Effect of a Chiral 4-Alkyl Substituent in Hallucinogenic Amphetamines

Robert Oberlender,† P. V. Ramachandran,‡ Michael P. Johnson, Xuemei Huang, and David E. Nichols*

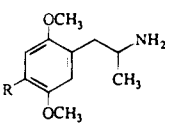
Departments of Medicinal Chemistry and Pharmacognosy and of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, and H. C. Brown and R. B. Wetherill Laboratories of Chemistry, Purdue University, West Lafayette, Indiana 47907

Received April 17, 1995®

The potency of hallucinogenic amphetamine derivatives of the 1-(2,5-dimethoxy-4-alkylphenyl)-2-aminopropane type drops dramatically when the length of the 4-alkyl substituent exceeds propyl or when the substituent is branched. This investigation was directed toward evaluating changes in behavioral and biochemical pharmacology resulting from introducing chirality into the 4-alkyl group of such analogues. Two diastereoisomeric derivatives of this class containing a 4-(*R* or *S*)-2-butyl substituent, **11a**,**b**, respectively, were studied. A slight but nonsignificant potency difference in *d*-lysergic acid diethylamide tartrate (LSD)-like discriminative stimulus properties and equal affinity for [^{125}I]-*(R)*-(2,5-dimethoxy-4-iodophenyl)isopropylamine-labeled serotonin 5-HT$_{2A/C}$ radioligand-binding sites were observed. Thus, the portion of the receptor that interacts with the 4-alkyl substituent on hallucinogenic amphetamines does not present a highly asymmetric environment to the ligand. However, since both test drugs had higher binding affinity but lower LSD-like behavioral potency than the prototype compound with a 4-methyl group ((2,5-dimethoxy-4-methylphenyl)isopropylamine, **2**), **11a**,**b** may differ in their receptor agonist efficacy from more behaviorally active compounds such as **2**.

A large number of hallucinogenic phenethylamine derivatives have now been evaluated in investigations of structure–activity relationships (SARs).[1–5] A majority of the analogues that have been studied are ring-methoxylated α-methylphenethylamine derivatives, also referred to as "substituted amphetamines." Recently, these compounds have been viewed with greater interest because of their selectivity and potency as agonists at the 5-HT$_2$ serotonin receptor subtypes.[6–8] The prototype compound (2,5-dimethoxy-4-methylphenyl)isopropylamine (DOM, **2**) and several more recently developed analogues containing 4-halogen substituents (**3** and **4**) are considered to be some of the best pharmacological examples of 5-HT$_2$ agonists.[9] In view of the significance of these compounds as tools to probe central serotonergic function, certain aspects of their SARs deserve further attention. In particular, we wished to expand on a previous study[10] which examined the effect of a methyl branch added to the 4-propyl group of **6**, by focusing on potential differences in biological activity resulting from chirality in the 4-alkyl substituent.

Within the homologous series of 4-alkyl derivatives, optimum clinical activity is associated with a straight alkyl chain two to three carbons in length.[11] Potency increases by 1 order of magnitude as the 4-hydrogen of **1** is replaced by a short alkyl chain and then decreases if chain length exceeds four carbons. In addition, the lack of (or greatly attenuated) hallucinogenic activity for the *tert*-butyl derivative **9** suggested that branching of the 4-alkyl substituent was not tolerated. Similar results were obtained in animal studies. For example, Pinder and co-workers[12] found that the 4-substituent



1 R = H
2 R = CH_3
3 R = Br
4 R = I
5 R = CH_2CH_3
6 R = $CH_2CH_2CH_3$
7 R = $CH(CH_3)_2$
8 R = $CH_2CH_2CH_2CH_3$
9 R = $C(CH_3)_3$
10 R = $CH_2CH(CH_3)_2$
11 R = $CH(CH_3)CH_2CH_3$
12 R = $CH_2CH_2CH_2CH_2CH_3$

was essential for high activity in the rabbit hyperthermia model. Activity remained high for **5** and **6** but decreased dramatically when branching in the form of an isopropyl group, as in **7**, or a *tert*-butyl group, as in **9**, was introduced at this position.[12] These results are consistent with a recent quantitative structure–activity relationship (QSAR) study in which activity was found first to increase with para substituent volume and then decrease as the volume passed a maximum value.[13]

Drug discrimination (DD) studies have provided further evidence for the deleterious effect of excessive chain length or branching on activity. The DD paradigm has been particularly useful in assessing the behavioral activity, or discriminative stimulus (DS) properties, of hallucinogens in animals and in identifying a critical role of 5-HT$_2$ receptors in mediating the cue produced by these drugs.[9] In rats trained to discriminate **2** from saline, it was found that **5**, **6**, and **8** were all perceived as similar to the training drug and had comparable potencies.[14] The lengthening by one methylene of the 4-butyl substituent of **8**, as in **12**, or rearrangement to a *tert*-butyl, as in **9**, resulted in the loss of **2**-like DS properties. In *d*-lysergic acid diethylamide tartrate (LSD)-trained rats, hallucinogen-like behavioral activity was observed to be sensitive to the position of a methyl branch on the 4-alkyl substituent since **10** was more potent and more qualitatively similar to LSD than was **11**.[10]

* Address all correspondence to this author at Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907. Telephone: (317)-494–1461. Fax: (317)-494–6790. Internet: drdave@sage.cc.purdue.edu.

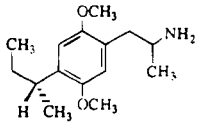
† Present address: Department of Pharmaceutics and Medicinal Chemistry, School of Pharmacy, University of the Pacific, Stockton, CA 95211.

‡ H. C. Brown and R. B. Wetherill Laboratories of Chemistry.

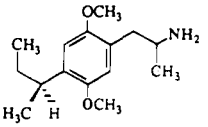
® Abstract published in *Advance ACS Abstracts*, August 1, 1995.

0022-2623/95/1838-3593$09.00/0

Steric factors, possibly related to the formation of a charge-transfer complex between hallucinogenic amphetamines and their binding sites, may be important in understanding the attenuation of activity resulting from branching in the 4-alkyl substituent.[10] The experiments described in this report were designed to investigate the effects of stereochemistry in the 4-alkyl substituent on the LSD-like DS properties of substituted amphetamine derivatives. Specifically, the simplest chiral alkyl substituent, a 2-butyl group, was examined. The [*(R)*- and *(S)*-4-(2-butyl)-2,5-dimethoxyphenyl]isopropylamines, **11a,b**, respectively, were prepared by asymmetric synthesis. These compounds can be viewed as analogues of the potent derivative **6**, in which a methyl branch is added to the benzylic carbon of the 4-*n*-propyl group. Behavioral activity was measured in stimulus generalization experiments using rats trained to discriminate 0.08 mg/kg LSD tartrate from saline in the two-lever DD paradigm. In addition, *in vitro* receptor binding experiments were performed using radioiodinated *(R)*-**4**, the 5-HT_2 agonist [^{125}I](2,5-dimethoxy-4-iodophenyl)isopropylamine ([^{125}I]-*(R)*-DOI), as the radioligand.



11a



11b

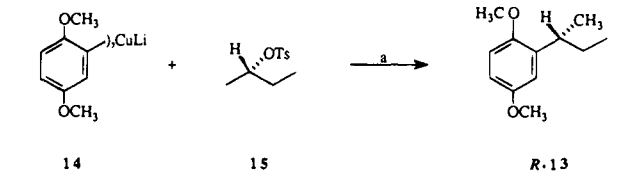
A difference in the interaction of these isomers with the receptor was anticipated, on the basis of a molecular mechanics analysis previously carried out in a model system.[15] In that study, the enantiomers of 2-(2-butyl)-anisole were used to model **11a,b**. This simplified system allowed a comparison of the steric factors in the interaction of the *(R)*- and *(S)*-2-butyl substituents with a hydrophobic surface, as might occur upon receptor binding. The results of that analysis demonstrated a clear difference between the conformational properties of the two enantiomers as studied in a chiral environment.[15] It was also found that **11a** can best approach a planar surface with its α-face, while **11b** can best approach with its β-face. Thus, in addition to gaining an understanding of stereochemical aspects of branching in the 4-substituent, the evaluation of these compounds was anticipated to provide data that would be useful in testing predictions of the orientation of substituted amphetamines in the drug–receptor interaction.

Chemistry

Racemic **11** was first prepared as described previously.[10] Although **11** is a mixture of diastereomers, they did not resolve on TLC, nor did repeated recrystallizations of the hydrochloride salt alter the melting point of this mixture. Several early attempts to prepare the individual isomers of **11** through chemical resolution of various intermediates also proved fruitless. Asymmetric syntheses were therefore considered.

The key intermediates for these syntheses were the *R*- and *S*-isomers of 2-(2,5-dimethoxyphenyl)butane, **13**. Hydrogenation of a prochiral olefin over asymmetric catalysts was rejected because of the generally low level of optical purity that could be expected for the product.[16] Three routes were then identified that could lead to the

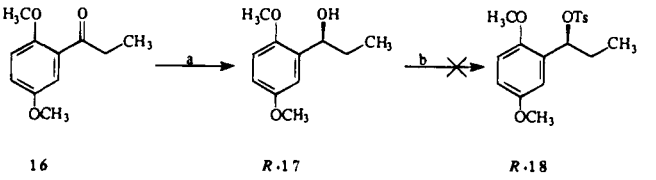
Scheme 1[a]



14 **15** ***R*-13**

[a] Reagent: Et_2O.

Scheme 2[a]



16 ***R*-17** ***R*-18**

[a] Reagents: asymmetric reduction; (b) tosyl Cl, pyridine.

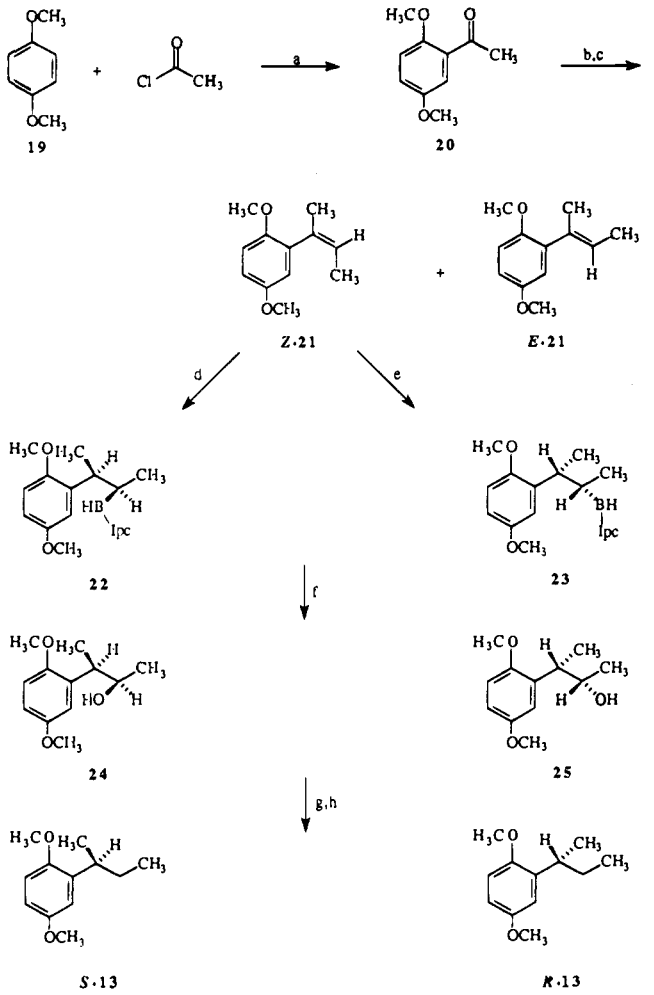
desired optically pure enantiomers of **13**. The first route, based on the work of Johnson and Dutra[17] and shown in Scheme 1, employed lithium bis(2,5-dimethoxyphenyl)cuprate, **14**, and *(S)*-(+)-2-butyl tosylate, **15**, and yielded the desired product *(R)*-(−)-**13**. This procedure served to confirm the absolute configuration of **13** as *R*-(−). Nevertheless, the separation of the product from unreacted dimethoxybenzene proved to be extremely tedious (in addition, at the time this work was done the optically pure enantiomers of 2-butanol were not commercially available), and other methods were examined.

The second route attempted to introduce chirality at the benzylic carbon with an asymmetric reduction of 1-(2,5-dimethoxyphenyl)propanone, **16**. It was anticipated that alcohol **17** could then be converted to tosylate **18**. Subsequent displacement in an S_N2 reaction with methyllithium would afford the enantiomers of **13**. This route is shown in Scheme 2. Although chiral alcohol **17** (96% ee) was successfully synthesized using the isomers of diisopinocampheylchloroborane,[18] careful attempts to convert the racemic alcohol to the tosylate or mesylate consistently resulted in elimination to the olefin.

The isomers of 2-(2,5-dimethoxyphenyl)butane were successfully prepared, as shown in Scheme 3, using an indirect method to accomplish an asymmetric hydrogenation of *(Z)*-2-(2,5-dimethoxyphenyl)-2-butene, **21**. The chiral benzylic center was formed by the hydroboration of the olefin with (+)- or (−)-monoisopinocampheylborane ($IpcBH_2$). Brown and co-workers[19,20] had previously demonstrated the synthetic utility of $IpcBH_2$ for phenyl-substituted tertiary olefins. Optical purities greater than 80% were reported, for example, in the hydroboration of 2-phenylbutene. In addition, a subsequently published report[21] described the possibility of improving enantiomeric excess of the product by simply recrystallizing the intermediate isopinocampheylalkylborane. Thus, the isopinocampheyl group is used for both optical induction and upgrading the dialkylborane products of lower optical purity.

The synthesis started with the Friedel–Crafts acylation of *p*-dimethoxybenzene, **19**, with acetyl chloride to form the ketone **20**. The Wittig reaction of **20** with ethyltriphenylphosphorane[22] afforded a 7:3 mixture of *Z*- and *E*-olefins, **21**, which were separated by spinning band distillation. The *Z*-olefin was then reacted with

Scheme 3[a]



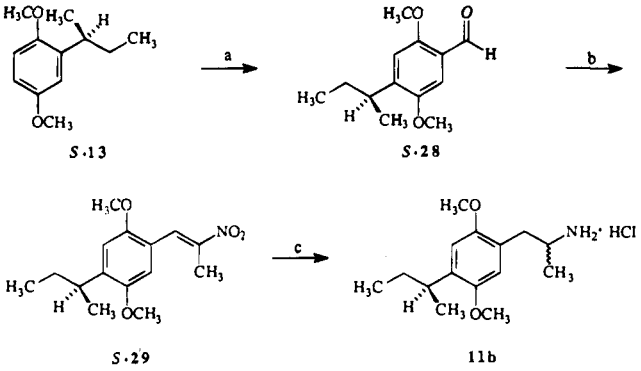
[a] Reagents: $SnCl_4$, CH_2Cl_2; (b) $EtP(PH)_3Br$, *n*-BuLi; (c) spinning band distillation; (d) (+)-$IpcBH_2$; (e) (−)-$IpcBH_2$; (f) H_2O_2, NaOH; (g) tosyl Cl, pyridine; (h) $LiAlH_4$, Et_2O.

optically pure (+)- or (−)-$IpcBH_2$,[23] yielding [(2*R*,3*S*)- and (2*S*,3*R*)-3-(2,5-dimethoxyphenyl)-2-butyl]boranes **22** and **23**, respectively. These products were solid, but unfortunately, crystallization did not improve their optical purity (as determined by the method described in the Experimental Section for **24**). Products with higher optical purity could not be obtained from the reactions of *E*-**21** with (+)- and (−)-$IpcBH_2$, since the intermediate dialkylboranes could not be induced to crystallize. However, the 82−83% ee that was achieved for **22** and **23** was felt to be more than adequate to determine pharmacological differences of any significant magnitude.

Attempts to replace the IpcBH group with hydrogen through protonolysis[24] with acetic, propionic, or isobutyric acid were unsuccessful, leading to decomposition. Instead, **22** and **23** were filtered and converted by hydrogen peroxide oxidation to the 2*R*,3*R*- and 2*S*,3*S*-alcohols **24** and **25**, respectively. Conversion of the alcohols to the tosylates was followed by reduction with lithium aluminum hydride, affording *(S)*-(+)- and *(R)*-(−)-**13**. The absolute configurations were confirmed by comparison of the sign of rotation of the products to material of unambiguous stereochemistry prepared by the previously described coupling reaction (Scheme 1).

The synthesis then proceeded with the formylation of the isomers of **13** using dichloromethyl methyl ether.

Scheme 4[a]



[a] Reagents: $SnCl_4$, Cl_2CHOCH_3, CH_2Cl_2; (b) NH_4OAC, $CH_3CH_2NO_2$; (c) $LiAlH_4$, Et_2O.

Initially, it was not clear whether the presence of tin(IV) chloride and free HCl in the reaction mixture would lead to racemization. To confirm that this did not occur, a sample of *(R)*-(−)-**13**, of known optical purity, was converted to the aldehyde by three different methods and the rotations were compared for the products obtained. The first method was just described. The second was the milder Vilsmeier-Haack reaction using *N*-methylformanilide and phosphorus oxychloride. The third method was that of Weyerstahl *et al.*,[25] by which 2-phenylbutane was iodinated, lithiated through a lithium−halogen exchange reaction, and then formylated by quenching with DMF, without loss of optical activity. All three methods resulted in an aldehyde of comparable rotation (within 0.5°), thereby demonstrating the lack of racemization with the more convenient first procedure. Completion of the synthesis was accomplished in the usual manner[10] which is illustrated in Scheme 4 for **11b**. The same procedure was used for **11a**. Because the critical stereochemical center for compound **11** was the benzylic carbon of the 4-alkyl substituent, it was important to determine whether chirality in the 4-substituent might effect any degree of asymmetric induction during the hydride reduction of the nitroolefin moiety. Such an event seemed unlikely, on the basis of the distance between the two centers, and it was indeed confirmed that the chiral center α to the nitrogen exists in the racemic form using HPLC analysis of the *(R)*-(*O*-methylmandeloyl)amide derivatives prepared from the final amines.

Pharmacology

Using methods described previously,[26] compound **2**, as well as **11** and **11a**,**b**, were evaluated in the two-lever drug discrimination assay in a group of rats trained to discriminate the behavioral state following injections of saline or LSD tartrate (0.08 mg/kg, ip). Potencies were measured, using ED_{50} values with 95% confidence intervals,[27] for those compounds that completely substituted for LSD. The methods used in binding experiments have also been described in an earlier report.[28] Briefly, the ability of the test compounds to displace 0.25 nM [^{125}I]-*(R)*-DOI ([^{125}I]-*(R)*-**4**) from binding sites in rat frontal cortex was measured. Using K_A values obtained from these studies, the free energy (ΔG) of binding was estimated from the equation $\Delta G = -RT \ln K_A$.

Results and Discussion

The results of substitution testing in rats trained to discriminate LSD tartrate from saline are presented in

Table 1. Results of Substitution Tests in LSD-Trained Rats

compd	dose (μmol/kg)	n^a	D^b	%SDL[c]	ED_{50} (μmol/kg)	(95% CI)
LSD	0.012	8	0	13	0.02	(0.01–0.04)
	0.024	8	0	13		
	0.047	8	0	38		
	0.093	9	0	89		
	0.186	9	0	100		
2	0.51	7	0	13	0.61	(0.38–0.95)
	0.77	9	0	67		
	1.02	9	0	67		
	2.04	9	1	100		
11a	0.87	11	1	0	3.08	(2.20–4.31)
	1.74	12	0	25		
	3.48	11	1	50		
	5.22	11	1	80		
11	1.74	9	1	18	3.69	(2.24–6.08)
	3.48	10	2	60		
	6.96	8	0	70		
	13.91	13	3	90		
11b	1.74	10	0	0	4.76	(3.06–7.42)
	3.48	11	1	60		
	5.22	11	1	30		
	6.96	11	1	70		
	13.92	14	2	90		
saline		8	0	0		

[a] Number of animals tested. [b] Number of animals disrupted. [c] Percentage of animals selecting drug lever.

Table 2. Results of 5-HT_2-Binding Studies with LSD, **2**, and the Diastereoisomers of **11**

drug	K_I (nM)	Hill coeff	$\Delta G°$ (kcal/mol)
LSD	6.31 ± 0.13	1.18 ± 0.08	−11.29
2	18.6 ± 2.00	0.89 ± 0.10	−10.65
11a	7.84 ± 1.08	1.00 ± 0.13	−11.16
11b	7.87 ± 0.63	1.06 ± 0.18	−11.16

Table 1. Complete substitution for the training stimulus was observed for each analogue tested. A dose of each test drug produced LSD-lever selection in at least 80% of the rats that emitted 50 presses on one lever. All of the eight rats tested with saline selected the saline lever. In quantitative terms, LSD was clearly the most potent of the test drugs, while **2** was of intermediate potency, and **11a,b** were least potent. Significant disruption of bar pressing, at the doses which mimicked the training dose of LSD, was not observed for any of the test compounds. While complete substitution of racemic **11** was observed in the present experiments, only partial substitution was obtained in a prior study.[10] The difference between these results is probably attributable to testing of more rats at each dose in the present work. This underscores the need for using a sufficient number of animals in DD experiments.

The potency of the test drug with the *(R)*-2-butyl group, **11a**, as measured by the ED_{50} value, was *ca.* 1.5 times greater than the potency of the same drug possessing the *(S)*-2-butyl substituent, **11b**, with the racemic mixture having an intermediate potency. In the DD paradigm, however, the practical limitations on the number of rats that are tested per dose of test compound results in large confidence intervals and a loss of statistical significance for relatively small differences (*e.g.*, less than 2-fold) in potency. Thus, the potency differences observed for the racemic and isomeric forms of **11** were not significant.

The data obtained in the receptor binding experiments are presented in Table 2. All of the test compounds displaced the 5-$HT_{2A/2C}$ agonist ligand [^{125}I]-*(R)*-DOI from binding sites in rat frontal cortex with high affinity. Interestingly, **11a,b** were virtually equipotent in displacing the radioligand, consistent with the lack of a significant difference in their behavioral activity. However, 5-$HT_{2A/2C}$ binding affinity of both **11a,b** is significantly greater than that of **2**. This result is especially noteworthy in light of the disparity observed when compared with the significant *decrease* in potency of either **11a,b** relative to **2** in producing LSD-like DS properties. The lack of correlation between behavioral activity and 5-HT_2 binding affinities at the agonist-labeled site can be rationalized in view of previously reported data. For example, the 4-*n*-butyl derivative **8** is only one-half as active as **2** in man[11] and in rats trained to discriminate **2** from saline.[14] Yet **8** had 2 times the affinity of **2** in displacing [^{3}H]ketanserin, an antagonist ligand, from rat cortical homogenate sites.[29] However, in radioligand displacement experiments where [^{3}H]DOB, a prototype 5-HT_2 agonist, was used as the radioligand, **8** had about 4-fold *lower* affinity than did **2**.[30]

In terms of our present level of understanding, if a single receptor type mediates the major effects of hallucinogens, the 5-HT_2 receptor appears to be the most likely candidate.[9,31] Although the affinity of substituted amphetamines for both the agonist and antagonist states of the 5-HT_2 receptor seems to increase with longer 4-alkyl substituents, the *in vivo* biological activity (potency for substituting for either LSD or **2**, as well as clinical activity) decreases when bulk or volume exceeds that of the *n*-propyl substituent. Therefore, it seems most likely that these compounds may have differing agonist efficacies at this receptor. Indeed, it has been suggested recently that certain hallucinogenic amphetamines that contain highly lipophilic 4-substituents may act as serotonin 5-HT_2 antagonists.[29]

We had originally hypothesized that one molecular face might be more critical for interaction with the receptor[15] and that studies of the sort presented here might help to elucidate this information. We gained no support for such a hypothesis from the data obtained from the present experiments. On the basis of our present understanding of the nature of G-protein-coupled receptors, of which the 5-HT_2 receptors are members, it seems possible that the 4-alkyl substituent of the substituted amphetamines may simply insert within various adjacent hydrophobic amino acid residues that do not create a highly asymmetric environment.

In conclusion, the results of the present study provide additional insight into the deleterious effect on the activity of 2,5-dimethoxyamphetamine (**1**) analogues due to branching in the 4-alkyl substituent.[10] The stereochemistry of the branching may exert some influence, but it does not appear to be a critical factor in the SAR. Further investigations could eventually lead to a better understanding of the specific features of subtypes of serotonin receptors that influence ligand binding. Finally, more work is needed to elucidate clearly the relationship between the DS properties, serotonin receptor interactions, and clinical activity of hallucinogenic agents. It should be noted that while the latter is obviously the most relevant, it is at present perhaps the least understood of the three. As noted previously,[32] there is a critical need for additional clinical studies in this field that will help to place

results, such as those presented here, into a meaningful perspective.

Experimental Section

General. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H-NMR spectra (200 MHz) were obtained in $CDCl_3$ with a Chemagnetics A-200 spectrometer. Chemical shifts are reported in δ values (ppm) relative to an internal reference of tetramethylsilane (δ 0). Abbreviations used in NMR analysis are as follows: br s = broad singlet, d = doublet, dd = doublet of doublets, dq = doublet of quartets, m = multiplet, q = quartet, s = singlet, t = triplet. Chemical ionization mass spectra were obtained with a Finnegan 4000 spectrometer. Elemental analyses were performed by the Purdue Microanalysis Laboratory or Galbraith Laboratories, Knoxville, TN, and are within ±0.4% of the calculated values unless otherwise noted.

(*Z*)- and (*E*)-1,4-Dimethoxy-2-(1-methyl-1-propenyl)-benzene ((*Z*)-21 and (*E*)-21). Following the procedure of Schlosser *et al.*,[22] a solution of 20.33 g (54.76 mmol) of ethyltriphenylphosphonium bromide in 100 mL of dry THF was stirred in a flame-dried 250 mL three-neck round bottom flask equipped with mechanical stirrer, condenser, and addition funnel with cooling in an ice bath. After the addition of 23.54 mL (54.8 mmol) of a 2.36 M solution of *n*-BuLi in hexane over 10 min, and 20 min of additional stirring, 9.85 g (54.76 mmol) of 2,5-dimethoxyacetophenone (**20**)[33] was added dropwise over 15 min. The ice bath was removed, and the mixture was stirred at room temperature for 61 h. The THF was removed by rotary vacuum evaporation, and the residue was dissolved in 50 mL of $CHCl_3$ and filtered through Celite. After rotary vacuum evaporation, the product was purified by flash chromatography, eluting with 5% EtOAc in hexanes to yield 5.79 g (55%) of olefin. Analysis of the NMR integrations of the methyl protons at δ 1.47 (*Z*-isomer) and 1.75 (*E*-isomer) indicated a 7/3 ratio of *Z*/*E*-isomers, which were separated by spinning band distillation.

(*Z*)-21: bp 58 °C (0.18 mmHg); ^{1}H-NMR ($CDCl_3$) δ 1.47 (d, 3, CH_3, J = 6.7 Hz), 1.98 (d, 3, CH_3, J = 1.5 Hz), 3.77 (s, 6, OCH_3), 5.59 (q, 1, H, J = 6.7 Hz), 6.63 (d, 1, ArH, J = 2.6 Hz), 6.73−6.86 (m, 2, ArH); MS *m*/*e* 193 (MH^+). Anal. ($C_{12}H_{16}O_2$) C, H.

(*E*)-21: bp 62 °C (0.18 mmHg); ^{1}H-NMR ($CDCl_3$) δ 1.75 (d, 3, CH_3, J = 6.7 Hz), 1.96 (s, 3, CH_3), 3.77 (s, 3, OCH_3), 5.56 (q, 1, H, J = 6.7 Hz), 6.73−6.86 (m, 3, ArH); MS *m*/*e* 193 (MH^+). Anal. ($C_{12}H_{16}O_2$) C, H.

(+)-Monoisopinocampheylborane. Following the procedure of Brown *et al.*,[20] a 500 mL flame-dried round bottom flask was charged with 24.34 mL (243.4 mmol) of 10 M borane-methyl sulfide and 160 mL of Et_2O. While the solution stirred at room temperature, 76.28 g (560 mmol) of (−)-α-pinene (Aldrich; freshly distilled from LAH, bp 155 °C; $[\alpha]_D$ = −41.11°) was added dropwise at a rate such that the reaction mixture refluxed gently. Following the addition, the mixture was heated at reflux for 0.5 h, and then 18.36 mL (121.7 mmol) of tetramethylethylenediamine (TMED, freshly distilled from CaH_2) was added and the mixture remained at reflux for 0.5 h. The 2IpcBH$_2$·TMED complex was crystallized by allowing the flask to remain in the oil bath after the heat was turned off so that the solution cooled slowly to room temperature. After sitting overnight at room temperature, the flask was cooled in a freezer for 7 h to ensure complete crystallization. A modification of the original procedure was used to separate the solid complex from the solution (Dr. B. Singaram, personal communication). The 2IpcBH$_2$·TMED complex is stable to air and does not require special handling. Therefore the crystalline material was simply filtered, washed well with pentane, and dried under vacuum (5 h at 0.1 mmHg) yielding 41.98 g (83%) of (+)-2IpcBH$_2$·TMED: $[\alpha]_D$ = +67.43° (*c* = 9.7, EtOH); mp 139−140 °C (lit.[20] $[\alpha]_D$ = +69.03°; mp 140.5−141.5 °C).

Liberation of free (+)-monoisopinocampheylborane was accomplished by a modified procedure of Brown and Singaram.[21] A solution of 41.7 g (100 mmol) of (+)-2IpcBH$_2$·TMED in 134 mL of Et_2O was stirred while 24 mL (195 mmol) of 8.13 M BF_3·OEt_2 was added over 20 min. Stirring was continued at room tempertaure for an additional 2 h. The moisture-sensitive (+)-monoisopinocampheylborane was removed from the 2BF_3·TMED as follows. A round hole was bored out of the center of a 24/40 rubber stopper so as to allow the tight fit of a flame-dried gas dispersion tube (ROBU microfilter candle, Aldrich; 20 mm length, 9 mm diameter, 100−160 pore size) which was closed off with a rubber septum. An 18 gauge double-ended needle was inserted through the septum so that the tip of the needle came within 1 cm of the filter end inside the candle. The other end of the needle was inserted through a rubber septum, into a dry 500 mL round bottom flask that was vented to a bubbler. The 24/40 rubber stopper with the filter candle and needle was quickly sealed on to the flask containing the reaction mixture, and nitrogen was used to force the (+)-IpcBH$_2$ solution through the filter and into the clean flask. To facilitate this procedure, the flask was hand-held while the filter candle was slowly stirred in the slurry. When the filtration was complete, an additional 72 mL of Et_2O was added by syringe to the original flask and filtered as before. This procedure was repeated once more with another washing of 72 mL of Et_2O. The combined filtrate was analyzed for (+)-IpcBH$_2$ by hydrolysis[31] with 1:1:1 glycerol, water, and THF as the hydrolyzing mixture and found to be 0.75 M (230 mL, 173 mmol, 86%): ^{11}B-NMR (decoupled) 22.4 (s); $[\alpha]_D$ = +38.72° (*c* = 11.2, Et_2O) (lit.[21] data for (−)-isomer, $[\alpha]_D$ = −39.93° (*c* = 11.9, Et_2O)).

(2*R*,3*R*)-(−)-3-(2,5-Dimethoxyphenyl)-2-butanol (24). Asymmetric hydroboration and oxidation were accomplished by the method of Brown and Singaram.[21] A solution of 5.5 g (28.7 mmol) of **21** was added to 40 mL (30 mmol) of (+)-IpcBH$_2$ in Et_2O cooled to −35 °C in a constant temperature bath. The mixture was swirled after the addition and then allowed to stand with cooling for 60 h. The monoisopinocampheylborane intermediate had crystallized from the ethereal solution which was removed using a gas dispersion tube and two-ended needle as described for (+)-IpcBH$_2$. The solid borane was washed and filtered twice with 20 mL of Et_2O. The solid was cooled in an ice bath, and 3 mL of EtOH was added dropwise over 10 min followed by 10 min of additional stirring. Then 5.27 mL of 6 N NaOH was added over 3 min, and stirring was continued for an additional 5 min. After the dropwise addition of 8.6 mL of 30% H_2O_2 over 5 min, the mixture was heated to 50 °C, stirred for 1 h, and then cooled in an ice bath. To this was added 30 mL of Et_2O, and the aqueous layer was saturated with K_2CO_3 and separated. The ether layer was washed with 50 mL of H_2O and 30 mL of saturated NaCl. The combined aqueous washes were extracted with 2 × 50 mL Et_2O, which was combined with the original Et_2O and dried (Na_2SO_4). After filtration and rotary vacuum evaporation of the solvent, the product was purified by silica gel flash chromatography, eluting with 5% EtOAC in hexane, to afford 7.8 g (71.4%) of **24**: $[\alpha]_D$ = −4.10° (*c* = 4.97, EtOH); ^{1}H-NMR ($CDCl_3$) δ 1.09 (d, 3, CH_3, J = 6.4 Hz), 1.26 (d, 3, CH_3, J = 7.2 Hz), 2.01 (br s, 1, OH, vanishes with D_2O), 3.19−3.32 (m, 1, H), 3.77 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 3.84−3.98 (m, 1, H), 6.67−6.82 (m, 3, ArH); MS *m*/*e* 211 (MH^+), 193 ($MH^+ - H_2O$). Anal. ($C_{12}H_{18}O_3$) C, H. Enantiomeric excess = 82.5% as determined by gas chromatography analysis of the corresponding ester derived from the reaction with *(R)*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[18] This was carried out on a Hewlett-Packard 5750 chromatograph using a 50 m methylsilicone-packed column; isothermal T = 205 °C. Similar analysis of the alcohol which remained in the ethereal filtrate separated from the crystallized monoisopinocampheylalkylborane indicated a 62% ee.

(−)-Monoisopinocampheylborane. Following a procedure identical to that used for the (+)-isomer, 61.3 g (450 mmol) of (+)-α-pinene (Aldrich; freshly distilled from LAH, bp 155 °C, $[\alpha]_D$ = +44.56°) was reacted with 19.6 mL (196 mmol) of borane-methyl sulfide and 14.75 mL (98 mmol) of TMED (freshly distilled from CaH_2) to afford 19.17 g (47%) of (−)-2IpcBH$_2$·TMED complex: $[\alpha]$ = −67.65° (*c* = 9.6, EtOH); mp 139−140 °C (lit.[20] values for the (+)-isomer, $[\alpha]_D$ = +69.03°; mp 140.5−141.5 °C).

Liberation of free (−)-monoisopinocampheylborane was accomplished with 18.9 g (45 mmol) of (−)-2IpcBH$_2$·TMED in 61

mL of Et_2O and 10.9 mL (89 mmol) of BF_3·OEt_2. The solid $2BF_3$·TMED complex was washed with an additional 2 × 33 mL of Et_2O, as described for the (+)-isomer. The resulting solution was analyzed for (−)-$IpcBH_2$ by hydrolysis with 1:1:1 glycerol, water, and THF[43] as the hydrolyzing mixture and found to be 1 M (68 mL, 68 mmol, 76%): ^{11}B NMR (decoupled) 22.4 (s); $[\alpha]_D = -38.64°$ (c = 11.1, Et_2O) (lit.[21] $[\alpha]_D = -39.93°$ (c = 11.9, Et_2O)).

(2S,3S)-(+)-3-(2,5-Dimethoxyphenyl)-2-butanol (25). Starting with 9 g (46.9 mmol) of *(Z)*-**21**, a procedure similar to that described for the preparation of **24** was used except that (−)-$IpcBH_2$ replaced the (+)-isomer. The purified yield of **25** was 6.9 g (70%): $[\alpha]_D = +4.18°$ (c = 5.26, EtOH); ee = 82.9% by GC analysis of MTPA ester; ^1H-NMR ($CDCl_3$) identical to **24**; MS m/e 211 (MH^+), 193 ($MH^+ - H_2O$). Anal. ($C_{12}H_{18}O_3$) C, H.

(2R,3R)-(−)-3-(2,5-Dimethoxyphenyl)-2-butyl *p*-Toluenesulfonate (26). Tosylation was accomplished by the method of Kabalka *et al.*[35] A solution of 7.4 g (35.24 mmol) of **24** in 35 mL of $CHCl_3$ was cooled in an ice bath and stirred while 5.7 mL (70 mmol) of pyridine was added followed by 10 g (52.86 mmol) of *p*-toluenesulfonyl chloride in small portions over 10 min. After 3 h, 100 mL of Et_2O and 25 mL of H_2O were added, and the organic layer was washed with 100 mL of 2 N HCl, 100 mL of 5% $NaHCO_3$, and 100 mL of H_2O and dried over $MgSO_4$. The solution was filtered, and the solvent was removed by rotary vacuum evaporation. The tosylate was purified by silica gel flash chromatography eluting with 5% EtOAc in hexane to afford 8.79 g (69%): $[\alpha]_D = -25.14°$ (c = 5.39, EtOH); ^1H-NMR ($CDCl_3$) δ 1.17 (d, 3, CH_3, J = 7.0 Hz), 1.25 (d, 3, CH_3, J = 6.4 Hz), 2.41 (s, 3, $ArCH_3$), 3.21 (pentet, 1, J = 7.0 Hz), 3.69 (s, 3, OCH_3), 3.72 (s, 3, OCH_3), 4.82 (pentet, 1, CH, J = 6.4 Hz), 6.59 (s, 1, ArH), 6.65 (s, 2, ArH), 7.21 (d, 2, ArH); MS m/e 193 (MH^+ − tosylate).

***S*-(+)-1,4-Dimethoxy-2-(1-methylpropyl)benzene ((*S*)-(+)-13).** To a solution of 7.8 g (21.43 mmol) of the 2*R*,3*R*-tosylate **26**, dissolved in 10 mL of dry ether, while stirring under nitrogen with cooling in an ice bath, was added over 2 min 40 mL of a 1 M solution of lithium aluminum hydride in THF. After an additional 5 min of stirring, the ice bath was removed, and the mixture was heated at reflux for 5 h. An additional 50 mL of ether was added, and the mixture was again cooled in an ice bath. With vigorous stirring, the lithium aluminum hydride was quenched by addition of 3.2 mL of water, and stirring was continued at room temperature for 20 min. The precipitate was filtered through Celite, and the ether was washed with 2 × 50 mL of 3 N HCl, 2 × 50 mL of 5% $NaHCO_3$, and 50 mL of saturated NaCl. After drying ($MgSO_4$), filtration, and concentration by rotary vacuum evaporation, the crude product was purified by centrifugal chromatography using a 4 mm silica gel plate and eluting with 1% EtOAc in hexane to afford 2.5 g (60%) of *(S)*-(+)-**13**: $[\alpha]_D = +16.63°$ (c = 5.01, EtOH); ^1H-NMR ($CDCl_3$) δ 0.84 (t, 3, CH_3, J = 7.3 Hz), 1.18 (d, 3, CH_3, J = 7.0 Hz), 1.48−1.62 (m, 2, CH_2), 3.07 (m, 1, H, J = 7.0 Hz), 3.77 (s, 6, OCH_3), 6.63−6.80 (m, 3, ArH); MS m/e 195 (MH^+). The NMR and mass spectra were substantially similar to those of the previously synthesized racemic mixture.[10]

(2S,3S)-(+)-3-(2,5-Dimethoxyphenyl)-2-butyl *p*-Toluenesulfonate (27). A procedure similar to that described for the 2*R*,3*R*-isomer was used, starting with 6.7 g (31.9 mmol) of 2*S*,3*S*-alcohol **25**, which afforded 8.25 g (71%) of the tosylate: $[\alpha]_D = +27.13°$ (c = 5.5, EtOH). The ^1H-NMR and mass spectra of the two tosylate enantiomers were identical.

***R*-(−)-1,4-Dimethoxy-2-(1-methylpropyl)benzene ((*R*)-(−)-13).** Using an identical procedure to the one used for the *S*-isomer, the reduction of 8.00 g (21.97 mmol) of the 2*S*,3*S*-tosylate **27** afforded 2.17 g (51%) of *(R)*-(−)-**13**: $[\alpha]_D = -17.46°$ (c = 5.23, EtOH). The ^1H-NMR ($CDCl_3$) and mass spectra were identical with those of *(S)*-(+)-**13**.

***R*-(−)-13: Alternate Method.** Following the procedure of Johnson and Dutra,[17] a solution of 13.8 g (100 mmol) of *p*-dimethoxybenzene, **19**, in 100 mL of dry THF was stirred at −78 °C while 0.12 mmol of *n*-BuLi in 48 mL of hexane was added over 10 min. The solution was then heated at reflux for 2 h, after which the solvent was removed by distillation, the flask was cooled in an ice bath, and 35 mL of dry Et_2O was added. The aryllithium solution was added dropwise over 1 h to a suspension of freshly purified CuI (9.52 g, 50 mmol) in 30 mL of Et_2O in an ice bath. The mixture was then cooled further in an acetone/dry ice bath, and 4 g (17 mmol) of *(S)*-(+)-2-butyl tosylate[17] in 25 mL of Et_2O was added dropwise over 30 min followed by overnight stirring. The specific rotation of the tosylate used indicated that the optical purity was 69% since the $[\alpha]_D$ was found to be identical (+9.9° (c = 5, EtOH)) with the $[\alpha]_D$ reported for the same compound of 69% ee in the literature.[17] The reaction was then quenched with 50 mL of a saturated solution of NH_4Cl added over 10 min, and the mixture was stirred for an additional 15 min. The Et_2O layer was separated, washed with 3 × 100 mL of a saturated NaCl solution, and dried over $MgSO_4$. The solution was filtered, and the solvent was removed under vacuum. Purification of the product was accomplished in two steps, beginning with flash chromatography over silica gel and elution with 40% Et_2O in hexanes. Finally, centrifugal chromatography (Chromatotron instrument) on a 4 mm silica gel rotor, eluting with 20% Et_2O in hexanes, afforded 1.25 g (38%) of *(R)*-(−)-**13**: $[\alpha]_D = -12.94°$ (c = 5, EtOH). The negative sign of rotation for the *R*-isomer confirms the assignment of absolute configuration inferred from the mechanism of the hydroboration reaction described above. The decreased rotation value for this product resulted from the low optical purity of the *(S)*-(+)-2-butanol obtained from a commercial source. The ^1H-NMR spectrum of this product was identical with that of the same product prepared through the asymmetric hydroboration/oxidation method.

***S*-(+)-2,5-Dimethoxy-4-(1-methylpropyl)benzaldehyde ((*S*)-(+)-28).** A solution of 770 mg (3.97 mmol) of *(S)*-(+)-**13** in 20 mL of dry methylene chloride was stirred under nitrogen in an ice bath for 10 min. After addition of 1.55 g (5.96 mmol) of $SnCl_4$, 685 mg (5.96 mmol) of Cl_2CHOCH_3 was added. The ice bath was removed, and following 10 min of additional stirring, 5 g of ice was added. The organic phase was separated, directly loaded on to a small silica gel flash column, and eluted with methylene chloride. The crude product was purified by centrifugal chromatography on a 4 mm silica gel plate, eluting with 5% EtOAc in hexane to afford 764 mg (87%) of the aldehyde: $[\alpha]_D = +18.6°$ (c = 5.53, EtOH); ^1H-NMR ($CDCl_3$) δ 0.86 (t, 3, CH_3, J = 7.3 Hz), 1.21 (d, 3, CH_3, J = 7.0 Hz), 1.52−1.68 (m, 2, CH_2), 3.15 (m, 1, H, J = 7.0 Hz), 3.82 (s, 3, OCH_3), 3.90 (s, 3, CH_3), 6.81 (s, 1, ArH), 7.28 (s, 1, ArH), 10.40 (s, 1, CHO); MS m/e 223 (MH^+). The NMR and mass spectra were substantially similar to those of the previously synthesized racemic mixture.[10]

Alternate Route. Following a modified procedure of Weyerstahl *et al.*,[25] a solution of 0.6 g (3.1 mmol) of *(S)*-(+)-**13**, identical with the material used above, was dissolved in 15 mL of acetic acid and stirred at room temperature while 0.55 g (3.4 mmol) of iodine monochloride was added over 2 min. After stirring for 2 h, the reaction mixture was diluted with 30 mL Et_2O and washed with 3 × 30 mL of 5% $NaHCO_3$, 3 × 30 mL 5% $Na_2S_2O_3$, and 3 × 30 mL of saturated NaCl. The solution was dried (Na_2SO_4), filtered, reduced by rotary vacuum evaporation, and purified by a Chromatotron instrument (2 mm silica gel rotor, eluting with CH_2Cl_2) to afford 679 mg (68%) of 4-iodo-*(S)*-(+)-**13**: $[\alpha]_D = +15.59°$ (c = 1, EtOH); ^1H-NMR ($CDCl_3$) δ 0.84 (3, t, CH_3), 1.17 (3, d, CH_3), 1.55 (2, m, CH_2), 3.02 (1, q, CH), 3.76 (3, s, OCH_3), 3.83 (3, s, OCH_3), 6.67 (1, s, ArH), 7.20 (1, s, ArH).

A solution of 675 mg (2.11 mmol) of this iodinated material in 5 mL of dry THF was stirred under N_2 and cooled to −78 °C. A solution of 2.32 mmol (0.98 mL of 2.36 M solution) of *n*-BuLi in hexane was then added over 3 min. The mixture was allowed to stir for an additional 3 min, after which 366 mg (5 mmol) of DMF was added. The reaction was complete in 30 min, as determined by TLC, AT which time 10 mL of saturated NH_4Cl was carefully added over 20 min followed by 10 min of stirring. The mixture was then diluted with 30 mL of pentane, washed with 3 × 30 mL of saturated NaCl, dried (Na_2SO_4), filtered, and reduced by rotary vacuum evaporation. Purification was accomplished by a Chromatotron instrument (2 mm silica gel rotor, eluting with CH_2Cl_2) to afford 120 mg

(26%) of the *S*-(+)-aldehyde: $[\alpha]_D = +18.14°$ ($c = 4.75$, EtOH), which is comparable to the value obtained for the same product obtained by the other formylation procedures; $[\alpha]_D = +18.6°$ ($c = 5.53$, EtOH) for the product of the Cl_2CHOCH_3 reaction, described above; $[\alpha]_D = +18.03°$ ($c = 4.52$, EtOH) for the product produced by a Vilsmeier–Haack reaction. All three products were identical by TLC, $R_f = 0.4$ (silica, CH_2Cl_2), and ^1H NMR.

***(R)*-(−)-2,5-Dimethoxy-4-(1-methylpropyl)benzaldehyde ((*R*)-(−)-28).** Using the first of the two procedures described for the *S*-isomer, 1.1 g (5.67 mmol) of *(R)*-**13** was formylated and purified to yield 996 mg (79%) of the aldehyde: $[\alpha]_D = -18.54°$ ($c = 5.5$, EtOH). The ^1H-NMR ($CDCl_3$) and mass spectra were identical with those of *(S)*-(+)-**28**.

(*S*)-(+)-1,4-Dimethoxy-2-(1-methylpropyl)-5-(2-nitropropenyl)benzene ((*S*)-(+)-29). A mixture of 764 mg (3.44 mmol) of (+)-**28** and 2.3 g (30 mmol) of ammonium acetate in 8 mL of nitroethane was heated at reflux under nitrogen for 4 h. The excess nitroethane was removed by rotary vacuum evaporation, and the residue was partitioned between methylene chloride and water. After purification over a small silica gel flash column and elution with methylene chloride, the product was further purified by centrifugal chromatography on a 4 mm plate, eluting with methylene chloride, to afford 879 mg (92%) of pure product: $[\alpha]_D = +17.07°$ ($c = 5$, EtOH); ^1H-NMR ($CDCl_3$) δ 0.87 (t, 3, CH_3, $J = 7.3$ Hz), 1.21 (d, 3, CH_3, $J = 7.0$ Hz), 1.52–1.65 (m, 2, CH_2), 2.43 (s, 3, CH_3), 3.13 (m, 1, H, $J = 7.0$ Hz), 3.80 (s, 3, OCH_3), 3.85 (s, 3, CH_3), 6.77 (s, 1, Ar H), 6.79 (s, 1, Ar H), 8.29 (s, 1, =CH); MS *m/e* 280 (MH^+), 223 ($MH^+ - C_4H_9$). The NMR and mass spectra were substantially similar to those of the previously synthesized racemic mixture.[10]

(*R*)-(−)-1,4-Dimethoxy-2-(1-methylpropyl)-5-(2-nitropropenyl)benzene ((*R*)-(−)-29). The condensation of 996 mg (4.49 mmol) of (−)-**28** with nitroethane was accomplished by the method described for the *(S)*-nitropropene to afford 819 mg (66%) of pure product: $[\alpha]_D = -17.71°$ ($c = 5$, EtOH). The ^1H-NMR ($CDCl_3$) and mass spectra were identical with those of *(S)*-(+)-**29**.

(−)-2,5-Dimethoxy-(α*RS*)-methyl-4-(1-(*R*)-methylpropyl)-benzeneethanamine Hydrochloride (11a). To a stirring, ice bath-cooled solution of 817 mg (2.93 mmol) of the nitropropene *(R)*-(−)-**29** in 10 mL of dry ether under nitrogen was added 10 mL of a 1 M solution of lithium aluminum hydride in THF over 5 min. The ice bath was removed, and the mixture was stirred at reflux for 2.5 h. Additional ether was then added (30 mL), and the mixture was cooled in an ice bath and then the reaction quenched by addition of 0.5 mL of water. After stirring at room temperature for 15 min, the mixture was filtered through Celite, and the crude amine was extracted into 3 × 30 mL of 3 N HCl. Following basification with 5 N NaOH, the free base was extracted into 3 × 50 mL of ether and dried (Na_2SO_4). The drying agent was removed by filtration, and the solvent was removed by rotary vacuum evaporation. The amine was converted into the hydrochloride salt by neutralization with HCl/EtOH, and recrystallization from acetonitrile afforded 610 mg (83%) of the salt: mp 164 °C: $[\alpha]_D = -15.74°$ ($c = 1$, H_2O); ^1H-NMR ($CDCl_3$) δ 0.84 (t, 3, CH_3, $J = 7.3$ Hz), 1.16 (d, 3, CH_3, $J = 6.7$ Hz), 1.37 (d, 3, CH_3, $J = 6.4$ Hz), 1.51–1.61 (m, 2, CH_2, $J = 7.0$ Hz), 2.87–3.10 (m, 4, CH_2, CH, CH), 3.77 (s, 3, OCH_3), 3.80 (s, 3, OCH_3), 6.68 (s, 1, ArH), 6.70 (s, 1, Ar H), 8.37 (br s, 3, NH_3); MS *m/e* 252 ($MH^+ - HCl$). Anal. ($C_{15}H_{26}ClNO_2$) C, H, N.

(+)-2,5-Dimethoxy-(α*RS*)-methyl-4-(1-*(S)*-methylpropyl)benzeneethanamine Hydrochloride (11b). The reduction of 760 mg (2.72 mmol) of the *(S)*-(+)-nitropropene **29** was carried out by the procedure described above for 11a. Recrystallization of the crude amine hydrochloride from acetonitrile afforded 394 mg (58%) of the hydrochloride salt: mp 162–164 °C; $[\alpha]_D = +15.27°$ ($c = 1.1$, H_2O); MS *m/e* 252 ($MH^+ - HCl$). Anal. ($C_{15}H_{26}ClNO_2$) C, H, N.

To confirm that the chiral 2-butyl group at the 4-position had no effect on the resulting stereochemistry of the racemic isopropylamine side chain, chromatographic analysis of the amide derived from an optically pure acid was performed. The acid chloride of *(R)*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA; Aldrich) was prepared by stirring 1 g of the acid with 10 mL of $SOCl_2$ at reflux for 12 h. The excess $SOCl_2$ was removed by rotary evaporation. To a stirred solution of 15 μL of MTPA chloride were added 5 mg of 11**a** and 0.5 mL of pyridine. After stirring overnight at room temperature, 2 mL of Et_2O was added, and the mixture was washed with water and then with 3 N HCl, saturated $NaHCO_3$, and saturated NaCl. The ether layer was dried, and the MTPA amide of 11a was analyzed on a Hewlett-Packard 5890 A gas chromatograph using a SPB-5 (30 m) capillary column at 250 °C. Two peaks with retention times of 19.5 and 20.8 min were found to have identical areas under their curves.

Pharmacology Methods. Drug Discrimination Studies. Twenty male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 200–240 g at the beginning of their use as experimental subjects were employed for this study. These rats had previously received drugs and behavioral training as part of our ongoing investigations.[45] Water was freely available in their individual home cages, and a rationed amount of supplemental feed (Purina Lab Blox) was made available after experimental sessions so as to maintain approximately 85% of the free-feeding weight. The temperature of the animal facility remained within the range of 22–24 °C. The humidity was maintained at 40–50%, and the lights were on between 6 a.m. and 8 p.m.

Apparatus. Six standard operant chambers (Coulbourn Instruments, Lehigh Valley, PA) consisted of modular test cages enclosed within sound-attenuated cubicles with fans for ventilation and background white noise. A white house light was centered near the top of the front panel of the test cage, which was also equipped with two response levers, separated by a food hopper, all positioned 2.5 cm above the floor. Solid state logic, in an adjacent room, interfaced through a Coulbourn Instruments Dynaport instrument to an IBM PC controlled reinforcement and data acquisition with locally written software.

Drug Administration. The training drug *d*-LSD tartrate (NIDA; 186 nmol/kg, 0.08 mg/kg) or saline was administered ip 30 min prior to sessions. All drugs were administered dissolved in saline such that a volume of 1 mL/kg of body weight was used. Solutions were sterilized prior to use by filtration through a sterile 0.2 μm filter (Millipore) into an autoclaved vial.

Discrimination Training. A fixed ratio (FR) 50 schedule of food reinforcement (Bioserv 45 mg dustless pellets) in a two-lever paradigm was used. Initially, rats were taught to lever press on an FR 1 schedule so that one food pellet was dispensed for each press. One-half of the rats were trained on drug-L, saline-R and the other half drug-R, saline-L, to avoid positional preference. Training sessions lasted 15 min and were conducted at the same time each day, Monday through Friday. Levers were cleaned with a 10% ethanol solution in order to avoid olfactory cues.[46] Both levers were present during all phases of training, but reinforcements were delivered only after responses on the stimulus-appropriate lever. Presses on the incorrect lever were recorded but had no programmed consequence. After initially learning to lever-press for food, saline and drug sessions were randomly ordered, with neither treatment given more than three consecutive sessions. As responding rates stabilized, the schedule of reinforcement was gradually increased from FR 1 to FR 50. Once at FR 50, training continued until an accuracy of at least 85% (number of correct presses × 100/number of total presses) was attained for 8 of 10 consecutive sessions.

Once criterion performance was attained, test sessions were interspersed between training sessions either one or two times per week. At least one drug and one saline session separated each test session. Rats were required to maintain the 85% correct responding criterion on training days in order to be tested. In addition, test data were discarded when the accuracy criterion of 85% was not achieved on the training sessions following a test session.[38] Test sessions were run under conditions of extinction, with rats removed from the operant box when 50 presses were emitted on one lever. If 50 presses on one lever were not completed within 5 min, the

session was ended and scored as a disruption (D). Treatments were randomized at the beginning of the study.

Data Analysis. The data were scored in quantal fashion with the lever on which the rat first emitted 50 presses in a test session scored as the "selected" lever. The percentage of rats selecting the drug lever (%SDL) for each dose of test compound was determined. If that drug was one which completely substituted for the training drug (at least one dose resulted in the %SDL = 80% or higher), the method of Litchfield and Wilcoxon[28] was used to determine the ED_{50} and 95% confidence interval (95% CI). This method also allowed for tests of parallelism between the dose–response curves for LSD and the test drugs.

Pharmacology Methods. Radioligand-Binding Studies. [^{125}I]-*(R)*-DOI was synthesized by the procedure of Mathis *et al.*[39] at a specific activity of 2000 Ci/mmol. The procedure of Johnson *et al.*[27] was employed with minor modifications. Briefly, the frontal cortex region from 10–20 male Sprague–Dawley rats (175–199 g; Harlan Laboratories, Indianapolis, IN) was pooled and homogenized (Brinkman Polytron homogenizer; setting 6 for 2 × 20 s) in 4 volumes of 0.32 M sucrose. The homogenate was centrifuged at 36500*g* for 10 min, and the resulting pellet was resuspended in the same volume of sucrose. Separate aliquots of tissue were then frozen at −70 °C until assay.

For each separate experiment, a tissue aliquot was thawed slowly and diluted 1 to 25 with 50 mM Tris HCl (pH 7.4). The homogenate was then incubated at 37 °C for 10 min and centrifuged twice at 36500*g* for 10 min. The resulting pellet was resuspended in 50 mM Tris HCl with 0.5 mM Na_2EDTA, 0.1% Na ascorbate, 10 mM $MgCl_2$, and 10 μM pargyline HCl (pH 7.4). A second preincubation for 10 min at 37 °C was conducted, and the tissues were then cooled in an ice bath.

All experiments were performed with triplicate determinations using the appropriate buffer, to which 200–400 μg of protein was added, giving a final volume of 1 mL. Displacement and saturation experiments were conducted with *(R)*-[^{125}I]DOI as described in Nichols *et al.*[36] Tubes were allowed to equilibrate for 15 min at 37 °C before filtering through Whatman GF/C filters. Specific binding was defined as that displaceable with 1 μM cinanserin. Under these conditions *(R)*-[^{125}I]DOI was found to bind to a single site (Hill coefficient of 0.99 ± 0.03) with a B_{max} of 46 ± 3 fmol/mg of protein and a K_D of 1.34 ± 0.12 nM. The ability of the test drugs to displace 0.25 nM *(R)*-[^{125}I]DOI was determined. Filters were allowed to air-dry before counting with a γ-counter. After counting at an efficiency of 79% for ^{125}I, binding parameters were determined using the computer programs EBDA and LIGAND as described elsewhere.[40] The values from three to four separate experiments were combined. Protein determinations were made using the procedure of Bradford.[41] Free energy of binding at 37 °C (310 K) was estimated from $\Delta G° = -RT \ln(1/K_I)$.

Acknowledgment. This work was supported by USPHS Grant DA-02189 from the National Institute on Drug Abuse. (+)-LSD tartrate was obtained from the National Institute on Drug Abuse. We also acknowledge the valuable technical assistance of Dr. Bakthan Singaram.

References

(1) Shulgin, A. T. The six trimethoxyphenylisopropylamine (trimethoxyamphetamines). *J. Med. Chem.* **1966**, *9*, 445–446.
(2) Shulgin, A. T. Psychotomimetic Drugs: Structure-activity relationships. In *Handbook of Pyschopharmacology*; Iversen, L. L., Iversen, S. D., Snyder, S. H., Eds.; Plenum Press: New York, 1980; pp 243–333.
(3) Nichols, D. E. Structure-activity relationships of phenethylamine hallucinogens. *J. Pharm. Sci.* **1981**, *70*, 839–849.
(4) Nichols, D. E.; Glennon, R. A. Medicinal chemistry and structure-activity relationships of hallucinogens. In *Hallucinogens: Neurochemical, Behavioral, and Clinical Perspectives*; Jacobs, B., Ed.; Raven Press: New York, 1984; pp 95–142.
(5) Nichols, D. E. Studies of the relationship between molecular structure and hallucinogenic activity. *Pharmacol. Biochem. Behav.* **1986**, *24*, 335–340.
(6) Glennon, R. A.; Titeler, M.; McKenney, J. D. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci.* **1984**, *35*, 2505–2511.
(7) Lyon, R. A.; Davis, K. H.; Titeler, M. ^{3}H-DOB (4-bromo-2,5-dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of the cortical 5-HT_2 receptors. *Mol. Pharmacol.* **1986**, *31*, 194–199.
(8) Johnson, M. P.; Hoffman, A. J.; Nichols, D. E.; Mathis, C. A. Binding to the 5-HT_2 receptor by the enantiomers of [^{125}I]-DOI. *Neuropharmacol.* **1987**, *26*, 1803–1806.
(9) Titeler, M.; Lyon, R. A.; Glennon, R. A. Radioligand binding evidence implicates the brain 5-HT_2 receptor as a site of action for LSD and phenylisopropylamine hallucinogens. *Psychopharmacology* **1988**, *94*, 213–218.
(10) Oberlender, R.; Kothari, P.; Nichols, D. E.; Zabik, J. E. Substitutent branching in phenethylamine-type hallucinogens: A comparison of 1-[2,5-dimethoxy-4-(2-butyl)phenyl]-2-aminopropane and 1-[2,5-dimethoxy-4-(2-methylpropyl)phenyl]-2-aminopropane. *J. Med. Chem.* **1984**, *27*, 788–792.
(11) Shulgin, A. T.; Dyer, D. C. Psychotomimetic phenylisopropylamines. 5. 4-Alkyl-2,5-dimethoxyphenylisopropylamines. *J. Med. Chem.* **1975**, *18*, 1201–1204.
(12) Aldous, F. A. B.; Barrass, B. C.; Brewster, K.; Buxton, D. A.; Green, D. M.; Pinder, R. M.; Rich, P.; Skeels, R. M.; Tutt, K. J. Structure-activity relationships in psychotomimetic phenylalkylamines. *J. Med. Chem.* **1974**, *17*, 1100–1111.
(13) Clare, B. Structure-activity correlations for psychotomimetics. 1. Phenylalkylamines: Electronic, volume, and hydrophobicity parameters. *J. Med. Chem.* **1990**, *33*, 687–702.
(14) Glennon, R. A.; Young, R.; Rosecrans, J. A. A comparison of the behavioral effects of DOM homologs. *Pharmacol. Biochem. Behav.* **1982**, *16*, 557–559.
(15) Nichols, D. E.; Weintraub, H. J. R. Effects of steric interactions on model charge-transfer complexes: A preliminary report. *Int. J. Quant. Chem. Quant. Biol. Symp.* **1982**, *9*, 205–211.
(16) Knowles, W. S. Asymmetric hydrogenation. *Acc. Chem. Res.* **1983**, *16*, 106–112.
(17) Johnson, C. R.; Dutra, G. A. Reaction of lithium diorganocuprates (I) with tosylates. II. Stereochemical, kinetic, and mechanistic aspects. *J. Am. Chem. Soc.* **1973**, *95*, 7783–7788.
(18) Brown, H. C.; Park, W. S.; Cho, B. T.; Ramachandran, P. V. Selective reductions. 40. A critical examination of the relative effectiveness of various reducing agents for the asymmetric reduction of different classes of ketones. *J. Org. Chem.* **1987**, *52*, 5406–5412.
(19) Mandal, A. K.; Jadhav, P. K.; Brown, H. C. Monoisopinocampheylborane: An excellent chiral hydroborating agent for phenyl-substituted tertiary olefins. Synthesis of alcohols approaching 100% enantiomeric excess. *J. Org. Chem.* **1980**, *45*, 3543–3544.
(20) Brown, H. C.; Jadhav, P. K.; Mandal, A. K. Hydroboration. 62. Monoisopinocampheylborane, an excellent chiral hydroborating agent for trans-disubstituted and trisubstituted alkenes. Evidence for a strong dependence in such asymmetric hydroborations. *J. Org. Chem.* **1982**, *47*, 5069–5074.
(21) Brown, H. C.; Singaram, B. Chiral synthesis via organoboranes. 1. A simple procedure to achieve products of essentially 100% optical purity in hydroboration of alkenes with monoisopinocampheylborane. Synthesis of boronic esters and derived products of very high enantiomeric purities. *J. Am. Chem. Soc.* **1984**, *106*, 1797–1800.
(22) Schlosser, M.; Schaub, B.; de Oliveira-Neto, J.; Jeganathan, S. Practical guidance for obtaining optimum cis-selectivities in Wittig reactions with triphenylphonoalkanides. *Chimia* **1986**, *40*, 244–245.
(23) Brown, H. C.; Mandal, A. K.; Yoon, N. M.; Singaram, B.; Scwier, J. R.; Jadhav, P. K. Organoboranes. 27. Exploration of synthetic procedures for the preparation of monoisopinocampheylborane. *J. Org. Chem.* **1982**, *47*, 5069–5074.
(24) Brown, H. C.; Murray, K. A convenient non-catalytic conversion of olefinic derivatives into saturated compounds through hydroboration and protonolysis. *J. Am. Chem. Soc.* **1959**, *81*, 4108–4109.
(25) Weyerstahl, V. P.; Zielke, R.; Nerdel, F. Synthese und optische eigenschaften von 4,4'-di-sek-butyl-benzil. (Synthesis and optical properties of 4,4'-di-sec-butylbenzil.) *Liebigs Ann. Chem.* **1970**, *731*, 18–22.
(26) Oberlender, R.; Nichols, D. E. Drug Discrimination Studies with MDMA and Amphetamine. *Psychopharmacology* **1988**, *95*, 71–76.
(27) Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.
(28) Johnson, M. P.; Mathis, C. A.; Shulgin, A. T.; Hoffman, A. J.; Nichols, D. E. [^{125}I]-2-(2,5-Dimethoxy-4-iodophenyl)aminoethane ([^{125}I]-2-CI) as a label for the 5-HT_2 receptor in rat frontal cortex. *Pharmacol. Biochem. Behav.* **1990**, *35*, 211–217.

(29) Seggel, M. R.; Yousif, M. Y.; Lyon, R. A.; Titeler, M.; Roth, B. L.; Suba, E. A.; Glennon, R. A. A structure-affinity study of the binding of 4-substituted analogues of 1-(2,5-dimethoxy-phenyl)-2-aminopropane at 5-HT_2 serotonin receptors. *J. Med. Chem.* **1990**, *33*, 1032–1036.
(30) Titeler, M.; Leonhardt, S.; Appel, N. M.; DeSouza, E. B.; Glennon, R. A. Receptor pharmacology of MDMA and related hallucinogens. In *The Neuropharmacology of Serotonin*; Whitaker-Azmitia, P. M., Peroutka, S. J., Eds.; The New York Academy of Sciences: New York, 1990; pp 626–639.
(31) Pierce, P.; Peroutka, S. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* **1989**, *97*, 118–122.
(32) White, F. J. Comparative effects of LSD and lisuride: Cues to specific hallucinogenic drug actions. *Pharmacol. Biochem. Behav.* **1986**, *24*, 365–379.
(33) Anderson, W. K.; Lee, G. E. Synthesis of C-ring functionalized A-ring-aromatic trichothecane analogues. *J. Org. Chem.* **1980**, *45*, 501–506.
(34) Brown, H. C.; Kramer, G. W.; Levy, A. B.; Midland, M. M. *Organic Syntheses via Boranes*; Wiley-Interscience: New York, 1975.
(35) Kabalka, G. W.; Varma, M.; Varma, R. S. Tosylation of alcohols. *J. Org. Chem.* **1986**, *51*, 2386–2388.
(36) Nichols, D. E.; Snyder, S. E.; Oberlender, R.; Johnson, M. P.; Huang, X. 2,3-Dihydrobenzo-furan analogues of hallucinogenic phenethylamines. *J. Med. Chem.* **1991**, *34*, 276–281.
(37) Extance, K.; Goudie, A. J. Interanimal olfactory cues in operant drug discrimination procedures in rats. *Psychopharmacology* **1981**, *73*, 363–371.
(38) Colpaert, F. C.; Niemegeers, C. J. E.; Janssen, P. A. J. A drug discrimination analysis of lysergic acid diethylamide (LSD): *In vivo* agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenpirone, a LSD-antagonist. *J. Pharmacol. Exp. Ther.* **1982**, *221*, 206–214.
(39) Mathis, C. A.; Hoffman, A. J.; Nichols, D. E.; Shulgin, A. T. Synthesis and high-specific activity ^{125}I- and ^{123}I-labelled enantiomers of 2,5-dimethoxy-4-iodophenylisopropylamine (DOI). *J. Labelled Compd. Radiopharm.* **1988**, *25*, 1255–1265.
(40) McPherson, G. Analysis of radioligand binding experiments: A collection of computer programs for the IBM PC. *J. Pharmacol. Methods* **1985**, *14*, 213–218.
(41) Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the priciple of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

JM9502899