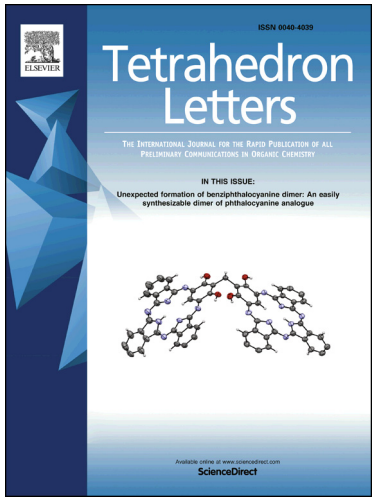

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Synthesis of Bicyclic β-Lactamase Inhibitor Relabactam Derivatives From a Relabactam Intermediate

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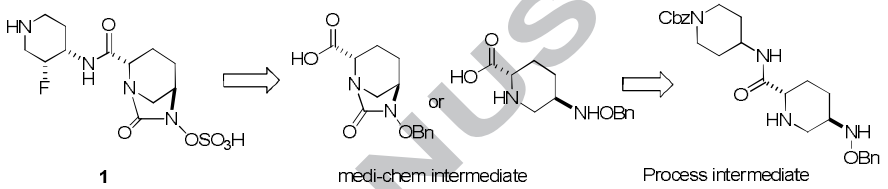
Supporting Information Placeholder

Graphical Abstract:



ABSTRACT: We have developed highly efficient chemistry to prepare two key intermediates from a Relabactam intermediate available from the process chemistry. The new intermediates enabled us to quickly synthesize other Ralebactam derivatives such as compound **1**.

The rise of multidrug resistant Gram-negative bacteria has posed a tremendous threat to human health.[1] One of the main mechanisms of the β-lactam antibiotic drug resistance is the over-expression of β-lactamases by the resistant strains, resulting in the degradation of β-lactam antibiotics that inhibit the bacterial cell wall synthesis.[2-4] β-Lactamase inhibitors (BLIs) such as clavulanic acid inhibit β-lactamases and restore the susceptibility of β-lactams against the resistant strains.[5-6] The combination of β-lactam antibiotics and BLIs has demonstrated restoration of the original efficacy spectrum of β-lactams.[7-8] One of the successful examples in the clinic is Augmentin, a combination of amoxicillin and clavulanic acid (Figure 1), which is widely prescribed for various bacterial infections. However there are hundreds of known β-lactamases, categorized into four classes (A, B, C, and D).[3] The clinically used BLIs only inhibit class A β-lactamases which deactivate penicillins and early cephalosporins. There is an unmet medical need for the treatment of serious bacterial infections caused by the resistant Gram-negative bacterial strains that produce other classes of β-lactamases, for example, AmpC by *pseudomonas aeruginosa*.[9] Relabactam (MK-7655 is a potent BLI currently in Phase III clinical trials in combination with imipenem for the treatment of severe bacterial infection. Relabactam displays inhibition against both classes A and C β-lactamases (e.g. KPC-2 and AmpC).[10] It effectively restored imipenem's activity against imipenem-resistant *Klebsiella and pseudomonasa* strains.[10] A similar compound avibactam combined with ceftazidime was approved by FDA in 2015.

In our BLI program, there was a need to prepare gram quantities of compound **1**, a close analog to MK-7655, for various purposes (Figure 2). A default solution was to use the original lengthy synthetic route described by Blizzard *et al*.[10] However, a substantial quantity of compound **2** was available as an intermediate from the process synthesis of MK-7655.[11,12] In this communication, we describe the highly efficient chemistry we developed to convert the process intermediate **2** to intermediate **5** and the subsequent synthesis of **1**.

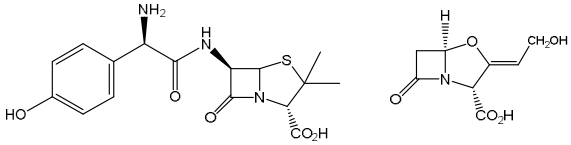


Figure 1. Example of BL/BLI used in clinic

Figure 3 illustrates both the chemical and enzymatic cleavage of the amide bond in compound **2**, which was available in kg quantity. The chemical method required the protection of the amide NH with the Boc group followed by the hydrolysis reaction. Thus intermediate **2** was protected with Boc using di-t-butyl dicarbonate and DMAP in THF. The yield for intermediate **3** was only 26% after optimization, and the major product was di-Boc protected **3a** in > 50% yield (Figure 4). While the tris-Boc protected **3** could be cleanly converted to intermediate **4** using the $LiOH/H_2O_2$ method, the conversion of the bis-Boc protected **3a** to its corresponding acid was much poorer. Our attention was turned to the enzymatic cleavage method and a biocatalytic screen for various enzymes was performed directly on compound **2**. The screening experiments quickly identified Amano protease P6 as the enzyme of choice for this amide bond cleavage step.

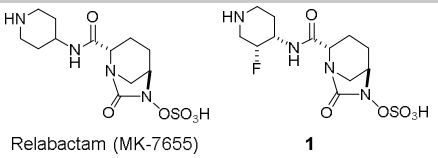


Figure 2. The structures of relabactam (MK-7655) and **1**

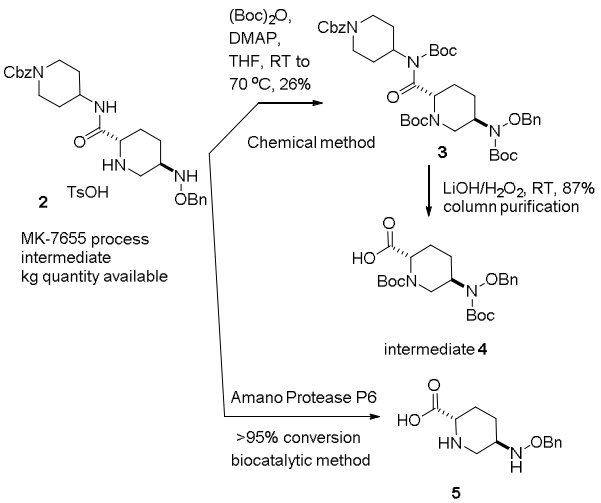


Figure 3. Chemical and enzymatic cleavage of the amide bond

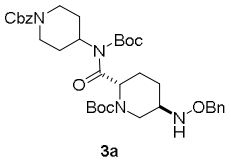


Figure 4. Major product of Boc-protection step

To further optimize the enzymatic cleavage condition, an additional screen with varying DMSO concentrations in the buffer (10% to 30%) and the temperature (30 to 40 °C) was performed. Only the condition with 10% DMSO in the buffer led to >97% conversion at 30 °C and 40 °C. Another screen varying the concentrations of substrate **2** (10 g/L, 20 g/L, 30 g/L, 40 g/L, and 50 g/L) and the temperature (30 to 40 °C) with fixed amount of Amano protease P6 (conc. ~10 mg/mL) was also performed to optimize the highest possible loading of **2**. The results are displayed in Table 1. No reactions reached completion at 20 or 29 h, except for the 10 g/L group, indicating longer reaction time was required for full conversion in the high concentration groups (20 g/L-50 g/L). Although temperature did not play a significant role, , for the high loading group (50 g/L), the conversion rate was higher at 35 °C (98.5%) and 40 °C (99%) thanthat at 30 °C (94%). All the tested conditions reached >98.5% conversion after 3 days at 35 °C and 40 °C. Therefore a large scale reaction (183 g of compound **2**) was performed at 50 g/L substrate concentration under 35-40 °C for three days. The desired product **5** was directly obtained from crystallization from the reaction solution (42 g, 43% yield). The rest of the product in the mother solution was not suitable for extraction due to the high polarity of **5**. Instead, the solution underwent direct Boc protection on the piperidine nitrogen of **5** (details described below) and delivered 38 g of **12** (Figure 7). Through such 2-step synthesis, >95% conversion was achieved.

Table 1. Optimization of biocatalytic conversion of **2** to **5**

Conc. of **2** (g/L)	Conversion Rates Incubation time[a]		
	20 h	29 h	3 days
10	>98%	ND	ND
20	90-94%	88-97%	>99%
30	88-95%	84-97%	>99%
40	90-91%	90-91%	>99%
50	46-93%	81-93%	94%-99%[b]

[a]The incubation was carried out at three temperatures (30 °C, 35 °C, and 40 °C) for each concentration. The ranges of the conversion rates were given based on the conversion at these three temperatures. [b]For 50 g/L concentration, the conversion rate was reached to 99% at 40 °C. The conversion was monitored by LC/MS.

To finish the synthesis of **1**, a short route was proposed using direct amide coupling without protecting the piperidine nitrogen (Figure 5). The reaction was undertaken using the BOP ((Benzotriazol-1-yloxy)tris(dimethylamino)phophonium hexafluorophosphate) coupling conditions with amine **6** to afford intermediate **7**. Since compound **7** was highly water soluble, it was not suitable for normal phase purification. Thus, the crude **7** was directly treated with triphosgene, and the key intermediate **8** was obtained in 38% yield after purification in two steps (Exp. 2 in Table 2). A self-coupling byproduct **11**[14]was identified as the major byproduct in the coupling reaction (Figure 6). Our strategy to improve the yield of the desired product **7** was to allow the hetero amide coupling to compete with the self-coupling early to improve the reaction yield. By mixing the acid **5** and BOP followed by the immediate addition of amine **6** and the Hunig's base, the self-coupling was minimized and the overall yield of compound **8** in 2 steps was significantly improved to 76% (Table 2). The benzyl protecting group of **8** was removed using Pd/C under hydrogen atmosphere (1 atm, hydrogen balloon) in methanol to obtain **9** in 93% yield. The hydroxyl group of **9** was converted to sulfate using sulfur trioxide pyridine complex. The sulfate **10** was converted to the *tert*-butyl ammonium salt to enhance its stability and to reduce its polarity for easy handling. Finally the Boc group was removed under TFA condition to obtain the desire product **1**. The pure compound **1** was obtained using the crystallization method or purification on a prep HPLC column without any acid or base modifier (see Supplemental material). In general this route was very effective and could avoid complicated protecting and de-protecting steps.

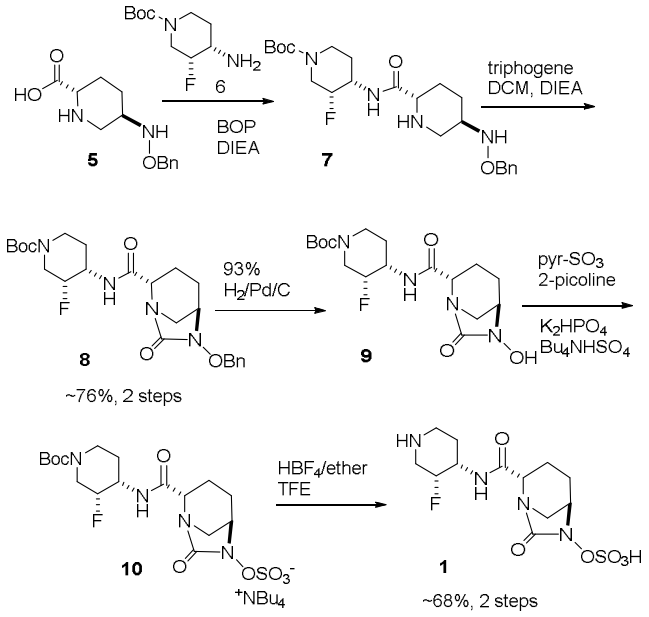


Figure 5. Synthetic scheme of **1** from intermediate **5**

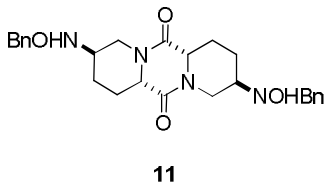


Figure 6. The structure of the byproduct, symmetric dimer **11**

While intermediate **5** served as an excellent intermediate to make new relabactam analogs for further structure-activity relationship (SAR) studies, we were also interested in a more advanced intermediate **16** for some practical reasons. After failed attempts on direct cyclization from **5** to bicyclic **16**, we adopted a uneventful synthetic route shown in Figure 7. Taking the crude mother liquid of **5** directly from the enzymatic amide cleavage step in Figure 3, the pH was adjusted to 10-11 using saturated sodium carbonate followed by the addition of Boc_2O.[15] The overnight reaction afforded compound **12** after the EtOAc extraction. Esterification with allyl alcohol in DMF at 60 °C for one hour provided the clean compound **13** which was immediately treated with 4 M HCl in dioxane/isopropanol. The desired intermediate **14** was obtained by crystallization without any column purification (60-70% yield in 3 steps from **5**). The excellent yield of these three steps further demonstrated the high yield of the enzymatic amide bond cleavage step was achivable (estimated as >95% yield, Figure 3).

To complete the synthesis of **16**, the HCl salt of compound **14** was first converted to the free base followed by the intramolecular cyclization with triphosgene. The optimal condition used 0.4 equivalent of triphosgene at -30 °C with pretreated Hunig's Base (2.5 eq.) in acetonitrile, followed by stirring at ~40-45 °C for one hour. Additional triphosgene could cause the formation of other unidentified byproducts. Removing the allyl group using sodium ethyl-hexanoate and $Pd(dppf)Cl_2$ gave rise to the final compound **16** in > 90% yield in two steps. No flash chromatography was required in this 6-step synthesis (Figure 7).

Table 2. Correlation of wait time versus yields of **8**

	Wait time [a]	Yield of **8** [b]
Exp 1	10 min	14%
Exp 2	5 min	38%
Exp 3	0 min	76%

[a] Wait time for amine **6** and Hunig's base addition after BOP addition. [b] Two steps from compound **5**. [a] All reactions were conducted at rt with 1.2 eq. BOP, 1.1 eq. amine **6** and 3 eq. Hunig's base in DMF. The yields described here are isolated yields after purification.

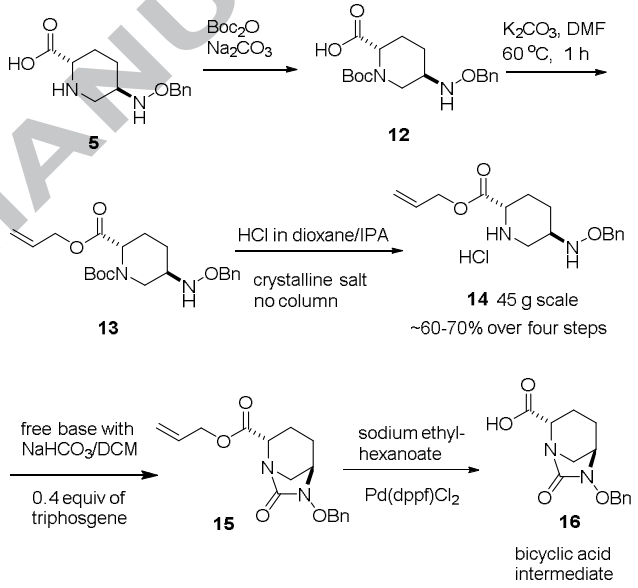


Figure 7. Conversion of intermediate **5** to bicyclic intermediate **16**

With the cyclized product **16** in hand, Compound **1** could be synthesized as shown in Figure 8. This route also allowed us to completely avoid the formation of byproduct **11**, enabling easy access to new relabactam analogs with modification on the piperdine ring.

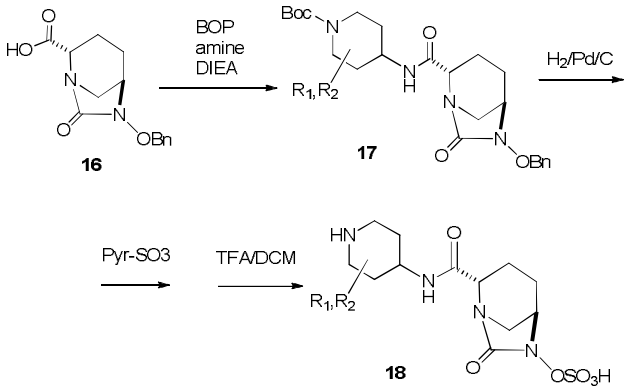


Figure 8. Synthetic route of new relabactam derivatives from intermediate **16**

In conclusion we have developed highly efficient chemistry for the synthesis of intermediates **5** and **16** from compound **2**. The novel chemistry empowered us to synthesize and scaled up an important analog **1** of relabactam in an effective manner. The key intermediates **5** and **16** also greatly advanced our SAR studies on relabactam to assist future development of its analogs for treatment of severe Gram negative bacterial infections.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, compound characterization, and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The biological data of **1** will be published elsewhere.
(14) The structure of the dimer by-product **11** was confirmed by NMR techniques, including COSY, HSQC, HMBC, and NOE.
(15) The Boc protection could not be achieved at pH ~8. The pH of the solution was adjusted to ~10-11 to achieve the Boc addition. Blizzard, T. A.; Chen, H.; Gude, C.; Hermes, J. D.; Imbriglio, J. E.; Kim, S.; Wu, J. Y.; Ha, S.; Mortko, C. J.; Mangion, I.; Rivera, N.; Ruck, R. T.; Shevlin, M. PTC WO 2009091856 A3, 2009.

- A very mild condition to enzymatically cleave an amide bond was identified.
- Amide coupling condition optimized without protecting other functional groups.
- An alternative approach to the bicyclic core was developed to enable convergent synthesis.
- All reactions shown above were done in > 80% yield each step.