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Conversion of Azides into Diazo Compounds in Water

Ho-Hsuan Chou[†] and Ronald T. Raines*[,†,∥]

[†]Department of Chemistry and [∥]Department of Biochemistry, University of Wisconsin—Madison, Madison, Wisconsin 53706, United States

Supporting Information

ABSTRACT: Diazo compounds are in widespread use in synthetic organic chemistry but have untapped potential in chemical biology. We report on the design and optimization of a phosphinoester that mediates the efficient conversion of azides into diazo compounds in phosphate buffer at neutral pH and room temperature. High yields are maintained in the presence of common nucleophilic or electrophilic functional groups, and reaction progress can be monitored by colorimetry. As azido groups are easy to install and maintain in biopolymers or their ligands, this new mode of azide reactivity could have substantial utility in chemical biology.

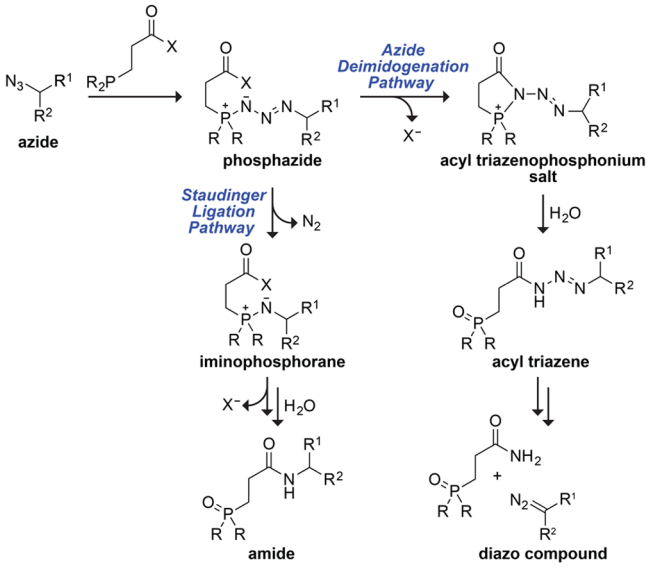
Diazo compounds are among the most versatile intermediates in organic synthesis. Because of their inherent dipolar nature, diazo compounds can readily participate in 1,3-dipolar cycloaddition reactions with a wide range of dipolarophiles.[1] Moreover, C-protonation gives rise to diazonium ions, which are highly reactive alkylating agents.[2] Thermal or photochemical generation of carbenes, along with transition metal-mediated carbenoid formation, facilitates addition to double bonds and insertion into C−H, O−H, and N−H bonds.[3] This broad reactivity makes diazo compounds attractive for applications in chemical biology, having special promise in the labeling of proteins[4,5] and as tunable reactants in 1,3-dipolar cycloaddition reactions with cycloalkynes.[1,6]

Although the first diazo compound was synthesized in the 19th century,[7] there are still few methods for their construction: diazo transfer to an activated C−H acceptor, diazotization of an amine, decomposition or oxidation of a hydrazone, rearrangement of an *N*-acyl-*N*-nitrosoamine, and fragmentation of a triazene.[5c,7,8] The harsh conditions required to access diazo compounds can provoke undesirable reactivity with common functional groups, and the need for organic solvents hampers the generation of diazo compounds in many molecules of biological interest.

Recently, we reported that an azide can be converted into the corresponding diazo compound by the generation and subsequent decomposition of an acyl triazene.[8f] The phosphinoester reagent that mediates this deimidogenation is, however, nearly insoluble in water and unstable to hydrolysis, and the preferred solvent is THF/H_2O (20:3). As azido groups have become a privileged functionality in chemical biology,[9] a stable, water-soluble reagent that converts azides into their corresponding diazo compounds under physiological conditions and that tolerates the sensitive functionalities in biological molecules could have high utility.

According to our putative mechanism (Scheme 1),[8f,10] azide deimidogenation begins with nucleophilic attack of the phosphine on the azide to form a phosphazide. Then, the reaction takes one of two possible pathways, depending on the ability of the pendant acyl group to trap the phosphazide intermediate prior to N_2(g) extrusion. We hypothesized that this ability correlates with the pK_a of the conjugate acid of the leaving group, and that more reactive acyl groups would favor diazo compound formation and less reactive groups would favor amides.

Scheme 1



To test our hypothesis, we surveyed the reactivity of a wide variety of potential phosphinoesters with α-azido-*N*-benzylacetamide. Reaction mixtures had equimolar reactants, which were incubated at room temperature for 4 h, quenched with saturated aqueous $NaHCO_3$, and then stirred for another 8 h. We found that the diazo compound/amide product ratio does indeed correlate with the pK_a of the conjugate acid of the leaving group (Table 1). When the pK_a was ≥9.2, the intermediate phosphazide underwent rapid N_2(g) extrusion rather than acyl transfer, yielding exclusively the Staudinger ligation product. As the pK_a was lowered from 9.0 to 7.6, the

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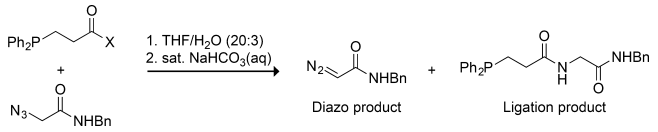
Table 1. Phosphinoester Reactivity and Stability



		product ratio[a]		% decomposition at pH			
XH	pK_a	diazo compound	amide	4.0	7.0	9.0	12.0
methanol	15.5	0	100				
ethanethiol	10.6	0	100				
4-fluorophenol	10.0	0	100				
benzylmercaptan	9.4	0	100				
3-(dimethylamino)phenol	9.2	0	100				
3-chlorophenol	9.0	33	67				
3,5-difluorophenol	8.7	63	27	<5	<5	5	21
3-hydroxypyridine	8.5	67	33	<5	<5	12	62
2,2,2-trifluoroethanethiol	7.6	83	17	<5	<5	13	35
4-nitrophenol	7.1	97	3	<5	<5	10	46
2,4,6-trifluorophenol	6.9	97	3	<5	<5	<5	13
N-hydroxysuccinimide	6.0	98	2	12	40	54	98
pentafluorophenol	5.5	97	3	<5	<5	<5	16

[a]Determined by ^{1}H NMR spectroscopy: δ 4.73 (1H, diazo compound) and 3.95 (2H, amide).

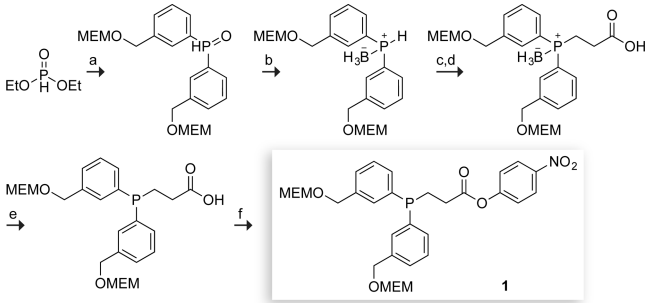
product ratio began to increase. When the pK_a was ≤7.1, the reaction produced the diazo compound, almost exclusively.

In addition to promoting diazo-compound formation, a useful reagent must exhibit high chemical stability. We were concerned about this attribute because esters formed with alcohols having pK_a ≤7.1 can be unstable to hydrolysis. Accordingly, we assessed stability by stirring a reagent in phosphate buffer containing 40% (v/v) THF (to enhance solubility) for 19 h and evaluating decomposition by ^{1}H NMR spectroscopy. Hydrolysis was not observed at pH 7.0 (Table 1), except for the *N*-hydroxysuccinimide (NHS) ester, which was the object of our previous study.[8f] Most of the phosphinoesters were likewise stable at pH 9.0. On the basis of these reactivity/stability screens, ease-of-synthesis, and chromogenicity, the 4-nitrophenyl ester was chosen as ideal.

Next, we sought a reagent that was soluble in water. We reported previously that *N,N*-dimethylamino groups imparted water solubility to phosphinothioesters and enabled the traceless Staudinger ligation in water.[11] *N,N*-Dimethylamino groups, however, led to rapid decomposition of the phosphinoesters used herein. After screening other functional groups that confer water solubility on similar phosphines,[11] we settled on methoxyethoxymethyl (MEM) as a preferred group.[12]

The synthesis of an optimized reagent for the conversion of azides into diazo compounds in water began from 3-bromobenzyl alcohol that was MEM-protected, converted into the corresponding Grignard compound, and added to diethyl phosphite to give the bis-aryl phosphine oxide (Scheme 2). Reduction of the phosphine oxide and subsequent protection with borane dimethylsulfide gave the borane-protected phosphine.[13] Direct deprotonation of the protected phosphine and subsequent conjugate addition with methyl acrylate followed by hydrolysis afforded the phosphine-carboxylic acid.[8f,14] Removing the borane protecting group with a mild methanol reflux,[15] and installing the 4-nitrophenylester using standard coupling conditions provided phosphinoester **1** in 6 steps and 45% overall yield.

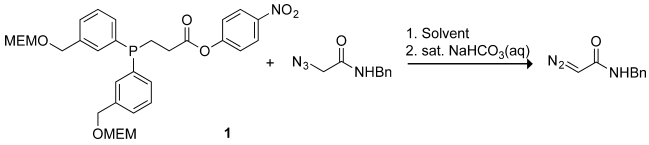
Scheme 2. Synthetic Route to Phosphinoester 1[a]



[a]Conditions: (a) Mg, aryl bromide, THF, 95%; (b) BH_3−SMe_2, THF, 98%; (c) NaOMe, methy acrylate, MeOH, 71%; (d) 7 N KOH, MeOH, 98%; (e) MeOH/toulene reflux, 97%; (f) 4-nitrophenol, EDC, CH_2Cl_2, 72%.

The ability of phosphinoester **1** to convert azides into their corresponding diazo compounds was assessed first in phosphate buffer (Table 2). α-Azido-*N*-benzylacetamide was treated with phosphinoester **1** in buffers of various pH for 24 h,

Table 2. Effect of pH and Organic Cosolvent on Diazo Compound Formation by Phosphinoester 1



entry	solvent[a]	yield (%)[b]	entry	solvent[c]	yield (%)[b]
1	pH 5.0	69	5	50% v/v MeOH	80
2	pH 6.0	88	6	50% v/v CH_3CN	74
3	pH 7.0	91	7	50% v/v DMF	75
4	pH 8.0	71	8	1% v/v DMSO	74
			9	20% v/v E.G.	78

[a]10 mM sodium phosphate buffer. [b]Isolated yield. [c]In 10 mM sodium phosphate buffer, pH 7.0. E.G. = ethylene glycol.

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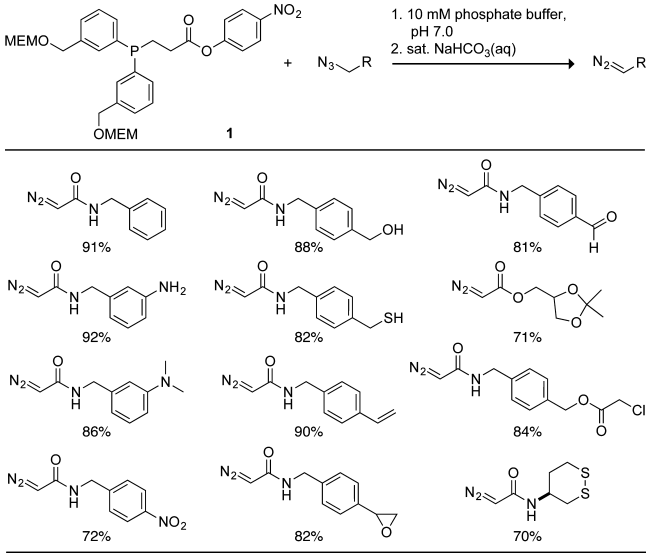
and the ensuing diazo compound was isolated by chromatography on silica gel. The reaction was found to be efficient under the conditions screened, with the optimal yield being achieved at neutral pH without the addition of an organic cosolvent (entry 3). This result bodes well for biological applications, as the optimal conversion was attained near physiological conditions. Under those conditions, reaction progress can be monitored by quantifying the yellow 4-nitrophenolate anion.[16]

Then, we probed functional group compatibility using derivatives of azido-glycine. The initial test was to probe the tolerance of azide deimidogenation by phosphinoester **1** to strong nucleophiles, such as alcohols, amines, and thiols, all of which are capable of undergoing acyl transfer reactions with 4-nitrophenyl esters.[17] We found that acyl transfer was not competitive with deimidogenation in phosphate buffer at pH 7.0 (Table 3).

Table 3. Chemoselectivity of Diazo-Compound Formation in Water by Phosphinoester 1[a]



[a]Isolated yields.

The next test was to probe the tolerance of electrophiles, such as aldehydes,[18] α-chloroesters,[19] and disulfide bonds,[20] and epoxides,[21] in the presence of the nucleophilic phosphorus of phosphinoester **1**. Again, we found phosphinoester **1** to be highly chemoselective, converting azido-glycine derivatives into their corresponding diazo compounds without notable side reactivity (Table 3). Moreover, both acetals (which are hydrolyzed readily) and styrenes (which are prone to polymerization) were quite tolerant of the reaction conditions.

The final but critical test was to assess the compatibility of the deimidogenation reaction with actual biomolecules. We found that α-azido-*N*-benzylacetamide was converted to a diazo compound with a high isolated yield (79%) in the presence of 20 equiv of oxidized L-glutathione, which is an abundant cellular component that contains both nucleophilic (amino and carboxyl) and electrophilic (disulfide) functional groups. The yield was likewise high (87% by ^{1}H NMR spectroscopy) in the presence of bovine pancreatic (RNase A), a well-known model protein,[22] at 14 mg/mL. Moreover, RNase A was not modified covalently by the procedure according to mass spectrometry and retained full enzymatic activity, indicative of its maintaining a proper three-dimensional conformation.

In conclusion, we have developed a phosphinoester that mediates the efficient conversion of azides into diazo compounds in phosphate buffer at neutral pH. This conversion is tolerant to the functional groups relevant to chemical biology. No other method for generating diazo compounds has these attributes. Azido groups, which can be introduced with a simple S_N2 reaction, have found widespread use in chemical biology.[9] Accordingly, a reagent capable of converting azides into their smaller and even more versatile diazo congeners in water could have substantial utility.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and spectral data for novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

rtraines@wisc.edu

Notes

The authors declare no competing financial interest.

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