Pergamon

Tetrahedron Letters 43 (2002) 7585–7587

TETRAHEDRON LETTERS

# Synthesis of optically pure (*S*)-2-acetylthio-3-benzenepropanoic acid via enzymatic resolution

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Received 22 July 2002; revised 20 August 2002; accepted 21 August 2002

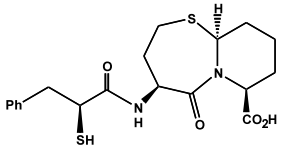
**Abstract**—A method of synthesizing optically pure (*S*)-2-acetylthio-3-benzenepropanoic acid has been developed and good to excellent enantiomeric excess achieved via enzymatic resolution. © 2002 Published by Elsevier Science Ltd.

Over the last several years, a number of compounds possessing both angiotensin converting enzyme (ACE) inhibitory activity and neutral endopeptidase (NEP) inhibitory activity, also known as vasopeptidase inhibitors, have been of interest as cardiovascular agents particularly in the treatment of hypertension, congestive heart failure, and renal disease.

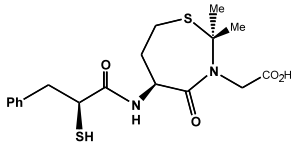
Omapatrilat and gemopatrilat are vasopeptidase inhibitors that are currently undergoing clinical evaluation.[1] A common intermediate for the synthesis of these compounds is (*S*)-2-acetylthio-3-benzenepropanoic acid. In the literature, (*S*)-2-acetylthio-3-benzenepropanoic acid is prepared from the unnatural D-phenylalanine through diazotization/bromination and thio-substitution reactions.[1b] However, this approach is expensive and undesirable for large-scale production. Recently, a method of preparing the compound using enzymatic resolution in aqueous buffer solution has been reported;[2] however, the enantiomeric excesses obtained were moderate.

Resolution of a racemic ester or acid using enzymes has been widely reported in the literature.[3] Generally, a high enantioselectivity can be obtained by selecting an appropriate enzyme, varying substituents in the substrate and/or changing reaction conditions. Here, we report our recent results on the synthesis of (*S*)-2-acetylthio-3-benzenepropanoic acid directly via enzymatic resolution. The synthetic route is depicted in Scheme 1.

Ethyl 2-benzylacetoacetate can be prepared from alkylation of ethyl acetoacetate with a benzyl halide. However, the reaction generally requires the use of a catalyst.[4] These methods have one or more limitations as regards to the use of toxic materials, as well as tedious and long reaction procedures and/or relatively low yields of products. For example, without a phase transfer catalyst, the alkylation reaction using less active benzyl chloride gave only ~25% yield after 8 h refluxing in benzene.[4b] Practically, it is of great advantage to use less expensive benzyl chloride as a starting material for large-scale manufacturing. We found that in the presence of an excess of ethyl acetoacetate, the alkylation reaction using benzyl chloride in the presence of a slight excess of sodium ethoxide solution without solvent gave high yield with exclusive mono *C*-alkylation selectivity at 80°C. The excess starting material and product were easily isolated by fractional distillation




Omapatrilat




Gemopatrilat

*Keywords*: enzymatic resolution; enzyme; 2-acetylthio-3-benzenepropanoic acid.
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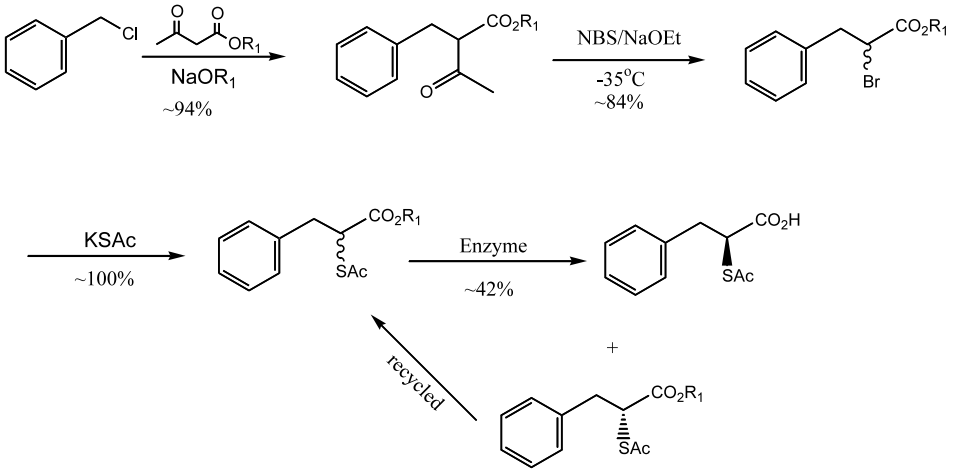
0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)01820-8




**Scheme 1.**

under reduced pressure. The recovered starting material was reusable.

The bromination–deacetylation reaction of ethyl 2-benzylacetoacetate to give the corresponding 2-bromo-3-benzenepropanoate was reported in literature.[5] We found that NBS (*N*-bromosuccinimide) can be replaced by cheaper DBDMH (N,*N*-dibromo-5,5-dimethylhydantoin) under similar reaction conditions. A comparable yield can be achieved. The product was easily purified and isolated by vacuum distillation.

Although there was no report for the preparation of ethyl 2-acetylthio-3-benzenepropanoate from the bromo ester, we found that it readily undergoes the nucleophilic substitution with potassium thioacetate at room temperature to give ethyl 2-acetylthio-3-benzenepropanoate. After stirring in ethyl acetate for 4–6 h, the reaction gave an almost quantitative yield.

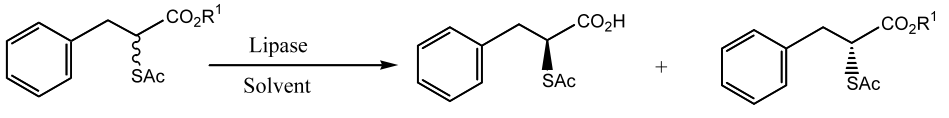
The synthesis of optically active 2-acetylthio-3-benzenepropanoic acid was achieved via enzymatic resolution using a hydrolytic enzyme on the racemic esters of 2-acetylthio-3-benzenepropanoates. Out of more than 80 enzymes screened (shaken at room temperature for 18 h in 0.2 M borate buffer, pH 7.0), nine enzymes were found to be active and preferentially to hydrolyze the *S* enantiomer. Among them, Mucor genus derived lipase (Lipozyme IM, Novo Nordisk Ltd.) was found to be the most active and selective. Therefore, further optimization was carried out using this commercially available enzyme.

Besides enzyme selection, the reaction media is another key factor for the high selectivity of enzymatic resolution. We found that the enzymatic resolution carried out in a mixture of an organic solvent and buffer (or water) gave better selectivity than in buffer alone (entries 1–3). The organic solvent can be acetonitrile, ketones, or alcohols. Other solvents did not work well. In some cases, a small amount of transesterification occurred when an alcohol was used. Results on the enzymatic resolution using Lipozyme IM are summarized in Table 1.

It is apparent that variation in $R^1$ (entries 3, 6–8) effected the enantioselectivity significantly. The highest enanatioselectivity (the greatest *E* value among entries 3, 6–8) was observed with ethyl ester, and the lowest with trifluoroethyl ester. Trifluoroethyl ester may be too labile to autohydrolyse and is not a desirable substrate for enzymatic resolution. It can also be seen that the higher the concentration, the slower the reaction (compare entry 2 to 4 and 3 to 5). The highest ee's of 94–97% ($E=57$–$102$) can be obtained in 90% acetonitrile or acetone or 2-propanol and 10% pH 4.0 phosphate buffer with 25–40% conversion (entries 9–11).

After removal of the enzyme by filtration, the unreacted ester (*R* isomer) was easily isolated by phase separation at pH 7 and extraction with methyl *t*-butyl ether (MTBE). The pH of the aqueous phase was then lowered to 1–2 and the product was extracted with MTBE. The crude product obtained after evaporation of solvent was purified by crystallization from heptane and MTBE. Greater than 99% ee was obtained in ~65% yield from crude product that has an ee of ~88% from the enzyme resolution.

In summary, we have developed a method for preparing optically pure (*S*)-2-acetylthio-3-benzenepropanoic acid via enzymatic resolution with good to excellent enantiomeric excess. This approach offers a potentially economical and environmentally-friendly process to this useful compound.




(S) (R)

**Table 1.** Enzymatic resolution[6]

| Entry | $R^1$ | Substrate concentration (mg/mL) | Solvent | Time (h) | Conversion (%) | Product (*S*) optical purity (% ee)* | *E*** |
|---|---|---|---|---|---|---|---|
| 1 | Et | 20 | pH 7.0 buffer | 6 | 40 | 74 | 10.8 |
| 2 | Et | 10 | pH 4.0 buffer | 4.5 | 34 | 85 | 19.1 |
| 3 | Et | 10 | 90% *t*-butyl alcohol, 10% pH 4.0 buffer | 7 | 34 | 92 | 35.1 |
| 4 | Et | 100 | pH 4.0 buffer | 10 | 10 | 85 | 13.7 |
| 5 | Et | 100 | 90% *t*-butyl alcohol, 10% pH 4.0 buffer | 18 | 38 | 88 | 26.6 |
| 6 | *n*-Bu | 10 | 90% *t*-butyl alcohol, 10% pH 4.0 buffer | 10 | 52 | 70 | 12.7 |
| 7 | *i*-Bu | 10 | 90% *t*-butyl alcohol, 10% pH 4.0 buffer | 16 | 35 | 86 | 21.1 |
| 8 | $CH_2CF_3$ | 10 | 90% *t*-butyl alcohol, 10% pH 4.0 buffer | 1 | 90 | 20 | – |
| 9 | Et | 10 | 90% acetonitrile, 10% pH 4.0 buffer | 40 | 42 | 96 | 101.9 |
| 10 | Et | 10 | 90% acetone, 10% pH 4.0 buffer | 40 | 25 | 97 | 84.9 |
| 11 | Et | 10 | 90% 2-propanol, 10% pH 4.0 buffer | 40 | 37 | 94 | 57.5 |

* The ee% was determined by area percentage on a chiral HPLC [column: Chiralcel AD from Daicel Chemical Industries; mobile phase: hexane: ethanol: trifluoroacetic acid (98:2:0.1); flow rate: 1 mL/min; Detector: UV; $\lambda$: 230 nm.

** Enantioselectivity constant.

## References


1. (a) Robl, et al. Bristol-Myers Squibb Patent, Patent No. US-5508272, April 16, 1996; (b) Karanewsky, et al. Bristol-Myers Squibb Patent, Patent No. US-5552397, September 3, 1996.
2. Yamagishi, et al. Mitsubishi Chemical K.K. Patent Application, Patent Application No. JP-2000-23693, January 25, 2000.
3. Wu, S.-H.; Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 1990 and references cited therein.
4. (a) Brandstrom, A.; Junggren, U. *Acta Chem. Scand.* **1969**, *23*, 2204; (b) Durst, H. D.; Liebeskind, L. *J. Org. Chem.* **1974**, *39*, 3271; (c) Ranu, B. C.; Bhar, S. *J. Chem. Soc., Perkin Trans. 1* **1992**, 365 and references cited therein.
5. Mignani, G.; Morel, D.; Grass, F. *Tetrahedron Lett.* **1987**, *28*, 5505.
6. A typical procedure for enzymatic resolution: To a 100 mL three-neck round-bottom glass vessel were charged 1.0 mL 0.01 M sodium acetate or sodium phosphate buffer, pH 4.0–7.0 (5%), 19.0 mL solvent (95%), 0.254g, (1.0 mmol) racemic thioacetate ethyl esters and 0.20 g Lipozyme lipase (1%, w/v). The reaction mixture was magnetically stirred at room temperature for a period of time (as specified in Table 1). The pH was maintained at specified values with 1N NaOH using an autotitrator. After the reaction was stopped, the enzyme was filtered. The enzyme cake was washed with water (2×20 mL). The filtrate was analyzed on HPLC against authentic samples.