Fullerene Immunoconjugates for Cancer Imaging and Treatment

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Talk abstract (*just FYI, not a real slide*)

Metallofullerene-paclitaxel-antibody conjugates are under investigation for combined cancer imaging and treatment. Fullerenes are non-toxic carbon cage molecules with a rich derivatization chemistry that is useful for generating covalently-conjugated therapeutic prodrugs [1]. Fullerene derivatives have been shown to spontaneously form non-covalent immunoconjugates with antibodies such as the anti-gp240 melanoma antibody ZME-018; a recent study revealed how ZME-018 forms non-covalent immunoconjugates with C_{60} derivatives without significant loss of antibody activity [2]. Endohedral gadofullerene derivatives have been shown in prior studies to function effectively as T_1-active magnetic resonance imaging (MRI) contrast agents, both for *in vivo* MRI [3] and for *in vitro* cellular labeling [4].

In this study, chemical routes for conjugation of paclitaxel and $Gd@C_{60}$ are being developed to form a combined therapeutic/imaging agent prodrug. Using the gadofullerene-paclitaxel conjugates, immunoconjugates with the anti-gp240 melanoma antibody ZME-018 will be tested for cellular uptake, for MR imaging efficacy and for cytotoxicity against melanoma cancer cells *in vitro*. The strategy of combining antibody targeting with a therapeutic MR imaging agent will improve melanoma diagnosis and treatment, and has the potential for targeting multiple drugs to cancerous cells at the same time to improve patient outcome. Recent progress toward the gadofullerene-paclitaxel immunoconjugates, including *in vitro* cell binding and internalization of conjugates with A375 melanoma cells, will be discussed.

1. Zakharian, T. Y.; Seryshev, A.; Sitharaman, B.; Gilbert, B. E.; Knight, V.; Wilson, L. J. "A Fullerene-Paclitaxel Chemotherapeutic: Synthesis, Characterization, and Study of Biological Activity in Tissue Culture," *J. Am. Chem. Soc.* **2005,** *127*, 12508-12509.

2. Ashcroft, J. M.; Tsyboulski, D. A.; Hartman, K. B.; Zakharian, T. Y.; Marks, J. W.; Weisman, R. B.; Rosenblum, M. G.; Wilson, L. J. "Fullerene (C_{60}) immunoconjugates: interaction of water-soluble C_{60} derivatives with the murine anti-gp240 melanoma antibody," *Chem. Commun.* **2006, 3004-3006.**

3. Bolskar, R. D.; Benedetto, A. F.; Husebo, L. O.; Price, R. E.; Jackson, E. F.; Wallace, S.; Wilson, L. J.; Alford, J. M. "First Soluble $M@C_{60}$ Derivatives Provide Enhanced Access to Metallofullerenes and Permit *in Vivo* Evaluation of $Gd@C_{60}[C(COOH)_2]_{10}$ as a MRI Contrast Agent," *J. Am. Chem. Soc.* **2003,** *125*, 5471-5478.

4. Sitharaman, B.; Tran, L. A.; Pham, Q. P.; Bolskar, R. D.; Muthupillai, R.; Flamm, S. D.; Mikos, A. G.; Wilson, L. J. "Gadofullerenes as nanoscale magnetic labels for cellular MRI," *Contrast Med. Mol. Imag.* **2007,** *2*, 139-146.

Overview: Fullerene Immunoconjugates

- Theranostics: combining therapy and imaging
- Using fullerenes for selective drug delivery and magnetic resonance imaging (MRI)
- *In vitro* study of $C_{60}(OH)_x$ immunoconjugates
- Synthesis of new conjugates: covalent linking of paclitaxel to C_{60} and gadofullerenes
- Ongoing studies and future directions

TDA
Research

Combining Drug Delivery with Selective Imaging

- **Theranostics**: combination of selective imaging with therapeutic drug delivery in one agent
- Goal: treat cancer (*or other disease*) with targeted drugs, while also monitoring with targeted imaging for-
  - -primary tumor location/margins
  - -metastases
  - -treatment progress
- Reduced side effects from systemic administration of toxic chemotherapeutics

diagnosis + therapy

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Gadofullerene-Drug Conjugates for Melanoma Treatment and MR Imaging

- We are combining three fullerene research areas:

Fullerene – ZME-018 immunoconjugates

C_{60} – taxol drug conjugates

Gadofullerene – MRI contrast agents

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Gadofullerene-Drug Conjugates for Melanoma Treatment and MR Imaging

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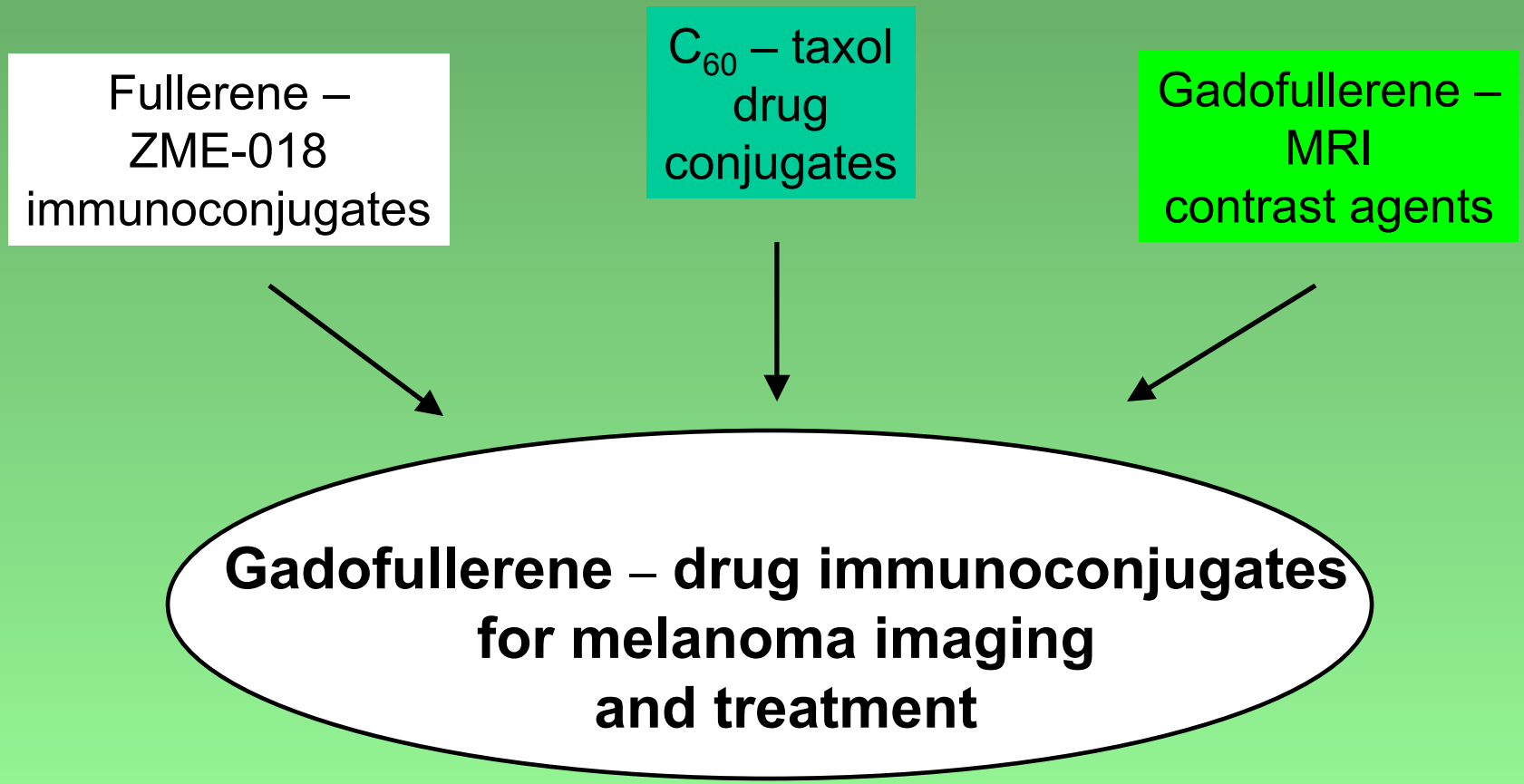

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Melanoma: an Important Target for Theranostics

- Melanoma: a potentially deadly skin cancer that has increased in frequency for ~ 30+ years
- Only ca. 5% of skin cancers are melanoma, but it causes over 75% of skin cancer deaths
- In 2009: 68,720 new cases; 8,650 deaths
- Early detection *and* treatment are key for good outcome
- New treatments are needed, especially for advanced and metastatic cases (traditional chemotherapy and radiation do not work well)

source: American Cancer Society, 2009 US estimates

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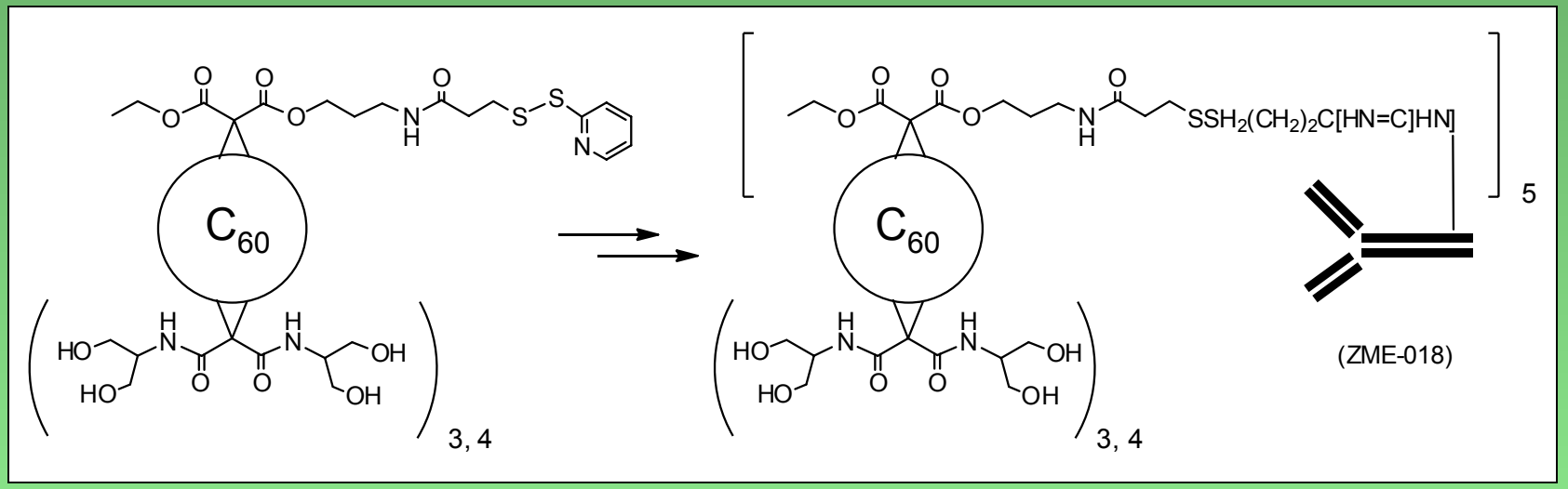
Fullerene Immunoconjugates

- Selective targeting of fullerenes to cancer with antibodies
- Antibody ZME-018 targets the gp240 antigen, present on over 80% of human melanomas



- C_{60} – (bis)serinol – ZME-018 derivative; avg. 5 disulfide bonds

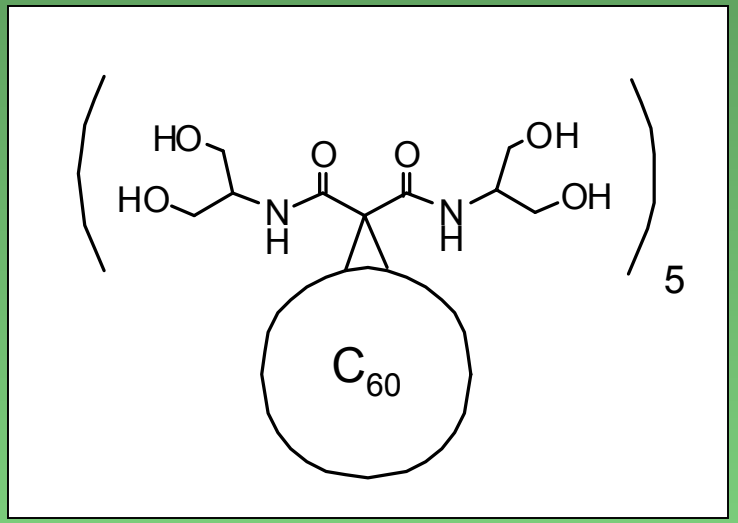
Rosenblum et al. *Cancer Res.* (2003), **63**, 3995-4002

Ashcroft et al. *Chem. Comm.* (2006), 3004-3006

TDA Research

Fullerene Immunoconjugates

- Non-covalent C_{60} – serinol – ZME-018 derivative forms spontaneously



- ELISA analyses showed high binding ratios (15:1 deriv:Ab)
- Even at these high levels, the antibody to antigen binding is not significantly decreased relative to controls
- Fullerenes (ca. 1 nm^3) likely non-covalently bind in the hydrophobic pockets (ca. 560 nm^3) of ZME-018

Ashcroft et al. *Chem. Comm.* (2006), 3004-3006

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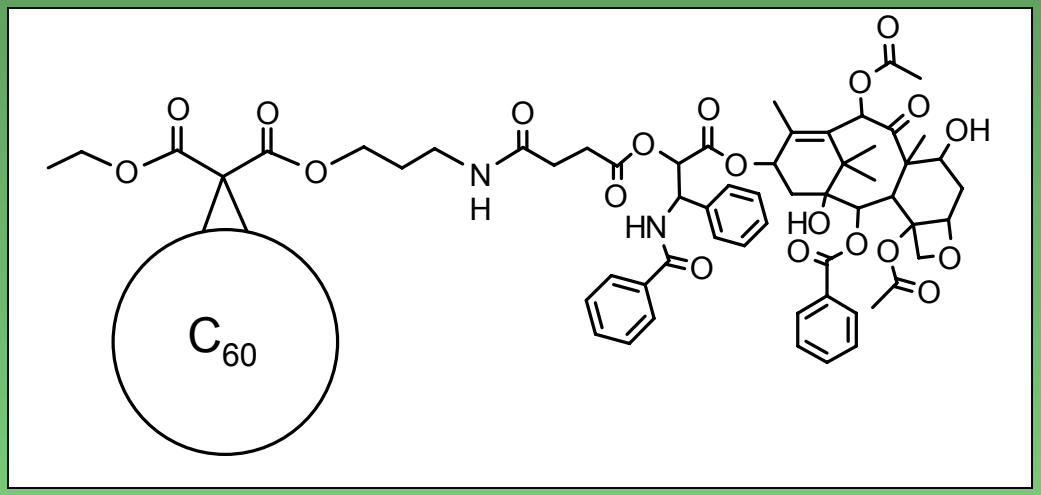
A Fullerene-Paclitaxel Conjugate

- Wilson and co-workers reported the first C_{60}-paclitaxel covalent conjugate (linked via 2'-OH position)



- Paclitaxel (Taxol®) induces apoptotic cell death (→ *cancer*)
- The conjugate, with a hydrolytic $t_{1/2}$ of ca. 80 min, serves as a prodrug for slow release of paclitaxel in plasma
- *In vitro* tests with a liposomal formulation showed significant efficacy against lung cancer cells with the above conjugate, comparable to a paclitaxel/liposome formulation alone

Zakharian et al. *J. Am. Chem. Soc.* (2005), **127**, 12508

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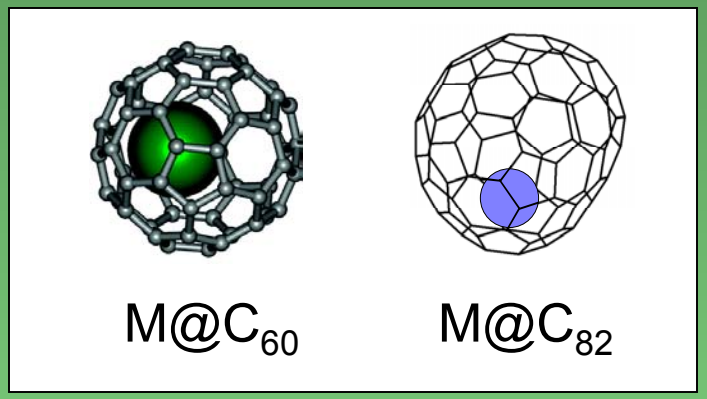
Metallofullerenes in Biomedicine

- Endohedral metallofullerenes sequester atom(s) in their hollow interiors



- Trapped metal ions are useful for imaging agents (MRI, X-Ray, radiotracers, etc.) and nuclear medicine for **safety** and **efficacy** reasons

- Metallofullerenes offer a unique “chelator” motif with the diverse surface chemistry of fullerene cages

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Magnetic Resonance Imaging (MRI) Contrast Agents and Gadofullerenes

- MRI: images tissue water protons, non-invasive, no ionizing radiation
- Gd contrast agents increase signal by increasing relaxation rates

- TDA's redox- and solubility-based processes access the arc-produced Gd metallofullerenes
- Gadofullerene derivatives are 5 to 25 times more efficient than current clinical Gd^{3+} chelate agents at water proton relaxation

Bolskar et al. *J. Am. Chem. Soc.* (2003), **125**, 5471-5478

Laus et al. *J. Phys. Chem. C* (2007), **111**, 5633-5639

Raebiger et al. *J. Phys. Chem. C* (2008), **112**, 6605-6612

TDA Research

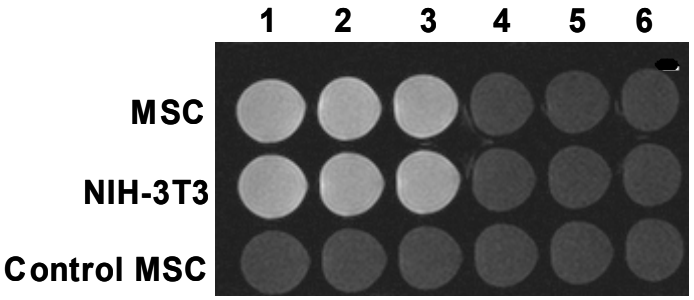
Gadofullerene MRI Contrast Agents

- Water-soluble $Gd@C_{2n}$ derivatives we have studied:

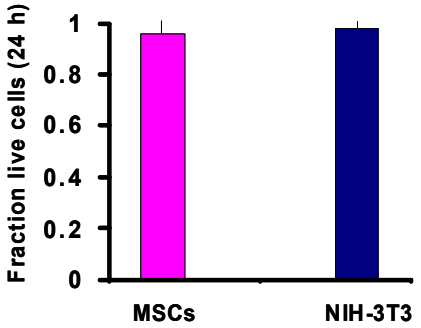
carboxylate – $Gd@C_{60}[C(COOH)_2]_{10}$

fullerol – $Gd@C_{60}(OH)_x$ ($x \approx 27$)

- *In vitro* MRI study of $Gd@C_{60}[C(COOH)_2]_{10}$ showed it to function as an efficient intracellular magnetic label:



T_1 weighted *in vitro* MR images show a ca. 250% MRI signal increase with gadofullerene



Excellent cell viability after 24 hr incubation

Tóth et al. *J. Am. Chem. Soc.* (2005), **127**, 799-805

Sitharaman et al. *Contr. Med. Mol. Imaging* (2007), **2**, 139-146

TDA Research

In Vitro Study of $Gd@C_{60}(OH)_x$ Immunoconjugates

- For *in vitro* studies: use metallofullerene derivatives in place of C_{60} derivatives for quantifying binding and cellular internalization
- Gd as a “metal ion tracer”; ICP-MS allows detection at the parts-per-trillion level
- Cellular internalization with gadofullerol $Gd@C_{60}(OH)_x$ immunoconjugates was studied first, without an attached drug cargo

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In Vitro Study of $Gd@C_{60}(OH)_x$ Immunoconjugates

- $Gd@C_{60}(OH)_x$ was non-covalently conjugated to ZME-018 and to MuIgG (as a control); for both, the fullerene:Ab ratio was ca. 5:1 (by ICP-MS and UV-Vis spectroscopy)
- With antigen-positive A375m melanoma cells, the gado-fullerene/ZME-018 immunoconjugate showed strong binding and internalization into the cells
- Antigen negative control cells (T24 bladder carcinoma) showed lower internalization of gado-fullerene/ZME-018, demonstrating specificity of the immunoconjugate

S. Berger, L. Wilson, Rice Univ.

TDA Research

Gadofullerenes for Combined Drug Delivery and MRI Contrast Agents

- Antibody targets the drug payload and gadofullerene MRI agent *together*
- Drug delivery goal is to increase *in vivo* efficacy of the drug, with specific (targeted) delivery to decrease side effects (chemotherapeutics kill rapidly dividing cells…)
- Covalent bonds link the fullerene to the payload for relative stability during the time of delivery / targeting / uptake, followed by hydrolytic release (esters, carbonates, etc.)
- Aqueous solubility of paclitaxel is poor… new options for its delivery are greatly desired

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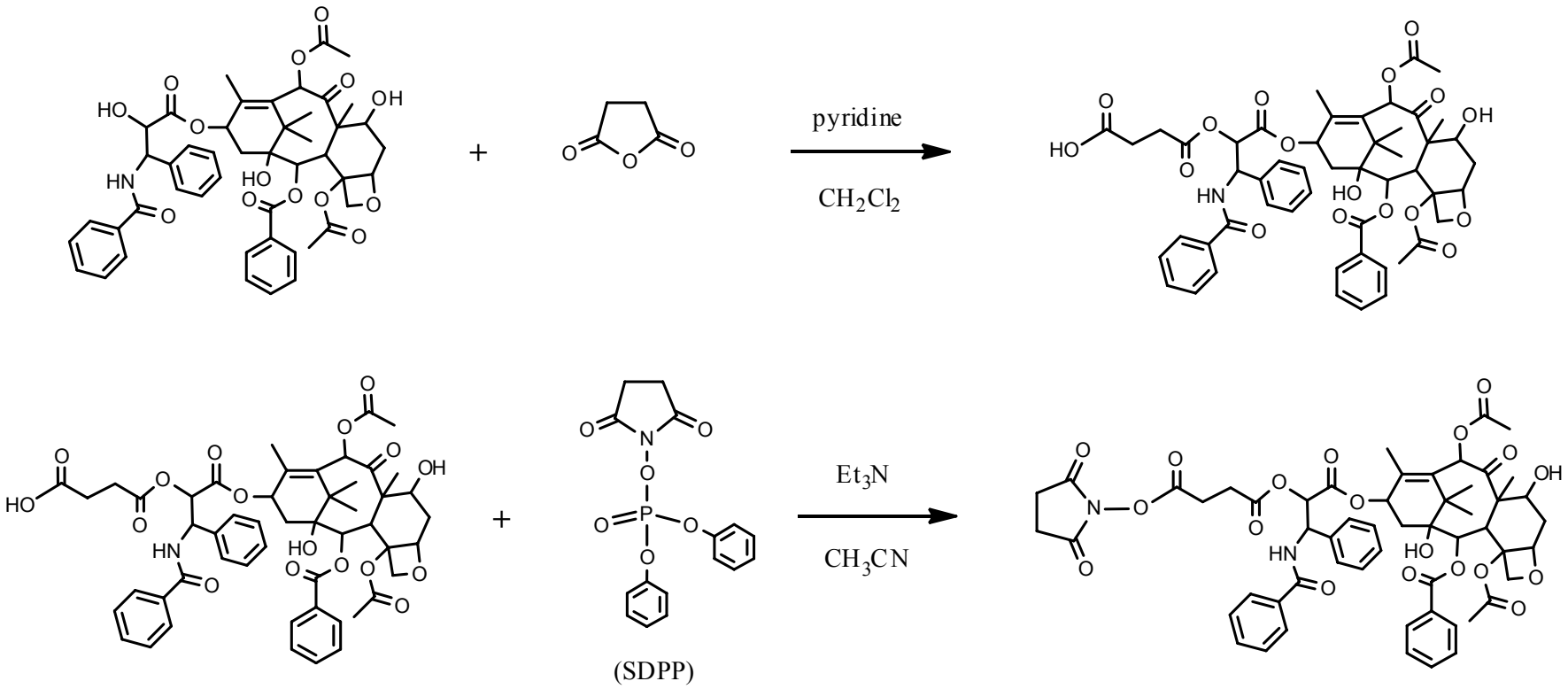
Synthesis of Paclitaxel Precursor

- 2'-hemisuccinate synthesis, followed by NHS ester formation

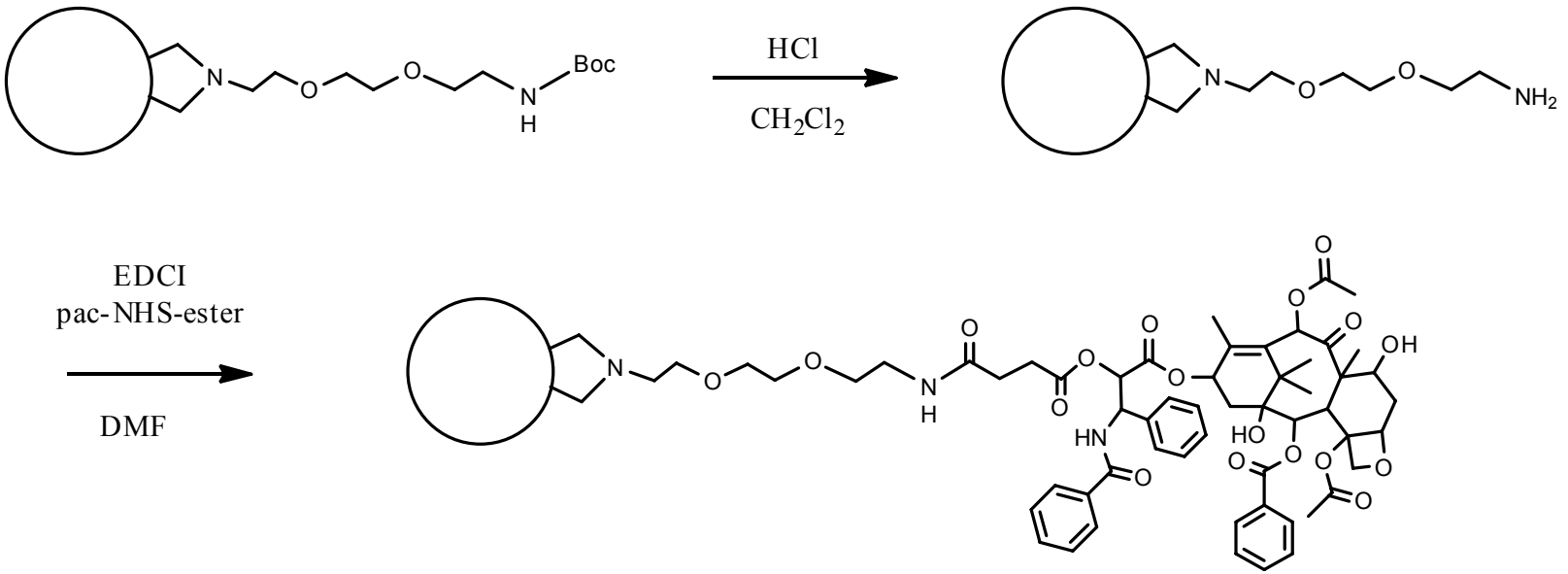


Luo et al. *Bioconj. Chem.* (1999), **10**, 755-763

TDA Research

Linking Paclitaxel to Fullerenes

- Pyrrolidine derivatization, followed by covalent paclitaxel linkage



fullerene = C_{60}, $Gd@C_{60}$

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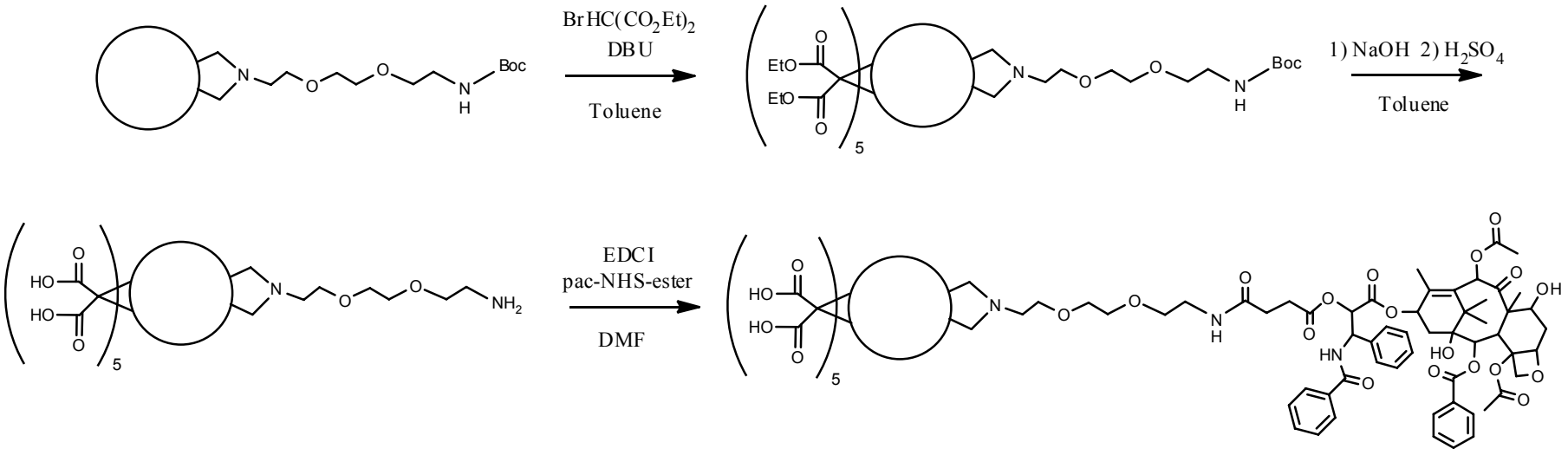
Linking Paclitaxel to Fullerenes

- Second derivatization (cyclopropanation), followed by pac-linkage

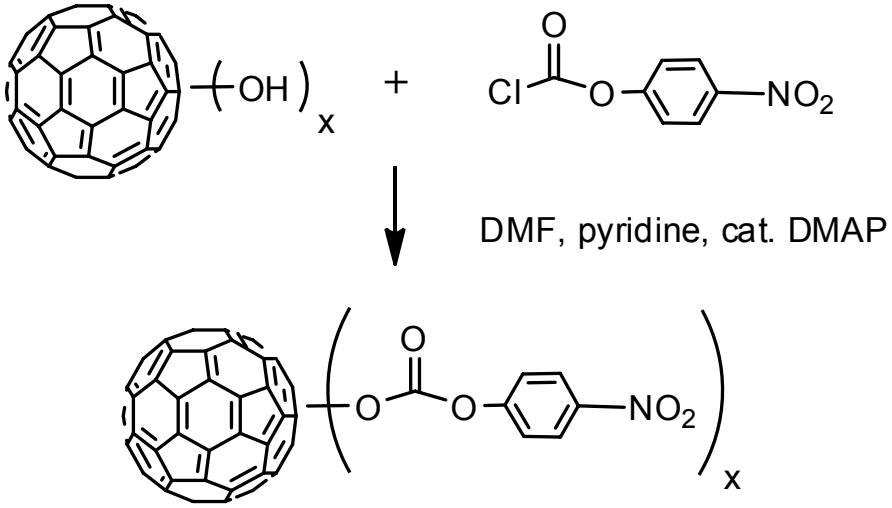


fullerene = C_{60}

TDA Research

Derivatizing Fullerols for Linkage of Paclitaxel and Water Solubility

- Step 1: Hydroxyl group activation with *p*-nitrophenyl chloroformate



fullerene = C_{60}, $Gd@C_{60}$

Chaudhuri et al. *ACS Nano* (2009), **3**, 2505

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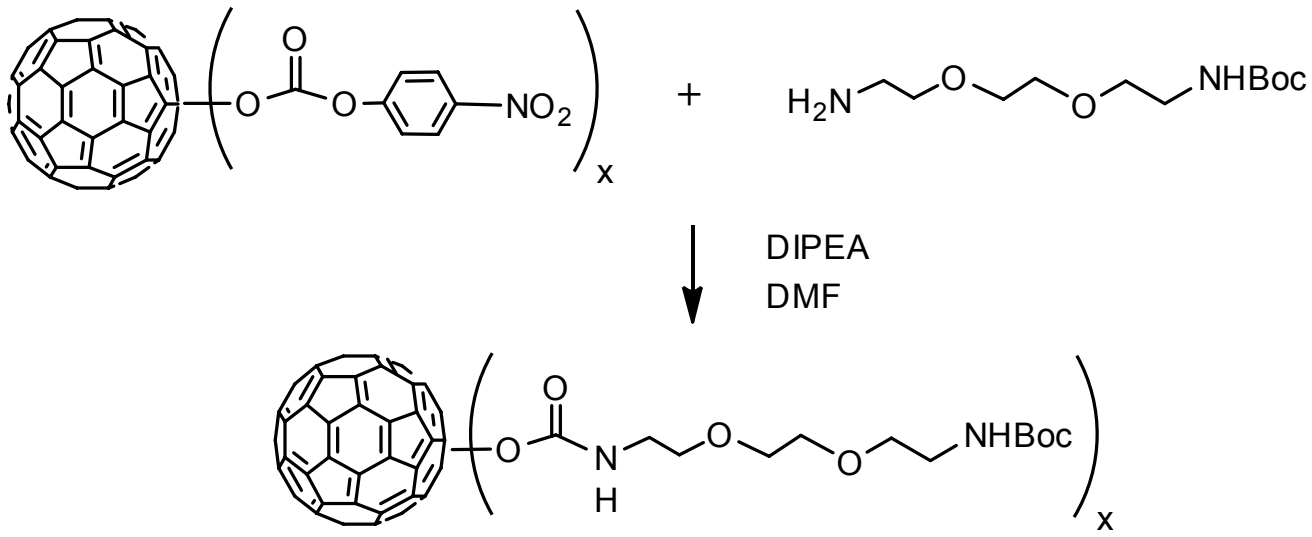
Derivatizing Fullerols for Linkage of Paclitaxel and Water Solubility

- Step 2: Derivatization with the Boc-protected “linker” unit



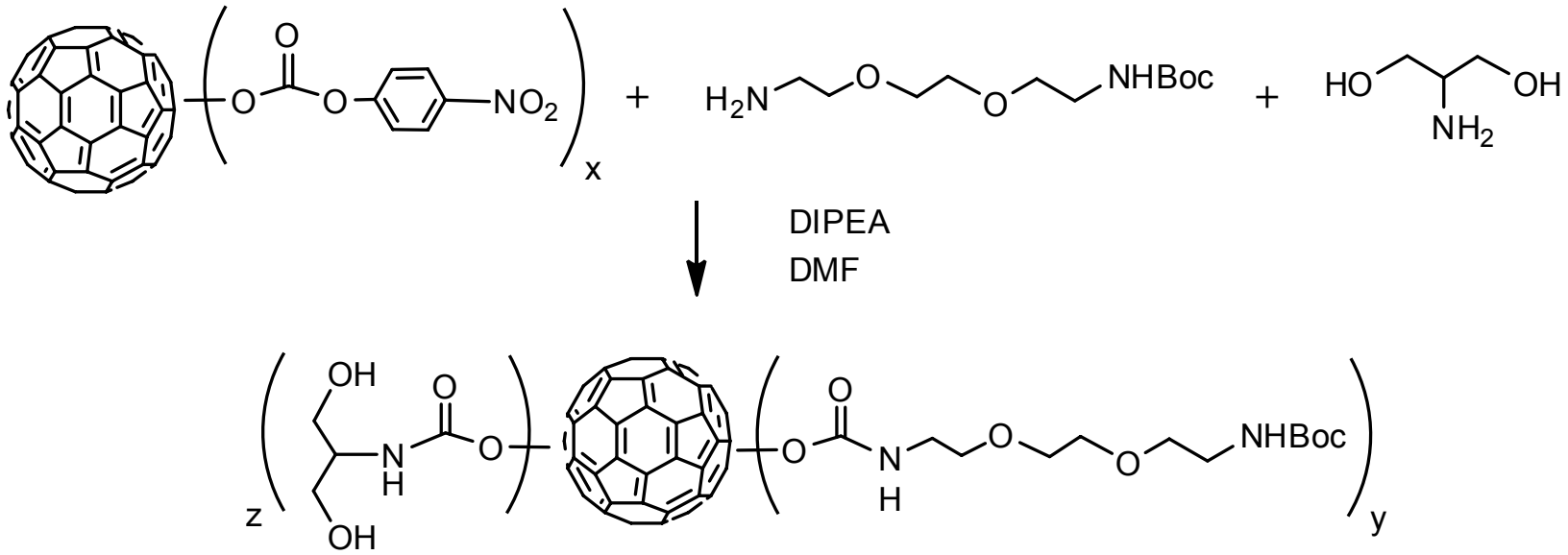
fullerene = C_{60}, $Gd@C_{60}$

Chaudhuri et al. *ACS Nano* (2009), **3**, 2505

TDA Research

Derivatizing Fullerols for Linkage of Paclitaxel and Water Solubility

- Step 2 option: Co-derivatization with the linker unit plus serinol

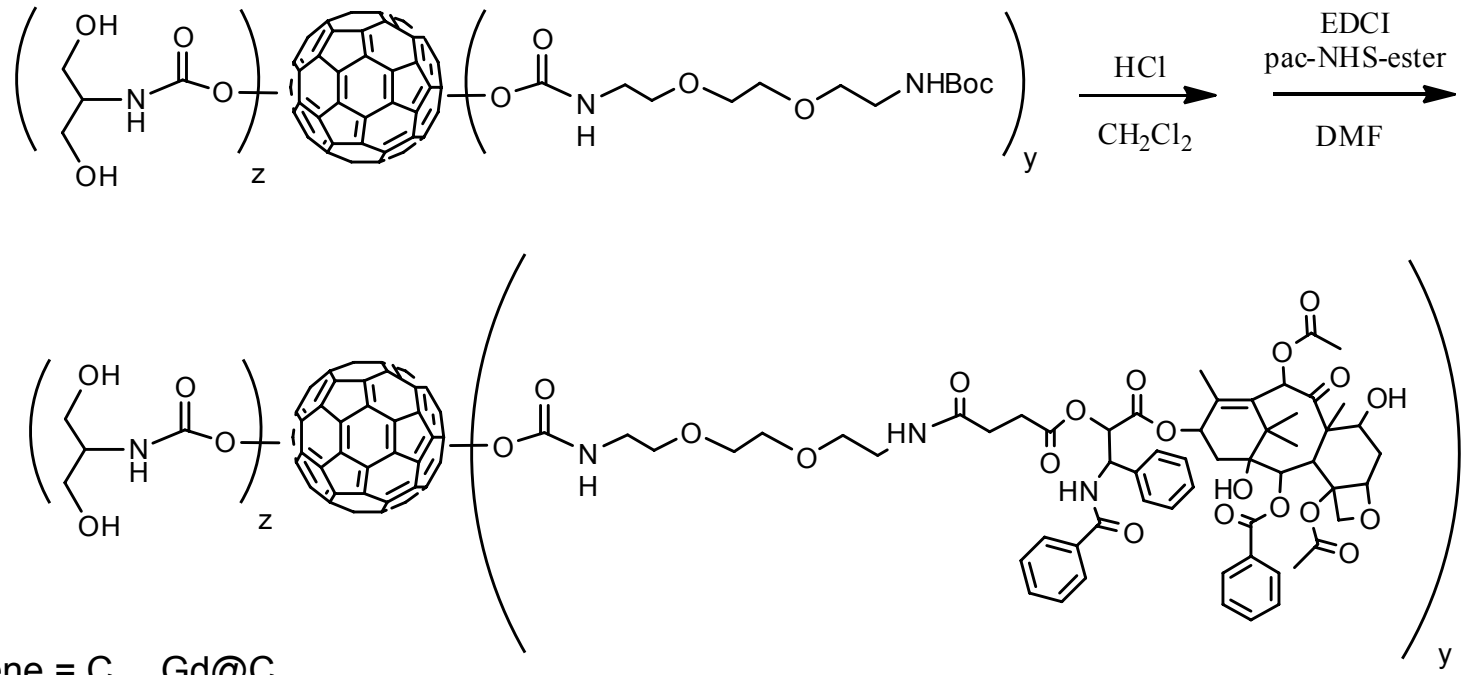


fullerene = C_{60}, $Gd@C_{60}$

TDA Research

Derivatizing Fullerols for Linkage of Paclitaxel and Water Solubility

- Step 3: Covalent linkage of Paclitaxel to the linker-amine terminus



fullerene = C_{60}, $Gd@C_{60}$

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Ongoing and Future Studies

- Paclitaxel covalently linked to C_{60} and gadofullerenes
- Co-linkage of serinol co-groups to benefit water solubility (Paclitaxel is hydrophobic – avoid overloading)
- *In vitro* testing of immunoconjugates with melanoma cells
- Drug release kinetics (hydrolysis)
- Derivatization strategy has broad utility - modifiable for linking different amines, functional groups, and drug payloads

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Summary

Fullerene
ZME-018
immunoconjugates

+

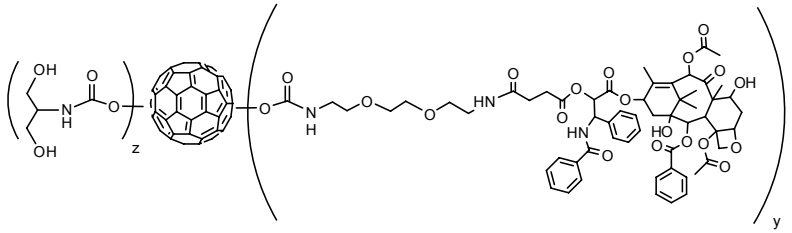
Fullerene
paclitaxel
covalent
conjugates

+

Gadofullerene
MRI
contrast agents

→

Gadofullerene-drug immunoconjugates for combined diagnosis & therapy of melanoma



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Research groups of Prof. Michael Rosenblum of the M. D. Anderson Cancer Center and Prof. Lon Wilson of Rice University *(both in Houston, TX)*

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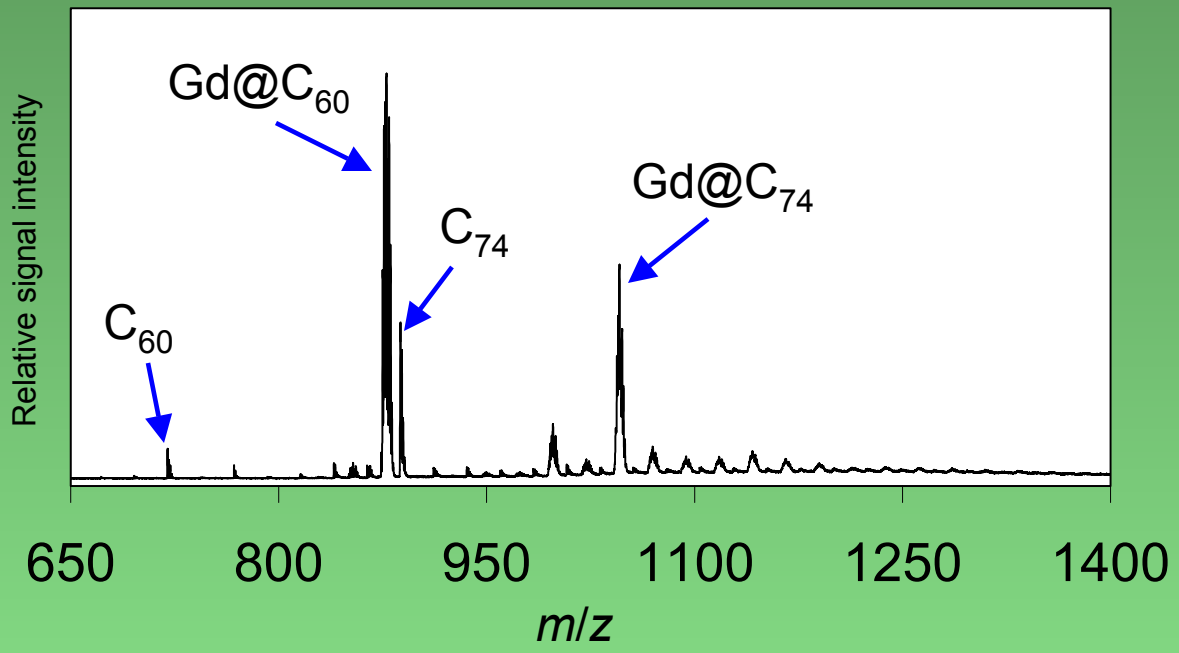
The Electrochemical Society

$Gd@C_{2n}$ source material used in gadofullerol syntheses



- LD-TOF mass spectrum (TCNQ matrix) of typical material that is polyhydroxylated
- Enriched $Gd@C_{60}$ class of fullerenes, ($Gd@C_{60}$, $Gd@C_{70}$, $Gd@C_{74}$, etc.) with decreased empty C_{2n} content

Raebiger et al. *J. Phys. Chem. C* (2008), **112**, 6605-6612

TDA Research