

# Asymmetric Synthesis of L-[ $\beta$ - $^{11}\text{C}$ ]Alanine Using A Glycine Derivative With Two Chiral Handles, (1*R*, 2*S*, 3*R*)-8-Phenylmenthan-3-yl *N*-[(1*R*, 2*R*, 5*R*)-2-Hydroxypinan-3-ylidene]glycinate

Karl Johan Fasth, Gunnar Antoni and Bengt Långström\*

Department of Organic Chemistry, Institute of Chemistry, Uppsala University, Box 531, S-751 21, Uppsala, Sweden

Fasth, K. J., Antoni, G. and Långström, B., 1990. Asymmetric Synthesis of L-[ $\beta$ - $^{11}\text{C}$ ]Alanine Using A Glycine Derivative With Two Chiral Handles, (1*R*, 2*S*, 3*R*)-8-Phenylmenthan-3-yl *N*-[(1*R*, 2*R*, 5*R*)-2-Hydroxypinan-3-ylidene]glycinate. - Acta Chem. Scand. 44: 527-530.

[ $\beta$ - $^{11}\text{C}$ ]-Labelled amino acids have been synthesized by various methods in either racemic, enantiomerically enriched or pure form.<sup>1-10</sup> Rapid resolution of enantiomers from a racemic mixture can be used but a disadvantage is that 50 % of the radioactivity is lost in the resolution procedure. Routes for asymmetric synthesis of [ $\beta$ - $^{11}\text{C}$ ]-labelled amino acids have thus been of interest. We have previously used [ $^{11}\text{C}$ ]-labelled alkyl iodides and Schiff bases of glycine esters for the asymmetric synthesis of [ $\beta$ - $^{11}\text{C}$ ]-labelled amino acids<sup>6,8</sup> with the chiral handles in the substrate being either in the imine (1) or the ester (2) part of the molecule (Fig. 1). When [(1*R*, 2*R*, 5*R*)-2-hydroxypinan-3-ylidene]glycine *tert*-butyl ester (1) was used as a substrate and the  $^{11}\text{C}$ -methylation was carried out under anhydrous conditions, the [ $\beta$ - $^{11}\text{C}$ ]alanine was obtained with an enantiomeric excess of 89%.<sup>6</sup>  $^{11}\text{C}$ -Methylation of (1*R*, 2*S*, 3*R*)-8-phenylmenthan-3-yl *N*-(diphenylmethylene)glycinate (2) could be achieved using mild phase-transfer conditions, but the [ $\beta$ - $^{11}\text{C}$ ]alanine was obtained with a lower enantiomeric excess (52 % e.e.).<sup>8</sup>

A glycine derivative containing both the above mentioned chiral handles, (1*R*, 2*S*, 3*R*)-8-phenylmenthan-3-yl *N*-[(1*R*, 2*R*, 5*R*)-2-hydroxypinan-3-ylidene]glycinate (3)

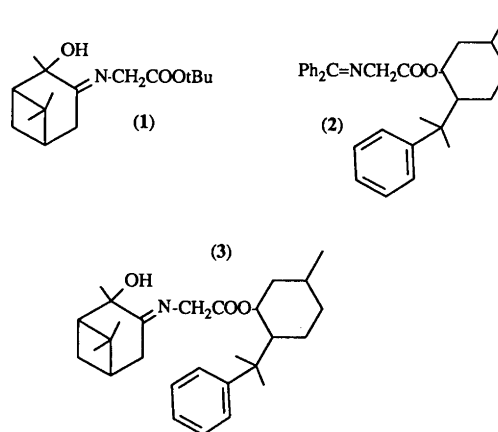
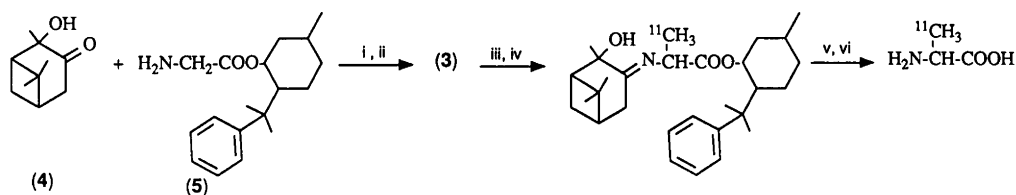


Fig. 1.

(Fig. 1) has now been synthesized and used together with  $^{11}\text{C}$ -methyl iodide in the asymmetric synthesis of [ $\beta$ - $^{11}\text{C}$ ]alanine (Scheme 1).

## Results and discussion

(1*R*, 2*S*, 3*R*)-8-Phenylmenthan-3-yl glycinate (5) and (+)-hydroxypinan-3-one (4) were obtained according to



Scheme 1. Reagents: i,  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ; ii, xylene; iii, Base; iv,  $^{11}\text{CH}_3\text{I}$ ; v,  $\text{NH}_2\text{OH}$ ; vi,  $\text{NaOH}/\text{H}_2\text{O}$ .

\* To whom correspondence should be addressed.

procedures described earlier.<sup>8,11</sup> The (1*R*, 2*S*, 3*R*)-8-phenylmenthan-3-yl *N*-[(1*R*, 2*R*, 5*R*)-2-hydroxypinan-3-ylidene]glycinate (**3**) was obtained in approximately 35% yield. Purification of **3** was difficult because of the lability of the imine bond, but with the 'dry column chromatography procedure'<sup>16</sup> a purity of 95% was achieved with benzophenone as the main impurity.

The [<sup>13</sup>C]methyl iodide was synthesized in a one-pot system by the standard procedure in our laboratory.<sup>13</sup> [<sup>13</sup>C]Methyl iodide was obtained in 60–80% radiochemical yield within 5 min (decay corrected and counted from <sup>13</sup>CO<sub>2</sub>) and with a radiochemical purity higher than 98%. The alkylation reactions were carried out under a number of different conditions (see the Experimental). Using analytical straight-phase HPLC it was possible to separate the diastereomers of the alkylation products and thus determine the amount of asymmetric induction in the alkylation step. To prove the identity, the peaks representing the two diastereomers were collected and hydrolysed separately giving in both cases [<sup>13</sup>C]alanine. The results are summarised in Table 1. Under the reaction conditions used the substrate was partly hydrolysed during the alkylation reaction.

The removal of the protecting groups was performed in two steps. The amino protecting group was removed, within 3 min, using hydroxylamine. This hydrolysis was most efficient around pH 4–6, at higher or lower pH the deprotection reaction was considerably slower. The hydrolysis of the ester was performed with aqueous sodium hydroxide and was complete within 5 min at 130 °C. According to previous studies, the alkaline hydrolysis does not cause any racemisation.<sup>1</sup> Purification using a Sep-Pak C-18 column gave the [β-<sup>13</sup>C]alanine in about 40% (decay corrected) radiochemical yield and with a radiochemical purity higher than 98%. The enantiomeric excess was determined using *N*-(5-fluoro-2,4-dinitrophenyl)-L-alaninamide (Marfey's reagent)<sup>8,12</sup> and was found to range from 36% to 80% e.e. corresponding to the d.e. values obtained in the <sup>13</sup>C-methylation. The total synthesis times, counted from the release of [<sup>13</sup>C]carbon dioxide from the molecular sieves to the purified product was 25–30 min.

The results of the asymmetric synthesis, using this glycine derivative with two chiral handles, did not differ significantly from previously described procedures in which

the two chiral handles had been used separately.<sup>6,8,14</sup> The phase-transfer alkylation (methods 1 and 5) gave similar asymmetric induction with this substrate as compared with **2** (56% d.e.). The use of a chiral phase-transfer catalyst did not influence the degree of asymmetric induction (method 5). The use of other bases such as potassium hydroxide or sodium hydride (methods 2 and 3) resulted in a lower enantiomeric excess. Methylation under anhydrous conditions (method 4) gave slightly lower asymmetric induction than that of **1** (89% e.e.) using the same conditions.

Under the above mentioned conditions and with these two chiral handles there seems to be no additive effect on the degree of asymmetric induction. The 2-hydroxypinan-3-yl part of the molecule governs the asymmetric induction of the reaction only in method 4 where the dianion in which the imine nitrogen complexes to a lithium counterion as described earlier<sup>14</sup> can be formed. It seems that under the other conditions the 8-phenylmenthan-3-yl part of the molecule directs the asymmetric induction, since no asymmetric induction was observed when **1** was [<sup>13</sup>C]methylated using these conditions.

## Experimental

**General.** The <sup>13</sup>C was prepared by the <sup>14</sup>N(p,α)<sup>13</sup>C nuclear reaction using a nitrogen-gas target and 10 MeV protons produced by the tandem Van de Graaff accelerator at the university of Uppsala. The <sup>13</sup>C carbon dioxide formed was trapped in a quartz tube containing 4 Å molecular sieves kept in a lead-shielded oven and transported to the chemistry laboratory. Analytical LC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a UV diode-array detector or a Waters system (pump 501, Data and control system 840, UV absorbance detector 440) in series with a β-particle flow detector<sup>15</sup> and using one of the following columns: Supelco NH<sub>2</sub> 250×4.6 mm (i.d.) 5 μ column (A), Alltech C-18 250×4.6 mm (i.d.) 10 μ column (B) or Nucleosil 50 250×4.6 mm (i.d.) 5 μ column (C). Ammonium formate (0.05 M, pH 3.5) (D), methanol (E), potassium dihydrogen phosphate (0.01 M, pH 4.6) (F), acetonitrile/water (500/70, v/v) (G), hexane/2-propanol 100/0.2 (H) or THF/2-propanol 100/0.2 (I) were used as mobile phases. Analytical GLC was carried out on a Hewlett-Packard 5880 A gas chromatograph equipped with a 70 cm 3% PS-400/Chrom W HP 80/100 column (K). Dichloromethane used as the solvent in the alkylation reaction was purified by passage through an aluminium oxide column (basic grade I). Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone and stored in a glass vessel containing activated 4 Å molecular sieves. NMR was performed on a Varian XL-300 NMR spectrometer.

(1*R*, 2*S*, 3*R*)-8-Phenylmenthan-3-yl *N*-[(1*R*, 2*R*, 5*R*)-2-hydroxypinan-3-ylidene]glycinate (**3**). (1*R*, 2*S*, 3*R*)-8-Phenylmenthan-3-yl glycinate (**5**) (1.85 g, 6.4 mmol) and

Table 1. Asymmetric induction in the various alkylation procedures.

Alkylation method No.	Asymmetric Induction (% d.e.)
1	56
2	36
3A	36
3B	36
4	80
5	56

(+)-hydroxy-pinane-3-one (**4**) (1.08 g, 6.4 mmol) (obtained according to Refs. 8 and 15) were dissolved in 15 ml toluene and 50  $\mu$ l of boron trifluoride etherate were added. The reaction mixture was heated at reflux for a period of 12 h, while the water formed was removed by azeotropic distillation. The solvent was then removed by evaporation. Purification by dry column flash chromatography<sup>16</sup> resulted in approximately 1 g (35 %) of compound **3** with a purity of about 95 % as determined by GLC (column K, flow 30 ml min<sup>-1</sup>, linear temperature gradient (10 °C min<sup>-1</sup>) from 150 to 180 °C, N<sub>2</sub>, retention time 16 min). NMR spectroscopy confirmed the structure of the product  $\delta_c$  (75.5 MHz; solvent CDCl<sub>3</sub>; standard CDCl<sub>3</sub>) 21.8, 22.9, 24.2, 27.3, 28.2, 28.7 (Me), 26.5, 28.1, 33.9, 41.7, 42.9 (CH<sub>2</sub>), 31.2, 38.2, 50.2, 50.4 (CH), 38.5, 39.5 (C), 52.4 (CH<sub>2</sub>N), 74.5 (OCH), 76.4 (COH), 124.9, 125.2, 125.3, 127.8, 127.9 (ArCH), 151.7 (ArC), 169.5 (C=N), 179.2 (C=O).

**[<sup>11</sup>C]Methyl iodide.** [<sup>11</sup>C]Methyl iodide was prepared by reduction of <sup>11</sup>CO<sub>2</sub> with LAH and treatment with hydriodic acid in the one-pot system as previously described,<sup>13</sup> distilled off and transferred in the nitrogen gas stream to a reaction vessel containing compound **3** in the appropriate solvent.

#### Methods for <sup>11</sup>C-methylation of compound **3**

**Method 1.** Compound **3** (10–20 mg, 23–45  $\mu$ mol) was dissolved in dichloromethane (0.3–0.4 ml) in a septum equipped vial. A solution of tetrabutylammonium hydrogen sulfate (40–50 mg) in 0.7 ml of a 2.5 M aqueous sodium hydroxide solution was added to generate the anion of compound **3**. The aqueous solution was removed and [<sup>11</sup>C]methyl iodide was transferred to the reaction vessel as described above. The reaction was performed at room temperature for 5 min.

**Method 2.** Two equivalents (9 to 18  $\mu$ l, 45–90  $\mu$ mol) of a 5 M aqueous solution of potassium hydroxide were used as the base and was added to compound **3** (10–20 mg, 23–45  $\mu$ mol) dissolved in *N,N*-dimethylformamide (DMF) (0.4 ml). [<sup>11</sup>C]Methyl iodide was transferred to the reaction vessel as described above. The reaction was performed at room temperature for 5 min.

**Method 3.** Two equivalents (2.2–4.3 mg, 45–90  $\mu$ mol) of sodium hydride (50 % in mineral oil) were added to **3** (10–20 mg, 23–45  $\mu$ mol) in 0.3 ml of DMF (A) or dichloromethane (B). [<sup>11</sup>C]Methyl iodide was transferred to the reaction vessel as described above. The reaction was performed at room temperature for 5 min.

**Method 4.** The reaction was carried out under anhydrous conditions using lithiated 2,2,6,6-tetramethylpiperidine (TMP) as the base and a mixture of THF and 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidinone (DMPU) as the solvent. To 20 mg (45  $\mu$ mol) of **3** in 0.4 ml of THF/DMPU

(95/5, v/v), were added two equivalents (128  $\mu$ l, 90 mmol) of a mixture of 1.5 ml butyllithium (1.5 M, in hexane), 390  $\mu$ l TMP and 1.05 ml of THF at –72 °C. The [<sup>11</sup>C]methyl iodide was added and the reaction was carried out at –72 °C for 5 min.

**Method 5.** Compound **3** (10–20 mg, 23–45  $\mu$ mol) and (–)-*N*-Benzylquininium chloride (40–50 mg) were dissolved in dichloromethane (0.3–0.4 ml) in a septum equipped vial. 2.5 M Aqueous sodium hydroxide (0.7 ml) was added to generate the anion of compound **3**. The aqueous phase was removed and [<sup>11</sup>C]methyl iodide was transferred to the reaction vessel as described above. The reaction was performed at room temperature for 5 min.

The alkylation reactions were followed by LC on column B (solvents D/E, 10/90 v/v, flow 2 ml min<sup>-1</sup>, column temperature 40 °C, wavelength 254 nm). The retention times were 1.9 min for [<sup>11</sup>C]methyl iodide and 6.2 min for the alkylation products.

**Analysis of the diastereomeric excess.** A small amount of the reaction mixture, from the various <sup>11</sup>C-methylations, was diluted with mobile phase and analysed using column C (solvent H/I 85/15, v/v, flow 2 ml min<sup>-1</sup>, column temperature 25 °C, wavelength 254 nm, retention times 2.8 and 3.2 min for the diastereomeric alkylation products).

**[ $\beta$ -<sup>11</sup>C]Alanine.** To the alkylation products from the above described methods 1–5, was added a 0.5 M solution of hydroxylamine in 80 % ethanol (2 ml) and the solution was heated at 45 °C for 5 min. After addition of 5 M sodium hydroxide in 70 % ethanol (2 ml) the mixture was heated at 130 °C for 5 min. The alkylation and hydrolysis reactions were followed by LC using the conditions described above. The [ $\beta$ -<sup>11</sup>C]alanine was purified using a C-18 Sep-Pak column. The analysis of the radiochemical purity was performed by LC (column A, solvents F/G, 5/95 v/v, linear gradient to 40/60 during 8 min, flow 2 ml min<sup>-1</sup>, column temperature 40 °C, wavelength 254 nm, retention time 5.9 min).

**Analysis of the enantiomeric excess.** For the determination of the enantiomeric excess, the [ $\beta$ -<sup>11</sup>C]alanine was converted into diastereomeric derivatives by reaction with *N*-(5-fluoro-2,4-dinitrophenyl)-*L*-alaninamide described in detail elsewhere.<sup>8,12</sup> The derivatives were separated by LC (column B, solvents D/E, 70/30 v/v, linear gradient to 50/50 during 0–10 min, flow 2 ml min<sup>-1</sup>, column temp. 40 °C, wavelength 340 nm). The retention times were 6.0 and 9.0 min for the diastereomeric derivatives of *L*- and *D*-[ $\beta$ -<sup>11</sup>C]alanine, respectively.

**Acknowledgements.** We are indebted to Fil. lic. Petter Malmberg for assistance with the radionuclide production. This work was financially supported by the Swedish Natural Science Research Council, grant K-KU 3463.

## References

1. Långström, B., Stridsberg, B. and Bergson, G. *Chem. Scr.* 13 (1979) 49.
2. Kilbourn, M., Dischino, D. and Welch, M. *Int. J. Appl. Radiat. Isot.* 35 (1984) 603.
3. Halldin, C. and Långström, B. *Int. J. Appl. Radiat. Isot.* 35 (1984) 779.
4. Halldin, C. and Långström, B. *Int. J. Appl. Radiat. Isot.* 35 (1984) 945.
5. Antoni, G. and Långström, B. *J. Labelled Compd. Radiopharm.* 24 (1987) 125.
6. Antoni, G. and Långström, B. *Acta Chem. Scand. B* 41 (1987) 511.
7. Antoni, G. and Långström, B. *Int. J. Appl. Radiat. Isot.* 38 (1987) 655.
8. Fasth, K. J., Antoni, G. and Långström, B. *J. Chem. Soc., Perkin Trans. I* (1988) 3081.
9. Bjurling, P., Watanabe, Y., Tokushige, M., Oda, T. and Långström, B. *J. Chem. Soc., Perkin Trans. I* (1989) 1331.
10. Bjurling, P., Watanabe, Y., Oka, S., Nagasawa, T., Yamada, H. and Långström, B. *Acta Chem. Scand.* 44 (1990) 183.
11. Carlson, R. and Pierce, J. *J. Org. Chem.* 16 (1971) 2319.
12. Marfey, P. *Carlsberg Res. Commun.* 49 (1984) 591.
13. Långström, B., Antoni, G., Gullberg, P., Halldin, C., Malmberg, P., Någren, K., Rimland, A. and Svärd, H. *J. Nucl. Med.* 28 (1987) 1037.
14. Oguri, T., Kawai, N., Shioiri, T. and Yamada, S. *J. Pharm. Bull.* 26(3) (1978) 803.
15. Långström, B. and Lundqvist, H. *Radiochem. Radioanal. Lett.* 41 (1979) 375.
16. Harwood, L. *Aldrichim. Acta* 18 (1985) No. 1.

Received December 27, 1989.