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Highly selective reactions of $C_{60}Cl_6$ with thiols for the synthesis of functionalized [60]fullerene derivatives†

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Chlorofullerene $C_{60}Cl_6$ undergoes highly selective reactions with thiols forming compounds $C_{60}[SR]_5H$ with high yields. These reactions open up straightforward synthetic routes to many functionalized fullerene derivatives, *e.g.* water-soluble compounds showing interesting biological activities.

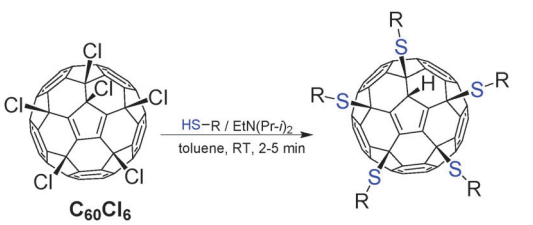
Fullerene chemistry has been intensively developed since 1991–1992, when pristine C_{60} and C_{70} became available in bulk quantities.[1] Many reactions discovered for C_{60} and C_{70} fullerenes are based on the addition of reagents across one of the double bonds of the carbon cage.[2] The formation of such monofunctionalized fullerene derivatives is selective only in the case of C_{60}, while C_{70} usually produces mixtures of regio- and stereoisomers with except for very few notable reactions.[3] Double, triple or multiple functionalization of C_{60} and C_{70} fullerenes yields typically barely separable mixtures of isomeric products.[4] Thus, selective derivatization of C_{60} and C_{70} molecules with multiple organic addends is a great challenge in the field of fullerene chemistry.

Halogenation of fullerenes is one of very few reactions that proceed with remarkably high selectivity. For example, chlorination of C_{60} or C_{70} using ICl solution as a reagent produces individual isomers of $C_{60}Cl_6$ or $C_{70}Cl_{10}$ in quantitative yields.[5] Availability of isomerically pure chlorofullerenes inspired researchers to perform their functionalization by replacement of halogen atoms with organic functional groups. Taylor *et al.* investigated reactions of chlorofullerenes with C- and O-nucleophiles: aromatic hydrocarbons in the presence of Lewis acids, MeLi, $H_2C{=}CHCH_2TMS$ and alcohols.[6] The reaction of $C_{60}Cl_6$ with organic cyanides was shown to produce salts of the stable pentacyanofullerene anion $[C_{60}(CN)_5]^-$.[7] Unfortunately, these reactions exhibited insufficient selectivity and required exhaustive chromatographic separation for isolation of the target products in low yields.

We investigated previously the reactions of $C_{60}Cl_6$ with N-nucleophiles. Initial studies revealed the formation of 1,4-diaminofullerenes as the only isolable products of the high-temperature $C_{60}Cl_6$ reactions with amines.[8] Our recent experiments showed that low-temperature syntheses employing salts of amines produce pentaaminofullerenes $C_{60}[NHR]_5X$ (X = H, Cl) with high yields.[9] Low stability of the aminofullerenes in aqueous solutions due to hydrolysis with the formation of fullerenols limits their practical applications.

Here we report the first reactions of halogenated fullerenes with S-nucleophiles. It has been shown that chlorofullerene $C_{60}Cl_6$ reacts readily with thiols producing a novel family of fullerene derivatives. These reactions proceed instantly (2–5 min) at room temperature in the presence of an organic base. The target products $C_{60}[SR]_5H$ are isolated with 80–98% yields using flash chromatography or simple non-chromatographic work-up (in the case of water-soluble compounds). Scheme 1 illustrates the potential of the discovered reaction. We believe that the reaction of $C_{60}Cl_6$ with thiols proceeds *via* a sequence of electron transfer, chlorine anion elimination and RS^- addition steps (a proposed mechanism is shown in Scheme S1, ESI†).

The synthetic procedures applied for the preparation of **1a–1i** are rather simple. Typically, chlorofullerene $C_{60}Cl_6$ (1000 mg) was dissolved in toluene (800 ml) under continuous



1a: R= -$C_{12}H_{25}$, 98%
1b: R= -CH_2COOMe, 80%
1c: R= -CH_2COOEt, 92%
1d: R= -$CH_2COOCH_2CH(CH_3)CH_2CH_3$, 83%
1e: R= -CH_2CH_2COOMe, 85%
1f: R= -CH_2CH_2NHBOC, 86%
1g: R= -CH_2COOH, 87%
1h: R= -CH_2CH_2COOH, 95%
1i: R= -$(CH_2)_{10}COOH$, 84%

Scheme 1

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† Electronic supplementary information (ESI) available: Spectroscopic data, HPLC profiles, NMR and ESI MS spectra for **1a–i**; crystallographic data for **1c**. CCDC 875844. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc32517a

stirring in air. The thiol reagent was added in a large excess (10–20 eq.) followed by 6 eq. of diisopropylethylamine (Hünig's base). The resulting mixture was stirred at room temperature for 2–5 min until TLC showed complete chlorofullerene conversion. The reaction mixtures were concentrated to the volume of 100 mL and poured on the top of a chromatographic column filled with silica gel in the cases of **1b–1f**. Elution with toluene/ethyl acetate mixtures produced first trace amounts of the by-products (brown $C_{60}(SR)_2$ and red-brown $C_{60}(SR)_4$) followed by bright orange-red fractions of the title products **1b–1f**. The concentration of the reaction mixture to the volume of 30 mL and addition of 300 mL of methanol resulted in the precipitation of a dark red oil of **1a**. The pentacarboxylic acids **1g–1i** are insoluble in toluene and precipitate from the reaction mixture. These precipitates can be easily collected by filtration or centrifugation. The dispersion of raw **1g–1i** samples in deionized water followed by titration with 0.1 M K_2CO_3 produces transparent aqueous solutions of potassium salts of **1g–1i**. Filtration and re-precipitation by addition of an excess amount of 1 M hydrochloric acid followed by vacuum drying produces pure carboxylic acids **1g–1i**.

High compositional purity of the prepared thiofullerenes **1a–f** was revealed by HPLC. Molecular compositions and structures of **1a–i** were confirmed by NMR spectroscopy and mass spectrometry. The exemplary NMR spectra of **1h** shown in Fig. 1 fully comply with the proposed molecular formula shown in Scheme 1. The spectra of all thiofullerenes are provided in the ESI (Fig. S1–S47†).

The thiofullerene **1c** formed well-shaped single crystals suitable for X-ray single crystal diffraction analysis. The molecular structure of **1c** is shown in Fig. 2 in two projections. Five sulfide groups are arranged around the central pentagon on the fullerene cage. The hydrogen atom is attached to the central pentagon, thus completing the cyclopentadienyl type framework.

The selectivity of the room-temperature reactions of $C_{60}Cl_6$ with thiols was quite remarkable. The by-products were formed

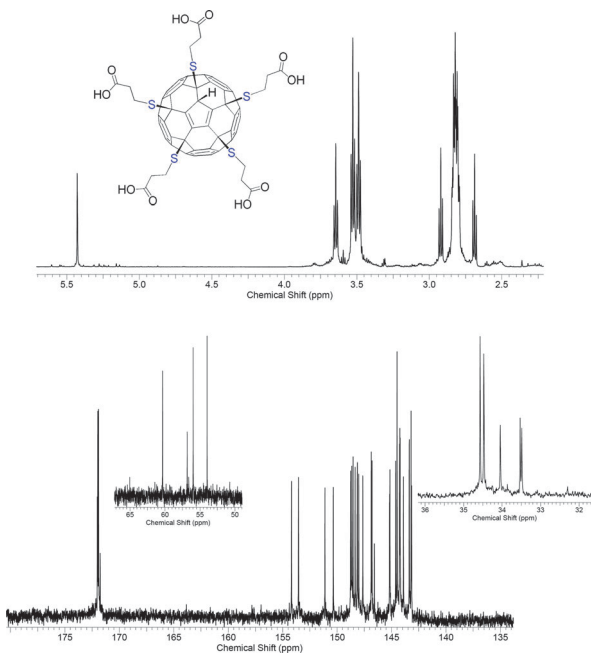

Fig. 1 ^1H (top) and ^{13}C (bottom) NMR spectra of **1h**.

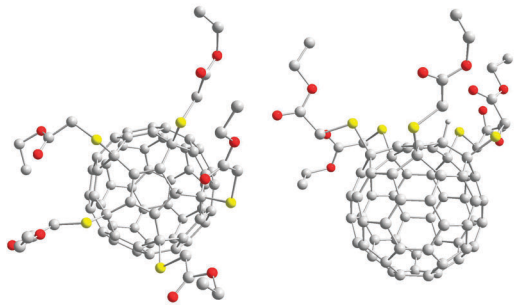

Fig. 2 Two projections of the molecular structure of **1c** according to the X-ray single crystal diffraction data. Disorder of some attached groups is not shown. Methylene and methyl H atoms are omitted for clarity.

in such low amounts that it was not possible to isolate and characterize them. Increasing the reaction temperature to 130 °C (boiling chlorobenzene) allowed us to decrease the selectivity of the reaction and promote the formation of the by-products (yields up to 20–30%). Exemplary compound **2e** bearing two sulfide ($-SCH_2CH_2COOMe$) groups attached at positions 1 and 4 of the fullerene cage has been isolated and spectroscopically characterized.

We emphasize that polycarboxylic acids **1g–i** have been produced on a large scale (2–6 g) in a single step starting from chlorofullerene $C_{60}Cl_6$ which is a readily available precursor (synthesized in 10–15 min from C_{60} with quantitative yield[5,10]). The syntheses of **1g–i** were performed in air within 2–5 min at room temperature. High-purity products were obtained without using time and solvent consuming chromatographic separation. Moreover, toluene applied as a solvent for $C_{60}Cl_6$ was recycled and reused many times. Therefore, the developed thiol-based approach seems to be the most efficient method for the synthesis of water-soluble polycarboxylic fullerene derivatives. This technology can be applied on an industrial scale for the production of water-soluble fullerene derivatives for biomedicinal applications.[11]

The potassium salts of **1g–i** (**1g–K**, **1h–K**, **1i–K**) showed high solubility in water (>200 mg ml^{-1}) and excellent stability. Heating their aqueous solutions in air at reflux for 2 h does not produce any detectable amounts of hydrolysis products. Note that aminofullerenes decompose almost immediately under such conditions. We have shown that the synthesized water-soluble fullerene derivatives have rather low acute toxicity determined *in vivo* using intraperitoneal injection in mice (Table 1).‡

Fullerene derivatives are known to be potent antiviral agents inhibiting viral enzymes such as HIV protease and gp-120 enzymes.[10,11] *In vitro* studies have been performed to reveal the antiviral activity of compounds **1h–K** and **1i–K** using a broad panel of DNA and RNA viruses (Table 2). Both compounds show a moderate activity against HIV-1. **1i–K** inhibits respiratory

Table 1 Acute toxicity of the water-soluble fullerene derivatives[a]

Compound	MTD, mg kg^{-1}	LD_{50}, mg kg^{-1}	LD_{100}, mg kg^{-1}
1g–K	800	900	1000
1h–K	150	250	350
1i–K	400	900	1400

[a] MTD is a minimal tolerated dose, LD_{50} is a median lethal dose, LD_{100} is a lethal dose.

Table 2 Antiviral activity of the water-soluble fullerene derivatives

Compound	Cell culture	MCC, μM[a]	Virus strain	EC_{50}, μM[a]
1h–K	CEM	> 100	HIV-1	45.2
			HIV-2	> 100
	MDCK	14.0	Influenza A (H3N2)	2.0
1i–K	CEM	> 100	HIV-1	24.0
			HIV-2	42.0
	HeLa	> 100	Respiratory syncytial virus	22.5
Ribavirin	MDCK	> 100	Influenza A (H3N2)	27.8
	HeLa	> 250	Respiratory syncytial virus	21.7
Oseltamivir carboxylate (Tamiflu®)	MDCK	> 100	Influenza A (H3N2)	13.8

[a] MCC or minimal cytotoxic concentration required to cause a microscopically detectable alteration of cell morphology; EC_{50} is a concentration required to reduce virus-induced cytopathicity by 50%.

Table 3 Inhibition of PTPs by water-soluble fullerene derivatives

Compound	PTB1B, IC_{50}[a], μM	TC-PTP, IC_{50}, μM	PTP-β, IC_{50}, μM	LAR-PTP, IC_{50}, μM
1h–K	0.20 ± 0.04	2.9 ± 0.01	4.0 ± 0.8	16.4 ± 1.7
1i–K	0.21 ± 0.01	1.6 ± 0.04	11.2 ± 1.7	2.1 ± 0.6

[a] IC_{50} is the concentration required to reduce the PTP activity by 50%.

syncytial virus with an efficacy comparable to that of ribavirin. Even more remarkable is the activity of **1h–K** against influenza A H3N2 virus subtype. It is shown in Table 2 that **1h–K** is a few times more active against this virus compared to the commercial drugs such as ribavirin and oseltamivir carboxylate. It is notable that water-soluble derivatives of [70]fullerene were also previously shown to be active against influenza viruses.[12] Therefore, further exploration of the antiviral activity of water-soluble fullerene derivatives may reveal new pharmaceutically interesting lead compounds.

Fullerene derivatives were also shown to be potent inhibitors of different enzymes.[13] Protein tyrosine phosphatases (PTPs) catalyze dephosphorylation of phosphotyrosine residues in proteins and are involved in a number of biochemical processes, including cell-signalling and metabolism pathways.[14] PTP1B is a widely expressed phosphatase, which is being considered as a promising target for new drug development to treat diabetes and obesity.[15] We have investigated activities of **1i–K** and **1h–K** as inhibitors of the protein tyrosine phosphatases PTP1B, TC-PTP, PTPβ and LAR-PTP. Both compounds inhibit the target PTP1B at high nanomolar concentrations (Table 3). Moreover, they show high selectivity with respect to PTP1B, thus discriminating it among other investigated PTPs (see Fig. S45, ESI†). These results suggest that fullerene derivatives might be particularly useful in the design of highly selective inhibitors of PTP1B, which might lead to pharmaceutical applications.

In conclusion, we have shown that thiols can be used as reagents for efficient derivatization of chlorofullerene $C_{60}Cl_6$, producing thiofullerenes, a novel family of fullerene derivatives. Remarkably high selectivity of the revealed reaction opens up wide opportunities for the synthesis of multifunctional fullerene derivatives for materials science and biomedicinal applications.

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Notes and references

‡ All experiments with live animals were performed in compliance with the relevant laws of Russian Federation, the institutional guidelines, and were approved by the board of directors of the Institute for Physiologically Active Compounds of Russian Academy of Sciences where these experiments were performed.

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