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Coupling of sterically hindered aldehyde with fluorinated synthons: Stereoselective synthesis of fluorinated analogues of salinosporamide A

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ABSTRACT

Salinosporamide A is an irreversible inhibitor of the β-subunits of the 20S proteasome. Its C-5 cyclohexenyl moiety is the key to its affinity and potency as an anticancer agent. Here we describe the synthesis of C-5 difluoromethylated and trifluoromethylated analogues of salinosporamide A and their biological evaluation as proteasome inhibitors against purified yeast 20S proteasome. The synthetic strategy featured the stereoselective coupling reaction of sterically hindered aldehyde **3** with fluorinated organolithium reagents.

1. Introduction

Currently more than half of the pharmaceuticals in use are derived from natural products [1–3]. The synthesis of natural product analogues by removing suspected sites of toxicity or by introducing additional functionalities may enhance potency or stability of natural medicines [4,5]. In this regards, many fluorinated analogues of natural compounds have been synthesized due to the special properties of the fluorine atom, such as strong electronegativity, capacity to enhance metabolic stability, small size and low polarisability of the C–F bond [6]. From 1957, more than 150 fluorinated drugs have come into the market and now make up about 20% of all pharmaceuticals [7,8].

Salinosporamide A (**1**), isolated by Fenical and co-workers in 2003 from the marine bacterium *Salinispora tropica* [9], is a potent anticancer agent that recently entered phase I human clinical trials for the treatment of multiple myeloma and other cancers [10]. Because of its novel chemical structure and promising biological properties, many synthetic and biosynthetic studies have been reported towards it and its analogues [11]. One of such studies involved fluorine substitution at C-13 to replace the reactive chlorine substituent that acts as a leaving group during the drug's covalent attachment to the proteasome β-subunits [12]. In that case, fluorine substitution slows down salinosporamide's two-step irreversible binding reaction pathway and increases its residence time in complex with the 20S proteasome core particle [12,13]. While the C-2 chloroethyl group is key to salinosporamide A's irreversible binding mechanism, its C-5 cyclohexenyl ring is integral to salinosporamide A's binding affinity to the β5-subunit of the 20S proteasome [14,15]. In view that the hydrophobic property of the cyclohexenyl ring is important to the biological activity of salinosporamide A [14], we sought to replace the cyclohexenyl ring with fluorinated groups to probe its structure–activity relationship since fluorinated groups possess significant hydrophobicity. Herein we report the stereoselective synthesis of difluoromethylated and trifluoromethylated analogues of salinosporamide A, compounds **1A**, **1B** and **1C** (Fig. 1).

2. Results and discussion

The retrosynthetic analysis of analogues **1A**–**1C** is shown in Scheme 1. The key step is the stereoselective reaction of aldehyde **3** with fluorinated alkyl metal reagents, affording alcohol **4′**. **1A**–**1C** could be accomplished by Tamao-Fleming oxidation, ester hydrolysis, lactone formation, chlorination of primary alcohol and PMB cleavage from **4′**. Aldehyde **3** can be synthesized from *cis*-fusedlactam **2** [16].

Our synthesis began with L-threonine, from which silyl ether **2** was prepared in 12 steps [16]. Hydrogenation of benzyl ether **2** and followed by Dess-Martin periodinane oxidation provided the aldehyde **3** in 85% overall yield (Scheme 2).

With aldehyde **3** in hand, the coupling of **3** with 3-bromo-3,3-fluoropropene **17** was investigated [17–20]. Indium or zinc mediated coupling of **17** with general aldehyde proceeds smoothly

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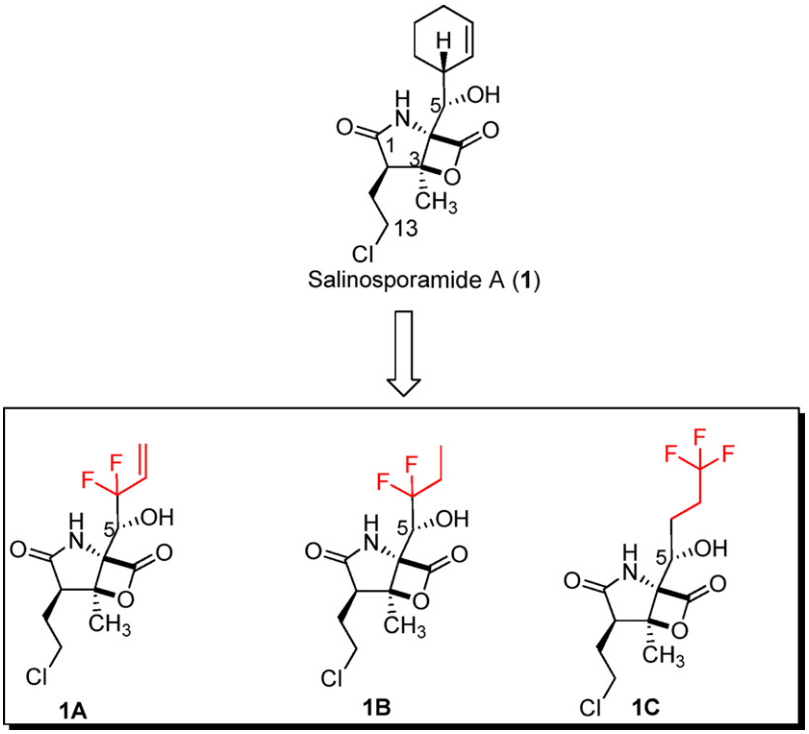
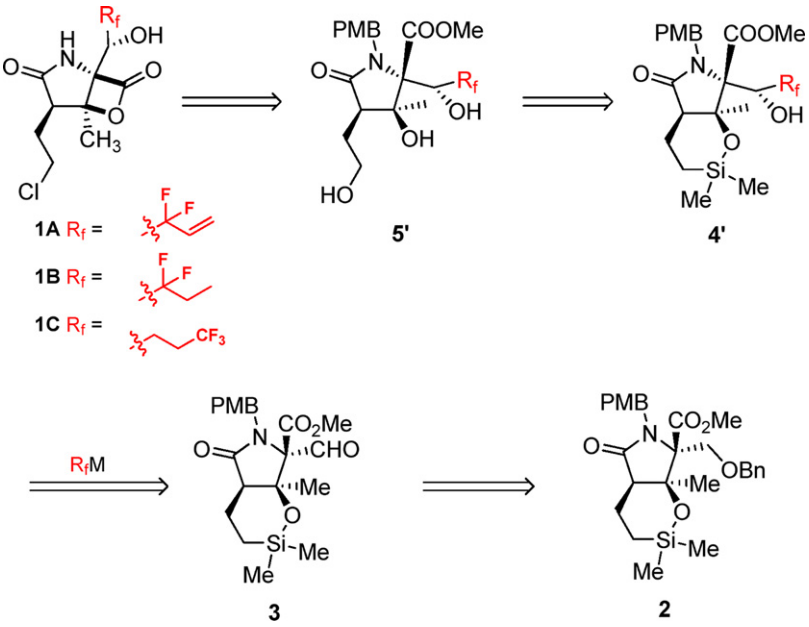


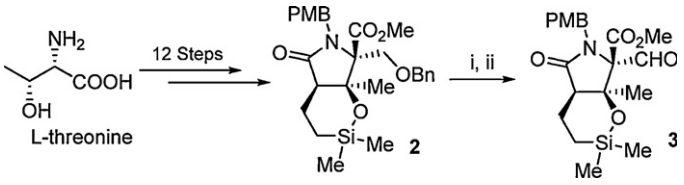
Fig. 1. Design of fluorinated analogues of salinosporamide A.



Scheme 1. Retrosynthetic analysis of target molecules.

to give an alcohol in high yield, but alcohol **4** could not be prepared by these easy handling methods from aldehyde **3** and **17** (Table 1, entries 1–4). This might be induced by the extreme steric hindrance of the aldehyde **3**. Thus a more reactive organolithium reagent, *gem*-difluoroallyllithium [21,22], was tested for the coupling reaction with aldehyde **3**. Two equiv. of *n*-butyllithium in *n*-hexane was added slowly to a mixture of **17** and aldehyde **3** in THF/Et_2O/pentane at −98 °C. To our delight, the *gem*-difluoroallyllithium in situ generated reacted with aldehyde **3** to give alcohol **4** was prepared in 50% yield (entry 5). The Diastereoisomer of alcohol **4** was not found, which meant the coupling of aldehyde **3** with organolithium is a stereoselective reaction. Furthermore, the yield of compound **4** was improved to 75% when 3.0 equiv. of both **17** and *n*-butyl lithium were used (entry 6).

Ring opening of silyl ether **4** with hydrogen peroxide and sodium hydrogen carbonate in a mixture of methanol and THF afforded triol **5** in 85% yield [23,24]. Accordingly, ester **5** was hydrolyzed to the corresponding lactam-carboxylic acid using 3 N lithium hydroxide in aqueous THF at 5 °C. The resulting crude acid was then treated with pyridine and bis-(2-oxo-3-oxazolidinyl) phosphinic chloride (BOPCl) at 23 °C under argon for 1 h to afford compound **7** in 52% yield in 2 steps. Chlorination of compound **7** with triphenylphosphine dichloride (Ph_3PCl_2) in anhydrous CH_3CN/pyridine (1:1) at 23 °C for 12 h provided **8** in 80% yield



Scheme 2. Synthesis of aldehyde **3**. *Reagents and conditions*: (i) H_2, Pd $(OH)_2$/C, THF; (ii) Dess-Martin periodinanes, CH_2Cl_2, 85%.

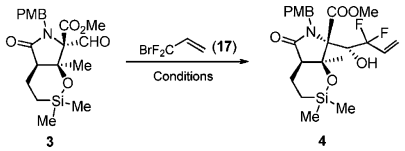
as a colourless oil. Finally, oxidative cleavage of the PMB group gave the target molecule **1A** in 40% yield (Scheme 3). Notably, if the PMB group was removed from **5**, the following hydrolyzation of the methyl ester **6** could not lead to desired acid and the starting material **6** was totally decomposed.

With the established synthetic route to **1A**, target molecules **1B** and **1C** were readily obtained from building blocks **9** and **12** correspondingly. As shown in Scheme 4, ester **9** was prepared from compound **5** by hydrogenation in the presence of $Pd(OH)_2$/C, and was eventually converted to target molecule **1B** by the same procedures for compound **5** as described in Scheme 3.

Building block **12** was synthesized stereoselectively by the coupling of aldehyde **3** with in situ generated 3,3,3-trifluoropropyllithium [25] in pentane at −78 °C (Scheme 5). As equivalent to compound **4** in Scheme 3, alcohol **12** was converted smoothly to target molecule **1C** by the same procedures.

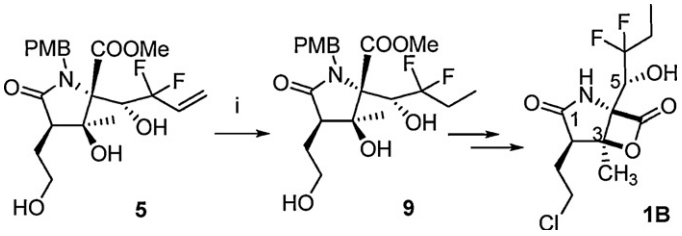
The absolute configuration of **12** at C-5 position was determined as *S* by the X-ray crystal structure (Fig. 2) [26], which is coincident with Cram–Felkin–Anh rule for the reaction of aldehydes with nucleophiles [27,28]. Therefore, the absolute configuration of target molecules**1A**, **1B** and **1C** at C-5 position could be determined as *R*, *R* and *S* correspondingly according to the absolute configuration of compound **12**, as the chiral centre at C-5 of all the compounds were generated by a similar stereoselective coupling of aldehyde **3** with organolithium reagents.

Table 1
Direct coupling of sterically hindered aldehyde 3 with *gem*-difluoroallyl synthon **17**.



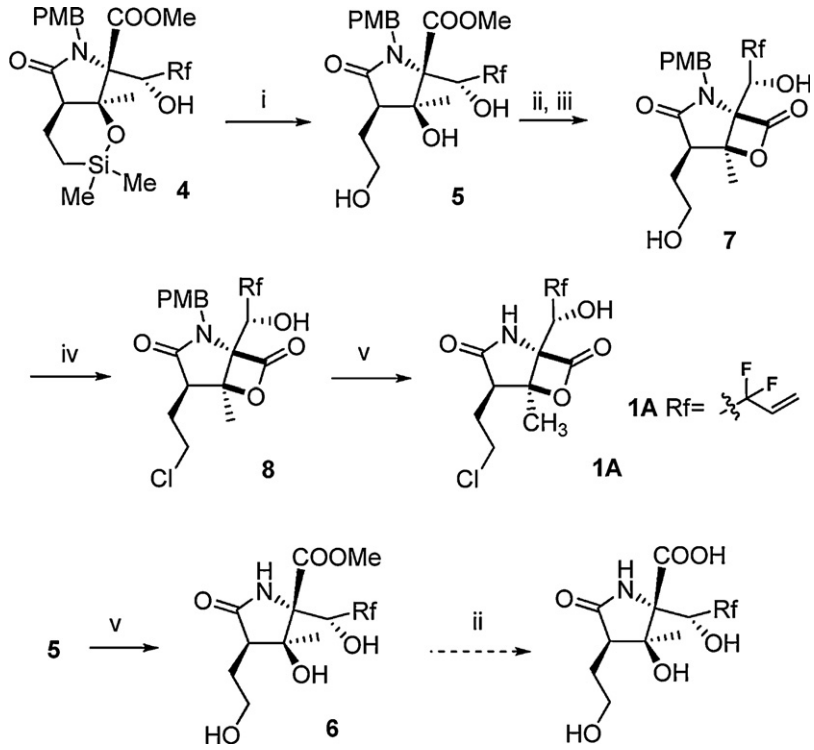
Entry	Metal	Solvent	Temp.	Yield[d]
1[a]	Zn (4.0 equiv.)	DMF	0 °C to rt	NR
2[a]	Zn (4.0 equiv.)	THF	0 °C to rt	NR
3[a]	In (4.0 equiv.)	DMF	0 °C to rt	NR
4[a]	In (4.0 equiv.)	THF:H_2O = 4:1	0 °C to rt	NR
5[b]	^{n}BuLi (2.0 equiv.)	THF:pentane:Et_2O = 10:1:1	−98 °C	50%
6[c]	^{n}BuLi (3.0 equiv.)	THF:pentane:Et_2O = 10:1:1	−98 °C	75%

[a] $BrCF_2CH{=}CH_2$ (4.0 equiv.).
[b] $BrCF_2CH{=}CH_2$ (2.0 equiv.).
[c] $BrCF_2CH{=}CH_2$ (3.0 equiv.).
[d] Isolated yield.

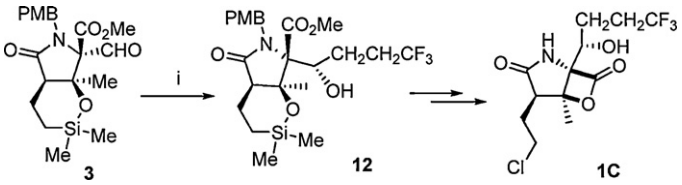


Scheme 4. Synthesis of target molecule **1B**. *Reagents and conditions*: (i) H_2, Pd $(OH)_2$/C, THF, 90%.

With the fluorinated analogues in hand, we next evaluated the biological activities of compounds **1A**, **1B** and **1C** in comparison with salinosporamide A in inhibition assays against the β5-subunit of the purified yeast 20S proteasome. The results are illustrated in



Scheme 3. Synthesis of target molecule **1A**. *Reagents and conditions*: (i) KF, $NaHCO_3$, H_2O_2, THF, MeOH, 85%; (ii) LiOH, THF, H_2O, 5 °C; (iii) BOPCl, CH_2Cl_2, Py; (iv) Ph_3PCl_2, CH_3CN, Py, 42% for 3 steps; (v) CAN, MeCN, H_2O, 0 °C, 1 h, 40%.



Scheme 5. Synthesis of target molecule **1C**. *Reagents and conditions*: (i) 3-bromo-1,1,1-trifluoropropane, ether, pentane, *tert*-butyllithium, −78 °C, 62%.

Table 2
IC_{50} for inhibition of chymotrypsin-like activity of the purified 20S proteasome of *Saccharomyces cerevisiae*.

Compound	Proteasome inhibition [nM]
Salinosporamide A (**1**)	0.80 ± 0.04
Difluorosalinosporamide (**1A**)	240 ± 21
Difluorosalinosporamide (**1B**)	94 ± 9
Trifluorosalinosporamide (**1C**)	220 ± 20

Table 2 and show >100-fold loss in activity as previously observed with aliphatic, straight chain C-5 salinosporamide derivatives [29]. Introduction of the fluorinated groups did not lead to any significant improvement in inhibitory activity in comparison to the biosynthtically prepared, non-fluorinated derivative of **1B** that had a reported 245 ± 38 nM IC_{50} value [29], as the *gem*-difluoromethylene at C-5 may increase the acidity of the vicinal hydroxyl group and subsequently decrease the hydrophobicity of the molecule. Therefore, fluorination of the salinosporamide C-5 side chain does not afford improved activity in this molecular series.

In summary, we accomplished the synthesis of three new fluorinated analogues of salinosporamide A. The key synthetic transformation involved the stereoselective coupling of aldehyde **3** with organolithium reagents for the introduction of fluorinated groups at the C-5 position.

3. Experimental

3.1. General description of materials and methods

All reactions were carried out using commercially available starting materials and solvents without further purification under an argon atmosphere. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled under argon with sodium/benzophenone ketyl and dichloromethane (CH_2Cl_2) with calcium hydride. Petroleum ether refers to the fraction of light petroleum ether with bp 60–90°C. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AM-300, Bruker AM-400 or Varian Mercury-300 spectrometers. ^{19}F NMR was recorded on a Bruker AM-300 spectrometer ($FCCl_3$ as outside standard and low field is positive). Chemical shifts (δ) are reported in parts per million, and coupling constants (J) are in hertz. Optical rotations were measured using a Perkin-Elmer 241 or 341 polarimeter. Crystallographic data were analyzed with Rigaku FCR Diffractimer. All melting points are uncorrected.

3.2. Preparation of (4aR,7R,7aS)-methyl7-((R)-2,2-difluoro-1-hydroxybut-3-en-1-yl)-6-(4-methoxybenzyl)-2,2,7a-trimethyl-5-oxooctahydro-[1,2]oxasilino[5,6-c]pyrrole-7-carboxylate (4)

A solution of **3** (230 mg, 0.57 mmol) and 3-bromo-3,3-difluoropropene (267 mg, 1.71 mmol) in THF/Pentane/Et_2O (6 mL/0.3 mL/0.3 mL) under nitrogen at −95 °C was added *n*-butyllithium slowly. After being stirred for 90 min, the reaction was quenched with water then extracted with ethyl acetate. The

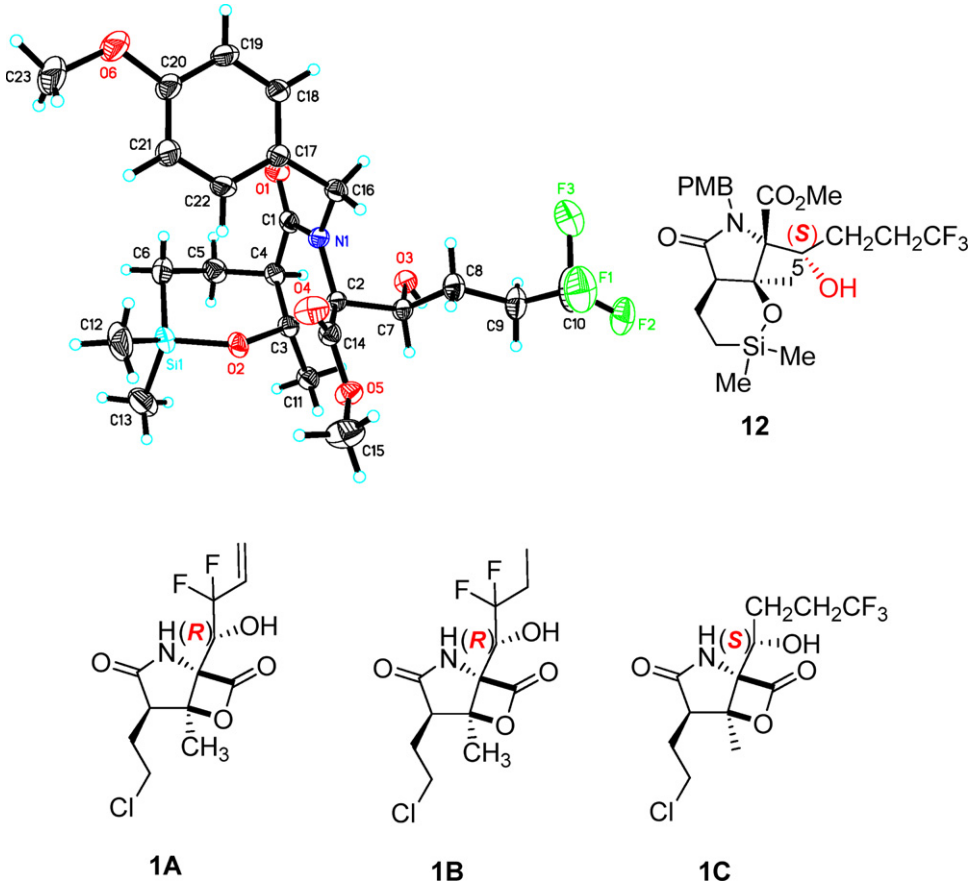


Fig. 2. The X-ray crystallographic structure of compound **12** and the absolute configuration of **1A**–**1C**.

combined solvents were evaporated by a rotary evaporator, and the obtained crude product was purified by column chromatography on silica gel (eluent:EtOAc/PE = 1:1) to afford compound **4** (207 mg, 75%) as a clear oil. $[\alpha]_D^{26} = -28.7$ (*c*1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.39 (br, 2H, ArH), 6.80 (d, *J* = 8.5 Hz, 2H, ArH), 6.09–5.91 (m, 1H, C*H*=CH_2), 5.64 (d, *J* = 17.3 Hz, 1H, CH=C*H*H), 5.49 (d, *J* = 10.9 Hz, 1H, CH=CH*H*), 4.76–4.43 (m, 3H, CF_2CHOH and NCH_2Ar), 4.17–4.14 (s, 1H, O*H*), 3.78 (s, 3H, ArOCH_3), 3.59 (br, 3H, $COOCH_3$), 2.79 (s, 1H, C*H*), 2.36–2.30 (m, 1H, C*H*HCH_2Si), 2.36–2.31 (m, 1H, CH*H*CH_2Si), 1.64 (s, 3H, CCH_3), 0.63–0.50 (m, 1H, CH_2C*H*HSi), 0.39–0.34 (m, 1H, CH_2CH*H*Si), 0.14 (s, 3H, CH_3Si CH_3), 0.05 (s, 3H, CH_3SiCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3 (CO), 158.1 (CAr), 131.4 (t, J_{C-F} = 25.4 Hz, *C*H=CH_2), 131.4 (CAr), 128.8 (CAr), 121.1 (t, J_{C-F} = 9.3 Hz, CH=CH_2), 121.1 (CAr), 119.0 (t, J_{C-F} = 246.5 Hz, CF_2), 113.1 (CAr), 84.2 (NCCOOMe), 79.2 (SiO*C*), 74.8 (t, J_{C-F} = 26.4 Hz, CF_2CHOH), 55.4 (ArOCH_3), 51.7 ($COOCH_3$), 48.3 (NCH_2), 48.0 (*C*H), 22.1 (CH_3), 17.3 ($SiCH_2CH_2$), 7.6 ($SiCH_2CH_2$), 0.2 ($SiCH_3$), 0.03 ($SiCH_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ −102.0 (br, 1F), −110.7 (d, *J* = 247.65 Hz, 1F); IR (thin film) ν_{max} 3323, 1865, 1680, 1514, 1053 cm^{-1}; MS (ESI) *m*/*z* 484.3 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{23}H_{31}F_2NO_6SiNa$: 506.1791, found 506.1781.

3.3. Preparation of (2R,3S,4R)-methyl2-((R)-2,2-difluoro-1-hydroxybut-3-en-1-yl)-3-hydroxy-4-(2-hydroxyethyl)-1-(4-methoxybenzyl)-3-methyl-5-oxopyrrolidine-2-carboxylate (5)

To a solution of **4** (280 mg, 0.145 mmol) in THF (0.4 mL) and MeOH (0.4 mL) at 23 °C was added $NaHCO_3$ (49 mg, 0.58 mmol) and KF (26 mg, 0.44 mmol). Hydrogen peroxide (30% in water, 0.4 mL) was then introduced to this mixture. The reaction mixture was vigorously stirred at 23 °C for 18 h, the reaction mixture was quenched carefully with $NaHSO_3$ solution. The mixture was extracted with ethyl acetate and the combined organic layers were washed with water and dried over Na_2SO_4. The solvents were evaporated by a rotary evaporator, and the resulting crude product was purified by column chromatography on silica gel (eluent:CH_2Cl_2/MeOH = 6:1) to yield **5** as a clear oil (200 mg, 85%). $[\alpha]_D^{26} = 11.0$ (*c*1.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (d, *J* = 7.3 Hz, 2H, ArH), 6.82 (d, *J* = 6.8 Hz, 2H, ArH), 6.02–5.89 (m, 1H, C*H*=CH_2), 5.66 (d, *J* = 17.4 Hz, 1H, CH=C*H*H), 5.51 (d, *J* = 10.9 Hz, 1H, CH=CH*H*), 4.70–4.41 (m, 3H, CF_2CHOH and NCH_2Ar), 4.32 (s, 1H, CF_2CHOH), 4.16 (s, 1H, O*H*), 3.77 (s, 3H, ArOCH_3), 3.77–3.77 (m, 2H, CH_2CH_2OH), 3.66 (s, 3H, $COOCH_3$), 3.11 (br, 1H, O*H*), 2.98–2.94 (m, 1H, C*H*), 1.88–1.72 (m, 2H, CH_2CH_2OH), 1.63 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.20 (CO), 168.41 (CO), 158.12 (CAr), 131.08 (t, J_{C-F} = 26.1 Hz, *C*H=CH_2), 130.58 (CAr), 128.29 (CAr), 122.34 (CAr), 121.41 (t, J_{C-F} = 9.5 Hz, CH=*C*H_2), 118.96 (t, J_{C-F} = 241.5 Hz, *C*F_2), 113.48 (CAr), 81.13 (NCCOOMe), 78.87 (HO*C*), 74.69 (t, J_{C-F} = 26.4 Hz, CF_2*C*HOH), 61.32 (CH_2CH_2OH), 55.36 (ArOCH_3), 52.25 ($COOCH_3$), 50.60 (NCH_2), 48.1 (*C*H), 26.88 (CH_2CH_2OH), 21.16 (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −102.5 (d, *J* = 249.23 Hz, 1F), −110.7 (ddd, *J* = 248.65, 19.36, 10.96 Hz, 1F); IR (thin film) ν_{max} 3339, 1763, 1672, 1514, 1038, 812 cm^{-1}; MS (ESI) *m*/*z* 444.1 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{21}H_{28}F_2NO_7$: 444.1839, found 444.1828.

3.4. Preparation of (2R,3S,4R)-methyl 2-((R)-2,2-difluoro-1-hydroxybut-3-en-1-yl)-3-hydroxy-4-(2-hydroxyethyl)-3-methyl-5-oxopyrrolidine-2-carboxylate (6)

To a solution of **5** (150 mg, 0.339 mmol) in acetonitrile (2.0 mL) at 0 °C was added a pre-cooled solution of ceric ammonium nitrate (CAN) (559 mg, 1.02 mmol in 0.7 mL H_2O). TLC analysis showed the complete consumption of starting material of **5**. The reaction mixture was diluted with ethyl acetate, washed with saturated NaCl solution and organic layers were dried over Na_2SO_4. The solvent was removed in vacuo to give the crude product which was purified by column chromatography on silica gel (eluent:ethyl acetate) giving the pure **6** (78 mg, 72%) as a white solid. $[\alpha]_D^{25} = 29.7$ (*c*0.8, MeOH); M.p. 207–208 °C. ^1H NMR (300 MHz, MeOD) δ 6.14–5.96 (m, 1H, CH=C*H*H), 5.66 (d, *J* = 17.3 Hz, 1H, CH=C*H*H), 5.55 (d, *J* = 11.0 Hz, 1H, CH=CH*H*), 4.39 (dd, *J* = 13.3, 7.0 Hz, 1H, CF_2CHOH), 3.74 (s, 3H, $COOCH_3$), 3.74–3.71 (m, 2H, CH_2CH_2OH), 2.86 (dd, *J* = 8.1, 4.9 Hz, 1H, C*H*), 1.90–1.69 (m, 2H, CH_2CH_2OH), 1.59 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, MeOD) δ 180.8 (CO), 170.8 (CO), 131.9 (t, J_{C-F} = 23.3 Hz, *C*H=CH_2), 121.5 (t, J_{C-F} = 7.5 Hz, CH=*C*H_2), 118.1 (t, J_{C-F} = 246.2 Hz, CF_2), 82.5 (NCCOOMe), 74.6 (CF_2CHOH), 61.9 (HO*C*), 52.6 (CH_2CH_2OH), 27.9 (CH_2CH_2OH), 20.4 (CH_3); ^{19}F NMR (282 MHz, MeOD) δ −101.3 (d, *J* = 253.31 Hz, 1F), −111.9 (d, *J* = 255.00 Hz, 1F); IR (thin film) ν_{max} 3316, 1737, 1676, 1421, 1042, 798 cm^{-1}; MS (ESI) *m*/*z* 324.1 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{13}H_{20}F_2NO_6$: 324.1265, found 324.1253.

3.5. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-1-((R)-2,2-difluoro-1-hydroxybut-3-en-1-yl)-2-(4-methoxybenzyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (8)

A solution of ester **5** (100 mg, 0.226 mmol) in 3 N aq LiOH (1.7 mL) and THF (0.58 mL) was stirred for 12 h until hydrolysis was complete. The reaction mixture was acidified with phosphoric acid (to pH 3.5). The solvent was removed in vacuo and the residue was extracted with EtOAc, separated, and concentrated in vacuo to give a crude carboxylic acid. The crude acid was suspended in dry CH_2Cl_2 (0.90 mL), treated with pyridine (0.28 mL) and stirred vigorously at 23 °C for 5 min. To this solution was added BOPCl (149 mg, 0.59 mmol) at 23 °C under argon, and stirring was continued for 3 h. The solvent was removed under high vacuum and the residue was suspended in dry CH_3CN (0.57 mL) and pyridine (0.57 mL). To this solution was added PPh_3Cl_2 (183 mg, 0.55 mmol) at 23 °C under argon with stirring. After 1 h the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (eluent:EtOAc/PE = 1:2.5) to yield **8** (38.6 mg, 42% for 3 steps) as a clear oil. $[\alpha]_D^{23} = 0.7$ (*c*0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.6 Hz, 2H, ArH), 6.82 (d, *J* = 8.6 Hz, 2H, ArH), 5.88–5.79 (m, 1H, C*H*=CH_2), 5.64 (d, *J* = 17.3 Hz, 1H, CH=C*H*H), 5.52 (d, *J* = 10.7 Hz, 1H, CH=CH*H*), 4.82 (d, *J* = 15.4 Hz, 1H, NC*H*HAr), 4.62 (dd, *J* = 18.9, 7.5 Hz, 1H, CF_2CHOH), 4.27 (d, *J* = 15.4 Hz, 1H, NCH*H*Ar), 4.30–4.25 (m, 1H, CH_2CHHCl), 3.87–3.82 (m, 1H, CH_2CH*H*Cl), 3.78 (s, 3H, ArOCH_3), 2.89 (dd, *J* = 8.1, 5.5 Hz, 1H, C*H*), 2.34–2.23 (m, 1H, C*H*HCH_2Cl), 2.16–2.04 (m, 1H, CH*H*CH_2Cl), 1.92 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1 (CO), 166.0 (CO), 159.3 (CAr), 130.0 (CAr), 129.6 (t, J_{C-F} = 24.9 Hz, *C*H=CH_2), 127.7 (CAr), 121.9 (t, J_{C-F} = 231.1 Hz, CH=*C*H_2), 121.60 (t, J_{C-F} = 9.6 Hz), 114.1 (CAr), 86.1 (NCCO), 80.0 (COO*C*), 68.41 (dd, J_{C-F} = 32.3, 28.2 Hz, CF_2*C*HOH), 55.2 (ArOCH_3), 45.4 (NCH_2), 44.9 (*C*H), 42.4 (CH_2CH_2Cl), 28.2 (CH_2CH_2Cl), 20.0 (d, J_{C-F} = 6.9 Hz, CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −103.5 (dt, *J* = 251.53, 12.70 Hz, 1F), −107.6 (dt, *J* = 254.07, 12.42 Hz, 1F); IR (thin film) ν_{max} 3392, 1834, 1682, 1514, 1034, 820 cm^{-1}; MS (ESI) *m*/*z* 430.1 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{22}H_{22}ClF_2NO_5Na$: 452.1059, found 452.1047.

3.6. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-1-((R)-2,2-difluoro-1-hydroxybut-3-en-1-yl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (1A)

Using the same conditions as described for compound **6**, compound **1A** (9 mg, 40%) was prepared as a white solid from compound **7** (40 mg, 0.093 mmol) (eluent:CH_2Cl_2/EtOAc = 10:1–4:1). $[\alpha]_D^{24} = -15.4$ (*c*0.5, MeOH); M.p. 145–147 °C. ^1H NMR (300 MHz, MeOD) δ 6.22–6.06 (m, 1H, C*H*=CH_2), 5.73 (d, *J* = 17.3 Hz, 1H, CH=C*H*H), 5.59 (d, *J* = 11.1 Hz, 1H, CH=CH*H*), 4.37

(dd, J = 16.8, 9.3, Hz, 1H, CF_2CHOH), 3.97–3.77 (m, 2H, CH_2CH_2Cl), 2.76 (d, J = 7.0 Hz, 1H, CH), 2.24–2.04 (m, 2H, CH_2CH_2Cl), 1.86 (s, 3H, CH_3); ^{13}C NMR (101 MHz, MeOD) δ 176.2 (CO), 166.8 (CO), 130.7 (t, J_{C-F} = 24.9 Hz, $CH{=}CH_2$), 120.2 (t, J_{C-F} = 10.6 Hz, $CH{=}CH_2$), 119.8 (t, J_{C-F} = 243.1 Hz, CF_2), 86.3 (NCCO), 76.7 (COOC), 68.5 (dd, J_{C-F} = 31.4, 27.2 Hz, CF_2CHOH), 45.3 (CH), 41.8 (CH_2CH_2Cl), 28.0 (CH_2CH_2Cl), 18.7 (d, J_{C-F} = 2.6 Hz, CH_3); ^{19}F NMR (282 MHz, MeOD) δ −104.6 (dt, J = 252.18, 11.58 Hz, 1F), −109.2 (dt, J = 251.05, 15.5 Hz, 1F); IR (thin film) ν_{max} 3358, 1834, 1710, 1421, 1082, 960, 831 cm^{-1}; MS (ESI) m/z 364.2 [M+MeOH+Na]$^+$; HRMS (ESI) Calcd. for $C_{13}H_{18}ClF_2NO_5$: 364.0743, found 364.0734.

3.7. Preparation of (2R,3S,4R)-methyl 2-((R)-2,2-difluoro-1-hydroxybutyl)-3-hydroxy-4-(2-hydroxyethyl)-1-(4-methoxybenzyl)-3-methyl-5-oxopyrrolidine-2-carboxylate (9)

A solution of **5** (170 mg, 0.384 mmol) in THF (5 mL) at 23 °C was treated with 20% $Pd(OH)_2$-C (90 mg). The mixture was treated with H_2 (1 atm, H_2 balloon) gas for 18 h. The resulting crude product was purified by column chromatography on silica gel (eluent:CH_2Cl_2/MeOH = 25:1) to **9** (158 mg, 90%) as a clear oil. $[\alpha]_D^{26}$ = 16.5 (c7.8, MeOH); M.p. 142–145 °C. ^{1}H NMR (300 MHz, MeOD) δ 7.27 (d, J = 8.2 Hz, 2H, ArH), 6.76 (d, J = 8.6 Hz, 2H, ArH), 4.60–4.50 (m, 3H, CF_2CHOH and NCH_2Ar), 3.71 (s, 5H, $ArOCH_3$ and CH_2CH_2OH), 3.58 (s, 3H, $COOCH_3$), 2.88 (t, J = 6.6 Hz, 1H, CH), 1.78–1.76 (m, 4H, $CF_2CH_2CH_3$ and CH_2CH_2OH), 1.63 (s, 3H, CCH_3), 1.00 (t, J = 7.4 Hz, 3H, $CF_2CH_2CH_3$); ^{13}C NMR (75 MHz, MeOD) δ 178.7 (CO), 168.7 (CO), 157.9 (CAr), 130.9 (CAr), 127.8 (CAr), 123.6 (t, J_{C-F} = 247.8 Hz, CF_2), 112.6 (CAr), 80.5 (NCCOOMe), 78.7 (HOC), 72.5 (t, J_{C-F} = 24.2 Hz, CF_2CHOH), 60.5 (CH_2CH_2OH), 54.4 ($ArOCH_3$), 50.8 ($COOCH_3$), 49.2 (NCH_2), 48.6 (CH), 28.2 (t, J_{C-F} = 21.3 Hz, $CF_2CH_2CH_3$), 27.4 (CH_2CH_2OH), 19.8 (CH_3), 4.9 (t, J_{C-F} = 4.8 Hz, $CF_2CH_2CH_3$); ^{19}F NMR (282 MHz, MeOD) δ −106.0 (br, 1F), −111.4 (d, J = 243.43 Hz, 1F); IR (thin film) ν_{max} 3399, 1763, 1670, 1514, 1040, 976, 831 cm^{-1}; MS (ESI) m/z 468.3 [M+Na]$^+$; HRMS (ESI) Calcd. for $C_{21}H_{29}F_2NO_7Na$: 468.1823, found 468.1804.

3.8. Preparation of (1R,4R,5S)-1-((R)-2,2-difluoro-1-hydroxybutyl)-4-(2-hydroxyethyl)-2-(4-methoxybenzyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (10)

A solution of triol ester **9** (75 mg, 0.167 mmol) in 3 N aq LiOH (1.3 mL) and THF (0.43 mL) was stirred for 12 h until hydrolysis was complete. The reaction mixture was acidified with phosphoric acid (to pH 3.5). The solvent was removed in vacuo and the residue was extracted with EtOAc, separated, and concentrated in vacuo to give the crude trihydroxy carboxylic acid. The crude acid was suspended in dry CH_2Cl_2 (0.67 mL), treated with pyridine (0.21 mL) and stirred vigorously at 23 °C for 5 min. To this solution was added BOPCl (110 mg, 0.44 mmol) at 23 °C under argon, and stirring was continued for 3 h. The solvent was removed under high vacuum and the crude product was purified by column chromatography on silica gel (eluent:EtOAc/PE = 2:3) to yield **10** (35.8 mg, 52% for 3 steps) as a clear oil. $[\alpha]_D^{23}$ = 4.5 (c1.0, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.4 Hz, 2H, ArH), 6.81 (d, J = 8.5 Hz, 2H, ArH), 4.90 (d, J = 15.0 Hz, 1H, $NCHHAr$), 4.74 (br, 1H, OH), 4.55 (d, J = 22.5 Hz, 1H, CF_2CHOH), 4.24 (d, J = 15.5 Hz, 1H, $NCHHAr$), 4.21 (br, 1H, OH), 3.97–3.81 (m, 2H, CH_2CH_2OH), 3.76 (s, 3H, $ArOCH_3$), 2.81 (t, J = 6.5 Hz, 1H, CH), 1.98–1.70 (m, 4H, $CF_2CH_2CH_3$ and CH_2CH_2OH), 1.86 (s, 3H, CCH_3), 0.97 (t, J = 7.4 Hz, 3H, $CF_2CH_2CH_3$); ^{13}C NMR (101 MHz, MeOD) δ 176.9 (CO), 167.7 (CO), 160.8 (CAr), 131.0 (CAr), 130.7 (CAr), 126.7 (t, J_{C-F} = 243.3 Hz, CF_2), 114.8 (CAr), 87.7 (NCCO), 82.0 (COOC), 67.7 (t, J_{C-F} = 28.0 Hz, CF_2CHOH), 60.4 (CH_2CHOH), 55.7 ($ArOCH_3$), 46.5 (NCH_2), 46.0 (d, J_{C-F} = 5.7 Hz, CH), 29.0 (CH_2CH_2OH), 28.5 (t, J_{C-F} = 24.6 Hz, $CF_2CH_2CH_3$), 20.5 (d, J_{C-F} = 10.1 Hz, CH_3), 5.8 (dd, J_{C-F} = 7.3, 5.1 Hz, $CF_2CH_2CH_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ −104.4 (dm, J = 250.77 Hz, 1F), −108.2 (dtd, J = 252.46, 19.77, 9.88 Hz, 1F); IR (thin film) ν_{max} 3379, 1832, 1678, 1514, 1040, 812 cm^{-1}; MS (ESI) m/z 436.1 [M+Na]$^+$; HRMS (ESI) Calcd. for $C_{20}H_{25}F_2NO_6Na$: 436.1545, found 436.1542.

3.9. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-1-((R)-2,2-difluoro-1-hydroxybutyl)-2-(4-methoxybenzyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (11)

Compound **10** (35.8 mg, 0.087 mmol) was suspended in dry CH_3CN (0.42 mL) and pyridine (0.42 mL). To this solution was added PPh_3Cl_2 (136 mg, 0.41 mmol) at 23 °C under argon with stirring. After 1 h the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (eluent:EtOAc/PE = 1:3) to yield **11** (30.7 mg, 82%) as a clear oil. $[\alpha]_D^{25}$ = 4.5 (c1.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (d, J = 8.4 Hz, 2H, ArH), 6.82 (d, J = 8.4 Hz, 2H, ArH), 4.83 (d, J = 15.5 Hz, 1H, $NCHHAr$), 4.54 (d, J = 22.2 Hz, 1H, CF_2CHOH), 4.27 (d, J = 15.5 Hz, 1H, $NCHHAr$), 4.05–3.97 (m, 1H, CH_2CHHOH), 3.87–3.81 (m, 2H, CH_2CHHOH and OH), 3.77 (s, 3H, $ArOCH_3$), 2.98–2.83 (m, 1H, CH), 2.34–2.23 (m, 1H, $CHHCH_2Cl$), 2.16–2.04 (m, 1H, $CHHCH_2Cl$), 1.90–1.65 (m, 2H, $CF_2CH_2CH_3$), 1.88 (s, 3H, CCH_3), 0.97 (t, J = 7.4 Hz, 3H, $CF_2CH_2CH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (CO), 167.0 (CO), 159.5 (CAr), 129.9 (CAr), 128.0 (CAr), 124.4 (dd, J_{C-F} = 249.3, 245.1 Hz, CF_2), 114.1 (CAr), 86.7 (NCCO), 80.5 (COOC), 67.5 (dd, J_{C-F} = 33.2, 26.3 Hz, CF_2CHOH), 55.4 ($ArOCH_3$), 45.3 (d, J_{C-F} = 4.2 Hz, NCH_2), 45.0 (d, CH), 42.6 (CH_2CH_2Cl), 28.4 (CH_2CH_2Cl), 27.9 (t, J_{C-F} = 23.6 Hz, $CF_2CH_2CH_3$), 19.9 (d, J_{C-F} = 8.3, Hz, CH_3), 5.6 (t, J_{C-F} = 5.6 Hz, $CF_2CH_2CH_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ −104.6 (dm, J = 251.05 Hz, 1F), −108.3 (dtd, J = 253.03, 22.87, 8.75 Hz, 1F); IR (thin film) ν_{max} 3397, 1834, 1681, 1515, 1040, 820 cm^{-1}; MS (ESI) m/z 432.1 [M+Na]$^+$; HRMS (ESI) Calcd. for $C_{20}H_{25}ClF_2NO_5$: 432.1395, found 432.1384.

3.10. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-1-((R)-2,2-difluoro-1-hydroxybutyl)-5-methyl-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (1B)

Using the same conditions as described for compound **1A**, compound **1B** (12 mg, 56%) was prepared from compound **11** (31 mg, 0.072 mmol) (eluent:CH_2Cl_2/EtOAc = 10:1–4:1). $[\alpha]_D^{27}$ = −19.3 (c0.40, MeOH); M.p. 148–150 °C. ^{1}H NMR (300 MHz, MeOD) δ 4.35 (dd, J = 20.7, 7.6 Hz, 1H, CF_2CHOH), 4.40–3.79 (m, 2H, CH_2CH_2Cl), 2.78 (t, J = 7.0 Hz, 1H, CH), 2.23–1.94 (m, 4H, $CF_2CH_2CH_3$ and CH_2CH_2Cl), 1.84 (s, 3H, CCH_3), 1.05 (t, J = 7.5 Hz, 3H, $CF_2CH_2CH_3$); ^{13}C NMR (75 MHz, MeOD) δ 177.7 (CO), 168.4 (CO), 126.1 (t, J_{C-F} = 246.5 Hz, CF_2), 87.7 (NCCO), 78.2 (COOC), 68.6 (dd, J_{C-F} = 31.8, 26.3 Hz, CF_2CHOH), 46.6 (CH), 43.2 (CH_2CH_2Cl), 29.4 (CH_2CH_2Cl), 28.3 (t, J_{C-F} = 25.0 Hz, $CF_2CH_2CH_3$), 20.0 (d, J_{C-F} = 4.2 Hz, CH_3), 5.9 (t, J_{C-F} = 6.9 Hz, $CF_2CH_2CH_3$); ^{19}F NMR (282 MHz, MeOD) δ −108.0 (dtd, J = 241.65, 18.91, 7.34 Hz, 1F), −111.9 (ddd, J = 248.99, 34.44, 18.91 Hz, 1F); IR (thin film) ν_{max} 3366, 1834, 1703, 1390, 985, 830 cm^{-1}; MS (ESI) m/z 366.1 [M+MeOH+Na]$^+$; HRMS (ESI) Calcd. for $C_{13}H_{20}ClF_2NO_5Na$: 366.0902, found 366.0890.

3.11. Preparation of (4aR,7R,7aS)-methyl 6-(4-methoxybenzyl)-2,2,7a-trimethyl-5-oxo-7-((S)-4,4,4-trifluoro-1-hydroxybutyl)octahydro-[1,2]oxasilino[5,6-c]pyrrole-7-carboxylate (12)

tert-Butyllithium (1.6 M in pentane, 2.2 mL) was slowly added at −78 °C to a stirred solution of 3-bromo-1,1,1-trifluoropropane (524.5 mg, 2.96 mmol) in ether (2 mL) and pentane (3 mL). The solution was then stirred 2 h at −78 °C. Then compound **3** (200 mg, 0.49 mmol) in ether (1.2 mL) was slowly added to the mixture.

After stirred at −78 °C for 3 h, the solution was quenched (NH_4Cl), extracted (EtOAc) and dried, then purified by silica gel column chromatography (eluent:EtOAc/PE = 1:3) to give **12** (152 mg, 62%) as a white solid. $[\alpha]_D^{25}$ = −19.2 (*c*1.2, $CHCl_3$); M.p. 67–69 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 7.33 (d, *J* = 8.4 Hz, 2H, ArH), 6.83 (d, *J* = 8.1 Hz, 2H, ArH), 4.76 (d, *J* = 15.0 Hz, 1H, NCH*H*Ar), 4.64 (d, *J* = 15.3 Hz, 1H, NC*H*HAr), 4.16 (t, *J* = 8.1 Hz, 1H, CF_3CH_2CHOH), 3.79 (s, 3H, ArOCH_3), 3.74–3.60 (br, 1H, OH), 3.67 (3H, s, COOCH_3), 2.17 (t, *J* = 3.0 Hz, 1H, C*H*), 2.35–152 (m, 6H, $CF_3CH_2CH_2$ and $SiCH_2CH_2$), 1.60 (3H, s, CCH_3), 0.59 (td, *J* = 14.4, 5.1, 1H, SiCH*H*CH_2), 0.41 (dt, *J* = 15.1, 3.7, 1H, SiC*H*HCH_2), 0.14 (3H, s, CH_3SiCH_3), 0.05 (3H, s, CH_3SiCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.8 (CO), 169.6 (CO), 158.6 (CAr), 131.2 (CAr), 128.7 (CAr), 125.7 (q, $J_{C\text{-}F}$ = 273.9 Hz), 113.8 (CAr), 83.1 (NCCOOMe), 82.9 (SiO*C*), 72.9 (CF_3CHOH), 55.4 (ArOCH_3), 51.8 (COOCH_3), 48.4 (NCH_2), 48.3 (*C*H), 31.5 (q, $J_{C\text{-}F}$ = 29.1 Hz, $CF_3CH_2CH_2$), 27.1 ($CF_3CH_2CH_2$), 22.5 (CCH_3), 17.0 ($SiCH_2CH_2$), 7.5 ($SiCH_2CH_2$), 0.3 ($SiCH_3$), 0.2 ($SiCH_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ −66.7 (t, *J* = 8.47 Hz, 3F); IR (thin film) ν_{max} 3359, 1757, 1676, 1514, 1026 cm^{-1}; MS (ESI) *m*/*z* 504.4 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{23}H_{32}F_3NO_6SiNa$: 526.1844, found 526.1843.

3.12. Preparation of (2R,3S,4R)-methyl 3-hydroxy-4-(2-hydroxyethyl)-1-(4-methoxybenzyl)-3-methyl-5-oxo-2- ((S)-4,4,4-trifluoro-1-hydroxybutyl)pyrrolidine-2-carboxylate (13)

Using the same conditions as described for compound **5**, compound **13** (92 mg, 77%) was prepared as a clear oil from compound **12** (130 mg, 0.26 mmol) (eluent:CH_2Cl_2/MeOH = 25:1). $[\alpha]_D^{24}$ = 4.4 (*c*1.6, MeOH); ^1H NMR (300 MHz, MeOD): δ 7.30 (d, *J* = 8.6 Hz, 2H, ArH), 6.80 (d, *J* = 8.6 Hz, 2H, ArH), 4.70 (d, *J* = 15.9 Hz, 1H, NCH*H*Ar), 4.57 (d, *J* = 15.9 Hz, 1H, NC*H*HAr), 4.27 (d, *J* = 10.2 Hz, 1H, CF_3 CH_2 CH_2CHOH), 3.76–3.72 (m, 2H, CH_2CH_2OH), 3.74 (s, 3H, ArOCH_3), 3.72 (s, 3H, COOCH_3), 2.89 (dd, *J* = 7.5, 5.5 Hz, 1H, C*H*), 2.36–2.03 (m, 2H, $CF_3CH_2CH_2$), 1.86–1.72 (m, 2H, CH_2CH_2OH), 1.68–1.41 (m, 2H, $CF_3CH_2CH_2$), 1.61 (s, 3H, CCH_3); ^{13}C NMR (101 MHz, MeOD) δ 180.2 (CO), 171.0 (CO), 159.7 (CAr), 128.6 (q, $J_{C\text{-}F}$ = 275.9 Hz, CF_2), 114.3 (CAr), 83.8 (NCCOOMe), 80.6 (COH), 72.7 ($CF_3CH_2CH_2CH$OH), 61.8 (CH_2CH_2OH), 55.7 (ArOCH_3), 52.3 (COOCH_3), 50.5 (NCH_2), 49.2 (*C*H), 32.0 (q, $J_{C\text{-}F}$ = 29.1 Hz, $CF_3CH_2CH_2$), 28.3 (CH_2CH_2OH), 28.0 ($CF_3CH_2CH_2$), 21.3 (CCH_3); ^{19}F NMR (282 MHz, MeOD) δ −66.2 (t, *J* = 9.88 Hz, 3F); IR (thin film) ν_{max} 3361, 1755, 1673, 1515, 1037, 815 cm^{-1}; MS (ESI) *m*/*z* 464.3 $[M+H]^+$; HRMS (ESI) Calcd. for $C_{21}H_{29}F_3NO_7$: 464.1889, found 464.1891.

3.13. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-2-(4-methoxybenzyl)-5-methyl-1-((S)-4,4,4-trifluoro-1-hydroxybutyl)-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (15)

Using the same conditions as described for compound **8**, compound **15** (16 mg, 37%) was prepared as a clear oil from compound **13** (40 mg, 0.09 mmol) (eluent:EtOAc/PE = 1:1). $[\alpha]_D^{25}$ = −61.8 (*c*0.75, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.21 (d, *J* = 8.5 Hz, 2H, ArH), 6.86 (d, *J* = 8.6 Hz, 2H, ArH), 5.11 (d, *J* = 15.3 Hz, 1H, NC*H*HAr), 4.13–4.04 (m, 2H, CH_2CHHCl and CF_3 CH_2CH_2CHOH), 4.00 (d, *J* = 15.2 Hz, 1H, NCH*H*Ar), 3.87–3.81 (m, 1H, CH_2CHHCl), 3.78 (s, 3H, ArOCH_3), 2.92 (dd, *J* = 7.8, 6.0 Hz, 1H, C*H*), 2.52 (d, *J* = 6.2 Hz, 1H, OH), 2.37–1.99 (m, 3H, $CF_3CH_2CH_2$ and C*H*HCH_2Cl), 1.89 (s, 3H, CCH_3), 1.48–1.38 (m, 1H, CH*H*CH_2Cl), 1.25–1.12 (m, 2H, $CF_3CH_2CH_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.3 (CO), 168.0 (CO), 160.0 (CAr), 129.5 (CAr), 128.8 (CAr), 126.5 (q, $J_{C\text{-}F}$ = 263.0 Hz, CF_2), 114.7 (CAr), 84.8 (NCCOOMe), 82.7 (COO*C*), 66.8 ($CF_3CH_2CH_2CH$OH), 55.5 (ArOCH_3), 45.5 (NCH_2), 45.1 (*C*H), 42.6 (CH_2CH_2Cl), 30.7 (q, $J_{C\text{-}F}$ = 29.4 Hz, $CF_3CH_2CH_2$), 28.3 (CH_2CH_2Cl), 22.7 (dd, $J_{C\text{-}F}$ = 5.5, 2.6 Hz, $CF_3CH_2CH_2$), 19.8 (CCH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −66.8 (t, *J* = 9.88 Hz, 3F); IR (thin film) ν_{max} 3224, 1826, 1687, 1514, 1024, 822 cm^{-1}; MS (ESI) *m*/*z* 504.2 $[M+MeOH+Na]^+$; HRMS (ESI) Calcd. for $C_{21}H_{27}ClF_3NO_6Na$: 504.1392, found 504.1371.

3.14. Preparation of (1R,4R,5S)-4-(2-chloroethyl)-5-methyl-1-((S)-4,4,4-trifluoro-1-hydroxybutyl)-6-oxa-2-azabicyclo[3.2.0]heptane-3,7-dione (1C)

Using the same conditions as described for compound **1A**, compound **1C** (10 mg, 68%) was prepared as white solid from compound **15** (20 mg, 0.045 mmol) (eluent:CH_2Cl_2/EtOAc = 10:1–4:1). $[\alpha]_D^{23}$ = −49.4 (*c*0.50, MeOH); M.p. 164–165 °C. ^1H NMR (300 MHz, MeOD): δ 4.15 (dd, *J* = 11.0, 2.4 Hz, 1H, $CF_3CH_2CH_2CH$OH), 3.95–3.77 (m, 2H, CH_2CH_2Cl), 2.83 (t, *J* = 7.1 Hz, 1H, C*H*), 2.50–1.93 (m, 5H, $CF_3CH_2CH_2$, CH_2CH_2Cl and CF_3CH_2CH*H*), 1.85–1.71 (m, 1H, CF_3CH_2CH*H*), 1.80 (s, 3H, CCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.1 (CO), 168.2 (CO), 127.4 (q, $J_{C\text{-}F}$ = 273.4 Hz, CF_2), 85.6 (NCCO), 78.5 (COO*C*), 64.5 ($CF_3CH_2CH_2CH$OH), 45.5 (*C*H), 41.2 (CH_2CH_2Cl), 29.9 (q, $J_{C\text{-}F}$ = 29.5 Hz, $CF_3CH_2CH_2$), 28.1 (CH_2CH_2Cl), 24.3 (t, $J_{C\text{-}F}$ = 3.3 Hz, $CF_3CH_2CH_2$), 18.1 (CCH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −68.3 (t, *J* = 11.00 Hz, 3F); IR (thin film) ν_{max} 3352, 1826, 1705, 1016, 809 cm^{-1}; MS (ESI) *m*/*z* 328.0 $[M-H]^-$; HRMS (ESI) Calcd. for $C_{12}H_{14}ClF_3NO_4$: 328.0560, found 328.0569.

3.15. 20S proteasome inhibition assays

Salinosporamide A and synthetic analogues **1A–1C** were assayed for inhibition of the *Saccharomyces cerevisiae* 20S proteasome β5-subunit as previously described except 0.5 g mL^{-1} proteasome was used [29]. Data were plotted on SigmaPlot 11.0 and fit with a "4 parameter logistic" curve to obtain IC_{50} values with standard errors.

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