127.8, 127.6, 127.2, 126.9, 119.0, 113.1; MS, *m/e* (relative intensity) 540 (M^+), 511 (M^+ - HCO), 435 (M^+ - PhCO, 100%); UV λ_{max} (MeOH) 214, 313 nm.

1-Hydroxy-1,1-diphenyl-2-propanone (0.5 g, 2.2 mmol) in chloroform (20 mL) was stirred with concentrated sulfuric acid (2 mL) at 0 °C for 4 h. After an aqueous workup a red oil was obtained which on preparative thin-layer chromatography (silica gel/benzene) gave the following compounds. 1,2-Diphenyl-2-propen-1-one (20%; R_f 0.79): mp 28 °C [lit. mp 28 °C];[40] IR (neat) 1680 cm^{-1} (C=O); ^1H NMR ($CDCl_3$, 60 MHz) 7.96 (2 H, d, J = 2.7 Hz), 7.27-7.64 (8 H, m), 6.07 (1 H, s), 5.64 (1 H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) 197.4 (C=O), 148.2, 137.1, 133.0, 129.9, 128.6; 128.4, 127.00, 120.7.[41] 6-[1-(1,1-Diphenyl-2-oxopropyl)-2-methyl-3-phenylbenzo[*b*]furan (R_f 0.55; 20%): mp 153-155 °C; IR (neat) 1710 cm^{-1} (C=O); ^1H NMR ($CDCl_3$, 60 MHz) δ 7.15-7.50 (18 H, m), 2.46 (3 H, s), 2.12 (3 H, s); UV λ_{max} (EtOH) 210, 234 (sh), 262 nm; MS, *m/e*[42] (relative intensity) 386, 373 (M^+ - $COCH_3$), 295, 252, 165, 43. Bis[1-(1,1-diphenyl-2-oxopropyl)]-substituted 2-methyl-3-phenylbenzo[*b*]furan (R_f 0.24; 51%): mp 145-148 °C; IR (neat) 1715 cm^{-1} (br, C=O); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.13 (3 H, s), 2.16 (3 H, s), 2.53 (3 H, s), 7.09-7.52 (27 H, m); UV λ_{max} (EtOH) 208, 234 (sh), 270 nm; MS, *m/e* (relative intensity) 581 (M^+ - CMe, 100%), 538 (M^+ - $2CCH_3$), 373, 165, 43.

α-Phenyl-α-(trifluoromethyl)benzenemethanol[43] (0.5 g, 2.00 mmol) in chloroform (50 mL) was stirred with concentrated sulfuric acid (2 mL) at 0 °C for 2 h. After an aqueous workup a yellow oil (0.5 g) was obtained which on preparative thin-layer chromatography ((silica gel/benzene)-hexane 1:1) gave 9-(trifluoromethyl)-9*H*-fluorene (25%; R_f 0.73): mp 95-97 °C [lit.[44] mp, 95.5-96.5 °C]; ^1H NMR ($CDCl_3$, 60 MHz) δ 7.10-7.73 (8 H, m), 4.54 (1 H, 9, $^1J_{F-H}$ = 9.5 Hz, CH-CF_3). Also obtained was unreacted starting material (50%; R_f 0.11).

Registry No. 6a, 89196-84-9; **6b**, 38252-89-0; **6c**, 87963-52-8; **6d**, 38252-97-0; **6e**, 38252-93-6; **6f**, 103192-03-6; 9-trifluoromethylfluorene, 1554-95-6; (2-oxo-1,1-diphenylpropyl)-2-methyl-3-phenylbenzofuran, 103150-67-0; bis(2-oxo-1,1-dimethylpropyl)-2-methyl-3-phenylbenzofuran, 103150-68-1; 1,2-diphenylprop-2-en-1-one, 4452-11-3; 2,3-diphenylbenzofuran, 13054-95-0; (2-oxo-1,1,2-triphenyl)-2,3-diphenylbenzofuran, 103150-69-2; 9-fluorenecarboxylic acid, 1989-33-9; 3,3,6,6-tetraphenyl-2,5-*p*-dioxanedione, 467-32-3; methyl 9-fluorenecarboxylate, 3002-30-0; 2-(α,α-diphenylacetic acid)-9-fluorenecarboxylic acid dimethyl ester, 103192-04-7; *N*,*N*-dimethyl-9-fluorenecarboxamide, 31859-84-4.

(40) Kopinski, R. P.; Pinhey, J. *Aust. J. Chem.* **1983**, *36*, 311.
(41) An authentic sample of 1,2-diphenyl-2-propen-1-one was obtained by phosphorus pentoxide dehydration of 2-hydroxy-1,2-diphenyl-1-propanone and was shown by ^1H NMR, IR, and ^{13}C NMR spectroscopies and TLC to be identical with our sample.
(42) Cardillo, G.; Cricchio, R.; Merlini, L. *Tetrahedron* **1971**, 1875.
(43) Olah, G. A.; Pittman, C. U., Jr. *J. Am. Chem. Soc.* **1966**, *88*, 3310.
(44) Streitwieser, A., Jr.; Marchand, A. P.; Pudjaatmaka, A. H. *J. Am. Chem. Soc.* **1967**, *89*, 693.

Mechanisms for the Removal of Benzyl Protecting Groups in Synthetic Peptides by Trifluoromethanesulfonic Acid-Trifluoroacetic Acid-Dimethyl Sulfide

James P. Tam,* William F. Heath, and R. B. Merrifield

Contribution from The Rockefeller University, New York, New York 10021.
Received November 13, 1985

Abstract: The need to improve peptide synthesis has led to the development of less acidic and milder S_N2 deprotection conditions to remove benzyl protecting groups and to avoid reactive carbocations associated with the conventional S_N1 conditions. To further understand the relationships of both mechanisms and the acidity of the reaction, the S_N1 and S_N2 deprotection mechanisms for synthetic peptides with *O*-benzyl protecting groups were studied in a ternary mixture of trifluoromethanesulfonic acid (TFMSA)-trifluoroacetic acid (TFA)-dimethyl sulfide (DMS). Kinetic studies of the deprotection rate-acid profiles of *O*-benzylserine in sets of experiments containing predetermined amounts of DMS revealed sharp changeover points in the mechanism from S_N2 ($A_{AL}2$) to S_N1 ($A_{AL}1$) when the concentration of TFMSA in TFA was increased. Similar changeover points in mechanism were also found in the deprotection product-acid profiles of *O*-benzyltyrosine. The activities of DMS required for the S_N2 reaction were determined by ^1H NMR, and the acidities of the reaction media were calculated from the Yates-McClelland equation. The changeover points were found to be in the range where DMS activities were approaching zero. In general, the reaction mechanism depended on the activity of DMS and the acidity of the reaction mixture. The S_N1 deprotection mechanism predominated at high acidities and low DMS activities. S_N2 reaction mechanisms were observed at moderate acidities and high DMS activities. On the basis of the changeover points, a mechanism-reagent composition diagram could be constructed in the form of an equilateral triangle in which the S_N1 and S_N2 regions could be defined as a function of reagent composition. Furthermore, from the mechanistic considerations, a practical mixture for the S_N2 deprotection reaction was found to be TFMSA-TFA-DMS-*m*-cresol (10:50:30:10 (v/v)). For the deprotection of Trp(For)-containing peptides, the reagent was adjusted to TFMSA-TFA-DMS-*m*-cresol-ethanedithiol (10:50:30:8:2 (v/v)) so that the N^{in}-formyl could be removed concomitantly with other protecting groups. Both deprotection mixtures also converted Met(O) to Met efficiently.

The fundamental design of most protecting group strategies in peptide synthesis is based on the differential lability of protecting groups in acids.[1,2] A popular and widely used version is the combination of N^{α}-*tert*-butyloxycarbonyl and side-chain benzyl protection. Usually, a strong anhydrous acid in an S_N1 mechanism is employed in the final step to remove all the benzyl protecting groups.[3,4] Because of the strong acidity attendant with the S_N1

(1) Related papers of this work: (a) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *Tetrahedron Lett.* **1982**, *23*, 2939-2942. (b) Heath, W. F.; Tam, J. P.; and Merrifield, R. B. *J. Chem. Soc., Chem. Commun.* **1982**, 896-897. (c) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *Tetrahedron Lett.* **1982**, *23*, 4435-4438. (d) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6442-6455. (e) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *Int. J. Peptide Protein Res.* **1983**, *21*, 57-65. (f) Tam, J. P.; Heath, W. F. In *Proceedings of the 7th American Peptide Symposium*; Hruby, V. J., Rich, D. H.; Ed.; Pierce Chemical Co.: Chicago, 1983; pp 83-86.

(2) (a) Merrifield, R. B. *J.* Am. *Chem. Soc.* **1963**, *85*, 2149-2154. (b) Merrifield, R. B.; Vizioli, L. D.; Boman, H. G. *Biochemistry* **1982**, *21*, 5020-5031. (c) Anderson, G. W.; Blodinger, J.; Welcher, A. D. *J. Am. Chem. Soc.* **1952**, *74*, 5309-5312. (d) Schwyzer, R.; Sieber, P. *Nature* (*London*) **1963**, *199*, 172-174.
(3) Sakakibara, S. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1971; Vol. 1, pp 51-85.

0002-7863/86/1508-5242$01.50/0

condition, peptides with nucleophilic side chains are strongly susceptible to modifications by the carbocations generated under the S_N1 deprotection condition. These include alkylation, arylation, and acylation side products of methionine, cysteine, tryptopan, tyrosine, and asprartic and glutamic acids. These side reactions often pose impediments toward the synthesis of large and complex peptides. Recently, we have reported the mechanistic investigations and application of an S_N2 condition for the acidolytic removal of benzylic protecting groups using a mixture of dimethyl sulfide in a low HF concentration to minimize many side reactions associated with the generation of carbocations under the S_N1 deprotection condition.[1]

Another strong acid being used in the deprotection of synthetic peptides is trifluoromethanesulfonic acid (TFMSA). TFMSA is an even stronger acid (acidity function, $H_0 = -13$) than HF ($H_0 = -11$).[5] Unlike HF, which is volatile, TFMSA is a viscous liquid that is difficult to handle and is not as readily removable as HF. However, the use of TFMSA does not require special apparatus for its handling in the laboratory and may be more amenable for the large-scale industrial synthesis of peptides.

Since 1974, Yajima and his colleagues have pioneered in the application of the combination of TFMSA in trifluoroacetic acid (TFA) as the deprotecting agent for many peptide syntheses.[5] More recently, two groups, including Yajima and his co-workers, have further refined the TFMSA–TFA deprotecting system by the addition of thioanisole as a weak base to give a push–pull type of deprotection mechanism.[6,7] Clearly, the push–pull mechanism for TFMSA–TFA–thioanisole proposed by both laboratories with use of the "hard acid and soft base" concept of Pearson[8] is broadly accepted as the S_N2 mechanism of Ingold.[9] However, the relationship of the S_N1 and S_N2 mechanisms in such a mixture has not been defined. Furthermore, the recommended condition is more acidic than the safe operational limits expected for the S_N2 deprotection condition. More importantly, thioanisole has been found to be an alkyating agent at the moderate acidity of the S_N2 condition and thus may not be the optimal weak base.[1c] Since dimethyl sulfide (DMS) does not possess this property, it is a more suitable choice as a weak base for the S_N2 studies. In this paper, we describe the S_N1 and S_N2 mechanistic profiles of two model reactions in the ternary system containing TFMSA, TFA, and DMS, as well as the relationship between the acidity of the deprotection medium and the activity of the weak base (concentration of the free base). From the results of these studies, an optimal and rational deprotecting condition for the removal of benzylic protecting groups from synthetic peptides is described.

Results

Methods of Approach. The approach to our study followed that of the previous study of HF–DMS.[1] The rate and product profiles for the removal of the benzylic protecting groups in TFA–TFMSA–DMS mixtures were examined in the widest possible range of TFMSA and DMS concentrations. The alkyl–oxygen cleavage of *O*-benzylserine was chosen as the model reaction for the acid–rate studies since the acid lability of *O*-benzylserine is comparable to that of most other benzylic protecting groups.[10]

Table I. Deprotection Rates[a] of *O*-Benzylserine in TFMSA–TFA–DMS

% TFMSA	10^3k_1	% TFMSA	10^3k_1
0% DMS		10% DMS	
0	0.0003	0	0.0032
0.5	34.6	0.5	0.756
1		1	0.208
1.5	147	1.5	0.292
2	266	2	0.367
2.5		2.5	0.606
5% DMS		3	0.833
0	0.0005	4	
0.5		5	1.1
1	0.063	7.5	0.9
1.5		9	0.882
2	0.29	10	1.13
2.5		11	1.97
3	0.43	12	4.93
4	1.18	12.5	6.0
5	25	13	
7.5	385	14	
20% DMS		15	20.5
0	0.0016	17.5	
2.5	0.208	20	50.4
5	0.417	30% DMS	
7.5	0.684	0	0.0016
10	1.67	2.5	0.09
15	3.0	5	0.89
20	2.96	7.5	0.99
25	3.14	10	2.81
30	3.64	20	5.0
30	3.64	30	9.1
32.5	4.67	40	13.3
35	12.30	50	17.8
40	48	60	40
50% DMS		65	15
0	0.0006	68.75	18.8
10		40% DMS	
20	6	0	0.0009
30	13.3	2.5	0.01
40		5	0.75
50		10	0.73
		20	2.14
		30	5.76
		40	7.5
		50	15.4
		58.5	26.7

[a] Pseudo-first-order rates.

These results will provide a practical guide to the selection of an optimal deprotecting condition. Similarly, the acid–product profiles of *O*-benzyltyrosine are also studied to provide the knowledge of the extent of side reactions such as alkylation of nucleophilic side chains of amino acids by reactive carbocations. The side product, 3-benzyltyrosine, from the deprotection mixture of *O*-benzyltyrosine could only be derived by the S_N1 deprotecting mechanism,[11] and the acid–product profiles will provide a direct approach to the determination of the S_N1 and S_N2 mechanisms. Thus, both of these studies will provide a realistic assessment of the purity of the synthetic peptide products due to the deprotection process. Finally, to correlate the distinction of the mechanisms based on the rate and product profiles, the acidity of the reaction medium and the activity of DMS required to participate in the S_N2 mechanism are also determined as acid–activity profiles by ^1H NMR titration.[12]

(A) Kinetic Studies of Deprotection of *O*-Benzylserine. The kinetic experiments were performed at 0 °C in a large excess of TFMSA–TFA–DMS, and pseudo-first-order rate constants (k_1) were obtained. The product mixture containing Ser and Ser(Bzl)

(4) Lenard, J.; Robinson, A. B. *J. Am. Chem. Soc.* **1967**, *89*, 181–182.
(5) (a) Gillespie, R. J. *Acc. Chem. Res.* **1968**, *1*, 202–209. (b) Howells, R. D.; McCown, J. C. *Chem. Rev.* **1977**, *77*, 69–92.
(6) (a) Yajima, H.; Fujii, N., Ogawa, H.; Kawatani, H. *J. Chem. Soc., Chem. Commun.* **1974**, 107–108. (b) Irie, H.; Fujii, N.; Ogawa, H.; Yajima, H.; Fujino, M.; Shinagawa, S. *J. Chem. Soc., Chem. Commun.* **1976**, 922–923. (c) Yajima, H.; Fujii, N. *J. Am. Chem. Soc.* **1981**, *103*, 5867–5871. (d) Yajima, H.; Minamitake, Y.; Funakoshi, S.; Katayama, I.; Fujii, N.; Segawa, T.; Nakata, Y.; Yasuhara, T.; Natajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 344–348.
(7) (a) Kiso, Y.; Nakamura, S.; Ito, K.; Ukawa, K.; Kitagawa, K.; Akita, T.; Moritoki, H. *J. Chem. Soc., Chem. Commun.* **1979**, 971–972. (b) Kiso, Y.; Ukawa, K.; Nakamura, S.; Ito, K.; Akita, T. (1980) *Chem. Pharm. Bull. Jpn.* **1980**, *28*, 673–676.
(8) Pearson, R. G. *J. Am. Chem. Soc.* **1963**, *85*, 3533–3539.
(9) (a) Datta, S. C.; Day, J. N. E; Ingold, C. K. *J. Chem. Soc.* **1939**, 838–840. (b) Ingold, C. K. In *Structure and Mechanism in Organic Chemistry*; Cornell University Press: Ithaca, NY, 1972; pp 1128–1164.
(10) Erickson, B. W.; Merrifield, R. B. *J. Am. Chem. Soc.* **1973**, *95*, 3750–3756.
(11) (a) Erickson, B. W.; Merrifield, R. B. *J. Am. Chem. Soc.* **1973**, *95*, 3757–3763. (b) Yamashiro, D.; Li, C. H. *J. Org. Chem.* **1973**, *38*, 591–592. (c) For a review in side reactions, see: Bodanszky, M.; Martinez, J. *Synthesis* **1981**, 333–356.
(12) Rochester, C. H. *Acidity Functions*; Academic Press: New York, 1970; pp 21–71.

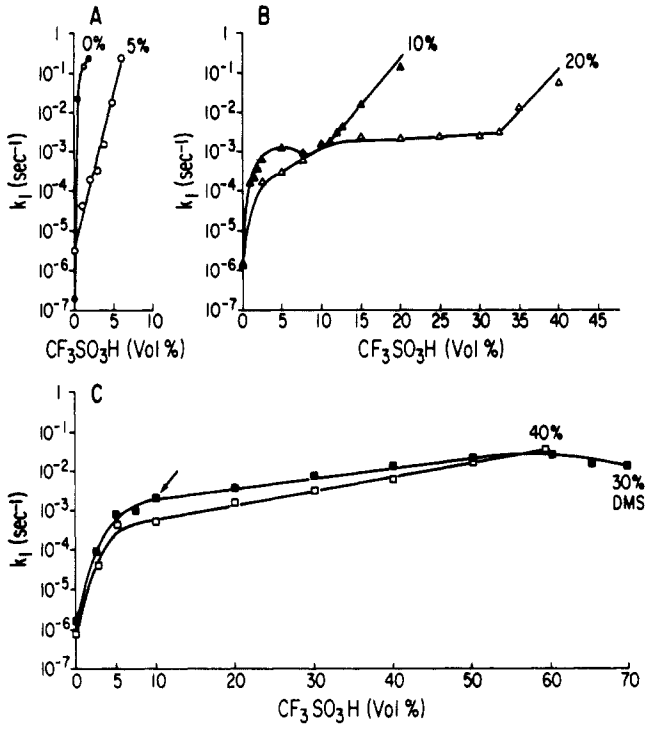

Figure 1. Kinetic rate–acid profiles of deprotection of Ser(Bzl) to Ser in CF_3SO_3H–CF_3CO_2H with fixed concentrations of dimethyl sulfide at 0 °C; K_1 is the pseudo-first-order rate constant in s^{-1}. Three types of rate profiles are shown (in vol % of dimethyl sulfide): A, type I, 0% and 5%; B, type II, 10% and 20%; C, type III, 30% and 40%.

was quantitated on the amino acid analyzer. Initial and average rate constants ($>10^{-2}$ s^{-1}) were measured at fixed concentrations of DMS (from 0 to 50% by vol) as the concentration of TFMSA was varied from 0 to the highest point experimentally possible, with the remaining volume made up with TFA (Table I). Each of the seven sets of experiments at fixed levels of DMS gave 6–22 rate constants which were then plotted as a function of TFMSA concentration (Figure 1). Within each rate profile, an observed sudden upward break in the curve was taken as the changeover point in mechanism from S_N2 to S_N1.

Three types of rate profiles were obtained. Type 1 (Figure 1A), obtained in O and 5% of DMS, showed a steady, and monotonical increase in rate with TFMSA concentration. There was no observable break and changeover point in the type I acid–rate profile. In the 0% DMS rate profile, the rate increased 10^5-fold from 0% of TFMSA–100% TFA ($k_1 = 3 \times 10^{-7}$ s^{-1}) to 0.5% TFMSA–99.5% TFA ($k_1 = 3.5 \times 10^{-2}$ s^{-1}) and more than 10^6-fold from 0 to 2.5% TFMSA (all concentrations are % by volume). Thus, the rate change of the Ser(Bzl) deprotection at zero DMS concentrations with TFMSA was 4500 s^{-1}/%. This rapid change of rates confirms that dilute TFMSA in TFA is a strong ionizing acid. More importantly, the steep acid–rate profile indicates the strong dependence of rate on the acid concentration and the S_N1 mechanism.

Type II (Figure 1B), in 10% and 20% of DMS, was characterized by a triphasic pattern. Initially, rates increased steadily with the increase in TFMSA concentration. Typically, the rate constants changed from 3×10^{-6} s^{-1} at 0% TFMSA to 2×10^{-3} s^{-1} at 5% TFMSA. The slope of the rate change with TFMSA concentration was about 100 s^{-1}/% but was considerably less steep than that of type I. In the middle phase, the rate profile entered into a steady and long plateau, with a slope of rate change of about 0.1 to 0.2 s^{-1}/% by vol of TFMSA. Finally, in the last phase, the rate sharply rose again with further increase of TFMSA, with a steep slope of 1.4 s^{-1}/% by volume of TFMSA. The beginning and middle phases of the type II rate profile are of the S_N2 type of mechanism, and the sudden upbreak in the rate at the last phase is the S_N1 type. The changeover point was taken to be point of the upbreak between the second and the third phase.

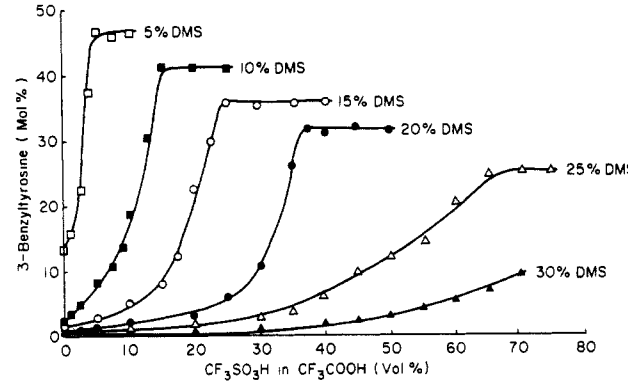

Figure 3. Product–acid profiles of 3-Bzl-Tyr from the deprotection of Tyr(Bzl) in CF_3SO_3H–CF_3CO_2H with fixed concentrations of dimethyl sulfide at 0 °C. The yield of Tyr can be calculated by difference.

Type III (Figure 1C), found in 30%, 40%, and 50% of DMS, was similar to type II except that the final sharp increase in rate characteristic of the changeover to the S_N1 mechanism was missing. Thus, the rate constants increased steadily with the initial addition of TFMSA but turned into a long plateau with further increase of TFMSA. This type of rate profile therefore produces predominantly the S_N2 deprotection mechanism and would be useful for synthetic purposes.

(B) Product Analysis of Deprotection of *O*-Benzyltyrosine. The kinetic reaction products from the TFMSA–TFA–DMS deprotection of *O*-benzyltyrosine were used for the plots of the acid–product profiles. Tyrosine, 3-benzyltyrosine, and benzyldimethylsulfonium salt produced from the deprotection mixture were quantitated by reverse-phase HPLC. The experimental procedure for studying the product analysis was similar to that for the Ser(Bzl) reaction, and predetermined amounts of DMS in eight sets of experiments from 5 to 40% of DMS (by volume) were used with increasing concentrations of TFMSA. To complete our data we have also examined mixtures containing 50 to 80% DMS (Table II).

The rate of *O*-benzyl cleavage of Tyr(Bzl) is about 80-fold faster than that of Ser(Bzl), and rate constants from Table I were used as a guide for all experiments of the product–acid profile studies. Most deprotection reactions were observed to be completed at 0 °C in 1 h. However, with DMS concentrations higher than 40% (Table II) the deprotection rates were slower and data were taken after 2 h. In the absence of TFMSA and in TFA alone, or when the rates were extremely slow (e.g., 70–80% DMS), the reactions were allowed to proceed for 1–7 days.

Since the products were analyzed only at end points, we also examined the stability of the products in the course of the deprotection (Figure 2 available in the supplementary material) to eliminate the possibility of side reactions such as methylation or sulfonation which might affect the results of our profiles. Thus, time points from 30 s to 90 min were taken to monitor the mol % of alkylation in different deprotection mixtures ranging from very acidic, high TFMSA concentrations to moderately acidic, low TFMSA concentrations. The results showed that the molar ratio between tyrosine and 3-benzyltyrosine remained constant (Figure 2), and methylation and other side reactions would not affect our results.

The yield of 3-benzyltyrosine formed as a result of the S_N1 process was plotted as a function of TFMSA concentration in a series of acid–product profiles (Figure 3). The observed amounts of 3-benzyltyrosine varied over 2250-fold from 0.02 to 45% (Table II). Another indicator product, benzyldimethylsulfonium salt which resulted from the weak base, DMS participating in the S_N2 reaction, was also plotted as a function of TFMSA concentrations (Figure 4). Both acid–product profiles (Figures 3 and 4) agreed well with each other and resembled those of acid–base titration curves.

Like the acid–rate profiles, three types of acid–product profiles were observed. Type I was characterized by the low DMS participating concentrations (<10%). In the absence of DMS and

Table II. Deprotection Products of *O*-Benzyltyrosine in TFMSA–TFA–DMS

vol % TFMSA	mol % 3BzlTyr	mol % BzlDMS
	0% DMS	
0	38	
5	45.8	
10	45	
15	45.4	
	5% DMS	
0	11.3	86
0.5	13.1	86
1	15.4	81
4	37.4	31
5	46	25
7.5	46.1	19.5
10	46.7	14.8
12.5	43.2	14.2
1	42	13.2
		13.8
	15% DMS	
0	3.2	94.3
5	3.7	93.8
10	5.2	90.7
15	8.2	87.8
17.5	13.9	85.3
20	22.5	68
22.5	29.8	38.3
25	36.3	33.1
30	35.3	25.2
	20% DMS	
0	1.3	95
5	1.5	94.4
10	2.1	90
20	3.2	86.5
25	6.7	83.3
30	10.9	78.6
32.5	25.4	62.6
35	30.8	48.5
37.5	33.1	44.5
40	34	42.8
45	34.7	41
50	32.5	40.9
60	34.5	38
		50
	30% DMS	
0	0.3	97.6
2.5	0.2	98
5	0.2	98
10	0.2	97
20	0.5	95.6
30	0.8	95
40	2.2	94
45	2.5	93
50	3.1	93
55	4	92
60	5.4	91
65	7.2	89
70	10.9	82
	50% DMS	
0	0.2	99
10	0.04	99
20	0.04	99
30	0.07	99
40	0.1	99
50	0.2	99
	70% DMS	
10	0.02	99
20	0.06	99
30	0.2	99
	10% DMS	
0	4.4	88
1	3.9	87
2.5	5.2	85.3
5	6.2	82.2
7.5	10.5	80.1
9	13.4	78.3
10	16.7	76.6
11	23	72.5
12.5	30.5	50.9
37	42	
15	40.6	35.2
20	42	16.1
25	41	14.9
	25% DMS	
0	0.6	96.9
2.5	0.5	97
5	0.5	96
10	1.2	95.6
20	1.8	94
30	2.6	94
40	6.7	89
45	9.7	84
47.5	12.5	82.2
15.4	77.3	
55	21.7	69.3
60	25.1	60.4
70	28.8	56.8
	35% DMS	
0	0.3	99
10	0.2	99
20	0.3	98
30	0.4	98
45	0.6	98
50	0.9	98
	40% DMS	
0	0.3	99
5	0.1	99
10	0.1	99
20	0.2	99
30	0.3	99
40	0.5	99
50	1.4	97
55	2	96
60	2.3	95
	60% DMS	
0	0.1	99
10	0.02	99
20	0.5	99
30	0.6	99
40	1.3	97
	80% DMS	
10	0.03	99
20	0.3	99

with or without TFMSA in the reaction mixture, the amounts of 3-benzyltyrosine averaged 42%. With low DMS concentrations such as the 5% DMS profile, the increase in 3-benzyltyrosine was steady and rapid with the increase in TFMSA concentration. At

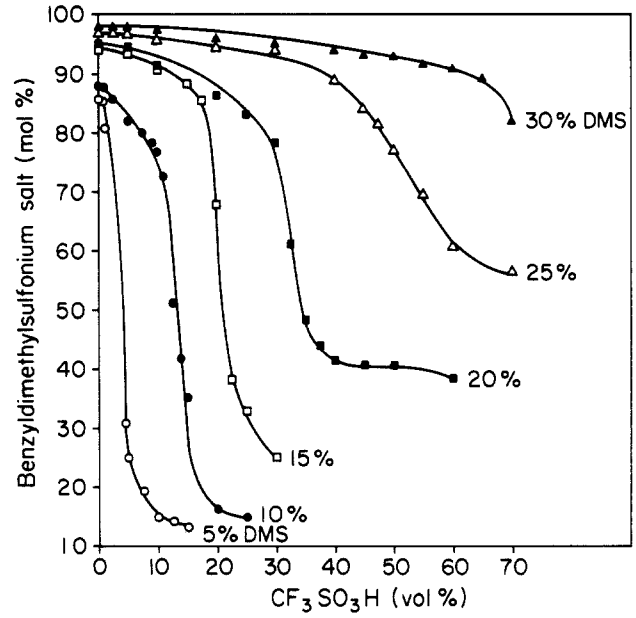

Figure 4. Product–acid profiles of benzyldimethylsulfonium salt from the deprotection of Tyr(Bzl) in CF_3SO_3H–CF_3CO_2H with fixed concentrations of dimethyl sulfide at 0 °C.

0% TFMSA, the amount of 3-benzyltyrosine was 11.3% and changed to 46% at 5% TFMSA. Since no visible break was observed in the slope of product profile, and since the amounts of 3-benzyltyrosine product were high and benzyldimethylsulfonium salt low, it was concluded that the S_N1 mechanism was predominant in this type of profile.

Type II profiles included those with the moderate DMS concentrations such as the 10 to 25% DMS profiles. The amount of 3-benzyltyrosine was small and increased only very slowly. Typically, the amount ranged from 0.6 to 4% at the initial addition of TFMSA concentration. Similarly, the amount of benzylsulfonium salt was high (>90%) and decreased slowly with the increase of TFMSA concentration. At the later stage of higher TFMSA concentrations and near the changeover point of the mechanism, the amount of 3-benzyltyrosine increased and the amount of benzyldimethylsulfonium salt decreased significantly and rapidly with small increases of TFMSA concentration. Both products leveled again at the high TFMSA concentrations. The sudden upbreak in the rate of product was taken as the changeover from an S_N2 to an S_N1 mechanism.

Type III acid–product profiles were exemplified by the high DMS concentrations such as those with 30% or more DMS. The amounts of 3-benzyltyrosine were found to be very small and the amounts of benzyldimethylsulfonium salt high throughout the acid–product profile. For example, at a TFMSA concentration of 20% or less, the amount of 3-benzyltyrosine was usually less than 1%, and the slope of product increase was less than 0.14%/% of TFMSA. The type III product profile is therefore characteristic of the S_N2 reaction mechanism.

(C) NMR Titration. The activity (concentration) of dimethyl sulfide that determined the cleavage mechanism in the TFMSA–TFA–DMS system was also examined in a wide range of TFMSA concentrations by measurement of the thermodynamic equilibrium of the protonated and unprotonated forms of DMS by ^1H NMR. Since the NMR time scale is relatively long and the protonation–deprotonation of DMS very rapid, a time and species averaged chemical shift is observed for a given proton resonance. Thus, at a particular acidity corresponding to partial protonation of DMS, the observed chemical shift of a methyl proton is a weighted average of the shift in the protonated and unprotonated molecules. The NMR technique[12] involved measurements, as a function of the TFMSA concentrations, of the chemical shift (Δ_ν in Hz, 300 MHz) of the methyl protons of DMS, relative to that of the internal standard, tetramethylammonium sulfate (Table III). The use of the internal standard minimizes errors in

Table III. Calculated Acidity Function of TFMSA–TFA–DMS Mixture

vol % TFMSA	$-H_0$	vol % TFMSA	$-H_0$
5% DMS		10% DMS	
2.5	5.46	1.0	4.63
5.0	6.41	2.5	5.06
		5.0	5.44
20% DMS		10.0	6.04
2.5	4.76	13.0	7.10
10.0	5.37		
20.0	5.71	15% DMS	
30.0	6.82	5.0	5.14
		10.0	5.56
30% DMS		10.0	5.33
2.5	4.56	20.0	5.70
5.0	4.93	30.0	6.27
10.0	5.20		
20.0	5.60	40% DMS	
30.0	6.03	5.0	4.76
40.0	6.24	10.0	4.94
50.0	6.68	20.0	5.24
		30.0	5.52

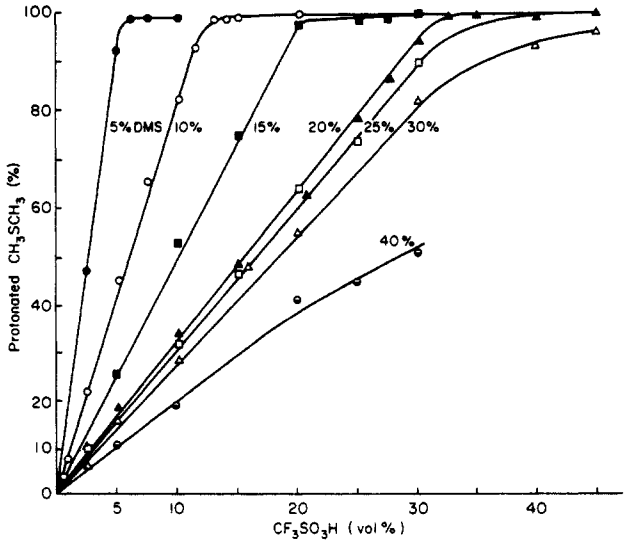

Figure 5. Proton NMR determination of protonation of dimethyl sulfide in CF_3SO_3H–CF_3CO_2H with fixed concentrations of dimethyl sulfide at 0 °C. Percent protonation of CH_3SCH_3 is calculated from eq 2, $I = [\Delta\nu]/[238.5 - \Delta\nu]$, where I is expressed in %, and $\Delta\nu$ is the observed chemical shift of the free base.

measurements due to the solvent effects. The acid–NMR titration profiles (Figure 5) in six sets of predetermined DMS concentrations were plotted from the observed chemical shift differences of the methyl protons of DMS as a function of TFMSA concentration. Our results showed that the chemical shift differences were proportional to the TFMSA concentrations and thus confirmed that both the activities of DMS and the acidities of the reaction media were dependent linearly with the TFMSA concentrations.

In order to obtain the acidity of the reaction medium, we calculated the ionization ratio (I) from the chemical shift of the free (Δ_ν(B)) and protonated (Δ_ν(BH^+)) base according to eq 1.

$$I = [BH^+]/[B]$$

$$I = [\Delta_\nu - \Delta_\nu(B)]/[\Delta_\nu(BH^+) - \Delta_\nu] \quad (1)$$

The Δ_ν(B) and Δ_ν(BH^+) were corrected with respect to the chemical shift of the tetramethylammonium ion to be 0 and 238.5 Hz, respectively. Thus, eq 1 can simply be rewritten as eq 2.

$$I = [\Delta_\nu]/[238.5 - \Delta_\nu] \quad (2)$$

The knowledge of the ionization ratio allows quantitative expression of the acidity of the reaction medium in terms of an acidity scale[13] such as the Hammett acidity function[13] (H_0) in eq 3. Since the Hammett acidity function pertains only to the

$$\log I = -H_0 + pK_{BH^+} \quad (3)$$

primary aromatic base, and since most acidity functions are known to be approximately linear with respect to each other, it is possible to generalize eq 3 by insertion of a factor m.[14] The slope m would in fact measure the response of the equilibrium of DMS to changing acid concentration in the TFMSA–TFA solution. The resulting eq 3 then becomes eq 4. Equation 4 now becomes the

$$\log I = -mH_0 + pK_{BH^+} \quad (4)$$

Yates–McClelland equation[14] that is the basis for their investigation to determine reaction mechanisms in various aqueous acidic media. Furthermore, it has also been applied for the determination of the pK_a's of many weak bases.[15,16] However, the pK_{BH^+} of DMS and m have not been determined in the TFMSA–TFA acidic medium. For our purpose, we used the values (m = 1.26, pK_{BH^+} = −6.95) of Bonvivinci et al.,[15] which were obtained from their studies of DMS in sulfuric acid solutions. When all the known values and eq 2 are substituted into eq 4, the acidity of the reaction medium, H_0, was then calculated from eq 5 (Table III; chemical-shift differences given in the supplementary material).

$$\log\left[\frac{\Delta_\nu}{238.5 - \Delta_\nu}\right] = 1.26H_0 - 6.95 \quad (5)$$

Equation 5 can then be rearranged as eq 6.

$$H_0 = -\frac{1}{1.26}\left[\log\left(\frac{\Delta_\nu}{238.5 - \Delta_\nu}\right) + 6.95\right] \quad (6)$$

Discussion

Mechanistic Framework. The theory of the acid-catalyzed S_N2 mechanism, its kinetics, and its other properties which allow it to be distinguished from the S_N1 mechanism have been set out in many published articles.[12-18] Almost all these involve mechanistic studies in aqueous acidic solutions since the kinetics, particularly k_1, which is the first-order rate constant in either mechanism, can be correlated well with the known acidity function of the reaction medium. However, little is known about nonaqueous strong acid cleavage mechanisms, and in particular, the much needed information concerning the nonaqueous strong acid catalyzed unimolecular or bimolecular cleavage at the alkyl–oxygen of benzyl ethers or esters encountered in synthetic peptides. Nevertheless, analogies can be drawn from the aqueous system for the nonaqueous system in which the weak base, DMS, is substituted for water as the nucleophile.

Invariably, the distinction between the two mechanisms in the aqueous acidic system is that the S_N2 mechanism predominates in dilute or moderately concentrated acid, and in turn depends on the specific rate k_1, on the concentration of the strong acid, and on the water activity.[9b] In the S_N1 mechanism, the specific rate rises with acid concentration, always faster than the acidity function, and on an ever-steepening slope. However, in the S_N2 mechanism, the rate rises less steeply than the acidity function, and with a diminishing slope of water activity. As the water activity reaches zero, or some low value, the S_N1 mechanism takes over.[9b]

Recently, using kinetics and product profiles, we have shown that such qualitative distinctions between the S_N1 and S_N2

(13) (a) Hammett, L. P.; Deyrup, A. J. *J. Am. Chem. Soc.* **1932**, *54*, 2721–2739. (b) Bunnet, J. F.; Olsen, F. P. *Can. J. Chem.* **1966**, *44*, 1899–1916. (c) Maroziano, N. C.; Traverso, P. G.; Passerini, R. C. *J. Chem. Soc., Perkin Trans. 2*, **1977**, 306–313. (d) Cox, R. A.; Yates, K. *J. Am. Chem. Soc.* **1978**, *100*, 3861–3867.
(14) Yates, K.; McClelland, R. A. *J. Am. Chem. Soc.* **1967**, *89*, 2686–2692.
(15) (a) Bonvicini, P.; Levi, A.; Lucchini, V.; Modena, G.; Scorrano, G. *J. Am. Chem. Soc.* **1973**, *95*, 5960–5964. (b) Bonvicini, P.; Levi, A.; Lucchini, V.; Scorrano, G. *J. Chem. Soc., Perkin Trans. 2* 1972, 2267–2269.
(16) Arnett, E. M.; Scorrano, G. *Adv. Phy. Org. Chem.* **1976**, *13*, 83–145.
(17) (a) Bunnett, J. *J. Am. Chem. Soc.* **1961**, *83*, 4956–4967. (b) Bunnett, J. *Ibid.* **1961**, *83*, 4968–4977. (c) Bunnett, J.; Olsen, F. P. *Can. J. Chem.* **1966**, *44*, 1899–1916.
(18) (a) Long, F. A.; Paul, M. A. *Chem. Rev.* **1957**, *57*, 935. (b) Paul, M. A.; Long, F. A. *Ibid* **1957**, *57*, 1–45.

Table IV. Analysis of Slope of Change in Rate Constants with CF_3SO_3H Concentrations

type of profile	CH_3SCH_3 profile (vol %)	range of CF_3SO_3H (vol %)	slope of rate change s^{-1}/% vol of CF_3SO_3H
I	0	0–2.5	5000
	5	0–7.5	4000
II	10	0–5	220
		6–10	0.06
		11–15	5
	20	0–15	200
		16–35	0.1
		36–40	6.5
III		0–10	280
		11–69	0.3

mechanisms can be extended to a binary nonaqueous mixture of HF–DMS.[1] In such a system, the deprotection mechanism proceeds through two distinct phases similar to those found in the aqueous acidic systems, with the S_N2 mechanism dominating at the low HF concentrations and the S_N1 mechanism at the high HF concentrations. However, because of technical difficulties of measuring the protonated and unprotonated forms of DMS in HF, we were unable to correlate DMS activity with the reaction profiles. In the present study, we were able to determine the DMS activities in reaction mixtures with TFMSA–TFA by ^1H NMR. Furthermore, on the basis of these determinations, the acidity functions were also calculated. Thus, the mechanistic distinctions between S_N1 and S_N2 in TFMSA–TFA–DMS can be discussed in terms of the correlation of the reaction profiles with the acidity functions and the activities of DMS of the reaction media.

Correlation of Acid–Rate and Acid–Product Profiles. As shown in Table I, the rate constants of Ser(Bzl) deprotection cover 10^7-fold, from 1×10^{-7} to 1 s^{-1}. Similarly, the amounts of 3-benzyltyrosine alkylation products (Table II) also vary more than 2000-fold from 0.02 to 45%. Such diverse behavior in the deprotection mixture of TFMSA–TFA–DMS would tend to indicate different mechanisms in different reaction media. In the Results section, we have tentatively assigned the deprotection mechanisms based on the acid–rate or acid–product profiles alone. Since mechanistic distinctions can be drawn from the dependence of the specific rate k_1 and the formation of S_N1 products on the concentration of the strong acid and since the slopes of the rate and product profiles measure the rate changes with the TFMSA concentrations, we are now in a position to confirm such assignments by the correlations of both profiles and by analyses of the slope of the profiles (Table IV). Thus, using this approach, we will first attempt to determine mechanisms qualitatively without the quantitative knowledge of the acidity functions and the activities of the weak base as nucleophile.

Essentially, the three types of profiles seen in this study can be classified into three broad categories in terms of the DMS concentrations: (1) low DMS concentration (<10%), (2) moderate DMS concentration (10–30%), and (3) high DMS concentration (>30%).

With low DMS concentration, the reaction profiles showed steady and monotonical increase in rates with a rate constant change over 4000 s^{-1}/% vol of TFMSA and alkylated tyrosine products with the increase in TFMSA concentrations. This is particularly true in the case when DMS is absent in the reaction mixture in which the rate constants increase more than 10^7-fold with only a small increase in TFMSA concentration (rate constant change of 5000 s^{-1}/% vol of TFMSA) and the amount of 3-benzyltyrosine product obtained was more than 45% (Table IV). No visible changeover point could be assigned from these reaction profiles. These types of reaction profiles are characteristics of the S_N1 mechanism and are expected from the strong acidic medium of TFMSA in TFA.

With moderate DMS concentration, the reacton profiles revealed three distinctive phases. At low TFMSA concentrations (<10% TFMSA), the deprotection rate constants increased quite

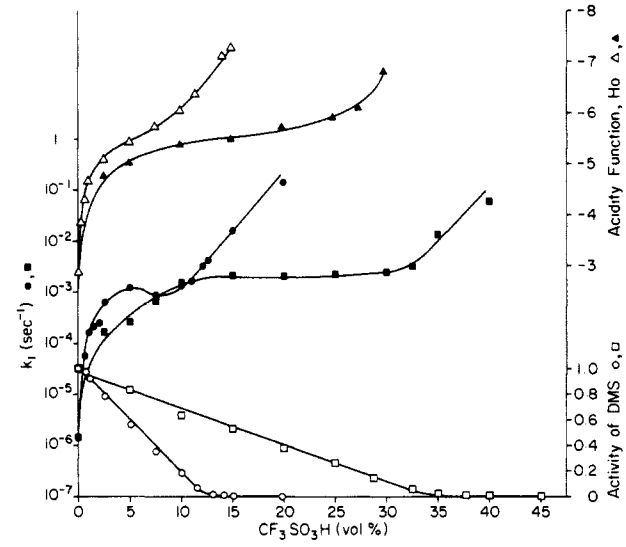

Figure 6. Kinetic rate–acid profiles (10% (●) and 20% (■) of dimethyl sulfide) vs. activities of dimethyl sulfide (10% (○) and 20% (□) of CH_3SCH_3) vs. acidity functions (10% (△) and 20% (▲).

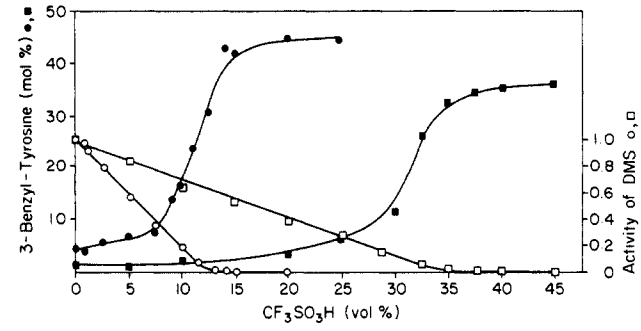

Figure 7. Product rate–acid profile (10% (○) and 20% (□) of dimethyl sulfide) vs. activities of dimethyl sulfide (10% (●) and 20% (■) of CH_3SCH_3).

rapidly with a slope of rate constant change of about 200 s^{-1}/% vol of TFMSA but reached plateaus with further increase in TFMSA concentrations and with a slope of rate constant change of <0.1 s^{-1}/% vol of TFMSA. Interestingly, the 3-benzyltyrosine profiles did not exhibit this behavior and only increased very slowly and steadily. Finally, both the rate and 3-benzyltyrosine profiles turned upward steeply at the high TFMSA concentrations with a slope of rate constant change >5% s^{-1}/% vol of TFMSA. Clearly, these behaviors are characteristic of mixed deprotection mechanisms with the S_N2 mechanism at the earlier phases and the S_N1 mechanism at the final phase.

Finally, with the high DMS concentration (>30%), both the rate and the 3-benzyltyrosine profiles exhibited behavior similar to the first two phases of the moderate DMS concentration just described, but the final phases of rapid rate or the sudden 3-benzyltyrosine alkylation increases were distinctly missing. No visible changeover point was observable. These types of profiles are again characteristic of the S_N2 mechanism.

Correlation of Rate and Product Profiles with DMS Activities. A clear and definitive approach to distinguish the S_N1 and S_N2 mechanisms is to correlate the rate and product profiles with the DMS activities and the acidity functions of the reaction mixture. The mole fraction, X_{DMS}, of "free and unprotonated" DMS in the reaction medium was taken as a measure of the activity of DMS. Thus, "free" DMS implies that all DMS which is not bound to ions in solvation can participate in the S_N2 reaction mechanism. Thus, X_{DMS} will vary between 1 and 0 with the low numbers indicating the low DMS activities.

When the rate and product profiles are replotted as a function of the DMS activities (Figures 6 and 7), the changover points of mechanisms between S_N1 and S_N2 can readily be seen as the DMS

Table V. A Summary of Changeover Points in TFMSA–TFA–DMS

vol % of DMS	changeover points determined by vol (%) of TFMSA		
	rate	prod anal.	NMR
10	11.0	12.0	12.5
15		17.5	
20	32.5	32.5	32.5
25		47.5	
30	62.5	63	60

activity approaches zero, the rate constants of Ser(Bzl) resume a steep increase (Figure 6), and the 3-benzyltyrosine alkylation products reach local maxima (Figure 7). Furthermore, when the acidity functions of the reaction media are added to the figures, the results of the rate and product profiles can be explained by the behaviors of the acidity functions and DMS activities. Thus, the initial rate increase seen in the 10 to 30% rate profiles with a slope of rate constant change >200 s^{-1} per vol % change of TFMSA are largely due to the rapid change of acidity functions because of the small addition of TFMSA into TFA. Such rapid change of acidity functions have been well documented. For example, small molar % addition of HF or H_2SO_4 to TFA will cause a change of 2 to 3 units in H_0. However, as the acidity of the reaction medium increases further, the activity of DMS decreases. Such behavior also explains the long plateau seen in the rate profiles as the opposing force of acidity and activity cancel each other. Finally, as the activity of DMS approaches zero, an alternative mechanism takes place. Thus, in the moderate DMS concentrations (10 to 25%), the S_N2 mechanisms are seen when DMS activities are >0, and the S_N1 mechanisms are seen when DMS activities are 0. Since the DMS activities do not approach 0 when the concentrations of DMS are higher than 30%, S_N2 mechanisms predominate with mixtures containing more than 30% DMS. Similarly, with TFMSA–TFA mixtures containing less than 5% DMS, the DMS activities will be low and S_N1 will be likely to predominate.

Changeover Points and Effects of Dimethyl Sulfide. The changeover point marks the beginning of predominance of one mechanism over the other. As shown from the rate, product, and NMR titration profiles, the changeover points to the S_N2 from the S_N1 mechanism are sharp and distinct. The changeover points from the rate profiles were determined by the intersection of the two slopes of the S_N1 and S_N2 rate curves. The changeover points of the product profiles were obtained from the curves when the 3-benzyltyrosine products reached their local maxima. Finally, the changeover points of the titration profiles were those when the activities of DMS approach 0. Table V shows that the changeover points obtained from all three methods agree well with each other.

The determinations of the changeover points not only define the boundary between the two mechanisms but also give insights into the effects of DMS as a nucleophile and as a nonpolar solvent in TFMSA–TFA. This is particularly apparent when the molar equivalents of DMS were plotted against the molar equivalents of TFMSA at each changeover point (Figure 8). At low DMS concentrations (<10%), the stoichiometric relation of TFMSA and DMS is close to 1. Since TFMSA–TFA is a strongly ionizing acid system, DMS is expected to be strongly protonated in such a solvent mixture. This also explains the low activities of DMS and the high amount of 3-benzyltyrosine seen in these solvent mixtures.

At DMS concentrations higher than 10%, the medium effect of DMS exerts its influence and the TFMSA–TFA–DMS mixture becomes increasingly less ionizing. As a result the changeover points require higher molar concentrations of TFMSA than DMS. At 20% DMS, the stoichiometric equivalency of TFMSA to DMS approaches 2 to 1 and at 30% the ratio approaches 3 to 1.

One general consequence of DMS in the TFMSA–TFA mixture is its effect to suppress the acidity and as a result the specific rate, k_1, of deprotection of Ser(Bzl) is also depressed (Figure 9A). A dramatic example is that the rate in 0.5% TFMSA in TFA is about the same as the rate in 60% TFMSA in 40% DMS. However,

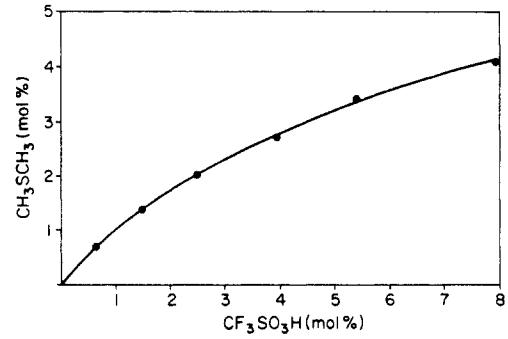

Figure 8. Mechanism changeover points plotted as molar equivalencies of CF_3SO_3H and dimethyl sulfide.

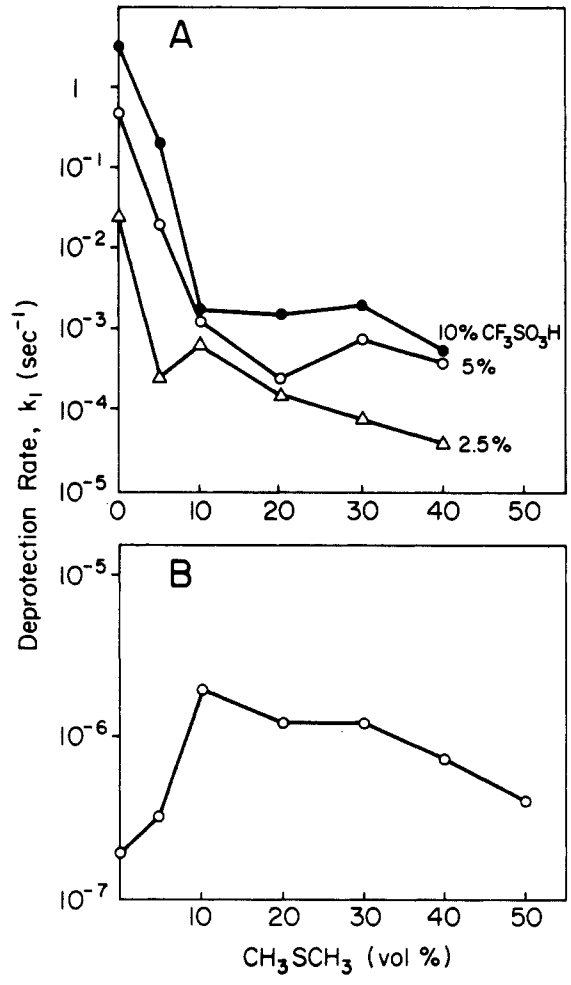

Figure 9. Effect of dimethyl sulfide in suppressing the specific rate, k_1, of deprotonation of Ser(Bzl) in CF_3SO_3H–CF_3CO_2H (A) and in accelerating the k_1 in CF_3CO_2H (B).

in contrast to its role in the TFMSA–TFA mixture, DMS does not depress k_1 in the TFA alone. In fact, k_1 is accelerated 6- and 10-fold with increases to 10% and 30% of DMS, respectively, in TFA, as compared to neat TFA alone (Figure 9B). These contrasting behaviors can readily be explained by the pK_{BH^+} of DMS and the acidity of the reaction medium. Since the pK_a of TFA is −3.1,[19] TFA is not sufficiently strong to protonate DMS (pK_{BH^+} −6.95).[15] Thus, DMS activity in TFA is high enough to participate as the nucleophile. However, it should be pointed out that even with the beneficial effect of DMS in TFA, the specific rate k_1 is still too slow for general application to peptide synthesis. With

(19) (a) Hyman, H. H.; Garber, R. A. *J. Am. Chem. Soc.* **1959**, *81*, 1847–1849. (b) Kilpatrick, M.; Jones, J. G. In *The Chemistry of Non-Aqueous Solvents II*; Lagowski, J. J., Ed.; Academic Press: New York, 1967; Vol. II, pp 43–99.

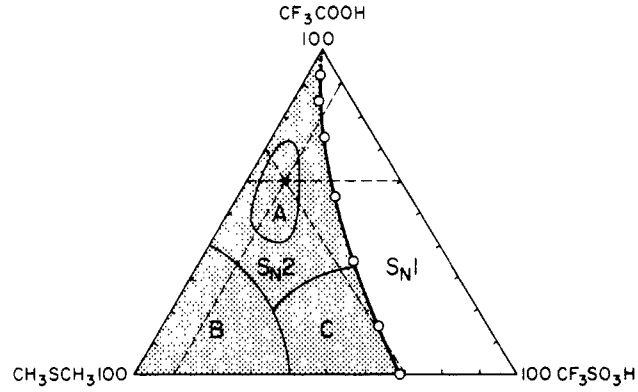

Figure 10. Mechanism–composition diagram of the S_N1 and the S_N2 mechanisms as plotted by the changeover points. The recommended condition is indicated by an asterisk. See text for explanations of areas A, B, and C.

minute amounts of TFMSA added to TFA, the TFMSA–TFA mixtures will have acidity functions approaching that of the pK_{BH^+} of DMS. In such mixtures DMS will be protonated and hence have lower activity. More importantly, DMS also acts as a base in the TFMSA–TFA mixture and thus further lowers the acidity of the TFMSA–TFA mixture.

The contrasting effects of DMS in different acidic solutions provide a firm basis to compare the different conditions recommended by the laboratories of Yajima[6] and Kiso.[7] Both groups have recommended the use of the more nucleophilic weak base, thioanisole, instead of DMS. However, the acidic conditions proposed by the two groups are vastly different. Kiso and his co-workers have recommended thioanisole in TFA alone while Yajima and his co-workers have chosen 1 M TFMSA–thioanisole in TFA solution (TFMSA:TFA:thioanisole, 8.9:79.4:11.7 (v/v)). Since thioanisole is an S-methylating agent in moderate acidities, and since methylation of methionine has not been reported under either condition, the acidity of these two conditions has to be substantially above or below the pK_{BH^+} of Met to prevent this side reaction. Thus, the TFA–thioanisole solution will likely have an acidity function of −3, and the acidity function of 1 M TFMSA–thioanisole in TFA will probably be greater than −6.95, and therefore under either condition a range of suitable acidity (between −3 to −6.95) is not being used. The proposed acidities of the two recommended conditions are supported by the specific rates k_1 reported by both groups as well as those seen in our laboratory. Furthermore, the two deprotection conditions differ greatly in their removal of different protecting groups. For example, the tosyl protecting group is removed only by the S_N1 mechanism under very acidic conditions, and thioanisole in TFA will not remove the tosyl protecting group of Arg(Tos) while 1 M TFMSA–thioanisole in TFA is capable of its completes removal. The different approaches by these laboratories provide an additional basis for our consideration of the criteria of an optimal condition using TFMSA–TFA–DMS that will operate under an S_N2 mechanism and thereby avoid many of the side reactions resulting from carbocations generated by S_N1 condition.

Mechanism–Composition Diagram of the S_N1 and S_N2 Mechanisms. A consequence of this study is its application to the construction of a mechanism–composition diagram that will define the relationship of the concentrations of all three components: TFMSA, TFA, and DMS with respect to the deprotection mechanism. The mechanism–composition diagram is contained in an equilateral triangle with the composition of each component from 0 to 100% (by volume) represented on each side (Figure 10). Thus, the composition of each reagent in percent by volume is defined at any point in the diagram by its perpendicular distance to the base of the triangle. When the changeover points (Table V) are plotted on the mechanism–composition diagram, a smooth curve which divides the diagram into two specific areas is obtained. The reagent compositions giving rise to an S_N1 mechanism are thus represented by one area and those giving an S_N2 mechanism are found in the remaining area.

Table VI. A Summary of Results for the Removal of Protecting Groups and Polymer Supports Using a Recommended Condition of CF_3SO_3H–CF_3CO_2H–CH_3SCH_3 (10, 60, 30 (v/v)), 4 h, at °C

	condition[a]	result
1.	acidity function	−5.2
2.	CH_3SCH_3	0.73
3.	% alkylation product (3-BzlTyr)[1]	0.4%
4.	% deprotection of	
	(a) Ser (Bzl)	100 ($t_{1/2}$ = 3.6 min)
	(b) Asp(OBzl), Glu(OBzl), Lys(ClZ), Thr(Bzl), and Tyr(BrZ)[2]	100 ($t_{1/2}$ < 20 min)
	(c) Met(O)[3] → Met	100 ($t_{1/2}$ < 40 min)
	(d) Trp(For)[4] → Trp	100 ($t_{1/2}$ < 20 min)
	(e) Boc-Ala-OCH_2-resin[5]	>90%
	(f) Boc-Ala-O-CH_2-Pam-resin[6]	>90%
	(g) Boc-Ala-benzhydrylamine-resin	<5%
	(h) Cys(4-MeBzl)[7]	<1%
	(i) Arg(Tos)[8]	<5%

[a] The superscripts 1–8 indicate the following: 1. from the deprotection of Tyr(Bzl); 2. aspartyl-β-benzyl ester, glutamyl-γ-benzyl ester, N^{ϵ}-2-chlorobenzoxycarbonyl-sysine, threonyl β-benzyl ether, tyrosyl-2-bromobenzoxycarbonyl ether; 3. conversion of methionine sulfoxide to methionine; 4. N^{i}-formyltryptophan to tryptophan, thiol adjuvant needed, see text; 5. from chloromethyl-resin; 6. -oxymethyl-phenylacetamidomethyl-resin; 7. cysteinyl--methyl-benzyl thiol ether; and 8. N^{ϵ}-tosyl-arginine.

The mechanism–composition diagram can also be visualized as the composite picture of the rate product and NMR titration profiles. An immediate application of the mechanism diagram is that any mixture of the TFMSA–TFA–DMS used in the deprotection of synthetic peptides can readily be classified as one promoting either the S_N1 or S_N2 mechanism. Thus, there will be no ambiguity concerning the mechanism–composition when a new mixture of TFMSA–TFA–DMS is used. Furthermore, the mechanism–composition diagram will also predict conditions to be employed in the deprotection. For example, in the regions defined by high DMS concentrations, the activity of DMS will likely be high particularly when the TFA concentration is higher than TFMSA (area A, Figure 10). This area will be expected to give low 3-benzyltyrosine alkylation products and reasonable deprotection rate constants (Table VI). However, when the concentration of DMS is higher than 50%, the reaction rates are too slow to be practical (area B, Figure 10).

General Conditions for the Removal of Protecting Groups and Polymer Supports. On the basis of many studies both in our laboratory and elsewhere, it is clear that many side reactions are catalyzed by strong acids under conditions that promote the formation of carbocations, which are then responsible for various alkylations and acylations. These can be avoided by operating under the S_N2 conditions. There are at least five requirements for such a condition: (1) the reaction should proceed by an S_N2 mechanism, with minimal S_N1 character; (2) the reaction should be rapid; (3) all or most of the common benzyl-based protecting groups should be removed; (4) the reagents should be easy to handled; and (5) the workup of the reaction mixture should be simple and efficient.

It is clear from Figure 10 that many but not all mixtures of TFMSA/TFA/DMS will meet these requirements and that there is not a single unique composition that is best. The most useful one will be a compromise that best balances the various requirements. The selection of the first condition is relatively easy since by inspection of the acid–rate profiles (Figure 1), the deprotection rate usually achieves a low maximum at the beginning of the plateau phase in the 20–40% DMS rate profiles. Upon further inspection of acidity of the medium, the activity of DMS, and the effect of DMS, it appears that a mixture of TFMSA–TFA–DMS (10:60:30 (v/v)) will best fulfill these requirements (Figure 10, area A). Under such a condition, the acidity of the reaction medium is relatively low and the activity of DMS is relatively high. Both of these requirements will allow reasonably fast deprotection rates of the benzylic groups but still maintain very low alkylation side products. Furthermore, the concentration of TFMSA is low. Since TFMSA is a viscous liquid, the recovery

of the peptide product at the end of the reaction is usually more conveniently achieved in dilute rather than concentrated TFMSA solution such as area C in Figure 10. As a bonus, under these conditions methionine sulfoxide, Met(O),[20] can be converted to Met and in the presence of an added thiol, the N^i-formyl protecting group of tryptophan, Trp(For),[21] can be smoothly removed to give Trp.

The recommended condition, TFMSA–TFA–DMS (10:60:30 (v/v)), is operationally named the "low-acidity TFMSA" deprotection procedure. It has an observed acidity function of −5.2, and the activity of DMS is 0.73. In other words, under such a condition 73 mol % of DMS will act as free base for participation in the S_N2 reaction. It is also far removed from the S_N1 region of Figure 10. The observed pseudo-first-order deprotection rate of Ser(Bzl) is 3.2×10^{-3} s^{-1} ($t_{1/2}$ = 3.6 min), and 30 to 60 min will give essentially complete reaction. These conditions will only give 0.4% ring alkylation side product in the deprotection of Tyr(Bzl). Interestingly, the recommended low TFMSA condition has about the same acidity and specific rate of benzyl group removal as the low HF cleavage condition (HF:DMS, 25/75 (v/v))[1] recommended earlier by us as the improved condition for the deprotection of synthetic peptides. The conditions will remove most common benzyl-based protecting groups and peptides anchored to polymeric supports by benzyl ester linkages but will not deprotect Cys(MeBzl), Arg(Tos), and Asp(OcHex) or cleave peptides from benzhydrylamine resins. The reagents are easy to handle and workup is simple. Essentially, these conditions can be handled as a continued step in the routine manual or automatic peptide synthesis. Furthermore, we have found that, to be fully compatible with the cleavage of protecting groups under the solid-phase condition, the addition of an aromatic solvent such as *m*-cresol, which serves as a swelling solvent for the peptidyl resin and as a scavenger for the possible sulfonation reactions, is beneficial to the deprotection mixture. Usually, *m*-cresol is added to make up 10% by volume of the recommended condition. A summary of the recommended condition is shown on Table VI.

Detailed Conditions for Deprotection of Synthetic Peptides or Peptidyl Resins. For syntheses using the normal existing protecting groups and polymeric supports, we have derived from our data the following deprotection conditions:

(1) For synthetic peptides or peptidyl resins not containing Arg(Tos) or Cys(4-MeBzl), and synthesized on a chloromethyl or Pam resin support,[22] a cleavage method with the low TFMSA deprotection condition (TFMSA–TFA–DMS–*m*-cresol, 10:50:30:10 (v/v)) at 0 °C for 4 h is generally sufficient. The crude peptide products are precipitated by pouring into it a 10- to 20-fold volume of dry ice cooled ethyl ether. For the precipitation process to be completed anhydrous pyridine is added dropwise to the acid–ether mixture. In general, the total volume of pyridine should not exceed 1% of the mixture. We have purposely recommended the use of *m*-cresol instead of *p*-cresol[1] since *m*-cresol is a liquid and usually will remain in the ether solution. The cleaved, deprotected peptide can be recovered by filtration or centrifugation.

(2) For peptides containing Trp(For), ethanedithiol (EDT) is added to the low TFMSA deprotection mixture to remove the N^i-formyl protecting group. The deprotection mixture now becomes TFMSA–TFA–DMS–*m*-cresol–EDT, 10:50:30:8:2 (v/v). The workup remains the same as in condition 1.

These conditions for the deprotection of synthetic peptides have been tested in our laboratory for the past several years and have resulted in many successful syntheses. In addition to the removal of benzyl protecting groups with minimal side reactions, the low TFMSA deprotecting procedures will convert Met(O) to Met and Trp(For) to Trp (procedure 2) efficiently. However, these mild deprotection conditions will not remove protecting groups such as Arg(Tos) or Cys(MeBzl). Synthetic peptides containing such amino acids will require either a separate S_N1 deprotection step or an alternative more acid labile protecting group. A dilute TFMSA solution operating under the S_N1 condition with 2–10% of TFMSA in TFA and in the presence of 10% *p*-cresol, "high-acidity" TFMSA deprotection condition (see Figure 10) has been used to remove the S_N2-resistant protecting groups.[25] Recently, more acid-sensitive protecting groups for both of these amino acids are available.[23] We have also investigated several other strategies to overcome these problems,[24] one of which is the use of the low-acidity TFMSA condition in combination with a multidetachable resin support such as the *p*-acyloxybenzhydrylamine resin, which gives the peptide α-carboxamide.[25]

In conclusion, we have investigated the mechanisms for the acidolytic deprotection of benzyl protecting groups in ternary mixtures of TFMSA–TFA–DMS. On the basis of these results, we have derived a mechanism–reagent composition diagram and have defined an optimal condition for the deprotection of synthetic peptides.

Experimental Section

Commercial protected amino acids were obtained from Peninsula Laboratoies, San Carlos, CA. 3-Benzyltyrosine hydrochloride salt was prepared according to Iselin[22] (mp 239–243 °C (lit. mp 239–243 °C)). Other reagents were trifluoroacetic acid (Halocarbon Products), trifluoromethanesulfonic acid (Aldrich Chemicals), dimethyl sulfide (Fluka–Tridom), and acetonitrile, HPLC grade (Jackson and Burdick).

Analytical high-pressure liquid chromatography was on a reverse-phase Altex C-18 column (4 × 300 mm) in a Waters Associates instrument fitted with a Schoeffel variable wavelength UV and fluorescence (Waters Assoc.) photometer and an automatic Wisp injector. The chromatograms were recorded and peak areas were integrated on a Hewlett Packard 3380A Integrator (1 mV full scale).

Kinetic Studies of Deprotection of *O*-Benzylserine. The desired amount of dimethyl sulfide was introduced into a precooled, magnetically stirring solution of predetermined amount of TFMSA-TFA solution at 0° C in a teflon-lined screw-capped 13 × 100 mm test tube. The final total volume of the TFMSA–TFA–DMS was always brought to 5 mL. After 30 min of equilibration at 0 °C, solid *O*-benzylserine (9.25 mg, 50 μmol) and alanine (2 mg, 23 μmol) were added separately to the acid solution. The temperature was maintained at 0 °C throughout the course of the reaction. Samples (25 μL, which contained 250 nmol of Ser plus Ser(Bzl)) were withdrawn at the required time intervals and then quenched in 1 mL of 0.1 N sodium acetate buffer, pH 5.9. The aqueous samples (5 to 20 μL) containing 1 to 5 nmol of Ser and Ser(Bzl) were analyzed on the amino acid analyzer with use of the reverse phase HPLC system by the orthophthaldehyde method of Jones et al.[23] Since the sensitivity of detection is 0.005 nmol, less than 1% of deprotection product can be detected. The elution times (45 min gradient run) for Ser, Ala, and Ser(Bzl) were 19.2, 29.1, and 39.3 respectively. The amount of deprotection of Ser(Bzl) was calculated from the serine to Ser(Bzl) ratio, normalized against the internal standard, alanine. The apparent first-order rate constants were obtained from the slope of the plot ln (X_0/X_t) vs. time (s), where X_0 and X_t represented the concentration of Ser(Bzl) at the star and at the time t, respectively. The results are summarized in Table I.

Studies of C-3-Benzylation from *O*-Benzyltyrosine. A typical experiment was as follows: Tyr(Bzl) (10 mg) with a measured volume of dimethyl sulfide was maintained at 0 °C. Precooled TFMSA–TFA was then added to the reaction mixture (5 mL of total volume) and the reaction was allowed to proceed to the required time. Portions of the sample (25 to 50 μL) were diluted with buffer A for HPLC analysis. The elution conditions was the following: solution A, 950 mL of H_2O, 50 mL of CH_3CN, 0.95 mL of H_3PO_4; solution B, 500 mL of H_2O, 500 mL of CH_3CN, 0.50 mL of H_3PO_4; linear gradient from 2 to 98% B into A in 45 min, 1 mL/min, at 215 or 280 nm. The elution times were (in min) the following: Tyr (4.6), benzyldimethylsulfonium salt (9.0), Tyr(Bzl) (20.3), and 3-Bzl-Tyr (17.4). Some of the experiments were repeated under a slightly different protocol because of very slow cleavage rate:

(20) Kessler, W.; Iselin, B. *Helv. Chim. Act* **1966**, *49*, 1330–1344.
(21) (a) Ohno, M.; Tsukamoto, S.; Sato, S.; Izumiya, N. *Bull. Chem. Soc. Jpn* **1973**, *46*, 3280–3285. (b) Yamashiro, D.; Li, C. H. *J. Org. Chem.* **1973**, *38*, 2594–2597.
(22) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B. *J. Am. Chem. Soc.* **1976**, *98*, 7357–7362.
(23) (a) Fujino, M.; Wakimasu, M.; Kitada *Chem. Pharm. Bull. Jpn.* **1981**, *29*, 2825–2831. (b) Yajima, H.; Funakoshi, S.; Akaji, K. *Int. J. Peptide Protein Res.* **1985**, *26*, 337–361.
(24) Tam, J. P.; Merrifield R. B. *J. Int. Peptide Protein Res.* **1985**, *26*, 262–273.
(25) Tam, J. P. *J. Org. Chem.* **1985**, *50*, 5291–5298.
(26) Iselin, B. *Helv. Chim. Acta* **1961**, *44*, 61–78.
(27) Jones, B N.; Gilligan, J. P. *J. Chromatogr.* **1983**, *266*, 471–482.

Tyr(Bzl) (10 mg), Ala (3 mg), and a known concentration of TFMSA-TFA-DMS were charged to a total volume of 5 mL. The reaction proceeded as above for 1 to 48 h at 0 °C. The reaction was stopped by withdrawing two separate 0.05-mL portions of solution and quenching one in NH_4OH solution (1 mL, 12% NH_3) and the other in sodium citrate buffer (1 mL, pH 2.2). The products in the NH_4OH solution were analyzed by HPLC and the other solution was analyzed on the Beckman 121 amino acid analyzer with alanine as the standard. The results are summarized in Table II.

NMR Titrations and Determinations of Dimethyl Sulfide Acitvities. The ^1H NMR spectra were taken on a Nicolet/Oxford NT-300 NMR spectrometer at 300 MHz. Trifluoromethanesulfonic acid-trifluoroacetic acid solutions were made up at 0 °C, and the volumes were checked against the weight increases of each addition. Tetramethylammonium sulfate (2% of weight) was added as the internal standard. Dimethyl sulfide was added to make up the solutions just before measurement to prevent unnecessary decomposition of the sulfide. The final weight increases of solutions due to DMS were again checked to correct for loss of DMS during mixing. The computation was made on the basis of eq 1 and 5.

Acknowledgment. We thank D. Rosberger for technical assistance and F. Picart for the NMR spectra. This work was supported in part by Grants AM 01260 and CA 34746 from the U.S. Public Health Service. NMR spectra were obtained on the 7T spectrometer at the Rockefeller University purchased in part with funds from the National Science Foundation (PCM-7912083) and from the Camile and Henry Dreyfus Foundation.

Registry No. TFMSA, 1493-13-6; TFA, 76-05-1; DMS, 75-18-3; Asp(OBzl), 2177-63-1; Glu(OBzl), 1676-73-9; Lys(Clz), 42390-97-6; Thr(Bzl), 4378-10-3; Tyr(BrZ), 37440-25-8; Met(O), 454-41-1; Trp(For), 74257-18-4; *O*-benzylserine, 4726-96-9; *O*-benzyltyrosine, 16652-64-5.

Supplementary Material Available: Table of chemical shift differences for dimethyl sulfide and a figure showing the stability of 3-benzyltyrosine in the course of deprotection of Tyr(Bzl) in CF_3SO_3H-CF_3CO_2H in 10% of CH_3SCH_3 at 0 °C (3 pages). Ordering information is given on any current masthead page.

Nucleophilic Addition to Olefins. 18.[1] Kinetics of the Addition of Primary Amines and α-Effect Nucleophiles to Benzylidene Meldrum's Acid

Claude F. Bernasconi* and Christopher J. Murray[2]

Contribution from the Thimann Laboratories of the University of California, Santa Cruz, California 95064. *Received December 9, 1985*

Abstract: Primary and secondary amines add reversibly to benzylidene Meldrum's acid (Scheme I), to form a zwitterionic adduct ($PhCH(N^+HRR')C^-(COO)_2C(CH_3)_2 = T_A^{\pm}$) which is in rapid acid-base equilibrium with the anionic form ($PhCH(NRR')C^-(COO)_2C(CH_3)_2 = T_A^-$). Rate constants for amine addition (k_1) and its reverse (k_{-1}), equilibrium constants ($K_1 = k_1/k_{-1}$), and pK_a values of $T_A^{\pm}$ ($pK_a^{\pm}$) were determined for *n*-butylamine, 2-methoxyethylamine, glycinamide, (cyanomethyl)amine, hydrazine, methoxyamine, and semicarbazide and compared with previously reported data on piperidine and morpholine addition. With glycinamide and 2-methoxyethylamine, rate constants for the protonation of $T_A^{\pm}$ on carbon (k_5^H) and for the collapse of $PhCH(NRR')CH(COO)_2C(CH_3)_2$ (T_A^0) into $PhCH{=}N^+RR'$ and $C^-H(COO)_2C(CH_3)_2$ (k_4, Scheme I) could also be estimated. log K_1 for the non-α-effect amines correlates linearly with amine basicity (pK_a^{AH}), with $\beta_{eq} = d \log K_1/dpK_a^{AH} \approx 0.83$. This β_{eq} is consistent with the stabilization of $T_A^{\pm}$ by intramolecular hydrogen bonding. K_1 for the three α-effect nucleophiles hydrazine, methoxyamine, and semicarbazide deviates positively from the correlation, but no such deviation is observed in a plot of log k_1 vs. pK_a^{AH}. The absence of a rate enhancement for the α-effect nucleophiles is probably related to the low $\beta_{nuc} = d \log k_1/dpK_a^{AH} = 0.22$, indicating an early transition state in which the product stabilizing factors are little developed. The β_{nuc} for primary amines, though small, is still substantially greater than $\beta_{nuc} = 0.07$ for the addition of piperidine and morpholine to benzylidene Meldrum's acid. Possible reasons for this difference in β_{nuc}, which has been observed with other electrophiles, are discussed.

The reaction of primary or secondary amines with benzylidene Meldrum's acid (BMA) involves many steps and, in aqueous solution, leads ultimately to the hydrolysis products benzaldehyde and Meldrum's acid (or its anion). This is shown in Scheme I.

In a previous paper[3] we reported detailed kinetic data for the reactions of BMA with piperidine and morpholine in aqueous solution. We were able to determine k_1, k_{-1}, and $K_a^{\pm}$ for both amines and showed that in the morpholine reaction carbon protonation of T_A^- is rate limiting in the overall hydrolysis. It was further concluded that the intramolecular proton switch (k_i) dominates over the direct carbon protonation of T_A^- by the hydronium ion (k_3^H).

The present and an accompanying paper[4] represent an extension of this work to the following primary amines: *n*-butylamine, 2-methoxyethylamine, glycinamide, (cyanomethyl)amine, hydrazine, methoxyamine, and semicarbazide.

There are several mechanistic and structure-reactivity problems we wish to address: (1) The most significant and intriguing one is whether the intramolecular proton switch, $T_A^{\pm} \rightarrow T_A^0$, which was observed for the morpholine adduct, is also detectable with other amines. If so, can we learn something about the transition state of this process by studying the dependence of k_i on the pK_a of the amine moiety in $T_A^{\pm}$ ($pK_a^{\pm}$)?[2]

(1) Part 17: Bernasconi, C. F.: Stronach, M. W. *J. Org. Chem.* **1986**, *51*, 2144.

(2) Department of Chemistry, University of California, Berkeley, CA 94720.

(3) Bernasconi, C. F.; Fornarini, S. *J. Am. Chem. Soc.* **1980**, *102*, 5329.

(4) Bernasconi, C. F.; Murray, C. J. *J. Am. Chem. Soc.*, following article in this issue.

0002-7863/86/1508-5251$01.50/0