followed by H_2O (1 × 25 mL). After drying over $MgSO_4$ the organic layer was evaporated to a tan solid. The crude product was recrystallized from MeOH to yield 317.0 mg (47.8%) of **13**: mp 118–121 °C; ^1H NMR ($CDCl_3$) δ 1.80 (br m, 6 H, tetrahydropyranyl $CH_2CH_2CH_2$), 2.10 (s, 3 H, $COCH_3$), 3.78 (overlapping m and s, 5 H, OCH_3 and THP–OCH_2), 5.28 (br s, 1 H, THP–OCH), 6.73 (dd, 1 H, $J_{6',2'}$ = 2 Hz, $J_{6',5'}$ = 9 Hz, aromatic $H_{6'}$), 7.02 (d, 1 H, $J_{5',6'}$ = 9 Hz, aromatic $H_{5'}$), 7.35 (d, 1 H, $J_{2',6'}$ = 2 Hz, aromatic $H_{2'}$), 7.70 (br s, 1 H, NH); IR (KBr) ν 3236 (m), 2928 (s, CH_2), 1648 (s, C=O), 1468 (m, CH_2), 1233 (s, C–O–C) cm^{-1}; UV (MeOH) λ_{max} 251 nm (ε 13600), 286 (4200); CIMS (CH_4), *m/e* 266 (MH^+), 182 ($C_9H_{11}NO_3$), 181 ($C_9H_{11}NO_3$) or ($MH^+ - C_5H_9O$), 139 ($C_9H_{11}NO_3$–CH_2CO), 85 ($C_5H_8OH^+$), 43 (CH_3CO^+).

3′-Methoxy-4′-(α-tetrahydropyranyloxy)-*N*-methylacetanilide (14). To a stirred solution of **13** (202.5 mg, 0.76 mmol) in dry Me_2SO (1 mL) was added pulverized KOH (174.0 mg, 3.10 mmol). The reaction mixture was stirred at room temperature for 5 min at which point a solution of CH_3I (0.43 g, 3.05 mmol) in Me_2SO (2 mL) was added over a 2-min period. After stirring for 90 min the reaction mixture was quenched with H_2O (10 mL) and then extracted with Et_2O (5 × 10 mL). The organic layers were combined, dried over $MgSO_4$, and evaporated to a white solid. Recrystallization from MeOH gave 72.9 mg (34.3% yield) of 14 as a white solid: mp 109–111 °C; ^1H NMR ($CDCl_3$) δ 1.80 (br m, 9 H, overlap of $COCH_3$ and tetrahydropyranyl $CH_2CH_2CH_2$), 3.17 (s, 3 H, NCH_3), 3.80 (m, 5 H, OCH_3 and THP–OCH_2), 5.33 (m, 1 H, THP–OCH), 6.67 (m, 2 H, aromatic H), 7.20 (m, 1 H, aromatic H); IR (KBr) ν 2945 (m, CH_2), 1650 (s, C=O), 1245 (s, C–O–C) cm^{-1}; UV (MeOH) λ_{max} 233 nm (ε 9400), 280 (2900); CIMS (CH_4), *m/e* 280 (MH^+), 196 ($C_{10}H_{13}NO_3$ + H), 195 ($MH^+ - C_5H_3O$), 85 ($C_5H_8OH^+$), 43 (CH_3CO^+).

3′-Methoxy-4′-hydroxy-*N*-methylacetanilide (4b). The THP ether was removed by the method of Beier and Mundy.[27] A mixture of **14** (212.3 mg, 0.76 mmol) and Dowex 50W (H^+) resin (325.0 mg) were stirred together in MeOH (2 mL) for 1 h at room temperature. The reaction mixture was then filtered and evaporated to a yellow solid under reduced pressure. The crude product was recrystallized twice from MeOH (charcoal) to yield 19.2 mg (13.2%) of **4b** as a white crystalline solid: mp 135–136 °C; ^1H NMR (CH_3OH-d_4) δ 1.87 (s, 3 H, $COCH_3$), 3.22 (s, 3 H, NCH_3), 3.88 (s, 3 H, OCH_3), 6.68 (dd, 1 H, $J_{6',2'}$ = 2.58 Hz, $J_{6',5'}$ = 7.74 Hz, aromatic $H_{6'}$), 6.78 (d, 1 H, $J_{2',6'}$ = 2.58 Hz, aromatic $H_{2'}$), 6.85 (d, 1 H, $J_{5',6'}$ = 8.59 Hz, aromatic $H_{5'}$); IR (KBr) ν 2500–3500 (m, OH), 1619 (s, C=O), 1601 (s), 1529 (s), 1459 (s), 1323 (m), 1284 (s), 1249 (s), 1220 (s), 1175 (s), 1129 (m) cm^{-1}; UV (MeOH) λ_{max} 235 nm (ε 8000), 283 (3200); EIMS, *m/e* 195 (M^+), 153 (M – CH_2CO), 152 (M – CH_3CO), 138 (M – CH_2CO – CH_3), 137 (M – CH_3CO – CH_3), 43 (CH_3CO^+). Calcd for $C_{10}H_{13}NO_3$, 195.0892. Found, 195.0891.

Registry No. 4, 29121-34-4; 4 (1-^{14}C), 102683-54-5; **4A**, 54826-77-6; **4B**, 102683-60-3; **5**, 39495-15-3; **6**, 16375-90-9; **7**, 182-55-8; **8**, 64179-40-4; **9**, 102683-55-6; **10**, 102683-56-7; 11, 3251-56-7; **12**, 102683-57-8; **13**, 102683-58-9; 14, 102683-59-0; 4-$H_3CNHC_6H_4OH{\cdot}H_2SO_4$, 55-55-0; 4-$HOC_6H_4NH^{14}COCH_3$, 60218-46-4; 4-$H_2NC_6H_4OH$, 123-30-8; 4-$H_2NC_6H_4SO_3H$, 121-57-3; 4-$^+N_2C_6H_4SO_3H{\cdot}Cl^-$, 6118-33-8; 2-$HOC_6H_4CH_3$, 95-48-7; 4-hydroxy-2-methylaniline, 2835-99-6; 4-hydroxy-3-methylaniline, 2835-96-3; catechol, 120-80-9; cyclohexanone, 108-94-1; 3,4-dimethoxynitrobenzene, 709-09-1.

(27) Beier, R.; Mundy, B. P. *Synth. Commun.* **1979**, *9*, 271.

Isozyme-Specific Enzyme Inhibitors. 12.[1] *C*- and *N*-Methylmethionines as Substrates and Inhibitors of Methionine Adenosyltransferases of Normal and Hepatoma Rat Tissues

Hong Lim, Francis Kappler, Ton T. Hai, and Alexander Hampton*

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received October 15, 1985*

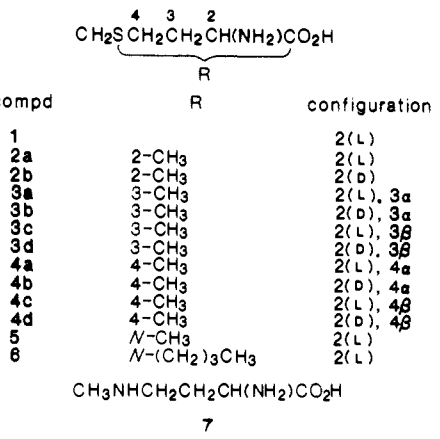
The 2-, 3-, and 4-mono-*C*-methyl derivatives of D,L-methionine (Met) have been resolved into the 10 possible enantiomeric forms having the configurations 2-Me-D, 2-Me-L, 3(α or β)-Me-D, 3(α or β)-Me-L, 4(α or β)-Me-D, and 4(α or β)-Me-L (the α designation was given to enantiomeric pairs that had higher R_f values on silica gel chromatograms than their diastereomeric counterparts). All compounds were weak, poorly selective inhibitors of the rat M-2 (normal tissue) and M-T (Novikoff ascitic hepatoma) variants of Met adenosyltransferase. Kinetic analysis of the three most effective showed them to be competitive inhibitors with respect to Met with both variants; the strongest inhibition (K_M(Met)/K_i = 0.03) was that of M-T by 3β-Me-L-Met. The Me-Met enantiomers had low substrate efficiencies (V_{max}/K_M) in the range (0.5–2.2) × 10^{-4} that of L-Met with M-2 and (0.2–1.3) × 10^{-3} with M-T among seven compounds studied. At a 4 mM level, seven of the enantiomers were converted to adenosylmethionine derivatives more rapidly by M-T than by M-2. Among these, 2-Me-L-Met, 3α-Me-L-Met, 3α-Me-D-Met, and 4β-Me-D-Met had little or no substrate activity with M-2. These differences in substrate specificity are potentially exploitable in the design of compounds with selective toxicity for rat tumor tissue. *N*-Me- and *N*-(*n*-Bu)-Met, and the Met analogue in which NH is substituted for S, were weak inhibitors of M-T and M-2 and showed no substrate activity at a level of 4 mM.

The isozyme composition of mammalian normal and neoplastic tissues indicates, on grounds discussed previously,[2] that isozyme-specific inhibitors are potentially useful as progenitors of new antineoplastic agents. In previous work in this series, we have studied several approaches to the design of such inhibitors. These studies were carried out with four enzymes, each of which occurs as a variant that is of interest as a possible target in cancer chemotherapy. It was found that monosubstituted substrate derivatives that bear short substituents at various positions frequently possess substrate or inhibitor properties that are isozyme selective.[3] In addition, it was found that such derivatives can sometimes serve as starting points in the development of compounds with enhanced selectivity and/or inhibitory potency.[4–6] Recently,[3] we described isozyme-selective inhibitory effects produced by monosubstituted adenosine 5′-triphosphate (ATP) deriv-

(1) For part 11 of this series see: Kappler, F.; Hai, T. T.; Cotter, R. J.; Hyver, K. J.; Hampton, A. *J. Med. Chem.* **1986**, *29*, 1030.
(2) Hampton, A.; Kappler, F.; Maeda, M.; Patel, A. D. *J. Med. Chem.* **1978**, *21*, 1137.
(3) Kappler, F.; Hai, T. T.; Hampton, A. *J. Med. Chem.*, in press.
(4) Hampton, A.; Kappler, F.; Picker, D. *J. Med. Chem.* **1982**, *25*, 638.
(5) Hampton, A.; Chawla, R. R.; Kappler, F. *J. Med. Chem.* **1982**, *25*, 644.
(6) Kappler, F.; Hai, T. T.; Abo, M.; Hampton, A. *J. Med. Chem.* **1982**, *25*, 1179.

0022-2623/86/1829-1743$01.50/0

Chart I



compd	R	configuration
1		2(L)
2a	2-CH3	2(L)
2b	2-CH3	2(D)
3a	3-CH3	2(L), 3α
3b	3-CH3	2(D), 3α
3c	3-CH3	2(L), 3β
3d	3-CH3	2(D), 3β
4a	4-CH3	2(L), 4α
4b	4-CH3	2(D), 4α
4c	4-CH3	2(L), 4β
4d	4-CH3	2(D), 4β
5	N-CH3	2(L)
6	N-(CH2)3CH3	2(L)

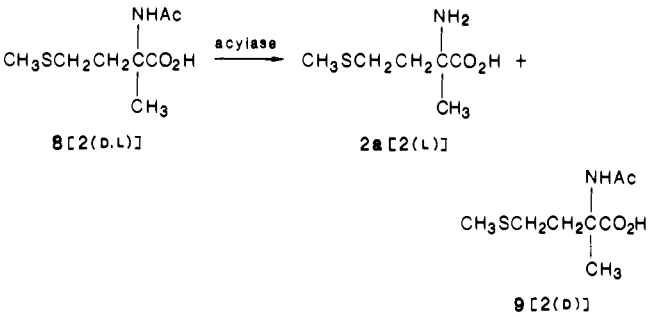
CH3NHCH2CH2CH(NH2)CO2H

7

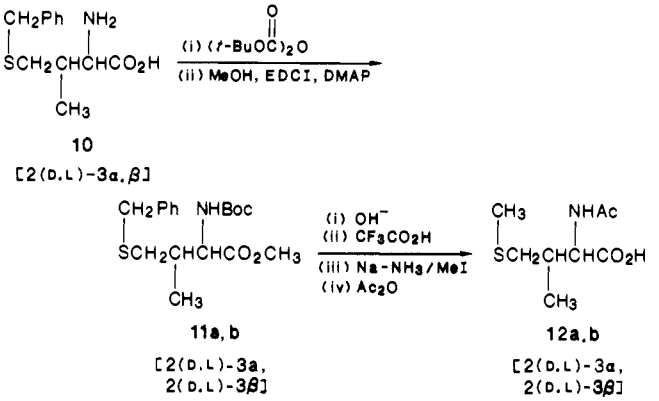
atives with the rat M-2 (normal tissue) and M-T (Novikoff ascitic hepatoma) variants of ATP: L-methionine *S*-adenosyltransferase (EC 2.5.1.6) (MAT), which catalyzes attack of the sulfur of L-methionine (1) on C5′ of ATP to form *S*-adenosylmethionine (AdoMet). We now report substrate and inhibitor properties toward the same two isozymes of the mono-*C*-methyl derivatives **2–4** of D- and L-methionine, of *N*-methyl- and *N*-(*n*-butyl)-L-methionine (**5** and **6**), and of an isostere of L-methionine in which S is replaced by NH (**7**). (See Chart I.) The *C*-methylmethionines had previously been obtained only as mixtures of enantiomers.[7] Described here is the enzymatic resolution of 2-methyl-D,L-methionine, via its *N*-acetyl derivative, into the L and D forms **2a** and **2b**, together with chemical, chromatographic, and enzymatic procedures leading to resolution of 3- and 4-methylmethionines, via derivatives of *S*-benzyl-3- or 4-methylhomocysteine, into their four enantiomers **3a–d** and **4a–d**, respectively. The C3 and C4 configurations of the enantiomers were not established in this study and were designated as either α or β, where α refers to enantiomeric pairs which had higher R_f values on silica gel chromatograms than their diastereomeric (β) counterparts.

It was reported recently that a number of methionine analogues, including 2-methyl-D,L-methionine, showed substrate and/or inhibitor properties that were different with MAT from rat Novikoff solid hepatoma than with MAT from normal rat tissues.[8,9]

Chemistry. 2-Methyl-D,L-methionine was converted to its mono-*N*-acetyl derivative **8** with acetic anhydride in acetic acid, using modifications of a reported procedure for the N-acetylation of methionine.[8] Traces of unacetylated 2-methylmethionines were removed by adsorption onto the sulfonic acid form of a cation-exchange resin, after which the mixed **8** enantiomers were obtained in crystalline form in 80% yield. Treatment of **8** with hog acylase I resulted in selective deacetylation of the L enantiomer. As expected for an α-methyl α-amino acid,[11] the rate was significantly slower than normally encountered in the resolution of *N*-acetylmethionines and other *N*-acetyl α-amino acids by this method. The reaction was stopped after hydrolysis of 30–40% of **8** had taken place, after which the residual **8** was separated from 2-methyl-L-methionine (**2a**) and re-treated with the same amount of the acylase for a similar period of time to ensure that *N*-acetyl-2-methyl-D-methionine (**9**) was free of its L enantiomer. The additional **2a** so obtained possessed the same optical rotation as **2a** produced in the initial action of the acylase on **8**, indicating that hydrolysis of *N*-acetyl-2-methyl-D-methionine (**9**) had not occurred to a detectable extent. This was supported by the finding that the specific rotation of 2-methyl-D-methionine (**2b**), obtained by acidic hydrolysis of **9**, was equal and opposite in sign to that of **2a**.



The four enantiomers **3a–d** of 3-methylmethionine were successfully derived from the previously described *S*-benzyl-3α,β-methyl-D,L-homocysteine (**10**).[12] Thin-layer chromatography on silica gel, using repeated developments, yielded no separation of **10** into pairs of enantiomers, whereas the *N*-acetyl or *N*-benzyloxycarbonyl derivative of **10** did separate, with difficulty, into two such enantiomeric pairs. Slightly better separation was observed with the *N*-*tert*-butyloxycarbonyl (Boc) derivative of **10**, and separability was further improved, and to roughly an equal extent, when the *N*-Boc derivative was converted into either its methyl or isopropyl ester. It was found that **10** could be converted readily, and in high overall yield, into the methyl ester, **11**, of its *N*-Boc derivative by treatment of **10** with di-*tert*-butyldicarbonate[13] followed by treatment of the resulting *N*-Boc derivative with a mixture of methanol, 1-ethyl-3-[(3-dimethylamino)-propyl]carbodiimide, and 4-(dimethylamino)pyridine.[14]



The ^{1}H NMR spectrum of **11** was consistent with the expected structure. Silica gel column chromatography by a dry-column technique was then used to separate the enantiomeric pair (**11a**) of higher R_f value (designated as 2(D,L)-3α) from the pair (**11b**) of lower R_f (designated as 2(D,L)-3β). The methyl ester groups of the separated **11a** and **11b** were hydrolyzed by the action of 1.1 equiv of sodium hydroxide at 22 °C, after which the Boc groups

(7) Zdansky, G. *Ark. Kemi* **1967**, *27*, 447.
(8) Sufrin, J. R.; Lombardini, J. B. *Mol. Pharmacol.* **1982**, *22*, 752.
(9) Lombardini, J. B.; Sufrin, J. R. *Biochem. Pharmacol.* **1983**, *32*, 489.
(10) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 3, p 2149.
(11) Reference 10; Vol. 1, p 748.
(12) Zdansky, G. *Ark. Kemi* **1968**, *29*, 47.
(13) Moroder, L.; Hallett, A.; Wunsch, E.; Keller, O.; Wersin, G. *Z. Physiol. Chem.* **1976**, *357*, 1651.
(14) Dhaon, M. D.; Olsen, R. K.; Ramasamy, K. *J. Org. Chem.* **1982**, *47*, 1962.

Table I. Substrate and Inhibitor (inhib) Activities of Methionine (Met) Derivatives with Normal Tissue (M-2) and Novikoff Hepatoma (M-T) Forms of Rat Methionine Adenosyltransferase

Met derivative		inhib activity				substrate activity							
		inhib by 3 mM Met derivative,[a] %		inhib constant (K_i),[b] mM		[^{14}C]AdoMet deriv from 4 mM Met deriv,[c] cpm		K_M,[d] mM		rel V_{max}		rel V_{max}/K_M	
no.	name and/or configuration	M-2	M-T	M-2	M-T	M-2	M-T	M-2	M-T	M-2	M-T	M-2	M-T
1	L-Met							0.005	0.0156	100	100	2×10^4	6.4×10^3
2a	2(L)	15	11			49	241		21		27		1.3
3a	2(L),3α	15	8.7			38	348		20		37		1.8
3c	2(L),3β	68	65	0.5	0.5	838	701	6.1	1.4	27	12	4.4	8.6
4a	2(L),4α	29	24	2.4	3.2	624	272	10		26		2.6	
4c	2(L),4β	36	23	1.5	2.4	368	343	15		22		1.5	
2b	2(D)	4.8	3.7			41	40						
3b	2(D),3α	17	6.6			−10	191						
3d	2(D),3β	21	15			333	381	29		26		0.9	
4b	2(D),4α	24	15			506	731	14		27		1.9	
4d	2(D),4β	9.7	14			30	173						
5	2(L)	2.9	0			−9	36						
6	2(L)	0	0			−15	13						
7	2(L)	6.6	0			37	33						

[a] The level of Met was 10 μM with M-2 and 32 μM with M-T. Other conditions are given in the Experimental Section. [b] All inhibitions were competitive with respect to Met and gave linear plots of slope vs. inhibitor level. [c] C-Methylated AdoMet derivatives formed in enzyme-catalyzed reactions were labeled with [^{14}C]ATP (see Experimental Section for details). Values are for reactions carried out for 1 h, 37 °C, with 1.6×10^{-5} units of enzyme. Each figure represents counts per minute, in 10 μL, of a reaction mixture, in excess of counts per minute in a control mixture lacking the Met derivative. Control values varied over 52–70 cpm for M-2 and 55–88 cpm for M-T. [d] K_M (Michaelis constant) = substrate level that produces half the maximal velocity (V_{max}).

were removed with trifluoroacetic acid to give, after purification, *S*-benzyl-3α-methyl-D,L-homocysteine and the corresponding 3β enantiomeric pair, each in 93% yield. Reintroduction of the two blocking groups into portions of these products produced, in both cases, material that moved as a single component with the expected R_f value on silica gel TLC, confirming that no racemization at C3 had occurred during the two deblocking operations. The benzyl group in each deblocked enantiomeric pair was replaced by a methyl group, in 80% yield, by a known procedure for the conversion of *S*-benzyl-L-homocysteine to L-methionine.[15] Subsequent N-acetylation under the conditions used with 2-methyl-D,L-methionine then furnished *N*-acetyl-3α-methyl-D,L-methionine (**12a**) and *N*-acetyl-3β-methyl-D,L-methionine (**12b**). Each of these pairs of enantiomers was subjected to the two-stage treatment with hog acylase described above for the *N*-acetyl-2-methylmethionines. Enzymatic deacetylation occurred approximately 4-fold more rapidly, and again appeared to be entirely selective for the L configuration and furnished the four desired enantiomers in chromatographically homogeneous and crystalline form, the specific rotation of 3α-methyl-L-methionine (**3a**) being equal and opposite in sign to that of 3α-methyl-D-methionine (**3b**), and that of 3β-methyl-L-methionine (**3c**) likewise equal and opposite to that of 3β-methyl-D-methionine (**3d**).

The four enantiomers **4a–d** of 4-methylmethionine were also obtained by the above route, starting from the known *S*-benzyl-4α,β-methyl-D,L-homocysteine.[12] Column chromatographic separation of the two pairs of enantiomers proved to be less difficult than in the case of the corresponding 3-methylhomocysteine derivatives **11a,b**. Deacetylation by hog acylase occurred at a similar rate as in the 3-methyl series, and was again selective for the 2(L) configuration. In confirmation of the assigned configurations, 4β-methyl-D-methionine, but not its L enantiomer, was found to be a substrate of D-amino acid oxidase.

N-Methyl-L-methionine (**5**) was prepared from D-methionine via 2(D)-bromo-4-(methylthio)butyric acid by a published procedure for synthesis of *N*-methyl-D-methionine from L-methionine.[16] *N*-(*n*-Butyl)-L-methionine (**6**) was prepared conveniently by the same route. The synthesis of γ-*N*-methyl-L-α,γ-diaminobutyric acid (**7**) has been reported by Yamamoto and his coworkers.[17]

Enzyme Studies. Studies with the rat M-2 (normal tissue) and M-T (hepatoma tissue) forms of MAT (termed MAT-2 and MAT-T in previous work[1,3]) were carried out in the presence of levels of the essential cations K^+ and Mg^{2+} and at a pH value that was the same for both forms and known to be near optimal for their activity.[18,19] Inhibition constants were determined in the presence of a saturating level (2 mM; $14 \times K_M$(ATP)] of ATP that was not of itself inhibitory; in the determination of substrate constants, which employed ^{14}C-labeled ATP, ATP was used more sparingly at $3 \times K_M$(ATP).

Three compounds, all of the L configuration, were sufficiently effective inhibitors to permit determination of their inhibition constants (Table I). All inhibited competitively with respect to L-methionine. The most effective, 3β-methyl-L-methionine (**3c**), was a weak inhibitor (K_M(methionine)/K_i = 0.01 and 0.03, respectively) of M-2 and M-T. All the methionine derivatives were tested, at a concentration of 3 mM, as inhibitors of the conversion of [^{14}C]methionine to [^{14}C]AdoMet catalyzed by the two MAT forms. The methionine level in all determinations was $2 \times K_M$(methionine). Although the level of the test compounds was thereby either 94 or 300 times higher than that of methionine, inhibitions were in general weak. Seven compounds (**2a,b**, **3a,b,d**, and **4b,c**) inhibited M-2 slightly more than M-T; one (**4d**) inhibited M-T slightly more than M-2; and two (**3c**, **4a**) appeared to be nonselective.

Substrate properties of the *C*- and *N*-alkylmethionines were also studied. Rates of conversion of [^{14}C]ATP to

(15) duVigneaud, V.; Patterson, W. I. *J. Biol. Chem.* **1935**, *109*, 97.
(16) Izumiya, N.; Nagamatsu, A.; Ota, S. *Kyushu Mem. Med. Sci.* **1953**, *4*, 1.
(17) Yamamoto, H.; Miyazaki, M.; Hayakawa, T. *Polymer* **1976**, *17*, 429.
(18) Liau, M. C.; Lin, G. W.; Hurlbert, R. B. *Cancer Res.* **1977**, *37*, 427.
(19) Sullivan, D. M.; Hoffman, J. L. *Biochemistry* **1983**, *22*, 1636.

[^{14}C]AdoMet derivatives were determined using a level (4 mM) of each methionine derivative that was high in relation to the K_M value of methionine with M-2 or M-T. The results obtained with identical activity levels of M-2 and M-T are given in Table I. The *N*-alkylmethionines **5** and **6** showed no substrate activity. The amino group of methionine appears to contribute to binding of methionine to MAT,[20,21] and the substrate and inhibitor properties of **5** and **6** suggest steric interference with that contribution, inasmuch as electronic effects of the added alkyl groups can be expected to be relatively small. Interactions of *N*-alkylmethionines with MAT from any biological source have apparently not been described previously; N-formylation or N-acetylation of methionine has, however, been reported to cause partial or total loss of substrate activity.[20] Likewise, no studies with the nitrogen analogue **7** of methionine appear to have been hitherto reported. This analogue showed no significant substrate activity with M-2 or M-T and was a feeble inhibitor of both forms. The isosteric nitrogen of **7** presumably exists predominantly in cationic form at the pH (8.2) of these studies. That no substrate activity (attack of the isosteric N on C5′ of ATP) was detected, despite the superior nucleophilicity of dialkylamine nitrogen over dialkyl thio ether sulfur and the virtual absence in **7** of additional bulk, suggests that **7** may suffer electrostatic repulsion from the methionine site, or is weakly bound as its cationic, unreactive species or in a manner that produces an unfavorable N-C5′ orientation.

Of the 10 *C*-methylmethionines (**2a–4d**), 9 were substrates of M-T and 5 were substrates of M-2. Seven of them, when tested at a level of 4 mM, were converted to AdoMet derivatives more rapidly by M-T than by M-2 (Table I). It was possible to determine K_M and V_{max} values of five substrates with M-2 and of three substrates with M-T, revealing in all cases a low substrate efficiency (V_{max}/K_M) in a range (0.5–2.2) $\times 10^{-4}$ that of methionine with M-2 and (0.2–1.3) $\times 10^{-3}$ with M-T (Table I). Of two compounds (**3c** and **4a**) that formed AdoMet derivatives significantly more rapidly with M-2 under the chosen test conditions, one (**3c**) was found to possess a relatively low K_M value with M-T and a substrate efficiency (V_{max}/K_M) that, resultingly, was 6 times higher with M-T than with M-2. Neither M-T nor M-2 showed a marked preference for the 2(D) or 2(L) configuration, as indicated by the velocity data with 4 mM *C*-methylmethionines and by the K_M and V_{max} values obtained in these studies. It should be noted that the specificity for L-methionine of MAT from various biological sources has been recognized to be only relative.[21]

Four of the *C*-methylmethionines (**2a**, **3a**, **3b**, and **4d**) showed significant substrate activity with M-T but little if any with M-2. The present finding with 2-methyl-L-methionine (**2a**) accords with the report of Sufrin and Lombardini[8] that 2-methyl-D,L-methionine exhibited substrate activity with rat Novikoff solid hepatoma MAT but not with rat liver M-2. It is of interest that in the present studies the enantiomeric 2-methyl-D-methionine (**2b**) exhibited no significant substrate activity with either M-T or M-2. Introduction of a methyl group in the α configuration at C3 of either D- or L-methionine (**3a** and **3b**) produced selective substrate activity with M-T. The same selectivity was shown by 4β-methyl-D-methionine (**4d**) but not by its L enantiomer, **4c**. Since **4d** inhibits M-T more effectively than M-2, the selectivity of **4d** could arise in part from a relatively high affinity for the methionine site of M-T. The remaining selective M-T substrates (**2a**, **3a**, and **3b**), in contrast, inhibit M-T less effectively than M-2. It is uncertain whether the inhibitions produced by the *C*-methylmethionines arise in all cases solely from their absorption to the enzymic methionine sites, although this appeared to be true in all six systems (Table I) that were analyzed kinetically, inasmuch as these were found to involve linear competitive inhibition with respect to methionine. These considerations argue against but do not eliminate the possibility that the selective substrate activities of **2a**, **3a**, and **3b** may arise from selective affinities for the M-T methionine site; on the other hand, they do tend to suggest that other factors may be operative, such as variations in the orientations of *C*-methylmethionines within the M-T and M-2 methionine sites, or selective effects occurring in later stages of the catalytic cycle.

Previously, we studied several series of monosubstituted substrate derivatives in which short substituents had been attached at a variety of substrate atoms and found that such derivatives frequently showed substrate or inhibitor properties that were isozyme selective. The present work, similarly, has shown that 7 of 10 mono-*C*-methylmethionines were converted to AdoMet derivatives more rapidly by M-T than by M-2, and, moreover, that 4 of these compounds had no detectable substrate activity with M-2. The substrate efficiencies of the selective M-T substrates were not high; e.g., the value for **3a** was 0.03% that of methionine. By structural modification of these compounds, however, it may be possible to increase substrate efficiency while retaining selectivity, and, through this approach, to generate compounds that are selectively toxic toward rat neoplastic tissue.

Experimental Section

General. Melting points were determined in capillary tubes and are uncorrected. Optical rotations were measured with a Perkin-Elmer polarimeter, Model 141 (10 cm cell length). ^{1}H NMR spectra were obtained with a Perkin-Elmer 24B spectrometer. Chemical shifts are given as parts per million downfield from $SiMe_4$. TLC was carried out on E. Merck silica gel 60F-254 plates, and components were visualized in iodine vapor or by ninhydrin spray. Systems used were (A) EtOAc–EtOH–aqueous 5% AcOH (5:2.5:1), (B) EtOAc–MeOH–hexane (1:0.1:1), (C) hexane–EtOAc (8:1), (D) EtOAc–MeOH–aqueous 5% AcOH (2:1:0.1), (E) EtOAc–MeOH–aqueous 5% AcOH (5:1:0.5), and (F) EtOAc–MeOH–aqueous 5% AcOH (2:1:0.5). Anion-exchange column chromatography was performed with AG 50W-X8 resin (200–400 mesh) from Bio-Rad. Reverse-phase flash chromatography was carried out with Baker C_{18}-bonded silica (40 μm) with the apparatus described by Still.[22] Modified dry column chromatography[23] was performed with E. Merck Kieselgel 60 (70–230 mesh) silica gel. This was presaturated with 20% v/w of eluting solvent by occasional shaking for 1 h, then packed into a glass column and eluted with the same solvent system; fractions of 5–10 mL were collected. Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, TN, on compounds dried at 78 °C; results were within ±0.4% of theoretical values.

Crystalline D-amino acid oxidase, acylase I (grade III, from hog kidney), di-*tert*-butyldicarbonate, D-methionine, and 2-methyl-D,L-methionine were purchased from Sigma Chemical Co. L-[*methyl*-^{14}C]Methionine (49 Ci/mol) was purchased from New England Nuclear. [8-^{14}C]Adenosine triphosphate (51 Ci/mol) was purchased from ICN Pharmaceuticals, Inc., and prior to use was chromatographed on Dowex-50 (NH_4^+) ion-exchange resin to remove a cationic radioactive impurity.

Resolution of 2-Methyl-D,L-methionine. 2-Methyl-D,L-methionine (490 mg, 3 mmol) was dissolved in 15 mL of hot AcOH. Ac_2O (344 mg, 3.3 mmol) was added dropwise. The solution was

(20) Mudd, S. H. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1973; Vol. VIII, Part A, pp 121–154.
(21) Tabor, C. W.; Tabor, H. *Adv. Enzymol.* **1984**, *56*, 251.
(22) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(23) Loev, B.; Snader, K. M. *Chem. Ind. (London)* **1965**, *15*.

boiled for 3 min, then allowed to cool to room temperature. Volatiles were evaporated in vacuo, and water was evaporated 3 times, in vacuo, from the residue. An aqueous solution of the residue was passed through a column (2 × 5 cm) of AG 50W (H^+ form) to remove traces of starting material. Water was evaporated in vacuo from the aqueous eluates, and the residue was crystallized twice from ethyl acetate to remove traces of less polar material, giving *N*-acetyl-2-methyl-D,L-methionine as a white crystalline powder (493 mg, 80% yield), mp 126–128 °C, which moved as a single component, R_f = 0.65, on TLC in system A.

A solution of the above *N*-acetyl derivative (300 mg) in H_2O (40 mL) was adjusted to pH 7.5–8.0 by addition of NH_4OH. Acylase I (3 mg) was added, and deacetylation was followed by TLC in system A. The solution was stored for 26 h at 37–38 °C, then brought to pH 5 with AcOH and passed through a column (4 × 8 cm) of AG 50W (H^+ form). The column was eluted with H_2O to remove unhydrolyzed starting material and then with 2 N HCl. HCl fractions containing ninhydrin-positive material were combined and evaporated to dryness in vacuo and again after the addition of H_2O. A solution in H_2O of the residual amino acid hydrochloride was applied to a column (3 × 20 cm) of C_{18}-bonded silica; elution with H_2O gave, firstly, HCl-containing fractions, then the HCl-free amino acid. Evaporation of appropriate fractions and recrystallization of the residue from H_2O–acetone afforded 81 mg (68% yield) of 2-methyl-L-methionine as a white crystalline powder that yielded a single component, R_f = 0.3, on TLC in system A, $[\alpha]^{27}_D$ +18° (*c* 1.8, 2 N HCl). Fractions from the above AG 50W column that contained *N*-acetyl amino acids were combined and evaporated to dryness in vacuo. The residue was treated with acylase I for 16 h under the conditions given above. The deacetylated product was isolated, as above, by use of an AG 50W column and was freed of HCl with a column of C_{18}-bonded silica and crystallized from water–acetone to give an additional 12 mg of 2-methyl-L-methionine: $[\alpha]^{27}_D$ +19° (*c*, 1.2, 2 N HCl). Anal. ($C_6H_{13}NO_2S$) C, H, N.

Fractions from the AG 50W column containing *N*-acetyl-2-methyl-D-methionine were combined and concentrated to dryness in vacuo. The residue was refluxed in 2 N HCl for 2–3 h until hydrolysis was complete as shown by TLC in system A. From this solution 2-methyl-D-methionine was isolated as described above for its L isomer and obtained as a white crystalline powder (104 mg, 87%) that exhibited a single spot, R_f = 0.3, on TLC in system A and had $[\alpha]^{26}_D$ −21° (*c* 2.1, 2 N HCl). Anal. ($C_6H_{13}NO_2S$) C, H, N.

Resolution of 3(α,β)-Methyl-D,L-methionine. (a) Separation of *S*-Benzyl-*N*-(*tert*-butyloxycarbonyl)-3(α,β)-methyl-D,L-homocysteine Methyl Esters. *S*-Benzyl-3(α,β)-methyl-D,L-homocysteine (850 mg) was treated with di-*tert*-butyldicarbonate (853 mg) and 1 N NaOH (7 mL) in 30 mL of dioxane–water (2:1) under conditions described by Moroder[13] for other amino acids. Workup as described[13] gave the *N*-Boc-*S*-benzyl-3-methylhomocysteines, R_f = 0.1 in system B. These were treated with MeOH, 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide, and 4-(dimethylamino)pyridine in CH_2Cl_2 according to Olsen.[14] The *N*-Boc-*S*-benzyl-3-methylhomocysteine methyl esters so obtained moved as a single spot of R_f = 0.16 on TLC in system C: ^1H NMR ($CDCl_3$, 60 MHz) δ 0.83–1.03 (m, 3, 3-CH_3), 1.43 (s, 9, *t*-Bu), 2.0–2.6 (m, 3, H-3 and H-4), 3.66 (s, 5, OCH_3 and SCH_2Ph), 4.22–4.66 (m, 1, H-2), 5.01 (br d, 1, *J* = 7 Hz, NH), 7.23 (s, 5, Ph). The preparation moved as two spots of apparent R_f = 0.38 and 0.43 after four developments in system C. Dry-column chromatography on silica gel (3 × 80 cm) in EtOAc–hexane (1:5) gave partial separation. Overlapping fractions were rechromatographed 3 times to complete the separation. This gave 250 mg of the enantiomeric mixture of the higher R_f value (designated as having a 2(D,L)-3(α) configuration) and 340 mg of the mixture of lower R_f [designated 2(D,L)-3(β)].

(b) Formation of 3(α)-Methyl-D,L-methionine and 3(β)-Methyl-D,L-methionine. Carbomethoxy groups in the above homocysteines were hydrolyzed by treatment for 4 h at 22 °C with NaOH (0.2 N, 1.1 equiv) in H_2O–dioxane (3:7).[24] The product was extracted into EtOAc after adjusting the pH to 3. The EtOAc was removed in vacuo. The residue was treated for 80 min at 22 °C with 100% trifluoroacetic acid to remove the Boc group.[25] The crude products were crystallized from MeOH–EtOAc–*n*-C_6H_{12} to give *S*-benzyl-3(α)-methyl-D,L-homocysteine and its 3(β) isomers in 93% yield. Each showed a single component, R_f = 0.38, in system A. The benzyl group in a 0.75-mmol portion was replaced by a methyl group by treatment with Na–liquid NH_3 followed by addition of methyl iodide.[15,26] Reverse-phase chromatography (3 × 20 cm, water) provided homogeneous 3(α)-methyl-D,L-methionine and its 3(β) isomers (R_f = 0.2 in system E) in 80–83% yield.

(c) Separation of the Four Stereoisomers of 3(α,β)-Methyl-D,L-methionine. N-Acetylation (92–95% yield) was performed as described for 2-methylmethionine. Volatiles were removed in vacuo, and traces of starting material were removed by passage of an aqueous solution of the residue through a column (2 × 5 cm) of Dowex 50 (H^+) per millimole of amino acid. The products (R_f = 0.7 in system E) were treated with acylase I according to the procedure described for 2-methylmethionines, except that the first acylase treatment was carried out for 4 h, at which time TLC in system E indicated that deacetylation was ca. 40% complete. The second acylase treatment was carried out for 16 h. The free amino acids were obtained in 90–96% yield by reverse-phase chromatography and were homogeneous on TLC in systems E and F (R_f = 0.2 and 0.5, respectively). They separated as white crystalline solids from solutions in H_2O–acetone. $[\alpha]^{26}_D$ values in 2 N HCl: 2(L)-3(α), +49° (*c*, 1.0); 2(D)-3(α), −47° (*c*, 0.4); 2(L)-3(β), −1.2° (*c*, 1.5); 2(D)-3(β), +1.2° (*c*, 1.5).

Resolution of 4(α,β)-Methyl-D,L-methionine. All procedures were the same as employed for the resolution of 3(α,β)-methyl-D,L-methionine. Thus, the *N*-Boc derivatives of *S*-benzyl-4(α,-β)-methyl-D,L-homocysteines (R_f = 0.75 in system D) were prepared from 900 mg of 11 and converted to their methyl esters (ΔR_f = 0.1 after developing twice in system C), and these were separated by dry-column chromatography and designated as enantiomeric mixtures with a 2(D,L)-4(α) configuration (490 mg; R_f = 0.38 after two developments with system C) and a 2(D,L)-4(β) configuration (670 mg; R_f = 0.28), respectively: ^1H NMR of the R_f = 0.38 mixture ($CDCl_3$, 60 MHz) δ 1.28 (d, 2, *J* = 6 Hz, 4-CH_3), 1.40 (s, 9, *t*-Bu), 1.86 (t, 2, $J_{2,3} = J_{3,4}$ = 6 Hz, H-3), 2.44–2.91 (m, 1, H-4), 3.66 and 3.69 (2s, 5, OCH_3, SCH_2Ph), 4.20–4.54 (m, 1, H-2), 4.84 (br d, 1, *J* = 7 Hz, NH), 7.24 (s, 5, Ph). The R_f = 0.28 mixture gave an almost identical ^1H NMR spectrum. Hydrolysis of blocking groups gave the *S*-benzyl-4-methylhomocysteines (R_f = 0.27 in system D) in 96% yield after crystallization from MeOH–acetone–H_2O. S-debenzylation and S-methylation of these gave the 4-methylmethionines (R_f = 0.2 in solvent E) in 90% yield. N-acetylation of these gave 96% yield of homogeneous products of R_f 0.7 in system E. These were treated with acylase I as described above for the *N*-acetyl-3-methylmethionines. The deacetylated D- and L-amino acids (R_f = 0.2 and 0.5 in systems E and F, respectively) were obtained in 90–98% yields after reverse-phase chromatography as materials homogeneous on TLC. Each separated as a white crystalline solid from a solution in H_2O–acetone. $[\alpha]^{26}_D$ values in 2 N HCl: 2(L)-4(α), +11° (*c*, 1.6); 2(D)-4(α), −12° (*c*, 2.0); 2(L)-4(β), −22° (*c*, 2.0); 2(D)-4(β), +21° (*c*, 3.0). Anal. (**4c**, **4d**) ($C_6H_{13}NO_2S$) C, H, N.

Treatment of 4(β)-Methyl-D- and L-Methionines with D-Amino Acid Oxidase. Each amino acid was treated overnight with the oxidase under described conditions.[27] TLC of the reaction mixtures in system F showed that the 2(L)-4(β) isomer (R_f = 0.5) was unaffected and that the 2(D)-4(β) isomer was completely converted to ninhydrin-negative material of R_f = 0.8.

***N*-Methyl-L-methionine (5).** This was prepared from D-methionine in 34% yield as irridescent micro needles using a procedure described for the preparation of *N*-methyl-D-methionine from L-methionine.[16] The product was homogeneous on TLC: R_f = 0.27 (system A) and 0.09 (system E); mp 237 °C dec; $[\alpha]^{28}_D$ +40° (*c* 2.0, 3 N HCl) (reported for *N*-methyl-D-methionine, mp 238–239 °C, $[\alpha]^{20}_D$ −40.7° (1.0, 3 N HCl));[16] ^1H NMR (60 MHz,

(24) Nguyen, D. L.; Dormoy, J-R., Castro, B.; Prevot, D. *Tetrahedron* 1981, *37*, 4229.

(25) Schwyzer, R.; Costopanagiotis, A.; Sieber, P. *Helv. Chim. Acta* 1963, *46*, 870.

(26) Reisner, D. B. *J. Am. Chem. Soc.* 1956, *78*, 2132.

(27) Reference 10; p 1738.

CF_3CO_2H) δ 2.05 (s, 3, SCH_3), 2.37 (m, 2, H-3), 2.75 (m, 2, H-4), 2.90 (s, 3, NCH_3), 4.20 (m, 1, H-2), 7.65 (br, 2, NH_2^+).

N-(n-Butyl)-L-methionine (6). To a solution of 2(D)-bromo-4-(methylthio)butyric acid (1.9 g, 9 mmol), prepared from D-methionine,[16] in DMF (25 mL) was added freshly distilled n-butylamine (2.2 g, 3 mL, 30 mmol). The solution was kept at 45 °C for 16 h, then poured into acetone (100 mL), and the resulting solid filtered and washed with acetone to give 906 mg (49%) of a white crystalline solid, homogeneous by TLC with R_f = 0.45 in system A. Recrystallization from water gave 743 mg of stout crystals: mp 255 °C dec; $[\alpha]^{26}_D$ +47° (c 2.0, 3 N HCl); ^1H NMR (60 MHz, CF_3CO_2H) δ 0.90 (t, 3 H, CH_3 in C_4H_9), 1.30 and 1.60 (m, 2 each, $(CH_2)_3$ in C_4H_9), 2.05 (s, 3, SCH_3), 2.35 (m, 2, H-3), 2.70 (m, 2, H-4), 3.20 (m, 2, NCH_2), 4.20 (m, 1, H-2), 7.20 and 7.75 (br, 1 each, NH_2^+). Anal. ($C_9H_{19}NO_2S$·0.1acetone) C, H, N.

Enzyme Studies. M-2 and M-T preparations were obtained and partially purified as given previously[3] except that the isolation and concentration of M-2 (final volume 0.14 mL/g of kidney extracted) and M-T (final volume 0.27 mL/g of Novikoff hepatoma cells extracted) were completed within 1 day.

Enzyme assays were conducted at 37 °C in a final volume of 0.1 mL containing 150 mM KCl–15 mM $MgCl_2$–5 mM dithiothreitol–50 mM Tris·HCl, pH 8.2. Studies of methionine derivatives as inhibitors employed [^{14}C]methionine as the radioactive substrate, 2 mM MgATP, and 8.6×10^{-6} units of M-2 or 7.9×10^{-6} units of M-T (10 μL of a 10-fold diluted stock solution prepared freshly each day). (One unit is defined as the amount that produces a V_{max} of 1 μmol of S-adenosylmethionine (AdoMet)/min under these conditions.) The values in Table I for percent inhibition of M-2 and M-T by 3 mM levels of the Met derivatives are averages of three separate determinations each done in duplicate. The values obtained were within 5–10% of the average value. Assays for inhibition studies were performed for 10 min and terminated by addition of 10 μL of 4 N $HClO_4$–10 mM L-methionine with cooling in an ice bath. Each suspension was centrifuged, and 50 μL of supernatant was applied to a 2.3-cm disc of phosphocellulose paper. Discs were washed as described,[28] then immersed in a toluene solution of phosphors and counted in a Packard liquid scintillation spectrometer (Model 2425). Controls were provided by incubations carried out in the absence of ATP. Reaction velocities were linear for at least 60 min and were proportional to the amount of enzyme added at the levels of enzyme activity employed.

Studies of inhibition kinetics were made with five or six levels of [^{14}C]methionine in the range (0.5–4) × K_M of methionine for each of two inhibitor levels and for control mixtures lacking inhibitor. Inhibition constants (K_i values) were obtained from replots of inhibitor concentrations (1–3 mM) vs. slopes of double-reciprocal plots of velocity vs. substrate level. All of the latter plots were linear.

Studies of the methionine derivatives as potential substrates were conducted for 60 min at 37 °C in the presence of 0.4 mM [^{14}C]ATP and 1.72×10^{-5} units of M-2 or 1.58×10^{-5} units of M-T. The velocity of formation of AdoMet was linear for more than 90 min when assayed with 0.4 mM unlabeled ATP and 32 μM [^{14}C]Met. Reactions (0.1 mL) were terminated by applying 10-μL aliquots onto Whatman 3 MM paper and carrying out paper electrophoresis at pH 5.0 (0.05 M acetate) at 20 V/cm for 3 h. Under these conditions, ATP migrated 13 cm toward the anode and AdoMet migrated 10.5 cm toward the cathode. Five areas of paper (each 1 × 2 cm) were cut out in a region extending from 1 cm ahead to 2 cm behind AdoMet, and ^{14}C in these was determined as described above. Controls were provided by incubations carried out in the absence of methionine analogues and then subjected to simultaneous electrophoresis analysis; control values were routinely 75 ± 11 cpm for M-2 and 71 ± 16 cpm for M-T. The production, by 4 mM levels of Met derivatives, of at least twice the control counts per minute in the AdoMet region was taken as evidence for substrate activity in the results given in Table I. In determinations of K_M and V_{max} values, the smallest value obtained in any assay was 135 cpm above the control value. These substrate constants (Table I) were determined from double-reciprocal plots of velocity vs. substrate concentration; all such plots were linear. This paper electrophoretic analysis was used instead of the phosphocellulose disc method above or an ion-exchange procedure of Chou and Lombardini[29] because these methods gave higher blank values with the present enzyme preparations.

Acknowledgment. This work was supported by Public Health Service Research Grant CA-11196 from the National Cancer Institute and by grants to the Institute for Cancer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania). We wish to thank Dr. Sidney Weinhouse, Temple University, for providing the rat Novikoff cells used in this work, Dr. John Lombardini, Texas Tech University, for carrying out studies with unresolved methylmethionines and variants of rat methionine adenosyltransferase, Dr. Goran Zdansky, University of Uppsala, for gifts of 3- and 4-methyl-S-benzylhomocysteines, and Dr. Hiroyuki Yamamoto, Shinshu University, for a gift of γ-N-methyl-L-α,γ-diaminobutyric acid.

Registry No. 1, 63-68-3; **2a**, 59013-75-1; **2b**, 98302-79-5; **3a** (isomer 1), 103129-08-4; **3a** (isomer 2), 103129-10-8; **3b** (isomer 1), 103129-09-5; **3b** (isomer 2), 103129-11-9; **4a** (isomer 1), 103129-12-0; **4a** (isomer 2), 103239-15-2; **4b** (isomer 1), 103129-13-1; **4b** (isomer 2), 103239-16-3; **5**, 42537-72-4; **6**, 103024-69-7; **7**, 4135-28-8; **8**, 103024-70-0; **9**, 103024-71-1; **10** (isomer 1), 103129-16-4; **10** (isomer 2), 103129-17-5; **11** (isomer 1), 103024-72-2; **11** (isomer 2), 103129-14-2; **12** (isomer 1), 103024-73-3; **12** (isomer 2), 103129-15-3; MAT, 9012-52-6; DL-$MeO_2CCH(NHBOC)CH_2CH(CH_3)SCH_2Ph$ (isomer 1), 103024-74-4; DL-$MeO_2CCH(NHBOC)CH_2CH(CH_3)SCH_2Ph$ (isomer 2), 103024-75-5; D-$MeS(CH_2)_2CHBrCO_2H$, 42990-67-0; DL-$HO_2CCH(NHBOC)CH(CH_3)CH_2SCH_2Ph$ (isomer 1), 103024-76-6; DL-$HO_2CCH(NHBOC)CH(CH_3)CH_2SCH_2Ph$ (isomer 2), 103129-18-6; DL-$HO_2CCH(NHBOC)CH_2CH(CH_3)SCH_2Ph$ (isomer 1), 103024-77-7; DL-$HO_2CCH(NHBOC)CH_2CH(CH_3)SCH_2Ph$ (isomer 2), 103024-78-8; DL-$HO_2CCH(NH_2)CH_2CH(CH_3)SCH_2Ph$ (isomer 1), 103129-19-7; DL-$HO_2CCH(NH_2)CH_2CH(CH_3)SCH_2Ph$ (isomer 2), 103129-20-0; DL-$HO_2CCH(NH_2)CH(CH_3)CH_2SMe$ (isomer 1), 103129-21-1; DL-$HO_2CCH(NH_2)CH(CH_3)CH_2SMe$ (isomer 2), 103129-22-2; DL-$MeS(CH_2)_2C(CH_3)(NH_2)CO_2H$, 2749-07-7; D-$MeS(CH_2)_2CH(NH_2)CO_2H$, 348-67-4.

(28) Hoffman, J. L. *Methods Enzymol.* **1983**, *94*, 223.

(29) Chou, T.-C.; Lombardini, J. B. *Biochim. Biophys. Acta* **1972**, *276*, 399.