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Design, total synthesis, and biological evaluation of neodysiherbaine A derivative as potential probes

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Abstract—To enable studies to elucidate the detailed biological function of dysiherbaine and neodysiherbaine A, potent and subunit-selective agonists for ionotropic glutamate receptors, the derivative with a hydroxymethyl substituent at the C10 position has been developed. Preliminary biological evaluation of the analogue showed that a C10 hydroxymethyl substituent produced significant alterations in binding affinities for the ionotropic glutamate receptor subtypes.

Glutamate receptors (GluRs) play a central role in the mammalian central nervous system (CNS), not only in excitatory neurotransmission but also in complex brain functions such as learning and memory. GluRs are broadly divided into ionotropic and metabotropic receptors. Ionotropic GluRs (iGluRs) are further subdivided into three subtypes on the basis of their pharmacological preference toward selective agonists: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainate, and *N*-methyl-D-aspartic acid (NMDA) receptors.[1] Molecular cloning studies demonstrated that iGluRs are encoded by at least six NMDA (NR1, NR2A-2D, and NR3A), four AMPA (GluR1-4), and five kainate (GluR5-7 and KA1-2) receptor genes.[2]

Dysiherbaine (**1**, Fig. 1),[3] isolated from the Micronesian sponge, *Dysidea herbacea*, is a remarkable excitatory amino acid with potent convulsant activity. Dysiherbaine activates AMPA and kainate receptors, with a higher affinity for kainate receptors, but shows no detectable affinity for NMDA receptors.[4] Furthermore, it has been revealed that dysiherbaine had extremely high affinity for recombinant GluR5 or GluR6 kainate receptors

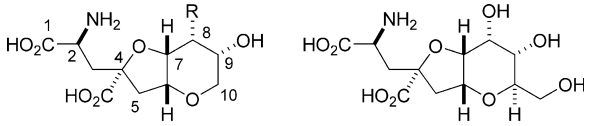

Figure 1. Structures of dysiherbaine (**1**), neodysiherbaine A (**2**), and 10-hydroxymethyl-neodysiherbaine A (**3**).

but very low affinity for KA2 receptors, which produced unusual biophysical behavior from heteromeric kainate receptors.[5] However, exact mode of interaction is still elusive. Neodysiherbaine A (**2**, Fig. 1), a closely related natural congener isolated as a minor constituent from the same sponge, differs from dysiherbaine in the C8 functional group and is also a selective agonist for AMPA and kainate receptors.[6] Recently, it has been shown that neodysiherbaine A is similar to dysiherbaine in its pharmacological activity on kainate receptors, albeit with slightly different binding affinities for individual receptor subunits.[7]

Due to their unprecedented molecular structures and unique biological profiles, dysiherbaine and neodysiherbaine A have attracted much attention among organic and biological communities.[6,8–11] Especially, the exceedingly high affinity of dysiherbaines for GluR5 receptors

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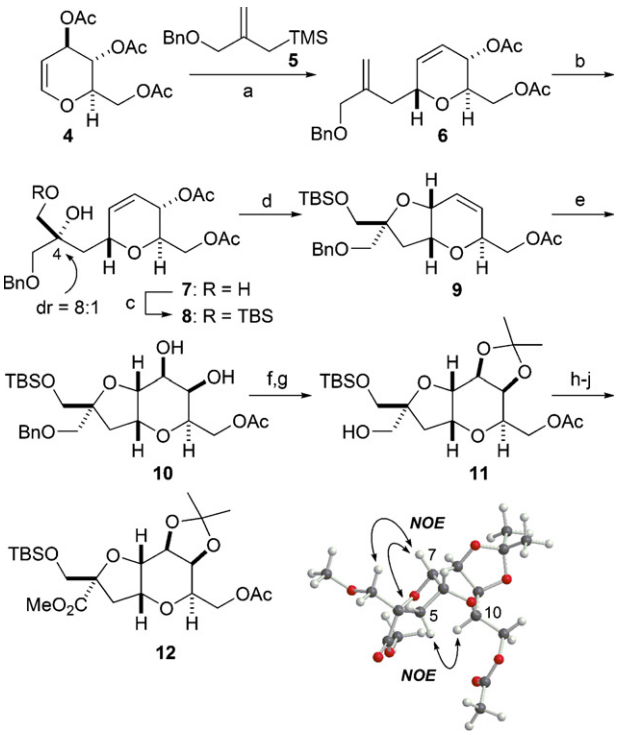
suggests that their designed analogues could be a useful probe in studying previously inaccessible synaptic sites, at which GluR5 receptors play a functional role. Accordingly, we have described the total synthesis of dysiherbaine, neodysiherbaine A, and their structural analogues, and structure–activity relationship (SAR) studies.[6,8b,10] These SAR studies showed that both the type and stereochemistry of the C8 and C9 functional groups affected the subtype selectivity of dysiherbaines for members of the kainate receptor family. Some analogues showed about 600-fold difference in affinities for GluR5 over GluR6 in the binding assay.[10c] Although these analogues could serve as interesting tools in neurobiology, appropriate labeling, preferably, non-radioisotope (RI) labeling would expand their use. However, selective small-molecule GluR agonists have resisted to be labeled externally, for example, by fluorescent groups, due to their small molecular size where no extra positions are available for labeling. In the case of dysiherbaine, however, earlier modeling experiments[7] suggested that the terminal methylene unit at C10 of dysiherbaines appears to contribute little to the binding for GluRs, and introduction of some functional group at C10 seems to be achieved without serious loss of its binding property to GluRs. Hence, we designed the C10 hydroxymethyl-substituted derivative **3** (Fig. 1) of neodysiherbaine A as a precursor of 'tagged' dysiherbaine.

In this letter, we describe a synthesis of the derivative with a hydroxymethyl substituent at the C10 position of neodysiherbaine A for the development of potential molecular probes and show that this molecule still retained high affinity for AMPA/kainate receptors while some unexpected group selectivity took place.

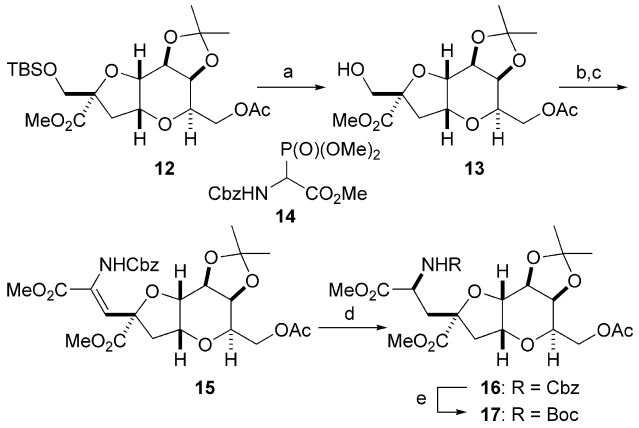
According to the previous total synthesis of neodysiherbaine A (**2**) and its analogues,[10b,c] the synthesis of **3** started with C-glycosylation of allylsilane **5**[12] with tri-*O*-acetyl-D-glucal (**4**). Reaction of **4** with **5** in the presence of $Yb(OTf)_3$ (10 mol %) in CH_2Cl_2 at room temperature gave the desired *C*-glycoside **6** in 83% yield as the sole product (Scheme 1).[13] Subsequent asymmetric dihydroxylation of **6** with $(DHQD)_2AQN$[14] as a chiral ligand proceeded smoothly to afford the desired diol **7** in good yield and diastereoselectivity (80%, 8:1 dr). Although the stereochemistry at the C4 position could not be determined at this stage, the major product was tentatively assigned based on the previous results.[10c] Selective protection of the primary hydroxy group of **7** gave the TBS ether **8** in 87% yield. Our first attempt to construct the bicyclic ether skeleton focused on stereoselective epoxidation of the double bond followed by acid-catalyzed 5-*exo* ring-closure.[10b] However, attempted epoxidation of **8** was not successful, resulting in recovery of the starting material. We next carried out the formation of a tetrahydrofuran ring by palladium(0)-catalyzed π-allyl ring closure.[15] After some experiments, it was found that the best result was obtained by treatment of **8** with catalytic $Pd_2(dba)_3$ in the presence of neocuproine as a ligand. Thus, the desired bicyclic ether **9** was obtained in excellent yield. Subsequent dihydroxylation of **9** with OsO_4/NMO produced *cis*-diol **10** exclusively, which was protected as the acetonide and then subjected to hydrogenolysis to give primary alcohol **11**. At this stage, the minor diastereomer at the C4 quaternary center was readily removed by silica gel chromatography. Oxidation of **11** by a two-step procedure led to the corresponding acid, which was then esterified with trimethylsilyldiazomethane to afford methyl ester **12**. The stereostructure of **12** was confirmed by NOE experiments as shown in Scheme 1.



Scheme 1. Reagents and conditions: (a) compound **5**, $Yb(OTf)_3$, CH_2Cl_2, rt, 83%; (b) cat OsO_4, $(DHQD)_2AQN$, K_2CO_3, $K_2[Fe(CN)_6]$, $MeSO_2NH_2$, *t*-BuOH/H_2O, 0 °C, 83% (8:1 dr); (c) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 92%; (d) $Pd_2(dba)_3$, neocuproine, toluene, 60 °C, 98%; (e) cat OsO_4, NMO, acetone/H_2O, rt, 92%; (f) DMP, CSA, CH_2Cl_2, rt, 92%; (g) H_2, Pd/C, hexane, rt, 85%; (h) SO_3·pyridine, Et_3N, DMSO, CH_2Cl_2, rt; (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O, rt; (j) $TMSCHN_2$, benzene/MeOH, rt, 75% (three steps).

For the construction of the amino acid side chain, **12** was converted to enamide ester **15**. After removal of the TBS ether of **12**, the resultant primary alcohol **13** was oxidized under Swern conditions and the derived aldehyde was subjected to Horner–Wadsworth–Emmons (HWE) reaction using phosphonate **14**[16] in the presence of tetramethylguanidine (TMG) (Scheme 2). The requisite enamide ester **15** was obtained in 77% yield over two steps. Subsequent asymmetric hydrogenation using Burk's (*S*,*S*)-EtDuPHOS Rh(I) catalyst[17] under high pressure conditions proceeded in a highly stereoselective manner to afford the desired amino acid derivative **16** in 74% yield as the sole product. Finally, the Cbz group was replaced with the Boc group (H_2, Pd/C, $(Boc)_2O$, MeOH, 85%) to afford intermediate **17**.

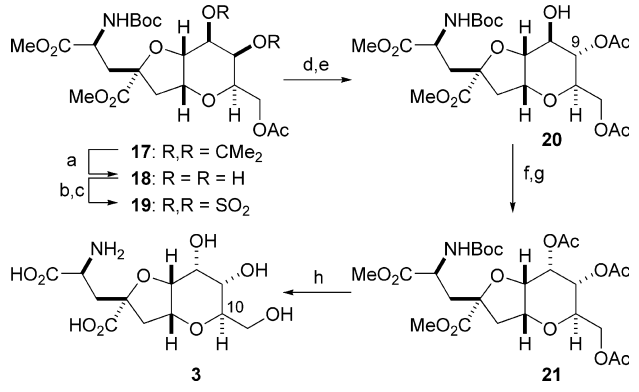
Selective removal of the acetonide of **17** with DDQ[18] provided diol **18** in modest yield (49%), which was then



Scheme 2. Reagents and conditions: (a) TBAF, THF, rt, 100%; (b) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, $-78 \rightarrow 0$ °C; (c) compound **14**, TMG, CH_2Cl_2, 0 °C, 77% (two steps); (d) H_2 (0.8 MPa), [Rh(I)(COD)(*S*,*S*)-Et-DuPHOS]$^+$OTf$^-$ (5 mol %), THF, rt, 74%; (e) H_2, Pd/C, $(Boc)_2O$, MeOH, rt, 85%.

converted to the cyclic sulfonate **19** by a two-step procedure in 69% overall yield (Scheme 3).[19] Treatment of **19** with cesium acetate effected regioselective substitution at the C9 position to generate alcohol **20** after acid hydrolysis of the derived sulfonate ester in 85% yield over two steps. Inversion of the hydroxy group of **20** was next carried out by nucleophilic substitution. Thus, alcohol **20** was converted to the triflate and subsequently treated with cesium acetate to afford diacetate **21** in 51% overall yield. Finally, acid hydrolysis of **21** (6 M HCl, 80 °C) completed the synthesis of the targeted 10-hydroxymethyl-neodysiherbaine A (**3**) in 90% yield.[20] The present synthesis is sufficiently efficient and allows us to prepare derivative **3** in several hundred milligrams.

The in vivo toxicity of derivative **3** against mice was tested by intracerebroventricular injection (Table 1). Compound **3** induced dose-dependent behavioral changes in mice with the ED_{50} value of 110 pmol/mouse, which is 7-fold less active than that for **2** (16 pmol/mouse). Interestingly, the behavioral profile of **3** was substantial-



Scheme 3. Reagents and conditions: (a) DDQ, MeCN/H_2O, 40 °C, 49%; (b) $SOCl_2$, Et_3N, CH_2Cl_2, −20 °C; (c) $RuCl_3$, $NaIO_4$, CCl_4/MeCN/H_2O, rt, 69% (two steps); (d) CsOAc, DMF, rt; (e) cat H_2SO_4, THF, rt, 85% (two steps); (f) Tf_2O, pyridine, DMAP, CH_2Cl_2, −20 °C; (g) CsOAc, DMF, rt, 51% (two steps); (h) 6 M HCl, 80 °C, 90%.

Table 1. Epileptogenic activity of natural dysiherbaines (**1** and **2**) and derivative **3**

Compound	ED_{50} pmol/mouse (icv)
1[a]	13
2[b]	16
3	110

[a] Ref. 4.
[b] Ref. 6.

ly different from those of the natural products **1** and **2**. Stereotyped behaviors, such as persistent scratching or clonic convulsions, frequently observed after administration of **1** and **2**, were absent in the case of **3**. Instead, transient jumping and running behaviors were apparent.

Next, the binding affinity of **3** was evaluated with native ionotropic glutamate receptors by radioligand binding assays using rat synaptic membrane preparation.[4] The results are summarized in Table 2. It is noteworthy that **3** displaced [^{3}H]AMPA more potently than [^{3}H]kainic acid from the receptors. Derivative **3** displaced the [^{3}H]AMPA with K_i value of 153 ± 35 nM, which is comparable to that of the natural neodysiherbaine A (**2**), whereas **3** displaced [^{3}H]kainic acid with 15-fold less potency than **2**. As expected, **3** did not exhibit detectable affinity for NMDA receptors.

Not expectedly, the affinity of **3** for kainate receptors was attenuated significantly, whereas that for AMPA receptors retained. This drastic shift in subtype selectivity was not predictable from our earlier model.[7] However, the present result suggested that introduction of an additional polar group created another site for hydrogen bonding with some amino acid residue in AMPA receptors. Thus, the C10 position of dysiherbaines is another interesting site for further modification as analogous result has been recently reported by Chamberlin and coworkers.[11]

In conclusion, we have developed the derivative **3** with a hydroxymethyl substituent at the C-10 position of neodysiherbaine A. Preliminary biological evaluation revealed that the hydroxymethyl derivative **3** shows in vivo epileptogenic activity in mice with about seven times less potency than the natural product **2** with significant shift in receptor selectivity. This result may suggest that the C10 position of dysiherbaines is another interesting site for modification. Nevertheless, this molecule could still

Table 2. Receptor binding affinities of natural dysiherbaines (**1** and **2**) and derivative **3**[a]

Compound	K_i (nM)	
	[^{3}H]AMPA	[^{3}H]kainic acid
1[b]	153 ± 11	26 ± 4
2[c]	151 ± 32	52 ± 4
3	153 ± 35	790 ± 100

[a] Affinities for receptors (K_i, nM) were determined by the displacement of [^{3}H]AMPA and [^{3}H]kainic acid.
[b] Ref. 4.
[c] IC_{50} value from Ref. 6 was converted to the K_i value.

be considered as an interesting precursor for the probe as only a few examples of non-RI probes for GluRs are known. Detailed neurophysiological studies on **3** and preparation of its biotinylated or fluorescently labeled probes are in progress and will be reported in due course.

Acknowledgments

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20. Data for compound **3**: $[\alpha]_D^{20}$ +1.4 (*c* 0.07, H_2O); ^1H NMR (600 MHz, D_2O) δ 4.28 (br s, 1H), 4.14 (br s, 1H), 3.94 (m, 1H), 3.80 (dd, J = 3.6, 3.6 Hz, 1H), 3.68 (dd, J = 12.0, 9.6 Hz, 1H), 3.57 (br m, 1H), 3.49 (d, J = 12.0 Hz, 1H), 3.48 (dd, J = 12.6, 1.8 Hz, 1H), 2.58 (dd, J = 15.0, 1.8 Hz, 1H), 2.50 (d, J = 14.4 Hz, 1H), 2.12 (dd, J = 14.4, 3.6 Hz, 1H), 1.87 (dd, J = 15.0, 12.6 Hz, 1H); ^{13}C NMR (125 MHz, D_2O/CD_3OD = 15:1) δ 179.4, 173.2, 87.1, 82.6, 82.5, 73.3, 69.0, 66.1, 59.4, 53.2, 46.4, 39.8; HRMS (FAB) calcd for $C_{12}H_{18}NO_9$ [$(M-H)^-$] 320.0982, found 320.0982.