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Isolation, Structure Determination, and Synthesis of Neodysiherbaine A, a New Excitatory Amino Acid from a Marine Sponge

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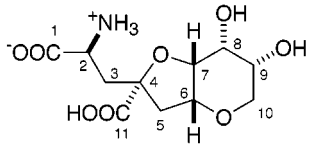
ABSTRACT



2 : neodysiherbaine A

A new excitatory amino acid, neodysiherbaine A (2), was isolated as a minor constituent of the aqueous extract from the marine sponge *Dysidea herbacea*. The structure was deduced by spectroscopic methods and established unambiguously by the total synthesis. The present synthesis, including as a key step cross-coupling of the 6/5-bicyclic core with an amino acid residue, is useful in constructing its structural analogues.

In the course of our study to find neurologically active secondary metabolites from marine organisms, we have isolated a novel glutamate receptor agonist, dysiherbaine (**1**), as a potent epileptogenic amino acid from the marine sponge *Dysidea herbacea*.[1] Dysiherbaine induces characteristic epilepsy-like seizures in mice and has been characterized to be the most potent epileptogenic excitatory amino acid yet identified. Dysiherbaine activates neuronal non-NMDA type glutamate receptors (GluR), namely, AMPA and kainic acid (KA) receptors with considerable preference over KA receptors.[2] Moreover, Swanson and co-workers have recently demonstrated highly unusual actions of **1** toward recombinant KA receptors; in that, **1** could differentially activate one of the activation sites within the subunit of a heteromeric GluR5/KA2 receptor complex.[3] This discrete affinity of **1** has enabled characterization of the unexpectedly complex behavior of the heteromeric KA receptors. Because of these unusual pharmacological properties of **1** to KA receptors, and its potent epileptogenic action, **1** is anticipated to be a useful tool to investigate GluR in the central nervous system. These biological properties of **1** as well as the unique structure have attracted the keen attention of synthetic

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(2) Sakai, R.; Swanson, G. T.; Shimamoto, K.; Contractor, A.; Ghetti, A.; Tamura-Horikawa, Y.; Oiwa , C.; Kamiya, H. *J. Pharm. Exp. Ther.* **2001**, *296*, 655−663.
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chemists. Recently, several groups, including us,[4] have independently accomplished the total synthesis of **1**.[5] This work not only confirmed its chemical structure but also defined the absolute stereochemistry. However, further chemical studies are required to uncover the structure−activity relationships (SAR) of **1**, which may lead to development of more selective GluR ligands.

We therefore searched for minor neuroactive components in the aqueous extract of *D. herbacea* using the mouse assay as a guide and found a new dysiherbaine analogue, neodysiherbaine A (**2**), as a minor constituent. We now report the isolation, structure determination, and total synthesis of **2** (Figure 1).

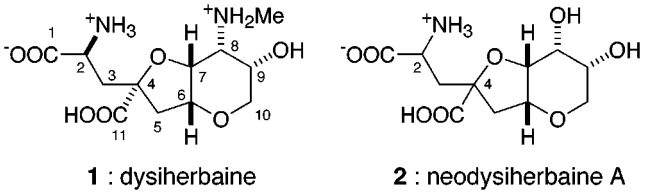
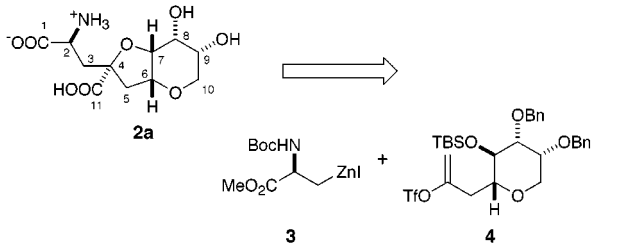


Figure 1. Structures of dysiherbaine (**1**) and neodysiherbaine A (**2**).

Dysidea herbacea collected in Yap State, Micronesia, in July 1998 was homogenized with water. The aqueous extract was separated by a series of gel filtration chromatography (Sephadex LH20 and BioGel P2). Fractions were pooled according to their TLC profile and bioactivity. Purification of the most active fraction afforded **1** (70 ppm of the wet sponge). Another bioactive fraction eluted before **1** on the BioGel P2 column was separated by DE52 anion exchange chromatography (Whattman). The UV (342 nm), ^{1}H and ^{13}C NMR, and ESIMS (*m*/*z* 330) spectra of the bioactive fraction indicated that it contained shinorine (or mytilin A), a ubiquitous mycosporine,[6] as a major component. However, an authentic compound presented by Prof. H. Nakamura of Nagoya University confirmed that shinorine was not the bioactive principal of this fraction. We thus suspected the presence of a minor active principal, and finally, purification by HPLC (C18) of this fraction afforded a small amount of the active component, neodysiherbaine A (**2**, 0.26 mg).[7] FABMS of **2** showed a molecular ion at *m*/*z* 290. From high-resolution FABMS data, *m*/*z* 290.0865 ([M − H]$^-$), its formula was deduced to be $C_{11}H_{17}NO_8$. In the ^{1}H NMR, the overall spectral pattern of **2** was similar to that of **1**, indicating that the structure of **2** is closely related to that of **1**, although a significant difference, lack of a methyl signal, was evident. A COSY experiment assigned all the non-exchangeable proton signals, whereby large differences in chemical shifts were observed in some signals for **1** and **2**; H-8 ($\Delta\delta_{1-2} = -0.2$), H-7 (+0.2), and H-9 (+0.3). These shifts along with the difference in molecular formulas between **1** and **2** (less CH_3N with an additional oxygen in **2** in comparison with **1**) allowed us to assign a hydroxyl group at C8. The relative stereochemistry of the perhydrofuro[*b*]-pyran ring is assigned to be the same as that for **1**, since the coupling pattern of all the protons on this ring system is nearly identical to that of **1**. However, we could not determine the stereochemistry at quaternary C4 and correlate stereochemistry at C2 to the bicyclic portion because a minute amount of **2** hampered further 2D NMR studies. We thus carried out the total synthesis of **2** to resolve the remaining stereochemical ambiguities and ultimately provide access to additional material for biological evaluation.

We decided to synthesize **2a** as the most likely candidate. The present synthesis relied on a strategy developed for the total synthesis of **1**,[5] which was designed to be applicable to a variety of its analogues. We envisioned that **2a** would be constructed by cross-coupling of organozinc compound **3** and vinyl triflate **4**, which would be accessed from a carbohydrate precursor (Scheme 1).

Scheme 1. Retrosynthesis of NeodysiherbaineA (**2a**)



Synthesis of vinyl triflate **4** commenced with tri-*O*-acetyl-D-glucal (**5**), which was converted into olefin **6** by a three-step sequence of reactions in 84% overall yield (Scheme 2). Dihydroxylation of **6** proceeded stereoselectively to give α-diol **7** in 79% yield along with 17% yield of the corresponding *β*-diol. Routine protective group manipulations led to alcohol **8** (76% overall), which upon oxidation and Wittig reaction provided terminal olefin **9** in 83% yield. Hydroboration followed by oxidative workup afforded primary alcohol **10** (95% yield), which was then converted to methyl ketone **11** in 83% overall yield. Kinetic deprotonation (KHMDS, THF, −78 °C) followed by treatment of the derived enolate with Tf_2NPh provided the desired enol triflate **4**, which was immediately used in the crucial coupling reaction.

Treatment of **4** with organozinc reagent **3**[8] in the presence of $PdCl_2(PPh_3)_2$ in THF−DMA (1:1) according to the reported method[4] furnished the desired cross-coupled product

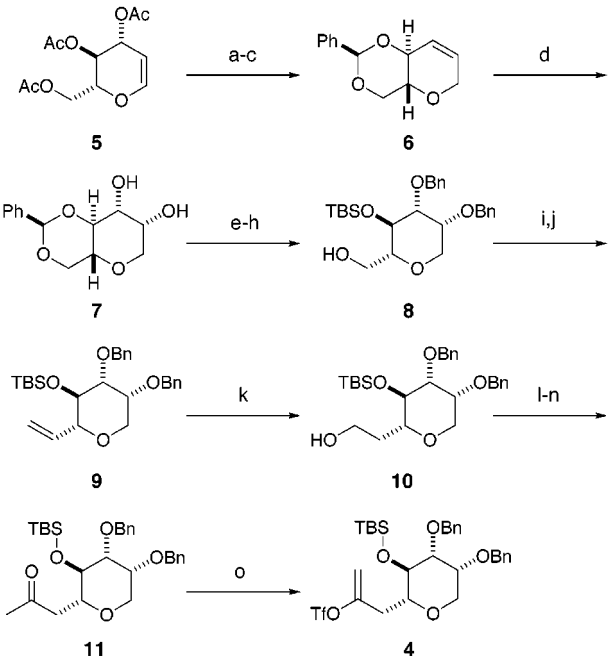
(4) Sasaki, M.; Koike, T.; Sakai, R.; Tachibana, K. *Tetrahedron Lett.* **2000**, 3923−3926.

(5) (a) Snider, B. B.; Hawryluk, N. A.*Org. Lett.* **2000**, *2*, 635−638. (b) Masaki, H.; Maeyama, J.; Kamada, K.; Esumi, T.; Iwabuchi, Y.; Hatakeyama, S. *J. Am. Chem. Soc.* **2000**, *122*, 5216−5217.

(6) Chioccara, F.; Misuraca, G.; Novellino, E.; Prota, G. *Tetrahedron lett.* **1979**, 3181−3182.

(7) CD (H_2O) λ_{ext} 204 nm, $\Delta\epsilon = 1.7$; ^{1}H NMR (400 MHz, D_2O at 20 °C, HOD at δ 4.65 as an internal reference) δ 4.07 (brs, 1H, H-7), 3.99 (brs, 1H, H-6), 3.76 (t, 1H, $J = 3.7$ Hz, H-8), 3.71 (dd, 1H, $J = 1$ 2.6, 2.5 Hz, H-10a), 3.55 (brs, 1H, H-9), 3.42 (brd, 2H, $J = 12.7$ Hz, H-10b, H-2), 2.50 (dd, 1H, $J = 14.9$, 1.5 Hz, H-3a), 2.42 (d, 1H, $J = 14.2$ Hz, H-5a), 2.01(dd, 1H, $J = 14.0$, 3.4 Hz, H-5b), 1.81 (dd, 1H, $J = 15.1$, 12.1 Hz, H-3b).

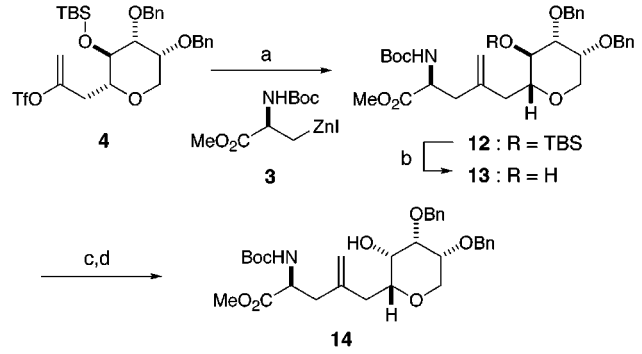
Scheme 2. Synthesis of Enol Triflate **4**[a]



[a] Reagents and conditions: (a) Et_3SiH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0 °C; (b) NaOMe, MeOH, rt; (c) $PhCH(OMe)_2$, CSA, CH_2Cl_2, rt, 84% (three steps); (d) OsO_4, NMO, acetone−H_2O, rt, 79% (+ β-diol, 17%); (e) NaH, BnBr, DMF 0 °C → rt; (f) CSA, CH_2Cl_2−MeOH, 0 °C, 99% (two steps); (g) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt; (h) CSA, CH_2Cl_2−MeOH, 0 °C, 85% (two steps); (i) $SO_3{\cdot}$Pyr, Et_3N, DMSO, CH_2Cl_2, rt; (j) $Ph_3P^+CH_3Br^-$, NaHMDS, THF, 0 °C, 83% (two steps); (k) 9-BBN, THF, rt, then $NaHCO_3$, H_2O_2, rt, 95%; (l) $SO_3{\cdot}$Pyr, Et_3N, DMSO, CH_2Cl_2, rt; (m) MeMgBr, THF, −78 → 0 °C; (n) TPAP, NMO, 4 Å molecular sieves, CH_2Cl_2, rt, 83% (three steps); (o) KHMDS, THF, −78 °C, then Tf_2NPh, −78 → 0 °C.

12 in 52% yield from **11** (Scheme 3). Subsequent desilylation provided secondary alcohol **13** in 83% yield. Oxidation with Dess−Martin periodinane[9] followed by $NaBH_4$ reduction at low temperature led to alcohol **14** as a single stereoisomer in 84% yield for the two steps.

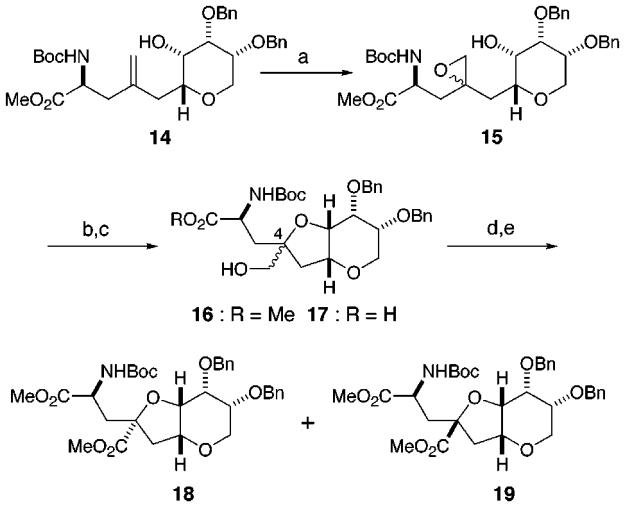
Scheme 3[a]



[a] Reagents and conditions: (a) **3**, $PdCl_2(PPh_3)_2$, THF−DMA, 60 °C, 52% from **11**; (b) *n*-Bu_4NF, THF, rt, 83%; (c) Dess−Martin periodinane, $NaHCO_3$, CH_2Cl_2, rt; (d) $NaBH_4$, THF−MeOH, −78 → 0 °C, 84% (two steps).

Elaboration of **14** to the fully protected neodysiherbaine A (**18**) was carried out according to the previous synthesis of **1**.[4] Thus, epoxidation of **14** with *m*-chloroperbenzoic acid (*m*CPBA) in CH_2Cl_2−pH 7.0 phosphate buffer (1:1) produced epoxide **15** as a 1:1 mixture of diastereomers in 88% yield (Scheme 4). Upon treatment of this mixture with

Scheme 4[a]



[a] Reagents and conditions: (a) *m*CPBA, CH_2Cl_2−pH 7.0 phosphate buffer, rt, 88%; (b) CSA, CH_2Cl_2, rt; (c) 1 N NaOH, THF, rt, 46% (two steps); (d) TPAP, NMO, 4 Å molecular sieves, CH_3CN, rt; (e) $TMSCHN_2$, MeOH, rt **18**: 43% (two steps) **19**: 24% (two steps).

camphorsulfonic acid (CSA), 5-*exo*-ring closure proceeded to yield an inseparable mixture of 6/5-bicyclic alcohol **16** and its δ-lactone, which was then hydrolyzed with aqueous NaOH to give hydroxy acid **17** as an approximately 2:1 mixture of diastereomers at C4 (46% yield from **15**). The mixture of isomers was subjected to oxidation with TPAP/NMO[10] followed by treatment with trimethylsilyldiazomethane and chromatography on silica gel to give protected neodysiherbaine A (**18**) and its C4 epimer **19** in 43% and 24% overall yields, respectively. The stereochemistry at C4 of **18** was unambiguously determined by NOEs between H_2-3/H-5β and H-5β/H-6.

Finally, hydrogenolysis of the benzyl groups of **18** provided diol **20** (77% yield), which was hydrolyzed with 6 N HCl to obtain the targeted **2a** as its hydrochloride salt in 99% yield (Scheme 5). The ^{1}H NMR spectra and biological activity of synthetic **2a** were identical with those of the natural neodysiherbaine A. In addition, the CD spectrum measured for synthetic **2a** showed close agreement with that for the natural sample. Thus, the complete structure, includ-

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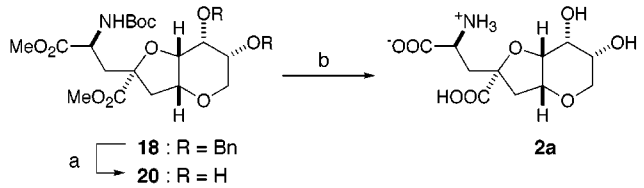
(9) (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7278. (b) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899−2900.
(10) Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639−666.

Scheme 5[a]



[a] Reagents and conditions: (a) H_2, Pd/C, MeOH, rt, 77%; (b) 6 N HCl, 60 °C, 99%.

ing the absolute configuration, of neodysiherbaine A was unambiguously determined as depicted in structure **2a**. 4-Epineodysiherbaine A was also synthesized from **19** in 84% overall yield in the same manner as described for the synthesis of **2a** from **18**.

Neodysiherbaine A (**2**) induced dose-dependent behavioral changes in mice. The ED_{50} value of **2** (15 pmol/mouse) was comparable to that of **1** (13 pmol/mouse), although some behavioral patterns observed for **2** were distinguishable from that for **1**. Epileptogenic activity of 4-epineodysiherbaine A (ED_{50} = 11.4 nmol/mouse) was much less potent than that of **2**. In our radioligand binding study using rat synaptic membrane preparation, **2** displaced [^{3}H]KA and [^{3}H]AMPA at IC_{50} values of 66 ± 5.2 and 227 ± 40 nM, respectively, but not [^{3}H]CGP39653, an NMDA receptor ligand. In the same assay, the corresponding values for **1** were 33 ± 5.1 and 230 ± 15 nM, respectively. It is of particular interest whether **2** shows discrete affinity within the subtypes of the recombinant hetereomeric KA receptors. Detailed neurological properties of **2** will be published elsewhere.

In summary, we have isolated a new excitatory amino acid, neodysiherbaine A (**2**). The structure as well as the absolute configuration was determined by spectroscopic methods. The synthesis described herein should allow for the preparation of various analogues of this excitatory amino acid, including radiolabeled compounds. Further studies along this line are underway and will be reported in due course.

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Supporting Information Available: Experimental procedures for the isolation of **2**; FABMS, ^{1}H NMR, and COSY spectra of **2**; experimental procedures for the synthesis of **2a**; comparison of ^{1}H NMR and CD spectra for **2** and **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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