Synthesis of New β-Lactam Analogs and Evaluation of Their Histone Deacetylase (HDAC) Activity

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A simple synthesis of the β-lactams **11** – **13** and **16** – **17** as novel histone deacetylase (HDAC) inhibitors is described. The key synthetic strategies involved the *O*-alkylation of 6-APA and the coupling reactions of freshly prepared *N*-carbobenzyloxy-L-prolines **5** and **6** and 6-aminopenicillanates **8** – **10** and **15** in high yields. It was found that all compounds show potent growth inhibitory activity on human tumor cell lines, the most potent compound **16** exhibiting an IC_{50} = 2.1 μM *in vitro*.

Key words: β-Lactams, Histone Deacetylase, Coupling Reaction, Anticancer, Synthesis

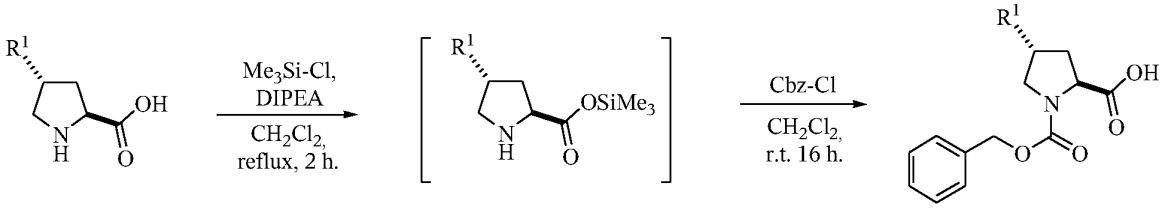
Introduction

Since the introduction of β-lactam antibiotics, β-lactam substrates have stimulated significant interest due to their wide range of intriguing biological activities [1] such as antibiotic [2], antioxidant [3], antiviral [4], and anticancer [5] properties. The variety of the pharmacological activities of these β-lactams and their unique structural features, including azetidin-2-one rings, generated a great deal of interest among synthetic chemists and biologists. Recently, β-lactam substrates with significant biological activities such as serine-dependent enzyme inhibitors [6], matrix-metalloprotease inhibitors [7], cysteine protease inhibitors [8], and apoptosis inductors [9] were reported in the literature. Furthermore, they have served as synthons in the preparation of various heterocyclic compounds and potent anticancer agents such as paclitaxel, epothilones, and their analogs [10]. Current research priorities for the β-lactam moieties are focused on providing better antibacterial efficacy and on biochemical features as enzyme inhibitors including apoptosis-inducing properties. In addition, efforts to develop optimal β-lactams as anticancer agents are underway. More recently, histone acetylation was reasonably accepted as a mechanism of chromatin remodeling. It is highly governed by the antagonistic activity of histone acetyltransferases (HAT) and histone deacetylase (HDAC) as well as its acetylase inhibitors [11]. The small lactam (β and γ) derivatives are privileged structures for enzyme inhibition, mainly due to their ability to trap serine or cysteine residues in the DNA binding domain.

In a continuation of our medicinal chemistry program connected with the synthesis of new β-lactam moieties and evaluation of their biological properties, we required Cbz-protected L-prolines **5** – **6** and 6-aminopenicillanates **8** – **10**, **15** as important fragments in order to generate novel histone acetylase inhibitors. We wish to report herein a simple synthesis and the evaluation of the anticancer activity of the 6-acylaminopenicillanates **11** – **13** and **16** – **17**, starting from 6-aminopenicillanic acid (6-APA, **1**) *via O*-alkylation and coupling reactions.

Results and Discussion

To generate the Cbz-protected L-prolines **5** and **6**, which are well known as a pharmacophore for HDAc inhibitors, the commercially available L-proline (**1**) and *trans*-4-hydroxy-L-proline (**2**) were treated with chlorotrimethylsilane (Me_3SiCl) in the presence of diisopropylethylamine (DIPEA) in dichloromethane to yield the silyl-protected L-prolines **3** and **4**, which were used in the next step without purification. Intermediates **3** and **4** were subsequently treated with benzyloxycarbonyl chloride (Cbz-Cl) to give the Cbz-protected

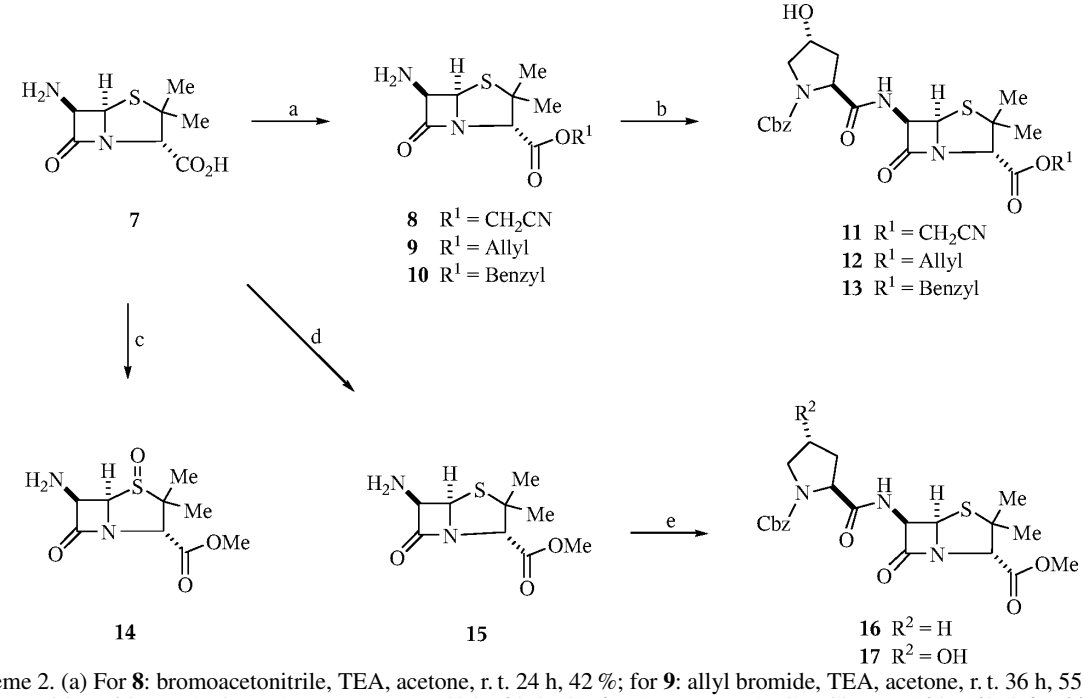


1 R^1 = H
2 R^1 = OH

3 R^1 = H
4 R^1 = $OSiMe_3$

5 R^1 = H (97 %)
6 R^1 = OH (95 %)

Scheme 1. Synthesis of Cbz-protected L-prolines **5** and **6**.



7

8 R^1 = CH_2CN
9 R^1 = Allyl
10 R^1 = Benzyl

11 R^1 = CH_2CN
12 R^1 = Allyl
13 R^1 = Benzyl

14

15

16 R^2 = H
17 R^2 = OH

Scheme 2. (a) For **8**: bromoacetonitrile, TEA, acetone, r. t. 24 h, 42 %; for **9**: allyl bromide, TEA, acetone, r. t. 36 h, 55 %; for **10**: benzyl bromide, TEA, 4-DMAP, acetone, r. t. 48 h, 65 %; (b) **6**, HATU, DIPEA, CH_2Cl_2, r. t. 16 h, (68 % for **11**, 75 % for **12**, 76 % for **13**); (c) dimethyldioxirane, CH_2Cl_2, 0 °C to r. t. 1 h; then, CH_2N_2, CH_2Cl_2, r. t. 1 h; (d) CH_2N_2, CH_2Cl_2, r. t. 1 h, 95 %; (e) **5** – **6**, HOBt, EDCI, TEA, CH_2Cl_2, r. t. 3 h, (80 % for **16**, 82 % for **17**).

L-prolines **5** and **6** in 97 and 95 % two-step yields, respectively (Scheme 1).

The β-lactam moieties **11** – **13** were prepared from readily generated 6-aminopenicillanates **8** – **10** [12] with *N*-carbobenzyloxy-L-proline (**5**) and *N*-carbobenzyloxy-L-4-hydroxyproline (**6**) *via* common condensation reactions. 6-Aminopenicillanic acid (6-APA, **7**) was treated with several alkylating reagents such as bromoacetonitrile, allyl bromide, and benzyl bromide to give esters **8** – **10**. At this stage, we tried to generate the various esters using several alkylating reagents such as bromochloromethane, chloromethyl methyl ether, 4-methoxybenzyl bromide, and benzyl chloromethyl ether under SN2-type reaction conditions including K_2CO_3/DMF [13], DBU/CH_3CN [14], and NaH/THF [15], but these reactions were all unsatisfactory, and for the most part the starting material was recovered. Compounds **8** – **10** were readily coupled with **6** in the presence of 2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and DIPEA in dichloromethane to afford **11** – **13** in good yields (Scheme 2).

In addition, 6-APA (**7**) was treated with freshly prepared diazomethane [16] in dichloromethane to afford ester **15**, in high yield. On the other hand, in

an effort to prepare the sulfoxide **14**, 6-APA (**7**) was smoothly treated with 3,3-dimethyldioxirane (DMDO) [17] in dichloromethane to generate a sulfoxide, which was then readily treated with diazomethane [18]. Unfortunately, these reactions failed to afford sulfoxide **14**, leaving only starting material and/or decomposed products. Compound **15** was condensed with acids **5** (*Z*-Hyp) or **6** (*Z*-Hyp-OH) using ethyl(dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) to generate **16**, **17** in high yields [13]. At this stage, coupling of **15** with **5**, **6** was also accomplished by dicyclohexylcarbodiimide (DCC)/CH_2Cl_2, HATU/CH_2Cl_2, and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) in CH_2Cl_2. Although the latter conditions were more convenient, the HOBt/EDCI method afforded a superior yield.

In vitro inhibition of histone deacetylase

A histone deacetylase fraction was prepared as described by Yoshida *et al*. [19]. Human leukemia K562 (2.5×10^8) cells were disrupted in buffer-A [15 mM of potassium phosphate buffer (pH 7.5) containing 5 % glycerol and 0.2 mM EDTA (15 mL)]. The nuclei were collected by centrifugation (35000 g, 10 min) and resuspended with buffer-A (15 mL) containing 1 M $(NH_4)_2SO_4$. After sonication, the supernatant was collected by centrifugation, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A (2000 mL). The dialysate was loaded onto a mono Q HR 5/5 column (Pharmacia) equilibrated with buffer-A, and eluted with a linear gradient of 0 – 1 M NaCl in buffer-A (30 mL). A single peak of histone deacetylase activity was eluted around 0.4 M NaCl, and the fraction was stored at −80 °C until use. Inhibition of histone deacetylase was estimated as described by Yoshida *et al.* with slight modifications. ^{3}H-labeled histone was prepared as reported, K562 cells (10^8 cells) were incubated in growth medium (25 mL) containing 0.5 mCi mL^{-1} [^{3}H]sodium acetate (152.8 GBq mmol^{-1}; NEN) and 5 mM sodium butyrate at 37 °C [18]. Histone deacetylase inhibitory activity of the test compound was measured as follows: The mixture (total volume 50 μL) containing the above histone deacetylase fraction (2 μL), ^{3}H labeled histone (100 μg mL^{-1}), and the test compound (5 μL) was incubated for 10 min at 37 °C. [^{3}H]Acetic acid, which was liberated from ^{3}H-labeled histone, was extracted with ethyl acetate, and radioactivity was measured by a liquid scintillation counter.

Table 1. HDAC and growth inhibiting potency of novel β-lactam moities **6** – **10**.

Compound	IC_{50} enzyme (μM)[a]	IC_{50} cells (μM)[b]
11	44.0	68.4
12	40.0	32.7
13	12.8	4.0
16	6.3	2.1
17	11.8	5.5
Sodium butyrate[c]	–	140
Trichostatin A[c]	–	0.0046

[a] HDAC enzyme assay; [b] the values are means of three experiments [20]; [c] materials for comparison.

The *in vitro* anticancer activity of β-lactam moieties **11** – **13** and **16**, **17** were evaluated in human tumor cell lines, and the results are summarized in Table 1. It was found that all compounds showed potent growth inhibitory activity on human tumor cell lines with the most potent compound **16** exhibiting IC_{50} = 2.1 μM. In addition, the methoxy esters **16**, **17** (Table 1, entries 4, 5) or benzyl ester **13** (Table 1, entry 3) exhibited higher *in vitro* growth inhibitory activity when compared to cyanomethyl or allyl esters **11**, **12**. In addition, the novel β-lactam moieties **11** – **13** and **16**, **17** showed better HDAC activity than sodium butyrate. However, all prepared sulfonamides exhibited less HDAC activity than trichostatin A.

In conclusion, a simple preparation of new histone deacetylase (HDAC) inhibitors has been described. Compound **16** exhibited the most potent anticancer activity among these analogs. We expect that simple syntheses of new β-lactam moieties and key fragments are useful for the modification of histone acetylase inhibitors.

Experimental Section

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran was distilled from sodium benzophenone ketyl prior to use. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with 254 nm UV light. Flash chromatography was carried out on silica gel 60 (Scientific Adsorbents Incorporated (SAI), particle size 32 – 63 μM, pore size 60 Å). ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on Bruker DPX 500 instruments at 500 MHz (^{1}H) and 125 MHz (^{13}C). The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC-Mass system and

high-resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). When necessary, chemicals were purified according to the reported procedures [21].

*(4R,2S)-4-Hydroxypyrrolidine-1,2-dicarboxylic acid 1-benzyl ester (**6**) [22]*

To a stirred solution of **4** (7.5 g, 57.2 mmol) in dry CH_2Cl_2 (120 mL) was added DIPEA (24.3 g, 188.7 mmol) under argon atmosphere at r. t., followed by addition of Me_3SiCl (27.9 g, 257.2 mmol). The mixture was refluxed for 2 h, and then the reaction mixture was cooled to 0 °C using an ice-salt bath. Cbz-Cl (9.3 g, 54.4 mmol) was added dropwise to the mixture and the resulting mixture was stirred at r. t. for 16 h. The reaction mixture was concentrated under reduced pressure, and the residue was dissolved in 3 % aqueous sodium bicarbonate solution (120 mL). The aqueous layer was separated and acidified to pH = 2 by 5 % aqueous HCl solution. The aqueous phase was extracted with ethyl acetate (100 mL × 3) and the combined organic layer was washed with saturated aqueous NH_4Cl solution (200 mL), water (200 mL) and brine (150 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 70 : 25 : 5; *n*-hexane : ethyl acetate : methanol, v/v) to give **6** (14.5 g, 95 %) as a beige oil. R_f = 0.1 (60 : 30 : 10, *n*-hexane : ethyl acetate : methanol, v/v). – $[\alpha]_D^{24}$ = −71.6 (*c* = 6.0, CH_2Cl_2). – IR (neat, NaCl): ν = 3385, 2986, 1691, 1550, 1462, 1218, 1176, 842 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 12.01 (br s, 1H), 7.35 – 7.28 (m, 5H), 5.10 (s, 2H), 4.33 – 4.29 (m, 2H), 3.48 (d, *J* = 10.8 Hz, 1H), 3.38 (dd, *J* = 10.8, 4.7 Hz, 2H), 2.20 (d, *J* = 7.5 Hz, 1H), 1.19 (d, *J* = 7.5 Hz, 1H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 172.1, 153.6, 136.9, 127.9, 127.1, 126.8, 67.9, 65.5, 57.4, 54.2, 38.1. – HRMS: *m/z* = 288.0859 (calcd. 288.0848 for $C_{13}H_{15}NO_5Na$, $[M+Na]^+$).

*General procedure for the preparation of compounds **11** – **13** via condensation reaction of acid **6** and amino esters **8** – **10***

To a stirred suspension of acid **6** (0.45 mmol), DIPEA (0.50 mmol) and HATU (0.54 mmol) in dry dichloromethane (8.0 mL) were added amino esters **8** – **10** (0.45 mmol) at 5 °C. The mixture was stirred at r. t. for 16 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with sat. aqueous NH_4Cl solution (10 mL) and brine (12 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate : *n*-hexanes : methanol = 20 : 75 : 5, v/v) to give pure carboxamides **11** – **13**.

*(2S,4R)-Benzyl 2-{(2S,5R,6R)-2-(2-cyanoacetyl)-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptan-6-ylcarbamoyl}-4-hydroxypyrrolidine-1-carboxylate (**11**)*

Yield: 58 %. Semisolid. R_f = 0.3 (*n*-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – $[\alpha]_D^{24}$ = 170.1 (*c* = 0.29, $CHCl_3$). – IR (neat, NaCl): ν = 3342, 3066, 3034, 2960, 1778, 1756, 1682, 1521, 1426, 1358, 1200, 1178, 1083, 771 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.65 (br s, 2/3H), 7.39 – 7.18 (m, 10H), 6.96 (br s, 1/3H), 5.66 – 5.46 (m, 1H), 5.14 (br s, 2H), 4.85 (dd, *J* = 18.8, 21.2 Hz, 1H), 4.71 – 4.55 (m, 1H), 4.54 – 4.35 (m, 2H), 3.78 – 3.53 (m, 4H), 3.10 (dd, *J* = 7.5, 7.0 Hz, 1H), 2.53 – 2.04 (m, 2H), 1.58 (s, 3H), 1.49 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 176.5, 166.4, 155.9, 136.1, 128.6, 128.2, 127.7, 114.0, 113.7, 69.9, 67.7, 67.6, 65.8, 64.8, 49.0, 48.8, 26.8. – HRMS: *m/z* = 525.1423 (calcd. 525.1420 for $C_{23}H_{26}N_4O_7SNa$, $[M+Na]^+$).

*(2S,4R)-Benzyl 2-{(2S,5R,6R)-2-but-3-enoyl-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptan-6-ylcarbamoyl}-4-hydroxypyrrolidine-1-carboxylate (**12**)*

Yield: 65 %. White solid. R_f = 0.4 (*n*-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – $[\alpha]_D^{24}$ = 80.1 (*c* = 0.28, $CHCl_3$). – IR (neat, NaCl): ν = 3341, 3065, 3034, 2963, 1785, 1744, 1682, 1531, 1422, 1358, 1206, 1128, 1085, 877 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.67 (br s, 2/3H), 7.41 – 7.16 (m, 10H), 6.99 (br s, 1/3H), 5.98 – 5.80 (m, 1H), 5.66 – 5.43 (m, 1H), 5.34 (dd, *J* = 16.8, 9.6 Hz, 2H), 5.13 (s, 2H), 4.71 – 4.33 (m, 5H), 3.76 – 3.45 (m, 3H), 3.15 (d, *J* = 7.5 Hz, 1H), 2.48 – 2.00 (m, 2H), 1.58 (s, 3H), 1.46 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 173.5, 167.4, 156.8, 136.2, 131.3, 131.1, 128.5, 128.1, 127.8, 119.7, 70.4, 69.9, 67.9, 67.5, 66.2, 58.8, 55.7, 54.4, 52.6, 39.7, 38.6, 36.8, 31.8, 30.3, 26.9. – HRMS: *m/z* = 504.1787 (calcd. 504.1804 for $C_{24}H_{30}N_3O_7S$, $[M+H]^+$).

*(2S,4R)-Benzyl 2-{(2S,5R,6R)-3,3-dimethyl-7-oxo-2-(2-phenylacetyl)-4-thia-1-aza-bicyclo[3.2.0]heptan-6-ylcarbamoyl}-4-hydroxypyrrolidine-1-carboxylate (**13**)*

Yield: 66 %. White solid. R_f = 0.4 (*n*-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – $[\alpha]_D^{24}$ = 130.9 (*c* = 0.26, $CHCl_3$). – IR (neat, NaCl): ν = 3342, 3065, 3034, 2958, 1785, 1746, 1694, 1519, 1420, 1357, 1205, 1127, 1084, 733 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.64 (br s, 2/3H), 7.46 – 7.13 (m, 10H), 6.91 (br s, 1/3H), 5.53 (d, *J* = 8.0 Hz, 1H), 5.15 (d, *J* = 13.2 Hz, 4H), 4.62 – 4.35 (m, 3H), 3.80 – 3.43 (m, 3H), 3.00 (dd, *J* = 7.0, 6.8 Hz, 1H), 2.49 – 2.01 (m, 2H), 1.55 (s, 3H), 1.38 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 173.3, 167.2, 156.7, 136.2, 134.9, 134.7, 128.8, 128.7, 128.5, 128.1, 127.8, 70.4, 69.9, 67.9, 67.5, 64.7, 58.8, 55.7, 54.4, 52.6, 39.7, 38.6, 36.8, 31.8,

30.3, 26.8. – HRMS: *m/z* = 554.1973 (calcd. 554.1961 for $C_{28}H_{32}N_3O_7S$, $[M+H]^+$).

*General procedure for the preparation of compounds **16, 17** via condensation reaction of acids **5, 6** and amino ester **15***

To a stirred suspension of acids **5**, **6** (1.0 mmol) in dry dichloromethane (15 mL) were added HOBt (1.1 mmol), EDCI (1.1 mmol), TEA (1.2 mmol) and aminoester **15** (1.0 mmol) at 5 °C. The mixture was stirred at r.t. for 16 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with sat'd. aqueous NH_4Cl solution (10 mL) and brine (12 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate: *n*-hexanes : methanol = 20 : 75 : 5, v/v) to give pure β-lactams **16**, **17**.

*(2S,5R,6R)-Methyl 6-[(S)-1-(benzyloxycarbonyl) pyrrolidine-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylate (**16**)*

Yield: 70 %. Semisolid. R_f = 0.4 (*n*-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – $[\alpha]_D^{26}$ = 91.1 (*c* = 0.20, $CHCl_3$). – IR (neat, NaCl): ν = 3345, 3013, 2956, 1785, 1750, 1698, 1531, 1421, 1358, 1214, 1125, 1084, 754 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.64 (br s, 2/3H), 7.43 – 7.15 (m, 10H), 6.91 (br s, 1/3H), 5.55 (d, *J* = 8.5 Hz, 2H), 5.14 (s, 2H), 4.55 (d, *J* = 22.8 Hz, 2H), 3.84 – 3.43 (m, 6H), 2.95 (br s, 1H), 2.50 – 1.94 (m, 2H), 1.58 (s, 3H), 1.44 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 173.5, 167.1, 156.7, 136.2, 128.6, 128.1, 127.8, 70.5, 67.9, 67.6, 65.8, 64.6, 58.8, 52.4, 39.7, 38.6, 36.8, 31.6, 26.9. – HRMS: *m/z* = 478.1657 (calcd. 478.1648 for $C_{22}H_{28}N_3O_7S$, $[M+H]^+$).

*(2S,5R,6R)-Methyl 6-[(2S,4R)-1-(benzyloxycarbonyl)-4-hydroxypyrrolidine-2-carboxamido]-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylate (**17**)*

Yield: 78 %. White solid. R_f = 0.4 (*n*-hexane : ethyl acetate : methanol = 65 : 30 : 5, v/v). – $[\alpha]_D^{26}$ = 144.3 (*c* = 0.20, $CHCl_3$). – IR (neat, NaCl): ν = 3300, 3018, 2957, 1785, 1751, 1699, 1586, 1416, 1357, 1301, 1123, 1089, 754 cm^{-1}. – ^1H NMR ($CDCl_3$, 500.14 MHz): δ = 7.66 (br s, 2/3H), 7.41 – 7.16 (m, 10H), 6.91 (br s, 1/3H), 5.56 (d, *J* = 44.2 Hz, 2H), 5.15 (s, 2H), 4.41 (d, *J* = 10.8 Hz, 2H), 3.74 (s, 3H), 3.61 – 3.34 (m, 3H), 2.45 – 2.31 (m, 1H), 2.20 – 2.06 (m, 1H), 1.91 (s, 2H), 1.57 (s, 3H), 1.43 (s, 3H). – ^{13}C NMR ($CDCl_3$, 125.67 MHz): δ = 173.5, 168.2, 156.7, 136.3, 128.6, 128.1, 127.9, 70.5, 67.9, 67.6, 65.8, 64.6, 58.8, 52.4, 39.7, 38.6, 36.8, 31.6, 26.9; 24.5. – HRMS: *m/z* = 462.1717 (calcd. 462.1699 for $C_{22}H_{28}N_3O_7S$, $[M+H]^+$).

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