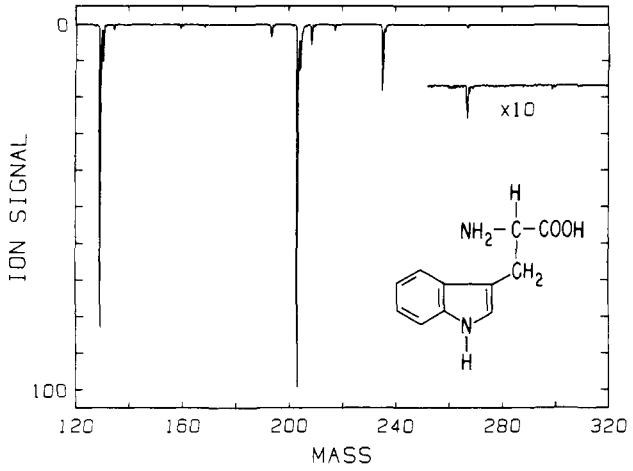

Figure 1. Mass spectrum of tryptophan obtained from a thermospray jet of 10^{-4} M tryptophan in methanol expanded in a supersonic molecular beam of helium. The data are an average over 1200 laser shots. Prominent features are assigned to ions of CH_2-indole (mass 130), tryptophan (mass 204), Trp-CH_3OH (mass 236), and Trp-$(CH_3OH)_2$ (mass 268). The mass spectrum was produced by photoionization at 285 nm.

produced by the second harmonic of a Nd:YAG pumped pulsed dye laser operating at a 10-Hz repetition rate. Thus far, we have observed nonresonant two-photon ionization with the laser operating at various wavelengths in the region 265-295 nm. The entire mass spectrum was collected at each laser shot by a 10 ns/channel transient digitizer, and the results of many laser shots were averaged by a computer. Appropriate synchronization of the valve opening, the laser pulse, and the transient digitizer was produced by digital delay generators.

The mass spectrum of the molecular beam generated by this apparatus, as shown in Figure 1, is dominated by a large peak at mass 204, the parent mass of tryptophan, with a second intense peak appearing at mass 130, the mass of the CH_2-indole fragment. Under the conditions used to produce Figure 1, no significant peaks are seen at masses higher than those shown. In particular, we were unable to observe a peak at mass 408, the mass of the tryptophan dimer. Satellite peaks appear at masses 205 and 131 with intensities consistent with naturally abundant ^{13}C substitution in ions containing 11 and 9 carbon atoms, respectively.[9] When we use a CH_3OH-D_2O solvent, the mass spectrum contains peaks at masses 130, 131, 206, 207, and 208 with relative intensities appropriate for deuterium substitution of the four exchangeable hydrogens of tryptophan[10] or the single exchangeable hydrogen of CH_2-indole. The mass spectrum produced under identical conditions but using a pure methanol-water solvent without tryptophan produces none of the above mass peaks.

Of particular interest are the mass peaks at 236 and 268. These are the parent ions of the clusters Trp-CH_3OH and Trp-$(CH_3OH)_2$, respectively. The existence of these small, weakly bound clusters suggests that the supersonic expansion has indeed produced the cooling necessary for the production of these clusters. The nature of these partially solvated molecules is a matter of some interest.

We believe that the mass 204 peak is produced by photoionization of single uncomplexed neutral tryptophan molecules that are present in the supersonic molecular beam. An alternative explanation would be that the molecular beam contains only large clusters or droplets of solvated tryptophan and that the mass 204 ion is produced by laser desorption ionization from the surface of these large clusters. We find that when we greatly increase the methanol flow rate, we do observe cluster ions containing up to 20 methanol molecules and the fraction of mass 204 ion decreases substantially. This observation indicates that when larger clusters are produced in the jet, the clusters survive the ionization process and appear as high-mass ions. This being the case, it seems highly unlikely under the conditions used to obtain Figure 1 that larger neutral clusters would fragment during ionization to produce only mass 204 and lighter ions without also producing larger cluster ions.

It should be noted that the fringing field of the mass spectrometer grids will prevent ions from entering the mass spectrometer, and in fact we see no ions that are not synchronous with the firing of the laser. In the experiments of Vestal and coworkers,[5] the thermospray of ionic solutions produced gas-phase ions and allowed the observation of mass spectra with no auxiliary source of ionization. Our results indicate that, although under our conditions ions may be produced, there is a significant neutral component to the molecular beam.

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Registry No. DL-Tryptophan, 54-12-6.

(9) The resolution of the mass spectrometer is insufficient to completely resolve the ^{13}C-substituted peaks and to accurately determine the number of carbon atoms in the various ions.

(10) Gudgin, E.; Lopez-Delgado, R.; Ware, W. R. *J. Phys. Chem.* **1983**, *87*, 1559 The methanol-water solution was 85 mol % exchangeable deuterium, and the expected intensities at masses 205 and 204 were below our limit of detection.

1-Methyl-2-(2-hydroxyphenyl)imidazole: A Catalytic Phosphate Protecting Group in Deoxyoligonucleotide Synthesis†

Brian C. Froehler and Mark D. Matteucci*

Department of Molecular Biology
Genentech, Inc., South San Francisco, California 94080
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Protecting groups serve a key function in the chemical synthesis of deoxyoligonucletides.[1,2] DNA synthesis, via the phosphotriester method, typically utilizes an aryl protecting group at the phosphate diester to prevent unwanted reactions at that site (Figure 1).[3] The blocking moiety, commonly an *o*- or *p*-chlorophenyl ester (**1a**,**1b**, Figure 1), is positioned at the site of reaction when forming the internucleotide bond between the electrophilic 3′-phosphate (**1**, Figure 1) and the nucleophilic 5′-hydroxyl (**2**, Figure 1). We report the use of a phenyl protecting group bearing a 1-methylimidazole moiety in the proper position to significantly enhance the rate of internucleotide bond formation.

Rate enhancement of chemical reactions by neighboring-group participation is well-known[4] the ultimate example of such phenomenon being enzyme catalysis.[5] The mechanism for hydrolysis of phosphorylethanolamine triesters has also been well studied, and a large rate enhancement due to neighboring-group participation of the amino function was observed.[6] The condensation reaction of a nucleotide 3′-phosphate diester and a nucleotide 5′-hydroxyl using an aryl sulfonyl chloride (Figure 1) is catalyzed by addition of heterocyclic amines such as 1-methylimidazole, 4-(dimethylamino)pyridine, and 5*H*-tetrazole.[7] While the mechanism of such catalysis has not been rigorously elucidated, the heterocyclic amine presumably serves as a nucleophilic catalyst. We investigated whether the positioning of a 1-methylimidazolyl moiety ortho to the phosphate ester (**1c**, Figure 1) would lead to increased rate of condensation, possibly via an active cyclic intermediate.

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(1) Greene, T. W. "Protecting Groups in Organic Synthesis"; Wiley: New York, 1981.
(2) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183.
(3) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143.
(4) Page *Chem. Soc. Rev.* **1973**, *2*, 295-323.
(5) Bruice, T. C. "The Enzymes, Kinetics and Mechanisms"; Academic Press: New York, 1970; Vol. 2, pp 217-279.
(6) Lazarus, R. A.; Benkovic, S. J. *J. Am. Chem. Soc.* **1979**, *101*, 4300-4312.

0002-7863/85/1507-0278$01.50/0

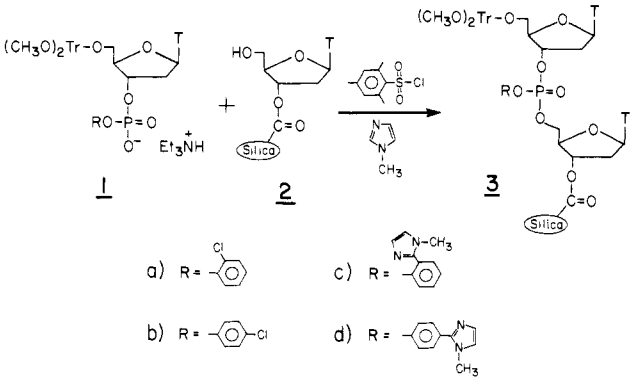

Figure 1. Phosphotriester method of deoxyoligonucleotide synthesis.

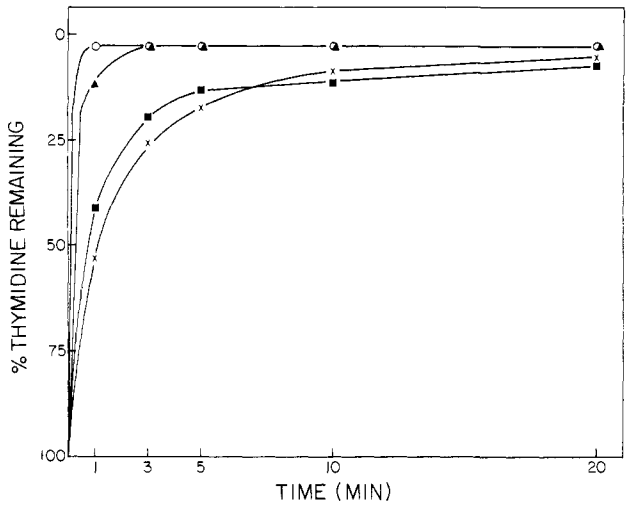

Figure 2. Relative reactivities of 3'-phosphate phenyl diesters of 5'-(dimethoxytrityl)thymidine: (A) (○) 50 mM *o*-(1-methylimidazoyl) (**1c**), (B), (▲) 5 mM *o*-(1-methylimidazoyl) (**1c**), (C) (■) 50 mM *p*-(1-methylimidazoyl) (**1d**), (D), (×) 50 mM *p*-chloro (**1b**).

1-Methyl-2-(2-hydroxyphenyl)imidazole was synthesized from methyl salicylate (Aldrich) and *N*-methylethylenediamine (Aldrich).[8] The corresponding 3'-phosphate phenyl diester of 5'-(dimethoxytrityl)thymidine (**1c**, Figure 1) was initially synthesized as a 2,4-dichlorophenyl triester[9] which was then hydrolyzed to the diester (concentrated NH_4OH/dioxane, 1/1, 60 °C, 12 h). The product diester was purified on silica gel (CH_3CN/H_2O, 9/1, v/v) then converted to the triethylammonium salt by evaporation from 5% Et_3N/CH_3CN and washing of a CH_2Cl_2 solution of this salt with 0.1 M aqueous triethylammonium bicarbonate (pH 8.5).

Relative reactivities of the different diesters toward an immobilized nucleoside 5'-hydroxyl were measured by reaction of diester (0.05 or 0.005 M), mesitylenesulfonyl chloride (0.15 M) and 1-methylimidazole (0.45 M) in pyridine with silica gel bearing a 3'-ester linked thymidine (**2**, Figure 1). The polymer support was loaded to approximately 30 μmol of thymidine per g of silica gel,[10] and a large excess (100 equiv) of the diester was used relative to immobilized thymidine. Reactions were quenched at various times with CH_3CN/H_2O (9/1, v/v), and the dimethoxytrityl group was removed (0.1 M *p*-TSA/CH_3CN).[10] After extensive washings and 2 h concentrated NH_4OH cleavage of the product from the silica gel, unreacted starting thymidine was quantitated by reverse-phase HPLC. A comparison of curves A and D in Figure 2 demonstrates that 1-methyl-2-(2-hydroxyphenyl)-imidazole diester (**1c**, Figure 1) is significantly more reactive than the known *p*-chlorophenyl derivative (**1b**, Figure 1). When the experiment is repeated using 5 mM 1-methyl-2-(2-hydroxyphenyl)imidazole diester (curve B, Figure 2) a rate increase of approximately 5-10-fold over 50 mM *p*-chlorophenyl diester (curve D, Figure 2) is observed.

Omission of 1-methylimidazole from the above condensation reaction results in a large decrease (90-fold) in rate of diester coupling with the *p*-chlorophenyl diester (**1b**, Figure 1), while a negligible effect is observed with the catalytic protecting group diester (1c, Figure 1) (data not shown). Curve C in Figure 2 demonstrates that 1-methyl-2-(4-hydroxyphenyl)imidazole diester (**1d**, Figure 1) shows no rate enhancement over the *p*-chlorophenyl diester. This diester has the 1-methylimidazoyl moiety positioned para to the phosphate, thereby precluding neighboring-group participation. These results strongly suggest the rate enhancement in curves A and B (Figure 2) is due to the ortho positioning of 1-methylimidazole and its participation in the rate-limiting step.

1-Methyl-2-(2-hydroxyphenyl)imidazole protecting group is removed from a phosphate triester by treatment with concentrated ammonium hydroxide (12 h, 60 °C) or by treatment with tetramethylguanidinium 2-pyridinealdoximate in dioxane/water (12 h, 60 °C).[11] The potential synthetic utility of this protecting group for the rapid synthesis of deoxyoligonucleotides was assessed by the synthesis of pentadecathymidylic acid (T_{15}) using short condensation times (5 min). HPLC analysis of the fully deprotected crude products confirmed a high-yield synthesis of T_{15}. This result and those shown above clearly demonstrate that catalytic protecting groups can effect a dramatic improvement of phosphorylation kinetics in the chemical synthesis of deoxyoligonucleotides.

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(7) Efimov, V. A.; Reverdatto, S. V.; Chakhmakhcheva, O. G. *Nucleic Acids Res.* **1982**, *10*, 6675.
(8) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1974**, *96*, 2463.
(9) Broka, C.; Hozami, T.; Arentzen, R.; Itakura, K. *Nucleic Acid Res.* **1980**, *8*, 5461.
(10) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185.
(11) Reese, C. B.; Zard, L. *Nucleic Acids Res.* **1981**, *9*, 4611.

A Novel Metal–Metal Bonded Iridium(II) Dimer

P. G. Rasmussen,* J. E. Anderson, O. H. Bailey, and Milton Tamres

Department of Chemistry, University of Michigan Ann Arbor, Michigan 48109

J. C. Bayón

Department de Química Inorgànica Facultat de Ciències Universitat Autònoma de Barcelona Bellaterra, Barcelona, Spain

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As part of an investigation of the ligand tetracyanobiimidazole (H_2Tcbiim)[1] we report the synthesis and structure of a novel dimer of iridium(II) containing the first example of an iridium–iridium bond without bridging ligands. Although such dimers are well-known in rhodium chemistry[2] the number of iridium(II) dimers previously reported is very small, and all have bridging ligands.[3]

The precursors to the iridium(II) dimer are salts of general formula M'[Ir$(CO)_2$Tcbiim] which have planar anions. The colors of the solids vary with the cation, e.g., when M' = $N(Et)_4^+$, red, $N(Me)_4^+$, green, and $C(NH_2)_3^+$, Na^+, or K^+, blue-black. In dilute solutions of acetonitrile all the salts are yellow, implicating an intermolecular association. We have studied this by the methods of charge-transfer spectroscopy and find ΔH = -27 kJ/mol and

(1) Rasmussen, P. G.; Hough, R. L.; Anderson, J. E.; Bailey, O. H.; Bayon, J. C. *J. Am. Chem. Soc.* **1982**, *104*, 6155.
(2) Felthouse, T. R. *Prog. Inorg. Chem.* **1982**, *29*, 73.
(3) Richards, R. L.; Leigh, G. J. "Comprehensive Organometallic Chemistry"; Wilkinson, G., Ed.; Pergamon Press: Oxford, 1982; Vol. 5, p 541.

0002-7863/85/1507-0279$01.50/0