Selective alkylation by photogenerated aryl and vinyl cation

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Chapter 8 | **Perspectives**

> *"As many of the reactions are described poorly, if at all understood, this is not as unsatisfactorily as it may seem. Indeed, many photogenerated reagents have proved extremely useful in the absence of any detailed knowledge of the way in which they react!"*
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> Hagan Bayley in: Photogenerated reagents in biochemistry and molecular biology; Work, T. S.; Burdon, R. H. Burdon, Eds., Elsevier, Amsterdam, 1983.

Photogeneration of aryl and vinyl cations

In Chapter 2 of this thesis it is proposed that photolysis of 4-substituted phenyliodonium salts gives a singlet phenyl cation-iodobenzene pair by heterolytic C-I bond cleavage in the singlet excited state, and a triplet phenyl cation-iodobenzene pair by heterolytic cleavage in the triplet excited state. This proposal is different from the mechanism embraced in the literature where homolytic cleavage in the triplet excited state, resulting in a phenyl radical and a iodobenzene radical cation, is an important intermediate-forming route next to heterolysis in the singlet excited state. The strongest evidence for the occurrence of homolytic cleavage is the observation of the iodobenzene radical cation (not the phenyl radical) by flash-photolysis on the picosecond time scale.

It is expected that flash-photolysis experiments on an even shorter time scale (<500 fs) will reveal the formation of extremely short-lived triplet aryl cation-iodobenzene pairs, which upon electron transfer form the radical/radical cation pair. Both the direct and the indirect formation of the phenyl radical account for the radical-derived productformation, but selectivity in the trapping of the leaving group by the reactive intermediate formed upon photolysis is best understood by assuming heterolytic cleavage from the triplet excited state.

Vinyl cations, like phenyl cations, probably can be formed in their singlet or triplet manifold. Triplet reactivity, however, is seldom or never considered in the discussion of the vinyl cations produced upon photolysis of vinyl(pseudo)halides. It is of interest to subject vinyl halides and vinyl(phenyl)iodonium salts to femtosecond range flash photolysis experiments, to establish a foothold for the interpretation of product patterns in terms of singlet or triplet product-forming reactive intermediates.

Hitherto, little or no attention has been paid to the issue that phenyl cations are generated either from the singlet or the triplet excited state, that both routes may yield cations, and that these cations may display different chemistry. The resulting chemo- and regioselectivity depend on the intersystem crossing ability of the electronically excited precursors and on the multiplicity of the cations. Photolysis of a variety of phenyl cation precursors in acetonitrile/anisole gives product patterns that are indicative of the spin-multiplicity of the reacting cationic intermediate ("fingerprinting") (Chapters 2 and 4). Also the cations can be distinguished from the corresponding phenyl radicals ("profiling"). That same method also gives indicative

product patterns for the 1-naphthyl, 9-anthryl and 9-phenanthryl cation (Chapters 6 and 7). It is expected that this indicative reaction can be applied to all types of phenyl and aryl cations.

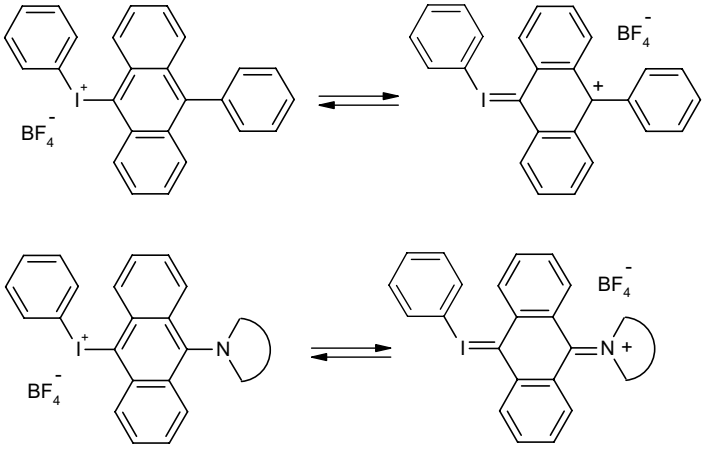
B3LYP/6-31G* calculations on the electronic ground state of a series of polynuclear aryl cations give singlet/triplet gaps which agree with the results of higher level calculations. In the case of small singlet/triplet gaps as for the 1- and 2-naphthyl and the 9-phenanthryl cation, the order of the states may invert by the presence of a polar solvent as observed for the naphthyl cations. For the 1-pyrenyl cation a quite large singlet/triplet gap is calculated, which is not inverted by the presence of any solvent. This ion is predicted to show typical triplet cation behavior. Because the synthesis of the precursors is time-consuming it may pay off to base the design of any such ions on the results of quantum mechanical calculations.

The photolysis of 1-naphthyl(phenyl)iodonium tetrafluoroborate and 2-naphthyl(phenyl)iodonium tetrafluoroborate yields 2- and 1-naphthyl substituted products, respectively (Chapter 5). This *cine*-substitution product pattern is attributed to the ability of the triplet naphthyl cations to undergo a 1,2-H-shift or lose a β-proton and form a triplet naphthyne. *Cine* substitution is also expected to occur the photolysis of 1-pyrenyl(phenyl)iodonium tetrafluoroborate because the 1-pyrenyl cation has a triplet ground state which will be generated from the triplet state of the iodonium salt.

Photolysis of appropriately (e.g. N,N-dimethylamino) substituted diphenyliodonium and benzenediazonium salts, produces mainly singlet, open-shell cations as product-forming intermediates with particular chemistry (Chapters 2 and 3). In *para* amino substituted phenyl cations this is probably due to the phenomenon that the initially formed singlet closed-shell phenyl cation resembles the singlet open-shell cation in energy. In the photolysis of 9-anthryl(phenyl)-iodonium tetrafluoroborate (Chapter 7), an open-shell aryl cation is also proposed as product-forming intermediate. For the 9-anthryl cation intermediate the same relation between the singlet closed shell and the singlet open cation is proposed as for the 4-amino phenyl cation.

It is predicted that the importance of the singlet open-shell cation as product-forming intermediate will be even more outspoken in the photolysis of 9-(10-phenylanthryl)(phenyl)-iodonium salt, (9-[1-anthracen-10-ylpyrrolidinyl])(phenyl)iodonium salt and (9-[1-anthracen-10-ylpiperidine])(phenyl) iodonium salt (Scheme 1). The first compound has already been prepared in our laboratory and is a mint green salt of which only solid state NMR and IR measurements could be made because of the insolubility of the material. Is is proposed that the isolated salt is the chinoid isomer of the iodonium salt that upon photolysis will mainly produce a singlet carbenoid cation next to some triplet carbenoid cation.

Scheme 1: Isomers of iodonium salts with an extended electron-rich ligand.



Photoalkylation of nucleic acids

In the photoalkylation of nucleic acids four types of precursors are commonly used: 1) azides, producing nitrenes, 2) photolabile compounds yielding carbocations, 3) photolabile compounds yielding quinone methides and, 4) psoralens, which form [2+2] photocycloaddition constructs. Precursors yielding radical intermediates are barely even used for alkylation purposes because radicals preferentially abstract a hydrogen atom from the (deoxy)ribose moiety, thereby inducing strand cleavage.

The use of photogenerated nitrenes, carbocations and quinone methides suffers from side reactions that are non-productive for photoalkylation. Singlet nitrenes produced in the photolysis of azides, isomerise in part to a less reactive intermediate prior to reaction with substrate. Triplet nitrenes react predominantly through hydrogen atom abstraction (from the sugar moiety). Carbocations alkylate the phosphate group rather than the nucleobases and also react readily, non-productively, with hydroxylic solvents such as water. Quinone methides react faster with hydroxylic solvents than with the nucleobases (at the exo-amino functions of guanine and adenine).

Aryl cations, in particular the triplet species, are expected to be more efficient in their reaction with the nucleobases of nucleic acids than the conventional intermediates and also to be more selective:

(1) Aryl cations, trapped by a nucleophilic substrate, yield alkylation products in large excess over hydrogen abstraction products (Chapter 4).
(2) The possibility to prepare singlet and triplet isomers of aryl cations (Chapter 6) provides chemoselectivity, leading to a preference for π over n nucleophiles (i.e. nucleo-

bases over phosphate groups and hydroxylic solvents). This feature is usually not compatible with high reactivity.

To illustrate this prospect a selection of typical aryl cation precursors (Chapter 4) was irradiated at 0.01 M in DMSO in the presence of an equimolar amount of guanosine at λ_{exc} = 300 or 350 nm (Scheme 2, Table 1). In experiment 1 and 4 the formation of 8-phenylguanosine (R = H), in experiment 3 the formation of 8-acetylphenylguanosine (R = $C(O)CH_3$, and in experiment 6 the formation 9-hydroxyphenylguanosine (R=OH) was confirmed by coinjection with independently prepared samples. Alkylation at position 8 of guanosine is in agreement with literature data that cationic intermediates (eventually) as well as radical intermediates alkylate C-8. (NB: the regioselectivity of triplet aryl cations is that of a phenyl radical (Chapter 4)). The preliminary results of Table 1 indicate that triplet phenyl cations, specially the ones destabilised by the electron-withdrawing carbonyl group, are significantly more reactive toward guanosine than singlet phenyl cations (entries 5, 6 and especially 3 versus 1 and 4).

Scheme 2: Indicative photoreactions of a selection of aryl cation precursors with guanosine.

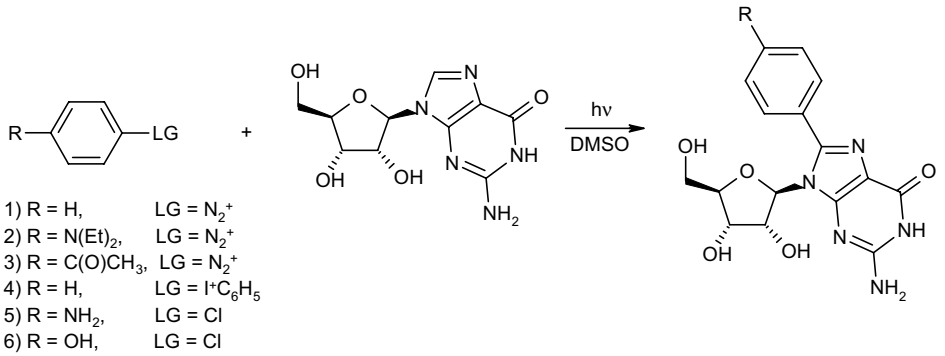


Table 1: Indicative photoreactions of a selection of aryl cation precursors with guanosine.

Exp.	Photolabile compound	λ_{exc} (nm)	Product[a]
1	benzenediazonium BF_4^-	300	4
2	N,N-diethylaminophenyldiazonium BF_4^-	350	0
3	4-acetyl benzenediazonium BF_4^-	300	36
4	diphenyliodonium BF_4^-	300	3
5	4-chloroaniline	300	20
6	4-chlorophenol	300	10

[a] Arylation product as percentage of total amount of LC-MS observed product after 3 hrs of irradiation.

Photoalkylated residues of nucleic acids are usually analyzed by mass spectrometry, by using radioactive tracers (mainly iodine) and by fluorescence. Fluorescence nowadays is the method

of choice. It couples high sensitivity with the appealing possibility of fluorescence microscopy. Photolysis of iodonium salts substituted with a large aromatic ligand (e.g. anthryl, phenanthryl, pyrenyl) releases the corresponding anthryl and phenanthryl cations and probably also the pyrenyl cation. Reaction of these cations with nucleic acids, is predicted to produce fluorescent DNA or RNA residues that can be visualised or separated from the bulk. The best results are expected for tagging with the pyrenyl moiety which is an effective fluorescer and less sensitive to (photo)oxidation than e.g. the anthryl moiety.

A major disadvantage of all precursors discussed thus far is the unselectivity of their photoreactions with respect to the base sequence of the nucleic acids. This disadvantage has been addressed by coupling photolabile precursors to a strand-recognizing peptide nucleic acid (PNA) molecule. Provided that the photolabile compound can be excited at a wavelength beyond the absorption of nucleic acids and the PNA, such constructs, upon irradiation, give alkylations in a desired region of nucleobases. Coupling of photolabile triplet aryl cation precursors, which can be excited above λ= 320 nm, to molecules with base sequence-recognizing ability (e.g. PNA) is expected to result, upon photolysis, in a specifically tagged nucleic acid molecule that is traceable by means of fluorescence detection.

The method of synthesis of iodonium compounds described in this thesis may be used in a post-synthetic strategy to prepare constructs of a target identifying molecule and a photoactivatable aryl cation releasing agent. After synthesis and purification of the identifying strand in which one position is used to carry a (masked) phenyl boronic acid functionality the molecule is treated with (hydroxy(tosyloxy))iodoarene.

A covalent coupling leading to fluorescence between a recognizing molecule and a target molecule can also be achieved by employing a vinyl halide as 9-bromomethylene-*9H*-fluorene (Chapter 7) attached to a recognition unit. In this case a recognizing molecule has to be prepared in which one of the positions is still available for coupling the vinyl halide through a handle on one of the aromatic rings.

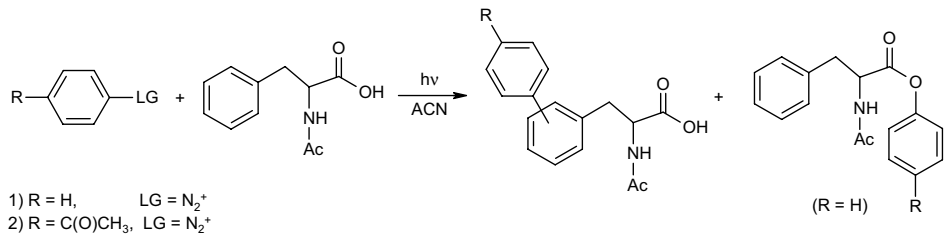
Photoalkylation of proteins

Photoalkylation of proteins is used extensively in photoaffinity labeling, a technique employed to study ligand-receptor interactions and membrane or protein topographies. The photolyses of azides (producing nitrenes), diazo-compounds (yielding carbenes), diazonium salts (producing carbocations or radicals) and diazirines (yielding carbenes) have found wide application. Disadvantages of these types of intermediates are: important side reactions such as hydrogen atom abstraction and formal insertion reaction with hydroxylic solvents, as has been outlined above. Because (triplet) aryl cations do not suffer these disadvantages (also see above), a high

yielding photoreaction of aryliodonium salts with amino acids is expected. Pre-complexation will be an extra beneficial factor. The positively charged iodonium salts are able to coordinate with a hydroxyl or a carboxyl group in amino acids such as phenylalanine, tyrosine or tryp - tophane. Photolysis of the iodonium salt then liberates a highly reactive triplet aryl cation in close proximity to an amino acid bearing an aromatic substructure.

To illustrate this prospect a selection of aryl cation precursors (Chapter 4) was irradiated at 0.01 M in acetonitrile in the presence of an equimolar amount of acetylated phenylalanine at λ_{exc} = 254 or 300 nm (Scheme 3, Table 2). These preliminary results indicate that the triplet phenyl cations generated in experiments 2, 4 and 5 have similar reactivity as the carbenes generated in experiment 6. Purely judged on overall reactivity they are no match for the par - ent phenyl cation generated in experiment 1. But the chemoselectivity (C- and O- alkylation in experiments 1 and 3 versus only C-alkylation in experiments 2 and 4 to 6) makes the triplet phenyl cations more useful because they do not react with the carboxylic acid group.

Scheme 3: Indicative photoreactions of a selection of aryl cation precursors with N-acetylated phenylalanine.



1) R = H, LG = N_2^+
2) R = C(O)CH_3, LG = N_2^+
3) R = H, LG = $I^+C_6H_5$
4) R = NH_2, LG = Cl
5) R = OH, LG = Cl
6) 4-diazoquinone

Table 2: Indicative photoreactions of a selection of aryl cation precursors with N-acetylated phenylalanine.

Exp.	Photolabile compound	λ_{exc} (nm)	Product[a]
1	benzenediazonium BF_4^-	254	22[b]
2	4-acetyl benzenediazonium BF_4^-	254	8
3	diphenyliodonium BF_4^-	254	7[b]
4	4-chlorophenol	254	5
5	4-chloroaniline	300	6
6	4-diazoquinone	254	11

[a] Alkylated product as percentage of total amount of LC-MS observed product after 3 hrs of irradiation.
[b] Combined yield of the products of arylation at the phenyl ring and the carboxyl group.

Selective C-alkylation of amino acids is also predicted to occur upon photolysis of iodonium salts substituted with a large aromatic ligand (e.g. anthryl, phenanthryl, pyrenyl). These salts release the anthryl, phenanthryl and probably the pyrenyl cation, which will react at the soft nucleophilic spots on a protein molecule and produce fluorescent polypeptides that can be visualised or separated from the bulk.