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+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\cite{footnote} (Figure 2).

In this report, we describe details of their synthesis and biological activities using cell-based colorimetric assay.

Our synthetic plan towards the simplified crambscin 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.\cite{footnote} 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.\cite{footnote} 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.\cite{footnote}
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 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·SMe₂ to furnish the corresponding adduct 8a in 84% yield. Deprotection of Boc group of 8a and subsequent guanylation under conventional conditions 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\(_3\)-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.

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\text{Scheme 3. Synthesis of hydroxypropyl analogue (R)-5}
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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\(_3\)SiH resulted in the formation of cyclic guanidinium analogue \(3\) in 79% yield (entry 1). Similar reactions with anisole and potassium triethylsilyl ethynyl 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

<table>
<thead>
<tr>
<th>entry</th>
<th>nucleophile</th>
<th>solvent</th>
<th>R</th>
<th>X</th>
<th>product</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et$_3$SiH</td>
<td>CH$_2$Cl$_2$</td>
<td>3</td>
<td>HCO$_2$</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>OMe</td>
<td>CH$_2$Cl$_2$</td>
<td>13</td>
<td>Cl</td>
<td>84 (dr = &gt;95:&lt;5)</td>
<td></td>
</tr>
<tr>
<td>3°</td>
<td>TES-——BF$_3$·K</td>
<td>MeCN</td>
<td>14</td>
<td>HCO$_2$</td>
<td>21 (dr = &gt;95:&lt;5)</td>
<td></td>
</tr>
</tbody>
</table>

*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
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-butylidimethylsiloxy)propylmagnesium bromide and dry CeCl\textsubscript{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.\textsuperscript{6a,b

\textbf{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\textsuperscript{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\textsuperscript+K\textsuperscript+-ATPase, respectively. And then, the inhibitory activity on VGSCs of a synthesized analogue is evaluated by the concentration of EC\textsubscript{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),\textsuperscript{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\textsubscript{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\textsubscript{11}-alkyl side chain probably be essential for the potent inhibitory activity.

![Figure 4. Inhibitory activities (EC\textsubscript{50}) values of synthesized crambescin analogues\textsuperscript{a,b}](image)
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.

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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 online version, as URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/27208/105/1.

REFERENCES AND NOTES


