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A Sulfotransferase Model

Covalent Participation of a Macrocyclic Oxime in the Hydrolysis of 2,4-Dinitrophenyl Sulfate

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The liberation of 2,4-dinitrophenolate ion from 2,4-dinitrophenyl sulfate (DNPS) in aqueous organic solvent with 0.1 *N* sodium hydroxide was accelerated upon addition of an equimolar amount of Oxime-I (10-hydroxy-11-hydroxyimino[20]-paracyclophane) to the sulfate ester. Oxime-I was found to undergo covalent participation at the oxime group to afford oxime *O*-sulfonate. The rate acceleration with Oxime-I was larger than that with β-CD (cycloheptaamylose). The catalytic efficiency of Oxime-I has been ascribed primarily to the tighter inclusion of the substrate ester into the more hydrophobic Oxime-I cavity provided by the effective apolar paracyclophane skeleton, as well as to the greater nucleophilicity of the oxime group than of the hydroxyl group in β-CD. Consequently, Oxime-I may be considered as a conventional model for arylsulfatases and sulfotransferases, providing the effective binding process for the substrate.

INTRODUCTION

Aryl sulfates are widely distributed in animal and plant tissues and are usually decomposed by arylsulfatases through fission of the S–O bond of the substrate esters (*1*). The sulfatases, then, would be expected to act as sulfate acceptors (sulfotransferases) during the course of catalytic reactions, although there is no well-documented example of such a transfer process (*2*). On the other hand, the bimolecular nucleophilic participation of different amines has been well documented for the nonenzymatic hydrolysis of aryl sulfates (*3,4*), and spontaneous hydrolyses were found to proceed via the unimolecular cleavage of S–O bond (*5*).

Congdon and Bender have reported that the hydrolyses of 4-nitrophenyl and 2,4-dinitrophenyl sulfates were accelerated by the addition of cycloamyloses (*6*). They suggested that the rate acceleration was brought about by both induced strain within a substrate molecule and a microsolvent effect due to the cycloamylose cavity, rather than by the covalent participation of secondary hydroxyl groups situated in the interior cavity of the host molecule (*6*).

As a consequence of the increasing interest in the development of cyclic compounds as conventional enzyme-models, Murakami and co-workers have recently reported that 10-hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) showed a significant catalytic effect, via formation of an inclusion complex between substrate and catalyst, on

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the decomposition of *p*-nitrophenyl carboxylates (*7,8*). Since Oxime-I has a nucleophilic oxime group and a suitably hydrophobic binding cavity, it is also expected to behave as a transferase model of another type. In this work, the catalytic effect of Oxime-I on the hydrolytic decomposition of 2,4-dinitrophenyl sulfate has been investigated in order to compare and contrast it with those of the cycloamyloses previously studied by Bender and Congdon (*6*).

EXPERIMENTAL

The potassium salt of 2,4-dinitrophenyl sulfate (DNPS) was prepared according to the method of Fendler and Fendler (*5*), but with some modification. Chlorosulfonic acid (0.076 mol, 5 ml) was added dropwise to a stirred solution of *N,N*-dimethylaniline (0.201 mol, 25.5 ml) in 27.0 ml of carbon disulfide cooled by an ice bath. After slowly warming to 37°C, 2,4-dinitrophenol (0.0543 mol, 10.0g) was added and the reaction mixture was stirred for 9 hr at 41°C. After cooling to 6°C, 55 ml of 4 *M* potassium hydroxide was added very rapidly to the vigorously stirred and ice-cooled reaction mixture. The precipitate formed was filtered immediately, washed first with cold absolute ethanol and then with acetone, and dried *in vacuo* at ambient temperature, giving a crude yield of 11.5 g. The crude product contained inorganic salts and 2,4-dinitrophenol which were removed by further washing with absolute ethanol to remove the phenol, followed by dissolution in dry acetonitrile, filtration, and evaporation of the solvent to remove the inorganic salts. White crystalline potassium 2,4-dinitrophenyl sulfate was obtained in good yield. Its purity was established by infrared and uv-visible spectrophotometry and by elemental analysis: Calcd for $C_6H_3O_8N_2SK$: C, 23.84; H, 1.00; N, 9.27. Found: C, 24.21; H, 0.92; N, 9.30. 10-Hydroxy-11-hydroxyimino[20]paracyclophane (Oxime-I) was the same as that used in previous studies (*7,8*). Cycloheptaamylose (*β*-CD) was a gift of the Research Institute of Teijin Co., Ltd., and was purified by recrystallization from hot water (*9*): dp. 269–270°C. *Anal.* Calcd. for $C_{42}H_{70}O_{35}$: C, 43.08; H, 6.36. Found: C, 42.94; H, 6.41. Other inorganic and organic reagents were commercially purchased as analytical grade and were used without further purification.

The spectrophotometric procedures used for the kinetic determinations have been described previously in the literature (*3–5*). A Shimadzu–Bausch and Lomb Spectronic 88 or a Hitachi 124 recording spectrophotometer equipped with a thermostatted cell (±0.1°C) was used. Spectrophotometric analysis of DNPS in stock solutions indicated that more than 95% of the initial salt remained as the unhydrolyzed sulfate ester. For all the systems containing Oxime-I and/or other additives such as detergents, no precipitation occurred during the course of the kinetic runs. Because of the rather poor solubility of Oxime-I in aqueous media, higher concentrations of Oxime-I could not be attained. Therefore, most of the experimental runs were carried out under conditions where the concentration ratio of Oxime-I to substrate was close to or less than two. Under these reaction conditions, good first-order kinetics were attained only over the range of 30–40% conversion of the substrate, after which the first-order rate plots deviated negatively due to formation of oxime *O*-sulfonate. Pseudo-first-order rate constants, k_{obs}, were usually calculated from data obtained during the first

halflife. Determination of the spectral change for the reaction of DNPS with Oxime-I was run on a Hitachi 323 recording spectrophotometer.

RESULTS AND DISCUSSION

Second-order rate constants for the liberation of 2,4-dinitrophenolate ion in the presence or absence of two macrocyclic compounds, Oxime-I and β-CD, are given in Table 1. At relatively lower concentrations of β-CD no significant rate acceleration was

TABLE 1

EFFECTS OF MACROCYCLIC COMPOUNDS IN THE HYDROLYTIC DECOMPOSITION OF 2,4-DINITROPHENYL SULFATE AT 40.0°C AND $\mu = 0.10$ (KCl) IN 0.99% (v/v) ACETONITRILE–9.9% (v/v) ETHANOL WITH 0.100 *M* SODIUM HYDROXIDE[a]

10^5 [DNPS] (*M*)	10^5 [Macrocycle] (*M*)	k'_u (M^{-1} min^{-1})	$10^{-2}k_c$ (M^{-1} min^{-1})	k_c/k_u^1
1.03	None	0.18 ± 0.03	—	—
1.03	Oxime-I: 1.97	—	3.0 ± 0.3	1,600
1.03	Oxime-II: 1.99	—	0.10 ± 0.25	60
1.03	β-CD: 30.4	—	0.03 ± 0.02	20

[a] Each observed first-order rate constant (k_{obs} or k'_{obs}) is estimated to be accurate within ±3%, and experimental error agrees within ±3% for duplicated runs. The second-order rate constant for the specific base-catalyzed hydrolysis, k'_u, is calculated from the observed pseudo first-order rate constant, according to $k_{obs}/[HO^-]$. The second-order rate constant for the macrocycle catalysis, k_c, is calculated according to $(k'_{obs} - k_{obs})/$[macrocycle], where k'_{obs} stands for the observed pseudo first-order rate constant of the reactions with macrocyclic catalysts.

observed. This may be primarily the consequence of the considerably weak interaction between β-CD and DNPS ($K_s \simeq 2$–3×10^{-2} *M*) (*6*). Thus, in order to obtain a measurable rate acceleration, it was necessary to use a large excess of β-CD (more than 30 times that of the substrate, see Table 1). In the system with Oxime-I, on the other hand, rate acceleration was observed even at relatively low concentrations of Oxime-I. When Oxime-II, 2-hydroxycyclodecanone oxime, was used instead of Oxime-I, no significant catalytic effect was observed. This result is explicable in terms of the smaller size of the cavity into which DNPS can be incorporated (*7,8*).

That the catalytic effects of both macrocycles, β-CD and Oxime-I, are primarily due to the hydrophobic interaction (*6*) between substrate and catalyst was confirmed by the experiments carried out in the presence of a detergent in addition to either of the macrocycles. Table 2 shows effects of Im-I (*N,N*-dimethyl-*N*-hexadecyl-*N*-(4-imidazolium)methylammonium dichloride) and CTAB (hexadecyltrimethylammonium bromide) as a cofactor in the β-CD- or Oxime-I-catalyzed hydrolysis of DNPS. The aqueous micellar catalysis by CTAB upon the hydrolysis of DNPS has been investigated by Fendler and co-workers (*4*). It is of interest that CTAB can catalyze the reaction even at concentrations below its cmc (*4*), as seen in Table 2. Nevertheless, the rate of DNPS decomposition, which is catalyzed by macrocyclic compounds alone,

TABLE 2

EFFECTS OF COFACTORS IN THE HYDROLYTIC DECOMPOSITION OF 2,4-DINITROPHENYL SULFATE WITH MACROCYCLIC COMPOUNDS IN 0.99% (v/v) ACETONITRILE–9.9% (v/v) ETHANOL WITH 0.100 *M* SODIUM HYDROXIDE AT 40.0°C[a]

10^5 [Macrocycle] (*M*)	10^5 [Cofactor] (*M*)	$10^3 k_{obs}$ (min^{-1})
None	None	17.8
None	CTAB: 1.99	24.9
None	Im-I: 2.09	25.1
β-CD: 40.6	None	19.5
β-CD: 40.6	CTAB: 1.99	18.7
β-CD: 40.6	Im-I: 2.09	21.0
Oxime-I: 1.97	None	23.5
Oxime-I: 1.97	CTAB: 1.99	19.5
Oxime-I: 1.97	Im-I: 2.09	21.0

[a] Initial concentration of the substrate was 1.030×10^{-5} *M*.

is inhibited upon the addition of CTAB, though to a minor extent. Im-I also exhibits a similar effect. Since Im-I has a nucleophilic imidazole group, it would be expected to behave as a catalyst for the decomposition of sulfate ester (*4*). Nevertheless, in the presence of both catalysts, Im-I and β-CD or Oxime-I, rate accelerations greater than those in the presence of these respective catalysts used independently could not be obtained. This is attributable to the competitive inclusion of the cofactor and substrate molecules into the macrocyclic cavity, with the incorporation of the former predominating over that of the substrate due to its greater hydrophobic nature (*10*). Hence, these results suggest that the rate acceleration of the hydrolytic decomposition of DNPS with both macrocycles is primarily due to the extent of hydrophobic incorporation.

There are two reasons for the difference in catalytic efficiency between Oxime-I and β-CD: (i), the tighter incorporation of DNPS into the Oxime-I cavity than that into the β-CD, causing a larger strain within the substrate molecule, and/or a larger microsolvent effect caused by the greater hydrophobicity of the former cavity relative to the latter; and (ii), the additional effect due to the strong nucleophilic character of the oxime group within the inclusion complex, involving the covalent participation of the catalyst in the course of sulfate transfer which was not seen in the β-CD-catalysis (*6*). First, judging from the reaction of *p*-nitrophenyl hexadecanoate with Oxime-I (*8*) one may assume that Oxime-I has greater binding properties than those of cycloamyloses, due to its strongly hydrophobic paracyclophane skeleton (*11*). The CPK molecular model also indicates that the inner size of Oxime-I cavity (5–5.5 Å) (*11*) is comparable to that of cyclohexaamylose (4.5 Å) and less than that of cycloheptaamylose, β-CD (~7.0 Å) (*11*). The slightly larger catalytic effect of Oxime-I, therefore, may be ascribed to the tighter inclusion of the sulfate into the efficient hydrophobic Oxime-I cavity, relative to that of β-CD. Even in about 10% aqueous organic solvent, saturation-type kinetics were obtained as a function of concentration of Oxime-I. Using the data in

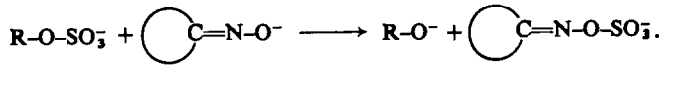
TABLE 3

OBSERVED FIRST-ORDER RATE CONSTANTS IN THE HYDROLYTIC DECOMPOSITION OF 2,4-DINITROPHENYL SULFATE WITH DIFFERENT CONCENTRATIONS OF MACROCYCLES AT 40.0°C AND $\mu = 0.10$ IN 0.99% (v/v) ACETONITRILE–9.9% (v/v) ETHANOL WITH 0.100 *M* SODIUM HYDROXIDE[a]

	10^5 [Macrocycle] (*M*)	$10^2 k_{obs}$ (min^{-1})
Oxime-I	0	1.79 ± 0.03
	0.394	1.88 ± 0.02
	0.592	2.03 ± 0.01
	0.789	2.09 ± 0.02
	0.986	2.13 ± 0.03
	1.97	2.27 ± 0.01

[a] Initial concentration of DNPS was 1.04×10^{-5} *M*.

Table 3, the rate constant for the sulfate group transfer reaction, $k_{transfer}$, and the binding constant, K_b, could be estimated graphically from the double reciprocal plots: for the reaction of DNPS with Oxime-I in 9.9% (v/v) ethanol–0.99% (v/v) acetonitrile at 40.0°C, $k_{transfer} = (2.65 \pm 0.05) \times 10^{-2}$ min^{-1} and $K_b = (6.6 \pm 0.2) \times 10^4$ M^{-1}, respectively. The rate constant for sulfate transfer from the aryl sulfate to the oxime group in the cavity is not necessarily large in comparison with that of simple alkaline hydrolysis under the same reaction conditions ($k_u = 1.8 \times 10^{-2}$ min^{-1}). This means that the catalytic effect may be mostly the result of strong incorporation (*7,8*). Secondly, Oxime-I has two nucleophilic centers, the oxime group and neighboring secondary hydroxyl group. Upon reviewing the results obtained in reactions between cycloamylose and aryl sulfates (*6*), and Oxime-I and *p*-nitrophenyl carboxylates (*7,8*), the latter nucleophile is not exclusively effective on the S–O cleavage of aryl sulfates. On the other hand, it is well known that sinigrin, which is a mustard oil glycoside and carries a moiety of oxime *O*-sulfonate, is hydrolyzed by myrosulfatase to afford merosinigrin, having a free oxime group (*12*). It has been recently reported, in addition, that 4-methylthiobutylaldoxime is a direct precursor of sinigrin (*13*) and that the sulfate moiety of sinigrin is derived from sulfate ion by sulfate transfer (*14*). Moreover, several stable oxime *O*-sulfonates have been isolated (*15*). These previous investigations suggest that the oxime group of Oxime-I may act as an acceptor for sulfur trioxide liberated from the ester to afford oxime *O*-sulfonate;



In order to examine the formation of Oxime-I *O*-sulfonic acid in the reaction of DNPS with Oxime-I, the determination of spectral change during the course of reaction has been made. Figure 1 shows the spectral changes in the 210–360-nm range for

(a), the simple alkaline hydrolysis of DNPS and (b), the reaction of DNPS with Oxime-I, respectively. In the simple alkaline hydrolysis of the sulfate ester, only one isosbestic point at 302 nm was observed (Fig. 1a), while in the presence of Oxime-I there appeared an additional isosbestic point at 227.5 nm, and the absorption at 219 nm gradually

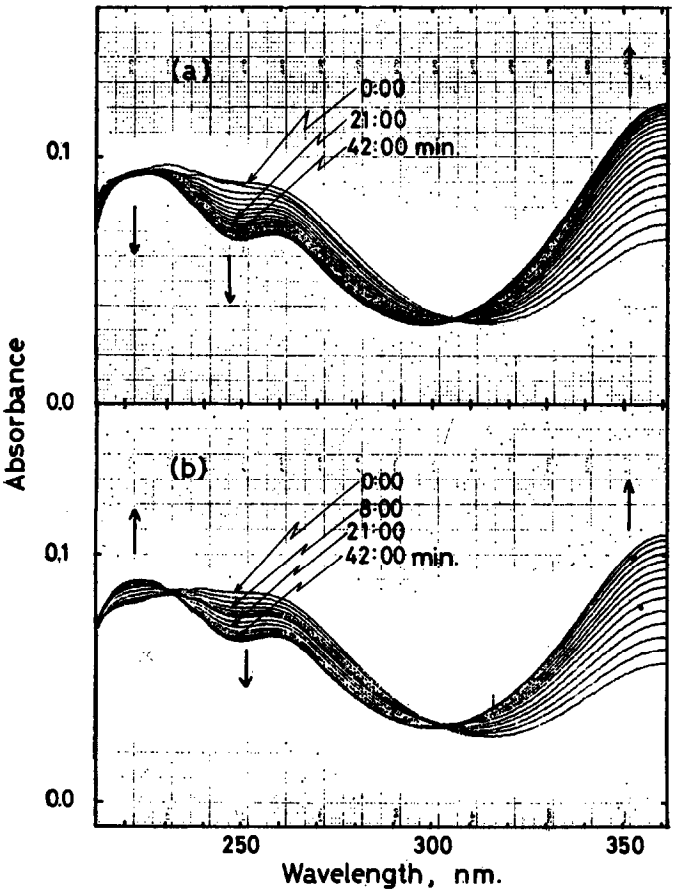


FIG. 1. Spectral changes in the 210–360-nm range during the course of reactions of 2,4-dinitrophenyl sulfate (DNPS); (a), at $\mu = 0.10$ (KCl), pH 12 (0.012 *M* sodium hydroxide ion-catalyzed hydrolysis of DNPS (0.960×10^{-5} *M*)) and (b), in the presence of Oxime-I (1.972×10^{-5} *M*). Vertical arrows in figures indicate the direction of change in intensity during the course of reaction.

increased in its intensity during the course of reaction (Fig. 1b). In order to understand the nature of the oxime *O*-sulfonate, acetoxime was used as a simple oxime moiety and acetoxime *O*-sulfonic acid[1] was prepared by the reaction of acetoxime with chlorosulfonic acid according to a method described in the literature (*16*), and the correlation

[1] Identification of acetoxime *O*-sulfonic acid: acetoxime *O*-sulfonic acid, which was isolated by ion exchange–column chromatography technique using Dowex 50W × 8 from the sulfonation mixture, was identified by tlc (silica gel), developed by methanol:benzene = 1:3 (v/v), and detected by oxidation with acidic bichromate; $R_f = 0.15$ for acetoxime *O*-sulfonic acid and 0.85 for acetoxime, respectively.

in spectra between acetoxime and its *O*-sulfonic acid was investigated in conjunction with those of the reaction mixture of DNPS and Oxime-I. As seen in Fig. 2, at pH 10–12 in 10.9% aqueous ethanol solution, acetoxime *O*-sulfonate has an absorption maximum at 223 nm, while acetoxime has no absorption maxima in the ultraviolet region under these conditions. Similar behavior has been observed for acetoxime and

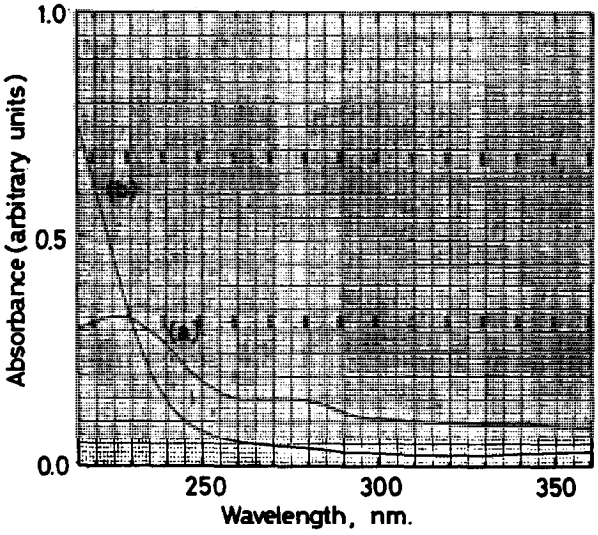


FIG. 2. The uv spectra of acetoxime *O*-sulfonic acid (a) and acetoxime (b) in 0.12 *M* sodium hydroxide at 20.0°C.

its *O*-acetate (*17*). Consequently, it is quite reasonable to assume that the Oxime-I *O*-sulfonate is responsible for the increased absorption at 219 nm during the course of the reaction of DNPS with Oxime-I in alkaline media.

Additional evidence for nucleophilic participation comes from studies on the effect of metal ions in the reaction. The addition of the copper(II) or nickel(II) ion to the system of Oxime-I catalysis profoundly retarded the release of the 2,4-dinitrophenolate ion (Table 4), while it did not have any effect on the uncatalyzed hydrolysis of DNPS.

TABLE 4

EFFECTS OF THE COPPER(II) AND NICKEL(II) IONS IN THE HYDROLYTIC DECOMPOSITION OF 2,4-DINITROPHENYL SULFATE CATALYZED BY OXIME-I IN 0.7% (V/V) ACETONITRILE–9.9% (V/V) ACETONE WITH 0.012 *M* SODIUM HYDROXIDE AT 40.0°C AND $\mu = 0.10$ (KCl)

10^5 [Oxime-I] (*M*)	10^5 [Metal ion] (*M*)	$10^3 k_{obs}$ (min^{-1})
None	None	7.63
0.989	None	10.0
0.989	Ni(II): 1.069	8.92
0.989	Cu(II): 0.904	7.29

In the case of the copper(II) ion, addition of an equimolar amount of the metal ion relative to the ester and Oxime-I decreased the reaction rate to less than that in the absence of the catalyst. A similar effect with the copper(II) ion has been previously observed in the acyl transfer from *p*-nitrophenyl carboxylates to Oxime-I (*7*). The coordination of the oxime group with metal ions should reduce its nucleophilicity and subsequently cause deceleration of the reaction rate.

In their entirety, these results strongly suggest that Oxime-I certainly undergoes covalent participation in its catalysis of the S–O fission of aryl sulfates through the incorporation of a substrate into the Oxime-I cavity. Thus, the present investigation provides and describes useful sulfotransferase- and aryl sulfatase-model systems.

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REFERENCES

1. B. SPENCER, *Biochem. J.* **69**, 155 (1958).
2. A. B. ROY, "The Enzymes," 3rd ed., Vol. 5, Chap. 1. Academic Press, New York, 1971.
3. S. J. BENKOVIC AND P. A. BENKOVIC, *J. Amer. Chem. Soc.* **88**, 5504 (1966).
4. J. H. FENDLER, E. J. FENDLER, AND L. W. SMITH, *J. Chem. Soc. Perkin II*, 2097 (1972).
5. E. J. FENDLER AND J. H. FENDLER, *J. Org. Chem.* **32**, 3852 (1968).
6. W. I. CONGDON AND M. L. BENDER, *Bioorg. Chem.* **1**, 424 (1971).
7. Y. MURAKAMI, J. SUNAMOTO, AND K. KANO, *Bull. Chem. Soc. Japan* **47**, 1238 (1974).
8. Y. MURAKAMI, J. SUNAMOTO, H. OKAMOTO, AND K. KAWANAMI, *Bull. Chem. Soc. Japan* **48**, 1537 (1975).
9. D. FRENCH, M. L. LEVINE, J. H. PAGUR, AND E. NORBERG, *J. Amer. Chem. Soc.* **71**, 353 (1949).
10. J. SUNAMOTO, H. OKAMOTO, K. TAIRA, AND Y. MURAKAMI, *Chem. Lett.*, 371 (1975).
11. D. W. GRIFFITHS AND M. L. BENDER, *Advan. Catalysis* **23**, 209 (1973).
12. Z. NAGASHIMA AND M. UCHIYAMA, *Nippon Nogei Kagaku Kaishi* **33**, 1068 (1959).
13. M. MATSUO, *Tetrahedron Lett.* 4101 (1968).
14. L. R. WETTER, *Phytochemistry* **3**, 57 (1964).
15. R. D. GAINES AND K. J. GOERING, *Arc. Biochem. Biophys.* **96**, 13 (1962)
16. (a) F. SOMMER AND H. G. TEMPLIN, *Ber.* **47**, 1221 (1914).
    (b) P. A. S. SMITH, *J. Amer. Chem. Soc.* **70**, 323 (1948).
17. J. SUNAMOTO, H. OKAMOTO, H. KONDO, AND Y. MURAKAMI, *Tetrahedron Lett.* 2761 (1975).