ACS chemical biology

Reviews

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Diazo Compounds: Versatile Tools for Chemical Biology

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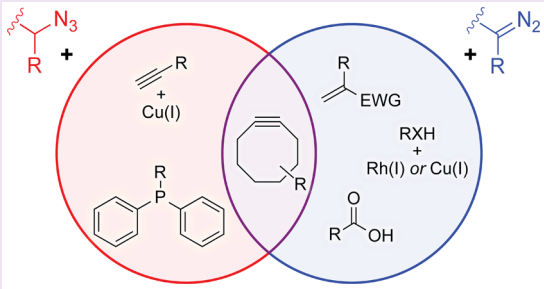
ABSTRACT: Diazo groups have broad and tunable reactivity. That and other attributes endow diazo compounds with the potential to be valuable reagents for chemical biologists. The presence of diazo groups in natural products underscores their metabolic stability and anticipates their utility in a biological context. The chemoselectivity of diazo groups, even in the presence of azido groups, presents many opportunities. Already, diazo compounds have served as chemical probes and elicited novel modifications of proteins and nucleic acids. Here, we review advances that have facilitated the chemical synthesis of diazo compounds, and we highlight applications of diazo compounds in the detection and modification of biomolecules.



Azido groups dominate the current landscape of chemoselective reactions in chemical biology. Yet, diazo groups have attributes that are even more desirable than those of azido groups. For example, diazo groups ($R^1R^2C{=}N_2$) are smaller than analogous azido groups ($R^1R^2HC{-}N_3$), and diazo groups display a broader range of reactivity.[1,2]

The simplest diazo compound, diazomethane, is a yellow gas that was discovered by von Pechmann in 1894[3,4] and is a common reagent in synthetic organic chemistry. Diazomethane and other diazoalkanes are, however, highly toxic[5−7] and explosively reactive[8,9] and have little utility in the context of chemical biology. The problem arises from their high basicity, as protonation of the α carbon of a diazo group leads to the formation of a diazonium species ($R^1R^2HC{-}N_2^+$) poised for a rapid S_N2 reaction that releases nitrogen gas.

Recent advances in synthetic methodology provide ready access to "stabilized" diazo compounds that are compatible with living systems. The stability arises from diminished basicity due to delocalization of the electrons on the α carbon to another functional group. Such stabilized diazo compounds have the potential for widespread application in chemical biology.

Here, we review the use of diazo compounds in chemical biology. We begin with an overview of natural products and amino acids that contain a diazo group. That is followed by a summary of methods for the chemical synthesis of diazo compounds. We then highlight the remarkable versatility of diazo compounds in the context of chemical biology, and we end with a brief prospectus for the future.

■ NATURAL PRODUCTS

In contrast to azido groups,[10] diazo groups are found in many natural products.[11] Isotopic labeling studies and genome mining have provided insight into their biosynthesis.[12−15] No enzyme is known to catalyze the formation of a N−N bond, though a gene cluster that encodes a nitrous acid-producing enzyme could be a source.[16] Intrinsic antitumor and antibiotic activities endow some natural diazo compounds with potential clinical utility, but mechanisms of action *in vivo* are unclear. As the isolation and synthesis of diazo-containing natural products has been reviewed extensively elsewhere,[17,18] we summarize only key findings and recent advances. We focus, in particular, on the kinamycins and lomaiviticins, two classes of natural products with unusual structures and intriguing mechanisms of reactivity (Figure 1A and B).

The kinamycins were isolated from *Streptomyces murayamaensis* in 1970 and displayed antimicrobial activity against Gram-positive bacteria.[19] Initially, the compounds were thought to contain a cyanamide group due to their infrared absorption near ∼2155 cm^{-1} but were later established to have a diazo moiety.[20] The complex architecture of these molecules, which consist of a four-ring carbocyclic skeleton that contains several stereogenic centers, challenged synthetic chemists until routes were developed a decade ago.[21−23]

Like the kinamycins, the lomaiviticins are analogs of 9-diazofluorene (Figure 1A). Lomaiviticins A and B were isolated in 2001 from the marine ascidian symbiont *Salinispora pacifica* and displayed antitumor activity at submicromolar concentrations.[24] Lomaiviticins C−E were isolated in 2012 from *Salinispora pacifica* and demonstrated similar potency.[25] Although synthetic routes to the lomaiviticins are unrealized to date, progress has been made toward intermediates and analogues.[26−30]

Diazofluorene analogues have long been used to investigate possible mechanisms of DNA cleavage *in vitro*. Using 9-diazofluorene, Arya and Jebaratnam were among the first to suggest that a diazo group could mediate DNA cleavage.[31] Kinafluorenone, which contains a ketone oxygen in lieu of a diazo group, displayed no antibiotic activity and thus supported

Received: September 16, 2016
Accepted: October 14, 2016
Published: October 14, 2016

DOI: 10.1021/acschembio.6b00810
ACS Chem. Biol. 2016, 11, 3233−3244

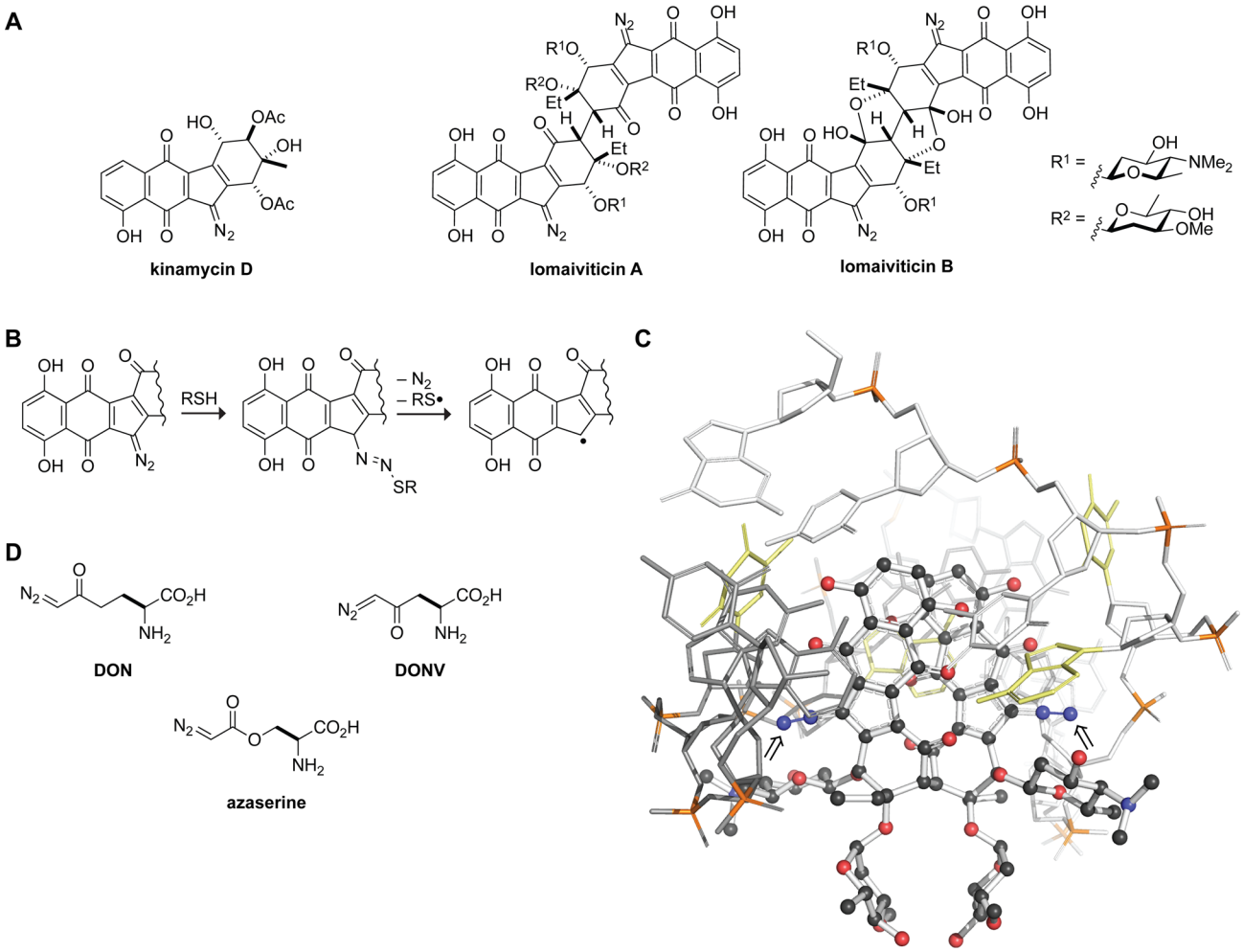


Figure 1. Structure and reactivity of some natural products that contain diazo groups. (A) Kinamycin D, lomaiviticin A, and lomaiviticin B. (B) Putative mechanism for the generation of a reactive vinylogous radical from lomaiviticin A.[34] (C) Solution structure of the complex of lomaiviticin A with a G-C-T-A-T-A-G-C duplex.[39] Displaced A·T basepairs are depicted in yellow. Phosphorus atoms are depicted in orange. Hydrogen atoms are not shown. Arrows point to the two diazo groups. Image was created with Protein Data Bank entry 2n96 and the program PyMOL from Schrödinger (New York, NY). (D) Amino acids that contain diazo groups.

the hypothesis that the diazo moiety is the active pharmacophore.[32] A variety of reactive intermediates that elicit cytotoxicity have been proposed, including a covalent adduct,[33,34] *ortho*-quinone methide,[34,35] acylfulvene,[36] or vinyl radical[33−35,37,38] (Figure 1B). Certain lomaiviticins, such as (−)-lomaiviticin A, are nearly 100-fold more toxic to cancer cells than are kinamycins,[38] despite similar reactive intermediates being accessible from both kinamycins and lomaiviticins. (−)-Lomaiviticin A is especially potent, exhibiting cytotoxic activity at nanomolar−picomolar concentrations.

To reveal the basis for the superior cytotoxicity of (−)-lomaiviticin A, Herzon and co-workers performed a thorough comparison of (−)-lomaiviticin A, (−)-lomaiviticin C, and (−)-kinamycin C.[38] They found that the reduction of (−)-lomaiviticin A *in vitro* proceeds more rapidly than does that of (−)-kinamycin C. Moreover, only (−)-lomaiviticin A causes double-stranded breaks in DNA and activates the double-strand break repair pathway in cells. This combination of attributes likely accounts for the superior potency of (−)-lomaiviticin A. Further, these authors provided evidence that DNA cleavage is instigated by a vinylic carbon radical (Figure 1B) and is independent of iron and reactive oxygen species. A solution structure of (−)-lomaiviticin A in complex with DNA revealed that both subunits of lomaiviticin A intercalate into DNA at AT-rich sequences and cause base pairs to be twisted out of the duplex (Figure 1C).[39] The α carbon of the diazo group lies in close proximity to the DNA strand, facilitating hydrogen abstraction by an incipient radical.

One challenge in the investigation and application of lomaiviticins is their limited availability. Smaller analogues that are easier to synthesize provide a partial solution.[40] One such analogue, a monomeric lomaiviticin aglycon, is capable of inducing DNA damage, albeit at higher concentrations than does (−)-lomaiviticin A. Both (−)-lomaiviticin A and this monomeric lomaiviticin aglycon activate homologous recombination and the nonhomologous end-joining repair of DNA in cells.[41] Dysfunctional DNA-repair pathways underlie many human cancers,[42] rendering lomaiviticins as a potential treatment strategy. In support of this strategy, cell lines with defective DNA-repair pathways (e.g., BRCA2- and PTEN-deficient cells), are more sensitive to (−)-lomaiviticin A and monomeric lomaiviticin aglycon than are isogenic cell lines with intact damage repair pathways.

DOI: 10.1021/acschembio.6b00810
ACS Chem. Biol. 2016, 11, 3233−3244

AMINO ACIDS

Some natural amino acids contain diazo groups.[43,44] Notable examples include azaserine and 6-diazo-5-oxo-norleucine (DON), which are nearly isosteric to glutamine (Figure 1D).[45] Both amino acids were isolated initially from *Streptomyces* cultures and exhibit antibiotic and tumor inhibitory properties.[43,46] These diazo compounds effectively inhibit amidotransferases involved in the biosynthesis of pyrimidines and purines.[47−49] DON entered early stage clinical trials based on its beneficial activity against various carcinomas, lymphomas, and Hodgkin's disease.[50] The ability of DON to inhibit amidotransferases revealed the mechanism by which γ-glutamyl transferase acts in tandem with aminopeptidase M to transfer the glutamyl group of glutathione to amino acids and peptides.[51−53] DON was also used to determine the catalytic nucleophile and characterize the substrate specificity of glutaminase−asparaginases from various organisms.[54,55]

Likewise, diazo-containing analogs of asparagine have found utility in medicine as well as enzymology. 5-Diazo-4-oxo-norvaline (DONV; Figure 1D) inhibits the growth of asparagine-dependent tumors by interfering with the synthesis and utilization of asparagine.[44,56] DONV is also a specific inhibitor of L-asparaginase, which is used routinely in the treatment of leukemia.[57] Clinical assays that aim to determine the blood concentration of asparagine in patients treated with L-asparaginase suffer from degradation of asparagine in the serum sample due to L-asparaginase. The addition of DONV to the assay mixture improves the reliability of asparagine detection.[57]

PREPARATION

The synthesis of diazo compounds has become facile. Common methods include (i) diazo transfer,[58,59] (ii) diazotization,[60,61] (iii) hydrazone decomposition[62,63] or hydrazone oxidation,[64,65] (iv) rearrangement of *N*-alkyl *N*-nitroso compounds,[8,66] (v) 1,3-disubstituted acyl (or aryl) triazine fragmentation,[67,68] and (vi) elaboration of other diazo compounds (Figure 2).[69−73] Most of these routes have been reviewed extensively for their merits in the context of synthetic chemistry.[74,75] Nevertheless, the preparation of diazo compounds for applications in chemical biology entails additional challenges because of restrictions on the compatibility of ancillary functional groups and on solubility.

Diazo transfer is a simple and effective way to introduce the diazo group when the pK_a of a proton on the acceptor carbon is low enough to be extracted with a mild base, as is necessary in the stabilized diazo compounds useful in chemical biology. For example, 1,8-diazabicycloundec-7-ene (DBU) can generate α-diazocarbonyl groups after a diazo transfer reaction using sulfonyl azide reagents (e.g., *p*-acetamidobenzenesulfonyl azide and imidazolesulfonyl azide).[59,76,77] The electronic delocalization that enables diazo transfer also stabilizes the ensuing diazo compound.

Recently, our group reported on a general method to prepare a stabilized diazo group directly from a parent azide.[78,79] Fragmentation of acyl triazines uses a phosphinoester to convert an azido group into its corresponding diazo group. The reactivity underlying this loss of NH, or "deimidogenation," was derived from insight into the mechanism of the Staudinger ligation.[80−84] In the Staudinger ligation as well as the Staudinger reaction,[85,86] the incipient phosphazide quickly extrudes molecular nitrogen to generate an iminophosphorane. A highly reactive acylating group subverts nitrogen extrusion by

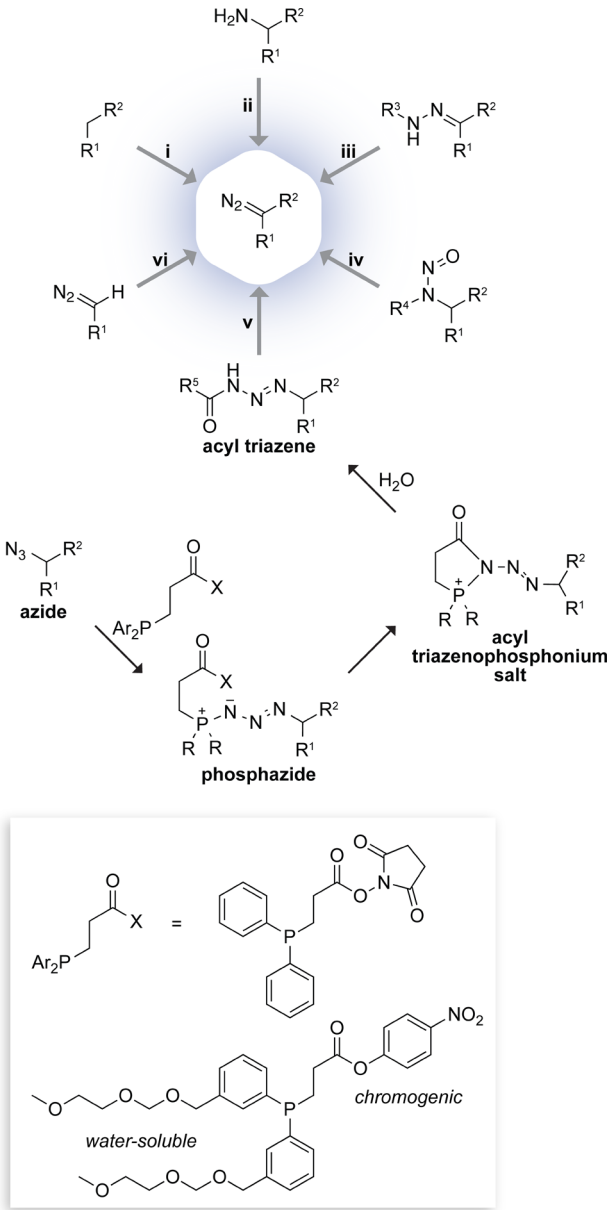


Figure 2. Preparation of diazo compounds by (i) diazo transfer,[58,59] (ii) diazotization,[60,61] (iii) hydrazone decomposition[62,63] or hydrazone oxidation,[64,65] (iv) rearrangement of *N*-alkyl *N*-nitroso compounds,[8,66] (v) 1,3-disubstituted acyl or aryl triazine fragmentation,[67,68] and (vi) elaboration of other diazo compounds. Diazo compounds can be accessed from azides *via* acyl triazenes in a process mediated by a phosphinoester.[78,79]

trapping the phosphazide (Figure 2). The ensuing triazenophosphonium intermediate hydrolyzes quickly in water to form an acyl triazene, which is a known precursor to a diazo group.[67,68]

Azide deimidogenation benefits from the extraordinary chemoselectivity of phosphine for an azide. This approach has a high tolerance for other functional groups, including ketones, esters, aldehydes, thiols, α-chloroesters, epoxides, and disulfide bonds. Chemoselectivity was demonstrated by converting an azido group into a diazo group in aqueous solution containing an enzyme, which was not modified covalently and retained full catalytic activity.[79] Notably, appropriate azides for deimidogenation (that is, azides with

DOI: 10.1021/acschembio.6b00810
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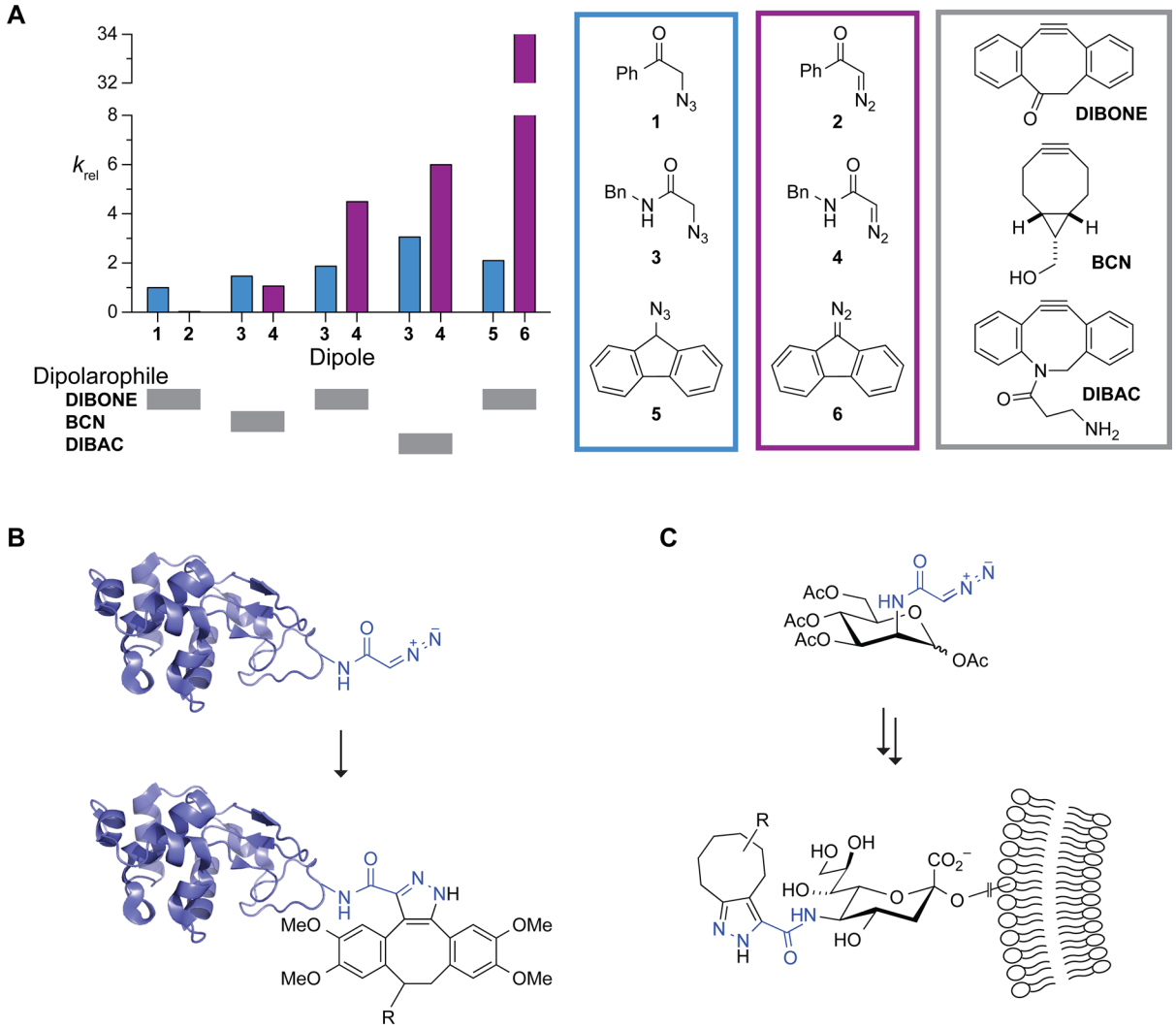


Figure 3. Diazo compounds in dipolar cycloadditions with strained alkynes. (A) Relative rate constants of diazo compounds and analogous azides with various cyclooctynes.[92,109] (B) Labeling of a diazo-modified lysozyme with a cyclooctyne.[93] (C) Labeling of a metabolized diazo sugar displayed on the surface of human cells with a cyclooctyne.[92]

an electron-withdrawing group on the α carbon) are readily accessible via S_N2 reactions with inorganic azide.[87]

Finally, diazo compounds that contain sensitive functional groups can be prepared by the late-stage installation of a prefabricated diazo group. This strategy typically relies on acyl transfer. In 1962, Westheimer and co-workers introduced the concept of photoaffinity labeling by acylating chymotrypsin with *p*-nitrophenyl diazoacetate and then forming an intramolecular cross-link upon photolysis.[88] Most recent late-stage installations have employed an *N*-hydroxysuccinimide (NHS) ester containing a pendant α-diazocarbonyl group. Badet and co-workers developed a clever synthetic route to the simplest reagent of this class, *N*-hydroxysuccinimidyl diazoacetate.[89] Such NHS esters have been used to install diazo groups on small molecules[90,91] as well as biomolecules of varying complexity, including biotin,[92] mannosamine,[93] heparan-sulfate fragments,[94] lysozyme,[93] and bovine serum albumin (BSA).[95]

■ CYCLOADDITIONS

The archetypal reaction for the diazo group is the 1,3-dipolar cycloaddition. Soon after the synthesis of ethyl diazoacetate by Curtius,[60] Buchner observed its reaction with an α,β-unsaturated carboxylic ester to form a pyrazole.[96] Over the past century, the reactivity of diazo groups in cycloadditions has engaged theoretical, synthetic, and biological chemists, and these explorations have been reviewed for their use and merits in synthetic chemistry.[97,98] Here, we focus on recent work that is relevant to biological systems.

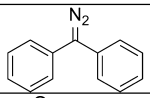
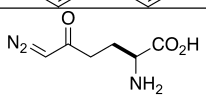
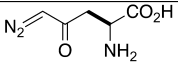
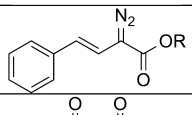
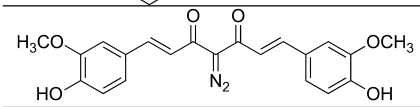
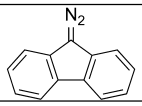
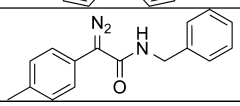
Copper-catalyzed azide−alkyne cycloadditions (CuAAC)[99,100] and strain-promoted azide−alkyne cycloadditions (SPAAC)[101−103] are two of the most enabling advances in the field of chemical biology.[83,104,105] The diazo group shares the ability of the azido group to undergo cycloadditions with alkynes, forming a pyrazole rather than a triazole.[95,106,107] The reactivity of diazo groups is remarkably predictable and tunable[108]—the diazo compounds can react with a strained alkyne at much higher or much lower rates than analogous azides (Figure 3A).[106,107,109] Because a diazo group can be generated directly from an azido group[78,79] and reacts with strained alkynes in common use, the diazo group fits easily into extant methodology.

In addition to reacting with strained alkynes, diazo groups undergo uncatalyzed cycloadditions with unstrained dipolarophiles, including terminal alkenes and alkynes. Moreover,

DOI: 10.1021/acschembio.6b00810
ACS Chem. Biol. 2016, 11, 3233−3244

Table 1. Diazo Compounds That Esterify Proteins

Diazo compounds that esterify proteins.

Diazo Compound	Protein	Year	Reference
	caseinogen insulin β-lactoglobulin lysozyme	1914 1958	125 130
	polyclonal antibody chymotrypsinogen ribonuclease A pepsin acid proteases prorenin *O*-sulfotransferase	1960 1961 1965 1966–1968 1972–1973 1980 2015	131 126 127 137,139,140 146-149 151 94
	pepsin	1966	135
	phosphoribosyl pyrophosphate amidotransferase glutaminase A glutamyl transpeptidase	1963 1973 1978	47 48 52-54
	asparaginase	1977	56
	myoglobin subtilisin Yes kinase	2004 2015	54 165
	β-lactoglobulin	2007	174
	ribonuclease A red fluorescent protein	2015	128
	ribonuclease A	2015	129

diazo compounds can react chemoselectively with certain alkenes and alkynes in the presence of an azide. In essence, a diazo group is more electron-rich and thus a better nucleophile in normal-electron-demand cycloadditions with electron-deficient dipolarophiles.[110−113] Detailed insight is attainable from computational analyses. Distortion energies account for a majority (80%) of the activation energy for 1,3-dipolar cycloadditions. Due to their increased nucleophilicity and higher HOMO energy, diazo compounds have lower distortion energies than do their azide analogues.[110,113] The reactions can occur at ambient temperature in aqueous cosolvent with reaction rates similar to or greater than those of SPAACs with azides. Notably, a diazo group can react chemoselectively with the naturally occurring amino acid dehydroalanine (Dha), which contains an electronically activated alkene.[110] Selective biotinylation of activated alkenes could enable enrichment and isolation of compounds from a complex lysate, facilitating the discovery of new natural products.

■ PROBES

The diazo group is found in the natural products of microorganisms (*vide supra*). In contrast, its absence in higher organisms enables its utility there as a chemical reporter. The reactivity of the diazo group with many common SPAAC dipolarophiles spawned the use of a diazo group as a chemical reporter for cell-surface glycosylation.

Leeper and co-workers prepared an *N*-diazoacetyl galactosamine and incubated this synthetic sugar with LL2 cells.[93] Treatment with a biotin-bearing cyclooctyne and subsequent addition of an avidin fluorophore produced some increase in fluorescence of cells incubated with the diazo-bearing glycan compared to untreated cells. In the same study, an α-diazo NHS ester was reacted with a lysine residue on lysozyme to append the diazo group. Following modification, the appendage was used to attach a fluorophore to the protein *via* a cycloaddition between the diazo group and a cyclooctyne (Figure 3B).

Our group demonstrated the suitability of a diazoacetamide derivative of *N*-acetyl mannosamine as a chemical reporter of glycosylation on the surface of CHO K1, Jurkat, HEK293T, and HeLa cells (Figure 3C).[92] The degree of labeling was determined by SPAAC between the diazo group and a biotin-bearing cyclooctyne, followed by treatment with an avidin fluorophore. Metabolic incorporation of the diazo-bearing sugar was evidenced through live-cell microscopy and flow cytometry, and labeling was abolished by treatment with a sialidase. Diazo and alkynyl sugars could be labeled independently on the cell surface. Notably, such dual labeling was not possible on cells displaying azido and alkynyl sugars due to the reactivity of the azide in both CuAAC and SPAAC reactions.

Diazo compounds have long been incorporated into biomolecules as photoaffinity probes.[114,115] Upon irradiation with ultraviolet light, the diazo group fragments into molecular nitrogen and a carbene, which can undergo either an insertion reaction or a Wolff rearrangement[116,117] followed by nucleophilic attack on the ensuing ketene, both of which

DOI: 10.1021/acschembio.6b00810
ACS Chem. Biol. 2016, 11, 3233−3244

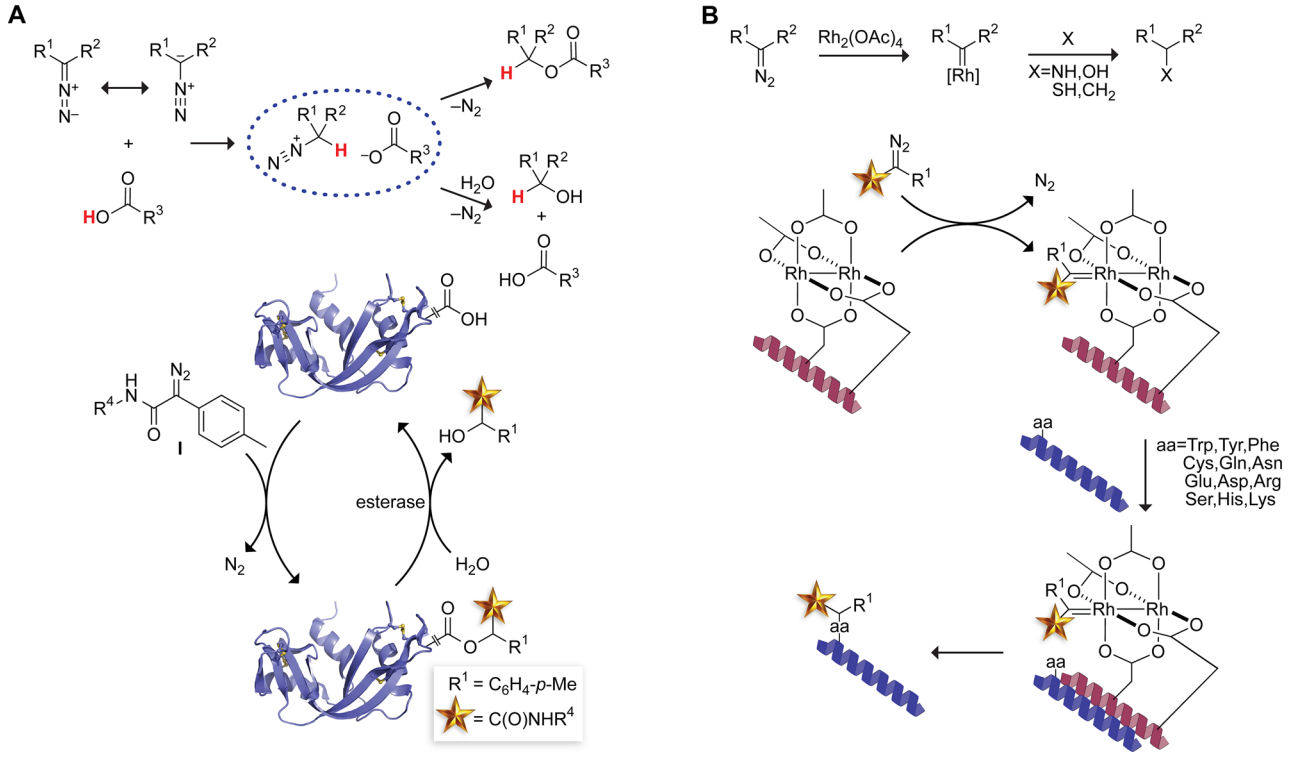


Figure 4. Diazo compounds for covalent modification of proteins. (A) Putative mechanism for the esterification of carboxylic acids with a diazo compound,[134] and its application to the bioreversible labeling of a protein.[128,129] Diazo compound **I** is optimized for protein esterification.[129] (B) Putative mechanism of a diazo carbenoid insertion reaction, and its application to the site-specific modification of a proximal amino acid residue.[161]

cross-link the diazo compound to proximal functional groups. This strategy has been used to map the architecture of chymotrypsin (*vide supra*),[88] reveal antibody combining sites,[118] examine the structure of lipid membranes,[119] and identify isoprenoid-binding sites on proteins.[120]

■ PROTEIN ALKYLATION

The ability of diazo reagents to alkylate oxygen, nitrogen, sulfur, and even carbon exemplifies their diverse reactivity.[1,121−124] When applied to protein modification, these reactions are typically catalyzed by acid or transition metals. Despite the apparent promiscuity of this mode of reactivity, even highly reactive compounds such as diazomethane have historically found utility in elucidating structural and functional aspects of proteins.[125] Stabilized diazo reagents enable *O*-alkylation of carboxyl groups and were valuable tools in classical protein chemistry and enzymology.[126,127] Later, the discovery of diazo-containing amino acid analogues led medicinal chemists and structural biologists to employ these compounds as covalent inhibitors of metabolic enzymes.[45] Modern applications of diazo chemistry in chemical biology aim to capitalize on the versatility of diazo compounds to access linkages that cannot be achieved by other methods. Maintaining chemoselectivity in the presence of water and other biological nucleophiles has been a primary challenge in developing diazo compounds as useful tools for protein chemistry.[128,129]

The earliest uses of diazo reagents for protein labeling sought to characterize structural features of proteins. In 1914, Geake and Nierenstein used diazomethane to alkylate caseinogen so as to characterize the structure of amino acid side chains (Table 1).[125] By comparing the methylated and unmethylated protein, they identified and quantified side chains that contain amino or hydroxyl groups. Later studies addressed large-scale structural characterization of proteins, such as quantification of the number of peptide chains in a protein and identification of carboxyl groups in the binding region of the antihapten antibody.[130,131]

The past 100 years have seen many attempts to limit the promiscuity of the diazo reagent by using stabilized α-diazo amides (Table 1). Doscher and Wilcox used α-diazoacetamide to label chymotrypsin in work that laid the foundation for modern protein-labeling endeavors.[126] They demonstrated that, although the rate of esterification was much greater than the rate of diazo-compound hydrolysis, the large excess of water molecules limits the efficiency of esterification. The authors suggested that employing a mixed aqueous−organic solvent could increase esterification efficiency by both limiting diazo hydrolysis and increasing the pK_a of enzymic carboxyl groups. This idea was later explored and did indeed increase the efficiency of protein esterification.[128] Although α-diazoacetamide was more selective than diazomethane, it still *S*-alkylated sulfhydryl groups.

In 1917, Staudinger and Gaule became the first to use a diazo compound, diphenyldiazomethane, to form an ester.[132] The mechanism of this reaction was established in elegant work by Roberts and co-workers in 1951 (Figure 4A).[133,134] The heightened reactivity of carboxyl groups versus carboxylates inspired subsequent esterification experiments. Riehm and Scheraga used α-diazo acetoglycinamide to esterify the carboxyl groups in ribonuclease A.[127] They found that one aspartic acid residue was esterified preferentially and proposed that this residue resides in a solvent-accessible area of local negative charge, which would raise its pK_a value and lead to its selective esterification. Shortly thereafter, Delpierre and Fruton used an α-diazoketone to label a single residue in the active site of pepsin, causing near-complete inhibition of the enzyme.[135]

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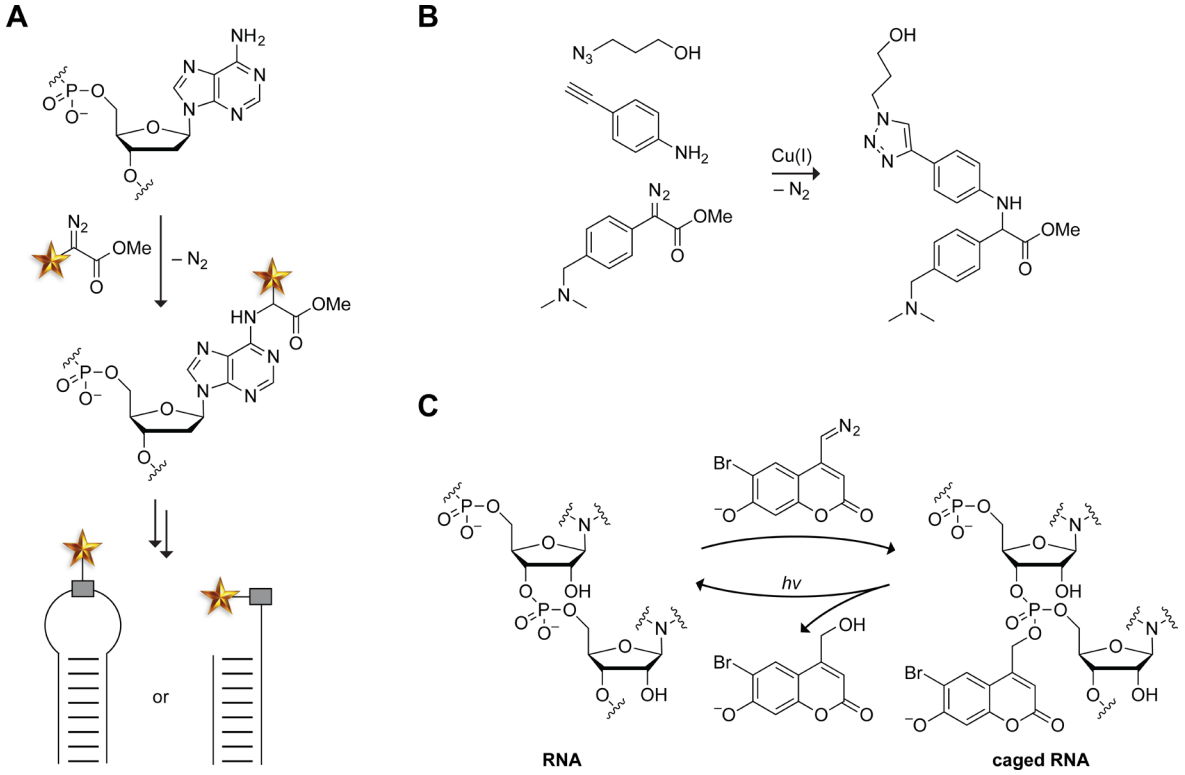

Figure 5. Covalent modification of nucleic acids using diazo compounds. (A) Representative alkylation of DNA by a diazo compound. Alkylation occurs on solvent-accessible nucleobases.[166] (B) One-pot N−H insertion and azide−alkyne cycloaddition with a copper(I) catalyst.[168] (C) Photoreversible *O*-alkylation of a phosphoryl group in RNA by a diazo coumarin.[169]

These workers proposed that this residue was in a privileged environment that enabled its selective labeling, as was posited for the aspartic acid in ribonuclease A,[127] though neither of these speculations has been explored further. Instead, the inhibition of pepsin using α-diazoketones gave rise to a breadth of studies characterizing the active site of pepsin and comparing pepsin to its zymogen form (i.e., pepsinogen), in which the active-site residue is inaccessible to solvent and thus does not react with the diazo reagent.[136−144] The combination of covalent labeling using a diazo reagent with Edman degradation (which was invented concurrently) provided a robust method for determining the identity of a catalytically important residue and its surrounding sequence.[145] Using these techniques, novel acid proteases were classified based on their propensity to be inactivated by a diazo compound.[146−152] Nonetheless, with the advent of site-directed mutagenesis, the use of diazo compounds to characterize proteins became rare.

■ BIOREVERSIBLE PROTEIN MODIFICATION

The abundance and promiscuity of cellular esterases has been utilized in prodrug strategies in which chemotherapeutic agents are masked as esters and converted to their active forms upon cellular uptake.[153−155] Our group envisioned a similar strategy for proteins in which carboxyl moieties are esterified by a diazo compound to install a molecular tag, such as a pharmacokinetic-enhancing, cell-type-targeting, or cell-penetrating moiety. Upon cellular uptake, the ester-linked tags are removed by endogenous esterases to recreate the native protein (Figure 4A). This strategy would be especially valuable for the delivery of proteins whose activities decrease significantly upon irreversible modification.[156]

In an initial study, structurally and electronically diverse diazo compounds were screened for their reactivity and selectivity in an aqueous environment.[128] Of these compounds, only 9-diazofluorene esterified a panel of carboxylic acids efficiently in the presence of water. This diazo compound was used to label two model proteins, ribonuclease A and red fluorescent protein. The nascent esters were hydrolyzed upon treatment with a HeLa-cell extract, regenerating wild-type protein.

Later, a more systematic study investigated the rate and selectivity of a series of structurally similar but electronically diverse α-diazo amides.[129] A Hammett analysis of these compounds, which were derived from phenylglycine, revealed that electron-donating or electron-withdrawing groups on the aryl ring had a dramatic effect on the rate of esterification. Still, the compounds were similar in their selectivity for ester formation over hydrolysis of the diazo reagent. The comparable selectivity among the compounds in this study supports the proposed mechanism in which the diazonium and carboxylate species, formed as intermediates, are held together in a solvent cage as an intimate ion pair (Figure 4A),[134] and the ratio of ester to alcohol product is determined by the diffusion out of this solvent cage rather than the reactivity of the diazo compound.[133,134,157] An α-diazo(*p*-methylphenyl)-glycinamide (**I**) demonstrated the fastest rate while maintaining selectivity, and esterifies proteins more efficiently than any known diazo reagent. The amide of compound **I** allows for facile incorporation of an amine of interest.

■ PEPTIDE AND PROTEIN MODIFICATION WITH CARBENOIDS

An early example of asymmetric catalysis employed a chiral transition-metal catalyst to generate a carbenoid from a diazo

DOI: 10.1021/acschembio.6b00810
ACS Chem. Biol. 2016, 11, 3233−3244

compound.[158] Carbenoids generated similarly can access a broad scope of insertion reactions and are hence powerful reagents for modifying peptides and proteins. In a seminal study, Francis and Antos used vinylic α-diazo esters to modify tryptophan residues in horse heart myoglobin.[76] Then, Ball employed metallopeptides to combine proximity-driven and transition-metal-driven catalysis.[159,160] In this system, the rhodium catalyst is displayed on a peptide, which is designed to bind a second peptide or protein of interest by forming a coiled coil (Figure 4B).[161] The catalyst on the metallopeptide is oriented such that the incipient carbenoid is generated proximal to the target residue, focusing its high reactivity and enabling modification of many types of amino acids.[162] For example, although tryptophan can be modified by the addition of a diazo compound and rhodium acetate catalyst alone, employing a metallopeptide to orient the catalyst enables modification of the phenyl group of phenylalanine, imidazolyl group of histidine, and guanidinium group of arginine.

In a proof-of-concept study, Popp and Ball alkylated the aromatic amino acid side chains by tethering the dirhodium center to a lysine-rich K3 peptide, which binds to and reacts with a glutamate-rich E3 peptide at a specific tryptophan residue.[161] In a follow-up investigation, the scope of the E3/K3 system was extended to the alkylation of a broad range of functional groups, including a carboxamide.[162] This system has since been used to modify maltose-binding protein fused to the E3 peptide,[163] as well as for the site-selective modification of the native Fyn protein using a peptide ligand bearing the rhodium catalyst.[164,165]

NUCLEIC ACID ALKYLATION

Natural nucleobases can be modified *in situ* with diazo compounds. Gillingham and co-workers used rhodium(II) to catalyze the conversion of a diazo ester into a carbenoid that inserted into exocyclic N−H bonds (Figure 5A).[166] Because this reactivity does not extend to double-helical regions, the strategy can target hairpins and single-stranded regions (Figure 5B). This selectivity is useful, for example, in studies on the mechanism of RNA interference, which entails 3′ overhangs.

Rhodium(II) has been used most widely as a catalyst for the generation of carbenoids in chemical biology.[167] Gillingham and co-workers showed, however, that copper(I)-carbenoid chemistry for N−H insertion is likewise effective.[168] Their work demonstrated novel synergy of the diazo group with "copper-click" chemistry by combining N−H insertion with CuAAC in a one-pot single-catalyst process (Figure 5B).

An alternative strategy for nucleic acid modification involves *O*-alkylation of the phosphoryl group. Okamoto and co-workers employed this method to modify an mRNA using a photolabile derivative of coumarin bearing a diazo moiety (Figure 5C).[169] The ensuing "caged" mRNA, which encoded green fluorescent protein, was delivered to zebrafish embryos, where its translation could be modulated spatially and temporally by uncaging using ultraviolet light. Photolabile diazo groups have also been used to control RNA interference, in which a double-stranded precursor to an siRNA is inactivated upon modification with the diazo reagent and then uncaged with ultraviolet light.[170] Diazo compounds have been employed to label and detect nucleic acids on microarrays without disrupting base pairing.[171] Recently, Gillingham and co-workers reported on a diazo compound that modifies the phosphoryl groups of nucleic acids selectively in the presence of carboxylic acids.[172] Their methodology could be useful for the labeling and detection of phosphorylated peptides and proteins as well.

OUTLOOK

Diazo compounds were discovered over 120 years ago. Recent advances in chemical synthesis have enabled the facile preparation of stabilized diazo compounds that are compatible with living systems. Like azido groups, diazo groups are chemoselective. Unlike azido groups, diazo groups have reactivity with natural and nonnatural functional groups that is tunable. The ability to tune their reactivity by delocalization of the electrons on the α carbon renders diazo compounds as attractive reagents in physiological contexts. Moreover, the versatility of diazo-group reactivity is extraordinary. Their ability to react rapidly, selectively, and autonomously with nonnatural functional groups (e.g., strained alkynes) as well as natural carboxyl groups, phosphoryl groups, and even the alkene in dehydroalanine residues anoints diazo groups as special. Accordingly, we envision an expansion in the use of diazo compounds to probe biological phenomena and to treat human disease and even foresee an era of "diazophilia."[173]

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ACKNOWLEDGMENTS

We are grateful to C. L. Jenkins for comments on the manuscript. K.A.M. was supported by Molecular Biosciences Training Grant T32 GM007215 (National Institutes of Health). Work on diazo compounds in the Raines laboratory is supported by Grant R01 GM044783 (National Institutes of Health).

KEYWORDS

bioreversible esterification: *O*-alkylation of carboxylic acids to form an ester that is a substrate for a cellular esterase; for example, using a tuned diazo compound

carbenoid: reactive intermediate, often generated from the metal-catalyzed decomposition of a diazo compound, that contains a divalent carbon with an unshared electron pair

chemical reporter: non-natural functional group appended to a biomolecule of interest for detection or derivatization

deimidogenation: loss of an NH moiety as in the phosphinoester-mediated conversion of an azido group to a diazo group

diazo compound: compound that contains the functional group $-C{=}N^{+}{=}N^{-}$

1,3-dipolar cycloaddition: chemical reaction between a 1,3-dipole (such as a diazo group) and a dipolarophile (such as an alkyne or alkene) to form a five-membered ring

lomaiviticin: diazofluorene-based natural product with antiproliferative activity

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DOI: 10.1021/acschembio.6b00810
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DOI: 10.1021/acschembio.6b00810
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