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S-Adenosylmethionine: Stability and Stabilization[1]

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With a developed HPLC technique for the separation of both (+)- and (−)-*S*-adenosylmethionine (AdoMet) and ^{1}H NMR analysis of the epimeric S-CH_3 chemical shifts, a kinetic study on the stability of (−)-AdoMet in solution to decomposition and epimerization is described. The results obtained from the effects of pH, temperature, and sulfonium counterions on the stability of AdoMet indicate that the epimerization appears to proceed through pyramidal inversion of the sulfonium pole. The optimal conditions for AdoMet to be stable in solution to decomposition and epimerization is to keep the compound at pH 3–5, containing an excess of large-size, nonnucleophilic counterions such as tosylate or sulfate. © 1987 Academic Press, Inc.

INTRODUCTION

As part of our efforts to develop practical routes to obtain valuable biochemicals (*1–3*), we have reported improved chemical and enzymatic procedures for the synthesis of (−)-*S*-adenosylmethionine (AdoMet) (*4*). We described here studies on the stability of this compound to decomposition and epimerization at the sulfonium center under different pH's, temperatures, and sulfonium counterions.

With respect to the studies done related to the stability of (−)-AdoMet (see scheme 1), major efforts have been focused on studying its stability to decomposition (*5–11*). Only a limited amount of work has been directed toward studying the chiral instability at the sulfonium center (12–13). The most recent study of the latter character of AdoMet indicates that the rate constant for the epimerization of (−)-AdoMet to (+)-AdoMet (the biologically inactive form) at the sulfonium center at 37°C and pH 7.5 ($k_r = 8 \times 10^{-6}$ s^{-1}, $t_{1/2} \simeq 24$ h) is comparable to that for the decomposition ($k_h = 6 \times 10^{-6}$ s^{-1}, $t_{1/2} = 32$ h) (*12*). It has been speculated that (−)-AdoMet in biological systems may have a rapid turnover or be stabilized through macromolecular binding, if a serious waste of biosynthetic energy is to be avoided (*12*). Indeed, a study of the conformation of *S*-adenosylhomocysteine (SAH) and its competitive binding with (−)-AdoMet-utilizing enzymes indicates that decomposition via intramolecular displacement can be avoided through binding (*14*).

Although mechanistic studies on the decomposition of (−)-AdoMet through

[1] After this paper was submitted, a paper on a similar subject was reported: Hoffman, J. L. (1986) *Biochemistry* **25**, 4444–4449.

[2] NSF Predoctoral Fellow, 1984–1987.

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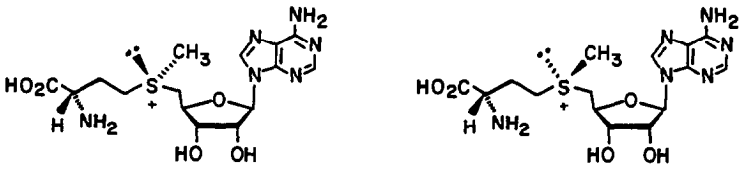


(−)-AdoMet (+)-AdoMet

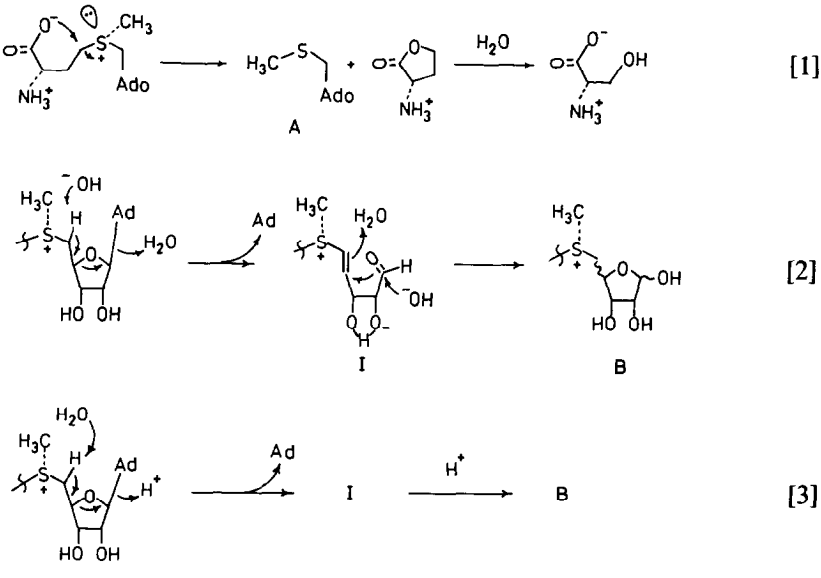
SCHEME 1

hydrolysis have been reported and conditions for use to improve the stability of AdoMet to decomposition have been suggested, no information is available regarding the mechanism and stabilization to epimerization. A purpose of this work is to suggest a possible mechanism for the epimerization and provide possible ways of improving the chiral stability at the sulfonium pole of this important biochemical.

RESULTS AND DISCUSSION

Decomposition of S-Adenosylmethionine

Several mechanisms have been proposed for the decomposition of AdoMet. They are summarized in Eqs. [1]–[3]. The results are obtained from various literature studies for the decomposition of AdoMet under neutral (Eq. [1]), basic (Eq.



[1]

[2]

[3]

[2]), and acidic (Eq. [3]) conditions. Under neutral conditions, the most probable mechanism for the decomposition as shown in Eq. [1] has the first-order rate constant of 6×10^6 s^{-1} at 37°C, corresponding to a half-life of 32 h. Assuming that the forward and the reverse epimerization rate constants are equal and that the rate constants for the decomposition of (−)- and (+)-AdoMet are equal (*12*), the kinetic law for the decomposition and epimerization as shown in Eq. [4] is given by Eqs. [5]–[7]

$$\begin{array}{ccc} \text{(−)-AdoMet} & \underset{k_r}{\overset{k_r}{\rightleftharpoons}} & \text{(+)-AdoMet} \\ \downarrow k_h & & \downarrow k_h \\ \text{P} & & \text{P} \end{array} \qquad [4]$$

$$2[\text{(−)-AdoMet}]_t = [\text{(−)-AdoMet}]_0 \, (e^{-(k_h t} + e^{-(k_h + 2k_r)t}) \qquad [5]$$

$$2[\text{(+)-AdoMet}]_t = [\text{(−)-AdoMet}]_0 \, (e^{-(k_h t} - e^{-(k_h + 2k_r)t}) \qquad [6]$$

$$[\text{P}]_t = [\text{(−)-AdoMet}]_0 \, (1 - e^{-k_h t}), \qquad [7]$$

where k_r is the epimerization rate constant, k_h is the decomposition rate constant, and P is the decomposition product. The concentration of (−)- or (+)-AdoMet can be determined by HPLC or by NMR analysis of the S–CH_3 peaks (see Experimental). Extrapolation of the reported k_h at 37 to 23°C gave $k_h = 1.2 \times 10^{-6}$ s^{-1}, corresponding to a half-life of 7 days. This value is in agreement with our observed value at 23°C. Comparison of this value with our observed first-order rate constant at different pH values (Table 1, Fig. 1) indicates that AdoMet is quite stable under slightly acidic conditions (pH 3–5) but unstable under extremely basic or acidic aqueous solutions. This is consistent with the mechanism proposed as shown in Eqs. [1]–[3].

TABLE I

KINETIC CONSTANTS FOR THE DECOMPOSITION AND EPIMERIZATION OF ADOMET

	Decomposition, k_h (s^{-1})		Epimerization k_h (s^{-1})	
pH	23°C	37°C	23°C	37°C
2.1	2.0×10^{-4}	—	—	—
2.8	2.2×10^{-6}	—	3.0×10^{-7}	3.2×10^{-6}
5.3	6.5×10^{-7} (2.9×10^{-7})[a]	1.6×10^{-6}	3.0×10^{-7} (1.1×10^{-7})[a]	3.2×10^{-6}
7.5	—	1.8×10^{-5} (9.6×10^{-6})[b]	—	3.2×10^{-6} (2.5×10^{-6})[b]
8.0	2.8×10^{-6}	—	—	—
10.5	7.4×10^{-5}	—	—	—

Note. Those at 23°C are done by HPLC analysis and those at 37°C by NMR analysis. For details see the Experimental.

[a] Containing 0.1 M Na-tosylate, 0.5 mM AdoMet.

[b] Containing 0.02 M Na-Tosylate, 10 mM AdoMet.

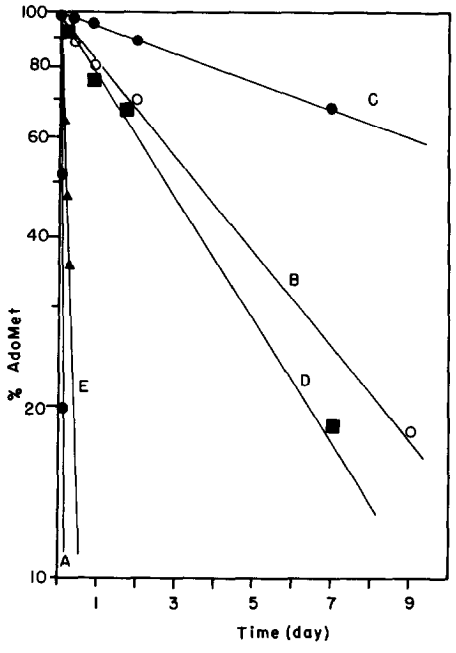


FIG. 1. First-order plots of AdoMet stability at 23°C as a function of pH. Reactions are monitored by HPLC. The buffer concentrations are 0.1 M, that of AdoMet sulfate is 1.0 mM. A, HCO_2H for pH 2.1; B, blank for pH 2.8; C, triethanolamine for pH 5.3; D, triethanolamine for pH 8.0; E, TRIZMA base for pH 10.5.

Effect of Sulfonium Counterions on Decomposition

Changing the counterion for the sulfonium center also affects the stability of AdoMet to decomposition in solution. As shown in Fig. 2, tosylate and sulfate anions stabilize AdoMet the best. For the halide salts, a decrease of stability in the order of $I^- > Br^- > Cl^-$ was observed. Based on these results, it appears that the maximal stability of AdoMet in solution can be obtained using large-size, nonnucleophilic anions such as tosylate or sulfate at pH 3–5.

Epimerization of S-Adenosylmethionine

The rate of epimerization was determined by measuring the peak intensities of $S-CH_3$ shift with a 200-MHz instrument and by HPLC analysis. Figure 3 shows a typical example. Table 1 summarizes kinetic constants.

We have studied the stability of AdoMet to epimerization as a function of pH and temperature. As seen, changing pH from 2.8 to 7.5 has very little effect on the rate of epimerization. The rate of epimerization, however, is quite sensitive to temperature. The estimated rate constant at 37°C is about 3.2×10^{-6} s^{-1} ($t_{1/2}$ = 60 h) and 3×10^{-7} s^{-1} ($t_{1/2}$ = 590 h) at 22°C. The concentration and the type of counterion also affects the epimerization rate. As shown in Fig. 4, the rate of

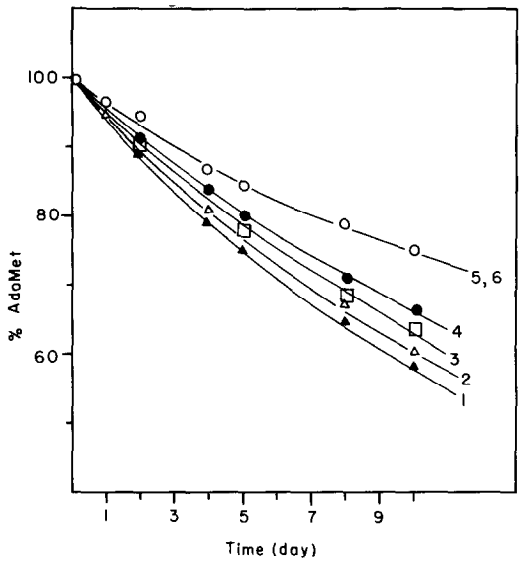


FIG. 2. AdoMet sulfate stability at pH 5.3, 23°C, to decomposition in the presence of different counterions. The solution contains 0.01 M triethanolamine, 0.5 mM AdoMet sulfate, and 0.1 M counterion. 1, blank; 2, KBr, 3, KCl; 4, KI; 5, Na-tosylate; 6, Na_2SO_4.

epimerization decreases with an increase in the concentration of tosylate, and tosylate and sulfate stabilize to epimerization the best. Using the halides, a change in the order is seen in the decreasing stabilizing ability: $Br^- > Cl^- > I^-$. This difference in order of stabilizing ability versus decomposition and epimerization

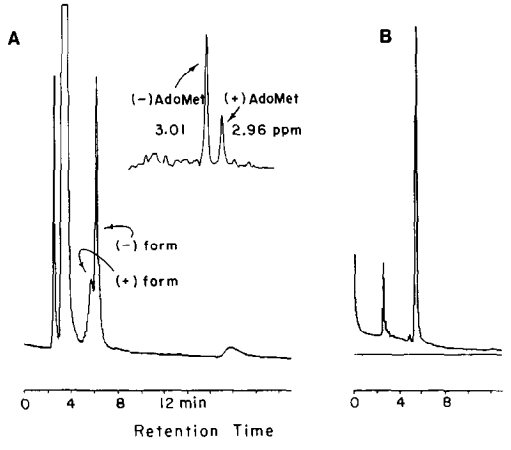


FIG. 3. (A) HPLC analysis of standard AdoMet tosylate from Sigma. The chromatography conditions are described in the experimental section. Inserted is the ^1H NMR spectrum of the sulfonium methyl group. $\delta = 2.96$ ppm for the (−)-form and 3.01 ppm for the (+)-form. (B) HPLC analysis of the enzyme product from 1 mM reaction after denaturation of enzyme and centrifugation.

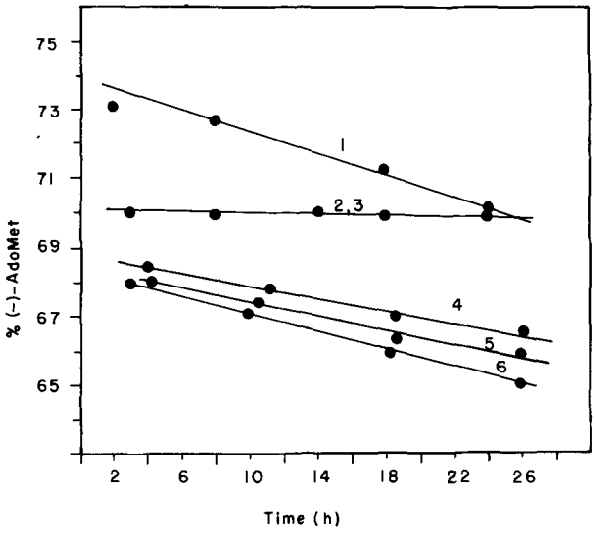


FIG. 4. AdoMet (10 mM sulfate) stability to epimerization at 23°C in the presence of different counterions (0.1 M) at pH 5.3 (triethanolamine, 0.1 M in D_2O). Percentage of (−)-AdoMet was determined by ^1H NMR by measuring the ratio of (−):(+)-AdoMet. 1, blank; 2, Na-tosylate; 3, Na_2SO_4; 4, KBr; 5, KCl; 6, KI.

could presumably be due to different electronic and steric effects (see below). It is seen that a large nonnucleophilic counterion present in excess is necessary for stabilization to epimerization.

By measuring the absolute intensity change of the S–CH_3 shifts at 2.97 and 3.01 ppm, the rates of decomposition at 37°C under different conditions can also be obtained. Figure 5 indicates a first-order plot of decomposition at 37°C. The

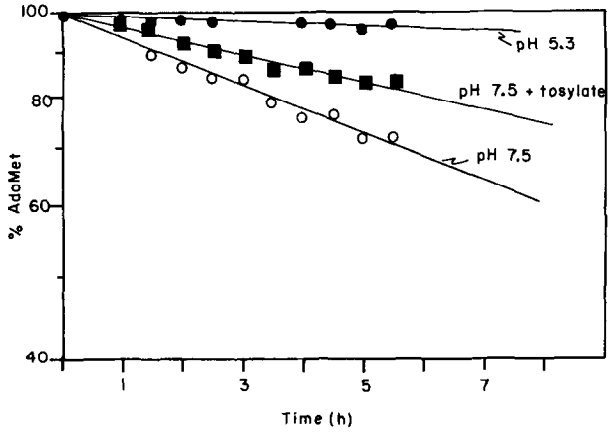
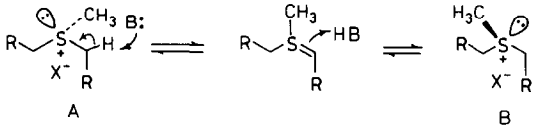


FIG. 5. AdoMet stability to decomposition determined by NMR analysis of the S–CH_3, group. Conditions are the same as those used in Fig. 4. ●, pH 5.3; ■, pH 7.5 with 0.02 M Na-tosylate added; ○, pH 7.5.

results are in reasonably agreement with those reported obtained at 23°C based on HPLC analysis. The data obtained in experiments are consistent with Eqs. [5]–[7] based on the assumption that k_r and k_h for both isomers are equal. The same conclusion was reported previously (*12*).

Mechanism of Epimerization

Previous studies on the thermal racemization of small sulfonium species indicate that it may proceed by four routes: ylide formation via base catalyzed deprotonation of carbons next to the sulfonium center (Eq. [8]) (*15*), dissociation of one of the alkyl groups from the sulfur, forming an ion-neutral molecular complex, followed by reassociation (SN_1 mechanism) (Eq. [9] (*16*), dissociation of one of the alkyl groups from the sulfur assisted by a nucleophile (SN_2 mechanism) (Eq. [10], (*17*), and thermally induced pyramidal inversion (Eq. [11]) (*15, 18, 19*). Each of these mechanisms has been suggested as the major pathway for the racemization of representative small sulfonium species. The mechanism for the epimerization of AdoMet, however, has not been described. Based on the results obtained, we suggest that the most probable mechanism for the epimerization of AdoMet be pyramidal inversion. The ylide mechanism is eliminated because the epimerization is pH independent. The SN_2 mechanism is unlikely because tosylate and sulfate are not nucleophiles. Large-size halides such as I^- may have a very small contribution to this mechanism due to the relatively unstable iodide compared to chloride. The SN_1 mechanism is unlikely too because none of the dissociated primary cations would be a stable intermediate, and no solvolysis was observed in the condition of epimerization. Racemization accompanied by solvolysis at a relatively equal rate has been observed for those proceeding by SN_1 mechanism



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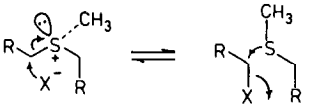

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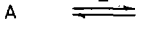

[11]

(*16, 19*). The proposed pyramidal inversion is consistent with all the experimental results: it is independent of pH and sensitive to temperature and the size of counterions. The opening of the C–S–C angle in the transition of pyramidal inversion, which is the most obvious requirement of the mechanism, would suggest that large-size, nonnucleophilic counterions may inhibit the inversion process and thus increase the chiral stability. This was indeed observed in the case of tosylate species. Further, the fact that steric factors rather than electronic factors have a significant effect on the inversion mechanism also supports the proposed mechanism: unlike heterocyclic sulfur systems, the sulfur pyramid in AdoMet is not constrained, and the large-size substituent such as the adenosyl group could help stabilize the transition state of the inversion process. Finally, the activation energy for the epimerization of Adomet calculated from the observed rate constants (~25–26 Kcal/mol) is in the same range as that obtained for the small chiral sulfonium species proceeding through the same mechanism (*19, 20*).

CONCLUSIONS

Based on the kinetic results obtained in this study, the stability of AdoMet in solution to decomposition and epimerization can be improved by adding large-size, nonnucleophilic anions. Although large nucleophilic species (e.g., I^-) also have a slight stabilization effect on decomposition, they have a negative effect on epimerization. The compromised condition for AdoMet to be stable in solution with minimum decomposition and epimerization is to keep the compound at pH 3.5–5.5, containing an excess of large-size nonnucleophilic counterions such as tosylate. With the HPLC technique developed together with ^1H NMR analysis of the S–CH_3 shifts, the kinetics of decomposition and epimerization can be studied conveniently without the use of radioactive AdoMet. Although the kinetic results we obtained are slightly different from those reported previously using radioactive material (*12*), they are on the same order of magnitude. The information presented in this study should help those who intend to prepare AdoMet on a large scale and those who use this compound for various biochemical studies.

EXPERIMENTAL

The following materials were obtained from the mentioned sources: (−)-AdoMet tosylate and biochemicals, Sigma; (−)-AdoMet sulfate, Boehringer; (−)-AdoMet was also prepared enzymatically according to the procedure described in another paper (*4*). ^1H NMR spectra were obtained on a Varian XL200 (200-MHz) instrument. The AdoMet solution in D_2O-containing buffer components were monitored at δ2.5–3.5 ppm for the following resonances δ2.96 (s, 3H, (+)-S–CH_3); 3.01 (S, 3H, (−)-S–CH_3). The shifts of methyl group varied slightly with different counterions. HPLC analyses were done on a Gilson chromatograph integrated to an Apple IIe computer and equipped with a VYDAC C-18 reverse-phase column

under the following conditions: 1.0 ml/min, 1.70 k_{psi}, 5% ethanol, 243 mM EDTA, 4 mM heptanesulfonic acid, pH 3.75, with NaOH. Before the HPLC analyses were run with the heptanesulfonic acid buffer, the column was preequilibrated with a 2.3 mM (1*S*)-(+)-10-camphorsulphonic acid buffer (using the same concentration of other chemicals as described) for 1 h at 1.0 ml/min. This camphorsulfonic acid-preequilibrated column can be used with the heptanesulfonic acid buffer for 2.5–3.0 h and must then be reequilibrated with the chiral camphorsulfonic acid solution as before. Even though a 0.4- to 0.6-min resolution is achieved, peak overlap does not allow for complete quantitation of the epimers. R_t(+)-SAM = 10.6 min, R_t(−)SAM = 11 min, R_tSAH = 22–24 min. The column is monitored at 259 nm (ε_{AdoMet} = 15,400 M^{-1}).

AdoMet Stability to Decomposition and Epimerization (HPLC Analysis)

(+)- or (−)-AdoMet tosylate or sulfate salt was dissolved in D_2O buffer to a concentration of 1 or 10 mM. The buffer (0.1–0.4 M) pH was adjusted to the desired value at 23°C: pH 2.1 (dilute HCO_2H), pH 2.8 (no buffer solution, just D_2O), pH 5.3 ($(EtOH)_3NHCl$), pH 8.0 ($(EtOH)_3NHCl$ with NaOD), pH 10.5 (TRIZMA base). The total initial AdoMet concentration measured by HPLC was taken as 100% for each ^1H NMR tube. The methylsulfonium resonances were monitored and initial epimer mole ratios determined. The tubes were monitored versus time by both HPLC and ^1H NMR as a means of determining the rates of both decomposition and epimerization versus pH.

Adomet Epimerization versus Temperature

A solution was made 10 mM in AdoMet sulfate or tosylate (pH 2.8) and 100 mM in Na_2SO_4 or other salts. This solution was separated into two ^1H NMR tubes and incubated separately (one at 23°C, the other at 37°C). The methyl sulfonium resonances were monitored frequently and the percentage of (−)-SAM calculated according to the relative peak intensities (epimer mole ratio was measured by peak intensities of the S–CH_3 shift).

For studies at different pH values, triethanolamine (final concn. 0.4–0.5 M) was added to the AdoMet solution (pH 2.8) and the pH adjusted with NaOD to the desired values.

AdoMet Decomposition and Epimerization versus Counterions

A stock solution of 10 mM AdoMet sulfate or tosylate was made to pH 5.3 by adding triethanolamine (final concn. of triethanolamine was 0.1 M) and adjusted with NaOH. ^1H NMR tubes were filled with 0.5 ml of this solution and each of the following salts (Na-tosylate, Na_2SO_4, KCl, KBr, KI) was added to 0.1 M concentration and epimerization was monitored by ^1H NMR. The decomposition study was done on a dilute stock solution (0.5 mM AdoMet sulfate, pH 5.3, $(EtOH)_3NHCl$ 0.01 M, with NaOD) again made 0.1 M in the respective counterions. Total AdoMet concentration was quantitated by HPLC.

ACKNOWLEDGMENT

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REFERENCES

1. WHITESIDES, G. M., AND WONG, G.-H. (1985) *Angew. Chem. Int. Ed. Eng.* **24,** 617.
2. ROOT, R. L., DURRWACHTER, J. R., AND WONG, C.-H. (1985) *J. Amer. Chem. Soc.* **107,** 2297.
3. WONG, C.-H., DRUECKHAMMER, D. G., AND SWEERS, H. M. (1985) *J. Amer. Chem. Soc.* **107,** 4028.
4. MATOS, J. R., RAUSHEL, F. M., AND WONG, C.-H. (1987) *Biotechnol. Appl. Biochem.* **9,** 39–52.
5. CANTONI, G. L. (1953) *J. Biol. Chem.* **204,** 403.
6. PARKS, L. W., AND SCHLENK, F. (1958) *J. Biol. Chem.* **230,** 295.
7. BORCHARDT, R. T. (1979) *J. Amer. Chem. Soc.* **101,** 458.
8. BADDILEY, T., CANTONI, G. L., AND JAMIESON, G. A. (1953) *J. Chem. Soc.*, pp. 2662–2664.
9. ZAPPIA, V., GALLETTI, P., OLIVIA, A., AND DESANTIS, A. (1977) *Anal. Biochem.* **79,** 535.
10. ZAPPIA, V., CARTENI-FARINA, M., AND PORCELLI, M. (1979) *in* Transmethylation (Usdin, E., Borchardt, R. T., and Creveling, C. R. Eds.), pp. 95–104, Elsevier/North-Holland, New York/Amsterdam.
11. MUDD, S. H. (1959) *J. Biol. Chem.* **234,** 87; Mathew, A. N. (1958) *Fed. Proc.* **17,** 721.
12. WU, S. E., HUSKEY, W. P., BORCHARDT, R. T., AND SCHOWEN, R. L. (1983) *Biochemistry* **22,** 2828; according to our NMR results, the equilibrium constant of the epimerization is 1.4 in favor of the (−)-form.
13. STOLOWITZ, M. L., AND MINCH, J. J. (1981) *J. Amer. Chem. Soc.* **103,** 6015.
14. ISHIDA, T., TANAKA, A., INOUE, M., FUJIWARA, T., AND TOMITA, K. I. (1982) *J. Amer. Chem. Soc.* **104,** 7239.
15. MENON, B. C., AND DARWISH, D. (1973) *Tetrahedron Lett.* **42,** 4119.
16. DARWISH, D., HUI, S. H., AND TOMILSON, R. (1968) *J. Amer. Chem. Soc.* **90,** 5631.
17. DARWISH, D., AND TOURIGNY, G. J. (1966) *Amer. Chem. Soc.* **88,** 4303.
18. DARWISH, D., AND TOMILSON, R. L. (1968) *J. Amer. Soc.* **90,** 5938.
19. SCARTAZZINI, R., AND MISLOW, K. (1967) *Tetrahedron Lett.* **28,** 2719.
20. MARYANOFF, C. A., HAYES, K. S., AND MISLOW, K. (1977) *J. Amer. Chem. Soc.* **99,** 4412.