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Toward fullerene-based X-ray contrast agents: design and synthesis of non-ionic, highly-iodinated derivatives of C_{60}

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Abstract—An X-ray contrast agent precursor based on C_{60} has been designed, synthesized, and characterized. The compound is the monoadduct of a malonodiamide containing six iodine atoms and eight acetal-protected alcohols with multiple hindered rotations at ambient temperature.

The use of contrast agents has become routine in contemporary medical imaging. Although technological advances have increased the popularity of ultrasound and magnetic resonance imaging techniques, X-ray related imaging still accounts for 75–80% of all diagnostic imaging procedures. Water-soluble, non-ionic X-ray contrast agents such as Iohexol[1] are based on the 1,3,5-triiodobenzene structure (Fig. 1). Administered intravenously, the agents enhance radiographic image contrast by increasing X-ray attenuation via their multiple electron-rich iodine atoms. In the US alone, iodinated X-ray contrast agents are currently used in approximately 20 million procedures annually.[2]

Today's X-ray contrast agents are reasonably safe and effective. However, there is current interest in novel compounds that could give superior performance (i.e. tungsten clusters[3]), and specialized compounds for niche applications (i.e. liver-specific[2] and blood-pool agents[4]). In view of the increasing interest in the medical application of fullerene materials,[5] we report here the synthesis and characterization of the first X-ray contrast agent precursor, **5**, based on the biologically compatible[6] and versatile C_{60} scaffolding (Fig. 2).

Starting from 5-aminoisophthalic acid (Scheme 1), diacid dichloride **1** was prepared in two steps as previously reported.[7] Condensation of **1** with malonyl dichloride in refluxing THF gave **2** as colorless microcrytals in 83% yield.[8] Given the solubility limitations of C_{60}, it was necessary for the 1,3-diols of **4** to be

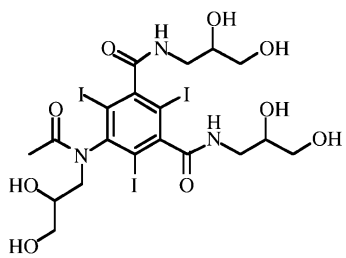
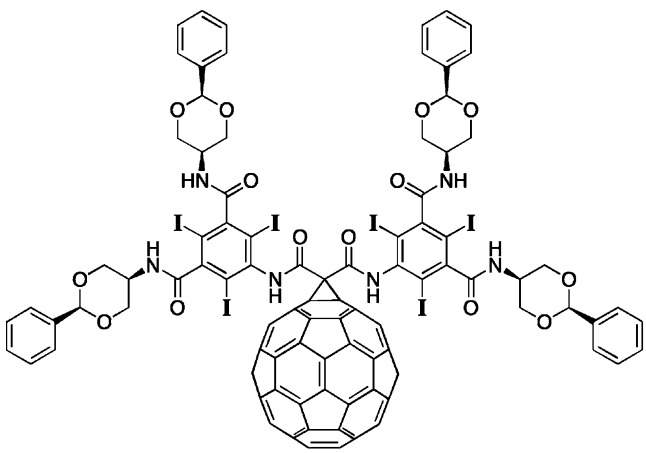


Figure 1. Structure of Iohexol.



5

Figure 2. Structure of the C_{60}-based X-ray contrast agent precursor, **5**.

Keywords: X-ray contrast agent; fullerenes; fullerene derivatives.

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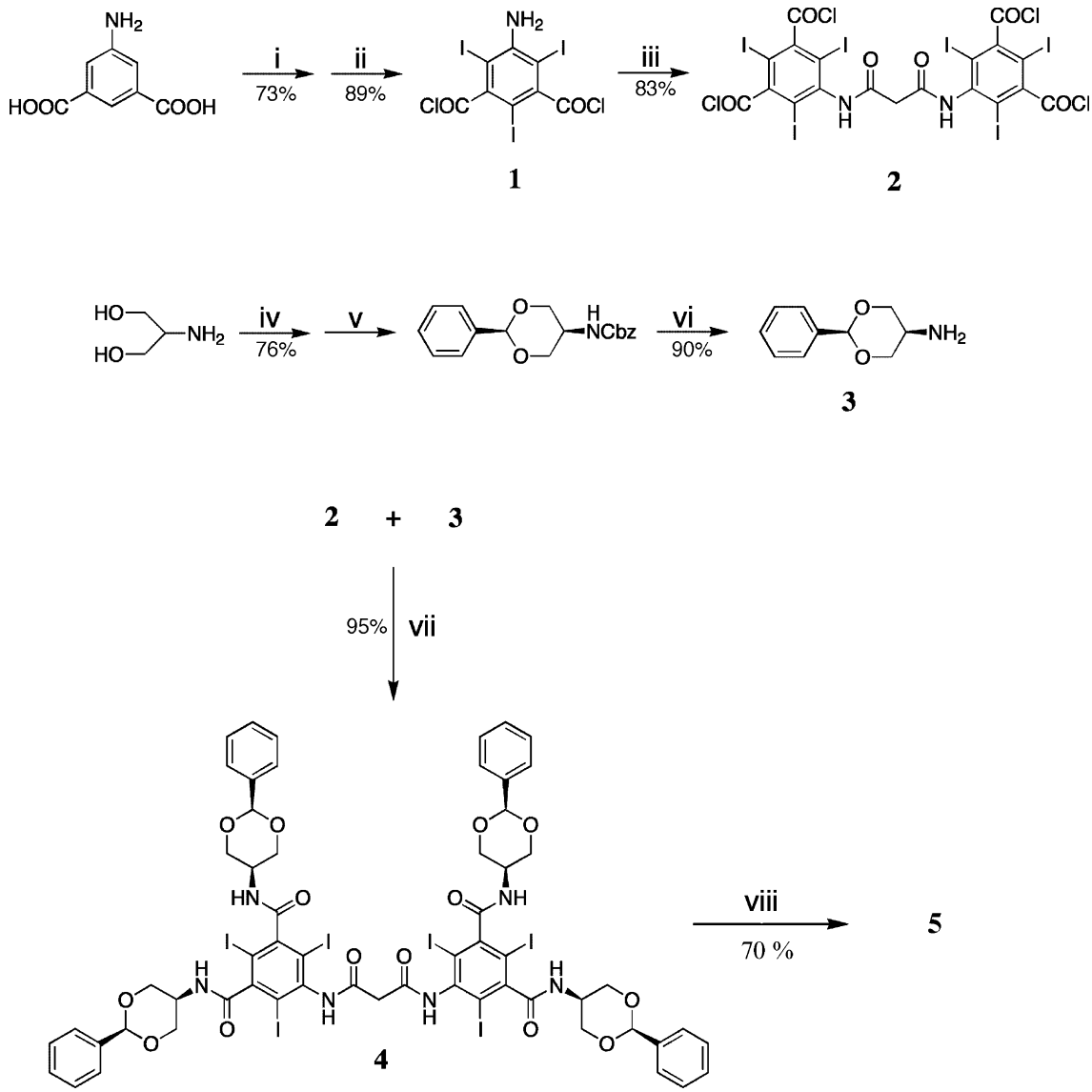

Scheme 1. Synthesis of **5**. *Reagents and conditions*: (i) $KICl_2$, H_2O, 55°C, 24 h; (ii) $SOCl_2$, reflux 6 h; (iii) $CH_2(COCl)_2$, THF, reflux 6 h; (iv) benzyl chloroformate, NEt_3, EtOH; (v) PhCHO, cat. H_2SO_4, toluene, reflux 6 h; (vi) 300 psi H_2, Pd/C, EtOH, 50 min; (vii) DMA, NEt_3, 24 h; (viii) C_{60}, CBr_4, DBU, toluene:pyridine 4:1.

protected so that a mutual solvent system could be used when appending **4** to the fullerene. This was most easily achieved by *O*,*O*′-protection of serinol (3-amino-1,2-propanediol) prior to condensation with **2**, and this was carried out in three steps. Protection of the serinol amine as the benzyl carbamate was followed by benzylidene acetal formation with benzaldehyde (cat. H_2SO_4) to give an equilibrium mixture of *cis*:*trans* substituted 1,3-dioxane in a ratio of 8:2. The *cis* isomer selectivity can be rationalized by the axial preference for the *N*-benzyl carbamate group due to intramolecular N–H···O hydrogen bonding.[9] The *cis* isomer of **3** has also been shown to react as a nucleophile at twice the rate of the corresponding *trans* isomer.[10] Thus, the intermediate *cis* isomer of *N*- and *O*,*O*′-protected serinol was purified by fractional recrystallization and the *trans* isomer was recycled in subsequent runs. Removal of the benzoxycarbonyl group by catalytic hydrogenation of the *cis* intermediate gave *O*,*O*′-benzylidene protected serinol, **3**, as the *cis* isomer exclusively. The condensation of **2** with **3** proceeded smoothly in dimethyl acetamide (DMA) with NEt_3 in 95% yield to give **4**.[11] For the final addition of **4** to C_{60}, the method of Hirsch was used.[12] Accordingly, DBU (1,8-diazabicyclo[5.4.0]-undec-7-ene) was added to a solution of CBr_4, **4**, and C_{60} in 4:1 toluene:pyridine. After chromatographic workup, **5** was obtained as a brown solid in 70% yield.

Compounds **4** and **5** are interesting examples of sterically-congested hexasubstituted benzenes[13] with as many as ten hindered rotations at ambient temperature. Variable-temperature 500 MHz ^1H NMR experiments of **4** (see Ref. 11) indicated that although most rotations are free at 70°C, some rotations remain hindered even at 130°C in DMSO-d_6 as evidenced by a still changing spectrum. However, thermal decomposition of the benzylidene acetals prohibited even higher tem-

perature experiments. An interpretable ^{13}C NMR spectrum (500 MHz) of **4** was achieved at 70°C with the expected 14 signals.[11] However, the 500 MHz ^{13}C spectrum of **5** (0.05 M in pyridine-d_5) at 70°C was still very broadened and the S/N ratio of most of the signals was too small to interpret even after 17 h of data collection. This indicates that some of the rotations of the addend (**4**) are significantly more hindered on addition of the malonodiamide to C_{60}.

Anilide and substituted anilide malonodiamide derivatives of C_{60} have been reported to be paramagnetic.[14] However, solid-state Evan's balance measurements indicated that **5** is essentially diamagnetic, ruling out paramagnetism as a major source of signal broadening in the NMR spectra. In the case of **5**, it is likely that the fully-substituted anilide, with it's large iodine atoms, is too sterically encumbered to allow a significant charge-transfer type interaction between the phenyl ring and the cage of C_{60} that are thought to give rise to paramagnetism in related derivatives.[14] In fact, it is somewhat surprising that **4** reacts with C_{60} in such good yield, considering its significant steric demands.

In vitro studies have shown[15] that **5** provides X-ray attenuation comparable to the commercial agent Iohexol as expected based on its 30% iodine content. However, a contrast agent that is based on C_{60} offers potential advantages over conventional X-ray contrast agents. For example, such fullerene-based agents when used in vivo, could prove to be specific for liver or kidney tissue and thus serve as tissue-specific imaging agents. The versatility of the fullerene core allows a 'tuning' of the properties of an X-ray contrast agent by varying the type or degree of functionalization. In addition, the larger, pseudo-spherical, molecular volume of a C_{60}-based agent could increase blood-pool retention time,[4] and may be confined to the vascular spaces, leading to a novel 'blood-pool' contrast agent.

In conclusion, the first X-ray contrast agent precursor based on C_{60} has been synthesized and characterized. Water solubilizing **5** with malonodiserinolamide moieties,[16] followed by benzylidene acetal cleavage, should lead to the first fullerene-based X-ray contrast agent for in vivo imaging.[17] Efforts in this direction are continuing.

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11. Spectral data: for **4**; TLC R_f 0.54 in 1:1 THF:toluene; ^1H NMR (500 MHz, DMSO-d_6, 70°C) δ (ppm, solvent std.) 3.57 (s, 2H, -COCH_2CO-), 3.99 (distorted d, $J=6.5$ Hz, 4H, -NHCH(CH_2-)$_2$), 4.22 (distorted q, $J=8.4$ Hz, 16H, -CH(CH_2O-)$_2$), 5.63 (s, 4H, PhCH(O-)$_2$), 7.35 (m, 12H, PhH), 7.50 (m, 8H, PhH), 7.98 (bs, 1H, NH), 8.7–9.3 (bm, 3H, NH), 10.08 (s, 2H, NH); ^{13}C NMR (500 MHz, DMSO-d_6, 70°C) δ (ppm, solvent std.) 41.95 (-CO-CH$_2$CO-), 43.70 (-NHCH(CH$_2$-)$_2$, 68.45 (-CH(CH$_2$O-)$_2$), 90.21, 98.10 (arom. CI), 100.40 (PhCH(O-)$_2$), 126.14, 127.40, 128.23 (arom. CH), 138.27 (arom. CCH(O-)$_2$), 142.07 (arom. CNH-), 149.22 (arom. CCONH-), 164.55, 168.90 (C=O); FT-IR 1654 (s), 1103 (s); MALDI-TOF MS calcd for $C_{59}H_{52}N_6O_{14}I_6$ [M]$^-$ 1829.8, found 1828.7 [M−1]$^-$; Anal. ($C_{59}H_{52}N_6O_{14}I_6$) calcd: I, 41.60; C, 38.71; H, 2.86; N, 4.59. Found: I, 41.86; C, 39.17; H, 3.34; N, 4.41; UV λ_{max} 285; for **5**, TLC R_f 0.75 1:1 THF:toluene; ^1H NMR (500 MHz, pyridine-d_5, 70°C) δ (ppm, solvent std.) 4.16–5.10 (bm, 20H, -NHCH(CH$_2$O-)$_2$, -NHCH-(CH_2O-)$_2$), 5.78–6.05 (bs, 4H, PhCH(O-)$_2$), 7.33–7.68, 7.68–7.96 (bm, 20H, PhH), 8.89 (1H, NH); MALDI-TOF MS calcd for $C_{119}H_{50}N_6O_{14}I_6$ [M]$^-$ 2547.8. Found: 2547.5; FT-IR 1654 (s), 1103 (s), 526 (m); UV λ_{max} (nm) 273, 328; Anal. ($C_{119}H_{50}N_6O_{14}I_6$) calcd: I, 29.87; C, 56.07; H, 1.98; N, 3.30; O, 8.79, found: I, 31.26; C, 54.76; H, 2.60; N, 3.05; O, 8.34 (O by subtraction).
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15. In 1 mL syringes, a 0.197 M (150 mg I/mL) solution of **5** in 5:1 DMF:toluene, 4 standard aqueous solutions of Omnipaque® at concentrations of 300 mg I/mL, 225 mg I/mL, 150 mg I/mL, and 75 mg I/mL (diluted with saline), and 2 samples containing the saline (0.9% NaCl) and the DMF:toluene backgrounds were exposed to diagnostic wavelength (0.087–8.5 nm) X-rays in a Faxitron 43855A imager. The image was captured on standard Kodak MIN-R 2000 film. The density of the film was determined for each of the samples with a Noritsu Model 810 densitometer. The solution of **5** showed X-ray attenuation comparable to that of the standard.
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