°C (from acetone–petroleum ether). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

***cis*- and *trans*-4-Benzyl-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (40 and 41).** The enamide **39** (9.30 g, 40.6 mmol) was *N*-benzylated as described for **20** to yield an oil (13.6 g), which was reduced using first $LiAlH_4$ (4.00 g, 105 mmol) and then $NaBH_3CN$ (3.38 g, 53.8 mmol) as described for **21** and **22** to leave an oil (11.5 g) with a cis/trans ratio 86:14 according to GLC. Repeated treatment of the hydrochloride of the mixture with boiling acetone gave several crops of the pure cis isomer **40**·HCl (totally 3.15 g), mp 250–253 °C. Anal. ($C_{21}H_{25}NO$·HCl) C, H, N. The mother liquors were chromatographed on SiO_2 with CH_2Cl_2/acetone (5:1) as eluent, and 0.177 g of the trans isomer **41**·HCl was isolated, mp 100–140 °C (not recrystallized due to the small amount isolated). Anal. ($C_{21}H_{25}NO$·HCl) C, H, N.

***cis*-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (42).** This compound was obtained from **40**·HCl (0.55 g, 1.7 mmol) as described for **23**: yield 0.40 g (95%); mp 260–262 °C (from ethanol–ether). Anal. ($C_{14}H_{19}NO$·HCl) C, H, N.

***trans*-10-Methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (43).** This compound was obtained from **41**·HCl (0.170 g, 0.490 mmol) as described for **23**: yield 0.050 g (40%); mp 266–269 °C (not analyzed due to the small amount isolated).

***cis*-10-Methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (44).** This compound was prepared from **42**·HCl (0.27 g, 1.1 mmol) as described for **26**, yielding 0.22 g (68%) of the hydrochloride, mp 223.5–224.5 °C (from ethanol–ether). Anal. ($C_{17}H_{25}NO$·HCl) C, H, N.

***trans*-10-Methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline (45).** This compound was prepared from **43**·HCl (0.05 g, 0.20 mmol) as described for **27**, yielding 0.050 g (86%) of an oil, which was demethylated with 48% aqueous HBr without further purification (see Table I).

Demethylation of Methoxy Compounds. The phenols were obtained by heating the appropriate methoxy compounds in 48% aqueous HBr for 2 h at 125 °C under nitrogen. The hydrobromic acid was evaporated, and the residue was recrystallized at least twice.

Pharmacology. Animals used in the biochemical and motor activity experiments were male rats of Sprague–Dawley strain (Anticimex, Stockholm) weighing 200–300 g.

All substances to be tested were dissolved in saline immediately before use, occasionally with the addition of a few drops of glacial acetic acid and/or moderate heating in order to obtain complete dissolution. Reserpine was dissolved in a few drops of glacial acetic acid and made up to volume with 5.5% glucose. Injection volumes were 5 or 10 mL/kg and were all neutral (except for the solutions of reserpine).

Biochemistry. The biochemical experiments and the spectrophotometric determinations of Dopa and 5-HTP were performed as previously described.[10,17]

Separate dose-response curves based on four to six dose levels for each substance (sc administration) and brain area were constructed (cf. ref 14). From these curves was estimated the dose of the drug yielding a half-maximal decrease of the Dopa level, the ED_{50} value (presented in Table I).

Motor Activity. The motor activity was measured by means of photocell recordings ("M/P 40 Fc Electronic Motility Meter", Motron Products, Stockholm) as previously described.[10,17]

Six hours prior to the motility testing (carried out between 1 and 6 p.m.), the rats were intraperitoneally injected with reserpine (10 mg/kg), and 1 h before the test, DL-α-methyl-*p*-tryosine methyl ester hydrochloride (250 mg/kg) was injected intraperitoneally. The different compounds under investigation were administered subcutaneously in the neck region ($n = 4$) in a dose of 1 mg/kg (3100 nmol/kg) for the trans derivatives and 10 mg/kg (31 000 nmol/kg) for the cis derivatives.

Immediately after drug administration the rats were placed in the test cages (one rat per cage) and put into the motility meters. Motor activity was then followed and recorded for the subsequent 60 min. Each box was equipped with a semitransparent mirror that allowed gross behavior observations of the animals during the experiments. The motor activity results are shown in Table I.

Acknowledgment. The authors thank Ingrid Bergh, Lucia Gaete-Valderrama, and Boel Göransson for their assistance in the pharmacological testing. Dr. Magnar Ervik (AB Hässle, Mölndal, Sweden) is gratefully acknowledged for performing GC/MS recordings. The financial support from Astra Läkemedel AB, Södertälje, Sweden, and the Swedish Board for Technical Development, "Svenska Läkarsällskapet", "Wilhelm & Martina Lundgrens Vetenskapsfond", and the Medical Faculty, University of Göteborg, is gratefully acknowledged.

Use of (*S*)-(Trifloxymethyl)oxirane in the Synthesis of a Chiral β-Adrenoceptor Antagonist, (*R*)- and (*S*)-9-[[3-(*tert*-Butylamino)-2-hydroxypropyl]oximino]fluorene

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Two synthetic approaches were used to prepare, in chirally pure form, the β-adrenoceptor antagonist 9-[[3-(*tert*-butylamino)-2-hydroxypropyl]oximino]fluorene (**1a**). One of these employed the oxazolidine (*S*)-**6** generated from D-mannitol, while the other utilized (*S*)-[[(trifluoromethanesulfonyl)oxy]methyl]oxirane (**4**) as the chiral three-carbon fragment. This latter synthesis was designed to incorporate the amino function in the last step. In vitro, a β_2 selectivity of only 2.2 was observed for **1a**. The example, (*S*)-9-[[3-(*tert*-amylamino)-2-hydroxypropyl]oximino]fluorene (**1b**), was also prepared and found to be selective for the β_1 receptor by a factor of 2.5. In contrast to other β-adrenoceptor antagonists, the enantiomers of **1a** exhibited no chiral preference; i.e., (*S*)-**1a** and (*R*)-**1a** possessed a similar order of β-adrenoceptor antagonistic activity.

Since the β-adrenoceptor blocking agent 9-[[3-(*tert*-butylamino)-2-hydroxypropyl]oximino]fluorene [(*RS*)-**1a**] has been reported to be a selective antagonist for the β_2 receptor,[1] it was felt that the *S* enantiomer [(*S*)-**1a**] or a

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(1) Imbs, J. L.; Miesch, F.; Schwartz, J.; Velly, J.; LeClerc, G.; Mann, A.; Wermuth, C. G. *Br. J. Pharmacol.* **1977**, *60*, 357.

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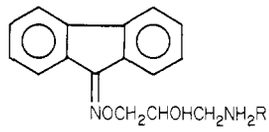


1a, R = $C(CH_3)_3$
b, R = $C(CH_3)_2C_2H_5$

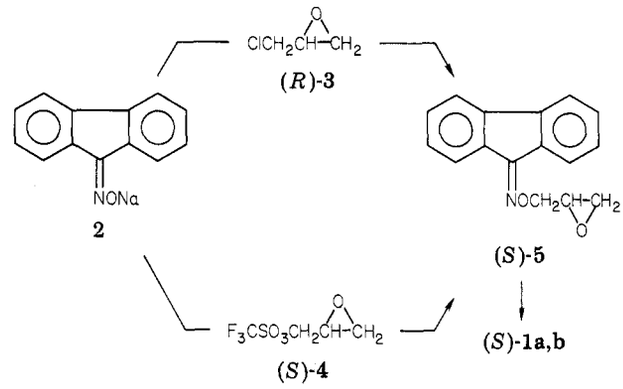
structurally similar analogue would be a particularly attractive candidate for use as a radioligand in studies of β_2-adrenergic receptors. Before such a ligand could be considered, it was necessary first to develop a method of synthesis that would allow for both stereochemical control and the introduction of the [^{3}H]alkylamino function in the last step and, secondly, to validate the in vitro β_2 selectivity of the chosen example. Since methods for the synthesis of [^{3}H]*tert*-butylamine have not been reported, it was decided to investigate the *tert*-amyl derivative **1b** as a viable alternative. In this case, the required *tert*-amylamine could, in principle, be derived from 1,1-dimethylpropargylamine via catalytic hydrogenation, a method suitable for tritium introduction. This report describes the successful development of such a synthetic method using (trifloxymethyl)oxirane and also outlines the comparative in vitro pharmacology of **1a**, (*S*)-**1a**, (*R*)-**1a**, and **1b** in regard to β-receptor affinity and specificity.

Chemistry. We have recently reported that (*S*)-[(aryloxy)methyl]oxirane can be conveniently prepared in high chiral purity from the reaction of phenoxide with either (*R*)-(chloromethyl)oxirane (**3**) in acetone or (*S*)-(trifloxymethyl)oxirane (**4**) in THF/CH_2Cl_2.[2] Under these conditions, phenoxide reacts with **3** primarily via a mechanism involving initial opening of the epoxide ring and with **4** by direct displacement of the triflate group. As an approach to chiral **1a** and **1b**, we therefore investigated the O-alkylation of fluorenone oxime with **3** and **4**. The projected reaction sequence is outlined in Scheme I.

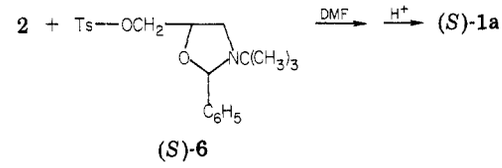
Initially, the reaction of racemic **3** with fluorenone oxime was studied in acetone/K_2CO_3, the conditions found to give chiral control with phenols. The crude reaction mixture on treatment with *tert*-butylamine gave **1a** in low yield, along with a nearly quantitative recovery of unreacted oxime. Due to these unsatisfactory results, the reaction of the sodium salt **2** was studied under the conditions summarized in Table I. In all cases, the intermediate epoxide **5** was not purified but was allowed to react directly with 1.5 equiv of *tert*-butylamine in ethanol, and the resulting (*S*)-**1a** was isolated by chromatography. As presented in Table I, the solvent was important in determining the mode of nucleophilic attack on (*R*)-**3**. In DMF, essentially no selectivity was observed, while in either acetone or THF, there was a 3.5:1 preference for reaction at the epoxide ring over direct chloride displacement. In contrast to this multidirectionality of attack seen with (*R*)-**3**, use of the triflate (*S*)-**4** in the reaction sequence yielded enantiomerically pure (*S*)-**1a**. This retention of chirality is in agreement with the results previously found in the reaction of **4** with phenols.[2]

Optical rotations were determined prior to recrystallization to assure that no change in enantiomeric composition induced by purification would be encountered. Chiral purity was determined by comparison of optical rotations and by examination of NMR spectra of the product in the presence of the chiral shift reagent Eu(hfbc)$_3$. In these spectra the aromatic syn peri hydrogen

Scheme I



Scheme II



Scheme III

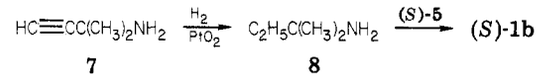
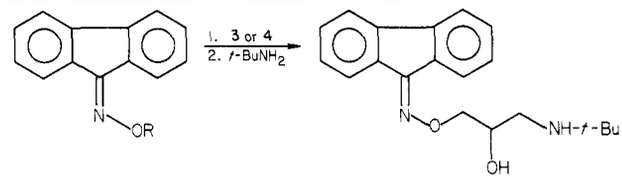


Table I



R	solvent	reactant	yield, %	$[\alpha]^{25}_D$ (CH_3OH)	S/R^a
H	acetone/K_2CO_3	(*RS*)-**3**	9[b]		50/50
Na	DMF	(*R*)-**3**	41	1.0	52/48
Na	THF	(*R*)-**3**	9	12.84	78/22
Na	acetone	(*R*)-**3**	27	12.75	78/22
Na	THF/CH_2Cl_2	(*S*)-**4**	21	20.38	⩾98/2

[a] These *S*/*R* ratios were determined through the use of chiral-shift NMR analysis. Samples were not recrystallized prior to analysis in order to ensure that no increase in enantiomeric excess occurred by purification. Recrystallized (*S*)-**1a** exhibited $[\alpha]^{25}_D$ 22.7° (*c* 0.6, CH_3OH) (see Experimental Section). Optical rotations were also used to determine the enantiomeric *S*/*R* ratios and generally corroborated the chiral-shift NMR results. [b] This reaction gave racemic product,[1] mp 101–104 °C.

was used as the analytical signal; a separation of the enantiomeric signals of 30 Hz was achieved in the presence of about 0.2 molar equiv of the shift reagent.

The assignment of the absolute configuration and enantiomeric ratios of **1a** generated under the various reaction conditions required correlation with **1a** of high optical purity and known configuration. This was accomplished by reaction of **2** with the tosylate (*S*)-**6**[3,4,5] according to

(2) (a) McClure, D. E.; Arison, B. H.; Baldwin, J. J. *J. Am. Chem. Soc.* **1979**, *101*, 3666. (b) Baldwin, J. J.; Raab, A. W.; Mensler, K.; Arison, B. H.; McClure, D. E. *J. Org. Chem.* **1978**, 43, 4676.

(3) (a) Baldwin, J. J.; Engelhardt, E. L.; Hirschmann, R.; Lundell, G. F.; Ponticello, G. S.; Ludden, C. T.; Sweet, C. S.; Scriabine, A.; Share, N. N.; Hall, R. *J. Med. Chem.* **1979**, *22*, 687. (b) Baldwin, J. J.; Hirschmann, R.; Lumma, P. K.; Lumma, W. C., Jr.; Ponticello, G. S.; Sweet, C. S.; Scriabine, A. *J. Med. Chem.* **1977**, *20*, 1024.

(4) Weinstock, L. M.; Mulvey, D. M.; Tull, R. *J. Org. Chem.* **1976**, *41*, 3121.

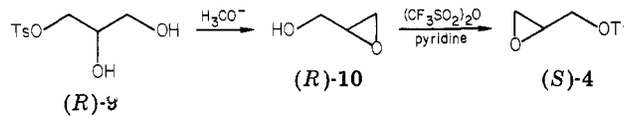
(5) Reinhold, D. F. U.S. Patent 4 097 490, June 27, 1978.

Scheme II. The enantiomer (*R*)-**1a** was synthesized by a similar process from the (*R*)-mesylate corresponding to **6**[5] as described under Experimental Section.

Acidic workup of the reaction mixture to remove the oxazolidine protecting group, followed by chromatography, provided the pure enantiomers, (*S*)-**1a** and (*R*)-**1a**. Racemic **1a** was prepared as previously described.[1] These materials were used in all comparative studies.

A reaction sequence similar to that used for (*S*)-**1a** was employed for the preparation of the *tert*-amyl derivative, (*S*)-**1b**, and is shown in Scheme III. The reduction of the propargylamine **7** proceeded smoothly following the procedure described for the hydrogenation of 1,1-dimethylallylamine.[6] The use of diglyme as solvent allowed for room temperature, vacuum distillation of **8** directly from the reaction mixture in a purity suitable for conversion to **1b**. The reaction of **8** with (*S*)-**5**, prepared from redistilled (*S*)-**4**, led to a 60% overall yield of (*S*)-**1b**. Chiral-shift NMR analysis of the free base indicated a chiral purity of >98% *S*. The corresponding racemic **1b** was prepared in a manner analogous to that used for racemic **1a**.

Since (*S*)-**4** is a somewhat labile reagent, comments should be made in regard to its preparation and storage. The conversion of (*R*)-glycidol to (*S*)-**4** can be readily accomplished according to procedures similar to those used for (*R*)- and (*S*)-**3** and (*S*)-[(mesyloxy)methyl]oxirane.[2] Treatment of (*R*)-**9** with base gave crude (*R*)-**10**, which was



(*R*)-**9** (*R*)-**10** (*S*)-**4**

used without further purification in the reaction with triflic anhydride. Distillation under reduced pressure gave (*S*)-**4** in 55–60% yield. Storage of the crude triflate at −20 °C overnight, followed by distillation, resulted in yields of only 30–45%. Distilled (*S*)-**4** decomposed within 2 days at room temperature but could be stored for 2–4 weeks at −20 °C. The redistillation of material stored longer than 2–4 weeks provided (*S*)-**4** with substantial loss.

Discussion

With the development of synthetic methods (Schemes I and II) for (*R*)- and (*S*)-**1a** and the *tert*-amyl analogue **1b**, it was possible to assess some of the critical structure–activity relationships in the oximic series of β-adrenoceptor antagonists, including β_2 selectivity, enantiomeric specificity, and relative equivalency of the *N*-*tert*-amyl substituent. In vitro methods were chosen as the approach to answer these questions of relative adrenoceptor affinities. The basis for assessment was pA_2 values determined using guinea pig atria (β_1) and tracheal chains (β_2); these data were supplemented with IC_{50} values for **1a** determined by radioligand binding studies obtained using heart and lung homogenates with [^{3}H]dihydroalprenolol as radioligand.

As indicated in Table II, only a 2.2-fold β_2 selectivity was found for **1a**; this is in contrast to the studies of Imbs et al.,[7] who reported a 155-fold β_2 selectivity. However, our results are in agreement with those of O'Donnell and Walduck[8] who found a β_2 selectivity of 3.3. The discrepancy does not lie in atrial pA_2 values but rather in the tracheal pA_2 determinations. Imbs observed a pA_2 of 9.23 using carbachol-contracted trachea, while O'Donnell's value of 7.98,[8] based on intrinsic tone, and ours of 7.81, obtained with $PGF_{2\alpha}$-induced tone, are in good agreement. In addition, Imbs' high selectivity was based on Schild plots with widely different slopes (1.32 for atria vs. 0.66 for trachea). Our results and those of O'Donnell indicate that **1a** is β_2 selective, but not nearly to the extent previously reported. To independently confirm the atria–trachea results, relative in vitro selectivity for **1a** was determined at the level of β receptor by its ability to displace the nonselective ligand [^{3}H]dihydroalprenolol from heart (β_1) and lung membranes (β_2). The IC_{50} values listed in Table III indicate only a slight degree of selectivity for **1a**, with (*R*)-**1a** showing a twofold selectivity for the β_2 receptor, and confirm the results found with the atria–trachea preparations. The sensitivity of the method to selective agents is, however, demonstrated by the 18-fold β_1 selectivity found for betaxolol and the 11-fold β_2 selectivity observed with ICI-118551 (**13**). Using iodinated IHYP as radioligand, Minneman et al.[9] obtained curvilinear Hofstee plots indicative of the presence of mixed β_1/β_2 receptor populations in several tissue preparations. From com-

Table II. In Vitro Activity of 1a and Its Enantiomers on Guinea Pig Atria and Tracheal Chains

compd	pA_2[a] atria (β_1)	pA_2[a] trachea (β_2)
1a	7.47 ± 0.103 (7.23-7.72)	7.81 ± 0.026 (7.75-7.87)
(*S*)-**1a**	7.43 ± 0.076 (7.25-7.61)	7.77 ± 0.044 (7.67-7.88)
(*R*)-**1a**	7.88 ± 0.102 (7.64-8.12)	7.74 ± 0.053 (7.62-7.87)
1b	7.46 ± 0.077 (7.28-7.65)	7.07 ± 0.059 (6.93-7.21)
(*S*)-**1b**	7.78 ± 0.080 (7.60-7.97)	7.13 ± 0.051 (7.01-7.25)
13	7.01 ± 0.086 (6.80-7.21)	7.50 ± 0.023 (7.44-7.56)
12 (metoprolol)	7.83 ± 0.105	6.79 ± 0.040 (6.70-6.88)
(*S*)-**11** (timolol)	9.44 ± 0.074 (9.28-9.61)	9.62 ± 0.102 (9.38-9.86)
(*R*)-**11**	7.57 ± 0.107 (7.31-7.82)	7.86 ± 0.101 (7.62-8.10)
(*S*)-**12**	8.12 ± 0.056 (7.98-8.25)	7.02 ± 0.027 (6.95-7.08)
(*R*)-**12**	6.13 ± 0.044 (6.02-6.23)	5.24 ± 0.026 (5.18-5.30)

[a] Mean plus or minus standard error of the mean with 95% confidence limits in parentheses.

Table III. [^{3}H]DHA Binding in Dog Heart and Calf Lung Membranes

compd	IC_{50},[a] nM heart (β_1)	IC_{50},[a] nM lung (β_2)	β_2/β_1
(−)-alprenolol	4.88 ± 1.25	2.98 ± 0.09	0.61
betaxolol	37.1 ± 8.4	657 ± 137	17.71
13	186 ± 12	17.6 ± 1.5	0.09
1a	37.9 ± 11	24.6 ± 4.1	0.65
(*S*)-**1a**	29.4 ± 2.2	30.8 ± 4.4	1.05
(*R*)-**1a**	43.3 ± 6	20.1 ± 3.5	0.46

[a] Results are the mean plus or minus standard deviation for three to five separate observations. Drugs were run at three or more concentrations in each experiment, and IC_{50}'s were determined by linear regression of log probit analysis of radioligand displacement.

(6) Semenow, D.; Shih, C.-H.; Young, W. G. *J. Am. Chem. Soc.* **1958**, *80*, 5472.
(7) Imbs, J. L.; Miesch, F.; Schwartz, J.; Velly, J.; LeClerc, G.; Mann, A.; Wermuth, C. G. *Br. J. Pharmacol.* **1977**, *60*, 357.
(8) (a) O'Donnell, S. R.; Walduck, K. *J. Pharm. Pharmacol.* **1981**, *33*, 223. (b) O'Donnell, S. R.; Wonstall, J. C. *Life Sci.* **1980**, *27*, 671.
(9) Minneman, K. P.; Hedberg, A.; Molinoff, P. B. *J. Pharmacol. Exp. Ther.* **1979**, *211*, 502.

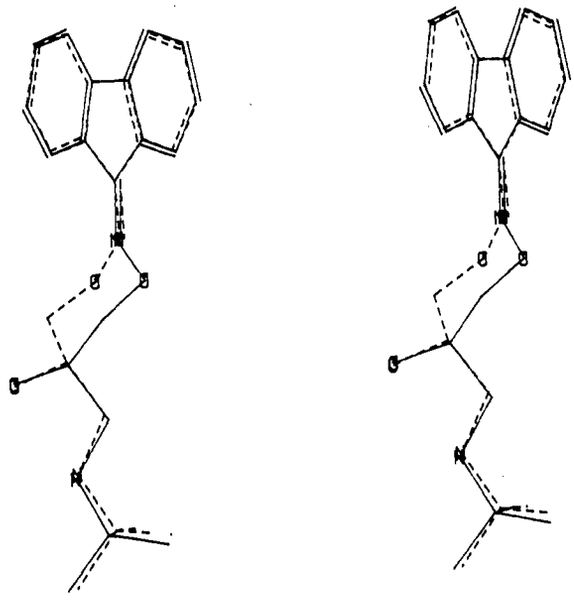
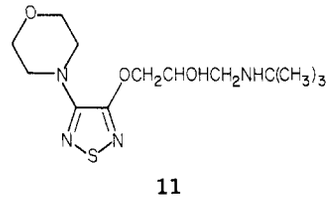


Figure 1. Superposed stereoscopic view A of (*R*)-**1a** (solid) and (*S*)-**1a** (dotted) illustrating spacial relationship of the aryl–hydroxyl–amine triad.

puter-assisted fits of such Hofstee plots, a β_2/β_1 ratio of 25 was obtained for **1a**, a finding in contrast to that observed in the present study. Based on our in vitro studies, **1a** would not be likely to serve as a satisfactory β_2-specific ligand, and hence its synthesis in tritiated form was not pursued.

Despite our failure to demonstrate β_2 selectivity in vitro, **1a** has been found to exhibit such selectivity in vivo.[10] At this time it would appear prudent to cautiously evaluate both in vitro and in vivo results by taking specific experimental conditions into account before concluding that receptor selectivity exists. This is supported by the studies of O'Donnell and Wonstall,[8b] who demonstrated the need for β_2-selective agonists in the evaluation of the β_2-selective antagonist ICI 118551, **13**. In our hands, with isoproterenol as agonist, this compound was only modestly β_2 selective.



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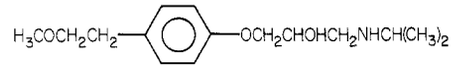

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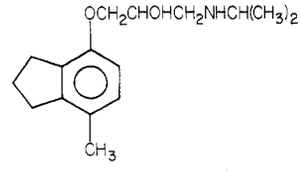

13

The second key point to emerge from this study was the observation that both *R* and *S* enantiomers of **1a** had similar potency as nonselective β-adrenoceptor antagonists (Table II). This is in contrast to other β-adrenergic blocking agents which exhibit a separation in potency between stereoisomers; thus, this represents the first enantiomeric pair to exhibit an absence of stereochemical selectivity. These differences are illustrated with timolol (*S*)-**11** and metoprolol (**12**). A conformational analysis of (*R*)- and (*S*)-**1a** provides a possible explanation for this anomaly. As indicated in the stereoscopic representations, Figures 1 and 2, conformations of the enantiomers exist where the aryl–hydroxy–amino triad are identically positioned in space. This unusual conformational superposition of the two enantiomers is due to the pseudoasymmetry introduced by the oxime function. However, of the two possible conformations, we prefer A, since the bulk requirement on either side of the aryl binding site imposed by B seems unrealistic. It may be possible to distinguish between A and B through the evaluation of selected, enantiomerically pure analogues of **1a**. Although there is at present insufficient structural evidence to select between A or B or to gauge the contribution of the imino double bond, the molecular superpositions offer an explanation for the recognition of both enantiomers by the β-adrenergic receptor. Thus, the oximino class of antagonist, in contrast to the (aryloxy)propanolamines, have no chiral requirement in the case of symmetrical fluorenones.

(10) D. M. Gross, unpublished observation.

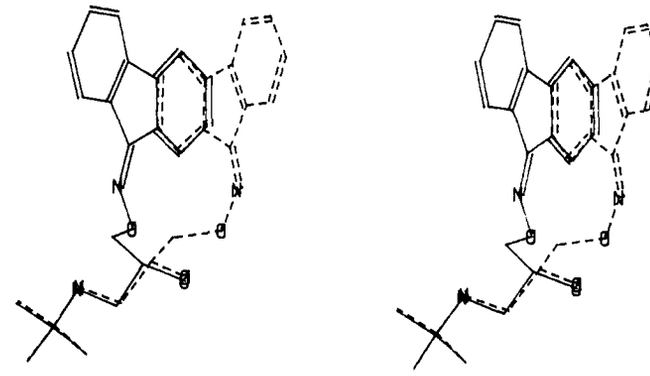

Figure 2. Stereoscopic view B of (*R*)-**1a** (solid) and (*S*)-**1a** (dotted) illustrating an alternate superposition.

As indicated in Table II, the *tert*-amyl group can serve as a replacement for the *tert*-butyl group in potential nonselective ligands. Since the *tert*-amyl derivative (*S*)-**1b** is β_1 selective as opposed to the β_2 selectivity seen with (*S*)-**1a**, when relative subreceptor selectivity is critical, as with **1a**, effects of this structural modification on β_2/β_1 ratio will have to be determined on an individual basis.

In summary, the chirally controlled (trifloxymethyl)-oxirane procedure outlined in Scheme I is a viable method for the preparation of the aminohydroxypropoxy side chain in which the amino function is introduced in the last step. This approach provides a chiral epoxide synthon which can be used to facilitate studies of the N-substituent in β-adrenoceptor antagonists. Although the level of in vitro selectivity for the β-receptor subtypes suggests that tritiated **1a** would not be of value, conceptually the general approach from dimethylpropargylamine offers a promising avenue to the synthesis of other potentially useful radioligands.

Experimental Section

NMR spectra were determined in the indicated solvent on a Varian T-60 using tetramethylsilane as an internal standard. The NMR studies to determine the chiral purity of various products were conducted on a Varian SC-300 operating in the Fourier transform mode. Optical rotations were determined using a Perkin-Elmer 141 polarimeter. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Concentration of solutions was accomplished on a Buchi rotary evaporator at water aspirator pressure (20–25 mm). Analyses are within 0.4% of theoretical values when indicated by symbols of the elements.

(S)-[[(Trifluoromethanesulfonyl)oxy]methyl]oxirane [(S)-4]. To a cooled solution of (R)-9[2b] (25.0 g, 0.10 mol) in ether (100 mL) and methanol (200 mL) was added the Na spheres (2.2 g, 0.10 mol). The reaction mixture was then stirred for 1 h with ice cooling before removal of the solvent on a rotary evaporator at 30 °C (25 mm). Ether was added, the salts were removed by filtration, and the solvent was removed at 30 °C (25 mm). Chloroform was added and evaporated from the resulting oil twice to remove residual methanol. Crude (R)-glycidol (**10**, 80%) was used without further purification; NMR analysis indicated the amount of **9** present.

Triflic anhydride (26.8 g, 0.095 mol, 1 equiv for each equivalent of **10** and 2 equiv for each equivalent of **9**) was added dropwise to pyridine (7.60 g, 0.096 mol) in CH_2Cl_2 (500 mL) while cooling at −23 °C in a dry ice/CCl_4 bath. When this addition was complete, **10** was added slowly. After stirring for 15 min at −23 °C and 30 min without cooling, an equal volume of hexane was added, and the solution was filtered and concentrated at 30 °C (25 mm). The residue was distilled using a short-path distillation head with dry ice/acetone cooling of the receiver to give 9.4 g (57%) of (S)-[[(trifluoromethanesulfonyl)oxy]methyl]oxirane: bp 36–39 °C (0.2 mm); $[\alpha]^{24}_D$ 14.7° (*c* 3.90, $CHCl_3$); NMR ($CDCl_3$) δ 2.7 (1 H, d of d, *J* = 5 and 2 Hz), 2.9 (1 H, t, *J* = 5 Hz), 3.3 (1 H, m), 4.3 (1 H, d of d, *J* = 6 and 12 Hz), 4.7 (1 H, d of d, *J* = 3 and 12 Hz). The use of $Eu(hfbc)_3$ in chiral-shift NMR analysis of this material indicated a chiral purity of ≥98% *S*.

(RS)-[[(Trifluoromethanesulfonyl)oxy]methyl]oxirane [(RS)-4]. The use of (RS)-glycidol in place of (R)-glycidol in the above procedure gave (RS)-**4** (60%): bp 35–40 °C (0.2 mm). Anal. ($C_4H_5F_3SO_4$) H; C: calcd, 23.30; found, 22.74.

(S)-9-[[(3-(tert-Butylamino)-2-hydroxypropyl]oximino]fluorene [(S)-1a]. Method A. To 9-fluorenone oxime (970 mg, 5 mmol) in dry THF (15 mL) was added NaH (50% in mineral oil, 240 mg, 5 mmol). After stirring for 15 min at room temperature, (S)-**4** (1 g, 0.005 mol) in CH_2Cl_2 (5 mL) was added dropwise. The mixture was stirred at ambient temperature for 1 h and then concentrated under reduced pressure. Ether and water were added, the layers were separated, and the aqueous phase was extracted with ether. After drying, the organic layer was concentrated to yield (S)-**5** as an oil: NMR ($CDCl_3$) δ 2.8 (2 H, m), 3.4 (1 H, m), 4.35 (1 H, d of d, *J* = 13 and 6 Hz), 4.6 (1 H, d of d, *J* = 13 and 4 Hz), 7.2–7.9 (7 H, m), 8.35 (1 H, m).

The resulting oil was dissolved in ethanol (10 mL), and *tert*-butylamine (600 mg, 8 mmol) was added. After standing overnight at ambient temperature, the mixture was concentrated. The crude product was chromatographed on silica gel using $CHCl_3$ saturated with ammonia as eluant. The product fractions were collected and concentrated, and the residue was triturated with hexane to give 350 mg (20% overall) of solid (S)-**1a**: mp 83–84 °C; $[\alpha]^{24}_D$ 20.38° (*c* 1.6, MeOH); NMR ($CDCl_3$) δ 1.1 (9 H, s), 2.7 (2 H, m), 4.1 (1 H, m), 4.4 (2 H, d, *J* = 6 Hz), 7.1–7.8 (7 H, m), 8.3 (1 H, m). The use of $Eu(hfbc)_3$ in an NMR chiral-shift analysis indicated a chiral purity of ≥98% *S*. Recrystallization from hexane gave (S)-**1a** having the same properties as the material prepared by method B: $[\alpha]^{23}_D$ 22.70° (*c* 0.63, CH_3OH). Anal. ($C_{20}H_{24}N_2O_2$) C, H, N.

Method B. To fluorenone oxime (2.5 g, 0.013 mol) in DMF (20 mL) was added NaH (600 mg, 50% in mineral oil, 0.013 mol). The tosylate (S)-**6**,[7] prepared from 2-phenyl-3-*tert*-butyl-5-(hydroxymethyl)oxazolidine (3.2 g, 0.014 mol), was added in DMF (5 mL) and the mixture was warmed for 18 h at 100 °C. Water was added and the mixture was extracted with $CHCl_3$. The organic extract was concentrated and suspended in 1.5 N HCl (25 mL). After warming for 30 min over steam, the mixture was cooled and extracted with $CHCl_3$. The organic layer was concentrated, and the residue was treated with 1.5 N NaOH solution. The mixture was extracted with $CHCl_3$; the extract was dried over Na_2SO_4 and chromatographed on silica gel using ammonia-saturated $CHCl_3$ to yield **1a** after recrystallization from hexane (1.2 g, 29%): mp 84.5–85.5 °C; $[\alpha]^{23}_D$ 22.76° (*c* 1.6, MeOH). The free base was converted to the hydrochloride salt: mp 154–155 °C; $[\alpha]^{25}_D$ 10.7° (*c* 0.634, CH_3OH). Anal. ($C_{20}H_{24}N_2O_2$·HCl) C, H, N.

1,1-Dimethylpropylamine (8).[6] A mixture of **7** (9.2 g 90%, 0.10 mol), diglyme (50 mL), and PtO_2 was hydrogenated at 40 psi on a Parr apparatus until the theoretical amount of H_2 had been absorbed. The volatile material was distilled directly from the reaction mixture at ambient temperature (0.2 mm) while trapping these materials in a dry ice/acetone bath. The desired **8** was produced essentially quantitatively, although it showed some contamination with H_2O and traces of ethers related to diglyme. The product was sufficiently pure for subsequent use. NMR ($CDCl_3$) δ 0.9 (3 H, t), 1.1 (6 H, s), 1.4 (2 H, m).

(S)-9-[[3-tert-(Amylamino)-2-hydroxypropyl]oximino]fluorene [(S)-1b]. The use of **8** in place of *tert*-butylamine in method A for the preparation of (S)-**1** gave (S)-**1b** (60% overall) after chromatography. The free base [(S)-**1b**] was crystallized as the hydrochloride of (S)-**1b** in ethanol/ether: mp 140–142 °C; $[\alpha]^{23}_D$ 10.6° (*c* 1.05, MeOH); NMR (Me_2SO-d_6) δ 0.85 (3 H, t, *J* = 7.5 Hz), 1.25 (6 H, s), 1.65 (2 H, q, *J* = 7.5 Hz), 3.0 (2 H, m), 4.4 (3 H, m), 7.25–8.05 (7 H, m), 8.35 (1 H, m). The maleate salt was generally easier to handle and could be prepared from the free base: mp 142–144 °C; $[\alpha]^{25}_D$ 9.80° (*c* 1.49, CH_3OH). The $Eu(hfbc)_3$ chiral-shift NMR analysis of (S)-**1b** regenerated from its hydrochloride or maleate salts indicated a chiral purity of ≥98% *S*. Anal. ($C_{21}H_{26}N_2O_2$·HCl) C, H, N. Anal. ($C_{21}H_{26}N_2O_2$·$C_4H_4O_4$) C, N; H: calcd, 6.66; found, 7.09.

(R)-9-[[3-(tert-Butylamino)-2-hydroxypropyl]oximino]fluorene [(R)-1a]. To an ice-cooled solution of (R)-2-phenyl-3-*tert*-butyl-5-(hydroxymethyl)oxazolidine[5] (6.44 g, 0.0274 mol) and triethylamine (4.2 mL, 0.03 mol) in CH_2Cl_2 (100 mL) was added methanesulfonyl chloride (2.2 mL, 0.028 mol) dropwise over a few minutes. Stirring was continued for 0.5 h with cooling and for 1 h without. Potassium carbonate (4.0 g, 0.029 mol) was added, and the layers were separated. After drying and concentration, the crude (R)-mesylate (94%) was used without further purification.

To fluorenone oxime (5.07 g, 0.026 mol) in DMF (30 mL) was added NaH (1.25 g, 50% in mineral oil, 0.026 mol), and the mixture was briefly heated. After the mixture cooled, the (R)-mesylate from above was added dropwise over a few minutes, and the mixture was then heated on a steam bath for 60 h. Water was added, and the mixture was extracted with ethyl acetate. The ethyl acetate was backwashed with water and then dried (Na_2SO_4). After concentrating, the residue was chromatographed following the procedures given for the corresponding *S* isomer. The hydrochloride salt was formed in ethanol/ether and recrystallized from CH_3CN to give (R)-**1a**·HCl (34%): mp 153–156 °C; $[\alpha]^{25}_D$ −10.2° (*c* 0.62, CH_3OH). The free base was regenerated from the HCl salt: mp 85–87 °C; $[\alpha]^{25}_D$ −22.36° (*c* 0.626, CH_3OH). Examination using $Eu(hfbc)_3$ for chiral-shift NMR analysis indicated a chiral purity of ≥98% *R*. Anal. ($C_{20}H_{24}N_2O_2$·HCl) C, H, N.

9-[(2,3-Epoxypropyl)oximino]fluorene (5). To fluorenone oxime (9.75 g, 0.050 mol) in DMF (100 mL) was added NaH (2.5 g, 50% in mineral oil, 0.052 mol) in portions. The mixture was then stirred for 1 h prior to the addition of epichlorohydrin (9.25 g, 7.8 mL, 0.10 mol) and continued stirring for an additional 3 h. The DMF was evaporated at 50 °C/2 torr on a rotary evaporator. The residue was taken up in CH_2Cl_2 and washed with H_2O. After drying and concentration, the residual oil, **5**, was used without further purification: NMR ($CDCl_3$) δ 2.4 (1 H, m), 2.7 (1 H, t, *J* = 5 Hz), 3.3 (1 H, m), 4.2 (1 H, d of d, *J* = 12 and 6 Hz), 4.5 (1 H, d of d, *J* = 12 and 4 Hz), 7.0–7.8 (7 H, m), 8.2 (1 H, m).

9-[[3-(tert-Amylamino)-2-hydroxypropyl]oximino]fluorene (1b). Crude **5** (6.2 g, 0.025 mol) was stirred with *tert*-amylamine (5 mL) in ethanol (100 mL) overnight.[1] After concentration, the residue was chromatographed as described for the *S* isomer. The maleate salt was prepared in, and recrystallized from, ethanol/ether to give **1b**-maleate, mp 145–148 °C. Anal. C, H, N.

Pharmacology. To determine in vitro β_1-adrenoceptor activity, female Duncan–Hartley guinea pigs (200–300 g body weight) were killed by cervical dislocation. The extirpated hearts were placed in warm Krebs buffer solution, and the left and right intact atria were isolated from the ventricles and major blood vessels. The left atrium was sutured to a glass mounting rod, and the right atrium was attached to the force-displacement transducer. The preparations were set up in water-jacketed, 10-mL isolated tissue baths using a modified Krebs buffer (pH 7.2, mM): NaCl, 106.1; KCl, 4.63; $CaCl_2$, 2.51; $MgSO_4$, 1.2; NaH_2PO_4, 0.88; $NaHCO_3$, 11.9; dextrose, 5.6; ascorbic acid, 0.051. The temperature of the baths

was maintained at 37 °C, and the buffer was continuously aerated with 95% O_2 and 5% CO_2. The initial tension of 1 g was applied to the atria following their attachment to the force transducer. The tissues were washed at 0 and 15 min. Fifteen minutes after the second wash, isoproterenol was added to the bath. Seven cumulative concentrations were given at 1-min intervals in volumes of 0.1 mL each. At the end of the first concentration–response, the tissues were washed at 0 and 15 min and allowed to recover for 30 min, at which time the isoproterenol concentration–response series was repeated. The atria were again washed, and the antagonist being tested was added to the bath at 0 and 15 min with a wash in between. At the end of 30 min, isoproterenol was added to the bath but at 5 times the original series concentration; i.e., instead of 1×10^{-9} M, it was added at 5×10^{-9} M in the presence of the antagonist.

To determine in vitro β_2-adrenoceptor blockade, female Duncan–Hartley guinea pigs (200–400 g body weight) were also used. They were killed by a blow to the head, and the trachea were excised and placed in a petri dish containing normal saline. The extraneous tissue was trimmed away, and the tracheal tube was cut lengthwise through the cartilage opposite the line of smooth muscle. Segments of trachea were cut approximately 2–3 mm wide. One segment from each of the four guinea pigs per assay was used in each tracheal chain. Segments were placed end to end and tied securely, taking care not to tie any of the smooth muscle in the knots. One end of the chain was attached to a glass tissue holder and the other end to a force-displacement transducer. The tracheal chains were then placed in 10-mL water-jacketed organ baths containing a modified Krebs buffer solution at 37 °C containing (mM) NaCl, 106.1; KCl, 4.63; $CaCl_2$, 1.89; $MgSO_4$, 1.16; NaH_2PO_4, 1.0; $NaHCO_3$, 25.0; dextrose, 11.1; ascorbic acid, 0.051; indomethacin, 0.0014; and $PGF_{2\alpha}$, 0.0014. The baths were constantly aerated with 95% O_2 and 5% CO_2. An initial 2.0-g tension was applied to each of the chains for exactly 5 min, after which the tension was lowered to 1.0 g. The chains were washed several times and allowed to stabilize for 60 min. After the chains had gained a degree of tension over the 60-min period, cumulative concentrations of the agonist (isoproterenol) were added to the baths at 5-min intervals. After the concentration–response curve was established, the tracheal chains were washed, then washed again 15 min later, and allowed to stabilize for a total time of 30 min. The concentration–response curve was repeated, the tissues were washed, and the antagonist was added to the bath. The chains were washed after 15 min exposure to the antagonist, which was then readministered to the bath for a total exposure for 30 min. A concentration–response curve was repeated in the presence of the antagonist.

Data Analysis. The data were analyzed by calculating the mean isoproterenol-induced relaxation of the tracheal chain (five to seven concentrations) for six to eight tracheal chain preparations. With the atria, the mean isoproterenol-induced chronotropic responses of the preparation (seven to eight concentrations) were calculated from seven to eight atria. These were done for the isoproterenol concentration–response in the absence of antagonist and in the presence of antagonist. The concentration–response relationship was analyzed by nonlinear symmetrical curve fitting routines to yield a calculated EC_{50}. If only a single concentration of antagonist was being studied, the data resulting from that analysis was used to calculate a local pA_2. All computations were performed on a DEC MINC-II computer.

Peripheral β-Adrenoceptor Binding Assays. The radioligand [^{3}H]dihydroalprenolol (DHA) was also used to define adreoceptor populations of the subtypes β_1 and β_2 using dog heart and calf lung, respectively. Dog heart ventricular membranes were prepared as described by Alexander.[11] The heart from a mongrel dog was removed and placed in "STM" buffer (0.25 M sucrose, 5 mM Tris-HCl, and 1 mM $MgCl_2 \cdot 6H_2O$, pH 7.4). The ventricular tissue was dissected from the intact heart and, after weighing, placed in 4 volumes of ice-cold STM buffer. The tissue was minced with scissors and homogenized in a Waring blender by 3×60 s at high speed, chilling the blender chamber between successive runs. The resultant homogenate was then rehomogenized with a Polytron (setting 6.0 for 60 s) and filtered through four layers of cheesecloth. The filtered homogenate was centrifuged at 700g for 12 min at 4C, and the resultant supernatant was centrifuged at 10000g for 12 min. The second supernatant was then centrifuged for 15 min at 29000g, and the resultant pellet was quick frozen in an acetone/dry ice bath and stored at −70 °C until needed. Protein determinations were performed on the second supernatant after thoroughly washing the suspension to remove the sucrose.

Binding was measured in polypropylene tubes in a final volume of 0.5 mL. Each assay tube contained 0.05 mL of [^{3}H]DHA to a final concentration of 0.2 nM, 0.05 mL of either (−)-alprenolol to a final concentration of 10 μm or 0.05 mL of 50 mM Tris-HCl (pH 8.0) containing drugs under evaluation to determine nonspecific and drug-dependent binding, respectively, and 50 mM Tris-HCl (pH 8.0) buffer to a volume of 0.3 mL. Binding was initiated by the addition of 0.2 mL of the tissue suspension in buffer (1 mg of protein/mL) and continued for 30 min at room temperature. The reaction was terminated by placing the assay tubes in an ice–water bath and adding 1 mL of ice-cold incubation buffer. Bound radioactivity was isolated by vacuum filtration on Whatman GF/F glass-fiber filters, and unbound radioactivity was removed with 3×5 mL washes of ice-cold buffer. Calf lung tissue was obtained immediately following death and placed on ice. Within 1–2 h, the lungs were freed of major bronchi, minced with scissors, and homogenized in 50 volumes of 50 mM Tris-HCl (pH 7.8) using a Polytron (setting 6.0 for 10 s). The resultant homogenate was rehomogenized with 12 complete strokes of a Teflon–glass homogenizer at 500 rpm. The homogenate was then filtered through a single layer of cheesecloth to remove connective tissue. The filtrate was then centrifuged at 30000g for 10 min at 4C. The pellet was washed three times with 50 mM Tris-HCl buffer (pH 7.8). An aliquot of the final suspension was used for protein determinations. The final pellet was frozen until needed for the assay.

The binding assay was carried out in polypropylene tubes. The frozen membranes were resuspended using the Polytron (setting 5.5 for 10 s) in 50 mM Tris-HCl (pH 7.8) to a final concentration of 1 mg of protein/mL. The binding assay was conducted as described above for dog ventricular membranes.

Following vacuum filtration and washing of the glass-fiber filters, they were placed in scintillation vials to which was added 10 mL of Aquassure 2 (New England Nuclear) liquid scintillation cocktail. The vials were capped and mechanically shaken for 30 min at room temperature. After equilibration, bound radioactivity was determined at an efficiency of approximately 38–40% in a Packard liquid scintillation spectrometer.

All determinations were conducted in triplicate. Total binding and nonspecific binding were determined for each binding assay, and specific binding was obtained as the difference between the two. This represented 100% binding. Specific binding was then calculated in the presence of each of three or more concentrations of the test agent and expressed as a percentage of the total specific binding. IC_{50}'s were then determined by linear regression analysis (Hewlett Packard HP-97 SD 03A program) of the log concentration vs. % probit inhibition of binding.

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