further stirred for 5 min and directly analyzed by GC. The competitive hydroxylation of cyclohexane and [D_{12}]cyclohexane was performed with a mixture of cyclohexane (0.3 mmol) and [D_{12}]cyclohexane (1 mmol).

Labeled water ($H_2{}^{18}O$) experiment: H_2O_2 (0.04 mmol, diluted in CH_3CN (0.5 mL)) was slowly added over a period of 1 h to a stirred solution containing [Fe(tpfpp)(NO_3)] (1×10^{-3} mmol), substrate (1 mmol), and $H_2{}^{18}O$ (50 μL, 95% ^{18}O enriched) in a solvent mixture (1 mL) of CH_3CN and CH_2Cl_2 (3:1). The reaction mixture was further stirred for 5 min and directly analyzed by GC/MS. The ^{16}O and ^{18}O compositions in cyclohexanol and cyclohexene oxide were determined by the relative abundances of mass peaks at *m*/*z* 57 and 59 for cyclohexanol and at *m*/*z* 83 and 85 for cyclohexene oxide. Control reactions, performed by stirring cyclohexanol-^{16}O or cyclohexene oxide-^{16}O in a solution containing [Fe(tpfpp)(NO_3)] and $H_2{}^{18}O$, showed that the oxygen of the products did not exchange with labeled water under the reaction conditions.

Electrochemical measurements: All electrochemical experiments were performed under an N_2 atmosphere in a glove box using a BAS 50W voltammetric analyzer. The cyclic voltammetric measurements were carried out in a solvent mixture of CH_3CN/CH_2Cl_2 (1:1) containing iron porphyrin (0.2 mM) and tBu$_4$NPF$_6$ (40 mM) as a supporting electrolyte in one compartment. The working electrode was a glassy carbon disk and the counter electrode was a platinum wire. The potential was measured by using a Ag/Ag^+ (0.01 M) reference electrode and reported versus a Fc/Fc^+ couple. The cyclic voltammograms were run at a scan rate of 50 mV s^{-1}.

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Dynamics of Hole Trapping by G, GG, and GGG in DNA**

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Oxidative damage to DNA by ionizing radiation, carcinogenic agents, and photosensitizers occurs predominately at guanine (G) bases,[1, 2] a result which can be rationalized by the hierarchy of in vitro oxidation potentials of the isolated nucleobases (G < A ≪ C,T).[3] Strand cleavage reactions, induced, for instance, by piperidine treatment,[4] have shown consistently that multiple guanine tracts in DNA are more susceptible to oxidative damage than isolated guanine bases.

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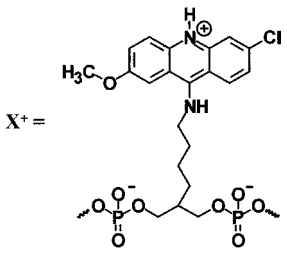
This feature has been utilized in long-range hole transport studies,[5] which are based on GGG being a better trap than a single G base. In addition, an orientational dependence of the oxidation site, which leads to strand cleavage, is observed because the energetics of guanine are dependent upon their immediate environment. In a GG trap, the 5′-guanine is the preferred cleavage site,[6, 7] and in GGG either the 5′- or central guanine is the preferred cleavage site depending upon the nucleobases immediately neighboring the trap.[8, 9] This view of preferred sites of charge localization in GG and GGG traps is supported independently by the quantum mechanical calculations of Voityuk et al.[10] The interesting question is now whether a distant hole donor populates the preferred cleavage site(s) in either GG or GGG in a direct unistep reaction or whether a sequential kinetic scheme must be envisioned, in which the nearest G is oxidized first and trapping of the charge at the preferred G occurs in a subsequent step (two-step mechanism).

To analyze the dynamics of hole trapping in multiple G tracts, we investigate here the kinetics of hole transfer (HT) from a photoexcited donor to either a G, GG , or GGG site. In order to optimize the sensitivity, we chose protonated 9-amino-6-chloro-2-methoxyacridine ($\mathbf{X}^+$)[11] which has been shown to undergo activated hole transfer to G in cases where one or more A:T base pair(s) serve as a bridge.[12] The most interesting result from the present study is that hole transfer proceeds in all cases to the closest guanine base. Subsequently relaxation of the hole occurs, which in multiple guanine tracts such as GG or GGG, leads to localization of the hole within the G tract. The site of localization may differ from initially oxidized state, and may be that responsible for strand cleavage.



The chemical structure of the $\mathbf{X}^+$ oxidant and the DNA sequences studied are shown in Scheme 1. All samples were characterized by their steady-state absorption and fluorescence spectra (Figure 1). Photoexcitation of $\mathbf{X}^+$ in all seven duplexes with ~2 ns, 450 nm pump pulses[13] results in two transient kinetic features (Figure 2). The first feature is the decay of the excited state absorption probed in the spectral region from 570 → 800 nm, characteristic for the $S_1 \rightarrow S_n$ absorption of $\mathbf{X}^+$. The second feature is the ground state recovery probed at 420 nm. As a representative for all duplexes under study, the transient features of 3′-$\mathbf{X}^+$-A-GG are shown in Figure 2. The equivalence of the two kinetic traces indicates that the rate determining step in the kinetic Scheme (Scheme 2) is the forward hole-transfer rate and that charge recombination cannot be resolved. This phenomenon is a necessary consequence of the high thermal activation of the forward rate[12] and a zero, or small, activation energy for the back transfer.[14]

Monoexponential rates obtained from least-squares fitting of these data are compiled in Table 1. The monoexponentiality, together with the structural characterization by Tanaka et al.,[11] points to the fact that the acridine must be intercalated in a well defined site.[15]

Duplex	*x*	*y*	*z*
$\mathbf{X}^+$-(AT)	A	A	T
5′-$\mathbf{X}^+$-A-G	G	A	T
5′-$\mathbf{X}^+$-A-GG	G	G	T
5′-$\mathbf{X}^+$-A-GGG	G	G	G
3′-$\mathbf{X}^+$-A-G	T	A	G
3′-$\mathbf{X}^+$-A-GG	T	G	G
3′-$\mathbf{X}^+$-A-GGG	G	G	G

Duplexes $\mathbf{X}^+$-(AT) and 5′-$\mathbf{X}^+$-A

5′-GCG TTA TAT A($\mathbf{X}^+$)A ***xyz*** TAT GCG-3′

Duplexes 3′-$\mathbf{X}^+$-A

5′-GCG TTA ***xyz*** A($\mathbf{X}^+$)A TAA TAT GCG-3′

Scheme 1. Structure of the $\mathbf{X}^+$ dye and the studied DNA sequences. An adenine base was placed opposite $\mathbf{X}^+$ in all duplexes similar to those in the studies of Tanaka et al.[11] and all other bases were paired with their normal Watson–Crick complements. DNA single strands were purchased from Eurogentec Köln (Germany) and delivered lysophilized after polyacrylamide gel electrophoresis (PAGE) purification. Stock solutions were made by dissolving the single strands in a buffer solution (10 mM Na_2HPO_4/NaH_2PO_4, 100 mM NaCl, pH 7.2). $\mathbf{X}^+$-labeled strands were mixed with a 10% excess of counter-strand to help ensure that all $\mathbf{X}^+$ molecules were incorporated into duplex DNA. Hybridization was performed by heating the mixtures of single strands to 80 °C, followed by slow cooling over 2 h to room temperature. All samples had an adenine base opposite to $\mathbf{X}^+$ on the counter-strand, similar to the studies of Tanaka et al.[11] All samples were placed in a quartz cuvette (1 mm pathlength) and had an optical density of ~0.3 at 450 nm.

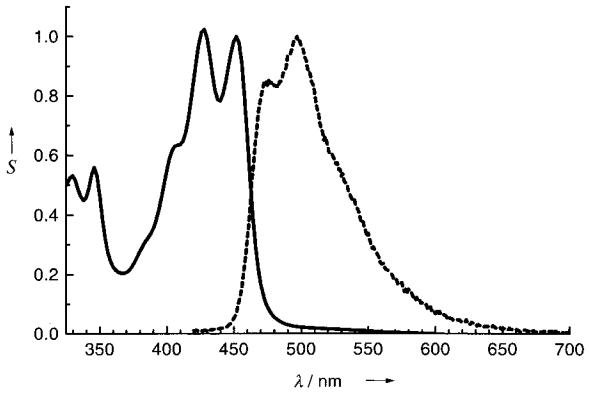


Figure 1. Ground state absorption (——) and static fluorescence spectra (- - - -) of $\mathbf{X}^+$ in duplex 5′-$\mathbf{X}^+$-A-G at 283 K. The absorption spectra shows $S_0 \rightarrow S_1$ absorption bands at 452 and 428 nm, and $S_0 \rightarrow S_2$ bands at 346 and 330 nm. The steady-state fluorescence has two bands at 474 nm and 497 nm.

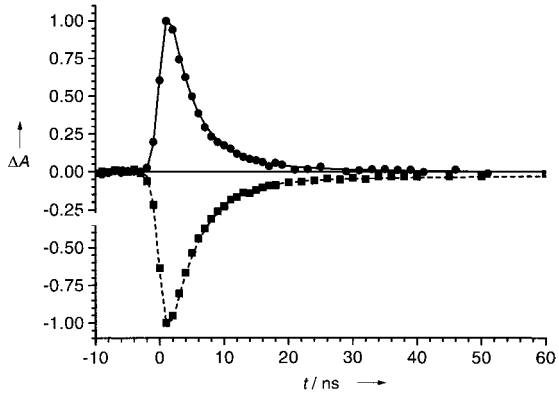
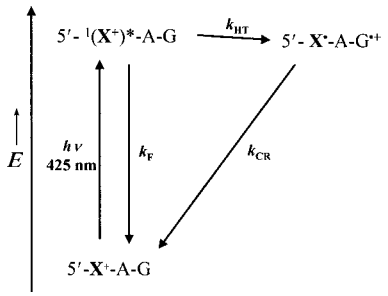


Figure 2. Transient absorbtion kinetics of duplex 3′-$\mathbf{X}^+$-A-GG (283 K) monitoring ground state recovery (425 nm, - - - -) and excited state decay (575 nm, ——) after excitation of $\mathbf{X}^+$ at 450 nm. Both states evolve on the same time scale and there is no evidence for a long-lived ground state recovery due to trapping by GG.

  0044-8249/00/11220-3796 $ 17.50+.50/0



Scheme 2. Kinetic scheme for the photoinduced hole-transfer reaction in all six hole-transfer duplexes using 5′-**X**$^+$-A-G as an illustrative example. k_{HT} is the rate of hole transfer, k_F is the fluorescence lifetime of ^{1}(**X**$^+$)*, k_{CS} is the rate of the forward charge-shift reaction, and k_{CR} is the rate of charge recombination. k_{CS} is a highly activated reaction in this duplex leading to $k_{CR} > k_{CS}$; therefore there are no observable intermediate features in the transient absorption data (Figure 2).

Table 1. Measured excited-state decay lifetimes (τ_{ES})[a] and the calculated hole-transfer lifetimes (τ_{HT})[b] for all seven duplexes at 283 K.

Duplex	τ_{ES} [ns]	τ_{HT} [ns]
X$^+$-(AT)	18.0	
5′-**X**$^+$-A-G	12.0 (12.7)	36.0
5′-**X**$^+$-A-GG	8.9 (9.6)	17.6
5′-**X**$^+$-A-GGG	9.0 (9.4)	18.0
3′-**X**$^+$-A-G	6.9 (7.4)	11.2
3′-**X**$^+$-A-GG	4.5 (4.2)	6.0
3′-**X**$^+$-A-GGG	5.7 (5.3)	8.3

[a] The numbers in parentheses are the measured rates of ground state recovery. [b] τ_{HT} was calculated from the formula $1/\tau_{HT} = 1/\tau_{ES} - 1/\tau_0$, where τ_0 is the excited state lifetime of the model duplex, **X**$^+$-(AT).

If we compare the sequence 5′-**X**$^+$-A-G with 5′-**X**$^+$-A-GG and 5′-**X**$^+$-A-GGG (Table 1), we observe that the oxidation rates in all cases are similar to within a factor of two. In fact, the oxidation of both GG and GGG occurs slightly faster. The small, factor of two variance in the rates is in line with recent calculations of the energetics of guanine bases in such combinations.[10] Because we do not expect different electronic couplings between **X**$^+$ and the closest guanine, this result indicates that it is this closest guanine which is in all cases the initially oxidized site.

If we change the directionality of the hole transfer, by comparison of the sequences 3′-**X**$^+$-A-G, 3′-**X**$^+$-A-GG, and 3′-**X**$^+$-A-GGG (Table 1), we observe again that the hole transfer rates are similar, in which the oxidation rates of GG and GGG are slightly faster. The similarity of the hole-transfer rates points to the fact that, in this case also, the hole-accepting site is the proximate G.

As a final comparison, we look at the differences in rates between 5′-**X**$^+$-A-G and 3′-**X**$^+$-A-G and between their GG and GGG counterparts. What we conclude from the data compiled in Table 1 is a systematic factor of three difference in rates, with transfer in the 3′-**X**$^+$-A sequences consistently faster. This result can be rationalized by the difference in electronic coupling between G and A in the two directions of the helix because 5′-G-A (valid for 3′-**X**$^+$-A duplexes) coupling is predicted to be slightly stronger than 3′-G-A coupling (valid for 5′-**X**$^+$-A duplexes).[16] However, one caveat on the validity of the theoretical argument is that the difference in rates between the two helical directions could also arise from asymmetrical electronic coupling of the acridine chromophore with the neighboring adenine bases.

Since the G proximal to a hole-injection site in GGG tracts is initially populated, there must be a subsequent process which leads to long-lived hole trapping in GGG. This process is expected to involve charge transfer as a consequence of structural and energetic relaxation. This entire process is what we refer to as the trapping reaction in GGG. The lifetime data reported in Table 1 are in excellent accord with the yield data of a reduced hole donor that we have described recently.[17] In these experiments, the donor species is the enolether radical cation.

In recent experiments, Lewis et al.[18] used delayed ground-state recovery dynamics of a photoexcited stilbene chromophore to measure the one-AT superexchange mediated, forward and back hole-transfer rates between G and GG in a hairpin DNA duplex. By fitting a simple kinetic model to their data, their published rate for forward transfer exceeds the one for back transfer by a factor of eight. The difference in rates was interpreted in a model, where GG is, a priori, a deeper hole trap than G and the asymmetry between the forward- and back-transfer rates is due to energetic differences. The results of our study may add an important detail to the understanding of this trapping reaction. We postulate that the lower energetics, characteristic of GG, are the result of an intraguanine tract-relaxation process subsequent to the primary oxidation kinetics. The order of magnitude similarity between the Lewis hopping rate to that extracted from a detailed model[19] of the chemical yield experiments[5] confirms the validity of the chemical yield approach to measuring charge transfer rates in DNA. In addition, it is rewarding to see that direct kinetic measurements and the dynamics modeled from chemical yield data support each other.

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From Glycals to Glycopeptides: A Convergent and Stereoselective Total Synthesis of a High Mannose N-Linked Glycopeptide**

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Given the elaborate machinery required for the biosynthesis of glycoproteins in cells, it seems likely that such systems perform significant biological functions.[1, 2] Indeed, protein glycosylation has been implicated in mediating protein folding,[3] in protecting against proteolysis,[4] in cellular differentiation,[5] and in cell–cell communication.[6] Major breakthroughs in the detection, purification, sequencing, and spectroscopic analysis of glycans have enabled a growing appreciation of the role of glycobiology in vital life processes.[7] Chemical synthesis[8–10] can play an important role in our understanding of glycobiology by providing access to well-selected, homogeneous, but realistically complex, probe structures for elucidating the relationship of glycoarchitecture and function.[11, 12]

Broadly speaking, glycoproteins are of two major types. In one motif, the terminal galNAc hexose of the saccharide domain is joined to the polypeptide through an α-*O*-glycosidic linkage to the hydroxyl group of a serine (or threonine).[13] The target systems which prompted the research described herein are N-linked glycoproteins, wherein the two domains are joined through a β-N linkage of an asparagine group to a glcNAc unit at the reducing end of the oligosaccharide.[14]

Specifically, we focused on a target where the consensus core high mannose pentamer sequence (see below) would be joined to the peptide domain through a carboxyl group of an Asp side chain (**1**, Scheme 3). Our goals in reaching **1** by chemical synthesis included a concise and efficient assembly of the required oligosaccharide.[15] Clearly, global deprotection of diversely protected functionalities would eventually be required. To this set of specifications we added another, namely, that the fashioning of the asparagine linkage be conducted in a maximally convergent sense with high stereocontrol by joining a fully mature high mannose saccharide to a fully mature peptide. In this way we hoped to pave the way for

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