Transient Protection: Efficient One-Flask Syntheses of Protected Deoxynucleosides[1]

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Abstract: Application of the concept of transient protection to the synthesis of protected deoxynucleosides is described. The deoxynucleosides are first treated with trimethylchlorosilane in pyridine for protection of the hydroxyl groups, and then immediately reacted with an acylating agent—benzoyl chloride for **1a** and **1b** and isobutyric anhydride for **1c**—to effect N-acylation. Hydrolysis of the trimethylsilyl groups takes a few hours in aqueous pyridine or a few minutes with dilute ammonia. The ammonia also effects selective hydrolysis of the initially formed *N*,*N*-dibenzoyldeoxyadenosine derivative (**3a**) to the desired *N*-benzoyldeoxyadenosine (**4a**). This one-flask procedure gives crystalline *N*-acyl deoxynucleosides **4a** and **4b** in 95% yield and **4c** in 75% yield, in only a few hours. The 5′-*O*-dimethoxytrityl deoxynucleosides **8a** and **8b** are also obtained in a one-flask procedure by initial reaction of the deoxynucleosides with 4,4′-dimethoxytrityl chloride, followed by treatment with trimethylchlorosilane and then benzoyl chloride. Although with deoxycytidine some of the 4-*N*,5′-*O*-bis(dimethoxytrityl) derivative (**5c**) is formed, benzoyl chloride effects conversion to the 4-*N*-benzoyl derivative (**7b**). After simple purification by flash chromatography **8a** and **8b** are each obtained in 80–90% overall yield from **1a** or **1b**.

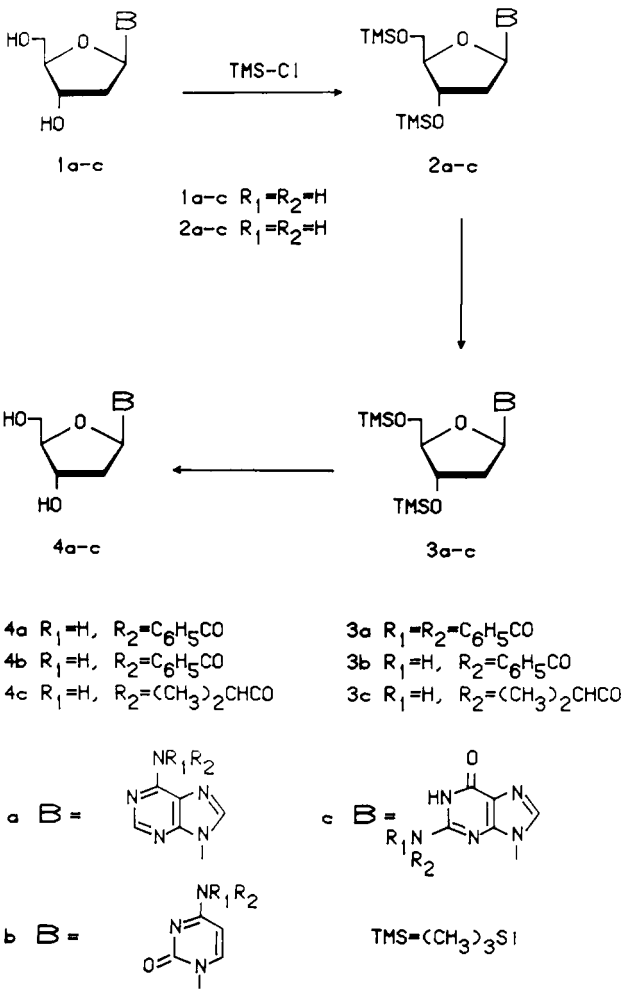
Synthetic oligonucleotides have proved to be invaluable tools of modern molecular biology.[2] Their uses include enzymatic construction of genes,[3-7] most recently an interferon gene,[8] physical studies of the stability and conformational behavior of nucleic acids,[9-14] and investigation of drug–nucleic acid[15] and protein–nucleic acid interactions.[16] Recent advances in chemical synthesis,[17-19] especially polymer-supported methods,[20-22] have markedly reduced the enormous amount of time and effort formerly required for oligonucleotide synthesis.[23,24] The effort devoted to protection of the monomers has become a significant aspect of a total synthesis. In fact, it may now be possible to synthesize an oligomer, by solid phase, in less time than it takes to protect the deoxynucleosides used in the synthesis.

The protecting group methodology and procedures still employed, in either solution or solid-phase synthesis, by either the triester or phosphite methods, are those originally developed by Khorana and co-workers for their diester method.[25,26] In this scheme the nucleosides are peracylated and the *O*-acyl groups then selectively cleaved with controlled hydroxide treatment to give the *N*-acyl derivatives **4a–c**. The 5′-hydroxyl is then protected with either an acid labile trityl derivative, usually the 4,4′-dimethoxytrityl group, or with the 2-(dibromomethyl)benzoyl group introduced recently by Reese.[17] Although this is a proven, reliable procedure, it is multi-step. As a result it is time consuming and gives only moderate overall yields. Attempts at selective N-acylation with use of benzoic anhydride,[27] 2-(chloromethyl)-4-nitrophenyl benzoate,[28] *O*-ethyl *S*-benzoyl dithiocarbonate,[29] *p*-nitrophenyl benzoate/1-hydroxybenzotriazole,[30] or pentafluorophenyl benzoate[31] have been successful only with cytosine nucleosides. With other nucleosides concomitant O-acylation invariably accompanies N-acylation.

Several ongoing projects in our laboratory require that we incorporate into oligonucleotides specifically deuterated or otherwise modified nucleosides which we have synthesized. We therefore sought a method for protecting nucleosides that would be both faster and more nucleoside efficient than the Khorana procedure. An obvious way to achieve overall selective N-acylation would be to protect the hydroxyl groups prior to the acylation reaction. This approach, however, would offer no improvement over the classic procedure unless the hydroxyl protecting groups could be much more easily removed than *O*-acyl groups.

We now wish to report application to this problem of the concept of *transient protection*. In this case such protection requires a protecting group which can be introduced quantitatively and is selective for the hydroxyl groups. Further, while it must be stable during N-acylation in anhydrous pyridine it should be hydrolyzed in aqueous pyridine. High-yield synthesis of the *N*-acyl nucleosides

Scheme I



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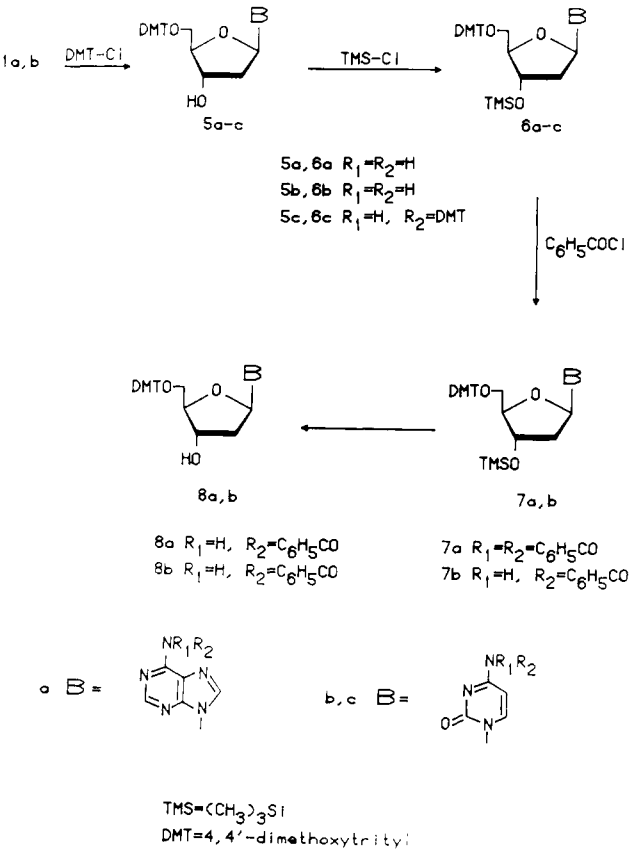
4a–c in one-flask reactions, using trimethylchlorosilane for transient hydroxyl protection, is shown in Scheme I.

Formation of the putative 3′,5′-*O*-bis(trimethylsilyl) derivatives **2a–c** occurs in minutes (TLC) upon addition of trimethylchlorosilane[32] to a suspension of the deoxynucleoside (**1a–c**) in dry pyridine. These necessarily labile compounds have not been isolated and the structures shown must therefore be presumed on the basis of subsequent reactions. Implicit in the criteria for transient protection is that the reagent, in this case trimethylchlorosilane, must not interfere with subsequent reactions. In fact, N-acylation is unaffected by the presence of even a substantial excess of trimethylchlorosilane. We have used benzoyl chloride for protection of deoxyadenosine and deoxycytidine and isobutyric anhydride for protection of deoxyguanosine. In each case the acylation is complete within 2 h. The reaction mixture is then cooled to 0 °C and quenched with water. Under these conditions hydrolysis of the trimethylsilyl groups requires about 4 h.

Reaction of adenine nucleosides with benzoyl chloride is known[26] to give *N*,*N*-dibenzoyl derivatives, in this case **3a**, and we have isolated *N*,*N*-dibenzoyl-2′-deoxyadenosine from this reaction. However, selective hydrolysis of one of the benzoyl groups is achieved by brief treatment with 2 M aqueous ammonia, with no trace of complete N-deprotection. Ammonia also effects instant removal of the trimethylsilyl groups and for this reason is employed with **3b** and **3c** as well. In each case concentrated aqueous ammonia is added to the reaction mixture to give a 2 M solution. Finally, the N-protected nucleosides **4a–c** are obtained by crystallization from water in yields of 95% for **4a** and **4b** and 75% for **4c** from, respectively, **1a–c** in about 4 h.

Scheme II



Compounds **4a–c** are immediately usable, without further protection, as the hydroxy component in the triester schemes reported by Agarwal[33] and later by Reese[17] for synthesis of di- and trinucleoside blocks. The extent of undesired 3′,3′ joining is quite small and pure products are obtained by chromatography. Although the 5′-*O*-dimethoxytrityl derivatives of **4a–c** may be prepared by known procedures,[25,26] the success of our transient protection approach to synthesis of **4a–c** prompted us to explore its use for direct synthesis of the 5′-*O*-(dimethoxytrityl) derivatives. As shown in Scheme II, the deoxynucleosides are first treated with dimethoxytrityl chloride to give the 5′ protected derivatives **5a** and **5b**. In addition to **5b**, deoxycytidine gives a significant and unavoidable amount of the 4-*N*,5′-*O*-bis(dimethoxytrityl) compound **5c**. However, upon treatment of **5c** with trimethylchlorosilane and benzoyl chloride the desired product, **8b**, is obtained. Presumably **6c** reacts with benzoyl chloride to give a *N*-benzoyl-*N*-(dimethoxytrityl) derivative, which would be expected to be unstable and to lose the dimethoxytrityl group. Trimethylchlorosilane alone does not effect cleavage of the *N*-(dimethoxytrityl) group, nor does addition of anhydrides such as acetic and isobutyric. These anhydrides are considerably less reactive than benzoyl chloride and may be unable to acylate the hindered amino group of **6c**. Even using benzoyl chloride, conversion of **6c** to **7b** is much slower than simple benzoylation of **6b**. Nevertheless, treatment of either **5a** or the mixture of **5b** and **5c**, without isolation, gives **8a** or **8b** in a one-flask procedure, sufficiently pure for immediate phosphorylation. If desired they may be further purified by flash chromatography[34] in overall yields from **1a** or **1b** of 80–90%. This approach does not work well with deoxyguanosine, which undergoes preferential reaction at N-2

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upon treatment with 4,4'-dimethoxytrityl chloride.[25] Moreover, it has become clear that protection of guanine nucleosides is presently inadequate, and that an entirely new concept for the protection of guanine is required. Work along these lines is in progress.

Successful chemical synthesis of oligonucleotides relies on precise manipulation of hydroxy and amino protecting groups. In addition, lipophilic hydroxyl protecting groups have been shown to markedly enhance separation and thereby greatly aid purification of oligonucleotides.[35] Yet the chemical differentiation of nucleoside hydroxyl and amino groups necessary for selective protection has proved surprisingly difficult to achieve. Other than our transient protection approach, only the classic Khorana procedure[25] has allowed such differentiation. Our original goal was only to optimize methods for handling our modified nucleosides. However, the concept of transient protection we developed appears to be widely applicable to a variety of synthetic problems in multifunctional molecules. Some of these applications, principally in nucleoside and nucleotide synthesis, are under investigation in our laboratory and will be reported shortly.

Experimental Section

General Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-360 with Me_4Si as internal reference. Ultraviolet (UV) spectra were recorded on a Cary 118-C spectrophotometer. Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, Tenn. Thin-layer chromatography (TLC) was performed on Eastman Chromatogram sheets (silica gel No. 18181, indicator No. 6060) in methylene chloride containing from 1 to 10% methanol, as appropriate. Evaporations were carried out at 40 °C or lower using aspirator or oil pump vacuum.

Flash chromatography[34] was performed on EM silica gel 60, 230–400 mesh. Pyridine was refluxed over and then distilled from calcium hydride and stored over dried 4A molecular sieves. Trimethylchlorosilane, obtained from Aldrich Chemical Co., was handled by syringe and stored in an Aldrich Storage Flask, type C. Deoxynucleosides were purchased from Sigma Chemical Co., Leon Industries, or the United States Biomedical Corp.

6-*N*-Benzoyl-2'-deoxyadenosine (4a). To 2.5 g (10 mmol) of **1a** dried three times by evaporation of pyridine and suspended in 50 mL of dry pyridine was added 6.4 mL (50 mmol) of trimethylchlorosilane.[32] After the mixture was stirred for 15 min 5.8 mL (50 mmol) of benzoyl chloride was added and the reaction was maintained at room temperature for 2 h. The mixture was then cooled in an ice bath and 10 mL of water was added. After 5 min 20 mL of 29% aqueous ammonia was added and the mixture was stirred at room temperature for 0.5 h. The reaction was then evaporated to near dryness and the residue was dissolved in 150 mL of water. The solution was washed once with a 50-mL portion of ethyl acetate. Crystallization began immediately after separation of the layers. After the solution was cooled, filtration gave 3.33 g (94%) of **4a**: mp, UV, and NMR were identical with those of material prepared according to the literature.[25] Anal. ($C_{17}H_{17}N_5O_4 \cdot {}^1/_4H_2O$) C, H, N.

6-*N*,*N*-Dibenzoyl-2'-deoxyadenosine. Reaction was carried out as described above for compound **4a** except that the reaction was quenched by addition of 100 mL of cold saturated $NaHCO_3$. The solution was stirred for 4 h and then extracted with 2 × 100 mL portions of ethyl acetate. The combined ethyl acetate layers were evaporated to give the product as a foam in 87% yield. UV_{max} (MeOH) 249 mm (ε 20 100), sh 272 (ε 16 600); NMR ($CDCl_3$, D_2O) δ 8.67 (s, 1, H_8), 8.38 (s, 1, H_2), 7.28–8.08 (m, 10, Ar), 6.42 ("t", J = 7 Hz, 1, $H_{1'}$), 4.65 (m, 1, $H_{3'}$), 4.12 (m, 1, $H_{4'}$), 3.80 (br s, 2, $H_{5',5''}$), 2.08–2.88 (m, 2, $H_{2',2''}$). Anal. ($C_{24}H_{21}N_5O_5$) C, H, N.

4-*N*-Benzoyl-2'-deoxycytidine (4b). To 2.27 g (10 mmol) of **1b** dried three times by evaporation of pyridine and suspended in 50 mL of dry pyridine was added 6.4 mL (50 mmol) of trimethylchlorosilane.[32] After the solution was stirred 15 min 5.8 mL (50 mmol) of benzoyl chloride was added and the reaction maintained at room temperature for 2 h. The mixture was then cooled in an ice bath and 10 mL of water was added. After 5 min 10 mL of 29% aqueous ammonia was added and the mixture was stirred at room temperature for 15 min. The reaction was then evaporated to near dryness and the residue was dissolved in 150 mL of water. The solution was washed once with a 50-mL portion of ethyl acetate. Crystallization began immediately after separation of the layers. After the solution was cooled, filtration gave 3.11 g (94%) of **4b**: mp, UV, and NMR were identical with those of material prepared according to the literature.[25] Anal. ($C_{16}H_{17}N_3O_5$) C, H, N.

2-*N*-Isobutyryl-2'-deoxyguanosine (4c). To 1.34 g (10 mmol of **1c** dried three times by evaporation of pyridine and suspended in 50 mL of dry pyridine was added 6.4 mL (50 mmol) of trimethylchlorosilane.[32] After the solution was stirred 15 min 8.2 mL (50 mmol) of isobutyric anhydride was added and the solution was maintained at room temperature for 3 h. The reaction was then cooled in an ice bath and 10 mL of water was added. After 5 min 10 mL of 29% aqueous ammonia was added and the reaction was stirred for 15 min. The solution was then evaporated to near dryness and the residue was dissolved in 50 mL of water. The solution was washed once with a 50-mL portion of ethyl acetate:ether (1:1). The organic layer was extracted with a 25-mL portion of water and the combined aqueous layers were concentrated to about 30 mL. Crystallization generally occurred quickly. Concentration of the filtrate gave additional material for a total yield of 2.5 g (75%) of **4c**: mp (softens at 130 °C, darkens at 235 °C, does not melt below 300 °C, UV, and NMR were identical with those of material prepared according to the literature.[26] Anal. ($C_{14}H_{19}N_5O_5 \cdot {}^3/_4H_2O$) C, H, N.

5'-*O*-(Dimethoxytrityl)-2'-deoxyadenosine (5a). To 2.5 g (10 mmol) of **1a** dried three times by evaporation of pyridine and suspended in 50 mL of dry pyridine was added 5 g (15 mmol) of 4,4'-dimethoxytrityl chloride, 2.1 mL (15 mmol) of triethylamine, and 30 mg (0.25 mmol) of 4-dimethylaminopyridine.[36] After 4 h TLC (CH_2Cl_2:CH_3OH, 95:5) showed complete reaction.

The reaction was cooled in an ice bath and 50 mL of 5% aqueous $NaHCO_3$ was added. The mixture was then extracted with two 150-mL portions of ethyl acetate. The combined organic layers were evaporated to dryness and the residue purified by flash chromatography on silica gel.[34] The appropriate fractions were combined and evaporated and a methylene chloride solution of the residue was added dropwise to petroleum ether to precipitate 4.0 g (77%) of **5a**: UV_{max} (CH_3OH) 259, 236 nm (ε 17 900, 27 300), UV_{min} 254, 225 nm (ε 17 500, 22 700); ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1, H_8), 7.92 (s, 1, H_2), 7.5–7.0 (m, 9, aryl), 6.9–6.5 (m, 4, aryl), 6.37 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 6.07 (br s, 2, NH_2), 4.60 (m, 1, $H_{3'}$), 4.12 (m, 1, $H_{4'}$), 3.68 (s, 6, OCH_3), 3.32 (m, 2, $H_{5',5''}$), 3.1–2.3 (m, 2, $H_{2',2''}$). Anal. ($C_{31}H_{31}N_5O_5$) C, H, N.

5'-*O*-(Dimethoxytrityl)-6-*N*-benzoyl-2'-deoxyadenosine (8a). To the solution obtained at the end of the first paragraph above was added 0.4 mL (50 mmol) of trimethylchlorosilane.[32] After 30 min 5.8 mL (50 mmol) of benzoyl chloride was added and the reaction was stirred at room temperature for 2 h. The mixture was then cooled in an ice bath and 10 mL of water was added. After 5 min 20 mL of 29% aqueous ammonia was added and the mixture was stirred at room temperature for 30 min. The reaction was then evaporated to a gum and the gum partitioned between 150 mL of CH_2Cl_2 and 150 mL of 5% aqueous $NaHCO_3$. The organic layer was evaporated to dryness and the residue purified by flash chromatography on silica gel.[34] The appropriate fractions were combined and evaporated and a methylene chloride solution of the residue was added dropwise to petroleum ether to precipitate 5.5 g (88%) of 8a: UV and NMR were identical with those of material prepared according to the literature.[25] Anal. ($C_{38}H_{35}N_5O_6 \cdot {}^2/_3H_2O$) C, H, N, O.

5'-*O*-(Dimethoxytrityl)-2'-deoxycytidine (5b) and 5'-*O*,4-*N*-Bis(dimethoxytrityl)-2'-deoxycytidine (5c). To 2.6 g (10 mmol) of **1b** dried three times by evaporation of pyridine and suspended in 50 mL of dry pyridine was added 6.7 g (20 mmol) of 4,4'-dimethoxytrityl chloride, 2.8 mL (20 mmol) of triethylamine, and 50 mg (0.42 mmol) of 4-(dimethylamino)pyridine.[36] After 3 h TLC (CH_2Cl_2:CH_3OH, 95:5) showed that no unreacted **1b** was present.

The mixture was then poured into 250 mL of cold, saturated $NaHCO_3$ and the solution was extracted with three 150-mL portions of ethyl acetate. The combined organic layers were evaporated to dryness and the residue purified by flash chromatography on silica gel.[34] Appropriate fractions of the less polar compound were combined and evaporated and a methylene chloride solution of the residue was added dropwise to petroleum ether to precipitate 3.4 g (40%) of 5c: UV_{max} (CH_3OH) 280, 230 nm (ε 18 300, 45 300); UV_{min} 259 nm (ε 17 900); NMR ($CDCl_3$, D_2O) δ 7.58 (d, $J_{5,6}$ = 8 Hz, 1, H_6), 7.5–6.5 (m, 26, aryl), 6.32 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 4.83 (d, $J_{5,6}$ = 8 Hz, 1, H_5), 4.47 (m, 1, $H_{3'}$), 4.07 (m, 1, $H_{4'}$), 3.75 (s, 12, OCH_3), 3.33 (m, 2, $H_{5',5''}$), 2.8–1.9 (m, 2, $H_{2',2''}$). Anal. ($C_{51}H_{49}N_3O_8 \cdot {}^1/_2H_2O$) C, H, N, O.

Appropriate fractions of the more polar compound were combined and evaporated and a methylene chloride solution of the residue was added dropwise to petroleum ether to precipitate 1.7 g (32%) of **5b**: UV_{max} (CH_3OH) 274, 234 nm (ε 11 900, 28 800); UV_{min} 258 nm (ε 9100); NMR ($CDCl_3$, Me_2SO-d_6, D_2O) δ 7.82 (d, $J_{5,6}$ = 8 Hz, 1, H_6), 7.5–7.2 (m, 9,

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aryl), 7.0–6.7 (m, 4, aryl), 6.17 (t, $J_{apparent}$ = 7 Hz, 1, $H_{1'}$), 5.58 (d, $J_{5,6}$ = 8 Hz, 1, H_5), 4.40 (m, 1, $H_{3'}$), 4.23 (m, 1, $H_{4'}$), 3.80 (s, 6, OCH_3), 3.37 (m, 2, $H_{5',5''}$), 2.8-1.9 (m, 2, $H_{2',2''}$). Anal. ($C_{30}H_{31}N_3O_6 \cdot {}^1/_2H_2O$) C, H, N, O.

5′-*O*-(Dimethoxytrityl)-4-*N*-benzoyl-2′-deoxycytidine (8b). To the solution obtained at the end of the first paragraph above was added 6.4 mL (50 mmol) of trimethylchlorosilane.[32] After 15 min 5.8 mL (50 mmol) of benzoyl chloride was added and the reaction stirred at room temperature for 10 h. The reaction was then cooled in an ice bath and 10 mL of water was added. After 5 min 20 mL of 29% aqueous ammonia was added and the solution stirred at room temperature for 30 min. The mixture was then evaporated to a gum and the gum partitioned between 200 mL of CH_2Cl_2 and 200 mL of 5% aqueous $NaHCO_3$. The aqueous layer was extracted with two 100-mL portions of CH_2Cl_2, the combined organic layers evaporated to dryness, and the residue purified by flash chromatography on silica gel.[34] Appropriate fractions were combined and evaporated and a methylene chloride solution of the residue was added dropwise to petroleum ether to precipitate 5.1 g (81%) of **8b**: UV and NMR were identical with those of material prepared according to the literature.[25] Anal. ($C_{37}H_{35}N_3O_7$) C, H, N.

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Registry No. 1a, 958-09-8; **1b**, 951-77-9; **1c**, 961-07-9; **4a**, 4546-72-9; **4b**, 4836-13-9; **4c**, 68892-42-2; **5a**, 17331-22-5; **5b**, 76512-82-8; **5c**, 80594-29-2; **8a**, 80594-30-5; **8b**, 80594-31-6; 6-*N*,*N*-dibenzoyl-2′-deoxyadenosine, 6711-37-1.

Oxidation–Reduction Mechanisms. Inner-Sphere and Outer-Sphere Electron Transfer in the Reduction of Iron(III), Ruthenium(III), and Osmium(III) Complexes by Alkyl Radicals

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Abstract: Alkyl radicals are readily oxidized by the tris(phenanthroline) and tris(bipyridine) complexes ML_3^{3+} of iron(III), ruthenium(III), and osmium(III) in acetonitrile solution, the second-order rate constants easily exceeding 10^6 M^{-1} s^{-1} at 25 °C. Two oxidative processes are identified as (a) *ligand substitution* on the coordinated 1,10-phenanthroline to yield various alkylphenanthrolines and (b) *cation formation* to afford alkenes and *N*-alkylacetamides (after hydrolysis). Cation formation is characterized by extensive skeletal rearrangement of neopentyl, isobutyl, and *n*-propyl groups, whereas ligand substitution by the same alkyl radicals occurs without any rearrangement. Steric effects hinder ligand substitution since the rate constant k_L increases in the order neopentyl < isobutyl < *n*-propyl and $Fe(4,7\text{-}Ph_2phen)_3^{3+} < Fe(4,7\text{-}Me_2phen)_3^{3+} < Fe(phen)_3^{3+}$. By contrast, cation formation is not subject to steric effects and the rate constant k_R is invariant for neopentyl, isobutyl, and *n*-propyl radicals, which all have essentially the same ionization potentials. An outer-sphere mechanism for electron transfer is described for the oxidative process leading to cation formation, in accord with the fit of k_R to the linear free energy relationship established by Marcus theory. An inner-sphere mechanism for the oxidative process leading to phenanthroline substitution is discussed in the context of steric effects on the rate constant k_L for ligand substitution.

Oxidation–reduction processes mediated by transition-metal complexes are playing an increasing role in organic chemistry.[1] However, the mechanistic distinction in such processes between outer-sphere and inner-sphere electron transfer has not been established, largely owing to inadequately developed criteria.[2] By contrast, electron transfer mechanisms in wholly inorganic systems have been considered for some time,[3] and reasonably reliable experimental and theoretical guidelines have been developed.[4]

The oxidation–reduction of organic free radicals by transition-metal complexes provides an excellent opportunity to examine the mechanism of electron transfer in organic systems, by relying on some of the mechanistic criteria developed in inorganic chemistry. Thus the various alkyl radicals (R·) by their paramagnetic nature are necessarily constrained to undergo one-electron changes, i.e.[5,6]

$$R:^- \xleftarrow{-e^-} R\cdot \xrightarrow{-e^-} R^+$$

Likewise, in the family of coordinatively saturated tris(polypyridine) complexes of the iron triad ML_3^{3+}

we have a structurally and chemically homologous series of one-electron oxidants with graded redox potentials. Furthermore, the availability of ligands, especially with L = substituted 1,10-phenanthrolines, allows further fine tuning of the redox potentials and the steric properties of these oxidants.[7,8] Coupled with the

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