Photosensitised Switchable Nitric Oxide Donor Furoxans

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Photosensitised Switchable Nitric Oxide Donor Furoxans
(光増感作用を利用した光応答性一酸化窒素ドナーフロキサンの開発)

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Kobe University

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Table of contents

Abbreviations

Acac	acetylacetonate
AcOH	acetic acid
AIBN	2,2-azobisisobutylronitrile
Ar	aryl
a.u.	arbitrary units
Br	broad
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
Bu	butyl
Calcd	calculated
c-GMP	cyclic gaunylate monophosphate
Cy	cyclohexyl
DART	direct analysis in real time
Dba	dibenzylideneacetone
DCM	dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-*p*-benzoquinone
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMI	1,3-Dimethyl-2-imidazolidinone
DMSO	dimethyl sulfoxide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone
DNB	dimethylnitrobenzene
eNOS	endothelial nitric oxide synthase
EtOAc	ethyl acetate
EtOH	ethanol
eq.	equivalents
Furoxan	1,2,5-oxazadiazole-2-oxide
GSNO	S-nitroso-glutathione
GTN	glyceral trinitrate
GTP	guanylate triphosphate

HGN	hollow gold nanoparticle
HMPA	hexamethylphosphoramide
HOMO	highest occupied moleclular orbital
HRMS	high resolution mass spectrometry
HTMP	2,2,6,6-tetramethylpiperidine
IBX	2-iodoxybenzoic acid
iNOS	inducible nitric oxide synthase
IR	infra-red
LRMS	low resolution mass spec
LUMO	lowest occupied molecular orbital
Me	methyl
MeCN	acetonitrile
mp	melting point
MS	Molecular sieves
NBS	*N*-bromosuccinimide
n.d.	not determined
NDI	naphthalenediimide
NIR	near infra-red
NIS	*N*-iodoosuccinimide
NMO	*N*-methylmorpholine *N*-oxide
NMP	*N*-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
nNOS	neuronal nitric oxide synthase
NOC7	3-(2-hydroxy-1-methyl-2-nitrosohydrazino)-*N*-methyl-1-propanamine
NOF	nitroimidazole framework
NO-NSAIDs	nitric oxide-releasing nonsteroidal anti-inflammatory drugs
NONOate	diazeniumdiolate
o	ortho
OAc	acetate
p	para
PBS	phosphate-buffered saline

Ph	phenyl
PINOD	photoinduced nitric oxide donor
Ppm	parts per million
PS	photostationary state
PTLC	preparative thin layer chromatography
R_f	Retention factor
RNS	reactive nitrogen species
rt or r.t.	room temperature
SCE	saturated calomel electrode
sGC	soluble gunaylate cyclase
SNAP	S-nitroso-N-acetylpenicillamine
S_NAr	nucleophilic aromatic substitution
SNP	sodium nitroprusside
TBAF	tetrabutylammonium fluoride
TBDMS or TBS	*tert*-butyldimethylsilyl
TBSCl	*tert*-Butyldimethylsilyl chloride
TCF	thiol functionalized cupferron
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	tetramethylethylenediamine
Tol	tolyl
TPEG	thiolated polyethylene glycol
UV	ultraviolet
Xphos	2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl

Abstract

Nitric oxide (NO) is a reactive free radical with a short lifetime and area of effect. NO behaves as a cellular signalling molecule involved in vasodilation, cell death and neurotransmission. Diseases resulting from a deficiency of NO can be treated with stable NO donor molecules, however current clinical NO donors are non-specific and have issues with toxicity and tolerance.

Our research was focussed on the NO donor furoxan, which releases NO in presence of thiols. Furoxan molecules have been shown to isomerize under irradiation from inactive to active NO donors. Previous research into these photoswitchable furoxan NO donors was limited by the furoxan absorption being within the UV range which is damaging to organic tissue.

The core of this research investigated visible light absorbing photoswitchable NO donors. We hypothesized that a visible light absorbing sensitizer could transfer energy to the furoxan moiety and induce isomerization and subsequent NO release.

Following screening experiments, we selected anthraquinone as a sensitizer to conjoin with fluorofuroxan. Initial synthetic routes included ethylene glycol linker chains and connection via carbon-carbon bonds formed by palladium catalysed cross coupling. However, we encountered regioselectivity and solubility issues. Consolidating on these approaches we utilized an organocatalysed [4 + 2] reaction to furnish the insoluble anthraquinone core and carbon bridge to furoxan in a single step. We demonstrated in vitro photoswitchable NO release under a high degree of control. The hybrid molecule also displayed the ability to release NO in the absence of thiol, a rare phenomenon for furoxans, but we could not observe whether the molecule was taken into cells during testing.

To address the cellular uptake issue, we designed and synthesised hybrid furoxan-sensitizer molecules which displayed theranostic properties (the ability to fluoresce and sensitize). In our first design we synthesised a fluorescent alkoxyfuroxan-aminofluorenone hybrid which induced sensitized isomerization in organic solvent but not in aqueous solution. A second theranostic sensitizer-furoxan design made use of naphthalenediimide (NDI) sensitizer cores. The NDI-furoxan displayed in vitro

fluorescent photoswitchable NO release and enabled us to observe cellular uptake of the molecule. Unfortunately, NO was not released from within a cell culture system due to biothiols, which possibly quenched the excited state of the sensitizer.

Finally, we suggest an improved design of NDI-furoxans which would hopefully release NO under spatiotemporal control from within a cell culture system.

Acknowledgements

I would firstly like to thank the Japanese government for awarding me the MEXT Scholarship which enabled me to travel to Japan and undertake this study. I am deeply thankful to all of the Japanese people who have welcomed me into their country and shared their magnificent culture with me.

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I acknowledge Professor Masahiko Hayashi for his contributions to my research and his kindness during my time in his research laboratory.

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This study in Japan would not have been possible without the contributions and encouragement of the people back home who helped get me here, to list but a few would be unfair to the countless others.

Finally, I want to thank with all my heart my loving partner Becky, who during my study period worked so hard to teach English to Japanese preschool children. Whom despite missing her family and her home, stayed in Japan for several years to support and encourage me. Thank you for being my Samwise Gamgee.

Ⅰ: Introduction

1.1 An introduction to nitric oxide

Nitric oxide (NO) is a gaseous reactive radical species which mediates a variety of cellular functions, the most notable of which are vasodilation,[1,2] platelet aggregation,[3] neuromodulation,[4,5,6] wound repair[7] and immune response[8] (Figure 1). These cellular effects were originally attributed to endothelium-derived relaxing factor which was later found to be NO,[9] much to the surprise of chemists at the time since NO was regarded as an environmental pollutant.[10]

The importance of NO as a cellular signalling molecule was highlighted in 1992 when it was awarded 'molecule of the year',[11] and once again in 1998 when the Nobel Prize was awarded for the discovery of its role as a cardiovascular signalling molecule.[9]

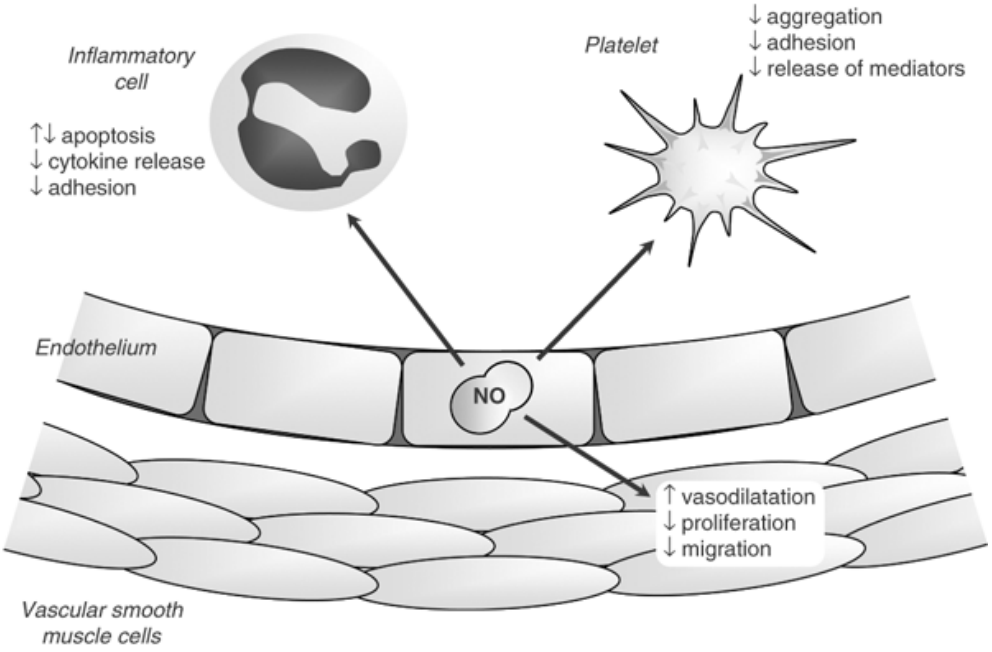


Figure 1: Effects of NO in the cardiovascular system. Figure is reproduced from Miller et al,.[12]

NO can passively diffuse throughout cells and cross intracellular distances with ease, the radical nature renders the molecule highly reactive towards other radical species and leads to short lifetimes limited within a small area of effect (approximately 100 μM).[12]

NO is biosynthesised from L-arginine which is transformed into cytrulline and NO by nitric oxide synthases enzymes.[13] There are three types of NO synthases, neuronal nNOS, inducible iNOS and endothelial eNOS.[14] NO activates soluble guanylate cyclase (sGC) leading to the dephosphorylation of guanylate triphosphate (GTP) to cyclic gaunylate monophosphate (c-GMP).[15] c-GMP acts through a variety of pathways such as protein kinase activation to produce the effects observed in the cardiovascular, central nervous and peripheral nervous systems (Figure 2).[16]

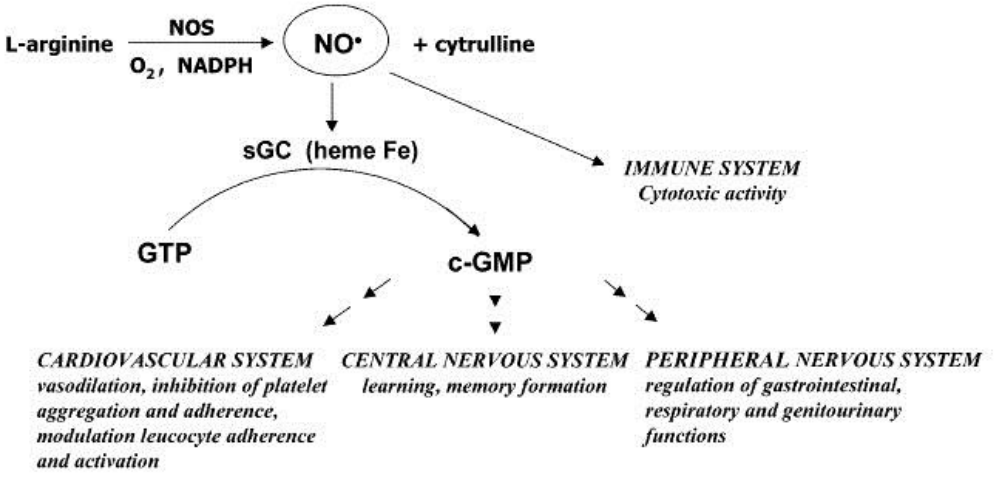


Figure 2: Biosynthesis of NO and action upon sCG. Figure is reproduced from Gasco et al.[16]

High concentrations (μM) of NO produced by macrophages under oxidative stress can lead to cytotoxic effects through the formation of reactive nitrogen species (RNS) such as peroxynitrite, a product of superoxide and NO radicals.[17] These reactive radicals can inhibit cellular respiration and lead to cell apoptosis in inflammatory cells. [18]

NO availability is implicated in the development of diseases and disorders such as arthritis,[19] cardiovascular diseases,[20] male impotence[21] and neurodegeneration such as in Alzheimer's disease.[22]

1.2 Nitric oxide donors

Delivery of exogenous NO is a therapeutic option for the treatment and prevention of diseases which are characterised by both reduced availability and overproduction of NO. Duel functionalities are possessed by NO, at endogenous concentrations beneficial effects are observed but at superphysiological concentrations NO is detrimental.[23] Small quantities of exogenous NO have demonstrated inhibition of iNOS expression via feedback mechanisms to abrogate upregulation of NO biosynthesis.[24]

Given that NO and its synthesis is almost ubiquitous and the biological effects of NO are concentration dependent, it is clear that NO therapeutics can affect and treat a broad range of disorders. The administration of NO as a gas is possible, however impractical due to instability, requirement for oxygen exclusion and nonspecific nature of gaseous NO.[12]

1.2.1 Clinical nitric oxide donors

Since NO has a short lifetime and area of effect the use of prodrugs has been developed to stabilise NO until its desired release. Currently there are few NO donors in clinical use, the most notable of which are sodium nitroprusside (SNP), a nitrosyl metal complex and glycerine trinitrate (GTN), an organic nitrate (Figure 3). Both of these compounds amongst others are known as 'accidental NO donors' since they were used before their effects involving NO were understood.[16]

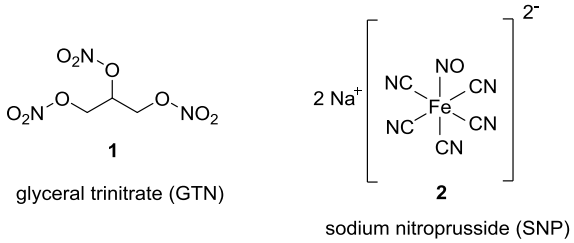


Figure 3: Clinical NO donors

GTN is mainly used for pain relief, one use is in the treatment of angina and it can also be used topically to treat anal fissures.[12] The main limitation of GTN and other organic nitrate donors is the build-up of tolerance with prolonged use.[25,26]

SNP is used on site in hospitals for rapid lowering of blood pressure during hypertensive crisis.[27] The caveat of long term use of SNP is the risk of developing cyanosis due to release of cyanide groups,[28] although reports of cyanide build up are rare, several other inconveniences such as intravenous administration and sensitivity to light highlights precedent for the development of alternative donors.

1.2.2 Non-clinical nitric oxide donors

Although not used clinically there are many promising NO donors under investigation, the most notable of which are the diazeniumdiolates (NONOates) and the S-nitroso thiols.

The NONOates feature a diolate group from which up to two molar equivalents of NO are released. The NONOates decompose spontaneously in physiological pH solutions and can be tailored to have specific half-lives from seconds to hours depending on the structure.[29] Often a requirement of NO donors is the presence of thiols to induce NO release, one admirable feature of NONOates is the release of NO independent of thiol cofactors.[30] Notable examples of NONOates include PROLI/NO, DEA/NO, SPER/NO and DETA/NO to name a few (Figure 4)

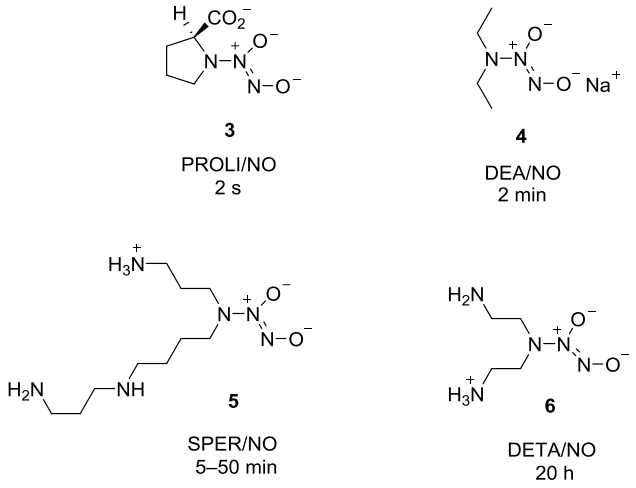


Figure 4: Examples of NONOates and the half-lives of NO release in physiological solutions. SPER/NO exhibits a tuneable concentration dependant NO release range as a less reactive dimer forms at higher concentrations.[29]

The second prominent group of NO donors is the S-nitrosothiols which contain a single bond between a sulphydryl and NO moiety. S-Nitrosothiols are not only synthesized and administered exogenously but also found endogenously.[31] S-Nitrosothiols decompose under a variety of conditions such as UV-light, heat and enzymic release triggers[12,32] and they are particularly susceptible to copper catalysed decomposition.[32] S-Nitrosothiols can also undergo transnitrosation in which NO^+ is transferred from S-nitrosothiol to other cellular thiols.[33] The transnitrosation protects the NO under conditions of oxidative stress.[12] S-Nitrosothiols have an advantage over other NO donors, such as the organic nitrates, in that they do not induce a build-up of tolerance with prolonged use.[34] Notable examples of synthetic S-nitrosothiols include GSNO and SNAP (Figure 5).

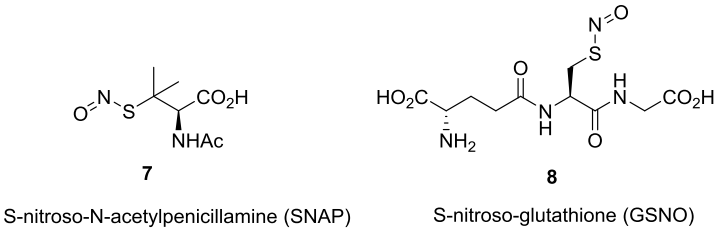
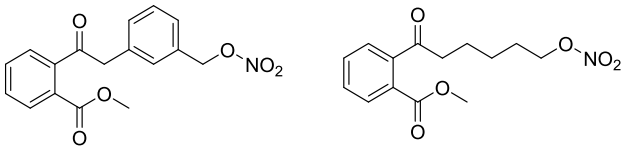


Figure 5: Notable S-nitrosothiols SNAP and GSNO

1.2.3 Hybrid nitric oxide donors

NO donors have been attached to established drugs and are known as NO hybrid drugs. The established drug retains its pharmacological properties whilst also showcasing the biological activity of the NO donor. Often the NO donating ability of the hybrid drug can offset the negative side effects associated with the parent non-hybrid drug. A wide variety of hybrid drugs have been developed which include NO donor moieties such as NONOates, s-nitrosothiols, nitrates and furoxans and are projected to be used in the treatments of inflammatory, cardiovascular and neurodegenerative diseases as well as showing antibacterial, antiparasitic and anti-cancer properties.[35] Perhaps the most renown examples of NO-donor hybrids are the nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) of aspirin 'nitroaspirins', NicOx compounds **9** and **10** were the first aspirin NO-NSAIDs to be produced commercially (Figure 6).



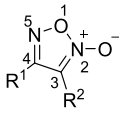
9 NCX4215 **10** NCX4016

Figure 6: NO-donor hybrid drugs combining aspirin with an organonitrate.

Aspirin has several uses from pain relief to the treatment of arthritis, however prolonged use can lead to gastrointestinal damage and ulcer formation.[36] Combining the aspirin with a NO donor helps alleviate the negative effects on the gastric tract by increasing the production of gastric mucus, increasing blood flow towards gastric mucosa, promoting repair and removal of toxins amongst other benefits.[12]

1.3 Furoxan as a nitric oxide donor

Furoxan (1,2,5-oxazadiazole 2-oxide) is a heterocyclic NO prodrug that is susceptible to attack from thiols leading to the release of NO, although in some rare cases NO can be released in the absence of thiol (Figure 7).[37,38] The quantity and rate of NO release from each furoxan is related to the substituents on the 3- and 4- positions of the ring,[39,40] for example one furoxan regioisomer may be stable to NO release but the other regioisomer may be highly reactive. In general, electron withdrawing groups help induce nucleophilic attack at the electron deficient positions of the furoxan ring leading to enhanced quantities of NO release.[41]



11
furoxan

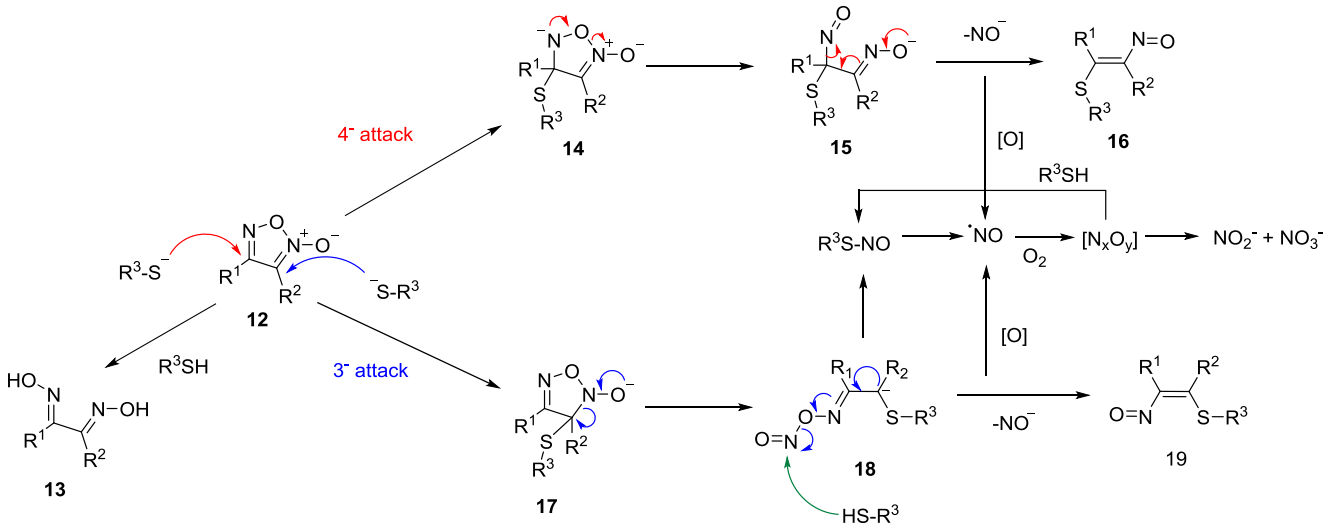
Figure 7: A general structure of 1,2,5-oxazadiazole 2-oxide complete with numbering convention.

Furoxans have exhibited a wide range of biological activities such as anti-parasitic, anti-microbial, immunosuppressive and anticancer effects.[42] Furoxan being an NO prodrug also shows anti-aggregating and vasorelaxant activity with one derivative, CAS 1609, reaching animal testing in which it featured no build-up of tolerance with prolonged use.[43] Unfortunately, clinical studies with CAS 1609 have been hindered due to mutagenic effects.[16]

1.3.1 Thiol mediated nitric oxide release from furoxan

The mechanism of thiol mediated release under physiological conditions is thought to occur by initial attack of a thiolate anion to the furoxan ring which leads to ring opening to a tetrahedral intermediate (Scheme 1).[38] Depending on whether the 3- or 4- position is attacked governs the mechanism on release. Attack at the 4-position via

14 leads to posterior loss of a nitroxyl anion from the tetrahedral intermediate **15** which is subsequently oxidised in situ. Thiolate attack at the electron rich 3- position leads to release of a nitoxyl anion but may also form S-nitrothiols directly via intermediates **17** and **18** interacting with thiol. Only one equivalent of NO is released despite there being potential for two. It is suggested that thiol attack on the furoxan ring can also lead to ring opening and dioxime formation **13**.[38] It should be noted that thiol mediated NO release mechanisms are speculative at the time of writing and are probably substrate specific.

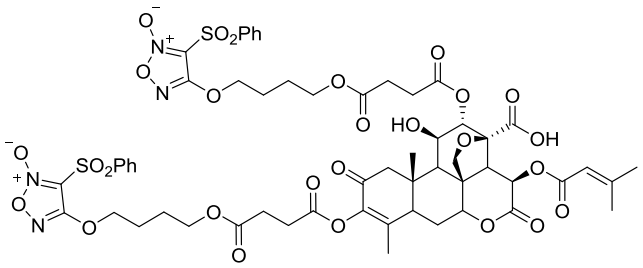


Scheme 1: Proposed NO release mechanism of furoxan under physiological conditions. Scheme is modified from Gasco et al.[16]

1.3.2 Furoxans as NO donor hybrids

As mentioned previously, furoxan motifs have appeared in a wide variety of natural product and synthetic NO donor hybrids demonstrating anticancer, antiparasitic, antimicrobial, antimalarial and gastroprotective activities.[44,45] One example of particular interest is the hybrid of natural product brusatol containing phenylsulphonyl furoxans (Figure 8).[23] Brusatol in its natural form exhibits an assortment of pharmacological activities and it has generated particular interest due to its anti-inflammatory effects, however it was found to be too toxic for application.

Connection of phenysulphonyl furoxans enhanced the pharmacological profile of brusatol. The NO release from **20** inhibited iNOS expression in macrophages via feedback mechanisms whilst simultaneously reducing toxicity by 100-fold relative to brusatol. The hybrid demonstrated in vivo anti-inflammatory activity and was used in the treatment of chronic obstructive pulmonary disease-like lung inflammation in mice presenting a promising lead compound.[23]



20

Figure 8: Brusatol-furoxan hybrid molecule

1.3.3 Furoxan synthesis

Furoxans can be synthesized by a variety of methods (Figure 9),[46] one such method is the dimerization of benzonitrile N-oxides to form symmetrically substituted furoxans,[47] although this approach is limited in diversity as both N-oxides are indistinct. Asymmetric synthetic methods include the dehydration of glyoximes to afford substituted furoxans,[48] reaction of dinitroolefins with NaN_3[49] and acid catalysed cyclization of α-nitro-oximes.[50] These methods often require the tedious preparation of pre-functionalized intermediates prior to the formation of the furoxan ring. Perhaps the most convenient preparation of furoxan is the treatment of styrene with $NaNO_2$ under acidic conditions to form nitrofuroxans via dinitrogen trioxide, nitrofuroxan products can then be further functionalized via aromatic nucleophilic substitution and thermal isomerization.[51,52,53] Aside from simplicity, the advantage of using the styrene and acidic $NaNO_2$ method lies with the wide range of commercially available, readily synthesized and cheap styrene starting materials.[54] Recently attempts have been made to increase the diversity and utility of furoxan synthesis, especially in the

Matsubara research group where the treatment of styrenes with $NOBF_4$ enabled the synthesis of acid sensitive derivatives under basic conditions.[54]

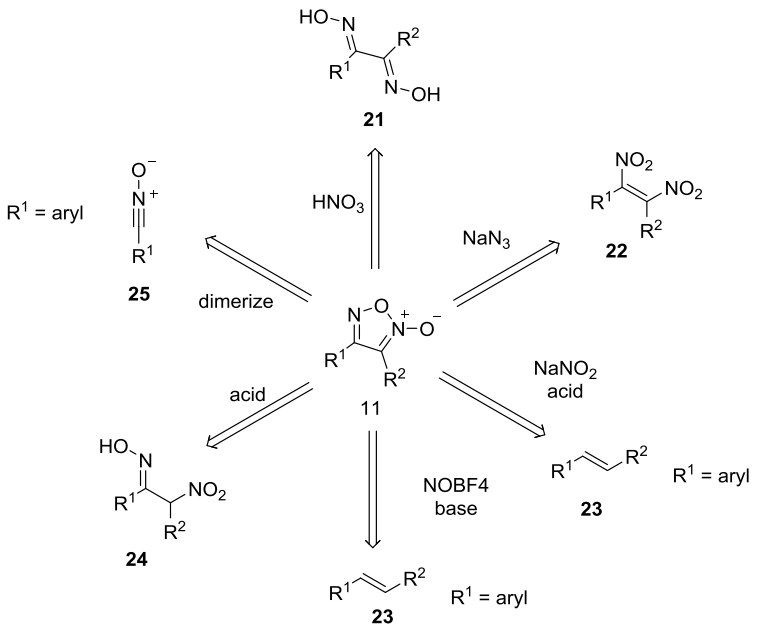
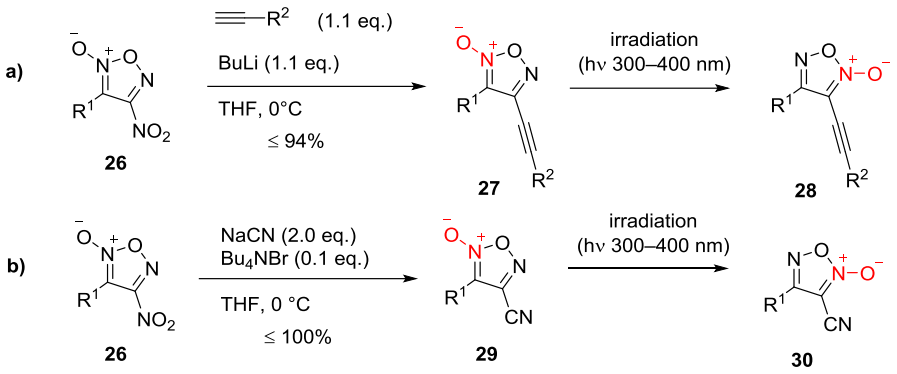


Figure 9: Established furoxan synthetic methods

1.3.4 Furoxan ring modification

Furoxan chemistry is largely underdeveloped due to difficult synthesis and limited synthetic utility. Aside from furoxan ring synthesis under basic conditions recent work in the Matsubara lab has led to the facile introduction of multiple functional groups directly onto the furoxan ring, including carbon-carbon bonds enhancing the accessibility of furoxan chemistry. Synthetic methods introducing carbon substituents on the furoxan ring prior to this work were rare and non-trivial with only two examples using Grignard reagents available and no further evolution of scope.[55] Introduction of alkynyl groups under mild conditions via alkyl lithiums led to an expansion of carbon-based substituents in high yields (Scheme 2a).[56] The alkynyl furoxans also showed photoswitchable character granting access to the 3-isomers. A second contribution to carbon-carbon bond forming reactions onto the furoxan ring featured the cyanation of nitrofuroxans, once again photoisomerization granted access to the regioisomer

(Scheme 2b).[40] The cyano and alkynyl functionalities can be easily modified further to grant libraries of biologically active compounds.



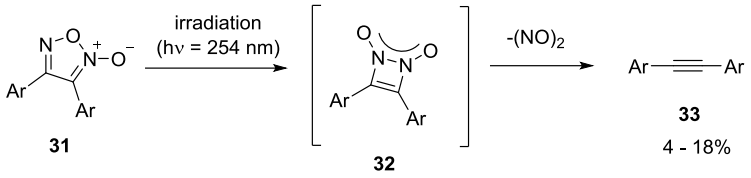
Scheme 2: S_NAr reaction of (a) alkyl lithiums and (b) sodium cyanide on nitrofuroxan and subsequent photoisomerization.

1.3.5 Photoswitchable furoxan nitric oxide release

Many options exist when selecting an appropriate NO donor but in the so far discussed examples all suffer from a lack of site specific selectivity and general instability. The molecules are not activated with a significant element of control. Spatiotemporal control over NO release is important since the concentration and location of NO release can determine the kind of biological response, whether it is simple vasodilation or signalling cell apoptosis.[8] This kind of selectivity can be achieved through external stimuli and perhaps the most promising of stimuli is light. Light can be used to induce reactivity without remaining in the system afterwards, furthermore light is highly characterizable and at longer wavelengths is non-invasive. Irradiating the intended sight of release for a specific period of time could ideally trigger a specific quantity of NO to be released. These kinds of molecules are known as photoinduced NO donors (PINODs).

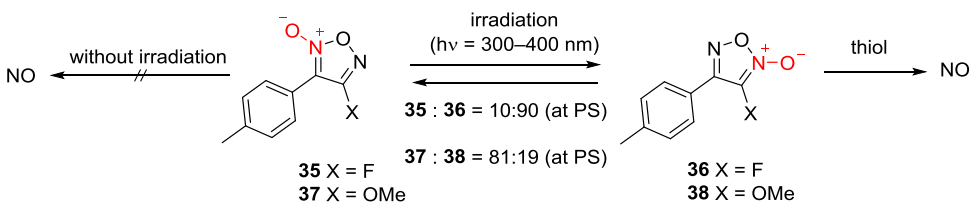
Reports of furoxan PINODS prior to work in the Matsubara group are rare and speculative. For example, it has been suggested that diarylfuroxans form diaryacetylenes under UV irradiation and generate low yields of NO via azo-N,N'-

dioxides (Scheme 3).[57] It is of note that Hwang et al. similarly reported UV photolysis of 3,4-bis-2'-chlorophenylfuroxan with implied release of NO.[58] NO has also been released, albeit unquantified, from self-assembled monolayers of furoxans under UV irradiation.[59]



Scheme 3: Proposed mechanism of NO liberation from diarylfuroxan under UV irradiation.

Recently efforts have been made towards the realisation of furoxan PINODs (Scheme 4).[41,60] Fluorofuroxans displayed a high isomeric ratio at the photostationary state (PS) under UV irradiation (approximately 10 : 90 of the 4- : 3- regioisomers respectively). The 4-fluoro regioisomer was stable to thiol cofactor but once isomerized to the 3-regioisomer underwent thiol mediated NO release in up to 40%.[41] The fluorofuroxans presented the first quantitative photoswitchable NO release under physiological conditions. Shortly after, alkoxy furoxans displayed photoswitchable NO release in moderate yields under UV irradiation albeit lower total isomeric ratio at the PS. Despite the negligible isomeric ratios, the alkoxy furoxans underwent rapid NO release with the reactive 3-regioisomer being replenished upon consumption enabling further discharge of NO.[60]



Scheme 4: Photoswitchable NO release from fluoro- and alkoxyfuroxans.

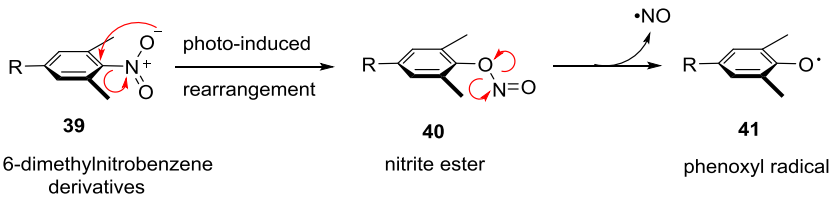
The limitations of furoxan PINODs were clear; the necessity of UV irradiation prevented PINOD furoxans from finding biological application since the high energy light required was cytotoxic and poorly penetrating.

1.4 Non-furoxan photoswitchable nitric oxide donors

At the time of writing to the best of our knowledge, other than methoxy and fluorofuroxans developed by Matsubara et al. there are no other furoxan PINODs. However, other PINOD systems with diverse structures and release mechanisms have been developed and switchable NO release has been achieved using photoirradiation ranging from UV to near infra-red (NIR) wavelengths.[10,61–68] In this section such PINODs will be briefly discussed.

NO has been 'uncaged' from *o*-nitrobenzyl[69] and *N*,*N*'-dinitroso-*p*-phenylenediamine substrates in a switchable manner under UV irradaiton.[61] S-Nitosothiols became thermally stable once adsorbed onto gold surfaces and generated NO under UV irradiation.[62]

UV light has been utilized to release NO from sterically hindered 2,6-dimethylnitrobenzene (2,6-DNB) derivatives, which when irradiated demonstrated anti-cancer activity in a cell culture system under spatiotemporal control (Scheme 5).[10] The irradiation wavelength required to induce NO release from 2,6-DNB has recently been increased to within the visible range (400–430 nm) by conjugation with a coumarin moiety.[70]



Scheme 5: Proposed mechanism of photoinduced rearrangement of the twisted nitro group and subsequent NO release following isomerization from 2,6-dimethyl-nitrobenzene derivatives. Scheme is modified from Kitamura et al.[70]

Visible light has been used to decage NO from bycyclic *N*-pyramidal nitrosoamines.[64] The strained bridged nitroso moiety features attenuated p-orbital overlap expressed as greater antibonding character compared to monocyclic nitrosoamines. This lowers the HOMO–LUMO energy gap of the n–π* transition, thus extending the decaging wavelength into the visible region (Figure 10).

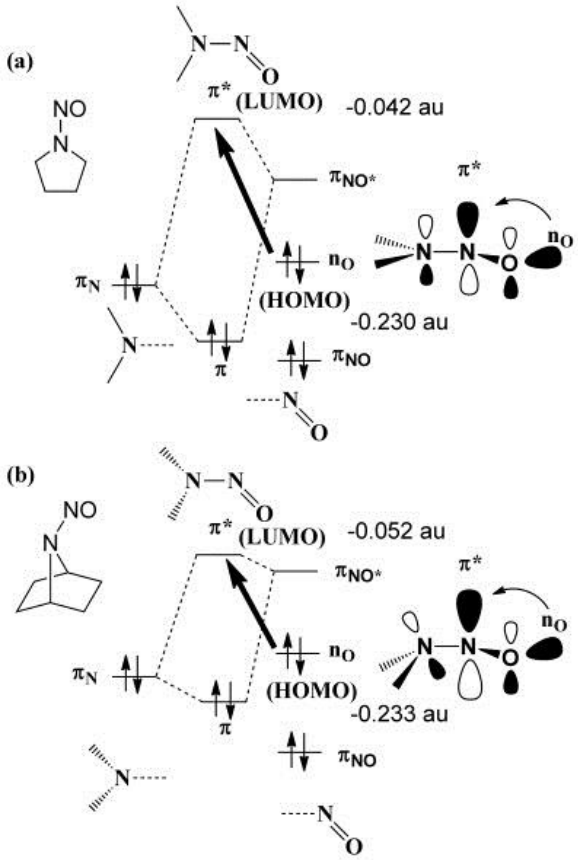
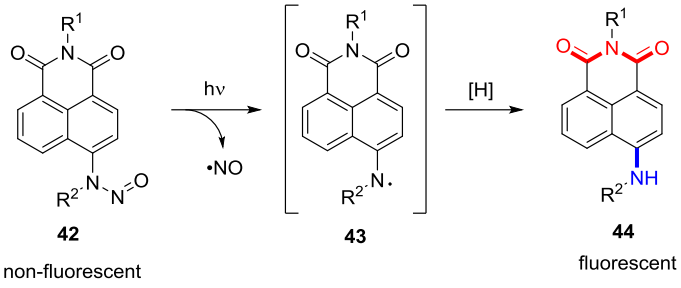


Figure 10: Orbital interactions within the nitrosoamino functionality and HOMO–LUMO transition; (a) monocyclic and (b) bicyclic nitrosoamines. Figure is reproduced from Karaki et al.[64]

Recently NO release from *N*⁻ nitrosated napthalimides via UV or near-red two photon irradiation was accompanied by fluorescence turn-on and enabled quantitation release analysis (Scheme 6).[67]



Scheme 6: Pathway of NO release and fluorophore generation upon photolysis of nitrosoamine.[67]

Early metal nitrosyl compounds released NO under irradiation but suffered from short UV wavelength requirements and poor stability in biological media, it is of note that the clinically relevant SNP is considered amongst them.[68] Efforts have been made to increase both the stability and irradiation wavelength of ruthenium nitrosyl compounds via ligand modification and the attachment of light harvesting dyes, of particular merit is a resorufin coordinated Ru-NO '1-Resf' (Figure 11).[63] Resorufin improves the light harvesting ability of the complex and assists in the sensitization of the nitrosyl. Uptake by cancer cells was indicated via trackable red fluorescence (which was quenched upon NO release) and following a brief period of irradiation with visible light cell apoptosis occurred.[63]

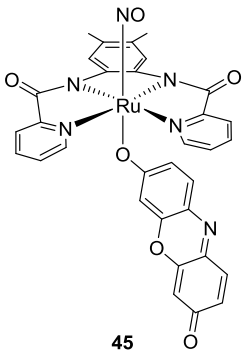


Figure 11: Resorufin coordinated ruthenium nitrosyl complex '1-Resf'.

An unusual yet effective approach to PINODs is the synthesis of zeolite porous coordination polymers from self-assembled nitroimidazole frameworks 'NOFs' (Figure 12).[65] Nitroimidazoles tend to undergo photoexcited deactivation, however immobilization as the porous frameworks of the NOF complex prevented quenching of the ligand reactive excited state. NO flux could be tuned by irradiation intensity and quantity of irradiated material and it was demonstrated the NOFs showed spatiotemporal release from within a cell culture system under NIR two-photon laser absorption.

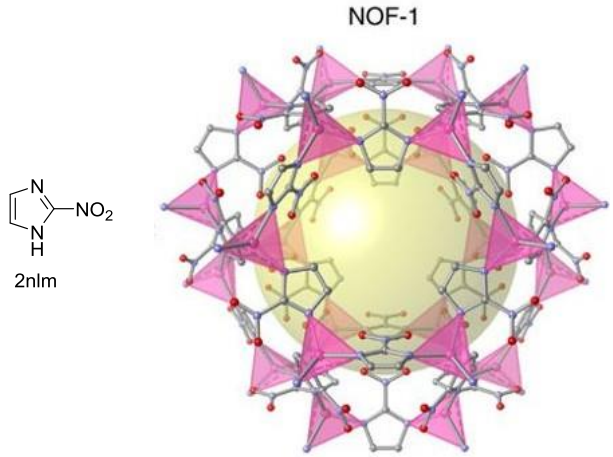
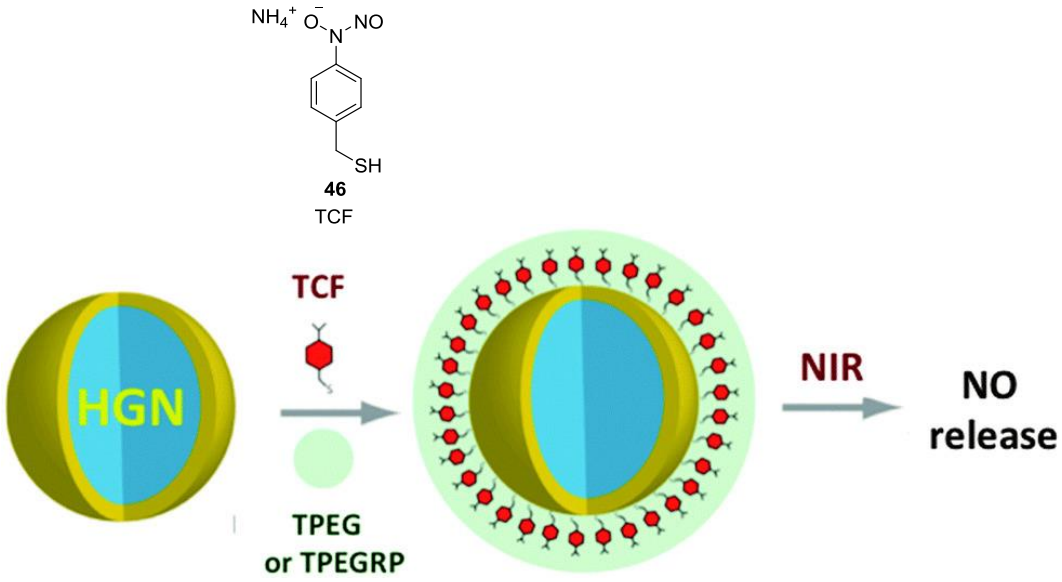


Figure 12: Self assembled nitrosoimidazole zeolite complex from 2nlm. Figure reproduced from Diring et al.[65]

Perhaps the most remarkable PINOD recently reported is the NIR uncaging of NO from fluorescent hollow gold nanoparticles (HGN) (Scheme 7).[66] NIR irradiation induces thermochemical release of NO from thiol functionalized cupferron (TCF) absorbed onto HGN. Thiolated polyethylene glycol (TPEG) absorbed onto the HGN improved water solubility, targeting peptides were also absorbed which induced internalization and switchable NO release within cancer cells.



Scheme 7: Thiol functionalozed cupferron absorbed onto Hollow gold nanoparticles and subsequent NIR NO release. Figure is reproduced from Levy et al.[66]

1.5 Summary of considerations

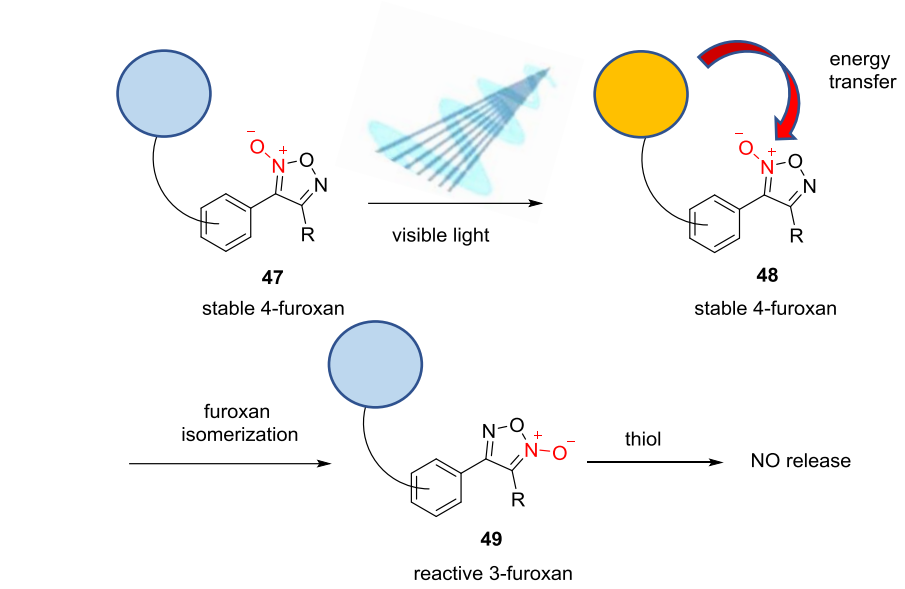
The exogenous delivery of NO via stable NO prodrugs is necessary for studying the biological effects of NO and for the treatment of cancer, cardiovascular and neurological diseases. Current clinical donors are hindered by their toxicity and induction of tolerance, whereas non-clinical donors such as S-nitro thiols and NONOates appear promising, like their clinical predecessors they still struggle with lack of controlled site-specific NO release. To counter the inadequacies of NO-donors PINODs have been developed ranging from the impractical UV absorbing caged nitrosyls to the fantastical NIR photothermochemical HGN. Despite recent progress, the diversity of PINODs and their release mechanisms are sparse and the development of new PINODs with novel release mechanisms are required in pursuit of controllable discharge of NO under biologically inert conditions.

1.6 Hypothesis: visible light furoxan photoswitchable nitric oxide donors

As mentioned previously, visible light is preferable to UV since visible light can penetrate further into tissues and is biologically benign whereas UV is toxic. Ideally NIR light should be used which can penetrate deeper than surface tissues and into organs, although this can often be difficult to achieve without extended conjugation, nanoparticles or two photon absorption techniques.

The recent research into fluoro- and alkoxyfuroxan isomerization were the first steps towards spatiotemporal controlled NO release from furoxans. The initial results were promising but since the furoxan absorption tends not to extend beyond 320 nm they are hindered by the necessity of UV light. Visible to NIR light induced NO release is necessary for PINOD furoxans to have any tangible biological application.

The requirement for visible light furoxan PINODs led us to contemplate how the NO release could be induced from the UV absorbing furoxan. We hypothesized that a light harvesting moiety could absorb light in the visible range and then transfer the energy to the furoxan whereupon it would isomerize to the reactive 3-isomer and become vulnerable to thiol attack and release NO (Scheme 8).



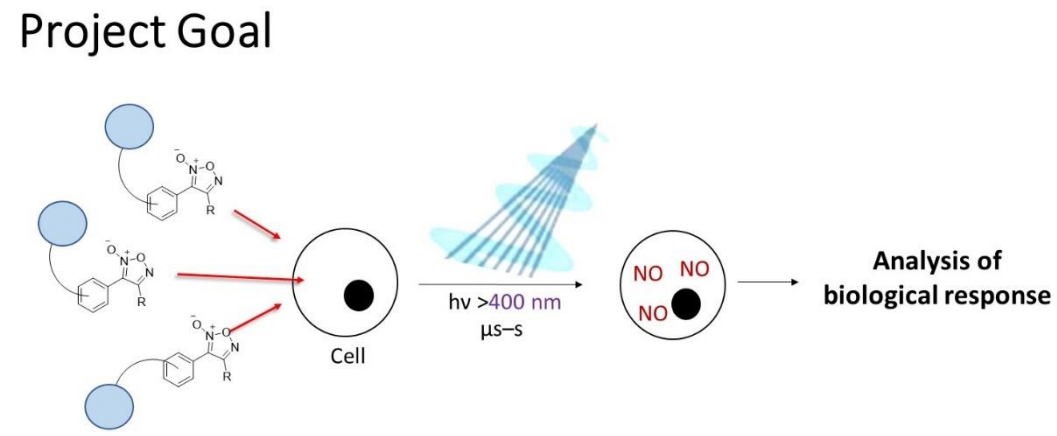
Scheme 8: Envisioned mechanism of furoxan isomerization and NO release mediated by visible light absorbing moieties.

Compared with established NO donors such as S-nitroso thiols and NONOates the choice of furoxan NO donor may seem questionable, however since furoxan chemistry is underdeveloped there is huge potential for furoxans to equal or even surpass the current 'in vogue' donors and lead to further application in biology and neuroscience. Furoxans are small molecules with low molecular weight making them drug like, furthermore furoxans can easily exhibit high solubilities in aqueous solutions with simple modifications. Furoxans are also highly stable and have potential for acutely controlled NO release with suitable functionalisation.

1.6.1 Research aim

The aim of the research is to synthesize a water soluble furoxan PINOD that releases NO under spatiotemporal control from within a cell (Scheme 9). Following NO release, the immediate biological response could then be analysed. Once a simple prototype

has been synthesized the molecular complexity could be increased to include fluorescent markers to show the region of cellular uptake and recognition moieties to target specific cell types or sites within the cell.



Switchable **release of NO under spatiotemporal control** using **visible** or **near-IR** light

- visible light harvesting moiety

Scheme 9: Model application of visible light switchable furoxan PINOD.

Herein we report on the development of visible light-triggered NO donors based on the photosensitization of furoxans.

Ⅱ: Studies towards anthraquinone-fluorofuroxan

2.1 The photosensitization of fluorofuroxan

In our hypothesis we suggested that a light harvesting moiety could absorb irradiation and transfer the energy to the furoxan inducing NO release (Scheme 8). One family of light harvesting moieties are the photosensitizers, which can transfer energy via the triplet excited state.[71]

In order for energy transfer to occur, the furoxan would also need to be active in the triplet state. An indication of triplet activity within a compound is if the molecule displays phosphorescence emission.

2.1.1 Phosphorescence of compound 35

The triplet energy level of 3-tolyl-4-fluorofuroxan **35** was investigated by detecting the phosphorescence emission. The spectrum was measured in glassy frozen 2-methyltetrahydrofuran (2-MeTHF) at 77 K. To our delight excitation at 280 nm produced a well resolved emission peak with λ_{max} at 420 nm (Figure 13). The emission was broad and ranged between 370–570 nm suggesting that light with wavelength longer than 400 nm could be used in conjunction with a suitable triplet sensitizer to induce isomerization.

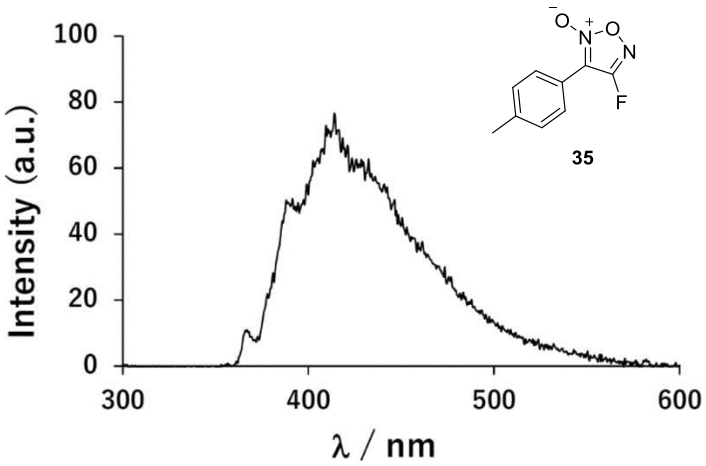
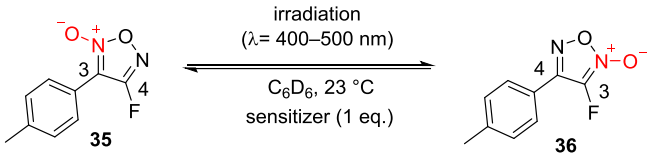


Figure 13: Phosphorescence spectra of **35** in 2-MeTHF at 77 K with excitation wavelength of 280 nm.

2.1.2 Photosensitizer screening of compound 35

Potential photosensitizers were selected based on their photophysical properties as recorded in 'The Handbook of Photochemistry'.[72] The selected sensitizers were screened by irradiating a 5 mM 1:1 ratio of **35** and sensitizer in deaerated C_6D_6 and measuring the isomerization ratio relative to a dodecane internal standard (Table 1 and Figure 14) . Samples were irradiated with 400–500 nm light and isomerization ratio measured at 15-minute intervals by ^{1}H NMR up to 90 minutes.

Table 1: Photosensitized furoxan isomerization under 400–500 nm irradiation. Sensitizer (2.4 µmol) and furoxan (2.4 µmol) 1 : 1 eq. in deaerated C_6D_6 (0.5 mL). [a]Determined by ^{1}H NMR analysis using dodecane as an internal standard. [b]Phenothiazine decomposed and byproduct started to form after 1 h of irradiation. [c]DDQ reacted with furoxan. [d]PS was not reached after 1.5 h of irradiation.



		Yield of 36 (%)[a]		
Entry	**Sensitizer**	**15 min**	**30 min**	**PS**
1	anthraquinone	59	75	76
2	1,2-benzanthraquinone	96	96	96
3	benzil	79	92	94
4	phenothiazine[b]	8	14	-
5	DDQ[c]	0	0	-
6	benzophenone[d]	1	2	>6
7	none	0	0	0

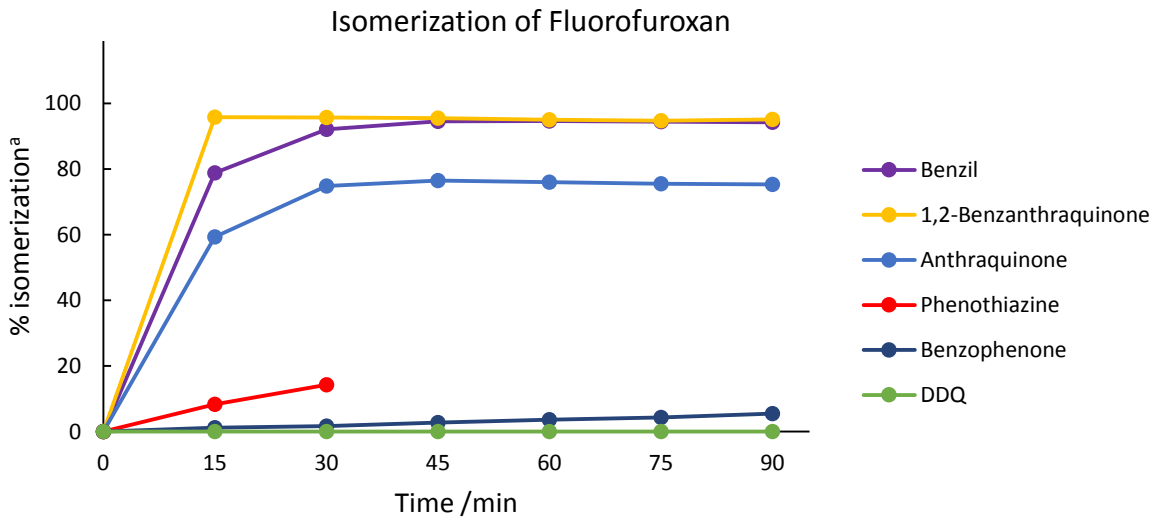


Figure 14: Photosensitized furoxan isomerization under 400–500 nm irradiation. Sensitizer (2.4 µmol) and furoxan (2.4 µmol) 1 : 1 eq. in deaerated C_6D_6 (0.5 mL). [a]Determined by ^{1}H NMR analysis using dodecane as an internal standard.

The quinone sensitizers showed exceptional sensitizing ability. 1,2-Benzanthraquinone and benzil had the fastest rates of isomerization and the highest yields at the PS (Table 1, Entry 2 and 3 respectively). Benzil reached the PS at around 30 minutes of irradiation and 1,2-benzanthraquinone reached PS in 15 minutes. Following 30 minutes of irradiation anthraquinone produced a good yield of 76% at the PS (Table 1, Entry 1). Phenothiazine induced a slow rate of isomerization in the furoxan but after 30 minutes decomposition of both sensitizer and furoxan were observed (Table 1, Entry 4). Benzophenone was ineffective at sensitizing the furoxan with a sluggish rate of isomerization which did not reach the PS after 90 minutes of irradiation (Table 1, Entry 5). 3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) did not cause isomerization but instead reacted with the furoxan under irradiation (Table 1, Entry 6). In the absence of a sensitizer no isomerization was observed due to the furoxan not absorbing within the irradiation window (Table 1, Entry 7).

Due to their admirable sensitizing properties the quinones 1,2-benzanthraquinone, benzil and anthraquinone were considered for a suitable fluorofuroxan-sensitizer conjoined molecule. The ratio of isomerization at the PS was not a significant factor in selecting the sensitizer, previous research into furoxan PINODs had shown successful NO release from molecules which displayed negligible isomerization at the PS.[60] This is due to rapid replenishment of isomerized furoxan to maintain said PS. Benzil would have provided a challenging synthesis due to the biacetyl nature, furthermore we were concerned about stability in biological applications. Possible synthetic routes towards 1,2-benzanthraquinone were investigated and found to be non-trivial, furthermore the unsymmetrical nature of the molecule would lead to regioisomeric complexities during synthesis rendering the sensitizer as unattractive in this instance. The symmetry and synthetic utility of the anthraquinone were a positive factor in selecting it for synthetic application, although the photophysical properties were not as good as other screened quinones the anthraquinone presented a greater chance of successful synthesis.

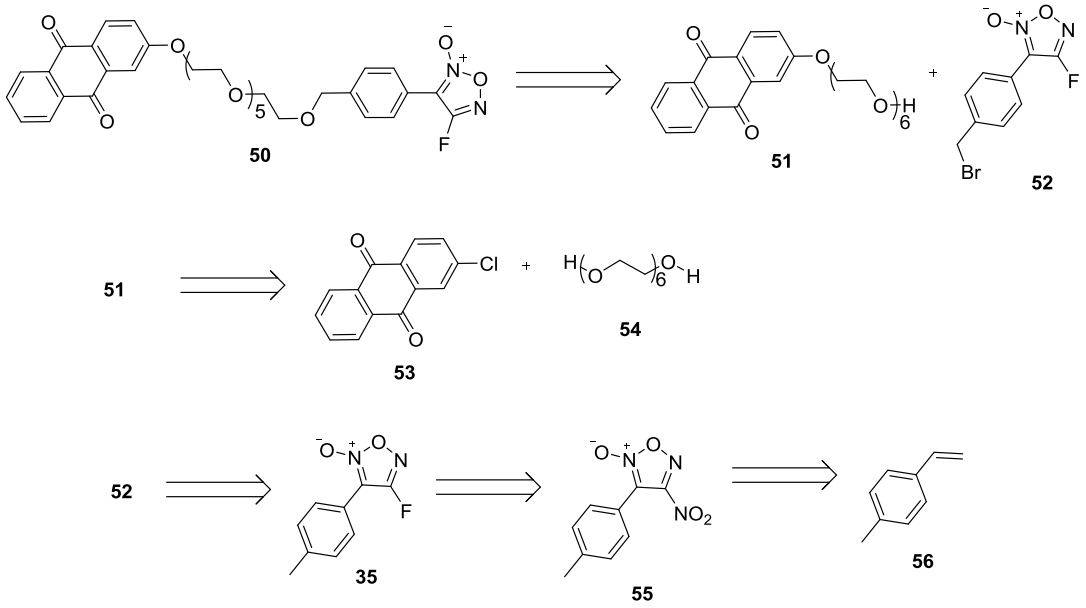
2.2 Nucleophilic aromatic substitution towards compound 50

Our first synthetic stratagem was the direct linking between anthraquinone and fluorofuroxan via a glycol linker.

The anthraquinone photosensitizer core is known for its poor solubility and the fluorofuroxan is only marginally soluble in phosphate buffer, therefore aqueous solubilizing moieties would need to be installed in the molecule for successful application. Ethylene glycol and longer chain glycols are known to have a solubilizing effect due to the polar ether linkages and terminal alcohol groups. We suspected that water solubility may be granted by a suitably long linker between the furoxan and anthraquinone. Water solubility has previously been endowed to anthraquinone molecules using glycol chains to synthesize antifungal agents for use in catfish ponds.[73]

2.2.1 Synthesis towards compound 50

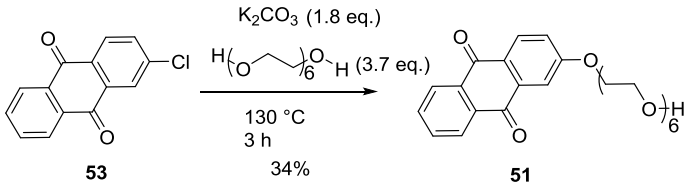
With solubility in mind our first synthetic plan utilizing hexaethylene glycol is shown in scheme 10.



Scheme 10: Proposed synthesis of glycol tethered anthraquinone-furoxan **50**.

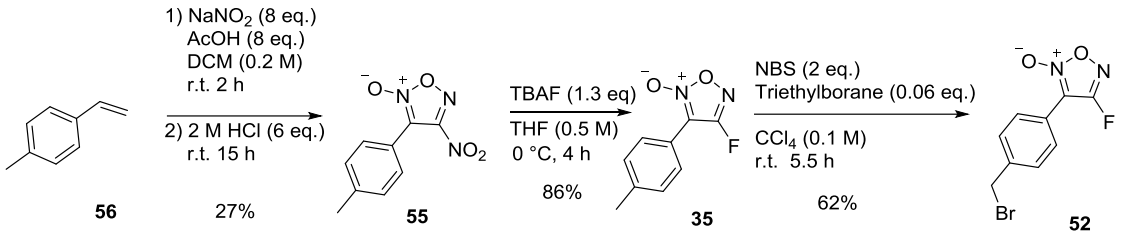
The glycol-tethered anthraquinone furoxan **50** can be synthesized by benzylic substitution of fluorofuroxan **52** with anthraquinone-tethered alcohol **51**. Anthraquinone **51** is synthesized using commercially available 3-chloroanthraquinone and hexaethylene glycol.[73] Furoxan **52** is synthesized by bromination of fluorofuroxan **36** which in turn is synthesized by nucleophilic aromatic substitution of the corresponding nitrofuroxan. The Weiland procedure grants nitrofuroxan **55** from styrene **56**.[52]

Stirring 3-chloroanthraquinone in hexaethylene glycol at 130 °C granted **51** in 34% yield (Scheme 11)



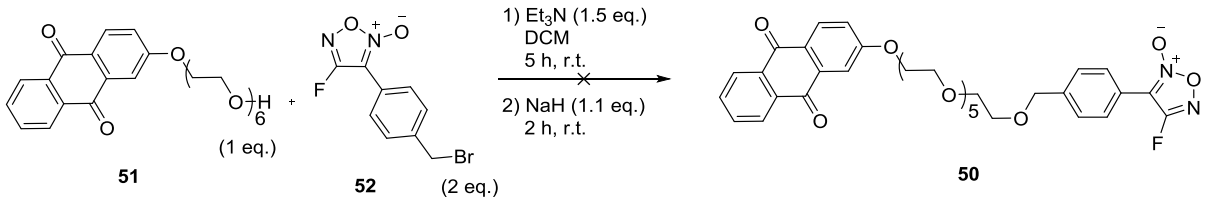
Scheme 11: Nucleophilic aromatic substitution reaction to form **51**.

The furoxan partner **52** was prepared firstly by fluorination of nitrofuroxan and subsequent bromination of the benzylic position (Scheme 12).



Scheme 12: Synthesis of brominated fluorofuroxan **52**.

With both reaction partners in hand we attempted to join the furoxan to anthraquinone via alkoxy linker (Scheme 13).

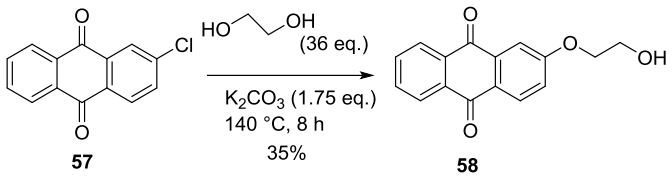


Scheme 13: Attempted conjoining of **51** and **52** firstly under mild conditions and then hard conditions.

After 5 hours of stirring the reagents in the presence of weak base no reaction was observed by TLC. NaH was added to deprotonate the alcohol and induce reaction. After a further 2 hours consumption of furoxan was observed, however crude analysis suggested a complex mixture had formed. Purification attempts with both silica chromatography and PTLC were unsuccessful and only unreacted anthraquinone starting material was successfully recovered. It was clear that optimisation studies were required.

2.2.2 Synthesis of compound 58

Rather than risk expensive hexaethylene glycol-anthraquinone starting material we decided to use a cheaper ethylene glycol model compound **58** for use in screening experiments.



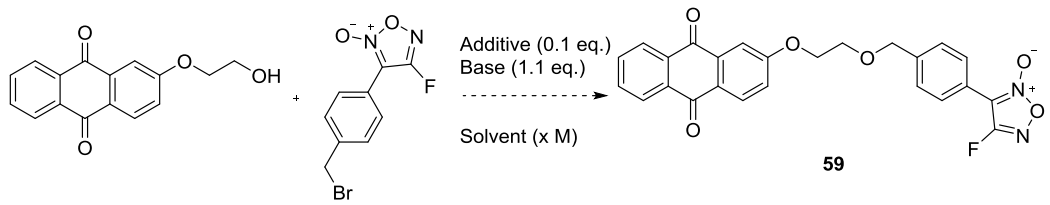
Scheme 14: Synthesis of model compound **58.**

Ethylene glycol was not as solubilizing as hexaethylene glycol and so had to be used in excess to adequately dissolve anthraquinone. The product was obtained in moderate yield after stirring at 140 °C for 8 hours (Scheme 14).

2.2.3 Screening experiments towards compound 59

With starting materials in hand, we conducted screening experiments between **58** and **52** (Table 2).

Table 2: Screened conditions for the benzylic nucleophilic substitution of **52**.



Entry	Solvent (x)	Base	Additive	Time (h)	Yield (%)
1	DMSO (0.5 M)	Et_3N	KI	15	0
2	DMF (0.5 M)	Et_3N	KI	15	0
3	MeCN (0.5 M)	Et_3N	KI	18	0
4	DMSO (0.5 M)	Et_3N	-	18	0
5	MeCN (0.2 M)	Et_3N	-	18	0
6	DMF (0.4 M)	Et_3N	-	18	0
7	DMSO (0.4 M)	Pyridine	-	19	0
8	DMSO (0.4 M)	*N*,*N*-Diisopropylethylamine	-	19	0
9	DMSO (0.4 M)	HTMP	-	19	0
10	DMSO (0.4 M)	Na_2CO_3	-	19	0

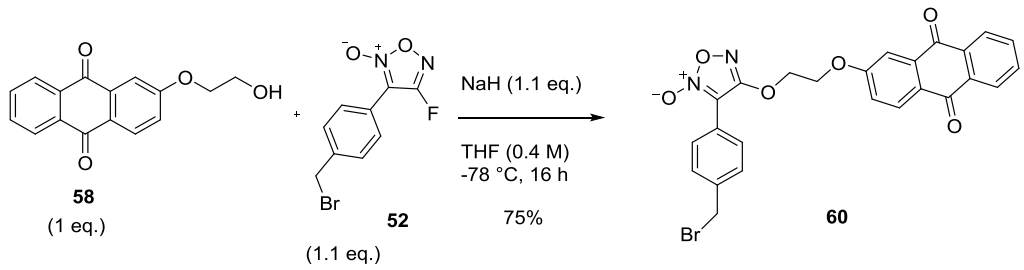
For the nucleophilic substitution reaction high polarity solvents were selected along with catalytic KI and Et_3N base to enhance S_N2 reactivity. Solubility was an issue and solvents other than DMF and DMSO struggled to dissolve the anthraquinone.

In all cases the desired compound was not observed (Table 2). This is due to significant and exclusive decomposition of the furoxan starting material, we also observed unreacted anthraquinone. Acetonitrile did not adequately solubilise the mixture and after 18 hours furoxan had undergone significant decomposition (Table 2, Entry 3). Omission of the KI salt did not lead to desired reactivity which led us to suspect that the probable cause of decomposition was due to the nature of the base. Changing the base to pyridine (Table 2, Entry 7) resulted in the same furoxan decomposition, however when more sterically hindered amine bases were used different

decomposition products became observable by TLC and ^{1}H NMR (Table 2, Entries 8 and 9). Using a non-amine base (Table 2, Entry 10) also lead to decomposition of the furoxan. The variety of decomposition products suggested that careful choice of base was required in future experiments with fluorofuroxan.

In summary, due to the reactivity of furoxan in the presence of selected bases we were unable to connect furoxan **52** and anthraquinone **58** using mild S_N2 conditions.

Finally, attempted hard nucleophilic conditions by deprotonating the alcohol with sodium hydride (Scheme 15).



Scheme 15: Reaction between **58** and **52** using NaH as a base.

Slow addition of the deprotonated **58** into a solution of **52** in THF at -78 °C resulted in incorrect regioselectivity and granted **60**. Reaction occurred directly at the furoxan ring rather than the benzylic position, the byproduct was obtained and the structure was assigned based on the absence of a fluorine peak in ^{31}F NMR.

2.2.4 Conclusions

During the synthesis towards compound **50** we encountered stability and regioselectivity issues due to the reactive nature of the fluoro- substituted furoxan 4-position. Interestingly, byproduct **60** may have been a competent alkoxy photoswitchable NO donor in its own right, further solubility could have been granted

by substitution at the benzylic position with pendant solubilising groups. Unfortunately, we were limited to investigating only the anthraquinone and fluorofuroxan moiety at this point in the project due to competing research into alkoxy furoxan PINODs within the group, so regrettably this route was not further investigated. Incorrect regioselectivity aside, the length of the hexaethylene glycol chain could have been detrimental to a successfully synthesized anthraquinone-fluorofuroxan molecule as it places the two partners relatively far apart so excited state energy transfer via electron exchange may have been slow if occurring at all.

We decided to take an alternative approach towards a water soluble anthraquinone tethered fluorofuroxan molecule.

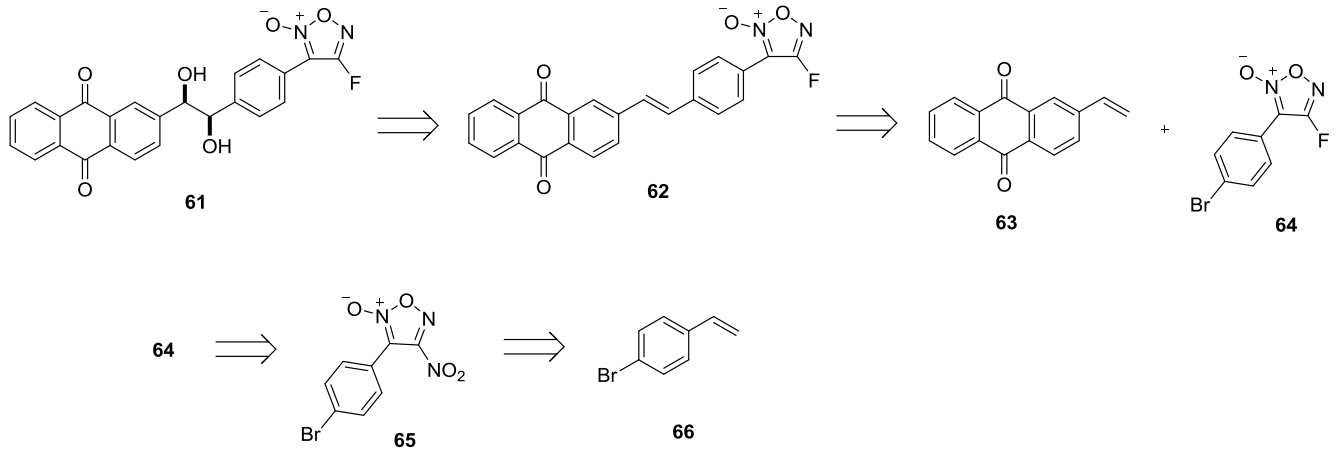
2.3 Palladium catalysis towards compound 61

2.3.1 Heck reaction

In order to avoid regioselectivity issues and also to shorten the linker between sensitizer and furoxan we decided to investigate the Heck reaction as linker formation method

2.3.1.1 Synthesis towards compound 64

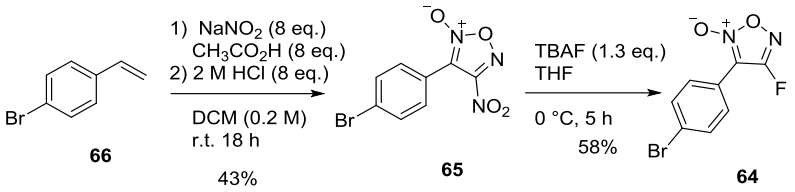
The retrosynthetic analysis of compound **64** is shown in Scheme 16.



Scheme 16: Retrosynthetic analysis of Heck catalysed formation of **61**.

Bearing two bridge alcohol groups we hypothesized that the water solubility of the connected molecule **61** would be adequate for NO release testing. The completed molecule can be accessed by dihdroxylation of **62,** which would in turn be furnished by Heck reaction between commercially available 2-vinylanthraquinone and fluorofuroxan coupling partner **64**. The furoxan **64** would be synthesized by fluorination of nitrofuroxan **65** which would be constructed by Wieland conditions applied to commercially available 4-bromostyrene **66**.

The synthesis of **64** began with formation of **65** upon treatment of 4-bromostyrene with $NaNO_2$ under acidic conditions (Scheme 17). Fluorination of the nitrofuroxan **65** yielded coupling partner **64** in moderate yield.

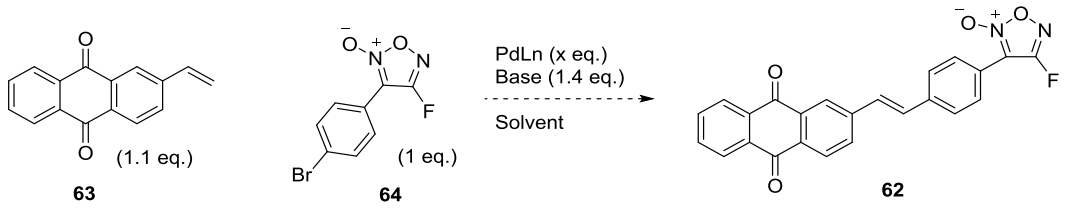


Scheme 17: Preparation of fluorofuroxan **64**.

2.3.1.2 Screening experiments towards compound 62

After obtaining the furoxan starting material, the Heck reaction conditions were investigated (Table 3).

Table 3: Screened Heck reaction conditions



Entry	Solvent	Base	Catalyst/Ligand (x)	Temp. (°C)	Time (h)	Yield (%)
1	DMF (0.35 M)	NaOAc	$Pd(OAc)_2$ (0.01 eq.)	90		0
2	DMF (0.35 M)	-	$Pd(OAc)_2$ (0.01 eq.)	90		0
3	Dioxane (0.35 M)	KOAc	$Pd(OAc)_2$ (0.05 eq.)	60		0
4	Dioxane (0.8 M)	Cy_2NMe	$Pd_2(dba)_3$ (0.05 eq.)	r.t.	40	0
5	DMSO (0.8 M)	Cy_2NMe	$Pd_2(dba)_3$ (0.005	r.t	40	0

We were unable to form the desired product using the Heck conditions in table 3. In each case the main observable byproduct was 4-bromobenzontrile. The 2-

vinylanthraquinone remained unreacted but the furoxan was sensitive to the reaction conditions and decomposed to 4-bromobenzonitrile. The omission of base resulted in decomposition of both anthraquinone and furoxan starting materials into a complex mixture (Table 3, Entry 2). The use of less polar dioxane (Table 3, Entry 3) led to longer reaction time and after consumption of furoxan starting material, the product was not isolated. In an effort to prevent furoxan decomposition we next employed conditions developed for room temperature Heck reaction (Table 3, entry 3).[74] Gratifyingly the furoxan did not decompose at room temperature after 40 hours, in fact neither the anthraquinone nor the furoxan reacted. Warming the reaction mixture to 60 °C for 6 hours also did not invoke reaction, however when heating to 120 °C we observed full decomposition after 14 hours with both dioxane (Table 3, Entry 4) and DMSO (Table 3, Entry 5) solvents.

Our current hypothesis is that the furoxan is attacked by base in the presence of a Lewis acid chelated palladium species. This is supported by the longer lifetime of the furoxan when using sterically hindered base species Cy_2NMe, which required higher temperatures to decompose the furoxan.

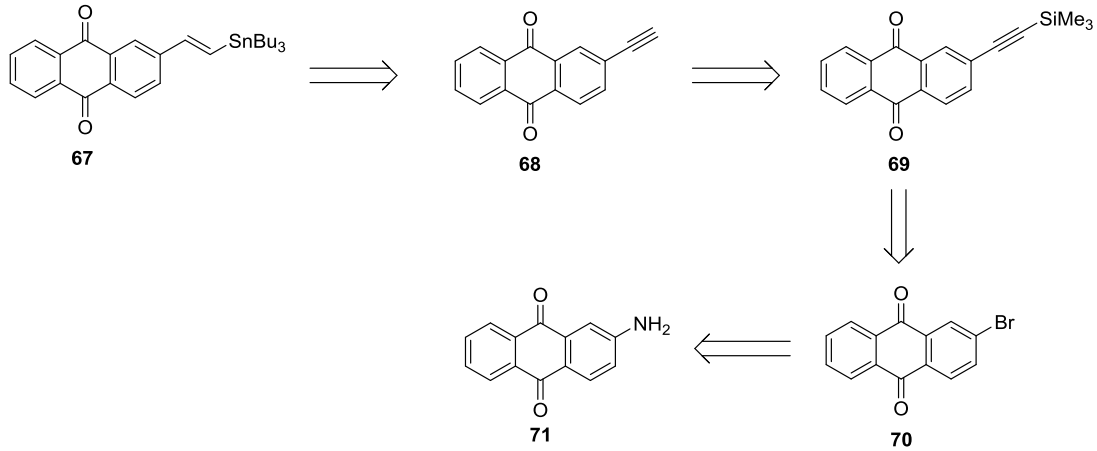
We concluded that the instability of the furoxan under investigated conditions so far was due to the presence of base and decided to try milder palladium catalysed conditions which did not require it.

2.3.2 Stille coupling

Based on the encountered difficulties with furoxan instability in the presence of base and palladium catalyst, we hypothesized that the Stille reaction may provide the mild conditions necessary for cross coupling between anthraquinone and furoxan partners.

2.3.2.1 Synthesis towards compound 67

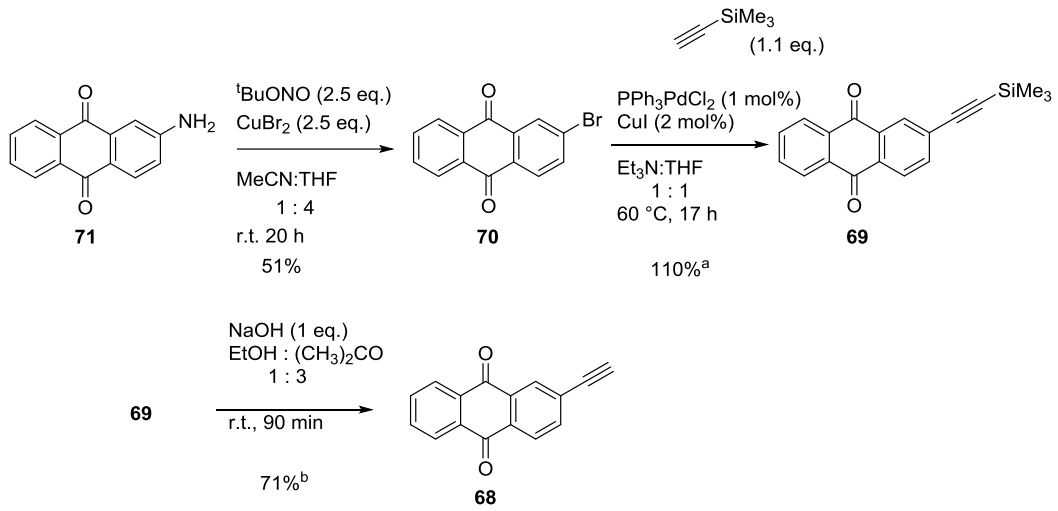
With compound **64** already synthesized we designed a synthesis for vinylstannane anthraquinone **67** (Scheme 18).



Scheme 18: Retrosynthetic analysis of vinyl stannane anthraquinone **67**.

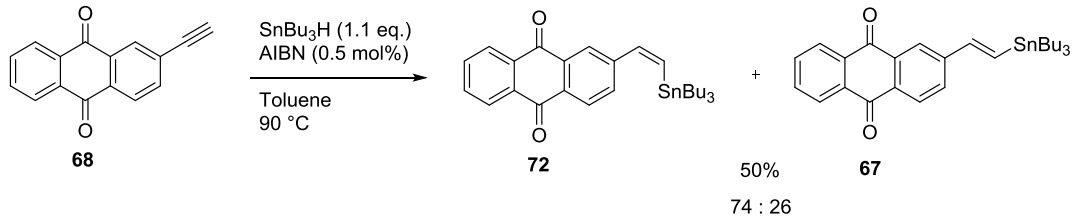
Compound **67** is accessed by hydrostannylation of alkyne **68** which in turn in synthesized by desilylation of **69** with base. The silyl alkyne **69** is synthesized by Sonogashira coupling of **70** with trimethylsilaneacetylene. Compound **70** is granted by Sandmeyer reaction on commercially available 2-aminoanthraquinone.

Our synthesis began by employing modified Sandmeyer conditions which granted **70** in moderate yield (Scheme 19).[75] The Sonogashira coupling of **70** with trimethylsilaneacetylene formed the intermediate **69** which upon treatment with base, desilylated to 2-alkenylanthraquinone **68** in 71% yield over two steps.[76]



Scheme 19: Synthesis towards **68**. [a]Crude yield. [b]Two steps from **70**.

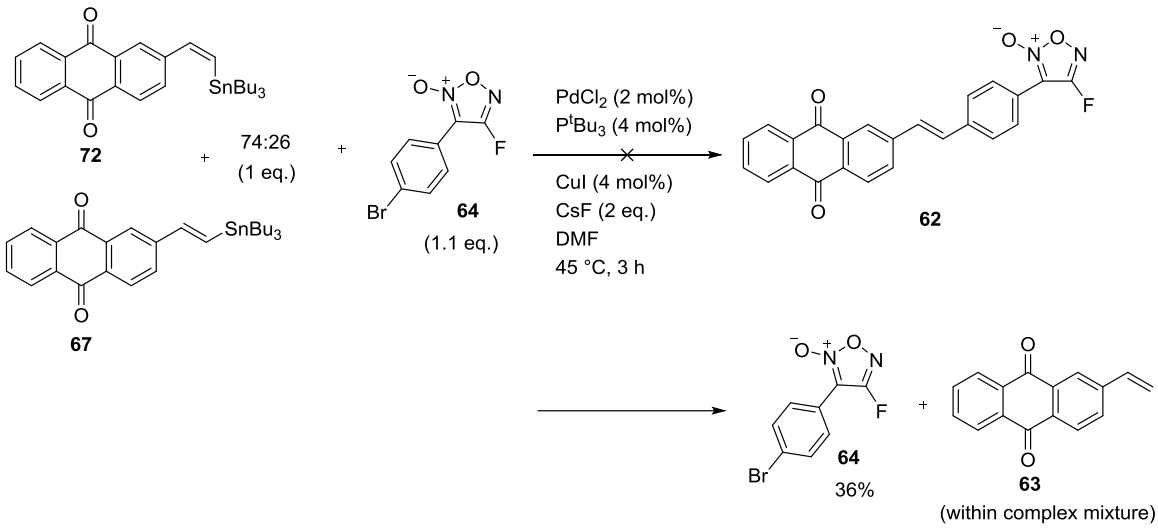
Hydrostannylation of **68** produced a moderate yielding mixture of isomers that were inseparable by column chromatography.[77] based upon coupling constants in the ^{1}H NMR, we deduced that the desired E stereoisomer **67** was unexpectantly obtained as the minor isomer and the Z isomer **72** was present as the major (26 : 74 respectively) (Scheme 20)



Scheme 20: Hydrostannylation of **68**.

2.3.2.2 Reaction between compound 67 and 72 with compound 64

Although we had obtained the undesired isomer of the vinly stannylanthraquinone we decided to test if the Stille reaction could work with this substrate (Scheme 21).



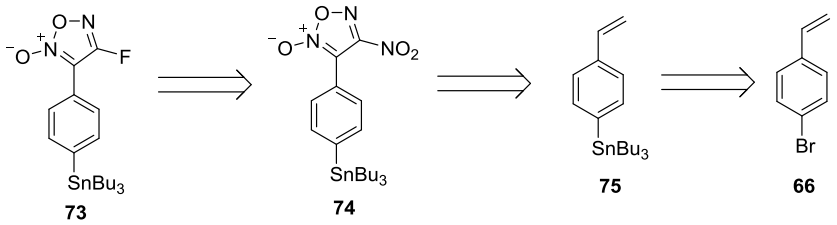
Scheme 21: Attempted Stille cross coupling between a mixture of **67** and **72** with **64**.

Following purification of the crude mixture the desired product **62** was not isolated, instead 36% of furoxan starting material was recovered along with destannylated 2-vinylanthraquinone **63** as the major component within a complex mixture.

This reaction showed that although vinylstannanes **67** and **72** are susceptible to destannylation, under these conditions the furoxan **64** is not fully decomposed and suggested that fluorofuroxans may be suitable for Stille coupling under optimised conditions.

2.3.2.3 Synthesis of compounds 73 and 77

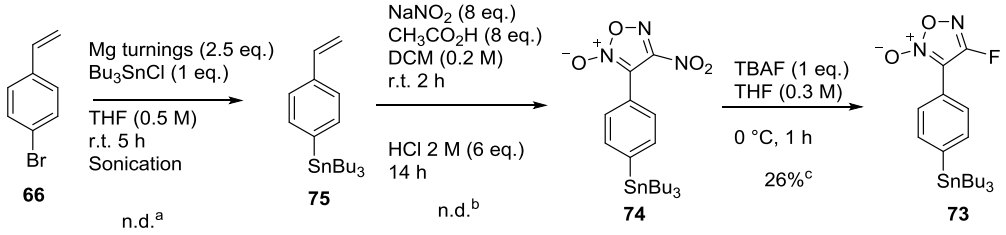
To address the incompatibility of **67** as a substrate in the Stille reaction we decided to swap the coupling partner roles in the reaction. Stannyl furoxan **73** would need to be synthesised, scheme 22 shows the retrosynthetic analysis.



Scheme 22: Retrosynthetic analysis of Stannyl furoxan **73**.

Compound **72** could be synthesised by fluorination of nitrofuroxan **74**, which in turn could be constructed by using the standard Wieland procedure on **75**. The synthesis of **75** had been reported from 4-bromostyrene in an unusual fashion by sonicating the styrene in THF with magnesium turnings for two hours.[78] The stannylation of **66** is reportedly low yielding with standard Grignard type preparation due to polymerisation of the starting material.

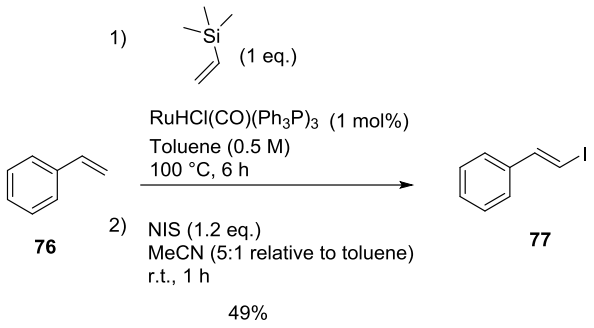
Following the sonication procedure, using silica chromatography we were unable to cleanly isolate the stannylated styrene due to the highly unpolar nature of the product and byproducts. We were concerned that distillation would decompose the product or induce polymerisation of the styrene so we decided to carry the material forward with minor impurities. To our delight the nitrofuroxan was formed in good yield after chromatographic purification and fluorination proceeded smoothly to give us the desired coupling partner **73** in 26% yield over 3 steps (Scheme 23).



Scheme 23: Synthesis of stannyl furoxan **73**. [a]Due to inseparable byproducts after silica chromatography, approximately 76% yield. [b]Due to impure starting material approximately 42% yield.[c]Yield is calculated over 3 steps, 85% from **74**.

Due to the difficulty in handling and purifying the anthraquinones we decided to use

β-iodostyrene as a model in the Stille reactions. This was synthesised according to literature procedure and was isolated in 49% yield (Scheme 24).[79]



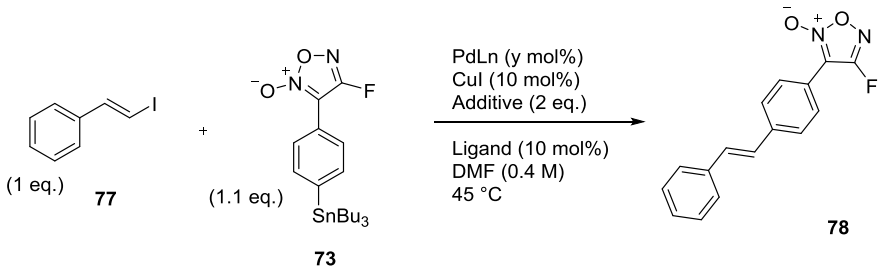
Scheme 24: Synthesis of model coupling partner for Stille reaction.

2.3.2.4 Screening experiments towards compound 78

With the coupling partners in hand we began screening conditions and reagents for reaction optimization. The conditions were derived from a comprehensive review on Stille coupling by Farina et. al[80]

We began by screening different palladium catalysts with 10% CuI in DMF at 45 °C (Table 4).

Table 4: Catalysts screened in the Stille coupling between **77** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug. [b] Reaction length for convenience. [c] Impure product.

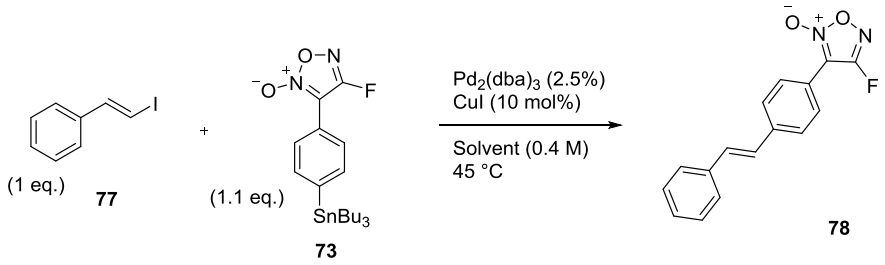


Entry	Catalyst (y mol%)	Ligand	Additive	Time (h)	Yield[a] (%)
1	$Pd(PPh_3)_4$ (5%)	-	CsF	2	9
2	$Pd_2(dba)_3$ (2.5%)	-	CsF	1	9
3	$Pd(PPh_3)Cl_2$ (5%)	-	-	5	4
4	$Pd(CNCH_3)_2Cl_2$ (5%)	-	-	23	10
5	$Pd(OAc)_2$ (5%)	$P(o\text{-}tol)_3$	-	22	14[c]
6	$Pd_2(dba)_3$ (2.5%)	-	-	[b]69	14
7	$Pd(PPh_3)_4$ (5%)	-	-	6.5	5

The screening reactions suggested that the best catalyst was $Pd_2(dba)_3$ (Table 4, Entry 6). The yield was equally high using $Pd(OAc)_2$ (Table 4, Entry 5) but in this case the product was not isolated cleanly. The addition of CsF proved to be detrimental to the reaction with the use of $Pd_2(dba)_3$, CsF supposedly can help by precipitating tin residues from the reaction solution (Table 4, Entry 2).[81] It is possible that in this case it reacted as a fluoride nucleophile and decomposed some of the furoxan.

Having selected $Pd_2(dba)_3$ as the catalyst of choice for the Stille coupling we next moved on to investigating the solvent.

Table 5: Solvents screened in the Stille coupling between **77** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug. [b]Reaction length for convenience. [c]Reaction did not reach completion. [d]Impure product.

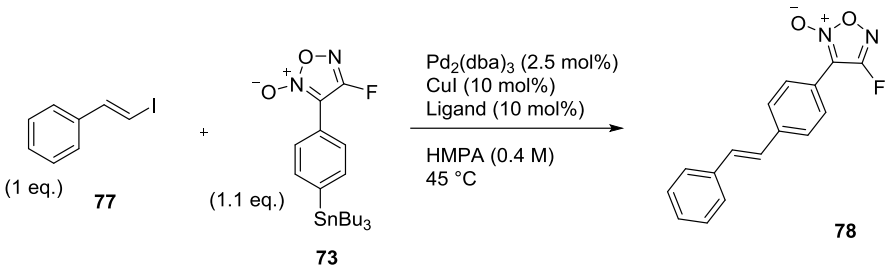


Entry	Solvent	Time (h)	Yield[a] (%)
-	DMF	[b]69	14
1	Toluene	65	13[c]
2	THF	65	30[c]
3	NMP	24	15[d]
4	HMPA	6	34

Analysis of the screened solvents shows that DMF (Table 4, Entry 6), NMP (Table 5, Entry 3) and toluene (Table 5, Entry 1) provided low yields, whereas THF (Table 5, Entry 2) and HMPA (Table 5, Entry 4) provided moderate yields. For convenience HMPA was selected because the reaction time was considerably shorter and yield slightly higher. It would also be easier to investigate temperature later in the screening due to the lower volatility and higher boiling point of HMPA relative to THF.

With a suitable solvent selected we moved on to the investigation of ligand screening (Table 6).

Table 6: Ligands screened in the Stille coupling between **77** and **77**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug. [b]Reaction incomplete after 8 h, reaction length for convenience.

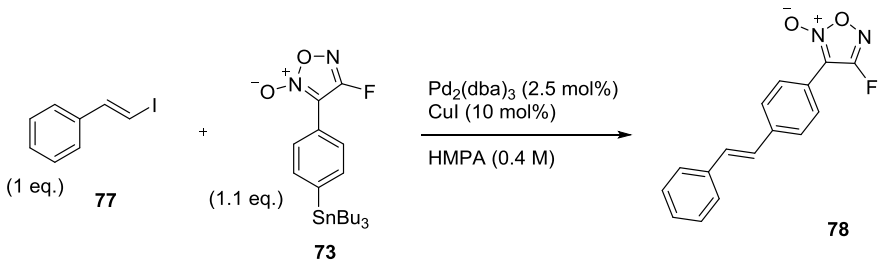


Entry	Ligand	Time (h)	Yield[a] (%)
-	-	6	34
1	Ph_3As	48	16[b]
2	XPhos	48	30[b]

The addition of ligands retarded the reaction rate. Ph_3As is supposed to dissociate from the metal centre more readily than other ligands creating an active site and promoting reactivity, however under the investigated conditions the yield was diminished (Table 6, Entry 1). Bulky ligands are also supposed to enhance the Stille coupling however XPhos did not provide a significant improvement (Table 6, Entry 2). Further ligand screening was not conducted due to the slow reaction rate and poor performance of archetypical Stille coupling ligands.

The effect of temperature on the Stille coupling was investigated next (Table 7).

Table 7: Reaction temperature screened in the Stille coupling between **77** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug.

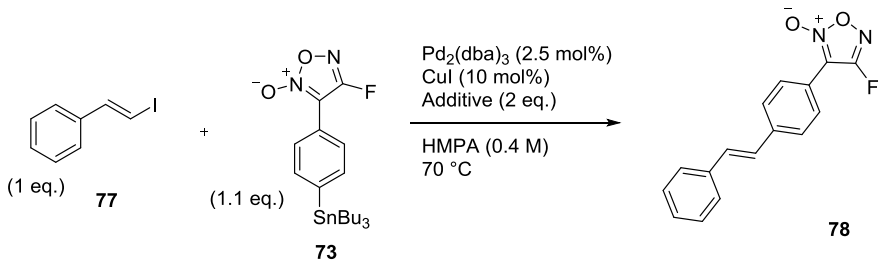


Entry	Temperature (°C)	Time (h)	Yield[a] (%)
-	45	6	34
1	70	1	35
2	100	1	31

The temperature effect was not significant with respect to the yield; however, the reaction rate was increased at higher temperatures. Following this data, we decided to conduct the reaction at 70 °C (Table 7, Entry 1).

We next investigated the effect of additives (Table 8).

Table 8: Additives screened in the Stille coupling between **77** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug.



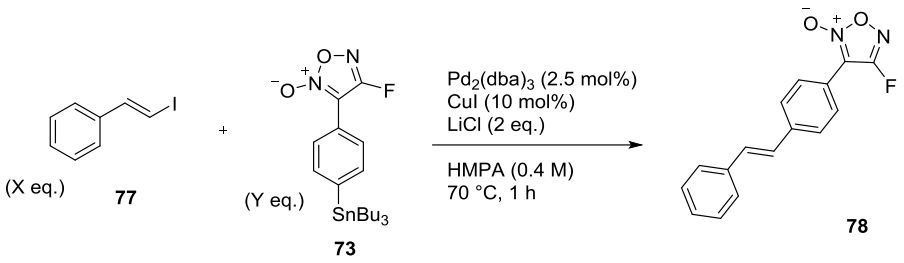
Entry	Additive	Time (h)	Yield[a] (%)
-	**-**	1	35
1	LiCl	1	57
2	CsF	1.5	21

Both LiCl and CuI are common additives used to enhance the Stille coupling, the CuI assists with transmetallation of the stannane in polar solvents and the LiCl is reported to have an effect on the oxidative addition.[80] CsF is suspected to have an accelerating effect on the transmetallation and also removes the tin residues in situ by forming insoluble polymeric compounds to drive the equilibrium forward.[81]

Similar to the results in table 1, entry 2 we found that CsF depreciates the yield (Table 8, Entry 2). LiCl has a significant effect increasing the yield by 22% (Table 8, Entry 1). This suggests that the oxidative addition step may be rate limiting or the palladium complex may be less stable without the presence of chloride ions.

Finally reversing the reagent ratios granted the product in good yield (Table 9).

Table 9: The effect of reagent ratio on the Stille coupling reaction between **77** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug. [b]Isolated by silica column chromatography.



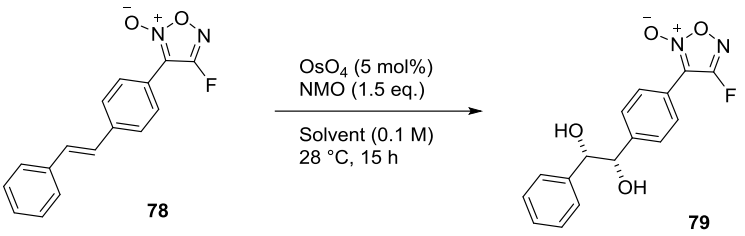
Entry	Reagent Eq. (X:Y)	Yield (%)
1	1 : 1.1	57[a]
2	1.14 : 1	67[b]

The yield was improved by using a slight excess of β-iodostyrene relative to stannyl furoxan. With an optimised yield of 67% we decided to move onto the dihydroxylation reaction to break the conjugation and complete the model synthesis.

2.3.2.5 Synthesis of compound 79

We applied standard conditions of the Upjohn Dihydroxylation in two different solvents (Table 10). Using THF instead of acetone gave a slightly higher yield and less residual osmium contamination (Table 10, Entry 2).

Table 10: Dihydroxylation of **78**. [a]Osmium contamination. [b]Minor osmium contamination

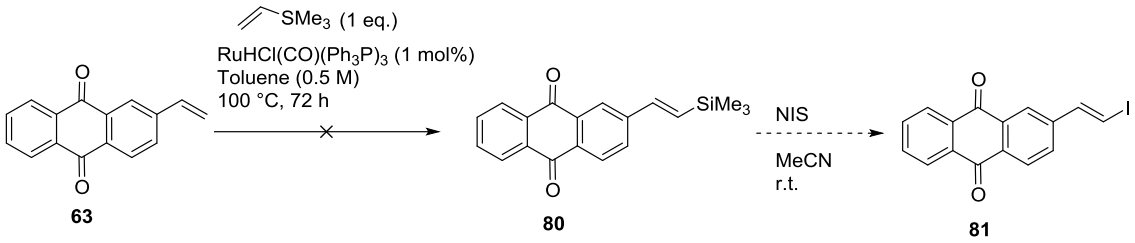


Entry	Solvent	Yield[a] (%)
1	Acetone : Water	86[a]
2	THF : Water	91[b]

2.3.2.6 Synthesis of compound 81

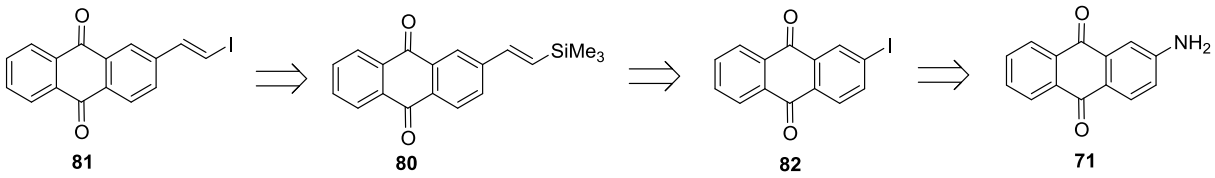
We were successfully able to synthesise the model compound **79** and decided to apply the developed synthetic route to coupling anthraquinone and stannlyfuroxan.

Firstly, we needed to synthesize the β-iodo-vinylanthraquinone, our initial attempt made use of the metathesis reaction used in the synthesis of **77** (Scheme 25).[79] Despite using the same conditions as the iodination of styrene there was no reaction even after 3 days. This is most likely due to the electron deficient nature of the anthraquinone conjugated alkene. Instead the vinylanthraquinone starting material was recovered.



Scheme 25: Attempted synthesis of β-iodo-vinylanthraquinone.

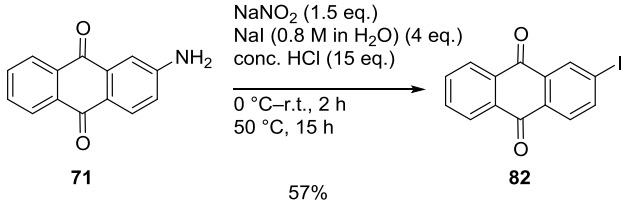
A new method needed to be devised for the synthesis of **81**, the retrosynthetic route is shown in scheme 26.



Scheme 26: Retrosynthetic analysis of β-iodo-vinylanthraquinone

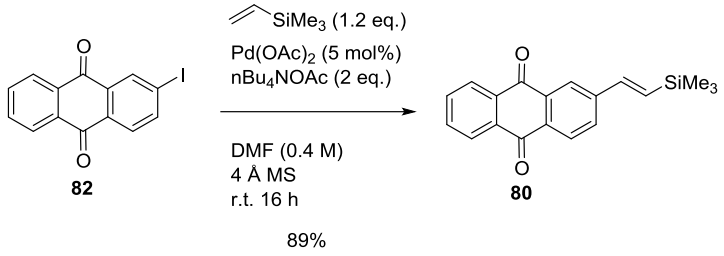
The iodostyrene is granted by halodesilylation of **80** which in turn is accessed by Heck reaction from 2-iodoanthraquinone. Sandmeyer reaction with **71** completes the retrosynthetic analysis.

2-Iodoanthraquinone was synthesised using Sandmeyer type conditions and was obtained in good yield (Scheme 27).[82]



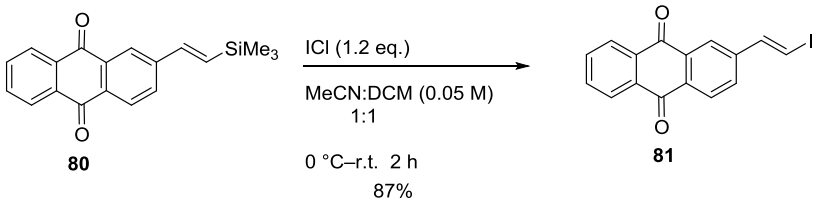
Scheme 27: Sandmeyer type reaction to form **82**.

The 2-iodoanthraquinone was then coupled to trimethylvinylsilane via Heck reaction to afford **80** in 89% yield (Scheme 28).[83]



Scheme 28: Palladium catalysed Heck reaction of **82** with vinylsilane.

Halodesilylation using iodinemonochloride granted the desired coupling partner **81** (Scheme 29).



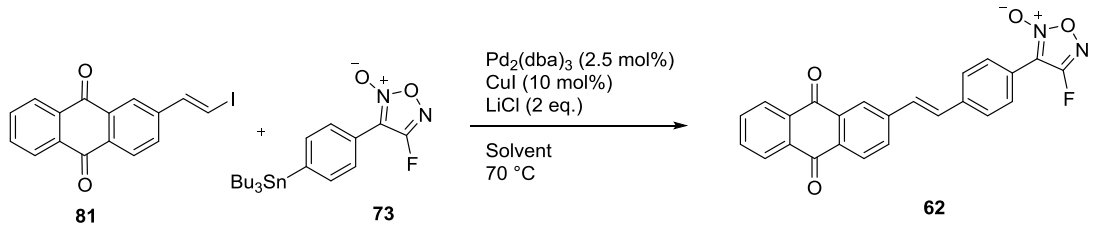
Scheme 29: Halodesilylation of **80** with ICl.

2.3.2.7 Synthesis of compound 61

Next, we attempted the Stille coupling between furoxan **73** and anthraquinone **81** (Table 11).

Our first attempt at the Stille coupling (Table 11, Entry 1) utilised our optimised conditions for the Stille coupling between **77** and **73** (Table 9, Entry 2).

Table 11: Stille coupling between **81** and **73**. [a]Isolated yield by silica PTLC after crude filtration through 10% w/w K_2CO_3 silica plug. [b]Product observed by ^1H NMR but not successfully isolated.



Entry	Solvent	Conc. (M)	Time (h)	Yield (%)[a]
1	HMPA	0.4	1	0
2	HMPA	0.1	1.5	12
3	DMSO	0.1	3	0[b]
4	DMI	0.1	1	0[b]
5	DMPU	0.1	1	0[b]

The solubility of the anthraquinone coupling partner was so poor that in HMPA at 0.4 M a thick paste formed preventing stirring and efficient reaction, this resulted in a complex mixture (Table 11, Entry 1).

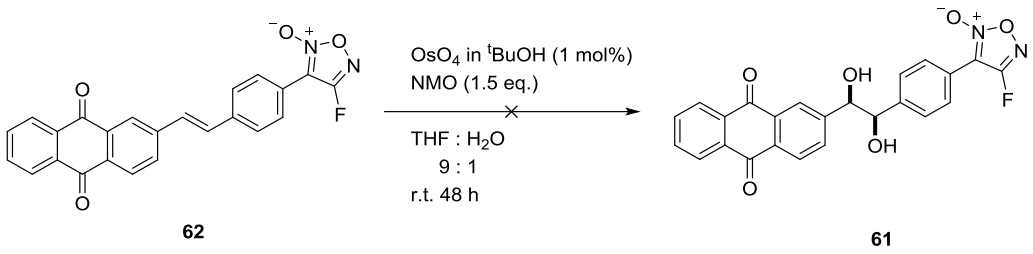
When the concentration was reduced to 0.1 M the reaction proceeded but in low yield providing **62**, however we encountered significant purification issues and we were not able to cleanly isolate the product (Table 11, Entry 2). The reaction conditions did not seem suitable since 49% of **81** starting material was recovered and furoxan homocoupled byproducts were detected showing that the anthraquinone was not particularly reactive.

We next changed the solvent of the reaction in the hopes that the reaction yield could be improved (Table 11, Entries 3-5). Unfortunately, despite observing some product formation by ^1H NMR we were unable to cleanly isolate the product.

The difficulty with purification raised concerns and we concluded that in future reactions it may be preferable to use **62** as a crude mixture in the hopes that the dihydroxylated product could be isolated more easily.

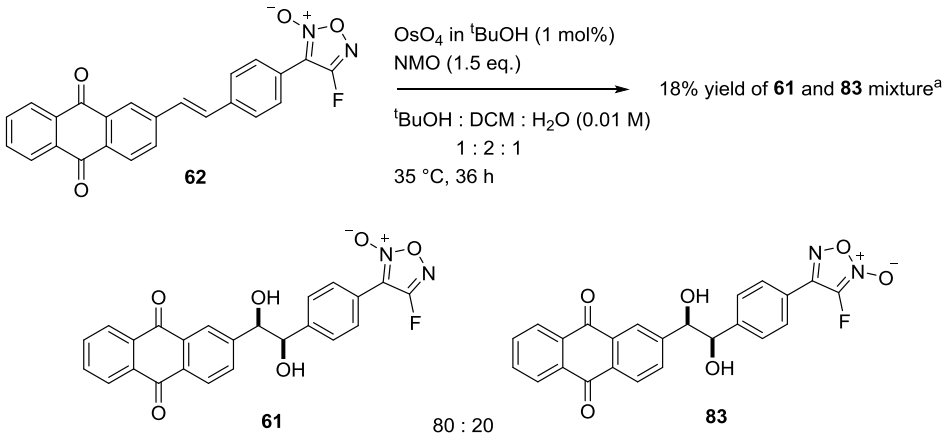
The dihydroxylation step was designed to break the conjugation in the molecule but also to improve the solubility of the product.

The same conditions used in the dihydroxylation of the model molecule **78** were not applicable since **62** had significant solubility issues in THF and so the reaction mixture was diluted to a 9:1 THF to water mixture (Scheme 30). Unfortunately, after 48 hours TLC and ^{1}H NMR suggested that no reaction had occurred.



Scheme 30: Attempted dihydroxylation of **62**.

Changing the solvent mixture to more polar ^{t}BuOH, DCM and H_2O lead to the formation of a small amount of *probable* product after 36 hours (Scheme 31). Most of the starting material remained unreacted. The product was isolated as part of a mixture by PTLC as a white solid (only 1.9 mg) using a highly polar solvent mixture (1% Et_3N, 5% MeOH and 94% $CHCl_3$). By heating the sample in deuterated methanol, a small quantity dissolved so we were able to obtain a proton and fluorine NMR.



[a]Isolated by silica PTLC. Remaining material is unreacted starting material.

Scheme 31: Dihydroxylation of precursor **62**.

The ^{1}H NMR suggested that the product was obtained as a mixture of isomers. The ^{19}F NMR chart supported this with two peaks, these peaks were in agreement with the spectra of the two regioisomers of fluorofuroxan, **35** and **36**. The integration of the peaks suggested that approximately 80% of the sample was the 4-fluorofuroxan isomer **35** and the remaining 20% was the isomerized 3-fluorofuroxan **36**.

We irradiated the sample with 400–600 nm of light for 15 minutes to see if the sample would isomerize further, perhaps favouring the formation of one regioisomer. Following irradiation, inspection of the ^{1}H NMR suggested decomposition had occurred.

2.3.3 Conclusions

After encountering regioselectivity issues with ethyleneglycol functionalized anthraquinones and fluorofuroxans we attempted palladium catalysed coupling reactions to ensure stereospecificity. Our attempts with the Heck reaction were limited by the poor stability of furoxan under the conditions. Changing our synthetic stratagem to utilize the milder Stille coupling enabled us to connect stannyl furoxan with β-iodovinyl functionalized moieties, leading to a coupled anthraquinone-fluorofuroxan hybrid. Although the synthesis of **83** could be optimised and larger quantities obtained, the problem of low solubility and tedious purification remained.

We decided that the practical application of the design was limited, even if further functionalisation of the dihydroxy linker was a possibility. We concluded that a new design was required, one in which solubility could be guaranteed via a salt functionality.

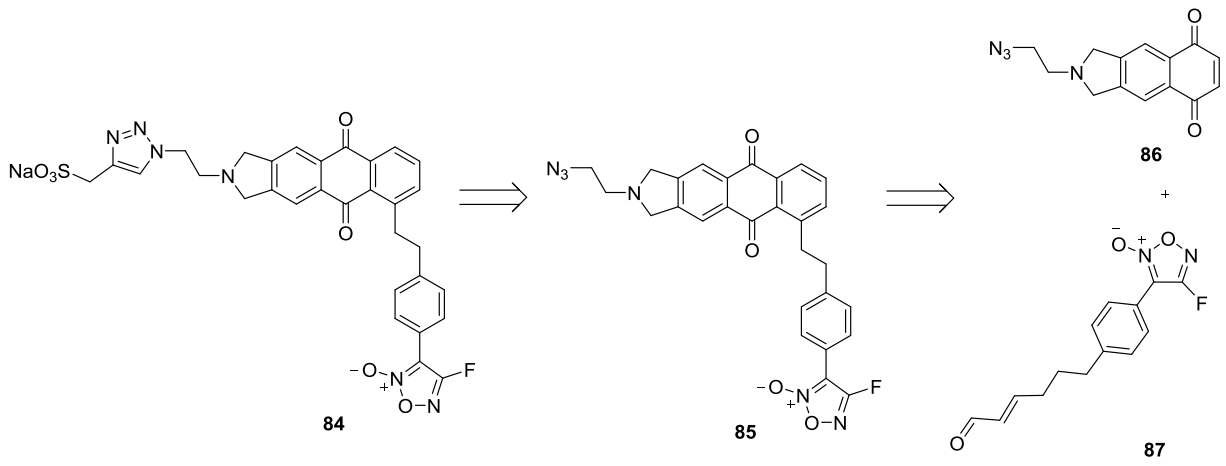
It is of note that the Stille coupling between **77** and **73** presented an unprecedented new range of Stille coupling mediated functionalisation available to furoxan chemistry and may find application in future research.

2.4 Organocatalysed [4 + 2] reaction towards compounds 104, 122 and 126 and evaluation of NO releasing abilities

It was noted during the previous synthetic routes that the handling, purification and characterisation of anthraquinone was difficult and time consuming due to poor solubility, so we wanted to synthesise the anthraquinone ring as one of the final steps. We also required a modular design that could be easily modified and retrofitted as needed. The final requirement was the presence of a salt group to assist with aqueous solubility.

2.4.1 Synthesis towards compound 84

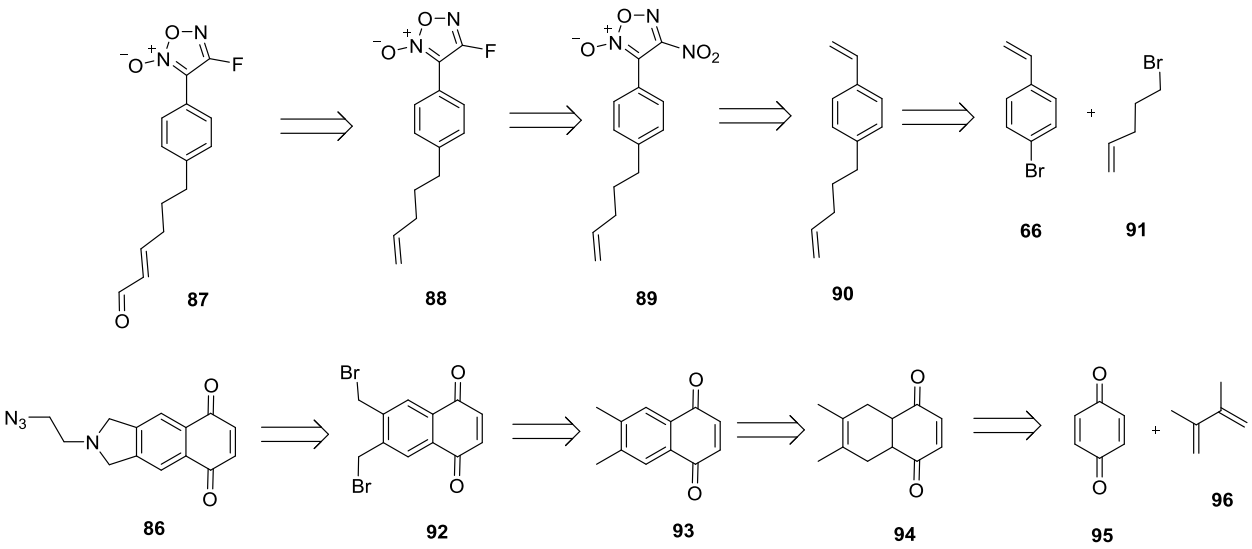
We designed a molecule which exhibited all of the above features and hypothesized upon a facile synthetic route. The key step features an organocatalysed [4 + 2] reaction based upon work by Magar et al.[84] in which the anthraquinone core is furnished with connection to the furoxan preprogramed during benzannulation. Water solubility would be endowed by sulfonate salt via 'click' triazole formation. The retrosynthetic analysis is shown in schemes 32 and 33.



Scheme 32: Retrosynthetic analysis of compound **84**

The desired product **84** could be obtained by click reaction triazole formation from anthraquinone **85**, which in turn could be synthesised by an organocatalyzed [4 + 2] and in situ oxidation reaction between modular building blocks **86** and **87**.[84]

Intermediate **87** could be obtained in 4 steps from 4-bromostyrene and 5-bromopentene (Scheme 33). A carbon-carbon bond formed *via* Grignard reaction would grant **90**, followed by Wieland furoxan synthesis to afford **89.** Subsequent fluorination and Grubbs metathesis with acrolein leads to the desired compound **87**.

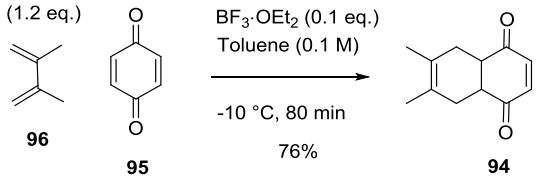


Scheme 33: Synthesis of organocatalyzed [4 + 2] reaction coupling partners **87** and **86**.

Intermediate **86** could be obtained in 4 steps from 2,3-dimethylbutadiene and 1,4-benzoquinone. Diels Alder reaction grants **94** which can be oxidised to **93**. Benzylic bromination leads to **92** which can be substituted with amino-azide to form the desired intermediate **86**.

2.4.1.1 Synthesis of compound 86

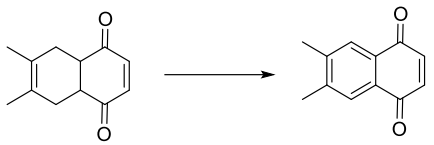
Firstly, a Diels-Alder reaction between commercially available 2,3-dimethylbutadiene and 1,4-benzoquinone in the presence of $BF_3{\cdot}OEt_2$ Lewis acid yielded the adduct in high yield following recrystallization in toluene (Scheme 34).



Scheme 34: Synthesis of **94** by Diels-Alder reaction.

Following successful Diels-Alder adduct formation we next attempted the oxidation of **94** to naphthoquinone **93** (Table 12).

Table 12: Reagents and conditions used in the oxidation of **94**. [a]Isolated yield by silica gel column chromatography.



94 **93**

Entry	Reagents	Conditions	Yield (%)[a]
1	Pd/C (5 mol%), acetone (0.25 M)	Reflux, 15 h	0
2	DDQ (3 eq.), dioxane (0.1 M)	Reflux, 18 h	28
3	IBX (3 eq.), DMSO (0.1 M)	80 °C, 18 h	22
4	MnO_2 (10 eq.), toluene (0.1 M)	Reflux, 2 h	68

Firstly, we attempted dehydrogenation by stirring **94** with Pd/C in refluxing acetone overnight, however it resulted in no reaction (Table 12, Entry 1).

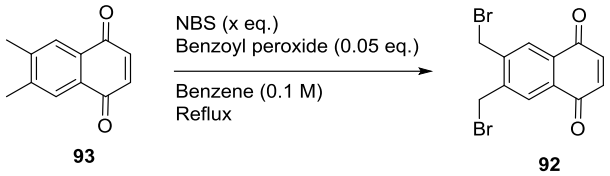
We next attempted oxidation of **94** with DDQ (Table 12, Entry 2). Refluxing **94** with DDQ in dioxane overnight granted **93** in 28% yield following purification by column chromatography.[85]

Stirring **94** and IBX in DMSO overnight yielded **93** in 22% yield (Table 12, Entry 3).[86]

We suspected that the starting material and product may be susceptible to side reactions, possibly due to over oxidation, so we decided to use the mild oxidising agent MnO_2. Refluxing **94** and MnO_2 in toluene for 2 hours granted **93** in high yield (Table 12, Entry 4).[87]

The naphthoquinone was then subjected to bromination conditions (Table 13).

Table 13: Benzylic bromination of **93** and respective purification attempts. [a]Impure product after chromatography. [b]Product obtained following recrystallization with minor impurities. [c]Yield obtained following two recrystallization attempts of crude material, minor impurities observed.

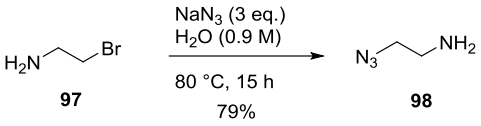


93 **92**

Entry	X	Time (h)	Purification Method	Yield (%)
1	2.3	18	Silica gel chromatography	78[a]
2	2.1	16	Silica gel chromatography followed by recrystallisation in	62[a]/30[b]
3	2.1	16	Recrystallization in EtOAc/hexane	52[c]

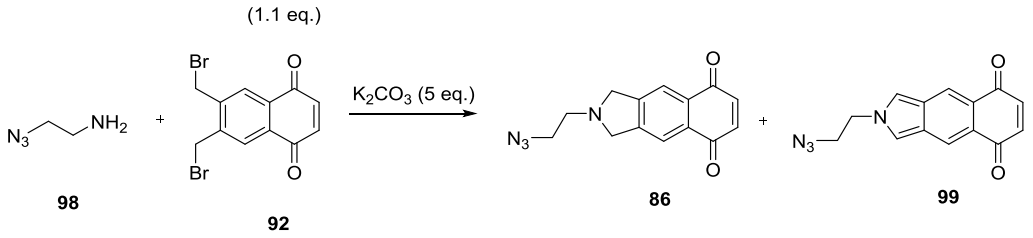
The reaction proceeded reasonably well but difficulty was encountered with purification. Purification by column chromatography alone resulted in coelution of byproducts, thought to be mono-brominated or over brominated material (Table 13, Entry 1). Attempts at recrystallization helped to reduce the severity of contamination but not remove it completely and at the cost of reduced isolated yield (Table 13, Entry 2). Abstaining from chromatography and utilizing only recrystallization yielded reasonably pure material but with successive recrystallization attempts of the remaining filtrate the purity diminished and clean product could not be further isolated (Table 13, Entry 3).

With recrystallized compound **92** we began screening conditions for the nucleophilic substitution of the brominated benzylic positions. Firstly, the amino-azide was synthesised from 2-bromoethylamine hydrochloride (Scheme 35), given the volatile nature of **98** we used the crude product without further purification.



Scheme 35: Synthesis of azide **98**.

Table 14: Conditions investigated in the substitution of benzyl bromide with amine **98**. [a]Isolated yield by silica PTLC.



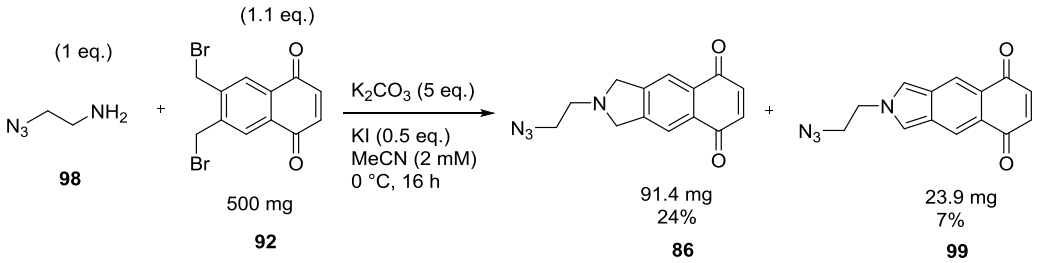
Entry	Additive (0.1 eq.)	Solvent (2 mM)	Temp. (°C)	Time. (h)	Yield 86[a] (%)	Yield 99[a] (%)	Comment
1	-	MeCN	60	2.5	8	5	-
2	-	MeCN	0	22	14	11	-
3	-	DMSO	r.t.	0.5	-	-	Decomposition
4	KI	MeCN	0	18	26	11	-
5	KI	DCM	0	18	-	-	No reaction
			r.t.	68	7	n.d.	-
6	KI	Toluene	r.t.	18	-	-	No reaction
			50	20	-	-	No reaction
7	KI	THF	r.t.	18	12	4	-
8	KI	MeCN (10 mM)	0	18	14	4	-
9	KI (0.5 eq.)	MeCN	0	18	28	12	-
10	KI (2 eq.)	MeCN	0	18	7	5	-

We began our investigation using similar conditions as reported for the nucleophilic substitution of (mono) benzylic bromide substituted naphthoquinones with amines (Table 14, Entry 1).[88] The reaction reached completion in only 2.5 hours turning from a bright yellow to a dark red colour. Aqueous workup was required for fear of remaining amine species attacking the desired product upon concentration. After

PTLC purification we obtained the product as a crystalline orange solid **86** in 8% yield along with a more polar oxidised byproduct **99** in 5% yield (Table 14, Entry 1). Lowering the reaction temperature to 0 °C increased the yield to 14% and 11% of **86** and **99** respectively (Table 14, Entry 2). Changing the solvent to more polar DMSO had a detrimental effect with almost immediate decomposition into an insoluble black solid (Table 14, Entry 3). The addition of catalytic potassium iodide improved the yield considerably to 26% and 11% of **86** and **99** respectively (Table 14, Entry 4). Changing the solvent to less polar DCM and toluene resulted in no reaction at 0 °C after 18 h (Table 14, Entries 5 and 6). Warming the DCM mixture allowed 7% of product to be obtained after prolonged reaction time (Table 14, Entry 5). The toluene reaction was warmed to 50 °C but no reaction was observed after 20 h, warming to 100 °C led to decomposition (Table 14, Entry 6). Changing the solvent to THF at r.t. led to a lower yield (Table 14, Entry 7). Increasing the concentration was detrimental to the reaction (Table 14, Entry 8). Increasing the equivalents of KI had a negligible effect at 0.5 equivalents, when increased to stoichiometric levels the yield was low, possibly due to attack from the iodide to the electron deficient alkene (Table 14, Entries 9 and 10).

Our best results gave us a yield of 28% which is reasonable considering the unstable nature of the product, which appeared to decompose if kept at room temperature for long periods. (Table 14, Entry 9)

With optimised procedure in hand we decided scale up to 500 mg (Scheme 36).



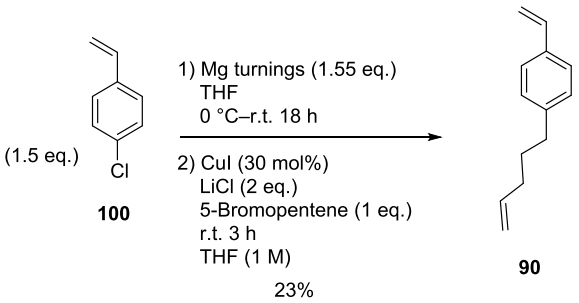
Scheme 36: Scale up of nucleophilic substitution reaction to form **86**.

Stirring overnight granted the desired product in 24% yield and the oxidised product in 7% yield. The products were purified by silica chromatography. The slightly lower yield compared to table 14, entry 9 can be attributed to decomposition. The instability of the compound became immediately apparent as it began to turn brown upon standing in air during unavoidable prolonged handling and transfer times.

2.4.1.2 Synthesis of compound 87

The synthesis of the furoxan intermediate **87** began with the Grignard reaction between halostyrene and 5-bromopent-1-ene.

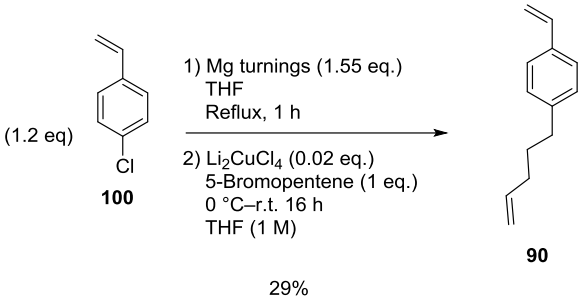
We were concerned about polymerization of bromostyrenes so decided to employ 4-chlorostyrene in the hopes that it was stable to the reaction conditions (Scheme 37). We used CuI catalyst with LiCl additive.[89] Once the Grignard reagent had been prepared from chlorostyrene and magnesium turnings the CuI and LiCl were added along with 5-bromopentene. Significant polymerisation occurred but we were able to isolate the desired product after column chromatography as the polymer was filtered off.



Scheme 37: Copper catalysed Grignard reaction to form **90**.

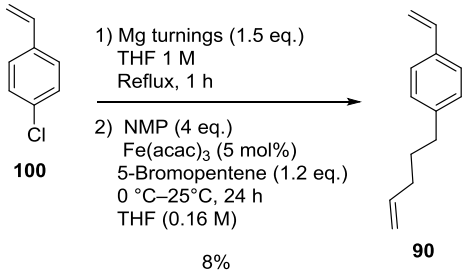
In order to improve the yield, we attempted the reaction using Li_2CuCl_4 as the catalyst (Scheme 38). This time we allowed the reaction to reflux gently during Grignard

formation. The Grignard solution was added dropwise to a solution of 5-bromopentene and Li_2CuCl_4 in THF then stirred at room temperature overnight. Following work up and purification on silica the product was obtained in 29% yield, once again significant polymerization was observed.



Scheme 38: Li_2CuCl_4 catalysed Grignard reaction.

We found that iron could be a good catalyst for sp^2-sp^3 Grignard reactions. We first tried $Fe(acac)_3$ in THF with NMP additive (Scheme 39).[90] After dropwise addition of Grignard reagent and overnight stirring the product was obtained in only 8% yield as determined by internal standard. The low yield was likely due to the poor reactivity of the chlorostyrene as significant unreacted material remained.



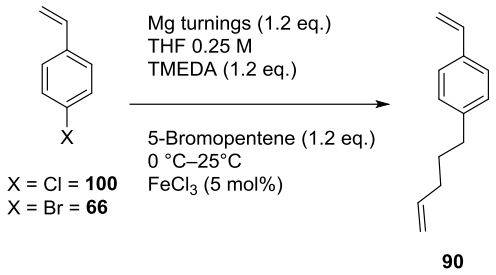
Scheme 39: Iron catalysed Grignard reaction.

Next, we tried using a different procedure where TMEDA which is used as an additive as it is known to improve iron catalysed reactions (Table 15, Entry 1).[91] In this procedure, no separate formation of Grignard reagent is required as it forms and reacts in situ. Following overnight stirring after warming to room temperature only a

small quantity of product was formed and once again the low reactivity of chlorostyrene was held accountable.

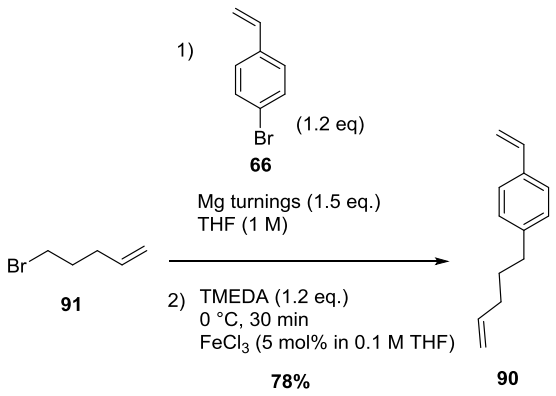
Changing the 4-chlorostyrene for 4-bromostyrene resulted in a significant increase in yield (Table 15, Entry 2).

Table 15: $FeCl_3$ catalysed Grignard reaction with chloro- or bromo- styrene.



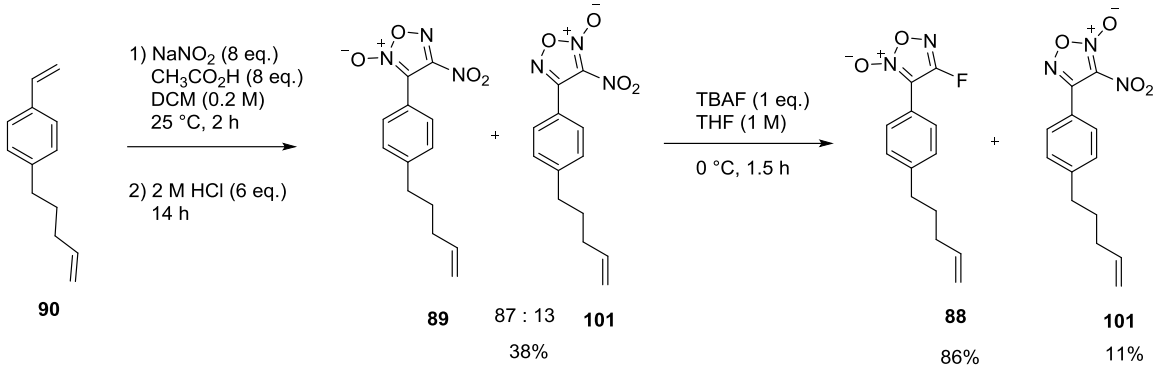
Entry	X	Time (h)	Yield (%)
1	Cl	22	8
2	Br	3	44

Still unsatisfied with the product yield we used a procedure developed my Nakamura.[92] In this procedure pre-formed Grignard reagent and TMEDA is added dropwise to a solution of bromopentene and $FeCl_3$ catalyst (Scheme 40). To our delight the reaction was rapid and high yielding.



Scheme 40: Nakamura's conditions applied to the synthesis of **90**.

Applying Weiland conditions to the dialkene granted the desired nitrofuroxan product as a mixture of 3- and 4- regioisomers in good yield (Scheme 41). Gratifyingly, only the conjugated alkene reacted and the terminal alkene remained untouched.

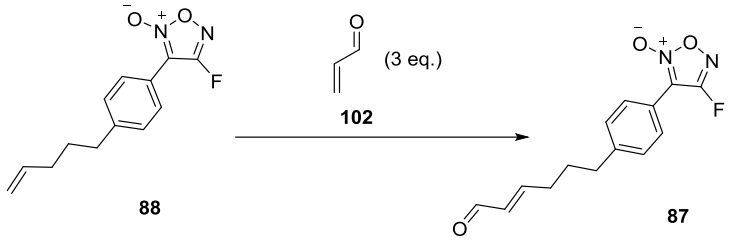


Scheme 41: Synthesis of nitrofuroxan **89** and **101** as a mixture of isomers followed by fluorination.

Fluorination of the mixture of nitrofuroxan isomers **89** and **101** with TBAF granted **88** in high yield (Scheme 41). Compound **101** remained unreacted due to the increased electron density on the 3- position and was easily separated from the product by column chromatography.

The final step in the synthesis of the furoxan module was the cross metathesis of **88** with acrolein. This was firstly attempted with Grubbs-Hoveyda first generation catalyst but the reaction was sluggish and the yield was poor, 37% of the starting material remained unreacted after 44 hours and crude ^{1}H NMR showed several byproducts as part of a complex mixture (Table 16, Entry 1).[93]

Table 16: Hoveyda-Grubbs catalysed cross metathesis of **88** with acrolein.



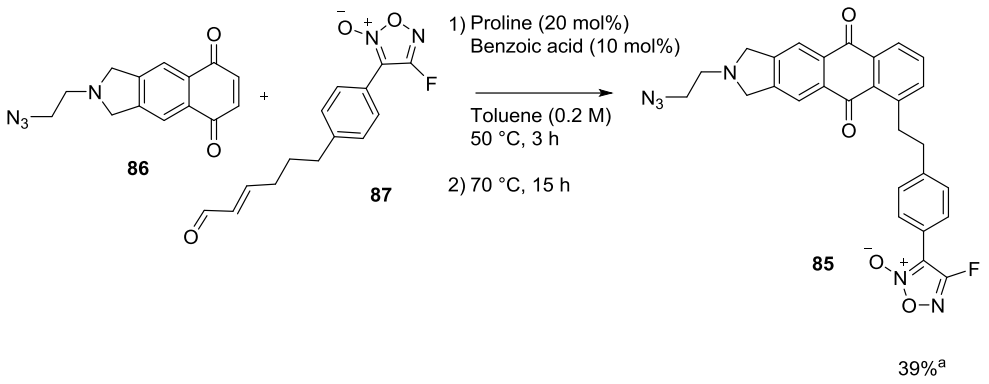
Entry	Catalyst	Solvent	Temp. (°C)	Time (h)	Yield (%)
1	Hoveyda-Grubbs 1st generation (10 mol%)	DCM (0.35 M)	r.t.	44	7
2	Hoveyda-Grubbs 2nd generation (3 mol%)	Toluene (6 mM)	100	28	76

Acrolein is rarely reported in cross metathesis reactions as it is notoriously unreactive. We did however happen upon a publication which successfully reacted it under high dilution and high temperature.[94] Using Grubbs-Hoveyda second generation catalyst granted us the desired product in 76% yield along with 18% recovered starting material (Table 16, Entry 2).

2.4.1.3 Synthesis of compound 85

Finally we combined the recently synthesised naphthoquinone **86** with the furoxan aldehyde **87** using organocatalyzed [4 + 2] reaction conditions (Scheme 42).[84] After stirring for 3 hours ^{1}H NMR of an aliquot suggested little or no reaction had occurred so the reaction temperature was increased to 70 °C. After a further 15 hours the reaction was complete, purification attempts were partially successful and we obtained **85** along with inseparable by-products. The product appeared to precipitate out during column chromatography using a variety of solvents (EtOAc/hexane and MeOH/DCM). The product also dragged along a PTLC plate and recrystallization

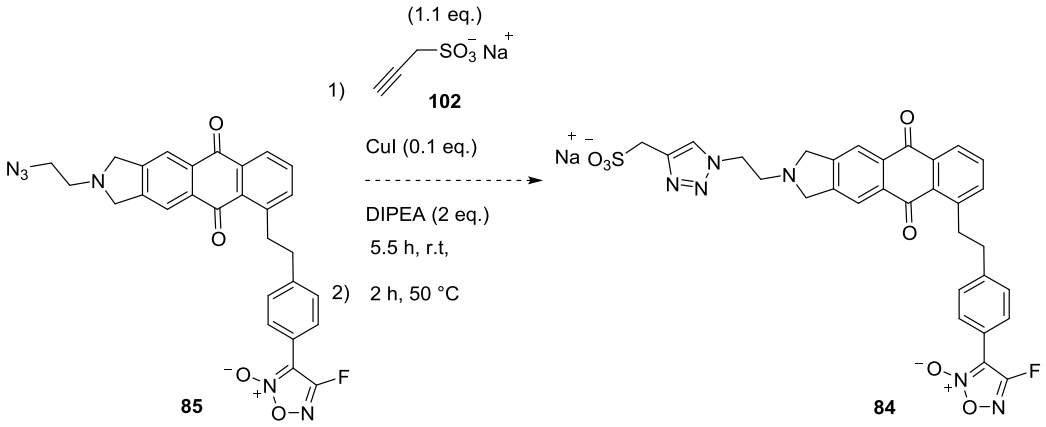
attempts were unsuccessful. Furthermore, suspected that the **85** may be slightly unstable since each purification attempt resulted in the production of insoluble brown material, diminishing the yield further.



Scheme 42: Oraganocatalyzed [4 + 2] reaction. [a]Isolated yield with impurities by silica column chromatography.

2.4.1.4 Synthesis of compound 84

We decided to carry the semi-pure **85** forward to the copper catalysed click triazole formation with alkenyl sulfonate **102** (Scheme 43).



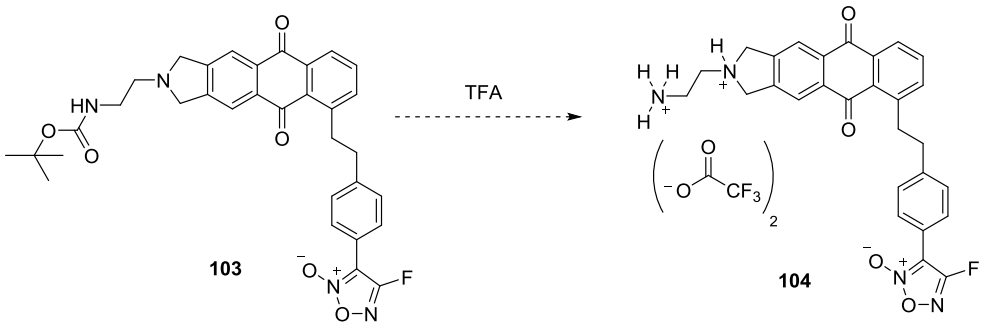
Scheme 43: Attempted triazole formation.

After stirring for 5.5 hours ^{1}H NMR suggested that no reaction had occurred so the temperature was increased to 50 °C. After a further 2 hours no reaction had occurred so the solvent was removed in an attempt to recover the starting material. To our surprise most of the material had become an insoluble orange solid. The material was insoluble in deuterated solvents (including $CDCl_3$ and D_2O) and could not be analysed. We suspect that the material had decomposed or if it was the desired product, it was too insoluble to purify or obtain structural data.

2.4.2 Synthesis towards compound 104

The purification and isolation of **84** had proven to be challenging and the azide may not be suitable for further reactions, so we decided to investigate an alternative amine tethered side chain in the hopes that we could easily purify the products and induce water solubility in subsequent steps.

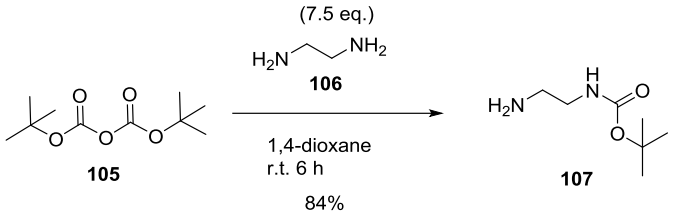
We envisioned that a pair of quaternary ammonium salts would provide the required aqueous solubility. Furthermore, we suspected that an amine tethered Boc group would assist with purification. The Boc group could be cleaved using trifluoroacetic acid as a final step, providing **104** as a trifluoroacetate salt after removal of solvent (Scheme 44).



Scheme 44: Envisioned intermediate **103** and simultaneous deprotection and acidification of amines.

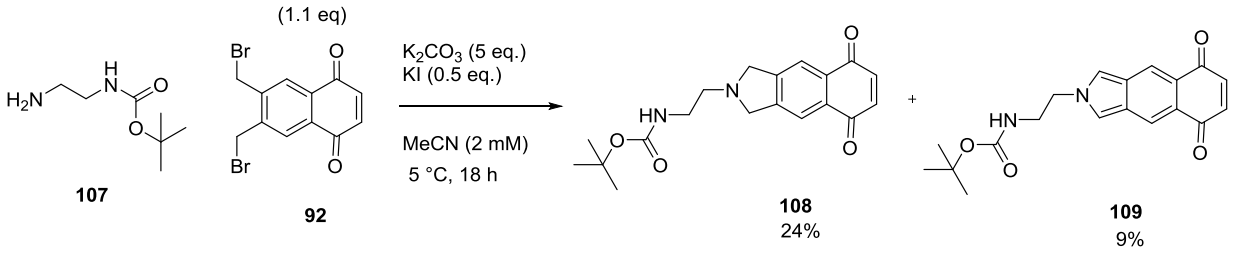
2.4.2.1 Synthesis of compound 108

Boc protected amine **107** was obtained in good yield (Scheme 45)



Scheme 45: Boc protection of **106**.

Amine **107** was then reacted with recrystallized **92**, to our delight the Boc tethered naphthoquinone **108** was obtained in moderate yield, we also obtained oxidised byproduct **109** (Scheme 46).

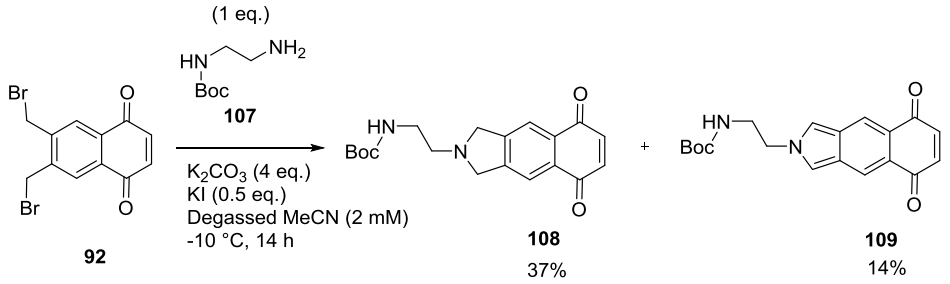


Scheme 46: Synthesis of Boc tethered naphthoquinone **108**.

It is of note that as with the azide tethered naphthoquinone, the Boc tethered intermediate **108** appears to be inherently unstable and readily oxidises and decomposes if left at room temperature. Storing under argon in a freezer prevents

excessive decomposition but best results are obtained in the organocatalyzed [4 + 2] reaction if **108** is used immediately after synthesis.

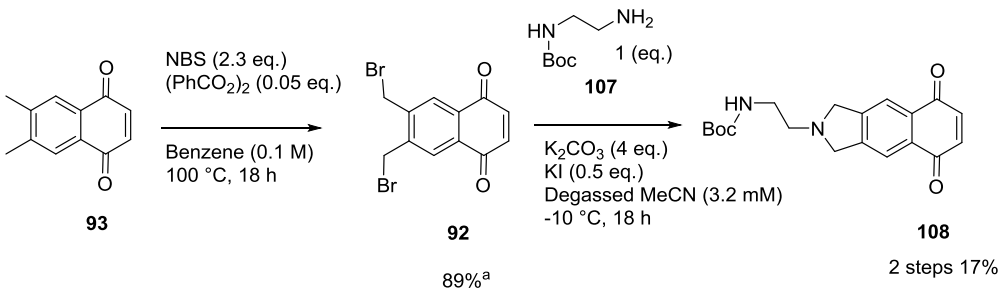
We suspected that we could prevent the oxidative side reaction by degassing the MeCN solvent prior to reaction by freeze pump thaw method (Scheme 47).



Scheme 47: Synthesis of **108** using degassed MeCN.

The use of degassed solvent led to a significant improvement in the yield of desired product **108** and to our surprise we still obtained oxidised byproduct **109** in moderate yield. One problem with degassing is that the low concentration of the reaction necessitates large volumes of solvent, so the process is somewhat time consuming and impractical.

The purification of dibrominated compound **92** had proven difficult, obtaining partially pure product following column chromatography and sequential recrystallization in moderate yields. We decided to try a two-step synthesis towards **108** to bypass the purification of **92** and hopefully improve the overall yield of the synthesis (Scheme 48).



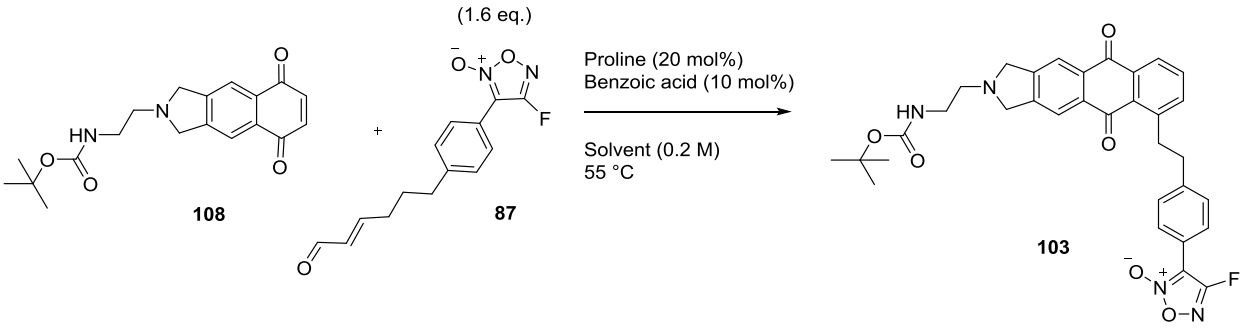
Scheme 48: Two step synthesis towards **108**. [a]Impurities present.

Bromination of **93** with NBS granted 89% yield of semi pure **92** following silica chromatography. ^{1}H NMR showed the product was impure with mono-brominated and multi brominated byproducts present. The intermediate was used in the next reaction with Boc tethered amine. Overnight stirring at -10 °C granted **108** in 17% over two steps. In previous syntheses of **108** we have used recrystallized **92** which is approximately 90% pure and grants **108** in 37% yield if solvent is degassed. The yield over the two steps is fairly average yielding but does avoid time consuming recrystallization of **92**.

2.4.2.2 Synthesis of compound 104

In our first reaction between **87** and **108** (Table 17, Entry 1) we noted that the reagents were not fully soluble in toluene which we attributed the low yield to. In the following reactions we changed the solvent to MeCN (Table 17, Entry 2) and chloroform (Table 17, Entry 3) which improved both the solubilities of the reagents and the product yields.

Table 17: Organocatalyzed [4 + 2] reaction between **87** and **108**. [a]Isolated by silica gel chromatography.

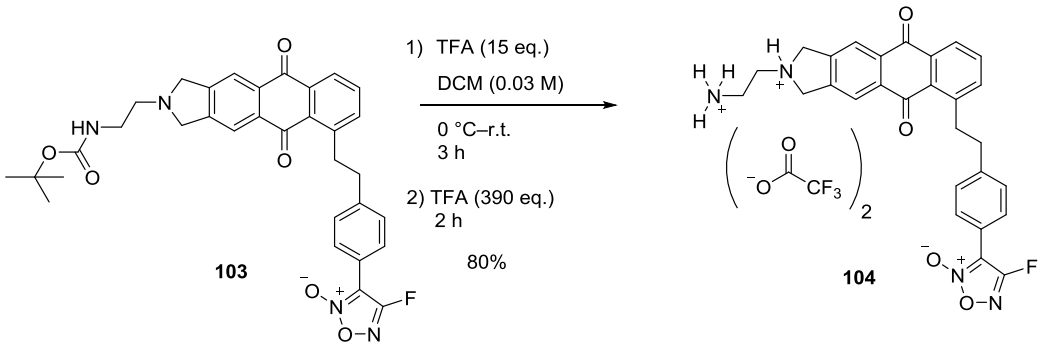


Entry	Solvent	Time (h)	Yield (%)[a]
1	Toluene	16	26
2	MeCN	10	36

3	$CHCl_3$	15	38

As per our design, the presence of the Boc group enabled easy purification of the product on silica gel column eluting with 20% EtOAc in hexane. The yields were moderate and the product was cleanly isolated. We attribute the moderate yields to the instability of compound **108** during the reaction.

The deprotection of **103** was conducted with excess TFA (Scheme 49).

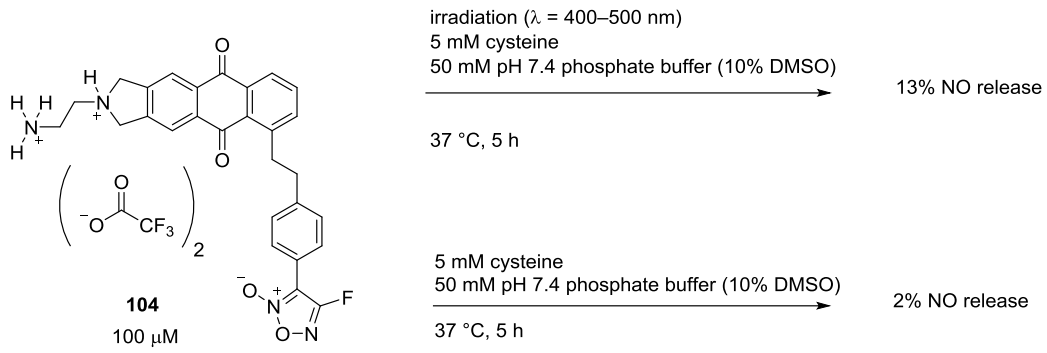


Scheme 49: Deprotection of **103** and salt formation.

Initially we used 15 equivalents of TFA which after 3 hours served only to protonate the tertiary amine of **103**. After increasing the concentration of TFA to make a 1 : 1 solvent with DCM, the Boc deprotection proceeded to yield **104**. The solvent was removed in vacuo and the crude brown insoluble material was filtered and washed several times with chloroform. The product was isolated as the double TFA salt following sonication in Et_2O whereupon it precipitated out as a brown solid. Compound **104** was sparingly soluble in water and fully soluble in DMSO, we expected that at 100 µM concentration **104** would be soluble in aqueous phosphate buffer solution.

2.4.3 NO releasing ability of compound 104

The NO releasing ability **104** was analysed by Griess test (Scheme 50). The NO release was sluggish with only 13% released after 5 hours of continuous irradiation. The release however was switchable as only residual amounts of NO are released under the same conditions without irradiation.



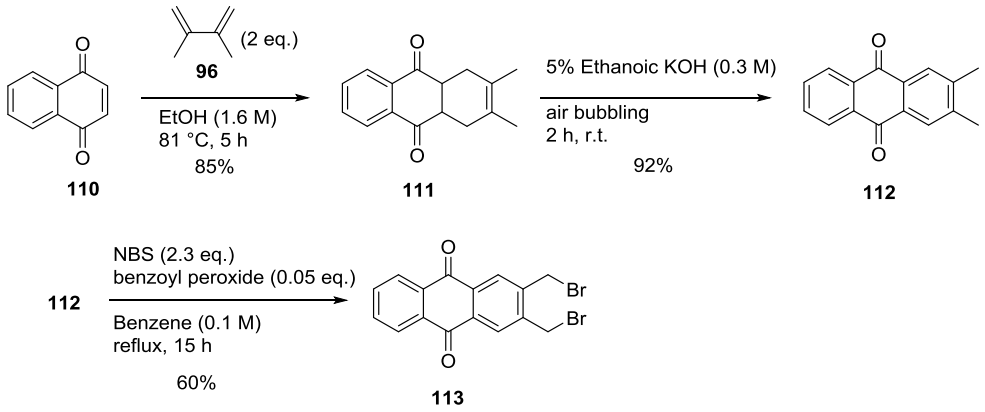
Scheme 50: NO release of **104** in 50 mM pH 7.4 phosphate buffer.

2.4.4 Investigation into NO release of compound 104

We decided to investigate the reason for the slow release and how to resolve it.

2.4.4.1 Synthesis of compound 115

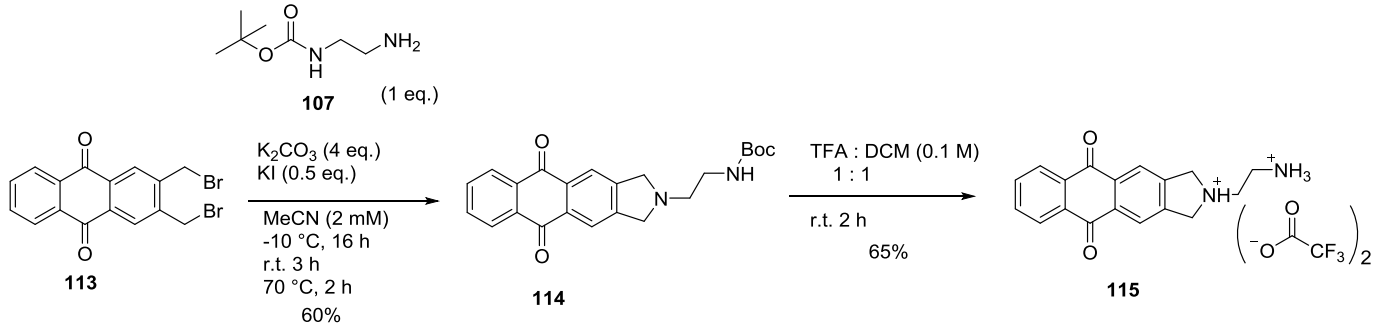
We started by synthesising a disconnected anthraquinone model to test if the proximity between the furoxan and anthraquinone had a significant effect (Scheme 51).



Scheme 51: Synthesis towards disconnected sensitizer model.

Diels-Alder reaction between naphthoquinone and 2,3-dimethylbutadiene yielded **111**, which was in turn treated with ethanoic KOH solution whilst air was streamed through the solution to oxidise to **112** (Scheme 51).[95] Benzylic bromination of **112** gave **113** along with minor brominated byproducts in 60% yield following column chromatography.

Treatment of **113** with Boc protected primary amine **107** yielded **114** in excellent yield (Scheme 52). Clearly the anthraquinone was much more stable to benzylic substitution than naphthoquinone.



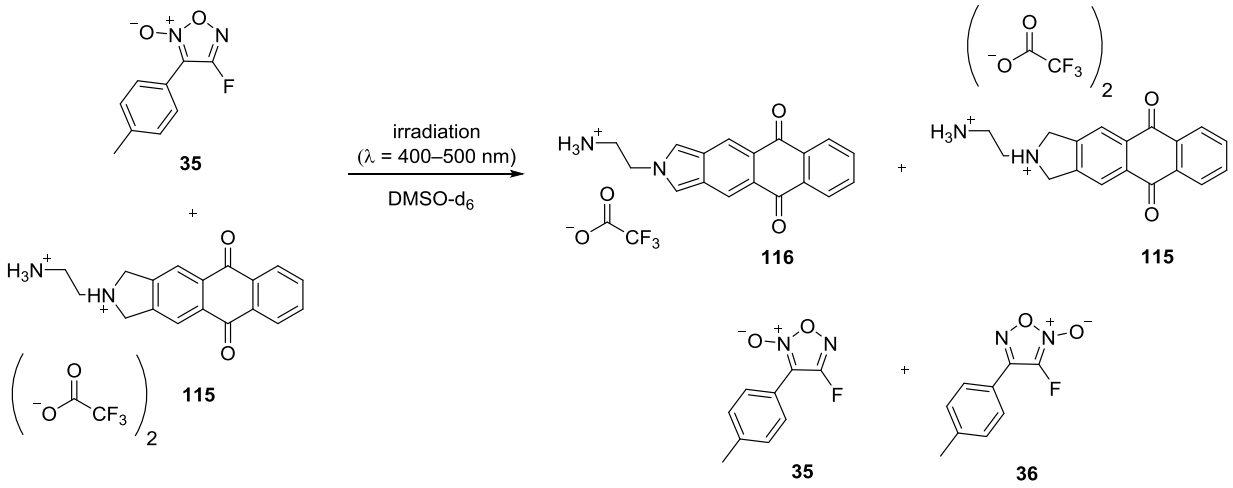
Scheme 52: Formation of **114** and subsequent Boc deprotection and acidification to **115**.

Treatment of **114** with TFA in DCM lead to the deprotected salt form following filtration and washing with chloroform.

2.4.4.2 Sensitized isomerization of compound 35 with compound 115

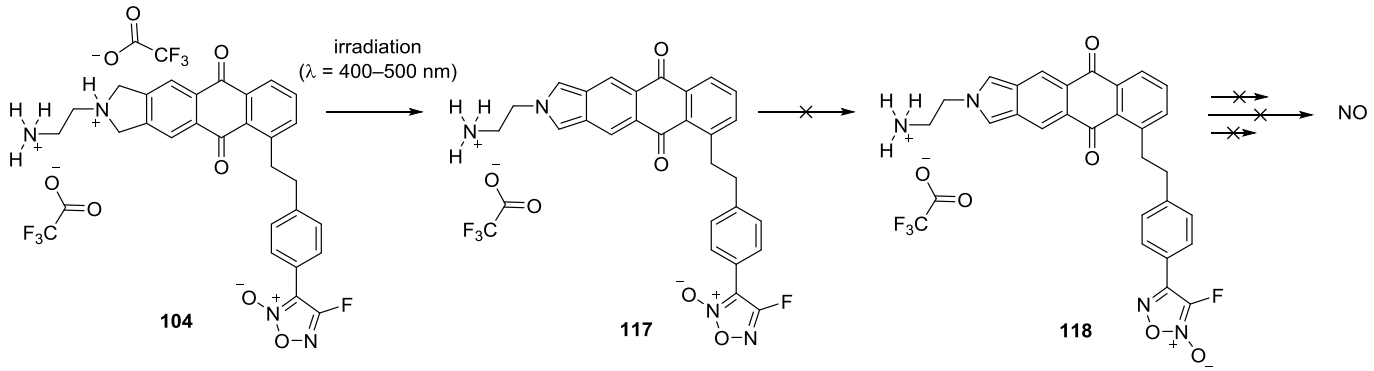
With the disconnected anthraquinone synthesised we performed a control experiment with fluorofuroxan (Table 18).

Table 18: Irradiation of **155** (30 mM) and **35** (30 mM) in DMSO-d_6 with 400–500 nm light at rt. Isomerization of **35** and oxidation of **115** was observed. [a]Measured by ^{1}H NMR using relative peak integration.



Time (min)	116 : 115[a]	35 : 36[a]
10	31 : 69	91 : 9
30	48 : 52	83 : 17
180	70 : 30	35 : 65

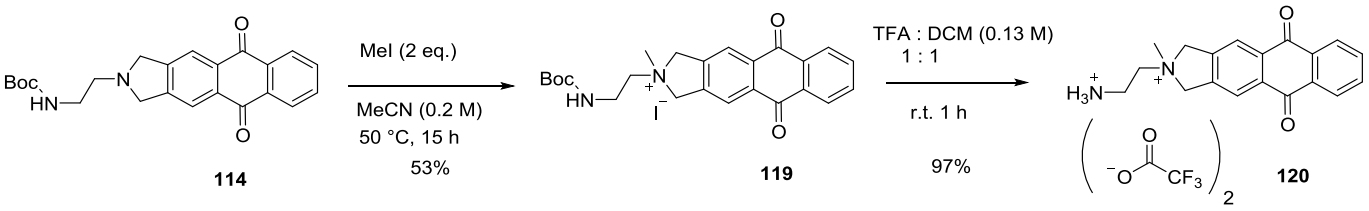
Irradiation of a 1 : 1 mixture of **115** and **35** in DMSO-d_6 lead to partial isomerization of the furoxan but the anthraquinone also reacted and became oxidised (Table 18). Following oxidation, the solution in the NMR tube became fluorescent red. The rate of anthraquinone oxidation (**116** : **115**) was faster than the rate of furoxan isomerization (**35** : **36**). One explanation for the sluggish release of NO from compound **104** under irradiation is that in the triplet state, the anthraquinone oxidises rather than transfers energy to the furoxan. Once in the oxidised form **117**, the molecule fluoresces and cannot efficiently transfer energy to the furoxan. This means that furoxan isomerization is hindered or even stopped and the NO cannot be released (Scheme 53).



Scheme 53: Proposed reason for hindered NO release from **104** under irradiation.

2.4.4.3 Synthesis of compound 120

In order to prevent the oxidation of the pyrrolidine type ring we methylated the amine of **114** prior to Boc deprotection and treated crude **119** with TFA in DCM to grant **120** (Scheme 54).

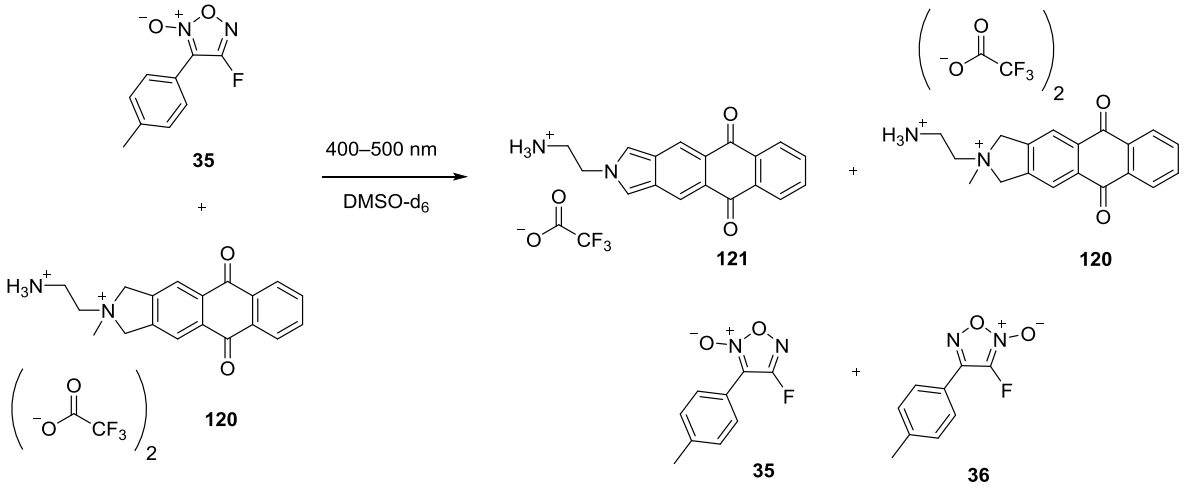


Scheme 54: Methylation of **114** and subsequent deprotection and acidification to **120**.

2.4.4.4 Sensitization of compound 35 with compound 120

Following the synthesis of **120** we then decided to check the isomerizing ability of **120** with fluorofuroxan (Table 19) in DMSO-d_6.

Table 19: Irradiation of **120** (20 mM) and **35** (20 mM) in DMSO-d_6 with 400–500 nm light at rt. [a]Measured by ^{1}H NMR using relative peak integration.

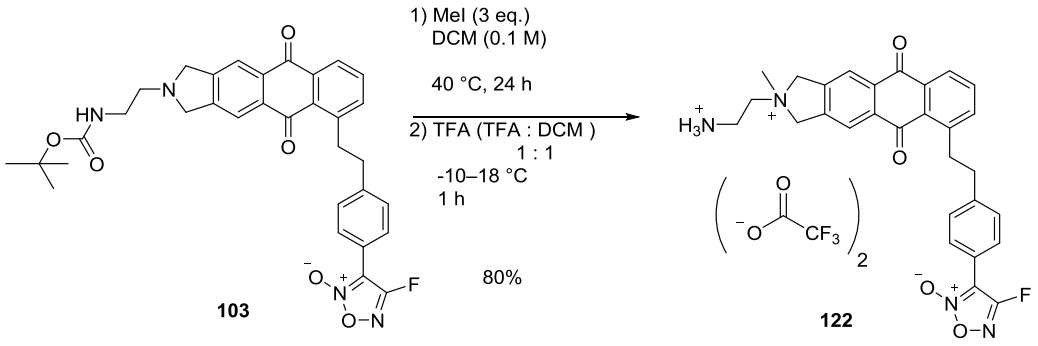


Time (min)	121 : 120[a]	35 : 36[a]
5	0 : 100	100 : 0
15	0 : 100	100 : 0
60	0 : 100	100 : 0

To our delight the sensitizer no longer oxidised under irradiation but the furoxan no longer isomerized either. Previous work with alkoxyfuroxans had suggested that observable isomerization is not necessary for reliable NO release. The photostationary state may be almost fully towards the non-isomerized configuration but some of the isomerized furoxan is present to release NO. We decided to synthesise the *N*-methylated anthraquinone-furoxan connected molecule and see if the NO release was improved relative to **104**.

2.4.5 Synthesis of compound 122

The methylation of **103** and treatment with acid were performed in one step (Scheme 55). Simply heating the anthraquinone-furoxan overnight in DCM with MeI methylates the amine which can then be diluted with equal portions of TFA to DCM after cooling to -10 °C. Compound **122** is isolated by removal of solvent, washing with organic solvents and then sonication in Et_2O which precipitates out a brown solid.



Scheme 55: Methylation and Boc deprotection of **103**.

2.4.6 NO releasing ability of compound 122

Compound **122** was then tested for NO releasing ability using the Griess method (Figure 15).

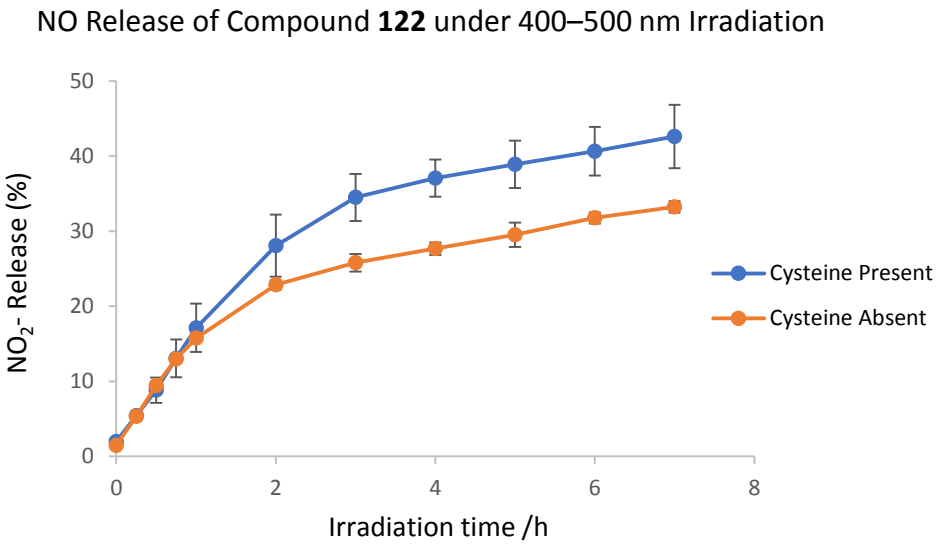


Figure 15: NO release curve of **122** (100 µM) in 1% DMSO 50 mM phosphate buffer containing or omitting 5 mM of L-cysteine under 400–500 nm irradiation at 37 °C. Error determined by standard deviation calculations (n=3).

We were delighted to observe NO release from compound **122** under visible light irradiation in reasonable quantities. This showed the value of methylation of the pyrrole amine which increased total NO release to 39% after 5 hours. The total released NO was comparable to 3-toly-4-fluorofuroxan **35** under 300–400 nm irradiation, although the rate was slightly slower (Figure 15, blue line).[41] Rapid release of NO was observed within the first hour of irradiation (up to 20%), however under prolonged irradiation the rate of NO-release slowed. This retarded rate of NO release is a common trend shared by other NO donors which seem to have intrinsic NO-release limits. A negative control experiment which omitted irradiation presented only background NO-release (up to 2%) in the presence of cysteine after 1 h (not shown).

Irradiation in the absence of cysteine led to an unexpected NO release with initial rates comparable to that with thiol present (Figure 15, orange line). Reports of furoxans that release significant quantities of NO in the absence of thiol are rare and the exact reason for NO-release is unknown.[37,58,59]

The ability of compound **122** to release NO in the absence of cysteine indicated that in addition to the irradiation-independent thiol-mediated NO-release from the 3-regioisomer, there may be another pathway where the photochemically excited fluorofuroxan or secondary transient intermediate can release NO without thiol cofactor assistance.

After demonstrating photoswitchable NO-release, we decided to investigate the level of control over release (Figure 16). Compound **122** was irradiated for 1-hour intervals with 400−500 nm of light, in between the irradiation periods the sample remained under ambient light, aliquots were taken at intervals. As soon as irradiation is ceased, the NO-release almost completely stops apart from some minor background release (Figure 16). This demonstrates a high level of control over NO-release with compound **122**; therefore, it may be possible to release a specific quantity of NO with selected sample concentration and irradiation time.

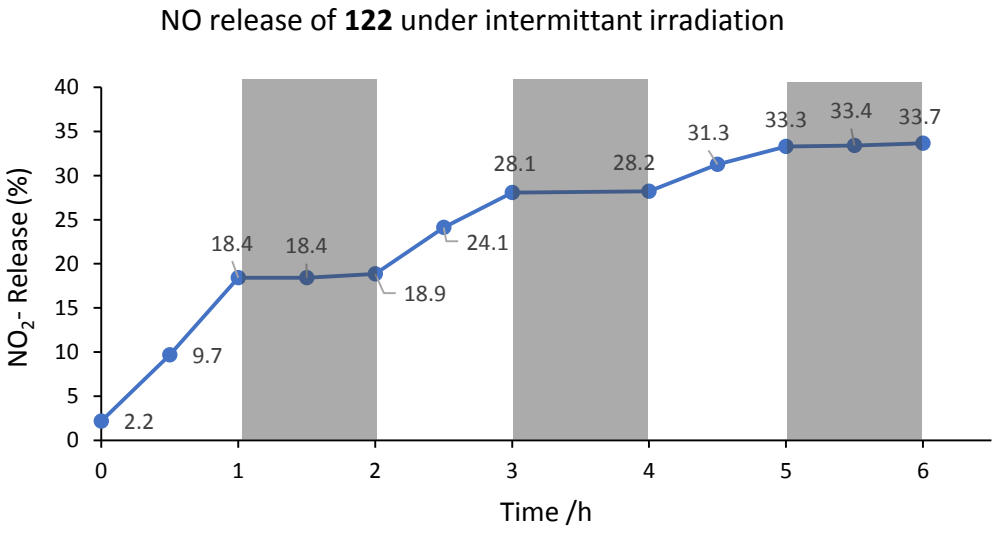


Figure 16: NO-release curve of **122** (100 µM) in 1% DMSO 50 mM phosphate buffer containing 5 mM of L-cysteine under 1-h rotations of 400−500 nm irradiation and

ambient light at 37 °C. Grey areas indicate periods of non-irradiation in ambient light.

2.4.7 Cellular studies with compound 122

With **122** showing NO release in both the presence and absence of cysteine and to a high degree of control we next submitted the sample for studies within a cell culture system in the hopes of achieving spatiotemporal NO release (Figure 17).

HeLa cancer cells were initially incubated with NO_{550},[96] a fluorescent probe for NO detection and then treated with **122** for 30 min. After being washed with PBS buffer, the cells were irradiated with 400–500 nm light for 1 hour. Disappointingly the cells exhibited no significant fluorescence derived from the reaction of NO_{550} and NO. Due to the non-fluorescent nature of **122**, it could not be determined whether or not **122** permeated and entered the cells. The failure in NO-release observation may be due to the low cellular uptake of **122**, possibly due to the charged nature of the molecule and the high molecular weight.

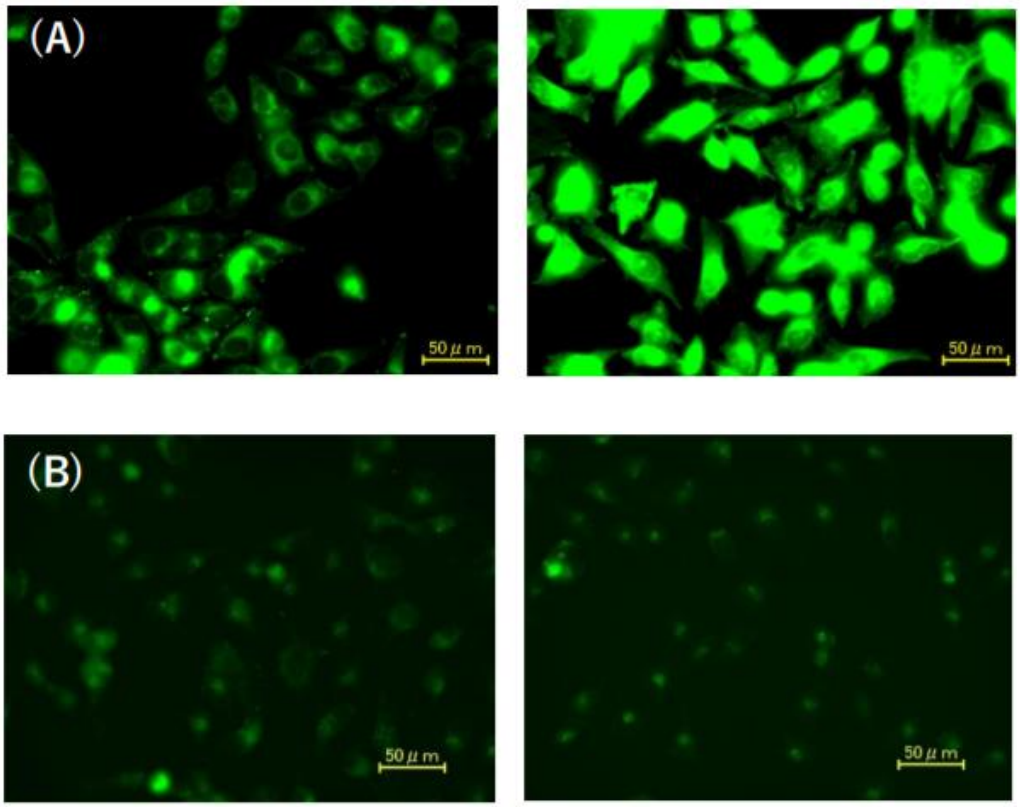


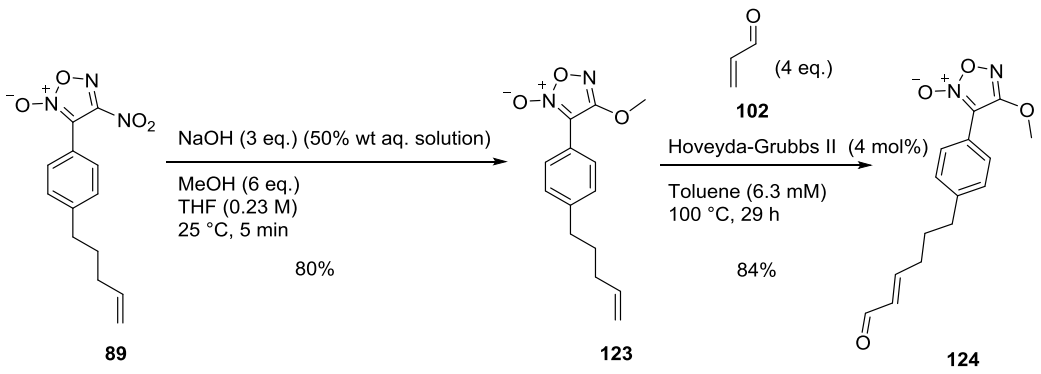
Figure 17: Fluorescence imaging of liberated NO in cultured living HeLa cells. (A) HeLa cells were incubated with 10 μM NO_{550} alone (left; exposure time 1 sec) followed by the addition of 30 μM NOC7 in the medium (right; exposure time 1 sec). Comparison of the images showed that the fluorescent brightness was much greater in the presence of NOC7, indicating that NO_{550} in the cells reacted with liberated NO. (B) Fluorescence image of HeLa cells preincubated with NO_{550} and 5 (left; exposure time 1 sec), and the image after the exposure of light in the wavelength of 400–500 nm for 1 hour (right; exposure time 1 sec). Comparison of the images showed no significant difference.

2.4.8 Synthesis towards compound 126

Recent work within the research group had shown that alkoxyfuroxans exhibited switchable PINOD properties and were able to release NO in quantities greater than fluorofuroxans under UV irradiation.[60] We suspected that an alkoxyfuroxan-tethered anthraquinone may release NO via sensitized mechanism in greater quantities than **122**. Prior to cell culture investigations with compound **122** we synthesised an alkoxy derivative.

2.4.8.1 Synthesis of compound 124

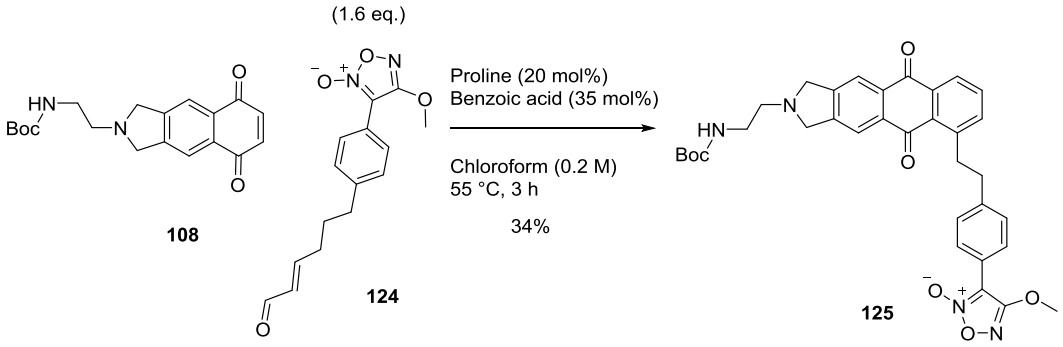
Firstly, starting from **89** we synthesised the alkoxy derivative **123** under basic conditions in high yield (Scheme 56). Compound **123** was then reacted with acrolein under Grubbs metathesis catalysis to grant furoxan intermediate **124**.[94]



Scheme 56: Synthesis of organocatalyzed [4 + 2] furoxan starting material **124**. Compound **89** is as an 87:13 mixture with isomer **101** (not shown).

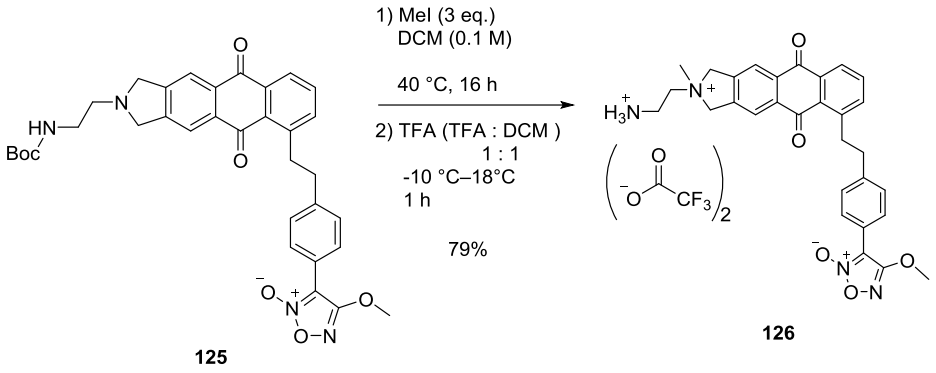
2.4.8.2 Synthesis of compound 126

The union of alkoxy furoxan and anthraquinone was made possible by organocatalysed [4 + 2] reaction which granted **125** in 34% after column chromatography (Scheme 57)



Scheme 57: Organocatalyzed [4 + 2] reaction between **108** and **124**.

Finally, the pyrrolidine amine was methylated with excess MeI overnight and the Boc group deprotected with TBAF to produce an oily residue. The residue was sonicated in Et_2O for 1 hour which formed a semi-crystalline solid that ^1H NMR revealed to be **126** complexed with an ether molecule (Scheme 58).



Scheme 58: Methylation and deprotection of **125**.

2.4.9 NO releasing ability of compound 126

The NO releasing ability of **126** was tested under 400–500 nm irradiation using the Griess method (Figure 18).

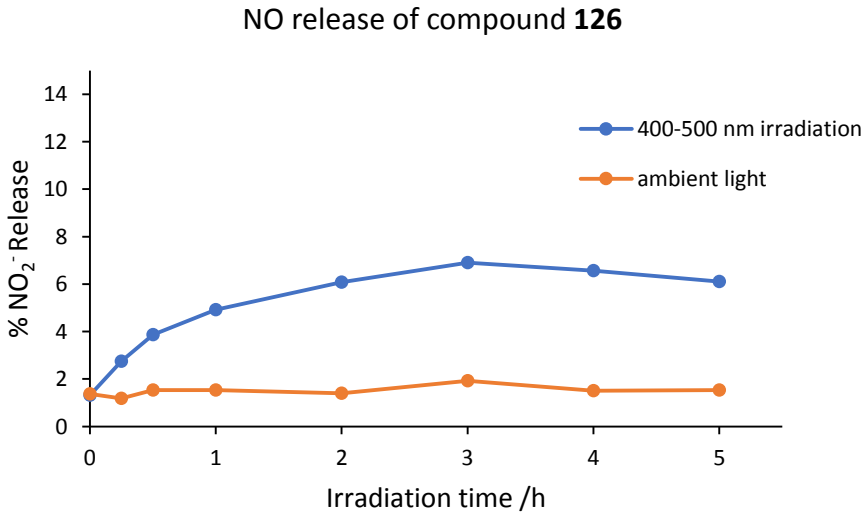


Figure 18: NO release curve of **126** (100 μM) in 1% DMSO 50 mM phosphate buffer containing 5 mM L-cysteine under 400–500 nm irradiation or ambient conditions at 37 °C.

Disappointingly, the NO release of **126** was low compared to the fluoro- isomer **122**, the NO release plateaued at 7% (Figure 18, blue line). The negative control shows only marginal NO release which is attributed to background discharge (Figure 18, orange line).

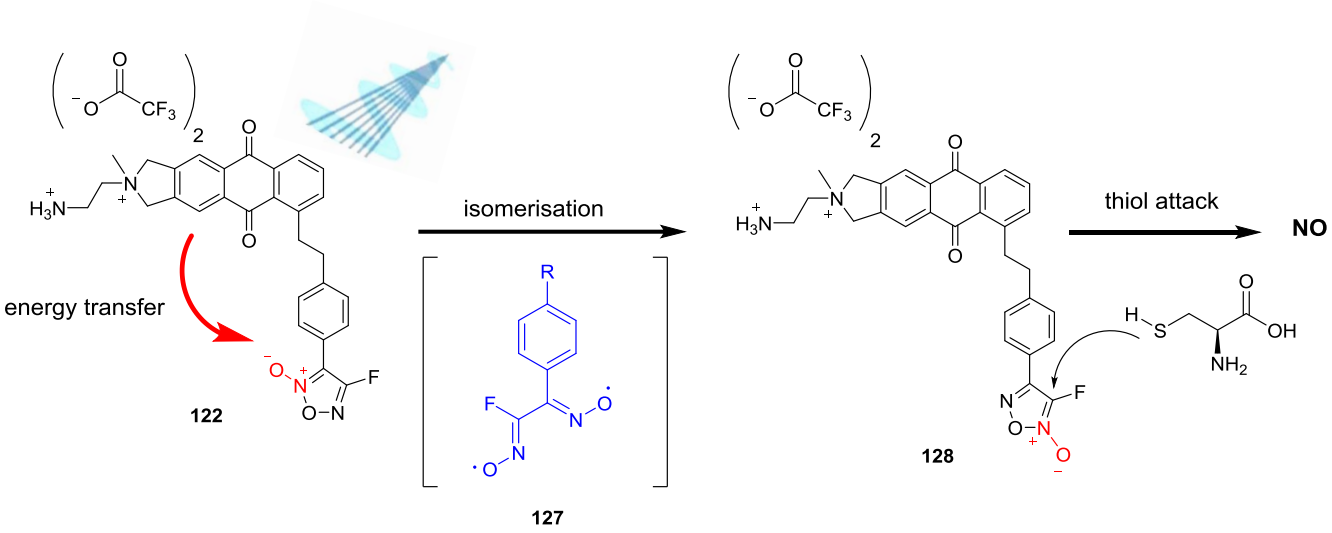
It is likely that the triplet energy level of the methoxyfuroxan is much higher than the fluorofuroxan due to the lower relative electronegativity of the oxygen and greater electron donation into the ring raising the LUMO. Therefore, successful energy transfer from sensitizer to furoxan is perhaps slower due to a misalignment of energy levels. It is also possible that rather than isomerizing after sensitized energy transfer, the alkoxy furoxan reacts in such a way that NO is not released, potentially reacting with the sensitizer itself.

The exact reason for the poor NO release from **126** is unknown but the fact that alkoxyfuroxan can release NO via sensitization was encouraging. The increased stability of alkoxyfuroxans relative to fluorofuroxan opened up the synthetic toolbox for future designs.

2.4.10 Mechanism of sensitized NO release from furoxan

2.4.10.1 Proposed mechanism of sensitized NO release

Fluorofuroxan shows switchable NO release by isomerizing from 4-fluorofuroxan to 3-fluorofuroxan. In the isomerized 3-fluorofuroxan state, the molecule is susceptible to attack from cysteine whereupon NO is released (Figure 19). The release mechanism of furoxans by thiol attack was discussed in the introduction (Scheme 1), here we take a closer look at the sensitized release of NO.



Scheme 59: Proposed thiol mediated sensitized NO release mechanism.

Since ^{1}H NMR of irradiation experiments showed furoxan isomerization in the presence of sensitizer under 400–500 nm irradiation (Table 1) it is logical that the same thiol dependant release mechanism occurs (Scheme 59). Energy transfer via electron exchange between the excited state anthraquinone and ground state fluorofuroxan induces isomerization through an intermediate **127**. Once isomerized the furoxan is immediately attacked by cysteine and NO is released.

This thiol dependant mechanism does not explain however, how NO is released in the absence of cysteine. It is of note that the experiments conducted in the absence of cysteine resulted in lower quantities of NO released (Figure 15). We suspect that the water molecules in the phosphate buffer solution may react with the furoxan in the transient state between isomerized forms, from here NO may be released. A second theory is that once in the excited state a separate sensitization event occurs in which the transient furoxan species releases NO. The sensitization of furoxan may not directly lead to NO release but instead the formation of intermediates that react with solvent and cysteine to release NO. This could explain why experiments conducted with cysteine resulted in a greater quantity of NO released, since cysteine may attack non-furoxan NO-releasing byproducts. At this point however, we do not have

evidence to support or disprove any of the described theories and the exact NO release mechanism is unknown.

2.4.10.2 Cyclic voltammetry studies of compound 35 and anthraquinone

Another possible energy transfer mechanism is sensitized electron transfer, we decided to test this by measuring the electrochemical properties of anthraquinone and fluorofuroxan **35** using cyclic voltammetry (Figure 19). The experiments were conducted in degassed MeCN using 0.1 M Bu_4NClO_4 as a supporting electrolyte and revealed the reduction potentials of anthraquinone/ anthraquinone•− and **35**•+/**35** couples to be −0.95 V and +2.11 V versus saturated calomel electrode (SCE), respectively. The triplet energy of anthraquinone was reported to be 2.73 eV.[97] Following the Rehm−Weller formalism[98,99] with the assumption that the work function ω is a small contribution,[100] the excited-state reduction potential of anthraquinone was estimated to be +1.78 V versus SCE, which is not comparable to the reduction potential of **35**•+/**35** couple (+2.11 V versus SCE). Thus, the ΔG^0 for the electron transfer from **35** to the excited anthraquinone is a large positive value, suggesting that this pathway is unlikely.

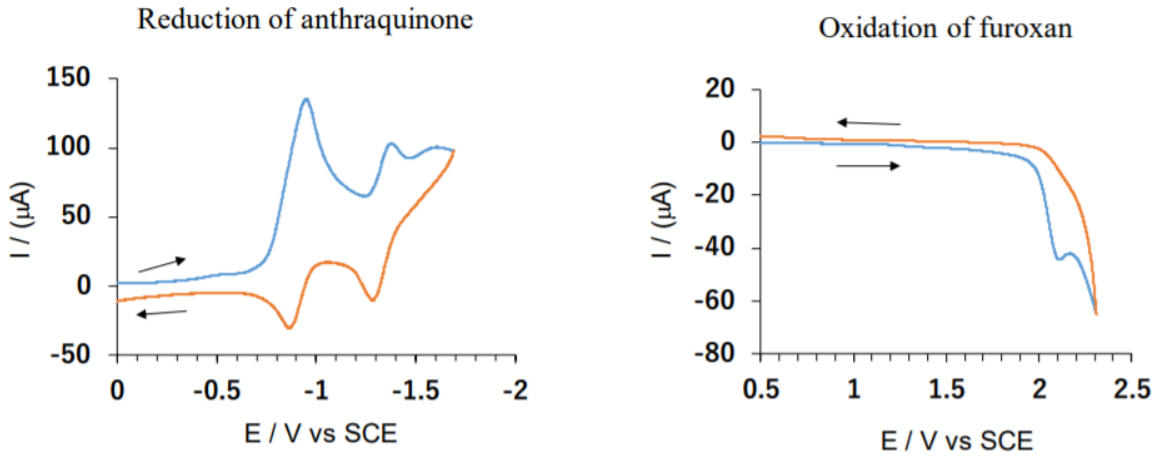


Figure 19: Cyclic voltammograms of 1.0×10^{-3} M anthraquinone and 4-fluorofuroxan **35** for the measurement of their reduction and oxidation potentials, respectively. Solvent: MeCN, WE: glassy carbon, RE: Ag/Ag+ with 0.1 M $TBAClO_4$ in MeCN, CE:

platinum wire, electrolyte: 0.1 M $TBAClO_4$, sweep rate: 50 mV/s. The redox potentials corrected to the SCE scale are shown.

2.4.11 Conclusions

By considering the solubility issues of anthraquinone and reactivity of fluorofuroxan we were able to devise a convergent synthetic strategy. The organocatalysed [4 + 2] reaction with an *N*-Boc protected naphthoquinone and terminal aldehyde fluorofuroxan ensured that the anthraquinone core and carbon linker to the furoxan was furnished in one pot under mild conditions. Simple deprotection and saponification granted the hybrid PINOD. We were able to show for the first time photoswitchable NO release from a furoxan under visible light irradiation which used a novel release mechanism. The synthesis was not without its flaws however, and we were unable to determine whether the hybrid permeated the cells. This research served as a good proof of concept and was the first step towards photoswitchable NO release from a furoxan within a cell culture system.

Ⅲ： Improvement to visible light PINOD furoxan: studies towards aminofluorenone-alkoxyfuroxan

3.1 Consideration of compound 122 and required improvements

With a sound prototype in hand we analysed the shortfalls of **122** and considered necessary requirements to synthesise an improved molecule. Previously, we were unable to determine whether compound **122** was localized within a cell culture system and **122** was only sparingly soluble in phosphate buffer solution. Furthermore, the synthesis presented restrictions due to the reactive fluorofuroxan requiring mild conditions. We also decided that although 400 nm irradiation wavelength is largely biologically benign, the absorption wavelength of the sensitizer should be as long as possible to assist with tissue penetration.

This led to new requirements for the synthesis of an improved molecule (Figure 20):

- The molecule should be fluorescent as well as sensitizing
- The molecule should have an absorption wavelength >400 nm
- The molecule should be cell permeable
- The synthesis should be simplified by finding a more stable furoxan than fluorofuroxan.

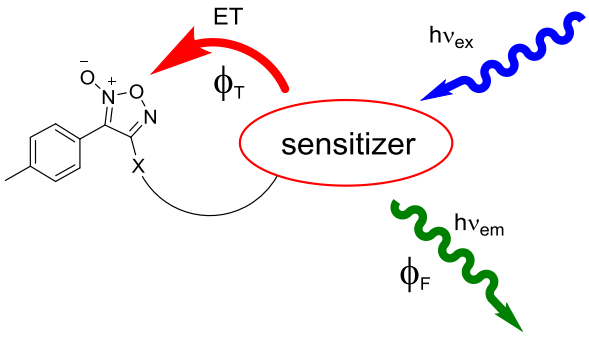


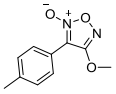
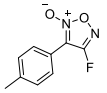
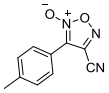
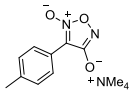
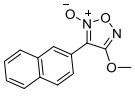
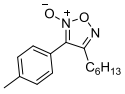
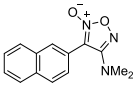
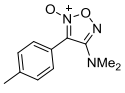
Figure 20: Concept for an improved sensitized furoxan PINOD.

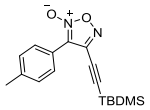
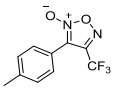
3.2 Investigation into furoxan NO releasing ability

With the knowledge that compound **122** released NO under visible irradiation in the absence of thiol cofactor, we decided to test whether furoxan molecules could release NO in the absence of thiol under UV irradiation. Previously it had been assumed that isomerization was required followed by thiol attack, but it had not been examined whether furoxans can release NO under continuous UV irradiation.

We made the assumption that furoxans that could release significant quantities of NO under UV irradiation may also release similar amounts of NO via a sensitized mechanism under visible irradiation. To find a suitable furoxan for the next design we irradiated furoxans in pH 7.4 50 mM phosphate buffer with 300–400 nm light in the absence of cysteine (Table 20). The NO release was determined by Griess test.

Table 20: NO release of furoxans (100 μM) in 50 mM pH 7.4 phosphate buffer under 300–400 nm irradiation. Structures are shown at 50% scale.

Entry	Structure no.	Furoxan	%NO release after 2 h continuous irradiation	Comment
1	**37**		31	Decrease after 2 h
2	**35**		28	Decrease after 2 h
3	**129**		10	14% after 5 h
4	**130**		-	41% After 0.5 h then decrease
5	**131**		12	
6	**132**		7	
7	**133**		12	
8	**134**		17	22% After 5 h

9	**135**		5	10% DMSO in buffer
10	**136**		2	

The first molecule to be tested was 3-tolyl-4 methoxy furoxan **37** (Table 20, Entry 1). To our delight the furoxan released a large quantity of NO in the absence of cysteine. However, we observed the NO release decreasing with prolonged irradiation (time > 2 hours) suggesting that NO_x species were reacting with furoxan post release byproducts. Because of this unusual phenomenon we decided to quantify NO release only up to two hours irradiation with subsequent experiments.

3-Toly-4-fluorofuroxan **35** was included as a control and showed reasonably high levels of NO release (Table 20, Entry 2). 3-Tolyl-4-cyanofuroxan **129** released low levels of NO at a sluggish rate, this was disappointing since a method for cyanation of nitrofuroxan had recently been developed within the group (Table 20, Entry 3).[40] To our surprise 3-tolyl-4-tetrabutylammonium oxide furoxan **130** released an exceptional 41% NO within the first 30 minutes of irradiation (Table 20, Entry 4). However, compound **130** was hindered by non-selective spontaneous NO release in both isomers and so it was not truly a PINOD molecule.[101] Increasing the conjugation of the furoxan with 3-napthylen-2-yl-4-methoxy furoxan **131** resulted in lower quantities of NO release (Table 20, Entry 5). Aliphatic furoxan 3-tolyl-4-hexanfuroxan **132** released only 7% (Table 20, Entry 6). Amino furoxans **133** and **134** released low to moderate quantities of NO with 12% and 17% respectively (Table 20, Entries 7 and 8). Alkynyl **135** and trifluoromethyl **136** derivatives released negligible quantities of NO under irradiation (Table 20, Entries 9 and 10).

Compounds **37**, **35**, **130** and **134** released moderate quantities of NO under irradiation. This suggested that electronegative heteroatoms directly bonded to the furoxan ring are required to induce significant NO release. The more conjugated naphthylfuroxans appeared to release a lower quantity of NO, perhaps a stabilizing effect of conjugation reduced the reactivity of the transient excited state intermediates towards NO release pathways.

Following the screening experiments shown in table 20, methoxyfuroxan **37** was selected as the candidate for connection with a fluorescent sensitizer.

3.3 Investigation into potential fluorescent sensitizers of compound 37

With the NO release of furoxans under UV irradiation investigated and a suitable furoxan PINOD selected, we explored potential sensitizers which exhibited both sensitizing and fluorescent properties. Before beginning the search, we surveyed the literature for potential sensitizers of furoxan which held favourable photophysical properties. We defined 'favourable' properties by first reviewing the successful sensitizers for fluorofuroxan (Table 21).[72] Note: Following initial sensitizer screening at the beginning of the project and after the synthesis of **122**, it was found that 9-fluorenone was also an exceptional photosensitizer of fluorofuroxan **35** inducing 91% isomerization in 15 minutes.

Table 21: Photophysical properties of successful sensitizers of fluorofuroxan. Data extracted from 'The Handbook of Photochemistry'.[72]

Entry	Sensitizer	E_s/Kj mol^{-1}	Φ_{fl}	Φ_T	E_T/Kj mol^{-1}	τ_T/μs
1	Benzil	247	0.0013	0.92	233	5
2	Anthraquinone	284	-	0.90	261	0.11
3	9-Fluorenone	266	0.0027	0.48	211	100

Compounds **35** and **37** are different molecules and would therefore have different HOMO/LUMO energy levels, so these properties were used as a reference rather than a definitive range.

Analysis of the sensitizers in table 21 suggested an acceptable range of triplet energy levels between 211 Kj mol^{-1} and 261 Kj mol^{-1} to sensitize isomerization in fluorofuroxan **35**. A triplet quantum yield as low as 0.48 was sufficient to induce rapid isomerization (90% in 15 minutes with 9-fluorenone) so perhaps even lower values would suffice depending on the respective absorption coefficients. As long as the sensitizer absorbs

in the visible light range (E_s < 300 Kj mol^{-1}) it would be sufficient, although longer wavelengths were preferred. Even a short triplet lifetime was acceptable when sensitizing **35** (0.11 μs for Anthraquinone) (Table 21, Entry 2).

Following exhaustive searching we found that the requirements were limiting but a few potential sensitizers had been discovered (Table 22).

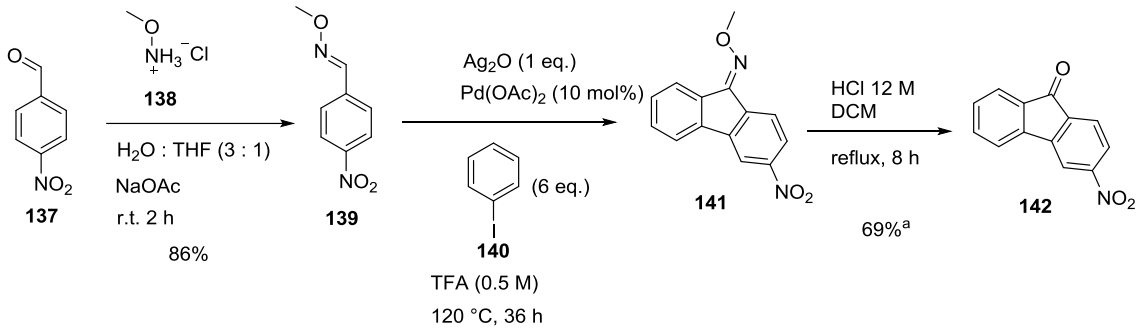
Table 22: Sensitizers showing triplet sensitizing and fluorescent properties suitable for sensitization of **35** and perhaps **37**.[72] Omitted data indicates that it had not been recorded in the literature. [a]0.064 in polar solvent. [b]0.08 in polar solvent.

Entry	Sensitizer	E_s/Kj	Φ_{fl}	Φ_T	E_T/Kj mol^{-}	τ_T/μs
1	Proflavin	250	0.40	0.22	205	20
2	3-Amino-9-fluorenone	262	0.16[a]	0.26[b]	193	-
3	Aminoanthracene	264	0.57	-	184	-
4	Riboflavin	263	0.12	0.4	-	19

Proflavin, aminoanthracene and riboflavin were commercially available and showed favourable properties. Proflavin (Table 22, Entry 1) showed attractive fluorescent and triplet sensitizing properties and similar photophysical values to the successful sensitizers of **35** in table 21. Aminoanthracene did not have a recorded triplet quantum yield but did have a recorded triplet energy, albeit a potentially low one (Table 22, Entry 3). Riboflavin showed reasonable fluorescence and good triplet quantum yield but the triplet energy level was not recorded, since it was commercially available we decided to test it anyway. 3-Amino-9-fluorenone had potential, but worryingly the favourable fluorescence and triplet sensitizing values decreased in

polar solvents (Table 22, Entry 2), nevertheless we decided to synthesise and test the sensitizer (Scheme 60).

3.3.1 Synthesis of compound 143

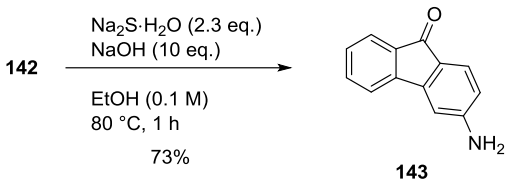


Scheme 60: Synthesis of **142**. [a]Yield after 2 steps from **139**.

The first step in the synthesis towards **142** was the formation of oxime **139** from 4-nitrobenzaldehyde **137** (Scheme 60). Treatment of **137** with methoxylamine hydrochloride formed **139** in 86% yield following purification by column chromatography.

3-Nitro-9-fluorenone was synthesised using a one-pot palladium catalysed Heck and C-H insertion reaction.[102] Coupling of **139** with iodobenzene **140** formed a mixture of oxime intermediate **141** and nitrofluorenone **142**. Refluxing the mixture in conc. HCl

and DCM helped to form more of the nitrofluorenone but some oxime still remained unhydrolyzed. The insoluble product was isolated by pre-binding to silica prior to chromatography, the compound eluted with a $CHCl_3$/MeOH mobile phase and was obtained in 69% over two steps from compound **139**.



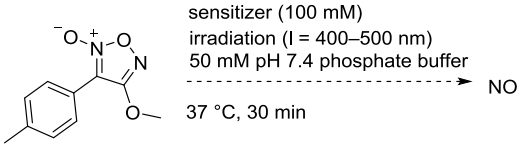
Scheme 61: Reduction of **142** to grant 3-amino-9-fluorenone.

Compound **142** was reduced with sodium sulphide to grant the sensitizer **143** (Scheme 61).[103]

3.3.2 Investigation into sensitized NO release of compound 37

With **143** and the commercially available sensitizers in hand we began screening experiments to see if NO was released from **37** under visible light irradiation in the presence of a sensitizer (Table 23). All of the sensitizers were soluble in phosphate buffer solution at 100 µM concentration, the NO release experiments were conducted in the absence of cysteine.

Table 23: Sensitized NO release experiments of **37**. Furoxan (100 µM) and sensitizer (100 µM) in 1% DMSO 50 mM pH 7.4 phosphate buffer at 37 °C. Irradiated with 400–500 nm light for 30 minutes. Aliquots treated with Griess reagent and %NO release determined spectroscopically.



37

100 μM

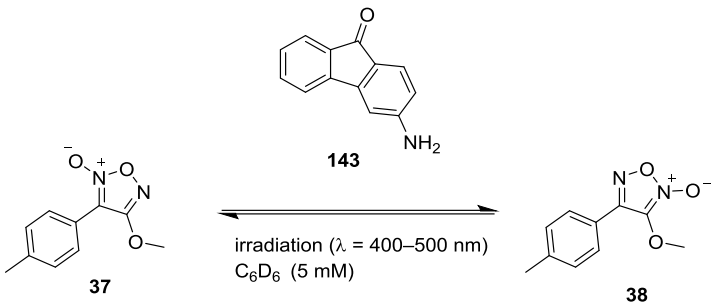
Entry	Sensitizer	%NO release	Comment
1	Proflavin	0	Photobleached
2	3-Amino-9-fluorenone	0	Photostable
3	Aminoanthracene	0	Photobleached
4	Riboflavin	0	Photobleached

Unfortunately, none of the sensitizers induced observable NO release (Table 23). We suspected that the lack of NO release was either due to direct photobleaching of the sensitizer or that the furoxan and sensitizer were too far apart in solution to interact effectively. Proflavin, aminoanthracene and riboflavin all photobleached within 30 minutes and were not suitable for connection with **37** (Table 23, Entries 1, 3 and 4 respectively). Interestingly, 3-amino-9-fluorenone was photostable since the UV/vis absorption spectrum remained unchanged after an hour of irradiation (Table 23, Entry 2).

3.3.3 Sensitized isomerization of compound 37 with compound 143

To check whether **143** could sensitize **37** we conducted an isomerization experiment. A solution of deaerated deuterated benzene containing a 1 : 1 mixture of **37** and **143** was irradiated under 400–500 nm and the isomerization ratio between 3- and 4-methoxyfuroxan was determined. To our delight the quantity of 3- isomer in the sample increased to 10.7% after 45 minutes of irradiation suggesting that **143** could indeed sensitize the **37**, at least in benzene (Table 24).

Table 24: Sensitized isomerization of methoxyfuroxan. 5 mM C_6D_6 solution containing 1 : 1 **37** and **143**. Irradiated with 400–500 nm light. [a]Determined relative to dodecane internal standard by ^1H NMR.

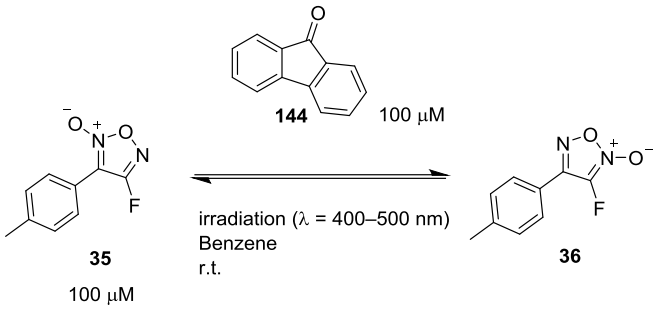


Time (min)	Isomerization (%)[a]	Comment
0	3.5	Residual Isomerization in sample
15	7.5	
30	9.1	
45	10.7	PS
60	10.7	

3.3.4 Sensitized isomerization of compound 35 with compound 144

A second isomerization experiment was conducted at 100 µM concentration in benzene to test whether sensitized isomerization can occur at low concentration. This time we used 9-fluoreneone **144** and fluorofuroxan **35** because fluorofuroxan indicates isomerization more clearly than alkoxyfuroxan (fluorofuroxan isomerizes up to 90% whereas alkoxyfuroxan shows less than 11%) (Table 25).

Table 25: Sensitized isomerization of **35** under 400–500 nm irradiation at 100 µM concentrations of sensitizer and furoxan.

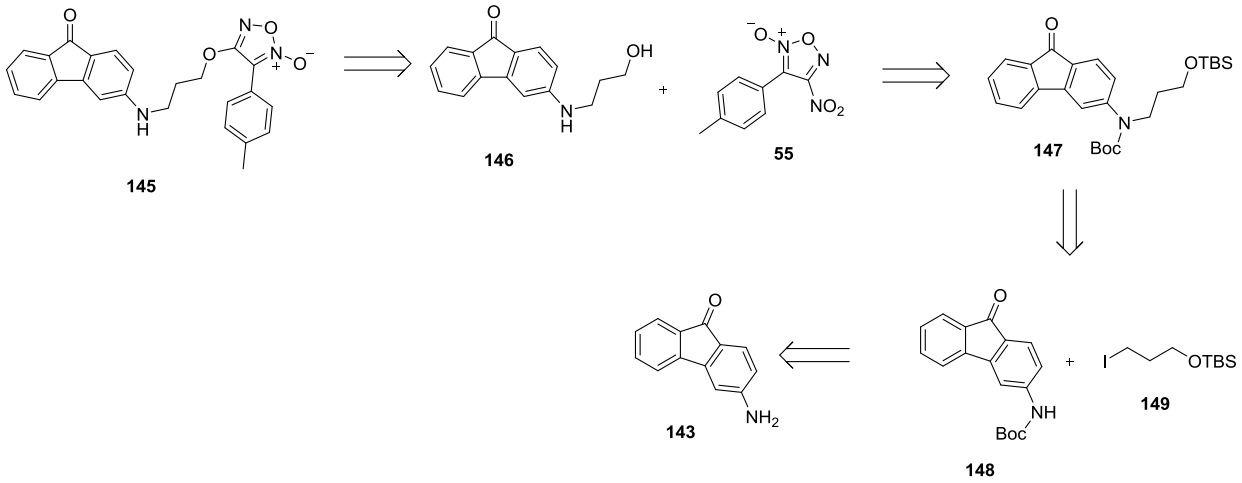


Time (min)	Isomerization% (35 : 36)
0	Trace
30	69
60	88

To our delight we observed significant sensitized isomerization of **35** to **36** after one hour of irradiation. This is a slower rate of isomerization compared to previous experiments which have been conducted at 5 mM concentration but these results indicated that the desired photochemical processes occur at 100 μM in benzene.

3.4 Synthesis towards compound 145

With the knowledge that 3-amino-9-fluorenone induces isomerization in methoxyfuroxan under visible light irradiation, we decided to connect the two molecules, the retrosynthetic analysis is shown in scheme 62.

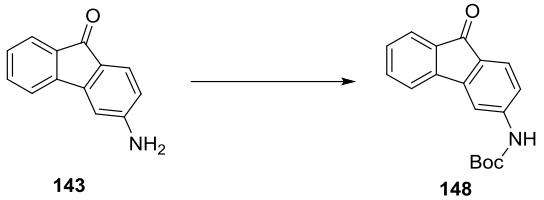


Scheme 62: Retrosynthetic analysis of aminofluorenone tethered alkoxyfuroxan **145**.

Compound **145** is accessed by nucleophilic aromatic substitution of nitrofuroxan **55** with **146**. Compound **146** is formed from global deprotection of **147** which in turn is granted by nucleophilic substitution of silyl ether protected alkyl iodide **149** with Boc protected amine **148**. A propyl linker was selected over an ethyl linker to discourage any intramolecular 5-membered cyclisation between the amine and furoxan.

Our synthesis began with the Boc protection of **143** (Table 26).

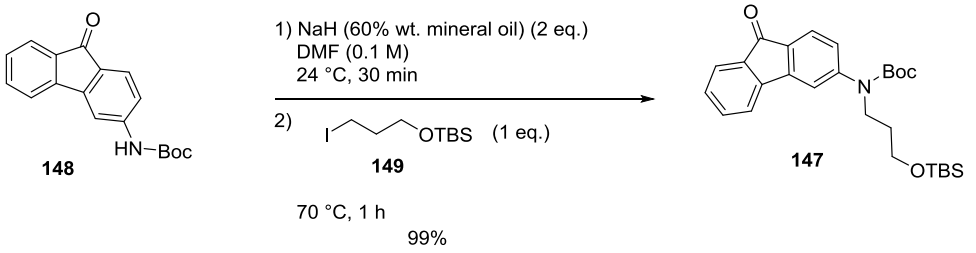
Table 26: Attempted Boc protection of **143**. [a]Isolated yield.



Entry	Reagents	Conditions	Yield (%)[a]
1	Boc_2O (1.1 eq.), MeOH (0.3 M)	50 °C, 12 h	21
2	DMAP (1 eq.), Boc_2O (1.1 eq.), Et_3N (1.1 eq.) THF (0.3 M)	50 °C, 40 h	38

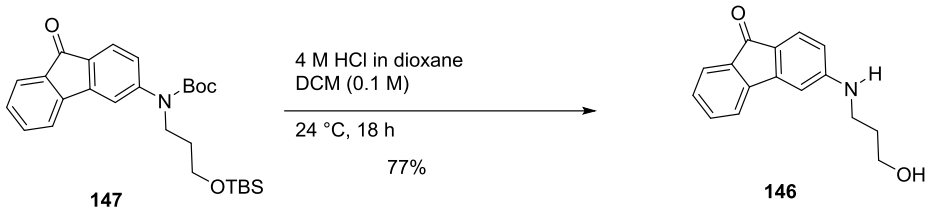
The reaction between **143** and Boc_2O in MeOH overnight did not reach completion (Table 26, Entry 1). ^1H NMR suggested only a small portion of product had formed so the unreacted starting material was recovered. The low reactivity of **143** was due to the amine being conjugated with the aromatic system and electron deficient ketone, which had a strong deactivating effect. The next attempt utilized DMAP to activate the Boc anhydride to nucleophilic attack from the deactivated amine, once again the reaction was slow and after 40 hours significant starting material remained (Table 26, Entry 2).

Given the low reactivity of the amine to Boc protection we suspected that base would be necessary to alkylate **148**. A solution of compound **148** was pre-treated with NaH and then **149** was added dropwise (Scheme 63). Gratifyingly the reactivity was enhanced with deprotonation and **147** was isolated in near quantitative yield.

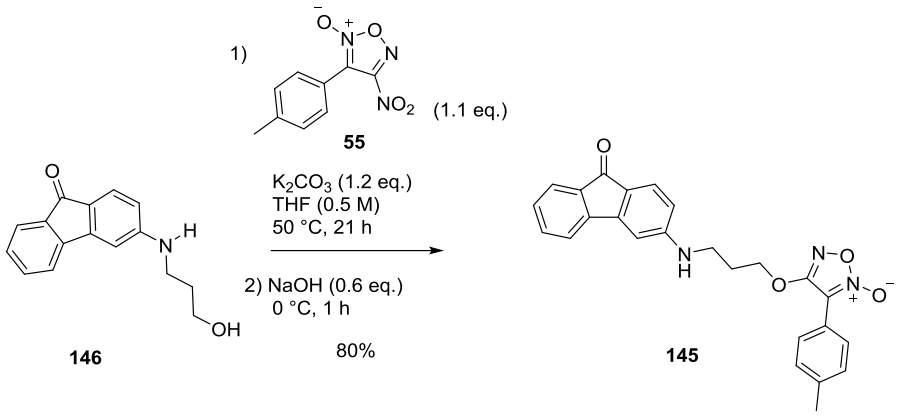


Scheme 63: Alkylation of **148**.

Global deprotection of the silyl ether and Boc protecting groups using 4 M HCl in dioxane granted **146** (Scheme 64).



Scheme 64: Global deprotection of **147**.



Scheme 65: Nucleophilic aromatic substitution reaction between **146** and nitrofuroxan **55**.

Finally, reaction of nitrofuroxan with **146** granted **145** (Scheme 65). Initially we tried to use a K_2CO_3 as a mild base but addition of NaOH was necessary to see the reaction to completion.

3.5 Absorption and fluorescence of compound 145

To our delight compound **145** showed excellent absorption with a λ_{max} of 456 nm and fluorescence with a λ_{max} 545 nm in 50 mM pH 7.4 phosphate buffer solution. However, 20% DMSO was necessary to keep the molecule from precipitating out of solution (Figure 21).

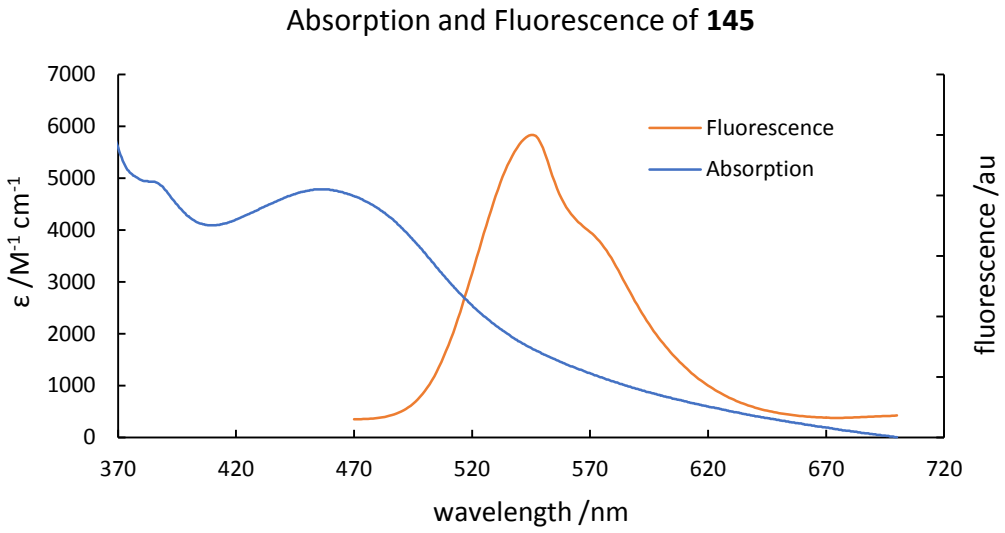
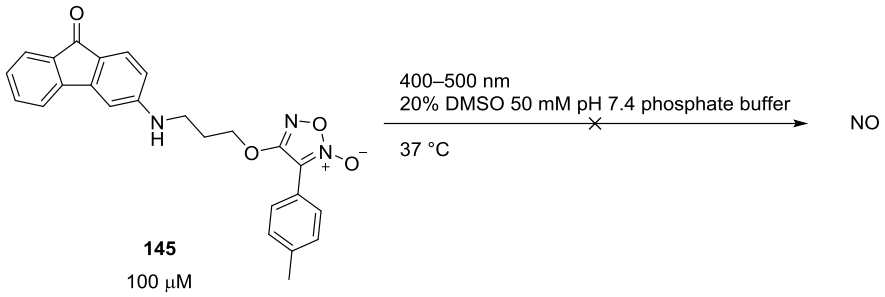


Figure 21: Absorption and fluorescence of compound **145** (100 µM) in 20% DMSO pH 7.4 phosphate buffer at 25 °C. Fluorescence λ_{ex} 470 nm.

3.6 NO releasing ability of compound 145

Compound **145** exhibited limited solubility in the phosphate buffer so 20% DMSO phosphate buffer was used instead. This was not too much of an issue as the molecule could be further functionalized with water soluble moieties later. Unfortunately, even though the sensitizer and furoxan were connected the molecule did not release NO under 400–500 nm irradiation (Scheme 66). The absorption spectrum showed a decrease in the fluorenone absorption over time so we suspected that the molecule may either react unfavourably under irradiation and photobleach, or simply precipitate out of solution (Figure 22). It is of note that the molecule appears to be initially soluble in the phosphate buffer, under irradiation the precipitation appeared to occur at a faster rate.



Scheme 66: Unsuccessful NO release from compound **145**.

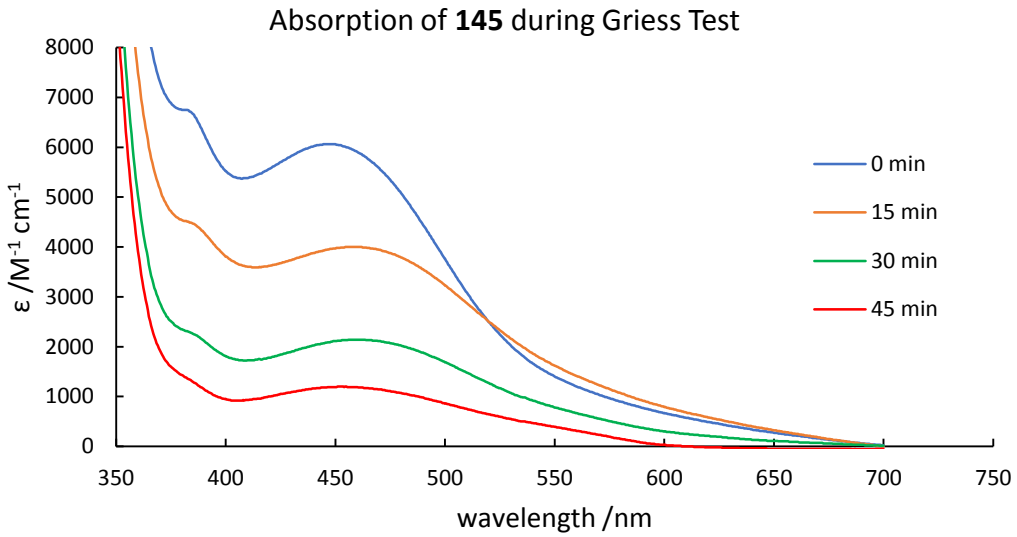
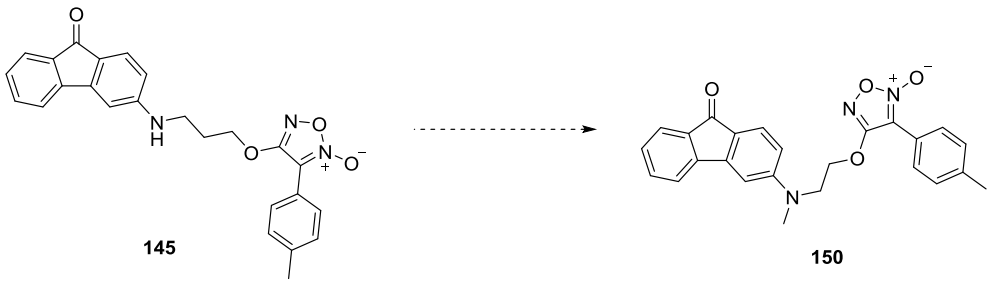


Figure 22: Absorption of **145** (100 μM) in pH 7.4 phosphate buffer during Griess test. Irradiation with 400–500 nm light.

3.7 Synthesis towards compound 150

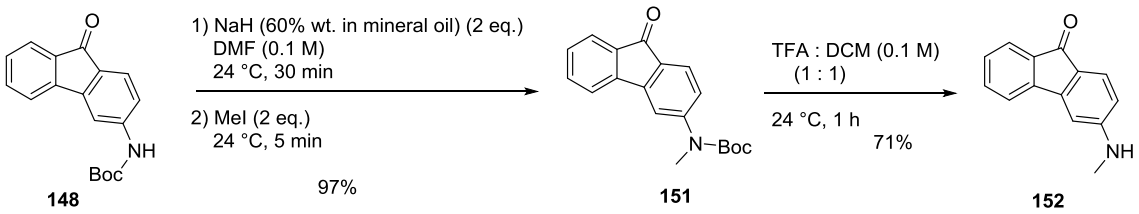
We suspected that the molecule was reacting unfavourably under irradiation and rationalised that the N-H moiety may be responsible for photobleaching, quenching or undesired reactivity. We decided to modify the molecule by methylating the amine, although this would come at the cost of reduced solubility, we wanted to check if any NO could be released and then graft a water-soluble moiety onto the molecule upon release confirmation. Since solubility was already an issue and we now knew the

amine was fairly unreactive when in resonance with the fluorenone core, we also decided to shorten the tether from propyl to ethyl (Scheme 66).



Scheme 67: Modification of **145** by alkylating the amine and reducing linker length.

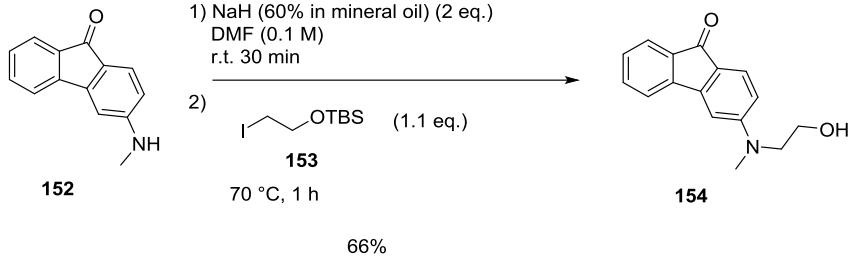
The synthetic route followed the same scheme as **145** but the Boc protected **148** was methylated prior to forming the fluorenone-furoxan bridge (Scheme 68). The methylation occurred in high yield and Boc deprotection afforded **152**.



Scheme 68: Methylation of compound **148** and Boc deprotection. 7

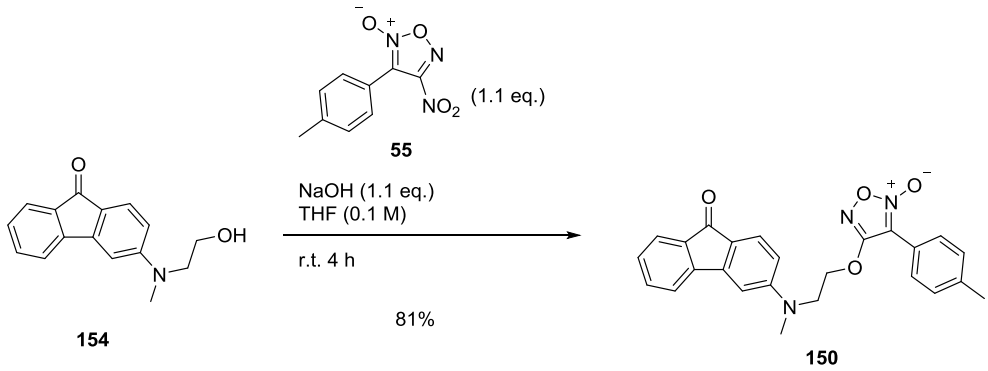
In the next step we attempted to synthesize the bridge between furoxan and fluorenone. Following deprotonation of **152** with NaH we added dropwise silyl either protected ethyl iodide **153**. After one hour of reaction we were surprised to observe the consumption of **153** but remaining **152**. Proton NMR of an aliquot revealed complete removal of the silyl protecting group (Scheme 69). For fear of oligomerization we did not add further base or silyl ether. We were pleasantly surprised that **154** was obtained without oligomers in 66% yield and 34% starting

material recovery following chromatography. The basic amide anion is likely able to deprotect the sily ether leading to partial deactivation of starting material.



Scheme 69: Alkylation of **152** with unexpected -OTBS deprotection.

We next connected fluorenone and furoxan in high yield (Scheme 70).



Scheme 70: Connection of **154** and furoxan **55**.

3.8 Absorption and fluorescence of compound 150

Like the non-methylated isomer **145**, compound **150** showed a promising albeit less intense spectra with absorption λ_{max} of 450 nm and fluorescence λ_{max} of 545 nm in 20% DMSO 50 mM pH 7.4 phosphate buffer (Figure 23).

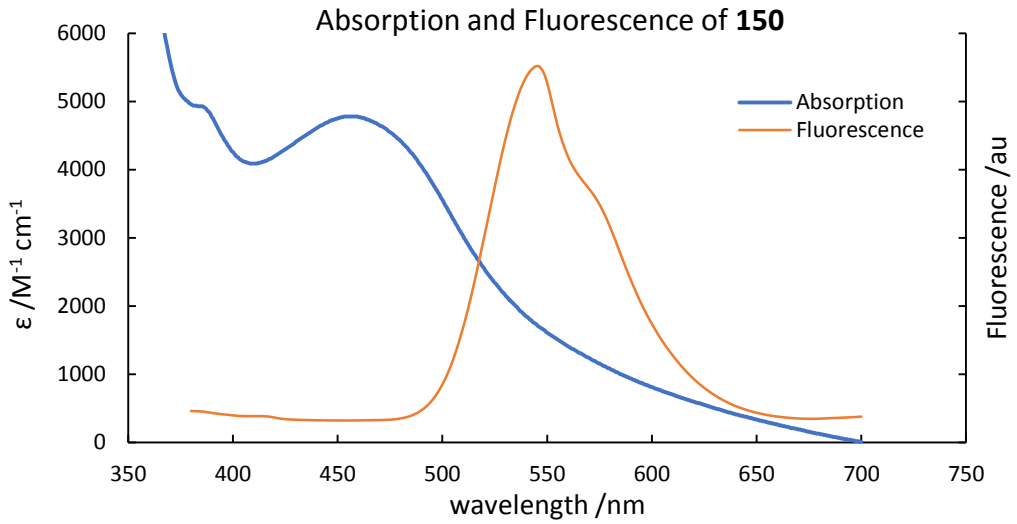
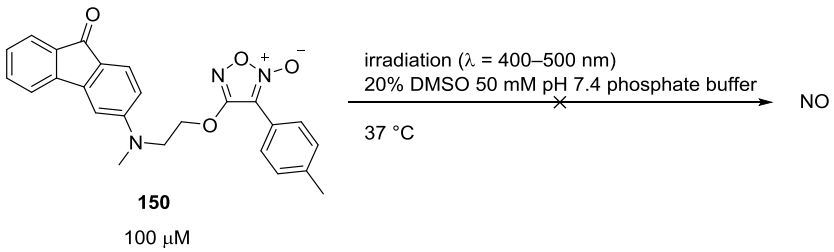


Figure 23: Absorption and fluorescence of compound **150** in 20% DMSO pH 7.4 phosphate buffer at 25 °C. Fluorescence λ_{ex} of 380 nm.

3.9 NO releasing ability of compound 150

Next the NO releasing ability was analysed by Griess test in 20% DMSO 50 mM pH 7.4 phosphate buffer under 400–500 nm irradiation (Scheme 71). Unfortunately, despite methylation of the amine no discernible NO was released under 400–500 nm irradiation and the molecule underwent rapid photobleaching at a rate faster than compound **145** (Figure 24).



Scheme 71: Unsuccessful NO release from compound **150**.

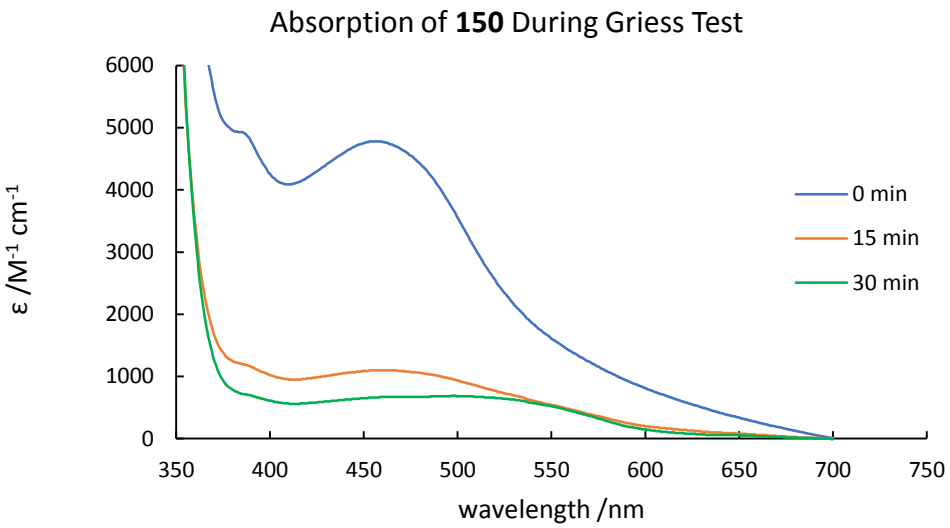
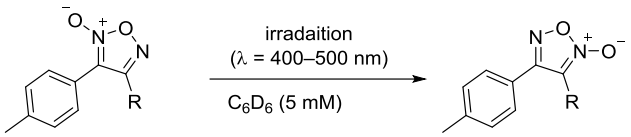


Figure 24: Absorption of **150** (100 µM) in pH 7.4 phosphate buffer during Griess test. Irradiation with 400–500 nm light.

3.10 Sensitized isomerization of compounds 145 and 150

With the fluorenone-tethered alkoxyfuroxans being non-NO releasing during Griess assays, we decided to check if **145** and **150** truly possessed sensitizing ability by irradiating a sample in deuterated benzene and observing any isomerization of the furoxan (Table 27).

Table 27: Isomerization of **145** and **150** under irradiation in C_6D_6 (5 mM).



Compound	Time (min)	Isomerization%	Comment
150	0	2	Residual isomerization
	15	7	PS
	30	7	
	45	7	
145	0	0	
	15	13	
	30	21	PS
	45	21	

The isomerization experiments were conducted on a 5 mM scale in deaerated benzene (Table 27). Irradiation with visible light at room temperature for 15-minute intervals confirmed that the molecules both possess sensitizing ability. Compound **150** reached the PS within 15 minutes showing only minor isomerization and compound **145** showed a greater isomerization ratio reaching PS after 30 minutes.

This data meant that although the molecules do photochemically interact with themselves in non-polar organic solution, they do not photochemically interact in aqueous solution.

One possibility for this is that the aminofluorenone excited state is quenched by the solvent cage of polar molecules. Supposedly aminofluorenones can be quenched by the solvent rearranging itself around the intramolecular charge transfer excited state.[104]

Another possibility is that the excited energy levels of the furoxan and sensitizer align favourably when in non-polar solvents but are no longer suitably aligned in polar solvents. The El-Sayad rule states that the rate of intersystem crossing is relatively large if the radiationless transition involves a change of orbital type.[105] It is known that

the fluorenone triplet T_3 energy level is destabilized in polar solvent relative to the singlet S_1 excited energy level so intersystem crossing (S_1 π-π* to T_3 n- π*) becomes inefficient and nonradiative relaxation becomes the primary method of deactivation,[106] this was not however known to us at the time of design. Note that the fluorenone T_1 and T_2 energy levels are of π-π* character so ISC is not easily accessible from the S_1 state. This theory aligns with the observation of isomerization occurring in deuterated benzene but observing no NO release in phosphate buffer solution. This does not explain however the observed photobleaching, this may be due to excited state quenching by the furoxan or undesired reactivity, another possibility is that the molecule simply precipitates out of solution, although we suspect that any precipitation is assisted by the irradiation process.

3.11 Conclusions

By considering the shortfalls of our proof of concept anthraquinone-fluorofuroxan hybrid we were able to define criteria that an improved molecule would meet. By selecting 3-amino-9-fluorenone we acquired theranostic properties and a more accessible synthetic route. We were delighted that the 3-amino-9-fluorenone-furoxan hybrids worked as intended in benzene but the lack of NO release and difficulty encountered when in phosphate buffer solution rendered the design impractical. Of course, further modification may have led to a reasonable and functioning molecule but modification was tedious and the literature theory behind fluorenones in polar protic solvents projected the success of the design unfavourably.

We decided to continue the search for a fluorescent, triplet sensitizing, water soluble, cell permeable and NO releasing molecule by referring once again to literature on the topic.

Ⅳ: Improvement to visible light PINOD furoxans: Naphthalenediimide-alkoxyfuroxan

4.1 Naphthalenediimides as fluorescent photosensitizers

Naphthalenediimides (NDIs) find application in a variety of fields such as fluorescence spectroscopy, electronic devices, supramolecular structures, ion and pH sensors and organonucleotide targeting.[107] Functionalization with heteroatoms via lateral core substitution creates highly tuneable fluorescent NDI analogues depending on the nature of functionalization (Figure 25).[108]

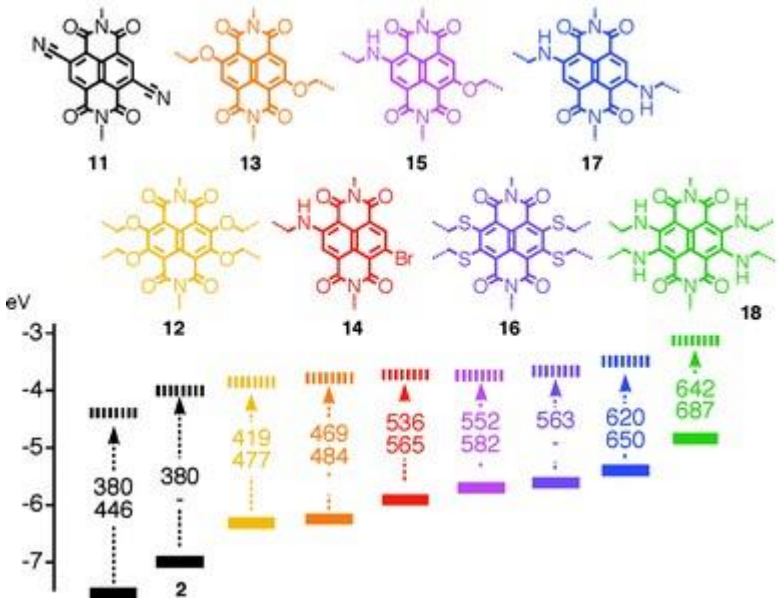
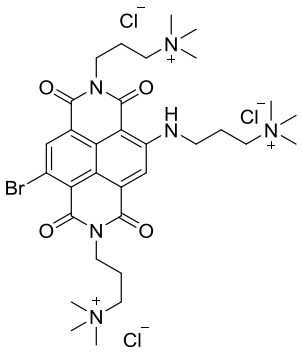


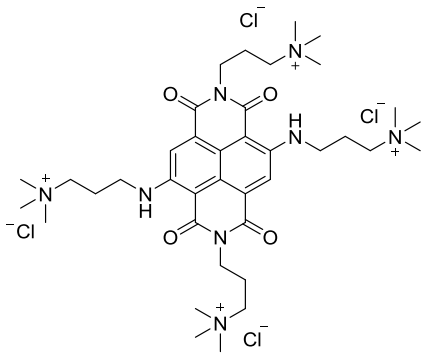
Figure 25: Core substituted NDI and their respective absorption wavelengths (lower number), emission wavelengths (upper number), HOMO (bold) and LUMO (dashed) energies. Figure reproduced from Sakai et al.[108]

Recently Doria et al. reported the synthesis of several water soluble fluorescent NDI derivatives which were able to sensitize the formation of singlet oxygen under irradiation with visible light via introduction of a bromine atom onto the NDI core (Table 28, Entry 1).[109] One NDI derivative was particularly remarkable and exhibited sensitizing ability without a heavy atom, the tetrasubstituted NDI **156** was later applied to cellular studies and reduced cancer cell viability to 40% under visible light irradiation (Table 28, Entry 2).[110]

Table 28: Sensitizing and fluorescent NDI derivatives. [a]Using $Ru(bpy)^{3+}$ as reference with a value of 0.028 in aerated water [b]Excitation at 373 nm. [c]Determined from phosphorescence of $^1O_2$ at 1270 nm in D_2O, under air-equilibrated conditions. All solutions are isosbestic at the 532 nm excitation wavelength. [d]Triplet lifetime in argon-saturated solution. Excitation at 532 nm. Data adapted from Doria et al.[109]



155



156

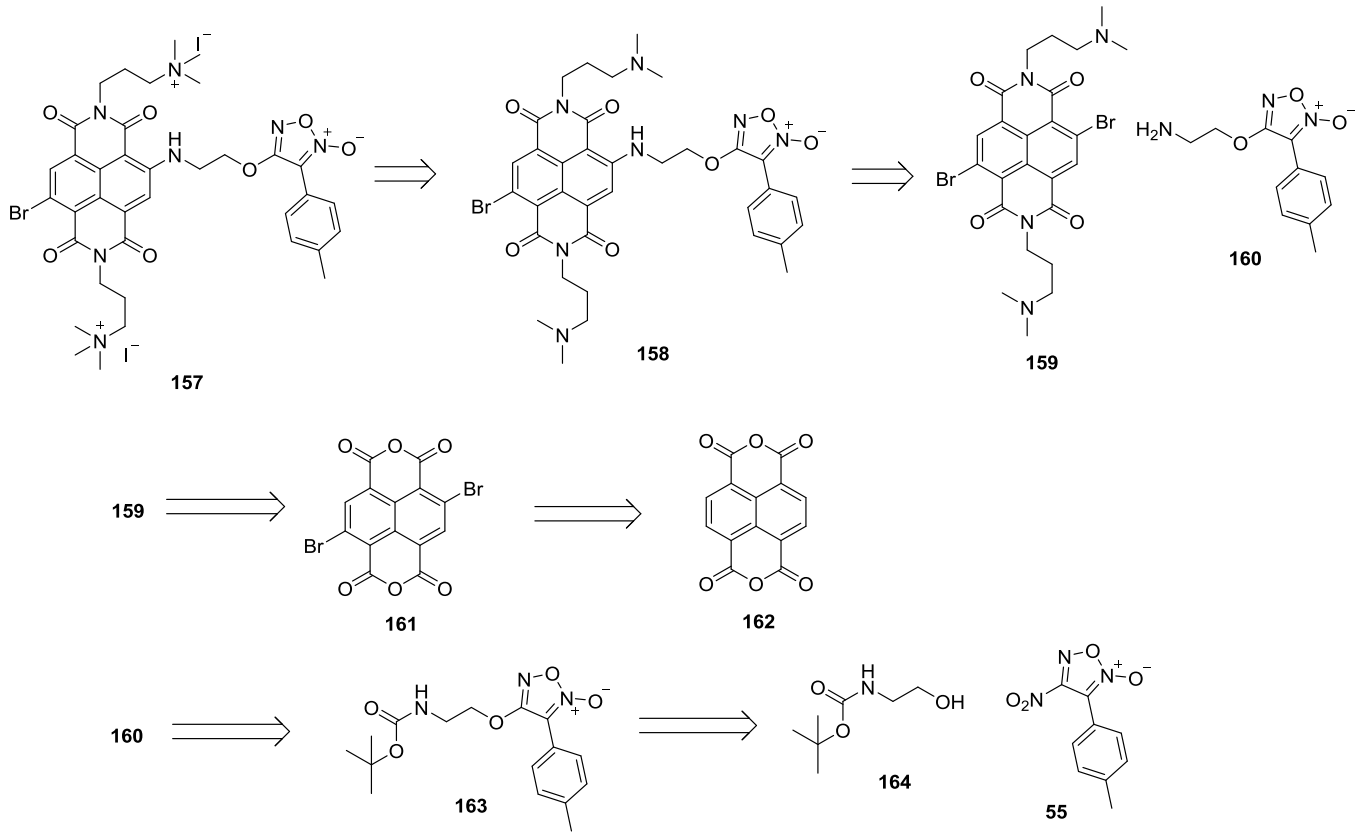
NDI	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})	ϕ_F[a]	τ_f (ns)[b]	ϕ_Δ[c]	τ_T (µs)[d]
155	530	7600	0.12	3.33	0.46	22
156	613	10700	0.17	4.00	0.30	32

4.2 Synthesis towards compound 157

Encouraged by the ability of NDIs **155** and **156** to sensitize singlet oxygen through the triplet state and the theranostic nature of the molecules, we hypothesized that a suitably substituted NDI tethered to a furoxan may induce NO release under spatiotemporal control. The hybrid molecule would fluoresce under irradiation indicating the cellular uptake and perhaps even localize in DNA as NDIs have been known to behave as reversible ligands for G4-quadruplexes often observed in telemorase, a key target overexpressed by cancerous cells.[110]

One advantage of NO induced cell apoptosis over $^1O_2$ induced cell death is that cell death via $^1O_2$ occurs by necrosis and induces an immune response resulting in an undesirable and painful inflammatory event, whereas apoptosis does not.[63]

We designed a prototype molecule which utilized the bromo-facilitated spin orbit coupling to induce sensitized NO release from alkoxyfuroxan (Scheme 72).



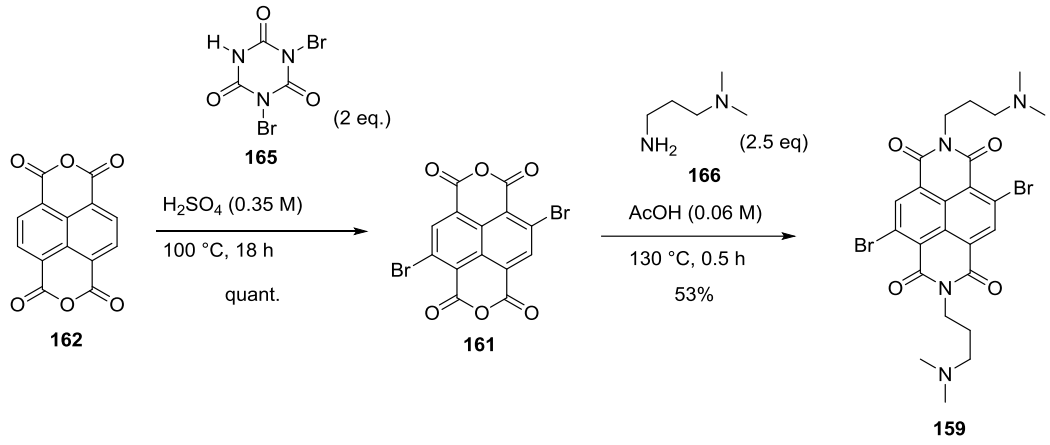
Scheme 72: Retrosynthetic analysis of NDI-alkoxyfuroxan hybrid **157**.

The desired compound **157** contains two terminal quaternary ammonium salts which serve a dual function. Quaternarization is necessary to prevent amine single electron transfer from quenching the excited state NDI (at pH 7.4 the amine would not be in a continuous protonated state), furthermore the water solubility would be enhanced.

Compound **157** is accessed by methylation of the terminal amines of **158** which in turn is synthesised by nucleophilic aromatic substitution of diimide **159** with pendant amino alkoxyfuroxan **160** (Scheme 72). NDI **159** is synthesised by imidization of dianhydride **161** with *N*,*N*-dimethylaminopropylamine. Compound **161** can be constructed by bromination of commercially available anhydride **161** using dibromocyanuric acid. Furoxan **160** is synthesized by Boc deprotection of **163**

previously furnished by nucleophilic aromatic substitution of nitrofuroxan **55** with Boc protected 2-aminoethanol **164**.

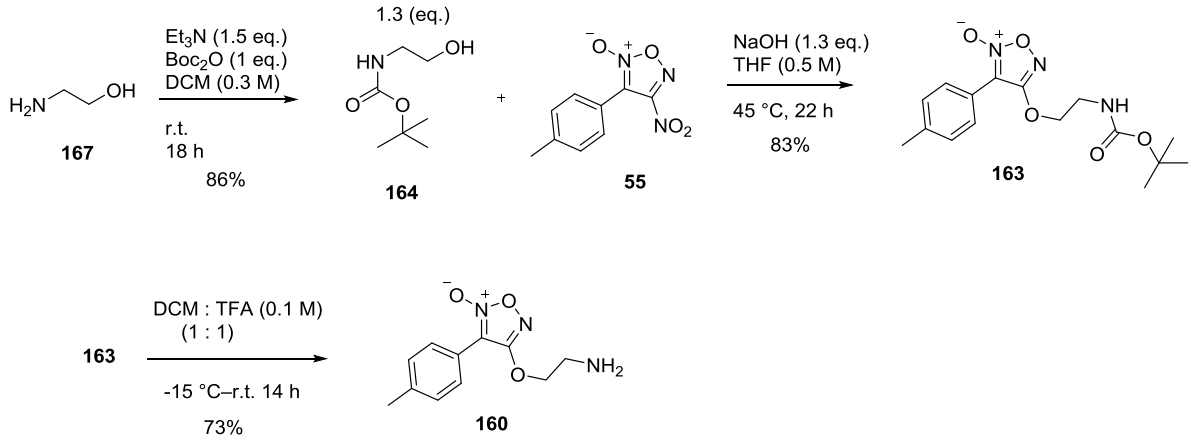
The NDI synthesis up to compound **159** was obtained from previous work by Doria et al. in their synthesis towards NDIs that target telomeric G-quadruplex structures (Scheme 73).[111]



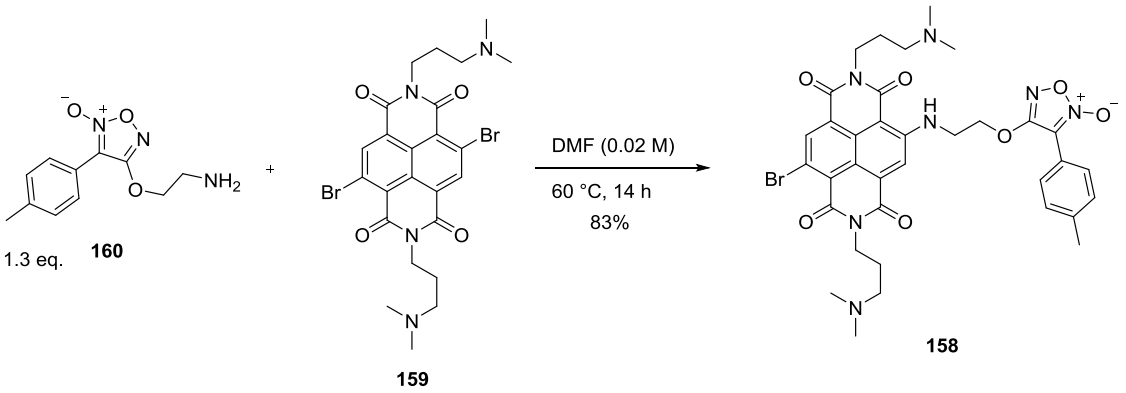
Scheme 73: Synthesis of NDI **159**.

Starting from commercially available 1,4,5,8-naphthalenetetracarboxylic dianhydride **162** we brominated the core twice using dibromocyanuric acid **165**, the product **161** is known for being notoriously insoluble and was filtered following precipitation from ice water and then washed with hot methanol to afford a crude mixture containing **161** in quantitative yield. The brominated dianhydride underwent imidization with *N*,*N*-dimethylaminopropylamine **166** following 1 h reflux in acetic acid, the product **159** was obtained by silica chromatography in 53% yield.

The furoxan partner **160** was synthesized by Boc protection of 2-aminoethanol **167** and S_NAr reaction onto nitrofuroxan followed by deprotection (Scheme 74).



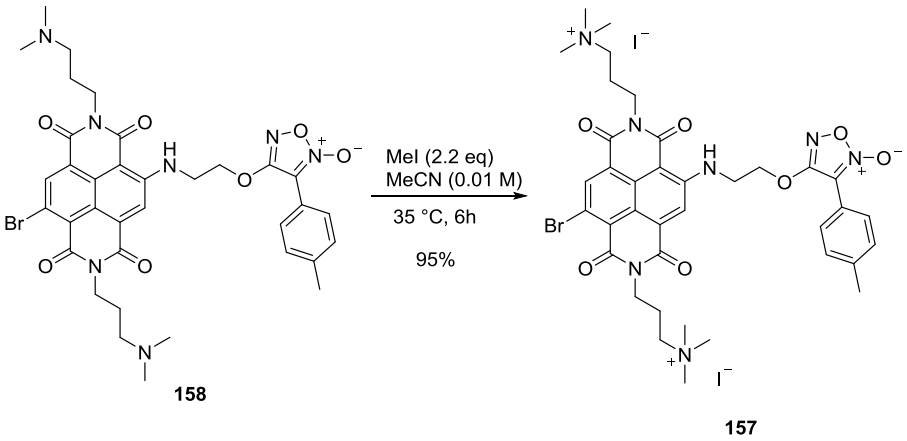
Scheme 74: Synthesis of **160**.



Scheme 75: Connection of furoxan and NDI via alkylamino bridge.

Furoxan **160** was connected to NDI **159** by S_NAr reaction in dilute DMF. The product was highly polar but deactivation of silica gel with triethylamine (5% MeOH, 95% $CHCl_3$, 0.5% v/v Et_3N) enabled the pure red solid to be collected.

Finally, methylation of the pendant amino groups granted **157** as a purple solid following filtration and washing with $CHCl_3$ (Scheme 76). Curiously, the product was reasonably soluble in acetonitrile.



Scheme 76: Methylation of **158** to afford the desired NDI-furoxan **157**.

4.3 Absorption and fluorescence of compound 157

To our delight, even with the furoxan substitution, **157** was water soluble and we were able to obtain the absorption and fluorescence spectra in pH 7.4 phosphate buffer (Figure 26).

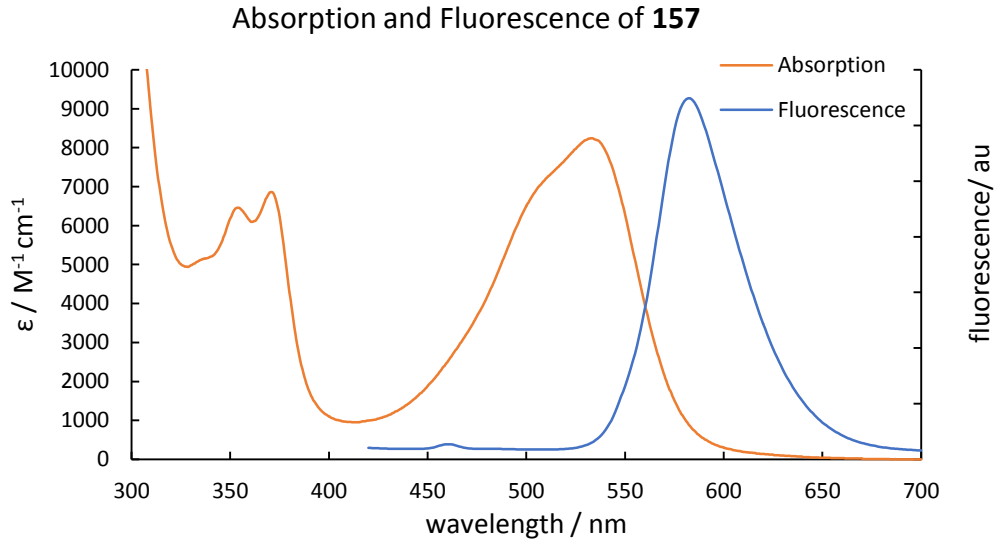


Figure 26: Absorption and fluorescence spectrum of **157** in 50 mM pH 7.4 phosphate buffer at 25 °C. Fluorescence λ_{ex} of 420 nm.

Compound **157** showed a broad absorption above 400 nm, the intramolecular charge transfer can be seen as an intense peak with a λ_{max} of 533 nm. The fluorescence spectrum has a λ_{max} of 581 nm and extends beyond 650 nm which is useful for identifying cellular uptake locations.

4.4 NO releasing ability of compound 157

We next investigated the NO releasing ability of **157** under visible irradiation both in the presence and absence of excess cysteine.

In the absence of cysteine compound **157** releases up to 16 µM of NO after 2 hours of irradiation with 420–600 nm light (Figure 27). When irradiating with a longer wavelength (500–600 nm) the NO release rate is comparatively more gradual, reaching 10 µM over 4 hours. Under ambient lighting conditions there is no significant NO release observed during 4 hours of irradiation.

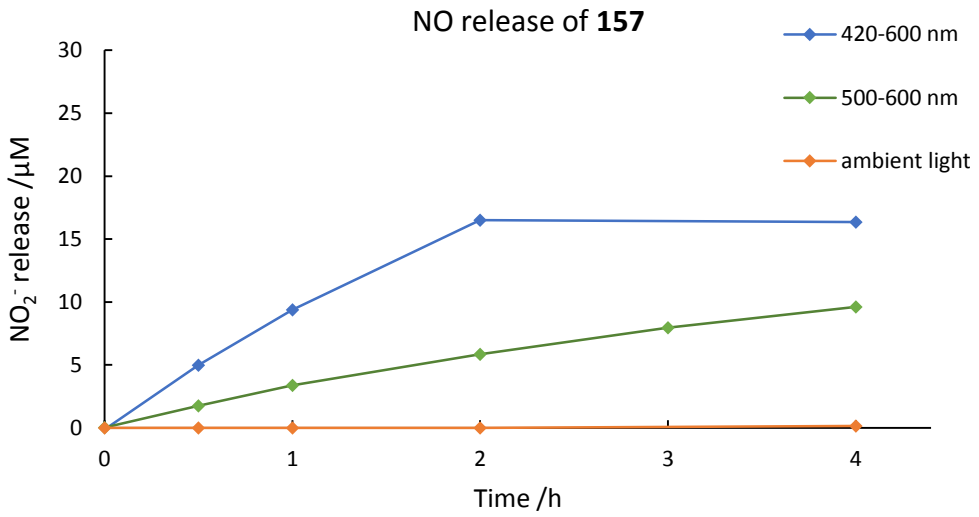


Figure 27: NO release curve of **157** (100 µM) in 50 mM pH 7.4 phosphate buffer under 420–600 nm, 500–600 nm irradiation or under ambient light at 37 °C. The experiment was conducted in the absence of cysteine.

When compound **157** was irradiated in phosphate buffer containing 5 mM cysteine we were disappointed by the lack of NO release (Figure 28). We suspect that cysteine can quench the excited state, possibly by single electron transfer. We have also not ruled out the possibility of nucleophilic substitution of the core bromo or amino functionalities by the excess thiol.

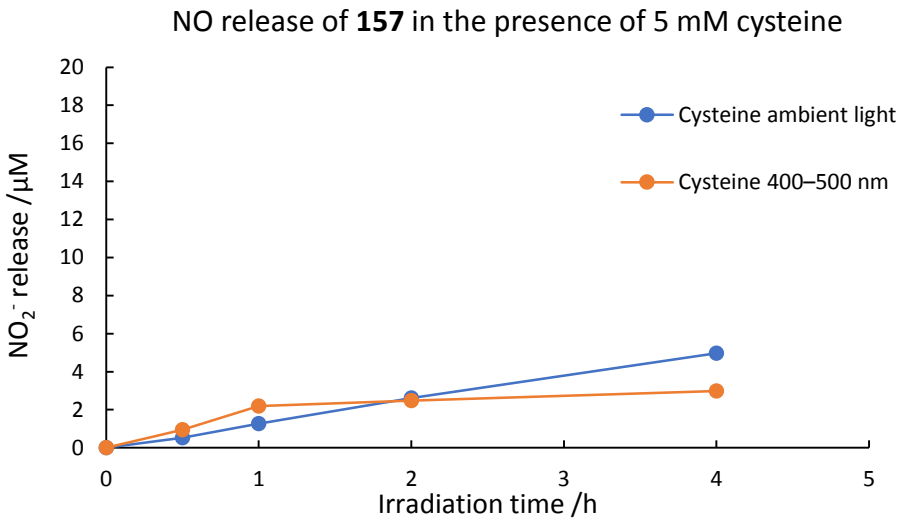


Figure 28: NO release curve of **157** (100 µM) in 50 mM pH 7.4 phosphate buffer containing 5 mM cysteine under 400–500 nm irradiation or under ambient light at 37 °C.

The lower quantity of NO release in the absence of thiol compared to anthraquinone-tethered fluorofuroxan **122** can be explained by considering a number of factors; compound **157** exhibits theranostic properties so a portion of the absorbed energy will be used for fluorescence rather than sensitizing the furoxan. We also suspect that alkoxyfuroxan is capable of reacting with the sensitizer inducing side reactivity, whereas fluorofuroxan, to the best of our knowledge, does not react with anthraquinone. Compound **157** unexpectedly undergoes photobleaching, so only partial release of NO can be achieved.

Compound **157** is almost fully photobleached within 3 hours of continuous irradiation (Figure 29a). Furthermore, the fluorescence spectrum undergoes a hypsochromic shift from λ_{max} of 581 nm to λ_{max} of 521 nm with a significant increase in fluorescence

intensity under prolonged irradiation (Figure 29b). Despite the possibility of limiting NO release, this can be a useful property since the molecule behaves as a fluorometric indicator to signal when the NO release event has finished. The change in absorption of compound **157** with irradiation shows an overall decrease in intensity at both 533 nm and 371 nm with prolonged irradiation time. The decrease at 371 nm is attributed to a perturbation of the NDI core conjugation, perhaps due to decomposition of the diimide moieties. The decrease at 533 nm could also be attributed to cleavage of the core substituted NDI-amino bond preventing intramolecular charge transfer.

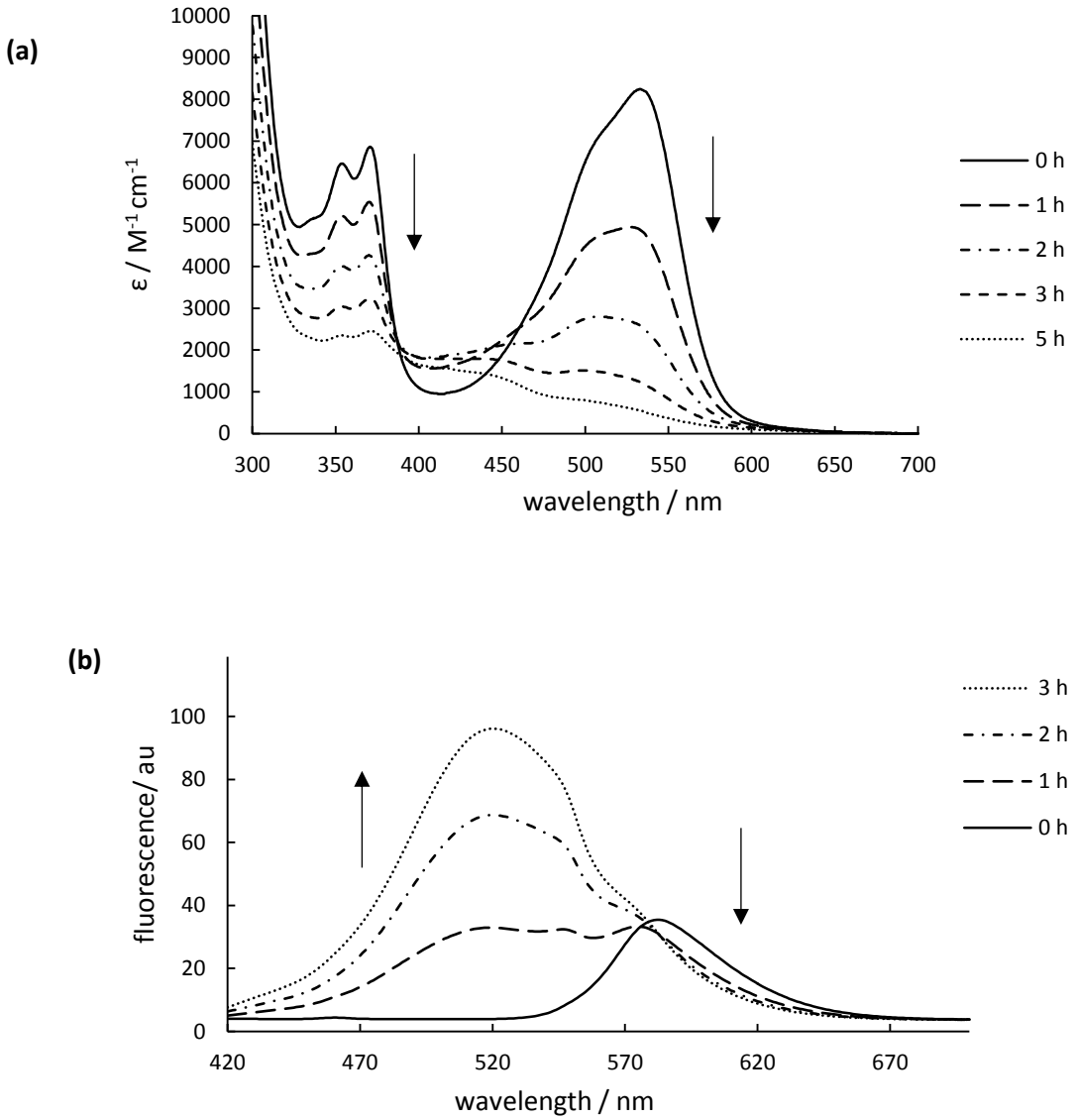


Figure 29: (a) Photobleaching of compound **157** under 420–600 nm continuous irradiation in 50 mM pH 7.4 phosphate buffer solution at 37 °C. At intervals a 2 mL aliquot was taken and the absorption spectra measured at 25 °C (b) Change in

fluorescence spectrum of compound **157** under 420–600 nm continuous irradiation in 50 mM pH 7.4 phosphate buffer solution at 37 °C. At intervals a 2 mL aliquot was taken and the fluorescence intensity measured at 25 °C with λ_{ex} of 420 nm.

The instability of compound **157** in the presence of thiol was unfortunate but it should be noted that 5 mM of cysteine is a significant excess compared with cellular levels (30–250 µM), however total cellular thiol concentration is much greater.[112] It is of note that monobrominated core substituted NDIs have been used in cancer cell studies previously with no reports of denaturization, so it is likely that the decomposition occurs due to the irradiation process rather than any inherent instability. In fact, alkylating NDIs showed cytotoxicity towards telemorase positive cells with IC_{50} values in the µM range.[111]

4.5 Cellular studies with compound 157

We decided to test compound **157** for cell permeability and hoped that despite disappointing in vitro experiments, NO may still be released under irradiation from within a cell (Figure 30).

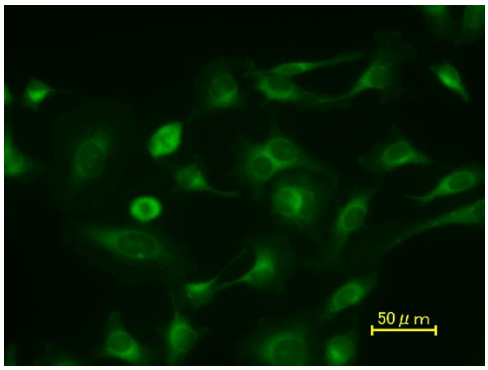


Figure 30: Fluorescence imaging of **157** taken up into living HeLa cancer cells (exposure time 1/3 second). HeLa cells were incubated with **157** (20 µM) for 20 minutes before washing thrice with PBS buffer solution. Exposure of light in the wavelength of 500–600 nm shows successful cellular uptake.

Previous research into NDI cellular uptake had shown successful cell permeability despite tri- and tetrasubstituted quaternary ammonium moieties, so we were pleased to see that even with the dual quaternary ammonium ions and the tethered furoxan molecule, the hybrid permeates the cells.[110,111] Although the uptake mechanism is not fully understood, it has been suggested that similarities between tetracationic porphyrins and NDIs indicate endocytosis as a possible pathway.[110] Unfortunately, when irradiated with 500–600 nm light for 20 minutes there was no indication of NO release (not shown). This is probably due to thiol quenching the excited state, the same as with the in vitro experiments.

4.6 Conclusions

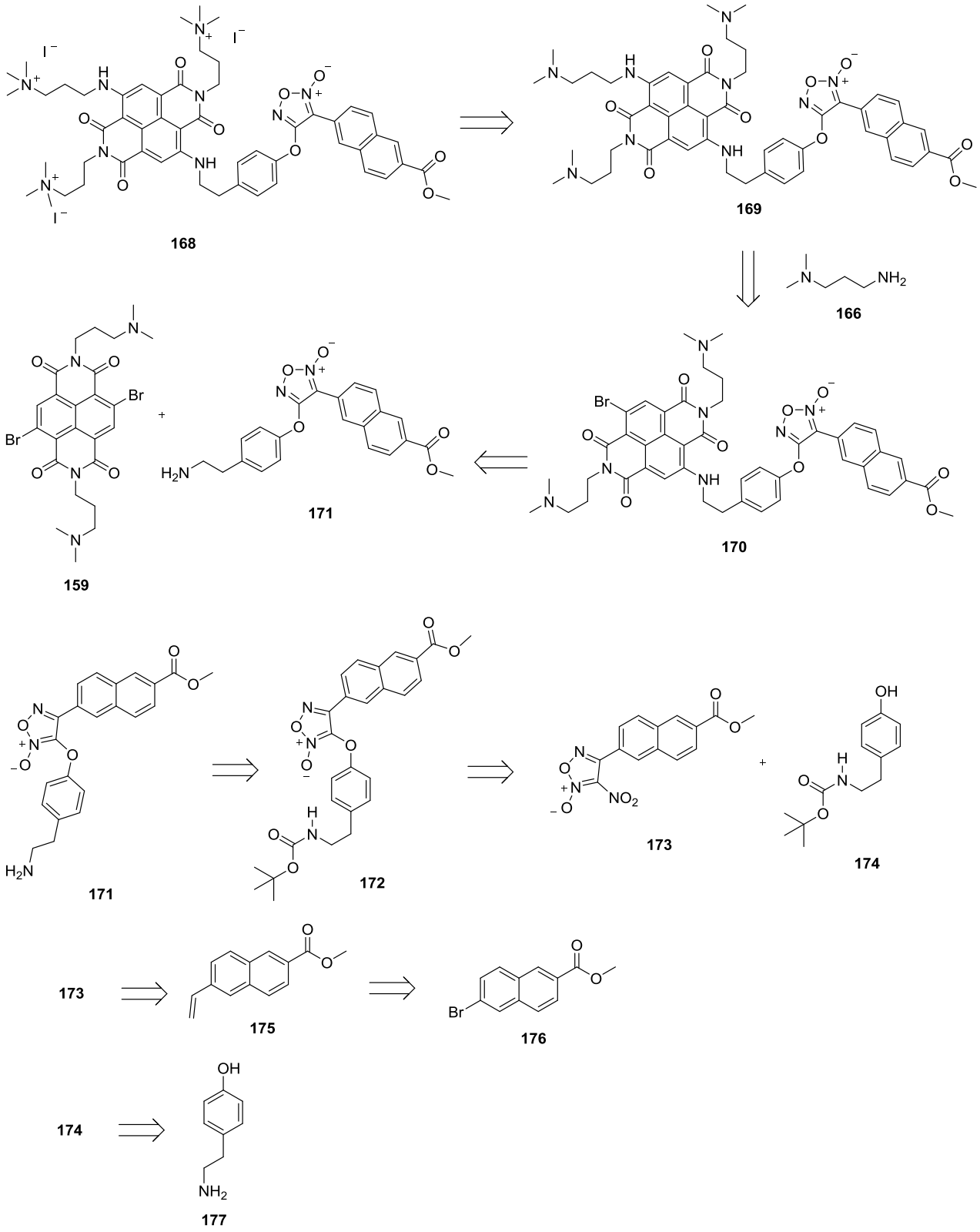
We consolidated on our previous designs by selecting NDI as a theranostic sensitizer core. Furthermore, we were able to successfully connect an alkoxyfuroxan. The synthesis of the NDI-furoxan hybrid was much simpler when compared with previous sensitizer-furoxan designs. Compound **157** demonstrated photoswitchable fluorescent sensitized NO release in phosphate buffer. Unfortunately, when cysteine was present the NO release was prevented. In spite of this we were able to observe localization within a cell culture system but photoswitchable NO release from within the cells was not achieved, likely due to the local thiol concentration. This strategy was a further step towards the overall project goal of photoswitchable NO release from within a cell culture system.

4.7 Future research

In previous studies tetrasubstituted compound **156** showed photostability and singlet oxygen generation after 6 hours of continuous irradiation,[109] we hypothesized that this is due to increased electron density and the absence of potentially photoreactive bromine on the NDI core. We also envisioned that by increasing the conjugation of the furoxan partner and introducing electron withdrawing substituents we could lower

the orbital energies (HOMO and LUMO) and promote facile sensitization without the suspected malign photobleaching interactions between sensitizer and furoxan.

With the initial results of prototype **157** being promising we designed a furoxan tethered tetrasubsituted NDI molecule (Scheme 77).



Scheme 77: Retrosynthetic analysis of tetrasubstituted furoxan tethered NDI **168**.

Compound **168** can be accessed by exhaustive methylation of the terminal amines of intermediate **169**. The tetrasubstitution is notoriously difficult from trisubstituted NDIs unless microwave irradiation is used, so microwave assisted reaction of **170** with a third *N*,*N*-dimethylaminopropylamine constructs **169**. Connection of furoxan **171** and NDI **159** is undertaken using the same S_NAr approach previously completed. The extended conjugation on the furoxan **171** is accessed by S_NAr reaction between nitrofuroxan **173** and Boc protected tyramine **174**. Nitrofuroxan **173** is furnished by Wieland procedure on a Stille coupled vinyl naphthalene **175** which is constructed from commercially available methyl 6-bromo-2-naphthoate **176**.

We hope that in the future research of this project, NO will be released under spatiotemporal control from an NDI-furoxan localized within a cell culture system.

V: Conclusions

In conclusion we have developed a number of photosensitized switchable furoxan NO donors with varying degrees of NO donating ability, however so far successful designs have been hindered by limited biological application.

Initial attempts at synthesising an anthraquinone-tethered fluorofuroxan encountered regioselectivity issues between the fluoro- and benzylic positions with anthraquinone-tethered alkoxy nucleophiles. That being said, the formed product may have made an excellent NO donor as it was alkoxy in nature and the remaining benzylic position could be functionalized further with water soluble pendant groups. However, at the time of conducting the research we were not fully aware of alkoxyfuroxans as PINODs and competing research within the group led to the need for a different approach which remained focused on fluorofuroxans.

A second approach utilized a novel Stille coupling reaction between bromo-functionalized fluorofuroxans and β-iodostyrenes. The synthesis of a model compound was successful following extensive reaction optimisation. However, application to anthraquinone β-iodostyrene encountered solubility issues and low reactivity due to the extended and unreactive conjugated anthraquinone core. The route was also plagued with purification issues due to the insoluble nature of the anthraquinone. Despite requiring a redesign, the Stille coupling applied to electron deficient and reactive furoxans under mild conditions is a highlight of the research and enables expansion of the available reactivity for furoxans. We hope that the furoxan Stille coupling reaction conditions will find application within the research group in future work, perhaps even expansion of reaction scope.

To address the difficulty with handling and purification of the anthraquinone we designed a combinatorial synthetic route which formed the anthraquinone core at the final stages of the synthesis via a proline catalysed [4 + 2] cycloaddition. Initial attempts were troublesome once again due to purification issues with the anthraquinone. These were addressed by utilizing a Boc protecting group which significantly enhanced the solubility of the formed anthraquinone hybrid molecule and enabled facile isolation. Simple methylation and acid catalysed deprotection granted the product as a water-soluble salt which released NO in a photoswitchable manner. To our surprise the molecule did not require cysteine to release NO, however cysteine being present did result in greater quantities released. The mechanism of release was briefly investigated by electrochemical methods and suggested that

electron transfer is unfavourable leading us to propose that the energy transfer is via Dexter exchange of electrons. Unfortunately, we could not ascertain whether the molecule was cell permeable and therefore could not determine whether NO could be released from within a cell culture system. Attempts to improve the NO donating ability by utilizing an alkoxy furoxan were lower yielding, possibly due to a higher energy level of the (relatively) electron rich alkoxy furoxan.

Evaluation of the limitations of the anthraquinone tethered fluorofuroxan led us to set a series of requirements for a successful furoxan PINOD. The molecule needed to be water soluble, cell permeable, fluorescent and triplet sensitizing leading to spatiotemporal NO release. We began screening for suitable furoxans and sensitizers and found that alkoxy- and fluoro- furoxans release high quantities of NO under irradiation, whereas amino furoxans release moderate and alkyl furoxans release negligible quantities of NO. This research was especially valuable as it showed that almost all furoxans release NO under irradiation in the absence of cysteine, the requirement of which is a major limitation for biological application as cellular levels vary. We were unsuccessful in finding a wide variety of fluorescent sensitizers with only 3-aminofluorenone being photostable under prolonged visible irradiation. The aminofluorenone sensitizer showed photosensitizing ability of furoxan in non-polar solvents and we synthesized two hybrid molecules. Unfortunately, once submerged in phosphate buffer solution the sensitizing ability was quenched. This was possibly due to rearrangement of hydrogen bonds around the excited state and difficulty accessing the triplet energy level due to destabilization of the triplet energy level relative to the excited singlet excited state. The hybrid was also limited by poor solubility and possibly underwent furoxan mediated side reaction or photobleaching.

Finally, we investigated naphthalenediimides as potential fluorescent, water soluble and cell permeable photosensitizers of alkoxyfuroxans. Our initial prototype displayed favourable properties being able to release moderate quantities of NO under irradiation in the absence of cysteine. However, in the presence of cysteine the molecule was non-NO releasing and appeared to be quenched, perhaps by thiol single electron transfer. The NDI-furoxan hybrid was taken up into HeLa cancer cells and could be observed due to fluorescence but NO was not released upon irradiation. This limited the biological application so we proposed a further functionalized tetrasubstituted NDI which was connected to a highly conjugated and electron

deficient furoxan in the hopes that NO release within a cell culture system could be achieved in the future.

As a final comment, the furoxan offers a versatile platform as a photoswitchable NO donor being susceptible to photosensitization by a variety of sensitizers and releasing NO in moderate quantities. However, when compared to other NO donor platforms the furoxan perhaps falls short. S-nitrosothiols, organonitrates and NONOates release NO in greater quantities (up to two molar equivalents!). With new photoswitchable NO donor platforms being developed and appearing in the literature at an increasing frequency, it does make one wonder; *is* ***furoxan*** *really competitive enough to* ***match*** *or* ***supersede*** *the* ***competition****?*

That will be the challenge faced by my successors.

VI: Experimental

Unless otherwise noted, all reactions were carried out in well cleaned glasswares with magnetic stirring. Operations were performed under an atmosphere of dry argon using Schlenk and vacuum techniques. All starting materials were obtained from commercial sources or were synthesized using standard procedures. Melting points were measured on a Yanaco MP-500D and are not corrected. ^{1}H and ^{13}C NMR (400 and 100 MHz, respectively) were recorded on a Bruker Avance III HD 400 using TMS (0 ppm) and $CDCl_3$ (77.0 ppm) as an internal standard. The following abbreviations are used in connection with NMR; s = singlet, d = doublet, t = triplet, q = quartet, quin =

quintet, sep = septet, and m = multiplet. Mass spectra were measured using a JEOL JMS-T100LP (DART method, ambient ionization) or a Thermo Orbitrap Elite (ESI method) or Thermo Finnigan LCQ TRACE GC ULTRA (EI method). Preparative column chromatography was performed using Kanto Chemical silica gel 60 N (spherical, neutral), Fuji Silysia BW- 4:10MH silica gel or YMC_GEL Silica (6 nm I-40−63 μm). Thin layer chromatography (TLC) was carried out on Merck 25 TLC silica gel 60 F254 aluminum sheets. Phosphorescence spectrum was recorded on a spectrofluorometer (Jasco FP-6500) using phosphorescence measurement mode. Cyclic voltammetric measurements were performed using an ALS CHI606S electrochemical analyzer.

Phosphorescence Measurement of 35

Phosphorescence of 4-fluorofuroxan **35** was measured for a 1 mM 2-MeTHF solution of **35** kept in a quartz ESR tube (Φ 5 mm) at 77 K. The delay time, gate time and excitation wavelength were set to be 9 ms, 9 ms and 280 nm, respectively.

Absorption and Fluorescence Spectra

Photoluminescence spectra were recorded on a spectrofluorometer (Jasco FP-6500) with a quartz absorption cuvette (light path: 1 cm). UV–visible spectra were recorded on a Shimadzu UV-1800 spectrometer with a quartz absorption cuvette (light path: 1 cm).

Photosensitized isomerization of 4-furoxan regioisomer to 3-furoxan regioisomer general procedure

A solution of 4-furoxan (2.6 μmol) and sensitizer (2.6 μmol) (1 : 1) in deaerated deuterated solvent (0.5 mL) was prepared in a Pyrex NMR tube. A small quantity of dodecane (approximately 1 μL) was added as an internal standard. Before irradiation of the sample, the integration ratio of the ^{1}H NMR peaks corresponding to the 4-furoxan and dodecane were measured. The solution was irradiated with light (a 300 W xenon lamp, Asahi Spectra MAX-303 equipped with a 300 to 600 nm

ultraviolet−visible module, and a combination of a long-pass and short-pass filters). The reaction progress was monitored by ^{1}H NMR analysis. The yield was determined from the peak integration relative to dodecane internal standard under the assumption that the internal standard was not affected by the experiment or by relative peak integration of pre and post irradiated sample.

Redox property experiments

Cyclic voltammetric measurements were performed at 298 K with solvents being deaerated by Ar bubbling for 30 min before each measurement. The supporting electrolyte was 0.10 M $TBAClO_4$. A conventional three-electrode cell was used with a platinum working electrode and a platinum wire as a counter electrode. The cyclic voltammograms were recorded with respect to the $Ag/AgNO_3$ (10 mM) reference electrode at a sweep rate of 50 mV/s. The oxidation potential (determined as the peak potential) was corrected to the SCE scale by adding 0.31 V, on the basis of the measurement of the redox potential (+0.09 V in the cell system used) of the Fc/Fc+ couple as the internal standard.

Cellular uptake and NO-release experiment with 122

HeLa cells (Riken BioResource Center) were cultured in high glucose DME medium (Sigma-Aldrich) containing 10 %(v/v) FBS, 1 %(v/v) Penicillin-Streptomycin (P/S) (Sigma-Aldrich) at 37 °C in a CO_2 incubator (5% CO_2). Then, the HeLa cells were seeded at a density of 1.5 $\times$ 105 cells/mL in DME medium in a 35 mm glass bottom dish. After 24 hours of culture, the cells were incubated with 10 μM NO550 for 30 min in a CO_2 incubator (5 % CO_2) at 37 °C. After the incubation, the cells were washed thrice with PBS buffer and incubated with 30 μM compound **122** or 30 μM NOC7 (DOJINDO) in DME medium for 30 min. After 30 min of the incubation, the DME medium was removed and the cells were washed thrice with PBS buffer. The cells incubated with **122** were exposed to the light in the wavelength of 400–500 nm for 1 hour to liberate NO and were then washed thrice with PBS buffer. The fluorescence derived from NO550 in the cells was then analysed using a fluorescence microscope (BZ-X700;

KEYENCE) equipped with BZ-X filter GFP and a 40x objective lens with the exposure time of 1 second.

Cellular uptake experiment with 157

HeLa cells (Riken BioResource Center) were cultured in high glucose DME medium (Sigma-Aldrich) containing 10 %(v/v) FBS, 1 %(v/v) Penicillin-Streptomycin (P/S) (Sigma-Aldrich) at 37 °C in a CO_2 incubator (5% CO_2). Then, the HeLa cells were seeded at a density of 1×10^5 cells/mL in DME medium in a 35 mm glass bottom dish. After 24 hours of culture, the cells were incubated with 20 μM **157** for 20 min in a CO_2 incubator (5 % CO_2) at 37 °C. After the incubation, the cells were washed thrice with PBS buffer. The fluorescence derived from **157** in the cells was then analysed using a fluorescence microscope (BZ-X700; KEYENCE) equipped with BZ-X filter GFP and a 40x objective lens with the exposure time of 1/3 second.

Measurement of the NO-releasing ability with light irradiation general procedure

A 100 μM of NO-release compound was prepared as a 10 mL solution of 50 mM phosphate buffer (pH 7.4) containing or omitting 5 mM of cysteine in a pyrex vial. The vial was placed into a 37 °C oil bath and then irradiated. At intervals aliquots (0.5 mL) were taken and treated with Griess reagent (40 μL). After the samples were stood for 30 min, the absorbance of the samples at 520 nm was measured. The percent nitrite (NO_2^-) (mol/mol) was determined from a calibration curve prepared in advance by using $NaNO_2$ standard solutions (20−100 μM) treated with the Griess reagent.

The Griess reagent was prepared by diluting a mixture of 1 g of sulfanilamide, 50 mg of N-naphthylethylenediamine dihydrochloride, and 2.5 mL of 85% phosphoric acid with distilled water to a final volume of 25 mL.

Measurement of the NO-releasing ability without light irradiation general procedure

A 100 μM solution of NO release compound was prepared as a 10 mL solution of 50 mM phosphate buffer (pH 7.4) containing or omitting 5 mM of cysteine in a pyrex vial.

The vial was placed into a 37 °C oil bath. At intervals aliquots (0.5 mL) were taken and treated with Griess reagent (40 μL). After the samples were stood for 30 min, the absorbance of the samples at 520 nm was measured. The percent nitrite (NO_2^-) (mol/mol) was determined from a calibration curve prepared in advance by using $NaNO_2$ standard solutions (20−100 μM) treated with the Griess reagent.

Measurement of the NO-releasing ability of compound 104

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) containing 5 mM cysteine was added to a 10 mM solution of **104** in DMSO. Further DMSO was added to bring the total concentration to 100 μM (10% DMSO). The irradiated experiment used wavelength of 400–500 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 5 h.

Measurement of the NO-releasing ability of compound 122

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) either containing or omitting 5 mM cysteine was added to a 10 mM solution of **122** in DMSO to bring the total concentration to 100 μM (1% DMSO). The irradiated experiment used wavelength of 400–500 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 7 h.

Measurement of the NO-releasing ability of compound 126

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) containing 5 mM cysteine was added to a 10 mM solution of **126** in DMSO to bring the total concentration to 100 μM (1% DMSO). The irradiated experiment used wavelength of 400–500 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 5 h.

Measurement of the NO-releasing ability of compound 145

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) and DMSO was added to a solution of **145** to bring the total concentration to 100 μM (20% DMSO). The irradiated experiment used wavelength of 400–500 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 45 min.

Measurement of the NO-releasing ability of compound 150

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) and DMSO was added to a solution of **150** to bring the total concentration to 100 μM (20% DMSO). The irradiated experiment used wavelength of 400–500 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 30 min.

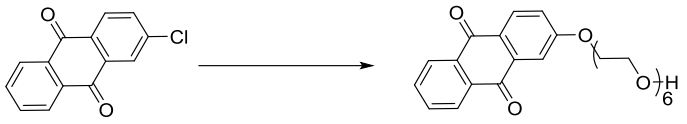
Measurement of the NO-releasing ability of compound 157

As per the general NO-releasing procedures but with the following modifications; 50 mM phosphate buffer solution (pH 7.4) containing or omitting 5 mM cysteine was added to a solution of **157** to bring the total concentration to 100 μM. At intervals two aliquots (0.5 mL) were taken and one treated with Griess reagent (40 μL) and the difference in absorption at 520 nm compared. The irradiated experiment used wavelength of 420–500 nm or 500–600 nm. The non-irradiated control was performed in ambient light. The experiments were performed over 4 h.

2 Studies towards anthraquinone-fluorofuroxan

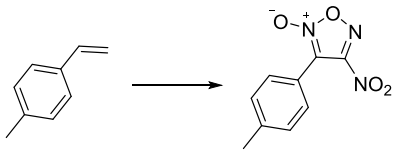
2.2 Nucleophilic aromatic substitution towards compound 50

2-[2-[2-[2-[2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]anthracene-9,10-dione (51)[73]



Compound **51** was synthesized according to the reported method.[73] A mixture of 2-chloroanthraquinone (0.20 g, 0.824 mmol) and K_2CO_3 (0.20 g, 1.45 mmol) were stirred in hexaethyleneglycol (0.75 mL, 3.01 mmol) and heated to 130 °C for 3 h. The reaction was cooled and crude mixture purified by silica chromatography (5% MeOH in CH_2Cl_2) to grant **51** as a yellow solid (136 mg, 0.276 mmol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.32–8.28 (2H, m), 8.27 (1H, apparent d, *J* = 8.8 Hz), 7.82–7.50 (2H, m), 7.74 (1H, apparent d, *J* = 1.4 Hz), 7.31 (1H, dd, *J* = 12, 2.8 Hz), 4.33 (2H, apparent t, *J* = 4.8 Hz), 3.94 (2H, apparent t, *J* = 4.8 Hz), 3.77–3.60 (20H, m), 2.58 (1H, t, 6.4 Hz); δ_C (100 MHz, $CDCl_3$) 183.1, 182.1, 163.6, 135.5, 134.1, 133.7, 133.6, 133.5, 129.7, 127.1, 127.1, 121.6, 110.6, 72.5, 70.9, 70.6, 70.6, 70.6, 70.6, 70.6, 70.5, 70.3, 69.4, 68.1, 65.8, 61.7; IR (neat) 3450, 2883, 1662, 1588, 1578, 1492, 1449, 1345, 1328, 1304, 1237, 1132, 1101, 1087, 1051, 992, 951, 921, 884, 851, 842, 723, 715; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{26}H_{33}O_9$ 489; Found 489; mp 60–62 °C.

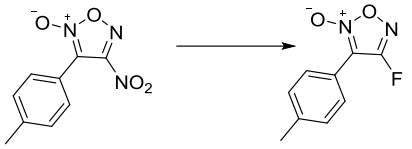
3-(4-Methylphenyl)-4-nitrofuroxan (55)



Compound **55** was synthesized according to the reported method.[113] To a stirred suspension of $NaNO_2$ (105 g, 1.52 mol) in a 0.2 M CH_2Cl_2 solution of 4-methylstyrene (25 mL, 0.19 mol) was added AcOH (86.7 mL, 1.52 mol) over 30 min at rt. After 2 h from starting the AcOH addition, a 2 M solution of HCl (570 mL, 1.14 mol) was added. The reaction mixture was stirred for 14 h at rt, and then extracted thrice with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography (2% EtOAc in hexane), the semi-pure material was then recrystallized in EtOAc/hexane to yield yellow needles (11.4 g, 51.5 mmol, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.50 (2H, apparent d, *J* = 8.4 Hz), 7.37 (2H, apparent d, *J* = 8.4 Hz), 2.45 (3H, s); δ_C (100 MHz, $CDCl_3$) 142.7, 130.0, 128.6, 120.9, 116.3, 109.2, 21.7; IR (neat) 1606, 1557, 1520, 1488, 1362, 1287, 1272, 1120, 1072, 988, 819, 777, 741; LRMS (EI)

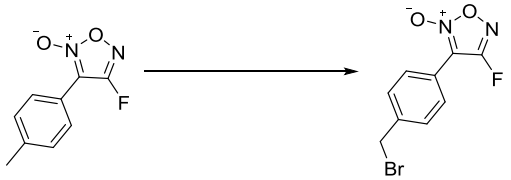
m/z [M – CN_2O_4] Calcd for C_8H_7N 171; found 171. [M]+ was not observed due to facile fragmentation; mp 78–80 °C.

4-Fluoro-3-(4-methylphenyl)furoxan (35)



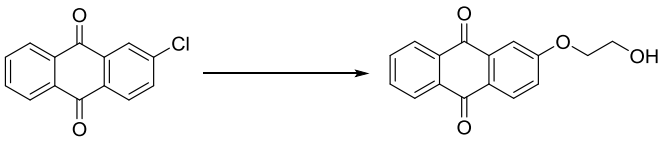
Compound **35** was synthesized according to the reported method.[41] To a solution of 3-(4-methylphenyl)-4-nitrofuroxan (2.00 g, 9.04 mmol) in THF (18 mL) was added dropwise a 1 M solution of TBAF in THF (12.0 mL, 11.8 mmol) at 0 °C. After stirring for 4 h, the reaction was quenched by the addition of saturated aqueous solution of NH_4Cl. The mixture was extracted thrice with Et_2O. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica chromatography (3% EtOAc in hexane) to give compound **35** as a white solid (1.51 g, 7.78 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.86 (2H, d, J = 8 Hz), 7.36 (2H, d, J = 8 Hz), 2.43 (3H, s); δ_C (100 MHz, $CDCl_3$) 162.1 (d, $J_{C\text{-}F}$ = 258.4 Hz), 142.1, 130.1, 125.8 (d, $J_{C\text{-}F}$ = 3.8 Hz), 117.6 (d, $J_{C\text{-}F}$ = 4.2 Hz), 107.6, 21.6; IR (neat) 1663, 1610, 1570, 1520, 1472, 1404, 1340, 1317, 1155, 1124, 1094, 1046, 978, 834, 815, 730, 581; LRMS (DART) *m/z* [M + H]+ Calcd for $C_9H_8FN_2O_2$ 195; Found 195; mp 77–79 °C.

3-(4-Bromomethylphenyl)-4-fluorofuroxan (52)



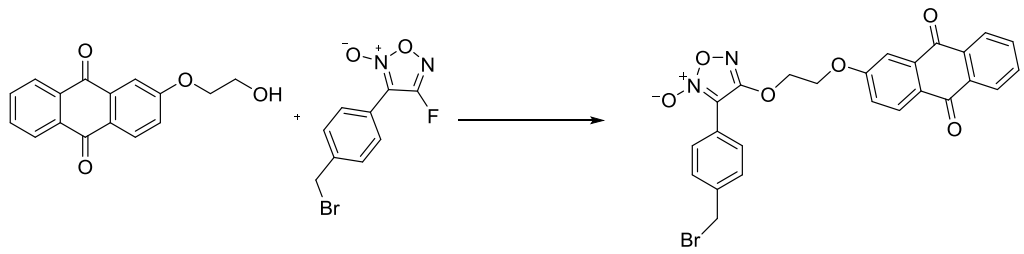
4-Fluoro-3-(4-methylphenyl)furoxan (0.20 g, 1.03 mmol) and NBS (0.24 g, 2.06 mmol) were stirred in CCl_4 at rt. Triethyl borane (62.0 µL of a 1 M solution in THF) was added and the mixture stirred for 5.5 h at rt. The reaction mixture was filtered and washed with 5% EtOAc in hexane solution. The solvent was removed in vacuo and then diluted with water and extracted thrice with Et_2O, dried over $MgSO_4$, filtered and concentrated. The crude mixture was purified by silica chromatography (5% EtOAc in hexane) to yield **52** as a white solid (172 mg, 0.634 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.97 (2H, d, *J* = 8 Hz), 7.58 (2H, apparent d, *J* = 8 Hz), 4.52 (2H, s); δ_C (100 MHz, $CDCl_3$) 161.9 (d, $J_{C\text{-}F}$ = 258.6 Hz), 141.2, 130.0, 126.2 (d, $J_{C\text{-}F}$ = 3.9 Hz), 120.5 (d, $J_{C\text{-}F}$ = 4.1 Hz), 31.9; IR (neat) 1615, 1570, 1523, 1475, 1411, 1345, 1319, 1228, 1196, 1155, 1131, 1098, 1090, 990, 835, 812, 741, 730, 637, 601; LRMS (DART) *m/z* [M + H]+ Calcd for $C_9H_7BrFN_2O_2$ 273; Found 273; mp 110–113 °C.

(2-Hydroxyethoxy)anthracene-9,10-dione (58)



A suspension of 2-chloroanthraquinone (2.00 g, 8.24 mmol) and K_2CO_3 (1.99 g, 14.4 mmol) were stirred in 25 mL of ethyleneglycol and heated to 140 °C for 8 h. The reaction mixture was diluted with water and extracted thrice with EtOAc, the organics were dried over Na_2SO_4, filtered and solvent removed in vacuo. The crude material was pre-bound to silica and purified by silica chromatography (30% EtOAc in hexane–50% EtOAc in hexane) to yield **58** as a yellow solid (770 mg, 2.85 mmol, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.32–8.28 (2H, m), 8.28 (1H, d, *J* = 8.8Hz), 7.83–7.75 (2H, m), 7.75 (1H, d, 2.8 Hz), 4.29 (2H, apparent t, *J* = 5.0 Hz), 4.06 (2H, apparent q, 5.0 Hz), 2.03 (1H, t, *J* = 6.4 Hz); δ_C (100 MHz, $CDCl_3$) 183.2, 182.1, 163.4, 135.6, 134.2, 133.8, 133.6, 133.5, 129.9, 127.4, 127.2, 121.5, 110.6, 69.9, 61.2; IR (neat) 3473, 1667, 1585, 1493, 1436, 1327, 1243, 1175, 1153, 1103, 1079, 1040, 931, 903, 842, 803, 712, 661; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{13}O_4$ 269; Found 269; mp 168–171 °C.

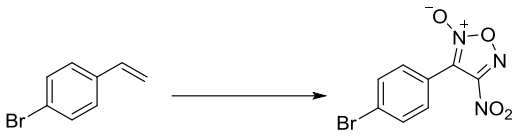
2-[2-({4-[4-(Bromomethyl)phenyl]furoxan-3-yl}oxy)ethoxy]anthracene-9,10-dione (60)



NaH (60% in mineral oil) (16.3 mg, 0.407 mmol) was added to a solution of compound **58** (0.10 g, 0.37 mmol) in THF (1 mL) at -78 °C and was stirred for 1 h. The deprotonated material formed a brown suspension which was added dropwise to a solution of **52** (100 mg, 0.37 mmol) in THF (1.5 mL). The reaction mixture allowed to warm to rt and stirred for 16 h. The mixture was diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude mixture was purified by silica chromatography ($CHCl_3$) to yield **60** as a yellow solid (145 mg, 0.278 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.33–8.29 (3H, m), 8.80 (2H, apparent d, *J* = 8.6 Hz), 7.84–7.77 (3H, m), 7.48 (2H, apparent d, 8.6 Hz), 7.34 (1H, dd, *J* = 8, 2.8 Hz), 4.95–4.93 (2H, m), 4.66–4.64 (2H, m), 4.45 (2H, s); δ_C (100 MHz, DMSO-d_6) 182.3, 181.4, 163.0, 162.0, 140.6, 135.1, 134.7, 134.3, 133.1, 129.8, 129.6, 126.8, 126.7, 126.5, 121.7, 121.5, 110.9, 107.5, 69.3, 66.4, 33.3; IR (neat) 1665, 1590, 1551, 1519, 1471, 1408, 1325, 1292, 1240, 1214, 1169, 1150, 1086, 1007, 928, 849, 841, 724, 590; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{25}H_{18}BrN_2O_6$ 521; Found 521; mp 203–206 °C.

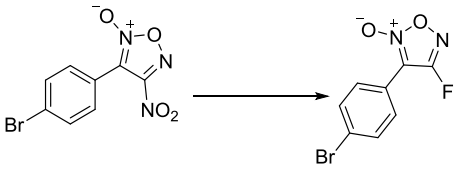
2.3 Palladium catalysis towards compound 61

4-(4-Bromophenyl)-3-nitrofuroxan (65)



Compound **65** was synthesized according to the reported method.[113] To a stirred suspension of $NaNO_2$ (1.51 g, 21.9 mmol) in a 0.2 M CH_2Cl_2 solution of 4-bromostyrene (0.36 mL, 2.73 mmol) was added AcOH (1.25 mL, 21.9 mmol) over 30 min at rt. After 2 h from starting the AcOH addition, a 2 M solution of HCl (8.2 mL, 16.4 mmol) was added. The reaction mixture was stirred for 18 h at rt and then diluted with water and extracted thrice with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo and the residue was purified by silica chromatography (4% EtOAc in hexane), the semi-pure material was then recrystallized in EtOAc/hexane to yield **65** as a yellow oil (0.37 g, 1.18 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.72 (2H, apparent d, *J* = 8.6 Hz), 7.49 (2H, apparent d, *J* = 8.6 Hz); δ_C (100 MHz, $CDCl_3$) 157.7, 132.7, 130.3, 126.8, 118.3, 108.7; IR (neat) 3104, 1633, 1586, 1563, 1514, 1486, 1401, 1327, 1330, 1262, 1202, 1182, 1110, 1071, 1009, 967, 940, 811, 742, 725; LRMS (EI) *m*/*z* [M – CN_2O_4]+ Calcd for C_7H_4BrN 181; found 181. [M]+ was not observed due to facile fragmentation; mp 145–147 °C.

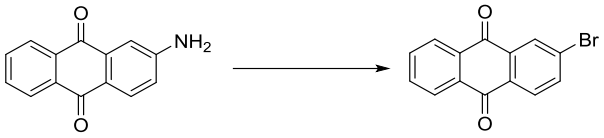
4-(4-Bromophenyl)-3-fluorofuroxan (64)



Compound **64** was synthesized according to the reported method.[41] To a solution of 3-(4-bromophenyl)-4-nitrofuroxan (99.2 mg, 0.348 mmol) in THF (0.2 mL) was added dropwise a 1 M solution of tetrabutylammonium fluoride in THF (386 µL, 0.386 mmol) at 0 °C. After stirring for 5 h, the reaction was quenched by the addition of saturated aqueous solution of NH_4Cl. The mixture was extracted thrice with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica chromatography (5% EtOAc in hexane) to give **64** as an orange solid (57.2 mg, 0.204 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.87 (2H, apparent d, *J* = 8.4 Hz), 7.70 (2H, apparent d, *J* = 8.4 Hz); δ_C (100 MHz, $CDCl_3$) 161.7 (d, $J_{C\text{-}F}$ = 258.6 Hz), 132.8, 127.1 (d, $J_{C\text{-}F}$ = 4.0 Hz), 125.9, 119.5 (d, $J_{C\text{-}F}$ = 4.2 Hz), 105.4

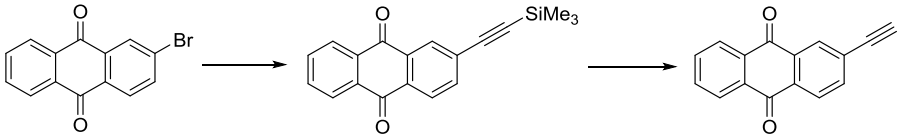
(d, $J_{C\text{-}F}$ = 28.8 Hz); IR (neat) 3102, 1614, 1587, 1569, 1498, 1465, 1396, 1338, 1308, 1155, 1116, 1073, 1008, 975, 823, 750, 636; LRMS (DART) *m/z* [M + H]+ Calcd for $C_8H_5BrFN_2O_2$ 259; Found 259; mp 94–96 °C.

2-Bromoanthracene-9,10-dione (70)



Compound **70** was synthesized according to the reported method.[75] To a solution of $CuBr_2$ (7.26 g, 32.5 mmol) and *tert*-butylnitrite (3.35 g, 32.5 mmol) in MeCN (50 mL) was added dropwise a suspension of 2-aminoanthraquinone (2.90 g, 13.0 mmol) in THF (110 mL) and the reaction was stirred at rt for 20 h. Upon completion the mixture was diluted with water and extracted thrice with $CHCl_3$, the organics were washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated and purified by silica chromatography (1 : 1 CH_2Cl_2/hexane) to yield **70** as a yellow solid (1.67 g, 5.83 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.45 (1H, s), 8.33–8.31 (2H, m), 8.19 (1H, apparent d, *J* = 8.4 Hz), 7.93 (1H, apparent d, *J* = 8Hz), 7.84–7.82 (2H, m); δ_C (100 MHz, DMSO-d_6) 182.3, 181.9, 137.6, 135.3, 135.2, 134.9, 133.4, 133.2, 132.4, 129.5, 129.5, 129.1, 127.3, 127.3; IR (neat) 3084, 2921, 1676, 1575, 1401, 1317, 1287, 1257, 1163, 1068, 958, 929, 913, 897, 853, 806, 706, 695, 643; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{14}H_8BrO_2$ 287; Found 287; mp 207–210 °C.

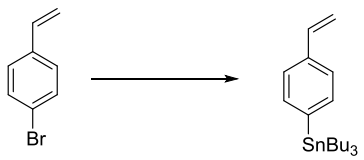
2-Ethynylanthracene-9,10-dione (68)



Compound **68** was synthesized according to the reported method.[114] To a solution of 2-bromoanthraquinone (1.50 g, 5.22 mmol) and PPh_3PdCl_2 (36.6 mg, 52.0 µmol) in 1 : 1 THF/Et_3N (17.4 mL) was added CuI (19.8 mg, 0.10 mmol) and stirred at 60 °C for 15 min. Trimethylsilylacetylene (546 mg, 5.74 mmol) was added dropwise and the reaction stirred for 17 h. The reaction mixture was cooled, filtered through Celite and washed with 4 : 1 $CHCl_3$/hex. The filtrate was washed with water, 1 M HCl and then

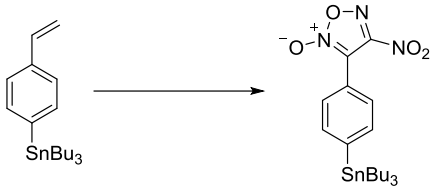
dried over $MgSO_4$, filtered and solvent removed in vacuo to yield a crude yellow solid (1.74 g). The solid was dissolved in 2 : 1 acetone/ethanol and NaOH (210 mg, 5.22 mmol) was added, then the reaction mixture stirred at rt for 90 min. The mixture was diluted with water and extracted thrice with $CHCl_3$ and organics dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude solid was purified by silica chromatography (20% hexane in $CHCl_3$) to yield **68** (858 mg, 3.69 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.42 (1H, d, *J* = 1.6 Hz), 8.35–8.31 (2H, m), 8.23 (1H, d, *J* = 11.2 Hz), 7.88 (1H, dd, *J* = 8, 1.6 Hz), 7.85–7.80 (2H, m), 3.37 (1H, s); δ_C (100 MHz, $CDCl_3$) 182.4, 182.4, 137.1, 134.4, 134.3, 133.4, 133.4, 133.4, 132.9, 130.9, 128.3, 127.4, 127.3, 127.3, 82.0, 81.9; IR (neat) 3266, 1673, 1588, 1407, 1329, 1318, 1285, 1172, 1135, 981, 932, 858, 720, 707, 651; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{16}H_9O_2$ 233; Found 233; mp 200 °C (decomposed).

4-Tributylstannylstyrene (75)



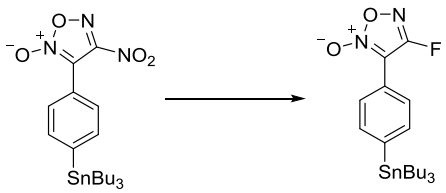
Compound **75** was synthesized according to the reported method.[78] To a suspension of magnesium turnings (329 mg, 13.7 mmol) in THF (11 mL) was added tributyltinchloride (1.49 mL, 5.49 mmol) and 4-bromostyrene (714 µL, 5.49 mmol) and the mixture sonicated at rt for 2 hours. The mixture was filtered, diluted with water and extracted thrice with CH_2Cl_2. The organics were washed with brine, dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude mixture was purified by silica chromatography (hexane) to yield **75** as a colourless oil (1.73 g, 4.38 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.43 (2H, apparent d, *J* = 8.0 Hz), 7.36 (2H, apparent d, *J* = 8.0 Hz), 6.70 (1H, dd, *J* = 17.6, 10.8 Hz), 5.76 (1H, dd, *J* = 17.6, 0.8 Hz), 5.22 (1H, dd, *J* = 10.8, 0.8 Hz), 1.60–1.50 (6H, m), 1.37–1.22 (6H, m), 1.13–0.96 (6H, m), 0.883 (9H, t, *J* = 7.2 Hz); δ_C (100 MHz, $CDCl_3$) 141.9, 137.2, 137.1, 136.7 ($J_{Sn\text{-}C}$ = 45.5 Hz), 125.7 ($J_{Sn\text{-}C}$ = 20.7 Hz), 113.6, 29.1 ($J_{Sn\text{-}C}$ = 10.0 Hz), 27.4 ($J_{Sn\text{-}C}$ = 28.3 Hz), 13.7, 9.6 ($J_{Sn\text{-}C}$ = 165.4, 7.4 Hz); IR (neat) 2955, 2922, 2870, 2851, 1627, 1462, 1455, 1417, 1386, 1376, 1338, 1290, 1247, 1182, 1149, 1071, 987, 960, 904, 873, 865, 822, 688, 662, 582; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{20}H_{35}Sn$ 395; Found 395.

4-Nitro-3-[4-(tributylstannyl)phenyl]furoxan (74)



Compound **74** was synthesized according to the reported method.[113] To a stirred suspension of $NaNO_2$ (30.8 g, 0.45 mol) in a 0.2 M CH_2Cl_2 solution of 4-tetrabutylstannylstyrene (22.0 g, 55.8 mol) was added AcOH (25.5 mL, 0.45 mol) over 30 min at rt. Two hours after starting the AcOH addition, a 2 M solution of HCl (100 mL, 0.34 mol) was added. The reaction mixture was stirred for 18 h at rt, then diluted with water and extracted thrice with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica chromatography (25% benzene in hexane) to yield **74** as a yellow oil (11.8 g, 23.7 mmol, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.65 (2H, apparent d, J = 8.2 Hz), 7.51 (2H, apparent d, J = 8.2 Hz), 1.65–1.45 (6H, m), 1.34 (6H, sex, J = 7.6 Hz), 1.19–1.02 (6H, m), 0.895 (9H, apparent t, J = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 149.1, 137.1 ($J_{Sn\text{-}C}$ = 14.8 Hz), 127.3 ($J_{Sn\text{-}C}$ = 18.7), 123.1, 118.6, 109.2, 29.0 ($J_{Sn\text{-}C}$ = 10.2 Hz), 27.4 ($J_{Sn\text{-}C}$ = 28.8 Hz), 13.7, 9.7 ($J_{Sn\text{-}C}$ = 163.6, 7.5 Hz).

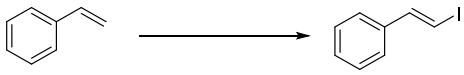
4-Fluoro-3-[4-(tributylstannyl)phenyl]furoxan (73)



Compound **73** was synthesized according to the reported method.[41] To a solution of 3-(4-tributylstanylphenyl)-4-nitrofuroxan (300 mg, 0.60 mmol) in THF (1.2 mL) was added dropwise a 1 M solution of TBAF in THF (600 µL, 0.60 mmol) at 0 °C and was stirred for 1 h. The reaction was quenched by the addition of saturated aqueous solution of NH_4Cl and extracted thrice with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (10% benzene in hexane) to yield **73** as a yellow oil (232 mg, 0.494 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.81 (2H, apparent dd, J =

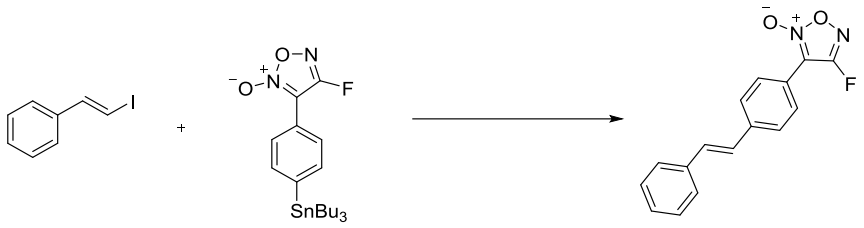
8.4, 1.2 Hz), 7.64 (2H, apparent d, *J* = 8.4 Hz), 1.634–1.448 (6H, m), 1.34 (6H, sex, *J* = 7.4 Hz), 1.19–1.02 (6H, m), 0.89 (9H, apparent t, *J* = 7.4 Hz); δ_C (100 MHz, $CDCl_3$) 162.3 (d, $J_{C\text{-}F}$ = 308.6 Hz), 148.3, 137.3 ($J_{C\text{-}Sn}$ = 14.7 Hz), 124.6 (d, $J_{C\text{-}F}$ = 3.6 Hz, $J_{C\text{-}Sn}$ = 28.5 Hz), 119.8 (d, $J_{C\text{-}F}$ = 4.1 Hz), 106.0 (d, $J_{C\text{-}F}$ = 26.5 Hz), 29.0 ($J_{C\text{-}Sn}$ = 10.3 Hz), 27.3 ($J_{C\text{-}Sn}$ = 28.5 Hz), 11.4, 8.1 ($J_{C\text{-}Sn}$ = 7.6 Hz); LRMS (DART) m/z [M – F + H]+ Calcd for $C_{20}H_{32}N_2O_2Sn$ 452; Found 452. [M + H]+ was not observed due to facile fragmentation.

(E)-(2-Iodovinyl)benzene (77)



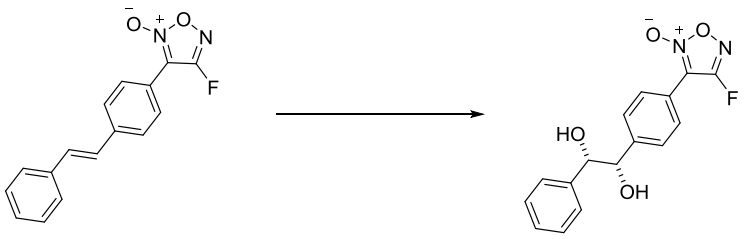
Compound **77** was synthesized according to the reported method.[115] To a stirred solution of $RuH(CO)Cl(PPh_3)_3$ (267 mg, 0.28 mmol) in toluene (100 mL) was added styrene (3.31 mL, 28.8 mmol) and trimethylvinylsilane (4.15 mL, 28.8 mmol) and the reaction heated to 100 °C for 6 h. The reaction was cooled to rt and MeCN (400 mL) was added followed by *N*-iodosuccinamide (7.78 g, 34.6 mmol) and the reaction stirred at rt for 1 hour. The solvent was removed in vacuo and crude material purified by silica column chromatography (hexane) to yield **77** (3.28 g, 14.2 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.43 (1H, d, *J* = 14.8 Hz), 7.35–7.26 (5H, m), 6.83 (1H, d, *J* = 14.8 Hz); δ_C (100 MHz, $CDCl_3$) 145.0, 137.7, 128.7, 128.4, 126.0, 76.7; IR (neat) 3057, 3045, 3021, 1595, 1568, 1494, 1444, 1299, 1277, 1210, 1169, 1028, 944, 791, 725, 687, 548; LRMS (DART) *m/z* [M + H]+ Calcd for C_8H_8I 231; Found 231.

4-Fluoro-3-{4-[(*E*)-2-phenylethenyl]phenyl}furoxan (78)



CuI (189 mg, 0.62 mmol), $Pd_2(dba)_3$ (142 mg, 0.16 mmol), LiCl (527 mg, 12.4 mmol) and **73** (2.92g, 6.22 mmol) were placed into a flame dried flask which was then evacuated and refilled with argon thrice. HMPA (17.2 mL) followed by **77** (1.72 g, 7.06 mmol) were added and the reaction stirred and heated to 70 °C for 90 min. The mixture was cooled, filtered and purified by chromatography (10% w/w K_2CO_3 silica gel) (35% $CHCl_3$ in hexane) to yield **78** as pale-yellow crystals (1.17 g, 4.14 mmol, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.98 (2H, d, *J* = 8.0 Hz), 7.68 (2H, apparent d, *J* = 8.4 Hz), 7.68 (2H, apparent d, *J* = 8.4 Hz), 7.39 (2H, apparent t, *J* = 7.4 Hz), 7.31 (1H, apparent tt, *J* = 7.4, 1.2 Hz), 7.24 (1H, d, *J* = 16.0 Hz), 7.13 (1H, d, *J* = 16.0 Hz); δ_C (100 MHz, $CDCl_3$) 162.0 (d, $J_{C\text{-}F}$ = 258.5 Hz), 140.4, 136.5, 131.5, 128.8, 128.4, 127.2, 127.0, 126.8, 126.1, 126.1, 119.1 (d, $J_{C\text{-}F}$ = 4.3), 105.9 (d, $J_{C\text{-}F}$ = 26.6 Hz); IR (neat) 1611, 1601, 1522, 1476, 1448, 1411, 1337, 1308, 1154, 1113, 1094, 970, 956, 872, 837, 823, 758, 722, 692, 633; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{12}FN_2O_2$ 283; Found 283; mp 169–172 °C.

1-[4-(4-Fluorofuroxan-3-yl)phenyl]-2-phenylethane-1,2-diol (79)



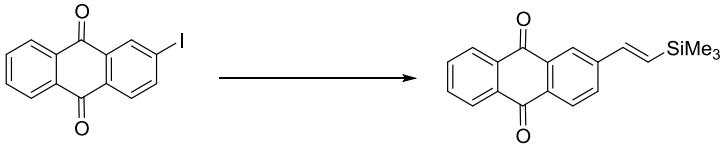
To a stirred solution of **78** (100 mg, 0.35 mmol) in a mixture of acetone (3.2 mL) and water (0.35 mL) was added NMO (62.2 mg, 0.53 mmol) followed by OsO_4 (0.04 M in ^{t}BuOH, 0.44 mL, 17.7 µmol) and stirred at 28 °C for 15 h. The mixture was quenched with aqueous Na_2SO_3 and extracted thrice with EtOAc. The organics were washed with brine, dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude solid was purified by silica chromatography (5% MeOH in $CHCl_3$) to yield **79** as a white solid (94.6 mg, 0.30 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.84 (2H, apparent d, *J* = 8.0 Hz), 7.30–7.25 (5H, m), 7.14–7.11 (2H, m), 4.81 (1H, dd, *J* = 7.8, 2.2 Hz), 4.68 (1H, dd,

J = 7.8, 3.0 Hz). 3.09 (1H, d, *J* = 2.2 Hz), 2.71 (1H, d, *J* = 3.0 Hz); δ_C (100 MHz, DMSO-d_6) 161.8 (d, $J_{C\text{-}F}$ = 256.3 Hz), 145.6, 141.4, 127.4, 126.7, 126.5, 126.2, 124.5 (d, $J_{C\text{-}F}$ = 3.1 Hz), 118.0 (d, $J_{C\text{-}F}$ = 27.3 Hz), 106.2 (d, $J_{C\text{-}F}$ = 27.3 Hz), 76.5, 76.3; IR (neat) 3407, 3212, 1668, 1615, 1574, 1521, 1474, 1449, 1410, 1383, 1342, 1300, 1248, 1225, 1197, 1155, 1087, 1068, 1042, 1029, 835, 820, 776, 709, 695; LRMS (DART) m/z [M − OH + H]+ Calcd for $C_{16}H_{13}N_2FN_2O_3$ 300; Found 300. [M + H]+ was not observed due to facile fragmentation; mp 156–158 °C.

2-Iodoanthracene-9,10-dione (82)

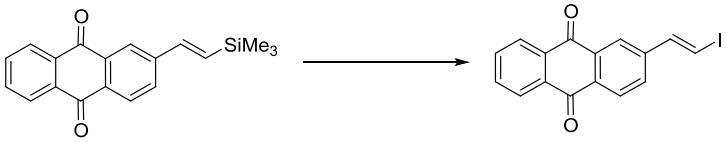
Compound **82** was synthesized according to the reported method.[75] To a stirred solution of 2-aminoanthraquinone in concentrated HCl (10 mL) was added a solution of NaI (4.94 g, 33.0 mmol) in water (40 mL) dropwise at 0 °C and allowed to warm to rt over 2 h. The reaction was then heated to 50 °C for 15 h. Upon completion the mixture was diluted with aqueous NH_4Cl and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude solid was purified by silica chromatography (CH_2Cl_2/hexane 1 : 1) to yield **82** as a yellow solid (1.48 g, 4.43 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.66 (1H, d, *J* = 1.6 Hz), 8.34–8.28 (2 H, m), 8.16 (1H, dd, *J* = 8.6, 1.8 Hz), 8.01 (1H, d, *J* = 8.6 Hz), 7.85–7.806 (2H, m); δ_C (100 MHz, DMSO-d_6) 181.5, 180.8, 142.4, 134.4, 134.1, 134.0, 133.1, 132.3, 132.0, 131.5, 127.8, 126.2, 126.1, 102.7; IR (neat) 3080, 1672, 1567, 1397, 1325, 1315, 1280, 1163, 1096, 1062, 952, 927, 915, 848, 801, 706, 689, 705, 689, 650, 639, 630; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{14}H_8IO_2$ 335; Found 335; mp 178–181 °C.

(E)-2-(2-(Trimethylsilyl)vinyl)anthracene-9,10-dione (80)



Compound **80** was synthesized according to the reported method.[116] 2-Iodoanthraquinone (400 mg, 1.20 mmol), tetrabutylammonium acetate (721 mg, 2.39 mmol), $Pd(OAc)_2$ (13.4 mg, 60.0 µmol) were added to the reaction vessel which was then evacuated and refilled with argon thrice. DMF (3 mL) was added followed by vinyltrimethylsilane (208 µL, 1.44 mmol) and the reaction stirred at rt for 16 h. The reaction mixture was diluted with $CHCl_3$ and washed with 10% aqueous NH_4Cl thrice, organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (20% hexane in CH_2Cl_2) to yield **80** as a yellow solid with minor inseparable impurities present (327 mg, 1.07 mmol, 89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.34–8.30 (3H, m), 8.27 (1H, d, *J* = 8.0 Hz), 7.83–7.87 (3H, m), 7.01 (1H, d, *J* = 19.2 Hz), 6.83 (1H, d, *J* = 19.2 Hz), 0.21 (9H, s); δ_C (100 MHz, $CDCl_3$) 183.4, 182.9, 144.0, 141.7, 136.0, 134.3, 134.1, 133.9, 133.8, 132.6, 131.6, 128.0, 127.4, 127.3, 125.0, -1.3; IR (neat) 2954, 2894, 1675, 1588, 1326, 1289, 1245, 1205, 1174, 982, 933, 834, 744, 729, 704; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{19}H_{19}O_2Si$ 307; Found 307; mp 90–93 °C.

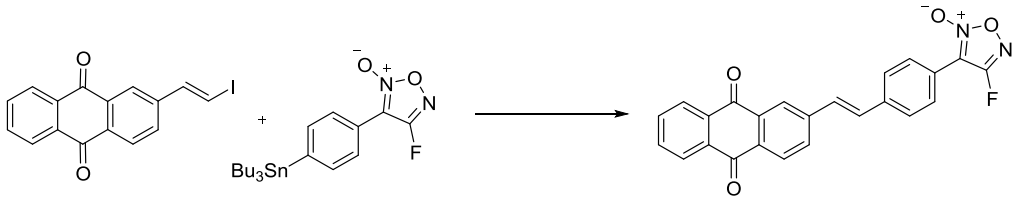
2-[(*E*)-2-Iodoethenyl]anthracene-9,10-dione (81)



To a suspension of **80** (100 mg, 0.33 mmol) in MeCN (3 mL) was added iodine monochloride (19.7 µL, 0.39 mmol) dropwise at 0 °C. To the mixture was added CH_2Cl_2 (3 mL) and allowed to warm to rt over 2 h. The reaction was quenched by aqueous Na_2SO_3, diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was bound to silica and purified by silica chromatography (CH_2Cl_2/hexane 1 : 1) to yield **81** as yellow solid (103 mg, 0.286 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.35–8.30 (2H, m), 8.28 (1H, d, *J* = 8.4 Hz), 8.23 (1H, apparent d, *J* = 1.6 Hz), 7.84–7.79 (2H, m), 7.70 (1H, dd, *J* = 8.4, 1.6 Hz), 7.59 (1H, d, *J* = 15.0 Hz), 7.30 (1H, d, *J* = 15.0 Hz); δ_C (100 MHz,DMSO-d_6) 181.7,

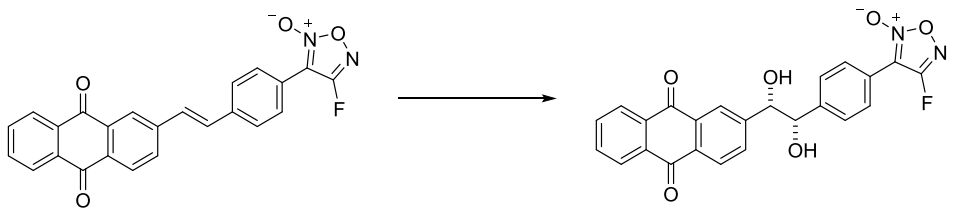
181.3, 142.2, 142.1, 134.0, 133.9, 132.9, 132.5, 131.5, 130.6, 126.7, 126.2, 126.1, 123.7, 86.1; IR (neat) 3063, 3051, 1670, 1587, 1421, 1326, 1288, 1209, 1175, 1159, 1142, 990, 977, 944, 932, 905, 855, 796, 771, 705, 657, 635; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{10}IO_2$ 361; Found 361; mp 222-225 °C

2-{(*E*)-2-[4-(4-Fluorofuroxan-3-yl)phenyl]ethenyl}anthracene-9,10-dione (62)



Compound **81** (50.0 mg, 0.138 mmol), $Pd_2(dba)_3$ (3.16 mg, 3.45 µmol) and LiCl (10.7 mg, 0.26 mmol) were added to the reaction vessel which was then evacuated and filled with argon thrice. Compound **73** (59.3 mg, 0.13 mmol) was added followed by HMPA (1.4 mL) and the reaction heated to 70 °C for 90 min. The crude mixture was filtered through a plug of 10% K_2CO_3 w/w silica with EtOAc. The filtrate was washed with 1 : 1 brine/water thrice and organics dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica PTLC (CH_2Cl_2) to yield **62** as a yellow solid (6.20 mg, 15.0 µmol, 12%) which contained inseparable impurities. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.48 (1H, apparent d, *J* = 1.2 Hz), 8.36–8.33 (3H, m), 8.04 (2H, d, *J* = 8.4 Hz), 7.93 (1H, dd, *J* = 8, 2 Hz), 7.85–7.81 (2, m), 7.76 (2H, d, *J* = 8.4 Hz), 7.39 (2H, d, J = 12.4 Hz); IR (neat) 2962, 1670, 1609, 1587, 1520, 1475, 1410, 1328, 1303, 1294, 1260, 1233, 1178, 1157, 1092, 1047, 1018, 980, 963, 932, 848, 709, 658, 642; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{24}H_{14}FN_2O_4$ 413; Found 413; mp 283 °C (decomposed).

2-{2-[4-(4-fluorofuroxan-3-yl)phenyl]-1,2-dihydroxyethyl}anthracene-9,10-dione (61)

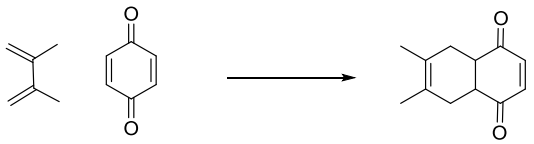


Compound **62** (10.0 mg, 24.0 µmol), NMO (7.50 µL, 36.0 µmol) and OsO_4 (0.04 M in ^{t}BuOH, 60.0 µL, 2.40 µmol) were added to the reaction vessel followed by ^{t}BuOH (0.6 mL), CH_2Cl_2 (1.2 mL) and H_2O and the reaction stirred at 35 °C for 36 h. The reaction

was quenched with aq. Na_2SO_3, diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was purified by silica PTLC (5% MeOH, 1% Et_3N in $CHCl_3$) to yield an insoluble white solid (1.90 mg, 4.25 µmol, 18%). Due to the insoluble nature of the compound, the obtainable data is tentatively assigned as a mixture of 3- and 4-fluorofuroxan isomers. Discernible major 4-fluorofuroxan isomer peaks are reported. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.32–8.28 (2H, m), 8.22–8.18 (1H, m), 8.11 (1H, apparent d, *J* = 1.6 Hz), 7.88 (2H, d, *J* = 8.2 Hz), 7.83–7.79 (2H, m), 7.54 (1H, dd, *J* = 8 Hz, 1.6 Hz), 7.35 (2H, d, *J* = 8.2 Hz), 4.92 (1H, d, *J* = 7.0 Hz), 4.87 (1H, d, *J* = 7.0 Hz); ^{19}F -117.4, -162.1.

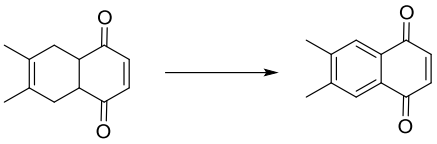
2.4 Organocatalyzed [4 + 2] reaction towards compounds 104, 122 and 126 and evaluation of NO releasing abilities

6,7-Dimethyl-4a,5,8,8a-tetrahydronaphthalene-1,4-dione (94).



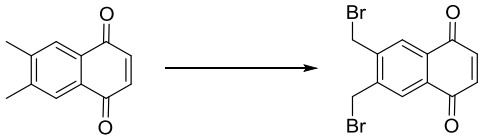
To a stirred solution of 1,4-benzoquinone (10.0 g, 92.5 mmol) and $BF_3 \cdot OEt_2$ (1.14 mL, 9.25 mmol) in toluene was added 2,3-dimethyl-1,3- butadiene (12.6 mL, 111 mmol) dropwise at −16 °C over 10 min. After addition completion the reaction was allowed to warm to rt over 80 min. Upon completion the mixture was washed twice with brine and the organics dried over Na_2SO_4, filtered and solvent condensed under vacuum. The product was recrystallized from toluene to yield **94** as yellow crystals (13.3 g, 70.0 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 6.65 (2H, s), 3.22−3.17 (2H, m), 2.40 (2H, apparent d, *J* = 16.9 Hz), 2.08 (2H, apparent d, *J* = 16.9 Hz), 1.63 (6H, s); δ_C (100 MHz, $CDCl_3$) 200.3, 139.3, 123.3, 47.1, 30.3, 18.9; IR (neat) 2919, 2876, 2828, 1679, 1602, 1441, 1427, 1373, 1354, 1262, 1196, 1095, 887, 864, 848, 805, 749, 682 cm^{-1}; HRMS (DART) m/z [M + H]+ Calcd for $C_{12}H_{15}O_2$ 191.1067; Found 191.1072; mp 119−121 °C.

6,7-Dimethylnaphthalene-1,4-dione (93)



Naphthoquinone **93** was synthesized according to the reported method.[87] To a stirred solution of **94** (3.54 g, 18.6 mmol) in toluene (140 mL) was added portion wise activated MnO_2 (17.4 g, 200 mmol) and refluxed for 2 h. The reaction mixture was cooled and filtered through Celite by washing with EtOH. The solvent was condensed and crude material purified by column chromatography to yield **93** as a yellow solid (2.42 g, 12.7 mmol, 68%). R_f = 0.30 (5% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.83 (2H, s), 6.90 (2H, s), 2.41 (6H, s); δ_C (100 MHz, $CDCl_3$) 185.3, 143.8, 138.5, 129.9, 127.4, 20.2; IR (neat) 2948, 2920, 1664, 1595, 1447, 1392, 1341, 1326, 1307, 1221, 1144, 1057, 1017, 994, 972, 835, 769, 712, 626 cm^{-1}; HRMS (DART) m/z [M + H]+ Calcd for $C_{12}H_{11}O_2$ 187.0754; Found 187.0759; mp 121−123 °C.

6,7-bis(bromomethyl)naphthalene-1,4-dione (92)

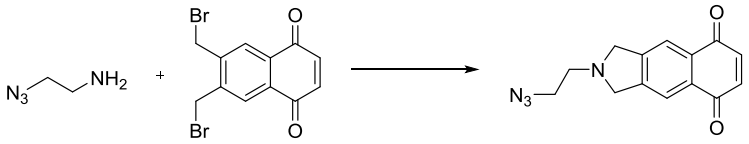


A stirred solution of **93** (4.50 g, 24.2 mmol), NBS (10.8 g, 60.5 mmol) and benzoyl peroxide (293 mg, 1.21 mmol) in benzene (242 mL) was heated to reflux for 22 h. Upon reaction completion the mixture was washed twice with distilled water, the organic layer was dried over $MgSO_4$, filtered and solvent condensed in vacuo. The crude residue was recrystallized from a minimum of hot benzene twice to yield **92** as a semi-crystalline yellow solid (4.33 g, 12.6 mmol, 52%). Minor impurities were observed. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.08 (2H, s), 7.01 (2H, s), 4.71 (4H, s); δ_C (100 MHz, $CDCl_3$) 183.9, 142.7, 138.8, 132.0, 129.1, 28.0; IR (neat) 3035, 1659, 1599, 1456, 1333, 1309, 1221, 1209, 1171, 1144, 1051, 945, 849, 769, 711, 644, 628, 605; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{12}H_9{}^{79}Br^{81}BrO_2$ 345; Found 345; mp 173–176 °C.

2-Azidoethanamine (98)

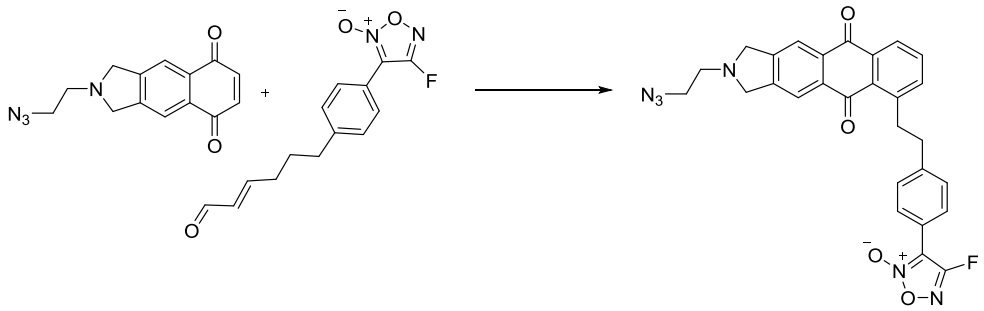
2-Chloroethylamine hydrochloride (5.00 g, 43.1 mmol) and NaN_3 (8.41 g, 129 mmol) were stirred in water (48 mL) at 80 °C for 15 h. The reaction mixture was basified with KOH and extracted with Et_2O 5 times. The organics were washed with brine, dried over $MgSO_4$, filtered and solvent removed in vacuo to yield a colourless oil (2.95 g, 34.2 mmol, 79%). Compound **98** was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 3.37 (2H, t, *J* = 5.6 Hz), 2.88 (2H, t, *J* = 5.6 Hz); δ_C (100 MHz, $CDCl_3$) 54.6, 41.3; IR (neat) 3368, 2936, 2865, 2092, 1595, 1446, 1344, 1285, 1094, 1038, 904, 855, 801, 628.

2-(2-azidoethyl)-2,3-dihydro-1*H*-benzo[*f*]isoindole-5,8-dione (86)



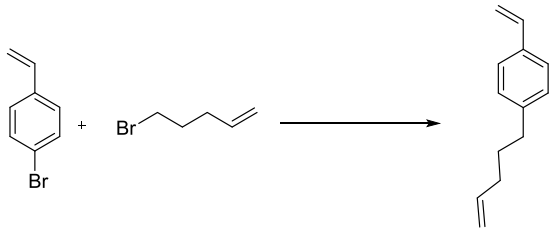
Compound **92** (50.0 mg, 0.145 mmol), K_2CO_3 (91.0 mg, 0.66 mmol) and KI (11.0 mg, 66.0 µmol) were stirred in MeCN (60 mL) and sparged with argon for 15 min. The reaction mixture was then cooled to 0 °C, compound **98** (20.2 µL, 0.132 mmol) was added and the mixture stirred for 18 h. Upon reaction completion the reaction mixture was concentrated, diluted with water and extracted thrice with CH_2Cl_2. The combined organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was purified by silica PTLC (EtOAc/hexane 1 : 1) to yield **86** as an orange solid (9.90 mg, 36.9 µmol, 25%). Compound **86** should be used immediately in the next step as it is unstable and prone to spontaneous oxidation. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.92 (2H, s), 6.96 (2H, s), 4.13 (4H, s), 3.51 (2H, t, *J* = 6.0 Hz), 3.02 (2H, t, *J* = 6.0 Hz); δ_C (100 MHz, $CDCl_3$) 185.0, 146.4, 138.6, 131.6, 120.4, 58.9, 54.4, 50.0; IR (neat) 2926, 2887, 2807, 2104, 1660, 1607, 1451, 1327, 1303, 1268, 1191, 1154, 1130, 1047, 972, 933, 840, 644; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{14}H_{13}N_4O_2$ 269; Found 269; mp 87 °C (decomposed).

2-(2-azidoethyl)-6-{2-[4-(4-fluorofuroxan-3-yl)phenyl]ethyl}-2,3-dihydro-1*H*-naphtho[2,3-*f*]isoindole-5,10-dione (85)



Compound **85** was synthesized according to the reported method.[84] A mixture of **86** (90.0 mg, 0.335 mmol), **87** (148 mg, 0.537 mmol), L- proline (7.70 mg, 67.0 µmol) and benzoic acid (3.81 mg, 34.0 µmol) in toluene (1.7 mL) was stirred at 50 °C for 3 h and then 70 °C for a further 15 h. After reaction completion the mixture was diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The residue purified by silica chromatography (3% EtOAc in $CHCl_3$) to afford **85** as an orange solid (68.3 mg, 0.13 mmol, 39%). Minor impurities observed. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.30 (1H, dd, *J* = 8.0, 1.6 Hz), 8.13 (1H, s), 8.11 (1H, s), 7.92 (2H, d, *J* = 8.0 Hz), 7.65 (1H, t, *J* = 7.6 Hz), 7.53 (2H, apparent d, *J* = 8.0 Hz), 7.48 (1H, dd, *J* = 7.6, 1.2 Hz), 4.16 (4H, s), 3.58–3.51 (4H, m), 3.07–3.03 (4H, m).

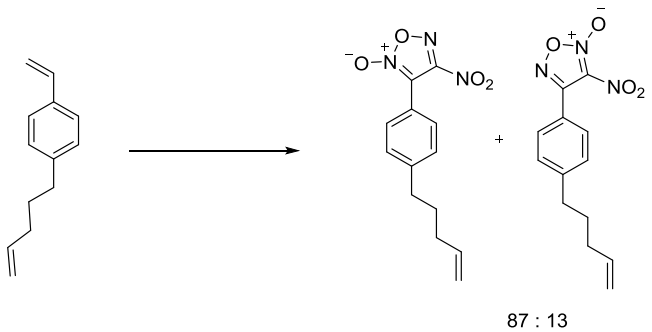
1-Ethenyl-4-(pent-4-en-1-yl)benzene (90)



Compound **90** was synthesized according to the reported method.[92] To a mixture of 5-bromopent-1-ene (11.3 mL, 95.6 mmol), $FeCl_3$ (47.8 mL of a 0.1 M THF solution, 4.78 mmol) was added at 0 °C a mixture of 4-ethenylphenylmagnesium bromide (115 mL of a 1M THF solution, 115 mmol) and TMEDA (17.2 mL, 115 mmol) dropwise over 2.5 h at a rate that keeps the reaction mixture yellow (4-ethenylphenylmagnesium bromide was prepared by dropwise addition of a 1 M solution of 4-ethenylphenyl

bromide in THF to Mg turnings at a rate which maintained gentle reflux, following addition the mixture was stirred for a further 30 min). Once addition was completed the reaction was stirred for a further 20 min. Saturated aqueous NH_4Cl was added followed by 1 M HCl and water, the reaction mixture was extracted thrice with EtOAc and the organics washed with brine, dried over $MgSO_4$ and filtered. Solvent was removed in vacuo and the residue purified by silica column chromatography (hexane) to yield **90** as a colourless oil (11.8 g, 68.6 mmol, 72%). R_f = 0.47 (hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.33 (2H, d, *J* = 8.0 Hz), 7.15 (2H, d, *J* = 8.0 Hz), 6.70 (1H, dd, *J* = 17.6, 11.0 Hz), 5.84 (1H, ddt, *J* = 17.2, 10.2, 6.8 Hz), 5.71 (1H, dd, *J* = 17.6, 1.0 Hz), 5.20 (1H, dd, *J* = 11.0, 1.0 Hz), 5.03 (1H, dq, *J* = 17.2, 2.0 Hz), 4.98 (ddt, *J* = 10.2 Hz, 3.2 Hz, 1.2 Hz), 2.62 (2H, t, *J* = 7.6 Hz), 2.01 (2H, q, *J* = 6.8 Hz), 1.72 (2H, quin, *J* = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 142.3, 138.6, 136.7, 135.2, 128.7, 126.2, 114.8, 112.9, 35.1, 33.3, 30.6; IR (neat) 3081, 3000, 2973, 2927, 2853, 1641, 1620, 1511, 1438, 1406, 988, 902, 836, 817 cm^{-1}. HRMS (DART) m/z [M + H]+ Calcd for $C_{13}H_{17}$ 173.1325; Found 173.1330.

4-Nitro-3-[4-(pent-4-en-1-yl)phenyl]furoxan (89)

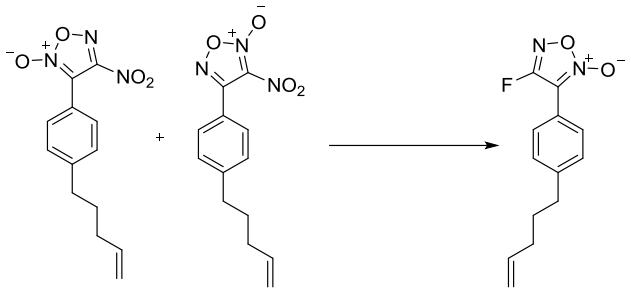


87 : 13

Nitrofuroxans **89** and **101** were synthesized according to a reported method.[113] To a stirred suspension of $NaNO_2$ (961 mg, 13.9 mmol) in a 0.2M CH_2Cl_2 solution of **90** (300 mg, 1.74 mmol) was added AcOH (796 µL, 13.9 mmol) over 30 min at room temperature. Two hours after starting the AcOH addition, a 2 M solution of HCl (5.2 mL, 10.5 mmol) was added. The reaction mixture was stirred for 14 h at rt, and then extracted three times with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica chromatography (30% CH_2Cl_2 in hexane) to yield **89** and **101** (181 mg, 0.66 mmol, 38%) as an 87:13 (**87**:**13**) mixture. The reaction can be conducted on a larger scale at the cost of diminished yield as byproduct formation increases. Data

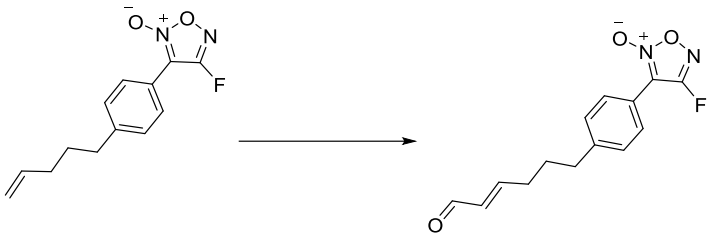
reported is for the major isomer. R_f = 0.25 (30% CH_2Cl_2 in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.52 (2H, apparent dt, *J* = 8.6, 2.0 Hz), 7.37 (2H, apparent d, *J* = 8.6 Hz), 5.83 (1H, ddt, *J* = 17.0, 10.4, 6.4 Hz), 5.04 (1H, dq, *J* = 17.0, 2.0 Hz), 5.01 (1H, ddt, *J* = 10.4, 2.0, 1.2 Hz), 2.71 (2H, t, *J* = 8.0 Hz), 2.11 (2H, q, *J* = 6.4 Hz), 1.76 (2H, quin, *J* = 7.2 Hz); δ_C (100 MHz, $CDCl_3$) Major isomer **87**; 158.2, 147.1, 138.1, 129.4, 128.6, 116.5, 115.2, 109.2, 35.3, 33.2, 30.2; distinguishable minor isomer **101**; 147.4, 138.1, 129.2, 128.6; IR (neat) 3075, 2924, 2856, 1612, 1560, 1519, 1492, 1357, 1270, 1115, 1069, 983, 909, 839, Article 812, 785, 755 cm^{-1}. HRMS (DART) m/z [M + H]+ Calcd for $C_{13}H_{14}N_3O_4$ 276.0979; Found 276.0984.

4-Fluoro-3-[4-(pent-4-en-1-yl)phenyl]furoxan (88)



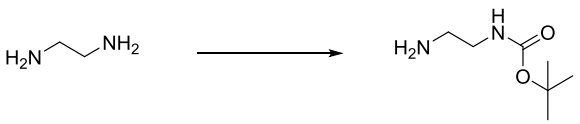
Fluorofuroxan **88** was synthesized according to the reported method.[41] To a solution containing the mixed isomers of **89** and **101** (1.00 g, 3.63 mmol) in THF (7.3 mL) was added dropwise a 1 M solution of tetrabutylammonium fluoride (TBAF) in THF (3.63 mL, 3.63 mmol) at 0 °C. After 1 h the reaction was diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The residue was purified by silica chromatography (30% CH_2Cl_2 in hexane) to yield **88** as a colourless oil (652 mg, 2.63 mmol, 72%). R_f = 0.36 (30% DCM in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.89 (2H, d, *J* = 8.6 Hz), 7.37 (2H, d, *J* = 8.6 Hz), 5.83 (1H, ddt, *J* = 17.2, 10.4, 6.4 Hz), 5.06−4.98 (2H, m), 2.70 (2H, t, *J* = 8.0 Hz), 2.10 (2H, q, *J* = 7.6 Hz), 1.75 (2H, quin, *J* = 8.0 Hz); δ_C (100 MHz, $CDCl_3$) 161.0 (d, J_{C-F} = 258.4 Hz), 145.5, 137.1, 128.5, 124.8 (d, J_{C-F} = 3.8 Hz), 116.9, 114.1, 104.9 (d, J_{C-F} = 26.4 Hz), 34.2, 32.1, 29.1; IR (neat) 3072, 2975, 2924, 2856, 1612, 1568, 1522, 1476, 1411, 1338, 1321, 1154, 1124, 1093, 977, 909, 825, 800, 733, 634, 608, 590 cm^{-1}. HRMS (DART) m/z [M + H]+ Calcd for $C_{13}H_{14}FN2O_2$ 249.1034; Found 249.1039.

(2E)-6-[4-(4-Fluoro-furoxan-3-yl)phenyl]hex-2-enal (87)



Furoxan **87** was synthesised according to the reported method.[94] To a stirred solution of **88** (300 mg, 1.21 mmol) and Hoveyda-Grubbs second generation catalyst (22.7 mg, 36.3 µmol) was added acrolein monomer and the mixture heated to 100 °C for 28 h. Upon reaction completion the solvent was removed in vacuo and crude material purified by column chromatography on silica gel (35% EtOAc in hexane) to give **87** (252 mg, 0.913 mmol, 76%) as a pale yellow crystalline solid. R_f = 0.44 (35% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 9.52 (1H, d, *J* = 7.8 Hz), 7.91 (2H, d, *J* = 8.4 Hz), 7.37 (2H, d, *J* = 8.4 Hz), 6.85 (1H, dt, *J* = 15.6, 6.8 Hz), 6.14 (1H, ddt, *J* = 15.6, 7.8, 1.2 Hz), 2.75 (2H, t, *J* = 8.0 Hz), 2.39 (2H, qd, *J* = 8.0, 1.6 Hz), 1.89 (2H, quin, *J* = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 193.8, 162.0 (d, J_{C-F} = 258.4 Hz), 157.4, 145.4, 133.4, 129.4, 126.0 (d, J_{C-F} = 3.7 Hz), 118.3, 105.8 (d, J_{C-F} = 26.5 Hz), 35.2, 32.0, 29.0; IR (neat) 2951, 2935, 2870, 2829, 2739, 1679, 1612, 1568, 1522, 1473, 1408, 1340, 1326, 1305, 1292, 1150, 1120, 1091, 974, 831, 814, 725, 598 cm^{-1}. HRMS (DART) m/z [M + H]+ Calcd for $C_{14}H_{14}FN_2O_3$ 277.0983; Found 277.0989; mp 53−56 °C.

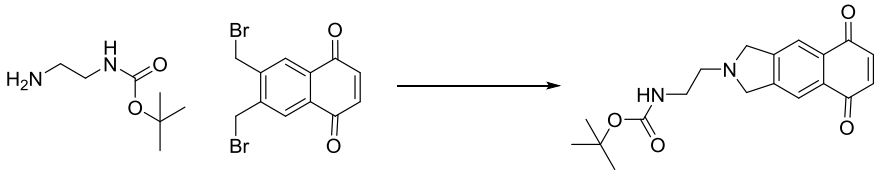
***tert*-butyl-*N*-(2-aminoethyl)carbamate (107)**



To a solution of 1,2-diaminoethane (12.0 mL, 180 mmol) in 1,4-dioxane (60 mL) was added dropwise a solution of Boc_2O (5.24 g, 24.0 mmol) in 1,4-dioxane (60 mL) over 1 h at rt. Following addition, the reaction was stirred for a further 2h. Upon completion the reaction mixture was filtered, solvent removed in vacuo and then diluted with brine and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo to yield **107** as a colourless oil (3.21 g, 20.1 mmol, 84%)

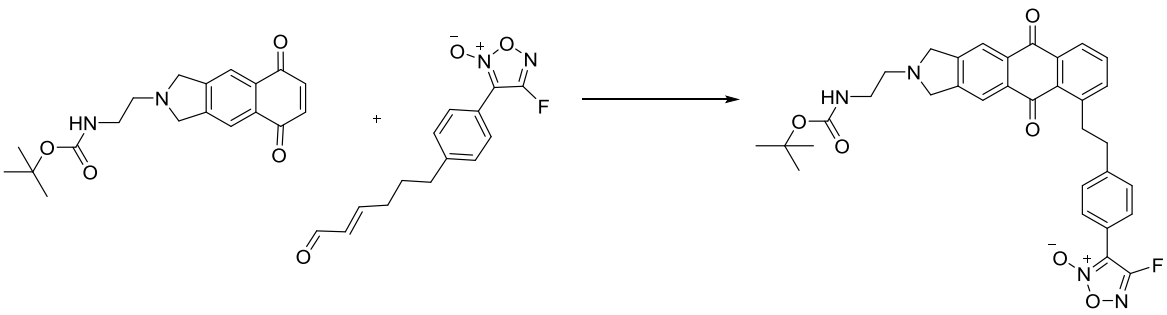
without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 4.85 (1H, br s), 3.17 (2H, apparent q, *J* = 6.0 Hz), 2.80 (2H, t, *J* = 6.0 Hz), 1.45 (9H, s); δ_C (100 MHz, $CDCl_3$) 156.3, 79.1, 43.2, 41.7, 28.4; IR (neat) 3360, 2975, 2932, 2867, 1683, 1593, 1529, 1455, 1391, 1365, 1270, 1250, 1167, 1039, 954, 870, 781, 629; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_7H_{17}N_2O_2$ 161; Found 161.

tert-Butyl [2-(5,8-dioxo-1,3,5,8-tetrahydro-2*H*-benzo[*f*]isoindol- 2-yl)ethyl]carbamate (108)



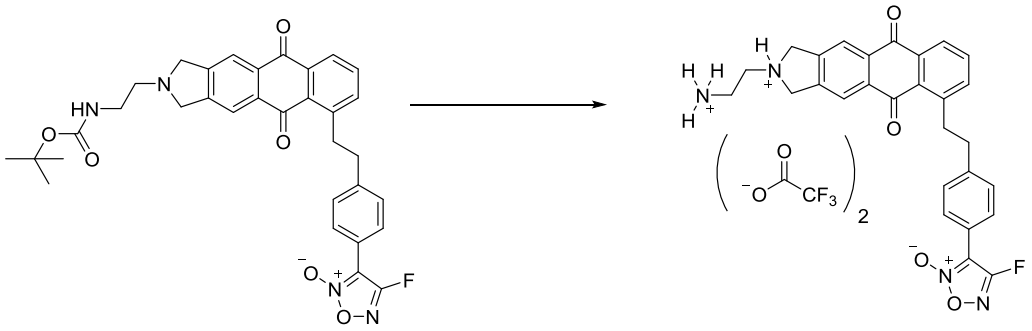
Compound **92** (500 mg, 1.45 mmol) was stirred in a mixture containing K_2CO_3 (729 mg, 5.28 mmol) and KI (110 mg, 0.66 mmol) in MeCN (750 mL). To the mixture was added **107** (212 mg, 1.32 mmol) at −10 °C and stirred for 16 h. Upon reaction completion the reaction mixture was concentrated and the crude material was purified by silica chromatography (35% EtOAc in CH_2Cl_2) to yield **108** as an orange solid (107 mg, 0.313 mmol, 24%). Compound **108** should be used immediately in the next step as it is unstable and prone to spontaneous oxidation. R_f = 0.31 (40% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.91 (2H, s), 6.96 (2H, s), 5.01 (1H, broad s), 4.06 (4H, s), 3.37–3.32 (2H, m), 2.90 (2H, t, *J* = 6.0 Hz), 1.45 (9H, s); δ_C (100 MHz, $CDCl_3$) 185.0, 156.0, 149.7, 146.6, 138.5, 131.5, 120.4, 58.6, 54.7, 39.0, 28.4; IR (neat) 3368, 3048, 3013, 2980, 2980, 2930, 281, 2761, 1688, 1663, 1608, 1524, 1435, 1385, 1362, 1327, 1305, 1273, 1240, 1153, 1129, 1040, 964, 838 cm^{-1}; HRMS (DART) m/z [M + H]+ Calcd for $C_{19}H_{23}N_2O_4$ 343.1652; Found 343.1658; mp 110 °C (decomposed).

tert-Butyl [2-(6-{2-[4-(4-fluorofuroxan-3-yl)phenyl]ethyl}-5,10-dioxo-1,3,5,10-tetrahy- dro-2H-naphtho[2,3-*f*]isoindol-2-yl)ethyl]carbamate (103)



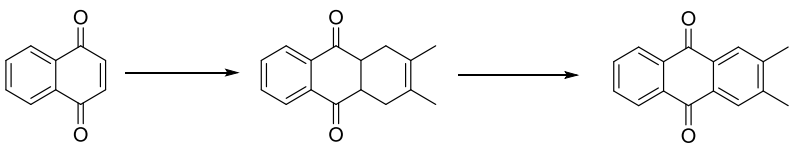
Compound **103** was synthesized according to the reported method.[84] A mixture of **108** (100 mg, 0.292 mmol), **87** (129 mg, 0.468 mmol), L- proline (6.70 mg, 58.0 µmol) and benzoic acid (3.40 mg, 30.0 µmol) in $CHCl_3$ (1.5 mL) was stirred at 50 °C for 15 h. After reaction completion the solvent was condensed in vacuo and the residue purified by silica chromatography (20% EtOAc in hexane) to afford **103** (67.1 mg, 0.11 mmol, 38%) as an orange solid. R_f = 0.35 (20% EtOAc in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.31 (1H, dd, *J* = 7.8, 1.2 Hz), 8.12 (1H, s), 8.10 (1H, s), 7.92 (2H, d, *J* = 8.0 Hz), 7.65 (1H, t, *J* = 7.8 Hz), 7.52 (2H, d, *J* = 8.0 Hz), 7.48 (1H, dd, *J* = 8.0, 1.2 Hz), 5.05 (1H, br s), 4.10 (4H, s), 3.56 (2H, t, *J* = 7.8 Hz), 3.40−3.33 (2H, m), 3.05 (2H, t, *J* = 7.8 Hz), 2.93 (2H, apparent t, *J* = 6.4 Hz), 1.46 (9H, s); δ_C (100 MHz, $CDCl_3$) 184.9, 183.4, 162.1 (d, J_{C-F} = 258.3 Hz), 156.0, 147.0, 146.4, 145.9, 144.6, 137.8, 135.5, 134.5, 133.3, 132.3, 131.0, 129.8, 126.8, 125.9 (d, J_{C-F} = 3.7 Hz), 121.2, 120.5, 118.1 (d, J_{C-F} = 4.2 Hz), 105.9 (d, J_{C-F} = 26.6 Hz), 79.4, 58.7, 58.6, 54.8, 39.1, 37.6, 37.2 28.4; IR (neat) 2958, 2927, 2846, 1712, 1666, 1606, 1584, 1522, 1471, 1321, 1298 1252, 1168, 1153, 1115, 981, 726 cm^{-1}; HRMS (DART) m/z [M + H]+ Calcd for $C_{33}H_{32}FN_4O_6$ 599.2300; Found 599.2306; mp 209 °C (decomposed).

2-(2-azaniumylethyl)-6-{2-[4-(4-fluorofuroxan-3-yl)phenyl]ethyl}-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium bis(trifluoroacetate) (104)



To a stirred solution of compound **103** (50.0 mg, 83.5 µmol) in CH_2Cl_2 (2 mL) at -10 °C was added TFA (2 mL) and the reaction was allowed to warm to rt over 1 h. Upon reaction completion the solvent was removed in vacuo to grant a brown oil. The oil was sonicated in Et_2O for 1 h whereupon it solidified and was filtered and washed with $CHCl_3$ to afford **104** as a brown solid (45.6 mg, 62.8 µmol, 75%). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H(ppm) 8.13–8.08 (3H, m), 7.80 (2H, d, *J* = 8.2 Hz), 7.74 (1H, t, *J* = 8.0 Hz), 7.68 (1H, apparent d, 6.8 Hz), 7.54 (2H, d, *J* = 8.2 Hz), 4.55 (4H, br s), 3.46 (2H, apparent t, *J* = 7.6 Hz), 3.31 (2H, br s), 3.10 (2H, br s), 2.93 (2H, apparent t, *J* = 7.6 Hz); δ_C (100 MHz, DMSO-d_6) 184.7, 183.0, 145.5 ($J_{C\text{-}F}$ = 111.1 Hz), 138.5, 135.2, 135.0, 134.2, 132.7, 130.9, 129.9, 126.7, 126.7, 126.6, 121.8, 121.0, 118.5 ($J_{C\text{-}F}$ = 4.0 Hz), 115.5, 107.3 ($J_{C\text{-}F}$ = 27.4 Hz), 79.8, 79.4, 58.6, 58.5, 51.9, 37.0, 36.8, 36.6; IR (neat) 3016, 2561, 2673, 1609, 1524, 1476, 1435, 1416, 1327, 1294, 1177, 1128, 1046, 1009, 980, 890, 836, 794, 717;); mp 145 °C (decomposed).

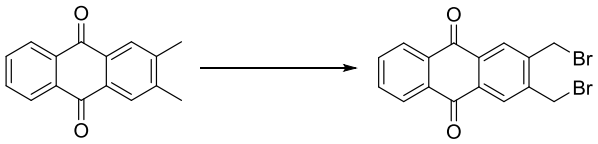
2,3-Dimethylanthracene-9,10-dione (112)



Furoxan **112** was synthesised according to the reported method.[95] To a stirred solution of 1,4-naphthaquinone (1.00 g, 6.32 mmol) in EtOH (4.14 mL) was added 2,3-dimethylbutadiene (1.43 mL, 12.7 mmol) and the mixture heated to 80 °C for 5 h.

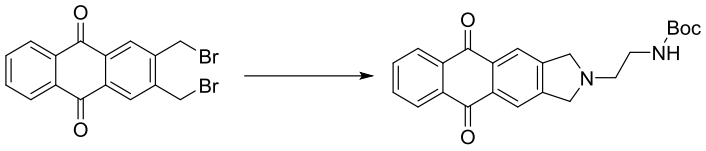
Upon completion the reaction was cooled, filtered and washed with cold ethanol to grant white crystals (1.29 g, 5.36 mmol, 85%). The crude intermediate (1.00 g, 4.17 mmol) was stirred in a 5% KOH ethanolic solution (13.8 mL) and air bubbled through the solution for 2 h at rt. The reaction mixture was filtered and washed with cold EtOH, water and finally Et_2O before drying to grant **112** as a yellow solid (913 mg, 3.86 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.31–8.27 (2H, m), 8.06 (2H, s), 7.80–7.76 (2H, m), 2.44 (6H, s); δ_C (100 MHz, $CDCl_3$) 183.3, 144.1, 133.8, 133.6, 131.4, 128.1, 127.0, 20.3; IR (neat) 3062, 2921, 2852, 1668, 1588, 1446, 1327, 1294, 1260, 1222, 1101, 1024, 955, 903, 799, 788, 710, 614; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{13}O_2$ 237; Found 237; mp 215–217 °C.

2,3-bis(Bromomethyl)anthracene-9,10-dione (113)



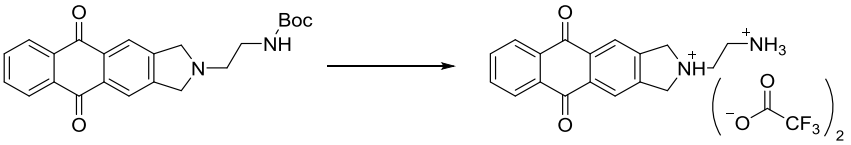
A stirred solution of **112** (600 mg, 2.54 mmol), NBS (1.04 g, 5.83 mmol) and benzoyl peroxide (30.0 mg, 0.127 mmol) in benzene (25.4 mL) was heated to reflux for 15 h. Upon reaction completion the mixture was washed twice with distilled water, the organic layer was dried over $MgSO_4$, filtered and solvent condensed in vacuo. The crude residue was purified by silica chromatography to yield **113** as a yellow solid (593 mg, 1.51 mmol, 60%). Minor impurities were observed. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.34–8.31 (2H, m), 8.30 (2H, s), 7.86–7.81 (2H, m), 4.76 (4H, s); IR (neat) 3037, 1676, 1588, 1329, 1296, 1227, 1189, 967, 932, 797, 712, 617; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{11}{}^{81}Br_2O_2$ 397; Found 397; mp 228 °C (decomposed).

tert-butyl [2-(5,10-dioxo-1,3,5,10-tetrahydro-2*H*-naphtho[2,3-*f*]isoindol-2-yl)ethyl]carbamate (114)



Compound **113** (500 mg, 1.28 mmol) was stirred in a mixture containing K_2CO_3 (704 mg, 5.10 mmol) and KI (106 mg, 0.638 mmol) in MeCN (638 mL). To the mixture was added **107** (204 mg, 1.28 mmol) at −10 °C and stirred for 16 h. The reaction was not complete and so was allowed to warm to rt for 3 h and finally heated to 70 °C for 2 h. Upon reaction completion the reaction mixture was concentrated. The crude material was diluted with water and extracted thrice with $CHCl_3$. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude solid was purified by silica chromatography (1 : 1 EtOAc/CH_2Cl_2) to yield **114** as an orange solid (300 mg, 0.77 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.33–8.30 (2H, m), 8.14 (2H, s), 7.81–7.79 (2H, m), 5.04 (1H, br s), 4.10 (4H, s), 3.38–3.40 (2H, m), 2.92 (2H, t, *J* = 6.0 Hz), 1.45 (9H, s); δ_C (100 MHz, $CDCl_3$) 183.0, 156.0, 146.8, 134.1, 133.4, 133.1, 127.2, 121.0, 79.3, 58.6, 54.8, 39.0, 28.4; IR (neat) 2975, 2932, 1705, 1672, 1589, 1493, 1454, 1365, 1326, 1300, 1249, 1213, 1160, 1082, 1003, 954, 861, 794, 709; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{23}H_{25}N_2O_2$ 393; Found 393; mp 139 °C (decomposed).

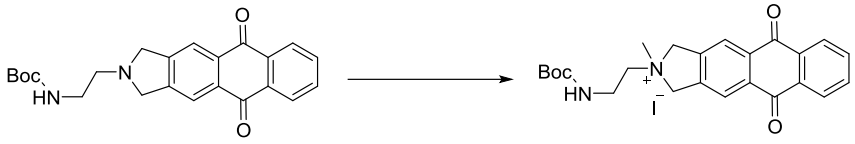
2-(2-ammonioethyl)-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium 2,2,2-trifluoroacetate (115)



To a solution of compound **114** (100 mg, 0.255 mmol) in CH_2Cl_2 (1.3 mL) was added TFA (1.3 mL) and the reaction stirred for 2 h at rt. Upon reaction completion the solvent was removed in vacuo to grant a brown oil. The oil was sonicated in Et_2O for 1 h whereupon it solidified and was filtered and washed with $CHCl_3$ to afford **115** as a brown solid (86.8 mg, 0.166 mmol, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H(ppm) 8.25–8.23 (2H, m), 8.17 (2H, s), 7.97–7.95 (2H, m), 4.39–4.24 (4H, m), 3.18–3.04 (4H, m); δ_C (100 MHz, DMSO-d_6) 181.6, 157.8 ($J_{C\text{-}F}$ = 33.2 Hz), 134.1, 132.6, 132.3, 126.2, 120.5,

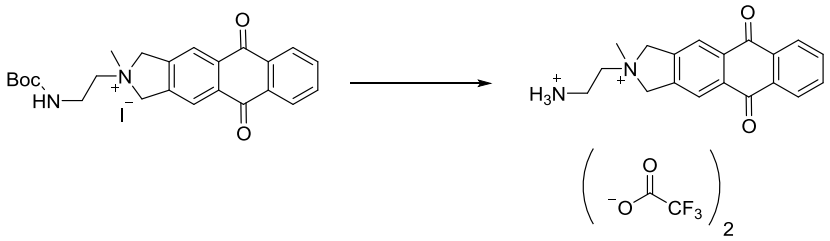
57.5, 50.8, 35.3; IR (neat) 3012, 2568, 1672, 1615, 1590, 1435, 1326, 1311, 1295, 1197, 1176, 1122, 957, 836, 795, 710; LRMS (DART) *m*/*z* [M − $2CF_3CO_2$ - H]+ Calcd for $C_{18}H_{18}N_2O_2$ 293; Found 293. [M + H]+ was not observed due to facile fragmentation; mp 185 °C (decomposed).

2-(2-((*tert*-butoxycarbonyl)amino)ethyl)-2-methyl-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium iodide (119)



To a solution of **114** (100 mg, 0.255 mmol) in MeCN (1.3 mL) was added MeI (31.7 µL, 0.51 mmol) and the reaction stirred at 50 °C for 16 h. Upon reaction completion the solvent was removed in vacuo and the crude residue filtered, washed with CH_2Cl_2 and dried to grant **119** as a brown solid (74.2 mg, 0.134 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.37–8.31 (4H, m), 7.88–7.84 (2H, m), 5.83 (1H, t, *J* = 6.6 Hz), 5.57 (2H, d, *J* = 15.2 Hz), 5.18 (2H, d, *J* = 15.2 Hz), 4.43 (2H, apparent t, *J* = 6.0 Hz), 3.87 (2H, apparent q, *J* = 6.0 Hz), 3.57 (3H, s), 1.47 (9H, s); IR (neat) 2959, 2922, 2853, 1699, 1674, 1622, 1589, 1507, 1456, 1367, 1328, 1302, 1254, 1163, 1096, 1021, 958, 927, 801, 712; mp 185 °C (decomposed).

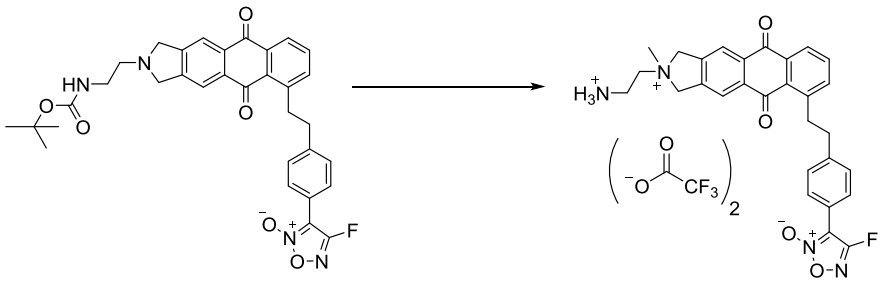
2-(2-ammonioethyl)-2-methyl-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium 2,2,2-trifluoroacetate (120)



To a stirred solution of **119** (50.0 mg, 94.0 µmol) in CH_2Cl_2 was added TFA (0.75 mL) at -10 °C. After 1 h the solvent was removed in vacuo and the crude solid filtered and washed with $CHCl_3$ and Et_2O to yield a black solid (48.6 mg, 91.0 µmol, 97%). ^{1}H NMR (400 MHz, D_2O) δ_H(ppm) 8.24 (2H, s), 8.22–8.18 (2H, m), 7.88–7.84 (2H, m), 5.18 (2H,

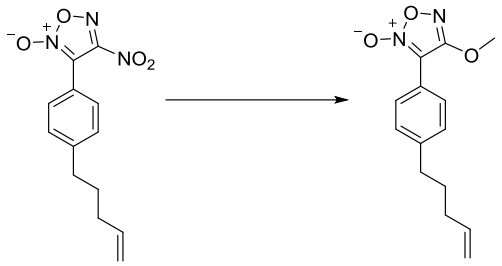
apparent d, *J* = 15.2 Hz), 5.12 (2H, apparent d, *J* = 15.2 Hz), 4.04–4.00 (2H, m), 3.59–3.55 (2H, m), 3.33 (3H, s); δ_C (100 MHz, DMSO-d_6) 181.4, 157.7 ($J_{C\text{-}F}$ = 33.9 Hz), 139.1, 134.3, 133.2, 132.3, 126.4, 121.7, 68.3, 59.0, 49.3, 32.6; IR (neat) 3203, 1673, 1591, 1429, 1328, 1302, 1199, 1128, 957, 835, 799, 711; LRMS (DART) m/z [M−2CF_3CO_2−$C_2H_4NH_2$]+ Calcd for $C_{17}H_{14}NO_2$ 264; Found 264. [M + H]+ was not observed due to facile fragmentation.

2-(2-Aminoethyl)-6-[4[(4-fluorofuroxan-3-yl)phenyl]ethyl]-2-methyl-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium bistrifluoroacetate (122)



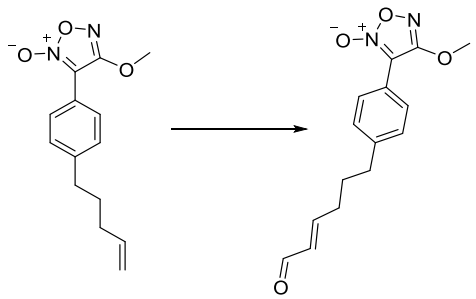
Compound **103** (60.0 mg, 0.10 mmol) was stirred in CH_2Cl_2 (1 mL) and MeI (32.0 µL, 0.50 mmol) was added and the reaction warmed to 40 °C for 20 h. The solvent and excess MeI were removed under vacuum. To the crude material was added CH_2Cl_2 (0.5 mL) and cooled to −10 °C, TFA (0.5 mL) was added the mixture stirred for 1 h. The solvent and excess TFA were removed in vacuo and the brown oil sonicated in Et_2O for 1 h whereupon it solidified and was filtered to afford **122** (58.9 mg, 80.0 µmol, 80%) as a brown solid. ^{1}H NMR (400 MHz, D_2O) δ_H(ppm) 8.14 (1H, s), 8.09 (1H, dd, *J* = 7.6, 1.2 Hz), 8.03 (1H, s), 7.68−7.64 (3H, m), 7.56 (1H, t, *J* = 7.6 Hz), 7.23 (2H, d, *J* = 8.0 Hz), 5.17−5.06 (4H, m), 4.05−4.01 (2H, m), 3.62−3.58 (2H, m), 3.51–3.46 (2H, m), 3.33 (3H, s), 2.94 (2H, t, *J* = 7.2 Hz); δ_C (100 MHz, DMSO-d_6) 183.9, 182.4, 162.5 (d, J_{C-F} = 256.3 Hz), 158.2 (q, J_{C-F} = 32.5 Hz), 145.5, 144.6, 139.8, 139.2, 138.2, 135.2, 134.6, 134.0, 133.0, 130.4, 129.4, 126.3 (d, J_{C-F} = 3.1 Hz), 122.6, 121.8, 118.2, 106.9 (d, J_{C-F} = 27.4 Hz), 68.9, 59.6, 49.9, 36.6, 36.3, 33.3; IR (neat) 3006, 2929, 2860, 1667, 1614, 1523, 1473, 1415, 1327, 1298, 1197, 1173, 1125, 976, 836, 796, 719 cm^{-1}. HRMS (DART) m/z [M−2CF_3CO_2−$C_2H_4NH_2$]+ Calcd for $C_{27}H_{21}FN_3O_4$ 470.1511; Found 470.1516. $[M + H]^+$ was not observed due to facile fragmentation

4-Methoxy-3-[4-(pent-4-en-1-yl)phenyl]furoxan (123)



To a solution of compound **89** (0.70 g, 2.54 mmol) and MeOH (616 µL, 75.3 mmol) in THF (12.7 mL) was added dropwise NaOH 50% wt. aqueous solution (460 µL) and the reaction stirred at rt for 10 min. Upon reaction completion the mixture was diluted with water and extracted thrice with CH_2Cl_2, organics dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude oil was purified by silica chromatography (1 : 1 benzene/hexane) to yield **123** as a colourless oil (526 mg, 2.02 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.04 (2H, apparent d, *J* = 8.6 Hz), 7.31 (2H, d, 8.6 Hz), 5.82 (1H, ddt, *J* = 17.2, 10.4, 6.4 Hz), 5.06–4.97 (2H, m), 4.21 (3H, s), 2.68 (2H, t, *J* = 7.6 Hz), 2.10 (2H, q, *J* = 7.6 Hz), 1.74 (2H, quint, *J* = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 162.9, 145.4, 138.3, 129.0, 126.2, 119.8, 115.0, 107.8, 57.5, 35.2, 33.2, 30.3; IR (neat) 3075, 2934, 2858, 1602, 1568, 1554, 1520, 1475, 1455, 1405, 1340, 1295, 1199, 1163, 1122, 1088, 995, 953, 911, 840, 810, 740, 701, 616; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{14}H_{17}N_2O_3$ 261; Found 261.

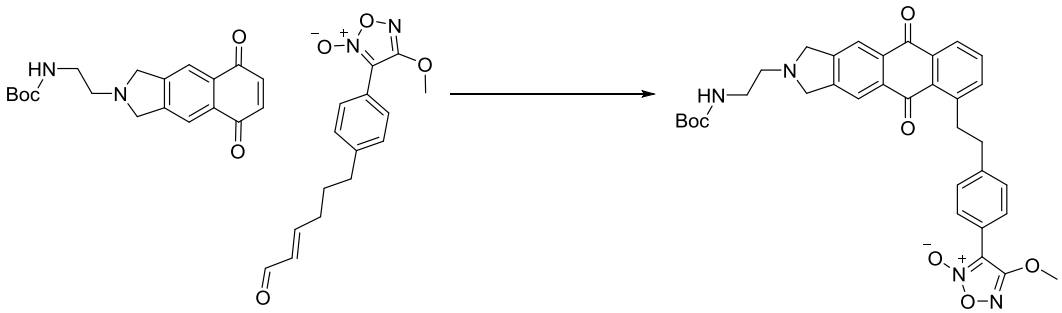
(2E)-6-[4-(4-Methoxy-furoxan-3-yl)phenyl]hex-2-enal (124)



Furoxan **124** was synthesised according to the reported method.[94] To a stirred solution of **123** (350 mg, 1.35 mmol) and Hoveyda-Grubbs second generation catalyst (33.7 mg, 54.0 µmol) was added acrolein monomer (359 µL, 5.38 mmol) and the mixture heated to 100 °C for 29 h. Upon reaction completion the solvent was removed in vacuo and crude material purified by column chromatography on silica gel (35% EtOAc in hexane) to give **124** (323 mg, 1.12 mmol, 83%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$)

δ_H(ppm) 9.52 (1H, d, *J* = 7.6 Hz), 8.06 (2H, apparent d, *J* = 8.4 Hz), 7.31 (2H, d, *J* = 8.4 Hz), 6.84 (1H, dt, *J* = 15.8, 6.8 Hz), 6.14 (1H, ddt, *J* = 15.8, 7.6, 1.2 Hz), 4.22 (3H, s), 2.73 (2H, t, *J* = 7.6 Hz), 2.38 (2H, apparent q, *J* = 7.6 Hz), 1.88 (2H, quint, *J* = 7.6 Hz); δ_C (100 MHz, $CDCl_3$) 193.9, 162.9, 157.7, 144.2, 133.4, 128.9, 126.4, 120.2, 107.7, 57.5, 35.1, 32.0, 29.1; IR (neat) 2941, 2859, 2826, 2743, 1682, 1634, 1598, 1568, 1556, 1519, 1472, 1455, 1403, 1340, 1325, 1296, 1200, 1160, 1125, 1112, 1088, 995, 972, 952, 841, 818, 741, 699, 599; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{15}H_{17}N_2O_4$ 289; Found 289.

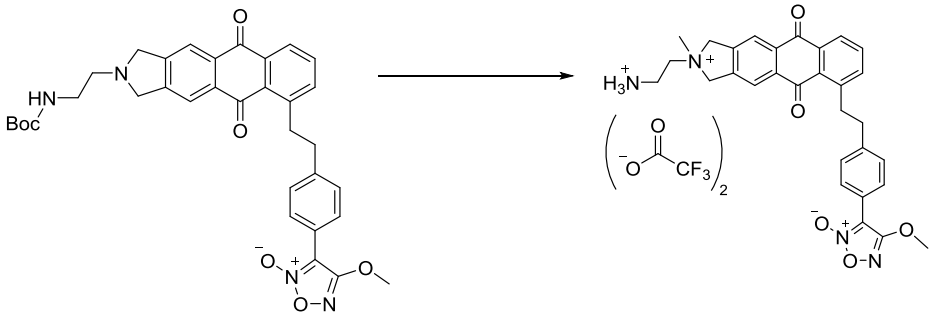
tert-Butyl [2-(6-{2-[4-(4-methoxyfuroxan-3-yl)phenyl]ethyl}-5,10-dioxo-1,3,5,10-tetrahy-dro-2*H*-naphtho[2,3-*f*]isoindol-2-yl)ethyl]carbamate (125)



Compound **125** was synthesized according to the reported method.[84] A mixture of **108** (80.0 mg, 0.234 mmol), **124** (108 mg, 0.374 mmol), L- proline (5.40 mg, 47.0 µmol) and benzoic acid (9.17 mg, 0.82 mmol) in $CHCl_3$ (1.2 mL) was stirred at 50 °C for 3 h. After reaction completion the solvent was condensed in vacuo and the residue purified by silica chromatography (25% EtOAc in CH_2Cl_2) to afford **125** (48.2 mg, 79.0 µmol, 34%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.29 (1H, dd, *J* = 8.0, 1.6 Hz), 8.11 (1H, s), 8.09 (1H, s), 8.07 (2H, d, *J* = 8.4 Hz), 7.64 (1H, t, *J* = 7.6 Hz), 7.49–7.44 (3H, m), 5.05 (1H, br s), 4.22 (3H, s), 4.09 (4H, s), 3.56 (2H, apparent t, *J* = 8.0 Hz), 3.38–3.34 (2H, m), 3.03 (2H, apparent t, *J* = 7.8 Hz), 2.92 (2H, apparent t, *J* = 6.0 Hz), 1.45 (9H, s); δ_C (100 MHz, $CDCl_3$) 184.8, 183.4, 162.9, 156.1, 146.9, 146.2, 144.8, 144.8, 137.8, 135.3, 134.5, 133.3, 143.3, 131.0, 129.2, 126.7, 126.2, 121.2, 120.5, 120.0, 107.8, 79.3, 58.7, 58.6, 57.5, 54.8, 39.0, 37.6, 37.1, 28.4; IR (neat) 2969, 2930, 1712, 1666, 1598, 1568, 1556, 1520, 1471, 1407, 1365, 1321, 1298, 1251, 1205, 1163, 1117, 1087, 1045, 995, 981, 860, 844, 820, 799, 726, 714; LRMS (DART) m/z [M - $CH_2N(H)CO_2C(CH_3)_3$ -

CNO_2OCH_3 + H]+ Calcd for $C_{26}H_{20}N_2O_2$ 392; Found 392. [M + H]+ was not observed due to facile fragmentation. mp 148 °C (decomposed).

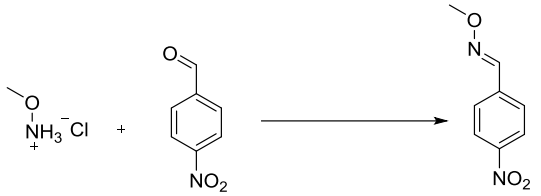
2-(2-Aminoethyl)-6-[4[(4-methoxyfuroxan-3-yl)phenyl]ethyl]-2-methyl-5,10-dioxo-2,3,5,10-tetrahydro-1*H*-naphtho[2,3-*f*]isoindol-2-ium bistrifluoroacetate (126)



Compound **125** (35.0 mg, 57.0 µmol) was stirred in CH_2Cl_2 (0.6 mL) and MeI (10.7 µL, 0.172 mmol) was added and the reaction warmed to 40 °C for 16 h. The solvent and excess MeI were removed under vacuum. To the crude material was added CH_2Cl_2 (0.6 mL) and cooled to −10 °C, TFA (0.6 mL) was added the mixture stirred for 1 h. The solvent and excess TFA were removed in vacuo and the brown oil sonicated in Et_2O for 1 h whereupon it solidified and was filtered to afford **126** (38.0 mg, 45.0 µmol, 79%) as a brown solid. The product appeared to precipitate out as a complex with one equivalent of Et_2O. Discernible peaks are reported. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H(ppm) 8.34 (1H, s), 8.29 (1H, s), 8.21 (1H, dd, *J* = 7.6, 1.6 Hz), 7.99 (2H, d, *J* = 8.4 Hz), 7.84 (1H, t, *J* = 7.6 Hz), 7.77 (1H, apparent dd, *J* = 7.6, 1.2 Hz), 7.57 (2H, d, *J* = 8.4 Hz), 5.19 (1H, apparent d, *J* = 15.4 Hz), 5.14 (1H, apparent d, *J* = 15.4 Hz), 4.18 (3H, s), 3.88–3.84 (2H, m), 3.51 (4H , br s overlapping), 3.38 (6H, q, *J* = 7.0 Hz), 3.30 (3H, s), 2.99 (2H, apparent t, *J* = 8.0 Hz), 1.09 (4H, t, *J* = 7.0 Hz); δ_C (100 MHz, DMSO-d_6) 183.3, 181.8, 162.3, 157.6 ($J_{C\text{-}F}$ = 32.0 Hz), 144.2, 144.1, 139.1, 138.5, 137.6, 134.6, 134.0, 133.4, 132.3, 129.7, 128.5, 125.7, 122.0, 121.2, 119.0, 107.0, 68.3, 64.3, 58.9, 57.3, 49.3, 36.0, 35.7, 32.7, 26.5, 14.6; IR (neat) 2929, 1668, 1600, 1567, 1557, 1520, 1472, 1455, 1406, 1327, 1298, 1198, 1127, 991, 924, 838, 798, 719; LRMS (DART) m/z [M – $2CF_3CO_2$ - NH_2CH_2 - CH_3 - CNO_2OCH_3 + H]+ Calcd for $C_{26}H_{20}N_2O_2$ 392; Found 392. [M + H]+ was not observed due to facile fragmentation. mp 119 °C (decomposed).

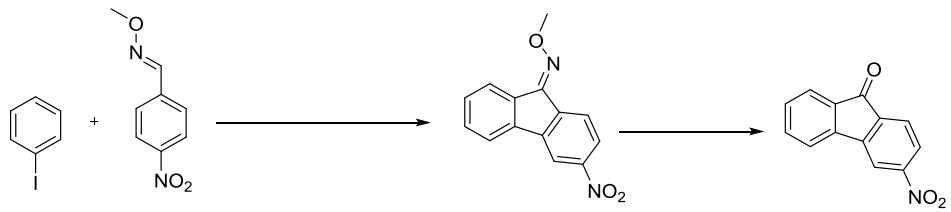
3. Improvements to visible light photoinduced nitric oxide donor furoxans: studies towards aminofluorenone-alkoxyfuroxan

4-Nitrobenzaldehyde-*O*-methyl oxime (139)



To a solution of methoxylamine hydrochloride (1.42 g, 17.0 mmol) and sodium acetate (1.23 g, 15.0 mmol) in 3 : 1 water/THF was added *p*-nitrobenzaldehyde (1.51 g, 10.0 mmol) and the reaction stirred for 2 h at rt. Upon reaction completion the mixture was diluted with water and extracted thrice with EtOAc. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (3% EtOAc in hexane) to yield a white solid (1.55 g, 8.60 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.23 (2H, apparent d, *J* = 9.0 Hz), 8.10 (1H,s), 7.75 (2H, apparent d, 9.0 Hz), 4.04 (3H, s); δ_C (100 MHz, $CDCl_3$) 148.3, 146.3, 138.4, 127.6, 124.0, 62.7; IR (neat) 3110, 3089, 3012, 2989, 2948, 2906, 2826, 1613, 1596, 1585, 1513, 1464, 1442, 1409, 1385, 1344, 1312, 1296, 1217, 1182, 1172, 1107, 1046, 1009, 931, 868, 850, 829, 748, 689, 630; LRMS (DART) *m/z* [M + H]+ Calcd for $C_8H_9N_2O_3$ 181; Found 181; mp 107–108 °C.

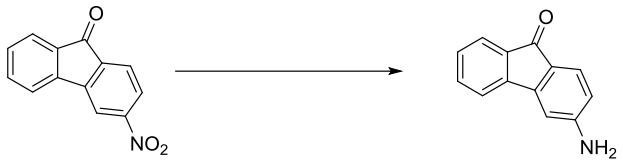
3-Nitro-9*H*-fluoren-9-one (141)



Compound **141** was synthesized according to the reported method.[102] Trifluoroacetic acid (13.2 mL), **139** (1.20 g, 6.66 mmol) and iodobenzene (4.48 mL, 40.0 mmol) were added to $Pd(OAc)_2$ (22.4 mg, 0.10 mmol) and silver(I) oxide (232 mg, 1.00 mmol) and the mixture stirred at 120 °C for 36 h. The mixture was cooled and filtered through a pad of Celite and washed with CH_2Cl_2. The filtrate was condensed to 5 mL and 12 M

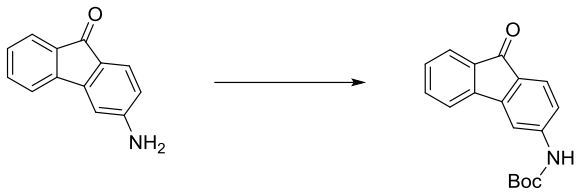
HCl was added (2.0 mL) and the reaction heated to 95 °C for 8 h. The reaction mixture was cooled, diluted with water and extracted thrice with EtOAc. The organics were dried over $MgSO_4$, filtered and concentrated. The crude was purified by silica chromatography (5% EtOAc in hexane) to yield **141** as a yellow solid (1.04 g, 4.61 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.35 (1H, d, *J* = 1.6 Hz), 8.21 (1H, dd, *J* = 8.0, 2.0 Hz), 7.82 (1H, d, *J* = 8.4 Hz), 7.76 (1H, apparent d, 7.2 Hz), 7.67 (1H, apparent d, *J* = 7.6 Hz), 7.62 (1H, td, *J* = 7.4, 1.2 Hz), 7.43 (1H, td, *J* = 7.4, 1.2 Hz); δ_C (100 MHz, $CDCl_3$) 191.5, 152.2, 145.7, 142.5, 138.4, 135.7, 134.1, 130.5, 125.0, 124.8, 124.7, 121.2, 115.3; IR (neat) 3091, 1711, 1605, 1527, 1444, 1347, 1300, 1260, 1192, 1152, 1104, 925, 912, 872, 785, 763, 727, 672, 656; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{13}H_8NO_3$ 226; Found 226; mp 229–232 °C.

3-Amino-9*H*-fluoren-9-one (143)



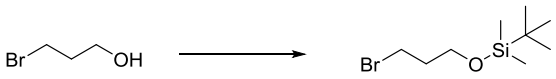
Compound **143** was synthesized according to the reported method.[103] A solution of compound **141** (900 mg, 3.97 mmol), NaOH (1.60 g, 40.0 mmol) and sodium sulphite nonohydrate (2.21 g, 9.20 mmol) in EtOH (40 mL) and water (40 mL) was stirred at 80 °C for 1 h. Upon reaction completion the reaction was cooled, concentrated in vacuo and extracted with EtOAc thrice. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was prebound to silica and purified by silica chromatography (2.5% EtOAc in $CHCl_3$) to yield **143** as a red solid (564 mg, 2.89 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.61 (1H, apparent d, *J* = 8.4 Hz), 7.51 (1H, d, *J* = 8.0 Hz), 7.46–7.41 (2H, m), 7.31–7.27 (1H, m), 6.77 (1H, d, *J* = 2.0 Hz), 6.46 (1H, dd, *J* = 8.0, 2.0 Hz), 4.26 (2H, br s); δ_C (100 MHz, $CDCl_3$) 192.3, 152.9, 147.4, 143.2, 136.0, 133.6, 129.1, 126.8, 124.6, 123.5, 119.8, 113.3, 106.3; IR (neat) 3485, 3388, 3211, 2921, 2855, 1687, 1608, 1584, 1495, 1489, 1449, 1376, 1292, 1249, 1205, 1155, 916, 867, 827, 771, 761, 743, 675; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{13}H_{10}NO$ 196; Found 196; mp 148–151 °C.

tert-butyl (9-oxo-9*H*-fluoren-3-yl)carbamate (148)



To a solution of compound **141** (150 mg, 0.256 mmol), DMAP (31.3 mg, 0.256 mmol) and Et_3N (39.3 µL, 0.282 mmol) in THF (0.85 mL) was added Boc_2O (61.6 mg, 0.282 mmol) and the reaction heated to 50 °C for 40 h. The reaction mixture was diluted with water and extracted thrice with EtOAc, the organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (5% EtOAc in $CHCl_3$) to yield **148** as a yellow solid (28.9 mg, 97.9 mol, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.85 (1H, apparent d, *J* = 1.6 Hz), 7.63 (1H, d, *J* = 7.2 Hz), 7.59 (1H, d, *J* = 8.0 Hz), 7.53 (1H, d, *J* = 7.2 Hz), 7.47 (1H, td, *J* = 7.2, 1.2 Hz), 7.30 (1H, td, *J* = 7.4, 0.8 Hz), 7.03 (1H, dd, *J* = 8.2, 1.6 Hz), 1.55 (9H, s); δ_C (100 MHz, $CDCl_3$) 192.8, 152.1, 146.5, 144.6, 143.6, 135.1, 134.3, 129.3, 128.7, 125.5, 123.9, 120.4, 117.4, 109.9, 81.5, 28.3; IR (neat) 3296, 3249, 3192, 3122, 3058, 2976, 1723, 1690, 1593, 1537, 1490, 1490, 1474, 1451, 1428, 1391, 1365, 1297, 1281, 1233, 1149, 1110, 1052, 1027, 908, 888, 858, 835, 764, 731, 670, 647; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{18}H_{18}NO_3$ 296; Found 296; mp 177–179 °C.

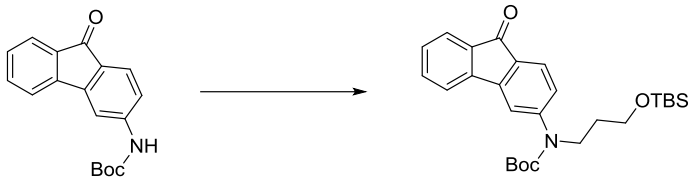
1-Bromo-3-(*tert*-butyldimethylsiloxy)propane (149)



To a stirred solution of 3-bromopropanol (649 µL, 7.19 mmol) and imidazole (951 µL, 14.4 mmol) in CH_2Cl_2 (18 mL) was added TBSCl (1.08 g, 7.19 mmol) at 0 °C and allowed to warm to rt after 1 h. After a further 5 h the reaction was diluted with water and extracted thrice with CH_2Cl_2, the organics were washed with $NaHCO_3$, brine and then dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was purified by silica chromatography (hexane) to yield **149** as a colourless oil (1.36 g, 5.40 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 3.67 (2H, t, *J* = 5.8 Hz), 3.45 (2H, t, *J* = 6.6 Hz), 2.00–1.94 (2H, m), 0.90 (9H, s), 0.07 (6H, s); δ_C (100 MHz, $CDCl_3$) 60.4, 35.6, 30.6, 25.9, 18.3, -5.4; IR (neat) 2954, 2928, 2857, 1471, 1462, 1385, 1361, 1254, 1211,

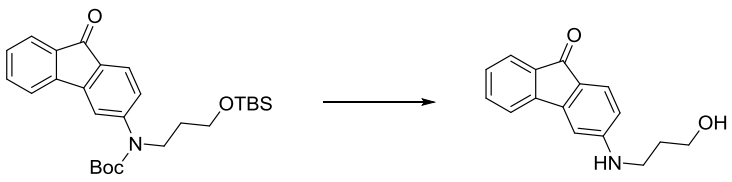
1147, 1099, 1061, 1030, 951, 939, 832, 774, 717, 662; LRMS (DART) *m/z* [M + H]+ Calcd for $C_9H_{22}BrOSi$ 253; Found 253.

tert-butyl (3-((tert-butyldimethylsilyl)oxy)propyl)(9-oxo-9*H*-fluoren-3-yl)carbamate (147)



To a solution of NaH (60% in mineral oil) (32.0 mg, 0.80 mmol) in DMF (3 mL) was added **148** (200 mg, 0.68 mmol) and stirred for 30 min at rt. A solution of **149** (101 mg, 40.0 mmol) in DMF (3 mL) was added dropwise and the reaction heated to 70 °C for 1 h. The reaction was quenched with aq. NH_4Cl and diluted with CH_2Cl_2 and H_2O. The organics were washed with water thrice and dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude oil was purified by silica chromatography (10% EtOAc in hexane) to yield **147** as an orange oil (186 mg, 0.38 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.64 (1H, d, *J* = 7.2 Hz), 7.61 (1H, d, *J* = 8.0 Hz), 7.50–7.43 (3H, m), 7.31–7.27 (1H, m), 7.14 (1H, dd, *J* = 8.0, 2.0 Hz), 3.79 (2H, t, *J* = 7.2 Hz), 3.65 (2H, t, *J* = 6.0 Hz), 1.88–1.83 (2H, m), 1.48 (9H, m), 0.84 (6H, m); δ_C (100 MHz, $CDCl_3$) 192.9, 154.0, 149.0, 145.3, 143.8, 134.7, 134.5, 130.9, 129.2, 126.0, 124.8, 124.2, 120.3, 118.3, 81.0, 60.5, 47.2, 32.0, 28.4, 25.9, 18.3, -5.4; IR (neat) 2953, 2927, 2852, 1702, 1610, 1471, 1448, 1387, 1365, 1297, 1248, 1161, 1141, 1100, 996, 916, 832, 764, 737, 682; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{27}H_{38}NO_4Si$ 468; Found 468.

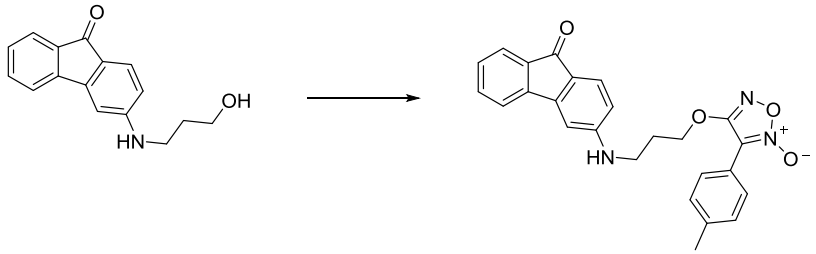
3-[(3-hydroxypropyl)amino]-9*H*-fluoren-9-one (146)



To a stirred solution of **147** (145 mg, 0.31 mmol) in CH_2Cl_2 (3 mL) was added 4 M HCl in dioxane (320 µL, 1.28 mmol) and stirred at rt for 18 h. Upon reaction completion

the mixture was diluted with EtOAc and washed with 1 M NaOH. Water was added to the organics and extracted thrice with EtOAc. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude material was purified by silica chromatography (40% EtOAc in $CHCl_3$) to yield **146** as an orange solid (59.9 mg, 0.24 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.60 (1H, apparent d, *J* = 7.2 Hz), 7.53 (1H, d, *J* = 8.4 Hz), 7.45–7.39 (2H, m), 7.29–7.27 (1H, m), 6.71 (1H, d, *J* = 2.0 Hz), 6.38 (1H, dd, *J* = 8.0, 2.0 Hz), 4.79 (1H, br s), 3.89–3.85 (2H, m), 3.45–3.41 (2H, m), 1.95 (2H, apparent quin, *J* = 6.2 Hz), 1.50 (1H, t, *J* = 4.8 Hz); δ_C (100 MHz, $CDCl_3$) 192.1, 154.1, 147.3, 143.3, 136.3, 133.3, 129.0, 126.8, 123.3, 123.2, 119.6, 110.9, 104.1, 61.3, 41.4, 31.4; IR (neat) 3293, 1676, 1612, 1584, 1551, 1475, 1452, 1428, 1384, 1361, 1312, 1297, 1242, 1210, 1196, 1156, 1122, 1100, 1066, 1021, 1007, 919, 867, 767, 734, 676, 651; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{16}NO_2$ 254; Found 254; mp 146–149 °C.

4-(3-((9-oxo-9*H*-fluoren-3-yl)amino)propoxy)-3-(*p*-tolyl)-1,2,5-oxadiazole 2-oxide (145)



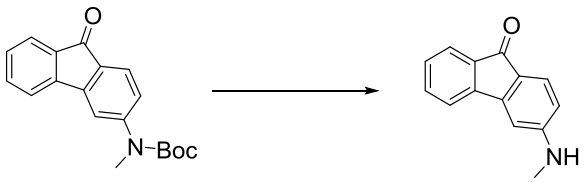
Compound **55** (24.0 mg, 109 µmol), **146** (25.0 mg, 98.9 µmol) and K_2CO_3 (16.4 mg, 0.12 mmol) were stirred in THF (1.0 mL) at 50 °C for 21 h. NaOH (2.40 mg, 60.0 µmol) was added and the reaction stirred for a further 1 hour. Upon reaction completion the mixture was diluted with water and extracted thrice with $CHCl_3$. The organics were washed with brine, dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude mixture was purified by silica chromatography (5% EtOAc in $CHCl_3$) to yield **145** an orange solid (33.6 mg, 78.6 µmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.99 (2H, apparent d, *J* = 8.4 Hz), 7.60 (1H, d, *J* = 7.2 Hz), 7.51 (1H, d, *J* = 8.0 Hz), 7.40 (1H, dt, *J* = 7.4, 1.2 Hz), 7.34 (1H, apparent d, *J* = 7.2 Hz), 7.32–7.29 (2H, m), 7.26 (1H, dd, *J* = 7.2, 0.8 Hz), 6.66 (1H, d, *J* = 2.0 Hz), 6.36 (1H, dd, *J* = 8.0, 2.0 Hz), 4.66 (2H, t, *J* = 6.2 Hz), 4.61–4.58 (1H, m), 3.55–3.50 (2H, m), 2.40 (3H, s), 2.30 (2H, quin, *J* = 6.2 Hz); δ_C (100 MHz, $CDCl_3$) 192.1, 162.2, 153.4, 147.3, 143.1, 141.1, 136.2, 133.4, 129.7, 129.1, 126.8, 126.0, 123.8, 123.5, 119.7, 119.4, 110.8, 107.6, 104.2, 68.6, 40.4, 28.5, 21.6; IR

(neat) 3432, 3358, 2964, 2925, 1682, 1588, 1551, 1512, 1473, 1454, 1367, 1335, 1312, 1300, 1262, 1202, 1165, 1122, 1099, 1025, 1007, 973, 911, 851, 818, 791, 766, 732, 676; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{25}H_{22}N_3O_4$ 428; Found 428; mp 175–178 °C.

3-(Boc-methylamino)-9*H*-fluoren-9-one (151)

To a solution of compound **148** (50.0 mg, 0.169 mmol) in THF was added NaH (60% wt. in mineral oil) (136 mg, 0.339 mmol) and stirred at rt for 30 min. Iodomethane (42.6 µL, 0.339 mmol) was added dropwise and after 5 min the mixture was diluted with water and extracted thrice with EtOAc. The organics were washed with brine, dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (15% EtOAc in hexane) to yield **151** as yellow oil (50.5 mg, 0.163 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.65 (1H, d, *J* = 7.6 Hz), 7.62 (1H, d, *J* = 8.0 Hz), 7.52–7.46 (3H, m), 7.30 (1H, dt, *J* = 7.2, 1.6 Hz), 7.14 (1H, dd, *J* = 8.0, 2.0 Hz), 3.34 (3H, s), 1.51 (9H, s); δ_C (100 MHz, $CDCl_3$) 192.9, 154.1, 149.8, 145.1, 143.8, 134.7, 134.5, 130.4, 129.2, 124.7, 124.4, 124.2, 120.3, 117.1, 81.3, 37.0, 28.3; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{19}H_{20}NO_3$ 310; Found 310.

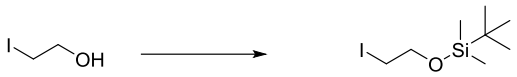
3-(Methylamino)-9*H*-fluoren-9-one (152)



To a solution of compound **151** (50.0 mg, 0.162 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.8 mL) and the mixture stirred at rt for 1 h. The reaction mixture was diluted with 1 M NaOH and extracted thrice with $CHCl_3$, the organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude residue was purified by silica chromatography (2.5% EtOAc in hexane) to yield **152** as an orange solid (24.0 mg,

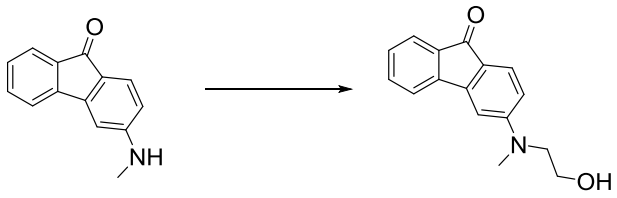
0.115 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.60 (1H, d, *J* = 7.2 Hz), 7.53 (1H, d, *J* = 8.0 Hz), 7.46–7.40 (2H, m), 7.28 (1H, dd, *J* = 7.2, 1.6 Hz), 6.69 (1H, d, *J* = 2.0 Hz), 6.37 (1H, dd, *J* = 8.0, 2.0 Hz), 4.21 (1H, br s), 2.98 (3H, d, *J* = 5.2 Hz); δ_C (100 MHz, $CDCl_3$) 192.2, 154.9, 147.2, 143.2, 136.3, 133.3, 129.0, 126.7, 123.3, 123.2, 119.7, 110.6, 103.8, 30.3; IR (neat) 3299, 1675, 1609, 1581, 1463, 1453, 1417, 1370, 1299, 1244, 1207, 1151, 1097, 1057, 1023, 1005, 917, 857, 817, 764, 733, 674; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_{14}H_{12}NO$ 210; Found 210; mp 116–119 °C.

1-Iodo-2-(*tert*-butyldimethylsilyloxy)ethane (153)



To a stirred solution of 2-iodoethanol (454 µL, 5.81 mmol) and imidazole (769 µL, 5.81 mmol) in CH_2Cl_2 (14.5 mL) was added TBSCl (876 mg, 11.6 mmol) at 0 °C and allowed to warm to rt. After 1 h the reaction was diluted with water and extracted thrice with CH_2Cl_2. The organics were washed with $NaHCO_3$, brine and then dried over $MgSO_4$, filtered and solvent removed in vacuo to yield **153** a colourless oil (727 mg, 2.54 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 3.84 (2H, t, *J* = 7.2 Hz), 3.20 (2H, t, *J* = 7.2 Hz), 0.91 (9H, s), 0.09 (6H, s); δ_C (100 MHz, $CDCl_3$) 64.3, 25.9, 18.3, 7.0, -5.2; IR (neat) 2954, 2928, 2885, 2856, 1471, 1463, 1276, 1254, 1189, 1166, 1120, 1081, 997, 939, 833, 775, 708, 667; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_8H_{20}IOSi$ 287; Found 287.

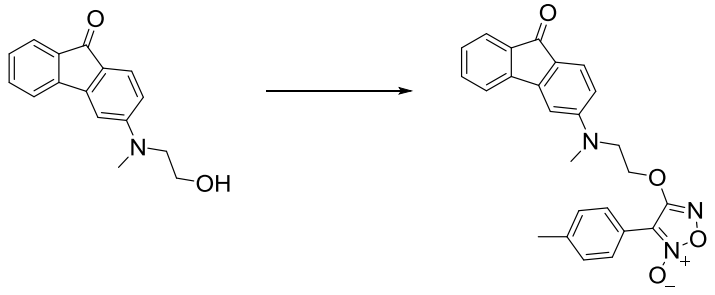
3-[(2-hydroxyethyl)(methyl)amino]-9*H*-fluoren-9-one (154)



To a solution of compound **152** (22.4 mg, 107 µmol) in DMF (1.0 mL) was added NaH (60% wt. in mineral oil) (8.6 mg, 214 µmol) and stirred at rt for 30 min. To the mixture was added dropwise **153** (33.7 mg, 118 µmol) and the reaction heated to 70 °C for 1 h. The mixture was cooled, diluted with $CHCl_3$ and washed with water 5 times. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude

was purified by silica chromatography (25% EtOAc in $CHCl_3$) to yield **154** as a red solid (17.8 mg, 70.3 µmol, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.60 (1H, d, *J* = 7.2 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.47 (1H, apparent d, *J* = 7.2 Hz), 7.42 (1H, td, *J* = 7.2, 1.2 Hz), 7.28 (1H, apparent dd, *J* = 7.2, 1.2 Hz), 6.86 (1H, s), 6.53 (1H, dd, *J* = 8.4 Hz, 2.4 Hz), 3.92 (2H, q, *J* = 5.6 Hz), 3.67 (2H, t, *J* = 5.6 Hz), 3.17 (3H, s); δ_C (100 MHz, $CDCl_3$) 192.3, 154.7, 147.1, 143.4, 136.4, 133.3, 128.9, 126.5, 123.2, 122.2, 119.6, 110.4, 103.4, 60.3, 54.7, 39.5; IR (neat) 3393, 2924, 2872, 1673, 1608, 1586, 1507, 1454, 1377, 1308, 1298, 1259, 1209, 1188, 1156, 1106, 1049, 1025, 1006, 917, 838, 810, 785, 764, 732, 674; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{16}NO_2$ 254; Found 254; mp 125–128 °C.

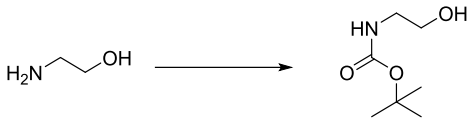
4-(2-(methyl(9-oxo-9*H*-fluoren-3-yl)amino)ethoxy)-3-(*p*-tolyl)-1,2,5-oxadiazole 2-oxide (150)



To a solution of compound **154** (16.8 mg, 66.3 µmol) and **55** (16.2 mg, 73.2 µmol) in THF (0.7 mL) was added NaOH (2.92 mg, 73.0 µmol) and the reaction stirred at rt for 4 h. The mixture was diluted with water and extracted thrice with $CHCl_3$. The organics dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (2% EtOAc in $CHCl_3$) to yield **150** as a yellow solid (22.9 mg, 53.6 µmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 7.84 (2H, apparent d, *J* = 8.2 Hz), 7.61 (1H, d, *J* = 7.6 Hz), 7.56 (1H, d, *J* = 8.4 Hz), 7.44–7.39 (2H, m), 7.31–7.26 (1H, m), 7.24 (2H, d, *J* = 8.2 Hz), 6.91 (1H, d, *J* = 2.4 Hz), 6.56 (1H, dd, *J* = 8.0, 1.2 Hz), 4.74 (2H, t, *J* = 5.4 Hz), 4.03 (2H, t, *J* = 5.4 Hz), 3.21 (3H, s), 2.29 (3H, s); δ_C (100 MHz, $CDCl_3$) 192.1, 162.1, 154.0, 147.1, 143.3, 141.0, 136.2, 133.4, 129.5, 129.1, 126.5, 126.0, 123.4, 123.0, 119.7, 118.9, 110.6, 107.6, 103.6, 67.4, 50.6, 39.0, 21.5; IR (neat) 1688, 1608, 1590, 1547, 1506, 1474, 1451, 1375, 1334, 1314, 1267, 1243, 1212, 1165, 1108, 1026, 994, 917, 843, 816, 766, 734, 676; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{25}H_{22}N_3O_4$ 428; Found 428; mp 155–158 °C.

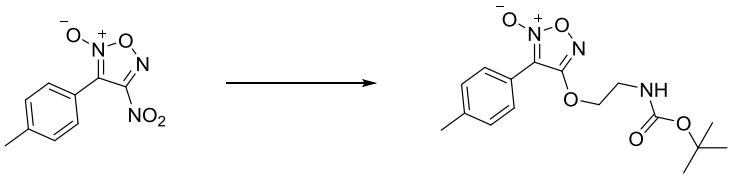
4. Improvement to visible light photoinduced nitric oxide donor furoxans: studies towards naphthalenediimide-talkoxyfuroxan

N-Boc-ethanolamine (164)



To a stirred solution of 2-aminoethanol (1.02 mL, 16.4 mmol) in CH_2Cl_2 was added di-*tert*-butyl dicarbonate (3.57 g, 16.4 mL) and stirred for 16 h at rt. The reaction mixture was diluted with water and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo to yield **164** as a colourless oil (2.28 g, 14.1 mmol, 86%). The product was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 4.94 (1H, br s), 3.71 (2H, q, *J* = 5.2 Hz), 3.29 (2H, q, *J* = 5.2 Hz), 2.39 (1H, br s); δ_C (100 MHz, $CDCl_3$) 156.9, 79.6, 43.1, 28.4, 8.61; IR (neat) 3341, 2977, 2929, 2874, 1681, 1514, 1454, 1389, 1366, 1279, 1247, 1167, 1064, 862 cm^{-1}; LRMS (DART) *m*/*z* [M + H]+ Calcd for $C_7H_{16}NO_3$ 162; Found 162.

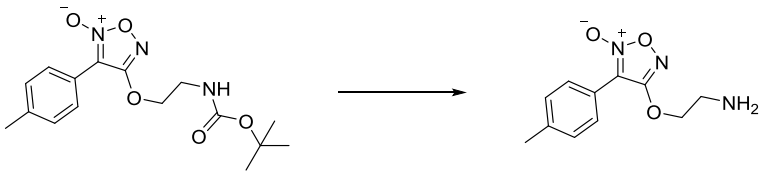
tert-Butyl-2-((3-(4-methylphenyl))-4-furoxany-4-yl)oxy)ethylcarbamate (163)



To a solution of **55** (250 mg, 1.13 mmol) and K_2CO_3 (468 mg, 3.39 mmol) in THF was added **164** (218 mg, 1.36 mmol) and stirred at 45 °C for 18 h. Upon reaction completion the mixture was diluted with water and extracted thrice with $CHCl_3$. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo. The crude was purified by silica chromatography ($CHCl_3$) to yield **163** as an off white solid (290 mg, 0.87 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.01 (2H, d, *J* = 8.0 Hz), 7.32 (2H, d, *J* = 8.0 Hz), 4.84 (1H, br s), 4.55 (2H, t, *J* = 5.2 Hz), 3.67 (2H, apparent q, *J* = 5.2 Hz), 2.42 (3H, s), 1.44 (9H, s); δ_C (100 MHz, $CDCl_3$); 162.2, 155.7, 141.0, 129.6, 126.1,

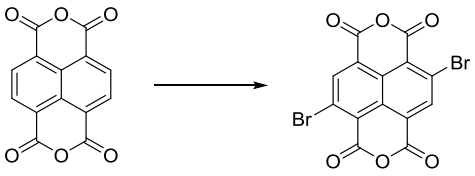
119.4, 107.7, 80.0, 70.0, 39.5, 28.4, 21.6; IR (neat) 3349, 2983, 2927, 1681, 1604, 1556, 1537, 1519, 1481, 1442, 1383, 1359, 1332, 1314, 1287, 1274, 1253, 1163, 1115, 1046, 1030, 998, 966, 860, 839, 822, 631 cm^{-1}; HRMS (DART) *m/z* [M + H]+ Calcd for $C_{16}H_{22}N_3O_5$ 336.1566; Found 336.1560; mp 157–159 °C.

4-(2-Aminoethyloxy)-3-(4-methylphenyl)furoxan (160)



To a solution of **163** (270 mg, 0.805 mmol) in CH_2Cl_2 (4 mL) was added TFA (4 mL) and the reaction stirred at rt for 1 h. The mixture was diluted with K_2CO_3 basified water (pH 8) and extracted thrice with CH_2Cl_2. The organics were dried over $MgSO_4$, filtered and solvent removed in vacuo to yield an orange solid (98.4 mg, 0.42 mmol, 56%). The product was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 8.01 (2H, d, *J* = 8.4 Hz), 7.32 (2H, d, *J* = 8.4 Hz), 4.53 (2H, t, *J* = 5.2 Hz), 3.23 (2H, t, *J* = 5.2 Hz), 2.41 (3H, s); δ_C (100 MHz, $CDCl_3$) 162.4, 141.0, 129.6, 126.1, 119.4, 107.7, 72.8, 40.8, 21.6; IR (neat) 3386, 2954, 2922, 2862, 1691, 1597, 1550, 1516, 1441, 1396, 1310, 1159, 1123, 990, 857, 818, 797, 732 cm^{-1}; HRMS (DART) *m/z* [M + H]+ Calcd for $C_{11}H_{14}N_3O_3$ 236.1034; Found 236.1035; mp 97–99 °C.

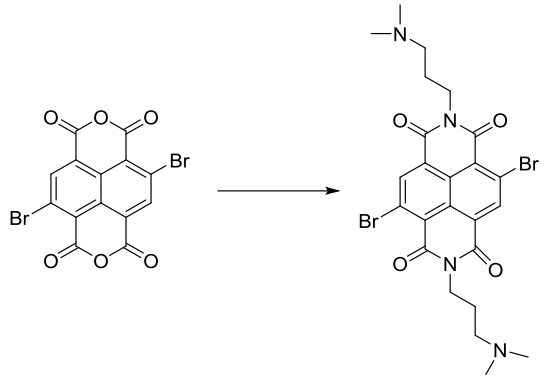
2,6-Dibromonaphtalene-1,4,5,8-tetracarboxylic dianhydride (161)



Compound **161** was synthesized according to the reported method.[117] To a solution of dibromocyaburic acid (9.56 g, 33.3 mmol) in concentrated H_2SO_4 (0.3 M) was added

portionwise 1,4,5,8-naphthalenetetracarboxylic dianhydride (4.47 g, 16.7 mmol) at room temperature, following complete addition the reaction was heated to 130 °C for 18 h. Upon completion the reaction was cooled, poured into ice water and stirred until yellow precipitate formed. The precipitate was filtered and washed with water and hot MeOH then dried to yield a yellow crude mixture containing **161** (7.37 g, 17.3 mmol, 104%). Due to the insoluble nature of **161**, the mixture is used without further purification.^{1}H NMR (400 MHz, DMSO-d_6) δ_H(ppm) 8.79 (2H, s) proton data is in agreement with the literature[117] ; IR (neat) 3203, 3081, 2832, 1782, 1744, 1692, 1460, 1400, 1262, 1189, 1137, 1069, 977, 776, 698 cm^{-1}; LRMS (DART) *m/z* [M + H]+ Calcd for $C_{28}H_2Br_2O_6$ 426; Found 426.

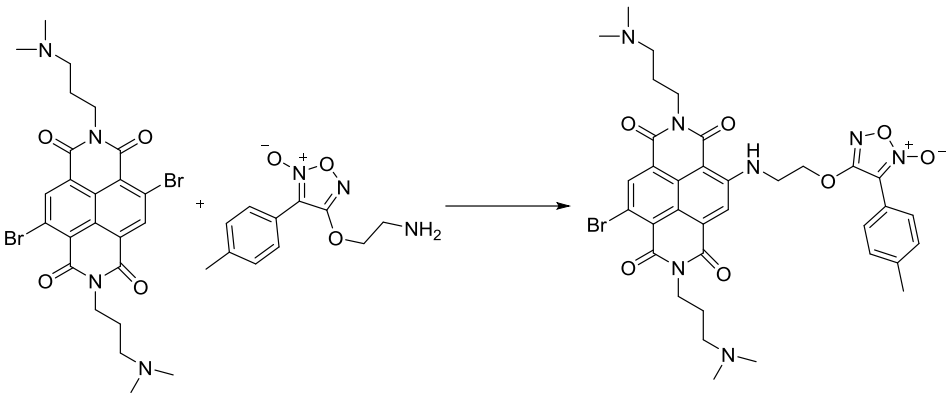
_N,N'_-Bis((dimethylamino)propylamino)-2,6-dibromonaphthalene-1,4,5,8- tetra-carboxylic acid bisimide (159)



Compound **159** was synthesized according to the reported method.[118] To a solution of compound **161** (1.00 g, 2.34 mmol) in acetic acid (40 mL) was added dropwise 3-(dimethylamino)-1-propylamine (736 µL, 5.85 mmol) and the reaction heated to 130 °C for 30 minutes. Upon reaction completion the mixture was poured into ice water, basified with K_2CO_3 (pH 8) and extracted 6 times with $CHCl_3$. The organics were dried over Na_2SO_4, filtered and solvent removed in vacuo. The crude solid was purified by silica chromatography (5% MeOH, 0.5% Et_3N in $CHCl_3$) to yield **159** as an orange solid (734 mg, 1.24 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 9.00 (2H, s), 4.27 (4H, t, *J* = 7.2 Hz), 2.45 (4H, t, *J* = 7.2 Hz), 2.24 (12H, s), 1.93 (4H, quin, J = 7.2 Hz); δ_C (100 MHz, $CDCl_3$) 160.8, 160.7, 139.0, 128.3, 127.7, 125.3, 124.1, 57.1, 45.4, 40.0, 25.6; IR (neat) 3057, 2943, 2815, 2762, 1707, 1651, 1561, 1434, 1420, 1364, 1309, 1216,

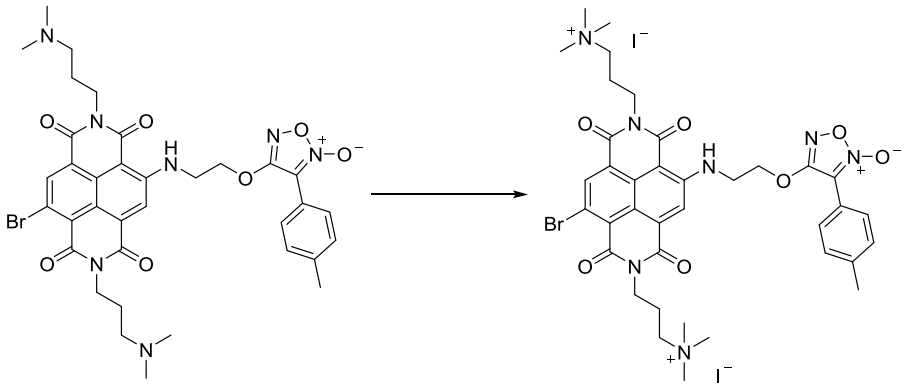
1178, 1040, 857, 785, 759, 714 cm^{-1}; mp 210 °C (decomposed) data is in agreement with the literature.[118]

Synthesis of alkoxyfuroxan tethered NDI 6 (Scheme 5). *N,N'* -Bis(3-(dimethylamino)propylamino)-2-bromo-6-(2-{[3-(4-methylphenyl)furoxan-4-yl]oxy}ethylamino)-1,4,5,8-naphthalenetetracarboxylic bisimide (158)



Compound **158** was synthesized according to the reported method.[111] NDI **159** (1.00 g, 1.68 mmol) and furoxan **160** (515 mg, 2.19 mmol) were stirred in DMF (0.02 M) at 60 °C 14 h. The reaction mixture was cooled and poured into Na_2CO_3 (pH 8) basified water, diluted with EtOAc and washed gently 5 times with an excess of aq. Na_2CO_3. The organics were dried over Na_2SO_4, filtered and solvent removed in vacuo. The crude was purified by silica chromatography (5% MeOH, 1% Et_3N, 94% $CHCl_3$) to yield **158** as a red solid (1.04 g, 1.39 mmol, 83%). ^1H NMR (400 MHz, $CDCl_3$) δ_H(ppm) 10.44 (1H, t, *J* = 7.2 Hz), 8.84 (1H, s), 8.28 (1H, s), 7.89 (2H, d, *J* = 8.4 Hz), 7.15 (2H, d, *J* = 8.4 Hz), 4.86 (2H, t, *J* = 5.2 Hz), 4.24–4.14 (6H, m), 2.46 (4H, q, *J* = 6.8 Hz), 2.33 (3H, s), 2.27 (6H, s), 2.26 (6H, s), 1.95–1.86 (4H, m); δ_C (100 MHz, $CDCl_3$) 166.0, 161.9, 161.7, 161.7, 161.2, 151.7, 141.0, 138.5, 129.5, 128.4, 127.5, 126.0, 123.6, 123.4, 121.6, 121.1, 119.6, 119.1, 107.6, 101.1, 68.7, 57.1, 45.3, 45.2, 41.5, 39.8, 38.9, 25.8, 25.6, 21.6; IR (neat) 2937, 2812, 2759, 1707, 1686, 1662, 1633, 1604, 1580, 1439, 1312, 1256, 1256, 1218, 1178, 1162, 1016, 817, 791 cm^{-1}; HRMS (ESI) m/z [M + H]+ Calcd for $C_{35}H_{39}BrN_7O_7$ 750.2068; Found 750.2076; mp 163 °C (decomposed).

N*,*N **’ -Bis(3-(trimethylamino)propylamino)-2-bromo-6-(2-{[3-(4-methylphenyl)furoxan-4-yl]oxy}ethylamino)-1,4,5,8-naphthalenetetracarboxylic bisimide diiodide (159)**



To a solution of compound **158** (20.0 mg, 26.7 μmol) in MeCN (2.60 mL) was added MeI (3.65 μL, 58.8 μmol) and the reaction heated to 35 °C for 6 h. Upon completion the solvent was removed in vacuo and the crude solid diluted with $CHCl_3$, filtered and washed with hexane and $CHCl_3$ to yield a purple solid (22.5 mg, 21.5 μmol, 95%). ^{1}H NMR (400 MHz, acetonitrile-d_3) δ_H(ppm) 10.27 (1H, apparent t, *J* = 7.6 Hz), 8.73 (1H, s), 8.28 (1H, s), 7.70 (2H, d, *J* = 8.4 Hz), 7.01 (2H, d, *J* = 8.4 Hz), 4.85 (2H, t, *J* = 5.2 Hz), 4.24–4.20 (4H, m), 4.11 (2H, t, *J* = 6.8 Hz), 3.45–3.38 (4H, m), 3.04 (9H, s), 3.00 (9H, s); δ_C (100 MHz, acetonitrile-d_3) 166.9, 16.2, 162.9, 162.7, 162.2, 153.4, 141.9, 138.1, 130.1, 129.5, 128.2, 126.7, 124.6, 124.4, 122.6, 121.4, 120.4, 108.7, 101.5, 70.7, 65.2, 65.0, 53.9, 42.0, 38.7, 37.9, 22.8, 22.5, 21.4; IR (neat) 3435, 2954, 2917, 2847, 1709, 1670, 1637, 1584, 1517, 1475, 1444, 1311, 1267, 1241, 1191, 1163, 1124, 960, 785, 738, 720 cm^{-1}; HRMS (ESI) m/z [M – 2I]2+ Calcd for $C_{37}H_{44}BrN_7O_7$ 389.6227; Found 389.6232; mp 188 °C (decomposed).

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Appendix Ⅰ: list of publications

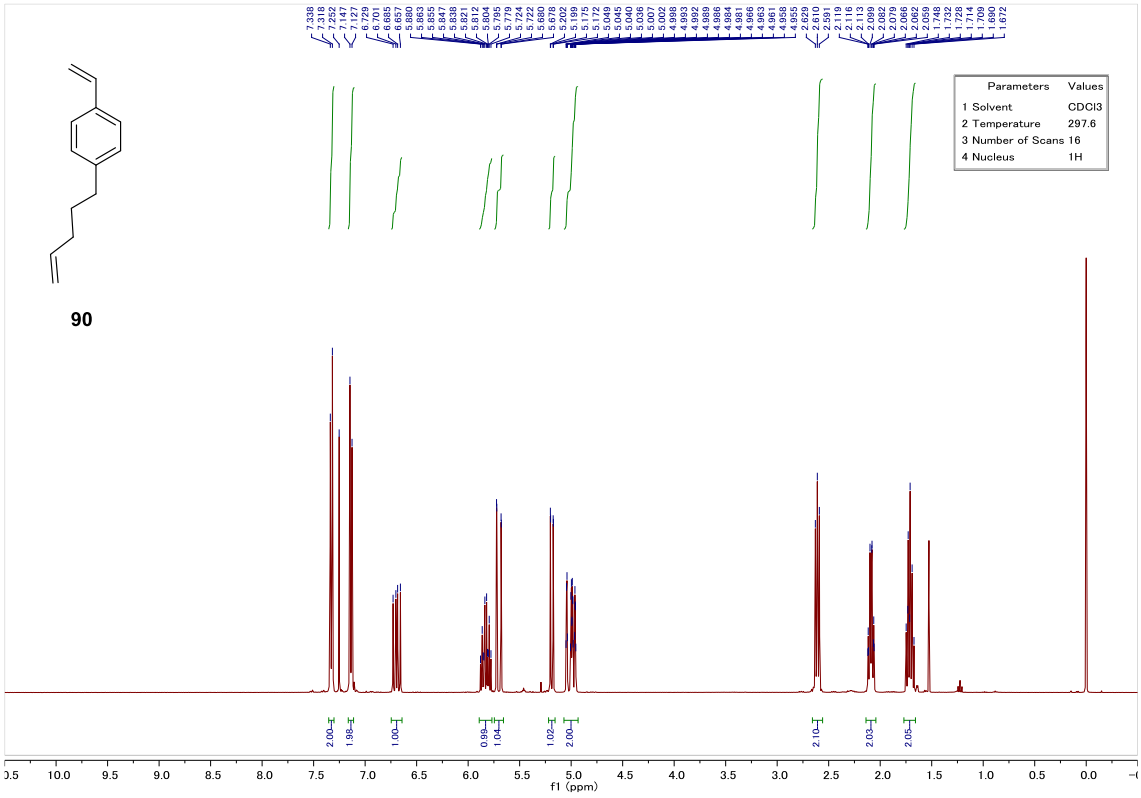
1 Photosensitization of Fluorofuroxans and Its Application to the Development of Visible Light-Triggered Nitric Oxide Donor
C. P. Seymour, R. Tohda, M. Tsubaki, M. Hayashi, R. Matsubara, *J. Org. Chem.,* **82**, 2017, 9647–9654.

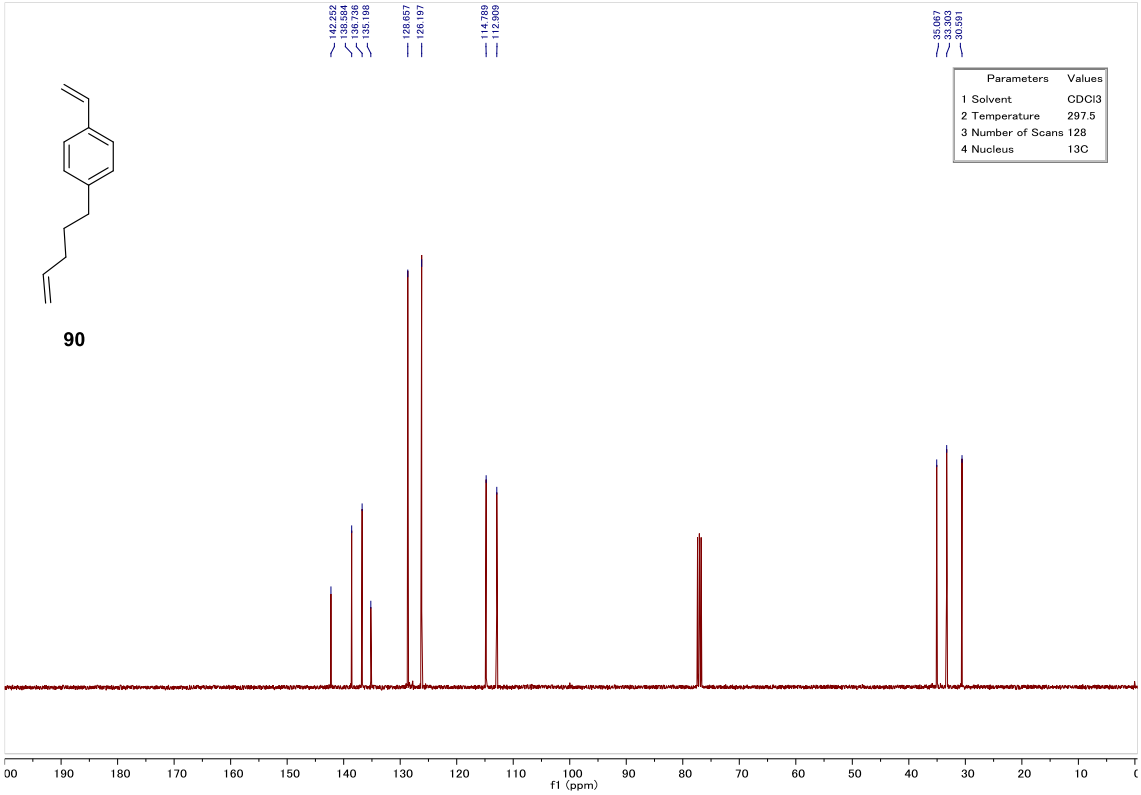
2 A Fluorescent Photoswitchable Water-Soluble Naphthalenediimide-Alkoxyfuroxan Visible Light Nitric Oxide Donor
C. P. Seymour, Akito Nakata, Motonari Tsubaki, Masahiko Hayashi and Ryosuke Matsubara, *Bull. Chem. Soc. Jpn*., Submitted.

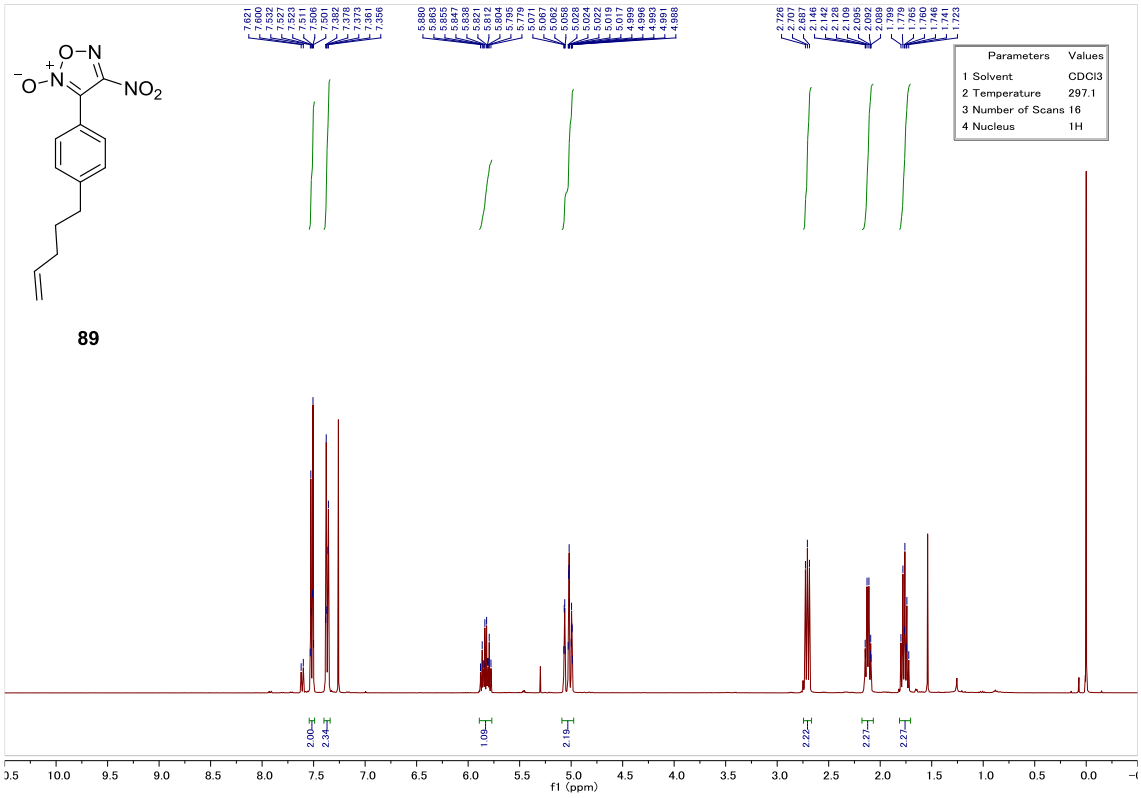
Appendix Ⅱ: ^{1}H and ^{13}C spectra of published compounds

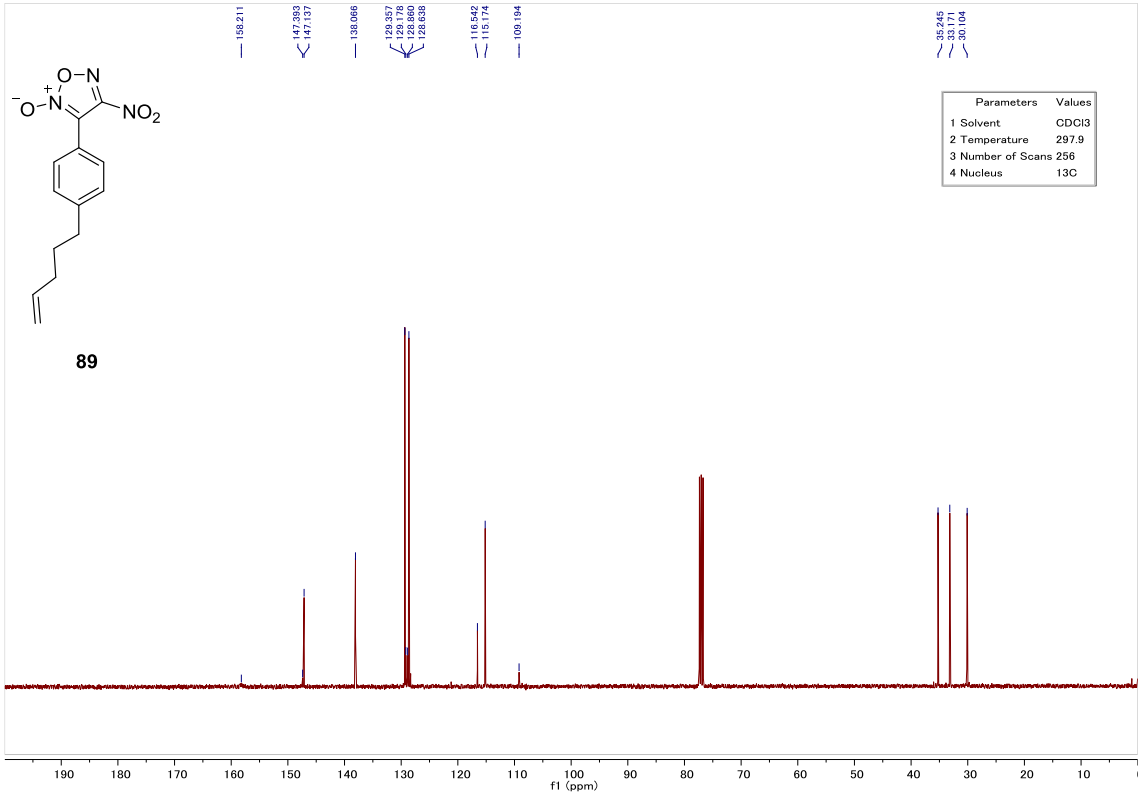
1. Photosensitization of Fluorofuroxans and Its Application to the Development of Visible Light-Triggered Nitric Oxide

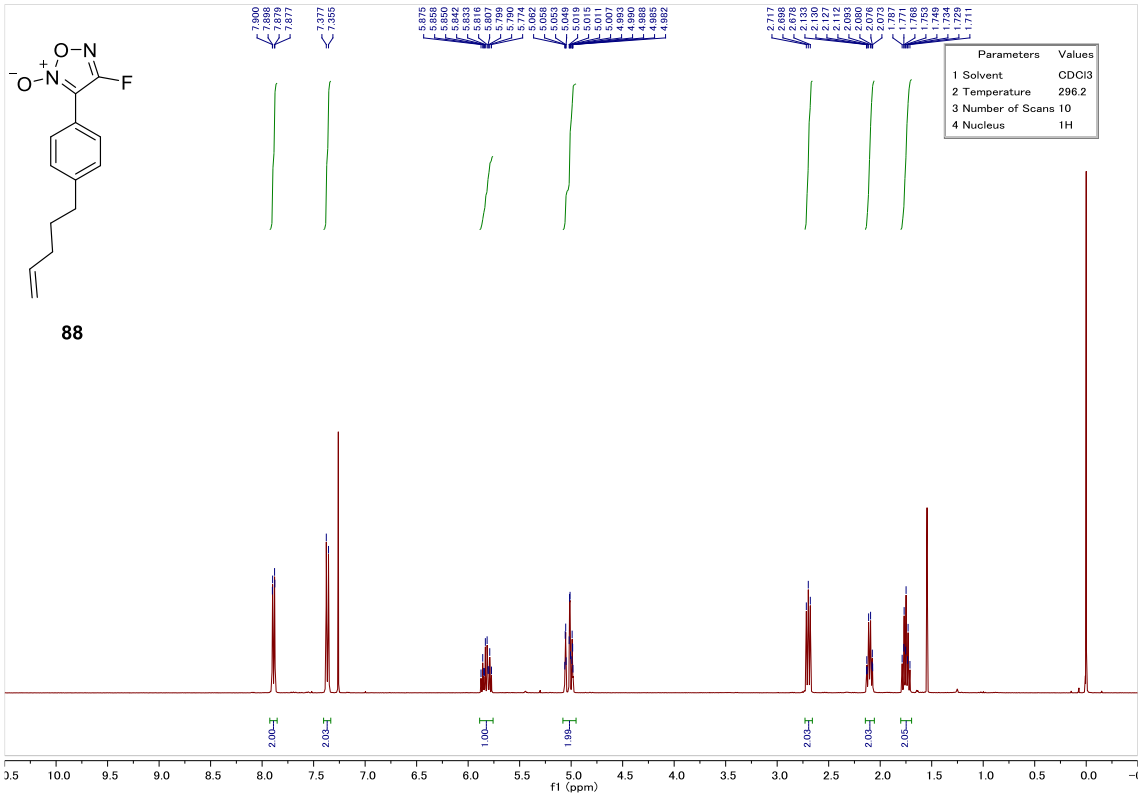
Donor

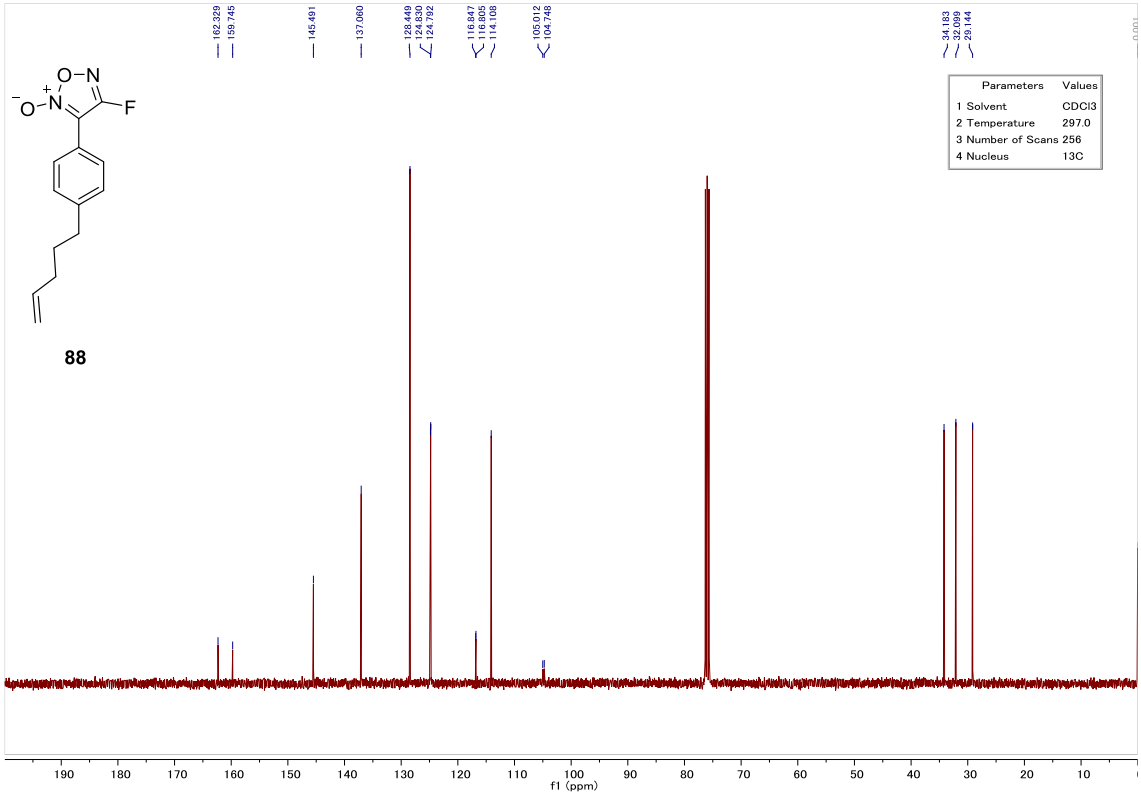

90
Parameters Values
1 Solvent CDCl3
2 Temperature 297.6
3 Number of Scans 16
4 Nucleus 1H
f1 (ppm)

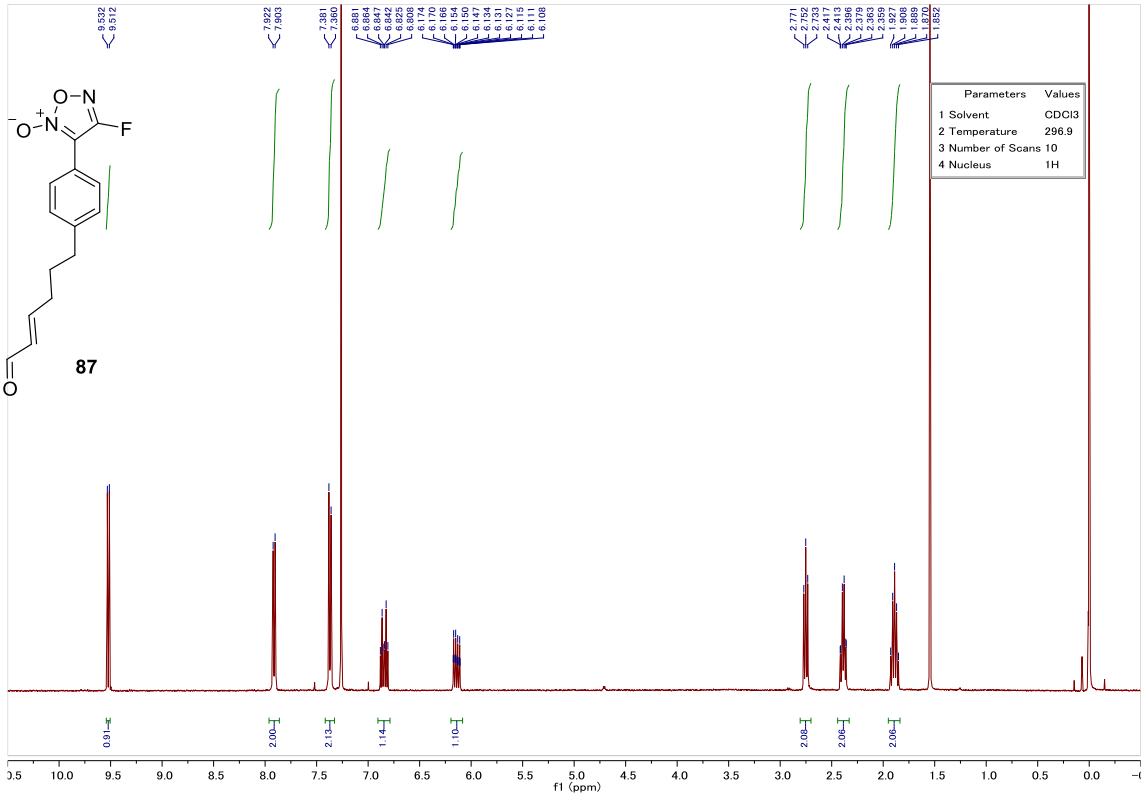

90
Parameters Values
1 Solvent CDCl3
2 Temperature 297.5
3 Number of Scans 128
4 Nucleus 13C
f1 (ppm)

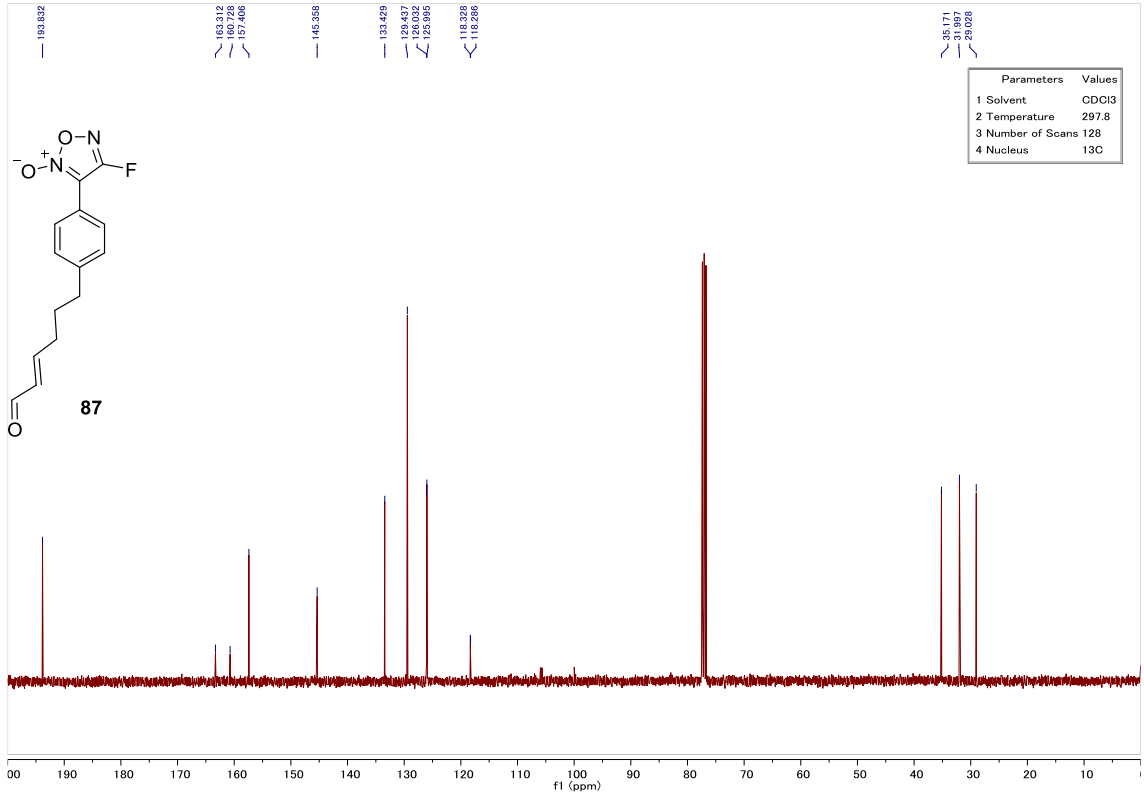

89
Parameters Values
1 Solvent CDCl3
2 Temperature 297.1
3 Number of Scans 16
4 Nucleus 1H
f1 (ppm)

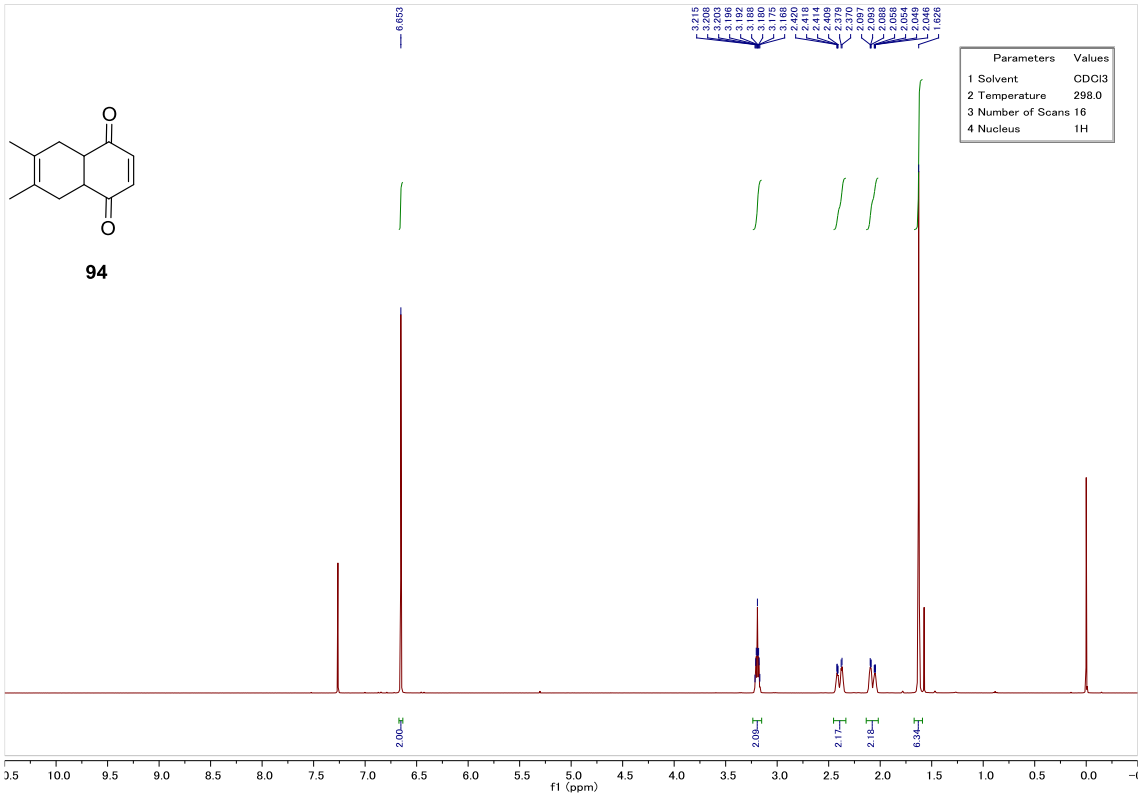

89
Parameters Values
1 Solvent CDCl3
2 Temperature 297.9
3 Number of Scans 256
4 Nucleus 13C
f1 (ppm)

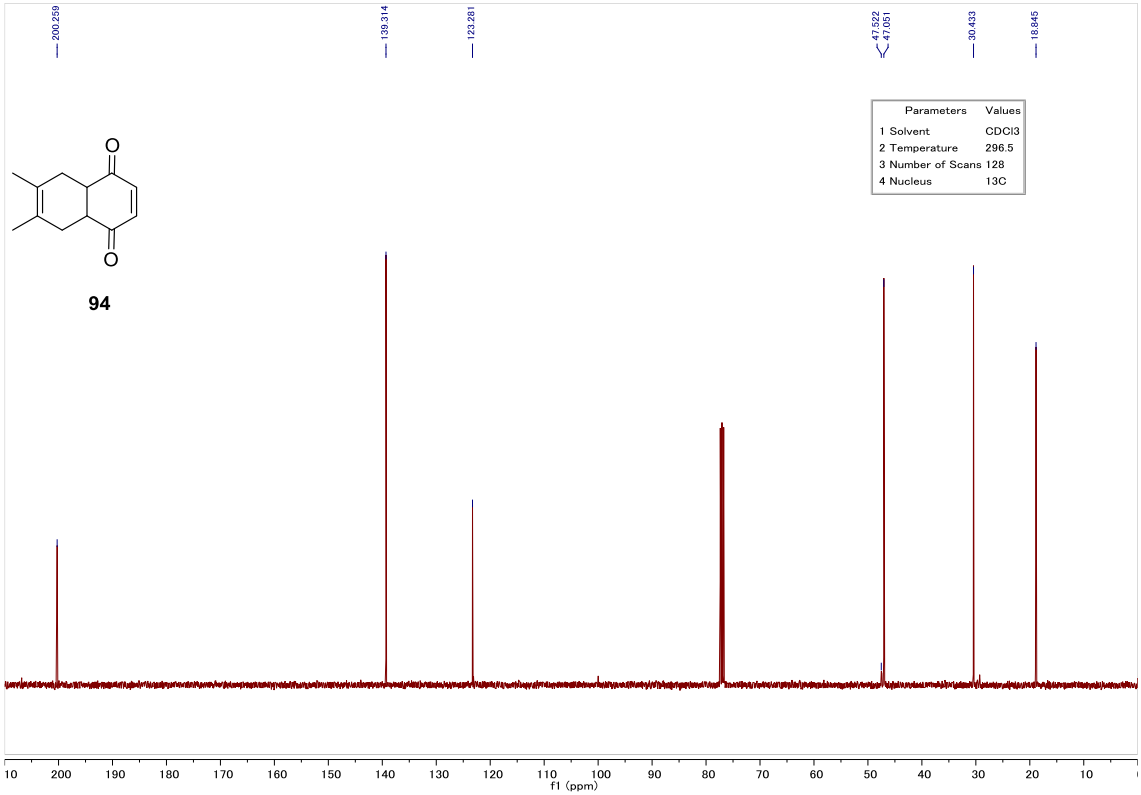

88
Parameters Values
1 Solvent CDCl3
2 Temperature 296.2
3 Number of Scans 10
4 Nucleus 1H
f1 (ppm)

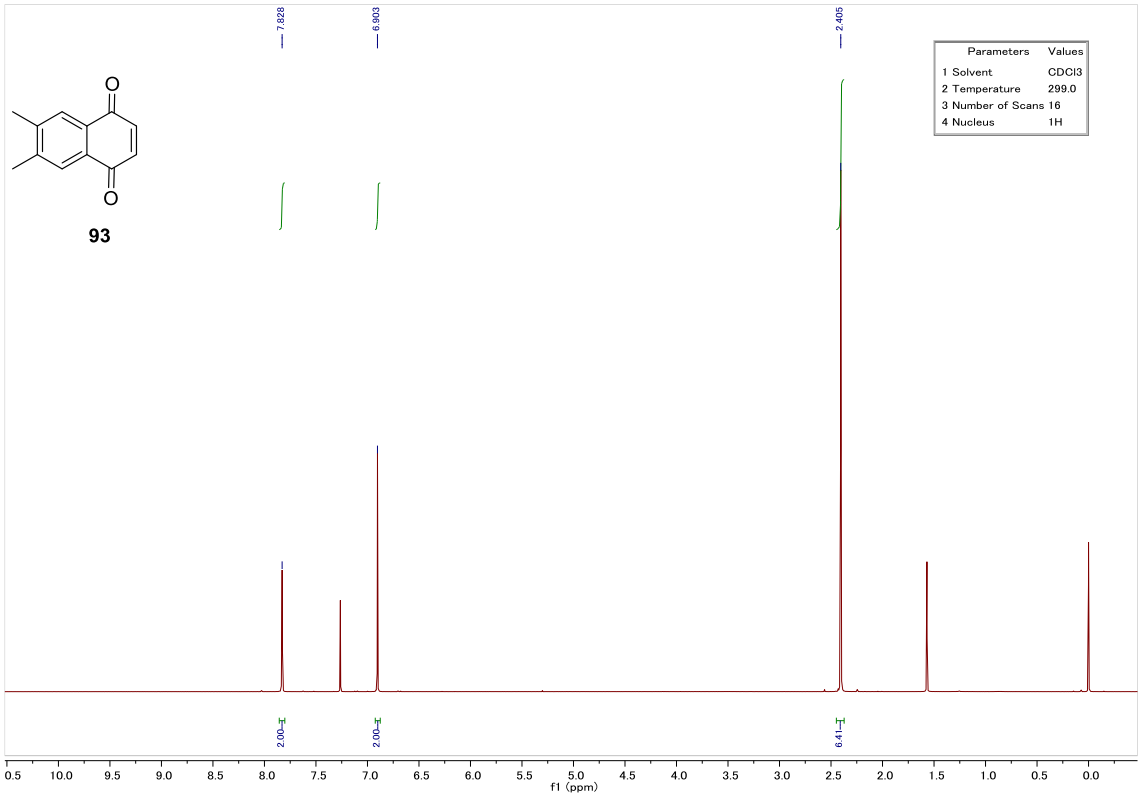

88
Parameters Values
1 Solvent CDCl3
2 Temperature 297.0
3 Number of Scans 256
4 Nucleus 13C
f1 (ppm)

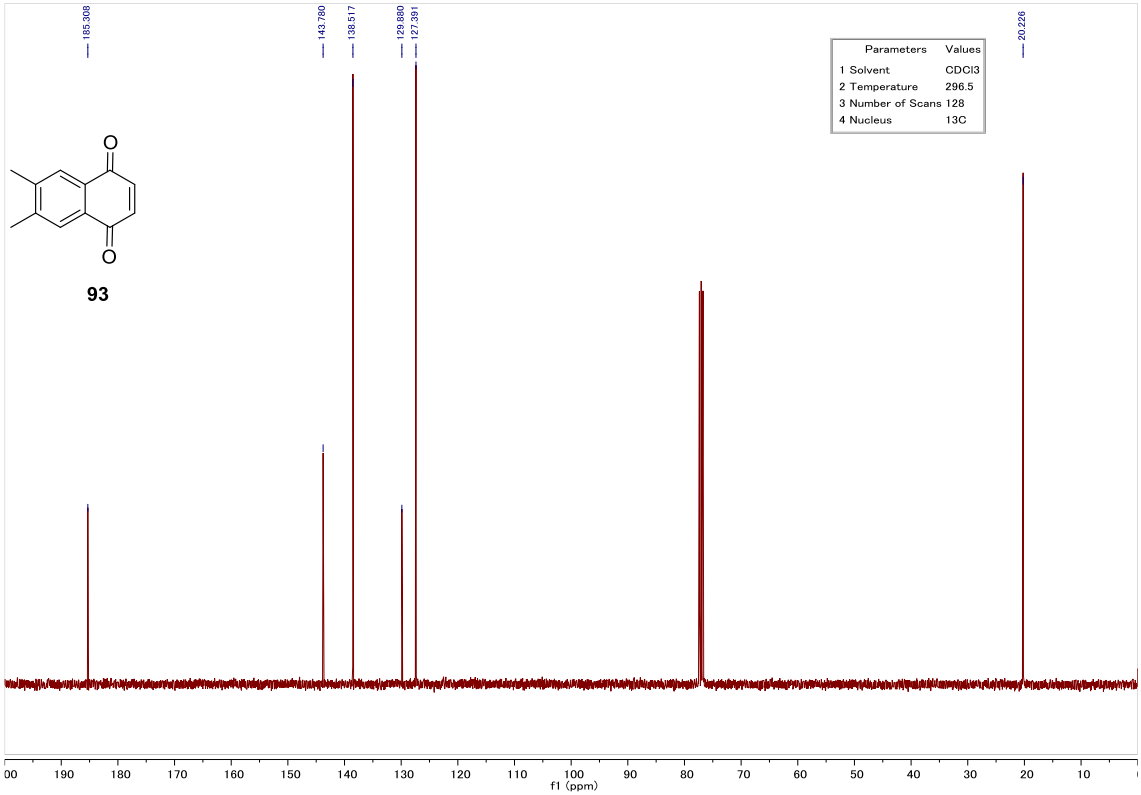

9.532
9.512
7.922
7.903
7.381
7.360
6.881
6.864
6.847
6.842
6.825
6.808
6.174
6.170
6.166
6.154
6.150
6.147
6.134
6.131
6.127
6.115
6.111
6.108
2.771
2.752
2.733
2.417
2.413
2.398
2.379
2.363
2.359
1.927
1.908
1.889
1.870
1.852
Parameters Values
1 Solvent CDCl3
2 Temperature 296.9
3 Number of Scans 10
4 Nucleus 1H
87
0.91
2.00
2.13
1.14
1.10
2.08
2.06
2.06
f1 (ppm)

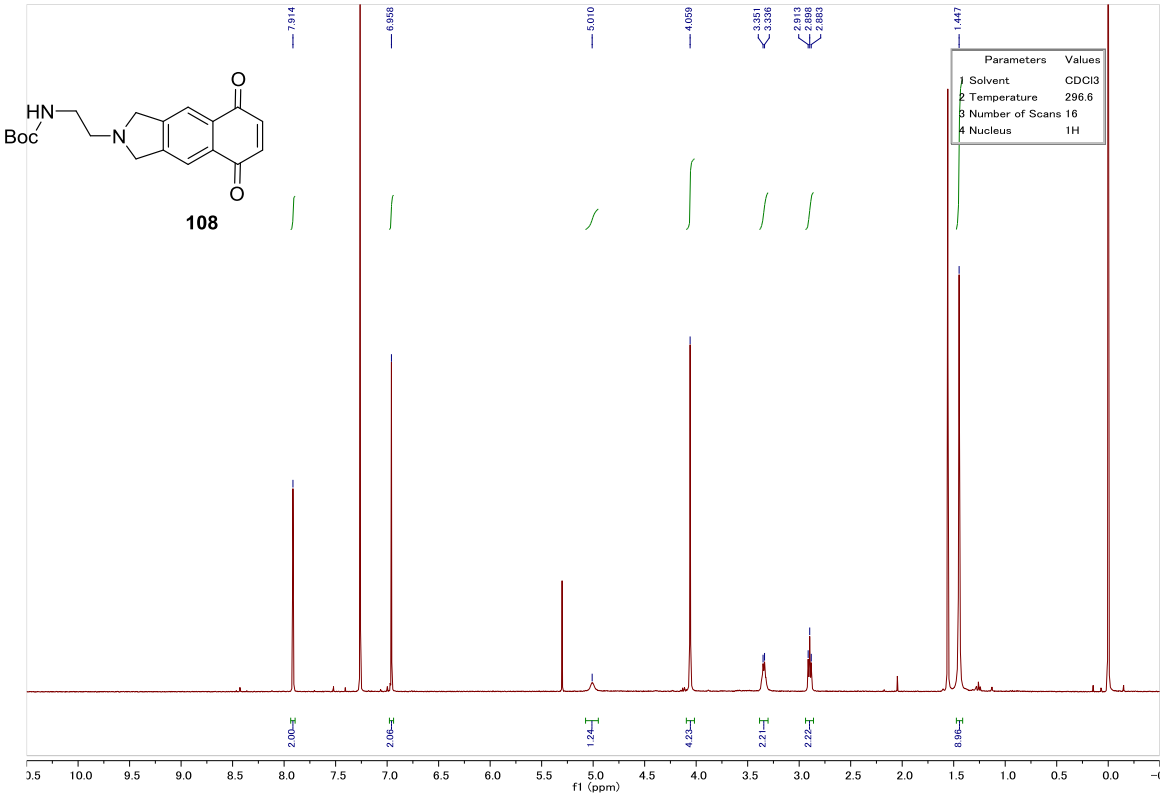

193.832
163.312
160.728
157.406
145.358
133.429
129.437
129.032
125.995
118.328
118.286
35.171
31.997
29.028
Parameters Values
1 Solvent CDCl3
2 Temperature 297.8
3 Number of Scans 128
4 Nucleus 13C
87
f1 (ppm)

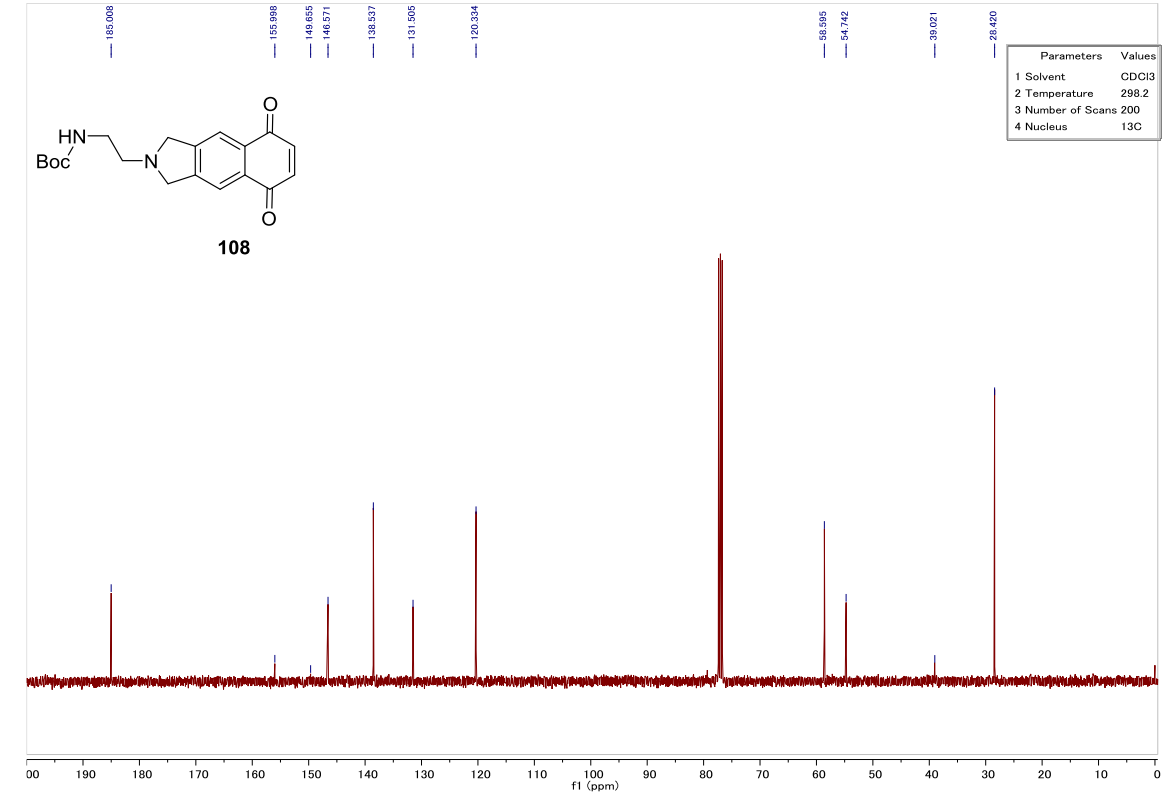

6.653
3.215
3.208
3.203
3.196
3.192
3.188
3.180
3.175
3.168
2.420
2.416
2.414
2.409
2.379
2.370
2.093
2.088
2.058
2.054
2.049
2.046
1.626
Parameters Values
1 Solvent CDCl3
2 Temperature 298.0
3 Number of Scans 16
4 Nucleus 1H
O
O
94
2.00
2.09
2.17
2.18
6.34
f1 (ppm)

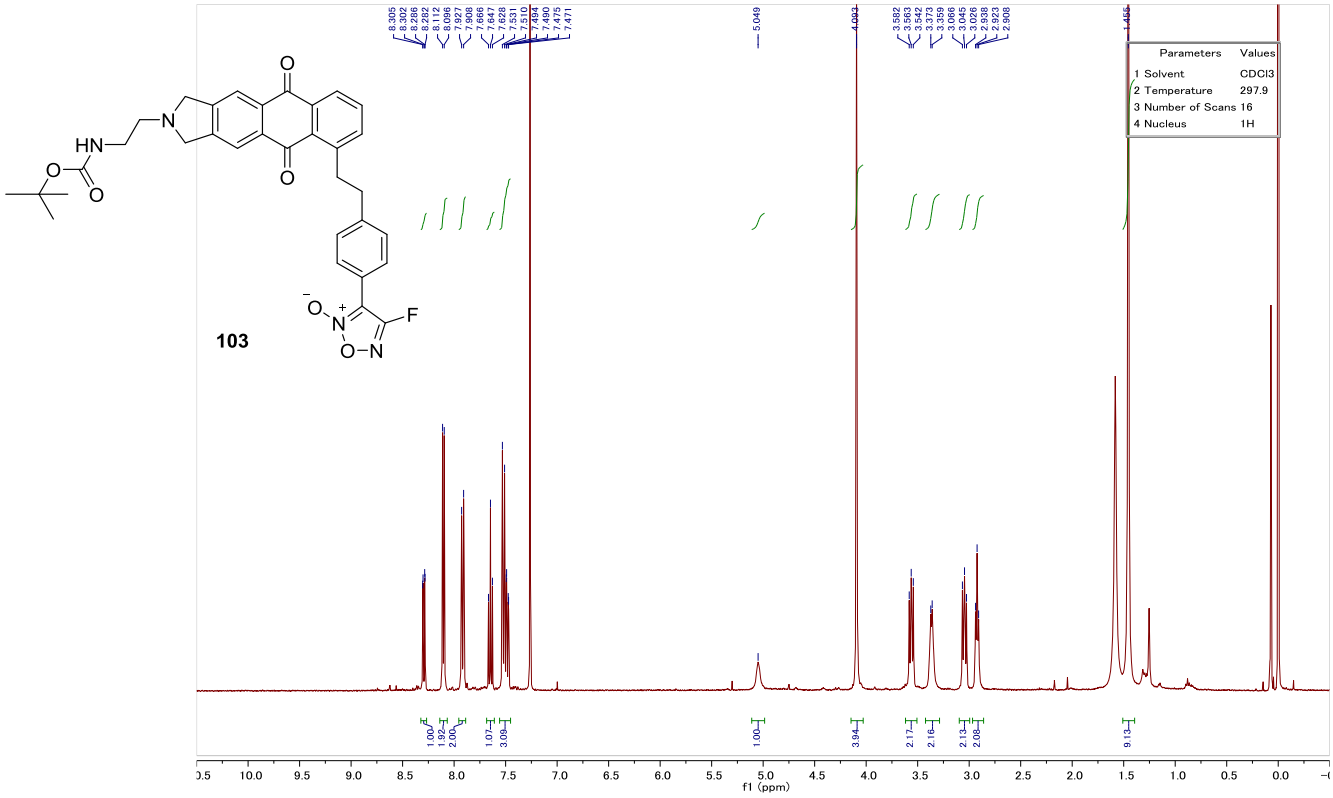

200.259
139.314
123.281
47.522
47.051
30.433
18.845
Parameters Values
1 Solvent CDCl3
2 Temperature 296.5
3 Number of Scans 128
4 Nucleus 13C
O
O
94
f1 (ppm)

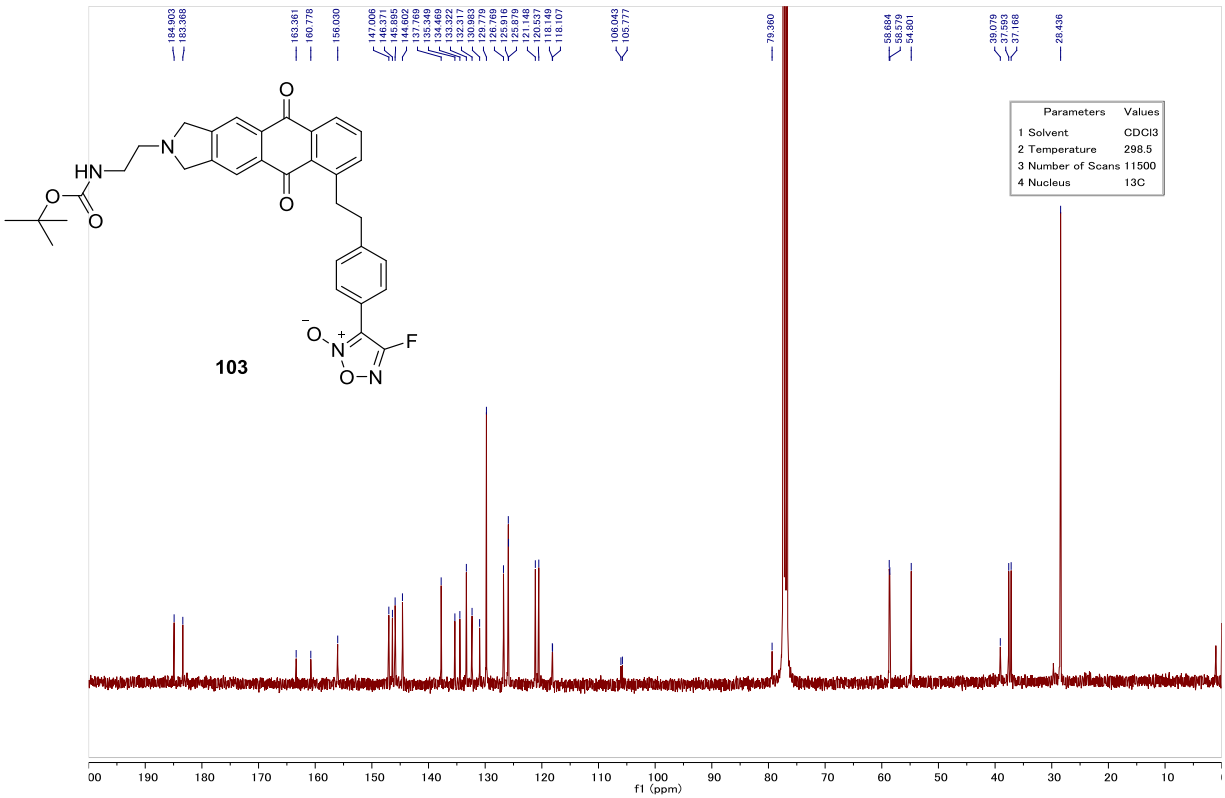

7.828
6.903
2.405
Parameters Values
1 Solvent CDCl3
2 Temperature 299.0
3 Number of Scans 16
4 Nucleus 1H
93
2.00
2.00
6.41
f1 (ppm)

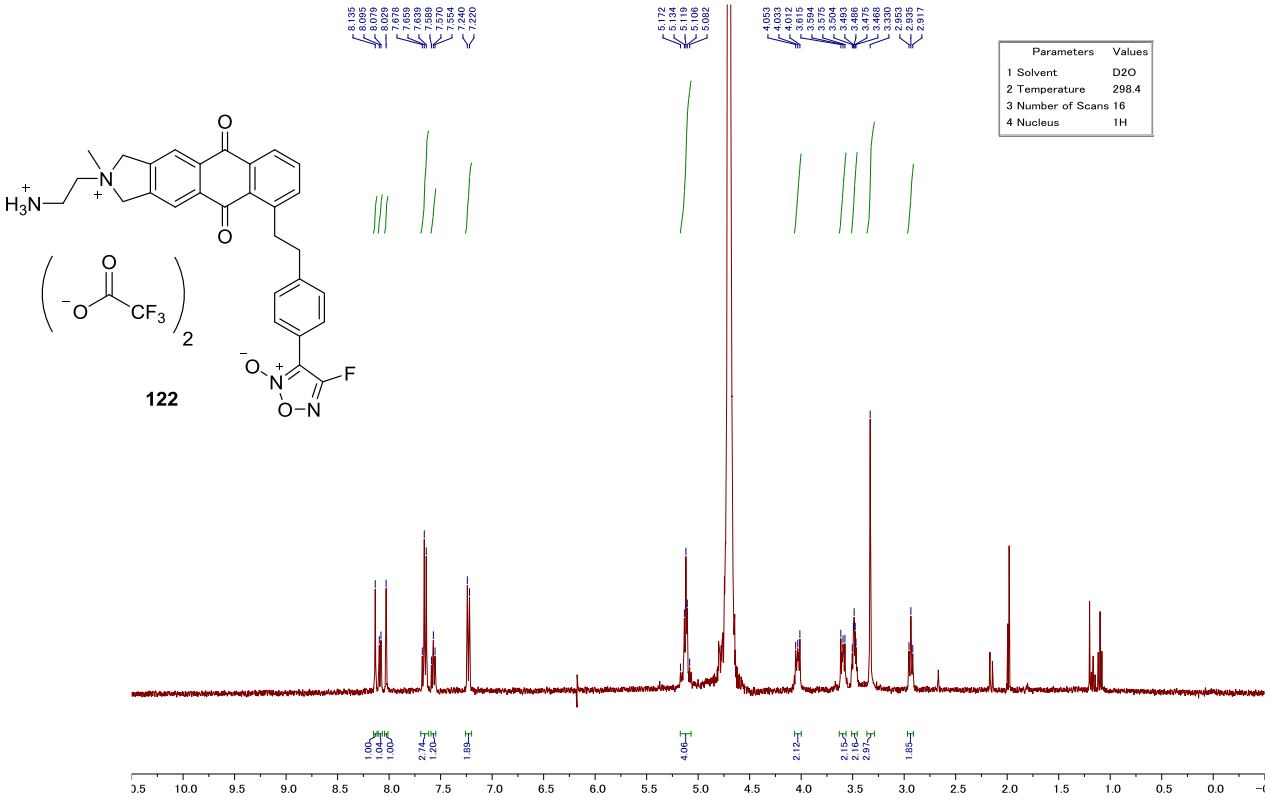

185.308
143.780
138.517
129.860
127.391
20.226
Parameters Values
1 Solvent CDCl3
2 Temperature 296.5
3 Number of Scans 128
4 Nucleus 13C
93
f1 (ppm)

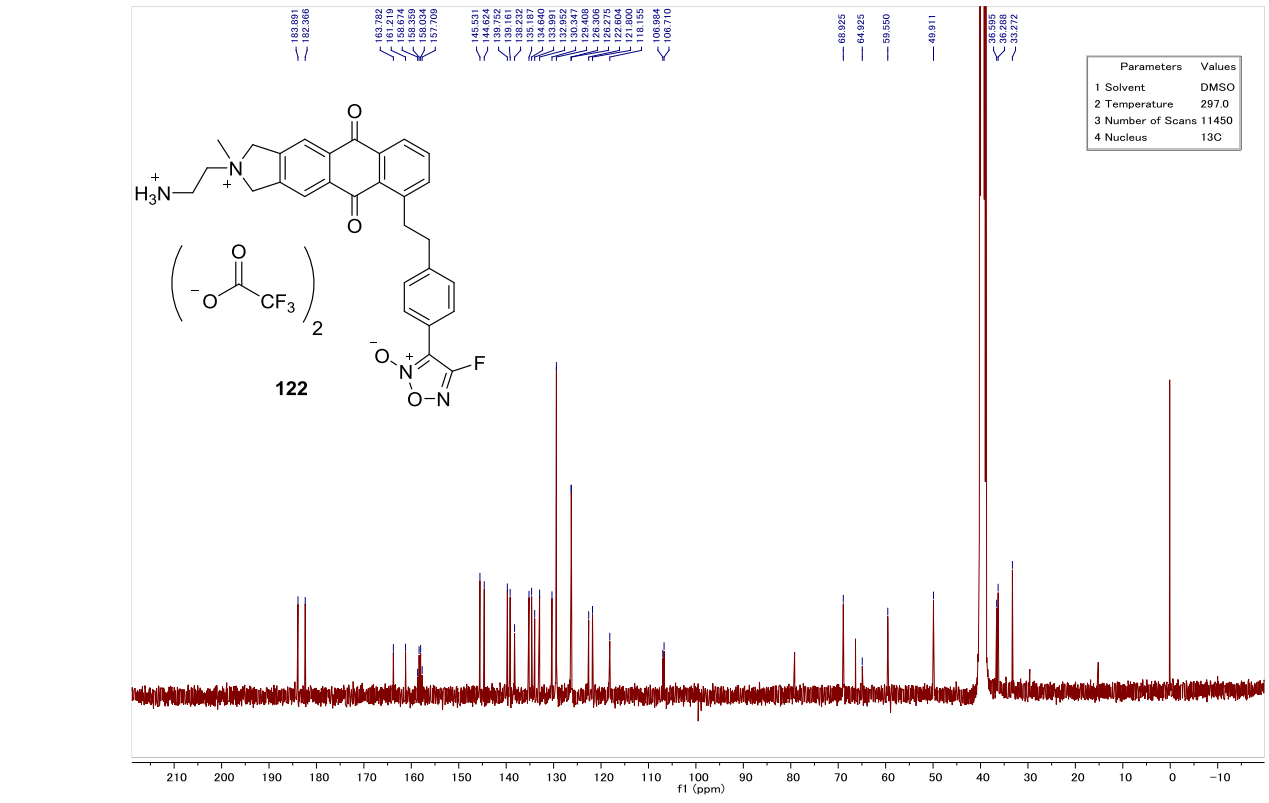

7.914
6.958
5.010
4.059
3.351
3.336
2.913
2.898
2.883
1.447
Parameters Values
1 Solvent CDCl3
2 Temperature 296.6
3 Number of Scans 16
4 Nucleus 1H
HN
Boc
N
O
O
108
2.00
2.06
1.24
4.23
2.21
2.22
8.96
f1 (ppm)


185.008
155.998
149.655
146.571
138.537
131.505
120.334
58.595
54.742
39.021
28.420
Parameters Values
1 Solvent CDCl3
2 Temperature 298.2
3 Number of Scans 200
4 Nucleus 13C
HN
Boc
N
O
O
108
f1 (ppm)

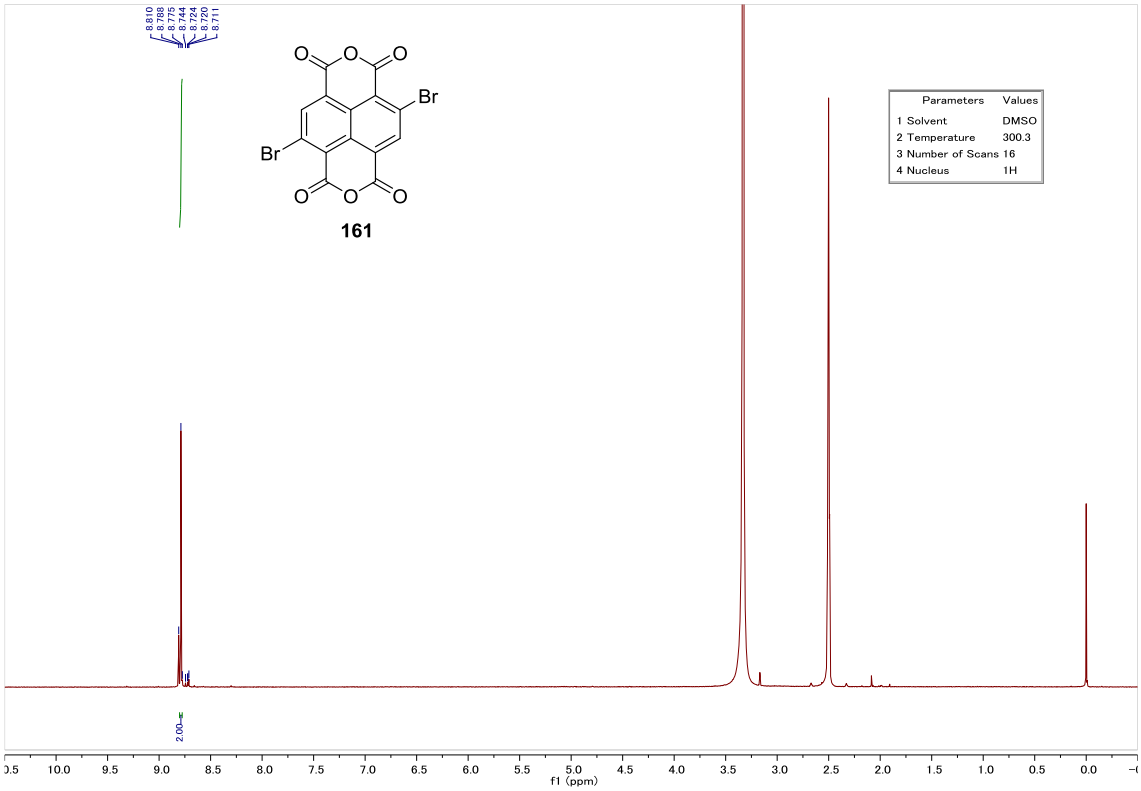

103
Parameters Values
1 Solvent CDCl3
2 Temperature 297.9
3 Number of Scans 16
4 Nucleus 1H
f1 (ppm)

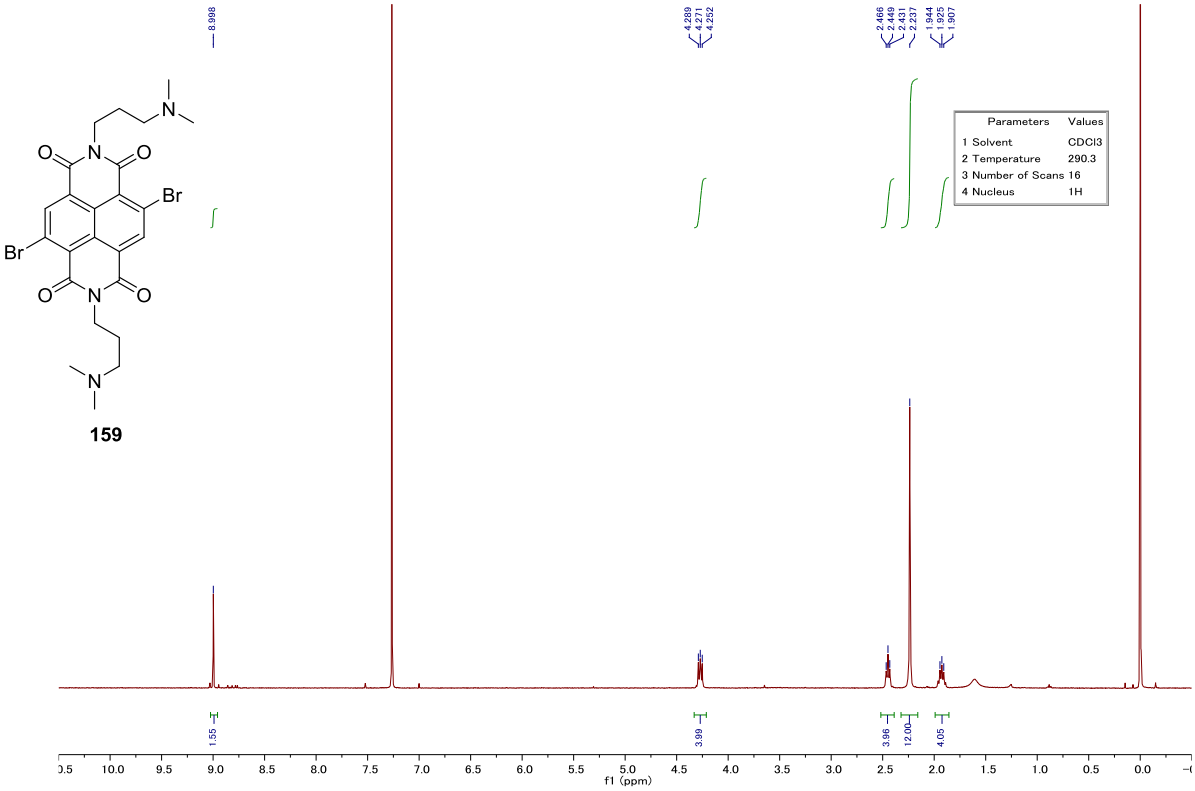

103
Parameters Values
1 Solvent CDCl3
2 Temperature 298.5
3 Number of Scans 11500
4 Nucleus 13C
f1 (ppm)

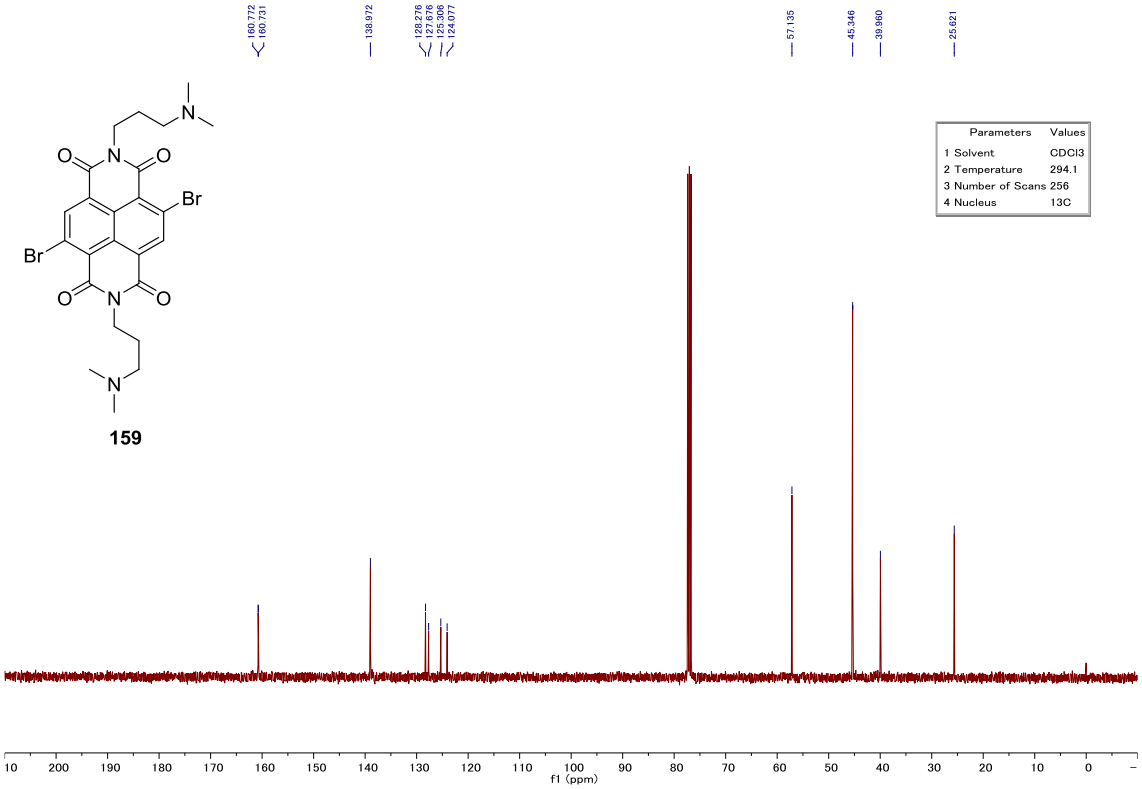

Parameters Values
1 Solvent D2O
2 Temperature 298.4
3 Number of Scans 16
4 Nucleus 1H
122

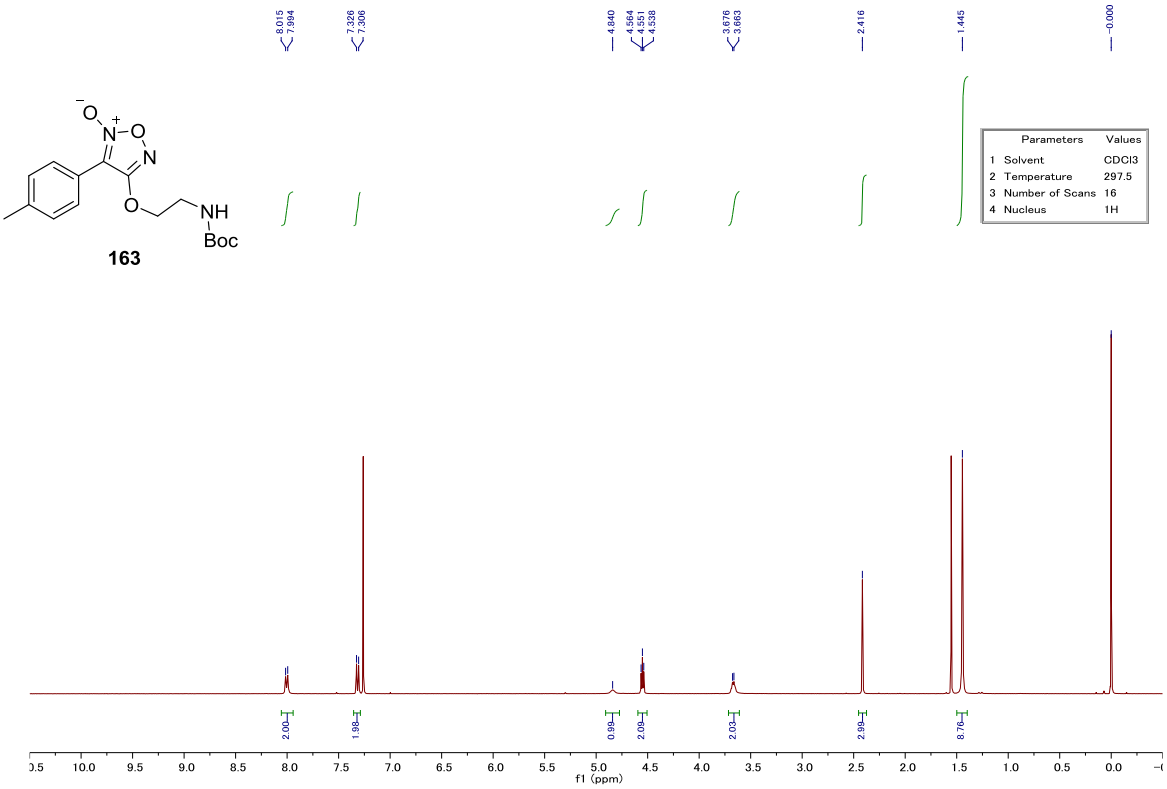

Parameters Values
1 Solvent DMSO
2 Temperature 297.0
3 Number of Scans 11450
4 Nucleus 13C
122
f1 (ppm)

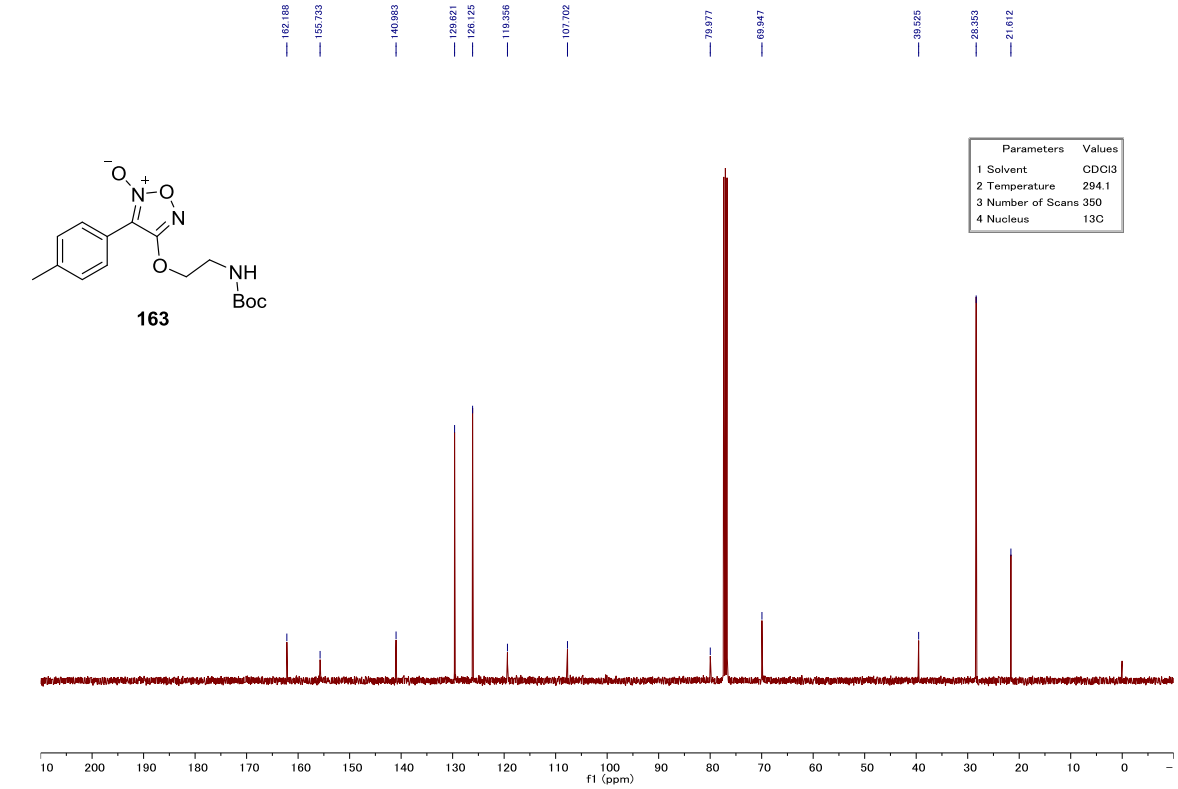
2. A Fluorescent Photoswitchable Water-Soluble Naphthalenediimide-Alkoxyfuroxan Visible Light Nitric-Oxide Donor

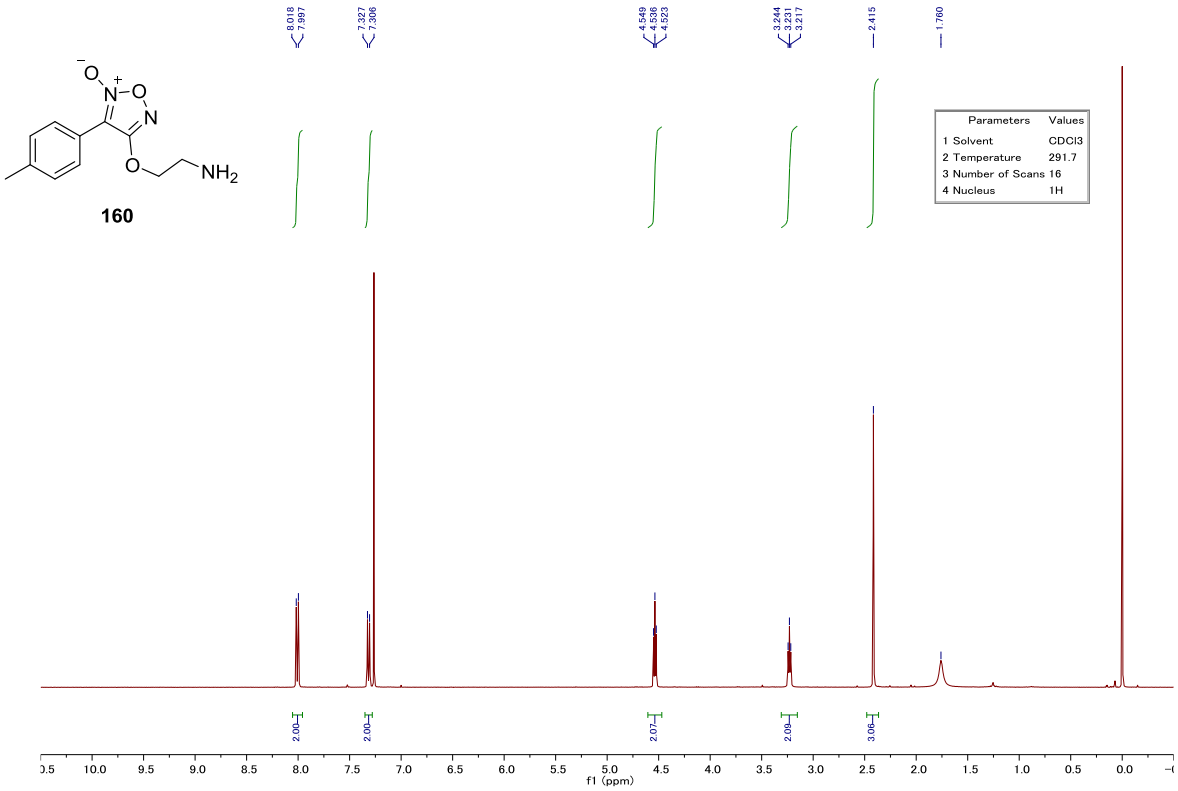

8.810
8.808
8.775
8.744
8.724
8.720
8.711
Br
Br
O
O
O
O
O
O
161
Parameters Values
1 Solvent DMSO
2 Temperature 300.3
3 Number of Scans 16
4 Nucleus 1H
2.00
f1 (ppm)

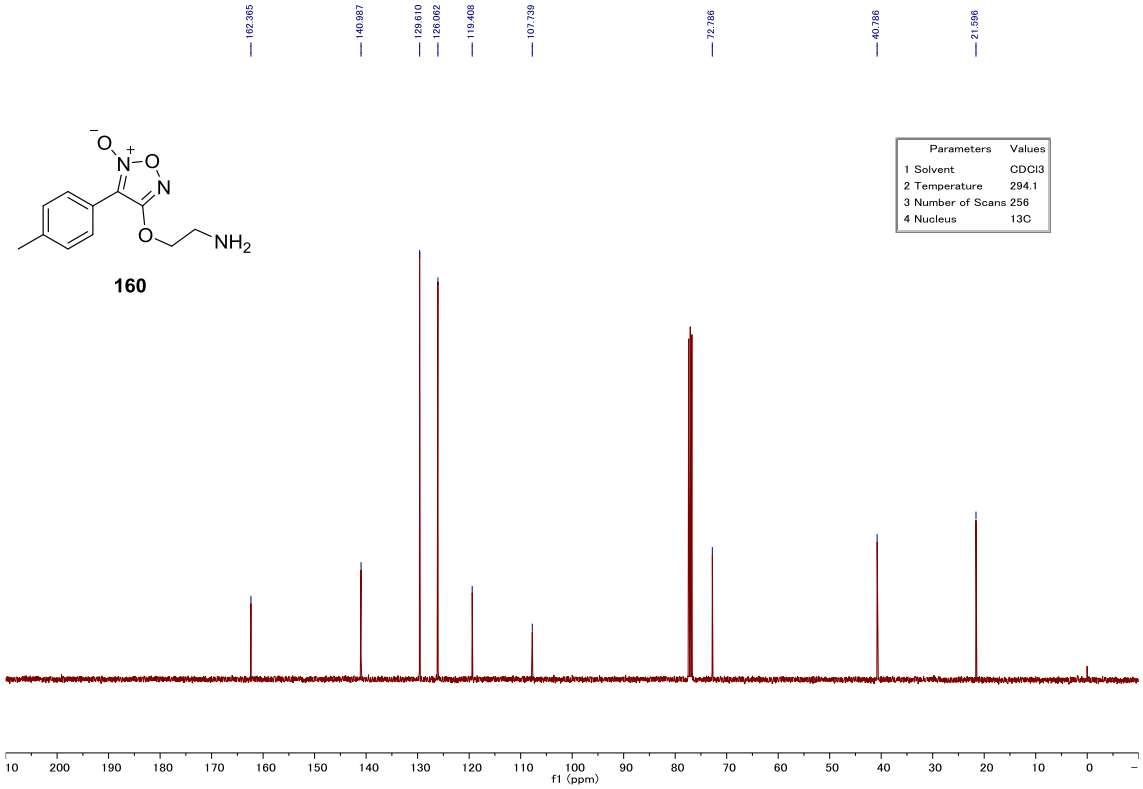

8.998
4.289
4.271
4.252
2.466
2.449
2.431
2.237
1.944
1.925
1.907
N
O
O
Br
Br
O
O
N
N
159
Parameters Values
1 Solvent CDCl3
2 Temperature 290.3
3 Number of Scans 16
4 Nucleus 1H
1.55
3.99
3.96
12.00
4.05
f1 (ppm)

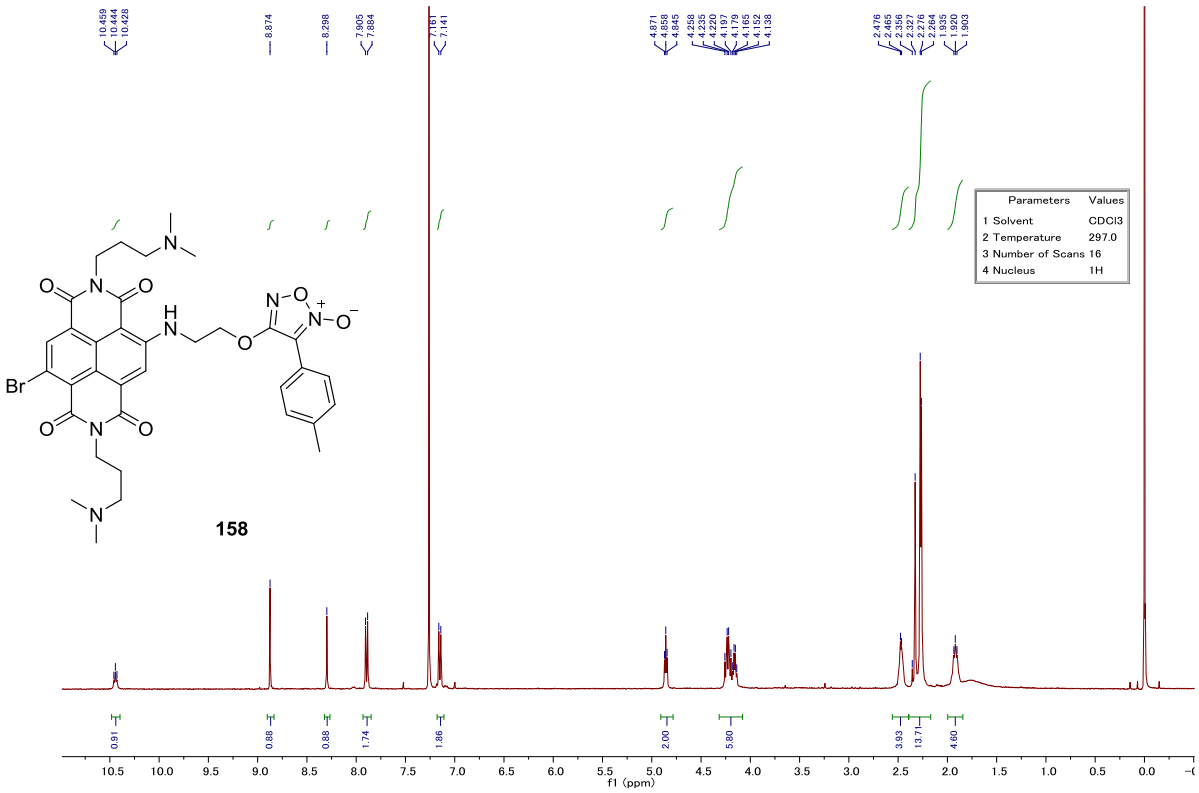

160.772
160.731
138.972
128.276
127.676
125.098
124.077
57.135
45.346
39.960
25.821
Parameters Values
1 Solvent CDCl3
2 Temperature 294.1
3 Number of Scans 256
4 Nucleus 13C
Br
Br
159
f1 (ppm)

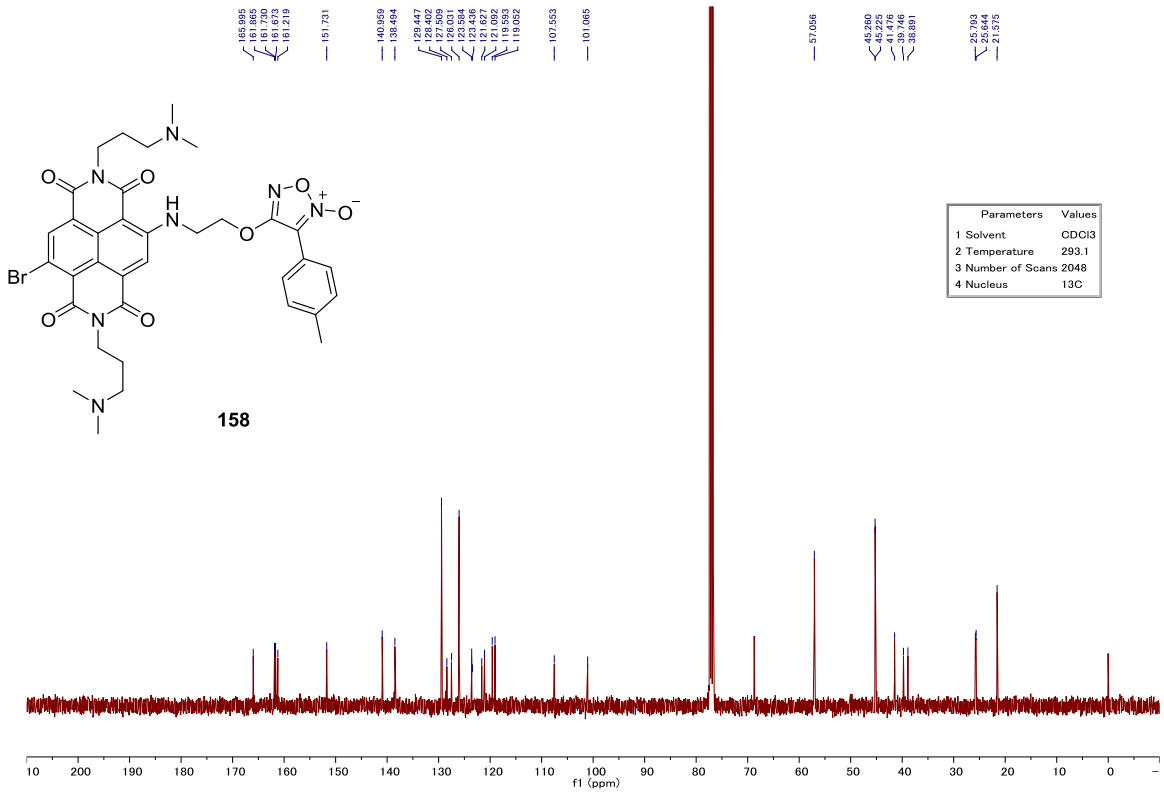

8.015
7.994
7.326
7.306
4.840
4.564
4.551
4.538
3.676
3.663
2.416
1.445
0.000
Parameters Values
1 Solvent CDCl3
2 Temperature 297.5
3 Number of Scans 16
4 Nucleus 1H
NH
Boc
163
2.00
1.98
0.99
2.09
2.03
2.99
8.76
f1 (ppm)

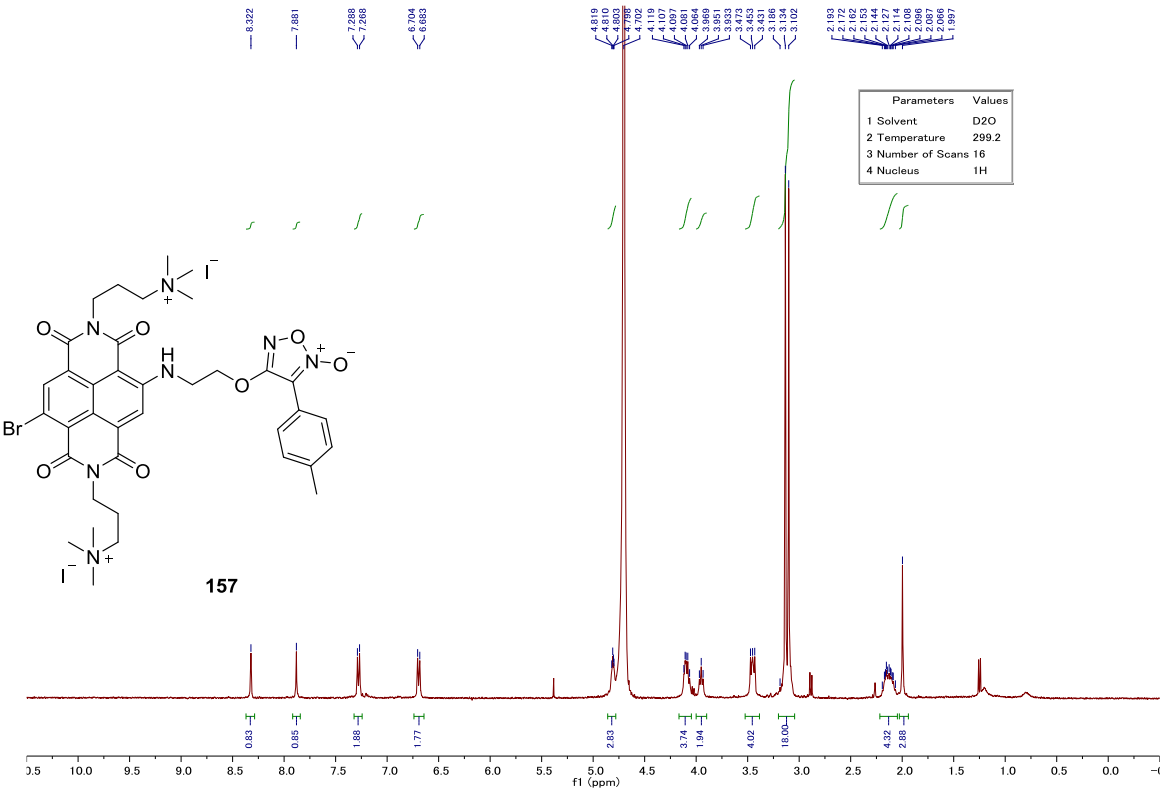

162.188
155.733
140.983
129.621
126.125
119.356
107.702
79.977
69.947
39.525
28.353
21.612
Parameters Values
1 Solvent CDCl3
2 Temperature 294.1
3 Number of Scans 350
4 Nucleus 13C
NH
Boc
163
f1 (ppm)

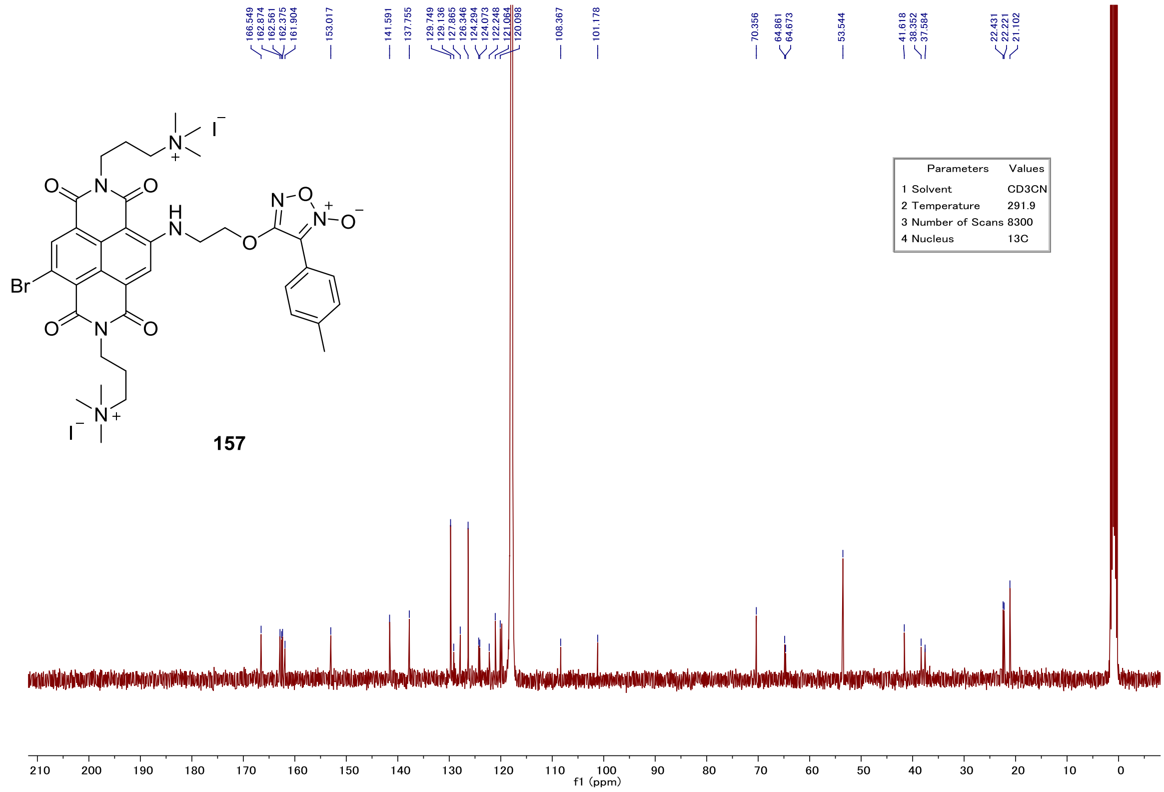

8.018
7.997
7.327
7.306
4.549
4.536
4.523
3.244
3.231
3.217
2.415
1.760
Parameters Values
1 Solvent CDCl3
2 Temperature 291.7
3 Number of Scans 16
4 Nucleus 1H
NH2
160
2.00
2.00
2.07
2.09
3.06
f1 (ppm)


162.365
140.987
129.610
126.082
119.408
107.739
72.788
40.766
21.596
Parameters Values
1 Solvent CDCl3
2 Temperature 294.1
3 Number of Scans 256
4 Nucleus 13C
160
f1 (ppm)


Parameters Values
1 Solvent CDCl3
2 Temperature 297.0
3 Number of Scans 16
4 Nucleus 1H
158
f1 (ppm)


Parameters Values
1 Solvent CDCl3
2 Temperature 293.1
3 Number of Scans 2048
4 Nucleus 13C
158
f1 (ppm)


Parameters Values
1 Solvent D2O
2 Temperature 299.2
3 Number of Scans 16
4 Nucleus 1H
157
f1 (ppm)



166.549
162.874
162.561
162.375
161.904
153.017
141.591
137.755
129.749
129.136
127.865
126.346
124.294
124.073
122.248
121.064
120.098
108.367
101.178
70.356
64.861
64.673
53.544
41.618
38.352
37.584
22.431
22.221
21.102
Parameters Values
1 Solvent CD3CN
2 Temperature 291.9
3 Number of Scans 8300
4 Nucleus 13C
f1 (ppm)

157