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Synthesis and biological evaluation of optically active Ki16425

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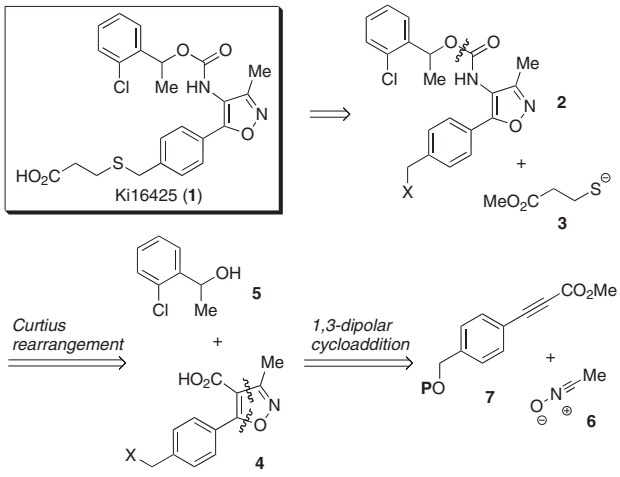
ABSTRACT

An enantionselective synthesis of both enantiomers of Ki16425, which possesses selective LPA antagonistic activity, was achieved. The isoxazole core was constructed by a 1,3-dipolar cycloaddition of nitrile oxide with alkyne and condensation with the optically active α-phenethyl alcohol segment, which was prepared by an enantioselective reduction of arylmethylketone. Biological evaluation of both enantiomers of Ki16425 revealed that the (*R*)-isomer showed much higher antagonistic activity for LPA_1 and LPA_3 receptors.

Lysophosphatidic acid (1- or 2-*O*-acyl-*sn*-glycero-3-phosphate, LPA) represents biologically active lipid mediators, which shows broad range of biological effects in vitro such as stimulation of cell proliferation and migration, promotion of cell survival, platelet aggregation, apoptosis, and smooth muscle contraction.[1,2] To date, six types of receptors, LPA_{1-6}, have been identified and are responsible for most of the biological activities of LPA.[3–8] Recent studies on gene targeting in mice and family diseases of these receptors revealed that LPA is involved in various patho-physiological states.[9] Because of its important biological activities, LPA antagonists have attracted considerable attention and numerous small molecules having LPA antagonistic activity have been reported. However, most compounds show poor oral bioavailability due to their lipid-like structures.[1,10–15] On the other hand, Ki16425 (**1**), reported by Okajima et al., is a small molecule LPA antagonist with oral activity and high selectivity toward LPA_{1-3}.[16–19] Therefore, Ki16425 (**1**) has been used as a standard compound for evaluation of potency of LPA antagonists. However, availability of this compound from commercial suppliers has recently become quite limited despite its importance to the research field of biologically active lipid mediators. In addition, the previously established synthesis is inefficient regarding the key construction of the isoxazole core.[17] Furthermore, no optically active compound is available and thus differences in biological activity between two enantiomers have not been fully investigated. With this background, we have initiated investigation to develop an efficient synthetic route to racemic as well as optically active Ki16425 (**1**). We report herein an efficient synthesis of Ki16425 (**1**) both in racemic and optically active form and an evaluation of its LPA antagonistic activity.



Scheme 1. Retrosynthetic analysis of Ki16425.

Retrosynthetic analysis of Ki16425 is shown in Scheme 1. The 3-mercaptopropionic acid chain would be introduced at a later stage of the synthesis by a S_N2 reaction between a benzyl halide **2** and a thiolate anion **3**. The urethane moiety on the isoxazole ring would be constructed by Curtius rearrangement of isoxazolyl carboxylic acid **4** and in situ trapping of the resultant isocyanate with phenethyl alcohol **5**. The isoxazole core would be formed by a 1,3-dipolar cycloaddition of acetonitrile oxide **6** with arylpropiolate **7**.[20–27]

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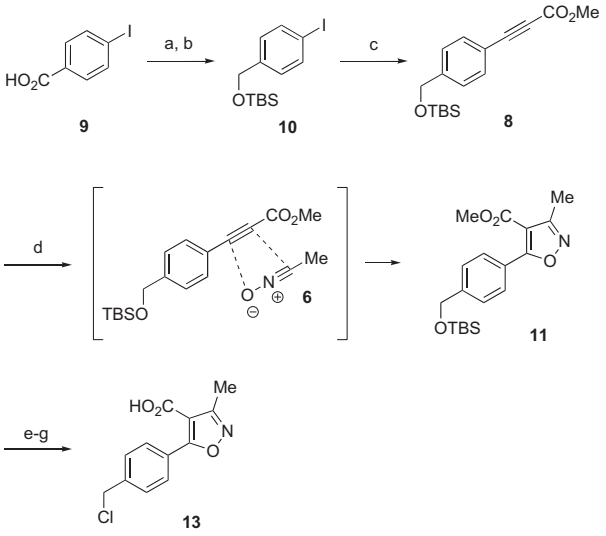
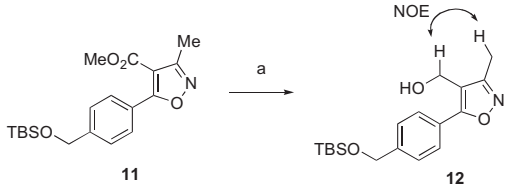
We started our synthetic studies on racemic Ki16425 by preparation of arylpropiolate **8** as a substrate for the 1,3-dipolar cycloaddition reaction (Scheme 2). After borane reduction of *p*-iodobenzoic acid (**9**), the resultant benzyl alcohol was protected as TBS ether to give **10**.[28] Iodobenzene derivative **10** was then coupled with methyl propiolate under Negishi coupling conditions to provide alkyne **8**.[29] At this point, we examined the crucial 1,3-dipolar cycloaddition. Upon generation of acetonitrile oxide (**6**) using the Mukaiyama protocol by treatment of nitroethane with phenyl isocyanate in the presence of alkyne **8**,[30] in situ generation of acetonitrile oxide (**6**) and the expected 1,3-dipolar cycloaddition took place smoothly to furnish cycloadduct **11** as a sole product. The structure of the product **11** was unambiguously established by NOESY experiments after reduction of methyl ester **11** to the corresponding alcohol **12** (Scheme 3). Substantial NOE effects were observed between the methyl and methylene protons, indicating that the desired isoxazole was generated by an exclusive mode of 1,3-dipolar cycloaddition via the transition state as depicted in Scheme 2. This excellent regiochemical control would be explained by the steric repulsion between the methyl group on 1,3-dipole and the aryl group on dipolarophile.[20–27] The cycloadduct **11** was then converted to the key isoxazole carboxylic acid **13** by three step transformations including desilylation, chlorination at the benzylic position, and saponification of the methyl ester.

With the requisite trisubstituted isoxazole **13** in hand, we then installed two side chains in this compound to complete synthesis of racemic Ki16425 based on the Fujita's procedure (Scheme 4).[19] Carboxylic acid **13** was subjected to Curtius rearrangement using DPPA in the presence of α-phenethyl alcohol **5**, which was prepared by $NaBH_4$ reduction of commercially available 2-chloroacetophenone. Generation of isocyanate intermediate and in situ condensation with alcohol **5** occurred efficiently to afford carbamate **14**. Elongation of the lower side chain was executed by S_N2 reaction of benzyl chloride **14** with commercially available methyl 3-mercaptopropanoate to afford the corresponding sulfide, followed by saponification of the methyl ester leading to racemic Ki16425 (**1**) (33% overall yield in total 10 steps from **9**).

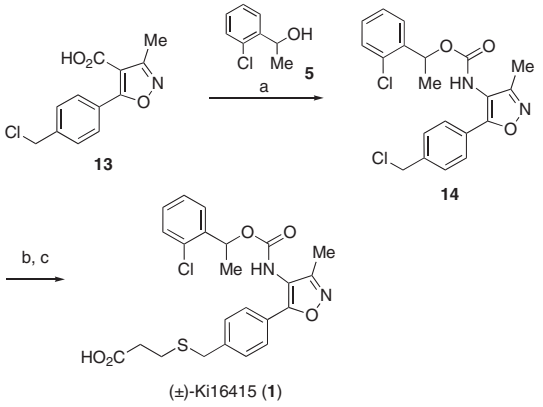
Having established a synthetic route to racemic Ki16425, we then applied it to an enantioselective synthesis of two enantiomers of Ki16425 corresponding to the stereocenter of the α-phenethyl



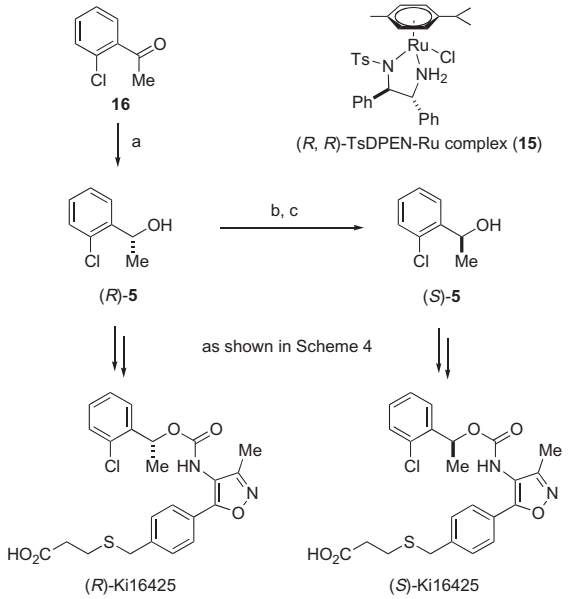
Scheme 2. Preparation of isoxazole carboxylic acid **13**. Reagents and conditions: (a) BH_3·THF, THF, reflux, 2 h, quant.; (b) TBSCl, imidazole, DMF, rt, 30 min, quant.; (c) methyl propiolate, cat. $Pd(PPh_3)_4$, $ZnBr_2$, LDA, THF, 35 °C, 12 h; (d) $EtNO_2$, PhNCO, cat. Et_3N, toluene, 80 °C, 1 h, 88%; (e) TBAF, THF, 0 °C, 35 min, 95%; (f) CCl_4, PPh_3, CH_2Cl_2, reflux, 20 min, 85%; (g) LiOH, THF–H_2O, rt, 19 h, 98%.



Scheme 3. Structural determination of cycloadduct **11**. Reagent and conditions: (a) DIBALH, CH_2Cl_2, −78 °C, 35 min, quant.



Scheme 4. Synthesis of (±)-Ki16425 (**1**). Reagents and conditions: (a) DPPA, Et_3N, toluene, reflux, 30 min, 75%; (b) methyl 3-mercaptopropanoate, TBAI, Et_3N, CH_2Cl_2, reflux, 2 h, 88%; (c) LiOH, THF–H_2O, rt, 4 h, 87%.



Scheme 5. Synthesis of both enantiomers of Ki16425. Reagents and conditions: (a) **15** (6 mol %), HCO_2H, Et_3N, 2 days, 90%, 92% ee; (b) *p*-nitrobenzoic acid, DEAD, PPh_3, THF, 0 °C, 10 min, 95%; (c) LiOH, THF–H_2O, rt, 9.5 h, 79%.

segment (Scheme 5). For preparation of (*R*)-α-phenethyl alcohol **5**, we examined a catalytic asymmetric reduction under the conditions developed by Noyori and co-workers.[31] Thus, under the transfer hydrogenation conditions with a chiral ruthenium catalyst **15**, aryl methyl ketone **16** was converted to the corresponding (*R*)-phenethyl alcohol **5** in 92% ee. (*S*)-Phenethyl alcohol **5**, on the other hand, was prepared by Mitsunobu inversion of (*R*)-phenethyl alcohol **5** and saponification.[32] By using (*R*)-**5** and (*S*)-**5**, both

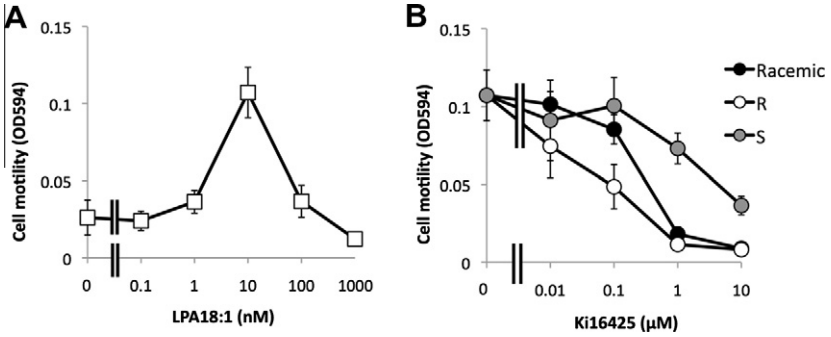


Figure 1. Evaluation of (±)-, (*R*), and (*S*)-Ki16425 in the cell migration assay.

enantiomers of Ki16425 were synthesized, respectively, according to the synthetic route we established.

Biological activity of both enantiomers of Ki16425 was evaluated by both the cell migration assay with PC-3 cells [33] and the TGFα shedding assay.[34] PC-3 cells are sensitive to LPA-dependent cell migration and Ki16425 inhibits the migratory response of PC-3 cells, as described previously.[35] In this assay, PC-3 cells migrated in response to LPA (Fig. 1A). When PC-3 cells preincubated with Ki16425 were stimulated with 10 nM LPA, Ki16425 showed antagonistic activity against cell migration (Fig. 1B). The rank order of antagonistic activity was (*R*)- > racemic > (*S*)-Ki16425. AP-TGFα release assay confirmed that *(R)*-Ki16425 was more potent in anatagonizing LPA_1 and LPA_3 than *(S)*-Ki16425, and the rank order was *(R)*- > racemic > *(S)*-Ki16425.

In conclusion, we have established a synthesis of Ki16425 via 1,3-dipolar cycloaddition of nitrile oxide as the key step. By utiliing the synthetic route, both enantiomers of Ki16425 were synthesized through Noyori's catalytic asymmetric reduction. In comparison to the known synthetic route to **1**,[19] our 1,3-dipolar cycloaddition strategy should be advantageous since facile preparation of isoxazole core possessing a various substituted pattern is possible simply by switching nitroalkanes and substituted propiolates. Evaluation of LPA antagonistic activity of both enantiomers of Ki16425 revealed that (*R*)-isomer possessed higher activity.[38]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.012.

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33. *Chemotaxis assay:* PC-3 (prostate cancer) cells were cultured in RPMI 1640 (Nissui, Japan) supplemented with 5% fetal bovine serum. Cell migration was determined by a modified Boyden chamber assays as described previously.[36] In brief, polycarbonate filters with 8-μm pores (Neuro Probe, Inc.) were coated with 0.001% of fibronectin (Sigma). PC-3 cells (1×10^5 cells in 200 μL/well) were loaded into upper chambers and incubated at 37 °C for 3 h to allow migration. The cell migration to the bottom side of the filter was evaluated by measuring optical densities at 594 nm. For Ki16425 treatment, cells were preincubated with each concentration of Ki16425 for 30 min.
34. *Evaluation of LPA receptor activation:* Activation of LPA_1 and LPA_3 are performed essentially as described previously.[37] cDNAs for alkaline phosphatase-tagged transforming growth factor-α (AP-TGF-α) and for LPA receptor (human LPA1 or LPA3) were introduced into HEK293 cells using lipofectamine 2000 as transfection reagent. Twenty-four hours after transfection, cells were re-plated in 96-well plates (1×10^5/well) stimulated with LPA (1-oleoyl). After 60 min amount of AP-TGF-α released from cells or remained in cells were determined by measuring the AP activity using *p*-nitrophenyl phosphate (*p*-NPP) as a substrate. Activation of each LPA receptor was expressed as % release of AP activity using the following formula: (AP activity in the culture supernatant)/(AP activity in the culture supernatant + AP activity remained

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