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S-[1-(2,3-Diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol – Simplified Asymmetric Synthesis with CD and Chiral HPLC Analysis

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Abstract: *S*-[1-(2,3-Diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol is important as a precursor in the radiosynthesis of a ^{11}C-labelled radioligand (*S*-[*carbonyl*-^{11}C]CGP 12177) for the study of β-receptors in living man. The asymmetric synthesis of this precursor has been simplified based on the reaction of readily available 2-amino-3-nitrophenol and the chiral auxiliary, *S*-glycidyl-3-nitrobenzenesulphonate, followed by treatment of the resultant *S*-epoxide with *t*-butylamine and reduction with hydrogen in the presence of palladium on carbon. Chiral HPLC methods have been successfully developed to monitor the enantiomeric purity of the chiral auxiliary and of all intermediates in the asymmetric synthesis. All intermediates and products have been studied by CD. It has been demonstrated by chiral HPLC that *S*-CGP 12177 can be prepared in > 98.4% e.e. from the synthesised *S*-precursor. *R*-CGP 12177 (e.e. > 96.2%) was prepared analogously.

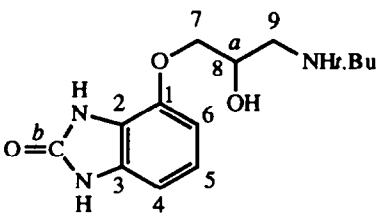
INTRODUCTION

R,S-CGP 12177 (I) is a potent hydrophilic antagonist at β-adrenergic receptors. It has low non-specific binding to membranes and low cellular uptake[1,2] and so only binds to cell-surface receptors rather than to internalised receptors.[1] The labelling of *R,S*-CGP 12177 (I) with the cyclotron-produced positron-emitting radionuclide, carbon-11 ($t_{1/2}$ = 20.4 min),[3] was soon found to provide a promising radioligand for studying cell-surface β-adrenergic receptors in human heart *in vivo* by the quantitative and atraumatic technique of positron

emission tomography (PET).[4,5] Application of such a radioligand now promises to provide new insights into the role of β-receptors in the progress of diseases in human heart and lung.[5]

Studies *in vitro* have shown that the *S*-enantiomer of CGP 12177 (*S*-I) has about eighty-fold greater affinity than the *R*-enantiomer (*R*-I) for β-adrenergic receptors.[6] Also, *S*-[^{3}H]CGP 12177, compared to the tritiated racemate, gives an approximately two-fold greater ratio of receptor-bound radioligand to non-receptor bound radioligand in heart and lung after intravenous injection into rats.[7] It is known that the relatively less potent enantiomers of stereoselective receptor ligands may exhibit differential metabolism,[8] protein-binding,[9] biodistribution,[8] toxicity,[10] pharmacokinetics[11] and pharmacological effect.[10,12] Such differences are particularly well documented for β-receptor ligands.[13-18] Many of these effects could exacerbate the difficulty of interpreting quantitative PET studies of β-receptors in man. On this basis, it would clearly be preferable to use the *S*-enantiomer of [^{11}C]CGP 12177 (*S*-[^{11}C]I), rather than its racemate, for such PET studies.

The only reported method for labelling *R,S*-CGP 12177 (I) with carbon-11 is based on the reaction of [^{11}C]phosgene with *R,S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol.[3] Here, we describe a simplified asymmetric synthesis (Scheme 1) of the *S*-enantiomer of this diamino precursor (*S*-VI) from a readily available starting material, 2-amino-3-nitrophenol (II), and a readily available chiral auxiliary, *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III), that permits *S*-[*carbonyl*-^{11}C]CGP 12177 (*S*-[^{11}C]I) to be obtained in greater than 98.4% enantiomeric excess. Compounds along the pathway of asymmetric synthesis have been studied by circular dichroism. We also describe convenient chiral HPLC methods for monitoring the progress of the asymmetric synthesis and for assuring the quality of this increasingly important precursor (*S*-VI) and of derived *S*-CGP 12177 (*S*-I).

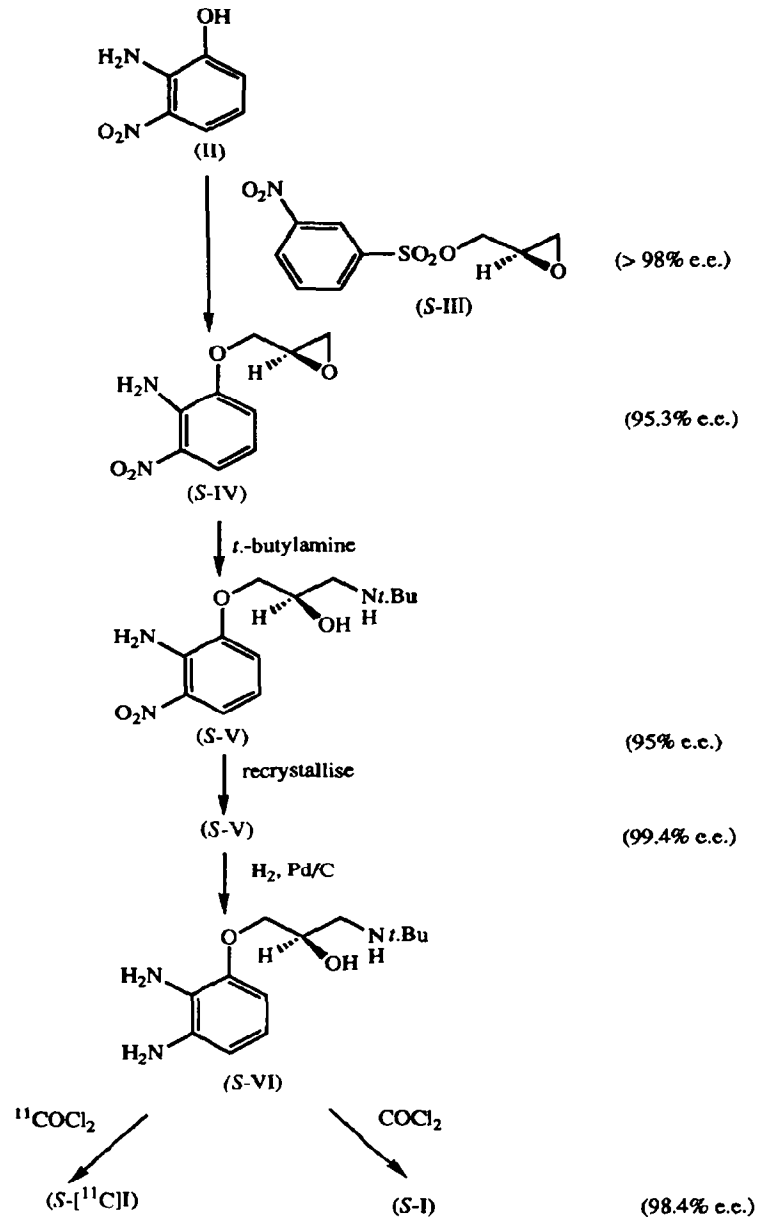


(I)	*a* = *R,S*	*b* = $^{12/13}$C
(*S*-I)	*a* = *S*	*b* = $^{12/13}$C
(*R*-I)	*a* = *R*	*b* = $^{12/13}$C
(*S*-[^{11}C]I)	*a* = *S*	*b* = ^{11}C

EXPERIMENTAL AND RESULTS

Materials

Authentic *R,S*-[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (m.p. = 102 °C; Lit. m.p.s = 95 - 97 °C,[19] 98 - 100 °C[20]) was prepared by reacting 2,3-dinitrophenol with allyl bromide followed by treatment of the resultant allyloxy ether with hydrogen peroxide and benzonitrile.[19] *R*-[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (*R*-VIII) was prepared from 2,3-dinitrophenol (VII) and *R*-glycidyl-3-nitrobenzenesulphonate (*R*-III) by analogy with the synthesis of *S*-III reported previously.[21] These compounds served to establish retention



Scheme 1: Simplified asymmetric synthesis of the *S*-diamino compound (*S*-VI) for the preparation of *S*-CGP 12177 (*S*-I) and the PET radioligand *S*-[*carbonyl*-^{11}C]CGP 121177 (*S*-[^{11}C]I).

times in chiral HPLC (*vide infra*). Authentic *S*-(3'-*t*-butylamino-2'-hydroxypropoxy)benzimidazol-2-one (*S*-CGP 12177; *S*-I) as the hydrochloride, $[\alpha]_D$ = - 13 ± 1°, and its *R*-isomer (*R*-I) also as the hydrochloride, $[\alpha]_D$ = + 14 ± 1°, were gifts from Ciba-Geigy AG and had been synthesised[6] from 2,3-diaminophenol and either *S*- or *R*-benzyl-2,3-epoxy-propyl ether,[22] respectively. Other chemicals and solvents were purchased as follows: 2-amino-3-nitrophenol (II), *S*-glycidyl-3-nitrobenzenesulphonate (99%) (*S*-III), its *R*-isomer (*R*-III), methyl ethyl ketone (MEK), sodium hydride, *N,N*-dimethylformamide (DMF), *t*-butylamine, 5% palladium on carbon and potassium carbonate (Aldrich Chemical Co. Ltd); chloroform, ethanol, ethyl acetate and propan-2-ol (IPA), all of HPLC grade (Fisons). Other reagents were of 'Analar' quality. Chiralcel™ OD columns [silica-based cellulose *tris*-(3,5-dimethyl-phenyl)carbamate, Daicel Chemical Industries Ltd, Tokyo; 25 cm X 4.6 mm i.d.] were purchased from Baker Ltd, U.K. Ultron™ ES-OVM columns (150 X 4.6 mm i.d.; Shinwa Chemical Industries) were purchased from Jones Chromatography Ltd, U.K.

Methods

Mass spectrometry was performed with a quadrupole mass spectrometer (Nermag R10/10C). Samples were introduced into the ionisation source of the spectrometer using the probe facility and vaporised by passing a current through the probe filament. The spectrometer was calibrated conventionally using FC-43 (perfluorotributylamine) and run in the electron impact (EI) mode or tuned for positive or negative ions in the chemical ionisation (CI +ve or -ve) mode, using ammonia as reactant gas. Spectral data were collected using a PDP 11/23 (Digital Computers) and analyzed using the Sidar software programme (Nermag).

^{1}H- and proton-decoupled ^{13}C-NMR spectroscopy were performed on a Brucker 250 (250 MHz) instrument at North London Polytechnic, London. Chemical shifts are relative to the internal standard, TMS (δ = 0 ppm). Peak splitting is described as s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Tentative assignments are given according to the numbering shown for the carbon skeleton of formula I.

UV absorption and CD (circular dichroism) spectra were measured with a Varian 2390 spectrophotometer and a Jasco J-600 spectropolarimeter respectively at 20 °C, using methanol as the solvent and cell pathlengths of 10.0, 0.5 and 0.2 mm as appropriate.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 20 °C using methanol as solvent and a decimetre cell at the National CD Service (SERC), Birkbeck College, University of London.

Chiral HPLC (high performance liquid chromatography) was performed on a Chiralcel™ OD column (25 X 4.6 mm i.d.) or an Ultron™ ES-OVM column (25 X 4.6 mm i.d.) using appropriate pre-columns and a detector for absorbance. Chromatographic conditions and separation parameters for analysed compounds (I, *R*-I, *S*-I, *R*-III - *R*-V, *S*-III - *S*-V, VIII, *S*-VIII and *R*-VIII) are given in Table 1. Percent enantiomeric excess was calculated as:

$$\text{e.e. (\%)} = 100(\text{Peak area}_{Isomer} - \text{Peak area}_{Antipode})/(\text{Peak area}_{Isomer} + \text{Peak area}_{Antipode}).$$

In analyses, with closely eluting peaks of congruent shape, peak height was used as an index of peak area. Otherwise peak areas were obtained either by instrumental or manual integration.

Table 1: Chiral HPLC Conditions and Separation Parameters.

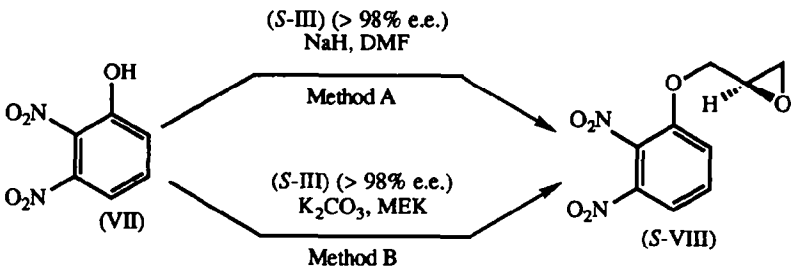
Enantiomeric pair	Column	Eluent	Flow rate (mL/min)	Ret. time[a] *S* (min)	Ret. time[a] *R* (min)	k *S*	k *R*	R_s	Limit of detection *R* in *S* (%)	Limit of detection *S* in *R* (%)
VIII	Chiralcel™ OD	Hexane/IPA (3:1 v/v)	1.0	17.2	19.0[b]	3.3	3.75	1.63	2	0.1
III	Chiralcel™ OD	Hexane/IPA (3:1 v/v)	1.0	19.9	19.2[b]	4.0	3.82	0.54	0.5	4
IV	Chiralcel™ OD	Hexane/IPA (4:1 v/v)	1.0	15.2	16.8	2.6	3.0	1.00	2	0.1
V	Chiralcel™ OD	Hexane/IPA (4:1 v/v)	1.0	12.1	8.1	2.55	1.4	2.2	0.1	0.1
I	Ultron™ ES-OVM	*aq.* KH_2PO_4 (10 mM; pH 4.6)	0.5	7.29	9.66	0.93	1.56	1.41	0.5	0.5

[a] Samples (10 - 20 µL) were injected as 0.1 - 0.6% w/v solutions in eluent and detected by absorbance at 212 or 254 nm.
[b] These close retention times required *R*-VIII to be free of *R*-III before measurement of % e.e. Absence of *R*-III was checked by M.S.

Asymmetric syntheses

The following methods describe the preparation of compounds with *S*-configuration. The corresponding *R*-enantiomers were prepared by the same methods from starting materials having the *R*-configuration.

Preparation of S-*[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (S-VIII).* Two methods were used based on the reaction of 2,3-dinitrophenol (VII) with *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III) (Scheme 2).



Scheme 2: Synthesis of *S*-[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (*S*-VIII) from 2,3-dinitrophenol (VII) and *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III).

Chiral HPLC on Chiralcel™ OD (Table 1) confirmed that the *S*-III used had > 98% e.e. (Table 2). The first (Method A) used sodium hydride in DMF as reaction medium as described previously[21] and the second (Method B) used potassium carbonate in MEK, as follows.

A solution of *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III) (e.e. > 98% by chiral HPLC; 1.0 g, 3.85 mmol) and 2,3-dinitrophenol (VII) (0.71 g, 3.85 mmol) in MEK (15 mL) was stirred with anhydrous potassium carbonate (0.58 g, 4.2 mmol) at 60 °C for 18 h. The solvent was removed under reduced pressure. The yellow residue was extracted with chloroform (150 mL), washed with water (3 x 15 mL) and then dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to give *S*-[1-(2-3-dinitrophenoxy)]-2',3'-epoxypropane (*S*-VIII) as a light yellow solid (0.86 g, 85 %). M.p. = 105 - 106 °C.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.76 (1H, dd, *J* = 5 and 3 Hz; C(9)H_2), 2.93 (1H, dd, *J* = 5 and 5 Hz; C-(9)H_2), 3.34 (1H, m; C(8)H), 4.13 (1H, dd, *J* = 12 and 6 Hz; C(7)H_2), 4.52 (1H, dd, *J* = 12 and 3 Hz; C(7)H_2), 7.52 (1H, dd, *J* = 8 and 2 Hz; C(6)H), 7.61 (1H, dd, *J* = 8 and 8 Hz; C(5)H), 7.81 (1H, dd, *J* = 8 and 1 Hz; C-(4)H).

Mass spectrometry in CI +ve mode: *m/z* = 258 $[M+NH_4]^+$, 275 $[M+NH_3+NH_4]^+$ and 292 $[M+2NH_3+NH_4]^+$; and in EI mode: *m/z* = 240 $[M]^+$ (12%), 184 (27%), 167 (4%), 164 (5%), 121 (4%), 107 (3%), 93 (13%) and 57 (100%). These mass spectra are in accord with those previously reported.[21]

Chiral HPLC analysis was performed on the products from Methods A and B, on analogously prepared antipode (*R*-VIII) and also on authentic *R,S*-1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (VIII), according to the conditions described in Table 1. Results are shown in Table 2. CD spectra were also recorded for *S*-VIII prepared by Method B, and also for its analogously prepared antipode (*R*-VIII). g-Numbers calculated from CD and also measured specific optical rotations are recorded in Table 2.

Preparation of S-[1-*(2-amino-3-nitrophenoxy)]-2',3'-epoxypropane (S-IV).* A solution of *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III) (3.0 g, 11.6 mmol; e.e. > 98% by chiral HPLC) and 2-amino-3-nitrophenol (II) (1.79 g, 11.6 mmol) in MEK (30 mL) was stirred with anhydrous potassium carbonate (1.75 g, 12.7 mmol) at 60 °C for 18 h. The solvent was removed under reduced pressure. The orange residue was extracted with chloroform (150 mL), washed with water (3 x 30 mL) and then dried over anhydrous magnesium sulphate. The solvent was removed under reduced pressure to give *S*-[1-(2-amino-3-nitrophenoxy)]-2',3'-epoxypropane (*S*-IV) as a dark orange solid (2.2 g, 90 %). M.p. 99 °C.

^{1}H-NMR ($CDCl_3$): δ (ppm) = 2.77 (1H, dd, *J* = 6 and 3 Hz; C(9)H_2), 2.96 (1H, dd, *J* = 5 and 5 Hz; C-(9)H_2), 3.41 (1H, m; C(8)H), 3.95 (1H, dd, *J* = 11 and 5 Hz; C(7)H_2), 4.37 (1H, dd, *J* = 11 and 3 Hz; C(7)H_2), 6.48 (2H, br; NH_2), 6.59 (1H, dd, *J* = 9 and 8 Hz; C(5)H), 6.93 (1H, dd, *J* = 9 and 1 Hz; C(4)H), 7.75 (1H, dd, *J* = 9 and 1 Hz; C(6)H). This closely agrees with the spectrum previously reported.[23,24]

^{13}C-NMR ($CDCl_3$): δ (ppm) = 44.5 C(9), 49.9 C(8), 70.4 C(7), 114.5 C(4), 115.5 C(6), 118.2 C(5), 131.9 C(2), 137.2 C(3), 147 C(1).

Mass spectrometry in CI +ve mode: *m/z* = 258 $[M+H]^+$, 275 $[M+NH_4]^+$ and 292 $[M+NH_3+NH_4]^+$.

Chiral HPLC analysis was performed on *S*-IV and analogously prepared antipode (*R*-IV) as described in Table 1. Figure 1 shows the separation achieved for a mixture of *S*-IV and *R*-IV. Results of chiral analysis are shown in Table 2. CD spectra were also recorded for *S*-IV and its antipode (*R*-IV) (Figure 2). g-Numbers calculated from CD and also measured specific optical rotation values are shown in Table 2.

Table 2: Specific Optical Rotations, Circular Dichroism g-Numbers and e.e. Measured by Chiral HPLC.

Compound	$[\alpha]_D$[a]	Lit. $[\alpha]_D$[a]	Ref. to Lit. $[\alpha]_D$	CD g-number[b] (x 10^5)	Lit. CD g-number (x 10^5)	Ref. to Lit. g-number	e.e.[c] (%)
S-III	n.d.			n.d			> 98[d]
R-III	n.d.			n.d			> 92[d]
S-IV	+ 17.1 ± 0.1			g_{234}^{235} = + 4.2			95.3
R-IV	- 17.2 ± 0.1			g_{234}^{235} = - 4.2			95
S-V (crude)	n.d.			n.d			95
R-V (crude)	n.d.			n.d.			95.5
S-V (recryst.)	+ 32 ± 4	+ 22.5[f]	23	g_{238}^{227} = + 4.3			> 99.4
R-V (recryst.)	- 32 ± 4			g_{238}^{227} = - 4.4			> 98.6
S-VI	n.d.[e]	+ 6.9[g]	24	g_{240}^{210} = + 10			n.d.
R-VI	n.d.[e]			g_{240}^{210} = - 9.7			n.d.
S-I (HCl)	- 8.0 ± 2	- 10.8[h]	24	g_{271}^{275} = + 1.95	g_{271}^{275} = + 2.06[i]	21	> 98.4
R-I (HCl)	+ 8.7 ± 2			g_{271}^{275} = - 2.04	g_{271}^{275} = - 2.06[i]	21	> 96.2
S-I (HCl)[j]	- 13 ± 1[k]			g_{271}^{275} = + 2.1			99.6
R-I (HCl)[j]	+ 14 ± 1[k]			g_{271}^{275} = - 2.1			90.3
S-VIII[l]	n.d.			n.d.			96
R-VIII[l]	n.d.			n.d.			94
S-VIII[m]	+ 8.1 ± 2			g_{260}^{210} = + 12.7			94
R-VIII[m]	- 8.1 ± 2			g_{260}^{210} = - 12.9			97

[a] Measured in methanol at 20 °C.

[b] None of the listed compounds gave a CD g-number at 350 nm.

[c] Determined by the chiral HPLC methods described in Table 1.

[d] Claimed to be > 98% e.e. by supplier.

[e] Equal and opposite specific optical rotation values were not obtained, possibly because of previously noted[24] instability.

[f] For preparation claimed to be enantiomerically pure.

[g] For preparation claimed to have 95% e.e.

[h] For preparation claimed to have 99% e.e.

[i] For compounds prepared by a different route[21] and calculated from the reported CD values of + 0.68 and - 0.68 mdeg/absorbance unit for the *S*- and *R*- enantiomer respectively.

[j] Reference compounds prepared[6] from *S*- and *R*-benzyl-2,3-epoxy-propyl ether.[22]

[k] Values were obtained on a different instrument from the other values in this column.

[l] Prepared by Method A (see Experimental and Scheme 2).

[m] Prepared by Method B (see Experimental and Scheme 2).

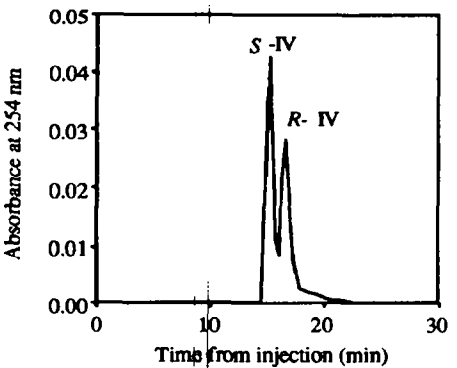


Figure 1. Chromatogram from the chiral HPLC of a mixture of *R*-IV and *S*-IV on Chiralcel™ OD (see Table 1 for elution conditions).

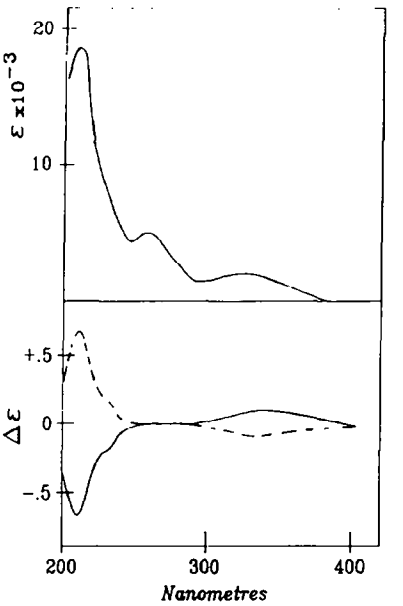


Figure 2. UV absorption spectrum of IV (upper panel) and CD spectra (lower panel) for *S*-IV (- - -) and *R*-IV (___).

*Preparation of S-[1-(2-amino-3-nitrophenoxy)]-3'-(*N-t-*butylamino)propan-2'-ol (S-V).* A solution of *S*-[1-(2-amino-3-nitrophenoxy)]-2',3'-epoxypropane (*S*-IV) (2.0 g, 9.53 mmol) and *t*-butylamine (6.67 g, 91.4 mmol) in ethanol (130 mL) was stirred for 20 h at ambient temperature. The solvent was removed under reduced pressure to leave an orange residue which was dissolved in hydrochloric acid (1M; 100 mL). This solution was washed with diethyl ether (3 x 30 mL). Then potassium hydroxide solution (1M; 110 mL) was added to give an orange precipitate. The mixture was extracted with chloroform (3 x 30 mL). The chloroform extract was dried with anhydrous magnesium sulphate, filtered and evaporated under reduced pressure to give an orange solid. Recrystallisation from chloroform-hexane gave *S*-[1-(2-amino-3-nitrophenoxy)]-3'-(*N-t*-butylamino)propan-2'-ol (*S*-V) (1.5 g, 55 %). M.p. = 70 °C. Lit. m.p.s = 60 - 61 °C (for e.e. = 63% by optical rotation),[24] 60 - 62 °C (for e.e. = 82% by optical rotation),[24] 62 - 64 °C (for e.e. = 62% by optical rotation),[23] 61 - 62 °C (for e.e. = 95% by optical rotation)[24] and 61 - 63 °C (for e.e. = 100% by optical rotation).[23]

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.13 (9H, s; $C(Me)_3$), 2.2-2.5 (1H, br; OH), 2.66 (1H, dd, J = 12 and 8 Hz; $C(9)H_2$) 2.87 (1H, dd, J = 12 and 4 Hz; $C(9)H_2$), 3.9-4.05 (3H, br; $C(7)H_2$ + C(8)H), 6.50 (2H, br; NH_2), 6.58 (1H, dd, J = 9 and 8 Hz; C(5)H), 6.93 (1H, dd, J = 8 and 1 Hz; C(4)H), 7.74 (1H, dd, J = 9 and 1 Hz; C(6)H). This is in close agreement with the spectrum recorded for *S*-V previously.[23,24]

^{13}C-NMR ($CDCl_3$): δ (ppm) = 29.1 $C(\underline{C}H_3)_3$, 44.5 C(9), 50.5 $\underline{C}(Me)_3$, 68.4 C(8), 72.2 C(7), 114.5 C(4), 115.7 C(6), 118.1 C(5), 131.8 C(2), 137.6 C(3), 147.4 C(1).

Mass spectrometry in CI +ve mode: m/z = 284 $[M+H]^+$.

Chiral HPLC analysis was performed on *S*-V and on its antipode (*R*-V) as described in Table 1. Figure 3 shows the separation achieved on a mixture of *S*-V and *R*-V. Results of chiral analysis are shown in Table 2.

CD spectra were also recorded for *S*-V and its analogously prepared antipode (*R*-V) (Figure 4). g-Numbers calculated from CD and also measured specific optical rotations are shown in Table 2.

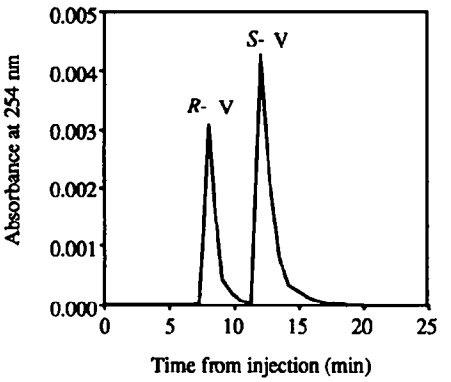


Figure 3. Chromatogram from the chiral HPLC of a mixture of *R*-V and *S*-V on Chiralcel™ OD (see Table 1 for elution conditions).

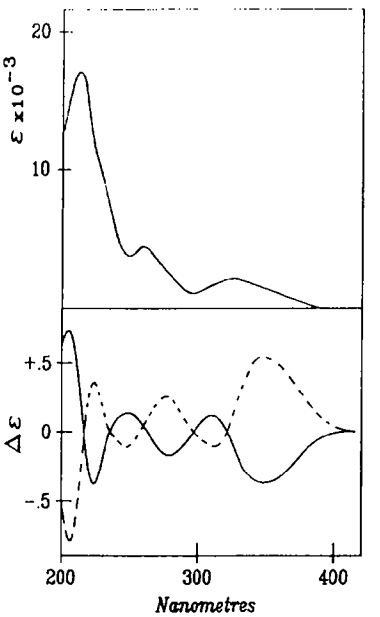


Figure 4. UV absorption spectrum of V (upper panel) and CD spectra (lower panel) for *S*-V (- - -) and *R*-V (——).

Preparation of S-[1-(2,3-diaminophenoxy)]-3'-(N-t-butylamino)propan-2'-ol (S-VI). To a solution of *S*-[1-(2-amino-3-nitrophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (*S*-V) (0.5 g, 1.77 mmol) in ethanol (70 mL) was added palladium on carbon (5%; 0.4 g) under nitrogen. Hydrogen was passed through the reaction mixture for *ca* 1 h at room temperature. The catalyst was removed by filtration and washed with hot ethanol. The ethanol was removed under reduced pressure giving a brown oil. Recrystallisation from dichloromethane-ether gave a brown solid of *S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (*S*-VI) (0.36 g, 80%).

^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.11 (9H, s; $C(Me)_3$), 1.8-2.5 (1H, br; OH), 2.67 (1H, d d, J = 12 and 7 Hz; $C(9)H_2$), 2.85 (1H, d d, J = 12 and 4 Hz; $C(9)H_2$), 3.3-3.7 (2H, br; NH_2), 3.9-4.05 (3H, br; $C(7)H_2$ + C(8)H), 6.40 (1H, d d, J = 2 and 1Hz; C(6)H), 6.43 (1H, d d, J = 3 and 1 Hz; C(5)H), 6.65 (1H, t, J = 8 Hz; $C(4)H_2$). A comparable spectrum has been recorded[24] for *S*-VI in CD_3OD.

Mass spectrometry in CI +ve mode: *m/z* = 254 $[M+H]^+$; and in EI mode: *m/z* = 253 $[M]^+$ (22%), 238 (8%), 166 (5%), 163 (5%), 130 (10%), 124 (100%), 112 (27%) and 95 (41%), in accord with that reported previously.[21]

Chiral HPLC was attempted on Chiralcel™ OD according to the methods described in Table 1. However,

racemic [1-(2,3-diaminophenoxy)]-3'-(*N*-t-butylamino)propan-2'-ol (VI) was not eluted, and hence was not resolvable under these conditions. CD spectra were also recorded for *S*-VI and its analogously prepared antipode (*R*-VI). g-Numbers calculated from CD are shown in Table 2.

Preparation of S-*CGP 12177* (S-*I*) *hydrochloride.* The *S*-diamino precursor (*S*-VI) (0.2 mg, 0.8 μmol) was treated with phosgene and gave *S*-CGP 12177 (*S*-I) hydrochloride as described previously.[24]

^{1}H-NMR (CD_3OD): δ (ppm) = 1.41 (9H, s; $C(Me)_3$), 3.17 (1H, dd, *J* = 12, and 9 Hz; $C(9)H_2$), 3.33 (1H, dd, *J* = 10 and 3 Hz; $C(9)H_2$), 4.16 (2H, dd, *J* = 5 and 2 Hz; $C(7)H_2$), 4.24 (1H, m; C(8)H) 6.71 (1H; C(6)H), 6.74 (1H, s; C(5)H), 6.99 (1H, dd, *J* = 8 and 8 Hz; $C(4)H_2$). A comparable spectrum has been recorded for *S*-I in CD_3OD.[24]

Mass spectrometry of *S*-VI gave spectra identical to those for authentic *R,S*-CGP 12177 (I) *i.e.* in CI +ve mode: *m/z* = 280 $[M+H]^+$ and 297 $[M+NH_4]^+$; and in EI mode: *m/z* = 279 $[M]^+$ (20%), 264 $[M-CH_3]^+$ (12%), 189 (8%), 150 (13%), 149 (8%), 121 (12%), 86 (100%) and 71 (27%).

Chiral HPLC analysis was performed on *S*-I, on its antipode (*R*-I) and on its racemate (I) as described in Table 1. Figure 5 shows a chromatogram of the separation achieved on racemic I. Results of chiral analysis are shown in Table 2. CD spectra were also recorded for *S*-VI and its analogously prepared antipode (*R*-VI) (Figure 6). g-Numbers calculated from CD and also the measured specific optical rotations are shown in Table 2.

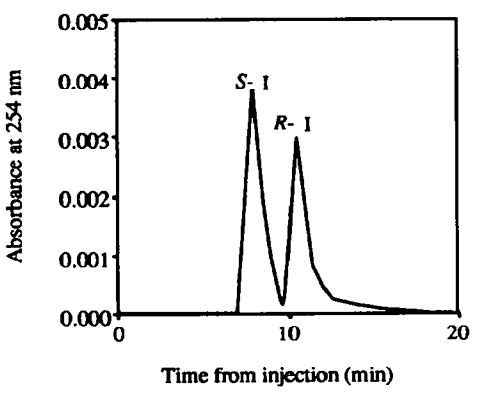


Figure 5. Chromatogram from the chiral HPLC of racemic I (see Table 1 for elution conditions).

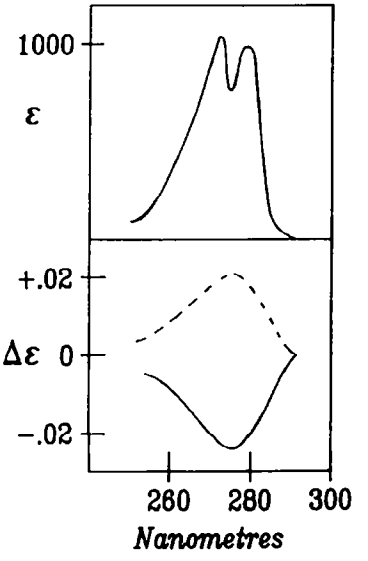
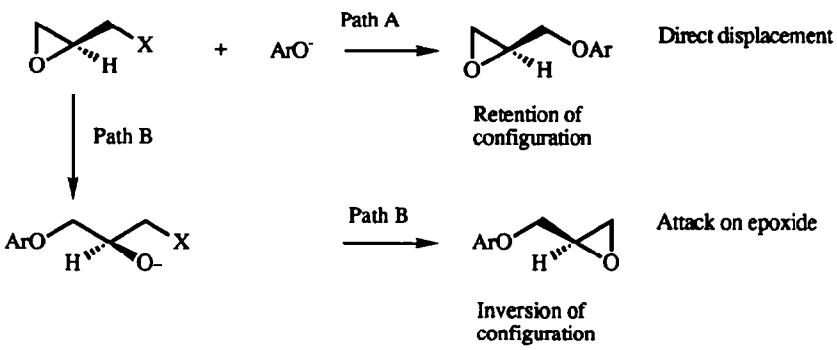


Figure 6. UV absorption spectrum of I (upper panel) and CD spectra (lower panel) for *S*-I (- - -) and *R*-I (——).

DISCUSSION

The only method so far available for labelling CGP 12177 (I) with carbon-11 is the reaction of [^{11}C]phosgene[25] with the diamino precursor, *R,S*-[1-(2,3-diaminophenoxy)]-3'-(*N*-*t*-butylamino)propan-2'-ol (VI).[3] Thus, in order to obtain *S*-[*carbonyl*-^{11}C]CGP 12177 (*S*-I), either the *S*-diamino precursor (*S*-VI) must be used in the radiosynthesis or the racemic radioligand must be resolved. The latter approach, though not entirely excluded by the short physical half-life of carbon-11 (20.3 min), would be wasteful of half the radioactivity. We were previously unable to resolve the diamino precursor (VI) by HPLC[21] using any one of a number of different chiral columns, including Chiralcel™ OD and Cyclobond™ I. We[21,26] and others [23,24] have therefore sought a convenient synthetic route to the *S*-enantiomer of the precursor (*S*-VI) for use in the radiosynthesis of *S*-[^{11}C]CGP 12177 (*S*-I). All these approaches have involved the reactions of phenoxides with chiral auxiliaries, mostly[21,23,26] chiral glycidyls with terminal leaving groups. Direct displacement in such chiral glycidyls results in retention of configuration whereas ring opening followed by ring closure leads to inversion of configuration (Scheme 3).[27] The ratio of direct substitution of leaving group to ring opening varies widely and is controlled by factors such as leaving group, nucleophile and the subtleties of the reaction, particularly the choice of base and solvent.[27]



Scheme 3: Reactions of homochiral *S*-glycidyl compounds with aryl oxides leading to retention of configuration (Path A: direct displacement of terminal leaving group, X) and inversion of configuration (Path B: attack on epoxide ring, followed by ring closure).

Hammadi and Crouzel[23,24] have described two approaches to the asymmetric synthesis of the *S*-diamino compound (*S*-VI), using 2-amino-3-nitrophenol (II) as starting material. In their first approach[23,24] they used *S*-glycidyl tosylate (91% e.e) as chiral auxiliary. However, reaction of sodium 2-amino-3-nitrophenoxide in DMF, followed by addition of *t*-butylamine gave the expected *S*-arylpropanolamine (*S*-V) in only 84% e.e., indicating 96% selectivity for direct tosyl displacement (Table 3).[23,24] Also reaction of the phenol (II) with *S*-glycidyl tosylate in the presence of potassium carbonate in refluxing acetone followed by addition of *t*-butylamine led to the arylpropanolamine (*S*-V) in only 63% e.e., corresponding to only 85% selectivity for direct tosyl displacement *versus* epoxide attack (Table 3).[24]

Table 3: Selectivities for Direct Substitution in Homochiral Glycidyl Compounds by Phenols.

Homochiral glycidyl compound	Phenol	Solvent	Base	Selectivity[a] (%)	Reference
Tosylate (91% e.e)	II	DMF	NaH	96[b]	24
Tosylate (91% e.e.)	II	Acetone	K_2CO_3	86[b]	"
S-III (> 98% e.e.)	II	MEK	K_2CO_3	98.6	This work
R-III (> 98% e.e.)	II	MEK	K_2CO_3	98.5	"
S-III (> 98% e.e.)	VII	DMF	NaH	99	"
R-III (> 98% e.e.)	VII	DMF	NaH	98.5	"
S-III (> 98% e.e.)	VII	Acetone	K_2CO_3	98.5	"
R-III (> 98% e.e.)	VII	Acetone	K_2CO_3	99.5	"

[a] For route A versus route B in Scheme 3. Unless otherwise indicated this is calculated from the chiral HPLC measurement of e.e. on product as:

$50(\%\ e.e._{Glycidyl\ compound} + \%\ e.e._{Product\ of\ same\ configuration})/(\%\ e.e._{Glycidyl\ compound})$.

[b] Calculated by the authors[24] from the e.e. of the ring-opened product (*S*-V) which is derived from the measured specific optical rotation, taking a value of + 22.5 ° to represent 100% e.e.[23] Our value for the specific optical rotation of *S*-V is 32 ± 4 ° (Table 2).

A quite recent report[28] shows that the asymmetric synthesis of β-receptor ligands with *S*-configuration is greatly improved by the use of *S*-glycidyl-3-nitrobenzenesulphonate (*S*-III) in place of *S*-glycidyl tosylate. Under appropriate conditions, the reaction of an aryl oxide with *S*-III proceeds not only faster than with *S*-glycidyl tosylate but also with almost complete retention of configuration. This rate enhancement is advantageous since the aryl epoxy ether is exposed to nucleophilic aryl oxide for a shorter period leading to higher yields. These considerations prompted us to use *S*-III as the chiral auxiliary in the asymmetric synthesis of the required *S*-diamino compound (*S*-VI). During this work we also noted that the chiral auxiliary, *S*-III, had been used successfully in the asymmetric synthesis of another arylpropanolamine, the β-blocker *S*-metoprolol, and had been preferred to the use of *S*-glycidyl tosylate for this purpose.[29]

Our initial approach[26] started with the reduction of 2,3-dinitrophenol (VII) to 2,3-diaminophenol followed by acetylation to give 2,3-diacetylaminophenol. Its phenoxide was reacted with the chiral auxiliary, *S*-III, to give *S*-[1-(2,3-diacetylaminophenoxy)]-2',3'-epoxypropane, which when refluxed with *t*-butylamine gave *S*-[1-(2,3-diacetylaminophenoxy)]-3'-(*N*-*t*-butylamino)-propan-2'-ol. Finally, reflux with potassium hydroxide gave the desired *S*-diamino compound (*S*-VI). However, the instability of this product to deprotection resulted in very low overall yield (< 1.5 %). To avoid this problem and shorten the synthesis we then adopted a second approach, as follows.[21] The phenoxide of 2,3-dinitrophenol (VII) was reacted with *S*-III in DMF to give *S*-[1-(2,3-dinitrophenoxy)]-2',3'-epoxypropane (*S*-VIII), which was then treated with *t*-butylamine to give *S*-[1-(2,3-dinitrophenoxy)]-3'-(*N*-*t*-butylamino)-propan-2'-ol. Finally, hydrogenation in the presence of palladium on

carbon gave the *S*-diamino compound, *S*-VI, in 24% overall yield and in > 95% e.e. [as estimated[21]by CD on derived *S*-CGP 12177 (*S*-I)].

Hammadi and Crouzel, in their second approach,[24] switched to *S*-3-tosyloxy-1,2-propanediol acetonide as chiral auxiliary. Though this change lengthens the asymmetric synthesis by two steps, it is claimed that the intermediate *S*-arylpropanolamine (*S*-V) was initially obtained in > 95% e.e. and could be recrystallised easily to > 99% e.e. The overall yield was 54% from the phenol (II). *S*-V was readily reduced to the required product (*S*-VI).

The simplified asymmetric synthesis of the *S*-diamino compound (*S*-VI) that we report here (Scheme 1) is a hybrid of two of these former methods[21,24] and embodies their best features for practical ease, efficiency (45% yield overall), and very high enantiomeric excess (e.e. in the range 98.6 - 99.4%). These features are:

a) the use[23,24] of readily available 2-amino-3-nitrophenol (II), in preference to 2,3-diaminophenol,[26] which requires extra reaction stages, or to 2,3-dinitrophenol (VII),[21] which is not readily available.

b) the use[21,26] of readily available[30] *S*-glycidyl-3-nitrobenzensulphonate (*S*-III) as a chiral auxiliary, so permitting rapid direct attack by the phenol (II) on the carbon bearing the leaving group, resulting in almost complete retention of configuration (*c.f.* Path A in Scheme 3). This is preferable to the former use of *S*-glycidyl tosylate,[23] which is slower reacting[23] and less-selectively attacked[23,24] so resulting in significant inversion[23,24] (*c.f.* Path B in Scheme 3; Table 3), or to *S*-3-tosyloxy-1,2-propanediol acetonide,[24] which requires extra stages in synthesis). It should also be noted that *S*-III can be recrystallised to very high enantiomeric purity,[30] which we have shown here can be checked easily by chiral HPLC on a Chiralcel™ OD column (Tables 1 and 2).

c) use of MEK and potassium carbonate as a practically attractive medium for highly selective (98.6%) direct substitution in the chiral auxiliary (*S*-III) by the phenol (II) (Table 3). The selectivity for reactions between the phenols (II and VII) with the chiral epoxides, *S*-glycidyl tosylate and *S*-III, under various reaction conditions are compared in Table 3. 2-Amino-3-nitrophenol (II) shows generally very high selectivity for direct substitution on *S*-III, equivalent to the formerly used 2,3-dinitrophenol (VII). Though sodium hydride-DMF is clearly a superior reaction medium for the reaction of the phenol, II, with *S*-glycidyl tosylate,[24] no significant gain in selectivity was observed from the use of sodium hydride-DMF for reaction between the phenol (II) and the chiral auxiliary (*S*-III) (Table 3)

d) recrystallisation of the stable intermediate *N*-*t*-butylamino compound (*S*-V) for enhancement of e.e.[24] Chiral HPLC confirmed that the e.e. of *S*-V could be improved from 95% to 99.4% by a single recrystallisation (Table 2).

e) 3-stage synthesis,[21] in preference to 5 stages from *S*-3-tosyloxy-1,2-propanediol acetonide.[24]

Methods that have previously been used to establish the e.e. of the diamino compound (*S*-VI), its precursors or of derived *S*-CGP 12177 (*S*-I) include optical rotation (*S*-V[23] and *S*-I[24] only), CD (*S*-I only)[21] and NMR with chiral shift reagents (*S*-V only).[24] In principle, optical measurements, such as CD and optical rotation, can characterise absolute stereochemistry and enantiomeric purity. Absolute stereochemistry is derived from the sign pattern and relative intensities of peaks in a CD spectrum and labelled simply by an optical rotation measurement, typically at 589 nm. Enantiomeric purity can be determined from the magnitudes of the measured values (*versus* that of reference material). However, the compounds presented here have exceedingly weak CD spectra because of the large preferred ordinary absorption of the aromatic moiety that is also remote from the centre of chirality. This exacerbates the errors that naturally arise when comparing two independently determined optical parameters for an enantiomeric pair; these errors also depend on the amounts of sample and their purity.

Precision can be greatly improved by quoting the CD (or optical rotation) per absorption unit at a particular wavelength, usually corresponding to a maximum in the spectra. This parameter is formally defined as the dissymmetry number or g-number.[31] Using Beer's Law:

$$g_\lambda = CD_\lambda/Absorption_\lambda = \Delta A_\lambda/A_\lambda = \Delta\varepsilon_\lambda cl/\varepsilon_\lambda cl = A\varepsilon_\lambda/\varepsilon_\lambda$$

Hence, the g-number is a dimensionless constant, and independent of concentration (c) and pathlength (l), provided that the latter is the same in both measurements; moreover the sign and magnitude of a g-number characterise a chiral molecule and its optical purity. In practice, as optical purity is a signed quantity, overlap cancellation can frequently lead to non-coincident CD and ordinary absorption peaks. Separate analytical wavelengths must then be used. A more general g-number is therefore required, written as $g^{\lambda 1}_{\lambda 2}$, with the superscript indicating the wavelength of CD (or optical rotation) measurement and the subscript indicating the wavelength of ordinary absorption measurement. The g-number provides quantitative values when CD, optical rotation or ordinary absorption cannot themselves be quantified due to, for example, lack of material, difficult to extricate oils and variations in solvent of crystallisation.

Some of the obtained CD spectra (*e.g.* Figures 2 and 4) were necessarily quite complex. Hence, the choice of analytical wavelengths was sample-dependent. In Table 2, g-numbers are recorded to provide the best spectroscopic inter-comparisons and correlations of absolute stereochemistry and enantiomeric purity. In general, the wavelength of the prominent absorption in the 230-260 nm region provided the most reliable value for the subscript, with the associated CD maximum taken for the superscript. For CGP 12177 (I), only CD data peaking at 275 nm, associated with the lowest energy ordinary absorption at 271 nm, could be observed.

The precision of the g-numbers is difficult to judge. They were found to be reproducible to within ± 5%. Thus, the g-number for CGP 12177 (I), reported previously,[21] has now been measured six times from three separate preparations of the enantiomers to give values that range from 2.04×10^{-5} to 1.9×10^{-5}. These represent the smallest g-numbers in the present set of compounds (Table 2). Further, careful correlation of the CD spectra indicates that no inversion of absolute configuration has occurred in the synthetic sequence leading from *S*-III to *S*-I (Scheme 1) or similarly from *R*-III to *R*-I. It should be noted that the specific optical rotation obtained for *S*-V (Table 2) agrees with that[24] for *S*-V prepared *via* reaction of the phenol (II) with the tosylate of *S*-2-phenyl-3-*tert*-butyl-5-hydroxymethyloxazolidine,[32] so confirming the assignment of absolute configuration in the series *S*-IV, *S*-V, S-VI and *S*-I.

Optical rotation measurements were rendered difficult by colour, low solubility or the small quantity available for measurement. The diamino compounds (*S*-VI and *R*-VI) are particularly unstable and insufficient quantities were available for conventional optical rotation measurements. Table 2 compares the specific optical rotation data for compounds *S*-V and *S*-I obtained in this study with those reported by Hammadi and Crouzel.[23,24] Our values are equal in magnitude and opposite in sign for *R*-V and *S*-V and correspond to compounds giving equal and opposite g-numbers in CD. However, Hammadi and Crouzel[23] have reported a much lower $[\alpha]_D$ value for *S*-V claimed to be enantiomerically pure. Also, concerning *S*-I, it is noteworthy that the $[\alpha]_D$ value obtained by Hammadi and Crouzel[24] is -10.8 °, slightly less than the value of -13 ± 1 ° we obtained on *S*-I that had earlier been prepared by a route[6] independent to that reported here, but slightly greater than the value (8 ± 2 °) measured for *S*-I prepared by Scheme 2. Equal and opposite values were obtained for the specific optical rotation and g-number of

the *R*-isomer for each enatiomeric pair in our possession (Table 2). Nonetheless, these variations in measured specific optical rotations, which are not matched by any variation in CD g-number (Table 2), and their quite large associated errors precluded their use for reliable and accurate estimation of e.e.

We were concerned to have robust method for measuring enantiomeric purity that would be less dependent on the use of reference compounds as in the optical rotation and CD methods. We therefore re-explored chiral HPLC for this purpose, and in particular HPLC on Chiralcel™ OD, which had previously been shown[29,33,34] to resolve arylpropanolamines. Elution conditions were established on Chiralcel™ OD for measuring the e.e. in all of the homochiral intermediates in the asymmetric synthesis of *S*-VI. However, the diamino compound (VI) and CGP 12177 (I) could not be resolved on this column. It was found possible to resolve CGP 12177 (I), though again not the diamino compound (VI), on an Ultron™-ES OVM column (Tables 1 and 2). The chiral HPLC methods that we report here (Table 1) are useful as follows: 1) for checking the e.e. of the commercially available chiral auxiliary (*S*-III), 2) for estimating e.e. for compound *S*-IV, so permitting the crucial reaction between the phenol (II) and chiral auxiliary (*S*-III) to be monitored for selectivity, 3) for measuring the improvement in e.e. on recrystallisation of the *N*-*t*-butyl compound (*S*-V) and 4) for directly measuring the e.e. of derived *S*-CGP 12177 (*S*-I) (Table 2). Examples of chromatograms for enantiomeric pairs (either mixtures or racemates) generated in these analyses are shown in Figures 1, 3 and 5. Separation parameters calculated from such chromatograms are shown in Table 1. The assignment of order of elution for each enantiomeric pair (Table 1) is on the basis that there is no change in configuration in the reaction sequence from *S*-III to *S*-I, in accord with mechanistic considerations and with the accumulated CD data (Table 2). These methods of chiral HPLC analysis are sensitive, accurate and convenient. Unlike the optical techniques, they do not require reference compounds of absolute or known optical purity and are intrinsically less prone to error from any optically active contaminants.

S-[^{11}C]CGP 12177 (*S*-[^{11}C]I), prepared with greater than 98.4 % e.e. from the precursor (*S*-VI), is now being applied in clinical research to the study of β-receptors in human heart and lung with PET, and also in ancillary biological experiments.

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