

WATER-SOLUBLE FULLERENES & MOLECULAR MACHINES:
SUPRAMOLECULAR APPROACHES TO NANOMEDICINE

A thesis submitted for the degree of Doctor of Philosophy



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ABSTRACT

Water-Soluble Fullerenes & Molecular Machines: Supramolecular Approaches to Nanomedicine

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The incursion of the physical sciences into the medical sphere has led to myriad investigations in the use of nanomaterials for diagnostic and therapeutic purposes. In particular, the fullerenes show great promise in a number of areas because of their unique molecular properties. The chemistry of these carbon building blocks can be harnessed through covalent functionalisation and incorporation into supramolecular architectures such as extended self-assembled structures and molecular machines based on mechanically interlocked molecules. This thesis describes the synthesis and characterisation of such supramolecular structures incorporating fullerenes, whose potential utility in nanomedicine is demonstrated.

Water-solubilisation of fullerene C_{60} is achieved in the first part of this thesis by covalent functionalisation with triethylene glycol monoethyl ether. The resulting complex molecular structure is elucidated and a new method developed for interpreting elemental and thermal gravimetric analyses. It is highly soluble in water (37 mg/mL) and exhibits concentration-dependent photoluminescent behaviour suitable for biomedical applications. Ultraviolet-Visible absorption data points to the formation of self-assembled structures. Over time, these self-assembled structures grow considerably into hydrogels. Optical and scanning electron microscopies show the hydrogels have a tricontinuous hierarchical structure of great promise for use in drug delivery. The same functionalisation protocol is applied to higher fullerenes C_{70} , C_{84} and C_{90-92} , and extensive hierarchical structures are formed for the latter two. It is proposed that the presence of corannulene-like hydrophobic regions in the functionalised fullerenes affects the formation of hierarchical structures. Atomic force microscopy nano-indentation shows that the strongest of the formed hydrogels (C_{90-92} derivative, $\bar{E} = 432 \pm 286$ kPa) fares remarkably well against other supramolecular hydrogel competitors.

In the second part of this thesis, fullerene C_{60} is functionalised for incorporation into a rotaxane-based molecular machine. Two new iodo-alkyne functionalised fullerene derivatives are isolated in this process. C_{60} is incorporated into a four-station [3]rotaxane with two peripheral naphthalene diimide stations and two ferrocene-functionalised macrocycles via a copper-catalysed click reaction. The anion-induced motion of the macrocycles leads to a tuneable photoluminescent response due to photoinduced electron transfer. C_{60} plays a crucial electron-accepting role in this setup. In this way, this fullerene-containing mechanically interlocked architecture demonstrates a unique platform for the sensing of chloride ions. The utility of this [3]rotaxane is expanded into a theranostic tool by the demonstration of tuneable singlet oxygen generation by the central fullerene moiety concomitant with the tuneable photoluminescence.

To the memory of my uncle and my aunt

Savo Rašović

and

Mara Borovac

У сјећање на мог стрица и моју тетку

Саво Рашовић

и

Мара Боровац

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PRESENTATIONS

CHAPTER 1

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Sample Number	Sample
1	TEGylated C ₆₀
2	TEGylated C ₆₀ hydrogel
3	TEGylated C ₇₀
4	TEGylated C ₈₄
5	TEGylated C ₉₀₋₉₂
6	C ₆₀ -functionalised [3]rotaxane
7	Bisalkyne C ₆₀ methanofullerene
8	Bisalkyne malonate
9	Monoiodo-bisalkyne C ₆₀ methanofullerene
10	Bisiodoalkyne C ₆₀ methanofullerene
11	Azide-functionalised NDI precursor
12	Bis-triazole axle
13	Bis-triazolium axle salt
14	Ferrocene-functionalised macrocycle precursor

Нека будућност каже истину и свакога оцјени по раду и заслуги.

Њихова је садашњост а моја будућност за коју сам и радио.

Let the future tell the truth and evaluate each one according to his work and accomplishments. The present is theirs; the future, for which I really worked, is mine.

— NIKOLA TESLA

LITERATURE REVIEW

The fullerenes and their salient properties are introduced. Popular water-solubilisation methods are summarised. A variety of applications of fullerenes in medicine are reviewed. Molecular machines are briefly introduced, and some such systems containing fullerenes are highlighted.

1.1 FULLERENES

1.1.1 Why So Interesting?

The fullerenes constitute a distinct allotrope in the family of carbon nanomaterials. Their unique cage-like structure and electron-deficient nature lead to fascinating properties which have made them a promising focus of research in an array of fields. The most abundant (and therefore most studied) fullerene, C_{60} , has a van der Waals diameter of 10.18 Å [1], positioning it well within the realm of nanotechnology. The origin of the fullerenes' key properties and rich chemistry lies in the unique hybridisation of the strained carbon-carbon bonds; whilst the carbon atoms in graphene and carbon nanotubes are sp^2 -hybridised to give hexagonal networks, and the tetrahedral network of diamond is sp^3 -hybridised, the fullerenes are made

up of a network of both hexagons (1,3,5-cyclohexatriene¹) and pentagons ([5]radialene), as shown in Fig. 1, leading to an average hybridisation of $sp^{2.278}$ [3]. The presence of pentagonal

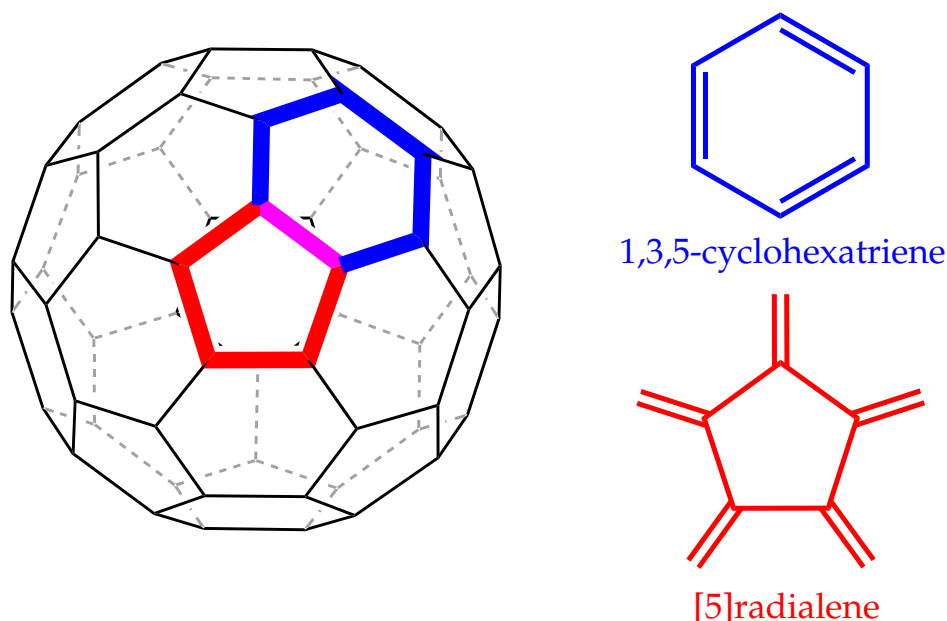


Figure 1: 1,3,5-Cyclohexatriene and [5]radialene sub-units, as found in C₆₀ (double bonds are emitted for clarity).

rings introduces a significant amount of strain into the network,² pyramidalising each carbon atom to give a rehybridised three-dimensional structure as π orbitals with pure p character are only allowed in planar configurations. This rehybridisation underpins three crucial properties of fullerenes due to the fact that the π orbitals are now asymmetric and so they extend further outwards than inwards—perhaps counter-intuitively, their chemical behaviour is thus akin to electron-deficient alkenes rather than electron-rich aromatic systems [4]. First, the fullerenes are outstanding electron acceptors, with C₆₀ exhibiting six reversible reductions [5]. Second, the exterior of the cages can be decorated using a vast range of chemical reactions—this will be discussed further in Section 1.1.2. Finally, the relative inertness of the interior of the cages facilitates the ability of fullerenes to capture and thus isolate ionic, atomic and even small

¹ Differentiated from benzene due to the difference in bond lengths: the [6,6] bonds (double) between adjacent hexagons are 1.38 Å, while the [6,5] bonds (single) which form the border between hexagons and pentagons are 1.45 Å [2].

² This strain accounts for approximately 80% of the heat of formation of C₆₀ [3].

molecular species—this will be discussed further in Section 1.1.3. Considerations of both topology and orbital rehybridisation give the fullerenes their unique chemical properties.

1.1.1.1 *Size Differences*

Whilst the fullerenes are by themselves a distinct allotrope of carbon, there is considerable variation within the family: the cages can be made up of different numbers of carbon atoms, giving rise to different molecular shapes and sizes. This only increases their versatility and gives researchers considerably greater freedom in picking and tailoring the molecules' properties. C_{60} is the smallest stable fullerene which obeys the isolated pentagon rule (IPR)—its beautiful, football-like truncated icosahedron structure (I_h symmetry point group) leads to every carbon atom environment being identical, as elegantly shown by the sole peak in the ^{13}C NMR spectrum. Higher fullerenes exist, with adherence to the IPR a necessity for stability. Bigger cages have an increased number of isomers, making isomeric isolation difficult and costly. Isomeric variation also leads to differences in shape and symmetry; as an example, C_{80} has seven IPR isomers each with a different symmetry group, and C_{84} has 24 IPR isomers spread over nine symmetry groups [4].

These differences in size and shape have significant effects on both the optical properties and chemical behaviour of the fullerenes. The absorption spectra of the fullerenes change as the size of the conjugated system increases: with slight variations depending on solvent, C_{60} is an intense purple colour, C_{70} red like wine, and C_{76-84} gradations of brown-green-yellow [6]. Generally speaking, the gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) decreases with increasing cage size leading to optical absorptions of lower energy, i.e. longer wavelength.

With regards to solubility, this decreases with increasing cage size [7]. Fullerenes are generally soluble in aromatic hydrocarbons and halogenated solvents, as well as carbon

disulphide (CS_2) [8]. Typically one of toluene, benzene, *o*-xylene, *o*-dichlorobenzene (*o*-DCB) or CS_2 is used due to the reasonable solubility offered and their ubiquity [9].

The final size effect regards the reactivities of the fullerenes. This will be covered in more depth in Section 1.1.2. For now it suffices to mention that the fullerenes generally exhibit reduced reactivity as the cage size increases; the departure from a curved C_{60} surface to a more planar, graphitic topology explains this.

1.1.1.2 Synthesis

The mechanism of fullerene formation still remains a mystery despite numerous attempts at explanation [10]. That hasn't, however, precluded our ability to produce tens of tons of C_{60} per year [11]. This process put forward by Murayama *et al.* for Frontier Carbon Corporation (FCC) gives a soluble product mixture of 60% C_{60} and 25% C_{70} , while the remaining 15% are higher fullerenes up to C_{96} . These are soluble in the organic solvents used for fullerene separation and constitute only 20% of the solid mass retrieved from the reaction chamber; the remaining 80% is insoluble carbonaceous soot. This compares favourably to the first process to produce fullerenes on the gram-scale, the standard Krätschmer-Huffman arc discharge, where the fullerene content was $\leq 5\%$ and the relative intensities of the fullerene species were similar [12]. In this process, which has become the standard method for fullerene synthesis, electrical discharge creates a plasma which evaporates graphite in a helium atmosphere. That pioneering work built upon the initial breakthrough discovery of C_{60} five years earlier where it was produced using a low-yield laser ablation method [13]. Whilst it is now evident that the production of C_{60} and C_{70} has been successfully scaled-up, thus significantly driving down their cost,³ access to large quantities of higher fullerenes still remains an issue. Even

³ C_{60} can now be easily bought in large quantities at a price comparable to any standard laboratory chemical. MER Corporation of Arizona, USA sells 99+% pure batches of >100 g at \$20 per gram. As a good example to see how the price and rarity increases with cage size, the same company sells C_{70} of the same purity for \$325 per gram, up to 25 g. (Prices correct as of September 2018).

more pressing is the difficulty in scalably synthesising endohedral fullerenes, something of crucial importance to numerous future technologies, which will be discussed in Section 1.1.3.

Both size and synthesis issues impact the processability of different fullerenes and, as will be seen, their derivatives. Practical considerations of solubility, reactivity and scalable production methods play a great role in determining whether a fullerene-based technology can feasibly go to market; this is of particular importance in the pharmaceutical industry and will be touched upon throughout this review.

1.1.2 *Exohedral Functionalisation*

The primary driving force for the chemical addition of moieties to the outside of fullerene cages is the preferential release of the immense amount of strain (see Footnote 2). Strain is released because the carbon atoms of the cage which form the covalent bridge to the external moiety now have their tetravalency saturated and so rehybridise to the un-strained sp^3 configuration, breaking the conjugated system. The number of different reactions which have been carried out on fullerenes is immense and barely a fraction of them can be covered in this section.⁴ The spotlight will be shone here on a selection of three reactions which are particularly relevant to the development of water-soluble fullerene derivatives for biomedical applications. It should be noted that, unless stated otherwise, all reactions in this section were done with C_{60} .

The Prato [15] and Bingel [16] reactions, named after their discoverers, are very popular fullerene reactions lauded for their ability to introduce a vast array of complex functional groups to the fullerene derivative in simple, one-pot processes. For this reason they have been

⁴ Readers are directed to the excellent monograph by Hirsch and Brettreich for a more complete overview of the library of fullerene reactions [14].

employed in numerous application areas⁵ besides water-solubilisation [14]. Their general characteristics are described here first before some specific examples of their exploitation for imparting water-solubility are given. The third reaction covered—hydroxylation—is possibly the most popular when it comes to synthesising water-soluble fullerene derivatives. The introduction of multiple hydroxyl groups to the fullerene cage leads to a final multi-adduct product which is a mixture of a number of different regioisomers. Similar uncontrolled reactions to produce water-soluble derivatives can be achieved using amino groups [19] and amino acids [20]. However, the advent of stoichiometrically controllable fullerene reactions led to the ability to synthesise and isolate regio-isomerically pure derivatives more easily, thus, function in a biomedical context can be more explicitly correlated to the molecular structure.

1.1.2.1 *Water-Solubilisation*

Pristine fullerenes are highly hydrophobic and so cannot be deployed in the body without being functionalised with hydrophilic groups first, given that water is the primary constituent of blood plasma, which is itself the main component of blood, into which medicines are delivered intravenously.⁶ Polar groups which are capable of hydrogen bonding with water molecules are the key to achieving water-solubility; in turn, water-solubility is the key to a longer retention time in the bloodstream due to decreased recognition as a foreign body by the immune system.

Covalent functionalisation is the surest way to producing fullerene-based systems viable for biomedical application as the structures of such molecules can be precisely defined (using X-ray crystallography) and their pharmaco-kinetic and -dynamic behaviour better

⁵ They are such robust reaction protocols that they have also been applied to functionalise carbon nanotubes [17, 18].

⁶ The favoured method of administering medicines as it is fastest and gives 100% bioavailability of the administered species.

understood and fine-tuned if need be. This is in comparison with two other methods of water-solubilisation:

1. *Encapsulation in water-soluble hosts*—Calixarenes are a good example of this type [21];
2. *Supramolecular/covalent incorporation into water-soluble polymers*—Giacalone *et al.* give a good overview [22].

The major issues with these two methods are: the lack of definition of molecular structure in solution; inhomogeneity of samples;⁷ and unpredictable and undesired side effects from the water-solubilising component(s).

1.1.2.2 Prato Reaction

This 1,3-dipolar cycloaddition of an azomethine ylide to a fullerene gives a stable pyrrolidinofullerene product in good yield. The ylide is a reactive intermediate produced *in situ* by the condensation of an α -amino acid with an aldehyde or ketone, followed by decarboxylation. The reaction mechanism is given in Fig. 2. The thermodynamically favoured product of this reaction involving C₆₀ has the pyrrolidine ring attached across a [6,6] bond, with the kinetically favoured attachment being across the [6,5] bond. Cardona *et al.* showed that these products were reversed (i.e. [6,6] adduct is kinetically favoured, [6,5] thermodynamically) in the case of endohedral metallofullerenes (EMFs) M₃N@C₈₀, leading to drastically different electrochemical behaviour [23]. This is surprising given that the difference between the mono-adducts is simply the addend location.⁸

The beauty of the Prato reaction is its ability to introduce multiple functional groups to the fullerene moiety. Referring to Fig. 2, groups R₁, R₂ and R₃ can be tailored to the

⁷ This is particularly true when long-chain hydrophilic polymers are involved and control over the precise length of polymer chains is impossible. Whilst more ethylene glycol units, for example, will lead to an increased solubility in polyethylene glycol (PEG)-based systems, the pharmacokinetic behaviour grows in complexity with increasing chain length due to increasing size and configurational entropy of the polymer chain. With a mixture of chain lengths in the sample then predicting behaviour within the body becomes even more difficult.

⁸ The effect of incarcerated endohedral species on the reactivities of fullerenes is discussed further in Section 1.1.3.

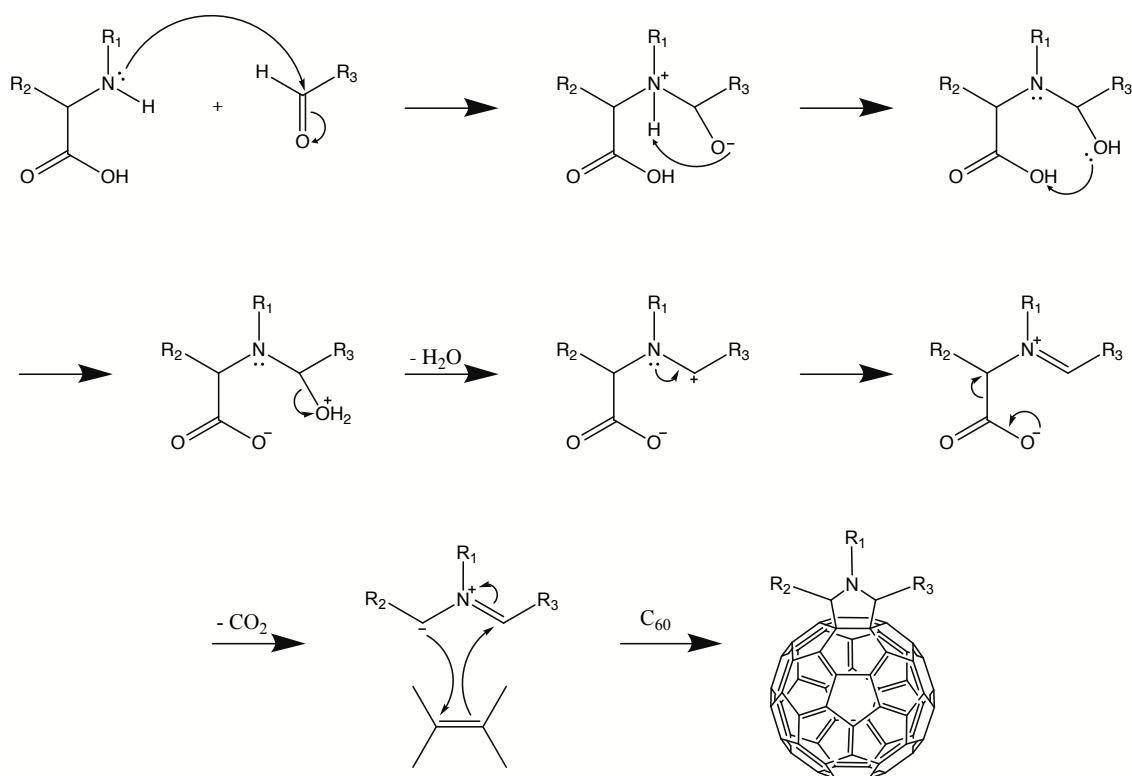


Figure 2: **Prato reaction mechanism.** Initial attack of the aldehyde/ketone's polar carbonyl group by the nitrogen lone pair of the amino acid leads to the expulsion of water, before decarboxylation gives the reactive ylide intermediate.

desired function. In particular, the possible variability of R_1 means the Prato reaction can proceed not only with primary but also secondary amino acids, thus expanding the reaction's applicability.

In contrast with the lack of control of the number of addends in the amination and hydroxylation reactions, good control can be achieved with the Prato reaction by tuning the stoichiometry of reagents and reaction time. Given that all [6,6] bonds on pristine C_{60} are identical, all mono-adducts of the same addend are likewise identical. Significant regioisomerism and lack of control is, however, introduced on addition of a second addend to form a bis-adduct [24]. The second addend has a choice of nine different [6,6] bonds at which to attach, as shown in Fig. 3, leading to a possible eight regioisomers (presuming the second addend is the same as the first). The difficulty in product purification which this regioisomerism introduces is compounded by the *cis/trans* isomerism present in

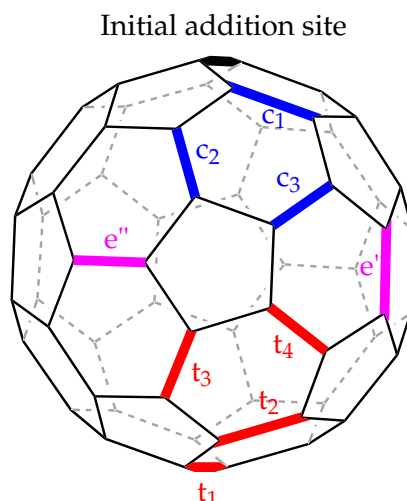


Figure 3: **Possible second addend addition sites** (adapted after [24]). There are three sites in the same hemisphere as the initial site of addition, giving rise to *cis*-regioisomers c_1 , c_2 and c_3 ; two equatorial sites, e' and e'' ; and four addition sites in the other hemisphere, giving trans-regioisomers t_1 , t_2 , t_3 and t_4 .

pyrrolidinofullerenes due to the chiral carbon atoms in the ring either side of the amino group (provided $R_2, R_3 \neq H$). This could be of significance when looking at using fullerene derivatives in biomedical contexts as regioisomerism is known to have a significant effect on pharmaco-kinetics and -dynamics [25]. In a critical study—significant in deciding which route to take to well-defined fullerene multi-adducts—Lu *et al.* concluded that bis-addition using the Prato reaction cannot be as well controlled as with the Bingel reaction [26]. However, Zhou *et al.* used a rigid tether between two azomethine ylides to controllably and selectively synthesise regioisomeric bisadducts [27]. This technique has found plenty of use with the Bingel reaction but came up slightly short here due to the chiral centres in the pyrrolidine rings introducing stereoisomeric complications.

The first attempts to utilise the Prato reaction for imparting water-solubility to fullerenes yielded products that only exhibited moderate solubility in 9:1 H₂O-DMSO binary mixtures [28]. These mono-functionalised derivatives all used N-functionalised glycine as the amino acid required in the Prato reaction. The choice of aldehyde affected the solubility of the final

product, with triethylene glycol functionalisation expectedly giving a higher solubility than pyridine derivatives.

Since, a key water-solubilisation route using the Prato reaction has been the creation of artificial fullerene-containing peptides, *fulleropeptides*. Selective bis-functionalisation at the equatorial or trans- positions can lead to the incorporation of C₆₀ into the backbone of a peptide rather than simply as a side-chain [24]. Prato mono-adducts can still be considered useful too, with a bis-carboxylic acid derivative seemingly more stable than the malonic acid derivatives achieved by the Bingel reaction [29]. This particular symmetric molecule has proved to be a useful building block for further derivatisation to yield water-soluble fullerenes via conjugation with polyethylene glycol (PEG) and peptides. Recently, reversible conjugation of peptides and amino acids to C₆₀ has been achieved via hydrophosphination with secondary phosphine boranes [30]. The use of a phase transfer catalyst led to reactions with very good yields of up to 80%, better than the generally <50% possible with the Bingel and Prato protocols.

1.1.2.3 Bingel Reaction

Arguably the most popular reaction for exohedral functionalisation of fullerenes, this cyclopropanation involves the addition of an α -halo ester/ketone to the cage under strongly basic conditions (1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and NaH are most commonly used), yielding a methanofullerene [16]. The mechanism of the classic reaction using a bromomalonate is given in Fig. 4. As with the Prato reaction, functional groups R₁ and R₂ can be specifically tailored to purpose; on the downside, the issue of regioisomerism in bis-adducts is also common. The popularity of the Bingel reaction, however, arises from versatile modifications of the original reaction, not least the ability to produce the halogenated intermediates *in situ*, which negates the need for their often time-consuming

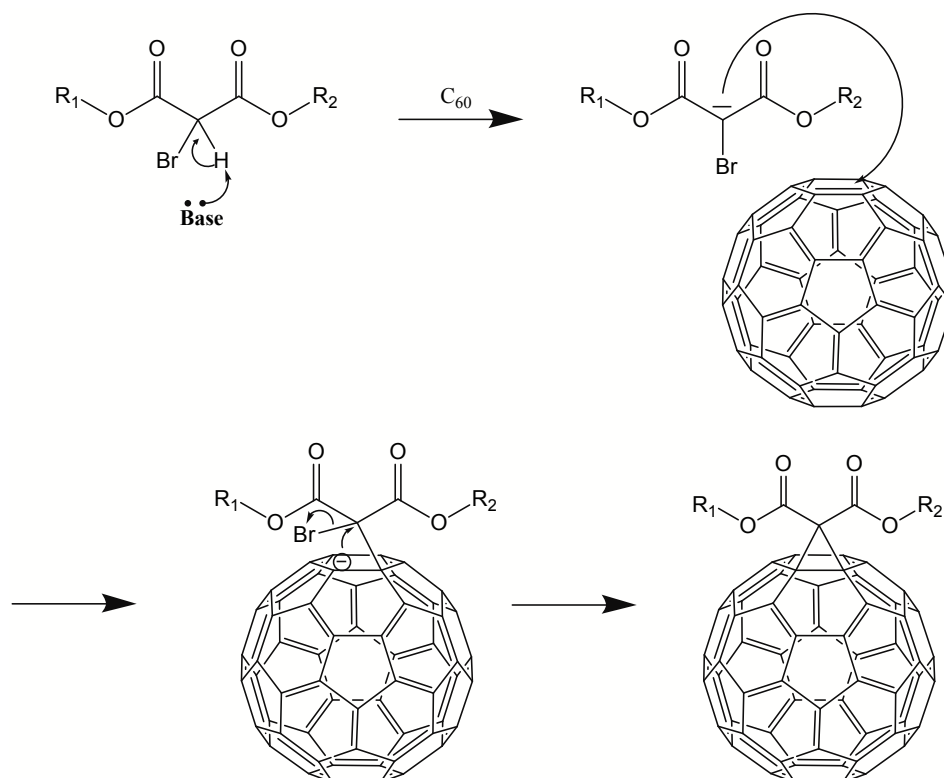


Figure 4: **Bingel reaction mechanism.** Deprotonation by the strong base gives the nucleophilic malonate anion which then attacks the [6,6] bond. Bromine is then expelled as cyclisation is completed.

purification. Iodine [31] and tetrabromomethane (CBr_4) [32] have been effectively used for this purpose, with reaction yields of generally 30–60%. Also, unlike the Prato reaction which proceeds at $\sim 120^\circ C$, the Bingel reaction needs mild room temperature conditions—this is particularly useful when functionalising the nitrogen-containing endohedral fullerene, $N@C_{60}$ [33].

Hirsch *et al.* addressed the issue of regioisomerism arising from the Bingel reaction using a somewhat circuitous route to well-defined C_{60} tris-adducts [34]. After first synthesising the expected range of bis-adducts, the *trans*-3 (12.0% yield) and *e* (15.5% yield) regioisomers were isolated and further cyclopropanated. This yielded the tris-adducts seen in Fig. 5 in an impressive 40% yield. The tris-adduct from the *trans*-3 bis-adduct has all attachments at the *trans*-3 position (*trans*-3,*trans*-3,*trans*-3) and so D_3 symmetry; that from the *e* bis-adduct has all

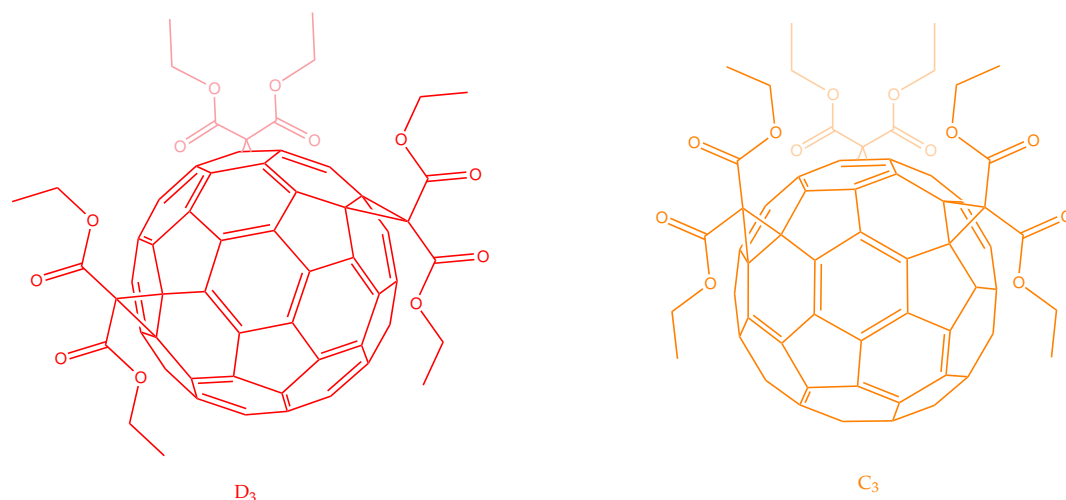


Figure 5: **C₆₀ diethyl malonate tris-adducts**. Addition at the *trans*-3 positions imparts D₃ symmetry and produces a red solution; at the equatorial positions, C₃ symmetry and an intense orange solution.

attachments at the equatorial positions (*e,e,e*) and so C₃ symmetry. A visible colour difference was also observed: the C₃ product being a more intense orange compared to the red of the D₃ product. These results indicate that functionalisation history affects the subsequent addition of other groups onto the fullerene cage and so can be used to control spatial functionalisation.

As mentioned for the Prato reaction analogue in Section 1.1.2.2, tethered moieties can be used to direct functionalisation to specific points on the cage. Sigwalt *et al.* successfully used *tert*-butyl(trialkoxysilane tris-malonates to synthesise *e,e,e* tris-adducts (Fig. 6) in 8–26% yield [35]. The silane derivative was chosen as it is readily available in a pure form and, after attachment of all three malonate groups to the fullerene cage, the Si–O bond can be easily cleaved (67–99% yield) to leave isolated methano-bridges. The variation in yields originates from the different number ($2 < n < 4$) of methylene units in the trismalonate— $n = 3$ gave optimum yields for both cyclopropanation and removal of the *tert*-butylsilane group. This method gives a notably increased yield in pure *e,e,e* tris-adduct final product compared to the functionalisation route via isolation of bis-adducts of Hirsch *et al.* above. Another attempt at *e,e,e* tris-adduct isolation, involving a protection-deprotection sequence based on

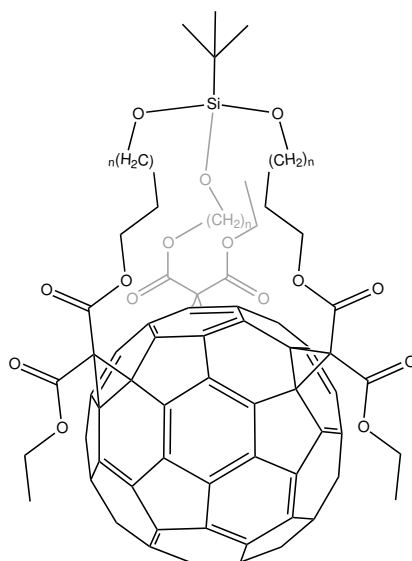


Figure 6: *tert*-Butyl(trialkoxy)silane–tethered cyclopropanation *e,e,e* tris-adduct ($2 < n < 4$).

the high-temperature (180 °C) Diels-Alder reaction, achieved a meagre 4.2% yield in much harsher conditions [36].

Fullerene derivatives with even more adducts might be desired for biomedical applications as a greater number of polar groups should give increased solubility. Hexakis-adducts of high T_h symmetry with structurally complex malonates⁹ have been synthesised in good yield (40–68%) by Li *et al.* who used a deceptively simple modification of the standard Bingel reaction [37]. Rather than using precise stoichiometric ratios for the desired product (e.g. for hexakis-adducts, a 1:6:6:6 ratio of C_{60} : malonate: base: halogenating agent), they found that a 100-fold excess of the halogenating agent (in this case, CBr_4) preferentially yielded the hexakis-adduct. A 10-fold excess of malonate and DBU were used, as is standard procedure when trying to synthesise multi-adducts: an excess of base and the moiety to be attached are added in order to encourage the reaction to go to completion. This was found not to be the factor leading to high yielding hexakis-addition, as a 100-fold excess of CBr_4 with reagents in stoichiometric ratio for just mono-addition still gave the hexakis-adduct in 15% yield.

⁹ Compared to the simplicity of the prototypical diethyl malonate used in Bingel reactions.

The first examples of water-soluble C_{60} derivatives with multiple additions were synthesised via the Bingel reaction [38]. The quintessential addition of diethyl bromomalonate to the fullerene core occurred under basic conditions, with stoichiometric conditions chosen to yield mono-, bis- and tris-adducts. The subsequent diethyl derivatives required transformation to their malonic acid forms in order to impart water solubility. This was achieved via hydrogenolysis using excess NaH at 60 °C, rather than saponification with NaOH, which would cause unwanted hydroxylation of the fullerene cage. As expected, water-solubility increases with increasing number of malonic acid groups—only the tris-adduct exhibited very good solubility in neutral water. This tris-adduct has its addends at the equatorial positions, putting it in symmetry point group C_{3v} , for which reason the molecule has subsequently been called C_{3v} . It is in many ways a model example of a water-soluble fullerene owing to its well-defined structure and relatively easy synthesis, and indeed has been used extensively in biomedical studies [39–41].

A highly symmetric (T_h point group) fullerene malonic acid hexakis-adduct has also been formed [42]. Such highly polar fullerene species are unsuitable for traditional separation via reverse-phase HPLC, so ion chromatography was instead used to isolate the final product. This innovative technique also allowed for the separation and isolation of the products of degradation of these malonic acid derivatives (they are, unfortunately, prone to decarboxylation). In an effort to produce water-soluble derivatives with only one addend, the Bingel reaction has been employed to attach a dendritic structure with 18 carboxylic acid groups [43]. This second-generation polyamide dendron imparts significant water-solubility: 34 mg/mL in neutral water.

1.1.2.4 Hydroxylation

The first attempt to synthesise and characterise water-soluble C_{60} derivatives employed the ever since popular hydroxylation method, which involves the direct addition of polar hydroxy groups to the carbons of the fullerene cage, yielding products interchangeably called *fullerols* (sometimes *fullerenols*) or *polyhydroxylated fullerenes* (PHFs) [44]. This first synthesis involved stirring C_{60} in an aqueous acid solution containing concentrated sulphuric and nitric acids (each 40% by volume). Upon completion of the reaction, the solution turned a yellow-brown colour—a practical tell-tale sign of the presence of water-soluble fullerene derivatives. Fourier transform infrared (FTIR) and X-ray photoelectron (XPS) spectroscopies were used to characterise the extent of oxidation of the fullerene cage, indicating the presence of other functional groups alongside hydroxyls, such as ketones and hemiketals [45]. Mass spectrometry provided no evidence of molecular ion peaks. Thus, in order to calculate the number of hydroxyl groups present, esterification with alkanoyl chlorides was carried out—it was determined that an average of 14–15 hydroxyl groups were present per C_{60} molecule. It should be noted now that the nature of “bulk” reactions such as hydroxylation means that there is inevitably a spread in the number of additions to the cage, and a product with precisely defined molecular structure is not possible.

This initial fullerol was “moderately soluble in water and very soluble in acidic water at $\text{pH} < 5.0$ ”. An increased number of hydroxyl groups, and thus solubility, can be achieved by the direct reaction between C_{60} fullerene and sodium hydroxide (NaOH) [46]. A solution of C_{60} in benzene was stirred with aqueous NaOH, and the reaction was accelerated in the presence of the phase transfer catalyst, tetrabutylammonium hydroxide (TBAH), taking only three minutes. The reaction can be tracked to completion by following the loss of the purple colour of the benzene solution and resulting precipitation of a brown sludge. Removal of

benzene was followed by addition of more water and further stirring before the final product was recovered via precipitation with methanol (MeOH). Elemental analysis (EA) indicated an average of 24–26 hydroxyl groups per C_{60} cage. This basic method (cf. the first acidic method) has since become popular owing to its speed and direct access to highly functionalised cages. This is in comparison with other methods which involve hydrolysis of intermediate species [47].

Hydroxylation can also be achieved under solvent-free reaction conditions [48]. An average of 16 hydroxyl groups was found to attach to C_{60} when ground with NaOH in the presence of hydrogen peroxide (H_2O_2), with excellent yields achievable in only fifteen minutes.

Hydrogen peroxide can be used to even greater efficacy to produce PHFs with 36–40 hydroxyl groups per C_{60} molecule, starting from poorly soluble $C_{60}(OH)_{12}$ [49]. It is suspended in 30% aqueous H_2O_2 and stirred at 60 °C under air for several days. Precipitation, centrifugation and decantation were used to isolate the final product. Thermal gravimetric analysis (TGA) and EA were used to determine the number of tightly bound secondary water molecules, the presence of which would reasonably be expected of such a highly functionalised molecule. $C_{60}(OH)_{36}$ was estimated to have eight secondary water molecules; $C_{60}(OH)_{40}$, nine secondary water molecules. Mass spectrometry of these PHFs proved inconclusive—a common theme with such highly functionalised fullerene species. These highly functionalised PHFs had high water solubilities up to 58.9 mg/mL in neutral water. Importantly, this method negated any chance of sodium contamination.

Combining the two methods of using H_2O_2 and TBAH as a phase transfer catalyst leads to the formation of the most highly functionalised PHFs, $C_{60}(OH)_{44} \cdot 8 H_2O$, in a one-pot process starting from pristine C_{60} [50]. The reaction proceeds well at 60 °C in air, taking 16 hours to yield a product with a water solubility of 64.9 mg/mL. Dynamic light scattering (DLS) measurements showed a narrow particle size distribution (1–2 nm), suggestive of a

highly functionalised cage with excellent dispersion in aqueous solution—the predominance of favourable hydrogen bonding with water negates any significant hydrophobicity-driven aggregation effects.

Recently, a “green” approach has been proposed for the formation of the PHF, $C_{60}(OH)_8 \cdot 2H_2O$, via sonication in dilute H_2O_2 at room temperature [51]. Sonication of aqueous H_2O_2 produces the hydroxyl radical (OH), which subsequently attacks C_{60} —after one hour, the product with eight hydroxyl groups was synthesised. This is, however, below the oft-quoted minimum number of hydroxyl groups needed to impart meaningful water solubility (twelve).

During the development of methods for efficient syntheses of fullerenes with large numbers of hydroxyl groups, evidence from FTIR, XPS and ^{13}C NMR spectroscopies, in particular, clearly suggested the presence of other oxygen-containing functional groups on the surface of the fullerenes, such as carbonyls, hemiketals and epoxides [48, 50].

Whilst increasing the number of hydroxyl moieties increases the water solubility, it has been hypothesised that there is a sweet spot to the degree of hydroxyl functionalisation (number of hydroxyl groups, $n = 36$) with regards to the stability of PHFs due to the presence of these other oxygenated functional groups [52]. The reaction conditions necessary for synthesising PHFs with $n > 36$ are conducive to the introduction of large numbers of carbonyl groups also, and it was found that those PHFs with $n > 36$ were less stable under the irradiation of a matrix-assisted laser desorption/ionisation time-of-flight mass spectrometer (MALDI-TOF MS), breaking into a larger number of fragments than their less functionalised analogues. In fact, no linear relationship was found between the stability and the number of hydroxyl groups, rather between the stability and the ratio of the number of hydroxyl groups to the number of other oxygenated groups (as determined by FTIR and XPS). Such an important

factor as molecular stability has potentially profound implications for the decision of which PHFs to use in biomedical applications.

And PHFs have indeed found application in various areas of biomedical fullerene research [53, 54]. A fundamentally important factor in this area is their interactions with lipid bilayers, used as mimics for cell walls [55]. The number and positions of hydroxyl groups affect these interactions [56], and it has been shown that the presence of PHFs in a lipid bilayer affects its ionic permeability [57].

Not yet mentioned is the generality of these hydroxylation reactions—they have been heavily employed not only on empty cages but also in the functionalisation of gadolinium-containing endohedral metallofullerenes (Gd-EMFs). These species can be used as magnetic resonance imaging contrast agents (MRI CAs) and are discussed further in Section 1.2.2.1.

1.1.3 Endohedral Functionalisation

The relatively inert interiors of the fullerenes render them able to capture atoms and atomic clusters within. The standard notation of $X@C_{2n}$ was introduced by Chai *et al.* to denote endohedral fullerenes, where X is the incarcerated species and n specifies the cage size [58]. Recently, a “molecular surgery” approach, using a series of cage-opening and -closing chemical reactions, has led to the encapsulation of single molecules of hydrogen and water in C_{60} to give $H_2@C_{60}$ and $H_2O@C_{60}$ (60–78% incorporation respectively) [59]. The focus of this section, however, will be on two types of endohedral fullerenes synthesised by more established procedures: namely the endohedral metallofullerenes (EMFs) and nitrogen-containing fullerene, $N@C_{60}$.

ENDOHEDRAL METALLOFULLERENES (EMFs) This is a broad family of endohedral fullerenes where the cage plays host to either one (monometallic) or two (dimetallic) metal atoms or a cluster of metal atoms with another atom, often nitrogen. The presence of large metal atoms within the cage leads to differing reactivities and properties which can be exploited for numerous applications, including those in the biomedical realm [60]. In that field, the majority of literature covers the use of gadolinium-containing EMFs as contrast agents in magnetic resonance imaging (MRI), which will be further discussed in Section 1.2.2.1.

SYNTHESIS Much like C_{60} , the first EMFs were synthesised using very expensive and low-yielding laser ablation [61]. Adaptations of the now-standard Krätschmer-Huffman arc discharge method have led to scaled-up synthesis of EMFs: composite graphite rods containing the desired metal oxide (most often lanthanide oxides) are used, however, yields are still generally less than 2% of the total carbonaceous material produced [62].¹⁰ The major product of such a synthesis is monometallic fullerene, $M@C_{82}$, which has good solubility in common organic solvents and so can be separated from other products using HPLC. It should be noted that the first step of EMF isolation before HPLC separation is its extraction, using a standard Soxhlet method, from the carbon soot mixture by use of polar solvents such as dimethyl formamide (DMF). This is because the incarcerated metal atom doesn't sit centrally in the elongated cages of larger fullerene cages, giving the molecule an overall electric dipole moment. Kozlov *et al.* optimised this standard process to produce $Gd@C_{82}$ of excellent purity (99%) [64].

¹⁰ EMF yields are found to be dependent on many different parameters in the arc discharge reactor, with a strong dependence on the pressure of the He atmosphere [63]. Reactor size thus obviously has a major effect, as do rod size, distance between rods and current.

Stevenson *et al.* synthesised $\text{Sc}_3\text{N@C}_{80}$, the first trimetallic nitride template (TNT) EMF, in a marginally better 3–5% yield by introducing a small amount of nitrogen to the arc reactor [65]. Their synthesis was vastly improved by Dunsch *et al.* whose “reactive gas atmosphere” concept—the addition of ammonia into the reactor—gave EMFs as the majority fullerene product for the first time [66]. The composition of the TNT EMFs can be generalised to $\text{X}_x\text{Y}_y\text{M}_{3-x-y}\text{N@C}_{80}$, where X, Y and M are different metallic species. These species exist because the nitride clusters have been found to stabilise the high-symmetry $I_h\text{-C}_{80}$ cage.

A further step towards commercial synthesis of TNT EMFs was achieved by Bezmelnitsyn *et al.* whose novel approach to arc discharge, they estimate, has reduced the cost of lutetium-containing TNT EMF synthesis by 70–80% [67]. A three-fold increase in yield was achieved by using a pyramidal three-phase AC arc discharge setup (*cf.* the standard two-electrode DC setup) along with a pure nitrogen atmosphere (negating the need for expensive helium) and metal oxide powder injection with solid graphite electrodes.

These improved synthesis routes are important as gadolinium-containing TNT EMFs have shown great promise as MRI contrast agents (see Section 1.2.2.1).

EXOEDRAL FUNCTIONALISATION If the exohedral functionalisation of empty-cage fullerenes is non-trivial, then that of EMFs is akin to a lottery.¹¹ Metallic species within the cage transfer electrons to the carbon cage, with this excess electron density inhomogeneously distributed over the cage, and even extending out of the cage—a phenomenon dubbed “spin leakage” [69]. This is expected to have profound effects on the chemistry of the EMFs. Prato reactions have been carried out on La@C_{82} to yield mono- and bis-adducts, with electron paramagnetic resonance (EPR)¹² measurements showing slight modification of the electronic

¹¹ This does an injustice to the numerous researchers who have attempted to make sense of this complex field in both theory [68] and experiment.

¹² Sometimes referred to in the literature as electron spin resonance (ESR).

structure upon mono-addition, and significant modification upon bis-addition [70]. Bingel cyclopropanation was used by Bolskar *et al.* to isolate a derivative of the otherwise insoluble Gd@C₆₀ [71]. This is of value as the Gd@C₆₀ fraction of the arc discharge soot is more abundant than Gd@C₈₂ but could never be isolated due to its insolubility. A 15-fold excess of diethyl bromomalonate yielded primarily the decakis-adduct, Gd@C₆₀[C(COOCH₂CH₃)₂]₁₀, which was hydrolysed to the water-soluble Gd@C₆₀[C(COOH)₂]₁₀. This has been investigated as an MRI contrast agent, as discussed in Section 1.2.2.1. Other Gd-EMF MRI contrast agents which have been studied, and which are examined later, have primarily been hydroxylated to achieve water-solubility.

1.1.3.1 Nitrogen-Containing Fullerenes

The nitrogen-containing fullerene, N@C₆₀, is a fascinating species because it isolates highly reactive atomic nitrogen with minimal interaction with the cage—unlike the EMFs, 98% of nitrogen's electron density remains away from the cage [72]. This has interesting consequences for its chemical behaviour (see below). ¹⁴N@C₆₀ has electron spin $S = \frac{3}{2}$ and nuclear spin $I = 1$ coupled by an isotropic hyperfine interaction, leading to a 12-level energy splitting when placed inside a magnetic field. The continuous wave (CW) EPR spectrum thus shows three distinct peaks due to the triple degeneracy of the allowed electron spin transitions. These peaks are uniquely narrow, with a linewidth of $< 0.3 \mu\text{T}$ [73]. Upon exohedral functionalisation, Zhang *et al.* showed that anisotropy of the hyperfine interaction and a zero-field splitting (ZFS) effect were introduced to the EPR spectrum [74].

SYNTHESIS N@C₆₀ is produced in a low-yielding¹³ ion implantation process, where low-energy nitrogen ions are ionised and fired at a thin layer of freshly sublimed C₆₀ [73].

¹³ The N@C₆₀:C₆₀ yield is typically 10^{-5} – 10^{-4} .

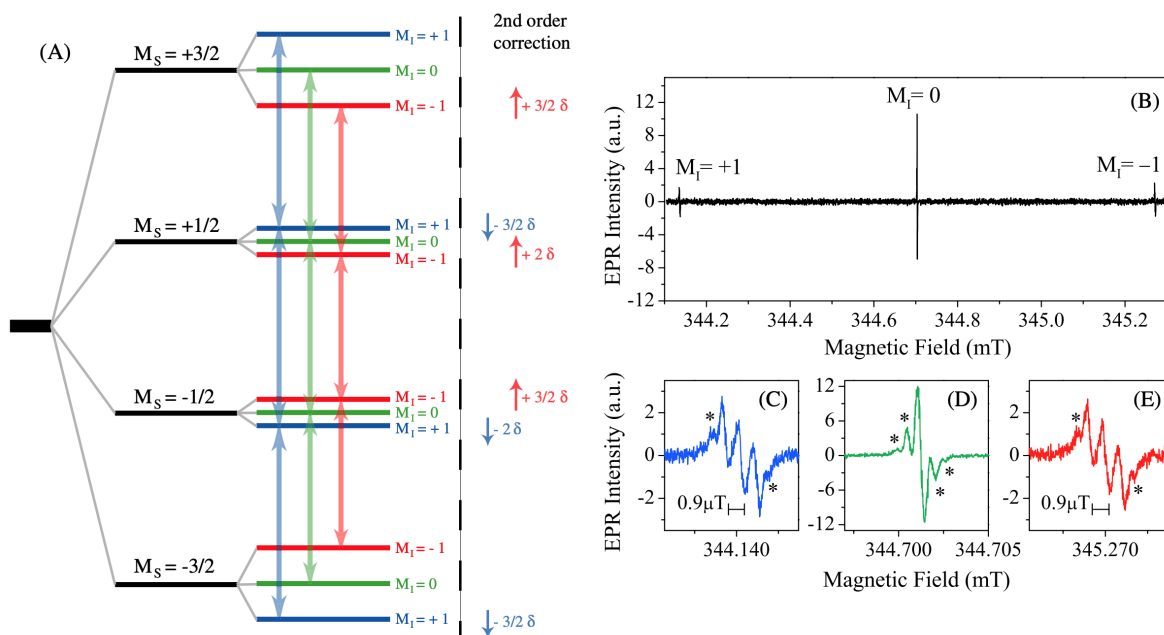


Figure 7: The origin of $^{14}\text{N}@C_{60}$'s unique EPR spectrum. (A) shows the energy levels of $^{14}\text{N}@C_{60}$; (B) its CW spectrum in carbon disulphide (CS_2); (C–E) the fine structures of each hyperfine line. Reprinted with permission from J. Phys. Condens. Matter 2006, 18, S867–S883. Copyright 2006 Institute of Physics Publishing.

Lengthy recycling HPLC is then used to isolate $\text{N}@C_{60}$ from empty C_{60} [75]. The process is so lengthy due to the low yield of ion implantation, and the fact that the two species are chemically so similar (the lack of electron density transfer from nitrogen to the cage accounts for this) and the mass difference between the two is only 2%. Thus, resolution of their separate peaks requires many passes through the HPLC column. Plant and Porfyrakis's work has led to an efficient, high-throughput method of producing high-purity $\text{N}@C_{60}$ by using in-line EPR spectroscopy along with an impressive flow-rate of 18 L h^{-1} [76]. Of course, a downside is that this process uses high volumes of solvent (toluene in this case), however, this shouldn't be a barrier when looking to scale-up to commercial production as toluene can be very easily recycled for further use.

EXOEDRAL FUNCTIONALISATION

The literature on exohedral functionalisation of $\text{N}@C_{60}$ is limited, with the Prato [77, 78] and Bingel [79, 80] reactions being most explored.

Arguably the most important factor to consider when functionalising N@C₆₀ is the retention of the nitrogen spin within the cage, particularly when aiming to scale-up production for application. Quantitative EPR spectroscopy is used to measure spin retention ratios (SRRs) of the final derivative compared with the starting pristine N@C₆₀. In the past, both Bingel [81] and Prato [74, 77] reactions were found to give SRRs of 50–79%; this is highly unscalable given the already small amounts of N@C₆₀ available. A significant breakthrough was achieved by our group, whereby an SRR of 94% was achieved in a Bingel reaction by employing a diluted base catalyst (0.5% DBU-toluene solution) [33]. As such, the Bingel reaction is now a viable route to scaled-up synthesis of N@C₆₀ derivatives. Indeed, this methodology has subsequently been used by our group to synthesise the first water-soluble N@C₆₀ derivative [82].

1.2 FULLERENES IN MEDICINE

The above has been a brief introduction to some of the studies that have been carried out focusing on the synthesis of water-soluble fullerene derivatives. What follows is a deeper exploration into those structures that have been investigated for specific biomedical applications—particular attention is given to the relationship between molecular structure and function. The applications are numerous and cover both therapeutic and diagnostic capabilities.

Of all posited applications of fullerenes, the space for exploration and eventual exploitation is arguably ripest in the biomedical field [7, 83–92]. As this chapter and the rest of the literature show, there are a multitude of applications in this area that are and/or have already been the topic of intense fullerene-based research. The simple reason for such breadth is

that their three key properties—electron deficiency, reactive exteriors, and inert interiors—endow the fullerenes with the ability to be used for both *diagnostic* and *therapeutic* purposes. The nature of the unfunctionalised carbon cage itself is useful in areas such as enzyme inhibition, singlet oxygen generation and radical scavenging. The ability to functionalise the carbon cage exohedrally and effectively use it as a scaffold for grafting functional groups means the fullerenes can easily be incorporated into complex three-dimensional and multi-functional molecular architectures—this gives rise to the notion of synthesising *theranostic* platforms, where both diagnostic and therapeutic functionalities can be achieved in one molecule. Endohedral functionalisation gives an elegant way of incorporating atoms that serve a diagnostic purpose. Each of these unique benefits, and others, are discussed below.

The incorporation of various different fullerene species into increasingly complex (and often elegant) architectures can only be achieved because of the uniqueness of this family of molecules. In the treatment of disease, these systems can act in a more global approach *cf.* traditional drugs, by disrupting the molecular machinery of the body—such an approach has given rise to the idea of “nanologicals” [93].

While there have been many discoveries in terms of understanding the intimate links between molecular design and function in various biomedical application areas, no intravenously administered fullerene-based pharmaceutical product exists yet on the market.

1.2.1 Therapeutics

1.2.1.1 HIV-1 Protease Inhibition

The unique spherical shape of C₆₀ offers an elegant potential solution to producing effective enzyme inhibitors for the treatment of deadly diseases such as human immunodeficiency

virus (HIV) infection. This, the first posited medical application of C_{60} , was first proposed and investigated for HIV-1 protease (HIVP) as its active site is predominantly lined by hydrophobic amino acids and has a radius comparable to that of C_{60} [94]. Inhibition is driven by favourable hydrophobic interactions between the fullerene cage and the active site lining, as predicted by computational modelling (Fig. 8). A mono-functionalised bis(phenethylamino-succinate) methanofullerene derivative was synthesised in order to test the hypothesis experimentally [95]. This derivative was so chosen because the mono-addition retained the majority of the fullerene's hydrophobic surface while conferring suitable water-solubility so that the derivative could actually remain in solution with HIVP. It was hypothesised that the efficacy of the HIVP inhibition could be increased via the introduction of two appropriately placed amino groups, which could form salt bridges with the aspartate groups present in the active site, thus improving binding.

Other computational studies have focused on designing novel fullerene derivatives from scratch in silico [96]. Steric, electrostatic, hydrophobic and hydrogen bonding interactions were all taken into account—it was shown that that highest contributions to the binding energy came from hydrophobic interactions and hydrogen bonding.

HIVP inhibition has also been experimentally verified with fullerene-based amino acids [97]. Functionalisation of C_{60} -phenylalanine with 9-fluoromethoxycarbonyl (Fmoc) was particularly potent as the Fmoc protecting group interacts favourably with the hydrophobic active site. It must be noted, however, that even this derivative is not as effective as protease inhibitors already on the market.

1.2.1.2 Other Antiviral Activity

Recently there has been an intense research effort investigating novel *glycofullerenes* for their antiviral activity. These multivalent globular structures are highly soluble in water thanks

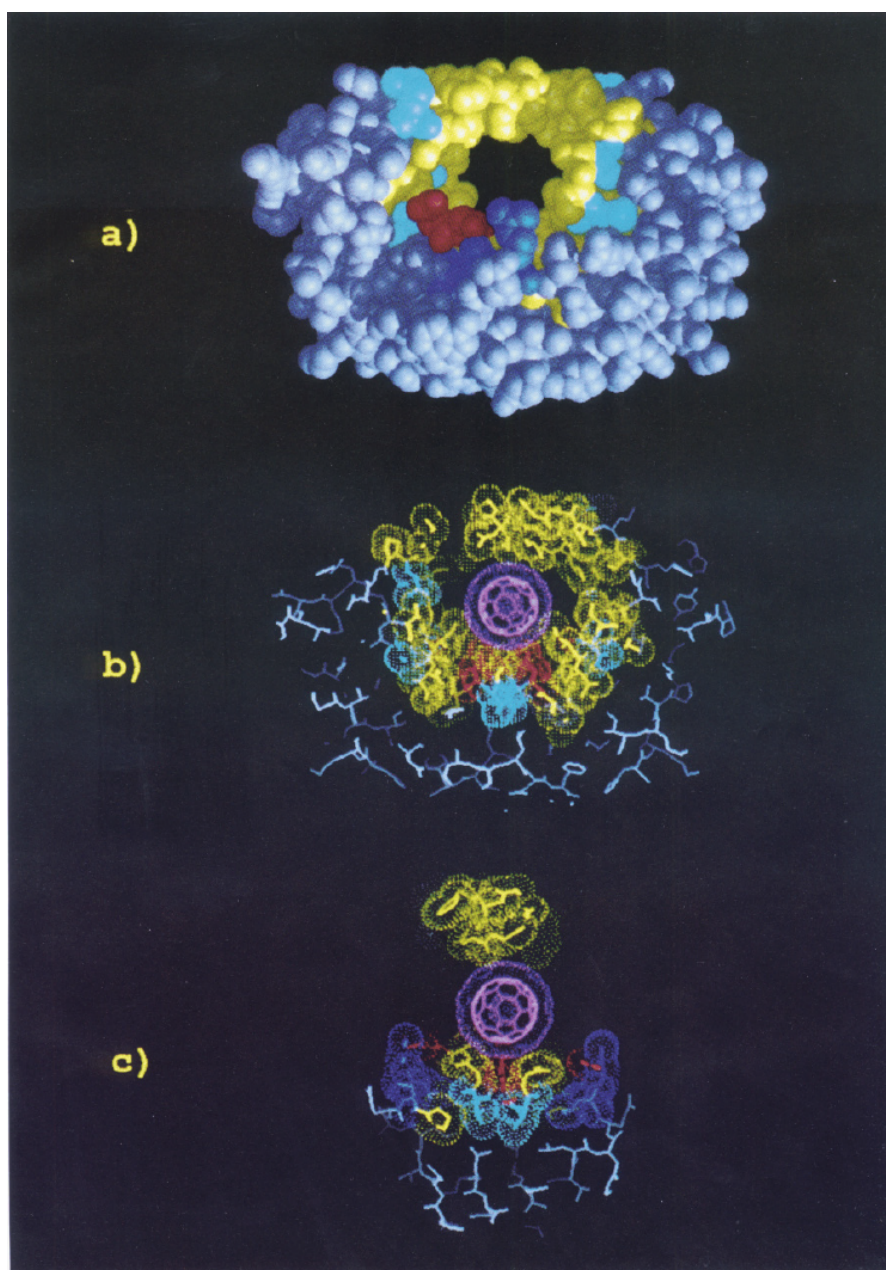


Figure 8: Computational model of C₆₀ favourably interacting with, and thus inhibiting, the active site of HIV-1 protease (HIVP). (a) The active site of HIVP is highlighted in yellow, blue and red. (b) The same view with C₆₀ shown in the orientation calculated to give strongest van der Waals interaction with the active site; the van der Waals surfaces of C₆₀ and the active site are represented by surrounding dots. (c) The same complex as in (b) is represented in 90° cross section. Reprinted with permission from J. Am. Chem. Soc. 1993, 115, 6506-6509. Copyright 1993 American Chemical Society.

to the large number of sugar units conjugated to the fullerene surface. Not only do these carbohydrates solubilise the fullerene but they also inherently act as targeting moieties for certain lectins, carbohydrate-binding proteins found at the surfaces of cells. DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) is one such lectin often used by viruses, such as Ebola, to trick the immune system and enter the cell. Blocking DC-SIGN would therefore inhibit a virus' ability to penetrate a cell. C_{60} acts as a perfect scaffold on which to attach multiple sugar units in three dimensions. The first such glycofullerene to have its antiviral properties tested began life as a T_h -symmetrical hexakis-adduct, formed via the Bingel reaction, with functional terminal alkyne groups [98]. These could then undergo a ubiquitously used click reaction—the copper-catalysed azide-alkyne cycloaddition (CuAAC)—with terminal azide-functionalised sugar-containing moieties. As such, glycofullerenes with 12, 24 and 36 mannose units were formed. The derivative with the fewest units exhibited the best antiviral activity against a model Ebola system, likely due to a negation of steric hindrance. Its inhibition activity was further improved by lengthening the distance of the mannose unit from the central fullerene cage via a glycol linker. This has been attributed to the flexibility of the linker and the slower translational diffusion of the resulting larger product, thus increasing the binding efficiency [99].

The same synthetic method of combining Bingel hexakis-adducts and CuAAC can also be used to create giant multivalent derivatives [100], such as tridecafullerene “sugar balls” containing a central C_{60} surrounded by 12 other C_{60} moieties, leading to a maximum of 120 sugar units per molecule [101]. The seeming structural complexity of these dendritic macromolecules (56 kDa) belies their straightforward synthesis. Even more encouragingly, by increasing the possible multivalent interactions with DC-SIGN, these sugar balls show dramatically increased inhibitory performance against a model Ebola system compared to the glycofullerenes with only 12 mannose units, with efficacy in the sub-nanomolar concentration

range. A highly symmetric C_{60} hexakis-adduct that can undergo copper-free click chemistry (important for biomedical applications as copper can prove very toxic) via its maleimide and cyclooctyne unites has recently been synthesised [102].

Moving away from highly functionalised multi-adducts, glycofullerene monoadducts have been shown to self-assemble into micelles, as is to be expected of such amphiphilic molecules [103]. These 50–60 μm structures showed an improved inhibitory effect in a model Ebola system: as such, it has been shown what a crucial role size as well as multivalency plays in antiviral inhibition via DC-SIGN blocking.

1.2.1.3 Chemotherapy

In the above antiviral studies, it was found that the fullerene derivatives exhibited minimal to no cytotoxicity, an important prerequisite to ensure that the designed molecule only acts on its intended pathway. This has also been found in studies using fullerenes as antineoplastic agents, and is of particular importance as common chemotherapeutics, such as cyclophosphamide (CTX) and cisplatin, come hand-in-hand with deleterious toxic effects. A fullerene derivative which has been extensively studied in this field is the polyhydroxylated Gd-EMF, $\text{Gd}@C_{82}(\text{OH})_{22}$ [104]. While the role of Gd-EMFs as MRI CAs will be explored later in this chapter, we will first look at the antitumor effects of these derivatives. One of the early studies in this field showed that these molecules form 22 nm-sized aggregates in saline solution and can achieve the same antineoplastic activity as cisplatin and CTX at concentrations one and two orders of magnitude lower respectively [105]. The lack of cytotoxicity implies that the antineoplastic activity arises indirectly—indeed, in vivo experiments showed that only 0.05% of the administered dose reached tumour tissue. It was therefore hypothesised that, rather crudely, the size of the $\text{Gd}@C_{82}(\text{OH})_{22}$ aggregates led to blockage of pores into the tumour, thus blocking off its nutrient and oxygen supply.

The actual mechanism of action is, naturally, far more complex and multi-faceted [106]. First, it was shown that $\text{Gd@C}_{82}(\text{OH})_{22}$ stimulated maturation of dendritic cells, which are key to immune defence [107]. This Gd-EMF has also been shown to inhibit tumour metastasis in breast cancer [108] and pancreatic cancer [109], for which a key mechanism is inhibition of matrix metalloproteinases 2 and 9 (MMP-2/9) [110]. Much like the inhibition of HIVP by C_{60} , this relies on favourable hydrophobic, electrostatic and hydrogen bonding interactions. The strength of this binding interaction can be improved by the introduction of charged functional groups such as PO_4^{2-} [111]. Another posited mechanism is that $\text{Gd@C}_{82}(\text{OH})_{22}$ increases fibroblast activity and thus collagen expression, encasing tumorous tissue [112].

Recently, however, caution has been urged: molecular dynamics (MD) simulations have shown that binding of $\text{Gd@C}_{82}(\text{OH})_{22}$ by other normal proteins, such as human monooxygenase CYP2C8, is favourable and could lead to unwanted toxic side effects [113].

1.2.1.4 Antioxidants

The role of oxidative stress in the pathogenesis of human disease cannot be overstated, as demonstrated by the vast body of literature related to this phenomenon [114]. While reactive oxygen species (ROS), created by the partial reduction of oxygen,¹⁴ are naturally produced by metabolic processes within the body and have even been found to play a key part in cellular signalling, deviation from homeostasis due to an increase in levels of ROS¹⁵ or a decrease in inherent antioxidant capability leads to problems [116]. Such problems include promotion of tumor metastasis [117], neurodegeneration [118] and other degenerative diseases associated with aging [119].

¹⁴ There are many ROS but two of the most studied are the superoxide ($\text{O}_2^{\cdot-}$) and hydroxyl ($\text{OH}^{\cdot}$) radicals. Reduction of molecular oxygen yields the superoxide radical ($\text{O}_2 \xrightarrow{+e^-} \text{O}_2^{\cdot-}$) which can dismutate to hydrogen peroxide ($2\text{H}^+ + 2\text{O}_2^{\cdot-} \longrightarrow \text{H}_2\text{O}_2 + \text{O}_2$), which can then be fully reduced to water or partially reduced to the hydroxyl radical ($\text{H}_2\text{O}_2 \longrightarrow 2\text{OH}^{\cdot}$) [115].

¹⁵ This increase can be a result of endogenous generation (i.e. standard cellular activity) or exogenous sources (e.g. through smoking tobacco).

A widely pursued application of water-soluble fullerene derivatives regards their antioxidant activity. Once again, this arises thanks to the fullerene's unique structure: it is known as a "radical sponge" because of its high electron affinity and numerous double bonds that can be attacked by free radical species, thus "absorbing" them from the surrounding environment [120]. Indeed, pristine C_{60} was shown early on to protect rat livers against oxidative stress without any acute or subacute toxicity [121]. Soon after, however, pristine C_{60} was shown to exert a strong pro-oxidant effect, leading to ROS-induced cytotoxicity (this ability of fullerenes to produce ROS is covered in more detail in the section on photodynamic therapy (PDT) below), while PHF exerted a strong antioxidant effect [122].

This duality of the fullerenes as exerting both *pro*- and *anti*-oxidant effects has been the topic of intense research to determine what structural characteristics determine their behaviour [123]. For example, an investigation on eight different types of functionalisation—ranging from covalent exohedral functionalisation to inclusion in complexes and liposomes—concluded that the preference for pro-/anti-oxidant activity depends on the aggregation behaviour of the derivative [124]. Those derivatives that dispersed well in aqueous solution were found to be more effective ROS producers, while those that aggregated were more effective antioxidants.

PHFs have attracted particular attention as effective antioxidants [125], having been shown to be more effective than a bis-carboxylic acid derivative [126]. Spin-trapping and electron paramagnetic resonance (EPR) spectroscopy were used to determine the efficacy of antioxidant activity against 1O_2 , O_2^- and OH. For all these ROS, $Gd@C_{82}(OH)_{22}$ performed better than $C_{60}(OH)_{22}$, which performed better than $C_{60}(C(COOH)_2)_2$. Examples of deployment of PHFs for their antioxidant properties include preventing premature birth in mice models [127] and negating oxidative stress-induced toxicity from chemotherapeutics such as doxorubicin [128, 129]. They have also been shown to suppress inflammatory responses related to lower back

pain by reducing the levels of ROS through radical scavenging [130, 131] and antagonising the activity of tumour necrosis factor- α (TNF- α) [132]. PHFs also act as effective radioprotective agents [133]—during radiotherapy for the treatment of malignant tumours, radiolysis of water within the body produces ROS which can severely damage surrounding non-cancerous tissue, and currently clinically used radioprotective agents typically induce toxic side effects in a majority of patients. The water-soluble malonic acid derivative, C_3 , is also an effective radioprotective agent [39].

With respect to the absorption of O_2^- specifically, the C_{60} mono-adduct dendrofullerene introduced earlier on outperformed tris-malonic acid and other tris-malonate anionic derivatives, which themselves outperformed multiply functionalised cationic derivatives [134]. Coupled with the evidence above, it is clear that the antioxidant activity of fullerene derivatives is not only a function of aggregation but also of conjugation of the fullerene cage, hydrophilicity, and surface charge.

Antioxidant activity is not limited to C_{60} though. In fact, bis- and tris-malonic acid (carboxyfullerene) derivatives of C_{70} have been shown to give the same protective effect against oxidative stress as C_{60} -carboxyfullerenes but at lower concentrations (2.5 μ M *cf.* 40 μ M) [135]. Other efforts have been made to employ C_{70} as a targeted antioxidant with the use of tetraglutamate-functionalisation to target specific cellular substrates [136]. Recently, even a Gd-EMF has been functionalised in such a way as to improve its antioxidant capability [137]. An average of 8 ethylenediamine (EDA) groups were attached to each fullerene simply by stirring in EDA at room temperature for three hours. The cytoprotective effect of these amino-fullerenes at 2.5 μ M was the same as polyhydroxylated $Gd@C_{82}(OH)_{26}$ at 40 μ M. The improvement in performance of EDA derivatives could be attributed to both the observed better cellular uptake and the more intact π -system of the fullerene cage.

The remarkable antioxidant and anti-inflammatory properties of fullerenes have long attracted the attention of researchers in diseases concerning the brain and central nervous system (CNS). Tris-carboxyfullerene, C_3 , was earmarked early on as a promising neuroprotective candidate, outperforming its more symmetrical analogue, D_3 [40]. This difference in activity was rationalised by C_3 's better ability to penetrate lipid bilayer membranes. The ability to pass the blood-brain barrier (BBB) and scavenge hydroxyl and superoxide radical species is of importance for a swathe of CNS-related diseases, as oxidative stress is implicated in various disease pathways—and indeed, C_3 has shown promise in amyotrophic lateral sclerosis (ALS) and Parkinson's models [138]. In fact, in *in vivo* primate Parkinson's models, a two-month treatment with C_3 significantly improved motor function without any toxicity [139]. It has been found that carboxyfullerenes exert their potent neuroprotection not only through ROS scavenging but also via modulating mitochondrial dynamics [140].

PHFs have also shown promise in the treatment of neuroinflammation after ischemic stroke [141]. Cellular damage and inflammation were both reduced, leading to a smaller infarct size after five days. Alongside the antioxidant approach, structural differences between fullerene derivatives have also been investigated for their differing effects in antineoplastic behaviour and ability to induce neural repair via neural stem cell (NSC) growth [142]. A suite of well-defined water-soluble C_{60} derivatives were synthesised starting from the chlorinated precursor $C_{60}Cl_6$ (with five chlorine atoms attached at the end of the radialene arms of one pentagon, and the sixth on the pentagon itself [143]). Specifically, a derivative with phenylbutyrate groups was found to promote neural repair without affecting glioblastoma growth; a phenylalanine-based derivative did the opposite, inhibiting glioblastoma growth with no effect on neural repair. This thus shows that the surface chemistry of the fullerene derivative drastically affects its biomedical action.

1.2.1.5 Photodynamic Therapy

PDT is lauded as an attractive cancer therapy, complimentary to traditional radiotherapy, for localised smaller lesions and carcinomas, as well as as an aid during surgery of larger masses, thanks to its relative non-invasiveness, precise targeting and lack of long-term side effects [144]. It works by irradiating an intravenously administered photosensitising molecule at a particular wavelength, which excites it into its very short-lived (nanosecond scale) first excited singlet state, S_1 . Non-radiative decay of this S_1 state gives the longer-lived (up to milli-second scale) triplet state, T_1 which is subsequently quenched [145]. In the case of molecular oxygen, quenching by energy transfer¹⁶ leads to the formation of a highly reactive and cytotoxic ROS: singlet oxygen (1O_2) [146]. Thus, the effectiveness of PDT relies on the evolution of singlet oxygen in areas with a high density of cancerous cells.

Fullerene derivatives hold great promise as PDT photosensitisers due to their unprecedented efficiency of 1O_2 formation. In benzene, under irradiation at 532 nm, pristine C_{60} has a 1O_2 generation efficiency (Φ) of 0.96 [147]. This high efficiency is due to intersystem crossing (ISC) from the excited singlet state to the triplet state, which occurs in C_{60} at an efficiency of near unity, and makes C_{60} a promising candidate as a highly effective photosensitiser in PDT [148, 149].

There are, however, some drawbacks. The first is that C_{60} absorbs well in the UV range and poorly in the visible range, particularly at the red end of the electromagnetic spectrum; this matters as these are the wavelengths of the light sources typically used in PDT. The second potential drawback concerns the effect of multiple functionalisation of the fullerene cage. Hamano *et al.* showed that increasing the number of addends decreased the efficiency of 1O_2 production [150]. At the extreme end, a PHF exhibited zero photosensitisation. A final

¹⁶ This is the Type II photosensitisation pathway; the Type I pathway involves the transfer of an electron to molecular oxygen, yielding superoxide radical $O_2^{\cdot-}$.

drawback is the solvent dependency of $^1\text{O}_2$ generation. Yamakoshi *et al.* showed that, while $^1\text{O}_2$ is easily generated in nonpolar solvents, $\text{O}_2^{\cdot-}$ and $\text{OH}^\cdot$ are the preferential products in polar solvents such as water, and that these are the species responsible for DNA cleavage in physiological conditions [151], thus casting shadow over the precise mechanism(s) of PDT. Readers are directed to the review of Mroz *et al.* for a fuller treatment of this topic [152].

1.2.1.6 Drug Delivery Vehicles

Despite the bountiful opportunities that fullerenes offer for use as therapeutics in their own right thanks to their unique chemical, physical and photophysical properties, they can also be employed as agents and carriers for the delivery of other drugs. This can be achieved in two distinct ways: small molecule species can be conjugated to the cage via covalent linkage, or the fullerene can be functionalised in such a way as to give an amphiphilic structure which self-assembles into a micellar structure, suitable for action as a drug delivery vehicle (DDV).

Examples of conjugation are numerous in the literature, and PHFs have shown particular promise due to the abundance of easily functionalisable hydroxyl groups [153]. Cancer treatment is a particular area where conjugation of drugs to fullerenes is of vital importance: many of these drugs suffer from poor solubility, poor bioavailability, and severe undesirable side-effects. Seemingly across the board, the efficacy and availability of these drugs are improved via conjugation with fullerenes, irrespective of the drug. Doxorubicin (DOX) is a “gold standard” chemotherapeutic agent. DOX and PEG with Mw ~ 2000 can be conjugated to PHF C_{60} in a simple two-step synthetic procedure to yield derivatives which can deliver a high concentration of DOX to the tumour [154]. The therapeutic efficacy was found to be the same as free DOX but the fullerene conjugate exhibited no unwanted cytotoxic side effects on healthy tissue. A non-covalent conjugation approach has also been achieved by oxidising the surface of C_{60} with nitric acid before conjugating DOX via favourable hydrogen bonding and

π - π interactions [155]. Studies relying solely on non-covalent interactions of cisplatin with pristine C₆₀ have also shown an improved antineoplastic effect [156].

Simple covalent conjugation between PHFs and anti-cancer drug, methotrexate (MTX), has also been achieved [157]. The resultant ester bond could be hydrolysed within cells leading to a slower drug-release profile than for MTX by itself—indeed, in the acidic conditions of tumorous tissue, the drug release was “switched on” as compared to when circulating in the blood at normal physiological pH (85.7% *cf.* 38.2% respectively). Carboxyfullerenes can also be used as starting scaffolds for conjugating anti-cancer drugs such as docetaxel (DTX) [158]. A crudely functionalised carboxylated C₆₀ was synthesised by mixing in concentrated sulphuric and nitric acids. Acylation then preceded conjugation with DTX in the presence of anhydrous tetrahydrofuran (THF) and pyridine. A four-fold increase in bioavailability of DTX for the treatment of breast cancer cells was observed using this conjugation method. Glycine has also been attached to C₆₀, followed by acylation, to create a scaffold for conjugation of the anti-cancer drug, tamoxifen (TAM) [159]. A tetraethylene glycol linker was incorporated between the glycine addend and TAM to act as an intracellular release mechanism, due to its biodegradability. This led to a three-fold increase in bioavailability of TAM and a marked increase in the efficacy of the conjugated system *cf.* the drug by itself.

The efficacy of anti-cancer therapeutics can be improved even further by the targeting of specific receptors. Folate receptors (FR) are often overexpressed in malignant tumour cells and so the use of folic acid (FA) as a targeting agent is a viable strategy. Fullerenes offer the perfect scaffold onto which to graft such a targeting moiety—combined with the conjugation of an anti-cancer drug, this is a promising platform for greatly improving cancer therapeutics. FA has been conjugated to C₆₀ functionalised first by EDA and then polyethyleneimine (PEI) [160]. DTX was then simply incorporated into the structure by physisorption after room temperature sonication for two hours. The FR-targeting system achieved better treatment

efficiency in tumour-bearing mice than both free DTX and C₆₀-DTX conjugate without the FA targeting moiety.

FA-functionalised C₆₀ has also been used as a DOX-releasing therapy [161]. Starting with PHF, succinic acid was attached via the hydroxyl groups before DOX and FA were covalently linked using biodegradable triethylene glycol spacers. An average of five DOX molecules were conjugated to each C₆₀. Along with improved DOX bioavailability, two additional functionalities are possible with this system. First, PDT thanks to irradiation of the C₆₀ core supplemented the chemotherapeutic use of DOX, thus increasing the anti-cancer activity. Second, DOX is a fluorescent molecule. When attached close to C₆₀, electron transfer to the strongly electron-accepting fullerene is possible and thus DOX's fluorescence is quenched. Upon biodegradation of the triethylene glycol linker, DOX increases its distance from the fullerene and so its fluorescence is switched on—thus the intracellular release of DOX can be elegantly tracked using fluorescence microscopy.

The second technique of fullerene-based drug delivery involves functionalising the cage in such a way as to produce an amphiphilic molecule which can self-assemble in aqueous solution into structures such as micelles and liposomes, on the order of nanometres to micrometres in size [162]. So-called “buckysomes” are self-assembled structures formed from a Bingel-modified C₆₀ hexakis-adduct, where one of the malonates is a hydrophilic dendrimer while the other five are simple long aliphatic chains [163]. The hydrophobic core of these buckysomes is a suitable environment for embedding hydrophobic anti-cancer drugs such as paclitaxel [164]. Indeed, this system is as effective as a commercially nanoparticle-based paclitaxel formulation, Abraxane. Uniformly sized bilayer vesicles can also be formed using the structural template offered by chlorofullerene, C₆₀Cl₆ [165]. Methyl esters of 4-aminobutyric/glutamic acid or phenylalanine were attached at five of the chlorine positions. The resultant spherical vesicles, self-assembled in 4:1 acetonitrile-water mixtures,

had diameters of 80–135 nm and successfully encapsulated three different anti-cancer drugs. In the case of 5-fluorouracil, its release rate was three times slower than that of the non-encapsulated drug.

1.2.2 *Diagnostics*

Magnetic resonance imaging (MRI) dominates research on fullerenes for non-invasive imaging techniques¹⁷ and, as such, are the key focus of this section. Interesting studies on other imaging modalities and some theranostic systems are also included.

1.2.2.1 *Magnetic Resonance Imaging*

The paramagnetic gadolinium(III) ion (Gd^{3+}) is used in MRI contrast agents (CAs) because its large magnetic moment and seven unpaired, isotropically distributed $4f$ electrons (the highest number for any element) dramatically reduce the T_1 and T_2 relaxation times¹⁸ of any surrounding water protons, thus giving increased contrast in images [167, 168]. The percentage change in T_1 is greater than in T_2 and so the best visualisation is in so-called T_1 -weighted images [169]. In order for Gd^{3+} to enter the body, chelating agents are used to strongly bind the ion; several different chelating agents are currently available on the market and are used every day in hospitals across the world [170]. However, there is a growing body of evidence that the chelates currently used as CAs do not bind the gadolinium as strongly as thought in physiological conditions: repeated administration leads to gadolinium deposition in the brain [171], and environmental gadolinium concentrations have increased with increasing use of MRI CAs [172]. Most concerning is the link between use of gadolinium-containing CAs and the onset of nephrogenic systemic fibrosis (NSF)

¹⁷ Readers are directed elsewhere for a comprehensive discussion of such techniques [166].

¹⁸ Thus increasing the relaxivities, respectively r_1 and r_2 .

in patients with renal failure [173]. Given these concerns, the European Medicines Agency (EMA) has recently suspended and restricted the clinical use of certain gadolinium-chelating MRI CAs [174]. The stage is seemingly set for fullerenes to be exploited as MRI CAs [175] as the endohedral gadolinium species cannot escape the cage and, as will be shown, many of the Gd-EMFs outperform currently used agents, giving the same T_1 -weighted contrast at lower concentrations.

One of the earliest examples of a Gd-EMF CA was polyhydroxylated $\text{Gd}@\text{C}_{82}(\text{OH})_{20}$ [176]. The observed T_1 relaxivity (r_1) of $47 \text{ mM}^{-1}\text{s}^{-1}$ (at 9.4 T magnetic field) is an order of magnitude greater than clinically used CAs. These chelating agents typically allow one water molecule to interact directly (inner-sphere mechanism) with Gd^{3+} at a time, whereas water protons can exchange with the twenty hydroxyl groups on the fullerene—this was posited to be the reason for the higher r_1 value, even though there is no direct interaction between Gd^{3+} and the water protons (second-sphere mechanism). This effect is likely enhanced by the transfer of gadolinium's three electrons to the fullerene cage [177]. A more highly functionalised cage, $\text{Gd}@\text{C}_{82}(\text{OH})_{40}$ gave a higher r_1 value of $81 \text{ mM}^{-1}\text{s}^{-1}$ at the clinically used 1.0 T field [178]. Given, however, that highly hydroxylated cages have been shown to lead to open structures [52] which may let the endohedral species escape, a less hydroxylated derivative, $\text{Gd}@\text{C}_{82}(\text{OH})_{16}$ has also been synthesised [179]. Even this species, with r_1 of $19.3 \text{ mM}^{-1}\text{s}^{-1}$ (4.7 T field), outperformed a clinically used chelate. Other examples of synthetic routes to $\text{Gd}@\text{C}_{82}$ derivatives with better r_1 values *cf.* clinical agents include a one-pot reaction with β -alanine ($r_1 = 9.1 \text{ mM}^{-1}\text{s}^{-1}$ at 1.5 T) [180] and functionalisation with bone-targeting organophosphates ($r_1 = 37 \text{ mM}^{-1}\text{s}^{-1}$ at 0.35 T) [181].

Another Gd-EMF which has been extensively studied as an MRI CA is the TNT EMF, $\text{Gd}_3\text{N}@\text{C}_{80}$. It would be expected that this fullerene, with three gadoliniums per cage, would outperform $\text{Gd}@\text{C}_{82}$ with one per cage, and indeed this was shown to be the case [182]. This

derivative was functionalised with a PEG-functionalised malonate (PEG average $M_w = 5000$) via the Bingel reaction, before the introduction of multiple hydroxyl groups. At 2.4 T, r_1 was measured to be $143 \text{ mM}^{-1}\text{s}^{-1}$. The replacement of the “stealth” PEGylated addend with a carboxylic acid-functionalised moiety increases the r_1 value to $207 \text{ mM}^{-1}\text{s}^{-1}$ (2.4 T) [183]. This was ascribed to a unimodal distribution of larger aggregates of 78 nm in pure water (cf. PEG-Gd₃N@C₈₀), as measured by DLS. In phosphate buffer solution (PBS), this aggregate size decreased to 44 nm, accompanied by a decrease in r_1 to $35 \text{ mM}^{-1}\text{s}^{-1}$. This dependence of r_1 on aggregate size has been further confirmed by the conjugation of lower molecular weight PEG chains (average $M_w = 350$), which gave 95 nm aggregates (cf. 37 nm for PEG₅₀₀₀) and r_1 of $237 \text{ mM}^{-1}\text{s}^{-1}$ [184].

Other examples of Gd-EMF MRI CAs include the mixed-metal EMFs, Sc_xGd_{3-x}N@C₈₀O_m(OH)_n [185] and the exotic, egg-shaped Gd₃N@C₈₄(OH)_x [186]. The mixed-metal EMFs were produced in higher yield than solely Gd₃N@C₈₀ during arc discharge, and gave r_1 values (14.1 T) of 20.7 ($x = 1$) and $17.6 \text{ mM}^{-1}\text{s}^{-1}$ ($x = 2$), still better than clinically used agents. The unusual egg-shaped Gd-EMF outperformed its more highly symmetric C₈₀ analogue at all measured field strengths (e.g. 173 vs. 140 $\text{mM}^{-1}\text{s}^{-1}$ at 1.4 T). This is likely due to, as observed, larger aggregate size and a larger number of hydroxyl groups on the surface of the cage available for exchange with surrounding water protons.

Application-wise, the carboxylated Gd₃N@C₈₀ derivative has proved a useful scaffold for the conjugation of IL-13 peptides, known for their ability to target glial tumours due to overexpression of the IL-13 receptor [187]. The resultant structure is thus an efficient imaging tool for brain tumour cells. Hydroxylated Gd₃N@C₈₀ has been used to track stem cells in the lung [188] and has been incorporated into targeted liposomes for the detection of atherosclerotic plaque lesions [189]. This Gd-EMF has also been used as a scaffold for the grafting of radioactive label species in order to produce unique theranostic and dual-modal

imaging systems. Functionalisation of the cage with chelate, tetraazacyclododecane tetraacetic acid (DOTA) labelled with β -emitter, ^{177}Lu , allows for both MR imaging and delivery of brachytherapy¹⁹ for the treatment of brain tumours [190]. Hydroxylating the cage instead, followed by iodination with ^{124}I , gives a dual-modal positron emission tomography (PET) and MR imaging agent [191].

1.3 MOLECULAR MACHINES

In line with examination regulations, only a cursory introduction to molecular machines, specifically rotaxanes and those containing fullerenes, is given here as this was the topic of a thesis I have previously submitted for a degree at this institution [192].

A molecular machine is defined as a molecular device whose function involves the controlled mechanical motion of one molecular component relative to another, in response to some stimulus [193]. Like machines in the macroscopic world, the process should be reversible and give a signal to indicate operation [194]. A plethora of synthetic strategies and theoretical designs exist for molecular machines [195, 196], but arguably the most promising are those that attempt to mimic Nature in harnessing, rather than fighting against, Brownian motion [197, 198]. In order for the relative motion of molecular machines to take place, *mechanically interlocked molecules* (MIMs) must be synthesised so as to stop components disassociating from each other. Central to the synthesis of MIMs is the idea of the *mechanical bond*—this is the association of two or more molecular components by topological constraints. Classic examples include molecular knots [199, 200], catenanes [201, 202] and, the focus of this work, rotaxanes [203–206].

¹⁹ Delivery of a radioactive source within the body so as to allow localised tumor targeting with minimal radiation side-effects *cf.* traditional radiotherapy using external X-ray sources.

1.3.1 Rotaxanes

A rotaxane is described as an $[n]$ rotaxane where n is the number of mechanically interlocked components. A macrocycle encompasses the linear axle of the dumbbell-like structure and is prevented from sliding free of the rotaxane architecture by two bulky stopper groups (Fig. 9).

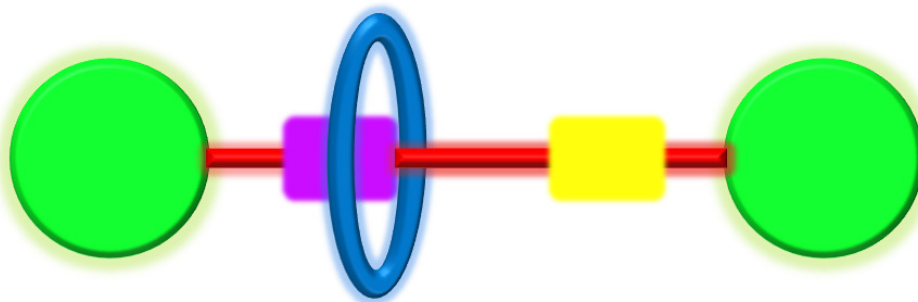


Figure 9: Schematic representation of a $[2]$ rotaxane. The dumbbell structure comprises a molecular axle (red) bookended by two bulky stopper groups (green) which prevent the macrocycle (blue) from sliding free. The macrocycle would move freely along the axle buffeted by the Brownian motion of the surrounding medium were it not for the presence of two “stations” (purple and yellow). The rotaxane system is designed such that non-covalent interaction between the macrocycle and a given station are favourable under certain conditions; upon changing those conditions (e.g. pH, presence of anions), the non-covalent interaction between the macrocycle and that station becomes less favourable than between the macrocycle and the other station. Thus, the macrocycle preferentially shuttles to reside at the other station.

External stimuli [207–210] can induce shuttling between two stations, which have favourable non-covalent interactions with the macrocycle, thus thermodynamically anchoring the macrocycle in place.

Fullerenes have been specifically incorporated into rotaxanes, not only as bulky stopper groups, but also for their excellent electron-accepting properties; by also including a donor into the rotaxane, controllable charge-separated states can be created [211–216]. The linear motion of rotaxanes have been investigated for biomedical applications (e.g. intracellular transport [217]) as well as as biomimetic functionalities (e.g. like muscle tissue) [218]. However, until now, the explicit investigation of combining these two systems—fullerene-containing rotaxanes and rotaxanes for biomedical applications—has not been meaningfully pursued.

WATER-SOLUBILISATION OF FULLERENES

C₆₀ is functionalised with multiple groups of the oligomer, triethylene glycol monoethyl ether (TEG-MEE). The resultant molecular structure is characterised by a suite of techniques; in the absence of reliable mass spectrometry data, EA and TGA are processed using a tabular method to determine the average stoichiometric composition and molecular weight. The highly water-soluble derivative shows a bathochromic shift in its photoluminescent behaviour, suggesting potential utility as a fluorescent probe.

2.1 INTRODUCTION

There now exists a whole library of water-soluble fullerene derivatives, many of which have been specifically synthesised for use in biomedical applications (Chapter 1). The starting point of this thesis is the synthesis of a novel highly water-soluble derivative of C₆₀ via covalent exohedral functionalisation with cheap and readily available materials. Oligomeric ethylene glycols have been used previously to functionalise nanoparticles in order to make them resistant to protein adsorption [219], thus increasing their retention in the body and making them useful for biomedical applications.

2.2 MOTIVATION

The primary aim of this chapter is the synthesis of a neutral and highly water-soluble C_{60} fullerene derivative based on the modification of a previously reported synthetic procedure using oligomeric ethylene glycol [220]. This procedure was chosen as 1) the resultant structures were reported to show photoluminescent behaviour which varied with the starting concentration of C_{60} in the reaction mixture, and 2) I believed that the synthesis could be improved and, thus, the photoluminescent behaviour better understood. The improvement involves the introduction of a monoethyl ether functionality at one end of the ethylene glycol chain, rather than a hydroxyl group. In this way, it is anticipated that any TEG-MEE chain can only be covalently linked to one C_{60} , giving the target structure, **1**, in Fig. 10; previously, the presence of two hydroxyl groups meant that the bridging of two fullerenes could not be precluded. The target structure shows not only x TEG-MEE chains but also the presence of y hydroxyl groups [221] and n oxygen atoms in functional groups other than TEG-MEE and hydroxyl, such as e.g. carbonyl [222, 223], that are expected to be introduced during the synthesis procedure.

2.3 FUNCTIONALISATION OF C_{60} WITH TEG-MEE

2.3.1 *Synthesis of TEGylated C_{60}*

C_{60} (40 mg, 55.6 μ mol, 1 eq) was dissolved in toluene (25 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (5 mL, 28.6 mmol, 515 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (217 mg, 5.4 mmol, 97 eq) was

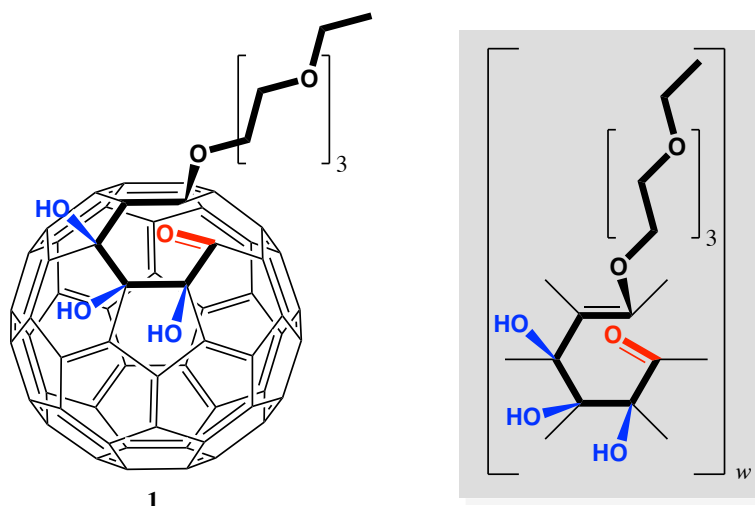


Figure 10: Target structure of TEGylated C₆₀ **1** with the functionalisation motif of one hexagon highlighted for clarity (grey box). A motif such as this would expect to repeat several (w) times across the surface of the fullerene. The presence of a carbonyl group automatically leads to an open-cage structure, which can reasonably be expected from the hydroxylation of C₆₀ under alkaline conditions [223]. All wedge bonds radiate *out* from the fullerene surface.

added and the stoppered reaction solution was stirred for 21 hours at room temperature (a visible change in solution colour from purple to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (75 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 µm), leaving a brown precipitate on the filter and a cloudy yellow filtrate. EtOAc (150 mL) was added to the cloudy filtrate and placed in the fridge overnight. On filtering through a Büchner funnel again, brown precipitate was left on the filter and the filtrate was a clear pale yellow. The brown precipitates on the PTFE filters were redispersed in EtOH (35 mL) with reasonable solubility, before EtOAc (70 mL) was again added and the solution left in the fridge overnight, giving a brown precipitate. This EtOH redispersion–EtOAc precipitation–filtration process was repeated once again, and the resulting precipitate dried *in vacuo* to give **1** as a brown powder (121 mg).

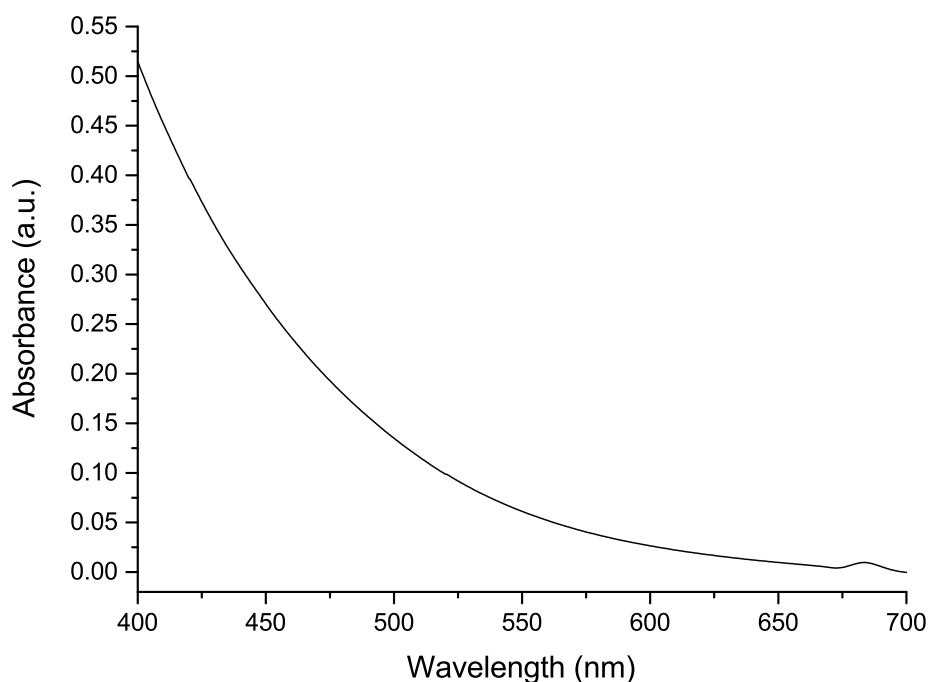


Figure 11: Visible absorption spectrum of TEGylated C₆₀ **1** (0.1 mg/mL, H₂O).

2.3.2 Structural Characterisation of TEGylated C₆₀

The following structural characterisation results are discussed *in situ* as well as collated and summarised in Section 2.3.2.7. Discussion section 2.5.2 covers the solubility and photoluminescent measurements of Sections 2.4 and 2.5.

2.3.2.1 Absorption Spectrum of TEGylated C₆₀

The visible absorption spectrum of aqueous solution of **1** (Fig. 11) does not show the 425 nm shoulder diagnostic of a fullerene functionalised with few groups, instead displaying a featureless spectrum whose absorbance strongly increases towards the blue, indicative of a highly functionalised cage [224].

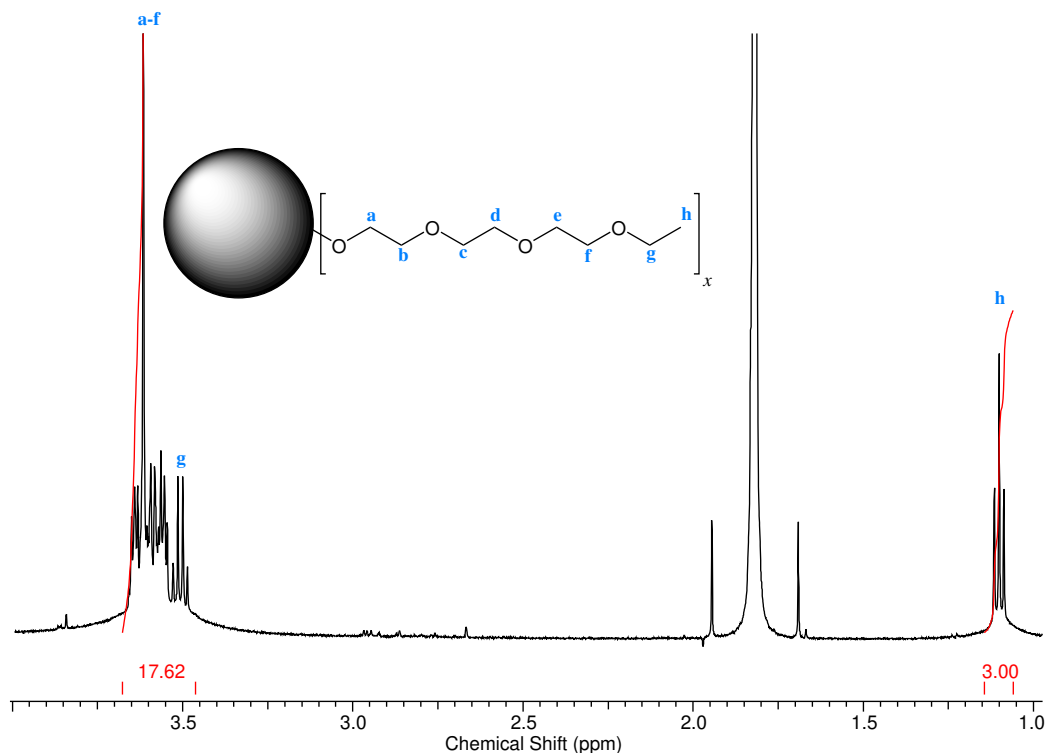


Figure 12: ^1H NMR spectrum of **1** (500 MHz, D_2O) δ 3.67–3.54 (12H, m, CH_2), 3.51 (2H, q, $J = 7.1$ Hz, CH_2), 1.10 (3H, t, $J = 7.1$ Hz, CH_3). A solid sphere is used to represent the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in ^1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains attached around the fullerene cage.

2.3.2.2 NMR Characterisation of TEGylated C₆₀

The ^1H NMR spectrum of **1** (Fig. 12) confirms the presence of TEG-MEE. The triplet (δ 1.10) is assigned to the terminal alkyl protons and the partially hidden quartet (δ 3.51) to those of the adjacent carbon. The large multiplet cluster corresponds to the rest of TEG-MEE's protons, whose environments are all very similar. Assignment of the triplet and quartet to the five ethyl protons (on C_g and C_h in the TEG-MEE chain) is confirmed by COSY NMR (Fig. 13a). Closer inspection of the highlighted region of the COSY spectrum (Fig. 13b) shows at least one more coupling (δ 3.64 and δ 3.55), which is tentatively assigned to the protons on C_a and C_b . The above assignments are confirmed by the multiplicity-edited HSQC spectrum (Fig. 14). Terminal C_h (δ 14.0) is easily identified by its positive peak, indicative of an odd number

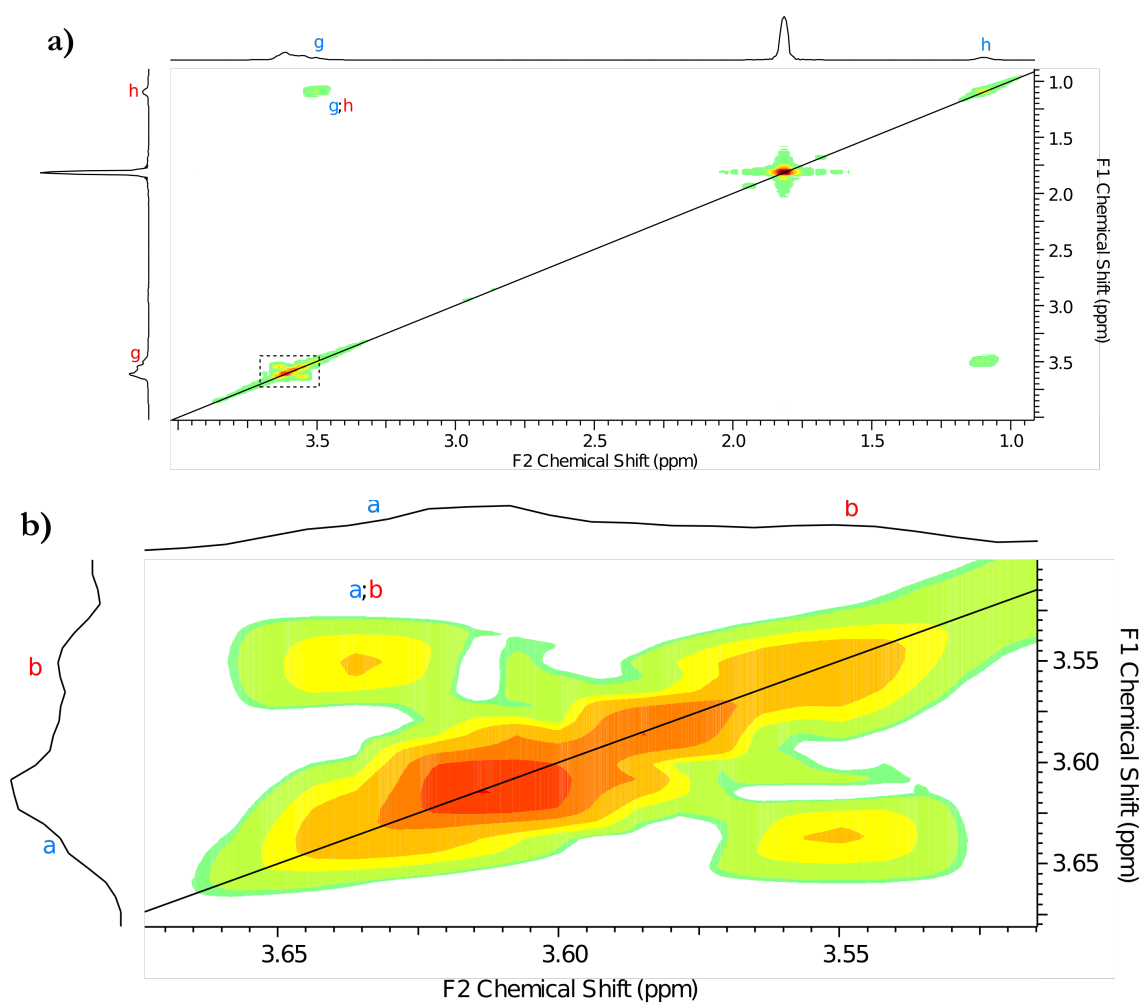


Figure 13: COSY NMR spectrum of TEGylated C₆₀ **1** (500 MHz, D₂O) showing (a) coupling between adjacent ethyl protons in TEG-MEE, and (b) a zoom-in of coupling between protons on adjacent glycol carbons.

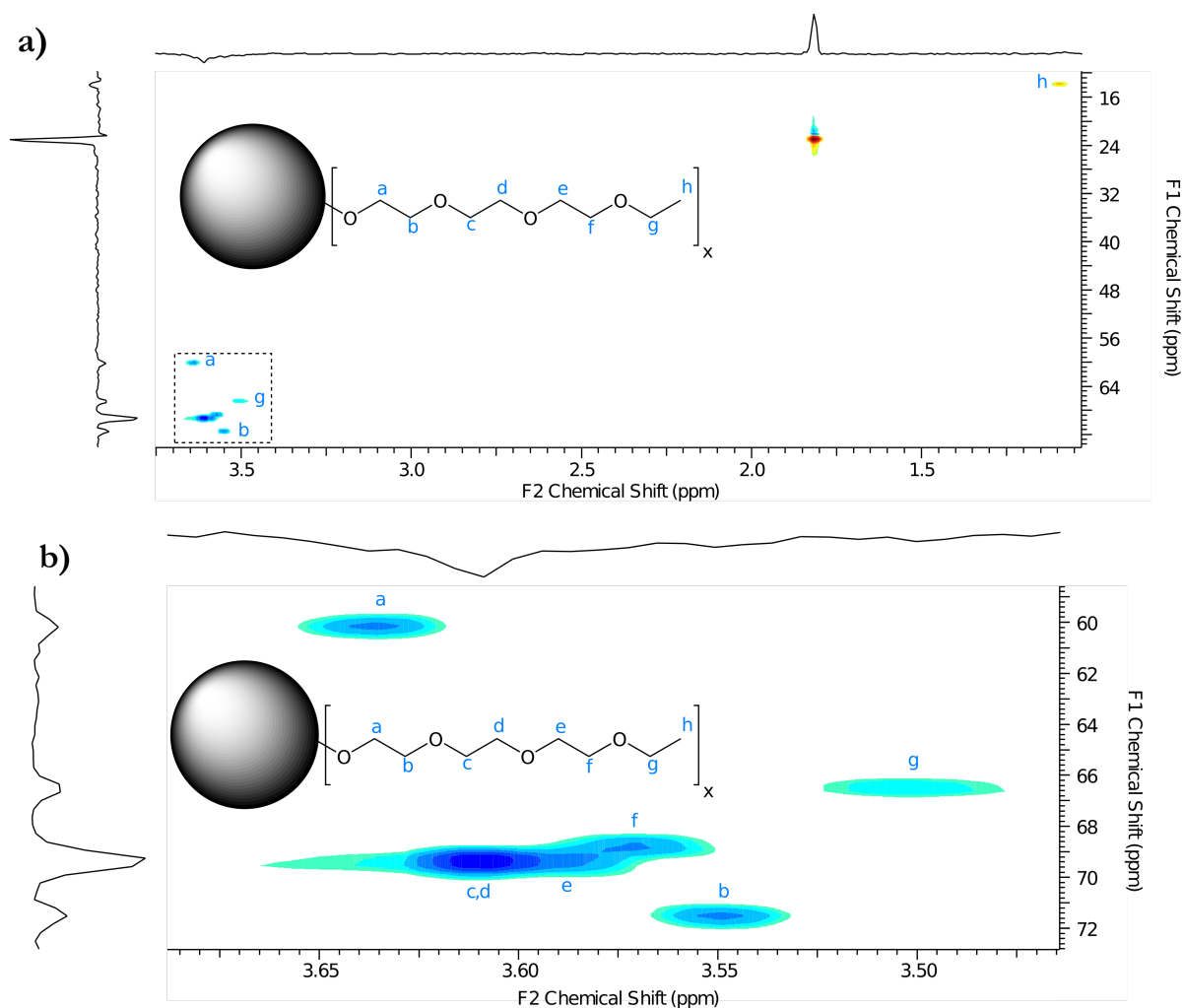


Figure 14: (a) Multiplicity-edited HSQC spectrum of TEGylated C₆₀ **1** (500 MHz, D₂O) with (b) a zoom-in of $-\text{CH}_2$ groups in the highlighted region. A solid sphere is used to represent the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in ^1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains attached around the fullerene cage.

of associated protons ($-\text{CH}_3$). The rest of the peaks in the highlighted region are negative, indicative of an even number of protons, thereby confirming that these peaks belong to the remaining $-\text{CH}_2$ carbons **a–g** in the TEG-MEE chain(s). C_g can be assigned (δ 66.6) based on its protons' relatively upfield chemical shift, as confirmed by COSY; this is corroborated by the HMBC spectrum (Fig. 15) wherein the ^2J coupling between C_g and the protons of C_h (and vice versa) are clearly visible. Confirmation of C_a (δ 60.2) being bonded to the protons at δ 3.64 is given by HMBC as these protons couple with only one carbon, namely C_b (δ 71.6),

whose protons can now be confidently assigned to δ 3.55, confirming COSY. Given that the proton environment of C_g has been confirmed, it is reasonable to search in HMBC for 3J coupling to C_f, and indeed this is seen.¹ In the same vein, now that the chemical shift of C_f is known (δ 68.8), its 2J coupling to the protons of C_e (δ 3.59) can be found. At this point, the poor resolution of the HMBC experiment somewhat hinders a full labelling of all possible 2J and 3J couplings. However, the equivalence of C_c and C_d (being symmetrically sandwiched between ethylene glycol units), means that their 1H , HSQC and HMBC peaks overlap to give a strong signal, and so C_e's peak (δ 69.4) can be isolated in HSQC. Indeed, the ^{13}C NMR signals of C_c and C_d are almost indistinguishable (Fig. 16), thus confirming identification of C_e. HMBC adds the final confirmation of the TEG-MEE structure as there is not only a strong peak associated with 2J coupling between C_c and C_d and the respective protons, but also 3J coupling between C_c and the protons of C_b, and C_d and the protons of C_e.

Aside from confirmation of the presence of TEG-MEE, the ^{13}C NMR spectrum shows some interesting features. The usual cluster of peaks in the range 130-155 ppm, which correspond to non-equivalent sp^2 fullerene carbons in functionalised derivatives [225], is wholly absent. Discussion of this is covered in Section 2.3.2.7. Instead, at least three unique carbon environments are observed downfield (δ 171.0, 164.8 and 163.21), indicative of oxidised carbons in the fullerene cage, such as carbonyl [222, 223] and enol ether [226] groups, and carbons adjacent to those directly attached to hydroxyl groups [227, 228]. On comparison with the ^{13}C NMR spectrum of neat TEG-MEE (Fig. 17), two new peaks (δ 68.3 and 63.8) appear in the region dominated by TEG-MEE's carbons. Crucially, the more downfield of these peaks (δ 68.3) is diagnostic of an alkoxy linkage to the fullerene cage [229], thus confirming a covalent attachment of TEG-MEE to C₆₀. The upfield peak (δ 63.8) can therefore reasonably be associated with a hydroxyl group directly attached to the cage [221].

¹ The appearance of this coupling as two peaks is simply down to the poor resolution of the measurement and the quadruplet shape of C_g's proton signal.

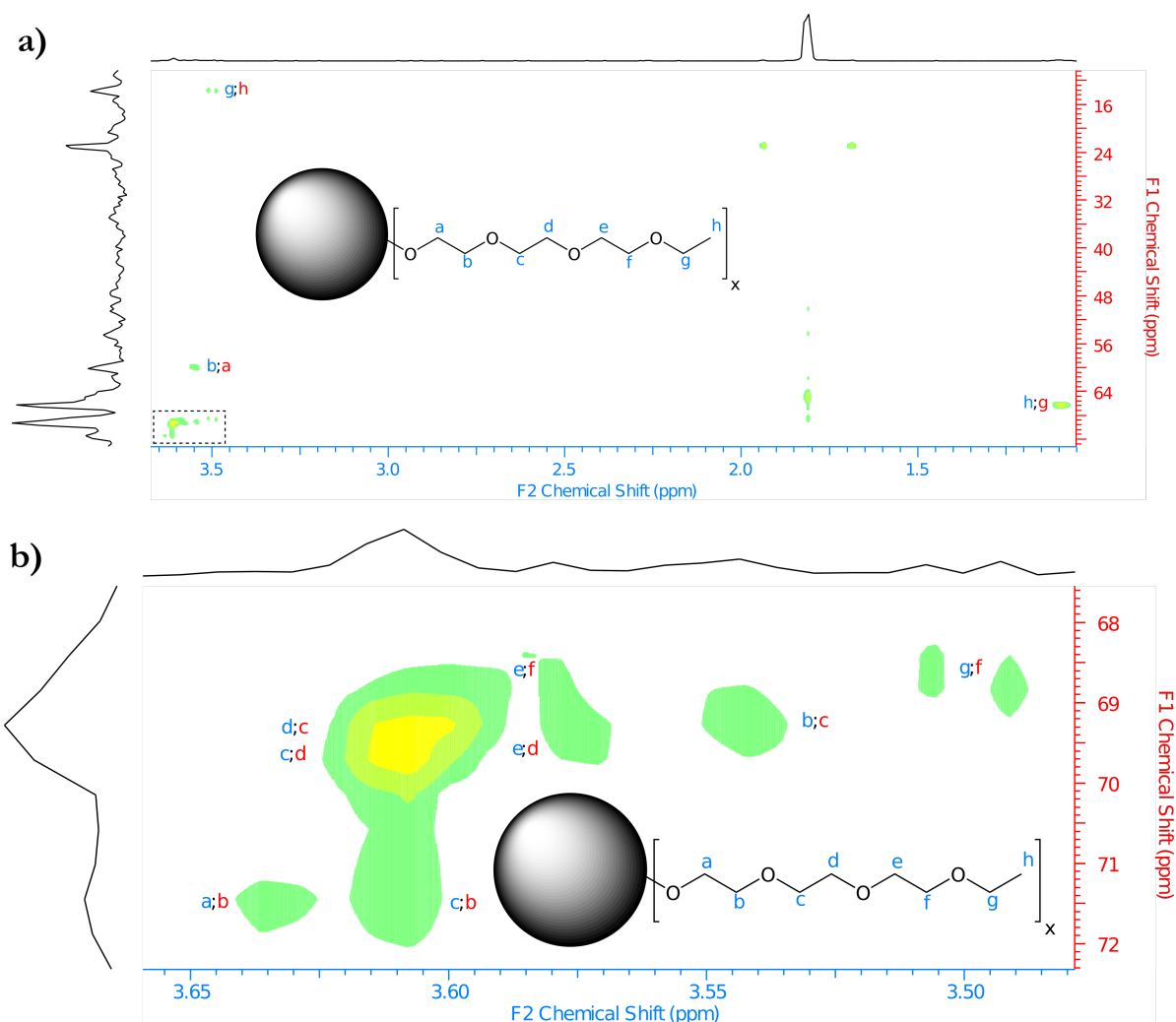


Figure 15: (a) HMBC spectrum of TEGylated C₆₀ **1** (500 MHz, D₂O) with (b) a zoom-in of the highlighted region. Red and blue axes are used to help differentiate contributions to the cross-peaks. A solid sphere is used to represent the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in ¹H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. *x* represents the as yet unknown number of TEG-MEE chains attached around the fullerene cage.

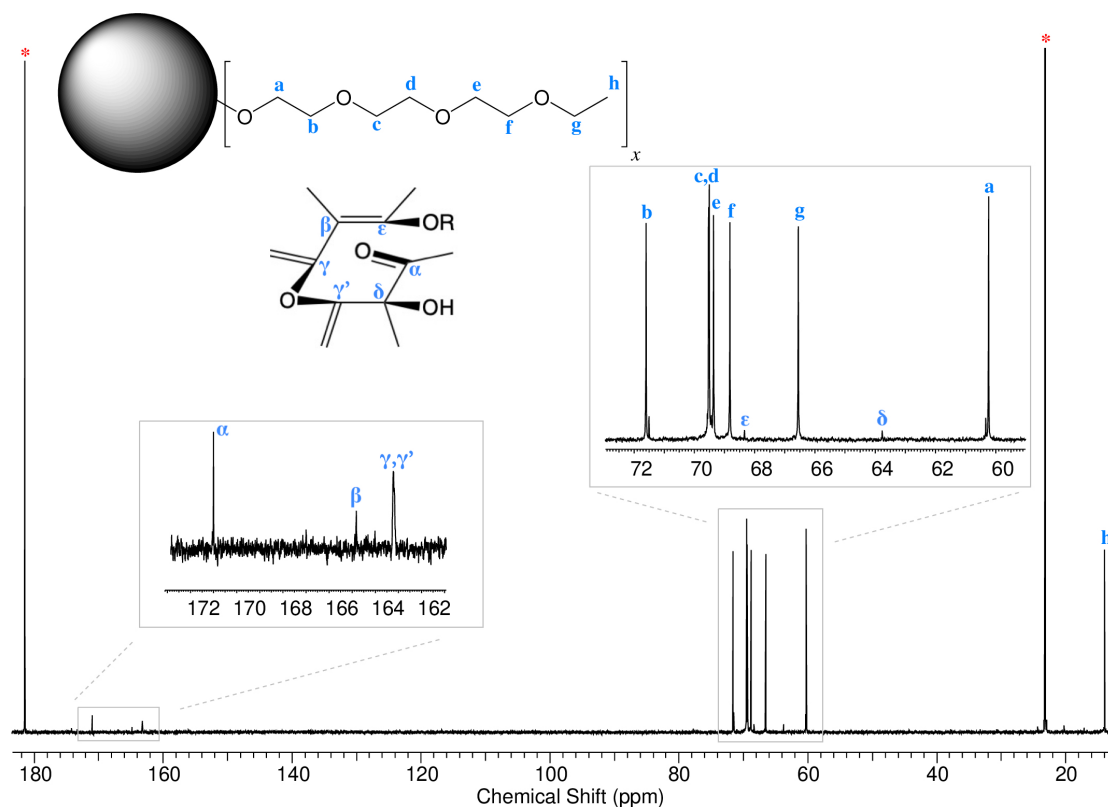


Figure 16: ^{13}C NMR spectrum of TEGylated C₆₀ **1** (126 MHz, D₂O) δ 171.03, 164.84, 163.24, 163.19, 71.61, 69.54, 69.52, 69.37, 68.83, 68.35, 66.56, 63.78, 60.25, 13.97, * residual EtOAc. A solid sphere is used to represent the fullerene cage, which likely contains numerous hydroxyl groups and other oxygen-containing groups such as carbonyls in a non-trivial arrangement. Assignments for such groups are, however, given (in Greek lettering) in the inset with reference to a feasible configuration on the fullerene surface (shown on one hexagon for clarity). x represents the as yet unknown number of TEG-MEE chains attached around the fullerene cage.

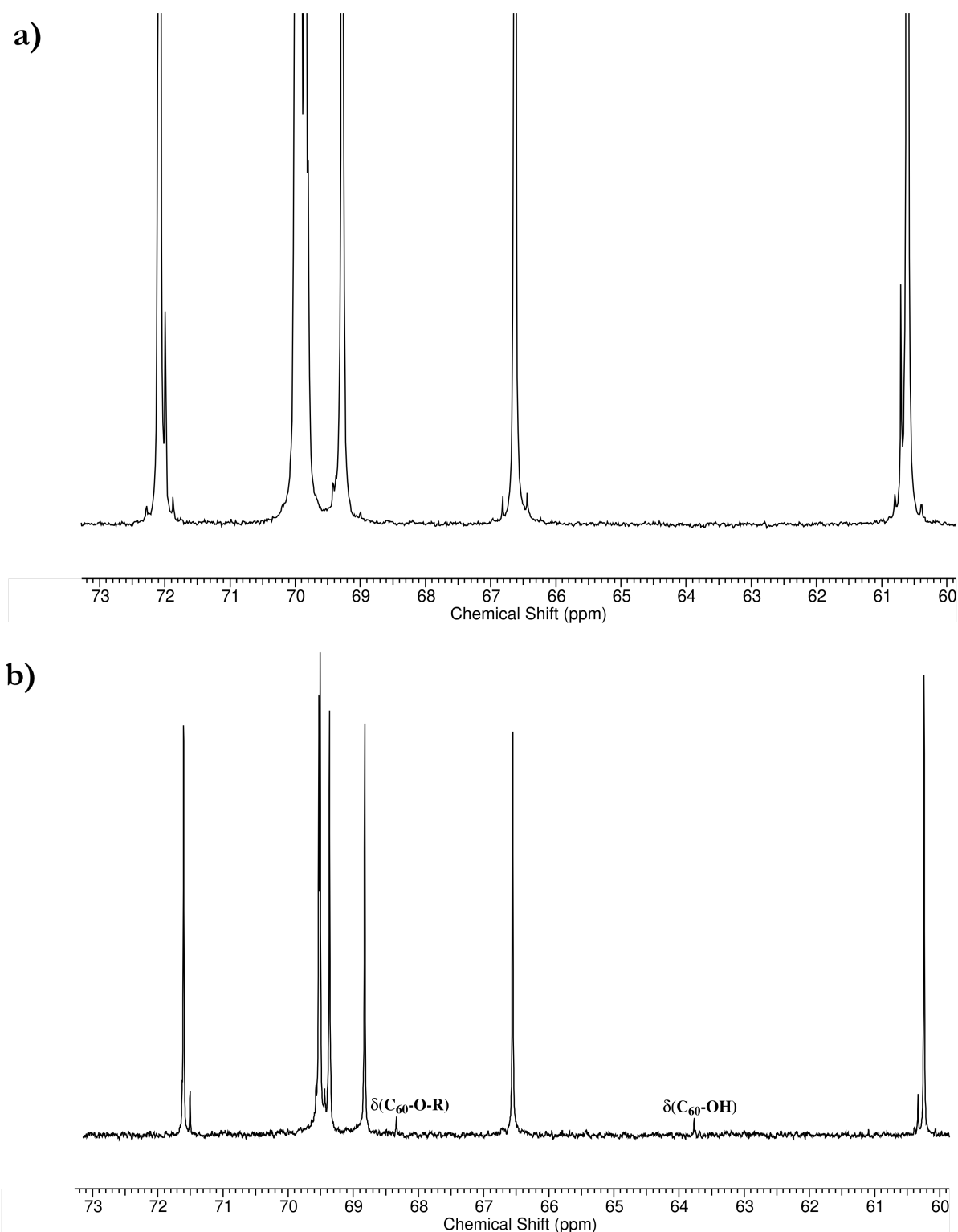


Figure 17: ¹³C NMR spectrum of (a) neat TEG-MEE in comparison with (b) TEGylated C₆₀ **1** (500 MHz, D₂O). New peaks unique to **1** correspond to alkoxy (δ 68.3) and hydroxy (δ 63.8) groups attached to the C₆₀ cage.

2.3.2.3 FTIR Characterisation of TEGylated C₆₀

The FTIR spectrum of neat TEG-MEE is shown as a basis for comparison (Fig. 18a). The broad peak with a maximum at 3443 cm⁻¹ corresponds to the stretching vibration of the hydroxyl group. The collection of peaks with a maximum at 2868 cm⁻¹ correspond to the symmetric and antisymmetric stretching vibrations of the methylene groups in the TEG-MEE chain and its terminal methyl group. Other than a carbon dioxide artefact, no other functional groups appear before the fingerprint region, as expected. The peak with maximum at 1458 cm⁻¹ and small shoulder corresponds to the in-plane bending vibrations of the methylene and methyl groups. The wagging and twisting vibration modes for the methylene groups appear at the maxima 1324 and 1294 cm⁻¹ [230]. The bending vibration mode of the glycol ether bridges has a maximum at 1376 cm⁻¹ while their asymmetric stretching mode corresponds to the maximum at 1247 cm⁻¹; the carbon-hydroxyl bond appears at a maximum of 1348 cm⁻¹. The bending and symmetric stretching vibration modes of these same groups appear as strong peaks with maxima at 1067 and 1103 cm⁻¹ respectively.² Finally, the three remaining peaks (maxima 939, 885 and 843 cm⁻¹) correspond to the rocking vibration modes of the methylene groups [231] and the stretching vibration mode of single carbon bonds [232].

In comparison, the FTIR spectrum of **1** (Fig. 18b) has a broader set of peaks with a maximum at 3407 cm⁻¹ corresponding to hydroxyl stretching vibrations, indicative of hydroxylation of the fullerene cage [233]. Beneath this broad stretch are the methylene and methyl stretching vibrations of the TEG-MEE chains (maxima at 3003, 2984, 2936 cm⁻¹). The most striking changes appear as we enter the fingerprint region. The strong peak with maximum at 1565 cm⁻¹ corresponds to the stretching vibrational mode of the sp² carbons of the fullerene cage [234]. The shoulder(s) in the range 1600—1700 cm⁻¹ could well correspond to a small number

² It is likely that the stretching vibration mode of the carbon-hydroxyl bond is hidden beneath these strong peaks.

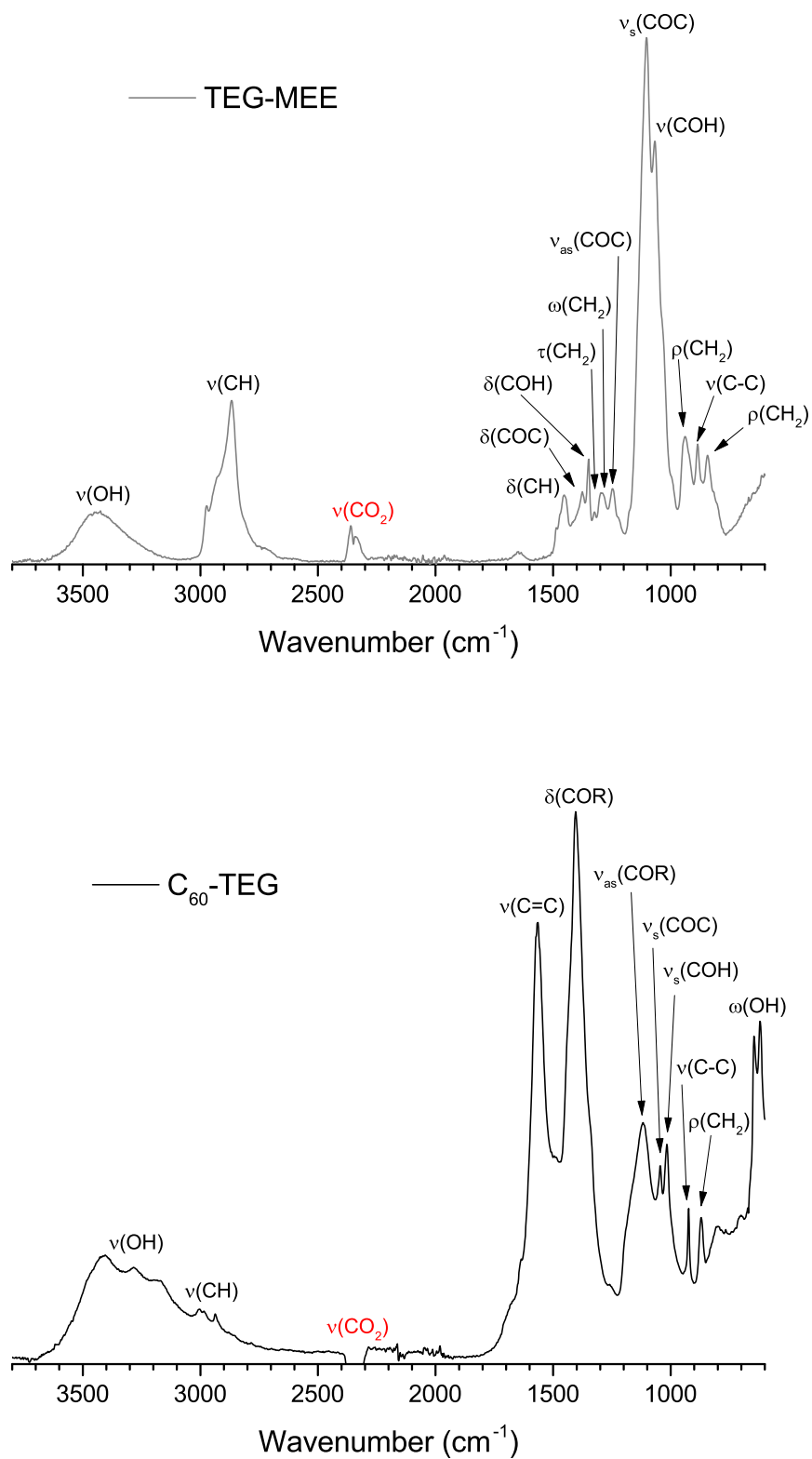


Figure 18: FTIR spectra of (top) TEG-MEE and (bottom) **1**. Nomenclature: ν_s/ν_{as} symmetric/asymmetric stretching; δ bending; ρ rocking; τ twisting; ω wagging.

of carbonyls [235], as also suggested by the ¹³C NMR spectrum in Fig. 16. The strongest peak at a maximum of 1404 cm⁻¹ corresponds to the bending vibrational mode of ether bridges [228]; the strength of this signal (particularly when compared to its counterpart in the spectrum of TEG-MEE by itself) is strong evidence of covalent functionalisation of the fullerene surface via oxygen bridges. The profile of this signal shows it is comprised of at least two peaks, suggesting that both TEG-MEE and hydroxyl groups are attached to the fullerene. Further evidence of this covalent functionalisation is given by the relatively broad signal (again of at least two peaks) for the asymmetrical stretching modes of ether bridges with maximum at 1118 cm⁻¹; this is, again, stronger than in the TEG-MEE spectrum.³ The symmetric stretching vibrational mode of the ether bridges is again present (maximum at 1044 cm⁻¹), along with the stretching vibrational mode of hydroxylated carbons (maximum at 1016 cm⁻¹). Below 1000 cm⁻¹, the stretching vibration mode of single carbon bonds (maximum at 924 cm⁻¹) is still present, which is a good indicator of the presence of TEG-MEE. Crucially, only one rocking vibration mode of the methylene groups is present (maximum at 870 cm⁻¹)—it has previously been shown that the frequency of the rocking mode of methylene groups adjacent to the hydroxyl group in ethylene glycols differs from that of the other methylene groups present in the chain [237], good evidence that the TEG-MEE chains have indeed been covalently grafted to the fullerene cage. Final evidence of hydroxylation of the cage is given by the presence of hydroxyl's wagging vibration modes with maxima at 646 and 620 cm⁻¹.

2.3.2.4 MS of TEGylated C₆₀

A first attempt to quantify the number of TEG-MEE chains appended to the fullerene core was attempted using MALDI-TOF MS. Obtaining a mass spectrum of **1**, however, proved to

³ It is also possible that this peak contains one of the four vibrational modes seen in pristine C₆₀ [236].

be very challenging. Spectra suffered variously from terrible signal-to-noise ratios and/or predominant matrix artefact peaks, making assignment of peaks nigh on impossible. A summary of the matrices, matrix solution solvents, sample solution solvents, solution volume ratios, and laser powers is given in Table 1. DCTB and dithranol were first deployed as standard matrices for obtaining MALDI-TOF spectra of fullerene derivatives; polar solvents were used that would be miscible with the sample solution but still dissolve the matrix. Having failed to obtain meaningful spectra with these matrices, a set of other matrices were chosen. These matrices and associated solvent mixtures (see Table 1) are standards in MALDI-TOF analysis of non-fullerene samples: sinapic acid, 2-(4-hydroxyphenylazo)benzoic acid (HPABA), 2,6-dihydroxyacetophenone (DHAP) and 2',4',6'-trihydroxyacetophenone monohydrate (THAPM). Again without success, final attempts were made to obtain a mass spectrum using no matrix at all, with sample **1** dissolved in three different solvents—H₂O, MeOH and DCM—but to no avail.

nESI of aqueous solutions of **1** was also attempted but spectra only showed low molecular weight distribution profiles associated with the TEG chains.⁴ The inability to obtain a comprehensible mass spectrum of **1** can be rationalised by it not being able to crystallise well with any of the tested matrices. This could be explained by three effects:

1. Matrices routinely used for MALDI-TOF MS of fullerene derivatives—namely DCTB and dithranol—might not be effective because of **1**'s highly functionalised and water-soluble structure which is very much unlike the hydrophobic nature of pristine fullerene and less highly functionalised derivatives. On the other hand, typical matrices for the analysis of water-soluble biological structures such as proteins—for example, sinapic

⁴ These measurements were carried out by the National Mass Spectrometry Facility at Swansea University. The qualitative description given in the text was provided in their report but not the spectra themselves.

Table 1: Unsuccessful MALDI-TOF MS measurements of 1. * Experiments run by the National Mass Spectrometry Facility, Swansea University.

MATRIX	MATRIX SOLVENT	SAMPLE SOLVENT	IONISATION MODE	LINEAR, REFLECTOR MODE	LASER POWER
DCTB	AcN	H ₂ O	Positive & negative	Linear & reflector	10–100%
		MeOH			
	THF + 1% TFA (aq.) (2:1 vol.)	H ₂ O			
		MeOH			
	MeOH	H ₂ O	Negative	Unknown	Unknown
		MeOH			
	DCM *	DCM			
		DCM			
Dithranol	THF	H ₂ O	Positive & negative	Linear & reflector	10–100%
		MeOH			
	THF + 1% TFA (aq.) (2:1 vol.)	H ₂ O			
		MeOH			
Dithranol (+NaOAc) *	THF	DCM	Positive	Unknown	Unknown
2,5-Dihydroxybenzoic acid [DHB] *	MeOH	DCM	Positive	Unknown	Unknown
Sinapic acid	AcN:H ₂ O (1:1 vol.)	H ₂ O			
		MeOH			
	AcN:H ₂ O + 1% TFA (aq.)	H ₂ O			
		MeOH			
2-(4-Hydroxyphenylazo)benzoic acid [HPABA]	MeOH	H ₂ O	Positive & negative	Linear & reflector	10–100%
		MeOH			
	AcN:H ₂ O (1:1 vol.)	H ₂ O			
		MeOH			
2,6-Dihydroxyacetophenone [DHAP] 2',4',6'-Trihydroxyacetophenone monohydrate [THAPM]	AcN:H ₂ O + 1% TFA (aq.)	H ₂ O			
		MeOH			
	—	H ₂ O			
		MeOH			
No matrix		DCM			
		H ₂ O			
	+ 1% TFA (aq.) (2:1 vol.)	H ₂ O			
		MeOH			

acid, HPABA, THAPM and DHAP (see Table 1)—might not be effective precisely because of **1**'s carbonaceous nature.

2. The employed functionalisation method is inherently uncontrolled (see Section 1.1.2.4). This would give rise to a range of similar molecular structures whose regioisomerism and spread of number of TEG-MEE and hydroxyl addends would make them difficult to crystallise. Similarly, any possibly obtained spectra would likely be non-trivial to assign due to the expected spread of m/z peaks and unpredictable fragmentation behaviour.
3. The presence of any salt or surfactant in the sample [238]. Although the observed pH (7–8) of aqueous solution of **1** suggests a minimal degree of saponification, any sodium ions present could affect sample crystallisation. Likewise, TGA results (see Section 2.3.2.6) support the claim that any TEG-MEE present in the sample is covalently bonded to the fullerene cage rather than physisorbed, such that it would have little effect as a surfactant. Indeed, sample **1** itself is amphiphilic like a surfactant and could explain its inability to crystallise well with the matrices.

In light of no reliable mass spectrometric data, the average structure of **1** has to be estimated (Section 2.3.2.7) using evidence from other complimentary techniques.

2.3.2.5 EA of TEGylated C₆₀

Elemental analysis was carried out to determine **1**'s percentage content of carbon, hydrogen and oxygen. The results are shown in Table 2. The sum of the percentage values (65.33 ± 0.52%) suggests that the sample did not wholly degrade during the dynamic flash combustion process, and so the observed values correspond only to groups appended to the fullerene cage. This is particularly important with respect to the %C measurement as then an estimated

Table 2: % content of C, H & O of TEGylated C₆₀ **1** as determined by EA. %N was determined at the same time as %C and %H, and was <0.1%. Absolute accuracy provided by Medac Ltd. is given in the table; duplicate measurements gave identical values.

%C	%H	%O
30.83 ± 0.30	3.37 ± 0.30	31.13 ± 0.30

number of additions to the cage can be made (Section 2.3.2.7). This assumption is backed up by the TGA results presented in Section 2.3.2.6, where it can be seen that indeed there is still a significant proportion (38%)⁵ of sample remaining at 900 °C, the operating temperature of the EA furnace for CHN analysis.

2.3.2.6 TGA of TEGylated C₆₀

Dynamic TGA of **1** was carried out by heating from room temperature at a rate of 10 °C/min under nitrogen flow of 200 mL/min, collecting data points every 0.2 s. The trace of **1**, showing at least four distinct mass losses as confirmed by the plot of its first derivative (Fig. 19), is in keeping with results seen for a tetraethylene glycol derivative synthesised using an analogous method [220]. Each mass loss is characterised by three parameters, here defined as: T_O (initial decomposition or onset temperature), T_M (temperature at maximum rate of decomposition), and Δm (percentage point change in mass). The first decrease, Δ_1 , (T_O 106 °C, T_M 122 °C, Δm 3.77 ± 0.57 %) corresponds to the loss of secondary bound water [45, 49], as would be expected of such a highly functionalised and hydrophilic structure. The lack of any appreciable mass loss which correlates to the TEG-MEE control sample is good evidence that any of its chains present must be covalently linked to the fullerene cage, rather than simply being loosely and non-covalently associated. The second mass loss, Δ_2 , (T_O 450 °C, T_M 471 °C, Δm 35.29 ± 1.84 %) corresponds to groups connected to the fullerene core via an oxygen atom, *i.e.* hydroxyl groups and TEG-MEE chains. This loss has previously

⁵ This is in reasonable agreement with the ~35% missing from the EA sample

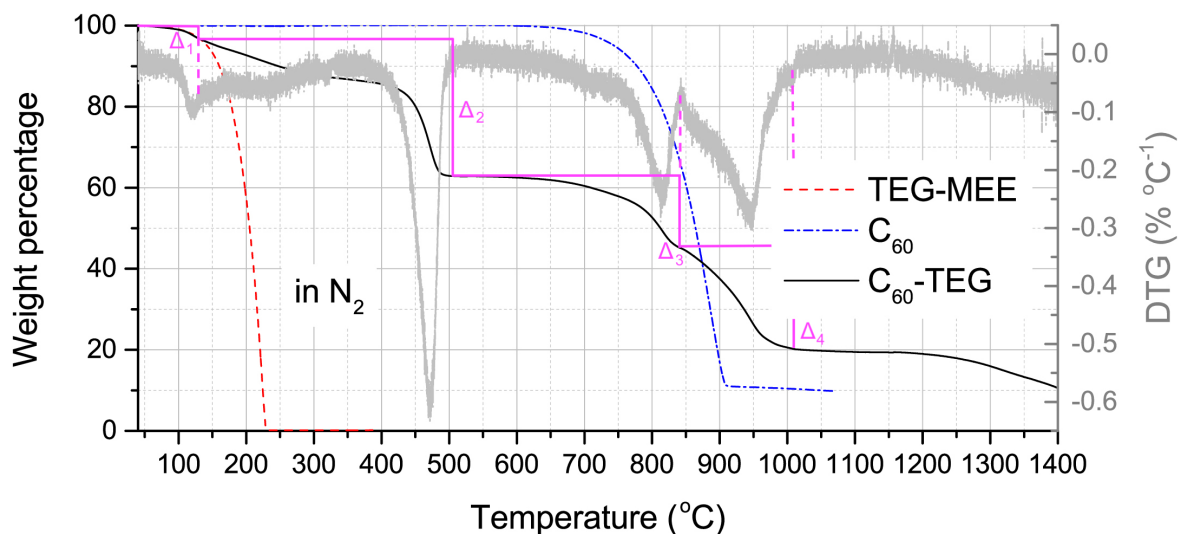


Figure 19: TGA trace of TEGylated C₆₀ **1** in nitrogen atmosphere. Traces for control samples of **TEG-MEE** and pristine C₆₀ are also shown, along with the derivative trace of **1** (DTG = differential thermogravimetry). The solid pink lines define each weight loss (Δ_x) up until the end of its associated DTG peak (dotted pink lines connect to the DTG curve for clarity). Part of these lines for Δ_4 are covered by the legend.

been attributed to the dehydration of hydroxyl groups [49, 51] but, importantly, at lower temperatures and with significantly smaller percentage point changes in mass. This gives further credence to the presence of covalently attached TEG-MEE chains, which contribute a greater proportion to the overall molecular weight per addend than a hydroxyl group, and whose ether bridge requires a greater amount of energy to decompose [239]. The third mass loss, Δ_3 , (T_O 771 °C, T_M 815 °C, Δm 17.74 ± 0.05 %) correlates with the C₆₀ control and can be assigned to the degradation of fullerene-like structures remaining after removal of hydroxyl and TEG-MEE groups [240]. The fourth and final characterisable mass loss, Δ_4 , (T_O 904 °C, T_M 946 °C, Δm 25.44 ± 0.24 %) can be assigned to the decomposition or sublimation of likely remaining carbonaceous structures [241].

2.3.2.7 Estimated Average Structure of TEGylated C₆₀

The above characterisation techniques can be combined to estimate an average composition of the isolated C₆₀ derivative **1**. Qualitatively, evidence of a fullerene with numerous functional

groups attached is given by the featureless visible absorption spectrum. NMR and FTIR techniques confirm the presence of TEG-MEE which has been covalently linked to the fullerene cage; indeed, they show that hydroxylation of the cage has also occurred, and that other oxygen-containing functional groups are likely present [242]. ¹³C NMR corroborates the evidence from the absorption spectrum that the cage is highly functionalised, due to the lack of sp² carbon peaks in the range 130-155ppm. It is, therefore, reasonable to speculate on possible structures contained within **1**, where the overwhelming majority of fullerene carbons are functionalised. In the most extreme case (Fig. 20.a), all six carbons of any given hexagon would be functionalised with either a TEG-MEE chain or hydroxyl group—such a highly functionalised fullerene derivative, where every carbon atom has a functional group attached, has never been synthesised before and is very unlikely to exist due to the high strains introduced [49]. However, it is feasible that instances of these fully functionalised hexagons do exist in **1**—indeed, in a previous study on highly hydroxylated C₆₀ derivatives, it has been computationally demonstrated that the lowest energy structure is obtained when the hydroxylated carbons are on the same hexagon as each other, isolated from other such hydroxylated “islands” [243]. Another possibility (Fig. 20. b), in line with ¹³C NMR results presented herein, sees the TEG-MEE chain attached as an enol ether [226], with a carbonyl [222, 223] across from it in a now open-cage configuration. A final configuration (Fig. 20. c) supported by ¹³C NMR incorporates an open-cage epoxide group [229].

Quantitative analysis of the number of functional groups attached could not be achieved using MS, so EA and TGA are leveraged instead. The overall “master” equation for determining a molecular weight of **1**, M_w , sums up the contributions from all possible functionalities,

$$M_w = M_{C_{60}} + M_{TEG} \cdot x + M_{OH} \cdot y + M_{ONa} \cdot z + M_{H_2O} \cdot m + M_O \cdot n \quad (1)$$

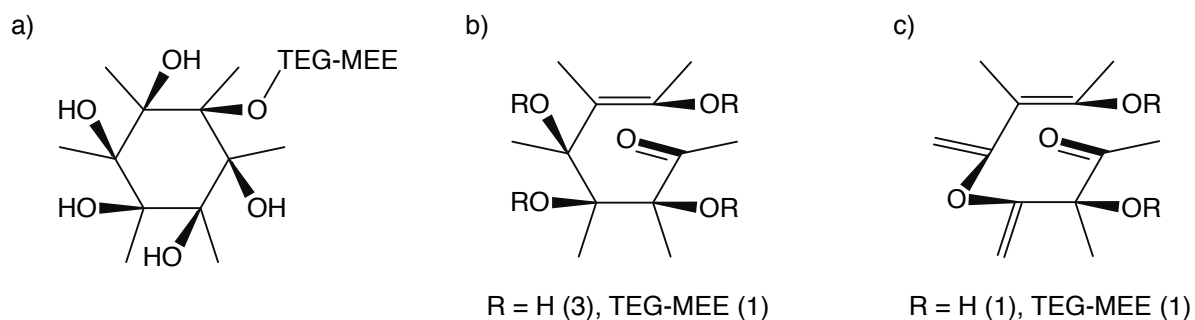


Figure 20: Representative oxygenated groups in TEGylated C₆₀ derivative **1**. All groups are represented on one hexagon for clarity and in keeping with computational observations that highly functionalised fullerenes have lowest energy structures when their functional groups are grouped together in hexagonal “islands” isolated from other such islands [243]. (a) All six carbons are functionalised with either a hydroxyl group or alkoxy linkage to TEG-MEE; (b) the introduction of an enol ether and opposing carbonyl group leads to an open-cage configuration (hydroxyl groups left in the same configuration as in (a) for the sake of comparison); (c) the cage can be further opened by the presence of an epoxide group.

where

$M?$ is the molecular weight of the subscript chemical species⁶,

x is the number of TEG-MEE addends,

y is the number of surface hydroxyl groups,

z is the number of surface hydroxyl groups in their saponified form [125],⁷

m is the number of water of hydration molecules [51],

n is the number of oxygen atoms not occurring in hydroxyl or TEG-MEE groups.

Making some assumptions about the thermal degradation behaviour of **1**, seven simultaneous equations (Eqs. 1 to 8) are set up for the six unknowns in its molecular structure and molecular weight. From EA, assuming that all the observed carbon is from TEG-MEE chains and not from C₆₀ itself,

⁶ $M_{C_{60}}$ 720, M_{TEG} 177, M_{OH} 17, M_{ONa} 39, M_{H_2O} 18, M_O 16.

⁷ The subscript for the chemical species should of course be the ionic O^-Na^+ but is simplified for formatting purposes.

$$(\% \text{ C}) \cdot M_w = (M_C \cdot N_C)x \quad (2)$$

$$(0.3083 \pm 0.0030) \cdot M_w = 96x$$

where N_C is the number of carbon atoms in each TEG-MEE chain (eight). Similarly, assuming that all oxygen atoms in TEG-MEE chains, bound water and those attached to the fullerene cage contribute to the oxygen EA measurement,

$$(\% \text{ O}) \cdot M_w = M_O \cdot (N_{O_{TEG}} \cdot x + y + z + m + n) \quad (3)$$

$$(0.3113 \pm 0.0030) \cdot M_w = 16(4x + y + z + m + n)$$

where $N_{O_{TEG}}$ is the number of oxygen atoms in each TEG-MEE chain. Alternatively, if the oxygen contribution only comes from TEG-MEE and bound water then Eq. 3 takes the new form,

$$(0.3113 \pm 0.0030) \cdot M_w = 16(3x + m) \quad (4)$$

This situation would arise if, during combustion, dehydrated hydroxyl groups and carbonyl groups underwent thermal pinacol rearrangements [49, 125] and remained attached to the fullerene core. Similarly, the coefficient of x is reduced from 4 (Eq. 3) to 3 (Eq. 4) on the assumption that degradation of TEG-MEE occurs up to, but not including, the ether bridge to the fullerene core. This assumption is based on the presence of two mass losses in the TGA trace between 600 °C and 1000 °C (Fig. 19), one of which is assigned to degradation of oxidised fullerene structures, compared with one mass loss for pristine fullerene.

Further, if all hydrogens in TEG-MEE, hydroxyl groups and bound water are assumed to contribute to the hydrogen EA measurement,

$$(\% \text{ H}) \cdot M_w = (M_H \cdot N_{H_{TEG}})x + M_H \cdot y + (M_H \cdot N_{H_{H_2O}})m \quad (5)$$

$$(0.0037 \pm 0.0030) \cdot M_w = 17x + y + 2m$$

where $N_{H_{TEG}}$ is the number of hydrogen atoms in each TEG-MEE chain and $N_{H_{H_2O}}$ the number of hydrogen atoms in water. A final assumption from EA—that the “missing” mass corresponds exclusively to C₆₀, sodium (from saponified hydroxyl groups) and residual oxygen from hydroxyl groups, carbonyl and other oxygen-containing groups, and ether bridges between TEG-MEE and C₆₀—yields

$$(\% \text{ fullerene, Na \& O}) \cdot M_w = M_{C_{60}} + M_O \cdot (x + y + n) + M_{ONa} \cdot z \quad (6)$$

$$(0.3467 \pm 0.0052) \cdot M_w = 720 + 16(x + y + n) + 39z$$

On the basis of the same assumption as for Eq. 4—namely that all oxygens directly attached to the fullerene cage remain so—then the second loss in TGA corresponding to the degradation of TEG-MEE and dehydration of hydroxyl groups gives

$$(\% \text{ TEG \& OH}) \cdot M_w = (M_{TEG} - M_O)x + y \quad (7)$$

$$(0.3529 \pm 0.0184) \cdot M_w = 161x + y$$

Similarly, TGA nicely informs on the loss of water of hydration,

$$(\% \text{ water}) \cdot M_w = M_{H_2O} \cdot m \quad (8)$$

$$(0.0282 \pm 0.0062) \cdot M_w = 18m$$

Given the quoted errors in this over-determined system of equations, a quasi-graphical tabular approach is used to determine an average stoichiometric composition of **1**, using a series of Excel spreadsheets. Tables are set out as demonstrated in Table 3. Table 3 calculates the percentage quantities defined by Eqs. 2–8 and plugs these in to Eq. 1 in order to calculate an average M_w for **1**. The two independent variables are the number of hydroxyl groups (y , up to 44) and the number of bound water molecules (m , up to 14). One Table 3 exists per TEG-MEE chain attached (x , up to eight), thus yielding an array of nine versions of Table 3. This array is repeated for three values of n (the number of oxygen atoms not occurring in hydroxyl or TEG-MEE groups): 0, 8 and 16. Finally, these three arrays are produced for two different values of the ratio $z:y$ (degree of saponification): 0 and 0.25—this upper limit was chosen on the basis of the observed pH (7–8) of aqueous solutions of **1**, suggesting a minimal degree of saponification and thus alkalinity. The tables are populated by inputting independent variables x , y , z , m and n , and values which fall within the ranges designated by experimental error are highlighted green (via conditional formatting in Excel). In this way, an average structure and molecular weight of **1** can be estimated by finding compositions over which highlighted areas in different columns coincide (i.e. those measurements independently correspond to the same stoichiometric composition). An example is given in Fig. 21.

Table 3: Correlating EA and TGA results to average molecular structure of **1** using a quasi-graphical tabular approach. The equation number(s) used for each column is given. Each of these tables is repeated to cover all possible combinations of given values of independent variables x (number of TEG-MEE groups), y (number of hydroxyl groups), z (number of saponified hydroxyl groups), m (number of water of hydration molecules) and n (number of oxygen atoms not occurring in hydroxyl or TEG-MEE groups). As such, a table such as this exists for each value of x and n . Those values which fall within the range measured experimentally are highlighted in green, as in Fig. 21.

# hydroxyl ($y + z$)	# water (m)	M _w	% C	% O	% H	% fullerene & Na & O	% TEG & hydroxyl	% water
2		"	"	"	"	6	7	8
4		"	"	"	"	"	"	"
6	0	"	"	"	"	"	"	"
...		"	"	"	"	"	"	"
44		"	"	"	"	"	"	"
2		"	"	"	"	"	"	"
4		"	"	"	"	"	"	"
6	1	"	"	"	"	"	"	"
...		"	"	"	"	"	"	"
44		"	"	"	"	"	"	"
2		"	"	"	"	"	"	"
4		"	"	"	"	"	"	"
6	...	"	"	"	"	"	"	"
...		"	"	"	"	"	"	"
44		"	"	"	"	"	"	"
2		"	"	"	"	"	"	"
4		"	"	"	"	"	"	"
6	14	"	"	"	"	"	"	"
...		"	"	"	"	"	"	"
44		"	"	"	"	"	"	"

M _w	% C	% O	% H	% C ₆₀ +Na+O	% TEG+OH	%H ₂ O
1868	0.2570	0.2998	0.0463	0.4516	0.4317	0.0482
1913	0.2509	0.3095	0.0460	0.4409	0.4224	0.0470
1958	0.2451	0.3187	0.0457	0.4308	0.4134	0.0460
2003	0.2396	0.3275	0.0454	0.4211	0.4049	0.0449
2048	0.2344	0.3359	0.0452	0.4119	0.3967	0.0439
2093	0.2293	0.3440	0.0449	0.4030	0.3889	0.0430
2138	0.2245	0.3517	0.0447	0.3945	0.3814	0.0421
2183	0.2199	0.3591	0.0444	0.3864	0.3743	0.0412
2228	0.2154	0.3662	0.0442	0.3786	0.3674	0.0404
2273	0.2112	0.3731	0.0440	0.3711	0.3608	0.0396
2318	0.2071	0.3796	0.0438	0.3639	0.3544	0.0388
2363	0.2031	0.3860	0.0436	0.3570	0.3483	0.0381
2408	0.1993	0.3920	0.0434	0.3503	0.3424	0.0374
2453	0.1957	0.3979	0.0432	0.3439	0.3367	0.0367
2498	0.1922	0.4035	0.0430	0.3377	0.3313	0.0360
2543	0.1888	0.4090	0.0429	0.3317	0.3260	0.0354
2588	0.1855	0.4142	0.0427	0.3259	0.3209	0.0348
2633	0.1823	0.4193	0.0425	0.3204	0.3160	0.0342
2678	0.1792	0.4242	0.0424	0.3150	0.3112	0.0336
2723	0.1763	0.4289	0.0422	0.3098	0.3066	0.0331
2768	0.1734	0.4335	0.0421	0.3047	0.3022	0.0325
2813	0.1706	0.4380	0.0419	0.2999	0.2979	0.0320

Figure 21: Sample of tabular method for calculating the average stoichiometric composition and molecular weight of **1**. Values of independent variables for this particular iteration: number of TEG-MEE groups, $x = 5$; ratio of saponified to protonated hydroxyl groups, $\frac{z}{y} = 0.25$; number of water of hydration molecules, $m = 5$; number of fullerene-oxygen groups not in hydroxyls or TEG-MEE, $n = 8$. The emboldened and italicised columns are those whose values can validly be correlated (as discussed in the main text). Green highlighted cells are values which lie within the experimental error parameters for that particular column's equation (as outlined in Table 3). In this example, the molecular weight for which the three cells bordered in red match is 2453. This corresponds to a structure of C₆₀O₈((OC₂H₄)₃)OC₂H₅)₅(OH)₂₁(O⁻Na⁺)₇.

Table 4: Table of stoichiometric compositions and associated molecular weights of TEGylated C₆₀ **1** that correspond to observed experimental data. Independent variables given in black; **calculated coincident values given in green**. $\frac{z}{y}$ is the ratio of saponified (z) to protonated (y) hydroxyl groups and is 0.25 here; n is the number of fullerene-oxygen groups not in hydroxyls or TEG-MEE; m is the number of water of hydration molecules; x is the number of TEG-MEE groups. The sum of x , y , z and n gives the number of carbon atoms in the fullerene cage which have been covalently functionalised. TEG in the structural formulae refers to TEG-MEE.

n	m	x	$y + z$	$x + y + z + n$	M_w	Structural Formula
0			34	39	2460	C ₆₀ (TEG) ₅ (OH) ₂₅₋₂₆ (O ⁻ Na ⁺) ₈₋₉ ·5 H ₂ O
8	5	5	28	41	2453	C ₆₀ O ₈ (TEG) ₅ (OH) ₂₁ (O ⁻ Na ⁺) ₇ ·5 H ₂ O
16			22	43	2446	C ₆₀ O ₁₆ (TEG) ₅ (OH) ₁₆₋₁₇ (O ⁻ Na ⁺) ₅₋₆ ·5 H ₂ O

Using this method, it becomes immediately clear across all iterations that the assumptions used to rationalise the % C, % O⁸ and % H measurements are not valid as there are no compositions for which all three columns coincide. This is not surprising as TGA results indicate that the temperatures used for EA would lead to incomplete combustion. Therefore, correlations between the columns calculating % H₂O, % TEG & hydroxyl and % C₆₀, Na & O are only considered instead. Compositions for which these columns coincide are summarised in Table 4.

The high total number of functionalised carbons is consistent with the observed featureless absorption spectrum, as well as with previous studies utilising basic conditions for the hydroxylation of C₆₀ [49–51, 125, 244]. Given evidence from ¹³C NMR, the structure with no oxygenated groups other than hydroxyl or TEG-MEE ($n = 0$) is unlikely to be a dominant component. As such, an average molecular structure of C₆₀O₈(TEG)₅(OH)₂₁(O⁻Na⁺)₇·5 H₂O (M_w 2453, TEG = TEG-MEE) is assigned to **1**. This corresponds nicely with 121 mg (49.3 μmol) of **1** collected from the synthesis procedure.⁹ It is important to note, however, that this is an *average* structure—in such an inherently uncontrolled reaction, there will be a spread of

⁸ This includes calculating % O both using Eqns. 3 and 4.

⁹ 4.5 mg (6.3 μmol) of C₆₀ that didn't convert to a sufficiently water-soluble product seems reasonable given the colour of the EtOAc filtrate solution observed during the synthesis procedure.

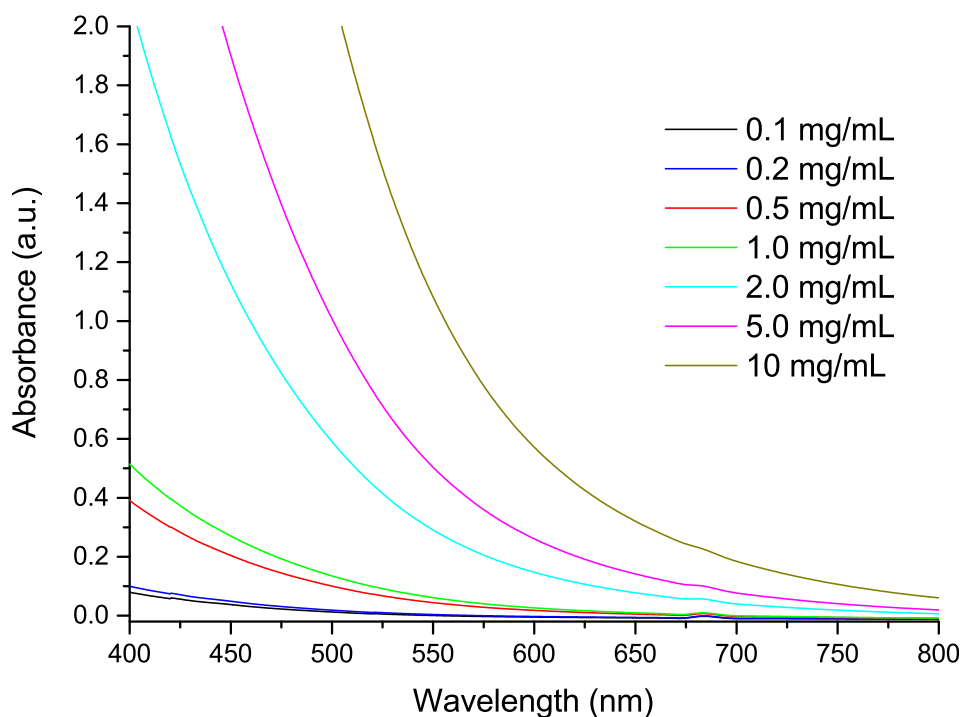


Figure 22: Absorption spectra of serial dilutions of TEGylated C₆₀ **1** in deionised water.

compositions and molecular weights corresponding to varying numbers of functional groups attached to the fullerene cage. In the absence of mass spectrometry data to quantify this spread, an average molecular weight and structure is informative.

With the complex molecular structure of **1** elucidated, its applicability for biomedical applications could begin to be explored.

2.4 SOLUBILITY OF TEGYLATED C₆₀

The solubility of **1** in deionised water was determined using absorption spectroscopy. A series of dilutions were prepared from a stock solution of 10 mg/mL and their absorption spectra recorded in the visible region (Fig. 22). A log-log plot of concentration versus absorbance

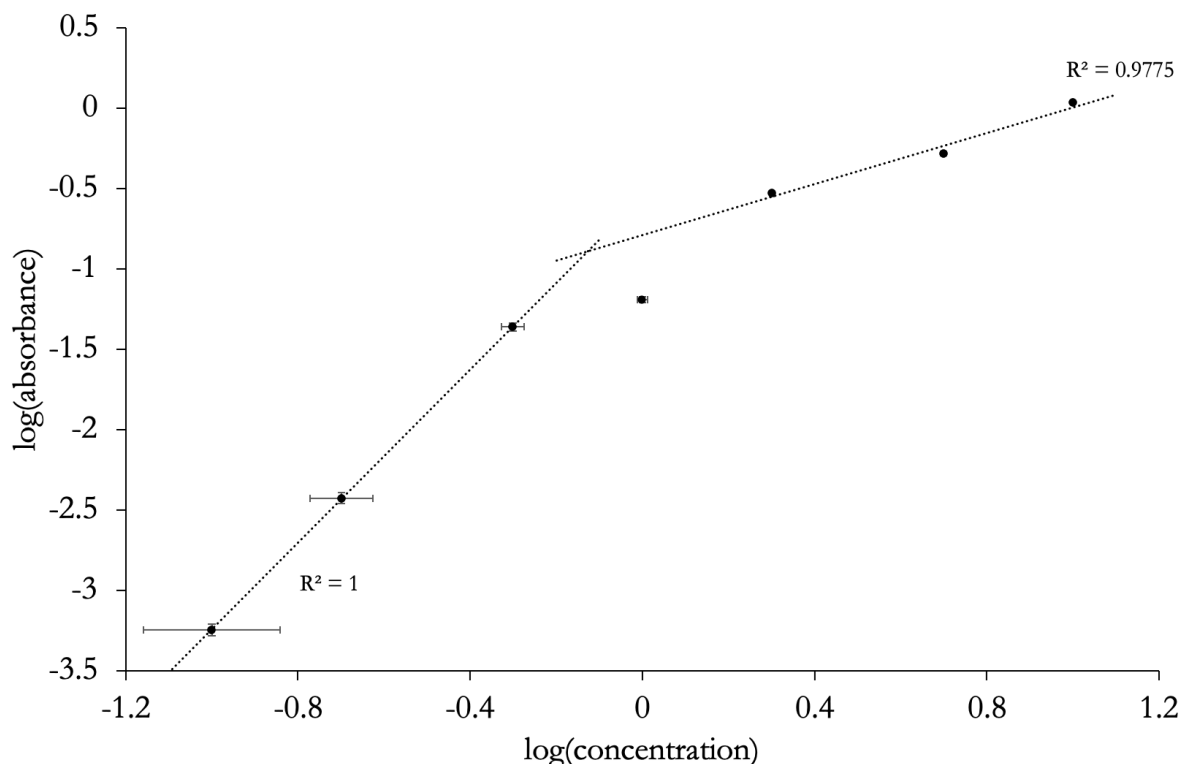


Figure 23: Serial dilutions of TEGylated C₆₀ **1** in deionised water show two distinct linear regions in a log-log plot of concentration versus absorbance. Absorbance measurements were carried out on three independently prepared dilution series—vertical error bars correspond to one standard deviation above and below the mean value; horizontal error bars arise from cumulative error (calculated in Section 2.6.2.1).

at 550 nm shows a definite kink between two linear gradients occurring in the region of 1.0 mg/mL concentration (Fig. 23).¹⁰ This is indicative of the formation of micelles at a critical micelle concentration (CMC) of around 1.0 mg/mL [245, 246]. This behaviour can reasonably be expected of a derivative such as **1**, with an amphiphilic character derived from its hydrophobic fullerene core and hydrophilic addends, and is explored further in Chapter 3. In order to measure the solubility of **1** in deionised water, 20 mg were added to 1 mL in a vial and sonicated. The very dark brown solution was hard to see through but, on inversion of the vial, no solid was seen to have crashed out. In roughly 5 mg steps, more **1** was added to the solution until 50 mg had been added in total—at this point, some solid could be observed

¹⁰ In the absence of any prominent features in the absorption spectrum, this wavelength was chosen as the absorbance of all sample solutions in the calibration curve was maximised (to minimise error) but still less than 1 (within the linear absorbance range of the spectrophotometer). The 10 mg/mL sample, with an average absorbance of 1.08 ± 0.01 , was deemed acceptably close to include.

at the bottom of the vial upon inversion. The saturated solution was left undisturbed for 1 hr at room temperature, before 0.3 mL was extracted, being careful not to disturb or extract any solid from the bottom of the vial. This extraction was diluted four times by 50% to give a solution that, by eye, had an optical transparency appropriate for measurement via absorbance spectroscopy. The measured absorbance at 550 nm (0.3146) corresponds to a concentration of 2.31 mg/mL, which gives an estimated aqueous solubility of **1** of 37 mg/mL based on the assumption that the linear relationship for higher concentrations (the grey line of the log-log plot in Fig. 23) continues.

2.5 PHOTOLUMINESCENT BEHAVIOUR OF TEGYLATED C₆₀

The concentration-dependent photoluminescent behaviour of **1** was also investigated using fluorescence spectroscopy. The same solutions from the serial dilutions in Section 2.4 above were used and their emission spectra recorded (10 nm slit width, medium sensitivity) immediately after measuring the absorbance spectra. Emission spectra were measured for a range of excitation wavelengths (250–570 nm), giving “3-D” PL maps (Fig. 24). Six sets of emission peaks could be seen to evolve over the range of concentrations. Group A consists of two peaks accessible by excitation in the UV (λ_{ex} 280 nm)—one (λ_{em} 336 nm) broader than the other (λ_{em} 307 nm). The intensity of this emission decreases with increasing concentration (Fig. 25). Group B, also accessible via excitation at 280 nm, consists of two emissions (λ_{em} 383 and 400 nm) which increase in intensity up to a concentration of 0.1 mg/mL, before dramatically decreasing (Fig. 26). Analogously, Group C, accessible via 345 nm excitation, consists of one definite emission peak (λ_{em} 387 nm) and a shoulder peak, which increase in intensity up to a concentration of 0.1 mg/mL, before again dramatically decreasing (Fig. 27). Peak D is

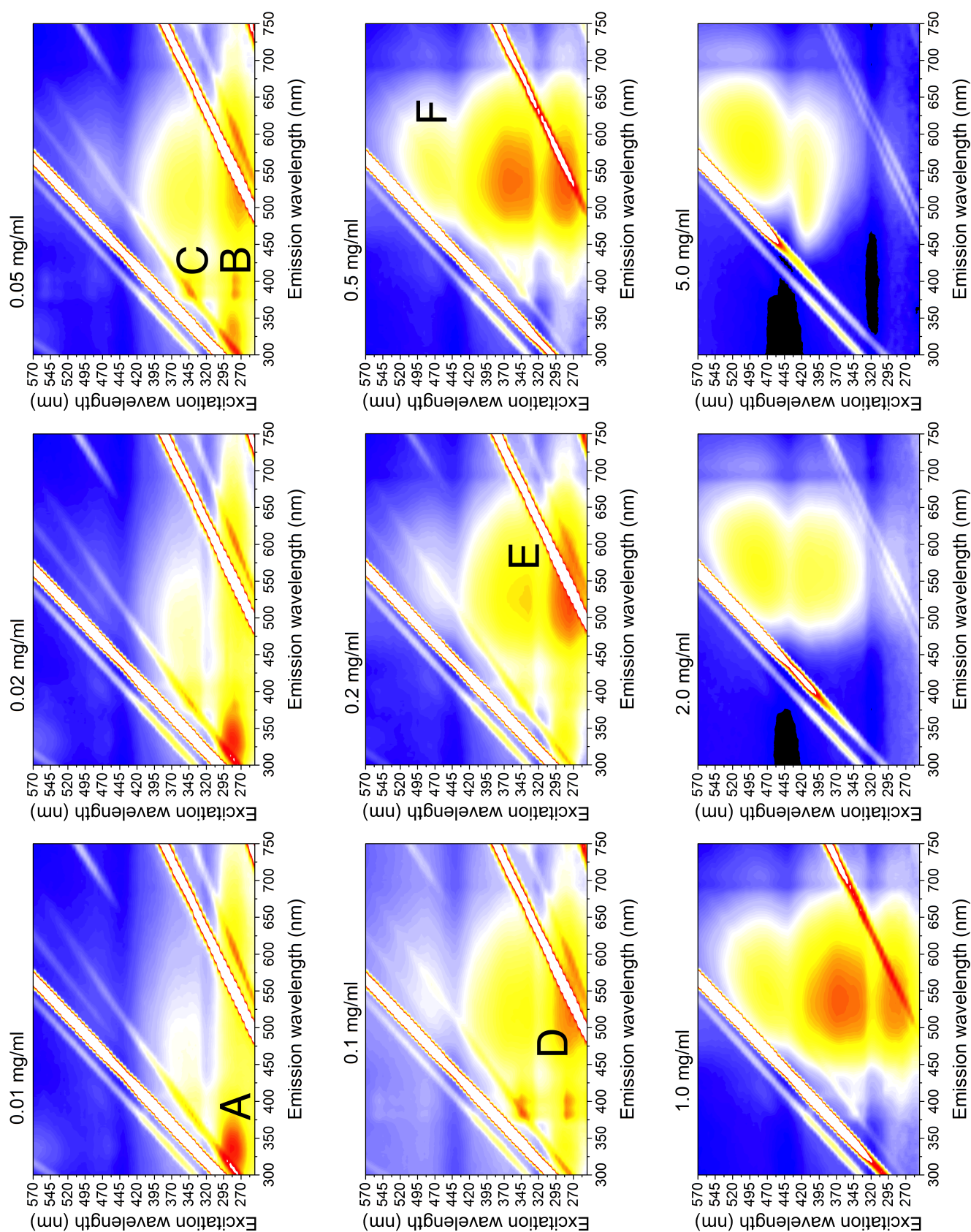


Figure 24: “3-D” photoluminescence maps of aqueous solutions of TEGylated C₆₀ **1** at different concentrations, normalised to the maximum observed emission intensity over all spectra. Colour key (lowest to highest intensity): **blue**, white, **yellow**, **orange**, **red**. Thick wavelength-dependent bands correspond to the excitation beam and its reflection. Other wavelength-dependent streaks correspond to scattering processes.

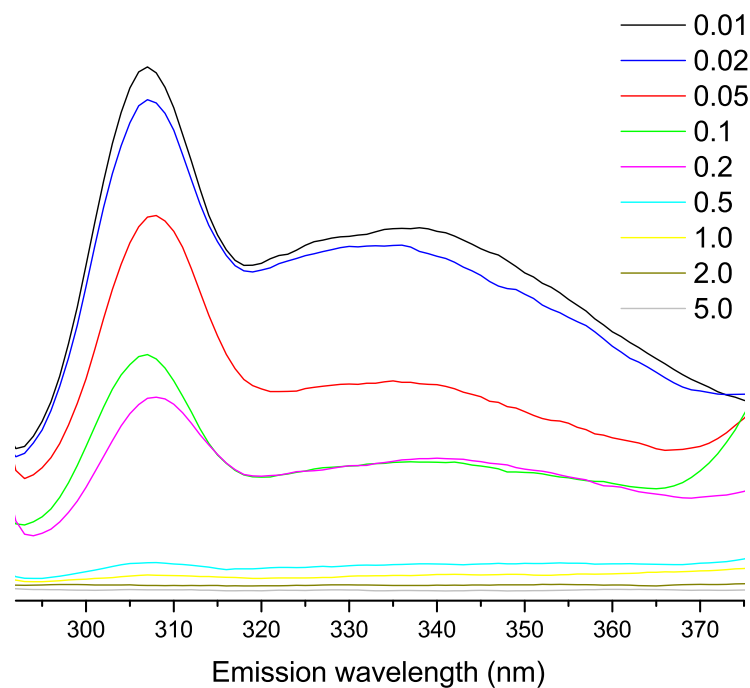


Figure 25: Peak group A of emission spectra (λ_{ex} 280 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL.

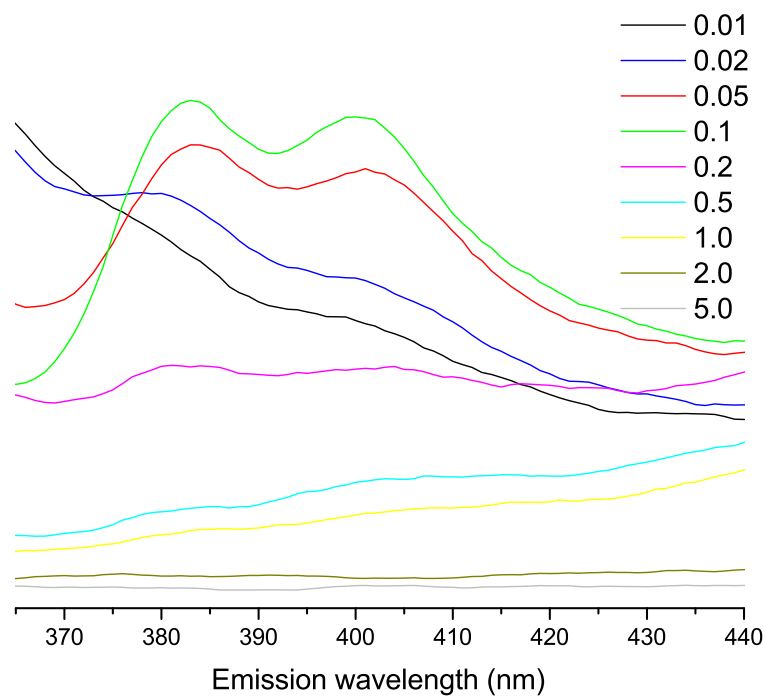


Figure 26: Peak group B of emission spectra (λ_{ex} 280 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL.

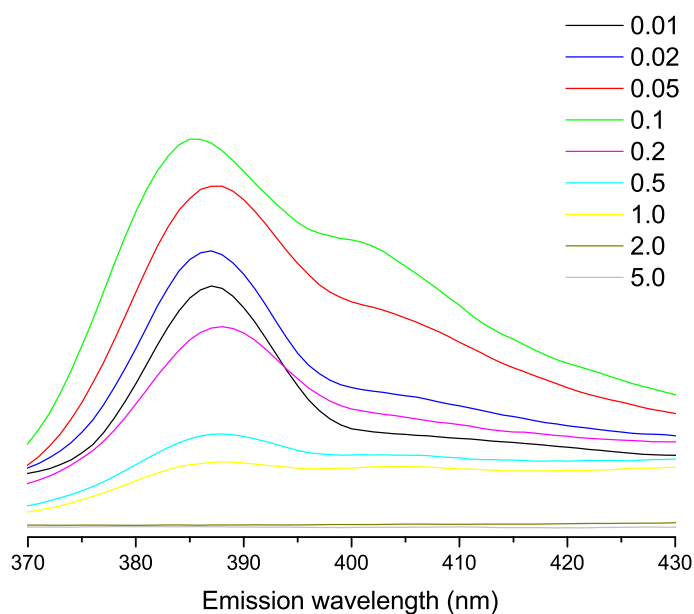


Figure 27: Peak group C of emission spectra (λ_{ex} 345 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL.

somewhat hidden behind the excitation beam (λ_{ex} 280 nm) and associated scattering (Fig. 28) but has the largest Stokes shift of all observed emissions (258 nm). Again, the intensity of this peak reaches a maximum in intensity at a concentration of 0.2 mg/mL. Peak E increases in intensity up to a concentration of 1.0 mg/mL before dramatically decreasing (Fig. 29). Intriguingly, the Stokes shift of this emission increases by over 100 nm from the least to most concentrated solutions, following a logarithmic relationship (Fig. 30). Finally, peak F develops only at higher concentrations (>0.2 mg/mL), increasing in intensity with increasing concentration (Fig. 31). As with peak E, the relationship between concentration and Stokes shift is logarithmic (Fig. 32).

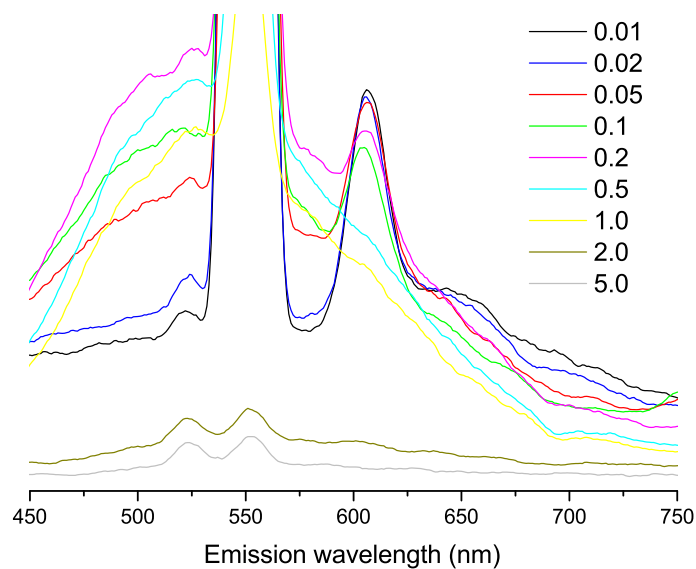


Figure 28: Peak D of emission spectra (λ_{ex} 280 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL. Well-defined peaks are due to the excitation beam (λ_{em} 552 nm) and associated scattering (607 nm).

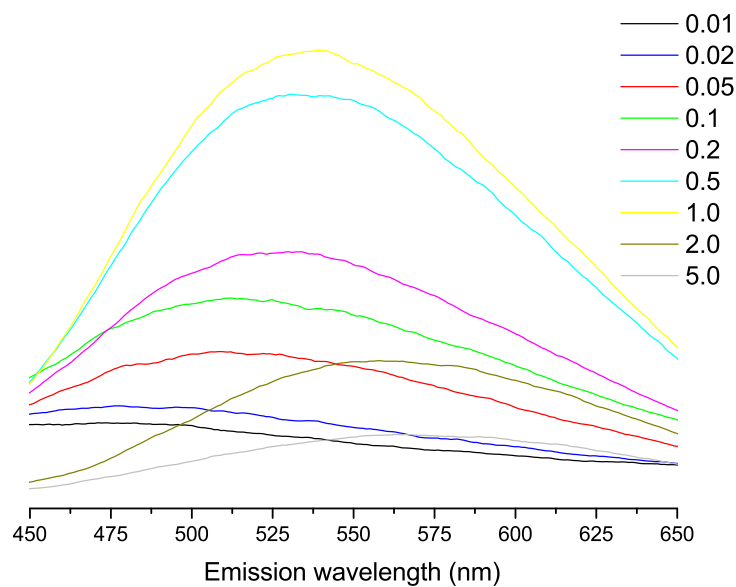


Figure 29: Peak E of emission spectra (λ_{ex} 370 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL.

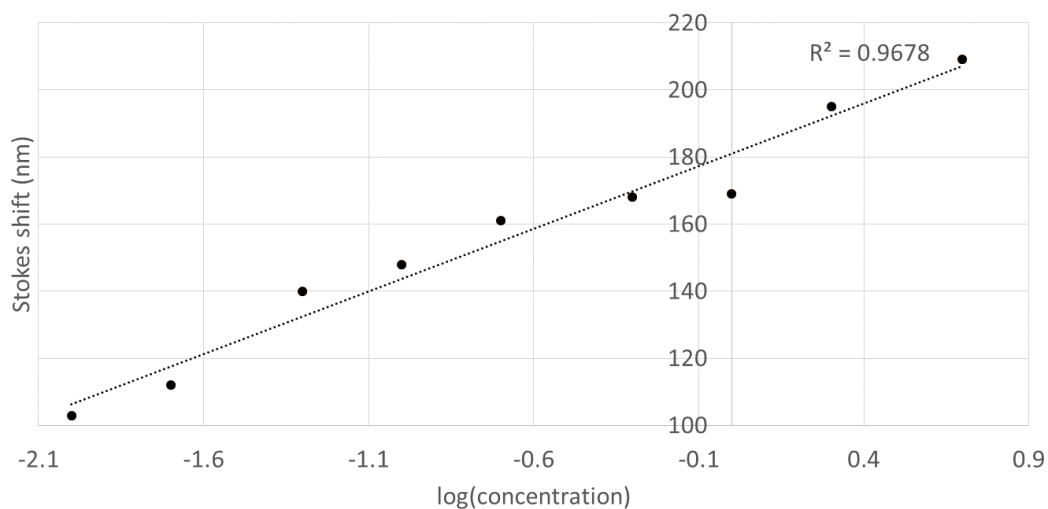


Figure 30: Logarithmic relationship between concentration of aqueous solutions of TEGylated C₆₀ **1** and the Stokes shift of emission peak E (λ_{ex} 370 nm).

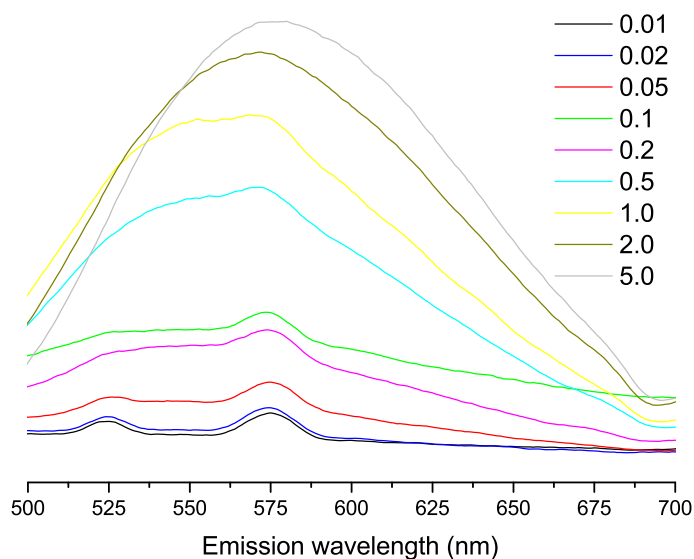


Figure 31: Peak F of emission spectra (λ_{ex} 485 nm) of aqueous solutions of TEGylated C₆₀ **1** at different concentrations. Concentrations in mg/mL. Two scattering peaks are observed (λ_{em} 525 and 575 nm).

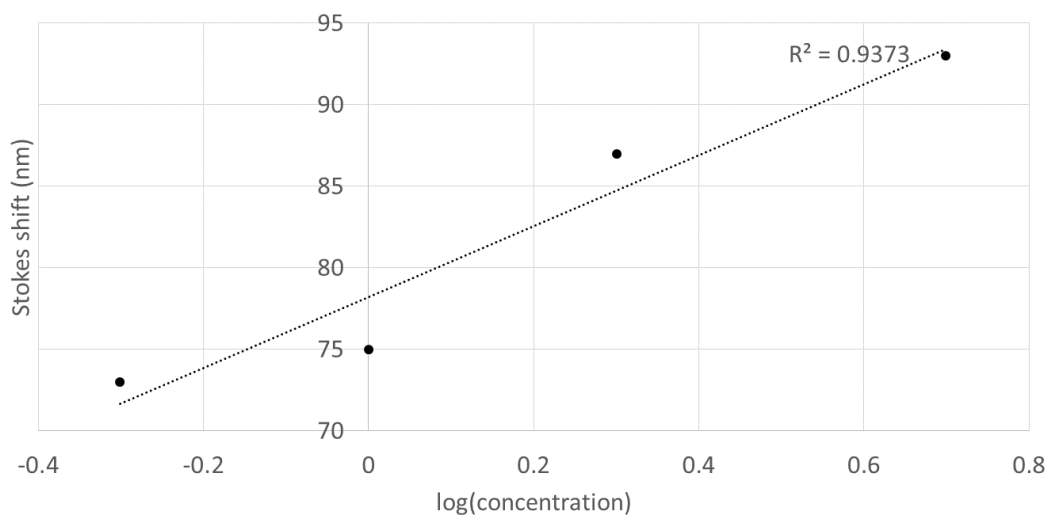


Figure 32: Logarithmic relationship between concentration of aqueous solutions of TEGylated C₆₀ **1** and the Stokes shift of emission peak F (λ_{ex} 485 nm).

2.5.1 Quantum Yield of Fluorescence of TEGylated C₆₀

The quantum yield of fluorescence (Φ) was calculated for the emission at peak E using Williams' relative method [247]. Briefly, this involves plotting the absorbance of several optically dilute solutions against the integrated fluorescence intensity (IFI) of the desired emission, and doing the same with a standard compound with a well-known Φ . The Φ of the test compound can then be determined using

$$\Phi_X = \Phi_S \left(\frac{\text{grad}_X}{\text{grad}_S} \right) \left(\frac{\eta_X^2}{\eta_S^2} \right) \quad (9)$$

where $\Phi_{X/S}$ is the quantum yield of fluorescence of the test (X) or standard compound (S); $\text{grad}_{X/S}$ is the gradient of the absorbance–IFI plot; and $\eta_{X/S}$ is the refractive index of the solvent.

Coumarin 153 dissolved in EtOH was used as the standard sample ($\Phi = 0.58$, $\eta = 1.36$) [248], with the IFI calculated by integrating from 450–700 nm. The absorbance of **1** at 400 nm was noted for concentrations where fluorescent peak E was present and increasing in intensity

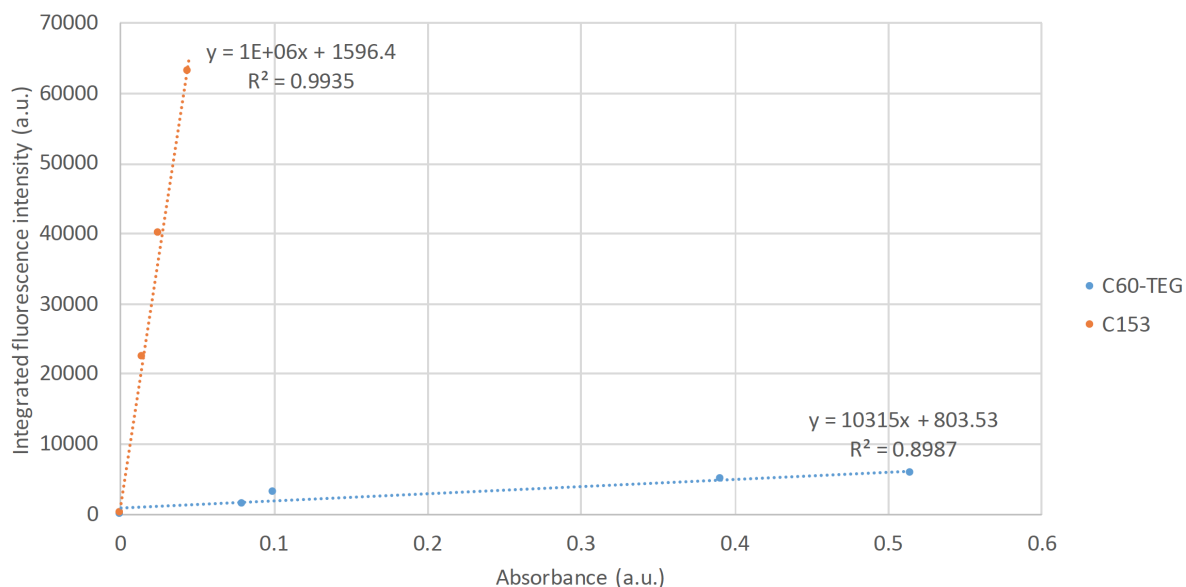


Figure 33: IFI vs. absorbance plots for calculating fluorescence quantum yield, Φ , at $\lambda_{\text{ex}} = 400$ nm for **1** (H₂O) relative to a coumarin 153 standard (EtOH).

(i.e. 0.1, 0.2, 0.5 and 1.0 mg/mL). The IFI of these solutions ($\eta = 1.33$) was then calculated by integrating from 417–685 nm. Plotting IFI against absorbance for each sample (Fig. 33) and using equation 9, Φ_1 was calculated to be 0.57%.

2.5.2 Discussion of Photoluminescent Behaviour

The aqueous solubility of **1** at neutral pH (37 mg/mL) is in excellent agreement with similarly highly functionalised water-soluble C₆₀ derivatives, for example, 17.5 mg/mL for C₆₀(OH)₃₆·8H₂O [49] and 64.9 mg/mL for C₆₀(OH)₄₄·8H₂O [50].

The fluorescent behaviour of **1** at low concentrations (emission peak groups A, B and C) is not too dissimilar to that of polyhydroxylated fullerenes [249]. The emergence of peaks E and F (emitting at roughly the same wavelength as peak D) at higher concentrations, and their accompanying bathochromic shifts, are suggestive of an aggregation-induced emission (AIE) effect [250, 251]. This is corroborated by the concomitant decrease in intensity of peaks A, B

and C, which can be assigned to molecular or very small aggregates of **1**, which would be consumed by larger aggregates in an Ostwald ripening-type process [252]. This is further corroborated by the existence of a critical micelle concentration at 1.0 mg/mL.

The remarkably large Stokes shift (258 nm) observed for peak D at λ_{ex} 280 nm means its emission is bathochromically shifted—similar emission in the far-visible region can also be accessed at non-UV excitation (peaks E and, particularly, F). This demonstrates a useful property for exploitation as a fluorescent probe for e.g. cell tagging [253], as the inner-filter effect is negated and absorption by surrounding tissue would be reduced [254]. Furthermore, the Φ value for peak E demonstrates an order of magnitude increase in the quantum yield of this species *cf.* pristine fullerene [255]. Indeed, this fluorescent behaviour could be harnessed as part of a theranostic tool where, for example, the fullerene could be tracked by its fluorescence while also acting as a PDT/antioxidant therapeutic. Such a technique has recently been demonstrated using organic nanoparticles with AIE [256].

2.6 MATERIALS AND METHODS

All commercial solvents, reactants and reagents were purchased from Sigma-Aldrich (United Kingdom) and used as purchased unless otherwise stated. Fullerene C₆₀ (99+% purity) was used as purchased from Materials and Electrochemical Research (MER) Corporation (Arizona, USA). Grade 2 deionised water adhering to ISO3696 purchased from Chemiphase (United Kingdom) was used unless otherwise stated. ¹H, ¹³C, COSY, HSQC and HMBC NMR spectra were recorded using Bruker AVIII400 and Bruker AVIII500 (with He cryoprobe) spectrometers. Mass spectra were recorded on a Bruker microflex LT matrix-assisted laser desorption/ionisation time-of-flight mass spectrometer (MALDI-TOF MS). FTIR spectra

were recorded at room temperature on a Varian Excalibur FTS 3500 FT-IR with golden gate diamond attenuated total reflection (ATR). Liquid samples were simply pipetted onto the ATR crystal. Solid samples were measured as fine powders, with the golden gate setup ensuring compaction on the ATR crystal surface.

2.6.1 Synthesis and Structural Characterisation of TEGylated C_{60}

C_{60} (40 mg, 55.6 μmol , 1 eq) was dissolved in toluene (25 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (5 mL, 28.6 mmol, 515 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (217 mg, 5.4 mmol, 97 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (a visible change in solution colour from purple to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (75 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 μm), leaving a brown precipitate on the filter and a cloudy yellow filtrate. EtOAc (150 mL) was added to the cloudy filtrate and placed in the fridge overnight. On filtering through a Büchner funnel again, brown precipitate was left on the filter and the filtrate was a clear pale yellow. The brown precipitates on the PTFE filters were redispersed in EtOH (35 mL) with reasonable solubility, before EtOAc (70 mL) was again added and the solution left in the fridge overnight, giving a brown precipitate. This EtOH redispersion–EtOAc precipitation–filtration process was repeated once again, and the resulting precipitate dried *in vacuo* to give **1** as a brown powder (121 mg, 49.3 μmol , 89%).¹¹

¹¹ These molar and percentage yields are given based on the calculated average M_w of 2453 (see Sections 2.3.2.7 and 2.6.3).

^1H NMR (500 MHz, D_2O) δ 3.67–3.54 (12H, m, CH_2), 3.51 (2H, q, $J = 7.1$ Hz, CH_2), 1.10 (3H, t, $J = 7.1$ Hz, CH_3).

^{13}C NMR (126 MHz, D_2O) δ 171.03, 164.84, 163.24, 163.19, 71.61, 69.54, 69.52, 69.37, 68.83, 68.35, 66.56, 63.78, 60.25, 13.97.

FTIR 3407 m , 3284 m , 3169 m [br., $\nu(\text{OH})$]; 3003 w , 2984 w , 2936 w [br., $\nu(\text{CH})$]; 1565 s [$\nu(\text{C}=\text{C})$]; 1404 s [$\delta(\text{COR})$]; 1118 m [$\nu_{\text{as}}(\text{COR})$]; 1044 m [$\nu_{\text{s}}(\text{COC})$]; 1016 m [$\nu(\text{COH})$]; 924 w [$\nu(\text{C}-\text{C})$]; 870 w [$\rho(\text{CH}_2)$]; 646 m , 620 m [$\omega(\text{COH})$] cm^{-1} . All peaks referenced to maxima. Nomenclature: $\nu_{\text{s/as}}$ symmetric/asymmetric stretching; δ bending; ρ rocking; τ twisting; ω wagging; s strong; m medium; w weak; br. broad.

2.6.1.1 Thermal Gravimetric Analysis

Dynamic TGA measurements were carried out on a Perkin Elmer Pyris 1 under nitrogen atmosphere (200 mL/min), at a constant rate of change of temperature of 10 $^{\circ}\text{C}/\text{min}$ in the range 40 to 1200/1400 $^{\circ}\text{C}$, collecting data points every 0.2 s. Samples (at least 2 mg) were loaded in ceramic TGA pans (Perkin Elmer, United Kingdom) and calibrated against an empty pan. The onset temperature (T_{O}) of each weight loss (Δ_x) was defined as the temperature at which the tangents to the TGA curve at T_{M} (see next sentence) and T_{B} (the baseline temperature, defined here as the temperature at the maximum DTG value immediately before that weight loss) intersect. The temperature at maximum rate of decomposition (T_{M}) of each weight loss was defined as the temperature at the minimum DTG value for that weight loss (i.e. at the DTG peak). The percentage point change in mass (Δm) for each weight loss was calculated as the difference between weight percentages at the beginning and end of the weight loss. The beginning of each weight loss (differentiated from the onset temperature)

was defined as the temperature at the end of the previous weight loss (see pink solid lines in Fig. 19), except for the first weight loss, Δ_1 , where the starting temperature was used. Due to the noisy DTG signal, the end of each weight loss was poorly defined (except in the case of Δ_3 , whose end overlaps sharply with the beginning of Δ_4) and, in the absence of peak fitting software, was judged by eye as a point where the signal to noise ratio of the DTG curve became roughly constant. The upper and lower bounds of the weight percentage at both the beginning and end of each weight loss were then read off the TGA curve based on an instrumental error in temperature measurement of ± 5 °C. Maximum and minimum values of Δm were then calculated from these and Δm values quoted as the average $\pm$ the difference with their bounding values.

2.6.1.2 *Elemental Analysis*

All samples were sent to MEDAC Ltd. (Surrey, United Kingdom) and measurements carried out in duplicate. For CHN analysis, samples were held in a tin capsule, purged by a continuous flow of helium and dropped into a vertical quartz tube maintained at 900 °C, at which point the helium stream was temporarily enriched with pure oxygen, causing the tin to promote a violent reaction (flash combustion). The resulting gases are passed over catalysts and the mixture then passed through a chromatographic column where the individual components were separated and eluted as nitrogen, carbon dioxide and water. For O analysis, samples were held in a silver container and dropped inside the reactor furnace maintained at 1060 °C instead.

2.6.2 Photophysical Characterisation of TEGylated C_{60}

All steady state UV-vis absorption spectra were recorded in H_2O at 298 K on a dual-beam JASCO V-570 spectrophotometer, with reference to a solvent blank. All fluorescence spectra were recorded in H_2O at 298 K on a JASCO FP-6200 spectrofluorometer. Quartz cuvettes with 1 cm path length were used in all cases. Scan rate for all absorption and fluorescence measurements was 200 nm/min. 3-D fluorescence spectra were collected for excitation wavelengths at intervals of 5 nm. Fluorescence quantum yields were calculated using Williams' relative method [247], with coumarin 153 dissolved in EtOH as the standard sample ($\Phi = 0.58$, $\eta = 1.36$) [248].

2.6.2.1 Solubility Determination of TEGylated C_{60}

Serial dilutions of **1** in deionised water were prepared from a stock solution of 10 mg/mL and the absorption spectra of each concentration recorded three times in the visible region; errors in absorbance are thus given to one standard deviation of these measurements. The error in concentration for each dilution was calculated as follows. The instrumental errors in creating the stock solution are ± 0.05 mg and ± 0.01 mL. This mass error (δm , mg) remains the same throughout as the mass was only measured once for the stock solution, while the volumetric error (δV , mL) is defined as a summative error,

$$\delta V_n = \sqrt{(2n + 1)(0.01)^2}$$

for the n^{th} dilution. This is because, for each dilution there are three volumetric errors to consider: the error in measuring the volume of the previous dilution (± 0.01 mL), the error in measuring the volume of fresh solvent added (± 0.01 mL), and the cumulative volumetric

errors (each ± 0.01 mL) for previous dilutions up to that point. The concentration error (δc , mg/mL) is then defined as

$$\delta c_n = \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V_n}{V}\right)^2}$$

for the n^{th} dilution from a stock solution of concentration c made with mass m and volume V . Error bars for the log-log plot were calculated using the standard logarithmic error relationship,

$$\delta(\log(c_n)) = 0.434 \cdot \frac{\delta c_n}{c_n}$$

for the n^{th} dilution.

For solubility determination, 20 mg of **1** was added to 1 mL of deionised water in a vial and sonicated. In roughly 5 mg steps, more **1** was added to the solution until some solid could be observed at the bottom of the vial upon inversion. The saturated solution was left undisturbed for 1 hr at room temperature, before 0.3 mL was extracted, being careful not to disturb or extract any solid from the bottom of the vial. This extraction was diluted four times by 50% and its absorbance measured at 550 nm.

2.6.3 Structure Determination of TEGylated C_{60}

A quasi-graphical tabular approach was used, using a series of Excel spreadsheets. Tables were constructed as demonstrated in Table 3. Table 3 calculates the percentage quantities defined by Eqs. 2–8 and plugs these in to Eq. 1 in order to calculate an average M_w for **1**. The two independent variables are the number of hydroxyl groups (y , up to 44) and the number of bound water molecules (m , up to 14). One Table 3 exists per TEG-MEE chain attached (x , up to eight), thus yielding an array of nine versions of Table 3. This array was repeated

for three values of n (the number of oxygen atoms not occurring in hydroxyl or TEG-MEE groups): 0, 8 and 16. Finally, these three arrays were produced for two different values of the ratio $z:y$ (degree of saponification): 0 and 0.25—this upper limit was chosen on the basis of the observed pH (7–8) of aqueous solutions of **1**, suggesting a minimal degree of saponification and thus alkalinity. The tables were populated by inputting independent variables x , y , z , m and n , and values which fell within the ranges designated by experimental error were highlighted green (via conditional formatting in Excel). Coincident highlighted cells in different columns correspond to a stoichiometric composition for which those measurements independently matched (see Fig. 21). In this way, the average structure of **1** was found to be $C_{60}O_8(((OC_2H_4)_3)OC_2H_5)_5(OH)_{21}(O^-Na^+)_7 \cdot 5H_2O$ (M_w 2453).

2.7 CONCLUSIONS

A novel highly water-soluble fullerene derivative, **1**, has been synthesised in a simple one-pot procedure by covalent attachment of oligomeric triethylene glycol monoethyl ether (TEG-MEE) to a C_{60} fullerene core. The presence of NaOH in the synthesis, carried out under aerobic conditions, leads to simultaneous hydroxylation of the cage. The resulting complex molecular structure was determined using absorption, NMR and FTIR spectroscopies, along with elemental and thermal gravimetric analyses (EA and TGA). A generic tabular approach for the determination of TEGylated fullerene derivatives by comparison with observed thermal degradation data was devised, prescribing an average structure of $C_{60}O_8(((OC_2H_4)_3)OC_2H_5)_5(OH)_{21}(O^-Na^+)_7 \cdot 5H_2O$ (M_w 2453) to **1**.

The solubility of **1** in neutral water was determined, using absorption spectroscopy of serial solutions, to be 37 mg/mL, which is in keeping with the highest water solubilities

observed for fullerene derivatives and is very promising for its application in a biomedical context. Notably, **1** is observed to possess a critical micelle concentration of around 1.0 mg/mL, strongly suggesting the presence of self-assembly at the molecular level. **1** displays interesting photoluminescent behaviour, with different transitions accessible at different excitation wavelengths, and a fluorescence quantum yield of 0.57%: an order of magnitude increase *cf.* pristine fullerene. Based on spectroscopic evidence, aggregation-induced emission (AIE) is suspected to be the cause for bathochromically shifted emissions. Access to this part of the spectrum is of great importance in biomedical settings as **1** could be used as a fluorescent probe in cell tracking, with minimal absorption by surrounding tissue. Furthermore, given that this is a fullerene-based material, its other properties could be harnessed to give a theranostic tool, providing therapeutic (e.g. PDT or antioxidant activity) and diagnostic (fluorescence tracking) effects.

SELF-ASSEMBLY OF WATER-SOLUBLE FULLERENES

C₆₀ functionalised with TEG-MEE self-assembles in aqueous solution into hydrogels easily visible to the naked eye and manipulable with tweezers. These are freeze-dried and, under SEM, observed to be hierarchical structures comprised of tubules forming mesoporous networks. The self-assembly processes involved, never before seen at these length scales in a system based on highly functionalised fullerenes, are rationalised in light of related systems in the literature and previous theoretical studies. Higher fullerenes, C₇₀, C₈₄ and C_{90–92}, are functionalised in the same manner—their self-assembly behaviour is observed to depend on the size of the fullerene cage. The Young's moduli of the self-assembled structures are measured using environmental AFM and found to depend on the size of the fullerene.

3.1 INTRODUCTION

Self-assembled structures made from fullerene derivatives show promise in biomedical applications, particularly as drug delivery vehicles (Section 1.2.1.6). Typically, the self-assembled structures investigated to this end are limited to micelles and liposomes [163], and the predominant driving force for self-assembly in aqueous/polar solutions is based on hydrophobicity/amphiphilicity [257]. Examples of structures self-assembled from fullerene derivatives in organic solvents include columns [258], fibres [259], ribbons [260] and hollow

tubes [261], but examples of such extended structures in aqueous solution are rare [262, 263]. C_{60} -based supramolecular polymers [264] and hierarchical structures formed from fullerenes are coming under ever closer scrutiny [265–267] as interest in such processes also develops with other amphiphilic materials systems such as metallic nanoparticles [268], carbon nanotubes [269], DNA [270] and particularly peptides [271–273].

The vast majority of studies on fullerene self-assembly focus on C_{60} derivatives. Indeed, the chemistry of empty-cage higher fullerenes above C_{70} is relatively unexplored [224, 274] owing to the scarcity of the starting materials [6, 275], so investigating their potential in biomedical applications and self-assembly processes is fertile ground.

TEGylated C_{60} **1** can be thought of as an oligomeric star-shaped polymer [276], with an average of five arms radiating from its core. Similar fullerene-based systems functionalised with long-chain polystyrene [277] and polyethylene glycol [278, 279] have been shown to form clusters greater than 1 μm in diameter in aqueous solution, but there are few studies on oligoethylene glycol derivatives, despite their use in the creation of hierarchical assemblies of other materials, particularly polymers [280–282].

3.2 MOTIVATION

Having observed evidence of a critical micelle concentration (CMC) for **1** in the previous chapter, aqueous solutions of **1** were left undisturbed with the simple aim of seeing what self-assembled structures, if any, might be produced. The results presented herein chronicle a serendipitous exploration stemming from this initial premise, with exciting ramifications for the use of fullerenes in biomedical applications and beyond.

3.3 SELF-ASSEMBLY OF TEGYLATED C₆₀

Serial dilutions of **1** (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10 and 20 mg/mL) were prepared in aqueous solution (pH 7–8). 2 mL of each sample were pipetted into 20 mL glass vials, the lids screwed on and sealed with Parafilm M, and the vials stored undisturbed in a dark cupboard. Within two weeks, solutions with concentration >0.5 mg/mL could be seen with the naked eye to contain floating gels, with a largest dimension in solution of 2 mm. Typically, only one such structure existed per solution. Within six weeks, the gel in solution of concentration 2.0 mg/mL had increased in size to have a largest dimension in solution of 10 mm; gels in 1.0, 10 and 20 mg/mL solutions had not increased in size, while solutions with concentration <1.0 mg/mL still contained no aggregates visible to the naked eye. This correlates well with the observed CMC for **1** of 1.0 mg/mL. Within six months, the gel in 2.0 mg/mL solution (henceforth, sample **2**) had increased in size to have a largest dimension in solution of 23 mm; the gel in solution at 10 mg/mL had only increased in its largest dimension to 4 mm, that in 1.0 mg/mL to 8 mm, while the 20 mg/mL solution contained a thin structure with longest dimension 10 mm. Sample **2** could be picked up and manipulated using tweezers without fracture (Fig. 34)—when held between two pinning points, it displayed viscoelastic behaviour (Fig. 35). When plucked from solution, the gel was evidently swollen with water—draining this water by dabbing on absorbent paper led to a visible decrease in the volume of the structure. Because of this water content, sample **2** can fairly be referred to as a hydrogel [283]. No noticeable increase in size of any hydrogel sample was observed beyond six months.

Subsequent iterations of the reaction (Section 2.3.1) and serial dilutions yielded broadly the same results: no structures visible to the naked eye were formed in solutions of concentration

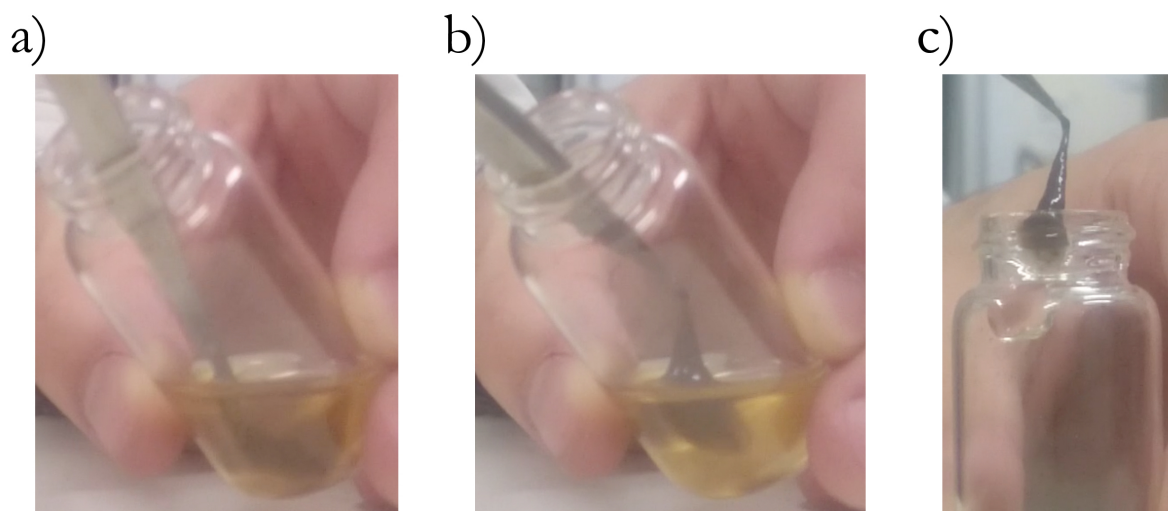


Figure 34: Freeze-frames from a video (time proceeding from left to right) of TEGylated C₆₀ **1** hydrogel being picked from aqueous solution (2.0 mg/mL) with tweezers.

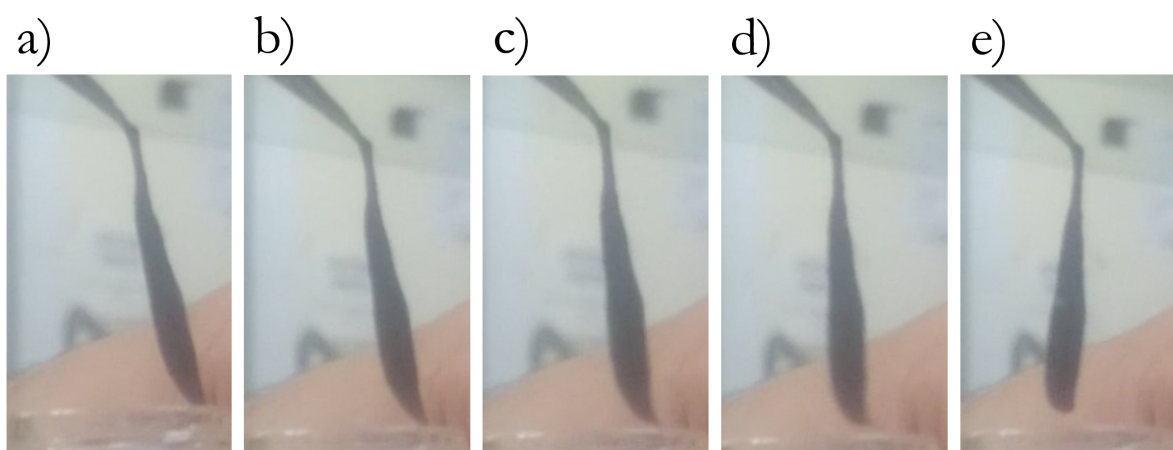


Figure 35: Consecutive freeze-frames from a video (time proceeding from left to right) of TEGylated C₆₀ **1** hydrogel being picked from aqueous solution (2.0 mg/mL) with tweezers. Stretching of the hydrogel due to slight adhesion to the wall of the glass vial can be seen in frames a) to c). Break-off, when the upward force from the pulling of the tweezers exceeds the adhesive force at the vial wall, is shown in d). The hydrogel supports its own weight under the force of gravity (e).

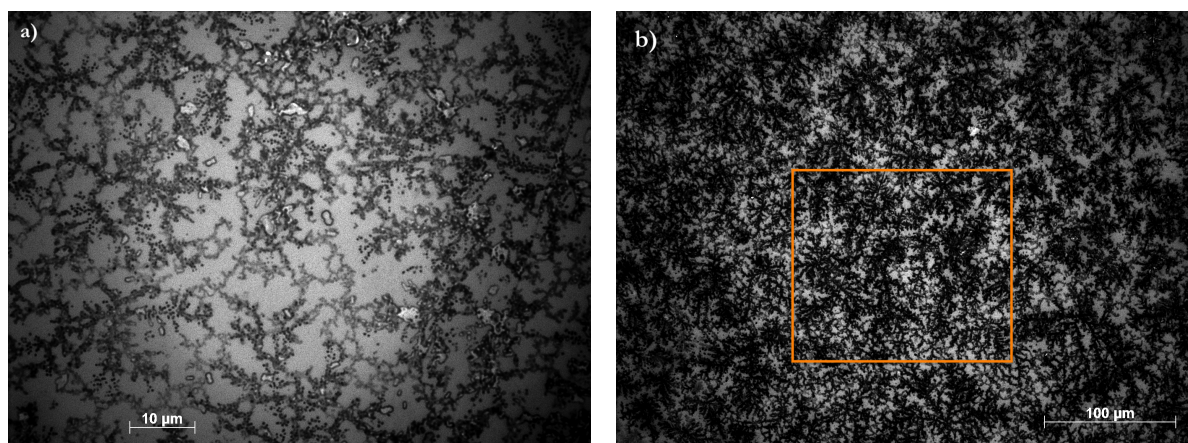


Figure 36: Transmission optical micrographs of a dried aqueous solution of TEGylated C₆₀ **1** (0.02 mg/mL). Upon drying, the formation of (a) chains and (b) fractal patterns of spherical particles (diameter ~400 nm and ~1300 nm, respectively) can be seen. Fractal dimension (D_b) of highlighted area, 1.83.

<1.0 mg/mL; the largest hydrogels formed were in solutions of 1.0 and 2.0 mg/mL concentrations; no hydrogels in further 10 and 20 mg/mL solutions exceeded 10 mm in their largest dimension.

Given the observed distinction of two concentration regimes, delineated by a CMC of 1.0 mg/mL, the self-assembly processes involved in each are approached separately.

3.3.1 Self-Assembly of TEGylated C₆₀ **1** at Low Concentrations

Sessile drops of aqueous solution of **1** at a concentration (0.02 mg/mL) below the CMC were dropped onto glass microscope slides and left at room temperature to dry out before undergoing inspection on a Motic BA210 binocular optical microscope. Chains (Fig. 36a) and fractal patterns (Fig. 36b) of spherical particles, diameter ~400 nm, formed as the aqueous solvent front receded. The box-counting fractal dimension, D_b , of the highlighted area in Fig. 36b was calculated using the FracLac plugin in ImageJ and found to be 1.83. The fractal nature of the two-dimensional aggregation pattern formed by evaporating the solvent of

an aqueous solution of **1** below its CMC is strong evidence of this process taking place in the diffusion-limited aggregation (DLA) regime [284, 285]. Further, the calculated fractal dimension, $D_b = 1.83$, is characteristic of aggregates formed under DLA [286]; this behaviour has previously been observed in the self-assembly of an alkyl-functionalised C₆₀ derivative in THF:water solution [287], as well as in other self-assembling systems that lead to hierarchical structures based on functionalised peptides [288, 289, 272] and carbon nanotubes [290]. In the DLA regime, every collision results in particles sticking together, thus implying that there are strong non-covalent interactions occurring between the constituent building blocks. In this case, those building blocks appear to be spherical particles of diameter ~400–1300 nm, which are themselves composed of building blocks of TEGylated C₆₀ **1**. The appearance of such large particles below the measured CMC is not an unusual phenomenon, with these aggregate structures being dubbed *premicelles* [291–293].

3.3.1.1 Non-Covalent Interactions Governing the Self-Assembly of TEGylated C₆₀

Given the evidence of DLA and premicelle formation, what are the causes of these strong non-covalent intermolecular interactions? Previous work on star-shaped C₆₀ derivatives functionalised with hydrophilic polymers such as polyethylene glycol has shown that the long polymer chains physically cross-link with each other to form self-assembled structures [278]. Given that **1** consists of much shorter oligomeric ethylene glycol chains grafted to a C₆₀ core, at first glance it is unclear whether these chains are long enough to form such cross-links. However, the covalent grafting of six oligomeric ethylene glycol monomethyl ether (OEGMME) chains to C₆₀ has been shown via molecular dynamics simulations to induce favourable non-covalent intermolecular interactions in water, leading to the formation of linear chain-like aggregates [294, 295], just as observed here. This study highlighted several key phenomena related to this aggregation behaviour:

1. The presence of covalently attached OEGMME chains induces a weak long-range (> 12 Å) interaction between molecules, despite the chains' hydrophilicity.
2. The short-range (< 12 Å) attraction between fullerenes in aqueous solution due to their hydrophobicity [296] is increased by the presence of covalently attached OEGMME—this is mediated by a favourable hugging interaction between OEGMME and the equator of an adjacent fullerene. Crucially, because the oligomeric chains are short, they do not sterically cover their own fullerenes to which they are attached, thus allowing this interaction between adjacent molecules to occur. Increasing the number of attached chains per fullerene leads to dispersion in solution rather than aggregation, as the OEGMME chains can no longer wrap around neighbouring fullerenes.
3. The even distribution of covalent attachment of OEGMME chains across the surface of C₆₀ leads to anisotropic aggregation.

Given the average molecular structure of **1** (C₆₀O₈(TEG)₅(OH)₂₁(O[−]Na⁺)₇·5 H₂O) deduced in Section 2.3.2.7 containing five TEG-MEE chains, it is reasonable to expect that there will be examples of molecules with six chains that might behave in this way. However, it is important to note that the average structure of **1** consists of a high number of hydroxyl groups: 28. Therefore, this proposed interaction between TEG-MEE and hydrophobic regions of adjacent fullerenes is likely further enhanced by strong hydrogen bonding between TEG-MEE chains and hydroxyl groups adjacent to non-functionalised carbons on the fullerene cage, evidence for the existence of which is seen in ¹³C NMR (Fig. 16 in Section 2.3.2.2).

In summary, the observation of premicelles in concentrations of **1** below its CMC can be rationalised by the cooperative effects of a series of strong non-covalent interactions between individual molecules of **1** at short range, namely:

1. Hydrophobicity from carbons in adjacent fullerenes;

2. Interaction of TEG-MEE chains with hydrophobic regions of adjacent functionalised fullerene cages;
3. Strengthening of this interaction via hydrogen bonding between TEG-MEE chains and hydroxyl groups bordering hydrophobic regions on adjacent fullerenes.

It is therefore reasonable to propose that these premicelles are “close-packed” aggregate particles formed of molecules of **1**.

3.3.2 Self-Assembly of TEGylated C₆₀ at High Concentrations

In contrast, the slow rate of aggregation in solutions of concentration > 1.0 mg/mL giving rise to large hydrogel structures, such as **2**, is indicative of reaction-limited aggregation (RLA) [297]. In this regime, not every collision between particles due to Brownian motion results in their sticking together. This situation arises above the CMC because the particles colliding are already formed into fairly stable micellar or similar self-assembled structures. The long time (weeks) taken to see formation of hydrogel structures from **1** is in good agreement with previous experimental observation of self-assembly of C₆₀ functionalised with polyethylene glycol [298]. As well as the kinetics of aggregation, the RLA regime is characterised by highly polydisperse solutions [297], evidence of which was seen by carrying out nanoparticle tracking analysis (NTA) on a 2.0 mg/mL solution of **1** (Fig. 37).

The self-assembly of an amphiphilic structure such as **1** in aqueous solution is driven by a balance between the enthalpic gain of its hydrophilic components (here, TEG-MEE and surface groups such as hydroxyl and carbonyl) solvating with water via hydrogen bonding, and the entropic gain of its hydrophobic components (here, C₆₀) disrupting the water network [299]. In the classical theory of self-assembly of amphiphiles that are made up of a hydrophilic

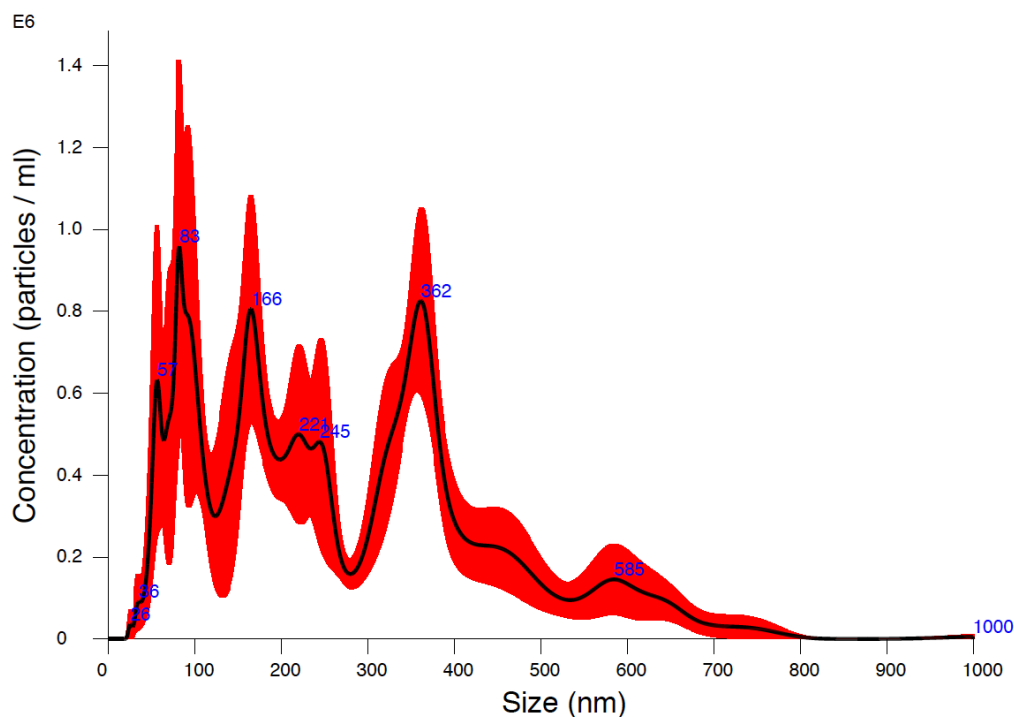


Figure 37: Nanoparticle tracking analysis of aqueous solution of TEGylated C₆₀ **1** (2.0 mg/mL). The red envelope traces one standard deviation either side across eight measurement runs.

head group and a long hydrophobic tail [300], the expected shape of the aggregates can be predicted from the *critical packing parameter*,

$$C_{pp} = \frac{V}{a_0 \cdot l_c} \quad (10)$$

where V is the effective volume occupied by the hydrophobic tail, a_0 the effective surface area of the hydrophilic head group at the interface with the solution, and l_c the maximum effective length of the amphiphile. The gamut of predictable structures is shown in Fig. 38. **1**, however, is not a simple amphiphile in this vain, thus rendering calculation of C_{pp} ineffective. Indeed, in order to get a better idea of the structure of the formed hydrogels, scanning electron microscopy (SEM) was used.

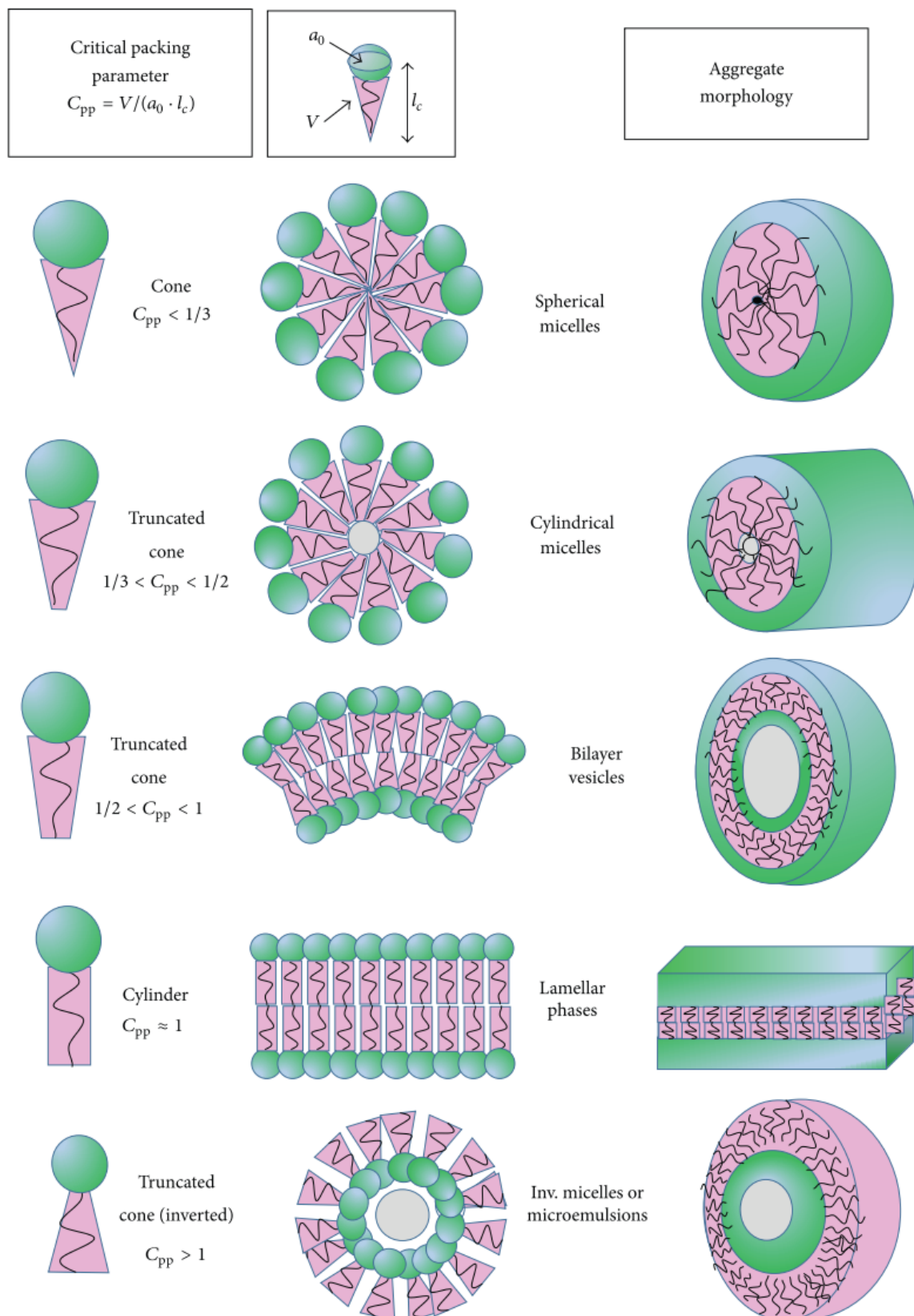


Figure 38: Summary of aggregate structures predicted from an amphiphile's critical packing parameter. V is the effective volume occupied by hydrophobic chains; l_c is the maximum effective length; a_0 is the effective hydrophilic headgroup surface area at the aggregate-solution interface. Reprinted from [299] with permission under the Creative Commons Attribution License. Copyright © 2015 Domenico Lombardo et al.

3.3.2.1 Structural Characterisation of Hydrogel of TEGylated C₆₀

TEGylated C₆₀ hydrogel sample **2** was picked out of 2.0 mg/mL solution with tweezers and left to dry overnight on an SEM stub, giving a flat xerogel [301] structure due to evaporative loss of water and resulting capillary forces (Fig. 39). Although flattened *in vacuo*, a network of fibrillar strands, 1.5–2 µm wide, are seen to project from spherical node junctions, ~5–8 µm in diameter (Fig. 39a). Crucially, these are hollow fibres, with internal diameters of ~900 nm (Fig. 39b). This is larger than any fullerene-based supramolecular hierarchical structure before. Fibres less than 100 µm long and 500 nm wide have previously been synthesised using molecular recognition of C₆₀ with calixarenes [302], and “nanowhiskers” (> 100 µm long) with outer diameters of 100–300 nm and inner diameters consistently of 65 nm [261].

Another hydrogel sample of TEGylated C₆₀ (from another 2.0 mg/mL solution) was pipetted onto an SEM stub and freeze-dried to produce an aerogel [303] in order to maintain its three-dimensional structure for imaging purposes. SEM showed the interconnected porous network in three dimensions (Fig. 40), with an average pore size of 60 ± 15 µm, as determined by random sampling of 20 pores in ImageJ.

The self-assembled hydrogel of TEGylated C₆₀ **1** can be defined as a supramolecular hydrogel [303–307], its extended hierarchical structure formed via the favourable non-covalent interaction(s) of its constituent components, namely TEGylated C₆₀ **1**. The hierarchical structure observed for **1** is comprised of a porous network (pore size 60 ± 15 µm) of hollow tubular fibres (inner diameter ~900 nm, outer diameter 1.5–2 µm). The presence of three-/four-arm junctions is strong evidence of a complex tricontinuous phase, where three unconnected continuous zones can be defined: within the tubes, the walls of the tubes themselves, and without the tubes [300]. This unique structure derives from the structure of its fundamental building block: TEGylated C₆₀ **1**. A schematic representation of a tentatively assigned packing

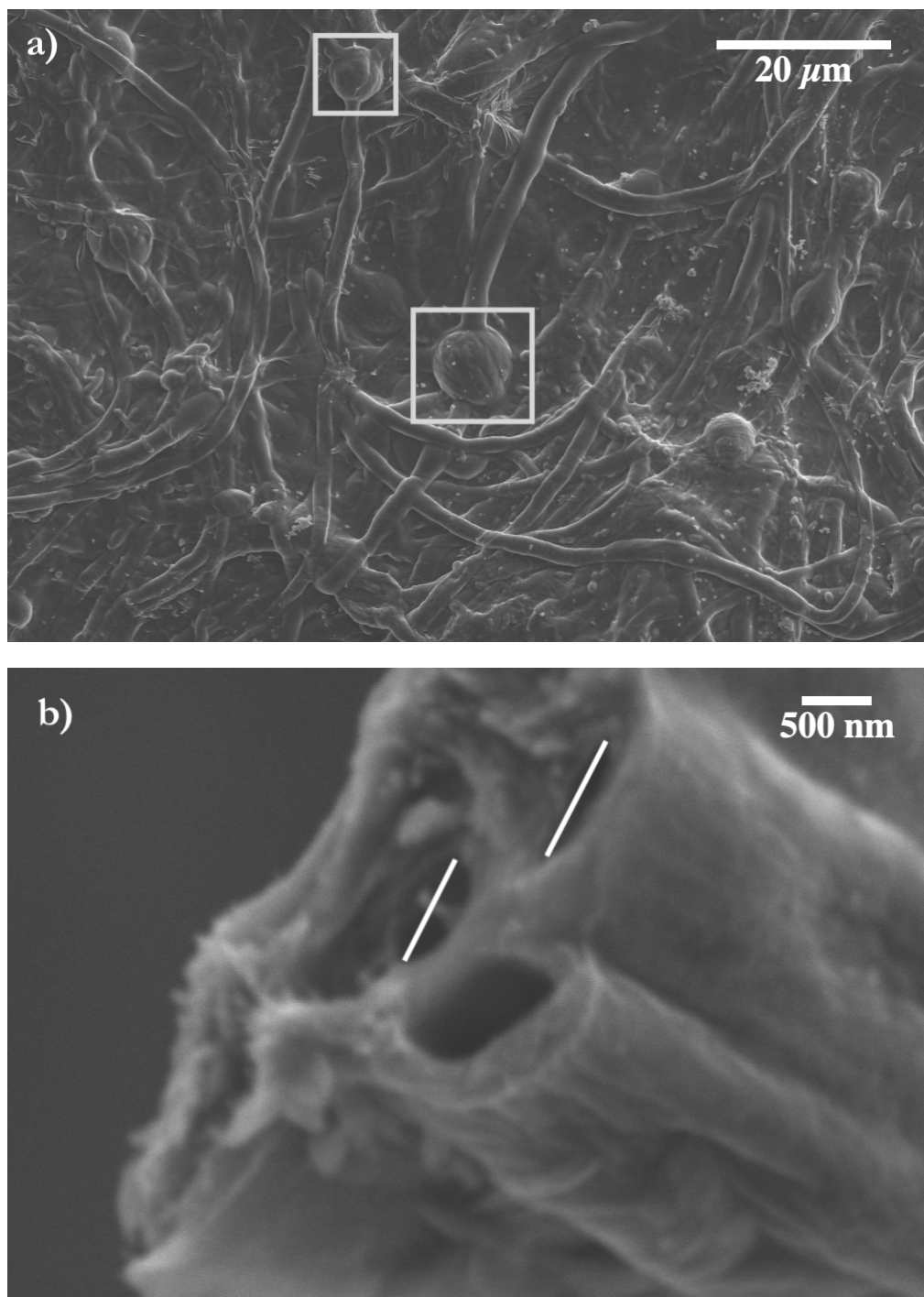


Figure 39: SEM micrographs of xerogel of TEGylated C₆₀ **1**. a) Nodes (5.6 μm and 8.3 μm diameter) typical of a tri-continuous phase can be seen linking fibrillar strands (1.5–2 μm wide) in a wider network. Image acquired with 5 kV accelerating voltage, 50 pA probe current. b) By looking at the edge of the xerogel, the fibres are found to be hollow tubules of inner diameter ~900 nm (calculated as the average of the two diameters highlighted). Image acquired with 5 kV accelerating voltage, 30 pA probe current.

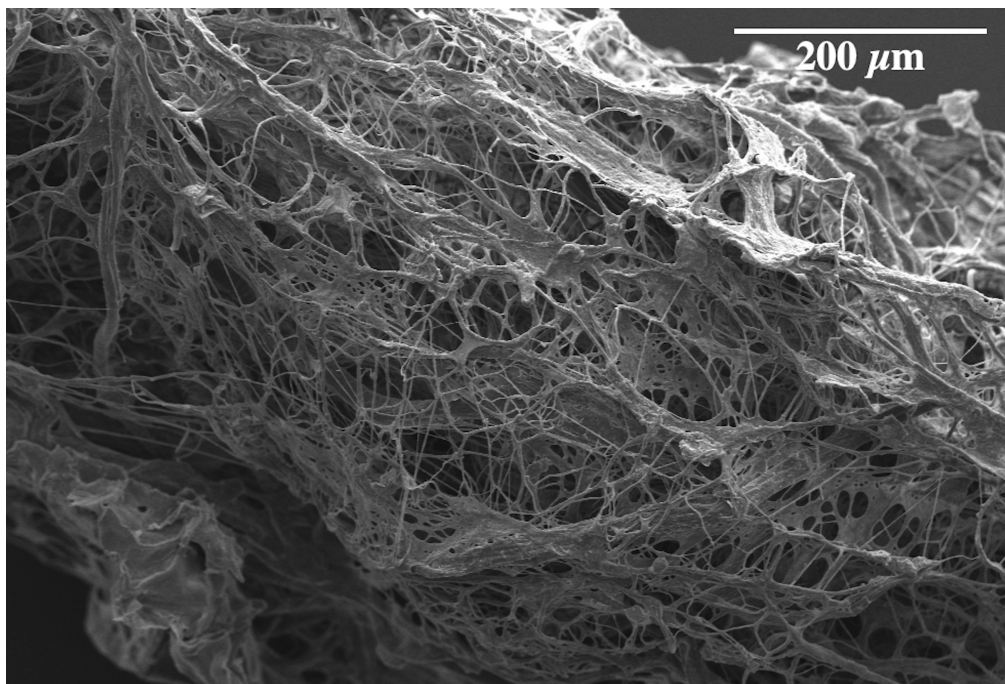


Figure 40: SEM micrograph of aerogel of TEGylated C₆₀ **1** after freeze-drying. The highly porous structure, has an average pore size of $60 \pm 15 \mu\text{m}$. Flat regions near the edges were unintentionally introduced upon squeezing the sample into a pipette before freeze-drying. Image acquired with 10 kV accelerating voltage and 100 pA probe current.

structure is shown in Fig. 41. Molecules with six TEG-MEE chains attached are proposed to form the core of the fibre wall due to their favourably strong intermolecular interactions and subsequent close packing (as outlined in Section 3.3.1). Moving out from the centre of the wall in both directions, molecules with a lower ratio of TEG-MEE chains to hydroxyl groups join the structure as their TEG-MEE chains can interact with those molecules of **1** closer to the centre, while their hydroxyl groups can hydrogen bond more easily with surrounding water.

Extended networks of this type of tricontinuous structure are notoriously difficult to make, and have been achieved with silica-based [308] and ternary polymer [309] systems. In fact, such tricontinuous systems have been theorised to form preferentially when the branches of the star-shaped building block radiate from a central core which is both larger than a single atom and structurally rigid [310]—C₆₀ fulfills both of these criteria. Never before, to the best of my knowledge, have such structures been achieved, manipulable on the macroscale,

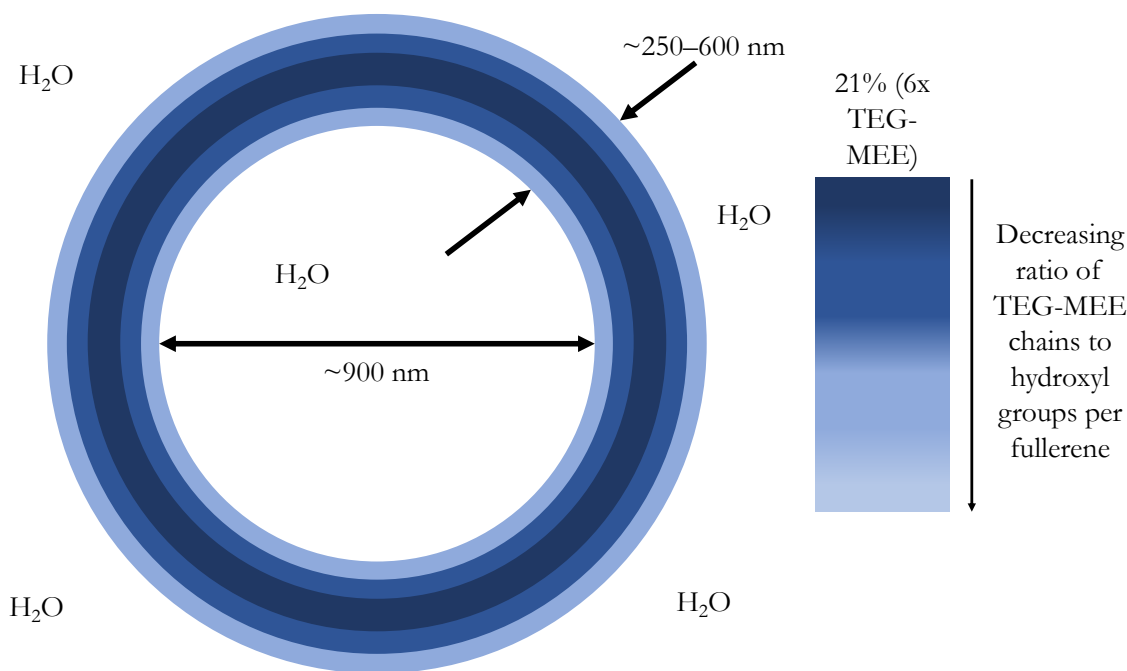


Figure 41: Proposed packing structure of TEGylated C_{60} **1** within its hydrogel. Those molecules with the highest number of TEG-MEE chains (up to 6, in accordance with computational studies) form the core of the tubular walls while the outermost molecules are those with a lower ratio of TEG-MEE chains to hydroxyl groups.

directly from a non-polymeric covalent derivative of C_{60} as described here. Porous materials such as this are highly sought after in the biomedical field for drug delivery purposes [311]. Furthermore, this is an example of a very porous material with a predominantly carbonaceous composition—such materials are particularly sought after for electrochemical applications such as next-generation capacitors [312].

3.4 SELF-ASSEMBLY OF TEGYLATED HIGHER FULLERENES

With TEGylated C_{60} seen to self-assemble into extended hierarchical supramolecular hydrogel structures in aqueous solution, the same functionalisation method was carried out on higher fullerenes, C_{70} , C_{84} and C_{90-92} , to see if they too would self-assemble into similar structures.

3.4.1 Synthesis of TEGylated Higher Fullerenes

3.4.1.1 TEGylated C_{70}

C_{70} (30 mg, 41.7 μmol , 1 eq) was dissolved in toluene (18 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (3.75 mL, 21.4 mmol, 515 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (192 mg, 4.80 mmol, 115 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (as with C_{60} , a visible change in solution colour from red to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (55 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 μm), leaving a dark grey precipitate on the filter and a very dilute yellow filtrate. This dark grey precipitate was rinsed with EtOH (30 mL), giving a brown filtrate and leaving a brown precipitate on the filter paper. This precipitate was dried *in vacuo* to give **3** as a brown powder (98 mg).

3.4.1.2 TEGylated C_{84}

C_{84} (5 mg, 4.96 μmol , 1 eq) was dissolved in toluene (8 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (1 mL, 5.72 mmol, 1154 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (68 mg, 1.70 mmol, 343 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (as with C_{60} and C_{70} , a visible change in solution colour from dark green to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (25 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a light brown precipitate. The contents of the flask were then filtered using a Büchner funnel

(PTFE filter, 0.2 μm), leaving a dark grey precipitate on the filter and a dilute yellow filtrate. This dark grey precipitate was rinsed with acetone (20 mL), giving a very pale yellow filtrate and leaving a light brown precipitate on the filter paper. This precipitate was dried *in vacuo* to give **4** as a brown powder (23 mg).

3.4.1.3 TEGylated C_{90-92}

C_{90-92} (2 mg, 1.85 μmol , 1 eq) was dissolved in toluene (4 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (0.7 mL, 4.01 mmol, 2165 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (52 mg, 1.30 mmol, 703 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (the colour change associated with solutions of C_{60} , C_{70} and C_{84} could be seen within two minutes of adding NaOH, but was less discernible given the dilute concentration and small amount of C_{90-92} used). A volumetric excess of EtOAc (15 mL) was added to the reaction flask, which was then placed in the fridge (2 $^{\circ}\text{C}$) overnight, giving a light brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 μm), leaving a light grey precipitate on the filter and a dilute yellow filtrate. This light grey precipitate was dried *in vacuo* to give **5** as a brown powder (12 mg).

3.4.2 Structural Characterisation of TEGylated Higher Fullerenes

3.4.2.1 TEGylated C_{70}

ABSORPTION As with **1**, the visible absorption spectra of solutions of **3** in deionised water (Fig. 42) are featureless with absorbance strongly increasing towards the blue, indicative of a highly functionalised cage. A log-log plot of concentration versus absorbance at 400 nm

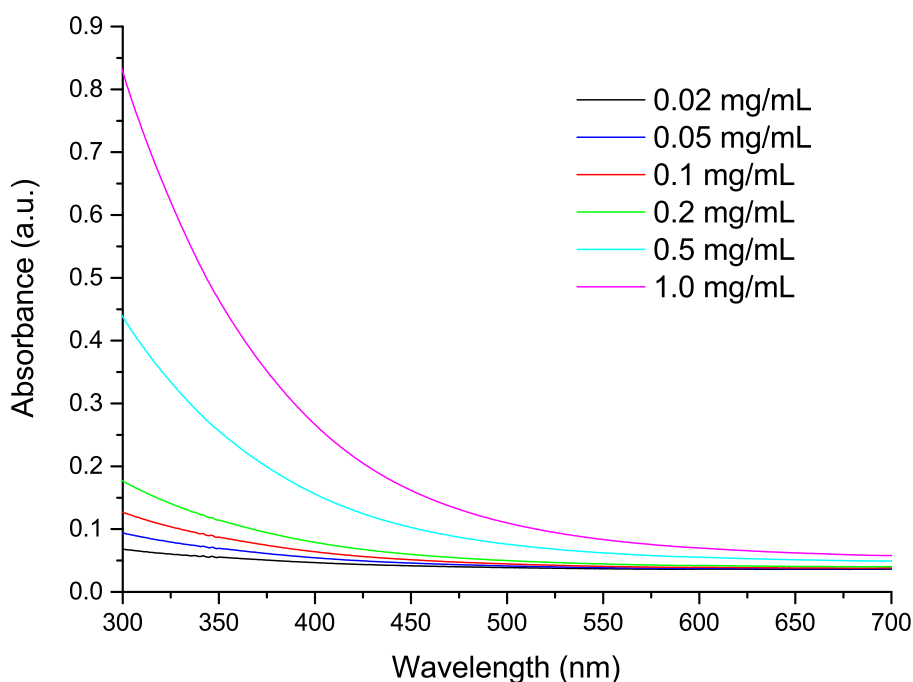


Figure 42: Absorption spectra of serial dilutions of TEGylated C_{70} **3** in deionised water.

shows a definite kink between two linear gradients occurring between 0.05 and 0.1 mg/mL concentration (Fig. 43).¹ As with **1**, this is indicative of the formation of micelles at a CMC in this region [245, 246]. It should be noted, however, that this value is stated with little confidence due to the large dilution errors (as evidenced in Fig. 43) that arise from working with serial dilutions at such low concentrations. In order to measure the solubility of **3** in deionised water, 10 mg were added to 1 mL in a vial and sonicated, giving a dark yellow solution. Not all of **3** dissolved. The saturated solution was left undisturbed for 1 hr at room temperature, before 0.3 mL was extracted, being careful not to disturb or extract any solid from the bottom of the vial. This extraction was diluted once by 50%. The measured

¹ As with **1**, in the absence of any prominent features in the absorption spectrum, a wavelength had to be chosen where the absorbance of all sample solutions in the calibration curve was maximised (to minimise error) but still less than 1 (within the linear absorbance range of the spectrophotometer). The second criterion is met for all concentrations in the calibration curve. However, the wavelength used for determination of solubility of **1** (550 nm) did not maximise the absorbance of all sample solutions (very low and similar absorbance values for concentrations below 0.5 mg/mL). As such, an alternative wavelength of 400 nm was used as the absorbance of lower concentrations could be differentiated and, crucially, the same light source was in use (the light source changed over at 350 nm).

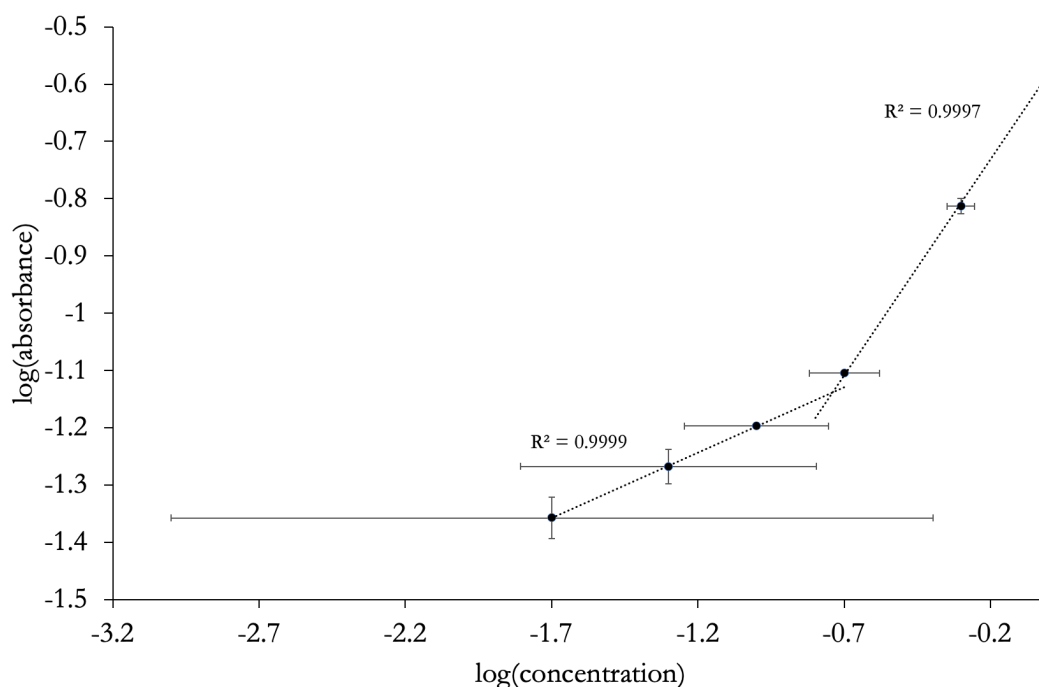


Figure 43: Serial dilutions of TEGylated C_{70} **3** in deionised water show two distinct linear regions in a log-log plot of concentration versus absorbance. Absorbance measurements were carried out on three independently prepared dilution series—vertical error bars correspond to one standard deviation above and below the mean value; horizontal error bars arise from cumulative error (calculated in Section 2.6.2.1).

absorbance at 400 nm of this solution (0.6442) corresponds to a concentration of 0.52 mg/mL, which gives an estimated aqueous solubility of **3** of 1.04 mg/mL. This would suggest that C_{70} derivative **3** is more hydrophobic than C_{60} derivative **1**. The observed CMC value for **3** (0.05–0.1 mg/mL) being an order of magnitude lower than that for **1** (1.0 mg/mL) is consistent with this as amphiphiles with a greater proportion of hydrophobicity exhibit lower CMC values [300].

NMR ^1H (Fig. 44), COSY (Fig. 45), HSQC (Fig. 46) and HMBC (Fig. 47) NMR spectra of **3** confirm the presence of TEG-MEE, as per assignments in Section 2.3.2.2. ^{13}C NMR also confirms the presence of TEG-MEE (Fig. 48). As with C_{60} derivative **1**, no peaks are observed in the typical fullerene range. Again, peaks likely corresponding to carbonyl carbons and carbons adjacent to hydroxyl/TEG-MEE groups (δ 171.1 and 166.3, respectively) are present.

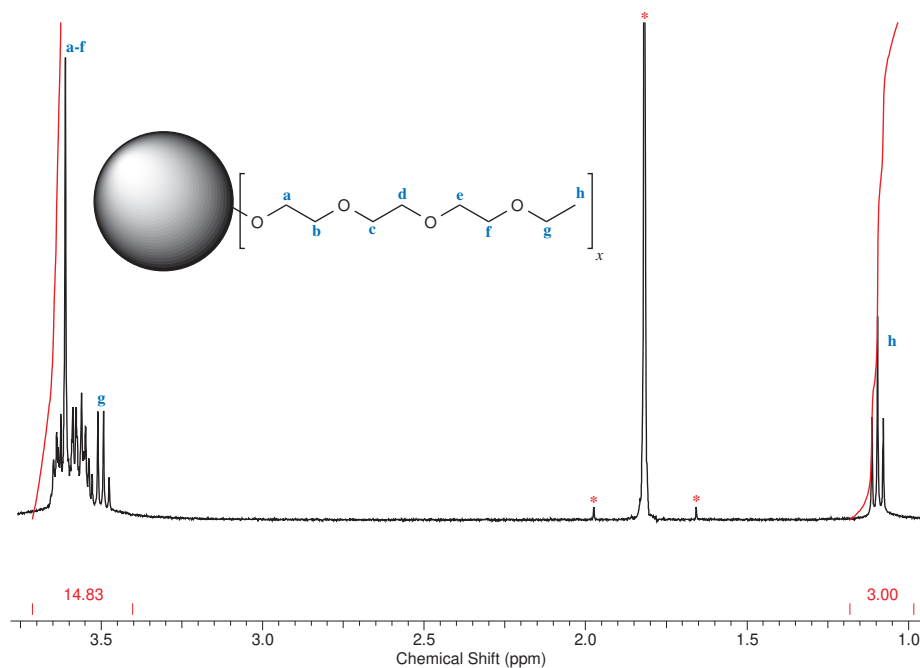


Figure 44: ^1H NMR spectrum of TEGylated C_{70} **3** (500 MHz, D_2O) δ 3.69–3.53 (12H, m, CH_2), 3.50 (2H, q, $J = 7.0$ Hz, CH_2), 1.10 (3H, t, $J = 7.1$ Hz, CH_3). A solid sphere is used to represent schematically the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in ^1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains presumed to be attached or associated around the fullerene cage.

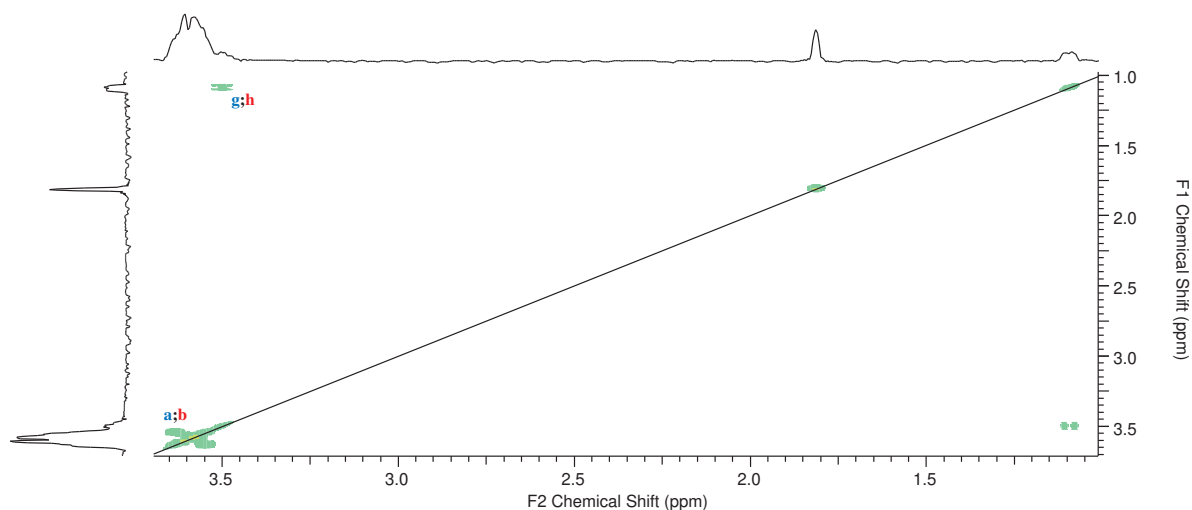


Figure 45: COSY NMR spectrum of TEGylated C_{70} **3** (500 MHz, D_2O).

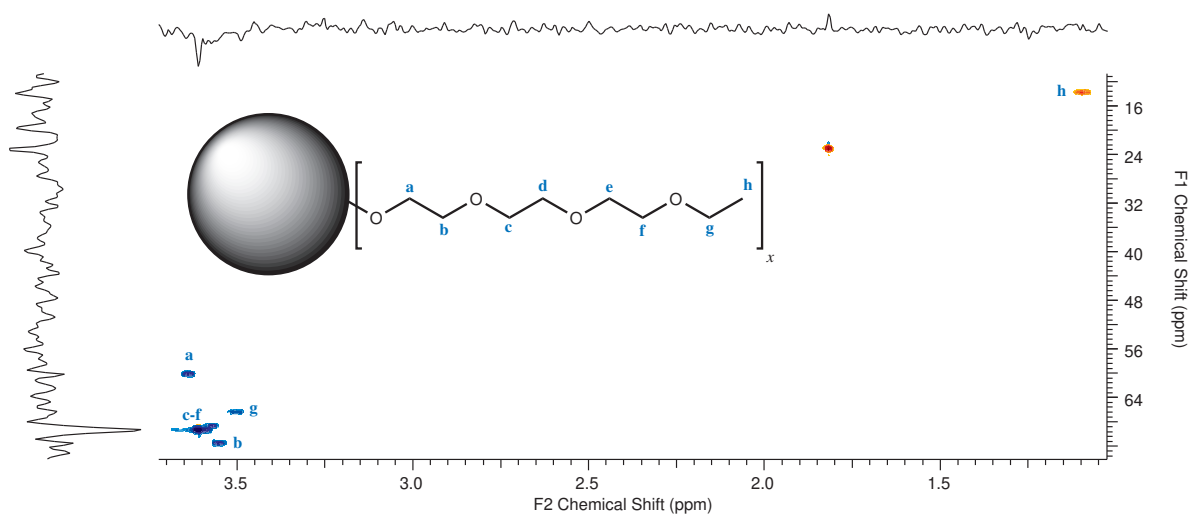


Figure 46: HSQC NMR spectrum of TEGylated C_{70} **3** (500 MHz, D_2O). A solid sphere is used to represent schematically the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in 1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains presumed to be attached or associated around the fullerene cage.

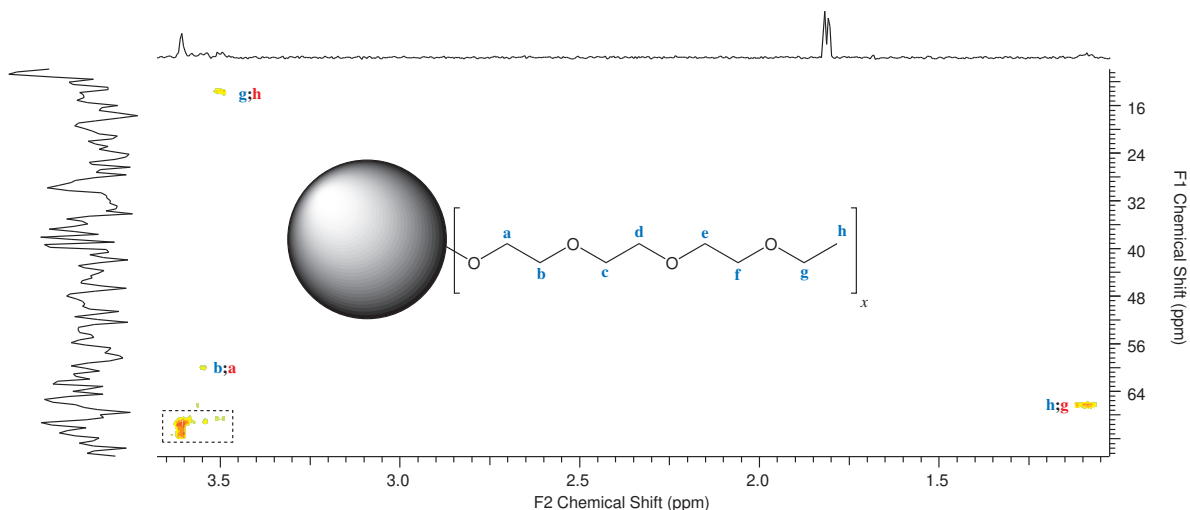


Figure 47: HMBC NMR spectrum of TEGylated C_{70} **3** (500 MHz, D_2O). Assignments of cross-peaks in the highlighted region are as per Section 2.3.2.2. A solid sphere is used to represent schematically the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in 1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains presumed to be attached or associated around the fullerene cage.

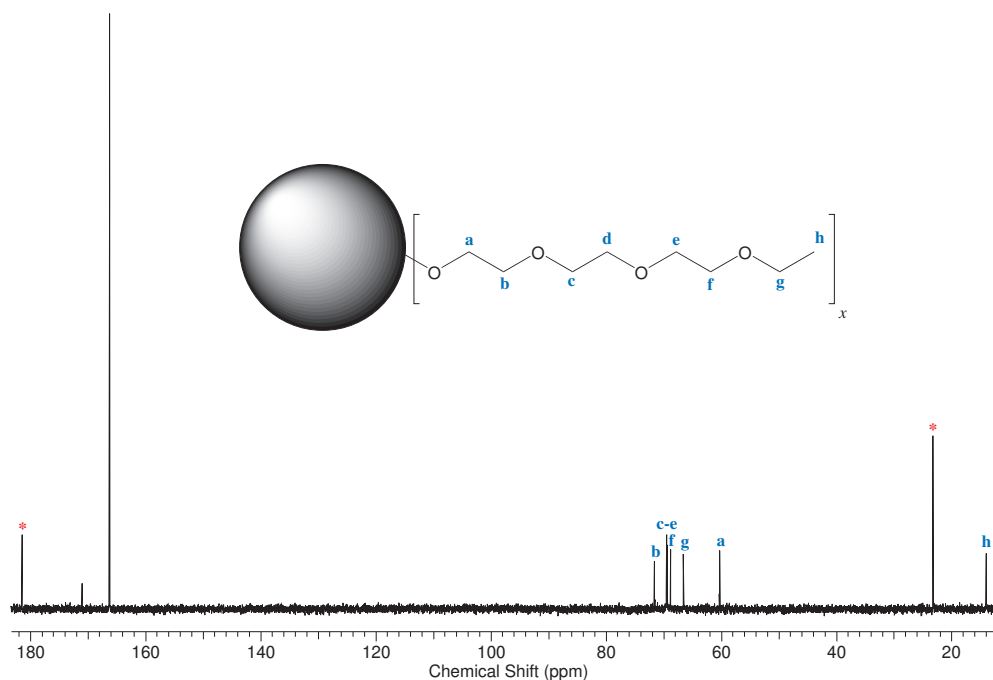


Figure 48: ^{13}C NMR spectrum of TEGylated C_{70} **3** (126 MHz, D_2O) δ 171.08, 166.31, 71.66, 69.58, 69.56, 69.43, 66.60, 60.31, 14.02, * residual EtOAc. A solid sphere is used to represent schematically the fullerene cage, which likely contains numerous hydroxyl groups and other oxygen-containing groups such as carbonyls in a non-trivial arrangement. Indeed, analogously to C_{60} derivative **1**, the peaks at δ 171.08 and δ 166.31 are respectively assigned to 1) carbons on the fullerene cage attached to carbonyl groups and 2) carbons adjacent to others that are themselves bonded to hydroxyl or TEG-MEE groups. x represents the as yet unknown number of TEG-MEE chains presumed to be attached or associated around the fullerene cage.

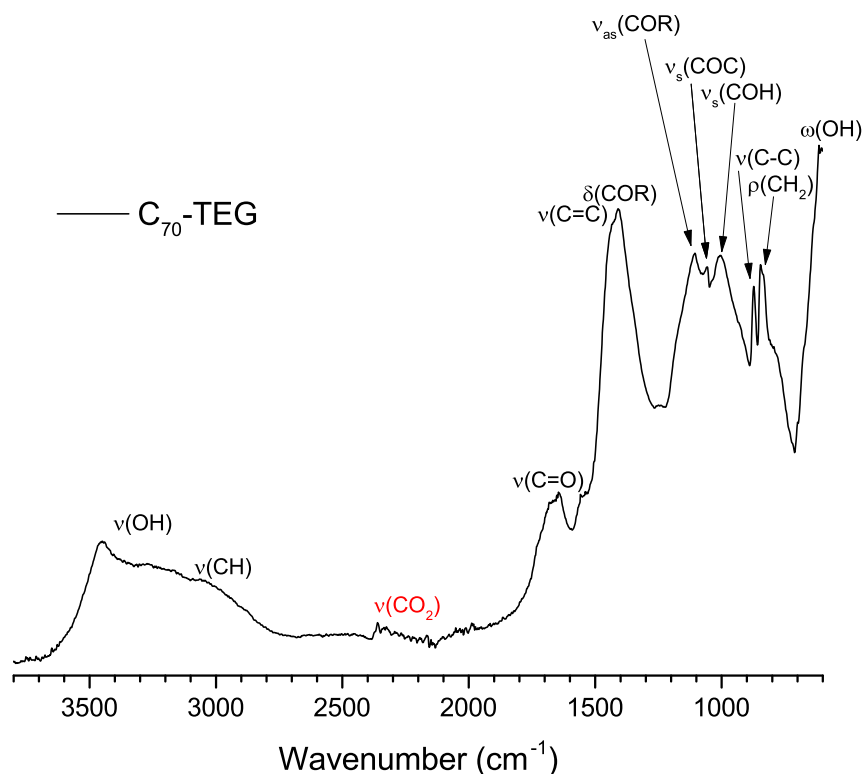


Figure 49: FTIR spectrum of TEGylated C_{70} **3**. 3449, 3273 [$\nu(\text{OH})$]; 3055 [$\nu(\text{CH})$]; 1645 [$\nu(\text{C}=\text{O})$]; 1429 [$\nu(\text{C}=\text{C})$]; 1410 [$\delta(\text{COR})$]; 1105 [$\nu_{\text{as}}(\text{COR})$]; 1058 [$\nu_{\text{s}}(\text{COC})$]; 1006 [$\nu(\text{COH})$]; 874 [$\nu(\text{C}-\text{C})$]; 847 [$\rho(\text{CH}_2)$]; 615, 604 [$\omega(\text{COH})$] cm^{-1} . All peaks referenced to maxima. Nomenclature: $\nu_{\text{s/as}}$ symmetric/asymmetric stretching; δ bending; ρ rocking; τ twisting; ω wagging.

FTIR The FTIR spectrum of **3** (Fig. 49) shares many of the same features as that of C_{60} derivative **1**. Once again, the broad peak above 3000 cm^{-1} is evidence of hydroxylation of the fullerene cage. Due to its reduced symmetry (D_{5h} *cf.* I_h for C_{60}), C_{70} has an increased number of vibrational modes—31 of which are IR active [313]. The highest wavenumber vibration for pristine C_{70} is at 1429 cm^{-1} , which overlaps with the bending vibrational mode of ether bridges (1410 cm^{-1}). Crucially, unlike with **1**, this means that evidence of carbonyl stretching bands can be seen at 1645 cm^{-1} . All other peaks are analogous to the spectrum of **1**.

EA The results of elemental analysis to determine the percentage content of carbon, hydrogen and oxygen in **3** are shown in Table 5. The sum of the percentage values (46.54

Table 5: % content of C, H & O of **3** as determined by EA. %N was determined at the same time as %C and %H, and was <0.1%. Absolute accuracy provided by Medac Ltd. is given in the table; duplicate measurements gave identical values.

%C	%H	%O
13.96 ± 0.30	1.91 ± 0.30	30.67 ± 0.30

$\pm 0.52\%$) once again suggests that the sample did not wholly degrade during the dynamic flash combustion process. Again, this assumption is backed up by the TGA results presented in Section 3.4.2.1, where it can be seen that indeed the majority (54%)² of sample remains at 900 °C, the operating temperature of the EA furnace for CHN analysis. Given that this incomplete combustion suggests that EA measurements primarily arise from groups attached to the fullerene, rather than the fullerene itself, the lower %C value would suggest that the ratio of covalently bound TEG-MEE chains to hydroxyl groups is lower in **3** than **1**.

TGA Dynamic TGA of **3** was carried out by heating from room temperature at a rate of 10 °C/min under nitrogen flow of 200 mL/min, collecting data points every 0.2 s. The trace of **3** (Fig. 50) is markedly different from that of C₆₀ derivative **1** (Fig. 19). The first mass loss (T_O 92 °C, T_M 95 °C, Δm 2.90 ± 0.91 %) corresponds to evaporation of free water. The second mass loss (T_O 121 °C, T_M 132 °C, Δm 18.05 ± 0.56 %) is considerably greater than for **1** and can reasonably be assigned to the loss of secondary bound water [45, 49] or non-covalently bound TEG-MEE—the latter is expected to dominate this contribution overwhelmingly as **3** is less functionalised than C₆₀ derivative **1**, whose secondary bound water mass loss (Δm 3.77 ± 0.57 %) is a much smaller percentage than present here. A third, shallow mass loss spanning a wide temperature range from 150 to 650 °C (Δm 7.07 ± 0.27 %) can be assigned to the dehydration of hydroxyl groups [49, 51]; unlike **1**, there is no well-defined loss at 450 °C, and so no compelling evidence for loss of covalently bound TEG-MEE chains. Like **1** though, the

² As in the case of C₆₀ derivative **1**, this is in excellent agreement with the ~53% missing from the EA sample.

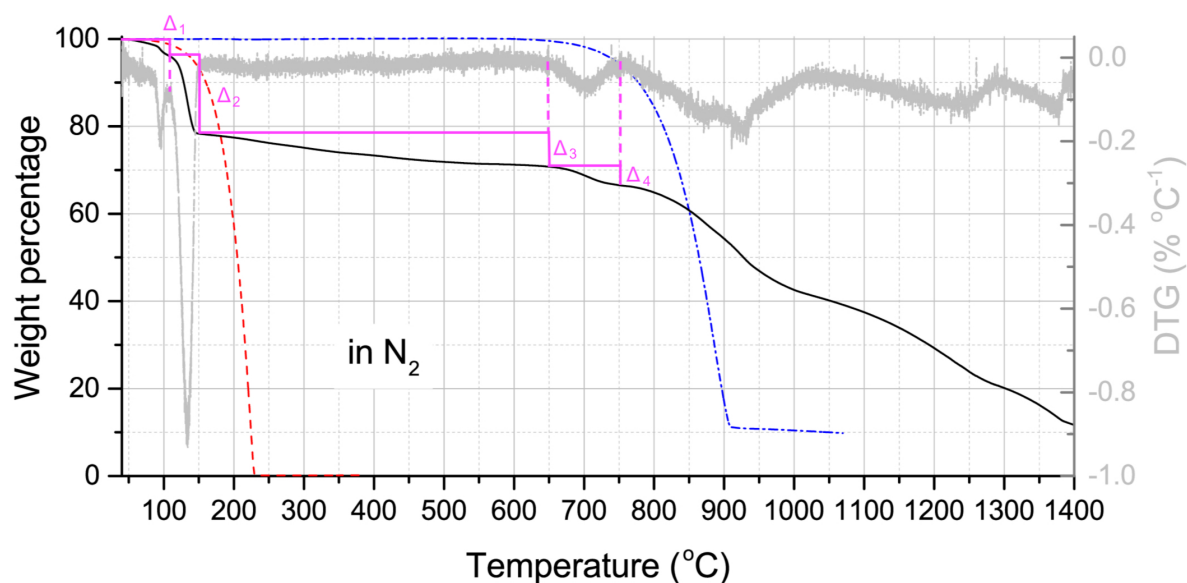


Figure 50: TGA trace of TEGylated C_{70} **3** in nitrogen atmosphere. Traces for control samples of **TEG-MEE** and pristine C_{60} (sublimation behaviour of C_{60} and C_{70} are known to be very similar under inert conditions [314]) are also shown, along with the derivative trace of **3** (DTG = differential thermogravimetry). The solid pink lines define each weight loss (Δ_x) up until the end of its associated DTG peak/beginning of the next DTG peak (dotted pink lines connect to the DTG curve for clarity).

fourth (T_O 669 °C, T_M 700 °C, Δm 4.64 ± 0.50 %) and subsequent mass losses correspond to sublimation of fullerene-like structures and decomposition of associated oxidised structures, respectively.

ESTIMATED AVERAGE STRUCTURE OF TEGYLATED C_{70} Qualitatively, evidence of a fullerene with numerous functional groups attached is given by the featureless visible absorption spectrum. NMR and FTIR techniques confirm the presence of TEG-MEE; hydroxylation of the cage has also occurred, and other oxygen-containing functional groups are likely present, as with the functional motif in **1**. Unlike **1**, there is little compelling evidence in TGA that TEG-MEE has been covalently bonded to the fullerene cage in **3**. This is perhaps not surprising as it has previously been noted that addition of oxygen-containing groups to C_{70} occurs at slower rates than to C_{60} [315]. The average structure of **3** can be estimated using the same quasi-graphical method as that used for C_{60} derivative **1** in Section

2.3.2.7, with substitution of $M_{C_{70}} = 840$ and experimental values from EA and TGA. Similarly, all terms in x (number of TEG-MEE chains) are removed from Equations 6 and 7 (because they are not covalently bonded to the cage) and Equation 8 referring to loss of secondary bound water is altered to correspond to loss of non-covalently bound TEG-MEE instead, giving

$$(\% \text{ TEG}) \cdot M_w = M_{\text{TEG}} \cdot x \quad (11)$$

No stoichiometric composition was found which coincided with all observed experimental values, however, the greatest overlap was seen for structures with 4 TEG-MEE chains (x) per C_{70} ($\frac{z}{y} = 0.5$; $n = 16$; $m = 16-20$). As such, an average molecular structure of $C_{70}O_{16}(OH)_9(O^-Na^+)_9 \cdot 4 \text{ TEG} \cdot 18 \text{ H}_2\text{O}$ ($M_w = 2668$) is assigned to **3**. The lower ratio of functional groups to fullerene cage carbons compared to C_{60} derivative **1** ($34:70 = 49\%$ for **3** versus $41:60 = 68\%$ for **1**) corresponds well with the observed lower water solubility of **3**, as well as with previous work suggesting that oxidation of C_{70} is not as fast as C_{60} [315]. The proposed average structure also corresponds nicely with 98 mg ($36.7 \mu\text{mol}$, from $41.7 \mu\text{mol}$ of C_{70} starting material) of **3** collected from the synthesis procedure.

3.4.2.2 TEGylated C_{84} and C_{90-92}

Owing to the small amounts of material available, full characterisation of the structures of TEGylated C_{84} **4** and TEGylated C_{90-92} **5** was not carried out. ^1H (Fig. 51) and COSY (Fig. 52) NMR spectra of **4** were, however, obtained, thus confirming the presence of TEG-MEE. Due to the appreciable gains in mass of the final products compared to their parent fullerenes (460% for **4**; 600% for **5**), and the evidence that C_{70} derivative **3** (mass increase 327%) contained non-covalently associated TEG-MEE rather than covalently grafted TEG-MEE, it is reasonable

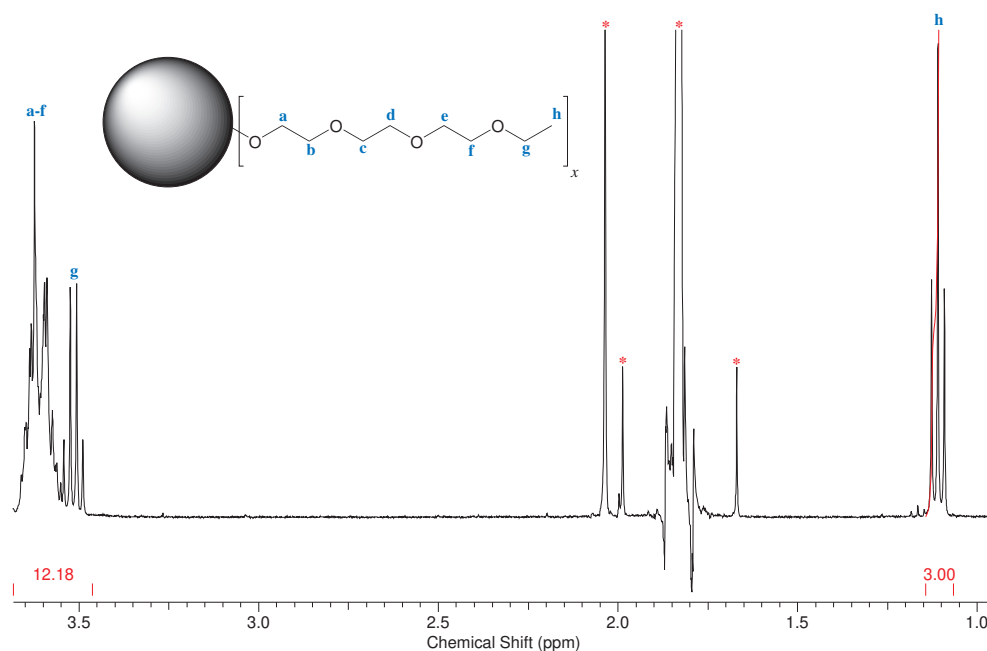


Figure 51: ^1H NMR spectrum of C_{84} derivative **4** (400 MHz, D_2O) δ 3.68–3.55 (12H, m, CH_2), 3.52 (2H, q, $J = 7.0$ Hz, CH_2), 1.11 (3H, t, $J = 7.1$ Hz, CH_3), * residual EtOAc and acetone. A solid sphere is used to represent schematically the fullerene cage, which likely contains numerous hydroxyl groups (whose exchangeable protons do not show in ^1H NMR) and other oxygen-containing groups such as carbonyls (which do not have protons) whose number and positions are not yet known. x represents the as yet unknown number of TEG-MEE chains presumed to be attached or associated around the fullerene cage.

to suggest that these higher fullerene derivatives, **4** and **5**, are non-covalently associated with even greater amounts of TEG-MEE than **3**.

Each sample was dissolved in 2 mL of deionised water, sonicated, and left to stand for an hour. Almost all of **4** had dissolved, with only a small amount of sediment—by eye, the solution was more concentrated than the equivalent saturated solution of **3**. Conversely, the saturated solution of **5** (also with a little sediment) was less concentrated than that of **3**. Their ready solubility in water suggests the presence of hydroxyls and other oxygen-containing groups, as with **1** and **3**.

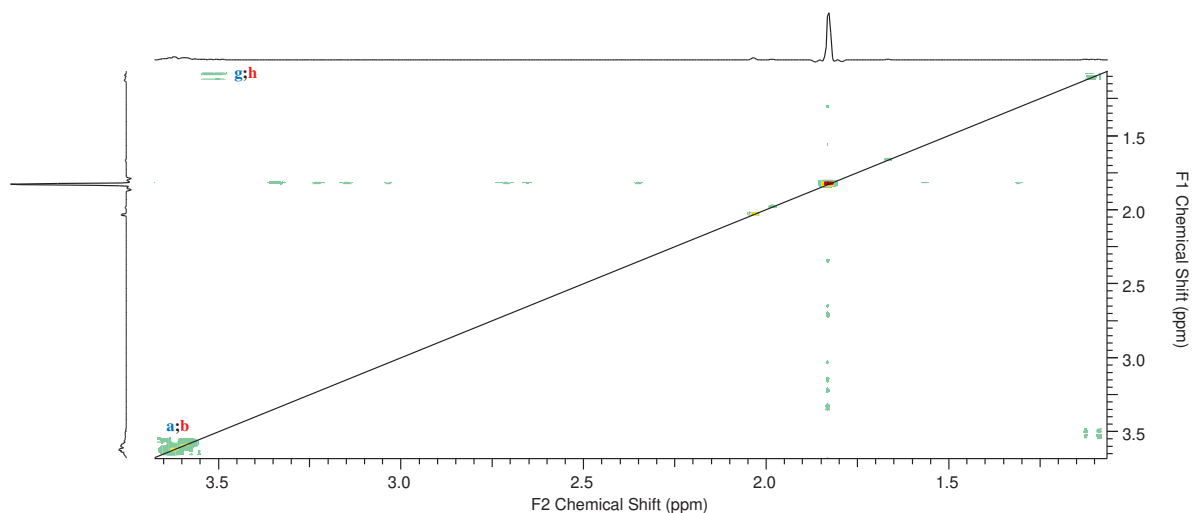


Figure 52: COSY NMR spectrum (400 MHz, D₂O) of C₈₄ derivative **4**.

3.4.3 Self-Assembly of TEGylated Higher Fullerenes

Serial dilutions of **3** (0.02, 0.05, 0.1, 0.2, 0.5 and 1.0 mg/mL) were prepared in aqueous solution (pH 8–9). 2 mL of each sample were pipetted into 20 mL glass vials, the lids screwed on and sealed with Parafilm M, and the vials stored in a dark cupboard. Within two weeks, solutions with concentration >0.2 mg/mL had several small aggregates visible to the naked eye (<0.5 mm), which had flocculated and sunk to the bottom of the vial; solutions with concentration <0.5 mg/mL showed no obvious aggregation. Within six months, no change in the shape or size of the aggregates had occurred as judged by the naked eye, but the number of particles at the bottom of vials with solutions >0.2 mg/mL had increased. When agitated, the aggregates whirled in solution, much like a snowglobe, before settling down. They were too small to pick up with tweezers and displayed none of the viscoelasticity of the TEGylated C₆₀ hydrogel (tested by prodding with tweezers).

The saturated solutions of TEGylated C₈₄ **4** and TEGylated C_{90–92} **5** were further diluted by addition of 6 mL of deionised water each to give solutions of 3.3 and 1.7 mg/mL concentration respectively. Their storage vials were sealed with Parafilm M and stored in a dark cupboard.

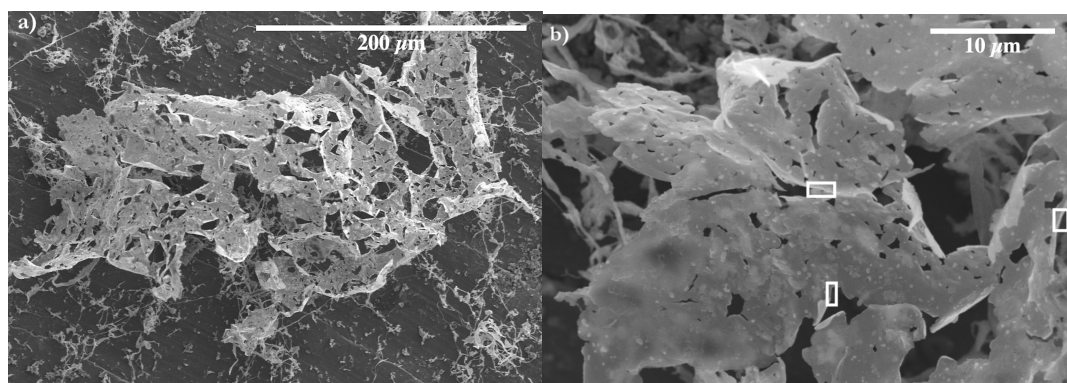


Figure 53: SEM micrographs of one example of a) an aggregated structure of TEGylated C_{70} derivative **3**. b) A close-up allows more accurate measurement of the thickness of the flakes at the locations highlighted (~ 110 nm). Both images acquired with 10 kV accelerating voltage, 100 pA probe current.

Within two weeks, four very small black floating aggregates could be seen in solution of **4**, while a thin and wispy aggregate had started to form in the **5** solution. After six months, several black quasi-spherical aggregates (2-6 mm in solution) were floating in the **4** solution—the biggest of these could be picked up with tweezers and displayed viscoelastic behaviour like the C_{60} hydrogel. Meanwhile, one large (21 mm in its largest dimension), white and wispy aggregate had formed in **5** solution—it could also be picked up and showed viscoelastic behaviour.

3.4.3.1 Structure of TEGylated C_{70} Self-Assembled Structures

A sessile drop of parent solution containing aggregates of TEGylated C_{70} **3** was pipetted onto an SEM stub and allowed to dry overnight before imaging under the SEM.

The short-range structures observed for **3** comprised of small patchy flakes extending no more than $400\text{ }\mu\text{m}$ in two dimensions, with a thickness of only ~ 110 nm (Fig. 53). Unlike cycloadditions to the C_{70} cage (which attack the more strained polar α bonds [316]), hydroxylation is known to preferentially occur across the equatorial belt [315, 317]. Given the lack of compelling evidence for covalent attachment of TEG-MEE chains to C_{70} (Section 3.4.2.1), it is reasonable to assume that hydroxylation (and introduction of other groups such

as carbonyls) is the primary functionalisation route of **3**, as demonstrated in its calculated average molecular structure, $C_{70}O_{16}(OH)_9(O^-Na^+)_9 \cdot 4$ TEG. This would give rise to a “reverse bolaamphiphilic” structure [318] wherein a hydrophilic equator region is capped by two hydrophobic corranulene-like poles (Fig. 54). The flat, flake-like morphology of aggregates of **3** can then be explained by extension of the concepts used to rationalise the self-assembly of **1**: the hugging interaction between TEG-MEE (this time non-covalently bound) and fullerene is favoured around the equatorial region [294], and so, in the case of **3**, this could lead to a patchwork-like aggregate morphology extending in two dimensions (Fig. 54). The limited size of these **3** structures (*cf.* **2**) can thus be rationalised, as the hydroxyl-filled equator of **3** is primarily involved in non-covalent interactions with TEG-MEE chains in the patchwork. Upon reaching a critical size limit, the aggregate does not have enough available hydrophilic groups to interact strongly with the surrounding water and it precipitates out of solution, as observed.

3.4.3.2 Structure of TEGylated C_{84} & C_{90-92} Self-Assembled Structures

Hydrogel samples of **4** and **5** were freeze-dried, as with C_{60} **1**, to create an aerogel before being imaged in the SEM. The self-assembled structures of both **4** and **5** more closely resemble the hierarchical structure of C_{60} **1** aerogel. An overview of the entirety of C_{84} **4** aerogel shows its dense structure extending over a maximum of 2 mm (Fig. 55a). On closer inspection, this is seen to be a dense interpenetrated network (average pore size 19 ± 5 μm) (Fig. 55b) of hollow fibres (1–2 μm outer diameter, 0.6–0.9 μm inner diameter, 0.4–1.1 μm wall thickness) (Fig. 55c). In places, the length of the fibres is stunted and flaky patches similar to those seen for C_{70} derivative **3** co-exist (Fig. 55d). C_{84} has 24 structural isomers that obey the IPR (Fig. 56) [4]; of these, the D_2 and D_{2d} isomers are the most abundant and have the highest sphericity [320]. Thus, it is expected that these two isomers are the primary components of **4**.

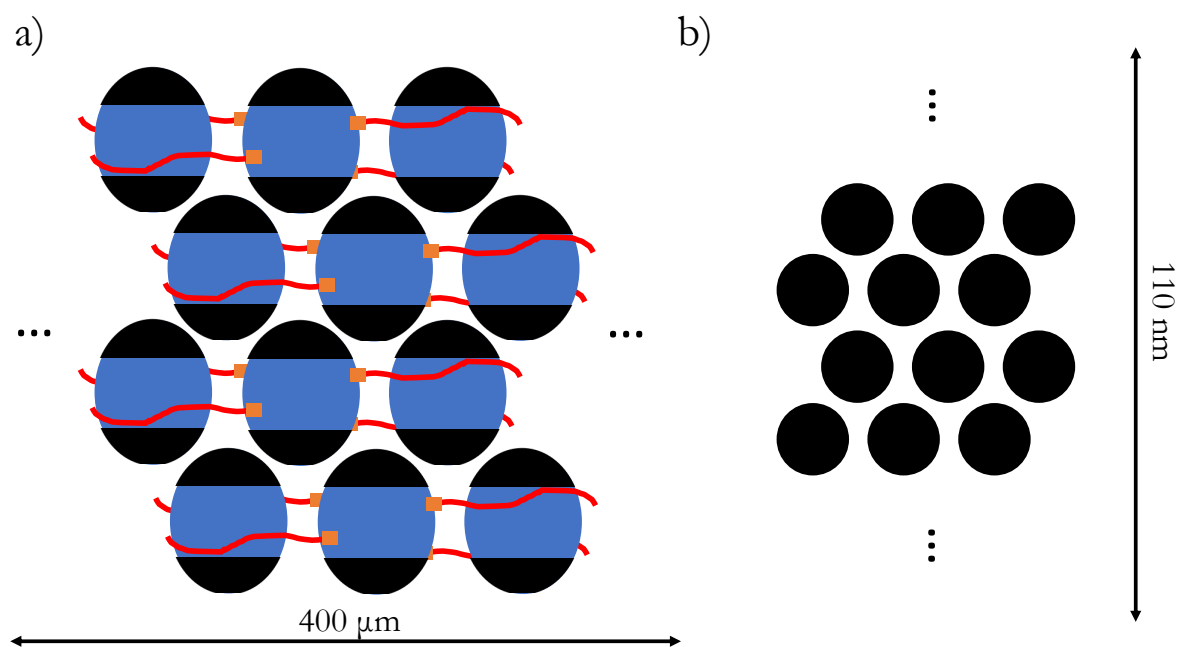


Figure 54: Cartoon representation of proposed self-assembly mechanism of C_{70} derivative **3**. a) Looking down at the surface of a flat flake extending up to $\sim 400\ \mu\text{m}$ in two dimensions (4 non-covalently associated TEG-MEE chains are shown only for the middle C_{70} in each row for clarity). b) Looking end-on through the thickness of a flake (black circles represent hydrophobic polar regions of C_{70} ; TEG-MEE chains are omitted for clarity). Ellipses are used to show that the pattern repeats in the given dimension up to the length stated. Blue regions represent hydrophilic surfaces due to hydroxyl groups; black regions represent hydrophobic unfunctionalised carbon. The favourable hugging interaction between TEG-MEE (red chains) and C_{70} is likely further strengthened, as with **1**, by hydrogen bonding with surface hydroxyl groups [319]. Given an average of four associated TEG-MEE groups per fullerene, two of these interactions could be in the plane of the page, and the other two out of the plane; this is represented in the cartoon by the two TEG-MEE chains below the plane representing in-plane interactions, and the two TEG-MEE chains above the plane representing interactions to the next patchwork layer. The hydrophobic polar caps are attracted to each other in aqueous solution. The terminal hydroxyl group of TEG-MEE is non-covalently associated to the oxidised fullerene surface through hydrogen bonding (indicated by orange squares). The relatively high hydrophobicity of this derivative (as evidenced by its low solubility) corresponds to its self-assembled structure's limited range.

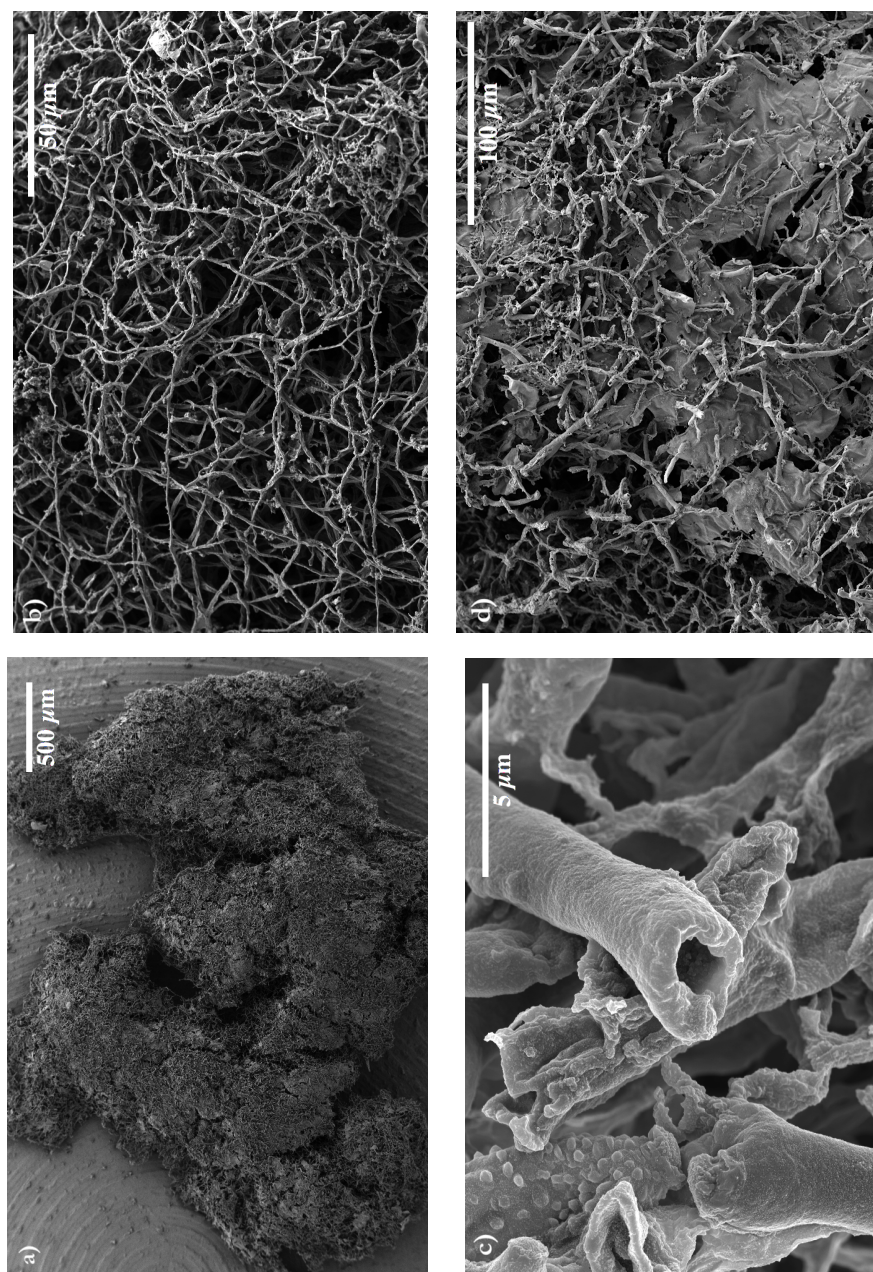


Figure 55: SEM micrographs of aggregate of C_{84} derivative **4**. a) The aggregate (longest dimension of 3.3 mm) can be seen to be far more densely packed than the hydrogel for C_{60} derivative **1**. b) The dense tangle has an average pore size of $19 \pm 5\text{ }\mu\text{m}$. c) The network is comprised of hollow fibres ($1\text{--}2\text{ }\mu\text{m}$ outer diameter, $0.6\text{--}0.9\text{ }\mu\text{m}$ inner diameter, $0.4\text{--}1.1\text{ }\mu\text{m}$ wall thickness), but there are also d) regions of flat flake-like morphologies.

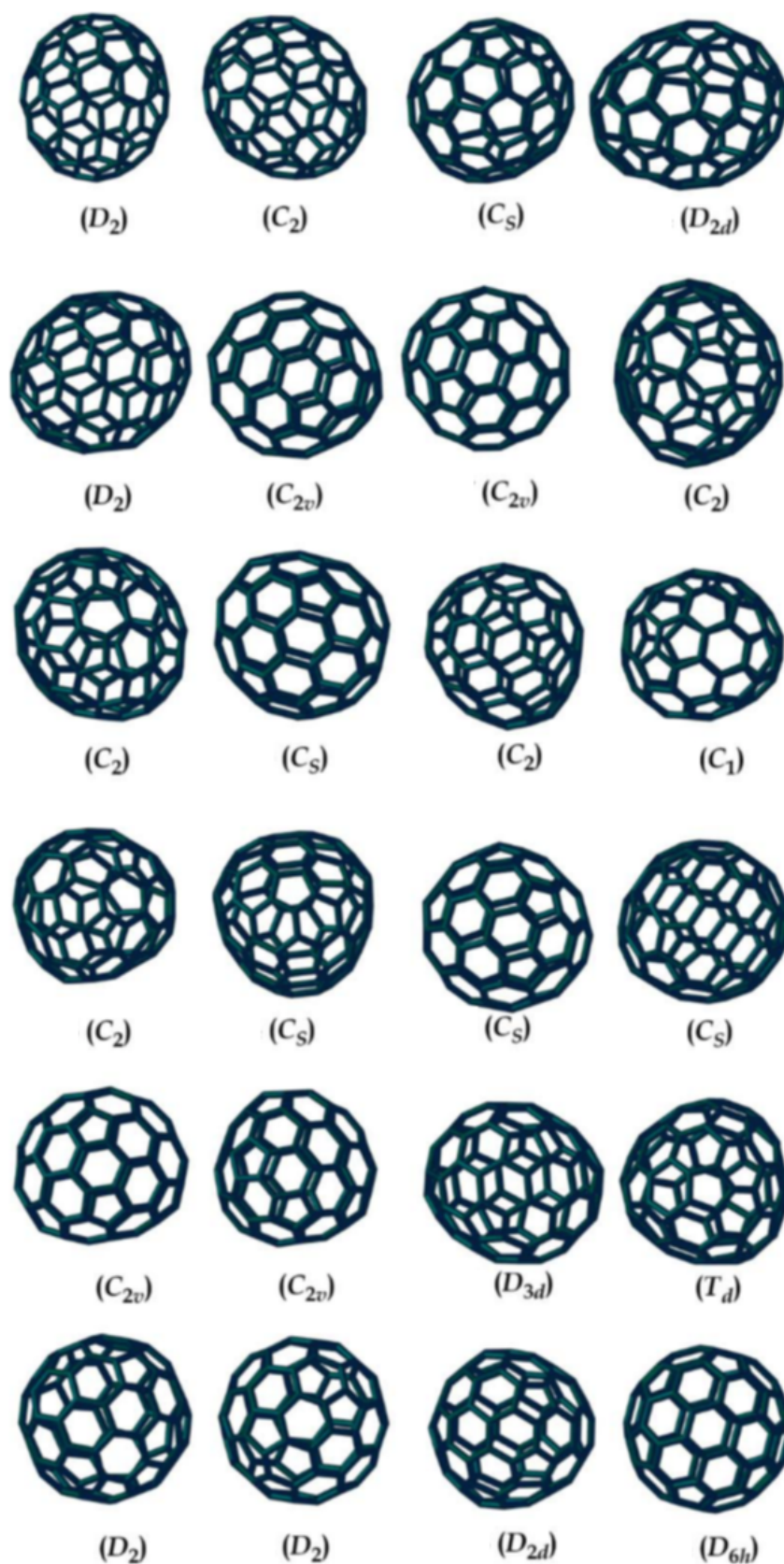


Figure 56: The 24 IPR isomers of C₈₄ with their respective point groups in brackets. Reprinted with permission from Sabirov, Ori and Laszlo, 2018, Fullerenes, Nanotubes and Carbon Nanostructures, 100–110. Copyright 2018 Taylor & Francis.

The high sphericity of the major isomers is a plausible explanation for the formation of the interpenetrated network of hollow fibres—this is analogous to highly spherical C_{60} leading to the interpenetrated network for **1**. It has previously been shown that highly functionalised C_{84} derivatives have “ribbons” of unfunctionalised hexagons across their surface [321]—in the presence of multiple hydroxylation and TEGylation here, these ribbons likely form and provide appropriate sites for the hugging interaction between TEG-MEE and fullerene, found to be so key for the aggregation process. Again, this hugging interaction is very likely enhanced by hydrogen bonding between TEG-MEE chains and surface hydroxyl groups. The presence of flaky regions in aerogel of **4** could be due to structural isomers whose functionalisation patterns lead to hydrophobic corannulene-like regions across the cage, similar to C_{70} derivative **3**, but crucially not diametrically opposed to each other.

The network of the aerogel of C_{90-92} **5** (average pore size $26 \pm 11 \mu\text{m}$, which extended in two dimensions to $\sim 2 \text{ mm}$) is visibly denser than for both C_{60} **1** and C_{84} **4**, with a global morphology intermediate of the fibrous networks of **1** and **4**, and the flakes of C_{70} derivative **3** (Fig. 57). The fibres have an outer diameter of $\sim 5 \mu\text{m}$, although the increased curvature of the pores makes this a less exact series of measurements than for the C_{60} and C_{84} aerogels.

C_{90} has 46 structural isomers that obey the IPR [4], 11 of which are kinetically and thermodynamically stable [322]; these are typically very low symmetry [323], with a highest symmetry point group of C_{2v} [324], excluding the D_{5h} “nanotube” isomer that is synthesised via non-standard arc discharge conditions [325]. C_{92} has 86 IPR-compliant structural isomers, predominantly low symmetry [4]. The positions of the most reactive sites on C_{90} (Fig. 58) suggest that under the TEGylation reaction conditions structures could be formed where hydrophobic corannulene-like regions exist on opposite sides of the cage to each other, similar to C_{70} derivative **3** but, as with C_{84} derivative **4**, crucially not diametrically opposed to each other. Given these assumptions, the observed structure of the aerogel of C_{90-92} **5** is

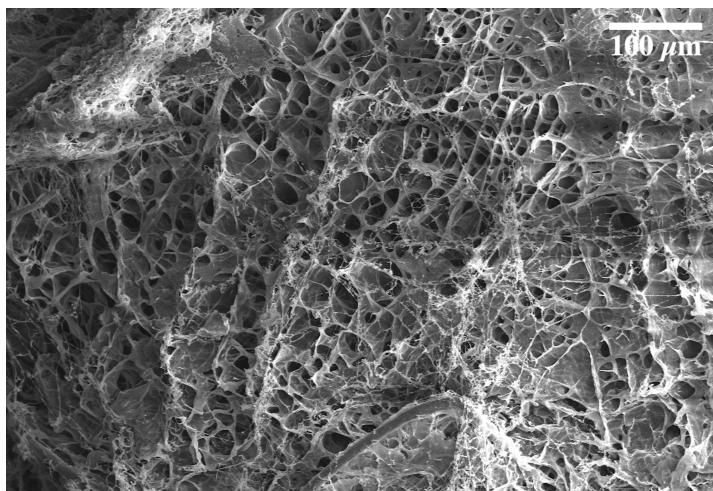


Figure 57: SEM micrograph of aggregate of C_{90} 5 aerogel showing a morphology (average pore size $26 \pm 11 \mu\text{m}$) intermediate of the fibrous networks of C_{60} derivative 1 and C_{84} derivative 4, and the flakes of C_{70} derivative 3.

reasonable, comprised of a dense interpenetrated network intermediate between a fibrous and flat morphology.

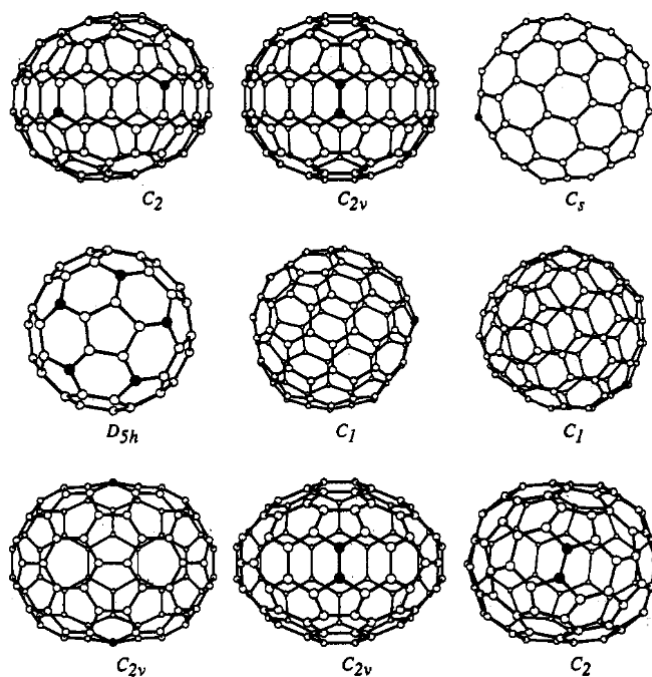


Figure 58: Some IPR isomers of C_{90} and their respective point groups, with most reactive sites highlighted in **black**. Reprinted with permission from Murry and Scuseria, 1994, J. Phys. Chem., 4212–4214. Copyright 1994 American Chemical Society.

3.4.4 Comparing Self-Assembled Structures Across Fullerene Sizes

The hierarchical self-assembled structures of TEGylated C_{84} **4** and TEGylated C_{90-92} **5** bear a striking resemblance to that of TEGylated C_{60} **1**, though with subtle differences. Table 6 summarises the key properties of each of the self-assembled structures formed by the different sized fullerene derivatives.

From Table 6, three related conclusions can be inferred:

1. Morphology and density (inversely proportional to pore size) are related, via the type of fullerene. This might sound obvious but, given that the morphology of a hierarchically self-assembled material is a function of its fundamental building block, and the density of the ensuing product affects its performance in applications (such as a drug delivery agent or a gas-separating membrane [277]), being able to tune the final product simply by doing the same procedure but with one different starting material is very powerful. In this respect, there is a stark difference between the behaviour of C_{60} and C_{70} in that the former yields the most porous and least dense material on an unprecedentedly large scale, while the latter yields structures at least an order of magnitude smaller and with minimal porosity under the same conditions.
2. The same cannot be said with as much certainty for C_{84} and C_{90-92} . The difference in the synthesis procedure (more TEG-MEE added³ and fewer rinse steps⁴) means that the collected product almost certainly has quite large amounts of non-covalently bound TEG-MEE. Indeed, this is suggested by the large weight gains during synthesis. It is feasible, therefore, that partially hydroxylated C_{84} and C_{90-92} are contained within micelles, with TEG-MEE as a surfactant. What is remarkable though is that both of these samples form

³ So done to compensate for the known lesser reactivity of higher fullerenes.

⁴ For fear of washing away too much product.

Fullerene	Oxidised Coverage of Fullerene ^a	Opposed Hydrophobic Corannulene-Like Regions After Functionalisation?	Average Number of Associated TEG-MEE Chains	Covalently Bound TEG-MEE?	Synthesis Weight Gain	Hydrogel Size (Largest Dimension)	Hydrogel Morphology	Average Pore Size (μm)	Fibre Diameter (μm)
C ₆₀	60%, evenly distributed	No	5	Yes	203%	Large, 23 mm	Porous	60 $\pm$ 15	1.5–2
C ₇₀	49%, around the equator	Yes, diametrically opposed	4	No	227%	Small, 400 μm	No hydrogel, flat flakes	N/A	N/A
C ₈₄	Unknown, likely presence of hydrophobic ribbons	Possible	Unknown	Unknown, unlikely	360%	Medium, 2 mm	Porous, small regions of flakes	19 $\pm$ 5	1–2
C _{90–92}	Unknown, likely isolated hydrophilic islands	Possible	Unknown	Unknown, unlikely	500%	Medium, 2 mm	Porous, some flat regions	26 $\pm$ 11	4–5

Table 6: Summary of properties of self-assembled structures of TEGylated fullerenes of different sizes.

^a Ratio of functionalised to non-functionalised carbons.

hydrogels of similar large size and porosity as C_{60} covalently functionalised with TEG-MEE. It suggests that the combination of hydroxylated fullerene cages and TEG-MEE (covalently bound or not) is key for the creation of extended macroscopic self-assembled structures. As has been proposed above, based on evidence from previous studies and the experiments contained within, there is a strong intermolecular interaction between hydroxylated fullerene cages and TEG-MEE that has, for the first time, been exploited to create supramolecular hydrogels of varied morphology from multiple fullerene building blocks.

3. This protocol also appears to convey properties from the molecular level such that they emerge at a higher level of the hierarchy: namely, the reactivity of the fullerene cages related to their shape. To demonstrate, highly spherical and highly functionalised C_{60} yields an aerogel with minimal flat-flake regions; C_{70} , with its isolated hydrophobic polar regions, around which TEG-MEE can't envelop, yields nothing but flat-flake regions. C_{84} and C_{90-92} , which reasonably do have isolated hydrophobic corannulene-like regions but, importantly, not at diametrically opposing poles (due to their lower symmetry *cf.* C_{70}), are then capable of interacting with TEG-MEE across most of their surface, despite their lower symmetry, thus yielding extensive hierarchical structures which do exhibit some areas of planarity reminiscent of the flat flakes of C_{70} .

To the best of my knowledge, there is only one other study relating the shape of nanocarbons to their ability to form supramolecular hydrogels [326]. In it, oxidised nanocarbons of three different dimensionalities—1-D nanotubes, 2-D graphene oxide and 3-D nanohorns—were functionalised with a known tripeptide gelator, and the ensuing supramolecular structures characterised. This is the first investigation into the supramolecular self-assembly of fullerenes bigger than C_{70} , and a rare exploration of the chemistry of the higher fullerenes [275].

3.5 AFM FORCE MEASUREMENTS OF TEGYLATED FULLERENE HYDROGELS

Using AFM, the Young's moduli of hydrogels of C_{60} derivative **1**, C_{84} derivative **4** and C_{90-92} derivative **5** were measured in their hydrated state in an aqueous environment, in order to determine any correlation between their mechanical properties and structures. The self-assembled structures of C_{70} derivative **3** were too small to be isolated and too thin for this technique.

Aside from imaging, AFM is also a diverse characterisation technique which has been used for the characterisation of the mechanical properties of hydrogels via nano-rheology [327] and nano-indentation [328]. The nano-indentation, or force measurement, technique was employed here.

3.5.1 Method

AFM was used here as a force microscopy technique for characterisation of the mechanical properties of samples. Deflection of a cantilever, with an attached tip that interacts with the sample, is measured via a piezoelectric or interferometric method as it is brought into contact with the sample surface. As the tip approaches the sample surface, the forces at play shift from long-range electrostatic interactions to van der Waals and solvation forces, and finally to direct indentation of the sample, from which the mechanical properties can be determined.

Hydrogels of TEGylated C_{60} **1**, C_{84} **4** and C_{90-92} **5** were picked in their hydrated state from storage in deionised water using tweezers, and placed on a glass microscope slide covered with a piece of double-sided sticky tape.⁵ A few drops of water were then pipetted onto the

⁵ The thickness of this tape is negligible with respect to the size of the hydrogel samples and so its effects on the hydrogels' mechanical properties are negated.

sample to keep it hydrated throughout the duration of the experiment. Force measurements on all samples were performed at room temperature with an MFP-3D AFM (Asylum Research, Oxford Instruments, Santa Barbara, CA). AC240 cantilevers (Olympus, Japan) were used with an attached colloidal polystyrene bead of diameter $20 \pm 0.3 \mu\text{m}$ (Sigma Aldrich). The optical lever sensitivity of the cantilever was determined by the indentation of a glass slide followed by the calculation of the spring constant using the thermal oscillation method [329]. Force measurements were performed with a maximum loading force of 70 nN at $40 \mu\text{m/s}$ to avoid time-dependent behaviour due to poroelasticity and/or viscoelasticity. At least seven positions were indented per hydrogel; 100 force curves were recorded at each position.

The force (F) and indentation (δ) of a spherical indenter of radius R can be related by the Hertz model [330] (which assumes no adhesion between indenter and sample),

$$F = \frac{4}{3} E^* \sqrt{R} \delta^{\frac{3}{2}} \quad (12)$$

where E^* is the effective Young's modulus of the sample,

$$E^* = \frac{E}{1 - \nu^2} \quad (13)$$

where E and ν are the Young's modulus and Poisson's ratio of the sample, respectively. Poisson's ratio for each of the hydrogels is unknown and so results are reported in terms of E^* .

Data analysis—contact point determination and Hertz fitting—was performed using a Python code developed in-house at the laboratory of Prof. Sonia Contera [331]. Force curves were fit from 30 nN to 45 nN.

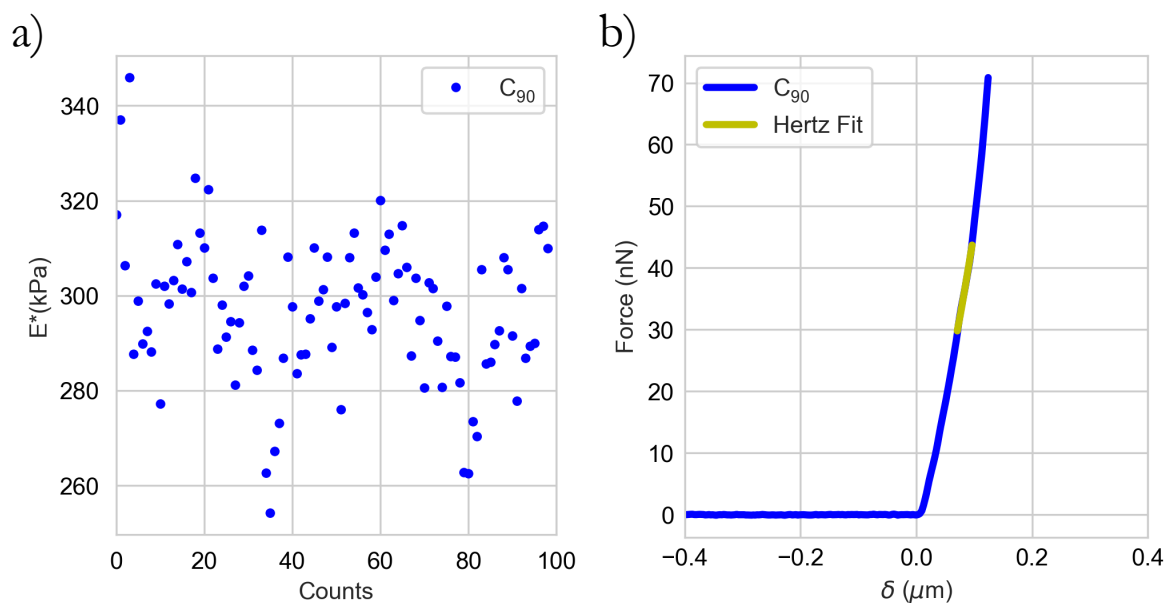


Figure 59: a) Typical AFM force measurement curve plotting the number of the measurement against the effective Young's modulus. b) effective Young's modulus distribution for hydrogel of TEGylated C_{90-92} .

3.5.2 Results

An example of a force curve obtained is shown in Fig. 59.a. Fig. 59.b shows a typical distribution of effective Young's modulus over 100 measurements at a single point. E^* was calculated for each force curve and the mean E^* calculated over the 100 measurements for each position; the average effective Young's modulus, $\bar{E}$, of each sample was then calculated as the mean of the E^* values for each position (Fig. 60). $\bar{E}$ for C_{60} **1** hydrogel (8.2 ± 6.2 kPa) is two orders of magnitude less than that for both C_{84} **4** hydrogel (183 ± 29 kPa) and C_{90-92} **5** hydrogel (432 ± 286 kPa).

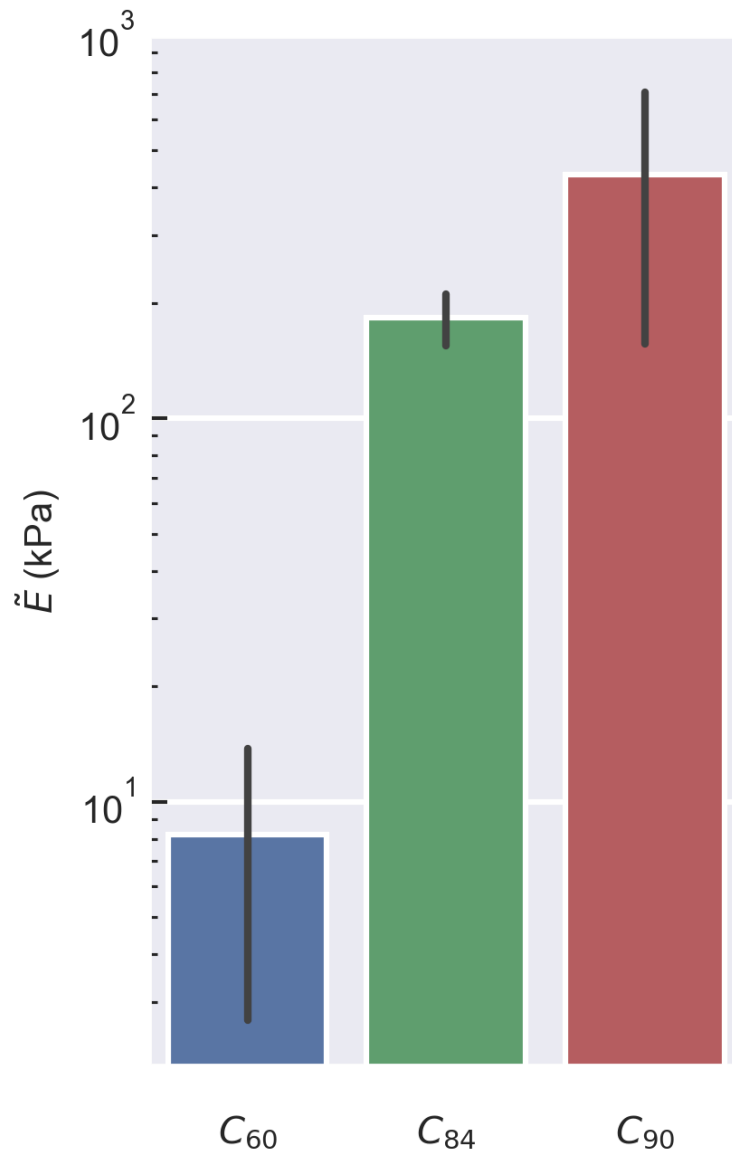


Figure 60: Effective Young's moduli of hydrogels of TEGylated C_{60} (8.2 ± 6.2 kPa), C_{84} (183 ± 29 kPa) and C_{90-92} (432 ± 286 kPa), as determined by AFM force measurements. Error bars at 95% confidence interval.

3.5.3 Discussion

There is a stark difference in the effective Young's moduli for each of the fullerene hydrogels. The $\tilde{E}$ of C₆₀ **1** hydrogel can be classed as intermediate in the realm of biological tissues and is comparable to that of breast tumour, kidney and lung tissue [332]. It is about average for similar supramolecular hydrogels [333]. $\tilde{E}$ of C₈₄ **4** hydrogel and C₉₀₋₉₂ **5** hydrogel can be classed as hard in the realm of biological tissues and is comparable to skeletal muscle and articular cartilage tissue [332]. Indeed, these are both an order of magnitude higher than for some "tough" polymeric supramolecular hydrogels whose mechanical properties deteriorate in the hydrated state [334].

The differences in measured $\tilde{E}$ values can be rationalised based on the porosity of the hydrogel structures:

1. C₆₀ **1** hydrogel is the most porous and least dense of the hydrogels. When, for example, one of its fibres is bent by the AFM indenter, there is a small number of fibres behind it to share the load. The anisotropy and irregular porosity of this sample is the reason for the large error in $\tilde{E}$.
2. C₈₄ **4** hydrogel is a far denser network and so the load is transferred more easily to other fibres. Similarly, the presence of flat flaky regions increases the surface area of the sample—any applied load is thus spread over a larger surface area, meaning it must be increased in order to achieve the same strain (i.e. a higher effective Young's modulus). The high density and regular porosity of this sample minimises the error in $\tilde{E}$.
3. C₉₀₋₉₂ **5** hydrogel is the extreme example amongst these samples, with the flattest morphology of the interpenetrating networks. Thus, it has the highest effective Young's

modulus. The large error arises because of differences in morphology across the sample's area.

A direct link between the fullerene building block and the Young's modulus of its hydrogel resulting from functionalisation with TEG-MEE can be inferred. However, given the differences in amount of non-covalently bound TEG-MEE for the higher fullerene samples, one should proceed with caution. Qualitatively, a reasonable relationship between fullerene building block (and the likelihood of the presence of corannulene-like hydrophobic regions) and presence of flat flake-like morphologies can be made. It is unclear though if this extends to the density of the whole hydrogel or if amount of non-covalently bound TEG-MEE plays a significant role; as such, quantitative comparisons between the mechanical properties of these fullerene hydrogels are dangerous. It is encouraging though to note that the strongest of these fullerene hydrogels fares very well against supramolecular polymer opposition.

3.6 MATERIALS AND METHODS

All commercial solvents, reactants and reagents were purchased from Sigma-Aldrich (United Kingdom) and used as purchased unless otherwise stated. Fullerenes C_{60} (99+% purity) and C_{70} (95+% purity) were used as purchased from Materials and Electrochemical Research (MER) Corporation (Arizona, USA). Fullerenes C_{84} and C_{90-92} were provided by Dr. Panagiotis Dallas (synthesis method described below). Grade 2 deionised water adhering to ISO3696 purchased from Chemiphase (United Kingdom) was used unless otherwise stated. 1H , ^{13}C , COSY, HSQC and HMBC NMR spectra were recorded using Bruker AVIII400 and Bruker AVIII500 (with He cryoprobe) spectrometers. Mass spectra were recorded on a Bruker microflex LT matrix-assisted laser desorption/ionisation time-of-flight mass spectrometer

(MALDI-TOF MS). FTIR spectra were recorded at room temperature on a Varian Excalibur FTS 3500 FT-IR with golden gate diamond attenuated total reflection (ATR) for fine powder solid samples. Steady state UV-vis absorption spectra were recorded in H₂O at 298 K on a dual-beam JASCO V-570 spectrophotometer (scan rate 200 nm/min), with reference to a solvent blank; quartz cuvettes with 1 cm path length were used in all cases. Nanoparticle tracking analysis was carried out on a Malvern Panalytical Nanosight LM10 at room temperature (638 nm laser); eight measurement runs were conducted per sample.

3.6.1 *Synthesis and Isolation of Higher Fullerenes C₈₄ and C₉₀₋₉₂*

Standard Krätschmer-Huffman arc discharge [12] was employed. Rods (10 x 10 x 100 mm) of pure graphite or Gd-doped (2 wt% Gd₂O₃) graphite were purchased from Toyo Tanso (Osaka, Japan) and used as electrodes—the anode-cathode distance was < 1 cm. Optimal conditions for fullerene production were set: the water-cooled arc discharge chamber was evacuated (10⁻⁴ Torr) before being filled with He gas (50 mbar), and a 200 A direct current was used for the arc [335]. Being careful not to exceed the maximum operating temperature (~3700 °C) for more than ten seconds at a time, the rods were burnt down. The resulting soot was collected from the chamber and dissolved in toluene. This solution then underwent Soxhlet extraction typically for 48 hours in order to extract soluble empty-cage fullerenes from other insoluble carbonaceous material. Reverse-phase HPLC (Japan Analytical Industry (JAI) LC-9103 Preparative HPLC with a modular Hitachi L-7150 pump and JAI UV Detector 3702 at 312 nm absorption) with a Cosmosil Buckyprep-M column (20.0 mm ID x 250 mm, toluene, 16 mL/min flow rate) was then used on this concentrated solution to isolate the

different sized cages. Structural isomers of the same-sized cages with different symmetries [4] were not separated from each other [336].

3.6.2 Synthesis and Structural Characterisation of TEGylated C_{70}

C_{70} (30 mg, 41.7 μ mol, 1 eq) was dissolved in toluene (18 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (3.75 mL, 21.4 mmol, 515 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (192 mg, 4.80 mmol, 115 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (as with C_{60} , a visible change in solution colour from red to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (55 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 μ m), leaving a dark grey precipitate on the filter and a very dilute yellow filtrate. This dark grey precipitate was rinsed with EtOH (30 mL), giving a brown filtrate and leaving a brown precipitate on the filter paper. This precipitate was dried *in vacuo* to give **3** as a brown powder (98 mg).

$^1\text{H NMR}$ (500 MHz, D_2O) δ 3.69–3.53 (12H, m, CH_2), 3.50 (2H, q, $J = 7.0$ Hz, CH_2), 1.10 (3H, t, $J = 7.1$ Hz, CH_3).

$^{13}\text{C NMR}$ (126 MHz, D_2O) δ 171.08, 166.31, 71.66, 69.58, 69.56, 69.43, 66.60, 60.31, 14.02.

FTIR 3449 m , 3273 m [br., $\nu(\text{OH})$]; 3055 m [br., $\nu(\text{CH})$]; 1645 m [$\nu(\text{C}=\text{O})$]; 1429 s [$\nu(\text{C}=\text{C})$]; 1410 s [$\delta(\text{COR})$]; 1105 m [$\nu_{\text{as}}(\text{COR})$]; 1058 m [$\nu_{\text{s}}(\text{COC})$]; 1006 m [$\nu(\text{COH})$]; 874 m [$\nu(\text{C}-\text{C})$]; 847 m

[$\rho(\text{CH}_2)$]; 615s, 604s [$\omega(\text{COH})$] cm^{-1} . All peaks referenced to maxima. Nomenclature: $\nu_{\text{s/as}}$ symmetric/asymmetric stretching; δ bending; ρ rocking; τ twisting; ω wagging; s strong; m medium; w weak; br. broad.

3.6.2.1 Thermal Gravimetric Analysis

Dynamic TGA measurements were carried out on a Perkin Elmer Pyris 1 under nitrogen atmosphere (200 mL/min), at a constant rate of change of temperature of 10 °C/min in the range 40 to 1200/1400 °C, collecting data points every 0.2 s. Samples (at least 2 mg) were loaded in ceramic TGA pans (Perkin Elmer, United Kingdom) and calibrated against an empty pan. The onset temperature (T_{O}) of each weight loss (Δ_x) was defined as the temperature at which the tangents to the TGA curve at T_{M} (see next sentence) and T_{B} (the baseline temperature, defined here as the temperature at the maximum DTG value immediately before that weight loss) intersect. The temperature at maximum rate of decomposition (T_{M}) of each weight loss was defined as the temperature at the minimum DTG value for that weight loss (i.e. at the DTG peak). The percentage point change in mass (Δm) for each weight loss was calculated as the difference between weight percentages at the beginning and end of the weight loss. The beginning of each weight loss (differentiated from the onset temperature) was defined as the temperature at the end of the previous weight loss (see pink solid lines in Fig. 50), except for the first weight loss, Δ_1 , where the starting temperature was used. Due to the noisy DTG signal, the end of each weight loss was poorly defined and, in the absence of peak fitting software, was judged by eye as a point where the signal to noise ratio of the DTG curve became roughly constant. The upper and lower bounds of the weight percentage at both the beginning and end of each weight loss were then read off the TGA curve based on an instrumental error in temperature measurement of ± 5 °C. Maximum and

minimum bounding values of Δm were then calculated from these and Δm values quoted as the average $\pm$ the difference with the bounding values.

3.6.2.2 *Elemental Analysis*

All samples were sent to MEDAC Ltd. (Surrey, United Kingdom) and measurements carried out in duplicate. For CHN analysis, samples were held in a tin capsule, purged by a continuous flow of helium and dropped into a vertical quartz tube maintained at 900 °C, at which point the helium stream was temporarily enriched with pure oxygen, causing the tin to promote a violent reaction (flash combustion). The resulting gases are passed over catalysts and the mixture then passed through a chromatographic column where the individual components were separated and eluted as nitrogen, carbon dioxide and water. For O analysis, samples were held in a silver container and dropped inside the reactor furnace maintained at 1060 °C instead.

3.6.2.3 *Structure Determination of TEGylated C_{70}*

A quasi-graphical tabular approach was used, using a series of Excel spreadsheets. Tables were constructed as for C_{60} derivative **1** (Table 3 in Section 2.3.2.7) but with substitution of $M_{C_{70}} = 840$ in Eqns. 1–5 and removal of terms in m . To account for loss of non-covalently bound TEG-MEE, terms in x were removed from Eqns. 6 and 7, and Eqn. 8 was altered, giving Eqn. 11 (Section 3.4.2.1). The independent variable is the number of hydroxyl groups (y , up to 44). One Table 3 exists per TEG-MEE chain associated (x , up to eight), thus yielding an array of nine versions of Table 3. This array was repeated for three values of n (the number of oxygen atoms not occurring in hydroxyl or TEG-MEE groups): 0, 8 and 16. Finally, these three arrays were produced for four different values of the ratio $z:y$ (degree of saponification): 0, 0.25, 0.5 and 0.75. The tables were populated by inputting

independent variables x , y , z and n , and values which fell within the ranges designated by experimental error were highlighted green (via conditional formatting in Excel). Coincident highlighted cells in different columns correspond to a stoichiometric composition for which those measurements independently matched. In this way, the average structure of **3** was found to be $C_{70}O_{16}(OH)_9(O^-Na^+)_9 \cdot 4(H(OC_2H_4)_3OC_2H_5) \cdot 18H_2O$ (M_w 2668).

3.6.2.4 Solubility Determination of TEGylated C_{70}

Serial dilutions of **3** in deionised water were prepared from a stock solution of 1.0 mg/mL and the absorption spectra of each concentration recorded three times in the visible region; errors in absorbance are thus given to one standard deviation of these measurements. The error in concentration for each dilution was calculated as follows. The instrumental errors in creating the stock solution are ± 0.05 mg and ± 0.01 mL. This mass error (δm , mg) remains the same throughout as the mass was only measured once for the stock solution, while the volumetric error (δV , mL) is defined as a summative error,

$$\delta V_n = \sqrt{(2n + 1)(0.01)^2}$$

for the n^{th} dilution. This is because, for each dilution there are three volumetric errors to consider: the error in measuring the volume of the previous dilution (± 0.01 mL), the error in measuring the volume of fresh solvent added (± 0.01 mL), and the cumulative volumetric errors (each ± 0.01 mL) for previous dilutions up to that point. The concentration error (δc , mg/mL) is then defined as

$$\delta c_n = \sqrt{\left(\frac{\delta m}{m}\right)^2 + \left(\frac{\delta V_n}{V}\right)^2}$$

for the n^{th} dilution from a stock solution of concentration c made with mass m and volume V . Error bars for the log-log plot were calculated using the standard logarithmic error relationship,

$$\delta(\log(c_n)) = 0.434 \cdot \frac{\delta c_n}{c_n}$$

for the n^{th} dilution.

For solubility determination, 10 mg of **3** were added to 1 mL of deionised water in a vial and sonicated, giving a dark yellow solution. Not all of **3** dissolved. The saturated solution was left undisturbed for 1 hr at room temperature, before 0.3 mL was extracted, being careful not to disturb or extract any solid from the bottom of the vial. This extraction was diluted once by 50% and its absorbance measured at 400 nm.

3.6.3 *Synthesis and Structural Characterisation of TEGylated C₈₄*

C₈₄ (5 mg, 4.96 μmol , 1 eq) was dissolved in toluene (8 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (1 mL, 5.72 mmol, 1154 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (68 mg, 1.70 mmol, 343 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (as with C₆₀ and C₇₀, a visible change in solution colour from dark green to brown could be seen within two minutes of adding NaOH). A volumetric excess of EtOAc (25 mL) was added to the reaction flask, which was then placed in the fridge (2 °C) overnight, giving a light brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, 0.2 μm), leaving a dark grey precipitate on the filter and a dilute yellow filtrate. This dark grey precipitate was rinsed with acetone (20 mL), giving a very pale yellow filtrate

and leaving a light brown precipitate on the filter paper. This precipitate was dried *in vacuo* to give **4** as a brown powder (23 mg).

^1H NMR (400 MHz, D_2O) δ 3.68–3.55 (12H, m, CH_2), 3.52 (2H, q, $J = 7.0$ Hz, CH_2), 1.11 (3H, t, $J = 7.1$ Hz, CH_3).

3.6.4 Synthesis of TEGylated C_{90-92}

C_{90-92} (2 mg, $1.85\ \mu\text{mol}$, 1 eq) was dissolved in toluene (4 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. TEG-MEE (0.7 mL, 4.01 mmol, 2165 eq) was added to the solution along with a magnetic stirrer bar. A pellet of NaOH (52 mg, 1.30 mmol, 703 eq) was added and the stoppered reaction solution was stirred for 21 hours at room temperature (the colour change associated with solutions of C_{60} , C_{70} and C_{84} could be seen within two minutes of adding NaOH, but was less discernible given the dilute concentration and small amount of C_{90-92} used). A volumetric excess of EtOAc (15 mL) was added to the reaction flask, which was then placed in the fridge ($2\ ^\circ\text{C}$) overnight, giving a light brown precipitate. The contents of the flask were then filtered using a Büchner funnel (PTFE filter, $0.2\ \mu\text{m}$), leaving a light grey precipitate on the filter and a dilute yellow filtrate. This light grey precipitate was dried *in vacuo* to give **5** as a brown powder (12 mg).

3.6.5 *Synthesis and Structural Characterisation of Supramolecular Hydrogels*

3.6.5.1 *Hydrogel Synthesis*

Serial dilutions of TEGylated C₆₀ **1** (0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10 and 20 mg/mL) and TEGylated C₇₀ **3** (0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 mg/mL) were prepared in deionised water. 2 mL of each sample were pipetted into separate 20 mL glass vials, the lids screwed on and sealed with Parafilm M, and the vials stored undisturbed in a dark cupboard for up to six months. Saturated aqueous solutions of TEGylated C₈₄ **4** and TEGylated C_{90–92} **5** (23 mg and 12 mg, respectively, in 2 mL) were further diluted by addition of 6 mL of deionised water each to give solutions of 3.3 and 1.7 mg/mL concentration respectively. Their storage vials were sealed with Parafilm M and stored undisturbed in a dark cupboard for up to six months.

3.6.5.2 *Optical Microscopy*

Samples were pipetted onto glass microscope slides and imaged in transmission mode on a Motic BA210 binocular optical microscope.

3.6.5.3 *Scanning Electron Microscopy*

Samples (floating aggregates in aqueous solution) were pipetted or, if large enough, placed with tweezers onto an SEM stub then frozen for 24 hours at -20 °C before being freeze-dried for 24 hours at 0 °C and 0.05 atm, as per the Czernuszka group procedure for collagen tissue scaffolds [337, 338]. The freeze-dried samples were then placed on another SEM stub covered with double-sided conductive copper tape before being coated with 5–10 nm of gold by a Bio-Rad E5000 sputter coater. Secondary electron images were acquired on a Zeiss EVO MA10 with an accelerating voltage of 5–10 kV and a probe current of 30–100 pA.

3.6.5.4 *Image Analysis*

Lengths were measured using ImageJ image processing software, with respect to scale bars that had been obtained on microscope-specific software. Pore sizes were calculated as an average measured over 20 random pores. Box-counting fractal dimensions, D_b , were calculated using the box-counting method of the FracLac plugin in ImageJ; the image was converted to a binary image, a maximum of 12 grid positions was selected, and box sizes were selected using the default sampling size, with the minimum box size unspecified and the maximum at 45% of the sampled area.

3.6.6 *Mechanical Characterisation of Supramolecular Hydrogels*

Force measurements on all samples were performed at room temperature with an MFP-3D Atomic Force Microscope (Asylum Research, Oxford Instruments, Santa Barbara, CA). AC240 cantilevers (Olympus, Japan) were used with an attached colloidal polystyrene bead of diameter $20 \pm 0.3 \mu\text{m}$ (Sigma Aldrich). The optical lever sensitivity of the cantilever was determined by the indentation of a glass slide followed by the calculation of the spring constant using the thermal oscillation method [329]. Force measurements were performed with a maximum loading force of 70 nN at $40 \mu\text{m/s}$ to avoid time-dependent behaviour due to poroelasticity and/or viscoelasticity. Samples were picked in their hydrated state from storage in deionised water using tweezers, and placed on a glass microscope slide covered with a piece of double-sided sticky tape.⁶ A few drops of water were then pipetted onto the sample to keep it hydrated throughout the duration of the experiment. At least seven positions were indented per sample; 100 force curves were recorded at each position. Data

⁶ The thickness of this tape is negligible with respect to the size of the hydrogel samples and so its effects on the hydrogels' mechanical properties are negated.

analysis—contact point determination and Hertz fitting—was performed using a Python code developed in-house at the laboratory of Prof. Sonia Contera [331].

3.7 CONCLUSIONS

TEGylated C_{60} **1** self-assembles in aqueous solution over a period of weeks to months into large hierarchical supramolecular hydrogels composed of an interpenetrating porous (average pore size $60 \pm 15 \mu\text{m}$) network of hollow fibres (inner diameter $\sim 900 \text{ nm}$, outer diameter $1.5\text{--}2 \mu\text{m}$). This aggregation process occurs thanks to strong non-covalent interactions between covalently bound TEG-MEE chains and the central hydroxylated fullerene moiety.

The same functionalisation method was applied to higher fullerenes, C_{70} , C_{84} and C_{90-92} , yielding water-soluble derivatives **3**, **4** and **5** respectively. The self-assembly behaviour of these derivatives was found to depend strongly on the size and shape of the parent fullerene, with **3** not forming any hierarchical structures at any scale, and C_{84} derivative **4** and C_{90-92} derivative **5** both forming hydrogels of similar, but distinct, morphologies to C_{60} derivative **1**. Such millimetre-sized hierarchical structures are unprecedented for covalently functionalised water-soluble fullerene derivatives.

Just as striking is the discovered dependence of the mechanical properties of these hydrogels on their structural characteristics. It was found that C_{90-92} **5** hydrogel was the strongest structure, closely followed by C_{84} **4**, while C_{60} derivative **1** had an effective Young's modulus two orders of magnitude lower. Although a correlation can be made between the macroscopic mechanical properties of the produced hydrogels and their nanoscopic molecular building blocks, the uncertainty in amount of non-covalently bound TEG-MEE makes quantitative comparisons between types of fullerene unreliable.

Numerous hydrogel systems are finding varied application in the biomedical field [307, 339–341], including drug delivery [342] and tissue engineering [343, 344]. It is important to note that the mechanical properties of tissue engineering scaffolds and matrices dramatically affect the proliferation of different types of cells [345]. As such, the hydrogels produced in this chapter demonstrate that one family of starting materials, the fullerenes, can be functionalised via one simple method, TEGylation, to give a range of mesoporous materials with broad potential in the biomedical field [346].

A FULLERENE-CONTAINING [3]ROTAXANE CHLORIDE SENSOR & SINGLET OXYGEN GENERATOR

C₆₀ is functionalised with a bisalkyne malonate via the Bingel reaction. Recycling HPLC is used to separate the desired mono-adduct from newly discovered iodo-functionalised analogues, thus increasing the purity of the final product. This bisalkyne C₆₀ derivative is clicked with two NDI-containing components via copper-catalysed azide-alkyne cycloaddition (CuAAC) to give a four-station axle with three electron-accepting units. Anion templation is then used to introduce two Fc-containing macrocycles and thus complete the synthesis of the [3]rotaxane. Once structurally characterised, the photophysical behaviour of the rotaxane in its two co-conformations is investigated and rationalised. A striking visible fluorescent response is seen from the chloride ion-induced co-conformation. The ability of the two co-conformations of the [3]rotaxane to generate singlet oxygen (¹O₂) is investigated using an EPR spin-trapping method. Control measurements are taken with the constituent parts of the rotaxane. A difference in efficiency of ¹O₂ generation is seen between the two rotaxane co-conformations, in line with PET fluorescence quenching results. Thus, a second functionality with biomedical applicability of this fullerene-containing rotaxane is introduced and its utility as a theranostic tool is demonstrated.

4.1 INTRODUCTION

Syntheses of MIMs, specifically rotaxanes, have been rendered far easier by the advent of anion templation [347]—in particular, the 1,2,3-triazolium motif formed during the commonly used CuAAC reaction is an excellent binding site for small anionic species [348]. Thus, the incorporation of several molecular components into an axle via CuAAC can come hand-in-hand with an easy subsequent path to rotaxane formation.

Incorporating a fullerene into a rotaxane (Section 1.3) along with an electron donating moiety, such as ferrocene (Fc), offers a route to electron transfer controllable via motion of mechanically interlocked molecular components [212, 214, 215, 349, 350]. Naphthalene diimide (NDI), a versatile electron-accepting moiety [351–353] with tunable fluorescence [354–357], is often included in rotaxanes for its favourable non-covalent interactions with macrocycles [205]. Combining the two into a functional rotaxane-based sensor has, to the best of my knowledge, never been achieved before, though a non-fullerene [3]rotaxane for fluorescent sensing of other anions has been synthesised [358].

The sensing of chloride ions in a biomedical context is of particular importance as chloride transport into and out of cells plays a key role in regulating intracellular pH and extracellular fluid volume—deficiencies in this transport of chloride can lead to diseases such as cystic fibrosis [359]. Detection of excessive chloride can lead to earlier diagnosis [360]. Attempts have been made at developing nanoparticle-based fluorescent sensors to this end [361], and one other rotaxane-based chloride sensor also exists [362].

Fullerenes are also excellent photosensitisers for the generation of ROS (Section 1.2.1.5), which are highly cytotoxic. This photosensitisation property is desired for PDT, whereby a photosensitiser is delivered into a part of the body where certain cells need to be killed—this

is most commonly adopted as a therapy in cancer treatment, complimentary to traditional radiotherapy and chemotherapy [144]. Examples of fullerenes being used as PDT agents are numerous in the literature [146, 363, 364]. Rotaxanes, on the other hand, have rarely been investigated for application in PDT [365]. The introduction of C₆₀ fullerene into [3]rotaxane **6**, therefore, offers an attractive avenue for such investigation into producing a MIM with dual functionality: fluorescent chloride detection and singlet oxygen generation.

4.2 MOTIVATION

The primary aim of this chapter is the design, synthesis and photophysical characterisation of a multi-station rotaxane with a central fullerene moiety. The fullerene derivative will be incorporated into the rotaxane's axle using CuAAC. The photophysical characterisation will be the first assessment of the system's applicability for potential biomedical applications. Leveraging the expertise of the group of Prof. Paul Beer (Chemistry Research Laboratory), chloride anion templation will be used to create a mechanically interlocked architecture and the translational motion of its molecular components will be controlled depending on the anion present (discussed further in Section 4.4). In this way, the aim is to create a rotaxane-based chloride sensor.

4.2.1 Rotaxane Design

In order to achieve the desired chloride sensing capability, a [3]rotaxane is designed to exploit photoelectron transfer (PET) between acceptor and donor units, controlled by the spatial translocation of the rotaxane's macrocycles (Fig. 61). As such, ferrocene (Fc), a good electron

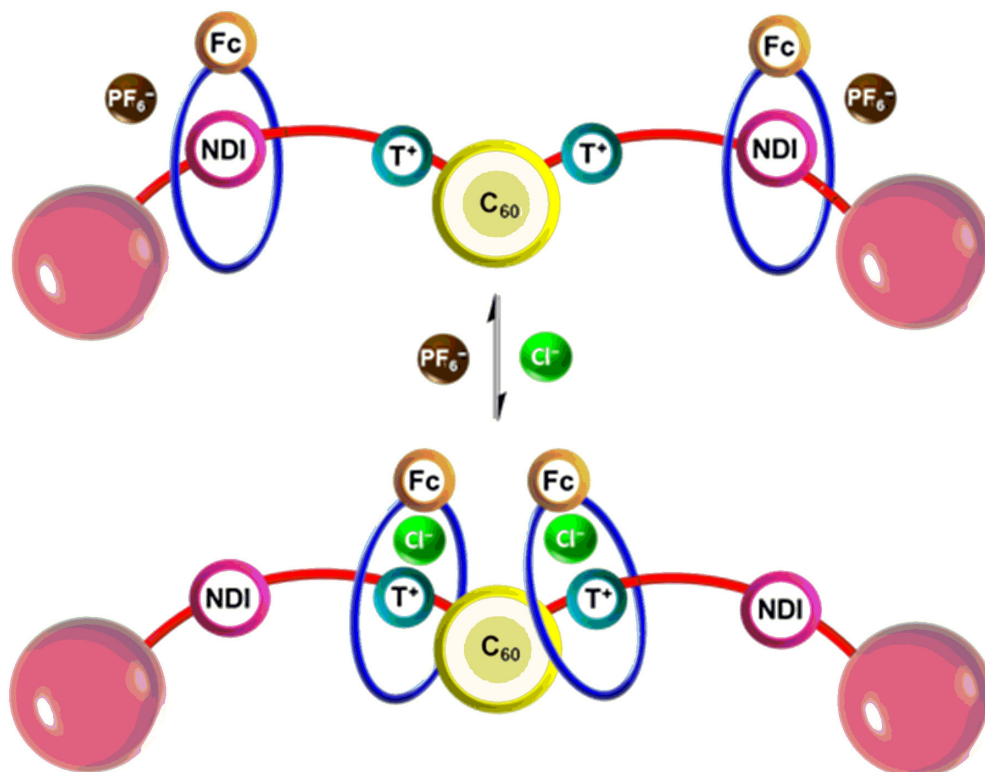


Figure 61: Schematic cartoon of the design of [3]rotaxane **6**. The shuttling of the electron-donating ferrocene (Fc)-containing macrocycles between a central electron-accepting fullerene (C_{60}) moiety and two peripheral electron-accepting naphthalene diimide (NDI) stations is controlled by the presence of coordinating (Cl^-) or non-coordinating (PF_6^-) anions. Cl^- ions have a favourable non-covalent interaction with the positively charged triazolium (T^+) station and macrocycle, their small size allowing them to sit in the associated cavity. Larger PF_6^- ions cannot fit in this cavity and so the macrocycles move to the peripheral NDI stations with which they interact via a preferable aromatic stacking interaction.

donor, is to be attached to the mobile macrocycles. The two unique acceptor moieties are to have dual functionality. The central fullerene acts as a mechanical stopper, allowing for the division of the rotaxane into two identical halves and the introduction of two non-interacting macrocycles; it is also a potent electron acceptor. The two peripheral NDIs act as stations at which the macrocycles can reside due to favourable non-covalent interactions (the other stations being triazolium linkages); they are also good electron acceptors. Further details of the operation of this device follow in Sections 4.4 and 4.5.

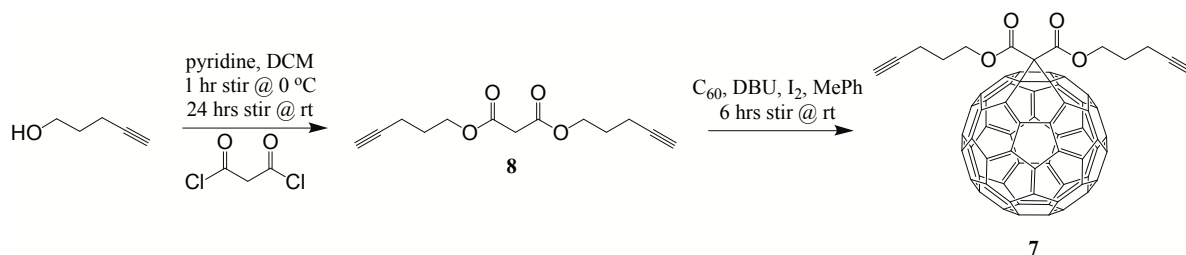


Figure 62: Synthesis scheme for bis-alkyne C₆₀ methanofullerene building block 7.

4.3 BISALKYNE FULLERENE FUNCTIONALISATION

A target C₆₀ derivative building block, 7, was first selected from the literature [366] (Fig. 62). Given that the derivative was to be incorporated as the central moiety in a [3]rotaxane's axle via CuAAC, it had to bear a pair of either terminal azide or alkyne groups. Derivatives with terminal azides, however, are unstable, having a propensity to undergo [3+2] cycloadditions with the fullerene cage to yield bridged azafullerenes [367]. Thus, bisalkyne mono-adduct 7, to be synthesised using the Bingel reaction, was selected.

4.3.1 Bisalkyne Malonate Synthesis & Characterisation

Bisalkyne malonate 8 was synthesised according to a literature procedure [366]. DCM (45 mL) and a magnetic stirrer bar were added to a stoppered round-bottomed flask, which was placed for 15 minutes in an ice bath on a stirring plate. Salt (NaCl) was added to the bath to maintain a temperature of 0 °C. 4-pentyn-1-ol (660 µL, 7.10 mmol, 2 eq) and pyridine (572 µL, 7.10 mmol, 2 eq) were added to the DCM and left to stir for 15 minutes. Malonyl dichloride (345 µL, 3.55 mmol, 1 eq) was added to this solution and the reaction stirred at 0 °C for one hour. After one hour, the ice bath was removed and the stoppered solution left to stir for 24 hours. Aqueous liquid-liquid extraction was used to begin the isolation of bisalkyne malonate

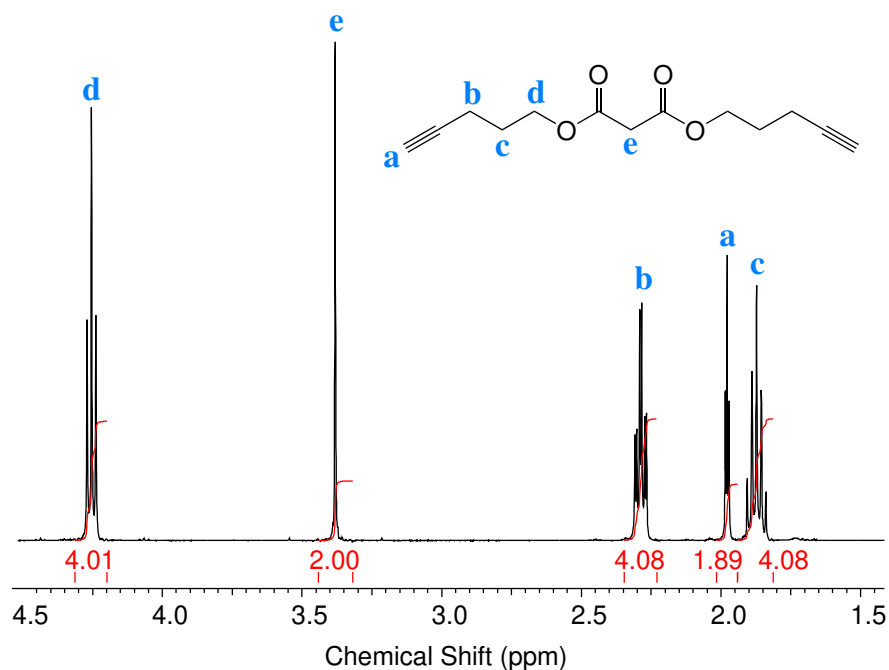


Figure 63: Bisalkyne malonate **8** ^1H NMR (400 MHz, CDCl_3) δ 4.25 (4H, t, J = 6.3 Hz, CH_2), 3.38 (2H, s), 2.29 (4H, td, J = 6.9, 2.7 Hz, CH_2), 1.98 (2H, t, J = 2.6 Hz, alkyne), 1.87 (4H, quint, J = 6.6 Hz, CH_2).

8: after addition of distilled water, the funnel was gently shaken and periodically vented until no further gas was audibly expelled; the funnel was placed upright in a suitable clamp, and the yellow DCM and colourless water layers left to separate. The denser DCM layer was isolated into a beaker, to which was added several spatulas of MgSO_4 . After 20 minutes, the contents of the beaker were passed through a funnel with GE Healthcare WhatmanTM Grade 1 filter paper, collected in a round-bottomed flask and the solution concentrated *in vacuo*. The resulting solution was purified using silica gel column chromatography (DCM), and bisalkyne malonate **8** was isolated as a faint yellow viscous oil (588 mg, 2.49 mmol, 70%). The structure of **8** was confirmed by ^1H NMR (Fig. 63).

4.3.2 Mono-adduct Synthesis

Standard Bingel reaction conditions were used (Fig. 62). C_{60} (1.17 g, 1.63 mmol, 1 eq) was dissolved in toluene (700 mL) in a stoppered round-bottomed flask under sonication for 10 minutes. A magnetic stirrer bar was added and the solution purged with N_2 for 15 minutes. Bisalkyne malonate **8** (385 mg, 1.63 mmol, 1 eq) and I_2 (622 mg, 2.45 mmol, 1.5 eq) were added and left to dissolve for two minutes before DBU (729 μ L, 4.89 mmol, 3 eq) was added dropwise. A visible change in solution colour from purple to red-brown could be seen within five minutes of adding DBU. The reaction was stirred for six hours at room temperature before being passed through a flash silica column (toluene). The resulting solution was concentrated down *in vacuo*. In a departure from the literature, this solution was then injected into reverse-phase HPLC. Using a toluene mobile phase (16 mL/min flow rate) and Buckyprep-M column, three groups of peaks were isolated and collected: α ($t_R \sim 4.5\text{--}5.7$ mins, 27%), β ($t_R \sim 5.8\text{--}7$ mins, 55%) and γ ($t_R \sim 7.4\text{--}8$ mins, 18%) (Fig. 64). Yields were calculated using the built-in integration tool in the Datalys Azur HPLC software. Fraction γ , with its fingerprint purple colour and characteristic retention time under the given conditions, was immediately assigned to unreacted C_{60} .

4.3.3 Mono-adduct Characterisation

MALDI-TOF MS of fraction β (DCTB, THF, negative ionisation) confirmed that it contained the desired bisalkyne mono-adduct **7** ($m/z = 954$), however, the HPLC trace in Fig. 64 evidently displays at least one shoulder on the main peak of fraction β . As such, once isolated, fraction β was injected back into HPLC and recycled in order to separate out any hidden

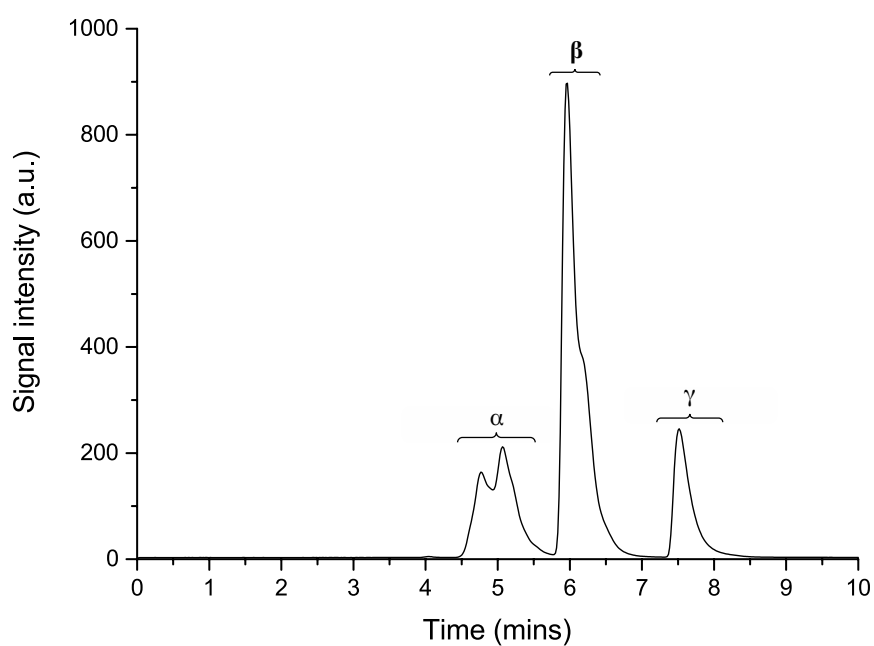


Figure 64: HPLC trace of reaction solution for Bingel mono-addition of bisalkyne malonate **8** (Buckyprep-M, toluene, 16 mL/min). Fraction γ ($t_R \sim 7.4$ –8 mins, 18%) has the characteristic purple colour and retention time of C_{60} . Fraction α ($t_R \sim 4.5$ –5.7 mins, 27%) very likely contains multi-adducts and their regio-isomers. Fraction β ($t_R \sim 5.8$ –7 mins, 55%) contains the desired bisalkyne mono-adduct **7** and newly discovered iodinated analogues.

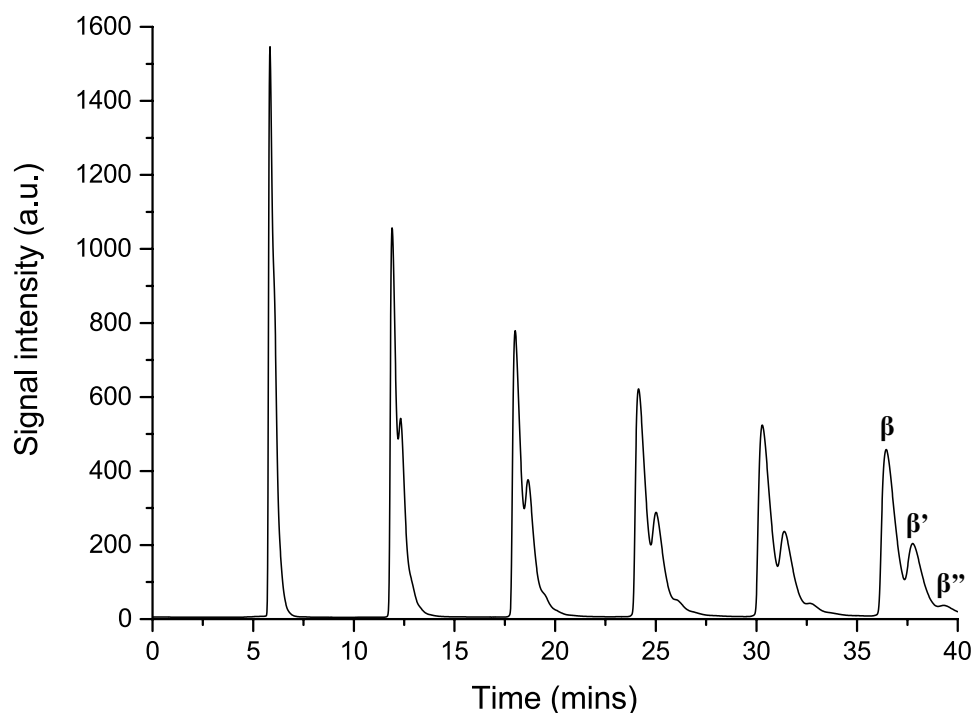


Figure 65: Recycling HPLC trace for separation of fraction β , containing bisalkyne mono-adduct **7**, into constituent peaks β , β' and β'' (Buckyprep-M, toluene, 16 mL/min).

peaks. After 40 minutes, three distinct peaks, β (68%), β' (27%) and β'' (5%), were separated enough to be individually isolated (Fig. 65).

MALDI-TOF MS (DCTB, THF, negative ionisation) (Fig. 66a), ^1H NMR (Fig. 66b) and ^{13}C NMR (Fig. 67a) confirmed peak β to be **7**. MALDI-TOF MS (DCTB, THF, negative ionisation) of peak β'' suggested an addition of two iodine atoms to this structure (Fig. 66e). Indeed, ^1H NMR confirmed that each of the protons of the terminal alkynes had been replaced by an iodine atom: the triplet of the terminal proton (δ 1.98 ppm) had disappeared in the spectrum for β'' , and the triplet of doublets (δ 2.35 ppm) had reduced to a triplet shifted to δ 2.55 ppm due to the presence of only two equivalent neighbours and no terminal proton (Fig. 66f). Extra confirmation of this bis-iodoalkyne functionalisation comes from ^{13}C NMR: most notably, the signal of the terminal alkyne carbon (δ 69.75 ppm) in **7** disappears and a

new peak appears in **10** drastically upfield (δ -4.80 ppm), which is diagnostic of terminal iodoalkyne functionalisation [368, 369] (Fig. 67b). Further, the two adjacent carbon atoms to the iodine attachment are deshielded and experience downfield shifts proportional to their proximity to the iodine [370] (δ 15.25 ppm to δ 17.60 ppm ($\Delta\delta$ = 2.35 ppm) and δ 82.45 ppm to δ 92.47 ppm ($\Delta\delta$ = 10.02 ppm), respectively). The appearance of two new peaks around 129 ppm can be assigned to fullerene peaks upfield-shifted thanks to a through-space heavy atom interaction with iodine [371, 372, 373]. MALDI-TOF MS (DCTB, THF, negative ionisation) of peak β' suggested that only one iodine atom had replaced an alkyne proton (Fig. 66c) and indeed this is confirmed by the ^1H NMR spectrum, which is a halfway house between the two extreme species (Fig. 66d). Again, ^{13}C NMR supports this with the presence of at least both peaks δ 15.25 ppm and δ 17.60 ppm (Fig. 67c). The absence of the singlet (δ 3.38) (present in the spectrum of malonate **8**) in all ^1H NMR spectra of species **7**, **9** and **10** confirmed that the malonate had successfully undergone Bingel addition to form the corresponding methanofullerene. Confirmation of a successful reaction could also be seen in the UV-vis spectrum of **7** (Fig. 68), wherein the sharp shoulder at 425 nm is evidence of C_{60} 's conjugated system being broken and characteristic proof of addition to the fullerene cage across a [6,6] bond.

4.3.3.1 Discussion

Using HPLC in the isolation of bisalkyne mono-adduct **7**, as introduced above, led to the discovery of two new iodinated methanofullerene analogues, **9** and **10**, bearing terminal iodoalkynes. Their appearance as shoulders of the main product peak in the HPLC trace indicates that they could not have previously been isolated with silica gel column chromatography and toluene as the eluent. Their synthesis here can reasonably be rationalised in two ways. First, iodoalkynes have previously been formed from terminal

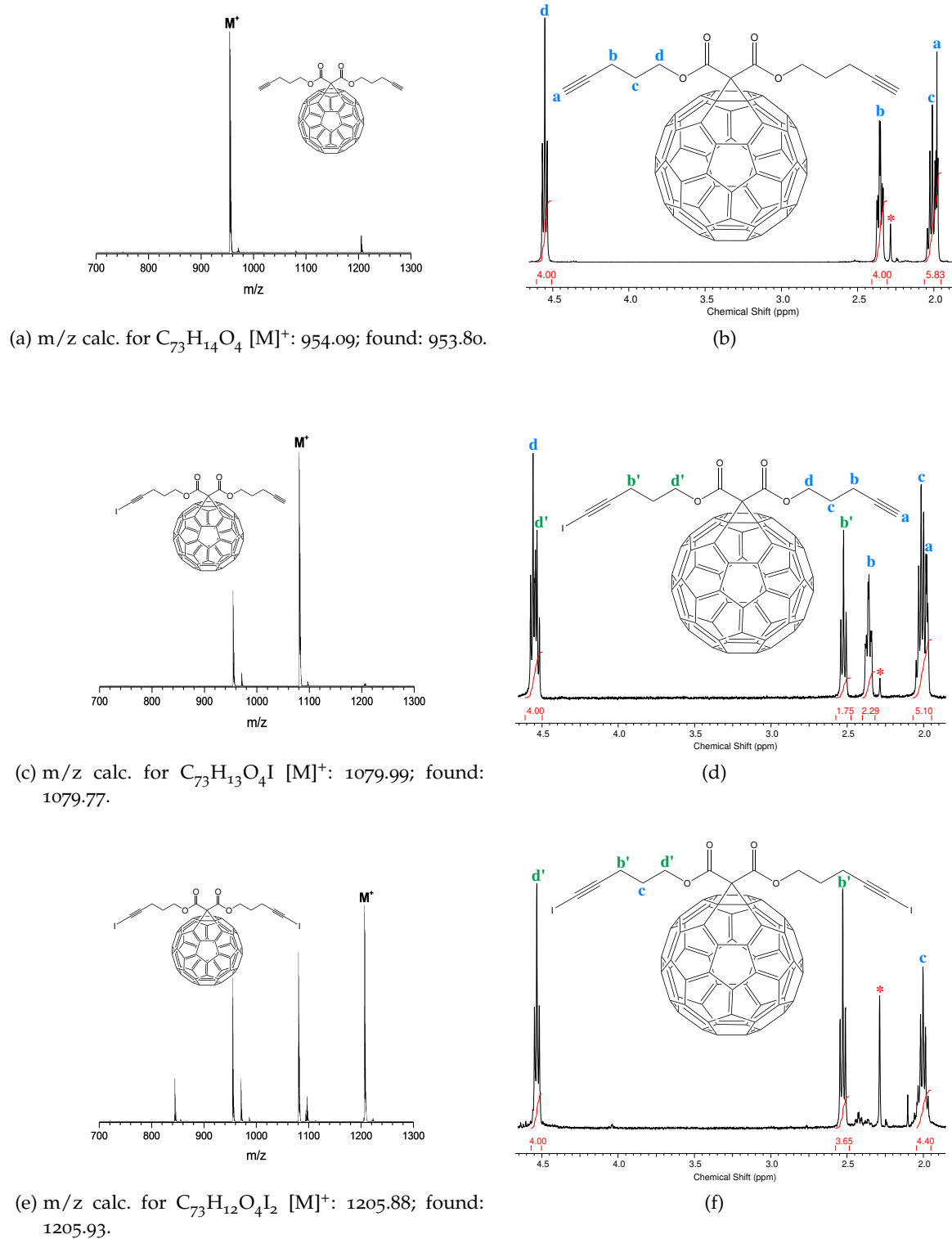


Figure 66: MALDI-TOF MS and 1H NMR (400 MHz, $CDCl_3$) spectra of bisalkyne monoadduct **7** found in peak β (66a and 66b), its mono-iodo analogue **9** found in peak β' (66c and 66d), and its bis-iodo analogue **10** found in peak β'' (66e and 66f). * residual toluene.

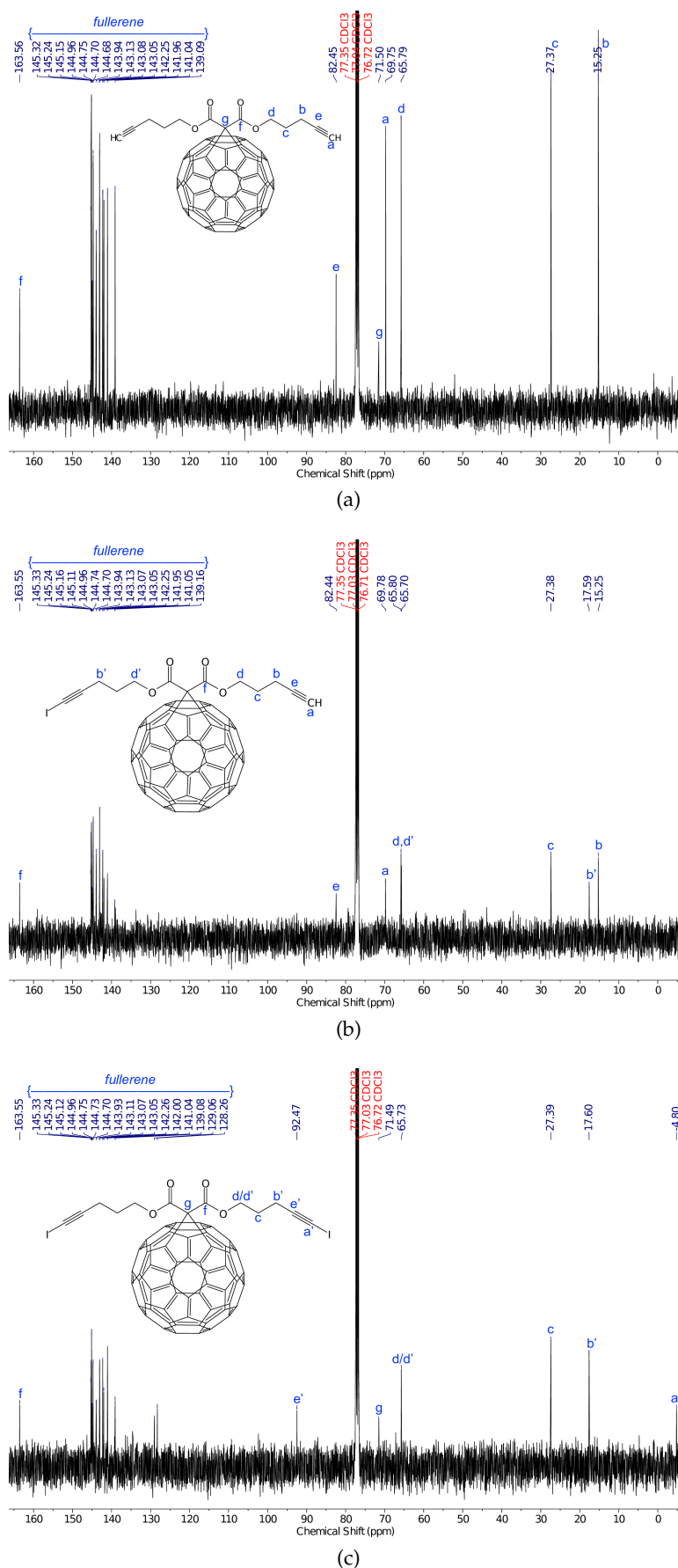


Figure 67: ^{13}C NMR spectra of (a) bisalkyne monoadduct **7**, (b) its mono-iodo analogue **9**, and (c) its bis-iodo analogue **10**. The 15 peaks in each spectra in the range 139–146 ppm correlate with the expected symmetry of a methanofullerene mono-adduct [374].

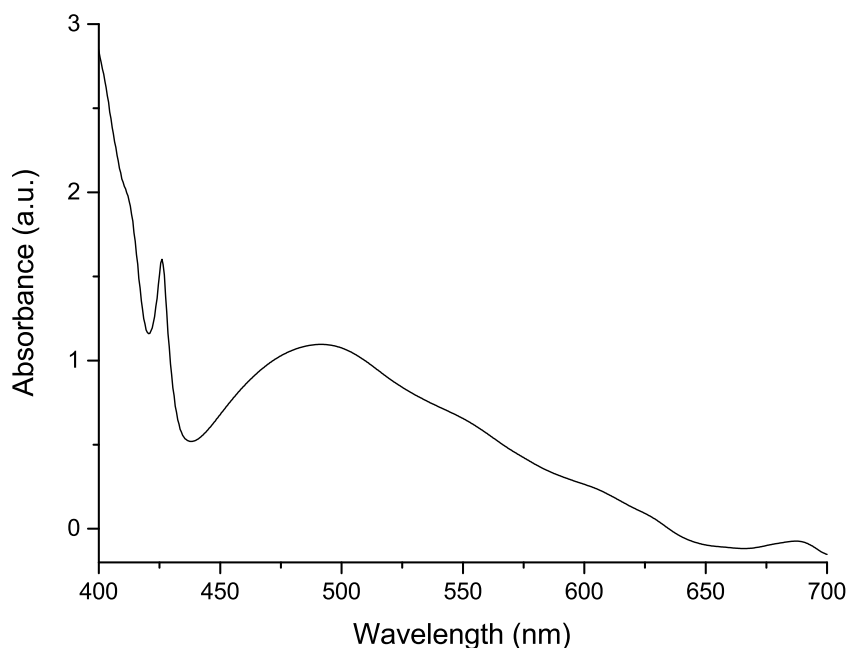


Figure 68: UV-vis spectrum of bisalkyne mono-adduct **7** (toluene, high but unknown concentration).

alkynes in the presence of electrophilic reagents [375–378]; C_{60} could well act as an excellent electrophile here. Terminal alkynes have also been turned into iodoalkynes in the presence of molecular iodine and base [379]; both I_2 and DBU are present here in slight excess.

The presence of these iodinated analogues is not a mere curiosity. These derivatives themselves have great potential in materials chemistry,¹ and particularly with respect to anion-templated rotaxane synthesis. CuAAC with iodoalkynes has been shown to lead preferentially to the formation of 5-iodo-1,2,3-triazole linkages, *cf.* terminal alkynes leading to standard 1,2,3-triazoles (Fig. 69) [380]. The power of this 5-iodo-1,2,3-triazole linkage has been demonstrated in an exemplary [2]rotaxane system (Fig. 70), whereby anion binding was significantly improved (even in competitive aqueous solution) *cf.* a standard hydrogen-

¹ Haloalkynes are versatile building blocks in organic synthesis [235], and so these iodoalkyne-functionalised derivatives offer the possibility of easily incorporating fullerenes into more complex structures. From a more physical chemistry point of view, early experiments are under way with Prof. Henry Snaith's group (Clarendon Laboratory) to see if these iodinated fullerenes can improve the performance of perovskite-based solar cells (discussed further in Outlook chapter 5).

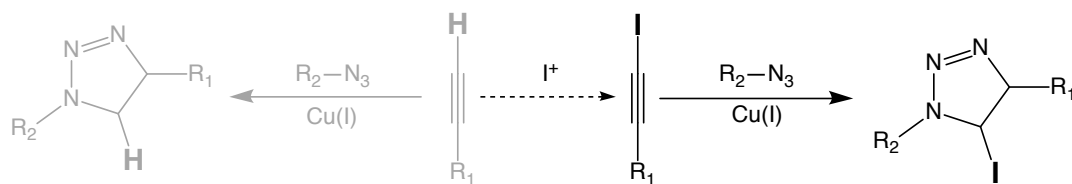


Figure 69: CuAAC with terminal alkynes leads to the formation of 1,2,3-triazoles; with iodoalkynes, 5-iodo-1,2,3-triazoles are formed instead.

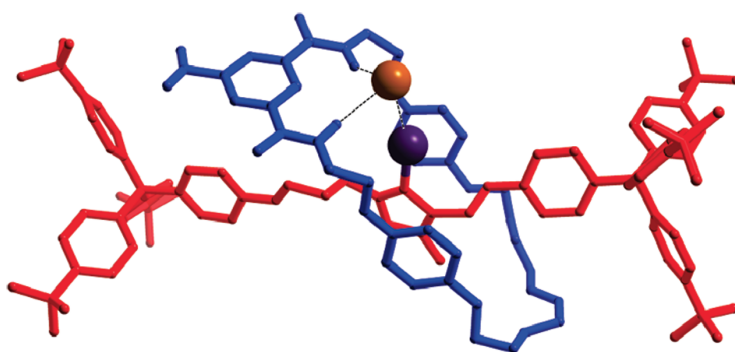


Figure 70: X-ray crystal structure of an exemplary [2]rotaxane formed via anion templation, aided by a 5-iodo-1,2,3-triazole linkage. The iodine donor (purple) forms a strong halogen-bond with the bromide acceptor (brown). Reproduced from [381] with permission of American Chemical Society.

bonding analogue, thanks to strong halogen-bonding between the iodine donor and halo-anion acceptor [381].

4.4 ROTAXANE SYNTHESIS AND STRUCTURAL CHARACTERISATION

Having synthesised functionalised fullerene **7**, the next step was to incorporate it as the central moiety in a four-station NDI-containing axle via CuAAC, before anion-templated mechanical bond formation to give the final [3]rotaxane. Most of the synthesis and all of the NMR shuttling characterisation was carried out by Dr. Timothy Barendt in the research group of Prof. Paul Beer (Chemistry Research Laboratory); a brief overview is included here to provide the reader with a logical progression through to the subsequent characterisation. Fuller details in Experimental Section 4.7.

The scheme for the following set of reactions is shown in Fig. 71. CuAAC between bisalkyne methanofullerene **7** (20 mg, 0.02 mmol, 1 eq) and azide-functionalised NDI-containing stopper component **11** (43 mg, 0.04 mmol, 2 eq) (i) yielded bis-triazole axle component **12** as a brown solid (46 mg, 0.02 mmol, 73%) following silica gel column chromatography (CH_2Cl_2 :MeOH 99:1). **12** was converted via an alkylation reaction (ii) to the positively charged bis-triazolium axle salt **13**·(BF_4)₂ (45 mg, 0.01 mmol, 92%). **13**·(BF_4)₂ then underwent anion exchange (iii) through a column of chloride-loaded Amberlite® resin to yield bis-chloride axle salt **13**·Cl₂ (44 mg, 0.01 mmol, quant). Finally, Fc-containing macrocycle **14** (49 mg, 62 μmol , 3 eq) was added to **13**·Cl₂ (67 mg, 21 μmol , 1 eq) in the presence of Grubbs' II catalyst (iv) to yield [3]rotaxane **6**·Cl₂ as a brown solid (11 mg, 2.3 μmol , 11%) after preparative TLC (EtOAc:MeOH 95:5 then CH_2Cl_2 :MeOH 95:5).

4.4.1 Discussion

The production of positively charged bis-triazolium analogue **13** via alkylation step (ii) was necessary in order for the introduction of anions to the system—there is a favourable hydrogen-bonding interaction between the C₅ proton of the 1,2,3-triazolium ring and the anion [382]. This binding ability was then used in order to template the formation of a mechanically interlocked molecular structure—in this case [3]rotaxane **6**—as described in introduction section 4.1. Anion exchange from **13**·(BF_4)₂ to **13**·Cl₂ (iii) was necessary for rotaxane formation as the smaller chloride ions fit nicely into the cavities formed by the axle **13** and macrocycles **14**. Indeed, stronger anion binding (*cf.* the axle itself) is present due to the cooperative effects of hydrogen-bonding between the chloride ions and both the triazolium stations of the axle and the isophthalamide groups of the macrocycles. This crucial distinction

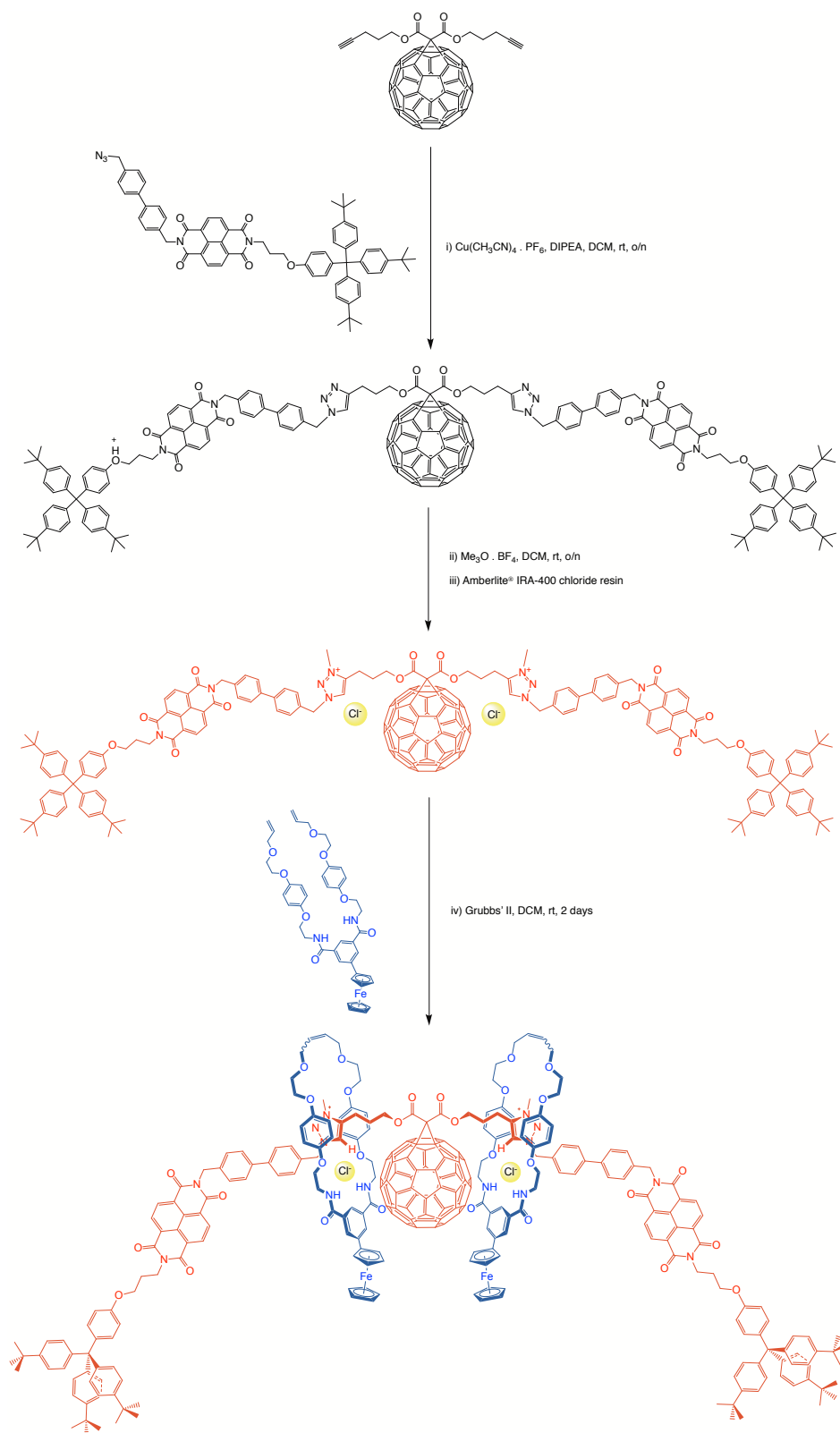


Figure 71: Synthesis scheme for [3]rotaxane **6**·Cl₂. (i) CuAAC between alkyne-functionalised methanofullerene **7** and azide-functionalised NDI precursor **11** gives axle component **12** (73%). (ii) Alkylation then turns this into the positively charged triazolium salt **13**·(BF₄)₂ (92%) which undergoes (iii) anion exchange to give chloride salt **13**·Cl₂. (iv) Macrocycle **14** is introduced via anion templation and the structure mechanically interlocked by ring-closing metathesis to give [3]rotaxane **6**·Cl₂ (11%).

between the two salts is explored further in sections 4.4.2 and 4.5 below. Final step (iv) was a ring-closing metathesis of the macrocycles to complete the formation of the mechanical bonds, and thus [3]rotaxane $6\cdot\text{Cl}_2$.

4.4.2 Rotaxane Shuttling

With the rotaxane synthesised, attention could turn to characterising its behaviour as a linear molecular shuttle. A portion of the bis-chloride salt $6\cdot\text{Cl}_2$ was converted to its bis-hexafluorophosphate salt $6\cdot(\text{PF}_6)_2$ using Amberlite® anion-exchange resin. As anticipated, the change in anion led to a co-conformational change whereby the rotaxane's macrocycles resided over a different station, due to the difference in anion size. In $6\cdot\text{Cl}_2$, the smaller chloride ion, as mentioned above, coordinates well with the axle's triazolium and macrocycles' isophthalamide groups and so, in this co-conformation, the macrocycles reside over the triazolium station, close to the central fullerene moiety; in $6\cdot(\text{PF}_6)_2$, the larger hexafluorophosphate ion cannot coordinate in the pocket formed by the triazolium and isophthalamide groups and so the macrocycles are driven to translocate so as to reside over the NDI stations, away from the fullerene, thanks to favourable aromatic stacking interactions between the macrocycles' hydroquinone groups and the axle's NDI groups. This evidence of linear shuttling between stations was confirmed by ^1H NMR (Fig. 72). Specifically, anion exchange to the $6\cdot(\text{PF}_6)_2$ salt produces large upfield shifts of macrocycle hydroquinone $\text{H}_{\gamma,\gamma'}$ ($\Delta\delta = 0.18$ ppm) and axle NDI $\text{H}_{e,e'}$ resonances ($\Delta\delta = 0.18$ ppm) due to the formation of strong aromatic stacking interactions between the components, while perturbation of the nearby methylene peak (H_f) provides further support for this co-conformational change. The removal of chloride also produces diagnostic upfield shifts of

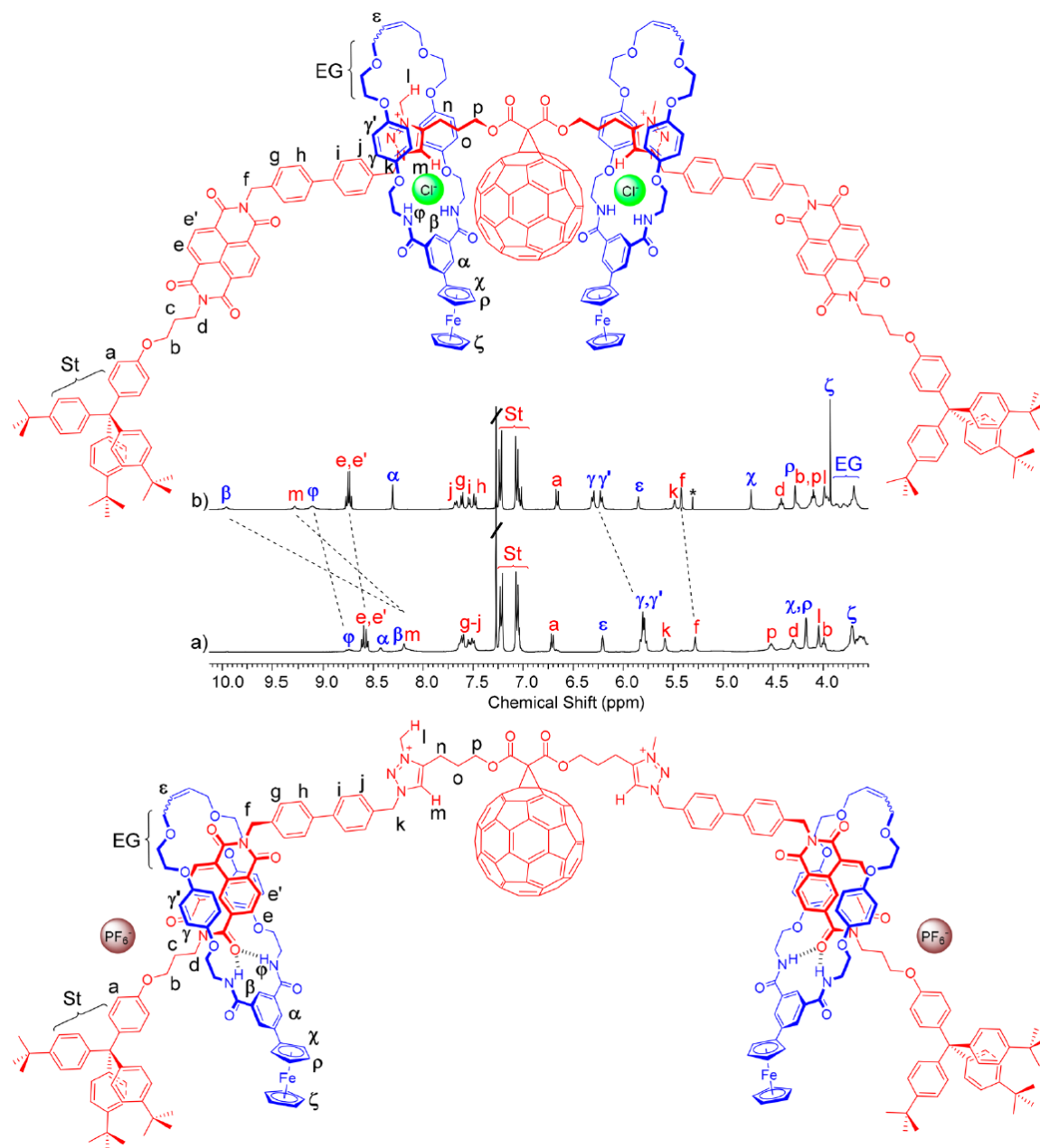


Figure 72: Comparison of ^1H NMR spectra of [3]rotaxanes (a) $6 \cdot (\text{PF}_6)_2$ and (b) $6 \cdot \text{Cl}_2$ reveals a co-conformational change via translational motion of ferrocenyl macrocycle components (CDCl_3 , 298 K, 400 MHz). Additional proton signals: St = axle stopper, EG = macrocycle ethylene glycol, and * = trace residual solvent.

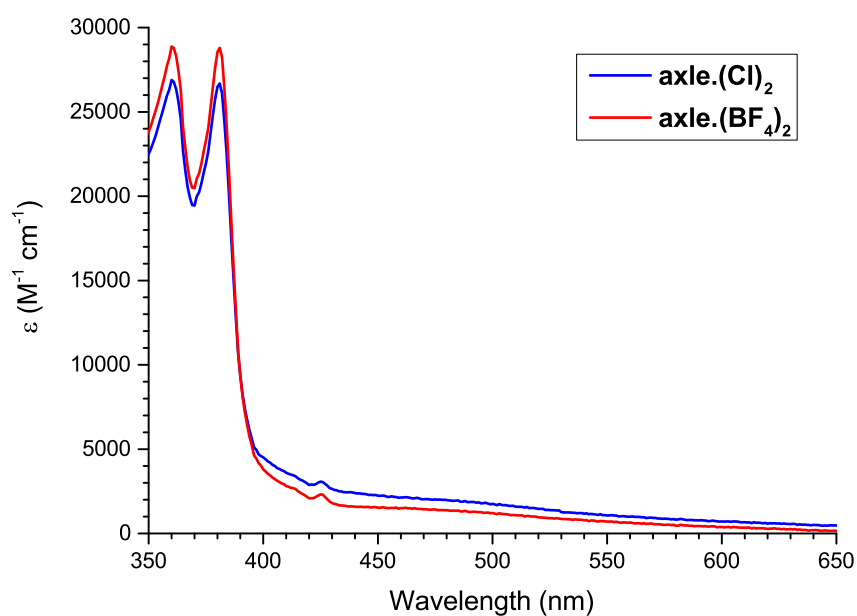
acidic protons H_m and $H_{\beta,\varphi}$ at the anion binding station. Following a previously developed method [205], 1H NMR was also used for quantitative assessment of the station occupancies in the two co-conformations: 95% of macrocycles resided over the triazolium stations in $6 \cdot Cl_2$, with 85% over the NDI stations in $6 \cdot (PF_6)_2$ [383].

4.5 [3]ROTAXANE CHLORIDE SENSOR WITH VISUAL OUTPUT

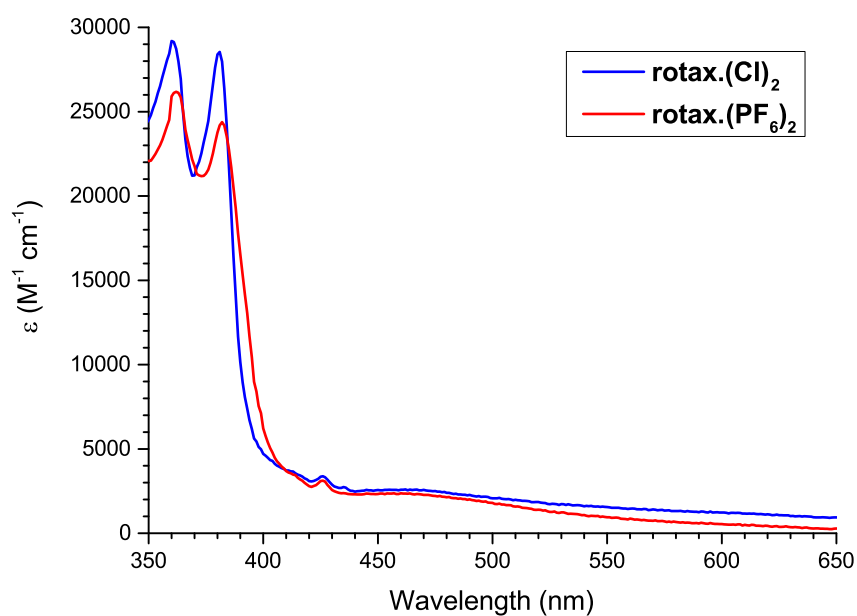
Confirmation of [3]rotaxane **6**'s anion-dependent translocation of its macrocycles between two unique axle stations meant that the system's performance as a molecular machine could be more thoroughly investigated; a given input had been shown to induce mechanical motion, but does that motion lead to the anticipated functional output? Namely, does the movement of electron-donating ferrocenyl groups between two unique electron-accepting moieties (C_{60} and NDI) lead to a change in **6**'s photophysical properties due to PET effects?

4.5.1 Steady-State Absorption of [3]Rotaxane Chloride Sensor

The absorption spectra of axle salts $13 \cdot Cl_2$ and $13 \cdot (BF_4)_2$ were measured in the UV-visible region in 10 μM chloroform solutions (Fig. 73a). The functionalised fullerene fingerprint is visible in both at 425 nm, as in the spectrum of mono-adduct **7** itself (Fig. 68). The strong and structured absorption below 400 nm is characteristic of *N,N*-dialkyl-substituted NDI derivatives [354]. Comparing each axle salt, there is no difference in either the shapes or ratios of these NDI peaks nor the fullerene peak, showing that the anions used do not affect the absorption behaviour of the axle component. In the case of the [3]rotaxane salts $6 \cdot Cl_2$ and $6 \cdot (PF_6)_2$ (Fig. 73b), the overall spectral profiles are very similar in form to the axle, with a



(a)



(b)

Figure 73: UV-vis absorption spectra of (a) axle salts $13 \cdot \text{Cl}_2$ and $13 \cdot (\text{BF}_4)_2$ and (b) [3]rotaxane salts $6 \cdot \text{Cl}_2$ and $6 \cdot (\text{PF}_6)_2$ (both CHCl_3 , 10 μM , 1 cm path length).

slight change in peak ratio of the vibronic fine structure of the NDI band suggesting only a small degree of electronic communication between the mechanically interlocked components. There is a subtle difference between the spectra of the two rotaxane salts: while the fullerene shoulder remains unaffected by the type of anion, on exchanging to the non-coordinating hexafluorophosphate anion² the NDI band is slightly broadened, perhaps indicative of intramolecular charge transfer involving the NDI unit [202] caused by aromatic stacking interactions with the macrocycles' hydroquinone groups (see Section 4.4.2 above).

4.5.2 Photoluminescence of [3]Rotaxane Chloride Sensor

Emission spectra were all recorded with 10 nm slit width, medium sensitivity and 1000 nm/min scan rate. Spectra of 50 μM chloroform solutions of rotaxane salts $\mathbf{6}\cdot\text{Cl}_2$ and $\mathbf{6}\cdot(\text{PF}_6)_2$ were recorded at an excitation wavelength of 300 nm (Fig. 74a). The marked difference in fluorescence intensity of the structured emission band from ~400–550 nm sparked a curiosity to observe the rotaxane solutions irradiated outside of the spectrometer; indeed, upon excitation with a 405 nm laser pen, $\mathbf{6}\cdot(\text{PF}_6)_2$ gave no visible response while $\mathbf{6}\cdot\text{Cl}_2$ fluoresced a brilliant blue, easily visible in a darkened room (Fig. 74b). Control emission spectra were also measured for the axle components $\mathbf{13}\cdot\text{Cl}_2$ and $\mathbf{13}\cdot(\text{BF}_4)_2$ (Fig. 75).

The difference in fluorescence intensity of [3]rotaxane **6** between its two co-conformations was quantified by calculating the fluorescence quantum yields, Φ , of the observed emission band in each species. The relative method introduced in Section 2.5 was once again employed, using coumarin 153 dissolved in EtOH as the standard sample ($\Phi = 0.58$, $\eta = 1.36$). Absorption spectra of each sample were recorded for at least four optically dilute solutions in CHCl_3 ($\eta =$

² It should be noted that the use of different non-coordinating anions in the axle and rotaxane systems (namely tetrafluoroborate in axle $\mathbf{13}\cdot(\text{BF}_4)_2$ and hexafluorophosphate in rotaxane $\mathbf{6}\cdot(\text{PF}_6)_2$) is not significant, as they both play the same functional role based on their size.

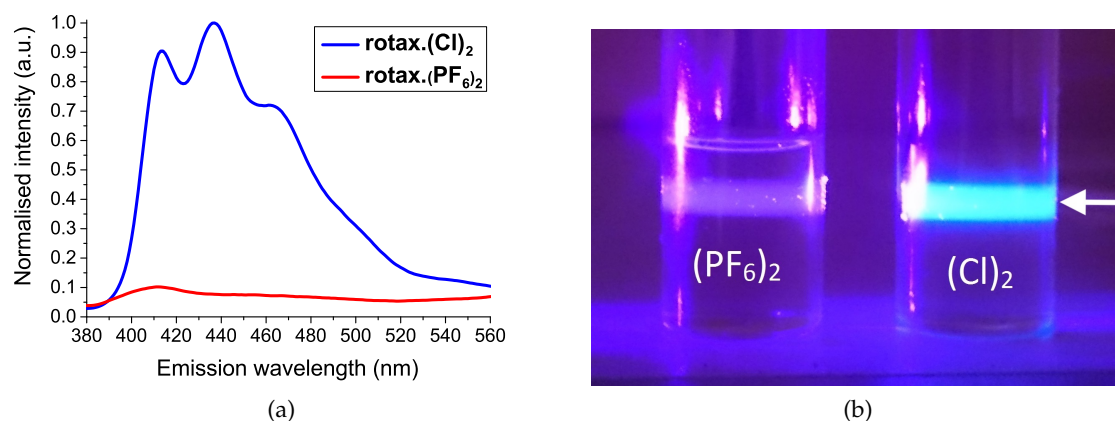


Figure 74: a) Emission spectra of [3]rotaxane salts $6 \cdot \text{Cl}_2$ and $6 \cdot (\text{PF}_6)_2$ ($50 \mu\text{M}$, CHCl_3 , $\lambda_{\text{ex}} = 300 \text{ nm}$), normalised to the maximum emission at 437 nm ; b) under 405 nm excitation from a laser pen (arrow indicates incidence), a striking blue fluorescent response is visible with the naked eye from $6 \cdot \text{Cl}_2$.

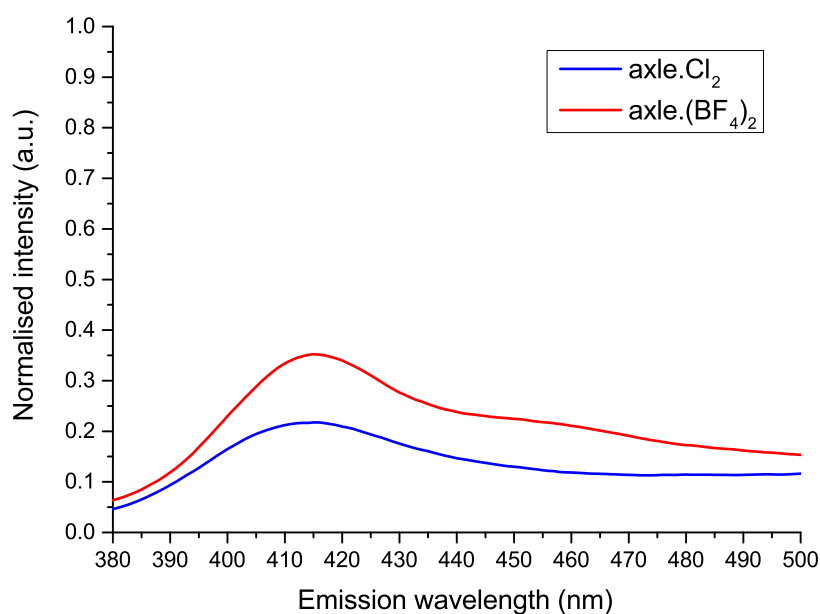


Figure 75: Emission spectra ($50 \mu\text{M}$, CHCl_3 , $\lambda_{\text{ex}} = 300 \text{ nm}$) of axle components $13 \cdot \text{Cl}_2$ and $13 \cdot (\text{BF}_4)_2$ (normalised to intensity at $\lambda_{\text{em}} = 415 \text{ nm}$ for $6 \cdot \text{Cl}_2$).

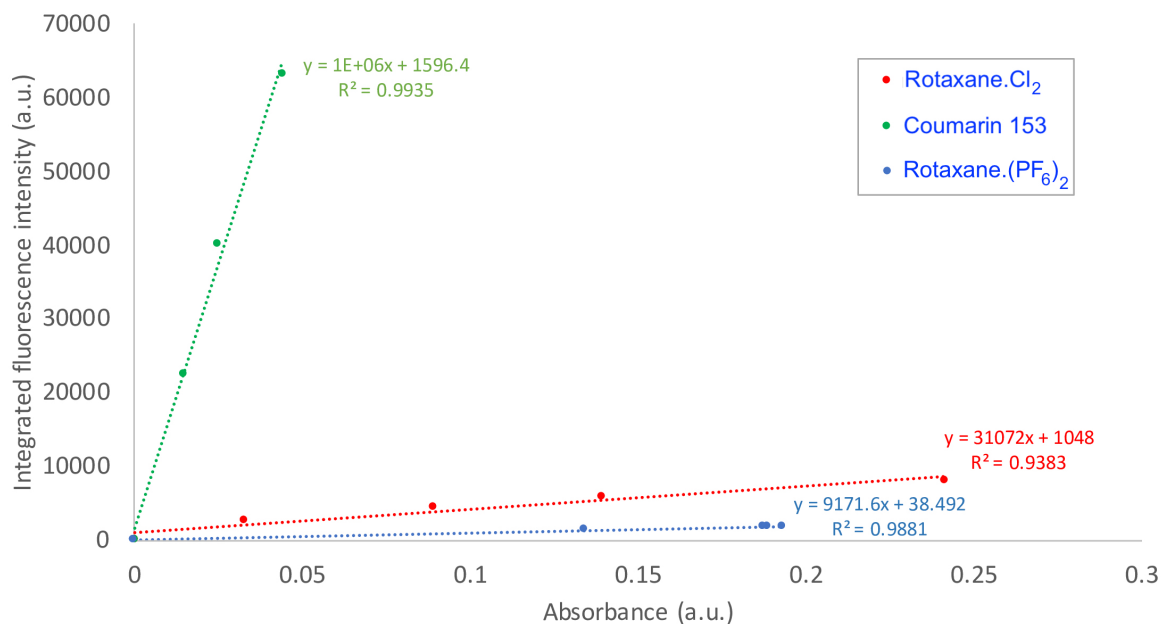


Figure 76: IFI vs. absorbance plots for calculating fluorescence quantum yield, Φ , at $\lambda_{\text{ex}} = 400$ nm for rotaxanes $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ (CHCl_3) relative to a coumarin 153 standard (EtOH).

1.44), including a solvent blank, and the absorbance at 400 nm recorded. Emission spectra at 400 nm excitation were then recorded for the same solutions, and the integrated fluorescence intensity (IFI) calculated by integrating between 420–580 nm (for $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$) or 450–700 nm (for coumarin 153). Plotting IFI against absorbance for each sample (Fig. 76) and using equation 9, $\Phi_{6\cdot\text{Cl}_2}$ and $\Phi_{6\cdot(\text{PF}_6)_2}$ were calculated to be 2.0% and 0.6% respectively.

4.5.3 Electrochemical Characterisation of [3]Rotaxane Chloride Sensor

Cyclic and square wave voltammograms were recorded by Dr. Maria Lebedeva in dimethylformamide containing 0.2 M LiCl or $[\text{nBu}_4\text{N}][\text{BF}_4]$ as supporting electrolytes (for compounds containing coordinating or non-coordinating anion salts, respectively). Measurements were made on [3]rotaxane salts $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$, as well as control measurements on axles $13\cdot\text{Cl}_2$ and $13\cdot(\text{BF}_4)_2$ and appropriate building blocks: C_{60} bisalkyne

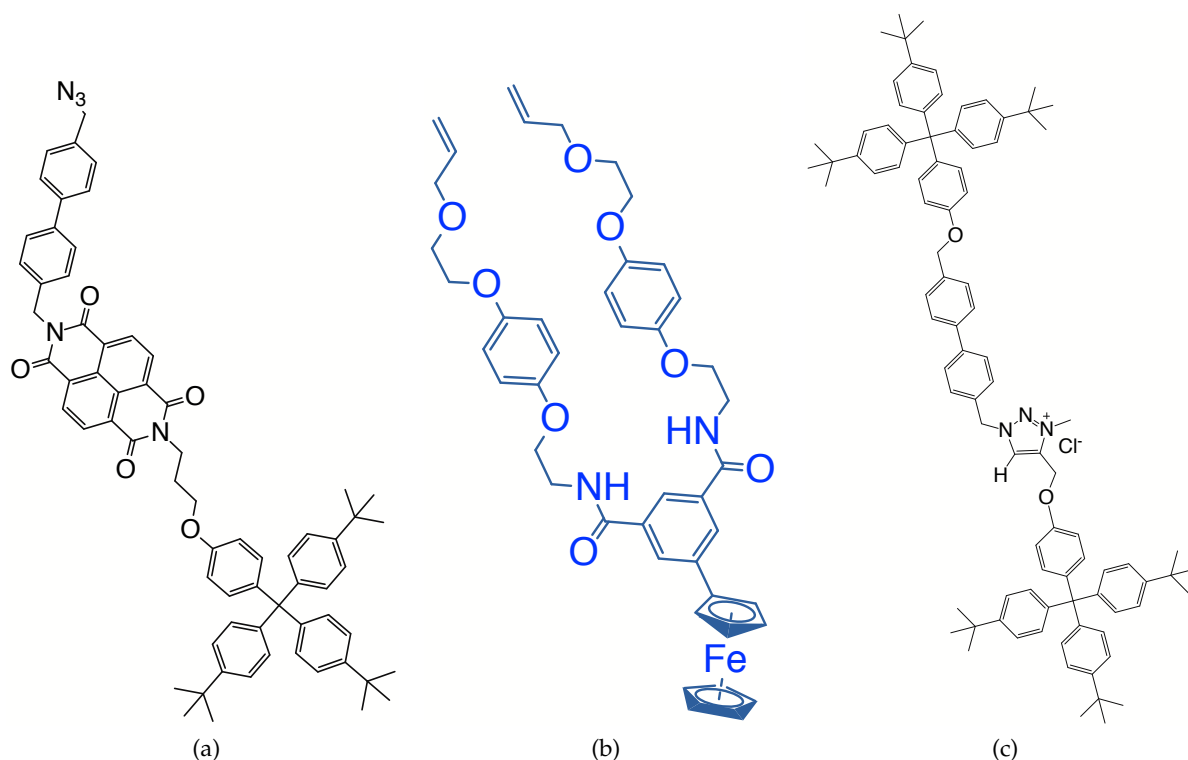


Figure 77: Structures of rotaxane building blocks: (a) NDI precursor **11** (b) Fc-macrocycle precursor **14** (c) triazolium analogue **15**.

mono-adduct **7**, NDI precursor **11** (Fig. 77a), Fc-macrocycle precursor **14** [384] (Fig. 77b), and triazolium analogue **15** [205] (Fig. 77c). Control measurements on the building blocks were taken in the presence of both coordinating (Fig. 78) and non-coordinating (Fig. 79) anion salts.

Both rotaxanes $6 \cdot \text{Cl}_2$ and $6 \cdot (\text{PF}_6)_2$ exhibited a similar voltammographic response (direct comparison shown in Fig. 80). A number of closely spaced reduction processes were observed in each case, corresponding to sequential addition of an electron on either a C_{60} or NDI moiety. Square wave voltammetry was used to determine the redox potential values for closely overlapping peaks. This permits assignment of processes at -0.81 V (-0.82 V), -1.25 V (-1.27 V) and -1.82 V as fullerene $\text{C}_{60}^- / \text{C}_{60}$, $(\text{C}_{60})_2^- / \text{C}_{60}^-$, and $(\text{C}_{60})_3^- / (\text{C}_{60})_2^-$ redox couples for non-coordinating (and coordinating) anion salts respectively [385]. Similarly, peaks at -0.96 V and -1.41 V (-1.32 V) are assigned as $\text{NDI}^- / \text{NDI}$ and $\text{NDI}^{2-} / \text{NDI}^-$ redox couples

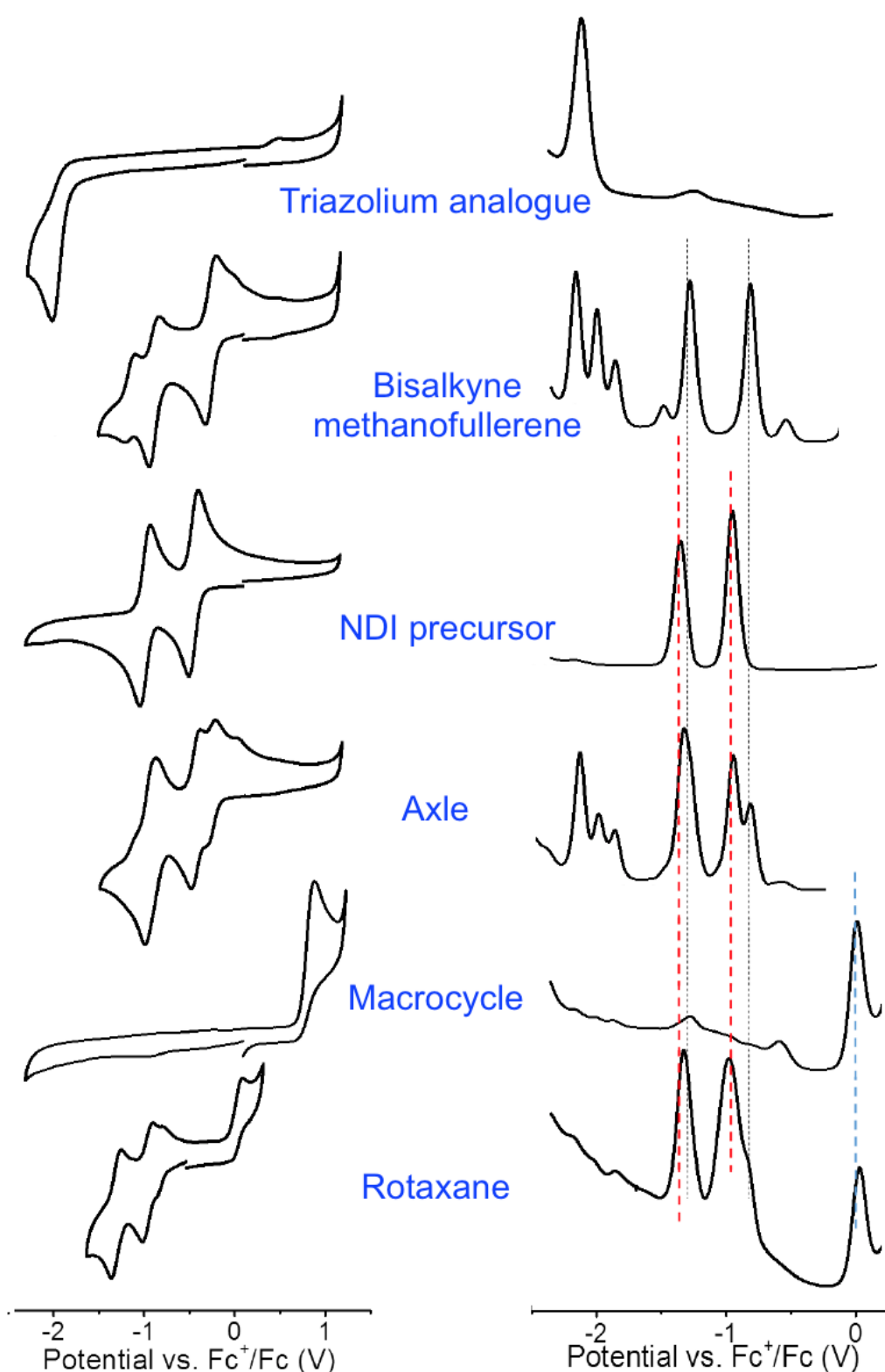


Figure 78: Cyclic voltammograms (left) and square wave voltammograms (right) of the chloride salt of triazolium analogue **15**·Cl, C₆₀ bisalkyne mono-adduct **7**, NDI precursor **11**, axle **13**·Cl₂, macrocycle **14** and rotaxane **6**·Cl₂ recorded in DMF containing 0.2 M LiCl as supporting electrolyte at 0.1 V/s. Blue dashed lines indicate the relative position of the ferrocene oxidation; red dashed lines indicate the relative position of the NDI reduction processes; and black dashed lines indicate the position of the fullerene-based reduction processes in each sample.

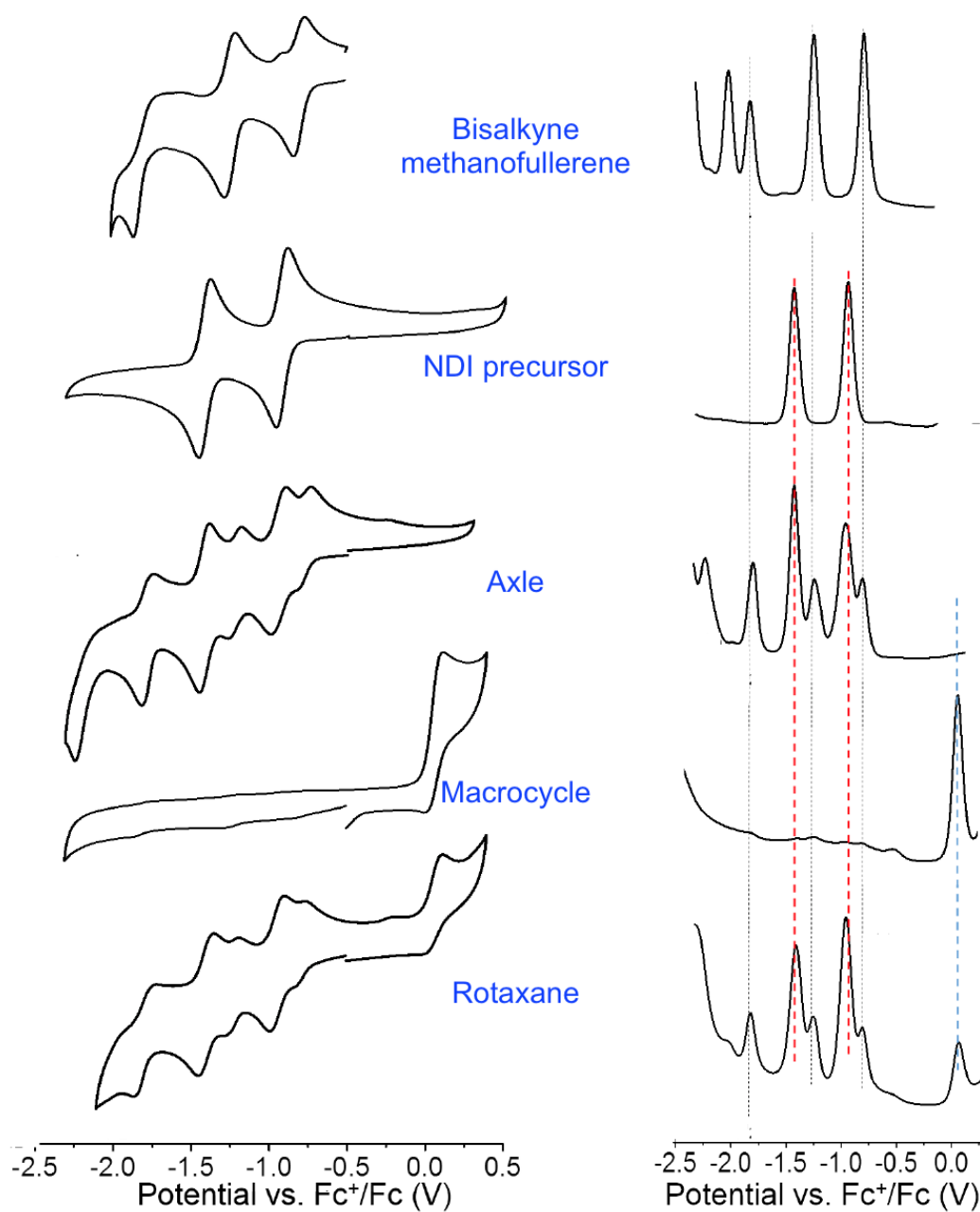


Figure 79: Cyclic voltammograms (left) and square wave voltammograms (right) of C_{60} bisalkyne mono-adduct **7**, NDI precursor **11**, axle **13**·(BF_4)₂, macrocycle **14** and rotaxane **6**·(BF_4)₂ recorded in DMF containing 0.2 M [nBu_4N][BF_4] as supporting electrolyte at 0.1 V/s. Blue dashed lines indicate the relative position of the ferrocene oxidation; red dashed lines indicate the relative position of the NDI reduction processes; and black dashed lines indicate the position of the fullerene-based reduction processes in each sample.

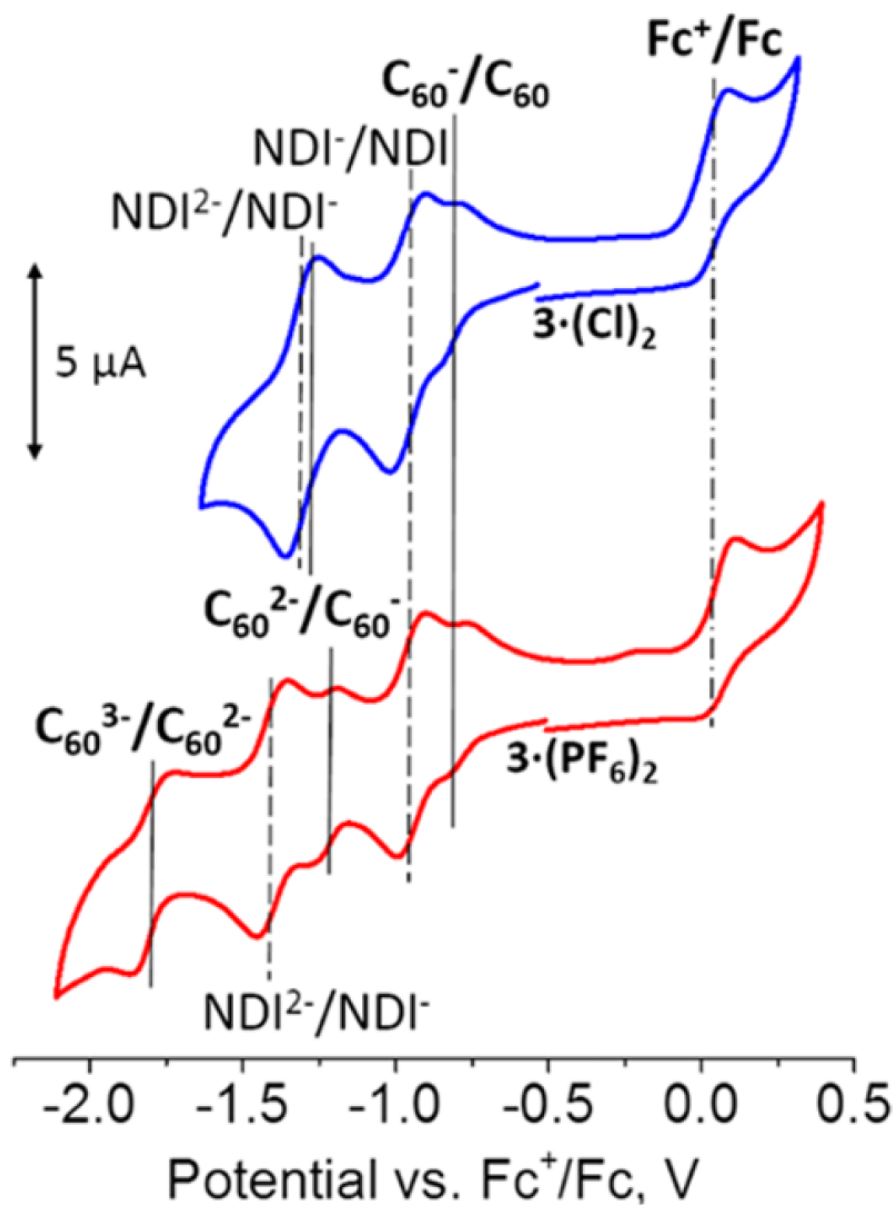


Figure 8o: Cyclic voltammograms of $6 \cdot \text{Cl}_2$ (top) and $6 \cdot (\text{PF}_6)_2$ (bottom) recorded in DMF containing either 0.2 M LiCl or $[\text{nBu}_4\text{N}][\text{BF}_4]$, respectively, as supporting electrolyte at 0.1 V/s. Solid lines indicate the $E_{1/2}$ of the fullerene-based redox processes, dashed lines indicate the $E_{1/2}$ position of the NDI-centered redox processes, and the dash-dotted line shows the $E_{1/2}$ of ferrocene oxidation in both samples.

[386], while the single oxidation process observed at +0.07 V (+0.03 V) is associated with the Fc^+/Fc redox couple of the ferrocenyl macrocycle components. Importantly, the oxidation of NDI, biphenyl, or hydroquinone units was not observed, and the triazolium unit reduction was only observed at -2.09 V, which is a significantly more cathodic potential value than the third reduction potential of the fullerene cage. These redox couples are listed in Table 7.

The potential of each redox couple in the rotaxanes was largely unperturbed when compared to the individual methanofullerene, NDI, and ferrocene building blocks such that the voltammographic responses of both $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ are effectively a superposition of the redox processes of the individual donor/acceptor components. Comparison of each redox couple in rotaxanes $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ with the corresponding axle or macrocycle component redox couple values also reveal only small cathodic or anodic shifts for the reduction or oxidation processes, respectively. This, therefore, confirms the presence of only a weak ground-state electronic interaction between interlocked components within [3]rotaxane **6**, consistent with observations from steady-state absorption spectroscopy. A 40 mV cathodic shift of the Fc^+/Fc redox couple when comparing $6\cdot(\text{PF}_6)_2$ to $6\cdot\text{Cl}_2$ is seen due to chloride binding in the conjugated ferrocenyl–isophthalamide-containing cavity of the macrocycle [384]. However, because anion recognition does not occur directly at any of the acceptor C_{60} and NDI motifs, anion exchange of rotaxane **6** produces only a minor difference in the redox potential position of the first and second reduction processes, confirming that anion exchange induces only a small effect on the ground-state electronic structure of the system.

Salt	Sample	$E_{1/2, ox_1}$ (Fc), V	$E_{1/2, red_1}$ (C ₆₀), V	$E_{1/2, red_1}$ (NDI), V	$E_{1/2, red_2}$ (C ₆₀), V	$E_{1/2, red_2}$ (NDI), V	$E_{1/2, red_3}$ (C ₆₀), V	$E_{1/2, red_1}$ (triazolium), V
Cl	NDI precursor 11	-	-	-0.94	-	-1.35	-	-
	Methanofullerene 7	-	-0.80	-	-1.27	-	-	-
	Triazolium 15	-	-	-	-	-	-	-2.09
	Axle 13 ·Cl ₂	-	-0.80	-0.94	-1.26	-1.32	-	-
	Fc-macrocycle precursor 14	+0.02	-	-	-	-	-	-
	Rotaxane 6 ·Cl ₂	+0.03	-0.82	-0.96	-1.27	-1.32	-	-
PF ₆	NDI precursor 11	-	-	-0.93	-	-1.43	-	-
	Methanofullerene 7	-	-0.80	-	-1.26	-	-1.84	-
	Axle 13 ·(BF ₄) ₂	-	-0.80	-0.95	-1.23	-1.42	-1.79	-
	Fc-macrocycle precursor 14	+0.06	-	-	-	-	-	-
	Rotaxane 6 ·(PF ₆) ₂	+0.07	-0.81	-0.96	-1.25	-1.41	-1.82	-

Table 7: Electrochemical data of rotaxanes **6**·Cl₂ and **6**·(PF₆)₂, axles **13**·Cl₂ and **13**·(BF₄)₂ and corresponding building blocks **7**, **11**, **14** and **15**. All potentials are reported against the Fc⁺/Fc couple for 0.5–1.0 mM solutions in DMF containing 0.2 M of the corresponding supporting electrolyte (LiCl or [tⁿBu₄N][BF₄]).

4.5.4 Discussion of Photoluminescent Behaviour

The strong emission band observed for [3]rotaxane $6\cdot\text{Cl}_2$ is of a shape and over a wavelength range characteristic of non-core-functionalised NDI derivatives [354]. These non-core-functionalised NDIs typically exhibit very low fluorescence quantum yields, however, increased fluorescence emission has previously been explained by solvent-fluorophore interactions specific to the excited state [387]. This is also supported by the ostensibly similar absorption spectra of the two rotaxane salts (Fig. 73b). Indeed, strong and structured emission in the same range as $6\cdot\text{Cl}_2$ has previously been displayed by a simple NDI derivative in chlorinated solvent [388].

Of paramount importance here, however, is the stark difference in the same emission for $6\cdot(\text{PF}_6)_2$, which has been significantly quenched. The relative difference between the two [3]rotaxane co-confirmations is a greater than three-fold increase in the fluorescence quantum yield, when moving from the non-coordinating to coordinating anion³, which gives a response visible to the naked-eye (something not seen in any other control solution, including of the NDI component itself). All control spectra show broad and unstructured emissions with significantly lower intensities than $6\cdot\text{Cl}_2$ and negligible anion dependence—in fact, a *decrease*

³ It is worth noting here that the *relative* change in quantum yield between the two co-confirmations, rather than their absolute values, is of greatest importance. Claims of accurate absolute quantum yield values should be taken with a pinch of salt. In this particular case, great care was taken when carrying out the measurements, weighing samples and diluting solutions (as evidenced by the good R^2 values for all samples). However, there are two sources of systematic error which, because of their relationship to each other, make it impossible to quantify the certainty of these quantum yields in a meaningful way. The first, crucially, arises because of the lack of the necessary spectral correction software on the used spectrofluorometer—thus, emission spectra were not corrected for inherent spectral artefacts. Second, the 400 nm excitation wavelength used means that the excitation beam slightly overlaps the NDI emission of the [3]rotaxanes, thus the range over which the emission peaks for samples $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ was integrated was slightly less than the true width of the emission (see Fig. 74a). Even with the deconvolution and subtraction of this excitation beam peak, accurate absolute quantum yield values would still not be achieved due to the spectra not having been corrected. Cross-calibration of standards with well-documented quantum yields (i.e. with a minimal spread in reported values) was attempted, however, anthracene was not suitable as it doesn't absorb at 400 nm and 9,10-dimethyl anthracene was not suitable as it was too strongly fluorescent and saturated the detector under the prescribed experimental conditions.

in the emission intensity is seen on moving to the chloride salts, which could possibly be indicative of collisional quenching [389].

The remarkable “on-off” NDI fluorescent response of [3]rotaxane **6** is defined by the controlled, reversible, and large-amplitude anion-induced translational motion of its ferrocenyl-macrocycle components. In **6**·(PF₆)₂, the quenching of the NDI emission can be explained by PET from the ferrocenyl donor on the macrocycles to the NDI stations at which the macrocycles reside. In contrast, in **6**·Cl₂, the ferrocenyl donors are spatially removed from the peripheral NDI stations, residing instead near the central fullerene moiety, and so long-distance through-space PET to the NDI stations is kinetically unfavoured *cf.* PET to the nearby fullerene.

Thermodynamic driving forces for PET must also be taken into account. As such, electrochemical characterisation offers several useful insights. For both **6**·Cl₂ and **6**·(PF₆)₂, the LUMO⁴ is located on the central fullerene moiety (-4.29 eV), with NDI lying very close energetically (-4.14 eV), while the HOMO is located on ferrocene (-5.13 eV for **6**·Cl₂ and -5.17 eV for **6**·(PF₆)₂). Thus, both [3]rotaxanes can be considered co-conformations of an acceptor–donor–stronger acceptor (NDI–Fc–C₆₀) system, with HOMO-LUMO gaps of 0.85 eV (**6**·Cl₂) and 0.88 eV (**6**·(PF₆)₂). Importantly, these small gaps indicate that excited state charge-transfer is energetically favourable over the formation of C₆₀’s triplet state, ³C₆₀^{*} (1.56 eV) [390]—therefore, in **6**·Cl₂, PET to the fullerene is both kinetically and thermodynamically favoured. Similarly, this triplet energy is also greater than the energy for charge transfer from Fc to NDI (0.99 eV for **6**·Cl₂ and 1.04 eV for **6**·(PF₆)₂), thus adding thermodynamic credence to PET as the mechanism for the quenching of **6**·(PF₆)₂’s fluorescence when the Fc groups are close to the NDI stations.

⁴ HOMO and LUMO values are calculated from the half-wave oxidation and reduction potentials respectively using $E_{HOMO}(eV) = E_{\frac{1}{2},ox_1} + 5.1$ and $E_{LUMO}(eV) = E_{\frac{1}{2},red_1} + 5.1$

4.6 SINGLET OXYGEN GENERATION OF [3]ROTAXANE CHLORIDE SENSOR

The above has demonstrated that controllable anion-induced molecular motion of rotaxanes **6**·Cl₂ and **6**·(PF₆)₂ leads to a switchable fluorescent response thanks to competitive PET processes. As such, the system is a proof-of-concept for chloride sensing, which is of biomedical relevance. The aim of this section is to measure the ability of each co-conformation to generate ¹O₂. C₆₀ is a devastating ¹O₂ sensitizer and PET a mechanism for modulating ¹O₂ generation. Thus, is the switching on of NDI's fluorescent response concomitant with a decrease in the generation of ¹O₂ by the central fullerene moiety?

Which ROS is produced upon irradiation of C₆₀ is known to depend on the polarity of the solvent used [391] (Section 1.2.1.5). The production of ¹O₂ is favoured in non-polar solvents. For this reason, CS₂ was selected as the solvent for these experiments—the other key reason for using CS₂ is that all materials required for this experiment show excellent solubility in it.

The direct detection of the phosphorescence of ¹O₂ at 1270 nm can be achieved via laser flash photolysis with an appropriate NIR photodetector [392, 393]. Access to such a setup was, however, not at my disposal during this project. Thus, a spin-trapping EPR spectroscopy method was employed, as described below.

4.6.1 Spin-Trapping Method

The commonly used spin trap, 2,2,6,6-tetramethylpiperidine (TEMP), was used as it is selective towards ¹O₂ rather than any other ROS [394]. ¹O₂ reacts with TEMP (Fig. 81) to give the persistent radical, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO), which is spin active and gives a characteristic nitroxide line pattern in its EPR spectrum.

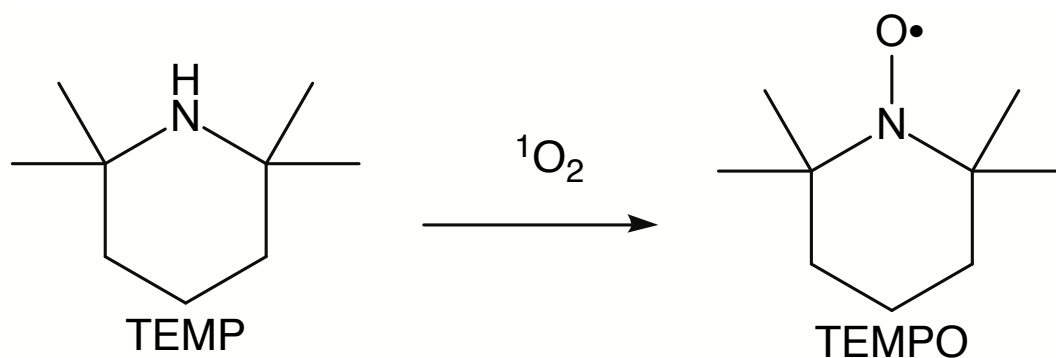


Figure 81: Formation of TEMPO by reaction of ${}^1\text{O}_2$ with TEMP.

A previously reported method was modified [395], with continuous wave (CW) X-band (9.4 GHz) EPR spectra recorded at room temperature for all samples. A 2 M solution of TEMP in CS_2 was freshly prepared before each measurement session, and stored in a Parafilm-sealed vial in the dark; 0.5 mM solutions of each sample in CS_2 were also prepared and stored in Parafilm-sealed vials in the dark. Four spectra were recorded for each sample in standard X-band EPR tubes:

1. 400 μL of sample solution
2. 400 μL of sample solution after irradiation with a 200 W broadband halogen photoreflector lamp for 2.5 minutes at a distance of 30 cm
3. 400 μL of sample solution plus 200 μL of TEMP solution
4. 400 μL of sample solution plus 200 μL of TEMP solution after irradiation with a 200 W broadband halogen photoreflector lamp for 2.5 minutes at a distance of 30 cm

Further measurement parameters: 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power. Dr. Shen Zhou fit all spectra and determined g factor values by performing simulations using the EasySpin software package [396].

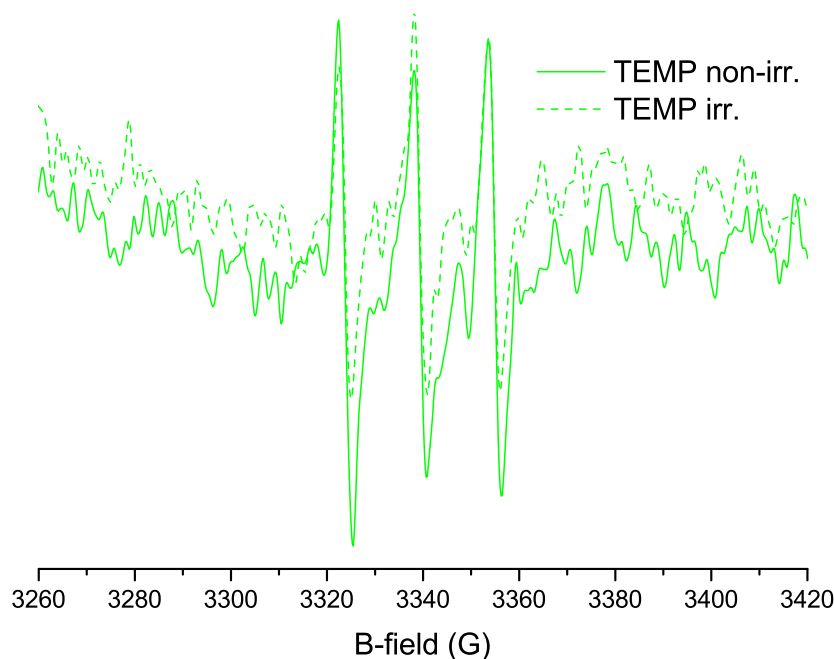


Figure 82: X-band EPR spectra of TEMP (2 M, CS₂) before and after irradiation. There is no significant change in the observed TEMPO signal between the two cases, and these signals barely rise above the background noise. Experimental parameters: 298 K temperature; 9.378390 GHz microwave frequency; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power.

4.6.2 Results & Discussion

A first control was carried out to determine that TEMP, both before and after irradiation, did not produce an appreciable TEMPO signal. That was indeed found to be the case (Fig. 82). The method outlined above was then tested to ensure that only the irradiated sample with TEMP present produced a TEMPO signal. The spectra of C₆₀ samples show this to be the case, the sample irradiated in the presence of TEMP giving a characteristic TEMPO signal centred at 3339.5 G ($g = 2.0065$), with a peak-to-peak line width of 2.2 G [394] (Fig. 83). The other functional components of rotaxane 6—NDI precursor **11** (Fig. 84a) and Fc-macrocycle precursor **14** (Fig. 84c)—underwent the same set of measurements (Figs. 84b and

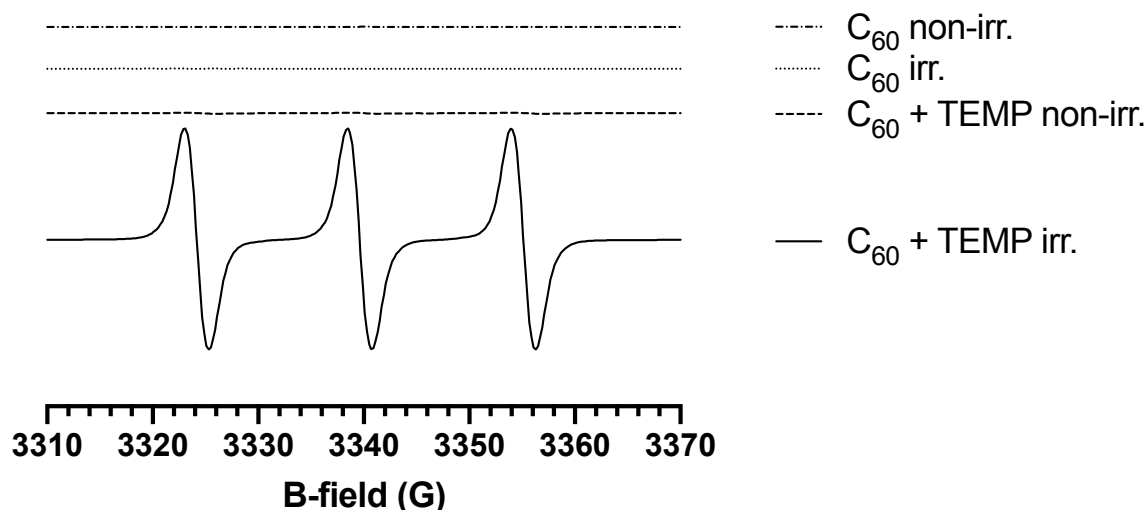


Figure 83: X-band EPR spectra of C_{60} with and without TEMP, before and after irradiation. Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.379014 GHz microwave frequency for C_{60} + TEMP irr.

84d respectively). The presence of a TEMPO signal in both spectra would suggest that both NDI precursor **11** [397] and Fc-macrocycle precursor **14** [398] sensitise the production of 1O_2 but, crucially, to nowhere near the same extent as C_{60} (Fig. 85). The 1O_2 yield is proportional to the double integral of the observed TEMPO peaks [33]; as such, a ratio of 1O_2 yields was calculated as 1:0.15:0.06 (C_{60} :Fc:NDI).

Axle salts **13**·Cl₂ and **13**·(BF₄)₂ followed the same trend as the preceding samples: TEMPO production was only observed in irradiated samples which contained TEMP. This is to be expected with both C_{60} and NDI present. The 1O_2 yield of **13**·(BF₄)₂ was found to be 85% that of **13**·Cl₂ (Fig. 86).⁵ The increased 1O_2 yield for the chloride salt **13**·Cl₂ could well be due to chloride quenching [389] of NDI's fluorescence (as observed in Fig. 75)—non-radiative pathways of relaxation could then be favoured, such as 1O_2 sensitisation, which would contribute additional sensitisation to that of the axle's central fullerene moiety. The

⁵ For these and all other spectra henceforth, the 1O_2 yields were calculated as the double integral of the leftmost TEMPO peak, as this is the only of the three TEMPO peaks which does not overlap with any other observed signal.

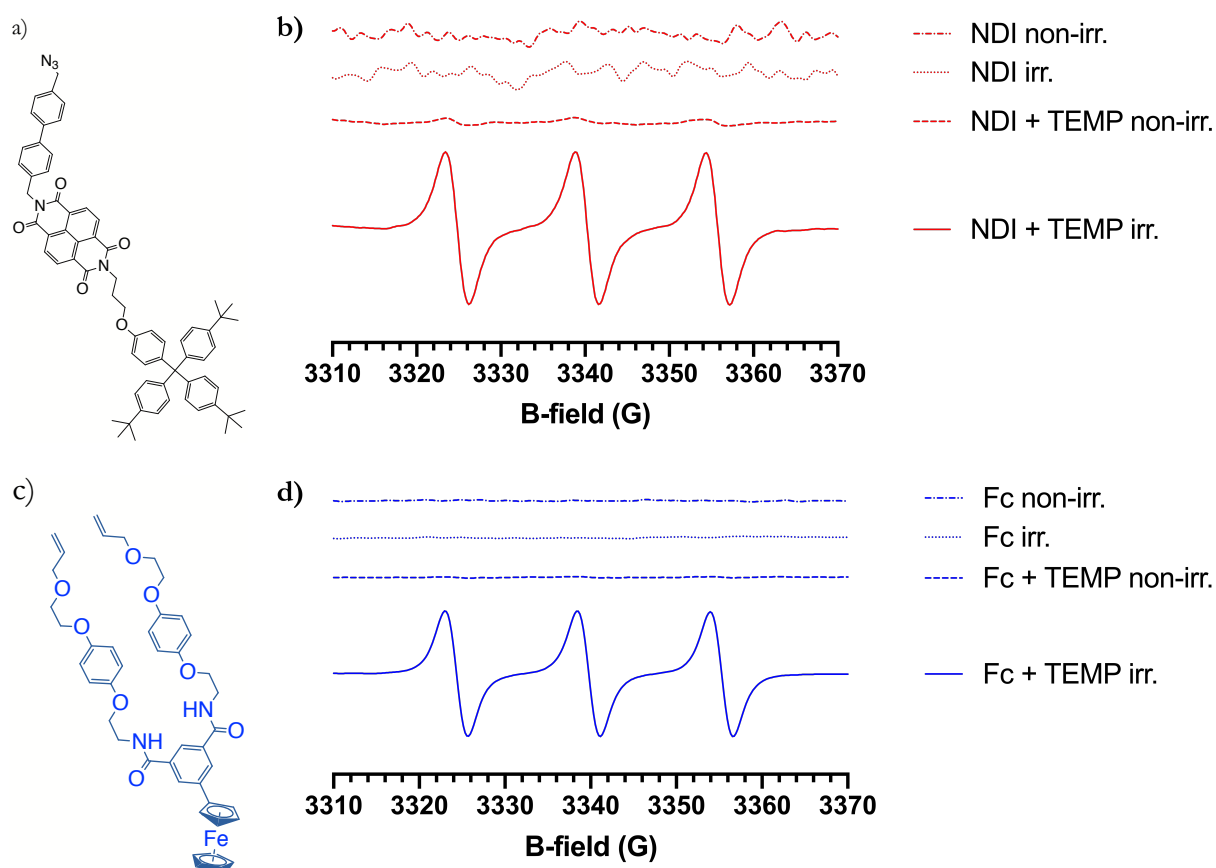


Figure 84: X-band EPR spectra of (a & b) NDI- and (c & d) Fc-containing precursors with and without TEMP, before and after irradiation. Spectra are translated in the y-direction for clarity. Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.380850 GHz (b) and 9.379511 GHz (c) microwave frequency for samples + TEMP irr.

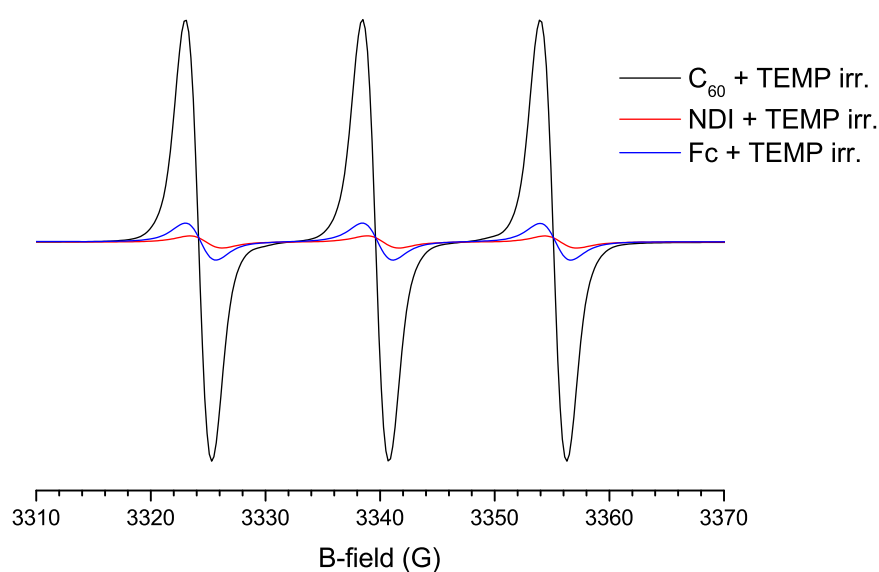


Figure 85: X-band EPR spectra of C_{60} and precursors **11** and **14** in the presence of TEMP, after irradiation. The ratio of 1O_2 yields, calculated as the double integral of the peaks, is 1:0.15:0.06 (C_{60} :Fc:NDI). Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.379014 GHz (C_{60}), 9.380850 GHz (NDI) and 9.379511 GHz (Fc) microwave frequency.

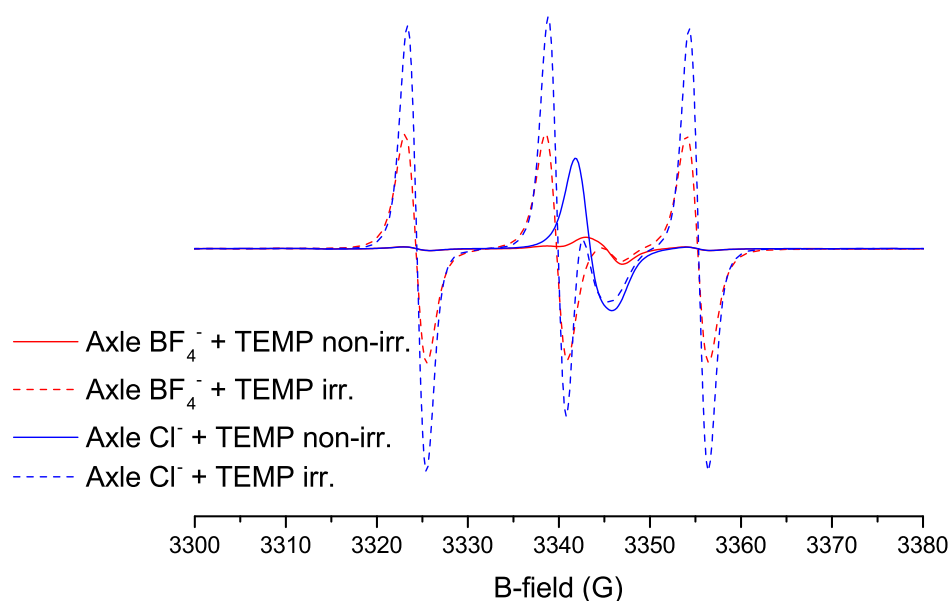


Figure 86: X-band EPR spectra of axle salts $\mathbf{13} \cdot \text{Cl}_2$ and $\mathbf{13} \cdot (\text{BF}_4)_2$ in the presence of TEMP, before and after irradiation. The $^1\text{O}_2$ yield of $\mathbf{13} \cdot (\text{BF}_4)_2$ is 85.2% that of $\mathbf{13} \cdot \text{Cl}_2$. Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.379370 GHz ($\mathbf{13} \cdot (\text{BF}_4)_2$ + TEMP non-irr.), 9.379418 GHz ($\mathbf{13} \cdot (\text{BF}_4)_2$ + TEMP irr.), 9.379620 GHz ($\mathbf{13} \cdot \text{Cl}_2$ + TEMP non-irr.) and 9.379650 GHz ($\mathbf{13} \cdot \text{Cl}_2$ + TEMP irr.) microwave frequency.

non-TEMPO signal in each sample ($\mathbf{13} \cdot \text{Cl}_2$: $g_x = 2.0039$, $g_y = 2.0049$, $g_z = 2.0042$; $\mathbf{13} \cdot (\text{BF}_4)_2$: $g = 2.0039$) can reasonably be assigned to oxidised fullerene [399], likely introduced during the click procedure between azide and alkyne components. The origin of the anisotropy of the g -factor of $\mathbf{13} \cdot \text{Cl}_2$ is as yet unclear, but this anisotropy accounts for the observed line broadening [400].

The spectra of [3]rotaxane salts $\mathbf{6} \cdot \text{Cl}_2$ and $\mathbf{6} \cdot (\text{PF}_6)_2$, in the absence of TEMP, display almost identical features to each other. The centremost signals in both rotaxane salts ($\mathbf{13} \cdot \text{Cl}_2$: $g_x = 2.0039$, $g_y = 2.0049$, $g_z = 2.0042$; $\mathbf{13} \cdot (\text{BF}_4)_2$: $g = 2.0032$) correspond, respectively, to the anisotropic and isotropic oxidised fullerene signals observed in the axle salts. The two peaks flanking both spectra correspond to the presence of Cu(II) ions ($g = 2.0451$) [401], an impurity

remaining from the click procedure.⁶ The addition of TEMP, without irradiation, does not give a discernible TEMPO signal in either case.

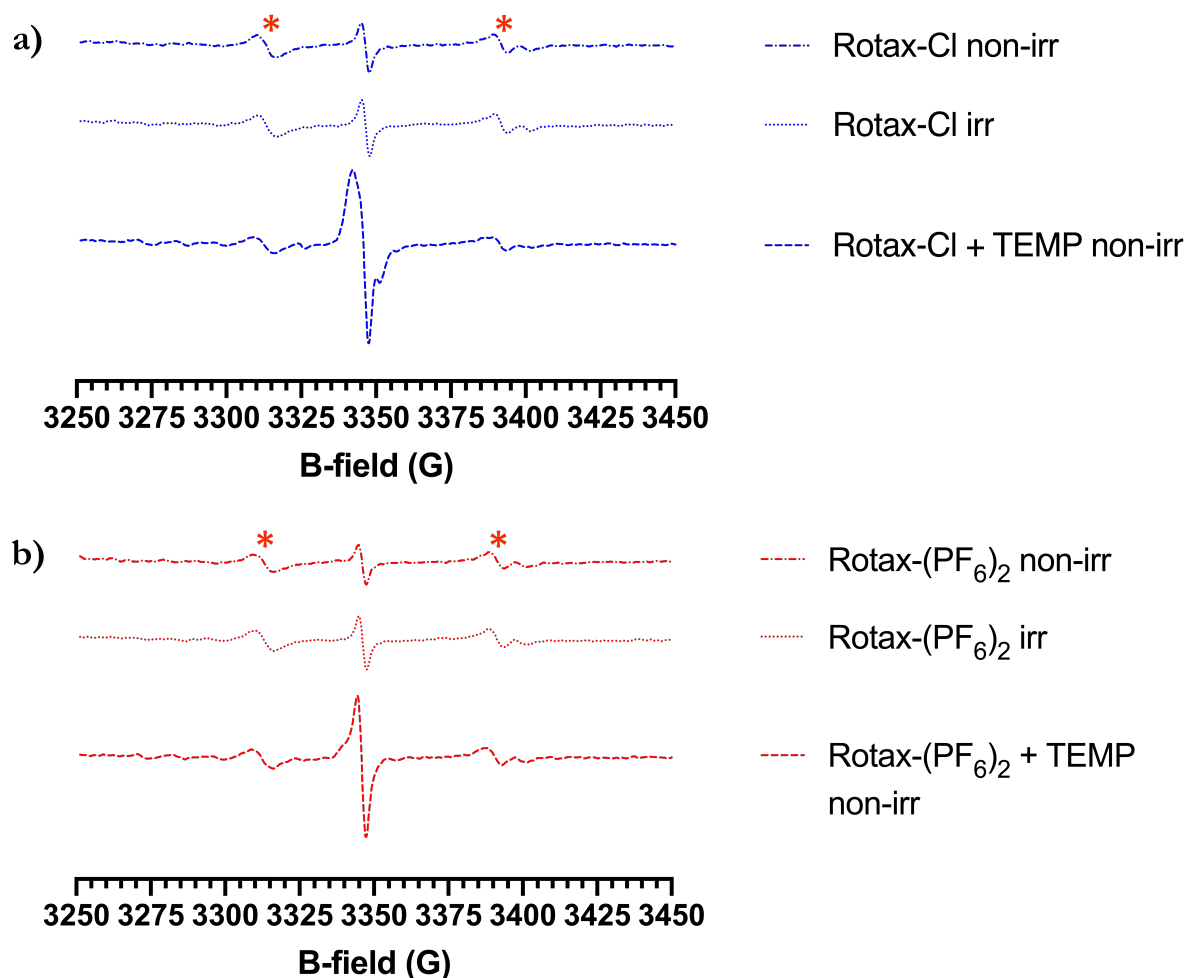


Figure 87: X-band EPR spectra of [3]rotaxane salts (a) $6\cdot\text{Cl}_2$ and (b) $6\cdot(\text{PF}_6)_2$ in the absence of TEMP, and also with TEMP (but not irradiated). The starred peaks correspond to Cu(II) impurity ($g = 2.0451$). Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.382304 GHz ($6\cdot\text{Cl}_2$ non-irr.), 9.382406 GHz ($6\cdot\text{Cl}_2$ irr.), 9.382211 GHz ($6\cdot\text{Cl}_2$ + TEMP non-irr.), 9.380998 GHz ($6\cdot(\text{PF}_6)_2$ non-irr.), 9.381120 GHz ($6\cdot(\text{PF}_6)_2$ irr.) and 9.380960 GHz ($6\cdot(\text{PF}_6)_2$ + TEMP non-irr.) microwave frequency.

When TEMP is added, and the samples irradiated, a difference in the strength of the TEMPO signal is observed (Fig. 88): the $^1\text{O}_2$ yield of $6\cdot\text{Cl}_2$ is 43% that of $6\cdot(\text{PF}_6)_2$. This difference in $^1\text{O}_2$ sensitisation is in reasonable agreement with the observed three-fold difference in fluorescence quantum yield measured in Section 4.5. This can be rationalised

⁶ The other two peaks of this Cu(II) signal, arising from hyperfine coupling, lie outside the measured field range.

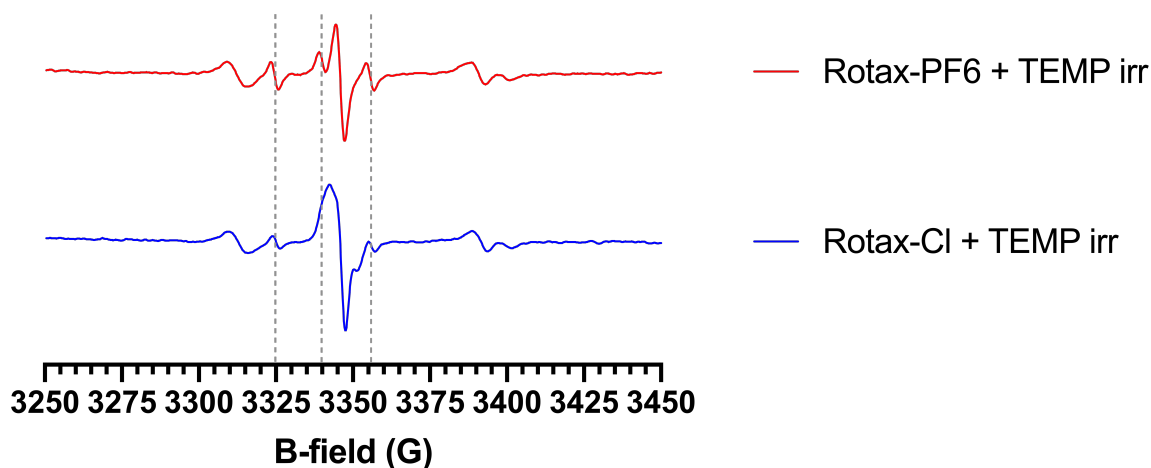


Figure 88: X-band EPR spectra of irradiated [3]rotaxane salts $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ in the presence of TEMP. Grey dashed lines indicate the centre of each hyperfine signal corresponding to TEMPO. The rest of the spectrum is just a superposition of oxidised fullerene and Cu(II) signals as observed in Fig. 87, hence the hidden central TEMPO peak in $6\cdot\text{Cl}_2$. Experimental parameters: 298 K temperature; 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; 100 kHz modulation frequency; three scans per measurement; 23 dB attenuation; 1 mW power; 9.382242 GHz ($6\cdot\text{Cl}_2$) and 9.381016 GHz ($6\cdot(\text{PF}_6)_2$ irr.).

by PET acting as a quenching mechanism for the sensitisation of $^1\text{O}_2$ [402]. This, as with the fluorescent response, is mediated by the anion-induced translocation of the ferrocenyl donor-appended macrocycles towards and away from the central fullerene moiety—when in close proximity (as in $6\cdot\text{Cl}_2$), PET from Fc to C_{60} is favoured, unlike when the macrocycles are spatially removed to the NDI stations (as in $6\cdot(\text{PF}_6)_2$). It should be noted that this result is opposite to what is seen with the axle salts (i.e. $13\cdot\text{Cl}_2$ shows stronger sensitisation than $13\cdot(\text{BF}_4)_2$), and so adds credence to the difference in $^1\text{O}_2$ sensitisation for [3]rotaxane salts $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ arising due to tuneable PET.

4.7 MATERIALS AND METHODS

All commercial solvents, reactants and reagents were purchased from Sigma-Aldrich (United Kingdom) and used as purchased unless otherwise stated. Anhydrous solvents were degassed with N_2 and dried by passing them through an MBraun-800 column. Fullerene C_{60}

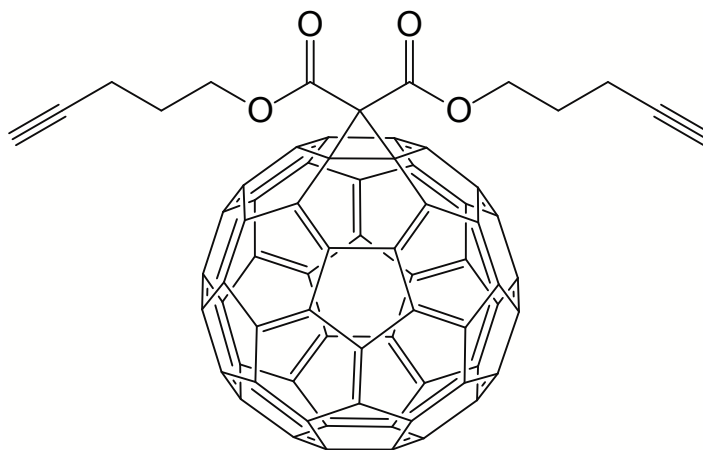
(99+% purity) was used as purchased from Materials and Electrochemical Research (MER) Corporation (Arizona, USA). Water was distilled and microfiltered using a Milli-Q Millipore machine. Chromatography was undertaken using silica gel (particle size: 40-63 μm) or preparative TLC plates (20 $\times$ 20 cm, 1 cm silica thickness) unless otherwise stated. ^1H , ^{13}C , ^{19}F and ^{31}P NMR spectra were recorded using Bruker AVIII400 and Bruker AVIII500 (with He cryoprobe) spectrometers. Mass spectra were recorded on a Bruker microflex LT matrix-assisted laser desorption/ionisation time-of-flight mass spectrometer (MALDI-TOF MS) with DCTB as the matrix.

4.7.1 *Bisalkyne Methanofullerene Synthesis and Structural Characterisation*

Bisalkyne malonate **8** and methanofullerene bisalkyne mono-adduct **7** were synthesised according to literature procedures [366]. **7** was further purified using reverse-phase HPLC (Japan Analytical Industry (JAI) LC-9103 Recycling Preparative HPLC with a modular Hitachi L-7150 pump and JAI UV Detector 3702 at 312 nm absorption). 2.7 mL of sample solution was injected per run through a Cosmosil Buckyprep-M column (20.0 mm ID $\times$ 250 mm, toluene, 16 mL/min flow rate). Recycling HPLC of isolated fractions was carried out on the same equipment with the same sample volume; the recycling pump was activated at the onset of the peak. Data was collected and analysed using Datalys Azur software. Sample solutions were concentrated *in vacuo* as much as possible before injection into HPLC in order to minimise the number of injections.

4.7.1.1 *HPLC Isolation of Methanofullerene Bisalkyne Mono-Adducts*

The peak containing **7** was first isolated (τ_{R} 5.8–7 mins, 55%) from unreacted C_{60} (τ_{R} 7.4–8 mins, 18%) and bisalkyne multi-adducts [33] (τ_{R} 4.5–5.7 mins, 27%). This peak (containing **7**)

Figure 89: Bisalkyne methanofullerene **7**.

was then recycled through the HPLC six times leading to the separation and isolation of **7** (68%), mono-iodo derivative **9** (27%) and bis-iodo derivative **10** (5%).

4.7.1.2 Bisalkyne Methanofullerene **7** Structural Characterisation

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.55 (4H, t, $J = 6.3$ Hz, CH_2), 2.35 (4H, td, $J = 6.9, 2.7$ Hz, CH_2), 2.01 (4H, quint, $J = 6.6$ Hz, CH_2), 1.98 (2H, t, $J = 2.6$ Hz, alkyne).

$^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ 163.56, 145.32, 145.24, 145.15, 144.96, 144.75, 144.70, 144.68, 143.94, 143.13, 143.08, 143.05, 142.25, 141.96, 141.04, 139.09, 82.45, 71.50, 69.75, 65.79, 27.37, 15.25.

MS (MALDI) m/z calc. for $\text{C}_{73}\text{H}_{14}\text{O}_4$ $[\text{M}]^+$: 954.09; found: 953.80.

4.7.1.3 Bisalkyne Methanofullerene Mono-Iodo Derivative **9** Structural Characterisation

$^1\text{H NMR}$ (400 MHz, CDCl_3) δ 4.56 (2H, t, $J = 6.2$ Hz, CH_2), 4.53 (2H, t, $J = 6.1$ Hz, CH_2), 2.53 (2H, t, $J = 6.9$ Hz, CH_2), 2.36 (2H, td, $J = 7.0, 2.6$ Hz, CH_2), 2.02 (4H, quint, $J = 6.4$ Hz, CH_2), 1.98 (1H, t, $J = 2.4$ Hz, alkyne).

^{13}C NMR (101 MHz, CDCl_3) δ 163.55, 145.33, 145.24, 145.16, 145.11, 144.96, 144.74, 144.70, 143.94, 143.13, 143.07, 143.05, 142.25, 141.95, 141.05, 139.16, 82.44, 69.78, 65.80, 65.70, 27.38, 17.59, 15.25.

MS (MALDI) m/z calc. for $\text{C}_{73}\text{H}_{13}\text{O}_4\text{I} [\text{M}]^+$: 1079.99; found: 1079.77.

4.7.1.4 Bisalkyne Methanofullerene Bis-Iodo Derivative **10** Structural Characterisation

^1H NMR (400 MHz, CDCl_3) δ 4.53 (4H, t, J = 6.2 Hz, CH_2), 2.53 (4H, t, J = 6.9 Hz, CH_2), 2.00 (4H, quint, J = 6.5 Hz, CH_2).

^{13}C NMR (101 MHz, CDCl_3) δ 163.55, 145.33, 145.24, 145.12, 144.96, 144.75, 144.73, 144.70, 143.93, 143.11, 143.07, 143.05, 142.26, 142.00, 141.04, 139.08, 129.06, 128.26, 92.47, 71.49, 65.73, 27.39, 17.60, -4.80.

MS (MALDI) m/z calc. for $\text{C}_{73}\text{H}_{12}\text{O}_4\text{I}_2 [\text{M}]^+$: 1205.88; found: 1205.93.

4.7.2 Rotaxane Synthesis and Structural Characterisation

Grubbs' second generation catalyst and $\text{Cu}(\text{MeCN})_4\cdot\text{PF}_6$ were stored in a desiccator with P_2O_5 . Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) was prepared following a literature procedure [403]. An anion exchange procedure was used in preference to the addition of tetrabutylammonium salts because it allows repetitive anion switching to be performed without the build-up of unwanted inorganic salts that must be removed by additional purification techniques. Axle and rotaxane components were fully anion exchanged using a column containing an Amberlite[®] ion exchange resin that had been loaded with

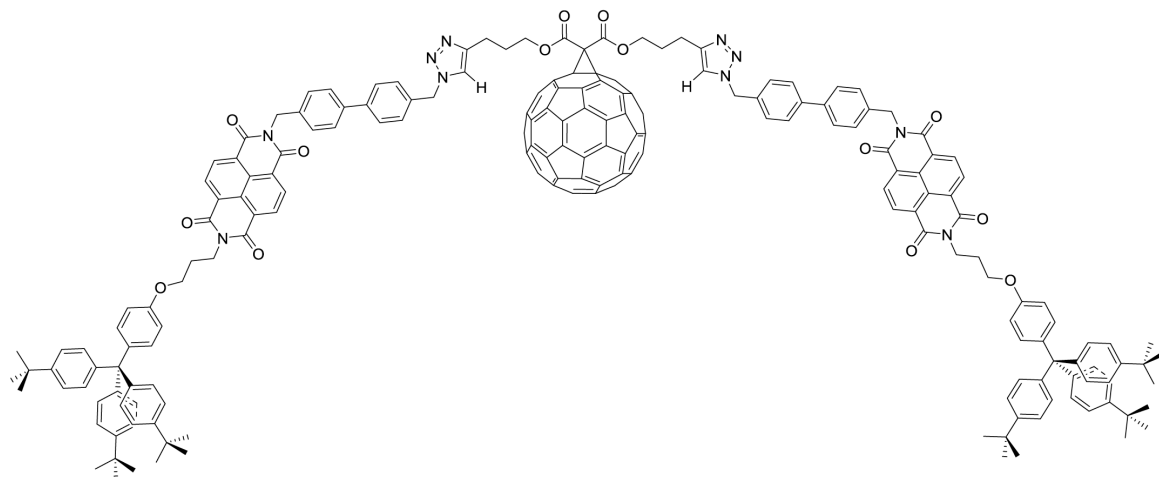


Figure 90: Bis-*proto*-triazole bis-NDI C₆₀ fullerene axle **12**.

the desired anion. Amberlite[®] was loaded by washing the resin with NaOH_(aq) (10%), H₂O, and either NH₄Cl_(aq) (1 M), or NH₄PF_{6(aq)} (0.1 M), followed by further H₂O, and the solvent (45:45:10 CHCl₃:MeOH:H₂O) to be used in the anion exchange. The compound was then dissolved in 5–10 mL of 45:45:10 CHCl₃:MeOH:H₂O and passed through the column at least three times to achieve complete anion exchange. After this the solvent was removed *in vacuo*, the residue redissolved in CHCl₃ (5–10 mL) and washed with H₂O (5–10 mL). After drying the organic phase over anhydrous MgSO₄ the solvent was removed *in vacuo* to give the product.

NDI precursor **11** [205] and Fc-macrocycle precursor **14** [384] were synthesised according to literature procedures.

4.7.2.1 Bis-Triazole Axle Component **12** Synthesis and Structural Characterisation

Azide-functionalised NDI precursor **11** (43 mg, 0.04 mmol, 2 eq) and C₆₀ methanofullerene bisalkyne mono-adduct **7** (20 mg, 0.02 mmol, 1 eq) were dissolved in dry CH₂Cl₂ (3 mL). Then TBTA (5 mg, 0.01 mmol, 0.4 eq), Cu(MeCN)₄·PF₆ (3 mg, 0.01 mmol, 0.4 eq) and *N,N*-diisopropylethylamine (15 µl, 0.08 mmol, 4 eq) were added to the mixture and left to stir at

room temperature for 2 days, monitoring by thin layer chromatography (CH₂Cl₂:MeOH 99:1). The mixture was diluted with CH₂Cl₂ and washed with a 0.02 M EDTA/0.1 M NH₄OH_(aq) solution, the organic layer was dried over anhydrous MgSO₄, and the solvent was removed *in vacuo*. The resultant crude was purified by silica gel column chromatography (CH₂Cl₂:MeOH 99:1) to give axle **12** as a brown solid (46 mg, 0.02 mmol, 73%).

¹H NMR (400 MHz, CDCl₃) δ 8.69–8.82 (8H, m, ArH NDI), 7.63 (4H, d, *J* = 8.2 Hz, ArH biphenyl), 7.45–7.56 (10H, m, ArH biphenyl, triazole), 7.18–7.26 (16H, m, ArH stopper, biphenyl), 6.98–7.13 (16H, m, ArH stopper), 6.66 (4H, d, *J* = 8.7 Hz, ArH stopper), 5.50 (4H, s, CH₂), 5.42 (4H, s, CH₂), 4.50 (4H, t, *J* = 6.1 Hz, CH₂), 4.43 (4H, t, *J* = 6.8 Hz, CH₂), 4.10 (4H, t, *J* = 5.9 Hz, CH₂), 2.84 (4H, t, *J* = 7.5 Hz, CH₂), 2.14–2.30 (8H, m, CH₂), 1.28 (54H, s, CH₃ stopper).

¹³C NMR (101 MHz, CDCl₃) δ 15.2, 23.6, 27.3, 28.5, 31.4, 33.2, 34.3, 52.0, 54.5, 63.0, 65.8, 66.0, 67.6, 69.7, 71.5, 82.4, 98.4, 108.0, 112.9, 124.0, 126.6, 127.4, 128.6, 130.7, 131.1, 139.0, 134.5, 135.8, 141.0, 141.9, 142.2, 143.0, 143.0, 143.1, 143.9, 144.6, 144.7, 144.9, 145.1, 145.2, 145.3, 148.3, 162.9, 163.5.

MS (MALDI) m/z calc. for C₂₀₉H₁₄₅N₁₀O₁₄ [M + H]⁺: 3020.100; found: 3020.190.

4.7.2.2 Bis-Triazolium Axle Salt **13**·(BF₄)₂ Synthesis and Structural Characterisation

Axle **12** (46 mg, 0.02 mmol, 1 eq) was dissolved in CH₂Cl₂ (4 mL) with Me₃O·BF₄ (7 mg, 0.06 mmol, 3 eq) and the mixture was left stirring overnight at room temperature. The reaction was quenched with three drops of MeOH and the solvent was removed *in vacuo*. The resultant

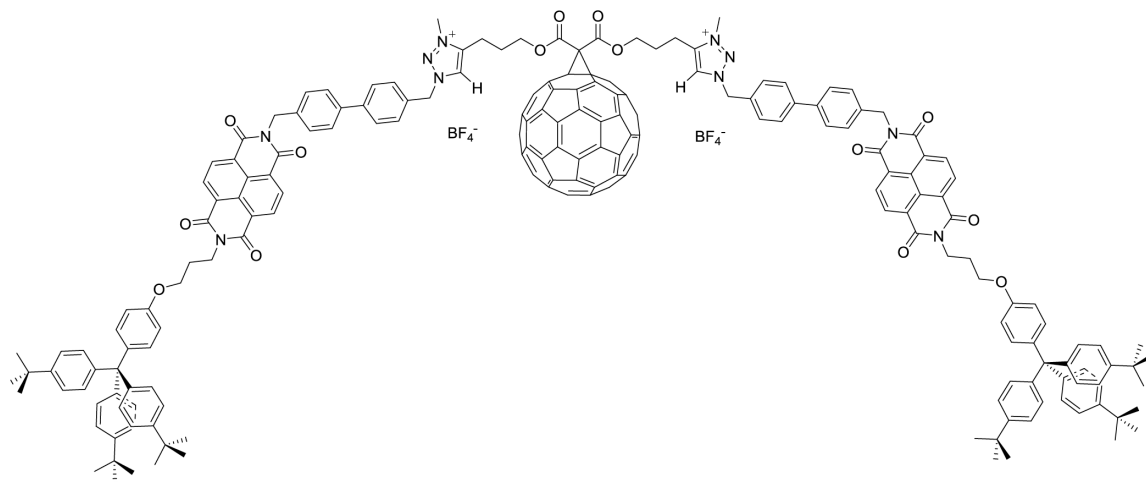


Figure 91: Bis-proto-triazolium bis-NDI C₆₀ fullerene axle salt **13**·(BF₄)₂.

solid was purified by preparative silica TLC (CH₂Cl₂:MeOH 97:3) to give **13**·(BF₄)₂ as a brown solid (45 mg, 0.01 mmol, 92%).

¹H NMR (400 MHz, CDCl₃) δ 8.67–8.79 (8H, m, ArH NDI), 8.56 (2H, br. s., ArH triazolium), 7.60 (4H, d, *J* = 8.1 Hz, ArH biphenyl), 7.53 (8H, s, ArH biphenyl), 7.48 (4H, d, *J* = 8.1 Hz, ArH biphenyl), 7.23 (12H, d, *J* = 8.6 Hz, ArH stopper), 6.97–7.10 (16H, m, ArH stopper), 6.65 (4H, d, *J* = 8.8 Hz, ArH stopper), 5.67 (4H, br. s., CH₂), 5.38 (4H, s, CH₂), 4.63 (4H, br. s., CH₂), 4.42 (4H, t, *J* = 7.0 Hz, CH₂), 4.18–4.26 (6H, m, CH₃ triazolium), 4.10 (4H, t, *J* = 5.9 Hz, CH₂), 3.04 (4H, br. s., CH₂), 2.34 (4H, br. s., CH₂), 2.19–2.30 (4H, m, CH₂), 1.28 (54H, s, CH₃ stopper).

¹³C NMR (101 MHz, CDCl₃) δ 15.2, 23.6, 27.3, 28.5, 31.4, 31.7, 33.2, 34.2, 37.8, 52.0, 54.5, 63.0, 65.8, 66.0, 67.6, 69.9, 71.5, 82.4, 98.6, 108.0, 112.9, 124.0, 126.6, 127.6, 128.6, 130.7, 130.8, 131.1, 139.0, 134.6, 135.8, 141.0, 141.9, 142.2, 143.0, 143.4, 143.6, 143.9, 144.6, 144.7, 144.9, 145.1, 145.2, 145.3, 148.8, 162.9, 163.5.

MS (MALDI) m/z calc. for $C_{211}H_{150}BF_4N_{10}O_{14}$ $[M - BF_4]^+$: 3136.142; found: 3136.465.

UV-VIS $\lambda_{\max}$ (nm, $CHCl_3$) 360 nm (ϵ 28,880 $M^{-1} cm^{-1}$), 381 (28,880), 425sh (2,330).

4.7.2.3 Bis-Triazolium Axle Salt **13**· Cl_2 Synthesis and Structural Characterisation

Subsequent anion exchange was achieved by dissolving **13**· $(BF_4)_2$ in (CH_2Cl_2 :MeOH 90:10) and passing the solution three times through a column of Amberlite® resin loaded with chloride to yield axle **13**· Cl_2 (44 mg, 0.01 mmol, quant.).

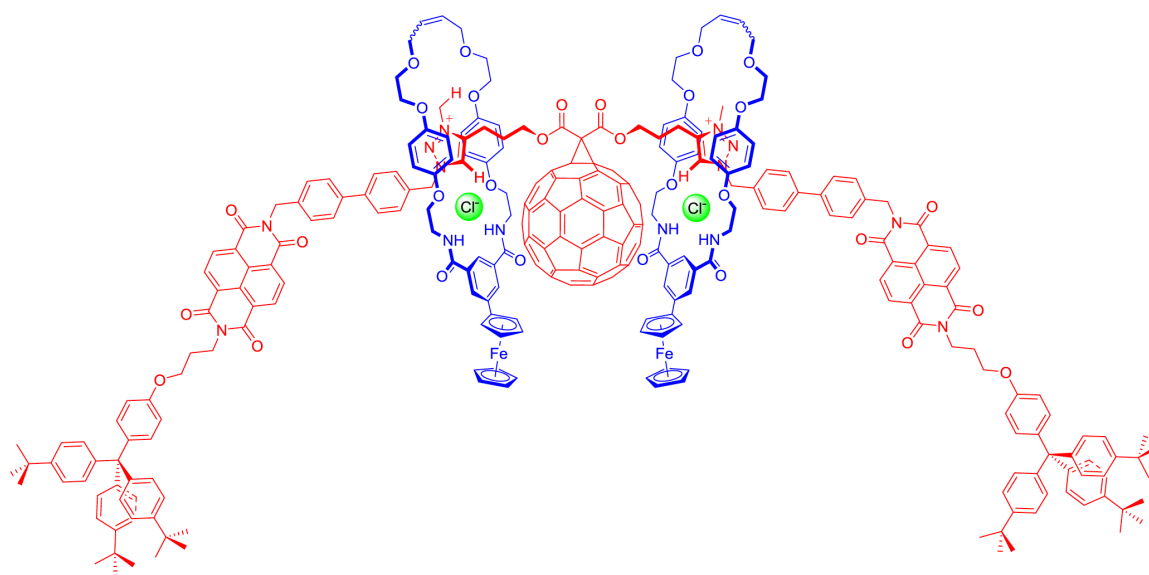
1H NMR (400 MHz, $CDCl_3$) δ 9.98 (2H, s, ArH triazolium), 8.63–8.80 (8H, m, ArH NDI), 7.67 (4H, d, J = 8.2 Hz, ArH biphenyl), 7.61 (4H, d, J = 8.3 Hz, ArH biphenyl), 7.52 (4H, d, J = 8.2 Hz, ArH biphenyl), 7.48 (4H, d, J = 8.2 Hz, ArH biphenyl), 7.22 (12H, d, J = 8.6 Hz, ArH stopper), 6.96–7.13 (16H, m, ArH stopper), 6.65 (4H, d, J = 8.9 Hz, ArH stopper), 5.95 (4H, s, CH_2), 5.40 (4H, s, CH_2), 4.73 (4H, s, CH_2), 4.42 (4H, t, J = 7.0 Hz, CH_2), 4.37 (6H, s, CH_3 triazolium), 4.10 (4H, t, J = 5.9 Hz, CH_2), 3.30 (4H, br. s., CH_2), 2.25 (4H, quint., J = 6.5 Hz, CH_2), 1.29 (54H, s, CH_3 stopper).

MS (MALDI) m/z calc. for $C_{211}H_{151}Cl_2N_{10}O_{14}$ $[M + H]^+$: 3120.085; found: 3120.591.

UV-VIS $\lambda_{\max}$ (nm, $CHCl_3$) 360 nm (ϵ 26,890 $M^{-1} cm^{-1}$), 381 (26,680), 425sh (3,070).

4.7.2.4 [3]Rotaxane Salt **6**· Cl_2 Synthesis & Structural Characterisation

Axle **13**· Cl_2 (67 mg, 20.8 μ mol, 1 eq) and Fc-macrocycle precursor **14** (49 mg, 62.1 μ mol, 3 eq) were dissolved in dry CH_2Cl_2 (15 mL), the mixture was left to stir for 30 minutes. Grubbs' II catalyst (5 mg, 10 wt. %) was added and the solution stirred at room temperature,

Figure 92: [3]Rotaxane Salt **6**·Cl₂.

monitoring by TLC (CH₂Cl₂:MeOH 95:5) and ESI mass spectrometry throughout. After one day a further addition of Grubbs' II catalyst (5 mg, 10 wt. %) was made and after a further day the solvent was removed *in vacuo*. The crude was purified by iterative preparative silica TLC (EtOAc:MeOH 95:5 then CH₂Cl₂:MeOH 95:5) to yield [3]rotaxane **6**·Cl₂ as a brown solid (11 mg, 2.3 μmol, 11%).

¹H NMR (400 MHz, CDCl₃) δ 9.96 (2H, br. s., ArH int. macro), 9.29 (2H, br. s., ArH triazolium), 9.11 (4H, br. s., NH macro), 8.65–8.84 (8H, m, ArH NDI), 8.30 (4H, s, ArH ext. macro), 7.67 (4H, d, *J* = 7.8 Hz, ArH biphenyl), 7.61 (4H, d, *J* = 8.3 Hz, ArH biphenyl), 7.55 (4H, m, ArH biphenyl), 7.48 (4H, d, *J* = 8.1 Hz, ArH biphenyl), 7.23 (12H, d, *J* = 8.6 Hz, ArH stopper), 6.97–7.13 (16H, m, ArH stopper), 6.66 (4H, d, *J* = 9.0 Hz, ArH stopper), 6.30 (8H, d, *J* = 8.8 Hz, ArH hydroquinone macro), 6.22 (8H, d, *J* = 8.8 Hz, ArH hydroquinone macro), 5.85 (4H, br. s., CH alkene macro), 5.48 (4H, br. s., CH₂ axle), 5.42 (4H, s, CH₂ axle), 4.72 (4H, s, ArH ferrocene), 4.42 (4H, t, *J* = 7.0 Hz, CH₂ axle), 4.21–4.33 (8H, m, ArH ferrocene, CH₂ axle), 4.05–4.19 (16H, m, CH₂ axle, macro), 3.83–4.04 (23H, m, ArH ferrocene, CH₃ triazolium,

CH₂ axle, macro), 3.60–3.83 (20H, m, CH₂ macro), 2.19–2.31 (4H, m, CH₂ axle), 2.05 (4H, br. s., CH₂ axle), 1.30 (54H, s, CH₃ stopper).

¹³C NMR (101 MHz, CDCl₃) δ 15.2, 23.4, 27.3, 28.5, 31.4, 31.7, 33.2, 34.2, 37.8, 39.9, 52.0, 54.5, 63.0, 65.8, 66.0, 66.7, 67.0, 67.6, 68.0, 68.5, 69.7, 69.9, 71.5, 72.3, 74.2, 82.4, 98.6, 108.0, 112.9, 115.3, 115.6, 116.6, 117.2, 120.6, 121.4, 124.0, 126.6, 127.6, 128.6, 130.7, 130.8, 131.1, 139.0, 134.5, 134.6, 135.8, 136.3, 141.0, 141.4, 141.9, 142.2, 143.2, 143.4, 143.6, 143.9, 144.6, 144.7, 144.9, 145.1, 145.2, 145.3, 148.1, 148.8, 152.6, 153.3, 162.9, 163.5, 165.7, 166.0.

MS (MALDI) m/z calc. for C₂₉₅H₂₃₈ClF₆Fe₂N₁₄NaO₃₀P [M - Cl + NaPF₆]⁺: 4770.545; found: 4770.505.

UV-VIS λ_{max} (nm, CHCl₃) 360 nm (ε 29,190 M⁻¹ cm⁻¹), 382 (27,970), 426sh (3,380).

4.7.2.5 [3]Rotaxane Salt **6**·(PF₆)₂ Synthesis & Structural Characterisation

6·Cl₂ was anion-exchanged to the bis-hexafluorophosphate salt **6**·(PF₆)₂ using Amberlite[®] anion exchange resin. The solvent used was 45:45:10 CHCl₃:MeOH:H₂O (10 mL).

¹H NMR (400 MHz, CDCl₃) δ 8.53–8.65 (8H, m, ArH NDI), 8.43 (2H, br. s., ArH int. macro), 8.19 (4H, br. s., ArH ext. macro, triazolium), 7.44–7.68 (16H, m, ArH biphenyl), 7.22 (12H, d, J = 8.6 Hz, ArH stopper), 7.00–7.12 (16H, m, ArH stopper), 6.71 (4H, d, J = 8.8 Hz, ArH stopper), 6.20 (3H, br. s., CH alkene macro), 5.71–5.87 (16H, m, ArH hydroquinone macro), 5.58 (4H, br. s., CH₂ axle), 5.28 (4H, br. s., CH₂ axle), 4.52 (4H, br. s., CH₂ axle), 4.25–4.35 (8H, m, CH₂ axle), 4.17 (8H, br. s., ArH ferrocene), 3.93–4.09 (20H, m, ArH ferrocene,

CH₃ triazolium, CH₂ axle), 3.50–3.80 (40H, m, CH₂ macro), 2.12–2.28 (8H, m, CH₂ axle), 1.33 (54H, s, CH₃ stopper).

¹⁹F NMR (470 MHz, CDCl₃) δ -72.7 (d, ¹J = 707 Hz, PF₆).

³¹P NMR (202 MHz, CDCl₃) δ -144.3 (sept, ¹J = 709 Hz, PF₆).

MS (MALDI) m/z calc. for C₂₉₅H₂₃₈ClF₆Fe₂N₁₄NaO₃₀P [M - PF₆ + NaCl]⁺: 4770.545; found: 4770.505.

UV-VIS λ_{max} (nm, CHCl₃) 362 nm (ε 26,180 M⁻¹ cm⁻¹), 382 (24,380), 426sh (3,120).

4.7.3 Photophysical Characterisation

All steady state UV-vis absorption spectra were recorded in CHCl₃ at 10 μM concentration at 298 K on a dual-beam JASCO V-570 spectrophotometer, with reference to a solvent blank. All fluorescence spectra were recorded in CHCl₃ at 50 μM concentration at 298 K on a JASCO FP-6200 spectrofluorometer. Quartz cuvettes with 1 cm path length were used in all cases. Scan rate for all absorption and fluorescence measurements was 200 nm/min.

4.7.4 Electrochemical Characterisation

Cyclic voltammetric studies were carried out using an Autolab PGSTAT302N potentiostat, in a three-electrode arrangement in a single compartment cell. A glassy carbon working electrode,

a Pt wire secondary electrode and a saturated Ag/AgCl reference electrode (chemically isolated from the test solution via a bridge tube containing electrolyte solution and fitted with a porous Vycor frit) were used in the cell. Experiments were performed under an atmosphere of argon and in anhydrous solvents. Sample solutions were prepared under an atmosphere of argon using Schlenk line techniques and consisted of a 0.2 M solution of the corresponding supporting electrolyte (either LiCl or $[\text{Bu}_4\text{N}][\text{BF}_4]$) and a 0.5–1 mM solution of the test compound. Redox potentials were referenced vs. the Fc^+/Fc couple either directly by adding an equal amount of ferrocene to the test solution where applicable, or indirectly by adding an equal amount of decamethyl ferrocene to the test solution, and then measuring the position of the $(\text{Me})_{10}\text{Fc}^+ / (\text{Me})_{10}\text{Fc}$ redox couple vs. ferrocene in a separate experiment. Compensation for internal resistance was not applied.

4.7.5 *Electron Paramagnetic Resonance Characterisation*

All EPR spectra were recorded in CS_2 on a Bruker EMXmicro X-band continuous wave (CW) EPR spectrometer at room temperature with the following measurement parameters: 200 G sweep width; 40 s sweep time; 50 dB receiver gain; 2 G modulation amplitude; three scans per measurement; 23 dB attenuation; 1 mW power. All solutions (0.5 mM for each sample, 2 mM for TEMP) were prepared in the dark. Samples were irradiated in their EPR tubes with a 200 W broadband halogen photorelector lamp for 2.5 minutes at a distance of 30 cm. Simulations of CW EPR spectra were performed using the EasySpin software package [396].

4.8 CONCLUSIONS

This chapter has charted a fullerene-containing rotaxane-based molecular machine from its design, through the synthesis of its pivotal fullerene building block, to its photophysical characterisation. First, the synthesis of bisalkyne methanofullerene **7** was improved by employing HPLC in order to separate the mono-adduct product from higher adducts and pristine C₆₀. In this way, two new iodo-alkyne derivatives, **9** and **10**, were discovered, which are themselves useful building blocks. CuAAC was used to incorporate **7** as the central moiety in a four-station axle with two peripheral NDI stations, before two ferrocene-containing macrocycles were introduced via anion-templation to furnish a mechanically interlocked system, **6**·Cl₂. This [3]rotaxane contained three static strongly electron-accepting units (one central fullerene and two peripheral NDIs) and two mobile electron-donors (ferrocene). Fluorescence spectroscopy showed this system's applicability as a fluorescent sensor, with chloride ions inducing molecular shuttling of the macrocycles, resulting in a dramatic enhancement of NDI's fluorescent emission that could be seen with the naked eye. On addition of a large non-coordinating anion, shuttling of the macrocycles back to the NDI stations caused the NDI emission to switch off due to efficient quenching via PET from the macrocycles' ferrocenyl units to NDI.

The fullerene's key role in this system was two-fold. First, its large size meant that it could be used as a central mechanical stopper, enabling the introduction of two macrocyclic units to the axle. Second, its powerful ability to accept electrons meant that, in chloride-induced co-conformation **6**·Cl₂, PET from Fc to C₆₀ precluded PET from Fc to NDI, and so a strong emissive response was turned on. As such, this [3]rotaxane was shown to be an efficient

and easily distinguishable chloride ion sensor, whose function is dependent on controlled molecular motion.

The ability of [3]rotaxane salts $6\cdot\text{Cl}_2$ and $6\cdot(\text{PF}_6)_2$ to sensitise the production of $^1\text{O}_2$ was assessed using a spin-trapping EPR spectroscopy method. The selective oxidation of TEMP by $^1\text{O}_2$ produces TEMPO, which could be detected by its characteristic three-line EPR spectrum. Control measurements were carried out on constituent components, C_{60} , **11** and **14**, as well as axle salts $13\cdot\text{Cl}_2$ and $13\cdot(\text{BF}_4)_2$, in order to check the validity of the spin-trapping experiment. Four spectra were taken for each sample—before irradiation, after irradiation, with TEMP before irradiation, and with TEMP after irradiation—with only the final one ever exhibiting an appreciable TEMPO signal. In line with fluorescence results, $6\cdot\text{Cl}_2$ exhibits $^1\text{O}_2$ sensitisation 43% that of $6\cdot(\text{PF}_6)_2$, which arises because PET—which quenches $^1\text{O}_2$ generation—from Fc on the macrocycles to C_{60} in $6\cdot\text{Cl}_2$ is favoured.

In this way, [3]rotaxane **6** is demonstrated to act as a dual-response system: a visible on-off fluorescent response is concomitant with tuning of its ability to sensitise the production of cytotoxic $^1\text{O}_2$, thus demonstrating its possible utility as a theranostic tool. To the best of my knowledge, this is the first example of such dual functionality being achieved with a rotaxane of any kind.

CONCLUSIONS & OUTLOOK

The final part of this thesis summarises the key conclusions from each experimental chapter. Future possible experiments are suggested.

The overarching ultimate goal, common to all experimental work carried out in this thesis, is material- and application-based: namely to create new fullerene-based materials that show promise for exploitation in nanomedicine [404], existing examples of which were critically evaluated in the Literature Review. The two classes of materials investigated to this end were water-soluble fullerenes and fullerene-containing rotaxanes. In this way, two different aspects of the supramolecular chemistry of fullerenes were investigated—molecular self-assembly and mechanically interlocked molecules (MIMs). For molecular self-assembly, covalent functionalisation of the fullerene cages introduced a cocktail of non-covalent interactions between these molecular building blocks, which were exploited to create extended hierarchical structures. For MIMs, the fullerene was covalently incorporated into an extended structure around which a mechanical bond was introduced via templated non-covalent interactions; the MIM architecture and associated non-covalent interactions could then be manipulated to tune the structure and function. In different ways, non-covalent supramolecular interactions govern the structure and function of both of these systems, which both show great promise for nanomedicine applications.



5.1 CONCLUSIONS

In Chapter 2, a novel highly water-soluble (37 mg/mL) C₆₀ derivative, **1**, was synthesised in a one-pot reaction with cheap and readily available materials: C₆₀, NaOH and triethylene glycol monoethyl ether (TEG-MEE). Its average molecular structure (Fig. 93) of C₆₀O₈(TEG)₅(OH)₂₁(O⁻Na⁺)₇·5 H₂O (*M_w* 2453, TEG = TEG-MEE) was determined on the basis of evidence from UV-Vis, FTIR and NMR (¹H, ¹³C, COSY, HSQC and HMBC) spectroscopies, and elemental and thermal gravimetric analyses. In the absence of reliable mass spectrometry data, a new quasi-graphical method for interpreting thermal decomposition data from elemental and thermal gravimetric analyses was developed to help in this regard. Incomplete combustion during EA led to the negation of some assumptions in these calculations but the average structure was ultimately determined from considerations

of: the total mass left after EA; the mass loss corresponding to fullerene-bound TEG-MEE and hydroxyl groups; and the mass loss corresponding to secondary bound water. The high degree of hydroxylation of the fullerene cage is consistent with its high water solubility. The fluorescence quantum yield of **1** (0.57%) was found to be an order of magnitude higher than pristine C₆₀, and it displayed a remarkably large Stokes shift (258 nm at λ_{ex} 280 nm). This bathochromic shift is very promising for potential exploitation in cell labelling and other biomedical tracking applications as the inner-filter effect is negated and absorption by surrounding tissue would be reduced. UV-Vis absorption measurements of aqueous solutions of **1** at various concentrations suggested the existence of a critical micelle concentration at 1.0 mg/mL. This suggested that the photoluminescent behaviour of **1** could well be down to aggregation-induced emission. Further, it implied the presence of favourable non-covalent interactions causing molecules of **1** to self-assemble and form larger structures.

This self-assembly process was explored further in Chapter 3. Within two weeks of being stored undisturbed in the dark, aqueous solutions of **1** >0.5 mg/mL could be seen with the naked eye to contain floating gels, with a largest dimension in solution of 2 mm. Across all iterations and concentrations only one such hydrogel was observed per vial. These hydrogels, swollen with water, could be picked from solution and manipulated with tweezers, displaying viscoelastic behaviour. In order to gain an understanding of how these hydrogels were forming, optical microscopy was first used to investigate dried solutions of **1** at concentrations below the critical micelle concentration (<1.0 mg/mL). The observed two-dimensional fractal patterns ($D_b = 1.83$) of premicellar particles (~400–1300 nm diameter) strongly suggested that they were formed in the diffusion-limited aggregation regime, consistent with other systems that self-assemble into three-dimensional hierarchical structures, and indicative of strong non-covalent interactions between molecular building blocks. These strong non-covalent interactions are proposed to be, based on evidence from the average molecular

structure and computational studies in the literature, a combination of hydrophobic effects and favourable TEG-MEE—fullerene hugging interactions, enhanced by hydrogen bonding with surface hydroxyl groups. The hydrogels formed above the critical micelle concentration were imaged using SEM and indeed found to be hierarchical structures consisting of a porous network (average pore size $60 \pm 15 \mu\text{m}$) of hollow tubular fibres (inner diameter $\sim 900 \text{ nm}$, outer diameter $1.5\text{--}2 \mu\text{m}$). This extended tricontinuous structure is unprecedented for a non-polymeric covalent derivative of C_{60} , and its porous structure bodes well, in the context of nanomedicine, for drug delivery and cell scaffolding purposes.

Having observed self-assembly on a grand scale for C_{60} derivative **1**, the same functionalisation protocol was carried out on higher fullerenes C_{70} , C_{84} and $\text{C}_{90\text{--}92}$. The average structure of C_{70} derivative **3** was calculated using a similar method as devised for C_{60} derivative **1** and found to be $\text{C}_{70}\text{O}_{16}(\text{OH})_9(\text{O}^-\text{Na}^+)_9 \cdot 4 \text{ TEG} \cdot 18 \text{ H}_2\text{O}$, i.e. no convincing evidence of covalent attachment of TEG-MEE to the fullerene core was observed as for C_{60} derivative **1**. Its lesser extent of functionalisation compared to C_{60} derivative **1** was mirrored in its lower water solubility (1.04 mg/mL). Indeed, both are rationalised by the propensity of C_{70} to undergo oxidation reactions at a slower rate across its less strained and less reactive equatorial belt. This structural motif was thus observed to impact directly upon its self-assembly behaviour: **3** did not form any hydrogels at any concentration. Instead, small flakes ($< 400 \mu\text{m}$ in two dimensions, $\sim 110 \text{ nm}$ thickness) flocculated from solutions $> 0.2 \text{ mg/mL}$. This behaviour was ascribed to the presence of a hydrophilic hydroxylated equatorial belt (around which non-covalently bound TEG-MEE could wrap itself, as in C_{60} derivative **1**) and unfunctionalised hydrophobic poles, the combination of which, it is proposed, leads to a short-range patchwork-like structure within the flakes. Remarkably, C_{84} and $\text{C}_{90\text{--}92}$ functionalised using the same method (**4** and **5**, respectively) formed hydrogels whose hierarchical structures extended over 1 mm in at least two dimensions. Hydrogels of

Fullerene	Opposed Hydrophobic Corannulene-Like Regions After Functionalisation?	Covalently Bound TEG-MEE?	Hydrogel Morphology	Average Pore Size (μm)
C_{60} 1	No	Yes	Porous	60 ± 15
C_{70} 3	Yes, diametrically opposed	No	No hydrogel, flat flakes	N/A
C_{84} 4	Possible	Unknown, unlikely	Porous, small regions of flakes	19 ± 5
C_{90-92} 5	Possible	Unknown, unlikely	Porous, some flat regions	26 ± 11

Table 8: Relationship between fullerene shape and self-assembled structure: the density of the supramolecular hydrogel (proportional to the inverse of average pore size) increases with the likelihood of opposed corannulene-like hydrophobic regions existing in the fullerene building block after functionalisation.

both C_{84} derivative **4** (average pore size $19 \pm 5 \mu\text{m}$, hollow fibre inner diameter $0.6\text{--}0.9 \mu\text{m}$, outer diameter $1\text{--}2 \mu\text{m}$) and C_{90-92} derivative **5** (average pore size $26 \pm 11 \mu\text{m}$, fibre outer diameter $\sim 5 \mu\text{m}$) bore similarity to the hydrogels of C_{60} derivative **1** in their hierarchical fibrillar structures but with additional flat regions reminiscent of the flake-like morphology of the structures formed by C_{70} derivative **3**. Given the lack of covalent grafting of TEG-MEE to C_{70} cages and assumed lesser reactivity of C_{84} and C_{90-92} , these two higher fullerenes were expected to be hydroxylated in part but only non-covalently associated with TEG-MEE. Given the observed morphologies, a relationship between fullerene shape and self-assembled structure after this functionalisation protocol is proposed: the area of flat flake-like morphologies increases with the likelihood of opposed corannulene-like hydrophobic regions existing in the fullerene building block after functionalisation. As Table 8 demonstrates, C_{60} derivative **1** and C_{70} derivative **3** are the two extreme cases, with C_{84} derivative **4** and C_{90-92} derivative **5** intermediate of the two. The proposed link between molecular fullerene building block and hydrogel structure suggests an ability to tune the final properties of a desired supramolecular entity based on its starting material. Aside from

this, a more general conclusion can be drawn that hydroxylation of fullerene cages of different sizes in the presence of TEG-MEE, whether covalently bound or not, leads to extended hierarchical supramolecular architectures. Most excitingly, this has been shown to be applicable to higher fullerenes C_{84} and C_{90-92} , providing the first example of a route to synthesising macroscopic materials with potential medical utility from these rare building blocks.

AFM nanoindentation of all these supramolecular hydrogels yielded Young's modulus values in keeping with the structures observed in SEM: the most porous material (C_{60} **1** hydrogel) was the weakest while the least porous (C_{90-92} **5** hydrogel)¹ was the strongest. Due to uncertainty in the role the concentration of non-covalently bound TEG-MEE plays, a direct link between molecular building block and hydrogel Young's modulus cannot be confidently made. However, the observed strongest hydrogel (C_{90-92} derivative **5**, 432 ± 286 kPa) is very strong compared with other reported supramolecular structures in their hydrated state.

Chapter 4 moved away from molecular self-assembly to another type of supramolecular system: a mechanically interlocked fullerene-containing [3]rotaxane (Fig. 94). Recycling HPLC was used to improve upon an existing procedure for the isolation of bisalkyne methanofullerene building block **7**—in so doing, two new iodo-alkyne derivatives **9** and **10** were isolated and characterised. Methanofullerene **7** was incorporated via CuAAC as the central moiety in a four-station axle with two peripheral NDI stations before anion templation was used to introduce two ferrocene-containing macrocycles, giving [3]rotaxane **6**·Cl₂. Shuttling of the macrocycles between the centrally located triazolium stations and peripheral NDI stations was achieved by changing the size of the anion present: small coordinating chloride ions could fit within the cavity between the triazolium stations and the macrocycles; larger non-coordinating hexafluorophosphate ions could not fit within this cavity

¹ Within the designated error in average pore size, C_{84} **4** hydrogel and C_{90-92} **5** hydrogel are quite similar. The difference arises from C_{90-92} **5** hydrogel having a greater proportion of flat flake-like regions.

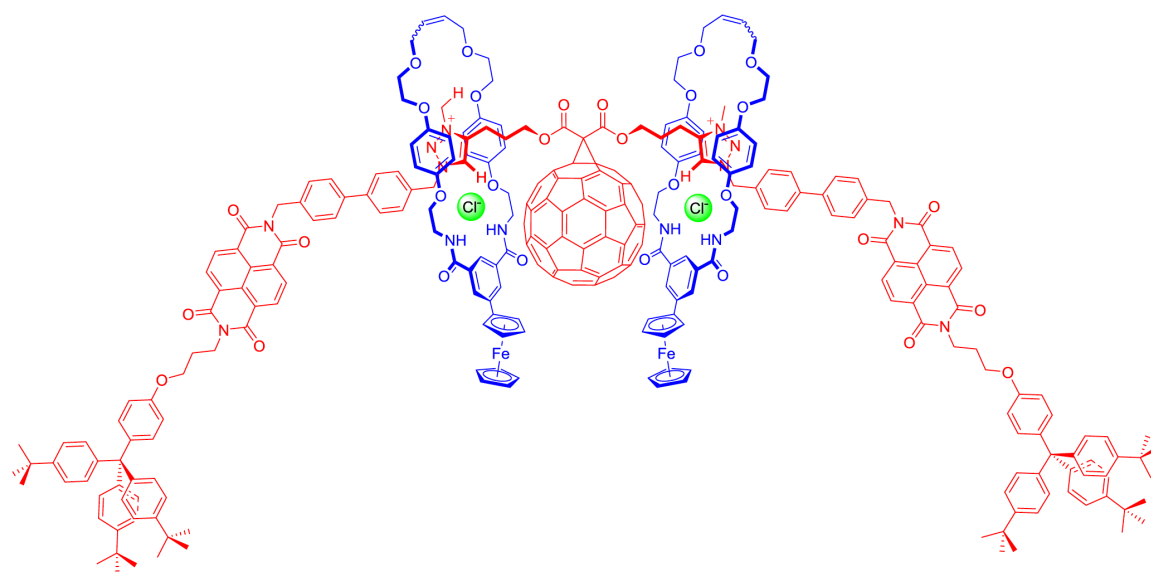


Figure 94: Structure of the chloride salt co-conformation of [3]rotaxane **6**·Cl₂.

and so the macrocycles shuttled to the NDI stations with which they could favourably interact via aromatic stacking. This controllable large amplitude motion meant that the ferrocene electron donors on the macrocycles could be controllably moved into proximity of either the C₆₀ or NDI electron acceptors depending on the anion present. Accordingly, this motion could be tracked using fluorescence spectroscopy, with a strong fluorescent response from the NDI moieties observed for **6**·Cl₂ while none was observed for **6**·(PF₆)₂, due to photo-induced electron transfer from ferrocene to NDI quenching the response from this co-conformation. As such, [3]rotaxane **6** was shown to act as an effective chloride sensor with visual output. Given the essential role played by chloride ions in the body with regards to cell water content, this is a proof of principle of a fullerene-containing rotaxane with potential biomedical applicability. Further, the ability of the two co-conformations of [3]rotaxane **6** to sensitise the production of cytotoxic ¹O₂ was investigated using EPR. Accordingly, the ¹O₂ yield of **6**·Cl₂ was 43% that of **6**·(PF₆)₂ due to photoinduced electron transfer to the fullerene moiety quenching its ¹O₂-generating ability. [3]rotaxane **6** was thus shown to have dual functionality based on the controlled motion of its mechanically interlocked molecular components. This

is the first example of a rotaxane with such dual functionality. The presence of fullerene in the supramolecular structure is essential to its function and, as such, demonstrates a complimentary approach to the self-assembly experiments as to how the supramolecular chemistry of fullerenes can be leveraged for application in the context of nanomedicine.

5.2 FUTURE WORK

WATER-SOLUBILISATION From a structural viewpoint, a first port of call would be the development of a small piece of software that encodes the new method for structural determination of TEGylated derivatives (e.g. as a MATLAB toolbox), such that experimental data would have to be input only once and the need for manual perusal of several huge Excel spreadsheets would be negated. Going forward, it would be interesting to try and calibrate the method to other uncontrolled fullerene functionalisation procedures (e.g. amination). As such, a useful tool could be created for distribution to researchers working on highly functionalised fullerene derivatives. To further this new functionalisation motif into more biomedical applications, several routes are possible. The first would be to verify the suitability of **1** for these applications by assessing its toxicity *in vitro* and *in vivo*. Alongside this, to compliment its promising fluorescence properties and investigate it as a theranostic platform, its ability to generate $^1\text{O}_2$ and also absorb ROS should be assessed as these are known to depend strongly on the degree of functionalisation. Another route would be to investigate the TEGylation protocol on endohedral fullerenes, particularly gadolinium-containing EMFs such as $\text{Gd}_3\text{N@C}_{80}$ for their potential use as MRI CAs.

SELF-ASSEMBLY The discovery of these fullerene-based hierarchical self-assembled hydrogels poses further questions and also opens up many doors for research in the nanomedicine sphere and beyond. At a fundamental level, the need (or lack thereof) for covalent TEGylation of C_{60} in order to achieve supramolecular gelation could be investigated. As apparently with the higher fullerenes, can such structures be formed just by mixing hydroxylated fullerenes with TEG-MEE? Further, it is not yet clear if there is a single molecular species responsible for the extended hierarchical structures, or if a distribution of molecular structures is a necessary prerequisite. For emergent properties from complex systems of this sort, the answer is not immediately obvious and never trivial. Separation (e.g. by HPLC) and individual characterisation of the constituent building blocks could be carried out potentially to try and isolate a prime culprit. Arguably more pressingly, the hierarchical structures formed need to be characterised using small angle scattering techniques such as SANS and SAXS in order to gain insight into their internal structure—this would help inform as to whether the packing structures proposed herein are on the right tracks or not. Excitingly, given the presence of hierarchical structures for fullerenes beyond C_{70} , it could be interesting to try out the TEGylation protocol on a mixture of fullerenes obtained from the arc discharge process—would the supramolecular structures formed have hybrid morphologies of those observed herein? Would they even form? This could potentially lead to two very interesting outcomes:

1. The ability to functionalise fullerenes *en masse* (without needing the costly procedures of separating and isolating them individually) to give porous carbonaceous materials;
2. The ability to tune the hierarchical structure formed based on the particular blend of fullerenes used (i.e. would hybrid morphologies be controllable?)

Both of these would pose promising ramifications for the development of fullerene-based materials on industrial scales. Of course, before this, another avenue of investigation is optimisation of the gelation process so as to minimise the times involved. In more applied studies, these hydrogels could be investigated as scaffolds for growing cells²—their differing mechanical properties would imply that cell proliferation would also differ. The porous nature of these materials, with their small radius hollow fibres, also holds great potential for investigation as drug delivery vehicles so their ability to first load drugs should be investigated.

ROTAXANES With regards to the iodo-alkyne derivatives **9** and **10**, they could be exploited for the synthesis of 5-iodo-1,2,3-triazoles using CuAAC, which could be used to exploit even stronger halogen bonding in anion-templated rotaxane synthesis [405, 406]. Halogen bonding in rotaxanes has been shown to aid in sensing of analytes even in competitive aqueous solution [407], so this is of particular importance for biomedical applications. It would be sensible to synthesise a simpler [2]rotaxane analogue³ of [3]rotaxane **6** containing C₆₀ and NDI stations and Fc on the macrocycle and compare its fluorescence and photosensitisation properties with those presented herein. The simpler structure could then be modified in a modular manner to assess various properties—for example, the fullerene could be TEGylated for water solubility and the self-assembly properties of the system investigated. In this way, we might anticipate to synthesise self-assembled structures with a fullerene core and radiating rotaxane unit—similar structures with gold nanorod [408] and mesoporous silica [409] cores have proved effective as multi-modal theranostic nanomedicine platforms.

² Early promising conversations have already taken place for a collaboration with researchers at the University of Manchester.

³ This would require a fullerene derivative with only one terminal alkyne group, which is possible via the Prato reaction [192] or with an asymmetric malonate for the Bingel reaction.

5.3 FINAL WORD

Particularly pertinent to the work presented in this thesis, one must never forget the “cultural context of nanomedicine” [410]. Drawing upon such a breadth of source material in order not only to carry out appropriate experiments, but also to contextualise the results in a meaningful way, has been no easy feat. Arguably even harder is the task of relaying these results to relevant audiences: different disciplines speak different languages and, in some of my experience, can be easily dismissive of work not set in their traditional mould. This barrier has to be overcome if the promise of nanomedicine is to live up to its hype and actually be used in clinic. As with any work with a medical slant, the aim should be the betterment of patients. Being open and willing to engage in conversations across disciplines, and perhaps even to adopt experimental techniques less familiar, means that research of a more fundamental nature is given the space to breathe and find a broader audience, who may just know how to take things forward in a more applied direction.

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