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Preparations of Antifungal Sch 42427/SM 9164: Preparative Chromatographic Resolution, and Total Asymmetric Synthesis via Enzymatic Preparation of Chiral α-Hydroxy Arylketones.

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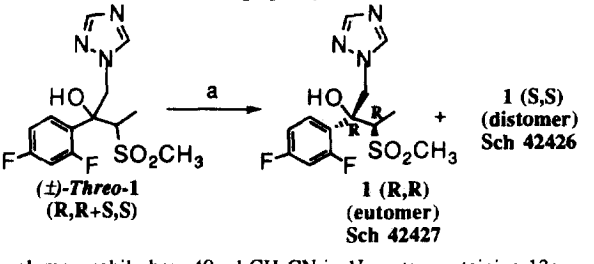
Abstract: Efficient approaches towards the preparation of chiral azole antifungals Sch 42427/ SM 9164 (**1**) via large scale chromatographic separation of its enantiomers, or via enzymatic syntheses of key chiral intermediates α-hydroxy arylketones **5** in excellent enantiomeric excesses (*ees*) are described.

Due to the potential role in AIDS related complications, the azole antifungals such as Sch 42427 (**1**) are of high current interest.[1] This literature suggests that in many cases, as exemplified by Sch 42427/SM 9164, only one of several isomers carry the eutomeric properties.[2] In the initial phases of the development of these compounds, small quantities of these eutomers have been obtained by resolving advanced intermediates (diasteromeric chemical derivatization/salt formation with optically active acids/bases). Although these approaches utilize efficient synthesis of *(±)-threo* (i.e. 1:1 mixture of R,R+S,S isomers) intermediates **7**(OR=SMe) or **8**, the resolution yields are often low. Furthermore, these processes are tedious requiring additional steps to obtain the final product in high chemical and optical purities. Once the absolute stereochemistry of the eutomer is determined, the total asymmetric syntheses of the compounds have been initiated[3,4]. In this report we describe a rapid, quantitative chromatographic process for obtaining the optically as well as chemically pure quantities of these compounds needed for the initial toxicology studies. We also disclose an enzymatic route for obtaining highly enantiomerically pure key intermediates, the α-hydroxy ketones, and their use for the total chiral synthesis of the antifungals through the intermediacy of chiral epoxide **7** and chiral diol **8**. This route is an attractive alternate to the Sharpless epoxidation, or dihydroxylation for the preparation of **7** and **8**, respectively[5].

As for the isolation of Sch 42427 (R,R-**1**), an efficient synthesis of racemic *(±)-threo* -**1** in five steps is known[2a]. Since chirally and chemically this compound is quite stable, we considered it to be a good candidate for large scale separation of its enantiomers. In fact, as depicted in **Scheme I**, a chromatographic procedure[6] utilizing readily available, inexpensive β-cyclodextrin as a resolving reagent in the mobil phase was developed that allowed for the use of commercially available preparative HPLC columns. This procedure led to the rapid isolation of 150+g of each of both enantiomers of *(±)-threo* -**1** in high purity.

Scheme I



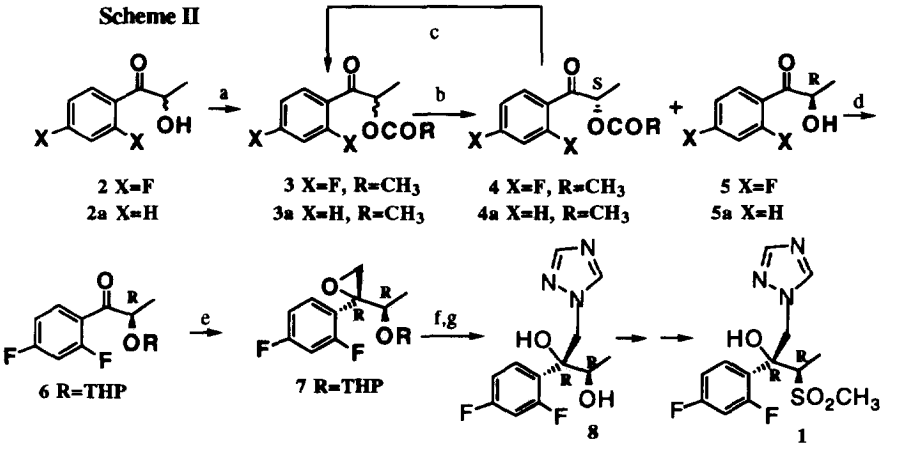
a) C-4 column, mobil phase:40 ml CH_3CN in 1L water containing 13g β-cyclodextrin + 6.5g KH_2PO_4; UV 210nm (see note 6 for details).

Although the above resolution was efficient, the undesired isomer (S,S)-**1** could not be conveniently converted to the desired Sch 42427 or to *(±)-threo* -**1**. In fact, the daunting task of maintaining the *threo*

stereochemistry while inverting/epimerizing a tertiary benzylic hydroxy group as well as doing the same to the adjacent hydrogen on the carbon bearing methylsulfonyl group (either simultaneously or in a stepwise manner), without generating rearrangement/elimination products, is not well precedented in the literature. This lack of recyclabilty of (S,S)-**1** led to a 50% waste[7] of all the reagents used in the preparation of *(±)-threo* **-1**. From environmental, and commercial perspective this was undesirable. Hence an alternate approach where such loss is minimized was developed which is depicted in **Scheme II**.

At the commencement of this work only the instability of α-hydroxyketones (**2**, and **2a**) was known[8,9]. With time it was realized that when free of acid or oxidizing reagents, compounds **2** and **2a** have a good shelf life (up to a year) provided they are stored properly (i.e. in a cold place and preferably under inert atmosphere away form light). Encouraged by this finding the enzymatic synthesis of **5** was undertaken .

Scheme II



a) Ref. 4a; b) See Table 1; c) i) Hexane,0.3mole % DBU, 35°C,18h ii) silica plug/ether, 65%; or i)THF/ 0.1mole% DBN, RT, 48h, ii) silica plug/ether, 65% d) DHP, PPTS, RT, quant.; e) (i) Me_3SOI/DMSO/60% NaH heated to 55°; add **6** in THF; f) DMF/Na -Triazole, 70°C; g) aq. HCl or pTSA/MeOH/H_2O, 60% for three steps (from **6**)

Based on the preliminary information available to us from previous work[4], for this enzymatic approach to be a practical synthesis, **5** with an *ee* of ~95% was needed. Here a series of enzymes were screened to identify several that led to the desired results (Table 1). Compared to compound **3a**, substitution of two aromatic hydrogens with flourines made the enantiomeric hydrolysis of **3** less discriminating (compare entries #1, and #2 with #7, and #8, respectively in Table 1), which required the screening of several dozen additional enzymes prior to identifying ones which led to an *ee* of >94 for (**R**)-**5**. A summary of useful enzymes resulting from this study is shown in Table 1. For this work two complementary, chiral HPLC procedures[10,11] were developed. One monitored[9] the consumption of two enantiomers of esters **4** and **4a**. When an enzyme indicated good (i.e. *ee* > 65%) discrimination of these enantiomers, the reactions were allowed to attain completion, and the products were isolated. The *ee* of the α-hydroxyketones (**5**, and **5a**) was then confirmed by second HPLC[10]. The application of some of these enzymes for compound **5a** was conducted as an aside.

The chiral alcohol **5** was converted to Sch 42427 as follows. Of several protecting groups evaluated for the conversion of **5** to **7** (R= TMS or Ac, unstable; TBDMS led to a slow conversion of **6** to **7**), THP was found the most attractive. In the latter case, due to the instability of **5/5a** to acids, essentially neutral PPTS was used as a catalyst for the protection of **5**. The use of DHP as a solvent allowed for mild conditions which led to a

quantitative yield of **6**. The addition of **6** as a THF solution to the preheated Me_3SOI/NaH/DMF mixture resulted in only a small amount (ca. 3-5%) of epimerization of the chiral center of **6** with a >7:1 ratio (NMR) of the desired threo (R,R) to undesirable erythro (S,R) **7**. Due to its basicity, the reaction of Na-triazole in DMF with epoxide **7** resulted in the epoxide opening at the non-benzylic center only (NMR, HPLC). The majority of the product from this reaction was the desired 1-substituted triazole, with only ~5% of 4-substituted triazole regio- isomer. Most of the undesirable traizole isomer and the *erythro* byproducts were removed by crystallization of diol **8**. The conversion of diol **8** to Sch 42427 was accomplished using previously reported conditions[4]. The enantiomeric purity of the final product was improved (>98%) by its crystallization from boiling water in which insoluble racemic **1** (i.e. 1:1 mixture of RR:SS isomers) was removed conveniently by filtration.

Table 1: Enzymatic Hydrolysis of Esters 3 and 3a:

#	Substrate	Enzyme (Reaction Time, h)[a]	Alcohol (*ee* %), yield[b]
1	**3**	Lipase AK, 72h	**(R) 5** (*92*), 46%
2	**3**	Lipase PS-30, 72h	**(R) 5** (*94*), 47%
3	**3**	Lipase AP-12 (*Aspergillus niger*), 28h	**(S) 5** (*64*), 50%
4	**3**	Urease type X (*Bacillus pasteruri*), 96h	**(R) 5** (*86*), 56%
5	**3**	LPL-80S *Pseudonomas sp.*, 5h	**(R) 5** (*94*), 54%
6	**3**	LPL-200S *Pseudonomas sp.*, 3h	**(R) 5** (*>98*), 51% [c]
7	**3a**	Lipase AK, 72h	**(R) 5a** (*98*), 45% [d]
8	**3a**	Lipase PS-30, 72h	**(R) 5a** (*96*), 48%

Notes: (a) All reactions were conducted at room temperature in a 0.2M phosphate buffer, pH 7.0 with a substrate concentration of 10%. The ratio of substrate to enzyme was form 3:1 (for crude enzymes) to 25:1 (for purer enzymes). These are unoptimized conditions. (b) The *ee*s were determined as described in ref. 10. Yield is shown as a mole % of **3**/**3a**. The rest of the mass was accounted for by the left over chiral esters **4** or **4a** of complimentary chirality (HPLC, note. 10). (c) $[\alpha]_D^{27}$ +73°; *c*:1, $CHCl_3$: (d) $[\alpha]_D^{RT}$ +85°; *c*:1, $CHCl_3$: (see ref: 12).

Having accomplished the goal of converting **5** with an *ee* ≥ 94% to Sch 42427, the focus of this work was shifted towards the incorporation of ester **4** (which was essentially of the same *ee* as its counterpart **5** for a given enzyme) towards the synthesis of **1**. Although hydrolysis followed by Mitsunobu inversion[13] could achieve this goal, we sought alternative enzymatic ways of utilizing **4**. The proton on the stereogenic carbon of esters **4** and **4a** is flanked by two electron withdrawing groups (i.e. the ester moiety, and the phenacyl moiety) which make it acidic. This intrinsic property of the compound was utilized to epimerize the undesired isomers of ester **4** and **4a** under basic conditions. It was found that bases such as K-O*t*Bu (aprotic solvents), NaOAc, DMAP, or DABCO led to negligible epimerization, whereas triethylamine led to a very slow epimerization. Quartenary bases such as $(Bu)_4NOH$ or $(Me)_4NOH$ caused hydrolysis. (These reactions were conducted for a period of 24 to 72hrs, with hexane, THF, or H_2O as solvent.) On the other hand hindered bases such as DBN, and DBU led to more rapid epimerization. For the purpose of subjecting thus racemized esters to enzymatic hydrolysis, they were purified by silica gel column chromatography. The yields reported in **Scheme II** reflect the yield of isolated epimerized **3** and **3a**[14]. Enzymatic hydrolysis of these epimerized esters progressed uneventfully. The recycling of these esters makes the enzymatic route depicted in **Scheme II** quite efficient for the synthesis of α-hydroxyketone **5** and hence for the synthesis of antifungals such as Sch 42427.

In summary, an HPLC method for the rapid, quantitative separation of enantiomers of *(±)-threo* -**1**, and enzymatic preparations of key chiral intermediate α-hydroxy arylketones in very good yields with excellent enantiomeric excesses for the preparation of chiral azole antifungals such as Sch 42427 (**1**) has been achieved.

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5 Since the allylic epoxidation is expected to lead to the wrong chirality at the benzylic center, the epoxide with chirality similar to **7** would entail further manipulations. No reports for the preparation of the diols similar to **8** via the dihyroxylation are known, presumably due to the difficulties in preparing the requisite trisubstituted olefins.

6 In a typical experiment ~3g of *(±)-threo*-**1** in 10ml TFA was injected on the preparatory column (4"x 24") and eluted with the mobil phase at a rate of ~350ml/min. The appropriate fractions were pooled and extracted with EtOAc . The extracts were concentrated to half volume, washed with aq. $NaHCO_3$ (to remove TFA), dried over anhyd. $MgSO_4$ and concentrated to obtain the separated isomers. Finally, each isomer was recrystallized from EtOAc/hexane to obtain 175g each of Sch 42427, and Sch 42426.

7 After the extraction of **1**, the mobil phase replenished with CH_3CN can be recycled on a larger scale.

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10 The *ees* of esters were determined with Chiracel AS®, 254nm, 7-7.5% *i*-PrOH/hexane, flow: 0.7-0.9 ml/min. The base line separation of the enantiomers was established by using the racemic esters.

11 The *ees* of alcohols were determined with Chiracel OB®, 220nm, 4-7% *i*-PrOH/hexane, flow: 0.7-1.1 ml/min. The base line separation of the enantiomers was established by using the racemic alcohols.

12 For chirally pure (NMR) (S)-**9a**, $[\alpha]_D^{RT}$ -86.7° *c*: 2, $CHCl_3$ has been reported by Davis, F.A.; Haque, M. S. *J. Org. Chem.* **1986**, *51*, 4085. Our *ees* (verified by chiral LC), and specific rotation agree with this result.

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14 The yields reported in this manuscript represent the product content in the worked up reactions. The small amounts of unreacted substrates and the regio isomers accounted for the rest of the mass. A detailed account will be available in a full paper.

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