# Mechanism of Reaction of an Arenediazonium Ion in Aqueous Solutions of Acetamide, *N*-Methylacetamide, and *N*,*N*-Dimethylacetamide. A Potential Method for Chemically Tagging Peptide Bonds at Aggregate Interfaces

**Laurence S. Romsted,* Jianbing Zhang,† and Lanzhen Zhuang‡**

*Contribution from the Department of Chemistry, Wright and Rieman Laboratories, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903*

*Received February 18, 1998. Revised Manuscript Received July 7, 1998*

**Abstract:** The mechanism of dediazoniation of 2,4,6-trimethylbenzenediazonium ion, 1-$ArN_2^+$, in concentrated aqueous solutions of acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide (peptide bond models) was probed by a combination of techniques including HPLC, GC/MS, and $H_2{}^{18}O$ isotopic labeling. The kinetics and product distributions are completely consistent with the heterolytic dediazoniation mechanism, i.e., rate-determining loss of $N_2$ followed by trapping of the aryl cation intermediate, 1-Ar+, by $H_2O$ and the oxygens and nitrogens of the amides. Aryl imidates formed from trapping by amide O hydrolyze rapidly into aryl ester/amine and amide/phenol product pairs. The results were used to estimate the selectivity of 1-Ar+ toward the amide oxygens and nitrogens versus $H_2O$. 1-Ar+ is only 10−40% more selective toward $H_2O$ than amide O, but it is more than 10 times more selective toward $H_2O$ than the amide N. 1-Ar+ is slightly more selective toward the N of acetamide than *N*-methylacetamide. However, within the HPLC detection limit, 1-Ar+ does not give a product from reaction with the *N*,*N*-dimethylacetamide nitrogen. The selectivities are interpreted by using a preassociation model, i.e., selective solvation by the different nucleophiles of the reactive diazonio group in the ground state. These results indicate that chemical tagging (trapping by N) and cleaving (trapping by O) of the peptide bonds and the weakly basic side chains of polypeptides and proteins bound to association colloids, vesicles and biomembranes, and emulsions may provide new information on their topologies and orientations at the aggregates' interfaces.

## Introduction

We have developed a novel method for estimating the compositions of the interfacial regions of amphiphilic aggregates based on product yields from chemical trapping by an aggregate-bound arenediazonium ion, 4-hexadecyl-2,6-dimethylbenzenediazonium ion, 16-$ArN_2^+$.[1] Recently the chemical trapping method was used to estimate chloride ion concentrations at the surfaces of zwitterionic phospholipid micelles and vesicles,[2] hydration numbers of nonionic micelles,[3] alcohol distributions in reverse microemulsions,[4] halide concentrations at the surfaces of anionic micelles,[5] and, with the short-chain analogue, 1-$ArN_2^+$, degrees of ionization of cationic micelles.[6] The basic protocols were also used to obtain new information on the pH dependence of $CuCl_2$-catalyzed dediazoniation of *p*-nitrobenzenediazonium ion.[7] The logic of chemical trapping is grounded in the pseudophase model of aggregate effects on chemical reactivity.[8,9] The success of the method stems from the low selectivity of dediazoniation reactions toward weakly basic nucleophiles such that reasonable product yields are obtained from competing reactions with each nucleophile, including $H_2O$, present in the interfacial region.[1] Arenediazonium ions are known to react with very weak nucleophiles, including $N_2$ and CO,[10] but an extensive search of the current literature and reviews and classic texts[11−19] for information on reactions of

* To whom correspondence should be addressed. E-mail: romsted@rutchem.rutgers.edu. Web: http://chmwww.rutgers.edu/faculty/romsted/romsted.html.

† E-mail: jiazhang@rutchem.rutgers.edu.

‡ Current address: Columbia University, 630 W. 168th St., Room 11-401, New York, NY 10032. E-mail: lz90@columbia.edu.

(1) Chaudhuri, A.; Loughlin, J. A.; Romsted, L. S.; Yao, J. *J. Am. Chem. Soc.* **1993**, *115*, 8351.

(2) Jain, M. K.; Rogers, J.; Yu, B.-Z.; Yao, J.; Romsted, L. S.; Berg, O. G. *Biochemistry* **1997**, *36*, 14512.

(3) Romsted, L. S.; Yao, J. *Langmuir* **1996**, *12*, 2425.

(4) Yao, J.; Romsted, L. S. *Colloids Surf. A: Physicochem. Eng. Aspects* **1997**, *123−124*, 89.

(5) Cuccovia, I. M.; Agostihno-Neto, A.; Wendel, C. M. A.; Chaimovich, H.; Romsted, L. S. *Langmuir* **1997**, *13*, 5302.

(6) Cuccovia, I. M.; da Silva, I. N.; Chaimovich, H.; Romsted, L. S. *Langmuir* **1997**, *13*, 647.

(7) Bravo-Diaz, C.; Romsted, L. S.; Harbowy, M.; Romero-Nieto, E.; Gonzalez-Romero, E. *J. Phys. Org. Chem.*, in press.

(8) Bunton, C. A.; Nome, F.; Quina, F. H.; Romsted, L. S. *Acc. Chem. Res.* **1991**, *24*, 357.

(9) Bunton, C. A.; Yao, J.; Romsted, L. S. *Curr. Opin. Colloid Interface Sci.* **1997**, *2*, 622.

(10) Zollinger, H. *J. Org. Chem.* **1990**, *55*, 3846.

(11) Zollinger, H. *Diazo Chemistry I: Aromatic and Heteroaromatic Compounds*; VCH Publishers: Weinheim, 1994; p 1.

(12) Zollinger, H. *Acc. Chem. Res.* **1973**, *6*, 335.

(13) Zollinger, H. *Angew. Chem., Intl. Ed. Engl.* **1978**, *17*, 141.

(14) Hegarty, A. F. In *The Chemistry of the Diazonium and Diazo Groups, Part 2*; Patai, S., Eds.; John Wiley & Sons: New York, 1978; p 511.

(15) Wulfman, D. S. In *The Chemistry of Diazonium and Diazo Groups, Part 1*; Patai, S., Ed.; John Wiley and Sons: New York, 1978; p 247.

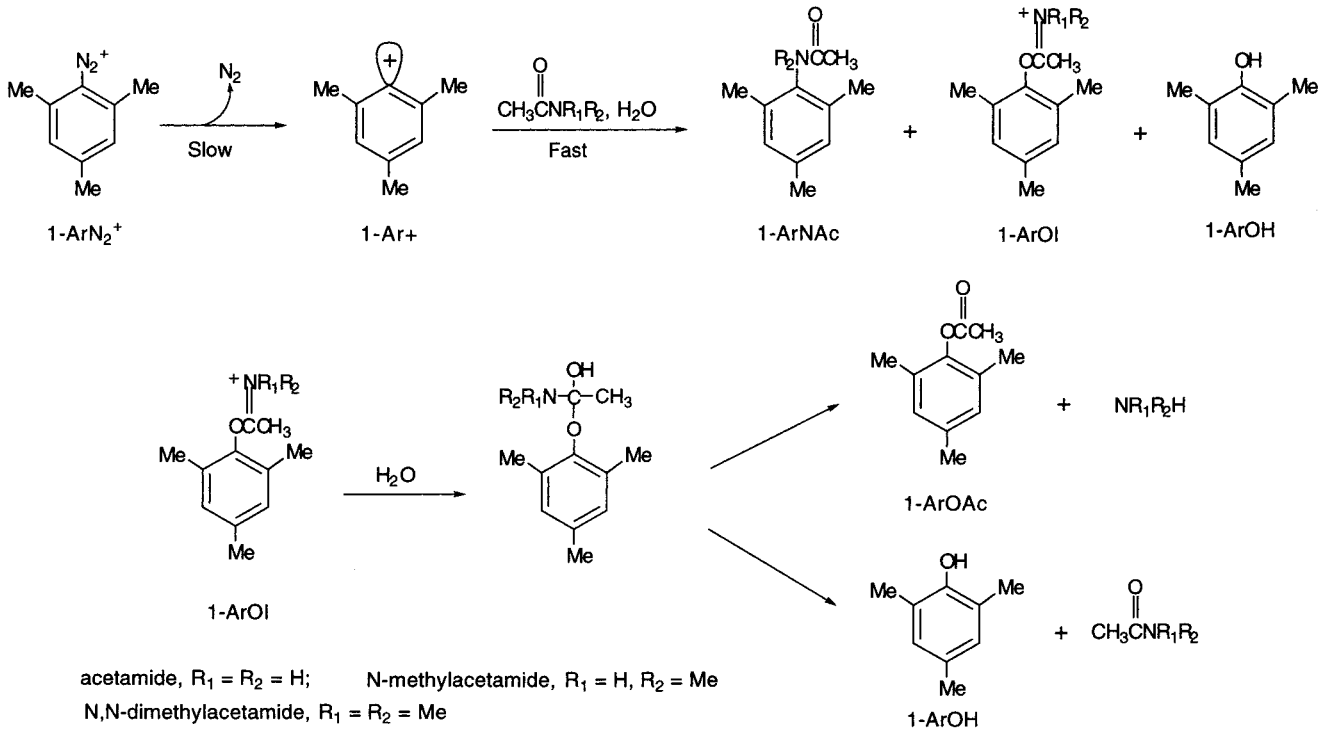
(16) Ambroz, H. B.; Kemp, T. J. *Chem. Soc. Rev.* **1979**, *8*, 353.

(17) Zollinger, H. In *Supplement C: The Chemistry of Triple-Bonded Functional Groups, Part 1*; Patai, S., Rappoport, Z., Eds.; John Wiley & Sons: New York, 1983; p 603.

(18) Saunders, K. H.; Allen, R. L. M. *Aromatic Diazo Compounds*, 3rd ed.; Edward Arnold: London, 1985.

S0002-7863(98)00556-3 CCC: $15.00 
Published on Web 09/19/1998

**Scheme 1**




arenediazonium ions with amide bonds drew a blank. Here we report the results of a study of the mechanism of reaction of 1-$ArN_2^+$ with some simple amides in aqueous solution, propose a preassociation model for interpreting the results, and suggest how chemical trapping might be used to obtain information on polypeptide topology and orientation at aggregate interfaces.

## Results

Scheme 1 summarizes the basic mechanism for dediazoniation of 1-$ArN_2^+$ in aqueous solutions of acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide that is consistent with our results. Evidence for this mechanism comes from dediazoniation kinetics, identification of products by FT-IR, NMR, HPLC, and GC/MS, and $^{18}O$ isotopic labeling experiments. A detailed interpretation is given in the Discussion. Scheme 1 is based on the well-established heterolytic pathway for reactions of arenediazonium ions with weakly basic nucleophiles.[11] The rate-determining step is loss of $N_2$ to generate an aryl cation, 1-Ar+,[20] followed by product formation in a subsequent fast step. 2,4,6-Trimethylphenol, 1-ArOH, is formed from reaction with $H_2O$. *N*-(2,4,6-Trimethylphenyl)acetamide and *N*-(2,4,6-trimethylphenyl)-*N*-methylacetamide, 1-ArNAc, are formed by reaction with the nitrogen (amide N) of acetamide and *N*-methylacetamide, respectively, but no product was observed from reaction with the amide N of *N*,*N*-dimethylacetamide. Trapping of 1-Ar+ by the amide N of *N*,*N*-dimethylacetamide should give acetyl *N*,*N*-dimethyl-2,4,6-trimethylanilinium ion as an intermediate that should hydrolyze rapidly to acetic acid and *N*,*N*-dimethylaniline, 1-$ArNMe_2$. No 1-$ArNMe_2$ was observed in the product mixtures above the detection limit of the HPLC, about 0.05%. Control experiments were run to check for possible competing reactions between 1-$ArN_2^+$ and 1-$ArNMe_2$ that would remove 1-$ArNMe_2$ from the product mixture, but none were found (see Experimental Section).

The formation of the ester product, 1-ArOAc, from all three amides, which was isolated from the reaction mixture and characterized (see Experimental Section), was initially a surprise. The most sensible explanation for formation of 1-ArOAc is that amide O traps 1-Ar+ to give 2,4,6-trimethylphenyl imidates, 1-ArOI, as reactive intermediates that hydrolyze to give 1-ArOAc/amine and 1-ArOH/amide product pairs, consistent with published reports of the chemistry of aryl imidates.[21−26] 1-ArOI is represented as a cation because aryl imidates are weak acids; e.g., for protonated *p*-tolyl imidates, $pK_a \geq 6.7$.[21] The rate constant for the spontaneous hydrolysis of *p*-tolyl *N*,*N*-dimethylacetimidate, which is structurally similar to 1-ArOI (which has two additional ortho methyls on the phenyl ring), is 0.056 $min^{-1}$ at 50 °C.[21] We estimate the half-life for this reaction to be ca. 50 min at 40 °C, which is close to the half-life for dediazoniation at this temperature, $t_{1/2} = 35.2$ min (see below). Because dediazoniations were carried out for ≥24 h, no 1-ArOI should be observed in the HPLC chromatograms.

Tables 1−3 list normalized product yields obtained from HPLC chromatograms for products formed from reaction of 1-$ArN_2^+$ with $H_2O$ and acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide, respectively. The major product is 1-ArOH, which is typically ≥92% of the total normalized yield. All other products are formed in ca. 1−5% yield. Products yields are measured in triplicate, with a reproducibility of ±5% or better. Normalized product yields are used in all calculations instead of total measured yields because of weighing errors in the small amounts of 1-$ArN_2BF_4$ used in preparation of stock solutions, because all relevant products and side products could be accounted for, because formation of these products does not significantly affect the normalized yields of the phenol, amide, and ester products (see below), and because the reproducibility

(19) Zollinger, H. *Color Chemistry: Syntheses, Properties and Applications of Organic Dyes and Pigments*, 2nd ed.; VCH: Weinheim, 1991.

(20) The plus sign is on the line (not superscripted) to represent the positively charged $sp^2$ orbital that remains after $N_2$ is lost.

(21) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031.
(22) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018.
(23) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7045.
(24) Lee, Y.-N.; Schmir, G. L. *J. Am. Chem. Soc.* **1978**, *100*, 6700.
(25) Lee, Y.-N.; Schmir, G. S. *J. Am. Chem. Soc.* **1979**, *101*, 3026.
(26) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover: New York, 1987.

**Table 1.** Normalized Product Yields for Reaction of 1-$ArN_2^+$ with Acetamide in Water at 40 ± 0.1 °C[a]

| $N_W/N_A$[b] | $10^3$[1-$ArN_2^+$][c] (M) | 1-ArNHAc (%) | 1-ArOH total (%) | 1-$ArOH_h$[d] (%) | 1-$ArOH_w$[d] (%) | 1-ArOAc (%) | ester[e] (%) |
|---|---|---|---|---|---|---|---|
| 2 | 1.00 | 2.70 | 94.22 | 25.16 | 69.16 | 3.08 | 10.9 |
| 2 | 0.50 | 2.68 | 94.23 | 25.16 | 69.16 | 3.08 | 10.9 |
| 2 | 4.76 | 2.99 | 94.22 | 25.16 | 69.16 | 2.79 | 10.0 |
| 2 | 2.00 | 2.70 | 94.71 | 25.29 | 69.52 | 2.60 | 9.3 |
| 4 | 1.00 | 1.82 | 96.26 | 16.46 | 79.80 | 1.92 | 10.4 |
| 4 | 0.50 | 2.06 | 96.19 | 16.45 | 79.74 | 1.75 | 9.6 |
| 4 | 4.76 | 1.97 | 96.28 | 16.46 | 79.82 | 1.75 | 9.6 |
| 4 | 2.00 | 1.52 | 96.70 | 16.54 | 80.16 | 1.78 | 9.7 |

[a] Normalized yield: %1-ArX (X = OH, OAc, NHAc) = 100{%(1-ArX)}/{%(1-ArOH) + %(1-ArOAc) + %(1-ArNHAc)} [b] The molar ratio of water to acetamide in each solution. [c] Cyclohexane was layered over all solutions except those containing $4.76 \times 10^{-3}$ M 1-$ArN_2^+$. [d] Normalized yields from hydrolysis of 1-ArOI, 1-$ArOH_h$, and by direct trapping of water, 1-$ArOH_w$. [e] Yield of ester from hydrolysis of 1-ArOI: % ester = 100{%(1-ArOAc)}/{%(1-ArOAc) + %(1-$ArOH_h$)}. Average = 10.1 ± 0.6%.

**Table 2.** Normalized Yields for Reaction of 1-$ArN_2^+$ with *N*-Methylacetamide in Water at 40 ± 0.1 °C[a]

| $N_W/N_A$[b] | $10^3$[1-$ArN_2^+$] (M) | 1-ArNMeAc (%) | 1-ArOH total (%) | 1-$ArOH_h$[c] (%) | 1-$ArOH_w$[c] (%) | 1-ArOAc (%) | ester[d] (%) |
|---|---|---|---|---|---|---|---|
| 2 | 7.81 | 1.18 | 92.90 | 17.74 | 75.16 | 5.92 | 25.0 |
| 2 | 7.81 | 1.30 | 92.72 | 17.71 | 75.01 | 5.98 | 25.2 |
| 2 | 4.76 | 1.57 | 92.45 | 17.66 | 74.79 | 5.98 | 25.3 |
| 2 | 4.76 | 1.84 | 92.11 | 17.59 | 74.52 | 6.05 | 25.6 |
| 2 | 4.76 | 1.94 | 91.97 | 17.57 | 74.40 | 6.09 | 25.7 |
| 4 | 4.76 | 1.13 | 95.43 | 10.40 | 85.03 | 3.44 | 24.9 |
| 4 | 4.76 | 0.88 | 96.09 | 10.47 | 85.62 | 3.02 | 22.4 |
| 4 | 4.76 | 1.10 | 95.76 | 10.44 | 85.32 | 3.13 | 23.1 |
| 4 | 4.76 | 0.90 | 96.10 | 10.47 | 85.62 | 3.00 | 22.3 |
| 4 | 4.76 | 1.13 | 95.93 | 10.46 | 85.47 | 2.95 | 22.0 |

[a] Normalized yield: %1-ArX (X = OH, OAc, NMeAc) = 100{%(1-ArX)}/{%(1-ArOH) + %(1-ArOAc) + %(1-ArNMeAc)}. [b] The molar ratio of water to *N*-methylacetamide. [c] Normalized yields from hydrolysis of 1-ArOI, 1-$ArOH_h$, and by direct trapping of water, 1-$ArOH_w$. [d] Yield of ester from hydrolysis of 1-ArOI: % ester = 100{%(1-ArOAc)}/{%(1-ArOAc) + %(1-$ArOH_h$)}. Average = 24.2 ± 0.34%.

**Table 3.** Normalized Yields for Reaction of 1-$ArN_2^+$ with *N*,*N*-Dimethylacetamide in Water at 40 ± 0.1 °C[a]

| $N_W/N_A$[b] | $10^3$[1-$ArN_2^+$][c] (M) | 1-ArOH total (%) | 1-$ArOH_h$[d] (%) | 1-$ArOH_w$[d] (%) | 1-ArOAc (%) | ester[f] (%) |
|---|---|---|---|---|---|---|
| 2 | 4.76 | 92.70 | 16.41 | 76.29 | 7.30 | 30.8 |
| 2 | 4.76 | 92.41 | 16.36 | 76.05 | 7.59 | 31.7 |
| 2 | 3.75 | 92.58 | 16.39 | 76.19 | 7.42 | 31.2 |
| 2[e] | 4.71 | 93.64 | 16.57 | 77.07 | 6.36 | 27.7 |
| 4 | 4.73 | 95.51 | 9.07 | 86.44 | 4.49 | 33.1 |
| 4 | 4.76 | 95.19 | 9.04 | 86.15 | 4.81 | 34.7 |
| 4 | 4.76 | 95.05 | 9.03 | 86.02 | 4.95 | 35.4 |
| 4 | 3.75 | 95.24 | 9.05 | 86.19 | 4.76 | 34.5 |
| 4[e] | 4.71 | 95.98 | 9.12 | 86.86 | 4.02 | 30.6 |

[a] Normalized yield: %1-ArX (X = OH, OAc) = 100{%(1-ArX)}/{%(1-ArOH) + %(1-ArOAc)}. [b] The molar ratio of water to *N*,*N*-dimethylacetamide. [c] Cyclohexane was layered only on solutions with $3.75 \times 10^{-3}$ M 1-$ArN_2^+$. [d] Normalized yields from hydrolysis of 1-ArOI, 1-$ArOH_h$, and by direct trapping of water, 1-$ArOH_w$. [e] Experiments aimed at detecting 1-$ArNMe_2$ (see Experimental Section). [f] Ester yield from hydrolysis of 1-ArOI: % ester = 100{%(1-ArOAc)}/{%(1-ArOAc) + %(1-$ArOH_h$)}. Average = 32.2 ± 0.64%.

is excellent at the same molar ratio of $H_2O$, $N_W$, to amide, $N_A$, but different 1-$ArN_2^+$ concentrations. The greatest variations occur for products formed in low yields. The largest average deviation is ca. ±15% for 1-ArNMeAc yields at $N_W/N_A = 2$, Table 2. Tables 1−3 also list the yields of 1-ArOH obtained from direct trapping of 1-Ar+ by $H_2O$, 1-$ArOH_w$, and from hydrolysis of 1-ArOI, 1-$ArOH_h$, which were determined by $^{18}O$ isotopic labeling experiments combined with HPLC results (see below). The last column lists the percent yield of ester obtained from 1-ArOI hydrolysis. No trends are observed in the product yields with variation of the 1-$ArN_2^+$ concentration, but increasing the concentration of $H_2O$, i.e., increasing the $H_2O$ to amide molar ratios, reduces the amount of amide and ester products compared to 1-ArOH. Increasing the number of methyl groups on the amide nitrogen increases the percent yield of ester.

Values of $k_{obs}$ for dediazoniation of 1-$ArN_2^+$ were determined by standard methods under the experimental conditions used for measuring product distributions; i.e., at two different amide-to-$H_2O$ ratios, $N_W/N_A$, at 40 °C to check for composition and medium effects on the rate-determining step (see Supporting Information). The average value of $k_{obs}$, $(3.28 \pm 0.33) \times 10^{-4}$ $s^{-1}$, is constant within 10% and a little lower than $k_{obs}$ in water, ca. $5 \times 10^{-4}$ $s^{-1}$ at 40 °C.[1] The value of $k_{obs}$ increases slightly with the concentration of $H_2O$ and decreases slightly with added *N*-methyl groups on the amide. This insensitivity of $k_{obs}$ to amide type and concentration is consistent with the characteristic insensitivity of thermal dediazoniations to solvent polarity.[1,27,28]

Estimating the selectivity of the 1-Ar+ toward the amide O relative to $H_2O$ and the amide N (see below) requires determination of the yield of 1-ArOI. However, the yield of 1-ArOI could not be measured directly because it hydrolyzes too rapidly, and the phenol from hydrolysis of 1-ArOI, 1-$ArOH_h$, cannot be measured by HPLC because its peak area in the chromatograms cannot be separated from the peak area for phenol formed by direct reaction of 1-Ar+ with $H_2O$. A series of experiments were carried out in 43.85% $H_2{}^{18}O$ to determined the yield of 1-ArOH from hydrolysis of 1-ArOI. Scheme 2 shows the expected distribution of the $^{18}O$ label for trapping of amide O and $H_2O$. The underlined numbers are the molecular weights of the products whose yields were measured by GC/MS and HPLC. The variables, *x*, *y*, and *z*, are needed in product yield calculations (see Supporting Information). The amount of the phenol produced by each pathway was determined from analyses

(27) Swain, C. G.; Sheats, J. E.; Harbison, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 783.
(28) Szele, I.; Zollinger, H. *Helv. Chim. Acta* **1978**, *61*, 1721.

**Scheme 2**

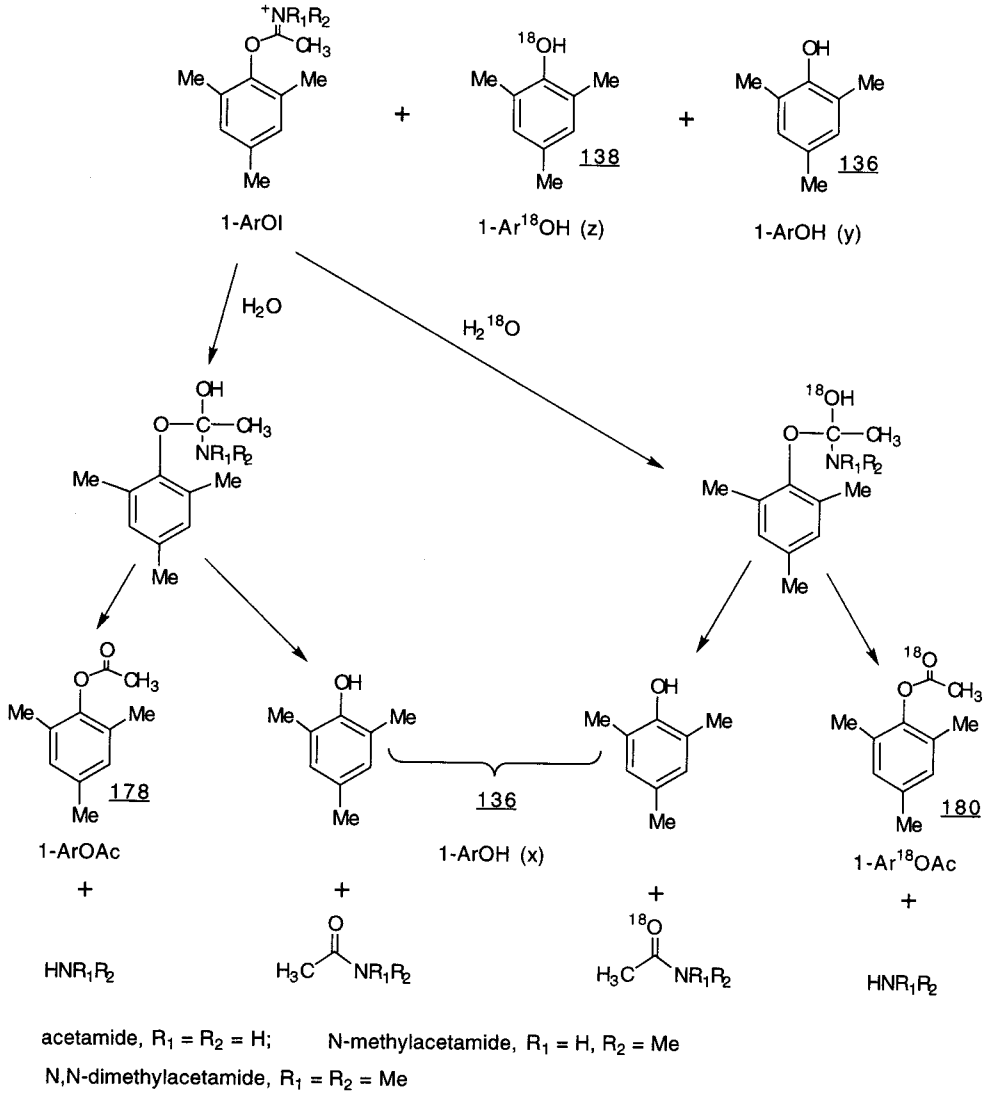



**Table 4.** GC/MS Peak Abundance Ratios, $h_{m/z}$,[a] for Reaction of 5 × $10^{-3}$ M 1-$ArN_2^+$ in Aqueous Amide Solutions Containing 43.85% $H_2{}^{18}O$ at 40 ± 0.1 °C

| amides | $N_W/N_A$[b] | $h_{121}/h_{123}$ | $h_{136}/h_{138}$ | $K$[d] |
|---|---|---|---|---|
| acetamide | 2 | 2.10, 2.10[c] | 2.10, 2.15[c] | 2.11 |
| acetamide | 4 | 1.75, 1.74[c] | 1.78, 1.73[c] | 1.75 |
| *N*-methylacetamide | 2 | 1.82 | 1.82 | 1.82 |
| *N*-methylacetamide | 4 | 1.53 | 1.59 | 1.56 |
| *N*,*N*-dimethylacetamide | 2 | 1.75 | 1.79 | 1.77 |
| *N*,*N*-dimethylacetamide | 4 | 1.51 | 1.54 | 1.52 |

[a] Peak abundance at particular mass-to-charge ratios, *m*/*z*. [b] Molar ratio of water to amide. [c] A different preparation of 1-$ArN_2BF_4$ at a different concentration, 3.62 × $10^{-4}$ M. [d] $K$ is the average peak abundance ratios of $h_{121}/h_{123}$ and $h_{136}/h_{138}$.

of peak abundance ratios for the base and molecular ion peaks of 1-ArOH and 1-Ar$^{18}$OH obtained by GC/MS and analyses of total phenol yields by HPLC of the same samples.

Table 4 lists phenol peak abundance ratio results for dediazoniation of 1-$ArN_2^+$ in aqueous amide solutions containing $^{18}$O-labeled $H_2O$, 43.85%. Control experiments demonstrated that the peak abundance ratios for the molecular ions and base peaks are very reproducible; i.e., they gave the same percentage of $^{18}$O as that of the labeled water within experimental error and provided reliable estimates of the amount of $^{18}$O in the phenol product (see Supporting Information). The peak abundance ratios for the base peak, $h_{121}/h_{123}$, and the parent ion, $h_{136}/h_{138}$, for 1-ArOH are very similar, and triplicate runs gave good reproducibility, typically ±3% or better. The average peak abundance ratios, $K$, are used in calculations of selectivities (see below). The minimum value of $K$ is 1.28; i.e., the 1-ArOH:1-Ar$^{18}$OH ratio = (100% − 43.85%)/43.85%, for 1-ArOH produced only by reaction of 1-$ArN_2^+$ with 43.85% labeled $H_2O$. All measured values of $K$ are greater than 1.28, showing that a significant fraction of unlabeled 1-ArOH is produced by the hydrolysis of 1-ArOI.

The selectivities of 1-Ar+ toward amide O and toward amide N compared to $H_2O$, eqs 1 and 2 respectively, were calculated from the stoichiometric molar ratio $N_W/N_A$ and the normalized yields of 1-$ArOH_w$, 1-ArOI (amide O), and 1-ArNAc (amide N) listed in Tables 1−3.

$$S_W^O = \frac{\%(\text{1-ArOI})}{\%(\text{1-ArOH}_w)} \frac{N_W}{N_A} \quad (1)$$

$$S_W^N = \frac{\%(\text{1-ArNAc})}{\%(\text{1-ArOH}_w)} \frac{N_W}{N_A} \quad (2)$$

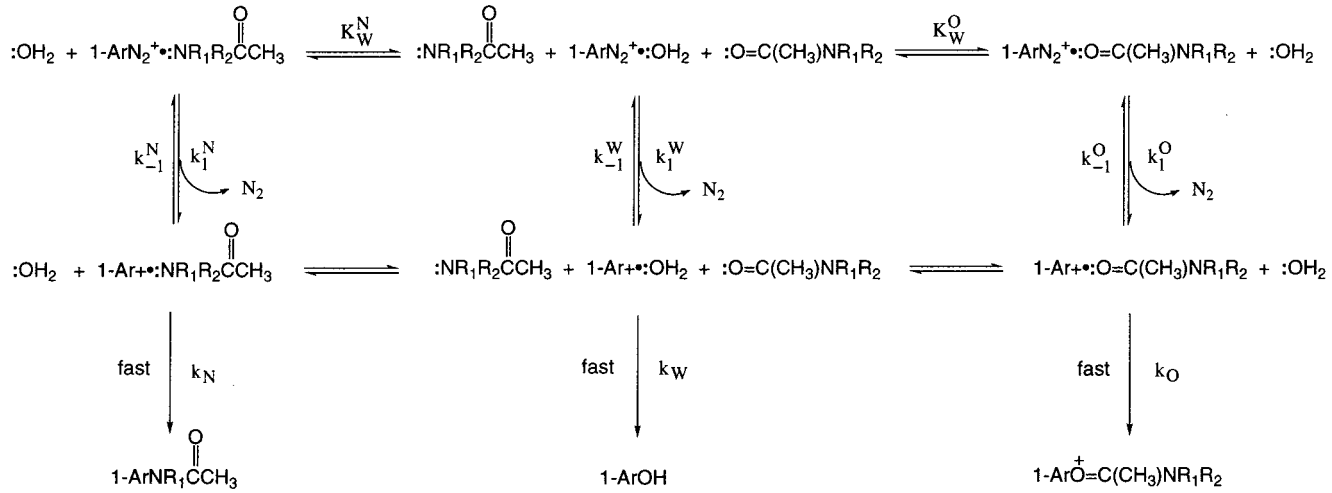
The selectivity of 1-Ar+ toward the amide O compared to the

**Table 5.** %(1-$ArOH_h$) Yields from 1-ArOI Hydrolysis and Average Selectivities Determined by Chemical Trapping at Two Water/Amide, $N_W/N_A$, Molar Ratios at 40 °C

| amide | $N_W/N_A$ | % (1-$ArOH_h$)[a] | $S_W^O$[b] | $S_W^N$[c] | $S_N^O$[d] |
|---|---|---|---|---|---|
| acetamide | 2 | 26.7 | 0.81 (4) | 0.080 (4) | 10.4 |
| acetamide | 4 | 17.1 | 0.92 (4) | 0.092 (4) | 9.94 |
| *N*-methylacetamide | 2 | 19.2 | 0.63 (5) | 0.042 (4) | 15.1 |
| *N*-methylacetamide | 4 | 10.9 | 0.64 (5) | 0.048 (4) | 13.3 |
| *N*,*N*-dimethylacetamide | 2 | 17.7 | 0.62 (4) | ($\leq$0.0001)[e] | |
| *N*,*N*-dimethylacetamide | 4 | 9.5 | 0.63 (4) | | |

[a] Based on total phenol, 1-$ArOH_T$, from 1-ArOI hydrolysis and from reaction with water. [b] Defined by eq 1. Number of averaged values is given in parentheses. [c] Defined by eq 2. Number of averaged values is given in parentheses. [d] Defined by eq 3. [e] Estimate based on the detection limit of the HPLC.

**Scheme 3**




amide N, $S_N^O$, is given by the $S_W^O/S_W^N$ ratio, eq 3. Values of %(1-ArNAc) are obtained directly from HPLC data.

$$S_N^O = \frac{S_W^O}{S_W^N} = \frac{\%(1\text{-ArOI})}{\%(1\text{-ArNAc})} \qquad (3)$$

%(1-$ArOH_w$) is equal to the total phenol yield obtained by HPLC minus the amount of unlabeled phenol produced by hydrolysis of 1-ArOI, Scheme 2. %(1-ArOI) is equal to the sum of the yields of 1-ArOAc and 1-$ArOH_h$. Calculations of %(1-ArOI) and %(1-$ArOH_w$) for each amide require defining the selectivities in terms of the average peak abundance ratios, *K*, listed in Table 4 and the total product yields obtained by HPLC (see Supporting Information).

Table 5 lists the average values of $S_W^O$, $S_W^N$, and $S_N^O$ obtained for acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide for all the results in Tables 1−3. Table 5 also gives the average percent yields of 1-$ArOH_h$ from aryl imidate hydrolysis based on the average total phenol yield, %(1-$ArOH_T$). Several trends are apparent. The values of %(1-$ArOH_h$) decrease with added $H_2O$, indicating an increase in the fraction of %(1-$ArOH_T$) that comes from reaction with $H_2O$. %(1-$ArOH_h$) decreases with the number of *N*-methyl groups, indicating that adding methyl groups makes the amine a better leaving group from the tetrahedral intermediate, Scheme 2. The selectivities for each amide at $N_W/N_A$ ratios of 2 and 4 are essentially the same, and adding one or two methyl groups reduces $S_W^O$ modestly, about 20%. However, adding methyl groups has a much greater effect on $S_W^N$. One methyl reduces $S_W^N$ about 50%, and there is no evidence of trapping of the amide N of *N*,*N*-dimethylacetamide. Based on the assumption that the yield of %(1-$ArNMe_2$) from reaction of 1-Ar+ with *N*,*N*-dimethylacetamide was equal to the detection limit of the HPLC, about 0.05%, $S_W^N$ can be no greater than 0.0001, about 100 times smaller than that for acetamide. Note that the amide O is trapped more efficiently than the amide *N*; i.e., $S_N^O$ is about 10 and 14 for acetamide and *N*-methylacetamide, respectively.

## Discussion

Arenediazonium ions have a rich complex chemistry that is still not completely understood.[11,14] Dediazoniation, i.e., loss of $N_2$ during product formation, proceeds by both homolytic and heterolytic pathways.[11] In the absence of strongly basic nucleophiles, in aqueous solutions containing acid and $O_2$, and in the dark, arenediazonium ions lose $N_2$ spontaneously in a slow step to give a highly reactive aryl cation that is trapped in a subsequent fast step by a nucleophile, Scheme 3 (see below). Zollinger discussed in detail the evidence for the formation of two intermediates leading to product formation, a solvated aryl cation·$N_2$ pair and a free (solvated) aryl cation leading to product formation.[11] Sidhu and Thibblin used the concept of a rate-determining, irreversibly formed carbocation·nucleophile pair to explain the competitive formation of nucleophilic substitution and elimination products in solvolyses of compounds with good leaving groups that form tertiary carbocation intermediates.[29,30]

The single most compelling piece of evidence for rate-determining loss of $N_2$ followed by fast subsequent product-forming steps is the extraordinary insensitivity of the rates of heterolytic dediazoniation reactions to solvent polarity.[1,11,27,28] For example, $k_{obs}$ for dediazoniation of benzenediazonium tetrafluoroborate varies by only 9-fold in solvents such as methylene chloride, dioxane, $H_2O$, fuming sulfuric acid, and

(29) Thibblin, A.; Sidhu, H. *J. Phys. Org. Chem.* **1993**, *6*, 374.
(30) Sidhu, H.; Thibblin, A. *J. Phys. Org. Chem.* **1994**, *7*, 578.

MeOH that vary from about 2 to over 100 in their dielectric constants. This extreme insensitivity of reactivity to solvent polarity stands in dramatic contrast to the extraordinary solvent sensitivity of other reactions, such as decarboxylations that vary by $\sim 10^8$ [31,32] and solvolyses of alkyl halides or arenesulfonates that vary by a factor of $\sim 10^{16}$.[33]

The almost complete insensitivity of $k_{obs}$ to medium effects suggests that selective solvation of the ground state, in particular its interactions with its immediate environment of ions and neutral molecules, remains unchanged in the vicinity of the transition state and the aryl cation intermediate; i.e., the free energy of activation is not significantly affected by solvent. Recent ab initio calculations of bonding in benzenediazonium ions in the gas phase[34] and electron density analyses of gas-phase heterolytic dediazoniations[35] support this interpretation. The calculated binding energy for $N_2$ to the aryl cation is in excellent agreement with experiment,[34] and virtually all the positive charge is distributed among the hydrogens and carbons of the aromatic ring, indicating that the solution chemistry of the benzendiazonium ion depends on its intrinsic properties and not its interactions with the medium.[34] Glaser, Horan and Zollinger conclude that "...[d]iazonium ions can best be thought of as carbenium ions closely associated with an $N_2$ molecule that is internally polarized in the fashion $N_\alpha^{\delta-}-N_\beta^{\delta+}$."[35]

Substantial evidence suggests that the aryl cation must be extremely short-lived, probably less than 500 ps,[36−38] consistent with the reactivity−selectivity principle.[39] The formation of a highly reactive charged delocalized cation is also consistent with the extremely low selectivity typically shown by arenediazonium ions toward competing nucleophiles. The selectivity of 1-$ArN_2^+$ toward alcohols versus $H_2O$ is about 0.3−0.6,[1,3] and it is only about 10 times more selective toward anions over $H_2O$.[1,27] The results in Table 5 show that the selectivities toward amide O compared to $H_2O$ are a little less than 1 and amide N compared to $H_2O$ are about 10 times less.

Scheme 3 is an adaptation of Zollinger's ion·molecule pair mechanism for dediazoniation to reactions in aqueous amide solutions.[40,41] The selectivities of the aryl cation·$N_2$ pair and the free aryl cation toward nucleophiles are assumed to be the same. A preassociation equilibrium[42] in the ground state is included to describe the selectivities of the dediazoniation reaction toward different nucleophiles. This approach is consistent with Rys's detailed analysis of the factors that may contribute to chemical selectivity, including nonstoichiometric reactant distribution within the solvent cage of an encounter complex.[43] The equilibrium distributions of arenediazonium cation·nucleophile pairs represent selective solvation of the reactive diazonio group of 1-$ArN_2^+$ by the nucleophiles, water, amide N, and amide O in the ground state. Nucleophile exchange is assumed to occur at the diffusion-controlled limit. The remainder of Scheme 3 illustrates rate-determining formation of the selectively solvated aryl cation·nucleophile intermediates which collapse in a subsequent fast steps to give stable products. Selective solvation of the diazonio group is represented by the equilibrium constants, $K_W^N$ and $K_W^O$. Product yields are proportional to the concentration of each nucleophile in solution and to the equilibrium constants for formation of each arenediazonium cation·nucleophile pair. The value of $k_{obs}$ reflects the rate of spontaneous decomposition of the entire ensemble of ground-state arenediazonium ion·nucleophile pairs. The lifetimes of the aryl cation intermediates are currently unknown. If their lifetimes are significantly longer than the diffusion-controlled limit, then exchange of nucleophiles between these intermediates is possible, as shown for the aryl cation·nucleophile intermediates, Scheme 3. However, because charge distributions in the ground, transition, and intermediate states are very similar (see above), the distributions of nucleophiles in the aryl cation·nucleophile pairs are assumed to be the same as in the ground state. Relationships between measured selectivities and equilibrium constants for nucleophile exchange have been derived and applied to alcohol and halide ion exchange with water.[1] The relationships indicate that measured selectivities are equal to the exchange constants, provided the rate constants for collapse of the aryl cation·nucleophile pairs to products in the fast steps are the same, i.e., $k_N = k_W = k_O$.

The interaction of 1-$ArN_2^+$ with weakly basic nucleophiles can be viewed as the formation of a complex and the selectivities as a measure of relative basicities of the nucleophiles. In this sense, $H_2O$ and the amide O solvate the diazonio group to about the same extent, i.e., $S_W^O \approx 0.6-0.9$, consistent with the similarities in their interactions with a proton; i.e., the p$K_a$ of water is $-1.74$, and those of amides are in the range from 0 to $-4$.[39] The greater solvation of 1-$ArN_2^+$ by amide O over amide N, i.e., $S_N^O \geq 10$, is consistent with significant contribution from the charged resonance form; i.e., a negative charge on O, a positive charge on N, and a double bond between C and N. Recent semiempirical and ab initio calculations seem at variance with this picture because, regardless of the basis set used, the calculations always give greater negative charge density on the amide N than on the amide O.[44] However, the negative charge density on the amide O is always greater than that of the entire $-NH_2$ group, which in some basis sets has a net charge near zero. Thus, these calculations are consistent with the amide O solvating 1-$ArN_2^+$ better than the $-NH_2$ and probably better than the $-NHMe$ and $-NMe_2$ groups.

The results with *N*,*N*-dimethylacetamide are strikingly different in that no product appears to be formed from reaction with amide N. Chemical trapping of 1-Ar+ by the nitrogen of *N*,*N*-dimethylacetamide should give the acetyl *N*,*N*-dimethyl-2,4,6-trimethylanilinium ion. This ion should be a reactive intermediate, similar to acyl pyridinium ions,[45] and hydrolyze rapidly into acetic acid and 1-$ArNMe_2$, in its protonated or unprotonated form. The absence of a significant yield of 1-$ArNMe_2$ suggests that the $-NMe_2$ group may be sufficiently hydrophobic that it does not complex (solvate) the diazonio group of 1-$ArN_2^+$ significantly, and no product is formed from reaction with 1-Ar+. Alternatively, 1-Ar+ may not react with tertiary amides because concerted loss of a proton is a fundamental requirement for aryl-N bond formation. This explanation is consistent with our chemical trapping results in

(31) Kemp, D. S.; Paul, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 7305.
(32) Kemp, D. S.; Cox, D. D.; Paul, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 7312.
(33) Szele, I.; Zollinger, H. *J. Am. Chem. Soc.* **1978**, *100*, 2811.
(34) Glaser, R.; Horan, C. J. *J. Org. Chem.* **1995**, *60*, 7518.
(35) Glaser, R.; Horan, C. J.; Zollinger, H. *Angew. Chem., Intl. Ed. Engl.* **1997**, *36*, 2210.
(36) Scaiano, J. C.; Kim-Thuan, N. *J. Photochem.* **1983**, *23*, 269.
(37) Chateauneuf, J. E. *J. Chem. Soc., Chem. Commun.* **1991**, 1437.
(38) Cacace, F. *Science* **1990**, *250*, 392.
(39) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper and Row: New York, 1987.
(40) Hashida, Y.; Landells, R. G. M.; Lewis, G. E.; Szele, I.; Zollinger, H. *J. Am. Chem. Soc.* **1978**, *100*, 2816.
(41) Ravenscroft, M. D.; Takagi, K.; Weiss, B.; Zollinger, H. *Gazz. Chim. Ital.* **1987**, *117(b)*, 353.
(42) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345.
(43) Rys, P. *Acc. Chem. Res.* **1976**, *9*, 345.
(44) Estiu, G. L. *Theochem* **1997**, *401*, 157.
(45) Carey, R. A. *Advanced Organic Chemistry. Part A: Structure and Mechanism*, 3rd ed.; Plenum Press: New York, 1993.

nonionic oligooxyethylene monoalkyl ether micelles and in aqueous oligooxyethylene glycol solutions.[3] Product is formed from reaction with the terminal −OH group which has a removable proton, but no products are observed from trapping by ether oxygens within the oligooxyethylene chains; i.e., no C−O bond cleavage is observed.

The mechanism of hydrolysis of imidate esters has been studied in detail.[21−26] The yield of ester formed by hydrolysis of 1-ArOI, Scheme 2, at $H_2O$:amide molar ratios of 2 and 4 is independent of solution composition but increases with the number of methyl groups on the amide nitrogen, Tables 1−3. The average ester yields for acetamide, *N*-methylacetamide, and *N*,*N*-dimethylacetamide are respectively 10.1 ± 0.6%, 24.2 ± 0.34%, and 32.2 ± 0.64%. This increase in ester yield with the number of methyl groups compares favorably with the results of Satterthwait and Jencks[21] for the hydrolysis of *p*-tolyl *N*-methylacetimidate (∼10% ester) and *p*-tolyl *N*,*N*-dimethylacetimidate (∼20% ester) in dilute aqueous solution between pH 3 and 6, in which the aryl imidate is in its protonated form at 25 °C. Thus, the mechanism for aryl imidate hydrolysis in aqueous amide solutions and in dilute aqueous solution is probably the same. The high reproducibility of 1-$ArOH_h$ and 1-ArOAc yields shows that selectivities determined by the chemical trapping method from these data, Table 5, are reliable.

## Chemical Trapping of Aggregate-Bound Polypeptides

Structures and orientations of polypeptides at aggregate surfaces are difficult to determine by CD, 2D NMR, IR, electrophoretic mobility, gel filtration, and calorimetry[46−51] because their structures are often not fixed by multiple cross-links and internal hydrogen bonds but are induced by multiple hydrophobic, electrostatic, hydration, and hydrogen bonding interactions at interfaces.[46,49] Chemical tagging of aggregate-bound polypeptides should identify those sections of the polypeptides in the interfacial region of the aggregate. Chemical tagging of proteins at interfaces has been attempted by using a variety of reagents,[52−54] including arenediazonium ions,[55−57] but without much success. We recently demonstrated by HPLC that amphiphilic 16-$ArN_2^+$ gives significant yields of products from reaction with the terminal carboxylate groups and amide O (but not amide N) in micelles of sodium lauroylsarcosinate and sodium lauroylglycinate. These surfactants have *N*-methylglycine and glycine headgroups, respectively, and are reasonable models of peptide bonds at aggregate interfaces.[58] 16-$ArN_2^+$ should tag amino acid side chains, and reaction with the peptide bond should give fragments after hydrolysis (O cleavage). The tagging and fragmentation data should identify which peptide bonds are located in the interfacial region of the aggregates and provide information on the topology and orientation of the original polypeptide at the aggregate surface. Small yields of multiple peptide fragments can be determined by mass spectroscopy or by HPLC using fluorescence detection after tagging the amino terminus of the cleaved peptide bond with a fluorescent tag such as 9-fluorenylmethyl chloroformate (FMOC-Cl).[59]

## Conclusions

Chemical trapping of acetamide and *N*-methylated acetamides with 2,4,6-trimethylbenzenediazonium ion, 1-$ArN_2^+$, in aqueous solution works. Observed rate constants and product yields are completely consistent with the heterolytic dediazoniation mechanism, i.e., rate-determining loss of $N_2$ to give a highly reactive/unselective aryl cation, 1-Ar+, that traps the weakly basic nucleophiles in a separate, subsequent step, in aqueous amide solutions: $H_2O$, amide N, and amide O. Recent ab initio calculations support this model. Trapping of the amide nitrogen gives stable aryl amides with acetamide and *N*-methylacetamide, However, no observable product is obtained from trapping of the nitrogen in *N*,*N*-dimethylacetamide, suggesting either that the $-NMe_2$ group is too nonpolar to solvate the cationic diazonio group or that a removable proton is required for product formation. Trapping of amide oxygens gives aryl imidate intermediates that hydrolyze within the dediazoniation reaction time to give ester/amine and phenol/amide product pairs, consistent with literature results. The traditional heterolytic dediazoniation mechanism is combined with a preassociation mechanism to describe the competitive solvation of the diazonio group by $H_2O$, amide O, and amide N. The chemical trapping method provides a potentially novel approach for determining the topologies and orientations of aggregate-bound polypeptides.

## Experimental Section

**Methods.** $^1H$ and $^{13}C$ NMR spectra were recorded on a Gemini-200 spectrophotometer. Mass spectra of synthesized compounds were obtained on a HP 5890 Series II GC interfaced with a HP 5971 mass-selective detector using a 12-m × 0.2-mm HP-1 capillary column (cross-linked methyl silicone on fused silica), 0.33-mm particle size. IR spectra were recorded on a Mattson Genesis Series FT-IR spectrophotometer. Product yields were determined on a Perkin-Elmer LC-235 HPLC equipped with a Ranin Microsorb-MV C-18 reversed-phase column (5-mm particle size, 4.6 mm i.d. × 25 cm), a 200-μL sample loop, a LC-235 diode array detector, and a PE-Nelson 900 series interface attached to a Ultra PC computer. The mobile phase for product separation was 60%/40% MeOH/$H_2O$ with a flow rate of 0.8 mL/min for all runs. HPLC chromatograms were analyzed by using PE Nelson Turbochrom 3 software. Absorbances were measured at 220 nm, and reported peak areas are averages of triplicate injections. Kinetics were monitored on Perkin-Elmer UV/visible 559A spectrophotometer fitted with electronic temperature control. Melting points were determined by using a Mel-Temp apparatus and are uncorrected.

**Materials.** HPLC grade MeOH, 2,4,6-trimethylphenol, 1-ArOH (99%), acetamide (99+%), *N*-methylacetamide (99+%), *N*,*N*-dimethylacetamide (99.9+%), 1,3,5-trimethylbenzene (98%), 1-ArH, *N*,*N*-dimethylaniline (99.5+%), 1-$ArNMe_2$, ethyl acetate, EA, petroleum ether, PE, and inorganic reagents were purchased from Aldrich, and 43.85% $H_2{}^{18}O$ was purchased from Icon Services, Inc. (Summit, NJ). All reagents were used as received. Preparations of 2,4,6-trimethylbenzenediazonium tetrafluoroborate, 1-$ArN_2BF_4$, and 2,4,6-trimethylfluorobenzene, 1-ArF, have been described.[1,3] Dediazoniation products

(46) DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622.
(47) Shon, K.-J.; Kim, Y.; Colnago, L. A.; Opella, S. J. *Science* **1991**, *252*, 1303.
(48) de Jongh, H. H. J.; Goormaghtigh, E.; Killian, J. A. *Biochemistry* **1994**, *33*, 14521.
(49) Taylor, J. W.; Osapay, G. *Acc. Chem. Res.* **1990**, *23*, 338.
(50) Buser, C. A.; Sigal, C. T.; Resh, M. D.; McLaughlin, S. *Biochemistry* **1994**, *33*, 13093.
(51) Flach, C. R.; Brauner, J. W.; Taylor, J. W.; Baldwin, R. C.; Mendelsohn, R. *Biophys. J.* **1994**, *67*, 402.
(52) Aggeler, R.; Chicas-Cruz, K.; Cai, S.-X.; Keana, J. F. W.; Capaldi, R. A. *Biochemistry* **1992**, *31*, 2956.
(53) Davison, M. D.; Findlay, J. B. C. *Biochem. J.* **1986**, *234*, 413.
(54) Andres, S. F.; Atassi, M. Z. *Biochemistry* **1973**, *12*, 942.
(55) Prochaska, L.; Bisson, R.; Capaldi, R. A. *Biochemistry* **1980**, *19*, 3174.
(56) Ludwig, B.; Prochaska, L.; Capaldi, R. A. *Biochemistry* **1980**, *19*, 1516.
(57) Eytan, G. D.; Broza, R. *J. Biol. Chem.* **1978**, *253*, 3196.
(58) Unpublished results.
(59) Einarsson, S.; Folestad, S.; Josefsson, B.; Lagerkvist, S. *Anal. Chem.* **1986**, *58*, 1638.

have been prepared previously, and literature references are given. All aqueous solutions were prepared by using distilled water which was passed over activated carbon and deionizing resin and then redistilled.

**Methyl 2,4,6-Trimethylphenyl Ether, 1-ArOMe.**[60] The ether was prepared by alkylation of 1-ArOH with MeI according to the procedure of Miller et al.[61] The crude product was purified by column chromatography on alumina eluted with *n*-pentane giving a colorless liquid; 65% yield: IR (neat) $\nu_{max}$ ($cm^{-1}$) 2938, 1599, 1484, 1451, 1225, 1144, 1019, 853, 762; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 6.92 (2H, s), 3.82 (3H, s), 2.31 (3H, s), 2.38 (6H, s); GC/MS 150 (*m*/*e*), 135, 119, 105, 91, 79, 65.

***N*-(2,4,6-Trimethylphenyl)acetamide, 1-ArNHAc.**[62] The aryl amide was prepared by acylation of 2,4,6-trimethylaniline, 1-$ArNH_2$, with acetyl chloride according to a procedure for the preparation of benzanilide.[63] The crude product was recrystallized twice from EtOH/$H_2O$ to give white crystals; 80% yield (mp 215−216 °C): IR (film in $CH_2Cl_2$) $\nu_{max}$ ($cm^{-1}$) 3425.5, 3019.1, 1668.7, 1215.9, 770.2; $^1H$ NMR ($CDCl_3$:DMSO-$d_6$ 1:1) $\delta$ (ppm) 8.99 (1H, s), 6.81 (2H, s), 2.21 (3H, s), 2.10 (6H, s), 2.04 (3H, s); GC/MS 177 (*m*/*e*), 135, 120, 91, 77, 65, 43, 39.

***N*-(2,4,6-Trimethylphenyl)-*N*-methylacetamide, 1-ArNMeAc.**[64] The aryl methyl amide was prepared by the phase-transfer catalysis procedure of Kalkote et al. for the N-alkylation of aryl amides.[65] 1-ArNHAc was methylated with dimethyl sulfate in a stirred mixture of aqueous $NaOH/K_2CO_3$ and toluene at ca. 30 °C for ca. 2 h using tetra-*n*-butylammonium hydrogen sulfate as the catalyst. The white flat crystals were recrystallized from EtOH/$H_2O$ and further purified by chromatography on silica (eluting solvent PE:EA:$Et_2O$ 1:1:1) and by preparative HPLC (C-18 reversed-phase, 40% $H_2O$/60% MeOH, 220 nm). The overall yield was 80% (mp 53−54 °C, lit.[64] mp 52−52.5): IR (film in $CH_2Cl_2$) $\nu_{max}$ ($cm^{-1}$) 3053.6, 1650.3, 1264.9, 1064.9, 1034.2, 896.0, 738.0; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 6.93 (2H, s), 3.11 (3H, s), 2.30 (3H, s), 2.16 (6H, s), 1.73 (3H, s); GC/MS 191 (*m*/*e*), 176, 148, 134, 119, 105, 91, 77, 65, 56, 43.

**2,4,6-Trimethylphenyl Acetate, 1-ArOAc.**[66] The ester was prepared from 1-ArOH and acetyl chloride using a procedure based on the preparations of phenyl acetate and phenyl benzoate.[63] Vacuum distillation gave an impure product (GC/MS), which was further purified by column chromatography on silica (eluting solvent EA:hexane 1:6 or $CH_2Cl_2$:PE 1:3). The overall yield of the colorless liquid was 90% (bp 85−90 °C, 3−4 mmHg): IR (neat) $\nu_{max}$ ($cm^{-1}$) 3011.5, 1754.6, 1607.1, 1370, 1222.7, 1191.0, 1137.1, 910.6, 854.5, 733.0; $^1H$ NMR ($CDCl_3$) $\delta$ (ppm) 6.91 (2H, s), 2.36 (3H, s), 2.30 (3H, s), 2.15 (6H, s); $^{13}C$ NMR, broad-band decoupled ($CDCl_3$) $\delta$ (ppm) 169.6, 146.4, 135.8, 130.1, 129.7, 21.3, 21.0, 16.7; GC/MS 178 (*m*/*e*), 136, 121, 91, 77, 65, 43.

**HPLC Experiments/Product Yields.** Products were identified and their yields obtained by the following general procedures. Reaction was initiated by injecting small amounts, ca. 20 $\mu$L, of freshly prepared, ice cold, stock solutions of 0.1 M 1-$ArN_2BF_4$ in MeOH into aqueous amide solutions of varying molar ratios in 5−10-mL volumetric flasks thermostated at 40 °C. Note that MeOH was used as the solvent for 1-$ArN_2BF_4$ instead of MeCN because the peak in the chromatograms for amide product formed from reaction of 1-$ArN_2^+$ with MeCN has the same retention time as that of some products from reaction with the amides.[3] MeOH concentations in the reaction mixtures varied from a low of 0.5% (v/v) at $5 \times 10^{-4}$ M to 8.2% at $7.81 \times 10^{-3}$ M 1-$ArN_2^+$, see Tables 1−3. After 24−72 h (the half-life for dediazoniation is ca. 35 min at 40 °C), products were separated by HPLC.

1-ArOH, 1-ArNAc, and other products (see below) were identified in HPLC chromatograms by spiking experiments using independently prepared and characterized compounds. One significant new peak consistently appeared in the HPLC chromatograms. Product mixtures from reaction of 1-$ArN_2^+$ in neat liquid *N*-methylacetamide were extracted with $Et_2O$, and GC/MS analysis showed that this peak was 1-ArOAc. This assignment was confirmed by isolating 1-ArOAc from the product mixture by column chromatography and characterizing it by $^1H$ and $^{13}C$ NMR, and by a spiking experiment with independently synthesized 1-ArOAc. In previous work, we found that some products, particularly aryl halide products, have significant vapor pressures and that their yields decrease on standing.[1] This problem was solved by layering small amounts of cyclohexane on the top of the solutions to dissolve volatile products. The cyclohexane method was used here to check for product loss; small increases in the total yield were found, but the product ratios were unchanged.

Several other products (not shown in Scheme 1) are observed in the HPLC chromatograms. All have been identified previously and synthesized,[1,3] if not available commercially: 2,4,6-trimethylbenzene, 1-ArH, 2,4,6-trimethylfluorobenzene, 1-ArF, and 2,4,6-trimethylanisole, 1-ArOMe. The observed yields of these products are small and variable. Typical yields are as follow: 1-ArH, <2%; 1-ArF, <2%; and 1-ArOMe, <3%. 1-ArF is formed via the Schiemann reaction[67] from 1-$ArN_2BF_4$ in the solid state or in solution.[3] 1-ArOMe is formed in the MeOH stock solutions and possibly in the reaction mixture. Formation of these two products reduces the amount of 1-$ArN_2^+$ available to react but does not affect the relative yields of 1-ArOH, 1-ArNAc, and 1-ArOAc. 1-ArH is formed by reaction of 1-ArOH with unreacted 1-$ArN_2^+$,[3] but its effect on the total phenol yield is small, and it was ignored in all calculations. Typical retention times in minutes for dediazoniation products are as follow: 1-ArNHAc, 6−7; 1-ArOH, 11−12; 1-ArNMeAc, 12−13; 1-ArOAc, 17−18; 1-ArOMe, 25−26; 1-ArH, 45−48; and 1-ArF, 52−54. Concentrations of products were obtained from peak areas using standard calibration curves obtained from independently prepared or commercial products. Percent yields reported in the Supporting Information are based on the concentration of added 1-$ArN_2BF_4$. Normalized yields based on the total yields of phenol, ester and amide products were used in the calculations of selectivities.

**The Search for *N*,*N*-Dimethylaniline.** Dediazoniations were carried out in two different aqueous *N*,*N*-dimethylacetamide solutions with $N_W/N_A$ molar ratios of 2 and 4 containing $4.71 \times 10^{-3}$ M 1-$ArN_2BF_4$ (ca. 2.5% MeOH v/v) at 40 °C for 24 h. No peak was observed at the retention time for 1-$ArNMe_2$, but the areas of all other peaks were consistent with earlier results (see Table 3). In a separate set of experiments, 0.05, 0.1, and 1 equiv (compared to 1-$ArN_2^+$) of 1-$ArNMe_2$ were added to $5 \times 10^{-3}$ M 1-$ArN_2^+$ in a 2:1 molar ratio of $H_2O$:*N*,*N*-dimethylacetamide at 40 °C. Increasing the amount of 1-$ArNMe_2$ did not affect the total product yield or the 1-ArOH:1-ArOAc product yield ratio significantly within experimental error, i.e., ±5%. A special gradient eluting system was used to minimize broadening of the 1-$ArNMe_2$ peak: 0−7 min, 0.05% TFA/80% $H_2O$/20% MeCN; 7−37 min, the ratio was changed linearly to 0.05% TFA/20% $H_2O$/80% MeCN and held constant for 5 min, followed by wash cycles, and returned to the original condition. Typical retention times were as follow: 1-$ArNMe_2$, 10 min; 1-ArOH, 30 min; 1-ArOAc, 36 min; and 1-ArOMe, 39.5 min. The smallest peak observable by our detector has an area of ca. $5 \times 10^3$ $\mu$V·s, which is equivalent to ca. $2.49 \times 10^{-6}$ M 1-$ArNMe_2$ or about 0.05% of the initial concentration of 1-$ArN_2BF_4$ in the aqueous *N*,*N*-dimethylacetamide reaction mixture.

**Isotopic Labeling Experiments.** Experiments using 43.85% $H_2{}^{18}O$ were prepared by adding successively to 500-$\mu$L cone-shaped flasks (Weaton), 45 $\mu$L of $H_2{}^{18}O$, sufficient weight or volume (based on densities) of the amide, i.e., acetamide, *N*-methylacetamide, or *N*,*N*-dimethylacetamide, to give the needed $N_W/N_A$ molar ratio (see Table 4), and sufficient volume of a freshly prepared stock solution of 1-$ArN_2BF_4$ in MeOH (0.05 M) to give a final concentration of $5 \times 10^{-3}$ M 1-$ArN_2^+$. The final concentration (by volume) of MeOH in the solutions was about 10%. The solutions were thermostated at 40 °C overnight and then analyzed by GC/MS. Reported peak abundances were average values of triplicate injections.

(60) Miller, B. *J. Org. Chem.* **1977**, *42*, 1402.
(61) Miller, J. M.; So, K. H.; Clark, J. H. *Can. J. Chem.* **1979**, *57*, 1887.
(62) Bradshaw, J. S.; Knudsen, R. D.; Loveridge, E. L. *J. Org. Chem.* **1970**, *35*, 1219.
(63) Furniss, B. S.; Hannaford, A. J.; Rogers, V.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 4th ed.; Longman: London, 1979; p 1368.
(64) Volz, H.; Ruchti, L. *Justus Liebigs Ann. Chem.* **1972**, *763*, 184.
(65) Kalkote, U. R.; Choudhary, A. R.; Natu, A. A.; Lahoti, R. J.; Ayyangar, N. R. *Synth. Commun.* **1991**, *21*, 1889.
(66) Baciocchi, E.; Rol, C.; Mandolini, L. *J. Org. Chem.* **1977**, *42*, 3682.
(67) Swain, C. G.; Rogers, R. J. *J. Am. Chem. Soc.* **1975**, *97*, 799.

**Acknowledgment.** We appreciate Jianping Lou's assistance with the synthesis of some of the analytical standards, Arabinda Chaudhuri for preparing 1-ArOMe; helpful discussions with Santanu Bhattacharya, Clifford Bunton, Douglass G. Dalgleish, Robert Moss, Ron Sauers, John Taylor, and Heinrich Zollinger; and very useful comments by the reviewers. L.Z. thanks Rutgers University for the Bevier Fellowship that provided partial support of her Ph.D. research. L.S.R. thanks the National Science Foundation, Organic Dynamics Division (CHE 95-26206), NSF-U.S.-Brazil Cooperative Science Program (INT-9722458). This is publication no. D10535-1-98 of the New Jersey Agricultural Experiment Station, supported by State Funds and the Center for Advanced Food Technology (CAFT).

**Supporting Information Available:** Appendix S1, showing the derivation of the equations used to analyze the product yields in the $^{18}O$ labeling experiments; Tables S1−S3, containing peak areas, measured and normalized product yields, and HPLC calibration curves for Tables 1−3; Table S4, completely listing of phenol yields and selectivities for Table 5; Table S5, listing the dediazoniation rate constants and the experimental method for measuring $k_{obs}$; and Table S6, summarizing the control experiments for the $^{18}O$ labeling experiments (8 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA980556A