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An asymmetric synthesis of Taniborbactam enantiomer

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ABSTRACT: Taniborbactam (VNRX-5133) is a novel cyclic boronate β-lactamase inhibitor in clinical development in combination with cefepime for the treatment of infections caused by carbapenem-resistant Enterobacterales (CRE) and Pseudomonas aeruginosa (CRPA). Taniborbactam is the first β-lactam inhibitor that inhibits Ambler class A, B, C, and D. Here we describe the improved total synthesis of a taniborbactam enantiomer utilising a previously reported synthetic protocol. The key steps employed were the Grignard reaction followed by Matteson homologation. All the compounds are characterized by spectral analysis.

KEYWORDS:β-lactam inhibitor,Taniborbactam, VNRX-5133, asymmetric synthesis, Grignard reaction,Matteson homologation.

I. INTRODUCTION

The majority of gram-negative infections are treated with beta-lactam antibiotics, such as penicillins, cephalosporins, monobactams, and carbapenems (Figure **1**). Rapidly increasing antibiotic resistance in gram-negative bacteria, which is usually linked with healthcare-associated infections, is a growing public health problem in worldwide.[1] While the cephalosporin family of β-lactams constituted the cornerstone of therapy in the 1980s, the spread of extended-spectrum β-lactamases during the last two decades has significantly weakened the effectiveness of this class, resulted in a comparable dependence on carbapenems.[2]Although carbapenems are generally considered as a safe and effective class of antiviral drugs, carbapenem-resistant enterobacteriaceae (CRE) caused by the Klebsiella pneumoniae carbapenemase and other β-lactamases is increasingly threatening the effectiveness of all β-lactam antibiotics.[3]

Carbapenem-resistant Enterobacteriaceae (CRE) are bacterial strains that are resistant to a kind of antibiotic (carpabenem) used to treat severe infections. CRE are also resistant to the majority of other widely used antibiotics, and in certain instances, all antibiotics. The Centers for Disease Control and Prevention (CDC) believes CRE to be an urgent antibiotic resistance issue that has now been discovered in virtually, with an alarming rise in prevalence over the last five years.[4]The inability to develop antibiotic drugs to control CRE risks having a negative effect on the healthcare system.[5]

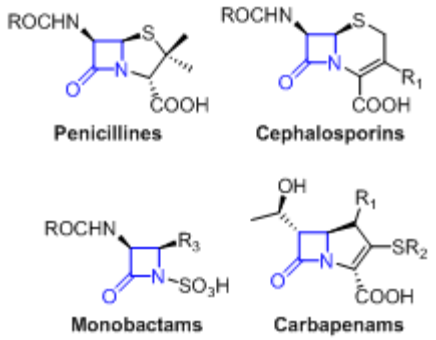

Fig 1: Common beta-lactam antibiotics.

To combat resistance to β-lactam antibiotics, a well-known and effective method has been to combine them with an inhibitor of the β-lactamase enzymes responsible for their breakdown. Clinically relevant β-lactamase inhibitors include clavulanic acid **1** (in combination with amoxicillin), sulbactam **2** (in combination with ampicillin), and tazobactam **3** (in combination with piperacillin) (Figure **2**). Because these β-lactamase inhibitors inhibit KPC-lactamase weakly, they are ineffective in the treatment of CRE infections. The diazabicyclooctane inhibitors (i.e,. Avibactam **4**, relebactam **5** and zidebactam **6**) have recently entered clinical trials in conjunction with ceftazidime and imipenem, respectively.[6-8] Both compounds resist a wide range of β-lactamases, including the KPC enzyme.

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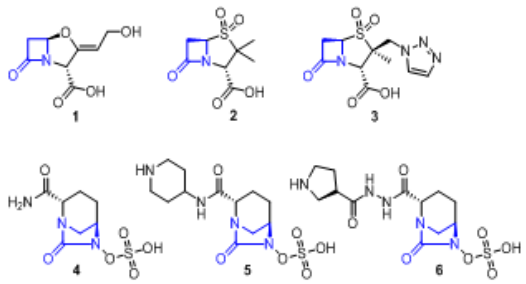


Fig 2: New β-lactamase inhibitors.

Boronic acids have long been researched as serine protease inhibitors.[9]Researchers at Oxford University reported that borate ions inhibit β-lactamase I.[10] Researchers subsequently carried on to report that boronic acids, such as phenylboronic acid **7** inhibit β-lactamase (Figure **3**).[11]Jones (Toronto) disclosed a rationally developed inhibitor **8** after publishing the high-resolution X-ray crystal structure of E. coli RTEM-1 β-lactamase.[12-14] Structure-guided design provided very powerful inhibitors **9** and **10**.[15]

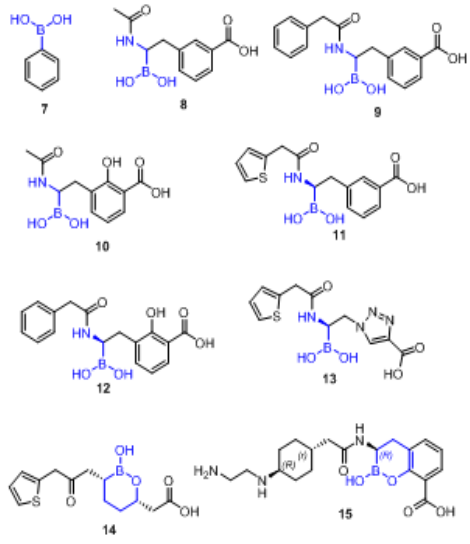
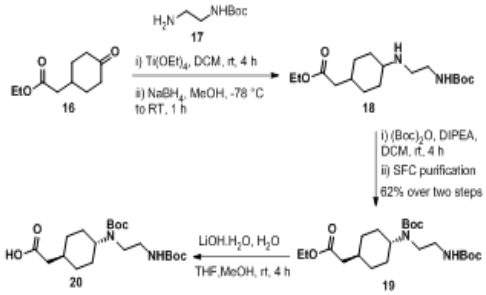


Fig 3: Boronic acid β-lactamase inhibitors.

Several publications from B. Shoichet's lab describe structure-based design approaches in this class (e.g., compounds **11**).[16, 17] Recent patent applications describe analogues of **9** and **10**,[18-20] as well as heterocyclic versions like **13** [21] (Figure 2). Vaborbactam is a recent cyclic boronic acid beta-lactamase inhibitor. The FDA approved it on August 29, 2017, for the treatment of complicated bacterial urinary tract infections.[22] Taniborbactam (VNRX-5133), developed by Venatorx Pharmaceuticals, is a broad-spectrum serine and metallo β-lactamase inhibitor used for carbapenem-resistant bacterial infections.[23-26] In this communication, we disclose our findings on the improved synthesis of taniborbactam enantiomer.

II. RESULTS AND DISCUSSION:

Our synthetic approach commenced with the preparation of trans acid compound **19**. The trans acid **20**,prepared by reductive amination of ketone compound **16** with N-Boc-ethylene diamine **17** followed by reduction with $NaBH_4$, gives the amine ester compound **18**. The crude mixture of cis and trance compounds was carried to the next step without purification. The secondary amine in compound **18** was protected with boc anhydride to give diboc compound with a mixture of trans and cis compounds in the ratio of 8:2. The cis and trans compounds are separated by SFC purification. The structure of trans compound **19** was confirmed by 2D NMR and nOesy spectroscopy. The ethyl ester was then hydrolyzed with aqueous LiOH to give the enantiomerically pure trans acid compound **20** with a 90% yield,as illustrated in **Scheme-1**.



Scheme 1: Synthesis of trans acid derivative **(20).**

Chloroidomethane **21** with n-BuLi, trimethylborate in THF at -78 °C, followed by the addition of (-)-pinanediol **23** at room temperature, gives chloromethyl boronate ester in a 64% yield.[27] The tert-butyl ester compound **25**,was prepared with commercially available 3-Iodo-2-methoxybenzoic acid with tert-butanol in the presence of H_2SO_4 at 120 °C to give the tert-butyl ester compound **25**, with 88% of the yield. The Grignard reaction of obtained tert-butyl ester compound **25** with chloromethyl boronate ester **24** in the presence of isopropyl magnesium chloride at -78 °C give aromatic boronate ester compound **26** in 70% yield (**Scheme-2**).

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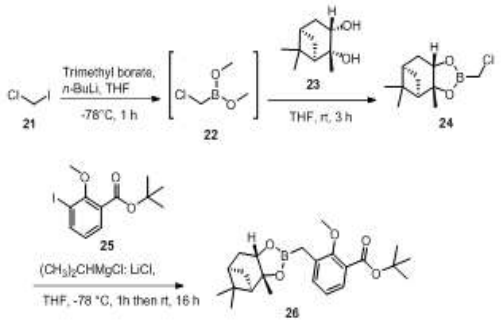
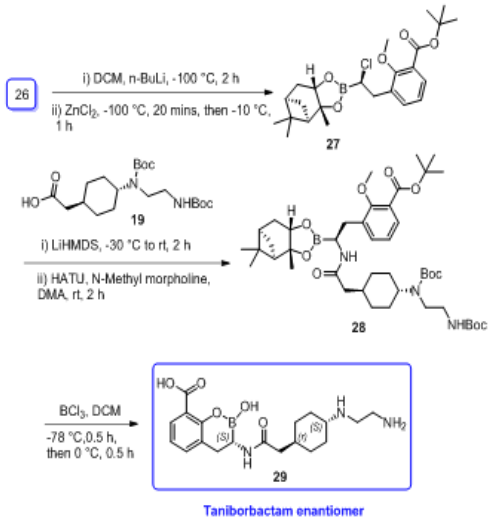


Scheme 2: Synthesis of boronate ester **(26):**

The diastereoselective matteson homologation of in situ-generated (dichloromethyl)lithium with aromatic boronate ester **26** at -100 °C in THF, followed by treatment with zinc chloride, resulted in chain extension to afford α-chloro derivative **27** in a 80% yield.[28] The stereospecific displacement of the chloro group with hexamethyldisilazide followed by in situ reaction with trans acid compound **20** affords amido boronate ester **28** with a 66% yield, as shown in **scheme-3**.



Scheme 3: Synthesis of taniborbactam enantiomer **(29).**

The amido boronate ester **28** was treated with BCl_3 to remove all the protective groups, cyclization giving taniborbactam enantiomer **29**. The purity of Taniborbactam enantiomer was found to be > 99% by HPLC and chiral HPLC.

III. CONCLUSION:

An efficient synthesis of clinical candidate taniborbactam enantiomer has been demonstrated. The synthesis was achieved in eight steps. In our laboratories, we are now working on further improvisation as well as the development of taniborbactam derivatives, which will be published in due time.

IV. EXPERIMENTAL SECTION:

All reactions were carried out in an oven or flame-dried glass-wares under a moisture-free atmosphere. All the reagents and solvents used were purchased from commercial sources and used without further purification. All moisture-sensitive reactions were carried out under nitrogen. TLC plates were visualised either with UV or $KMnO_4$ spray, unless noted otherwise. Crude products were purified by column chromatography on silica gel of 100-200 mesh. ^1H NMR spectra were recorded on a Bruker Avance 300 MHz, 400 MHz, 500 MHz respectively, using $CDCl_3$, DMSO-d6 and TMS as reference unless otherwise indicated. Chemical shifts are reported in parts per million relative to $CDCl_3$ (^1H, δ7.26), and DMSO-d_6 (^1H, 2.50ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd= doublet of doublet, t = triplet, q = quartet, quint = quintet, sep = septet, m = multiplet, br = broad), coupling constant and integration. LC-Mass spectra were obtained from Agilent 6400 Series Triple Quad system using electrospray ionization (ESI).

Ethyl 2-(4-((2-((tert-butoxycarbonyl) amino) ethyl) amino) cyclohexyl)acetate (18):

To a stirred solution of ethyl 2-(4-oxocyclohexyl) acetate **16** (5.0 g, 27.13 mmol), 2-(N-[2-(amino)-ethyl]-amino) pyridine **17** (5.43 g, 33.92 mmol) in dichloromethane was added titanium ethoxide (3.0 g, 13.56 mmol) dropwise at room temperature. The resulting mixture was stirred for 4 hours, and then concentrated completely under reduced pressure at 40 °C to give a gummy compound. The resulting gummy compound was dissolved in methanol (40 mL) and cooled to -78 °C. Sodium borohydride (1.53 g, 40.69 mmol) was added lot-wise over a period of 20 minutes at -78 °C. On complete addition, the cold bath was removed and stirring continued for 1 hour at room temperature. The reaction mixture was diluted with dichloromethane (50 mL) and washed with saturated sodium bicarbonate solution (50 mL). The organic phase was separated, dried over Na_2SO_4, evaporated solvent completely under reduced pressure to give ethyl 2-(4-((2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl) acetate **18** (8.4 g, crude) as a colourless gummy compound with mixture of cis and trans isomers. This crude mixture of isomers was used in the next step without further purification.

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Ethyl 2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl) amino)ethyl)amino)cyclohexyl)acetate (19) :

To a stirred solution of ethyl 2-(4-((2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl) acetate **18** (8.0 g, 24.36 mmol) in dichloromethane (120 mL) were added diisopropyl ethylamine (10.6 mL, 60.90 mmol) followed by Boc anhydride (13.3 mL, 60.90 mmol) at room temperature. The resulting solution was stirred for 4 hours at this temperature. The reaction mixture was diluted with dichloromethane (60 mL), washed with water (100 mL) and brine solution (100 mL), dried over Na_2SO_4 and evaporated solvent under reduced pressure at 40 °C to give the crude compound. The crude compound was purified by flash column chromatography over silica gel (80 g) using gradient elution of 0-20% EtOAc in pet ether to give the title compound as a 2:8 mixture of cis and trans isomers. The cis and trans isomers were separated by SFC purification to give ethyl 2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tertbutoxycarbonyl)amino) ethyl)amino)cyclohexyl)acetate**19** (7.2 g, 62% over two steps) as a white solid.
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.34 (q, J=7.2 Hz, 2H), 3.85 (s, 4H), 3.41-3.35 (m, 1H), 2.44 (d, J=6.8 Hz, 2H), 2.31 (d, J=10.8 Hz, 2H), 2.07 (d, J=12.8 Hz, 2H), 1.98-1.93 (m, 1H), 1.66-1.51 (s, 19H), 1.37 (t, J=7.2 Hz, 3H), 1.31-1.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 172.9, 156.5, 156.2, 80.0, 60.3, 42.6, 41.7, 41.1, 34.1, 32.3, 30.4, 28.6, 28.5, 14.4; LC-MS (ESI): 95.0%, m/z 429.3 (M+H), HPLC purity: 98.3% and Chiral purity: 98.9%.

2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl) acetic acid (20): :

To a stirred solution of ethyl 2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tert butoxycarbonyl)amino)ethyl)amino)cyclohexyl)acetate**19** (2.5 g, 5.83 mmol) in THF (10 mL), methanol (10 ml.) and water (20 mL) was added lithium hydroxide monohydrate (1.22 mg, 29.15 mmol) at room temperature. The resulting solution was stirred for 4 hours at room temperature. The reaction mixture was acidified (pH ~5) with 1N HCl solution and extracted with dichloromethane (2x20 mL). The combined organic layer was dried over Na_2SO_4, evaporating solvent completely under reduced pressure at 40 °C to give the crude compound. The crude compound was purified by flash column chromatography over silica gel (24 g) using gradient elution of 0–50% EtOAc in pet ether to afford 2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tert-butoxy carbonyl)amino)ethyl)amino)cyclohexyl) acetic acid **20** (2.1 g, 90%) as an off-white solid.
^{1}H NMR (500 MHz, DMSO-d6, VT at 100 °C): δ (ppm) 12.01 (s, 1H), 6.29 (brs, 1H), 3.56 (brs, 1H), 3.11-2.93 (m, 4H), 2.08 (d, J=7.0 Hz, 2H), 1.78 (d, J=12.5 Hz, 2H), 1.63-1.48 (m, 5H), 1.41-1.38 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.4, 177.1, 80.2, 80.1, 41.2, 33.8, 32.2, 30.4, 28.6, 28.5, 20.6; LC-MS (ESI): 93.5%, m/z 399.1 $(M-H)^-$.

(3aR,4R,6R,7aS)-2-(chloromethyl)-3a,5,5-trimethylhexahydro-4,6-methano benzo[d][1,3,2]dioxaborole (24)

To a stirred solution of trimethyl borate (1.83 g, 17.76 mmol), chloroiodomethane **21** (1.53 mL, 21.14 mmol) in dry THF (25 ml.) was added n-BuLi (7.8 mL, 2.5 M in hexane, 19.38 mmol) drop wise over a period of 30 minutes at -78 °C. After 1 h, the mixture was quenched with trimethylsilyl chloride (2.2 g, 20.26 mmol) and allowed to warm to room temperature and stirred overnight at this temperature. Then, (-)-pinanediol **23** (3.0 g, 17.62 mmol) dissolved in dry THF (5 mL) was added to this reaction mixture. The reaction mixture was stirred for 3 hours at room temperature. The mixture was partitioned between water (50 mL) and diethyl ether (50 mL). The organic layer was separated, dried over Na_2SO_4, and evaporated under reduced pressure at 30 °C to give the crude compound. The crude compound was purified by flash column chromatography over silica gel (40 g) using gradient elution of 5% ethyl acetate in pet ether to afford (3aR,4R,6R,7aS)-2-(chloromethyl)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborole **24** (2.5 g, 64%) as a colourless gummy compound.
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 4.39-4.36 (m, 1H), 3.01 (s, 2H), 2.38-2.38 (m, 2H), 2.10-2.07 (m, 1H), 1.95-1.89 (m, 3H), 1.43 (s, 3H), 1.30 (s, 3H), 1.16 (d, J=11.0 Hz, 1H), 0.84 (s,3H).^{13}C NMR: δ (ppm) 86.8, 78.6, 51.3, 39.4, 38.3, 35.3, 28.5, 27.1, 26.3, 24.0.

Tert-butyl 3-iodo-2-methoxybenzoate (25):

Sulphuric acid (6.5 mL, 118.7 mmol) was added dropwise over 15 minutes to a solution of 3-iodo-2-methoxy-benzoic acid (30 g, 107.8 mmol) in tert-butanol (150 mL) at 0 °C. The reaction mixture was heated to 120 °C and stirred for 16 hours at that temperature. The reaction mixture was neutralised with a saturated $NaHCO_3$ solution and extracted with ethyl acetate (200 mL). The organic

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layer was washed with water (50 mL) and brine (100 mL), dried over Na_2SO_4 and evaporated solvent under reduced pressure to give the crude compound. The crude compound was purified by column chromatography over silica gel (100–200 mesh) using gradient elution of 0–10% EtOAc in pet ether to afford tert-butyl 3-iodo-2-methoxybenzoate **25** (32 g, 88%) as a pale yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.52 (dd, J=7.6, 1.6 Hz, 1H), 7.32 (dd, J=7.6, 2.0 Hz, 1H), 7.01 (t, J=7.6 Hz, 1H), 4.28-4.26 (m, 1H), 3.82 (d, J=4.0 Hz, 1H), 2.31-2.29 (m, 3H), 2.26-2.17 (m, 1H), 2.03 (t, J=5.2 Hz, 1H), 1.59 (s, 9H), 1.38 (s, 3H), 1.27 (s, 3H), 1.19 (d, J=10.8 Hz, 1H), 0.83 (s,3H).LC-MS purity (ESI): 94.21%; m/z 423.4 $(M+Na)^{+.}$

Tert-butyl 2-methoxy-3-(((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo [d][1,3,2]dioxaborol-2-yl)methyl)benzoate (26):

To a stirred solution of tert-Butyl 3-iodo-2-methoxy-benzoate **25** (2.5 g, 7.48 mmol) in dry THF (20 mL) was added isopropyl magnesium chloride lithium chloride complex solution (6.3 mL, 1.3 M in THF, 8.22 mmol) drop-wise over a period of 30 minutes. On completing the addition, the solution was stirred for 30 minutes. The reaction mixture was then cooled to -78 °C. Chloromethyl boronic acid (-)-pinanediolato diester **24** (2.5 g, 11.22 mmol) in THF (4 mL) was added over a period of 20 mins at -78 °C. The reaction mixture was slowly warmed to room temperature and stirred for 16 hours at this temperature. After completion of the reaction, the reaction mixture was diluted with diethyl ether (40 ml), washed with 0.1 M HCI solution (20 mL), brine solution (20 mL), dried over Na_2SO_4, and evaporated solvent completely under reduced pressure at 40 °C to give the crude compound. The crude compound was purified by flash column chromatography over silica gel (24 g) and gradient elution of 3-5% ethyl acetate in pet ether to yield tert-butyl 2-methoxy-3-(((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)methyl)benzoate **26** (2.1 g, 70%) as a colorless gummy compound.

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.52 (dd, J=7.5, 1.5 Hz, 1H), 7.32 (dd, J=8.0, 1.5 Hz, 1H), 7.01 (t, J=8.0 Hz, 1H), 4.26 (dd, J=9.0, 2.0 Hz, 1H), 3.81 (s, 3H), 2.31 (s, 2H), 2.30-2.18 (m, 2H), 2.03 (t, J=5.5 Hz, 1H), 1.90-1.80 (m, 2H), 1.59 (s, 9H), 1.38 (s, 3H), 1.27 (s, 3H), 1.19 (d, J=11.0 Hz, 1H), 0.83 (s,3H).^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 166.3, 157.5, 134.5, 134.2, 128.7, 126.5, 123.6, 86.0, 81.3, 78.1, 61.6, 51.5, 39.8, 39.7, 38.4, 35.5, 28.8, 28.5, 27.3, 26.6, 24.3; LC-MS purity (ESI): 93.2%; m/z 423.1 $(M+Na)^+$.

tert-butyl 3-((R)-2-chloro-2-((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2] dioxaborol-2-yl)ethyl)-2-methoxybenzoate (27):

To a stirred solution of dichloromethane (1.27 g , 14.98 mmol) in THF (12 mL) was added n-BuLi (2.6 mL, 2.5M in hexanes, 6.49 mmol) drop-wise over a period of 30 minutes at -100 °C. On complete addition, the resulting white cloudy solution was stirred for 30 minutes at -100 °C, then a solution of (3-tert-butoxycarbonyl-2-methoxyphenyl) methyl boronic acid (-)-pinanediolato diester **26** (2.0 g, 4.99 mmol) in THF (4 mL) was added drop-wise over a period of 30 minutes. After the addition was completed, the reaction mixture was allowed to stir for 30 minutes at -100 °C. Then, a solution of $ZnC1_2$ solution (6.5 mL, 1 M in diethyl ether, 6.59 mmol) was added drop-wise over a period of 15 minutes at -100 °C. The reaction mixture was gradually warmed to -10 °C, and the resulting reaction mixture was stirred for 1 hour. The resulting yellow solution was diluted with diethyl ether (20 mL), washed with 0.1M HCI solution (25 mL), and brain solution (30 mL). The organic layer was dried over Na_2SO_4, and concentrated completely under reduced pressure at 40 °C to give the crude compound. The crude compound was purified by flash column chromatography over silica gel (24 g) using gradient elution of 2-5% EtOAc in pet ether to afford tert-butyl 3-((R)-2-chloro-2-((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)ethyl)-2-methoxybenzoate **27** (1.8 g, 80%) as a colourless gummy compound.

^{1}H NMR (500 MHz, CDCl3): δ (ppm) 7.61 (dd, J = 8.0, 2.0 Hz), 7.40 (dd, J = 7.5, 2.0 Hz), 7.05 (t, J = 8.0Hz), 4.38 (dd, J = 8.3, 2.0 Hz), 3.86 (s, 3H), 3.74-3.71 (m, 1H), 2.27 (dd, J = 13.2, 8.0 Hz), 2.26-2.18 (m, 1H), 2.11 (m, 1H), 1.95-1.91 (m, 1H), 1.85-1.81 (m, 1H), 1.79-1.75 (m, 1H), 1.46 (s, 9H), 1.28 (s, 3H), 1.23 (s, 3H) 1.20 (d, J = 9.7 Hz, 1H), 0.85 (s, 3H); 13C NMR (125 MHz, CDCl3): δ (ppm) 166.2, 157.8, 137.0, 134.4, 128.6, 125.5, 123.5, 86.0, 81.2 , 78.0, 61.6, 51.3, 39.6, 39.2, 35.6, 28.7, 28.3, 27.2, 26.5, 24.1. LC-MS purity (ESI): 98.2%; m/z 470.21 $(M+Na)^+$.

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tert-butyl 3-((S)-2-(2-((1r,4S)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl) amino)ethyl) amino)cyclohexyl)acetamido)-2-((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)ethyl)-2-methoxybenzoate (28):

To a stirred solution of tert-butyl 3-((R)-2-chloro-2-((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6 methanobenzo[d][1,3,2]dioxaborol-2-yl)ethyl)-2-methoxybenzoate **27** (1.1 g, 2.45 mmol) in THF (6 mL) was added lithium bis(trimethyl silyl)amide solution (2.5 mL, 1M in THF, 2.45 mmol) at -30 °C. On complete addition, the cold bath was removed and stirring continued for 2 hours at room temperature. The resulting solution was used for the next step immediately. In a separate RB flask, ethyl 2-((1r,4r)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl)acetate**19** (1.0 g, 2.49 mmol) in DMA (6 mL) was added N-methylmorpholine (277 mg, 2.74 mmol) followed by HATU (1.08 g, 2.74 mmol) at room temperature. The reaction mixture was stirred for 2 hours at this temperature. The above prepared solution was added to this reaction mixture at room temperature. The resulting mixture was stirred for 4 hours at room temperature. After completion of the reaction, the reaction mixture was diluted with ethyl acetate (20 mL), washed with water (20 mL), brine (20 mL), dried over sodium Na_2SO_4 and concentrated under reduced pressure at 35 °C to give the crude compound. The crude compound was purified by flash reverse phase column chromatography over C18 column (40 g) using gradient elution of 0-80 %, mobile phase A: 0.1% HCOOH in H_2O, mobile phase B: acetonitrile. The pure fractions are freeze dried to give tert-butyl 3-((S)-2-(2-((1r,4S)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl)acetamido)-2 ((3aR, 4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)ethyl)-2-methoxybenzoate **28** (1.3 g, 66%) as an off-white solid.

^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 9.33 (s, 1H), 7.44 (dd, J=8.0, 2.0 Hz, 1H), 7.49 (dd, J=7.5, 1.5 Hz, 1H), 7.09 (t, J=8.0 Hz, 1H), 6.76 (brs, 1H), 4.0-4.3.98 (m, 1H), 3.71 (s, 3H), 3.12-2.99 (m, 5H), 2.52-2.49 (m, 5H), 2.16 (d, J=7.0 Hz,1H), 1.78 (d, J=6.0 Hz,1H), 1.74-1.61 (m, 18H), 1.40-1.36 (m, 21H), 1.32-1.30 (m, 8H), 1.21-1.20 (m, 2H), 0.80 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d6): δ (ppm) 174.8, 173.1, 170.4, 158.5, 157.6, 144.2, 141.6, 133.3, 131.2, 130.4, 126.6, 125.7, 124.8, 81.3, 80.2, 78.6, 70.0, 61.6, 59.9, 55.4, 52.9, 52.0, 42.1, 41.9, 40.9, 38.8, 36.9, 35.3, 33.3, 30.2, 29.2, 29.1, 28.9, 28.8, 28.5, 28.3, 27.6, 27.3, 24.7, 24.4, 21.0, 14.6; LC-MS purity (ESI): 93.2%; m/z 812.5 $(M+H)^+$.

(S)-3-(2-((1r,4S)-4-((2-aminoethyl)amino)cyclohexyl)acetamido)-2-hydroxy-3,4-dihydro-2Hbenzo[e][1,2] oxaborinine-8-carboxylic acid (taniborbactam enantiomer) (29):

To a stirred solution of tert-butyl 3-((S)-2-(2-((1r,4S)-4-((tert-butoxycarbonyl)(2-((tert-butoxycarbonyl)amino)ethyl)amino)cyclohexyl)acetamido)-2-((3aR,4R,6R,7aS)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)ethyl)-2-methoxybenzoate **28** (1.2 g, 1.48 mmol) in dichloromethane (6 mL) was added to the BCl_3 solution (5.6 mL, 1M in dichloromethane, 5.54 mmol) drop-wise over a period of 20 minutes at -78 °C. The reaction mixture was stirred for 30 minutes at -78 °C, and then slowly warmed to 0 °C, stirred for 30 mins at 0 °C. The reaction mixture was quenched by water (12 mL) and stirred for 20 minutes at RT. The aqueous layer was separated and liquid injected on a C18 column (80 g), eluted with gradient elution of 0-30 % of 0.1% TFA in H_2O, acetonitrile. The pure fractions are freeze dried to give the taniborbactam enantiomer **29** (414 mg, 72%) as an off-white solid.

^{1}H NMR (DMSO-d6+D_2O, 500 MHz): δ (ppm) 7.73 (dd, J=8.0, 1.5 Hz, 1H), 7.32 (d, J=7.0 Hz, 1H), 6.93 (t, J=8.0 Hz, 1H), 3.10-3.03 (m, 5H), 2.84-2.75 (m, 3H), 2.25-2.24 (m, IH), 2.02-1.97 (m, 1H), 1.78-1.76 (m, 1H), 1.68-1.65 (m, 1H), 1.40-1.37 (m, 1H), 1.24-1.23 (m, 1H), 1.08-1.05 (m, 1H), 0.92-082 (m, 1H), 0.75-0.72 (m, 1H), 0.58-054 (m, 1H).^{13}C NMR (125 MHz, DMSO-d6+D_2O): δ (ppm) 179.4, 170.0, 156.5, 136.7, 129.7, 128.7, 122.0, 116.8, 56.9, 41.2, 35.6, 35.5, 33.7, 30.5, 28.8, 28.4, 28.1, 28.0; LC-MS (ESI): 93.2%; m/z 390.04 $(M+H)^+$,HPLC purity: 99.67%, Chiral HPLC purity: 99.89%.

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