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# DIAZABICYCLOOCTANE FUNCTIONALIZATION FOR INHIBITION OF $\beta$ -LACTAMASES FROM ENTEROBACTERIA

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10 ENTEROBACTERIA  
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16 *Mélanie Ethève-Quelquejeu,<sup>†\*</sup> Laura Iannazzo<sup>†\*</sup>*  
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29 **ABSTRACT:** Second-generation  $\beta$ -lactamase inhibitors containing a diazabicyclooctane  
30 (DBO) scaffold restore the activity of  $\beta$ -lactams against pathogenic bacteria, including those  
31 producing class A, C, and D enzymes that are not susceptible to first-generation inhibitors  
32 containing a  $\beta$ -lactam ring. Here, we report optimization of a synthetic route to access triazole-  
33 containing DBOs and biological evaluation of a series of 17 compounds for inhibition of five  
34  $\beta$ -lactamases representative of enzymes found in pathogenic Gram-negative bacteria. A strong  
35 correlation (Spearman coefficient of 0.87;  $p = 4.7 \cdot 10^{-21}$ ) was observed between the inhibition  
36 efficacy of purified  $\beta$ -lactamases and the potentiation of  $\beta$ -lactam antibacterial activity  
37 indicating that DBO functionalization did not impair penetration. In comparison to reference  
38 DBOs, avibactam and relebactam, our compounds displayed reduced efficacy likely due to the  
39 absence of hydrogen bonding with a conserved asparagine residue at position 132. This was  
40 partially compensated by additional interactions involving certain triazole substituents.  
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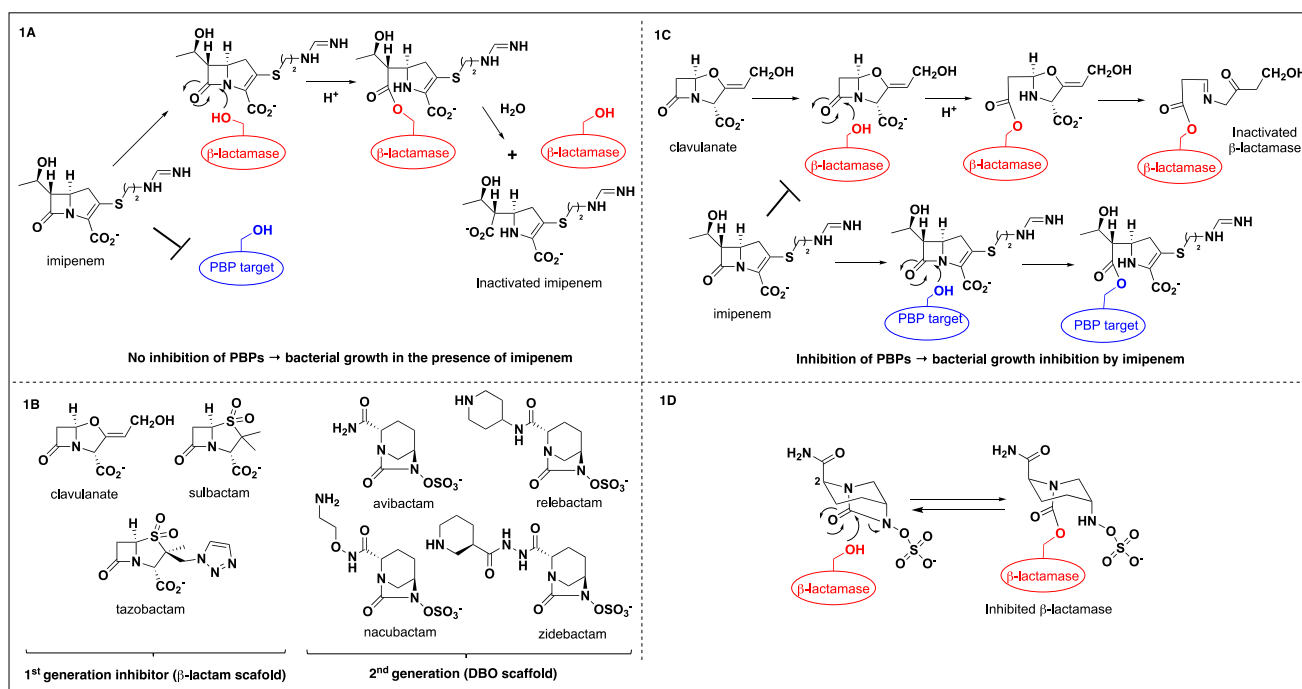
## INTRODUCTION

Penicillin is the first broadly used antibiotic introduced in medicine and the  $\beta$ -lactams remain the most widely used drug family in spite of the emergence of various resistance mechanisms.<sup>1</sup>

The targets of  $\beta$ -lactams are the D,D-transpeptidases, commonly referred to as penicillin-binding proteins (PBPs), that catalyze the last cross-linking step of peptidoglycan synthesis.<sup>2</sup>

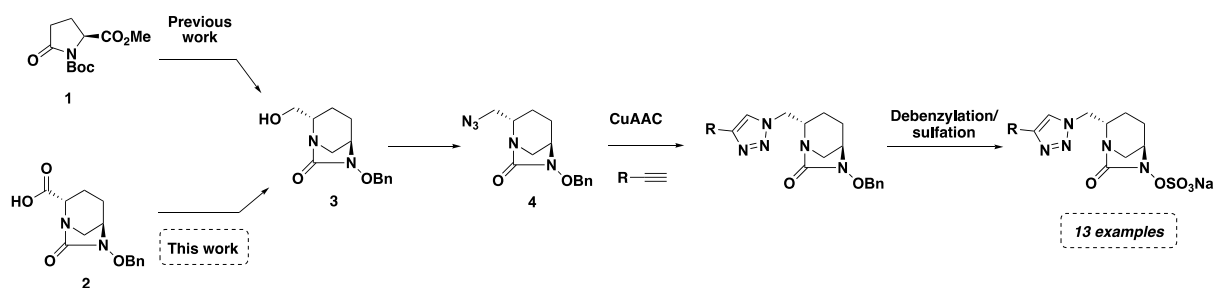
The most common  $\beta$ -lactam resistance mechanism in Gram-negative bacteria is the production of one or more  $\beta$ -lactamases, which catalyze the hydrolysis of the amide bond of the four-membered  $\beta$ -lactam ring (Figure 1A).<sup>3</sup> The hydrolysis product is not active accounting for the absence of inhibition of the PBP targets. This resistance mechanism has been successfully defeated by  $\beta$ -lactamase inhibitors, clavulanate, sulbactam, and tazobactam (Figure 1B).<sup>3,4</sup> In the presence of these inhibitors, the drug (imipenem in Figure 1C) remains active for acylation of the PBPs. First-generation inhibitors contain a  $\beta$ -lactam ring and act as suicide substrates forming stable adducts with  $\beta$ -lactamases (Figure 1C).<sup>3</sup> Avibactam (Figure 1B), clinically approved in combination with ceftazidime in 2015, is the first representative of second-generation inhibitors. Its structure is based on a distinct scaffold, diazabicyclooctane (DBO), which contains a five-membered cyclic urea rather than a  $\beta$ -lactam ring.<sup>5,6</sup> The mechanism of action of avibactam relies on nucleophilic attack of the DBO carbonyl by the active serine of class A, C and D  $\beta$ -lactamase leading to regioselective opening of the cyclic urea and nitrogen protonation (Figure 1D).<sup>6</sup> This reaction affords a stable carbamoyl-enzyme adduct. In contrast to  $\beta$ -lactam-containing inhibitors, formation of the carbamoyl-enzyme is fully reversible regenerating native  $\beta$ -lactamase and avibactam.<sup>6-8</sup> The efficacy of avibactam relies on the stabilization of the adduct that displaces the equilibrium toward the covalent carbamoyl-enzyme. Stabilization is also important to prevent carbamoyl-enzyme hydrolysis, which proceeds through an initial desulfonation for certain  $\beta$ -lactamases.<sup>9-11</sup> Compared to the first-generation inhibitors, avibactam inhibits a wider range of  $\beta$ -lactamases (class A, C, and some

class D enzymes).<sup>3</sup> In particular, avibactam inhibits  $\beta$ -lactamases that are fully insensitive to inhibition by clavulanate, such as *Klebsiella pneumoniae* carbapenemases (KPC) and *Mycobacterium abscessus* Bla<sub>Mab</sub>. Avibactam does not effectively inhibit zinc-dependent metallo- $\beta$ -lactamases (class B enzymes) since it binds only weakly to most tested enzymes and it is slowly hydrolyzed in certain cases.<sup>12,13</sup> In parallel to DBOs, unrelated  $\beta$ -lactamase inhibitors based on a cyclic boronic acid pharmacophore are currently developed, including vaborbactam,<sup>14,15</sup> recently approved by the FDA in combination with meropenem.<sup>16,17</sup> Discovery of additional  $\beta$ -lactamase inhibitors, including DBOs and boronates, is needed to broaden inhibition spectra, in particular to include inhibition of class B and D enzymes. In addition, mutations in  $\beta$ -lactamase genes have emerged *in vitro* and under treatment by the ceftazidime-avibactam combination.<sup>18-21</sup> Interestingly, the potency of vaborbactam was reported to be less affected than that of avibactam in strains producing KPC-2 carbapenemase variants that confer resistance to the ceftazidime-avibactam combination.<sup>17,22</sup>



**Figure 1.** Structure and mode of action of antibiotics and  $\beta$ -lactamase inhibitors. (1A)  $\beta$ -lactamase-mediated resistance to imipenem. (1B) Structure of 1<sup>st</sup> and 2<sup>nd</sup> generation  $\beta$ -

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3 lactamase inhibitors based on  $\beta$ -lactam and diazabicyclooctane (DBO) scaffolds, respectively.  
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5 (1C) Mode of action of a  $\beta$ -lactamase inhibitor (clavulanate), which prevents imipenem  
6 hydrolysis enabling inactivation of the penicillin-binding protein (PBP) targets. (1D)  
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8 Avibactam-mediated  $\beta$ -lactamase inhibition by reversible formation of a carbamoyl-enzyme.  
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13 Functionalization of the DBO scaffold has been extensively explored to improve their  $\beta$ -  
14 lactamase inhibition spectra.<sup>23-31</sup> In addition, introduction of side chains was reported to afford  
15 dual inhibitors that act both on  $\beta$ -lactamases and on essential penicillin-binding proteins  
16 required for cell wall peptidoglycan polymerization.<sup>23</sup> Since DBOs are chemically challenging  
17 molecules in terms of their structures, we recently developed a versatile method using copper-  
18 catalyzed alkyne-azide cycloaddition (CuAAC) to introduce 1,2,3-triazole-containing  
19 substituents at the C<sub>2</sub> position of the DBO scaffold.<sup>32</sup> Here, we report modification of our  
20 synthetic scheme (Scheme 1), which enabled synthesis of a series of compounds in sufficient  
21 amount for biological evaluation of a panel of five representatives of class A, C, and D  $\beta$ -  
22 lactamases. This evaluation involved both the determination of kinetic parameters for  $\beta$ -  
23 lactamase inhibition and evaluation of antibacterial activity against isogenic strains producing  
24 specific  $\beta$ -lactamases.  
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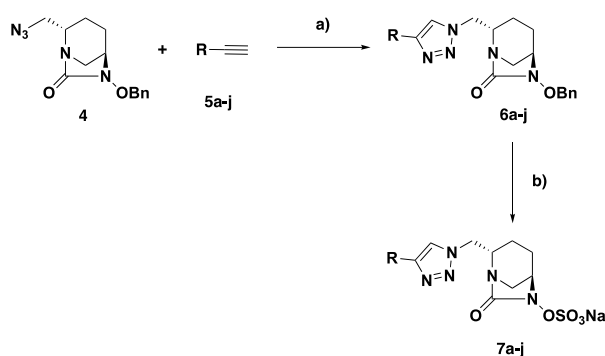


52 **Scheme 1.** Strategies to access functionalized DBOs

## 53 RESULTS AND DISCUSSION

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55 **Chemical synthesis.** We recently reported the synthesis of 2-azido-diazabicyclooctane **4**  
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58 starting from commercially available protected oxypyrrolidine **1** (Scheme 1).<sup>32</sup> This procedure  
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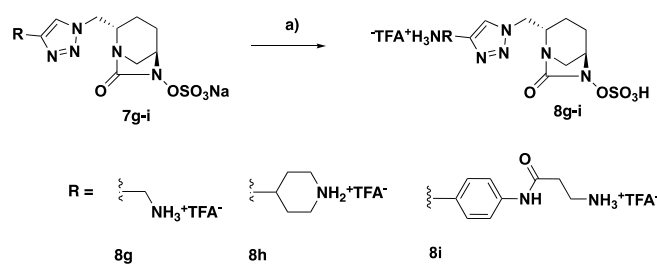
proceeds through the synthesis of the alcohol intermediate **3** and provides access to the key azido-intermediate **4** for the copper(I)-catalyzed Huisgen-Sharpless cycloaddition reaction<sup>33</sup> with an overall yield of 4%. Here, we propose a modification of our approach starting with the carboxylic acid DBO derivative **2**, which has recently been made commercially available. DBO **2** was activated with *iso*-butylchloroformate and directly reduced with NaBH<sub>4</sub> to afford the corresponding alcohol **3** (Scheme 1). By a classical strategy, the activation of **3** with mesylchloride followed by a nucleophilic substitution in the presence of sodium azide provided azido derivative **4**.



**Scheme 2.** a) CuSO<sub>4</sub> 30 mol %, sodium ascorbate 60 mol %, THF/H<sub>2</sub>O (3/1), rt, overnight; b) i. Pd/C, H<sub>2</sub>, MeOH, rt, 48 h, ii. SO<sub>3</sub>-Pyr, pyridine, rt, overnight, iii. DOWEX Na<sup>+</sup>.

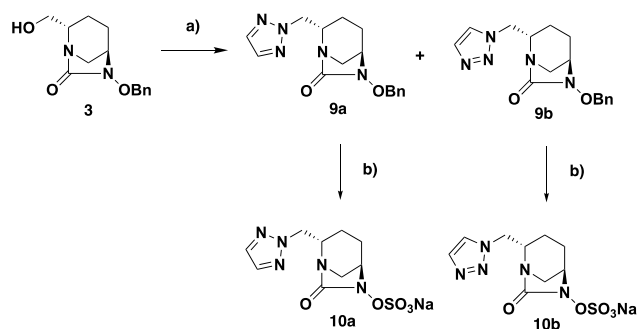
The functionalization of **4** was then explored with various alkynes to afford a series of new DBOs (Table 1). The rationale behind the study was to validate the synthetic approach for substituents containing various functional groups. Briefly, alkynes **5a**, **5b**, **5c**, **5d**, **5e**, **5h**, and **5j** were commercially available whereas **5f**, **5g**, and **5i** were prepared according to standard procedures (See supporting information). Compound **4** and alkynes **5a-j** were subjected to CuAAC conditions using CuSO<sub>4</sub> (30 mol %) and sodium ascorbate (60 mol %) in THF/H<sub>2</sub>O overnight at room temperature to afford compounds **6a-j** in 30 to 97% yield (Scheme 2). A wide range of alkynes was compatible with this procedure including compounds containing aromatic rings, such as pyridine (**5a**) and phenyl (**5b**, **5i**), Si, O, or N heteroatoms (**5c-f**), a

protected amine (**5g-i**), or a carboxylic acid function (**5j**). The deprotection of the benzyl group of compounds **6a-j** was performed by hydrogenolysis followed by sulfation with sulfur trioxide pyridine complex. The purification of the final compounds **7a-j** has been optimized by elution through DOWEX Na<sup>+</sup> followed by the dissolution of the residue in ethanol and filtration. This additional step was helpful for an efficient purification of DBOs over HPLC and allowed increasing the overall sulfation yield (from 10% for compounds **7a** and **7c** according to the previous method<sup>32</sup> to 21% and 14%, respectively, in the current study). The final deprotection step for Boc-containing DBOs (**7g**, **7h**, and **7i**) was performed by treatment with TFA in DCM at 0°C for 1 h (Scheme 3). Unprotected DBOs **8g**, **8h**, and **8i** were isolated in 36, 34 and 17% yield after HPLC purification, respectively.



**Scheme 3.** a) TFA, DCM, 0°C, 1 h; **8g** (36%); **8h** (34%); **8i** (17%).

To get access to unsubstituted triazoles **10a** and **10b** (Scheme 4), a new strategy has been designed. The alcohol **3** was activated with mesylchloride and treated with 1H-1,2,3-triazole under basic conditions<sup>34</sup> to afford regioisomers **9a** and **9b** in 44 and 37% yield, respectively. After separation of the two regioisomers by chromatography, each compound was submitted to hydrogenolysis conditions to remove the benzyl group followed by the sulfation step and elution on DOWEX Na<sup>+</sup>, as described above.

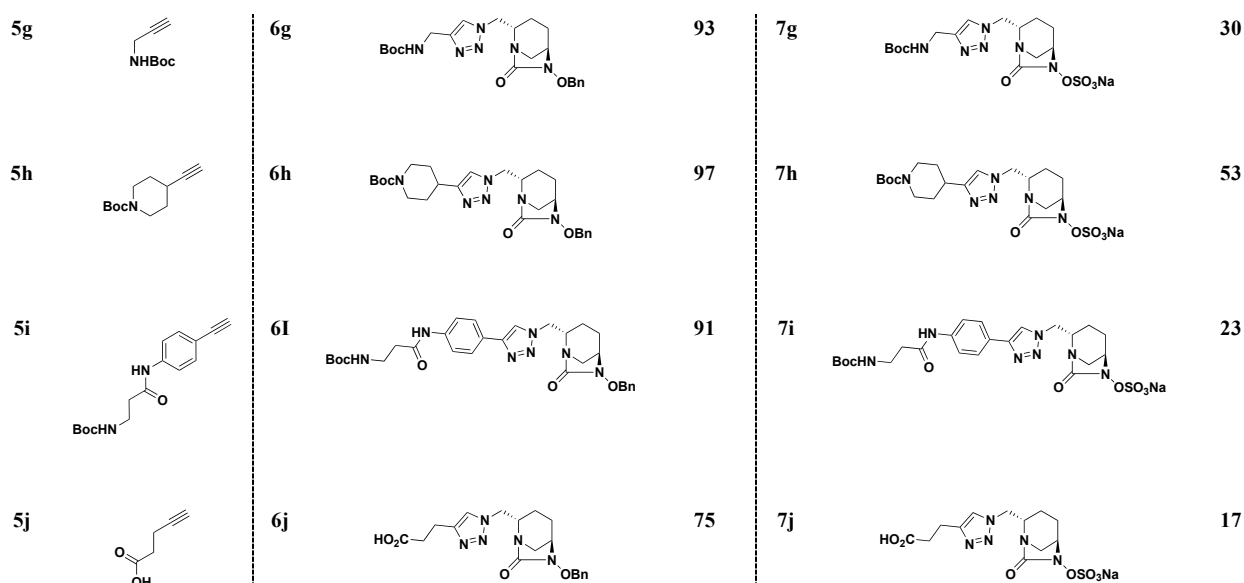


**Scheme 4.** a) i. MsCl, NEt<sub>3</sub>, DMAP, DCM, 0°C, 1h, ii. 1H-1,2,3-triazole, tBuOK, MeCN, reflux, 15h; **9a** (44%); **9b** (37%); b) i. Pd/C, H<sub>2</sub>, MeOH, rt, 48h, ii. SO<sub>3</sub>-Pyr, pyridine, rt, overnight, iii. DOWEX Na<sup>+</sup>; **10a** (27%); **10b** (8%).

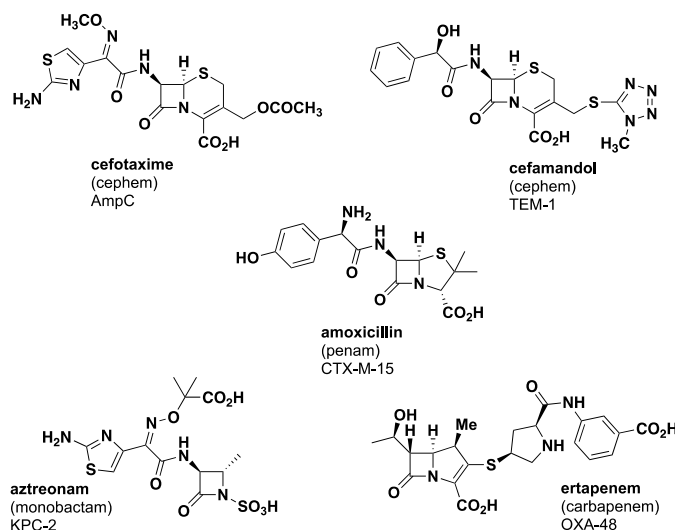
**Table 1.** Structure of alkynes used for CuAAC, resulting triazole-containing DBOs, and final sulfated compounds

Alkyne 5a-j	Compounds 6a-j	Yield [%]	Compounds 7a-j	Yield [%]
5a	6a	86	7a	21
5b	6b	85	7b	19
5c	6c	30	7c	14
5d	6d	86	7d	13
5e	6e	87	7e	18
5f	6f	90	7f	8





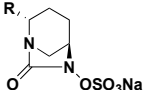
**Rationale for cell-based assays of DBOs.** We chose a panel of five  $\beta$ -lactamases that are clinically relevant, belong to different structural classes, and differ by their hydrolysis spectrum.<sup>3</sup> KPC-2 (class A) and OXA-48 (class D) hydrolyze carbapenems (carbapenemases), which are often the last resort antibiotics against multi-drug-resistant bacteria. CTX-M-15 and AmpC from *Enterobacter cloacae* were chosen as representatives of class A extended-spectrum  $\beta$ -lactamases and of class C cephalosporinases (preferential hydrolysis of cepheems). TEM-1 is a wide-spread representative of penicillinases (preferential hydrolysis of penams). Genes encoding these five  $\beta$ -lactamases were cloned under the control of an inducible promoter into the vector pTR99k and introduced in *Escherichia coli* Top10. The efficacy of the DBOs was tested by determining the minimal inhibitory concentration (MIC) of  $\beta$ -lactams in the presence or absence of a fixed concentration of inhibitor (15  $\mu$ M corresponding to 4  $\mu$ g/ml for avibactam). Since the TEM-1, KPC-2, CTX-M-15, AmpC, and OXA-48  $\beta$ -lactamases display different substrate spectra, preliminary experiments were performed to choose a suitable antibiotic to compare the efficacy of the inhibitors (Figure 2).


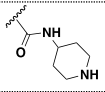
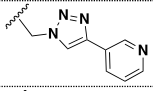
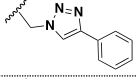
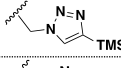
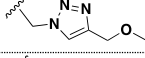
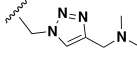
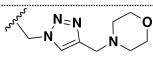
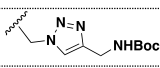
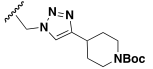
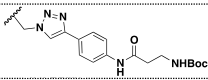
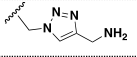
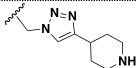
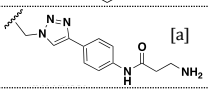
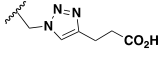
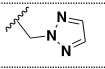
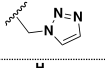
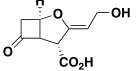


**Figure 2.** Structure of  $\beta$ -lactam antibiotics used as substrates to determine the activity of  $\beta$ -lactamases TEM-1, KPC-2, CTX-M-15, AmpC, and OXA-48. The drugs belong to the cephem, monobactam, penam, or carbapenem classes of  $\beta$ -lactams, as indicated in parenthesis.

For each of the five chosen  $\beta$ -lactam/ $\beta$ -lactamase combinations,  $\beta$ -lactamase production led to large MIC increases, ranging from 256 to 4,096 fold. For the cefamandole/TEM-1 combination for example, production of TEM-1 in the *E. coli* Top10 host resulted in a 1,024-fold increase in the MIC of cefamandole (from 2 to 2,048  $\mu\text{g/ml}$ ) (Table 2). Such a high dynamic range offers the possibility to compare inhibitors with various efficacies leading to partial inhibition of the  $\beta$ -lactamases and various MICs in the 2 to 2,048  $\mu\text{g/ml}$  range. Complete *in vivo* inhibition of TEM-1 requires very efficacious DBOs able to lead to the 1,024-fold decrease in the MIC needed to restore full susceptibility. For such inhibitors, the MICs of cefamandole against *E. coli* TOP10 and its isogenic TEM-producing derivative should be the same (2  $\mu\text{g/ml}$ ). For TEM-1 and cefamandole, this is the case for the first-generation inhibitor clavulanate (Table 2).

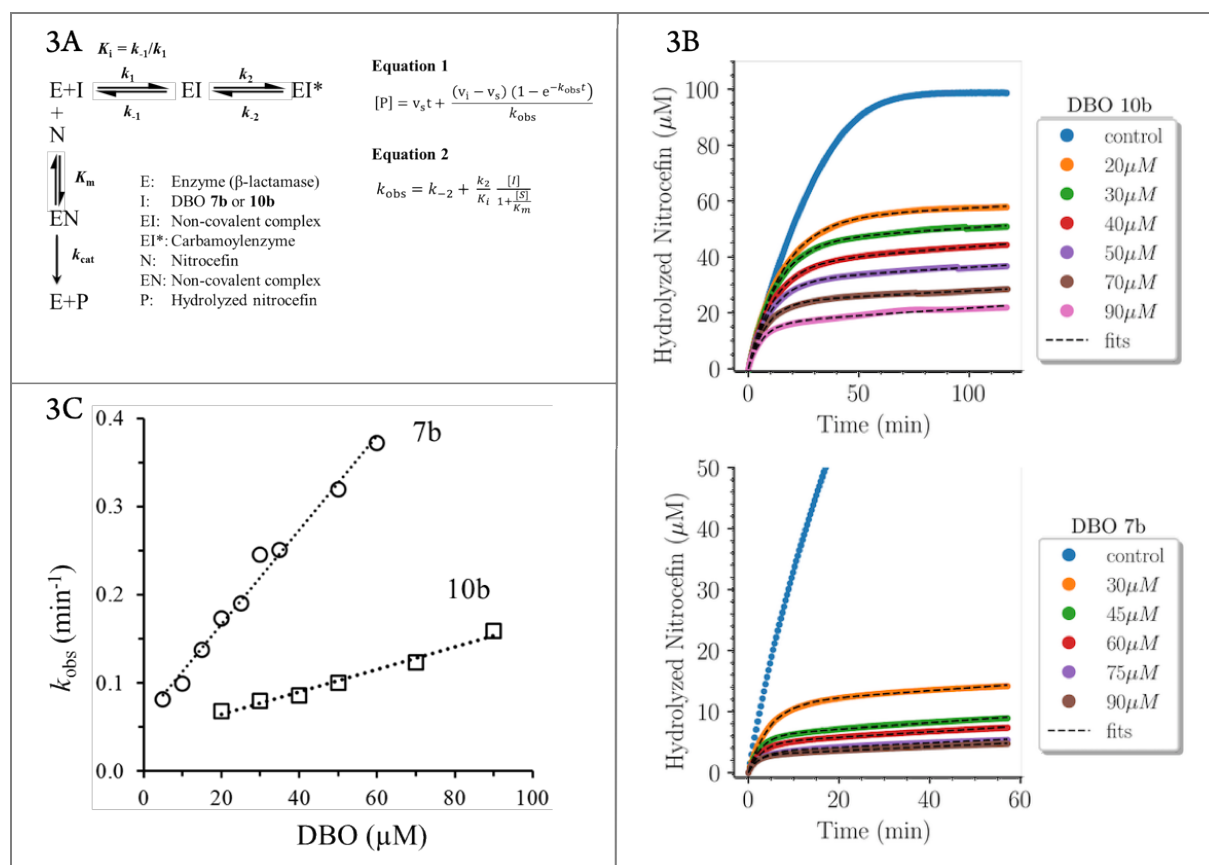
**Table 2.** Inhibition of penicillinase TEM-1 by DBOs and clavulanate

	Carbamoylation efficacy ( $\text{M}^{-1} \text{s}^{-1}$ )	MIC of cefamandole ( $\mu\text{g/ml}$ )	
		Without <sup>[b]</sup> TEM-1	With TEM-1

R =				
no inhibitor		NA	2	2,048
	Avibactam	88,000 ± 2,000	2	2
	Relebactam	43,000 ± 1,000	2	4
	7a	1,400 ± 100	2	8
	7b	4,700 ± 1,000	4	16
	7c	370 ± 50	2	64
	7d	830 ± 50	2	16
	7e	780 ± 80	2	32
	7f	1,200 ± 100	2	8
	7g	2,800 ± 400	2	128
	7h	1,700 ± 200	2	256
	7i	3,700 ± 500	2	256
	8g	3,100 ± 200	4	4
	8h	3,600 ± 300	2	4
	8i	940 ± 150	2	32
	7j	720 ± 60	2	32
	10a	2,600 ± 300	2	16
	10b	1,200 ± 100	2	8
	Clavulanate	NA	2	2

NA, not applicable as clavulanate and DBOs have different modes of action. Kinetics constants were deduced from a minimum of six progress curves obtained in a minimum of two independent experiments. Reported MICs are the medians from five biological repeats obtained in two independent experiments. <sup>[a]</sup> Zwitterionic species have been tested. <sup>[b]</sup> Avibactam alone was moderately active (MIC = 32 µg/ml) against *E. coli* Top10 harboring the vector pTRC-99k but none of our DBOs displayed activity in the absence of β-lactam (MIC >128 µg/ml).

**Rationale for enzymatic inhibition assays of DBOs.** Genes encoding soluble fragments of TEM-1, KPC-2, CTX-M-15, AmpC, and OXA-48 were cloned into the pET-TEV vector.  $\beta$ -lactamases were produced in *E. coli* BL21 and purified from clarified lysates by metal affinity and size-exclusion chromatography. For *in vitro* evaluation of inhibition efficacy, purified  $\beta$ -lactamases were incubated with DBOs at various concentrations with a fixed concentration of the chromogenic cephalosporin nitrocefin (Figure 3).<sup>7</sup> Estimates of the inhibition efficacy were provided by the ratio of the kinetic constants  $k_2$  and  $K_i$ , which takes into account the first order constant  $k_2$  for the chemical step of the reaction (carbamoylation) and the dissociation constant  $K_i$  for the preceding formation of a non-covalent complex. Since  $k_2$  and  $K_i$  could not be independently determined the changes in efficacy might arise from either or both tighter binding and placement of the groups in a more reactive conformation in the active site.



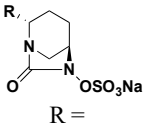


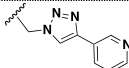
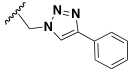
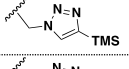
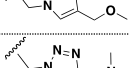
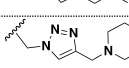
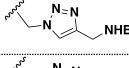
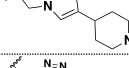
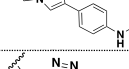
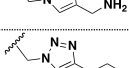
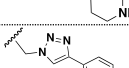
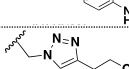
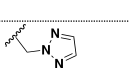
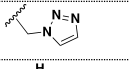
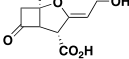
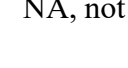
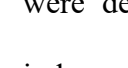
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3 **Figure 3.** Inhibition of  $\beta$ -lactamases by DBOs. (A) Reaction scheme and definition of rate  
4 constants.  $v_i$  and  $v_s$  are the initial and final velocities of the reactions. (B) Time-dependent  
5 inhibition of KPC-2 by **7b** and **10b**. KPC-2 (2.5 nM) was incubated with nitrocefirin (100  $\mu$ M)  
6 and various concentrations of **7b** and **10b** (20-90  $\mu$ M or 30-90  $\mu$ M, respectively). Equation 1  
7 was fitted to progress curves to obtain the pseudo-first order rate constant  $k_{obs}$ . Representative  
8 results from one experiment are shown. (C) Determination of carbamoylation rate constant  
9  $k_2/K_i$ .  $k_{obs}$  was plotted as a function of the DBO concentration. Equation 2 was fitted to data to  
10 determine the value of the  $k_2/K_i$  ratio and of  $k_2$ . Kinetic constant  $k_2$  was low for all DBOs (See  
11 supplementary Table S1). Kinetics constants were deduced from a minimum of six progress  
12 curves obtained in a minimum of two independent experiments.

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27 **Efficacy of inhibition of penicillinase TEM-1 by DBOs.** The two reference DBOs, avibactam  
28 and relebactam, afforded 1,024- and 512-fold reductions in the MIC of cefamandole indicating  
29 that inhibition of TEM-1 was complete or nearly complete, respectively. Our synthetic DBOs  
30 offered the possibility to investigate the impact of aromatic rings, carboxylic acids, amines, and  
31 the corresponding Boc-protected compounds on the efficacy of DBOs. Two amine-containing  
32 compounds, **8g** and **8h**, were as active as relebactam, also producing a 512-fold reduction in  
33 the MIC of cefamandole. A 256-fold reduction was observed for compounds **7a** and **7f**, which  
34 contain a pyridine or a morpholine group in their side chain, respectively. The *in vivo* efficacy  
35 of the remaining compounds was lower, in particular that of Boc-protected compounds **7g**, **7h**,  
36 and **7i** (4- or 8-fold reduction in the MIC). The *in vitro* efficacy ( $k_2/K_i$ ) of the latter compounds  
37 was in the 1,600 to 3,700  $M^{-1} s^{-1}$  range. These values are similar to those of DBOs **8g**, **8h**, and  
38 **8i** (3,100 to 3,700  $M^{-1} s^{-1}$  range) that afforded a 512-fold reduction in the MICs of cefamandole.  
39 The difference between the antibacterial activity of the two series of compounds (**7g-7i versus**  
40 **8g-8i**) may therefore result from a poor penetration of the Boc-containing compounds through  
41 the outer membrane of the tested strain. To further investigate the impact of the

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3 functionalization of the triazole ring, we synthesized regioisomers **10a** and **10b** containing  
4 unsubstituted triazoles. The inactivation efficacy of these compounds ( $k_2/K_i$  of 2,600 and 1,200  
5  $M^{-1} s^{-1}$ , respectively) was similar to that of compounds containing a substituted triazole ring (up  
6 to 4,700  $M^{-1} s^{-1}$ ), but *ca.* 19- and 9-fold lower than that of avibactam (88,000  $M^{-1} s^{-1}$ ) and  
7 relebactam (43,000  $M^{-1} s^{-1}$ ). These results indicate that the triazole linkage is disfavored in  
8 comparison to the carboxamide of avibactam or to the amide of relebactam. None of the tested  
9 side chains fully compensated this effect *in vitro*. Of note, inhibition of TEM-1 by avibactam  
10 and **10b** was reversible with similar off-rate constants (Supplementary Figure S1).

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22 **Efficacy of carbapenemase inhibition by DBOs.** KPC-2 was fully inhibited by the two  
23 reference DBOs, avibactam and relebactam, affording a 4,096-fold reduction in the MIC of  
24 aztreonam used as the reporter drug for this  $\beta$ -lactamase (Table 3). Clavulanate was inactive as  
25 expected.<sup>35</sup> Among the DBOs synthesized in this study, **7a**, **7f**, **8g**, and **8h** were the most active  
26 *in vivo*, affording a 64-fold reduction in the MIC of aztreonam. However, the MIC of aztreonam  
27 in the presence of these inhibitors (MIC = 32  $\mu\text{g/ml}$ ) was much higher than that obtained with  
28 avibactam and relebactam (MIC = 0.5  $\mu\text{g/ml}$ ) indicating that KPC-2 retained substantial  
29 hydrolytic activity when the inhibitors were added to the culture medium. The difference in the  
30 MICs conveyed by **7a**, **7f**, **8g**, and **8h** in comparison to avibactam and relebactam correlated  
31 with a >20-fold lower inhibition efficacy *in vitro* ( $k_2/K_i$  in the 120 to 550  $M^{-1} s^{-1}$  range for **7a**,  
32 **7f**, **8g**, and **8h** *versus* 26,000 and 10,000  $M^{-1} s^{-1}$  for avibactam and relebactam, respectively).  
33 Comparisons of the data obtained with DBOs containing unsubstituted triazole rings (**10a** and  
34 **10b**;  $k_2/K_i$  of 350 and 280  $M^{-1} s^{-1}$ , respectively) indicate that the side chain of three DBOs, **7a**,  
35 **7b**, and **7i** had a favorable impact on the inactivation efficacy ( $k_2/K_i$  of 550, 930, and 1,100  $M^{-1}$   
36  $s^{-1}$ , respectively). Several side chains were poorly tolerated by KPC-2, including a  
37 trimethylsilyl in **7c** ( $k_2/K_i = 97 M^{-1} s^{-1}$ ), a methoxy in **7d** (18  $M^{-1} s^{-1}$ ), and a carboxylate in **7j**  
38 (70  $M^{-1} s^{-1}$ ).

**Table 3.** Inhibition of class A  $\beta$ -lactamases by DBOs and clavulanate

 R =	Inhibition of KPC-2			Inhibition of CTX-M-15			
	Carbamoylation efficacy ( $M^{-1} s^{-1}$ )	MIC of aztreonam ( $\mu g/ml$ )		Carbamoylation efficacy ( $M^{-1} s^{-1}$ )	MIC of amoxicillin ( $\mu g/ml$ )		
		Without KPC-2	With KPC-2		Without CTX-M-15	With CTX-M-15	
no inhibitor	NA	0.5	2,048	NA	8	2,048	
	Avibactam	26,000 $\pm$ 1,000	0.25	0.5	130,000 $\pm$ 10,000	2	2
	Relebactam	10,000 $\pm$ 1,000	0.25	0.5	960 $\pm$ 80	2	16
	7a	550 $\pm$ 10	0.25	32	1,700 $\pm$ 400	2	32
	7b	930 $\pm$ 40	0.25	64	6,900 $\pm$ 2,000	8	64
	7c	97 $\pm$ 8	0.5	512	400 $\pm$ 10	8	1,024
	7d	18 $\pm$ 2	0.5	512	120 $\pm$ 40	8	256
	7e	120 $\pm$ 50	0.5	64	29 $\pm$ 5	4	256
	7f	190 $\pm$ 20	0.25	32	100 $\pm$ 10	8	64
	7g	540 $\pm$ 60	0.25	512	130 $\pm$ 10	8	1,024
	7h	830 $\pm$ 90	0.25	2,048	230 $\pm$ 30	8	4,096
	7i	1,100 $\pm$ 200	0.5	512	2,400 $\pm$ 200	8	768
	[a] 8g	120 $\pm$ 10	0.25	32	88 $\pm$ 13	4	128
	[a] 8h	340 $\pm$ 30	0.25	32	350 $\pm$ 60	4	64
	[a] 8i	180 $\pm$ 33	0.25	64	150 $\pm$ 10	4	128
	7j	70 $\pm$ 8	0.25	256	110 $\pm$ 10	2	96
	10a	350 $\pm$ 40	0.5	512	120 $\pm$ 10	4	512
	10b	280 $\pm$ 30	0.25	512	280 $\pm$ 10	8	64
	Clavulanate	NA	0.5	512	NA	8	2

NA, not applicable as clavulanate and DBOs have different modes of action. Kinetics constants were deduced from a minimum of six progress curves obtained in a minimum of two independent experiments. Reported MICs are the medians from five biological repeats obtained in two independent experiments. <sup>[a]</sup> Zwitterionic species have been tested.

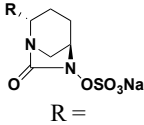

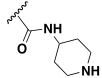
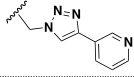
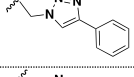
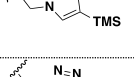
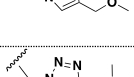
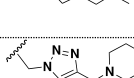
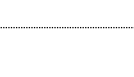
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3 **Efficacy of extended-spectrum  $\beta$ -lactamase inhibition by DBOs.** Inhibition of CTX-M-15  
4 was tested with amoxicillin as the reporter drug (Table 3). Avibactam fully inhibited CTX-M-  
5 15 *in vivo* (MIC = 2  $\mu\text{g/ml}$ ) but CTX-M-15 retained partial activity in bacteria exposed to  
6 relebactam (MIC = 16  $\mu\text{g/ml}$ ). In comparison with unsubstituted DBOs **10a** and **10b** ( $k_2/K_i$  of  
7 120 and 280  $\text{M}^{-1} \text{s}^{-1}$ , respectively), the pyridine and phenyl groups present in **7a**, **7b**, and **7i**  
8 enhanced CTX-M-15 inactivation ( $k_2/K_i$  of 1,700, 6,900, and 2,400  $\text{M}^{-1} \text{s}^{-1}$ , respectively).  
9 However, these compounds displayed >18-fold lower *in vitro* efficacies in comparison to  
10 avibactam and, accordingly, **7a** and **7b** did not fully restore the antibacterial activity of  
11 amoxicillin (MIC of 32 and 64  $\mu\text{g/ml}$ , respectively). Relebactam was less active than **7a** and  
12 **7b** *in vitro* (960 versus 1,700 and 6,900  $\text{M}^{-1} \text{s}^{-1}$ ). Accordingly, relebactam failed to fully inhibit  
13 CTX-M-15 *in vivo* (MIC = 16  $\mu\text{g/ml}$ ). DBO **7i** only afforded an 8-fold reduction in the MIC of  
14 amoxicillin in agreement with the proposed negative impact of the Boc group on permeability  
15 described above. Of note, four DBOs (avibactam, relebactam, **7a** and **7j**) slightly increased (4-  
16 fold) the activity of amoxicillin against the *E. coli* TOP10 host, which does not produce CTX-  
17 M-15. These results suggest that these DBOs may inhibit PBP targets in addition to the  $\beta$ -  
18 lactamase. This would imply that the DBOs and amoxicillin may inhibit the targets in a  
19 cooperative manner leading to a higher antibacterial activity in comparison to amoxicillin alone.  
20 Of note, inhibition of class B PBP2 has been documented for several DBOs.<sup>23,24</sup>

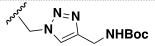
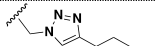
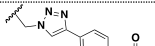
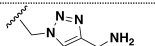
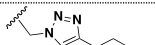
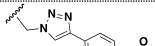
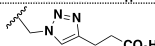
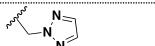
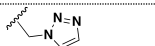
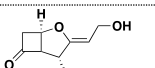
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45 **Inhibition of class C and D  $\beta$ -lactamases by DBOs.** Production of AmpC in the *E. coli* Top10  
46 host led to a 2,048-fold increase in the MIC of cefotaxime (from 0.125 to 256  $\mu\text{g/ml}$ ). *In vivo*  
47 inhibition of AmpC (class C) was partial for avibactam (MIC = 1  $\mu\text{g/ml}$ ) and nearly complete  
48 for relebactam (MIC = 0.25  $\mu\text{g/ml}$ ). The DBOs synthesized in this study achieved moderate  
49 MIC reduction and their *in vitro* inhibition efficacy was *ca.* 100-fold lower than that of the  
50 reference DBOs (avibactam and relebactam) (Table 4). The largest fold reductions in the MICs  
51 were obtained with **10a** and **10b** containing an unsubstituted triazole ring (from 256 to 32 and  
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16  $\mu\text{g/ml}$ , respectively). Accordingly, these two compounds were the most active *in vitro* (200 and 210  $\text{M}^{-1} \text{s}^{-1}$ ) indicating that none of the side chains had a positive impact on inhibition efficacy. DBOs **7b** and **7i** were as active *in vitro* as DBOs **10a** and **10b** containing unsubstituted triazole rings. The side chain of other compounds had a negative impact leading to 5- to 13-fold lower inhibition efficacies. Avibactam was the only DBO that significantly reduced the MIC of ertapenem against *E. coli* Top10 producing class D OXA-48 although the drug retained substantial antibacterial activity (MIC = 0.5  $\mu\text{g/ml}$  versus < 0.03  $\mu\text{g/ml}$  in the absence of OXA-48 production). *In vitro* evaluation indicated that four DBOs (**7b**, **7g**, **7h**, and **7i**) inhibited OXA-48 with efficacies similar to those of compounds **10a** and **10b**, which contained unsubstituted triazole rings ( $k_2/K_i$  ranges of 110 to 210 versus 140 to 150  $\text{M}^{-1} \text{s}^{-1}$ , respectively). The side chains of other DBOs, including that of relebactam, were detrimental since no inhibition was detected at the highest concentration that was tested (100  $\mu\text{M}$ ).

**Table 4.** Inhibition of class C (AmpC) and class D (OXA-48)  $\beta$ -lactamases by DBOs and clavulanate

 R =	Inhibition of AmpC				Inhibition of OXA-48		
	Carbamoylation efficacy ( $\text{M}^{-1} \text{s}^{-1}$ )	MIC of cefotaxime ( $\mu\text{g/ml}$ )		Carbamoylation efficacy ( $\text{M}^{-1} \text{s}^{-1}$ )	MIC of ertapenem ( $\mu\text{g/ml}$ )		
		Without AmpC	With AmpC		Without OXA-48	With OXA-48	
no inhibitor	NA	0.125	256	NA	< 0.03	8	
 Avibactam	18,000 $\pm$ 1,000	0.125	1	980 $\pm$ 60	< 0.03	0.5	
 Relebactam	15,000 $\pm$ 2,000	0.125	0.25	No inhibition	< 0.03	8	
 <b>7a</b>	85 $\pm$ 7	0.125	128	No inhibition	< 0.03	8	
 <b>7b</b>	200 $\pm$ 10	0.25	128	110 $\pm$ 30	< 0.03	8	
 <b>7c</b>	24 $\pm$ 1	0.125	64	No inhibition	< 0.03	16	
 <b>7d</b>	15 $\pm$ 5	0.125	128	No inhibition	< 0.03	16	
 <b>7e</b>	20 $\pm$ 8	0.125	64	No inhibition	< 0.03	8	
 <b>7f</b>	31 $\pm$ 3	0.125	128	No inhibition	< 0.03	8	

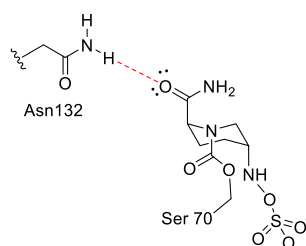
	7g	51 ± 8	0.125	128	210 ± 50	< 0.03	16
	7h	29 ± 6	0.125	128	210 ± 30	< 0.03	16
	7i	180 ± 20	0.125	128	150 ± 40	< 0.03	16
	8g	16 ± 2	0.125	128	No inhibition	< 0.03	8
	8h	26 ± 2	0.125	128	No inhibition	< 0.03	8
	8i	28 ± 13	0.25	64	No inhibition	< 0.03	4
	7j	99 ± 9	0.25	64	No inhibition	< 0.03	8
	10a	200 ± 20	0.125	32	150 ± 30	< 0.03	8
	10b	210 ± 20	0.125	16	140 ± 20	< 0.03	8
	Clavulanate	NA	0.0625	128	NA	< 0.03	8

NA, not applicable as clavulanate and DBOs have different modes of action. No inhibition, no inhibition at the highest inhibitor concentration that was tested (100  $\mu$ M). Kinetics constants were deduced from a minimum of six progress curves obtained in a minimum of two independent experiments. Reported MICs are the medians from five biological repeats obtained in two independent experiments. <sup>[a]</sup> Zwitterionic species have been tested.

**Evaluation of antibacterial activity against clinical isolates.** The capacity of DBOs to restore the activity of  $\beta$ -lactams against  $\beta$ -lactam-producing clinical isolates of enterobacteria was tested for a subset of the compounds synthesized in this study (Supplementary Table S2 and S3). The data obtained with clinical isolates were in agreement with the inhibition profiles determined by *in vitro* inhibition of  $\beta$ -lactamases and antibacterial evaluation in isogenic strains of *E. coli* (Tables 2, 3, and 4). In particular, the most active compounds, such as **7a**, restored the activity of  $\beta$ -lactams against clinical isolates producing TEM-1, KPC-2, and CTX-M-15, alone or in combination.

### Role of asparagine 132 in the carbamoylation efficacy of KPC-2 and CTX-M-15 by DBOs.

The crystal structure of KPC-2 and CTX-M-15 acylated by avibactam revealed hydrogen interaction between the oxygen of the avibactam carboxamide group and the amine in the carboxamide group of Asn at position 132 of the  $\beta$ -lactamases (Ambler numbering) (Figure 4).<sup>10, 36,37</sup> To assess the role of this interaction, we further characterized derivatives of KPC-2 and CTX-M-15 harboring an N<sup>132</sup>G substitution in the conserved SDN motif. This substitution was previously shown to impair (>1,000-fold) the efficacy of carbamoylation of KPC-2 and CTX-M-15 by avibactam whereas hydrolysis of nitrocefin was moderately ( $\leq 3$  fold) affected.<sup>38</sup> In this study, we compared the impact of the N<sup>132</sup>G substitution on the carbamoylation efficacy of the  $\beta$ -lactamases by avibactam, **10b** (unsubstituted triazole), and **7b** (phenyl-substituted triazole) (Table 5).



**Figure 4.** Hydrogen interaction between the carboxamides of avibactam and Asn at position 132 of  $\beta$ -lactamases (Ambler numbering).

**Table 5.** Impact of the N<sup>132</sup>G substitution on the carbamoylation efficacy ( $k_2/K_i$ ) of KPC-2 and CTX-M-15 by DBOs

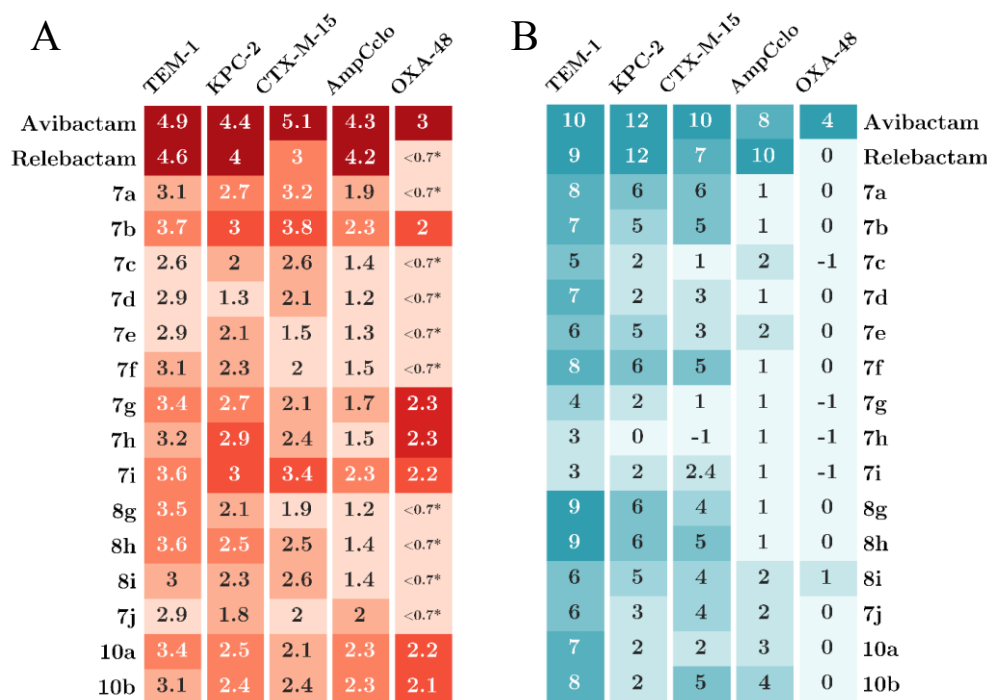
$\beta$ -lactamase	$k_2/K_i$ ( $M^{-1} s^{-1}$ ) for indicated DBO		
	Avibactam	<b>10b</b>	<b>7b</b>
KPC-2	26,000 $\pm$ 1,000	280 $\pm$ 30	930 $\pm$ 40
KPC-2 N <sup>132</sup> G	53 $\pm$ 3	31 $\pm$ 6	21 $\pm$ 3
CTX-M-15	130,000 $\pm$ 10,000	280 $\pm$ 10	6,900 $\pm$ 200
CTX-M-15 N <sup>132</sup> G	70 $\pm$ 6	6.6 $\pm$ 2.0	140 $\pm$ 10

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3 In comparison to avibactam, the substitution had a lower impact on the efficacy of  
4 carbamoylation of KPC-2 and CTX-M-15 by **10b** and **7b**. The latter compounds were much  
5  
6 less active than avibactam against the wild-type enzymes but as active as avibactam against  
7  
8 KPC-2 and CTX-M-15 harboring N<sup>132</sup>G. These observations suggest that the hydrogen  
9  
10 interaction between avibactam and N<sup>132</sup> is critical for efficacious carbamoylation of KPC-2 and  
11  
12 CTX-M-15 by avibactam and that the absence of this interaction in the case of triazole-  
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14 containing DBOs could be largely responsible for their lower efficacy in comparison to  
15  
16 avibactam. The modest  $k_2/K_i$  reductions observed for **10b** and **7b** following introduction of  
17  
18 N<sup>132</sup>G could be accounted for by additional roles of N<sup>132</sup> or a weak interaction of the latter  
19  
20 residue with the triazole ring. The role of the hydrogen interaction involving N<sup>132</sup> is also  
21  
22 highlighted by comparison of relebactam and compound **8h** that contain the same side chain  
23  
24 connected to the DBO scaffold by an amide or triazole, respectively.  
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## 31 CONCLUSIONS

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33 Efficacies of the 17 DBOs for inhibition of TEM-1, KPC-2, CTX-M-15, AmpC, and OXA-48  
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35 were recapitulated in a heat map (Figure 5A) to highlight key differences. Compounds **10a** and  
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37 **10b**, containing unsubstituted triazole rings, were less active than the reference DBOs  
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39 (avibactam and relebactam) against TEM-1, KPC-2, CTX-M-15, and AmpC. Loss of the  
40  
41 hydrogen interaction between the carboxamide of avibactam and conserved residue N<sup>132</sup> is  
42  
43 likely to be responsible for reduced activity that could not be fully compensated by any of the  
44  
45 substituents introduced in the triazole ring. In comparison to **10a** and **10b**, two DBOs, **7a** and  
46  
47 **7b**, displayed improved inhibition efficacies, in particular against the KPC-2 carbapenemase  
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49 and the CTX-M-15 extended-spectrum  $\beta$ -lactamase, indicating that the pyridine and  
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51 phenylgroups present in **7a** and **7b** had a positive impact on the inhibition of these class A  $\beta$ -  
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53 lactamases. Several side chains had a deleterious effect, in particular the methoxy and carboxyl  
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55 groups present in **7d** and **7j**. *In vivo* antibacterial susceptibility data were recapitulated in a heat  
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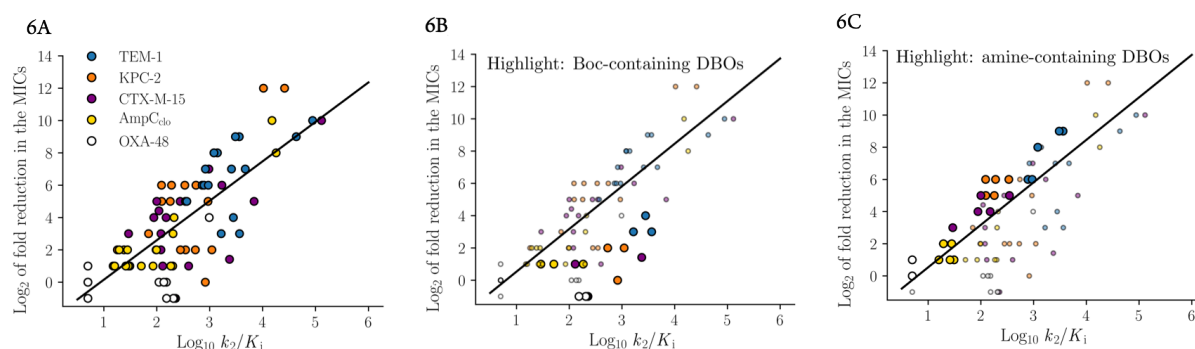
map presented in Figure 5B. The most active DBOs *in vitro* (**7a** and **7b**) were also among the most active compounds for restoring the activity of  $\beta$ -lactams against KPC-2- or CTX-M-15-producing *E. coli* strains.



**Figure 5.** Heat maps highlighting differences in (A) the efficacy of *in vitro* inhibition of  $\beta$ -lactamases ( $\text{Log}_{10}$  of  $k_2/K_i$ ) and (B) the antibacterial activity ( $\text{Log}_2$  of the fold reduction in the MICs). \*: No inhibition at the highest inhibitor concentration that was tested (100  $\mu\text{M}$ ). The color code shows the activities relative to the most active compound for each  $\beta$ -lactamase. Darker color indicates higher activity.

The correlation between antibacterial activity and *in vitro* inhibition efficacies did not appear to apply to all DBOs, in particular for DBOs containing protected amines, which appeared to be less active *in vivo* than expected from their *in vitro* activity. This was explored by plotting the *in vitro* efficacy of the DBOs against the fold reduction of the MICs they afforded (Figure 6A). Plotting the complete set of data, *i.e.* the inhibition of five  $\beta$ -lactamases by 17 DBOs, revealed a positive correlation between the  $\text{Log}_{10}$  of the inhibition efficacy ( $\text{Log}_{10}$  of  $k_2/K_i$ ) and

the  $\text{Log}_2$  of the fold reduction in the MIC of the reporter drugs (Spearman correlation coefficient of 0.72;  $p = 5.3 \cdot 10^{-14}$ ). DBOs harboring a Boc group in their side chain clustered below the regression line indicating that their *in vitro* efficacy was not translated into antibacterial activity due to impaired penetration through the outer membrane (Figure 6B). The Spearman correlation coefficient calculated without Boc-containing compounds was higher than that calculated with the entire set of data (0.87;  $p = 4.7 \cdot 10^{-21}$ ). In contrast, amine-containing compounds clustered above the regression line indicating a facilitated access to the periplasm (Figure 6C).



**Figure 6.** Correlation between the efficacy of DBOs in reducing MICs and in inhibiting  $\beta$ -lactamases. The trend line was obtained by linear regression. (A) Complete set of data. (B) The regression line was calculated by omitting Boc-containing DBOs (highlighted with large circles). (C) Clustering of amine-containing DBOs above the regression line (highlighted with large circles).

To summarize, we have developed an efficient strategy based on copper(I)-catalyzed Huisgen-Sharpless cycloaddition reaction for the synthesis of new DBOs. To achieve this goal, a modification of the original synthesis of azido derivative **4** has been realized. Our methodology is compatible with a wide range of functional groups for the modification of the C2 position of the DBO scaffold. The synthetic route provides a versatile approach to explore the chemical space for novel interactions with the catalytic cavity of potential targets.<sup>3,23,24,32</sup> Although the first series of triazole-containing compounds reported in this study did not lead to inhibitors

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3 with improved characteristics for inhibition of  $\beta$ -lactamases of Gram-negative bacteria. The  
4 reduced efficacy could be due to the absence of hydrogen bonding with a conserved asparagine  
5 residue at position 132. This could only be partially compensated by additional interactions  
6 involving the triazole substituents.  
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## 12 13 **EXPERIMENTAL SECTION**

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16 *General procedures.* Reactions were carried out under argon atmosphere and solvents were  
17 dried using standard methods and distilled before use. Unless otherwise specified, materials  
18 were purchased from commercial suppliers and used without further purification. TLC was  
19 performed using Merck commercial aluminum sheets coated with silica gel 60 F<sub>254</sub>. Compounds  
20 were detected by charring with phosphomolibdic acid in ethanol followed by heating.  
21 Purification was performed by flash chromatography on silica gel (60 Å, 180-240 mesh; Merck)  
22 or by preparative high-performance liquid chromatography (HPLC) using Shimadzu  
23 Prominence system with a Zorbax Extend-C18 prepHT column (150 x 21.2 mm, 5  $\mu$ m;  
24 Agilent). Compounds were eluted with a linear gradient (from 100% of H<sub>2</sub>O to 100% of  
25 CH<sub>3</sub>CN) that was applied between 5 and 30 min at a flow rate of 15 ml/min. Products were  
26 detected by UV absorption at 214 nm. NMR spectra were recorded on Bruker spectrometers  
27 (AM250, Avance II 500 and Avance III HD 4000). Chemical shifts ( $\delta$ ) are reported in ppm and  
28 referenced to the residual proton or carbon resonance of the solvents: CDCl<sub>3</sub> ( $\delta$  7.26), D<sub>2</sub>O ( $\delta$   
29 4.79) or (CD<sub>3</sub>)<sub>2</sub>SO ( $\delta$  2.50) for <sup>1</sup>H and CDCl<sub>3</sub> ( $\delta$  77.16) or (CD<sub>3</sub>)<sub>2</sub>SO ( $\delta$  39.52) for <sup>13</sup>C. Signals  
30 were assigned using 1D (<sup>1</sup>H and <sup>13</sup>C) and 2D (HSQC, COSY, and HMBC) spectra. NMR  
31 coupling constants (J) are reported in hertz (Hz). High-resolution mass spectroscopy (HRMS)  
32 was recorded with an ion trap mass analyzer under electrospray ionization (ESI) in the negative  
33 or positive ionization detection mode. HRMS was performed using Thermo Scientific LTQ  
34 Orbitrap XL and Bruker MaXis II ETD spectrometers. The purity of final compounds ( $\geq$ 95%)  
35 was established by analytical HPLC, which was performed on a Shimadzu Prominence system  
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with a Hypersil® BDS C18 column (150 x 4.6 mm, 5  $\mu$ m) or an Agilent Zorbax Extend C18 column (250 x 4.6 mm, 5  $\mu$ m) with UV detection at 214 and 220 nm. Optical rotations were measured with a sodium lamp (589 nm) at 20°C on a Perkin Elmer polarimeter.

**Compound (4).** Isobutyl chloroformate (6.26 ml, 47.1 mmol) was added dropwise at 0°C to a solution of (2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octane-2-carboxylic acid **2** (6.5 g, 23.5 mmol) and triethylamine (3.64 ml, 25.9 mmol) in THF (75 ml). The solution was stirred at 0°C for 2 h and NaBH<sub>4</sub> (2.72 g, 70.6 mmol) was gradually added. The reaction mixture was stirred for 3 additional hours at 0°C. Water (110 ml) and ethyl acetate (110 ml) were slowly added and the heterogeneous mixture was stirred for 30 min at room temperature. The organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure to afford (2S,5R)-6-(benzyloxy)-2-(hydroxymethyl)-1,6-diazabicyclo[3.2.1]octan-7-one **3**, which was used in the next step without further purification. Triethylamine (9.94 ml, 70.6 mmol), DMAP (4.36 g, 35.3 mmol) and Mesyl Chloride (2.76 ml, 35.3 mmol) were added at 0°C to a solution of crude product (23.5 mmol) in DCM (71 ml). The reaction mixture was stirred at 0°C for 2 h. DCM was then added and the organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under vacuum to afford ((2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)methyl methanesulfonate, which was used in the next step without further purification. Sodium azide (7.69 g, 117 mmol) was added to a solution of ((2S,5R)-6-(benzyloxy)-7-oxo-1,6-diazabicyclo[3.2.1]octan-2-yl)methyl methanesulfonate (23.5 mmol) in DMF (95 ml). The solution was stirred at 90°C for 20 h. After being cooled to room temperature, ethyl acetate was added and the organic layer was washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduce pressure. Purification by flash chromatography using cyclohexane/ethyl acetate (7/3) as the eluent gave compound **4** as a white solid (2.4 g, 35% over 3 steps). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.43-7.33 (m, 5H, H<sub>10,11,12</sub>), 5.04 (d,  $J$  = 11.5 Hz, 1H, H<sub>8</sub>), 4.89 (d,  $J$  = 11.5 Hz, 1H, H<sub>8</sub>), 3.61-3.56 (m, 1H, H<sub>1</sub>), 3.53 (dd,  $J$  = 12.5, 7.4 Hz,



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3 1H, H<sub>7</sub>), 3.35-3.32 (m, 2H, H<sub>4</sub>,  $\gamma$ ), 2.97 (s, 2H, H<sub>5</sub>), 2.06-2.02 (m, 1H, H<sub>3</sub>), 2.01-1.95 (m, 1H,  
4 H<sub>2</sub>), 1.63-1.55 (m, 1H, H<sub>3</sub>), 1.54-1.49 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  169.7 (C<sub>6</sub>),  
5 136.0 (C<sub>9</sub>), 129.3 (2C, C<sub>10</sub>), 128.8 (C<sub>12</sub>), 128.6 (2C, C<sub>11</sub>), 78.3 (C<sub>8</sub>), 58.6 (C<sub>4</sub>), 56.6 (C<sub>1</sub>), 53.3  
6 (C<sub>7</sub>), 44.1 (C<sub>5</sub>), 20.5 (C<sub>2</sub>), 20.0 (C<sub>3</sub>). HRMS calculated for C<sub>14</sub>H<sub>18</sub>N<sub>5</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 288.14605;  
7 found 288.14523. [ $\alpha$ ]<sub>D</sub>: -53.2° (7.8 mg/ml, MeOH).  
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15 **General procedure for Copper(I)-catalyzed azide-alkyne cycloaddition reaction.** To a  
16 solution of azido **4** in THF, were successively added alkyne **5** (2 eq), sodium ascorbate (0.6 eq,  
17 in water) and CuSO<sub>4</sub> (0.3 eq, in water). The heterogeneous mixture was stirred overnight at  
18 room temperature. Ethyl acetate was added and the organic layer was washed with brine, dried  
19 over MgSO<sub>4</sub>, and concentrated under reduced pressure. The crude product was purified by flash  
20 chromatography to afford product **6**.  
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31 **General procedure for introduction of sodium sulphite.** 10 wt. % Pd/C (1 eq) was added to  
32 a solution of **6** in MeOH and the reaction mixture was stirred under H<sub>2</sub> for 48 h at room  
33 temperature. Palladium was removed by filtration through celite and the filtrate concentrated.  
34 Sulfur trioxide pyridine complex (6 eq) was added to a solution of deprotected compound in  
35 pyridine and the reaction mixture was stirred for 2 h at room temperature. Additional SO<sub>3</sub>-  
36 pyridine complex (2 eq) was added, stirred overnight at room temperature, and pyridine was  
37 removed under reduced pressure. The crude product was solubilized in water, filtered, eluted  
38 on Dowex-Na resin with H<sub>2</sub>O, and lyophilized. The residue was dissolved in EtOH, filtered,  
39 and concentrated under vacuum. HPLC purification afforded product **7**.  
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52 **General procedure for Boc deprotection.** TFA (12 eq) was added dropwise at 0°C to a  
53 solution of **7** (1 eq) in DCM. The reaction mixture was stirred at 0°C for 1 h and concentrated  
54 under vacuum. HPLC purification afforded product **8**.  
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3 **Compound (6a)**. Following the general procedure for CuAAC, starting from compound **4** (200  
4 mg, 0.70 mmol) and 3-ethynylpyridine **5a** (144 mg, 1.39 mmol), and using DCM/MeOH (96/4)  
5 as eluent for flash chromatography purification, compound **6a** was obtained as a colorless oil  
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8 as eluent for flash chromatography purification, compound **6a** was obtained as a colorless oil  
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10 (234 mg, 86%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 9.01 (bs, 1H, H<sub>14</sub>), 8.57 (bd, *J* = 3.8 Hz, 1H,  
11 H<sub>13</sub>), 8.17 (dt, *J* = 7.9, 2.0 Hz, 1H, H<sub>11</sub>), 8.05 (s, 1H, H<sub>8</sub>), 7.42-7.33 (m, 6H, H<sub>12, 17, 18, 19</sub>), 5.02  
12 (d, *J* = 11.5 Hz, 1H, H<sub>15</sub>), 4.88 (d, *J* = 11.5 Hz, 1H, H<sub>15</sub>), 4.62 (dd, *J* = 14.3, 8.3 Hz, 1H, H<sub>7</sub>),  
13 4.56 (dd, *J* = 14.3, 6.5 Hz, 1H, H<sub>7</sub>), 3.92-3.87 (m, 1H, H<sub>1</sub>), 3.38-3.36 (m, 1H, H<sub>4</sub>), 2.99-2.92  
14 (m, 2H, H<sub>5</sub>), 2.12-2.07 (m, 1H, H<sub>3</sub>), 2.06-1.99 (m, 1H, H<sub>2</sub>), 1.73-1.67 (m, 1H, H<sub>3</sub>), 1.65-1.59  
15 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.4 (C<sub>6</sub>), 149.4 (C<sub>13</sub>), 147.3 (C<sub>14</sub>), 145.3 (C<sub>9</sub>),  
16 135.8 (C<sub>16</sub>), 133.2 (C<sub>11</sub>), 129.4 (2C, C<sub>17</sub>), 128.9 (C<sub>19</sub>), 128.7 (2C, C<sub>18</sub>), 126.8 (C<sub>10</sub>), 123.9 (C<sub>12</sub>),  
17 120.5 (C<sub>8</sub>), 78.4 (C<sub>15</sub>), 58.4 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 52.1 (C<sub>7</sub>), 43.8 (C<sub>5</sub>), 20.5 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS  
18 calculated for C<sub>21</sub>H<sub>23</sub>N<sub>6</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 391.18825; found: 391.18886. [α]<sub>D</sub>: -17.9° (8.0 mg/ml,  
19 MeOH).  
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33 **Compound (6b)**. Following the general procedure for CuAAC, starting from compound **4** (200  
34 mg, 0.70 mmol) and phenylacetylene **5b** (154 μl, 1.40 mmol), and using cyclohexane/ethyl  
35 acetate (4/6) as eluent for flash chromatography purification, compound **6b** was obtained as a  
36 colorless oil (232 mg, 85%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.97 (s, 1H, H<sub>8</sub>), 7.83 (d, *J* = 7.2  
37 Hz, 2H, H<sub>11</sub>), 7.43-7.32 (m, 8H, H<sub>12, 13, 16, 17, 18</sub>), 5.03 (d, *J* = 11.5 Hz, 1H, H<sub>14</sub>), 4.88 (d, *J* = 11.5  
38 Hz, 1H, H<sub>14</sub>), 4.63-4.54 (m, 2H, H<sub>7</sub>), 3.91-3.86 (m, 1H, H<sub>1</sub>), 3.36-3.35 (m, 1H, H<sub>4</sub>), 2.97-2.90  
39 (m, 2H, H<sub>5</sub>), 2.10-2.06 (m, 1H, H<sub>3</sub>), 2.03-1.96 (m, 1H, H<sub>2</sub>), 1.72-1.68 (m, 1H, H<sub>3</sub>), 1.66-1.60  
40 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.3 (C<sub>6</sub>), 148.2 (C<sub>9</sub>), 135.8 (C<sub>15</sub>), 130.3 (C<sub>10</sub>),  
41 129.4 (2C, C<sub>16</sub>), 129.0 (2C, C<sub>12</sub>), 128.9 (C<sub>18</sub>), 128.7 (2C, C<sub>17</sub>), 128.5 (C<sub>13</sub>), 126.0 (2C, C<sub>11</sub>),  
42 120.3 (C<sub>8</sub>), 78.4 (C<sub>14</sub>), 58.4 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 52.2 (C<sub>7</sub>), 43.9 (C<sub>5</sub>), 20.4 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS  
43 calculated for C<sub>22</sub>H<sub>24</sub>N<sub>5</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 390.19300; found: 390.19165. [α]<sub>D</sub>: -44.5° (5.2 mg/ml,  
44 DMSO).  
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**Compound (6c).** Following the general procedure for CuAAC, starting from compound **4** (200 mg, 0.70 mmol) and ethynyltrimethylsilane **5c** (388  $\mu$ l, 2.80 mmol), and using cyclohexane/ethyl acetate (3/7) as eluent for flash chromatography purification, compound **6c** was obtained as a colorless oil (82 mg, 30%).  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  7.64 (s, 1H, H<sub>8</sub>), 7.42-7.32 (m, 5H, H<sub>13,14,15</sub>), 5.01 (d,  $J$  = 11.5 Hz, 1H, H<sub>11</sub>), 4.86 (d,  $J$  = 11.5 Hz, 1H, H<sub>11</sub>), 4.55 (d,  $J$  = 7.3 Hz, 2H, H<sub>7</sub>), 3.85-3.75 (m, 1H, H<sub>1</sub>), 3.34-3.30 (m, 1H, H<sub>4</sub>), 2.95-2.82 (m, 2H, H<sub>5</sub>), 2.09-1.97 (m, 1H, H<sub>3</sub>), 1.96-1.85 (m, 1H, H<sub>2</sub>), 1.72-1.62 (m, 1H, H<sub>3</sub>), 1.62-1.50 (m, 1H, H<sub>2</sub>), 0.30 (s, 9H, H<sub>10</sub>).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.6 (C<sub>6</sub>), 147.3 (C<sub>9</sub>), 135.9 (C<sub>12</sub>), 129.4 (3C, C<sub>8</sub> and C<sub>13</sub>), 128.9 (C<sub>15</sub>), 128.7 (2C, C<sub>14</sub>), 78.4 (C<sub>11</sub>), 58.5 (C<sub>4</sub>), 56.8 (C<sub>1</sub>), 51.5 (C<sub>7</sub>), 44.0 (C<sub>5</sub>), 20.3 (C<sub>2</sub>), 19.9 (C<sub>3</sub>), -1.0 (3C, C<sub>10</sub>). HRMS calculated for C<sub>19</sub>H<sub>28</sub>N<sub>6</sub>O<sub>2</sub>Si [M+H]<sup>+</sup>: 386.20123; found: 386.20053.  $[\alpha]_D$ : -24.4° (5.6 mg/ml, MeOH).

**Compound (6d).** Following the general procedure for CuAAC, starting from compound **4** (200 mg, 0.70 mmol) and methyl propargyl ether **5d** (118  $\mu$ l, 1.40 mmol), and using DCM/MeOH (96/4) as eluent for flash chromatography purification, compound **6d** was obtained as a colorless oil (215 mg, 86%).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.70 (s, 1H, H<sub>8</sub>), 7.42-7.33 (m, 5H, H<sub>14,15,16</sub>), 5.02 (d,  $J$  = 11.5 Hz, 1H, H<sub>12</sub>), 4.87 (d,  $J$  = 11.5 Hz, 1H, H<sub>12</sub>), 4.57 (s, 2H, H<sub>10</sub>), 4.55-4.48 (m, 2H, H<sub>7</sub>), 3.81 (qd,  $J$  = 7.4, 4.2 Hz, 1H, H<sub>1</sub>), 3.40 (s, 3H, H<sub>11</sub>), 3.34 (q,  $J$  = 3.0 Hz, 1H, H<sub>4</sub>), 2.93 (bd,  $J$  = 11.9 Hz, 1H, H<sub>5</sub>), 2.88 (d,  $J$  = 11.9 Hz, 1H, H<sub>5</sub>), 2.09-2.03 (m, 1H, H<sub>3</sub>), 1.97 (dq,  $J$  = 15.1, 7.4 Hz, 1H, H<sub>2</sub>), 1.70-1.63 (m, 1H, H<sub>3</sub>), 1.60-1.54 (m, 1H, H<sub>2</sub>).  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta$  169.4 (C<sub>6</sub>), 145.8 (C<sub>9</sub>), 135.9 (C<sub>13</sub>), 129.4 (2C, C<sub>14</sub>), 128.9 (C<sub>16</sub>), 128.7 (2C, C<sub>15</sub>), 123.0 (C<sub>8</sub>), 78.4 (C<sub>12</sub>), 66.1 (C<sub>10</sub>), 58.5 (C<sub>11</sub>), 58.4 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 52.0 (C<sub>7</sub>), 43.9 (C<sub>5</sub>), 20.4 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS calculated for C<sub>18</sub>H<sub>24</sub>N<sub>5</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 358.18791; found: 358.218771.  $[\alpha]_D$ : -27.7° (6.6 mg/ml, MeOH).

**Compound (6e).** Following the general procedure for CuAAC, starting from compound **4** (150 mg, 0.52 mmol) and 3-dimethylamino-1-propyne **5e** (112  $\mu$ l, 1.04 mmol), and using

DCM/MeOH (9/1) + 1% NH<sub>4</sub>OH as eluent for flash chromatography purification, compound **6e** was obtained as a pale yellow oil (169 mg, 87%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.65 (s, 1H, H<sub>8</sub>), 7.38-7.28 (m, 5H, H<sub>14,15,16</sub>), 4.97 (d, *J* = 11.5 Hz, 1H, H<sub>12</sub>), 4.83 (d, *J* = 11.5 Hz, 1H, H<sub>12</sub>), 4.54-4.44 (m, 2H, H<sub>7</sub>), 3.79-3.74 (m, 1H, H<sub>1</sub>), 3.56 (s, 2H, H<sub>10</sub>), 3.36-3.34 (m, 1H, H<sub>4</sub>), 2.95-2.87 (m, 2H, H<sub>5</sub>), 2.22 (s, 6H, H<sub>11</sub>), 2.03-1.97 (m, 1H, H<sub>3</sub>), 1.95-1.87 (m, 1H, H<sub>2</sub>), 1.68-1.62 (m, 1H, H<sub>3</sub>), 1.55-1.49 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.4 (C<sub>6</sub>), 143.3 (C<sub>9</sub>), 135.9 (C<sub>13</sub>), 129.4 (2C, C<sub>14</sub>), 128.9 (C<sub>16</sub>), 128.7 (2C, C<sub>15</sub>), 124.2 (C<sub>8</sub>), 78.4 (C<sub>12</sub>), 58.4 (C<sub>4</sub>), 56.9 (C<sub>1</sub>), 53.9 (C<sub>10</sub>), 52.0 (C<sub>7</sub>), 44.5 (2C, C<sub>11</sub>), 43.7 (C<sub>5</sub>), 20.4 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS calculated for C<sub>19</sub>H<sub>27</sub>N<sub>6</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 371.21955; found: 371.21900. [α]<sub>D</sub>: -24.7° (7.5 mg/ml, MeOH).

**Compound (6f)**. Following the general procedure for CuAAC, starting from compound **4** (80 mg, 0.28 mmol) and compound **5f** (70 mg, 0.56 mmol), and using DCM/MeOH (96/4) as eluent for flash chromatography purification, compound **6f** was obtained as a colorless oil (104 mg, 90%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.61 (s, 1H, H<sub>8</sub>), 7.37 – 7.28 (m, 5H, H<sub>15,16,17</sub>), 4.96 (d, *J* = 11.5 Hz, 1H, H<sub>13</sub>), 4.83 (d, *J* = 11.5 Hz, 1H, H<sub>13</sub>), 4.49 (dd, *J* = 14.2, 8.1 Hz, 1H, H<sub>7</sub>), 4.44 (dd, *J* = 14.2, 6.9 Hz, 1H, H<sub>7</sub>), 3.78-3.73 (m, 1H, H<sub>1</sub>), 3.64 (t, *J* = 4.7 Hz, 4H, H<sub>12</sub>), 3.60 (s, 2H, H<sub>10</sub>), 3.33 (bs, 1H, H<sub>4</sub>), 2.88 (s, 2H, H<sub>5</sub>), 2.45 (t, *J* = 4.7 Hz, 4H, H<sub>11</sub>), 2.03-1.98 (m, 1H, H<sub>3</sub>), 1.95-1.87 (m, 1H, H<sub>2</sub>), 1.66-1.60 (m, 1H, H<sub>3</sub>), 1.54-1.48 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.3 (C<sub>6</sub>), 144.5 (C<sub>9</sub>), 135.7 (C<sub>14</sub>), 129.2 (2C, C<sub>15</sub>), 128.7 (C<sub>17</sub>), 128.5 (2C, C<sub>16</sub>), 123.0 (C<sub>8</sub>), 78.2 (C<sub>13</sub>), 66.8 (2C, C<sub>12</sub>), 58.2 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 53.6 (C<sub>10</sub>), 53.4 (2C, C<sub>11</sub>), 51.7 (C<sub>7</sub>), 43.7 (C<sub>5</sub>), 20.3 (C<sub>2</sub>), 19.6 (C<sub>3</sub>). HRMS calculated for C<sub>21</sub>H<sub>29</sub>N<sub>6</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 413.22957; found: 413.22753. [α]<sub>D</sub>: -17.3° (4.4 mg/ml, MeOH).

**Compound (6g)**. Following the general procedure for CuAAC, starting from compound **4** (200 mg, 0.70 mmol) and compound **5g** (217 mg, 1.40 mmol), and using cyclohexane/ethyl acetate (1/9) as eluent for flash chromatography purification, compound **6g** was obtained as a colorless oil (289 mg, 93%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.62 (s, 1H, H<sub>8</sub>), 7.39-7.30 (m, 5H,

H<sub>16,17,18</sub>), 4.98 (d,  $J = 11.5$  Hz, 1H, H<sub>14</sub>), 4.84 (d,  $J = 11.5$  Hz, 1H, H<sub>14</sub>), 4.51 (dd,  $J = 14.2, 8.1$  Hz, 1H, H<sub>7</sub>), 4.44 (dd,  $J = 14.2, 6.9$  Hz, 1H, H<sub>7</sub>), 4.34 (d,  $J = 5.9$  Hz, 2H, H<sub>10</sub>), 3.79-3.74 (m, 1H, H<sub>1</sub>), 3.33 (bs, 1H, H<sub>4</sub>), 2.89 (s, 2H, H<sub>5</sub>), 2.05-1.99 (m, 1H, H<sub>3</sub>), 1.97-1.89 (m, 1H, H<sub>2</sub>), 1.67-1.60 (m, 1H, H<sub>3</sub>), 1.54-1.48 (m, 1H, H<sub>2</sub>), 1.41 (s, 9H, H<sub>13</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  169.3 (C<sub>6</sub>), 155.9 (C<sub>11</sub>), 145.9 (C<sub>9</sub>), 135.8 (C<sub>15</sub>), 129.3 (2C, C<sub>16</sub>), 128.8 (C<sub>18</sub>), 128.6 (2C, C<sub>17</sub>), 122.3 (C<sub>8</sub>), 79.7 (C<sub>12</sub>), 78.2 (C<sub>14</sub>), 58.3 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 51.7 (C<sub>7</sub>), 43.8 (C<sub>5</sub>), 36.3 (C<sub>10</sub>), 28.4 (3C, C<sub>13</sub>), 20.3 (C<sub>2</sub>), 19.7 (C<sub>3</sub>). HRMS calculated for C<sub>22</sub>H<sub>31</sub>N<sub>6</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 443.24068; found: 443.23941. [ $\alpha$ ]<sub>D</sub>: -20.5° (5.4 mg/ml, MeOH).

**Compound (6h).** Following the general procedure for CuAAC, starting from compound **4** (200 mg, 0.70 mmol) and 1-boc-4-ethynylpiperidine **5h** (293 mg, 1.40 mmol), and using DCM/MeOH (96/4) as eluent for flash chromatography purification, compound **6h** was obtained as a colorless oil (336 mg, 97%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.37-7.27 (m, 5H, H<sub>18,19,20</sub>), 4.96 (d,  $J = 11.5$  Hz, 1H, H<sub>16</sub>), 4.82 (d,  $J = 11.5$  Hz, 1H, H<sub>16</sub>), 4.49 (bs, 2H, H<sub>7</sub>), 4.10 (bs, 2H, H<sub>12</sub>), 3.76 (bs, 1H, H<sub>1</sub>), 3.32 (s, 1H, H<sub>4</sub>), 2.88 (bs, 5H, H<sub>5,10,12</sub>), 2.01-1.98 (m, 3H, H<sub>3,11</sub>), 1.94-1.87 (m, 1H, H<sub>2</sub>), 1.68-1.62 (m, 1H, H<sub>3</sub>), 1.57-1.51 (m, 3H, H<sub>2,11</sub>), 1.42 (s, 9H, H<sub>15</sub>)\*H<sub>8</sub> not visible on the <sup>1</sup>H NMR spectrum. <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  169.2 (C<sub>6</sub>), 154.7 (C<sub>13</sub>), 135.7 (C<sub>17</sub>), 129.1 (2C, C<sub>18</sub>), 128.7 (C<sub>20</sub>), 128.5 (2C, C<sub>19</sub>), 79.4 (C<sub>14</sub>), 78.1 (C<sub>16</sub>), 58.3 (C<sub>4</sub>), 56.5 (C<sub>1</sub>), 52.1 (C<sub>7</sub>), 43.6 (3C, C<sub>5</sub> and C<sub>12</sub>), 33.6 (C<sub>10</sub>), 31.4 (2C, C<sub>11</sub>), 28.4 (3C, C<sub>15</sub>), 20.4 (C<sub>2</sub>), 19.6 (C<sub>3</sub>) \*C<sub>8</sub> and C<sub>9</sub> not visible on the <sup>13</sup>C NMR spectrum. HRMS calculated for C<sub>26</sub>H<sub>37</sub>N<sub>6</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 497.28763; found: 497.28723. [ $\alpha$ ]<sub>D</sub>: -6.3° (6.3 mg/ml, MeOH).

**Compound (6i).** Following the general procedure for CuAAC, starting from compound **4** (90 mg, 0.31 mmol) and **5i** (181 mg, 0.63 mmol) and using DCM/MeOH (96/4) as eluent for flash chromatography purification, compound **6i** was obtained as a pale yellow solid (163 mg, 91%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  8.35 (bd,  $J = 12.5$  Hz, 1H, NH), 7.85 (s, 1H, H<sub>8</sub>), 7.68 (d,  $J = 8.1$  Hz, 2H, H<sub>11</sub>), 7.57 (d,  $J = 8.2$  Hz, 2H, H<sub>12</sub>), 7.40-7.31 (m, 5H, H<sub>22,23,24</sub>), 5.31 (bs, 1H, NH),

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3 5.00 (d,  $J = 11.5$  Hz, 1H, H<sub>20</sub>), 4.86 (d,  $J = 11.5$  Hz, 1H, H<sub>20</sub>), 4.56 (dd,  $J = 14.2, 8.0$  Hz, 1H,  
4 H<sub>7</sub>), 4.50 (dd,  $J = 14.3, 6.9$  Hz, 1H, H<sub>7</sub>), 3.86-3.81 (m, 1H, H<sub>1</sub>), 3.49-3.45 (m, 2H, H<sub>16</sub>), 3.35  
5 (bs, 1H, H<sub>4</sub>), 2.94 (s, 2H, H<sub>5</sub>), 2.60 (t,  $J = 5.5$  Hz, 2H, H<sub>15</sub>), 2.07-2.02 (m, 1H, H<sub>3</sub>), 2.00-1.92  
6 (m, 1H, H<sub>2</sub>), 1.71-1.64 (m, 1H, H<sub>3</sub>), 1.60-1.54 (m, 1H, H<sub>2</sub>), 1.42 (s, 9H, H<sub>19</sub>). <sup>13</sup>C NMR (125  
7 MHz, CDCl<sub>3</sub>)  $\delta$  170.1 (C<sub>14</sub>), 169.5 (C<sub>6</sub>), 156.5 (C<sub>17</sub>), 147.8 (C<sub>9</sub>), 138.2 (C<sub>10</sub>), 135.7 (C<sub>21</sub>), 129.3  
8 (2C, C<sub>22</sub>), 128.9 (C<sub>24</sub>), 128.7 (2C, C<sub>23</sub>), 126.4 (3C, C<sub>11</sub> and C<sub>13</sub>), 120.2 (2C, C<sub>12</sub>), 119.9 (C<sub>8</sub>),  
9 79.7 (C<sub>18</sub>), 78.3 (C<sub>20</sub>), 58.4 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 51.9 (C<sub>7</sub>), 43.9 (C<sub>5</sub>), 37.6 (C<sub>15</sub>), 36.6 (C<sub>16</sub>), 28.5 (3C,  
10 C<sub>19</sub>), 20.3 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS calculated for C<sub>30</sub>H<sub>38</sub>N<sub>7</sub>O<sub>5</sub> [M+H]<sup>+</sup>: 576.29344; found:  
11 576.29582. [ $\alpha$ ]<sub>D</sub>: -13.5° (4.0 mg/ml, MeOH).

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24 **Compound (6j)**. Following the general procedure for CuAAC, starting from compound **4** (200  
25 mg, 0.70 mmol) and 4-pentynoic acid **5j** (137 mg, 1.40 mmol), and using DCM/MeOH (9/1)  
26 as eluent for flash chromatography purification, compound **6j** was obtained as a pale yellow  
27 solid (202 mg, 75%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  7.57 (s, 1H, H<sub>8</sub>), 7.41-7.33 (m, 5H, H<sub>15, 16,</sub>  
28 17), 5.01 (d,  $J = 11.5$  Hz, 1H, H<sub>13</sub>), 4.87 (d,  $J = 11.5$  Hz, 1H, H<sub>13</sub>), 4.53-4.44 (m, 2H, H<sub>7</sub>), 3.82-  
29 3.77 (m, 1H, H<sub>1</sub>), 3.35 (bd,  $J = 2.8$  Hz, 1H, H<sub>4</sub>), 3.03 (t,  $J = 6.4$  Hz, 2H, H<sub>10</sub>), 2.95-2.88 (m, 2H,  
30 H<sub>5</sub>), 2.75 (t,  $J = 6.2$  Hz, 2H, H<sub>11</sub>), 2.07-2.01 (m, 1H, H<sub>3</sub>), 1.94 (dq,  $J = 15.1, 7.5$  Hz, 1H, H<sub>2</sub>),  
31 1.69-1.63 (m, 1H, H<sub>3</sub>), 1.58-1.52 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  175.8 (C<sub>12</sub>), 169.5  
32 (C<sub>6</sub>), 146.8 (C<sub>9</sub>), 135.8 (C<sub>14</sub>), 129.4 (2C, C<sub>15</sub>), 128.9 (C<sub>17</sub>), 128.7 (2C, C<sub>16</sub>), 122.0 (C<sub>8</sub>), 78.4  
33 (C<sub>13</sub>), 58.4 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 51.9 (C<sub>7</sub>), 43.9 (C<sub>5</sub>), 33.5 (C<sub>11</sub>), 21.0 (C<sub>10</sub>), 20.3 (C<sub>2</sub>), 19.8 (C<sub>3</sub>).  
34 HRMS calculated for C<sub>19</sub>H<sub>24</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 386.18283; found: 386.18228. [ $\alpha$ ]<sub>D</sub>: -23.8° (7.9  
35 mg/ml, MeOH).

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52 **Compound (7a)**. Following the general procedure for the introduction of sodium sulphite,  
53 compound **7a** was obtained as a white powder (18 mg, 21%) starting from compound **6a** (83  
54 mg, 0.21 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  8.81 (bs, 1H, H<sub>14</sub>), 8.50 (bd,  $J = 4.8$  Hz, 1H, H<sub>13</sub>),  
55 8.44 (s, 1H, H<sub>8</sub>), 8.12 (bd,  $J = 8.0$  Hz, 1H, H<sub>11</sub>), 7.52 (dd,  $J = 7.9, 5.0$  Hz, 1H, H<sub>12</sub>), 4.97 (dd,  $J$   
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3 = 14.8, 10.6 Hz, 1H, H<sub>7</sub>), 4.71 (dd,  $J = 14.8, 5.6$  Hz, 1H, H<sub>7</sub>), 4.31-4.29 (m, 1H, H<sub>4</sub>), 4.01-3.96  
4 (m, 1H, H<sub>1</sub>), 3.56 (d,  $J = 12.3$  Hz, 1H, H<sub>5</sub>), 3.24 (bd,  $J = 12.2$  Hz, 1H, H<sub>5</sub>), 2.18-2.12 (m, 1H,  
5 H<sub>3</sub>), 2.09-1.99 (m, 2H, H<sub>2,3</sub>), 1.81-1.74 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  $\delta$  170.2 (C<sub>6</sub>),  
6 148.3 (C<sub>13</sub>), 145.5 (C<sub>14</sub>), 144.3 (C<sub>9</sub>), 134.1 (C<sub>11</sub>), 126.1 (C<sub>10</sub>), 124.5 (C<sub>12</sub>), 122.8 (C<sub>8</sub>), 60.1 (C<sub>4</sub>),  
7 57.9 (C<sub>1</sub>), 50.8 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 19.8 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>14</sub>H<sub>14</sub>N<sub>6</sub>O<sub>5</sub>S [M-H]<sup>-</sup>  
8 : 379.08246; found: 379.08392.  $[\alpha]_D$ : -44.6° (9.1 mg/ml, MeOH). HPLC purity = 98.7%; rt =  
9 9.4 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

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20 **Compound (7b)**. Following the general procedure for the introduction of sodium sulphite,  
21 compound **7b** was obtained as a white powder (44 mg, 19%) starting from compound **6b** (226  
22 mg, 0.58 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  8.28 (s, 1H, H<sub>8</sub>), 7.77 (d,  $J = 7.3$  Hz, 2H, H<sub>11</sub>),  
23 7.53 (t,  $J = 7.3$  Hz, 2H, H<sub>12</sub>), 7.46 (d,  $J = 7.3$  Hz, 1H, H<sub>13</sub>), 4.86 (dd,  $J = 14.8, 10.2$  Hz, 1H, H<sub>7</sub>),  
24 4.62 (dd,  $J = 14.7, 5.7$  Hz, 1H, H<sub>7</sub>), 4.30-4.28 (m, 1H, H<sub>4</sub>), 3.95-3.91 (m, 1H, H<sub>1</sub>), 3.50 (d,  $J =$   
25 12.3 Hz, 1H, H<sub>5</sub>), 3.22 (bd,  $J = 12.2$  Hz, 1H, H<sub>5</sub>), 2.15-2.09 (m, 1H, H<sub>3</sub>), 2.04-1.95 (m, 2H, H<sub>2,</sub>  
26 3), 1.77-1.68 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  $\delta$  170.1 (C<sub>6</sub>), 147.6 (C<sub>9</sub>), 129.4 (C<sub>10</sub>),  
27 129.2 (2C, C<sub>12</sub>), 128.8 (C<sub>13</sub>), 125.6 (2C, C<sub>11</sub>), 122.3 (C<sub>8</sub>), 60.1 (C<sub>4</sub>), 57.8 (C<sub>1</sub>), 50.8 (C<sub>7</sub>), 43.6  
28 (C<sub>5</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>15</sub>H<sub>16</sub>N<sub>5</sub>O<sub>5</sub>S [M-H]<sup>-</sup>: 378.08721; found:  
29 378.08804.  $[\alpha]_D$ : -36.7° (10.7 mg/ml, H<sub>2</sub>O). HPLC purity = 98.1%; rt = 10.4 min (CH<sub>3</sub>CN/H<sub>2</sub>O  
30 0:100 to 100:0 over 15 min).

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46 **Compound (7c)**. Following the general procedure for the introduction of sodium sulphite,  
47 compound **7c** was obtained as a white powder (12 mg, 14%) starting from compound **6c** (82  
48 mg, 0.21 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O)  $\delta$  8.15 (s, 1H, H<sub>8</sub>), 4.95 (dd,  $J = 14.7, 10.0$  Hz, 1H,  
49 H<sub>7</sub>), 4.72 (dd,  $J = 14.7, 5.9$  Hz, 1H, H<sub>7</sub>), 4.29-4.28 (m, 1H, H<sub>4</sub>), 3.97-3.92 (m, 1H, H<sub>1</sub>), 3.50 (d,  
50  $J = 12.3$  Hz, 1H, H<sub>5</sub>), 3.23-3.20 (m, 1H, H<sub>5</sub>), 2.17-2.09 (m, 1H, H<sub>3</sub>), 2.05-1.96 (m, 2H, H<sub>2,3</sub>),  
51 1.76-1.69 (m, 1H, H<sub>2</sub>), 0.35 (s, 9H, H<sub>10</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  $\delta$  170.2 (C<sub>6</sub>), 147.7 (C<sub>9</sub>),  
52 131.4 (C<sub>8</sub>), 60.0 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 50.1 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 19.6 (C<sub>2</sub>), 18.8 (C<sub>3</sub>), -2.3 (3C, C<sub>10</sub>). HRMS  
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3 calculated for  $C_{12}H_{19}N_5O_5SSi$  [M-H]<sup>-</sup>: 374.09544; found: 374.09427.  $[\alpha]_D$ : -52.0° (2.0 mg/ml,  
4 MeOH). HPLC purity = 96.1%; rt = 10.6 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).  
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8 **Compound (7d)**. Following the general procedure for the introduction of sodium sulphite,  
9 compound **7d** was obtained as a colorless foam (28 mg, 13%) starting from compound **6d** (210  
10 mg, 0.59 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.18 (s, 1H, H<sub>8</sub>), 4.96 (dd, *J* = 14.8, 10.3 Hz, 1H,  
11 H<sub>7</sub>), 4.73 (dd, *J* = 14.8, 5.7 Hz, 1H, H<sub>7</sub>), 4.68 (s, 2H, H<sub>10</sub>), 4.32-4.30 (m, 1H, H<sub>4</sub>), 4.00-3.95 (m,  
12 1H, H<sub>1</sub>), 3.55 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.45 (s, 3H, H<sub>11</sub>), 3.25 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 2.19-  
13 2.13 (m, 1H, H<sub>3</sub>), 2.08-1.99 (m, 2H, H<sub>2,3</sub>), 1.80-1.76 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O)  
14 δ 170.1 (C<sub>6</sub>), 144.0 (C<sub>9</sub>), 125.4 (C<sub>8</sub>), 64.4 (C<sub>10</sub>), 60.1 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 57.5 (C<sub>11</sub>), 50.7 (C<sub>7</sub>), 43.6  
15 (C<sub>5</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for  $C_{11}H_{16}N_5O_6S$  [M-H]<sup>-</sup>: 346.08213; found:  
16 346.08185. HPLC purity = 98.3%; rt = 13.5 min (CH<sub>3</sub>CN + 0.1% TFA / H<sub>2</sub>O + 0.1% TFA  
17 0:100 to 100:0 over 30 min).  
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31 **Compound (7e)**. Starting from compound **6e** (173 mg, 0.47 mmol) and following the general  
32 procedure for the introduction of sodium sulphite, product **7e** was obtained as a white powder  
33 (32 mg, 18%) in the form of a mixture of two isomers. <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.50 (s,  
34 0,5H, H<sub>8</sub>), 8.38 (s, 0,5H, H<sub>8</sub>'), 5.03-4.96 (m, 1H, H<sub>7,7'</sub>), 4.77-4.72 (m, 1H, H<sub>7,7'</sub>), 4.71 (s, 1H,  
35 H<sub>10</sub>), 4.52 (s, 1H, H<sub>10</sub>'), 4.30 (bs, 1H, H<sub>4,4'</sub>), 3.99-3.93 (m, 1H, H<sub>1,1'</sub>), 3.57 (dd, *J* = 12.3, 7.8  
36 Hz, 1H, H<sub>5,5'</sub>), 3.23 (bd, *J* = 12.3 Hz, 1H, H<sub>5,5'</sub>), 3.19 (s, 3H, H<sub>11</sub>), 2.94 (s, 3H, H<sub>11</sub>'), 2.15-2.10  
37 (m, 1H, H<sub>3,3'</sub>), 2.09-1.98 (m, 2H, H<sub>2,2',3,3'</sub>), 1.83-1.77 (m, 1H, H<sub>2,2'</sub>). <sup>13</sup>C NMR (125 MHz,  
38 D<sub>2</sub>O) δ 170.1 (2C, C<sub>6</sub> and C<sub>6'</sub>), 136.7 (C<sub>9</sub>'), 135.6 (C<sub>9</sub>), 129.1 (C<sub>8</sub>), 127.7 (C<sub>8</sub>'), 60.1 (C<sub>10</sub>), 59.8  
39 (2C, C<sub>4</sub> and C<sub>4'</sub>), 58.1 (C<sub>1 or 1'</sub>), 58.0 (C<sub>1 or 1'</sub>), 52.6 (2C, C<sub>11</sub>), 51.1 (C<sub>10</sub>'), 51.0 (C<sub>7 or 7'</sub>), 50.9 (C<sub>7</sub>  
40 or 7'), 43.4 (2C, C<sub>5</sub> and C<sub>5'</sub>), 42.2 (2C, C<sub>11</sub>'), 19.9 (C<sub>2 or 2'</sub>), 19.8 (C<sub>2 or 2'</sub>), 18.8 (2C, C<sub>3</sub> and C<sub>3'</sub>).  
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**Compound (7f).** Following the general procedure for the introduction of sodium sulphite, compound **7f** was obtained as a white powder (6 mg, 8%) starting from compound **6f** (74 mg, 0.18 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.12 (s, 1H, H<sub>8</sub>), 4.95 (dd, *J* = 14.7, 10.3 Hz, 1H, H<sub>7</sub>), 4.71 (dd, *J* = 14.7, 5.7 Hz, 1H, H<sub>7</sub>), 4.31-4.30 (m, 1H, H<sub>4</sub>), 3.99-3.94 (m, 1H, H<sub>1</sub>), 3.82-3.79 (m, 6H, H<sub>10, 12</sub>), 3.54 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.24 (bd, *J* = 12.1 Hz, 1H, H<sub>5</sub>), 2.67 (bs, 4H, H<sub>11</sub>), 2.16-2.12 (m, 1H, H<sub>3</sub>), 2.08-1.98 (m, 2H, H<sub>2, 3</sub>), 1.80-1.75 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.2 (C<sub>6</sub>), 142.0 (C<sub>9</sub>), 125.8 (C<sub>8</sub>), 66.0 (2C, C<sub>12</sub>), 60.1 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 51.9 (2C, C<sub>11</sub>), 51.6 (C<sub>10</sub>), 50.7 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>14</sub>H<sub>21</sub>N<sub>6</sub>O<sub>6</sub>S [M-H]<sup>-</sup>: 401.12433; found: 401.12557. [α]<sub>D</sub>: -37.2° (1.8 mg/ml, MeOH). HPLC purity = 95.1%; rt = 6.0 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (7g).** Following the general procedure for the introduction of sodium sulphite, compound **7g** was obtained as a white solid (88 mg, 30%) starting from compound **6g** (283 mg, 0.64 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.01 (s, 1H, H<sub>8</sub>), 4.92 (dd, *J* = 14.8, 10.3 Hz, 1H, H<sub>7</sub>), 4.69 (dd, *J* = 14.8, 5.7 Hz, 1H, H<sub>7</sub>), 4.39 (s, 2H, H<sub>10</sub>), 4.31-4.29 (m, 1H, H<sub>4</sub>), 3.97-3.92 (m, 1H, H<sub>1</sub>), 3.53 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.23 (bd, *J* = 12.2 Hz, 1H, H<sub>5</sub>), 2.16-2.11 (m, 1H, H<sub>3</sub>), 2.07-1.97 (m, 2H, H<sub>2, 3</sub>), 1.78-1.74 (m, 1H, H<sub>2</sub>), 1.47 (s, 9H, H<sub>13</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.1 (C<sub>6</sub>), 158.0 (C<sub>11</sub>), 146.0 (C<sub>9</sub>), 123.8 (C<sub>8</sub>), 81.4 (C<sub>12</sub>), 60.1 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 50.7 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 35.4 (C<sub>10</sub>), 27.6 (3C, C<sub>13</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>15</sub>H<sub>23</sub>N<sub>6</sub>O<sub>7</sub>S [M-H]<sup>-</sup>: 431.13489; found: 431.13669. [α]<sub>D</sub>: -35.6° (13.6 mg/ml, H<sub>2</sub>O). HPLC purity = 96.9%; rt = 10.2 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (7h).** Following the general procedure for the introduction of sodium sulphite, compound **7h** was obtained as a white powder (184 mg, 53%) starting from compound **6h** (336 mg, 0.68 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 7.94 (s, 1H, H<sub>8</sub>), 4.90 (dd, *J* = 14.7, 10.2 Hz, 1H, H<sub>7</sub>), 4.67 (dd, *J* = 14.7, 5.8 Hz, 1H, H<sub>7</sub>), 4.31-4.29 (m, 1H, H<sub>4</sub>), 4.13 (bd, *J* = 12.7 Hz, 2H, H<sub>12</sub>), 3.97-3.92 (m, 1H, H<sub>1</sub>), 3.51 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.22 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.08-3.01

(m, 3H, H<sub>10,12</sub>), 2.17-2.11 (m, 1H, H<sub>3</sub>), 2.07-1.98 (m, 4H, H<sub>2,3,11</sub>), 1.77-1.71 (m, 1H, H<sub>2</sub>), 1.66-1.59 (m, 2H, H<sub>11</sub>), 1.51 (s, 9H, H<sub>15</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.1 (C<sub>6</sub>), 156.6 (C<sub>13</sub>), 152.0 (C<sub>9</sub>), 122.4 (C<sub>8</sub>), 81.6 (C<sub>14</sub>), 60.1 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 50.6 (C<sub>7</sub>), 43.6 (3C, C<sub>5</sub> and C<sub>12</sub>), 32.5 (C<sub>10</sub>), 31.0 (2C, C<sub>11</sub>), 27.7 (3C, C<sub>15</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>19</sub>H<sub>30</sub>N<sub>6</sub>O<sub>7</sub>S [M+H]<sup>+</sup>: 487.19749; found: 487.19849. [α]<sub>D</sub>: -21.1° (11.8 mg/ml, H<sub>2</sub>O). HPLC purity = 95.1%; rt = 11.8 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (7i).** Following the general procedure for the introduction of sodium sulphite, compound **7i** was obtained as a white powder (37 mg, 23%) starting from compound **6i** (159 mg, 0.28 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.26 (s, 1H, H<sub>8</sub>), 7.71 (d, *J* = 8.4 Hz, 2H, H<sub>11</sub>), 7.52 (d, *J* = 8.2 Hz, 2H, H<sub>12</sub>), 4.85 (dd, *J* = 14.8, 10.2 Hz, 1H, H<sub>7</sub>), 4.61 (dd, *J* = 14.8, 5.8 Hz, 1H, H<sub>7</sub>), 4.29-4.27 (m, 1H, H<sub>4</sub>), 3.94-3.90 (m, 1H, H<sub>1</sub>), 3.49 (d, *J* = 12.2 Hz, 1H, H<sub>5</sub>), 3.46 (t, *J* = 6.1 Hz, 2H, H<sub>16</sub>), 3.21 (d, *J* = 12.1 Hz, 1H, H<sub>5</sub>), 2.59 (t, *J* = 5.9 Hz, 2H, H<sub>15</sub>), 2.15-2.09 (m, 1H, H<sub>3</sub>), 2.03-1.94 (m, 2H, H<sub>2,3</sub>), 1.74-1.67 (m, 1H, H<sub>2</sub>), 1.39 (s, 9H, H<sub>19</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 172.9 (C<sub>14</sub>), 170.1 (C<sub>6</sub>), 158.0 (C<sub>17</sub>), 147.2 (C<sub>9</sub>), 137.3 (C<sub>10</sub>), 126.3 (2C, C<sub>11</sub>), 126.2 (2C, C<sub>13</sub>), 122.1 (C<sub>8</sub>), 121.8 (2C, C<sub>12</sub>), 81.0 (C<sub>18</sub>), 60.1 (C<sub>4</sub>), 57.8 (C<sub>1</sub>), 50.8 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 37.2 (C<sub>15</sub>), 36.8 (C<sub>16</sub>), 27.6 (3C, C<sub>19</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>23</sub>H<sub>29</sub>N<sub>7</sub>O<sub>8</sub>S [M-H]<sup>-</sup>: 564.18766; found: 564.18766. [α]<sub>D</sub>: -12.2° (3.0 mg/ml, MeOH). HPLC purity = 99.1%; rt = 11.4 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (7j).** Following the general procedure for the introduction of sodium sulphite, compound **7j** was obtained as a colorless foam (34 mg, 17%) starting from compound **6j** (194 mg, 0.50 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 7.87 (s, 1H, H<sub>8</sub>), 4.89 (dd, *J* = 14.8, 10.1 Hz, 2H, H<sub>7</sub>), 4.66 (dd, *J* = 14.8, 6.0 Hz, 2H, H<sub>7</sub>), 4.29-4.28 (m, 1H, H<sub>4</sub>), 3.97-3.91 (m, 1H, H<sub>1</sub>), 3.51 (d, *J* = 12.4 Hz, 1H, H<sub>5</sub>), 3.21 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 2.99 (t, *J* = 7.6 Hz, 2H, H<sub>10</sub>), 2.57 (t, *J* = 7.6 Hz, 2H, H<sub>11</sub>), 2.15-2.09 (m, 1H, H<sub>3</sub>), 2.04-1.96 (m, 2H, H<sub>2,3</sub>), 1.76-1.69 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 181.7 (C<sub>12</sub>), 170.1 (C<sub>6</sub>), 148.0 (C<sub>9</sub>), 123.3 (C<sub>8</sub>), 60.0 (C<sub>4</sub>),

57.8 (C<sub>1</sub>), 50.5 (C<sub>7</sub>), 43.7 (C<sub>5</sub>), 36.7 (C<sub>11</sub>), 21.8 (C<sub>10</sub>), 19.6 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>12</sub>H<sub>16</sub>N<sub>5</sub>O<sub>7</sub>S [M-H]<sup>-</sup>: 374.07704; found: 374.07651. [α]<sub>D</sub>: -17.2° (16.4 mg/ml, H<sub>2</sub>O). HPLC purity = 96,0%; rt = 13.5 min (CH<sub>3</sub>CN + 0.1% TFA / H<sub>2</sub>O + 0.1% TFA 0:100 to 100:0 over 30 min).

**Compound (8g).** Following the general procedure for the deprotection of Boc, compound **8g** was obtained as a white powder (7.4 mg, 36%) starting from compound **7g** (21 mg, 0.05 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.26 (s, 1H, H<sub>8</sub>), 4.99 (dd, *J* = 14.8, 10.9 Hz, 1H, H<sub>7</sub>), 4.72 (dd, *J* = 14.8, 5.2 Hz, 1H, H<sub>7</sub>), 4.40 (s, 2H, H<sub>10</sub>), 4.32-4.30 (m, 1H, H<sub>4</sub>), 3.99-3.94 (m, 1H, H<sub>1</sub>), 3.57 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.24 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 2.17-2.12 (m, 1H, H<sub>3</sub>), 2.10-1.99 (m, 2H, H<sub>2,3</sub>), 1.82-1.78 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.1 (C<sub>6</sub>), 140.0 (C<sub>9</sub>), 125.5 (C<sub>8</sub>), 60.1 (C<sub>4</sub>), 58.0 (C<sub>1</sub>), 50.8 (C<sub>7</sub>), 43.5 (C<sub>5</sub>), 34.1 (C<sub>10</sub>), 19.8 (C<sub>2</sub>), 18.8 (C<sub>3</sub>). HRMS calculated for C<sub>10</sub>H<sub>15</sub>N<sub>6</sub>O<sub>5</sub>S [M-H]<sup>-</sup>: 331.08246; found: 331.08277. [α]<sub>D</sub>: -40.2° (2.5 mg/ml, H<sub>2</sub>O). HPLC purity = 97.7%; rt = 4.0 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (8h).** Following the general procedure for the deprotection of Boc, compound **8h** was obtained as a white powder (20 mg, 34%) starting from compound **7h** (59 mg, 0.12 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.00 (s, 1H, H<sub>8</sub>), 4.91 (dd, *J* = 14.8, 10.6 Hz, 1H, H<sub>7</sub>), 4.66 (dd, *J* = 14.8, 5.5 Hz, 1H, H<sub>7</sub>), 4.29-4.27 (m, 1H, H<sub>4</sub>), 3.96-3.91 (m, 1H, H<sub>1</sub>), 3.57-3.51 (m, 3H, H<sub>5,12</sub>), 3.25-3.18 (m, 4H, H<sub>5,10,12</sub>), 2.33-2.29 (m, 3H, 2H, H<sub>11</sub>), 2.16-2.09 (m, 1H, H<sub>3</sub>), 2.06-1.93 (m, 4H, H<sub>2,3,11</sub>), 1.77-1.71 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.1 (C<sub>6</sub>), 150.3 (C<sub>9</sub>), 122.6 (C<sub>8</sub>), 60.1 (C<sub>4</sub>), 57.9 (C<sub>1</sub>), 50.7 (C<sub>7</sub>), 43.6 (3C, C<sub>5</sub> and C<sub>12</sub>), 30.28 (C<sub>10</sub>), 27.8 (2C, C<sub>11</sub>), 19.7 (C<sub>2</sub>), 18.82 (C<sub>3</sub>). HRMS calculated for C<sub>14</sub>H<sub>21</sub>N<sub>6</sub>O<sub>5</sub>S [M-H]<sup>-</sup>: 385.12941; found: 385.13052. [α]<sub>D</sub>: -37.2° (13.7 mg/ml, H<sub>2</sub>O). HPLC purity = 97.6%; rt = 9.6 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (8i).** Following the general procedure for the deprotection of Boc, compound **8i** was obtained as a white solid (2.4 mg, 17%) starting from compound **7i** (14 mg, 0.02 mmol).

<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ 10.21 (s, 1H, NH), 8.49 (s, 1H, H<sub>8</sub>), 7.80 (d, *J* = 8.7 Hz, 2H, H<sub>11</sub>), 7.68 (d, *J* = 8.7 Hz, 2H, H<sub>12</sub>), 4.79 (dd, *J* = 14.3, 9.8 Hz, 1H, H<sub>7</sub>), 4.59 (dd, *J* = 14.4, 6.1 Hz, 1H, H<sub>7</sub>), 4.04-4.02 (m, 1H, H<sub>4</sub>), 3.68-3.63 (m, 1H, H<sub>1</sub>), 3.40 (d, *J* = 11.9 Hz, 1H, H<sub>5</sub>), 3.11 (t, *J* = 6.5 Hz, 2H, H<sub>16</sub>), 2.91 (bd, *J* = 11.9 Hz, 1H, H<sub>5</sub>), 2.71 (t, *J* = 6.5 Hz, 2H, H<sub>15</sub>), 1.87-1.75 (m, 3H, H<sub>2, 3</sub>), 1.56-1.50 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>SO) δ 168.4 (C<sub>14</sub>), 167.3 (C<sub>6</sub>), 146.1 (C<sub>9</sub>), 138.3 (C<sub>10</sub>), 126.0 (C<sub>13</sub>), 125.7 (2C, C<sub>11</sub>), 121.2 (C<sub>8</sub>), 119.4 (2C, C<sub>12</sub>), 58.1 (C<sub>4</sub>), 56.9 (C<sub>1</sub>), 50.5 (C<sub>7</sub>), 43.3 (C<sub>5</sub>), 34.8 (C<sub>16</sub>), 33.0 (C<sub>15</sub>), 19.8 (C<sub>2</sub>), 19.3 (C<sub>3</sub>). HRMS calculated for C<sub>18</sub>H<sub>22</sub>N<sub>7</sub>O<sub>6</sub>S [M-H]<sup>-</sup>: 464.13523; found: 464.13516. HPLC purity = 98.5%; rt = 10.5 min (CH<sub>3</sub>CN/H<sub>2</sub>O 0:100 to 100:0 over 15 min).

**Compound (3).** Ethyl chloroformate (115 μl, 1.20 mmol) was added dropwise at -10°C to a solution of compound **2** (300 mg, 1.09 mmol) and N-methylmorpholine (358 μl, 3.26 mmol) in THF (5 ml). The solution was stirred at -10°C for 1 h then at 5°C overnight. The reaction mixture was cooled again to -10°C and NaBH<sub>4</sub> (124 mg, 3.26 mmol) was added gradually. The solution was then stirred for 1 h 30 min at -10°C. Water (5 ml) and ethyl acetate (5 ml) were slowly added and the heterogeneous mixture was stirred for 30 min at room temperature. The phases were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure. Purification by flash chromatography using DCM/MeOH (96/4) as the eluent gave compound **3** as a colorless oil (88 mg, 31%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.40 – 7.29 (m, 5H, H<sub>10, 11, 12</sub>), 5.00 (d, *J* = 11.4 Hz, 1H, H<sub>8</sub>), 4.85 (d, *J* = 11.4 Hz, 1H, H<sub>8</sub>), 3.69 (dd, *J* = 11.3, 9.4 Hz, 1H, H<sub>7</sub>), 3.57 (dd, *J* = 11.4, 5.5 Hz, 1H, H<sub>7</sub>), 3.52-3.47 (m, 1H, H<sub>1</sub>), 3.33-3.31 (m, 1H, H<sub>4</sub>), 2.99 (d, *J* = 11.7 Hz, 1H, H<sub>5</sub>), 2.87 (bd, *J* = 11.6 Hz, 1H, H<sub>5</sub>), 1.99-1.93 (m, 1H, H<sub>3</sub>), 1.93-1.86 (m, 1H, H<sub>2</sub>), 1.57-1.51 (m, 1H, H<sub>3</sub>), 1.41-1.35 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 170.1 (C<sub>6</sub>), 136.1 (C<sub>9</sub>), 129.4 (2C, C<sub>10</sub>), 128.8 (C<sub>12</sub>), 128.7 (2C, C<sub>11</sub>), 78.4 (C<sub>8</sub>), 62.6 (C<sub>7</sub>),

58.8 (C<sub>1</sub>), 58.6 (C<sub>4</sub>), 43.5 (C<sub>5</sub>), 20.1 (C<sub>3</sub>), 19.4 (C<sub>2</sub>). HRMS calculated for C<sub>14</sub>H<sub>19</sub>N<sub>2</sub>O<sub>3</sub> [M+H]<sup>+</sup>: 263.13957; found: 263.13852. [α]<sub>D</sub>: -54.5° (4.0 mg/ml, MeOH).

**Compound (9a) and (9b).** N,N-Diisopropylethylamine (598 μl, 3.43 mmol), DMAP (14 mg, 0.11 mmol) and MsCl (133 μl, 1.72 mmol) were added at 0°C to a solution of **2** (300 mg, 1.14 mmol) in DCM (25 ml). The reaction mixture was stirred at 0°C for 1 h. DCM was then added and the organic layer was washed with brine, dried over MgSO<sub>4</sub> and concentrated under vacuum to afford compound **3** which was used in the next step without further purification. A solution of crude product **3** (1.14 mmol) in acetonitrile (18 ml) was added dropwise to a solution of 1H-1,2,3-triazole (133 μl, 2.29 mmol) and tBuOK (257 mg, 2.29 mmol) in acetonitrile (24 ml). The reaction mixture was stirred at 90°C for 15 h. DCM was then added and the organic layer was washed with H<sub>2</sub>O and brine, dried over MgSO<sub>4</sub> and concentrated under vacuum. Purification by flash chromatography using cyclohexane/ethyl acetate (9/1) as the eluent gave compounds **9a** (164 mg, 44% over 2 steps) and **9b** (136 mg, 37% over 2 steps) as orange solids. **9a**: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.55 (s, 2H, H<sub>8</sub>), 7.38-7.27 (m, 5H, H<sub>11, 12, 13</sub>), 4.98 (d, *J* = 11.5 Hz, 1H, H<sub>9</sub>), 4.84 (d, *J* = 11.5 Hz, 1H, H<sub>9</sub>), 4.66 (dd, *J* = 13.8, 7.7 Hz, 1H, H<sub>7</sub>), 4.52 (dd, *J* = 13.8, 7.7 Hz, 1H, H<sub>7</sub>), 4.03-3.98 (m, 1H, H<sub>1</sub>), 3.32 (q, *J* = 3.0 Hz, 1H, H<sub>4</sub>), 2.99 (d, *J* = 11.9 Hz, 1H, H<sub>5</sub>), 2.90 (dt, *J* = 11.9, 3.0 Hz, 1H, H<sub>5</sub>), 2.01-1.95 (m, 1H, H<sub>3</sub>), 1.93 – 1.86 (m, 1H, H<sub>2</sub>), 1.63-1.57 (m, 1H, H<sub>3</sub>), 1.46-1.40 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 169.2 (C<sub>6</sub>), 135.8 (C<sub>10</sub>), 134.5 (2C, C<sub>8</sub>), 129.1 (2C, C<sub>12</sub>), 128.6 (C<sub>13</sub>), 128.4 (2C, C<sub>11</sub>), 78.1 (C<sub>9</sub>), 58.2 (C<sub>4</sub>), 56.6 (C<sub>1</sub>), 55.9 (C<sub>7</sub>), 44.3 (C<sub>5</sub>), 19.9 (C<sub>2</sub>), 19.8 (C<sub>3</sub>). HRMS calculated for C<sub>16</sub>H<sub>20</sub>N<sub>5</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 314.16170; found: 314.16104. [α]<sub>D</sub>: -32.3° (6.0 mg/ml, MeOH). **9b**: <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.72 (d, *J* = 0.8 Hz, 1H, H<sub>8</sub>), 7.66 (d, *J* = 0.8 Hz, 1H, H<sub>9</sub>), 7.39-7.29 (m, 5H, H<sub>12, 13, 14</sub>), 4.98 (d, *J* = 11.5 Hz, 1H, H<sub>10</sub>), 4.84 (d, *J* = 11.5 Hz, 1H, H<sub>10</sub>), 4.56 (dd, *J* = 14.2, 8.0 Hz, 1H, H<sub>7</sub>), 4.51 (dd, *J* = 14.2, 6.9 Hz, 1H, H<sub>7</sub>), 3.81-3.76 (m, 1H, H<sub>1</sub>), 3.36-3.34 (m, 1H, H<sub>4</sub>), 2.90 (bs, 2H, H<sub>5</sub>), 2.05-1.99 (m, 1H, H<sub>3</sub>), 1.96-1.89 (m, 1H, H<sub>2</sub>), 1.69-1.62 (m, 1H, H<sub>3</sub>), 1.57-1.51 (m, 1H, H<sub>2</sub>). <sup>13</sup>C

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2  
3 NMR (125 MHz, CDCl<sub>3</sub>) δ 169.3 (C<sub>6</sub>), 135.7 (C<sub>11</sub>), 134.1 (C<sub>9</sub>), 129.2 (2C, C<sub>13</sub>), 128.8 (C<sub>14</sub>),  
4  
5 128.6 (2C, C<sub>12</sub>), 124.0 (C<sub>8</sub>), 78.2 (C<sub>10</sub>), 58.3 (C<sub>4</sub>), 56.7 (C<sub>1</sub>), 51.7 (C<sub>7</sub>), 43.7 (C<sub>5</sub>), 20.3 (C<sub>2</sub>), 19.7  
6  
7 (C<sub>3</sub>). HRMS calculated for C<sub>16</sub>H<sub>20</sub>N<sub>5</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 314.16170; found: 314.16113. [α]<sub>D</sub>: -32.9°  
8  
9 (3.4 mg/ml, MeOH).

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11  
12 **Compound (10a)**. Following the general procedure for the introduction of sodium sulphite,  
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14 compound **10a** was obtained as a yellow solid (44 mg, 27%) starting from compound **9a** (158  
15  
16 mg, 0.51 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 7.89 (s, 2H, H<sub>8</sub>), 5.04 (dd, *J* = 14.7,  
17  
18 10.4 Hz, 1H, H<sub>7</sub>), 4.77 (dd, *J* = 14.7, 5.8 Hz, 1H, H<sub>7</sub>), 4.33-4.31 (m, 1H, H<sub>4</sub>), 4.10-4.05 (m, 1H,  
19  
20 H<sub>1</sub>), 3.58 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.22 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 2.19-2.12 (m, 1H, H<sub>3</sub>), 2.08-  
21  
22 1.99 (m, 2H, H<sub>2,3</sub>), 1.81-1.73 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 169.9 (C<sub>6</sub>), 135.1 (2C,  
23  
24 C<sub>8</sub>), 60.0 (C<sub>4</sub>), 57.8 (C<sub>1</sub>), 54.6 (C<sub>7</sub>), 43.9 (C<sub>5</sub>), 19.5 (C<sub>2</sub>), 18.9 (C<sub>3</sub>). HRMS calculated for  
25  
26 C<sub>9</sub>H<sub>12</sub>N<sub>5</sub>O<sub>5</sub>S [M-H]<sup>-</sup>: 302.05591; found: 302.05685. [α]<sub>D</sub>: -37.4° (18.0 mg/ml, H<sub>2</sub>O). HPLC  
27  
28 purity = 97.0%; rt = 2.8 min (CH<sub>3</sub>CN / H<sub>2</sub>O 0:100 to 100:0 over 30 min).  
29  
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34 **Compound (10b)**. Following the general procedure for the introduction of sodium sulphite,  
35  
36 compound **10b** was obtained as a white foam (14 mg, 8%) starting from compound **9b** (132  
37  
38 mg, 0.42 mmol). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O) δ 8.13 (s, 1H, H<sub>8</sub>), 7.88 (s, 1H, H<sub>9</sub>), 5.00  
39  
40 (dd, *J* = 14.8, 10.5 Hz, 1H, H<sub>7</sub>), 4.74 (dd, *J* = 14.8, 5.6 Hz, 1H, H<sub>7</sub>), 4.32-4.30 (m, 1H, H<sub>4</sub>), 4.01  
41  
42 - 3.96 (m, 1H, H<sub>1</sub>), 3.57 (d, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 3.24 (bd, *J* = 12.3 Hz, 1H, H<sub>5</sub>), 2.19-2.12 (m,  
43  
44 1H, H<sub>3</sub>), 2.09-1.99 (m, 2H, H<sub>2,3</sub>), 1.82-1.74 (m, 1H, H<sub>2</sub>). <sup>13</sup>C NMR (125 MHz, D<sub>2</sub>O) δ 170.1  
45  
46 (C<sub>6</sub>), 134.2 (C<sub>9</sub>), 125.9 (C<sub>8</sub>), 60.0 (C<sub>4</sub>), 58.0 (C<sub>1</sub>), 50.4 (C<sub>7</sub>), 43.6 (C<sub>5</sub>), 19.7 (C<sub>2</sub>), 18.8 (C<sub>3</sub>).  
47  
48 HRMS calculated for C<sub>9</sub>H<sub>12</sub>N<sub>5</sub>O<sub>5</sub>S [M-H]<sup>-</sup>: 302.05591; found: 302.05670. [α]<sub>D</sub>: -44.6° (9.6  
49  
50 mg/ml, H<sub>2</sub>O). HPLC purity = 97.9%; rt = 2.8 min (CH<sub>3</sub>CN / H<sub>2</sub>O 0:100 to 100:0 over 30 min).  
51  
52  
53  
54

55 **Plasmid and strain construction**. For antibiotic susceptibility testing, the β-lactamase genes  
56  
57 were cloned into the pTRC-99k vector, which is a derivative of pTRC99a (Pharmacia) obtained  
58  
59 by replacing the β-lactamase resistance gene by a kanamycin resistance gene (Km, *lacI*, pTRC  
60

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2  
3 promoter, *oriVcolEI*; D. Mengin-Lecreulx, unpublished). Recombinant plasmids were  
4 introduced by electrotransformation into *E. coli* Top10. For  $\beta$ -lactamase production, fragments  
5 of the  $\beta$ -lactamase genes encoding soluble enzymes, *i.e.* devoid of the signal peptides, were  
6  
7  
8  
9  
10 cloned into the pET-TEV vector generating translational fusions with a vector-encoded N-  
11  
12 terminal 6 x His tag followed by a TEV cleavage site (MHHHHHHENLYFQGHEM).

13  
14  
15 **Production and purification of  $\beta$ -lactamases.** *E. coli* BL21 (DE3) harboring recombinant  
16  
17 plasmids were grown in brain heart infusion (BHI) broth supplemented with kanamycin (50  
18  
19  $\mu\text{g/ml}$ ) at 37°C under vigorous shaking until the optical density at 600 nm ( $\text{OD}_{600}$ ) reached 0.8.  
20  
21 Isopropyl  $\beta$ -D-1-thiogalactopyranoside IPTG (0.5 mM) was added and incubation was  
22  
23 continued at 16°C for 18 h. Bacteria were harvested by centrifugation, re-suspended in 25 mM  
24  
25 Tris-HCl (pH 7.5) containing 300 mM NaCl (buffer A), and lysed by sonication. The enzymes  
26  
27 were purified from clarified lysates by affinity chromatography (NiNTA agarose, Sigma-  
28  
29 Aldrich) and size exclusion chromatography in buffer A (Superdex 200 HL26/60, Amersham  
30  
31 Pharmacia Biotech). Protein concentration was determined by the Biorad protein assay using  
32  
33 bovine serum albumin as a standard.  
34  
35  
36  
37

38  
39 **Determination of kinetic parameters.** Kinetic parameters  $k_{\text{cat}}$ ,  $K_{\text{m}}$ , and  $k_{\text{cat}}/K_{\text{m}}$  for hydrolysis  
40  
41 of nitrocefin and CENTA were determined at 20 °C in 2-(*N*-morpholino)ethanesulfonic acid  
42  
43 (MES; 100 mM; pH 6.4) by spectrophotometry, as previously described.<sup>39,40</sup> Briefly, the initial  
44  
45 velocity ( $v_i$ ) was determined by spectrophotometry for various concentrations of  $\beta$ -lactams [S]  
46  
47 and a fixed concentration of  $\beta$ -lactamase [E]. The values of  $v_i$  were plotted as a function of [S].  
48  
49 The kinetic constants  $K_{\text{m}}$  and  $k_{\text{cat}}$  were determined by fitting the equation  $v_i = k_{\text{cat}} [\text{E}] [\text{S}] / K_{\text{m}} +$   
50  
51 [S] to the resulting curve. The molecular extinction coefficient was 15,200  $\text{M}^{-1}\text{cm}^{-1}$  at 486 nm  
52  
53 for nitrocefin and 7,380  $\text{M}^{-1}\text{cm}^{-1}$  at 415 nm for CENTA, respectively. Kinetic parameters for  
54  
55 the carbamoylation of  $\beta$ -lactamases by DBOs ( $k_2/K_i$  and  $k_2$ ) were determined at 20°C in MES  
56  
57 (100 mM; pH 6.4), as previously described.<sup>7</sup> The reporter substrate was nitrocefin (100  $\mu\text{M}$ )  
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59  
60

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2  
3 for TEM-1, KPC-2, CTX-M-15, and AmpC<sub>clo</sub> or CENTA (100  $\mu$ M) for OXA-48. Kinetics  
4  
5 constants were deduced from a minimum of 6 progress curves obtained in a minimum of two  
6  
7 independent experiments.  
8

9  
10 **MIC determination.** MICs of  $\beta$ -lactams were determined by the microdilution method in  
11  
12 Mueller-Hinton (cation-adjusted) broth according to Clinical and Laboratory Standards  
13  
14 Institute (CLSI) recommendations.<sup>41</sup> Diazabicyclooctanes were used at a fixed concentration  
15  
16 of 15  $\mu$ M (4  $\mu$ g/ml for avibactam). Clavulanate was tested at 4  $\mu$ g/ml. IPTG (500  $\mu$ M) was  
17  
18 added to the microdilution plates to induce production of the  $\beta$ -lactamase. The precultures were  
19  
20 grown in BHI broth containing IPTG (500  $\mu$ M) and kanamycin (50  $\mu$ g/ml) for plasmid  
21  
22 maintenance. Reported MICs are the medians from five biological repeats obtained in two  
23  
24 independent experiments.  
25  
26  
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## 28 29 30 ASSOCIATED CONTENT

### 31 32 Supporting Information

33  
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35 The Supporting Information is available free of charge on the ACS Publications website.

36  
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38 Synthesis and characterization of alkyne **5f**, **5g** and **5i** (PDF)

39  
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41 Synthesis and characterization of relebactam (PDF)

42  
43  
44 NMR analysis (PDF)

45  
46  
47 Table of Kinetic constant  $k_2$  ( $s^{-1}$ ) for decarbamylation of  $\beta$ -lactamases with synthesized  
48  
49 compounds (PDF)

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52 Molecular formula strings (CSV)

## 53 54 AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

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### ABBREVIATIONS

CuAAC, copper-catalyzed alkyne-azide cycloaddition; DBO, diazabicyclooctane; DCM, dichloromethane; DMAP, *N*-dimethylaminopyridine; DMF, *N*-dimethylformamide; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectroscopy; KPC, *Klebsiella pneumoniae* carbapenemases; MIC, minimal inhibitory concentration; NMR, nuclear magnetic resonance; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin layer chromatography.

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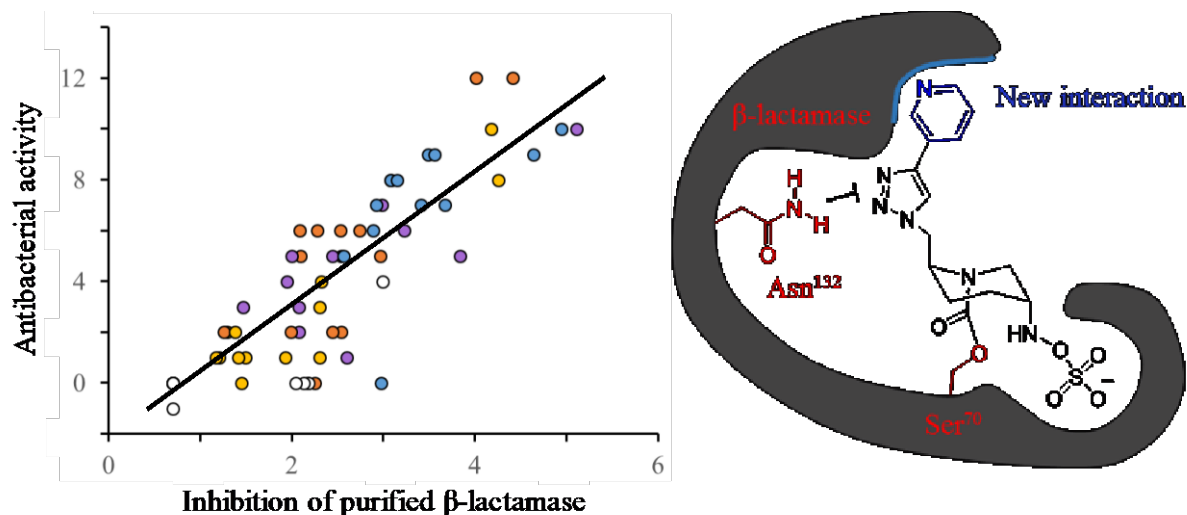
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