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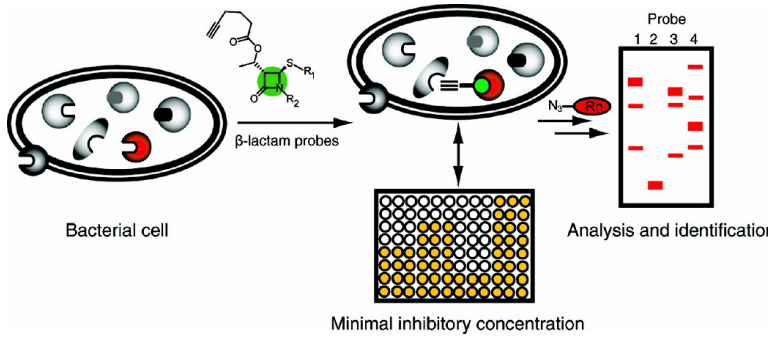

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β-Lactams as Selective Chemical Probes for the in Vivo Labeling of Bacterial Enzymes Involved in Cell Wall Biosynthesis, Antibiotic Resistance, and Virulence

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Abstract: With the development of antibiotic-resistant bacterial strains, infectious diseases have become again a life-threatening problem. One of the reasons for this dilemma is the limited number and breadth of current therapeutic targets for which several resistance strategies have evolved over time. To expand the number of addressable enzyme targets and to understand their function, activity, and regulation, we utilized a chemical proteomic strategy, called activity-based protein profiling (ABPP) pioneered by Cravatt, for the identification of β-lactam-binding enzymes under in vivo conditions. In this two-tiered strategy, we first prepared a selection of conventional antibiotics for labeling diverse penicillin binding proteins (PBPs) and second introduced a new synthetic generation of β-lactam probes, which labeled and inhibited a selection of additional PBP unrelated bacterial targets. Among these, the virulence-associated enzyme ClpP and a resistance-associated β-lactamase were labeled and inhibited by selected probes, indicating that the specificity of β-lactams can be adjusted to versatile enzyme families with important cellular functions.

Introduction

β-Lactams represent one of the most important groups of antibiotics prescribed today. They stop bacterial growth by inhibiting penicillin binding proteins (PBPs), which are indispensable for the cross-linking process during cell-wall biosynthesis.[1,2] These enzymes mistake β-lactam antibiotics for the C-terminal D-Ala-D-Ala end of nascent peptidoglycan and get inactivated by a nucleophilic attack of the active site serine on the electrophilic β-lactame ring, forming an inert acyl-enzyme intermediate. Although PBPs are well-characterized targets of many β-lactam antibiotics, not much is known about the regulation of their activity, which plays an important role during cell growth and division. Moreover, the precise function of some PBP classes such as low molecular weight PBPs (LMW) is still unknown.[3,4] Previously, β-lactams with radioactive or fluorescent tags were used to label and visualize active PBPs in membrane preparations.[5,6] However, these methods were limited either by their time-consuming and hazardous procedures (radioactivity) or by the attachment of large fluorescent dyes onto the core scaffold, which lower target affinity and reduce cell permeability important for in vivo studies. To explore the role of PBPs and other yet unknown targets of β-lactams in their native environment, new chemical-proteomic tools are needed for sensitive labeling and identification via mass spectrometry procedures.

Here, we utilize a novel chemical proteomic strategy referred to as "activity-based proteome profiling" (ABPP) pioneered by Cravatt and co-workers to design small β-lactam probes suitable for in vivo target labeling and identification.[7−10] Inspired by our recently introduced β-lactone probe strategy,[11] we appended the structurally related β-lactam core with a short alkyne handle as a benign tag for visualization and enrichment of labeled proteins. The modification of the alkyne tag via the 1,3-dipolar Huisgen cycloaddition (click chemistry, CC) allows the introduction of the bulky reporter group (e.g., rhodamine) after enzyme binding and cell preparation (Figure 1).[12] Labeled enzymes can be run on SDS gels, visualized by fluorescent scanning, and subsequently identified by mass spectrometry. The binding partners of several naturally occurring β-lactam antibiotics as well as novel synthetic analogues thereof were identified in diverse bacterial proteomes in vitro and in vivo. Interestingly, in addition to PBPs, several other targets with

(1) Macheboeuf, P.; Contreras-Martel, C.; Job, V.; Dideberg, O.; Dessen, A. *FEMS Microbiol. Rev.* **2006**, *30*, 673–91.
(2) Walsh, C. T. *Antibiotics: Actions, Origins, Resistance*; ASM Press: Washington DC, 2003.
(3) Buynak, J. D. *ACS Chem. Biol.* **2007**, *2*, 602–5.
(4) Popham, D. L.; Young, K. D. *Curr. Opin. Microbiol.* **2003**, *6*, 594–9.
(5) Preston, D. A.; Wu, C. Y.; Blaszczak, L. C.; Seitz, D. E.; Halligan, N. G. *Antimicrob. Agents Chemother.* **1990**, *34*, 718–21.
(6) Zhao, G.; Meier, T. I.; Kahl, S. D.; Gee, K. R.; Blaszczak, L. C.; Bocillin, F. L. *Antimicrob. Agents Chemother.* **1999**, *43*, 1124–8.
(7) Evans, M. J.; Cravatt, B. F. *Chem. Rev.* **2006**, *106*, 3279–301.
(8) Evans, M. J.; Saghatelian, A.; Sorensen, E. J.; Cravatt, B. F. *Nat. Biotechnol.* **2005**, *23*, 1303–7.
(9) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686–4687.
(10) Speers, A. E.; Cravatt, B. F. *Chem. Biol.* **2004**, *11*, 535–546.
(11) Böttcher, T.; Sieber, S. A. *Angew. Chem., Int. Ed.* **2008**, *47*, 4600–3.
(12) Sieber, S. A.; Niessen, S.; Hoover, H. S.; Cravatt, B. F. *Nat. Chem. Biol.* **2006**, *2*, 274–81.
(13) von Nussbaum, F.; Brands, M.; Hinzen, B.; Weigand, S.; Habich, D. *Angew. Chem., Int. Ed.* **2006**, *45*, 5072–129.

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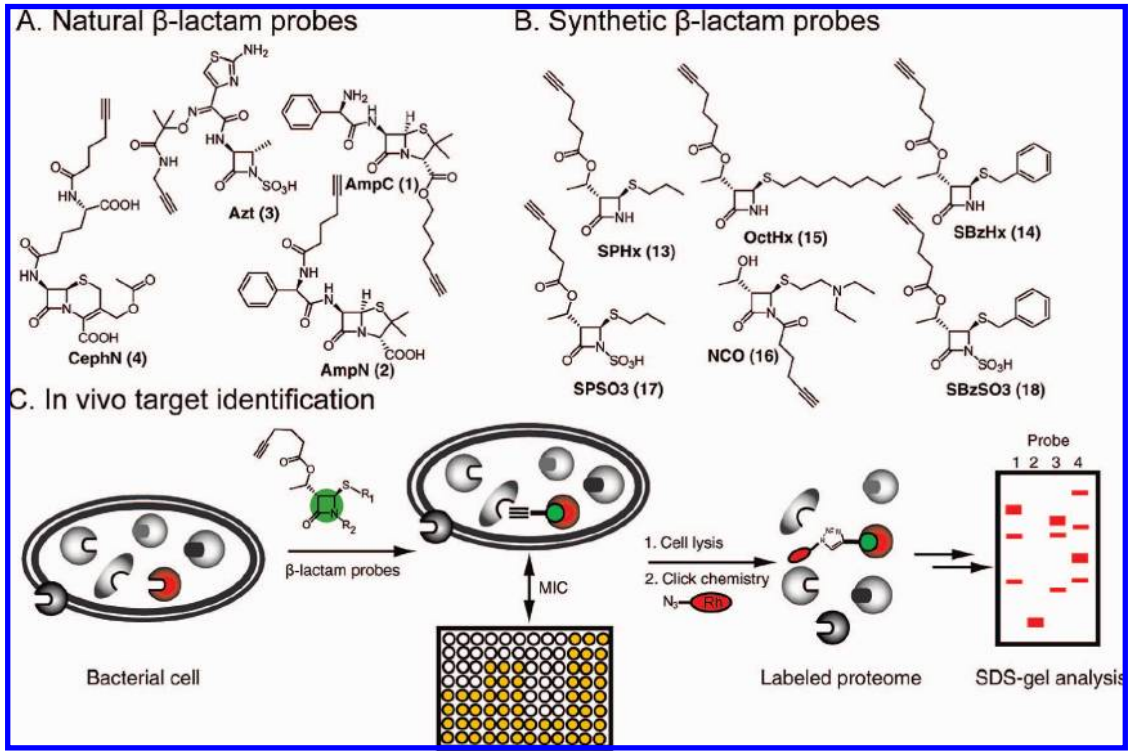
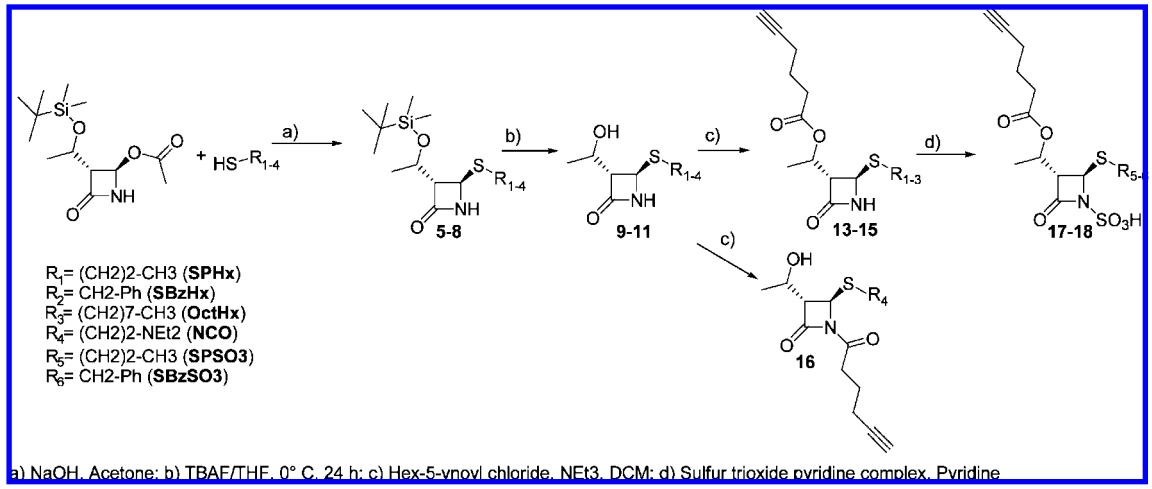


Figure 1. Natural and synthetic β-lactam probes for the labeling of bacterial enzymes in vivo. (A) Structures of natural β-lactam antibiotic probes. (B) Structures of synthetic probes. (C) Intact bacterial cells are first treated with the β-lactam probes followed by cell lysis and subsequent attachment of a fluorescent dye via CC. Labeled proteomes are run on SDS-PAGE and visualized by fluorescence scanning. To evaluate the potency of individual probes, the growth of bacteria is tested in the presence of probes in varying concentrations. The lowest probe concentration that inhibits bacterial growth is referred to as minimal inhibitory concentration (MIC).

Scheme 1. Synthesis of the β-Lactam Probe Library



crucial roles in resistance and virulence could be detected and characterized.

Results and Discussion

Design and Synthesis of β-Lactam Probes. To evaluate the potential of β-lactams to bind dedicated enzymes in proteomes, we first utilized three well-established antibiotics for modification with alkynes including cephalosporin, ampicillin, and aztreonam. This selection comprises three important classes of antibiotics, the chephems, penams, and monobactams,[13] and should contribute to different labeling preferences. The alkyne tag was coupled either on a free amino group (**CephN** (**4**) and **AmpN** (**2**)) or on a carboxylic acid (**Azt** (**3**) and **AmpC** (**1**)) (Figure 1A, Supporting Information Scheme 1).

In addition, we prepared a small library of diverse monocyclic β-lactams with no similarity to established natural products. These probes feature different side chain substitutions and reactivity profiles. We utilized a commercially available 2-azetedinone scaffold as a core structure and introduced different side chains in C4 position and the alkyne tag in C1′ position (Figure 1B, Scheme 1). The intrinsic β-lactam reactivity was modulated in some cases by the sulfonylation or acylation of the lactam amide (Experimental Section). We expect that these variations in structure and reactivity of a common scaffold will help to

increase the chance of selective enzyme labeling events in bacterial proteomes. For synthetic details, please refer to the Experimental Section.

Labeling Profiles of Antibiotic Probes in Bacterial Lysates. Antibiotic probes (Figure 1A) were first tested in *P. putida* proteome, which is phylogenetically related to the pathogenic *P. aeruginosa* strain. Labeling experiments with **CephN** (**4**), **AmpN** (**2**), and **AmpC** (**1**) were carried out by adding individual probes at 5 μM concentration, which was sufficient to achieve full saturation of most targets (Figure 2A, Supporting Information Figure S1). In case of the monocyclic β-lactam **Azt** (**3**), a 50 μM concentration was required for saturation probably due to its lower intrinsic reactivity as compared to the constrained bicyclic compounds. Interestingly, individual probes showed highly distinct reactivity profiles, indicating that individual modifications of the β-lactam core exerted a strong influence over specific probe−protein interactions. A preincubation of the proteome with a 100-fold excess of antibiotic (500 μM ampicillin and cephalosporin and 5 mM aztreonam) and subsequent labeling by the corresponding probe (5 μM **AmpN** (**2**) and **CephN** (**4**) and 50 μM **Azt** (**3**)) revealed that both compete for the same active sites in the great majority of target enzymes, indicating that the attachment of the small alkyne handle does not change preferences in enzyme selectivity (Supporting Information Figure S2). In general, we observed a high degree of specificity with the antibiotic probes. Only one unspecific binding event occurred in the heat denatured control of *P. putida*, emphasizing the preference of β-lactams predominantly for native proteins (Supporting Information Figure S3). In addition, the approach turned out to be very sensitive as most of the targeted proteins were of low abundance as shown by the direct comparison of the relative intensities observed with coomassie staining versus fluorescence scanning (Supporting Information Figure S4). Subsequent target identification by LC−MS analysis (Experimental Section, Supporting Information Table S2) revealed the labeling of several different high molecular weight (HMW) and low molecular weight (LMW) PBPs, but no other PBP unrelated targets, emphasizing the great class specificity of these antibiotics (Table 1). MS results were confirmed by recombinant expression of representative hits and subsequent labeling by the corresponding probes (Figure 2B). Interestingly, the direct comparison between the related **AmpC** (**1**) and **AmpN** (**2**) probes reveals that the alkyne modification via the free amine (**AmpN**) results in intense labeling of PBP4 and PBP5, while there is no binding observed with the carboxylic acid (**AmpC**)-modified probe (Supporting Information Figure S3). This is expected because the free acid of many bicyclic β-lactam antibiotics is a recognition element for PBPs mimicking the D-Ala, D-Ala portion of the natural peptide substrate.[2] A great coverage of different HMW and LMW PBPs was achieved by **Azt** (**3**), while **AmpN** (**2**) and **CephN** (**4**) showed stronger interactions with LMW PBPs only, which highlights the different substrate preferences of individual antibiotics.

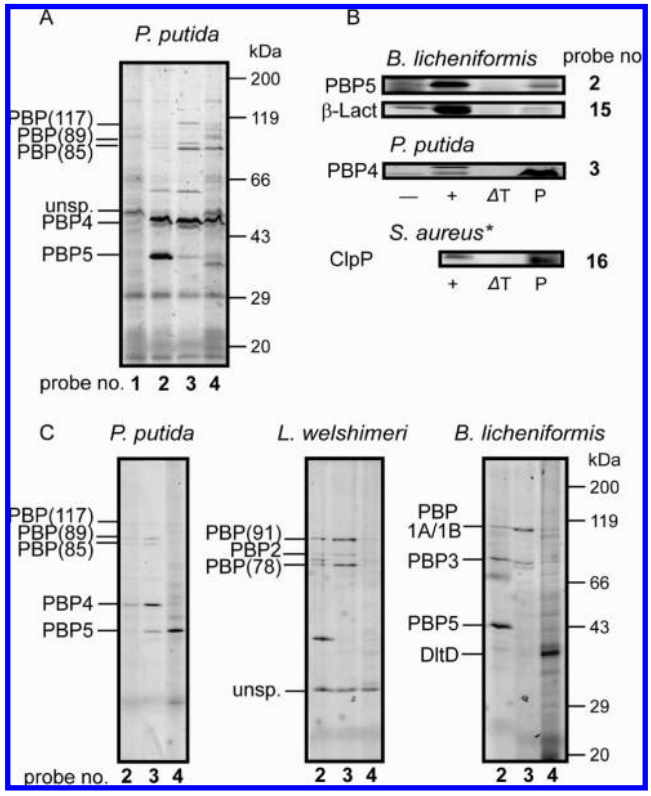


Figure 2. Labeling profiles of antibiotic β-lactam probes in bacterial proteomes. (A) Fluorescent gel of *P. putida* membrane proteome after in vitro treatment with 5 μM **AmpC** (**1**), **AmpN** (**2**), **CephN** (**4**), and 50 μM **Azt** (**3**). Enzyme identities are assigned to the corresponding gel band (for a list of abbreviations, please refer to Table 1). Numbers in brackets refer to the molecular weight (kDa) of unassigned PBPs. (B) Examples of recombinantly expressed enzymes (−, before induction; +, after induction; P, native proteome; ΔT, after induction/heat control). *In case of ClpP, the purified *S. aureus* enzyme was used and compared to ClpP from the *B. licheniformis* proteome. ClpP proteins are highly conserved among gram positive bacteria with a sequence identity of 82% between *B. licheniformis* and *S. aureus*. (C) Fluorescent gel of *P. putida*, *L. welshimeri*, and *B. licheniformis* after in vivo incubation with 250 μM **AmpN** (**2**), **Azt** (**3**), and **CephN** (**4**).

Table 1. Enzymes Identified by Antibiotic Probes

species	protein (kDa)	probe preference
P. putida	PBP(117)	**Azt** (**3**)
	PBP(89)	**Azt** (**3**), **AmpN** (**2**)
	PBP(85)	**Azt** (**3**), **CephN** (**4**), **AmpN** (**2**)
	PBP4(50)	**Azt** (**3**), **AmpN** (**2**), **CephN** (**4**)
	PBP5(42)	**AmpN** (**2**), **Azt** (**2**), **CephN** (**4**)
L. welshimeri	PBP(91)	**Azt** (**3**), **AmpN** (**2**)
	PBP2(82)	**Azt** (**3**), **AmpN** (**2**)
	PBP(78)	**Azt** (**3**), **AmpN** (**2**)
B. Licheniformis	PBP1A/1B(97)	**Azt** (**3**), **AmpN** (**2**), **CephN** (**4**)
	PBP3(73)	**Azt** (**3**), **AmpN** (**2**), **CephN** (**4**)
	PBP5(48)	**AmpN** (**2**)

Labeling of Enzymes in Vivo. The tracking of PBPs in bacterial lysates by individual probes may help to understand the regulation and drug interaction of these enzymes. However, enzyme activities in proteome preparations do often not reflect the situation in living cells.[10] One advantage of our approach is the low molecular weight of the β-lactam probes, which may allow for labeling in vivo prior to the attachment of the bulky reporter tag. To prove this concept, we incubated the three antibiotic probes (**AmpN** (**2**), **CephN** (**4**), and **Azt** (**3**), 250 μM each) with intact *P. putida*, *L. welshimeri*, and *B. licheniformis* for 2 h, lysed the cells after removing excess probe by washing procedures, and subsequently attached the fluorescent tag by CC. The corresponding SDS gels revealed strong labeling of individual targets, which were identified as diverse PBPs (Figure 2C, Table 1), indicating that this method is indeed successful for in vivo target analysis. Time course experiments revealed that the labeling intensity of individual enzymes increased over time, emphasizing that probe binding to dedicated targets occurred indeed in vivo and not after cell lyses (Supporting

Table 2. MIC Values of Bacterial Growth Inhibition Studies

strain	compound	MIC (μM)
P. putida	ampicillin	>100
	AmpN (**2**)	>100
	aztreonam	25
	Azt (**3**)	100
B. licheniformis	ampicillin	1
	AmpN (**2**)	8
	aztreonam	>350
	Azt (**3**)	>350
L. welshimeri	ampicillin	0.5
	AmpN (**2**)	1
	aztreonam	>100
	Azt (**3**)	>100

Information Figure 5). By a direct comparison between *P. putida* in vitro and in vivo labeling, one can see that the profiles are comparable in case of **Azt** (**3**), but differ significantly for **AmpN** (**2**) and **CephN** (**4**) (Supporting Information Table 1, Figure 6). Among the identified PBPs, several enzymes display uncharacterized functions such as PBP2 from *L. welshimeri*, which represents an important partner with a PASTA kinase domain.[1] This labeling approach could be a good starting point to investigate the role and regulation of this enzyme and other less characterized PBPs within the context of their native environments.

PBPs represent essential enzymes for bacteria, and it is well-known that their inhibition correlates with cell viability.[1,4] Because aztreonam and ampicillin are potent antibiotics, we investigated the effect on antibacterial potency after the alkyne tag attachment. MIC values for **AmpN** (**2**) were 8 and 1 μM for *B. licheniformis*[14] and *L. welshimeri*,[15] respectively (Table 2), which represents only a slight drop in potency as compared to the natural antibiotic (1 and 0.5 μM). Contrary to ampicillin and the corresponding **AmpN** (**2**) probe, aztreonam and the **Azt** (**3**) probe did not inhibit growth of *B. licheniformis* and *L. welshimeri* but showed activity against *P. putida* with MIC values of 25 and 100 μM, respectively. These results again suggest that the short tag does not severely interfere with antibiotic activity and that the observed labeling of several PBPs by antibiotic probes correlates well with their essential role for bacterial viability.

Enzyme Labeling Profiles with Novel β-Lactam Probes. Inspired by the great utility and selectivity of antibiotic probes for PBP detection in vivo, we designed and synthesized novel, antibiotic unrelated β-lactam probes to see whether additional enzyme classes could be explored by fine-tuning of the core scaffold (Figure 1B). Six different monocyclic molecules varying in substitution and reactivity were tested under in vivo conditions in all three bacterial systems. Several specific labeling events were detected on fluorescent gels and subsequently identified by MS. Interestingly, these modified β-lactams do not label any PBPs but have preferences for other important enzymes such as β-ketoacyl acyl carrier protein III (KAS III),[16] a β-lactamase (β-Lact), a lipase acylhydrolase (Lip/Ac), a thiol-specific antioxidant (AhpC),[17] and the virulence-associated

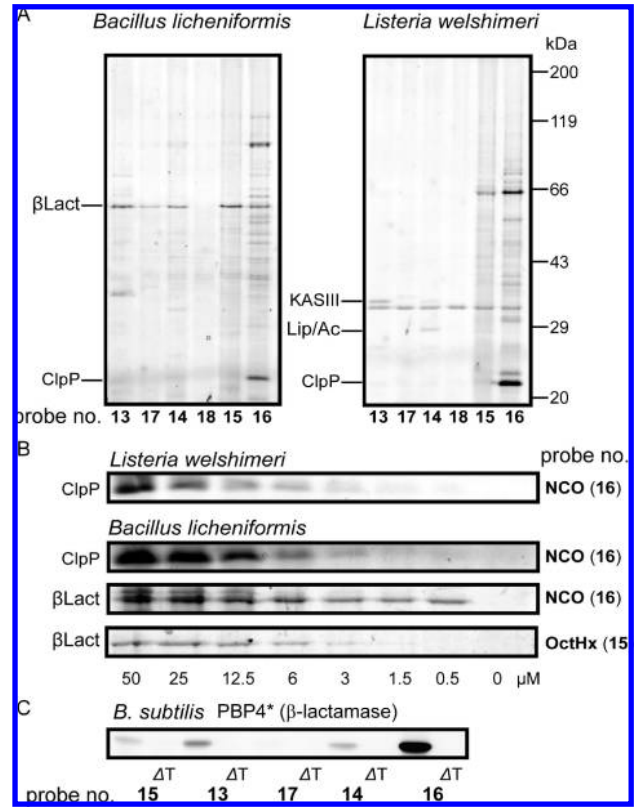


Figure 3. Screen of the synthetic β-lactam library against bacterial proteomes. (A) Fluorescent gel of *B. licheniformis* and *L. welshimeri* proteomes after in vivo treatment with 100 μM **SPHx** (**13**), 250 μM **SPSO3** (**17**), 250 μM **SBzHx** (**14**), 250 μM **SBzSO3** (**18**), 250 μM **OctHx** (**15**), and 10 μM **NCO** (**16**). Enzyme identities are assigned to the corresponding gel band (for a list of abbreviations, please refer to Table 1). (B) Dose down of selected probes for ClpP and β-Lact labeling in vivo. (C) Labeling of the *B. subtilis* β-lactamase by several probes with the corresponding heat controls Δ*T*.

Table 3. Enzymes Identified by Synthetic Probes

species	protein	probe preference
P. putida	KAS III	**SPHX** (**13**), **SBzHX** (**14**)
	AhpC	**NCO** (**16**)
L. welshimeri	KAS III	**SPHX** (13), **SBzHX** (**14**)
	Lip/Ac	**SBzHX** (**14**)
	ClpP	**NCO** (**16**)
B. Licheniformis	β-Lac	**SPHX** (**13**), **SPSO3** (**17**), **SBzHX** (**14**), **OctHX** (**15**), **NCO** (**16**)
	DltD	**CephN** (**4**)
	ClpP	**NCO** (**16**)

protein ClpP[18] (Figure 3A, Supporting Information Figure 7, Table 3), indicating that it is possible to tune the selectivity of monocyclic β-lactams by chemical modifications. Interestingly, these enzymes require a nucleophilic residue in their active site for catalysis (Cys in case of KASIII and AhpC or Ser in case of β-Lact, Lip/Ac, and ClpP), which is likely to attack the electrophilic β-lactam ring. In vivo time course and dose down experiments revealed fast labeling of *B. licheniformis* and *L. welshimeri* ClpP already after 15 min of incubation (Supporting Information Figure S4) and selective labeling down to a probe concentration of only 1.5 μM (Figure 3B). In previous experiments, we have identified ClpP as a specific target of β-lac-

(14) Weber, D. J.; Saviteer, S. M.; Rutala, W. A.; Thomann, C. A. *Antimicrob. Agents Chemother.* **1988**, *32*, 642–5.
(15) Troxler, R.; von Graevenitz, A.; Funke, G.; Wiedemann, B.; Stock, I. *Clin. Microbiol. Infect.* **2000**, *6*, 525–35.
(16) Wang, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7612–6.
(17) Chae, H. Z.; Robison, K.; Poole, L. B.; Church, G.; Storz, G.; Rhee, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7017–21.
(18) Michel, A.; Agerer, F.; Hauck, C. R.; Herrmann, M.; Ullrich, J.; Hacker, J.; Ohlsen, K. *J. Bacteriol.* **2006**, *188*, 5783–96.

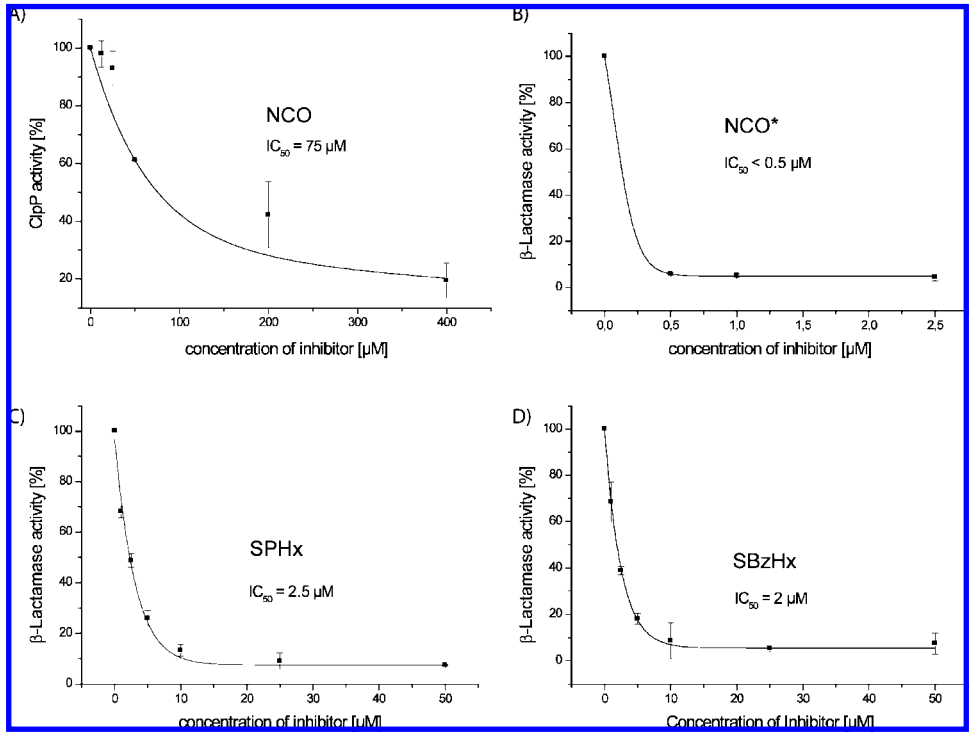


Figure 4. IC_{50} measurements. (A) Inhibition of the active $ClpP_{14}$ complex (116 nM) by **NCO** (**16**). (B) Inhibition of β-lactamase by **NCO** (**16**). *A 500 nM enzyme concentration was required to observe substrate turnover. Although at this concentration enzyme and inhibitor are in an equimolar ratio, full inhibition is still observed. The IC_{50} is therefore below 0.5 μM. (C) Inhibition of β-lactamase (500 nM) by **SPHx** (**13**). (D) Inhibition of β-lactamase (500 nM) by **SBzHx** (**14**).

tones.[11] Here, we show that in addition it can also be labeled and inhibited by the β-lactam probe **NCO** (**16**). ClpP is a central regulator of virulence, which is highly conserved in many pathogens such as *S. aureus*. In fact, labeling of recombinant *S. aureus* ClpP was achieved with the **NCO** (**16**) probe (Figure 2B). **NCO** (**16**)-mediated inhibition of the active $ClpP_{14}$ complex (116 nM) was measured by the enzymatic hydrolysis of the ClpP fluorogenic substrate *N*-succinyl-Leu-Tyr-7-amidomethylcoumarin as described previously,[19] and an IC_{50} of 75 μM was obtained (Figure 4A). Although this inhibition is moderate, the scaffold represents a good starting point for further optimization of potency. **NCO** (**16**) is the only probe of the library acylated at the amide group, which could explain the increase in reactivity comparable to that of β-lactones. All new compounds were tested for growth inhibition of the corresponding bacteria, but contrary to the previous antibiotic probes no effect on viability was observed. However, the lack of antibiotic activity can be explained by their preference to bind predominantly targets that are not essential for viability, with the exception of KASIII. Similar to our observations with lactones, it is likely that this enzyme is only partially inhibited by the lactam, which is not sufficient to observe a biological effect.

Inhibition of *B. licheniformis* β-Lactamase Activity. Several probes labeled a β-lactamase enzyme in *B. licheniformis* with high sensitivity and selectivity as shown by in vivo dose down experiments. Because β-lactamases represent a major reason for antibiotic resistance in many pathogenic bacterial strains, tools to monitor their activity and regulation are of paramount interest. We therefore characterized our best hits in terms of target sensitivity and selectivity. In these experiments, **OctHx** (**15**) turned out to be the most selective probe with no off targets observed even at a concentration of 50 μM in the background of the total proteome and a detection limit of 3 μM (Figure 3B, Supporting Information Figure 8A). In addition, the more reactive **NCO** (**16**) probe exhibited an even lower detection limit of <0.5 μM but showed labeling of additional targets at concentrations >3 μM (Figure 3B, Supporting Information Figure 8B). The selective and sensitive labeling of the *B. licheniformis* β-lactamase by several probes in vivo raised the question if these compounds would also impair the catalytic activity of the enzyme. To answer this question, the β-lactamase was recombinantly overexpressed, purified, and its activity measured by the hydrolysis of the chromogenic *p*-nitrophenylacetate substrate. Indeed, significant inhibition by **SPHx** (**13**), **SBzHx** (**14**), and **NCO** (**16**) could be obtained with corresponding IC_{50}-values of 2.5, 2, and <0.5 μM, respectively (Figure 4B,C). The long chain hydrophobic compound **OctHx** (**15**) was not soluble at concentrations above 25 μM in the lactamase assay buffer but revealed still significant inhibition below 10 μM. In addition, a different recombinant β-lactamase from *B. subtilis* (PBP4*) also revealed specific labeling with all probes except for the sulfonylated compound **SPSO3** (**17**) (Figure 3C), suggesting a broader applicability of the methodology in lactamase labeling. In general, these results emphasize the utility of our β-lactam method to identify potent inhibitors for bacterial targets such as β-lactamases and study their function and regulation in vivo. Future studies with β-lactam resistant pathogens will show whether these probes can help to restore

(19) Maurizi, M. R.; Thompson, M. W.; Singh, S. K.; Kim, S. H. *Methods Enzymol.* **1994**, *244*, 314–31.

(20) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. *Chem. Rev.* **2005**, *105*, 395–424.

or increase antibiotic susceptibility and help to overcome the pressing problem of antibiotic resistance.

Conclusion

Bacterial resistance has become a life-threatening problem in the treatment of infectious diseases. To understand the function, activity, and regulation of important bacterial target enzymes, we introduced a chemical proteomic strategy for the labeling and identification of β-lactam-binding enzymes under in vivo conditions. Interestingly, the small probe library did not only label PBPs, which are known β-lactam targets, but also PBP unrelated enzymes. This was achieved by a selection of novel substituted β-lactam compounds that exhibited distinct target preferences toward enzymes important for resistance and virulence, which shows that the electrophilic β-lactam ring is amenable for the inhibition of different enzyme classes by a suitable decoration of the core scaffold. This includes modifications of the amide nitrogen atom by sulfonation or alkylation, which fine-tunes the intrinsic reactivity of these compounds as we observed in our studies. Future studies with pathogenic and resistant[20] strains will show if these compounds are suitable to label targets with essential roles in pathogenesis and resistance and help to contribute to a better understanding of their in vivo activity and function.

Experimental Section

General. All chemicals were of reagent grade or better and used without further purification. Chemicals and solvents were purchased from Sigma Aldrich or Acros Organics. For all reactions, only commercially available solvents of ultra pure grade, dried over molecular sieve and stored under argon atmosphere, were used. Solvents for chromatography and workup purposes were generally of reagent grade and purified before use by distillation. All experiments were carried out under nitrogen. Column chromatography was performed on Merck silica gel (Acros Organics 0.035−0.070 mm, mesh 60 Å). ^{1}H NMR spectra were recorded on a Varian Mercury 200 (200 MHz), a Varian NMR-System 600 (600 MHz), or a Varian NMR-System 300 (300 MHz), and ^{13}C NMR spectra were measured with a Varian NMR-System 600 (600 MHz) and a Varian NMR-System 300 (300 MHz) and referenced to the residual proton and carbon signal of the deuterated solvent, respectively. ESI spectra were recorded with a Thermo Finnigan LTQ FT. HPLC analysis was accomplished with a Waters 2695 separations module, a X-Bridge BEH130 C18 column (4.6 × 100 mm), and a Waters 2996 PDA detector. Mobile phase (HPLC grade): A = water, 0.1% (v/v) TFA, B = acetonitrile, 0.1% (v/v) TFA.

Synthesis of β-Lactam Probes. AmpC (1). To a solution of ampicillin (185.7 mg, 0.50 mmol, 1.0 equiv) in absolute DMF (1.4 mL) was slowly added methanesulfonic acid hex-5-ynyl ester in DMF (0.5 mL). The mixture was stirred overnight, and then EtOAc (15 mL) was added. After the suspension was filtered, the EtOAc solution was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by preparative HPLC. From the obtained main fraction, the acetonitrile was removed under reduced pressure, and the residual aqueous suspension was dried by lyophilization to afford **AmpC** (10.8 mg, 5%). ^{1}H NMR (400 MHz, CD_3CN): δ 7.61 (d, J = 7.8 Hz, 1H, NH), 7.42−7.51 (m, 5H, Arom.), 5.57 (dd, J = 7.9, 4.1 Hz, 1H, *O*=C−CH), 5.46 (d, J = 4.1 Hz, 1H, N−CH), 5.23 (s, 1H, NH_2−CH), 4.36 (s, 1H, COO−CH), 4.16 and 4.15 (ABCD, J = 14.2, 10.9, 6.4 Hz, each 1H, COO−CH_2), 2.21 (dtr, J = 7.0, 2.7 Hz, 2H, CH_2−C′CH), 2.17 (tr, J = 2.6 Hz, 1H, C′CH), 1.74 (psquint, J = 7.2 Hz, 2H, COO−CH_2−CH_2), 1.57 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−C′CH), 1.45 (s, 3H, CH_3), 1.38 (s, 3H, CH_3). ^{13}C NMR (101 MHz, CD_3CN): δ 172.9, 168.5, 168.4, 133.4, 130.8, 130.0, 129.4, 84.8, 71.1, 70.0, 68.4, 65.9, 65.3, 60.2, 57.3, 32.2, 28.2, 26.9, 25.7, 18.3. HRMS calcd for $C_{22}H_{26}N_3O_4S^-$ ($M - H^+$) 428.16495, found 428.1651; calcd for $C_{22}H_{28}N_3O_4S^+$ ($M + H^+$) 430.17950, found 430.1823.

AmpN (2).[21] Isobutyl chloroformate (42.5 μL, 0.32 mmol, 1.3 equiv) was added under stirring at −13 °C to a solution of 5-hexynoic acid (35.8 μL, 0.32 mmol, 1.3 equiv) and *N*-methyl morpholine (35.7 μL, 0.32 mmol, 1.3 equiv) in absolute THF (5 mL). After the reaction mixture was kept for 45 min at −10 °C, a suspension of ampicillin sodium salt (92.8 mg, 0.25 mmol, 1.0 equiv) in tetrahydrofuran/water (3.7 mL, 20:1) was added. The mixture was stirred for 45 min at −10 °C and then allowed to warm to room temperature. The solution was concentrated under reduced pressure, and the residue was purified by preparative HPLC. From the obtained main fraction, the acetonitrile was removed under reduced pressure, and the residual aqueous suspension was dried by lyophilization to afford **AmpN** (4.7 mg, 4%). ^{1}H NMR (400 MHz, CD_3CN): δ 7.32−7.41 (m, 5H, Arom.), 7.25 (d, J = 8.7 Hz, 1H, *O*=C−CH−NH), 7.13 (d, J = 7.0 Hz, 1H, *O*=C−NH), 5.58 (dd, J = 8.7, 4.2 Hz, 1H, *O*=C−CH), 5.47 (d, J = 7.0 Hz, 1H, Ph−CH), 5.43 (d, J = 4.2 Hz, 1H, N−CH), 4.35 (s, 1H, N−CH−COOH), 2.35 and 2.31 (ABCD, each 1H, J = 19.5, 15.0, 7.4 Hz, NHCO−CHH), 2.20 (dt, J = 7.1, 2,6 Hz, 2H, CH_2−C′CH), 2.16−2.19 (m, 1H, CH_2−C′CH), 1.76 (psquint, J = 7.1 Hz, 2H, COO−CH_2−CH_2), 1.57 (s, 3H, CH_3), 1.47 (s, 3H, CH_3). ^{13}C NMR (101 MHz, CD_3CN): δ 174.2, 172.8, 170.8, 169.0, 138.5, 129.6, 129.1, 128.5, 84.7, 71.0, 70.1, 68.4, 65.0, 59.6, 58.0, 35.0, 31.7, 27.0, 25.2, 18.3. MS (MALDI-ToF, HCCA): 444.0 ($M + H^+$), 466.0 ($M + Na^+$), 482.0 ($M + K^+$).

Azt (3). To a solution of aztreonam (60.0 mg, 0.14 mmol, 1.0 equiv) in absolute DMF (3 mL) were added HOBt (27.9 mg, 0.21 mmol, 1.5 equiv), DIC (32.0 μL, 0.21 mmol, 1.5 equiv), and DIPEA (60.0 μL, 0.34 mmol, 2.5 equiv) under nitrogen at 0 °C. After the mixture was stirred for 30 min at 0 °C, propargylamine (13.2 μL, 0.21 mmol, 1.5 equiv) was added. The mixture was allowed to warm to room temperature after 30 min and then concentrated under reduced pressure. The residue was directly purified by preparative HPLC to give **Azt** (12.5 mg, 19%) as a white solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.40 (d, J = 8.2 Hz, 1H, NH), 7.76 (tr, J = 5.8, 1H, NH), 6.91 (s, 1H, SCH), 4.54 (dd, J = 8.2, 2.6 Hz, 1H, NH−CH−CO), 3.90 (dd, J = 5.9, 2.4 Hz, 2H, NH−CH_2), 3.72 (dqu, J = 6.2, 2.5 Hz, 1H, NSO_3H−CH), 3.01 (tr, J = 2.5 Hz, 1H, C′CH), 1.43 (d, J = 6.4 Hz, 3H, CH_3−CH−NSO_3H), 1.42 (s, 3H, C−(CH_3)$_2$), 1.40 (s, 3H, C−(CH_3)$_2$). ^{13}C NMR (101 MHz, d_6-DMSO): δ 173.3, 169.7, 162.3, 161.7, 148.2, 138.7, 111.3, 83.8, 81.6, 73.2, 60.6, 57.5, 28.6, 24.6, 24.1, 18.4. HRMS calcd for $C_{16}H_{19}N_6O_7S_2^-$ ($M - H^+$) 471.07621, found 471.0759; calcd for $C_{16}H_{21}N_6O_7S_2^+$ ($M + H^+$) 473.09077, found 473.0902.

CephN (4).[21] Isobutyl chloroformate (95.6 μL, 0.73 mmol, 1.4 equiv) was added under stirring at −13 °C to a solution of 5-hexynoic acid (83.1 μL, 0.73 mmol, 1.4 equiv) and *N*-methyl morpholine (80.3 μL, 0.73 mmol, 1.4 equiv) in absolute THF (10 mL). After the reaction mixture was kept for 1 h at −10 °C, a suspension of cephalosporin C zinc salt (250 mg, 0.52 mmol, 1.0 equiv) and triethylamine (70 μL) in tetrahydrofuran/water (3.6 mL, 6:1) was added. The mixture was stirred for 30 min at −10 °C and then allowed to warm to room temperature. The solution was concentrated under reduced pressure, and the residue was purified by preparative HPLC. From the obtained main fraction, the acetonitrile was removed under reduced pressure, and the residual aqueous suspension was dried by lyophilization to afford 24.1 mg of **CephN** (9%). ^{1}H NMR (400 MHz, MeCN/D_2O 10:1): δ 5.74 (d, J = 4.8 Hz, 1H, NHCO−CH−NHCO), 5.04 (d, J = 4.9 Hz, 1H, N−CH−S), 5.02 (d, J = 13.8 Hz, 1H, CHH−COOMe), 4.76 (d, J = 13.1 Hz, 1H, CHH−COOMe), 4.30−4.34 (m, 1H, COOH−CH−NHCO), 3.63 (d, J = 18.3 Hz, 1H, S−CHH), 3.45 (d, J = 18.4 Hz, 1H, S−CHH), 2.25−2.35 (m, 2H, CH_2−CONH), 2.19−2.24 (m, 3H, CH_2−C′CH), 2.04 (s, 3H, COO−CH_3),

(21) Heinisch, L.; Wittmann, S.; Stoiber, T.; Berg, A.; Ankel-Fuchs, D.; Mollmann, U. *J. Med. Chem.* **2002**, *45*, 3032–40.

1.60−1.86 (m, 6H, $C\underline{H}_2-CH_2-C'CH$, $COOH-CH-C\underline{H}_2-C\underline{H}_2$). ^{13}C NMR (101 MHz, CD_3CN/ D_2O 10:1): δ 173.8, 173.4, 173.3, 171.1, 165.4, 162.9, 126.1, 125.6, 84.1, 69.5, 63.1, 59.5, 57.6, 52.0, 34.6, 34.4, 30.7, 26.1, 24.6, 21.8, 20.2, 17.5. HRMS calcd for $C_{22}H_{26}N_3O_9S^-$ ($M - H^+$) 508.13953, found 508.1391; calcd for $C_{22}H_{28}N_3O_9S^+$ ($M + H^+$) 510.15408, found 510.1536.

Methanesulfonic Acid Hex-5-ynyl Ester.[22] To a stirred solution of hex-5-yn-1-ol (451.9 μL, 4.00 mmol, 1.0 equiv) and DMAP (48.9 mg, 0.40 mmol, 0.1 equiv) in DCM (50 mL) was added triethylamine (672.4 μL, 4.80 mmol, 1.2 equiv) under nitrogen at 0 °C. Subsequently, methanesulfonyl chloride (342.0 μL, 4.40 mmol, 1.1 equiv) was added dropwise. After 2 h of stirring at 0 °C, the solution was allowed to warm to room temperature overnight. The reaction mixture was washed with aqueous 0.5 m HCl and $NaHCO_3$ (saturated), dried over $MgSO_4$, and evaporated to afford methanesulfonic acid hex-5-ynyl ester (540.1 mg, 77%). ^1H NMR (200 MHz, $CDCl_3$): δ 4.27 (tr, J = 6.2 Hz, 2H, $MeSO_3C\underline{H}_2$), 3.01 (s, 3H, $C\underline{H}_3$), 2.27 (dt, J = 6.8, 2.6 Hz, 2H, $C\underline{H}_2-C'CH$), 1.98 (tr, J = 2.7 Hz, 1H, $C'C\underline{H}$), 1.86 (psquint, J = 6.8 Hz, 2H, $O-CH_2-C\underline{H}_2$), 1.62 (psquint, J = 6.8 Hz, 2H, $C\underline{H}_2-CH_2-C'CH$).

[3*R*(1′*R*,4*R*)]-3-[1-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-4-propylsulfanyl-azetidin-2-one (5).[23] To a solution of 1-propanethiol (230 μL, 189.3 mg, 2.49 mmol, 1.3 equiv) in acetone (2.3 mL) was added 1 M NaOH (2.6 mL) dropwise. The mixture was stirred for 15 min at room temperature, and then [3*R*(1′*R*,4*R*)]-(+)-4-acetoxy-3-[1-(*tert*-butyldimethylsilyloxy)ethyl]-2-azetidinone (550.0 mg, 1.91 mmol, 1.0 equiv) in acetone (4.5 mL) was added. After being stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and extracted with Et_2O. The Et_2O solution was washed with water and brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification of the residue by flash column chromatography on silica gel (DCM/MeOH 12:1) yielded **5** (520.3 mg, 90%) as a white solid. ^1H NMR (200 MHz, $CDCl_3$): δ 5.97 (br, 1H, $N-\underline{H}$), 4.83 (d, J = 2.3 Hz, 1H, $NH-C\underline{H}S$), 4.25 (dq, J = 6.3 Hz, 3.6 Hz, 1H, $C\underline{H}O$), 3.09−3.14 (m, 1H, $O{=}C-C\underline{H}$), 2.60 (tr, J = 7.3 Hz, 2, $S-C\underline{H}_2$), 1.64 (pssext, J = 7.3 Hz, 2H, $S-CH_2-C\underline{H}_2$), 1.24 (d, J = 6.3 Hz, 3H, $C\underline{H}_3-CHO$), 1.01 (tr, J = 7.3 Hz, 3H, $S-CH_2-CH_2-C\underline{H}_3$), 0.86 (s, 9H, Si−*t*-butyl), 0.06−0.08 (2s, 6H, $Si-C\underline{H}_3$).

[3*R*(1′*R*,4*R*)]-4-Benzylsulfanyl-3-[1-(*tert*-butyl-dimethyl-silanyloxy)-ethyl]-azetidin-2-one (6).[23] To a solution of benzylmercaptane (379 μL, 399.3 mg, 3.22 mmol, 1.4 equiv) in acetone (2.8 mL) was added 1 M NaOH (3.4 mL) dropwise. The mixture was stirred for 15 min at room temperature, and then [3*R*(1′*R*,4*R*)]-(+)-4-acetoxy-3-[1-(*tert*-butyldimethylsilyloxy)ethyl]-2-azetidinone (660 mg, 2.30 mmol, 1.0 equiv) in acetone (4.5 mL) was added. Reaction, workup, and purification followed the same procedure as described for compound **5**. Compound **6** (685.7 mg, 85%) was isolated as a yellow solid. ^1H NMR (200 MHz, $CDCl_3$): δ 7.28−7.35 (m, 5H, Ar), 5.30 (br, 1H, $N-\underline{H}$), 4.78 (d, J = 2.2 Hz, 1H, $NH-C\underline{H}S$), 4.18 (dq, J = 6.4 Hz, 3.2 Hz, 1H, $C\underline{H}O$), 3.86 (s, 2H, $S-C\underline{H}_2-Ph$), 3.02−3.06 (m, 1H, $O{=}C-C\underline{H}$), 1.16 (d, J = 6.4 Hz, 3H, $C\underline{H}_3-CHO$), 0.85 (s, 9H, Si−*t*-butyl), 0.01−0.06 (2s, 6H, $Si-C\underline{H}_3$).

[3*R*(1′*R*,4*R*)]-3-[1-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-4-octylsulfanyl-azetidin-2-one (7).[23] To a solution of 1-octanethiol (290 μL, 244.3 mg, 1.67 mmol, 1.2 equiv) in acetone (1.6 mL) was added 1 M NaOH (1.8 mL) dropwise. The mixture was stirred for 15 min at room temperature, and then [3*R*(1′*R*,4*R*)]-(+)-4-acetoxy-3-[1-(*tert*-butyldimethylsilyloxy)ethyl]-2-azetidinone (400 mg, 1.39 mmol, 1.0 equiv) in acetone (1.4 mL) was added. Reaction, workup, and purification followed the same procedure as described for compound **5**. Compound **7** (468.1 mg, 90%) was isolated as a white solid. ^1H NMR (200 MHz, $CDCl_3$): δ 5.92 (br, 1H, $N-\underline{H}$), 4.83 (d, J = 2.3 Hz, 1H, $NH-C\underline{H}S$), 4.25 (dq, J = 6.4 Hz, 3.4 Hz, 1H, $C\underline{H}O$), 3.09−3.14 (m, 1H, $O{=}C-C\underline{H}$), 2.61 (tr, J = 7.3 Hz, 2H, $S-C\underline{H}_2$), 1.60 (psquint, J = 7.3 Hz, 2H, $S-CH_2-C\underline{H}_2$), 1.26−1.45 (m, 10H, $S-CH_2-CH_2-C_5\underline{H}_{10}-CH_3$), 1.23 (d, J = 6.4 Hz, 3H, $C\underline{H}_3-CHO$), 0.88 (s, 9H, Si−*t*-butyl), 0.85−0.94 (m, 3H, $S-C_7H_{14}-C\underline{H}_3$), 0.07−0.09 (2s, 6H, $Si-C\underline{H}_3$).

[3*R*(1′*R*,4*R*)]-3-[1-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-4-(2-diethylaminoethylsulfanyl)-azetidin-2-one (8).[23] To a suspension of 2-diethylaminoethanethiol (391.2 mg, 2.07 mmol, 1.2 equiv) in acetone (2.0 mL) was added 1 M NaOH (4.3 mL) dropwise. The mixture was stirred for 15 min at room temperature, and then [3*R*(1′*R*,4*R*)]-(+)-4-acetoxy-3-[1-(*tert*-butyldimethylsilyloxy)ethyl]-2-azetidinone (496.1 mg, 0.17 mmol, 1.0 equiv) in acetone (1.7 mL) was added. After being stirred overnight at room temperature, the reaction mixture was concentrated under reduced pressure and extracted with EtOAc. The EtOAc solution was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification of the residue by flash column chromatography on silica gel (DCM/MeOH 12:1) yielded (329.2 mg, 53%) **8** as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 6.90 (br, 1H, $N-\underline{H}$), 4.77 (d, J = 2.4 Hz, 1H, $NH-C\underline{H}S$), 4.25 (dq, J = 6.3 Hz, 3.8 Hz, 1H, $C\underline{H}O$), 2.98−3.03 (m, 1H, $O{=}C-C\underline{H}$), 2.70−2.78 (m, 4H, $S-C\underline{H}_2-C\underline{H}_2$), 2.40−2.70 (m, 4H, $N-C\underline{H}_2-CH_3)_2$), 1.23 (d, J = 6.3 Hz, 3H, $C\underline{H}_3-CHO$), 1.06 (tr, J = 7.2 Hz, 6H, $N-(CH_2-C\underline{H}_3)_2$), 0.87 (s, 9H, Si−*t*-butyl), 0.07 (2s, 6H, $Si-C\underline{H}_3$).

[3*R*(1′*R*,4*R*)]-3-(1-Hydroxy-ethyl)-4-propylsulfanyl-azetidin-2-one (9). TBAF (5.1 mL, 1 M solution in THF, 5.14 mmol, 3.0 equiv) was added directly to **5** (520.3 mg, 1.71 mmol, 1.0 equiv) within 10 min at 0 °C under nitrogen. The reaction mixture was stirred overnight and then concentrated under reduced pressure and extracted with EtOAc. The EtOAc solution was washed with 1 M HCl and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (^{i}hexane/EtOAc 1:4) to afford **9** (151.1 mg, 47%) as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 6.73 (br, 1H, N−H), 4.83 (d, J = 2.3 Hz, 1H, $NH-C\underline{H}S$), 4.24 (dq, J = 6.4, 4.3 Hz, 1H, $C\underline{H}O$), 3.14 (ddd, J = 4.3, 2.3, 0.7 Hz, 1H, $O{=}C-C\underline{H}$), 2.60 (tr, J = 7.3 Hz, 2H, $S-C\underline{H}_2$), 1.64 (pssext, J = 7.3 Hz, 2H, $S-CH_2-C\underline{H}_2$), 1.29 (d, J = 6.4 Hz, 3H, $C\underline{H}_3-CHO$), 0.99 (tr, J = 7.3 Hz, 3H, $S-CH_2-CH_2-C\underline{H}_3$).

[3*R*(1′*R*,4*R*)]-4-Benzylsulfanyl-3-(1-hydroxy-ethyl)-azetidin-2-one (10). TBAF (6.3 mL, 1 M solution in THF, 6.30 mmol, 3.2 equiv) was added directly to **6** (685.7 mg, 1.95 mmol, 1.0 equiv) within 10 min at 0 °C under nitrogen. The reaction mixture was stirred overnight and then concentrated under reduced pressure, and the residue was extracted with EtOAc. The EtOAc solution was washed with 1 M HCl and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (^{i}hexane/EtOAc 1:2) to afford **10** (262.1 mg, 57%) as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ 7.28−7.37 (m, 5H, Ar), 5.41 (br, 1H, $N-\underline{H}$), 4.77 (d, J = 2.5 Hz, 1H, $NH-C\underline{H}S$), 4.20 (dq, J = 6.4, 4.7 Hz, 1H, $C\underline{H}O$), 3.88 (s, 2H, $S-C\underline{H}_2-Ph$), 3.10 (ddd, J = 4.7, 2.5, 0.9 Hz, 1H, $O{=}C-C\underline{H}$), 1.27 (d, J = 6.4 Hz, 3H, $C\underline{H}_3-CHO$).

[3*R*(1′*R*,4*R*)]-3-(1-Hydroxy-ethyl)-4-octylsulfanyl-azetidin-2-one (11). TBAF (4 mL, 1 M solution in THF, 4.00 mmol, 3.2 equiv) was added directly to **7** (468.1 mg, 1.25 mmol, 1.0 equiv) within 10 min at 0 °C under nitrogen. The reaction mixture was stirred overnight and then concentrated under reduced pressure, and the residue was extracted with EtOAc. The EtOAc solution was washed with 1 M HCl and brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel (^{i}hexane/EtOAc 1:4) to afford **11** (153.7 mg, 47%) as a yellow solid. ^1H NMR (200 MHz, $CDCl_3$): δ 6.23 (br, 1H, $N-\underline{H}$), 4.84 (d, J = 2.4 Hz, 1H, $NH-C\underline{H}S$), 4.26 (dq, J = 6.4 Hz, 4.6 Hz, 1H, $C\underline{H}O$), 3.18 (ddd, J = 4.6, 2.4, 0.7 Hz, 1H, $O{=}C-C\underline{H}$), 2.63 (tr, J = 7.3 Hz, 2H, $S-C\underline{H}_2$), 1.59 (psquint, J = 7.3 Hz, 2H, $S-CH_2-C\underline{H}_2$), 1.32 (d, J = 6.4 Hz, 3H, $C\underline{H}_3-CHO$), 1.22−1.70 (m, 10H, $S-CH_2-CH_2-C_5\underline{H}_{10}-CH_3$), 0.88 (tr, J = 6.4 Hz, 3H, $S-C_7H_{14}-C\underline{H}_3$).

(22) Murat Kizil, B. P.; Callaghan, O.; Murphy, J. A.; Hursthouse, M. B. *J. Org. Chem.* **1999**, *64*, 7856–7862.

(23) Moreira, R.; Santana, A. B.; Iley, J.; Neres, J.; Douglas, K. T.; Horton, P. N.; Hursthouse, M. B. *J. Med. Chem.* **2005**, *48*, 4861–70.

[3*R*(1′*R*,4*R*)]-4-(2-Diethylamino-ethylsulfanyl)-3-(1-hydroxy-ethyl)-azetidin-2-one (12). TBAF (2.4 mL, 1 M solution in THF, 2.40 mmol, 3.0 equiv) was added directly to **8** (286.2 mg, 0.79 mmol, 1.0 equiv) within 10 min at 0 °C under nitrogen. The reaction mixture was stirred overnight and then evaporated under reduced pressure. The residue was purified by HPLC to afford 127.8 mg (65%) of **12** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.77 (d, J = 2.3 Hz, 1H, NH−CHS), 4.23 (m, 1H, CHO), 3.07 (dd, J = 4.9, 2.3 Hz, 1H, *O*=C−CH), 2.60−2.80 (m, 4H, S−CH_2−CH_2), 2.40−2.60 (m, 4H, N−(CH_2−CH_3)$_2$), 1.30 (d, J = 6.4 Hz, 3H, CH_3−CHO), 1.03 (tr, J = 7.2 Hz, 6H, N−(CH_2−CH_3)$_2$. HRMS calcd for $C_{11}H_{23}N_2O_2S^+$ (M + H^+) 247.14747, found 247.1476.

[3*R*(1′*R*,4*R*)]-Hex-5-ynoic Acid 1-(2-Oxo-4-propylsulfanyl-azetidin-3-yl)-ethyl Ester (13, SPHx). Compound **9** (68.2 mg, 0.36 mmol, 1.0 equiv) was dissolved in DCM (8 mL) followed by the addition of hex-5-ynoyl chloride (70.6 mg, 0.54 mmol, 1.5 equiv) in nitrogen atmosphere. Hex-5-ynoyl chloride was prepared as described previously.[24] After the mixture was stirred for 10 min, NEt_3 (75.3 μL, 54.7 mg, 0.54 mmol, 1.5 equiv) was added and stirred for 1 h. Subsequently, the solution was evaporated and the residue directly purified by flash column chromatography on silica gel (^{i}hexane/EtOAc 2:1) to afford a colorless oil, which was in addition purified by HPLC to give **13** (9.8 mg, 10%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.09 (br, 1H, N−H),), 5.29 (psquint, J = 6.6 Hz, 1H, CHO), 4.75 (d, J = 2.4 Hz, 1H, NH−CHS), 3.24 (ddd, J = 7.1, 2.4, 0.9, 1H, *O*=C−CH), 2.57−2.61 (2tr, J = 7.3 Hz, 2H, S−CH_2), 2.45 (tr, J = 7.4 Hz, 2H, COOCH_2), 2.27 (dt, J = 6.9, 2.5 Hz, 2H, CH_2−C′CH), 1.97 (tr, J = 2.6 Hz, 1H, C′CH), 1.85 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−COO), 1.64 (psquint, J = 7.2 Hz, 2H, S−CH_2−CH_2), 1.39 (d, J = 6.4 Hz, 3H, CH_3−CHO), 1.01 (tr, J = 7.3 Hz, 3H, S−CH_2−CH_2−CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.1, 165.3, 83.2, 69.2, 67.6, 63.9, 56.3, 33.0, 32.2, 23.6, 23.3, 18.3, 17.8, 13.4. HRMS calcd for $C_{14}H_{22}NO_3S^+$ (M + H^+) 284.13149, found 284.1320; calcd for $C_{14}H_{21}NNaO_3S^+$ (M + Na^+) 306.11343, found 306.1140.

[3*R*(1′*R*,4*R*)]-Hex-5-ynoic Acid 1-(2-Benzylsulfanyl-4-oxo-azetidin-3-yl)-ethyl Ester (14, SBzHx). Compound **10** (58.3 mg, 0.25 mmol, 1.0 equiv) was dissolved in DCM (6 mL) followed by the slow addition of hex-5-ynoyl chloride (114.9 mg, 0.88 mmol, 3.6 equiv) in nitrogen atmosphere. After the mixture was stirred for 10 min, NEt_3 (114 μL, 82.76 mg, 0.82 mmol, 3.3 equiv) was added and stirred overnight. Subsequently, the solution was evaporated and the residue directly purified by flash column chromatography on silica gel (^{i}hexane/EtOAc 3:1) to afford **15** (24.8 mg, 30%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.26−7.36 (m, 5H, Arom.), 5.57 (br, 1H, N−H), 5.23 (psquint, J = 6.5 Hz, 1H, CHO), 4.69 (d, J = 2.5 Hz, 1H, NH−CHS), 3.85 (s, 2H, S−CH_2−Ph), 3.71 (ddd, J = 7.0, 2.5, 0.9, 1H, *O*=C−CH), 2.42 (tr, J = 7.4 Hz, 2H, COOCH_2), 2.25 (dt, J = 7.0, 2.6 Hz, 2H, CH_2−C′CH), 1.97 (tr, J = 2.6 Hz, 1H, C′CH), 1.82 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−COO), 1.31 (d, J = 6.4 Hz, 3H, CH_3−CHO). ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.1, 165.2, 137.7, 129.0, 128.7, 127.6, 83.2, 69.2, 67.3, 63.5, 56.2, 35.9, 33.0, 23.6, 18.2, 17.8. HRMS calcd for $C_{18}H_{22}NO_3S^+$ (M + H^+) 332.13149, found 332.1322.

[3*R*(1′*R*,4*R*)]-Hex-5-ynoic Acid 1-(2-Octylsulfanyl-4-oxo-azetidin-3-yl)-ethyl Ester (15, OctHx). Compound **11** (76.9 mg, 0.30 mmol, 1.0 equiv) was dissolved in 7 mL of DCM followed by the addition of hex-5-ynoyl chloride (58.0 mg, 0.44 mmol, 1.5 equiv) in nitrogen atmosphere. After the mixture was stirred for 10 min, NEt_3 (61.9 μL, 44.97 mg, 0.44 mmol, 1.5 equiv) was added and stirred for 1 h. Subsequently, the solution was concentrated under reduced pressure, and the residue was extracted with Et_2O. The organic phase was then washed with 1 M HCl and 10% $NaHCO_3$, and the combined organic solutions were dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by HPLC to afford **15** (19.5 mg, 19%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.04 (br, 1H, N−H), 5.30 (psquint, J = 6.5 Hz, 1H, CHO), 4.75 (d, J = 2.5 Hz, 1H, NH−CHS), 3.25 (ddd, J = 7.0, 1.7 Hz, 0.5 Hz, 1H, *O*=C−CH), 2.60 (2tr, J = 7.5 Hz, 2H, S−CHH), 2.45 (tr, J = 7.4 Hz, 2H, COOCH_2), 2.27 (dt, J = 7.0, 2.6 Hz, 2H, CH_2−C′CH), 1.97 (tr, J = 2.6 Hz, 1H, C′CH), 1.85 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−COO), 1.57−1.66 (m, 2H, S−CH_2−CH_2), 1.39 (d, J = 6.4 Hz, 3H, CH_3−CHO), 1.22−1.40 (m, 10H, S−CH_2−CH_2−C_5H_{10}−CH_3), 0.88 (tr, J = 7.0 Hz, 3H, S−C_7H_{14}−CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.1, 165.3, 83.6, 69.2, 67.5, 63.8, 56.2, 33.0, 31.8, 30.1, 29.9, 29.1, 29.1, 28.9, 23.6, 22.6, 18.3, 17.8, 14.1. HRMS calcd for $C_{19}H_{32}NO_3S^+$ (M + H^+) 354.20974, found 354.2105.

[3*R*(1′*R*,4*R*)]-4-(2-Diethylamino-ethylsulfanyl)-1-hex-5-ynoyl-3-(1-hydroxy-ethyl)-azetidin-2-one (16, NCO). Compound **12** (42.6 mg, 0.17 mmol, 1.0 equiv) was dissolved in 4 mL of DCM followed by the addition of hex-5-ynoyl chloride (45.0 mg, 0.34 mmol, 2.0 equiv) in nitrogen atmosphere. After the mixture was stirred for 10 min, NEt_3 (38.5 μL, 28.0 mg, 0.28 mmol, 1.6 equiv) was added and stirred for 1 h. Subsequently, the solution was concentrated under reduced pressure, and the residue was purified by HPLC to afford **16** (5.0 mg, 9%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.50 (d, J = 3.2 Hz, 1H, NH−CHS), 4.21 (psquint, J = 7.2 Hz, 1H, CHO), 3.48−3.54 (m, 1H, S−CH_2−CHH), 3.36−3.42 (m, 1H, S−CHH−CH_2), 3.24−3.32 (m, 2H, S−CHH−CHH), 3.13 (quart, J = 7.2 Hz, 4H, N−(CH_2−CH_3)$_2$, 3.13 (dd, J = 7.3, 3.3 Hz, 1H, *O*=C−CH), 2.85 (dt, J = 17.0, 7.4 Hz, 1H, N−CO−CHH), 2.81 (dt, J = 17.1, 7.3 Hz, 1H, N−CO−CHH), 2.28 (dt, J = 7.1, 2.6 Hz, 1H, CHH−C′CH), 2.28 (dt, J = 6.8, 2.7 Hz, 1H, CH−C′CH), 2.00 (tr, J = 2.6 Hz, 1H, C′CH), 1.88 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−CON), 1.38 (tr, J = 7.3 Hz, 6H, N−(CH_2−CH_3)$_2$, 1.38 (d, J = 6.4 Hz, 3H, CH_3−CHO). ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.6, 164.5, 83.1, 69.4, 65.8, 65.1, 58.1, 51.8, 46.7 (2×), 35.4, 27.6, 22.6, 21.3, 17.8, 8.8 (2×). HRMS calcd for $C_{17}H_{29}N_2O_3S^+$ (M + H^+) 341.18934, found 341.1894.

[3*R*(1′*R*,4*R*)]-Hex-5-ynoic Acid 1-(2-Oxo-4-propylsulfanyl-1-sulfo-azetidin-3-yl)-ethyl Ester (17, SPSO$_3$).[25] A solution of **13**(20.0 mg, 0.07 mmol, 1.0 equiv) in dry pyridine (150 μL) was treated with a pyridine−sulfur trioxide complex (44.9 mg, 0.28 mmol, 4.0 equiv) and immediately warmed to 90 °C. After being stirred for 1 h at 90 °C, the reaction mixture was allowed to cool to room temperature and was stirred overnight. The solution was then poured into 1 M aqueous KH_2PO_4 (1.9 mL) followed by the addition of n-Bu_4NHSO_4 (25.2 mg, 0.07 mmol, 1.1 equiv). After being stirred for 10 min, the solution was extracted with DCM, and the organic phase was evaporated. The residue was purified by flash column chromatography on silica gel (EtOAc → EtOAc/MeOH 98:2) to afford **17** (8.7 mg, 34%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.39 (psquint, J = 6.4 Hz, 1H, CHO), 5.13 (d, J = 2.9 Hz, 1H, NH−CHS), 3.26 (dd, J = 7.4, 2.9, 1H, *O*=C−CH), 2.74−2.82 (m, 1H, S−CHH), 2.82−2.91 (m, 1H, S−CHH), 2.46 (tr, J = 7.3 Hz, 2H, COOCH_2), 2.26 (dt, J = 7.0, 2.6 Hz, 2H, CH_2−C′CH), 2.00 (tr, J = 2.6 Hz, 1H, C′CH), 1.83 (psquint, J = 7.2 Hz, 2H, CH_2−CH_2−COO), 1.58−1.70 (m, 2H, S−CH_2−CH_2), 1.36 (d, J = 6.4 Hz, 3H, CH_3−CHO), 0.99 (t, J = 7.3 Hz, 3H, S−CH_2−CH_2−CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.8, 165.0, 83.4, 69.3, 67.5, 62.0, 61.9, 33.5, 33.0, 23.5, 22.7, 18.5, 17.8, 13.6. HRMS calcd for $C_{14}H_{20}NO_6S_2^-$ (M − H^+) 362.07375, found 362.0746.

[3*R*(1′*R*,4*R*)]-Hex-5-ynoic Acid 1-(2-Benzylsulfanyl-4-oxo-1-sulfo-azetidin-3-yl)-ethyl Ester (18, SBzSO^3).[25] A solution of **14** (20.0 mg, 0.06 mmol, 1.0 equiv) in dry pyridine (150 μL) was treated with a pyridine−sulfur trioxide complex (38.4 mg, 0.24 mmol, 4.0 equiv) and immediately warmed to 90 °C. After being stirred for 1 h at 90 °C, the reaction mixture was allowed to cool to room temperature. The solution was then poured into 1 M aqueous KH_2PO_4 (1.7 mL) followed by the addition of n-Bu_4NHSO_4

(24) Robert Luxenhofer, R. J. *Macromolecules* **2006**, *39*, 3509–3516.

(25) Luca Banfi, G. C. *Tetrahedron* **1994**, *50*, 11967–11982.

(22.5 mg, 0.06 mmol, 1.1 equiv). After being stirred for 10 min, the solution was extracted with DCM, and the organic phase was evaporated. The residue was purified by flash column chromatography on silica gel (EtOAc → EtOAc/MeOH 98:2) to afford **18** (16.8 mg, 68%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16−7.38 (m, 5H, Arom.), 5.22 (psquint, J = 6.5 Hz, 1H, C$\underline{H}$O), 5.08 (d, J = 1.7 Hz, 1H, NSO_3−C$\underline{H}$S), 4.11 (d, J = 13.1 Hz, 1H, S−C$\underline{H}$H−Ph), 3.97 (d, J = 13.1 Hz, 1H, S−CH$\underline{H}$−Ph), 3.10 (d, J = 5.0, 1H, O=C−C$\underline{H}$), 2.20−2.35 (m, 2H, COOC$\underline{H}\underline{H}$), 2.13 (dt, J = 7.0, 2.6 Hz, 2H, C$\underline{H}_2$−C′CH), 1.95 (tr, J = 2.6 Hz, 1H, C′C$\underline{H}$), 1.70 (psquint, J = 7.2 Hz, 2H, C$\underline{H}_2$−C$\underline{H}_2$−COO), 1.09 (d, J = 6.3 Hz, 3H, C$\underline{H}_3$−CHO). ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.6, 165.3, 137.5, 129.5, 128.6, 127.1, 83.4, 69.3, 67.1, 61.1, 60.5, 35.7, 32.9, 23.4, 18.2, 17.8. HRMS calcd for $C_{18}H_{20}NO_6S_2^-$ ($M - H^+$) 410.07375, found 410.0752.

Preparation of Proteomes. Proteomes of the bacterial strains *Bacillus licheniformis* ATCC 14580, *Listeria welshimeri* SLCC 5334 serovar 6b, and *Pseudomonas putida* KT2440 were prepared from 1 L liquid cultures harvested 1 h after transition in the stationary phase by centrifugation at 13 000 rpm. All strains were grown in LB (Luria−Bertani broth) medium except *Listeria welshimeri*, which was maintained in BHB (brain−heart broth) medium. The bacterial cell pellets were washed with PBS, resuspended in 20 mL of PBS, and lysed by French press.

Labeling of Bacterial Proteomes. Proteome samples were adjusted to a final concentration of 1 mg protein/mL by dilution in PBS prior to probe labeling. Experiments for visualization by 1D SDS-PAGE were carried out in 43 μL total volume and those for affinity enrichment in 1892 μL total volume, such that once CC reagents were added, the total reaction volume was 50 μL and 2 mL, respectively. Reactions were initiated by addition of the probe and allowed to incubate for 60 min at room temperature. For heat controls, the proteome was denatured with 1 μL of 43% SDS at 95 °C for 6 min and cooled to room temperature before the probe was applied. Following incubation, reporter tagged-azide reagents (13 μM rhodamine-azide for analytical or 20 μM rhodamine-biotin-azide for preparative scale) were added followed by 1 mM TCEP and 100 μM ligand. Samples were gently vortexed, and the cycloaddition was initiated by the addition of 1 mM $CuSO_4$. The reactions were incubated at room temperature for 1 h.[9] 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-3000 Fluoreszenz Darkbox with a Fujinon VRF 43LMD Lens, 605DF40 filter, and 520 nm EPI excitation wavelength. Reactions for enrichment were carried out together with a control lacking the probe to compare the results of the biotin−avidin-enriched samples with the background of unspecific protein binding on avidin−agarose beads. After CC, proteins were precipitated using an equal volume of prechilled acetone. Samples were stored on ice for 20 min and centrifuged at 13 000 rpm for 10 min. The supernatant was discarded, and the pellet was washed two times with 400 μL of prechilled methanol and resuspended by sonication. Subsequently, the pellet was dissolved in 1 mL of PBS with 0.2% SDS by sonication and incubated under gentle mixing with 50 μL of avidin−agarose beads (Sigma-Aldrich) for 1 h at room temperature. The beads were washed three times with 1 mL of PBS/0.2% SDS, twice with 1 mL of 6 M urea, and three times with 1 mL of PBS. 50 μL of 2 × SDS loading buffer was added, and the proteins were released for preparative SDS-PAGE by 6 min incubation at 95 °C. Gel bands were isolated, washed, and tryptically digested as described previously.[12] For analytical and preparative in vivo studies, bacteria were grown to stationary phase, pelleted by centrifugation (2 mL for analytical and 10 mL for preparative studies), resuspended with PBS, and incubated for 2 h with varying concentrations of probe at room temperature. Subsequently, the cells were lysed by sonication and separated into cytosolic and membrane fractions, followed by CC as described above.

IC_{50} Determination. The ClpP IC50 value for **NCO** (**16**) was measured by the enzyme mediated hydrolysis of the fluorogenic substrate *N*-succinyl-Leu-Tyr-7-amidomethylcoumarin (Succ-Leu-Tyr-AMC) that has been described as standard for ClpP activity assays.[19] Experiments were conducted in a ClpP activity buffer (50 mM Tris pH 8.0, 100 mM KCl, 1 mM dithiothreitol). 1.7 μg of ClpP (corresponds to 116 nM of the active $ClpP_{14}$ complex) was incubated in ClpP activity buffer with varying concentrations of **NCO** or DMSO for control experiments for 10 min. Next, 667 μM Succ-Leu-Tyr-AMC was added to give a total volume of 50 μL containing 2% DMSO. Fluorescence was recorded with 340 nm excitation and 450 nm emission by a TECAN GENios Pro. β-Lactamase-mediated hydrolysis of *p*-nitrophenylacetate leads to the release of *p*-nitrophenol, which can be monitored at 400 nm. Various concentrations of probes were added to a solution containing the substrate (2.4 mM) and enzyme (500 nM). Reactions were started by addition of the enzyme and subsequently monitored for 10 min. The mean average slopes of absorption (out of three independent experiments) versus probe concentration were plotted, and the concentration of 50% inhibition (IC_{50}) was determined.

MIC Determination. Antibiotic and β-lactam probe-mediated growth inhibition of *B. licheniformis*, *P. putida*, and *L. welshimeri* was obtained in 96 well plate-based assays. Various concentrations of β-lactams (1 μL dissolved in DMSO) were added to 99 μL of medium, which contained a fresh 1:1000-fold dilution of the corresponding bacterial overnight culture. The well plates were incubated at 37 °C for 14 h under shaking at 260 rpm. The MIC value was determined by several (>3) independent experiments (each experiment with at least triplicate runs for each concentration) and represented the lowest concentration of β-lactam at which no growth of bacteria could be observed.

Mass Spectrometry and Bioinformatics. Tryptic peptides were loaded onto a Dionex C18 Nano Trap Column (100 μm) and subsequently eluted and separated by a Dionex C18 PepMap 100 (3 μm) column for analysis by tandem MS followed by high-resolution MS using a coupled Dionex Ultimate 3000 LC-ThermoFinnegan LTQ-FT MS system. The mass spectrometry data were searched using the SEQUEST algorithm against the corresponding databases via the software "bioworks". The search was limited to only tryptic peptides, two missed cleavage sites, monoisotopic precursor ions, and a peptide tolerance of <10 ppm. Filters were set to further refine the search results. The X_{corr} versus charge state filter was set to X_{corr} values of 1.5, 2.0, and 2.5 for charge states +1, +2, and +3, respectively. The number of different peptides has to be ≥2, and the peptide probability filter was set to <0.001. These filter values are similar to others previously reported for SEQUEST analysis.[26] Maximum *P*-values and X_{corr} values of each run as well as the total number of obtained peptides are reported in Table S1.

Recombinant Expression. The major hits of MS analysis were recombinantly expressed in *E. coli* as an internal control of the MS results by using the Invitrogen Gateway Technology. Target genes were amplified from the corresponding genomes by PCR with an AccuPrime *Pfx* DNA Polymerase kit with 65 ng of genomic DNA, prepared by standard protocols. *att*B1 forward primer and *att*B2 reverse primer were designed to yield *att*B-PCR Products needed for Gateway Technology. Primer: D-alanyl-D-alanine carboxypeptidase *P. putida* KT2440. Forward primer: 5′-ggg gac aag ttt gta caa aaa agc agg cta cat gcc act tgc ctt gcc cag cca cg. Reverse primer: 5′-ggg gac cac ttt gta caa gaa agc tgg gtg tCA GTT GGC cGc tgT ACT gcC aGc C. D-alanyl-D-alanine carboxypeptidase (penicillin-binding protein 5) *Bacillus licheniformis* ATCC 14580. Forward primer: 5′-Ggg gac aag ttt gta caa aaa agc agg cta cat gaa aag caa gag att aaa gca g. Reverse primer: 5′-ggg gac cac ttt gta caa gaa agc tgg gtg tta aaa cca gcc ggt tac cat. β-Lactamase *Bacillus licheniformis* ATCC 14580. Forward primer: 5′-ggg gac aag ttt gta caa aaa agc agg cta cat gaa aca gtc att aaa gg. Reverse primer: 5′-ggg gac cac ttt gta caa gaa agc tgg gtg cta ttt ccg gac agc. PCR products were identified on agarose gels, and gel bands were

(26) Mirza, S. P.; Halligan, B. D.; Greene, A. S.; Olivier, M. *Physiol. Genomics* **2007**, *30*, 89–94.

isolated and extracted with an E.ZNA MicroElute Gel Extraction Kit. Concentrations of DNA were measured by a NanoDrop spectrophotometer ND-1000. 100 fmol of purified *att*B-PCR product and 50 fmol of *att*P-containing donor vector pDONR201 in TE buffer were used for in vitro BP recombination reaction with BP Clonase II enzyme mix to yield the appropriate *att*L-containing entry clone. After transformation in chemically competent One Shot TOP10 *E. coli* (Invitrogen), cells were plated on LB agar plates containing 25 μg mL^{-1} kanamycin. Clones of transformed cells were selected and grown in kanamycin LB medium. Cells were harvested and plasmids were isolated using an E.ZNA Plasmid Mini Kit. The corresponding *att*B-containing expression clone was generated by in vitro LR recombination reaction of approximately 50 fmol of the *att*L-containing entry clone and 50 fmol of the *att*R-containing destination vector pDest using LR Clonase II enzyme mix in TE buffer. The expression clone was transformed in chemically competent BL21 *E. coli* cells (Novagen) and selected on LB agar plates containing 100 μg mL^{-1} carbenicillin. Validity of the clones was confirmed by plasmid sequence analysis. Recombinant clones were grown in carbenicillin LB medium, and target gene expression was induced with anhydrotetracyclin.

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Supporting Information Available: Additional figures containing heat controls, competition studies with antibiotics, coomassie versus fluorescence intensities, IC_{50} graphs, mass spectrometry data, in vivo labeling time dependence, NMR data, and complete ref 16. This material is available free of charge via the Internet at http://pubs.acs.org.

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