Treatment of II in Liquid Ammonia.—A sample (3.3 mg) of II was stirred in *ca.* 20 ml of liquid ammonia for 2 hr at −60°, after which time the solution was allowed to evaporate to dryness at the boiling point. Residual ammonia was removed *in vacuo* and the product was subjected to partition chromatography on Sephadex G-25 in solvent system B. Two peaks were detected at 280 and 300 nm with R_f values of 0.19 and 0.15, the latter being the major peak and corresponding to peptide I. The smaller peak with R_f 0.19 was apparently the same aforementioned side product and represented about 19% of the total detectable material on the chromatogram.

In a second run a sample (1.9 mg) of II was treated in the same manner with the exception that hydroxylamine hydrochloride (16.7 mg) was present. Partition chromatography in the same manner gave a major peak with R_f 0.15 corresponding to peptide I and a very small peak with R_f 0.19 corresponding to side product. The latter represented about 5% of the total detectable material in the two peaks.

Registry No.—N^{α}-*tert*-Butyloxycarbonyl-N^{i}-formyltryptophan dicyclohexylamine salt, 40463-72-7; N^{i}-formyltryptophan hydrochloride, 38023-86-8; peptide I, 40463-74-9; peptide II, 40463-75-0.

Acknowledgment.—We thank Mr. Kenway Hoey and Mr. W. F. Hain for their skilled technical assistance.

In Vitro Decomposition of *S*-Methylmethioninesulfonium Salts

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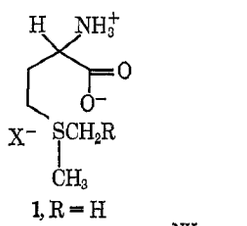
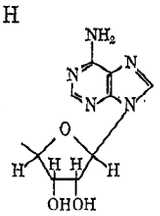
The *in vitro* decomposition of *S*-methylmethioninesulfonium salts (SMM) was studied in neutral, basic, and acidic aqueous solutions. The previously reported formation of dimethyl sulfide and homoserine (*via* its lactone) was verified. A new mode of self-destruction of SMM was discovered, *i.e.*, a nucleophilic attack by the dimethyl sulfide on one of the methyl groups of SMM with formation of trimethylsulfonium salt and methionine. The intermolecular demethylation of SMM was favored over the intramolecular decomposition to homoserine lactone with increasing acidity of the medium. Sodium thiosulfate effectively demethylates SMM in aqueous solution.

The *S*-methylmethioninesulfonium salts (SMM, **1**), the analogs of "active methionine" or *S*-adenosylmethioninesulfonium salts[1,2] (SAM, **2**), are of considerable interest biologically and medicinally. SMM is enzymatically synthesized from SAM and methionine in jack bean roots,[3] and can in turn be utilized as substrate by several methyl transferases.[4,5] SMM is widely distributed in nature and has been reported as a constituent of milk,[6] potatoes,[7] sweet corn,[8] soybean,[9] asparagus,[10] and cabbage.[11] Several reports have implicated SMM (vitamin U) in the prevention of ulcers of shay in rats,[12] of ulcers and of certain hepatic disorders in humans,[13] and of dietary hypercholesterolemia in rabbits.[14]



1, R = H



2, R =

In vitro syntheses of various SMM salts have been described,[15−17] and the pK values of the chloride have been measured.[18] McRorie, *et al.*,[11] reported the formation of homoserine (**5**, Scheme I) and of its lactone **3** (as hydroiodides) when an aqueous solution of SMM iodide was heated for 12 hr in an autoclave at unspecified temperatures. Challenger and Hayward[10] studied the decomposition of SMM in hot aqueous alkaline solution and reported the formation of dimethyl sulfide, homoserine, and methionine sulfoxide, which they regarded as the result of an oxidation of methionine. These authors[10] assumed that SMM decomposed by two paths: (1) formation of dimethyl sulfide and homoserine; (2) formation of methanol and methionine. Subsequently, Witkop and his coworkers[19] provided evidence for the initial formation of homoserine lactone (**3**) in the decomposition of SMM.

This paper describes an investigation of the behavior of SMM salts in aqueous solutions at neutral, basic, and acidic pH's, as a necessary first step in the elucidation of the much more complex behavior of SAM.

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(4) J. E. Turner and S. K. Shapiro, *Biochim. Biophys. Acta*, **51**, 585 (1961).

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(10) F. Challenger and B. J. Hayward, *Chem. Ind. (London)*, 729 (1959).

(11) R. A. McRorie, G. L. Sutherland, M. S. Lewis, A. D. Barton, M. R. Glazener, and W. Shive, *J. Amer. Chem. Soc.*, **76**, 115 (1954).

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(13) B. Colombo, *Minerva Med.*, 2944 (1960); *Chem. Abstr.*, **54**, 4881b (1960).

(14) (a) K. Nakamura and H. Ariyama, Tohoku *J. Agr. Res.*, **11**, 273 (1960); *Chem. Abstr.*, **55**, 7676d (1961); (b) *J. Agr. Res.*, **12**, 49 (1961); *Chem. Abstr.*, **55**, 27657d (1961); (c) *J. Arg. Res.*, **12**, 383 (1961); *Chem. Abstr.*, **57**, 5271b (1962).

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(18) W. A. Klee and S. H. Mudd, *Biochemistry*, **6**, 989 (1967).

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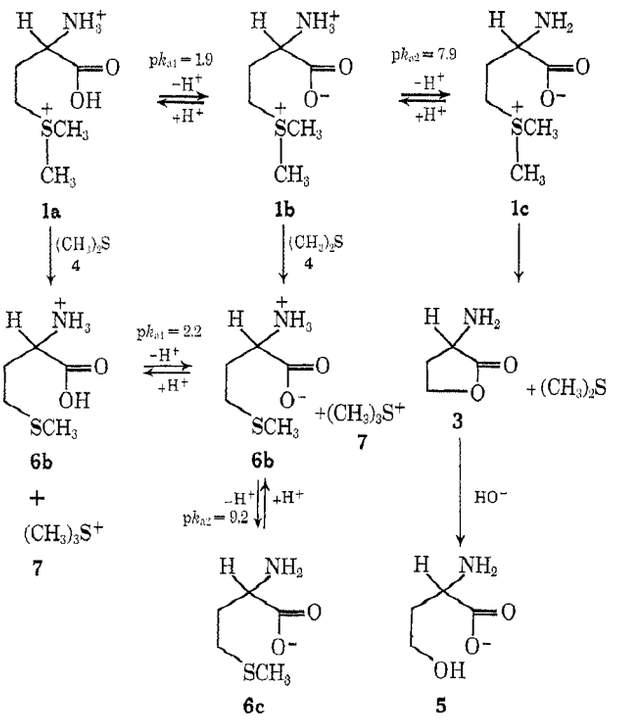
SCHEME I



Experimental Section

Starting Materials.—The DL amino acids were obtained from Mann Research. Trimethylsulfonium iodide (7, Scheme I) was made by the procedure of Pocker and Parker.[20] *S*-Methyl-DL-methioninesulfonium nitrate (1 nitrate) was made by addition of silver nitrate (3 mmol) to a solution of SMM chloride (1 chloride) (3 mmol) in 50 ml of water; the filtrate was evaporated to dryness under vacuum.

Experimental Conditions.—Tables I and II describe the experimental conditions and results. ^{1}H nmr spectra of authentic samples of the pertinent compounds were examined in D_2O and in H_2O solutions at 60 MHz. The chemical shifts of the CH_3 groups are listed in Table III. The composition of the various mixtures was determined by combined tlc and ^{1}H nmr analyses.

Reaction of Methionine (6) with Dimethyl Sulfide (4). A. With Added HCl.—DL-Methionine (0.075 g) and 6 *N* HCl were mixed in an nmr sample tube to a volume of 1 ml (0.05 *M* solution). An excess of $(CH_3)_2S$ was introduced, and the tube was sealed and heated to 90°. The ^{1}H nmr spectra were examined at ambient temperature after various time intervals. The first evidence for the appearance of $(CH_3)_3S^+$ (7) was obtained after 5 days.

B. With Added H_3PO_4.—The above procedure was repeated using 6 *N* H_3PO_4 instead of HCl. The spectrum of $(CH_3)_3S^+$ (7) was first noticeable after 10 days.

Reaction of Methionine (6) with Sodium Thiosulfate (10).—D_2O was added to a mixture of methionine (0.075 g, 0.05 mmol) and sodium thiosulfate (0.124 g, 0.5 mmol) to a volume of 1 ml in an nmr sample tube. The sealed tube was heated to 90° and the ^{1}H nmr spectra were determined at ambient temperature. The Bunte salt, $CH_3SSO_3^-$ (11), was first detectable only after 10 days.

Results and Discussion

The results of this investigation can be discussed with reference to Scheme I. In neutral and in basic aqueous solutions, the main pathway for the decomposition of SMM leads to homoserine (5) and dimethyl sulfide (4) *via* homoserine lactone (3), as had been previously reported.[10,11,17,19] The rate of decomposition of SMM increases markedly with increasing pH (*cf.* Table I). The lactone 3 is formed[19] by an intramolecular neigh-

(20) Y. Pocker and A. J. Parker, *J. Org. Chem.*, **31**, 1526 (1966).

TABLE I

HALF-LIVES ($t_{1/2}$) FOR THE DISAPPEARANCE OF *S*-METHYLMETHIONINESULFONIUM CHLORIDE (SMM Cl) IN 1 *M* AQUEOUS SOLUTIONS AT 90°

Reaction conditions	$t_{1/2}$
SMM Cl at pH 7[a]	50 min
SMM Cl at pH 11[a]	17 min
SMM Cl at pH 1[a]	25 hr
SMM Cl + dimethyl sulfide + HCl[b]	5 hr
SMM Cl + dimethyl sulfide[c] + methanol + HCl	100 min
SMM nitrate + dimethyl[d] sulfide + H_3PO_4	5 hr
SMM Cl + sodium thiosulfate[e]	20 min

[a] One millimole (0.200 g) of SMM Cl was diluted to a volume of 1 ml with 0.1 *M* NaOH, 1 *M* NaOH, and 1 *M* HCl, respectively, to give solutions with approximate pH's of 7, 11, and 1 in nmr sample tubes. The sealed tubes were heated to 90° and the ^{1}H nmr spectra were determined at 25° at various times. In the experiments at pH 11, the reaction was quenched by immersion of the tube in a Dry Ice–acetone bath prior to examination of the ^{1}H nmr. At the end of the reactions, the contents of the tubes were analyzed by tlc as indicated in Table II. [b] One millimole of SMM Cl was dissolved in 6 *N* aqueous HCl to a volume of 1 ml in a nmr sample tube. An excess of $(CH_3)_2S$ was introduced, and the sealed tube was heated to 90°. The ^{1}H nmr were examined at 25° at various times. [c] A mixture of 0.5 mmol of SMM Cl, 2.5 mmol of $(CH_3)_2S$, and 86 μl (1 mmol) of 38% aqueous HCl was diluted to a volume of 1 ml with methanol in an nmr sample tube. The sealed tube was kept at 90°, and the nmr spectra were determined at 25°. [d] As in *b* above except that SMM Cl was replaced by the nitrate salt and the HCl was replaced by 6 *N* H_3PO_4. [e] D_2O or water was added to a mixture of SMM Cl (0.200 g, 1 mmol) and sodium thiosulfate (0.248 g, 1 mmol) to a volume of 1 ml. The pH of this solution was 4.9. The sealed tube was heated to 90° and the ^{1}H nmr spectra were examined at 25° at various times.

TABLE II

THIN LAYER CHROMATOGRAPHY[a] OF THE PRODUCTS INVOLVED IN THE *in Vitro* DECOMPOSITION OF *S*-METHYLMETHIONINESULFONIUM CHLORIDE (1 Cl) IN AQUEOUS SOLUTIONS

		R_f values	
No.	Compd	Developing solution 1[b]	Developing solution 2[c]
1 Cl	*S*-Methyl-DL-methionine-sulfonium chloride	0.47	0.07
5	DL-Homoserine	0.61	0.50
6	DL-Methionine	0.80	0.70
8	DL-Homocysteine	0.67	0.70
9	DL-Homocystine	0.49	0.12
	Mixture from reaction at pH 7[d]	0.47, 0.61, 0.80	0.07, 0.49, 0.68
	Mixture from reaction at pH 11[e]	0.47, 0.62, 0.80	0.08, 0.50, 0.70
	Mixture from reaction at pH 1[f]	0.48, 0.63, 0.82, 0.68	0.07, 0.51, 0.70, 0.12

[a] The tlc plates were Eastman 6065 cellulose. The spots were developed with ninhydrin. [b] Developing solution 1: $CHCl_3$–CH_3OH–17% NH_4OH, 2:1:1 v/v. [c] Developing solution 2: *n*-C_4H_9OH–$(CH_3)_2CO$–$(C_2H_5)_3N$–H_2O, 10:10:2:5 v/v. [d] After 6.5 hr at 90°. Concentration of SMM = 0.11 *M*. (Initial SMM concentration = 1.0 *M*.) Major product was homoserine; minor product was methionine. [e] After 1.5 hr at 90°. Concentration of SMM = 0.045 *M*. (Initial SMM concentration = 1.0 *M*.) Major product was homoserine; minor product was methionine. [f] After 65 hr at 90°. Concentration of SMM = 0.26 *M*. (Initial SMM concentration = 1.0 *M*.) Major product was methionine; minor products were homoserine, homocysteine, and homocystine.

TABLE III

CHEMICAL SHIFTS OF CH_3 GROUPS[a]

Compd	λ, ppm
SMM Cl (1 Cl)	6.94
$(CH_3)_2S$ (4)	7.88
$(CH_3)_3S^+$ (7)	7.04
Methionine (6)	7.84
$^-OSO_2SCH_3$ (11)	7.35

[a] In parts per million from DSS = 10 (τ values), D_2O as solvent.

boring group participation[21a] of the carboxylate function (the nucleophile), and it is reasonable to expect higher nucleophilicity in the unprotonated form of SMM (**1c**) than in the zwitterion form (**1b**) owing to the respective charge distributions. For this reason, and taking into consideration the data on pK_a's of SMM, it seems probable that most or all of the lactone **3** arises from unprotonated SMM (**1c**) at all pH's above *ca.* 6. [In Scheme I, the acid–base equilibria for homoserine (**5**) and its lactone **3** have been omitted.]

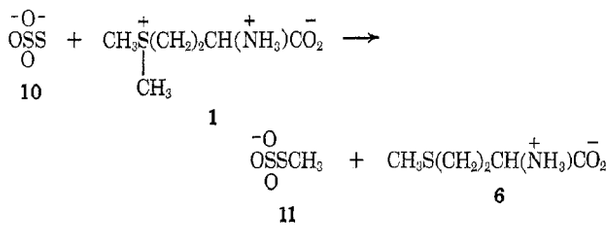
A second pathway for the disappearance of SMM can be detected in the neutral and in the basic solutions, namely, a nucleophilic attack by dimethyl sulfide (**4**) on the methyl group of SMM (**1**). This pathway, which represents the demethylation of SMM, leads to methionine (**6**, *cf.* Tables II and III) and trimethylsulfonium salt (**7**, *cf.* Table III). The overall rate of disappearance of SMM decreases significantly with decreasing pH (*cf.* Table I). This is due to a decrease in the rate of formation of lactone **3** and dimethyl sulfide (**4**) in acidic media, which is reasonable since under those conditions the less reactive zwitterion form of SMM (**1b**) is the source of the lactone (**3** in protonated form). (The amount of lactone formed from diprotonated SMM, **1a**, is probably negligible.) The specific reaction rate for the demethylation of SMM by dimethyl sulfide must be relatively large, because dimethyl sulfide has very little solubility in water, and yet the sulfide is quite effective in demethylating SMM (*cf.* Table I). (Note also the significant effect of methanol, whose role is mainly to increase the solubility of dimethyl sulfide in water.)

The formation of trimethylsulfonium salts (**7**) in the decomposition of SMM constitutes a novel observation; it accounts for the previously observed[10,17] formation of methionine in this decomposition. We failed to detect the formation of any methanol[10] at any pH from 1 to 11. Likewise, we found no methonine sulfoxide[10] in any of our experiments.

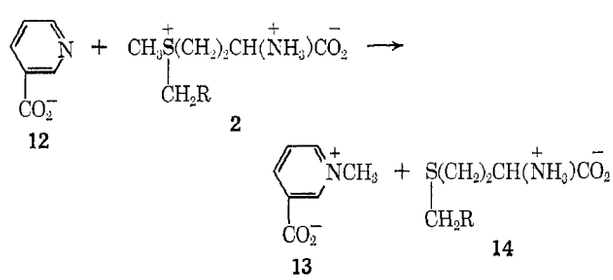
Demethylation by dimethyl sulfide plays an important role in the decomposition of SMM at pH's below 7. In this reaction, methionine (**6**) is the leaving group of a substitution by sulfur on carbon. It is reasonable to expect that the diprotonated form of methionine (**6a**) should be a better leaving group than the zwitterion **6b** or the unprotonated form **6c**, from their respective charge distributions. At the lowest pH's studied, most of the methionine (**6**) probably comes from diprotonated SMM (**1a**), while at higher pH's increasing amounts presumably arise also from the zwitterion **1b**. These considerations should be relevant to further analysis of the mechanisms of enzymatic methyl transfers from SMM (**1**) and from SAM (**2**).

To demonstrate that the demethylation of SMM chloride (**1** Cl) occurred by a nucleophilic attack of sulfur rather than chloride ion (which could give methyl chloride and thence trimethylsulfonium chloride), we determined the rate of demethylation of SMM nitrate (**1** NO_3) by dimethyl sulfide using H_3PO_4 as the acid, since both nitrate and phosphate are poor nucleophiles. The rate of disappearance of SMM was about the same as in the SMM chloride experiment (*cf.* Table I).

Thiosulfate (**10**) is a powerful nucleophile,[21b] and indeed it proved to be a most efficient demethylating agent for SMM (**1**), the products being methionine (**6**)



and the methyl Bunte[21c,22] salt, **11**. The facile *in vitro* demethylations of SMM (**1**) by sulfur nucleophiles, which compete favorably with the intramolecular decomposition to homoserine lactone (**3**) and dimethyl sulfide (**4**), find their counterpart in the enzymatic demethylation of SAM (**2**) by nitrogen nucleophiles as in the biosynthesis of trigonelline[2b] (**13**), where the by-product is *S*-adenosylhomocysteine (**14**).



CH_2R = 5′-adenosyl

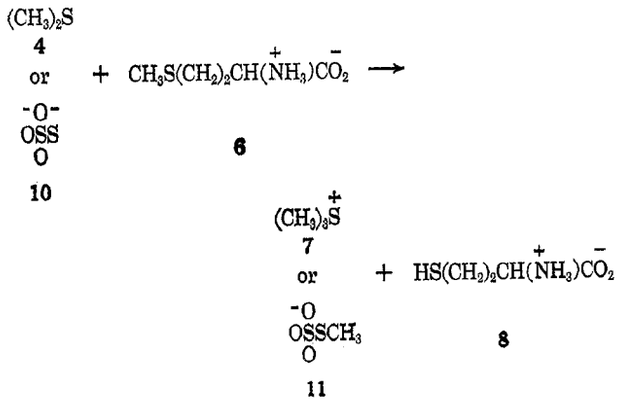
The corresponding intramolecular decomposition of SAM (**2**) would have given homoserine lactone (**3**) and *S*-methyl-5′-thioadenosine, CH_3SCH_2R (CH_2R = 5′-adenosyl). The enzyme function could be partly directed to discouraging this intramolecular decomposition and to favoring the intermolecular demethylation, both of which could be simultaneously achieved by the protonation of SAM (**2**), since the higher the state of protonation of the amino acid, the lower the nucleophilicity of the carboxyl function (lactone formation) and the higher the methylating power of the sulfonium group (*i.e.*, the better the leaving group, **14** or protonated **14**) as discussed above.

As shown in Table II, homocysteine (**8**) and its oxidation product homocystine (**9**) were observed as very minor by-products of the decomposition of SMM (**1**) in acidic solutions. Independent experiments showed that the formation of homocysteine (**8**) and trimethylsulfonium salt (**7**) from the reaction of methionine (**6**) with dimethyl sulfide (**4**) is extremely slow (see Experimental Section). Probably this reaction is susceptible

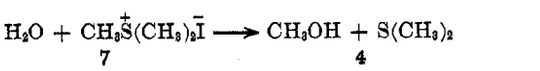
(21) (a) J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure," McGraw-Hill, New York, N. Y., 1968, p 263; (b) p 328; (c) p 330.

(22) J. Kice, *J. Org. Chem.*, **28**, 957 (1963).

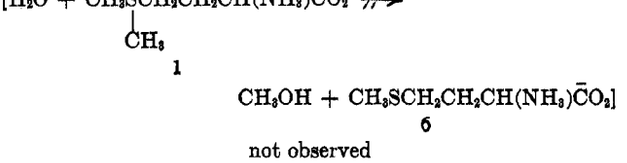
to acid catalysis in the form of protonation of the sulfur of methionine, since HCl seems to be more effective than H_3PO_4 at comparable normalities. As expected, thiosulfate **10** also caused the slow demethylation of methionine (**6**).



In conclusion, the formation of the lactone **3** as an intermediate in the hydrolytic decomposition of SMM (**1**) into homoserine (**5**) and dimethyl sulfide (**4**) is in accord with the observation that the rate of disappearance of SMM in water at neutral pH is significantly faster than solvolysis of trimethylsulfonium iodide[20] (**7**) under comparable conditions.



A neighboring group participation by the carboxylate anion would explain why the nucleophilic substitution at the methylene group of SMM (**1**) is faster than the substitution at the methyl group of the trimethylsulfonium cation (**7**). Moreover, the intermediacy of the lactone **3** also accounts for the nonoccurrence of the hydrolytic pathway for SMM that leads to methanol and methionine.



As the nucleophile becomes more effective, *i.e.*, when dimethyl sulfide is involved, the occurrence of the intermolecular nucleophilic substitution at the methyl group, with formation of trimethylsulfonium salt and methionine, becomes competitive with the intramolecular substitution at the methylene group.

Registry No.—**1** chloride, 3493-12-7; **1** nitrate, 33515-34-3; **4**, 75-18-3; **5**, 1927-25-9; **6**, 59-51-8; **7**, 676-84-6; **8**, 454-29-5; **9**, 870-93-9; **10**, 7772-98-7; **11**, 40463-71-6.

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Functionalization of Bis(phenylsulfonyl)methane

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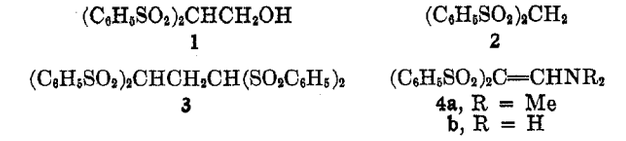
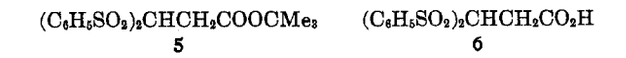
A convenient method is described for the single-carbon functionalization of bis(phenylsulfonyl)methane *via* thiomethylation with *N*-(benzoylthiomethyl)piperidine hydrochloride (**9**). The thiomethyl derivative **10** was easily converted to olefin **12** and thence to the disulfone alcohol **1**. In studying a similar approach to the tosyl analog of **12** some discrepancies with earlier structural assignments were noted and clarified.

As a possible precursor of reagents suitable for the development of new amino-protecting groups,[1] a disulfone alcohol such as **1** was of considerable interest. A readily available, logical precursor of **1** is **2** and

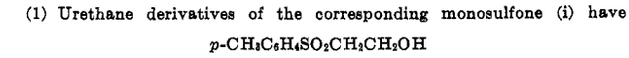


therefore a general study of the one-carbon functionalization of **2** was undertaken. Of the various techniques studied, only one proved suitable for the conversion to **1**. The most direct route, aldol condensation of **2** with formaldehyde,[1a] gave only the bis adduct **3**, which was also obtained as the sole product by alkylation of metallic salts of **2** with chloromethyl ether or *N*-chloromethylphthalimide or by application of the Mannich reaction to **2**.[2] Alkylations of the anion of **2** by means of *tert*-butyl α-bromoacetate or bromoacetic acid readily gave **5** and **6**, respectively. However, neither



of these compounds lent itself readily to conversion to **1** because of the presence of the extra carbon atom. Single-carbon functionalization of **2** was achieved *via*

(1) Urethane derivatives of the corresponding monosulfone (**i**) have been recommended as amino-protecting groups removable by base-catalyzed β elimination [A. T. Kader and C. J. M. Stirling, *J. Chem. Soc.*, 258 (1964)]. Use of the disulfone alcohol was expected to lead to much greater base sensitivity. For other examples of protective groups based on β-elimination processes, see (a) L. A. Carpino and G. Y. Han, *J. Amer. Chem. Soc.*, **92**, 5748 (1970); *J. Org. Chem.*, **37**, 3404 (1972); (b) T. Wieland, G. J. Schmitt, and P. Pfaender, *Justus Liebigs Ann. Chem.*, **694**, 38 (1966); (c) E. Wünsch and R. Spangenberg, *Chem. Ber.*, **104**, 2427 (1971).



(1a) NOTE ADDED IN PROOF (MAY 7, 1973).—After the submission of this work a paper appeared [H. Stetter and B. Riberi, *Monatsh. Chem.*, **103**, 1262 (1972)] which reported that the aldol condensation between **2** and formaldehyde gave 1,1-bis(phenylsulfonyl)ethene (**12**). However, the properties reported for **12** did not correspond to those we observed for this compound. Professor Stetter has kindly informed us that the compound obtained by his group is actually the isomeric 1,2-bis(phenylsulfonyl) analog.

(2) Lack of success with Mannich condensations involving **2** has previously been reported. See W. L. Nobles and B. B. Thompson, *J. Pharm. Sci.*, **54**, 576 (1965).