The Preparation of Carbonium Ions and Other High-Energy Alkylating Agents under Mild Conditions[1]

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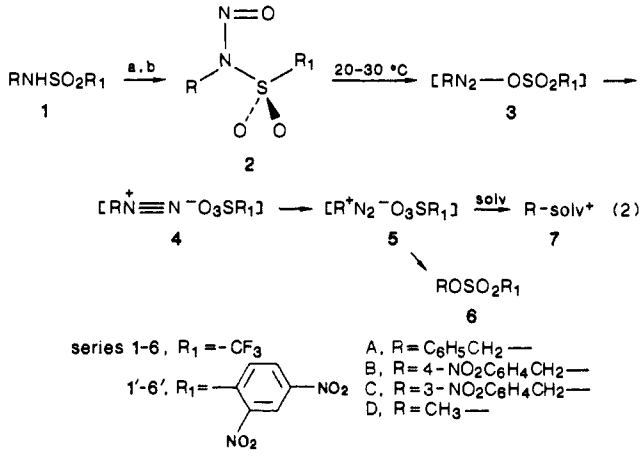
Highly reactive alkylating agents such as carbonium, nitrilium, and oxonium ion pair salts are usually prepared by means of highly reactive reagents (eq 1).[2] As salts, the alkylating agents are

$$RF + BF_3 + R_2O \rightarrow R_3O^+BF_4^- \quad (\text{also } SbCl_5,\ SbF_5,\ SnCl_4,\ \text{etc.}) \qquad (1)$$

normally employed in polar solvents or under heterogeneous conditions. We report here a method for the preparation of highly reactive alkylating agents under mild conditions in nonpolar solvents and in the absence of strong electrophilic reagents. The method lends itself to transformations of isotopically labeled intermediates and to alkylations on a microscale (although large scale operations are certainly feasible).

The method utilizes deaminatively produced carbonium ions formed via the decomposition of *N*-nitrososulfonamides selected to produce counterions of low basicity (eq 2).[3]

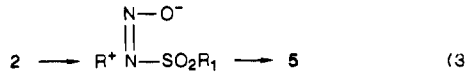


(2)

[a]NaOH and evaporation[4] [b]For **2**, N_2O_4 + Na_2CO_3 in CH_2Cl_2 at −10 °C;[6] for **2'**, N_2O_4 + NaOAc in CH_2Cl_2 at 20 °C.[7]

N-Benzyl-*N*-nitrosotrifluoromethanesulfonamides (**2**) decompose in $CDCl_3$ at 30 °C with half-lives of ~10–20 min; because of their instability,[8] they were freshly prepared for each run. The decomposition half-life for *N*-benzyl-*N*-nitrosodinitrobenzenesulfonamide **2'B** in $CDCl_3$ is ~6 h at 25 °C;[8] in this case, the crystalline nitrosoamide was readily isolated in yields of 90% (mp 103–4 °C); it was stored at −10 °C, and aliquots were used for different runs.

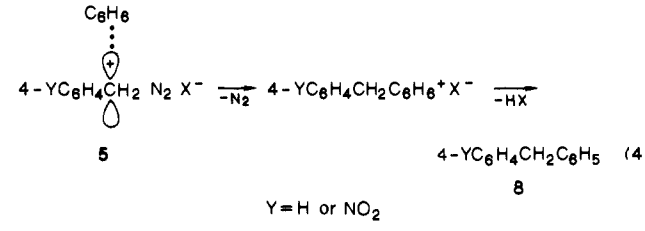
The decomposition rates of compounds **2A–C** are essentially the same ($T_{1/2}$ 13, 11, and 10 min, respectively, at 30 °C in $CDCl_3$), indicating that the typical rearrangement pathway is followed (eq 2)[9] rather than a direct ionization (eq 3), since the rates of the latter reaction would be expected to be sensitive to the nature of the substituents on the phenyl ring.



(3)

The decomposition of *N*-benzyl-*N*-nitrosotrifluoromethanesulfonamide (**2A**) [λ_{max} 375 (sh), 395, 410 in CH_2Cl_2][10] in the presence of an equivalent amount of the non-nucleophilic base 2,6-di-*tert*-butyl-4-methylpyridine (BMP) in $CDCl_3$ yields benzyl triflate (**6A**) cleanly.[6,11] The compound is labile, presumably because of a self-condensation of the Friedel–Crafts type. More stable were the nitrobenzyl triflates prepared from **2B** and **2C** in the presence of either Na_2CO_3 or BMP; these triflates showed no decomposition in solution over several days at 25 °C. In an analogous fashion, the *N*-nitrosodinitrobenzenesulfonamide **2'B** yielded the corresponding ester **6'B** on decomposition in $CDCl_3$, and nitrososulfonamide **2D** produced methyl triflate. The yields were quantitative[6] in the case of these stable triflates.

The decomposition of nitrososulfonamides **2B** and **2'B** in benzene in the presence of BMP at 25 °C yielded the alkylation product 4-nitrodiphenylmethane (**8**, Y = NO_2)[12] (55%) accompanied by 45% of sulfonate esters **6B** and **6'B**, respectively (no other products are formed). The product distributions for the reactions in C_6H_6 and C_6D_6 were the same indicating a negligible isotope effect. In the absence of bases, 4-nitrodiphenylmethane was the sole organic reaction product, (a result of acid catalysis of the Friedel–Crafts reaction). Similarly *N*-nitrosotriflamide **2A** in benzene yielded diphenylmethane. The sulfonate esters do not alkylate benzene in the presence of bases (BMP or Na_2CO_3); the alkylations stem from solvent interception of the carbonium ions of the nitrogen-separated ion pairs (**5**) formed in deamination[13] (eq 4).



(4)

The decomposition of nitrososulfonamides **2A** and **2B** in acetonitrile-d_3 at 25 °C in the absence of BMP yielded cleanly[6] the corresponding nitrilium salts $YC_6H_4CH_2N^+{\equiv}C{-}CD_3$ (Y = H and 4-NO_2, respectively).[14] The ^1H NMR resonances (Table I) were consistent with published values.[15] The addition of water

† Americal Chemical Society-Petroleum Research Fund Scholar.

(1) Paper 45 in a series on alkyl diazonium ion pairs and deamination (a listing is available from the senior author). Paper 44: White, E. H.; Lim, H. M. *J. Org. Chem.* **1987**, *52*, 2161–2166.

(2) Perst, H. In *Carbonium Ions* Olah, G. A., Schleyer, P. v. R., Eds.; Wiley-Interscience: New York, 1976; Vol. V, Chapter 34. Christie, J. J.; Lewis, E. S.; Casserly, E. F. *J. Org. Chem.* **1983**, *48*, 2531–2534.

(3) Amides (prepared from the corresponding sulfonyl chlorides) and stable products formed in the deamination reactions gave satisfactory elemental analyses and physical data.

(4) The sodium salt of dinitrobenzenesulfonamide **1'B** can be isolated only if the exposure of the amide to base is brief (few minutes); otherwise *N*-(4-nitrobenzyl)-2,4-dinitroaniline is formed [possibly via a Smiles rearrangement].[5]

(5) Bunnett, J. F.; Zahler, R E. *Chem. Rev.* **1951**, *49*, 273–412.

(6) The samples were filtered and evaporated to dryness at −10 °C. The solvent (and base if used) was added under strictly anhydrous conditions (under argon or on a vacuum line); otherwise, yields are depressed because of the formation of the amides, **1**, and N-alkylated derivatives formed from them.

(7) White, E. H. *J. Am. Chem. Soc.* **1955**, *77*, 6008. White, E. H.; Aufdermarsh, C. A., Jr. *J. Am. Chem. Soc.* **1961**, *83*, 1179. White, E. H. *Organic Synthesis*; Emmons, W. D., Ed.; John Wiley and Sons, Inc.: New York, 1967; Vol. 47, p 44.

(8) In stark contrast to these relatively labile derivatives of very strong acids, the related compound *N*-benzyl-*N*-nitroso-4-toluenesulfonamide exhibits a half-life of decomposition of ~75 days in $CDCl_3$ at 25 °C.

(9) White, E. H.; Woodcock, D. J. In *The Chemistry of the Amino Group*, Patai, S., Ed.; John Wiley and Sons, Inc.: New York, 1968; Chapter 8.

(10) For *N*-butyl-*N*-nitrosomethanesulfonamide, λ_{max} ($CDCl_3$) = 380 (sh), 395, and 413 nm (paper 44, cited in footnote 1).

(11) The reaction of benzyl alcohol with trifluoromethanesulfonic anhydride at −60 °C in the presence of trimethylpyridine has been reported to give a mixture containing benzyl triflate [δ CH_2 5.46 ppm in $CDCl_3$ (measured at −60 °C)]; (Lemieux, R. U.; Kondo, T. *Carbohydr. Res.* **1974**, *35*, C4-C6).

(12) Robinson, G. E.; Thomas, C. B.; Vernon, J. M. *J. Chem. Soc. B* **1971** *6*, 1273–82.

(13) White, E. H.; McGirk, R. H.; Aufdermarsh, C. A., Jr.; Tiwari, H. P.; Todd, M. J. *J. Am. Chem. Soc.* **1973**, *95*, 8107. White, E. H.; Field, K. W. *J. Am. Chem. Soc.* **1975**, *97*, 2148–2153.

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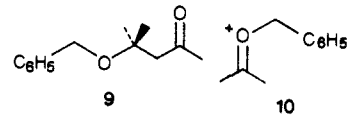
Table I. ^1H NMR Chemical Shifts for the Benzyl CH_2 Group of Labile Reactants and Products

compd	$\delta^{a,b}$
2A	4.91
2B	5.01 (5.13)[c]
2C	5.01
2D	3.26[d]
6A	5.47[e]
6B	5.56
6C	5.56
6D	4.20[d]
$C_6H_5CH_2N^+{\equiv}CCD_3$	5.31 (t, J_{NCH} = 2.5 Hz)
$4\text{-}NO_2C_6H_4CH_2N^+{\equiv}CCD_3$	5.46 (t, J_{NCH} = 2.5 Hz)
2'B	5.19 (5.13)[c] (5.30)[f] (4.76)[g]
6'B	5.47 (5.39)[c] (5.61)[f] (4.78)[g]

[a] Relative to TMS. [b] In $CDCl_3$ unless noted. [c] In CD_3CN. [d] For the CH_3 group. [e] The corresponding value for benzyl tosylate is 5.06 ppm. [f] In acetone-d_6. [g] In benzene-d_6.

to these salts produced the corresponding acetamides (80-90% yields).[16]

In acetone-d_6 in the absence of bases, the major product from the decomposition of **2A** was mesityl oxide [$(CH_3)_2C{=}CHCO\text{-}CH_3$], whereas in the presence of BMP the reaction product was compound **9**; the trapped benzyl cation **10** apparently reacts rapidly with the enol tautomer present in bulk acetone to form compound **9**.



9 **10**

A hierarchy of alkylating agents can thus be prepared conveniently and under mild reaction conditions: the method can produce—in decreasing order of reactivity the following: *carbonium ions*, *nitrilium salts* and other products formed from relatively unreactive solvents, *alkyl sulfonate esters*, and *alkyl derivatives* prepared from the sulfonates (quaternary ammonium salts, e.g.).

^1H NMR chemical shift values for the labile compounds mentioned in this article are listed in Table I.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(14) Unexpectedly, the decomposition of **2'B** in CD_3CN at 25 °C (~2 days required) yielded only traces of the nitrilium salt; the major products were recovered amide (**1'B**) and *N*-(4-nitrophenyl)acetamide. The runs were assembled on a vacuum line (solvent distilled from P_2O_5), and the NMR spectra showed no trace of the water resonance (2.1 ppm). The formation of amides may stem from the greater base strength of the dinitrobenzene sulfonate ion versus the triflate ion, but the pathway and the sources of the protons and oxygen atoms are unknown.

(15) Olah, G. A.; Kiovsky, T. E. *J. Am. Chem. Soc.* **1968**, *90*, 4666-72. Doyle, M. P.; Wierenga, W. *J. Am. Chem. Soc.* **1972**, *94*, 3896-3906.

(16) Yields not maximized.

Modification of Proteases to Esterases for Peptide Synthesis: Methylchymotrypsin[1]

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Recent investigations[2] have demonstrated the utility of protease-catalyzed synthesis of short peptides and semisynthesis of larger peptides via segment coupling. A serious drawback of this technique, however, is the potential loss of product due to the hydrolysis of sensitive peptide bonds by the protease. In our examinations of possible solutions to this problem, we have found certain target peptides that allow for the irreversible formation of product[3] as well as reaction conditions which selectively inhibit the amidase versus the esterase activities of several serine and cysteine proteases.[4] An alternate approach is the use of esterases which have no amidase activity,[5] although reaction rates are often slow.

A different approach was recently developed by Kaiser,[6] where an amidase-damaged protease, thiolsubtilisin, was used in conjunction with a mildly activated ester as acyl donor for segment coupling.

We report here the use of α-chymotrypsin methylated at $N^{\epsilon 2}$ of histidine 57[7] in peptide synthesis. This derivative, methylchymotrypsin (MeCT), is known to be some 4-5 orders of magnitude less active than α-CT toward ester substrates and inert toward amide substrates yet is known to have virtually identical binding properties to the native enzyme.[8] Although the modified enzyme is slowly active toward methyl esters, the use of cyanomethyl esters[9] increased rates considerably.

Kinetic parameters of the modified enzyme toward the two types of esters are shown in Table I. The lack of a common value for k_{cat} for the different esters indicates that with this enzyme, under these conditions, deacylation is no longer as strongly rate determining[10] so that both steps are kinetically significant. Both re-

Table I. Kinetic Parameters for the Hydrolysis of Z-L-Phe-OCH_2-X by Methylchymotrypsin[a]

X	k_{cat} (min^{-1})	K_M (mM)	k_{cat}/K_M (min^{-1} mM^{-1})
H	1.5	4.5	0.33
CN	5.1	4.5	1.1

[a] All reactions performed in 50 mM KCl/DMSO (1:1), pH 8.8. Initial velocities determined by Radiometer pH-Stat at four or more substrate concentrations. Enzyme concentration 29 μM. Parameters determined by Lineweaver-Burke plots, with correlation coefficients of 0.98 or greater. All velocities corrected for spontaneous hydrolysis of the respective ester at this pH. Enzyme purity assessed by FPLC (phenyl sepharose column, 1.7-0 M linear gradient of NaCl in 20 mM Tris-HCl, pH 7.8, in 35 min) and active site concentrations determined by measuring the burst of nitrophenol from Z-Tyr-ONp (pH 5.0, 5% MeCN) spectrophotometrically.

(1) Abbreviations: Y, Tyr: tyrosine; G, gly: Glycine; F, phe: phenylalanine; L, leu: leucine; Z: benzyloxycarbonyl; CT: α-chymotrypsin; MeCT: *N*-methylchymotrypsin; Np: *p*-nitrophenyl. All amino acids are of the L configuration unless otherwise specified. This work was supported by the NSF (CHE 8318217) to C.H.W. and NIH Grants GM 31960 and 32596 to J.L.H. and A.I.S., respectively.

(2) Jakubke, H.-D.; Kuhl, P.; Konnecke, A. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 85. Morihara, K. *Trends Biotechnol.* **1987**, *5*, 764. Fruton, J. *Adv. Enzymol.* **1982**, *53*, 239.

(3) West, J. B.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1986**, 417. West, J. B.; Wong, C.-H. *J. Org. Chem.* **1986**, *51*, 2728. Barbas, C. F.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1987**, 533.

(4) Barbas, C. F.; West, J. B.; Wong, C.-H. *J. Am. Chem. Soc.*, in press.

(5) West, J. B.; Wong, C.-H. *Tetrahedron Lett.* **1987**, *28*, 1629. Matos, J. R.; West, J. B.; Wong, C.-H. *Biotechnol. Lett.* **1987**, *9*, 233. Margolin, A. L.; Klibanov, A. M. *J. Am. Chem. Soc.* **1987**, *109*, 3802.

(6) Nakatsuka, T.; Sasaki, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1987**, *109*, 3808.

(7) MeCT was prepared by reaction of the enzyme with methyl *p*-nitrobenzenesulfonate (Ryan, D. S.; Feeney, R. E. *J. Biol. Chem.* **1975**, *250*, 843) as modified by M. S. Matta (personal communication to J. S.). To further remove possible contamination by native chymotrypsin, the methylated enzyme was again reacted with phenylmethylsulfonyl fluoride and purified by affinity chromatography with lima bean trypsin inhibitor agarose.

(8) Henderson, R. *Biochem. J.* **1971**, *124*, 13. Maehler, R.; Whitaker, J. R. *Biochemistry* **1982**, *21*, 4621. Byers, L. D.; Koshland, D. E. *Bioorg. Chem.* **1978**, *7*, 15. Based on structural considerations it is proposed that the nucleophilicity of Ser^{195}-OH is promoted by proton transfer to $N^{\epsilon 2}$. This mechanistic concept is supported by proton inventory studies.

(9) These esters are mildly activated and have the advantages of being readily prepared from chloroacetonitrile and the given carboxylate without prior activation of the acid. They also possess enhanced solubility in aqueous media.

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