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Synthesis and In Vivo Anti-Inflammatory Activity of Long-Chain 2-Amino-alcohols

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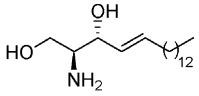
Abstract—The synthesis of optically pure long-chain 2-amino-alcohols and 1-*O*-dodecyl-2-deoxy-2-amino-*sn*-glycerol was carried out starting from L- or D-Boc-Ser(OBn)-ol by oxidation and consecutive Wittig reaction or etherification reaction. 2-Amino-oleyl alcohol was synthesized by reduction of the corresponding 2-amino-oleic acid. All the long chain amino-alcohols presented interesting inhibition of carrageenin-induced paw edema in rats (ED_{50} from 0.017 to 0.010 mmol/kg).

Introduction

Sphingosine, (2*S*,3*R*,4*E*)-2-amino-4-octadecene-1,3-diol, the lipid component that forms the structural backbone of all sphingolipids, has been shown to affect the activity of many important signaling enzymes, including serine/threonine protein kinases, phospholipase A_2, protein kinase C, protein phosphatase activity, and specific kinases that phosphorylate the epidermal growth factor (EGF) receptor.[1–3] The biological significance of sphingosine has stimulated much research on the synthesis of sphingosine analogues and study of their bioactivity (for examples, see refs 4–6).



Long-chain 2-amino alcohols are sphingosine analogues that have been reported to present interesting in vitro cytotoxicity,[7] immunosuppressive activity[8] and anti-Leishmanicidal activity.[9] Most recently it has been demonstrated that long-chain amino-alcohol and diamine derivatives induce apoptosis through a caspase-3 dependent pathway.[10] Long-chain 2-amino-alcohols may be prepared by reduction[11,12] of chiral lipidic α-amino acids[13–15] and may be used for the synthesis of long chain 1,2- and 1,3-diamines.[16] We present here a convenient method for the synthesis of saturated long-chain 2-amino-alcohols and *O*-alkyl ethers of 2-deoxy-2-amino-glycerol starting from serine, the synthesis of 2-amino-oleyl alcohol and the in vivo study of their anti-inflammatory and analgesic activity.

Chemistry

Commercially available L-Boc-Ser(Bn)-OH was chosen as starting material and the introduction of the long chain was based on a Wittig olefination reaction of the suitably protected serinal. Compound **1** was converted into alcohol **2** by reduction of its corresponding mixed anhydride with $NaBH_4$ (Fig. 1).[11] Alcohol **2** was oxidized in a two-phase system by NaOCl in the presence of a catalytic amount of 4-acetamido-TEMPO.[17] It is known that α-amino aldehydes present a high tendency for racemization.[18] However, it has been demonstrated that the oxidation of amino alcohols by NaOCl/TEMPO proceeds without racemization.[19]

The resulting aldehyde was immediately treated with the ylide, generated from tridecyl-triphenylphosphonium bromide and KHMDS, in toluene at 0 °C to afford the unsaturated derivative **4**. Under these reaction conditions, a *Z*/*E* (6:1) mixture of isomers was obtained, as shown by ^{1}H NMR spectroscopic analysis. Catalytic hydrogenation of the double bond of compound **4** with simultaneous removal of benzyl group produced 2-(*tert*-butoxycarbonylamino)-hexadecanol (**5**). After treatment of **5** with 4N HCl/Et_2O, (*R*)-2-amino-hexadecanol hydrochloride (**6**) was obtained. (*S*)-2-Amino-hexadecanol

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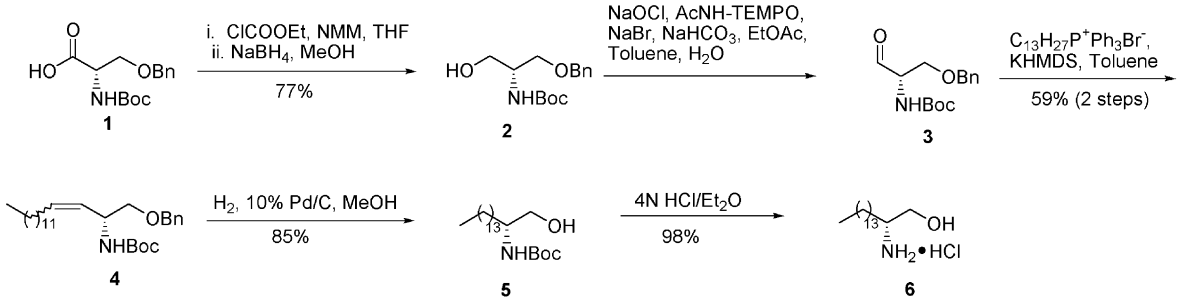

Figure 1. Synthesis of (*R*)-2-amino-hexadecanol.

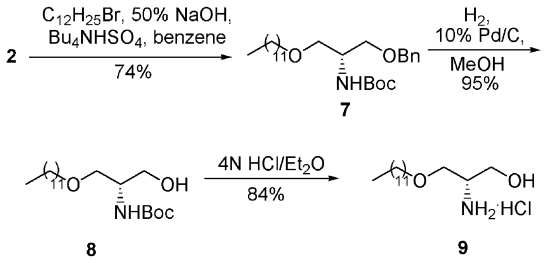

Figure 2. Synthesis of 1-*O*-dodecyl-2-deoxy-2-amino-*sn*-glycerol.

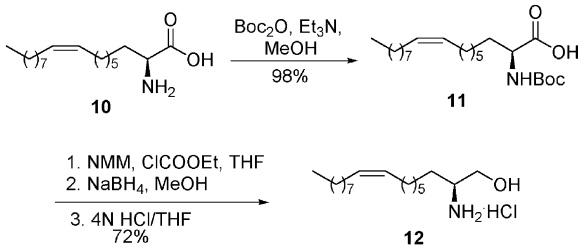

Figure 3. Synthesis of (*S*)-2-amino-oleyl alcohol.

was prepared by similar reactions, starting from D-Boc-Ser(Bn)–OH.

The proposed synthetic method is free of racemization as indicated by comparison of the value of specific rotation for (*R*)-alcohol **5** {$[\alpha]_D = -9.1$ (*c* 1.0 in $CHCl_3$)} with that reported in the literature {$[\alpha]_D = -8.5$ (*c* 2.0 in $CHCl_3$[13]}.

Protected serinol **2** is a chiral key-intermediate compound for the synthesis of mono-ethers of 2-deoxy-2-amino-glycerol structurally related to 2-amino alcohols (Fig. 2). Treatment of compound **2** with dodecylbromide in a biphasic system of benzene/aqueous sodium hydroxide in the presence of a catalytic amount of Bu_4NHSO_4 afforded the ether derivative **7** in good yield. Catalytic hydrogenation of **7** and treatment with 4N HCl/Et_2O yielded (*S*)-2-amino-3-dodecyloxy-propanol **9**.

α-Amino oleic acid exhibits interesting properties and it has been shown in in vivo studies to be a promising anti-calcification agent.[20] Recently, two synthetic procedures for the synthesis of enantiopure (*S*)-α-amino-oleic acid **10** have been reported.[21,22] Boc-protected derivative **11** was prepared by treatment of **10** with di-*tert*-butyl dicarbonate, and then was converted into alcohol **12** by reduction of its corresponding mixed anhydride with $NaBH_4$ (Fig. 3).

All products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.[23]

In vivo studies.

Inhibition of carrageenin-induced paw edema in rats. The experiment was conducted on Fisher 344 female rats weighing 200–260 g. Acute anti-inflammatory activity was measured after 3.5 h by reduction of rat paw carrageenin edema, induced by injection of 0.1 mL carrageenin 2% (K100, commercially available) in sterilized saline intradermally into the right footpad.[24] The examined compounds were administered ip simultaneously. Control animals accepted only vehicle. The experiment was repeated twice to compare the mean value in 2-amino-alcohols treated groups with that in groups of animals that were injected only with saline. Drug activities were expressed as percentage inhibition of edema formation compared to controls.

Analgesic activity

Acetic acid writhing test was used in rats.[24] In groups of five Fisher 344 female rats weighing 200–260 g, the title compounds' ED_{50} dose for the carrageenin test was given ip and 30 min later 1 mL/100 g body weight of 0.6% acetic acid was given ip. After 5 min, the number of stretches were counted each 5 min for a period of 30 min. The total number of writhes exhibited by each animal in the group was recorded and compared to that of the vehicle treated control group.

Results and Discussion

To access the anti-inflammatory activity of 2-amino-alcohols (*R*)-**6**, (*S*)-**6**, **9** and **12**, the rat carrageenin-induced paw edema assay was employed as a model for acute inflammation and indomethacin was included as a reference drug (47% inhibition at 0.011 mmol/kg), whereas the acetic acid writhing test was used to assess the analgesic activity in rats (sodium acetylsalicylate at 1 mmol/kg was administered as reference drug, 93.4%). The ED_{50} values obtained from the in vivo data and the results from the writhing test are summarized in Table 1. All compounds exhibited interesting inhibition of paw edema and analgesic activity. It seems that the stereochemistry does not influence the inhibition of

Table 1. In vivo anti-inflammatory and analgesic activity of long chain 2-amino-alcohols

Compd	ED_{50}, mmol/kg[a]
(*R*)-6	0.017 (72.6%)[b]
(*S*)-6	0.017 (89.2%)[b]
9	0.010 (95.6%)[b]
12	0.010 (88.1%)[b]

[a]Statistical significance of results was established using the Student's *t*-test, $P < 0.001$.
[b]Analgesic activity for the corresponding doses.

edema, since both enantiomers of 2-amino-hexadecanol exhibited the same anti-inflammatory activity. (*S*)-**6** Isomer exhibited slightly better analgesic activity than (*R*)-**6**. The replacement of a methylene group of the carbon chain by an oxygen atom proved to enhance the inhibition of paw edema and the protection against the acetic acid induced writhes (analgesic activity). The introduction of a *cis* double bond at C-9 caused a similar effect. (*S*)-2-Amino-3-dodecyloxy-propanediol (**9**) and (*S*)-2-amino-oleyl alcohol (**12**) presented the same anti-inflammatory activity (ED_{50} 0.010 mmol/kg). In addition, both compounds **9** and **12** exhibited strong analgesic activity at a dose of 0.010 mmol/kg corresponding to the ED_{50} dose of the carrageenin paw edema test.

Conclusion

An efficient method for the synthesis of enantiopure long chain 2-amino-alcohols starting from serine was developed. The length of the side chain depends on the alkyl-triphenylphosphonium bromide used for the olefination reaction or the alkyl bromide used for the etherification. 2-Amino-hexadecanol, the corresponding ether derivative and 2-amino-oleyl alcohol exhibit interesting in vivo anti-inflammatory and analgesic activity and constitute a class of 'lead' compounds for the development of novel pharmaceutical agents.

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23. For example: Compound **4**: $[\alpha]_D = +4.0$ (*c* 1.0, $CHCl_3$), ^1H NMR (200 MHz, $CDCl_3$) $\delta = 7.25$–7.40 (5H, m, Ph), 5.30–5.60 (2H, m, CHCH, *Z* isomer), 4.82 (1H, b, NH), 4.50–4.65 (3H, m, CH, CH_2Ph), 3.47 (2H, m, CH_2CH), 2.00–2.15 (2H, m, CH_2CH), 1.45 [9H, *s*, $C(CH_3)_3$], 1.20–1.40 (20H, m, $10\times CH_2$), 0.89 (3H, *t*, $J = 6.8$ Hz, CH_3), ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 155.2$ (CO), 138.0 (aromatic C), 133.4 (CH), 127.5–128.4 (aromatic CH and CH), 79.2 (C), 73.1 (CH_2), 72.7 (CH_2), 48.0 (CH), 31.9 (CH_2), 29.6 ($2\times CH_2$), 29.3 (CH_3), 28.4 ($3\times CH_2$), 28.3 ($3\times CH_2$), 27.8 (CH_2), 22.7 (CH_2), 14.1 (CH_3). Compound **6**: $[\alpha]_D = -4.5$ (*c* 0.5, CH_3OH), ^1H NMR (200 MHz, CD_3OD) $\delta = 3.75$ (1H, dd, C*H*HOH, $J_1 = 3.2$ Hz, $J_2 = 12.0$ Hz), 3.53 (1H, dd, CH*H*OH, $J_1 = 7.0$ Hz, $J_2 = 12.0$ Hz), 3.17 (1H, m, CH), 1.60 (2H, m, CH_2CHCH_2OH), 1.15–1.50 (24H, m, $12\times CH_2$), 0.90 (3H, t, $J = 7.0$ Hz, CH_3), ^{13}C NMR (50 MHz, CD_3OD) $\delta = 62.1$ (CH_2OH), 54.7 (CH), 33.1 (CH_2), 30.8 ($2\times CH_2$), 30.6 ($4\times CH_2$), 30.5 ($4\times CH_2$), 26.5 (CH_2), 23.7 (CH_2), 14.4 (CH_3). Compound **9**: $[\alpha]_D = +3.6$ (*c* 1.0, CH_3OH), ^1H NMR (200 MHz, CD_3OD) $\delta = 3.25$–3.80 (7H, m, $2\times CH_2O$, CH_2OH, CH), 1.58 (2H, m, CH_2CH_2O), 1.15–1.45 (18H, m, $9\times CH_2$), 0.87 (3H, t, $J = 6.8$ Hz, CH_3), ^{13}C NMR (50 MHz, CD_3OD) $\delta = 72.8$ (CH_2O), 68.7 (CH_2O), 60.3 (CH_2OH), 54.3 (CH), 33.1 (CH_2), 30.8 ($2\times CH_2$), 30.6 ($3\times CH_2$), 30.5 ($3\times CH_2$), 27.1 (CH_2), 23.8 (CH_2), 14.4 (CH_3). Compound **12**: $[\alpha]_D = +9.2$ (*c* 1.0, $CHCl_3$), MS (FAB) *m*/*z* (%): 284 ($M^+ + H$, 100), 252 (M−59, 10), 55 (52), ^1H NMR (200 MHz, $CDCl_3$) $\delta = 6.43$ (4H, m, NH_3^+, OH), 5.33 (2H, m, CH = CH), 3.62–4.00 (2H, m, CH_2OH), 3.41 (2H, m, CH, OH), 1.99 (4H, m, $2\times CH_2CH$), 1.66 (2H, m, CH_2CHCH_2OH), 1.10–1.45 (20H, m, $10\times CH_2$), 0.87 (3H, *t*, $J = 7.0$ Hz, CH_3), ^{13}C NMR (50 MHz, $CDCl_3$) $\delta = 129.5$ and 130.0 (CHCH), 62.0 (CH_2OH), 53.8 (CH), 31.9 ($2\times CH_2$), 29.7 (CH_2), 29.5 ($2\times CH_2$), 29.4 ($2\times CH_2$), 29.3 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 14.1 (CH_3).
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