were collected and like fractions were combined and lyophilized to afford a solid, which was generally >80% pure.

Cyclization. A. Via Air Oxidation. The partially purified acyclic peptide was dissolved in 0.1 M ammonium bicarbonate (1.5 mg/mL) and stirred open to the air. The course of the reaction was monitored via HPLC. After cyclization was complete (several hours to several days), the solution was acidified (30% AcOH) and lyophilized. The resulting solid was purified via HPLC on a C_{18} silica gel column (Vydac; 22 mm i.d. × 250 mm, 15–20 μm, 300 A) eluting with a linear gradient of 15–35% acetonitrile over 25 min at a flow rate of 9 mL/min.

B. Via Potassium Ferricyanide. To a magnetically stirred solution of 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (50 mL, pH 8–8.5) was added a solution of 0.1 N potassium ferricyanide (10 mL), followed by those pooled fractions collected from medium-pressure liquid chromatography (typically 36–40 mL) that have a purity >80%. The course of the reaction, as monitored by HPLC and Ellman's colorimetric assay,[12] was complete within 30 min. The solution was adjusted to a pH of 2.5–3.5 with acetic acid. Anion-exchange resin (Dowex SBR; nuclear grade, hydroxide form, strongly basic, 8% cross-linked, 20–50 dry mesh) was added until almost complete decoloration occurred. After filtration, lyophilization afforded a white solid, which was purified via HPLC as described in section Å.

C. Via Iodine. The cysteine residues of **6a** were protected with acetamidomethyl (ACM) groups during synthesis. Lyophilization of the aqueous fraction obtained from hydrogen fluoride induced cleavage of the resin-bound peptide afforded the crude acyclic peptide (57 mg, ~37% by HPLC integration). This material was dissolved in 80% acetic acid (20 mL). Solid iodine (40 mg) was added in one portion and the resulting dark brown solution was stirred at room temperature for 3 h. The reaction mixture was diluted with water (40 mL) and extracted with chloroform (3 × 40 mL). The organic layer was washed once with water (40 mL), and the aqueous layers were combined and concentrated in vacuo at room temperature to about half of the original volume. Lyophilization left a white solid, which was purified via HPLC as described in section A to afford **6a** as a white solid (3.7 mg; 97% purity). Additional, less pure material was also obtained.

Yields were unoptimized. Greater emphasis was placed on peptide purity, which resulted in decreased yields. Moreover, only a sufficient quantity of peptide was purified to complete the necessary analyses/assays. All peptides were purified to greater than 97% purity. Amino acid analyses and FABMS were in agreement with the expected results.

Receptor Binding Assay. Atrial peptide analogues were studied in a competitive binding assay using rabbit lung membranes as described previously.[8,9]

Acknowledgment. We thank E. W. Kolodziej, E. J. Reinhard, P. C. Toren, D. E. Whipple, and J. F. Zobel for excellent technical assistance.

Registry No. **2a**, 119414-80-1; **2b**, 119414-81-2; **2c**, 119435-36-8; **2d**, 119435-37-9; **2e**, 119435-62-0; **3a**, 119414-82-3; **3b**, 119414-83-4; **3c**, 119414-84-5; **3d**, 119414-85-6; **3e**, 119414-86-7; **3f**, 119435-63-1; **4**, 119435-64-2; **5a**, 119414-87-8; **5b**, 119414-88-9; **5c**, 119414-89-0; **5d**, 119414-90-3; **6a**, 119435-65-3; **6b**, 119435-66-4; **6c**, 119414-91-4; **6d**, 119414-92-5; **6e**, 119435-67-5; **7a**, 119414-93-6; **7b**, 119414-94-7; **7c**, 119414-95-8; **7d**, 119435-68-6.

Retinobenzoic Acids. 3. Structure–Activity Relationships of Retinoidal Azobenzene-4-carboxylic Acids and Stilbene-4-carboxylic Acids

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Alkyl-substituted azobenzene-4-carboxylic acids are potent differentiation inducers of human promyelocytic leukemia cell line HL-60 to mature granulocytes. Their structure–activity relationships are very similar to those of other retinoidal benzoic acids which are generally represented by 4 and named retinobenzoic acids. The structure–activity relationships of azobenzenecarboxylic acids can also be applied to the known retinoid TTNPB (**3**). Thus, (*E*)-4-[2-(3,4-diisopropylphenyl)-1-propenyl]benzoic acid (St30 (**28**)) and (*E*)-4-[2-(3-*tert*-butylphenyl)ethenyl]benzoic acid (St40 (**29**)), the acyclic alkyl analogues of TTNPB, are nearly as active as retinoic acid. Among the oxidatively derived compounds (Az90, Ep series and Ox series) of azobenzene- or stilbenecarboxylic acids, Az90 (**71**) and Ep80 (**61**) have strong activities. However, all the bishydroxylated derivatives of TTNPB are inactive, while a diketo analogue Ox580 (**69**) has only weak potency. The activities of conformationally restricted compounds of TTNPB offer some information on the stereochemistry of the active form of these retinoidal compounds.

Retinoids, retinoic acid (RA, **1**; Chart I) and its analogues, have a fundamental and essential role in various processes of life, that is, in the maintenance of growth and as morphogens, etc.[1-3] One of the most important activities is the control of cellular differentiation and proliferation.[2] Retinoic acid acts as a specific modulator in many types of cells, both normal and neoplastic. Mechanistic studies of the retinoidal actions on cellular modulation have been reported. Retinoids control several gene expressions, including the suppression of the expression of *c-myc*[4,5] and the gene for collagenase,[6] and the enhancement of the expression of the genes of epidermal growth factor receptor (EGFR).[7] Thus, retinoids are considered to affect directly the expression of genes which control cellular differentiation and proliferation. Only recently, some hypotheses were proposed based on gene technology studies[8-10] or direct attempts to isolate specific receptor(s).[11] Now, the term "retinoids", originally defined in

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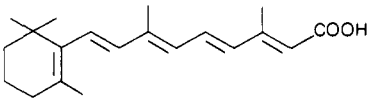
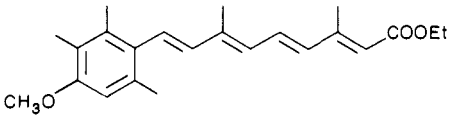
0022-2623/89/1832-1098$01.50/0

Chart I



retinoic acid (1)



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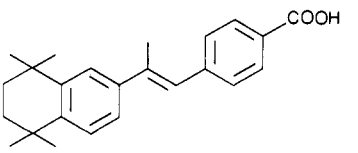


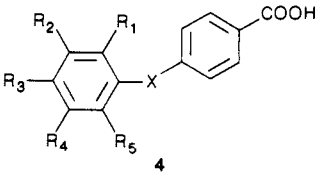
3

terms of chemical structures, has been redefined biologically as substances that elicit the specific responses through binding to the specific receptor(s), as proposed by Sporn et al.[12]

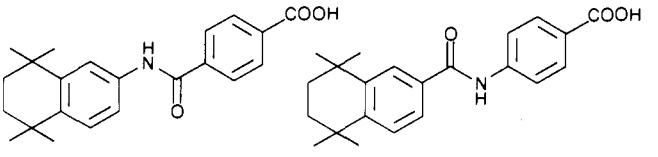
Another important aspect of retinoids, besides their fundamental roles in cell biology, is the possibility of applying them clinically in the fields of oncology and dermatology. They inhibit the neoplastic transformation by chemical carcinogens,[13] suppress the action of tumor promoters (TPA[14] or teleocidins[15]), and inhibit the induction of ornithine decarboxylase by tumor promoters. A number of retinoidal active compounds have been synthesized, and their clinical efficacy has been examined.[2] Parts of the structure of retinoic acid (cyclohexenyl ring, polyene chain, and terminal polar group) were modified, and among the synthetic compounds, etretinate (**2**)[16] and TTNPB (**3**)[17,18] may be useful clinically. However, the major disadvantage of these retinoids is their high toxicity (known as hypervitaminosis A), which is at least partially owing to the hydrophobicity of their hydrocarbon skeletons.

Recently, we reported that retinoidal activities had been demonstrated for various benzoic acids, whose structures are represented by 4 (Chart II),[19,20] where R is a medium-sized alkyl group(s) and the linking group X can be —NHCO—,[21] —CONH—,[22] —SO_2NH—, —N=N—,[23] —COCH=CH—,[24] and so on. These compounds, named "retinobenzoic acids", are structurally or physicochemically very different from the conventional retinoids, but they

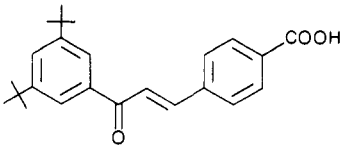
Chart II



4



Am80 (5) Am580 (6)



Ch55 (7)

have the same activities as retinoic acid in all cases.[12,20,25,26] The structure–activity relationships of two types of aromatic amides (X = —NHCO— or —CONH—)[27] and of chalconecarboxylic acids (X = —COCH=CH—)[28] are very similar, and therefore they are regarded as agonists with respect to each other. In particular, Am80 (**5**), Am580 (**6**), and Ch55 (**7**) are several times more active than retinoic acid in several assay systems. In this paper, the structure–activity relationships of azobenzene-4-carboxylic acids, another type of retinobenzoic acids (X = —N=N— in 4), and the relation to the bioisosteric compounds, stilbene-4-carboxylic acids, are discussed. As a measure of retinoidal activities, the ability to induce differentiation of human promyelocytic leukemia cell line HL-60 to mature granulocytes[29] was examined. This ability of retinoids correlates well with other retinoidal activities.[1,20] The morphological changes were examined after Wright-Giemsa staining, and the Nitroblue tetrazolium (NBT) reduction assay was employed as a functional marker of differentiation.[30] These two indexes of differentiation correlated well. Experiments were repeated more than three times in most cases, covering 5 orders of concentrations. The ED_{50} values of active compounds were

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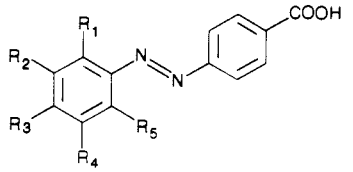
Table I. Differentiation-Inducing Activities of Azobenzene-4-carboxylic Acids



compd	R_1	R_2	R_3	R_4	R_5	ED_{50},[a] M	rel act.[b]
retinoic acid						2.4×10^{-9} [c]	100
Az00 (8)	H	H	H	H	H	inactive[d]	
Az10 (9)	H	Me	H	H	H	inactive	
Az20 (10)	H	Et	Et	H	H	2.2×10^{-7}	0.53
Az25 (11)	H	Et	H	H	H	$>10^{-6}$ [d]	$<10^{-2}$
Az30 (12)	H	H	iPr	H	H	$>10^{-6}$	$<10^{-2}$
Az32 (13)	H	iPr	H	H	H	3.8×10^{-7}	0.29
Az34 (14)	iPr	H	H	H	H	inactive	
Az40 (15)	H	tBu	H	H	H	4.0×10^{-7}	0.36
Az62 (16)	iPr	H	H	iPr	H	inactive	
Az64 (17)	iPr	H	iPr	H	H	$>10^{-6}$	$<10^{-2}$
Az66 (18)	H	iPr	H	iPr	H	4.1×10^{-7}	0.22
Az68 (19)	H	iPr	iPr	H	H	2.3×10^{-9}	39
Az70 (20)	H	$-(CH_3)_2CCH_2CH_2O-$		H	H	1.3×10^{-7}	3.5
Az75 (21)	H	$-(CH_3)_2CCH_2CH_2S-$		H	H	2.1×10^{-7}	2.1
Az80 (22)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	1.7×10^{-9}	130
Az160 (23)	H	Ph	H	H	H	$>10^{-6}$	$<10^{-2}$
Az162 (24)	H	cC_6H_{11}	H	H	H	1.3×10^{-7}	0.68

[a] ED_{50} values of active compounds were calculated from the NBT reduction assay data. Experiments were repeated more than three times in most cases. The values shown are representative ones or means (when more than five repetitions were done). This is also the case in the other tables. [b] The ratio of ED_{50} (retinoic acid) to ED_{50} (a test compound), both values having been obtained in concurrent experiments. This is also the case in the other tables. [c] The deviation (σ_{n-1}) of retinoic acid is estimated to be 1.8×10^{-9} M ($n = 90$). [d] "Inactive" means there was no activity at 10^{-6} M. "$>10^{-6}$ M" means there was slight activity at 10^{-6} M.

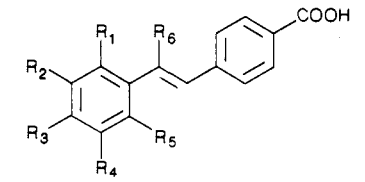
calculated from the NBT reduction assay data. Relative activity was defined as the ratio of ED_{50} of retinoic acid to ED_{50} of a test compound, both values having been obtained in concurrent experiments. These two values shown in tables are representative ones or means when more than five repetitions were done.

Results and Discussion

The differentiation-inducing activities of azobenzene-4-carboxylic acids are shown in Table I. Nonsubstituted azobenzene-4-carboxylic acid (Az00 (**8**)) is absolutely inactive at concentrations below 10^{-6} M in this assay. However, the introduction of one or more alkyl groups resulted in clear activity. The effect of alkyl substituents is very similar to those of other retinobenzoic acids.[19,27,28] That is, the compound with an isopropyl group (Az32 (**13**)) or a *tert*-butyl group (Az40 (**15**)) at the meta position has the activity. A smaller alkyl group (methyl or ethyl) does not have a significant effect on the activity, but a compound having a larger alkyl group, such as a cyclohexyl group (Az162 (**24**)), is active. Among three compounds with one isopropyl group, only the meta-substituted analogue (Az32 (**13**)) has the differentiation-inducing activity. A similar result was seen in a series of diisopropyl derivatives, and two significant features were noted. One is that the introduction of an *o*-isopropyl group on Az32 (**13**) (giving Az62 (**16**)) resulted in the disappearance of the activity. The other is that 3,4-diisopropyl-Az68 (**19**) is more active than 3,5-diisopropyl-Az66 (**18**), though the number of the *m*-alkyl groups (necessary for the activity) is one in the former and two in the latter. Thus, the para substituent, when it coexists with a *m*-alkyl group, is much more effective than the second (another) meta substituent. Similarly, the introduction of an additional *p*-ethyl group on the inactive compound Az25 (**11**) resulted in significant activity (Az20 (**10**)). This indirect effect of the para substituent on the activity is also seen in the retinoidal terephthalic anilides (X = –NHCO– in **4**).[27] In these amide compounds, it is considered that the para substituent interacts sterically with the *m*-alkyl group (such as an ethyl or isopropyl group), and the benzylic methyl groups consequently face opposite to the para position. The importance of the direction of the alkyl group is indicated by the stronger activity of Am80 (**5**), where the alkyl group conformation is restricted by the ring system and four methyl groups are facing away. A similar result was seen here. Thus, Az80 (**22**), corresponding to Am80 (**5**), is somewhat more active than Az68 (**19**) or retinoic acid and has the strongest activity among all the azobenzene-4-carboxylic acids synthesized so far. The exchange of the *p*-alkyl part of Az80 (**22**) to a polar heteroatom (Az70 (**20**) and Az75 (**21**)) reduced the activity by 2 orders of magnitude, and so some hydrophobicity is important for high activity.

The structure–activity relationships of azobenzene-4-carboxylic acids closely resemble those of retinoidal terephthalic anilides mentioned above and other retinobenzoic acids shown by the general structure **4**. Various types of compounds, though they have very different chemical and physical properties owing to the different linking group X, have very similar substituent effects. The known retinoid (*E*)-stilbene-4-carboxylic acid TTNPB (**3**)[17,18] can also be structurally represented by the structure **4** where X is an ethylenic bond. In particular, Az80 (**22**) and TTNPB (**3**) are bioisosteres. So, it is reasonable to consider that the results on the substituent effects in azobenzene-4-carboxylic acids can be applied to the stilbene-4-carboxylic acids. That is, the scission of the cyclic alkyl group of TTNPB will also keep the activity. Another presumption is that the methyl group on the olefinic carbon of TTNPB will not directly affect the activity. The role of the various linking groups X is considered to be in locating two benzene rings at the proper positions.[20,27] That is, the significant factor is the conformational character, regardless of the electronic properties. So the existence of the methyl group will only affect the activity to the extent that it alters the molecular conformation.

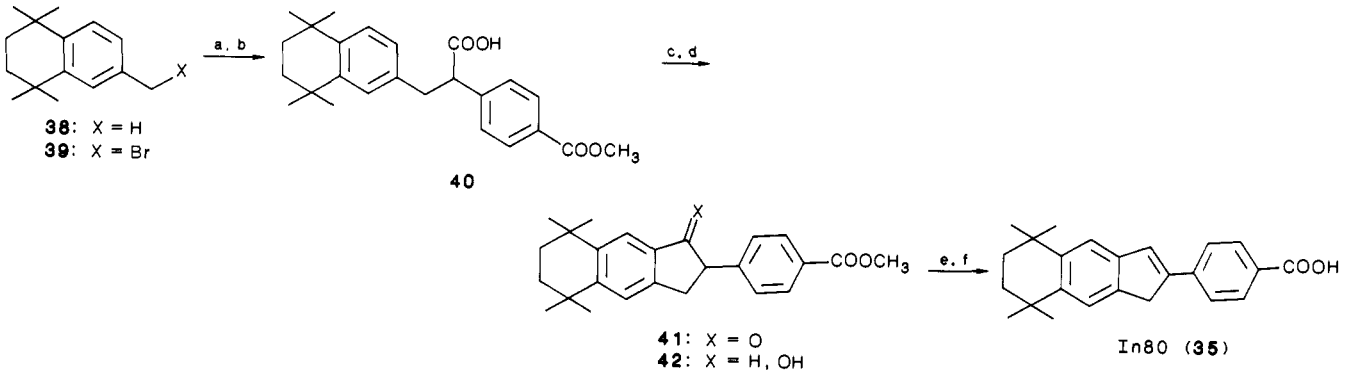
Table II. Differentiation-Inducing Activities of Stilbene-4-carboxylic Acids



compd	R_1	R_2	R_3	R_4	R_5	R_6	ED_{50}, M	rel act.
retinoic acid							2.4×10^{-9}	100
St00 (25)	H	H	H	H	H	H	inactive	
St10 (26)	H	Me	Me	H	H	Me	inactive	
St20 (27)	H	Et	Et	H	H	Me	8.7×10^{-8}	2.2
St30 (28)	H	iPr	iPr	H	H	Me	1.3×10^{-8}	15
St40 (29)	H	tBu	H	H	H	H	1.0×10^{-8}	13
St50 (30)	H	H	tBu	H	H	H	inactive	
St60 (31)	H	H	tBu	H	H	Me	inactive	
TTNPB (3)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	Me	2.5×10^{-9}	90
St80 (32)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	H	5.0×10^{-10}	260
St100 (33)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	CF_3	3.1×10^{-8}	5.7
St87 (34)[a]	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	H	1.3×10^{-7}	2.7

[a] St87 (34) is a derivative of St80 (32) in which the olefinic bond is hydrogenated.

Scheme I[a]



[a] (a) NBS/AIBN/$CHCl_3$; (b) $^-OOCCH^-Ph$-*p*-$COOCH_3$/THF; (c) $SOCl_2$/DMF; $AlCl_3/ClCH_2CH_2Cl$; (d) $NaBH_4/C_2H_5OH$; (e) MsCl/Pyr; (f) KOH/CH_3OH.

Several (*E*)-stilbene-4-carboxylic acids without the aliphatic cyclic ring were synthesized and their activities were examined in order to examine the above hypothesis (Table II). The effect of acyclic alkyl groups is the same as that observed in a series of azobenzene-4-carboxylic acids. The unsubstituted derivative (St00 (**25**)) is inactive, and substitution with a small alkyl group (St10 (**26**)) or at the para position (St50 (**30**) and St60 (**31**)) is of no effect. Compounds with a diethyl group (St20 (**27**)) or a *m-tert*-butyl group (St40 (**29**)) are more active than the corresponding azo compounds (Az20 (**10**) or Az40 (**15**)). St30 (**28**), having a diisopropyl group, also has significant activity, though its potency is somewhat weaker than that of the corresponding Az68 (**19**). Thus, the ring opening of the aliphatic ring of TTNPB (**3**) did not result in loss of the differentiation-inducing activity. Secondly, three derivatives of TTNPB (**3**) which all have the cyclic alkyl group but a different substituent on the olefinic carbon were compared. St80 (**32**), without any group on the olefin, is several times more active than TTNPB (**3**). On the other hand, St100 (**33**), having a CF_3 group instead of a methyl group, is less active than TTNPB (**3**) by 1 order of magnitude. The presence of a methyl group on the olefin is not required for the activity. The cause of the decreased activity of the compound with a CF_3 group remains to be identified.

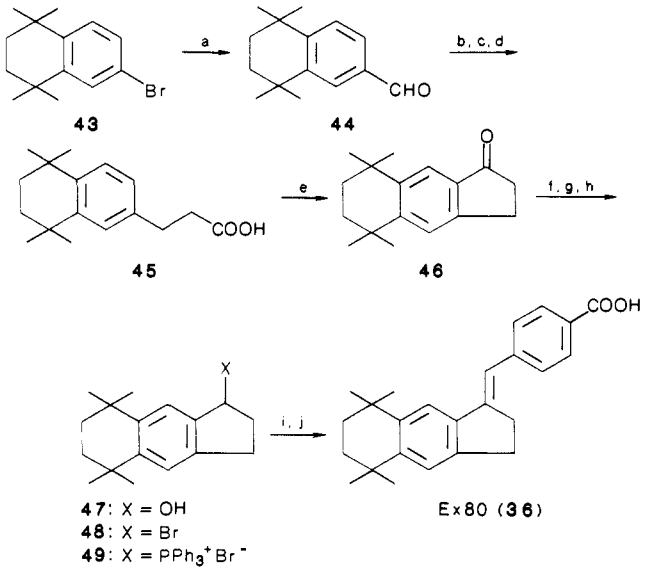
Strickland et al.[18] examined the differentiation-inducing activities of a series of 3-substituted TTNPBs on HL-60 and murine F9 teratocarcinoma cells. In this case, a large alkyl group at the position ortho to the olefinic bond reduced the activity. As mentioned above, the reduction of activity by a large alkyl group on or ortho to the olefinic bond, considering that the electronic properties of the linking group are not so significant, is due to the change of the conformation. These substituents should mostly affect the torsional angle of the Ar—C(=C) bond. Typically, there exist two conformers of TTNPB (**3**), s-trans and s-cis, as shown in Figure 1. Strickland et al. discussed the structure of TTNPB (**3**) and retinoic acid on the basis of X-ray crystallographic studies.[18] In the crystal, TTNPB exists as the s-trans form with some torsion. Though this structure can be superimposed on that of retinoic acid in the crystal, the biologically active conformation of TTNPB (**3**) is unknown. It is also unclear whether the substituent on (or ortho to) the olefin results in a preference for s-trans form or s-cis form. One method for the elucidation of the conformational problems would be fixation of a flexible bond by a ring system, and so two indene derivatives were designed. Indene derivative In80 (**35**) corresponds to a compound fixed in s-trans form and compound Ex80 (**36**) corresponds to s-cis form, as illustrated in Figure 1. These

Table III. Differentiation-Inducing Activities of Conformationally Restricted Analogues of TTNPB (3)

compd[a]	ED_{50}, M	rel act.
retinoic acid	2.4×10^{-9}	100
In80 (35)	(6.6×10^{-9})[b]	
Ex80 (36)	2.0×10^{-10}	570
Bf80 (37)	1.3×10^{-9}	120

[a] Structures: see Figure 1. [b] Maximum cellular response to In80 (**35**) is less than half of that of retinoic acid.

Scheme II[a]



[a] (a) tBuLi/ether; DMF; NH_4Cl; (b) *n*-BuLi/$(Et_2O)_2P(O)$-CH_2COOEt/ether; (c) H_2/10% Pd–C/EtOH; (d) NaOH/EtOH; (e) $SOCl_2$/DMF; $AlCl_3/CH_2Cl_2$; (f) $NaBH_4/CH_3OH$; (g) PBr_3/ether; (h) PPh_3/benzene; (i) OHCPh-*p*-$COOCH_3$/butylene oxide; (j) KOH/EtOH.

two compounds and an oxa analogue of In80 (**35**) (that is, the 2-phenylbenzofuran derivative Bf80 (**37**)) were synthesized by the route shown in Schemes I–III and their activities were compared (Table III). Of the two indene derivatives, Ex80 (**36**) is more active than In80 (**35**) and is several times more active than TTNPB (**3**). At a glance, Ex80 (**36**) seems to be close to the active form of TTNPB (**3**), but we cannot ignore the significant activity of In80 (**35**). Furthermore Bf80 (**37**), where the s-trans form was fixed by an O atom instead of a methylene in In80 (**35**), is as active as TTNPB (**3**) or retinoic acid. The reason for the difference of activity between In80 (**35**) and Bf80 (**37**) is not clear, but may involve a subtle difference of the torsion angle of the C(2)–Ar bond. Thus, the s-cis form seems to be the important conformation for activity, but the s-trans form also interacts with the receptor binding site, though not satisfactorily. Perhaps, some binding site flexibility makes the interaction with both compounds possible. Previously, we reported the highly potent activity

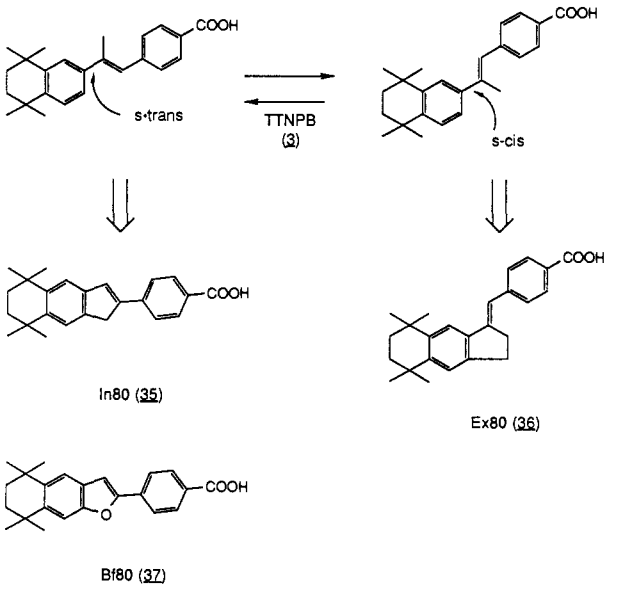
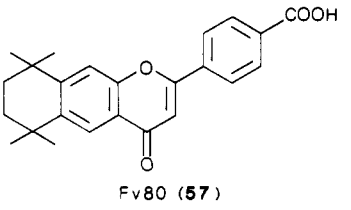


Figure 1.

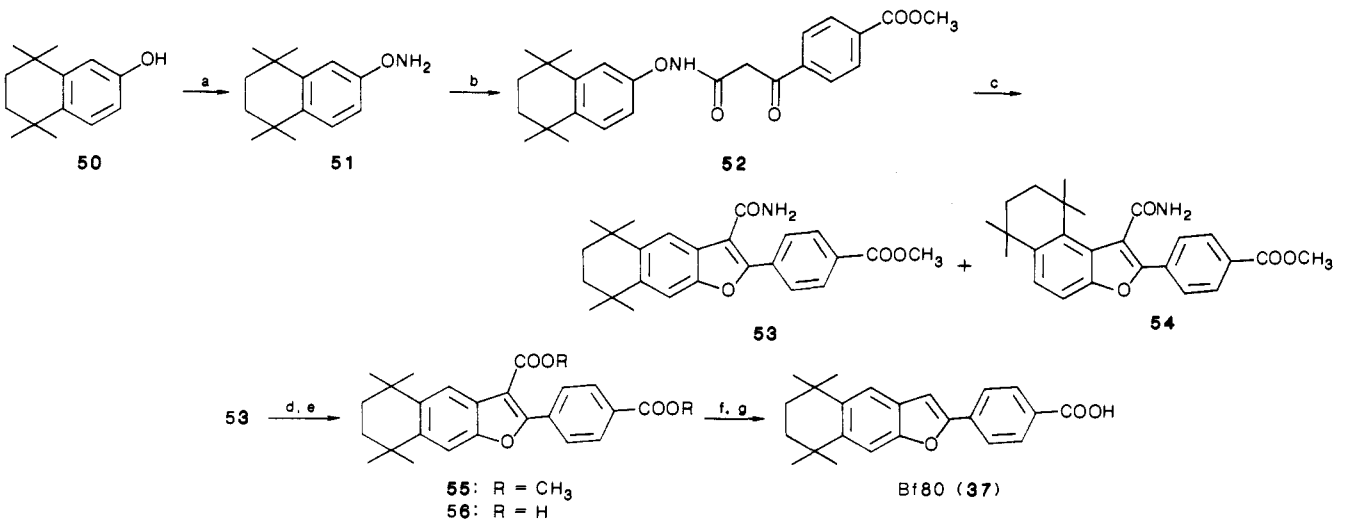
Chart III



of flavone-4′-carboxylic acid Fv80 (**57**; Chart III) whose ED_{50} is 4.6×10^{-11} M (27.4-fold more potent than retinoic acid).[28] Fv80 (**57**) is apparently stereochemically between Ex80 (**36**) and In80 (**35**), though the linking group corresponds to the s-cis form. Three-dimensional conformation analysis of retinobenzoic acids, including both flexible compounds and the restricted compounds, is in progress.

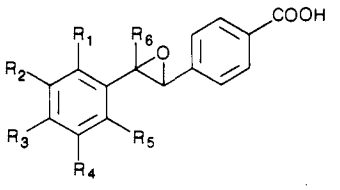
Finally, another point of interest is whether the metabolites of these azobenzene or stilbene derivatives are active or not. Oxidation is one of the possible routes of metabolism of these compounds; such oxidized derivatives are more polar than the parent compounds and so are expected to have reduced toxicities. Therefore, the ac-

Scheme III[a]



[a] (a) tBuOK/CH_3OH; MSH/DMF; (b) $HOOCCH_2COPh$-*p*-$COOCH_3$/DCC; (c) CF_3COOH; (d) HCl/CH_3OH; (e) $NaOH/CH_3OH$; (f) Cu/quinoline/HCl/CH_3OH; (g) $NaOH/CH_3OH$.

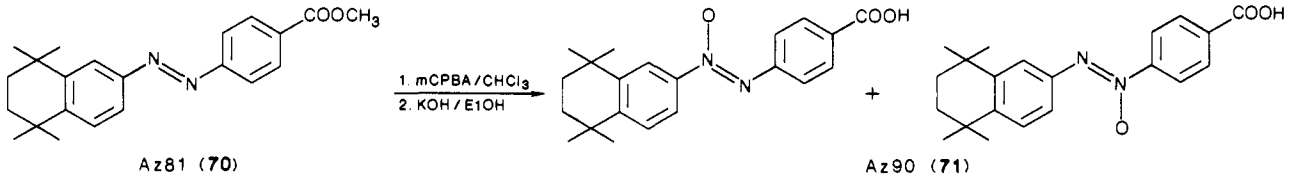
Table IV. Differentiation-Inducing Activities of Oxidized Analogues of Stilbene- and Azobenzene-4-carboxylic Acids



compd	R_1	R_2	R_3	R_4	R_5	R_6	ED_{50}, M	rel act.
retinoic acid							2.4×10^{-9}	100
Ep20 (58)	H	Et	Et	H	H	Me	inactive	
Ep40 (59)	H	tBu	H	H	H	H	$>10^{-6}$	$<10^{-2}$
Ep50 (60)	H	H	tBu	H	H	H	$>10^{-6}$	$<10^{-2}$
Ep80 (61)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	H	4.3×10^{-9}	22
Ep90 (62)	H	$-(CH_3)_2CCH_2CH_2C(CH_3)_2-$		H	H	Me	1.3×10^{-7}	0.65
Ox580 (69)[a]							1.4×10^{-7}	3.9
Az90 (71)[b]							2.5×10^{-10}	1960

[a] Structure: see Chart IV. [b] Structure: see Scheme IV.

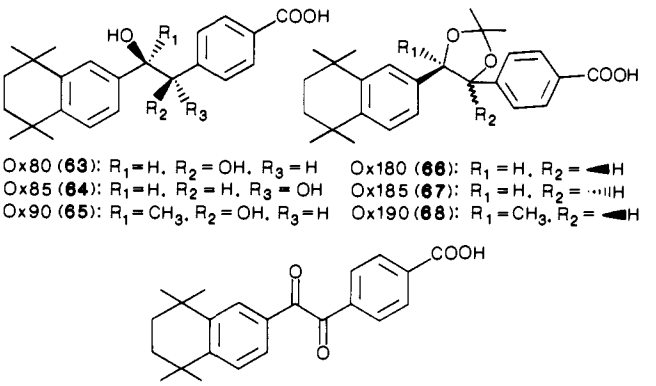
Scheme IV



tivities of some oxidative analogues of the linking group (—N=N— or —C≡C—) of Az80 (22) or stilbenecarboxylic acids were examined (Table IV). Epoxidation of the olefinic bond reduced the activity. Ep20 (58) and Ep40 (59) are inactive. Ep90 (62) is weaker than the original stilbene TTNPB (3) by 2 orders of magnitude. However, Ep80 (61), the epoxide derived from St80 (32), still has strong activity ($^1/_{10}$ as active as St80 (32)). The difference of the activities between Ep80 (61) and Ep90 (62) may be due to the change of the stereochemistry or the torsional angle of the Ar–C bond, as discussed above. Moreover, opening of the epoxide by hydration caused loss of the activity. Thus, two hydrated analogues of Ep80 (61), the *threo* diol Ox80 (63; Chart IV) and the *erythro* diol Ox85 (64), and one hydrated analogue Ox90 (65), derived from Ep90 (62) are all inactive. Interestingly, St87 (34), which is formed by hydrogenation of St80 (32), has the activity (Table II): the double bond character is not essential. In this case, also, the retention of the activity seems to be easily understood when considering that St87 (34) exists preferentially in a conformation where the two benzene rings will be placed in an antiperiplanar relationship. In the diol compounds, NMR studies (^1H–^1H coupling constant of two benzylic hydrogens) indicate that the two benzene rings do not favor the antiperiplanar arrangement owing to the interaction of the two hydroxyl groups, and the whole structures would be distorted. The same was seen when the two hydroxyl groups were connected and fixed by acetonization: the acetonides of all three diols (Ox180 (66), Ox185 (67), Ox190 (68)) are inactive. Further oxidation to the 1,2-dicarbonyl compound (Ox580 (69)) restored the activity to some degree, though it was weaker than we had anticipated.

On the other hand, oxidation of Az80 (22) gave an azoxy derivative Az90 (71), which has strong activity. A ^1H NMR study showed that Az90 (71) is a mixture of two regioisomers as to the position of the oxygen on the azo group (Scheme IV). Though it is unknown whether Az80 (22) can be metabolized to Az90 (71) (or vice versa) in HL-60 cells or other biological systems, both compounds have such potent activities that they should be important members of the retinobenzoic acids.

Chart IV



Conclusion

We have discussed the structure–activity relationships of azobenzene- and stilbene-4-carboxylic acids. These two types of compounds have similar substituent effects to other retinoidal benzoic acids previously reported. As expected, the cyclic alkyl group of TTNPB (3) can be cleaved to an acyclic alkyl group(s), such as an isopropyl or *tert*-butyl group, without significant reduction of the activity. As a whole, the stereochemistry of the compounds seems to determine the degree of activity. At present, it remains unclear what conformational properties are required for the retinoidal activities. More detailed conformational analyses of various retinobenzoic acids described here or previously would elucidate this problem. Furthermore, the newly synthesized retinobenzoic acids with different chemical structures possessing potent activities should have important roles in developing the clinical applications of retinoids in oncology and dermatology.

Experimental Section

Cells and Culture. The human promyelocytic leukemia cells HL-60 were provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and have been maintained in continuous suspension culture. The cells are cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal calf serum (FCS)

Table V. Chemical and Physical Properties of Azobenzene-4-carboxylic Acids

compd	mp, °C	crystal form	recrystn solvent	formula
Az00 (8)	246–247	red plates	AcOEt–*n*-hexane	$C_{13}H_{10}N_2O_2$
Az10 (9)	214–215	red flakes	AcOEt–*n*-hexane	$C_{14}H_{12}N_2O_2$
Az20 (10)	215–216	red needles	AcOEt–*n*-hexane	$C_{17}H_{18}N_2O_2$
Az25 (11)	191.5–192	red needles	AcOEt–*n*-hexane	$C_{15}H_{14}N_2O_2$
Az30 (12)	266.5–268.5	orange prisms	AcOEt–*n*-hexane	$C_{16}H_{16}N_2O_2$
Az32 (13)	186.5–188.5	orange needles	AcOEt–*n*-hexane	$C_{16}H_{16}N_2O_2$
Az34 (14)	195.5–197	orange needles	AcOEt–*n*-hexane	$C_{16}H_{16}N_2O_2$
Az40 (15)	245–246	orange prisms	AcOEt–*n*-hexane	$C_{17}H_{18}N_2O_2$
Az62 (16)	192.5–193	red needles	AcOEt–*n*-hexane	$C_{19}H_{22}N_2O_2$
Az64 (17)	206–208	red needles	AcOEt–*n*-hexane	$C_{19}H_{22}N_2O_2$
Az66 (18)	201–203	orange flakes	AcOEt–*n*-hexane	$C_{19}H_{22}N_2O_2$
Az68 (19)	230.5–232	red flakes	AcOEt–*n*-hexane	$C_{19}H_{22}N_2O_2$
Az70 (20)	285.5–286	orange needles	AcOEt–*n*-hexane	$C_{18}H_{18}N_2O_3$
Az75 (21)	280.5–281	orange flakes	AcOEt–*n*-hexane	$C_{18}H_{18}N_2O_2S$
Az80 (22)	287–288	red needles	AcOEt–*n*-hexane	$C_{21}H_{24}N_2O_2$
Az160 (23)	249–250	red prisms	AcOEt	$C_{19}H_{14}N_2O_2$
Az162 (24)	248–248.5	red prisms	AcOEt–*n*-hexane	$C_{19}H_{20}N_2O_2$

and antibiotics (penicillin G and streptomycin), at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Test compounds were dissolved in ethanol at 0.2 mM and added to the cells and seeded at about 8×10^4 cells/mL, while the final ethanol concentration was kept below 0.5%. Control cells were given only the same volume of ethanol. Retinoic acid, a positive control, was always assayed at the same time. The cells were incubated for 4 days and stained with Wright-Giemsa. Differential counts were then performed under a light microscope on a minimum of 200 cells. Nitroblue tetrazolium (NBT) reduction was assayed as described.[30] Cells were incubated for 20 min at 37 °C in RPMI1640 medium (5% FCS) and an equal volume of phosphate-buffered saline (PBS) containing NBT (0.2%) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA; 200 ng/mL). The percentage of cells containing blue-black formazan was determined on a minimum of 200 cells. The results of these two evaluations were always in good agreement.

The assays of test compounds were performed at least three times. ED_{50} values of active compounds were calculated from the NBT reduction assay data. Relative activities were calculated as the ratio of ED_{50} of retinoic acid to ED_{50} of the test compound obtained in concurrent experiments.

Chemistry. Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microanalytical Laboratory, University of Tokyo, and were within ±0.4% of the theoretical values. NMR spectra were recorded on JEOL FX 100-MHz and JEOL GX 400-MHz NMR spectrometers. Chemical shifts are expressed in ppm relative to tetramethylsilane.

General Procedure for Azobenzene-4-carboxylic Acids. An alkyl-substituted aniline (1 mmol) and methyl *p*-nitrosobenzoate[31] (0.9 mmol) were dissolved in 10 mL of AcOH, and the mixture was stirred at room temperature overnight, with shielding from light. The mixture was poured into water and extracted with AcOEt. The organic layer was washed successively with H_2O (twice), 1 N $NaHCO_3$ (three times), H_2O, and brine and dried over $MgSO_4$. After the removal of the solvent, the crude mixture was chromatographed on silica gel to give methyl azobenzene-4-carboxylate. This ester (1 mmol) was dissolved in 10 mL of EtOH under Ar gas. Then 2 mL of 2 N NaOH was added and the mixture was stirred overnight, with shielding from light. The mixture was poured into 1 N HCl and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. After evaporation, the crude product was recrystallized to give azobenzene-4-carboxylic acid. The chemical and physical properties of the azobenzene-4-carboxylic acids are listed in Table V.

(*E*)-4-[2-(3,4-Diethylphenyl)-1-propenyl]benzoic Acid (St20 (27)) (Method A). The mixture of *o*-diethylbenzene (5 g, 37.3 mmol) and AcCl (3.2 g, 40.7 mmol) in 30 mL of $ClCH_2CH_2Cl$ was added portionwise to a suspension of $AlCl_3$ (5.7 g, 42.7 mmol) in 30 mL of $ClCH_2CH_2Cl$ at 0 °C and then stirred for 1 h. The mixture was poured into ice water and extracted with ether. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After the removal of the solvent, the residue was distilled under vacuum to give 3,4-diethylacetophenone (62.1%). $NaBH_4$ (507 mg, 15.1 mmol) was added slowly to a solution of 3,4-diethylacetophenone (4.08 g, 23.1 mmol) in 15 mL of CH_3OH at 0 °C. The mixture was stirred for 1.5 h, poured into dilute HCl and ice, and extracted with ether. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After the removal of the solvent, the residue was distilled under vacuum to give 1-(3,4-diethylphenyl)ethanol (86.3%). This alcohol (3.56 g, 19.9 mmol) was dissolved in a mixture of 3 mL of ether, 30 mL of *n*-hexane, and 2 drops of pyridine. Then 1.05 mL of PBr_3 in 10 mL of *n*-hexane was added to the solution at 0 °C over 30 min. The mixture was stirred for 1.5 h, poured into ice, and extracted with ether. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. The solvent was removed to give the crude 4-(1-bromoethyl)-1,2-diethylbenzene (95.8%). A mixture of this bromide (4.61 g, 19.1 mmol) and triphenylphosphine (4.99 g, 19.0 mmol) in 30 mL of benzene was refluxed for 24 h. After cooling, the precipitates were collected to give [1-(3,4-diethylphenyl)ethyl]triphenylphosphonium bromide (64.7%). The mixture of this phosphonium salt (4.8 g, 9.53 mmol) and terephthalaldehydic acid methyl ester (1.56 g, 9.51 mmol) was dissolved in 40 mL of 1,2-butylene oxide and refluxed under Ar gas for 24 h. After concentration, the crude mixture was purified by silica gel column chromatography to give methyl 4-[2-(3,4-diethylphenyl)-1-propenyl]benzoate (83.1%, *E*/*Z* ratio, 5:1). The *E* isomer (St21; 98 mg, 0.29 mmol) was dissolved in 5 mL of EtOH and 3 mL of 2 N NaOH and heated at 70 °C for 2 h. The mixture was poured into 1 N HCl and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the crude product was recrystallized to give St20 (27) (95%). 3,4-Diethylacetophenone: colorless oil; bp 110–112.5 °C (2.5 mmHg); ^1H NMR (60 MHz, $CDCl_3$) δ 1.22 (t, 6 H, J = 7 Hz), 2.51 (s, 3 H), 2.68 (q, 4 H, J = 7 Hz), 7.17 (d, 1 H, J = 7 Hz), 7.65 (dd, 1 H, J = 2, 7 Hz), 7.74 (d, 1 H, J = 2 Hz). 1-(3,4-Diethylphenyl)ethanol: colorless oil; bp 114–115 °C (2.5 mmHg); ^1H NMR (60 MHz, $CDCl_3$) δ 1.21 (t, 6 H, J = 7 Hz), 1.43 (d, 3 H, J = 7 Hz), 2.25 (s, 1 H), 2.64 (q, 4 H, J = 7 Hz), 4.76 (q, 1 H, J = 7 Hz), 7.10 (s, 3 H). 4-(1-Bromoethyl)-1,2-diethylbenzene: ^1H NMR (60 MHz, $CDCl_3$) δ 1.40 (t, 6 H, J = 7 Hz), 2.21 (d, 3 H, J = 7 Hz), 2.87 (q, 4 H, J = 7 Hz), 5.35 (q, 1 H, J = 7 Hz), 7.32 (br s, 3 H). [1-(3,4-Diethylphenyl)ethyl]triphenylphosphonium bromide: ^1H NMR (60 MHz, $CDCl_3$) δ 1.04 (t, 3 H, J = 7 Hz), 1.25 (t, 3 H, J = 7 Hz), 1.88 (dd, 3 H, J = 7, 20 Hz), 2.55 (q, 4 H, J = 7 Hz), 5.8–6.7 (m, 1 H), 6.88 (br s, 1 H), 7.05 (s, 2 H), 7.6–8.3 (m, 15 H). St21: colorless prisms (from CH_2Cl_2–*n*-hexane); mp 50–50.5 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.27 (t, 6 H, J = 8 Hz), 2.30 (s, 3 H), 2.71 (q, 4 H, J = 8 Hz), 3.93 (s, 3 H), 6.81 (s, 1 H), 7.1–7.6 (m, 3 H), 7.41 (d, 2 H, J = 9 Hz), 8.03 (d, 2 H, J = 9 Hz); UV λ_{max} (nm) (log ϵ) 303 (4.32), 231 (4.11), 204 (4.36); IR (KBr) 1705 cm^{-1}. Anal. ($C_{21}H_{24}O_2$) C, H. St20 (27): colorless needles (from AcOEt); mp 193–194.5 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 1.26

(31) Nuttig, W. H.; Jewell, R. A.; Rapoport, H. *J. Org. Chem.* **1970**, *35*, 505.

Table VI. Chemical and Physical Properties of Stilbene-4-carboxylic Acids and Their Derivatives

compd	mp, °C	method	crystal form	recrystn solvent	formula
St00 (25)	257–258	B	colorless prisms	AcOEt–*n*-hexane	$C_{15}H_{12}O_2 \cdot {}^1/_3H_2O$
St10 (26)	213–215	A	colorless prisms	AcOEt	$C_{18}H_{18}O_2$
St20 (27)	193–194.5	A	colorless needles	AcOEt	$C_{20}H_{22}O_2$
St30 (28)	236.5–237	A	colorless prisms	AcOEt–*n*-hexane	$C_{22}H_{26}O_2$
St40 (29)	>300	B	colorless needles	AcOEt–*n*-hexane	$C_{19}H_{20}O_2$
St50 (30)	213.5–215	B	colorless needles	AcOEt–*n*-hexane	$C_{19}H_{20}O_2$
St60 (31)	243–244.5	A	colorless prisms	AcOEt	$C_{20}H_{22}O_2$
St80 (32)	274–276	B	colorless needles	*n*-hexane	$C_{23}H_{26}O_2$
St87 (34)	238–239		colorless flakes	AcOEt–*n*-hexane	$C_{23}H_{28}O_2$
St100 (33)	165–167	C	colorless prisms	AcOEt–*n*-hexane	$C_{24}H_{25}F_3O_2$
Ep20 (58)	146–148		colorless prisms	AcOEt–*n*-hexane	$C_{20}H_{22}O_3 \cdot {}^1/_6H_2O$
Ep40 (59)	199–200.5		colorless needles	AcOEt–*n*-hexane	$C_{19}H_{20}O_3 \cdot {}^1/_6H_2O$
Ep50 (60)	207–207.5		colorless plates	AcOEt–*n*-hexane	$C_{19}H_{20}O_3$
Ep80 (61)	215–216		colorless prisms	AcOEt–*n*-hexane	$C_{23}H_{26}O_3$
Ep90 (62)	202.5–203.5		colorless prisms	AcOEt–*n*-hexane	$C_{24}H_{28}O_3$
Ox80 (63)	207.5–209		colorless prisms	AcOEt–*n*-hexane	$C_{23}H_{28}O_4$
Ox85 (64)	205.5–206.5		colorless prisms	AcOEt–*n*-hexane	$C_{23}H_{28}O_4$
Ox90 (65)	115–117		colorless prisms	AcOEt–*n*-hexane	$C_{24}H_{30}O_4$

(t, 3 H, J = 7 Hz), 1.28 (t, 3 H, J = 7 Hz), 2.31 (s, 3 H), 2.4–2.8 (q, 4 H, J = 7 Hz), 6.83 (s, 1 H), 7.0–7.5 (m, 5 H), 8.04 (d, 2 H, J = 8 Hz); IR (KBr) 1670 cm^{-1}. Anal. ($C_{20}H_{22}O_2$) C, H.

(*E*)-4-[2-(3-*tert*-Butylphenyl)ethenyl]benzoic Acid (St40 (29)) (Method B). A mixture of *m*-*tert*-butyltoluene (400 mg, 2.70 mmol), *N*-bromosuccinimide (540 mg, 3.03 mmol), and azobisisobutyronitrile (50 mg) was dissolved in 10 mL of CCl_4 and refluxed for 2 h. After the filtration, the solvent was removed to give 3-*tert*-butylbenzyl bromide (98%). A mixture of 3-*tert*-butylbenzyl bromide (640 mg, 2.82 mmol) and triphenylphosphine (660 mg, 2.52 mmol) in 7 mL of benzene was refluxed for 5 h, and the precipitates were collected to give (3-*tert*-butylphenyl)triphenylphosphonium bromide (68.2%). This phosphonium salt (634 mg, 1.29 mmol) and terephthalaldehydic acid methyl ester (216 mg, 1.32 mmol) were dissolved in 15 mL of dry CH_3OH, and $NaOCH_3$ (80 mg, 1.48 mmol) was added to this solution. After stirring overnight, the mixture was purified by silica gel column chromatography to give methyl 4-[2-(3-*tert*-butylphenyl)-ethenyl]benzoate (85.7%; E/Z ratio, 7:5). The E isomer (St41; 50 mg, 0.16 mmol) was hydrolyzed by the method described in the section on St20 (27) to give St40 (29) (98.7%). St41: colorless needles (from CH_2Cl_2–*n*-hexane); mp 109.5–110.5 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.36 (s, 9 H), 3.92 (s, 3 H), 7.1–7.2 (m, 2 H), 7.33 (br s, 3 H), 7.5–7.6 (m, 3 H), 8.02 (d, 2 H, J = 8 Hz). Anal. ($C_{20}H_{22}O_2$) C, H. St40 (29): colorless needles (from AcOEt–*n*-hexane); mp 213.5–215 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) 1.37 (s, 9 H), 7.1–7.2 (m, 2 H), 7.2–7.6 (m, 6 H), 8.00 (d, 2 H, J = 8 Hz); IR (KBr) 1670 cm^{-1}. Anal. ($C_{19}H_{20}O_2$) C, H.

(*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-3,3,3-trifluoro-1-propenyl]benzoic Acid (St100 (33)) (Method C). A solution of *sec*-BuLi (5.16 mL of 1.45 M solution of cyclohexane; 7.48 mmol) was added portionwise to a solution of 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (1 g, 3.74 mmol) in 10 mL of anhydrous ether at 0 °C under Ar gas and the mixture was stirred for 30 min at room temperature. Then the mixture was cooled to 0 °C again and trifluoroacetic acid (0.29 mL, 3.79 mmol) in 3 mL of anhydrous ether was added slowly. The mixture was refluxed for 2 h and then poured into dilute HCl and ice and extracted with CH_2Cl_2. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After the removal of the solvent, the residue was chromatographed on silica gel and then distilled to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl trifluoromethyl ketone (25%). Wittig reaction of this trifluoroacetophenone was performed as follows.[32,33] A mixture of 18-crown-6 (147 mg, 0.41 mmol) and anhydrous KF (4.73 g, 81.41 mmol) was suspended in 30 mL of dry CH_3CN and stirred for 20 min at room temperature under Ar gas. To this mixture, a suspension of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl trifluoromethyl ketone (1.16 g, 4.08 mmol) and (4-carbomethoxybenzyl)triphenylphosphonium bromide (2 g, 4.07 mmol) in 20 mL of dry CH_3CN was added at 70–80 °C and the whole was heated for 2 h. After filtration and concentration, the mixture was chromatographed on silica gel to give methyl 4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-3,3,3-trifluoro-1-propenyl]benzoate (89.7%; E/Z ratio 7:1). The Z isomer (St101) was hydrolyzed by the method described in the section on St20 (27) to give St100 (33). 5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthyl trifluoromethyl ketone: colorless oil; bp 105–106 °C (3 mmHg); ^1H NMR (60 MHz, $CDCl_3$) δ 1.32 (s, 12 H), 1.72 (s, 4 H), 7.36 (d, 1 H, J = 9 Hz), 7.72 (br d, 1 H, J = 9 Hz), 7.96 (br s, 1 H); IR (KBr) 1715 cm^{-1}; MS M^+ 284. St101: colorless prisms (from CH_2Cl_2–*n*-hexane); mp 140–141.5 °C; ^1H NMR (60 MHz, $CDCl_3$) δ 1.32 (s, 12 H), 1.71 (s, 4 H), 3.77 (s, 3 H), 7.0–7.5 (m, 6 H), 7.96 (d, 2 H, J = 9 Hz); IR (KBr) 1720 cm^{-1}; HRMS ($C_{25}H_{27}F_3O_2$) 416.1966. St100 (33): colorless prisms (from AcOEt–*n*-Hexane); mp 165–167 °C; MS M^+ 402.

Other stilbene derivatives were prepared by the method described above (methods A–C), and their chemical and physical properties are listed in Table VI.

4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethyl]benzoic Acid (St87 (34)). Methyl (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-ethenyl]benzoate (St81) was hydrogenated (10% Pd-C in EtOH) and then hydrolyzed in the usual way to give St87 (34). St87 (34): colorless flakes (from AcOEt–*n*-hexane); mp 238–239 °C; ^1H NMR (100 MHz, $CDCl_3$–DMSO-d_6) δ 1.23 (s, 6 H), 1.27 (s, 6 H), 1.67 (s, 4 H), 2.92 (br s, 4 H), 6.95 (dd, 1 H, J = 2, 8 Hz), 7.02 (d, 1 H, J = 2 Hz), 7.21 (d, 1 H, J = 8 Hz), 7.24 (d, 2 H, J = 8 Hz), 7.93 (d, 2 H, J = 8 Hz); IR (KBr) 1685 cm^{-1}. Anal. ($C_{23}H_{28}O_2$) C, H.

4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-1*H*-benz[*f*]-inden-2-yl)benzoic Acid (In80 (35)). A solution of *n*-BuLi (9.2 mL of 1.5 M solution in *n*-hexane; 13.8 mmol) was added to a solution of diisopropylamine (1.52 g, 15.0 mmol) in 20 mL of THF at −78 °C under Ar gas, and the mixture was stirred for 20 min at 0 °C. A solution of 4-carbomethoxyphenylacetic acid (1.16 g, 6.0 mmol) in 25 mL of THF and 2 mL of HMPA was added at −78 °C, and the reaction mixture was stirred for 1.5 h. Then, a solution of 6-(bromomethyl)-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (39; 1.66 g, 6.6 mmol) in 10 mL of THF was added and the mixture stirred at −78 °C overnight. The mixture was poured into 30 mL of saturated NH_4Cl (aqueous) and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the mixture was chromatographed on silica gel and recrystallized from CH_2Cl_2–*n*-hexane to give 2-(4-carbomethoxyphenyl)-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propionic acid (40; 76.8%; mp 157.5–158.5 °C). 40 (1.48 g, 1.22 mmol) was dissolved in 15 mL of $SOCl_2$ containing one drop of DMF at 0 °C. After stirring of the mixture for 2 h, $SOCl_2$ was removed under vacuum and the residue was dissolved in 30 mL of dry $ClCH_2CH_2Cl$. This solution was added to a suspension of $AlCl_3$ (1.6 g, 12.0 mmol) in 150 mL of dry $ClCH_2CH_2Cl$ under Ar gas. The mixture was stirred for 15 min, poured into water, and extracted with CH_2Cl_2.

(32) Kossmehl, G.; Nuck, R. *Chem. Ber.* **1979**, *112*, 2342.
(33) Ruban, G.; Zobel, D.; Kossmehl, G.; Nuck, R. *Chem. Ber.* **1980**, 113, 3384.

The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the mixture was chromatographed on silica gel and recrystallized from AcOEt–*n*-hexane to give methyl 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-1-oxobenz[*f*]-indan-2-yl)benzoate (41; 70.5%; mp 122 °C). $NaBH_4$ (30.2 mg) was added to a solution of 41 (300 mg, 0.80 mmol) in 50 mL of EtOH at 0 °C and the mixture was stirred for 2 h, poured into dilute HCl, and extracted with ether. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the crude methyl 4-(5,6,7,8-tetrahydro-1-hydroxy-5,5,8,8-tetramethylbenz[*f*]indan-2-yl)benzoate (42) was obtained as colorless crystals (90.6%). Methanesulfonyl chloride (300 mg, 2.62 mmol) was added to a solution of 42 (210 mg, 0.56 mmol) in 10 mL of pyridine at 0 °C and the mixture was stirred for 1 h. H_2O was added dropwise and then the mixture was poured into water and extracted with AcOEt. The organic layer was washed successively with 1 N HCl, H_2O, and brine and dried over $MgSO_4$. After evaporation, the crude product was recrystallized to give methyl 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-1*H*-benz[*f*]inden-2-yl)benzoate (In81; 42.8%), which was hydrolyzed by the usual method (KOH (aq)/CH_3OH/60 °C) to give In80 (35) (74.4%). In81: colorless prisms (from CH_2Cl_2–*n*-hexane); mp 214 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.32 (s, 12 H), 1.70 (s, 4 H), 3.76 (s, 2 H), 3.90 (s, 3 H), 7.28 (br s, 1 H), 7.38 (s, 1 H), 7.42 (s, 1 H), 7.62 (d, 2 H, J = 8 Hz), 8.00 (d, 2 H, J = 8 Hz); IR (KBr) 1715 cm^{-1}. Anal. ($C_{25}H_{28}O_2$) C, H. In80 (35): colorless prisms (from AcOEt–*n*-hexane); mp >300 °C; ^1H NMR (100 MHz, acetone-d_6) δ 1.32 (s, 12 H), 1.72 (s, 4 H), 3.81 (s, 2 H), 7.44 (s, 1 H), 7.48 (br s, 2 H), 7.60 (d, 2 H, J = 7 Hz), 7.96 (d, 2 H, J = 7 Hz); IR (KBr) 1685 cm^{-1}. Anal. ($C_{24}H_{26}O_2 \cdot {}^1/_7H_2O$) C, H.

(*E*)-4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylbenz[*f*]-indan-1-ylidene)methyl]benzoic Acid (Ex80 (36)). A solution of *t*-BuLi (8.0 mL of 1.7 M solution in pentane; 13.6 mmol) was added to the solution of 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (43; 3.3 g, 12.3 mmol) in 50 mL of dry ether at −10 °C under Ar gas. The solution was stirred for 30 min at room temperature and refluxed for 30 min. Then 2.5 mL of DMF was added at 0 °C and the mixture was stirred for 30 min at room temperature. Saturated NH_4Cl (aqueous, 10 mL) was added and the whole was stirred for an additional 30 min and then diluted with H_2O and ether. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthaldehyde (44) (64.7%). A solution of *n*-BuLi (4.5 mL of 1.5 M solution in hexane; 6.75 mmol) was added to a solution of triethyl phosphonoacetate (1.5 g, 6.70 mmol) in 30 mL of absolute ether at 0 °C under Ar gas and the mixture was stirred for 30 min at room temperature. The solution of the aldehyde 44 (690 mg, 3.19 mmol) in 20 mL of absolute ether was added at 0 °C. The mixture was stirred for additional 5 h at room temperature, poured into water, and extracted with ether. The organic layer was washed with H_2O and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel to give ethyl (*E*)-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)acrylate (59.8%). The ester (546 mg, 1.91 mmol) was hydrogenated on 10% Pd–C (100 mg) for 30 min 90.9%) and then hydrolyzed (NaOH (aq)/EtOH; 88.6%) to give 3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propionic acid (45). Acid 45 (400 mg, 1.54 mmol) was dissolved 10 mL of $SOCl_2$ containing a drop of DMF and stirred for 1 h at 0 °C. After the removal of $SOCl_2$ under vacuum, the residue was dissolved in 10 mL of CH_2Cl_2. $AlCl_3$ (500 mg, 3.76 mmol) was added and the mixture was stirred for 2 h, poured into water, and extracted with CH_2Cl_2. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After evaporation, the crude product was purified by silica gel column chromatography to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethylbenz[*f*]indan-1-one (46; q.y). $NaBH_4$ (80 mg) was added to a solution of the ketone 46 (375 mg, 1.55 mmol) in 15 mL of CH_3OH at 0 °C and stirred for 30 min. The mixture was poured into dilute HCl and extracted with ether. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel and then recrystallized from CH_3OH to give 5,6,7,8-tetrahydro-5,5,8,8-tetramethylbenz[*f*]indan-1-ol (47; 59.5%). This product, 47 (225 mg, 0.92 mmol), was dissolved in 6 mL of ether and 4 mL of *n*-hexane, and 0.8 mL of PBr_3 was added slowly at 0 °C. After stirring for 1 h, the mixture was poured onto ice and extracted with ether. The organic layer was washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. The solvent was evaporated to leave the crude bromide 48 (95.8%). A mixture of crude 48 and triphenylphosphine (300 mg, 1.15 mmol) in 5 mL of benzene was stirred overnight at room temperature. After evaporation, the residue was chromatographed on silica gel to give (5,6,7,8-tetrahydro-5,5,8,8-tetramethylbenz-[*f*]indan-1-yl)triphenylphosphonium bromide (49; 38.9%). A mixture of 49 (204 mg, 0.36 mmol) and terephthalaldehydic acid methyl ester (60 mg, 0.37 mmol) was dissolved in 6 mL of 1,2-butylene oxide and was refluxed under Ar gas from 5 h. After removal of the solvent, the crude mixture was purified by silica gel column chromatography to give methyl 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylbenz[*f*]indan-1-ylidene)benzoate (74.6%, E/Z ratio, 6:1). The *E* isomer (Ex81) was hydrolyzed in the usual way to give Ex80 (36). Ex80 (36): colorless prisms (from AcOEt–*n*-hexane); mp 267–269 °C; ^1H NMR (400 MHz, CD_3OD) δ 1.29 (s, 6 H), 1.34 (s, 6 H), 1.72 (s, 4 H), 3.0–3.15 (m, 4 H), 6.99 (s, 1 H), 7.28 (s, 1 H), 7.57 (s, 1 H), 7.62 (d, 2 H, J = 8.5 Hz), 7.99 (d, 2 H, J = 8.5 Hz). Anal. ($C_{25}H_{28}O_2$) C, H.

4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphtho[2,3-*b*]-furan-2-yl)benzoic Acid (Bf80 (37)).[34] Potassium *tert*-butoxide (90% purity; 609 mg, 4.88 mmol) was added to a solution of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthol (50; 996 mg, 4.88 mmol) in 8 mL of CH_3OH. After the removal of the solvent, the residue was dissolved in 6 mL of DMF and a solution of (mesitylenesulfonyl)hydroxylamine[34] (MSH, 70% purity; 1.24 g, 4.05 mmol) in 4 mL of DMF was added at 0 °C. After stirring for 30 min, the mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. The residue was chromatographed on silica gel to give *O*-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)hydroxylamine (51; 57%) and 50 (24%). To a solution of 51 (127 mg, 0.58 mmol) in 1 mL of THF were added DCC (166 mg, 0.81 mmol) and then (4-carbomethoxybenzoyl)acetic acid (156 mg, 0.70 mmol), and the mixture was stirred for 15 min. After the addition of two drops of AcOH, the mixture was poured into 50 mL of AcOEt. The organic layer was filtered and the filtrate was washed with 1 N $NaHCO_3$ and brine and dried over $MgSO_4$. After the removal of the solvent at room temperature, crude methyl 4-[*N*-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)oxy]-malonamoyl]benzoate (52) was obtained. Crude 52 was dissolved in 4.5 mL of trifluoroacetic acid and the solution was stirred for 16 h, poured into water, and extracted with CH_2Cl_2. The organic layer was washed with 1 N $NaHCO_3$ and dried over $MgSO_4$. After evaporation, the mixture was chromatographed on silica gel to give methyl 4-(3-carbamoyl-5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphtho[2,3-*b*]furan-2-yl)benzoate (53; 36%) and its isomer, methyl 4-(3-carbamoyl-6,7,8,9-tetrahydro-6,6,9,9-tetramethylnaphtho[2,1-*b*]furan-2-yl)benzoate (54; 12%). The former product 53 (178 mg, 0.44 mmol) was dissolved in 20 mL of CH_3OH saturated with HCl gas and the solution was refluxed for 41 h, diluted with CH_2Cl_2, washed with H_2O and 1 N $NaHCO_3$, and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel to give the diester 55 (75%), which was hydrolyzed by the usual method (NaOH (aq)/CH_3OH) to give the diacid 56 (47%). Copper powder (15.6 mg) was added to a solution of 56 (66 mg, 0.17 mmol) in 1 mL of quinoline. The mixture was heated at 200–210 °C for 1 h, poured into 50 mL of concentrated HCl with ice, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. The residue was dissolved in 10 mL of CH_3OH-saturated HCl gas and the solution was stirred for 20 h, poured into H_2O, and extracted with CH_2Cl_2. The organic layer was washed with 1 N $NaHCO_3$ and dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel to give methyl 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethylnaphtho[2,3-*b*]-furan-2-yl)benzoate (Bf81; 30%), which was hydrolyzed by the usual method (NaOH (aq)/CH_3OH) to give Bf80 (37) (76%). 53: colorless needles (from benzene); mp 223–225 °C; ^1H NMR (100 MHz, $CDCl_3$) δ 1.36 (s, 6 H), 1.37 (s, 6 H), 1.76 (s, 4 H), 3.95 (s,

(34) Endo, Y.; Namikawa, K.; Shudo, K. *Tetrahedron Lett.* **1986**, *27*, 4209.

3 H), 5.7–5.9 (br s, 2 H), 7.50 (s, 1 H), 7.80 (s, 1 H), 8.04 (d, 2 H, J = 8 Hz), 8.14 (d, 2 H, J = 8 Hz). Anal. ($C_{25}H_{27}NO_4 \cdot {}^1/_3C_6H_6$) C, H, N. **54**: colorless flakes (from CH_2Cl_2–*n*-hexane); mp 266–267 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.34 (s, 6 H), 1.56 (s, 6 H), 1.74 (s, 4 H), 3.95 (s, 3 H), 5.7–5.9 (br s, 2 H), 7.39 (s, 2 H), 7.96 (d, 2 H, J = 8 Hz), 8.12 (d, 2 H, J = 8 Hz). Anal. ($C_{25}H_{27}NO_4$) C, H, N. **55**: colorless plates (from CH_2Cl_2–*n*-hexane); mp 168–169.5 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.36 (s, 6 H), 1.39 (s, 6 H), 1.76 (s, 4 H), 3.95 (s, 3 H), 3.96 (s, 3 H), 7.50 (s, 1 H), 8.00 (s, 1 H), 8.06 (d, 2 H, J = 8 Hz), 8.15 (d, 2 H, J = 8 Hz). Anal. ($C_{26}H_{28}O_5 \cdot {}^1/_6H_2O$) C, H. **56**: colorless needles (from EtOH–*n*-hexane); mp 292–293 °C; ^{1}H NMR (100 MHz, CD_3OD) δ 1.37 (s, 12 H), 1.78 (s, 4 H), 7.54 (s, 1 H), 8.05 (s, 1 H), 8.13 (s, 4 H). Anal. ($C_{24}H_{24}O_5 \cdot {}^1/_3H_2O$) C, H. Bf81: colorless needles (from CH_2Cl_2–*n*-hexane); mp 187–188.5 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.36 (s, 12 H), 1.75 (s, 4 H), 3.94 (s, 3 H), 7.06 (s, 1 H), 7.48 (s, 1 H), 7.54 (s, 1 H), 7.88 (d, 2 H, J = 8 Hz), 8.10 (d, 2 H, J = 8 Hz). Anal. ($C_{24}H_{26}O_3$) C, H. Bf80 (**37**): colorless flakes (from $CHCl_3$); mp >300 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.36 (s, 12 H), 1.75 (s, 4 H), 7.09 (s, 1 H), 7.50 (s, 1 H), 7.55 (s, 1 H), 7.92 (d, 2 H, J = 8 Hz), 8.17 (d, 2 H, J = 8 Hz). Anal. ($C_{23}H_{24}O_3$) C, H.

Epoxidation of Stilbene Derivatives (General Procedure for Ep Series). Alkyl-substituted (*E*)-stilbene-4-carboxylic acid methyl ester (St series; 1 mmol) was dissolved in 10 mL of $CHCl_3$. *m*-Chloroperbenzoic acid (180 mg, 1.04 mmol) was added and the mixture was refluxed for 2 h, then cooled, and filtered. The filtrate was diluted with CH_2Cl_2 and washed successively with 1 N $NaHCO_3$, H_2O, and brine and dried over $MgSO_4$. After evaporation, the crude product was chromatographed on silica gel or recrystallized to give methyl 4-(3-aryloxiranyl)benzoate, which was hydrolyzed as usual (NaOH (aq)/EtOH/room temperature) to give 4-(3-aryloxiranyl)benzoic acid. The chemical and physical properties of the Ep series are listed in Table VI.

***threo*-4-[1,2-Dihydroxy-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethyl]benzoic Acid (Ox80 (63)).** Methyl (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethenyl]benzoate (methyl ester of St80 (**32**), prepared by the method B; 290 mg, 0.83 mmol) was added slowly to a solution of OsO_4 (215 mg, 0.85 mmol) in 6 mL of dry pyridine and the mixture was stirred for 1 h. Then a solution of $NaHSO_3$ (600 mg, 6 mmol) in 4 mL of H_2O and 2 mL of pyridine was added. The mixture was stirred for 30 min and diluted with CH_2Cl_2 and brine. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum, and then the residue was chromatographed on silica gel to give methyl *threo*-4-[1,2-dihydroxy-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethyl]benzoate (Ox81, 94.2%). Next, 1.5 mL of 2 N NaOH was added to a solution of Ox81 (46 mg, 0.12 mmol) in 5 mL of CH_3OH. The mixture was stirred overnight, poured into dilute HCl and extracted with AcOEt. The organic layer was washed with H_2O and brine and dried over $MgSO_4$. The crude product was recrystallized to give Ox80 (**63**) (88%). Ox81: colorless needles (from CH_2Cl_2–*n*-hexane); mp 115.5–117 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.98 (s, 3 H), 1.17 (s, 3 H), 1.23 (s, 3 H), 1.25 (s, 3 H), 1.62 (s, 4 H), 2.58 (d, 1 H, J = 2.5 Hz), 3.00 (d, 1 H, J = 2.5 Hz), 3.89 (s, 3 H), 4.61 (dd, 1 H, J = 2.5, 7 Hz), 4.75 (dd, 1 H, J = 2.5, 7 Hz), 6.84 (d, 1 H, J = 2 Hz), 6.99 (dd, 1 H, J = 2, 8 Hz), 7.15 (d, 2 H, J = 8 Hz), 7.20 (d, 1 H, J = 8 Hz), 7.86 (d, 2 H, J = 8 Hz). Anal. ($C_{24}H_{30}O_4$) C, H. Ox80 (**63**): colorless prisms (from AcOEt–*n*-hexane); mp 207.5–209 °C; ^{1}H NMR (100 MHz, CD_3OD) δ 0.93 (s, 3 H), 1.13 (s, 3 H), 1.20 (d, 3 H), 1.21 (s, 3 H), 1.60 (s, 4 H), 4.56 (d, 1 H, J = 7.5 Hz), 4.66 (d, 1 H, J = 7.5 Hz), 6.76 (d, 1 H, J = 2 Hz), 6.96 (dd, 1 H, J = 2, 8 Hz), 7.12 (d, 2 H, J = 8 Hz), 7.16 (d, 1 H, J = 8 Hz), 7.79 (d, 2 H, J = 8 Hz). Anal. ($C_{23}H_{28}O_4$) C, H.

Other diol derivatives were also prepared according to this method and their chemical and physical properties are listed in Table VI.

4-[2,2,5-Trimethyl-5-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1,3-dioxolan-4-yl]benzoic Acid (Ox190 (68)). To a solution of methyl *threo*-4-[1,2-dihydroxy-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-propyl]benzoate (methyl ester of Ox90, prepared by the method described in the section of Ox80 (**63**); 40 mg, 0.10 mmol) in 2 mL of DMF were added *p*-TsOH (20 mg) and 2,2-dimethoxypropane (10 mg, 1.06 mmol), and the mixture was stirred for 3 h at 45 °C. After the removal of the solvent under vacuum, the residue was chromatographed on silica gel to give methyl 4-[2,2,5-trimethyl-5-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1,3-dioxolan-4-yl]benzoate (Ox191; q.y), which was hydrolyzed as usual (NaOH (aq)/CH_3OH) to give Ox190 (**68**) (95%). Ox190 (**68**): colorless prisms (from CH_2Cl_2–*n*-hexane); mp 206–207 °C; ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.19 (s, 3 H), 1.24 (s, 3 H), 1.31 (s, 12 H), 1.63 (s, 3 H), 1.72 (s, 4 H), 3.49 (s, 1 H), 5.00 (s, 1 H), 7.1–7.5 (m, 3 H), 7.37 (d, 2 H, J = 8 Hz), 8.05 (d, 2 H, J = 8 Hz). Anal. ($C_{27}H_{34}O_4$) C, H.

4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)glyoxyloyl]benzoic Acid (Ox580 (69)). A mixture of methyl (*E*)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethenyl]benzoate (methyl ester of St80, prepared by the method B; 207 mg, 0.59 mmol) and SeO_2 (180 mg, 1.64 mmol) was heated at 240 °C for 5 h. The mixture was chromatographed on silica gel to give methyl 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)glyoxyloyl]benzoate (Ox581, 22.2%), which was hydrolyzed as usual (NaOH (aq)/CH_3OH) to give Ox580 (**69**) (q.y). Ox580 (**69**): yellow needles (from AcOEt–*n*-hexane); mp 165.5–167.5 °C; ^{1}H NMR (100 MHz, CD_3OD) δ 1.29 (s, 6 H), 1.32 (s, 6 H), 1.76 (s, 6 H), 7.52 (d, 1 H, J = 8 Hz), 7.64 (dd, 1 H, J = 2, 8 Hz), 7.94 (d, 1 H, J = 2 Hz), 8.01 (d, 2 H, J = 8 Hz), 8.18 (d, 2 H, J = 8 Hz). Anal. ($C_{23}H_{24}O_4$) C, H.

4-[(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)azoxy]benzoic Acid (Az90 (71)). A mixture of methyl 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)azo]benzoate (Az81 (**70**); 222 mg, 0.64 mmol) and *m*-chloroperbenzoic acid (70% purity; 190 mg, 0.77 mmol) was dissolved in 10 mL of $CHCl_3$ and refluxed for 2 h. After concentration, the mixture was chromatographed on silica gel to give methyl 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)azoxy]benzoate (Az91), which was hydrolyzed as usual to give Az90 (**71**). Both Az90 (**71**) and Az91 are mixtures of two regioisomers as to the position of the oxygen on the azo group. Az91: pale yellow needles (from CH_2Cl_2–*n*-hexane); mp 114–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32, 1.33, 1.35, 1.36 (4 s, 12 H), 1.73, 1.74 (2 s, 4 H), 3.95 (s, 1.5 H), 3.97 (s, 1.5 H), 7.43 (dd-like, 1 H), 8.02 (dd, 0.5 H, J = 2, 8.5 Hz), 8.1–8.2 (m, 3 H), 8.18 (d, 1 H, J = 9 Hz), 8.23 (d, 0.5 H, J = 2.5 Hz), 8.37 (d, 1 H, J = 9 Hz). Anal. ($C_{22}H_{25}N_2O_3$) C, H, N. Az90 (**71**): pale yellow needles (from AcOEt); mp 261–262 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 1.32, 1.34, 1.36 (3 s, 12 H), 1.75, 1.77 (2 s, 4 H), 7.47 (d, 0.5 H, J = 8.5 Hz), 7.54 (d, 0.5 H, J = 8.5 Hz), 8.0–8.25 (m, 4.5 H), 8.26 (d, 0.5 H, J = 2 Hz), 8.38 (d, 1 H, J = 9 Hz). Anal. ($C_{21}H_{23}N_2O_3$) C, H, N.

Registry No. **3** (methyl ester), 102389-53-7; **8**, 37790-20-8; **8** (methyl ester), 119479-29-7; **9**, 119435-69-7; **9** (methyl ester), 119436-22-5; **10**, 119435-70-0; **10** (methyl ester), 119436-23-6; **11**, 119435-71-1; **11** (methyl ester), 119436-24-7; **12**, 119435-72-2; **12** (methyl ester), 119454-83-0; **13**, 119435-73-3; **13** (methyl ester), 119436-25-8; **14**, 119435-74-4; **14** (methyl ester), 119436-26-9; **15**, 119435-75-5; **15** (methyl ester), 119436-27-0; **16**, 119435-76-6; **16** (methyl ester), 119436-28-1; **17**, 119435-77-7; **17** (methyl ester), 119436-29-2; **18**, 119435-78-8; **18** (methyl ester), 119436-30-5; **19**, 119435-79-9; **19** (methyl ester), 119454-84-1; **20**, 119435-80-2; **20** (methyl ester), 119436-31-6; **21**, 119435-81-3; **21** (methyl ester), 119436-32-7; **22**, 119435-82-4; **22** (methyl ester), 119436-15-6; **23**, 119435-83-5; **23** (methyl ester), 119436-33-8; **24**, 119435-84-6; **24** (methyl ester), 119436-34-9; **25**, 13041-75-3; **25** (methyl ester), 1149-18-4; (*Z*)-**25** (methyl ester), 46925-32-0; **26**, 102405-27-6; **26** (methyl ester), 102405-26-5; (*Z*)-**26** (methyl ester), 119436-42-9; **27**, 102405-39-0; **27** (methyl ester), 102405-38-9; (*Z*)-**27** (methyl ester), 119436-20-3; **28**, 102405-28-7; **28** (methyl ester), 119436-46-3; (*Z*)-**28** (methyl ester), 119436-47-4; **29**, 102405-34-5; **29** (methyl ester), 102405-33-4; (*Z*)-**29** (methyl ester), 119436-36-1; **30**, 102405-29-8; **30** (methyl ester), 102405-30-1; (*Z*)-**30** (methyl ester), 119436-48-5; **31**, 102405-31-2; **31** (methyl ester), 102405-42-5; (*Z*)-**31** (methyl ester), 119436-51-0; **32**, 119454-82-9; **32** (methyl ester), 102121-54-0; (*Z*)-**32** (methyl ester), 119436-53-2; **33**, 119435-85-7; **33** (methyl ester), 119436-38-3; (*E*)-**33** (methyl ester), 119436-39-4; **34**, 119435-86-8; **34** (methyl ester), 119436-54-3; **35**, 119435-87-9; **36**, 119435-88-0; **36** (methyl ester), 119436-60-1; (*Z*)-**36** (methyl

ester), 119436-61-2; **37**, 119435-89-1; **37** (methyl ester), 119436-63-4; **38**, 6683-48-3; **39**, 119435-90-4; **39** (X = $PPh_3^+Br^-$), 119436-52-1; (±)-**40**, 119435-91-5; (±)-**40** (acid chloride), 119436-55-4; **41**, 119435-92-6; **42**, 119435-93-7; **42** (X = H, OMs), 119436-56-5; **43**, 27452-17-1; **44**, 92654-79-0; **45**, 119435-94-8; **45** (ethyl ester), 119436-58-7; **45** (acid chloride), 119436-59-8; **46**, 102296-82-2; (±)-**47**, 119435-95-9; (±)-**48**, 119435-96-0; (±)-**49**, 119435-97-1; **50**, 22824-31-3; **51**, 119435-98-2; **52**, 119435-99-3; **53**, 119436-00-9; **54**, 119436-01-0; **55**, 119436-02-1; **56**, 119436-03-2; (±)-**58**, 119436-04-3; (±)-**58** (methyl ester), 119436-64-5; (±)-**59**, 119454-60-3; (±)-**59** (methyl ester), 119436-65-6; (±)-**60**, 119436-05-4; (±)-**60** (methyl ester), 119436-66-7; (±)-**61**, 119436-06-5; (±)-**61** (methyl ester), 119436-67-8; (±)-**62**, 119436-07-6; (±)-**62** (methyl ester), 119436-68-9; (±)-**63**, 119436-08-7; (±)-**63** (methyl ester), 119436-69-0; (±)-**64**, 119436-09-8; (±)-**64** (methyl ester), 119436-70-3; (±)-**65**, 119436-10-1; (±)-**65** (methyl ester), 119436-71-4; (±)-**66**, 119436-11-2; (±)-**66** (methyl ester), 119436-72-5; (±)-**67**, 119436-12-3; (±)-**67** (methyl ester), 119436-73-6; (±)-**68**, 119436-13-4; (±)-**68** (methyl ester), 119436-74-7; (±)-**69**, 119436-14-5; (±)-**69** (methyl ester), 119436-75-8; **70**, 119436-15-6; **71** (regioisomer 1), 119436-16-7; **71** (regioisomer 2), 119436-76-9; **71** (regioisomer 1, methyl ester), 119436-77-0; **71** (regioisomer 2, methyl ester), 119436-78-1; $PhNH_2$, 62-53-3; 3-$MeC_6H_4NH_2$, 108-44-1; 3,4-$Et_2C_6H_3NH_2$, 54675-14-8; 3-$EtC_6H_4NH_2$, 587-02-0; 4-*i*-$PrC_6H_4NH_2$, 99-88-7; 3-*i*-$PrC_6H_4NH_2$, 5369-16-4; 2-*i*-$PrC_6H_4NH_2$, 643-28-7; 3-*t*-$BuC_6H_4NH_2$, 5369-19-7; 2,5-(*i*-$Pr)_2C_6H_3NH_2$, 91552-65-7; 2,4-(*i*-$Pr)_2C_6H_3NH_2$, 79069-41-3; 3,5-(*i*-$Pr)_2C_6H_3NH_2$, 7544-57-2; 3,4-(*i*-$Pr)_2C_6H_3NH_2$, 116233-13-7; 3-$PhC_6H_4NH_2$, 2243-47-2; 3-*c*-$C_6H_{11}C_6H_4NH_2$, 5369-21-1; 4-ONC_6H_4COOMe, 13170-28-0; *o*-$C_6H_4Et_2$, 135-01-3; 3,4-$Et_2C_6H_3COCH_3$, 102405-35-6; (±)-3,4-$Et_2C_6H_3CH(OH)CH_3$, 119436-17-8; (±)-3,4-$Et_2C_6H_3CHBrCH_3$, 119436-18-9; (±)-3,4-$Et_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-19-0; *p*-$OHCC_6H_4COOMe$, 1571-08-0; *m*-*t*-$BuC_6H_4Me$, 1075-38-3; *m*-*t*-$BuC_6H_4CH_2Br$, 102405-32-3; *m*-*t*-$BuC_6H_4CH_2PPh_3^+Br^-$, 119436-35-0; *p*-$MeOCOC_6H_4CH_2PPh_3^+Br^-$, 1253-46-9; *o*-$Me_2C_6H_4$, 95-47-6; 3,4-$Me_2C_6H_3COCH_3$, 3637-01-2; (±)-3,4-$Me_2C_6H_3CH(OH)CH_3$, 100646-15-9; (±)-3,4-$Me_2C_6H_3CHBrCH_3$, 119436-40-7; (±)-3,4-$Me_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-41-8; *o*-(*i*-$Pr)_2C_6H_4$, 577-55-9; 3,4-(*i*-$Pr)_2C_6H_3COCH_3$, 94291-81-3; (±)-3,4-(*i*-$Pr)_2C_6H_3CH(OH)CH_3$, 119436-43-0; (±)-3,4-(*i*-$Pr)_2C_6H_3CHBrCH_3$, 119436-44-1; (±)-3,4-(*i*-$Pr)_2C_6H_3CH(CH_3)PPh_3^+Br^-$, 119436-45-2; *p*-*t*-$BuC_6H_4Me$, 98-51-1; *p*-*t*-$BuC_6H_4CH_2Br$, 18880-00-7; *p*-*t*-$BuC_6H_4CH_2PPh_3^+Br^-$, 65413-33-4; *t*-BuPh, 98-06-6; *p*-*t*-$BuC_6H_4COCH_3$, 943-27-1; (±)-*p*-*t*-$BuC_6H_4CH(OH)CH_3$, 119479-30-0; (±)-*p*-*t*-$BuC_6H_4CHBrCH_3$, 119436-49-6; (±)-*p*-*t*-$BuC_6H_4CH(CH_3)PPh_3^+Br^-$, 119436-50-9; $PhCH_2Br$, 100-39-0; $PhCH_2PPh_3^+Br^-$, 1449-46-3; 4-$MeOCOC_6H_4CH_2CO_2H$, 22744-12-3; 4-$MeOCOC_6H_4COCO_2H$, 119436-62-3; 3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-amine, 109139-99-3; 3,4-dihydro-4,4-dimethyl-2*H*-1-benzothiopyran-6-amine, 119436-21-4; 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthylamine, 92050-16-3; 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl trifluoromethyl ketone, 119436-37-2; ethyl (*E*)-3-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)acrylate, 119436-57-6.

Peripherally Acting Enkephalin Analogues. 2.[1] Polar Tri- and Tetrapeptides[2]

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The design, synthesis, and biological activity of a series of D-Arg[2]-enkephalin-derived tetrapeptide amides and tripeptide aralkylamides are reported. These polar analogues were designed to be excluded from the central nervous system with their action thus limited to peripheral opioid receptors. The effects of the nature of the aromatic ring, aryl ring substitution, and aralkylamine chain length on activity were investigated; in a number of cases the N-terminal amino group of Tyr[1] was converted to a guanidino group to further increase hydrophilicity. The peptides were all synthesized by classical solution methodology. The opioid activity of the peptides was assessed in vitro on the guinea pig ileum and their antinociceptive activity was determined in vivo in chemically induced writhing models (peripheral activity) and in the hot-plate test (central activity), in rodents. That the analgesic effects were predominantly mediated in the periphery was demonstrated by antagonism of antinociception by the peripheral opioid antagonist *N*-methylnalorphine and by comparison of the activities in the writhing and hot-plate tests. As a class, the tetrapeptides were more potent than the tripeptides; N^{α}-amidination generally increased activity. A number of compounds exhibited very potent opioid activity and had the desired pharmacological profile, indicating a high degree of peripheral selectivity.

As part of a research program designed to investigate the potential of peripherally selective opioids as analgesic agents, we have examined the effect of the introduction of polar substituents on the activities of a variety of classes of opioids in order to restrict their passage across the blood–brain barrier.[1,3] Although the primary site of action of analgesic opioids is in the CNS, recent evidence suggests that there is a significant peripheral component to this activity;[4] inhibition of the cough reflex by opioids has also been shown to be peripherally mediated.[5] The serious side effects of respiratory depression, tolerance, and addictive liability associated with opiates, such as morphine, are mediated in the CNS.[6] It might be expected, therefore, that an effective peripherally acting opioid analgesic agent would be free from these undesirable side effects.

We have previously described the design and synthesis of a series of peripherally acting polar pentapeptide

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(1) Part 1: Hardy, G. W.; Lowe, L. A.; Pang, Y. S.; Simpkin, D. S. A.; Follenfant, R. L.; Smith, T. W. *J. Med. Chem.* **1988**, *31*, 960.
(2) Abbreviations used: acetic acid (AA), 1-amidino-3,5-dimethylpyrazole acetate (ADMP), central nervous system (CNS), dicyclohexylcarbodiimide (DCCI), dicyclohexylurea (DCU), dimethylformamide (DMF), guinea pig ileum (GPI), 1-hydroxybenzotriazole (HOBt), high-performance liquid chromatography (HPLC), 4A molecular sieve (MS4A), *N*-methylmorpholine (NMM), phenyl-*p*-benzoquinone (PBQ), structure–activity relationship(s) (SAR), tetrahydrofuran (THF), subcutaneous (sc), oral (po). All amino acids are of the L configuration unless otherwise noted.
(3) (a) Smith, T. W.; Buchan, P.; Parsons, D. N.; Wilkinson, S. *Life Sci.* **1982**, *31*, 1205. (b) Hardy, G. W.; Doyle, P. M.; Smith, T. W. *Eur. J. Med. Chem.* **1987** *22*, 331. (c) Doyle, P. M. In preparation.

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