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Enantioselective Synthesis and Profiling of Two Novel Diazabicyclooctanone β-Lactamase Inhibitors

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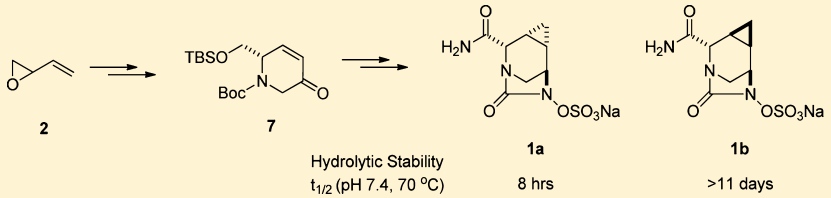
Supporting Information



ABSTRACT: The enantioselective synthesis of two novel cyclopropane-fused diazabicyclooctanones is reported here. Starting from butadiene monoxide, the key enone intermediate **7** was prepared in six steps. Subsequent stereoselective introduction of the cyclopropane group and further transformation led to compounds **1a** and **1b** as their corresponding sodium salt. The great disparity regarding their hydrolytic stability was rationalized by the steric interaction between the cyclopropyl methylene and urea carbonyl. These two novel β-lactamase inhibitors were active against class A, C, and D enzymes.

KEYWORDS: *β-lactamase inhibitor, asymmetric synthesis, hydrolytic stability*

Since the discovery of penicillin in the 1920s, β-lactam antibiotics have become one of the most important groups of antibiotics. The worldwide usage of these antibiotics has rapidly led to the development of bacterial resistance, mainly by the production and evolution of β-lactamases, which are enzymes responsible for efficiently catalyzing the hydrolysis of the β-lactam warheads. According to the Ambler classification,[1−3] β-lactamases are divided into four subfamilies: class A, C, and D enzymes have a key serine residue in the active site, whereas class B enzymes (also called metallo-β-lactamases) employ either one or two zinc ions.

The combination use of several mechanism-based β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) with β-lactam antibiotics is currently one of the most successful strategies in combating such resistance,[4−6] as evident by the wide clinical application of products such as Augmentin, Timentin, Unasyn, Sulperazone, and Zosyn. However, these β-lactamase inhibitors only have activity against class A β-lactamases and weak or no activity against class C and D enzymes.[4] In addition, β-lactamases are rapidly evolving, as evident by the rising number of new β-lactamases reported each year (over 1400 to date).[7] Therefore, this urgent medical need calls for firm commitment to the discovery and development of novel and more efficient inhibitors with broader coverage of β-lactamases.

Recently a novel series of β-lactamase inhibitors based on the diazabicyclooctanone (DBO) scaffold was reported.[8−10] Avibactam (NXL104)[11−13] and MK-7655,[14−16] with their common bicyclic urea structure (Figure 1), were reported to have limited intrinsic antibiotic activity, but are capable of inhibiting classes A and C and a limited number of class D β-lactamases.

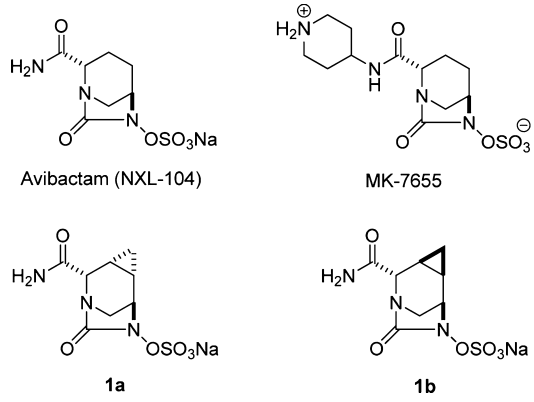


Figure 1. Chemical structures of selected examples of diazabicyclooctanones (DBO).

This letter describes our efforts toward the enantioselective synthesis of two new cyclopropane-fused diazabicyclooctanones **1a** and **1b** aiming to explore whether the introduction of additional ring strain can lead to higher reactivity of the urea carbonyl and broader β-lactamase coverage, while hoping to maintain sufficient hydrolytic stability.

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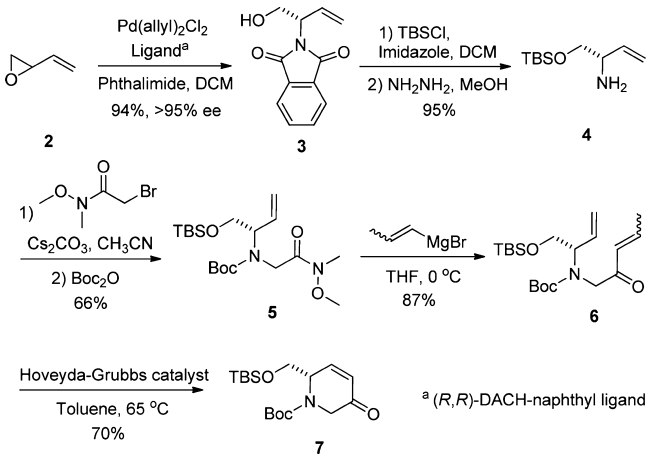
As shown in Scheme 1, the synthesis started with the Pd-catalyzed dynamic kinetic asymmetric transformation

Scheme 1. Synthesis of Key Enone Intermediate 7

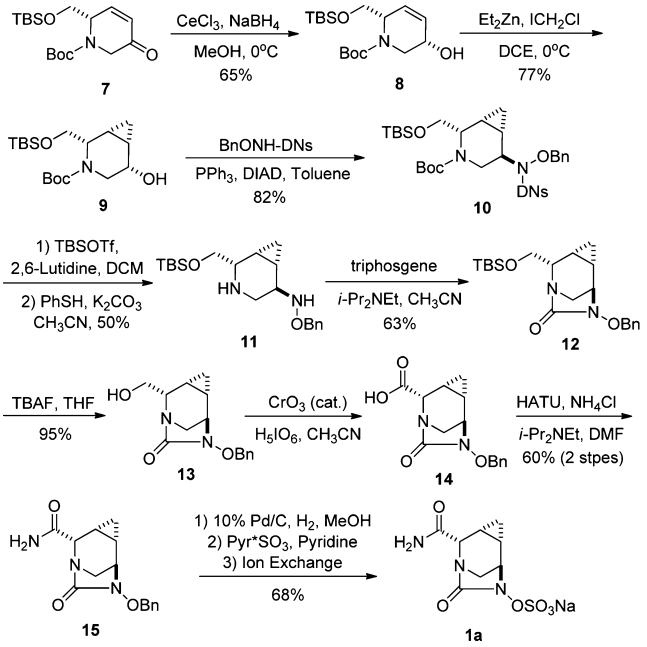


(DYKAT) of racemic butadiene monoxide **2** employing phthalimide, $Pd(allyl)_2Cl_2$, and the (*R*,*R*)-DACH-naphthyl ligand.[17] This reaction was highly reproducible and scalable, and alcohol **3** was consistently obtained in >90% yield and >95% ee on >100 g scale. Subsequent TBS protection and treatment with hydrazine in methanol gave amine **4** in high yield. Over reduction of the alkene by hydrazine was sometimes observed but could be controlled by limiting the reaction time. Monoalkylation of the primary amine, followed by Boc protection, afforded the Weinreb amide **5** in 66% yield. It is noteworthy that numerous attempts (various bases, solvents, additives, and temperatures) to alkylate the Boc carbamate of amine **4** led to low conversion. The addition of propenylmagnesium bromide to Weinreb amide **5** gave vinyl ketone **6**, which was then treated with 2 mol % of the second generation Hoveyda–Grubbs catalyst[18] in hot toluene to form the enone ring in good yield. The use of propenylmagnesium bromide led to higher yields in both the vinyl ketone formation and metathesis steps relative to vinylmagnesium bromide.

With enone intermediate **7** in hand, we next explored the introduction of the *cis*-cyclopropane ring (Scheme 2). Stereoselective Luche reduction[19] of the enone gave allylic alcohol **8** as a single diastereomer in 65% yield. The stereochemical outcome of this transformation was confirmed by an observed nuclear Overhauser effect (NOE) from the TBS ether methylene group to the axial proton H_{6a} (Figure 2). In addition, proton H_{6a} was determined to be axial due to its large trans diaxial coupling constant with proton H_{5a} (10.5 Hz). Hydroxyl-directed cyclopropanation of allylic alcohol **8** under Denmark's modified conditions[20] afforded compound **9** as a single diastereomer in 77% yield. The cyclopropanation of the double bond occurred on the same face of the alcohol group as confirmed by the presence of NOEs observed in compound **9** (Figure 2). Alcohol **9** was then converted to compound **10** under Mitsunobu conditions using dinitrobenzenesulfonyl protected *O*-benzylhydroxylamine.

Selective deprotection of the Boc group in the presence of the TBS ether was achieved employing either TBSOTf[21] or $ZnBr_2$[22,23] in comparable yields. Subsequent removal of the nitrobenzenesulfonyl group gave free diamine **11** in 50% yield over 2 steps.

Scheme 2. Synthesis of Compound 1a



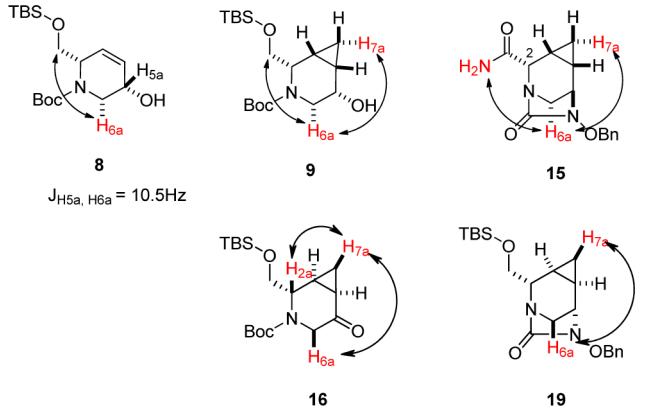


Figure 2. Assignment of relative stereochemistry by nuclear magnetic resonance (NMR). Each double arrow indicates a signal observed in the NOE experiments.

The bicyclic urea ring was formed via slow addition of a triphosgene solution to a dilute solution of diamine and Hünig's base in CH_3CN at 0 °C. The TBS ether was removed with TBAF. The resulting alcohol **13** (relative stereochemistry confirmed by X-ray analysis, see Supporting Information) was oxidized to the corresponding carboxylic acid **14** under mild conditions using catalytic CrO_3,[24] which was then converted to primary amide **15**. NOE experiments confirmed the stereochemistry at C2 remained intact through the oxidation and amide coupling steps (Figure 2). This could be explained by the rigid nature of the DBO scaffold where the highly strained system prevents the formation of the required tautomer to allow the epimerization to occur. Next the benzyl group was removed under catalytic hydrogenolysis conditions. After filtration and concentration, the *N*-hydroxide intermediate was treated with excess SO_3–pyridine complex in pyridine to drive the sulfate formation. Finally target compound **1a** was isolated as its sodium salt after treatment with NaOH preconditioned Dowex ion-exchange resin.

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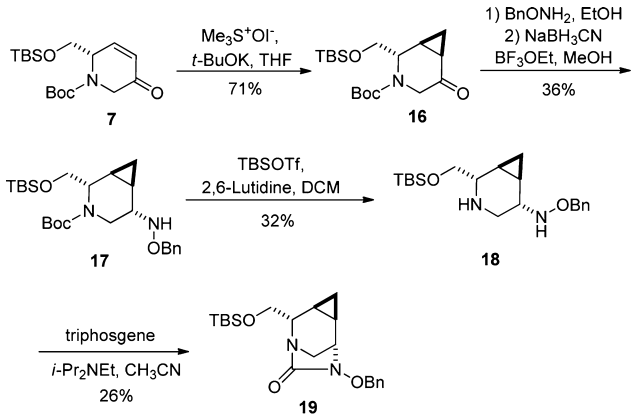
Next we turned our attention to the preparation of the other cyclopropane diastereomer **1b**. We reasoned that this could be accomplished by carrying out the cyclopropanation before formation of the alcohol, thus relying on steric hindrance to direct the stereochemical outcome of the reaction. As shown in Scheme 3, slow addition of enone **7** to a dilute solution (~0.05

Scheme 3. Synthesis of Urea 19

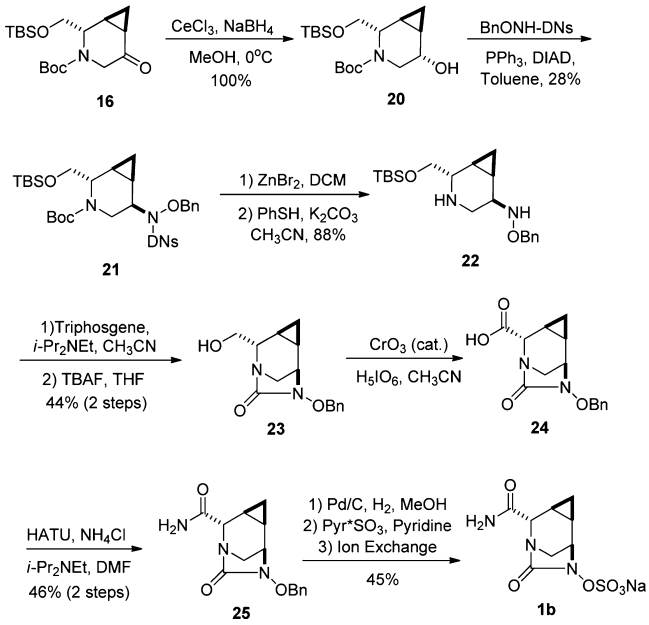


M) of preformed sulfoxonium ylide[25,26] in THF resulted in the formation of the cyclopropane **16** in good yield. The trans stereochemistry was established by NOE experiments (Figure 2). We hypothesized that the reduction of an oxime derived from ketone **16** would stereoselectively provide the desired *trans*-isomer due to the steric hindrance of the cyclopropane ring. To this end, ketone **16** was readily converted to the *O*-benzyl oxime, which was subsequently reduced to hydroxylamine **17** using a large excess of $NaBH_3CN$ and boron trifluoride-diethyl etherate, albeit in moderate yield. Selective removal of the Boc group, followed by urea formation, led to the formation of the bicyclic urea **19**. The relative stereochemistry of the rigid bicyclic urea **19** was readily determined by NOE experiments to be the undesired *cis* isomer (Figure 2). It is therefore clear that the presence or absence of the cyclopropane did not play a role in the stereoselectivity of the ketone reduction. As in the stereoselective reduction of enone **7** (Scheme 2), the large TBS ether group in its pseudoaxial position directed the axial attack of the hydride on the carbonyl/oxime group.

Alternatively, cyclopropanone **16** was reduced with a sterically undemanding $NaBH_4$ at 0 °C to provide the cis alcohol **20** in excellent yield and greater than 10 to 1 diastereoselectivity (Scheme 4). Following the previously established synthetic sequence used for the preparation of compound **1a**, alcohol **20** was converted to primary amide **25**. Catalytic hydrogenolysis of **25** to remove the benzyl protective group, sulfate formation, ion exchange, and RP-HPLC purification furnished the final compound **1b** as its sodium salt in 45% yield over the final 3 steps. As shown in Figure 3, confirmation of the structure of **1b** was obtained via a small molecule crystal structure.

With diastereomeric cyclopropane analogues **1a** and **1b** in hand, we examined their hydrolytic stability in pH 7.4 (aqueous phosphate buffer). Compound **1a** was stable with a half-life of 112 h at 37 °C. As expected, at elevated temperature (70 °C), the half-life of **1a** was significantly shorter (8.2 h). Compound **1b** was remarkably more stable, as evident by its long half-life (>264 h) even at 70 °C in pH 7.4 aqueous phosphate buffer. As

Scheme 4. Synthesis of compound 1b



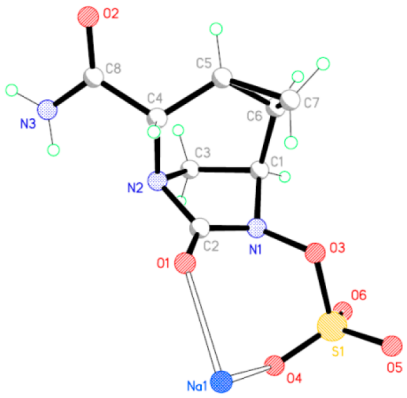


Figure 3. X-ray crystal structure of **1b**.

a reference, the half-life of the known β-lactamase inhibitor tazobactam in the same conditions was measured at 7.7 h, a value very similar to that of compound **1a**.

The exceptional stability of **1b** may be rationalized by its concave conformation (Figure 3). The rigid tricyclic structure and particular stereochemistry places the cyclopropane methylene group of **1b** in close proximity (C2–C7 distance = 2.48 Å) to the carbonyl group of the cyclic urea. This may provide a strong steric protection from the addition of water and also destabilize the tetrahedral intermediate that would be formed during hydrolysis.

Furthermore, the two novel cyclopropane-containing DBOs **1a** and **1b**, together with tazobactam were profiled for their inhibitory activities against a series of β-lactamases. As presented in Table 1, both analogues showed activity against class A, C, and D β-lactamases. This suggests that the measurement of hydrolytic stability is not enough to predict reactivity with β-lactamases. Additional factors like molecular recognition and acylation rates come into play in the stepwise mechanism of covalent inhibition.[27,28] While the levels of inhibition were variable across the 3 families of β-lactamases, the structure–activity relationship (SAR) between the two new analogues and tazobactam represents useful information to

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Table 1. Enzyme Inhibition against Different Classes of β-Lactamases

	class A IC_{50} (μM)		class C IC_{50} (μM)	class D IC_{50} (μM)
compound	CTX-M-15 *K. pneumoniae*	KPC-2 *E. cloacae*	AmpC *P. aeruginosa*	OXA-48 *K. pneumoniae*
Tazobactam	<0.007	39	3.4	3.6
1a	0.14	0.85	4.5	1.3
1b	0.48	6.7	2.0	28

design the next generation of DBO-based β-lactamase inhibitors. The increased hydrolytic stability for those new inhibitors will also represent an advantage in terms of formulation development and manufacturing.

In summary, the enantioselective synthesis of two novel tricyclic DBO analogues was accomplished in 17 linear steps (1.8% and 0.6% overall yield, respectively). Compound **1b** has demonstrated excellent aqueous stability at physiological pH, demonstrating the fundamental differences between ring strain and stability. Additionally, the biochemical data presented herein further illustrates the lack of direct correlation between reactivity and enzymatic activity for covalent inhibitors. Additional SAR exploration will be required to better understand the stability and improve the enzymatic activity of this exciting new class of β-lactamase inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

Representative assay protocols, experimental procedures, and spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): The authors are all current or former employees of AstraZeneca and may possess AstraZeneca stock.

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