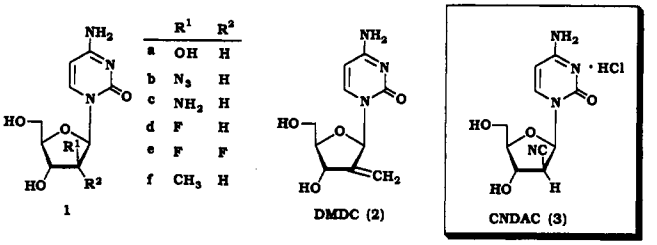
Communications to the Editor

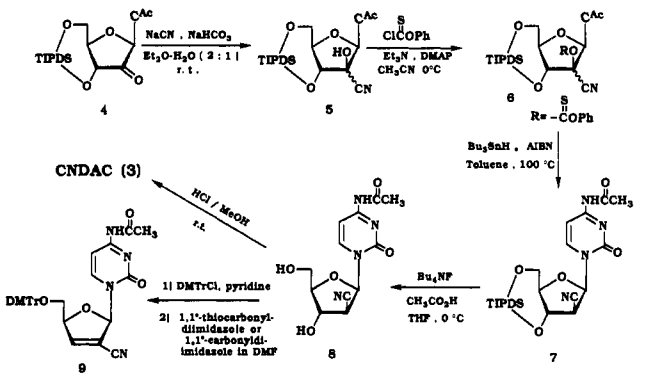
Nucleosides and Nucleotides. 100. 2′-*C*-Cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (CNDAC): Design of a Potential Mechanism-Based DNA-Strand-Breaking Antineoplastic Nucleoside[1,†]

Interest in certain 2′-deoxyribonucleosides and arabinofuranosyl nucleosides as potential anticancer chemotherapeutic agents was stimulated by the observation that 1-β-D-arabinofuranosylcytosine (*ara*-C, **1a**) had antileukemic activity; it is one of the most effective drugs for the treatment of adult acute myeloblastic leukemia.[2,3] However, *ara*-C has some drawbacks including its short half-life in plasma due to rapid deamination to chemotherapeutically inactive 1-β-D-arabinofuranosyluracil by cytidine deaminase, and it is not effective against solid tumors.[4-6] To overcome these problems, efforts have been made to substitute certain functional groups other than the hydroxyl group at the 2′-position in place of the hydrogen atom of 2′-deoxycytidine. As a result of these studies, several analogues of 2′-deoxycytidine **1b-e** in Chart I were found to be as potent as *ara*-C against leukemic cells.[7-10] As we have reported quite recently, (2′*S*)-2′-deoxy-2′-*C*-methylcytidine (**1f**, SMDC) was also found to be a potent inhibitor of the growth of mouse and human leukemic cells in vitro.[11-13] Additionally we have reported that 2′-deoxy-2′-methylidenecytidine (**2**, DMDC), which is resistant to the deaminase from mouse kidney, was effective on various human tumor cells in vitro as well as in vivo.[14-16] DMDC also showed therapeutic activity against human tumor xenografts.[16] One of the most important mechanisms related to their antineoplastic effects is to inhibit DNA polymerases in competition with dCTP after being metabolically activated as their 5′-triphosphates. We have also found that 5′-triphosphates of **1f** and **2** were incorporated into the 3′-terminus of a DNA primer, and they acted as chain terminators.[17] We sought to design a new

Chart I



Scheme I



(1) Part 99: Matsuda, A.; Akashi, M.; Ohara, Y.; Wataya, Y.; Hayatsu, H.; Ueda, T. Mutagenicity of (*p*-nitrophenyl)-adenines in *Salmonella typhimurium*. *Mutat. Res.* **1991**, *263*, 93-100.

(2) Keating, M. J.; McCredie, K. B.; Bodey, G. P.; Smith, T. L.; Gehan, E.; Freidreich, E. J. Improved prospects for long-term survival in adults with acute myelogenous leukemia. *JAMA* **1982**, *248*, 2481-2486.

(3) Livingston, R. B.; Carter, S. K. Cytosine arabinoside (NSC-63878)-Clinical brochure. *Cancer Chemother. Rep., Part 3* **1968**, *1* (No. 1), 179-205.

(4) Ellison, R. R.; Holland, J. F.; Weil, M.; Jacquillant, C.; Bernard, J.; Sawitsky, A.; Rosner, F.; Gussoff, B.; Silver, R. T.; Karanas, A.; Cuttner, J.; Spurr, C. L.; Hayes, D. M.; Blom, J.; Leone, L. A.; Haurani, F.; Kyle, R.; Huchison, J. L.; Forcier, R. J.; Moon, J. H. Arabinosyl cytosine: a useful agent in the treatment of acute leukemia in adults. *Blood* **1968**, *32*, 507-523.

(5) Prince, H. N.; Grumberg, E.; Buck, M.; Cleeland, R. A comparative study of antitumor and antiviral activity of 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA) and 1-β-D-arabinofuranosylcytosine (CA). *Proc. Soc. Exp. Biol. Med.* **1969**, *130*, 1080-1086.

(6) Ho, W. D. H. Distribution of kinase and deaminase of 1-β-D-arabinofuranosylcytosine in tissues of man and mouse. *Cancer Res.* **1973**, *33*, 2816-2820.

(7) Bobek, M.; Cheng, Y. C.; Block, A. Novel arabinofuranosyl derivatives of cytosine: Resistant to enzymatic deamination and possessing potent antitumor activity. *J. Med. Chem.* **1978**, *21*, 660-661.

(8) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. Synthesis and antiherpes virus activity of some 2′-fluoro-2′-deoxyarabinofuranosylpyrimidine nucleosides. *J. Med. Chem.* **1979**, *22*, 21-24.

(9) Su, T-L.; Watanabe, K. A.; Shinazi, R. F.; Fox, J. J. Synthesis and antiviral effects of several 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-alkyluracils. Some structure-activity relationships. *J. Med. Chem.* **1986**, *29*, 151-154 and references cited therein.

(10) Hertel, L. W.; Boder, G. B.; Stan Kroin, J.; Rinzel, S. M.; Poore, G. A.; Todd, G. C.; Grindey, G. B. Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). *Cancer Res.* **1990**, *50*, 4417-4422.

(11) Matsuda, A.; Takenuki, K.; Itoh, H.; Sasaki, T.; Ueda, T. Radical deoxygenation of *tert*-alcohols in 2′-branched-chain sugar pyrimidine nucleosides: Synthesis and antileukemic activity of 2′-deoxy-2′(*S*)-methylcytidine. *Chem. Pharm. Bull.* **1987**, *35*, 3967-3970.

(12) Ueda, T.; Matsuda, A.; Yoshimura, Y.; Takenuki, K. Synthesis and biological activity of branched chain-sugar nucleosides. *Nucleosides Nucleotides* **1989**, *8*, 743-752.

(13) Matsuda, A.; Takenuki, K.; Sasaki, T.; Ueda, T. Radical deoxygenation of *tert*-alcohols in 1-(2-*C*-alkylpentofuranosyl)pyrimidines: Synthesis of (2′*S*)-2′-deoxy-2′-*C*-methylcytidine, and antileukemic nucleoside. *J. Med. Chem.* **1991**, *34*, 234-239.

(14) Takenuki, K.; Matsuda, A.; Ueda, T.; Sasaki, T.; Fujii, A.; Yamagami, K. Design, synthesis, and antineoplastic activity of 2′-deoxy-2′-methylidenecytidine. *J. Med. Chem.* **1988**, *31*, 1063-1064.

(15) Matsuda, A.; Takenuki, K.; Tanaka, M.; Sasaki, T.; Ueda, T. Synthesis of new broad spectrum antineoplastic nucleosides, 2′-deoxy-2′-methylidenecytidine (DMDC) and its derivatives. *J. Med. Chem.* **1991**, *34*, 812-819.

(16) Yamagami, K.; Fujii, A.; Arita, M.; Okumoto, T.; Sakata, S.; Matsuda, A.; Ueda, T.; Sasaki, T. Antitumor activity of 2′-deoxy-2′-methylidenecytidine, a new 2′-deoxycytidine derivatives. *Cancer Res.* **1991**, *51*, 2319-2323.

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nucleoside that has a chemical reactivity that would cleave a DNA strand after its incorporation into the DNA molecule. This mechanism would not differ from the usual competitive inhibition of DNA polymerases or chain termination, and thus could exert a unique antineoplastic activity, much like the strand breaks in DNA produced by ionizing radiation therapy that have been hypothesized to result in tumor cell death.

In this communication, we describe the synthesis of 2′-*C*-cyano-2′-deoxy-1-β-D-arabinofuranosylcytosine (**3**, CNDAC). The inhibitory activity of CNDAC to human tumor cell lines in vitro and its antitumor activity on mouse leukemia P388 cells in vivo is also described. Introduction of the cyano group into the 2′-"up"-position of 2′-deoxycytidine would increase the acidity of the 2′-"down"-proton. If this analogue were enzymatically incorporated into DNA molecules, the cyano group would be β to the phosphate diester in the DNA molecule. Following the precedent that a β-cyanoethyl group on a phosphate diester of a nucleoside is easily deblocked with alkaline treatment through β-elimination,[18] a similar process could occur when a 2′-*C*-cyano nucleoside analogue is incorporated into the DNA molecule resulting in strand scission. Moreover, the sugar structure of the 3′-terminal nucleotide should be a good Michael acceptor, and possibly react with certain nucleophiles.

Chemistry. Recently, we have developed a method for introducing alkyl substituents into the 2′-"up"-position of pyrimidine nucleosides using radical deoxygenation of the methyl oxalyl ester of the tertiary alcohol.[11-13] This methodology was essentially adopted to synthesize CNDAC as outlined in Scheme I. N^4-Acetyl-1-[3,5-*O*-(tetraisopropyldisiloxane-1,3-diyl)-β-D-*erythro*-pentofuran-2-ulosyl]cytosine (**4**) was treated with sodium cyanide in the presence of sodium bicarbonate in aqueous ether[19,20] to afford an epimeric mixture of 2′-cyanohydrins **5**. Compound **5** was further treated with phenyl chlorothionocarbonate in the presence of 1 equiv of *p*-(dimethylamino)pyridine in acetonitrile to give thiocarbonate **6**. Deoxygenation with Bu_3SnH and 2,2′-azobis(isobutyronitrile) in toluene at 100 °C gave the crystalline nucleoside **7** in 57% yield from **4**.[21] The configuration at the 2′-position of **7** was confirmed by NOE experiments. When H-2′ (δ 3.72, dd) was irradiated, a NOE was observed at H-1′ (δ 6.34, d) about 15%. Also the H-6 (δ 8.07, d) was irradiated and a NOE was observed at H-1′. These indicate that radical deoxygenation proceeded stereospecifically to give the arabinonucleoside **7** due to the steric hindrance of the β-face. This is consistent with our previous observations.[11-13] Deprotection of the 3′,5′-*O*-tetraisopropyldisiloxanediyl group of **7** by tetrabutylammonium fluoride in the presence of acetic acid in tetrahydrofuran afforded N^4-acetyl derivative **8**[22] in 84% yield, which was further treated with HCl in MeOH (3%) at room temperature to give CNDAC as a hydrochloride in 58% yield.[23]

The acid-catalyzed deprotection of **8** to **3** was used because treatment of **8** with NH_3/MeOH to remove the N^4-acetyl group was unsuccessful, and the only isolable product from the reaction mixture was cytosine. This provided additional chemical evidence for the sensitivity of **3** to β-elimination. Also, reaction of the 5′-dimethoxytrityl derivative of **8** with 1,1′-thiocarbonyldiimidazole or 1,1′-carbonyldiimidazole in *N*,*N*-dimethylformamide furnished a β-elimination product, the 2′-*C*-cyano-2′,3′-didehydro-2′,3′-dideoxy derivative **9**. Such chemical reactivity would be expected from CNDAC if in its nucleotide form it were to be incorporated into DNA.

Biological Activity. When CNDAC was initially tested against mouse leukemic L1210 cells (1×10^4) for 72 h, IC_{50} (the concentration required for 50% inhibition of cell growth using the MTT assay[24]) was 0.21 μg/mL. This value was comparable to those of DMDC (**2**),[15] SMDC (**1f**),[13] and cytarazid (**1b**),[7,25] which we have recently reported. We then compared the in vitro cytotoxic spectrum of CNDAC with that of *ara*-C in the various human tumor

Table I. Inhibitory Effects of CNDAC on the Growth of Various Human Tumor Cell Lines in Vitro (IC_{50}, μg/mL)[a,b]

	cell line	CNDAC	*ara*-C
ST-KM	stomach adenocarcinoma	2.8	>100
NUGC-4	stomach adenocarcinoma	5.2	>100
MKN-1	stomach adenocarcinoma	2.1	ND[c]
MKN-7	stomach adenocarcinoma	1.4	ND[c]
MKN-28	stomach adenocarcinoma	1.7	4.5
MKN-74	stomach adenocarcinoma	0.04	ND[c]
OST	osteosarcoma	6.4	>100
KHOS-321H	osteosarcoma	4.5	0.27
SK-ES-1	osteosarcoma	2.2	0.09
MNNG-HOS	osteosarcoma	6.8	2.6
PC-3	lung adenocarcinoma	5.6	>100
PC-8	lung adenocarcinoma	4.6	0.28
PC-9	lung adenocarcinoma	4.4	1.6
PC-13	lung large-cell carcinoma	>100	>100
QG-56	lung squamous-cell carcinoma	40.0	>100
SW-480	colon adenocarcinoma	0.27	>100

[a] Cytotoxic activity assay in vitro was done following the method of Carmichael et al.[24] Each tumor cells (1×10^4/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and the OD (570 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 − OD (570 nm) of sample well/OD (570 nm) of control well] × 100. [b] IC_{50} (mg/mL) was given as the concentration at 50% inhibition of cell growth. [c] Not determined.

(17) Takenuki, K.; Matsuda, A. Unpublished results.
(18) Tener, G. M. 2-Cyanoethyl phosphate and its use in the synthesis of phosphate esters. *J. Am. Chem. Soc.* **1961**, *83*, 159–168.
(19) Calvo-Mateo, A.; Camarasa, M-J.; Diaz-Ortiz, A.; De las Heras, F. G. 3′-*C*-Cyano-3′-deoxythymidine. *Tetrahedron Lett.* **1988**, *29*, 941–944.
(20) Camarasa, M-J.; Diaz-Ortiz, A.; Caivo-Mateo, A.; De las Heras, F. G.; Balzarini, J.; De Clercq, E. Synthesis and antiviral activity of 3′-*C*-cyano-3′-deoxynucleosides. *J. Med. Chem.* **1989**, *32*, 1732–1738.
(21) Mp 209–211 °C (Et_2O–hexane); IR (KBr) 2260 cm^{-1} (–CN); ^1H NMR ($CDCl_3$) δ 9.92 (1 H, br s, 4-NH), 8.07 (1 H, d, H-6), 7.55 (1 H, d, H-5), 6.34 (1 H, d, H-1′, $J_{1'2'}$ = 7.0 Hz), 4.63 (1 H, t, H-3′), 4.18 (1 H, dd, H-5′a), 4.06 (1 H, dd, H-5′b), 3.89 (1 H, ddd, H-4′), 3.72 (1 H, dd, H-2′), 2.30 (3 H, s, Ac), 1.13–1.03 (28 H, m, *i*-Pr). Anal. ($C_{24}H_{40}N_4O_6Si_2$) C, H, N.
(22) Mp 210 °C dec (EtOH); IR (KBr) 2260 cm^{-1} (–CN); ^1H NMR (DMSO-d_6) δ 10.97 (1 H, br s, 4-NH), 8.36 (1 H, d, H-6), 7.26 (1 H, d, H-5), 6.27 (1 H, d, 3′-OH), 6.22 (1 H, d, H-1′, $J_{1'2'}$ = 7.1 Hz), 5.25 (1 H, t, 5′-OH), 4.43 (1 H, ddd, H-3′), 3.92 (1 H, t, H-2′), 3.84 (1 H, ddd, H-4′), 3.76 (1 H, br d, H-5′a), 3.63 (1 H, br d, H-5′b), 2.11 (3 H, s, Ac). Anal. ($C_{12}H_{14}N_4O_5 \cdot {}^1/_4H_2O$) C, H, N.
(23) Mp 175–176 °C (EtOH–Et_2O); IR (KBr) 2260 cm^{-1} (–CN); ^1H NMR (DMSO-d_6) δ 9.80 (1 H, br s, 4-NHa), 8.75 (1 H, br s, 4-NHb), 8.30 (1 H, d, H-6), 6.21 (1 H, d, H-1′, $J_{1'2'}$ = 7.2 Hz), 6.12 (1 H, d, H-5), 4.43 (1 H, dd, H-3′), 3.97 (1 H, t, H-2′), 3.83 (1 H, ddd, H-4′), 3.76 (1 H, dd, H-5′a), 3.62 (1 H, br d, H-5′b). Anal. ($C_{10}H_{12}N_4O_4 \cdot HCl \cdot {}^1/_2EtOH$) C, H, N.
(24) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. *Cancer Res.* **1987**, *47*, 936–942.
(25) Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. Improved synthesis of 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (Cytarazid) and -thymine. Inhibitory spectrum of cytarazid on the growth of various human tumor cells in vitro. *J. Med. Chem.* **1991**, *34*, 999–1002.

cell lines including six stomach adenocarcinomas, four osteosarcomas, three lung adenocarcinomas, a lung large-cell carcinoma, a lung squamous-cell carcinoma, and a colon adenocarcinoma. As summarized in Table I, CNDAC had potent cytotoxicity in 14 tumor cell lines with IC_{50} values ranging from 0.04 to 6.8 μg/mL, but not against PC-13 lung large-cell carcinoma and QG-56 lung squamous-cell carcinoma. On the other hand, *ara*-C showed good cytotoxicity to only six of the cell lines (0.09–4.5 μg/mL) tested in this study. It is important to note that CNDAC shows a potent cytotoxicity in all tested the human stomach adenocarcinomas, the incidence of which is high in Japan.

In vivo antitumor activity of CNDAC and *ara*-C was also examined against intraperitoneally implanted mouse leukemia P388 (10^6 cells) in CDF_1 mice, with a dose of 100 mg/kg per day intraperitoneally given on days 1 and 5 from the day after tumor transplantation. The antitumor effects of CNDAC were measured by comparison of the median survival time of the treated group and that of an untreated group, with six CDF_1 female mice in each group. The survival time was greatly increased; the ratio of treated vs the control in median survival time was 183%, while that of *ara*-C was 163%. Median survival time of the untreated control group was 10.0 days. When a 20 mg/kg dose of CNDAC was administered intraperitoneally once each day on days 1–10, five out of six mice survived over 60 days ($T/C > 600\%$) after initial treatment (one mouse died at day 53). Although the optimal therapeutic schedule of CNDAC is not yet known, such excellent activity suggests that CNDAC is a promising agent for further evaluation for therapy of human cancer. Whether the mechanism responsible for its antitumor properties is related to the DNA-strand-breakage hypothesis is being studied. If CNDAC indeed acts as a "chemical X-ray", it would demonstrate a new approach for anticancer chemotherapy.

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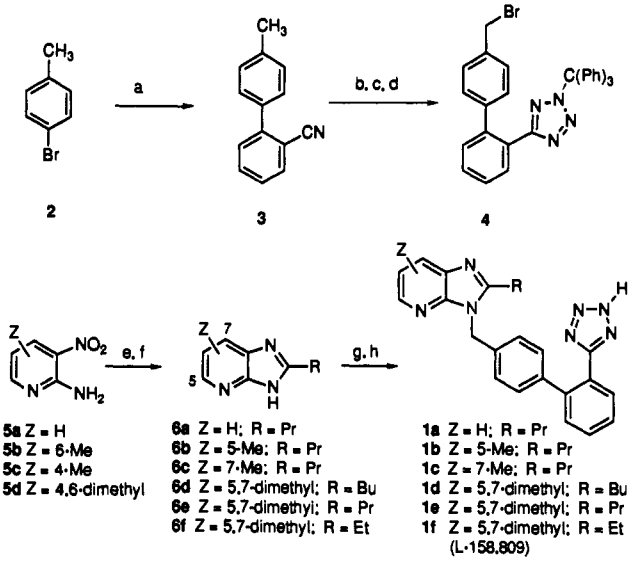
Potent, Orally Active Imidazo[4,5-*b*]pyridine-Based Angiotensin II Receptor Antagonists

Angiotensin II is the principle pressor agent of the renin–angiotensin system (RAS). This system, a proteolytic cascade that regulates hemodynamics and water and electrolyte balance, can contribute to hypertensive states in man. The effects of the octapeptide angiotensin II (AII), which include vasoconstriction, stimulation of aldosterone synthesis and release, cardiac stimulation, and renal reabsorption of sodium, are mediated through specific membrane-bound receptors. Reducing AII levels with angiotensin converting enzyme (ACE) inhibitors such as captopril and enalapril has confirmed the therapeutic benefit of inhibiting the RAS in hypertension and heart failure.

An alternative mode of inhibiting AII is to antagonize its interaction with the receptor. Although peptide analogues of AII inhibit the action of AII by competitively binding to the receptor,[1] their prospects as clinical agents are limited due to short duration, poor oral bioavailability, and partial agonist activity.

Recently, nonpeptidic, orally active AII receptor antagonists, have been reported,[2] and one of these, 2-*n*-butyl-4-chloro-5-(hydroxymethyl)-1-[[2′-1*H*-tetrazol-5-yl)bi-

Scheme I[a]



[a] Reagents: (a) ^{t}BuLi, pentane–THF, −78 °C, 30 min; $ZnCl_2$–Et_2O, 25 °C, 2 h; 2-bromobenzonitrile, 2 mol % $Ni(PPh_3)_2Cl_2$, 25 °C, 18 h, (88%); (b) Me_3SnN_3, toluene, reflux, 24 h; dilute HCl, 25 °C; (c) $(Ph)_3CCl$, Et_3N, CH_2Cl_2, reflux, 1.5 h; (d) *N*-bromosuccinimide, 10 mol % dibenzoyl peroxide, CCl_4, reflux, 2 h; (e) H_2, (1 atm), 10% Pd–C (10 w/w %), MeOH; (f) butyric acid (**6a–c,e**), valeric acid (**6d**), or propionic acid (**6f**), polyphosphoric acid, 80–90 °C, 3–8 h (75–95%, 2 steps); (g) NaH, DMF, 25 °C, 20 min; **4**, 2–12 h (30–75 %); (h) HCO_2H, 25 °C, 12 h (85–95%).

(1) Khosla, M. C.; Smeby, R. R.; Bumpus, F. M. Structure Activity Relationship in Angiontensin II Analogs. In *Angiotensin*; Page, I. H., Bumpus, F. M., Eds.; Springer Verlag: Heidelberg, 1973; pp 126–161. Streeten, D. H. P.; Anderson, G. H. Angiotensin-Receptor Blocking Drugs. In *Clinical Pharmacology of Antihypertensive Drugs, Handbook of Hypertension*, Doyle, A. E., Ed.; Elsevier: New York, 1984; Vol. 5, pp 246–271. Bovy, P. R.; Blaine, E. H. Peptidic and Non-peptidic Angiotensin II Competitive Antagonists. *Current Cardiovascular Patents* **1989**, *1*, 2044–2056.

(2) Matsumura, K.; Hashimoto, N.; Furakawa, Y. U.S. patent 4,207,324, 1980. Furakawa, Y.; Kishimoto, S.; Nishikawa, K. U.S. patent 4,340,598, 1982. Furakawa, Y.; Kishimoto, S.; Nishikawa, K. U.S. patent 4,355,040, 1982. Duncia, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. The Discovery of Potent Nonpeptide Angiotensin II Receptor Antagonists: A New Class of Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1312–1329. Weinstock, J.; Keenan, R. M.; Samanen, J.; Hempel, J.; Finkelstein, J. A.; Franz, R. G.; Gaitanopoulus, D. E.; Girard, G. R.; Gleason, J. G.; Hill, D. T.; Morgan, T. M.; Peishoff, C. E.; Aiyar, N.; Brooks, D. P.; Fredrickson, T. A.; Ohlstein, E. H.; Ruffolo, R. R., Jr.; Stack, E. J.; Sulpizio, A. C.; Weidley, E. F.; Edwards, R. M. 1-(Carboxybenzyl)imidazole-5-acrylic Acids: Potent and Selective Angiotensin II Receptor Antagonists. *J. Med. Chem.* **1991**, *34*, 1514–1517.

0022-2623/91/1834-2919$02.50/0