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Design and Synthesis of New Secretory Phospholipase A_2 Inhibitor of a Phospholipid Analog

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Abstract: All stereoisomers of N-acyl-4,5-disubstituted oxazolidinone phospholipid analogs were synthesized by regio and stereoselective epoxide ring opening accompanied by introduction of an amino group. The (4R,5S)-derivative showed stronger inhibitory activity toward type II phospholipase A_2 than the 4-substituted oxazolidinone phospholipid analog previously reported.

Keywords: phospholipase A_2; stereoselective synthesis; stronger inhibitory activity; oxazolidinone phospholipid analog

Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the ester linkage at the *sn*-2 position of glycerophospholipids **1a**, resulting in the release of arachidonic acid which is known to be the rate-limiting step in the production of eicosanoid mediators of inflammation.[1] Previously, we reported the synthesis and inhibitory activity of an oxazolidinone phospholipid analog PLA_2 inhibitor **2**.[2] This molecule showed strong inhibitory activity toward both type I and type II PLA_2, and can be regarded as a conformationally restricted amide analog of compound **3**, which was developed by de Haas and co-workers and has recieved significant attention because of its powerful inhibitory activity toward PLA_2. It is known that the powerful inhibitory activity of **3** is based on the hydrogen bonding between a nitrogen proton of C-2 amide group and the His-48 nitrogen of PLA_2 catalytic site.[3,4] On the other hand, the binding mode of our phospholipid analog **2** into the PLA_2 catalytic site was proposed to be very similar to that of the genuine glycerophospholipid substrate **1b** based on the results of its inhibitory activity.[5] The topological similarity in binding between our compound **2** and the authentic substrate **1b** encouraged the idea that our compound would be a very useful molecule for the elucidation of the catalytic mechanism of PLA_2.

Taking cues from strong binding affinity displayed by inhibitor **3**, and encouraged by the promising binding mode evidenced by compound **2**, we investigated the second-generation design of a PLA_2 inhibitor based on molecular modeling of the conformation of the acyclic analog **3** and our oxazolidinone bound to

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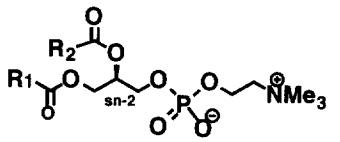
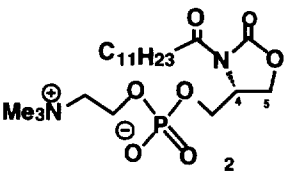


1a; R_1=alkyl, R_2=alkenyl
1b; R_1=R_2=hexyl



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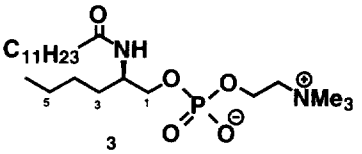

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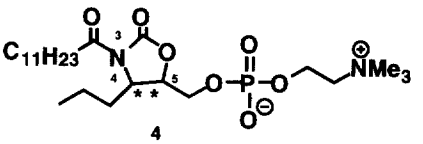

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phospholipid **2** the human secretory PLA_2. In this paper, we describe the synthesis of all four stereoisomers of 3-acyl-4,5-disubstituted oxazolidinone **4** and their inhibitory activities toward type II secretory PLA_2.

In our rational design, the complex between compound **3** and human secretory PLA_2 is superimposed with that of oxazolidinone analog **2** and human secretory PLA_2 based on enzyme structure. The carbon center at the 5-position of new oxazolidinone analog **4** using oxazolidinone numbering can consequently be superimposed with the 2-position of compound **3** using phospholipid numbering based on molecular modeling. We, then, chose to incorporate the three carbons at the 4-position of oxazolidinone **4** to correspond with carbons 4, 5, and 6 of compound **3** by using trans-2-hexen-1-ol as the starting material. Our synthetic strategy for all stereoisomers of the 3-acyl-4,5-disubstituted oxazolidinone phospholipid analog was based on the Sharpless asymmetric epoxidation protocol (Scheme 1). The synthesis of both cis oxazolidinone derivative **8** and trans oxazolidinone derivative **12** started with asymmetric epoxidation of trans-2-hexen-1-ol according to a reported procedure,[6] which gave optically pure (2R,3R)-epoxy-1-hexanol (**5**). Syn and anti stereoselective introduction of an amino group at the C-3 position *versus* the C-2 hydroxy group of **5** was accompanied by both regio and stereoselective opening of the epoxide. For cis oxazolidinone, treatment of (2R,3R)-epoxy alcohol **5** with trimethylsilyl azide in the presence of titanium tetraisopropoxide in benzene produced azide diol **6**.[7] Considering the difficulty of the isolation of the amino diol resulting from reduction of azide group, we tried to obtain the protected amino diol in a one pot procedure as follows.[8] Azide diol **6** was stirred with palladium on charcoal in the presence of di-*tert*-butyl dicarbonate under hydrogen atmosphere in methanol to yield the desired N-protected amino diol **7** in 82% yield, mp 90-91 °C. After selective protection of the primary hydroxyl group of **7**, the oxazolidinone derivative **8**, possessing substituents of cis stereochemistry, was obtained in good yield by treatment of the alcohol with sodium hydride. For the trans oxazolidinone derivative **12**, the regio- and stereoselective ring opening of **5** at the 2-position was accomplished in an intramolecular fashion. Phenyl carbamate **9**, mp 29-30 °C, derived from epoxy alcohol **5** by reaction with phenylisocyanate was treated with aqueous 5% perhydrochloric acid to produce carbonate **10**.[9] After treatment of **10** with trifluoromethanesulfonic anhydride, the resulting unstable triflate was not isolated and was allowed to directly react with sodium azide to yield the desired azide. Reduction and protection of the azide group using the one pot procedure previously described, followed by base treatment yielded the N-protected amino diol **11**, mp 62-63 °C. The trans derivative **12** was obtained by the same sequences, protection of the primary hydroxy group and base treatment, as the case of the cis derivative. The synthesis of new oxazolidinone phospholipid analog **4** was achieved by introduction of phosphatidylcholino part into the cis and trans oxazolidinone derivatives obtained, respectively. Thus, introduction of dodecanoyl group into the oxazolidinone nitrogen (cis; 89%, trans; 90% yield) and then acid treatment gave the corresponding N-acyl alcohol **13** (quantitatively). Allowing **13** to react with 2-chloro-2-oxo-1,3,2-

dioxaphospholane followed by a reaction with trimethylamine in a sealed tube, produced the desired (4S,5S)-**4** and (4S,5R)-**4** as foams (quantitatively), respectively.[2,10] Two other enantiomers, (4R,5S)-**4** and (4R,5R)-**4** were also synthesized starting from (2S, 3S)-epoxy-1-hexanol by the same procedures, respectively.

Scheme 1

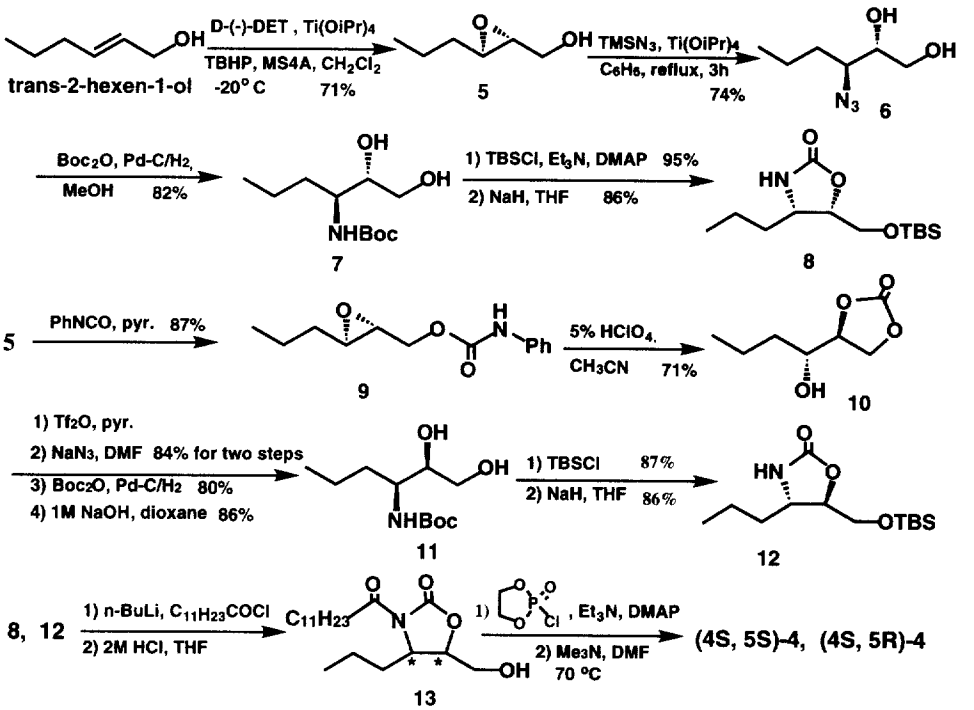

Figure 1

comp.	(4S, 5S)-4	(4S, 5R)-4	(4R, 5S)-4	(4R, 5R)-4
IC_{50}	52.9μM	40.1μM	19.8μM	45.8μM

comp.	(R)-2	(R)-3
IC_{50}	31.3μM	5.3μM

With all stereoisomers of new oxazolidinone phospholipid **4** in hand, we tested their inhibitory activities toward type II PLA_2 which was isolated from Japanese mamushi (*A. halys blomhoffii*) venom PLA_2 along with the previously synthesized oxazolidinone analog (R)-**2** and the linear amide analog (R)-**3** as a positive controls.[11] The 50% inhibition concentration of the six phospholipid amide analogs are summarized in Figure 1. The (4R,5S)-isomer showed stronger inhibitory activity than the previously synthesized 4-substituted analog (R)-**2**, though it showed less inhibitory activity than the linear amide analog (R)-**3**. The results obtained suggest that orientation of the side chain of (4R,5S)-**4** at these positions may be important for the lipophilic interaction of both the C-3 N-acyl group and the C-4 side chain with the hydrophobic channel which consists of lipophilic amino acid residues of PLA_2. In molecular modeling studies, docking energy

between (4R,5S)-**4** and PLA_2 is lower than that of others.[12] It is also worthy of note that molecular modeling showed that the side chains of (4R,5S)-**4** at both C-3 and C-4 position could be placed in the hydrophobic channel of the PLA_2 catalytic site and its phosphoryl group could coordinate with Ca^{2+} more favorably than those of the other isomers.

In conclusion, we synthesized a new and stronger PLA_2 inhibitor of oxazolidinone phospholipid analog, which may be an instructive compound for understanding the binding conformation of glycerophospholipid into PLA_2 catalytic site.

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10. Data for **(4S, 5S)-4**; ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 0.95 (t, 3H, J = 7.3 Hz), 1.25-1.40 (m, 18H), 1.62-1.76 (m, 4H), 2.78 (m, 1H), 2.94 (m, 1H), 3.23 (s, 9H), 3.66 (m, 2H), 4.18 (m, 2H), 4.30 (m, 2H), 4.61 (m, 1H), 4.81 (m, 1H) ; ^{13}C NMR (100 MHz, CD_3OD) δ 12.8, 12.9, 18.5, 22.2, 24.0, 28.7, 28.9, 29.1, 29.2, 29.9, 31.5, 34.8, 53.1, 55.5, 58.9, 59.0, 62.6, 65.9, 77.2, 153.5, 173.0; IR (neat, cm^{-1}) 2926, 2856, 1781, 1708, 1093, 968,; $[\alpha]_D$ +50.1 (c = 0.617, CH_3OH); FAB-HR-MS 507.3201(M+H)$^+$ calcd. for $C_{24}H_{48}O_7N_2P$ 507.3187. Data for **(4S, 5R)-4** ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (t, 3H, J = 6.8 Hz), 0.97 (t, 3H, J = 7.4 Hz), 1.29-1.38 (m, 18H), 1.60-1.63 (m, 2H), 1.73-1.76 (m, 2H), 2.75 (m, 1H), 2.95 (m, 1H), 3.22 (s, 9H), 3.63 (t, 2H, J = 4.8 Hz), 3.95 (m, 1H), 4.04 (m, 1H), 4.25 (br, 2H), 4.37 (dt, 1H, J = 3.2, 7.6 Hz), 4.51 (br, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 14.2, 14.5, 23.7, 25.6, 30.3, 30.5, 30.6, 30.7, 33.1, 35.3, 36.6, 54.7, 57.1, 60.5, 67.3, 78.8, 78.9, 155.2, 174.7; IR ($CHCl_3$, cm^{-1}) 3318, 2932, 1774, 1705, 1091, 969; $[\alpha]_D$ -4.89 (c = 0.624, CH_3OH); FAB-HR-MS 507.3196(M+H)$^+$ calcd. for $C_{24}H_{48}O_7N_2P$ 507.3187.
11. The enzyme activity was measured toward a monodispersed substrate **1b** in the presence of 3.3mM calcium chloride by using the pH-stat method at 25 °C, pH 8.2, and ionic strength 0.1.
12. Model building and structural analysis were accomplished using InsightII / Discover.[13] In order to use molecular dynamics (MD) simulation, the consistent valence force field (CVFF) was used. MD simulation of each of the 4 isomers complexed with human PLA_2 was performed at a constant temperature (300K) with a dielectric constant of 80 in gas phase.
13. The model building and structure analysis followed by molecular mechanics and dynamics simulation were performed with Insight II 2.1.0. Program and Discover 2.9 Program Biosym Technol Inc. San Diego, USA.