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Boronate, Trifluoroborate, Sulfone, Sulfinate and Sulfonate Congeners of Oseltamivir Carboxylic Acid: Synthesis and Anti-influenza Activity

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Abstract:

Tamiflu readily undergoes endogenous hydrolysis to give oseltamivir carboxylic acid (OC) as the active anti-influenza agent to inhibit the viral neuraminidase (NA). GOC is derived from OC by replacing the 5-amino group with a guanidino group. In this study, OC and GOC congeners with the carboxylic acid bioisosteres of boronic acid, trifluoroborate, sulfone, sulfinic acid, sulfonic acid and sulfonate ester were first synthesized, starting with conversion of OC to a Barton ester, followed by halodecarboxylation to give the iodocyclohexene, which served as a pivotal intermediate for palladium-catalyzed coupling reactions with appropriate diboron and thiol reagents. The enzymatic and cell-based assays indicated that the GOC congeners consistently displayed better NA inhibition and anti-influenza activity than the corresponding OC congeners. The GOC sulfonic acid congener (**7a**) was the most potent anti-influenza agent, showing EC_{50} = 2.2 nM against the wild-type H1N1 virus, presumably because the sulfonic acid **7a** was more lipophilic than GOC and exerted stronger interactions on the three arginine residues (R118, R292 and R371) in the NA active site. Although the trifluoroborates, sulfones and sulfonate esters did not have acidic proton, they still exhibited appreciable NA inhibitory activity, indicating that the polarized B−F and S→O bonds still made sufficient interactions with the tri-arginine motif.

Keywords:

Influenza; Neuraminidase inhibitor; Oseltamivir; Bioisosteres; Sulfonic acid; Boronic acid; Trifluoroborate; Sulfone; Sulfinic acid.

Research highlights

• First synthesis of boronate and sulfonate related OC and GOC bioisosteres **2a**−**9b**.

• OC derived iodocyclohexene **16a** is a key intermediate for syntheses of bioisosteres.

• Acidity and lipophilicity of bioisosteres are important to anti-influenza activity.

• GOC congeners show better NA inhibition and anti-influenza activity than OC congeners.

• GOC sulfonic acid is a potent inhibitor against H1N1 virus (EC_{50} = 2.2 nM).

Abbreviations:

Boc, *tert*-butoxycarbonyl; $(BPin)_2$, bis(pinacolato)diboron; CCK, cholecystokinin; cLogD, calculated distribution coefficient; CPE, cytopathic effect; dba, dibenzylideneacetone; DMAP, (4-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; GABA, γ-aminobutyric acid; GOC, guanidino oseltamivir carboxylic acid; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; MDCK, Madin−Darby canine kidney; MUNANA, 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid; NA, neuraminidase; OC, oseltamivir carboxylic acid; SD, standard deviation; $TCID_{50}$,

50% cell culture infectious dose; Tf, trifluoromethanesulfonyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran; Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

1. Introduction

Influenza is a highly contagious airborne disease, which has become a persistent global public health issue; effective drugs are needed for treatment of influenza infection. Influenza neuraminidase (NA), a glycoprotein on the surface of the virus, is responsible for the enzymatic cleavage of the terminal sialic acid residue from the sialo-receptors on host cells to facilitate the release of progeny viruses for propagation and infectivity. NA is a good target for development of anti-influenza drug because NA is located on the virus surface, and its active site is conserved across various influenza viral strains. Several anti-influenza drugs, such as zanamivir, [1, 2] oseltamivir, [3, 4] peramivir [5, 6] and laninamivir octanoate, [7, 8] have been developed as NA targeting agents. Tamiflu (the phosphate salt of oseltamivir) is an oral prodrug, which is hydrolyzed by endogenous esterase to oseltamivir carboxylic acid (OC, **1a** in Fig. 1) as the active NA inhibitor. The cyclohexene core structure of OC is designed to mimic the oxocarbenium-like intermediate in the NA catalyzed hydrolysis of the sialoside. [9, 10] The carboxyl group of OC exhibits strong electrostatic interactions with the three arginine residues (R118, R292 and R371) in the S1 site of influenza NA. [1, 11] In addition, OC has a 3-pentoxy substituent at C-3, in lieu of the glycerol side chain in sialoside, to attain hydrophobic interactions with NA. GOC (**1b**), [3] the guanidino analog of OC, has a more basic guanidino group at C-5 to enhance the electrostatic interactions with the acidic residues (E119, D151 and E227) in the S2 site of NA. However, GOC and its ethyl ester have not been

developed for therapeutic use due to their poor pharmacokinetic properties.

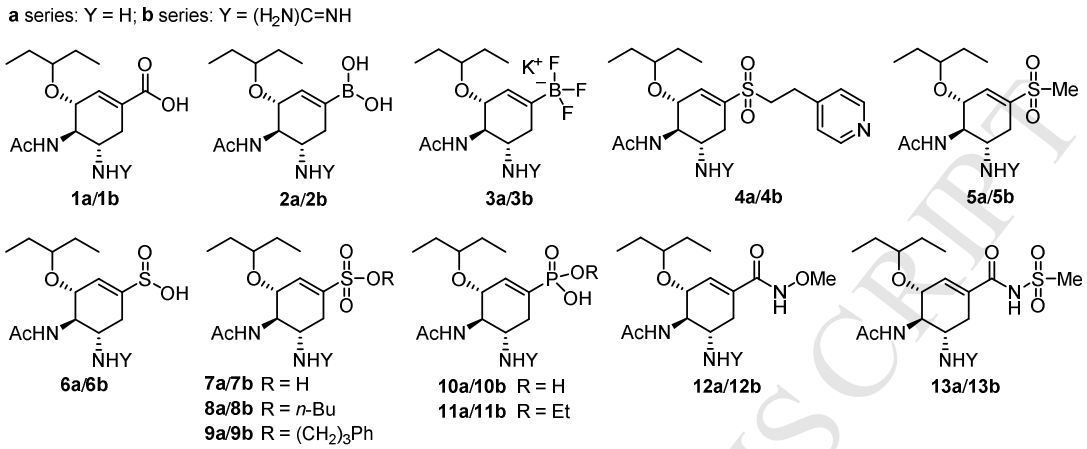


Fig. 1. Chemical structures of oseltamivir carboxylic acid (**1a**, OC), guanidino OC (**1b**, GOC), and their carboxyl bioisosteres (**2a**−**13b**).

Bioisosteres are substituents through rational modifications to mimic the structure of an active ingredient with similar chemical, physical, electronic, conformational and biological properties. [12, 13] As an approach to explore new anti-influenza drugs, we have previously developed the OC and GOC congeners by replacing the carboxylic acid with bioisosteres, [14] including phosphonic acid (**10a**/**10b**), [15−18] phosphonate monoester (**11a**/**11b**), [16, 18, 19] hydroxamate (**12a**/**12b**) [20] and acyl sulfonamide (**13a**/**13b**) [20]. Compounds **10a** (tamiphosphor) and **10b** are more potent NA inhibitors than their carboxyl congeners OC and GOC, because the phosphonate group exhibits stronger electronic interactions with the tri-arginine (R118, R292 and R371) motif in NA. [15] Phosphonate monoalkyl esters **11a** and

11b also exhibit high NA inhibitory activities because these compounds still contain a negatively charged group to render sufficient electrostatic interactions with the three arginine residues in the NA active site. [18, 19] The hydroxamate (**12a**/**12b**) and acyl sulfonamide (**13a**/**13b**) bioisosteres are less potent than OC and GOC, but still showed the IC_{50} values in nanomolar range against the NA of wild-type influenza virus (H1N1). [20]

Compared with carboxylic acid (pK_a ≈ 4.5), boronic acid is a weaker acid (pK_a ≈ 10.0) whereas sulfinic acid ((pK_a ≈ 2.0) and sulfonic acid (pK_a ≈ −0.5) are stronger acids. Use of boronic acid and sulfonic acid as the bioisosteres of carboxylic acid has been demonstrated in drug development. For example, bortezomib (velcade^{TM}) is a synthetic boronic acid drug used as protease inhibitor in cancer treatment. [21] In protein degradation, a specific peptide bond (−CONH−) of planar structure is attacked by a nucleophilic amino acid (e.g. serine) to form an intermediate of tetrahedral structure. Boron atom has an empty *p* orbital to accommodate nucleophile (e.g. water, alcohol, amine and thiol), thus becoming a negatively charged tetrahedral structure to mimic the intermediate during enzymatic degradation of peptide bond. The trifluoroborate salt with three electronegative fluorine atoms also exists in a tetrahedral structure to act as protease inhibitor. [22] In comparison with boronic acid and boronate ester, the corresponding trifluoroborate salts are usually easier to purify and preserve. In some cases, trifluoroborate salts are even more potent than boronic acid in inhibition of serine proteases. [22]

In an antagonist of cholecystokinin (CCK) receptor the carboxylic acid is replaced by sulfonic acid to give a dipeptoid hormone, which is more active and selective to CCK receptor. [23] As a surrogate of γ-aminobutyric acid (GABA), 3-aminopropylsulfonic acid also improves the activity to agonize $GABA_A$ receptor. [24] Some sulfo-sialic acid analogues are reported to have good inhibitory activity against influenza and bacterial neuraminidases. [25] Bren, Miertus and coworkers have applied molecular dynamics simulations in conjunction with a linear interaction energy method to predict that sulfonate **7a**, a putative compound in their publication, should be a very strong binder to N1 subtype neuraminidase. [26] The negatively charged sulfonate group of **7a** is considered to have great hydrogen bonding capacity to gain strong electrostatic interactions with the three arginine residues in the S1 site of NA.

As a continuing study to get a better picture for the relationship between the oseltamivir carboxylic acid and bioisosteres, we designed and synthesized new OC and GOC congeners (Fig. 1), including boronates **2a**/**2b**, trifluoroborates **3a**/**3b**, sulfones **4a**−**5b**, sulfinic acids **6a**/**6b**, sulfonic acids **7a**/**7b** as well as sulfonate esters **8a**/**8b** and **9a**/**9b**.

2. Results and discussions

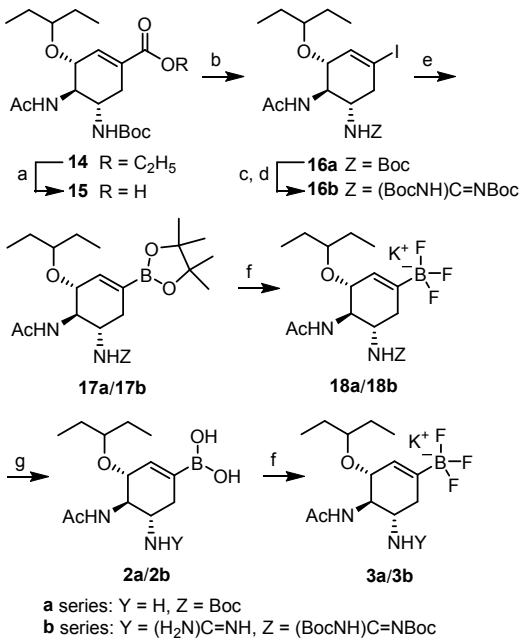
2.1. Chemical synthesis

Scheme 1 shows the synthesis of OC and GOC boronic acids (**2a**/**2b**) and trifluoroborates

(**3a**/**3b**). The *tert*-butoxycarbonyl (Boc) protected oseltamivir (**14**) was prepared by the previously reported procedure. [16, 19, 27] After saponification, the carboxylic acid product was reacted with 3-hydroxy-4-methyl-2(3*H*)-thiazolethione in the presence of HBTU, DMAP and Et_3N to give a Barton ester, which underwent photolysis in the presence of CF_3CH_2I to give the decarboxylative halogenation product **16a**. [16, 27−30] After testing several palladium catalysts, $Pd(PPh_3)_4$ was chosen for the Pd-catalyzed coupling reaction between the iodocyclohexene **16a** and bis(pinacolato)diboron to furnish the boronate ester **17a**. [31]

On the other hand, the Boc protecting group in **16a** was removed by trifluoroacetic acid (TFA), and the amine intermediate was treated with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine to give the guanidino derivative **16b**, which was subjected to the palladium-catalyzed boronation to afford compound **17b**. Attempts to hydrolyze boronate ester **17a** (or **17b**) in various conditions (e.g. using 1−3 M HCl solutions in Et_2O or 1,4-dioxane, BBr_3 in CH_2Cl_2, $NaIO_4$−HCl in THF−H_2O) failed to give the desired boronic acid **2a** (or **2b**). Alternatively, the boronate esters **17a** and **17b** were first treated with KHF_2 to give the trifluoroborate intermediates **18a** and **18b**, [22, 32−34] which were then successfully converted to the corresponding boronic acids **2a** and **2b** in 1 M aqueous HCl solution by concomitant removal of the Boc group. Treatment of **2a** and **2b** with KHF_2 afforded the trifluoroborates **3a** and **3b**, respectively.

Scheme 1. Synthesis of boronic acids **2a**/**2b** and trifluoroborates **3a**/**3b**

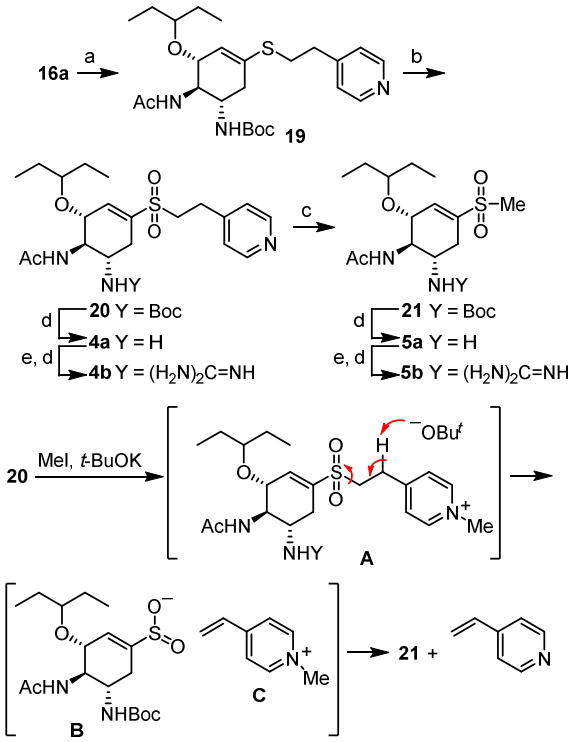


Reagents and conditions: (a) 1 M $KOH_{(aq)}$, MeOH, rt, 2 h; (b) 3-hydroxy-4-methyl-2(3*H*)-thiazolethione, HBTU, Et_3N, DMAP (cat.), CH_2Cl_2, rt, 3 h; then CF_3CH_2I, CH_2Cl_2, hv, 30 min; (c) CF_3COOH, CH_2Cl_2, rt, 3 h; (d) (BocNH)(TfNH)C=NBoc, NEt_3, CH_2Cl_2, rt, 10 h; (e) $(BPin)_2$, $Pd(PPh_3)_4$, KOAc, DMF, 90 ^oC, 4 h; (f) KHF_2, H_2O, MeOH, rt, 10 h; (g) 1 M HCl in H_2O, rt, 8 h. Yields: **15** (90% from oseltamivir), **16a** (54%), **16b** (72%), **2a** (48% from **16a**), **2b** (38% from **16b**), **3a** (72%), **3b** (67%). Boc = *tert*-butoxycarbonyl, $(BPin)_2$ = bis(pinacolato)diboron, DMAP = (4-dimethylamino)pyridine, HBTU = 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, rt = room temperature, Tf = trifluoromethanesulfonyl.

Using iodocyclohexene **16a** as the pivotal compound, OC and GOC sulfones (**4a**/**4b** and

5a/**5b**), sulfinic acids (**6a**/**6b**) and sulfonic acids (**7a**/**7b**) were synthesized. By the catalysis of $Pd_2(dba)_3$ and xantphos, [35] the iodine atom in **16a** was readily substituted by 2-(4-pyridyl)ethylthiolate to give the thioether **19**, which was then oxidized by *m*-chloroperbenzoic acid (*m*-CPBA) to give the sulfone product **20** (Scheme 2). Treatment of sulfone **20** with an alkylating agent CH_3I in the presence of a strong base *t*-BuOK produced methylsulfone **21**. The reaction possibly proceeded with a β-elimination that was promoted by prior methylation of the pyridine moiety, giving the intermediates of sulfinate ion **B** and 1-methyl-4-ethenylpyridium ion **C**. [36] The subsequent transfer of methyl group from the intermediate **C** to sulfinate **B** would yield the observed product **21**. On the other hand, removal of the Boc protecting group from **20** yielded compound **4a**. By the procedure similar to that for conversion of **2a** to **2b**, guanidination of **4a** and **5a** afforded **4b** and **5b**, respectively.

Scheme 2. Synthesis of sulfones **4a**/**4b** and **5a**/**5b**

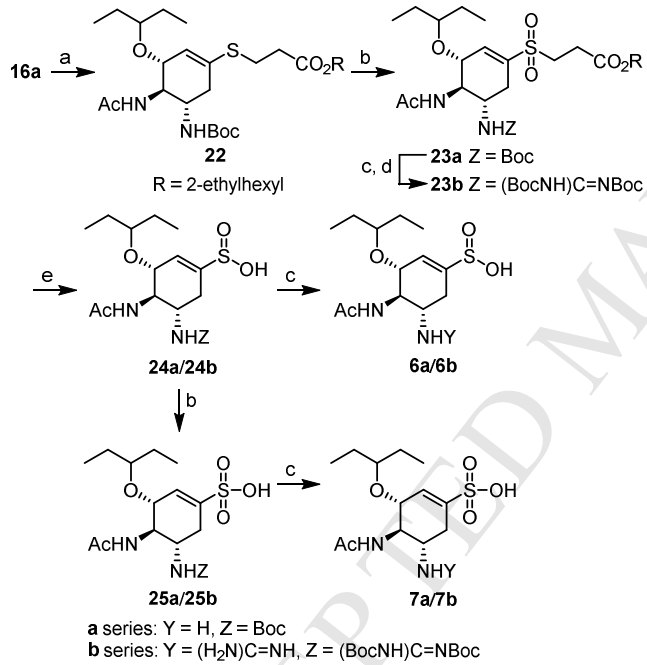


Reagents and conditions: (a) $HS(CH_2)_2Py$, $Pd_2(dba)_3$, xantphos, *i*-Pr_2NEt, DMF, 90 ^oC, 4 h; (b) *m*-$ClC_6H_4CO_3H$, CH_2Cl_2, 0 ^oC, 1 h; (c) MeI, *t*-BuOK, Me_2CO, H_2O, rt, 10 h; (d) CF_3CO_2H, CH_2Cl_2, rt, 3 h. (e) (BocNH)(TfNH)C=NBoc, NEt_3, CH_2Cl_2, rt, 10 h; Yields: **20** (62% from **16a**), **4a** (37%), **4b** (32%), **5a** (67% from **20**), **5b** (28%). dba = dibenzylideneacetone, DMF = *N*,*N*-dimethylformamide, xantphos = 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene.

The sulfone compound **23a** was obtained by the Pd-catalyzed coupling reaction of **16a** with 2-ethylhexyl 3-sulfanylpropanoate, followed by oxidation of the thioether intermediate **22** with *m*-CPBA (Scheme 3). The β-elimination reaction of **23a** occurred on treatment with *t*-BuOK to give the sulfinic acid **24a**, which was converted to the desired OC sulfinate bioisostere **6a** by removal of the Boc protecting group. Alternatively, oxidation of **24a** with

m-CPBA afforded the sulfonic acid **25a**, and the subsequent removal of Boc group gave the OC sulfonic acid **7a**. By the procedure similar to that for **6a** and **7a**, the Boc protecting groups of guanidine analogs **23b** and **25b** were removed to afford GOC sulfinic acid **6b** and GOC sulfonic acid **7b**.

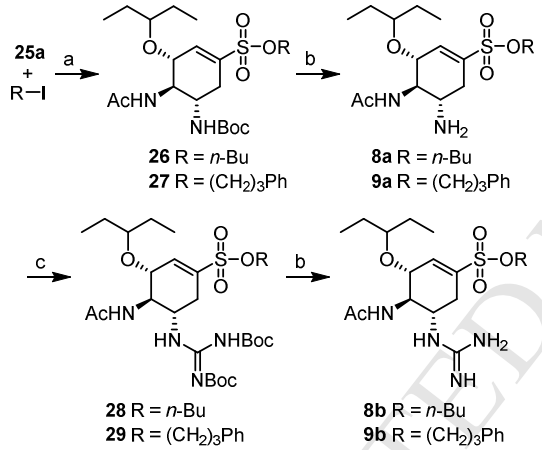
Scheme 3. Synthesis of sulfinic acids **6a**/**6b** and sulfonic acids **7a**/**7b**



Reagents and conditions: (a) $HS(CH_2)_2CO_2R$, $Pd_2(dba)_3$, xantphos, *i*-Pr_2NEt, DMF, 90 ^oC, 4 h; (b) *m*-$ClC_6H_4CO_3H$, CH_2Cl_2, 0 ^oC, 1 h; (c) CF_3CO_2H, CH_2Cl_2, rt, 3 h; (d) (BocNH)(TfNH)C=NBoc, NEt_3, CH_2Cl_2, rt, 10 h; (e) *t*-BuOK, Me_2CO, H_2O, rt, 10 h. Yields: **23a** (71% from **16a**), **23b** (72%), **6a** (61% from **23a**), **6b** (48% from **23b**), **7a** (47% from **23a**), **7b** (31% from **23b**).

The alkylation reactions of sulfonic acid **25a** with 1-iodobutane and 1-iodo-3-phenylpropane were carried out by using Ag_2O as the promoter to give the sulfonate esters **26** and **27**, respectively (Scheme 4). After removal of the Boc protecting group, the desired OC sulfonate esters **8a** and **9a** were obtained. The corresponding GOC sulfonate esters **8b** and **9b** were then prepared by guanidination of **8a** and **9a**, respectively.

Scheme 4. Synthesis of sulfonate esters **8a**–**9b**



Reagents and conditions: (a) Ag_2O, MeCN, 80 ^oC, 3 h; then *n*-BuI or $Ph(CH_2)_3I$, 80 ^oC, 10 h; (b) CF_3CO_2H, CH_2Cl_2, rt, 3 h; (c) (BocNH)(TfNH)C=NBoc, NEt_3, CH_2Cl_2, rt, 10 h; Yields: **26** (38%), **27** (45%), **28** (52%), **29** (65%), **8a** (29%), **8b** (60%), **9a** (25%), **9b** (58%).

2.2. Neuraminidase inhibition and anti-influenza activity

Table 1 shows the NA inhibition (IC_{50}) and anti-influenza activity (EC_{50}) against A/WSN/33 (H1N1) virus. The NA inhibitory activity was measured by using

2-(4-methylumbelliferyl)-α-D-*N*-acetyl-neuraminic acid (MUNANA) as the fluorogenic substrate. The anti-influenza activity was evaluated by the cytopathic effect of Madin-Darby canine kidney (MDCK) cells under viral infection. In general, GOC (**1b**) and other **b**-series compounds bearing a guanidino group at the C-5 position displayed better NA inhibition and anti-influenza activity than the corresponding OC (**1a**) and **a**-series compounds having a C-5 amino group. This result was attributable to the stronger interactions generated by the more basic guanidino group with the acidic residues (E119, D151 and E227) in NA active site.

In comparison with OC, the NA inhibitory activity of boronic acid **2a** dramatically decreased by 400 fold with a boronate group replacing the carboxyl group. Boronate **2a** was also 60-fold less effective than OC to protect MDCK cells from the infection of H1N1 viruses. However, by introduction of a C-5 guanidino substituent, compound **2b** regained the NA inhibition and anti-influenza activities, showing the IC_{50} and EC_{50} values of 28 and 580 nM, respectively. Furthermore, the trifluoroborate **3b**, with IC_{50} = 20 nM and EC_{50} = 270 nM, were more potent than boronate **2b**. This result indicated that the fluorine atoms having high electronegativity could render appreciable electrostatic interactions with the tri-arginine motif in the S1 site of NA. Since trifluoroborate compound tends to be more stable and easier in isolation than the corresponding boronate compound, it is worthwhile to exploit trifluoroborate as a general carboxyl bioisostere in drug discovery.

Because boron atom has an empty *p* orbital that may provide opportunity for covalent

bond formation. For this concern, we also incubated the boronate and trifluoroborate compounds (**2a**, **2b**, **3a** and **3b**) respectively with NA in various lengths of time (0, 0.5, 1, 2, 8, 16, 24, 42 and 48 h) to monitor the possibility of covalent modifications, which would result in irreversible inhibition and time-dependent shift in IC_{50} values. The NA inhibitory activities were measured, and the resulted IC_{50} values showed no shift with incubation time. Therefore, the inhibition of NA by compounds **2a**, **2b**, **3a** and **3b** may not proceed with an irreversible process caused by covalent bond formation.

Table 1. Neuraminidase inhibition (IC_{50}) and anti-influenza activity (EC_{50}) against A/WSN/33 (H1N1) virus[a]

Compound	IC_{50}[b] (nM)	EC_{50}[c] (nM)	CC_{50}[d] (μM)	cLogD[e]	pK_a[e]
1a (OC)	1.8 ± 0.6	28 ± 4	ND[f]	−1.84	4.4
1b (GOC)	1.0 ± 0.2	14 ± 4	ND[f]	−1.55	4.6
2a	730 ± 200	1700 ± 380	ND[f]	−1.75	10.0
2b	28 ± 2	580 ± 130	ND[f]	−2.48	10.0
3a	3400 ± 600	860 ± 730	ND[f]	NA[h]	NA[g]
3b	20 ± 6	270 ± 80	ND[f]	NA[h]	NA[g]
4a	330 ± 40	1500 ± 790	ND[f]	−0.93	NA[g]
4b	9.8 ± 4.5	100 ± 60	> 100	−2.56	NA[g]

5a	180 ± 10	1200 ± 250	ND[f]	−1.88	NA[g]
5b	2.4 ± 0.2	46 ± 13	> 100	−3.51	NA[g]
6a	22 ± 3	48 ± 13	> 100	−2.20	2.0
6b	2.2 ± 0.1	7.8 ± 2.5	> 100	−1.09	2.0
7a	1.8 ± 0.3	14 ± 3	> 100	−1.44	−0.5
7b	1.3 ± 0.1	2.2 ± 0.3	> 100	−1.15	−0.5
8a	27 ± 5	190 ± 100	ND[f]	−0.85	NA[g]
8b	3.3 ± 0.7	31 ± 4	> 100	−1.47	NA[g]
9a	45 ± 13	280 ± 30	ND[f]	1.55	NA[g]
9b	2.0 ± 0.1	24 ± 8	> 100	−0.33	NA[g]

[a] A/WSN/33 is a wild-type human H1N1 virus.

[b] A fluorescent substrate, 2-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA), was used to determine the IC_{50} values that are compound concentrations causing 50% inhibition of influenza neuraminidase. Data are shown as mean ± SD of three experiments.

[c] The anti-influenza activities against influenza virus were measured as EC_{50} values that are the compound concentrations for 50% protection of MDCK cytopathic effects due to the infection by influenza virus. Data are shown as mean ± SD of three experiments.

[d] The compound was nontoxic to human 293T cells (CC_{50} > 100 μM).

[e] The octanol−PBS distribution coefficient (cLogD) and acidity (pK_a) are calculated values

using MarvinSketch.

[f] ND: not determined (no cytotoxicity test was performed).

[g] NA: not available (cannot be calculated).

Based on molecular dynamics simulations, Bren and coworkers have proposed that the strength of electrostatic interactions provided by carboxylate ion and its bioisosteres might be correlated to their acidity. [26] The acidity in decreasing order is sulfonic acid (pK_a = −0.5), sulfinic acid (pK_a = 2.0), carboxylic acid (pK_a ≈ 4.5) and boronic acid (pK_a = 10.0). The sulfonic acid **7a** (EC_{50} = 14 nM) exhibited a higher anti-influenza activity than OC (EC_{50} = 28 nM), even they showed a similar NA inhibitory activity. Interestingly, compound **7a** was predicted to be more lipophilic than OC by comparing their distribution coefficients (cLogD in Table 1). Thus, compound **7a** might be more permeable to attain better effect in protecting cells from viral infection. Among the examined compounds, the GOC sulfonic acid bioisostere **7b** was the most potent anti-influenza agent with EC_{50} = 2.2 nM. The sulfinic acids **6a** and **6b** still possessed good NA inhibition and anti-influenza activities. The GOC sulfone congeners (**4b** & **5b**) as well as sulfonate esters (**8b** & **9b**) also inhibited influenza viral NA with IC_{50} in nanomolar range, indicating that the polarized S→O bonds could also provide sufficient electrostatic interactions with the tri-arginine motif. To our knowledge, there is no endogenous enzyme in mammal cells accountable for the hydrolysis of sulfonate

ester to sulfonic acid; thus sulfonate esters (e.g. **8b** & **9b**) may not act as the prodrugs of sulfonic acid (**7b**).

Lipophilicity is an important factor for the physicochemical properties and pharmacokinetic behavior of drugs. The partition of an ionic compound between octanol and PBS buffer (pH = 7.4) is represented as the distribution coefficient (LogD). Interestingly, Table 1 shows that compounds **1b** (GOC), **6b** and **7b** bearing a C5-guanidino group are predicted to be more lipophilic, with higher cLogD, than their corresponding compounds **1a** (OC), **6a** and **7a**. This result is in agreement with our previous observation in the phosphonate congeners **10a**/**10b** and **11a**/**11b**. [18] The increased lipophilic property of **1b**, **6b**, **7b**, **10b** and **11b** could be attributable to their zwitterionic structures, [37, 38] in which the C-5 guanidinium ion could pair with the carboxylate, sulfinate, sulfonate and phosphonate ions in either intra- or intermolecular manner. It is noted that Huryn, Ballatore and coworkers have previously found that phenylpropionic acid (LogD = −0.49) is more lipophilic than the corresponding sulfinic acid (LogD = −1.30) and sulfonic acid (LogD = −1.45) congeners. [39] In our case, compounds **1a**/**1b**, **6a**/**6b** and **7a**/**7b** may comprise zwitterionic structures in the presence of positively charged amino or guanidino substituents, which are absent in phenylpropionic acid. Since sulfonic acid is a stronger acid than carboxylic acid, congeners **7a**/**7b** will form the sulfonate−aminium/sulfonate−guanidinium ion-pairs more effectively than OC/GOC, thus ascribing to the higher lipophilicity of **7a**/**7b**.

3. Conclusion

In this study, we first synthesized the boronate, trifluoroborate, sulfone, sulfinate and sulfonate congeners of oseltamivir carboxylic acid and the guanidino derivatives. The synthesis started with elaboration of oseltamivir carboxylic acid (OC) to a Barton ester, followed by Hunsdiecker−Barton halodecarboxylation to give the iodocyclohexene **16a**, which served as the pivotal intermediate for the palladium-catalyzed coupling reactions with appropriate diboron and thiol reagents. The synthesized boronate, trifluoroborate, sulfone, sulfinate and sulfonate congeners of OC and its guanidino analogs were subject to influenza virus neuraminidase inhibition and cell-based anti-influenza assays. The GOC congeners (**b** series with C5-guanidino group) consistently showed better NA inhibition and anti-influenza activity than the corresponding OC congeners (**a** series with C5-amino group). Along with the interactions of the C5-amino (or guanidino), C-4 acetamide, and C3-pentoxy substituents in the S2–S5 sites of NA, the electrostatic interactions of carboxylate ion (or its bioisostere) with the three arginine residues (R118, R292 and R371) in the S1 site played an essential role in NA inhibition. The sulfonic acids **7a** and **7b** display high NA inhibitory activity, and **7b** is the most potent anti-influenza agent (EC_{50} = 2.2 nM) among the examined compounds. The order of NA inhibitory activity for sulfonic acids (**7a**/**7b**) > sulfinic acids (**6a**/**6b**) > boronic acids (**2a**/**2b**) seems to correlate with their strength of acidity; thus the sulfonic acid can make the

strongest interactions with the tri-arginine motif in the S1 site of NA. However, the acidity may not be the sole factor to be accounted for the affinity toward NA and the ability in virus suppression. For example, the NA inhibitory activity of the sulfinic acid congener **6a** (IC_{50} = 22 nM) is inferior to OC (IC_{50} = 1.8 nM), even though sulfinic acid (pK_a = 2.0) is a stronger acid than carboxylic acid (pK_a = 4.5). In another aspect, the sulfinic acid congener **6b** displays the anti-influenza activity comparable to GOC in the cell-based assay; even **6b** has lower NA inhibitory activity. This result is presumably related to the higher lipophilicity of **6b** for enhanced cell permeability. In agreement with this rationale, OC and the sulfonic acid congener **7a** have a similar NA inhibitory activity, but **7a** is more lipophilic to show a higher anti-influenza activity. Our study also indicates that trifluoroborate (**3b**), sulfones (**4a**/**4b**/**5a**/**5b**) and sulfonate esters (**8a**/**8b**/**9a**/**9b**) exhibit good NA inhibitory activity with IC_{50} values in nanomolar range, because the electronegative fluorine and oxygen atoms on the polarized B–F and S→O bonds can also make sufficient electrostatic interactions with the tri-arginine motif.

4. Experimental section

4.1. General part

All the reagents were commercially available and used without further purification unless indicated otherwise. All solvents were anhydrous grade unless indicated otherwise. Reactions

were magnetically stirred and monitored by thin-layer chromatography on silica gel. Flash chromatography was performed on silica gel (40–63 μm particle size) and LiChroprep RP-18 (40–63 μm particle size). Yields were reported for spectroscopically pure compounds. Melting points were recorded on a Yanaco melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity Plus-400 (400 MHz) spectrometer. ^{19}F NMR spectra were recorded on Varian Unity Plus-400 (400 MHz) spectrometer. Chemical shifts (δ) are given in parts per million (ppm) relative to residual signals of solvents as the internal standards: $CHCl_3$ (δ_H = 7.24), $CDCl_3$ (δ_C = 77.0 for the central line of triplet), CD_2HOD (δ_H = 3.31), CD_3OD (δ_C = 49.0), DHO (δ_H = 4.81), $(CH_3)_2SO$ (δ_H = 2.50) and $(CD_3)_2SO$ (δ_C = 39.5). Coupling constants (*J*) are given in hertz (Hz) and the splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad), and dd (doublet of doublets). IR spectra were recorded on Varian 640 or Nicolet Magna 500-II. Optical rotations were recorded on digital polarimeter of Japan JASCO Co. DIP-1000. $[\alpha]_D$ values were given in units of 10^{-1} deg cm^2g^{-1}. ESI mass spectra were recorded on a high-resolution mass spectrometer.

4.2. Materials

Influenza A/WSN/1933 (H1N1) was from Dr. Shin-Ru Shih at Chang Gung University in Taiwan. All viruses were cultured in the allantoic cavities of 10-day-old embryonated chicken

eggs for 72 h, and purified by sucrose gradient centrifugation. Madin–Darby canine kidney (MDCK) cells were obtained from American Type Culture Collection (Manassas, Va.), and were grown in DMEM (Dulbecco's modified Eagle medium, GibcoBRL) containing 10% fetal bovine serum (GibcoBRL) and penicillin-streptomycin (GibcoBRL) at 37 ^{o}C under 5% CO_2.

4.3. Determination of influenza virus $TCID_{50}$

The $TCID_{50}$ (50% tissue culture infectious dose) was determined by serial dilution of the influenza virus stock solution onto 100 μL MDCK cells at 1×10^5 cells/mL in 96-well microplates. The infected cells were incubated at 37 ^{o}C under 5.0% CO_2 for 48 h and added to each well with 100 μL of CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay reagent (Promega). After the incubation at 37 ^{o}C for 15 min, absorbance at 490 nm was read on a plate reader. Influenza virus $TCID_{50}$ was determined using Reed–Müench method. [40, 41]

4.4. Determination of neuraminidase activity by a fluorescent assay

The NA activity was measured by using diluted allantoic fluid harvested from influenza virus infected embryonated eggs or by recombinant neuraminidase proteins. A fluorometric assay was used to determine the NA activity with the fluorogenic substrate

2-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid (MUNANA; Sigma). The fluorescence of the released 4-methylumbelliferone was measured in Envision plate reader (Perkin-Elmer, Wellesley, MA) by using excitation and emission wavelength of 365 and 460 nm, respectively. Neuraminidase activity was determined at 200 μM of MUNANA (for diluted allantoic fluid) or at 5 μM of MUNANA (for recombinant influenza neuraminidases). Enzyme activity was expressed as the fluorescence increase during 15 min incubation at room temperature.

4.5. Determination of IC_{50} of neuraminidase inhibitors

Neuraminidase inhibition was determined by mixing inhibitor (0.02–83000 nM) and NA for 10 min at room temperature followed by the addition of substrate. The IC_{50} values were determined from the dose–response curves by plotting the percent inhibition of NA activity versus inhibitor concentrations using Graph Pad Prism 4.

4.6. Determination of EC_{50} and CC_{50} of neuraminidase inhibitors

The anti-influenza activities of neuraminidase inhibitors were measured by the EC_{50} values, which were the concentrations of NA inhibitor for 50% protection of the influenza virus infection-mediated cytopathic effects (CPE). Fifty microliters of diluted influenza virus at 100 $TCID_{50}$ was mixed with equal volumes of NA inhibitors at varied concentrations. The

mixtures were used to infect 100 μL of MDCK cells at 1×10^5 cells/mL in 96-wells. After 48 h of incubation at 37 ^oC under 5.0% CO_2, the cytopathic effects were determined with CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay reagent as described above. The EC_{50} values were determined by fitting the curve of percent CPE versus the concentrations of NA inhibitor using Graph Pad Prism 4. The CC_{50} values (50% cytotoxic concentrations) of NA inhibitor to MDCK cells were determined by the procedures similar to the EC_{50} determination but without virus infection.

4.7. Synthesis and characterization of compounds

4.7.1. Compound characterization

New compounds were characterized by their physical and spectroscopic properties (mp, TLC, [α], IR, ESI−MS, ^1H, ^{13}C and ^{11}B NMR). Purity of synthetic compounds was assessed to be ≥95% by HPLC analysis on an HC-C18 Agilent column (4.6 × 250 mm, 5 μm particle size) with the indicated eluent at a flow rate of 1 mL/min and detection at 254 nm wavelength.

4.7.2.

*4-Acetamido-5-(tert-butoxycarbonyl)amino-3-(1-ethylpropoxy)-1-cyclohexenecarboxylic acid (**15**) [19]*

To a mixture of oseltamivir (as the phosphate salt, 0.75 g, 1.83 mmol) and $NaHCO_3$ (0.77 g, 9.14 mmol) in THF/H_2O (10 mL/10 mL) was added di-*tert*-butyl dicarbonate [$(Boc)_2O$] (0.52 g, 2.38 mmol). The mixture was stirred for 2 h at room temperature, and concentrated under reduced pressure. The residue was diluted with CH_2Cl_2, and washed with water. The aqueous phase was extracted with CH_2Cl_2 (3 ×). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **14** (0.82 g), the Boc derivative of oseltamivir, which was used in the next step without further purification.

The above-prepared Boc derivative **14** (0.82 g) was stirred with KOH (3.5 mL, 1.0 M) in MeOH (6.6 mL, 0.3 M) at room temperature for 2 h, and then neutralized with Dowex 50W×8. The mixture was filtered, washed with MeOH, and the filtrate was concentrated under reduced pressure to give acid **15** (0.63 g, 90% yield from oseltamivir). $C_{19}H_{32}N_2O_6$; white solid; mp 217–219 ^oC; TLC (EtOAc/hexane, 2:1) R_f = 0.10; $[\alpha]_D^{23}$ –109.6 (*c* = 1, MeOH); IR (film) 3442, 3321, 2936, 1690, 1656, 1537, 1294, 1052, 1013 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.79 (1 H, s), 4.11 (1 H, d, *J* = 8.4 Hz), 3.88–3.83 (1 H, m), 3.75–3.68 (1 H, m), 3.41 (1 H, t, *J* = 5.6 Hz), 2.71–2.66 (1 H, m), 2.28–2.19 (1 H, m), 1.96 (3 H, s), 1.56–1.49 (4 H, m), 1.44 (9 H, s), 0.90–0.85 (6 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 168.1, 155.3, 135.0, 131.6, 81.0, 77.7, 75.4, 54.5, 49.3, 31.0, 28.4 (3 ×), 26.0, 25.4, 23.0, 9.6, 9.2; HRMS calcd for $C_{19}H_{33}N_2O_6$: 385.2339, found: *m/z* 385.2337 $[M + H]^+$.

*4.7.3. 4-Acetamido-5-(tert-butoxycarbonyl)amino-3-(1-ethylpropoxy)-1-iodo-1-cyclohexene (**16a**) [19]*

To a solution of carboxylic acid **15** (0.25 g, 0.65 mmol) in anhydrous CH_2Cl_2 (8 mL) were added Et_3N (0.14 mL, 0.98 mmol), HBTU (0.37 g, 0.98 mmol), and DMAP (catalytic amount). The mixture was stirred at room temperature for 30 min, and 3-hydroxy-4-methyl thiazolethione (0.13 g, 0.86 mmol) was added. The mixture was stirred for another 3 h at room temperature, and concentrated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (EtOAc/hexane, 1:1 to 2:1) to afford an intermediate Barton ester (0.3 g, 90% yield).

A solution containing the intermediate Barton ester (0.3 g, 0.58 mmol) and trifluoroethyl iodide (2 mL, 1.5 mmol) in anhydrous CH_2Cl_2 (0.3 M, 2 mL) was irradiated by 500 W lamp at room temperature for 0.5 h. The mixture was concentrated under reduced pressure, and purified by flash chromatography on a silica gel column (EtOAc/hexane, 1:3 to 1:1) to afford compound **16a** (164 mg, 0.35 mmol, 54% overall yield). $C_{18}H_{31}IN_2O_4$; pale yellow solid, mp 161–163 ^{o}C; TLC (EtOAc/hexane, 1:1) R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.31 (1 H, s), 5.61 (1 H, d, *J* = 16.4 Hz), 5.26 (1 H, d, *J* = 8.8 Hz), 4.08 (1 H, q, *J* = 9.6 Hz), 3.84–3.75 (2 H, m), 3.28 (1 H, t, *J* = 5.6 Hz), 2.86 (1 H, dd, *J* = 4.4, 5.8 Hz), 2.60 (1 H, dd, *J* = 1.7, 4.08 Hz), 1.98 (3 H, s), 1.54–1.41 (13 H, m), 0.90–0.85 (6 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 155.8, 138.0, 94.5, 82.0, 79.6, 77.5, 53.3, 50.5, 45.1, 28.4 (3 ×), 26.2, 25.8, 23.4, 9.7, 9.4;

HRMS calcd for $C_{18}H_{32}IN_2O_4$: 467.1407, found: *m/z* 467.1403 $[M + H]^+$.

4.7.4.

*(3R,4R,5S)-4-Acetamido-5-[N^2,N^3-bis(tert-butoxycarbonyl)guanidino]-3-(1-ethylpropoxy)-1-iodocyclohexene (**16b**)*

Trifluoroacetic acid (TFA, 0.03 mL, 0.4 mmol) was added to a solution of the iodo compound **16a** (300 mg, 0.32 mmol) in anhydrous CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 3 h, and then concentrated under reduced pressure to give an amine product as brown oil. The amine compound, 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (156 mg, 0.4 mmol) and NEt_3 (0.05 mL, 0.4 mmol) were dissolved in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 10 h, concentrated under reduced pressure, and purified by silica gel column chromatography (EtOAc/hexane = 2:1) to give the guanidination product **16b** (140 mg, 72% yield). $C_{24}H_{41}IN_4O_6$; colorless oil; $[\alpha]_D^{21}$ –18.0 (*c* = 1, CH_2Cl_2); IR (film) 3417, 2979, 2881, 2366, 1734, 1637, 1373, 1233, 1152 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.72 (1 H, d, *J* = 8.0 Hz), 6.28 (1 H, s), 6.21 (1 H, d, *J* = 8.8 Hz), 4.39 (1 H, quint, *J* = 8.0 Hz), 4.13–4.05 (1 H, m), 3.79 (1 H, br), 3.24 (1 H, quint, *J* = 5.6 Hz), 2.90 (1 H, dd, *J* = 18.0, 5.6 Hz), 2.70–2.63 (1 H, m), 1.90 (3 H, s), 1.45 (22 H, br), 0.87–0.80 (6 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 163.0, 156.4, 152.4, 137.8, 94.5, 83.4, 82.7, 79.7, 52.5, 48.9, 43.9, 28.2 (3 ×), 27.9 (3 ×), 26.0,

25.7 (2 ×), 23.1, 9.6, 9.3; HRMS calcd for $C_{24}H_{42}IN_4O_6$: 609.2141, found: *m/z* 609.2149 [M + H]$^+$.

*4.7.5. (3R,4R,5S)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexeneboronic acid (**2a**)*

Tetrakis(triphenylphosphine)palladium(0) (6 mg, 5 μmol) was added to a solution of iodo compound **16a** (47 mg, 0.1 mmol), KOAc (29 mg, 0.3 mmol), and bis(pinacolato)diboron (51 mg, 0.2 mmol) in anhydrous DMF (1 mL). The solution was heated to 90 ^{o}C and stirred for 4 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure to give a crude boronate ester **17a** as yellow oil. A solution of **17a** and potassium hydrogenfluoride (KHF_2, 31 mg, 0.4 mmol) in MeOH (2 mL) and H_2O (1 mL) was stirred for 10 h at room temperature, and then concentrated under reduced pressure to give white solids, which were dissolved in MeOH (5 mL) and filtered. The filtrate was concentrated under reduced pressure to give a crude trifluoroborate product **18a** as white solids. Compound **18a** was dissolved in 1 M HCl (2 mL), stirred for 8 h at room temperature, and concentrated under reduced pressure. The residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product of boronic acid **2a** (14 mg, 48% yield from **16a**). The purity of product **16a** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 μm particle size), t_R = 10.6 min (MeOH/H_2O = 3:7). $C_{13}H_{25}BN_2O_4$; hygroscopic solid; $[\alpha]_D^{23}$ –57.5 (*c* = 1, MeOH); IR (film) 3434, 2792, 2156,

1870, 1684, 1637, 1480, 1334, 1024, 867 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD)* δ 4.11 (1 H, d, *J* = 8.4 Hz), 3.92 (1 H, t, *J* = 9.6 Hz), 3.46–3.40 (1 H, m), 3.39–3.33 (1 H, m), 2.68 (1 H, dd, *J* = 17.2, 5.2 Hz), 2.26 (1 H, br), 2.04 (3 H, s), 1.58–1.46 (4 H, m), 0.98–0.87 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD)* δ 174.7, 83.6, 77.2, 54.9, 51.8, 27.3 (2 ×), 26.7, 23.2, 9.8, 9.7; ^{11}B NMR (128 MHz, CD_3OD) δ 28.3; HRMS calcd for $C_{13}H_{26}BN_2O_4$: 285.1986, found: *m/z* 285.1984 $[M + H]^+$.

*Note: The olefenic proton and carbons were not observed in the NMR spectra.

*4.7.6. (3R,4R,5S)-4-Acetamido-5-guanidino-3-(1-ethylpropoxy)-1-cyclohexeneboronic acid (**2b**)*

By a procedure similar to that for **2a**, the iodo compound **16b** (61 mg, 0.1 mmol) and bis(pinacolato)diboron (51 mg, 0.2 mmol) were treated with tetrakis(triphenylphosphine)palladium(0) (6 mg, 5 μmol) and KOAc (29 mg, 0.3 mmol)at 90 ^{o}C in anhydrous DMF (1 mL) for 4 h to give the coupling product of boronate ester **17b**. The solution of **17b** in MeOH (2 mL) and H_2O (1 mL) was treated with KHF_2 (31 mg, 0.4 mmol) to give trifluoroborate compound **18b**. Removal of the Boc protecting group in **18b** was performed in 1 M HCl (2 mL) at room temperature for 8 h to give the desired product of boronic acid **2b** (16 mg, 38% yield from **16b**) after purification on a reversed-phase RP-18 column ($MeOH/H_2O$ = 1:9 to 1:4). . The purity of product **2b** was > 99% as shown by HPLC

on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 μm particle size), t_R = 9.5 min (MeOH/H_2O = 3:7). $C_{14}H_{27}BN_4O_4$; hygroscopic solid; $[\alpha]_D^{23}$ –37.4 (*c* = 0.5, MeOH); IR (film) 3443, 2681, 2512, 2057, 1829, 1636, 1467, 1304, 1076, 978, 860 cm^{-1}; ^1H NMR (400 MHz, CD_3OD)* δ 4.09 (1 H, s), 3.88 (2 H, s), 3.67–3.60 (1 H, m), 2.64 (1 H, d, *J* = 16 Hz), 2.16–2.23 (1 H, m), 1.98 (3 H, s), 1.57–1.427 (4 H, m), 0.94–0.84 (6 H, m). ^{13}C NMR (100 MHz, DMSO-d_6)* δ 170.7, 154.6, 130.7, 81.4, 76.0, 55.1, 50.5, 26.2 (2 ×), 25.7, 23.0, 9.7, 9.5; ^{11}B NMR (128 MHz, DMSO-d_6) δ 30.4; HRMS calcd for $C_{14}H_{28}BN_4O_4$: 327.2204, found: *m/z* 327.2207 [M + H]$^+$.

*Note: The olefenic proton was not observed in the ^1H NMR spectrum, and one olefinic carbon was not observed in the ^{13}C NMR spectra.

*4.7.7. Potassium (3R,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-trifluoroborane (**3a**)*

A solution of boronic acid **2a** (14 mg, 0.05 mmol) and KHF_2 (15 mg, 0.2 mmol) in MeOH (2 mL) and H_2O (1 mL) was stirred at room temperature for 10 h. The mixture was concentrated under reduced pressure to give white solids, which were dissolved in MeOH (5 mL) and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product of trifluoroborate **3a** (12 mg, 72% yield). The purity of product **3a** was 95.8% as

shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 μm particle size), t_R = 9.9 min (MeOH/H_2O = 3:7). $C_{13}H_{23}BF_3KN_2O_2$; hygroscopic solid; $[\alpha]_D^{23}$ –50.4 (*c* = 1, MeOH); IR (film) 3423, 2932, 2886, 2365, 2331, 1631, 1561, 1391, 1118, 931, 744 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 5.75 (1 H, s), 3.97 (1 H, s), 3.95–3.89 (1 H, m), 3.41–3.34 (1 H, m), 3.28–3.22 (1 H, m), 2.59 (1 H, dd, *J* = 15.6, 8.8 Hz), 2.24 (1 H, dd, *J* = 14.4, 9.6 Hz), 2.02 (3 H, s), 1.54–1.50 (4 H, m), 0.93–0.86 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD)* δ 174.8, 122.2, 83.3, 76.8, 55.1, 52.5, 27.5 (2 ×), 27.4, 23.4, 9.94, 9.88; ^{11}B NMR (128 MHz, CD_3OD) δ 27.1; HRMS (negative mode) calcd for $C_{13}H_{23}BF_3N_2O_2$: 307.1805, found: *m/z* 307.1800 $[M - K]^-$.

*Note: One olefenic carbon was not observed in the ^{13}C NMR spectrum.

4.7.8. Potassium (3R,4R,5S)-4-acetamido-5-guanidino-3-(1-ethylpropoxy)-1-cyclohexene-trifluoroborane (3b)

A solution of boronic acid **2b** (16 mg, 0.05 mmol) and KHF_2 (15 mg, 0.2 mmol)in MeOH (2 mL) and H_2O (1 mL) was stirred at room temperature for 10 h. The mixture was concentrated under reduced pressure to give white solids, which were dissolved in MeOH (5 mL) and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product of trifluoroborate **3b** (14 mg, 67% yield). The purity of product **3b** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 μm particle size), t_R = 12.1

min (MeOH/H_2O = 3:7). $C_{14}H_{25}BF_3N_4O_2$; hygroscopic solid; $[\alpha]_D^{23}$ –20.9 (*c* = 0.5, MeOH); IR (film) 3432, 2973, 2384, 2068, 1181, 1632, 1286, 1205, 1065, 943 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 5.76 (1 H, s), 4.01 (2 H, s), 3.76 (1 H, s), 3.41 (1 H, s), 2.57 (1 H, dd, *J* = 16.8, 4.4 Hz), 2.18 (1 H, dd, *J* = 16.8, 8.8 Hz), 1.97 (3 H, s), 1.56–1.41 (4 H, m), 0.93–0.86 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD)* δ 173.7, 156.4, 128.0, 83.5, 78.5, 56.2, 53.2, 33.6, 27.5, 27.1, 23.0, 10.0, 9.8; ^{11}B NMR (128 MHz, CD_3OD) δ 22.2; HRMS (negative mode) calcd for $C_{14}H_{25}BF_3N_4O_2$: 349.2023, found: *m/z* 349.2036 $[M]^-$.

*Note: One olefenic carbon was not observed in the ^{13}C NMR spectrum.

4.7.9.

*(3R,4R,5S)-4-Acetamido-5-tert-butoxycarbonylamino-3-(1-ethylpropoxy)-1-(2-pyridin-4-yl)ethylsulfonyl-1-cyclohexene (**20**)*

A mixture of iodo compound **16a** (47 mg, 0.1 mmol), 4-(2-mercaptoethyl)pyridinium hydrochloride (21 mg, 0.12 mmol), xantphos (3 mg, 5 μmol), tris(dibenzylideneacetone)dipalladium(0) (5 mg, 5 μmol) and *N*,*N*-diisopropylethylamine (52 μL, 0.3 mmol) in anhydrous DMF (2 mL) was heated to 90 °C and stirred for 4 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (5 mL × 3) and H_2O (5 mL), and the organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by

silica gel column chromatography (EtOAc/hexane = 4:1) to give thioether **19** (38 mg, 80% yield) as a brown oil. The above-prepared compound **19** was dissolved in anhydrous CH_2Cl_2 (5 mL), followed by addition of *m*-chloroperbenzoic acid (*m*-CPBA, 38 mg, 2.2 mmol). The mixture was stirred at 0 ^oC for 1 h, and then washed by saturated $NaHCO_3$ (1 mL × 3). The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography (CH_2Cl_2/MeOH = 15:1) to give the sulfonyl compound **20** (32 mg, 78%). $C_{25}H_{39}N_3O_6S$; white solid; mp 191–194 ^oC; $[\alpha]_D^{24}$ –97.7 (*c* = 1, CH_2Cl_2); IR (film) 3291, 2971, 2935, 2879, 1686, 1654, 1603, 1533, 1457, 1367, 1311, 1168, 1121, 1082, 943, 807, 774, 605 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.50 (2 H, d, *J* = 6.0 Hz), 7.22 (2 H, d, *J* = 6.0 Hz), 6.72 (1 H, s), 6.45 (1 H, br), 6.54 (1 H, d, *J* = 8.4 Hz), 4.04 (1 H, quint, *J* = 8.4 Hz), 3.98 (1 H, d, *J* = 6.0 Hz), 3.87–3.79 (1 H, m), 3.35 (1 H, quint, *J* = 5.6 Hz), 3.23–3.19 (2 H, m), 3.07–2.94 (2 H, m), 2.78 (1 H, dd, *J* = 17.2, 4.8 Hz), 2.40 (1 H, dd, *J* = 17.2, 8.8 Hz), 1.94 (3 H, s), 1.53–1.42 (4 H, m), 1.35 (9 H, s), 0.84 (6 H, td, *J* = 7.2, 2.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 156.0, 150.1 (2 ×), 146.4, 138.9, 136.9, 123.7 (2 ×), 82.5, 79.8, 74.6, 53.2, 51.9, 48.7, 29.4, 28.2, 27.4 (3 ×), 25.9, 25.5, 23.1, 9.5, 9.1; HRMS calcd for $C_{25}H_{40}N_3O_6S$: 510.2638, found: *m/z* 510.2640 $[M + H]^+$.

4.7.10.

*(3R,4R,5S)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-(2-pyridin-4-yl)ethylsulfonyl-1-cycloh exene (**4a**)*

A solution of compound **20** (51 mg, 0.1 mmol) and TFA (77 µL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure and purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product **4a** (15 mg, 37% yield). The purity of product **4a** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 14.6 min (MeOH/H_2O = 3:7). $C_{20}H_{31}N_3O_4S$; hygroscopic solid; $[\alpha]_D^{24}$ –50.2 (*c* = 1, MeOH); IR (film) 3068, 2969, 2937, 2880, 1683, 1558, 1419, 1372, 1311, 1202, 1127, 939, 801, 721 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.50 (2 H, dd, *J* = 4.8, 1.6 Hz), 7.46 (2 H, d, *J* = 6.0 Hz), 6.77 (1 H, t, *J* = 2.4 Hz), 4.28 (1 H, d, *J* = 8.4 Hz), 3.94 (1 H, dd, *J* = 11.2, 8.4 Hz), 3.67–3.61 (1 H, m), 3.55–3.50 (2 H, m), 3.48–3.40 (1 H, m), 3.16 (2 H, t, *J* = 8.0 Hz), 2.98 (1 H, dd, *J* = 16.8, 5.6 Hz), 2.66–2.58 (1 H, m), 2.04 (3 H, s), 1.59–1.46 (4 H, m), 0.94–0.88 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 152.7 (2 ×), 128.7 (2 ×), 141.1, 137.0, 126.6, 84.2, 75.3, 54.2, 52.6, 50.5, 29.0, 28.9, 27.2, 26.7, 23.3, 10.0, 9.6; HRMS calcd for $C_{20}H_{32}N_3O_4S$: 410.2114, found: *m/z* 410.2112 $[M + H]^+$.

4.7.11.

*(3R,4R,5S)-4-Acetamido-3-(1-ethylpropoxy)-5-guanidino-1-(2-pyridin-4-yl)ethylsulfonyl-1-cyclohexene (**4b**)*

The guanidination of compound **4a** (41 mg, 0.1 mmol) was performed with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (47 mg, 0.12 mmol) in the presence of NEt_3 (0.041 mL, 0.3 mmol) in CH_2Cl_2 solution (1 mL). The mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane = 1:1) to give the Boc-protecting derivative (44 mg, 68% yield). $C_{31}H_{49}N_5O_8S$; colorless oil; $[\alpha]_D^{24}$ –67.3 (*c* = 1, CH_2Cl_2); IR (film) 3271, 2985, 2938, 2880, 1646, 1609, 1418, 1370, 1311, 1233, 1189, 1153, 1053, 948, 803, 610 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.64 (1 H, d, *J* = 8.0 Hz), 8.48 (2 H, d, *J* = 5.2 Hz), 7.17 (2 H, d, *J* = 5.6 Hz), 6.70 (1 H, s), 6.46 (1 H, d, *J* = 8.8 Hz), 4.31 (1 H, quint, *J* = 8.8 Hz), 4.07 (1 H, dd, *J* = 16.0, 5.6 Hz), 3.91 (1 H, d, *J* = 6.0 Hz), 3.33–3.17 (3 H, m), 3.10–3.00 (2 H, m), 2.88 (1 H, dd, *J* = 17.4, 5.2 Hz), 2.37 (1 H, dd, *J* = 17.4, 8.4 Hz), 1.90 (3 H, s), 1.49–1.41 (22 H, m), 0.84 (6 H, dt, *J* = 15.2, 7.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 162.9, 156.4, 152.5, 149.5 (2 ×), 147.0, 139.4, 136.3, 124.0 (2 ×), 83.7, 83.2, 79.8, 74.6, 52.8, 51.5, 47.7, 29.1, 28.1 (4 ×), 27.9 (3 ×), 25.6, 25.5, 22.9, 9.5, 9.1; HRMS calcd for $C_{31}H_{50}N_5O_8S$: 652.3380, found: *m/z* 652.3366 $[M + H]^+$.

The above-prepared Boc-protecting compound (44 mg) was treated with TFA (77 µL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) at room temperature for 3 h. The mixture was concentrated under reduced pressure, and purified on a reversed-phase RP-18 column (MeOH/ H_2O = 1:9 to 1:4) to give the desired product **4b** (21 mg, 47% yield). The purity of product **4b** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 11.7 min (MeOH/H_2O = 4:6). $C_{21}H_{33}N_5O_4S$; hygroscopic solid; $[\alpha]_D^{24}$ – 67.5 (*c* = 1, MeOH); IR (film) 3160, 2969, 2933, 2876, 1672, 1609, 1547, 1422, 1376, 1309, 1270, 1202, 1123, 1074, 992, 721 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.46 (2 H, dd, *J* = 4.8, 1.6 Hz), 7.39 (2 H, d, *J* = 6.4 Hz), 6.76 (1 H, t, *J* = 2.0 Hz), 4.32 (1 H, d, *J* = 8.0 Hz), 4.08–4.01 (1 H, m), 3.88 (1 H, dd, *J* = 16.8, 8.4 Hz), 3.52–3.48 (2 H, m), 3.43 (1 H, quint, *J* = 5.6 Hz), 3.14–3.10 (2 H, m), 2.94 (1 H, dd, *J* = 16.8, 4.8 Hz), 2.57–2.49 (1 H, m), 1.99 (3 H, s), 1.58–1.48 (4 H, m), 0.94–0.87 (6 H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 158.8, 150.4, 150.2 (2 ×), 140.7, 138.0, 125.9 (2 ×), 84.2, 75.7, 55.9, 52.7, 51.6, 30.7, 28.8, 27.3, 26.8, 23.0, 10.0, 9.7; HRMS calcd for $C_{21}H_{34}N_5O_4S$: 452.2332, found: *m/z* 452.2336 $[M + H]^+$.

*4.7.12. (3R,4R,5S)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-methylsulfonyl-1-cyclohexene (**5a**)*

A solution of compound **20** (51 mg, 0.1 mmol) and potassium *tert*-butoxide (17 mg, 0.15 mmol) in acetone (5 mL) and H_2O (1 mL) was stirred at room temperature for 10 min, followed by addition of iodomethane (19 µL, 0.3 mmol). The mixture was stirred at room temperature for 10 h, concentrated under reduced pressure, and extracted with EtOAc (5 mL × 3) and H_2O (5 mL). The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give methylsulfone **21** (35 mg, 84%).

A solution of **21** (35 mg) in CH_2Cl_2 solution (5 mL) was treated with TFA (77 µL, 1.0 mmol) at room temperature for 3 h. The mixture was concentrated under reduced pressure, and purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product **5a** (21 mg, 80% yield). The purity of product **5a** was 97.9% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 17.3 min (MeOH/H_2O = 3:7). $C_{14}H_{26}N_2O_4S$; hygroscopic solid; $[\alpha]_D^{22}$ –104.7 (*c* = 1, MeOH); IR (film) 3277, 2969, 2880, 1677, 1547, 1434, 1381, 1303, 1203, 1132, 960, 841 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.77 (1 H, s), 4.32 (1 H, d, *J* = 8.4 Hz), 4.01 (1 H, dd, *J* = 11.2, 5.6 Hz), 3.74–3.67 (1 H, m), 3,45 (1 H, quint, *J* = 5.6 Hz), 3.02 (3 H, s), 2.99 (1 H, d, *J* = 5.6 Hz), 2.71–2.62 (1 H, m), 2.04 (3 H, s), 1.49–1.48 (4 H, m), 0.98–0.86 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 175.0, 138.9, 138.4, 84.1, 75.2, 54.1, 50.5, 40.9, 28.7, 27.2, 26.7, 23.3, 10.0, 9.6; HRMS calcd for $C_{14}H_{27}N_2O_4S$: 319.1688, found: *m/z* 319.1692 $[M + H]^+$.

4.7.13.

(3R,4R,5S)-4-Acetamido-3-(1-ethylpropoxy)-5-guanidino-1-methylsulfonyl-1-cyclohexene

(5b)

The guanidination of compound **5a** (32 mg, 0.1 mmol) was performed with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (47 mg, 0.12 mmol) in the presence of NEt_3 (0.041 mL, 0.3 mmol) in CH_2Cl_2 solution (1 mL). The residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give a pale yellow oil, which was dissolved in CH_2Cl_2 (5 mL) and treated with TFA (77 µL, 1.0 mmol) at room temperature for 3 h to give the desired product **5b** (13 mg, 28% yield) after purification on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4). The purity of **5b** was 95.4% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 19.6 min (MeOH/H_2O = 3:7). $C_{15}H_{28}N_4O_4S$; hygroscopic solid; $[\alpha]_D^{22}$ –47.6 (*c* = 1, MeOH); IR (film) 3352, 3189, 2979, 2880, 1701, 1684, 1532, 1374, 1299, 1199, 1123, 1083, 832 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.76 (1 H, s), 4.29 (1 H, d, *J* = 6.4 Hz), 4.06–3.99 (1 H, m), 3.92 (1 H, dd, *J* = 10.4, 5.2 Hz), 3.43 (1 H, quint, *J* = 5.6 Hz), 3.01 (3 H, s), 2.93 (1 H, dd, *J* = 17.2, 8.4 Hz), 2.60–2.52 (1 H, m), 1.99 (3 H, s), 1.58–1.46 (4 H, m), 0.93–0.87 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 158.8, 139.5, 138.4, 84.1, 75.6, 55.6, 51.4, 40.9, 30.4,

27.2, 26.9, 23.0, 10.0, 9.7; HRMS calcd for $C_{15}H_{29}N_4O_4S$: 361.1909, found: *m/z* 361.1910 [M + H]$^+$.

*4.7.14. 2-Ethylhexyl (3R,4R,5S)-3-[4-(acetamido-5-tert-butoxycarbonylamino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonylpropanoate (**23a**)*

A mixture of iodo compound **16a** (47 mg, 0.1 mmol), 2-ethylhexyl 3-sulfanylpropanoate (26 mg, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0) (5 mg, 5 µmol), xantphos (3 mg, 5 µmol) and *N*,*N*-diisopropylethylamine (52 µL, 0.3 mmol) in anhydrous DMF (2 mL) was heated to 90 ^{o}C and stirred for 4 h. The mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was extracted with CH_2Cl_2 (5 mL × 3) and H_2O (5 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure, and purified by silica gel column chromatography (EtOAc/hexane = 1:1) to give the thioether **22** (48 mg, 86% yield) as a brown oil.

The above-prepared compound **22** (48 mg) was dissolved in anhydrous CH_2Cl_2 (5 mL), and treated with *m*-CPBA (38 mg, 2.2 mmol) for 1 h at 0 ^{o}C. The mixture was washed with saturated $NaHCO_3$ (1 mL × 3). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1:1) to give **23a** (42 mg, 83% yield). $C_{29}H_{52}N_2O_8S$; white

solid; mp 165–168 °C; $[\alpha]_D^{24}$ –60.5 (*c* = 1, CH_2Cl_2); IR (film) 3324, 2963, 2933, 2875, 1741, 1685, 1655, 1534, 1460, 1369, 1314, 1296, 1253, 1177, 1118, 1087, 1023, 939, 868, 729, 607 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.72 (1 H, s), 6.23 (1 H, d, *J* = 9.2 Hz), 5.45 (1 H, d, *J* = 8.8 Hz), 4.15–4.03 (1 H, m), 4.03–3.94 (3 H, m), 3.92–3.86 (1 H, m), 3.36 (1 H, quint, *J* = 5.6 Hz), 3.30–3.18 (2 H, m), 2.82–2.67 (3 H, m), 2.48 (1 H, dd, *J* = 15.6, 8.0 Hz), 1.95 (3 H, s), 1.55–1.24 (22 H, m), 0.86–0.83 (12 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.5, 155.9, 138.4, 137.3, 82.4, 79.8, 74.5, 68.0, 52.5, 48.2, 47.3, 38.5, 30.15, 29.1, 28.8 (3 ×), 28.2, 27.4, 25.9, 25.5, 23.5, 23.1, 22.8, 14.0, 10.83, 9.4, 9.1; HRMS calcd for $C_{29}H_{53}N_2O_8S$: 589.3523, found: *m/z* 589.3521 $[M + H]^+$.

*4.7.15. 2-Ethylhexyl (3R,4R,5S)-[4-acetamido-5-[N^2,N^3-bis(tert-butoxycarbonyl)-guanidino]-3-(1-ethylpropoxy)-1-cyclohexene]sulfonylpropanoate (**23b**)*

A solution of compound **23a** (59 mg, 0.1 mmol) and TFA (77 µL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure to give a brown oil, which was treated with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (47 mg, 0.12 mmol) and NEt_3 (28 µL, 0.2 mmol) in CH_2Cl_2 (5 mL) at room temperature for 10 h. The mixture was concentrated under reduced pressure, and purified by silica gel column chromatography (EtOAc/hexane = 1:1) to

give **23b** (42 mg, 72% yield). $C_{35}H_{62}N_4O_{10}S$; $[\alpha]_D^{24}$ –39.6 (*c* = 1, CH_2Cl_2); IR (film) 3271, 2965, 2938, 2873, 1731, 1641, 1611, 1561, 1414, 1368, 1251, 1128, 1056, 1030 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.34 (1 H, s), 8.82 (1 H, d, *J* = 8.0 Hz), 6.75 (1 H, s), 6.39 (1 H, d, *J* = 8.8 Hz), 4.50 (1 H, quint, *J* = 6.8 Hz), 4.23 (1 H, dd, *J* = 14.4, 8.4 Hz), 3.99–3.94 (3 H, m), 3.33–3.26 (2 H, m), 3.20–3.13 (1 H, m), 2.83 (1 H, dd, *J* = 14.4, 5.2 Hz), 2.79–2.65 (2 H, m), 2.57 (1 H, dd, *J* = 17.6, 8.4 Hz), 1.89 (3 H, s), 1.51–1.38 (22 H, m), 1.33–1.17 (9 H, m), 0.85–0.80 (12 H, m). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.0, 163.1, 156.2, 152.3, 138.1, 136.9, 83.5, 83.2, 79.4, 74.3, 68.0, 51.6, 47.3, 46.9, 38.5, 30.13, 30.09, 28.8, 28.2 (3 ×), 27.7 (3 ×), 27.5, 25.9, 25.6, 23.5, 23.0, 22.8, 13.9, 10.8, 9.6, 9.2; HRMS calcd for $C_{35}H_{63}N_4O_{10}S$: 731.4265, found: *m/z* 731.4264 $[M + H]^+$.

*4.7.16. (3R,4R,5S)-[4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfinic acid (**6a**)*

A mixture of compound **23a** (59 mg, 0.1 mmol) and potassium *tert*-butoxide (17 mg, 0.15 mmol) in acetone (5 mL) and H_2O (1 mL) was stirred at room temperature for 10 h. The mixture was concentrated under reduced pressure, and extracted with EtOAc (5 mL × 3) and H_2O (5 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude product **24a**. The Boc protecting group in **24a** was removed by treatment with TFA (77 μL, 1.0 mmol) in CH_2Cl_2 (5 mL) at room temperature for 3 h. The

mixture was concentrated under reduced pressure and purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4) to give the desired product **6a** (19 mg, 61% yield from **23a**). The purity of product **6a** was 100% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 12.7 min (MeOH/H_2O = 3:7). $C_{13}H_{24}N_2O_4S$; hygroscopic solid; $[\alpha]_D^{22}$ –41.8 (*c* = 0.5, MeOH); IR (film) 3649, 2965, 2884, 1734, 1653, 1540, 1457, 1374, 1290, 1012, 948 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.11 (1 H, s), 4.12 (1 H, d, *J* = 7.6 Hz), 3.99 (1 H, dd, *J* = 10.4, 5.2 Hz), 3.47–3.38 (2 H, m), 2.88 (1 H, dd, *J* = 16.8, 8.4 Hz), 2.41 (1 H, dd, *J* = 16.8, 8.4 Hz), 2.02 (3 H, s), 1.59–1.46 (4 H, m), 0.92–0.85 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 174.7, 152.1, 126.1, 83.5, 76.1, 54.9, 51.4, 27.4, 26.8, 23.6, 23.2, 10.0, 9.7; HRMS calcd for $C_{13}H_{25}N_2O_4S$: 305.1540, found: *m/z* 305.1535 $[M + H]^+$.

*4.7.17. (3R,4R,5S)-[4-Acetamido-3-(1-ethylpropoxy)-5-guanidino-1-cyclohexene]sulfinic acid (**6b**)*

By a procedure similar to that for **6a**, compound **23b** (73 mg, 0.1 mmol) was treated with potassium *tert*-butoxide (17 mg, 0.15 mmol) in acetone−water solution at room temperature for 10 h to give a crude product **24b**. The Boc protecting group in **24b** was removed by treatment with TFA in CH_2Cl_2, and the desired product **6b** (17 mg, 48% yield from **23b**) was obtained after purification on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4). The

purity of product **6b** was 96.2% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 8.8 min (MeOH/H_2O = 3:7). $C_{14}H_{26}N_4O_4S$; hygroscopic solid; $[\alpha]_D^{22}$ –29.4 (*c* = 0.23, MeOH); IR (film) 3269, 2966, 2881, 1658, 1563, 1502, 1441, 1376, 1311, 1184 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.08 (1 H, s), 4.16 (1 H, d, *J* = 6.8 Hz), 3.94–3.90 (1 H, m), 3.84–3.78 (1 H, m), 3.39 (1 H, quint, *J* = 5.6 Hz), 2.78 (1 H, dd, *J* = 17.2, 4.8 Hz), 2.34–2.27 (1 H, m), 1.98 (3 H, s), 1.56–1.46 (4 H, m), 0.93–0.86 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 174.2, 158.7, 152.6, 126.3, 83.6, 76.7, 56.4, 52.2, 27.5, 27.0, 25.6, 23.0, 10.0, 9.9; HRMS calcd for $C_{14}H_{27}N_4O_4S$: 347.1756, found: *m/z* 347.1753 $[M + H]^+$.

*4.7.18. (3R,4R,5S)-[4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonic acid (**7a**)*

The β-elimination reaction of **23a** (59 mg, 0.1 mmol) with potassium *tert*-butoxide (17 mg, 0.15 mmol) gave a potassium salt of **24a**, which was subsequently treated with *m*-CPBA (26 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C for 1 h. The mixture was extracted with H_2O (1 mL × 3). The aqueous phase was concentrated under reduced pressure to obtain a crude sulfonic acid compound **25a**. The Boc protecting group in **25a** was removed by treatment with TFA in CH_2Cl_2, and the desired product **7a** (15 mg, 47% yield from **23a**) was obtained after purification on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4). The purity of

product **7a** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 11.5 min (MeOH/H_2O = 3:7). $C_{13}H_{24}N_2O_5S$; hygroscopic solid; $[\alpha]_D^{22}$ – 65.6 (*c* = 1, MeOH); IR (film) 3273, 2965, 2937, 2878, 1684, 1670, 1559, 1534, 1457, 1372, 1218, 1181, 1094, 1051, 940 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.47 (1 H, s), 4.15 (1 H, d, *J* = 8.0 Hz), 3.98 (1 H, dd, *J* = 11.2, 8.0 Hz), 3.54–3.47 (1 H, m), 3.41 (1 H, quint, *J* = 5.6 Hz), 2.95 (1 H, dd, *J* = 16.8, 5.6 Hz), 2.63–2.54 (1 H, m), 2.03 (3 H, s), 1.58–1.46 (4 H, m), 0.87–0.93 (6 H, m). ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 140.3, 129.4, 83.7, 75.7, 54.4, 51.1, 29.2, 27.4, 26.8, 23.3, 10.0, 9.7; HRMS calcd for $C_{13}H_{25}N_2O_5S$: 321.1482, found: *m/z* 321.1484 $[M + H]^+$.

*4.7.19. (3R,4R,5S)-[4-Acetamido-3-(1-ethylpropoxy)-5-guanidino-1-cyclohexene]sulfonic acid (**7b**)*

The obtained by treatment The β-elimination reaction of **23b** (73 mg, 0.1 mmol) with potassium *tert*-butoxide (17 mg, 0.15 mmol) gave a potassium salt of **24b**, which was subsequently treated with *m*-CPBA (26 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) at 0 ^oC for 1 h to give a crude compound **25b**. The Boc protecting group in **25b** was removed by treatment with TFA in CH_2Cl_2, and the desired product **7b** (11 mg, 31% yield from **23b**) was obtained after purification on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 1:4). The purity of product **7b** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5

μm particle size), t_R = 22.3 min (MeOH/H_2O = 3:7). $C_{14}H_{26}N_4O_5S$; hygroscopic solid; $[\alpha]_D^{22}$ –30.6 (*c* = 0.3, DMSO); IR (film) 3420, 3003, 2918, 2595, 2136, 1972, 1907, 1652, 1437, 1407, 1316, 1200 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.81 (1 H, *J* = 7.6 Hz), 7.35 (1 H, d, *J* = 7.6 Hz), 6.07 (1 H, s), 4.00 (1 H, s), 3.69 (2 H, s), 2.64 (1 H, d, *J* = 14.8 Hz), 2.27–2.23 (1 H, m), 1.82 (3 H, s), 1.44–1.33 (4 H, m), 0.85–0.77 (6 H, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8, 156.6, 141.7, 125.0, 81.0, 74.7, 54.0, 50.3, 30.2, 25.8, 25.3, 22.8, 9.4, 9.1; HRMS calcd for $C_{14}H_{27}N_4O_5S$: 363.1712, found: *m/z* 363.1702 $[M + H]^+$.

*4.7.20. n-Butyl (3R,4R,5S)-[4-acetamido-5-(tert-butoxycarbonyl)amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonate (**26**)*

A mixture of sulfonic acid **25a** (42 mg, 0.1 mmol) and Ag_2O (0.15 mmol, 34 mg) in MeCN (5 mL) was stirred at 80 °C for 3 h. After addition of 1-iodobutane (34 μL, 0.3 mmol), the mixture was stirred for another 10 h at 80 °C, and then filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, and the residue was extracted with CH_2Cl_2 (5 mL × 3) and H_2O (5 mL). The organic phase was dried over $MgSO_4$, filtered, concentrated under reduced pressure, and purified by silica gel column chromatography (EtOAc/hexane = 1:1) to give compound **26** (18 mg, 38% yield). $C_{22}H_{40}N_2O_7S$; white solid; mp 172–175 °C; $[\alpha]_D^{24}$ –105.2 (*c* = 1, CH_2Cl_2); IR (film) 3358, 3289, 3117, 2970, 2938, 2872, 1683, 1646, 1581, 1528, 1454, 1368, 1295, 1258, 1180, 1164, 1144, 1062, 1017, 943, 784,

726, 627 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (1 H, s), 6.26 (1 H, br d, *J* = 8.8 Hz), 5.36 (1 H, br d, *J* = 9.2 Hz), 4.11–3.98 (4 H, m), 3.90–3.82 (1 H, m), 3.35 (1 H, quint, *J* = 5.6 Hz), 2.77 (1 H, dd, *J* = 17.4, 5.0 Hz), 2.38 (1 H, dd, *J* = 17.4, 8.8 Hz), 1.95 (3 H, s), 1.66 (2 H, quint, *J* = 6.8 Hz), 1.50–1.32 (15 H, m), 0.92–0.82 (9 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 156.0, 137.7, 134.2, 82.5, 79.9, 74.6, 70.8, 53.4, 48.4, 30.9, 29.5, 28.3 (3 ×), 26.0, 25.5, 23.2, 18.7, 13.4, 9.5, 9.1; HRMS calcd for $C_{22}H_{41}N_2O_7S$: 477.2634, found: *m/z* 477.2627 [M + H]$^{+}$.

4.7.21. *3-Phenylpropyl (3R,4R,5S)-[4-acetamido-5-(tert-butoxycarbonyl)amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonate (**27**)*

By a procedure similar to that for **26**, the sulfonic acid compound **25a** (42 mg, 0.1 mmol) was treated with Ag_2O (0.15 mmol, 34 mg) and 1-iodo-3-phenylpropane (48 μL, 0.3 mmol) in MeCN at 80 ^{o}C to give a sulfonate ester **27** (24 mg, 45% yield). $C_{27}H_{42}N_2O_7S$; white solid; mp 156–158 ^{o}C; $[\alpha]_D^{24}$ –74.0 (*c* = 1, CH_2Cl_2); IR (film) 3347, 3295, 2968, 2932, 2868, 1683, 1654, 1562, 1531, 1365, 1296, 1182, 1081, 1017, 931, 800, 746 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.26 (2 H, m), 7.22–7.17 (3 H, m), 6.73 (1 H, s), 6.02 (1 H, d, *J* = 8.4 Hz), 5.22 (1 H, *J* = 8.8 Hz), 4.14–4.04 (3 H, m), 4.03–3.99 (1 H, m), 3.92–3.84 (1 H, m), 3.35 (1 H, quint, *J* = 6.4 Hz), 2.81 (1 H, dd, *J* = 17.3, 5.0 Hz), 2.37 (1 H, dd, *J* = 17.3, 9.2 Hz), 2.04 (2

H, quint, *J* = 6.4 Hz), 1.98 (3 H, s), 1.51–1.46 (4 H, m), 1.41 (9 H, s), 0.88 (6 H, t, *J* = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 156.1, 140.2, 138.6, 134.2, 128.6 (2 ×), 128.4 (2 ×), 126.3, 82.5, 80.0, 74.9, 70.0, 53.7, 48.6, 31.5, 30.5, 29.7, 28.3 (3 ×), 26.0, 25.5, 23.2, 9.5, 9.1; HRMS calcd for $C_{27}H_{43}N_2O_7S$: 539.2785, found: *m/z* 539.2812 $[M + H]^+$.

*4.7.22. n-Butyl (3R,4R,5S)-[4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonate (**8a**)*

A solution of compound **26** (48 mg, 0.1 mmol) and TFA (77 µL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure, and the residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 6:4) to give the desired product **8a** (11 mg, 29% yield). The purity of product **8a** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 8.5 min (MeOH/H_2O = 6:4). $C_{17}H_{32}N_2O_5S$; hygroscopic solid; $[\alpha]_D^{23}$ – 69.6 (*c* = 1, MeOH); IR (film) 2970, 2938, 2876, 1679, 1540, 1458, 1442, 1364, 1295, 1209, 1176, 1131, 1058, 935, 890, 837, 808, 727 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.77 (1 H, t, *J* = 2.0 Hz), 4.33 (1 H, br d, *J* = 8.4 Hz), 4.14 (2 H, t, *J* = 6.4 Hz). 3.97 (1 H, dd, *J* = 11.4, 8.6 Hz), 3.70–3.63 (1 H, m), 3.44 (1 H, quint, *J* = 6.0 Hz), 2.94 (1 H, dd, *J* = 12.8, 5.6 Hz), 2.62–2.54 (1 H, m), 2.04 (3 H, s), 1.72 (2 H, quint, *J* = 6.8 Hz), 1.59–1.50 (4 H, m), 1.49–1.40 (2 H, m), 0.98–0.86 (9 H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 140.3, 133.7, 84.1, 75.3, 72.7,

54.5, 50.3, 32.2, 28.8, 27.2, 26.7, 23.3, 19.9, 14.0, 10.1, 9.6; HRMS calcd for $C_{17}H_{33}N_2O_5S$: 377.2110, found: *m/z* 377.2104 $[M + H]^+$.

*4.7.23. 3-Phenylpropyl (3R,4R,5S)-[4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonate (**9a**)*

By a procedure similar to that for **8a**, compound **27** (54 mg, 0.1 mmol) was treated with TFA in anhydrous CH_2Cl_2 at room temperature for 3 h, and then purified on a reversed-phase RP-18 column (MeOH/ H_2O = 1:9 to 4:6) to give the desired product **9a** (11 mg, 25% yield). The purity of product **9a** was > 99% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 8.9 min (MeOH/H_2O = 5:5). $C_{22}H_{34}N_2O_5S$; hygroscopic solid; $[\alpha]_D^{22}$ –55.6 (*c* = 1, MeOH); IR (film) 3032, 2974, 2938, 2880, 1679, 1552, 1458, 1356, 1295, 1205, 1180, 1136, 997, 923, 849, 800, 747 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.30–7.27 (2 H, m), 7.21–7.16 (3 H, m), 6.76 (1 H, d, *J* = 2.0 Hz), 4.31 (1 H, br d, *J* = 8.0 Hz), 4.14 (2 H, t, *J* = 6.2 Hz), 4.02–3.95 (1 H, m), 3.70–3.63 (1 H, m), 3.44 (1 H, quint, *J* = 5.6 Hz), 2.95 (1 H, dd, *J* = 16.8, 5.2 Hz), 2.73 (2 H, t, *J* = 7.6 Hz), 2.63–2.57 (1 H, m), 2.08–2.01 (5 H, m), 1.56–1.46 (4 H, m), 0.92–0.87 (6 H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 142.0, 140.3, 133.6, 129.8 (2 ×), 129.6 (2 ×), 127.4, 84.0, 75.1, 72.1, 54.3, 50.4, 32.7, 32.1, 28.9, 27.2, 26.7, 23.3, 10.0, 9.6; HRMS calcd for $C_{22}H_{35}N_2O_5S$: 439.2267, found: *m/z* 439.2253 $[M + H]^+$.

4.7.24. *n-Butyl (3R,4R,5S)-[4-acetamido-5-guanidino-3-(1-ethylpropoxy)-1-cyclohexene]sulfonate (**8b**)*

A solution of compound **8a** (38 mg, 0.1 mmol), 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (47 mg, 0.12 mmol) and NEt_3 (0.041 mL, 0.3 mmol) in CH_2Cl_2 (1 mL) solution was stirred at room temperature for 10 h. The mixture was concentrated under reduced pressure, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane = 1:1) to give an intermediate product **28** (32 mg, 52% yield). $C_{28}H_{50}N_4O_9S$; colorless oil; $[\alpha]_D^{20}$ –67.5 (*c* = 1, CH_2Cl_2); IR (film) 3273, 2970, 2978, 2880, 1736, 1638, 1614, 1556, 1417, 1368, 1311, 1254, 1176, 1054, 1021, 939, 813, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.39 (1 H, s), 8.74 (1 H, d, *J* = 8.0 Hz), 6.78 (1 H, s), 6.23 (1 H, d, *J* = 8.8 Hz), 4.56–4.48 (1 H, m), 4.22–4.16 (1 H, m), 4.14–3.99 (3 H, m), 3.35 (1 H, quint, *J* = 5.6 Hz), 2.85 (1 H, dd, *J* = 17.6, 5.2 Hz), 2.55–2.48 (1 H, m), 1.93 (3 H, s), 1.72–1.65 (2 H, m), 1.56–1.47 (22 H, m), 1.45–1.37 (2 H, m), 0.97–0.85 (9 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 162.8, 156.5, 152.5, 138.1, 133.6, 83.7, 83.2, 79.8, 74.8, 71.0, 53.2, 47.4, 30.9, 29.0, 28.2 (3 ×), 28.0 (3 ×), 25.9, 25.6, 23.2, 18.7, 13.5, 9.6, 9.2; HRMS calcd for $C_{28}H_{51}N_4O_9S$: 619.3377, found: *m/z* 619.3377 $[M + H]^+$.

A solution of the above-prepared compound **28** (32 mg) and TFA (77 μL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h. The mixture was

concentrated under reduced pressure, and the residue was purified on a reversed-phase RP-18 column (MeOH/ H_2O = 1:9 to 4:6) to give the desired product **8b** (13 mg, 60% yield). The purity of product **8b** was 98.2% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 3.4 min (MeOH/H_2O = 5:5). $C_{18}H_{34}N_4O_5S$; hygroscopic solid; $[\alpha]_D^{22}$ –58.3 (*c* = 1, MeOH); IR (film) 3334, 3273, 3175, 2966, 2938, 2880, 1675, 1548, 1458, 1430, 1352, 1205, 1180, 1136, 1074, 1054, 833, 796, 727 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.75 (1 H, br t, *J* = 2.0 Hz), 4.31 (1 H, br d, *J* = 2.8 Hz), 4.18–4.11 (2 H, m), 4.06–4.00 (1 H, m), 3.94–3.87 (1 H, m), 3.44 (1 H, quint, *J* = 5.6 Hz), 3.31–3.30 (2 H, m), 2.86 (1 H, dd, *J* = 16.8, 5.2 Hz), 2.52–2.45 (1 H, m), 1.99 (3 H, s), 1.76–1.69 (2 H, m), 1,58–1.40 (6 H, m), 0.99–0.87 (9 H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 158.8, 139.7, 134.9, 84.1, 75.4, 72.6, 55.8, 51.3, 32.3, 30.6, 27.3, 26.9, 22.9, 20.0, 14.3, 10.0, 9.7; HRMS calcd for $C_{18}H_{35}N_4O_5S$: 419.2328, found: *m/z* 419.2314 $[M + H]^+$.

*4.7.25. 3-Phenylpropyl (3R,4R,5S)-[4-acetamido-5-guanidino-3-(1-ethylpropoxy)-1-cyclohexane]sulfonate (**9b**)*

By a procedure similar to that for **8b**, compound **9a** (44 mg, 0.1 mmol) was treated with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine (47 mg, 0.12 mmol) to give an intermediate product **29** (44 mg, 65%yield) after purification by flash silica gel column chromatography (EtOAc/hexane = 1:1). $C_{33}H_{52}N_4O_9S$; colorless oil; $[\alpha]_D^{20}$ –44.1 (*c* = 1,

CH_2Cl_2); IR (film) 3391, 3301, 2978, 2938, 2876, 1785, 1732, 1626, 1561, 1462, 1417, 1377, 1340, 1213, 1172, 1127, 1062, 931, 813, 784 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 11.39 (1 H, s), 8.72 (1 H, d, *J* = 8.0 Hz), 7.32–7.25 (2 H, m), 7.22–7.16 (3 H, m), 6.77 (1 H, s), 6.23 (1 H, d, *J* = 8.8 Hz), 4.55–4.47 (1 H, m), 4.21–4.01 (4 H, m), 3.34 (1 H, quint, *J* = 5.6 Hz), 2.86 (1 H, dd, *J* = 17.1, 5.2 Hz), 2.74–2.66 (2 H, m), 2.49 (1 H, dd, *J* = 17.1, 8.6 Hz), 2.07–1.99 (2 H, m), 1.93 (3 H, s), 1.52–1.46 (22 H, m), 0.90–0.85 (6 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 156.5, 152.5, 151.4, 140.2, 138.4, 133.5, 128.6 (2 ×), 128.4 (2 ×), 126.3, 83.7, 83.2, 79.9, 74.8, 70.1, 53.4, 47.5, 31.5, 30.5, 29.1, 28.2 (3 ×), 28.0 (3 ×), 25.9, 25.6, 23.2, 9.6, 9.2; HRMS calcd for $C_{33}H_{53}N_4O_9S$: 681.3533, found: *m/z* 681.3531 $[M + H]^+$.

The above-prepared compound **29** (44 mg) and TFA (77 µL, 1.0 mmol) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure, and the residue was purified on a reversed-phase RP-18 column (MeOH/H_2O = 1:9 to 4:6) to give the desired product **9b** (18 mg, 58% yield). The purity of product **9b** was 99.4% as shown by HPLC on an HC-C18 column (Agilent, 4.6 × 250 mm, 5 µm particle size), t_R = 3.4 min (MeOH/H_2O = 5:5). $C_{23}H_{36}N_4O_5S$; hygroscopic solid; $[\alpha]_D^{22}$ – 42.6 (*c* = 1, MeOH); IR (film) 3273, 3175, 2970, 2929, 2872, 1663, 1548, 1454, 1426, 1360, 1201, 1180, 1140, 1078, 993, 931, 837, 809, 751, 727, 617 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.30–7.27 (2 H, m), 7.22–7.16 (3 H, m), 6.75 (1 H, s), 4.29 (1 H, br d, *J* = 7.2 Hz), 4.18–4.09 (2 H, m), 4.02–3.97 (1 H, m), 3.89 (1 H, dd, *J* = 10.8, 8.0 Hz), 3.43 (1 H, quint, *J* =

5.6 Hz), 2.87 (1 H, dd, *J* = 17.4, 4.8 Hz), 2.73 (2 H, t, *J* = 7.6 Hz), 2.48 (1 H, dd, *J* = 17.4, 5.4 Hz), 2.09–2.02 (2 H, m), 1.98 (3 H, s), 1.58–1.48 (4 H, m), 0.93–0.87 (6 H, m); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 158.8, 142.1, 140.0, 134.9, 129.7 (2 ×), 129.6 (2 ×), 127.4, 84.1, 75.4, 72.0, 55.9, 51.3, 32.7, 32.0, 30.7, 27.3, 26.9, 23.0, 10.1, 9.7; HRMS calcd for $C_{23}H_{37}N_4O_5S$: 481.2485, found: *m/z* 481.2477 $[M + H]^+$.

Notes

The authors declare no competing financial interest.

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Supplementary data

^{1}H and ^{13}C NMR spectra. Supplementary data related to this article can be found at https://

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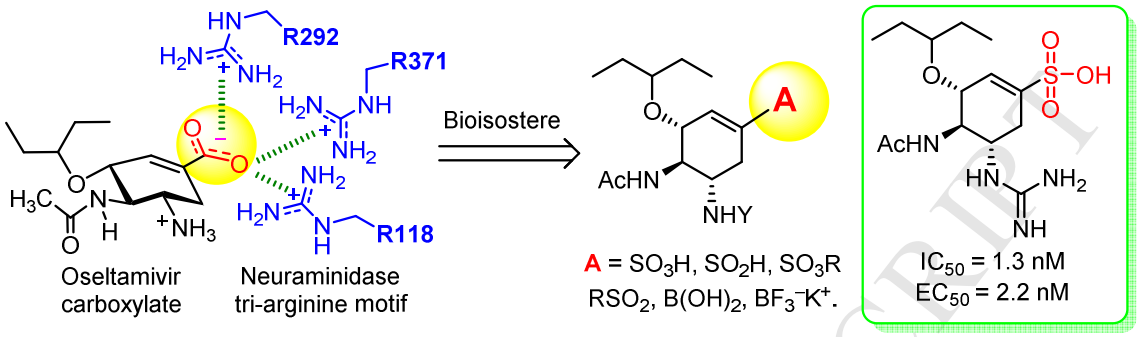
Graphic abstract



Legends of Figures, Schemes, and Tables.

Fig. 1. Chemical structures of oseltamivir carboxylic acid (**1a**, OC), guanidino OC (**1b**, GOC), and their carboxyl bioisosteres (**2a**−**13b**).

Scheme 1. Synthesis of boronic acids **2a**/**2b** and trifluoroborates **3a**/**3b**

Scheme 2. Synthesis of sulfones **4a**/**4b** and **5a**/**5b**

Scheme 3. Synthesis of sulfinic acids **6a**/**6b** and sulfonic acids **7a**/**7b**

Scheme 4. Synthesis of sulfonate esters **8a**–**9b**

Table 1. Neuraminidase inhibition (IC_{50}) and anti-influenza activity (EC_{50}) against A/WSN/33 (H1N1) virus[a]

Boronate, Trifluoroborate, Sulfone, Sulfinate and Sulfonate Congeners of Oseltamivir Carboxylic Acid: Synthesis and Anti-influenza Activity

Research highlights

• First synthesis of boronate and sulfonate related OC and GOC bioisosteres **2a**−**9b**.

• The OC derived iodocyclohexene **16a** is a key intermediate for syntheses of bioisosteres.

• Acidity and lipophilicity of bioisosteres are important to anti-influenza activity.

• GOC congeners show better NA inhibition and anti-influenza activity than OC congeners.

• GOC sulfonic acid is a potent inhibitor against H1N1 virus (EC_{50} = 2.2 nM).