Enantioselective Synthesis of α-Phosphono Sulfonate Squalene Synthase Inhibitors: Chiral Recognition in the Interactions of an α-Phosphono Sulfonate Inhibitor with Squalene Synthase

R. Michael Lawrence,† Scott A. Biller,*,†
John K. Dickson, Jr.,† Janette V. H. Logan,†
David R. Magnin,† Richard B. Sulsky,† John D. DiMarco,‡
Jack Z. Gougoutas,‡ Barbara D. Beyer,§ Susan C. Taylor,§
Shih-Jung Lan,|| Carl P. Ciosek, Jr.,⊥ Thomas W. Harrity,⊥
Kern G. Jolibois,⊥ Lori K. Kunselman,⊥ and
Dorothy A. Slusarchyk⊥

Division of Discovery Chemistry, Department of Solid State Chemistry, Analytical Research and Development Department of Pharmacokinetics and Metabolism Department of Metabolic Diseases Bristol-Myers Squibb Pharmaceutical Research Institute P.O. Box 4000, Princeton, New Jersey 08543

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Inhibitors of squalene synthase[1] have great potential as selective cholesterol lowering agents due to the strategic location of the enzyme at the first committed step in the cholesterol biosynthetic pathway. We recently reported the rational design and discovery of a potent squalene synthase inhibitor, α-phosphono sulfonate triacid **1**,[2] and the orally bioavailable prodrug ester **2**[3] (Chart 1). α-Phosphono sulfonates **1** and **2** exist as pairs of enantiomers. An important question remained unresolved: Could squalene synthase discriminate between the two enantiomers of **1**? This is a critical issue, both with respect to understanding the molecular interactions of α-phosphono sulfonates with squalene synthase and to selecting a single enantiomer for further pharmaceutical development.

1-Substituted phosphono methylsulfonates were virtually unknown in the chemical literature prior to our studies.[4] A major concern in our synthetic planning was the configurational stability of the chiral center, which is geminally substituted with two electron-withdrawing groups. Prior to attempting the enantioselective syntheses, deuterium incorporation studies were performed by ^{1}H NMR to assess the configurational stability of the chiral centers of **1**−**3**. While triacid **1** was resistant to D-incorporation over a wide range of pD values, the racemic prodrug **2** exhibited a slow H/D exchange at basic pD ($t_{1/2}$ = 110 h at pD 9.3, room temperature (rt)). Under the mild solvolytic conditions utilized to deprotect the cyclohexyl sulfonate ester of **3** (10:1 CF_3CH_2OD/D_2O, 3 equiv of KOAc, 40 °C, 20 h), **2** was obtained with complete D-incorporation. When **2** was subjected to these same solvolytic conditions, no D-incorporation was detected. We concluded that both the triacid **1** and diester **2** were isolable as single enantiomers, whereas triester **3** was very sensitive to racemization.

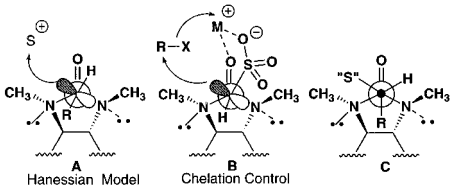
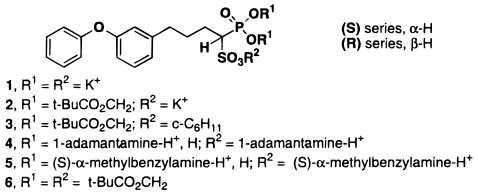
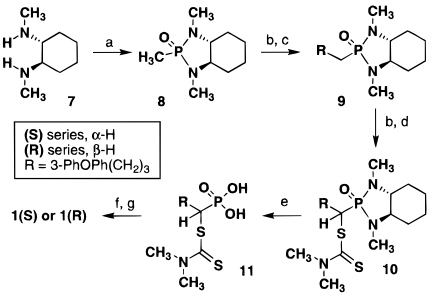


Figure 1. Newman projections of transition states (**A** and **B**) and product **(C)** of the reaction of chiral phosphonic diamide anions with electrophiles.

Chart 1



Scheme 1[a]



[a] (a) $CH_3P(O)Cl_2$, Et_3N, toluene, 81%; (b) *n*-BuLi, THF, −78 °C; (c) 3-(3′-phenoxyphenyl)propyl iodide, −78 °C to rt, 89%; (d) $[(CH_3)_2NCS_2]_2$, −90 to −70 °C, 84%; (e) aqueous HCl, CH_3CN, 99%; (f) 30% H_2O_2, HOAc/formic acid (9:1) ratio; (g) aqueous KOH, 82%.

Our strategy for the introduction of the asymmetric center relied upon the sulfuration or alkylation of a chiral phosphorus carbanion (Figure 1).[5,6] The chiral auxiliary that appeared most promising was the C2-symmetric phosphonic diamide derived from *trans*-1,2-cyclohexanediamine.[5] The sulfuration of anion **A** was predicted to provide **C** having the (*S*)-configuration, on the basis of the sterically favored approach to the carbanion as depicted in the Hanessian transition state model. Alkylation of the dianion **B** was predicted to provide **C** with the same configuration, on the basis of the sterically favored approach to the chelated dianion.[5c] Thus, the stereochemical outcome for both routes should be identical, since a change in the geometry of the anion (**A** versus **B**, Figure 1) is accompanied by a change in the order of group introduction.

In the sulfuration approach (Scheme 1), the diamine **7**[5a,d] was converted to phosphonic diamide **8**, which was deprotonated and alkylated with 3-(3′-phenoxyphenyl)propyl iodide to yield **9**. Anion **A** was formed by deprotonation of **9** with *n*-BuLi,

* Corresponding Author: Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543; tel. 609-252-5585; fax 609-252-6601; email biller@bms.com.
† Division of Discovery Chemistry.
‡ Department of Solid State Chemistry.
§ Analytical Research and Development.
|| Department of Pharmacokinetics and Metabolism.
⊥ Department of Metabolic Diseases.

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(7) Control studies indicate that the reduced diastereoselectivity in the sulfuration is not the result of epimerization of sulfurated phosphonate **10**.

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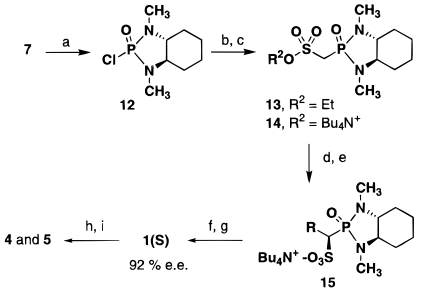
followed by sulfuration with $[(CH_3)_2NCS_2]_2$ (dithiuram) at −90 °C to provide **10(*S*)/10(*R*)** in 84% yield as a 3:1 mixture of diastereomers, which were readily separated by silica gel chromatography. The lower diastereoselectivity observed for the sulfuration as compared to that reported for alkylation[5] of anions such as **A** is presumably due to the long C−S bond length in the transition state, which would serve to attenuate the steric hindrance sensed by dithiuram relative to an alkyl halide.[7] Indeed, alkylation of **A** with benzyl bromide at −90 °C led to a 10:1 mixture of diastereomers. The separated diastereomers **10(*S*)** and **10(*R*)**, respectively, were hydrolyzed with mild acid to remove the chiral auxiliary, and the resulting dithiocarbamates were oxidized with H_2O_2 in HCO_2H/CH_3CO_2H to provide triacids **1(*S*)** [99.8% ee,[8] $[\alpha]_D$ −10.7° (*c* = 0.88, H_2O)] and **1(*R*)** [(95.5% ee,[8] $[\alpha]_D$ +9.5° (*c* = 0.89, H_2O)].

Scheme 2[a]



[a] (a) $P(O)Cl_3$, Et_3N, 85%; (b) CH_3SO_3Et, *n*-BuLi, THF, −78 °C to rt, 85%; (c) Bu_4NI, THF, 100%; (d) *n*-BuLi, THF, −90 °C; (e) 3-(3′-phenoxyphenyl)propyl iodide, THF, −90 to −78 °C; (f) HCl (aqueous); (g) AG-50 ×8 (K^+ form), 57% from **14**; (h) AG-50 ×8 (H^+ form); (i) R^1NH_2 (2 equiv); for **4**, R^1 = 1-adamantyl (99.5% ee); for **5**, R^1 = (*S*)-α-methylbenzyl.

For the chelation-controlled alkylation approach (Scheme 2), the precursor to anion **B** was synthesized by converting the diamine **7** to the Bu_4N^+ salt **14**.[9] Treatment of **14** with *n*-BuLi followed by alkylation with 3-(3′-phenoxyphenyl)propyl iodide at −90 °C provided **15** as a >20:1 ratio of diastereomers by ^{31}P NMR. Removal of the chiral auxiliary afforded **1(*S*)** (92% ee[8]) in 57% overall yield from **14**. The enantiomeric purity could be increased to 99.5%[8] by recrystallization of the bis-adamantylamine salt **4(*S*)**. The major enantiomers from the sulfuration and alkylation routes proved to be identical, which is consistent with the proposed transition state models (Figure 1). The absolute configuration of the major enantiomer was confirmed by single crystal X-ray analysis of the bis-(*S*)-α-methylbenzylamine salt **5(*S*)**.[10]

Both enantiomers of **1** were tested for their ability to inhibit the conversion of farnesyl diphosphate to squalene by rat microsomal squalene synthase *in vitro*.[11] Enantiomer **1(*S*)** was 16-fold more potent than **1(*R*)** as an inhibitor of squalene synthase (IC_{50} values of 68 and 1120 nM, respectively). Furthermore, **1(*S*)** was found to be considerably more potent as an inhibitor of the biosynthesis of cholesterol in rats.[11] On intravenous dosing, **1(*S*)** had an ED_{50} of 0.16 mg/kg, while **1-(*R*)** was inactive at 1 mg/kg.

Alkylation of the trisilver salt of **1(*S*)** with iodomethyl pivalate[12] (anisole, CH_2Cl_2, 4 Å sieves), followed by solvolysis of the labile triester **6** (CH_3CN, H_2O) and careful neutralization of the sulfonic acid with potassium phosphate provided **2(*S*)** in 98% ee[8] [57% yield, $[\alpha]_D$ −6.6° (*c* = 1.0, CH_3OH)], indicating minimal racemization. Following oral dosing of **2(*S*)** to rats, **1(*S*)** was recovered from plasma and bile without significant racemization, proving that **2(*S*)** possessed suitable chiral stability to deliver **1(*S*)** *in vivo*.[13]

The asymmetric routes to α-phosphono sulfonates described herein efficiently provide the potent squalene synthase inhibitor **1** and prodrug **2** in nonracemic form and are generalizable to other members of this class of molecules. Of particular interest is the enantioselectivity observed for the inhibitory potency of **1(*S*)** and **1(*R*)**, indicating that squalene synthase is able to discriminate between the phosphonate and sulfonate moieties in distinct binding sites. The dibasic phosphonate group and the monobasic sulfonate group have considerable structural similarity. Both are tetrahedral, second-row functions with a C_{3v} display of negatively charged oxygen atoms. In addition, they show significant similarity, but not identity, in their interaction with Lewis acids.[14] Certain proteins can discriminate between binding phosphonyl and sulfonyl groups,[15] whereas others bind both functions comparably well.[16] Bond angle and bond length data from the X-ray crystal structure of **5(*S*)** and a related α-phosphono sulfonate[10,17] support the close isosteric relationship between the phosphonate and sulfonate groups. Further structural studies will be required to understand the basis for this selective binding. The enantioselectivity displayed by squalene synthase reinforces the importance of single enantiomer drug development,[18] where the "inactive" enantiomer can possess unwanted pharmacologic and toxicologic properties.

Acknowledgment. We acknowledge helpful discussions with Dr. Youssef L. Bennani, currently of Abbott Laboratories. In addition, the assistance of Bristol-Myers Squibb Analytical Research and Development and the Discovery Chemistry NMR Group is appreciated in the determination of analytical and spectral data.

Supporting Information Available: Details of the X-ray structural analysis of compound **5(*S*)**, as well as the related α-phosphono sulfonate **i** of ref 2 (18 pages). See any current masthead page for ordering and Internet access instructions.

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(8) Chiral analytical HPLC was utilized to determine the enantiomeric enrichment in **1** and **2**. Both sets of enantiomers (**1** and **2**) were separated using the α_1-acid glycoprotein column (Chiral-AGP) available from ChromTech (Sweden), using an 100 × 4.0 mm i.d. column (10 μm particle size) at 1 mL/min flow rate. For the separation of **1(*S*)** and **1(*R*)**, the mobile phase was 85% 0.1 M KH_2PO_4 (pH = 4.6) and 15% acetonitrile. The retention times were 10.3 and 18.8 min, respectively. For the separation of **2(*S*)** and **2(*R*)**, the mobile phase was 78% 0.1 M KH_2PO_4 (pH = 4.6), 16% 2-propanol, and 6% methanol. The retention times were 24.5 and 15.7 min, respectively.

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(10) Details of the X-ray structural analysis for **5(*S*)** and the related compound **i** of ref 2 are included as Supporting Information. Atomic coordinates for both compounds have been deposited in the Cambridge Crystallographic Database and can be obtained upon request to the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

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(13) Compound **1(*S*)** was isolated from the 3 and 6 h plasma samples and the 0−12 h bile samples of rats that had received single 40 μmol/kg oral doses of **2(*S*)** and analyzed for **1(*R*)** and **1(*S*)** by HPLC using the assay reported in ref 8. The results showed that the isolated samples contained greater than 99.5% of **1(*S*)**.

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(17) Crystallographic assignment of the stereochemical configuration of **5(*S*)**[10] required a distinction between two strictly isosteric groups at the chiral center: the PO_3 and SO_3 groups of the α-phosphono sulfonate dianion (the remaining acidic OH was not observed experimentally). The C−S (1.78 Å) and O−S (av 1.44 Å) bonds are only several hundredths of an angstrom shorter than the corresponding bonds to phosphorus, although angles C−S−O (av = 107°) and O−S−O (105°, 109°, 121°) are not significantly different for the phosphonate group. The phosphonate is intermolecularly H-bonded *to itself* and therefore bears the only non-ionized acidic hydrogen: O3−P−O4−H···O3, where the O4···O3 distance is 2.61 Å. Phosphonate oxygen O4 is assigned as the hydroxyl on the basis of its longer bond length (P−O4 = 1.56 Å versus P−O3 = 1.48 Å). The other phosphonate oxygen and the three sulfonate oxygens are involved in H-bonds only as acceptors with the protonated amine counterions. Similar isosteric geometries have been observed in the crystal structure[10] of the related α-phosphono sulfonate (structure **i** of ref 2).

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