Strategies for Access to Enantiomerically Pure Ecadotril, Dexecadotril and Fasidotril: A Review

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Abstract: Ecadotril and dexecadotril are powerful and selective inhibitors of neprilysin (NEP, EC 3.4.24.11) and are being developed as therapeutic agents, since they behave as prodrugs of the enantiomers of thiorphan. They exhibit different pharmaceutical profiles (intestinal antisecretatory action for the (*R*) enantiomer, *i.e.* dexecadotril, and cardiovascular activity for the (*S*) enantiomer, *i.e.* ecadotril). Fasidotril is a related compound which has special interest as an equipotent dual inhibitor of NEP and ACE (EC 3.4.15.1). This behavior confers on fasidotril powerful pharmaceutical properties in the cardiovascular field. This review deals with various synthetic approaches, either published or patented, for access to the enantiomerically pure or highly enriched forms of these drugs. Thus, different methods have been studied, which are taken from different methodologies of resolution procedures and asymmetric synthesis, namely :

i- Synthesis from a chiron from the chiral pool

ii- Chemical resolution of racemic precursors

iii- Enzymatic resolution and desymmetrization of *meso* starting materials

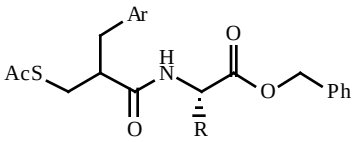
iv- Asymmetric synthesis, including enantioselective catalytic hydrogenation, alkaloid catalyzed asymmetric Michael additions, and diastereoselective alkylation of a chiral derivative.

Some of these methods are used in industrial processes leading to the indicated compounds.

INTRODUCTION

Racecadotril (***RS*)-1a** (previously named acetorphan) is used as a therapeutic agent against diarrhea, and was launched in France in 1993 [1]. Actually, it plays the role of a prodrug of thiorphan (*N*-[(*RS*)-1-oxo-2-(mercaptomethyl)-3-phenypropyl]-glycine) which acts as a powerful inhibitor of NEP (EC 3.4.24.11). It has been shown that the two enantiomers of thiorphan have a strong and equipotent *in vitro* affinity on the target enzyme [2]. However, the corresponding prodrugs display different pharmaceutical profiles. Indeed, the (*R*) enantiomer (dexecadotril (***R*)-1a**) is under clinical evaluation as an intestinal antisecretatory agent while the (*S*) enantiomer (ecadotril (***S*)-1a**) is useful in the cardiovascular field (Figure **1**) [3]. Fasidotril (***S,S*)-1b** is a related compound which has special interest as an equipotent dual inhibitor of NEP and ACE (EC 3.4.15.1) (Figure **1**). The compound is in clinical trial, since its dual mechanism of action, attenuation of angiotensin II and potentiation of atrial natriuretic factor gives useful activity for the treatment of hypertension and congestive heart failure [4].

The major challenge for the synthesis of these compounds is in the construction of the chiral mercaptoacyl part of the drugs, since a simple peptide coupling with either glycine or alanine (natural amino acids) leads to the drug (or the corresponding prodrug). In alternative approaches, the intrinsic chirality of the amino acid residue can be exploited for obtaining the stereogenic center in β-position of the thiol function. The different retrosyntheses are shown in Figure **2**.



(*S*)-1a, Ecadotril, R=H, Ar=Ph

(*R*)-1a, Dexecadotril, R=H, Ar=Ph

(*S,S*)-1b, Fasidotril, R=Me, Ar=piperonyl

Fig. (1).

Indeed, the industrial synthesis of both enantiomers of racecadotril involves the peptide coupling of benzyl glycinate with the optically pure 3-acetylthio-2-benzylpropionic acid **2a** of the required configuration, which thus constitutes the key intermediate for the synthesis of (***S***) and (***R*)-1a**. Fasidotril **1b** is obtained either by the same approach or by asymmetric Michael addition onto the acrylic peptide **6b**.

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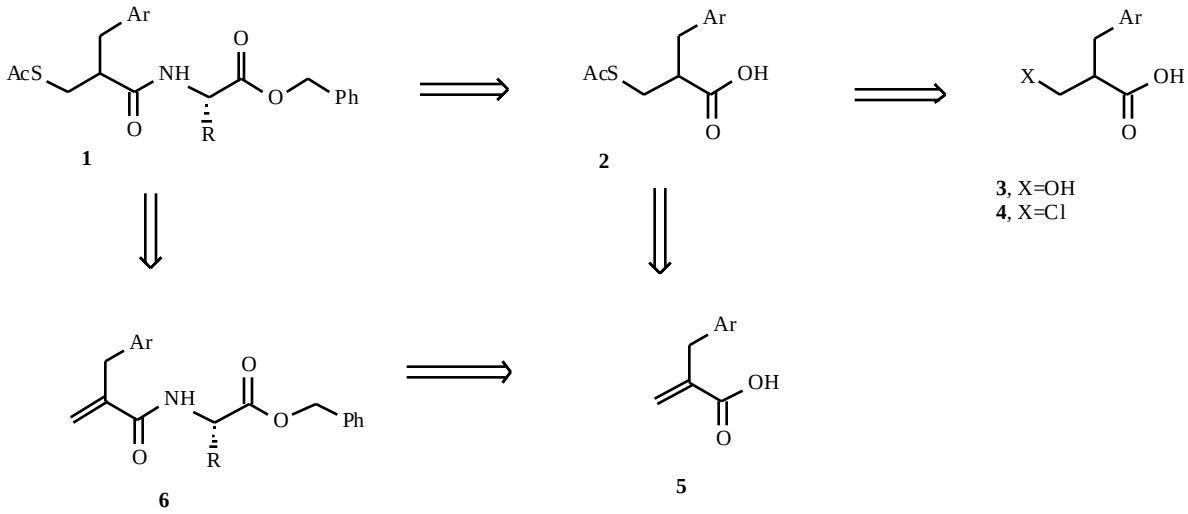


Fig. (2).

I. Chiral Pool Approach

In this type of method, chemists start from an available chiral compound bearing chirality by itself, and then modify it in order to obtain the desired chiral target.

In the chemistry of chiral mercapto acids, a report from Rüchardt *et al* suggests access to interesting precursors starting from (*S*)-phenylalanine [5]. Indeed, phenylalanine was transformed into a chiral azetidinone, *via* a stereospecific isonitrile-nitrile rearrangement by flash pyrolysis (up to multigram reactions). The first reaction involved the reduction of the starting material into (*S*)-phenylalaninol, which was then converted into the corresponding isonitrile in a two step sequence (Scheme 1). The key rearrangement step yielded the isomeric nitrile, which was transformed into the compounds (***S*)-3a** and (***R*)-4a**. These chiral molecules are direct precursors of the mercapto acid (***R*)-2a** required for the synthesis of dexecadotril, by a simple substitution of the leaving group (hydroxy or chloro function) by means of thioacetic acid [6,7].

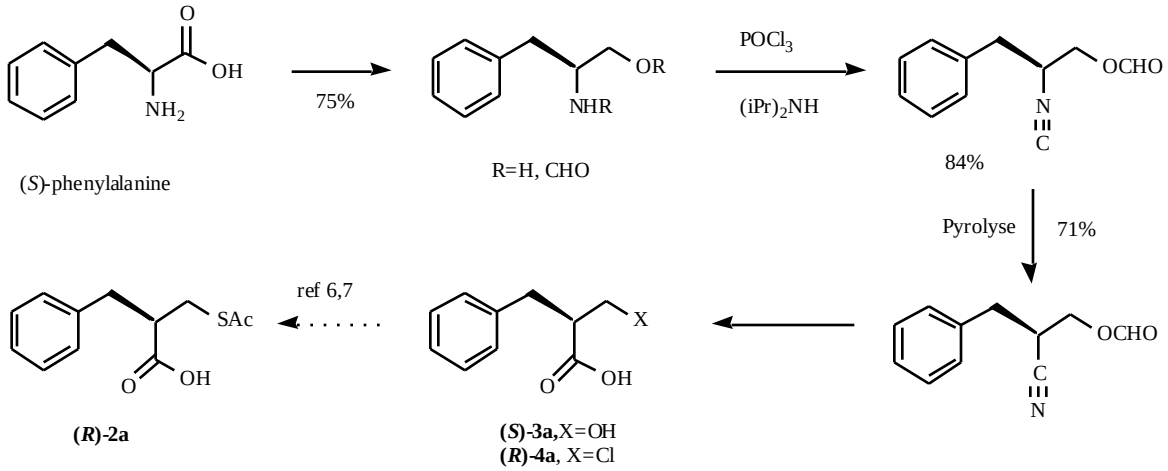
One can imagine access to ecadotril (***S*)-1a** by a similar procedure starting from unnatural (*R*)-phenylalanine: obviously, such a procedure would be considerably more expensive. This common drawback constitutes the major limitation of chiral pool approaches and justifies the necessity of alternative methods (*vide infra*).

II. Resolution Procedures

II.1. Chemical Resolutions

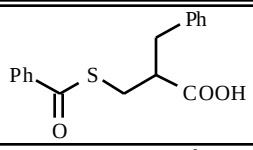
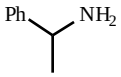
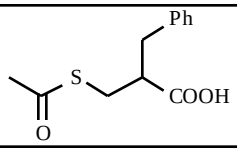
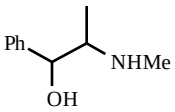
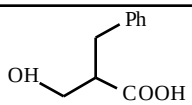
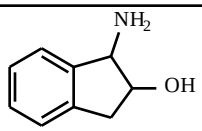
The first approach described in this field was disclosed in a patent from Pfizer [8]. Indeed, Bindra realized the resolution of the *S*-benzoyl mercapto acid by means of chiral phenylethylamine. This procedure was successfully applied by Fournié-Zaluski *et al*, who then utilized the resulting optically pure acid in order to prepare the two enantiomers of thiorphan (deprotected drug) [9].

In an independent study, our group decided to examine the resolution of the *S*-acetyl derivatives **2a** [2]. This approach has the advantage to give directly the required protected mercapto acid, which can be used for the coupling reaction with glycine benzyl ester without further modification. Experiments were carried out using the same chiral amine as Bindra and Fournié-Zaluski, without any success in the resolution. Nevertheless, we succeeded in a



Scheme 1.

Table 1.

Racemic substrate	Resolving agent	ee %	Yield% [a]	Ref
		80	10-15	8,9
		100	27 *ca* 40[b]	2,11
		100	35	10

a) Yield calculated from the racemic starting material; b) calculated from ephedrine.

practical and efficient resolution when using ephedrine as the resolving agent [2]. The best results were observed with 0.5-0.7 equiv. of the chiral amine in diethyl ether. One of the advantages of the method consists in the utilisation of ephedrine which is a cheap chiral reagent. Moreover, this synthon is available in both enantiomeric forms: this allows the preparation of both enantiomers of the protected mercaptoacid **2a**, and consequently, the preparation of ecadotril (***S***)-**1a** as well as dexecadotril (***R***)-**1a** by means of the same process.

Our work and that of Fournié-Zaluski led to both enantiomers of thiorphan, which were tested on neprilysin: an unexpected and interesting conclusion of these studies was the observation of the equipotency of the two enantiomers [2,9].

More recently, the Ajinimoto company described the resolution of a related compound, the corresponding β-hydroxyacid **3a**. The chiral resolving agent used in this study was a more exotic amino alcohol, *i.e.* cis-1-amino-2-indanol [10]. Again, the enantiomerically pure hydroxy acid **3a** thus obtained constitutes a direct precursor of the acetylthio derivative [6,7].

The three studies led efficiently to either the desired product **2a** or a direct precursor. However, enantiomeric purity is obtained only by recrystallization of the diastereomeric salts, thus lowering final yields. Our procedure presents the obvious advantages of avoiding any further modifications for obtaining **2a**, and can be applied to fasidotril precursor (***S***)-**2b** (yield 26%, ee 100%) [11].

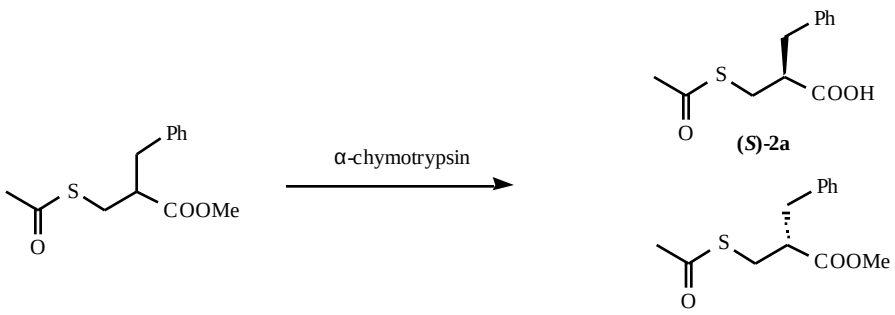
II.2. Enzymatic Resolution

In 1993 Kim *et al* described the only enzymatic resolution applied to date to this series of compounds. Indeed, they synthesized both enantiomers starting with the racemic acetylthio precursor **2a**, using a kinetic resolution with α-chymotrypsin as a key step [12]. This enzyme is an endopeptidase which hydrolyses with *L*-stereospecificity the carboxyl site of a peptide bond, and which also catalyses the hydrolysis of amino esters bearing a hydrophobic residue. Thus, the authors submitted the methyl ester of racemic **2a** to the action of α-chymotrypsin, and obtained the complete hydrolysis of the (*S*)-enantiomer, affording the corresponding acid (***S***)-**2a** in high optical purity (Scheme 2).

Clearly this procedure does not allow access to the acetylthioacid (***R***)-**2a**, since both ester and thioester functions are hydrolysed in the same step. Moreover, yields and detailed experimental conditions are not specified [12].

III. Desymmetrization of *meso* Compounds

In this part we describe our results in the preparation of both enantiomers (***S***)-**2a** and (***R***)-**2a** starting from the same prochiral precursor (Figure **3**) [6].



Scheme 2.

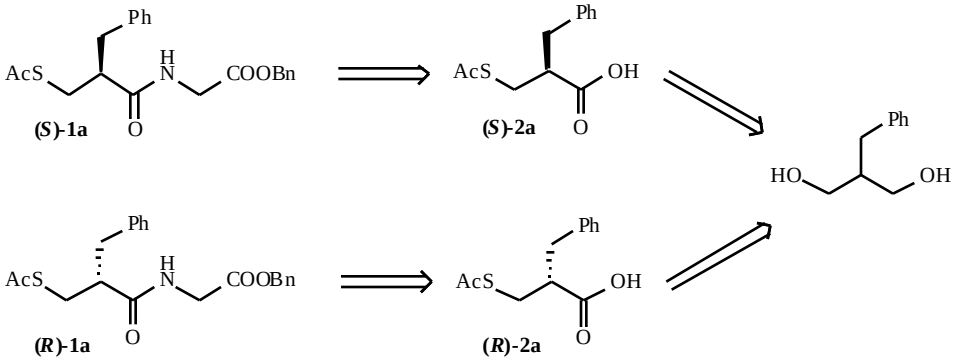


Fig. (3).

The key step of this work was the desymmetrization of 2-benzyl-1,3-propanediol by means of a lipase catalyzed transesterification (Scheme 3). Indeed, the known (*R*)-2-benzyl-1-hydroxypropylacetate was readily prepared from the prochiral diol using either the lipase PS Amano or the lipase P Fluka. After Jones oxidation, hydrolysis by means of aqueous lithium hydroxide provided the corresponding hydroxy acid (***S***)-**3a**, precursor of (***R***)-**2a,** *via* thioacetylation by the Mitsunobu procedure.

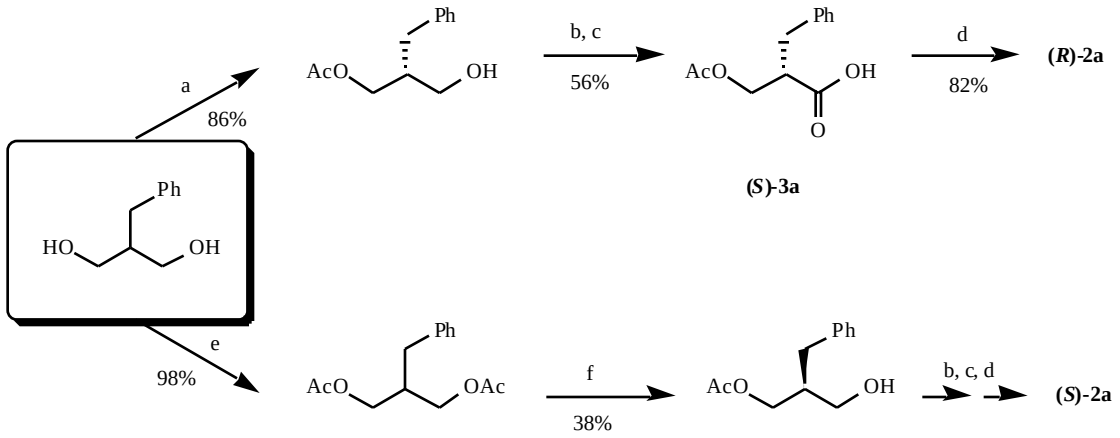
For access to (***S***)-**2a**, we turned to another strategy, in which the first step consisted of the diacetylation of the diol by means of Novozym 435 as the catalyst, in vinyl acetate to afford the corresponding prochiral diacetate. (*S*)-2-Benzyl-1-hydroxypropyl acetate was obtained *via* an enantioselective hydrolysis using Lipase P Fluka. A similar synthetic pathway as above allowed the transformation into (***S***)-**2a**. This gives the first convenient access to both enantiomers of **2a** by an enzymatic process starting from a single prochiral precursor. The low amount of catalyst needed and the efficiency of the synthetic steps allows the method to be used on a multigram scale.

Shortly after the Bioprojet patent [6b], a similar approach was disclosed by the Kaneka group [13]. The main improvement consisted of access to the enantiomer (***S***)-**2a**, by an elegant reaction sequence as described in Scheme 4.

The same approach allowed access to a direct precursor of fasidotril (Figure **4**).

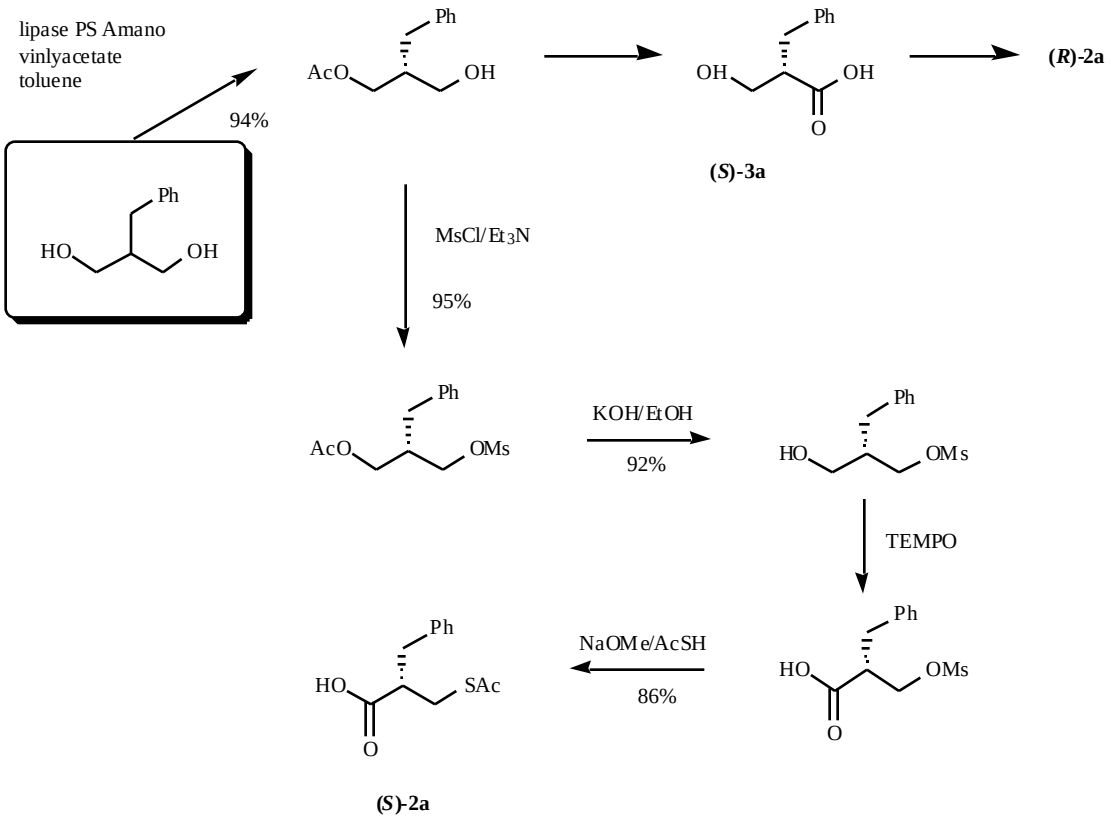
IV. ASYMMETRIC SYNTHESES

Resolution processes as described above are not actually asymmetric syntheses, since they do not give access to newly stereogenic units. This is not always true for chiral pool approaches which can yield the target molecule either by « simple » functionnal arrangement, as in the case described in chapter I (see Scheme 1) or by exploiting the initial chirality of the substrate in order to elaborate new stereogenic units. The latter case is known as first generation asymmetric synthesis, and one example is given below (diastereoselective Michael addition, see Scheme 11). Second generation methods also involve diastereoselective reactions, after coupling a chiral auxiliary, which can then be removed after the asymmetric step (for example, see diastereoselective addition onto enolates of chiral oxazolidinones, Scheme 5). Enantioselective syntheses require the use of either a chiral reagent (third generation) or



a: Lipase PS Amano, vinylacetate; b: Jones reagent; c: LiOH, THF/water=75/25; d: PPh_3, DIAD, thioacetic acid, THF; e: Novozym 435, vinyl acetate; f: Lipase P Fluka, 0.1 M KPB (pH7)/acetone (30%)

Scheme 3.



Scheme 4.

a chiral catalyst (fourth generation, see examples shown in Schemes 6 to 10). Desymmetrization by means of an enzyme is a special case of fourth generation asymmetric synthesis (see Schemes 3 and 4). This classification of asymmetric syntheses has previously been reviewed [14].

Interestingly, the synthesis of chiral compounds **2** described in the literature exemplify almost all the possible strategies, from resolution (either chemical or kinetic enzymatic resolutions, *vide supra*) to fourth generation catalytic enantioselective synthesis (*vide infra*). The only undescribed approach, to the best of our knowledge, is the case of third generation synthesis, which would necessitate the use of a stoichiometric chiral reagent. For example, one can imagine the enantioselective addition of a chiral thiol onto an acrylic precursor, thus giving a new asymmetric center (Figure **5**).

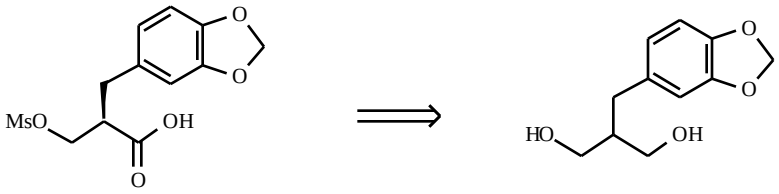


Fig. (4).

IV.1. Diastereoselective Alkylation

Evans *et al* proposed to synthesize both enantiomers of thiorphan by using the methodology previously discovered by the author [15]. Indeed, they prepared chiral enolates of

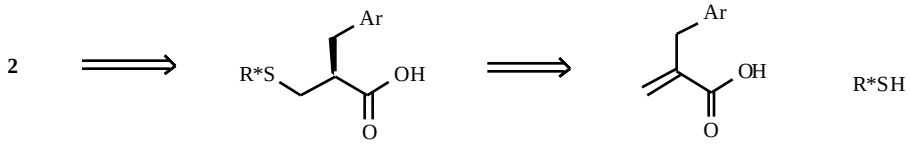
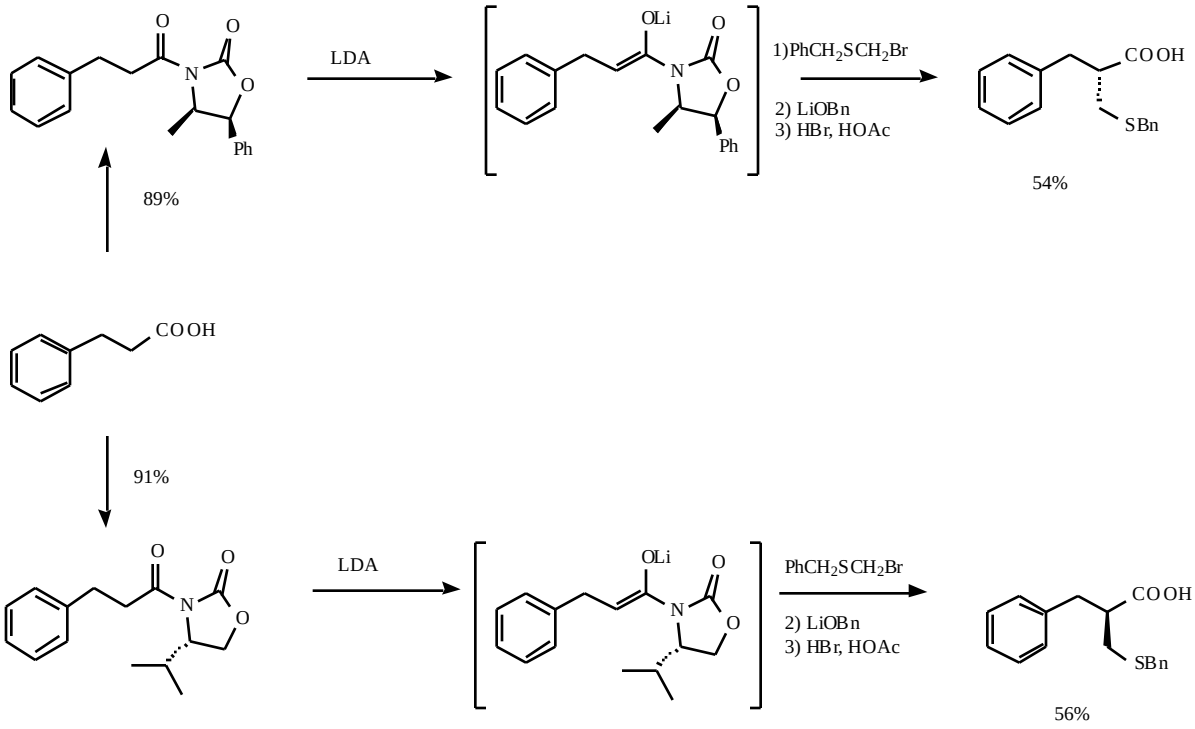


Fig. (5).



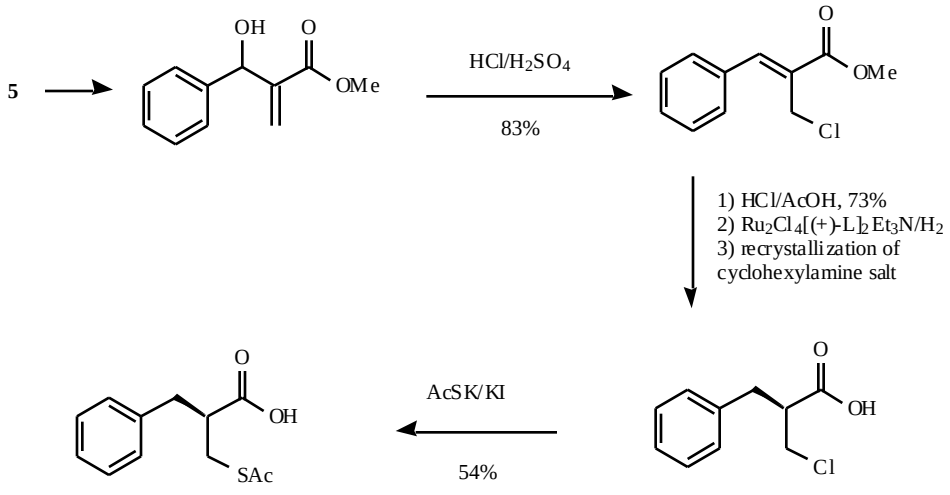
Scheme 5.

hydrocinnamic acid, after derivatization into chiral oxazolidinones. Diastereoselective alkylation of the enolates derived from either norephedrine or valinol led to the opposite configuration of the newly created stereogenic center (Scheme 5).

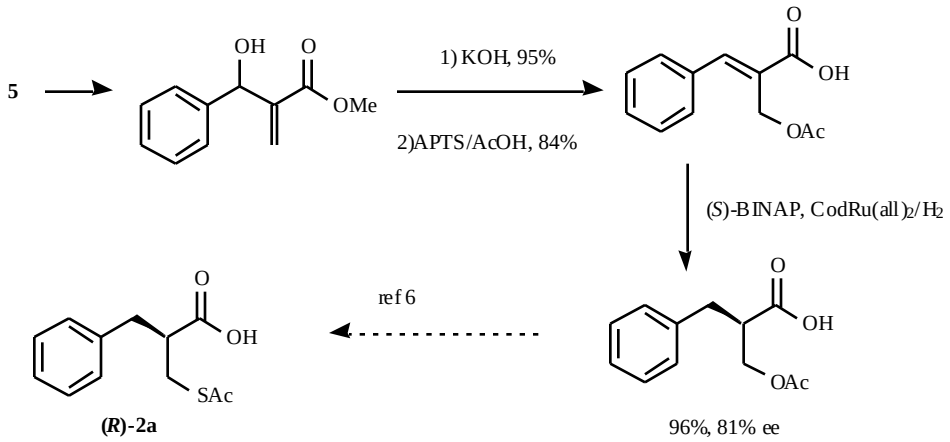
Starting from these two chiral intermediates, enantiomers of thiorphan were obtained in two steps, *i.e.* peptide coupling with glycine benzyl ester followed by double debenzylation. This method is rather attractive, but is probably difficult to use on a large scale, and moreover does not give a direct acces to the required *S*-acetyl prodrugs. Nevertheless, such asymmetric syntheses are useful tools on a laboratory scale: for example, the synthesis of mixampril (a dual inhibitor of ACE and NEP) by Turcaud *et al* involved such a procedure [16].

IV.2. Catalytic Enantioselective Hydrogenation

Two independent and closely related studies were simultaneously disclosed. Both of these syntheses used the same precursor **5**. Yuasa *et al* focused on the asymmetric hydrogenation of the chloro derivative [7], while Binay *et al* (Fournier Industrie) used the acetoxy compound [17]. The enantioselective key step involved in both cases a chiral Ru



Scheme 6.



Scheme 7.

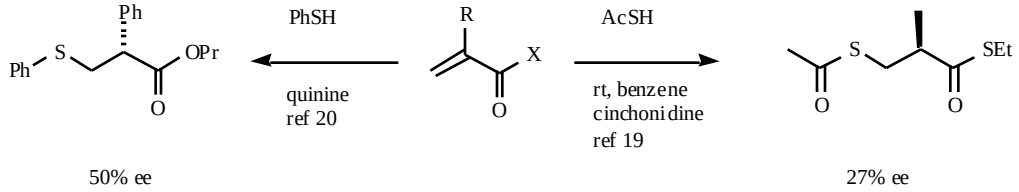
catalyst. The two approaches yielded the precursor with *ca.* 80% ee, which was improved by further recrystallization in the case of the chloro compound (***R*)-4a** (Scheme 6) [7].

In the case of the work of Fournier group, the published procedure stops at the chiral acetoxy stage (Scheme 7).

Such an approach, involving catalytic enantioselective hydrogenation, had been previously reported by Jendralla, for a β-mercapto acid derivative bearing an α-naphtyl residue in place of the phenyl ring [18].

The chiral catalysts used in these experiments were of the *Cinchona* alkaloid family, and enantiomeric excesses, while modest, were promising. One of the advantages of such a method is the mildness of experimental conditions, the use of a catalytic amount of the chiral auxiliary and possible access to both enantiomers of the target compound by exploiting the pseudoenantiomeric effect of the *Cinchona* alkaloids [21].

In a first series of experiments, we examined the hetero-Michael addition of thioacetic acid onto the corresponding enone [22].



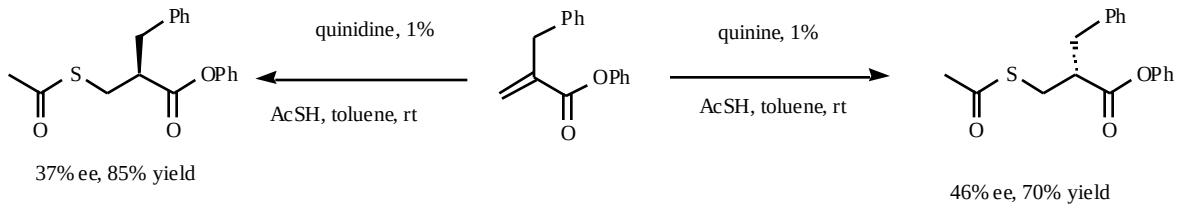
Scheme 8.

IV.3 Asymmetric Michael Additions

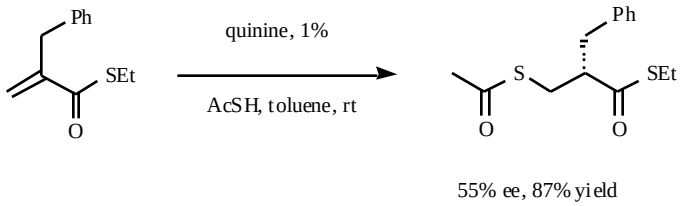
Our group was involved in a large programme for asymmetric synthesis of the *S*-protected β-mercapto acids, in parallel with our studies in the resolution field [2]. In this context, we preferably examined asymmetric Michael additions, which appeared to us an especially promising approach. Indeed, related studies had been previously reported in the literature, regarding access to either the precursor of captopril [19], or an aryl analogue (Scheme 8) [20].

The experiments reported in scheme 9 show the expected pseudoenantiomeric excess. The same procedure was applied to the corresponding ethylthioester, giving enantiomeric excess as high as 55% (*R* configuration, 87% yield), when using quinine as catalyst (Scheme 10).

In this case, the reaction time was shorter than in the ester series (1 week). In these studies, the origin af asymmetric induction remains unclear, but most of the published hypotheses rely on an enantioselective protonation of the primary adduct by the ammonium salt derived from the alkaloid [19,20]. Shortly after our work, Iritani *et al*

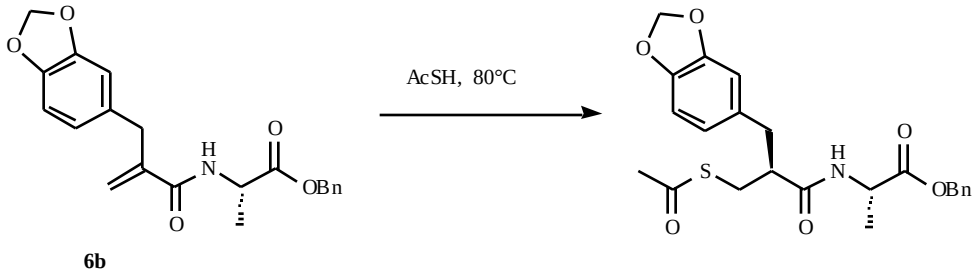


Scheme 9.



Scheme 10.

reported in a patent closely related results [23]. Thioacetic acid was added onto benzylacrylic ethylester, using *O*-acetyl interesting alternatives because of the high enantiomeric purity obtained and of the low amount of chiral catalyst



6b

(*S,S*)-1b, quantitative, 30% ed
98% ed after recrystallization

Scheme 11.

quinine as catalyst (10%). Similar results to ours were thus obtained (*S* enantiomer, 36% ee, 81 % yield).

Nevertheless, the asymmetric inductions observed remain moderate. We thus considered another approach, which could be more efficient, i.e. the diastereoselective hetero-Michael addition onto a substrate bearing a covalently bounded chiral moiety. In this context, we chose to examine the asymmetric synthesis of fasidotril, in which we wanted to take advantage of the chirality of the second amino acid residue, *i.e.* (*S*)-alanine [24,25].

For this purpose, we synthesized an acrylic peptide bearing the methyl-piperonyl residue **6b**, onto which we added thioacetic acid (Scheme 11).

We were pleased to observe a significant diastereoselectivity (about 30% depending on experimental conditions), even in the absence of a chiral catalyst [24,25]. The final adduct being a mixture of (*S,S*) and (*R,S*) diasteroisomers of fasidotril, we were able to reach optical purity simply by means of two recrystallizations in *iso*propanol (total yield 30%) [24,25].

CONCLUSION

This short review summarizes various approaches for the construction of a family of enzyme inhibitors in clinical trials for various therapeutic applications. Chemical resolution processes, despite the loss of at least half of the starting racemic material, give robust and reproductible results and are « easy » to exploit at an industrial scale. Nevertheless, enzymatic syntheses and resolutions could be needed. Finally, chemical asymmetric syntheses still need to be improved, but the very promising results reported herein should incite chemists to pursuit their investigations in this field.

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