ir (KBr disk) 1770 (lactam C=O), 1675, 1610 (-COO⁻) cm⁻¹; nmr (60 Mcps, D_2O) δ 1.55 (s, 3, CH_3), 1.67 (s, 3, CH_3), 4.29 (s, 1, C_3-H), 5.58 (s, 1, CH), 5.58 (2, C_5-H, C_6-H), 7.49 (5, Ph-H-). The MIC values in the *in vitro* tests are shown in Table II.

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Semisynthetic β-Lactam Antibiotics. 2.[1] Synthesis and Properties of D- and L-α-Sulfobenzylpenicillins[2]

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D(−)- and L(+)-α-sulfobenzylpenicillin (D- and L-I) were obtained by the following two methods: (1) acylation of 6-aminopenicillanic acid with D(−)- and L(+)-α-sulfophenylacetyl chloride and (2) separation of the diastereoisomeric mixture by means of column chromatography or by fractional crystallization from EtOH. D-I showed much more potent antibacterial activities than did L-I against *Pseudomonas aeruginosa* and other Gram-positive and Gram-negative organisms tested. A convenient nmr method was devised for estimating the optical purity of a partially isomerized mixture of D- and L-I. Stabilities of D-I, as measured in aqueous solutions, demonstrated that D-I is more stable than carbenicillin.

In the previous paper of this series[1] we reported the synthesis of a novel semisynthetic penicillin, *i.e.*, α-sulfobenzylpenicillin (DL-I), which showed potent antibacterial activity against a wide variety of Gram-positive and Gram-negative microorganisms including *Pseudomonas aeruginosa*. α-Sulfobenzylpenicillin, which has one asymmetric carbon in the acyl side chain, can exist as two diastereoisomers (D-I, L-I), which are expected to differ in antibacterial activity as in previous examples.[3] This paper deals with the syntheses of D- and L-α-sulfobenzylpenicillin and their physicochemical and biological properties.

DL-α-Sulfophenylacetic acid (DL-II) was successfully resolved by introducing basic natural amino acids into each enantiomorph (D-II, L-II) as described in the Experimental Section. The combination of sulfo and carboxyl group on the same carbon atom renders the α-hydrogen more acidic to the extent that each enantiomer is readily racemized in an alkaline solution. Addition of 1 equiv of NaOH to the enantiometric acid (D-II and L-II) yielded the optically active monosodium salt. However, the subsequent addition of another equivalent gave the disodium salt with complete loss of optical rotation. The absolute configuration assignment of each enantiomer (D-II, L-II) was accomplished on the monosodium salt of α-sulfophenylacetic acid by X-ray crystallographic studies in these laboratories.[4] Under conditions similar to those described in the preceding paper, the optically pure α-sulfophenylacetyl chloride (D-III, L-III) was synthesized by treatment of the optically pure acid with $SOCl_2$ without loss of optical rotation. After recrystallization from ether-hexane, completely resolved α-sulfophenylacetyl chloride, [α]D −23.7°, was obtained. The racemization of D- or L-III occurred in this reaction to some extent, the degree of racemization being smaller with a higher rate of reaction. When much solvent was used, the reaction time required for the conversion extended to a week or more at 30°, and the resulting product of III showed no optical rotation.

By treating 6-aminopenicillanic acid (6-APA) with D-III and L-III, each diastereomer of α-sulfobenzylpenicillin (D-I and L-I) was obtained with an optical purity of not less than 80%. Chromatography on XAD-2 was useful for purification of the reaction mixture and, moreover, for separation into each pure diastereoisomer. Thus, the L(+) epimer of the penicillin which showed a shorter retention time on the column was satisfactorily separated from the D(−) isomer. An alternative technique useful for resolving the diastereomeric mixture (DL-I) was fractional crystallization from ethanol. The colorless needles obtained proved to be the pure D(−) isomer, and the mother liquor contained L-I as the main component.

The 100-Mcps spectrum of D-I (D_2O, δ value) shows 2α- and 2β-methyl[5] protons at 1.70 and 1.76, H_3 at 4.47 (s), H_5 and H_6 at 5.74 (d, *J* = 4.0 cps) and 5.79 ppm (d, *J* = 4.0 cps), respectively, whereas the spectrum of L-I exhibits methyl protons at 1.76 and 1.84, H_3 at 4.51 (s), H_5 and H_6 at 5.80 (d, *J* = 4.0 cps) and 5.84 (d, *J* = 4.0 cps), respectively

The spectrum of DL-I shows clearly the presence of the two diastereoisomers, D-I and L-I (Figure 1). As for the protons attached to the acyl side chain, both D-I and L-I show equal signals at 5.33 (s) for H_{10} and at 7.5-7.9 (m, centered at 7.6) for phenyl protons. The assignment of the β-lactam ring protons was confirmed by the nmr spectra of the triethylamine salt in $CDCl_3$, which yielded a single-proton doublet centered at 5.58 and a single-proton quartet centered at 5.64 for H_5 and H_6, respectively. The H_6 quartet, existent in the lower field, consists of the coupling with the H_5 on the one side (*J* = 4.0 cps) and with the imino proton on the other (*J* = 10.5 cps). This is based on the fact that the quartet for H_6 collapsed to a doublet upon irradiation of the imino proton (δ 8.53), while irradiation of the peak at 5.58 changed the imino proton doublet (centered at 8.53) to a singlet.

The nmr spectra of D(−)- and L(+)-α-sulfobenzylpenicillin were significantly nonequivalent, presumably due to the dif-
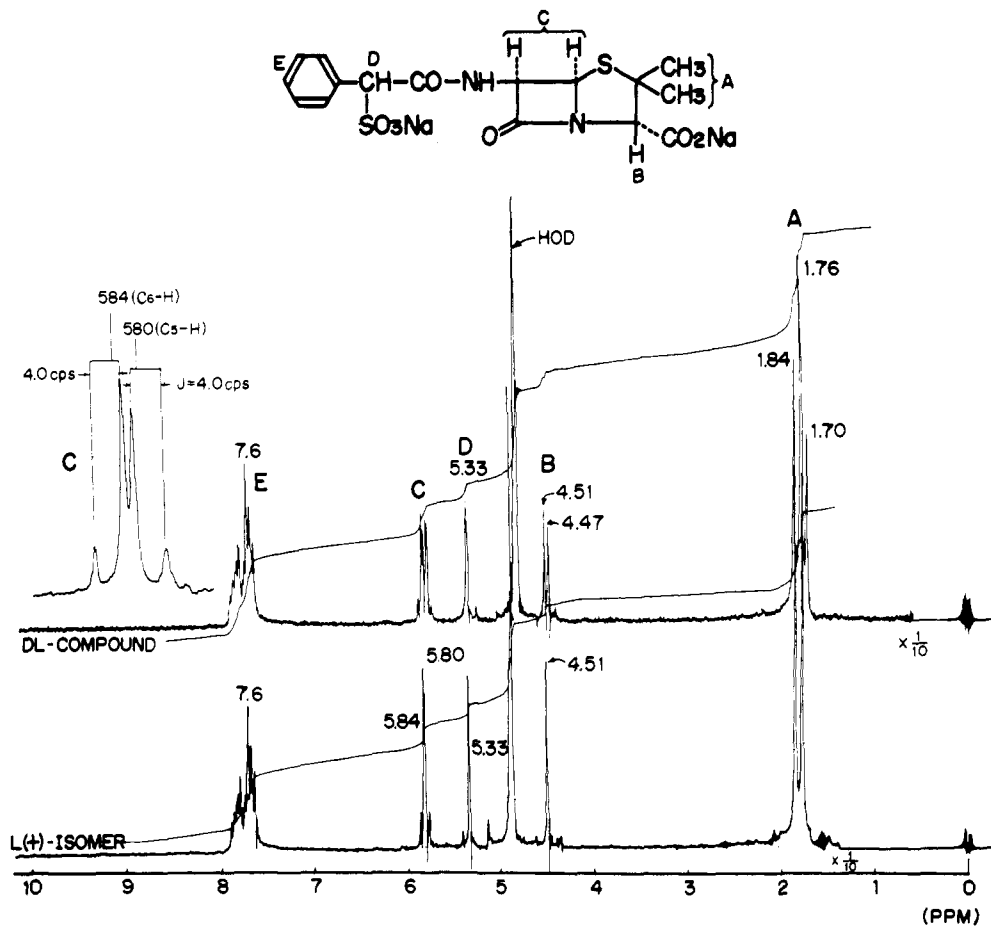
Figure 1. Nmr spectra of α-sulfobenzylpenicillin.

ferent environment of the hydrogens attached to the penicillin nucleus. The differences in chemical shifts between each pair of the protons bound to the nucleus were large enough ($\Delta\delta = 0.04 - 0.08$ ppm) to be used for the determination of the enantiometric purity of the given samples.

For instance, the ratio of the peak areas due to the methyl protons at 1.70 and 1.84 was confirmed to be proportional to the relative amounts of D-I and L-I present in a sample made up by compounding each enantiomer. Therefore, the optical purity of partially epimerized I can be rationally estimated by the ratio of the signal intensities due to the methyl protons corresponding to each epimer. The technique is very similar to that exemplified by O-methylmandelic acid derivatives[6] and, in addition, justified by the fact that over the range studied, the D-I content in a

sample linearly correlated with increase in the antibacterial activity against *Sarcina lutea*.

The results of a number of *in vitro* comparisons of D-I and L-I with other penicillins are listed in Table I. D-I was about 5 times more active against many organisms than the L(+) isomer. Previous examples showed a similar relationship to the result that the D(−) epimers of the penicillins derived from α-amino, α-azido-, and α-hydroxyphenylacetic acids were much more active than the corresponding L(+) isomers.[3] It was noteworthy that each of the carbenicillin diastereoisomers in the *in vitro* and *in vivo* tests was almost indistinguishable due to the rapid epimerization of the α-substituent under the conditions of the biological tests.[7]

In the present penicillin, the D(−) epimer was similar to carbenicillin in its range and order of antibacterial activity;

Table I. Antibacterial Activity of D- and L-α-Sulfobenzylpenicillins in Comparison with Other Semisynthetic Penicillins

	MIC, μg/ml			
Test organism	D(−)-C$_6$H$_5$C(SO$_3$H)H-[a]	L(+)-C$_6$H$_5$C(SO$_3$H)H-[a]	DL-C$_6$H$_5$C(CO$_2$H)H-[a]	D(−)-C$_6$H$_5$C(NH$_2$)H-
Pseudomonas aeruginosa IFO 3080	12.5	100	25	>100
P. aeruginosa IFO 3448	25	100	50	>100
Escherichia coli IFO 3044	12.5	100	12.5	12.5
Proteus vulgaris IFO 3045	1.56	12.5	1.56	3.13
P. morganii IFO 3168	3.13	25	1.56	100
P. mirabilis IFO 12255	1.56	12.5	1.56	3.13
Staphylococcus aureus FDA 209P	0.78	6.25	0.39	0.025
Staph. aureus Pc-R	6.25	100	50	100
Bacillus subtilis PCI 219	0.10	1.56	0.10	<0.025
Sarcina lutea PCI 1001	0.20	3.13	0.05	<0.025

[a]Used as the sodium salt.

namely, D-I showed almost the same degree of activity as carbenicillin against many species of Gram-negative bacteria. However, the antipseudomonal and antistaphylococcal (penicillinase +) activities proved to be significantly higher than those of carbenicillin.

Stabilities of D(−)-α-sulfobenzylpenicillin were measured in aqueous solutions by the biological determination using *Proteus morganii* and compared with those of carbenicillin.

At 37° and pH 4 in 0.1 M citrate buffer solution containing 1% of D-I, the half-life was about 23.2 hr, while at 50° and pH 6.5 the half-life was about 6 days. The corresponding values of carbenicillin are 4.0 hr and 2.3 days, respectively. In a more acidic solution, at pH 2, the half-life of D-I was found to be 13 hr at 21° and 1.75 hr at 37° *vs.* 2.3 hr and 0.43 hr for carbenicillin. These results show that the acid stability of I is superior to that of carbenicillin.

D-I was quite stable on prolonged storage and showed little tendency to degrade on standing or to produce benzylpenicillin. Additional microbiological investigations[8] of D-I have been reported, together with physicochemical studies.[9] D(−)-α-Sulfobenzylpenicillin is an effective drug for treatment of the infections of the urinary tract due to *Pseudomonas* in man.[10]

Experimental Section†

Minimal Inhibitory Concentrations. The minimal inhibitory concentrations (MIC) of the penicillins were determined by the agar dilution method. Nutrient agar was used as the assay medium. The test organism was grown for 18–24 hr on nutrient agar and one loopful of a suspension containing about 1 mg/ml of test organism was used as inoculum. The MIC's were determined after incubation at 37° for 18 hr.

Resolution of α-Sulfophenylacetic Acid (II). (a) D(−)-α-Sulfophenylacetic Acid (D-II) Resolved with L(+)-Lysine. To a solution of DL-α-sulfophenylacetic acid (DL-II) ($C_8H_8O_5S \cdot 2H_2O$, 7.5 g) in H_2O (15 ml) was added a solution of L(+)-lysine (5.1 g) in H_2O (12.7 ml). MeOH (100 ml) was added slowly over a period of 30 min, and the solution was left standing at 10° for 1 hr in an ice water bath. The colorless needles were filtered off and recrystallized from MeOH–H_2O (2:1): yield, 5.7 g of the lysine salt; [α]D −9.0° (*c* 1.0, H_2O).

After repeated recrystallization, the lysine salt of D-II showed a constant rotation: [α]^{28}D −9.8° (*c* 1.0, H_2O); mp 218°. *Anal.* ($C_{14}H_{22}N_2O_7S$) C, H, N, S.

The lysine salt (2.5 g) was dissolved in 10 ml of H_2O and passed through a column of Amberlite IR-120 (2 × 6.3 cm, 20 ml), followed by washing with H_2O. The eluate was collected and evaporated to a small portion *in vacuo* at 30°. The colorless syrup of the concentrate was dried in a vacuum desiccator at 1 mm over P_2O_5. After several days, 1.8 g of colorless needles of D(−)-α-sulfophenylacetic acid (D-II) was obtained. This was very hygroscopic and therefore the melting point could not be accurately measured: [α]D −19.4°; nmr (60 Mcps, D_2O) 4.98 (s, 1, methine), 7.3–7.9 ppm (m, 5, phenyl proton); ir (KBr) 2930 (broad, OH, CH), 2785, 2670, 2550 (OH), 1735 (-COOH), 1505 (phenyl), 1460 (CH), 1350 (CH), 1295, 1260, 1220, 1195, 1170 (SO₂), 1033 (-SO₃H), 700 cm⁻¹. *Anal.* ($C_8H_8O_5S \cdot 2H_2O$) C, H, S.

(b) D(−)-α-Sulfophenylacetic Acid (D-II) Resolved with L(+)-Histidine. The histidine salt of D-II was obtained as needles by a similar procedure to that described above. The histidine salt showed as follows: mp 148–149°, [α]^{25}D −12.1° (*c* 1.01, H_2O).

The histidine salt was dissolved in water and passed through a column of IR-120. After work-up as described above, D-II was obtained as a colorless hygroscopic crystalline powder: [α]D −19.4° (*c* 1.00, H_2O). Ir and nmr were identical with those described above.

(c) L(+)-α-Sulfophenylacetic Acid (L-II). After the lysine salt of the D(−)-acid was crystallized and filtered off, the resulting solution was concentrated to a small portion and five times the volume of MeOH was added to yield the lysine salt of L(+)-α-sulfophenylacetic acid as a crystalline powder (1.5 g). This was dissolved in a small amount of H_2O and freed of lysine by passage down a column

of Amberlite IR-120 (H-form). The eluate was concentrated *in vacuo* to a small portion and dried over P_2O_5 yielding 0.9 g of α-sulfophenylacetic acid as hygroscopic pale brownish needles: [α]D +15.6° (optical purity 65%). This was dissolved in 3 ml of H_2O and 0.66 g of D(−)-lysine added. After the addition of MeOH (14 ml) the resulting solution was left overnight at room temperature whereupon colorless needles of the D(−)-lysine salt of the L enantiomer separated: [α]D +10°. The nmr and ir spectra and the melting point were identical with those of the L(+)-lysine salt of D-II. The salt was converted to the acid as described above. The hygroscopic needles of L-II obtained showed [α]D +19.3° (*c* 1.00, H_2O).

D(−)- and L(+)-α-Sulfophenylacetyl Chloride (D-III and L-III). D(−)-Acid and L(+)-acid were individually allowed to react with thionyl chloride in a similar manner to that described in the previous paper yielding the corresponding acid chlorides, D-III and L-III, respectively. Each enantiomer of III showed [α]D −23.7 and +23.7° (Et₂O), respectively, as the maximum optical rotation. These were assumed to be optically pure.

D(−)-α-Sulfobenzylpenicillin (D-I). A solution of D-III (2.58 g, 0.011 mole) in 5 ml of Et₂O was added dropwise to a stirred solution of 6-APA (2.16 g, 0.01 mole), NaHCO₃ (1.76 g, 0.021 mole), and NaOH (0.4 g, 0.01 mole) in H_2O (17 ml) at 0°. The mixture was stirred at 0° for 30 min. Then, the organic layer was separated and the aqueous solution, after being degassed *in vacuo*, was applied to a column of Amberlite XAD-2 (100–200 mesh, 5 × 85 cm). Elution was begun with H_2O at a rate of 5.6 ml/min. The penicillin appeared after the first 1.8 l. of eluate and was eluted in the next 4.5 l. The combined penicillin fractions were evaporated to one-third the volume *in vacuo* and then lyophilized to yield a colorless powder (3.2 g). This was shown by nmr analysis to be 93% in optical purity. Ethanol crystallization gave essentially pure D-I as colorless needles: mp 250–255°; [α]D +162.8°. *Anal.* ($C_{16}H_{16}O_7N_2S_2Na_2 \cdot H_2O$) C, H, N, S. The ir spectrum was essentially identical with that of DL-I as described in the preceding paper.[1]

L(+)-α-Sulfobenzylpenicillin (L-I). By using L-III in the coupling reaction with 6-APA, L-I was obtained by a similar procedure to that described above. The optical purity was estimated to be 85% by nmr analysis. Attempts to crystallize it from several kinds of organic solvents failed. Rechromatography on a XAD-2 column and the subsequent lyophilization of the penicillin fraction yielded optically pure L(+) epimer as a colorless powder. [α]D +182.9° (*c* 1.01, H_2O). The ir spectrum was identical with that of DL-I. *Anal.* ($C_{16}H_{16}O_7N_2S_2Na_2 \cdot H_2O$) C, H, N, S.

Acid Stabilities of D(−)-α-Sulfobenzylpenicillin (D-I). The stabilities of D-I and carbenicillin were determined at 21, 24, 37, and 50°. Exactly 500 mg of the sample was accurately transferred to a series of 50-ml volumetric flasks and diluted with 0.1 M citrate buffer to make up to 100 ml, respectively. The flasks were stored in a constant temperature bath (21, 24, 37, 50°) which was regulated by a thermostat to within ±0.3°. At appropriate intervals samples were taken out and cooled in ice water. The remaining intact penicillin was determined by biological assay (*Proteus morganii*, IFO 3848). The pH of the solution was measured at the beginning and the end of each run. The pH drift was generally less than ±0.05 unit.

Periodical Stabilities of D(−)-α-Sulfobenzylpenicillin (D-I). The dry crystalline disodium salt (D-I) as well as the lyophilized powder‡ was stored in sealed tubes and stored at 40°. The residual D-I was determined by the agar diffusion assay with *P. morganii*, IFO 3848. After 3 months, approximately 10% loss of potency was observed. While stored at 25°, full potency was kept unchanged for 1 year.

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†Melting points were determined in open capillaries in a Mel-Temp apparatus. Optical rotations were obtained using a Perkin-Elmer 141 polarimeter and a 1-dm cell.

‡The water content of the sample estimated by the Karl-Fisher method was 3.0%.

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1-Substituted Vinyl-1-phthalanpropylamines as Potential Antidepressant Agents

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The preparation of a series of 1-substituted vinyl-1-phthalanpropylamines (5) and their ability to antagonize the effects of tetrabenazine in mice are described. The subject compounds were obtained from diethyl phthalate in three stages. Reaction of this ester with excess methylmagnesium bromide gave 1,3,3-trimethyl-1-phthalanol (7) which on treatment with perchloric acid afforded 1,1,3-trimethyl-1*H*-isobenzofurylium perchlorate (8) in 60% overall yield. The last substance condensed with a variety of arylaldehydes to give a series of 3-substituted vinyl-1,1-dimethyl-1*H*-isobenzofurylium perchlorates (9), which reacted with certain substituted aminopropylmagnesium halides to give the title compounds.

The clinical utility of amitriptyline (1), imipramine (2), and structurally similar substances has prompted the preparation of a variety of related compounds for testing as potential antidepressants.† The 11-substituted-5,10-epoxy-5*H*-dibenzo[*a,d*]cycloheptene-5-propylamines (3)[2] are particularly interesting, for they represent a departure from the tricyclic system characteristic of many of the analogs of amitriptyline, and one clinical report indicates that daily doses of 8–24 mg of *trans*-11-hydroxy-3 (R = α-OH) stimulate depressed patients.[3] Phthalan (4),[4] which can be viewed as an analog of 3 arising from cleavage of the 11–11a bond, also possesses thymoleptic properties.[5] The activity of 4 in reversing tetrabenazine-induced depression in mice (Table I) prompted us to search for a more interesting congener, and in the present paper we describe the synthesis and certain biological properties of a series of related 1-substituted vinyl-1-phthalanpropylamines (5) and a congener in which the phthalan system is completely reduced, e.g., 6.

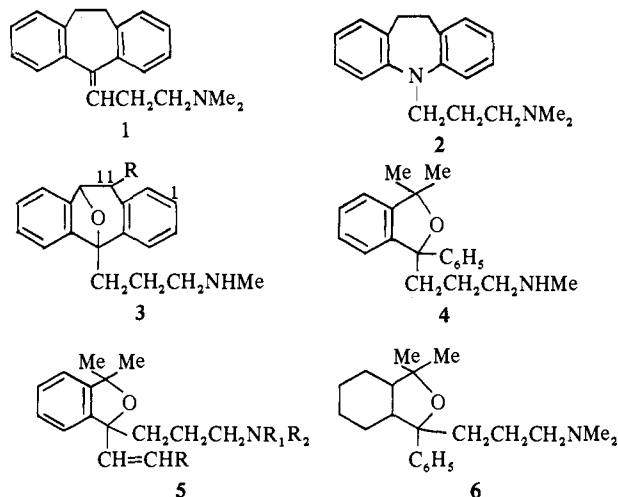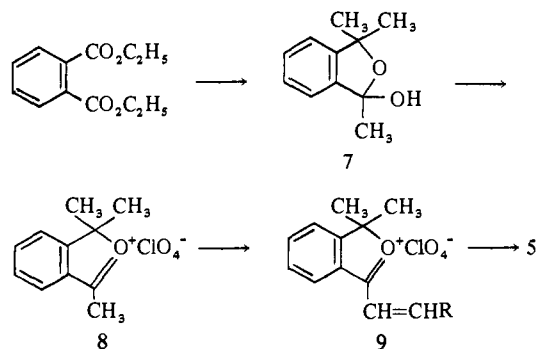Table I. Activity of Phthalans in Reversing Tetrabenazine Effects in Mice[a]

Compound	Lowest effective dose, mg/kg, ip
Amitriptyline (1)	3
Imipramine (2)	1.3
N,3,3-Trimethyl-1-phenyl-1-phthalanpropylamine (4) hydrochloride	0.8
N,N,3,3-Tetramethyl-1-styryl-1-phthalanpropylamine (14b) oxalate	0.8
N,N,3,3-Tetramethyl-1-styryl-1-phthalanpropylamine (14b) fumarate	1.6
N,3,3-Trimethyl-1-styryl-1-phthalanpropylamine (16) fumarate	1.6
N,N,3,3-Tetramethyl-1-(p-methoxystyryl)-1-phthalanpropylamine (5j) hydrochloride	0.8
N,N,3,3-Tetramethyl-1-(p-methylstyryl)-1-phthalanpropylamine (5h) oxalate	3
N,N,3,3-Tetramethyl-1-(o-methylstyryl)-1-phthalanpropylamine (5j) oxalate	6
N,N,3,3-Tetramethyl-1-(p-isopropylstyryl)-1-phthalanpropylamine (5m)	3
N,N,3,3-Tetramethyl-1-(3,4-dimethoxystyryl)-1-phthalanpropylamine (5k)	12.5

[a]See Greenblatt and Osterberg[9] for details of this assay.

The former compounds were prepared in three stages from diethyl phthalate (see Scheme I). Reaction of this ester with methylmagnesium bromide gives the phthalanol

1

2

3

4

5

6

Scheme I