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Chemical Tagging of Bioactive Amides by Cooperative Catalysis: Applications in the Syntheses of Drug Conjugates

Cunyuan Zhao,[†] Yuankai Wang,[†] Quan Pham, Changhang Dai, Abhishek Chatterjee, and Masayuki Wasa*

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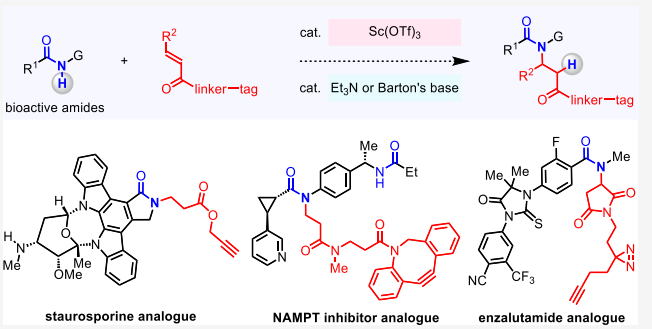
ABSTRACT: We disclose a practical catalytic method for arming bioactive amide-based natural products and other small-molecule drugs with various functional handles for the synthesis of drug conjugates. We demonstrate that a set of readily available Sc-based Lewis acids and N-based Brønsted bases can function cooperatively to deprotonate amide N−H bonds in polyfunctional drug molecules. An aza-Michael reaction between the resulting amidate and α,β-unsaturated compounds produces an array of drug analogues that are equipped with an alkyne, azide, maleimide, tetrazine, or diazirine moiety under redox and pH-neutral conditions. The utility of this chemical tagging strategy is showcased through the production of drug conjugates by the click reaction between the alkyne-tagged drug derivatives and an azide-containing green fluorescent protein, nanobody, or antibody.



1. INTRODUCTION

The late-stage functionalization of bioactive organic molecules permits facile access to drug analogues while omitting the need for potentially cumbersome total syntheses.[1−23] Protocols for the chemical tagging of natural products and pharmaceuticals are central to drug discovery as they expand the scope of small-molecule drugs that can be linked to biomolecules or other therapeutic reagents.[21−57] Processes of particular importance are those that incorporate chemical handles for azide−alkyne cycloaddition,[21−23,38−48,54−61] thiol-Michael addition (e.g., maleimide),[21,22,49,62−66] hetero Diels−Alder reaction (e.g., tetrazine),[47,50,67−70] and photoaffinity labels for chemoproteomics (e.g., arylazide and diazirine).[51,52,71−78] Functionalized derivatives generated by these transformations have been utilized to access drug conjugates (e.g., antibody−drug conjugates and bifunctional drugs)[21−33,79−86] or probes for mechanism-of-action and structure−activity-relationship studies.[76−78,87,88] However, the state-of-the-art tagging methods often involve chemoselective modifications of a native functional group within a drug molecule (e.g., alcohol, amine, and carboxylic acid) and are still limited in the scope of available arming sites.[21−57] Thus, strategies for the late-stage functionalization of ubiquitous, and otherwise unreactive, moieties within a bioactive entity offer highly efficient methods for the generation of new analogues.[1−23] Such approaches increase the range of accessible positions for the installation of chemical tags and enhance the possibility of identifying derivatives that exhibit desirable pharmacological properties.[54,87,88]

Secondary amide-based molecules are prevalent in pharmaceuticals (e.g., enzalutamide **1a**, NAMPT inhibitor **1b**, and staurosporine **1c**; Figure 1A).[89−94] To generate a bifunctional drug through the tethering of **1a** with another small-molecule drug, or to convert **1b** into an antibody−drug conjugate, the analogues of **1a** and **1b** equipped with a handle for the click reaction (**2a**) or thiol-Michael addition (**2b**) have been prepared through multistep synthesis.[95,96] In another study, **1c** was transformed into a probe for chemical proteomics (diazirine/alkyne-equipped **2c**) through the acylation of a secondary amine unit within **1c**.[73] Complementary to these chemical tagging strategies, we envisioned the development of a practical method for chemo- and site-selective N−H bond functionalization of a secondary amide unit within **1** with alkene-based tagging agents **3** (Figure 1B). Using a readily accessible catalyst system, such a process would produce novel tertiary amide analogues **4** possessing a wide variety of bioorthogonal handles in a single step. Another practical advantage of the N-alkylation strategy is that Michael acceptors that contain a plethora of "clickable" handles or photoaffinity labels are commercially available (due to the widespread use of thiol-Michael additions in bioconjugation) and can also be

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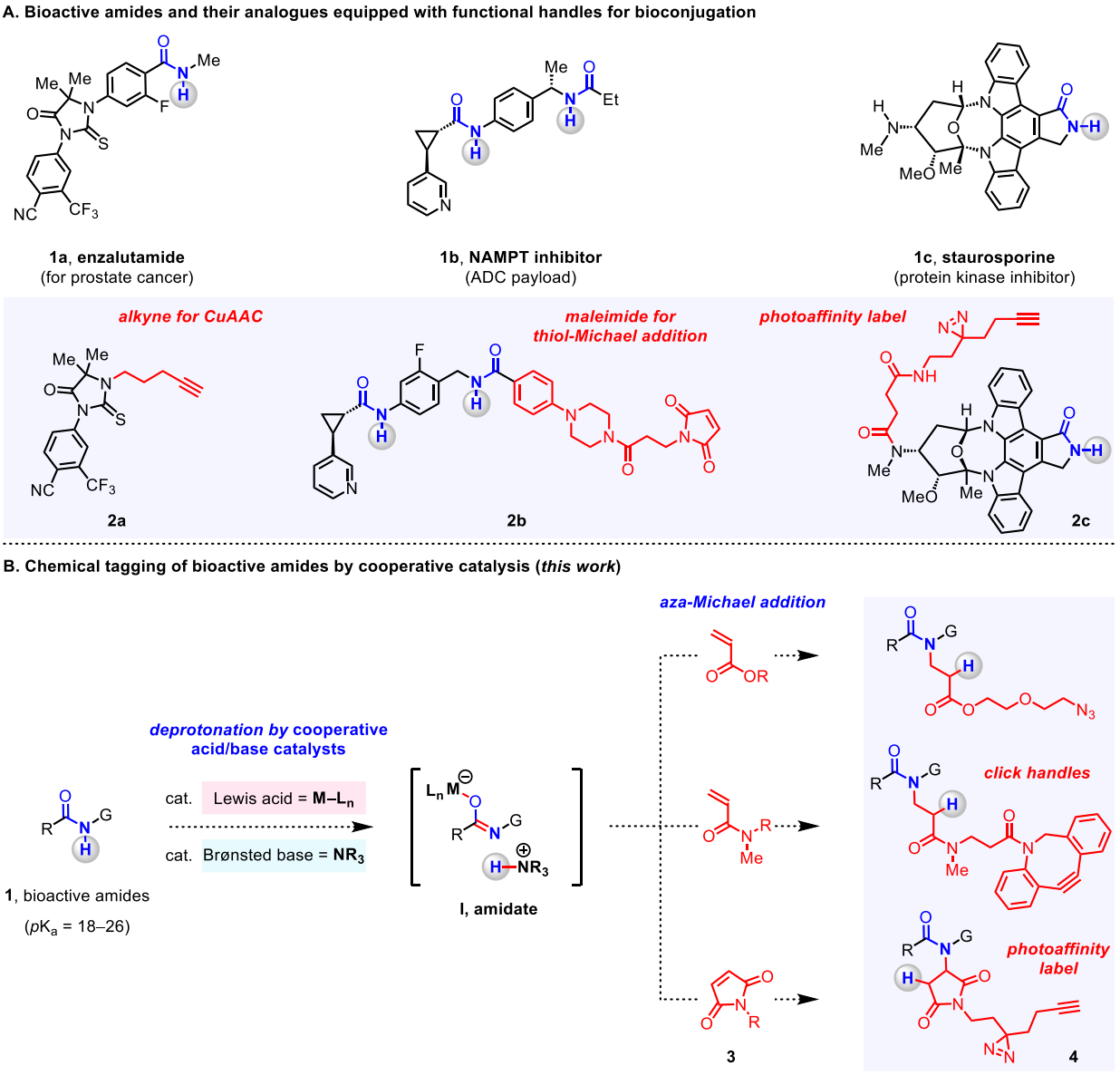

Figure 1. Strategies for chemical tagging of bioactive amides.

easily prepared.[62−66] However, relative to the more nucleophilic functional groups utilized for chemical tagging (e.g., amines, alcohols, and thiols),[21−28,62−66] amides have seldom been used as arming sites,[39,43,97] and many bioactive amides lack any apparent site for functionalization (e.g., **1a**−**1b**, Figure 1A). Breaking an amide N−H bond, which plays important roles as a H-bond donor,[90−94] is difficult (pK_a = 18−26 in DMSO,[98,99] BDE = >100 kcal/mol),[100,101] often demanding the use of strong bases,[102−110] oxidants,[111−116] or photoirradiation.[117−120] Thus, developing a catalyst system which can facilitate the efficient union of bioactive amides and tagging agents possessing a variety of base-, oxidant-, and/or photosensitive functional groups stands as a major challenge.

To arm amide-based small-molecule drugs under pH- and redox-neutral conditions, we sought to develop a cooperative Lewis acid/Brønsted base catalyst system (Figure 1B).[121−139] Cooperative catalysis facilitates the bond formation between nucleophiles and electrophiles that are generated in situ from otherwise unreactive substrates; such dual activation allows for various catalytic processes to occur under mild reaction conditions and often in a functional-group-tolerant manner.[121−143] Thus, we hypothesized that a Lewis acid could delocalize the electron density of an amide moiety, rendering its N−H bond more acidic for deprotonation by a Brønsted base. This collaborative operation would afford an amidate (**1** → **I**), and its addition to a Lewis acid-activated α,β-unsaturated compound (**3**) would produce an analogue equipped with a variety of functional handles (**I** → **4**).

A significant yet unresolved problem in cooperative catalysis is the formation of tightly associated acid−base adducts which can engender catalyst deactivation.[121−143] One strategy for circumventing such acid−base complexation is to avoid combinations of catalysts, pronucleophiles, and electrophiles that have a strong affinity for each other (i.e., hard−hard or soft−soft pairing).[135−139,142,143] However, such approaches are inherently limited in scope. Specifically, while cooperative catalysis has been used to promote the union of carbonyl pronucleophiles and electrophiles loaded with a click handle (e.g., alkyne[144,145] and azide[146]), such processes have been confined to α-functionalization of readily enolizable alde-

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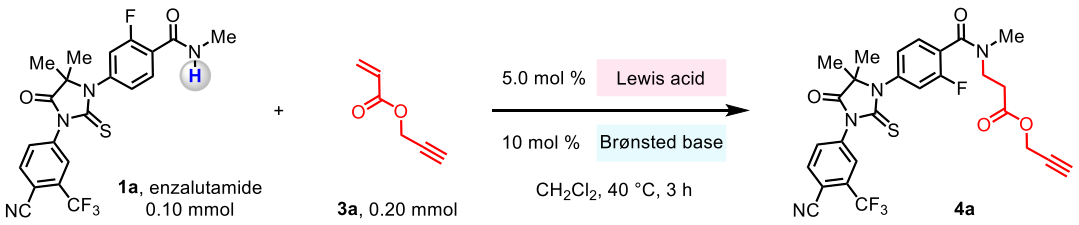
Table 1. Chemical Tagging of Enzalutamide[a,b,c]



entry	Lewis acid	Brønsted base	yield of **6a** (%)
1	none	Et_3N	<3
2	none	DBU	<3
3	none	KO*t*-Bu	<3
4	$Sc(OTf)_3$	Et_3N	92
5	$Sc(OTf)_3$	PMP	70
6	$Sc(OTf)_3$	DBU	53
7	$Sc(OTf)_3$	Barton's base	<3
8	$Sc(OTf)_3$	none	<3
9	$B(C_6F_5)_3$	Et_3N	<3
10	$Mg(OTf)_2$	Et_3N	<3
11	$Cu(OTf)_2$	Et_3N	<3
12	$Zn(OTf)_2$	Et_3N	<3

[a]Conditions: enzalutamide (**1a**, 0.10 mmol), propargyl acrylate (**3a**, 0.20 mmol), Lewis acid (5.0 mol %), Brønsted base (10 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 3 h. See the Supporting Information for details. [b]Yield values were determined by the ^1H NMR analysis of unpurified reaction mixtures with mesitylene as the internal standard. [c]DBU, PMP, and Barton's base denotes 1,8-diazabicyclo[5.4.0]undec-7-ene, 1,2,2,6,6-pentamethylpiperidine, and 2-*tert*-butyl-1,1,3,3-tetramethylguanidine, respectively.

hydes[145] and ketones[146] that do not possess other highly Lewis acid- and/or base-sensitive functional groups. Consequently, processes during which a polyfunctional secondary amide-based drug must be converted to a nucleophilic amidate through the selective deprotonation of a N−H bond (pK_a = 18−26; e.g., Figure 1A),[98,99] which then reacts with an electrophile armed with a wide variety of bio-orthogonal handles (Figure 1B), are not within the purview of the state of the art. Herein, we report that a catalyst system consisting of a hard Lewis acid and a sterically encumbered N-based Brønsted base can promote the union between various bioactive amides and tagging agents while addressing the mutual quenching issue that arises with cooperative catalysis. Furthermore, we demonstrate that adopting a strategy involving independent, unbound catalytic fragments can ameliorate the optimization processes for catalyst candidates, as opposed to the tedious and costly tethering of catalytic units required for bifunctional catalysts.[127−134]

2. RESULTS AND DISCUSSION

2.1. Method Development. *2.1.1. Identification of Optimal Conditions.* We set out to introduce a catalyst system which promotes the union of enzalutamide **1a** (0.10 mmol) and propargyl acrylate **3a** (0.20 mmol), generating **4a** (Table 1). To begin, we investigated if the reaction of **1a** and **3a** can be catalyzed solely by 10 mol % of Et_3N, DBU,[108] or KO*t*-Bu[104] to find that none of these Brønsted bases give **4a** in more than a 3% yield (entries 1−3). We then evaluated Lewis acids that could selectively activate the carbonyl units within **1a** and **3a** and are able to operate in concert with Brønsted bases to facilitate the aza-Michael reaction. We began by testing combinations of $Sc(OTf)_3$ and various Brønsted bases (entries 4−7). $Sc(OTf)_3$, a strong and hard Lewis acid,[147−150] has a unique affinity to preferentially activate a hard Lewis base, such as an oxygen atom of a carbonyl unit, within peptides[149,150] and other polyfunctional molecules possessing soft Lewis basic moieties such as nitriles,[151,152] alkynes,[153,154] and various heterocycles.[151,155] Moreover, cooperative catalyst systems comprising $Sc(OTf)_3$ and Lewis bases promote a range of C−C and C−heteroatom bond-forming processes.[145,156−162] Notably, however, such catalyst systems have not been extended to the development of C−N bond-forming reactions involving amide-based pronucleophiles.[163−165]

We discovered that a combination of $Sc(OTf)_3$ (5.0 mol %) and Et_3N (10 mol %) facilitates efficient deprotonation of **1a** and the ensuing addition of **1a**-derived amidate onto **3a**, giving **4a** in a 92% yield (entry 4). Such a process can be performed using Schlenk techniques, with **1a**, $Sc(OTf)_3$ (>98% purity), Et_3N (99.5% purity), and CH_2Cl_2 (anhydrous, >99.8% purity) purchased from commercial sources without further purification and freshly distilled propargyl acrylate **3a** (see the Supporting Information for further details). The tagging of **1a** was less efficient when more hindered PMP, more Lewis basic DBU, or Barton's base, were paired with $Sc(OTf)_3$ (70−<3% yield, entries 5−7). With only $Sc(OTf)_3$, **4a** was not generated (entry 8). Then, we tested the sets of different Lewis acids and Et_3N (entries 9−12). A "frustrated" Lewis pair comprising 5.0 mol % $B(C_6F_5)_3$ and 10 mol % Et_3N, which can deprotonate ketone- and ester-based pronucleophiles (pK_a = 20−

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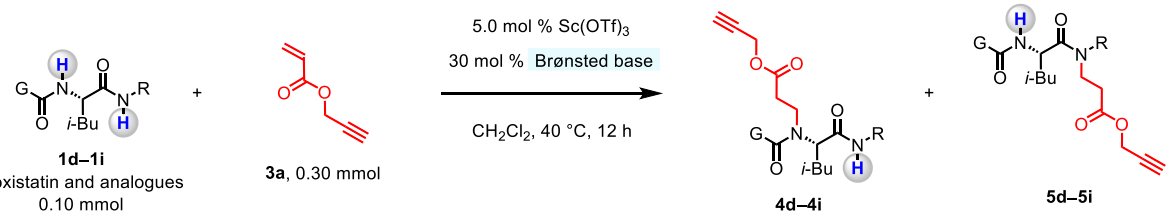
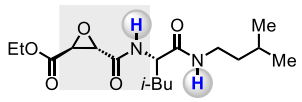
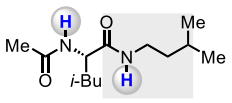
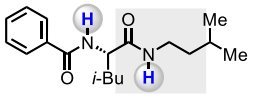
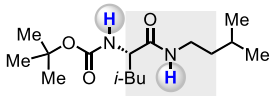
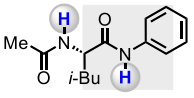
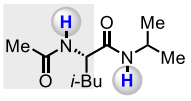
Table 2. Chemical Tagging of Aloxistatin and Analogues[a,b,c]



entry	1d–1i	Brønsted base	yield of 4d–4i (%)	yield of 5d–5i (%)
1	 **1d**, aloxistatin (cysteine protease inhibitor)	Et_3N	<3	<3
2	**1d**	DBU	<3	<3
3	**1d**	Barton's base	20	7
4	 **1e**	Barton's base	16	72
5	 **1f**	Barton's base	<3	64
6	 **1g**	Barton's base	<3	35
7	 **1h**	Barton's base	<3	88
8	 **1i**	Barton's base	70	<3

[a]Conditions: diamide (**1**, 0.10 mmol), propargyl acrylate (**3a**, 0.30 mmol), $Sc(OTf)_3$ (5.0 mol %), Brønsted base (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C. See the Supporting Information for details. [b]Yield values were determined by the ^1H NMR analysis of unpurified reaction mixtures with mesitylene as the internal standard. [c]DBU and Barton's base denote 1,8-diazabicyclo[5.4.0]undec-7-ene and 2-*tert*-butyl-1,1,3,3-tetramethylguanidine, respectively.

25),[135−139] failed to produce **4a** (entry 9). Moreover, the combinations of $Mg(OTf)_2$, $Cu(OTf)_2$, or $Zn(OTf)_2$ with Et_3N gave **4a** in <3% yield (entries 10−12; see the Supporting Information for details). These findings support the notion that $Sc(OTf)_3$ in combination with electron-rich *N*-alkylamines constitutes a uniquely potent and functional group-tolerant catalyst system for promoting the chemical tagging of **1a**.

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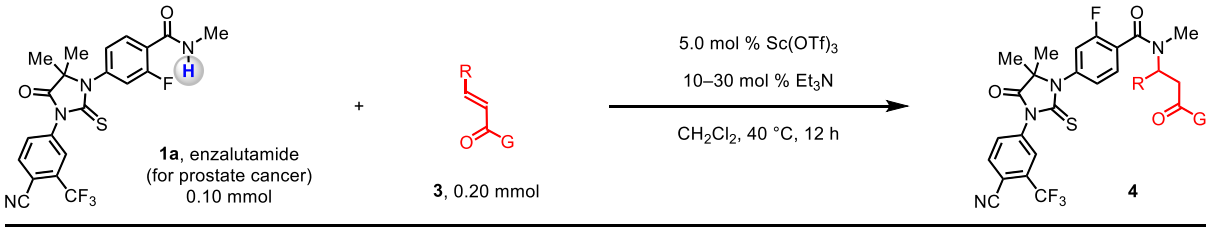
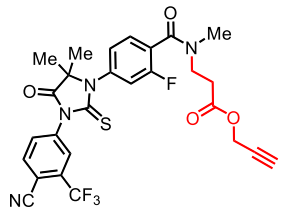
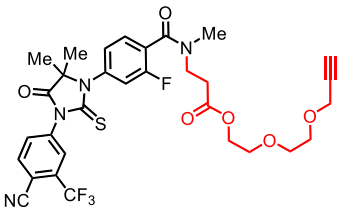
Table 3. Arming of Enzalutamide with Various Chemical Tags[a,b]

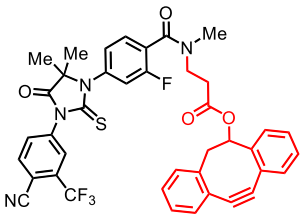




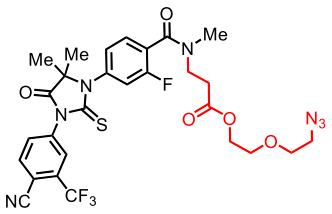
4a, 92% yield[c]



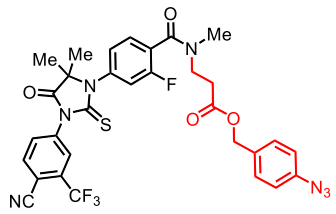
4j, 95% yield



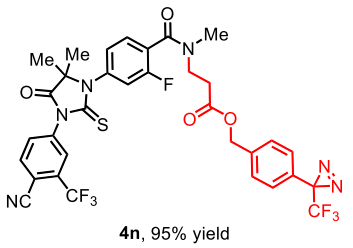
4k, 90% yield



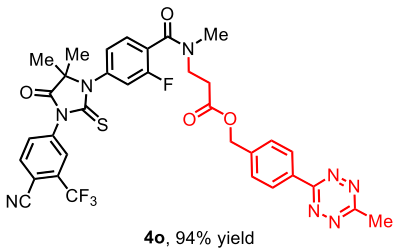
4l, 70% yield[d]



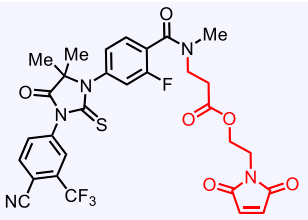
4m, 95% yield



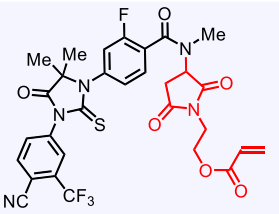
4n, 95% yield



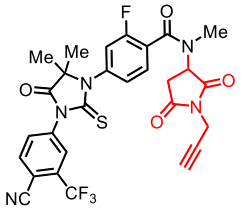
4o, 94% yield



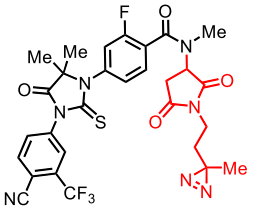
4p, 51% yield[e]



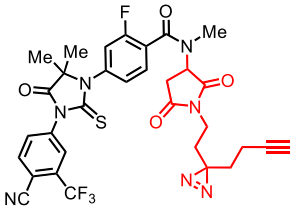
6p, 22% yield[e]



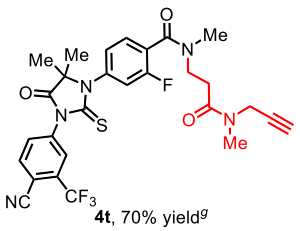
4q, 83% yield[f]



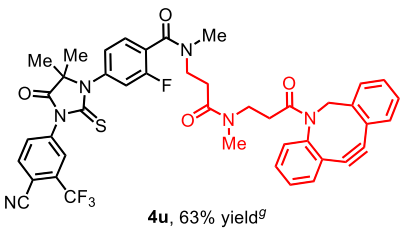
4r, 92% yield[f]



4s, 95% yield[f]



4t, 70% yield[g]



4u, 63% yield[g]

[a]Conditions: enzalutamide (**1a**, 0.10 mmol), α,β-unsaturated compound (**3**, 0.20 mmol), $Sc(OTf)_3$ (5.0 mol %), Et_3N (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 12 h. [b]Yield of isolated and purified products. See the Supporting Information for details. [c]The solution was allowed to stir for 3 h using 10 mol % Et_3N for the production of **4a**. [d]For the preparation of **4l**, 30 mol % Barton's base was used. [e]To synthesize **4p** and **6p**, 10 mol % Et_3N was used. [f]**4q**, **4r**, and **4s** were furnished using 10 mol % $Sc(OTf)_3$ and 1.0 equiv Et_3N. [g]For the production of **4t** and **4u**, 20 mol % Et_3N was used. See the Supporting Information for details.

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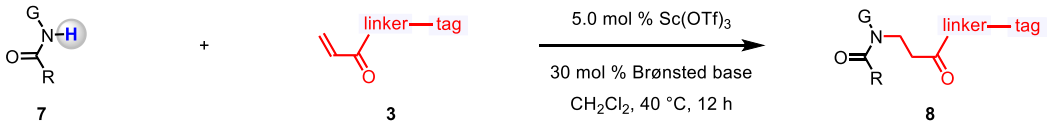
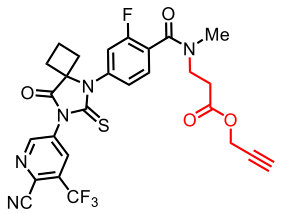
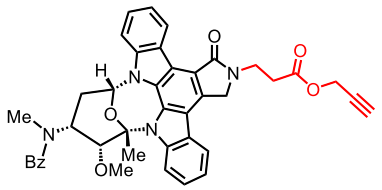
Table 4. Arming of Bioactive Amides by Aza-Michael Addition[a,b]

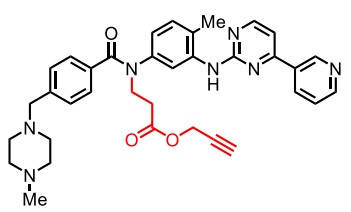




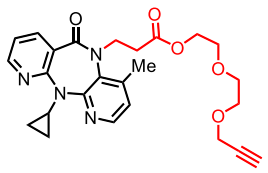
8a, 94% yield
from **apalutamide**
(for prostate cancer)



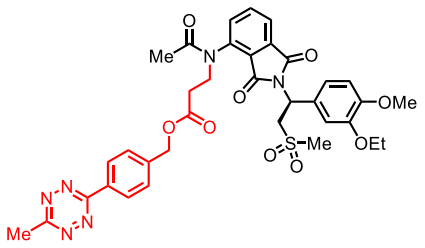
8b, 56% yield[c]
from **midostaurin**
(protein kinase inhibitor)



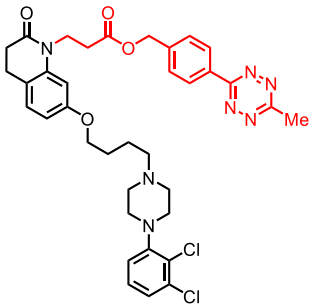
8c, 80% yield[d]
from **imatinib**
(for leukemia)



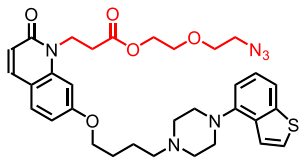
8d, 95% yield
from **nevirapine**
(for HIV/AIDS)



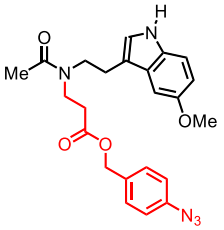
8e, 46% yield
from **apremilast**
(for psoriasis)



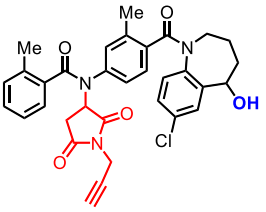
8f, 94% yield[c]
from **aripiprazole**
(for partial-onset seizures)



8g, 81% yield[c]
from **brexpiprazole**
(for schizophrenia)



8h, 50% yield[c]
from **melatonin**
(for insomnia)



8i, 43% yield[e]
from **tolvaptan**
(for hyponatremia)

[a]Conditions: amide (**7**, 0.10 mmol), α,β-unsaturated compound (**3**, 0.20−0.30 mmol), $Sc(OTf)_3$ (5.0 mol %), Et_3N (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 12 h. [b]Yield of isolated and purified products. See the Supporting Information for details. [c]For the preparations of **8b**, **8f**, **8g**, and **8h**, 30 mol % Barton's base was used. [d]To access **8c**, 10 mol % $Sc(OTf)_3$ and 50 mol % Barton's base were used. [e]To synthesize **8i**, 10 mol % $Sc(OTf)_3$ and 1.0 equiv Et_3N were used. See the Supporting Information for details.

Next, we examined if the conditions optimized for arming enzalutamide **1a** (Table 1) can be applied for efficient and site-selective N-functionalization of a diamide **1d** (aloxistatin, Table 2). However, the catalyst combination of $Sc(OTf)_3$ (5.0 mol %) and Et_3N (30 mol %) did not promote the addition of **1d** to **3a** (in CH_2Cl_2, 40 °C, 12 h; entry 1). We reasoned that the two secondary amide units of **1d** are less acidic in comparison to the aryl-substituted amide moiety of **1a** and that a stronger Brønsted base might be necessary for their deprotonation. While the use of $Sc(OTf)_3$ and DBU gave no desired product (entry 2), with the more potent Barton base, **4d** and **5d** were obtained in 20 and 7% yields, respectively (entry 3). A control experiment performed with only Barton's base (30 mol %) gave no desired product (see the Supporting Information for details). These findings support the notion that the highly Lewis acidic $Sc(OTf)_3$ together with a sterically demanding and electron-rich N-based Brønsted base represents the most effective combination for promoting the aza-Michael reaction. Nonetheless, as we thought to extend the arming strategy to various bioactive entities that consist of two or more secondary amide units (e.g., **1d**), the ability to predict which type of amide would undergo more efficient N-functionalization became crucial.

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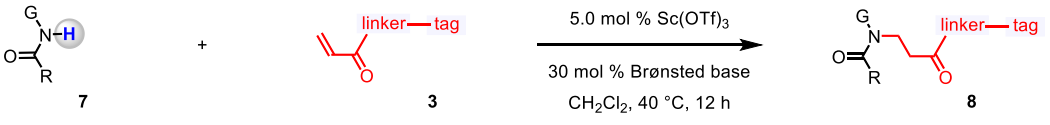
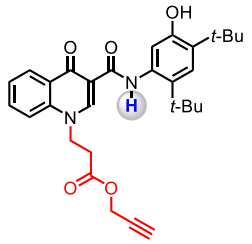
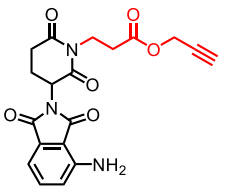
Table 5. Arming of Medicinal Agents That Contain Different Acidic Functional Groups[a,b]

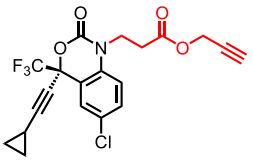




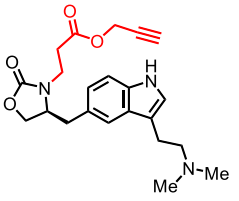
8j, 85% yield[c]
from **ivacaftor**
(for cystic fibrosis)



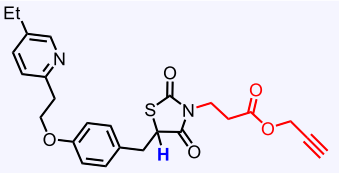
8k, 56% yield
from **pomalidomide**
(for myeloma)



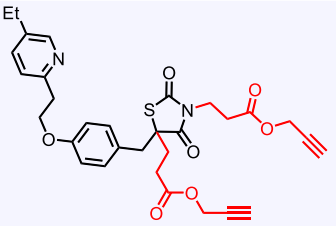
8l, 99% yield
from **efavirenz**
(for HIV/AIDS)



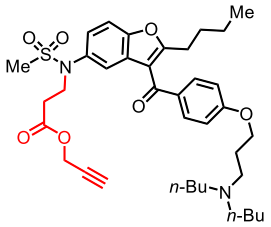
8m, 50% yield[c]
from **zolmitriptan**
(for migraine)



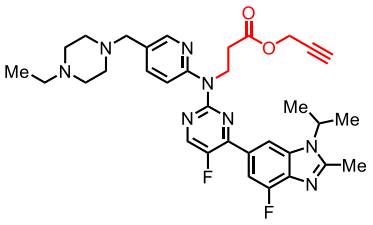
8n, 45% yield
from **pioglitazone**
(for type 2 diabetes)



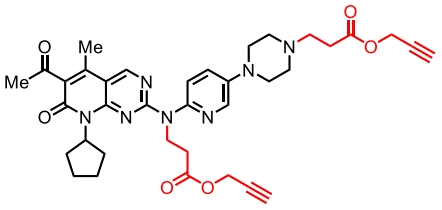
9n, 22% yield



8o, 77% yield[c]
from **dronedarone**
(for arrhythmias)



8p, 38% yield[d]
from **abemaciclib**
(for breast cancer)



8q, 75% yield[c]
from **palbociclib**
(for breast cancer)

[a]Conditions: amide (**7**, 0.10 mmol), α,β-unsaturated compound (**3**, 0.20−0.30 mmol), $Sc(OTf)_3$ (5.0 mol %), Et_3N (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 12 h. [b]Yield of isolated and purified products. See the Supporting Information for details. [c]For the preparations of **8j**, **8m**, **8o**, and **8q**, 30 mol % Barton's base was used. [d]To access **8p**, 20 mol % $Sc(OTf)_3$ and 1.0 equiv Barton's base were used. See the Supporting Information for details.

2.1.2. Factors That Influence Site Selectivity. In order to establish a preliminary model for predicting the site selectivity of aza-Michael reactions, we reacted a series of aloxistatin analogues possessing sterically and electronically disparate amide and/or carbamate units (**1e**−**1i**, entries 4−8, Table 2) with propargyl acrylate **3a** in the presence of $Sc(OTf)_3$ and Barton's base. To begin, we investigated the impact of the changing epoxypropionic amide moiety within **1d** into sterically and/or electronically different groups by comparing the reactivity of **1d**, **1e**, **1f**, and **1g** (entries 3−6). The N-alkylation of aloxistatin **1d** (entry 3) occurs more selectively at its epoxypropionic amide (**4d**, 20% yield) than at the *N*-isopentyl amide unit (**5d**, 7% yield). In contrast, the *N*-isopentyl amide moiety within **1e** was found to react efficiently with **3a** (vs N-alkylation at its acetamide unit), giving **4e** and **5e** in 16 and 72% yields, respectively (entry 4). With benzamide- or N-Boc-substituted analogues (**1f**, **1g**), N-alkylation only took place at its *N*-isopentyl amide unit, furnishing **5f** (64% yield, entry 5) or **5g** (35% yield, entry 6). Tagging of **1h**, which consists of *N*-phenylamide (pK_a = ca. 20) and acetamide (pK_a = ca. 25) units, was found to proceed exclusively at the more acidic *N*-phenylamide, giving **5h** in an 88% yield (entry 7). As for the union of **1i** and **3a**, the sterically encumbered *N*-isopropyl amide unit of **1i** remained intact, giving **4i** as the only product (70% yield, entry 8). Thus, these results indicate that $(TfO)_3Sc$/Barton's base-catalyzed tagging of a given diamide occurs selectively at an amide which is more sterically accessible (e.g., **1i**) and/or at a unit substituted to a *N*-aryl group (e.g., **1h**). Furthermore, more selective formation of **4d** over **5d** from aloxistatin **1d** is likely facilitated by bis-chelation of $Sc(OTf)_3$ with epoxypropionic amide of **1d**[166,167] (vs N-alkylation proceeding through Lewis acid activation of the *N*-isopentyl amide moiety within **1e**−**1g** that lack the epoxide moiety).

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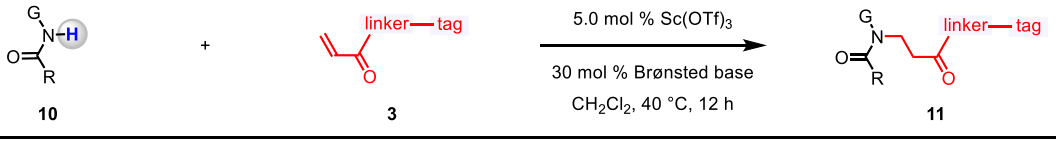
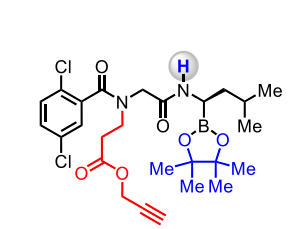
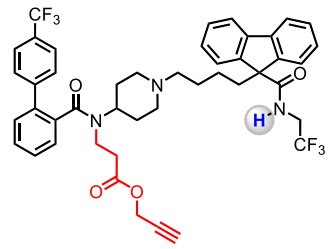
Table 6. Arming of Bioactive Diamides and Tetrapeptide with Different Chemical Tags[a,b]

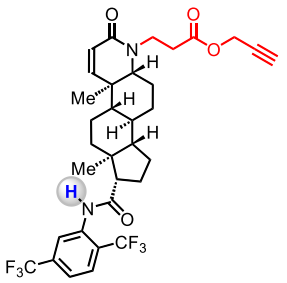




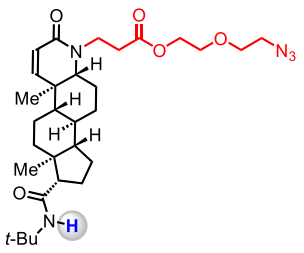
11a, 65% yield[c]
from **ixazomib**
(for myeloma)



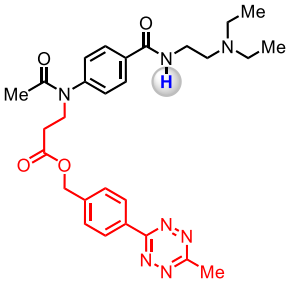
11b, 75% yield[d]
from **lomitapide**
(for hypercholesterolemia)



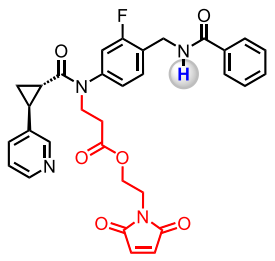
11c, 80% yield[d]
from **dutasteride**
(for prostatic hyperplasia)



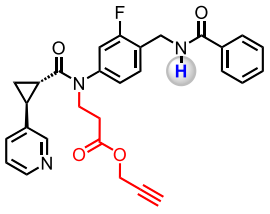
11d, 80% yield[d]
from **finasteride**
(for prostatic hyperplasia)



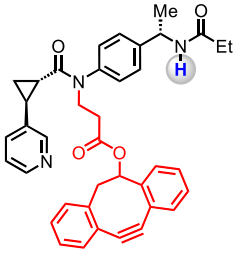
11e, 91% yield
from **acecainide**
(for arrhythmias)



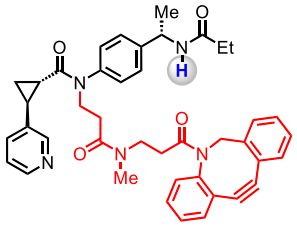
11f, 55% yield
from **NAMPT-inhibitor**
(cytotoxic)



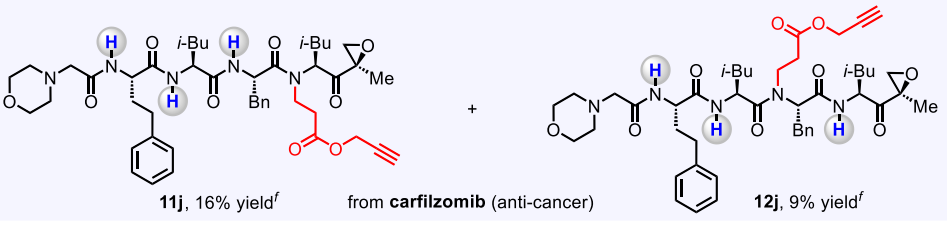
11g, 70% yield
from **NAMPT-inhibitor**



11h, 38% yield
from **NAMPT-inhibitor**



11i, 81% yield[e]
from **NAMPT-inhibitor**



11j, 16% yield[f] from **carfilzomib** (anti-cancer) **12j**, 9% yield[f]

[a]Conditions: amide (**10**, 0.10 mmol), α,β-unsaturated compound (**3**, 0.10−0.30 mmol), $Sc(OTf)_3$ (5.0 mol %), Et_3N (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 12 h. [b]Yield of isolated and purified products. See the Supporting Information for details. [c]For the preparation of **11a**, 1.0 equiv Et_3N was used. [d]The syntheses of **11b**, **11c**, and **11d** used 30 mol % Barton's base. [e]To access **11i**, 20 mol % Et_3N was used. [f]To synthesize **11j** and **12j**, 10 mol % $Sc(OTf)_3$ and 50 mol % Barton's base were used. See the Supporting Information for details.

2.1.3. Scope. $Sc(OTf)_3/Et_3N$-catalyzed aza-Michael additions of enzalutamide **1a** onto various acrylates (**3**) produce its tertiary amide analogues equipped with "clickable" handles (**4a**, **4j**−**4m**, Table 3), photoaffinity labels (**4m** and **4n**), tetrazine for hetero Diels−Alder reaction (**4o**), and alkenes for conjugate addition (**4p** and **6p**). Derivatives of **1a** that are tethered by poly(ethylene glycol) linkers to alkyne (**4j**, 95% yield), or azide (**4l**, 70% yield), could be prepared. The dibenzocyclooctynyl group (DIBO) used in strain-promoted azide−alkyne cycloaddition was readily incorporated,[60,61]

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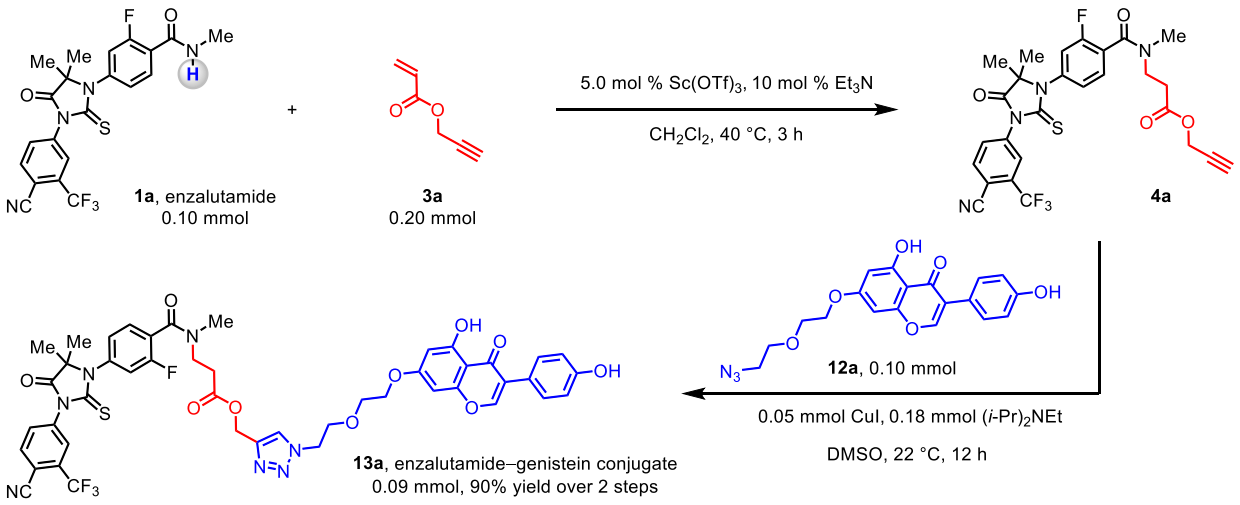

Figure 2. Synthesis of the enzalutamide−genistein conjugate.

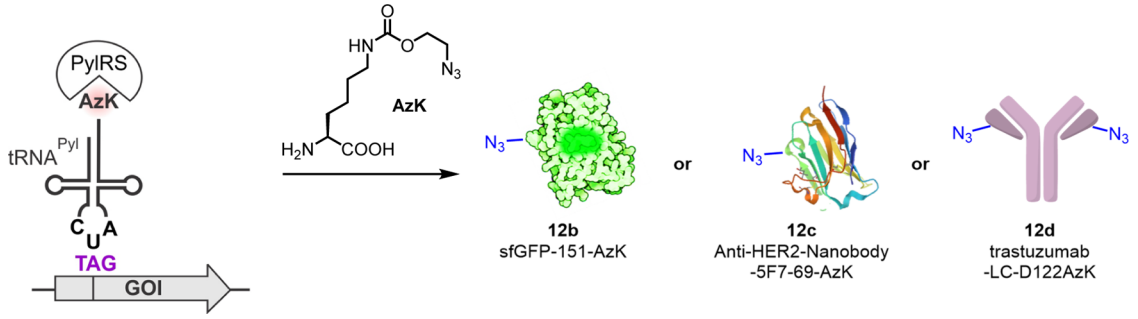

Figure 3. Access to sfGFP-151-AzK, nanobody-5F7-69-AzK, and trastuzumab-LC-D122AzK.

furnishing **4k** (90% yield). Due to the instability of azide-containing acrylates (**3l** and **3m**) in the neat form, they were used as 1.0 M solutions in CH_2Cl_2, giving **4l** and **4m** in 70 and 95% yields, respectively. When **1a** was reacted with an electrophile in which an acrylate is linked to a maleimide unit (**3p**), both alkenes were found to function as appropriate Michael acceptors, thereby giving a mixture of **4p** (51% yield) and **6p** (22% yield). Based on this finding, a series of N-substituted maleimides, as well as *α,β*-unsaturated amides, were tested. Such reactions furnished **1a** derivatives armed with a terminal alkyne (**4q**, **4s**, and **4t**), a diazirine (**4r** and **4s**), and/or a dibenzo-aza-cyclooctyne (DIBAC, **4u**) tag.[60,61] A bifunctional diazirine- and alkyne-containing photoaffinity probe (**3s**), which is used widely in chemoproteomics,[71−74] could also be installed with high efficiency to give **4s** in a 95% yield.

A broad range of bioactive amides, as well as other medicinal agents that contain acidic N−H bonds, could be armed by the Lewis acid/Brønsted base-catalyzed aza-Michael reaction (**7a**−**7q**, Tables 4 and 5; **10a**−**10j**, Table 6). The catalyst combination of $Sc(OTf)_3$ and Et_3N was found to successfully promote the N-alkylation of benzamide- and/or *N*-arylamide-based drugs (e.g., apalutamide **7a**, nevirapine **7d**, apremilast **7e**, aripiprazole **7f**, and tolvaptan **7i**; Table 4). With less acidic benzamide derivatives (e.g., midostaurin **7b** and imatinib **7c**), *α,β*-unsaturated lactam (e.g., brexpiprazole **7g**), and *N*-alkylamide (e.g., melatonin **7h**), $Sc(OTf)_3$ and Barton's base were used (see the Supporting Information for details). Substrates containing a variety of Lewis acid-sensitive moieties, such as pyridine (**1b**, **7a**, **7c**, **7d**, **7n**, **7p**, **7q**, and **10f**), piperazine (**7c**, **7f**, **7g**, **7p**, and **7q**), piperidine (**10b**), benzothiophene (**7g**), indole (**7h** and **7m**), benzofuran (**7o**), secondary alcohol (**7i**), phenol (**7j**), and/or aniline (**7k**), could be tagged efficiently. A range of *α,β*-unsaturated esters or amides could be merged with acyclic and cyclic bioactive amides to afford their tertiary amide derivatives linked to a terminal alkyne (**8a**−**8d**, **8i**−**8n**, **9n**, **8o**−**8q**, **11a**−**11c**, **11g**, **11j**, and **12j**), azide (**8g**, **8h**, and **11d**), tetrazine (**8e**, **8f**, and **11e**), maleimide (**11f**), or strained alkyne (**11h** and **11i**). Notably, with tolvaptan **7i** which contains a secondary alcohol moiety, chemoselective N-alkylation of the amide unit could be achieved (vs oxo-Michael addition involving the alcohol in **7i**). Specifically, the reaction of propargyl maleimide **3q** and tolvaptan **7i** furnished **8i** as the only product in a 43% yield; with propargyl acrylate **3a** as a tagging agent, *O*-TBS protection of **7i** was necessary to obtain its tertiary amide derivative (see the Supporting Information for details).

Furthermore, compounds featuring functional groups with an acidic N−H bond (besides amides) could also be tagged (Table 5). For instance, while a sterically encumbered *N*-arylamide group of ivacaftor **7j** was unreactive, the reaction with **3a** occurred at its 4-quinolone moiety to afford **8j** in an 85% yield. In addition, N−H bond functionalization of piperidine-2,6-dione (**7k**), 1,3-oxazinan-2-one (**7l**), oxazolidin-2-one (**7m**), or thiazolidine-2,4-dione (**7n**) provided **8k**−**8n** and **9n**. The reaction of pioglitazone **7n** with **3a** gave **8n** (45% yield), together with a dialkylation product **9n** (22% yield), which was likely formed by $(TfO)_3Sc/Et_3N$-catalyzed *α*-C−H alkylation of the thiazolidine-2,4-dione moiety. Other acidic functional groups, such as the *N*-arylmethanesulfona-

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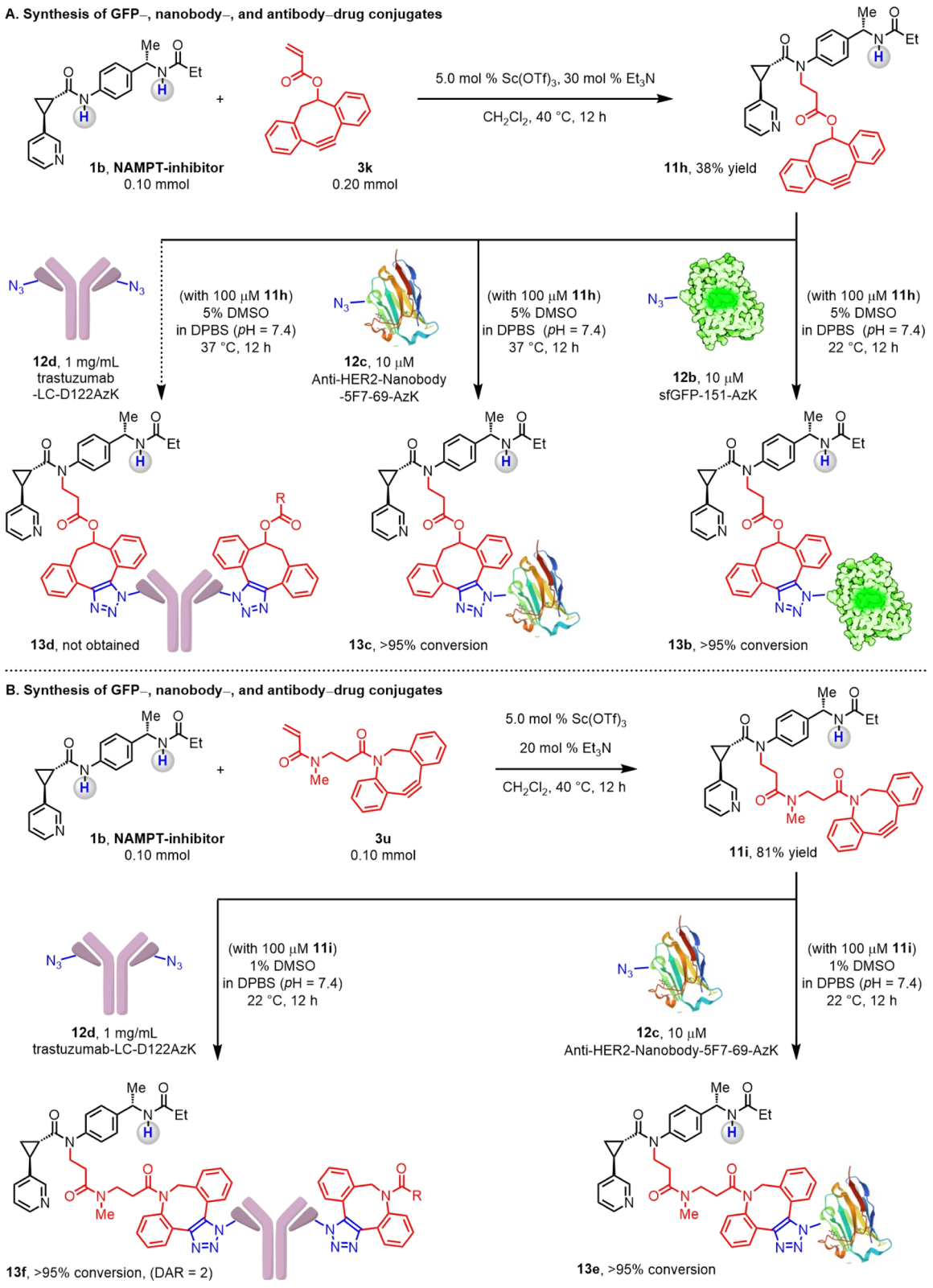

Figure 4. Syntheses of GFP–, nanobody–, and antibody–drug conjugates.

mide unit of dronedarone **7o**, as well as *N*-(pyridin-2-yl)pyrimidin-2-amine group of abemaciclib **7p**, could be tagged using **3a**, giving **8o** and **8p** in 77 and 38% yields, respectively. With palbociclib **7q**, N–H bonds of piperazine and *N*-(pyridin-2-yl)pyrimidin-2-amine moieties underwent efficient N-alkylation to afford **8q** in a 75% yield.

The site-selective chemical tagging of a series of bioactive diamides could be achieved (Table 6). The union of **3a** with diamides, such as ixazomib derivative **10a** and lomitapide **10b**, was found to proceed more efficiently at the more acidic aryl-substituted amides, giving **11a** and **11b** in 65 and 75% yields, respectively. With dutasteride **10c** and finasteride **10d**, their

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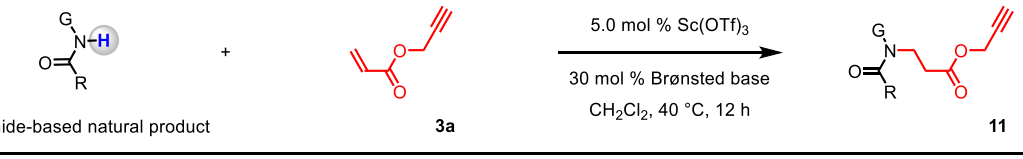
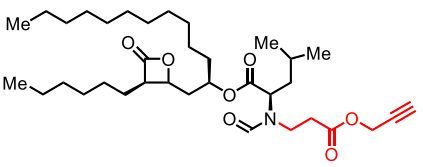
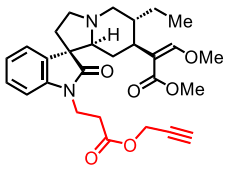
Table 7. Chemical Tagging of Bioactive Natural Products[a,b]

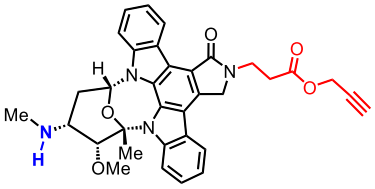




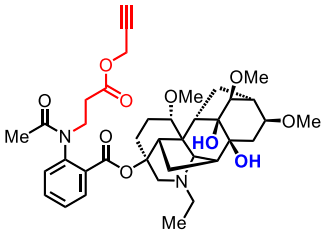
11k, 66% yield[c]
from **orlistat**
(lipase inhibitor)



11l, 65% yield
from **rhynchophylline**
(calcium channel blocker)



11m, 28% yield[d]
from **staurosporine**
(protein kinase inhibitor)



11n, 58% yield[e]
from **lappaconitine**
(sodium channel blocker)

[a]Conditions: amide (**10**, 0.10 mmol), propargyl acrylate (**3a**, 0.30−0.50 mmol), $Sc(OTf)_3$ (5.0 mol %), Et_3N (30 mol %), CH_2Cl_2 (0.5 mL), under N_2, 40 °C, 12 h. [b]Yield of isolated and purified products. [c]For the preparation of **11k**, 30 mol % Barton's base was used. [d]To synthesize **11m**, 30 mol % Barton's base was used, and THF was employed as the solvent. [e]To access **11n**, 10 mol % $Sc(OTf)_3$ and 50 mol % Barton's base were used. See the Supporting Information for details.

lactam N−H bonds were alkylated to afford **11c** (80% yield) or **11d** (80% yield), while the sterically hindered *N*-(2,5-bis(trifluoromethyl)phenyl)amide of **10c**, and *N*-*tert*-butylamide of **10d**, remained intact.

N-Arylamide moieties of acecainide **10e** and NAMPT inhibitor **10f** were tagged selectively (vs *N*-alkylbenzamide units within **10e** and **10f**) to furnish **11e** (91% yield) or **11f** (55% yield). This approach allows for the arming of NAMPT inhibitors **10f** and **1b** with a variety of bioconjugation handles to afford **11f**−**11i** in 38−81% yields (for the utility of these derivatives, see Figure 4). Carfilzomib **10j**, a cytotoxic tetrapeptide epoxyketone and an analogue of epoxomicin,[168] could be tagged with propargyl acrylate **3a**, giving **11j** and **12j** in a 16% yield and a 9% yield, respectively (50% of unreacted **10j** was recovered, and <3% of the dialkylation product was produced. See the Supporting Information for details).

A series of bioactive natural products, such as formamide-based orlistat **10k**, as well as amide-based rhynchophylline **10l**, staurosporine **1c**, and lappaconitine **10n**, could be tagged with **3a** (Table 7). With staurosporine **1c** and lappaconitine **10n** that contain a secondary amine, and two tertiary alcohols, respectively, chemoselective N-alkylation of the amide unit could be achieved to provide **11m** in a 28% yield (70% of unreacted **1c** was recovered) and **11n** in a 58% yield. For the synthesis of **11m** through the reaction of **1c** and **3a**, the use of THF as a solvent was found to be crucial; with less polar CH_2Cl_2, **1c** was hardly soluble, giving **11m** in a 14% yield (for the evaluation data of other solvents and cosolvents, see the Supporting Information). Other more complex secondary amide-based natural products were also reacted with **3a** (e.g., taxol, vancomycin, and geldanamycin); however, these experiments resulted in the full recovery of starting materials or gave an O-alkylation product (see the Supporting Information for details). Further optimization in the catalyst structures is underway to expand the scope of taggable drugs and to improve the chemoselectivity.

2.2. Applications. *2.2.1. Synthesis of a Bifunctional Drug.* The utility of the chemical tagging protocol is highlighted through the concise and efficient syntheses of drug conjugates from enzalutamide (**1a**, Figure 2) and a NAMPT inhibitor (**1b**, Figure 4). As shown in Tables 3 and 6, functional handles for copper-catalyzed azide−alkyne cycloaddition, strain-promoted azide−alkyne cycloaddition, hetero Diels−Alder reaction, or thiol-Michael addition can be readily incorporated onto enzalutamide (**1a** → **4a**, **4j**−**4u**) and NAMPT inhibitors (**1b**, **10f** → **11f**−**11i**). The ability to install such a wide variety of chemical tags provides an opportunity to evaluate/select the most efficient, chemo- and site-selective method for conjugation of the bioactive amides to another small-molecule drug or biomolecules.

Enzalutamide **1a** is a widely prescribed drug for the treatment of prostate cancer.[169,170] Studies aimed at addressing the drug resistance issues of **1a** and/or improving its pharmacological properties have been extensively re-

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ported.[95,169−173] These investigations often involve bifunctional drugs that are accessed by linking the *N*-aryl thiohydantoin component of **1a** with a variety of therapeutic agents (e.g., genistein, taxol, von Hippel-Lindau E3 ligase ligand, pomalidomide, and doxorubicin).[95,169−175] Despite that enzalutamide is readily available from commercial sources, such drug conjugates are seldom prepared through its late-stage functionalization.[176] Our amide N−H bond functionalization approach affords an enzalutamide−genistein conjugate **13a** in a two-step operation from **1a** (Figure 2). $(TfO)_3Sc$/Et_3N-catalyzed chemical tagging of enzalutamide **1a** with propargyl acrylate **3a** affords a tertiary amide derivative **4a**. ICu-catalyzed azide−alkyne cycloaddition of **4a** with genistein (tyrosine kinase inhibitor) linked to an azide (**12a**) gives the bifunctional drug **13a** in a 90% overall yield.

2.2.2. Bioconjugation. Next, we investigated the methods for the sequential tagging of NAMPT inhibitor **1b** and bioconjugation of the derivatives with macromolecules (Figures 3 and 4). **1b** (and its analogue **10f** used to produce **11f** and **11g**; cf., Table 6) were identified by a team at Novartis as lead molecules for the development of a maleimide-substituted ADC payload **2b** (cf., Figure 1A).[96] The ADC was assembled by a thiol-Michael reaction between **2b** and free cysteine residues generated through the reduction of interchain disulfide bonds within a native antibody.

However, heterogeneous products with different drug-to-antibody conjugation ratios (DAR = 2−4) are formed as the number of conjugation sites varies due to the nonselective nature of disulfide reduction.[177−183] Moreover, the loss of disulfide bonds and the retro-Michael reaction can cause stability issues in the ADC in vivo.[184−186] Thus, as a means to assemble various drug conjugates from **10f** and/or **1b** by sequential tagging and bioconjugation, we opted to not use the thiol-Michael addition (i.e., **11f** → ADC, Table 6). Instead, to achieve site-selective and irreversible bioconjugation,[186−188] we decided to merge an engineered azide-containing sfGFP-151-AzK (**12b**), nanobody-5F7-69-AzK (**12c**), or trastuzumab-LC-D122AzK (**12d**), with the NAMPT inhibitor derivatives armed with DIBO (**11h**) or DIBAC (**11i**) by a strain-promoted azide−alkyne cycloaddition (Figures 3 and 4). These proteins were recombinantly expressed either in *E. coli* (sfGFP and nanobody) or Expi293 mammalian suspension culture (trastuzumab), and the unnatural amino acid AzK was incorporated at the indicated site in response to an engineered UAG nonsense codon using an engineered pyrrolysyl-tRNA synthetase/tRNA pair (Figure 3).[189−192] As a result, the union of a DIBO unit of **11h** and an azide linked to GFP (**12b**) provided **13b** with >95% conversion (in 5% DMSO in DPBS, 22 °C, Figure 4A), as evidenced by whole-protein MS analysis. The union of **11h** and an azide-containing nanobody (**12c**) produced **13c** with >95% conversion; however, an elevated reaction temperature of 37 °C was required. However, the preparation of the full-length antibody−drug conjugate **13d** using a similar strategy proved to be problematic due to a lower reaction rate and the instability of the protein under more forcing conditions. We discovered that this limitation can be overcome through the use of **11i** equipped with a more reactive DIBAC unit (Figure 4B).[61] With **11i**, bioconjugation proceeded smoothly at 22 °C using a solvent mixture of 1% DMSO in DPBS, thereby giving the nanobody−drug (**13e**) or antibody−drug (**13f**, DAR = 2.0) conjugates with >95% conversion.

3. CONCLUSIONS

In summary, we have developed a catalytic strategy for the chemical tagging of bioactive amides and other related small-molecule drugs through their late-stage N−H bond functionalization. We identified that through the cooperative action of readily available $Sc(OTf)_3$ and Et_3N or Barton's base, it is possible to deprotonate a poorly to moderately acidic N−H bond in a wide array of pharmaceuticals and then promote the addition of the resulting N-based nucleophile to alkene-based tagging agents. The cooperative catalyst system facilitates such transformations under redox and pH-neutral conditions, and a wide variety of Lewis acid- and/or base-sensitive moieties can be tolerated. Thus, incorporation of photoaffinity labels, and functional handles for azide−alkyne cycloaddition, hetero Diels−Alder reaction, and Michael addition can be readily achieved. Utility of the tagged derivatives was demonstrated through the syntheses of a bifunctional drug, and also GFP−, nanobody−, or antibody−drug conjugates. The principles outlined above show that the proper combination of Lewis acidic and Brønsted basic catalysts may be used for chemo- and site-selective N−H bond functionalization, providing a rational basis for the future development of processes for derivatization of more complex peptides, proteins, and other amide-based natural products. Studies aimed at achieving these objectives are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.3c00169.

Experimental procedures and spectral data for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Masayuki Wasa − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0002-3309-9129; Email: wasa@bc.edu

Authors

Cunyuan Zhao − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Yuankai Wang − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Quan Pham − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Changhang Dai − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States*

Abhishek Chatterjee − *Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, Massachusetts 02467, United States;* orcid.org/0000-0002-6231-5302

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.3c00169

Author Contributions

†C.Z. and Y.W. contributed equally to this paper.

Notes

The authors declare no competing financial interest.

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