Supplementary Material Available: Listings of experimental details and spectroscopic characterization for compounds **2–11** and analytical data and stereoview of the optimized bound conformations of Ro 31-8959 and inhibitor **12** superimposed in the inhibited HIV-1 protease active site (8 pages). Ordering information is given on any current masthead page.

(18) Proton NMR and infrared spectra are consistent with assigned structures. Satisfactory (±0.4%) elemental analyses were obtained for new compounds, and all melting points were determined on a Thomas-Hoover capillary melting apparatus and are uncorrected.

Observation of a Small Oligonucleotide Duplex by Electrospray Ionization Mass Spectrometry

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Electrospray ionization (ESI) has been demonstrated to be a "soft" ionization technique, allowing accurate molecular weight determination for biopolymers due to gas-phase multiple charging.[1] Recent results have demonstrated that noncovalent associations can be preserved upon transfer into the gas phase with ESI, providing a new approach to the determination of both structurally-specific[2] and nonspecific[3] noncovalent associations in solution. The mass spectrometric experimental conditions necessary to preserve such noncovalent associations, and the physical constraints upon such, have yet to be elucidated, although it is clear that gentle interface conditions minimizing internal excitation of noncovalent complexes are helpful.[1c,2,3] Base-paired oligonucleotide hybridization constitutes one of the most important and thoroughly studied noncovalent associations of biopolymers. Our initial attempts to observe duplex oligonucleotides resulted in detection of only the monomeric constituents. Since then, we have developed interface conditions that are more gentle and yet still provide sufficient molecular ion desolvation to preserve such associations using new instrumentation with a greatly extended m/z range. In this communication, we report the successful ionization of duplex oligonucleotides and the conditions necessary for detection by negative ion ESI-MS.

Two complementary 20-base single-stranded oligodeoxyribonucleotides were prepared with a DNA synthesizer (Applied Biosystems Model 381A) by phosphoramidite chemistry. The samples were purified by reversed-phase HPLC followed by Centricon 3 (Amicon) ultrafiltration to remove residual salt impurities. Oligonucleotide I contained pyrimidine bases with the sequence 5′-dCCTTCCTCCCTCTCTCCTCC-3′ (M_r 5826.9), and oligonucleotide II was composed of the complementary purine bases with the sequence 5′-dGGAGGAGAGAGGGAGGAAGG-3′ (M_r 6410.3), chosen to avoid ambiguous hybridization of the complementary single strands. Also, their significant differences in M_r allowed potential nonspecific self-association to be resolved under the low-resolution mass spectrometric conditions employed. The purity of the single strands was confirmed by mass spectrometry, and their concentrations were determined by UV spectrophotometry. The single-stranded oligonucleotides (~1×10^{-4} M) were annealed in an aqueous 10 mM NH_4OAc solution by heating to 90–100 °C for 5 min and then slowly cooling to room temperature over 3 h. This solution was chosen to provide sufficient counterions to shield the negatively-charged phosphate groups and facilitate hybridization.

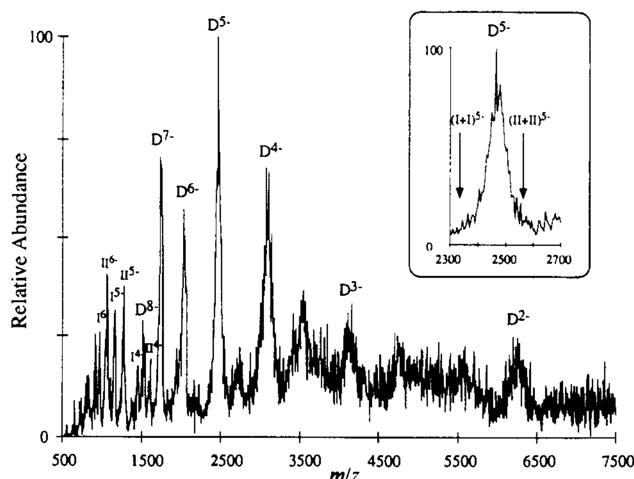

Figure 1. Negative ion ESI mass spectrum of a 20-mer oligodeoxyribonucleotide duplex obtained on the extended m/z range quadrupole mass spectrometer with a mildly heated capillary interface. Capillary–skimmer voltage = 68 V and capillary heating = 23 W (~180 °C at external surface). This spectrum is from the average of five scans consuming approximately 100 pmol. Peaks labeled D are from the duplex ions, and I and II are from the indicated charge states of the complementary single-stranded ions. The insert shows the 5− charge state region of the hybrid duplex ion at m/z ~2446 and the absence of the nonspecific association ions $(I + I)^{5-}$ at m/z 2330 and $(II + II)^{5-}$ at m/z 2563.

Negative ion ESI mass spectra were obtained using a new extended m/z range (~45 000) quadrupole mass spectrometer developed in our laboratory employing a heated capillary interface.[4] All mass spectra were obtained under conditions intended to minimize heating of molecular ions (i.e., low capillary–skimmer interface voltage, low capillary temperature), but which still provide sufficient excitation to give sufficient desolvation.[5] Figure 1 shows a low-resolution mass spectrum of the hybridized sample in 10 mM NH_4OAc showing multiply charged ions (D^{8-} to D^{2-}) corresponding to the intact duplex (D) dominating at m/z ~2500. It is noteworthy that, while both single-stranded constituents (I and II) are observed at low m/z, the duplex form is predominant at higher m/z. The insert in Figure 1 shows an expansion of the m/z region of the 5^- charge state of the double-stranded duplex ion (D^{5-}) at m/z 2446 corresponding to the specific hybridization of the complementary single-stranded species (I + II). Homodimeric association of each strand during the electrospray process would be expected to produce additional 5− ions at m/z 2330 (I + I) and m/z 2563 (II + II) which are not observed, indicating that a more specific association in solution was involved in producing the duplex ions. As expected, the intact duplex was not observed when distilled deionized water was substituted as the

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(5) Electrospray conditions utilized a 0.4 μL/min sample flow, a methanol sheath (3.0 μL/min), a coaxial flow of SF_6 (~100 mL/min) which serves to suppress electrical discharges during negative ion production, and a countercurrent flow of heated N_2 for desolvation. An aqueous sheath flow gave similar mass spectra but weaker ion signals.

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solvent. The measured molecular weights for the individual species of 5827 ± 2 Da (I) and 6410 ± 2 Da (II) were consistent with the sequence within experimental uncertainty. The measured molecular weight of the duplex (12 297 ± 10 Da) is higher than that calculated (M_r 12 237.1). This may be the result of insufficient desolvation of these higher molecular weight, lower charge state ions compared to their single-stranded counterparts or the result of greater counterion association (e.g., residual Na^+, NH_4^+, etc., which may remain and indeed may be necessary to stabilize the duplex structure in the gas phase).

The present results are the first observation of duplex oligonucleotides from solution by mass spectrometry, a potentially significant first step toward the study of oligonucleotide and nucleic acid associations. The results show that careful choice of both solution and ESI-MS interface conditions are crucial, consistent with earlier reports for the analysis of noncovalent complexes.[2,3] An extended *m/z* range (>*m/z* 2000) may also be useful for such observations since the intact duplex is observed with significant intensity only in relatively low charge states. It is uncertain whether the monomeric constituents observed in the mass spectra are representative of the solution or whether they are products of dissociation of Coulombically destabilized higher charge states of the duplex. Indeed, one expects decreased stability for the gas-phase duplex in higher charge states as desolvation removes the dielectric shielding provided by the solvent. Higher charge state ions are also collisionally activated to a greater extent in the interface than lower charge state ions[6] and so are subject to dissociation by the same processes required to desolvate the lower charge state ions. Further studies of larger duplex structures and other noncovalent nucleotide complexes are in progress.

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Chiral Poisoning: A Novel Strategy for Asymmetric Catalysis

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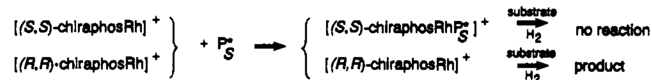
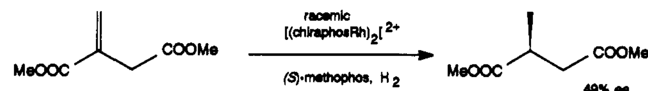
Asymmetric synthesis is an important goal in contemporary pharmaceutical and agricultural chemistry.[1] Catalytic asymmetric hydrogenation in particular has proven useful in many situtations, such as the industrial production of L-Dopa.[2] Asymmetric chelating bisphosphines, the key ligands in many of these metal-catalyzed reactions, are costly and can degrade with time. Our new approach offers the possibility of using the readily available racemic ligands and selectively poisoning one hand of the chiral catalyst with an inexpensive chiral poison.

We have investigated the racemic version of [(chiraphos)Rh(NBD)]BF_4, which was developed and extensively studied in the enantiomerically pure form by Bosnich [chiraphos = $Ph_2P(CHMe)_2PPh_2$].[3] Reduction with hydrogen releases a catalytically active species $[(chiraphos)Rh]^+$, which may be solvated or a dimer, $[(chiraphos)Rh]_2^{2+}$.[4,5] The dimer can be isolated and is a useful catalyst precursor, as it can dissociate in solution to yield $[(chiraphos)Rh]^+$. If racemic chiraphos is used to prepare the dimer, dissociation should provide equal amounts of $[(S,S)\text{-chiraphosRh}]^+$ and $[(R,R)\text{-chiraphosRh}]^+$. If a poison selectively deactivated all of one enantiomer, then the hydrogenation due to the remaining active catalyst would yield a product of high enantiomeric purity. In the ideal case one-half an equivalent of poison per equivalent of racemic catalyst would leave one-half of an equivalent of active homochiral catalyst.



In our initial screening, we found an effective poison for the asymmetric hydrogenation of dimethyl itaconate. It demonstrates the principles involved, and we report our observations here since we believe the strategy will be applicable to many asymmetric catalyst systems. Our chiral poison, which we call (*S*)-methophos, (*S*)-$[Ph_2POCH_2CH(NMe_2)CH_2CH_2SMe]$, is readily prepared from methionine.[7] Although this ligand can be effective in other types of asymmetric synthesis, it is a very poor ligand for the asymmetric hydrogenation of dimethyl itaconate and yields dimethyl methylsuccinate in <2% ee. We find that it can act as an effective chiral poison, however.

In a typical experiment, a solution of the racemic dimeric catalyst was stirred in THF under nitrogen with 0.7 equiv of (*S*)-methophos ((*S*)-mtp:Rh = 0.7:1) for 2 h. Dimethyl itaconate (DMI:Rh ≈ 15:1) was then added and the reaction mixture was transferred to a pressure reactor. The reaction was complete within 3 h at moderate pressures (~800 psi). The enantiomeric purity of the dimethyl methylsuccinate product was determined by chiral NMR shift experiments using $Eu(hfc)_3$.



As expected $[(chiraphos)Rh]_2^{2+}$ dimers produced from racemic chiraphos and hydrogenation in the presence of achiral diphos produced dimethyl methylsuccinate in 0% ee. Since the $[(S)\text{-methophosRh}]^+$ produced a product with <2% ee, the poisonong has dramatically enhanced the enantioselectivity of the asymmetric catalysis.

Hydrogenation using the pure (*R,R*)-chiraphos rhodium complex yields the (*S*)-methylsuccinate in high enantiomeric purity (>98% ee). Since (*S*)-methylsuccinate predominates in our system, we assume that the principal hydrogenation path involves $[(R,R)\text{-chiraphosRh}]^+$ and that the primary role of the (*S*)-methophos is to bind to $[(S,S)\text{-chiraphosRh}]^+$ and so reduce its equilibrium concentration.[10]

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(5) There are actually several dimers present here,[4] as well as in the diphos analogue.[6] In the dimers of chiraphos a phenyl group in one monomer bonds to the Rh of the other, which renders the phosphorus center chiral. This further complicates the ^{31}P NMR since diastereomers are formed owing to chirality at the carbon as well as at phosphorus. Studies of the poisoning are complicated by the multiplicity of diastereomeric species, as well as exchange phenomena which lead to broadening of the NMR spectra of species interacting with the methophos.

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