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First synthesis of the enantiomerically pure α-hydroxy analogue of S-*tert*-butyl cysteine

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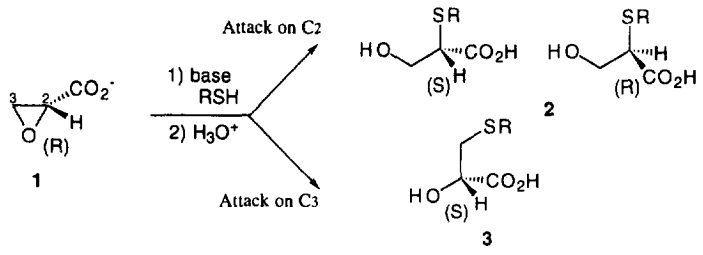
Abstract: The first synthesis of enantiomerically pure 2-hydroxy 3-*tert*-butylthio propionic acid in a one-pot reaction from chiral glycidate has been achieved with total conservation of stereochemistry. © 1997, Elsevier Science Ltd. All rights reserved.

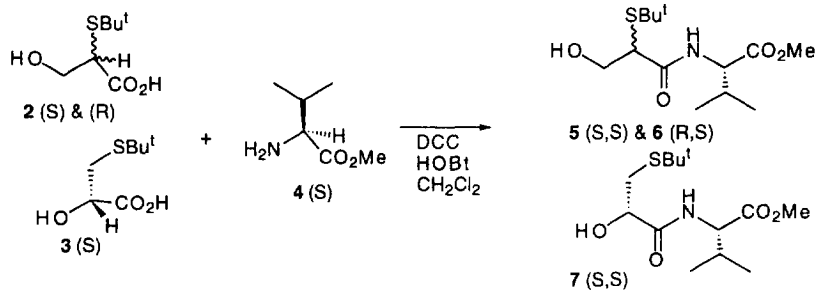
Chiral hydroxy acids are among the most important moieties in asymmetric synthesis. They are of considerable use as chiral auxiliaries or as constituents of biologically active molecules. In particular, the synthesis of depsipeptidic analogues of peptides requires the availability of the α-hydroxy acids corresponding to the natural amino acids. With many amino acids, nitrous acid deamination results in replacement of the amino group by an hydroxy group with efficient retention of configuration.[1] However, this reaction starting from cysteine does not lead to 2-hydroxy 3-mercapto propionic acid exclusively, but to a mixture containing carboxythiirane[2] and unsaturated compounds, resulting from β-elimination. Our attempts to reproduce the first described nitrous acid deamination of cystine,[3] to avoid unwanted reactions occurring with cysteine, revealed the presence of many sulphur containing compounds, presumably due to oxidative side-reactions. The thiirane formation has also been observed when starting from cystine, which first undergoes S–S bond cleavage.[4] Apart from nitrous acid deamination, several syntheses have been proposed in the literature but all give racemic products. Procedures leading to unprotected 2-hydroxy 3-mercapto propionic acid as a racemate are described starting from the very expensive 3-mercapto pyruvic acid sodium salt[5] or from a glycidate salt[6]. The racemic S-benzyl derivative was formerly prepared[7] by nitrous acid deamination of S-benzyl cysteine. Since nitrous acid deamination is not suitable for cysteine substrates, a different synthesis was described[8] years after, using a condensation of β-chlorolactic acid with toluene α-thiol and subsequent resolution by fractional crystallization with brucine or quinine. Teodori *et al.*[9] used the same pathway, but reported difficulties in repeating these experiments. Thus, the literature does not offer any asymmetric synthesis leading to enantiomeric forms of both unprotected and protected compounds.

We then decided to explore the patented racemic synthesis[6] via an epoxide, but starting from homochiral material. According to the well-documented chemistry of epoxide ring-opening, it was our expectation to be able to synthesise stereochemically pure hydroxy acid in a stereo- and regioselective manner in a one-pot sequence from homochiral potassium (R)-glycidate **1**,[10] readily available from (L)-serine,[11] by successive treatment with base and thiol. The reaction can lead to two regioisomers by competitive attack on C-2 and C-3, as depicted in Scheme 1. In the case of attack on C-2, the substitution generally undergoes a mechanism with inversion of configuration, the main compound being **2** (S) compared to **2** (R).

Under basic conditions, the less substituted C-3 is normally preferred, but a study regarding reactions of racemic α,β-epoxy carbonyl compounds clearly demonstrated that the carboxylic group activates the α-carbon toward nucleophilic substitution.[12] In this published work, electronic effects balance steric factors and methanethiolate attacks both α and β carbons, significantly leading to the two expected compounds, comparable to **2** and **3** in racemic forms, in the proportion of 36:64 respectively.



Scheme 1.



Scheme 2.

In order to obtain a useful protected derivative of (S)-2-hydroxy 3-mercapto propionic acid, corresponding to the hydroxy analogue of (S)-cysteine S-protected with the *tert*-butyl group, which is extensively used in peptide synthesis, we selected *tert*-butyl mercaptan as the nucleophilic reagent. Moreover, we felt that the steric bulk of the *tert*-butyl group might improve the regioselectivity exhibited for ring opening at C-3, thus minimising the amount of unwanted compounds **2**. Using KOH as base,[13] the reaction afforded the two expected regioisomers **2** and **3** in 80% yield with a ratio of 15:85 respectively, determined by HPLC and ^{1}H NMR spectroscopy. Although these compounds are structurally similar, they can be easily discriminated by ^{1}H NMR spectroscopy with the chemical shift of the methylene group, at higher field in the case of the expected compound **3**: the methylene group bearing the thiol function is shielded compare to the one bearing the hydroxyl group.[14] The regioisomers are separated by preparative HPLC,[15] allowing characterisation of 2-hydroxy 3-*tert*-butylthio propionic acid **3** (R=tBu).[14,16]

One very important aspect of epoxide ring-opening reactions is that they are usually stereospecific. The enantiomeric purity was found to be >99% by HPLC after derivatization of the compound with valine methyl ester according to our previously described method.[17] A first analysis on the crude coupling mixture of **3** with (D,L)Val-OMe allowed us to optimise the conditions to display clearly the two separate diastereomers. The coupling mixture of the obtained compound with (L)Val-OMe was then analyzed under these optimum HPLC conditions to establish the racemization rate. When the same analysis was carried out starting from the mixture of hydroxy acids **2** and **3** (Scheme 2), we undoubtedly proved a total inversion of configuration in the case of C-2 attack, since one single diastereomer **5** (S,S) could be detected, attesting that **2** (R) was not present, and ascertained the enantiomeric purity of **3** in the case of attack at C-3.

The two compounds **5** and **7** can now be separated by chromatography on silica gel, thus avoiding preparative HPLC to isolate **3** in a pure form. Indeed, the mixture of **2** and **3** can be involved in the coupling with the suitable amino acid as the first step of the synthesis leading to the targeted

depsipeptide. At this stage, the purification becomes much easier since a classical column is sufficient, rendering the more expensive preparative HPLC technique unnecessary.

In summary, we have developed an efficient one-pot reaction to prepare (S)-2-hydroxy 3-*tert*-butylthio propionic acid, corresponding to the hydroxy analogue of (D)-cysteine in a protected version useful in peptide synthesis, from homochiral glycidate with complete conservation of configuration. The targeted compound **3** was fully characterised in enantiomerically pure form for the first time. Starting from the commercially available (D)-serine would give rise to the enantiomeric glycidate, subsequently affording the hydroxy analogue of (L)-cysteine. More investigations to improve the regioselectivity are in progress in our laboratory.

Acknowledgements

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References

1. Brewster, P.; Hiron, F.; Hugues, E. D.; Ingold, C. K. and Rao, P. A. D. S. *Nature* **1950**, *166*, 179.
2. Maycock, C. D. and Stoodley, R. J. *J. Chem. Soc. Perkin I* **1979**, 1852.
3. Neuberg, C. and Ascher, E. *Biochem. Z.* **1907**, *5*, 451.
4. Gouesnard, J.-P. *Bull. Soc. Chim. France* **1989**, *1*, 88.
5. Costa, M.; Pensa, B.; Ivarone, C. and Cavallini, D. *Preparative Biochemistry* **1983**, *12*, 417.
6. Belg. P. 643752, **1964**.
7. Stekol, J. A. *J. Biol. Chem.* **1941**, *40*, 827.
8. Hope, D. B. and Wälti, M. *J. Chem. Soc. C* **1970**, 2475.
9. Teodori, E.; Guallieri, F.; Angeli, P.; Brasili, L.; Gianella, M. and Pigine, M. *J. Med. Chem.* **1986**, *29*, 1610.
10. NMR 250 MHz (D_2O) δ (ppm): 2.79 (dd, 1H, *H*CH, J_1 5.8Hz J_2 2.9Hz); 2.96 (dd, 1H, HC*H*, J_1 5.8Hz J_3 4.8Hz); 3.47 (dd, 1H, CH, J_2 2.9Hz J_3 4.8Hz). m.p. 150–152°C; α_D+33 (c=5, H_2O). α_D(lit)+32 (c=5, H_2O) Larchevêque M. and Petit Y. *Org. Syn.* in press.
11. Larchevêque M. and Petit Y. *Bull Soc. Chim. France* **1989**, *1*, 130.
12. Bihovsky R. *J. Org. Chem.* **1992**, *57*, 1029.
13. Typical procedure: *tert*-butyl mercaptan (25 ml, 235 mmol, 15 eq.) and 2M KOH (118ml) were added to the aqueous solution of potassium glycidate (2g, 15.7 mmol) and the reaction mixture was refluxed under stirring for 5 hours. The excess of thiol was removed by washing (3 times) the reaction mixture with ethyl acetate. The aqueous phase was acidified to pH 2 then the product was extracted into ethyl acetate (3 times). The combined organic phases were dried over Na_2SO_4 and concentrated *in vacuo* to afford an oil, which solidified upon standing overnight in the cold. Yield 80%.
14. NMR 250 MHz ($CDCl_3$) δ (ppm): compound **2**: 1.40 (s, 9H, $(CH_3)_3C$); 3.48 (dd, 1H, CHα, J_1 6.0Hz J_2 8.5Hz); 3.79 (dd, 1H, *H*CH, J_1 6.0Hz J_3 11.5Hz); 3.90 (dd, 1H, HC*H*, J_2 8.5Hz J_3 11.5Hz); 6.0 (br s, 1H, OH). compound **3**: 1.35 (s, 9H, $(CH_3)_3C$); 2.91 (dd, 1H, *H*CH, J_1 13.2Hz J_2 6.5Hz); 3.09 (dd, 1H, HC*H*, J_1 13.2Hz J_3 4.4Hz); 4.45 (dd, 1H, CHα, J_2 6.5Hz J_3 4.4Hz); 6.0 (br s, 1H, OH).
15. HPLC: Column Nucleosil C18 5μ (250×10 mm) Flow: 5ml/min. RT. Conditions: 20% ACN/80% H_2O/0.1% TFA. Retention times: **2**=6.00 mn; **3**=11.43 mn.
16. M.p. 67–68°C; $[\alpha]_D$+5.1 (c=1, H_2O); mass spectra (EI) m/z: $[M+2]^{+\cdot}$ 180 (15); $[M]^{+\cdot}$ 178 (20); $[(CH_3)_3C–S–CH_2]^+$ 103 (45); $[(CH_3)_3C]^+$ 57 (100).
17. Cavelier, F.; Gomez, S.; Jacquier, R.; Llinarès, M.; Mercadier, J.-L.; Pétrus, C. and Verducci, J. *Tetrahedron Asymm.* **1993**, *12*, 2495.

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