A Pyridinium Ylide-Alkylation Strategy for the Structural Diversification of *N*-Carbamoyl Pyridinium Salts

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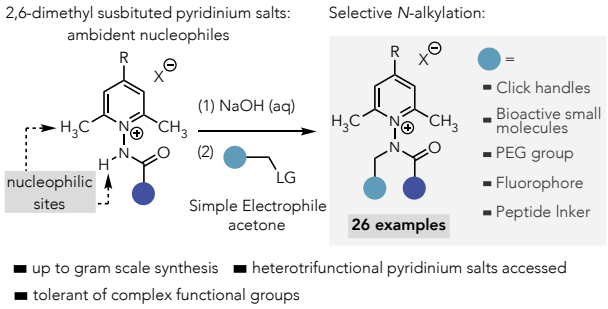
Supporting Information Placeholder



ABSTRACT: A pyridinium-ylide alkylation strategy has been developed for accessing *N,N*-disubstituted carbamoyl pyridinium salts that possess multiple nucleophilic sites. The method was shown to be tolerant towards an array of different pyridinium scaffolds and common electrophiles; enabling access to structurally diverse pyridinium salts. The potential versatility of the approach was demonstrated through the synthesis of chemically complex, heterotrifunctional pyridinium salts containing a pyridinium warhead, a click chemistry handle, and a third, high-value, payload.

Owing to their wide range of reactivity, *N*-substituted pyridinium salts have found myriad applications in organic synthesis. *N*-substituted pyridinium salts can be leveraged to access to a wide array of oxidation states between pyridine and piperidine type structures, which is achieved by the addition of either nucleophiles or electrons into the electron deficient aromatic ring[1]. Beyond redox cycling, the cationic charge on the pyridinium ring also imparts additional acidity to pendant functional groups; a property that makes these reagents ideally suited for generating ylides under mild conditions, and pyridinium ylides have found extensive use in cycloaddition chemistry[2]. The reactive properties of pyridinium salts are also becoming increasingly appreciated in chemical biology. We recently reported an optically triggered approach for Tryptophan (Trp)-selective protein modification in which we exploit the inherent photolability of Trp by pairing Trp-containing biomolecules with *N*-carbamoyl pyridinium salts that engage Trp in photo-induced electron transfer (PET) to yield a net carbamylation of the C2-position on the indole ring of Trp[3]. This ligation approach was found to be biocompatible and mechanistically tunable; traits which allowed us to exploit the mildness of this process to engage in live cell Trp-labelling[3c].

As part of our program to expand the utility of this chemistry, we sought here to increase the generality of the approach by developing methods for structural diversification of *N*-carbamoyl pyridinium salts that would ultimately enhance the scope of incorporable functionality into peptides and proteins. Additionally, *N*-carbamoyl pyridinium salts and structurally analogous N-carbonyl pyridinium salts have been shown by Studer and others to engage in photoredox catalyzed C–N bond formation with small molecule aromatics, and therefore general approaches to increasing pyridinium molecular diversity could be applied in this context as well[4].

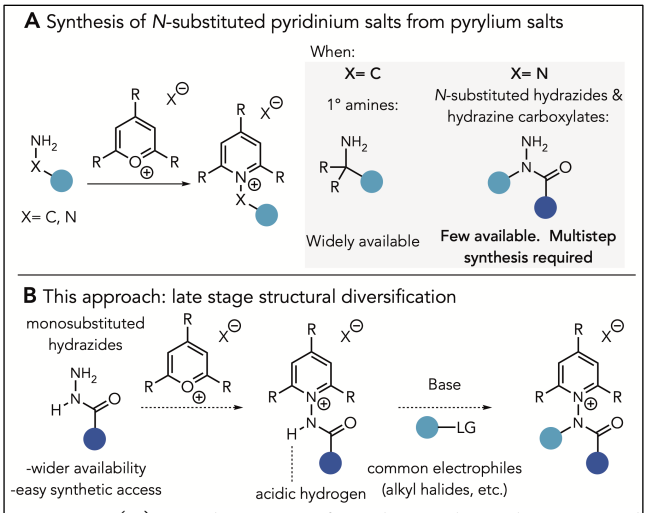


Figure 1. (A) Condensation of pyrylium salts with amine and hydrazides to form complex pyridinium salts. (B) This work: complex carbamoyl pyridinium salt synthesis using a pyridinium-ylide alkylation strategy.

Pyridinium salts that are used as radical reservoirs typically possess substitution at the 2,4, and 6 positions of the pyridinium ring in order to promote N-X bond fragmentation[5] and are typically synthesized by a condensation of a primary amine with a pyrylium salt to add the N-X bond that fragments[6] (Figure 1A). Whilst primary amines are widely available with extensive structural diversity, these only enable access to C-centered radical reservoirs. Accessing *N*-centered radical reservoirs require condensation of a pyrylium salt with either substituted hydrazine or hydrazide precursors that may require multi-step syntheses and extensive protecting group manipulations to access. We therefore considered a late stage diversification strategy, in which a single, monosubstituted hydrazide could be condensed onto the pyrylium salt, and then this structure could be derivatized by alkylation with exogenous electrophiles (Figure 1B). By virtue of being adjacent to a quaternized nitrogen, any X-H bonds on the atom adjacent increase notably in acidity and can thus be readily deprotonated under mild conditions to form pyridinium-ylides. We sought to use pyridinium ylides as nucleophiles that could be alkylated with a wide range of electrophiles. Whilst several groups have reported *N*-alkylation of amidopyridinium ylides, these reports use pyridinium ylides in which the pyridinium moiety lacks additional base-sensitive functional groups[7].

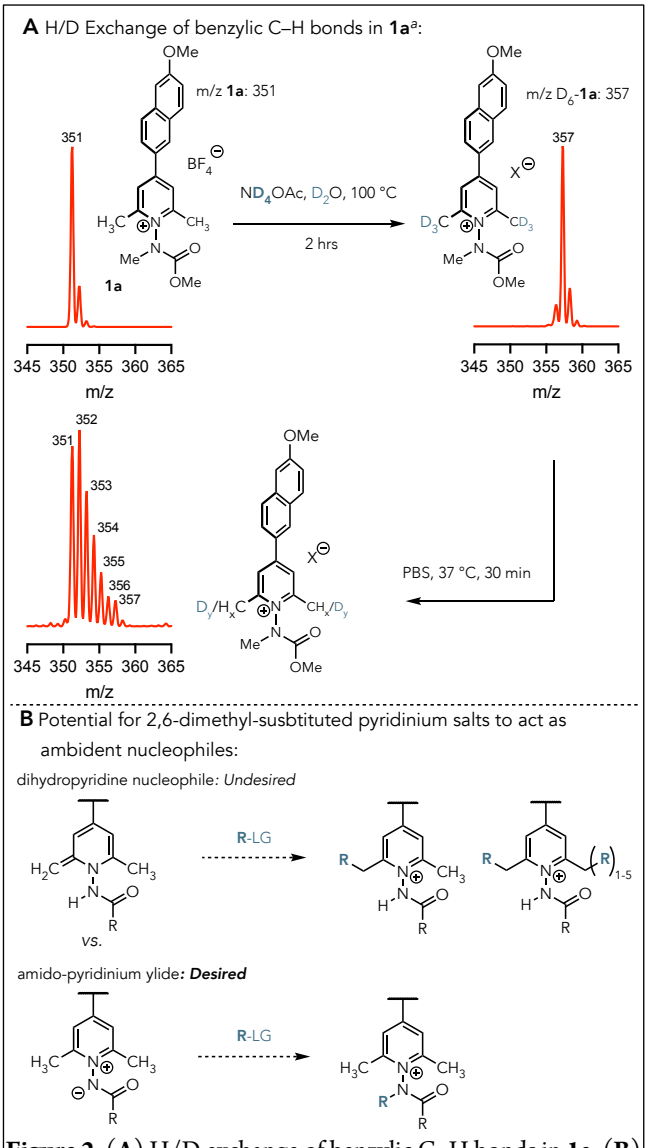
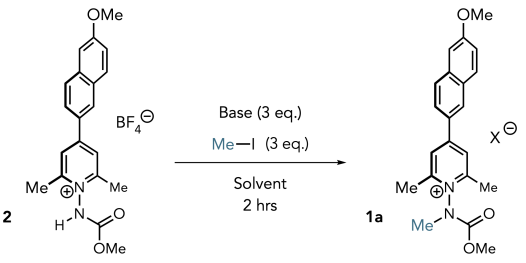


Figure 2. (**A**) H/D exchange of benzylic C–H bonds in **1a**. (**B**) Potential off-target alkylation of 2,6-dimethyl-substituted pyridinium salts. [a]H/D exchange monitored by LC/MS. PBS=phosphate buffered saline.

By contrast, our recently reported salt **1a** features two benzylic methyl groups that flank the *N*-methyl-*N*-carbamoyl transferring group and are essential for function and thermal stability of the salt in complex biological mixtures. When **1a** was incubated in buffered D_2O (ND_4^+OAc) at 100 °C, we observed near complete H/D exchange by LC/MS as evidenced by an increase by 6 m/z of **1a** (Figure 2A). When D_6-**1a** was incubated phosphate-buffered saline (PBS) at 37 °C for 30 minutes, we observed D/H exchange to give a mixture of deuteration states of **1a**; ranging from +0 to +6 deuterium atoms. H/D exchange has been previously reported for pyrylium structures, and these results show the same occurs with related pyridinium salts as expected[6]. Moreover, this experiment confirmed that **1a** and related pyridinium salts possessing benzylic methyl groups can exist as a mixture of pyridinium salt and nucleophilic 1,2-dihydropyridines through rapid and reversible protonation/deprotonation. Thus, **1a** and related pyridinium salts could potentially act as ambident nucleophiles (*N*-centered nucleophile vs. *C*-centered dihydropyridine nucleophile) for a potential electrophile (Figure 2B).

We sought conditions that would select for *N*-alkylation over *C*-alkylation by exploring the methylation of pyridinium salt **2** to give **1a**. Our studies commenced by exploring combinations of solvent, base (3 eq.), and methyl iodide as an electrophile (3 eq.). We opted to monitor these preliminary experiments using LC/MS as changes in alkylation state could readily be detected and semi-quantitated with this technique. Thus, treatment of **2** with NaOH in either ethanol or water (Table 1, entries 1 and 2) only gave trace conversion. Higher conversion was observed in polar aprotic solvents such as acetonitrile and DMF. However, when Cs_2CO_3 was used as base, we observed a wide distribution of alkylation products in both acetonitrile (Table 1, entry 3) and DMF (Table 1, entry 4) ranging from mono-methylation to penta-methylation (entries 3,4) and with very little selectivity for mono-methylation. These results confirmed that the benzylic methyl groups of **2** are reactive and viable nucleophiles

Table 1. Attempted optimization of a one pot alkylation



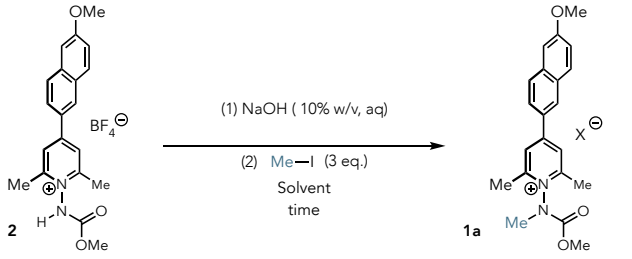
Entry	Base	Solvent	%Conversion[a]	Alkylation distribution ratio: (+1,+2,+3,+4,+5)
1	NaOH	EtOH	Trace	Not Measured
2	NaOH	H_2O	Trace	Not Measured
3	Cs_2CO_3	CH_3CN	46	1:1.8:1.8:1.7:1.3
4	Cs_2CO_3	DMF	80	2.1:1.5:1.4:1:0
5	TEA	CH_3CN	18	+1 observed only
6	KOtBu	Acetone	75	32:17:11:4:1

[a]Conversions and alkylation ratios were estimated using total ion count (TIC) from LC/MS

under these conditions. The use of triethylamine in acetonitrile gave solely mono-methylation (Table 1, entry 5), but only in modest conversion. Based on a report from Hong[7d], in which mono-alkylation of pyridinium ylides was observed in acetone, we attempted a one-pot alkylation of **2** using potassium t-butoxide as base in acetone and observed moderate conversion and modest selectivity for mono-methylation (Table 1, entry 6).

Given the inability to control alkylation states with one-pot conditions, we next considered a stepwise sequence featuring generation and isolation of the pyridinium ylide followed by alkylation with an electrophile. Reports from Charette[7b] and Davies[8] , in which simple *N*-benzyliminopyridinium ylides were isolated, suggested to us that our *N*-carbamoyl pyridinium ylides may also be sufficiently stable for isolation. Thus, **2** was treated with a solution of NaOH (aq., 3 eq.) for 1 hour, followed by extraction with CH_2Cl_2, removal of the organic solvent, and direct subjection of the crude residue to methyl iodide (3 eq.) (Table 2). Solvent and time conditions were varied as shown in Table 2 and results were assessed by LC/MS analysis of crude reaction mixtures. Under all solvent conditions tested, we observed significant conversion and with exclusive selectivity for mono-methylation; confirming that ylide of **2** was formed under the employed conditions. Between the solvents assayed, DMF gave the highest conversion within 2 hrs. (>95%, entry 3) and CH_2Cl_2 gave the lowest (56%, entry 2) with CH_3CN and acetone giving moderate conversion (70% and 64% respectively, entries 1 and 4). Extension of the reaction time in acetone to 6 hrs. resulted in full conversion of **2** (>95%, entry 5). Performance of the ylide-alkylation sequence in acetone on a 0.1 mmol scale reaction enabled isolation of **1a** in 67% yield (Scheme 1). The spectral qualities of **1a** isolated from this experiment were in excellent agreement with samples synthesized *via* hydrazide condensation[3c]. Whilst DMF gives complete conversion in a shorter timeframe compared to acetone, we opted to use acetone as the solvent of choice for this process owing to its practical ease of use and its superior properties as a green solvent compared to DMF[9].

Table 2. Optimization of an iterative ylide formation-alkylation procedure

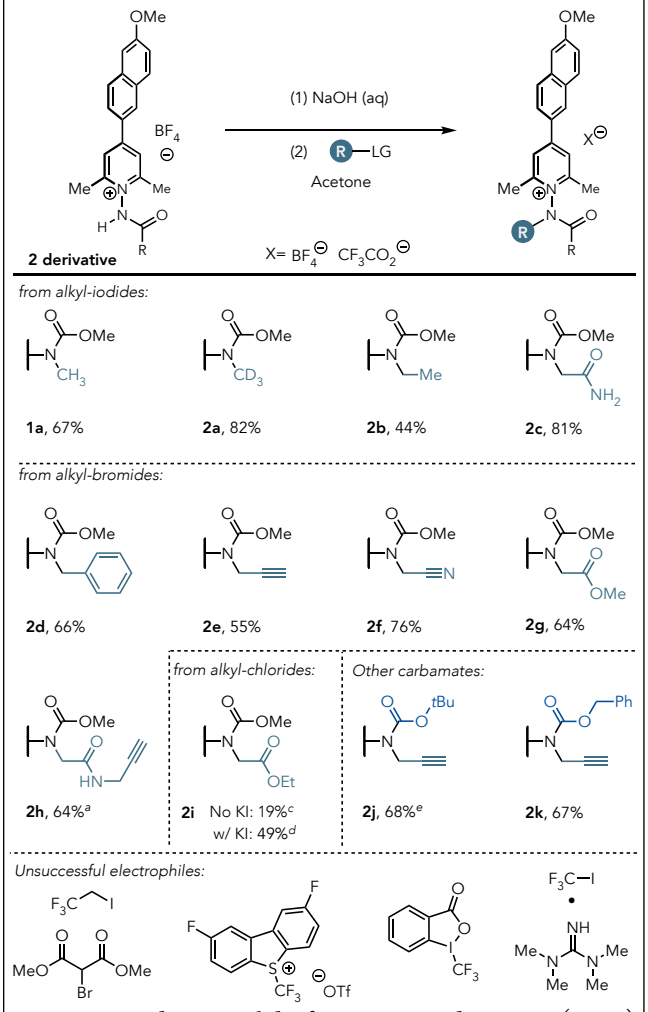


Entry	Solvent	t (hrs)	%Conversion[a]	Alkylation distribution ratio: (+1,+2,+3,+4,+5)[a]
1	CH_2Cl_2	2	56	+1 observed only
2	CH_3CN	2	70	+1 observed only
3	DMF	2	>95	+1 observed only
4	Acetone	2	64	+1 observed only
5	Acetone	6	>95	+1 observed only

[a]Conversions and alkylation ratios were estimated using total ion count (TIC) from LC/MS.

With optimal conditions in hand, we next sought to explore the scope of electrophiles tolerated through this approach. Beyond Me-I, alkyl iodides are well-tolerated for pyridinium alkylation and enabled perdeuteromethylation (**2a**, 82%), ethylation (**2b**, 44%) and acetamidylation (**2c**, 81%). Alkyl bromides also readily alkylate **2**, enabling benzylation (**2d**, 66%), propargylation (**2e**, 55%), as well as addition of nitriles (**2f**, 76%), and acetyl groups (**2g**, 64%). α -chloroacetates proved less reactive (19% conversion by LC/MS) and required the use of KI as an additive to achieve a competent yield (49% isolated yield). The ylide-alkylation method also tolerates carbamates that are frequently used as protecting groups, with boc-(**2j**) and cbz (**2k**) protecting groups tolerated in pyridinium-ylide propargylation in 68% and 67% respectively. Taken together, the diversity of tolerated electrophiles allowed for the potential incorporation of a wide array of functionality into the pyridinium scaffold. For example, perdeuteromethylation enables the synthesis of probes that are potentially useful for mass spectrometry-based workflows[10] and protein NMR spectroscopy[11], whilst α-haloacetamides and α-haloacetates are very mild electrophiles that are compatible with sensitive structures such as a drugs, drug fragments, peptides, and fluorophores. The method does have limitations, with unsuccessful electrophiles including dimethyl bromomalonate, 2,2,2-trifluoromethyl-1-iodo ethane, Umemoto's reagent, Togni's reagent, or the Ritter

Scheme 1. Electrophile & *N*-substituent substrate scope[a,b]

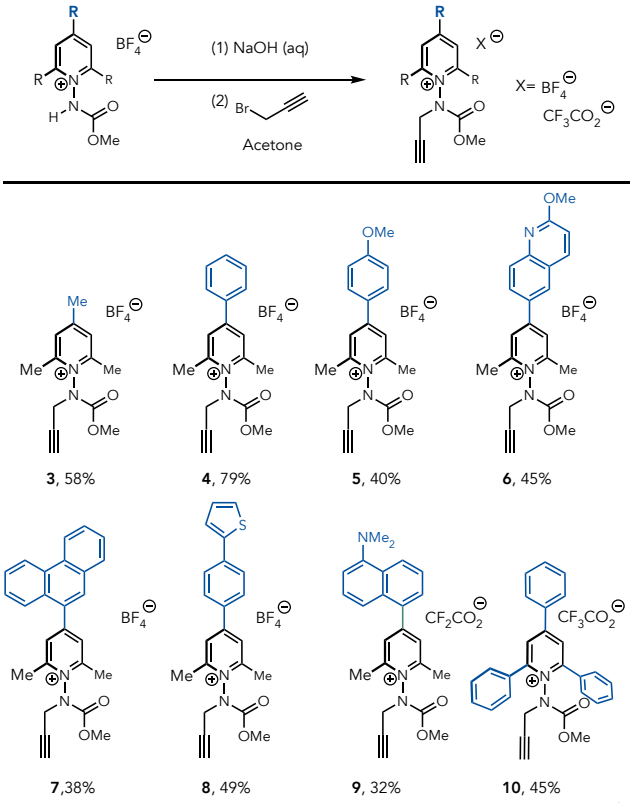


[a]Reaction conditions: Ylide formation: **2** derivative (1 eq.), NaOH (aq) (3-10 eq.). Ylide alkylation: electrophile (2-4 eq.), acetone ([ylide] = 0.3M). [b]Yields are averages of two experiments. [c]conversion by assessed by LC-MS. [d] 1:1 KI:electrophile ratio.[e] isolated with bromide counterion.

trifluoroiodomethane-TMG reagent. In these instances, no discernable alkylation or trifluoromethylation was observed.

Next, we explored the scope of pyridinium salt structures that would be compatible with this chemistry by synthesizing a small battery of *N*-carbamoyl pyridinium salts and studying their propargylation under our ylide conditions (Scheme 2). Analogs of our first generation pyridinium salts were readily propargylated to yield trimethyl pyridinium salt **3** (58%) and phenyl pyridinium salt **4** (79%) in good yields. Biaryl pyridinium salts containing diverse functionality are well tolerated by this process. *p*-methoxyphenyl substituted salt **5** was readily propargylated in 45% yield, whilst 2-methoxyquinoline-substited **6** was accessed in 40% yield. Anthracenyl substituted **7** was synthesized in 38% yield whilst 4-thiophenyl-phenyl-subsituted salt **8** was accessed in 49% yield. 5-dimethylamino naphthyl-substituted salt was accessed in 32% yield; a salt which bears structural/electronic similarities to dansyl-based fluorophores. Finally, 2,4,6-aryl substituted pyridinium salts have become essential radical reservoirs in small molecule organic synthesis, and we show that this motif is well tolerated with our ylide alkylation sequence by accessing salt **10** in a reasonable yield (45%).

Scheme 2. Pyridinium Substrate Scope[a,b]



[a]Reaction conditions: Ylide formation: pyridinium derivative (1 eq.), NaOH(aq) (3 eq.). Ylide alkylation: propargyl bromide (1.7-4.3 eq.), acetone ([ylide]=0.3M). [b]Yields are averages of two experiments.

Pyridinium salts **3-10** vary substantially in their substitution patterns, which can have a marked effect on photophysical properties. Photophysical data are provided in Table 3 and show that these salts vary in their absorption maxima from Uv-B (**3**,**4**) to the Uv-A region (**5**-**10**) and significant absorption into violet/blue spectrum (**8**, **9**)(see supplementary information for full spectra). As observed previously[2b], biaryl pyridinium salts display large Stokes shifts (>100 nm for **4**, **6**, and >140 nm for **7** and **8**). Notably, **9** displays a comparatively modest Stokes shift; likely due to the steric hindrance of the bulky 5-dimethylamino-naphthyl group causing hindered rotation about the biaryl bond.

Table 3. Photophysical properties of pyridinium salts **3-10**.[a]

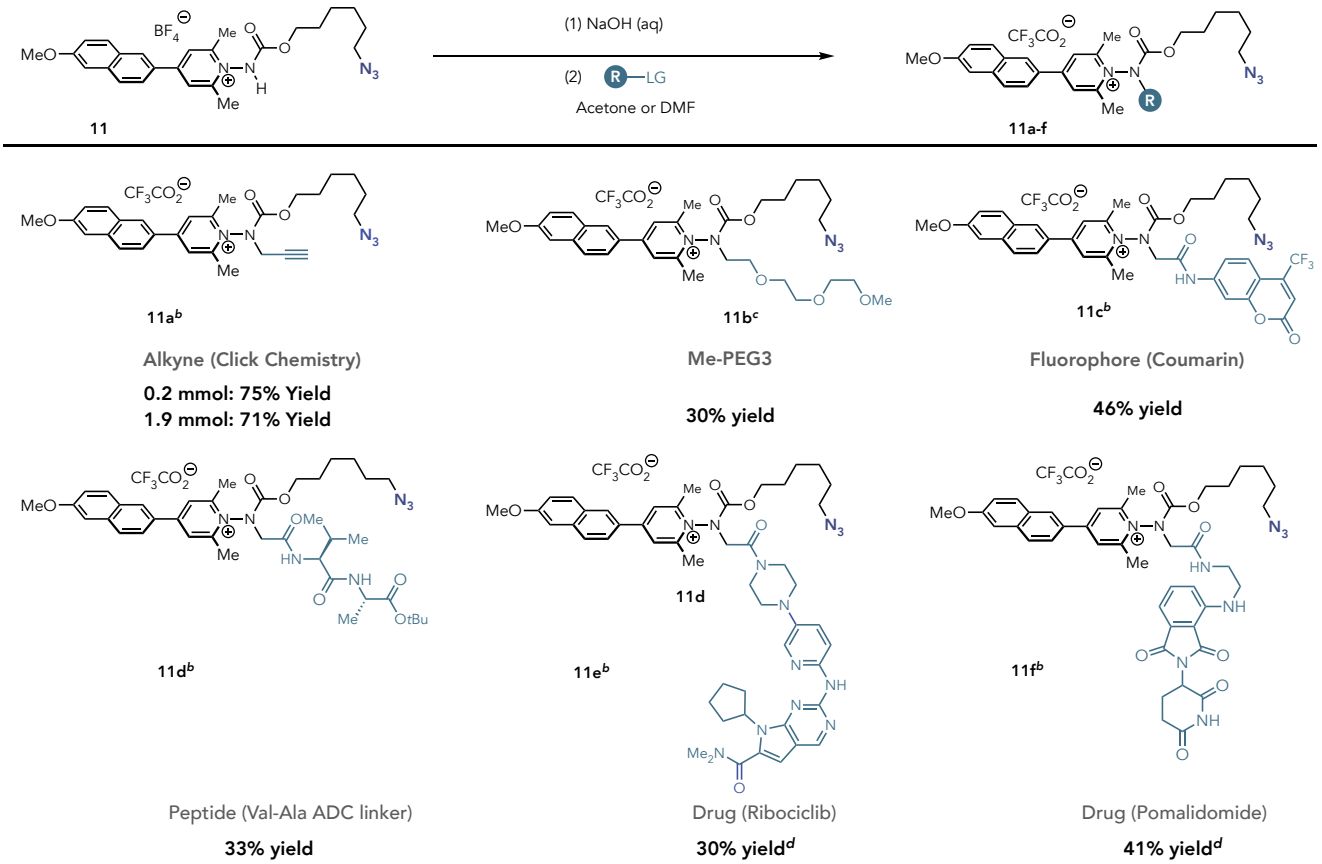
Compound	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Stokes shift (nm)	ε (Lmol^{-1}cm^{-1})
3	268	409	141	$3.4x10^4$
4	273	393	120	$1.0 x10^5$
5	350	441	91	$2.0x10^4$
6	345	449	104	$1.4x10^4$
7	362	504	142	$6.9x10^3$
8	376	523	147	$3.6x10^4$
9	387	447	60	$6.3x10^3$
10	321	386	65	$2.0x10^4$

[a]Data acquired in 20 mM NH_4OAc (pH 6.9) buffer.

Encouraged by the general nature of the alkylation procedure, we then sought to apply this approach to generate hetero-trifunctional pyridinium salts. Reagents of this class are appreciated as they provide platforms in which several different functionalities/capabilities can be incorporated into peptides and proteins in a single reaction and have found application in assembling theranostic-type compounds[12]. To achieve this, we selected heterobifunctional ylide precursor **11** as a suitable substrate (Scheme 3). **11** features two unique chemical warheads: the pyridinium warhead, as well as the azide click handle. Thus, treatment of **11** with NaOH (aq), followed by alkynylation with propargyl bromide yielded hetero-trifunctional pyridinium salt **11a** in 75% isolated yield. We then performed this reaction on a one gram scale (1.9 mmol); yielding **11a** in 71% yield. This result hints at the potential to perform larger scale probe synthesis. Encouraged by these results, we next sought to alkylate **11** with complex electrophiles to create highly bespoke pyridinium probes. Thus, alkylation of **11** with Me-PEG-3-Br in DMF and with 3 eq. of potassium iodide yielded Pegylated **11b** in a useful 30% yield. Our data in Scheme 2 clearly indicate that α-halo-acetates and acetamides are competent electrophiles for this process. A key advantage of these electrophiles is the ease with which they can be incorporated into many types of structures in a single step from simple α-haloacetyl halides and an appropriate amine or alcohol nucleophile. Using this simple approach, we prepared an α-bromoacetylated coumarin analog SI**29**. Treatment of **11** with **SI29** resulted in highly substituted **11c** in 46% isolated yield. We then extended this demonstration to peptides, showing that ADC linker Val-Ala[13] could readily be incorporated into the **11** scaffold in 33% isolated yield. Next, we synthesized α-bromoacetylated analog (**SI27**) of kinase inhibitor Ribociclib[14]. Treatment of **11** with **SI27** enabled access to **11e** in 30% yield. Finally, α-bromoacetylated pomalidomide analog **SI38** reacted smoothly with ylide **11** to yield **11f** in 40% isolated yield. Whilst each of the **11** analogs was purified by reverse phase chromatography, analogs **11e-f** each possessed identical retention times to **11**. Thus, to separate alkylated products from unreacted **11**, we quenched the alkylation reactions with benzyl bromide, which converted unreacted ylide to compound **2d**; which has a sufficiently different polarity to enable facile purification by reverse phase chromatography.

In summary, we have developed a pyridinium ylide alkylation approach that enables the assemblage of functionally diverse and, in

Scheme 3. Synthesis of heterotrifunctional pyridinium salts[a]



[a]Yields are averages of two experiments. [b] acetone used as solvent. [c]DMF used as solvent. [d]Reactions were quenched with excess benzyl bromide.

some cases, highly complex structures pyridinium salts. We anticipate that this method will enable the synthesis of a wide array of radical precursors that can be applied to both small molecule synthesis as well as protein ligation.

ASSOCIATED CONTENT

Supporting Information

Experimental data underpinning this study is available in this manuscript and the supporting information.

The Supporting Information is available free of charge on the ACS Publications website.
General considerations, experimental procedures, photophysical data, NMR spectra (pdf).

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Author Contributions

AMS: designed, performed, and analyzed experiments, contributed to writing the manuscript and associated documents. BG: designed, performed, and analyzed experiments. MTT: conceived the project, designed and analyzed experiments, contributed to writing the manuscript and associated documents.

Notes

The authors declare no competing financial interest.

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