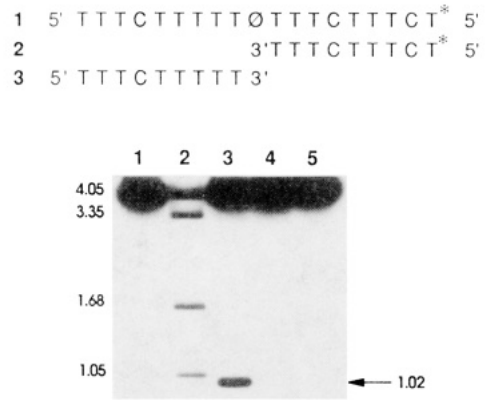

Figure 3. Site-specific double-strand cleavage of plasmid pHMPQ2 (4.05 kbp). In a typical experiment, a buffered solution containing tris(acetate) (25 mM, pH 7.0), oligonucleotide–EDTA (2 μM), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (2 μM), NaCl (100 mM), spermine (1 mM), ^{32}P-labeled pHMPQ2, and calf-thymus DNA (100 μM bp) was equilibrated at 37 °C. After 1 h, sodium isoascorbate (1 mM) was added to the reaction mixture, initiating strand cleavage. The reaction was allowed to proceed for 8 h (37 °C), followed by ethanol precipitation. Gel electrophoresis (1% agarose) separated the double-strand-cleavage products, which were visualized by autoradiography. Product yields were determined by scintillation counting of isolated bands. Lane 1: pHMPQ2 linearized with *Sty*I and uniquely labeled with [α-^{32}P]TTP at the 3′ end. Lane 2: DNA size markers obtained by digestion of linear pHMPQ2 with *Eco*RI (1055 bp), *Pvu*I (1680), *Pvu*II (3350), and undigested DNA (4049). Lane 3: plasmid with oligonucleotide **1** (2 μM). Lane 4: plasmid with oligonucleotide **2** (2 μM). Lane 5: plasmid with oligonucleotides **2** and **3** (2 μM each). Arrow indicates the labeled 1.02-kbp double-strand-cleavage product.

5′-(purine)$_m$(pyrimidine)$_n$-3′ and 5′-(pyrimidine)$_m$(purine)$_n$-3′ sequences. Essential to the design of these bidirectional oligonucleotides is the nature of the covalent linker. Based on model-building studies, an abasic deoxyribose analogue[5] (ϕ) should maintain overall structural continuity in the 3′–3′ motif for alternate-strand binding. The stereochemistry of the (2*R*,3*S*)-3-hydroxy-2-(hydroxymethyl)tetrahydrofuran moiety properly orients the two subunits for the necessary crossover within the major groove of the Watson–Crick double helix (Figure 1). The linking distance appears to be optimal for bridging two base pairs, in a nonspecific manner, at the junction of a 5′-(purine)$_m$(pyrimidine)$_n$-3′ target sequence.

The 3′-3′ oligonucleotide (5′-T_3CT_5-3′–3′-ϕT_3CT_3CT*-5′) **1** incorporating nine pyrimidines flanking the 1,2-dideoxy-D-ribose was synthesized by automated methods starting with the 5′-oxygen of thymidine attached to the solid support (Figure 2).[6] After synthesis of the first nine bases in the 5′–3′ direction, the 10th residue was introduced in opposite orientation by a 3′–3′ coupling using 5-*O*-(4,4′-dimethoxytrityl)-1,2-dideoxy-D-ribose 3-(β-cyanoethyl *N*,*N*-diisopropylphosphoramidite). The next eight bases were added in the conventional 3′–5′ orientation, and the protected thymidine–EDTA (T*) was incorporated as the last nucleotide.[7] As controls, oligonucleotides (**2** and **3**) capable of binding to only one half-site within the target sequence were synthesized with and without T*, respectively.

Oligonucleotide–EDTA·Fe **1** at 2 μM concentration (37 °C, pH 7.0) produced specific double-strand cleavage at the target site, 5′-$A_3GA_5GCT_3CT_3CT$-3′, 1.02 kbp from the end of plasmid DNA, 4.05 kbp in size[8] (14% yield) (Figure 3). No additional cleavage at partially homologous sequences was detected. No detectable cleavage was observed (<1.5%) with 9-mer **2** (lane 4) or the combination of oligonucleotides **2** and **3** (lane 5). These results indicate that oligonucleotide **1** occupies both half-sites in a one-to-one complex. In data not shown, if the 1,2-dideoxy-D-ribose is replaced by an acyclic chain, 1,3-propanediol, binding is 3 times less efficient, indicating that the rigidity and stereochemistry of the dideoxyribose ring are likely important.

This work demonstrates that binding to alternate strands of double-helical DNA by oligonucleotide-directed triple-helix formation extends recognition to mixed DNA sequences of the type (purine)$_m$NN(pyrimidine)$_n$.[10] This result is one example of a larger class of potential *multimeric crossover oligonucleotides*. One could imagine the synthesis of 5′–5′ linked oligonucleotides for binding (pyrimidine)$_m$(purine)$_n$ tracts of duplex DNA. Combinations of 5′–5′ and 3′–3′ linked oligonucleotides should then make multiple crossovers in the major groove possible for binding DNA sequences of the type 5′-(purine)$_m$(pyrimidine)$_n$-(purine)$_p$-3′ and 5′-(pyrimidine)$_m$(purine)$_n$(pyrimidine)$_p$-3′. Incorporation of triplet specificities for TA[1f] and CG base pairs within the alternate-strand triple-helix motif should allow targetng to an even broader range of mixed DNA sequences.

Acknowledgment. We are grateful for grant support from the National Institutes of Health (GM-42966), an unrestricted research grant from Burroughs-Wellcome, and a National Institutes of Health postdoctoral fellowship from the National Institute of General Medical Science (D.A.H.).

(5) (a) Takeshita, M.; Chang, C.-N.; Johnson, F.; Will, S.; Grollman, A. P. *J. Biol. Chem.* **1987**, *262*, 10171. (b) Eritja, R.; Walker, P. A.; Randall, S. K.; Goodman, M. F.; Kaplan, B. E. *Nucleosides Nucleotides* **1987**, *6*, 803.

(6) van de Sande, J. H.; Ramsing, N. B.; Germann, M. W.; Elhorst, W.; Kalisch, B. W.; Kitzing, v. E.; Pon, R. T.; Clegg, R. C.; Jovin, T. M. *Science* **1988**, *241*, 551.

(7) Dreyer, G. B.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 968.

(8) The plasmid containing the target sequence was prepared by ligation of a short duplex containing the 20-base-pair site into the 4.02-kilobase-pair (kbp) *Bam*HI–*Hin*dIII fragment of pBR322.[9] This sequence occurs once in 4049 base pairs and lies 1.02 kbp upstream from the *Sty*I restriction site.

(9) Mendel, D.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 910.

(10) In a formal sense, alternate strand triple helix formation of the type (purine)$_n$NN(pyrimidine)$_m$ (n = 1–7 and $n + m$ = 14) affords recognition of 967 044 sequences.

[2-(Benzyloxy)-1-(*N*-2,6-xylylimino)ethyl]samarium as a Synthetic Equivalent to α-Hydroxyacetyl Anion

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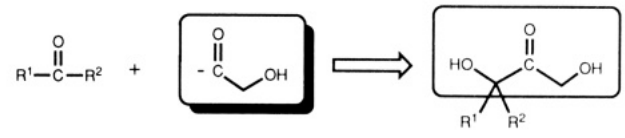
A synthetic equivalent to acyl anion is of great interest as an intermediate for nucleophilic introduction of the acyl group into organic molecules. In particular, α-hydroxyacetyl anion is attractive because the dihydroxyacetone unit, which would result from an addition reaction of α-hydroxyacetyl anion to a carbonyl compound, is a feature of a broad range of natural products such as keto sugars, corticosteroids, and anthracycline antibiotics.



Although a variety of acyl anion equivalents have been reported,[1] a synthetic equivalent to α-hydroxyacetyl anion is not well developed so far, and therefore, multistep procedures have been used for the introduction of a dihydroxyacetone side chain in the syntheses of corticosteroids[2] and adriamycin.[3] We now report

(1) Ager, D. J. In *Umpoled Synthons*; Hase, T. A., Ed.; Wiley: New York, 1987; p 19.

(2) For synthetic study of corticosteroids, see: Horiguchi, Y.; Nakamura, E.; Kuwajima, I. *J. Am. Chem. Soc.* **1989**, *111*, 6257 and references cited therein.

(3) Smith, T. H.; Fujiwara, A. N.; Henry, D. W.; Lee, W. W. *J. Am. Chem. Soc.* **1976**, *98*, 1969.

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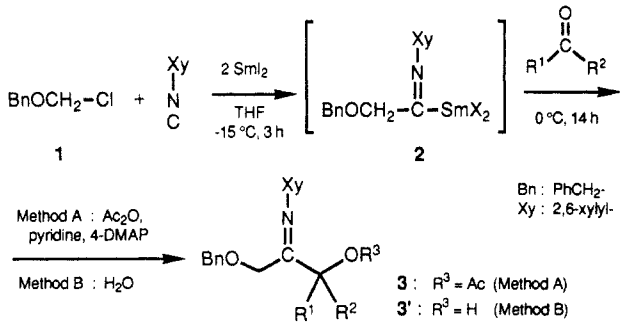
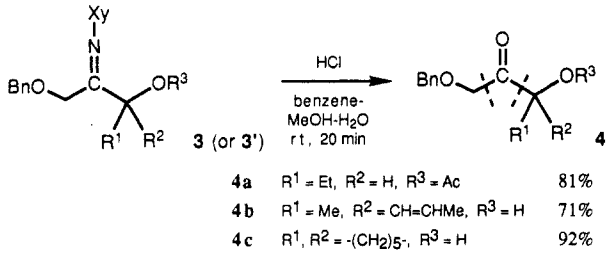
a facile preparation of an α-hydroxyacetyl anion equivalent, i.e., [2-(benzyloxy)-1-(*N*-2,6-xylylimino)ethyl]samarium, by the samarium(II) iodide mediated coupling reaction[4] of benzyl chloromethyl ether[5] with 2,6-xylyl isocyanide and its addition reaction to carbonyl compounds, giving α,α′-dioxygenated imines, which are easily transformed to the corresponding α,α′-dioxygenated ketones by hydrolysis. The usefulness of the present methodology is demonstrated by the stereoselective and simple synthesis of D-*erythro*-2-pentulose (D-ribulose).

Benzyl chloromethyl ether (**1**) was treated with 2,6-xylyl isocyanide and SmI_2 in the presence of HMPA in THF at −15 °C for 3 h, and subsequent treatment of the reaction mixture with aldehydes followed by the addition of acetic anhydride[6] afforded α,α′-dioxygenated imines **3** in good yields (Table I). This result suggested that [2-(benzyloxy)-1-(*N*-2,6-xylylimino)ethyl]samarium (**2**) may be formed as an intermediate. Because of the low basicity, **2** could react also with ketones, which are prone to enolization, to give the corresponding addition products (**3′**) after hydrolysis (entries 5–8). With α,β-unsaturated ketone, **2** selectively underwent 1,2-addition (entry 8).

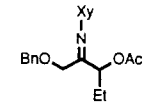
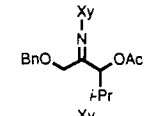
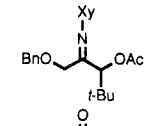
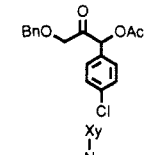
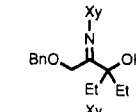
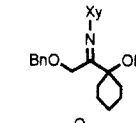
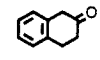
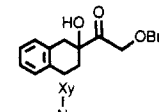
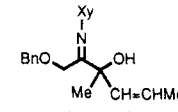


α,α′-Dioxygenated imines (**3** and **3′**) thus obtained could be easily transformed to the corresponding α,α′-dioxygenated ketones (**4**) in good yields by treatment with HCl in benzene–methanol–water at room temperature. Thus, the construction of the dihydroxyacetone unit was achieved by the coupling of three components, i.e., benzyl chloromethyl ether (**1**), isocyanide, and carbonyl compound.



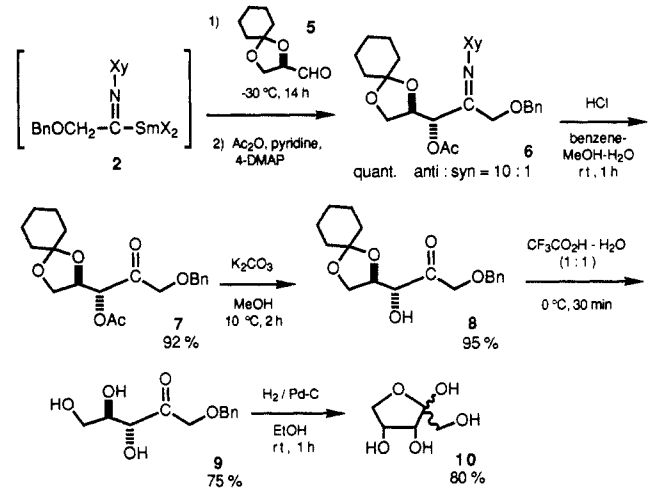
Next, the synthetic utility of the reaction mentioned above was demonstrated by the application to the synthesis of a 2-keto sugar. [2-(Benzyloxy)-1-(*N*-2,6-xylylimino)ethyl]samarium (**2**), generated by the SmI_2-mediated coupling reaction of **1** with 2,6-xylyl isocyanide, was treated with a protected D-glyceraldehyde (**5**) at −30 °C for 14 h to give **6** in good diastereoselectivity (10:1) with the anti isomer predominating. The stereoselectivity may be explained by Felkin's dipole model[7] in the transition state. It is noteworthy that the stereoselective construction of the carbon framework of 2-pentulose was accomplished in one step. The imino group of **6** was selectively hydrolyzed by treatment with HCl to give ketone **7**. After separation of *anti*-**7** by HPLC, deprotection of the acetyl, cyclohexylidene, and benzyl groups was performed

Table I. Reaction of **2** with Carbonyl Compounds[a]

entry	carbonyl compound	quenching[b]	product[c]		yield (%)
1	Et-CHO	A		**3a**	86
2	*i*-Pr-CHO	A		**3b**	90
3	*t*-Bu-CHO	A		**3c**	63
4	*p*-Cl-C6H4-CHO	A		**4d**	68[d]
5	3-pentanone	B		**3′a**	86
6	cyclohexanone	B		**3′b**	quant.
7		B		**4e**	70[d,e]
8	MeCH=CH-COMe	B		**3′c**	quant.[e]

[a] Unless otherwise noted, a mixture of SmI_2 (1.5 mmol) in THF (15 mL), HMPA (4 mmol), **1** (0.75 mmol), and isocyanide (0.50 mmol) was stirred at −15 °C for 3 h, and then carbonyl compound (0.25 mmol) was added. The resulting mixture was stirred at 0 °C for an additional 14 h. [b] A: Ac_2O (1.5 mmol), pyridine (2 mmol), 4-(dimethylamino)pyridine (cat.). B: H_2O. [c] Satisfactory NMR (^1H, ^{13}C) and IR spectra and combustion analyses or high-resolution mass spectra were obtained for all coupling products. [d] The product was isolated after hydrolysis to ketone **4**. [e] The reaction of **2**, generated in situ, with carbonyl compound was performed at −10 °C for 14 h.

Scheme I. Synthesis of D-*erythro*-2-Pentulose (**10**)



to afford D-*erythro*-2-pentulose (D-ribulose) (**10**) as shown in Scheme I. The structure of the product (**10**) was confirmed by its ^{13}C NMR spectra[8] and derivation to (*o*-nitrophenyl)hydrazone.[9]

(4) For a review on SmI_2-mediated synthetic reactions, see: Kagan, H. B.; Namy, J. L. *Tetrahedron* **1986**, *42*, 6573.

(5) Samarium(II) iodide mediated coupling of benzyl chloromethyl ether with carbonyl compounds: Imamoto, T.; Takeyama, T.; Yokoyama, M. *Tetrahedron Lett.* **1984**, *25*, 3225.

(6) With aldehydes, the addition products in the form of **3′** were unstable and difficult to purify while acetylated products **3** were rather stable.

(7) Chelest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199.

(8) Suzuki, K.; Yuki, Y.; Mukaiyama, T. *Chem. Lett.* **1981**, 1529.

(9) (*o*-Nitrophenyl)hydrazone of **10**: mp 168–169 °C; $[\alpha]^{21}_D$ −48.4° (*c* 0.345, MeOH); lit.[10] mp 168–169.5 °C, $[\alpha]^{20}_D$ −48.3° (*c* 0.32, MeOH).

Further application of the present methodology to syntheses of corticosteroids and aglycon of anthracycline antibiotics is in progress in our laboratory.

Acknowledgment. This work was supported in part by the Ministry of Education, Science and Culture, Japan [Grant-in-Aid for Scientific Research on Priority Areas (01649005, Multiplex Organic Systems)].

Supplementary Material Available: Experimental details of reaction of **2** with aldehydes and hydrolysis of **3** or **3'** to **4** and spectral and analytical data for **3a-c**, **3'a-c**, **4a-e**, **6**, *anti*-**7**, **8**, and **9** (4 pages). Ordering information is given on any current masthead page.

(10) Glatthaar, C.; Reichstein, T. *Helv. Chim. Acta* **1935**, *18*, 80.

Observation of the Cation Radicals of Pyrrole and of Some Substituted Pyrroles in Fast-Scan Cyclic Voltammetry. Standard Potentials and Lifetimes

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Polypyrroles and polysubstituted pyrroles have attracted considerable and increasing attention over the past 10 years in view of their remarkable conducting and electrocatalytic properties.[1] Oxidative electropolymerization of pyrrolic monomers is a convenient and attractive route to polypyrrole electrode coatings and free-standing films. In this connection, rather little is known about the mechanism of the electrochemical reactions involved in the first stages of the electropolymerization process. Although valuable information has been gained about the nucleation processes following the initial generation of dimeric and polymeric species,[2] the mechanism by which these dimers are formed has not been ascertained.[3] Likewise, the standard potentials at which the cation radicals are formed as well as their lifetimes are not known. The reason for this lack of information concerning the reactivity of the electrochemically generated pyrrole cation radical is that the measurement times employed in the experimental studies[2] carried out by potential-step and cyclic voltammetric techniques were too long to allow the observation of the cation radical by means of its rereduction current. Follow-up processes, such as polymer formation from oligomers and polymer growth through nucleation, were the only events that could be observed within the investigated time scales.

In the present preliminary report, we show that it is possible to overcome these difficulties by use of recently developed ultramicroelectrode techniques[4] and thus to observe the pyrrolic cation radicals through their rereduction wave in fast-scan cyclic

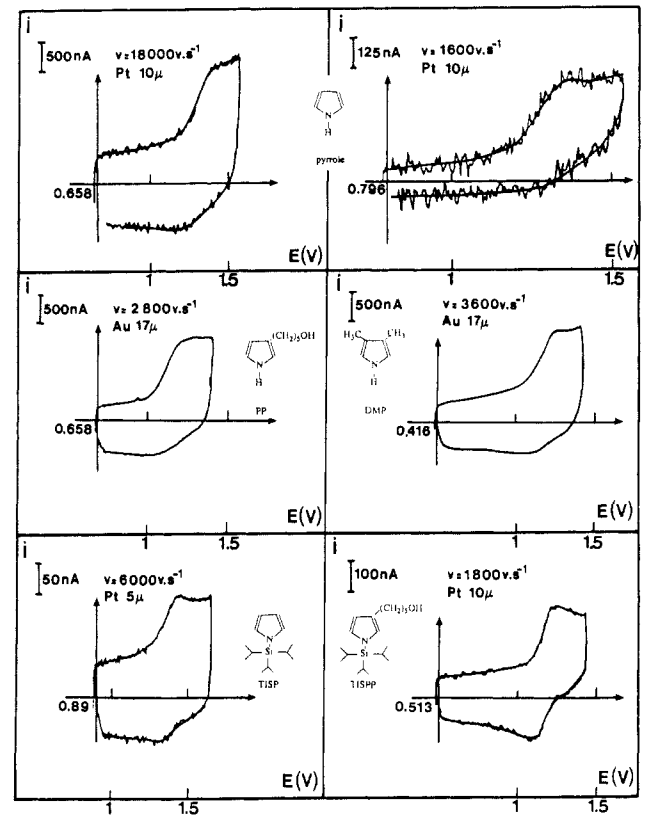

Figure 1. Cyclic voltammetry of pyrrole and substituted pyrroles (4 mM, except for TISPP: 10 mM) in acetonitrile + 0.6 M Et_4NClO_4 at 20 °C. The compound, the nature and diameter of the ultramicroelectrode, and the scan rate are indicated in each diagram. The instrumentation was the same as previously described.[4k]

Table I

compd	std potential	lifetime, μs
pyrrole	1.31 ± 0.02	30
PP	1.11 ± 0.02	150
TISP	1.35 ± 0.01	250
DMP	1.08 ± 0.01	300
TISPP	1.17_3 ± 0.005	2000

(1) (a) The literature on polypyrroles contains over 1000 references. For a general review, see ref 1b, and for a recent review on their electrocatalytic properties, see ref 1c. (b) Street, G. B. *Handbook of Conducting Polymers*; Skotheim, T. A., Ed.; Marcel Dekker: New York, 1986; pp 265-291. (c) Deronzier, A.; Moutet, J. C. *Acc. Chem. Res.* **1989**, *22*, 249.

(2) (a) Genies, E. M.; Bidan, G.; Diaz, A. F. *J. Electroanal. Chem.* **1983**, *149*, 101. (b) Inoue, T.; Yamase, T. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 985. (c) Asavapiriyamont, S.; Chandler, G. K.; Gunuwardena, g. A.; Pletcher, D. *J. Electroanal. Chem.* **1984**, *177*, 229. (d) Asavapiriyamont, S.; Chandler, G. K.; Gunuwardena, G. A.; Pletcher, D. *J. Electroanal. Chem.* **1984**, *177*, 245. (e) Downard, A. J.; Pletcher, D. *J. Electroanal. Chem.* **1986**, *206*, 139. (f) The situation is similar for polythiophenes,[2g-j] another class of interesting conducting polymers usually synthesized in the same electrolytic way.[2k] (g) Hillman, A. R.; Mallin, E. F. *J. Electroanal. Chem.* **1987**, *220*, 351. (h) Lang, P.; Chao, F.; Costa, M.; Garnier, F. *Polymer* **1987**, *28*, 668. (i) Downard, A. J.; Pletcher, D. *J. Electroanal. Chem.* **1986**, *206*, 147. (j) Lang, P.; Chao, F.; Costa, M.; Garnier, F. *J. Chim. Phys.* **1989**, *86*, 107. (k) Tourillon, G. *Handbook of Conducting Polymers*; Skotheim, T. A., Ed.; Marcel Dekker: New York, 1986; pp 293-350.

(3) (a) In the case of pyrrole, the dimerization has been assumed sometimes to result from the radical-radical coupling of two cation radicals[2a] and sometimes to involve the reaction of one cation radical with a pyrrole molecule.[2b-d] The latter mechanism has also been hypothesized in the case of thiophene.[2g-j] (b) While the mechanism of the dimerization of electrochemically generated anion radicals is reasonably understood and has been shown to be of the radical-radical coupling time in most cases (see refs 3c,d and references cited therein), much less is known in the case of cation radicals. The dimerization of the cation radical of 4-methoxybiphenyl, a rather slow reaction, is the only case that has been investigated in some detail.[3e] The reaction was first thought to proceed via radical-radical coupling of two cation radicals[3e] and later shown to involve the coupling of one cation radical with one molecule of the starting 4-methoxybiphenyl, followed by the oxidation of the ensuing dimeric cation radical by another monomeric cation radical.[3f] (c) Savéant, J.-M. *Acta Chem. Scand. B* **1983**, *37*, 365. (d) Savéant, J.-M. *Acta Chem. Scand. B* **1988**, *42*, 721. (e) Aalstad, B.; Ronlan, A.; Parker, V. D. *Acta Chem. Scand. B* **1981**, *35*, 874. (f) Amatore, C.; Savéant, J.-M. *J. Electroanal. Chem.* **1983**, *144*, 59.

(4) (a) Howell, J. O.; Wightman, R. M. *Anal. Chem.* **1984**, *56*, 524. (b) Howell, J. O.; Goncalves, J.; Amatore, C.; Klasinc, L.; Kochi, J.; Wightman, R. M. *J. Am. Chem. Soc.* **1984**, *106*, 3968. (c) Montenegro, M. I.; Pletcher, D. *J. Electroanal. Chem.* **1986**, *200*, 371. (d) Fitch, A.; Evans, D. H. *J. Electroanal. Chem.* **1986**, *202*, 83. (e) Amatore, C.; Jutand, A.; Pflüger, F. *J. Electroanal. Chem.* **1987**, *218*, 361. (f) Andrieux, C. P.; Garreau, D.; Hapiot, P.; Pinson, J.; Savéant, J.-M. *J. Electroanal. Chem.* **1988**, *243*, 321. (g) Andrieux, C. P.; Garreau, D.; Hapiot, P.; Savéant, J-M. *J. Electroanal. Chem.* **1988**, *248*, 447. (h) Andrieux, C. P.; Hapiot, P.; Savéant, J.-M. *J. Phys. Chem.* **1988**, *92*, 5987. (i) Wightman, R. M.; Wipf, D. O. *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1989; Vol. 15, pp 267-353. (j) Wipf, D. O.; Wightman, R. M. *J. Phys. Chem.* **1989**, *93*, 4286. (k) Garreau, D.; Hapiot, P.; Savéant, J.-M. *J. Electroanal. Chem.*, in press.

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