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HIGHLY-IODINATED FULLERENE AS A CONTRAST AGENT
FOR X-RAY IMAGING

by
TIM WHARTON

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE

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ABSTRACT

**Highly-iodinated Fullerene as a Contrast Agent
For X-ray Imaging**

by

Tim Wharton

The first fullerene-based X-ray contrast agent (CA) has been designed, synthesized, and characterized.

The new CA is an externally functionalized derivative of C_{60} that is conceptually based on contemporary X-ray CA, all of which use iodine as the X-ray attenuating vehicle and are built on the 2,4,6-triiodinated-benzene-ring substructure. Aqueous solutions of the agents are injected intravenously via catheter into patients followed by X-ray imaging. The CA is then eliminated rapidly through the kidneys.

A modified Bingel-type reaction (nucleophilic cyclopropanation) was developed in which 6 iodine atoms can be appended to C_{60} (per addend) to form a cyclopropane ring exclusively across one of the [6,6] double bonds of C_{60} . Each addend contains two 2,4,6-triiodinated-benzene-ring moieties attached to a malonodiamide functionality through a nitrogen in the 5 ring

position, with water-solubilizing 1,3-diol-containing serinol-benzamide substituents in the remaining ring positions (1 and 3). When the malonodiamide is reacted with C_{60} in excess (≥ 3 molar excess), however, only the fullerene monoadduct forms in good yield, with only small amounts of the diadduct detected.

A general method for producing highly water-soluble, non-ionic fullerene materials was simultaneously developed. The synthetic approach also utilizes the new malonodiamide addend methodology to form multiple Bingel adducts with C_{60} to give $C_{60}[C(COSer)_2]_n$ ($n = 4, 5, 6$, Ser = 2-amino-1,3-propanediol). The compound is the most water soluble fullerene material reported to date ($> 240 \text{ mg } C_{60} \text{ mL}^{-1}$). In addition, the aqueous solubility has no notable pH dependence.

Due to the lack of water solubility of the amphiphilic iodinated C_{60} monoadduct, the water-solubilization methodology was combined with the iodination methodology to prepare a "hybrid" material that contains both the water-solubilizing groups and the iodine containing groups. The resulting fullerene-based X-ray CA is a fully water-soluble, non-ionic pentaadduct with one hexaiodinated addend containing 8 hydroxy groups and four additional addends each containing 4 hydroxy groups. The new, first generation fullerene-based CA contains 24 % iodine by weight.

The ideological development of the new CA, the successful (and some of the unsuccessful) synthetic pathways and the spectral characterization of the new products is presented and discussed.

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INTRODUCTION

1. X-ray Contrast Agent Background

Since the fortuitous discovery of X-rays by Wilhelm C. Roentgen in 1895, X-ray radiography has evolved into the foundation of contemporary medical imaging. The term “X-ray radiography” can be taken to encompass all of the technology involved in the creation of medically useful images using X-rays, from the production of X-ray radiation to the processing of raw photographic, or more recently, digital data. Although the past two decades have experienced an explosive growth in the ultrasound and magnetic resonance imaging (MRI) modalities (due largely to the advent of the microchip), fully 75-80% of all imaging procedures still entail the use of X-rays.¹

Contemporary X-ray radiography is intimately dependent upon the use of X-ray contrast agents (also called X-ray contrast media, radiopaque agents, and roentgenographic agents). With the exception of orally-ingested, barium sulfate slurries for gastrointestinal imaging, all commonly employed X-ray contrast agents (CA) are based on the 2,4,6-triodinated-5-aminoisophthalic acid substructure.^{1,2,3,4} The substructure, along with a typical, commercially-available X-ray CA, Iohexol,⁵ is shown in Figure 1. Such iodinated CA are currently used in approximately 20 million procedures in the US annually.^{6,7}

Figure 1. (1) The 2,4,6-triodinated-5-aminoisophthalic acid-derived substructure of contemporary X-ray contrast agents. (2) The structure of commercially-available Iohexol⁵ with typical 1,3-diol water-solubilizing substituents.

2. X-ray imaging in theory

The fractional decrease in X-ray radiation intensity (X-ray attenuation) upon passing through a tissue of interest can be expressed by Beer's law as shown in equation 1.

$$I/I_0 = e^{-\mu x} \quad \text{(Equation 1)}$$

where I and I_0 are the transmitted and incident radiation intensities, respectively. The tissue thickness is x (in cm), and μ is the linear attenuation

coefficient defined as the fraction of X-ray radiation intensity that is lost per cm of tissue thickness. In terms of interactions with the tissue of interest, μ increases with (a) an increase in tissue density and/or (b) an increase in the mean atomic number, Z_{eff} , of the tissue. (Note that the relationship between Z_{eff} and μ is a complex function due to the presence of atomic absorption edges which leads to a dependence of μ on the energy of the incident X-ray radiation.)^{8,9,10}

The coefficient μ has three contributions at the clinical X-ray energies of 40 - 140 keV, each specifying an independent interaction of the X-ray radiation with matter: Rayleigh or coherent scattering (ω), Compton or incoherent scattering (δ), and the photoelectric event (τ). Thus, μ is simply a summation of each type of interaction at a given X-ray energy as expressed in equation 2.

$$\mu = \omega + \delta + \tau \quad (\text{Equation 2})$$

The contribution from coherent scattering is never more than 10% (usually much less) and generally considered negligible. Therefore, the major contributors to X-ray attenuation are incoherent scattering and the photoelectric event, especially in the absence of contrast agent. In applications involving the use of X-ray CA (high Z_{eff}), however, τ is the dominant term.⁹ Equation 3 shows the proportionality of τ with the cube of Z_{eff} .

$$\tau \propto Z_{\text{eff}}^3 \quad (\text{Equation 3})$$

Thus, in the presence of iodinated contrast agents with relatively high atomic weights (Iodine, $Z = 53$), the approximation in Equation 4 holds true.¹⁰

$$\mu \propto Z_{\text{eff}}^3 \quad (\text{Equation 4})$$

The proportionality of the linear attenuation coefficient, μ , to the cube of the effective atomic number, Z_{eff} , approximates the significant contrast enhancement that is observed in the final x-ray radiographs due to the presence of the CA.

3. X-ray imaging in practice

Iodinated, water-soluble X-ray CA are technically known as uroangiographic agents since they are most commonly used (and best suited) for imaging of the blood vessels and/or the urinary system. An example of angiographic contrast enhancement is shown in

Figure 2. Typically, an aqueous CA commercial formulation is injected rapidly via catheter directly into the blood stream. For conventional radiography, about 30 mg iodine/cm² is necessary for adequate contrast enhancement. Therefore, a blood vessel that is 1 mm in diameter requires 300 mg iodine/mL

and for a 10 mm vessel, 30 mg iodine/mL. However, contemporary data processing techniques such as digital subtraction angiography (DSA) have been able to reduce the amount of CA needed by up to an order of magnitude.¹¹ (Other less common applications include imaging of the spinal fluid, the nasal sinuses, and the salivary gland ducts.)¹²

Figure 2. Digital Subtraction Angiography (DSA) using an iodine-based uroangiographic X-ray CA (A) X-ray radiograph of lateral view of human carotid artery area prior to injection of contrast agent (B) Same view after injection of iodinated X-ray contrast agent (C) Digital subtraction of images A and B showing blood vessel detail (D) Digital subtraction of images A and B with reintroduction of some anatomical data and digital edge enhancement (Siemens Medical Systems, Inc.).

All water-soluble CA are highly hydrophilic and, *in vivo*, are restricted to the extracellular spaces. Clinical formulations typically contain a near saturated solution of the agent at a concentration of 300 mg iodine/mL. Upon injection, the agents diffuse throughout the bloodstream and are rapidly excreted (typically, $t_{1/2} = 1.5\text{-}2\text{ h}$)¹³ primarily through the kidneys by glomerular filtration. The excretion of the CA by the kidneys allows imaging of the urinary system.

In addition to simple diffusion throughout the blood pool, partial extravasation (diffusion mechanism) through the intercellular junctions of the vascular endothelium occurs everywhere except where an organ/blood barrier exists (e. g. brain and prostate).^{13,14} In the presence of organ/blood barriers, the CA is completely confined to the vascular spaces and any detected leakage in these areas into the interstitial spaces is of significant diagnostic importance.¹⁵

Today's X-ray CA have evolved to the point where it is unlikely that a simple modification of the R groups in Figure 1 will lead to any significant improvement in tolerability or performance. Accordingly, much of the current research in X-ray CA is focused on novel systems such as tungsten clusters,^{16,17} metal chelates,^{18,19} iodinated dicarbon carborane cages,^{20,21} and fullerenes (this work).

One very active area of investigation is the search for a suitable "blood pool" CA. As mentioned above, rapid equilibrium between the vascular and interstitial spaces (known as extravasation) occurs with the commonly employed uroangiographic X-ray CA. The pores in the vascular endothelium allow the diffusion of molecules with molecular weights lower than about 70,000 g/mol which results in a lowering of the contrast between the vascular structures and the surrounding tissues.²² In addition, CA dilution into the extracellular fluid space may necessitate further injections of CA. A true blood-pool CA, which is confined entirely to the vascular spaces, would eliminate both of these complications. Examples of macromolecules investigated for use as blood pool agents include iodinated polysaccharides,^{23,24} polymer-bound triiodobenzenes,^{25,26} liposomes encapsulating triiodinated monomeric²⁷ and hexaiodinated dimeric agents,²⁸ nanoparticles,²⁹ micelles,³⁰ and emulsions.³¹ However, a true blood pool agent has yet to make it to the market.

4. A Fullerene-based X-ray Contrast Agent

The use of C₆₀-derived materials for biological applications is currently an active area of research and several reviews have been written on the subject.^{32,33,34} The idea of using the easily derivatized, non-toxic

scaffolding^{35,36} of C₆₀ for the development of a new X-ray contrast agent has not previously been explored. Thus, the design, synthesis, and characterization of a fullerene-based X-ray CA represents a novel undertaking. (The potential advantages of a C₆₀-based X-ray CA are explored in the Results and Discussion: Design Concepts section.)

This work details the ideological and synthetic development of a first-generation, highly-iodinated (24 % I by weight), water-soluble, non-ionic X-ray CA based on the scaffolding of C₆₀. The agent, as shown in Figure 3, is the result of the evolution of synthetic methodology in the light of practical design considerations. The full synthetic pathway to 27 is summarized in Figure 21-Figure 26.

Figure 3. First generation highly-iodinated, non-ionic, water-soluble, X-ray contrast agent built on the C_{60} scaffolding, $[C_{60}(MI)(MS)_4]$, 27; Top: 2-D view, Bottom: 3-D view, yellow = iodine, red = oxygen, blue = nitrogen, gray = carbon, H omitted for clarity.

EXPERIMENTAL

Materials and Methods

All compounds used were reagent grade or better. All purifications for rendering anhydrous materials were carried out under a dry N₂ or Ar atmosphere. The inert gases (Trigas, prepurified) were further purified by passing through a column containing R3-11 catalyst (Chemical Dynamics Corp.) on vermiculite to remove trace O₂ followed by trace H₂O removal with a column of Drierite (CaSO₄). For anaerobic reactions, solvents were degassed by bubbling dry N₂ or Ar through the solvent for several hours. Drierite brand dessicant was used in dessicators. Many of the solvent purification procedures were adapted from literature precedent.³⁷

The following reagents were used as received: C₆₀ (99.5+%, MER Corp.), diethyl bromomalonate (Aldrich), 1,8-Diazabicyclo[5.4.0]undec-7-ene (Aldrich), NaH (50 % dispersion in mineral oil, Aldrich), conc. H₂SO₄ (Fisher), KOH (Fisher), diethyl malonate (Aldrich), Br₂ (Aldrich), 2-amino-1,3-propanediol (Aldrich), Pd/C (Pd 10%, Aldrich), iodine monochloride (Aldrich), KCl (Baker), 5-amino-isophthalic acid (Aldrich), NaHSO₃ (Fisher), conc. HCl (Fisher), P₂O₅ (Fisher), NaCl (Fisher), NaHCO₃ (Fisher), malonyl dichloride (Aldrich), benzyl chloroformate (Aldrich), benzaldehyde (EM Science), MgSO₄ (Fisher), CuSO₄

(Baker), K_2CO_3 (Fisher), CBr_4 (Aldrich), $\text{BF}_3\text{Et}_2\text{O}$ (Aldrich), Na_2CO_3 (Fisher), CaH_2 (Acros), CaCl_2 (Fisher), Na metal (Aldrich), K Metal (Fisher), MgSO_4 (Fisher), CaSO_4 (Drierite).

The following reagents were purified as described: *p*-toluidine (Aldrich) was recrystallized from petroleum ether, NEt_3 (Acros) was refluxed and distilled from CaH_2 and stored in a dessicator, SOCl_2 (Acros) was distilled from linseed oil (Aldrich, 20 % v/v) and used immediately, acetic anhydride (Fisher) was distilled and stored in a dessicator.

The following solvents were used as received: petroleum ether (Fisher), dimethylacetamide (Aldrich, anhydrous), acetic acid (Fisher), Et_2O (Fisher, anhydrous), acetonitrile (Fisher), acetone (Fisher), and deionized (DI) water was used out of the laboratory DI tap.

The following solvents were purified as described: toluene (Fisher) was refluxed and distilled from Na (with benzophenone indicator), hexanes (Fisher) were dried by contact with CaCl_2 and either distilled or microfiltered, CS_2 (Fisher) was stirred with CaCl_2 4h then distilled from P_2O_5 , CHCl_3 (Fisher) was refluxed and distilled from CaCl_2 , pyridine (Fisher) was refluxed and distilled from Na or KOH, EtOAc (Fisher) was distilled from MgSO_4 , THF (Fisher) was distilled from a K/Na alloy (made by heating 1:1 Na and K metal until molten),

EtOH (Pharmco) was refluxed and distilled from CaSO_4 , and methanol (Fisher) was distilled.

Silica gel (Aldrich), grade 62, 62-200 mesh, 150 Å was used for column chromatography after activation at 200 °C for at least 24 h. TLC analyses were carried out on Whatman 250 µm layer silica gel with fluorescent indicator on polyester backing (PE SIL G/UV).

Cation exchange resin (Bio-Rad) AG 50W-X2 (H^+ form) was used for the removal of cations from the fullerene materials. The resin was prepared and/or converted to H^+ form by rinsing with at least 5 bed volumes of each: DI H_2O , 1 M HCl, DI H_2O .

All NMR solvents were from Cambridge Isotope Laboratories and were used as received.

NMR spectra were obtained on a Bruker Avance 250, 400, or 500 MHz NMR (for VT experiments) spectrometer. FT-IR spectra were collected on a Perkin Elmer Paragon 1000 PC spectrometer as KBr pellets.

Mass spectra were collected on either a Finnigan Mat 95 mass spectrometer or a Bruker Biflex III MALDI-TOF mass spectrometer. For the MALDI spectra, unless otherwise specified, an elemental sulfur matrix deposited from a slurry in CS_2 was used. The analyte was dissolved in an appropriate solvent (specified) and either mixed with the CS_2 slurry and then spotted on the

sample plate or spotted directly onto the previously deposited sulfur matrix. Where resolution permitted, the isotopic distribution of the mass spectral peaks was compared to theory.

HPLC analyses were performed on Hitachi L-6200A Intelligent Pump HPLC system with a Hitachi Model L-3000 UV-Vis photodiode array detector using various columns as specified in the synthesis section. Elemental analyses were carried out commercially by Galbraith Laboratories (Knoxville, Tennessee). Melting points were determined on a Mel-Temp apparatus and are uncorrected.

For the VT NMR experiments, the solvents were degassed, and using either regular- or medium-walled NMR tubes, the sample solutions were frozen and sealed under vacuum (10^{-5} Torr) with a flame. The temperature of the 500 MHz spectrometer was allowed to stabilize for 10 min. between temperature changes.

For anhydrous reactions, all glassware was either flame-dried or dried at 200 °C for 24 h. Solutions purified by microfiltration were filtered with a 0.2 μ m nylon (Fisherbrand) syringe filter.

The water-solubility of **14** was determined by UV-Vis spectrophotometry on a Cary 4 optical system at pH = 7. In a 1.0 mm quartz cell containing 400 μ L of HPLC quality H₂O, **14** was dissolved in ~ 5 mg increments and spectra were sequentially collected.

TGA analyses were carried out on a SEIKO 1 TG/DTA 200 instrument with an Al pan in air. The temperature ramp was 10 °C/min. For the dynamic light scattering experiment, a Coulter N4 Plus dynamic light scattering particle sizer was used. Data was taken at 90°.

Magnetic measurements were obtained with an Evan's Magnetic Susceptibility Balance (MSB-1; Johnson Matthey), using standard sample tubes (3.24mm ID; Johnson Matthey). Diamagnetic corrections were determined from pure C₆₀ and the free addend (prior to adding to C₆₀).

The *n*-octanol/water partition coefficient (K_{OW}) of **14** and **27** was determined at 25 °C by the method of Leo.³⁸ Accordingly, a solution (~1 mM) of **14** or **27** in water (pre-saturated with *n*-octanol) was vigorously stirred for 5 minutes with an equal volume of *n*-octanol, then centrifuged. The absorbance in the UV-vis spectra of the *n*-octanol "extracted" solutions was compared to the spectra of the starting solutions.

The qualitative determination of X-ray attenuation of compound **24** was carried out as follows: In 1 mL syringes, a 0.197 M (150 mg I/mL) solution of **24** 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.

Syntheses

All new compounds in the synthesis of the C₆₀-based X-ray CA (27) were fully characterized. The data are at the end of the section for each compound and the spectra can be found in Appendix II. Previously prepared compounds and dead-end products were either partially or fully characterized and compared to relevant literature data (when appropriate) and selected spectra are also presented in Appendix II.

1',1'-Bis(diethoxycarbonyl)-1,2-methano[60]fullerene (3)

[C₆₀(COOEt)₂] was prepared by a slightly modified literature procedure.⁴⁹ Accordingly, 597 mg of C₆₀ (0.83 mmol) was stirred at rt in 400 mL anhydrous toluene in a 1000 mL rbf. After 2 h, the solution was filtered to remove 120 mg of insoluble material which leaving 477 mg of C₆₀ (0.66 mmol) in solution. 56.7 mg (0.24 mmol) of diethylbromomalonate (DBM) was then added followed by 109 mg of DBU (0.72 mmol). A darkening of the purple solution was noted. After 30 min stirring at rt under Ar sparge, the solvent was removed and the

product purified on slurry packed (toluene) SiO_2 (20 cm x 2.2 cm ID) by eluting first with 1:1 $\text{C}_6\text{S}:\text{PhCH}_3$ to remove unreacted C_{60} followed by pure PhCH_3 which caused the deep amber red product band to elute as 167 mg (0.19 mmol) analytically pure dark reddish-brown solid, yield 80 % based on DBM. 258 mg of C_{60} was recovered. TLC R_f = 0.77 in toluene; ^1H NMR (250 MHz, CDCl_3 , TMS ref) δ (ppm) 1.42 (q, J = 3.13 Hz, 6 H, CH_3), 4.50 (t, J = 3.13 Hz, 4 H, CH_2); FT-IR (KBr) ν (cm^{-1}) 2977 (C-H), 1744 (C=O), 1233 (asym $-\text{OCOCH}_2-$), 526 (C_{60} monoadduct); MALDI-TOF MS (neg ion, toluene) calcd for $\text{C}_{67}\text{H}_{10}\text{O}_4$ $[\text{M}]^-$ 878.1, found 878.7.

1',1'-Bis(hydroxycarbonyl)-1,2-methano[60]fullerene (4)

$[\text{C}_{61}(\text{COOH})_2]$ was prepared by the hydrogenolysis of the diester **3**.⁵⁰ Accordingly, 65 mg of **3** (0.074 mmol) was dissolved in 50 mL anhydrous toluene in a 100 mL rbf. 30 mg of NaH (50% mineral oil dispersion, 0.63 mmol) was added and the mixture stirred under Ar sparge for 5 min. 5 drops of MeOH was added with evolution of H_2 . After 3 min., an additional 5 drops of MeOH was added. A brown ppt formed. The stirring was stopped and the mixture allowed to stand for 3 min. followed by the addition of 5 more drops of MeOH. The mixture was neutralized with 1 N H_2SO_4 and filtered to collect the ppt. The brown solid was purified by suspension in PhCH_3 by sonication,

followed by centrifugation. After drying, the material was suspended in DI H₂O, which was made slightly basic with 1 M KOH (which gave a colloidal suspension), then slightly acidic with 1 N H₂SO₄ which caused full precipitation. After centrifugation and rinsing with fresh DI H₂O, the brown solid was dried to give 24.4 mg of **4** (0.030 mmol), 40% yield. FT-IR (KBr) ν (cm⁻¹) 3418 (COOH), 1708 (C=O), 526 (C₆₀ monoadduct); MALDI-TOF MS (pos ion, MeOH/toluene) calcd for C₆₃H₂O₄ [M]⁺ 822.0, found 778.2 [M - CO₂]⁺.

1'-[N-(*p*-tolyl)carbamoyl]-1,2-methano[60]fullerene (6**)**

9.4 mg (0.011 mmol) of **4** was sonicated in 6 mL of anhydrous CS₂ in a 25 mL rbf until homogeneous (~ 1 min). 1.0 mL of SOCl₂ (14 mmol) was then added and the resultant brown solution stirred at reflux for 44 h under Ar sparge. After this time, the solvent was removed and the solid dried at 95 °C for 19 h under vacuum to give the acid chloride **5**, which was characterized by FT-IR, but not isolated. 7.8 mg of *p*-toluidine (0.073 mmol) was dissolved in 15 mL anhydrous DMA and the solution transferred onto the fullerene residue via a cannulating needle. The brown solution was stirred 18 h at rt under Ar sparge at 45 °C followed by removal of the solvent under reduced pressure to give a brown powder. for **5**: FT-IR (KBr) ν (cm⁻¹) 1776 (C=O), 525 (C₆₀ monoadduct). for **6**: FT-IR (KBr) ν (cm⁻¹) 3034 (Ph-H), 1619 (amide C=O), 810

(Ph-H), 524 (C_{60} monoadduct); FAB-MS calcd for $C_{96}H_9NO$ $[M]^+$ 867.1, found 868.2 $[M + H]^+$.

***N,N'*-Di-*p*-tolyl-malonamide (7)**

1.92 g of diethyl malonate (DEM, 12 mmol) and 3.7 g *p*-toluidine (35 mmol) were placed in a 25 mL Erlenmeyer flask. With stirring, the flask was heated to ~ 150 °C which caused the *p*-toluidine to melt giving a homogenous mixture followed by the evolution of a large amount of EtOH upon condensation.⁵² The solution color changed from clear to brown over 10 min. After this time, little additional evolution of EtOH was noted. The flask was removed from the heat and, upon cooling, a solid quickly formed. The product was recrystallized in two crops from boiling AcOH (60 mL, then 20 mL) to give 2.53 g (9.0 mmol) of colorless needle-like crystals, yield 75 % based on DEM. 1H NMR (250 MHz, $CDCl_3$, TMS ref) δ (ppm) 2.32 (s, 6 H, CH_3), 3.48 (s, 2 H, CH_2), 7.14 (d, 4 H, ArH), 7.42 (d, 4 H, ArH), 8.6 (bs, 2 H, NH); EI-MS (+ ion) calcd for $C_{17}H_{18}N_2O_2$ $[M]^+$ 282.1, found 282.1.

2-Bromo-*N,N'*-di-*p*-tolyl-malonamide (8)

875 μ L of Br_2 (17 mmol) was dissolved in 20 mL $CHCl_3$ and added dropwise to a stirring solution of 3.55 g 7 (13 mmol) in 70 mL AcOH at ~ 100 °C. The color

quickly changed from orange to colorless, but remained slightly yellow after complete addition of the Br_2 . The heat was removed (after complete addition of Br_2) and the solution allowed to cool to rt for several hours. Sand-dollar like crystals formed which were filtered and recrystallized from 40 mL AcOH to give 3.17 g (8.8 mmol) colorless crystals. A second crop of 0.54 g (1.5 mmol) was obtained from the mother liquor, yield 81 %. ^1H NMR (250 MHz, CDCl_3 , TMS ref) δ (ppm) 2.33 (s, 6 H, CH_3), 4.94 (s, 1 H, CH), 7.15 (d, 4 H, ArH), 7.43 (d, 4 H, ArH).

1',1'-Di[*N*-(*p*-tolyl)carbamoyl]-1,2-methano[60]fullerene (9)

(prepared as a mixture of mono-, di-, and triadducts) 453 mg C_{60} (0.63 mmol) and 108 mg (0.30 mmol) **8** were dissolved in 400 mL PhCH_3 in a 500 mL rbf. 44.5 μL DBU (0.30 mmol) in 40 mL PhCH_3 was added dropwise via an addition funnel over 30 min. During the addition of the base, a brown ppt formed. After complete addition, the stirring was stopped and the flask allowed to settle for 15 min and placed on an ice bath for 30 min. After filtration, 132 mg of a light reddish-brown solid was obtained. The material was purified by flash chromatography on SiO_2 with THF. ^1H NMR (400 MHz, THF-d_8 , TMS ref, multiple broadened signals were observed due to the paramagnetism of the material and/or the presence of isomers of the di- and

triadducts, however, the relative integrations were accurate) δ (ppm) 2.25-2.40 (CH_3), 7.08-7.22 (ArH), 7.53-7.78 (ArH) 10.22, 10.34, 10.41, 10.43, 10.51, 10.87 (NH); FT-IR (KBr) ν (cm^{-1}) 1681 (amide C=O), 1595 (ArC-C) 1514, 523 (C_{60} monoadduct) FAB-MS (THF) calcd for monoadduct $\text{C}_{77}\text{H}_{16}\text{N}_2\text{O}_2$ $[\text{M}]^+$ 1000.1, found 1000, calcd for diadduct $\text{C}_{94}\text{H}_{32}\text{N}_4\text{O}_4$ $[\text{M}]^+$ 1280.2, found 1281, calcd for triadduct $\text{C}_{111}\text{H}_{48}\text{N}_6\text{O}_6$ $[\text{M}]^+$ 1560.4, found 1561.

N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-malonamide (10)
MSA

In a loosely capped 16.5 mm x 3.7 mm ID Pyrex tube, 10.59 g of serinol (2-amino-1,3-propanediol, 120 mmol) and 7.94 mL diethyl malonate (52 mmol) were combined and heated on a sand bath with vigorous stirring at 200-225 °C for 45 min with evolution of EtOH. Only the bottom 5 mm of the tube was heated so that the top portion acted as a condensing column. After this time, the loose cap was removed and most of the EtOH was allowed to boil off. The heat was removed and, upon cooling, the solid, colorless residue was treated with 40 mL each of freshly distilled Ac_2O and pyridine (the solid was broken up with a spatula) and stirred 18 h at rt. Methanol (20 mL) was added carefully with stirring and cooling on an ice bath. The solvents were removed under reduced pressure to give a golden oil. 75 mL of EtOAc was added and the

solution poured into a 300 mL separatory funnel which was extracted with dilute CuSO_4 (5 x 10 mL) followed by H_2O , and saturated NaCl . The organic phase was dried by contact with MgSO_4 and filtered. Recrystallization from EtOAc:hexanes (~ 2:1) in two crops gave 11.57g (28 mmol) of pure **10** as a colorless crystalline solid, yield 53%. mp = 91.5-92.5 °C ; TLC R_f = 0.58 in 1:1 EtOAc: C_6S ; mp 91.5-92.5; HPLC (anal C-8) in 7:3 AcCN: H_2O , detection at 240 nm, retention time 3.06 min, flowrate 0.30 mL/min; ^1H NMR (400 MHz, CDCl_3 , TMS ref.) δ (ppm) 2.08 (s, 12H, CH_3), 3.21 (s, 2H, CH_2), 4.18 (m, 8H, CH_2), 4.43 (m, 2H, CH), 7.50 (d, J = 8.36, 2H, NH); ^{13}C NMR (400 MHz, CDCl_3 , solvent ref.) δ (ppm) 20.70 (4C, CH_3), 42.49 (1C, CH_2), 47.42 (2C, CH), 62.47 (4C, CH_2), 167.27 (2C, C=O), 170.68 (4C, C=O); FT-IR (KBr) ν (cm^{-1}) 3254 (N-H), 3085, 1740 (C=O, ester), 1646 (C=O, amide), 1241 (asym. $-\text{OCOCH}_3$); EIMS calcd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_{10}$ (M^+) 418.2, found 418.1.

2-Bromo-N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-malonamide (11) BrMSA

In a typical bromination, 7.19 g of (17 mmol) **10** was dissolved in 140 mL of distilled EtOAc. With stirring at rt, four 299 μL aliquots of Br_2 (23 mmol, 1.35 molar excess) were added in 4 min intervals with the orange color disappearing each time. After complete addition, some yellow color remained and 3.22 mL

of distilled NEt_3 (23 mmol) was quickly added with large amounts of salt precipitation and a return to a colorless mixture. The HNEt_3Br was removed by filtration and the solution rotavapped to an oil. Analysis by ^1H NMR indicated ~77% mono-bromination with no dibromo compound formed. The residue was redissolved in 150 mL EtOAc with warming and, after cooling to rt, 282 μL Br_2 added (5.5 mmol, 1.35 molar excess relative to unbrominated material) with stirring. After 15 min, 765 μL NEt_3 (5.5 mmol) was added. The slurry was filtered and rotavapped as before. ^1H NMR analysis of the residue indicated ~90% monobromination and ~10% dibromination. Recrystallization from 25:16 EtOAc:hexanes in two crops gave 6.75 g of pure **11** (14 mmol), 79% yield. mp 111.0 - 112.5 $^\circ\text{C}$; TLC R_f = 0.10 in 1:1 EtOAc:hexanes; ^1H NMR (400 MHz, CDCl_3 , TMS ref.) δ (ppm) 2.10 (s, 12H, CH_3), 4.15-4.26 (m, 8H, CH_2), 4.42-4.43 (m, 2H, CH), 4.71 (s, 1H, CH), 7.36 (br d, J = 5.25 Hz, 2H, NH); ^{13}C NMR (400 MHz, CDCl_3 , solvent ref.) δ (ppm) 20.90 (4C, CH_3), 43.02 (1C, CH_2), 48.46 (2C, CH), 62.39, 62.43 (4C, CH_2 , signal split), 165.66 (2C, NC=O), 170.91, 170.96 (4C, OC=O , signal split); FT-IR (KBr) ν (cm^{-1}) 3247 (m, N-H), 1741 (s, ester C=O), 1665 (s, amide C=O), 1225 (s, asym. $-\text{OCOCH}_3$); HPLC (anal. C-8 column) in 7:3 AcCN: H_2O , retention time 3.30 min., detection at 250 nm; UV (7:3 $\text{H}_3\text{CCN}:\text{H}_2\text{O}$) λ_{max} 225 nm; EI-MS calcd for $\text{C}_{17}\text{H}_{25}\text{N}_2\text{O}_{10}\text{Br}$ (M^+)

496.1, found 496.1 (M^+), 423.0 ($-\text{CH}_2\text{OCOCH}_3$), 357.1 (base peak $-\text{Br}$, $-\text{OCOCH}_3$).

1',1'-Bis[N-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]carbamoyl]-1,2-methano[60]fullerene (12, n = 1) $\text{C}_{60}(\text{MSA})$

In a typical preparation, 62.0 mg of C_{60} (0.086 mmol) was dissolved in degassed toluene over 2 h under Ar in a 100 mL rbf. 22.6 mg **11** (0.045 mmol) was added with sonication until dissolved. 8.84 μL DBU (0.059 mmol) in 8.0 mL toluene was added dropwise over 20 min with stirring at rt. After complete addition of DBU, stirring was continued for 40 min during which a color change from purple to reddish-purple was noted. The solvent was removed under reduced pressure. 10 mL of CHCl_3 was added to the flask with sonication for 10 min. After filtration, the solution was chromatographed on a toluene slurry-packed column (SiO_2 , 25 cm x 2.2 cm ID) with toluene to remove unreacted C_{60} followed by 2:8 acetone:toluene. The second, red band consisted of pure monoadduct, 22.5 mg after solvent removal (0.020 mmol), yield 44 % based on **11**. (A third band was eluted with 1:1 acetone:toluene weighing 18.2 mg after removal of solvent and was determined to be a mixture of bis- and tris-adducts by MALDI-TOF MS.) TLC R_f = 0.26 in 9:1 PhCH_3 :MeOH; ^1H NMR (400 MHz, CDCl_3 , TMS ref.) δ (ppm) 2.10 (s, 12H, CH_3), 4.34-4.41 (m,

8H, CH₂), 4.68-4.72 (m, 2H, CH), 7.37 (br d, $J = 6.4$ Hz, 2H, NH); ¹³C NMR (400 MHz, CDCl₃, solvent ref.) δ (ppm) 170.95 (ester OC=O), 162.90 (NC=O), 145.57, 145.44, 145.49, 145.40, 145.06, 144.99, 144.80, 144.01, 143.41, 143.32, 143.24, 142.40, 142.29, 141.30, 138.21 (C₆₀ sp² C), 73.45 (C₆₀ sp³ C), 62.72 (CH₂), 58.28 (bridgehead C), 49.30 (CH), 20.96 (CH₃); FT-IR (KBr) ν (cm⁻¹) 1745 (s, ester C=O), 1685 (m, amide C=O), 1228 (s, asym. -OCOCH₃), 526 (m); HPLC (Cosmosil 5 PBB column), retention time 3.43 min. in toluene:MeOH (40:1), detection at 290 nm; UV (40:1 toluene:MeOH) λ_{max} 285 nm; MALDI-TOF MS (acetone, + ion) calcd for C₇₇H₂₄O₁₀N₂ (M⁺) 1136.1, found 1136.1; Anal. (C₇₇H₂₄O₁₀N₂·acetone (C₃H₆O)) calcd: C, 80.4; H, 2.53; N, 2.34, found: C, 79.79; H, 2.41; N, 2.45.

***n*[1',1'-Bis[N-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]carbamoyl]]-1,2-methano[60]fullerene (multiadduct, 13, $n = 4-6$) C₆₀(MSA)_{*n*}**
Typically, 51.8 mg of C₆₀ (0.072 mmol) was dissolved in 50 mL of degassed toluene in a 250 mL rbf with stirring under Ar over at least 2 h at rt. 0.379 g of **11** (0.79 mmol) was dissolved in 20 mL of acetone and added to the C₆₀ solution. Over 1.5 h, 118.3 μ L DBU (0.79 mmol) in 100 mL 4:6 acetone:toluene was added dropwise. After stirring for 3 h at rt, the bright red solution was rotovapped to dryness. The residue was dissolved in 10 mL CHCl₃ and flash

chromatographed (slurry packed SiO_2 , 4:6 acetone:toluene) with 4:6 acetone:toluene to give 0.104 g of a red solid after removal of solvent and drying over P_2O_5 at 100 °C under reduced pressure. Yield = 69.2% assuming $n = 5$. HPLC (Econosil Silica 10 μ column), retention time of multiple peaks 3.5 - 6.75 min. in 20:1 toluene:MeOH, detection at 290 nm; FT-IR (KBr) ν (cm^{-1}) 1741.64 (s, ester C=O), 1685 (m, amide C=O), 1228 (s, asym. $-\text{OCOCH}_3$), 1045 (s); MALDI-TOF MS calcd for $\text{C}_{128}\text{H}_{96}\text{N}_8\text{O}_{40}$ (M^+ , tetraadduct) 2384.6, found 2384.7, calcd for $\text{C}_{145}\text{H}_{120}\text{N}_{10}\text{O}_{50}$ (M^+ , pentaadduct) 2801, found 2800, calcd for (M^+ , hexaadduct) 3217, found 3216; Anal. calcd for hexaadduct ($\text{C}_{162}\text{H}_{144}\text{N}_{12}\text{O}_{60}$) C, 60.45; H, 4.51; N, 5.22, found C, 59.94; H, 4.45; N, 5.15.

$n[1',1'\text{-Bis}[N\text{-(2-hydroxy-1-(hydroxymethyl)ethyl)carbamoyl}]]\text{-1,2-methano[60]fullerene}$ (14, $n = 4\text{-}6$) $\text{C}_{60}(\text{MS})_n$

Typically, 66.1 mg of 13 was dissolved in 5 mL degassed MeOH under Ar. 34.6 mg of K_2CO_3 was added followed by 1.0 mL degassed, DI H_2O . The red solution was stirred under Ar at rt for 1.5 h. Cation exchange resin (H^+ form) was added until pH=7, and then a slight excess. After 1.0 h additional stirring at rt under Ar, the solution was filtered and rotovapped to dryness to give the deprotected water-soluble 14 in quantitative yield. ^1H NMR indicated complete OH deprotection; FT-IR (KBr) ν (cm^{-1}) 3338 (s, OH), 1675 (s, amide C=O),

1558 (s), 1038 (m); MALDI-TOF MS calcd for $C_{96}H_{64}N_8O_{24}$ (M^+ , tetraaduct) 1712.4, found 1712.1, calcd for $C_{105}H_{80}N_{10}O_{30}$ (M^+ , pentaaduct) 1960.5, found 1960.1, calcd for $C_{114}H_{96}N_{12}O_{36}$ (M^+ , hexaaduct) 2208.6, peak not observed.

5-Amino-2,4,6-triiodo-1,3-benzenedicarboxylic Acid (15)

The preparation of **15** was modified from a literature procedure.⁸³ Thus, 98.11 g of ICl (600 mmol) was added to a solution of 72.11 g KCl (970 mmol) in 300 mL H_2O and stirred at rt for 5 min. The mixture was filtered to remove insolubles, and the orange solution of $KICl_2$ was added via addition funnel over 1 h to a stirring slurry of 33.22 g of 5-aminoisophthalic acid (180 mmol) in 1100 mL DI H_2O at 55 °C in a 2000mL Erlenmeyer flask. After ~ 30 min, the acid had completely dissolved to give a very dark brown solution, but precipitated during the second half of the addition. After complete addition, the tan slurry was stirred at 55 °C for 24 h. The slurry was cooled on an ice bath for 3 h and filtered to give a light tan solid. The crude product was rinsed on a fritted glass filter 2x with 40 mL H_2O , 1x 20 mL H_2O , 2x 20 mL 0.5 N $NaHSO_3$, 2x 10 mL H_2O , dissolved in minimal 0.66 M KOH to give a clear brown solution, neutralized with conc HCl, and treated repeatedly with 0.5 g activated charcoal at 40 - 50 °C for 10 min with stirring and filtered until nearly colorless (6 - 8x).

The product was then precipitated w/ conc HCl with stirring until no further precipitation was observed, allowed to stand for several hours, then filtered and dried at ~ 100 °C over P₂O₅ (~ 10⁻² Torr) to give 73.68 g (130 mmol) of analytically pure off-white tan solid, 73 % yield. TLC R_f = 0.60 in 2:1 MeOH:EtOAc; HPLC (anal. C-8) detection at 255 nm, retention time 2.79 min. in phosphate buffer (pH = 7): MeOH (7:3), flowrate 0.3 mL/min.; ¹³C NMR (400 MHz, DMSO-d₆, solvent ref.) δ (ppm) 70.6 (1C, C-I) , 77.9 (2C, C-I), 147.7 (2C, C-COOH), 148.9 (1C, C-NH₂), 170.1 (2C, COOH); FT-IR (KBr) ν (cm⁻¹) 3629, 3442, 3378, 3305 (N-H), 2990, 2480 (COO-H), 1719 (C=O); MALDI-TOF MS (MeOH, + ion) calcd for C₈H₄O₄NI₃ (M⁺) 558.7, found 558.6.

5-Amino-2,4,6-triiodo-1,3-benzenedicarbonyl dichloride (16)

52 g 15 (93 mmol) was refluxed for 6 h in 400 mL SOCl₂ (freshly distilled from linseed oil 5-10% v/v) in a 1000mL rbf during which all material dissolved to give a dark yellow-orange solution. The SOCl₂ was removed under reduced pressure and the product dispersed in 400 mL EtOAc by sonication (1 min). The EtOAc was removed under reduced pressure to give a dark yellow solid. The crude material was again dispersed in 425 mL EtOAc (the material dissolved fully upon washing) and washed with 40 mL portions of a saturated solution of 250 g NaCl / 100 g NaHCO₃ in 1.25 L H₂O until the aqueous phase

remained basic (5x), followed by satd NaCl. The clear dark yellow-orange organic phase was flash chromatographed on toluene slurry packed SiO₂ (10 cm height x 7.9 cm ID) with toluene and rotovapped to give 49.1 g (83 mmol) of a pure beige solid (in some runs, the solid was yellow), yield 89.3%. TLC R_f = 0.62 in 1:3 C₆:EtOAc; HPLC (anal C-8) detection at 285 nm, retention time 7.80 min. in 8:2 AcCN:H₂O, flowrate 0.25 mL/min; ¹³C NMR (400 MHz, acetone-d₆, solvent ref) δ (ppm) 64.8 (1C, C-I) 77.0 (2C, C-I), 150.3 (1C, C-NH₂), 150.8 (2C, C-COCl), 170.0 (2C, COCl); FT-IR (KBr) ν (cm⁻¹) 3469, 3370 (N-H), 1769 (C=O); MALDI-TOF MS (acetone, - ion) calcd for C₈H₂O₂Nl₃Cl₂ [(M-H)⁻] 593.7, found 593.3.

N,N'-Bis(2,4,6-triiodo-3,5-benzenedichlorocarbonyl)-malonamide

(17)

25.7 g **16** (43 mmol) was dissolved in 200 mL anhydrous THF (500 mL rbf) and brought to reflux under argon. 2.0 mL (21 mmol) malonyl dichloride was added dropwise over 5 min and the orange solution refluxed for 6 h (under Ar sparge). Anhydrous hexanes were added until slight precipitation was noted (~10 mL). Stirring was stopped and the heat removed. Crystals formed when the flask was allowed to cool overnight under Ar. It was then placed on an ice bath for 3 h and filtered to give 19.65 g (16 mmol) of a fluffy, white,

microcrystalline solid. The mother liquor was stirred and precipitated with hexanes followed by filtration to give crude product. The crude tan solid was dissolved in minimal boiling THF and hexanes added until near precipitation. Upon cooling to rt, followed by an ice bath (2 h), a second crop of 1.91 g (1.5 mmol) pure material was obtained. An additional 2.53 g of crude material was obtained upon full precipitation and filtration of the mother liquor. Pure yield 83%. TLC R_f = 0.66 in 2:1 EtOAc:C₆S; HPLC (anal. SiO₂) in 4:1 PhCH₃:THF, detection at 300 nm, retention time 5.92 min., flowrate 1.0 mL/min.; ¹H NMR (400 MHz, acetone-d₆, TMS ref.) δ (ppm) 3.85 (s, 2H, CH₂), 10.0 (bs, 2H, NH); ¹³C NMR (400 MHz, acetone-d₆, solvent ref.) δ (ppm) 43.0 (1C, CH₂), 83.1 (2C, C-I), 98.3 (4C, C-I), 146.2 (2C, C-NH₂), 151.8 (4C, C-COCl), 166.1 (2C, CONH), 169.9 (4C, COCl); FT-IR (KBr) ν (cm⁻¹) 3190 (N-H), 2960 (C-H), 1760 (C=O, acid chloride), 1650 (C=O, amide); MALDI-TOF MS (acetone, - ion) calcd. for C₁₉H₄O₆N₂I₆Cl₄ [(M-H)⁻] 1256.3, found 1256.7.

2-Acetyl-*N,N'*-Bis[2,4,6-triiodo-*N,N'*-bis-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-3,5-benzenedicarbamoyl]-malonamide
(18)

244 mg of 17 (0.19 mmol) was dissolved in 10 mL of anhydrous DMA. 93 mg of serinol (1.0 mmol) and 160 μ L of NEt₃ (1.2 mmol) were added and the

colorless solution stirred under N₂ sparge at rt for 20 h during which a white ppt formed. The solvent was removed under reduced pressure and the colorless residue treated with excess Ac₂O/pyridine (~2 mL ea) at rt for 18 h. The excess Ac₂O was removed by the careful addition of MeOH while cooling on an ice bath. After solvent removal, the pale yellow solid was purified by flash chromatography on SiO₂ with 3:1 EtOAc:THF to give a colorless solid. TLC R_f = 0.68 in 3:1 EtOAc:THF (band envelope is comprised of at least three spots due to atropisomers); ¹H NMR (400 MHz, DMSO-d₆, TMS ref.) δ (ppm) 2.03 (bm, 27 H, CH₃), 3.67 (s, ~0.5 H, CH(COR)₃), 4.15 (bm, 16 H, CH₂), 4.35 (bp, 4 H, NCH), 8.80 (d, *J* = 7.96 Hz, ~ 0.8 H, NH), 8.95 (m, ~ 2 H, NH), 9.94 (s, ~ 0.6 H, NH); MALDI-TOF MS (acetone, pos ion) C₄₉H₅₄O₂₃N₆I₆ [M]⁺ 1855.8, found 1877.8 [M - H + Na]⁺.

(2-Hydroxy-1-hydroxymethyl-ethyl)-carbamic acid benzyl ester (19)

11.03 g of serinol (120 mmol) and 18.5 mL NEt₃ was dissolved in 60 mL dry EtOH in a 150 mL rbf and placed on an ice bath. 19.2 mL of benzyl chloroformate was added via an addition funnel over 15 min without allowing the temperature to rise above 35 °C. After complete addition, stirring was continued for 2 h at rt with the formation of a colorless ppt (NEt₃HCl). The mixture was filtered and concentrated to 50 mL. Allowed to stand at rt

overnight, the product crystallized to give 26.72 g. A second crop of 2.5 g was obtained by seeding the mother liquor and cooling at -10 °C 18 h. Pure product was obtained by recrystallization from H₂O in two crops to give colorless crystals, 20.64 g, 76 % yield. mp = 104-105 °C; TLC R_f = 0.58 in 10:1 EtOAc:MeOH; ¹H NMR (400 MHz, acetone-d₆, TMS ref.) δ (ppm) 2.91 (s, 2H, OH), 3.66 (m, 4H, CH₂), 3.86 (p, 1H, CH), 5.06 (s, 2H, CH₂), 6.03 (bs, 2H, NH), 7.28-7.40 (m, 5H, ArH); ¹³C NMR (400 MHz, acetone-d₆, solvent ref.) δ (ppm) 55.70 (1C, CH), 62.39 (2C, CH₂), 66.60 (1C, CH₂), 128.66, 128.71, 129.26, 138.45 (ArC), 157.21 (1C, C=O); FT-IR (KBr) ν (cm⁻¹) 3320 (O-H), 1695 (C=O); EI-MS calcd. for C₁₁H₁₅N₁O₄ (M⁺) 225.1, found 225.1.

***Cis, trans*-(2-Phenyl-[1,3]dioxan-5-yl)-carbamic acid benzyl ester
(20 *cis*, 21 *trans*)**

To a refluxing solution of 17.13 g **19** (76 mmol) in a 500 mL flask in 290 mL toluene was added 7.34 mL benzaldehyde (72 mmol). 15 μL of conc H₂SO₄ was suspended in 3 mL toluene by sonication and added to the flask. After 1.5 h at reflux, 25 mL of the reaction solvent was distilled off to azeotropically remove water. A second aliquot of 15 μL conc H₂SO₄ suspended in 3 mL toluene was added and reflux was continued for 3 h and an additional 45 mL distilled off. The heat was removed and crystals allowed to form by cooling the flask to r t

overnight under an Ar atmosphere. The colorless crystals were filtered to give 11.5 g of 80-90 % starting material **19** and 10-20 % *trans*-(2-phenyl-[1,3]dioxan-5-yl)-carbamic acid benzyl ester as determined by ^1H NMR. The filtrate was treated with 0.5 g MgSO_4 , filtered, and the solvent removed under reduced pressure. The clear oil was recrystallized from 9:1 EtOH (200 proof): H_2O in two crops to give 6.13 g of pure **20** as colorless crystals. In some preparations, very small percentages (< 3 %) of the *trans* isomer **21** were detectable by ^1H NMR. For **20**: mp = 67.0- 69.0 $^\circ\text{C}$; TLC R_f = 0.39 in 7:3 $\text{C}_6\text{s}:\text{EtOAc}$; mp 67-69 $^\circ\text{C}$; ^1H NMR (400 MHz, CDCl_3 , TMS ref.) δ (ppm) 3.76 (d, J = 8.8 Hz, 1H, CH), 4.14 (m, 4H, CH_2), 5.18 (s, 2H, CH_2), 5.55 (s, 1H, CH), 5.95 (bd, 1H, NH), 7.36-7.45 (m, 8H, ArH), 7.54 (m, 2H, ArH); ^{13}C NMR (400 MHz, CDCl_3 , solvent ref.) δ (ppm) 46.04 (1C, NCH), 67.31 (1C, CH_2), 71.14 (2C, CH_2), 102.11 (1C, acetal CH), 126.38, 128.62, 128.76, 129.02, 129.56, 136.87, 138.31 (12C, ArC), 156.40 (1C, $\text{C}=\text{O}$); FT-IR (KBr) ν (cm^{-1}) 3337 (N-H), 1685 (C=O), 1524; EI-MS calcd. for $\text{C}_{18}\text{H}_{19}\text{N}_1\text{O}_4$ (M-H) $^+$ 312.1, found 312. For **21**: TLC R_f = 0.32 in 7:3 $\text{C}_6\text{s}:\text{EtOAc}$; ^1H NMR (400 MHz, CDCl_3 , TMS ref.) δ (ppm) 3.58 (t, J = 10.2 Hz, 2H, axial HCH), 4.06 (m, 1H, NCH), 4.35 (dd, J = 4.82 Hz, 6.30 Hz, 2H, equatorial HCH), 4.60 (bd, J = 7.04 Hz, 1H, NH), 5.11 (s, 2H, CH_2), 5.44 (s, 1H, CH) 7.33-7.46 (m, 8H, ArH), 7.48 (m, 2H, ArH); ^{13}C NMR (400 MHz, CDCl_3 , solvent ref.) δ (ppm) 43.90 (1C, NCH), 67.29 (1C, CH_2),

69.81 (2C, CH₂), 101.21 (1C, CH), 126.35, 128.43, 128.53, 128.56, 128.80, 129.27, 136.22, 137.54 (12C, ArC), 155.76 (1C, C=O); EI-MS calcd. for C₁₈H₁₉N₁O₄ (M-H)⁺ 312.1, found 312.

***Cis*-5-amino-2-phenyl-1,3-dioxane (22)**

In a typical preparation, 1.4 g of **20** (4.5 mmol) was dissolved in 50 mL anhydrous EtOH in a 50 mL Erlenmeyer flask. 0.14 g of 10 % Pd/C was added. The slurry was stirred at rt under 300 psi H₂ (in a Parr hydrogenator) for 50 min. The catalyst was removed by syringe microfiltration, followed by careful solvent removal under reduced pressure (bp **22**: 50 °C, 0.65 Torr³⁹). The colorless residue was dissolved in minimal anhydrous Et₂O (~5-10 mL) and microfiltered to remove insolubles. The Et₂O was removed under reduced pressure to yield 0.72 g of pure **22** as a colorless oil (89 %). Since **22** appeared to decompose to a solid residue upon storage, the material was prepared as needed and used immediately. ¹H NMR (400 MHz, CDCl₃, TMS ref.) δ (ppm) 1.98 (bs, 2 H, NH₂), 2.77 (m, 1 H, CHNH₂), 4.03-4.16 (m, 4 H, CH₂), 5.55 (s, 1 H, ArCH), 7.30-7.40 (m, 3 H, ArH), 7.48-7.51 (m, 2 H, ArH); ¹³C NMR (400 MHz, CDCl₃, solvent ref.) δ (ppm) 45.96 (1 C, CHNH₂), 73.49 (2 C, CH₂), 101.97 (1 C, acetal CH), 126.06 (2 C, ArC), 128.44 (2 C, ArC), 129.14 (1 C, ArC), 138.36 (1 C, ArC).

N,N'-Bis[2,4,6-triiodo-N,N'-bis-[*cis*-(2-phenyl-[1,3]dioxan-5-yl))-3,5-benzenedicarbamoyl]-malonamide (23) MIB

In a typical preparation, 0.94 g **17** (0.75 mmol) was dissolved in 40 mL anhydrous DMA in a 100 mL rbf. A solution of 0.72 g of **22** (4.0 mmol, 1.33 molar excess) and 560 μ L anhydrous NEt₃ (4.0 mmol) in 10 mL anhydrous DMA was added via a cannulating needle. A white ppt slowly formed as the colorless solution was stirred for 20 h under Ar at rt. After filtration to remove HNEt₃Cl, the DMA was removed under reduced pressure to give a colorless solid. The material was flash chromatographed on a slurry packed column (silica, THF) with 10:3 THF:pyridine, the solvent removed under reduced pressure and flash chromatographed a second time on a slurry packed column (silica, THF) with pure THF. Solvent removal under reduced pressure gave 1.30 g (0.71 mmol) of an analytically pure colorless to very pale yellow solid, yield 94.7%. TLC R_f = 0.50 in 1:1 PhCH₃:THF; ¹H NMR (500 MHz, DMSO-d₆, 70 °C, solvent ref) δ (ppm) 3.57 (s, 2 H, -COCH₂CO-), 3.99 (distorted d, *J* = 6.5 Hz, 4 H, -NHCH(CH₂-)₂), 4.22 (distorted q, *J* = 8.4 Hz, 16 H, -CH(CH₂O-)₂), 5.63 (s, 4 H, acetal CH), 7.35 (m, 12 H, ArH), 7.50 (m, 8 H, PhH), 7.98 (bs, 1 H, NH), 8.7-9.3 (bm, 3 H, NH), 10.08 (s, 2 H, NH); ¹³C NMR (500 MHz, DMSO-d₆, 70 °C, solvent ref) δ (ppm) 41.95 (1 C, -COCH₂CO-), 43.70 (4 C, -NHCH(CH₂-)₂), 68.45 (8 C, -CH(CH₂O-)₂), 90.21, 98.10 (ArC), 100.40 (4 C, acetal CH),

126.14, 127.40, 128.23, 138.27, 142.07, 149.22 (ArC), 164.55, 168.90 (C=O); FT-IR (KBr) ν (cm⁻¹) 1654 (s), 1103 (s); UV λ_{max} 285; MALDI-TOF MS calcd for C₅₉H₅₂N₆O₁₄I₆ [M]⁻ 1829.8, found 1828.7 [M-H]⁻; Anal. (C₅₉H₅₂N₆O₁₄I₆) 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.

1',1'-Bis[N-[2,4,6-triiodo-N,N'-bis-[*c/s*-(2-phenyl-[1,3]dioxan-5-yl))-3,5-benzenedicarbamoyl]carbamoyl]-1,2-methano[60]fullerene (24)
C₆₀(MIB)

All of the solvents in the preparation and purification of **24** were degassed. In a 2000 mL rbf, 800 mg of C₆₀ (1.1 mmol) was added to 500 mL PhCH₃ and 200 mL pyridine, degassed and stirred until dissolved (> 2 h). 2.0 g of **23** (1.1 mmol) in 80 mL pyridine was then added. 0.36 g of CBr₄ (1.1 mmol) was added followed by 245 μ L DBU (1.6 mmol). A darkening of the color of the solution was noticeable almost immediately. Under N₂ sparge, the reaction mixture was stirred 14 h at rt. The solvent was removed and 150 mL CHCl₃ and 10 mL MeOH added to the dark solid. After stirring and 20 s sonication, the mixture was filtered and the solution flash chromatographed on SiO₂ (slurry packed with CHCl₃, 10 cm x 7.7 cm ID) with 30:1 CHCl₃:MeOH (degassed) and the solvent removed under reduced pressure. The dark product was redissolved in ~ 100 mL CHCl₃ and rechromatographed on SiO₂ (slurry packed

with CHCl_3 , 10 cm x 7.7 cm ID) first with CHCl_3 to remove C_{60} and then with 30:1 CHCl_3 :MeOH. After solvent removal, 2.17 g of dark brown solid obtained (0.85 mmol), 77 % yield (based on C_{60}). TLC R_f = 0.75 in 1:1 THF:toluene; ^1H NMR (400 MHz, CDCl_3 , solvent ref) δ (ppm) 4.0-4.5 (bm, 20H, $-\text{NHCH}(\text{CH}_2\text{O}-)_2$, $-\text{NHCH}(\text{CH}_2\text{O}-)_2$), 5.6 (bs, 4H, $\text{PhCH}(\text{O}-)_2$), 7.2-7.6 (bm, 20H, ArH), 8.89 (NH); MALDI-TOF MS (CHCl_3 , - ion) calcd for $\text{C}_{119}\text{H}_{50}\text{N}_6\text{O}_{14}\text{I}_6$ $[\text{M}]^-$ 2547.8, found 2547.5; FT-IR (KBr) ν (cm^{-1}) 1654 (s), 1103 (s), 526 (m); UV λ_{max} (nm) 273, 328; Anal. ($\text{C}_{119}\text{H}_{50}\text{N}_6\text{O}_{14}\text{I}_6$) calcd: C, 56.07; H, 1.98; N, 3.30; O, 8.79; I, 29.87; found: C, 54.76; H, 2.60; N, 3.05; O, 8.34; I, 31.26 (O by subtraction).

$\text{C}_{60}(\text{MIB})(\text{MSA})_n$ (25)

All of the solvents in the preparation and purification of **25** were degassed. In a typical preparation, 1.28 g **24** (0.50 mmol) was dissolved in 250 mL CHCl_3 (degassed). 1.61 g of **11** (3.2 mmol) was added. 616 μL DBU (4.1 mmol) in 50 mL CHCl_3 was added dropwise via addition funnel over 1 h. The brown solution was stirred under N_2 for 40 h at rt. The solution darkened slightly over the course of the reaction and appeared burnt orange in color. After removal of the solvent under reduced pressure, the reddish-brown residue was dissolved in 50 mL acetone and then 25 mL toluene was added to the solution. The solution was chromatographed on SiO_2 (slurry packed with toluene, 10 cm

x 7.7 cm ID) first with 1:5 acetone:toluene causing a small amount of colored material to elute which was discarded. The eluent was then changed to 1:1 toluene:acetone and 1200 mL of a brown solution was collected (yellow when dilute). The solvent was removed under reduced pressure to give 1.4 g of brownish solid. 66% yield (calculated for $n = 4$). ^1H NMR (400 MHz, CDCl_3 , solvent ref) δ (ppm) 1.9-2.2 (multiple bs, CH_3), 4.1- 4.5 (bm, serinol CH and CH_2), 5.6 (bs, 4H, $\text{PhCH}(\text{O}-)_2$), 7.1 (bd, NH), 7.3-7.5 (bm, ArH), 7.6 (bd, NH); FT-IR (KBr) ν (cm^{-1}) 1740 (ester $\text{C}=\text{O}$), 1675 (amide $\text{C}=\text{O}$), 1234 (asym - OCOCH_3); MALDI-TOF MS avg calcd for $[\text{C}_{60}(\text{MIB})(\text{MSA})_4]$ $\text{C}_{187}\text{H}_{146}\text{N}_{14}\text{O}_{54}\text{I}_6$ 4214.7, found 4214.4, avg calcd for $[\text{C}_{60}(\text{MIB})(\text{MSA})_3]$ $\text{C}_{170}\text{H}_{122}\text{N}_{12}\text{O}_{44}\text{I}_6$ 3798.3, found 3798.5; anal calcd for $[\text{C}_{60}(\text{MIB})(\text{MSA})_4]$ $\text{C}_{187}\text{H}_{146}\text{N}_{14}\text{O}_{54}\text{I}_6$: C, 53.3; H, 3.49; N, 4.65; O, 20.5; I, 18.1, found: C, 49.6; H, 4.45; N, 5.40; O*, 22.0; I 18.6, *Oxygen by subtraction.

$\text{C}_{60}(\text{MI})(\text{MS})_n$ (27)

All of the solvents in the preparation and purification of **27** were degassed. In a typical preparation, 421.7 mg of **25** was dissolved in 50 mL degassed CHCl_3 in a 100 mL rbf. The dark reddish-brown solution was cooled for 5 min on an acetone/dry ice bath but not allowed to freeze. 210 μL $\text{BF}_3\text{ET}_2\text{O}$ was added with stirring under N_2 . After 15 min, the flask was allowed to room to rt over

1h during which some orange ppt formed. With stirring, an additional 110 μL of $\text{BF}_3\cdot\text{Et}_2\text{O}$ was then added. Increased precipitation occurred and stirring was continued at rt for 2.5 h. The product was collected by centrifuge and washed with 3 aliquots of fresh CHCl_3 (10 mL) by repeated sonication and centrifugation to give acetal deprotected **26** (as verified by ^1H NMR, see Figure 85 in App II). The brown solid was then washed with 5 aliquots of ice cold H_2O (3 mL) until neutral (with stirring followed by centrifugation and decanting of the yellow solution each time). The brown solid was then dissolved in 12 mL of 4:1 $\text{MeOH}:\text{H}_2\text{O}$. A solution of 200 mg Na_2CO_3 in 3 mL H_2O was then added. After 1h of stirring at rt under N_2 , the dark reddish-brown solution was diluted up to 75 mL with 50 mL H_2O and 10 mL MeOH and stirred with ~1g (dry weight) cation exchange resin (H^+ form) over 1 h. The solution was filtered and the cation exchange resin reconverted to H^+ form with 1 M HCl . The solution was again treated with the same resin (to minimize product adsorption, which caused loss of material) for 1 h. After this time, the complete removal of Na^+ was verified by flame test on a Pt wire. The solution was filtered and the solvent was removed to give 106 mg of deprotected material **27**. Yield for two steps 33 %. 400 MHz ^1H NMR (D_2O , solvent ref.) δ (ppm) 3.5-4.2 (bm, CH, CH_2); FT-IR (KBr) ν (cm^{-1}) 3382 (O-H), 1652 (amide C=O); MALDI-TOF MS (2,5-dihydroxy benzoic acid matrix from 1:1 $\text{H}_3\text{CCN}:\text{H}_2\text{O}$) avg. calcd for

$C_{127}H_{98}N_{14}O_{38}I_6$ $[M - H + Na]^+$ 3211.1, found 3211.1, base peak $[M - I + Na]^+$ 3085.1; anal calcd for $[C_{60}(MI)(MS)_4]$ $C_{127}H_{98}N_{14}O_{38}I_6$: C, 47.8; H, 3.10; N, 6.15; I, 23.9; O*, 19.1, found: C, 43.8; H, 3.20; N, 5.80; I, 27.6; O*, 19.6, * Oxygen by subtraction.

RESULTS AND DISCUSSION

1. Design Concepts

The concept of this work was to design, synthesize and evaluate a first generation X-ray contrast agent (CA) that, like other contemporary agents, uses iodine as the active X-ray attenuating vehicle, but also utilizes the unique and versatile properties of fullerene-C₆₀.

The spheroidal, truncated-icosahedral structure of C₆₀ confers a globular shape to its highly-functionalized derivatives. The advantage of a more globular shape (rather than the disk-like shape of contemporary agents) is reduced viscosity in clinical formulations, which allows a more rapid intravenous injection of the agent.³

A C₆₀-derived agent could also be expected to benefit from an enhanced masking effect in which one side of the tri-iodinated phenyl ring may be blocked by the fullerene core from having hydrophobic interactions with blood plasma proteins. This would lead to decreased protein binding and increased *in vivo* tolerability (this is known as the “hydrophilic sphere concept”).⁴⁰

The minimal requirements for the initial design of any new uroangiographic agent are very good water solubility (if iodine-based, 30 mg

iodine/cm² are necessary) and sufficient X-ray attenuation (using iodine or some other "heavy" element).

One clear goal was to develop a very effective method for the water-solubilization of the hydrophobic C₆₀ molecule. In addition to conferring extremely good water solubility, the method must also result in a non-ionic product to avoid hyperosmolality, a problem commonly encountered with the older, ionic CA.³

For the X-ray attenuation requirement, essentially any heavy atom could be used, provided it could be "packaged" in a way that it could be introduced to a biological system in sufficiently high concentration and then simply excreted after image collection without any (harmful) biological interaction. The endohedral metallofullerene Ho@C₈₂(OH)_x, which contains one Holmium atom ($Z = 67$) is expected to attenuate slightly more X-rays than a single iodine atom ($Z = 53$) in the X-ray energy range of 90-140 keV (purely based on its Ho content).^{41,42} However, the availability of endohedral fullerenes is currently too low to warrant further consideration of the materials as X-ray CA (which requires gram quantities of material per use).

Although C₆₀ has been shown to form isolable reaction products with the halogens F₂, Cl₂, and Br₂, the reaction of the fullerene with I₂ results in no

covalently-bonded products but rather results in the preferential formation of intercalates such as $C_{60}(I_2)_2$ or $C_{60}I_2C_6H_5CH_3$.⁴³

Based on the above considerations, an initial design concept for the fullerene-based X-ray CA was developed. The functionalization of C_{60} is based on the nucleophilic cyclopropanation reaction (Bingel reaction) to give a malonofullerene.⁴⁹ The malono groups are each attached to two 2,4,6-triiodinated phenyl rings with water-solubilizing 1,3-diol-containing serinol-benzamide substituents in the 1 and 3 ring positions (Figure 4). The design concept takes advantage of the stability (Ph-I bond strength 274 kJ/mol⁴⁴) and established *in vivo* tolerability of the tri-iodinated benzene ring moieties, yet as discussed above, also utilizes the unique structural properties conferred by the fullerene.

Figure 4. Initial design concept for X-ray contrast agent utilizing the scaffolding of C_{60} ($n \geq 1$). X-ray attenuation is provided by the iodine atoms while water-solubilization is conferred by the multiple 1,3-diol groups.

As designed, the C_{60} -based agent in Figure 4 is expected, like contemporary uroangiographic agents, to be nonspecific (or non tissue-specific). In addition, a MW of 5174, for the hypothetical trisadduct,^a that is, three malono groups attached in different locations to the C_{60} core, $n = 3$ in Figure 4) is too low for the material to remain confined to the vascular spaces (blood pool). However, the versatility of C_{60} allows for the possible additional attachment of tissue-specific groups (such as small peptides⁴⁵ or antibodies⁴⁶)

^a C_{60} can add as many as seven or eight groups depending on the steric bulk of the addend. For the trisadduct, $n = 3$ in Figure 4.

which may confer desirable tissue specificity for therapeutic applications using radioactive iodine (^{125}I or ^{131}I).⁴⁷ In addition, attaching the CA to a macromolecule or microparticle would be expected to greatly change the physical and chemical properties of the agent and may result in a CA that concentrates in a specific type of tissue or an agent that behaves as a blood pool CA. In a sense, the C_{60} -based X-ray CA is "tunable".

The increased number of iodine atoms per molecule (i.e. 6 iodine atoms for the monoadduct, 12 for the bisadduct, etc.) relative to the only three iodine atoms of the most commonly used agents (Figure 1) could give formulations with an equivalent iodine content yet a lower relative molarity. This would result in further reduced osmolality.³

The evolution from the initial design concept as shown in Figure 4, which is a multiadduct that uses only one type of addend for both water-solubilization and iodine delivery, to the actual C_{60} -based X-ray CA product of Figure 3, which uses two types of addends, is described in detail below.

2. Fullerene functionalization

Of the efficient methods available to functionalize C_{60} ,⁴⁸ one of the best approaches is nucleophilic cyclopropanation or the Bingel reaction.⁴⁹ The reaction proceeds under mild conditions at ambient temperatures and results in

the exclusive formation of a cyclopropane ring across one of the [6,6] double bonds of C_{60} in relatively high yields (> 50 %). The prototypical Bingel reaction which uses diethylbromomalonate and sodium hydride as base is shown in Figure 5.

Figure 5. Nucleophilic cyclopropanation with malonates proceeds exclusively across one of the [6,6] double bonds of C_{60} (Bingel reaction).⁴⁹ (i) NaH, PhCH₃, rt, 6.5 h, 45%.

The reaction also occurs easily with other, bulkier diester groups, and yields are improved in homogeneous systems when DBU (1,8-Diazabicyclo[5.4.0]undec-7-ene) is used as the base.⁸⁹ However, the malonodiester fullerene adducts are easily hydrolyzed to give the diacid product,⁵⁰ an undesirable attribute for materials requiring high *in vivo* stability. Accordingly, the C_{60} -based CA design incorporates the more hydrolytically

stable malonodiamide functionality rather than the malonodiester group. (No examples of malonodiamide fullerenes derivatives had been reported prior to this work.)

Synthetically, one clear approach to the malonodiamide fullerene was the conversion of the diester **3** into a diamide. For the initial experiments, a simpler analogue of the tri-iodinated isophthalic acid derivative was used to test the methodology. In this case, *p*-methylaniline was employed for the model studies.

As shown in Figure 6, hydrolysis of the diester **3** with NaH and methanol gave the diacid **4**.⁵⁰ Treatment of **4** with SOCl₂ gave a product which showed the characteristic acid chloride stretch in the FT-IR spectrum (C=O, 1781 cm⁻¹). The acid chloride **5** was then treated with an excess of *p*-methylaniline in anhydrous DMA. The product was analyzed by FAB mass spectrometry which showed no recognizable molecular ion or fragment peaks corresponding to the fullerene malonodiamide. However, a peak corresponding to the monoamide product **6** which could only have formed by decarboxylation was observed at *m/e* = 867.3 (calcd 867.1, see Figure 28 in App II). (As seen below, the peak at 867.3 is not simply due to fragmentation of the diamide.)

Figure 6. Formation of monoamide model compound by ester hydrolysis followed by amide coupling via an acid chloride intermediate. (i) NaH, MeOH, PhCH₃, rt, 15 min., 40 %. (ii) SOCl₂, CS₂. (iii) *p*-toluidine, DMA.

A similar attempt was made by Hirsch to prepare several fullerene malonodiamide compounds starting from the diacid **4** using the amide coupling reagent DCC. It was found that the exclusive product in each case was only the monoamide with accompanying decarboxylation.⁵¹ Hirsch's identical result further substantiates the observed formation of only the monoamide using an acid chloride intermediate. (In this case, it is not clear if the decarboxylation occurs during the formation of the acid chloride intermediate **5**, or during the condensation step to give **6**.)

Although the observed formation of the monoamide was a partial success and could in theory result in the attachment of 3 iodine atoms per addend when the triiodinated isophthalic acid derivative was used as the amine, the decarboxylation complication might be eliminated if the diamide were "pre-assembled" and then attached using Bingel chemistry. Attention then turned to this more convergent approach.

Again, using the model amine *p*-toluidine, the bromomalonodiamide **8** was prepared as shown in Figure 7. Compound **7** was assembled by the direct condensation of diethylmalonate with *p*-toluidine at elevated temperature.⁵² The resultant diamide was then brominated with elemental bromine at the central acidic carbon in AcOH. Compound **8** was found to react readily with C₆₀ under the conditions of the Bingel reaction (with DBU as base) at rt. This

resulted in the preparation of the first malonodiamide fullerene derivative, model compound **9**. Thus, the cyclopropanation reaction, not surprisingly, works nicely for bromo-malonodiamides as well bromo-malonodiesteres. By FAB-MS, molecular ion peaks for mono-, di- and triadducts were observed, however, no fragment peaks corresponding to the decarboxylation products ($M - 134.1$ [-CONHPhCH₃]) were observed (see Figure 29 in App II). Thus, the previously observed monoamide product **6** is most likely not due to fragmentation but rather due to the decarboxylated molecular ion. (Note: the cleavage of a single amide bond and the loss of -NHPhCH₃ was observed as a prominent fragment in the FAB-MS of **7**, see Figure 29 in App II.)

Figure 7. Model compound synthesis ($n = 1,2,3$). (i) $>150\text{ }^{\circ}\text{C}$, 15 min, 75 %. (ii) Br_2 , CHCl_3 , AcOH , 5 min, 81 %. (iii) C_{60} , DBU, PhCH_3 , 45 min.

It is noteworthy to mention that the C₆₀ adducts of the simple *N*-phenyl derived malonodiamides such as *p*-toluidine (model compound **9**) and *p*-nitroaniline were found to be paramagnetic with rt solid state Evan's balance measurements of the crude product mixtures being 1.2 BM and 3.4 BM at rt, respectively.⁵³ Molecular orbital calculations^b suggest that a charge transfer interaction between the anilido group (*N*-phenyl) and the C₆₀ core may be responsible for the observed magnetic properties and the results were published as a short communication (see ref. 53). However, as seen below, this complication is eliminated with *N*-alkyl malonodiamides and very sterically bulky anilido groups (such as the triiodinated-isophthalic acid derived groups). This curious observation was included for completeness, but is of no concern for the remainder of the work presented here.

With the successful synthesis of the model compound **9** using the convergent approach shown in Figure 7, attention was then turned to the assembly of the iodine containing malonodiamide addend. Simultaneously, a general method for water-solubilization of fullerene materials was developed with the help of undergraduate students Rachel Mortis and Vinay Kini. The water-solubilization approach also utilizes the newly developed malonodiamide

^b MO calculations made by J. Michael Alford of TDA, Inc., Wheat Ridge, Colorado, see ref. 53.

synthetic strategy,⁵⁴ and as discussed below, ultimately became an integral part of the final X-ray CA design.

3. Highly Water-soluble, Non-ionic Fullerene Derivatives

In general, prospective diagnostic/therapeutic agents, including CA, based on fullerenes are required to have sufficient biological availability, and thus, adequate water solubility. C_{60} itself may be coerced to form a colloidal suspension in water,^{55,56} but it is intrinsically insoluble.^{57,58,59} Finding effective ways to water-solubilize fullerene materials has been an active area of research. To date, the methods used to water solubilize fullerenes have taken one of four approaches:

1. chemical/electrochemical reduction of C_{60} or its derivatives to a water-soluble carbon-based anion (e.g. C_{60}^{n-} or $Ph_5C_{60}^-$)^{60,61}
2. complexation of C_{60} in a host-guest fashion within hosts such as the cyclodextrins^{62,63} and calixarenes,⁶⁴ effectively shielding the hydrophobic fullerene from the polar aqueous environment (included in this category are dispersions of C_{60} formed with surfactants^{65,66})
3. covalent modification of C_{60} with organic functionalities (one or more) that contain ionic groups (one or more)^{67,68,69,70,71,72,73,74}

4. covalent modification of C₆₀ with organic functionalities (one or more) that contain strongly hydrophilic groups (one or more) that are non-ionic ^{75,76,77}

The highest degree of water-solubility achieved prior to this work was accomplished by functionalizing C₆₀ with 6 Bingel addends each containing 2 primary alkyl amine groups. The trifluoroacetic acid salt of the material was found to have an aqueous solubility of 89 mg C₆₀ mL⁻¹.⁶⁸ However, the solubility is highly pH dependent, with a greatly decreased solubility expected at a biological pH of 7.4. (For a more complete discussion of each of the methods for water-solubilization see ref 54.)

It was thus desirable to develop a water-solubilization method for fullerenes that results in a highly water-soluble material that is well-defined and non-ionic. Accordingly, serinol (2-amino-1,3-propanediol, serinol has been shown to be biologically compatible⁷⁸) was condensed directly with diethylmalonate at elevated temperature as shown in

Figure 8.⁵² It was necessary to protect the alcohol groups so that the material would be soluble in a mutual solvent system with C₆₀. (Unprotected, the material was only soluble in very polar solvents like DMSO and H₂O.) Thus, the crude reaction residue was then treated with Ac₂O/pyridine to protect the 1,3-diols which gave **10**. Electrophilic bromination at the central methylene with

Br_2 in EtOAc gave **11**. Synthesis of the C_{60} monoadduct proceeded smoothly, to give the expected diamagnetic product **12**. The ^{13}C spectrum (Figure 9) of the malonodiamide derivative verified a structure completely analogous to comparable structures containing malonodiester addends. That is, the malonodiamide added exclusively across one of the 6-6 double bonds of C_{60} forming a cyclopropane ring and giving a structure of C_{2v} symmetry.⁴⁸ Of the expected 16 sp^2 C fullerene signals, 15 signals were resolved.

Figure 8. Synthesis of monoadduct of serinoldiamide water-solubilizing addend, C₆₀(MSA). (i) 200-225 °C, 45 min (ii) Ac₂O, pyridine, 18 h, 53 % (iii) Br₂, NEt₃, EtOAc, 79 %. (iv) C₆₀, DBU, PhCH₃, 1 h, 44 %.

Figure 9. 400 MHz ^{13}C NMR of 1',1'-Bis[N-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]carbamoyl]-1,2-methano[60]fullerene [$\text{C}_{60}(\text{MSA})$] in CDCl_3 , * = solvent impurity.

The degree of addition of addend 11 to C_{60} can be controlled by stoichiometry. The synthesis of a multiadduct mixture was accomplished by using a 10 fold molar excess of 11 relative to C_{60} as shown in Figure 10 (All attempts to control the regiochemistry of the adducts by using dimethyl anthracene as template⁷⁹ only resulted in complex mixtures of isomers.) It was necessary to add acetone to the reaction solution as the reaction

progressed to prevent product precipitation and incomplete functionalization. After workup, the inseparable mixture was analyzed by MALDI-TOF MS and found to consist of mostly pentaadduct (> 80 %) with some tri- and hexaadducts present as well (see Figure 42 in App II). Complete alcohol deprotection (as verified by ^1H NMR) was accomplished with K_2CO_3 in $\text{MeOH}/\text{H}_2\text{O}$ followed by removal of the potassium ions with a cation exchange resin (H^+ form) to give the fully water-soluble **14**. (Note: the deprotected form of monoadduct **12** was found to be nearly insoluble in H_2O .)

Figure 10. Water-soluble malonodiserinolamide fullerene $[\text{C}_{60}(\text{MS})_n]$, ($n = 4-6$). (i) C_{60} , DBU, acetone, PhCH_3 , 4.5 h, 69 %. (ii) K_2CO_3 , MeOH, H_2O , 1.5 h, near 100 %.

The water solubility of **14** (at pH = 7), as determined using UV-vis spectrophotometry, was found to correspond minimally to 240 mg C₆₀ mL⁻¹ (see Figure 47 in App II). This is the highest degree of water solubility reported to date for a fullerene material.⁵⁴ In addition to the very high aqueous solubility, the synthetic methodology results in a characterizable (many other water-solubilization methods give only ill-defined products, for example polyhydroxylation⁸⁰), non-ionic product whose solubility has no observable pH dependence. Also, the water-solubilizing methodology can be extended to fullerenes which are already functionalized with other groups (as shown below) and to other fullerene materials as well, empty or endohedral.^{32,81}

The *n*-octanol/water partition coefficient (K_{OW}) of compounds has proven to be a useful parameter for the determination of biological structure-activity relationships. It is defined as $K_{OW} = c_o c_w^{-1}$, where c_o and c_w are the equilibrium concentrations of an analyte in *n*-octanol and water, respectively. The partition coefficient has been used to predict the rate of biological uptake, the degree of biomagnification, and the degree of binding of solutes to serum albumin and other blood proteins.^{82, 38}

The partition coefficient of **14** was determined qualitatively by UV-vis spectrophotometry. For **14**, $K_{OW} \approx 0$ indicating that the compound is highly

hydrophilic and that the fullerene hydrophobic regions are effectively shielded by the multiple polar addends.

4. A Highly-iodinated Derivative of C₆₀

The highly-iodinated addend assembly was started by preparing the hexa-iodinated malonodiamide core as shown in Figure 11. Thus, starting from 5-aminoisophthalic acid, the three iodine atoms were introduced by electrophilic halogenation with KICl₂ in water, to give the 2,4,6-triiodo-5-aminoisophthalic acid **15**.⁸³ Treatment of the diacid with neat SOCl₂ at reflux for 6 h gave the diacid chloride **16**.

Figure 11. Synthesis of hexaiodinated malonodiamide core 17. (i) KICl_2 , H_2O , 55°C , 24 h, 73 %. (ii) SOCl_2 , reflux 6 h, 89 %. (iii) $\text{CH}_2(\text{COCl})_2$, THF, reflux 6 h, 83 %.

Diacid dichloride **16** was then directly condensed with malonyl dichloride in refluxing THF to give the tetraacid tetrachloride **17** in 83 % yield (Figure 11).⁸⁴ Kinetically, condensation with malonyl dichloride is greatly favored over polymerization. The sterically congested environment of the hexasubstituted benzene ring results in surprisingly non-reactive primary amine and acid chloride groups (due to the large flanking iodine atoms) and allows for such selectivity.⁸³

At this point, the malonodiamide substructure was intact and preliminary experiments showed that the brominated form of **17** did in fact react with C₆₀ as confirmed by MALDI-TOF MS. Although serinol could be reacted with the C₆₀ adduct of **17**, previous experience showed it was better to develop the more convergent approach by fully assembling the addend *prior* to reaction with C₆₀. This minimizes the number of steps carried out on the fullerene material which may suffer from poor solubility or give low yields and unwanted side products.

Continuing the addend assembly, tetracid tetrachloride **17** was condensed with serinol at rt in DMA and the colorless residue treated with excess Ac₂O/pyridine at rt for 18 h to protect the alcohol groups. After workup, characterization of the product indicated that condensation with serinol had occurred and that the 1,3-diol groups had also been protected. However, acetylation had also occurred at the central acidic methylene as shown in Figure 12. The only detectable product was **18** as evidenced by MALDI-TOF MS (calcd for C₄₉H₅₄O₂₃N₆I₆ [M]⁺ 1855.8, found 1877.8 [M - H + Na]⁺, see Figure 59 in App II) and ¹H NMR, which lacked the 2H methylene singlet at around $\delta = 3.55$ ppm in DMSO-d₆. Only a peak which integrated to approximately 0.5 H was observed at $\delta = 3.66$ ppm (see Figure 58 in App II). Additionally, the acetate methyl peak integrated to 27 H as expected for compound **18**.

Figure 12. Acetylation occurs at the central methylene during *O*-protection with Ac₂O/pyridine. (i) NEt₃, DMA, 20 h. (ii) Ac₂O, pyridine, 18 h.

The acetylation of the central methylene in **18** precludes the assembly of a Bingel adduct with C_{60} since it cannot form a cyclopropane ring. Attention was then turned to the *O,O'*-protection of serinol prior to reaction with **17**.

A suitable method for the *O,O'*-protection of serinol was developed based on the reported stability of both *cis*- and *trans*-5-amino-2-phenyl-1,3-dioxane.⁸⁵ As shown in Figure 13, serinol was first *N*-protected with benzylchloroformate (CBZCl) to give **19** and then *O,O'*-protected by condensation with benzaldehyde (cat. H_2SO_4) in refluxing toluene (H_2O was azeotropically removed) 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 the presence of intramolecular *N-H...O* hydrogen bonding.⁸⁶ In addition, the nucleophilicity of the *cis* isomer of 5-amino-2-phenyl-1,3-dioxane has been shown to be approximately twice that of the *trans* isomer.⁸⁷ Therefore, **20** (*cis*) was chosen as the more desirable protected-serinol isomer and was purified from the reaction mixture by fractional recrystallization from EtOH/ H_2O .

Figure 13. *O,O'*-protection of serinol to give *cis*-5-amino-2-phenyl-1,3-dioxane. (i) PhCHO, cat. H₂SO₄, PhCH₃, reflux, 4.5 h (ii) 300 psi H₂, Pd/C, EtOH, 50 min, 89 %.

Removal of the benzoxycarbonyl from the nitrogen of **20** under catalytic hydrogenation conditions (300 psi H₂, Pd/C) proceeded smoothly to give pure **22** as a colorless oil. Condensation of **22** with tetra-acid chloride **17** in DMA at rt, as shown in Figure 14, proceeded in excellent yield (95 %) to give the malonodiamide **23**.

Figure 14. Reaction of *O,O'*-protected serinol with the tetraacid chloride to give 23. (i) NEt_3 , DMA, 20 h, 95 %.

Compound **23** is the organic-soluble, iodine-containing malonodiamide addend that is ready for reaction with C_{60} (bromination was carried in situ, see below). The characterization of **23** was complicated by the presence of up to twelve hindered rotations at rt (Figure 15) caused by the sterically congested environment of the hexasubstituted benzene rings.⁸⁸ The large number of atropisomers caused the rt 1H NMR to be broadened and complex. The 500 MHz VT 1H NMR of **23** in $DMSO-d_6$ is shown in Figure 16.

Figure 15. Compound **23** showing the probable hindered rotations at rt due to steric congestion.⁸⁸

Figure 16. VT ^1H NMR (500 MHz) in DMSO-d_6 of 23. The y-axis scale was adjusted so that each spectrum is of approximately the same intensity.

Two general observations that can be made from the VT NMR experiment are: 1. even after heating the sample to 115 °C, there still remain some hindered rotations, and 2. the benzylidene acetals begin to thermally decompose/re-equilibrate slightly at around 100 °C and rapidly decompose/re-equilibrate at 130 °C (the data at 130 °C were taken after ~15 min at this temperature). Each significant region of the spectrum and the signal assignments are discussed below.

In the spectrum obtained at 25 °C, the N-H region has four distinct complex signals centered around $\delta = 10.2$, 9.41, 9.28, and 8.20. These peaks integrate to 2 H, 1.5 H, 1.5 H, and 1 H, respectively, giving the expected total 6 H integration. This interesting pattern was consistently observed in every preparation of the material. On heating, the downfield signal sharpens into a nearly symmetrical singlet. The assignment for the $\delta = 10.2$ is thus the two equivalent aromatic N-H. The three remaining signals broaden almost completely into the baseline at approximately 85 °C and begin to re-emerge together near $\delta = 8.3$ at higher temperature. The emerging signal integrates to 4 H and is assigned to the 4 equivalent alkyl N-H.

The benzylidene C-H signal is seen as a complex signal centered around $\delta = 5.63$ which quickly sharpens into a singlet at 55 °C and continues to sharpen with increasing temperature. The extra signal at $\delta = 5.52$ which is very

noticeable at the higher temperatures is due to the *trans* isomer of the benzylidene acetal which forms during the thermal decomposition and re-equilibration of the benzylidene acetals (*cis* $\leftrightarrow$ *trans*).

The central methylene signal at $\delta = 3.57$ is a broad singlet at 25 °C which sharpens somewhat on heating to reach a maximum sharpness at around 55-70 °C and then begins to broaden into the baseline. This rebroadening is likely due to a shift in the enol equilibrium at higher temperatures.

All of the remaining signals were easily assigned and sharpen considerably as the temperature is elevated. The extra signals that appear in the 130 °C spectrum can either be assigned to benzaldehyde ($\delta = 7.59, 7.69, 7.9, 10.05$) or the *trans*-benzylidene acetal isomer ($\delta = 3.86, 4.01, 4.36, 5.52$) which forms as a re-equilibration product. As mentioned above, all of the signals continue to change even up to 130 °C, but due to thermal decomposition, higher temperature experiments were prohibited. An interpretable ^{13}C NMR (500 MHz) was obtained at 70 °C (0.05 M, 1440 scans, see Figure 74 in App II).

To prepare the C_{60} monoadduct of the malonodiamide **23**, the newly published method of Hirsch for the *in situ* generation (with CBr_4 and DBU) of the brominated intermediate was used.^{89,90} The reaction, as shown in Figure

17, was found to proceed nicely at rt in 2:3 pyridine:toluene in which both **23** and C_{60} were suitably soluble to give the monoadduct **26**. The relatively good yield of 77 % was somewhat surprising, but indicates that the central methylene (methine after *in situ* bromination) in **23** is available for reaction with C_{60} in its more favored rt conformer(s).

Figure 17. Synthesis of C_{60} monoadduct X-ray contrast agent precursor. (i) CBr_4 , DBU, pyridine, $PhCH_3$, rt 18 h, 77 %.

The FTIR spectrum of adduct **24** was nearly superimposable on the IR spectrum of addend **23** (see Figure 75 and Figure 77 in App II), but additionally had the characteristic C₆₀-monoadduct band at 526 cm⁻¹ (compare to the IR spectrum of monoadduct C₆₀(MSA) (**12**) in Figure 40, also see IR data for **3**).^{91,92} A molecular ion peak at 2547.5 was found in the MALDI-TOF MS ([M]⁺ calcd for C₁₁₉H₅₀N₆O₁₄I₆, 2547.8), with the base peak at 2453.5 corresponding to the [M - I + S]⁺ ion^c (see Figure 78 in App II). Additionally, the material gave a single band by thin layer chromatography at R_f = 0.75 in 1:1 THF:toluene.

The NMR characterization, as with compound **23**, was hampered by the presence of multiple atropisomers. In the case of **24**, however, even after 17 h of data collection at 70 °C, the S/N ratio of the ¹³C NMR spectrum (500 MHz, pyridine-d₅, 0.05 M, 6128 scans) was still too low and the signals too broadened to interpret, indicating that some of the rotations of the addend **23** are even more hindered upon addition to C₆₀.

Compound **24** was found to be diamagnetic by rt solid state Evans balance measurements (~0 BM), ruling out paramagnetism as a major source of signal broadening in the NMR spectrum. In this case, presumably the large steric demands of the addend prevent the paramagnetism that is observed in

^c Adducts of sulfur are commonly observed when an elemental S₈ matrix is used in MALDI-TOF mass spectra. Peaks corresponding to plus 1 and 3 sulfur atoms are the most frequent. Alkali metal adducts (Na and K) are also frequently observed.

simpler anilide-derived malonodiamide fullerene derivatives⁵³ from occurring by not allowing a significant charge-transfer type interaction with the fullerene core to take place.

A qualitative *in vitro* determination of the degree of X-ray attenuation produced by hexa-iodinated **24** is shown in Figure 18. As expected based purely on the iodine content, **24** significantly enhances X-ray absorption relative to the DMF/toluene and saline blanks.

Figure 18. *In vitro* determination of X-ray attenuation of compound **24**. Syringe 4, 150 mg I/mL standard solution of Iohexol⁵ diluted with saline (0.9 % NaCl) solution; 5, 150 mg I/mL (0.197 M) solution of **24** in 5:1 DMF:toluene; 7, saline blank; 8, 5:1 DMF:toluene blank.

Attention then turned to the synthesis of the C₆₀ multiadduct to give the *O,O'*-protected form of the initially designed compound shown in Figure 4. However, when C₆₀ was treated with an excess of **23** (≥ 3 fold relative to C₆₀), only the monoadduct formed in good yield as determined by TLC and MALDI-TOF MS. Although some bisadduct product was observed, no higher adducts were detected, and the monoadduct was the major product. Apparently, the bulkiness of the addend does not allow the formation of adducts higher than the bis-, whose formation is also inhibited.

Additionally, the deprotection of monoadduct **24** resulted in a material that is only sparingly soluble in water. This result is not surprising considering the highly amphiphilic nature of the material (8 hydrophilic hydroxyls on one side and the highly hydrophobic fullerene-C₆₀ core on the other side). Given the very high water solubility requirements for X-ray CA and the lack of formation of the highly functionalized adducts, it seemed that water-solubilization of the monoadduct **24** (which already contained a substantial percentage of iodine and 8 hydroxy groups) with the previously established water-solubilization methodology (Figure 10) to give a "hybrid" material would most directly result in a material that could be evaluated in animal studies as an fullerene-based X-ray CA.

5. Water-solubilization of the Highly-iodinated C₆₀ Derivative

To accomplish water solubilization, **24** was treated with an 8 fold excess of **11** at rt over 18 h to give **25** as shown in Figure 19. (Again, all attempts to control the regiochemistry of the additions with the template dimethyl anthracene⁷⁹ were unsuccessful.) The orange-red product mixture, after workup, was found to consist of predominantly the pentaadduct ($n = 4$), with small amounts ($< 10\%$) of the tetraadduct ($n = 3$) present, as determined by MALDI-TOF MS (see Figure 81 in App II).

The two step deprotection scheme for **25** that was worked out is shown in Figure 20. The benzylidene acetals were first cleaved with $\text{BF}_3\cdot\text{Et}_2\text{O}$ in CHCl_3 which caused complete precipitation from solution to give **26**, which was not isolated but carried through to the next step. The precipitate was first thoroughly rinsed with fresh CHCl_3 then with DI H_2O (in which **26** is only sparingly soluble) until neutral to remove all of the residual inorganic ions. Complete removal of the acetal groups was verified by ^1H NMR (see Figure 85 in App II).

Deprotection of the acetate groups was then accomplished with Na_2CO_3 in $\text{MeOH}/\text{H}_2\text{O}$ to give **27**. In this case, Na_2CO_3 was used instead of K_2CO_3 since the presence of trace amounts of K^+ in the sample could cause toxicity problems in the animal studies. The removal of the sodium ions with a cation exchange resin (H^+ form) was verified by Na flame test and the residual carbonic acid was simply removed with the solvent under reduced pressure.

Redissolution of the extremely water soluble **27** in DI H_2O gave an ion free, neutral solution. (Due to the large amounts of material necessary to determine the degree of water solubility, it was not possible to accurately determine the solubility of **27** in water. However, from rough gravimetric determinations, the minimum observed solubility of **27** is around 460 mg/mL.)

A molecular ion peak of the deprotonated sodium adduct of **27** was observed at $m/e = 3211.1$ (calcd 3211.1, see Figure 86 in App II). After much trial and error, the MALDI-TOF conditions that ultimately resulted in observable peaks was a 2,5-dihydroxy benzoic acid matrix in solution with **27** in 1:1 $H_3CCN:H_2O$ with 1 % trifluoroacetic acid. The complete lack of peaks corresponding to residual protected species indicates complete alcohol deprotection. The clear presence of two higher mass peaks at $[M + Na + 16]^+$ and $[M + Na + 32]^+$ indicates the additional presence of oxygenated species (epoxides) which are commonly observed in C_{60} -derived materials.⁹³ Although very small oxygenated species peaks were observed in the mass spectra of the precursor compounds $C_{60}(MIB)$ **24** and $C_{60}(MIB)(MSA)_4$ **25**, the much greater intensity of the peaks in the mass spectrum of **27** indicate that epoxide formation most likely occurs predominantly during the deprotection step (despite anaerobic conditions). Alternatively, the oxygen may have been introduced during the mass spectrum sample preparation. Additionally, fragment peaks corresponding to the loss of up to all 6 iodine atoms are clearly seen with the loss of one iodine atom $[M - I + Na]^+$ being the base peak.

Figure 20. Deprotection of the C₆₀-based X-ray contrast agent. (i) BF₃·Et₂O, CHCl₃, -60 °C, 3 h (ii) Na₂CO₃, MeOH, H₂O, 1 h, 33 % (for steps i and ii)

Thermogravimetric analysis (TGA) in air of a dried sample showed only approximately 2 % weight loss by 100 °C followed by no additional weight loss until around 280 °C when **27** began to decompose (see Figure 89 in App II). This indicated that the sample contained very little residual solvent (water). Note that the protected form, compound **25**, began to decompose around 240 °C (see Figure 83 in App II) most likely due to the presence of the thermally labile benzylidene acetal groups.

The UV-vis spectra of both **25** and **27** (0.62 M, see Figure 84 and Figure 88 in App II) have no maxima, which is characteristic of a complex mixture of isomers. In the ¹H NMR of **27**, complete deprotection is apparent and only the methine and methylenes of the serinol residues are observed (see Figure 90 in App II). The elemental analysis is consistent with the composition of **27** and the characteristic amide carbonyl (1652 cm⁻¹) and hydroxy (3382 cm⁻¹) stretches were observed in the FT-IR spectrum (see Figure 87 in App II).

Several water-soluble, amphiphilic C₆₀ derivatives have been shown to form aggregates in aqueous solution.^{94,95} However, dynamic light scattering experiments with **27** indicated that the compound does not form aggregates in the detection range of 3-3000 nm in aqueous solution (pH ≈ 7) at concentrations of ≤ 2 mM. This result is indicative of the hydrophobic shielding

expected by multiple strongly hydrophilic addends, and at concentrations of around 2 mM, **27** exists as a monomer in solution.

Additionally, the *n*-octanol/water partition coefficient (K_{OW}) was determined, and like **14**, no detectable amount of **27** moved into the organic layer from the aqueous layer. Thus for **27**, $K_{OW} \approx 0$. This result compares well with the very small K_{OW} values ($< 10^{-3}$)⁵ of currently-used x-ray contrast agents which are also very hydrophilic.

The complete synthesis of compound **27** is summarized in Figure 21-Figure 26. Unless previously indicated, each product and intermediate shown was completely characterized. The spectra are in Appendix II and the data are listed at the end of the experimental section for each compound.

Figure 21. Synthesis of malonodiamide 17. (i) KI , H_2O , 55°C , 24 h, 73 % (ii) SOCl_2 , reflux 6 h, 89 % (iii) $\text{CH}_2(\text{COCl})_2$, THF, reflux 6 h, 83 %.

Figure 22. Synthesis of *O,O'*-protected serinol. (i) benzyl chloroformate (CBZCl), NEt_3 , EtOH, $< 35^\circ\text{C}$, 2.25 h, 76 % (ii) PhCHO , cat. H_2SO_4 , PhCH_3 , reflux, 4.5 h (iii) 300 psi H_2 , Pd/C, EtOH, 50 min, 89 %.

Figure 23. Synthesis of malonodiamide 23. (i) NEt_3 , DMA, 20 h, 95 %.

Figure 24. Synthesis of 11. (i) 200-225 °C, 45 min (ii) Ac_2O , pyridine, 18 h, 53 % (iii) Br_2 , NEt_3 , EtOAc , 79 %.

Figure 25 Assembly of O-protected X-ray contrast agent [C₆₀(MIB)(MSA)₄], 25. (i) CBr₄, DBU, pyridine, PhCH₃, 77 % (ii) 11, DBU, CHCl₃, 40 h, 66 %

Figure 26. Final deprotection to give C₆₀-based X-ray contrast agent 27, [C₆₀(MI)(MS)_n] (i) BF₃·Et₂O, CHCl₃, -60 °C → rt, 3 h (ii) Na₂CO₃, MeOH, H₂O, 1 h, 33 % (for steps i and ii).

6. Conclusions

The first fullerene-based X-ray contrast agent has been designed, synthesized, and characterized. Spin-offs of the synthesis include the development of a method for the preparation of the first malonodiamide-fullerene adducts and the development of an efficient method for producing extremely water-soluble, non-ionic C₆₀ derivatives with the highest degree of aqueous solubility reported to date. Additionally, the water-solubilization methodology has been shown to work well at solubilizing a C₆₀ adduct (the hexaiodinated derivative) to give a hybrid material and would be expected to be applicable to the water-solubilization of any reactive fullerene material, including endohedral fullerenes.

The synthetic pathway was designed to minimize the number of synthetic steps involving any C₆₀ material, which in general provides overall improved yields. Additionally, the evolution of the X-ray CA design, stemming from synthetic and practical design limitations, is described in detail. For completeness, some of the major unsuccessful synthetic pathways are also highlighted.

7. Future Directions

Most of the steps in the synthesis of the X-ray CA $C_{60}(MI)(MS)_4$, **27** give good yields. An improvement in the yield (33%) of the final two-step deprotection of **25**, however, would greatly increase the overall efficiency of the preparation. Several different types of cation exchange resin should be tried to find a type that minimizes product adsorption (and loss of material).

The yield may also be improved by using the same protecting group for both types of addends, reducing the final deprotection to one step. As discussed above, the acetate protecting group was not suitable for the protection of the hexaiodinated addend, which lead to the use of bezylidene acetals to protect the 1,3-diols. This same group could be used to protect the water-solubilizing addends (MSA, **10**) as shown in Figure 27, which could then be appended to C_{60} using the *in situ* bromination method.

Figure 27. C_{60} -based X-ray CA with only one type of protecting group, the benzylidene acetal.

As mentioned above, when an excess (> 3 fold) of addend **23** (MIB) was reacted with C_{60} , only small amounts of the bisadduct formed with no higher adducts detected (under the same conditions used for the synthesis of the monoadduct). However, more of the bisadduct may form under other experimental conditions. Isolation and purification of the monoadduct followed by a second exposure to the addend in the same or a different solvent system may improve the yield, or a simple increase in the reaction temperature may drive the reaction toward more bisadduct. The deprotected bisadduct,

whether sufficiently water-soluble on its own, or further solubilized with 11 (BrMSA), would have an iodine/molecule ratio of 12. As previously discussed, advantages of such a high ratio (compare to a ratio of 3 for Iohexol) are lowered osmolality and a decreased molarity necessary for contrast enhancement.

Compound 27 could be polyhydroxylated at some of the remaining reactive double bonds by treatment with strong base.⁹² This modification will undoubtedly increase water solubility and may lead to solution aggregation, as previously observed with some fulleranol materials.⁹⁶ Solution aggregation may help to slow extravasation, leading to a material that behaves as a much-sought-after blood pool CA.

Targeting of X-ray CA to specific tissues by appending cell-receptor-specific antibodies or small proteins is not likely to be a practical objective with the current technology. This is due to the fact that X-ray CA are only effective at producing contrast at or above concentrations in the mM range. However, the biochemistry of ligands and receptors occurs in the nanomolar range. Clearly enough biological receptors are not available to increase contrast even at saturated receptor levels.

Finally, further studies to determine the efficacy of **27** as an X-ray CA should be carried out. At the time of the completion of this work, preliminary animal studies were underway.

APPENDIX I**Abbreviations and Symbols**

ArC	¹³C NMR chemical shift notation indicating that a given signal is due to a carbon atom in an aromatic ring
ArH	¹H NMR chemical shift notation indicating that a given signal is due to a proton on an aromatic ring
anal	analytical or analysis (for elemental analysis)
asym	asymmetric
BM	Bohr Magneton
bp	boiling point
conc	concentrated
CBZ	benzoxycarbonyl (amine protecting group)
DBM	diethylbromomalonate
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DEM	diethylmalonate
DCC	1,3-dicyclohexylcarbodiimide
DI	deionized
DMA	dimethylacetamide
EtOAc	ethyl acetate

EtOH	ethanol
Et ₂ O	diethyl ether
ea	each
FAB	fast atom bombardment (MS technique)
g	gram(s)
h	hour(s)
ID	inner diameter
MeOH	methanol
MIB	N,N'-Bis[2,4,6-triiodo-N,N'-bis-[<i>cis</i> -(2-phenyl-[1,3]dioxan-5-yl))-3,5-benzenedicarbamoyl]-malonamide
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
mmol	millimole
mol	mole
MS	mass spectrum
MSA	N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-malonamide
Ph	phenyl
ppt	precipitate
rbf	round bottom flask

ref	reference
rt	room temperature (ambient temperature)
satd	saturated
THF	tetrahydrofuran
TFA	trifluoroacetic acid
μL	microliter(s)
μm	micrometer(s)
VT	variable temperature

APPENDIX II
(Selected Spectra)

(6) 1'-[N-(*p*-tolyl)carbamoyl]-1,2-methano[60]fullerene

Figure 28. FAB mass spectrum of 6 showing the $[M+H]^+$ peak at 868.2 m/e. No significant higher mass peaks were observed.

(9) 1',1'-Di[*N*-(*p*-tolyl)carbamoyl]-1,2-methano[60]fullerene

Figure 29. FAB mass spectrum of 9. The mono-, di-, and tri-adducts are labeled, * corresponds to $[M - \text{NHPhCH}_3]^+$.

(10) N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-malonamide (MSA)

Figure 30. 400 MHz ^1H NMR of 10 in CDCl_3 .

Figure 31. EI^+ mass spectrum of 10, $[\text{M}]^+$ at 418.1 m/e .

Figure 32. FT-IR (KBr) of 10.

Figure 33. 400 MHz ¹³C NMR of 10 in CDCl₃.

(11) 2-Bromo-N,N'-bis[2-(acetyloxy)-1-[(acetyloxy)methyl] ethyl]-malonamide (BrMSA)

Figure 34. ¹H NMR of 11 in CDCl₃.

Figure 35. EI+ mass spectrum of 11, [M]⁺ = 496.1.

Figure 36. 400 MHz ^{13}C NMR of 11 in CDCl_3 .

Figure 37. FT-IR (KBr) of 11.

(12) 1',1'-Bis[N-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]carbamoyl]-1,2-methano[60]fullerene

Figure 38. ^1H NMR of 12 in CDCl_3 .

Figure 39. ^{13}C NMR of 12 in CDCl_3 , * = impurity.

Figure 40. FT-IR (KBr) of 12.

Figure 41. MALDI TOF mass spectrum of 12 (CHCl₃), [M]⁺ = 1136.1.

(13) $n[1',1'$ -Bis[N-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl] carbamoyl]]-1,2-methano[60]fullerene (multiadduct, 11, $n = 4-6$)

Figure 42. MALDI-TOF mass spectrum of 13 ($n = 4-6$).

Figure 43. FT-IR (KBr) spectrum of 13.

**(14) $n[1',1'$ -Bis[N -(2-hydroxy-1-(hydroxymethyl)ethyl)carbamoyl]]
-1,2-methano[60]fullerene ($n = 4-6$) $C_{60}(MS)_n$**

Figure 44. MALDI-TOF mass spectrum of 14, $[M]^+ n = 5$, 1960.5; $[M]^+ n = 4$, 1712.4.

Figure 45. FT-IR (KBr) spectrum of 14.

Figure 46 TGA data for 14. 10 °C/min ramp in air.

Figure 47. Aqueous solubility determination of 14. Absorbance vs. mass of 14 in 400 μ L H₂O in 1.0 mm cell measured at 630 nm.

(15) 5-Amino-2,4,6-triiodo-1,3-benzenedicarboxylic Acid **Figure 48. ¹³C NMR of 15 in DMSO-d₆.** **Figure 49. MALDI-TOF mass spectrum of 15 (acetone), [M]⁺ = 558.7.**

Figure 50. FT-IR (KBr) of 15.

(16) 5-Amino-2,4,6-triiodo-1,3-benzenedicarbonyl dichloride **Figure 51. 400 MHz ^{13}C NMR of 16 in acetone- d_6 .** **Figure 52. MALDI-TOF mass spectrum of 16 (acetone), $[\text{M}]^+ = 593.7$.**

Figure 53. FT-IR (KBr) of 16.

(17) N,N'-Bis(2,4,6-triiodo-3,5-benzenedichlorocarbonyl)-malonamide

Figure 54. 400 MHz ^1H NMR of 17 in acetone- d_6 . * = THF

Figure 55. 400 MHz ^{13}C NMR of 17 in acetone- d_6

Figure 56. FT-IR (KBr) of 17.

Figure 57. MALDI TOF mass spectrum of 17 (acetone), $[M]^+ = 1256.3$.

(18) 2-Acetyl-*N,N'*-Bis[2,4,6-triiodo-*N,N'*-bis-[2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl]-3,5-benzenedicarbamoyl]-malonamide.

Figure 58. 500 MHz ^1H NMR of 18 in DMSO-d_6 .

Figure 59. MALDI-TOF mass spectrum of 18 (acetone), $[\text{M} - \text{H} + \text{Na}]^+ = 1877.8$.

(19) (2-Hydroxy-1-hydroxymethyl-ethyl)-carbamic acid benzyl ester **Figure 60. 400 MHz ¹H NMR of 19 in acetone-d₆.** **Figure 61. 400 MHz ¹³C NMR of 19 in acetone-d₆.**

Figure 62. FT-IR (KBr) of 19.

Figure 63. EI+ mass spectrum of 19, $[M]^+ = 225.1$.

(20) *Cis*-(2-Phenyl-[1,3]dioxan-5-yl)-carbamic acid benzyl ester **Figure 64. 400 MHz ^1H NMR of 20 in CDCl_3 .** **Figure 65. 400 MHz ^{13}C NMR of 20 in CDCl_3 .**

Figure 66. FT-IR (KBr) of 20.

Figure 67. EI+ mass spectrum of 20, $[M - H]^+ = 312.1$.

(21) *Trans*-(2-Phenyl-[1,3]dioxan-5-yl)-carbamic acid benzyl ester

Figure 68. 400 ^1H MHz NMR of 21 in CDCl_3 .

Figure 69. EI^+ mass spectrum of 21 $[\text{M} - \text{H}]^+ = 312.1$

Figure 70. 400 MHz ^{13}C spectrum of 21 in CDCl_3 .

(22) *Cis*-5-amino-2-phenyl-1,3-dioxane **Figure 71. 400 MHz ¹H NMR of 22 in CDCl₃.** **Figure 72. 400 MHz ¹³C NMR of 22 in CDCl₃.**

(23) N,N'-Bis[2,4,6-triiodo-N,N'-bis-[*cis*-(2-phenyl-[1,3]dioxan-5-yl)]-3,5-benzenedicarbamoyl]-malonamide [MIB]

Figure 73. 500 MHz ¹H NMR of 23 in DMSO-d₆ at 70 °C.

Figure 74. 500 MHz ¹³C NMR of 23 in DMSO-d₆ at 70 °C.

Figure 75. FTIR (KBr) of 23.

Figure 76. MALDI-TOF mass spectrum of 23 (THF), $[M]^+ = 1829.8$.

(24) 1',1'-Bis[N-[2,4,6-triiodo-N,N'-bis-[*c/s*-(2-phenyl-[1,3]dioxan-5-yl)]-3,5-benzenedicarbamoyl]carbamoyl]-1,2-methano[60]fullerene [C₆₀(MIB)]

Figure 77. FTIR (KBr) of 24.

Figure 78. MALDI-TOF mass spectrum of 24 (CHCl₃), [M]⁺ = 2547.8.

Figure 79. 400 MHz ¹H NMR of 24 in CDCl₃. The small signals at $\delta = 1.3$ and 2.4 are due to impurities.

(25) $C_{60}(MIB)(MSA)_x$

Figure 80. 400 MHz ^1H NMR of 25 in CDCl_3 . The small peaks at $\delta = 1.9$ and 3.8 are THF and the resonance at $\delta = 3.2$ is an impurity.

Figure 81. MALDI-TOF mass spectrum of 25 (CHCl_3). The labelled peaks are $C_{60}(\text{MIB})(\text{MSA})_4$ and $C_{60}(\text{MIB})(\text{MSA})_3$.

Figure 82. FT-IR spectrum (KBr) of 25.

Figure 83. TGA data for 25. 10 °C/min ramp in air.

Figure 84. UV-vis spectrum of 25 in CHCl_3 , 0.62 mM.

(26) C₆₀(MI)(MSA)₄

Figure 85. 400 MHz ¹H NMR of 26 in DMSO-d₆, showing the complete removal of the benzylidene acetal protecting groups. The acetate groups are still intact.

(27) $C_{60}(MI)(MS)_4$

Figure 86. MALDI-TOF mass spectrum of 27 from 1:1 $H_3CCN:H_2O$ (2,5-dihydroxy benzoic acid matrix, 1 % TFA). $C_{60}(MI)(MS)_4$ $[M - H + Na]^+$ 3211.1.

Figure 87. FT-IR spectrum (KBr) of 27.

Figure 88. UV-vis spectrum of 27 in H₂O, 0.62 mM.

Figure 89. TGA data for 27. 10 °C/min ramp in air.

Figure 90. 400 MHz ¹H NMR of 27 in D₂O showing complete alcohol deprotection.

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