Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 17, pp. 1893-1898, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00316-9

DIASTEREOSELECTIVE SYNTHESIS OF MIXANPRIL, AN ORALLY ACTIVE DUAL INHIBITOR OF NEUTRAL ENDOPEPTIDASE AND ANGIOTENSIN CONVERTING ENZYME.

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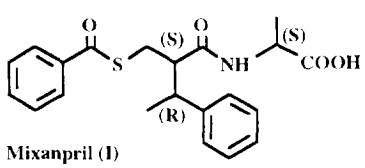
Abstract. A new diastereoselective synthesis of mixanpril, N-[(2S,3R)-2-benzoylthiomethyl-3-phenylbutanoyl]-L-alanine, a dual inhibitor of neutral endopeptidase and angiotensin converting enzyme, which could be used in the treatment of chronic hypertension and cardiac failure has been developed in order to obtain large quantities of this compound necessary for preclinical screening. As expected we showed that a complete inhibition of both enzymes was obtained after oral administration of mixanpril in anaesthesized rats.

In the search for new and efficient treatments of hypertension and cardiovascular diseases, we have proposed to inhibit both neutral endopeptidase-24.11, NEP and angiotensin converting enzyme, ACE [1]. ACE [2] is involved in the formation of angiotensin II, a vasoconstrictor peptide, and NEP [3] metabolizes atrial natriuretic peptide [4] which induces diuresis and natriuresis and possesses slight hypotensive properties. Moreover, both peptidases ensure the inactivation of bradykinin, a vasodilatory peptide [2,5]. Recent studies have shown the beneficial effects, in chronic hypertension and cardiac failure, resulting from the association of selective NEP and ACE inhibitors [6] or from dual mercapto inhibitors derived from the NEP inhibitor thiorphan [7] or containing a cyclic moiety [8]. These results have induced the synthesis of a large number of dual NEP/ACE inhibitors [9].

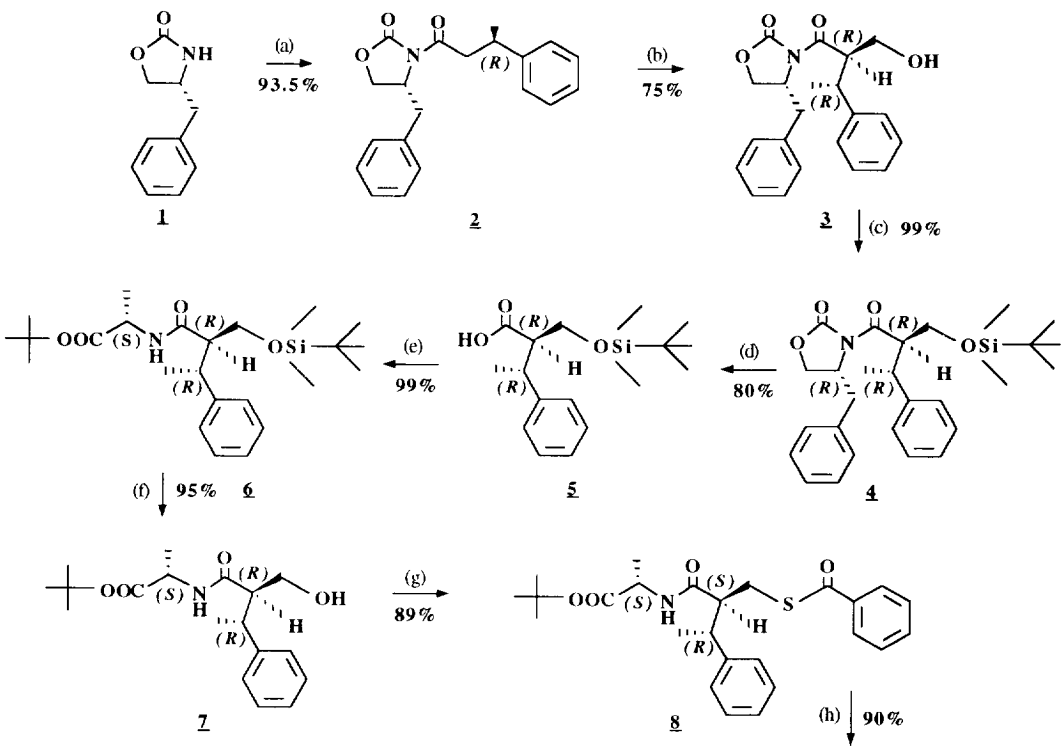
Among these compounds, mixanpril (I), an orally active inhibitor of NEP and ACE, designed using a model of NEP and ACE pharmacophores developed by molecular modeling [10], was shown to decrease blood pressure and increase diuresis in various models of hypertensive rats (SHR, Doca salt and renovascular rats) [11].



Mixanpril (I)

Compound **I** contains a 2-mercaptomethyl-3-phenylbutanoyl moiety in which the two asymmetric carbons have the 2S, 3R absolute configurations respectively. In order to obtain I, as an optically pure compound, two

synthetic pathways have been previously described [10]. In the first one, the precursor, 2-benzoylthiomethyl-3-phenyl butanoic acid, was prepared under racemic form and the (2S, 3R) isomer was isolated from the mixture by crystallization of the salt obtained with the chiral amine (S)(-)-α-methylbenzylamine [10]. In this case, the enantiomeric enrichment was greater than 95%, as measured by HPLC, but, at best, only 25% of the precursor could be used in the following steps of the synthesis.



(a) nBuLi/THF/(3R)-3-phenylbutanoyl chloride,
(b) $(n\text{-}Bu)_2$ BOTf/H_2CO/DIEA,
(c) $ClSi(CH_3)_2C(CH_3)_3$/Imidazole ;
(d) H_2O_2/LiOH
(e) DCC/HOBt/(L)AlaOtBu,
(f) $(nBu)_4N^+F^-$,
(g) PPh_3/PhCOSH/DIAD,
(h) TFA/CH_2Cl_2.

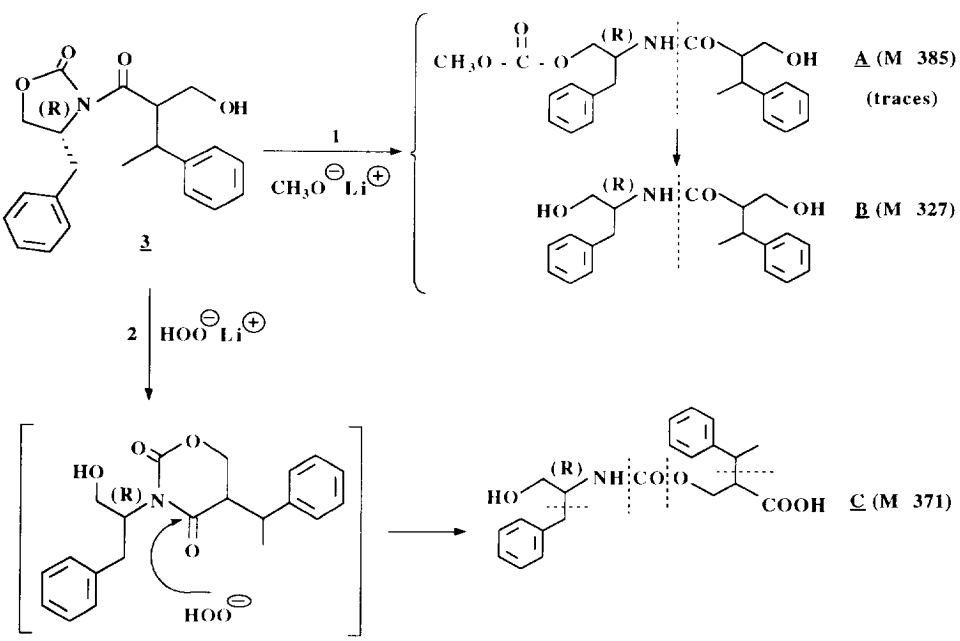
Scheme 1
Diastereoselective synthesis of N[(2S,3R)-2-benzoylthiomethyl)3-phenylbutanoyl]-L-alanine (Mixanpril)

The second procedure was derived from the enantiomeric synthesis of thiorphan, proposed by Evans [12] in which the introduction of a thioether group, as a precursor of the thiol function precedes the cleavage of the oxazolidinone. In the case of mixanpril, the cleavage of the chiral auxiliary required lithium hydroperoxide as a nucleophilic reagent, due to the steric hindrance of the methyl group in the ß position as regards to the exocyclic

carbonyl [13]. This induced major oxidation of the intermediary thioether with formation of sulfoxide and sulfone [10], leading to a relatively low yield (25-30%) for this step of the synthesis.

In this paper, we report a new synthetic approach, which prevents the problem of oxidation of the thioether group and improves the removal of the chiral auxiliary. This is summarized in Scheme 1.

The chiral synthon (4S)-4-phenylmethyl-2-oxazolidinone 1, was acylated by (3R)-3-phenylbutanoyl chloride [14], then hydroxymethylated by formaldehyde in the presence of di-n-butyl boron triflate [15] to yield compound 3. Before cleavage of the chiral auxiliary, the hydroxyl function of 3 was protected by a dimethyl-t-butylsilyl group (compound 4). Indeed in absence of the hydroxyl protecting group, undesirable reactions occured as shown in scheme 2. Thus, the treatment of 3 by lithium methylate gave essentially (98% yield) the product B corresponding to the endocyclic cleavage with traces of its precursor A, whose structures were characterized by mass spectrometry [16] (pathway 1, Scheme 2). Furthermore, with lithium hydroperoxyde as cleaving reagent, another product C, was isolated. Given the structure obtained for this compound [17], a rearrangement of the cyclic intermediate occurred, probably before cleavage of the amide carbonyl bond (pathway 2, Scheme 2).



Scheme 2

Side reactions observed during the cleavage of the chiral auxiliary of the hydroxymethyl derivative 3

(The dotted lines correspond to the fragmentation observed in mass spectra which allowed the structure of these compounds to be determined).

In contrast, the cleavage of the silyl ether **4** (scheme 1) by lithium hydroperoxide gave essentially the intermediate **5**. The protected ß-hydroxyacid **5** was condensed by the classical DCC/HOBt method with *t*-butylalaninate and then deprotected to give alcohol **7** in 95% yield. The introduction of the benzoylthiol group by a *Mitsunobu* reaction [18] followed by the cleavage of the *t*-butyl ester afforded mixanpril in 90% yield. The total yield was around 40% for the eight step synthesis, and analyses performed by HPLC and ^{1}H NMR showed an optical purity greater than 99%. This method allowed large quantities of mixanpril to be synthesized, enabling preclinical studies of this inhibitor to be achieved.

Thus, the *in vivo* inhibitory activities of mixanpril were tested in anaesthesized rats after oral administration [19]. The inhibition of urinary NEP, which is a good index of the inhibition of this enzyme in kidney, was almost complete at the lowest dose used (2.5 mg/kg) and continued during, at least, 3 hours after administration (not shown).

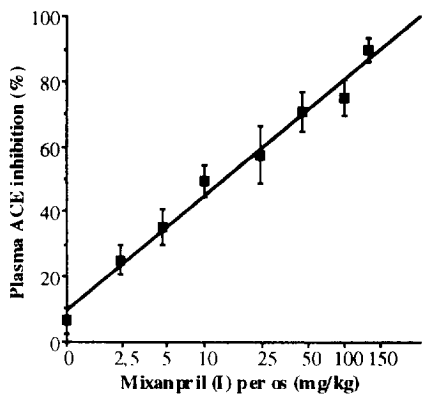


Figure 1.
Inhibition of Plasmatic angiotensin converting enzyme (ACE)
180 mn after oral administration of increased doses of mixanpril in rats.

For inhibition of ACE in plasma, a dose-effect curve was obtained (Fig. 1) with an ED_{50} of 12 mg/kg. At each dose, the maximal effect was obtained 30 min after oral administration and was maintained at least for 3 hours. These results confirm both the dual inhibitory action of mixanpril and its excellent bioavailability.

Acknowledgments :

The authors gratefully acknowledge C. Dupuis for the drafting of the manuscript and Dr. A. Beaumont for stylistic revision of the paper.

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16. Compound A was identified by the occurrence of peaks on the mass spectrum at m/z = 386 $(M+1)^+$ and 209. Compound **B** was characterized by the following peaks at m/z = 328 $(M+1)^+$, 178, 195, 152.

17. Compound **C** was characterized by the following fragmentations : 372 $(M+1)^+$, 195, 212, 178, 152, 105, 91.

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19. Wistar rats were anaesthesized with inactine at 30 mg/kg. Catheters were implanted in jugular vein, for blood sampling, in vesicle for urine collection and in oesophagus for mixanpril administration.

(*Received in Belgium* 14 *April* 1995; *accepted* 28 *June* 1995)