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Synthesis of Denosomin−Vitamin D_3 Hybrids and Evaluation of Their Anti-Alzheimer's Disease Activities

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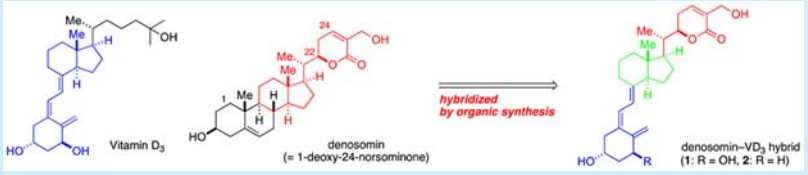
Supporting Information



ABSTRACT: As an extension of previously conducted studies on developing an anti-Alzheimer's disease agent, denosomin (1-deoxy-24-norsominone, an artificial inducer of neurite elongation), derivatives were designed and synthesized based on the hypothesis that our denosomin would exhibit axonal extension activity via a 1,25D_3-membrane-associated, rapid response steroid-binding protein (1,25D_3-MARRS) pathway. The biological assay revealed that the hybridization of characteristic δ-lactone in denosomin and the triene moiety in VD_3 was effective to enhance the nerve re-extension activity in amyloid β (Aβ)-damaged neurons.

Entering the era of an aging population, dementia has become one of the most serious problems in the world, and the number of affected patients has increased each year. The most common form of dementia is Alzheimer's disease (AD), and the number of patients in 2050 is predicted to reach 114 million worldwide, a 4-fold increase from 25 million in 2000.[1] Against such problems, several acetylcholinesterase inhibitors have been developed and prescribed for the clinical treatment of AD; however, those only address symptomatic treatment. Although recent studies revealed that amyloid β (Aβ) in the brain causes AD, Aβ targeting therapy could not recover from the advanced memory disorder. From the point of view of improving the injured brain function by reactivation and re-establishment of the damaged neuron network, neurotrophic agents are desirable, and a large number of potent natural products have been reported[2] since the isolation of lactacystin by Omura and co-workers.[3] In recent decades, steroids such as NGA0187[4] and withanolide A[5] were reported to exhibit potent neurite outgrowth activities. In the course of our studies on an Ayurvedic tonic medicine Ashwagandha, we also found that natural steroid sominone and its artificial, but more potent, derivative denosomin (1-deoxy-24-norsominone, Figure 1) showed significant axonal extension activity in Aβ-damaged neurons.[6] Recently, Tohda, one of the authors, revealed that diosgenin, a steroidal sapogenin, improved memory in AD model mice through the activation of the 1,25D_3-membrane-associated, rapid response steroid-binding protein (1,25D_3-MARRS).[7] Thus, we hypothesized that our denosomin also exhibited axonal extension activity via the 1,25D_3-MARRS pathway. If the hypothesis were correct, the CD-ring core equipped with a characteristic δ-lactone moiety in denosomin and the triene part mimicking VD_3 would possibly have a higher affinity with 1,25D_3-MARRS and would exhibit enhanced biological activity. In this manuscript, we describe the synthesis of a novel denosomin−VD_3 hybrid and the evaluation of axonal extension activity on Aβ-damaged neurons.

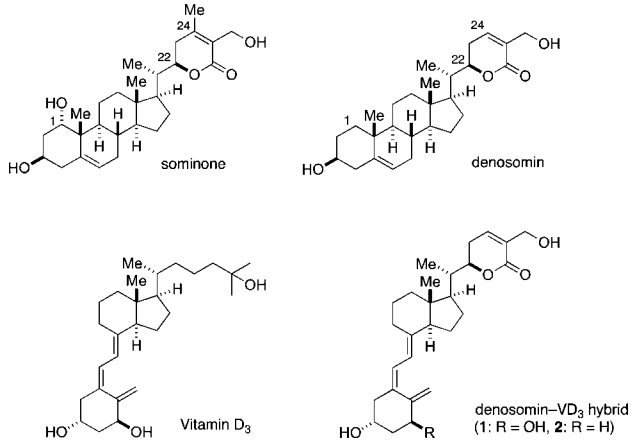


Figure 1. Denosomin and related compounds.

Our synthetic plan for denosomin−VD_3 hybrids (**1**: R = OH, **2**: R = H) is outlined in Scheme 1. A conjugated triene moiety would be constructed by a Trost coupling reaction[8] between CD-ring fragment **3** and known enyne **4**[9] (or **5**). Vinyl bromide **3** could be established from *trans*-hydrindan **6**[10] with

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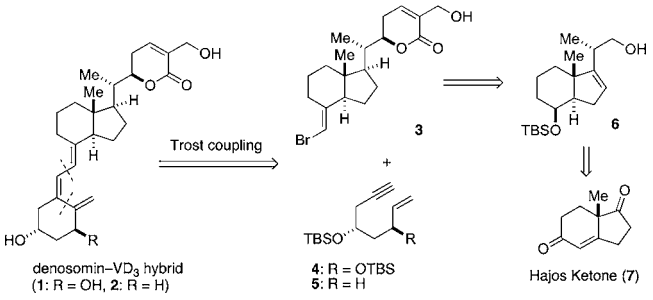
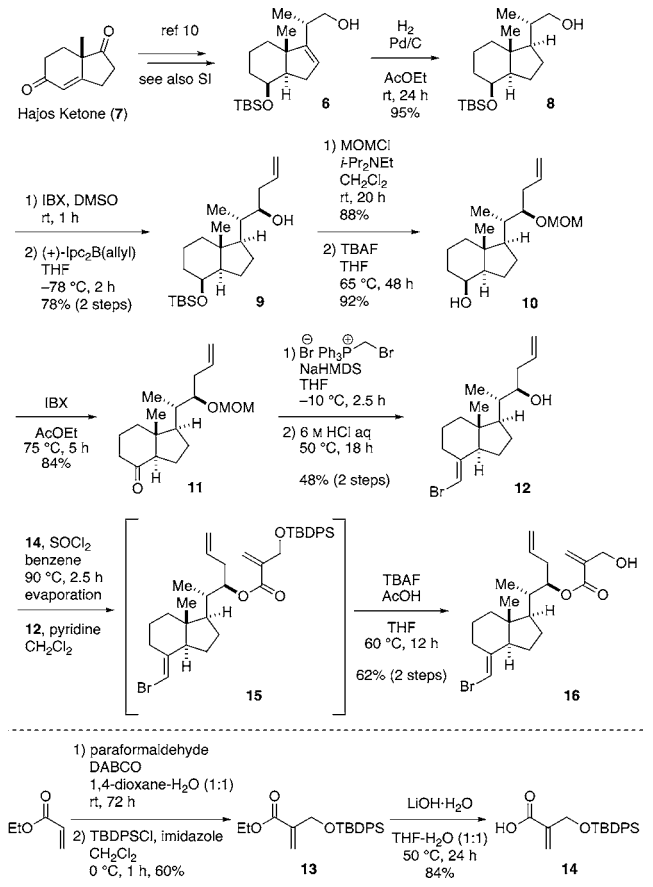
Scheme 1. Synthetic Plan for Denosomin−VD_3 Hybrids



modification of our previous work on the synthesis of denosomin.[6a] A ring precursor **4** and **5** could be prepared following the report by Takayama and co-workers.[9]

Hydrogenation of **6**, which was prepared from the Hajos Ketone (**7**) via known eight steps,[10] gave **8** in a diastereoselective manner (Scheme 2).[11] The primary hydroxyl

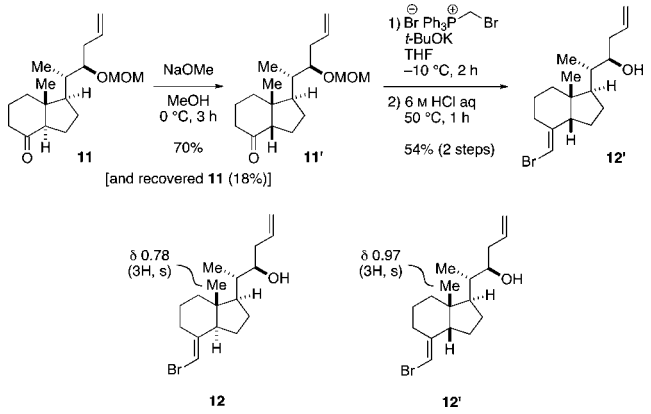
Scheme 2. Preparation of Diene 16



group was then oxidized with IBX, and subsequent asymmetric allylation of the resultant aldehyde afforded homoallylalcohol **9**. The ketone **11**, the precursor for vinyl bromide **12**, was obtained via MOM-protection of the secondary hydroxyl group, desilylation, and IBX oxidation. Wittig reaction of **11** with bromomethyltriphenylphosphonium bromide followed by acidic hydrolysis of MOM ether afforded **12**.[12] Silyloxymethacrylic acid **14**, which was readily prepared from ethyl acrylate through a Morita−Baylis−Hillman reaction, was condensed with **12**, and removal of the TBDPS group under neutral conditions gave diene **16**.

Despite the highly basic conditions included in the Wittig transformation from **11** to **12**, the *trans*-hydrindane scaffold in **11**, which occasionally isomerizes into thermodynamically stable *cis*-hydrindane under basic conditions,[13] was proven to be untouched through the process (Scheme 3). For stereo-

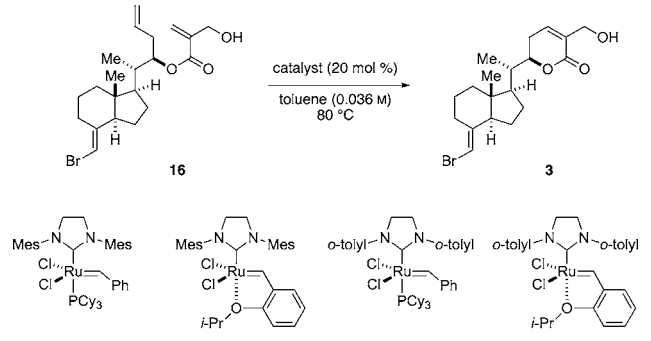
Scheme 3. Stereochemical Verification of Hydrindanes



chemical verification by ^{1}H NMR, *cis*-hydrindane **12′** was prepared by the consistent Wittig reaction/acidic deprotection sequence after the exposure of **11** to NaOMe/MeOH.[13] While the signal of the methyl group on the ring juncture in **12′** appears at 0.97 ppm as in the case of typical *cis*-hydrindanes, that for **12** was found at 0.78 ppm, which is typically observed in *trans*-hydrindanes.

Next, we examined a *δ*-lactone formation reaction of diene **16** by ring closing metathesis (RCM) with Ru-based catalysts (Table 1). Though Grubbs' second generation catalyst (**cat-1**)

Table 1. Ring Closing Metathesis for *δ*-Lactone



entry	catalyst	time (h)	yield (%)
1	**cat-1**	20	23
2	**cat-2**	7	57
3	**cat-3**	20	29
4	**cat-4**	5	61

gave the *δ*-lactone **3** in low yield (entry 1), Hoveyda−Grubbs' second generation catalyst (**cat-2**) smoothly afforded **3** in 57% yield. With sterically less demanding catalysts **cat-3**[14] and **cat-4**,[14] which were derivatives with the bis(*o*-tolyl) NHC ligand, the chemical yields were improved and desired **3** was furnished in 61% yield with **cat-4**.

On the other hand, enynes **4** and **5** were prepared from 3-buten-1-ol according to the procedures described by Takayama and co-workers (Scheme 4).[9] An eight-step transformation

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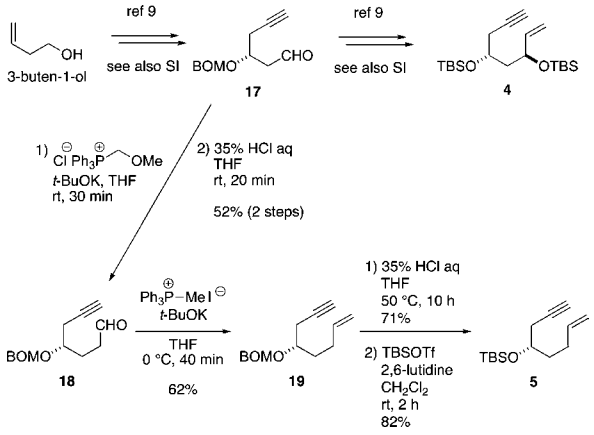
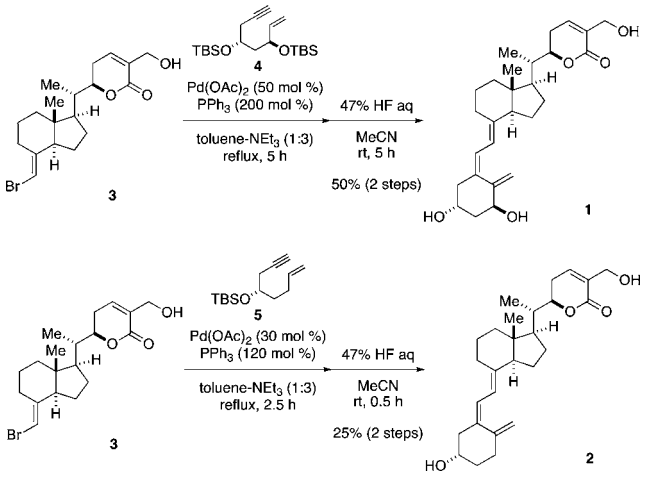
Scheme 4. Preparation of A Ring Precursors



from 3-butene-1-ol including Sharpless asymmetric dihydroxylation afforded the pivotal aldehyde **17**. Enyne **4**, the precursor of the dihydroxy A-ring unit, was synthesized through the literature methods, and monohydroxyenyne **5** could be obtained through chain elongation reactions of **17** via aldehyde **18**.

With the corresponding counterparts, a Trost-coupling reaction was examined between CD-ring core **3** and A ring precursor **4** or **5** (Scheme 5). In the presence of $Pd(OAc)_2$ and

Scheme 5. Synthesis of Denosomin–VD_3 Hybrids



PPh_3, the domino reaction proceeded smoothly to furnish desired coupling products, respectively. Removal of the TBS group with aqueous HF solution gave the hybrids **1** and **2**.

Finally, we evaluated the nerve re-extension activity of the hybrids in Aβ-damaged neurons compared with denosomin. Denosomin and hybrids **1** and **2** (0.1 μM or 1 μM each) were exposed to rat cortical neurons on the third day after treatment with 10 μM of Aβ (25–35), and the axonal densities were measured after further incubation for 4 days. As a result, we proved our hybrids exhibit more potent axonal re-extension activities than parent denosomin, especially in lower concentration, 0.1 μM (Figure 2A and B). These phenomena were consistent with our hypothesis that denosomin demonstrated the axonal extension activity via the 1,25D_3-MARRS pathway.

In conclusion, we designed and synthesized novel anti-Alzheimer's disease agents, denosomin–VD_3 hybrids, and proved their potency on axonal re-extension in rat cortical

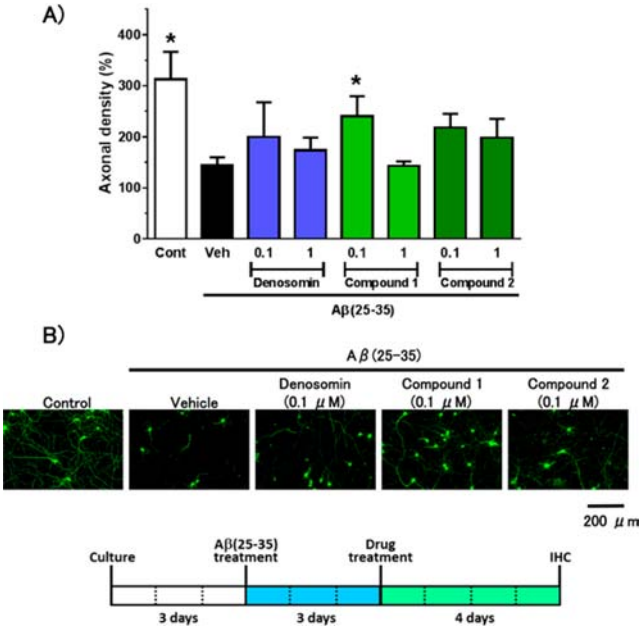


Figure 2. Biological evaluation of denosomin–VD_3 hybrids. (A) Effects on axonal density. (B) Representative fluorescent images of axons.

neurons. The result suggests that our anti-Alzheimer's disease agents exhibit nerve re-extension activity in Aβ-damaged neurons via the 1,25D_3-MARRS pathway. Based on these findings, further derivatizations and evaluations of denosomin–VD_3 hybrids are ongoing in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b03138.

Experimental procedure, spectral data, and copies of ^{1}H and ^{13}C NMR spectra for all novel compounds (PDF)

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Notes

The authors declare no competing financial interest.

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