mL of water and 100 mL of toluene–ethyl acetate (1:1 v/v). The aqueous layer was further extracted with two 50-mL portions of ethyl acetate. The combined organic layers were washed with two 50-mL portions of water and 50 mL of brine and dried over sodium sulfate. Removal of solvent at reduced pressure gave a deep amber oil, which was purified by flash chromatography on silica gel with 50% ethyl acetate in hexane to give 8.06 g of (*R*)-**2b** (77%) after exhaustive removal of solvent. ^{1}H and ^{13}C NMR were identical with previously isolated pure (*S*)-**2b**. HPLC on Chiracel OD using hexane–2-propanol (93:7) as eluant indicated a 93:7 *R*/*S* mixture of alcohols which showed that *no* racemization had taken place during deacylation.

Enzymatic Optical Enrichment of Methyl 7-(3(*R*)-Hydroxy-5-oxo-1-cyclopenten-1-yl)-4(*Z*)-heptenoate [(*R*)-2b]. Compound (*R*)-**2b** obtained above (7.5 g, 31.5 mmol) with 93:7 *R*/*S* ratio and 7.5 g of crude porcine pancreatic lipase in 180 mL of distilled vinyl acetate was stirred vigorously at room temperature for 45 h. HPLC of an aliquot on Chiralcel OD (using 93:7 hexane–2-propanol as solution system) showed excellent conversion of alcohol (*R*)-**2b** to the corresponding acetate (*R*)-**3b**. In fact, 98% of available (*R*)-alcohol had been consumed to give (*R*)-acetate with greater than 98.8% ee. The mixture was filtered through diatomaceous earth, and the filter cake washed with two 100-mL portions of methylene chloride. The combined filtrates were concentrated under reduced pressure to give 8.90 g of residue, which was purified by chromatography on silica with 20% ethyl acetate in hexane as eluant. By this technique, 7.53 g (85%) of (*R*)-**3b** was obtained in 98.8% ee, which was identical with the previously isolated (*R*)-acetate by ^{1}H and ^{13}C NMR, HPLC, and TLC.

Deacylation of Optically Enriched Methyl 7-[3(*R*)-(Acetyloxy)-5-oxo-1-cyclopenten-1-yl]-4(*Z*)-heptenoate [(*R*)-3b]. To a room-temperature solution of 7.47 g (26.6 mmol) of (*R*)-**3b** (98.8% of ee) in 25 mL of absolute methanol under argon was added dropwise via syringe 5.2 mL (2.6 mmol) of stock 0.5 M guanidine in methanol prepared above. The reaction was stirred at room temperature for 30 min. TLC on silica gel with 80% ethyl acetate in hexane showed complete conversion of acetate to free alcohol. The solvent was removed at reduced pressure, and the residue was partitioned between 150 mL of 1:1 toluene–ethyl acetate and 50 mL of water. The aqueous layer was further extracted with 50 mL of ethyl acetate. The combined organic layers were washed with two 25-mL portions of water and 25 mL of brine and dried over sodium sulfate to give 6.25 g of crude residue. This was purified by flash chromatography on silica gel with gradient elution of 50–75% ethyl acetate in hexane to give 4.89 g (77%) of (*R*)-**2b**. HPLC on Chiracel OD using 93:7 hexane–2-propanol as eluant indicated a 98.8% ee for the desired product. This product was identical to previously prepared (4*R*)-alcohol by HPLC, ^{1}H and ^{13}C NMR, and TLC.

Molecular Recognition in Macroporous Polymers Prepared by a Substrate Analogue Imprinting Strategy

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Polymers showing molecular recognition properties can be obtained by a technique called molecular imprinting.[1-5] In principle, imprinting of small molecules is carried out as follows.

(a) Functionalized monomers are bound, covalently or noncovalently, to a print molecule or template.

(b) The resulting print assembly is copolymerized with an excess of a cross-linking agent in an inert solvent to form a rigid polymer.

(c) The polymer is freed from print molecules, in most cases by hydrolysis or extraction.

(d) In binding experiments, the polymers thus formed are able to recognize selectively print molecules used in the polymerization step. The recognition observed has been ascribed to the formation of binding sites containing functional groups attached to the polymer network at defined positions.

Previously, we have developed an imprinting procedure for amino acid derivatives based on noncovalent interactions both in step a and in step d.[2] Following this procedure, polymers showing a high selectivity for the print molecule applied in the imprinting step could be prepared.[3,4] In some cases it may be of interest to use a substrate analogue as print molecule, in particular if the latter is expensive or difficult to synthesize. Here we wish to report on an imprinting procedure employing a print molecule with its configuration inverted, compared with that of the substrate interacting most strongly with the polymer in the binding assays. To our knowledge, this is the first example of the use of a substrate analogue as print molecule leading to a polymer showing inverse stereoselectivity.

Table I. Distributions and Enantioselectivities Found for Polymers A and B Prepared by Molecular Imprinting with Derivative 1 and the Print Assembly PVB:L-*p*-NH_2PheOEt (2:1 Molar Ratio), Respectively[a]

polymer	K_D	α (K_D/K_L)
A	0.59	1.26 ± 0.10
B	0.46	0.83 ± 0.01

[a] Imprinted polymers were equilibrated with substrate at room temperature using a batch procedure. The substrates D- and L-*p*-NH_2PheO[C-1,^{14}C]Et were applied separately in the reaction mixtures and the same polymer was used in two or three binding experiments. After completion of a binding cycle, polymers were freed from bound substrate by extraction (see Experimental Section) and then reapplied in further binding experiments. The K_D given is the distribution coefficient (ratio of the amount of bound and free enantiomer) for the D form in the first binding experiment. It was found that the values of both K_D and K_L decreased on using the polymers repeatedly. The separation factor $\alpha = K_D/K_L$ represents an average value, and the error limits given are standard deviations.

Results and Discussion

The derivatized print molecule, N^2-propionyl-O^1-acryl-

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(1) (a) For a review, see: Wulff, G. *Polymeric Reagents and Catalysts*; Ford, W. T., Ed.; ACS Symposium Series 308; Washington, DC, 1986; p 186–230 and references therein. (b) Wulff, G.; Sarhan, A.; Zabrocki, K. *Tetrahedron Lett.* **1973**, 4329–4332 and the following papers in this series. (c) Shea, K. J.; Thompson, E. A.; Pandey, S. D.; Beauchamp, P. S. *J. Am. Chem. Soc.* **1980**, *102*, 3149–3155. (d) Damen, J.; Neckers, D. C. *J. Am. Chem. Soc.* **1980**, *102*, 3265–3267. (e) Arshady, R.; Mosbach, K. *Makromol. Chem.* **1981**, *182*, 687–692. (f) Glad, M.; Norrlöw, O.; Sellergren, B.; Siegbahn, N.; Mosbach, K. *J. Chromatogr.* **1985**, *347*, 11–23. (g) Norrlöw, O.; Månsson, M.-O.; Mosbach, K. *J. Chromatogr.* **1987**, *396*, 374–377. (h) Wulff, G.; Heide, B.; Helfmeier, G. *J. Am. Chem. Soc.* **1986**, *108*, 1089–1091. (i) Shea, K. J.; Dougherty, T. K. *J. Am. Chem. Soc.* **1986**, *108*, 1091–1093.

(2) (a) Andersson, L.; Sellergren, B.; Mosbach, K. *Tetrahedron Lett.* **1984**, *25*, 5211–5214. (b) Sellergren, B.; Ekberg, B.; Mosbach, K. *J. Chromatogr.* **1985**, *347*, 1–10. (c) Andersson, L. *React. Polym.* **1988**, *9*, 29–41.

(3) (a) Sellergren, B.; Lepistö, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853–5860. (b) Lepistö, M.; Sellergren, B. *J. Org. Chem.* **1989**, *54*, 6010–6012.

(4) (a) Sellergren, B. *Chirality* **1989**, *1*, 63–68. (b) Sellergren, B. *Makromol. Chem.* **1989**, *190*, 2703–2711.

(5) Shea, K. J.; Sasaki, D. Y. *J. Am. Chem. Soc.* **1989**, *111*, 3442–3444.

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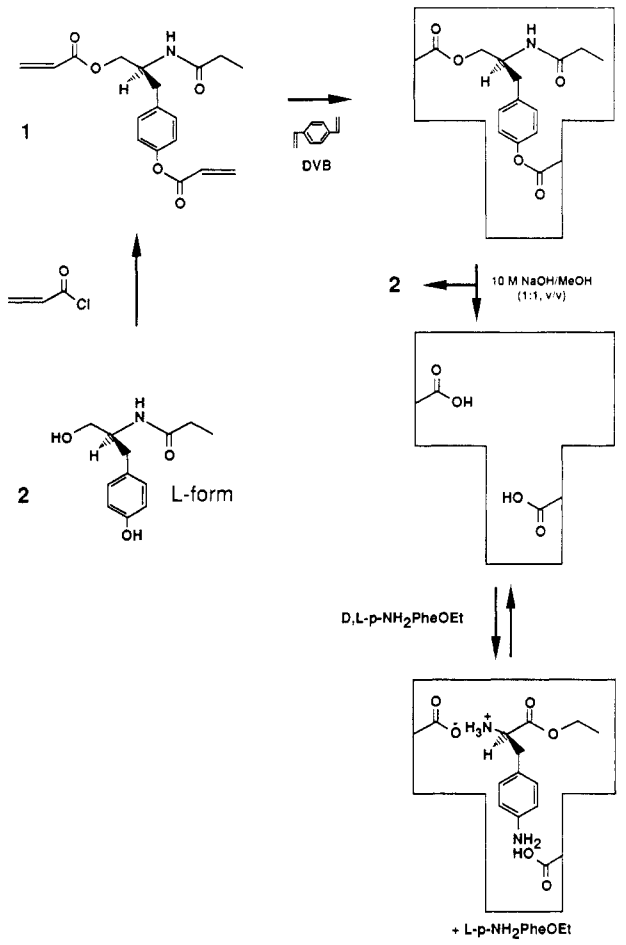
Scheme I



oyl-L-2-amino-3-(O^4-acryloyl-4-hydroxyphenyl)-1-propanol (1), was synthesized from L-2-amino-3-(4-hydroxyphenyl)-1-propanol by treating the latter successively with propionyl chloride and acryloyl chloride. 1 was then copolymerized with an excess of divinylbenzene (DVB) in acetonitrile, resulting in the formation of a macroporous polymer (Scheme I). The print molecule, N^2-propionyl-L-2-amino-3-(4-hydroxyphenyl)-1-propanol (2), was removed by subjecting the polymer to hydrolysis in a concentrated NaOH/methanol solution. The substrate selectivity of the polymers thus prepared was tested in a batch procedure by measuring the binding of radioactively labeled D- and L-*p*-aminophenylalanine ethyl ester (*p*-NH_2PheOEt) to the polymers. In the binding assays the esters were added separately to the print polymers. The amount of bound and free substrate molecules was determined, and the distribution coefficient (K) and the separation factor (α) were calculated (Table I). It is interesting to note that the polymers obtained preferred to interact with the D form of the substrate rather than with the L form, despite that L print molecules were used in the polymerization step.

Polymers showing the opposite binding selectivity (L-selective polymers) could be prepared by replacing the print molecules used carrying bisacrylic functional groups with a corresponding amount of *p*-vinylbenzoic acid (PVB) and L-*p*-NH_2PheOEt (2:1 molar ratio) during the polymerization. Furthermore, it was found that no substrate was bound to a reference polymer containing covalently attached print molecules (unhydrolyzed print polymer).

The inverse stereoselectivity observed in this study may be expected if electrostatic and hydrogen-bonding interactions take place stereospecifically between the aliphatic and aromatic amino groups of the added substrate and the carboxyl groups of the binding sites of the imprinted polymer (Scheme I). Such interactions in similar systems have been documented.[3,6] The structure of the bound substrate thus resembles the structure of the inverse form of the print molecule.

These results support the suggestions made previously[3,5] that imprinted polymers contain discrete binding sites that are complementary to added substrate molecules not only in terms of interacting functional groups but also in terms of size and shape. Moreover, we feel that the substrate analogue imprinting strategy described here may widen the scope of molecular imprinting.

Experimental Section

L-Tyrosine ethyl ester hydrochloride and the monohydrates of D- and L-*p*-aminophenylalanine monohydrochloride (D- and L-*p*-NH_2PheOH·HCl) were obtained from Sigma (St. Louis, MO). Divinylbenzene (DVB) and silica gel 60 were from Merck (Darmstadt), and *p*-vinylbenzoic acid (PVB) was bought from Polysciences, Inc. (Warrington, PA). Acryloyl chloride and propionyl chloride were purchased from Janssen (Beerse), and [C-1,^{14}C]ethanol (21 mCi/mmol) was bought from NEN (Boston).

N^2-Propionyl-O^1-acryloyl-L-2-amino-3-(O^4-acryloyl-4-hydroxyphenyl)-1-propanol (1). L-Tyrosine ethyl ester was reduced by $NaBH_4$ to form L-2-amino-3-(4-hydroxyphenyl)-1-propanol (L-tyrosinol).[7] The alcohol (8.32 g, 49.7 mmol) was dissolved in 180 mL of ice-cooled dimethylformamide (DMF) and 22.9 mL (164 mmol) of triethylamine (TEA). To this solution, kept at 0 °C, freshly distilled propionyl chloride (14.2 mL, 164 mmol) was added dropwise with stirring, and the mixture was allowed to react at room temperature for 3 h. The solution was then filtered and the filtrate was taken down to dryness. The residue was dissolved in ethyl acetate (250 mL), and the organic phase was successively washed with 0.5 M $NaHSO_4$ (3 × 125 mL), H_2O (1 × 125 mL), 0.5 M Na_2CO_3 (3 × 125 mL), and H_2O (1 × 60 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under vacuum. The product obtained, a mixture containing mono-, di-, and triacylated (major form) L-tyrosinol, was treated with 200 mL of ethanol/1 M NaOH (1:1, v/v) for 2 h at room temperature in order to remove the *O*-propionyl groups. The pH value of the solution was then brought to 6 with 1 M HCl, and ethanol was evaporated. Finally, the water phase was extracted with 1-butanol, and the butanol phase was taken down to dryness. The product obtained, N^2-propionyl-L-2-amino-3-(4-hydroxyphenyl)-1-propanol (2, *N*-propionyl-L-tyrosinol), was dried over P_2O_5 under vacuum. Yield: 20.1 mmol (4.49 g, 40.5%). TLC: R_f = 0.55 on silica in chloroform/methanol/acetic acid (10:2:1, v/v). In addition, the ^{1}H NMR data of the N-acylated alcohol were consistent with the proposed structure.

N-Propionyl-L-tyrosinol (2; 3.66 g, 16.4 mmol) was dissolved in a solution containing 150 mL of ice-cooled DMF and 9.14 mL (65.6 mmol) of TEA. To this solution, placed on ice, freshly distilled acryloyl chloride (5.33 mL, 65.6 mmol) was added dropwise. After reaction for 3–4 h at room temperature, the reaction mixture was filtered. The filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate (500 mL). The organic phase was washed with 0.5 M $NaHSO_4$ (3 × 250 mL), H_2O (1 × 250 mL), 0.5 M Na_2CO_3 (3 × 250 mL), and H_2O (1 × 130 mL). After the organic phase was dried (Na_2SO_4), ethyl acetate was evaporated under vacuum, leaving behind a yellow oil. The product was purified by subjecting the oil to three consecutive triturations with water (100, 150, and 900 mL for 0.5, 1.5, and 12 h, respectively). After filtration, the water phases of the second and third triturations were saved and the combined solution was taken down to dryness. During the evaporation, the product precipitated out as white crystals, which were filtered off and dried thoroughly over P_2O_5 under vacuum. Yield: 1.57 mmol (0.52 g, 9.6%). TLC: R_f = 0.76 on silica in benzene/ethyl acetate/methanol (1:1:1, v/v). Mp: 96–98 °C. FAB-MS: (M +

(6) Albrecht, G.; Zundel, G. *Z. Naturforsch.* **1984**, *39a*, 986–992.
(7) Seki, H.; Koga, K.; Matsuo, H.; Ohki, S.; Matsuo, I.; Yamada, S.-I. *Chem. Pharm. Bull.* **1965**, *13*, 995–1000.

H)$^+$ = 332, calculated molecular weight = 331.19. Elemental analysis: C, 65.2 (65.3); H, 6.3 (6.3); N, 4.2 (4.2); O, 24.4 (24.2) (calculated values are given in parentheses). Optical rotation: $[\alpha]^{23.5}_D$ = −17.1° (c 2.5; CH_3CN). ^1H NMR (200 MHz, $CDCl_3$): 1.1 (3 H, t, $J \sim 7.6$ Hz, CH_3), 2.2 (2 H, q, $J \sim 7.6$ Hz, CH_2CH_3), 2.9 (2 H, m, $CH_2C_6H_4$), 4.2 (2 H, m, CH_2O), 4.5 (1 H, m, >CH), 5.7 (1 H, d, $J \sim 9$ Hz, NH), 5.8–6.7 (6 H, m, $CH{=}CH_2$), 7.0–7.3 (4 H, m, C_6H_4).

Carbon-14-Labeled Substrates. ^{14}C-Labeled D- and L-*p*-aminophenylalanine ethyl esters were synthesized by esterification of free amino acid with [C-1,^{14}C]ethanol using a slightly modified standard procedure for amino acid ester synthesis,[8] a method known to yield no racemization of the product formed in the esterification step.

The following describes the method applied to make L-*p*-NH_2PheO[C-1,^{14}C]Et (D-*p*-NH_2PheO[C-1,^{14}C]Et was synthesized in an identical manner). The monohydrate of L-*p*-NH_2PheOH·HCl (126.6 mg, 0.54 mmol) was added to 7.5 mL of EtOH (99.5%) containing 250 μCi [C-1,^{14}C]ethanol. Dry HCl gas was bubbled through the mixture, placed on ice, to yield a saturated HCl solution. This solution was left for 40 h at room temperature, affording a product that had precipitated out from the solution during the reaction. The product formed was dissolved in "cold" ethanol (99.5%), and the solvent was evaporated under reduced pressure. This washing procedure using cold ethanol was repeated several times. The radiolabeled ethyl ester synthesized was purified by dissolving it in 15 mL of 0.2 M $NaHCO_3$ (pH adjusted to 9.5) and extracting the water phase with ethyl acetate (2 × 50 mL). The organic phase was saved and taken down to dryness, and the residue afforded in the evaporation step was dried over P_2O_5 under vacuum. Yield: 0.36 mmol (75 mg, 66%).

In order to remove small amounts of [^{14}C]ethanol remaining in the purified ^{14}C-labeled ester preparations, the product obtained was chromatographed twice on a silica gel column (1.6 × 20 cm, 33 mL) using chloroform/methanol (50:1, v/v) as the eluent. The product isolated was pure as judged from analysis by TLC, UV, specific radioactivity, and optical rotation. TLC: R_f = 0.51 on silica in chloroform/methanol (9:1, v/v); R_f = 0.63 on cellulose in 1-butanol/acetic acid/water (50:20:30, v/v). Optical rotation: $[\alpha]^{23}_D$ = +17.3° (c 2.5; CH_3CN) for the L enantiomer; $[\alpha]^{23}_D$ = −16.6° (c 4.2; CH_3CN) for the D-enantiomer. Specific radioactivity: L-*p*-NH_2PheO[C-1,^{14}C]Et, 1.79 nCi/μmol; D-*p*-NH_2PheO[C-1,^{14}C]Et, 2.11 nCi/μmol. UV: λ_{max} = 284 nm (in 0.1 M sodium phosphate, pH 7.5).

Nonlabeled L-*p*-NH_2PheOEt used as a print molecule was prepared similarly as described above for the preparation of the radiolabeled ethyl ester.

Preparation of Print Polymers. Polymer A. Following a procedure described previously,[2a] 1 (2.9 mol %) was copolymerized with an excess of DVB (technical grade containing 55.5 mol % of DVB and 41.6 mol % of ethylstyrene) in acetonitrile (1.28 mL/g of monomer mixture) in the presence of azobis(isobutyronitrile) (0.8% w/w of the monomers). After polymerization, the polymers were extracted continuously in toluene for 24 h using a Soxhlet extractor. Washing of the polymers was repeated once in acetonitrile. The polymers were then treated with 10 M NaOH/methanol (1:1, v/v) under reflux conditions for 20 h. After the hydrolysis step, they were washed successively in acetonitrile/water (1:1, v/v) and in acetonitrile using the Soxhlet extractor. Finally, the polymers were dried in an oven at 60 °C overnight. Prior to use, dried polymers were stored in a desiccator over P_2O_5 under vacuum. Elemental analyses of hydrolyzed and unhydrolyzed polymers were carried out. The nitrogen content of the polymers (0.44% before hydrolysis, 0.27% after hydrolysis, and about 0.1% for a blank polymer without print molecule) showed that approximately 50% of added print molecules had been split off from the polymers by the treatment with concentrated NaOH.

Polymer B. A print polymer was prepared by using instead of 1 a mixture of PVB (5.8 mol % of vinyl monomers) and L-*p*-NH_2PheOEt (2:1 molar ratio) in the polymerization step but otherwise under identical conditions as for polymer A except that the hydrolysis step was omitted.

Binding Experiments. Dry polymers (0.5 g) and acetonitrile (3 mL) containing D- or L-*p*-NH_2PheO[C-1,^{14}C]Et (1.7 mM) were equilibrated overnight at room temperature in centrifugation tubes placed on an end over end rocking table. The mixtures were then subjected to centrifugation. (In this context, it should be mentioned that in binding experiments similar to those described here, based on ammonium carboxylate ion-pair formation in highly cross-linked DVB polymers, the time dependence of ligand binding to the polymers indicated that equilibrium was reached within an hour at room temperature.) By measuring the radioactivity of the supernatant (in a liquid scintillation counter, LKB, rackbeta), the amount of bound and free substrate could be calculated. Used polymers were freed from bound ligand by washing the polymers with acetonitrile in the Soxhlet extractor. When the polymers were treated in this fashion, they could be reused two or three times.

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Registry No. 1, 126257-05-4; 2, 126257-06-5; H-L-(*p*-NH_2)-Phe-OH·*x*HCl, 120482-21-5; H-D-(*p*-NH_2)Phe-OH·*x*HCl, 126257-07-6; H-L-(*p*-NH_2)Phe-OEt, 114422-51-4; H-D-(*p*-NH_2)-Phe-OEt, 126257-08-7; L-tyrosinol, 5034-68-4; polymer A, 126257-09-8.

(8) Greenstein, J. P.; Winitz, M. In *Chemistry of the Amino Acids*; Wiley: New York, 1961; p 925.

Trifluoromethyl-Substituted Carbethoxy Carbene as a Novel CF_3-Containing a^2 Synthon Equivalent for the Preparation of 2-(Trifluoromethyl)-4-oxo Carboxylic Ester Derivatives: Highly Functionalized Synthetic Building Blocks Bearing a CF_3 Group

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The synthesis of specifically trifluoromethylated molecules is an ongoing area of research due to the unique physical and biological properties imparted by the CF_3 group.[1] While for the synthesis of trifluoromethylated aromatic compounds, direct transformations of certain functional groups to the CF_3 group have been employed,[2] the preparation of trifluoromethylated aliphatic compounds, on the other hand, is not straightforward because of the requirement of milder reaction conditions and limited intrinsic reactivity of various trifluoromethylating reagents. Therefore, the development of a simple method for the preparation of trifluoromethylated building blocks and their further utilization for the synthesis of desired CF_3-containing aliphatic compounds are essential to organofluorine chemistry. Previously, we reported the easy preparation of a novel CF_3-containing diazo compound 1 from readily available inexpensive starting materials and established its feasibility as a CF_3-substituted carbenoid

(1) (a) *Carbon-fluoride Compounds, A CIBA Foundation Symposium*; Elsevier: Amsterdam, 1972. (b) Filler, R.; Kobayashi, Y. In *Biomedicinal Aspects of Fluorine Chemistry*; Kodansha: Tokyo, 1983. (c) Welch, J. T. *Tetrahedron* **1987**, *43*, 3123. (d) Fuchigami, T. *J. Synth. Org. Chem. Jpn.* **1984**, *42*, 775.

(2) Hudlicky, M. In *Chemistry of Organic Fluorine Compounds*; Ellis Horwood: Chichester, 1976.

0022-3263/90/1955-3383$02.50/0