A SYNTHESIS OF 2-*ENDO*-AMINO-2-*EXO*-HYDROXYMETHYLNORBORNENES HAVING INHIBITORY ACTIVITY AGAINST PROTEIN KINASE C

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2-*endo*-Hexadecylamino-2-*exo*-hydroxymethylnorbornene (**1a**) was synthesized from 2-acetamidonorbornene-2-carboxylic acid methyl ester (**2**) in a good overall yield. 2-*endo*-Hexadecylamino-2,3-*exo*-bis(hydroxymethyl)norbornene (**1b**) was synthesized starting from dimethyl acetamidofumarate based on Diels-Alder strategy. **1a** and **1b** inhibited protein kinase C at the IC₅₀ values of 2×10^{-5} M and 1×10^{-5} M, respectively, but not protein kinase A at a concentration of 1×10^{-3} M. The structure-activity relationships are discussed.

KEYWORDS protein kinase C; aminonorbornene; Diels-Alder reaction; amino alcohol

Protein kinase C, a pivotal enzyme in signal transduction and cell regulation, is activated physiologically by diacylglycerol produced by the receptor-mediated turnover of inositol phospholipids.¹⁾ This enzyme is also the principal target of phorbol esters²⁾ and other tumor promoters.³⁾ To elucidate the physiological role of protein kinase C and to develop agents that can selectively prevent the effect of tumor promoters, a number of natural⁴⁾ and synthetic inhibitors⁵⁾ have been investigated. Of these inhibitors, UCN-1028A, isolated from the culture broth of *Cladosporium cladosporioides*, was reported to be a specific inhibitor of this enzyme.⁶⁾ Recently, on the other hand, Hannun *et al.*⁷⁾ have reported that sphingosin and liso-sphingolipids possessing an amino alcohol skeleton inhibit protein kinase C and may function as endogenous modulators of cell function. In connection with our studies in search of a new compound having intriguing biological activities, we now report a synthesis of 2-*endo*-amino-2-*exo*-hydroxymethylnorbornene derivatives (**1a**, **1b**), sterically defined amino alcohols, which inhibit protein kinase C. The critical structural features required for inhibition of protein kinase C for the norbornene derivatives are also described.

In Chart 1 and 2 are shown the synthetic routes of **1a** and **1b**, respectively. **1a**⁸⁾ was synthesized in 33% overall yield from 2-*endo*-acetamidonorbornene-2-carboxylic acid methyl ester (**2**)⁹⁾ by deacetylation

with the Meerwein's reagent, followed by alkylation with hexadecyl bromide in HMPA in the presence of K_2CO_3 and subsequent $LiAlH_4$ reduction of the resulting hexadecylamino ester (**3**).

1b⁸⁾ was synthesized starting from dimethyl acetamidofumarate¹⁰⁾ derived from dimethyl *N*-acetylaspartic acid. Our study began with experiments to evaluate the reactivity of dimethyl acetamidofumarate as a dienophile toward cyclopentadiene. We first examined the reaction of **4** with cyclopentadiene in the absence of a catalyst under the reaction conditions used in the Diels-Alder reaction of *N*-acetyl- α,β -dehydroalaninate with cyclopentadiene.⁹⁾ However, no desired Diels-Alder adducts were formed. After an experiments using a number of acidic catalysts, we found that the reaction proceeded smoothly in CH_2Cl_2 with the use of $SnCl_4$ as a catalyst to furnish a mixture of **5** and **6** in 61 % yield. Each isomer was separated by column chromatography on silica gel, the ratio of **5** to **6** being 3:1; the structure of each isomer was unambiguously determined based on the NMR spectrum¹¹⁾ in which the methyl proton of the acetyl group of **5** shifts to a higher field than that of **6**, due to the shielding effect of the 5,6-double bond.¹²⁾ Treating of **5** with NaOMe in MeOH caused the epimerization at the 3-position leading to the formation of **7**, which was converted to **1b** by using the same procedures as those employed in the preparation of **1a**.

i) a: $Et_3O^+BF_4^-/CH_2Cl_2$, r.t., b: aqueous $NaHCO_3$ solution; ii) $CH_3(CH_2)_{15}Br$, K_2CO_3 /HMPA, r.t.~70°C; iii) $LiAlH_4/THF$, 5°C~r.t.; iv) HCl ; v) cyclopentadiene, $SnCl_4/CH_2Cl_2$, r.t.; vi) *N*-NaOMe/MeOH, r.t.

The inhibitory activity of **1a** and **1b** on protein kinase C prepared from rat brain cytosole was measured under the assay conditions reported by Kikkawa *et al.*¹³⁾ **1a** and **1b** inhibited protein kinase C at the IC_{50} values of 2×10^{-5} M and 1×10^{-5} M, respectively. On the other hand, no inhibitory activities on protein kinase A were observed for **1a** and **1b** at a concentration of 1×10^{-3} M. These results indicate that **1a** and **1b** selectively inhibit protein kinase C. We also examined the inhibition of the binding of [3H]-phorbol dibutyl ester (PDBU) to protein kinase C by compound (**1a**) *in vitro* under the assay conditions reported by Blackshear *et al.*¹⁴⁾ **1a** competitively inhibited the [3H]-PDBU binding to protein kinase C at the IC_{50} value of 2×10^{-5} M.

To determine the critical structural features of these compounds required for inhibition of protein kinase C for the norbornene derivatives, we further synthesized **9c-d**¹⁵⁾, **10**¹⁵⁾ and **11**¹⁵⁾ starting from **2**, the

stereoisomer of **2**⁹⁾ and 2,2-dimethylaminoethanol, respectively. Inhibition of protein kinase C still occurred when the hexadecyl group of **1a** was replaced by the palmitoyl group, the IC₅₀ value of **9c** being 5×10^{-5} M.

No inhibitory activities were observed for **9d** and **10**, strongly indicating that the 2-*exo*-hydroxyl and 2-*endo*-amino groups are crucial for exhibiting the inhibitory activity. Furthermore, the activity of **11** (IC₅₀ = 2×10^{-4} M), in which the norbornyl group was replaced by the dimethyl group, was significantly reduced. The result implies the significance of the norbornyl skeleton which is capable of not only forming a rigid hydrophobic region but also sterically defining both the amino and hydroxyl groups.

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- 11) The NMR spectra of **5** and **6** are as follows: **5**, δ (CDCl₃) 1.89 (3H,s,NHCOCH₃), 3.68 (3H,s,C₃-CO₂CH₃), 3.76 (3H,s,C₂-CO₂CH₃); **6**, δ (CDCl₃) 1.97 (3H,s,NHCOCH₃), 3.67 (3H,s,C₃-CO₂CH₃), 3.69 (3H,s,C₂-CO₂CH₃).
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- 15) The synthetic methods are as follows. **9c** (mp 73-74°C): i) Et₃O⁺BF₄⁻/CH₂Cl₂, rt.; aqueous NaHCO₃; ii) CH₃(CH₂)₁₄COCl, Et₃N/THF, rt.; iii) LiAlH₄/THF. **9d** (syrup) was prepared by acetylation of **9c** with Ac₂O-Et₃N. **10** (mp 66°C) was prepared from the stereoisomer of **2** by the same procedures as described in the preparation of **1a**. **11** (mp 57-58°C) was prepared by acylation of 2,2-dimethylaminoethanol with CH₃(CH₂)₁₄COCl-Et₃N.

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