 H, s, H-2 and H-8); UV max 261 nm (ϵ 15 100) at pH 7.

5′-(*tert*-Butylthio)-5′-deoxy-5′-[[(hydroxypyrophosphoroxy)phosphinyl]methyl]adenosine (Mixture of 5′ Epimers, 5a + 5b). To a solution of **4a + 4b** (0.5 mmol) in DMF (5 mL) was added 1,1-carbonyldiimidazole (400 mg, 2.5 mmol). After 4 h at 22 °C, MeOH (0.17 mL) was added, and the solution was kept an additional 0.5 h at 22 °C. Bis(tri-*n*-butylammonium)-pyrophosphate (2.5 mmol) in DMF (5 mL) was added and the mixture stirred at 22 °C for 16 h. The precipitate was removed by centrifugation and washed with DMF. The combined DMF solutions were evaporated. The residue was dissolved in water (25 mL) and chromatographed at 5 °C on a column (4 × 25 cm) of DEAE (HCO_3^-) cellulose using a linear gradient of aqueous $NEt_3H{\cdot}HCO_3$ (0–0.4 M, 4 L). Fractions containing **5a + 5b** (4000 OD_{260} units, 53%) were combined, evaporated, and converted to the tetrasodium salts with an excess of NaI in MeOH–acetone (see the preparation of **4a + 4b**). HPLC analysis (Table I) showed only two UV-absorbing components; the more polar component comprised ca. 60% of the mixture. Anal. ($C_{15}H_{22}N_5O_{12}P_3SNa_4{\cdot}2H_2O{\cdot}MeOH$) C, H, N.

5′-Deoxy-5′-[[(hydroxypyrophosphoroxy)phosphinyl]methyl]-5′-mercaptoadenosine (Mixture of 5′ Epimers, 6a + 6b). These were prepared from **5a + 5b** in apparently quantitative yield (based upon UV spectrophotometric and HPLC analysis) using the conditions (see above) under which ATP proved stable. After removal of HgS, the solution was evaporated to dryness under reduced pressure (bath at 25 °C) and the residue, which contained sodium salts of **6a** and **6b** and sodium acetate, was stored under argon at −20 °C. HPLC analysis (Table I) showed only two UV-absorbing components of which the more polar comprised about 60% of the total. This material was used directly in the experiments aimed at effecting S-alkylation.

***S*-[5′-Deoxy-5′-[(diphenoxyphosphinyl)methyl]-2′,3′-*O*-isopropylideneadenosyl-5′]-L-homocysteine, 5′ Epimers (7a,b).** To an aqueous solution (40 mL) of freshly prepared sodium salt of L-homocysteine (18 mmol),[36] adjusted to pH 8.5 with 50% HI, was added 1 (3.2 g, 6 mmol) in THF (40 mL). HPLC analysis showed the reaction to be complete after 16 h at 22 °C. The mixture was evaporated and the residue suspended in MeOH–H_2O (3:1, 80 mL). The solid was filtered off and washed with MeOH–H_2O (25 mL). The filtrate was evaporated to a white foam, which was purified by preparative HPLC (MeOH–H_2O, 57:43, one recycle) to give 1.22 g (38%) of **7a** (first compound eluted from column), mp >200 °C (chars). Anal. ($C_{30}H_{35}N_6O_8PS{\cdot}2H_2O$) C, H, N. Subsequent elution gave 1.30 g (40.6%) of **7b**, mp 216–219 °C dec. Both compounds appeared homogeneous in two HPLC systems (Table I).

***S*-[5′-Deoxy-5′-[[bis(benzyloxy)phosphinyl]methyl]-2′,3′-*O*-isopropylideneadenosyl-5′]-L-*N*-(*tert*-butyloxycarbonyl)homocysteine Methyl Ester, 5′ Epimers (10a,b).** To compound **7a** or **7b** (335 mg, 0.5 mmol) in benzyl alcohol (20 mL) was added 1 M sodium benzoxide in benzyl alcohol (2 mL). After 0.5 h at 22 °C, diethyl ether (100 mL) was added and CO_2 was bubbled through the solution to give neutrality. The gelatinous mass was filtered and the filtrate was extracted with water (2 × 150 mL). The combined aqueous solutions were extracted with ether (3 × 200 mL) and then evaporated to dryness in vacuo. HPLC (Table I) showed the residue to contain **8a** or **8b** as a single component. The residue was dried by several additions and evaporations of dry DMF and was then suspended in DMF (20 mL), and NEt_3 (73 μL, 0.55 mmol) and di-*tert*-butyl pyrocarbonate (138 mg, 0.63 mmol) were added.[37] After 1 h at 22 °C, the solvent was evaporated in vacuo and the residue was partitioned between EtOAc (100 mL) and H_2O (50 mL). The mixture was cooled to 0 °C and the aqueous layer was adjusted to pH 2 with a dilute solution of $KHSO_4$. The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 50 mL). The EtOAc extracts were dried ($MgSO_4$) and evaporated to a white foam. HPLC analysis (Table I) showed **9a** or **9b** as a single component. Anal. (**9a**) ($C_{37}H_{47}N_6O_{10}PS{\cdot}H_2O$) C, H, N.

A solution of the above **9a** or **9b** in THF (30 mL) was treated at room temperature with an excess of ethereal diazomethane. After evaporation of volatiles, the residue was purified by silica gel flash chromatography[38] (EtOAc–acetone, 7:3) yielding **10a** (281 mg, 69.2% from **7a**) as a noncrystalline foam or **10b** (300 mg, 74.2%) also as a noncrystalline foam; **10a,b** were homogeneous (R_f 0.35) on TLC in EtOAc–acetone (7:3). ^1H NMR (60 MHz, $CDCl_3$): **10a**, δ 8.15 (s, 1, H-2), 7.83 (s, 1, H-8), 7.15 (s, 10, Ar), 6.55 (br s, 2, NH_2), 5.98 (s, 1, H-1′), 5.65 (br s, 1, NH), 5.20 (m, 2, H-2′,3′), 4.80 (d, 4, J_{POCH} = 8 Hz, CH_2 Ar), 4.20 (m, 2, H-4′, H-9′), 3.59 (s, 3, OCH_3), 3.15 (m, 1, H-5′), 2.60 (m, 4, H-6′,6″,7′,7″), 2.01 (m, 2, H-8′,8″), 1.53 and 1.38 (s, 15, CMe_2 and CMe_3); **10b**, δ 8.12 (s, 1, H-2), 7.89 (s, 1, H-8), 7.18 (s, 10, Ar), 6.36 (br s, 2, NH_2) 5.92 (d, 1, $J_{1',2'}$ = 3.5 Hz, H-1′), 5.47 (br s, 1, NH), 5.02 (m, 2, H-2′, H-3′), 4.90 (d, 4, J_{POCH} = 8 Hz, CH_2 Ar), 4.32 (m, 2, H-4′, H-9′), 3.60 (s, 3, OCH_3), 3.13 (m, 1, H-5′), 2.54 (m, 4, H-6′,6″, H-7′,7″), 1.95 (m, 2, H-8′,8″), 1.53 and 1.40 (s, 15, CMe_2 and CMe_3). Anal. (**10a**) ($C_{38}H_{49}N_6O_{10}PS{\cdot}H_2O$) C, H, N.

***S*-[5′-Deoxy-5′-[[(hydroxypyrophosphoroxy)phosphinyl]methyl]adenosyl-5′]-L-homocysteine, 5′ Epimers (14a,b).**

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A solution of **10a** (227 mg, 0.28 mmol) in acetic acid (10 mL) containing palladium black (420 mg) was shaken with hydrogen at 40 psi for 2.5 h, filtered, and evaporated to yield 169 mg (96%) of **11a** as a white solid which was homogeneous on HPLC and HVE (Table I). To a solution of **11a** in H_2O–pyridine (5 mL, 1:1) was added tri-*n*-butylamine (64 μL, 0.27 mmol). The solution was kept at room temperature for 10 min and evaporated. The residue was dried and dissolved in HMPA (4 mL) and to this was added 1,1′-carbonyldiimidazole (216 mg, 1.35 mmol). The solution was stirred for 1 h at 22 °C when paper electrophoresis (pH 7.6) showed conversion to a monoanionic species (mobility 0.32 vs. 0.67 for **11a**). MeOH (89 μL) was added, and after 0.5 h at 22 °C bis(tri-*n*-butylammonium) pyrophosphate (1.35 mmol) in HMPA (3.4 mL) was added and the mixture stirred for 18 h. The precipitated inorganic pyrophosphate was removed by centrifugation and washed with a small volume of HMPA. The HMPA solutions were combined, diluted with cold water (25 mL), and chromatographed on a column (3 × 25 cm) of DEAE (HCO_3^-) cellulose using a linear gradient of $NEt_3H{\cdot}HCO_3$ (0–0.4 M, 4L). Fractions containing **12a** (1700 OD_{260} units, 42%) were combined and evaporated. The residue was dissolved in aqueous 90% TFA (5 mL), and after 10 min at 22 °C volatiles were evaporated in vacuo. Toluene was evaporated twice from the residue to remove TFA. The residue was dissolved in 0.25 mL of buffer, pH 10.4, derived from 0.038 M $NaHCO_3$ and 0.025 M NaOH. Paper electrophoresis (pH 3.5) and PEI cellulose TLC (1 M LiCl) showed the reaction was complete after 6 h at 22 °C. The solution was chromatographed on a DEAE (HCO_3^-) cellulose column (2.5 × 15 cm) using a linear gradient of $NEt_3H{\cdot}HCO_3$ (0–0.4 M, 2 L). Fractions containing **14a** (1340 OD_{260} units, 34%) were combined, evaporated, and converted to the tetrasodium salt (40 mg) with excess of NaI (see the preparation of **4a–4b** disodium salts). UV max, pH 2, 257 nm (ε 15100); pH 11, 259 nm (ε 15400). FAB mass spectrum 727 $(M + H)^+$, 706 $(-Na + H)^+$, 684 $(-2Na + H)^+$, 661 $(-3Na + H)^+$, 625 $(-NaPO_3 + H)^+$, 603 $(-Na_2PO_3 + H)^+$. ^1H NMR (300 MHz, D_2O) δ 8.48 (s, 1, H-2), 8.26 (s, 1, H-8), 6.08 (d, J = 5.7 Hz, 1, H-1′), 4.55 (t, J=6.1, 1, H-3′), 4.48 (m, 1, H-4′), 3.85 (t, J=6.4Hz, 1, H-9′), 3.35 (m, 1, H-5′), 2.70 (m, 2, H-7′,7″), 2.25 (m, 2, H-6′,6″), 2.06 (m, 2, H-8′,8″). ^{31}P NMR (121.46 MHz, D_2O) δ 12.85 (d, J = 24.9 Hz, P^α), −9.68 (d, J = 18.7 Hz, P^γ), −23.74 (pseudotriplet, P^β); reported for ATP at pH 8: δ −10.85, −21.34, −5.63 for P^α, P^β, P^γ, respectively;[39] found for the P^α-CH_2-C5′ isostere of ATP[5] (Na_4 salt in D_2O), δ +17.61, −24.26, −11.52 for P^α, P^β, P^γ. Anal. ($C_{15}H_{21}N_6P_3SO_{14}Na_4{\cdot}3H_2O{\cdot}CH_3OH$) C, H, N.

Compound **11b** (0.16 mmol) was converted to **14b** by an identical procedure in 26% yield. Anal. ($C_{18}H_{21}N_6P_3SO_{14}Na_4{\cdot}3H_2O{\cdot}CH_3OH$) C, H, N. UV max, pH 2, 257 nm (ε 15000); pH 11, 259 nm (ε 15200). ^1H NMR (300 MHz, D_2O) δ 8.26 (s, 1, H-2), 8.18 (s, 1, H-8), 6.06 (d, J = 6 Hz, 1, H-1′), 4.50 (m, 1, H-4′), 3.83 (t, J = 6.5 Hz, 1, H-9′), 3.42 (m, 1, H-5′), 2.68 (m, 2, H-7′,7″), 2.15 (m, 4, H-6′,6″, H-8′,8″). ^{31}P NMR (121.46 MHz, D_2O) δ 13.11 (d, J = 25.1 Hz, P^α), −9.76 (d, J = 21.08 Hz, P^γ), −23.72 (dd, J = 20.2 Hz, P^β).

***P*$^\beta$,*P*$^\gamma$-Imido Isostere 15a of Adduct 14a.** To a solution of the tri-*n*-butylammonium salt of **11a** (50 μmol) in DMF (2 mL) was added 1,1′-carbonyldiimidazole (40 mg, 250 μmol). After 1 h at 22 °C, MeOH (17 μL) was added. The DMF was evaporated in vacuo after 0.5 h and the residue was extracted several times with dry ether to remove most of the imidazole. The residual oil was dissolved in DMF (1 mL) and added to a solution of the tri-*n*-butylammonium salt of imidodiphosphate (500 μmol) in DMF (2 mL). Paper electrophoresis (pH 6.8) showed the reaction was complete after 4 h at 22 °C. A solution of imidazole in DMF (1 mL, 1 M) was added. The resulting precipitate was removed by centrifugation and washed with a small volume of DMF. The DMF solutions were combined and evaporated in vacuo. The residue was dissolved in H_2O (10 mL) and applied to a column of C_{18} silica gel (1 × 15 cm). This was washed with H_2O (100 mL) to remove imidodiphosphate. Water–methanol (3:1, 100 mL) eluted **13a** (570 OD_{260} units; 77%); removal of volatiles in vacuo gave **13a** as a gum homogeneous by HVE at pH 7.5. The gum was dissolved in 90% TFA (2 mL), and after 10 min at 22 °C volatiles were removed in vacuo. Toluene was evaporated twice from the residue to remove residual TFA. A solution of the residue in 80 μL of the $NaHCO_3$–NaOH buffer (pH 10.4) used in the synthesis of **14a,b** was kept for 6 h at 22 °C when paper electrophoresis (pH 6.8) and PEI cellulose TLC (1 M LiCl) showed that hydrolysis was complete. The solution was chromatographed on a DEAE (HCO_3^-) cellulose column (2.5 × 15 cm) with a linear gradient of $Et_3NH{\cdot}HCO_3$ (0–0.4 M, 2 L). Fractions containing **15a** (320 OD_{260} units, 43%) were combined, evaporated, and converted to the tetrasodium salt in the usual manner. UV max, pH 2, 258 nm (ε 15200); pH 11, 259 nm (ε 15500); (ε values calculated for a tetrahydrated tetrasodium salt of **15a**). ^1H NMR (300 MHz, D_2O) δ 8.46 (s, 1, H-2), 8.24 (s, 1, H-8), 6.07 (d, J = 5.7 Hz, 1, H-1′), 4.54 (t, J = 6 Hz, 1, H-3′), 4.44 (m, 1, H-4′), 3.84 (t, J = 6.3 Hz, 1, H-9′), 3.33 (m, 1, H-5′), 2.69 (m, 2, H-7′,7″), 2.24 (m, 2, H-6′,6″), 2.05 (m, 2, H-8′,8″). ^{31}P NMR (121.46 MHz, D_2O) δ 13.02 (d, J = 24.8, P^α), −3.16 (s, P^γ), −12.28 (d, J = 22.3, P^β). Found for 5′-adenylyl imidodiphosphate tetralithium salt (Sigma) in D_2O: δ −11.75, −8.4, −1.84 for P^α, P^β, and P^γ, respectively.

***S*-[5′-Deoxy-5′-[[(hydroxypyrophosphoroxy)phosphinyl]methyl]adenosyl-5′]-L-homocysteine Sulfoxides (16a).** To a solution of **14a** tetrasodium salt (8 μmol) in H_2O (1 mL) was added 30% H_2O_2 (3 μL). HPLC showed that the reaction was complete after 4 days at 4 °C. Excess H_2O_2 was destroyed by hydrogenation over palladium black (2 mg). After filtration, **16a** was obtained in quantitative yield as determined spectrophotometrically. The water was evaporated in vacuo to give the tetrasodium salts of the two diastereomers of **16a** as a white solid which was homogeneous in the five analytical systems listed in Table I. λ_{max} was 259 nm at pH 7.5. FAB mass spectrum, 743 $(M + H)^+$, 721 $(-Na + H)^+$, 699 $(-2Na + H)^+$, 677 $(-3Na + H)^+$, 619 $(-Na_2PO_3 + H)^+$. ^1H NMR (300 MHz, D_2O) δ 8.40 (s, 1, H-2), 8.28 (s, 1, H-8), 6.11 (d, J = 4.2 Hz, 0.6, H-1′), 6.08 (d, J = 3.9 Hz, 0.4, H-1′), 3.82 (m, 1, H-9′), 3.64 (m, 1, H-5′), 3.13 (m, 2, H-7′,7″), 2.30 (m, 2, H-6′,6″), 2.10 (m, 2, H-8′,8″). ^{31}P NMR (121.46 MHz, D_2O) δ 11.14 (P^α), −9.72 (P^γ), −23.57 (P^β).

***S*-[5′-Deoxy-5′-[(dihydroxyphosphinyl)methyl]-2′,3′-*O*-isopropylideneadenosyl-5′]-L-*N*-(*tert*-butyloxycarbonyl)-homocysteine Sulfoxide Methyl Ester (17a).** A solution of **11a** (42 mg, 67 μmol) in acetic acid–H_2O (1:1, 10 mL) containing 30% H_2O_2 (8 μL, 72 μmol) was stored for 6 h at 22 °C. Excess of H_2O_2 was destroyed by hydrogenation for 5 min at 40 psi over palladium black (5 mg). Evaporation of solvents after filtration gave **17a** (42 mg, 97%) as an amorphous white powder which was homogeneous with HPLC and paper electrophoresis in the systems of Table I; UV max at pH 7, 260 nm.

Attempted Synthesis of 16a from 17a. Compound **17a** was converted to its pyrophosphoroxy derivative **18a** by the procedures used to convert **11a** to **13a**. By elution from C_{18} silica, **18a** was obtained in 65% yield as a $(n\text{-}Bu)_3NH^+$ salt that was homogeneous by paper electrophoresis at pH 3.5. This material was treated for 10 min at 22 °C with aqueous 90% CF_3CO_2H. Electrophoresis at pH 3.5 showed that the expected pyrophosphoryl phosphonate (a dianion) was not present but rather a neutral species that after deesterification at pH 10.4 for 6 h at 22 °C gave the deblocked monophosphonate **19a**, which migrated as a monoanion at pH 3.5. Treatment of **16a** with 90% CF_3CO_2H for 10 min at 22 °C gave total conversion to the same monophosphonate.

Enzyme Kinetic Studies. MAT-2 and MAT-T preparations were prepared and partially purified as described previously.[1] Enzyme assays were conducted at 37 °C for 10 min in a final volume of 0.1 mL. Each mixture was made up in duplicate. MAT-II and MAT-T were studied in 150 mM KCl–15 mM $MgCl_2$–5 mM dithiothreitol–50 mM Tris–HCl, pH 8.2.[6] L-[*methyl*-^{14}C]Methionine (New England Nuclear Co., 54 Ci/mol) and MgATP were included at the levels specified below and in Table II, footnote *a*. Reactions were started by addition of 10 μL of working enzyme solution (9.5–10.5 × 10^{-6} units of activity; 1 unit gives 1 μmol of product per min) and terminated by addition of 10 μL of 4 N $HClO_4$–10 mM L-methionine after cooling the solution in an ice bath. Each suspension was centrifuged and 50 μL of supernatant was applied to a 2.3-cm disk of phosphocellulose paper. Disks were washed as described,[29] then immersed in a toluene solution of phosphors and counted in a Packard liquid scintillation spectrometer (Model 2425). Blanks were provided by incubations carried out in the absence of ATP. Reaction

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velocities were linear for at least 30 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Inhibition studies were made with six to eight levels of MgATP or L-methionine in the range 0.5–4.0 × K_M for each of two inhibitor levels that were in the range 1–5 × K_M and for control mixtures lacking inhibitor. Inhibitors were tested as their 1:1 Mg complexes formed by admixture of stock solutions with equimolar amounts of $MgCl_2$. Inhibition constants (K_i values) were obtained to within ±15% from replots of inhibitor concentrations vs. slopes or intercepts on the vertical axis of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear, as were the replots.

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Registry No. 1, 89301-78-0; **2a**, 101249-43-8; **2b**, 101249-44-9; **3a**, 101249-45-0; **3b**, 101249-46-1; **4a** · 2Na, 101249-47-2; **4a** monophenyl ester, 101249-48-3; **4a** · Et_3N, 101249-50-7; **4b** · 2Na, 101249-51-8; **4b** monophenyl ester, 101249-52-9; **4b** · Et_3N, 101249-54-1; **5a** · 4Na, 101249-55-2; **5b** · 4Na, 101249-56-3; **6a** · XNa, 101249-57-4; **6b** · XNa, 101249-58-5; **7a**, 101249-59-6; **7b**, 101249-60-9; **8a**, 101249-61-0; **8b**, 101399-22-8; **9a**, 101249-62-1; **9b**, 101249-63-2; **10a**, 101249-64-3; **10b**, 101249-65-4; **11a**, 101249-66-5; **11a** · Bu_3N, 101249-67-6; **11b**, 101249-68-7; **12a**, 101249-69-8; **13a**, 101249-70-1; **14a**, 101249-71-2; **14a** · 4Na, 101314-63-0; **14b**, 101249-72-3; **14b** · 4Na, 101249-73-4; **15a**, 101249-74-5; **15a** · 4Na, 101249-75-6; **16a** (isomer 1), 101249-76-7; **16a** (isomer 2), 101399-23-9; **17a**, 101249-77-8; **18a** · Bu_3N, 101249-79-0; **19a**, 101249-80-3; *tert*-butyl mercaptan, 75-66-1; bis(tri-*n*-butylammonium) pyrophosphate, 5975-18-8; L-homocysteine sodium salt, 73292-23-6; di-*tert*-butyl pyrocarbonate, 24424-99-5; imidodiphosphate tri-*n*-butylammonium salt, 101249-81-4; methionine adenosyltransferase, 9012-52-6.

Synthesis and α-D-Glucosidase Inhibitory Activity of N-Substituted Valiolamine Derivatives as Potential Oral Antidiabetic Agents[1]

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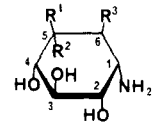
Various kinds of N-substituted valiolamine derivatives, including compounds **23a**, **24a**, and **34a**, which are structurally analogous to the key pseudodisaccharides (**25a** and **26a**) of naturally occurring oligosaccharide α-D-glucosidase inhibitors, have been synthesized and estimated by the measure of inhibitory activity against porcine sucrase and maltase. The N-substituted valiolamine derivatives evaluated in this study have been found to be more potent than the corresponding N-substituted valienamine derivatives as well as the parent valiolamine. It is noteworthy that even simple N-substituted valiolamine derivatives such as *N*-[2-hydroxy-1-(hydroxymethyl)ethyl]-, *N*-[(1*R*,2*R*)-2-hydroxycyclohexyl]-, and *N*-[(*R*)-(−)-β-hydroxyphenethyl]valiolamine (**6**, **8a**, and **9a**) have the stronger α-D-glucosidase inhibitory activity against porcine intestinal maltase and sucrase than naturally occurring oligosaccharide α-D-glucosidase inhibitors.

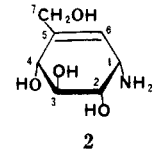
Since the middle 1970s, quite a few pseudooligosaccharides of microbial origin that exhibit a very pronounced inhibitory effect on intestinal α-D-glucosidase have been reported,[2-5] and some of them have aroused medical interest in the treatment of metabolic disease such as diabetes. In general, these microbial α-D-glucosidase inhibitors have valienamine (**2**)[6] as their key constituent, which was first found in validamycins. As previously reported, **2** itself is an inhibitor for α-D-glucosidase, and some *N*-alkyl- and *N*-aralkylvalienamine derivatives have stronger inhibitory activity against porcine sucrase and maltase than the parent valienamine.[7] These results suggest that the 4,6-dideoxy- and the 4-deoxy-D-glucopyranose units of **25a** and **26a**, found in the naturally occurring pseudooligosaccharide α-D-glucosidase inhibitors, such as the acarboses,[2] trestatins,[3] amylostatins,[4] and adiposins,[5] are not essential to sucrase and maltase inhibitory activity and are substitutable by some other structural unit.

We also found that the valiolamine (**1**),[8,9] (1*S*)-(1-(OH),2,4,5/1,3)-5-amino-1-*C*-(hydroxymethyl)-1,2,3,4-cyclohexanetetrol, has more potent α-D-glucosidase inhibitory activity than the other pseudo amino sugars such as **2**, validamine (**3**),[10] hydroxyvalidamine (**4**),[10] and epi-

Chart I



	R^1	R^2	R^3
1	$-^7CH_2OH$	-OH	-H
3	$-^7CH_2OH$	-H	-H
4	$-^7CH_2OH$	-H	-OH
5	-OH	$-^7CH_2OH$	-H



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(1) Part of this work and related experimental results are disclosed in the following patent applications: Horii, S.; Kameda, Y.; Fukase, H. (Takeda Chemical Industries, Ltd.), Eur. Pat. Appl. EP 56 194 (1982); *Chem. Abstr.* **1982**, *97*, 198515r; and EP 89 812 (1983); *Chem. Abstr.* **1984**, *101*, 38779c.

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