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Electrophilic Azides for Materials Synthesis and Chemical Biology

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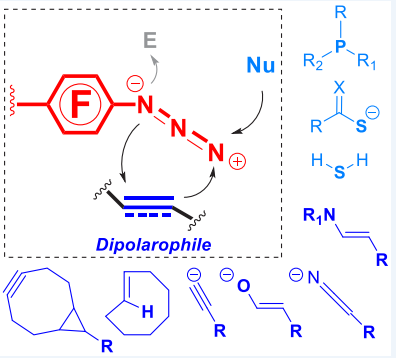
CONSPECTUS: Organic azides are involved in a variety of useful transformations, including nitrene chemistry, reactions with nucleophiles and electrophiles, and cycloadditions. The 1,3-dipolar cycloadditions of azides constitute a major class of highly reliable and versatile reactions, as shown by the development and rapid adoption of click chemistry and bioorthogonal chemistry. Metal-catalyzed azide−alkyne cycloaddition (Cu/RuAAC), the prototypical click reaction, has found wide utility in pharmaceutical, biomedical, and materials sciences. The strain-promoted, or distortion-accelerated, azide−alkyne cycloaddition eliminates the need for a metal catalyst.



In the azide-mediated 1,3-dipolar cycloaddition reactions, azides are ambiphilic, i.e., HOMO−LUMO-controlled dipoles where both the HOMO and LUMO interact strongly with the dipolarophile. Azide−alkyne cycloaddition proceeds primarily through the $HOMO_{azide}$−$LUMO_{dipolarophile}$ interaction, and electron-deficient dipolarophiles react more readily. The inverse-electron-demand reaction, involving the $LUMO_{azide}$−$HOMO_{dipolarophile}$ interaction, is less common because of the low stability of electron-deficient azides such as acyl, sulfonyl, and phosphoryl azides. Nevertheless, there have been reports since the 1960s showing enhanced reaction kinetics between electron-poor azides and electron-rich dipolarophiles. Our laboratory has developed the use of perfluoroaryl azides (PFAAs), a class of stable electron-deficient azides, as nitrene precursors and for reactions with nucleophiles and electron-rich dipolarophiles. Perfluorination on the aryl ring also facilitates the synthesis of PFAAs and quantitative analysis of the products by ^{19}F NMR spectroscopy.

In this Account, we summarize key reactions involving electrophilic azides and applications of these reactions in materials synthesis and chemical biology. These electron-deficient azides exhibit unique reactivity toward nucleophiles and electron-rich or strained dipolarophiles, in some cases leading to new transformations that do not require any catalysts or products that are impossible to obtain from the nonelectrophilic azides. We highlight work from our laboratories on reactions of PFAAs with enamines, enolates, thioacids, and phosphines. In the reactions of PFAAs with enamines or enolates, the triazole or triazoline cycloaddition products undergo further rearrangement to give amidines or amides as the final products at rates of up to 10^5 times faster than their nonfluorinated anlogues. Computational investigations by the distortion/interaction activation strain model reveal that perfluorination lowers the LUMO of the aryl azide as well as the overall activation energy of the reaction by decreasing the distortion energies of the reactants to reach the transition states. The PFAA−enamine reaction can be carried out in a one-pot fashion using readily available starting materials of aldehyde and amine, making the reaction especially attractive, for example, in the functionalization of nanomaterials and derivatization of antibiotics for the preparation of theranostic nanodrugs. Similar fast kinetics was also observed for the PPAA-mediated Staudinger reaction, which proceeds at 10^4 times higher rate than the classic Staudinger ligation, giving stable phosphoimines in high yields. The reaction is biorthogonal, allowing cell-surface labeling with minimal background noise.

INTRODUCTION

Organic azides are a unique class of reagents: small but energy-rich, reactive yet remarkably inert under physiological conditions. The stability is primarily due to resonance stabilization, while the size results from the small linear geometry of the azido group. Azides can engage in a rich variety of transformations (Scheme 1a): (1) release of N_2 leading to nitrene chemistry, (2) reaction with a nucleophile at the terminal nitrogen (N^3), (3) reaction with an electrophile at the substituted nitrogen (N^1), or (4) cycloaddition with a dipolarophile.[1]

1,3-Dipolar cycloaddition reactions are reliable and selective transformations. The transition-metal-catalyzed azide−alkyne cycloaddition (Cu/RuAAC) to form a triazole, the prototypical "click" reaction, provides a robust and reliable reaction to covalently conjugate chemical components and has found wide utilities in organic synthesis, bioconjugation, and material functionalization.[2] The reaction has been furthermore developed into catalyst-free click chemistry using strained alkynes, which is also compatible with physiological conditions; this reaction is deemed bioorthogonal.[3] Bioorthogonal

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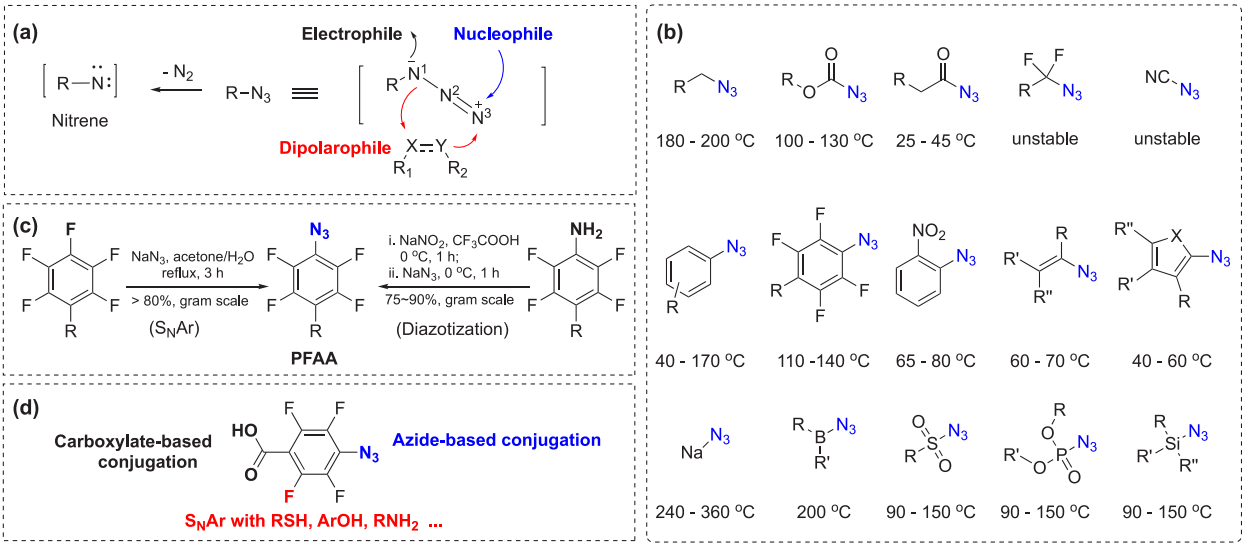
Scheme 1. (a) General Reactivities of Organic Azides;[2] (b) Azides and Their Thermal Decomposition Temperatures, at Which the Decomposition Rate Is in the Range of 10^{-5}−10^{-6} s^{-1} (Half-Life: 2−24 h);[4,5] (c) Synthesis of PFAAs via Nucleophilic Aromatic Substitution (S_NAr) Reactions (left) or Diazotization (right); (d) Example of a Multifunctional PFAA Coupling Reagent



reactions have since evolved as concise and reliable tools to manipulate chemical entities in the studies of biological processes in the past two decades.

Azides are both nucleophilic (HOMO-controlled) and electrophilic (LUMO-controlled) but are relatively unreactive as simple alkyl or aryl azides. Electron-withdrawing groups (EWGs) on the azide enhance their reactivities with nucleophiles and electron-rich dipolarophiles. However, EWGs decrease the stability of the azide in many cases (Scheme 1b). For example, the decomposition temperatures of acyl azides are in the range of 25−45 °C, limiting their reactions to low room-temperature conditions, while CF_3N_3 and N_3CN are reported to be unstable and explosive.[5] The reaction of acyl azides with amines to form amides, which was first described by Curtius in the early 1900s, proceeds only at low temperatures and has thus been mainly used for peptide coupling or protein modification.[6] In the reaction with alkynes, the Cu(I)−triazole intermediates formed from electron-deficient acyl azides, sulfonyl azides, and phosphoryl azides undergo ring-opening rearrangements to give a variety of diverse structures.[7] In contrast, aryl azides and aliphatic azides form stable triazoles.

Perfluoroaryl azides (PFAAs) constitute a special class of electron-deficient azides.[8,9] PFAAs are thermally stable (Scheme 1b) and are compatible with biological reagents and conditions. Perfluorination on the aryl ring facilitates the synthesis of PFAAs via nucleophilic aromatic substitution (S_NAr) from perfluoroaromatics using azide salts or by diazotization from the corresponding anilines (Scheme 1c). The ease of introducing additional functional groups on PFAAs makes them ideal candidates as multifunctional coupling agents. For example, the carboxyl derivative of pentafluorophenyl azide (PFPA), which can be straightforwardly synthesized on a gram scale in two steps from commercially available starting materials, enables classic carboxylate-based amide coupling to a variety of substances (Scheme 1d).[10] The azide-base conjugation can be accomplished by utilizing the azide in two different ways: as a nitrene precursor through a CH insertion or C═C addition reaction or as an electrophile through the reaction with a dipolarophile or a nucleophile.

We developed the PFAA nitrene-based coupling chemistry, which has been summarized in two previous Accounts,[8,9] for the functionalization of surfaces and nanomaterials, including pristine graphene. In this Account, we describe the chemistry of PFAAs as electrophilic azides developed in our laboratories. We discuss the origin of PFAA reactivity from computational studies and summarize the unique reactions of these electrophilic azides with electron-rich dipolarophiles, strained dipolarophiles, and nucleophiles, including their applications in materials synthesis and chemical biology.

REACTIVITY OF PFAAS: DISTORTION/INTERACTION MODEL

Azides are ambiphilic 1,3-dipoles, and both the HOMO and LUMO interact strongly with the frontier molecular orbitals (FMOs) of dipolarophiles (Scheme 2a). Perfluorination of the azide leads to sizably lowered $LUMO_{azide}$; the LUMO energy is 3.0 eV for PFPA versus 3.7 eV for phenyl azide (Scheme 2b). The result is significantly enhanced reactivity of PFAAs toward electron-rich dipolarophiles, where the reactions occur under metal catalyst-free and mild conditions. For example, in the azide−enamine reaction, PFAAs display rate enhancements of 3−4 orders of magnitude compared with phenyl azide (Scheme 2c).[5] Electron-withdrawing groups para to the azide group further accelerate the reaction.

To understand the origins of this reactivity, the distortion/interaction activation strain (D/I-AS) model developed by Houk, Bickelhaupt, and co-workers was applied (Scheme 2d, left).[11,12] In the D/I model, the activation energy is expressed as the sum of the distortion energy (i.e., the energy required to distort the reactants into their transition state (TS) geometries) and the stabilizing interaction energy: $\Delta E = \Delta E_d + \Delta E_i$. Computations on the azide−enamine cycloaddition correlated well with the experimental data; the activation energy is lower for the electron-deficient PFAAs, primarily

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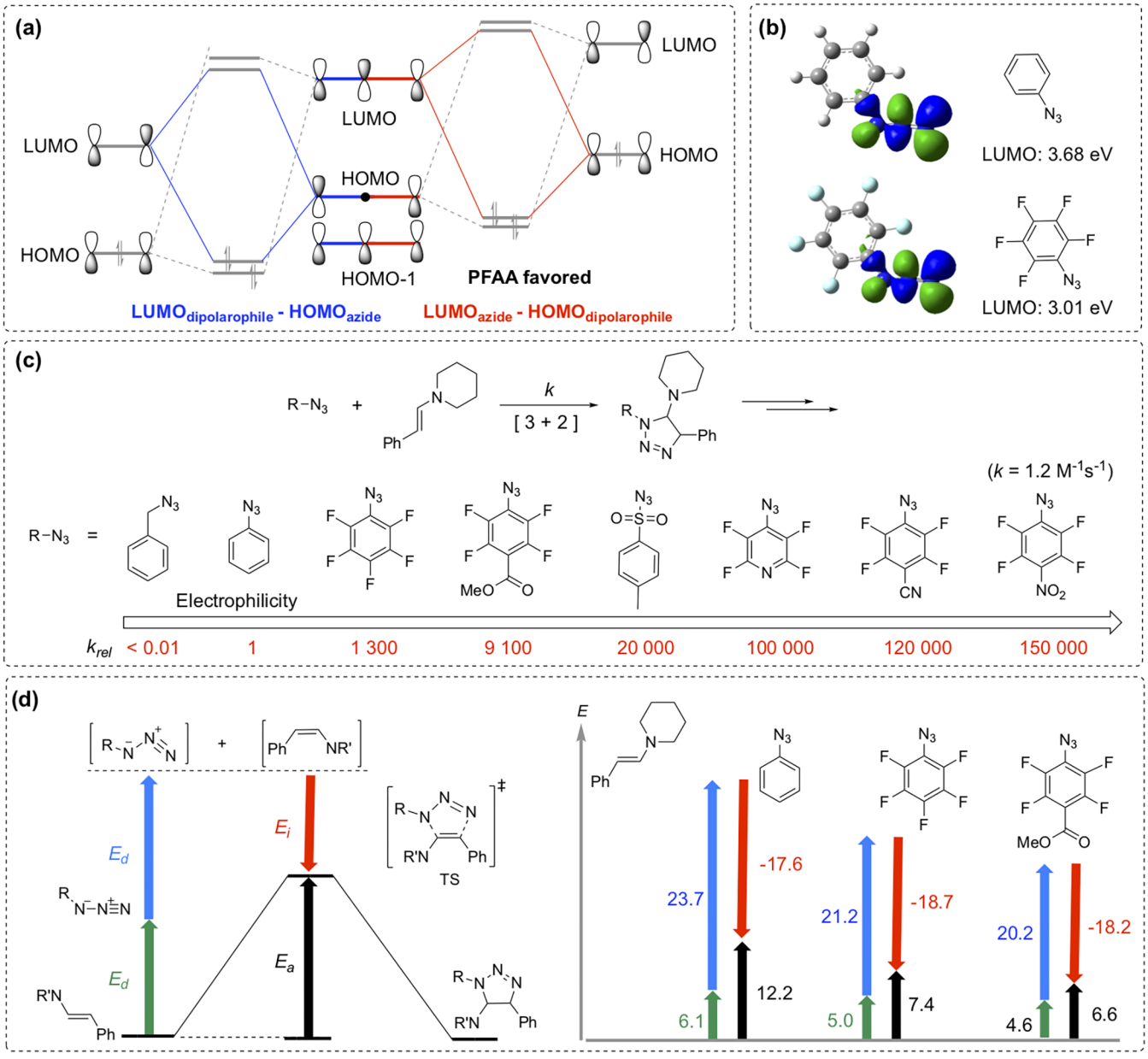
Scheme 2. (a) FMO Model for Azide−Dipolarophile Cycloaddition. (b) LUMOs of Phenyl Azide and Pentafluorophenyl Azide, Computed Using DFT;[5] (c) Relative Rates of Azide−Enamine Cycloaddition for Different Azides; (d) D/I Model in the Analysis of Azide−Alkyne (left) and Azide−Enamine (right) Cycloaddition Reactions



because of decreases in the distortion energies for both the alkene (green) and the azide (blue) upon perfluorination of phenyl azide (Scheme 2d). FMO analysis showed that the major stabilizing interaction was between the HOMO$_{enamine}$ and LUMO$_{azide}$, an outcome of the lowering of the LUMO$_{azide}$ due to the EWG on the azide, leading to a smaller HOMO−LUMO gap, an enhanced FMO interaction, and an earlier TS. Thus, the electrophilic azide not only lowers the distortion energy of the azide but also enhances the FMO interaction (HOMO$_{alkene}$−LUMO$_{azide}$) with electron-rich dipolarophiles.[13]

The regioselectivity of the 1,3-dipolar cycloaddition is also determined by the HOMO−LUMO interaction. This is exemplified by the higher regioselectivity favoring 1,4-triazoles in the reaction of electrophilic PFAAs with phenylacetylene, in contrast to phenyl azide, which gives almost equal amounts of 1,4- and 1,5-triazoles.

REACTIONS OF PFAAS WITH ELECTRON-RICH DIPOLAROPHILES

Enamines

The reaction of azides with electron-rich enamines showed unprecedented high reactivity and regioselectivity (Scheme 3a). The reaction was first described by Munk in the 1960s; aryl azides undergo cycloadditions with aldehyde enamines to form 5-amino-1,2,3-triazolines as the only regioisomer in high yield.[14] An EWG on the aryl azide and an electron-donating group (EDG) on the enamine generally enhance the rate of the cycloaddition reaction. Scheme 3b shows examples of enamine structures listed in order of decreasing nucleophilicity, together with the relative rates of reaction in the azide−enamine cycloaddition. The reaction generally involves concerted, asynchronous bond formation in the transition state.[15]

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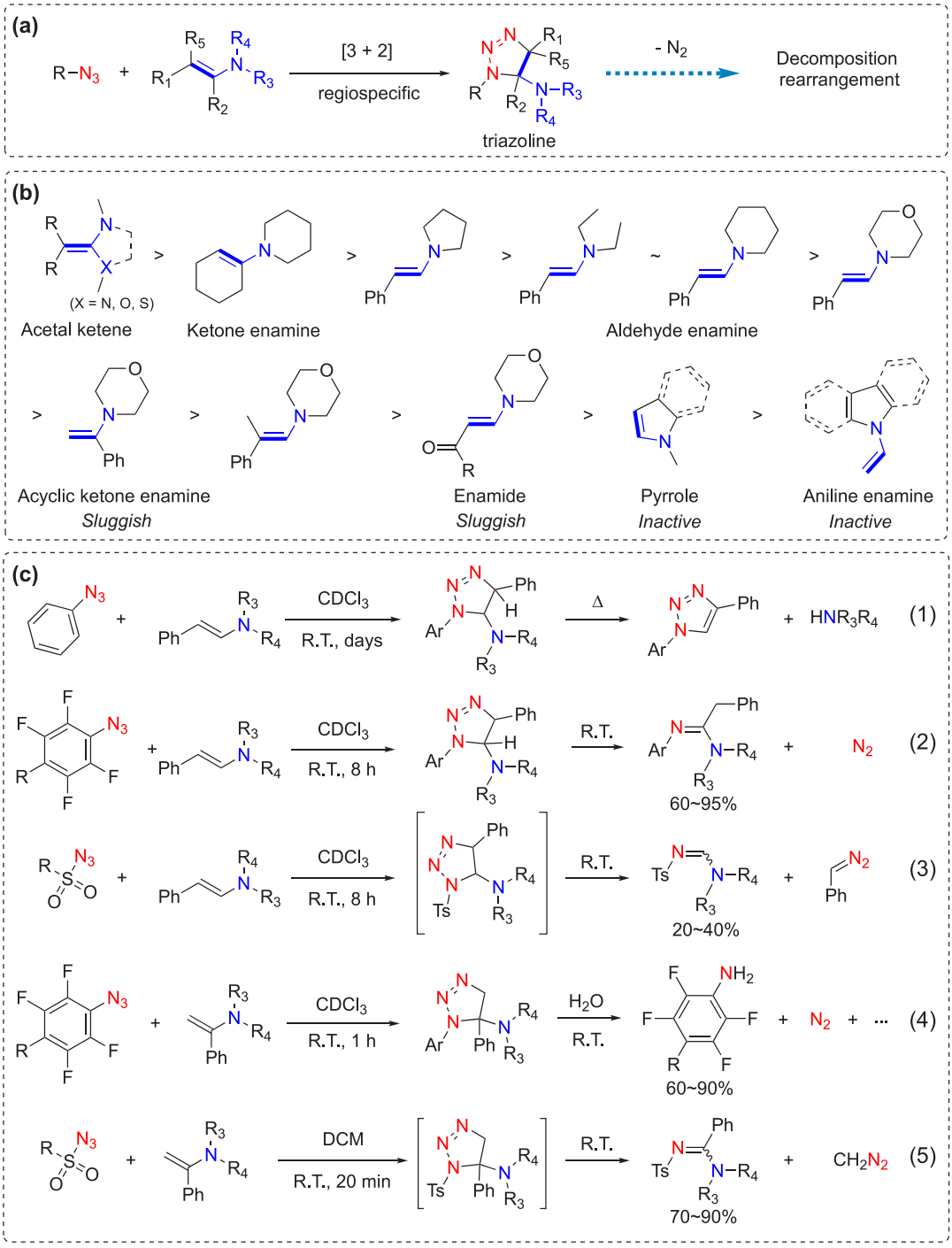
Scheme 3. (a) Azide−Enamine Cycloaddition Reactions; (b) Examples of Enamine Structures Listed in Order of Decreasing Nucleophilicity and the Relative Rates of Reaction with PFAAs; (c) Examples of the Reactions of Azides with Aldehyde Enamines (1−3) and Ketone Enamines (4 and 5)



The triazoline cycloaddition products display low stability, particularly in the case of electron-deficient azides, and can rearrange into a variety of products. Examples of the reactions of PFAAs and sulfonyl azides with enamines in comparison with phenyl azide are shown in Scheme 3c. Aldehyde enamines, formed from the condensation of an enolizable aldehyde and an amine, react with phenyl azide to form isolable 5-aminotriazolines. When refluxing in toluene or upon treatment with acid/base under hygroscopic conditions, the triazoline is converted primarily to the corresponding triazole upon elimination of the amine (reaction 1), although refluxing also leads to notable byproducts such as amidines, amides, and diazo compounds. When electron-deficient PFAAs were used, the triazoline products spontaneously decomposed to the corresponding amidines at room temperature with the release of N_2 (reaction 2).[5] For sulfonyl azide, the fate of the triazolines is very different, as they were observed to eliminate (diazomethyl)benzene to afford tosylformimidamides in 20−40% yield (reaction 3, unpublished results; see the Supporting

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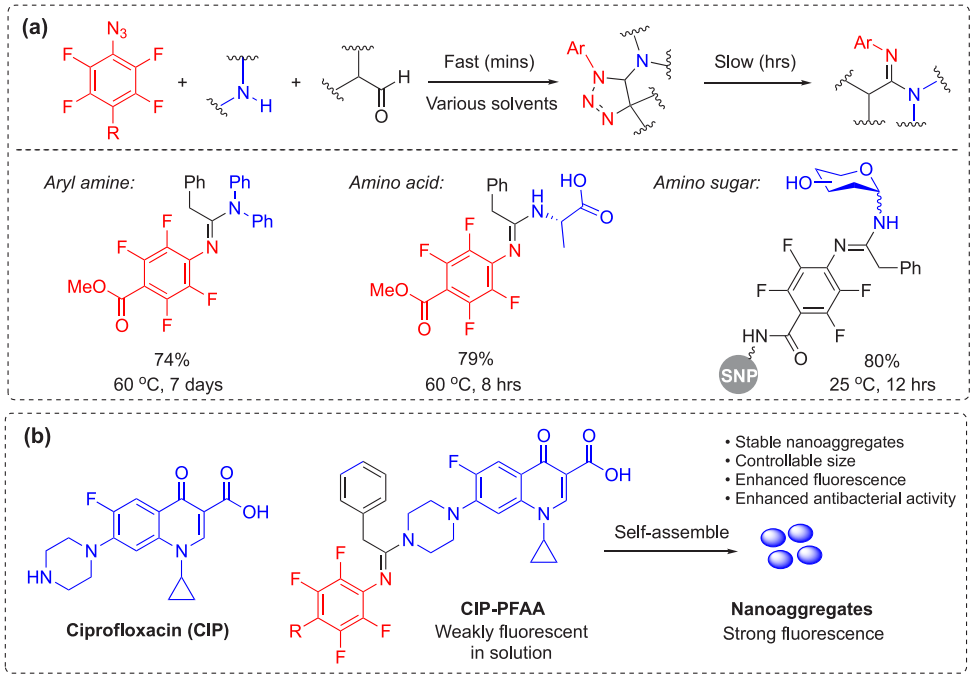
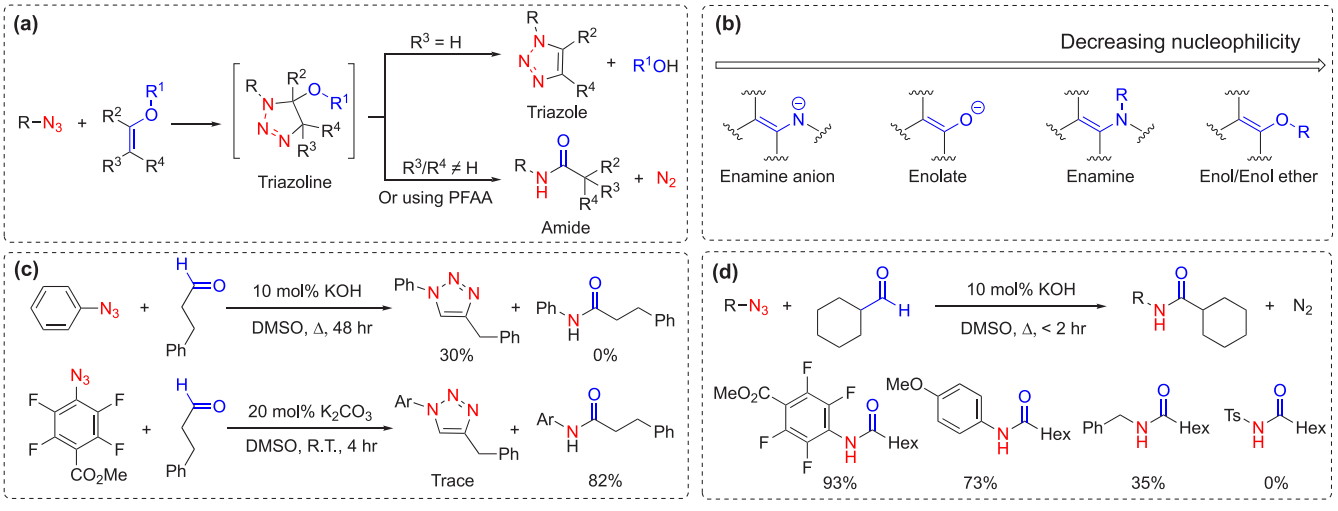
Scheme 4. (a) PFAA−Aldehyde−Amine Reaction and Selected Examples; (b) Synthesis of Ciprofloxacin-Containing Amidine That upon Aggregation into Nanoparticles Showed Enhanced Antimicrobial Activity and Fluorescence



Scheme 5. (a) Azide−Enol/Enolate Cycloaddition Reaction; (b) Nucleophilicities of Typical Electron-Rich Dipolarophiles; (c) Phenyl Azide and PFAAs in the Reaction with α-Monosubstituted Enolizable Aldehydes (R^3 = H); (d) Base-Promoted Amidation between Different Azides and α-Disubstituted Enolizable Aldehydes ($R^3/R^4 \neq H$)



Information for the detailed experimental procedure and Figures S1 and S2 for ^{1}H and ^{13}C NMR spectra).[16]

Interestingly, different enamines lead to distinct rearrangement pathways. Ketone enamines, formed by condensation of amines and ketones, are regioisomers of the corresponding aldehyde enamines. When an acyclic ketone enamine underwent cycloaddition with a PFAA (reaction 4), the resulting triazoline decomposed to the aniline cleanly instead of giving the amidine product as in the case of aldehyde enamines.[5] On the other hand, the reaction of ketone enamines with sulfonyl azide yielded amidines and diazomethane cleanly (reaction 5), in sharp contrast to the sluggish sulfonyl azide−aldehyde enamine reaction (reaction 3).[17] The triazoline intermediate was not detected experimentally and was proposed to decompose through a pericyclic retro-[3 + 2] cycloaddition mechanism.

The azide−enamine reaction can be carried out by mixing an azide, an enolizable aldehyde/ketone, and an amine, where the enamine dipolarophile is formed in situ between the aldehyde/ketone and the amine. As expected, the electron-deficient PFAA−aldehyde−amine reaction proceeds smoothly at room temperature without any catalysts to give the amidine product in high yield (Scheme 4a).[5,18] Importantly, the one-pot reaction essentially expands the substrate scope of the azide−enamine reaction to include those less nucleophilic aryl amines that hardly form isolable enamines with an aldehyde. The reaction also tolerates the carboxyl group and works efficiently for the amino group in unprotected amino acids

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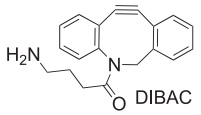
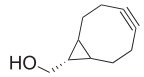
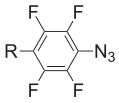
Table 1. Bimolecular Rate Constants for Cycloadditions of Alkyl Azide, Phenyl Azide, or PFAA and Different Strained Dipolarophiles

	 DIBAC		 BCN		 Norbornene		 TCO	
	k (M^{-1} s^{-1})	k_{rel}	k (M^{-1} s^{-1})	k_{rel}	k (10^{-4} M^{-1} s^{-1})	k_{rel}	k (10^{-2} M^{-1} s^{-1})	k_{rel}
	0.24	1	0.07	1	0.018	1	0.64	1
	0.033	0.14	0.20	2.9	0.15	8.3	1.7	2.7
	0.16 [R = C(O)NR]	0.67	1.23 [R = C(O)NR]	18	9.13 [R = C(O)OMe]	500	11 [R = Me]	17

(e.g., 0.1 mM L-alanine in 3:1 v/v DMSO/water, 79% yield after 8 h).

The PFAA−aldehyde−amine coupling reaction has been successfully employed to functionalize nanomaterials. For example, stirring an amino sugar, phenylacetaldehyde, and PFAA-functionalized silica nanoparticles (SNPs) in acetone/water at room temperature afforded sugar-decorated SNPs having high ligand density (10×10^{-16} nmol/nm^2 for D-mannose) and higher binding affinity to lectin (carbohydrate-binding protein) than similar SNPs prepared by the CuAAC reaction.[19] Another advantage of the reaction is the F atoms in PFAA, which allow quantification of the ligand density by quantitative ^{19}F NMR analysis.[19] As PFAA-functionalized materials can be readily prepared from various nanomaterials/surfaces and a heterobifunctional PFAA (Scheme 1d),[9] this coupling reaction constitutes a new and versatile tool for the preparation of multifunctional materials. One drawback of the reaction is its poor performance under aqueous conditions; the addition of an organic solvent such as DMSO is necessary to promote the enamine formation.

When phenylacetaldehyde is used, this modular coupling reaction introduces a phenyl ring and a perfluorinated aryl ring linked by an amidine bond. The product constitutes a supramolecular synthon that provides strong hydrophobic interactions for molecular assembly. In one example, a fluoroquinolone antibiotic having a secondary amine, ciprofloxacin or norfloxacin, reacted with phenylacetaldehyde and a PFAA to give a propeller-shaped amidine product that readily assembled into stable nanoparticles upon addition of its DMSO solution into water.[20] These nanoaggregates, made entirely of the drug molecules, showed enhanced antimicrobial activities compared with the simple molecule. The covalent functionalization furthermore turns on the otherwise weak fluorescence of ciprofloxacin.[20,21] The amidine product becomes strongly emissive upon aggregation, whereas its homogeneous solution is weakly fluorescent as a result of intramolecular charge transfer quenching. This example represents a novel approach to theranostic nanodrugs.

Enols and Enolates

Enols and enolates are also electron-rich dipolarophiles that can undergo cycloadditions with azides to form triazolines in a regiospecific manner (Scheme 5a). Many ketones and aldehydes are potential enol/enolate surrogates in the reaction (Scheme 5b).

Cycloaddition of an azide and enol/enolate gives a triazoline intermediate, whose fate follows one of two main pathways (Scheme 5a): (1) elimination of R^1OH to yield a triazole[22] or (2) release of N_2 and rearrangement to yield an amide.[23,24] The triazole formation requires a β-proton to facilitate the elimination−aromatization reaction, which is normally carried out under hygroscopic conditions. This transformation is more efficient for electron-deficient aryl azides than for electron-rich aryl azides and aliphatic azides. The reaction provides an alternative to access 1,4-triazoles for which metal-catalyzed or enamine-mediated click reactions either failed or gave the desired products in low yields.

When PFAA was used, the triazoline intermediate decomposed to form the amide product at room temperature, catalyzed by either a base or diethylurea.[23,24] Notably, no other azides behave like this, including sulfonyl azides and nitro-substituted aryl azides. In the example in Scheme 5c, the reaction between PFAA-COOMe and 3-phenylpropanal in the presence of K_2CO_3 occurred at room temperature to give the aryl amide in 82% yield, with only a trace amount of the triazole product observed.[24] The reaction between phenyl azide and 3-phenylpropanal at elevated temperature gave the triazole in 35% yield after 48 h, and no amide product was detected (Scheme 5c).[24] When the enolizable aldehyde is disubstituted at the α-posistion (i.e., $R^3/R^4 \neq H$), it is impossible to form the triazole by aromatization, and the triazoline intermediate rearranges to yield the aryl amide (Scheme 5d).[24−26] This transformation was more efficient for PFAAs than non-fluorinated aryl azides, sluggish for aliphatic azides, and absent for sulfonyl azides (Scheme 5d).[24]

These results clearly demonstrated that the highly electrophilic PFAA faciliated the spontaneous decomposition−rearrangement of the triazoline, similar to the PFAA−enamine reaction. The base-catalyzed PFAA−aldehyde reaction to form an aryl amide applies to different enolizable aldehydes, can be carried out under mild conditions to yield N_2 as the main byproduct, and thus is an efficient and clean method for the synthesis of the highly electron-deficient perfluoroaryl amides.[24] Perfluoaoryl amides, serving as unique ligand accelerators, are an emerging class of substrates for the rich Pd-catalyzed C−H activations.[27] These products are generally

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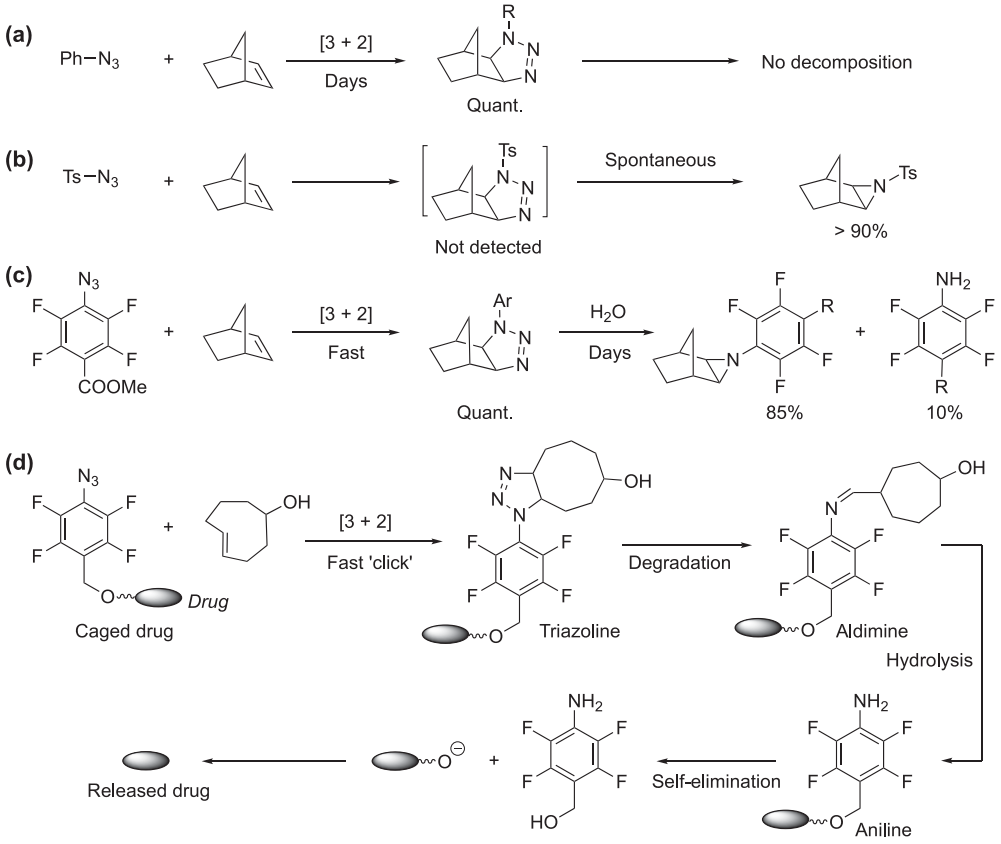
Scheme 6. (a–c) Azide–Norbornene Reactions; (d) PFAA–TOC Reaction in Prodrug Activation–Decaging Design for Controlled Drug Release



difficult to access by traditional methods, for example, amide coupling between a carboxylic acid and an amine.

REACTIONS WITH STRAINED DIPOLAROPHILES

Besides electron-rich enamines and enols/enolates, strained alkynes and alkenes having a distorted triple or double bond are also activated dipolarophiles in the cycloaddition reaction with azides. Table 1 summarizes the rates of cycloaddition reactions between different azides and strained alkynes (DIBAC, BCN) or alkenes (norbornene, *trans*-cyclooctene (TCO)).

Strained Alkynes

Several strained cyclooctynes react readily with azides without the use of any catalysts, leading to a major class of bioorthogonal reactions. These alkynes are activated by strain-promoted lowering of the distortion energy and enhanced interaction energy. Cycloadditions between an azide and an alkyne, including benzoannulated cyclooctynes, are primarily mediated by the $HOMO_{azide}$–$LUMO_{alkyne}$ interaction. As a result, electron-deficient aryl azides generally display lower rates than phenyl azide and alkyl azides (Table 1).[5,28] Surprisingly, the benzoannulated cyclooctyne DIBAC is more reactive toward PFAAs than phenyl azide (Table 1). Computational results from the D/I model indicate that the distortion energy is the major contribution to the overall activation energy, whereas the interaction energy is nearly constant in this case. The unprecedented reactivity of PFAAs toward benzoannulated cyclooctynes is likely associated with the two ortho F atoms, which distort the N_3 and further lower the distortion energy.

In the case of the aliphatic cyclooctyne BCN, electrophilic aryl azides showed better reactivity than both phenyl azide and alkyl azides (Table 1).[28] The enhanced reactivity can be explained by the strong FMO interaction between the $LUMO_{azide}$ and $HOMO_{alkyne}$. This inverse-electron-demand reactivity enables the development of highly accelerated strain-promoted AAC.

Strained Alkenes

The reactions between azides and strained alkenes proceed smoothly, and in many cases the resulting strained triazolines are converted into aziridines.

The azide–norbornene reaction has long been found to have unusual reactivity and high exo stereoselectivity.[29,30] Benzyl azide and phenyl azide form isolable triazoline compounds at relatively low rates (10^{-5}–10^{-6} M^{-1} s^{-1} at 25 °C) but can achieve full conversion given sufficient reaction time (Scheme 6a).[5] Tosyl azide yields the aziridine cleanly and more quickly (Scheme 6b).[31] The reaction is regarded as bioorthogonal and has been used in peptide and protein labeling.[32] PFAAs undergo fast cycloadditions to yield triazolines (~10 times faster than TsN_3), followed by fairly slow decomposition to give aziridines (>85%) with anilines as the minor byproduct (Scheme 6c). The high reactivity of PFAAs results from the activation by the two ortho fluorine atoms in addition to the electrophilic activation.[33]

The azide–TCO cycloaddition is almost 2 orders of magnitude faster than the corresponding azide–norbornene cycloaddition (Table 1).[34] The triazoline formed from PFAA and TCO is labile under physiological conditions and readily decomposes into the aziridine and aldimine, which further

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hydrolyzes to aniline (Scheme 6c). The unique reactivity of the PFAA−TCO reaction, i.e., fast cycloaddition followed by controllable decomposition, fits perfectly the criteria for bioorthogonal prodrug activation−decaging and has been used to design a controlled drug delivery system (Scheme 6d).[34]

REACTIONS WITH NUCLEOPHILES

Phosphine

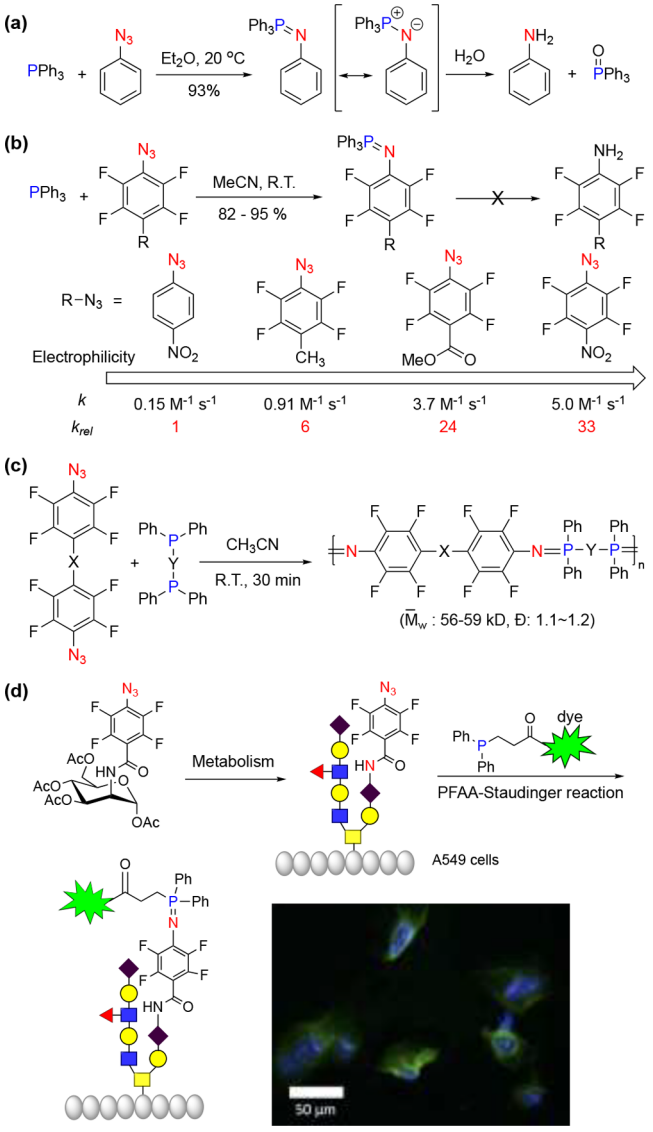
The Staudinger reaction is the reaction of an azide with a phosphine to form a phosphoimine. The reaction is efficient for different azides and can proceed in nearly full conversion without the need for any catalysts (Scheme 7a). The phosphoimines formed from aliphatic azides and phenyl azide are labile and can be further hydrolyzed to amines. Electrophilic azides generally show enhanced reactivity by increasing the stability of the phosphoimine. As a result, hydrolysis of the resulting phosphoimine is more challenging and usually must be done under harsh conditions, for example, at 100 °C in 3 M H_2SO_4. The use of electrophilic azides is nevertheless practical since the electron-rich phosphines are unstable.[35]

Early reports by Banks and co-workers showed that electrophilic aryl azides reacted readily with triphenylphosphine to yield isolable phosphoimines.[36,37] Recent studies from our lab have confirmed the enhanced reactivity of PFAAs with phosphines. The reaction occurs rapidly at room temperature to form stable phosphoimines that resist hydrolysis (Scheme 7b).[38,39] The reaction proceeds rapidly in polar solvents because of stabilization of the polar transition state.[39] Kinetic studies showed a rate of up to 18 M^{-1} s^{-1} in H_2O/MeCN, which is 3 orders magnitude higher than that of the classic Staudinger ligation (~10^{-3} M^{-1} s^{-1}).[40]

This catalyst-free, fast, and high yielding reaction was applied to synthesize polyphosphazenes by simple mixing of a bis-PFAA and a bisphosphine in MeCN (Scheme 7c).[41] Polyphosphazenes were obtained within 30 min with molecular weights of over 59 000 and a narrow dispersity ($Đ$) of 1.1−1.2, which is a significant improvement over the polyphosphazenes obtained from the classic Staudinger reaction. These fluorinated polyphosphazenes exhibited higher thermal stability, including high thermal decomposition temperatures (up to 441 °C) and char yields (up to 53%). The PFAA-Staudinger reaction can also be used to graft carbohydrates onto polymers, for example, by postsynthetic modification of PFAA-functionalized poly(lactic acid) with phosphine-derivatized mannose or maltoheptaose.[42]

The PFAA-Staudinger reaction is a bioorthogonal reaction. In an example involving imaging of cell-surface glycans, PFAA-derivatized monosaccharides (mannosamine/galactosamine) were treated with A549 cells, and the surface-expressed glycans were visualized by the PFAA-Staudinger reaction by treatment of the cells with phosphine-derivatized and fluorescein isothiocyanate (FITC)-labeled bovine serum albumin (BSA) (Scheme 7d).[38] The success of the PFAA-Staudinger reaction, confirmed by the bright green fluorescence on the cell surface and the dark background with minimal background noise, is due to the high rate of the reaction (15−30 min). The stable phosphoimine product does not undergo subsequent slow hydrolysis, which would cause sluggish labeling leading to high background noise.

Scheme 7. (a) Phenyl Azide and (b) PFAAs in the Staudinger reaction; (c) PFAA-Staudinger Polymerization to Yield Polyphosphazenes; (d) Use of the PFAA-Staudinger Reaction in Probing Cell-Surface Glycans[a]



[a]In (d), A549 cells were incubated with ManNAc-derivatized PFAA and then treated with phosphine-derivatized BSA. In the confocal fluorescence image, the cell nuclei were stained with Hoechst 33342 dye, which fluoresces blue. BSA was labeled with FITC, which fluoresces green.

Thioacids

Many azides react with thioacids (−CSOH or −COSH) to form amides, but the reaction rate varies to a large extent (Scheme 8). Electrophilic TsN_3 and PFAAs show the highest rates (10^{-3}−10^{-2} M^{-1} s^{-1}), which are more than 2 orders of magnitude higher than that for benzyl azide.[43] The reaction of electron-deficient azides, including PFAAs and sulfonyl azides, was proposed to proceed through a thiotriazoline intermediate via a stepwise cycloaddition followed by extrusion of N_2S (Scheme 8).[44] The reaction is carried out under mild conditions, is compatible with an aqueous environment (e.g., in pH 7.4 phosphate-buffered saline, 95% yield after 2 h), and shows a high degree of chemoselectivity. This reaction thus

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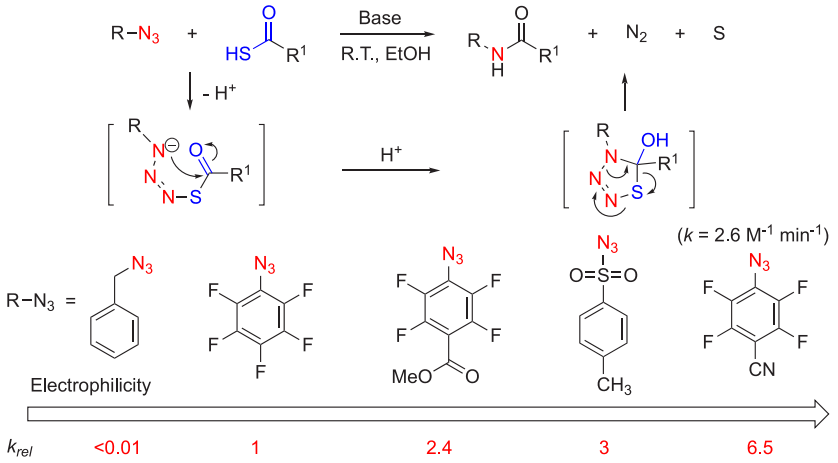
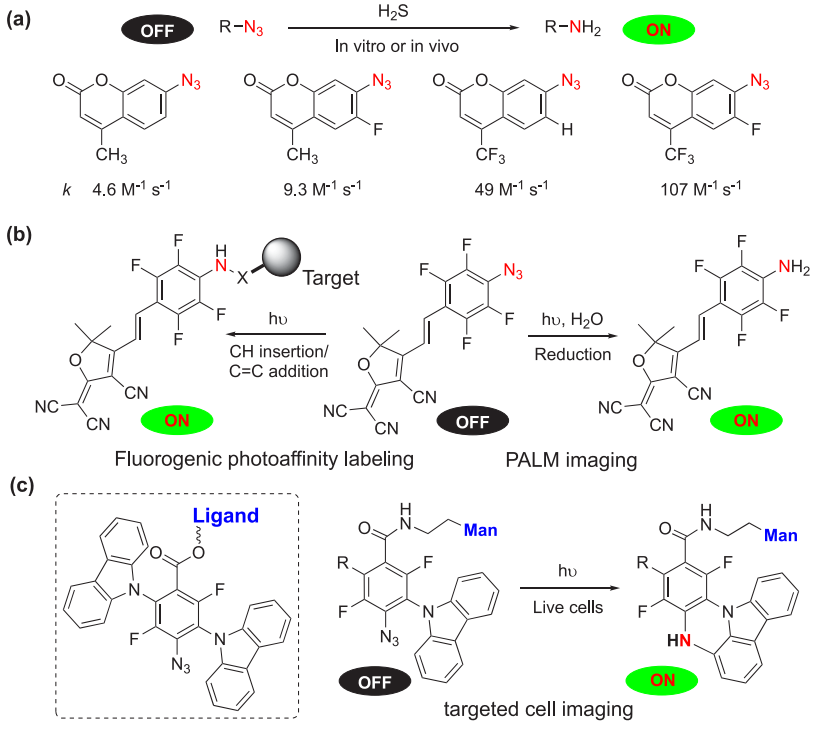
Scheme 8. Azide−Thioacid Amidation Reaction



Scheme 9. Azide−Amine Transformations Involving Azide-Based Fluorogenic Probes: (a) Reduction by H_2S; (b) Fluorogenic Photoaffinity Labeling[45] and PALM;[47] (c) Fluorogenic PFPA Probe for Cell Imaging via Intramolecular CH Insertion[48]



represents a general type of coupling reaction, offering an attractive alternative to the classic carboxy−amine coupling for bioconjugation. Furthermore, the ease of introducing an additional functional group is another clear advantage of PFAAs over sulfonyl azides in these transformations (Scheme 1d).

PHOTOACTIVATABLE FLUOROGENIC TRANSFORMATIONS

The azido group can deactivate fluorophores via photoinduced electron/charge transfer because of its low-lying LUMO orbital.[45] As a result, the azide-to-amine transformation leads to fluorescence turn-on, which offers the advantages of low background fluorescence and thus high detection sensitivity. The chemical inertness of the azides make them particularly useful molecular probes for bioimaging. Many of these azide-masked probes are electron-deficient azides, and they can be readily reduced to amines by H_2S and dithiols. EWGs on the azide significantly accelerate reduction (Scheme 9a),[46] which enables real-time visualization of the target, for example, endogenous H_2S fluxing events.

An emerging strategy is to use these fluorogenic azides as tunable molecular probes in chemical biology for fluorescence-based sensing, imaging, and labeling. PFAAs are particularly useful in this regard, since the photoactivated perfluoroaryl nitrenes undergo clean photoclick conjugation through efficient CH insertion reactions. The use of a PFAA-masked dihydrofuran fluorophore in photoaffinity labeling leads to self-reporting fluorogenic bioconjugation, which simplifies the subsequent detection, purification, and characterization of the target (Scheme 9b, left).[45] The photochemical azide-to-amine conversion process has also been successfully used in a single-

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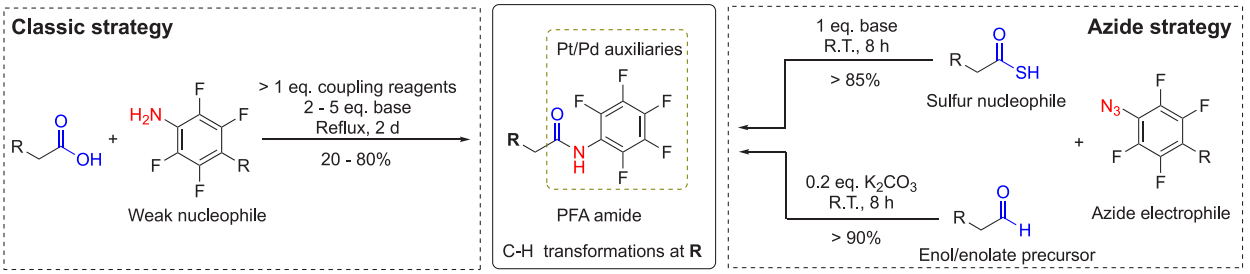
Scheme 10. Preparation of Perfluoroaryl Amides, A New Class of Auxiliaries for C−H Activation:[27] (left) the Classic Amide Coupling Strategy Using Weak Perfluoroaryl Aniline as the Nucleophile; (right) the Azide Strategy via the PFAA−Thioacid or PFAA−Aldehyde Amidation Reaction



molecule fluorescence microscopy technique known as photoactivated localization microscopy (PALM) (Scheme 9b, right).[47] The reaction offers high spatial resolution and high sensitivity, which was attributed to the efficient azide-to-amine transformation (87%). However, this system cannot accommodate ligands for targeted imaging. We developed a fluorogenic PFAA platform in which a third ligand (e.g., mannose) can be introduced on PFAA for specific targeting (Scheme 9c).[48] Importantly, the fluorogenic transformation was accomplished by an intramolecular CH insertion, which is clean, of high contrast, and unaffected by intracellular redox processes.

CONCLUDING REMARKS

We have summarized the reactions of electrophilic azides, particularly PFAAs, toward electron-rich dipolarophiles, strained dipolarophiles, and nucleophiles. Many of these reactions occur without the need of any catalysts, give clean and fast conversions, proceed under mild conditions, and mostly undergo extrusion of N_2 to yield stable products. The enhanced reactivities of PFAAs, analyzed by the D/I-AS model, have been shown to result from the markedly lowered LUMO and decreased distortion energy of the fluoroaryl azide. These properties are unique and are different from those electron-rich alkyl and aryl azides, which are much less chemically reactive and require specific catalysts when reacting with dipolarophiles. In the development of catalyst-free click chemistries, the activation of azides using electrophilic azides represents an important strategy that is just as valuable as activation of the dipolarophiles. PFAAs also display reactivities that are distinct from those of other electrophilic azides, such as sulfonyl azides.

The transformations of PFAAs provide a wide range of molecular tools for the development of new organic reactions, for nanomaterial functionalization, and for chemical biology. An example of a new reaction is the formation of useful electron-deficient perfluoraryl amides, as shown in Scheme 10. The PFAA−aldehyde reaction or the PFAA−thioacid amidation gives auxiliaries that enable C−H activation for difficult transformations and in green synthesis.[27] For comparison, the classic preparation by amide coupling suffers from harsh conditions and low yields becaue of the low nucleophilicity of the perfluoroaryl aniline.

PFAA reactions are highly suitable for surface and nanomaterial functionalization and can be directly adapted to surfaces and nanomaterials. Finally, the fast and catalyst-free reactions, such as the PFAA-Staudinger reaction, are excellent molecular tools to probe biological processes. Furthermore, the biocompatibility of the reagents and biorthogonality of the reactions make the PFAA-masked molecular probes new tools for reaction-based chemical sensing, bioimaging, $^{19}F/^{18}F$-based analysis, and drug delivery.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.accounts.0c00046.

Reaction of tosyl azide with styrylmorpholine: experimental protocol and NMR spectra of the product (PDF)

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The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Rolf Huisgen, the creator of the field of 1,3-dipolar cycloadditions, on the occasion of his 100th birthday.

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