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SYNTHESIS OF AN IODINE-LABELLED ANALOGUE OF PRACTOLOL: (*S*)-3-[4-(4-IODOBUT-3-ENCARBOXAMIDO)PHENOXY]-1-ISOPROPYLAMINOPROPAN-2-OL (AMI-9S)

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Summary

AMI-9S was synthesized in the *S* configuration with an enantiomeric purity of over 97% and labelled with iodine-123 with a specific activity of 300 Ci/mmol. Enantiomeric purity was determined by ^{19}F NMR spectroscopy following derivatisation using (*R*)-2-fluorophenylacetylchloride. Radio-iodination was carried out from a vinylic stannane in the presence of iodide and chloramine T.

Introduction

Preliminary biological studies revealed that **1** is a very interesting marker of β-adrenergic receptors (1). During these tests, the racemic mixture was used although it is known that only the *S* configuration compound is biologically active. On account of the synthesis route used (1), labelling was achieved by nucleophilic substitution. This reaction is generally difficult to perform on a vinylic

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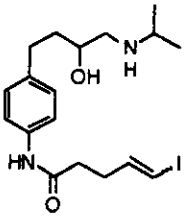
carbon (2). The rare documented examples of isotopic exchange take place under difficult experimental conditions which provide a product with low specific activity (3,4), incompatible with efficient external detection *in vivo*. We therefore decided to go back to the synthesis of **1** with two objectives : obtain the *S* configuration and significantly improve the labelling results.



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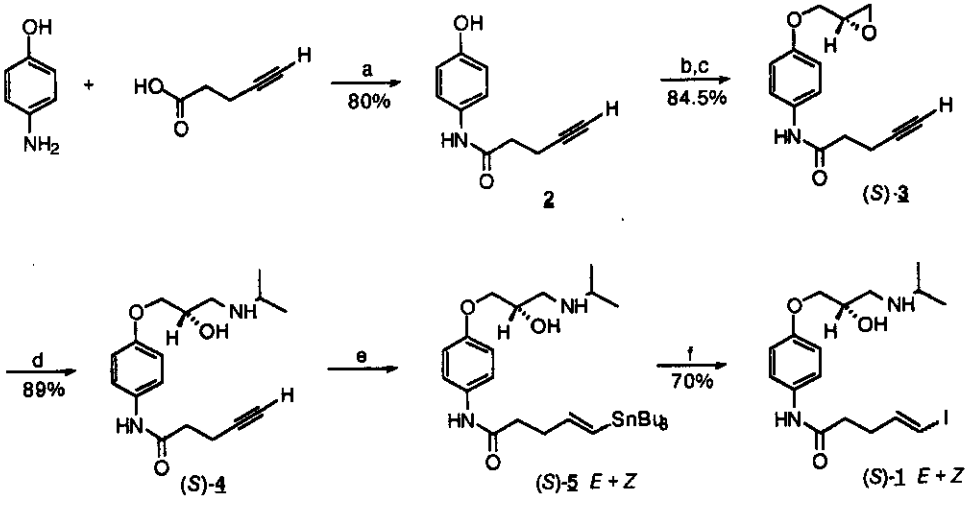
Results and Discussion

For iodine-123 to be used as an emitter of γ radiation, the radio-iodination stage has to be carried out at the end of the synthesis because of the short half-life of this element (13.2h). At the moment, the best procedure for preparing labelled vinylic iodides involves a stannane step (5,6). During the sequence

the Bu_3Sn/I exchange is very rapid, even instantaneous, and does not modify the stereochemistry around the double bond.

The action of a phenate on a glycidyl homochiral arenesulfonate is the best access route to chiral β-blockers. The two enantiomers of glycidyl 3-nitrobenzenesulfonate, which are commercialised with a purity of 99%, are the most interesting. Sharpless et al. (7) have shown that attack of the phenate ion occurs almost exclusively on carbon which bears the ester function. The subsequent step of ring-opening of the epoxide takes place only on the methylene and does not change the configuration of the chiral carbon.

The synthesis was thus developed from 4-aminophenol and pent-4-ynoic acid according to the route shown in Scheme 1. During the first step, the action of acetylenic acid on the aminophenol led almost exclusively to the formation of the amide function. The latter must be created before the β-aminoalcoholic part so as to remove the problem of chemoselectivity which would result from the

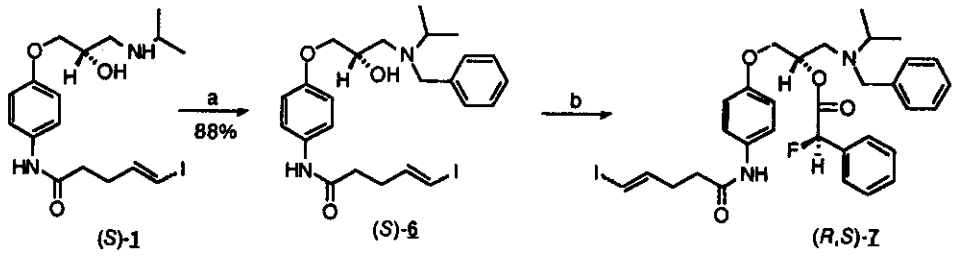


a: DCC, HOBT, THF, CH_3CN - b: NaH, DMF - c: (2S) - glycidyl 3-nitrobenzenesulfonate - d: $iPrNH_2$, propan-2-ol - e: $HSnBu_3$, AIBN, Toluene (reflux) - f: ICl, CH_2Cl_2.

Scheme 1

simultaneous presence of the two primary and secondary amine functions. Stannylation took place with no problems under argon atmosphere and in an anhydrous medium. The purification tests of (*S*)-5 by liquid phase chromatography on silica gel resulted in degradation of the compound. The final unlabeled derivative (*S*)-1, which had to be prepared for routine characterisations, was thus obtained from crude (*S*)-5. The action of ICl in dichloromethane was instantaneous at room temperature. Purification of (*S*)-1 took place without any problems; the desired compound was obtained in 70% yield from (*S*)-4. No iodination of the amine function was observed.

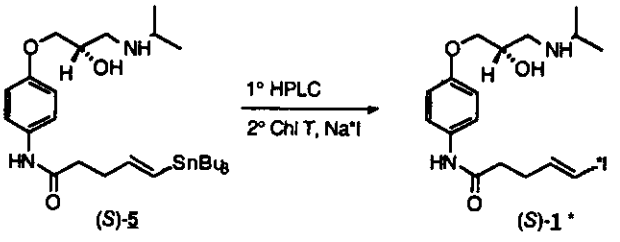
The enantiomeric purity of the final compound was determined according to the method developed by us for β-aminoalcohols (8) (Scheme 2). Following protection of the amine function by the benzyl group, the compound (*S*)-6 obtained was esterified by (*R*)-2-fluorophenylacetylchloride (9, 10). The fluorine NMR spectrum of (*R,S*)-7 presented two signals of very different intensity at -15.94 and -18.36 ppm (internal reference: C_6F_6) corresponding respectively to the *R,S* and *R,R* configurations. The ratio of the integral curves was 102/2.5, which corresponds for the *R,S* isomer to a purity of 97.5%, which conforms to the predicted value.



a: $C_6H_5CH_2Br$ (1.5 eq.), K_2CO_3, CH_3CN (reflux) - b: (R)-$C_6H_5CHFCOCl$, pyridine, rt.

Scheme 2

The passage from (*S*)-5 to labelled (*S*)-1 (Scheme 3) required much smaller amounts of material



Scheme 3

(a few tens of nanomoles) than the reaction with cold iodine. The stannic derivative could first be purified by reversed-phase HPLC. Labelling was then carried out at room temperature using an organometallic excess in relation to the iodides. The latter were oxidized using chloramine T, itself in large excess. The reaction was quenched by the addition of dithionite. The solution obtained was passed through an anionic resin in order to retain any radioactive iodides present while the organic products were easily eluted. Radioactivity counted in the resin and the filtered matter were used to determine labelling yield, which was the ratio between the radioactivity of the filtered matter and total radioactivity (filtered matter + resin). This radiochemical yield was over 70%. The final product analysed by reverse phase HPLC contained very few radiolabeled by-products (of the order of 5%). In the case of labelling with iodine-123, a satisfactory labelling yield could be obtained only through the addition of cold sodium iodide, in a $^{127}I^-/^{123}I^-$ ratio of the order of 100. This may seem surprising in that the $SnBu_3$-I exchange was rapid, unlike the isotopic exchange. The specific activity obtained was close to 2000Ci/mmol with ^{125}I. It was of the order of 300Ci/mmol in the case of ^{123}I,

much higher than we had obtained in the first tests on labelling by isotopic exchange (0.5Ci/mmol) (11).

Experimental

General procedures. Reactions were monitored by TLC using alumina plates coated with silica gel $60F_{254}$ (Merck) and visualized using either UV light, iodine or by charring with phosphomolybdic acid (5% solution in ethanol). Preparative chromatography was performed with Merck silica gel (0.063-0.200mm) using the same eluting systems as for TLC, unless otherwise noted. HPLC was carried out using Waters μ Bondapak C-18 reversed-phase columns (3.9x300mm - UV detection). DMF was distilled from (and stored over) 4Å molecular sieves. THF was distilled from sodium benzophenone ketyl immediately before use. Toluene was distilled from sodium. Acetonitrile and pyridine were distilled from CaH_2. Organic layers were dried with anhydrous Na_2SO_4. Sodium hydride (60% in oil) was washed with pentane, under argon, three times before use. Infrared spectra were recorded in nujol (Nicolet impact 400 Spectrophotometer). ^1H (^{13}C) NMR spectra were obtained on a 200 (50) δ MHz spectrometer (Brüker). Chemical shifts, for ^1H NMR spectra, are reported in δ units downfield from internal Me_4Si. ^{13}C NMR spectra were referenced to the $CDCl_3$ or CD_3OD peak at 77, 39.5 or 49 ppm relative to Me_4Si. For ^{19}F NMR spectra, C_6F_6 was used as internal reference. Multiplicities are reported as s (singlet), d (doublet), t (triplet), m (multiplet). Melting points were determined on a capillary melting point apparatus and are uncorrected. Optical rotations were determined at the sodium D line with a Perkin Elmer 341 polarimeter. Radioiodine-125 (IMS 30) was obtained from Amersham as a Na ^{125}I solution in NaOH (pH ~ 9) at 15-16 Ci/mg (1mCi/10μL) Iodine-123 (S1) was obtained from CIS bio international as a Na ^{123}I solution in 0.002N NaOH at 1mCi/10μL. Radiometric analyses were achieved by ionisation chamber (Medisystem). Elemental analyses were performed by the Service central d'analyses du CNRS.

4-(But-3-yncarboxamido)phenol 2

To a solution of 4-aminophenol (2g, 18.34 mmol) in THF-acetonitrile (1/1 - 100mL) was added dicyclohexylcarbodiimide (DCC) (3.88g, 18.34 mmol), hydroxybenzotriazole hydrate (HOBT) (2.50g, 18.34 mmol) and pent-4-ynoic acid (2.34g, 23.87 mmol). The mixture was stirred under argon at room temperature for 20h. After filtration of the dicyclohexylurea, the solvent was evaporated in vacuo and the residue purified by chromatography ($CHCl_3$/EtOAc : 3/2) to furnish 2

(2.77g, 80%) as a white solid: mp 146-147°C. IR (neat): 3333, 2123, 1654 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 2.32 (t, H, J=2.5Hz); 2.57-2.58 (m, 4H); 6.69-7.33 (m, 4H); ^{13}C NMR (50 MHz, CD_3OD) δ 15.8, 36.8, 70.5, 83.6, 116.3, 123.5, 131.7, 155.5, 172.1. Anal. Calcd for $C_{11}H_{11}NO_2$: C, 69.84; H, 5.82; N, 7.40. Found: C, 69.81; H, 5.96; N, 7.40.

(*S*)-1-[4-(But-3-yncarboxamido)phenoxy]-2,3-epoxypropane (*S*)-3

To a slurry of NaH (0.170g, 4.24 mmol) in dry DMF (10mL) at 0°C was added dropwise under argon phenol 2 (0.730g, 3.86 mmol in DMF (10mL). When the H_2 evolution stopped, (*S*)-glycidyl 3-nitrobenzensulfonate (1.00g, 3.86 mmol) in DMF (10mL) was added dropwise. After complete consumption of the phenol at room temperature, the solvent was carefully removed and the resulting crude product dissolved in EtOAc. The solution was washed three times with water (3x50mL), dried, concentrated in vacuo and the residue purified by flash chromatography ($CHCl_3$/EtOAc: 4/1) to furnish 3 (0.805g, 85%) as a white solid: mp 137°C; $[\alpha]_D^{20}$ = 9.85 (c 1.05, CH_3OH). IR (neat): 3271, 1647, 1252, 1036 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.03 (t, H, 2.6Hz); 2.51-2.60 (m, 4H); 2.71-2.75 (dd, H, J=3.7 and 2.6Hz); 2.86-2.91 (dd overlapping, H, J=4.6Hz); 3.31-3.33 (m, H); 3.87-3.95 (dd, H, J=11.0 and 5.7Hz); 4.15-4.22 (dd, H, J=11.0 and 3.1Hz); 6.84-6.88 and 7.37-7.41 (2m, 4H); 7.30 (s, H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.8, 36.1, 44.7, 50.1, 69.0, 69.7, 82.8, 115.6, 121.8, 131.3, 155.3, 169.0. Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.57; H, 6.12; N, 5.71. Found: C, 68.57; H, 5.97; N, 5.66.

(*S*)-3-[4-(But-3-yncarboxamido)phenoxy]-3-isopropylaminopropan-2-ol (*S*)-4

A solution of the preceeding epoxide (0.5g, 2.06 mmol) and isopropylamine (1.2g, 20.6 mmol) in isopropanol (50mL) was heated to reflux for 2h. After evaporation to dryness, the residue was purified by column chromatography ($CHCl_3$/CH_3OH/28% NH_4OH: 4/1/0.1) to give 0.558g (89%) of aminoalcohol (*S*)-4; mp 123°C; $[\alpha]_D^{20}$ = 1.4 (c 1.00, CH_3OH). IR (neat): 3308, 3278, 2123, 1666 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 1.13-1.17 (2d, 6H, J=6.3Hz); 2.33 (t, H, J=2.5Hz); 2.59 (m, 4H); 2.69-2.92 (m, 3H); 4.15 (m, 3H); 6.94-6.97 and 7.48-7.51 (2m, 4H); 7.20 (s, H); ^{13}C NMR (50 MHz, CD_3OD) δ 14.8, 23.0, 23.1, 36.1, 48.9, 49.2, 68.4, 69.6, 70.8, 83.9, 114.8, 121.8, 131.1, 155.6, 169.1. Anal. Calcd. for $C_{17}H_{24}N_2O_3$: C, 67.10; H, 7.89; N, 9.21. Found: C, 67.01; H, 8.03; N, 9.05.

(*S*)-3-[4-(4-Tributylstannylbut-3-encarboxamido)phenoxy]-1-isopropylaminopropan-2-ol (*S*)-5

A solution of (*S*)-4 (0.400g, 1.31 mmol), tributyltin hydride (0.48mL, 1.7 mmol) and AIBN (0.048g, 0.26 mmol) in dry toluene (10mL) was refluxed under argon. The reaction was monitored

by TLC ($CHCl_3$/MeOH/22% NH_4OH: 9/1/0.1). After 3h the solvent was evaporated in vacuo to give (*S*)-5 as an oil used as such for the next reaction.

(*S*)-3-[4-(4-Iodobut-3-encarboxamido)phenoxy]-1-isopropylaminopropan-2-ol (*S*)-1

To a solution of (*S*)-5 in CH_2Cl_2 (10mL) was added dropwise at room temperature ICl (0.234g, 1.44 mmol) in CH_2Cl_2. At the end of the reaction, monitored by TLC ($CHCl_3$/CH_3OH/27% NH_4OH: 9/1/0.1), the solvent was evaporated and the residue dissolved in EtOAc (20mL). A solution of KF (0.5g) in H_2O (5mL) was added and the mixture stirred for 1h. After filtration of the precipitate the separated aqueous phase was extracted with EtOAc and the combined organic layers were dried and concentrated. Chromatography of the residue on silica gel ($CHCl_3$/CH_3OH/27% NH_4OH: 4/1/0.1) afforded 0.396g (70%) of (*S*)-1 as a yellowish powder: mp 129-130°C; $[\alpha]_D^{20}$ = -7.4 (c 1.00, CH_3OH); ^1H NMR (200 MHz, CD_3OD) δ 1.11-1.15 (2d, 6H, J=6.3Hz); 2.35-2.45 (m, 6H); 2.62-2.95 (m, 3H); 3.92-4.10 (m, 3H); 6.10-6.40 (m and d, 1.2H, J=14.2Hz); 6.46-6.68 (m, 0.8H); 6.87-6.92 and 7.39-7.43 (m, 4H); 7.27 (s, H); ^{13}C NMR (50 MHz, CD_3OD) δ 22.5, 22.8, 32.6, 37.2, 33.6, 37.7, 48.7, 49.7, 69.9, 71.8, 76.9, 83.9, 115.8, 123.8, 134.1, 141.9, 146.1, 155.6, 169.1.

(*S*)-3-[4-(4-Iodobut-3-encarboxamido)phenoxy]-1-(N-benzyl)isopropylaminopropan-2-ol (*S*)-6

A solution of (*S*)-1 (0.033g, 0.08 mmol) and benzyl bromide (0.027g, 0.16 mmol) in CH_3CN (4mL) was heated to reflux in the presence of dry K_2CO_3 (0.022g, 0.16 mmol) for 2h. After filtration the solvent was evaporated and the residue purified by column chromatography ($CHCl_3$/EtOAc: 3/2) to give (*S*)-6 (0.035g, 88%) as a viscous oil; $[\alpha]_D^{20}$ = -29.5 (c 1.12, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.99-1.03 and 1.05-1.08 (2d, 6H, J=6.6Hz); 2.36-2.60 (m, 6H); 2.90-3.03 (sept, H, J=6.7Hz); 3.50-3.74 (2d, 2H, J=13.7Hz); 3.87-4.05 (m, 3H); 6.09-6.28 (m and d, 1.2H, J=14.5Hz); 6.48-6.59 (m, 0.8H); 7.06 (s, H); 6.79-6.83 (d, 2H, J=9.0Hz); 7.22-7.39 (m, 7H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 15.9, 20.0, 31.7, 35.8, 50.0, 51.6, 54.7, 65.9, 70.6, 76.4, 114.8, 121.7, 127.1, 128.4, 128.6, 130.8, 139.4, 139.6, 155.7, 169.3. Anal. Calcd for $C_{24}H_{29}IN_2O_3$: C, 55.17; H, 5.98; I, 24.29; N, 5.36. Found: C, 55.28; H, 6.33; I, 22.51; N, 5.20.

(*S*)-3-[4-(4-Iodobut-3-encarboxamido)phenoxy]-1-(N-benzyl)isopropylaminoprop-2-yl (*R*)-2-fluorophenylacetate (*R,S*)-7

A solution of (*S*)-6 (0.013g, 0.02 mmol), (*R*)-2-fluorophenylacetylchloride (0.009g, 0.05 mmol) and pyridine (0.004g, 0.05 mmol) in dry CH_2Cl_2 (6mL) was left under argon until reaction,as monitored by TLC (cyclohexane/acetone: 9/1) was complete. The mixture was successively washed

with a saturated $HNaCO_3$ solution and water. After drying and removing of the solvent, the residue was dissolved in the NMR solvent (methanol).

Radioiodinated (*S*)-1

After purification by HPLC (MeOH/H_2O/28% NH_4OH: 9/1/0.2) (*S*)-5 is dissolved in EtOH (1mg/mL). (*S*)-1 labelled with iodine 125 or 123 could be obtained from (*S*)-5 in one hour in ethanolic solution. Typical experiment: (*S*)-5 (20 µL, 34 nanomoles), chloramine T (100 µL, 355 nanomoles- solution: 1mg/mL in 0.1M phosphate buffer, pH=7.4), Na ^{125}I (50 µL, 2 nanomoles ~ 5 mCi). The mixture was left at room temperature for 1h. The reaction was quenched by addition of sodium dithionite (1mg/mL of the above buffer). The solution was passed through an anionic resin Dowex AG1x8 (Cl^- form) column (Pasteur pipette) which was eluted with acetone or phosphate buffer (NaH_2PO_4-Na_2HPO_4, 0.002M, pH=6). Finally, purification was carried out by HPLC (0.02M phosphate buffer -pH=4/acetonitrile: 65/35). The labelling yield exceeds 70%.

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