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THE SYNTHESIS OF SIMPLIFIED ANALOGUES OF CRAMBESCIN B CARBOXYLIC ACID AND THEIR INHIBITORY ACTIVITY OF VOLTAGE-GATED SODIUM CHANNELS: NEW ASPECTS OF STRUCTURE–ACTIVITY RELATIONSHIPS

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Abstract – We describe the synthesis of six new analogues of crambescin B carboxylic acid from L-aspartic acid and the elucidation of their structure-activity relationships by a cell-based colorimetric assay. All the synthesized analogues except for the C_4-analogue were found to have inhibitory activities against voltage-gated sodium channels (VGSCs) in nM order in a cell-based colorimetric assay.

Crambescin B is a guanidine alkaloid, originally isolated from the marine sponge *Crambe crambe* by Braekman in 1990[1] and established to have the structure depicted in Figure 1 based on a racemic synthesis by Snider.[2] In previous studies towards the development of a subtype selective inhibitor of voltage-gated sodium channels (VGSCs, Na_vs)[3] on the basis of natural products such as tetrodotoxin (TTX)[4] and saxitoxin,[5] we synthesized both enantiomers of crambescin B carboxylic acid (**1**) and its analogues (Figure 4 for detailed structures), determined the absolute stereochemistry of crambescin A, a natural analogue, and investigated their inhibitory activities of VGSCs.[6] A cell-based colorimetric assay revealed that the natural enantiomer of **1** was most active comparable to TTX beyond our expectation.[6a,b] In contrast, the electrophysiological assay revealed that **1** and its decarboxylate analogue **2b** did not inhibit VGSCs at a maximum concentration of 100 nM in a similar manner to TTX, but modulate the action of veratridine (VTD), an activator of VGSCs.[6c] To gain further insights of the structure–activity relationships (SAR) of **1**, we sought to synthesize six analogues having more simplified structures, the

monocyclic analogues **3**, **4**, (*R*)-**5**, and (*S*)-**5**, and bicyclic decarboxylate analogues **2a** and **2c**[7] (Figure 2). In this report, we describe details of their synthesis and biological activities using cell-based colorimetric assay.

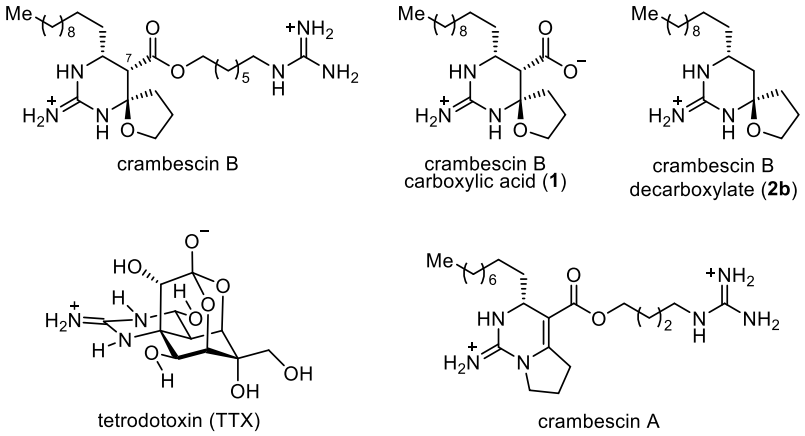


Figure 1. Structures of naturally occurring cyclic guanidine alkaloids

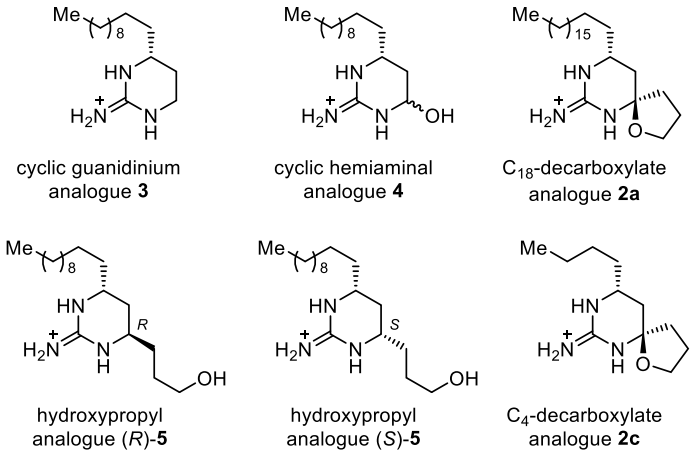
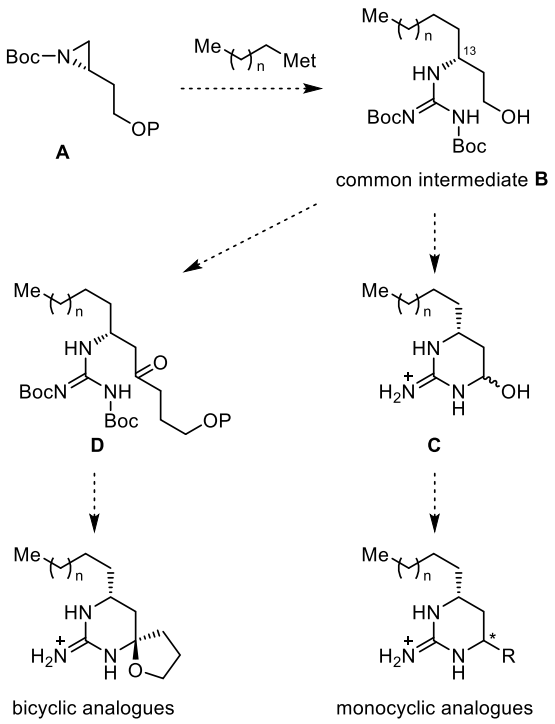


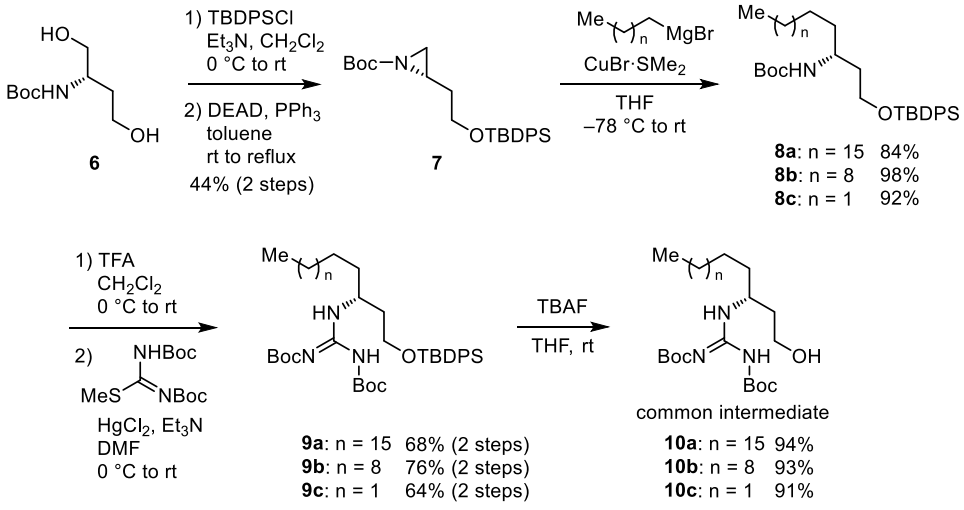
Figure 2. Six crambescin B analogues synthesized in this work

Our synthetic plan towards the simplified crambescin B analogues is depicted in Scheme 1. Alcohol **B** bearing a guanidine moiety and a stereogenic center at C13 was selected as a common intermediate from which both mono- and bicyclic analogues bearing alkyl chains of equivalent lengths could be synthesized. Its synthesis was anticipated from aziridine **A**, prepared from L-aspartic acid.[8] Alcohol **B** would allow to afford cyclic hemiaminal **C**, in which various substituents could be introduced to an aminal carbon using the *N*-acyliminium ion chemistry.[9] On the other hand, ketone **D**, also derived from **B**, would be a suitable precursor for the bicyclic analogues by sequential deprotection and acid-promoted cyclization. This approach allows for the facile introduction of variable length side chains to give both mono- and bicyclic analogues, which is one of its advantages compared to our previous approach.[6]



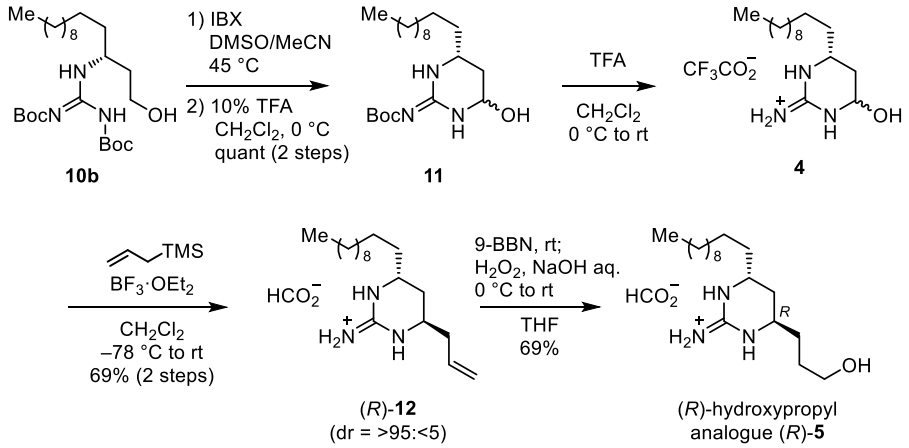
Scheme 1. Synthetic plan of monocyclic and bicyclic analogues of crambescin B

Synthesis of common intermediates **10a-10c** (**B** in Scheme 1, n = 15, 8, 1) commenced with the transformation of known diol **6**[8] to aziridine **7** via protection and cyclization under the Mitsunobu conditions (Scheme 2). Aziridine **7** was next treated with heptadecanylmagnesium bromide in the presence of freshly prepared CuBr·SMe_2 to furnish the corresponding adduct **8a** in 84% yield. Deprotection of Boc group of **8a** and subsequent guanylation under conventional conditions[5a] afforded **9a** in good overall yield. Removal of the TBDPS group with TBAF gave alcohol **10a**, one of the common intermediates. Other common intermediates **10b** and **10c** bearing different side chains were synthesized from **6** in the similar manner.



Scheme 2. Synthesis of the common intermediates **10a-10c**

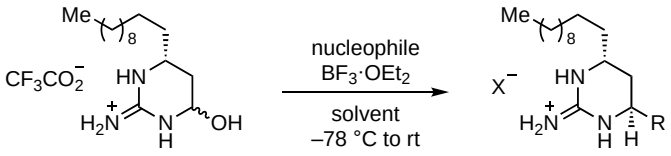
Synthesis of hydroxypropyl analogue (*R*)-**5** was first examined, as illustrated in Scheme 3. Oxidation of the primary alcohol in **10b** with IBX in DMSO and MeCN gave labile aldehyde, the Boc group of which was removed using TFA to provide *N*-Boc guanidine hemiaminal **11** in quantitative yield. Since attempted allylation of *N*-amidinyliminium ion generated from **11**[10] with Lewis acid failed probably due to a strong electron-withdrawing nature of the Boc group, the reaction of unprotected guanidinium hemiaminal **4** was therefore investigated. Indeed, the reaction of **4** with allyltrimethylsilane and $BF_3{\cdot}OEt_2$ afforded the desired (*R*)-**12** in 69% yield from **11** as a single diastereomer. Configuration of the newly created stereogenic center was confirmed by NOESY analysis to be *R* (Figure 3). Final hydroboration of the vinyl group in (*R*)-**12** was successfully achieved under conventional conditions to provide hydroxypropyl analogue (*R*)-**5** in 69% yield.



Scheme 3. Synthesis of hydroxypropyl analogue (*R*)-**5**

The above results establish the unprotected guanidinium hemiaminal such as **4** as a useful precursor of *N*-amidinyliminium ion for the purpose of nucleophilic addition, however, there are only several reports of similar reactions.[10] To evaluate the scope of this reaction, it was attempted with several different nucleophiles (Table 1). Reaction with Et_3SiH resulted in the formation of cyclic guanidinium analogue **3** in 79% yield (entry 1). Similar reactions with anisole and potassium triethylsilylethynyl trifluoroborate[11] gave the corresponding adducts **13** and **14**, respectively (entries 2 and 3). The attempted alkynylation of **4** (entry 3) was dominated by its undesired dehydration, presumably due to basicity of the borate. The relative stereochemistry of the newly generated stereogenic centers in **13** and **14** were determined by NOESY analysis (Figure 3). In the latter case, alkene **15**, derived by half reduction of **14** with Lindlar catalyst, was used for the structural determination.

Table 1. Substitution of hemiaminal **4** with several nucleophiles

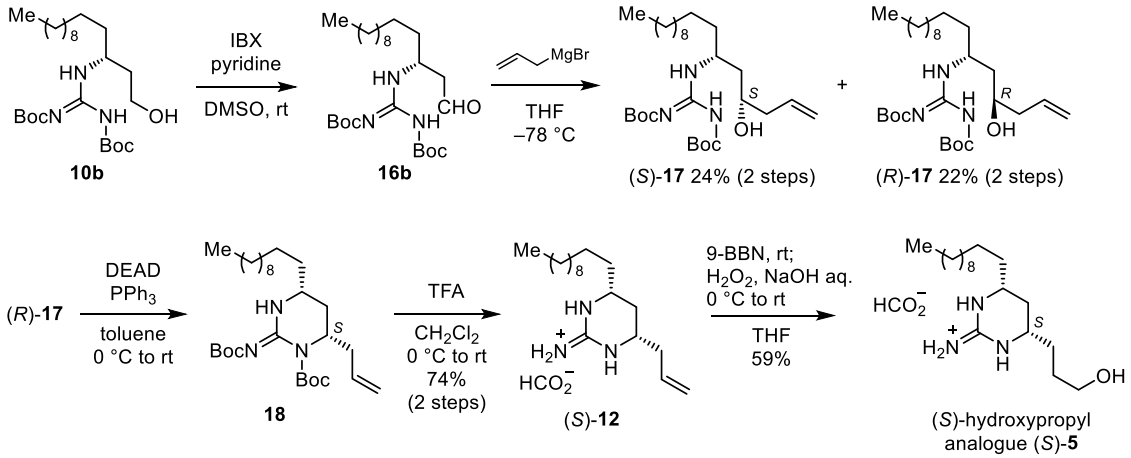


4 **3**, **13**, **14**

entry	nucleophile	solvent	product	R	X	yield (%)
1	Et_3SiH	CH_2Cl_2	**3**	H	HCO_2	79
2	OMe	CH_2Cl_2	**13**	OMe	Cl	84 (dr = >95:<5)
3[a]	TES—≡—BF_3K	MeCN	**14**	TES	HCO_2	21 (dr = >95:<5)

[a]Reaction was carried out at –40 °C to rt.

Next, the synthesis of hydroxypropyl analogue (*S*)-**5** was examined from the common intermediate **10b** (Scheme 4). Based on the extremely high diastereoselectivities shown in Scheme 3 and Table 1, it seems to be difficult to invert the facial selectivity of the allylation of *N*-amidinyliminium ion generated from **4**. We therefore decided to carry out the Mitsunobu reaction of (*R*)-**17**, obtained as a separable mixture of homoallyl alcohols (*R*)-**17** and (*S*)-**17** from **10b** by careful oxidation to the corresponding aldehyde **16b** with IBX followed by allylation with the requisite Grignard reagent, to give the cyclic guanidine **18**. This was transformed into unprotected cyclic guanidinium (*S*)-**12** in 74% yield in two steps from **18**. Finally, hydroboration was carried out in the similar manner as (*R*)-**12** to afford hydroxypropyl analogue (*S*)-**5**.



Scheme 4. Synthesis of hydroxypropyl analogue (*S*)-**5**

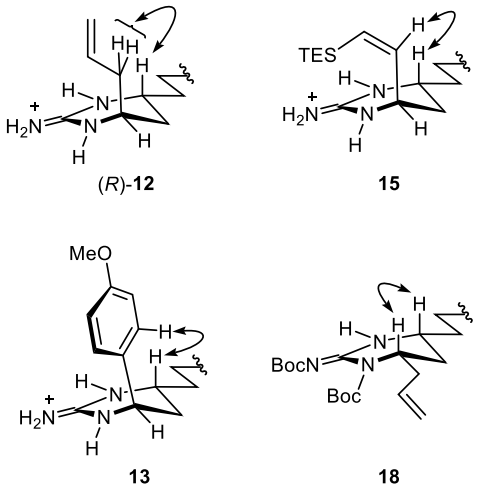
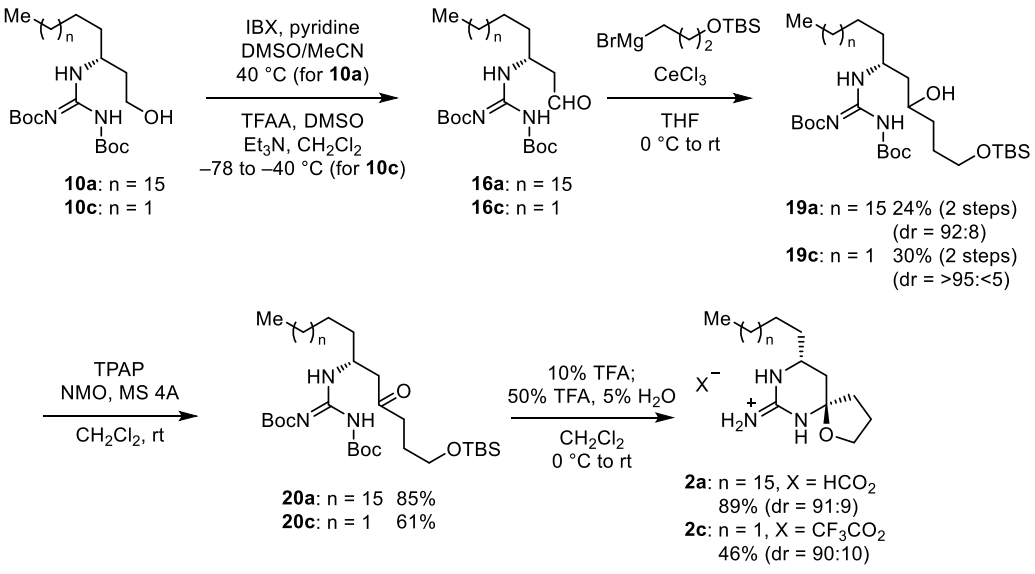


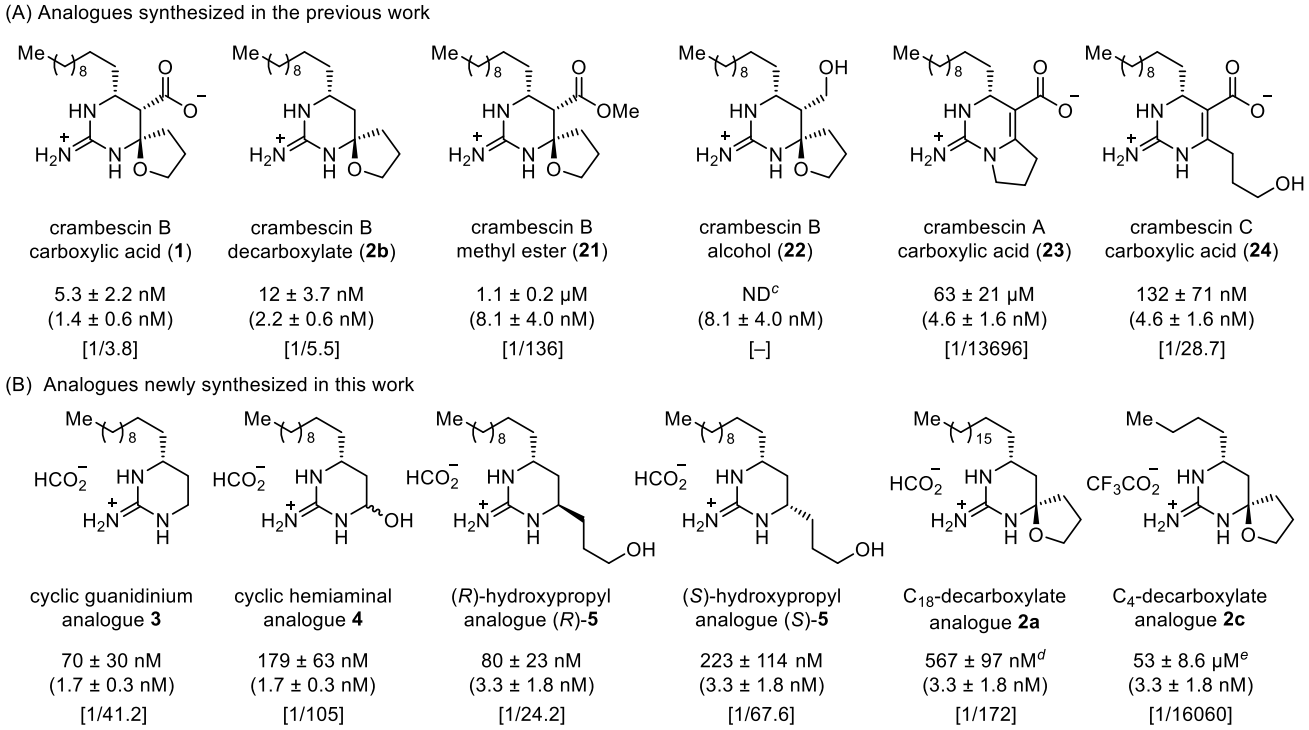
Figure 3. Diagnostic NOESY correlations of (*R*)-**12**, **13**, **15** derived from **14** after half reduction of the alkyne, and **18**

Attention then turned to the synthesis of bicyclic analogues **2a** and **2c** (bearing the C_{18}- or C_4-alkyl side chains, respectively) from common intermediates **10a** and **10c** (Scheme 5). Careful oxidation of common intermediate **10a** with IBX in the presence of pyridine afforded unstable aldehyde **16a**, which was then treated with freshly prepared (*tert*-butyldimethylsiloxy)propylmagnesium bromide and dry $CeCl_3$ to provide alcohol **19a** in 24% yield over two steps. Ley oxidation of **19a** gave ketone **20a**, which was treated with 10% TFA followed by 50% aqueous solution of TFA to provide *spiro*-hemiaminal **2a** in 89% yield with high diastereoselectivity (dr = 91:9). The synthesis of *spiro*-hemiaminal **2c** was similarly accomplished from the common intermediate **10c** except for the oxidation conditions in the first step. The relative stereochemistry of the spiro center in each of **2a** and **2c** was assumed based on the reaction mechanism involving axial attack of the hydroxy group to an iminium carbon.[6a,b]



Scheme 5. Synthesis of bicyclic analogues **2a** and **2c**

We next investigated inhibitory activities on VGSCs of these six analogues using a cell-based colorimetric assay[12] (Figure 4). Neuro-2a cells are forced to be fatal under the stimuli simultaneously with veratridine (VTD) and ouabain, an activator of VGSCs and an inhibitor of the Na^+/K^+-ATPase, respectively. And then, the inhibitory activity on VGSCs of a synthesized analogue is evaluated by the concentration of EC_{50}, at which the test compound restores the cell viability to 50%. In contrast to the potent inhibitory activities of crambescin B carboxylic acid (**1**) and its decarboxylate (**2b**),[6b] monocyclic analogues **3**, **4**, (*R*)-**5**, and (*S*)-**5** exhibited weaker inhibitory activities but the activity still retained in nM order [70 ± 30 nM, 179 ± 63 nM, 80 ± 23 nM, and 223 ± 114 nM, respectively (mean ± S.D.) (n = 3)], suggesting that the *spiro*-hemiaminal scaffold bearing the tetrahydrofuran ring is indispensable for the potency of inhibitory activity. By comparison with the inhibitory activities between hydroxypropyl analogues (*R*)-**5** and (*S*)-**5**, (*R*)-isomer displayed more potent inhibition [80 ± 23 nM and 223 ± 114 nM, respectively (mean ± S.D.) (n = 3)], and the presence of the polar substituent at the terminal of propyl group allows for the potent inhibitory activity. It is likely that the C_{11}-alkyl side chain of the bicyclic analogue **2b** contributes its potent inhibition, because the significant decrease of the activity was observed in the longer and shorter side chain analogues **2a** and **2c** [567 ± 97 nM and 53 ± 8.6 μM, respectively (mean ± S.D.) (n = 3)]. The present SAR suggests that bicyclic guanidine *spiro*-hemiaminal scaffold with the C_{11}-alkyl side chain probably be essential for the potent inhibitory activity.



[a]Mean ± S.D. (n = 3-5). EC_{50} of TTX in parentheses. [b]Relative inhibitory activity to TTX in brackets. [c]Not detected becacuse of its low inhibitory acitivity at concentrations above 100 μM (n = 5). [d]A 91:9 mixture of aminal epimers was used. [e]A 90:10 mixture of aminal epimers was used.

Figure 4. Inhibitory activities (EC_{50}) values of synthesized crambescin analogues[a,b]

In conclusion, we described the synthesis of six simplified analogues of crambescin B carboxylic acid staring from L-aspartic acid. Our synthetic route enables to supply a variety of analogues, modified on the alkyl-side chain as well as the tetrahydrofuran moiety, for SAR studies. Cell-based colorimetric assay revealed that those simplified analogues except for C_4-analogue **2c** retained inhibitory activities against VGSCs in nM order. We believe that an array of synthesized analogues of crambescin B carboxylic acid would be beneficial with further studies to disclose detailed mode of action, and their results will be described in due course.

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SUPPORTING INFORMATION

Supplementary data (Experimental procedures and details, characterization data for products, NMR spectra for all compounds) associated with this article can be found, in the onlineversion, as URL: https//www.heterocycles.jp/newlibrary/downloads/PDFsi/27208/105/1.

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