

Click and Release Chemistry for Activity-Based Purification of β -Lactam Targets

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Abstract: β -Lactams, the cornerstone of antibiotherapy, inhibit multiple and partially redundant targets referred to as transpeptidases or penicillin-binding proteins. These enzymes catalyze the essential cross-linking step of the polymerization of cell wall peptidoglycan. The understanding of the mechanisms of action of β -lactams and of resistance to these drugs requires the development of reliable methods to characterize their targets. Here, we describe an activity-based purification method of β -lactam targets based on click and release

chemistry. We synthesized alkyne-carbapenems with suitable properties with respect to the kinetics of acylation of a model target, the Ldt_{fm} L,D-transpeptidase, the stability of the resulting acylenzyme, and the reactivity of the alkyne for the cycloaddition of an azido probe containing a biotin moiety for affinity purification and a bioorthogonal cleavable linker. The probe provided access to the fluorescent target in a single click and release step.

Introduction

Bacterial resistance is a major threat to the efficacy of antibiotics requiring innovative strategies for maintaining the efficacy of antimicrobial chemotherapy. Identification and characterization of the biological targets of drugs is a key step for their development and the study of the mechanisms of resistance. This is particularly the case for antibiotics belonging to the β -lactam family since these drugs have multiple targets in bacterial cells with partially overlapping functions.^[1,2] The essential targets of β -lactams are the high-molecular weight (HMW) penicillin-binding proteins (PBP) that catalyze the last cross-linking step of peptidoglycan synthesis. This cell wall

polymer is essential for sustaining the osmotic pressure of the bacterial cytoplasm. It consists of glycan chains linked to each other by short stem peptides (Figure 1A). The cross-linking reaction catalyzed by HMW PBPs, the D,D-transpeptidases, proceeds by the cleavage of the C-terminal D-Ala-D-Ala peptide bond of a stem pentapeptide and linkage of the penultimate D-Ala to the third residue of a stem peptide carried by an adjacent glycan chain (4 \rightarrow 3 cross-links) (Figure 1B). β -lactams are structure analogues of the D-Ala-D-Ala extremity of peptidoglycan precursors and act as suicide substrates of the HMW PBPs^[3] (Figure 1C). Acylation of the active site Ser of the PBPs results in a long-lasting covalent adduct and irreversible inactivation of the target at the time scale of a bacterial generation.

The cross-linking reaction is performed by multiple HMW PBPs in most bacteria, typically 5 to 6 in the main human pathogens.^[1] In addition, β -lactams similarly inactivate low-molecular weight (LMW) PBPs that hydrolyze the D-Ala-D-Ala peptide bond of stem pentapeptides (D,D-carboxypeptidases) or the cross-links formed by the HMW PBPs (endopeptidases). A particular class of β -lactams, the carbapenems, also inactivate L,D-transpeptidases (Ldts) that form unusual cross-links connecting the third position on both stem peptides (3 \rightarrow 3 cross-links)^[4] (Figure 1B). Ldt inactivation results from acylation of their active-site Cys residue by the β -lactam ring of carbapenems (Figure 1C). Ldts are the main cross-linking enzyme in *Mycobacterium tuberculosis*^[5] and can be responsible for *in vitro* acquired resistance to β -lactams of the penam (penicillin) and cephem (cephalosporin) classes in other bacteria.^[6,7]

Historically, the targets of β -lactams have been identified by incubating membrane preparations with radiolabeled drugs followed by denaturing polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography.^[8] This method typically resolves a pattern of ca. 10 bands corresponding to proteins covalently labeled by the drug. Competitive binding with

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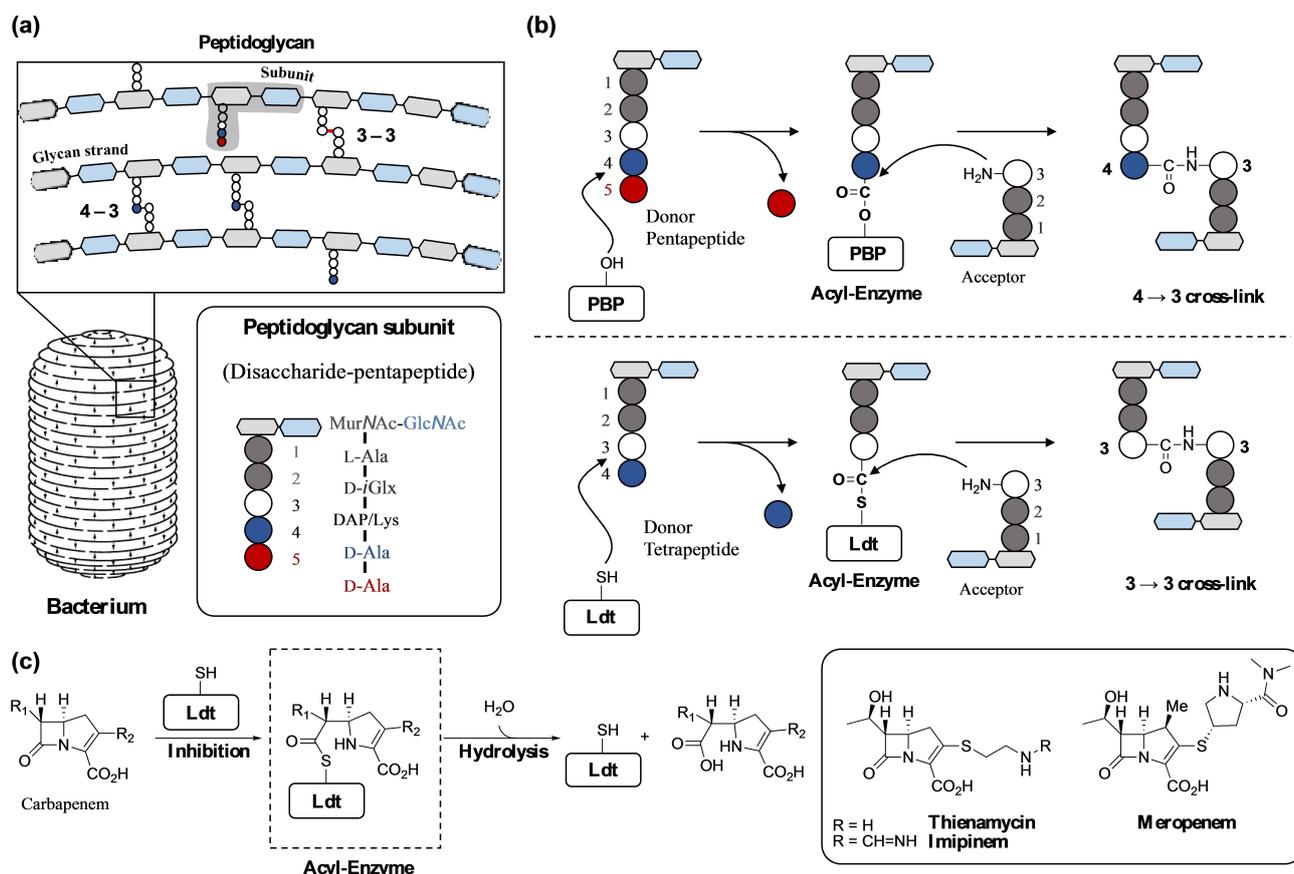


Figure 1. (a) Peptidoglycan structure. (b) Formation of peptidoglycan cross-links by PBPs and Ldts. (c) Inhibition of Ldts by carbapenems. The efficacy of Ldt inhibition depends upon slow hydrolysis of the acyl-enzyme. The inset shows the structure of carbapenems discussed in the text.

unlabeled drugs has been largely used to compare the reactivity of various β -lactams for the PBPs. For this approach, fluorescent β -lactams, such as bocillin, were developed to avoid the use of radioactive reagents.^[9–11] Further refinement of the PBP labeling methods afforded single cell imaging of bacteria exposed to the drugs.^[12] The key element of this application is the incorporation of a bioorthogonal active group in the β -lactam with minimal impact on drug penetration and on interaction of the drug with its multiple targets. Following labeling of the targets in the bacterium, the drug-target adducts were revealed by a bioorthogonal reaction with a functionalized dye.^[13,14] The most popular approach is the incorporation of an alkyne group on the drug allowing copper-catalyzed cycloaddition (CuAAC) with an azido-containing dye.^[13,14] This approach enables detection of known PBPs but present limitations concerning the identification of unknown targets since it only provides information on the electrophoretic mobility of the protein-dye adduct. This limitation has been addressed by the use of biotinylated azido-containing dyes for streptavidin affinity pull-down purification followed by mass spectrometry analysis.^[15,16] Selective penicillin-binding protein imaging probes have also been described using fluorescent β -lactams,^[17] such as fluorescent cephalosporins.^[18] Of note, fluorescent β -lactams were also used as fluorogenic molecular

probes for the detection of β -lactamases in vitro or in living bacteria.^[17,19,20]

In this study, we explore a strategy for combined affinity purification and fluorescence labeling of carbapenem targets. We report synthetic routes to alkynylated carbapenems that effectively labeled a model L,D-transpeptidase (Ldt_m) both with respect to the kinetics of acylation and the stability of the resulting acyl-enzymes. The alkyne group of the enzyme-bound carbapenem was subsequently exploited for CuAAC attachment of a biotin-containing iminosydnone linker, recently developed as a bioorthogonal cleavable spacer (Figure 2).^[21,22] After binding to streptavidin magnetic beads and purification, the iminosydnone mesoionic compound enabled the release and labeling of the captured carbapenem targets from the streptavidin support through a bioorthogonal click and release reaction,^[21] affording pure and fluorescent targets in a single step.

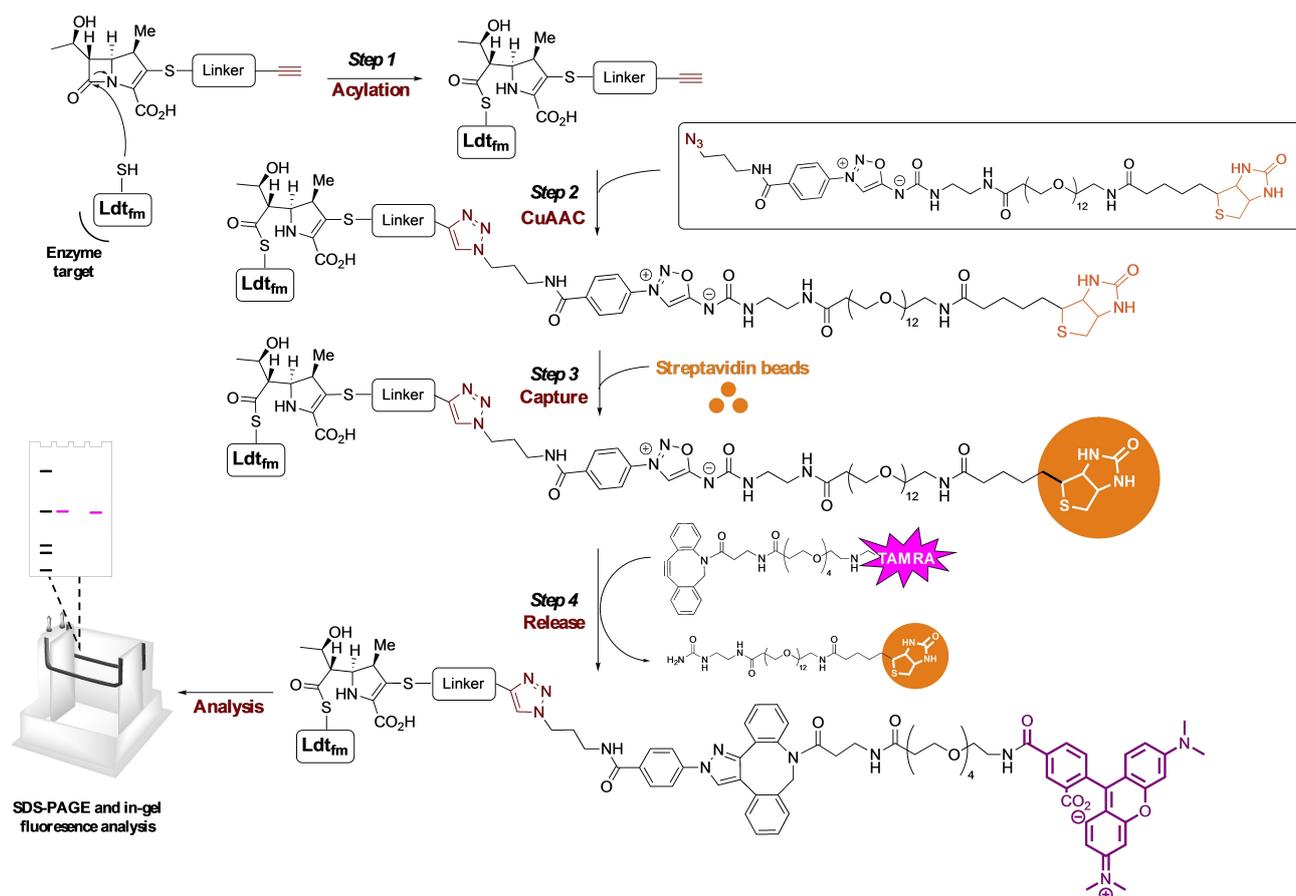


Figure 2. General strategy for activity-based purification of carbapenem targets.

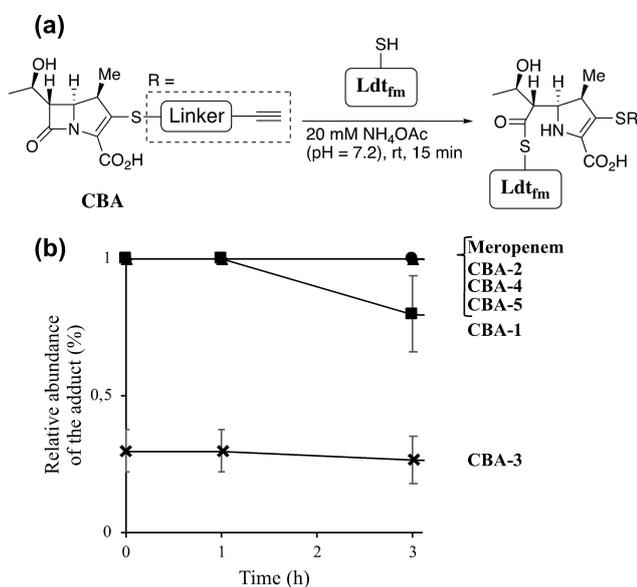


Figure 3. (a) Acylation of Ldt_{fm} by carbapenems. (b) Stability of Ldt_{fm} -carbapenem adducts determined by mass spectrometry (Supporting Information, Section 7.3).

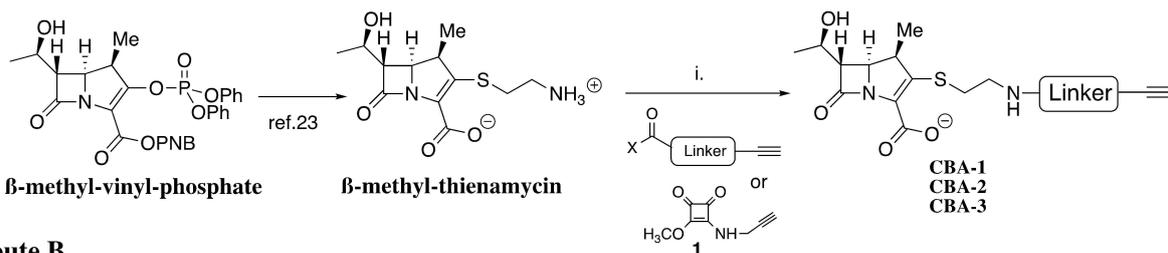
Results and Discussion

Synthesis of alkynylated carbapenems

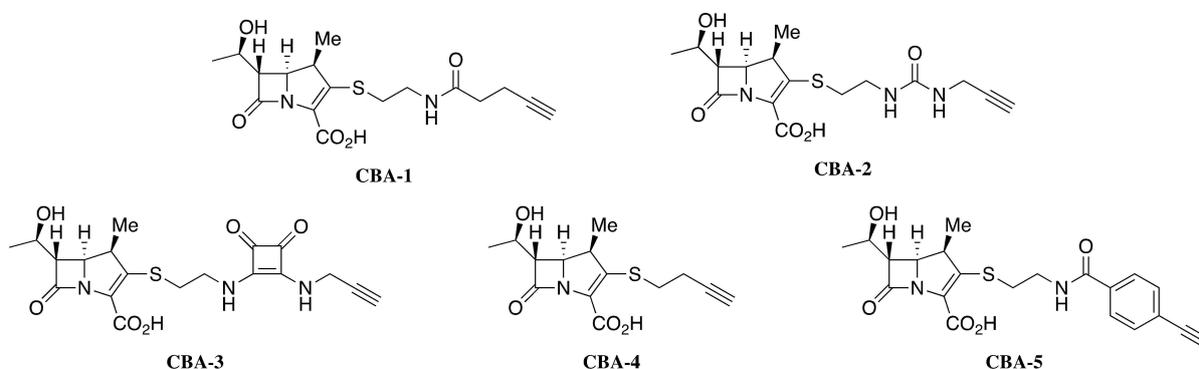
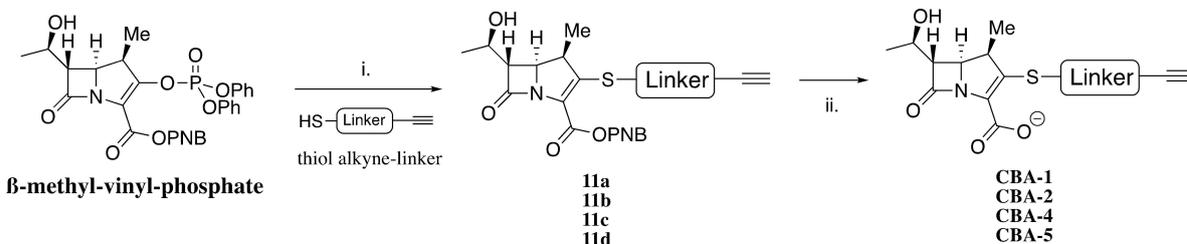
We started our synthesis by using a post-functionalization strategy of the fully deprotected methyl-thienamycin (Route A, Scheme 1). Methyl-thienamycin was obtained in two steps (21% yield) from commercially available β -methyl vinyl-phosphate.^[23] Methyl-thienamycin is renowned for its unstable chemical nature.^[24] Indeed, strong acidic, basic, or nucleophilic conditions as well as exposure to light, high temperature, or prolonged storage in solution lead to β -lactam ring opening. Thus, the reaction conditions for functionalizing methyl-thienamycin should be mild ($6.0 < \text{pH} < 8.5$; $< 25^\circ\text{C}$) and should not involve prolonged reaction time (< 1 hour). Despite these limitations, three alkyne-carbapenems were obtained, **CBA-1**, **CBA-2**, and **CBA-3**, using methyl-thienamycin in the presence of alkyne linkers, which were commercially available (for **CBA-1** and **CBA-2**) or synthesized in this study (Compound 1, Scheme 1). Alkyne-carbapenems **CBA-1**, **CBA-2**, and **CBA-3** were obtained with variable yields (49%, 67%, and 14%, respectively; Scheme 1, Route A).

Ring opening could not be fully prevented and subsequent purification of the desired compounds free of their hydrolyzed

Route A



Route B



Scheme 1. Two strategies for alkyne carbapenems synthesis. Reaction conditions for route A: i. 4-pentynoic acid succinimidyl ester or *N*-(prop-2-yn-1-yl)-1*H*-imidazole-1-carboxamide^[26] or compound 1. Reaction condition for route B: i. thiol alkyne-linkers **3**, **6**, 3-butynyl ethanethioate or **10** and DIPEA in DMF, ii. Zn dust in THF:PB (1:2). Compounds were obtained by route A (**CBA-3**), route B (**CBA-4** and **CBA-5**), or both routes (**CBA-1** and **CBA-2**).

products was cumbersome. For this reason, we explored an alternative approach starting from a protected carbapenem for direct addition of a thiol-alkyne onto the β -methyl vinyl-phosphate (Scheme 1, route B). Syntheses of the thiol alkyne linkers **3**, **6**, and **10** are described in the supporting information (Section 2). This first step provided the intermediate carbapenems **11a**, **b**, **c**, **d** in 97%, 77%, 78%^[23] and 54% yields, respectively. The critical step of the latter strategy was the final selective deprotection of the *para*-nitrobenzyl group (PNB), which was successfully achieved using zinc dust.^[25] Minimal β -lactam ring opening provided by this approach facilitated purification of the final compounds (**CBA-1**, **CBA-2**, **CBA-4**, and **CBA-5** in 11%, 77%, 40%, and 43% yields, respectively). Comparison of the NMR spectra of **CBA-1** and **CBA-2** obtained by routes A and B (Supporting Scheme S5) supported the superiority of route B with respect to the purity of the final compound.

Acylation of L,D-transpeptidase Ldt_{fm} by alkyne-carbapenems

The alkyne function of compounds **CBA-1** to **CBA-5** is connected to the C₂ position of the carbapenem core *via* variable links, including an amide (**CBA-1**), a urea (**CBA-2**), a squaramide (**CBA-3**), an alkyl (**CBA-4**), or a benzamide (**CBA-5**). These compounds were synthesized to explore the impact of the linkers on the kinetics of target acylation and the stability of the resulting covalent adduct. This was first investigated by mass spectrometry to detect Ldt_{fm}-carbapenem adducts (Figure 3). Incubation of Ldt_{fm} (6.5 μ M) with alkyne carbapenems **CBA-1** to **CBA-5** (39 μ M) for 15 min at 25 °C in 20 mM ammonium acetate (pH 7.2) led to full enzyme acylation, except for **CBA-3** (*ca.* 30% acylation). Stability of the Ldt_{fm}-carbapenem adducts was also monitored by mass spectrometry.

For this purpose, the Ldt_{fm}-carbapenem adducts were purified by gel filtration to remove the excess of unreacted carbapenem and incubated for 1 h and 3 h at 25 °C. This analysis showed that acylenzymes resulting from acylation of Ldt_{fm} by **CBA-2**, **CBA-4**, **CBA-5**, and the clinically-used carbape-

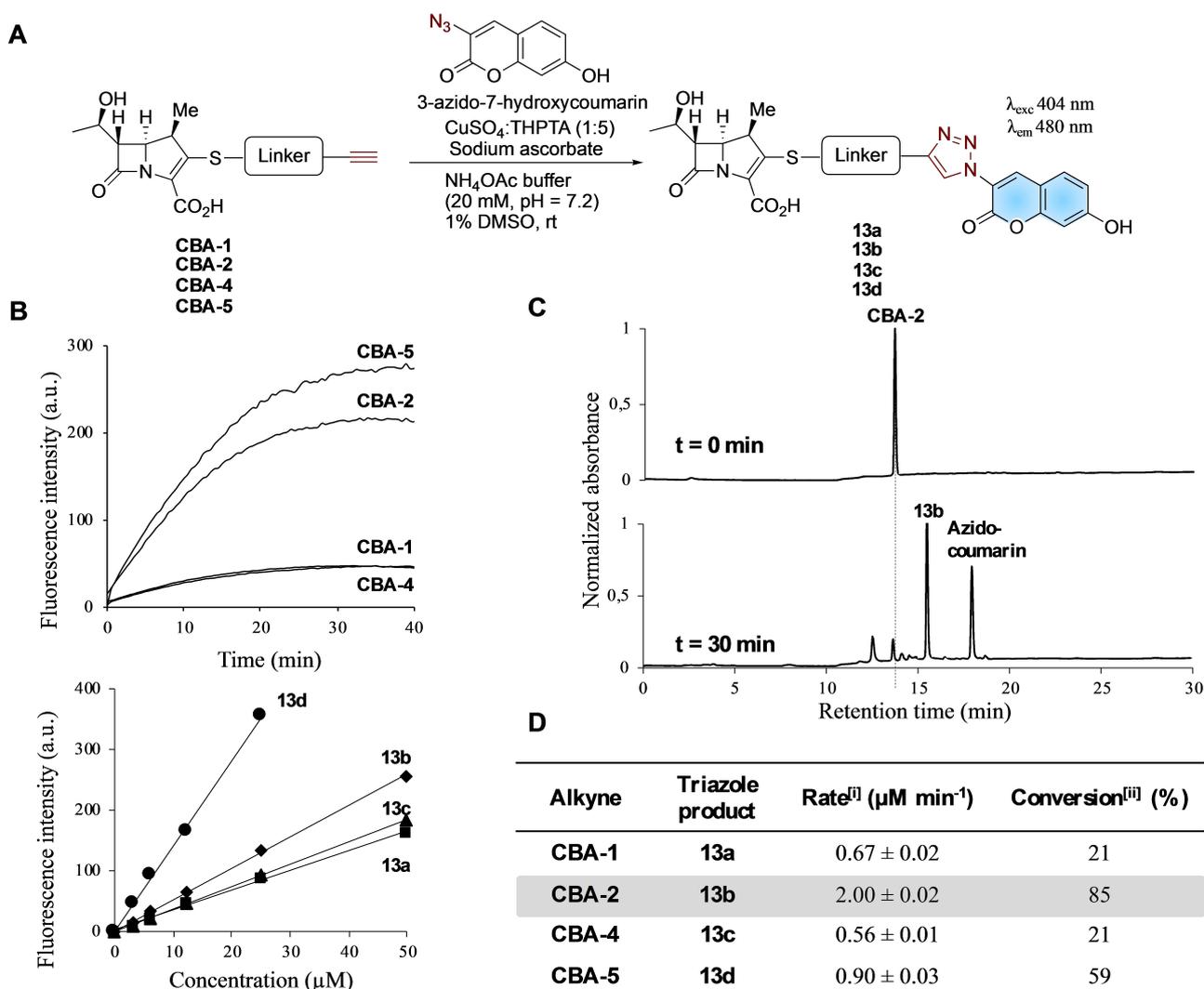


Figure 4. Impact of the linker of alkyne carbapenems on the efficiency of CuAAC. (a) CuAAC reaction with 3-azido-7-hydroxycoumarin. CuAAC performed with CBA-1, CBA-2, CBA-4, and CBA-5 provided compounds 13a, b, c, and d, respectively. (b) Upper panel: Fluorescence kinetics obtained with alkyne carbapenems CBA-1, CBA-2, CBA-4, and CBA-5. Lower panel: Calibration curves obtained with purified triazole compounds, 13a to 13d. Synthesis and characterization of these triazole compounds are reported in the Supporting Information, Section 4. Please note that the intrinsic fluorescence of 13d was higher than that of 13b. For this reason, the efficiency of the CuAAC reaction was higher for CBA-2 than for CBA-5 although the rate of increase in the fluorescence intensity was the highest for CBA-5. The efficiency of the reaction was evaluated based on the initial rate of the reaction, $2.00 \pm 0.02 \mu\text{M min}^{-1}$ versus $0.90 \pm 0.03 \mu\text{M min}^{-1}$ for CBA-2 and CBA-5, respectively. (c) The yield (%) of the reaction was evaluated based on the extent of the disappearance of the alkynes after 30 min of incubation (determined by HPLC analysis), as exemplified for CBA-2 (see Supporting Figure S1, S2, and S3 for CBA-1, CBA-4, and CBA-5). (d) Efficiency parameters. [i] Initial rate of CuAAC determined by fluorimetry (panel b). [ii] Conversion (%) was determined by HPLC (panel c). The conversion was higher for CBA-2 (85%) than for CBA-5 (59%), CBA-1 (21%), and CBA-4 (21%).

nem meropenem (Figure 1C) were stable (Figure 3). In addition, native Ldt_{fm} resulting from hydrolysis of the carbapenem-Ldt_{fm} thioester was not detected in the mass spectra. In contrast, partial hydrolysis ca. 20% was detected for CBA-1 since the ratio of peak intensities was in the order of 0.2 and 0.8 for native Ldt_{fm} and the Ldt_{fm}-CBA-1 acylenzyme, respectively. Since Ldt_{fm} was only partially acylated by CBA-3 this alkyne carbapenem was not selected for further studies.

Reactivity of alkyne-carbapenems in the Cu^I-catalyzed cycloaddition

The success of our click and release strategy relies on both the stability of the acylenzyme (above) and on the efficiency of the cycloaddition reaction (step 2; Figure 2). Kinetics of CuAAC were performed with a fluorogenic azido partner (3-azido-7-hydroxycoumarin)^[27,28] to compare the impact of the linkers present in the four alkyne carbapenems (Figure 4).

The rate of the cycloaddition reaction in the presence of CuSO₄ (at 25 μM), was higher for CBA-2 ($2.00 \pm 0.02 \mu\text{M min}^{-1}$) than for CBA-1, CBA-4, and CBA-5 (0.56 to $0.90 \mu\text{M min}^{-1}$)

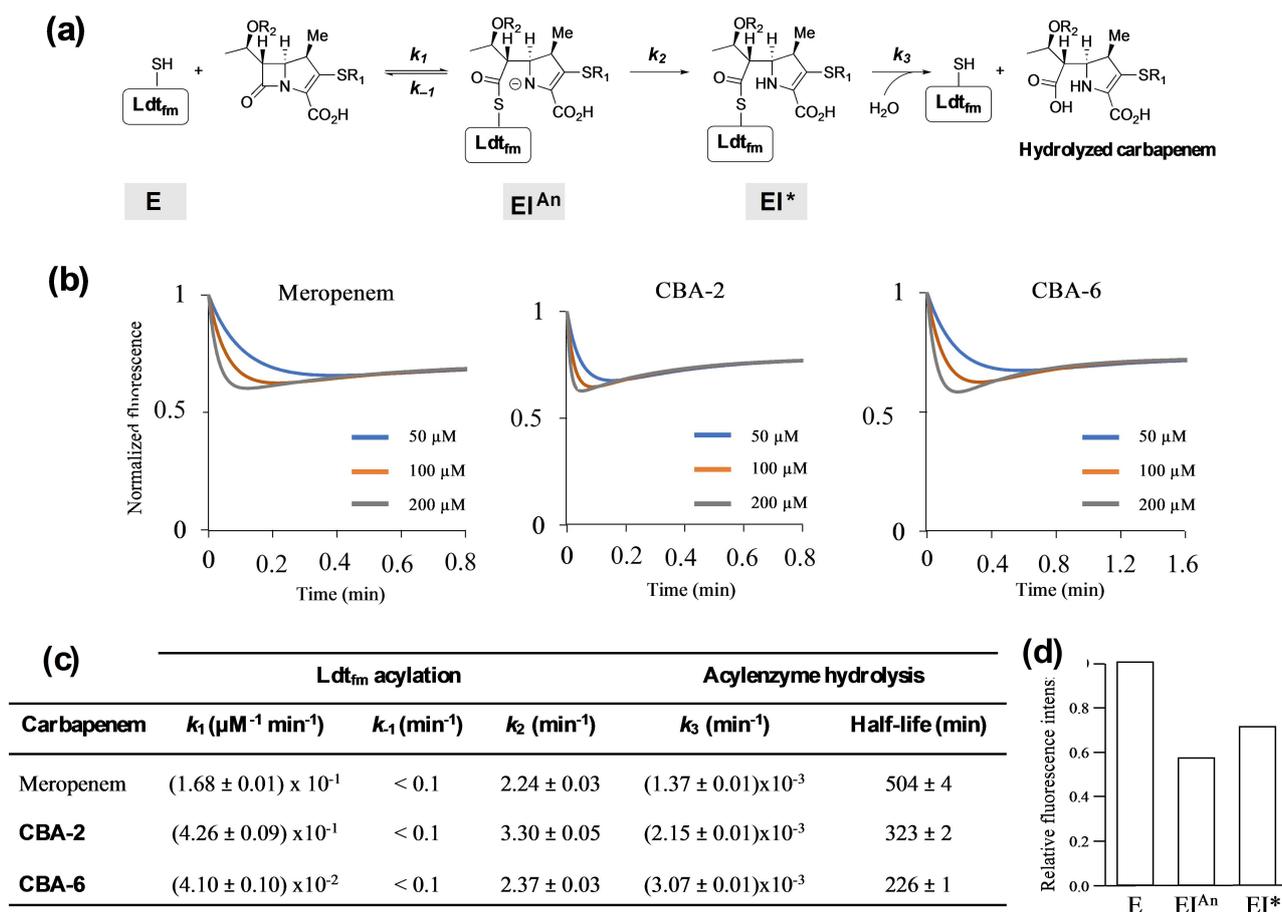


Figure 5. Kinetic parameters for Ldt_{fm} acylation by carbapenems. $R_2 = H$ for **CBA-2** and a diazirine moiety for **CBA-6**. (a) Reaction scheme for the two-step acylation reaction. $R_2 = H$ for **CBA-2** and a diazirine moiety for **CBA-6**. (b) Fluorescent kinetics obtained with three concentrations of carbapenem. (c) Kinetic parameters for the acylation reaction. (d) Relative fluorescence of the three forms of Ldt_{fm} . Biphasic fluorescence kinetics are accounted for by the low fluorescence intensity of the amine anion intermediate.^[29,30]

(Figure 4D). To evaluate the yield of the reaction for each alkyne carbapenem, 3-azido-7-hydroxycoumarin was allowed to react with alkyne carbapenems for 30 min and the progress of the reaction was followed by HPLC. The yield was the highest for **CBA-2** (85%), intermediate for **CBA-5** (59%), and the lowest for **CBA-1** and **CBA-4** (21%). The high reactivity of **CBA-2** might be accounted for by the chelating effect of the urea linker on the copper catalyst as observed for other chelating functions.^[28] This alkyne carbapenem was selected for further experiments as the best candidate based on stability of the acylzyme (Figure 3) and its efficiency in the cycloaddition reaction (Figure 4).

Kinetics of Ldt_{fm} acylation by alkyne carbapenem **CBA-2**

Our next objective was to evaluate the impact of the incorporation of a urea-linked alkyne function in **CBA-2** on the inactivation of Ldt_{fm} by this carbapenem.

Kinetics based on variation in the intrinsic fluorescence of tryptophan residues of Ldt_{fm} upon acylation were monitored for the reference carbapenem meropenem and **CBA-2** (Figure 5).

CBA-2 proved to be superior to meropenem both with respect to the rate constant k_1 for formation of the amine anion (0.426 ± 0.009 versus $0.168 \pm 0.001 \mu M^{-1} min^{-1}$) and the rate constant k_2 for formation of the acylzyme from this intermediate (3.30 ± 0.05 versus $2.24 \pm 0.03 min^{-1}$) (Figure 5). In agreement with mass spectrometry analysis (Figure 3), hydrolysis kinetics obtained by spectrophotometry revealed that the acylzymes formed with **CBA-2** and meropenem display a similar stability (half-lives 323 ± 2 and 504 ± 4 min, respectively). These values are higher than expected from the mass spectrometry analysis reported in Figure 3, which revealed that Ldt_{fm} remained fully acylated after 3 h of incubation. This discrepancy may indicate that the free unreacted carbapenems were not fully eliminated by the gel filtration step used in the mass spectrometry analysis (Figure 3) enabling re-acylation after acylzyme hydrolysis. These results indicate that we have successfully functionalized the carbapenem core without impairing the efficiency of Ldt_{fm} acylation or the stability of the resulting adduct.

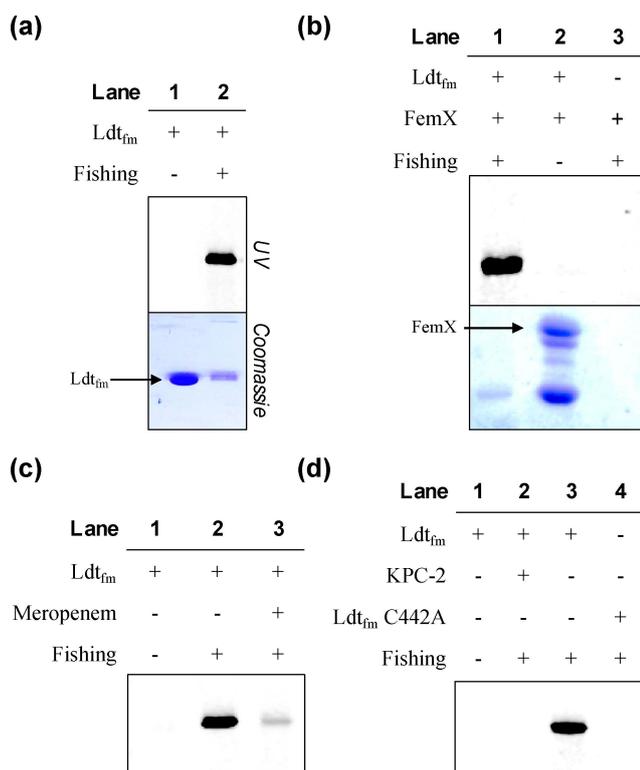


Figure 6. SDS-PAGE analyses of CBA-2-Ldt_{fm} adducts. (a) Click and release reactions applied to the Ldt_{fm} L,D-transpeptidase. Lane 1, 2 μg of native Ldt_{fm}; Lane 2, protein recovered from streptavidin beads. Proteins were detected by fluorescence (top panel) and Coomassie blue staining (bottom panel). (b) Assessing specificity by applying the click and release reactions to a mixture of equimolar amounts of Ldt_{fm} and FemX (Lane 1) and to FemX alone (Lane 3). Lane 2, native Ldt_{fm} and FemX proteins. (c) Assessing specificity by applying the click and release reactions to Ldt_{fm} (Lane 2) and to Ldt_{fm} preincubated with meropenem (Lane 3) to prevent acylation of the protein by the CBA-2 alkyne carbapenem. Lane 1, native Ldt_{fm}. (d) Assessing specificity by applying the click and release reactions to native Ldt_{fm} (Lane 1), to CBA-2 preincubated with carbapenemase KPC-2 (Lane 2) and to a derivative of Ldt_{fm} that cannot be acylated following replacement of the catalytic cysteine by alanine (Lane 4). Lane 3, click and release reactions applied to the Ldt_{fm} L,D-transpeptidase (as performed for panel A).

Capture of Ldt_{fm} and release of the fluorescent protein adduct

CBA-2 was used to explore the efficacy of step 3 (capture) and step 4 (release) of our click and release strategy (Figure 2). Ldt_{fm} (0.65 nmol) was acylated with 6 equivalents of CBA-2 (Figure 2, step 1). Excess alkyne-carbapenem was removed by gel filtration (G25 microspin). The CuAAC reaction was performed with 10.8 equivalents of the azido-platform^[21,22] leading to the CBA-2-Ldt_{fm} adduct (Figure 2, step 2). The platform provided a biotin handle for capture of the Ldt_{fm}-CBA-2 adduct by streptavidin-coated magnetic beads (Figure 2, step 3). Following extensive washing, the click and release reaction was performed with 60 equivalents of the TAMRA fluorescent cyclooctyne (Figure 2, step 4). The latter reaction enabled elution and labeling of the Ldt_{fm} adduct in a single step. SDS-PAGE analysis (Figure 6A) showed that TAMRA-labeled Ldt_{fm} was successfully eluted from the streptavidin beads.

Assessing the specificity of the capture and release reactions

As a negative control, we used FemX, a tRNA-dependent aminoacyl transferase that does not interact with carbapenems. This protein was not recovered from streptavidin beads indicating that the procedure was specific to the Ldt_{fm} carbapenem-target (Figure 6B). The procedure was also specific in recovering Ldt_{fm} from a reconstituted mixture containing a whole protein extract from *E. faecium* (18.6 μg of protein) and 0.65 nmol of Ldt_{fm} (18.8 μg) (Figure S5 in Supporting information). Specificity was further evaluated by preincubating Ldt_{fm} with meropenem to block the active-site Cys and prevent acylation by the CBA-2 alkyne carbapenem. This pretreatment almost completely abolished recovery of fluorescent Ldt_{fm} from streptavidin beads (Figure 6C). In addition, we showed that recovery of TAMRA-labeled Ldt_{fm} required the native carbapenem core of CBA-2 since preincubation of this compound with carbapenemase KPC-2, a hydrolytic enzyme of the β-lactamase family, abolished detection of the fluorescent protein band at 35 kDa (Figure 6D). Recovery of the TAMRA-labeled-Ldt_{fm} was also dependent upon acylation of the active-site Cys of the enzyme since no fluorescent band was observed for a Ldt_{fm} derivative devoid of the Cys nucleophile following substitution of this residue by Ala.

Bi-functionalization of the carbapenem scaffold with photoactivable and alkyne groups to improve acylenzyme stability

One possible limitation of the click and release procedure is the instability of the link connecting the drug to the target, a thioester bond in the case of Ldt_{fm}-carbapenem adducts.

Indeed, the link is exposed to multiple conditions during the four steps of the click and release procedure (Figure 2), which lasts for ca. 3 hours in its current version. Thus, our next objective was to add a photoactivable group to the alkyne carbapenem in order to stabilize the drug-target adduct (Figure 7). Diazirines have been successfully used for photoaffinity labeling^[31] and for studies aimed at detecting a variety of interactions, such as ligand-receptor,^[32] inhibitor-enzyme,^[33] and protein-protein,^[34] or for fishing of drug targets.^[35,36] The combined advantages of diazirines over other photoactivable cross-linking agents include their small size, long irradiation wavelength, short period of irradiation, and high stability in the presence of various nucleophiles.^[33,37] Upon irradiation in the 330 to 380 nm range, diazirines form reactive carbenes, which can insert into C–H, N–H, and O–H bonds.^[38]

In our approach, synthesis of the bi-functionalized carbapenem started from the protected methyl-thienamycin,^[23] which reacted with activated 3-(3-methyl-3H-diazirin-3-yl)propanoic acid^[39] to give compound **14** in 92% yield (Figure 7A). Removal of the PNB group using hydrogenolysis catalyzed by platinum on carbon, followed by the functionalization of compound **15** by *N*-(prop-2-yn-1-yl)-1H-imidazole-1-carboxamide, afforded compound CBA-6.

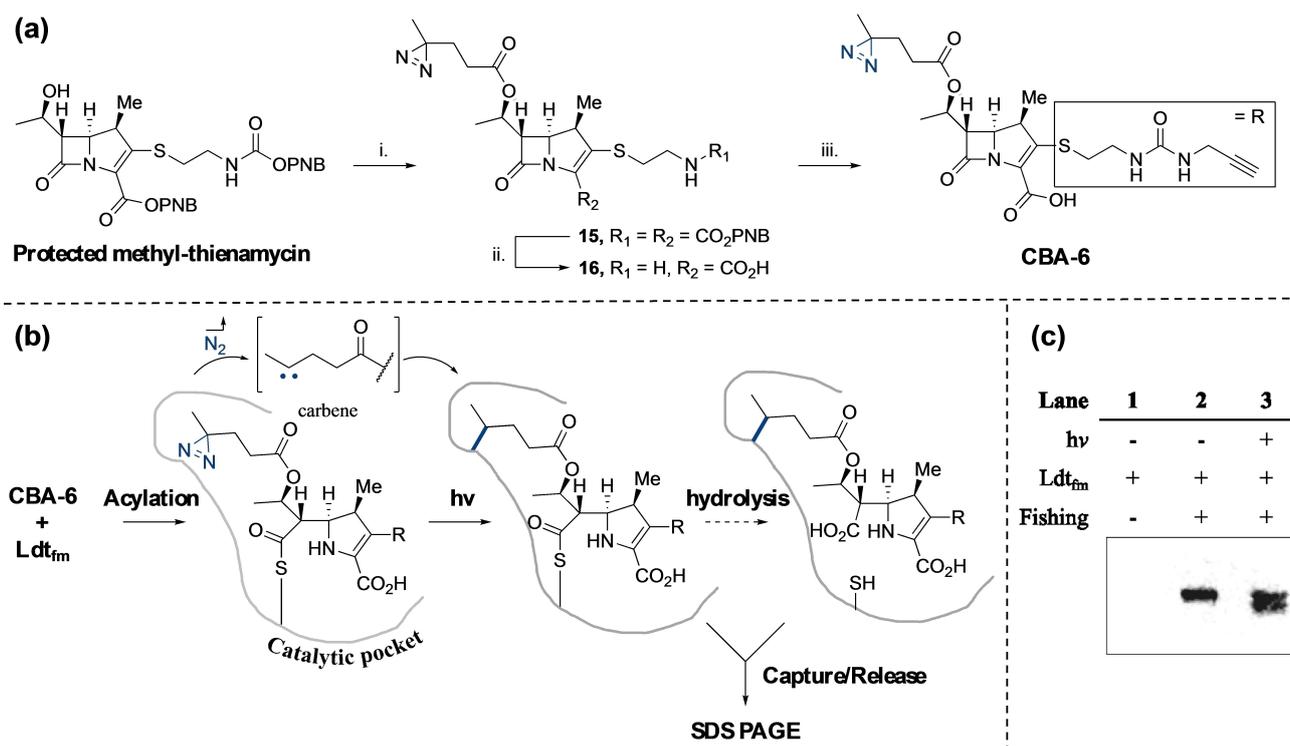


Figure 7. Synthesis and use of photoactivable carbapenem **CBA-6**. (a) Synthesis of **CBA-6**. i) 3-(3-methyl-3H-diazirin-3-yl)propanoic acid, DMAP, EDC, -20°C , 92%. ii) H_2 , 10% wt. Pt/C, THF/Buffer, 1 h, 25%. iii) *N*-(prop-2-yn-1-yl)-1H-imidazole-1-carboxamide, DMF/ H_2O , NaHCO_3 , 1 h, 25°C , 63%. (b) Acylation of **Ldt_{fm}** by **CBA-6** and photoactivation leading to formation of a second covalent bond stabilizing the adduct. The **Ldt_{fm}-CBA-6** interaction remains covalent even after hydrolysis of the Cys-**CBA-6** thioester bond. (c) SDS-PAGE of the click and release procedure applied to the **Ldt_{fm}-CBA-6** acyl-enzyme.

Capture and release of labeled **Ldt_{fm}** was performed as described above (Figure 2) with or without an additional photoactivation step between steps 1 and 2. Mass spectrometry analysis showed formation of the additional cross-link upon irradiation that resulted in the expected loss of two nitrogen atoms (-28.0 Da, see Table S1). **CBA-6** was functional in the click and release procedure (step 1 to 4 in Figure 2) but the irradiation did not improve recovery of labeled **Ldt_{fm}** (Figure 7C). This result suggests that hydrolysis of the thioester bond connecting **CBA-6** to **Ldt_{fm}** is not limiting to the recovery of fluorescent **Ldt_{fm}** after the final release step.

Conclusion

The targets of β -lactams are highly diverse and complex since they include both essential biosynthetic enzymes, the transpeptidases, as well as unessential hydrolases that participate in peptidoglycan metabolism.^[40] Assessing the essential nature of these transpeptidases is critical for drug development since it determines the killing activity of the drug. This task remains highly challenging despite decades of analyses of the mode of action and of the mechanisms of resistance to β -lactams. Since the enzymes have partially redundant functions, essentiality of the targets is defined by combinations of proteins rather than a single enzyme complement. These combinations differ both between and within bacterial species following the acquisition

of the so-called “low-affinity” PBPs responsible for β -lactam resistance. In addition, the complexity of these combinations includes evolutionary unrelated enzymes,^[5,41–43] including unidentified ones,^[44] in particular in *M. tuberculosis*.^[42,45] This context prompted us to develop here a new strategy for affinity purification of β -lactam targets. We functionalized the carbapenem core for affinity purification of β -lactam targets using a biotin-containing iminosydnone probe. First, we introduced at the carbapenem C_2 position an alkyne function connected by a series of five linkers. A urea linker was identified as the best candidate because (i) it did not impair the kinetics of target acylation and the stability of the resulting acyl-enzyme and (ii) it displayed the highest reactivity for copper-catalyzed cycloaddition of a fluorogenic probe. Second, we showed that the reaction of biotinylated **Ldt_{fm}** adducts captured on streptavidin magnetic beads with a TAMRA-cyclooctyne enables release and fluorescence labeling of **Ldt_{fm}** in a single step. Third, we synthesized bi-functionalized carbapenems containing both an alkyne and a photoactivable diazirine to introduce a second stabilizing covalent bond between **Ldt_{fm}** and the carbapenem. The strategy was compatible with mass-spectrometry detection of protein adducts in addition to fluorescent labeling. These tools should facilitate the characterization of the complex set of carbapenem targets, including the detection of unknown enzymes. Access to purification strategies based on acylation of active-site nucleophiles and photoactivation, alone or in combination offers specific advantages. Photoactivation pro-

vides very stable links but may decrease the specificity. Acylation of the nucleophile active-site is highly specific but the half-life of the adduct may compromise purification of certain targets.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: Activity-based probe · Antibiotic · Click and release · Iminosynone · Peptidoglycan

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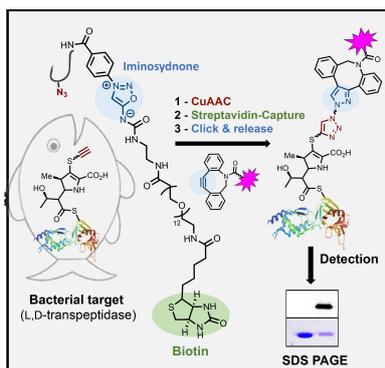
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FULL PAPER

In this study, we functionalized carba-penem β -lactams for the development of an original activity-based purification method of the complex targets of these drugs. The alkyne drug covalently bound to the target specifically reacted with an azido probe containing a biotin moiety for affinity purification and an iminosydnone for single-step elution and fluorescent labeling of the targets.



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Click and Release Chemistry for Activity-Based Purification of β -Lactam Targets

