SYNTHESIS, ATTEMPTED KINETIC RESOLUTION AND EVALUATION OF [^{123}I]-MK-447 ANALOGUES AS INFLAMMATION RADIOPHARMACEUTICALS

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SUMMARY

The non-steroidal anti-inflammatory agent 2-amino-methyl-4-tert-butyl-6-iodophenol hydrochloride (MK-447) and some of its analogues were labelled with ^{123}I and tested in an inflamed rat-paw model. Even though MK-447 has a significant anti-inflammatory activity, and a 2.3 ratio for inflamed site over muscle, its usefullness for scintigraphic detection of inflammation is hindered by its high uptake in the skin. The kinetic resolution of diastereomeric products with chiral dienyl iron complexes was unsuccessful as the signals from the respective ^{1}H and ^{13}C NMR spectra were coincident.

Keywords: Iodine-123, kinetic resolution of diastereoisomers, [MK-447]-chiral iron complexes, potiential radiopharmaceutical, inflammation

INTRODUCTION

MK-447 (2-aminomethyl-4-*tert*-butyl-6-iodophenol hydrochloride) 4 is a potent non-steroidal anti-inflammatory agent,[1,2] used for topical treatment of inflammation of the respiratory and intestinal mucosa, such as allergic rhinitis, bronchitis, bronchical asthma and cholitis. MK-447 is also effective for topical treatment of dermatological disorders. When administered in a therapeutic dosage, the drug

CCC 0362–4803/97/090711–19$17.50
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Received 3 February 1997
Revised 12 May 1997

effectively alleviates conditions usually associated with edema and hypertension. The mechanism of its therapeutic action in the body remains unclear[3,4,5]. Scintigraphic detection of foci of infection has been for over thirty years a useful methodology for managing sepsis and inflammation, through enabling a quicker diagnosis and a better localization of the disease. The major groups of radiopharmaceuticals used today for this purpose are mono- and polyclonal antibodies, and labelled leukocytes, agents with a variety of pitfalls in their preparation and use.

^{111}In-conjugated polyclonal immunoglobulins (hIgG) may have a superior localization to ^{99m}Tc-HSA or to ^{67}Ga-citrate, but have a high rate of binding to non-inflamed sites such as cancer tissue, and when labelled with ^{99m}Tc, a significant amount of the activity is found in the kidneys and the bladder, precluding detection of infection in the urogenital system[6]. Monoclonal antibodies have also been used for imaging inflammation, and abscesses can be detected with their images at 4-6 hours, but their specificity is relatively low: about 25 % remains in the spleen, liver and the bone marrow and a similar percentage is circulating, usually bound to the relevant cells' antigens or to damaged cells that become localized at the abscess site[7].

The sensitivity of detection of inflamed foci by labeled leukocytes varies between 50-95 %. There is also a relatively high-rate of false-positive results, due to cases such as aseptic chronic inflammation, osteoporosis, rheumatoid arthritis and some metastatic carcinomas[8]. In as much as labelled leukocytes can detect lesions of infections prior to formation of an abscess, this process requires 24 hours in order to detect sites of inflammation, an interval too long for managing an acutely ill patient. Moreover, techniques of harvesting and labelling these cells are time-consuming and require highly-trained personnel in order to maintain their viability. None of these proteins is the agent of choice today in detecting inflammation[9].

It occured to us that 2-aminomethyl-4-tert-butyl-6-iodophenol hydrochloride (MK-447), a potent non-steroidal anti-inflammatory agent[10-12] which is particularly effective in topical treatment of dermatological disorders and inflammations, could be successfully used for scintigraphic detection of inflammation. We decided to synthesize some of its analogues and tested two of them in an inflamed rat-paw model.

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RESULTS AND DISCUSSION

2-Aminomethyl-4-*tert*-butylphenol hydrochloride 1 was synthesized in 89% yield by the reaction of 4-*tert*-butylphenol with 2-chloro-*N*-(hydroxymethyl)acetamine in acetic acid with a catalytic amount of sulfuric acid at R.T. for 6 hr. The racemic amino acid *N*-benzyl-2-hydroxy-5-*tert*-butylphenylglycine 2 was obtained as an off-white powder in 81% yield, by a modification of the literature procedure[13-15]. This synthetic procedure involved refluxing a solution of sodium glyoxylic acid, 4-*tert*-butylphenol and benzylamine in methanol for 18 hr, followed by recrystallization from ethanol and water.

1: X = H
4: X = I

2: X = H
5: X = I

3: X = H
6: X = I

N-(5-*tert*-butyl-2-hydroxyphenyl)methylmorpholine 3 was obtained in 83% yield as a crystalline solid by refluxing a solution of 4-*tert*-butylphenol, paraformaldehyde and morphine in methanol for 18 hr, followed by recrystallization from ethanol and water. 2-Aminomethyl-4-*tert*-butyl-6-iodophenol hydrochloride (MK-447) 4 was synthesized in 87% yield by a similar procedure[1] to that for 1 by reaction of 4-*tert*-butyl-6-iodophenol with 2-chloro-*N*-(hydroxymethyl)acetamide in acetic acid with a catalytic amount of sulfuric acid at R. T. for 6 hr.

The monoiodinated derivative *N*-benzyl-2-hydroxy-5-*tert*-butyl-3-iodophenylglycine 5 was obtained as a crystalline solid by aromatic electrophilic iodination.[13] Treatment of *N*-benzyl-2-hydroxy-5-*tert*-butylphenylglycine 2 with molecular iodine in acetic acid or iodine in ethanol resulted in 46% and 57% yield, respectively of 5. However, use of sodium iodide and chloramine-T as the oxidizing agent enhanced the yield to 80%. The disappearance of the doublet at 6.72 ppm for H^3 (2) and the appearance of a doublet signal at 7.57 ppm {c.f. dd at 7.13 ppm for (2)} in the ^{1}H NMR spectrum (d_6-DMSO) of the product, indicated that the desired compound *N*-benzyl-2-hydroxy-5-*tert*-butyl-3-iodophenylglycine (5) was produced.

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N-(5-*tert*-butyl-2-hydrioxyphenyl-3-iodo)methylmorpholine (6) was synthesized by two different methods. The first method[12] involved the treatment of *N*-(5-*tert*-butyl-2-hydroxyphenyl)methylmorpholine (3) with 1.2 molar equivalents of sodium iodide and Chloramine-T in DMF to give a light yellow powder. Jones and Richardson[17], Bakker et al.[18,19] and Watts et al.[16,19] have reported that iodinations with sodium iodide and Chloramine-T could give intractable monoiodinated and diiodinated or chlorinated aromatic products. Purification by HPLC was tedious, and afforded low quantities of the desired product.

An alternative method for the preparation of *N*-(5-*tert*-butyl-2-hydrioxyphenyl-3-iodo)methylmorpholine 6 involved the treatment of 4-*tert*-butyl-2-iodo-phenol[13] with paraformaldehyde and morpholine in methanol with refluxing for 18 hr. The product mixture was then purified by column chromatography on silica gel to give 6 as a light yellow powder in 68% yield. Comparative ^{1}H NMR spectroscopy studies of 3 and 6 indicated that the disappearance of the doublet at 6.76 ppm corresponding to H^3 of the precursor 3, and the presence of a downfield doublet at 7.63 ppm corresponding to H^4 of 6.

Radiolabelling of MK-447 Analogues with ^{123}I

[^{123}I]-2-aminomethyl-4-*tert*-butyl-6-iodophenol **[^{123}I]-4** , [^{123}I]-*N*-benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine **[^{123}I]-5** and [^{123}I]-*N*-(5-*tert*-butyl-2-hydroxy-3-iodophenyl)-methylmorpholine **[^{123}I]-6** were obtained by radiolabelling of the precursors 1, 2, and 3, respectively, under mild conditions, by the production of electrophilic [^{123}I] iodine *in situ* from the oxidation of iodide with either Chloramine T, peracetic acid, or KIO_3 in 1M HCl at R. T. for 3 -5 min.

The radiochemical yields for **[^{123}I]-4**, **[^{123}I]-5** and **[^{123}I]-6** by the above reactions using KIO_3, peracetic acid or Chloramine-T as oxidising agent were similar. However, HPLC peak for the labelling reactions for **[^{123}I]-5** and **[^{123}I]-6** with peracetic acid were often very broad, and small peaks were observed with uv detection in the region where the radiolabelled product was eluted, suggesting that a low specific activity of the radiolabelled product was obtained. The specific activities of **[^{123}I]-5** and **[^{123}I]-6** were determined to be 181 and 180 Ci/mmol, respectively.

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Table 1: Radiochemical Yield[a] (%) with various oxidising agents

Substrate ($[^{123}I]$, oxidant)	Product	Radiochemical yields, % Chloramine-T	Peracetic acid	KIO_3
1 R= $-CH_2NH_2 \cdot HCl$	$[^{123}I]$-4	-	-	65
2 R= $-C(COOH)-NH-CH_2-C_6H_5$	$[^{123}I]$-5	48	53	52
3 R= $-C-N$ (morpholino)	$[^{123}I]$-6	64	65	69

[a] Average of 5 repeated reactions, average deviation ± 5%

Biological evaluation

Figure 1 demonstrates the dose-response of MK-447 in the inflamed rat-paw model[22]. The higher dose of the drug has a definitely stronger anti-inflammatory effect, with a significance level of $p < 0.05$ for most time periods.

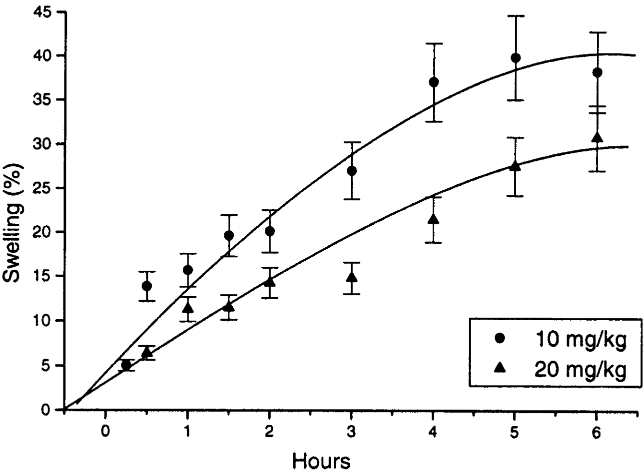

Fig. 1 Drug dose response to MK-447 as measured by reduction of swelling in rat paw (n = 10).

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Table 2: Uptake ratio of ^{123}I-MK-477 in inflamed tissue over muscle or blood

Ratio	0.25 h	0.5 h	1.0 h	3.0 h	6.0 h	24.0 h
Inflammation / muscle	<1	<1	<1	1.7	2.3	1
Inflammation / blood	1.5	1.2	0.8	0.6	0.6	0.5

Table 3. Biodistribution expressed as percent of injected dose per organ of ^{123}I-4 in rats with paw inflammed with turpentine[23]

Organ	0.25 h	0.5 h	1.0 h	3.0 h	6.0 h	24.0 h
Liver	14.14 ± 8.94	19.04 ± 1.83	22.43 ± 0.71	21.88 ± 0.36	21.93 ± 1.34	6.39 ± 1.02
Spleen	0.53 ± 0.37	0.42 ± 0.07	0.33 ± 0.02	0.16 ± 0.01	0.07 ± 0.01	0.01 ± 0
Kidney	1.73 ± 1.24	1.54 ± 0.3	1.61 ± 0.29	1.18 ± 0.45	0.83 ± 0.38	0.09 ± 0.01
Muscle	21.99 ± 14.37	17.25 ± 3.69	13.84 ± 1.18	6.43 ± 0.97	3.59 ± 0.36	0.53 ± 0.12
Skin	6.47 ± 4.78	7.14 ± 2.16	6.24 ± 0.65	5.73 ± 1.07	3.07 ± 0.37	0.84 ± 0.52
Bone	2.59 ± 1.76	2.14 ± 0.47	1.54 ± 0.07	0.9 ± 0.07	0.48 ± 0.03	0.08 ± 0.02
Lungs	6.44 ± 4.58	5.57 ± 1.35	5.21 ± 0.97	2.2 ± 0.39	0.62 ± 0.18	0.04 ± 0.01
Heart	0.41 ± 0.27	0.37 ± 0.06	0.3 ± 0.02	0.18 ± 0.01	0.09 ± 0	0.01 ± 0
Blood	1.49 ± 1.02	1.58 ± 0.22	2.12 ± 0.19	2.5 ± 0.17	1.7 ± 0.08	0.23 ± 0.02
Stomach	2.57 ± 1.92	2.38 ± 0.88	2.33 ± 0.47	1.82 ± 0.4	1.13 ± 0.27	0.12 ± 0.08
Gut	8.58 ± 5.63	8.94 ± 0.78	11.07 ± 1.23	15.36 ± 0.64	17 ± 0.46	5.78 ± 1.53
Brain	1.64 ± 1.12	1.41 ± 0.26	0.96 ± 0.05	0.48 ± 0.06	0.2 ± 0.01	0.01 ± 0
Thyroid	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.05 ± 0.02	0.11 ± 0.05	0.41 ± 0.22
Pancreas	0.22 ± 0.16	0.24 ± 0.03	0.17 ± 0.02	0.1 ± 0.01	0.04 ± 0.01	0 ± 0
Thymus	1.87 ± 1.24	1.9 ± 0.29	1.99 ± 0.4	1.28 ± 0.16	0.58 ± 0.13	0.01 ± 0
Inflammation	0.22 ± 0.13	0.21 ± 0.07	0.16 ± 0.03	0.13 ± 0.02	0.11 ± 0.05	0.01 ± 0.01

Table 2 details the percent of ^{123}I-MK-447 4 retained per organ in the turpentine-inflamed paws. Unfortunately, the highest values were recorded in the liver, muscle and skin, with low retention in the inflamed sites. When percentages of uptake were tabulated per gram of tissue (figure not shown), the inflammation-to-muscle ratios were above unity at 6 and 24 hours. The 2.3 ratio for the

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6 h uptake of 4 could not be used for scintigraphy of inflammation, due to the high uptake of the label in the skin, which masks it for the camera. The ratios for inflamed tissue over muscle and over blood are given in Table 2. We conclude that even though MK-447 has a significant anti-inflammatory activity (Fig. 1), and a 2.3 ratio for inflamed site over muscle (Table 2), its usefulness for scintigraphic detection of inflammation is hindered by its high uptake in the skin (Table 3).

The biodistribution of **^{123}I-4** and **^{123}I-6** were compared. The muscle to blood ratio for **^{123}I-6** at 3 h and 6 h was 1.0 and 1.1, respectively. The liver retention of **^{123}I-6** was lower than observed for **^{123}I-4**, i.e. 0.4 %/g for **^{123}I-6**, and 2.6 %/g for **^{123}I-4** at 3 h; while the skin uptake was 0.3 %/g and 0.15 %/g, respectively. The biological results did not support the use of the compound as an improved radiopharmaceutical for imaging inflammation. Consequently it was not justifiable to undertake further animal studies based upon analogues of the model compound 4.

Attempted Kinetic Resolution of MK-447 Analogues

To examine the feasibility of resolving the racemic MK-447 analogues 2 and 5 by kinetic resolution, *in situ* ^{1}H and ^{13}C NMR experiments were performed on the solutions obtained from the addition of 2 molar equivalents of racemic [2-MeOC_6H_6)Fe$(CO)_3$][PF_6] 7 to 1 molar equivalent of racemic *N*-benzyl-(5-*tert*-butylphenyl-2-hydroxy)glycine 2. If chiral discrimination occured in this reaction, one would expect to observe an unequal mixture of two diastereoisomeric products as shown in scheme 1. If enantiomerically pure 7 was used, this method could then be used to prepare optically active and radioactive 2 and 5. The optical purity of 2 or 5, however, would depend upon the degree of diastereoselection in the reaction of 7 with 2 or 5.

Initial studies involved the reactions of equimolar amounts of 7 with 2 and 5 to give the diene amine adducts 8 and 9, respectively. Scheme 1. These reactions were carried out in a solvent mixture of CD_3OD(CD_3CN (4 : 1) because the amino acids 2 and 5 were insoluble in CD_3CN. The ^{1}H NMR experiments showed that the reactions proceeded to completion, since unreacted 7 was not observed.

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Scheme 1

The ^{1}H NMR spectroscopic data confirmed the [(substituted diene)$Fe(CO)_3$] nature of products 8 and 9. The $J_{5',6'}$ coupling constants for 8 and 9 were found to be ca 9 Hz, indicating that they had 5-*exo* stereochemistry.

There were possibilities for the formation of other adducts arising from the addition of the phenol (OH) or carboxyl (COOH) groups in 2 or 5 to C-5 on the dienyl ring of the iron complex 7. However, the ^{1}H NMR spectroscopic data excluded the presence of such products. Thus, the N-CHCOOH signals at 5.11 ppm for 8 and at 5.21 ppm for 9 were strongly deshielded, as expected, as compared to their starting compounds 2 (at 4.55 ppm) and 5 (at 4.63 ppm), respectively, and a deshielding effect was also observed, as expected, for the NHCH2-Ph signals for 8 and 9. Quantitative interpretation of the deshielding effect is, however, complicated by the necessary change in solvent employed for the free and complex-bound pharmaceuticals.

Attachment of the MK-447 analogue 2 to the complex 7 via the N centre in 2 was also supported by the ^{13}C NMR spectrum of the desired product 8. Thus, the resonance for the benzylCH_2 atom adjacent to the N centre in 8, was shifted strongly downfield upon adduct formation. A small shift

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was observed for the COOH centre {from 169.4 ppm in free 2 to 170.4 ppm in the adduct 8}. The assignment of the diene ring carbon chemical shifts in adduct 8 was made by analogy with the ^{13}C NMR spectra of other [(2-$MeOC_6H_6$)$Fe(CO)_3$] complexes[21]. A DEPT experiment on the free ligand 2 assisted in the other assignments.

The ^{1}H and ^{13}C NMR spectra of 8 and 9 from these reactions revealed only one set of proton and carbon signals in each case. However, two diastereomeric products were expected in each reaction. This indicated fortuitous coincidence of the diastereomeric resonances.

Subsequent reactions performed on 2 : 1 and 1 : 2 molar ratio mixtures of 7 to 2 similarly showed only one set of the product signals for the adduct 8 in their *in situ* ^{1}H NMR spectra. The inability to distinguish the separate signals for the two diastereomeric products **8a** and **8b** unfortunately prevented the determination of the degree of diastereoselection in these reactions. Consequently these reactions were not further investigated for their use in the kinetic resolution of the MK-447 analogues.

The Compounds 2 and 5 were synthesized as racemic mixtures. The kinetic resolution of these compounds was not achieved by their reactions with the chiral dienyl iron complex 7.

EXPERIMENTAL

Materials

Glyoxylic acid monohydrate, 4-*tert*-butylphenol, morpholine, Chloramine-T and parafomaldehyde were commercial samples from Aldrich. 2-Chloro-*N*-(hydroxymethyl)acetamide was used as supplied by Lancaster. Sodium iodide-123 (in 0.1 N NaOH) was produced by the ^{124}Xe - ^{123}I production method. The radio-HPLC system consisted of a Waters 510 pump, a Linear UV/visible-200 variable wavelength spectrometer, and a modified Berthold LB 506C1 HPLC radioactivity monitor equipped with a NaI(Tl) detector.

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Anti-inflammatory Activity

The anti-inflammatory activity was evaluated by plethysmometry, using the Ugo Basile (Varese, Italy) differential volumeter model 7150. This is a volume meter specifically designed for accurate measurement of rat-paw swelling, due to inflammatory oedema. The volume, in ml to the nearest 0.01 ml, of both paws (up to the tibio-tarsal articulation) was determined for both the drug-injected and the control paws. Statistical significance of the average paw volume changes as compared to the zero increase, are established using the paired "t" test, and common standard errors of means are calculated by analysis of the variance.

Synthetic Studies

2-Aminomethyl-4-*tert*-butylphenol hydrochloride 1 : This compound was synthesized by the modified literature procedure.[1] A mixture of 4-*tert*-butylphenyl (7.5 g, 0.05 mol) in conc. H_2SO_4 (5 ml) and acetic acid (50 ml) and 2-chloro-*N*-(hydroxymethyl)acetamide (6.1 g, 0.05 mol) was stirred at R. T. for 6 hr. The resultant solution was then poured into ice water (200 ml) and the mixture was extracted with diethyl ether (5 x 50 ml). The ether phases were combined, saturated witih brine and evaporated under reduced pressure to give a light yellow oil. This crude oil was dissolved into a 1 : 1 mixture of 10 M HCl / ethanol (50 ml), and then heated to reflux for 3 hr, followed by stirring overnight. The solvent was removed by evaporation under reduced pressure and the resultant solid was recrystallized from ethanol / ethyl acetate to give the *title compound* as a white crystalline solid (9.4 g, 89 % yield). M.p. 228 - 230 C {c.f. lit[2]. 227 - 229 C (decomp.)}. IR (KBr): ν (OH) 3157, (C-N) 1267 cm^{-1}; ^1H NMR (d_6-DMSO): δ 9.96 (s, ^1H, OH), 8.2 (s, 2H, NH_2), 7.32 (d, $J_{3,5}$ = 2.4 Hz, 1H, H^3), 7.20 (dd, $J_{3,5}$ = 2.4 Hz, $J_{5,6}$ = 8.4 Hz, 1H, H^5), 6.80 (d, $J_{5,6}$ = 8.4 Hz, 1H, H^6), 3.92 (ABq, $J_{A,B}$ = 13.2 Hz, 2H, C<u>H</u>$_2$NH$_2$), 1.23 (s, 9H, Bu^t); ES MS (+ve, MeOH): *m/z* 180 ($[M + H]^+$ 55 %), 163 ($[M - NH_2]^+$, 100 %).

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N-(Benzyl-5-*tert*-butyl-2-hydroxy)phenylglycine 2 : A solution of glycoxylic acid monohydrate (0.39 g, 4.26 mmol) in water (2 ml) was treated with a solution of sodium hydroxide (0.17 g, 4.26 mmol) in water (1.5 ml) forming a pale yellow solution. A solution of 4-*tert*-butylphenol (0.60 g, 3.96 mmol) and benzylamine (0.42 g, 3.89 mmol) in methanol (3 ml) was then added and the resulting off-white mixture was heated to reflux for 18 hr. The mixture was then allowed to cool, extracted with ether (3 x 5 ml) and the aqueous layer was acidified with conc. HCl to pH 4. A fine off-white solid was readily separated from the solution (1.06 g, 87 % yield). This crude solid was purified by dissolving in hot EtOH (5 ml) with a few drops of conc. HCl, followed by treatment with ammonia (28 %) to pH 4.5. The white precipitate was collected and washed with 50 % aqueous EtOH to give the *title compound* as a white crystalline solid (0.98 g, 81 % yield). M.p. 219 - 220 C. IR (KBr): ν (OH) 3400, (C=O) 1628 cm^{-1}; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 7.36 -7.40 (m, 5H, Ar), 7.23 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^6), 7.13 (dd, $J_{4,6}$ = 2.4 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H^4), 6.72 (d, $J_{3,4}$ = 8.4 Hz, 1H, H^3), 4.55 (s, 1H, C$\underline{H}$COOH), 4.06 (ABq, $J_{A,B}$ = 13.2 Hz, 2H, C$\underline{H}_2$NH), 1.24 (s, 9H, Bu^t); ^{1}H NMR (CD_3OD): δ 7.39 - 7.45 (m, 5H, Ph), 7.33 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^6), 7.26 (dd, $J_{4,6}$ = 2.4 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H^4), 6.79 (d, $J_{3,4}$ = 8.4 Hz, 1H, H^3), 4.78 (s, 1H, C$\underline{H}$COOH), 4.12 (ABq, $J_{A,B}$ = 13.2 Hz, 2H, C$\underline{H}_2$NH), 1.27 (s, 9H, Bu^t); ^{13}C NMR (d_6-DMSO): δ 169.4 (COOH), 153.5 (C), 141.2 (C), 133.3 (C), 130.1 (CH), 128.6 (CH), 125.5 (CH), 123.7.3 (CH), 121.5 (CH), 116.4 (CH), 60.3 (CH), 50.1 ($\underline{C}H_2$Ph), 34.0 ($\underline{C}(CH)_3$), 31.5 ($\underline{C}(CH)_3$); FAB MS (+ve, glycerol): *m/z* 314 ($[M]^+$, 74 %); uv/vis (acetic acid): λ_{max} 281 nm.

N-(5-*tert*-Butyl-2-hydroxyphenyl)methylmorpholine 3 : Paraformaldehyde (0.68 g, 23 mmol) was added to a solution of morpholine (2.00 g, 23 mmol) in methanol (30 ml) with stirring. The mixture was heated to reflux for 1 hr until a homogeneous solution appeared. 4-*tert*-Butylphenol (3.46 g, 23 mmol) was then added slowly and the reaction mixture was refluxed for 18 hr. The mixture was then cooled and evaporated under reduced pressure to give a light orange-yellow oil (6.01 g). This crude oil crystallized from ethanol (25 ml) with a few drops of conc. HCl, diluted

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with H_2O (25 ml) and neutralised with saturated sodium bicarbonate to pH 9. A light creamy crystalline solid of the *title compound* was obtained (4.78 g, 83 % yield).). M.p. 136 - 138 C. IR (KBr): ν (OH) 3462, (C-N) 1257, (C-O) 1114 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.20 (dd, $J_{4,6}$ = 2.4 Hz, $J_{3,4}$ = 8.4 Hz, 1H, H^4), 6.97 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^6), 6.76 (d, $J_{3,4}$ = 8.4 Hz, 1H, H^3), 3.75 (t, J = 4.5 Hz, 4H, $(C\underline{H}_2)_2O$), 3.70 (s, 2H, $C\underline{H}_2N$), 2.57 (br s, 4H, $N(C\underline{H}_2)_2$), 1.27 (s, 9H, Bu^t); ^{13}C NMR ($CDCl_3$): δ 154.9 (C), 142.0 (C),

125.7 (CH), 125.5 (CH), 119.8 (C), 115.4 (CH), 66.8 ($2CH_2O$), 62.3 (CH_2N), 52.9 ($2CH_2N$), 33.9 ($\underline{C}(CH_3)_3$), 31.5 ($C(\underline{C}H_3)_3$); ES MS (+ve, CH_3CN): *m/z* 250 ($[M + H]^+$, 100 %); uv/vis (MeOH): λ_{max} 279 nm.

2-Aminomethyl-4-*tert*-butyl-6-iodophenol hydrochloride (MK-447) 4 : The compound was obtained as an off-white powder in 87 % yield, by the modified literature procedure[1] as described for the synthesis of **1**, except that 4-*tert*-butyl-6-iodophenol was used instead of 4-*tert*-butylphenol. M.p. 196 - 198 C {c.f. lit.[1] 200 - 210 C (decomp.)}. IR (KBr): ν (OH) 3000, (C=O) 1600, (C-N) 1232, (C-I) 516 cm^{-1}; ^1H NMR (d_6-DMSO): δ9.82 (s, 1H, OH), 8.23 (s, 2H, NH_2), 7.67 ($d_{3,5}$ = 2.4 Hz, 1H, H^3), 7.38 (d, $J_{3,5}$ = 2.4 Hz, 1H, H^5), 4.02 (Abq, $J_{A,B}$ = 13.2 Hz, 2H, $C\underline{H}_2NH_2$), 1.23 (s, 9H, Bu^t); ES MS (+ve, MeOH): *m/z* 306 ($[M]^+$, 100 %), 289 ($[M - NH_2 + H]^+$, 90 %).

***N*-Benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine 5** : *Method 1:* A solution of iodine (0.16 g, 0.63 mmol) in glacial acetic acid (6 ml) was added dropwise to a solution of *N*-benzyl-(*tert*-butyl-2-hydroxy)phenylglycine **2** (0.20 g, 0.64 mmol) in glacial acetic acid (2 ml) with stirring. The initial colour of iodine disappeared after the addition of each portion. The resultant permanent brown solution was stirred for 30 min, then neutralized with sodium hydroxide (10 %) to pH 4.5. A light yellow solid separated from the solution and was collected and rinsed with 50 % aqueous ethanol (0.14 g, 50 % crude yield). This crude solid was then recrystallized by dissolving in hot ethanol (5 ml), followed by the addition of conc. HCl (0.3 ml), and water (5 ml). A white precipitate was collected, and then rinsed with 50 % aqueous ethanol to give the desired *title compound* as a

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white powder (0.13 g, 46 % yield). M.p. 200 - 202 C. A similar yield (0.16 g, 57 % yield) was obtained when the reaction was carried out in ethanol.

Method 2: To a solution of *N*-benzyl-(5-*tert*-butyl-2-hydroxy)phenylglycine **2** (0.15 g, 0.48 mmol) in acetic acid (5 ml), was added sodium iodide (0.14 g, 0.94 mmol) in acetic acid (2 ml) and Chloramine-T (0.18 g, 0.79 mmol) in acetic acid (5 ml). The resulting brown solution was stirred at R. T. for 45 min until a light yellow precipitate formed. The mixture was allowed to stand at R. T. for several hr, and the resultant precipitate was then collected, washed with ethanol/water (1 : 1) and evaporated under reduced pressure. The pure *title compound* was obtained as a fine, white powder (0.28 g, 80 % yield). M.p. 201 - 202 C. Anal. Calcd for $C_{19}H_{22}O_3NI$: C, 52.0; H, 5.1; N, 3.2; Found: C, 52.2; H, 5.2; N, 2.9 %. IR (KBr): ν (OH) 3034, (C=O) 1631, (C-I) 513 cm^{-1}; ^1H NMR (d_6-DMSO, 400 MHz): δ 7.57 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^4), 7.39 - 7.49 (m, 5H, Ph), 7.27 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^6), 4.63 (s, 1H, C<u>H</u>COOH), 4.15 (Abq, $J_{A,B}$ = 14 Hz, 2H, NHC<u>H</u>$_2$Ph), 1.21 (s, 9H, Bu^t); ^{13}C NMR (d_6-DMSO): δ 168.9 (COOH), 153.3 (C), 143.0 (C), 134.1 (CH), 132.3 (C), 130.0 (CH), 128.7 (CH), 128.5 (CH), 123.1 (CH), 121.3 (C), 87.5 (C-I), 61.1 (CH), 50.1 (CH_2), 33.9 (<u>C</u>($CH_3)_3$), 31.2 ((<u>C</u>H3)3); FAB MS (+ve, thioglycerol / glacial acetic acid): *m/z* 440 ($[M + H]^+$, 72 %); uv/vis (acetic acid): λ_{max} 281 nm.

_N_-(5-_tert_-Butyl-2-hydroyphenyl-3-iodo)methylmorpholine 6 : *Method 1:* Chloramine-T (174 mg, 0.62 mmol) was added[16] to a solution of *N*-(5-*tert*-butyl-2-hydroxyphenyl)methylmorpholine **3** (129 mg, 0.52 mmol) and NaI (95 mg, 0.63 mmol) in dimethoxyethane (3 ml). The resulting light orange-yellow solution was stirred at 25 C for 1 hr, diluted with water (3 ml), acidified with HCl solution (5 %, 10 ml) and extracted with ethyl acetate (4 x 15 ml). The organic phases were combined, washed successively with 5 % sodium thiosulfate solution (2 x 25 ml) and brine, dried over anhydrous sodium sulphate and then filtered. The filtrate was evaporated under reduced pressure to give an orange-yellow crystalline solid (269 mg). This crude solid, as determined by ^1H NMR, ES MS and HPLC, was a mixture[20] of the desired compound *N*-(5-*tert*-butyl-2-hydroxyphenyl-3-iodo)methylmorpholine with a retention time on HPLC of 30 min {ES MS (+ve, CH_3CN): *m/z* 376

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[M + H]$^+$}, and a by-product *N*-(5-*tert*-butyl-3-chloro-2-hydroxyphenyl)methylmorpholine with a retention time on HPLC of 22 min {ES MS (+ve, CH_3CN): *m/z* 284 [M + H]$^+$}.

Method 2: 4-*tert*-Butyl-2-iodophenol was obtained as a light brown oil in 90 % yield according to the literature procedure[2] by the treatment of 4-*tert*-butylphenol with iodine monochloride. Column chromatographic purification of this crude oil on silica gel with ethyl acetate and hexane (19 : 1) yielded a white crystalline solid (23.13 g, 81 % yield). M.p. 73 - 74 C (c.f. lit[4] m.p. 73 - 75 C). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.62 (d, $J_{3,5}$ = 2.4 Hz 1H, H^3), 7.24 (dd, $J_{3,5}$ = 2.4 Hz, $J_{5,6}$ = 8.8 Hz, 1H, H^5), 6.90 (d, $J_{5,6}$ = 8.8 Hz 1H, H^6), 1.27 (s, 9H, Bu^t); ES MS (+ve, CH_3CN): *m/z* 276 ([M + H]$^+$, 52 %). Paraformaldehyde (91 mg, 3.03 mmol) was added to a solution of morpholine (260 mg, 3.01 mmol) in methanol (10 ml). The mixture was heated to reflux for 1 hr until the solution was homogeneous, 4-*tert*-butyl-2-iodophenol (830 mg, 3.00 mmol) was then added, and the resulting solution was refluxed for 20 hr. The reaction mixture was cooled and evaporated under reduced pressure to give an orange-brown oil (977 mg). Column chromatographic purification on silica gel with ethyl acetate and dichloromethane (1 : 9) yielded the *title compound* as a light yellow solid (760 mg, 68 % yield). M.p. 145 - 146 C. Anal. Calcd for $C_{15}H_{22}O_2NI$: C, 48.0; H, 5.9; N, 3.7; Found: C, 48.0; H, 6.0; N, 3.5 %. IR (KBr): ν (OH) 3025, (C-N) 1259, (C-O) 1113, (C-I) 514 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.63 (d, $J_{4,5}$ = 2.4 Hz, 1H, H^4), 6.95 (d, $J_{4,6}$ = 2.4 Hz, 1H, H^6), 3.76 (br t, J = 4.2 Hz, 4H, ($C\underline{H}_2)_2O$), 3.67 (s, 2H, $C\underline{H}_2N$), 2.59 (br s, 4H, $N(C\underline{H}_2)_2$), 1.26 (s, 9H, Bu^t); ^{13}C NMR ($CDCl_3$): δ 154.1 (C), 143.8 (C), 134.9 (CH), 125.7 (CH), 119.9 (C), 84.4 (C-I), 66.4 (CH_2O), 62.0 (CH_2), 52.6 ($2CH_2N$), 33.7 ($\underline{C}(CH_3)_3$), 31.2 ($C\underline{C}H_3)_3$); ES MS (+ve, MeOH): *m/z* 376 ([M + H]$^+$, 85 %), 375 ([M]$^+$, 100 %), 289 ([M - morpholine]$^+$, 95 %); uv/vis (MeOH): λ_{max} 279 nm.

Radiolabelling Studies

[^{123}I]-2-Aminomethyl-4-*tert*-butyl-6-iodophenol [^{123}I]-4: To a solution of 2-aminomethyl-4-*tert*-butylphenol 1 (1.0 mg) in 1N HCl (100 μl), was added $Na^{123}I$ (2.74 mCi), followed by KIO_3 (1.0 mg). The resultant solution was shaken at R. T. for 5 min, then injected onto the HPLC system. The activity corresponding to [^{123}I]-2-aminomethyl-4-*tert*-butyl-6-iodophenol [^{123}I]-4 was eluted at a

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retention time of 17 min (c.f. starting compound **1** at 11.5 min) with a radiochemical yield of 1.78 mCi, 65 %. The radioactivity purity was greater than 97 %. The HPLC system for this analysis carried out on an Econosil C_{18} column of size 10 μm x 10 mm x 25 cm, solvent mixture of 80 % methanol / 20 % 0.1 M NaH_2PO_4, flow rate of 2.5 ml / min, pressure at < 1000 psi and uv detection at 279 nm. Preparation and purification by HPLC took ≈ 20 min.

Preparation for in vivo *biodistribution study:* The solution of **[^{123}I]-4** (1.78 mCi) in 80 % methanol / 20 % 0.1 M NaH_2PO_4 was rotary evaporated and rinsed several time with distilled water to give a clear oily residue (0.86 mCi). This residue was then dissolved in 0.9 % saline (8.5 ml) and filtered through a cellulose filter into a vacuum sealed sample bottle. This saline solution of [^{123}I]-2-aminomethyl-4-*tert*-butyl-6-iodophenol **[^{123}I]-4** (0.86 mCi/8.5 ml, *i.e.*, 10 μCi / 100 μl) was ready for the biodistribution study.

[^{123}I]-*N*-Benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine 5: *Method 1:* To a solution of *N*-Benzyl-(5-*tert*-butyl-2-hydroxy)phenylglycine **2** (0.43 mg) in 1N HCl (100 μl) and methanol (50 μl), was added $Na^{123}I$ (108 μCi), followed by Chloramine-T (1 mM) in 0.1M NaH_2PO (100 μl). The resultant solution was shaken at R.T. for 3 min, then injected onto the HPLC system. The activity corresponding to [^{123}I]-*N*-Benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine **5** was eluted at a retention time of 15 min (c.f. starting compound **2** at 5 min) with a radiochemical yield of 45 μCi, 41 %. The radiochemical purity was > 97 % and the specific activity was 181 Ci/mmol. The HPLC analysis with a Goldpak C_{18} column of Exsil 100/5 ODS of size 5 μm x 4.6 mm x 25 cm, solvent mixture of 65 % methanol / 35 % 0.1 M NaH_2PO_4, flow rate of 1 ml/min, pressure at < 2500 psi and uv detection at 281 nm.

Method 2: Use of KIO_3 (50 μl of 1.5 mg in 400 μl 1N HCl) as the oxidising agent resulted in a 52% radiochemical yield, but with lower specific activity.

Method 3: Use of peracetic acid (0.5 %, 50 μl) as the oxidising agent resulted in a 53 % radiochemical yield, but with lower specific activity.

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[^{123}I]-*N*-(5-*tert*-Butyl-2-hydroxyphenyl-3-iodo)methylmorpholine 6: *Method 1*: To a solution of *N*-(5-*tert*-butyl-2-hydroxyphenyl)methylmorpholine 3 (0.50 mg) in 1N HCl (100 µl), was added Na^{123}I (200 µCi), followed by Chloramine-T (1 mM) in 0.1 M NaH_2PO_4 (100 µl). The resultant solution was shaken at R.T. for 4 min, then injected onto the HPLC system. The activity corresponding to [^{123}I]-*N*-(5-*tert*-Butyl-2-hydroxyphenyl-3-iodo)methylmorpholine 6 was eluted at a retention time of 17.5 min (c.f. starting compound 3 at 6.5 min) with a radiochemical yield of 128 µCi, 64 %. The radiochemical purity was > 98 % and the specific activity was 180 Ci/mmol. The analytical HPLC was made on a Goldpack C_{18} column with Exsil 100/5 ODS of size 5 µm x 4.6 mm x 25 cm, solvent mixture of 75 % methanol / 25 % 0.1M NaH_2PO_4, flow rate of 1 ml/min, pressure at < 2100 psi and uv detection at 279 nm. The preparative scale separation was on Econosil C_{18} column of size 10 µm X 10 mm x 25 cm, solvent mixture of 80 % methanol / 20 % 0.1M NaH_2PO_4, flow rate of 2.3 ml/min, pressure at < 1000 psi and uv detection at 279 nm.

Method 2: Use of KIO_3 (0.5 mg in 100 µl 1N HCl) as the oxidising agent resulted in a 69 % radiochemical yield, but with lower specific activity.

Method 3: Use of peracetic acid (0.5 %, 50 µl) as the oxidising agent resulted in a 65 % radiochemical yield, but with lower specific activity.

Method 4: A similar procedure as described in Method 1 was applied, except that this radiolabelling reaction was carried out on a preparative scale with higher level of radioactivity. To a solution of *N*-(5-*tert*-butyl-2-hydroxyphenyl)methylmorpholine 3 (0.60 mg) in 1M HCl (100 µl), was added Na^{123}I (2.56 mCi). The activity corresponding to [^{123}I]-*N*-(5-*tert*-Butyl-2-hydroxyphenyl-3-iodo)methylmorpholine 6 was eluted at a retention time of 32 min (c.f. starting compound 3 at 11.5 min) with a radiochemical yield of 1.26 µCi, 49 %. The radiochemical purity was greater than 98 %. The HPLC system for this analysis was methanol / 0.1M NaH_2PO_4 (80 : 20) with a flow rate of 2.3 ml/min.

Radioactive sample 6 preparation for in vivo *biodistribution study:* The solution of 6 (1.26 mCi) in 80 % methanol / 20 % 0.1 M NaH_2PO_4 was rotary evaporated and rinsed several times with distilled

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water to give a clear oily residue (0.50 mCi). This residue was then dissolved in 0.9 % saline (5 ml) and filtered through a cellulose filter into a vacuum sealed sample bottle. This saline solution of [^{123}I]-*N*-(5-*tert*-Butyl-2-hydroxyphenyl-3-iodo)methylmorpholine **6** (0.50 mCi/5 ml, *i.e.*, 10 μCi / 100 μl) was used in the biodistribution studies.

Kinetic Resolution Studies

Reaction of tricarbonyl(η^5-2-methoxycyclohexadiene)iron hexafluoroborate 7 with *N*-(Benzyl-5-*tert*-butyl-2-hydroxy)phenylglycine 2: *Reaction 1:* A mixture of tricarbonyl(η^5-2-methoxycyclohexadiene)iron hexafluoroborate **7** (5.6 mg, 0.014 mmol) and *N*-(Benzyl-5-*tert*-butyl-2-hydroxy)phenylglycine **2** (4.5 mg, 0.014 mmol) in CD_3OD (0.4 ml) and CD_3CN (0.1 ml) in an NMR tube was warmed up to *ca* 40 C for 15 min to give a pink solution. The *in situ* ^{1}H and ^{13}C NMR spectra of this solution indicated the formation of the desired diene amine adduct **8**. ^{1}H NMR (CD_3OD/CD_3CN, 4 : 1): δ 7.44 (d, $J_{12,14}$ = 2.4 Hz, 1H, H^{14}), 7.43 -7.36 (m, 5H, Ph), 7.25 (d, $J_{12,14}$ = 2.4 Hz, 1H, H^{12}), 6.85 (d, $J_{11,12}$ = 8.4 Hz, 1H, H^{11}), 5.39 (dd, $J_{1,3}$ = 2.4 Hz, $J_{3,4}$ = 6.4 Hz, 1H, H^3), 5.11 (s, 1H, C<u>H</u>COOH), 4.15 (br, s, 2H, NHC<u>H</u>$_2$Ph), 3.69 (s, 3H, MeO), 3.30 (dt, $J_{1,3}$ = 2.4 Hz, $J_{1,6'}$ = 3.8 Hz, 1H, H^1), 3.17 (dt, $J_{4,5'}$ = 3.6 Hz, 1H, $J_{5',6'}$ = 9.6 Hz, 1H, H^5), 2.80 (dd, $J_{4,5'}$ = 3.6 Hz, $J_{3,4}$ = 6.4 Hz, 1H, H^4), 2.24 (dddd, $J_{1,6'}$ = 3.8 Hz, $J_{5',6'}$ = 9.6 Hz, $J_{6,6'}$ = 15.2 Hz, 1H, $H^{6'}$), 1.59 (dt, $J_{1,6}$ = 2.4 Hz, $J_{6,6'}$ = 15.2 Hz, 1H, H^6), 1.28 (s, 9H, Bu^t); ^{13}C NMR (CD_3OD/CD_3CN, 4 : 1): δ 212.0 (CO), 170.4 (COOH), 154.5 (C), 144.3 (C^2), 142.4 (C), 131.8 (C), 131.2 (CH), 130.7 (CH), 130.2 (CH), 129.8 (CH), 116.3 (CH), 79.3 (<u>C</u>H$_2$Ph), 67.7 (C3), 60.0 (CH), 55.2 (C^1), 51.0 ($C^{5'}$), 50.3 (C^6), 50.1 (C^4), 35.0 (O<u>C</u>H$_3$), 33.3 (<u>C</u>(CH)$_3$), 31.7 (C(<u>C</u>H)$_3$).

Reaction 2: A similar procedure as in *Reaction 1* above was applied, except that the ratio of **7** : **2** was 2 : 1. The ^{1}H NMR spectrum gave the same signals for the desired product **8**, and unreacted **7**.

Reaction 3: A procedure as in *Reaction 1* above was used, except that the ratio of **7** : **2** was 1 : 2 . The ^{1}H NMR spectrum showed the same signals for the desired product **8**, and unreacted **2**.

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Reaction of tricarbonyl(η^5-2-methoxycyclohexadiene)iron hexafluoroborate 7 with *N*-Benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine 5: *Reaction 1:* A mixture of tricarbonyl(η^5-2-methoxycyclohexadiene)iron hexafluoroborate 7 (6.0 mg, 0.015 mmol) and *N*-Benzyl-(5-*tert*-butyl-2-hydroxy-3-iodo)phenylglycine 5 (4.8 mg, 0.015 mmol) in CD_3OD (0.4 ml) and CD_3CN (0.1 ml) in an NMR tube was warmed up to *ca* 40 C for 15 min to give a colorless solution. The *in situ* ^1H NMR spectrum of this solution indicated the formation of the desired diene amine adduct 9.

^1H NMR (CD_3OD/CD_3CN, 4 : 1): δ 7.82 (d, $J_{12,14}$ = 2.4 Hz, 1H, H^{12}), 7.46 -7.42 (m, 5H, Ph), 7.31 (d, $J_{12,14}$ = 2.4 Hz, 1H, H^{14}), 5.41 (dd, $J_{1,3}$ = 2.4 Hz, $J_{3,4}$ = 6.4 Hz, 1H, H^3), 5.21 (br s, 1H, C<u>H</u>COOH), 4.20 (ABq, $J_{A,B}$ = 13.6 Hz, 2H, NHC$\underline{H_A}\underline{H_B}$Ph), 3.71 (s, 3H, MeO), 3.31 (dt, $J_{1,3}$ = 2.4 Hz, $J_{1,6'}$ = 3.8 Hz, 1H, H^1), 3.19 (dt, $J_{4,5'}$ = 3.6 Hz, 1H, $J_{5',6'}$ = 9.2 Hz, 1H, $H^{5'}$), 2.81 (dd, $J_{4,5'}$ = 3.6 Hz, $J_{3,4}$ = 6.4 Hz, 1H, H^4), 2.25 (dddd, $J_{1,6'}$ = 3.8 Hz, $J_{5',6'}$ = 9.2 Hz, $J_{6,6'}$ = 14.8 Hz, 1H, $H^{6'}$), 1.60 (dt, $J_{1,6}$ = 2.4 Hz, $J_{6,6'}$ = 14.8 Hz, 1H, H^6), 1.28 (s, 9H, Bu^t).

ACKNOWLEDGEMENTS

This work was supported in part by an APRA (Industry) Award. Drs. Andrew Katsifis and Michael Kassiou are thanked for their assistance in training A.F.H.S. in radiochemical techniques and helpful discussions. The cooperation of Mrs Jeanette Chapman and Ms Joanne O'Brian is acknowledged with appreciation.

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