

HETEROCYCLES, Vol. 105, No. 1, 2022, pp. 343 - 351. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 16th October, 2021, Accepted, 19th November, 2021, Published online, 2nd December, 2021
DOI: 10.3987/COM-21-S(R)7

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 C₄-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 1990¹ 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.

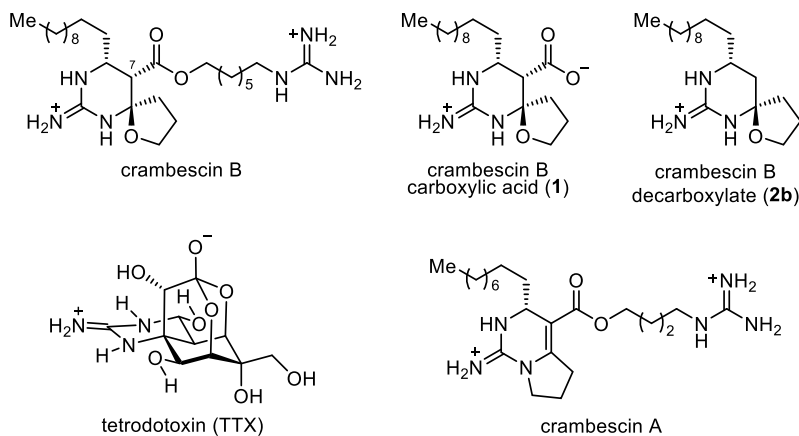


Figure 1. Structures of naturally occurring cyclic guanidine alkaloids

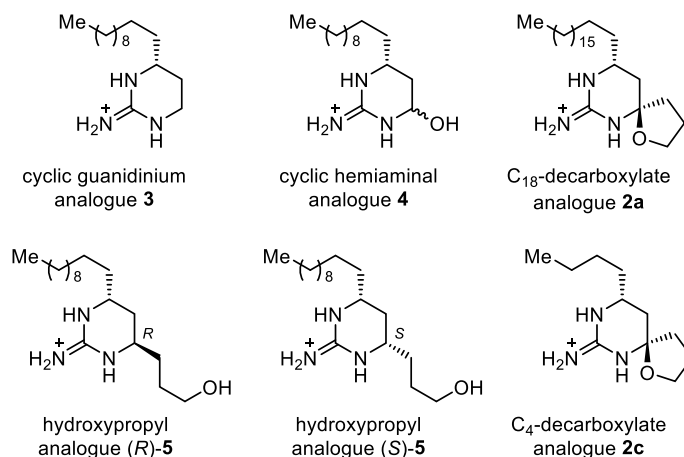
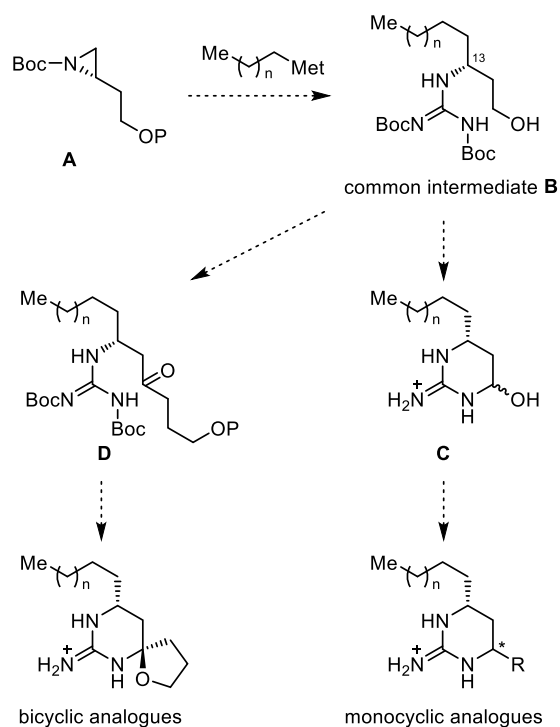


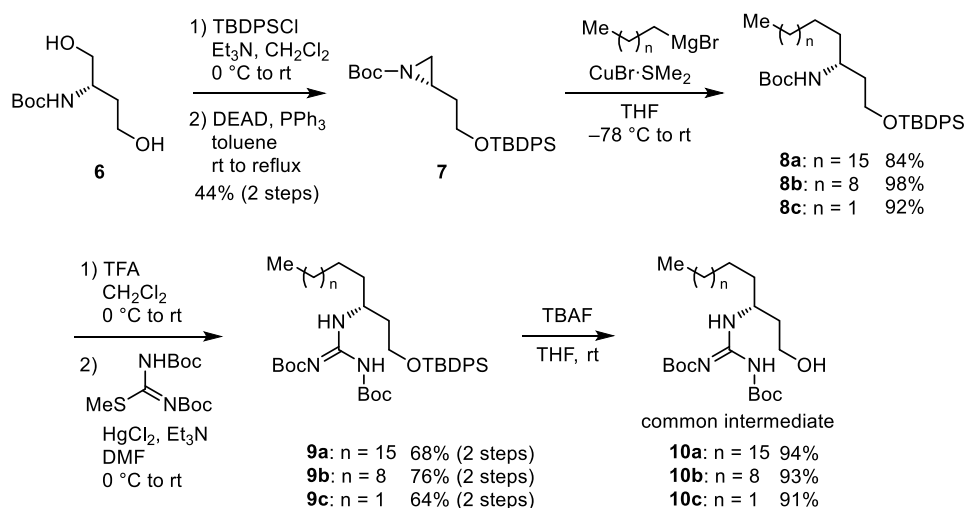
Figure 2. Six crambescin B analogues synthesized in this work

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



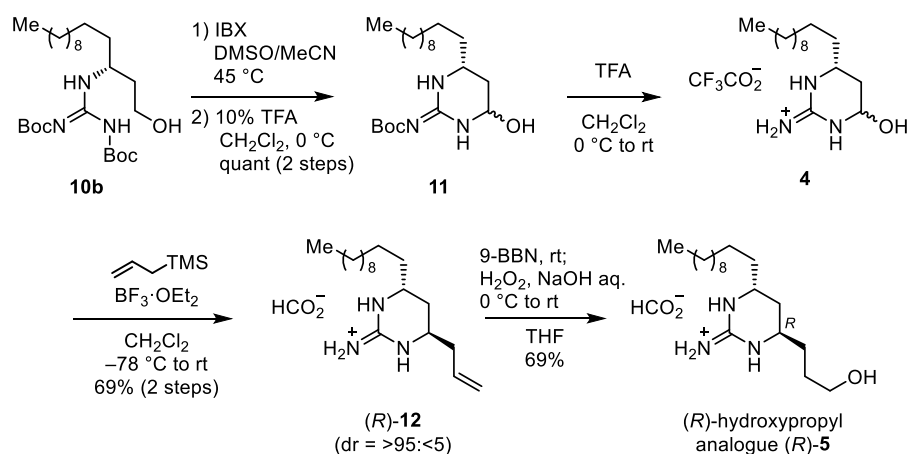
Scheme 1. Synthetic plan of monocyclic and bicyclic analogues of crambescin B

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 heptadecanymagnesium bromide in the presence of freshly prepared $\text{CuBr}\cdot\text{SMe}_2$ 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*-amidinylium 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 BF₃·OEt₂ 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**

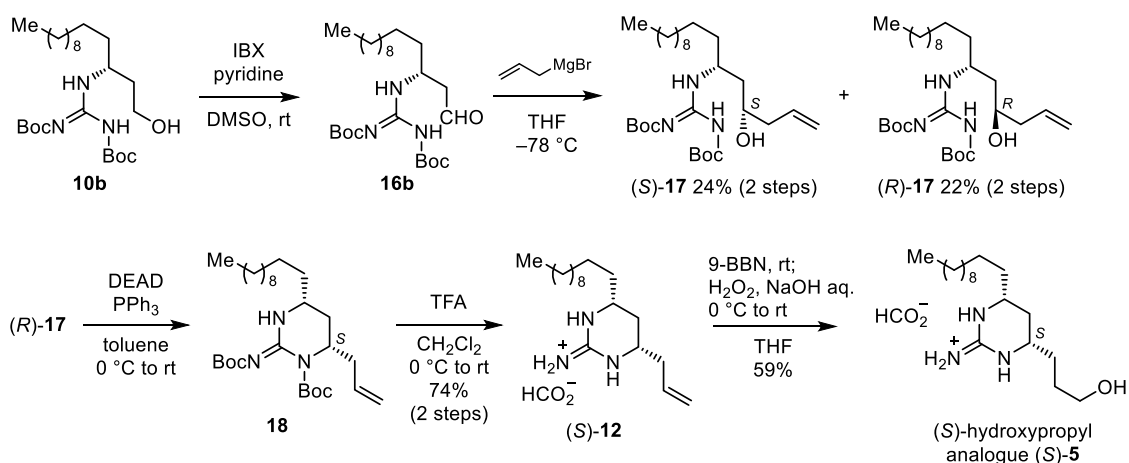
The above results establish the unprotected guanidinium hemiaminal such as **4** as a useful precursor of *N*-amidinylium ion for the purpose of nucleophilic addition, however, there are only several reports of similar reactions.¹⁰ To evaluate the scope of this reaction, it was attempted with several different nucleophiles (Table 1). Reaction with Et₃SiH resulted in the formation of cyclic guanidinium analogue **3** in 79% yield (entry 1). Similar reactions with anisole and potassium triethylsilylethynyl trifluoroborate¹¹ 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

entry	nucleophile	solvent	R	X	product	yield (%)
1	Et ₃ SiH	CH ₂ Cl ₂	3	HCO ₂	3	79
2		CH ₂ Cl ₂	13	Cl	13	84 (dr = >95:<5)
3 ^a	TES-C≡C-BF ₃ K	MeCN	14	HCO ₂	14	21 (dr = >95:<5)

^aReaction 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**

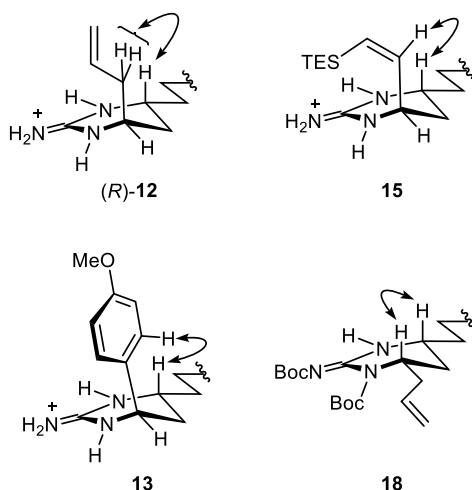
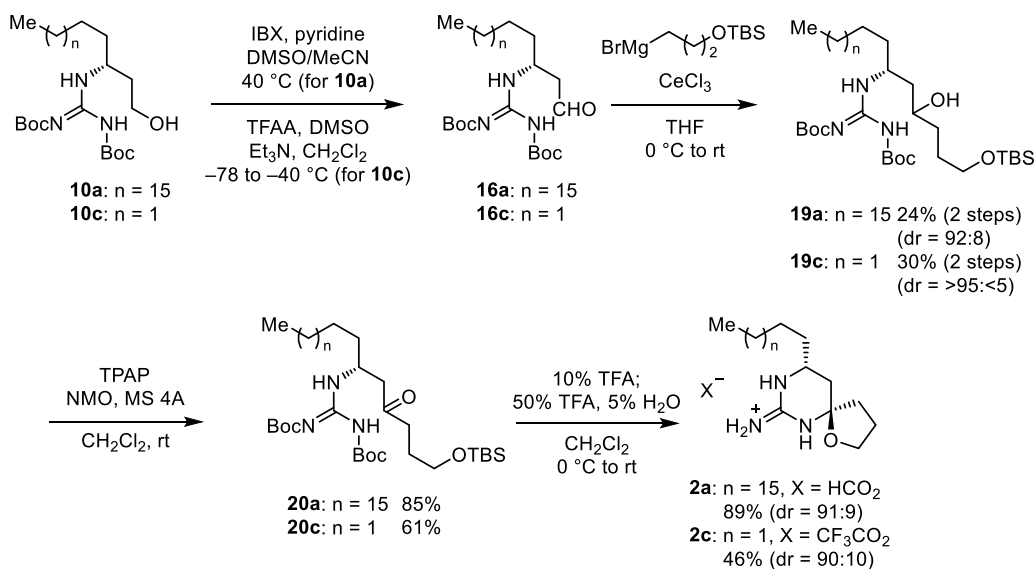


Figure 3. Diagnostic NOESY correlations of (*R*)-**12**, **13**, **15** derived from **14** after half reduction of the alkyne, and **18**

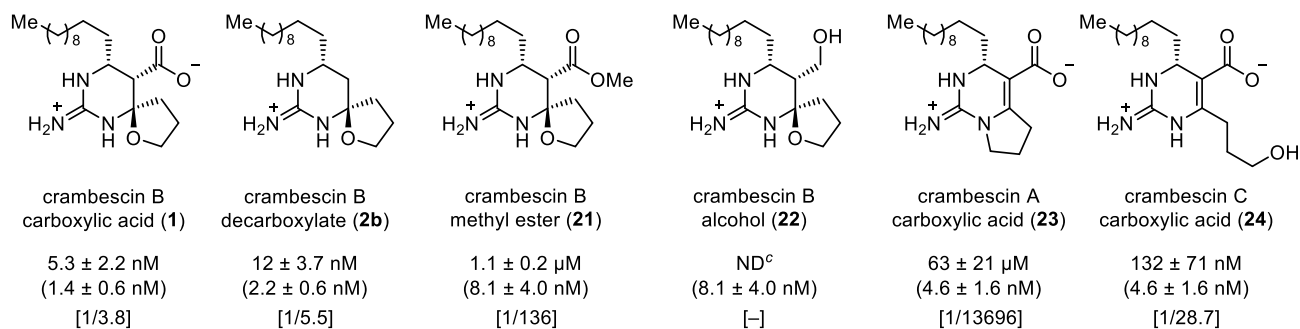
Attention then turned to the synthesis of bicyclic analogues **2a** and **2c** (bearing the C₁₈- or C₄-alkyl side chains, respectively) from common intermediates **10a** and **10c** (Scheme 5). Careful oxidation of common intermediate **10a** with IBX in the presence of pyridine afforded unstable aldehyde **16a**, which was then treated with freshly prepared (*tert*-butyldimethylsiloxy)propylmagnesium bromide and dry CeCl₃ 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}



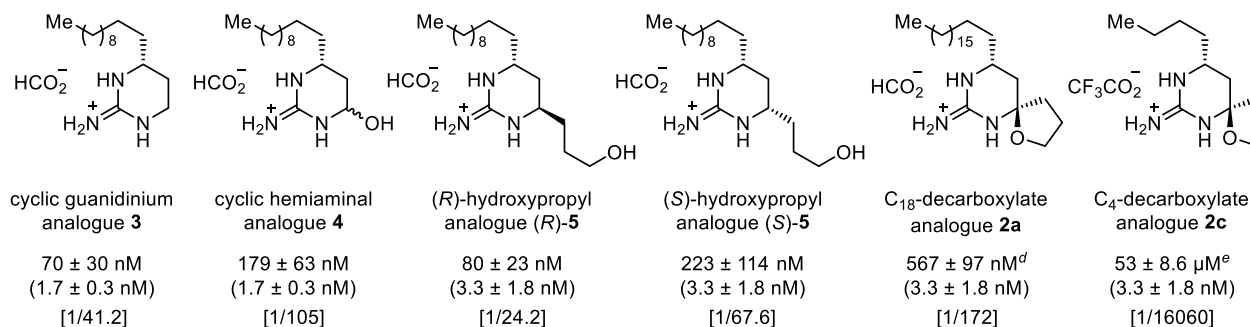
Scheme 5. Synthesis of bicyclic analogues **2a** and **2c**

We next investigated inhibitory activities on VGSCs of these six analogues using a cell-based colorimetric assay¹² (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 EC₅₀, 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 C₁₁-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 C₁₁-alkyl side chain probably be essential for the potent inhibitory activity.

(A) Analogues synthesized in the previous work



(B) Analogues newly synthesized in this work



^aMean ± S.D. (n = 3-5). EC₅₀ of TTX in parentheses. ^bRelative inhibitory activity to TTX in brackets. ^cNot detected because of its low inhibitory activity at concentrations above 100 μM (n = 5). ^dA 91:9 mixture of aminal epimers was used. ^eA 90:10 mixture of aminal epimers was used.

Figure 4. Inhibitory activities (EC₅₀) values of synthesized crambescin analogues^{a,b}

In conclusion, we described the synthesis of six simplified analogues of crambescin B carboxylic acid starting 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 C₄-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.

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Scientific Research (B) (No.16H04915) and Grants-in-Aid for Scientific Research on Innovative Areas “Frontier Research on Chemical Communication” (No.17H06406) from MEXT.

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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