# Synthesis and *in Vitro* and *in Vivo* Characteristics of an Iodinated Analogue of the β-Adrenoceptor Antagonist Carazolol

Eric A. Dubois,*[†,§] Jan C. van den Bos,[⊥,||] Tamme Doornbos,[⊥,||] Peter A. P. M. van Doremalen,[⊥]
G. Aernout Somsen,[‡] Jozef A. J. M. Vekemans,[||] Anton G. M. Janssen,[⊥] Harry D. Batink,[§] Gerard J. Boer,[†]
Martin Pfaffendorf,[§] Eric A. van Royen,[†] and Pieter A. van Zwieten[‡,§]

*Departments of Nuclear Medicine, Pharmacotherapy, and Cardiology, Academic Medical Center, University of Amsterdam,
Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, Cygne B.V., Eindhoven University of Technology, P.O. Box 513,
5600 MB Eindhoven, The Netherlands, and Laboratory of Organic Chemistry, Eindhoven University of Technology,
Eindhoven, The Netherlands*

*Received February 7, 1996*[⊗]

A new (radio)iodinated, β-adrenoceptor ligand, (*S*)-(−)-4-[3-[(1,1-dimethyl-3-iodo-(2*E*)-propenyl)-amino]-2-hydroxypropoxy]carbazole (CYBL8E, **1**), was prepared. **1** is an iodinated analogue of the high-affinity β-adrenoceptor antagonist carazolol (**2**). The asymmetric synthesis was achieved in four steps starting from 4-hydroxycarbazole. The iodine-123-labeled form was obtained by an iododestannylation reaction with [$^{123}$I]NaI in the presence of $H_2O_2$. Using classical *in vitro* displacement experiments with membrane fractions of cardiac left ventricular muscle, **1** proved to have a high affinity for the receptor ($K_i = 0.31 \pm 0.03$). Biodistribution studies performed in New Zealand white rabbits demonstrated the specificity of the binding *in vivo* to the receptor. Uptake of [$^{123}$I]**1** was reduced significantly in both atrial muscle, left ventricular muscle, frontal cortex, cerebellum, and striatum, by the pretreatment of the animals with different β-adrenoceptor antagonists. In conclusion, **1** is a potent nonselective β-adrenoceptor antagonist, which binds specifically to the β-adrenoceptor *in vivo*, and is therefore a promising radioligand for the imaging of β-adrenoceptors using single photon emission computerized tomography.

## Introduction

In chronic heart failure neuroendocrine changes of the cardiac sympathetic system and the renin–angiotensin–aldosterone system have been shown to be of great clinical importance for the pathophysiological understanding of the disease, the evaluation of therapy, and the management of patients. One of the important features of an increased cardiac sympathetic activity that is observed in these patients is the well-documented downregulation of cardiac β-adrenoceptors.[1–6] This dramatic decrease in cardiac β-adrenoceptor density has mainly been established in tissue samples, obtained during surgery or postmortem. Since a clear relationship between the degree of sympathetic activation and the severity of the disease and its prognosis has been established, it may be conceived that the *in vivo* measurement of cardiac β-adrenoceptor density can be used as a prognostic marker. The noninvasive measurement of cardiac β-adrenoceptor density in volunteers and subsequently in patients with chronic heart failure using single photon emission computerized tomography (SPECT) or positron emission tomography (PET) allows analysis of receptor characteristics. These techniques would allow followup after pharmacological intervention with drugs such as low-dose cardiac β-adrenoceptor antagonists, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, or other medications that may influence cardiac sympathetic activity.

Numerous cardiac β-adrenoceptor antagonists have been labeled with either $^{11}$C or $^{18}$F for PET imaging, with rather limited success due to low specific activity or high nonspecific binding. CGP12177 (4-[3-(*tert*-butylamin)-2-hydroxypropoxy]benzimidazol-2-one) has for instance been labeled with $^{11}$C for PET, but the specific activity of this compound showed a rather wide variety with values ranging from 400 to 1300 Ci/mmol.[7–9]

Carazolol, a potent, lipophilic β-adrenoceptor antagonist ($K_i < 0.2$ nM),[10–15] has been labeled with $^{11}$C by Berridge et al.[16] and more recently with $^{18}$F to image β-adrenoceptors *in vivo* using PET.[17,18] However, both ligands were not yet extensively applied in humans.

At present no optimal radioligands for the imaging of cardiac β-adrenoceptors using SPECT are available. We have recently synthesized a number of analogues of CGP12177 labeled with $^{123}$I.[19] Although some of the synthesized compounds had high affinity for the receptor *in vitro* with $K_i$ values of approximately 1 nM, their specific binding *in vivo* proved too low for imaging purposes.

In the present study the synthesis of an $^{123}$I-labeled analogue of carazolol is described (Figure 1). Furthermore, the affinity of this new compound for the β-adrenoceptor *in vitro* as well as the biodistribution and specificity of binding *in vivo*, in New Zealand white rabbits, is reported.

## Chemistry

It has been shown for a number of phenoxypropanolamines that the β-blocking activity of the two optical

---

* Author for correspondence. Telephone: (31)205663572. Fax: (31)-206976508. E-mail: E.A.Dubois@amc.uva.nl.
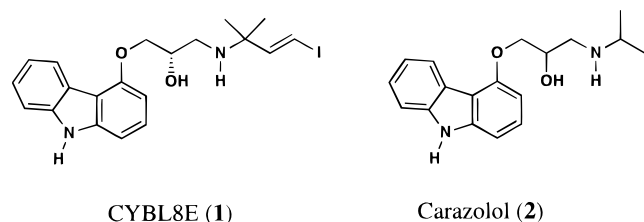† Department of Nuclear Medicine.
‡ Department of Cardiology.
§ Department of Pharmacotherapy.
⊥ Cygne B.V.
|| Laboratory of Organic Chemistry.
⊗ Abstract published in *Advance ACS Abstracts,* August 1, 1996.

CYBL8E (**1**)                 Carazolol (**2**)

**Figure 1.** Structural formulas of the nonselective β-adrenoceptor antagonist carazolol and the newly synthesized iodinated derivative **1**.

antipodes differs by a factor of at least 50.[20] The *S*-enantiomers generally appear to be the most potent ones. Furthermore, it is known that variations in the alkyl substituent on the nitrogen atom do not greatly affect the affinity. A prerequisite for a ligand suitable for SPECT is stability and a high affinity for the receptor.[21] Therefore, we wish to introduce a small entity in the alkylamino part of (*S*)-carazolol, which bears an iodine atom and has a reasonable chemical stability. In the present study we have chosen an iodovinyl group,[28] which is easily formed from the corresponding trialkylstannyl precursor by an iododestannylation reaction.

**Synthesis of 1 (Figure 2).** The asymmetric synthesis of **1** was achieved by reaction of **4** with **5**.[22] The intermediate **4** was synthesized in three steps from cyclohexane-1,3-dione according to Lauer et al.[23] and Berridge et al.,[16] but for the preparation of 1,2,3,4-tetrahydro-4-oxocarbazole (**3**), the improved procedure of Zinnes[24] was used. It is important to mention that the dehydrogenation of **3** only gave a satisfactory yield of **4** when freshly prepared Raney nickel was used. It is well known that the reactions of aryl oxides with the chiral auxiliary **5** results in the formation of the *S*-epoxide with almost complete retention of configuration.[22,25] The specific rotation of the formed epoxide **6** is significantly higher than that measured by Berridge et al.[16] on the same compound prepared with another method, which was approximately 90% enantiomerically pure. The propanolamine side chain was formed regioselectively by reaction of 1,1-dimethylpropargylamine with the epoxide **6**. Hydrostannylation of the propargylamino compound in the presence of the radical initiator AIBN in toluene resulted in the selective formation of the (*E*)-(tributylstannyl)vinyl isomer **8**, which was subsequently iododestannylated to the corresponding (*E*)-iodovinyl compound **1** with retention of configuration. The radiolabeled [$^{123}$I]**1** was obtained by

the treatment of **8** with [$^{123}$I]NaI in the presence of $H_2O_2$. The specific activity of the [$^{123}$I]**1** was deduced from that of the [$^{123}$I]NaI used and amounted to be 185 TBq/mmol (5000 Ci/mmol).

**Biological Results**

***In Vitro* Radioligand Displacement Experiments.** In Figure 3 the displacement curves for **1** and CGP12177 obtained with membrane preparations of cardiac left ventricular muscle and ICYP are shown. **1** proved to be slightly more effective in displacing ICYP from the β-adrenoceptor than CGP12177. The $K_i$ values (nmol/L, mean ± SEM, $n = 3-5$) were $0.31 ± 0.03$ for **1** versus $0.76 ± 0.10$ for CGP12177. The $K_i$ values of both compounds were not significantly different.
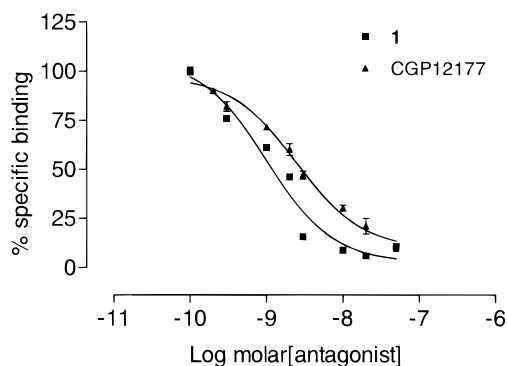
The displacement curves that were obtained with membrane preparations of pulmonary tissue are shown in Figure 4. A similar pattern was observed as for left ventricular muscle, with $K_i$ values for **1** and CGP12177 of $0.37 ± 0.10$ and $1.43 ± 0.21$, respectively. ICI118,-551 displaced ICYP less effectively ($K_i = 400 ± 21$).

***In Vivo* Biodistribution Studies.** In Table 1 the biodistribution pattern of [$^{123}$I]**1** is shown. All radioactivity levels are expressed as % ID × kg of body weight/g of tissue. After the intravenous administration of 50 μCi [$^{123}$I]**1**, radioactivity reached maximal values (average ± SEM, $n = 3$) of $0.52 ± 0.09$% in the cardiac left ventricular muscle at 5 min postinjection (pi). After this time point radioactivity dropped to $0.26 ± 0.02$ at 30 min pi. From 30 min pi until 2 h pi, radioactivity levels gradually decreased (values ranging from $0.26 ± 0.02$ to $0.21 ± 0.03$). At 24 h pi a level of $0.02 ± 0.0006$ was still present in the left ventricular muscle. In the right ventricular muscle a similar pattern was observed. In both left and right atrial muscle, substantial levels of radioactivity were found with virtually constant levels of radioactivity levels between 30 min and 2 h pi. High uptake was observed in the lungs of these animals. At 5 min pi a radioactivity level of $5.75 ± 0.37$ was present in the lungs. This level of radioactivity rapidly dropped to a value of $1.74 ± 0.14$ at 30 min pi. Between 30 min and 6 h pi uptake of [$^{123}$I]**1** was reduced to a level of $0.71 ± 0.05$. Radioactivity in the blood was low at all time points investigated with values ranging from $0.02 ± 0.003$ at 5 min pi to $0.01 ± 0.0009$ at 24 h pi. Fat and muscle showed levels of radioactivity < 0.10 at all time points and showed rather little variation over time.

As to be expected from the lipophilicity of **1**, considerable uptake in the brain of the animals was observed.
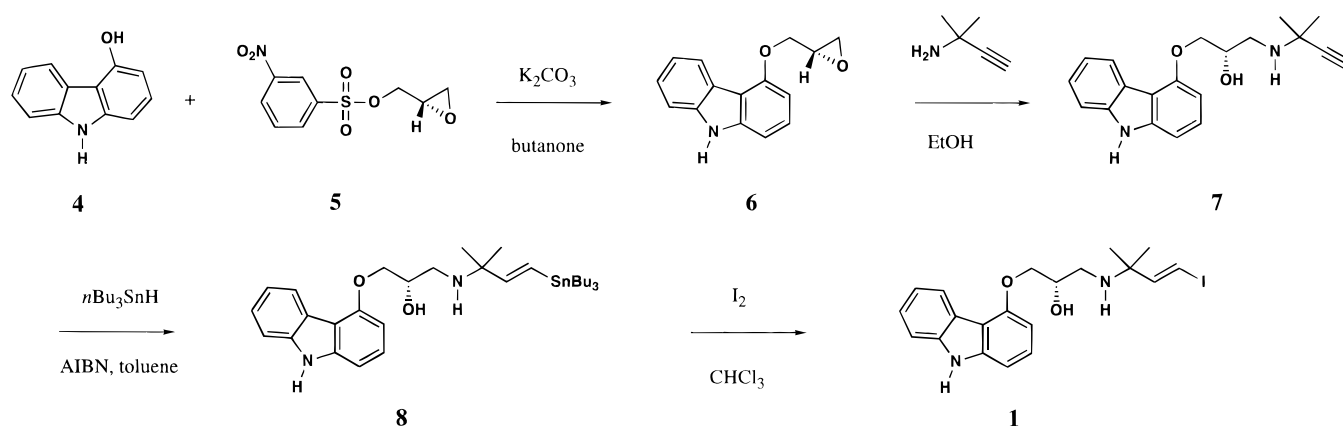


**Figure 2.** Schematic survey of the synthesis of the iodine-containing analogue of carazolol, **1**.

**Figure 3.** Displacement curves obtained with **1** and CGP12177 in membrane preparations of left ventricular muscle of New Zealand white rabbits with [$^{125}$I]iodocyanopindolol as a radioligand. Values are expressed as means ± SEM, $n = 3-5$.
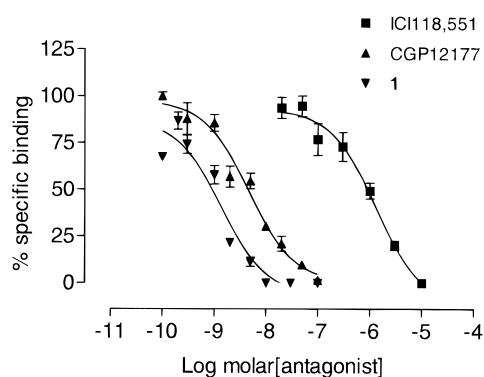


**Figure 4.** Displacement curves obtained with **1**, ICI118,551, and CGP12177 in membrane preparations of pulmonary tissue of New Zealand white rabbits with [$^{125}$I]iodocyanopindolol as a radioligand. Values are expressed as means ± SEM, $n = 3-5$.

Radioactivity levels reached maximal values at 5 min pi in several regions of the brain but rapidly diminished over time. The cerebral biodistribution of [$^{123}$I]**1** is summarized in Table 2.

**In Vivo Blocking Studies.** In order to determine specific binding of [$^{123}$I]**1** in the target organs, such as cardiac left ventricular muscle and lungs, *in vivo* blocking studies were performed, using different antagonists. The results of the *in vivo* blocking experiments are summarized in Table 3. In the left atrial muscle, uptake was significantly decreased by the preinjection of 0.1 $\mu$mol of CGP12177 or (−)-propranolol (0.22 ± 0.01 in the control group versus 0.05 ± 0.001 and 0.07 ± 0.002 in the CGP12177- and (−)-propranolol-treated groups, respectively). Atenolol tended to decrease atrial muscle uptake, although this decrease did not reach statistical significance (0.16 ± 0.07). ICI118,-551 slightly increased uptake of [$^{123}$I]**1**. Similar competition patterns were observed in right atrial muscle and left ventricular muscle.

In pulmonary tissue, all antagonists significantly reduced the uptake of [$^{123}$I]**1** (2.36 ± 0.64 in the control group versus 1.37 ± 0.13, 0.87 ± 0.18, 0.80 ± 0.18, and 1.07 ± 0.15 in the ICI118,551-, atenolol-, CGP12177-, and (−)-propranolol-treated groups, respectively). Muscular uptake of [$^{123}$I]**1** was significantly reduced by all antagonists used (0.09 ± 0.02 in the control group versus 0.06 ± 0.003, 0.06 ± 0.005, 0.03 ± 0.004, and 0.03 ± 0.002). In the liver a significant increase in uptake of the radioligand was observed after the pretreatment with ICI118,551 (0.08 ± 0.01 in the control group versus 0.15 ± 0.01 in the treated group). In other tissues, no changes in uptake were measured by the preinjection of any of the antagonists used.

The results of the *in vivo* cerebral blocking studies are shown in Table 4. In the pituitary gland, thalamus, and hypothalamus, no blocking of [$^{123}$I]**1** could be observed, with any of the antagonists used. In the frontal cortex a significant reduction in uptake of [$^{123}$I]-**1** was observed when animals were pretreated with atenolol or (−)-propranolol (0.13 ± 0.05 in controls versus 0.06 ± 0.003 and 0.05 ± 0.003 in the atenolol-

**Table 1.** Biodistribution Pattern of [$^{123}$I]**1** in Anesthetized New Zealand White Rabbits after Intravenous Injection of 50 $\mu$Ci of [$^{123}$I]**1**[a]

| | 5 min | 30 min | 1 h | 2 h | 4 h | 6 h | 24 h |
|---|---|---|---|---|---|---|---|
| left atrial muscle | 0.36 ± 0.06 | 0.16 ± 0.02* | 0.16 ± 0.02 | 0.17 ± 0.03 | 0.11 ± 0.02 | 0.10 ± 0.01 | 0.03 ± 0.001 |
| right atrial muscle | 0.19 ± 0.02 | 0.15 ± 0.04 | 0.13 ± 0.01* | 0.13 ± 0.01 | 0.08 ± 0.01 | 0.07 ± 0.003 | 0.03 ± 0.003 |
| left ventricular muscle | 0.52 ± 0.09 | 0.26 ± 0.02* | 0.20 ± 0.004 | 0.20 ± 0.03 | 0.14 ± 0.03 | 0.09 ± 0.004 | 0.02 ± 0.001 |
| right ventricular muscle | 0.43 ± 0.05 | 0.21 ± 0.01* | 0.18 ± 0.004 | 0.18 ± 0.003 | 0.12 ± 0.003 | 0.08 ± 0.003 | 0.02 ± 0.001 |
| lung | 5.75 ± 0.38 | 1.74 ± 0.14* | 1.43 ± 0.21 | 1.29 ± 0.21 | 0.82 ± 0.09 | 0.71 ± 0.05 | 0.18 ± 0.01 |
| intestine | 0.16 ± 0.01 | 0.15 ± 0.04* | 0.10 ± 0.01 | 0.13 ± 0.05 | 0.06 ± 0.02 | 0.05 ± 0.007 | 0.02 ± 0.004 |
| spleen | 0.39 ± 0.01 | 0.41 ± 0.05 | 0.28 ± 0.005 | 0.22 ± 0.02 | 0.10 ± 0.02 | 0.07 ± 0.02 | 0.03 ± 0.004 |
| liver | 0.14 ± 0.02 | 0.19 ± 0.03 | 0.13 ± 0.01 | 0.09 ± 0.004 | 0.04 ± 0.004 | 0.03 ± 0.003 | 0.01 ± 0.001 |
| kidney | 0.92 ± 0.11 | 0.29 ± 0.03* | 0.16 ± 0.008 | 0.11 ± 0.007 | 0.05 ± 0.004 | 0.04 ± 0.003 | 0.02 ± 0.001 |
| fat | 0.008 ± 0.001 | 0.08 ± 0.02* | 0.06 ± 0.01 | 0.04 ± 0.009 | 0.02 ± 0.003 | 0.02 ± 0.002 | 0.01 ± 0.002 |
| muscle | 0.08 ± 0.006 | 0.04 ± 0.01 | 0.07 ± 0.005 | 0.05 ± 0.01 | 0.04 ± 0.003 | 0.04 ± 0.004 | 0.05 ± 0.003 |
| blood | 0.03 ± 0.003 | 0.02 ± 0.001* | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.001 |

[a] Data are expressed as mean (% ID × kg of body weight/g of tissue) ± SEM, $n = 3$/time point. * $p < 0.05$ versus previous time point.

**Table 2.** Cerebral Biodistribution Pattern of [$^{123}$I]**1** in Anesthetized New Zealand White Rabbits after Intravenous Injection of 50 $\mu$Ci of [$^{123}$I]**1**[a]

| | 5 min | 30 min | 1 h | 2 h | 4 h | 6 h | 24 h |
|---|---|---|---|---|---|---|---|
| frontal cortex | 0.27 ± 0.02 | 0.14 ± 0.004* | 0.09 ± 0.005 | 0.09 ± 0.02 | 0.06 ± 0.01 | 0.05 ± 0.002 | 0.01 ± 0.006 |
| striatum | 0.27 ± 0.02 | 0.12 ± 0.009* | 0.07 ± 0.005* | 0.07 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.003 | 0.02 ± 0.001 |
| pituitary gland | 0.50 ± 0.09 | 0.14 ± 0.02* | 0.11 ± 0.01 | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.06 ± 0.006 | 0.03 ± 0.01 |
| thalamus | 0.31 ± 0.06 | 0.10 ± 0.01* | 0.07 ± 0.003 | 0.05 ± 0.005 | 0.03 ± 0.003 | 0.03 ± 0.005 | 0.02 ± 0.001 |
| cerebellum | 0.30 ± 0.03 | 0.15 ± 0.003 | 0.13 ± 0.008 | 0.15 ± 0.04 | 0.13 ± 0.01 | 0.14 ± 0.02 | 0.07 ± 0.004 |
| hypothalamus | 0.25 ± 0.03 | 0.09 ± 0.005* | 0.06 ± 0.004 | 0.05 ± 0.009 | 0.03 ± 0.004 | 0.03 ± 0.003 | 0.02 ± 0.0001 |

[a] Data are expressed as mean (% ID x kg of body weight/g of tissue) ± SEM, $n = 3$/time point. * $p < 0.05$ versus previous time point.

**Table 3.** *In Vivo* Blockade of [$^{123}$I]**1** Uptake in Anesthetized New Zealand White Rabbits upon Pretreatment with 0.1 $\mu$mol of the Antagonists CGP12177, Atenolol, (−)-Propranolol, or ICI118,551 10 min Prior to the Intravenous Administration of 50 $\mu$Ci of [$^{123}$I]**1**[a]

|  | control | ICI118,551 | atenolol | CGP12177 | (−)-propranolol |
|---|---|---|---|---|---|
| left atrial muscle | 0.22 ± 0.01 | 0.29 ± 0.05 | 0.16 ± 0.07 | 0.05 ± 0.001* | 0.07 ± 0.002* |
| right atrial muscle | 0.13 ± 0.05 | 0.24 ± 0.04 | 0.09 ± 0.004 | 0.05 ± 0.002* | 0.07 ± 0.004* |
| left ventricular muscle | 0.22 ± 0.09 | 0.29 ± 0.009 | 0.14 ± 0.03 | 0.08 ± 0.002* | 0.09 ± 0.005* |
| right ventricular muscle | 0.19 ± 0.08 | 0.25 ± 0.01 | 0.14 ± 0.03 | 0.08 ± 0.003* | 0.09 ± 0.002* |
| lung | 2.39 ± 0.64 | 1.37 ± 0.13* | 0.87 ± 0.18* | 0.80 ± 0.18* | 1.07 ± 0.15* |
| intestine | 0.36 ± 0.17 | 1.43 ± 0.62 | 0.20 ± 0.07 | 0.46 ± 0.14 | 0.29 ± 0.15 |
| spleen | 0.20 ± 0.09 | 0.20 ± 0.02 | 0.17 ± 0.02 | 0.12 ± 0.02 | 0.10 ± 0.01 |
| liver | 0.08 ± 0.01 | 0.15 ± 0.01* | 0.08 ± 0.008 | 0.10 ± 0.01 | 0.10 ± 0.02 |
| kidney | 0.14 ± 0.04 | 0.22 ± 0.02 | 0.07 ± 0.002 | 0.09 ± 0.04 | 0.10 ± 0.01 |
| fat | 0.04 ± 0.009 | 0.09 ± 0.03 | 0.05 ± 0.004 | 0.04 ± 0.004 | 0.04 ± 0.001 |
| muscle | 0.09 ± 0.02 | 0.06 ± 0.003* | 0.06 ± 0.005* | 0.03 ± 0.004* | 0.03 ± 0.002* |
| blood | 0.03 ± 0.008 | 0.02 ± 0.001 | 0.02 ± 0.001 | 0.01 ± 0.001 | 0.01 ± 0.0001 |

[a] Animals were sacrificed 2 h pi. Data are expressed as mean (% ID x kg of body weight/g of tissue) ± SEM, $n = 3$/time point. * $p < 0.05$ versus control animals.

**Table 4.** *In Vivo* Blockade of Cerebral Uptake of [$^{123}$I]**1** in Anesthetized New Zealand White Rabbits after Intravenous Injection of 0.1 $\mu$mol of the Antagonists CGP12177, Atenolol, (−)-Propranolol, or ICI118,551 10 min Prior to the Intravenous Administration of 50 $\mu$Ci of [$^{123}$I]**1**[a]

|  | control | ICI118,551 | atenolol | CGP12177 | (−)-propranolol |
|---|---|---|---|---|---|
| frontal cortex | 0.13 ± 0.05 | 0.12 ± 0.01 | 0.06 ± 0.003* | 0.10 ± 0.008 | 0.05 ± 0.003* |
| striatum | 0.08 ± 0.03 | 0.09 ± 0.008 | 0.05 ± 0.003 | 0.08 ± 0.003 | 0.04 ± 0.003* |
| pituitary gland | 0.12 ± 0.05 | 0.10 ± 0.004 | 0.10 ± 0.02 | 0.07 ± 0.002 | 0.06 ± 0.02 |
| thalamus | 0.06 ± 0.02 | 0.07 ± 0.008 | 0.04 ± 0.001 | 0.06 ± 0.004 | 0.04 ± 0.004 |
| cerebellum | 0.16 ± 0.07 | 0.12 ± 0.01 | 0.11 ± 0.007 | 0.17 ± 0.01 | 0.04 ± 0.002* |
| hypothalamus | 0.08 ± 0.03 | 0.08 ± 0.01 | 0.04 ± 0.004 | 0.06 ± 0.002 | 0.04 ± 0.004 |

[a] Animals were sacrificed 2 h pi. Data are expressed as mean (% ID x kg of body weight/g of tissue) ± SEM, $n = 3$/time point. * $p < 0.05$ versus control animals.

and (−)-propranolol-treated groups, respectively). In the cerebellum a significant decrease in uptake of [$^{123}$I]-**1** was observed when animals were pretreated with (−)-propranolol (0.16 ± 0.07 in the control group versus 0.04 ± 0.002 in the pretreated group). A similar pattern was observed in the striatum where (−)-propranolol reduced the uptake of [$^{123}$I]**1** significantly (0.08 ± 0.03 versus 0.04 ± 0.003).

## Discussion

***In Vitro* Radioligand Displacement Experiments.** Using classical *in vitro* displacement experiments, the affinity of **1** for the β-adrenoceptor was determined. **1** proved a potent β-adrenoceptor antagonist, as can be deduced from the $K_i$ values obtained in both pulmonary tissue and cardiac left ventricular muscle. It therefore fulfills one of the important criteria for being a possible candidate as a radioligand for cardiac β-adrenoceptor imaging: high affinity for the β-adrenoceptor, i.e., $K_i < 1$ nM.[16] In addition, the affinity of carazolol for the receptor remained almost unaltered after the introduction of the iodine moiety. Moreover, upon introduction of the iodinated substituent, a compound was synthesized showing no selectivity for either the β$_1$- or β$_2$-subtype.

***In Vivo* Biodistribution.** The *in vivo* biodistribution study with [$^{123}$I]**1** showed substantial uptake in cardiac left ventricular muscle, which proved stable for several hours and which may therefore be suggestive for specific binding. In pulmonary tissue initial uptake was very high, but after a dip untill 30 min, radioactivity levels slowly decreased over time.

The *in vivo* blocking studies, for which we used several antagonists (both lipophilic/hydrophilic and selective/nonselective) provided direct evidence for specific binding in the target organs. In both atrial and ventricular muscle, a significant reduction in uptake of [$^{123}$I]**1** was observed when the antagonists CGP12177

and (−)-propranolol were injected prior to the administration of the radioligand. Atenolol also tended to decrease the uptake in these organs. The decrease in uptake of the radioligand by using these antagonists clearly demonstrates the specificity of the binding *in vivo*. In addition, the β$_2$-subtype selective adrenoceptor antagonist ICI118,551 caused a slight increase in left ventricular uptake. This phenomenon is probably caused by the occupation of β$_2$-adrenoceptors in the lung, thus increasing the concentration of free radioligand in plasma available for binding to the β$_1$-adrenoceptor in the left ventricular muscle. This presumably also explains why radioactivity levels in the liver were enhanced; more radioligand was subjected to metabolism. In pulmonary tissue a significant decrease in uptake was observed for all antagonists, once again suggesting specific binding. Although radioactivity in blood and fat (nontarget tissues) remained unaltered upon the antagonists pretreatment, all antagonists caused a significant decrease in muscular uptake of [$^{123}$I]**1**. It is known that β-adrenoceptors are also present in muscular tissue. Therefore, the decreased muscular uptake once again suggests specific binding of [$^{123}$I]**1** *in vivo*.

As could be expected from the lipophilic character of [$^{123}$I]**1**, cerebral uptake was observed in all animals as well. Radioactivity levels were, however, rather low, <0.15% ID × kg of body weight/g of tissue, except at 5 min after injection, and they showed few changes during the time points investigated. Cerebral binding of [$^{123}$I]-**1** proved to be specific in the striatum, cerebellum, and frontal cortex, since the uptake of [$^{123}$I]**1** was significantly reduced by (−)-propranolol and/or atenolol.

In conclusion, the iodinated analogue of carazolol, **1**, is a radioligand which displays both high affinity and specificity for the β-adrenoceptor *in vitro* as well as specific myocardial, pulmonary, and cerebral uptake *in vivo*. Therefore, **1** may be a suitable radioligand for the

imaging of cardiac β-adrenoceptors *in vivo* using SPECT. Additional *in vivo* γ-camera studies are necessary to explore the possibilities of this compound.

## Experimental Section

**Chemical Synthesis. General Materials and Methods.** All chemicals used were of the purest grades commercially available. α,α′-Azoisobutyronitrile (AIBN) was obtained from Merck. 1,1-Dimethylpropargylamine was purchased from Acros Chimica NV. All other chemicals were obtained from Aldrich. All solvents used were of proanalysis grade and obtained from Merck.

$^1$H NMR spectra were measured on a Bruker AM-400 spectrometer at 400.13 MHz. $^{13}$C NMR spectra were run on the same apparatus at 100.62 MHz with proton noise decoupling. All δ values are given in ppm downfield from tetramethylsilane. Melting points were measured on a Jenaval melting point microscope and are given uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Mass spectroscopy was performed using a Hewlett Packard HP5790 GC instrument coupled with an HP5970 MSD. The EI spectrum was obtained with an electron energy of 70 eV. Ionspray mass spectroscopy was performed on a PE SCIEX API 300 MS/MS system. TLC was performed with Merck 60 F$_{254}$ silica gel plates, and compounds were visualized under UV light (λ = 254 nm) and with I$_2$ vapor. Flash chromatography was performed with Acros Chimica silica gel (35−70 μm, pore diameter 6 nm). High-performance liquid chromatography (HPLC) was performed on a reverse-phase column (C-18, Radpak 4 μm, Nova-Pak 10 × 5 mm i.d.) using a Waters HPLC system consisting of a Model 600 pump operated with a Model 600E system controller and a Model 490E wavelength detector set at 230 nm.

**Synthesis of 1 (Iodinated Analogue of Carazolol) and the Precursor for [$^{123}$I]1 (Figure 2).** The asymmetric synthesis of **1** is started by the reaction of 4-hydroxycarbazole as a synthetic intermediate. This compound was prepared according to procedures described by Berridge,[16] with the following modifications and additional characteristics.

**1,2,3,4-Tetrahydro-4-oxocarbazole (3).** A solution of cyclohexane-1,3-dione monophenylhydrazone (17.8 g, 80 mmol) in 100 mL of trifluoroacetic acid was boiled for 16 h. After cooling, the reaction mixture was slowly poured into ice water. The precipitate was collected and recrystallized from aqueous methanol to yield **3** as white crystals (13 mg, 80%): mp 228−230 °C (lit[26] mp 221−223 °C). The proton NMR spectrum was identical with that reported by Rodriguez.[26]

**4-Hydroxycarbazole (4).** 1,2,3,4-Tetrahydro-4-oxocarbazole (**3**) was dehydrogenated with freshly prepared Raney nickel according to Berridge et al.[16] **4**: mp 174−177 °C (lit.[16] mp 166−167 °C); $^1$H NMR (CDCl$_3$) δ 8.27 (dd, J = 0.8, and 7.8 Hz, 1H), 8.03 (br s, 1H), 7.40−7.35 (m, 2H), 7.29−7.18 (m, 2H), 6.97 (dd, J = 0.6, 8.1 Hz, 1H), 6.56 (d, J = 8.0 Hz, 1H), 5.62 (br s, 1H).

**(S)-(+)-4-(2,3-Epoxypropoxy)carbazole (6).** To a solution of 4-hydroxycarbazole (326 mg, 1.78 mmol) in 2-butanone (5 mL) were added (S)-glycidyl *m*-nitrobenzenesulfonate (465 mg, 1.78 mmol; **5** ([α]$_D$ = +21.7° (c = 1.1, CHCl$_3$)) and K$_2$CO$_3$ (275 mg, 2 mmol). After stirring for 40 h at 60 °C, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in CHCl$_3$ (50 mL) and washed with water (20 mL). The organic phase was separated, dried on Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. Recrystallization from CHCl$_3$−hexane yielded **6** as white crystals (300 mg, 70%): mp 159−161 °C (lit.[16] 160 °C); [α]$_D$ = + 39.1° (c = 1.03, acetone); $^1$H NMR (CDCl$_3$) δ 8.33 (d, J = 7.8 Hz, 1H), 8.09 (br s, 1H), 7.39 (m, 2H), 7.31 (t, J = 8.1 Hz, 1H), 7.24 (m, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 4.46 (dd, J = 3.3, 10.9 Hz, 1H), 4.25 (dd, J = 5.4, 10.9 Hz, 1H), 3.55 (m, 1H), 3.00 (dd, J = 4.2, 4.9 Hz, 1H), 2.89 (dd, J = 2.7, 4.9 Hz, 1H); $^{13}$C NMR (CDCl$_3$) δ 154.9, 140.9, 138.7, 126.5, 125.0, 123.2, 122.4, 119.7, 112.8, 109.9, 104.0, 101.3, 68.7, 50.3, 44.8.

**(S)-(−)-4-[3-[(1,1-Dimethylpropargyl)amino]-2-hydroxypropoxy]carbazole (7).** To a solution of **6** (105 mg, 0.44 mmol) in EtOH (7 mL) was added 1,1-dimethylpropargylamine

(1.0 mL, 8.5 mmol). The reaction mixture was stirred for 16 h at room temperature. Then the mixture was concentrated. Flash chromatography with EtOAc gave **7** (100 mg, 70%): TLC R$_f$ = 0.40 (EtOAc); [α]$_D$ = −19.5° (c = 0.25, MeOH); $^1$H NMR (CDCl$_3$) δ 8.29 (d, J = 8.2 Hz, 1H), 8.11 (br s, 1H), 7.45−7.36 (m, 2H), 7.33 (t, J = 8.0 Hz, 1H), 7.20 (m, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 4.35−4.25 (m, 3H), 3.17 (dd, J = 3.7, 12.0 Hz, 1H), 3.09 (dd, J = 7.0, 12.0 Hz, 1H), 2.31 (s, 1H), 1.42 (s, 6H); $^{13}$C NMR (CD$_3$OD) 156.4, 142.8, 140.6, 127.4, 125.6, 124.0, 119.7, 113.5, 111.0, 105.0, 101.4, 88.7, 72.1, 71.5, 70.4, 67.2, 50.8, 48.5, 30.0, 29.0; MS (EI) *m/z* (rel intensity) 323 ((M + H)$^+$, 0.8), 322 (M$^+$, 4), 184 (16), 183 (100), 155 (5.8), 154 (19.5), 140 (6.8), 127 (5.8), 96 (13), 80 (5.1), 41 (8.6), 30 (36).

**(S)-4-[[3-[1,1-Dimethyl-3-(tri-*n*-butylstannyl)-(2E)-propenyl]amino]-2-hydroxypropoxy]carbazole (8).** A solution of **7** (15 mg, 46 μmol), tri-*n*-butyltin hydride (0.1 mL, 0.37 mmol), and AIBN (3 mg) in toluene (0.5 mL) was stirred for 2 h at 90 °C. Then the reaction mixture was concentrated and purified by column chromatography over silica gel to yield **8** (13 mg, 46%): $^1$H NMR (CDCl$_3$) δ 8.26 (d, J = 7.8 Hz, 1H), 8.18 (br s, 1H), 7.46−7.36 (m, 2H), 7.31 (t, J = 8.0 Hz, 1H), 7.24−7.15 (m, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 5.97 (d, J = 19.5 Hz (with satellite peaks at 34.9, 36.5 Hz), 1H), 5.89 (d, J = 19.5 Hz (with satellite peaks at 34.9, 36.5 Hz), 1H), 4.31−4.25 (m, 1H), 4.25−4.15 (m, 2H), 2.92 (dd, J = 4.0, 12.2 Hz, 1H), 2.78 (dd, J = 7.1, 12.2 Hz, 1H), 1.55−1.4 (m, 6H), 1.35−1.25 (m, 6H), 1.21 (s, 6H), 0.97−0.75 (m, 15H).

**(S)-(−)-4-[3-[(1,1-Dimethyl-3-iodo-(2E)-propenyl)amino]-2-hydroxypropoxy]carbazole (1).** To a solution of **8** (9 mg, 15 μmol) in CHCl$_3$ (0.5 mL) was added dropwise a solution of 0.1 M iodine in methanol (150 μL). After 3 h the reaction mixture was diluted with CHCl$_3$ (10 mL), and to this mixture were added successively 1 M KF (0.5 mL) and 1 M NaHSO$_3$ (0.5 mL). The resulting mixture was stirred for 5 min, the organic phase was dried on Na$_2$SO$_4$ and filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography with EtOAc/hexane (3/2) to yield **1** (6 mg, 90%) as a glass: [α]$_D$ = −10.8° (c = 0.35, CHCl$_3$); $^1$H NMR (CDCl$_3$) δ 8.34 (d, J = 7.8 Hz, 1H), 8.20 (br s, 1H), 7.55−7.45 (m, 2H), 7.43 (t, J = 8.0 Hz, 1H), 7.37−7.30 (m, 1H), 7.17 (d, J = 8.9 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.64 (dd, J = 1.4, 14.7 Hz, 1H), 6.27 (dd, J = 1.4, 14.7 Hz, 1H), 4.45−4.28 (m, 3H), 2.94 (dd, J = 3.9, 12.0 Hz, 1H), 2.83 (dd, J = 7.0, 12.0 Hz, 1H), 1.22 (s, 6H); $^{13}$C NMR (CHCl$_3$) 155.0, 153.1, 140.9, 138.7, 126.7, 125.0, 122.8, 122.4, 119.8, 113.5, 110.0, 103.9, 101.2, 75.0, 70.4, 69.0, 57.8, 45.6, 26.9, 26.5; MS (ionspray) *m/z* 451 (M + H)$^+$. The enantiomeric purity of **1** (ee > 96%) was determined by separation of the enantiomers on a Chiralcel OD HPLC column with *n*-hexane/isopropyl (3/1) with a flow rate of 1.0 mL/min. Retention times for the *S*- and *R*-enantiomers were 21.6 and 24.0 min, respectively.

**Synthesis of [$^{123}$I]1.** To a solution of **8** (200 μg, 0.3 μmol) in ethanol containing 0.2% NH$_3$ (150 μL) was added [$^{123}$I]NaI in 0.05 N NaOH (250 μL, specific activity 185 TBq/mmol (5000Ci/mmol)). This mixture was concentrated *in vacuo*, and to the residue was added a mixture of acetic acid (450 μl) and 30% H$_2$O$_2$ (50 μL). After a reaction time of 15 min at room temperature, water was added. Then the mixture was loaded on a C-18 cartridge (Bond Elute; 1 mL) and washed with water. The [$^{123}$I]1 was eluted from the cartridge with EtOH/HCl (20/1, v/v, 2 mL). The eluate was concentrated *in vacuo*, and the residue was dissolved in ethanol and purified on a C-18 reverse-phase HPLC column. Elution with MeOH/acetate buffer (0.01 M, pH = 3.5) (30/70, v/v) yielded [$^{123}$I]1, which was loaded on a second C-18 cartridge, washed with water (1 mL), and again eluted with EtOH/HCl (20/1, v/v, 2 mL). Finally, the eluate was concentrated *in vacuo* and the residue dissolved in EtOH (200 μL) to which was added an isotonic acetate buffer (pH = 4.7, 4 mL). The resulting solution was sterilized by filtration on a 0.22 μm membrane (Millex FG). Identity and purity were further analyzed by TLC ([$^{123}$I]1 R$_f$ = 0.45 with CH$_2$Cl$_2$/MeOH/HOAc, 84/15/1, v/v/v) and HPLC (*vide supra*). The radioactive product [$^{123}$I]1 exhibited identical mobilities

on TLC and HPLC with those of the nonradioactive product. Radiochemical yield amounted to 15%. Radiochemical purity was >95%.

**In Vitro Analysis. Animals.** Male New Zealand white rabbits, weighing 1.5–2.5 kg, were anesthetized with an intramuscular injection of 5 mg/kg xylazine and 30 mg/kg ketamine and subsequently euthanized by 200 mg/kg pentobarbital im. The thorax was opened, and the heart was rapidly removed. The left ventricular muscle was dissected, rinsed in 10 mL ice-cold 1 mM $KHCO_3$, and frozen in liquid nitrogen. The lungs were rinsed in 10 mL of 20 mM $NaHCO_3$ and also frozen in liquid nitrogen. Both preparations were stored at −80 °C until used for radioligand binding experiments.

**Radioligand Displacement Experiments.** Membrane fractions of the left ventricular muscle and lungs were prepared for radioligand displacement experiments. The method described by Engel et al.[27] was used with some modifications: 100 mg of left ventricular muscle was minced with scissors and homogenized in 10 mL of ice-cold 1 mM $KHCO_3$ by a Polytron homogenizer (full speed, $3 \times 5$ s). The homogenate was centrifuged at $1000g$ for 20 min. The supernatant was filtered through four layers of cloth gauze and centrifuged at $45000g$ for 20 min. The pellet was resuspended, homogenized, and diluted to a level of 100 mL/g of wet weight in incubation buffer (10 mM Tris/HCl, 154 mM NaCl, and 0.01% ascorbic acid at pH = 7.4). Lung tissue was prepared using the method described above for the preparation of left ventricular muscle but with the use of 20 mM $NaHCO_3$ instead of 1 mM $KHCO_3$ and by diluting the fraction to 400 mL/g of wet weight instead of 100 mL/g of wet weight. Aliquots of the membrane suspension (150 $\mu$L) were incubated with 50 $\mu$L [$^{125}$I]iodocyanopindolol (ICYP) in a final concentration of $4.5 \times 1^{-11}$ M and 50 $\mu$L **1** in final concentrations ranging from $10^{-10}$ to maximally $10^{-3}$ M. The incubation was terminated by adding 3 mLof ice-cold incubation buffer to the entire mixture followed by rapid vacuum filtration through Whatman GF/C filters. Filters were washed twice with 3 mL of incubation buffer and counted for radioactivity at 74% efficiency in a Beckman $\gamma$-counter. Nonspecific binding of ICYP to the membrane suspension was defined as the radioactivity bound in the presence of $10^{-5}$ M ($\pm$)-CGP12177. Specific binding was defined as the total minus the nonspecific binding. Besides the newly synthesized iodinated compound, we also tested the affinity of CGP12177 in both pulmonary and left ventricular muscle and the $\beta_2$-adrenoceptor antagonist ICI118,551 in pulmonary tissue.

**Calculations.** Displacement curves were fitted to the individual binding data by a computer program based on a sigmoidal model for a single receptor subtype (GraphPad Software, San Diego, CA). The inhibition constant ($K_i$) was derived from the equation $K_i = EC_{50}/(1 + L/K_d)$, where $L$ equals the concentration of the radioligand ICYP and $K_d$ equals the affinity constant of ICYP for the $\beta$-adrenoceptor.

**In Vivo Biodistribution Studies.** Male New Zealand white rabbits, weighing 2.5–3.5 kg, were anesthetized with an intramuscular injection of 5 mg/kg xylazine and 30 mg/kg ketamine. Subsequently, the animals received 50 $\mu$Ci [$^{123}$I]**1** (specific activity > 5000 Ci/mmol) intravenously via an ear vein. Animals were euthanized at 5, 15, 30, 60, 120, and 240 min and 24 h ($n$ = 3/time point) after injection by intramuscular injection of 200 mg/kg pentobarbital. Blood was removed by a vacuum pump via an intracardial puncture. The organs were removed and weighed. Subsequently, radioactivity per individual organ tissue was measured using a $\gamma$-counter (Auto-Gamma 5000, Packard Instruments Co., Downers Grove, IL) at 51% efficiency, corrected for decay, weight, and body weight, and expressed as % injected dose (ID) $\times$ kg of body weight/g of tissue.

In an additional experiment, animals received an intravenous injection of 0.1 $\mu$mol of ICI118,551, CGP12177, (−)-propranolol, atenolol, or vehicle injection, respectively, 10 min prior to the administration of the radioligand to establish specificity of binding of [$^{123}$I]**1** to the $\beta$-adrenoceptor ($n$ = 3). These animals were euthanized 2 h after the intravenous administration of [$^{123}$I]**1**.

**Statistical Analysis.** Data are expressed as means $\pm$ SEM. Statistical analysis of the results was performed using a nonparametric Mann–Whitney test. For the biodistribution studies ANOVA with a Bonferroni test was performed. A $P$ value < 0.05 was considered to indicate statistical significance of differences.

**Drugs Used.** ($\pm$)-CGP12177 was obtained from Ciba-Geigy (Basel, Switzerland). ICYP (specific activity 2200 Ci/mmol) was provided by Du Pont de Nemours, New England Nuclear (Boston, MA). All other chemical compounds were obtained from Sigma Chemical Co. (St. Louis, MO).

## References

(1) Brodde, O. E.; Daul, A.; Michel, M. C.; Zerkowski, H. R. Importance of beta 2-adrenergic receptors in heart failure. *Z. Kardiol.* **1992**, *81* (Suppl. 4), 71–78.

(2) Brodde, O. E.; Zerkowski, H. R.; Doetsch, N.; Motomura, S.; Khamssi, M.; Michel, M. C. Myocardial beta-adrenoceptor changes in heart failure: concomitant reduction in beta 1- and beta 2-adrenoceptor function related to the degree of heart failure in patients with mitral valve disease. *J. Am. Coll. Cardiol.* **1989**, *14*, 323–331.

(3) Brodde, O. E. Chronic beta 1-blocker treatment and cardiac beta 2-adrenoceptor function [letter]. *Am. Heart J.* **1992**, *123*, 1727–1728.

(4) Brodde, O. E.; Zerkowski, H. R.; Borst, H. G.; Maier, W.; Michel, M. C. Drug-and disease-induced changes of human cardiac beta-1 and beta-2 adrenoceptors. *Eur. Heart J.* **1989**, *10* (Suppl. B), 38–44.

(5) Bristow, M. R.; Ginsburg, R.; Umans, V.; Fowler, M.; Minobe, W.; Rasmussen, R.; Zera, P.; Menlove, R.; Shah, P.; Jamieson, S. Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1- receptor down-regulation in heart failure. *Circ. Res.* **1986**, *59*, 297–309.

(6) Bristow, M. R.; Kantrowitz, N. E.; Ginsburg, R.; Fowler, M. B. Beta-adrenergic function in heart muscle disease and heart failure. *J. Mol. Cell Cardiol.* **1985**, *17* (Suppl. 2), 41–52.

(7) Delforge, J.; Syrota, A.; Lancon, J. P.; Nakajima, K.; Loc'h, C.; Janier, M.; Vallois, J. M.; Cayla, J.; Crouzel, C. Cardiac beta-adrenergic receptor density measured *in vivo* using PET, CGP 12177, and a new graphical method. *J. Nucl. Med.* **1991**, *32*, 739–748.

(8) Merlet, P.; Delforge, J.; Syrota, A.; Angevin, E.; Maziere, B.; Crouzel, C.; Valette, H.; Loisance, D.; Castaigne, A.; Rande, J. L. Positron emission tomography with 11C CGP-12177 to assess beta- adrenergic receptor concentration in idiopathic dilated cardiomyopathy. *Circulation* **1993**, *87*, 1169–1178.

(9) Brady, F.; Luthra, S. K.; Tochon Danguy, H. J.; Steel, C. J.; Waters, S. L.; Kensett, M. J.; Landais, P.; Shah, F.; Jaeggi, K. A.; Drake, A.; et al. Asymmetric synthesis of a precursor for the automated radiosynthesis of S-(3'-t-butylamino-2'-hydroxypropoxy)- benzimidazol-2-[11C]one (S-[11C]CGP 12177) as a preferred radioligand for beta-adrenergic receptors. *Int. J. Radiat. Appl. Instrum. A* **1991**, *42*, 621–628.

(10) Cohen, M. L.; Ruffolo, R. R.; Wiley, K. S. Antagonist dissociation constants and relative agonist efficacies for compounds interacting with beta-1 and beta-2 adrenergic receptors in the rat jugular vein. *J. Pharmacol. Exp. Ther.* **1980**, *215*, 325.

(11) Costin, B. I.; O'Donnell, S. R.; Wanstall, J. C. A comparison of the selectivity of carazolol with that of other beta-2 selective adrenoceptor antagonists. *J. Pharm. Pharmacol.* **1983**, *35*, 590.

(12) Innis, R. B.; Correa, F. M. A.; Snyder, S. H. Carazolol, an extremely potent $\beta$-adrenergic blocker: Binding to $\beta$-receptors in brain membranes. *Life Sci.* **1979**, *24*, 2255–2264.

(13) Lemoine, H.; Kaumann, A. J. The affinity of carazolol for myocardial and tracheal $\beta$-adrenoceptors activated by (−)-isoprenaline. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1978**, *302*, R53.

(14) Lemoine, H.; Ehle, B.; Kaumann, A. J. Direct labelling of beta-2-adrenoceptors. A comparison of binding potency of 3H-ICI118,-551 and blocking potency of ICI118,551. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1985**, *331*, 40..

(15) Manalan, A. S.; Besch, H. R.; Watanabe, A. M. Characterization of [3H] ($\pm$) carazololbinding to $\beta$-adrenergic receptors. Application to study of $\beta$-adrenergic receptor subtypes in canine ventricular myocardium and lung. *Circ. Res.* **1981**, *49*, 326.

(16) Berridge, M. S.; Cassidy, E. H.; Terris, A. H.; Vesselle, J. M. Preparation and in vivo binding of [11C]carazolol, a radiotracer for the beta-adrenergic receptor. *Int. J. Radiat. Appl. Instrum. B* **1992**, *19*, 563–569.

(17) Elsinga, P. H.; Vos, M. G.; Braker, A. H.; de Groot, T. J.; Van Waarde, A.; Anthonio, R. L.; Weemaes, A. M. A.; Brodde, O. E.; Visser, G. M.; Vaalburg, W. (S,S)- and (S,R)-1′-[18F]fluorcarazolol, ligands for the visualization of pulmonary beta-adrenoceptors. *Nucl. Med. Biol.* **1996**, *23*, 159−167.

(18) Zheng, L.; Berridge, M. S.; Ernsberger, P. Synthesis, binding properties, and 18F labeling of fluorocarazolol, a high-affinity beta-adrenergic receptor antagonist. *J. Med. Chem.* **1994**, *37*, 3219−3230.

(19) Dubois, E. A.; Somsen, G. A.; van den Bos, J. C.; Janssen, A. G. M.; Boer, G. J.; Batink, H. D.; van Royen, E. A.; Pfaffendorf, M.; van Zwieten, P. A. Pharmacological characterization in vitro and in vivo of 123I-labelled derivatives of the beta-adrenoceptor antagonist CGP12177, designed for the imaging of cardiac beta-adrenoceptors. *J. Nucl. Cardiol.* **1996**, in press.

(20) Morris, T. H.; Kaumann, A. J. Different steric characteristics of beta 1- and beta 2-adrenoceptors. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1984**, *327*, 176−179.

(21) Shu, Y.; Scarpace, P. J. Forskolin binding sites and G-protein immunoreactivity in rat hearts during aging. *J. Cardiovasc. Pharmacol.* **1994**, *23*, 188−193.

(22) Klunder, J. M.; Onami, T.; Sharpless, K. B. Arenesulfonate derivatives of homochiral glycidol: versatile chiral building-blocks for organic synthesis. *J. Org. Chem.* **1989**, *54*, 1295−1304.

(23) Lauer, K.; Kiegel, E. Verfahren zur Herstellung von 4-hydroxycarbazol. (A method for the synthesis of 4-hydroxycarbazole.) Ger. Offen DE 2928483, Boehringer Mannheim GmbH, Germany; *Chem. Abstr.* **1981**, *93*, 114325.

(24) Zinnes, H. Process for the preparation of 4-keto-1,2,3,4-tetrahydrocarbazole. U.S. Patent No. 3892766, Warner-Lamberts Co.; *Chem. Abstr.* **1975**, *83*, 178814v.

(25) Aigbirhio, F.; Pike, V. W.; Francotte, E.; Waters, S. L.; Banfield, B.; Jaeggi, K. A.; Drake, A. S-[1-(2,3-Diaminophenoxy)]-3′-(N-t-butylamino)propan-2′-ol. Simplified asymmetric synthesis with CD and chiral HPLC analysis. *Tetrahedron: Asymmetry* **1992**, *3*, 539−554.

(26) Rodriguez, J.-G.; Temprano, F.; Esteban-Calderon, C.; Martinez-Ripoll, M. Synthesis of 4-(N,N-dimethyl(aminoethyl)-1,2,3,4-tetrahydrocarbazole: molecular structure and reactivity of the 1,2-dihydrocarbazol-4(3H)-one and derivatives. *J. Chem. Soc., Perkin Trans. I* **1989**, 2117−2122.

(27) Engel, G.; Hoyer, D.; Berthold, R.; Wagner, H. (+/-)[125Iodo] cyanopindolol, a new ligand for beta- adrenoceptors: identification and quantitation of subclasses of beta-adrenoceptors in guinea pig. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1981**, *317*, 277−285.

(28) Musachio, J. L.; Lever, J. R. Vinylstannylated alkylating agents as prosthetic groups for radioiodination of small molecules; Design, synthesis and application to iodoallyl analogues of spiperone and diprenorphine. *Bioconjugate Chem.* **1992**, *3*, 167−175.