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Evaluation of α-Pyrones and Pyrimidones as Photoaffinity Probes for Affinity-Based Protein Profiling

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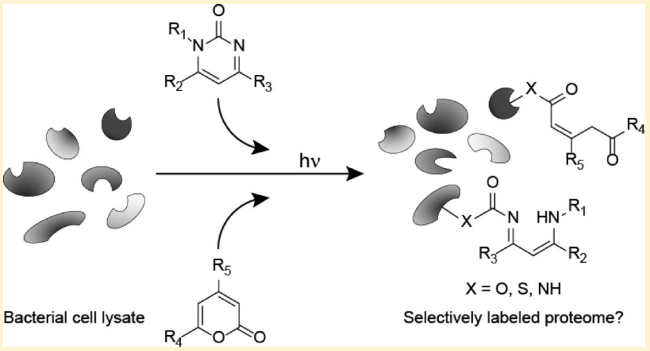
Supporting Information

ABSTRACT: α-Pyrones and pyrimidones are common structural motifs in natural products and bioactive compounds. They also display photochemistry that generates high-energy intermediates that may be capable of protein reactivity. A library of pyrones and pyrimidones was synthesized, and their potential to act as photoaffinity probes for nondirected affinity-based protein profiling in several crude cell lysates was evaluated. Further "proof-of-principle" experiments demonstrate that a pyrimidone tag on an appropriate scaffold is equally capable of proteome labeling as a benzophenone.



INTRODUCTION

Many disease states arise from aberrant protein function, and it is thus a major goal to determine the molecular, cellular, and physiological functions for the multitude of proteins encoded by eukaryotic and prokaryotic genomes, a goal that cannot be accomplished by analysis of genome sequences alone. Because proteins do not function in isolation but rather as parts of complex regulatory networks, a method to examine proteins in their natural state has been developed. Termed activity-based protein profiling (ABPP), this chemical-proteomic approach involves the interrogation of an entire native proteome with carefully designed probes.[1−4] These probes typically contain an active-site-directed group for binding and covalent modification of the target protein, a linker, and a tag that allows for visualization or enrichment of the labeled proteins.

Typically, ABPP probes achieve covalent protein modification of enzyme active sites either by electrophilic labeling of complementary protein nucleophilic sites or by photo-cross-linking, which is the strategy of choice for probes that lack an electrophilic function. Photo-cross-linking does not necessarily represent a mechanism-based labeling of target proteins and therefore can also be referred to as "affinity-based protein profiling".

Common examples of photo-cross-linking groups[5,6] are aryl azides,[7] diazirines,[8] and benzophenones.[9] Each group has its disadvantages and advantages; however, each involves the incorporation of a non-natural moiety into a probe structure that can drastically perturb probe−protein interaction. This limitation can be circumvented by the application of intrinsically photoreactive probes, i.e., probes whose natural structures generate highly reactive intermediates upon irradiation. Examples of intrinsic photoreactivity include steroid enones,[10,11] some aryl chlorides,[12] and some thioethers.[13,14]

α-Pyrones and pyrimidones are structural motifs found in many natural products and bioactive molecules. Examples include citreoviridin,[15] bufadienolides,[16] aureothins,[17] fusapyrone,[18] pyrimidone-thiazolidinediones,[19] and Zebularine[20] (Figure 1).

In addition to their presence in bioactive compounds, pyrones and pyrimidones also display well-documented photoreactivities. α-Pyrones (**1** or **4**), upon irradiation with UV light, undergo an isomerization to either ketene **2** or a bycyclic β-lactone **5**, depending on the nature of the substituents on the pyrone ring.[21−23] These isomerized forms can then undergo reactions with nucleophiles. In the case of 4-alkyl-substituted pyrone **1**, simple attack of the nucleophile, in this example, methanol, on ketene **2** leads to methyl β-acetonylcrotonate **3**. In the case of 4-alkoxy substituted pyrone, which initially forms bicyclic lactone **5** upon irradiation, the reaction pathway involves zwitterionic intermediate **6**.[24] MeOH addition to **6** followed by thermal conrotatory ring opening of **7** forms intermediate **8**, which can undergo ring closure to orthoester product **9**. Hydrolysis of **9** in aqueous media results in formation of β-carbomethoxymethylcrotonic acid **10** (Scheme 1).

Pyrimidones (**11**) upon irradiation undergo a Norrish type I reaction (**11** to **12**) to form either an isocyanate **13** or bicyclic intermediate **14**, which can then react with nucleophiles to form the tautomeric products **15** and **15a** as shown in Scheme 2.[25,26]

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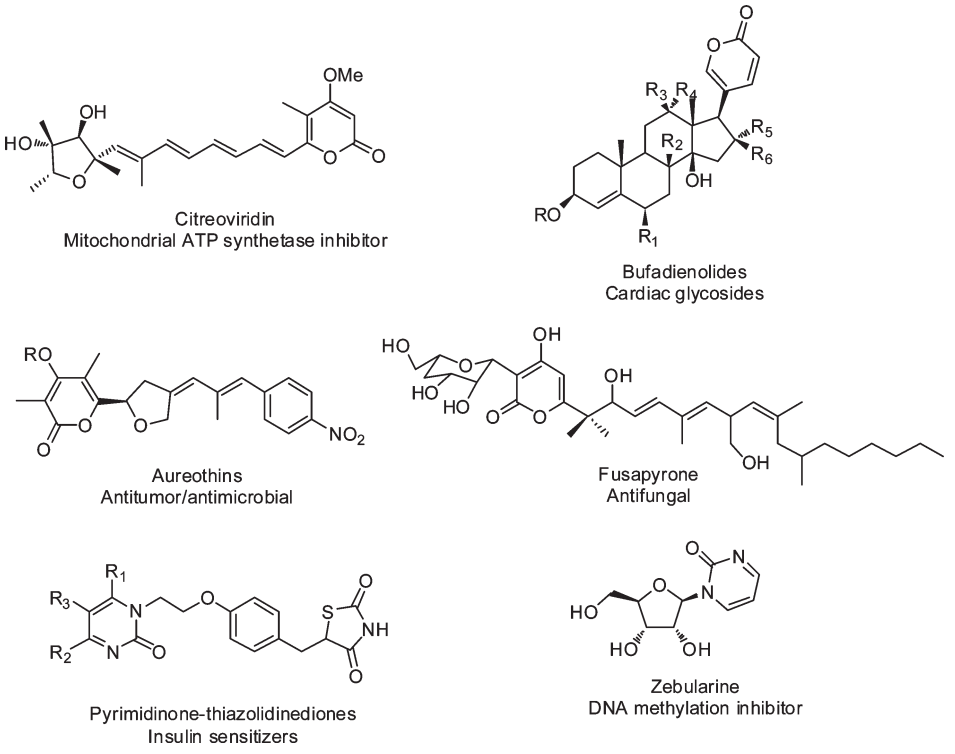
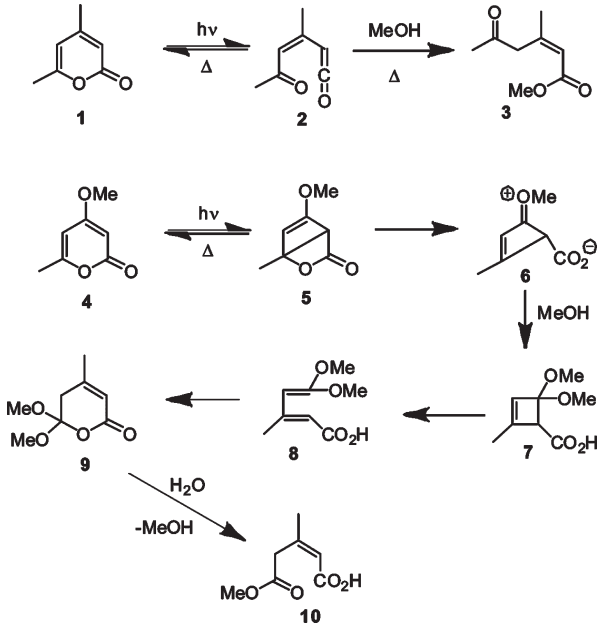


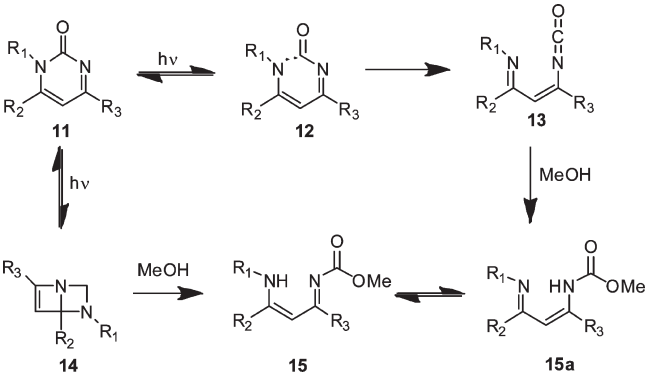
Figure 1. Pyrone and pyrimidone-containing bioactive compounds.

Scheme 1[23]



Kinetics of photoisomerization and reaction of the intermediates with nucleophiles has been the subject of several studies.[27−31] Whereas the above photoisomerizations to active species are fast, the reaction of these species with nucleophiles is relatively slow. However, given that the formation of the active species is largely reversible and in a protein−substrate complex, the nucleophile concentration is high, and a protein-labeling event is likely to occur.

Scheme 2[26]



We found this photoreactivity, coupled with the occurrence of α-pyrones and pyrimidones in various natural product and bioactive compounds to be an intriguing approach to intrinsic photoreactivity. Natural products and other bioactive compounds with α-pyrones or pyrimidones in their structures could be photoactivated to a reactive species as illustrated in Schemes 1 and 2. This reactive species could then react with a nearby amino acid to covalently modify the binding protein (Scheme 3).

In this report, we outline syntheses of α-pyrone- and pyrimidone-containing compounds and attempts to use these as nondirected photoactivatible affinity probes.

RESULTS AND DISCUSSION

The design of the probes required careful consideration for their structural elements. We sought to have a wide variety of

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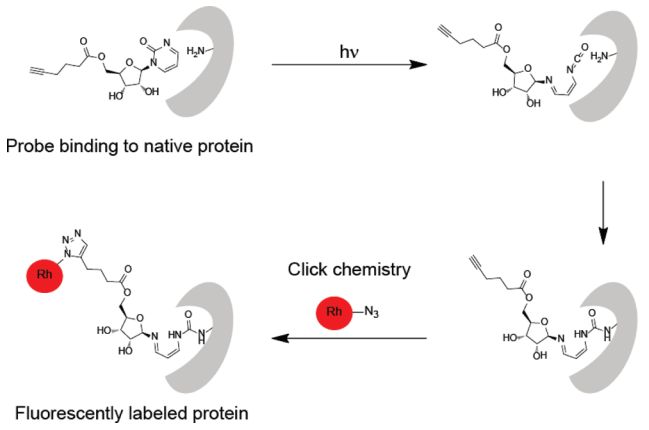
Scheme 3



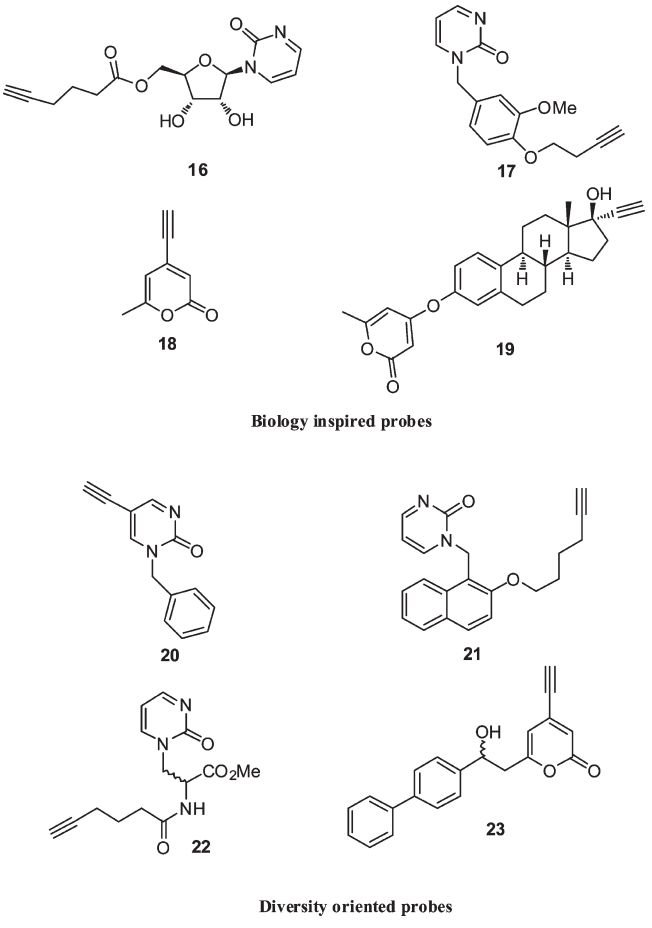


Figure 2. Biology- and diversity-inspired probes.

probe structures in order to maximize the opportunities for specific biological interactivity, in this case with bacterial proteomes. In addition to the α-pyrone or pyrimidone, within each structure is a sterically benign alkyne tag suitable for the Cu(I)-catalyzed Huisgen [3 + 2]-azide−alkyne cycloaddition,[32−34] which would allow the attachment of a fluorescent or a biotin tag for visualization and enrichment, respectively. Figure 2 shows the probes used in our proteome labeling experiments. These

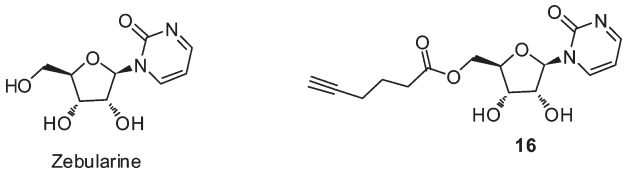
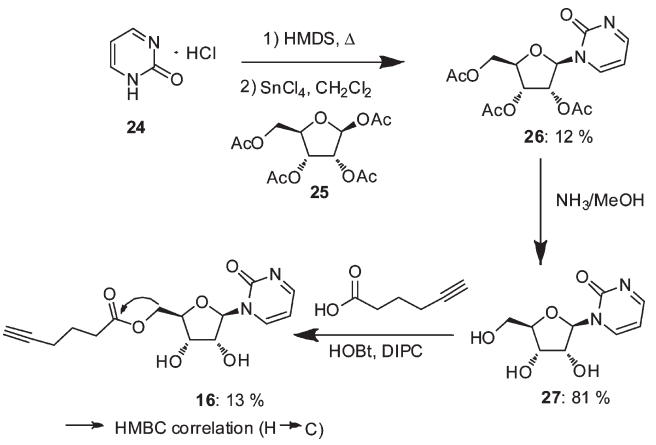


Figure 3. Zebularine and probe **16**.

Scheme 4



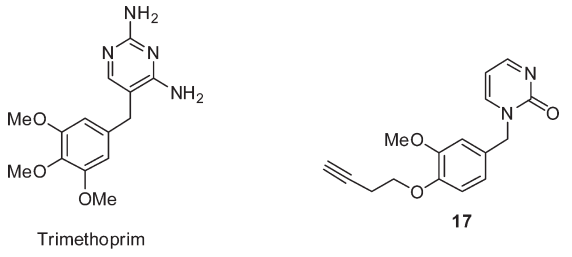


Figure 4. Trimethoprim and probe **17**.

probes can be broadly divided in two classes: those that are inspired by compounds with known bioactivity (biology-inspired probes) and those that are not (diversity-oriented probes).

Probe **16** was designed as a mimic of the DNA methylation inhibitor Zebularine[20] (Figure 3).

Formation of the glycosidic bond was accomplished by utilizing a one-pot procedure as outlined by Vorbrüggen et al.[35,36] between pyrimidine **24** and peracetylated ribofuranose **25** to give **26** in 12% yield. Deprotection of the acetyl groups by ammonia in MeOH gives nucleoside **27** in 81% yield.[37] At this point, the alkyne tag was installed by coupling of 5-hexynoic acid to nucleoside **27** in DMF. Probe **16** was isolated from a mixture of isomers, and its regiochemistry was confirmed by HMBC correlations between the 5′ ribofuranose methylene and the ester carbonyl (Scheme 4).

Probe **17** was designed to resemble the dihydrofolate reductase inhibitor trimethoprim (Figure 4).[38]

Therefore, vanillin was alkylated by tosylate **29** to yield aldehyde **30**.[39] Functional group interchanges[40,41] yielded bromide **31**, which was then substituted with pyrimidone **24** to yield probe **17** (Scheme 5).

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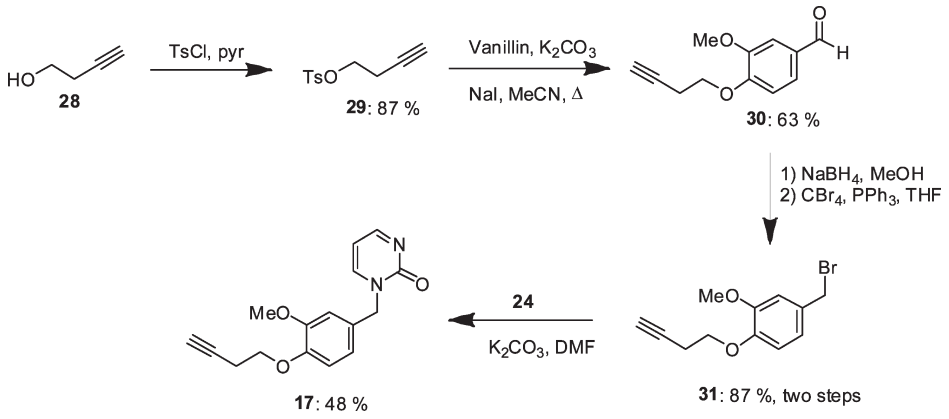
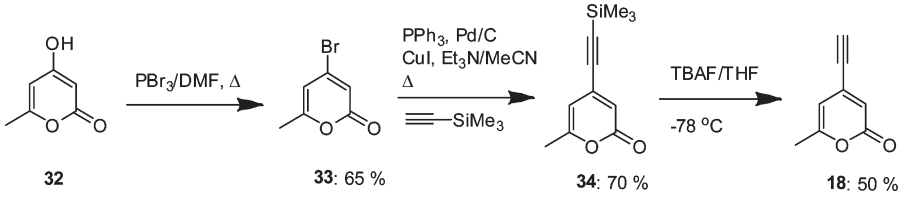
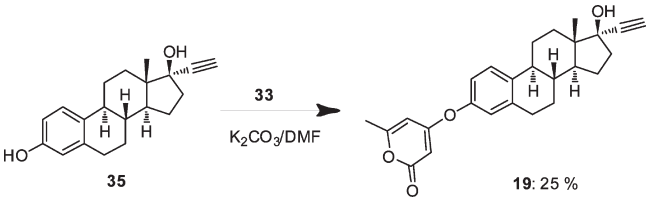
Scheme 5



Scheme 6



Scheme 7



α-Pyrone probe **18** has been reported to possess broad-spectrum antibacterial activity[42] and was prepared via bromination and subsequent Sonogashira reaction with trimethylsilylacetylene of the commercially available triacetic acid lactone **32** (Scheme 6).[43]

Probe **19** is based upon 17α-ethynylestradiol (**35**), a common estrogenic component in oral contraceptives that conveniently has a terminal alkyne in its structure. While 17α-ethynylestradiol will in all probability display no appreciable biological activity toward bacterial proteins, the core steroid structure is a common one and may impart specific protein binding in the bacterial proteome. Reaction of 17α-ethynylestradiol with bromide **33** under basic conditions results in a Michael addition−β-elimination to yield probe **19** (Scheme 7).[44]

The diversity-oriented class of probes were not based upon bioactive scaffolds but were chosen to present a variety of structural elements to the proteome. Probe **20** was synthesized by iodination of pyrimidone **24** with *N*-iodosuccinimide,[45] followed by installation of a benzyl group to yield **36**. Sonogashira reaction between iodide **36** and trimethylsilylacetylene yields **37**. Standard deprotection (TBAF/THF) then yields probe **20** (Scheme 8).

Probe **21** was based on a naphthalene core and was synthesized in a manner analogous to probe **17** starting with 2-hydroxy-1-napthaldehyde (**40**) (Scheme 9).

Probe **22** was based on an amino acid scaffold, in this case, serine. Protection of the C and N terminals of serine as a methyl ester and *tert*-butyl carbamate, respectively, gives **44**.[46] Bromination[47] of the primary alcohol to yield **45** followed by substitution with pyrimidone **24** gives **46**, albeit with racemization of the chiral center. Deprotection of the Boc group under standard conditions, followed by amide formation with 5-hexynoic acid, yields probe **22** (Scheme 10).

The final pyrone probe, **23**, was synthesized via deprotonation of bromide **33** at C7, followed by quenching of the anion with biphenyl-4-carboxaldehyde to yield **47**.[48,49] Sonogashira reaction with trimethylsilylacetylene and **47**, followed by standard deprotection, yields probe **23** (Scheme 11).

With probes **16**−**23** in hand, we wanted to verify their light-induced reactivity with nucleophiles. We picked two probes, **18** and **20**, as test compounds.

Irradiation of a methanolic solution of **18** at 350 nm resulted in the loss of starting material and the formation of a peak that corresponds to the expected ester photoproduct **49** (Figure 5). As LC−MS was problematic for this compound, it was isolated and the ^{1}H and ^{13}C NMR spectra of **49** were recorded. This reaction was complete after less than 10 min.

Irradiation of **20** in MeOH at 350 nm results in almost complete conversion after 30 min, accompanied by the formation of a peak that corresponds to the proposed photoproduct **50** as determined by LC−MS (Figure 6).

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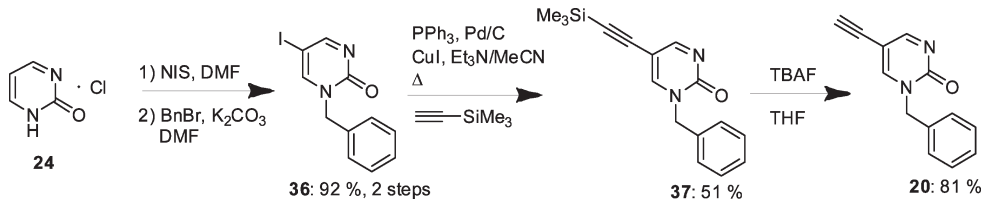
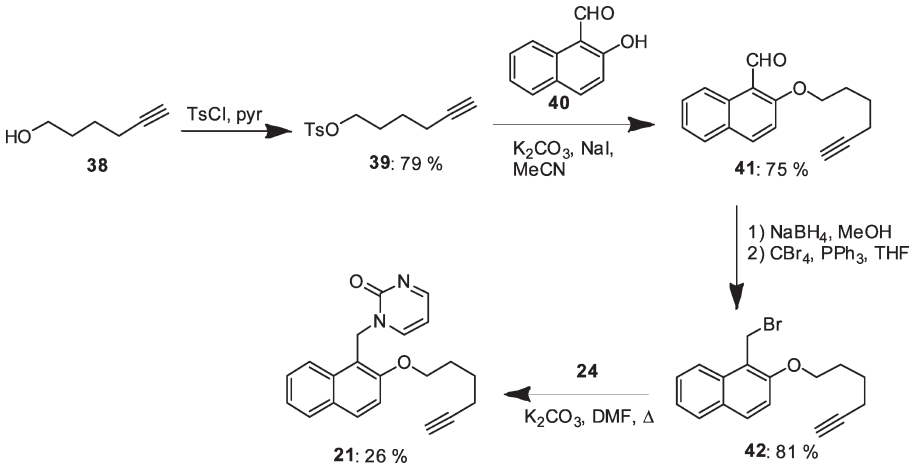
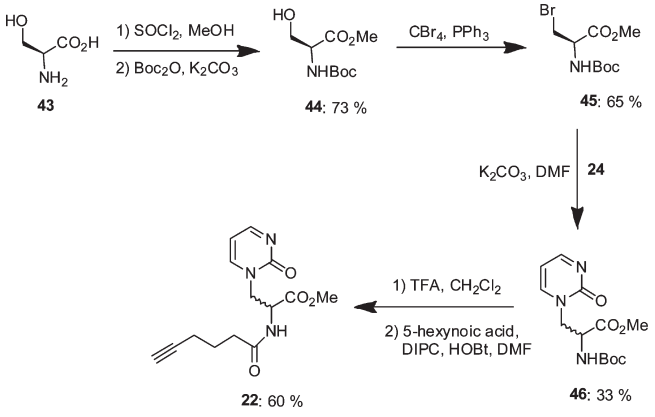
Scheme 8



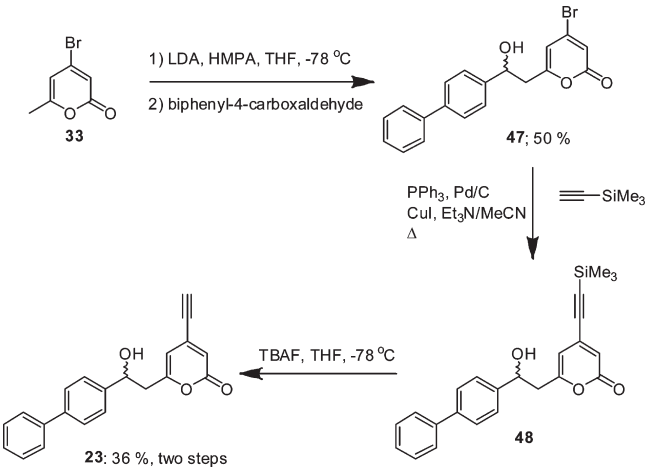
Scheme 9



Scheme 10



Scheme 11



Once we were satisfied that our probes were reacting sufficiently with weak nucleophiles, we set labeling parameters for optimal probe reaction with crude cell lysate preparations. Using either *E. coli* or *S. aureus* Mu50 cytosol preparations as our standard proteome preparations, we screened representative probes for time and concentration dependence with respect to photolabeling. In a typical experiment, a crude cell lysate (proteome) is diluted with PBS to give 43 μL of 1 mg/mL protein concentration. One microliter of the desired probe is then added in DMSO to give the final probe concentration. Irradiation is carried out over ice cooling in a 96-well plate. The irradiated solutions are then transferred back to a reaction vessel, and [3 + 2]-azide−alkyne "click chemistry" is performed on the solution with rhodamine azide for 1 h at rt. Figure 7 shows that for probe **19**, 100 μM and 1 h yields saturated protein labeling.

These conditions also result in saturated labeling for **17**; however, for **20**, while 100 μM remains an optimal concentration, only 20 min is necessary for saturated labeling (Figures S1 and S2, Supporting Information). Increases in irradiation time only increase background signal, and probe concentrations over

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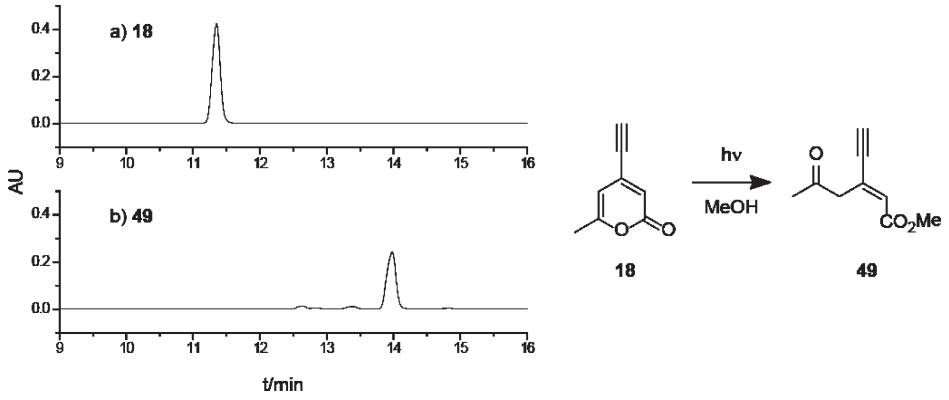

Figure 5. UV-dependent conversion of **18** to **49**.

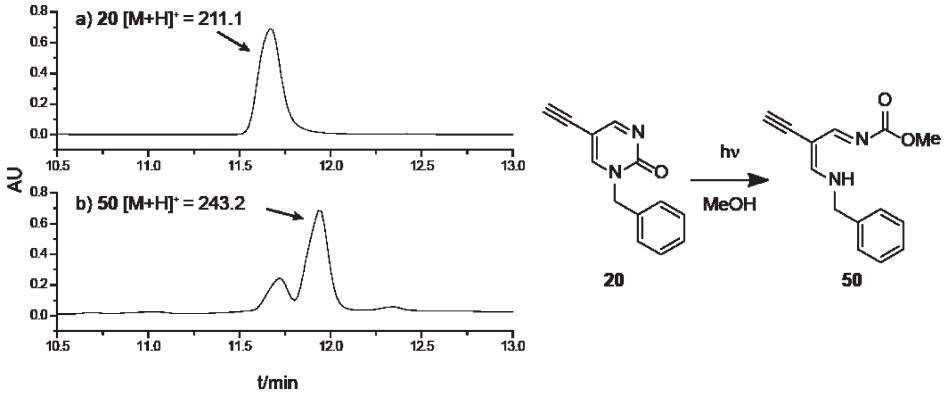

Figure 6. UV-dependent conversion of **20** to **50**.

100 μM do not result in significantly increased signal-to-noise ratios. Nonirradiated controls demonstrate a clear light-dependent labeling for some protein bands (Figure 7, lanes 5−8), emphasizing that photoinduced reactivity leads to protein labeling.

With the key parameters set, we then compared all probes for labeling patterns in both *L. welshimerii* and *E. coli* crude cell lysates using the optimized labeling parameters as described above. A variety of structures as shown in Figure 2 should, by virtue of their differing noncovalent interactions, show a wide variety of labeling patterns as well. However, Figure 8 demonstrates that while a variety of structural types are displayed to the bacterial proteomes, the labeling patterns show only a few differences (see also Figure S3, Supporting Information for *E. coli* labeling).

The similarity in labeling patterns may be due to the relatively long lifespan of the photogenerated intermediates of the α-pyrones or pyrimidones (Schemes 1 and 2) that allows for mobility from the probes original binding site. This mobile intermediate can then equilibrate among the reaction medium and then react with available protein nucleophiles, and hence the probes designed herein reflect a preference for type of reactant instead of type of binding site. However, a striking feature of cell lysate labeling with probes **16**−**23** is the moderate reactivity of the photogenerated species with respect to the native proteomes emphasizing that the photoinduced electrophiles display a certain selectivity for available protein nucleophiles. Concerned with this apparent lack of selectivity that probes **16**−**23** demonstrate toward bacterial lysates, we sought a more comparative approach toward demonstrating the feasibility of our hypothesis. Realizing that biology-based probes **16**−**19** display significant structural deviations from their original inspirations that may drastically reduce their target binding[50−52] and that diversity-based probes **20**−**23** may not possess enough structural elements to prevent diffusion after photoactivation, we chose to modify a substrate whereby the additional elements (photolabel and alkyne tag) would constitute a minimal structural change. A recent account from our group details the synthesis of vancomycin-based photoaffinity probes that selectively label autolysin (ATLam) and an ABC transporter protein (pABC) in *S. aureus* and *E. faecalis*, respectively (Figure 9).[53]

We reasoned that by substituting the benzophenone in probe **51** for a pyrone or pyrimidone, we could directly compare the labeling efficacy of a well-established photo-cross-linking moiety with a novel cross-linking group. For synthetic ease, we chose to examine a pyrimidone in this position. Compound **55** was prepared according to Scheme 12 by a coupling of primary amine **53** with modified vancomycin **54**.

With probe **55** in hand, we then compared the labeling patterns of **51** and **55** in BL21 *E. coli* recombinant clone cell lysates overexpressing both ATLam and pABP. To our gratification, the labeling patterns for both **51** and **55** were identical at 1 and 10 μM, respectively, while nonirradiated controls demonstrated little to no labeling at 100 μM **55**. Furthermore, attempts to photolabel the overexpressed *E. coli* cell lysates with 100 μM **22** gave no appreciable labeling, showing patterns similar to

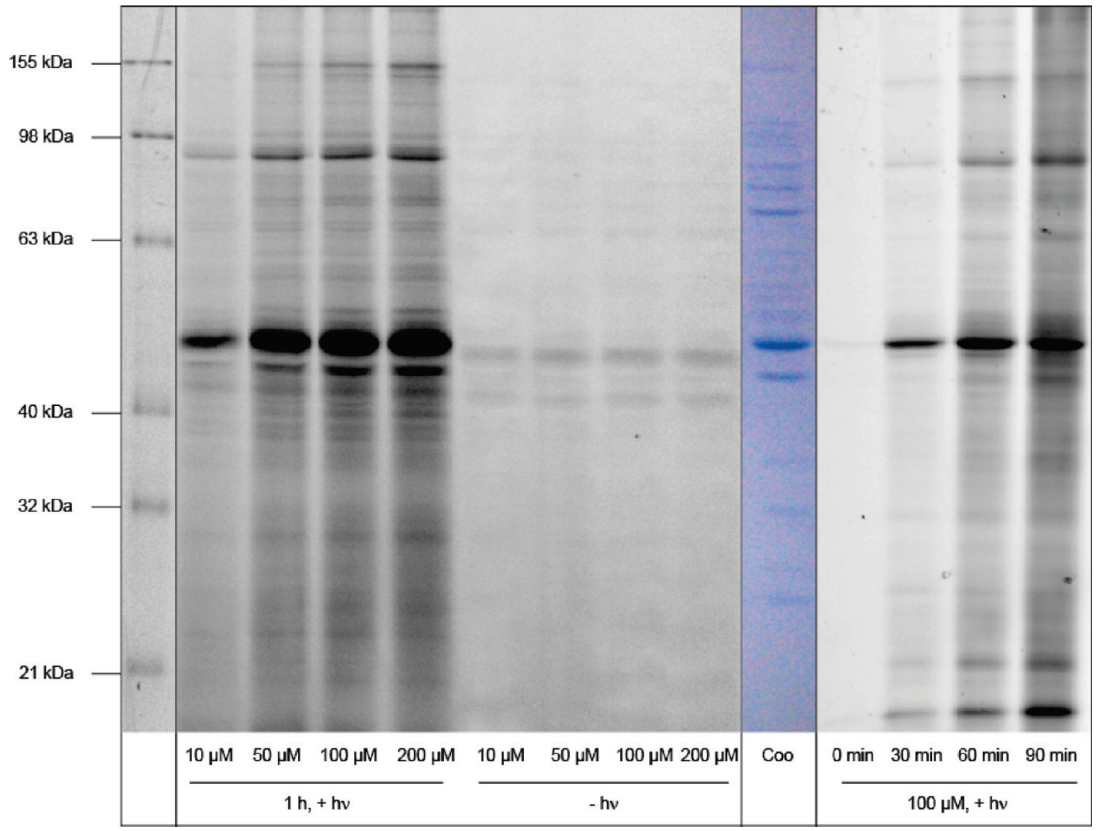

Figure 7. Time- and concentration-dependent labeling of *E. coli*. crude cellular lysate with **19**.

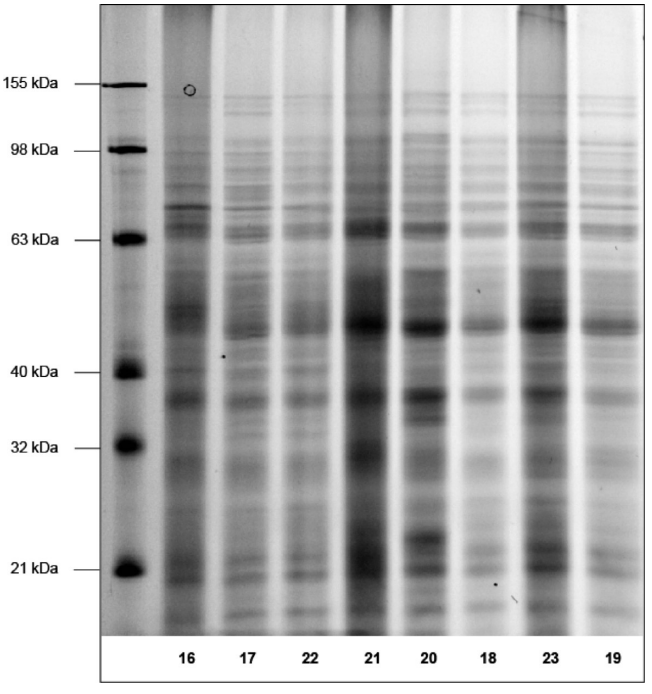

Figure 8. Labeling of *L. welshimerii* crude cell lysate with probes **16**−**23**.

Figure 8, demonstrating the key role of the vancomycin scaffold in the proteome labeling of **55** (Figure 10).

While **51** appears to label more effectively than **55**, both probes show identical selectivity toward the bacterial proteome. This result represents an important validation of the hypothesis that pyrimidones can be used as photo-cross-linkers. The selectivity of probe **55** relative to probes **16**−**23** can be explained by the much greater molecular complexity of vancomycin, which results in greater noncovalent protein binding, allowing the photoactivated species to remain target-bound while the isocyanate reacts the protein. Given the results displayed in Figure 10, we are confident that a high affinity natural product or bioactive compound containing a native pyrone or pyrimidone, appropriately modified with an alkyne tag, will display selective photolabeling of complex proteomes. Work is currently underway to adapt this approach to affinity labeling of proteomes with natural structures.

CONCLUSION

Using intrinsic photoreactivity represents a powerful approach to photoaffinity labeling that precludes the necessity of additional photoreactive groups, which can perturb native substrate−target interaction. We have attempted to use nondirected α-pyrone and pyrimidone probes to demonstrate the feasibility of these groups as intrinsic photolabels. We synthesized a series of biology- and diversity-inspired probes **16**−**23** and demonstrated that representative samples of these probes displayed light-induced reaction with MeOH and also displayed light-dependent labeling of crude bacterial lysates. However, these probes displayed little to no selectivity in bacterial proteomes, prompting us to attempt a secondary "proof of principle" strategy. In this strategy, vancomycin-based probe **55** displays selective labeling of ATLam and pABC proteins in *E. coli* in a manner identical to that for the similar benzophenone-containing vancomycin probe **51**.

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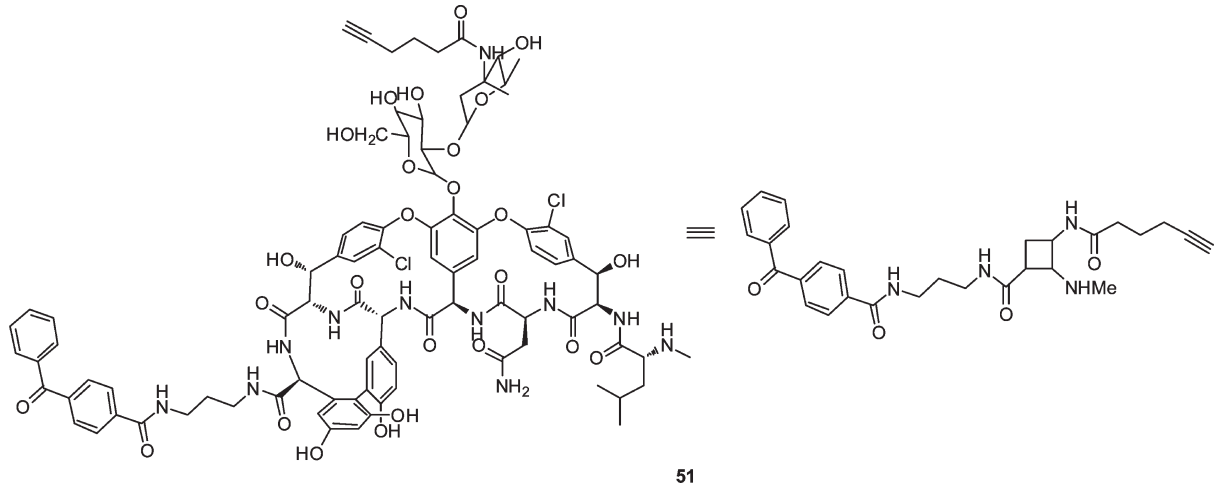
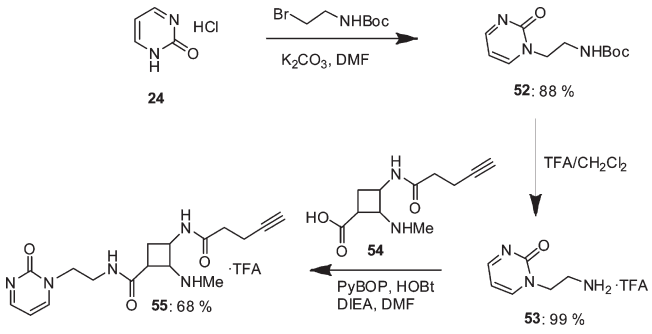


Figure 9. Full and shorthand illustrations of probe **51**.

Scheme 12



This result validates our hypothesis and encourages us to attempt this approach with natural product structures already containing a pyrone or pyrimidone.

EXPERIMENTAL SECTION

General Methods. All nonaqueous reactions were carried out in flame-dried glassware unless otherwise noted. Air- and moisture-sensitive liquid reagents were manipulated via a dry syringe. Anhydrous tetrahydrofuran (THF) was obtained from distillation over sodium. All other solvents and reagents were used as obtained from commercial sources without further purification. Flash chromatography was performed using Merck Silica Gel 60 (40−63 μm). HPLC analysis was accomplished with a C18 column 5 μm (4.6 × 100 mm) and a PDA detector. Mobile phase (HPLC grade): A, 0.1% (v/v) TFA in H_2O; B, 0.1% TFA in acetonitrile. Flow: 0.5 mL/min.

2′,3′,5′-Tri-*O*-acetyl-Zebularine (26). Pyrimidine **24** (8.9 g, 67 mmol) and a catalytic amount of $(NH_4)_2SO_4$ were refluxed for 4 h in HMDS (25 mL) under an inert atmosphere. Excessive HMDS was removed under reduced pressure, and the residue was dissolved in dry CH_2Cl_2 (10 mL). A solution of **25** (7.2 g, 23 mmol) and $SnCl_4$ (4 mL, 34 mmol) was then combined in 5 mL of dry CH_2Cl_2, and the solution was stirred at rt for 16 h. The mixture was washed with saturated $NaHCO_3$ solution, and the resulting emulsion was filtered over silica. The aqueous layer was extracted into CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography to yield **26** (2.9 g, 8 mmol, 12%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H), 1.96 (s, 6H), 4.23 (d, J = 3.4 Hz, 2H), 4.26−4.32 (m, 1H), 5.12−5.18 (m, 1H), 5.32 (dd, J = 3.4, 5.5 Hz, 1H), 5.88 (d, J = 3.3 Hz, 1H), 6.29 (dd, J = 4.1, 6.8 Hz, 1H), 7.94 (dd, J = 2.8, 6.8 Hz, 1H), 8.45 (dd, J = 2.9, 4.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.3, 20.3, 20.6, 62.4, 69.2, 73.6, 79.6, 90.1, 104.3, 143.4, 155.0, 166.6, 169.3, 169.3, 170.0. HRMS (ESI): [M + Na]$^+$ $C_{15}H_{18}O_8N_2Na$ calcd 377.0955, found 377.0954. R_f (10% $MeOH/CH_2Cl_2$) = 0.6.

Zebularine (27). Compound **27** was prepared by the removal of the acetyl groups in **26** with NH_3/MeOH to yield **27** (603 mg, 2.6 mmol, 81%) as a colorless solid. The spectral data matched with those reported in the literature.[20] ^{1}H NMR (400 MHz, CD_3CD) δ 3.81 (dd, J = 2.4, 12.5 Hz, 1H), 3.99 (dd, J = 2.1, 12.5 Hz, 1H), 4.09−4.19 (m, 3H), 5.87 (s, 1H), 6.59 (dd, J = 4.3, 6.7 Hz, 1H), 8.58 (dd, J = 2.7, 4.2 Hz, 1H), 8.79 (dd, J = 2.7, 6.7 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 59.5, 68.2, 75.2, 84.4, 92.2, 104.6, 145.3, 156.1, 165.9. HRMS (ESI): [2 M + Na]$^+$ $C_{18}H_{24}N_4NaO_{10}$ calcd 479.1385, found 479.1382.

5′-*O*-Hex-5-ynoyl-Zebularine (16). HOBt (432 mg, 3.2 mmol) and 5-hexynoic acid (353 μL, 3.2 mmol) were dissolved in DMF (5 mL), and *N*,*N*′-diisopropylcarbodiimide (496 μL, 3.2 mmol) was added. The reaction mixture was stirred for 30 min at rt. A solution of **27** (1.1 g, 3.2 mmol) in DMF (5 mL) was added. The mixture was stirred for 16 h at 50 °C. The reaction mixture was concentrated to dryness and purified by by column chromatography (CH_2Cl_2/MeOH) to yield **16** (121 mg, 0.4 mmol, 13%) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD) δ 1.79−1.88 (m, 2H), 2.24−2.30 (m, 3H), 2.54 (t, J = 7.4 Hz, 2H), 4.05 (dd, J = 5.0, 7.9 Hz, 1H), 4.21 (dd, J = 1.5, 4.9 Hz, 1H), 4.29−4.34 (m, 1H), 4.40−4.51 (m, 2H), 5.83 (d, J = 1.2 Hz, 1H), 6.64 (dd, J = 4.2, 6.7 Hz, 1H), 8.40 (dd, J = 2.7, 6.7 Hz, 1H), 8.61 (dd, J = 2.9, 4.1 Hz, 1H); ^{13}C NMR (90 MHz, CD_3OD) δ 17.0, 23.4, 32.2, 62.6, 69.0, 69.1, 74.6, 81.4, 82.6, 93.0, 104.7, 144.4, 156.0, 166.2, 172.8. HRMS (ESI): [M + H]$^+$ $C_{15}H_{18}N_2O_6$ calcd 323.1238, found 323.1220. R_f (10% $MeOH/CH_2Cl_2$) = 0.5.

But-3-yn-1-yl 4-Methylbenzenesulfonate (29)[39]. 4-Methylbenzenesulfonyl chloride (20.4 g, 110 mmol) was added to a solution of **28** (5.4 mL, 70 mmol) and pyridine (11.5 mL, 140 mmol) in CH_2Cl_2 at 0 °C, warmed to rt, and stirred for 16 h. The reaction mixture was treated with water and extracted with CH_2Cl_2. The combined organic layers were washed with 1 M HCl, saturated $NaHCO_3$, H_2O, and brine. The solution was dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by column chromatography to yield **29** (13.6 g, 61 mmol, 87%) as a colorless oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.98 (t, J = 2.7 Hz, 1H), 2.46 (s, 3H), 2.56 (td, J = 2.7, 7.0 Hz, 1H), 4.11 (t, J = 7.0

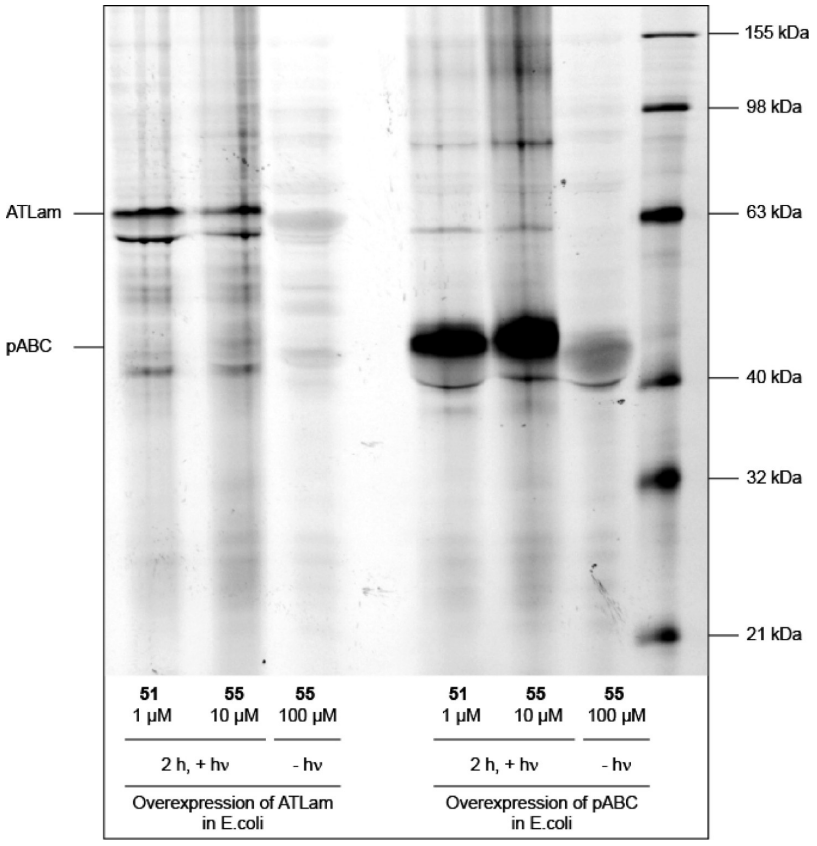


Figure 10. Labeling of *E. coli* overexpressing ATLam and pABC with **51** and **55**.

Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.3 Hz, 2H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 19.4, 21.6, 67.5, 70.8, 78.4, 127.9, 129.9, 132.8, 145.0. HRMS (EI): $[M]^+$ $C_{11}H_{12}O_3{}^{32}S$ calcd 224.0502, found 224.0504. R_f (hexanes) = 0.7.

4-(But-3-yn-1-yloxy)-3-methoxybenzaldehyde (30)[39]. A suspension of vanillin (5.1 g, 34 mmol), **29** (7.5 g, 33 mmol), K_2CO_3 (13.9, 101 mmol), and NaI (510 mg, 3.4 mmol) in CH_3CN was refluxed for 16 h. The solution was concentrated and treated with EtOAc/H_2O, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated to dryness to yield **30** (4.2 g, 21 mmol, 63%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.07 (t, J = 2.6 Hz, 1H), 2.76 (td, J = 2.6, 7.3 Hz, 2H), 3.90 (s, 3H), 4.21 (t, J = 7.3 Hz, 2H), 6.98 (d, J = 8.2 Hz, 1H), 7.37−7.45 (m, 2H), 9.83 (s, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 19.3, 56.0, 66.9, 70.5, 79.6, 109.6, 111.9, 126.5, 130.5, 149.9, 153.3, 190.8. HRMS (ESI): $[M + H]^+$ $C_{12}H_{13}O_3$ calcd 205.0856, found 205.0854. R_f (50% EtOAc/hexanes) = 0.3.

4-(Bromomethyl)-1-(but-3-yn-1-yloxy)-2-methoxybenzene (31). At 0 °C $NaBH_4$ (445 mg, 12 mmol) was slowly added to a cooled solution of **30** (1.2 g, 6 mmol) in dry THF. After 30 min the reaction mixture was treated with H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The crude product was used without further purification. The alcohol was added to a solution of CBr_4 (2.8 g, 8.5 mmol) and PPh_3 (2.2 g, 8.5 mmol) in THF and stirred for 16 h. The precipitate was removed by filtration, and the filtrate was concentrated to dryness. The crude product was purified by column chromatography to yield **31** (1.4 g, 5.2 mmol, 87%) as a yellow solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.06 (t, J = 2.7 Hz, 1H), 2.74 (td, J = 2.7, 7.4 Hz, 2H), 3.89 (s, J = 2.0 Hz, 3H), 4.16 (t, J = 7.4 Hz, 2H), 4.50 (s, J = 5.5 Hz, 2H), 6.81−6.98 (m, 3H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 19.4, 34.2, 56.0, 67.2, 70.2, 80.1, 112.9, 113.6, 121.6, 131.1, 148.0, 149.7. HRMS (EI): $[M]^+$ $C_{12}H_{13}{}^{79}BrO_2$ calcd 268.0093, found 268.0085. R_f (50% EtOAc/hexanes) = 0.4.

1-(4-(But-3-yn-1-yloxy)-3-methoxybenzyl)pyrimidin-2(1*H*)-one (17). Bromide **31** (675 mg, 2.5 mmol) was added to a suspension of K_2CO_3 (1.0 g, 7.5 mmol) and pyrimidine **24** (398 mg, 3.0 mmol) in DMF (25 mL). The reaction mixture was stirred for 16 h at 70 °C and concentrated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **17** (336 mg, 1.2 mmol, 48%) as a yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 2.33 (t, J = 2.7 Hz, 1H), 2.66 (td, J = 2.7, 6.9 Hz, 2H), 3.86 (s, 3H), 4.11 (t, J = 6.9 Hz, 2H), 5.15 (s, 2H), 6.64−6.71 (m, 1H), 6.99 (d, J = 0.9 Hz, 2H), 7.10 (s, 1H), 8.45−8.50 (m, 1H), 8.63−8.68 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 18.7, 54.2, 55.2, 67.2, 69.6, 79.9, 104.6, 112.9, 114.0, 121.4, 127.6, 148.5, 150.0, 153.2, 153.6, 163.3. HRMS (ESI): $[M + H]^+$ $C_{16}H_{17}N_2O_3$ calcd 285.1234, found 285.1228. R_f (10% MeOH/CH_2Cl_2) = 0.4

4-Ethynyl-6-methyl-2*H*-pyran-2-one (18). This compound was prepared according to Fairlamb et al.[43] The characterization data for this compound matched those reported in the literature.

3-(6-Methyl-2*H*-pyran-2-one)-17α-ethynylestradiol (19). 17α-Ethynylestradiol **35** (45.9 mg, 0.16 mmol), bromide **33** (30 mg, 0.16 mmol), and K_2CO_3 (21.4 mg, 0.16 mmol) were stirred in 1 mL of DMF at rt for 24 h. EtOAc was added, and the resulting suspension was extracted with 3 × H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by column chromatography to yield **19** (16.8 mg, 0.04 mmol, 25%) as a colorless solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.93 (s, 3H), 1.35−1.62 (m, 4H), 1.63−1.86 (m, 4H), 1.87−1.98 (m, 1H), 2.00−2.14 (m, 2H), 2,27 (s, 3H), 2.34−2.45 (m, 2H), 2.62 (s, 1H), 2.88 (m, 2H), 5.24 (s, 1H), 5.99 (s, 1H), 6.79 (s, 1H), 6.84, (dd, J = 2.2, 8.1 Hz, 1H), 7.34

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(d, J = 8.1 Hz, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 12.7, 20.0, 22.8, 26.3, 26.9, 29.6, 32.7, 38.9, 39.0, 43.7, 47.1, 49.5, 74.1, 79.8, 87.5, 90.9, 100.4, 118.0, 120.1, 127.1, 138.7, 139.2, 150.1, 163.1, 164.9, 171.1. HRMS (EI): $[M]^+$ $C_{26}H_{28}O_4$ calcd 404.1982, found 404.1971. R_f (40% EtOAc/hexanes) = 0.5.

1-Benzyl-5-iodopyrimidin-2(1*H*)-one (36). Pyrimidine **24** (304 mg, 2.3 mmol) and *N*-iodosuccinimide (541 mg, 2.4 mmol) in dry DMF (5 mL) were stirred at rt for 48 h. The reaction mixture was treated with Et_2O, and the resulting precipitate was collected by filtration. The crude product was washed with MeOH and used without further purification. The crude iodide, K_2CO_3 (934 mg, 6.7 mmol), and benzyl bromide (321 μL, 2.7 mmol) were dissolved in DMF (10 mL). The reaction mixture was stirred for 16 h at rt and concentrated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **36** (647 mg, 2.1 mmol, 92%, 2 steps) as a yellow solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.06 (s, 2H), 7.31−7.43 (m, 5H), 7.75 (d, J = 3.1 Hz, 1H), 8.59 (d, J = 3.1 Hz, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 54.3, 63.9, 128.8, 129.0, 129.3, 134.2, 151.2, 154.3, 170.3. HRMS (ESI): $[M + H]^+$ $C_{11}H_{10}ON_2I$ calcd 312.9832, found 312.9825. R_f (5% MeOH/CH_2Cl_2) = 0.5.

1-Benzyl-5-((trimethylsilyl)ethynyl)pyrimidin-2(1*H*)-one (37). A solution of **36** (298 mg, 1.0 mmol), ethynyltrimethylsilane (675 μL, 4.8 mmol), 10% Pd/C (2 mol % based on Pd), PPh_3 (2.5 mol %), and CuI (4 mol %) in 1:1 Et_3N/CH_3CN (10 mL) was refluxed for 4 h under an inert atmosphere. Solids were removed by centrifugation, and the remaining mixture was concentrated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **37** (134 mg, 0.5 mmol, 51%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.23 (s, 9H), 5.10 (s, 2H), 7.34−7.44 (m, 5H), 7.78 (d, J = 3.1 Hz, 1H), 8.62 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.3, 54.3, 96.4, 98.8, 102.2, 128.8, 128.9, 129.3, 134.2, 149.7, 154.6, 167.7. HRMS (ESI): $[M + H]^+$ $C_{16}H_{19}N_2OSi$ calcd 283.1261, found 283.1256. R_f (50% EtOAc/hexanes) = 0.5.

1-Benzyl-5-ethynylpyrimidin-2(1*H*)-one (20). **37** (124 mg, 0.4 mmol) was added to a solution of TBAF 3 H_2O (274 mg, 0.8 mmol) and AcOH (48 μL, 0.8 mmol) in THF (5 mL). The reaction mixture was stirred for 1 h at rt, concentrated to dryness, and purified by column chromatography (CH_2Cl_2/MeOH) to yield **20** (68 mg, 0.3 mmol, 81%) as a slightly yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.16 (s, 1H), 5.11 (s, 2H), 7.34−7.47 (m, 5H), 7.79 (d, J = 3.0 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 54.3, 75.7, 81.2, 101.0, 128.9, 129.1, 129.4, 134.0, 150.1, 154.6, 167.6. HRMS (ESI): $[M + H]^+$ $C_{13}H_{11}ON_2$ calcd 211.0866, found 211.0862. R_f (10% MeOH/CH_2Cl_2) = 0.5.

Hex-5-yn-1-yl 4-Methylbenzenesulfonate (39).[39] 4-Methylbenzenesulfonyl chloride (20.0 g, 105 mmol) was added to a solution of **38** (7.7 mL, 70 mmol) and pyridine (11.3 mL, 140 mmol) in CH_2Cl_2 (150 mL) at 0 °C, warmed to rt, and stirred for 16 h. The reaction mixture was treated with water and extracted with CH_2Cl_2.The combined organic layers were washed with 1 M HCl, saturated $NaHCO_3$, H_2O, and brine. The solution was dried over $MgSO_4$ and concentrated to dryness. The crude product was purified by column chromatography (hexane/EtOAc) to yield **39** (14.0 g, 55 mmol, 79%) as a colorless oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.53−1.63 (m, 2H), 1.75−1.86 (m, 2H), 1.94 (t, J = 2.7 Hz, 1H), 2.19 (td, J = 2.7, 6.9 Hz, 2H), 2.47 (s, 3H), 4.08 (t, J = 6.3 Hz, 2H), 7.34−7.40 (m, 2H), 7.78−7.84 (m, 2H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 17.7, 21.6, 24.2, 27.8, 69.0, 69.9, 83.4, 127.9, 129.8, 133.1, 144.7. HRMS (EI): $[M]^+$ $C_{13}H_{16}O_3{}^{32}S$ calcd 252.0815, found 252.0808. R_f (20% EtOAc/hexanes) = 0.4

2-(Hex-5-yn-1-yloxy)-1-naphthaldehyde (41). A suspension of aldehyde **40** (1.4 g, 8 mmol), **39** (3.0 g, 12 mmol), K_2CO_3 (3.3, 24 mmol), and NaI (120 mg, 0.8 mmol) in CH_3CN was refluxed for 16 h. The solution was concentrated and treated with EtOAc/H_2O, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated to dryness. The crude product was purified by column chromatography (hexane/EtOAc) to yield **41** (1.49 g, 6 mmol, 75%) as a slightly yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.75−1.82 (m, 2H), 1.99−2.07 (m, 3H), 2.29−2.36 (m, 2H), 4.24 (t, J = 6.2 Hz, 2H), 7.25 (d, J = 9.1 Hz, 1H), 7.43 (ddd, J = 1.1, 6.9, 8.0 Hz, 1H), 7.63 (ddd, J = 1.4, 6.9, 8.5 Hz, 1H), 7.77 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 9.30 (d, J = 8.7 Hz, 1H), 10.93 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.1, 25.0, 28.3, 68.8, 69.0, 83.8, 113.4, 116.6, 124.7, 124.9, 128.2, 128.4, 129.9, 131.5, 137.6, 163.5, 192.0. HRMS (EI): $[M]^+$ $C_{17}H_{16}O_2$ calcd 252.1145, found 252.1131. R_f (25% EtOAc/hexanes) = 0.5.

1-(Bromomethyl)-2-(hex-5-yn-1-yloxy)naphthalene (42). At 0 °C $NaBH_4$ (151 mg, 4.0 mmol) was slowly added to a cooled solution of **41** (504 mg, 2.0 mmol) in dry MeOH (5 mL). After 30 min the reaction mixture was treated with H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated. The crude product was used without further purification. The alcohol was added to a solution of CBr_4 (975 mg, 2.9 mmol) and PPh_3 (772 mg, 2.9 mmol) in THF (20 mL) and stirred for 16 h. The precipitate was removed by filtration, and the filtrate was concentrated to dryness. The crude product was purified by column chromatography (hexane/EtOAc) to yield the bromide **42** (515 mg, 1.6 mmol, 81%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.83−1.88 (m, 2H), 2.03−2.07 (m, 3H), 2.34−2.40 (m, 2H), 4.22 (t, J = 6.2 Hz, 2H), 5.13 (s, 2H), 7.23 (d, J = 9.1 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 7.81−7.87 (m, 1H), 8.06 (d, J = 8.6 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.3, 25.1, 25.5, 28.5, 68.6, 69.0, 84.2, 114.0, 118.3, 122.7, 123.9, 127.3, 128.7, 129.1, 129.9, 132.3, 154.6. HRMS (EI): $[M]^+$ $C_{17}H_{17}O^{79}Br$ calcd 316.0457, found 316.0447. R_f (25% EtOAc/hexanes) = 0.6.

1-((2-(Hex-5-yn-1-yloxy)naphthalen-1-yl)methyl)pyrimidin-2(1*H*)-one (21). Bromide **46** (201 mg, 0.63 mmol) was added to a suspension of K_2CO_3 (249 mg, 1.80 mmol) and pyrimidine **24** (100 mg, 0.75 mmol) in DMF (5 mL). The reaction mixture was stirred for 16 h at 70 °C and concentrated to dryness. The crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **21** (52 mg, 0.17 mmol, 26%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.67−1.76 (m, 2H), 1.94−2.03 (m, 3H), 2.25−2.32 (m, 2H), 4.24 (t, J = 6.3 Hz, 2H), 5.64 (s, 2H), 6.12 (dd, J = 4.1, 6.5 Hz, 1H), 7.35 (d, J = 9.1 Hz, 1H), 7.38−7.43 (m, 2H), 7.53 (t, J = 7.3 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 7.90−7.97 (m, 2H), 8.52 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.1, 24.9, 28.4, 43.1, 68.7, 69.0, 83.7, 104.2, 113.5, 114.3, 122.7, 124.3, 128.2, 128.7, 129.1, 131.8, 133.0, 146.4, 155.8, 156.8, 165.1. HRMS (ESI): $[M + H]^+$ $C_{21}H_{21}N_2O_2$ calcd 333.1598, found 333.1594. R_f (10% MeOH/CH^2Cl_2) = 0.5

(*S*)-Methyl 2-((*tert*-Butoxycarbonyl)amino)-3-hydroxypropanoate (44). Compound **44** was prepared according to the method of Chamberlain et al.[46] to yield **44** (7.2 g, 33 mmol, 73%) as a colorless oil. The spectral data matched with those reported in the literature. ^{1}H NMR (360 MHz, DMSO) δ 1.39 (s, 9H), 3.56−3.71 (m, 4H), 4.01−4.14 (m, 1H), 4.88 (t, J = 6.1 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H); ^{13}C NMR (90 MHz, DMSO) δ 28.6, 52.2, 56.7, 61.8, 78.8, 155.7, 171.9. HRMS (ESI): $[M + H]^+$ $C_9H_{18}NO_5$ calcd 220.1180, found 220.1170.

(*R*)-Methyl 3-Bromo-2-((*tert*-butoxycarbonyl)amino)propanoate (45). Alcohol **44** (4.0 g, 18.3 mmol), PPh_3 (7.2 g, 27.5 mmol), and CBr_4 (9.1 g, 27.5 mmol) were dissolved in CH_2Cl_2 (100 mL). The reaction mixture was stirred for 48 h at rt. Et_2O was added, and the resulting precipitate was removed by filtration. The organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to dryness. The crude product was purified by column chromatography to yield **45** (3.3 g, 12 mmol, 65%) as a yellow solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.40 (s, 9H), 3.59−3.87 (m, 4H),

4.62−4.80 (m, 1H), 5.36−5.54 (m, 1H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 28.2, 34.0, 52.9, 53.9, 80.4, 154.9, 169.6. HRMS (ESI): $[M + H]^+$ $C_9H_{17}BrNO_4$ calcd 282.0336, found 282.0336. R_f (30% EtOAc/hexanes) = 0.5.

(±)-Methyl 2-((*tert*-Butoxycarbonyl)amino)-3-(2-oxopyrimidin-1(2*H*)-yl)propanoate (46). A suspension of bromide **45** (2.3 g, 8.3 mmol), pyrimidine **24** (1.3 g, 10 mmol), and K_2CO_3 (3.5 g, 25 mmol) in DMF (50 mL) was stirred for 16 h at rt. The reaction mixture was concentrated to dryness and the crude product was purified by column chromatography (CH_2Cl_2/MeOH) to yield **46** (0.8 g, 2.8 mmol, 33%) as a slightly brown solid. Optical rotation measurements demonstrated that racemization of the chiral center had occurred during the reaction. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.35 (s, 9H), 3.74 (s, 3H), 4.15−4.29 (m, 1H), 4.38−4.51 (m, 1H), 4.56−4.68 (m, 1H), 5.89 (d, J = 6.8 Hz, 1H), 6.29 (dd, J = 4.2, 6.4 Hz, 1H), 7.69−7.80 (m, 1H), 8.53−8.58 (m, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.2, 52.1, 52.8, 80.3, 104.1, 149.2, 155.4, 156.5, 162.5, 166.4, 170.2. HRMS (ESI): $[M + H]^+$ $C_{13}H_{20}N_3O_5$ calcd 298.1398, found 298.1393. R_f (10% MeOH/CH^2Cl_2) = 0.6.

(±)-Methyl 2-(Hex-5-ynamido)-3-(2-oxopyrimidin-1(2*H*)-yl)propanoate (22). A solution of pyrimidone **46** (298 mg, 1.0 mmol) in 1:1 TFA/CH_2Cl_2 (2 mL) was stirred for 5 h (0 °C−rt). The reaction mixture was treated with saturated $NaHCO_3$, and the aqueous phase was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated. The crude product was used without further purification. HOBt (270 mg, 2.0 mmol) and 5-hexynoic acid (220 μL, 2.0 mmol) were dissolved in DMF (1 mL), and DIC (309 μL, 2.0 mmol) was added. The reaction mixture was stirred for 30 min at rt. A solution of the amine in DMF (1 mL) was added. The mixture was stirred for 2 h at rt. The reaction mixture was concentrated to dryness and purified by by column chromatography (CH_2Cl_2/MeOH) to yield **22** (175 mg, 0.6 mmol, 60%) as a yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 1.72−1.80 (m, 2H), 2.17−2.22 (m, 2H), 2.28 (t, J = 2.7 Hz, 1H), 2.32−2.36 (m, 2H), 3.78 (s, 3H), 4.13 (dd, J = 8.8, 13.3 Hz, 1H), 4.60 (dd, J = 5.2, 13.3 Hz, 1H), 4.93 (dd, J = 5.2, 8.8 Hz, 1H), 6.54 (dd, J = 4.3, 6.5 Hz, 1H), 8.02 (dd, J = 2.8, 6.5 Hz, 1H), 8.61 (dd, J = 2.8, 4.3 Hz, 1H); ^{13}C NMR (90 MHz, CD_3OD) δ 18.5, 25.6, 35.2, 51.6, 53.2, 53.2, 70.4, 84.0, 106.1, 151.9, 158.3, 168.0, 171.1, 175.6. HRMS (ESI): $[M + H]^+$ $C_{14}H_{18}O_4N_3$ calcd 292.1292, found 292.1287. R_f (10% MeOH/CH_2Cl_2) = 0.5

6-(2-([1,1′-bBiphenyl]-4-yl)-2-hydroxyethyl)-4-bromo-2*H*-pyran-2-one (47). Diisopropylamine (90.4 μL, 0.64 mmol) was dissolved under Ar in 5 mL of dry THF. ^{n}BuLi (0.27 mL, 2.4 M in hexanes, 0.64 mmol) was then added, and the solution was stirred at rt for 15 min. The reaction mixture was then cooled to −78 °C, and 100 μL HMPA was added. After stirring at −78 °C for 30 min, a solution of bromide **33** (100 mg, 0.53 mmol) in 1 mL of dry THF was slowly added. The reaction mixture was stirred cold for an additional 40 min, and then a solution of biphenyl-4-carboxaldehyde (116 mg, 0.64 mmol) in 1 mL of dry THF was slowly added. The reaction mixture was stirred at −78 °C for 1 h, then water was added to the cold solution, and the mixture was allowed to warm to rt. EtOAc was added, and the organic phase was washed with 2 × 1 M HCl and 1 × satd $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by flash chromatography to yield **47** (100 mg, 0.27 mmol, 50%) as a white solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.79−2.99 (m, 3H), 5.20 (m, 1H), 6.32 (s, 1H), 6.48 (s, 1H), 7.32−7.52 (m, 5H), 7.55−7.66 (m, 4H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 43.5, 71.0, 110.0, 115.4, 126.0, 127.1, 127.5, 128.9, 140.5, 141.1, 141.4, 141.9, 160.8, 162.0. HRMS (EI): $[M]^+$ $C_{19}H_{15}O_3{}^{79}Br_1$ calcd 370.0199, found 370.0181. R_f (20% EtOAc/hexanes) = 0.2.

6-(2-([1,1′-Biphenyl]-4-yl)-2-hydroxyethyl)-4-ethynyl-2*H*-pyran-2-one (23). Bromide **47** (100 mg, 0.27 mmol), 10% Pd/C (5.73 mg), PPh_3 (17.5, 0.067 mmol), and CuI (2.1 mg, 0.011 mmol) were combined in 1.5 mL of dry CH_3CN/2.2 mL of dry Et_3N. Trimethylsilylacetylene (64.5 mL, 0.46 mmol) was then added, and the solution was heated to 80 °C for 16 h. The reaction mixture was then cooled to rt, and the solids were filtered off and washed with EtOAc. The resulting organic solution was then washed with 2 × 1 M HCl and 1 × satd $NaHCO_3$. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was then passed through a silica plug and was used in the next step without further purification. Crude **48** was dissolved in 2 mL of THF and cooled to −78 °C. A solution of TBAF (300 μL, 1.0 M in THF, 300 mmol) was slowly added, and the solution was stirred for 1.5 h at −78 °C. The cold reaction was quenched by the addition of water, and the resulting solution was allowed to warm to rt. EtOAc was added, and the organic phase was washed with 1 × satd $NaHCO_3$. The organic phase was then dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by column chromatography to yield **23** (31 mg, 0.097 mmol, 36%, 2 steps) as a yellow solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.57 (s, br, 1H), 2.81−2.98 (m, 2H), 3.50 (s, 1H), 5.20 (m, 1H), 6.11 (s, 1H), 6.33 (s, 1H), 7.32−7.56 (m, 5H), 7.55−7.66 (m, 4H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 43.5, 71.1, 79.3, 86.6, 106.9, 116.9, 126.0, 127.0, 127.5, 128.9, 137.8, 140.6, 141.1, 141.9, 161.9, 162.1. HRMS (EI): $[M]^+$ $C_{21}H_{16}O_3$ calcd 316.1094, found 316.1087. R_f (20% EtOAc/hexanes) = 0.3.

(*E*)-Methyl 3-Ethynyl-5-oxohex-2-enoate (49). Compound **18** (16.2 mg, 0.12 mmol) was dissolved in 6 mL of MeOH and irradiated at 350 nm at a distance of 1 cm for 30 min. The solvent was then removed, and the resulting residue was purified by flash chromatography. The major product was **49** (9.9 mg, 0.060 mmol, 50%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.62 (s, 3H), 2.71 (m, 2H), 3.39 (s, 3H), 3.53 (s, 1H), 6.26 (s, 1H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 22.9, 39.3, 50.1, 80.4, 87.7, 103.7, 124.2, 135.4, 162.5. MS (EI): $[M]^+$ $C_9H_{10}O_3$ calcd 166.06, found 166.1. R_f (20% EtOAc/hexanes) = 0.35.

***tert*-Butyl (2-(2-Oxopyrimidin-1(2*H*)-yl)ethyl)carbamate (52).** A suspension of 2-(Boc-amino)ethylbromide (200 mg, 0.9 mmol), **24** (108 mg, 0.8 mmol) and K_2CO_3 (340 mg, 2.5 mmol) in DMF (5 mL) was stirred for 48 h at rt. The reaction mixture was concentrated to dryness, and the crude product was purified by column chromatography (CH_2Cl_2 /MeOH) to yield **52** (186 mg, 0.7 mmol, 88%) as a colorless solid. ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.33 (s, 9H), 3.46 (t, J = 5.5 Hz, 2H), 4.01 (t, J = 5.5 Hz, 2H), 6.25 (dd, J = 4.2, 6.4 Hz, 1H), 7.65−7.75 (m, J = 3.9 Hz, 1H), 8.45−8.55 (m, 1H). ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.3, 38.9, 51.2, 79.5, 104.0, 149.0, 156.2, 156.5, 166.0. HRMS (ESI): $[M + H]^+$ $C_{11}H_{18}O_3N_3$ calcd 240.1343, found 240.1344. R_f (10% MeOH/CH_2Cl_2) = 0.3.

1-(2-Aminoethyl)pyrimidin-2(1*H*)-one (53). A solution of pyrimidone **52** (97 mg, 0.4 mmol) in 1:1 TFA/CH_2Cl_2 (2 mL) was stirred for 5 h (0 °C−rt). The reaction mixture was poured into ice cold Et_2O. The precipitate was collected by centrifugation and dried under reduced pressure. The crude product **53** (101 mg, 0.4 mmol, quant) was used without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 3.48 (s, br, 2H), 4.40 (s, br, 2H), 6.74 (s, br, 1H), 8.50 (s, br, 1H), 8.74 (s, br, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 38.2, 49.5, 105.3, 153.3, 155.1, 164.9. HRMS (ESI): $[M + H]^+$ $C_6H_{10}ON_3$ calcd 140.0818, found 140.0819.

***N′*-(Hex-5-ynamide)-vancomycin (54).** This compound was prepared according to Eirich et al. The characterization data for this compound matched with those reported in the literature.[53]

***N′*-(Hex-5-ynamide)-*C*-[1-(2-aminoethyl)pyrimidin-2(1*H*)-one]-vancomycin (55).** *N′*-(Hex-5-ynamide)-vancomycin (**54**) (30 mg, 19 μmol), pyrimidone **53** (10 mg, 39 μmol), and DIPEA (16 μL, 95 μmol) were dissolved in DMF. PyBOP (11 mg, 21 μmol) and HOBt (4 mg, 21 μmol) were added, and the reaction mixture was stirred for 16 h at rt. The solution was poured into cold Et_2O, and the precipitate was collected by centrifugation and dried under reduced

pressure. The crude product was purified by HPLC to yield **55** (22 mg, 13 μmol, 68%) as a colorless solid. HRMS (ESI): $[M + H]^+$ $C_{78}H_{89}O_{25}N_{12}Cl^{37}Cl$ calcd 1665.5404, found 1665.5488.

Preparation of Proteome for Photolabeling Experiments. The proteomes of the bacterial strains *Staphylococcus aureus* Mu50, *Escherichia coli* K12, and *Listeria welshimeri* SLCC 5334 were prepared from 1 L liquid cultures in BHB or LB medium and harvested 1 h after transition in the stationary phase by centrifugation at 9000 rpm. The bacterial cell pellet was washed with PBS, resuspended in PBS, and lysed by sonication with a Bandelin Sonopuls instrument under ice cooling. Membrane and cytosol were separated by centrifugation at 9000 rpm for 45 min.

Recombinant Expression. ATLam and pABC were recombinantly expressed in BL21 *E. coli* as described by Eirich et al. For overexpression labeling, recombinant clones were grown in ampicillin LB medium, and target gene expression was induced with anhydrotetracyclin or IPTG depending on the expression vector used.

Proteome Photolabeling Experiments. Proteome samples were adjusted to a final concentration of 1 mg protein/mL by dilution with PBS prior to probe addition. Experiments were carried out in 43 μL total volume, such that once click chemistry reagents were added, the total volume was 50 μL. Probes were added at various concentrations in 1 μL of DMSO to achieve the desired final concentration. Photolysis was carried out over ice cooling in 96-well plates with either 2 × 8 W Hitachi FL8BL-B lamps centered at 350 nm or a 15 W Herolab lamp at 312 nm. The distance from lamp to sample was approximately 2 cm. For heat controls, the proteome was denatured with 2% SDS (4 mL of 21.5% SDS) at 95 °C for 6 min and cooled to room temperature before the probe was applied. Following incubation, reporter-tagged azide reagents and 13 μM rhodamine-azide (1 μL) were added, followed by 1 mM TCEP (1 μL) and 100 μM ligand (3 μL). Samples were gently vortexed, and the cycloaddition was initiated by the addition of 1 mM $CuSO_4$ (1 μL). The reactions were incubated at room temperature for 1 h. For analytical gel electrophoresis, 50 μL of 2 × SDS loading buffer was added, and 50 μL was applied on the gel. Fluorescence was recorded in a Fujifilm Las-4000 luminescent image analyzer with a Fujinon VRF43LMD3 lens and a 575DF20 filter.

■ ASSOCIATED CONTENT

Supporting Information. Copies of NMR spectra and SDS-PAGE gels for time and concentration proteome photolabeling with **17** and **20**, as well as labeling *E. coli* cytosolic proteins with probes **16**—**23**. This material is available free of charge via the Internet at http://pubs.acs.org.

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