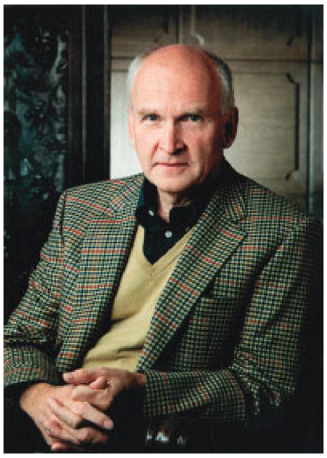
CHIRALITY 20:761 (2008)

Professor Wolfgang Lindner Winner of 2008 Chirality Medal



Wolfgang Lindner, Professor at the Department of Analytical Chemistry and Food Chemistry at the University of Vienna, Austria, has been selected as the recipient of the 2008 Chirality Medal in recognition of his outstanding achievements in the field of Separation Sciences and in particular in the domain of Stereoselective Separation Techniques.

During his academic life, Wolfgang Lindner has admirably succeeded in bridging both organic and analytical chemistry. Since the late 1970's, Wolfgang Lindner's research activities have been strongly focused on chromatographic stereoselective separation techniques. He is one of the pioneers in this field and greatly contributed to the dissemination of the technique around the world, making it the most popular method for the analysis of chiral compounds in biological media or those produced synthetically. He was one of the founding fathers of this new technology in many respects, including the design, synthesis and mechanistic understanding of anion- and cation-exchanger type chiral stationary phases, possessing unique features. His scientific career is driven by his fascination for the molecular recognition concept in chromatography.

With this award, Wolfgang Lindner joins the ranks of the 18 other distinguished scientists who have been awarded the prize since its institution in 1991 by the Societa Chimica Italiana (SCI). The Medal is given annually by the Chirality Medal Honor Committee and presented at a meeting of the International Symposium on Chirality. The formal presentation of the Chirality Medal to Professor Lindner will take place at the 20th International Symposium on Chirality, on July 6, in Geneva, Switzerland.

DOI: 10.1002/chir.20569

WILEY InterScience® DISCOVER SOMETHING GREAT CHIRALITY 20:762–770 (2008)

Review Article

A Consideration of the Patentability of Enantiomers in the Pharmaceutical Industry in the United States

CHRIS P. MILLER AND JOHN W. ULLRICH*

Department of Chemical and Screening Sciences, Wyeth Research, Collegeville, Pennsylvania

This article is part of the Special Issue on Chirality in the Pharmaceutical Industry

ABSTRACT During the last thirty years, concern over stereoselectivity of drug action has drawn a great deal of interest within the pharmaceutical field due to an improved understanding of the pharmacology and pharmacokinetics of enantiomers. Developing single enantiomers versus racemates or introducing a single enantiomer following the development of the racemic mixture appears to be the new trend. The intellectual property status of single enantiomers from racemates may be unclear. Drug discoverers and patent attorneys must examine the examples of the past to establish an appropriate pathway towards the development and intellectual property protection of chiral drugs. The review will focus on the patenting of an enantiomer in view of the prior art disclosure for the racemic mixture. *Chirality 20:762–770, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: chirality; selection patent; patentability; enantiomer; patent; eutomer; distomer

INTRODUCTION

In 2003, not a single drug was brought to the US market as a racemic or diastereomeric mixture[1] and 2004 saw the introduction of only one racemate, Gemifloxacin.[2] This is remarkable given that only 16 yr ago in 1990, more than 30% of the drugs brought to market were either racemates or diastereomers.[3,4] Keeping in mind the lag time between a drug's development and its market approval, these data indicate a clear sea change in the way that chirality has been handled in the drug discovery and development process.[5] Contributions to this seismic shift include a growing recognition of the important biological differences that can be manifested between each of a pair of the enantiomers and the growing technological prowess supporting the large-scale production of a single enantiomer.[6,7] At least partially as a consequence of these two factors, not only have completely new drugs tended to be single enantiomers, but also many enantiomers have been introduced to the market where the racemic correlate was already known. Since single enantiomers can often confer advantages in potency, efficacy, and/or safety over the corresponding racemate, the process of introducing an enantiomer when the racemate is already marketed can be very beneficial to the public.

In addition to the possible pharmaceutical benefits of a single enantiomer, the potential economic advantages need to be considered. One need only consider the tremendous amount of money that it can take to develop a new drug (est. >800 million dollars)[8] coupled with the fact that many of the drugs that finally do make it to market are not commercially successful. In this scenario, it makes sense that companies would look deep into their commercial portfolio (or other's portfolios) to see if there is any equity to be mined within the drugs that are already being successfully marketed. By taking a racemic product that is already marketed and improving it through developing and reintroducing it as a single enantiomer, the organization is engaging in what should be a lower risk proposition than drug discovery and development de novo since the racemate, at least to some extent, has already paved the way. A single enantiomer, if in fact it can demonstrate an actual pharmaceutical advantage[9] could have a quicker discovery turn around time, a more streamlined development, a smoother approval process, and a quicker uptake in the market (This strategy has sometimes been referred to as a "Chiral Switch" see: Refs. 3,4 for a detailed discussion). An example of such a process is shown in Figure 1.[10]

The process of introducing a single enantiomer to the market where before a racemic mixture had already been marketed has been commonly referred to as a "chiral switch."[3,4] The primary criterion necessary for a "chiral switch" is the change in the chirality status of the substance. Typically this is observed as a racemate to single enantiomer transformation but may also include alterations involving a nonracemic mixture of enantiomers.[11] Single enantiomers can confer advantages in potency, efficacy, and/or safety over the corresponding racemate, but

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*Correspondence to: John W. Ullrich, Department of Chemical and Screening Sciences, Wyeth Research, Collegeville, Pennsylvania 19426. E-mail: Ullricj@Wyeth.com

Received for publication 7 June 2007; Accepted 1 November 2007
DOI: 10.1002/chir.20520

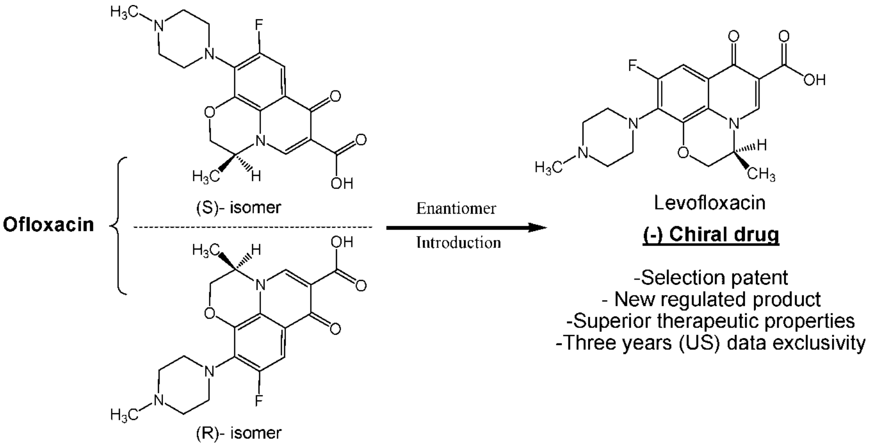

Fig. 1. An enantiomer introduction.

the possible advantages vary in a case-by-case basis and no generalizations can be made.

Some examples of drugs that have been re-introduced to the market as single enantiomers after they have already been marketed as their racemates are illustrated in Figure 2 (see the following: Levalbuterol, Levofloxacin, Dexketoprofen, Esomeprazole, Levobupivacaine, and Escitalopram).[12–17]

Although single enantiomers of previously approved racemates are not considered as a New Chemical Entity (NCE),[18] since they represent a previously approved active moiety, they are recognized by the FDA and assigned a CHE number.[19,20] These CHE numbers are assigned on a case-by-case basis, an example being CHE2, denoting "new derivative of an existing drug," which was assigned to esomeprazole magnesium (Nexium®).[15] As a result of the FDA's CHE classification, the introduction of an enantiomer, when the racemate has already been approved, probably does not obtain the five yr data exclusivity period, which is granted to new chemical entities but they should gain a three yr data exclusivity period after additional clinical trials are conducted.[18,*] Another potentially important advantage to bringing a single enantiomer to market is that the enantiomer may be patentable and, therefore, provide additional market exclusivity via a patent. The patentability aspect will be the focus of the remainder of our discussion.

PATENTS AND THE PHARMACEUTICAL INDUSTRY

Patents operate, as a "right to exclude" others from practicing one's claimed invention and, for patent applications filed after 1995, have a term of 20 yr from the date of their filing.[21–23] This patent right, along with freedom to operate, can allow the party holding the patent a period of time to develop their invention as well as market it.[†] In so doing, the patent laws can serve to encourage innovation and technological advancement. The exclusivity period is especially important where the cost or risk in engaging in a particular type of research is high, such as in the pharmaceutical industry. Furthermore, without patent protection, copying a marketed drug could occur very rapidly and few pharmaceutical companies would likely risk a long and expensive development process without the ability to exclusively market their product/invention.

To patent a compound in the United States, that compound must be:

- Useful;
- Novel;
- Nonobvious;
- Described in such a manner so that one of ordinary skill in the art can make and use the invention without undue experimentation; and
- Disclose the best mode for practicing the claimed invention.

We will now consider a scenario under which the patenting of an enantiomer sometimes occurs. In this scenario, we wish to patent the enantiomer where the racemate is already been known or disclosed (is in the prior art).

Since the racemate is part of the prior art, the prior art teachings about the racemate may be relevant to the determination of patentability of structures related to the racemate, including a single enantiomer. The prior art related to the racemate may include, for example, patents, publications, presentations, prior public use, sales, and the like. As stated earlier, in order for a compound to be patented, it must: (a) possess utility; (b) be novel (in view of the prior art); (c) be nonobvious (in view of the prior art); (d) it must be enabled so that one of ordinary skill in the art can make and use the invention without resorting to

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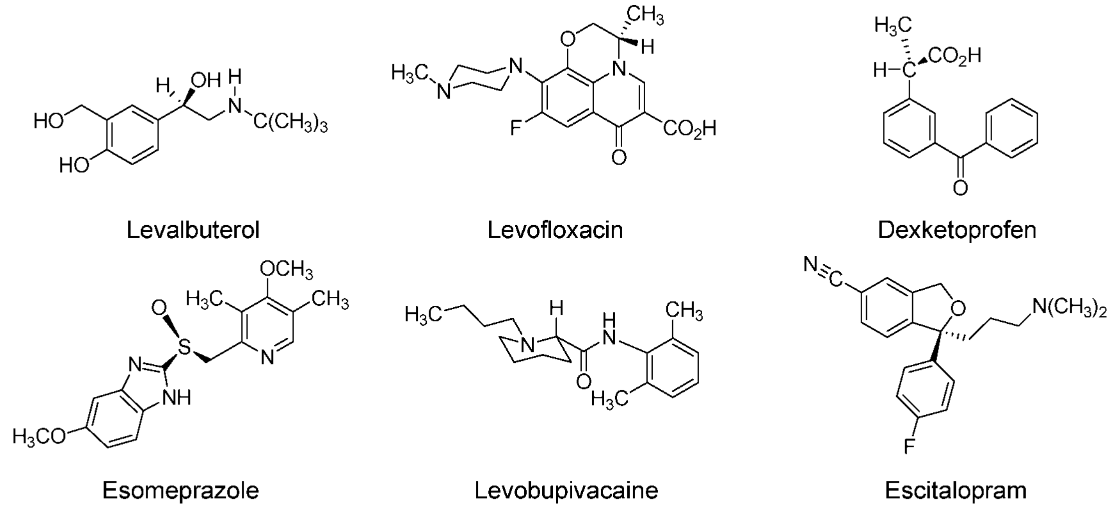
[*] Bear in mind that the three yr data exclusivity will not prevent a generic ANDA filing even as soon as the drug gets marketing approval, but it will prevent a generic drug from going to market prior to the expiration of the three yr period.

[†] A patent grants its holder a right to exclude but this does not necessarily mean that the holder can practice (make, use, or sell) the invention. For example, one might hold a patent that validly claims a molecule but another party may hold a patent that validly claims a genus of compounds that generically covers that molecule. Alternatively, legal or regulatory issues may prevent the practice of the invention.


Levalbuterol Levofloxacin Dexketoprofen

Esomeprazole Levobupivacaine Escitalopram

Fig. 2. Structure of some marketed single-enantiomer drugs.

undue experimentation; and (e) the patent must provide the best mode of practicing the claimed invention.

For the organization wishing to patent a single enantiomer in the shadow of the prior art on the racemate, some of the requirements for patentability will be in their control, while others will not. To further our understanding of these elements to allow a better appreciation of the reason why one element will be emphasized, these different criteria are summarized as they relate to the patentability of an enantiomer under this scenario.

Utility

For an invention to be patented, the invention must be "useful." This requirement is part of what is commonly referred to as the utility requirement in patent law. The utility requirement does not mean the claimed subject matter needs to possess exceptional or superior qualities. While there have been cases where chemical patent application claims have been rejected by the United States Patent and Trademark Office for a lack of utility and those rejections upheld by federal courts, such examples do not appear to be many and the subject matter of those claims were generally directed at chemical intermediates or starting materials, where those intermediates or starting materials were not alleged to have any useful activity in and of themselves but rather were alleged to be useful only in the production of final products where the final products themselves had only speculative utility.[24,25] As a general matter, the utility can be alleged in the patent application if it is substantial, specific and credible.[26,*] Actual data in the patent application is not always necessary to establish utility but if it is deemed to be necessary, *in vitro* and/or *in vivo* data may be used, depending on the particular circumstance. Since under our current scenario, the racemate is already known and possesses utility, demonstrating the utility of a closely related but putatively improved moiety should be relatively straightforward. Thus, the utility requirement is unlikely to present a significant hurdle to patentability for enantiomers provided it is properly asserted in the application.

Novelty

Novel means new and it is not surprising that one cannot patent something if it is not new. This requirement is strictly interpreted and as a result, novelty does not mean similar or functionally equivalent. The novelty requirement means that the claimed invention is not patentable if each and every element is disclosed by a single piece of prior art. Furthermore, novelty cannot be defeated by a combination of claim elements from different references. Accordingly, in order to determine if the novelty of an invention is defeated by a disclosure in the prior art, one must look at each and every element of the claimed invention and compare them to see if each and every element is found in the prior art being examined. If a claim contains 100 required elements and prior art reference "A" supplies 99 of the required elements and reference "B" supplies the other one, the invention is still novel, though it may suffer from other patent related infirmities. To demonstrate this principle, we now consider four separate claims from a hypothetical patent application, and then examine how a piece of prior art might affect the novelty of those four claims.

In Figure 3 below, claims are listed that appear in the hypothetical patent application that is claiming a compound, enantiomer, composition and method.

The identified prior art reference is a medicinal chemistry journal article that was published several years before the filing of the hypothetical patent application. The medicinal chemistry article contained the following relevant information. The authors disclosed that they had prepared 1.5 grams of racemic 1-(6-Fluoro-thiochroman-3-yl)-piperidine (compound **A** in Fig. 4) that was subsequently

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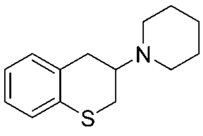
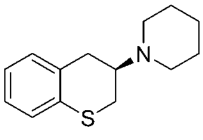
[*] "Specific" refers to the actual subject matter claimed and thus means the utility must relate to the claimed subject matter itself and not the broader class of the invention itself. "Substantial" refers to a "real-world" utility and thus means that the invention itself must be useful, not where the invention would require additional research to identify or confirm utility.

Claim 1: A compound having the structure **I**:



I

Claim 2: The compound of claim 1, wherein said compound comprises at least 95% the 3-(R)-isomer having the structure **II**:



II

Claim 3: A pharmaceutical composition, comprising a compound of claim 1 or 2 and at least one pharmaceutically acceptable excipient.

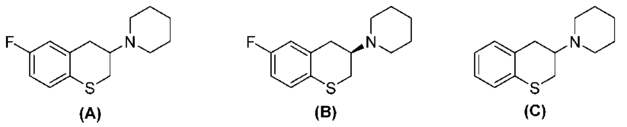
Claim 4: A method of preventing obesity comprising the administration of a composition according to claim 3 to a mammal in need thereof.

Fig. 3. Four claims from a hypothetical patent application.

resolved via fractional crystallization with a chiral acid to render the enantiomer **B**, which was reported to be free of its other enantiomer up to the level of detection (2%). Compound **A** was prepared from a bromo-precursor (compound not shown), and the bromo-precursor was prepared via electrophilic bromination of compound **C**. The preparation of compound **C** was disclosed, but the compound was apparently not tested.

The article further disclosed the testing of both the racemic mixture **A** and the single enantiomer **B**, which were administered in a cyclodextrin vehicle via gavage to Zucker obese rats in an established model where both compounds demonstrated significant anti-obesity effects at very low doses, although it appeared the single enantiomer **B** was approximately two times more potent. The authors further noted that the racemic mixture **A** appeared to cause tremors in many of the rats receiving it, even at doses below its weight-loss effective levels. The enantiomer was declared by the authors to be a very good candidate for further *in vivo* characterization and they suggested that it would be interesting to prepare and test additional analogues.

To determine the novelty-defeating effect of this reference on the cited claims, we begin with compound **A**. As we can see, compound **A** of Figure 4 is very similar in structure to the compound of claim 1 in Figure 3, differing by only the presence of a fluorine atom at the 6-position of the thiochroman. Is the compound of claim 1 novel in view of structure **A**? The answer is yes. While the two compounds differ only by the presence of a single fluorine atom, the prior art must be able to supply every claimed limitation and that is not the case here. Likewise, the disclosure of structure **A** does not render any of the other claims not novel either. Even though the compound has demonstrated efficacy for the same indication as the method of claim 4, it does not have the same structure since the method of claim 4 applies to the compounds and compositions of claims 1–3.



(A) (B) (C)

Fig. 4. Structures of compounds and compositions disclosed in prior art reference.

As for compound **B**, even though it is a single enantiomer and very similar to the compound of claim 2, its structure is not exactly the same and thus it does not meet the requirement for defeating novelty either. Interestingly, it is the intermediate used to prepare the tested compounds that could give this applicant some trouble with regard to novelty. In particular, the preparation of racemic mixture **C** was disclosed in this publication. Note that compound **C** was never tested for activity, but nevertheless its preparation has been described. Now consider claim 1. A literal reading of claim 1 indicates that it covers without further limitation, a compound of structure **C**. Thus, the description of **C** in the prior art publication meets all of the limitations of claim 1 since it describes the compound of formula **I**, as required in claim 1. Thus, claim 1 would be unpatentable for lack of novelty in view of the prior art reference description of **C**, but is this the case for claim 2? This aspect of the analysis is most relevant to the topic at hand. As we can see, there is a prior art disclosure of a racemic mixture that may affect a later claim to the compound as essentially one enantiomer. As can be seen from the specific facts of this case, a >95% single enantiomer of structure formula **II** is required to render claim 2 not novel. The prior art structure **C** has been described as a racemic mixture only and thus does not meet all of the limitations of claim 2 and thus claim 2 is novel. In other words, the requirement in claim 2 that at least 95% of the material must be the (*R*)-enantiomer must be met by the prior art disclosure for that disclosure to render the claimed subject matter not novel. Thus, the prior art disclosure of a racemic mixture should not defeat the *novelty* of a later-claimed single enantiomer or enhanced enantiomeric mixture.[27]

Claim 3 describes a compound of either structure **I** from claim 1 or a compound of structure **II** from claim 2, together with a pharmaceutical excipient. As can be discerned from the description of the prior art publication, a pharmaceutical excipient (cyclodextrin) was used, but only in combination with compounds **A** and **B** but not with **C**. Since the reference does not describe **C** together with a pharmaceutically acceptable excipient, the reference disclosure of **C** is not sufficient to defeat the novelty of claim 3, but is this so for claim 4? The method of claim 4 requires the administration to a *mammal* in need thereof, a compound of formula **I** or **II** together with at least one pharmaceutically acceptable excipient (claim 4 depends from claims 2 or 3 and thus includes all of the limitations from claim 2 or 3, which in turn depend from claim 1 and thus will include claim 1 limitations as well) (The use of the description "depends from" is the art-recognized equivalent of "depends on"). Since neither **A** nor **B** meet the structural requirement of claim 1 or 2, the disclosure of these compounds in the prior art publication does not defeat the novelty of claim 4 since claim 4 requires the use of the compound from claim 1 or 2. The disclosure of compound **C** thus does not meet all of the criteria of claim 4 either. Although **C** meets the structural requirement of claim 1, it was never disclosed in the prior art paper as being combined with a pharmaceutically acceptable excipient nor administered to a mammal in need thereof, and so claim 4 is novel as well.

Enablement

A patent cannot be granted if the invention described is not enabled. This means that the patent applicant must provide sufficient instruction in the description and claims of the invention so that one of ordinary skill in the art can make and use the invention without undue experimentation. It is not necessary that the application include every detail that is already known in the art and in fact, it is preferred that it does not.[28] The question of whether an amount of necessary experimentation is undue must take into account several guiding factors, among which the following eight are listed for your consideration. These factors are often referred to as the "Wands factors" (from the case containing the same name) and are listed below.[29,30]

A. The breadth of the claims;
B. The nature of the invention;
C. The state of the prior art;
D. The level of one of ordinary skill in the art;
E. The level of predictability in the art;
F. The amount of direction provided by the inventor;
G. The existence of working examples;
H. The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The question of whether a disclosure is enabling is really a broad inquiry that in turn is fact specific. While certain factors are outside the applicant's control when the application is drafted (factors B through E), for those within the applicant's (and/or their representative's) control, these factors should be kept in mind. For example, if one wished to include broad claims in the patent application, then one might be advised to provide representative description commensurate with the claim scope. Likewise, if one is claiming broadly, providing several working examples within, and representative of the scope of the claim is helpful. In contrast, if one were claiming a single enantiomer as a compound per se, then perhaps a single working example demonstrating how the compound is prepared might be sufficient. However, as mentioned previously, if the single enantiomer were to be broadly claimed in other ways, such as for use in the treatment of a large number of different diseases, then one might wish to provide a number of different working models and/or instructions regarding the utilization of the compounds to be commensurate with the scope of the claim. Finally, it should be appreciated that the enablement requires adequate description of "use" of the invention as well. The "use" portion of the enablement requirement overlaps with the utility requirement to the extent that one cannot teach how to use an invention if no credible utility is demonstrated or credibly asserted.

Best Mode

The best mode requirement exists to ensure that the patentee does not reap the benefits of a patent while hiding from the public the best way to practice the invention. One of the key features of the best mode requirement is that it does not mean that the applicant provides "the best mode" in an absolute objective sense but that the patentee provides what they reasonably believe to be the best mode at the time of the patent filing. It is possible that another way of practicing the invention is arguably a "better best mode," but if the applicant did not reasonably contemplate it as such, they have not violated the requirement.

Obviousness

Reviewing the brief description on novelty earlier, it is the case that even a very minor variation in, for example, a compound structure being claimed versus what is known in the prior art is sufficient to make the claimed compound novel. But note that it is not the case that a minor or insignificant change from what is known in the prior art would make the compound patentable. In fact, not only must a claimed invention be novel to be patentable, but it also must be nonobvious. In cases that have come before the federal courts where the issue of the patentability or validity of a patented enantiomer was at issue, the obviousness or nonobviousness of the enantiomer occupied a significant portion of the court's attention (see, for example, Refs. 31–33). As seen before, the prior art disclosure of a racemic mixture should not render a later claimed enantiomer not novel since each and every limitation of the claim may not have been met. So why is it not obvious to claim an enantiomer when the racemic mixture is already known? This is often the central question to be resolved and not surprisingly, the obviousness or nonobviousness of an enantiomer in view of the racemic mixture is going to depend upon, for example, what is known in the prior art and more importantly for our purposes, what the standard of obviousness means and how a court/jury applies it. To understand obviousness as it is applied to a later discovered enantiomer, we first review some general concepts of obviousness before turning to a recently issued federal court decision that applied these concepts to an actual litigation in the pharmaceutical industry.[34,*]

Obviousness, 35 U.S.C. 103 and the Graham Factors

The requirement that a patented invention be nonobvious originated in case law precedent and was eventually codified in 35 U.S.C. 103 of which the first section is produced below:

> "A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which such subject matter pertains. Patentability should not be negatived by the manner in which the invention was made."

In the above, "section 102" refers to the section of the United States Code requiring novelty of invention. The last sentence teaches that it does not matter how the invention was made, the invention will stand or fall on its own merits. This sentence was apparently added to reverse a perceived trend in the courts where it appeared that the inventor's state of mind was being considered.[35,†] Whereas legislative branches make the law, it is the job of the courts to interpret the laws and that is where we now turn our attention. Soon after the introduction of 35 U.S.C. 103, the case of Graham v. John Deere was decided by the United States Supreme Court and while the subject matter of the case itself is not relevant for present discussion purposes, its holdings is.[36] In particular, the Graham case has been useful since it provides an analytical framework for an obviousness inquiry. The Graham framework requires that an obviousness inquiry proceed with the following four steps:

Step 1: Determine the scope and content of the prior art.
Step 2: Ascertain the differences between the claimed invention and the prior art.
Step 3: Assess the level of skill in the art.
Step 4: Evaluate evidence of secondary considerations.

Step 1 requires that the obviousness inquiry consider the scope and content of the entire prior art. Thus, the art must be considered fairly for all that it teaches including art that not only might suggest the later claimed invention but also art that might teach against it. The second step requires that the differences between the claimed invention and the prior art be assessed. The third step is to consider the level of skill in the art. One of ordinary skill in the art is constructively assigned the knowledge of the art and thus any information in the art at the time of the invention is considered to be within their grasp and capability. Finally, and of very significant importance for the purposes of the present discussion is the evaluation of secondary considerations of nonobviousness. The use of the term secondary does not mean of secondary importance but rather refers to the consideration of important information regarding the nonobviousness of an invention including unexpected results, commercial success, long-felt need, copying by others, licensing, skepticism of experts, and independent development.

So, a common scenario for the patentability of an enantiomer is that the patent office will assert that the enantiomer is obvious where a prior art racemic mixture has been disclosed. This is probably not surprising given that the enantiomer is ½ of the racemic mixture and that it has the same structure. Furthermore, it is commonly appreciated that at least one of the two enantiomers (the eutomer), of a racemic mixture will often have useful activities (assuming such activity has been disclosed for the racemic mixture). The prior art disclosure of a racemic mixture, with desirable properties, will likely make a subsequent claim to either one or both of the separate enantiomers prima facie (on its face) obvious.

The inquiry is not over. Where such a prima facie case of obviousness has been made, it is still possible to rebut that case through a sufficient demonstration of nonobviousness via the introduction of secondary evidence of nonobviousness. Of these secondary factors, we focus on here evidence of unexpected and/or superior results. The reason that such results can in some cases rebut a prima facie case of obviousness is that they demonstrate that the compound refutes the normal expectation that a compound having a similar structure will have similar properties. Where a structural similarity is the cornerstone of the prima facie obviousness assertion, a successful rebuttal of that component can overcome the prima facie case. During the prosecution of a patent application, other forms of secondary evidence are often not available (commercial

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[*] The United States Supreme Court has very recently decided whether a claimed invention can be obvious in the absence of "some proven teaching, suggestion or motivation" that would have led a person of ordinary skill in the art to combine the relevant teachings. Although this case related to mechanical subject matter, it appears this holding could affect future chemical/pharmaceutical cases (see Ref. 38 and corresponding footnote).

[†] In this case, the United States Supreme Court instructed that the patentability of a device must reveal "the flash of creative genius, not merely the skill of the calling."

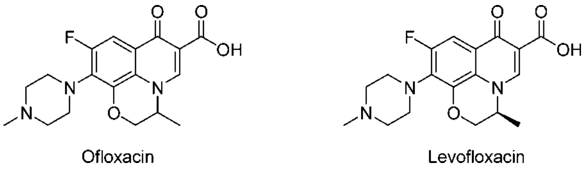

Fig. 5. Ofloxacin (Floxin®) and Levofloxacin (Levaquin®).

success, etc) but a comparison of the claimed invention with the prior art can be performed and that evidence is either provided in the application itself or submitted to the patent office in the form of a declaration.*

With that brief introduction in mind, we now review a case recently decided in the federal courts. Ofloxacin (marketed as Floxin®) is the racemic quinolone antibiotic having the structure shown in Figure 5. It was approved for marketing in 1985. Levofloxacin (marketed as Levaquin®) is the single enantiomer version of Ofloxacin (Floxin®) and was first approved in 1993.[10]

In the court case of *Ortho-McNeil Pharmaceuticals, Inc. v. Mylan Laboratories*, Inc. 348 F.Supp.2d 713 (United States District Court, N.D. West Virginia Dec 2004), the Federal District Court decided numerous issues related to an Abbreviated New Drug Application (ANDA) litigation† by generic challengers including several issues related to alleged invalidity of U.S. Pat. No. 5,053,407.[37] Among the asserted invalidity counterclaims made by the generic challengers was that the patented claims to levofloxacin were obvious in view of the prior art related to ofloxacin. In its extensive discussion of obviousness, the Court reviewed the data presented by the plaintiffs, which purported to demonstrate unexpected and superior results of levofloxacin when compared to the racemic compound, ofloxacin. These unexpected results were submitted to overcome any presumption of obviousness based on the structural similarity between the two compounds. In particular, the solubility of levofloxacin was highlighted by the Court as being especially surprising: The patent assignees provided evidence that demonstrated levofloxacin to be approximately 10 times more soluble than the racemic mixture. In addition to expert witness testimony documenting the significance of this large difference, the Court noted that prior to the discovery of levofloxacin, the largest reported difference in solubility between an enantiomer and its racemate was 5-fold. This difference alone, the Court indicated, was an unexpected result and presumably sufficient by itself to rebut the prima facie evidence presented against its patentability.

The Court also documented the fact that levofloxacin was approximately two times as potent as ofloxacin. While this fact alone might not have been persuasive, it was persuasive to the Court when coupled with the fact that levofloxacin was reportedly less toxic than ofloxacin. This appeared to contradict the conventional scientific wisdom in the art that the toxicity of the tricyclic quinolone antibiotics moved in parallel with the therapeutic activity.

The Court also noted the unexpectedly higher effectiveness of levofloxacin against *S. pneumoniae* when taking into account its exposure levels compared to oflaxocin as well as it's unexpected potency against quinolone-resistant *S. pneumoniae*. Taken altogether, the District Court found that the unexpected and superior results demonstrated for levofloxacin would be sufficient to rebut a prima facie case of obviousness (In this particular case, it appears that the District Court did not actually find that a prima facie of obviousness had been established).

The court also held that the generic defendant's additional defenses related to alleged invalidity of US 5,053,407 were not persuasive and the Court upheld the validity of the claims to levofloxacin and the Court's holding were affirmed without written opinion by the Court of Appeals of the Federal Circuit (see, for example, Ref. 38).‡ In addi-

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*Because of the typically lengthy time of drug development, a drug is not usually commercialized until well after a patent or patents issue(s) covering the product. Thus, while secondary evidence of nonobviousness, such as commercial success, may not be available at the time the patent is obtained, such evidence may be available and persuasive in subsequent litigation on the validity of the patent.

†A generic company seeking to market an innovator's product typically files an ANDA, Abbreviated New Drug Application. A paragraph IV filing must assert that the innovators patents are either invalid or not infringed. The filing of the ANDA is a constructive infringement for which the innovator has 30 days to file suit. Once the innovator files suit, a one-time 30 month stay will be initiated, during which time, the litigation will commence. During this litigation, if the generic company challenges the validity of the patent(s), the issues raised will be heard and decided by a federal district court. Any decision may be appealed to the Court of Appeals of the Federal Circuit (CAFC). Appeals from the federal CAFC may be made to the US Supreme Court, which may hear the case upon its discretion.

‡The Court rejected numerous alleged grounds of invalidity. In particular, the Court found that the prior art at the time of the invention coupled with the knowledge of one of ordinary skill in the art did not enable the production of the claimed single enantiomer (the prior art was not enabled). Moreover, the Court found the claimed enantiomer non-obvious in view of the prior art disclosing the racemic mixture. The racemic mixture is citalopram (Celexa®). Also, see Pfizer Inc. v. Ranbaxy Ltd., 405 F.Supp.2d 495 (D.Del.) 2005, where the Court upheld the validity of claims directed to Pfizer's very popularly prescribed LDL-lowering agent, atorvastatin calcium (Lipitor®). Among other issues decided, the Court found that the single enantiomer of the calcium salt was not prima facie obvious in view of a prior art patent application disclosing the racemate. The asserted claim of the patent was found invalid on appeal to the CAFC. 2006 WL 2137244, CAFC, August 02, 2006. However, the grounds for invalidation on appeal did not address the nonobviousness finding of the lower court. But importantly, please see Aventis et al. v. Lupin et al., 2006 WL 2008962 (E.D.Va.) July 17, 2006 where the district Court upheld the validity of 5,061,722, which claims ramipril (Altace®), a popularly prescribed ACE inhibitor useful for the treatment of hypertension. Among other issues decided in favor of patent validity, the district Court found that the enantiomer was not prima facie obvious in view of a prior art patent that disclosed a mixture of stereoisomers which included the claimed enantiomer. The district Court found that the prior art would not have motivated one of ordinary skill in the art to isolate the requisite enantiomer "substantially free of the other isomer" as required in the claim language. However, upon appeal to the Court of Appeals of the Federal Circuit, the claims to the enantiomer were found obvious and the relevant claims invalid. The Court of Appeals found there is a prima facie case of obviousness where "... it is known that some desirable property in a mixture derives in whole or in part from a particular one of its components, or if the prior art would provide a person of ordinary skill in the art with reason to believe that this is so, the purified compound is prima facie obvious over the mixture even without an explicit teaching that the ingredient should be concentrated or purified." Aventis Pharma Deutschland GMBH and King Pharmaceuticals, Inc. v. Lupin Ltd., CAFC (2006-1530, -1555). This CAFC decision came after the Supreme Court decision cited in footnote 30 and indicates that the US federal courts could more willingly find a claim to a single enantiomer prima facie obvious where the racemic mixture is known. Rebuttal of the prima facie case can still be made upon a sufficient showing, a showing which was deemed not sufficient in the instant case.

tion to the case just discussed, there has been at least two other, more recent federal district court decisions that have held that a single enantiomer was not obvious in view of prior art racemic mixtures.[39]

You might have noticed that the patentability of an enantiomer has been discussed primarily in the context of a situation where the prior art has already disclosed the racemic mixture. In the case where an enantiomer has been claimed and the prior art *does not* disclose the racemic mixture, the basic requirements of patentability do not change. What has changed is the prior art itself. We still must satisfy the basic elements of patentability. This does not mean that the patentee will be home free in terms of the prior art because the prior art still may contain compounds that are structurally very similar. In such a case, it still might be required for the patentee to submit secondary evidence of nonobviousness if the close teachings in the art are sufficient to make a prima facie case of obviousness.

CONCLUSION AND OUTLOOK

As will be seen from this brief exposition on the patentability of an enantiomer, an applicant needs to be aware of the requirements for patentability prior to the filing of the application to help ensure that those facets which might pose the greatest challenge to the patentability of their claimed invention are considered before the filing of the patent application. While the applicant cannot control what is already in the prior art, they can control the way that their application and claims are drafted in view of that prior art. Accordingly, an invention can be claimed in alternative ways that can help the invention to be further distinguished from the prior art. In this vein, consider the fact that we have primarily focused here on the patenting of an enantiomer per se (the compound itself) in view of the prior art disclosure for the racemic mixture. However, one can also consider claiming the enantiomer for specific therapeutic indications or pharmaceutical formulations that are not disclosed or suggested by the prior art.

With our increased knowledge of the role chirality plays in biological systems[40] coupled with significant technological advances for preparing and evaluating chiral molecules[5–7] we may continue to see a growth in the development of chiral drugs. Although it appears likely that we will see a decrease in the number of new chiral switches, single enantiomer additions to a marketplace already occupied by a racemic mixture of the same compound, since the introduction of racemic drugs into the market has dramatically declined, the patentability issues related to enantiomers in view of prior art containing racemic mixtures will be of ongoing interest. Furthermore, we may even see variations with formulations involving altered ratios of enantiomers to obtain optimal therapeutic profiles.[11] The patentability of such altered ratio enantiomeric mixtures will present a new twist on an old issue but the principles of patentability discussed herein should still apply.

ACKNOWLEDGMENTS

The Authors thank Dr. Oliver McConnell for providing valuable advice and guidance in the preparation of this manuscript.

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Chirality Forum

Pseudoasymmetry: A Final Twist?

SOSALE CHANDRASEKHAR*

Department of Chemistry, Middle East Technical University, 06531 Ankara, Turkey

ABSTRACT The original definition of "pseudoasymmetry" conveyed the apparent paradox that a tetrahedral center with four different groups did not result in overall chirality. However, there are problems in applying the concept to cyclic systems that do not contain chirotopic centers. Pseudoasymmetry appears most appropriate to acyclic systems with chirotopic carbon centers, e.g. the meso trihydroxyglutaric acids. Analogous cyclic cases, e.g. the isomeric 1,4-dimethylcyclobutanes, are best treated as diastereomers, and may indeed be described by an interesting extension of the *like–unlike* notation. Remarkably, in several tri- and tetramethylcyclohexanes, CIP descriptors cannot be applied even to chirotopic centers, which can only be described by the modified *l–u* notation. *Chirality 20:771–774, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: chirotopic; CIP; hyoscyamine; like–unlike; stereogenic; trihydroxyglutaric acid

INTRODUCTION

"Pseudoasymmetry" is an intriguing stereochemical concept the origins of which apparently date back to the 1890's. As the term implies, it refers to cases in which a molecule is achiral although it apparently possesses an asymmetric center.[1–3] The classic example of the concept is the case of the trihydroxyglutaric acids **1** (Scheme 1). Of the four possible stereoisomers, the two meso derivatives **1a** and **1b** are deemed to possess a pseudoasymmetric carbon atom at C_3. Although this center is connected to four different groups, the presence of the plane of symmetry passing through C_3 ensures that the molecule is achiral. Thus, inverting the configuration at C_3 only interconverts the diastereomers **1a** and **1b**, neither of which is chiral. "Pseudoasymmetry" has also been the subject of considerable debate and controversy in recent years (*vide infra.*)

It is important to note that in a molecule possessing several stereogenic centers, the inversion of one of them in a particular diastereomer would—in general—have one of three possible consequences for the overall chirality of the molecule: (i) retention of chirality, i.e. one chiral diastereomer is converted to another (e.g. D-glucose → D-mannose, α-D-glucose → β-D-glucose); (ii) creation of chirality, e.g. the conversion of meso tartaric acid to the optically active form; (iii) destruction of chirality, e.g. the conversion of optically active tartaric acid to the meso form.

A pseudoasymmetric center, however, submits to none of these consequences. Thus, inversion at a pseudoasymmetric center leads from one achiral form to another. This unique consequence—despite its linkage to four different groups—derives from the fact that the pseudoasymmetric center lies on a mirror plane as described earlier.

It is further intriguing that configurational descriptors can be assigned to a pseudoasymmetric center, despite the fact that it does not lead to enantiomerism as discussed earlier. Thus, the CIP nomenclature can be interestingly extended to the case of a pseudoasymmetric center to assign either the *r* or *s* descriptor as appropriate. (This is based on the assumed higher priority for the *R* configured center over the *S*, attached to the pseudoasymmetric atom.) Thus, "pseudoasymmetry" appears to define a conceptual middle ground between chirality and achirality.[†]

Recent decades have also witnessed a spirited debate on the precise definition of pseudoasymmetry.[2–5] The controversy essentially centers around the question of whether the molecule containing the pseudoasymmetric center can be chiral or not.[2] The currently accepted definition extends the pseudoasymmetry concept to (say) the case of the *l*-menthyl ether derivative **1c**, which is chiral by virtue of the menthyl moiety.

Although this seems to nullify the original definition of pseudoasymmetry (*vide supra*), it should be noted that even in cases such as **1c**, the overall chirality is not due to the pseudoasymmetric center. Thus, inversion at the pseudoasymmetric center still leads to a diastereomer (not

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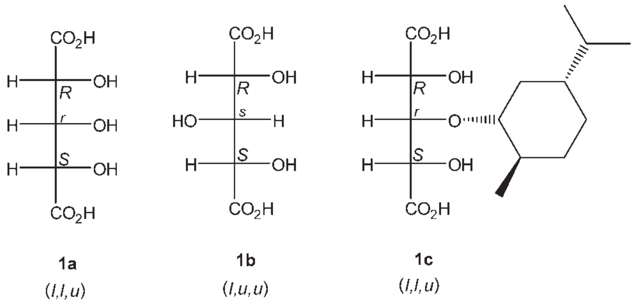
[†] The case of a carbon atom bearing two different sets of enantiomeric ligands, C(*R*)(*S*)(*R*′)(*S*′), is noteworthy. If *R* and *S*, as also *R*′ and *S*′, are enantiomeric but otherwise identical, the carbon centre would be "doubly-pseudoasymmetric," and it is easily verified that it would be chiral. "Double-pseudoasymmetry" would thus represent a transition from pseudoasymmetry to chirality. On the other hand, a carbon atom bearing two identical sets of enantiomeric ligands, C(*R*)$_2$(*S*)$_2$, would be achiral. Apparently, pseudoasymmetry depends on a delicate balance of symmetry, and occurs when a tetrahedral centre is bonded to two, and only two, enantiomerically related ligands.

Sosale Chandrasekhar while on Sabbatical leave from Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India.

*Correspondence to: Sosale Chandrasekhar, Department of Organic Chemistry, Indian Institute of Science, Bangalore 560012, India.
E-mail: sosale@orgchem.iisc.ernet.in

Received for publication 9 April 2007; Accepted 26 October 2007
DOI: 10.1002/chir.20515



Scheme 1. The trihydroxyglutaric acid diastereomers **1a–1c,** with the appropriate pseudoasymmetry descriptors (both *r-s* and *l-u*).

shown) but not an enantiomer (of the other diastereomer). Also, the expanded definition can be accommodated into the spirit of the earlier—more fundamentalist—view that pseudoasymmetry is the "duality arising out of the combination of two enantiomeric ligands with two enantiotopic half-spaces."[2,4] Thus, the enantiotopic half-spaces could be considered to exclude the additional chiral ligand (the chiral menthyl moiety in **1c**).

This author is largely in sympathy with the original formulation, which aptly conveyed the sense of paradox inherent in the pseudoasymmetry concept. However, to reiterate, a chiral moiety extraneous to the main framework bearing the pseudoasymmetric center, does not seriously affect the spirit of the classical definitions, and can be accommodated as discussed earlier.[‡]

Thus, the key requirement of pseudoasymmetry should be that despite the presence of chirotopic centers, the molecule as a whole is achiral by virtue of a plane of symmetry that also bears the pseudoasymmetric center. In the acyclic cases, this implies that pseudoasymmetry only occurs in certain meso isomers.[6] (Not all meso isomers, of course, bear a pseudoasymmetric center; cyclic systems can bear a pseudoasymmetric center without being strictly termed as meso, as discussed further below.)

The extension of the concept of pseudoasymmetry to cyclic systems, however, is not straightforward, as exemplified by the case of the symmetrically 1,3-disubstituted cyclobutanes **2** (Scheme 2).[1] These possess two stereogenic centers, but again by virtue of a plane of symmetry, are incapable of enantiomerism. However, there are important differences between the acyclic exemplars of pseudoasymmetry such as **1** and the cyclic analogs **2**, as discussed in the following section.

DISCUSSION

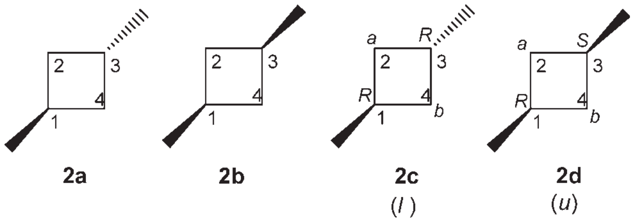
The 1,3-disubstituted cyclobutane system **2** possesses two stereogenic but achirotopic centers, C_1 and C_3. Indeed, the term "stereogenic" was introduced to describe such centers, as they cannot be termed "chiral centers," the molecule as a whole being achiral.[1,6] Thus, configurational inversion at these centers leads to diastereomers but not enantiomers. On the basis of this criterion, these centers have been considered to be pseudoasymmetric, and the pseudoasymmetric notation has been extended to them in an ingenious manner. This involves the tactic of "ligand complementation,"[1] originally employed to determine the priority order in the case of cyclic ligands at a chiral center.[7] Thus, the new descriptors r_n and s_n were proposed for systems such as **2**.

A problem with this approach is that the term "pseudoasymmetric" was (presumably) originally meant to convey the sense that even though such a center was bonded to four different groups, it did not lead to overall molecular chirality. This clearly does not apply to the case of **2**, in which the stereogenic centers are not bonded to four different groups. Indeed, there are no chirotopic carbon atoms in **2** at all.

One could, however, argue that in the case of the "trans" isomer **2a** the two "arms" of the cyclobutane ring that lead from one stereogenic center to the other, i.e. the two methylene groups, are enantiomorphic. Thus, although these two arms lead from (say) C_1 to the same stereogenic center C_3, one of them approaches C_3 from the left hand side and the other from the right hand side. (Thus, the molecule possesses a left-hand side and a right-hand side, despite being achiral overall. Note that **2a** possesses, in addition to the plane of symmetry passing through C_1 and C_3, a C_2 axis of symmetry passing though C_2 and C_4.) On this basis, an extension of the concept of pseudoasymmetry is perhaps admissible.

However, similar arguments cannot be made in the case of the "cis" isomer **2b**, which now possesses an additional plane of symmetry passing through C_2 and C_4 (orthogonal to the one passing through C_1 and C_3, and in lieu of the C_2 axis in the case of **2a**.) Accordingly, the two "methylene arms" of the cyclobutane ring (***vide supra***) are identical in the case of **2b**, so the stereogenic centers cannot be termed "pseudoasymmetric" in the true sense. Therefore, the validity of "pseudoasymmetry" is apparently dubious in the case of **2a** and clearly inadmissible in the case of **2b**. In any case, and decidedly, the concept of pseudoasymmetry cannot be applied uniformly to the two diastereomeric pair **2a** and **2b**, and thus loses much of its meaning and significance.

Thus, originally, "pseudoasymmetry" defined a phenomenon in which configurational change interconverted one "pseudoasymmetric form" to another. This clearly applies



Scheme 2. The 1,3-dimethylcyclobutane diastereomers **2a** and **2b**, with the appropriate diastereomer (*l-u*) descriptors, derived as indicated in **2c** and **2d**.

[‡] Mislow and Siegel have severely criticized the pseudoasymmetry concept as not being particularly meaningful.[5] They argue that a pseudoasymmetric centre can be classified satisfactorily on the basis of either its stereogenicity or its symmetry, and is thus neither novel nor useful. An alternative view, however, would be that pseudoasymmetry exemplifies the disconnect between stereogenicity and symmetry. (A pseudoasymmetric centre is rare in being stereogenic but achirotopic.)

to the case of **1a** and **1b**, but is tenuous at best in the case of **2a** and **2b**. Because of these ambiguities, it seems desirable to limit the use of the term pseudoasymmetric to refer only to the case of systems possessing chirotopic atoms. The case of **2a** and **2b** may perhaps be termed "quasi-asymmetry" to distinguish it from "pseudoasymmetry," but it appears best to treat it as a case of diastereomerism.

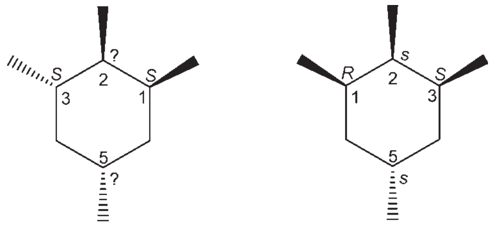
Therefore, "pseudoasymmetry" would generally appear to apply only to acyclic systems, as the additional symmetry elements that occur in cyclic systems complicate its application. Thus, the C_2 axis in **2a** and the mirror planes in **2b**, mentioned earlier, are absent in **1a** and **1b**. These symmetry elements relate to the absence of chirotopic atoms in the cyclic frameworks, and apparently conspire to invalidate the concept of pseudoasymmetry in them.

*Diastereomer Descriptors ('*l-u*') for 2*

In fact, the configurational descriptors employed for diastereomers[8,9] may be extended in an interesting way to the case of **2a** and **2b**. Firstly, the ring methylene centers are arbitrarily labeled as *a* and *b* (say, C_2 and C_4, respectively), thus (temporarily) converting C_1 and C_3 into chiral centers. Then, assuming a higher priority for the group labeled "*a*," the CIP chiral descriptors for C_1 and C_3 are determined (cf. **2c** and **2d**). The isomers are termed "*like*" (*l*) or "*unlike*" (*u*) depending on whether the descriptors are the same or different, respectively. Thus, the "trans" isomer **2a** would be *l* and the "cis" isomer **2b** would be *u*.

Note that this is a general result independent of the nature of the substituent: thus, (say) the "trans" and "cis" 1,3-dihydroxycyclobutanes would also be *l* and *u*, respectively. The configurations at C_1 and C_3 depend only on the order of C_2 and C_4, regardless of whether the substituent precedes or succeeds these. Note also that the configurations at the "temporary" chiral centers are not relevant to the final outcome: thus, the arbitrarily higher priority assigned to methylene group "*a*" implies the *R* configuration for C_1 and C_3 in the case of **2a;** the configurations would be *S* were methylene group "*b*" to be assigned the higher priority; however, the final outcome would be "*l*" in both the cases. Similar arguments can be made for the case of **2b:** C_1 and C_3 (respectively) would be either *R* and *S* ("*a*" higher priority) or *S* and *R* ("*b*" higher priority), the final outcome being "*u*" in both cases.

There are two main advantages of this notation over the current r_n–s_n notation.[1] Firstly, the *l–u* notation would clearly indicate that the case under consideration is one of diastereomerism rather than pseudoasymmetry; thus, it would distinguish **2** from a case of genuine pseudoasymmetry, e.g. **1**. Secondly, it is rather easier to apply, based on simple inspection ("at a glance"). This is because the the r_n–s_n notation is based on a rather elaborate disconnection scheme[1]: although it is valid, a simpler and equally rigorous notation should be preferred in practice.



3a

(1*S*,3*S*,2,5-*l*)

3b

(1*R*,3*S*,2,5-*l*)

Scheme 3. The 1,2,3,5-tetramethylcyclohexane diastereomers **3a** and **3b**, with the appropriate diastereomer (*l-u*) descriptors. (The *r-s* system is applicable only to **3b**, as indicated; however, in **3a** the CIP system itself fails at C_2 and C_5, as indicated by the question marks.)

In fact, the above disconnection scheme for the r_n–s_n notation depends on the tactic of "ligand complementation" of the CIP scheme, which leads to the priority order for multiply bonded and cyclic ligands. The disconnection "creates" chirality (where none existed), thus converting stereogenic centers into chiral centers, so the CIP system could be extended. In the present proposal, the arbitrary labeling of the ring methylene groups effectively raises the question "if the ring were to be chiral by virtue of substitution, what would be the configurations at C_1 and C_3"? Thus, both the previous r_n–s_n and the proposed *l-u* notations "temporarily create chirality." However, the latter approach is rather more straightforward as the rings need not be disconnected.[§]

Modified l-u *Notation for 1*

Interestingly, it is also possible to extend the *l–u* notation to systems bearing genuinely pseudoasymmetric centers such as **1**, by employing the modified *l–u* notation which arbitrarily assigns "*l*" to the lowest numbered chiral center (cf. Scheme 1).[8] This would be the *R* configured center attached to the pseudoasymmetric center. Furthermore, one may denote the analogous chiral and pseudoasymmetric descriptors as equivalent, i.e. $R = r$ and $S = s$. In the case of a pseudoasymmetric system with three stereogenic centers (e.g. **1**), the two possible stereoisomers would then be *l,l,u* and *l,u,u*.

All this, of course, is possible because pseudoasymmetry results in diastereomers. The extension of the *l–u* notation to pseudoasymmetry is a natural consequence of this fact. While in acyclic cases of genuine pseudoasymmetry (e.g. **1**) the *l–u* notation follows from the existing *r–s* one, in the "ambiguous" cyclic cases (e.g. **2**) extension of the *l–u* notation requires a departure from the existing r_n–s_n one, as discussed at length above.

Other Systems, Other Problems for the CIP System

It is also interesting to consider more elaborately substituted ring systems, e.g. the 1,2,3,5-tetramethylcyclohexane isomers **3a** and **3b** (Scheme 3). While **3a** is chiral, **3b** (a meso isomer) is not, so C_2 can be considered to be pseudoasymmetric in the case of **3b** but not **3a**, by the earlier definition. There is no problem in assigning pseudoasymmetric descriptors in the case of **3b**, but **3a** is rather simi-

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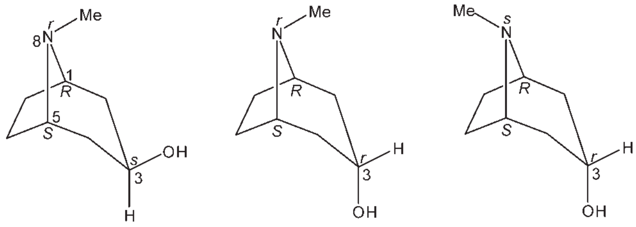
[§] It is noteworthy that both the r_n–s_n and *l–u* notations were proposed around the same time (1982),[1,8] so the suitability of the latter for cyclic cases (e.g. **2**) was not apparent.



4a **4b** **4c**

Scheme 4. Three 3-hydroxy-8-methyl-8-azabicyclo[3.2.1]octane diastereomers, **4a–4c**, with the appropriate pseudoasymmetry descriptors (*r-s*).

lar to the case of **2b** discussed at length above (pseudoasymmetry is inapplicable to both).

In fact, the case of **3a** is intriguing in that, despite its being chiral, the C_2 and C_5 centers cannot be assigned normal *R* or *S* configurations. Again, it would be inappropriate to consider them as pseudoasymmetric as the molecule is chiral, so the diastereomer notation described above needs to be employed, to arrive at "*l*." (This involves the arbitrary labeling of, say, the 1*S* center as "*a*" and the 3*S* center as "*b*," as described for the case of **2** above.) These examples again emphasize the problems of applying the pseudoasymmetry concept to ring systems, discussed above.

Thus, even with chirotopic centers in a cyclic system (i.e. **3a**), "pseudoasymmetry" cannot be applied in a straightforward manner. Note also the apparent breakdown of the CIP notation in the case of the C_2 and C_5 centers in **3a**, which can only be stereochemically designated by the above-described extension of the *l–u* notation. This is indeed a remarkable example in which chirotopic centers cannot be assigned CIP descriptors, but need to be assigned diastereomer descriptors. (Note that the case of the 1,2,3-trimethyl analog of **3a** would be similar.)[¶]

An Unambiguous Cyclic System

A genuine case of pseudoasymmetry in a cyclic system is that of 3-hydroxy-8-azabicyclo[3.2.1]octane (cf. **4a–4c**, Scheme 4). This is the hyoscyamine framework,[2] and interestingly, it contains two pseudoasymmetric centers, C_3 and N_8. Thus, **4a–4c** are diastereomers. The configurations at the C_1 and C_5 chirotopic centers, and the pseudoasymmetry descriptors at C_3 and N_8 have been indicated.

In the earlier discussed cases of **2** and **3**, the problem is that only one isomer of each pair possesses a pseudoasymmetric center, i.e. **2a** (possibly) and **3b**. This ambiguity is absent in the case of **4**, in which C_3 and N_8 are genuinely pseudoasymmetric, as they are flanked by two chirotopic centers (C_1 and C_5) and also lie on a mirror plane. (Note that a meso compound is defined as one of a set of stereoisomers, at least one of which is chiral[6]; thus **4** is not a meso compound as it does not possess a chiral stereoisomer.) Thus, "pseudoasymmetry" may also be applied in the case of cyclic systems, but with due care and caution.

CONCLUSIONS

The concept of pseudoasymmetry, despite its rarity, challenges the normal notions of chirality: although a pseudoasymmetric center is bonded to four different groups, it does not lead to the chirality of the molecule as a whole. Furthermore, pseudoasymmetry has played a seminal role in the development of the general concept of stereogenicity. (Thus, a pseudoasymmetric center may be termed as stereogenic but not chiral.) Apparently, however, "pseudoasymmetry" cannot be applied to certain ring systems in a consistent manner, because of the additional symmetry elements present in them (and the consequent absence of chirotopic ring atoms). These cases, in fact, can be treated as cases of diastereomerism, and the "*like–unlike*" nomenclature extended to them in an interesting manner. Therefore, it would be better to limit the use of the term "pseudoasymmetric" to atoms linked to chirotopic centers: in these the pseudoasymmetric center would lie on a mirror plane, and the pseudoasymmetry descriptors (*r* and *s*) can be employed for the resulting isomers. In certain cyclic chiral systems the limitations of the classical CIP notation become apparent; the modified *l–u* notation can be applied to these, which is remarkable in that chirotopic centers need to be assigned diastereomer descriptors.

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[¶] Interestingly, it may be argued that the case of **3a** supports the contention that chiral systems may be termed pseudoasymmetric. However, such an inclusive definition of pseudoasymmetry would be a travesty. Such ambiguous systems, whether achiral (e.g. **2a** and **2b**) or chiral (e.g. **3a**), can be described by the *l–u* notation, as discussed above.

CHIRALITY 20:775–780 (2008)

HPLC Enantioseparation and Absolute Configuration of Novel Anti-Inflammatory Pyrrole Derivatives

MARIANGELA BIAVA,[1] ROBERTO CIRILLI,[2]* VINCENZO FARES,[3] ROSELLA FERRETTI,[2] BRUNO GALLINELLA,[2] FRANCESCO LA TORRE,[2] GIOVANNA POCE,[1] GIULIO CESARE PORRETTA,[1] SIBILLA SUPINO,[1] AND CLAUDIO VILLANI[1]

[1]*Università "La Sapienza", Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, 00185 Rome, Italy*

[2]*Istituto Superiore di Sanità, Dipartimento del Farmaco, 00161 Rome, Italy*

[3]*Istituto di Cristallografia del C.N.R., Sez. di Monterotondo, 00016 Monterotondo Stazione (Rome), Italy*

ABSTRACT The assignment of the absolute configuration of novel anti-inflammatory pyrrole derivatives has been accomplished by a combined strategy based on independent physical methods. The key step of our stereochemical characterization approach is the production at mg-scale of enantiomerically pure forms by HPLC on Chiralpak IA stationary phase. *Chirality 20:775–780, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: anti-inflammatory pyrrole derivatives; stereoselective HPLC; polysaccharide-based chiral stationary phases; Chiralpak IA; semipreparative scale enantioseparation; chiroptical properties; absolute configuration

INTRODUCTION

The pharmacological and pharmacodynamic properties of biologically active chiral compounds are strictly related to their stereochemistry. Usually, only one enantiomer is found to have the desired effect while the other may be inactive or display other biological effects.

Knowledge of absolute configuration is thus of great importance to recognize the topology of receptorial site and it has become a key step in development of new chiral drugs.[1] For interpreting the absolute configuration (AC) several physical methods including nuclear magnetic resonance (NMR), circular dichroism (CD), vibrational circular dichroism (VCD), optical rotatory dispersion (ORD) or X-ray crystallography have been used.[2–10]

In this research area, empirical or more sophisticated theoretical treatments of spectroscopic data have been widely employed. However, to avoid misleading results the configurational assignment of a new family of compounds should be verified by comparing the findings of different methods or, in the case of solid or easily functionalizable samples, by X-ray structure analysis.

Here we describe the HPLC resolution and absolute configuration assignment of a series of novel anti-inflammatory alcohols and ethers incorporating a pyrrole nucleus directly linked to stereogenic center (Fig. 1) (Biava M, Porretta G, Poce, G, Supino S, Manetti F, Botta M, Sautebin L, Ghelardini C, Rossi A, Makovec F, Anzini M, Cirilli R, Ferretti R, Gallinella B, La Torre F. J Med Chem submitted). Enantiomerically pure samples, necessary as starting materials in stereochemical characterization studies, were obtained at mg-scale by HPLC on the amylose-based Chiralpak IA[11] stationary phase (CSP). We first determined the absolute stereochemistry of enantiomers of one of these compounds by X-ray crystallographic analysis. Then, the stereochemical study was extended to other pyrrole derivatives by CD spectroscopy.

MATERIAL AND METHODS

Compounds **1a,b-3a,b** and **4** were synthesized as reported elsewhere.[12] (Biava M, Porretta G, Poce, G, Supino S, Manetti F, Botta M, Sautebin L, Ghelardini C, Rossi A, Makovec F, Anzini M, Cirilli R, Ferretti R, Gallinella B, La Torre F. J Med Chem submitted).

Analytical and semipreparative HPLC enantioseparations were performed by using stainless-steel Chiralpak IA columns (250 × 4.6 mm and 250 × 10 mm I.D., 5 μm; Daicel Chemical Industries, Tokyo, Japan). Mobile phases for chromatography were prepared from HPLC grade solvents. Ethanol and ethyl acetate (EA) were obtained by Baker (Deventer, Holland), *n*-hexane and dichloromethane were purchased from Merck (Darmstadt, Germany). The hold-up timer marker 1,3,5-tri-*tert*-butylbenzene was purchased from Sigma (St. Louis, MO).

HPLC enantioseparations were carried out by using a Perkin Elmer (Norwalk, CT) 200 Lc pump equipped with a Rheodyne (Cotati, CA) injector with a 20 μL or 1 mL sample loop, a Perkin Elmer column oven and a Perkin Elmer 290 UV detector. Sign of the optical rotations of the resolved enantiomers was measured on-line at a wave-

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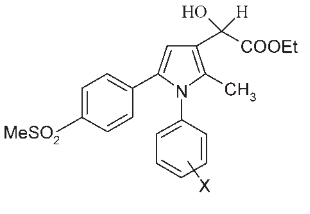
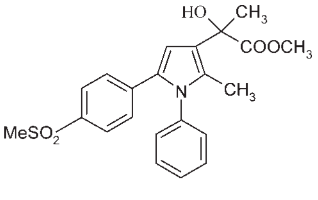
Contract grant sponsor: Rottapharm, Italy

*Correspondence to: Dr. Cirilli, R., Istituto Superiore di Sanità, Dipartimento del Farmaco, Viale Regina Elena 299, 00161 Rome, Italy.
E-mail: rcirilli@iss.it

Received for publication 29 October 2007; Accepted 1 November 2007
DOI: 10.1002/chir.20518



1a,b-3a,b



4

Compound	R	X
1a	H	H
1b	Et	H
2a	H	3-F
2b	Et	3-F
3a	H	4-OMe
3b	Et	4-OMe

Fig. 1. Structures of the chiral analytes **1a,b-3a,b** and **4**.

length of 365 nm by a Perkin Elmer polarimeter model 241 equipped with Hg/Na lamps and a 40 μL flow-cell. The system was at a temperature of 23°C. The signal was acquired and processed by Clarity (Data Apex, Prague, The Czech Republic) software.

Specific rotations of the single enantiomers of **1a,b-3a,b** and **4** dissolved in ethanol were measured on a Perkin Elmer 241 polarimeter equipped with a Na lamp operating at 589 nm. The volume of the cell was 1 mL and the length of the optical path 10 cm. The system was at 23°C.

The circular dichroism (CD) spectra of enantiomers of the chiral analytes **1a,b-3a,b** and **4,** dissolved in ethanol (concentration about 0.1 mg/mL) in a quartz cell (0.1 cm path length), were recorded by using a Jasco J-710 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan). The system was thermostated at 25°C. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

The ultraviolet (UV) spectra of the (+)-enantiomers of the chiral analytes **1a** and **4,** dissolved in ethanol (concentration about 0.01 mg/mL) in a quartz cell, were recorded by using a Unicam UV4 spectrophotometer.

Alcohol (−)-**2a** crystallized from acetone/EA as irregular transparent prisms. A crystal of dimensions 0.5 × 0.4 × 0.3 mm was selected and fixed on a glass fiber. Diffraction data were collected on a Rigaku four-circle diffractometer equipped with a rotating anode (graphite monochromated Cu-Kα radiation) by the θ-2θ scan method in the range 3.6° ≤ θ ≤ 62.1°. Data were corrected for Lorentz and polarization effects and from absorption by the semi-empirical ψ-scan method. The structure, consisting in two independent molecules in the asymmetric unit, was solved by direct methods and refined using the SIR2004 package of crystallographic programs.[13] Non-hydrogen atoms were refined anisotropically, while hydrogens were included at calculated positions and refined in a riding mode. The refinement of 541 parameters, carried out on F, with the molecules in the (*R*)-configuration, converged at $R_F = 0.053$ for 3077 reflections with $I > 3\sigma(I)$. Refinement of the Flack factor[14] × for the (*R*)-configuration gave the value of −0.006 (37), very close to the expected value for the right solution $x = 0.00$, while for the wrong (*S*)-configuration gave $x = 0.98$ (3) (expected 1.00). Such values allowed to assign unambiguously the (*R*)-configuration to the (−)-2a derivative.

RESULTS AND DISCUSSION

Analytical and Semipreparative Enantioselective HPLC

The first step of our study was addressed to develop enantioselective chromatographic conditions for obtaining enantiomerically pure samples at mg-scale. The enantiodiscrimination of pyrrole derivatives **1a,b-3a,b** was optimized by using the immobilized-type Chiralpak IA CSP, a "second generation" of polysaccharide-based CSPs for HPLC. The commercially available Chiralpak IA CSP contains amylose 3,5-dimethylphenylcarbamate as polymeric selector, which is immobilized and not coated onto a silica matrix (as in the Chiralpal AD CSP). The immobilization of the polymeric selector to the chromatographic matrix leads to high solvent stability and offers the possibility to develop multiple selectivity profiles with no constraint on the mobile phase composition.[15]

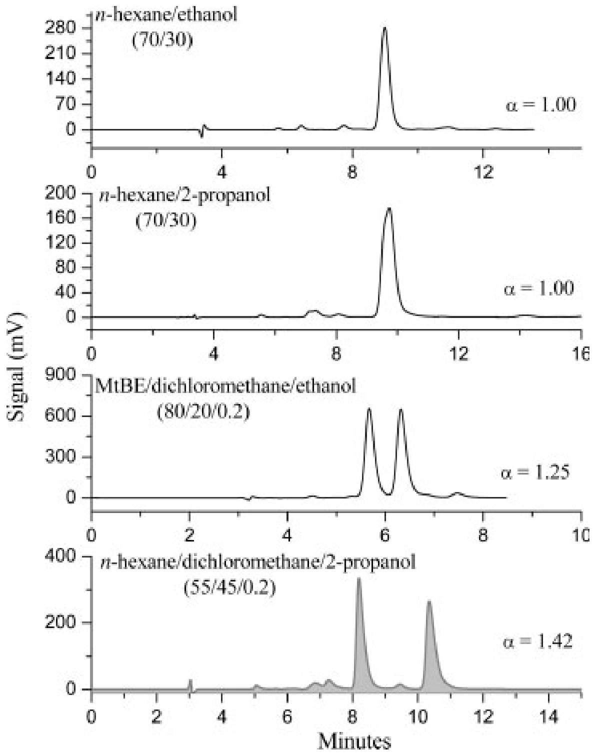


Fig. 2. Chromatograms concerning the resolution of **4** under various IA/eluent conditions. Flow rate: 1.0 mL/min; temperature: 25°C; detection: UV at 280 nm.

TABLE 1. Chromatographic, quantitative and polarimetric analysis of the pooled fractions containing the 1st (F1) and 2nd (F2) eluted enantiomers of 1a,b-3a,b and 4

						F1[e]		F2[e]	
Compound	Mobile phase	k_1[a]	α[b]	R_s[c]	SR[d]	*ee* (%)	$[\alpha]_D^{23}$	*ee* (%)	$[\alpha]_D^{23}$
1a	*n*-hexane-dichloromethane-ethanol 50-50-1	1.17 (+)	1.56	5.31	10	99.0	+70 (c = 0.05, EtOH)	99.0	−60 (c = 0.05, EtOH)
1b	*n*-hexane-ethyl acetate-ethanol 75-25-0.2	2.94 (+)	1.33	4.13	5	>99.0	+41 (c = 0.03, EtOH)	>99.0	−52 (c = 0.03, EtOH)
2a	*n*-hexane-dichloromethane-ethanol 50-50-1	1.19 (+)	1.47	4.50	13	>99.0	+88 (c = 0.02, EtOH)	>99.0	−81 (c = 0.05, EtOH)
2b	*n*-hexane-ethyl acetate-ethanol 75-25-0.2	2.67 (+)	1.32	4.12	5	>99.0	+45 (c = 0.04, EtOH)	99.0	−34 (c = 0.02, EtOH)
3a	*n*-hexane-dichloromethane-ethanol 50-50-1	1.22 (+)	1.93	7.66	25	>99.0	+57 (c = 0.09, EtOH)	>99.0	−58 (c = 0.09, EtOH)
3b	*n*-hexane-ethyl acetate-ethanol 75-25-0.2	3.10 (+)	1.30	2.90	3	99	+32 (c = 0.04, EtOH)	99	−43 (c = 0.04, EtOH)
4	*n*-hexane-dichloromethane-2-propanol 55-45-0.2	1.73 (+)	1.42	4.66	15	99	+58 (c = 0.10, EtOH)	99	−55 (c = 0.08, EtOH)

Column: Chiralpak IA (250 × 10 mm I.D); flow-rate: 4.7 mL/min; detector: UV at 360 nm; temperature: 25°C.

[a] Retention factor of the first eluted enantiomer, defined as $(t_1 - t_0)/t_0$ where t_0 is the void time of the column. (+) refers to the sign of polarimetric detector.

[b] Enantioselectivity factor defined as k_2/k_1.

[c] Resolution factor defined as $2(t_2 - t_1)/(w_1 + w_2)$ where t_1 and t_2 are retention times and w_1 and w_2 are band widths at the baseline in time units.

[d] Amount of sample (in mg) resolved in a single semipreparative run.

[e] Enantiomeric purity and polarimetric data for pooled fractions containing the 1st (F1) and 2nd (F2) eluted enantiomers.

Recently a paper dealing with HPLC enantioseparation of tertiary alcohols with 1,5-diarylpyrrole scaffolds functionalized at the 3-position of the pyrrole moiety on Chiralpak AD CSP has been published.[12] Figure 1 shows the structure of one of the analytes investigated (compound **4**). Using *n*-hexane-2-propanol 60:40 (v/v) as eluent, a baseline enantioseparation of **4** was not reached in spite of a long (35 min) analysis time. With the objective to achieve a more effective enantioseparation, Chiralpak IA was employed with conventional *n*-hexane-alcohol mixtures and unconventional normal phase eluents containing solvents of medium polarity. As shown in Figure 2, by switching from *n*-hexane-alcohol to dichloromethane-based eluents the chiral recognition ability of IA CSP was clearly demonstrated. With *n*-hexane-dicholoromethane-2-propanol (55:45:0.2) the enantiomers of **4** were separated with enantioselectivity and resolution factor values of 1.42 and 4.66, respectively, in a short analysis time (<12 min).

Coherently with these findings, the enantiomers of compounds **1a,b-3a,b** were either not separated or marginally separated (enantioselectivity factor, $\alpha < 1.15$) on IA CSP with *n*-hexane-2-propanol (70:30, v/v) or *n*-hexane-ethanol (70:30, v/v). On the contrary, non-standard eluents formed by mixing *n*-hexane and dichloromethane (in the cases of **1a-3a**) or ethyl acetate (EA) (in the cases of **1b-3b**) produced baseline enantioseparations with resolution factor, $R_s > 2.9$ (Table 1).

The advantage of this type of CSP was also evident in scale-up processes from analytical to semipreparative level. An enantioseparation is practical at mg-scale when the racemic compound shows an appreciable solubility in the eluent.[16,17] Pyrrole derivatives investigated revealed a very low solubility (<1 mg/mL) in *n*-hexane-alcohol mixtures and a good solubility in the optimized non-standard eluents containing EA or dichloromethane (>10 mg/mL). Thus, the scale-up of the optimized analytical conditions permitted us to isolate mg-amounts of single enantiomers with high enantiomeric purity and yields (*ee* ≥ 99%, recovery >70%), in less than 20 min.

Table 1 summarizes the analytical and semipreparative operative conditions and the chromatographic and polarimetric data pertinent to each enantiomer that was collected in a single fraction.

Figure 3 shows a typical semipreparative chromatogram of compound **3a** and the analytical check of the two collected enantiomers. The good chiral resolving ability of selected CSP/eluent system and high sample solubility permitted a complete resolution of 25 mg of racemate in a single step. In all of the enantioselective conditions employed, the elution order detected by polarimetry was consistent along the series **1a,b-3a,b** and

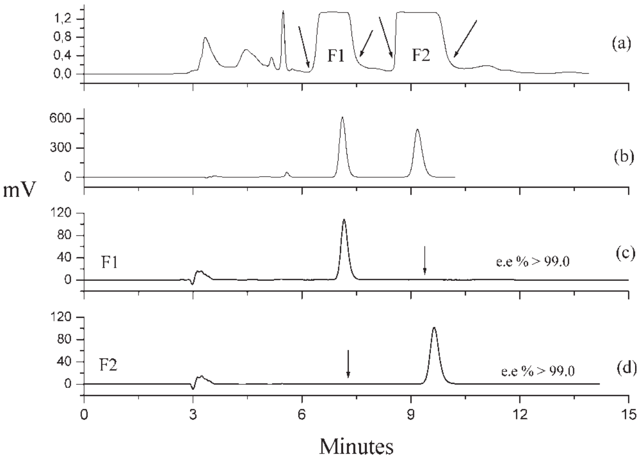


Fig. 3. Trace (**a**): semipreparative separation of 25 mg of **3a**. Column: Chiralpak IA 250 × 10 mm I.D.; eluent: *n*-hexane-dichloromethane-ethanol 50:50:0.2 (*v*/*v*/*v*); flow rate: 4.7 mL/min; temperature: 25°C; detection: UV at 360 nm. Trace (**b**): analytical separation of **3a**. Traces (**c**) and (**d**): purity control of the single fractions F_1 and F_2 collected at semipreparative scale. Column: Chiralpak IA (250 × 4.6 mm I.D); eluent: *n*-hexane-dichloromethane-ethanol 50:50:0.2 (*v*/*v*/*v*); flow rate: 1.0 mL/min; temperature: 25°C; detection: UV at 280 nm.

compound **4,** with the (+) enantiomer eluting before the (−)-enantiomer.

Determination of the Absolute Configuration

The AC of (−)-**2a** was determined by single-crystal X-ray diffraction analysis. The molecular structure is illustrated in Figure 4 and the crystallographic data are summarized in Table 2. The findings of X-ray structure analysis allow to assign unambiguously the (*R*)-configuration to the (−)-**2a** enantiomer.

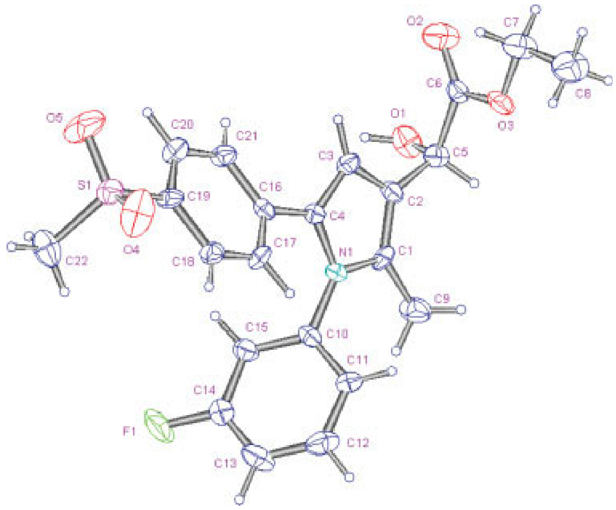


Fig. 4. A perspective view of the molecular structure of the (*R*)-(−)-**2a** enantiomer. Only one of the two crystallographically independent molecules is shown. Displacements ellipsoids are at 50% probability level. The complete set of experimental and computational data has been deposited with the Cambridge Crystallographic Data Center (CCDC # 603472). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

To arrive at the configurational determination of all the alcohols and ethers investigated, we recorded the circular dichroism (CD) spectra of the enantiomers of reference **2a** of known absolute configuration and then we compared them with those of the enantiomers of alcohol **1a, 3a,** and esters **1b-3b** (Fig. 5). The CD spectrum of the (*R*)-(−)-**2a** enantiomer showed a negative band with a maximum located at 236 nm and a weak and broad positive band at longer wavelengths centered around 330 nm. The CD spectrum of the (*S*)-(+)-**2a** enantiomer showed the same pattern, but, as expected, of opposite sign.

Defluorination of **2a** (compound **1a**) or substitution of 3-F by 4-OMe (compound **3a**) had no significant influence on the intensity and position of the CD band. These data indicate that the π → π* transition within the pyrrole fragment linked to the stereogenic center dominates the CD spectra of both alcohols and ethers. The CD spectra of the pyrrolyl carbinols appear to be independent on the alkylation of the hydroxy group, since the OH-free compounds showed very similar CD patterns to the corresponding ethyl ethers.

TABLE 2. Crystal data and structure refinement for (*R*)-(−)-2a enantiomer

Chemical formula	$C_{22}H_{22}FNO_5S$
Formula weight	431.48
Crystal system	Monoclinic
Space group	$P2_1$
Unit cell dimension	
a (Å)	11.173 (4)
b (Å)	15.480 (7)
c (Å)	12.288 (3)
β (°)	98.44 (2)
Volume (Å^3)	2102 (1)
Cell formula units Z	4
Radiation	CuKα
Wavelength (Å)	1.54178
Temperature (K)	293
F(000)	904
Crystal description	Transparent prisms
Crystal color	Colorless
Crystal size (mm)	0.5 × 0.4 × 0.3
D_c (Mg/m^3)	1.364
Absorption coefficient μ	1.713
Absorption correction	psi-scan
Min and max trasmission factors	0.9800–1.000
Radiation source	Rotating anode
Monochromator	Graphite
Data collection θ-range (°)	3.64–62.10
Index range	$0 \leq h \leq 12$; $0 \leq k \leq 17$; $-14 \leq l \leq 13$
Independent reflections	3451
Reflections with $F > 6.0\ \sigma(F)$	3077
Refinement method	Full matrix
Refined parameters	5 41
Final *R* indices	$R = 0.053$, wR = 0.071
Goodness of fit	1.29
Max and mean shift/esd	0.101; 0.022

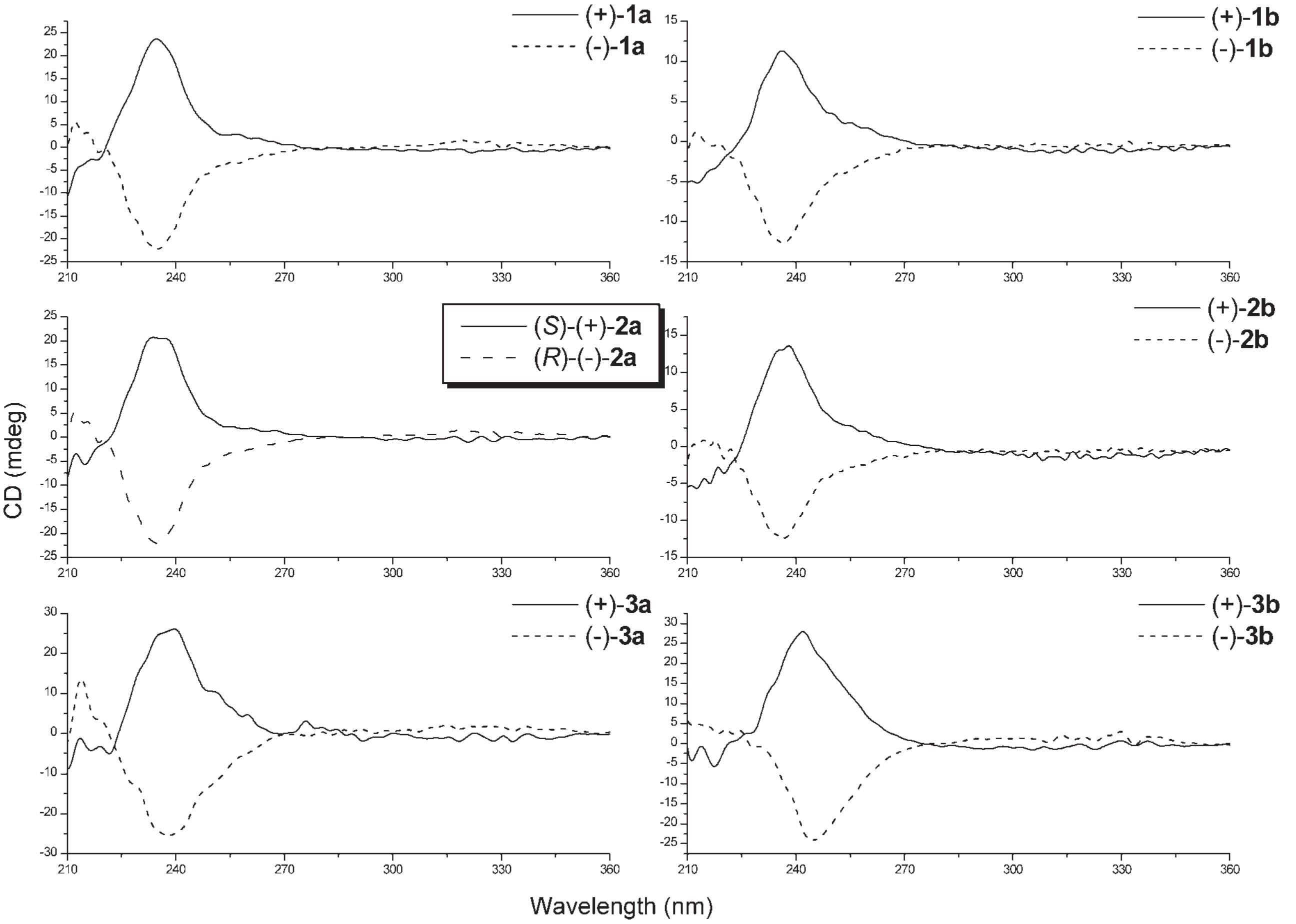


Fig. 5. CD spectra of the first eluted enantiomer (solid line) and second eluted enantiomer (dashed line) of the compounds **1a,b**-**3a,b** in ethanol at 25°C.

Based on our experimental study, we conclude that a negative CD band located at around 242–236 nm corresponds to the (*R*)-configuration of the (−)-enantiomers of pyrrole derivatives **1a,b**-**3a,b**, whereas a positive Cotton effect in the same spectral region corresponds to the (*S*)-configuration.

These findings are apparently at variance with the AC assignment of the enantiomers of compound **4** recently reported in literature.[12] By a theoretical CD study and conformational analysis, combined with CD on-line measurement during HPLC enantioseparation, the (*S*)-configuration was assigned to the enantiomer which showed a positive CD band at 330 nm and a negative peak centered around 245 nm. These spectral features are the averaged results of the individual spectra of four distinct conformers, each of which has its own contribution in sign and intensity. The CD spectra of enantiomers of **4** in ethanol are depicted in Figure 6. Replacement of the hydrogen on the stereogenic center of **1a** with a methyl group (compound **4**) drastically altered the CD spectrum profile. The (+)-**4** enantiomer, with assigned (*S*)-configuration,[12] shows strong positive CD signals at 329 and 221 nm and a negative CD signal at 239 nm. The position and intensity of CD bands measured off-line in ethanol are in good agreement with those recorded on-line in *n*-hexane-2-propanol 60:40. However, the similarity between the UV spectra of **1a** and **4** (Fig. 6) indicates that the arylpyrrole chromophore of both compounds absorbs similarly in the 200–400 nm region. Presumably, exchanging the Me group for the hydrogen atom on the stereogenic center induces a shift in the relative population of the various conformers of **1a** and **4**, leading to a sign inversion of the relevant CD absorptions.

In summary, we have shown that a combination of independent physical methods with HPLC on immobilized-type Chiralpak IA CSP may be used to unambiguously determine the absolute configuration of a series of pyrrole derivatives. As general trend, when we moved from so-called “standard” eluents formed by binary mixtures *n*-hexane-alcohol, to “non-standard” eluents containing solvents of intermediate polarity, such as ethyl acetate and dichloromethane, the resolution of analytes investigated on the IA CSP improved.

The scale-up of optimized Chiralpak IA/eluent system permitted to isolate shortly enantiopure forms of samples, which were employed in stereochemical characterization

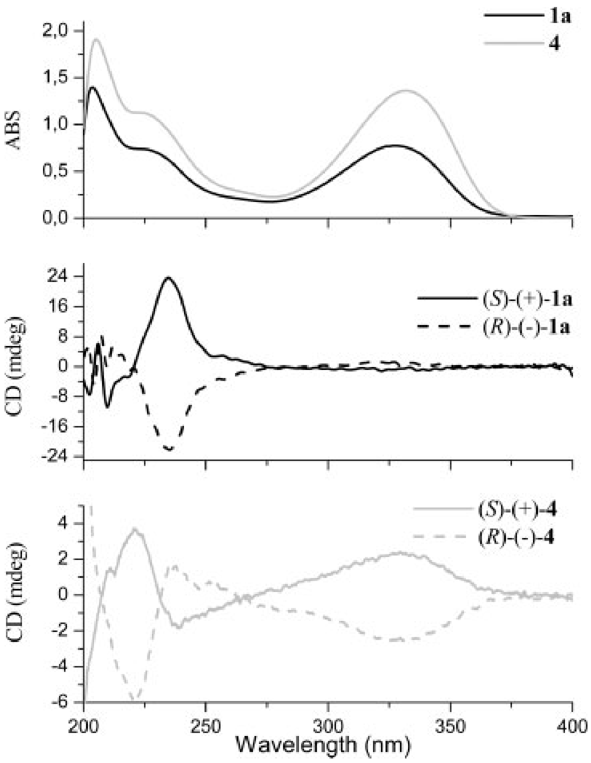


Fig. 6. UV and CD spectra of the first eluted enantiomer (solid line) and second eluted enantiomer (dashed line) of the compounds **1a** and **4** in ethanol at 25°C.

studies. Since pyrrole moieties are often present as substructures in naturally occurring and synthetic biologically active compounds, our results may find useful applications in bio-organic and medicinal chemistry.

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CHIRALITY 20:781–789 (2008)

Single Diastereomers of Unsymmetrical Tris-Spirocyclic Cyclotriphosphazenes Based on 1,1′-Bi-2-naphthol—Synthesis and Structures

N. N. BHUVAN KUMAR AND K. C. KUMARA SWAMY*

School of Chemistry, University of Hyderabad, Hyderabad, Andhra Pradesh, India

ABSTRACT Diastereoselective synthesis and characterization of chiral unsymmetrical tris-spirocyclic cyclotriphosphazenes based on chiral 1,1′-bi-2-naphthol (BINOL) are reported. Specifically, the chiral compounds $(-)N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](O\text{-}2,2'C_6H_4\text{-}C_6H_4O)Cl_2$ [(−)-**4**] and $(-)N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](OCH_2CH_2NMe)_2$ [(−)-**5**] are prepared by starting with the chiral mono-spiro compound $(-)N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2]Cl_4$ [(−)-**3**]. Synthesis of four other chiral spirocyclics, $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](OCH_2CH_2NMe)(O\text{-}2,2'C_6H_4\text{-}C_6H_4O)$ [(−)-**6** and (+)-**6**], $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](NMe_2)_4$ [(−)-**7**], $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](O\text{-}2,2'C_6H_4\text{-}C_6H_4O)(NMeCH_2CH_2OH)_2$ [(−)-**8** and (+)-**8**], and $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](O\text{-}2,2'C_6H_4\text{-}C_6H_4O)[NHCH_2CH_2CH_2Si(OEt)_3]_2$ (**9**) is also reported herein. Compounds **4–6** are obtained in the solid state diastereoselectively and their X-ray structures have been determined and discussed. The diastereoselectivity is also shown by structural characterization of two distinct isomers in the case of **6** [(−)-**6** and (+)-**6**, respectively] by starting with precursor of **3** having (*R*) or (*S*)-BINOL residue. The ^1H NMR spectra of **7** and **8** exhibit doublets with virtual coupling for the methyl protons, consistent with the chiral nature of the binaphthoxy residue. The potential of **9**, which hydrolyzes readily in $CDCl_3$ solution, as a useful precursor for chiral polymer applications is highlighted. *Chirality 20:781–789, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: chiral; cyclophosphazene; diastereoselective; spirocyclic; X-ray structure

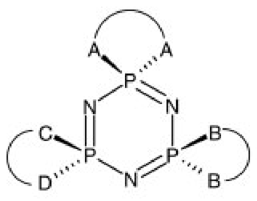
INTRODUCTION

Cyclophosphazenes $[NPX_2]_n$ [X = Cl, F], by virtue of the replaceable halogen substituents, are attractive substrates for a variety of applications resulting from the diversity of the products that can be obtained by simple halogen-substitution reactions.[1–5] They are also valuable precursors for specialty polymers.[6–9] Recently, liquid crystalline cyclophosphazenes, phosphazenium cations, and organo-linked cyclophosphazenes have also been investigated.[10–13] Reactions of halogenocyclophosphazenes with polyfunctional reagents can lead to a great variety of products that include "spiro," "ansa," or "intermolecular" condensation products in which the phosphazene ring is retained.[14–29] Thus it should be possible to introduce a variety of chiral functional groups onto the phosphazene backbone. Although not explored so far, *chiral* substituted cyclophosphazenes in principle should also be useful as bases in chiral catalysis and for chiral polymers. We have been interested in the chemistry of spirocyclic cyclophosphazenes,[23–26] and in particular, the unsymmetrically substituted ones of type **1**, because they could be new examples of potential molecularly chiral compounds that may be amenable for resolution and use in asymmetric synthesis. Although several tris-spirocyclic cyclotriphosphazenes are known,[24,25,30–32] only one (compound **2**) with three different difunctional residues (AA, BB, and CC type) is characterized.[25] We thought that the use of chiral 2,2′-binaphthoxy units could facilitate in obtaining diastereomers and this paper elaborates on this aspect. Another aspect of interest was to see if polymerizable groups could be introduced subsequent to the introduction of the chiral binaphthoxy group. Thus we report the synthesis and X-ray structural studies on chiral compounds **5** and **6** [both (+) and (−) forms] that are prepared by starting with the chiral precursors **3** and **4**. Compound **6** is formed with exclusive diastereoselectivity. The X-ray structure of **4** is also described herein. In addition to these, we also describe the synthesis of **7** and **8** that give an NMR spectrum consistent with the two naphthyl rings of the BINOL residue positioned differently. These compounds have been synthesized for possible use as bases in asymmetric synthesis at a later stage. We also report the synthesis of the silylamino substituted chiral compound **9** that has the potential to be used as a chiral polymer precursor.

Contract grant sponsor: DST, CSIR (New Delhi), UPE, CAS (UGC).

*Correspondence to: K. C. Kumara Swamy, School of Chemistry, University of Hyderabad, Hyderabad, Andhra Pradesh 500046, India.
E-mail: kckssc@yahoo.com

Received for publication 26 July 2007; Accepted 26 November 2007
DOI: 10.1002/chir.20528



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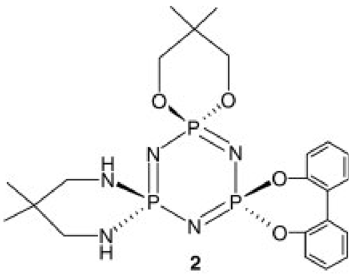

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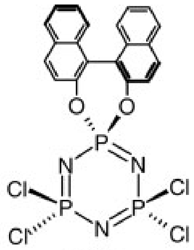

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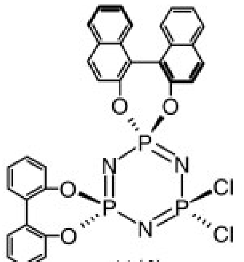

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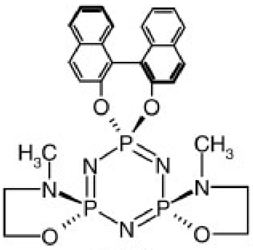

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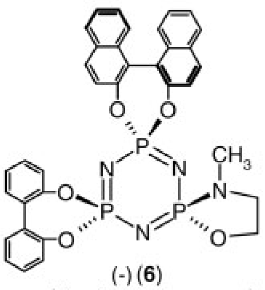

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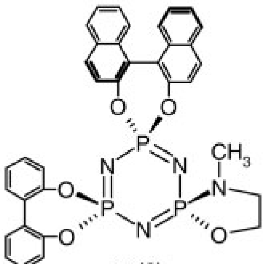
(single diastereomer)



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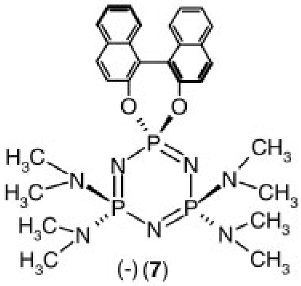
(single diastereomer)



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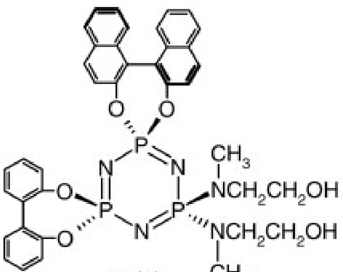

(-)(**8**)
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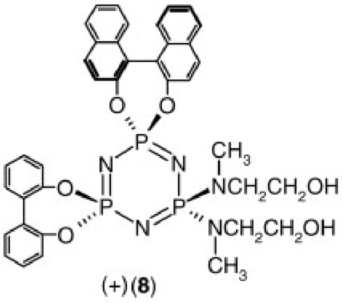
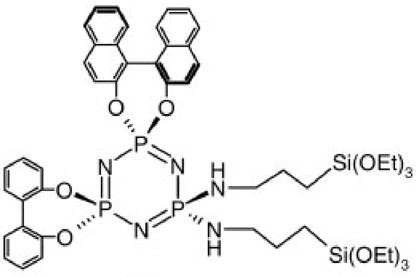


(+)(**8**)
(single diastereomer)



(**9**)

EXPERIMENTAL

Chemicals were procured from Aldrich or local manufacturers; they were purified when required according to standard procedures (see for example Ref. 33). All reactions, unless stated otherwise, were performed in a dry nitrogen atmosphere. ^{1}H, ^{13}C, and ^{31}P{H} NMR spectra were recorded using a 200 or a 400 MHz spectrometer in $CDCl_3$ (unless stated otherwise) with shifts referenced to $SiMe_4$ ($\delta = 0$) or 85% H_3PO_4 ($\delta = 0$). Infrared spectra were recorded on a JASCO FT/IR 5300 FTIR spectrometer. Melting points were determined using a local hot-stage melting point apparatus and are uncorrected. Microanalyses were performed using a Thermo Finnigan EA1112 analyzer. The optical rotations were measured on AUTOPOL-II automatic polarimeter (readability ±0.01).

Precursor **3** was prepared using a literature procedure using (*R*) (+) BINOL.[34] For further details, see our previous papers.[23,25]

Compound 4

To a stirred suspension of NaH (0.05 g, ~2.0 mmol) in THF (20 ml) at 0°C was added 2,2′-biphenol (0.19 g, 1.1 mmol). After ca. 0.5 h, **3** (0.6 g, 1.1 mmol) was added at 0°C and the mixture stirred for 5 h at rt. The reaction mixture was carefully quenched with ice, washed with water, extracted with dichloromethane (2 × 15 ml), and dried (Na$_2$SO$_4$). The solvent was removed to obtain a solid, which was purified by column chromatography (ethyl acetate–hexane, 1:10). Crystallization was done using dichloromethane–hexane mixture (1:2). Yield: 0.43 g (60%). M.p. > 270°C. IR (cm^{-1}): 2957, 2917, 1589, 1507, 1437, 1283, 1183, 972. ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.26–7.61 (m, 16H, Ar—*H*), 7.98–8.06 (m, 4H, Ar—*H*). ^{13}C NMR (50 MHz, CDCl$_3$): δ 121.0, 121.8, 122.0, 125.8, 126.4, 126.7, 127.2, 128.5, 128.8, 129.7, 129.9, 130.0, 131.0, 131.3, 131.9, 132.0, 132.5, 146.8, 147.8, 148.0 (all Ar—*C*). ^{31}P NMR (80 MHz, CDCl$_3$): δ 17.4–21.2 (m, O$_2$P(spiro, seven-membered)), 28.4 (dd → t, ^2J(P—P) ~ 77.6 Hz each, *P*Cl$_2$). Anal. calc. for $C_{32.5}H_{21}N_3O_4P_3Cl_3$ (with half molecule of dichloromethane): C, 54.8; H, 2.9; N, 5.8. Found: C, 54.8; H, 3.0; N, 5.8. LC-MS: 673 [M]$^+$ for ^{35}Cl (two chlorine pattern).

Compound 5

N-methyl ethanolamine (0.70 g, 0.74 ml, 9.3 mmol) and triethylamine (0.94 g, 9.3 mmol) were added via a syringe to a solution of **3** (0.87 g, 1.5 mmol) in THF (15 ml) and the mixture was then heated under reflux for 30 h. Filtration followed by the removal of solvent from the filtrate and passing through silica gel column (ethyl acetate hexane) led to compound **5**. The reaction mixture before passing through column, but after a water wash, showed **5** along with peaks in the region δ 25–30 in the ^{31}P NMR as the major components. We have also varied the molar ratios of the phosphazene to *N*-methyl ethanolamine. The yield of the product **5** ranged from 35 to 60%. Although the trans isomer may be formed, the spectrum was complicated and hence we could not clearly identify peaks due to them. Based on data for **5**, **8**, and the fact that δ[P(spiro)] for the cis- and trans-isomers of $N_3P_3Cl_2$(N(Me)CH$_2$CH$_2$O]$_2$ were within ±0.7 ppm,[35] we think that the peaks at δ 25–30 are due to open chain product(s). We were not able to isolate them in a pure state to confirm their identity. Crystallization of **5** was done using a mixture of dichloromethane, methanol, and acetonitrile (1:1:1). Yield: 0.52 g (60%). M.p. > 200°C (becomes black solid). IR (KBr, cm^{-1}): 3065, 2957, 2901, 1734, 1507, 1466, 1372, 1184, 1024, 974. ^{1}H NMR (200 MHz, CDCl$_3$): δ 2.62–2.82 (m, 6H, NC*H*$_3$), 3.28 −3.35 (br m, 4H, NC*H*$_2$), 4.12–4.35 (m, 4H, OC*H*$_2$), 7.21–8.00 (m, 12H, Ar—*H*). ^{13}C NMR (50 MHz, CDCl$_3$): δ 31.2 (s, N*C*H$_3$), 49.4 (d, ^2J(P—C) = 8.0 Hz, N*C*H$_2$), 64.2 (s, O*C*H$_2$), 121.4, 121.8, 125.2, 126.3, 127.2, 128.3, 130.6, 131.7, 132.6, and 147.4 (Ar*C*). ^{31}P NMR (162 MHz, CDCl$_3$): δ 30.4–34.1 (m, O$_2$*P*(spiro, seven-membered) and NO*P*(spiro)). Anal. calc. for $C_{26}H_{26}N_5O_4P_3$: C, 55.2; H, 4.6; N, 12.4. Found: C, 55.3; H, 4.7; N, 12.3. LC-MS: 566 [M + 1]$^+$.

Compounds 6 and 8

N-methylethanolamine (0.13 g, 0.14 ml, 1.8 mmol) and triethylamine (0.09 g, 0.12 ml, 0.9 mmol) were added via a syringe to a solution of (−)-**4** [prepared by using *R*(+) BINOL as described earlier; 0.40 g, 0.6 mmol] in THF (12 ml) and the reaction mixture stirred for 2 h at rt. Removal of solvent from the filtrate gave a solid, which was purified by column chromatography. First, compound (−)-**6** eluted (ethyl acetate–hexane, 3:7). This was followed by **8** (eluent: ethyl acetate–hexane, 7:3). Crystals of **6** were obtained from dichloromethane–toluene mixture.

Compound 6

Yield: 0.29 g (49%). M.p. > 260°C. IR (KBr, cm^{-1}): 3063, 2917, 2845, 1590, 1505, 1476, 1437, 1264, 1237, 1175, 1036, 968. ^{1}H NMR (400 MHz, CDCl$_3$): δ 2.89 (d, ^3J(P—H) = 11.9 Hz, 3H, NC*H*$_3$), 3.31–3.37 (m, 2H, NC*H*$_2$), 4.24–4.31 (m, 2H, OC*H*$_2$), 7.24–8.02 (m, 20H, ArC*H*). ^{13}C NMR (50 MHz, CDCl$_3$): δ 31.3 (s, N*C*H$_3$), 49.2 (s, N*C*H$_2$), 64.5 (s, O*C*H$_2$), 121.3, 122.1, 125.4, 126.0, 126.4, 127.2, 128.4, 129.6, 130.8, 131.8, 132.5, 147.3, and 148.3 (Ar*C*). ^{31}P NMR (160 MHz, CDCl$_3$): δ 32.8 (dd → t, ^2J(P—P) ~ 85.6 Hz each, NO*P*(spiro)), 27.2–29.8 (m, O$_2$*P*(spiro, seven-membered)). Anal. calc. for $C_{36}H_{29}N_4O_5P_3Cl_2$: C, 59.9; H, 3.9; N, 7.6. Found: C, 59.9; H, 3.9; N, 7.7. LC-MS: 677 [M + 1]$^+$. The other diastereomers (+)-**6** and (+)-**8** were also prepared similarly; (+)-**6** was crystallized using CH$_2$Cl$_2$-CH$_3$CN mixture.

Compound 8

Note: Using a stoichiometric amount of *N*-methylethanolamine resulted in an incomplete reaction. Yield: 0.18 g (40%). M.p. > 260°C. IR (KBr, cm^{-1}): 3420 (br), 3063, 2932, 1946, 1736, 1590, 1505, 1476, 1437, 1173, 1096, 961. ^{1}H NMR (400 MHz, CDCl$_3$): 2.75 (d with virtual coupling, ^3J(P—H) ~ 11.9 Hz, 3H, NC*H*$_3$), 2.91 (d with virtual coupling, ^3J(P—H) ~ 11.9 Hz, 3H, NC*H*$_3$), 3.24–3.33 (m, 4H, NC*H*$_2$), 3.71–3.85 (m, 4H, OC*H*$_2$), 7.28–8.04 (m, 20H, ArC*H*). ^{13}C NMR (50 MHz, CDCl$_3$): δ 33.5, 33.9 (2 s, N*C*H$_3$), 51.9 (s, N*C*H$_2$), 59.5 (s, O*C*H$_2$), 121.2, 121.6, 122.0, 125.4, 125.9, 126.08, 126.4, 127.2, 128.4, 128.9, 129.6, 130.7, 131.8, 132.6, 147.5, and 148.4 (Ar*C*). ^{31}P NMR (160 MHz, CDCl$_3$): δ 23.5–27.0 (m). Anal. calc. for $C_{38}H_{36}N_5O_6P_3$: C, 60.7; H, 4.8; N, 9.3. Found: C, 60.7; H, 4.8; N, 9.4. LC-MS: 752 [M + 1]$^+$.

Compound 7

Dry dimethylamine gas was bubbled through a stirred solution of **3** (1.00 g, 1.77 mmol) in dry THF (30 ml) for ~4 h at −78°C. The reaction mixture was allowed to room temperature, stirred for 2 h, filtered, and the solvent removed in vacuo to obtain a solid, which was purified by neutral alumina column chromatography (ethyl acetate–hexane, 15:85). Yield: 0.84 g (80%). M.p. 158–160°C. IR (KBr, cm^{-1}): 3056, 2988, 2870, 1742, 1620, 1591, 1510, 1460, 1175, 1069, 968. ^{1}H NMR (200 MHz, CDCl$_3$): δ 2.89 (d with virtual coupling, ^3J(P—H) ~ 11.9 Hz, 24H, NC*H*$_3$), 7.24–7.98 (m, 12H, ArC*H*). ^{13}C NMR (50 MHz, CDCl$_3$): δ 36.4 and 36.7 (s each, N*C*H$_3$), 121.8_6, 121.8_9, 122.0_9, 122.1_3, 124.9, 126.0, 127.3, 128.2, 130.2, 131.5, 132.7, and 148.4 (Ar*C*). ^{31}P NMR (80 MHz, CDCl$_3$): δ 26.0–28.4 (m, O$_2$*P*(spiro, seven-membered) and PNMe$_2$). Anal. calc. for $C_{28}H_{36}N_7O_2P_3$: C, 56.5; H, 6.0; N, 16.5. Found: C, 56.4; H, 6.1; N, 16.6. LC-MS: 596 [M + 1]$^+$.

Compounds 9 and 10

A mixture of **4** (0.107 g, 0.15 mmol) with an excess of $H_2NCH_2CH_2CH_2Si(OEt)_3$ (3 mol equiv. used) in the presence of triethylamine (6 mol equiv.) in THF (10 ml) was refluxed for 1 day. After filtering and removal of solvent by vacuo, compound **9** was obtained as a semisolid. It was evacuated at 100°C/0.3 mm to remove most of the silyl substituted amine, but traces still remained. ^{1}H NMR: (400 MHz, $CDCl_3$): 0.64–0.79 (2 m, 4H, CH_2), 1.16–1.31 ($\sim$2 t, 18H, CH_3), 1.55–1.76 (2 m, 4H, CH_2), 2.55 and 2.60 (2 m, 2H, NH), 2.92–3.33 (2 m, 4H, NCH_2), 3.75–3.93 (2 m, 12H, OCH_2), 7.26–8.01 (m, 20H, Ar—H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 7.5, 7.7, 18.2_6, 18.3_1, 25.1, 25.4, 43.7, 43.8, 58.3, 58.4, 121.6, 121.9, 125.2, 125.6, 125.7, 126.2, 127.2, 127.3, 128.3, 129.0, 129.3, 129.5, 129.6, 130.5, 130.5, 131.9, 132.6, 147.8. ^{31}P NMR: (160 MHz, $CDCl_3$): [close to ABX pattern] 19.2 (dd → t, ^2J(P—P) $\sim$ 65.7 Hz each, P(NH), 25.8–28.3 (m, O_2P(AB part; spiro, seven-membered)). Upon keeping in the NMR tube for a day, part of the sample got stuck to the inner walls of the tube and part was insoluble [IR (KBr, cm^{-1}): 3401, 2965, 1591, 1509, 1439, 1262, 1175, 1096, 959]. Because of the hydrolytic instability, column chromatography was not attempted. LC-MS: 1043 $[M]^+$.

A reaction of **3** (0.503 g, 0.89 mmol) with an excess (8 mol equiv. used) of $H_2NCH_2CH_2CH_2Si(OEt)_3$ in the presence of triethylamine was conducted in THF (20 ml). After filtering and removal of the silylamine by water wash, a semisolid (**10**) was obtained. We were unable to purify the sample, most likely because of hydrolysis at the $Si(OEt)_3$ end. ^{1}H NMR: (200 MHz, $CDCl_3$): 059–0.75 (m, 8H, CH_2), 1.20–1.30 ($\sim$2 t, 36H, CH_3), 1.55–1.76 (m, 8H, CH_2), 2.90–1.30 (m, 8H, CH_2), 3.60–4.00 ($\sim$2 qrt, 24H, OCH_2), 7.10–8.10 (m, ca. 10H, Ar—H). ^{31}P NMR (80 MHz, $CDCl_3$): 17.8 (mainly a doublet, ^2J(PP) $\sim$ 60.0 Hz), 27.8 (mainly a t, ^2J(PP) $\sim$ 60.0 Hz). Upon keeping in the NMR tube for a day, the sample got stuck to the inner walls of the tube.

Synthesis of α-Hydroxyphosphonate 12

To a mixture of $(OCH_2CMe_2CH_2O)P(O)H$ (0.19 g, 1.3 mmol) and benzaldehyde (0.13 g, 0.12 ml, 1.3 mmol) in xylene (5 ml) was added **7** (0.08 g, 0.13 mmol) in 25 ml RBF. The reaction mixture was stirred for 48 h at 60°C and the white solid obtained was filtered, washed with toluene (5 ml), and purified by silica gel column chromatography (ethyl acetate–hexane, 10:1). Compound **12** was characterized on the basis of physical and spectral data already available in the literature.[47] There was no observable optical rotation for the sample thus prepared.

X-ray Crystallography

Single crystal X-ray data were collected on an Enraf-Nonius MACH3 or on a Bruker AXS-SMART diffractometer, using Mo Kα (λ = 0.71073 Å) radiation. The structures were solved by direct methods and refined by full-matrix least squares method using standard procedures.[36–38] (The trans isomer is also known. See Ref. 35.) All nonhydrogen atoms were refined anisotropically; hydrogen atoms were fixed by geometry using a riding model except for the solvent molecules. The data quality was not that good in the case of **4**.1/2CH_2Cl_2, mainly because of the loss of solvent but otherwise the phosphazene bond parameters are reasonable. We could put the solvent as disordered half molecule of CH_2Cl_2 per molecule of **4**; it could not be modeled properly. There is a significant volume available for more solvent as shown by the checkcif but we could not find any significant peak above 0.7 Å of reasonable intensity to assign for a solvent.

Crystal Data

4.1/2CH_2Cl_2: $C_{32.5}H_{21}N_3O_4P_3Cl_3$, M = 716.78, Monoclinic, Space group $C2$, a = 26.3980(16), b = 7.3132(5), c = 18.9899(12) Å, β = 95.1540(10), V = 3651.3(4) Å^3, Z = 4, μ = 0.421 mm^{-1}, Flack parameter: −0.18(15), data/restraints/parameters: 4273/2/415, GOOF: 0.959, R indices ($I > 2\sigma(I)$): $R1$ = 0.0698, w$R2$ (all data) = 0.1971, max/min residual electron density [eÅ^{-3}]: 0.698/−0.366.

5. $C_{26}H_{26}N_5O_4P_3$, M = 565.43, Monoclinic, Space group $P2(1)$, a = 8.8573(10), b = 11.776(3), c = 12.628(2) Å, β = 100.535(14), V = 1294.9(4) Å^3, Z = 2, μ = 0.274 mm^{-1}, data/restraints/parameters: 2395/2/345, Flack parameter: −0.1(2), GOOF: 1.146, R indices ($I > 2\sigma(I)$): $R1$ = 0.0473, w$R2$ (all data) = 0.1674, max/min residual electron density [eÅ^{-3}]: 0.414/−0.308.

(−)-**6**. CH_2Cl_2: $C_{36}H_{29}Cl_2N_4O_5P_3$, M = 761.44, Monoclinic, Space group $P2(1)$, a = 12.820(3), b = 6.5000(13), c = 21.744(4) Å, β = 98.63(3)°, V = 1791.4(6) Å^3, Z = 2, μ = 0.364 mm^{-1}, data/restraints/parameters: 6225/1/452, Flack parameter: 0.02(14), GOOF: 1.247, R indices ($I > 2\sigma(I)$): $R1$ = 0.0731, w$R2$ (all data) = 0.1597, max/min residual electron density [eÅ^{-3}]: 0.550/−0.389.

(+)-**6**. CH_2Cl_2: $C_{36}H_{29}Cl_2N_4O_5P_3$, M = 761.44, Monoclinic, Space group $P2(1)$, a = 12.835(17), b = 6.5089(9), c = 21.739(3) Å, β = 98.525(2)°, V = 1796.2(4) Å^3, Z = 2, μ = 0.363 mm^{-1}, data/restraints/parameters: 6317/1/452, Flack parameter: 0.02(7), GOOF: 1.029, R indices ($I > 2\sigma(I)$): $R1$ = 0.0416, w$R2$ (all data) = 0.1128, max./min. residual electron dens. [eÅ^{-3}]: 0.401/−0.440.

RESULTS AND DISCUSSION

Synthesis and Spectra

Synthesis of all the compounds reported herein is straightforward and utilizes the reaction of the phosphazene precursor **3** with the amine/amino alcohol in the presence of a base or with a diol (as a sodium salt or in the presence of triethylamine). In the case of **7**, dimethylamine acts as the base as well as the nucleophile. It was found that for purification, neutral alumina works better than silica gel. In the synthesis of **6**, theoretical stoichiometry led to an incomplete reaction, but a higher stoichiometry gave the hydroxy-terminal compound **8** in addition to **6**.

Both the compounds **4** and **6** possess two spirophosphorus centers with a seven-membered 1,3,2-dioxophosphepin ring system that show up as closely spaced multiplets [AB portion of ABX pattern] in the region δ 17.4–21.2 or 27.2–29.8 in the ^{31}P NMR spectra. However, the $P$$Cl_2$ and P[N(Me)CH_2CH_2O)] region are well resolved and appear as near triplets (expected: doublet of doublet) due

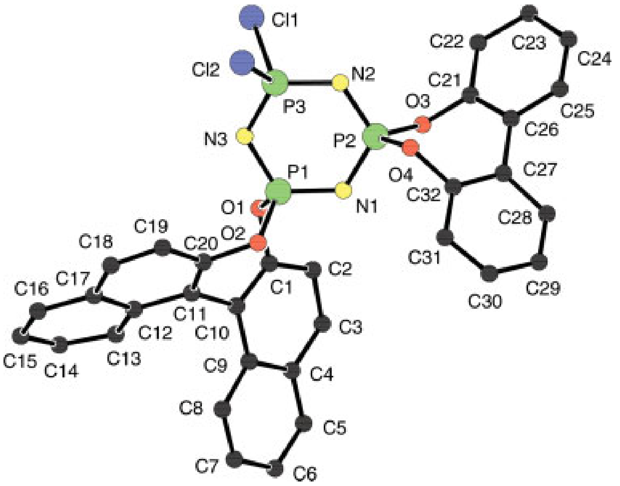
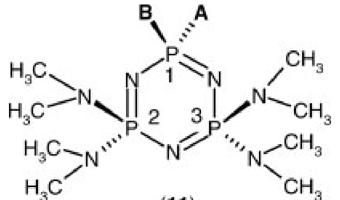


Fig. 1. A PLATON drawing for $\mathbf{4}.1/2CH_2Cl_2$. Only selected atoms shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to coupling to the other two phosphorus atoms. We recorded the low temperature ^{31}P NMR spectra [RT, −10, −20, and −30°C] for compounds **4** and **6**, but did not see perceptible changes that are indicative of interconversion. The ^{31}P NMR spectra of compounds **5** and **7** are not well resolved. What is perhaps more interesting is that in the ^{1}H NMR spectrum of compound **7**, methyl protons of the $—NMe_2$ group exhibit two doublets with virtual coupling. Normally, such a behavior is observed in species of type **11**.[39] (There are several reports on the structure of BINOL adducts/solvates wherein the dihedral angles are in the range 72–82°. For (−)-BINOL, a dihedral angle of 101.7° is reported. See Ref. 40.) This feature clearly reflects the fact that in solution the two naphthyl rings of the binaphthoxy residue are in different orientations with respect to the $—NMe_2$ groups [two above the plane and two below the plane of the phosphazene ring] on the remaining two phosphorus atoms. Consequently, the seven-membered ring containing phosphorus should have a rigid conformation in solution (vide infra). A similar feature was observed for **8**.



(11)

X-ray Crystal Structures of 4–6

The structures of **4–6** are shown in Figures 1–3; the bond parameters are given in Table 1. The phosphazenic P—N bond distances [1.555–1.592 Å] are in the normal range expected for such compounds.[23–25,41] The variation in the *mean* P—N distances is in the order: $Cl_2P—N$ < O_2P(spiro)—N < NOP(spiro)—N although the difference is not that large. However, the exocyclic P—N single bond distances are fairly close to the phosphazenic P—N bond distances; in particular, the phosphazenic P(2)—N(4) distance of 1.581(7) Å is shorter than some of the phosphazenic distances. As mentioned elsewhere,[25] this suggests substantial additional π-character at the exocyclic P—N bonds. It may be noted that in $N_3P_3[(Me)NCH_2CH_2O]_2Cl_2$, the mean phosphazenic P—N distance is 1.630 Å.[35,42] However this feature is *not* reflected in the sum of the bond angles at the exocyclic nitrogen atoms in **5** [ΣN(4) 347.4°; ΣN(5) 352.5°] and **6** [ΣN(4) 345.8°], which shows substantial pyramidal character. The latter observation is most likely a result of the fact that the nitrogen in question is a part of five-membered ring present in **5** and **6**. As regards phosphazenic bond angles, there is a marked deviation from the expected 120° at P(2) and P(3) in **5** and P(3) in **6** as a result of the strain due to the exocyclic five-membered ring. The disposition of the two

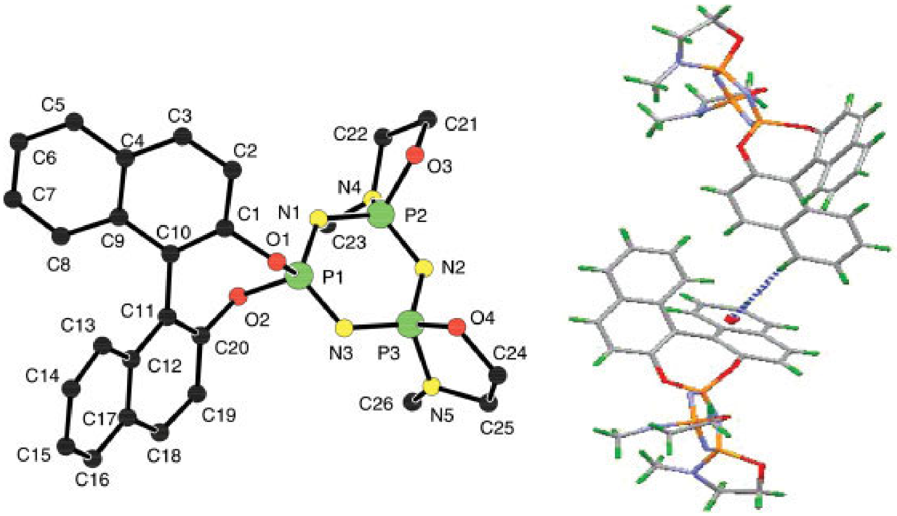


Fig. 2. A PLATON drawing for **5**. Only selected atoms shown. On the right-hand side is shown the C—H···π interaction. C—*H* to centroid distance is 2.836 Å. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

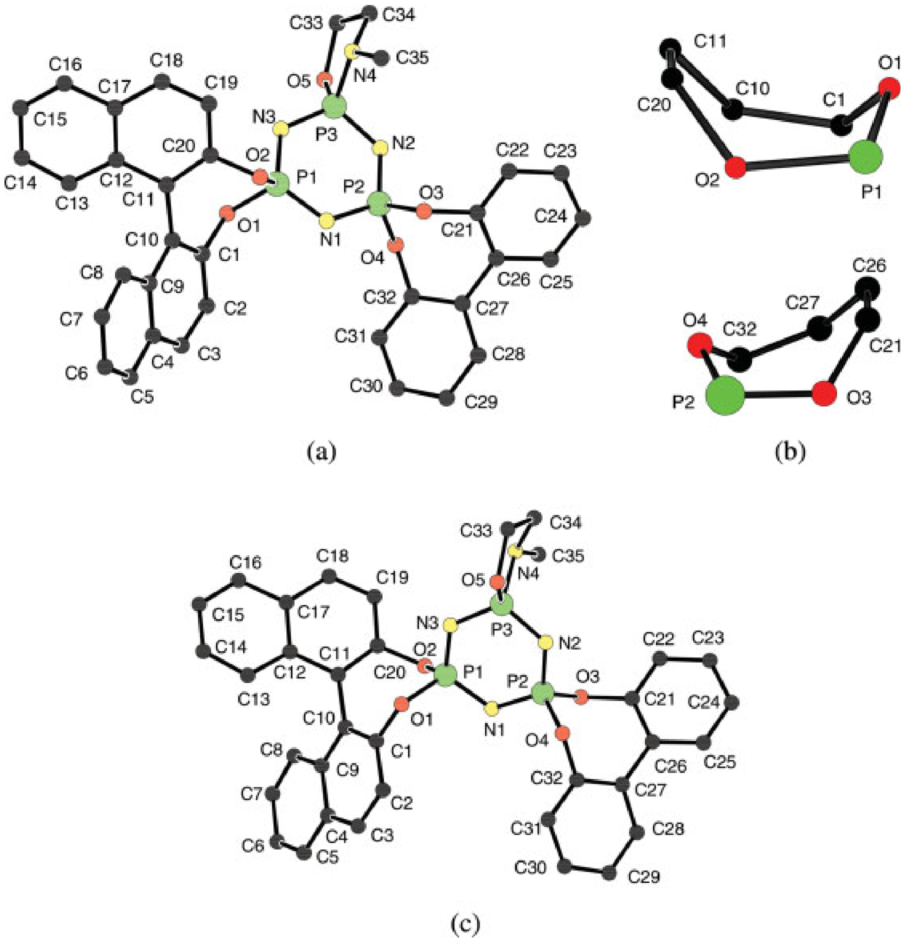


Fig. 3. PLATON drawings for (**a**) (−)-**6**.CH_2Cl_2, (**b**) conformations of the seven-membered rings involving the binaphthoxy (top) and biphenoxy (bottom) ring in (−)-**6**, and (**c**) (+)-**6**.CH_2Cl_2. Only selected atoms are shown. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

unsymmetrical five-membered rings in **5** is cis (i.e. two nitrogen atoms are cis with respect to each other); interestingly, this feature is the same as that observed before for $N_3P_3[(Me)NCH_2CH_2O]_2Cl_2]$.[42] The phosphazene ring in **4–6** is planar with a maximum deviation of 0.013–0.092 Å at P(3) from the mean plane in all the three compounds. There appears to be significant C—H···π interaction between aromatic hydrogen atoms and the naphthyl rings in compounds **4** and **5** (C—H to centroid distances 2.866 and 2.836 Å, respectively). This interaction is shown for compound **5** in Figure 2. The dihedral angle between the two naphthyl rings in the BINOL residue is in the range 58–61° which is significantly lower than that found in racemic BINOL (89.5°) itself.[43,44]

The conformation of the seven-membered 1,3,2-dioxophosphepin rings in the three compounds shows essentially the same rowboat. This is also depicted in Figure 3. In this context it is important to note that the same conformation for the biphenoxy ring is observed for several pentacoordinate phosphoranes.[45,46] Thus we believe that this ring is rigid in solution. This statement is also corroborated by the observation of virtual coupling for the NCH_3 protons in the ^1H NMR spectrum of **7**. If this is so, we believe that we should have observed chirality due to unsymmetrical substituted phosphazene ring leading to diastereomeric pairs for **4–6**, which was one of the interests in the present work.

Compound **6**, to our knowledge, is the first *chiral* unsymmetrical tris-spirocyclic cyclotriphosphazene to be structurally characterized. There should be at least four diastereomers possible using (*R*)-BINOL precursor **4** (*RRR*, *RRS*, *RSR*, *RSS*). We have checked several crystals of each of these but found that all of the crystals for each compound showed identical cell parameters. When (*S*)-BINOL derived **4** was used, again only one diastereomer was obtained. Thus it appears that the formation of the products **4–6** is *diastereoselective*.* The absolute configura-

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*One of the referees says "... The spiro biphenoxy group undergoes rapid interconversion at ambient temperatures and that is why diastereoisomeric pairs are not observed for compounds **4–6**. In fact there is X-ray evidence in this paper to support that conclusion...." The referee also thinks that the formation of these compounds is not diastereoselective. This is possible, but between 20 and −30°C we did not see any discernible change in the $^{31}P/^1H$ NMR spectra of **4** and **6**. Selectivity can also take place during crystallization. We think that more elaborate studies are required to clarify this point.

TABLE 1. Selected bond lengths (Å) and bond angles (°) for 4–6 with esd

4.1/2CH_2Cl_2	
P(1)—N(1)	1.562(6)
P(1)—N(3)	1.589(6)
P(1)—O(1)	1.575(5)
P(1)—O(2)	1.580(6)
P(2)—N(1)	1.584(7)
P(2)—N(2)	1.568(6)
P(2)—O(3)	1.578(6)
P(2)—O(4)	1.585(5)
P(3)—N(2)	1.560(6)
P(3)—N(3)	1.557(7)
P(3)—Cl(1)	1.987(3)
P(3)—Cl(2)	1.999(4)
N(1)—P(1)—N(3)	117.8(3)
N(1)—P(2)—N(2)	117.6(3)
N(2)—P(3)—N(3)	119.5(3)
P(1)—N(1)—P(2)	122.3(3)
P(2)—N(2)—P(3)	121.6(4)
P(1)—N(3)—P(3)	121.1(4)
5	
P(1)—N(1)	1.555(6)
P(1)—N(3)	1.569(6)
P(1)—O(1)	1.583(5)
P(1)—O(2)	1.599(5)
P(2)—N(1)	1.592(6)
P(2)—N(2)	1.580(6)
P(2)—N(4)	1.581(7)
P(2)—O(3)	1.595(7)
P(3)—N(2)	1.575(6)
P(3)—N(3)	1.582(6)
P(3)—N(5)	1.639(6)
P(3)—O(4)	1.601(6)
N(1)—P(1)—N(3)	119.4(3)
N(1)—P(2)—N(2)	114.7(3)
N(2)—P(3)—N(3)	115.6(3)
P(1)—N(1)—P(2)	122.4(4)
P(2)—N(2)—P(3)	125.4(3)
P(1)—N(3)—P(3)	121.5(3)
6.CH_2Cl_2[a]	
P(1)—N(1)	1.567(5) 1.573(2)
P(1)—N(3)	1.567(5) 1.567(2)
P(1)—O(1)	1.600(4) 1.595(2)
P(1)—O(2)	1.596(4) 1.597(2)
P(2)—N(1)	1.584(4) 1.581(2)
P(2)—N(2)	1.562(5) 1.570(2)
P(2)—O(3)	1.587(4) 1.588(2)
P(2)—O(4)	1.574(4) 1.584(2)
P(3)—N(2)	1.586(5) 1.586(2)
P(3)—N(3)	1.579(5) 1.580(2)
P(3)—N(4)	1.652(7) 1.656(3)
P(3)—O(5)	1.581(5) 1.578(2)
N(1)—P(1)—N(3)	118.6(2) 118.7(1)
N(1)—P(2)—N(2)	118.0(2) 118.4(1)
N(2)—P(3)—N(3)	114.6(3) 114.7(1)
P(1)—N(1)—P(2)	120.6(3) 120.3(2)
P(2)—N(2)—P(3)	123.4(3) 123.0(1)
P(1)—N(3)—P(3)	123.1(3) 123.0(1)

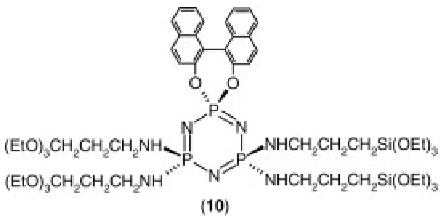
[a]First entries for (−) form and the second entries are for (+) form.

tion at the phosphorus centers (using checkcif/ WINGX) are (a) *R* at P(1) and *S* at P(2) for **4**, (b) *S* at P(2) and R at P(3) for **5** (the configuration at P(1) was not calculated), (c) *S* at P(1), *S* at P(2) and *R* at P(3) in (−)-**6**, and (d) *R* at P(1), *R* at P(2), and *S* at P(3) in (+)-**6**.

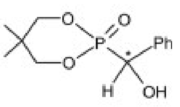
The optical rotations for compounds **3–8** in $CHCl_3$ at 25°C are given in Table 2. We have tried to use compound **7** for the optical induction in the synthesis of the α-hydroxyphosphonate $(OCH_2CMe_2CH_2O)P(O)CH(OH)Ph$ (**12**).[47] Although the product is formed, it did not show any observable optical rotation. A second point of interest we had was to use **3** and **4** for synthesizing chiral polymers. When we treated **3** with $H_2NCH_2CH_2CH_2Si(OEt)_3$/$Et_3N$ the product mixture had **10** as a major component, but a pure derivative could not be isolated. In the reaction using **4** we obtained a fairly pure derivative **9**. An interesting observation, however, was that both these products upon keeping in $CDCl_3$ in the NMR tube underwent ready hydrolysis to lead to an insoluble material, perhaps due to traces of acid.

TABLE 2. Optical rotation for compounds 3–8

Compound	*c*	$[\alpha]_D R(+)$	$[\alpha]_D S(-)$
3	1.0	−183.2	183.2
4	1.0	−140.1	140.1
5	0.5		−267.2
6	0.5	−202.9	202.9
7	1.0		−308.1
8	0.5	−186.8	186.8



(**10**)



(**12**)

SUMMARY AND OUTLOOK

In this paper, we have shown the diastereoselective formation of unsymmetrical tris-spirocyclic cyclotriphosphazenes by starting with a chiral precursor spirocycle, although conceptual realization of chiral isomers based simply on cyclophosphazene skeleton is not achieved unequivocally. By starting with either of the BINOL based chiral spirocycles, $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2]Cl_4$ [(−)-**3** or (+)-**3**], structural characterization of distinct diastereomers $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](OCH_2CH_2NMe)(O\text{-}2,2'C_6H_4\text{-}C_6H_4O)$ [(−)-**6** and (+)-**6**] is accomplished for the first time. The molecular structure is rigid in solution, as evidenced by NMR. Although our initial attempts to use these as chiral catalysts in the Pudovik reaction has not been successful, we believe that in favorable cases they could be effective and efforts directed towards this end are being pursued currently. Another possible application in chiral polymers or chiral adhesives to glass via derivatives of type $N_3P_3[1,1'\text{-}O_2(C_{10}H_6)_2](O\text{-}2,2'C_6H_4\text{-}C_6H_4O)[NHCH_2CH_2CH_2Si(OEt)_3]_2$ (**9**), is likely and is currently being investigated.

Crystallographic data for the structure(s) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC 644740-644743).

ACKNOWLEDGMENTS

This work was supported by the Department of Science and Technology (DST), New Delhi.

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CHIRALITY 20:790–795 (2008)

New Practical Synthesis of Tamsulosin

T. GIZUR,[1] E. FOGASSY,[2]* J. BÁLINT,[2] G. EGRI,[2] J. TÖRLEY,[1] Á. DEMETER,[1] AND I. GREINER[1]
[1]*Chemical Works of Gedeon Richter Ltd., Budapest, H-1475, Hungary*
[2]*Departmen of Organic Chemical Technology, Technical University of Budapest, Budapest, H-1521, Hungary*

ABSTRACT The medicine called Tamsulosin was produced 25 years ago and since then almost 10 new synthesis route has been known. Each process shows the researchers' workstyle, every year, which mainly differs in the way of separating the enantiomers. The applied reaction steps also reflect the development over the past 25 years and the new synthesis is influenced by the antecedents. The key-intermediate used in our new method is a racemic secondary amine derivative, which is unknown in the literature before and for resolving it, we worked out a quite advantegeous process. By using an optically active secondary amine, side reactions can be avoided. *Chirality 20:790–795, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: resolution; diastereomers; enantiomers; optically active amine

INTRODUCTION

Tamsulosin ***1*** (*R*-(−)-5-[2-[[2-(2-ethoxy)phenoxy)ethyl]-amino]propyl-2-methoxybenzene-sulfonamide] hydrochloride) (Scheme 1) is a well-known medicine for curing benignus prostata hyperplasia (BHP).[1]

The first synthesis (Scheme 2) of the optically active substance was achieved by the researchers of the Yamanouchi Pharma.[2]

Ten years later, *R*-(+)-α-methyl-benzylamine was used to form the chiral center by its reductive condensation with the key substance sulfamoyl acetone derivative ***2*** (Scheme 3).[3,4] The resulting two diastereomers were separated by fractional crystallization and they got the optically active amine ***3*** by hydrogenolysis of the desired diastereomer. In this reaction, the *R*-(+)-α-methyl-benzylamine used for the introduction of amino group and resolution was practically lost.

To get the final product, the amine ***3*** was reacted with a large excess of 2-(2-ethoxy-phenoxy)ethyl bromid.

Our aim was to develop a new way to obtain the (*R*-(−)-5-[2-[[2-(2-ethoxy)phenoxy)ethyl]amino]propyl-2-methoxybenzene-sulfonamide] hydrochloride) which is suitable for industrial reproduction and production (Scheme 4).

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer 1000 spectrophotometer in KBr pellet with 4 cm^{-1} resolution. NMR spectra were recorded on a Varian ^{UNITYI}*NOVA* NMR spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) with a $^{1}H\{^{13}C, ^{15}N\}$ PFG 5 mm probe. Chemical shifts are given relative to TMS ($\delta_{TMS} = 0.00$ ppm). Complete assigments were made by standard one- and two-dimensional NMR methods (1D: DPFGSENOE, 2D: PFG-HSQC, PFG-HMBC, and PFG-MQFCOSY). For quantitation of the enantiomeric purity by NMR, selective decoupling of the H-(8) proton was utilized with continuous low power pulse applied during acquisition. Control experiments were carried out to exclude the potential spill-over effects during decoupling. Sufficiently long relaxation delay (30 sec) was used to avoid distortion of the integral values over accumulation. All new compounds were analyzed correctly within 0.4% of theory for C, H, N, and S.

(±)-5-[2-(N-Benzylamino)propyl]-2-methoxybenzenesulphonamide hydrochloride (±)4

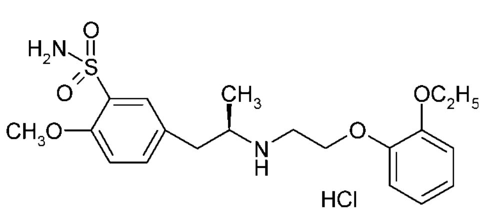
In a 500-ml flask, 18 g (0.074 mol) of (4-methoxy-3-sulfamoylphenyl)acetone, 200 ml of methanol, and 7.92 (0.074 mol) of benzylamine were added. The mixture was refluxed for 2 h, cooled to 35–40°C and the mixture of 2.74 g (0.074 mol) of sodium borohydride in 30 ml methanol and 1 ml of 40% sodium hydroxide was added dropwise. The reaction mixture was stirred for 2 h at this temperature. After the reaction was over 100 ml of water was added and the methanol solvent was evaporated under reduced pressure. The residual aqueous solution was extracted one time with 40 ml and twice with 25 ml of methyl isobutylketone. The combined organic phases were dried over sodium sulfate. The filtrate was acidified with ethanolic hydrochloric acid to pH 1 (approximately 10 ml). The precipitated crystal suspension is stirred at 8–10°C for 30 min, filtered and washed with methyl isobutylketone and dried at 80°C to give 15.12 g (yield: 55.2%) of the title compound.

(Found: C, 54.99; H, 6.11; N, 7.41; S, 8.49. Calc for $C_{17}H_{23}ClN_2O_3S$ (370.90): C, 55.05; H, 6.25; N, 7.55; S, 8.65%).

Contract grant sponsor: Hungarian Scientific Research Foundation; Contract grant number: OTKA T15 042725.

*Correspondence to: E. Fogassy, Department of Organic Chemical Technology, Technical University of Budapest, Budapest POB 91, H-1521, Hungary. E-mail: efogassy@mail.bme.hu

Received for publication 3 November 2007; Accepted 21 December 2007
DOI: 10.1002/chir.20544



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Scheme 1. Tamsulosin *1* (*R*-(−)-5-[2-[[2-(2-ethoxy.phenoxy)-ethyl]-amino]-propyl-2-methoxy-benzene-sulfonamide] hydrochloride).

IR (KBr, cm^{-1}): 3211–2400, 3330, 3161, 1606, 1590, 1328, 1281, 1161, 755, 705, 700; ^1H NMR (DMSO-d_6, 500 MHz, TMS): 1.21 (d, *J* = 6.5 Hz, 3H, H_3-9); 2.76 (dd, *J* = 12.9, 10.4 Hz, 1H, H_x-7); 3.30–3.45 (m, 2H, (H_y-7, H-8); 3.90 (s, 3H, OCH_3); 4.17–4.30 (m, 2H, H_2-11); 7.07 (s, 2H, SO_2NH_2); 7.19 (d, *J* = 8.5 Hz, 1H, H-6); 7.40–7.48 (m, 4H, H_2-14, H-15, H-5); 7.61 (d, *J* = 2.3 Hz, 1H, H-3); 7.66–7.71 (m, 2H, H_2-13); 9.68 (br s, 2H, N(10)H*HCl); ^{13}C NMR (DMSO-*d6*, 125 MHz, TMS): 14.8 (C-9), 37.1 (C-7), 47.2 (C-11), 54.5 (C-8), 56.1 (OCH_3), 112.9 (C-6), 128.0 (C-3), 128.3 (C-4), 128.5 (C-14), 128.7 (C-15), 130.0 (C-13), 131.2 (C-2), 132.3 (C-14), 134.4 (C-5), 154.8 (C-1).

(R)-5-[2-(N-Benzylamino)propyl]-2-methoxy-benzenesulphonamide (−)4

In a 500-ml flask, 18.5 g (0.05 mol) of (±)-5-[2-(*N*-benzyl-amino)propyl]-2-methoxy-benzenesulphonamide hydrochloride **(±)4** and 210 ml of methanol are added. The obtained suspension is warmed with stirring to 70°C. In another flask 9 g (0.024 mol) of dibenzoyl(d)tartaric acid monohydrate and 1.26 g (0.012 mol) of sodium carbonate are dissolved in 90 ml of water at 70°C and the mixture is poured to the methanol solution. The obtained suspension is dissolved quickly then allowed to cool to room temperature. The crystallization is started at 25°C and stirred for 48 h at 5–10°C. The diastereomer salt is filtered and recrystallized from the mixture of 120 ml methanol and water (2:1). The hot solution is cooled to 5–10°C and stirred for an hour at this temperature. The precipitated crystals are filtered and washed with 10 ml of water, to give 16.8 g of the wet product.

The specific rotation of the dry product is $[\alpha]_D^{20} = -70^\circ$ (*c* = 2-methanol).

The above wet product is suspended in 80 ml of water and then 80 ml of ethyl acetate and 7 ml of concentrated ammonium hydroxide are added. The mixture was stirred for dissolution. The phases are separated and the aqueous phase is extracted with 20 ml of ethyl acetate. The combined organic phases are dried over sodium sulfate, filtered and the solution is evaporated under reduced pressure. The residue is crystallized from isopropanol, filtered, dried to give 5.4 g (65% yield calculated on *R* enantiomer) of the title compound.

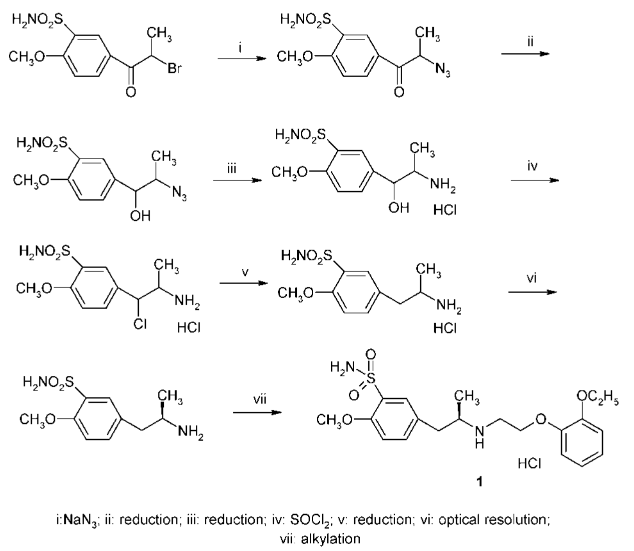
The melting point is 116–119°C, $[\alpha]_D^{20} = -21.4^\circ$ (*c* = 2-methanol).

(Found: C, 61.19; H, 6.49; N, 8.24; S, 9.42 Calc for $C_{17}H_{22}N_2O_3S$ (334.44): C, 61.05; H, 6.62; N, 8.38; S, 9.59%).

IR (KBr, cm^{-1}): 3100–2500, 3330, 3195, 1604, 1326, 1257, 1159, 795, 745, 705; ^1H NMR (DMSO-d_6, 500 MHz, TMS): 0.93 (d, *J* = 6.1 Hz, 3H, H_3-9); 1.86 (br s, 1H, N(10)H); 2.45–2.52 (m, 1H, H_x-7); 2.72–2.81 (m, 3H, H_y-7, H-8); 3.72 (d, *J* = 13.9 Hz, 1H, H_x-11); 3.77 (d, *J* = 13.9 Hz, 2H, H_2-11); 3.87 (s, 3H, OCH_3); 6.98 (br s, 2H, SO_2NH_2); 7.09 (d, *J* = 8.5 Hz, 1H, H-6); 7.18–7.22 (m, 1H, H-15); 7.26–7.32 (m, 4H, H_2-13, H_2-14); 7.34 (dd, *J* = 8.5, 2.4 Hz, 1H, H-5); 7.56 (d, *J* = 2.4 Hz, 1H, H-3); ^{13}C NMR (DMSO-d_6, 125 MHz, TMS): 19.5 (C-9), 41.5 (C-7), 50.3 (C-11), 53.4 (C-8), 56.0 (OCH_3), 112.3 (C-6), 126.3 (C-15), 127.8 (C-13), 128.0 (C-14, C-3), 130.8 (C-4), 131.2 (C-2), 134.3 (C-5), 141.2 (C-12), 154.2 (C-1).

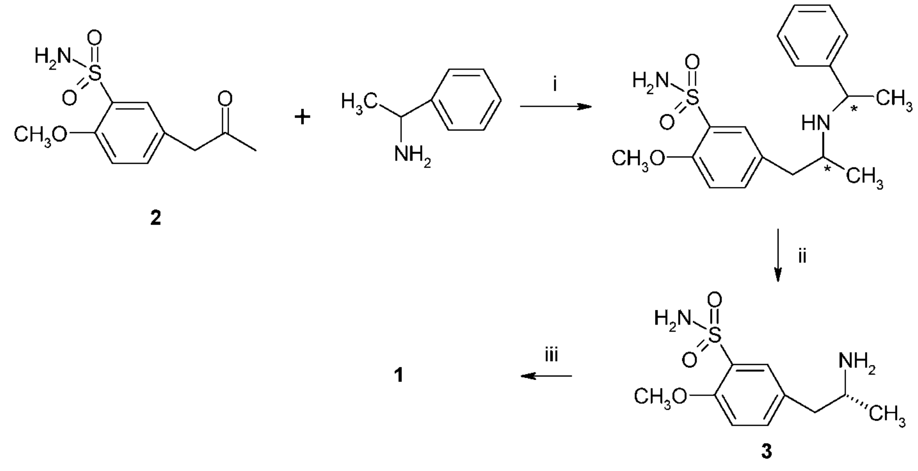
R-*5-[[2-[*N*-(2-Ethoxy-phenoxy)ethyl]-*N*-benzyl] amino]propyl-2-methoxy-benzenesulphonamide* 6

About 5.5 g (0.016 mol) of (*R*)-5-[2-(*N*-benzyl-amino)-propyl]-2-methoxy-benzenesulphonamide **(−)4,** 6.24 g (0.024 mol) of 5-ethoxy-1-(2-mesyloxyethyl)oxybenzene and 0.97 g (0.024 mol) of magnesium oxide are dissolved in 50 ml of xylene in a 250-ml flask. The mixture is refluxed for 37 h with intensive stirring. The reaction mixture is cooled to 20°C, the magnesium salts are filtered and washed twice with 5 ml of xylene. The combined xylene solution is washed twice with 10 ml of 1% aqueous acetic acid and then 80 ml of methanol is added. The mixture is cooled below 10°C and 80 ml of aqueous hydrochloric acid solution (74.5 ml of water and 5.5 ml of 10% hydrochloric acid) is added to adjust the pH 2–3. The phases are separated and the aqueous phase is washed with 10 ml of xylene. The aqueous phase is stirred with activated carbon at 20–25°C for half an hour, then filtered and washed with the mixture of 10 ml of methanol–water in the ratio of 1:1. Methanol is evaporated under reduced pressure and 75 ml of ethyl acetate is added to the aqueous residue. The solution is alkalified with 10% sodium hydroxide solution to pH 10 below 15°C. The phases are separated and the aqueous phase is extracted with 75 ml of ethyl acetate. The combined organic phases are washed with 40 ml of saturated sodium chloride solution and then with 40 ml of water. The solution is dried over sodium sulfate below



i:NaN_3; ii: reduction; iii: reduction; iv: $SOCl_2$; v: reduction; vi: optical resolution; vii: alkylation

Scheme 2. The first synthesis of Tamsulozin.



i: condensation and reduction, crystallization, ii: hydrogenolysis; iii: alkylation

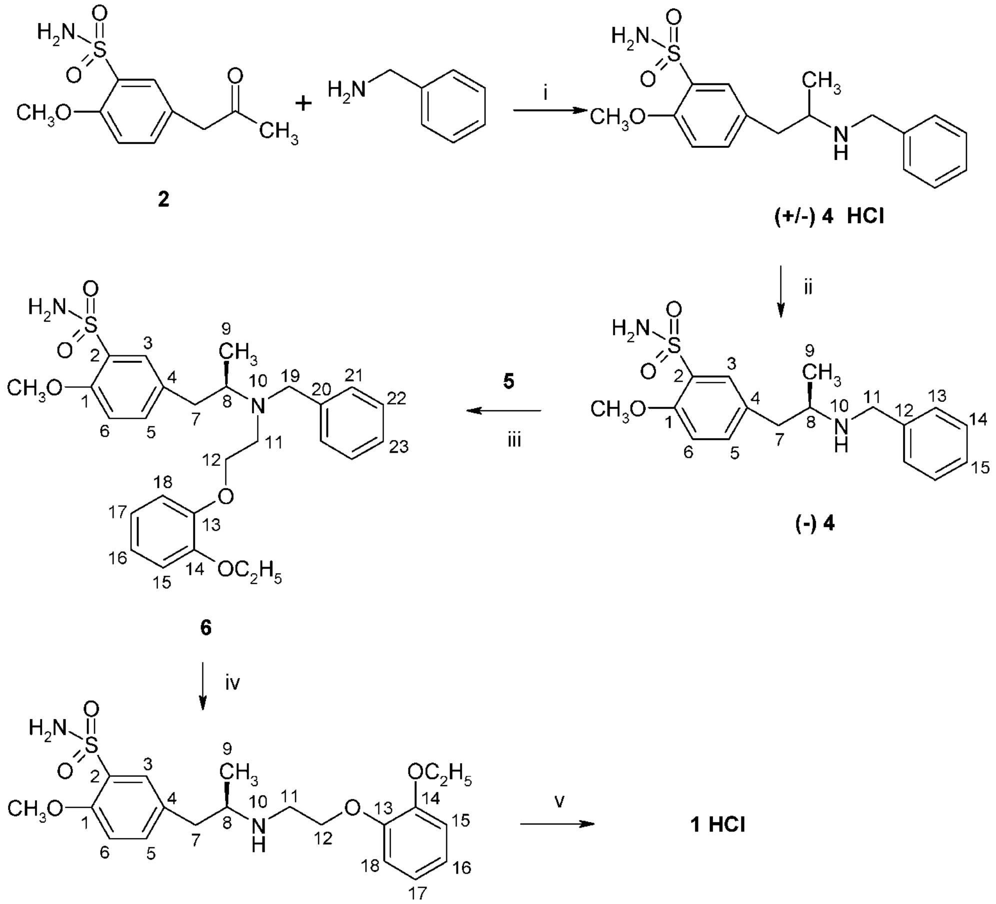
Scheme 3. An another procedure for synthesis of Tamsulozin using as key substance sulfamoyl aceton derivative 2.

15°C and filtered. The solution is evaporated under reduced pressure. The residue is crystallized from hexane, filtered off, and dried.

Yield: 5.1 g (63.9%).

The melting point is 65–68°C, $[\alpha]_D^{20} = -16.7°$ (c = 1-methanol).

(Found: C, 64.89; H, 6.74; N, 5.46; S, 6.29 Calc for $C_{27}H_{34}N_2O_5S$ (498.61): C, 65.04; H, 6.87, N, 5.62; S, 6.43%).



i: sodium-borohydride; ii: *R,R*-dibenzoyl-tartaric acid monohydrat; iii: 2-ethoxy-1-(2-mesyloxyethyl)oxybenzene magnesiumoxide, iv: hydrogen, palladium carbon; methanol, hydrochloric acid

Scheme 4. The rational synthesis, which is usable as an industrial process.

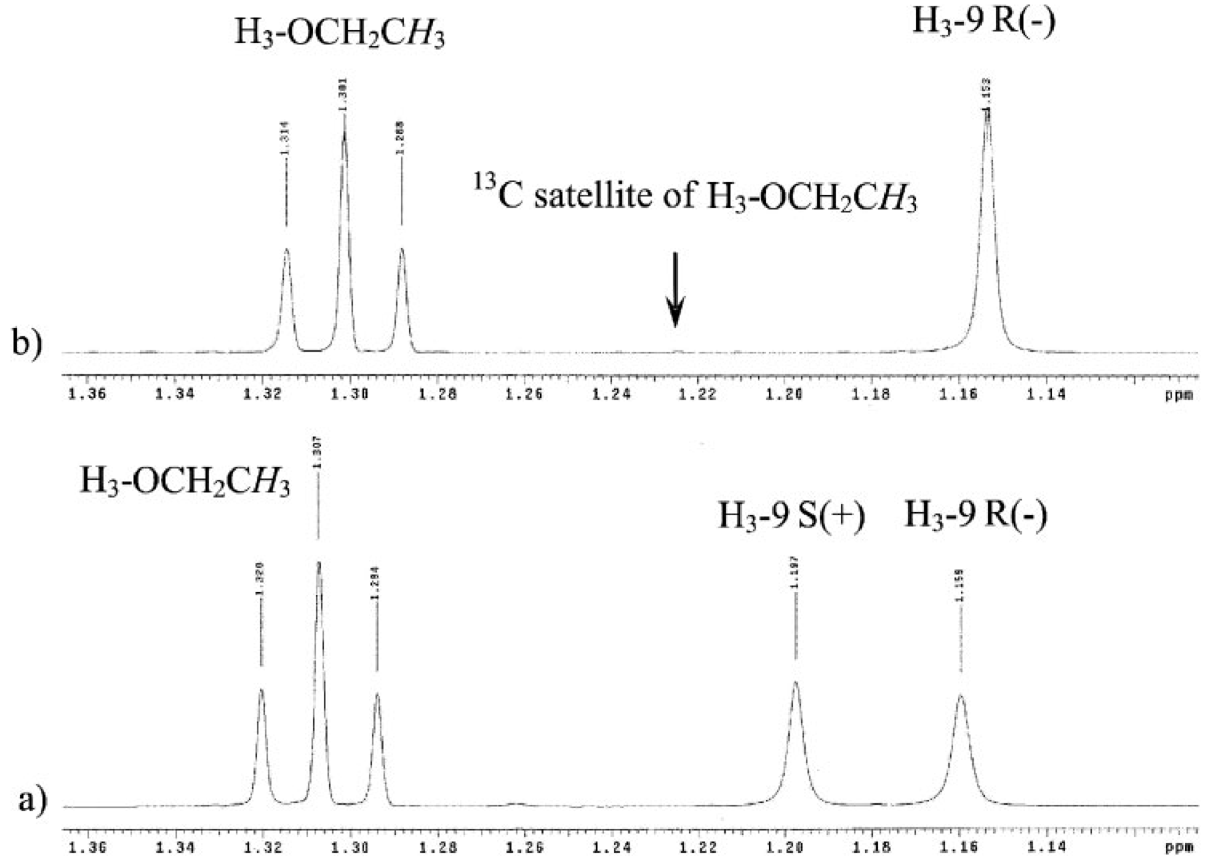


Fig. 1. Upfield region of the ^{1}H NMR spectrum of (**a**) racemic tamsulosin base, (**b**) pure $R(-)$ tamsulosin base in the presence of Mosher acid as a chiral additive (^{1}H 500 MHz, CD_2Cl_2, 21°C). Chemical shift difference is observed on H_3-9 methyl signals due to the $R(-)$ and $S(+)$ enantiomers. The H_3-(9) signals show up as singlet due to selective spin decoupling of the H-(8) proton during acquisition.

IR (KBr, cm^{-1}): 3200–2400, 3365, 1606, 1474, 1347, 1279, 1256, 1165, 827, 753, 701; ^{1}H NMR (Pyridine-d_5, 500 MHz, TMS): 1.03 (d, J = 6.0 Hz, 3H, H_3-9); 1.27 (t, J = 7.2 Hz, 3H OCH_2CH_3); 2.52 (dd, J = 12.9, 9.1 Hz, 1H, H_x-7); 3.10–3.38 (br m, 4H, H_y-7, H_2-11, H-8); 3.73 (s, 3H, OCH_3); 3.97 (q, J = 7.2 Hz, 2H, (OCH_2CH_3); 3.92–4.15 (br m, 2H, H_2-19); 4.27 (br, 2H, H_2-12); 6.95 (d, J = 8.1 Hz, 1H, H-6); 6.95–7.04 (m, 4H, H-15, H-16, H-17, H-18); 7.28 (t, J = 7.5 Hz 1H, H-23); 7.35 (dd, J = 8.1, 2.1 Hz, 1H, H-5); 7.39 (t, J = 7.5 Hz, 2H, H_2-22); 7.60–7.70 (br m, 2H, H_2-21); 8.15 (d, J = 2.1 Hz, 1H, H-3); 8.40 (br s, 2H, H_2-SO_2NH_2); 13.03 (br s, 1H, HCl); ^{13}C NMR (Pyridine-d5, 125 MHz, TMS): 14.3 (C-9), 15.1 (OCH_2CH_3), 38.1 (C-7), 49.2 (C-11), 55.6 (C-19), 56.1 (OCH_3), 58.5 (C-8), 64.5 (OCH_2CH_3), 68.1 (C-12), 112.9 (C-6), 114.1*, 114.5* (C-15, C-18), 121.4*, 121.8* (C-17, C-16), 127.8 (C-23), 128.8 (C-22), 129.4 (C-3), 129.8 (C-21), 132.2 (C-4), 132.4 (C-2), 134.6 (C-5), 138.8 br (C-20), 149.2 (C-13), 149.6 (C-14), 155.3 (C-1). *, interchangeable assignments.

(R)-(−)-Tamsulosine HCl 1

To the 7.8 g (0,0156 mol) of *R*-**6**, 230-ml of methanol is added and hyrogenated in the presence of 1.4 g of palladium carbon. The catalyst is filtered, washed thrice with 10 ml of methanol and the filtrate is evaporated under reduced pressure. The residue is crystallized from 80 ml of ethyl acetate with 0.25 g of activated carbon. The solution is cooled to 0–5°C and stirred for 1 h. The precipitated crystals are filtered, washed with 5 ml of ethyl acetate and then recrystallized again from 70 ml of ethyl acetate. The solution is cooled to 0–5°C and stirred for 1 hour. The crystals are filtered, washed with 5 ml of ethyl acetate and dried. The above residue is dissolved in 46 ml of methanol at 60°C and 0.92 ml of concentrated hydrochloric acid is added dropwise. (pH 2–3). The crystallization starts already at 60°C, it is cooled to 0°C in 1 h and stirred for further 1 h.

The precipitated product is filtered and washed twice with 20 ml of 0°C methanol and dried to give 4.54 g (65.2%).

Melting point is: 234°C, $[\alpha]_D^{20} = -4.4°$ (c = 1-methanol).

IR (KBr, cm^{-1}): 3400–2300, 1610, 1590, 1506, 1338, 1160, 1250, 819, 749, 577; ^{1}H NMR (DMSO-d_6, 500 MHz, TMS): 1.20 (d, J = 6.5 Hz, 3H, H_3-9); 1.26 (t, J = 7.0 Hz, 3H, OCH_2CH_3); 2.76 (dd, J = 11.5, 12.5 Hz, 1H, H_x-7); 3.36-3.50 (m, 3H, H_y-7 and H_2-11); 3.50-3.64 (br m, 1H, H-8); 3.91 (s, 3H, OCH_3); 4.02 (q, J = 7.0 Hz, 2H, OCH_2CH_3); 4.39 (t, J = 5.5 Hz, 2H, H_2-12); 6.90 (td, J = 8.0, 2.0 Hz, 1H, H-17); 6.96 (td, J = 8.0, 1.5 Hz, 1H, H-16); 7.00 (dd, J = 8.0, 2.0 Hz, 1H, H-15); 7.06-7.14 (m, 3H, SO_2NH_2 and H-18]; 7.19 (d, J = 8.5 Hz, 1H, H-6); 7.47 (dd, J = 8.5, 2.0 Hz,

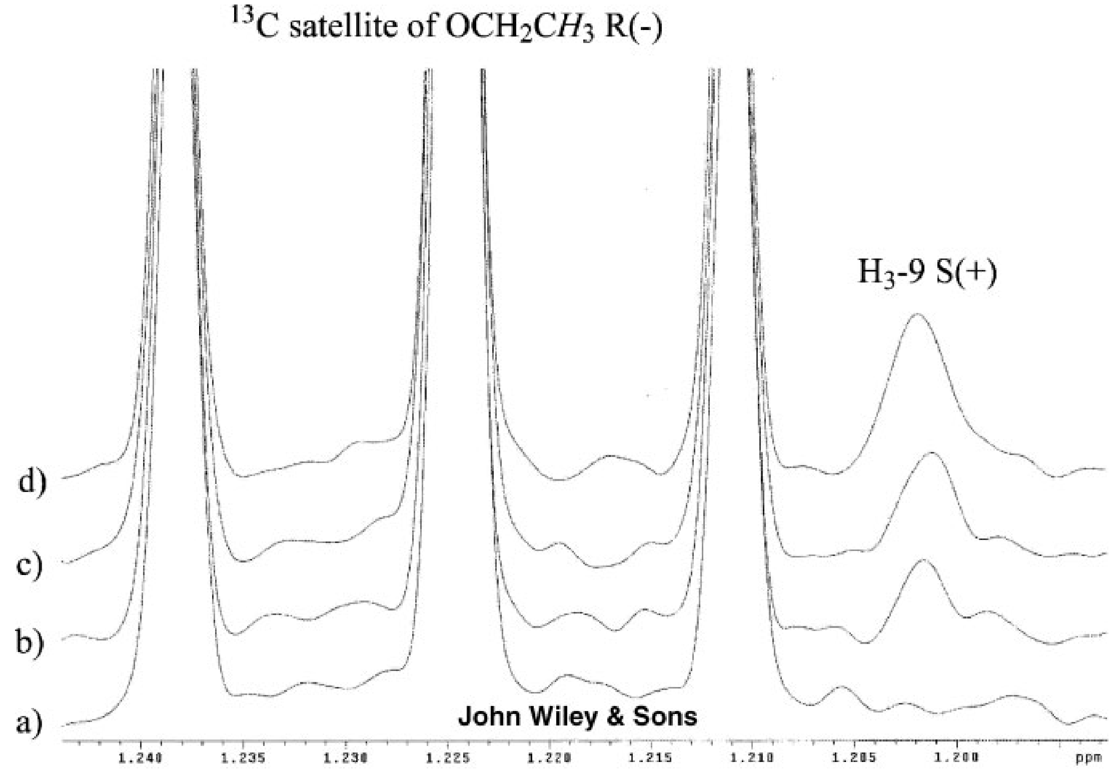


Fig. 2. (**a**) Upfield region of the ^{1}H NMR spectrum of pure $R(-)$ tamsulosin base in the presence of Mosher acid as a chiral additive (^{1}H 500 MHz, CD_2Cl_2, 21°C) by applying selective decoupling of the H-8 proton during acquisition. $R(-)$ tamsulosin base spiked with (**b**) 0.01%, (**c**) 0.03%, (**d**) 0.05% $S(+)$ tamsulosin base in the presence of Mosher acid.

1H, H-5); 7.67 (d, J = 2.0 Hz, 1H, H-3); 9.71 (br s, 2H, N(10)H*HCl); ^{13}C NMR (DMSO-d_6, 125 MHz, TMS): 14.6 (OCH$_2$CH$_3$), 14.8 (C-9), 37.2 (C-7), 43.0 (C-11), 54.7 (C-8), 56.1 (OCH$_3$), 63.7 (OCH$_2$CH$_3$), 65.1 (C-12), 112.9 (C-6), 113.6 (C-15), 115.4 (C-18), 120.7 (C-17), 122.3 (C-16), 128.1 (C-3), 128.3 (C-4), 131.2 (C-2), 134.4 (C-5), 147.3 (C-13), 148.7 (C-14), 154.9 (C-1).

RESULTS AND DISCUSSION

We also started from the sulfamoyl acetone derivative *2*. To introduce an amino group we used the cheap benzylamin in reductive condensation. The racemic secundary amine (±)**4** obtained is an unknown compound. **(±)4** having one asymmetric center was resolved with *R,R*-(−)-*O,O′*-dibenzoyl-tartaric acid. The optically pure enantiomer **(−)4** was N-alkylated with 2-ethoxy-1-(2-mesyloxyethyl)oxybenzene **5** boiling xylene in the presence of magnesium oxide. The use of stronger bases as an acid absorbent led to the alkylation of the sulfamid group as well. The magnesium oxide is such a weak base that we did not get the alkylated product on the sulfamid group (Scheme 4).

The optically pure tertiary amine **(−)6,** a new substance, was debenzylated by catalitic hydrogenation at atmospheric pressure to give tamsulosin base which was transformed into the hydrochlorid salt. The chemical purity of the product analyzed by HPLC was over 99.85%. The $S(+)$ enantiomer could not be detected at the 0.01% level by NMR analysis (see later); thus, the developed synthetic route results in the $R(+)$ tamsulosin with excellent enantiomeric purity.

A chiral NMR method was used to determine the enantiomeric purity of $R(-)$ tamsulosin base. Determination of the enantiomeric purity is based on the chemical shift difference between the $R(-)$ and $S(+)$ enantiomers observed on the H$_3$-(9) signal in the ^{1}H spectrum in the presence of Mosher acid[5] [(*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid] applied as chiral additive. Twenty five milligrams of tamsulosin base was dissolved in 0.7 ml of CD_2Cl_2 and equimolar Mosher acid was added to the solution. By applying selective decoupling of the H-(8) proton during acquisition the H$_3$-(9) proton gives two singlet signals due to the $R(-)$ and $S(+)$ enantiomers, respectively (see Fig. 1). The enantiomeric purity of the $R(-)$ enantiomer can be determined by comparing the integrated intensity of the H$_3$-(9) $S(+)$ signal with the intensity of the ^{13}C satellite signal of H$_3$—OCH$_2$CH$_3$ (see Fig. 2). Spectral deconvolution was used for measuring the $S(+)$ enantiomer content spiked in the optically pure $R(-)$ tamsulosin base, resulting in excellent agreement with the theoretical impurity content [$S(+)$ measured: 0.012%, 0.029%, 0.046%; $S(+)$ theoretical: 0,014%, 0.032%, 0.048%]. Even 0.01% $S(+)$ enantiomer can be detected with the developed method. The *S*(+) enantiomer could not be detected in the final *R*(−) tamsulosin base by NMR.

CONCLUSION

The most important step, the key-move to produce a chiral pharmaceutical-agent is the synthesis of a racemic compound suitable for selective substitution and which is separable into its enantiomers as well. In the new industrial synthesis of Tamsulozin **1,** the intermedier **4** fulfills both of these requirements.

ACKNOWLEDGMENTS

The authors are grateful for the useful assistance of Emese Pálovics (PhD student).

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CHIRALITY 20:796–804 (2008)

Stereoselective Disposition of *S*- and *R*-Licarbazepine in Mice

GILBERTO ALVES,[1] ISABEL FIGUEIREDO,[1] AMÍLCAR FALCÃO,[1]* MARGARIDA CASTEL-BRANCO,[1]
MARGARIDA CARAMONA,[1] AND PATRÍCIO SOARES-DA-SILVA[2]
[1]*Faculty of Pharmacy, Laboratory of Pharmacology, Coimbra University, Coimbra, Portugal*
[2]*Department of Research and Development, BIAL, S. Mamede do Coronado, Portugal*

ABSTRACT The stereoselective disposition of *S*-licarbazepine (*S*-Lic) and *R*-licarbazepine (*R*-Lic) was investigated in plasma, brain, liver, and kidney tissues after their individual administration (350 mg/kg) to mice by oral gavage. Plasma, brain, liver, and kidney concentrations of licarbazepine enantiomers and their metabolites were determined over the time by a validated chiral HPLC-UV method. The mean concentration data, attained at each time point, were analyzed using a non-compartmental model. *S*-Lic and *R*-Lic were rapidly absorbed from gastrointestinal tract of mouse and immediately distributed to tissues supplied with high blood flow rates. Both licarbazepine enantiomers were metabolized to a small extent, each parent compound being mainly responsible for the systemic and tissue drug exposure. The stereoselectivity in the metabolism and distribution of *S*- and *R*-Lic was easily identified. An additional metabolite was detected following *R*-Lic administration and *S*-Lic showed a particular predisposition for hepatic and renal accumulation. Stereoselective processes were also identified at the blood–brain barrier, with the brain exposure to *S*-Lic almost twice that of *R*-Lic. Another finding, reported here for the first time, was the ability of the mouse to perform the chiral inversion of *S*- and *R*-Lic, albeit to a small extent. *Chirality 20:796–804, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: stereoselectivity; licarbazepine enantiomers; eslicarbazepine acetate; oxcarbazepine; pharmacokinetics; chiral separation; mouse plasma and tissues

It is widely known that enantiomers of chiral drugs may have different pharmacodynamic, pharmacokinetic and toxicological effects upon biological systems.[1,2] Differences in the biological activity may be intrinsically related to the pharmacodynamics of each enantiomer or may be due to differences in their pharmacokinetics, with different blood or tissue concentration-time profiles. It is generally accepted that the drug metabolism introduces the greatest degree of chiral discrimination in pharmacokinetic processes, although other mechanisms in the body, among which the absorption, protein and tissue binding, distribution and excretion, may also contribute to the stereoselective drug disposition.[3–6] Hence, chirality has emerged as a critical issue in drug design, since the regulatory agencies now require pharmacological and toxicological studies not only on the racemate but also on both enantiomers before granting approval of a new chiral drug.[2]

Eslicarbazepine acetate (ESL), or *S*-licarbazepine acetate, previously known as BIA 2-093, is a new chiral drug currently undergoing clinical development for the treatment of epilepsy.[7] The molecule of licarbazepine acetate contains a chiral center at the tenth position, where an asymmetric carbon atom is attached to four different ligands, and accordingly exists as two enantiomers, ESL and its antipode *R*-licarbazepine acetate (BIA 2-059).[8,9] ESL, *R*-licarbazepine acetate and their racemic mixture, as well as other related compounds, were orally assessed in rats for anticonvulsant activity and compared with carbamazepine (CBZ) and oxcarbazepine (OXC). Surprisingly, the licarbazepine acetate enantiomers showed to possess strikingly different anticonvulsant properties. ESL was clearly the most potent with anticonvulsant potency similar to CBZ and higher than OXC, while *R*-licarbazepine acetate was almost devoid of activity and the racemic mixture demonstrated an intermediate potency.[8] These findings suggest that such differences could be due to the stereochemistry at the carbon-10 chiral center by itself or attributed to differences in their absorption, distribution, metabolism or excretion processes. Therefore, Hainzl et al.[9] performed some in vitro and in vivo experimental studies with ESL and its R-enantiomer in order to get enough pharmacokinetic data to understand their distinct pharmacodynamic properties. Subsequently, it was found that ESL and its stereoisomer *R*-licarbazepine acetate were rapidly hydrolyzed to their corresponding hydroxy deriva-

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Contract grant sponsor: Foundation for Science and Technology, Portugal; Contract grant number: SFRH/BD/12694/2003; Contract grant sponsor: BIAL, Portugal.

*Correspondence to: Amílcar Falcão, Ph.D., Faculty of Pharmacy, Laboratory of Pharmacology, Coimbra University, 3000-141 Coimbra, Portugal. E-mail: acfalcao@ff.uc.pt

Received for publication 19 November 2007; Accepted 10 January 2008
DOI: 10.1002/chir.20546

tives, *S*-licarbazepine (*S*-Lic) and *R*-licarbazepine (*R*-Lic), respectively. However, *R*-Lic undergoes a further oxidation to the *trans*-diol metabolite, demonstrating an increased predisposition to earlier inactivation. Indeed, the *trans*-diol is an inactive metabolite also produced from the metabolism of CBZ and OXC.[10]

Thus, ESL is being developed as a third generation to CBZ or a second generation to OXC,[11] but it is more closely related to OXC from a metabolic point of view. Briefly, OXC is an achiral prodrug which, in humans, is rapidly reduced in liver to the pharmacologically active metabolite licarbazepine or 10-hydroxy-carbazepine.[12] Nevertheless, OXC first-pass reduction to licarbazepine is stereoselective, appearing in plasma as *S*-Lic and *R*-Lic in approximately a 4:1 enantiomeric ratio.[13–15] Concerning to the metabolism of ESL, it is not yet well established if its deacetylation occurs in the gastrointestinal tract, in blood instantly after absorption or in liver.[9,16] In contrast, it is well known that in humans ESL is quickly and extensively metabolized to *S*-Lic (>95%) and, to a minor extent, to *R*-Lic and OXC.[17–19] Thereby, *S*-Lic is the most relevant metabolite of ESL and OXC, exemplifying chirality preservation and stereoselective metabolism, respectively. However, since low concentrations of *R*-Lic are also produced from ESL, the absolute enantioselectivity in the metabolism of ESL was not observed.

To date, in spite of OXC being used in clinical practice for several years and ESL being in its final phase of clinical trials, few studies explored the differential pharmacokinetics of licarbazepine enantiomers or the bi-directional chiral inversion.[9,20] As a result, taking into account the species-dependent metabolism of OXC and ESL, in this case among small laboratory animals, the mouse seems to be the most relevant species to humans from a metabolic point of view.[9,17,18,19] The current study was firstly designed to investigate the disposition and chiral inversion of *S*-Lic and *R*-Lic, and secondly, to examine the stereoselectivity in the pharmacokinetics of both enantiomers after their separate administration to mice by oral gavage.

MATERIALS AND METHODS

Chemicals

S-Lic (BIA 2-194, lot number PC020131B, 99.79% pure by HPLC), *R*-Lic (BIA 2-195, lot number PC040414, 100% pure by HPLC), OXC (lot number 97.12.17, >98% pure by HPLC) and BIA 2-265 (lot number PC050704, 97.4% pure by HPLC) used as internal standard (IS), were synthesized by BIAL (Porto, Portugal) (see Fig. 1). Methanol (HPLC grade, SDS), water milli-Q (HPLC grade, >15 MΩ, home-made), acetonitrile, ethyl acetate, sodium dihydrogen phosphate dihydrate, di-sodium hydrogen phosphate dehydrate and hydrochloric acid 37% were purchased from Merck KGaA (Darmstadt, Germany). Carboxymethylcellulose sodium salt for drug suspension was obtained from Sigma (St. Louis, USA).

Animals and Experimental Design

Adult male CD-1 mice obtained from Harlan-Interfauna (Barcelona, Spain) weighing 30–35 g were used. The animals were housed in local animal facilities, in a light (12 h light/dark cycle) and temperature (22°C ± 1°C) controlled environment, for at least 5 days prior to the experiments. A regular chow diet (4RF21, Mucedola, Italy) and tap water were available ad libitum before and immediately after the drug administration.

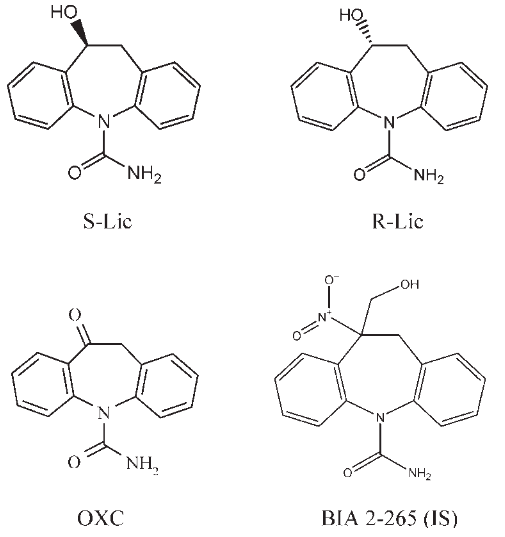

Fig. 1. Chemical structures of *S*-licarbazepine (*S*-Lic), *R*-licarbazepine (*R*-Lic), oxcarbazepine (OXC) and BIA 2-265 used as internal standard (IS).

S-Lic and *R*-Lic were suspended in a 0.5% carboxymethylcellulose aqueous solution. Groups of eight mice per time point received a single dose of *S*-Lic or *R*-Lic (350 mg/kg) by oral gavage (0.5 ml/30 g mouse weight). Blood samples, brain, liver and kidney tissues were taken at 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 16 and 24 h post-dose. Blood samples were collected into heparinized tubes by decapitation preceded of cervical dislocation. The plasma was separated by centrifugation at 4000 rpm for 10 min (4°C) and stored at −30°C until analysis. After exsanguination, brain, liver, and kidneys were quickly removed, weighed and then homogenized (4 ml/g) in a 0.1M sodium phosphate buffer (pH 5). The tissue homogenates were centrifuged at 4800 rpm for 15 min (4°C) and the supernatants were also stored at −30°C until analysis.

All animal experimentation was conducted in accordance with the European Directive (86/609/EEC) for the accommodation and care of laboratory animals and the experimental procedures were approved by the Portuguese Veterinary General Division.

Determination of Licarbazepine Enantiomers and OXC

Plasma and tissue concentrations of *S*-Lic, *R*-Lic and OXC were determined by an enantioselective HPLC-UV-SPE assay previously developed and validated.[21] Briefly, an aliquot of each plasma sample (250 μl) was added to 750 μl of 0.1M sodium phosphate buffer (pH 5) spiked with 2 μg of the IS. The samples were vortex-mixed and loaded into Waters Oasis® HLB extraction cartridges (30 mg, 1 ml; Milford, MA), which were previously conditioned with 1 ml of methanol, 1 ml of acetonitrile and 1 ml of water/acetonitrile (95:5, v/v). After sample elution, the loaded cartridges were submitted to −30 kPa and washed twice with 1 ml of water and twice with 1 ml of water/acetonitrile (95:5, v/v). After drying the sorbent under airflow for 5 min, the drugs were eluted with 1 ml of ethyl acetate under a gentle vacuum and then the cartridges were dried for 30 sec at −30 kPa. The eluates were evaporated to dryness under a nitrogen stream at 45°C and the residues reconstituted in 100 μl of water/methanol (88:12, v/v), vortexed for ∼30 sec and placed in an ultrasonic bath at room temperature for ∼1 min. Following this, the reconstituted extracts were transferred to 0.22 μm Spin-X centrifugal filters, centrifuged at 13,400 rpm for 2 min and 20 μl of the final filtered extract were used for HPLC analysis. The supernatants of brain, liver and kidney homogenates were centrifuged (13,400 rpm for 20 min) a second time to give clear supernatants of which 1 ml (∼250 μg of tissue) was also spiked with 2 μg of the IS. Next the drugs in the brain, liver, and kidney supernatants were extracted by the solid-phase extraction procedure already described for plasma samples, with some differences in the washing steps and vacuum conditions (−40 kPa). As tissue matrices are more complex than plasma, the loaded cartridges were washed with 1 ml of water and 1 ml of water/acetonitrile (95:5, v/v) three and four times in brain samples and in liver/kidney samples, respectively.

The HPLC analysis was performed on a BAS-480 Liquid Chromatograph equipped with a PM-80 pump, a Rheodyne manual injector with a 20-μl loop, a BAS UV-116 UV-Vis detector, a BAS LC-22C Temperature Controller, a BAS DA-5 Chromatography Control and a Data System Interface (all from Bioanalytical Systems, West Lafayette, IN). Data collection and integration were achieved by means of a BAS Chromgraph Control and Chromgraph Report software version 2.30. The chromatographic separation was carried out at 30°C by isocratic elution with water/methanol (88:12, v/v), at a flow rate of 0.7 ml/min, on a LiChroCART 250-4 ChiraDex (β-cyclodextrin, 5 μm) column protected by a LiChroCART 4-4 ChiraDex (β-cyclodextrin, 5 μm) guard column purchased from Merck KGaA (Darmstadt, Germany). The detector was set at 225 nm. The method was linear for OXC over concentration ranges 0.4–8 μg/ml in mouse plasma, 0.1–1.5 μg/ml in supernatant of brain homogenate and 0.1–2 μg/ml in supernatant of liver/kidney homogenates, and for each licarbazepine enantiomer in the ranges of 0.4–80 μg/ml, 0.1–15 μg/ml and 0.1–20 μg/ml in plasma, brain, and liver/kidney, respectively. The precision and accuracy were lower than 15%. No peaks due to the plasma and tissues interfered at the retention time of the analytes. The limit of quantification (LOQ) was 0.4 μg/ml in plasma and 0.1 μg/ml (∼0.4 μg/g) in supernatants of tissue homogenates.

Pharmacokinetic Analysis

The peak concentration of *S*-Lic, *R*-Lic and OXC in plasma and tissues ($C_{\max}$) and the time to reach $C_{\max}$ ($t_{\max}$) were obtained directly from the experimental data. Other pharmacokinetic parameters were computed from the mean concentration data ($n = 8$) obtained at each time point by non-compartmental analysis using the WinNonlin® Version 4.1 (Pharsight, Mountain View, CA): area under the concentration-time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the LOQ (AUC_{0-t}), calculated by the linear trapezoidal rule; AUC from time zero to infinite ($AUC_{0-\infty}$), calculated from $AUC_{0-t} + (C_{last}/\lambda_z)$, where C_{last} is the last quantifiable concentration and λ_z is the apparent terminal rate constant calculated by log-linear regression of the terminal segment of the concentration–time profile; apparent terminal elimination half-life ($t_{1/2}$) and mean residence time (MRT). Mean plasma and tissue concentrations lower than the LOQ of the assay were taken as zero for all calculations.

RESULTS

Plasma and Brain, Liver and Kidney Tissue Disposition of S*-Lic*

The mean plasma and brain, liver and kidney tissue concentration–time profiles of *S*-Lic and their metabolites (OXC and *R*-Lic), following oral administration of pure *S*-Lic enantiomer at 350 mg/kg to mice, are presented in Figure 2. The corresponding pharmacokinetic parameters estimated by non-compartmental analysis are summarized in Table 1. The peak concentrations of *S*-Lic were reached at the earliest sampling time point (0.25 h) in plasma, liver and kidneys, demonstrating that the parent drug is rapidly absorbed. As expected, the peak brain concentration of *S*-Lic was attained later (2 h) than in plasma. Figure 2 shows that in mice *S*-Lic is metabolized to a small extent with OXC and *R*-Lic being its major and minor metabolites, respectively. Indeed, following its own administration, *S*-Lic corresponded to approximately 85, 77, 90 and 92% of total drug exposure in plasma, brain, liver and kidneys, respectively (as assessed by $AUC_{0-\infty}$). In the same way, the metabolite OXC represented ∼15, 23, 10, and 8% in the corresponding matrices. The drug exposure to *R*-Lic was substantially lower representing less than 0.24% in liver, whereas in kidney tissue it was found in measurable amounts just at 0.25-h post-dose, not allowing the calculation of the corresponding $AUC_{0-\infty}$.

To assess the rate and the extent of tissue distribution of *S*-Lic and its metabolites, the corresponding $C_{\max}$ and $AUC_{0-\infty}$ tissue/plasma ratios were calculated for brain, liver and kidneys, and are displayed in the Table 2. First of all, the $C_{\max}$ and $AUC_{0-\infty}$ tissue/plasma ratios were not available for *R*-Lic, because its plasma levels were found below the LOQ of the analytical method. All estimated values for brain/plasma ratios were lower than the unity, demonstrating higher plasma drug concentrations. These data also indicated that the distribution of *S*-Lic into the brain was less favorable than that for OXC. In fact, the rate and the extension of OXC brain penetration were, respectively, 2.1 and 1.7 times greater than those for *S*-Lic. Moreover, as shown in the Table 1, the $t_{\max}$ of *S*-Lic in the brain was quite displaced to the right comparatively to that in plasma, whereas for OXC the $t_{\max}$ in brain and in plasma were coincident. On the other hand, in agreement with

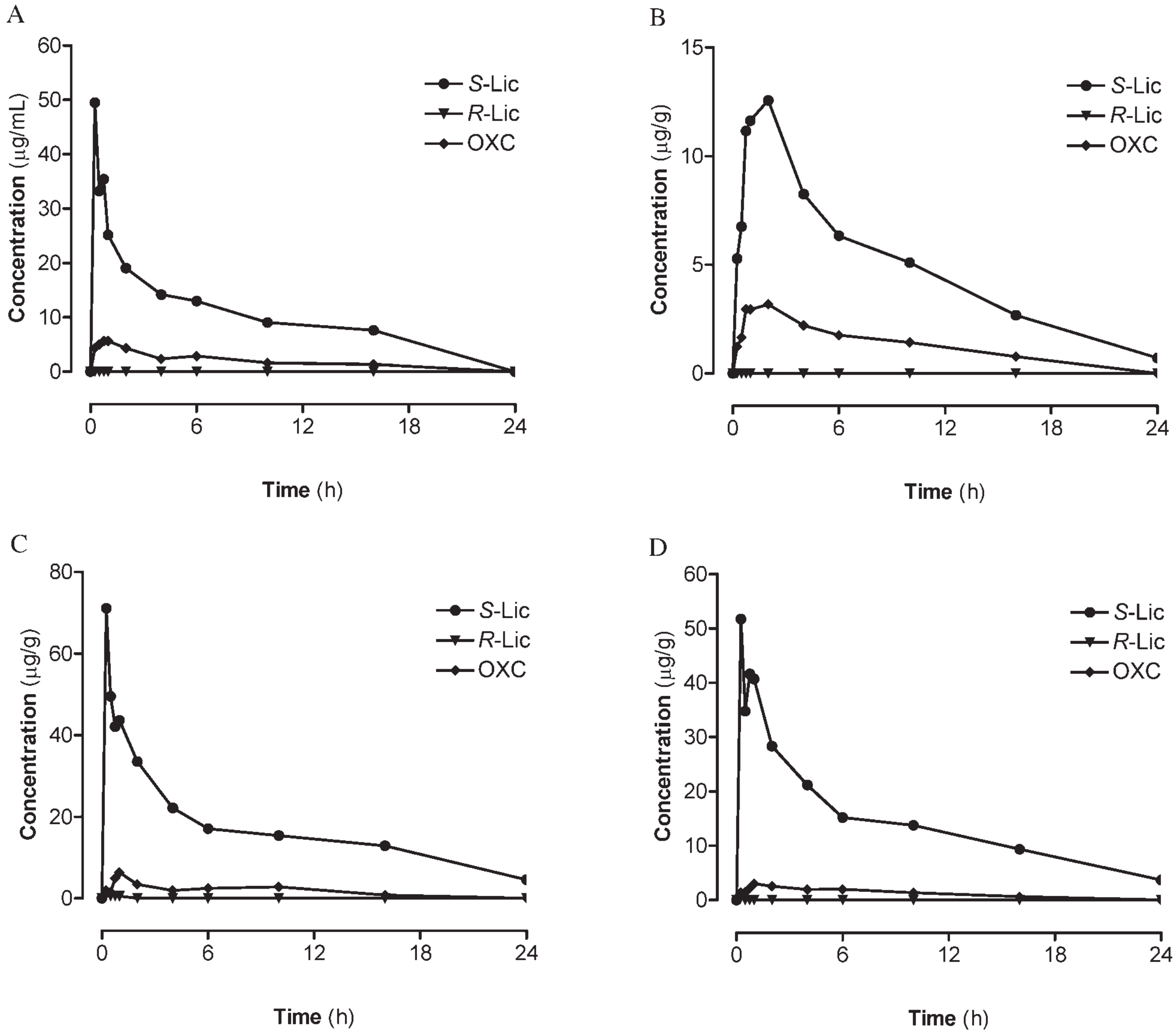

Fig. 2. Mean plasma (**A**), brain (**B**), liver (**C**), and kidney (**D**) concentration–time profiles of *S*-Lic, *R*-Lic, and OXC following a single oral administration of *S*-Lic 350 mg/kg to mice. Symbols represent the means of eight mice.

the values of $C_{\max}$ and $\mathrm{AUC}_{0-\infty}$ liver and kidney/plasma ratios, the predisposition for liver and kidney storage of *S*-Lic is evident in contrast to OXC, which appeared to in a larger extent in plasma. However, the $C_{\max}$ and $\mathrm{AUC}_{0-\infty}$ liver/plasma ratios for *S*-Lic were shown to be greater than those in kidneys, supporting that the liver will be its main deposit.

Regarding the $t_{1/2}$ and MRT parameters of all compounds in all matrices (Table 1), an aspect that deserves to be pointed out is the very low $t_{1/2}$ and MRT values estimated for *R*-Lic in liver.

Plasma and Brain, Liver and Kidney Tissue Disposition of R*-Lic*

The mean plasma and brain, liver and kidney tissue concentration–time profiles of *R*-Lic and its metabolites (OXC and *S*-Lic) were also determined in mice after a single oral dose of *R*-Lic enantiomer at 350 mg/kg (see Fig. 3). However, it is important to stress that in addition to OXC and *S*-Lic, another more polar metabolite was detected in considerable amounts, which was not quantified because the method was not validated for that purpose. Consequently, when possible, plasma and tissue pharmacokinetic parameters of *R*-Lic, *S*-Lic, and OXC were calculated by non-compartmental model and the most relevant parameters are summarized in Table 3. Taking into account the data presented, it is evident that the highest plasma, liver and kidney *R*-Lic concentrations were achieved at first sampling time point (0.25 h) and, at that moment, its brain levels were already elevated and close to the maximal brain concentration (Figure 3B). Hence, these findings showed that *R*-Lic is rapidly absorbed from the mouse gastrointestinal tract and is quickly distributed. Undoubtedly, following its oral administration to mice, *R*-Lic was found to be the major compound in plasma and in all assayed tissues over the full sampling time range. OXC and *S*-Lic were

TABLE 1. Plasma, brain, liver, and kidney pharmacokinetic parameters of *S*-Lic and its metabolites *R*-Lic and OXC in mice after a single oral dose of *S*-Lic 350 mg/kg

Pharmacokinetic parameters	*S*-Lic	*R*-Lic	OXC
Plasma			
t_{max} (h)	0.25	NA	0.75
C_{max} (μg/mL)	49.47	NA	5.65
AUC_{0-t} (μg h/mL)	209.18	NC	39.08
$AUC_{0-\infty}$ (μg h/mL)	310.49	NC	53.10
$t_{1/2}$ (h)	9.25	NC	7.22
MRT (h)	13.60	NC	11.37
Brain			
t_{max} (h)	2.00	NA	0.75
C_{max} (μg/g)	12.56	NA	2.95
AUC_{0-t} (μg h/g)	114.46	NC	27.20
$AUC_{0-\infty}$ (μg h/g)	120.41	NC	35.47
$t_{1/2}$ (h)	5.74	NC	7.40
MRT (h)	8.67	NC	11.01
Liver			
t_{max} (h)	0.25	0.25	1.00
C_{max} (μg/g)	71.13	0.87	6.38
AUC_{0-t} (μg h/g)	399.55	0.95	39.08
$AUC_{0-\infty}$ (μg h/g)	446.40	1.16	47.16
$t_{1/2}$ (h)	7.18	0.82	6.87
MRT (h)	10.96	1.24	9.87
Kidney			
t_{max} (h)	0.25	0.25	1.00
C_{max} (μg/g)	51.71	0.55	3.01
AUC_{0-t} (μg h/g)	336.89	NC	25.42
$AUC_{0-\infty}$ (μg h/g)	375.37	NC	31.79
$t_{1/2}$ (h)	7.26	NC	7.08
MRT (h)	10.62	NC	10.26

C_{max} and t_{max} are experimental values; AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, and MRT values were calculated by non-compartmental analysis from mean concentrations at each time point (n = 8 mice per group). NA, not available; NC, not calculated.

produced to a small extent possibly by *R*-Lic oxidation and isomerization, respectively. *S*-Lic concentrations were found above the LOQ of the assay in plasma, liver and kidneys, while OXC was quantified in all matrices.

Once again, to evaluate the rate and the extent of tissue distribution of *R*-Lic and its metabolites, the corresponding C_{max} and $AUC_{0-\infty}$ tissue/plasma ratios were estimated for brain, liver and kidneys (Table 4). The C_{max} and $AUC_{0-\infty}$ brain/plasma ratios for *R*-Lic and OXC were far lower than the unity, which support their poor penetration into the brain. However, in spite of the extent of brain distribution is similar for both *R*-Lic and OXC (as assessed by $AUC_{0-\infty}$), the value of the C_{max} brain/plasma ratio was 3.6 times higher for OXC, suggesting some differences in their blood–brain barrier (BBB) crossing rate. Actually, as shown in the Table 3, the t_{max} of *R*-Lic was observed former in plasma than in brain, whereas the t_{max} of OXC was synchronized in both matrices, indicating that OXC crosses the BBB more promptly than *R*-Lic. In this case, as *S*-Lic brain concentrations were lower than the LOQ of the assay, the corresponding C_{max} and $AUC_{0-\infty}$ brain/plasma ratios could not be calculated. Taking into consideration the C_{max} and $AUC_{0-\infty}$ liver/plasma ratios for the parent compound (*R*-Lic), its lack of ability for accumulation (ratios near the unity) is evident. In contrast, the *S*-Lic formed as a metabolite of *R*-Lic trends towards hepatic accumulation and the metabolite OXC undergoes hepatic release into the systemic circulation. Likewise, the kidney/plasma ratios support the renal storage of *S*-Lic produced but to a lesser degree than that occurring in liver. Finally, *R*-Lic and OXC appeared in greater amounts in plasma than in kidneys.

Stereoselective Distribution of S-Lic and R-Lic

The differential distribution of *S*-Lic and *R*-Lic enantiomers in plasma and brain, liver and kidney tissues, following their separate administration to mice, was studied based on their stereoselective index (enantiomeric ratio),[22] of the mean values of C_{max} and $AUC_{0-\infty}$ pharmacokinetic parameters. Levy and Boddy[22] considered a measurable difference in the parameter values for a pair of enantiomers when it is greater than or equal to 20%. In mice treated with 350 mg/kg of *S*-Lic or *R*-Lic by oral gavage, remarkable differences in the disposition of licarbazepine enantiomers were recorded (see Figure 4). The $AUC_{0-\infty}$ S/R ratios were higher than the unity in all matrices, indicating a greater exposure to *S*-Lic than *R*-Lic after equivalent dosing regime. In fact, the systemic exposure to *S*-Lic and *R*-Lic was comparable, but even so large differences in their brain, liver and kidney exposure were identified. Indeed these data support the hepatic and renal uptake of S-enantiomer as well as its larger distribution (almost twice) to the brain, demonstrating the chiral discrimination of *S*-Lic and *R*-Lic in a living system. Moreover, the corresponding C_{max} S/R ratios of 0.66 in plasma and 0.95 in liver and kidneys, observed at 0.25 h postdose, confirm an increased affinity of *S*-Lic to liver and kidney tissues from the earliest time points.

Stereoselective Metabolism of S-*Lic and* R-*Lic and Chiral Inversion*

Undoubtedly, analyzing the mean plasma and brain, liver and kidney tissue concentration–time profiles obtained after the administration of *S*-Lic and *R*-Lic to mice (Figures 2 and 3), it is obvious that each parent compound was the main responsible for the systemic or tissue drug exposure. It is also evident that OXC is a common metabolite produced either from *S*-Lic or *R*-Lic, which appeared in measurable amounts in all matrices. Thus, in order to compare the formation of OXC from both enantiomers, the corresponding C_{max} and $AUC_{0-\infty}$ ratios were calculated and

TABLE 2. Brain, liver, and kidney/plasma $AUC_{0-\infty}$ and C_{max} ratios of *S*-Lic, *R*-Lic, and OXC obtained after oral administration of *S*-Lic (350 mg/kg) by oral gavage to mice

	C_{max}			$AUC_{0-\infty}$		
Ratio	*S*-Lic	*R*-Lic	OXC	*S*-Lic	*R*-Lic	OXC
Brain/plasma	0.25	NA	0.52	0.39	NA	0.67
Liver/plasma	1.44	NA	1.13	1.44	NA	0.89
Kidney/plasma	1.05	NA	0.53	1.21	NA	0.60

NA, not available.

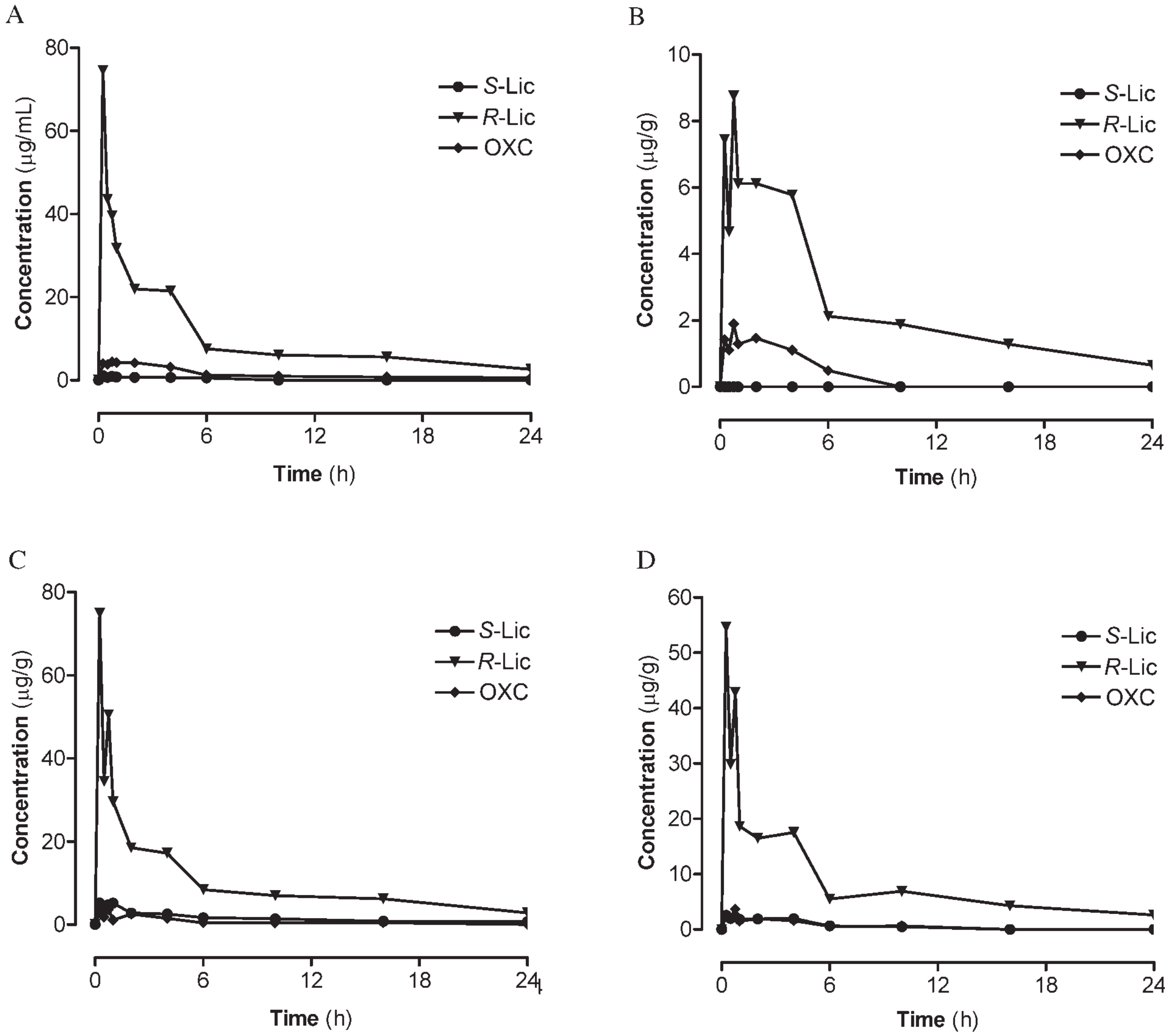

Fig. 3. Mean plasma (**A**), brain (**B**), liver (**C**), and kidney (**D**) concentration–time profiles of *R*-Lic, *S*-Lic, and OXC following a single oral administration of *R*-Lic 350 mg/kg to mice. Symbols represent the means of eight mice.

are represented in Figure 5. These data clearly support that *S*-Lic was metabolized to OXC to a greater extent than *R*-Lic. At this point it should be pointed out that the brain exposure to OXC is approximately four times higher after *S*-Lic administration. Finally, the occurrence of enantiomeric inversion of *S*-Lic and *R*-Lic in mice should be reported. By treatment with *S*-Lic, some amounts of *R*-Lic were produced and following *R*-Lic administration its antipode was also formed (Tables 1 and 3). Taking into account the pharmacokinetic profiles obtained, a strict parallel was observed between the parent compounds and their opposite enantiomer (Figures 2 and 3). Hence, these findings suggest the ability of the mouse to perform the interconversion of both licarbazepine enantiomers, albeit to a small extent. To identify the preferential direction of enantiomeric bioinversion, when possible, the $C_{\max}$ and $\mathrm{AUC}_{0-\infty}$ pharmacokinetic parameters of *S*-Lic and *R*-Lic produced by administration of their complementary enantiomer were compared (Table 5). As indicated, the production of *S*-Lic from *R*-Lic was much more favorable. In fact, the hepatic exposure to *S*-Lic after *R*-Lic administration was approximately 40 times greater than that observed for *R*-Lic following *S*-Lic administration (as assessed by $\mathrm{AUC}_{0-\infty}$). Finally, the stereoselectivity in the metabolism of licarbazepine enantiomers was also supported by the formation of an additional metabolite from *R*-Lic.

DISCUSSION

The interest in three-dimensional molecular structure of drugs was largely ignored during decades of pharmaceutical research, but recently, it has emerged as a key issue in drug design, discovery, development and regulatory fields.[5] In fact, chirality is one of the main features of living systems and the interactions of drugs with corresponding biological targets may be stereoselective.[1,2] Therefore, at the present time, the majority of the new drugs reaching

TABLE 3. Plasma, brain, liver, and kidney pharmacokinetic parameters of *R*-Lic and its metabolites *S*-Lic and OXC in mice after a single oral dose of *R*-Lic 350 mg/kg

Pharmacokinetic parameters	*R*-Lic	*S*-Lic	OXC
Plasma			
t_{max} (h)	0.25	0.25	0.75
C_{max} (μg/mL)	74.55	1.09	4.43
AUC_{0-t} (μg h/mL)	237.22	4.16	34.69
$AUC_{0-\infty}$ (μg h/mL)	282.68	11.67	42.21
$t_{1/2}$ (h)	12.24	8.98	8.47
MRT (h)	12.30	13.21	12.54
Brain			
t_{max} (h)	0.75	NA	0.75
C_{max} (μg/g)	8.76	NA	1.89
AUC_{0-t} (μg h/g)	57.21	NC	6.81
$AUC_{0-\infty}$ (μg h/g)	64.38	NC	9.10
$t_{1/2}$ (h)	7.63	NC	3.25
MRT (h)	10.27	NC	4.62
Liver			
t_{max} (h)	0.25	0.25	0.75
C_{max} (μg/g)	74.96	5.24	3.59
AUC_{0-t} (μg h/g)	235.88	36.76	14.05
$AUC_{0-\infty}$ (μg h/g)	263.44	46.25	18.04
$t_{1/2}$ (h)	6.74	9.06	6.14
MRT (h)	9.98	14.03	9.38
Kidney			
t_{max} (h)	0.25	0.25	0.75
C_{max} (μg/g)	54.62	2.52	3.66
AUC_{0-t} (μg h/g)	194.11	12.52	12.05
$AUC_{0-\infty}$ (μg h/g)	227.96	15.43	16.28
$t_{1/2}$ (h)	9.18	4.16	4.56
MRT (h)	11.59	5.92	6.99

C_{max} and t_{max} are experimental values; AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ and MRT values were calculated by non-compartmental analysis from mean concentrations at each time point (n = 8 mice per group); NA, not available; NC, not calculated.

TABLE 4. Brain, liver, and kidney/plasma $AUC_{0-\infty}$ and C_{max} ratios of *R*-Lic, *S*-Lic and OXC obtained after oral administration of *R*-Lic (350 mg/kg) by oral gavage to mice

	C_{max}			$AUC_{0-\infty}$		
Ratio	*R*-Lic	*S*-Lic	OXC	*R*-Lic	*S*-Lic	OXC
Brain/plasma	0.12	NA	0.43	0.23	NA	0.22
Liver/plasma	1.01	4.81	0.81	0.93	3.96	0.43
Kidney/plasma	0.73	2.31	0.83	0.81	1.32	0.39

NA, not available.

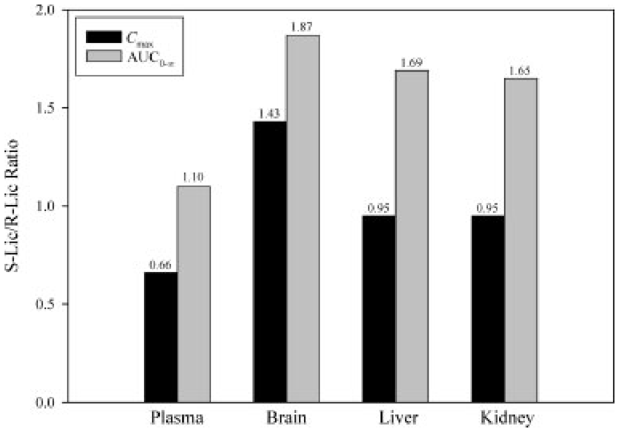

Fig. 4. The C_{max} and $AUC_{0-\infty}$ S/R enantiomeric ratios of *S*-Lic and *R*-Lic in plasma and brain, liver and kidney tissues after their separate administration (350 mg/kg) to mice by oral gavage.

the market are single enantiomers rather than racemic mixtures to improve the therapeutic index, given that one of the enantiomers may be inactive or even counterproductive to the therapeutic effect.[2,4]

It is widely accepted that the stereochemistry has little influence on passive processes such as diffusion across membranes. However, when the drug interacts with an enzyme or a transporter system the chiral discrimination may be seen, at least, if the drug stereogenic center(s) are important in its target interaction.[23] Metabolism has been shown to be the most important pharmacokinetic process in enantioselective drug disposition, but plasma and tissue protein binding as well as membrane permeability may also play a preponderant role.[24] Thereby, the liver and kidney pharmacokinetic behavior of *S*-Lic and *R*-Lic enantiomers and their brain uptake were investigated in mice. These biological matrices were chosen, firstly, because the brain is the therapeutic target of licarbazepine enantiomers, and secondly, the liver and kidneys are the most important organs from a pharmacokinetic point of view, being respectively, the primary organ for drug metabolism and drug excretion. In the present study, *S*-Lic and *R*-Lic plasma protein binding were not determined due to their low binding percentage (<30%) in dogs, which was almost identical for both enantiomers.[20] In humans, the licarbazepine plasma protein binding was found to be ~40%.[12,15]

Our results demonstrated that *S*-Lic and *R*-Lic are rapidly absorbed from the mouse gastrointestinal tract and rapidly distributed after their own administration, at least for the highly perfused tissues. From the overall data obtained, the enantioselectivity in licarbazepine absorption appears to be unlikely, because the liver concentrations of

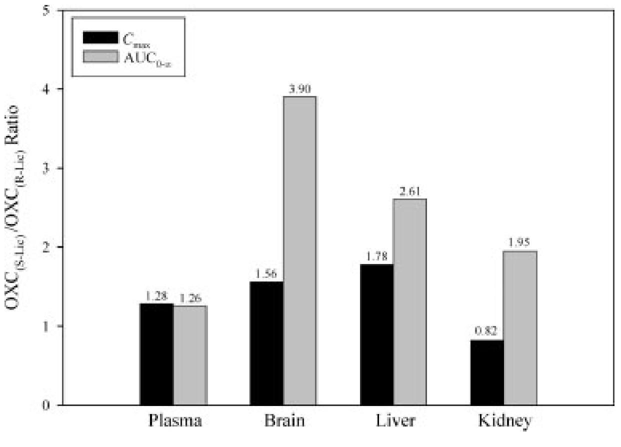

Fig. 5. The C_{max} and $AUC_{0-\infty}$ OXC ratios in plasma and brain, liver and kidney tissues after separate administration of *S*-Lic and *R*-Lic (350 mg/kg) to mice by oral gavage.

TABLE 5. Enantiomeric interconversion of *S*-Lic and *R*-Lic after their separate administration to mice by oral gavage (350 mg/kg)

	$S\text{-Lic}_{(R\text{-Lic})}/R\text{-Lic}_{(S\text{-Lic})}$	
Ratio	$C_{\max}$	$\text{AUC}_{0-\infty}$
Plasma	NA	NA
Brain	NA	NA
Liver	6.02	39.9
Kidney	4.58	NA

$S\text{-Lic}_{(R\text{-Lic})}$, *S*-Lic produced from *R*-Lic; $R\text{-Lic}_{(S\text{-Lic})}$, *R*-Lic produced from S-Lic; NA, not available.

both enantiomers after their separate administration are similar at first sampling time point (0.25 h). However, at that time, the value of the stereoselective index (1.51) between *R*-Lic and *S*-Lic plasma concentrations already reflect their stereoselective first-pass effect. It was demonstrated that *S*-Lic undergoes hepatic and renal accumulation either after its own administration or when formed as a metabolite of *R*-Lic. Hence, liver and kidneys act like reservoirs of *S*-Lic. From the metabolic data, it is also evident that OXC was produced from both licarbazepine enantiomers, being generated to a higher extent from *S*-Lic. However, less obvious may be the source of the small amounts of *S*-Lic and *R*-Lic produced following the administration of the corresponding antipode. At this moment, it is important to consider that the reduction of OXC not occurs effectively in mice, in contrast to man.[9] Moreover, the $t_{\max}$ values were coincident for both administered and produced licarbazepine enantiomers, whereas the $t_{\max}$ for OXC occurred later. Consequently, the bi-directional chiral inversion of licarbazepine enantiomers occurred in mice probably through a liver racemase, which favors the production of *S*-Lic from *R*-Lic, and not through OXC reduction. In fact, the chiral inversion is always mediated by enzymes and, in humans, was firstly demonstrated with ibuprofen.[25] The extra metabolite produced in mice after treatment with *R*-Lic, most likely, will correspond to the *trans*-diol. These results are in agreement with the observations performed by Hainzl et al.[9] demonstrating that the *trans*-diol is only produced from *R*-Lic. The formation of the *trans*-diol will probably contribute to an earlier inactivation of *R*-Lic. After all, taking into consideration the data presented and the information provided by Hainzl et al.,[9] the proposed metabolic pathways for *S*-Lic and *R*-Lic in mice are represented in Figure 6.

From this work, another relevant part of data was the stereoselective brain disposition of *S*- and *R*-Lic enantiomers. To our knowledge, these findings are reported here for the first time. Although their comparable systemic exposure ($\text{AUC}_{0-\infty}$ S/R plasma ratio close to unity), the extent of brain penetration for *S*-Lic was found to be approximately 2 times greater than that for *R*-Lic. Indeed, as other central nervous system drugs, *S*-Lic and *R*-Lic must overcome the BBB to reach the brain parenchyma, with their brain concentrations being much lower than those in plasma. Actually, the BBB consists of various transporter proteins involved in the active influx and efflux of drugs to and from the brain.[26] Thus, as with other drugs, licarbazepine enantiomers may be substrates for multidrug transporters (MDT) including *P*-glycoprotein at the BBB,[27] being subject to efflux transport and, possibly, *R*-Lic will be the preferential substrate. Previously, Clinckers et al.[28] demonstrated that OXC is a substrate for MDT at the BBB, which actively limits the penetration and accumulation of OXC into the brain.

In 1991, Levy and Boddy[22] considered three distinct organizational levels in the body (macromolecular, whole-organ and whole-body) and suggested that degree of stereoselectivity is more pronounced and easier to detect in front of macromolecular-specific or organ-specific parameters than in whole-body pharmacokinetic parameters. Indeed, our data appear to reflect this general theory: first of all an additional metabolite was formed from *R*-Lic, detecting stereoselectivity in the presence of metabolic enzymes; secondly, considering the $\text{AUC}_{0-\infty}$ pharmacokinetic parameter, the stereoselectivity between *S*- and *R*-Lic was greater in whole-organ level (brain, liver, and kidney) than in whole-body (see Fig. 4).

Despite the obvious species differences between mouse and man, these data give new information related to the pharmacokinetic disposition of licarbazepine enantiomers and their ability to cross the BBB and attain their therapeutic target. At last, the stereoselectivity identified in the disposition of *S*-Lic and *R*-Lic may also explain the differences observed in the systemic drug exposure to ESL and OXC. Indeed, Bialer et al.[29] reported that the bioavailability of ESL, measured in terms of *S*-Lic and *R*-Lic AUC, was 16% greater than that for OXC after intake of an equivalent molar dose.

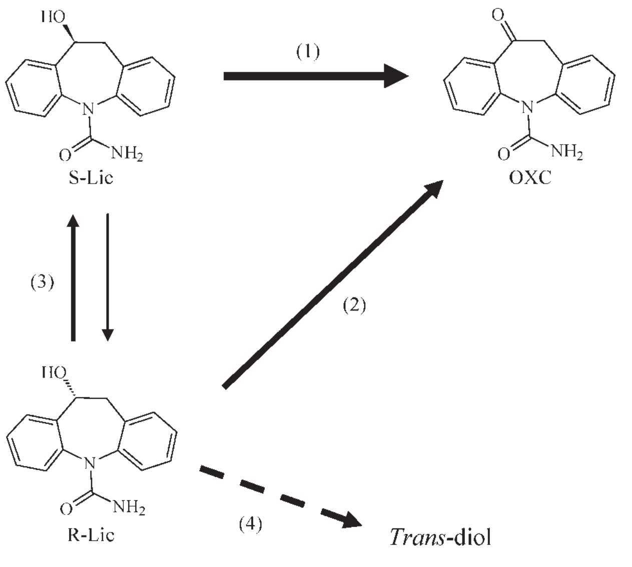

Fig. 6. Proposed metabolism of *S*-Lic and *R*-Lic in mice. The thickness of the arrows indicates the relative extent of the metabolic pathways: OXC is the major metabolite of *S*-Lic (1) and *R*-Lic (2), enantiomeric inversion is favorable to the formation of *S*-Lic (3) and conjugating the data of this study with the findings reported by Hainzl et al.[9] the *trans*-diol metabolite is produced only from *R*-Lic (4).

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Asymmetric Synthesis of Bicyclic Piperidines via L-Proline Catalyzed Aldol Reaction of 3-Phthalimidopropanal

FANGLIN ZHANG,[1] YIYUAN PENG,[2] AND YUEFA GONG[1]*
[1]*Department of Chemistry, Huazhong University of Science and Technology, Hubei, China*
[2]*Department of Chemistry, Jiangxi Normal University, Nanchang, China*

ABSTRACT A highly enantioselective approach for preparing optically active bicyclic piperidines is described. The key step for introducing chiral centers was a L-proline catalyzed direct enantioselective aldol reaction of 3-phthalimidopropanal with aliphatic ketones. In the reactions with alicyclic ketones, a highly enantioselective formation of *anti*-2-(3-phthalimido-1-hydroxypropyl)cycloketones **1a–1b** (>99% *ee*) was observed. The aldol products **1** could be subsequently converted into bicyclic piperidines **2** via a consecutive reductive deprotection, acylation, ring closure, and hydrolysis. *Chirality 20:805–811, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: enantioselective; bicyclic piperidines; L-proline; 3-phthalimidopropanal; aldol reaction

INTRODUCTION

Since the enantioselective aldol reaction is one of the most powerful and efficient methods for constructing stereocenters of some drugs and bioactive natural products,[1] L-proline catalyzed direct aldol reactions of ketones or aldehydes with aldehydes have been widely investigated in recent years.[2–10] A successful example reported by MacMillan and coworkers was the synthesis of different carbohydrates via the direct enantioselective aldol reaction of α-oxyaldehyde as the key step.[11] However, the substrate scope of this catalytic reaction is still narrow. In fact, the utility of functionalized aldehydes would expand the application of aldol reaction in the synthesis of valuable chemicals.

For our recent interest in catalytic reaction, we found that piperidine was an efficient catalyst for the direct cross aldol reactions of aldehydes and ketones with chloral or fluoral.[12,13] As a continuous work, we try to prepare chiral piperidine derivatives as potential efficient organocatalysts for the direct aldol reaction. To the best of our knowledge, there is no report referred to the L-proline catalyzed asymmetric aldol reaction of β-amino aldehyde hitherto. For L-proline-catalyzed aldol reaction of chiral 3-azido aldehydes with ketone, see Ref. 14. For aldolase-catalyzed asymmetric direct aldol reaction of N-Cbz-3-aminopropanal with dihydroxyacetone, see Ref. 15. In this work, 3-phthalimidopropanal was prepared and chosen as the functionalized aldehyde, and its direct asymmetric aldol or Mannich reaction with aliphatic ketones was investigated. Further conversion of the aldol products into bicyclic piperidines was also studied. The details are introduced as follows (Scheme 1).

EXPERIMENTAL SECTION

Optical rotation values were measured using WZZ-2S digital polarimeter using Na light. ^{1}H NMR spectra were measured on Bruker AC 200E (400 MHz) spectrometer at ambient temperature. Data were recorded by using TMS as the internal standard on the δ scale. ^{13}C NMR spectra were recorded on Bruker AC 200E (100 MHz) spectrometer at ambient temperature. Chemical shifts are recorded from the solvent resonance employed as the internal standard (deuterated chloroform at 77.07 ppm). Microanalyses for C, H, and N were performed on a Elementar Vario MICRO cube instrument. LC-MS was recorded using the electrospray ionization technique. The crude products were purified by preparative column chromatography on silica gel with 100–200 mesh. All the reagents were commercially available and used without further purification.

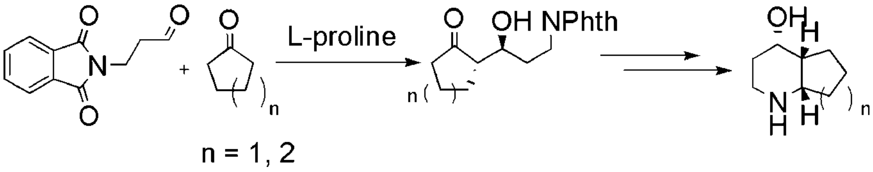
A Typical Procedure for L-Proline Catalyzed Cross Aldol Reaction

To a solution of acetone (1 ml) and 3-phthalimidopropanal (1 mmol, 203 mg) in solvent (4 ml) was added L-proline (0.3 mmol, 35 mg) in one portion at room temperature. The reaction mixture was stirred at room temperature for 48 h, and then partitioned between aqueous saturated NH_4Cl and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 2:1 (v/v) hexane/ethyl acetate to give the aldol product.

Contract grant sponsor: National Natural Science Foundation of China (NSFC); Contract grant number: B20572028.

*Correspondence to: YueFa Gong, Department of Chemistry, Huazhong University of Science and Technology, Hubei 430074, People's Republic of China. E-mail: gongyf@mail.hust.edu.cn

Received for publication 29 September 2007; Accepted 10 January 2008
DOI: 10.1002/chir.20549



Scheme 1. Outlined scheme for preparing bicyclic piperidines.

3-Hydroxy-5-phthalimido-2-(phthalimidomethyl)-pentanal (3). R_f = 0.21 (*n*-hexane/ethyl acetate, 2:1). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.89–2.01 (m, 2H, $\mathrm{NCH_2C}H_2$), 2.71–2.79 (m, 1H, $\mathrm{C}H\mathrm{CHO}$), 3.68 (br s, 1H, $\mathrm{CHO}H$), 3.82–4.03 (m, 4H, $\mathrm{NC}H_2$, $\mathrm{NC}H_2$), 4.16–4.24 (m, 1H, $\mathrm{C}H\mathrm{OH}$), 7.36–7.84 (m, 8H), 9.83 (d, *J* = 1.6 Hz, 1H, $\mathrm{C}H\mathrm{O}$), for its isomer 2.87–2.94 (m, 1H, $\mathrm{C}H\mathrm{CHO}$), 9.88 (d, *J* = 2.8 Hz, 1H, $\mathrm{C}H\mathrm{O}$).

5-Phthalimido-2-(phthalimidomethyl)-pent-2-enal (4). R_f = 0.51 (*n*-hexane/ethyl acetate, 2:1). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 3.04 (q, *J* = 6.8 Hz, 2H, $\mathrm{NCH_2C}H_2$), 3.90 (t, *J* = 6.8 Hz, 2H, $\mathrm{NC}H_2\mathrm{CH_2}$), 4.46 (s, 2H, $\mathrm{NC}H_2\mathrm{CCHO}$), 6.80 (t, *J* = 7.2 Hz, 1H, $\mathrm{C}H\mathrm{CCHO}$), 7.65–7.68 (m, 2H), 7.70–7.76 (m, 4H), 7.80–7.84 (m, 2H), 9.42 (s, 1H, $\mathrm{C}H\mathrm{O}$), ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$): δ = 28.7, 31.6, 36.3, 123.2, 123.3, 131.8, 131.9, 133.9, 134.1, 138.4, 153.6, 167.5, 168.2, 192.9.

6-Phthalimido-4-hydroxy-2-hexanone (5). R_f = 0.32 (*n*-hexane/ethyl acetate, 2:1). The *ee* value was determined by chiral HPLC (Daicel Chiralpak AD-RH, $\mathrm{CH_3CN/H_2O}$ = 30:70, flow rate: 0.5 ml/min, λ = 254 nm): t_R = 18.35 min (major enantiomer), t_R = 30.03 min (minor enantiomer). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.74–1.81 (m, 2H, $\mathrm{NCH_2C}H_2$), 2.17 (s, 3H, $\mathrm{COC}H_3$), 2.56 (dd, *J* = 3.2, 17.2 Hz, 1H, $\mathrm{CHOHC}H_\mathrm{a}\mathrm{H_bCO}$), 2.65 (dd, *J* = 8.8, 17.2 Hz, 1H, $\mathrm{CHOHCH_a}H_\mathrm{b}\mathrm{CO}$), 3.38 (br s, 1H, $\mathrm{CHO}H$), 3.84 (t, *J* = 6.8 Hz, 2H, $\mathrm{NC}H_2\mathrm{CH_2}$), 4.01–4.05 (m, 1H, $\mathrm{C}H\mathrm{OH}$), 7.70–7.74 (m, 2H), 7.82–7.84 (m, 2H), ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$): δ = 30.8, 34.5, 35.2, 49.7, 65.0, 123.3, 132.1, 134.0, 168.6, 208.7.

A Typical Procedure for L-Proline Catalyzed Mannich Reaction

A suspension of L-proline (35 mg, 0.3 mmol), acetone (1 ml), 3-phthalimidopropanal (203 mg, 1 mmol), and *p*-anisidine (135 mg, 1.1 mmol), in 4 ml of DMSO was stirred at room temperature for 24 h. The reaction was worked up by adding phosphate-buffered saline (PBS) solution (pH 7.4), extracting with ethyl acetate, drying the organic layer with anhydrous magnesium sulfate. The crude product was purified by silica gel column chromatography eluted with 2:1 (v/v) hexane/ethyl acetate to give **6**.

6-Phthalimido-4-(4-methoxyphenylamino)-hexan-2-one (6a). Yield, 71%. R_f = 0.36 (*n*-hexane/ethyl acetate, 2:1). The *ee* was determined by chiral HPLC (Daicel Chiralpak AS, hexane/*i*-PrOH = 70:30, flow rate: 0.5 ml/min, λ = 254 nm): t_R = 41.24 min (minor enantiomer), t_R = 46.36 min (major enantiomer). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.93 (q, *J* = 6.8 Hz, 2H, $\mathrm{NCH_2C}H_2$), 2.11 (s, 3H, $\mathrm{COC}H_3$), 2.64 (dd, *J* = 6.4, 16.8 Hz, 1H, $\mathrm{CHNHC}H_\mathrm{a}\mathrm{H_bCO}$), 2.73 (dd, *J* = 4.8, 16.8 Hz, 1H, $\mathrm{CHNHCH_a}H_\mathrm{b}\mathrm{CO}$), 3.73 (s, 3H, $\mathrm{OC}H_3$), 3.75–3.88 (m, 3H, $\mathrm{NC}H_2\mathrm{CH_2C}H\mathrm{NH}$), 6.57 (m, 2H), 6.64–6.66 (m, 1H), 6.71–6.75 (m, 2H), 7.69–7.71 (m, 2H), 7.80–7.92(m, 2H), ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$): δ = 30.1, 33.6, 35.5, 47.3, 49.3, 55.7, 114.9, 115.5, 123.2, 132.1, 134.0, 140.7, 152.4, 168.4, 207.9.

2-[3-Phthalimido-1-(4-methoxyphenylamino)propyl]-cyclohexanone (6b). Yield, 75%. dr = 2:1, R_f = 0.33 (*n*-hexane/ethyl acetate, 3:1). The *ee* was determined by chiral HPLC (Daicel Chiralpak AS, hexane/*i*-PrOH = 70:30, flow rate: 0.5 ml/min, λ = 254 nm): for anti isomer t_R = 19.60 min (major enantiomer), t_R = 21.76 min (minor enantiomer); for syn isomer t_R = 24.27 min (minor enantiomer), t_R = 38.24 min (major enantiomer). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.51–1.63 (m, 4H), 1.87–1.96 (m, 3H), 2.02–2.06 (m, 3H), 2.12–2.26 (m, 1H), 2.29–36 (m, 1H), 2.60–2.64 (m, 1H), 3.71 (s, 3H), 3.75–3.92 (m, 3H), 6.52–6.59 (m, 2H), 6.69–6.73 (m, 2H), 7.68–7.70 (m, 2H), 7.80–7.87(m, 2H).

Preparation of the Cross Aldol Adducts 1

To a solution of cycloketone (50 ml) and 3-phthalimidopropanal (10 mmol, 2.03 g) was added L-proline (3.0 mmol, 0.35 g) in one portion at room temperature. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was partitioned between aqueous saturated $\mathrm{NH_4Cl}$ and ethyl acetate. The organic layer was separated, and the aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by flash chromatography eluted with *n*-hexane/ethyl acetate to give **1**.

Anti-2-(3-Phthalimido-1-hydroxypropyl)-cyclopentanone (1a). Yield, 81%. anti:syn = 90:10, R_f = 0.38 (*n*-hexane/ethyl acetate, 2:1). $[\alpha]_\mathrm{D} = -42°$ (*c* 5.0, $\mathrm{CHCl_3}$). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.57–1.61 (m, 1H), 1.76–1.82 (m, 1H), 1.84–1.89 (m, 2H), 2.00–2.08 (m, 1H), 2.11–2.22 (m, 3H), 2.36 (m, 1H), 3.76 (q, *J* = 7.2 Hz, 1H, $\mathrm{C}H\mathrm{OH}$), 3.86–3.89 (m, 2H, $\mathrm{NC}H_2$), 7.70–7.73 (m, 2H), 7.83–7.85 (m, 2H), ^{13}C NMR (100 MHz, $\mathrm{CDCl_3}$): δ = 20.5, 26.6, 33.4, 34.6, 38.5, 53.4, 70.0, 123.2, 132.2, 133.9, 168.5, 222.7.

Anti-2-(3-Phthalimido-1-hydroxypropyl)-cyclohexanone (1b). Yield, 84%. anti:syn = 95:5, R_f = 0.39 (*n*-hexane/ethyl acetate, 2:1). $[\alpha]_\mathrm{D} = -16°$ (*c* 2.0, $\mathrm{CHCl_3}$). ^{1}H NMR (400 MHz, $\mathrm{CDCl_3}$): δ = 1.43–1.47 (m, 1H), 1.62–1.66 (m, 2H), 1.69–1.83 (m, 1H), 1.89–1.94 (m, 2H), 2.05–2.13 (m, 2H), 2.30–2.46 (m, 3H), 3.07 (br s, 1H), 3.80–3.90 (m,

TABLE 1. **Aldol reaction of 3-phthalimidopropanal with acetone in various solvents**

Entry	Solvent	Yield of 5[a]	Ee[b] (%)
1	DMSO	24	71
2	DMF	35	70
3	CH_2Cl_2	52	73
4	$CHCl_3$	64	73
5	CH_3CN	55	64
6	THF	69	72
7	$PhCH_3$	60	75
8	Acetone[c]	72	75

[a] Isolated yields.

[b] Determined by chiral stationary phase HPLC.

[c] Dry acetone (5 ml).

3H), 7.70–7.73 (m, 2H), 7.83–7.85 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.9, 27.7, 30.4, 32.1, 34.9, 42.7, 55.7, 69.3, 123.2, 132.2, 133.9, 168.5, 214.7.

Silylation of Cross Aldol Adducts 1a and 1b

In a three-necked round-bottomed flask were placed *tert*-butyldimethylsilyl chloride (TBSCl) (4 mmol) and imidazole (6 mmol) under N_2. Anhydrous DMF (5 ml) was added followed by **1** (2 mmol). The reaction mixture was stirred at room temperature for 48 h. Water (20 ml) was added and the mixture was then extracted with ether (3 × 20 ml). The organic extract was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The oily residue was applied to a silica gel column and eluted with (*n*-hexane/ethyl acetate, 5:1) to give **7**.

2-[3-Phthalimido-1-(tert-butyldimethylsilyloxy)-propyl]-cyclopentanone (7a). Yield, 78%. R_f = 0.31 (*n*-hexane/ethyl acetate, 5:1). The *ee* was determined by chiral HPLC (Daicel Chiralpak OD-H, hexane/*i*-PrOH = 98:2, flow rate: 0.5 ml/min, λ = 254 nm): for anti isomer t_R = 20.44 min (major enantiomer), t_R = 23.75 min (minor enantiomer). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.07 (s, 3H), 0.12 (s, 3H), 0.99 (s, 9H), 1.72–1.89 (m, 3H), 1.93–2.20 (m, 4H), 2.23–2.29 (m, 2H), 2.41–2.44 (m, 1H), 3.65–3.79 (m, 2H), 4.09–4.11 (m, 1H), 7.69–7.71 (m, 2H), 7.82–7.84 (m, 2H).

2-[3-Phthalimido-1(tert-butyldimethylsilyloxy)-propyl]-cyclohexanone (7b). Yield, 81%. R_f = 0.32 (*n*-hexane/ethyl acetate, 5:1). The *ee* was determined by chiral HPLC (Daicel Chiralpak AD-H, hexane/*i*-PrOH = 93:7, flow rate: 0.5 ml/min, λ = 254 nm): for anti isomer t_R = 11.58 min (major enantiomer), t_R = 15.74 min (minor enantiomer). ^1H NMR (400 MHz, $CDCl_3$): δ = 0.06 (s, 3H), 0.17 (s, 3H), 0.92 (s, 9H), 1.42–1.52 (m, 1H), 1.58–1.74 (m, 3H), 1.82–1.92 (m, 2H), 1.97–2.09 (m, 1H), 2.23–2.34 (m, 3H), 2.44–2.51 (m, 1H), 3.75–3.80 (m, 2H), 4.26–4.31 (m, 1H), 7.69–7.71 (m, 2H), 7.83–7.85 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = −4.6, 18.1, 24.9, 25.9, 27.1, 27.4, 32.4, 35.3, 42.2, 56.4, 68.3, 123.1, 132.3, 133.8, 168.4, 211.3.

Preparation of 11

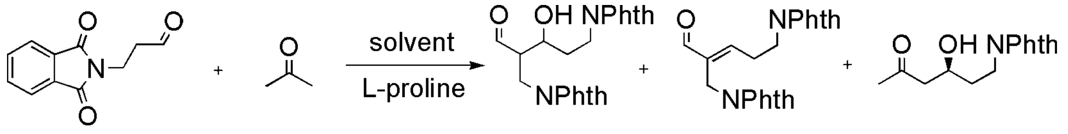
To a stirred solution of **1** (10 mmol) in 2-propanol (100 ml) and H_2O (17 ml) was added $NaBH_4$ (60 mmol). After stirring 24 h, TLC indicated complete consumption of starting material. Glacial acetic acid (12 ml) was added carefully and when the foaming subsided, the reaction mixture was heated to 80°C for 8 h. The crude reaction mixture was then evaporated under a reduced pressure and the residue was diluted with H_2O (30 ml). The aqueous layer was extracted with three 20-ml portions of Et_2O. The aqueous phase was made basic with Na_2CO_3 at 0°C. Then benzoyl chloride (20 mmol) was added successively over a period of 1 h. After being stirred for 4 h at 0°C, the reaction mixture was extracted with ethyl acetate (4 × 30 ml). The combined organic layers were dried over anhydrous sodium sulfate, evaporated, and the residue was purified using column chromatography (ethyl acetate) to give **11** as a colorless oil.

2-(3-Benzoylamino-1-hydroxypropyl)-cyclopentanol (11a). Yield, 73%. R_f = 0.34 (ethyl acetate). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.06–1.09 (m, 1H), 1.53–1.57 (m, 3H), 1.70–1.80 (m, 4H), 1.91–1.96 (m, 1H), 3.32–3.43 (m, 1H), 3.53–3.59 (t, *J* = 8.0 Hz, 1H), 3.76–3.88 (m, 1H), 3.97–4.05 (q, *J* = 7.6 Hz, 1H), 4.20 (br s, 2H), 7.35–7.40 (m, 2H), 7.45–7.49 (m, 1H), 7.52 (br s, 1H), 7.75–7.79 (m, 2H).

2-(3-Benzoylamino-1-hydroxypropyl)-cyclohexanol (11b). Yield, 78%. R_f = 0.35 (ethyl acetate). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.11–1.20 (m, 1H), 1.23–1.26 (m, 2H), 1.35–1.40 (m, 1H), 1.51–1.58 (m, 1H), 1.63–189 (m, 4H), 1.86–1.93 (m, 2H), 3.34–3.41 (m, 1H), 3.48–3.61 (m, 1H), 3.66–3.71 (m, 1H), 3.74–3.82 (m, 1H), 4.91 (br s, 2H), 7.33–7.41 (m, 2H), 7.43–7.47 (m, 1H), 7.76–7.80 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.5, 25.3, 27.2, 33.8, 35.2, 36.8, 48.5, 75.5, 76.0, 127.0, 128.5, 131.5, 134.1, 168.8.

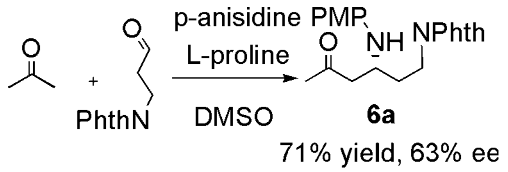
Tosylation of 11

The solution of **11** (5.0 mmol) in dichloromethane (25 ml) and pyridine (5 ml) was stirred at 0°C for 30 min. Then *p*-toluenesulfonyl chloride (1.05 g, 5.5 mmol) was added in several small portions over a period of 1 h. The reaction mixture was stirred at room temperature for 36 h.



3 **4** **5**

Scheme 2. Catalytic reaction between 3-phthalimidopropanal and acetone.



Scheme 3. Catalytic Mannich reaction between 3-phthalimidopropanal and acetone.

The reaction mixture was diluted with dichloromethane (20 ml) and poured into a mixture of 25 g of cracked ice, 10 ml of concentrated hydrochloric acid, and 10 ml of water. The organic layer was separated, and the aqueous layer was extracted further with two 20 ml portions of CH_2Cl_2. The organic layer was evaporated under a reduced pressure, and the residue was subjected to flash silica gel column chromatography (eluting with *n*-hexane/ethyl acetate, 3:2) to give **12** as a colorless oily liquid.

1-[2-(4-toluenesulfonyloxy)cyclopentyl]-3-benzoylaminopropanol (12a). Yield, 63%. R_f = 0.28 (*n*-hexane/ethyl acetate, 3:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.51–1.68 (m, 6H), 1.84–1.86 (m, 2H), 2.16–2.19 (m, 1H), 2.41 (s, 3H), 3.27–3.32 (m, 1H), 3.33 (br s, 1H), 3.44–3.49 (m, 1H), 3.76–3.82 (m, 1H), 5.01–5.02 (m, 1H), 7.12 (br s, 1H), 7.29–7.32 (m, 2H), 7.38–7.42 (m, 2H), 7.46–7.50 (m, 1H), 7.76–7.78 (m, 4H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6, 23.5, 28.0, 32.2, 35.1, 37.3, 52.1, 70.8, 86.5, 127.0, 127.8, 128.6, 129.8, 131.5, 134.1, 144.7, 168.4.

1-[2-(4-toluenesulfonyloxy)cyclohexyl]-3-benzoylaminopropanol (12b). Yield, 61%. R_f = 0.26 (*n*-hexane/ethyl acetate, 3:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.12–1.27 (m, 2H), 1.38–1.1.46 (m, 1H), 1.56–1.69 (m, 3H), 1.76–1.92 (m, 3H), 1.96–1.99 (m, 1H), 2.40 (s, 3H), 3.15 (br s, 1H), 3.29 (d, J = 13.2 Hz, 1H), 3.92–3.95 (m, 2H), 4.52 (dt, *J* = 4.4, 10.4 Hz, 1H), 7.01 (br s, 1H), 7.19 (d, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.6 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6, 24.4, 24.5, 24.8, 30.9, 32.8, 37.0, 48.1, 67.5, 82.6, 127.2, 127.5, 128.5, 129.7, 131.4, 134.1, 144.7, 168.61.

Preparation of 13

To a suspension of **12** (2.0 mmol) in dry THF (20 ml) was added NaH (120 mg, 5 mmol) at room temperature. The reaction was stirred for 12 h, quenched with water (10 ml), and extracted with ethyl acetate (3 × 20 ml). The combined organic layers were dried over magnesium sulfate and evaporated under vacuo. The residue was purified using column chromatography (*n*-hexane/ethyl acetate, 2:1) to give **13** as a colorless oil.

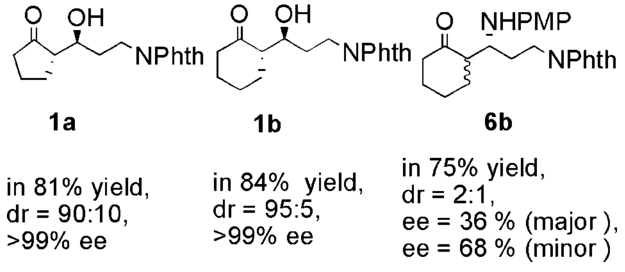


Fig. 1. Structures for Aldol adducts **1a-b** and Mannich base **6b**.

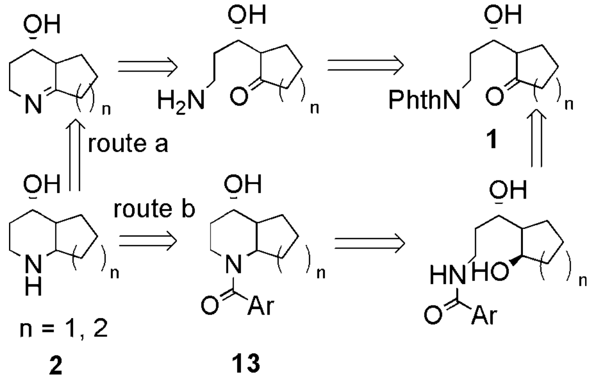


Fig. 2. Retrosynthetic analysis of bicyclic piperidines.

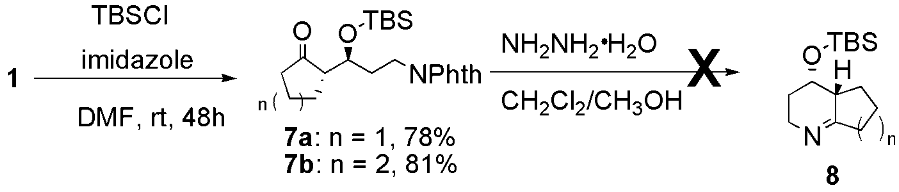
(cis, cis)-1-Benzoyl-octahydro-1H-cyclopenta[b]pyridin-4-ol (13a). Yield, 52%. R_f = 0.20 (*n*-hexane/ethyl acetate, 2:1), $[\alpha]_D$ = −12° (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.33–1.40 (m, 1H), 1.44–1.50 (m, 1H), 1.77 (dd, *J* = 6.4, 13.2 Hz, 1H), 1.92–2.00 (m, 3H), 2.06–2.14 (m, 2H, H-5), 2.83–2.87 (m, 1H, H-4a), 3.48 (m, 1H, H-2*a*), 3.74 (m, 1H, H-2*e*), 4.30 (dt, *J* = 4.0, 8.0 Hz, 1H, H-4), 5.18 (t, *J* = 4.6 Hz, 1H, H-7a), 7.17(br s, 1H), 7.38–7.42 (m, 2H), 7.45–7.47 (m, 1H), 7.75–7.77 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.9, 30.1, 34.7, 35.7, 37.7, 43.6, 85.2, 85.5, 126.9, 128.5, 131.2, 134.6, 167.3. Elemental Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.32; H, 7.93; N, 5.60. MS (ESI): m/z = 246.1 $[M+H]^+$.

(cis, cis)-1-Benzoyl-decahydro-4-quinolinol (13b). Yield, 54%. R_f = 0.18 (*n*-hexane/ethyl acetate, 2:1). $[\alpha]_D$ = +3.2° (*c* 2.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.42–1.45 (m, 2H), 1.60–1.64 (m, 1H), 1.68–1.82 (m, 5H), 1.95–2.03 (m, 1H), 2.08–2.12 (m, 1H), 2.61–2.67 (m, 1H, H-4a), 3.47 (m, 1H, H-2*a*), 3.75 (m, 1H, H-2*e*), 4.60 (ddd, *J* = 3.6, 5.2, 8.8 Hz, 1H, H-4), 4.89 (dt, *J* = 4.0, 8.8 Hz, 1H, H-8a), 7.15(br s, 1H), 7.39–7.43 (m, 2H), 7.46–7.50 (m, 1H), 7.76–7.78 (m, 2H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.8, 20.0, 24.5, 29.0, 34.6, 37.0, 37.9, 77.3, 85.4, 126.9, 128.5, 131.2, 134.6, 167.3. Elemental Anal. Calcd for $C_{16}H_{21}NO_2$: C, 74.10; H, 8.16; N, 5.40. Found: C, 73.97; H, 8.27; N, 5.52. MS (ESI): m/z = 260.1 $[M+H]^+$.

Preparation of 2a

A solution of **13a** (123 mg, 0.5 mmol) in 1*N* NaOH (10 ml) was heated under reflux for 6 h. The solution was cooled to room temperature and extracted with ethyl acetate (3 × 20 ml). The combined organic layers were dried over magnesium sulfate and evaporated. The residue was purified using column chromatography (ethyl acetate/methanol, 10:1) to give **2a**.

(cis, cis)-Octahydro-1H-cyclopenta[b]pyridin-4-ol (2a). Yield, 73%. R_f = 0.18 (ethyl acetate/methanol, 10:1). $[\alpha]_D$ = +3.6° (*c* 0.5, CH_3OH). ^{1}H NMR (400 MHz,



Scheme 4. Route (a) via hydrazine deprotection.

D_2O): δ = 1.39–1.45 (m, 2H), 1.70 (dd, *J* = 6.0, 13.2 Hz, 1H), 1.79–1.97 (m, 4H), 2.08 (m, 1H), 2.82–2.86 (m, 1H), 2.91 (t, *J* = 7.6, 2H), 4.23 (dt, *J* = 4.0, 8.0 Hz, 1H), 5.19 (dd, *J* = 4.0, 5.2 Hz, 1H), ^{13}C NMR (100 MHz, D_2O): δ = 23.3, 29.3, 33.8, 34.7, 35.8, 43.0, 84.0, 87.4.

RESULTS AND DISCUSSION

The catalytic activity of four common organocatalysts including L-prolinamide, L-prolinol, L-4-hydroxyproline, and L-proline was first assessed by utilizing the direct catalytic cross aldol reaction of 3-phthalimidopropanal with acetone. Among them, L-proline was the most appropriate for stereoselective aspect. As a typical procedure, the catalytic cross aldol reaction was performed by stirring a solution of 3-phthalimidopropanal (203 mg, 1 mmol), acetone (1 ml), and L-proline (35 mg, 30 mol%) in a solvent (4 ml) at room temperature. The reaction was followed by TLC and almost completed after 48 h. Products were isolated by silica gel column chromatography and characterized by NMR spectroscopy. All the results observed were given in Table 1. When the reaction was carried out in DMSO, the main products were self-aldol adduct **3**, dehydration product **4**, and cross-aldol adduct **5** (Scheme 2). The product distribution was apparently dependent upon the property of solvent (Entries 2–7). In THF, the cross-aldol adduct **5** was isolated in 69% yield. In fact, the best yield was achieved by using an excess of acetone without other solvent (Entry 8). In this case, only a little amount of the byproducts **3** and **4** were detected (<5%). In addition, the stereochemistry analysis of **5** by chiral HPLC clearly demonstrated that the above aldol reaction underwent with a moderate enantioselectivity (75%).

Next we assessed the L-proline catalyzed Mannich reaction between acetone, 3-phthalimidopropanal, and *p*-anisidine in different solvents. In contrast with the above cross aldol reaction, the Mannich reaction underwent smoothly in DMSO, affording the corresponding mannich base **6a** in good yield (71%) with a moderate enantioselectivity (63%) (Scheme 3).

In view of the considerable difference in stereoselectivity between alicyclic ketones and acetone,[16] the corresponding aldol and Mannich reactions of cyclopentanone were further investigated under the above reaction conditions. When the aldol reaction was carried out using an excess of cyclopentanone without any other solvent, the desired product **1a** was furnished in good yield (81%) with a high diastereoselectivity (anti:syn = 90:10). The *ee* value of the anti isomer of **1a** determined carefully by chiral HPLC was high up to 99% (see Fig. 1). A similar result was also observed when cyclohexanone was used as the substrate, and the yield and the stereoselectivity of the cross aldol adduct **1b** were listed in Figure 1.

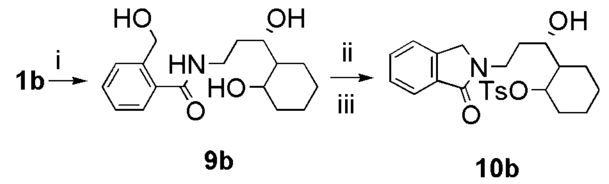
Similar reaction between cyclohexanone (1 ml), 3-phthalimidopropanal (1 mmol), and *p*-anisidine (1.1 mmol) in DMSO (4 ml) also furnished the corresponding β-amino ketone **6b** in good yields (75%). As illustrated in Figure 1, the stereoselectivity of this case, however, was not improved in comparison with acetone. For this reason, we have not continued to investigate the Mannich reaction of other alicyclic ketones.

Based on the above observations, we embarked on the synthesis of 2,3-cycloalkano-4-hydroxypiperidine. According to the retrosynthetic analysis, there are two possible synthetic routes to access the target bicyclic 4-hydroxypiperidine **2** starting from the optically active cross aldol adduct **1** as depicted in Figure 2.

The route (a) involved the deprotection via the hydrazinolysis of **1**, the ring closure, and the reduction of imine. As an alternative, the route (b) passed through the reductive cleavage of phthaloyl group of **1**, the intramolecular replacement, and the removal of protection group.

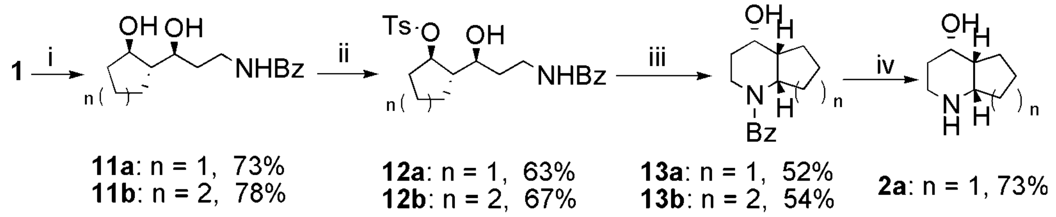
Initially, we started the synthesis of **2** along with route (a) shown in Scheme 4. The hydroxyl group of **1** was first protected by the reaction with *tert*-butyldimethylsilyl chloride to give **7** in good yields (78% for **7a**, 81% for **7b**). Next, the removal of phthaloyl group was tried by careful treatment of **7** with 80% aqueous hydrazine in CH_2Cl_2/CH_3OH. Unexpectedly, the desired cyclic imine **8** was not obtained (Scheme 4). Thus we have to turn our attention to the route (b).

As reported in the literature, the phthaloyl group was easily cleaved by treating **1b** with excess $NaBH_4$ in 2-propanol/H_2O at room temperature.[17] The hydroxyl groups of reduction product **9b** were then protected using an excess of toluenesulfonyl chloride in pyridine. As expected, subsequent intramolecular substitution took place predominantly at the site of benzyl tosylate (Scheme 5).



i: $NaBH_4$, 2-propanol/H_2O;
ii: excess TsCl, Py; iii: EtONa/THF

Scheme 5. Partial reductive deprotection route for **1b**.



i: $NaBH_4$, 2-propanol; then HOAc, 80℃; PhCOCl, aqueous Na_2CO_3, 0℃, 4h.
ii: TsCl, Py, CH_2Cl_2, 0℃ for 4h, then rt for 24 h.
iii: NaH, THF, rt for 12 h.
iv:NaOH 1N reflux for 5h.

Scheme 6. Synthesis of bicyclic piperidines **2**.

Therefore, we have to remove the phthaloyl group completely. After heating the reduction mixture of **1** in acetic acid at 80°C, the corresponding amino alcohol was obtained. The amino alcohol was further benzoylated without isolation with benzoyl chloride in Na_2CO_3 at 0°C, affording the desired amino protective product **11** in moderate yield (Scheme 6). Considering that the hydroxyl was a poor leaving group, it should be converted into its tosylate, a good leaving group. When the tosylation of **11** was performed with tosyl chloride and pyridine in dichloromethane, almost only the desired tosylates **12** was given in good yields, that is, the ring hydroxyl was preferentially tosylated. Subsequent cyclization carried out by treatment of **12** with NaH in THF at room temperature produced **13** in moderate yield (52% for **13a**, 54% for **13b**). Deprotection of **13a** was easily achieved by treatment with 1*N* NaOH aqueous solution, and the target bicyclic 4-hydroxypiperidine **2a** was obtained in 73% yield.

The stereochemical assignment of structures **13a–13b** was based on ^{1}H NMR coupling constants and NOE correlation as shown in Figure 3. As indicated by the NOE data of **13a**, there is a pronounced NOE between H-4/H-4a, H-4a/H-7a, and H-3(axial)/H-4a, whereas no distinct NOE between H-7a/H-2(axial) and H-7a/H-4. These data implied that H-4a is located at the axial position, while both H-4 and H-7a at the equatorial positions, which is rather consistent with the vicinal couplings ($J_{\text{H4-H4a}}$ = 4.0 Hz and $J_{\text{H4a-H7a}}$ = 4.6 Hz). The stereochemistry of **13a** was therefore assigned unequivocally to be *cis*, *cis*-isomer. Similar spectrum data was also applied to determine the stereochemistry of **13b**.

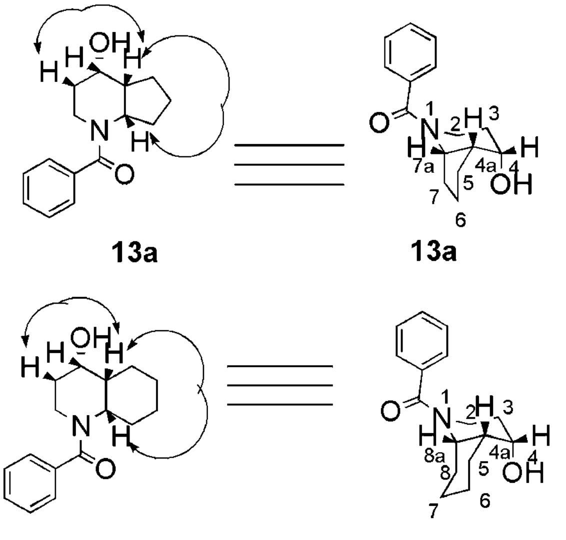


Fig. 3. Schematic representation and characteristic NOE diagram of **13a** and **13b**.

CONCLUSIONS

In conclusion, we have developed a novel four steps asymmetric route to (*cis*, *cis*)-2, 3- cycloalkano-4-hydroxypiperidine derivatives **13** starting from 3-phthalimidopropanal in 24% overall yield, where the key step to construct the chiral center is a L-proline catalyzed enantioselective cross aldol reaction. Compound **13** was easily converted into the target bicyclic 4-hydroxypiperidine. The application of this bicyclic 4-hydroxypiperidine **2** to the direct aldol reaction is underway.

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Book Review

On Chirality and the Universal Asymmetry—Reflections on Image and Mirror Image, by Georges H. Wagnière. 247 pp. HCA-Wiley/VCH: Zürich. 2007.

Chemists are often concerned with the molecular aspects of chirality, but usually forget that chirality is ubiquitous in our universe, affecting all aspects of life. The book "On Chirality and the Universal Asymmetry" takes an excursion through different fields of natural sciences where handedness is observed. Layout and design are excellent, and many figures come in color.

The book consists of 10 chapters. The first chapter introduces molecular chirality beginning with its manifestation in optical activity and how that helped to develop the concept of molecular structure in the 19th century. It goes on to the first determination of absolute configuration and asymmetric synthesis in the last century. The contributions of the important protagonists in the field are briefly presented, including Arago, Fresnel, Pasteur, van't Hoff, Lord Kelvin, Werner, Rosenfeld, Bijvoet, and the Nobel laureates of 2001, Noyori, Knowles, and Sharpless.

Chapter 2 deals with the violation of parity. After introducing the fundamental symmetry operations of parity (P), time reversal (T), and charge conjugation (C), parity violation is discussed. The consequences for nuclear physics, for atoms, as well as for the interconversion of molecular enantiomers are addressed. In particular the optical activity of atomic vapor and its experimental proof are well explained.

Chapter 3 gives a brief introduction into light, magnetism, and into natural and magnetic optical activity. One half of this chapter is dedicated to the magnetochiral effect. A professor emeritus in physical chemistry, the author can be considered as "the father" of this phenomenon. He was the first to predict that opposite enantiomers have different absorption coefficients for light propagating in the direction of a magnetic field—independently from the light's polarization—and proposed how this can be measured.[1] After his prediction was demonstrated experimentally,[2] he was the first to measure the effect in birefringence.[3]

Chapter 4 then connects the concept of chirality with space inversion (P) and time reversal, by introducing Barron's definition of chirality. In this light more manifestations of the magnetochiral effect are discussed and new effects are predicted.

Chapter 5 is a trip through outer space and the fundamentals of physics. It deals with the constitution of matter, the evolution of the universe, and astrophysics of galactic and stellar magnetic fields, all discussed in the frame of the basic CPT symmetries.

The next chapters are devoted to chirality in the solar system and on earth (Chapter 6) and to chirality at the micro- and nanometer scale (Chapter 7). Topics like chirality of crystals, liquid crystals, surfaces, and vortices are addressed in this latter Chapter.

Chapter 8 is named "Chiral Models." It actually deals with topological aspects of chirality, such as the dissection of geometric bodies into chiral objects ("La Coupe du Roi") and knot theory. Via the theory of chirality functions, sector rules to interpret CD and ORD spectra are derived.

Chapter 9 focuses on chiral recognition at the molecular level and treats enantioselective processes at phase boundaries, in chromatography, catalysis, and polymerization. Special emphasis is put here on surfaces and biopolymers, as regards to achieving homochirality.

Chapter 10, finally, is dedicated to the origin of life and prebiotic evolution, and discusses various reasons for homochirality in the biosphere.

This book provides an excellent overview of different manifestations of chirality. Despite its limited size, it is quite elaborate. It is not a text book and neither is it addressed to the specialist. While several chapters are accessible to the interested layperson—and the glossary is very helpful in this context—others require a basic knowledge of physics, crystallography, group theory, or algebra. The more complicated issues, however, are well explained and ought to be easily understood by persons with a scientific background. Equations are used strictly when necessary. A number of topics addressed here will be new to the trained chemist, and quite certainly to most physicists. It is not only highly recommended to students and teachers who are interested in chirality: This is a book for everyone with a background in natural sciences.

Karl-Heinz Ernst
Molecular Surface Science Group
Laboratory of Nanoscale Materials Science
Empa-Swiss Federal Laboratories of
Materials Testing and Research
Überlandstrasse 129
8600 Dübendorf, Switzerland

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DOI: 10.1002/chir.20536

CHIRALITY 20:813–814 (2008)

Erratum

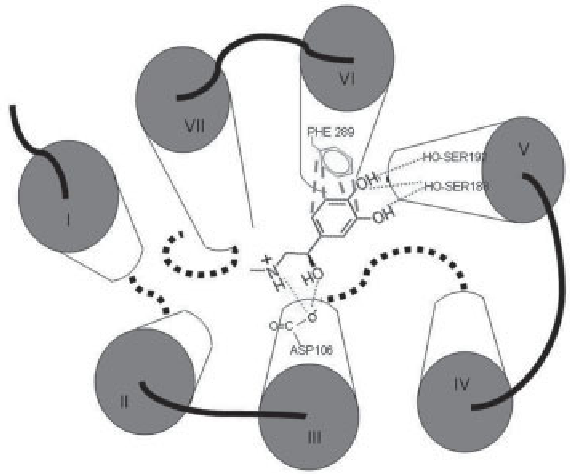
Analysis of Efficacy of Chiral Adrenergic Agonists by Popat N. Patil, Chenglong Li, Vandana Kumari, and J. Paul Hieble, *Chirality* 20:529-543, 2008.

In our recent article Figure 8 parts A and B were transposed. The corrected figure follows. Additionally, in the Acknowledgments, Dr. D.G. Patel was listed incorrectly and Dr. P.J. Rice was omitted. The corrected Acknowledgments appears directly below. We sincerely regret these errors.

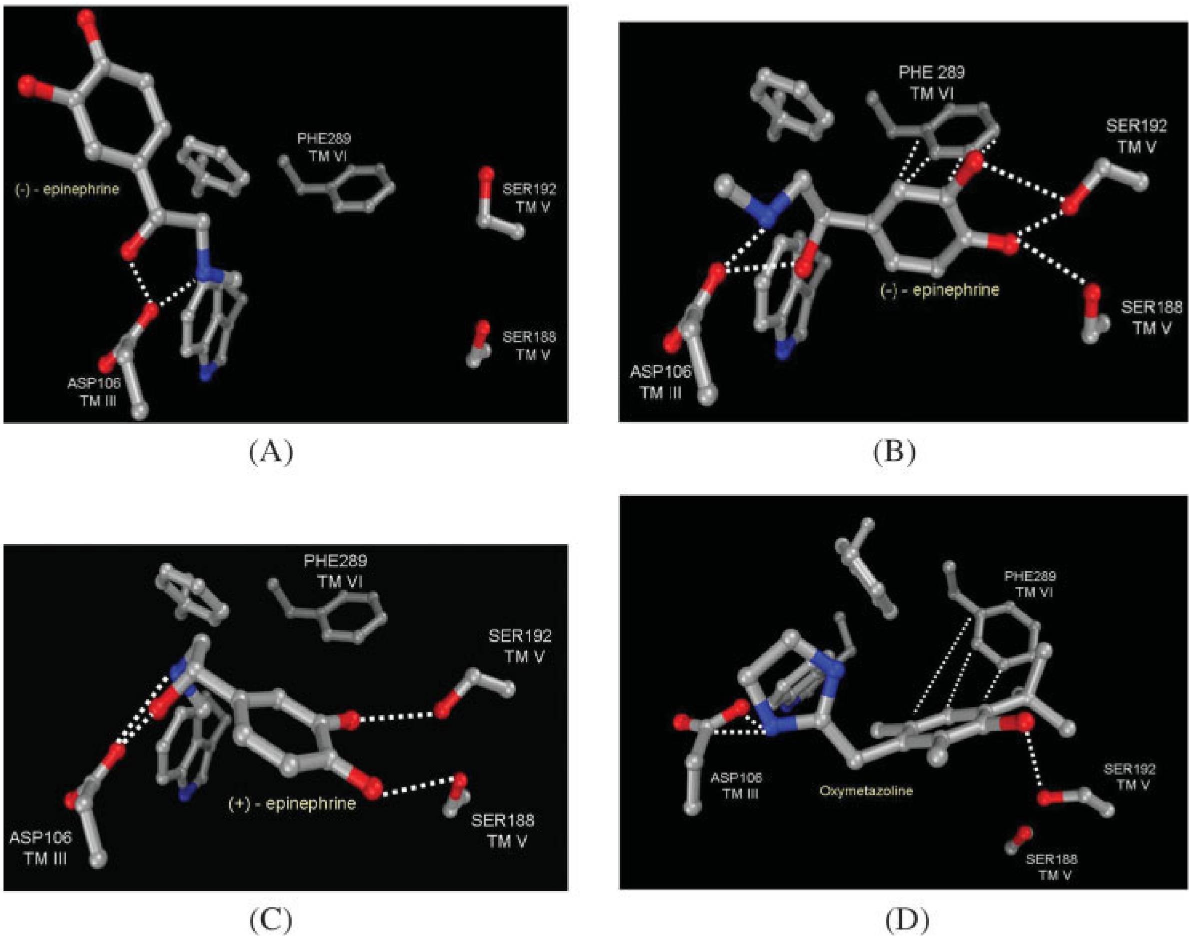
ACKNOWLEDGMENTS

The authors happily acknowledge the stimulating discussions, research collaborations, skills and scholarship exhibited over 35 years of investigation on this topic at the university by the wonderful faculty, colleagues and associates, namely Late Professor Emeritus Jules B. LaPidus, Professor Duane Miller, Professor Emeritus Dennis R. Feller, Professor Carl K. Buckner, Dr. R.D. Krell, Professor Emeritus B.D. Garg (India), Dr. D.G. Patel, Dr. A. Hamada, Mr. Jack Fowble, Dr. L.M. DeSantis, Dr. P. J. Rice, Dr. Robert R. Ruffolo, Jr., Dr. H.L. Komisky, Dr. Jon Banning, Dr. J. Sengupta, and the late Profs. Arthur Tye and Ullrich G. Trendelenburg (Germany). They also thank Dr. B.F. Tullar and Dr. S. Archer of Sterling-Winthrop Research Institute, Rensselaer, New York for the donation of pure enantiomers of catecholamines.

DOI: 10.1002/chir.20581



(-)Epinephrine docking at α_{1A}adrenoceptor



(A) (B)

(C) (D)

Fig. 8. Modeled binding modes of (–)-epinephrine, (+)-epinephrine and oxymetazoline to α_{1A} adrenoceptor. (**A**) Initial anchoring of (–)-epinephrine with Asp^{106}; (**B**, **C**, **D**) (–)-epinephrine, (+)-epinephrine, and oxymetazoline stable binding modes. Note in Figures 8b and 8c –$^{+}NH_3$ and chiral –OH of the enantiomers interact with Asp^{106} of the adrenoceptor. But in (+)-epinephrine, interaction with PHE^{289} of TM VI domain is lacking. The modeling of epinine remains to be examined.