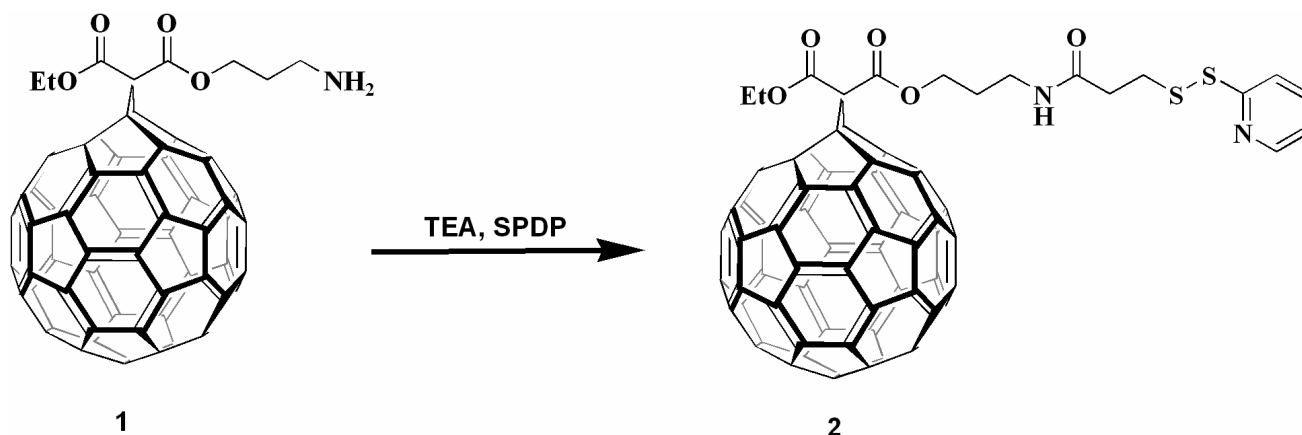


Supporting Information

General Synthetic Methods

The following reagents were used as received: C₆₀ (MER Corporation), N-succinimidyl-3-(2-pyridyldithio)propionate (PIERCE), *tert*-butyl-N-(3-hydroxypropyl)carbamate (Aldrich), ethylmalonyl chloride (Aldrich), DBU (Aldrich), CBr₄ (Aldrich), Serinol (Aldrich), Diethyl Malonate (Aldrich). All solvents were HPLC grade and purchased from Fisher. Flash chromatography was carried out using silica gel (70-230 mesh from EM Science). HPLC was performed on a Hitachi L-6200A Intelligent Pump HPLC system with a Hitachi Model L-3000 UV-VIS photodiode array detector. All ¹H and ¹³C NMR Spectra were recorded on a Bruker Avance 400 MHz spectrometer. Mass spectra were obtained on either a Finnigan Mat 95 mass spectrometer or Bruker Biflex III MALDI-TOF. For the MALDI-TOF spectra, unless otherwise specified, an elemental sulfur matrix deposited from a slurry of CS₂ was used.

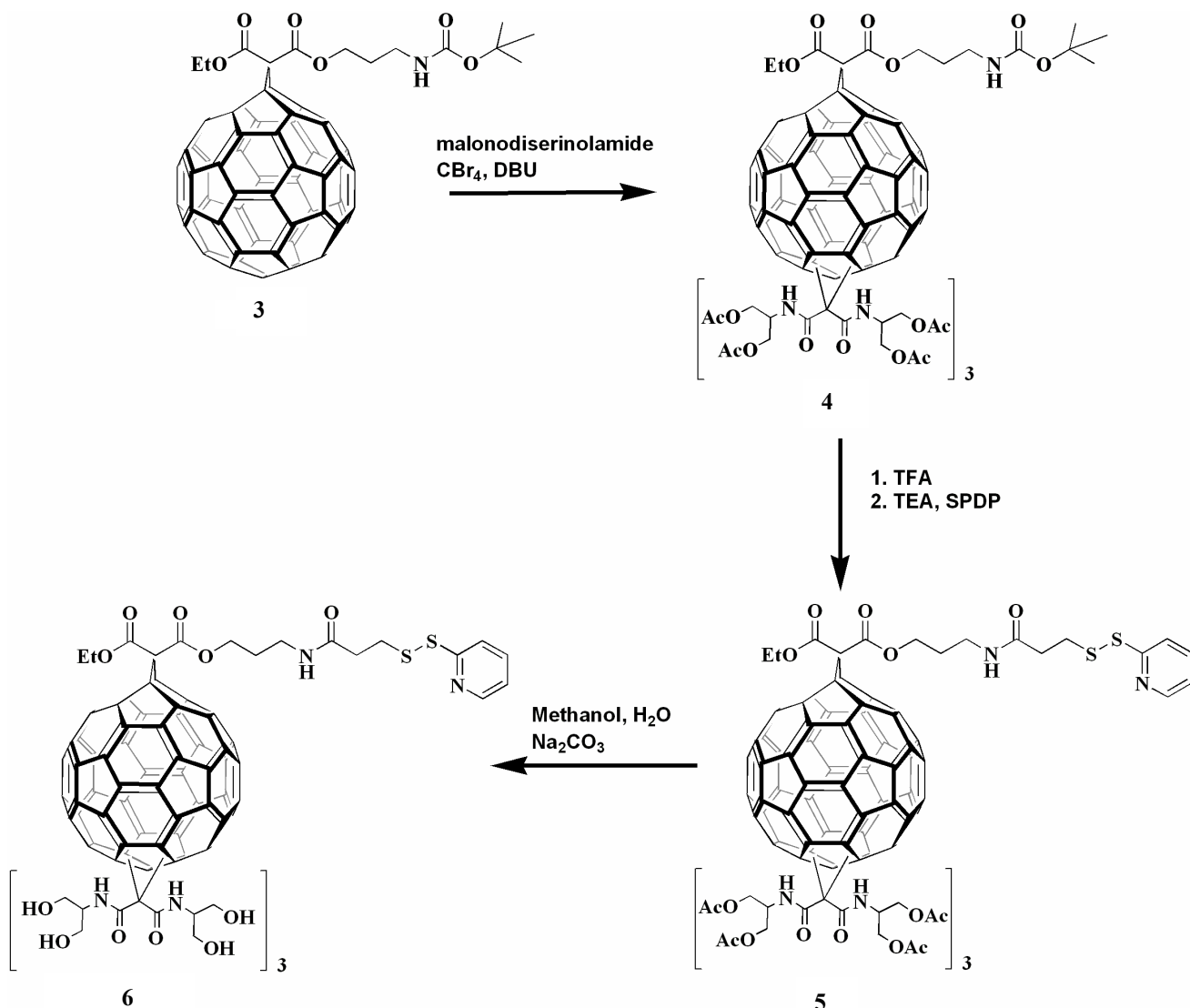
Reaction Schemes/Synthesis



Scheme 1: C₆₀-SPDP Monoadduct

1. Prepared by a published procedure.¹¹

2. TEA was added drop-wise to a solution of **1** (150 mg, 0.165 mmol) in 20 mL of CH₃Cl until the solid was dissolved. SPDP (50 mg, 0.160 mmol) was added and stirred at room temperature overnight. The resulting clear brown-red solution was purified via column chromatography on silica gel (toluene/EtOAc 1:1) to give **2** (40 mg, 23% yield) as a brown-red solid. It was further purified by HPLC on a silica column using toluene/methanol (15:1) as eluent. ¹H NMR (CDCl₃) δ 1.48 (s, 3H), 2.10 (p, 2H), 2.66 (t, 2H), 3.10 (t, 2H), 3.50 (q, 2H), 4.54-4.61 (m, 4H), 6.84 (bt, 1H), 7.16 (m, 1H), 7.62 (m, 2H), 8.48 (d, 1H). ¹³C NMR δ 14.52, 29.01, 35.33, 36.08, 36.68, 52.30, 63.87, 65.23, 120.69, 121.37, 125.51, 137.31, 139.08, 139.33, 142.08, 142.10, 142.42, 143.23, 143.26, 144.10, 144.86, 144.91, 145.12, 145.33, 145.40, 145.41, 145.50, 149.81, 163.88, 163.89, 171.35. MALDI-TOF MS calcd. for **2**: 1104; Found: 1105.



Scheme 2: Water-soluble C₆₀-SPDP

3. Prepared by a published procedure.¹¹

4. To a solution of **3** (100 mg, 0.10 mmol) in 100 mL of toluene, malonodiserinolamide (210 mg, 0.50 mmol) was added, followed by CBr₄ (170 mg, 0.51 mmol) and DBU (120 mg, 0.78 mmol). After stirring overnight, the solution was reduced to dryness under reduced pressure. Flash chromatography was then performed using silica gel (2:3 acetone:toluene) to give 70 mg of **4** (38% yield based on the trisadduct of **4** as a red-orange solid. Further purification using HPLC and a silica column was performed (20:1 toluene:methanol). MALDI-TOF MS calcd for **4**: 1424 for one adduct of malonodiserinolamide, 1840 for two adducts, and 2256 for three adducts, found: 1423, 1840, and 2257. For purposes of antibody conjugation, the various isomers of the derivatives were not separated. Deprotection of **4** gave the primary amine compound (not shown) in quantitative yield. MALDI-TOF MS calcd.: 1324, 1740, and 2156; found: 1325, 1741, and 2158.

5. After deprotection of **4** to give the primary amine compound (not shown) (100 mg, 0.057 mmol), the same method for preparing **2** with TEA and SPDP was used to give 45 mg of **5** (41% yield). Flash

chromatography on silica gel and HPLC on silica using methanol was then performed. MALDI-TOF MS calcd.: 1522, 1939, 2355; found: 1523, 1940, 2357.

6. 5 (25 mg, 0.013 mmol) was deprotected by first dissolving it in 5 mL degassed methanol under Ar. Na_2CO_3 (15 mg, 0.137 mmol) was then added, followed by 1.0 mL of degassed, DI H_2O . The reddish-orange solution was stirred under Ar for 1.5 h. Cation exchange resin (H^+ form) was added until pH = 7, then a slight excess. The solution was stirred an additional 1.0 h under Ar, then filtered and reduced to dryness under reduced pressure. An orange-red solid formed (12 mg, 58% yield). Flash chromatography on silica gel and HPLC on silica was performed. MALDI-TOF MS calcd.: 1600 for two dimalonoserinolamide and 1849 for three dimalonoserinolamide; found: 1637, 1651 ($\text{M}^+ + 2$ or 3 epoxide O's) and 1898 ($\text{M}^+ + 3$ epoxide O's). ^1H NMR (D_2O) was performed to verify that the pyridine moiety was still present on **6** after the deprotection reaction: δ 7.26 (m, 1H), 7.79-7.85 (m 2H), 8.42 (m 1H).

C₆₀-Ser. Synthesized according to a literature procedure.²

Spectra

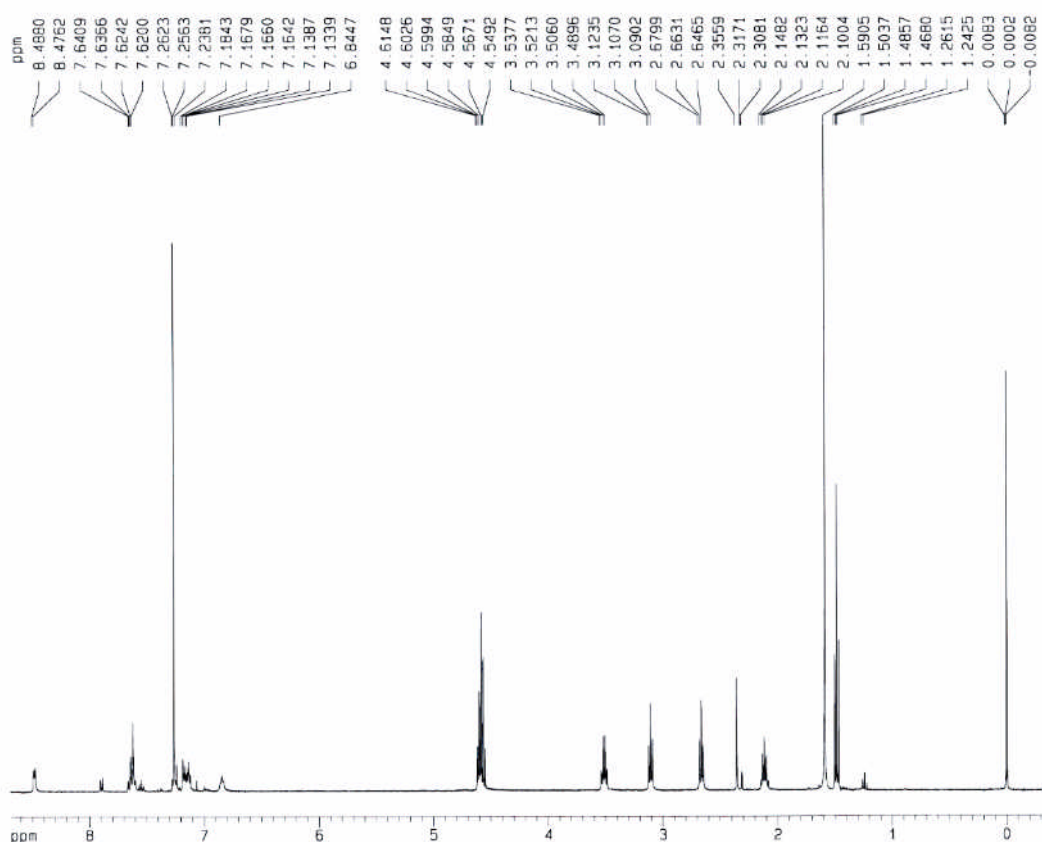


Figure 1. ^1H NMR of **2**.

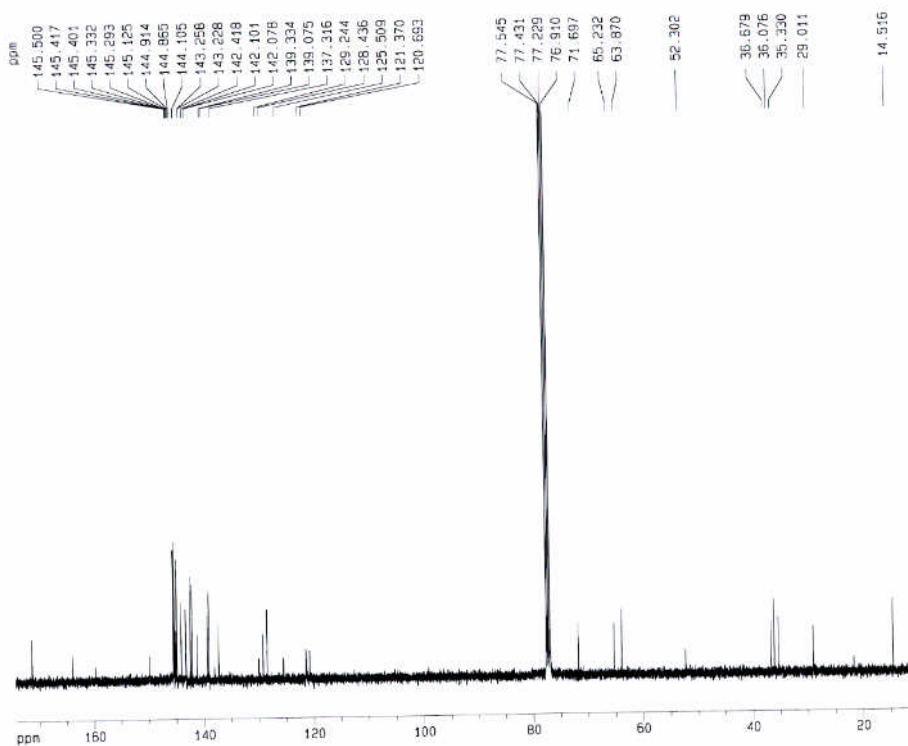


Figure 2. ^{13}C NMR of 2.

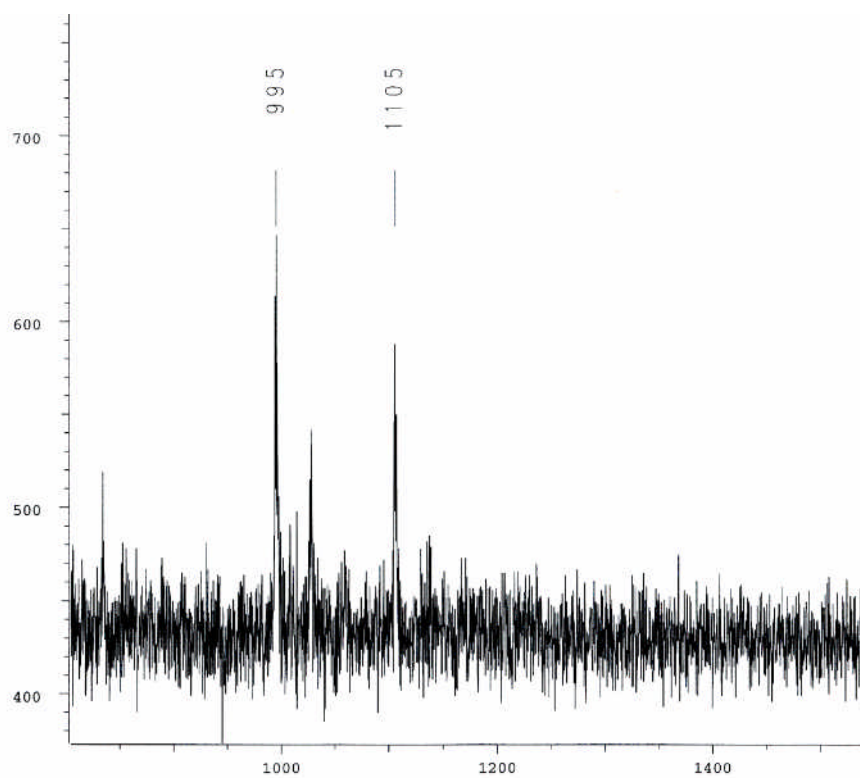


Figure 3. MALDI-TOF MS of 2.

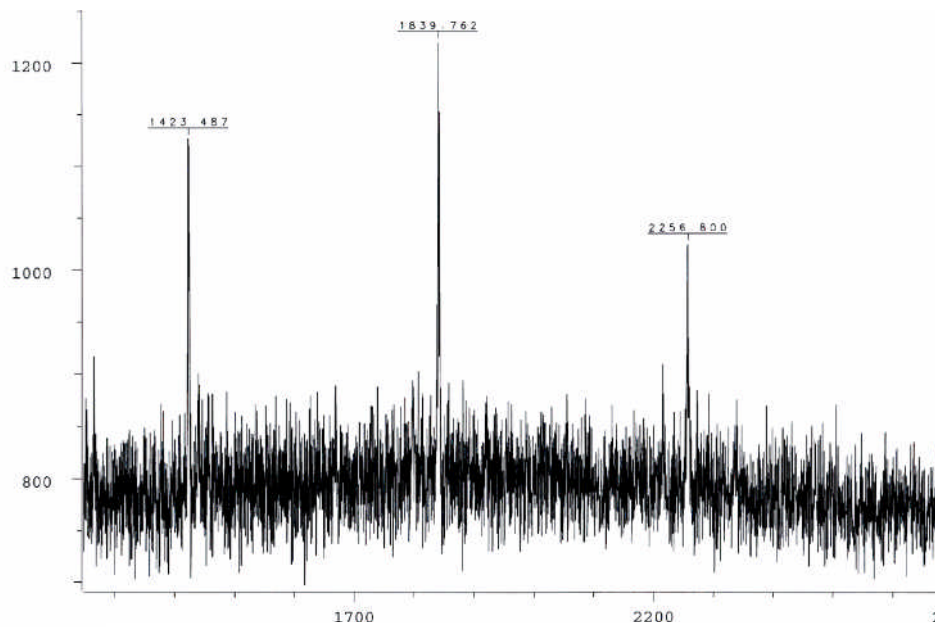


Figure 4. MALDI-TOF MS of **4**.

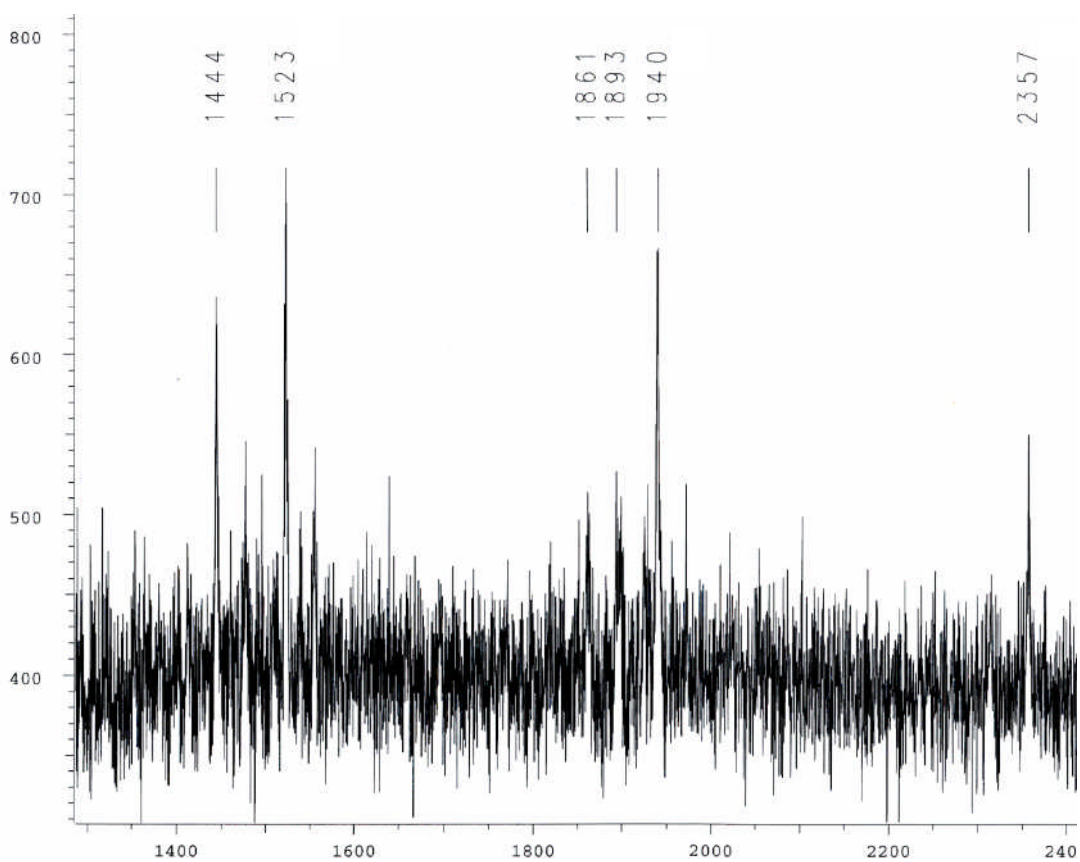
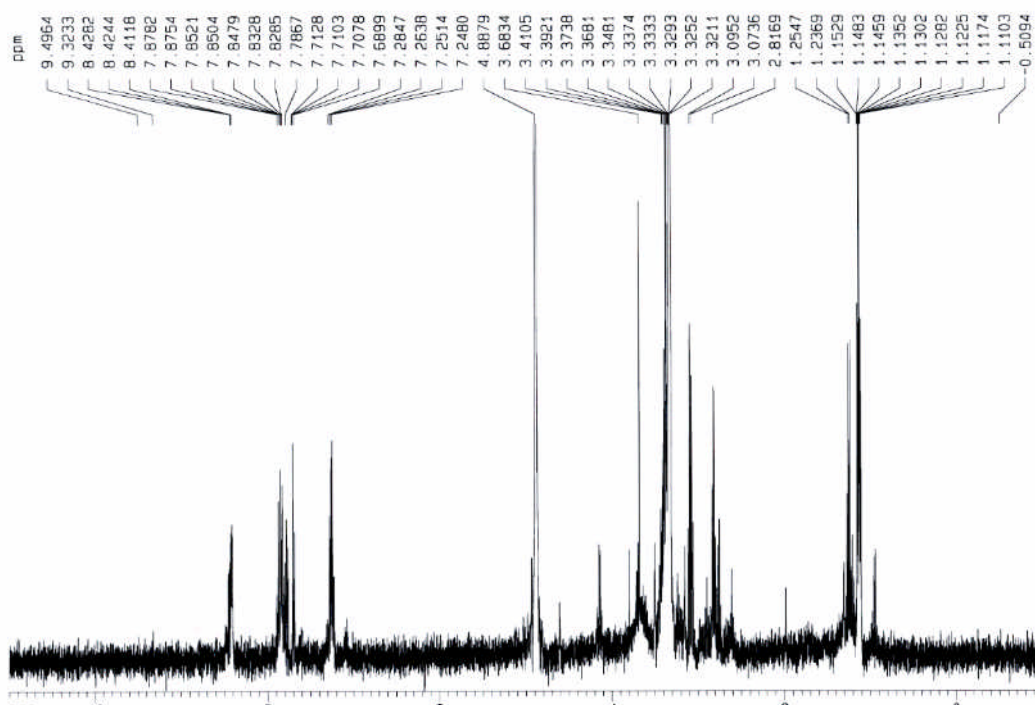
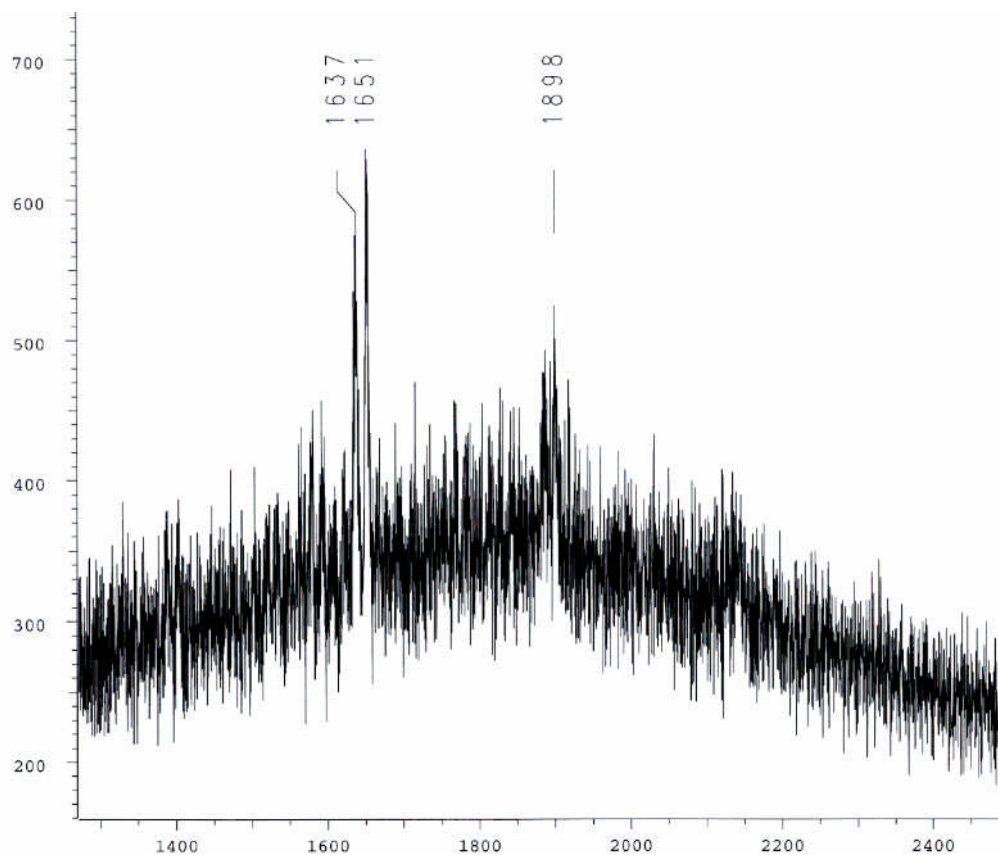


Figure 5. MALDI-TOF MS of **5**.



TEM Imaging

TEM—A single drop of the water-soluble fullerene-antibody conjugate was deposited on a 300 mesh copper grid, Lacey Carbon Type-A support film, manufactured by Ted Pella, Inc. The sample was allowed to air-dry for about 5 minutes under ambient conditions before imaging. A JEOL 2010 model TEM operating at 100 keV imaged samples at 30,000X and 80,000X magnification.

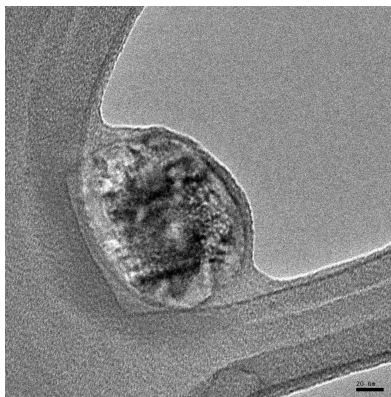


Figure 8. TEM of the C₆₀-SPDP-(ZME-018) immunoconjugate (Scale bar 20 nm)

C₆₀-ZME-018 Conjugation

ZME-018 (2 mg) was added to 3.42 mL of a phosphate/saline buffer. TEA was added until pH = 8.0, followed by addition of 1 mM EDTA. 2-Iminothiolane (7.8 μ L) was then added and stirred under nitrogen at 4°C for 90 minutes. The resulting solution was purified using a G-25 sephadex size-exclusion column and a solution consisting of 5 mM bis/tris, 50 mM NaCl and 1 mM EDTA at pH = 5.8. A biorad protein assay was performed to determine which fraction contained the derivatized antibody.

The antibody solution was then halved and the pH of each half brought to 7.0 with TEA. C₆₀-SPDP (123.7 μ L) and C₆₀-Ser (130.8 μ L) were then added to the antibody solution (10:1 C₆₀:antibody) and stirred overnight at 4°C. A white precipitate of unreacted antibody formed overnight, which was centrifuged and removed from the reaction vial. Iodoacetaamide (80 μ L) was added to the reaction and stirred for 1 hour. The precipitate was centrifuged for 10 minutes at 500 rpm. The supernatant was then removed and purified with the G-25 sephadex size-exclusion column using a buffer of 10 mM Na₃PO₄, 140 mM NaCl at pH = 7.2. This purification removed any free C₆₀ from the sample. A Biorad protein assay was used to determine which fraction contained the immunoconjugate. The sample was then halved. One half was dialyzed overnight with 6 M Urea, and the other half was set aside for analysis.

Triplet-Triplet Absorption Measurements

Triplet-triplet absorption measurements were performed with a home-built apparatus that used 532 nm pulses from a small Q-switched Nd:YAG laser for sample excitation and a monochromated tungsten lamp for probing. Three samples of C₆₀-SPDP-(ZME-018) conjugate, synthesized using molar ratios of C₆₀-SPDP:(ZME-018) equal to 1:1, 5:1, and 10:1, were carefully degassed by repeated freeze-pump-thaw cycles prior to measurement. The transient spectra shown in Fig. 2a of the paper were captured at a delay of 3 μs after excitation. Triplet decay kinetics measured at 690 nm are plotted in Figure 9 for C₆₀-SPDP and the C₆₀-SPDP-(ZME-018) immunoconjugate. The two species show identical triplet decays. The kinetics can be well-fit to a biexponential decay model with components having lifetimes of 14 and 55 μs and similar amplitudes.

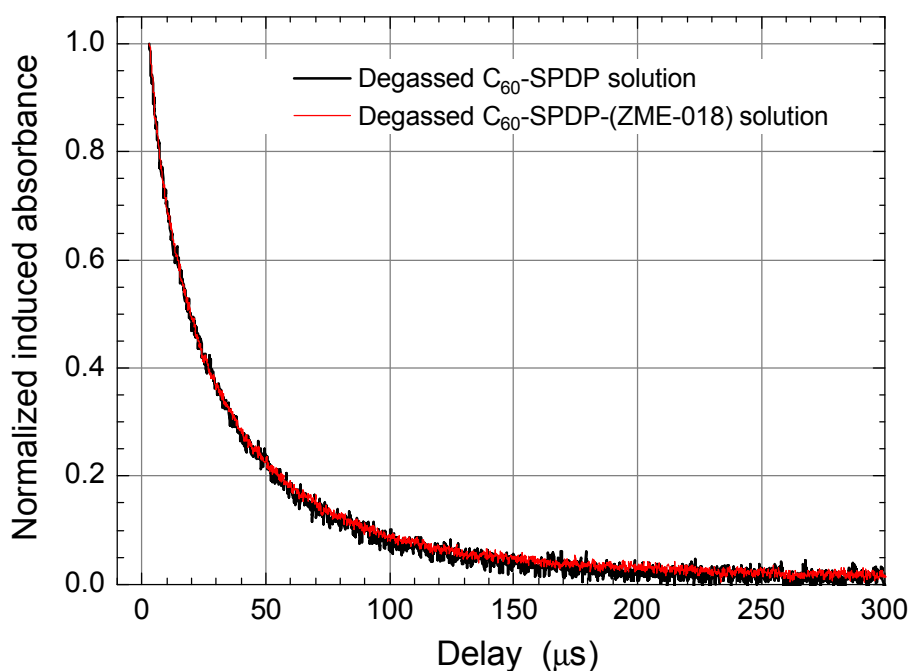


Figure 9. Triplet state decay kinetics of C₆₀-SPDP and C₆₀-SPDP-(ZME-018), as measured at 690 nm following 532 nm excitation.

UV-vis Absorption Measurements

UV-vis absorption spectra used to deduce the composition of the C_{60} -Ser-(ZME-018) conjugate are shown in Fig. 10.

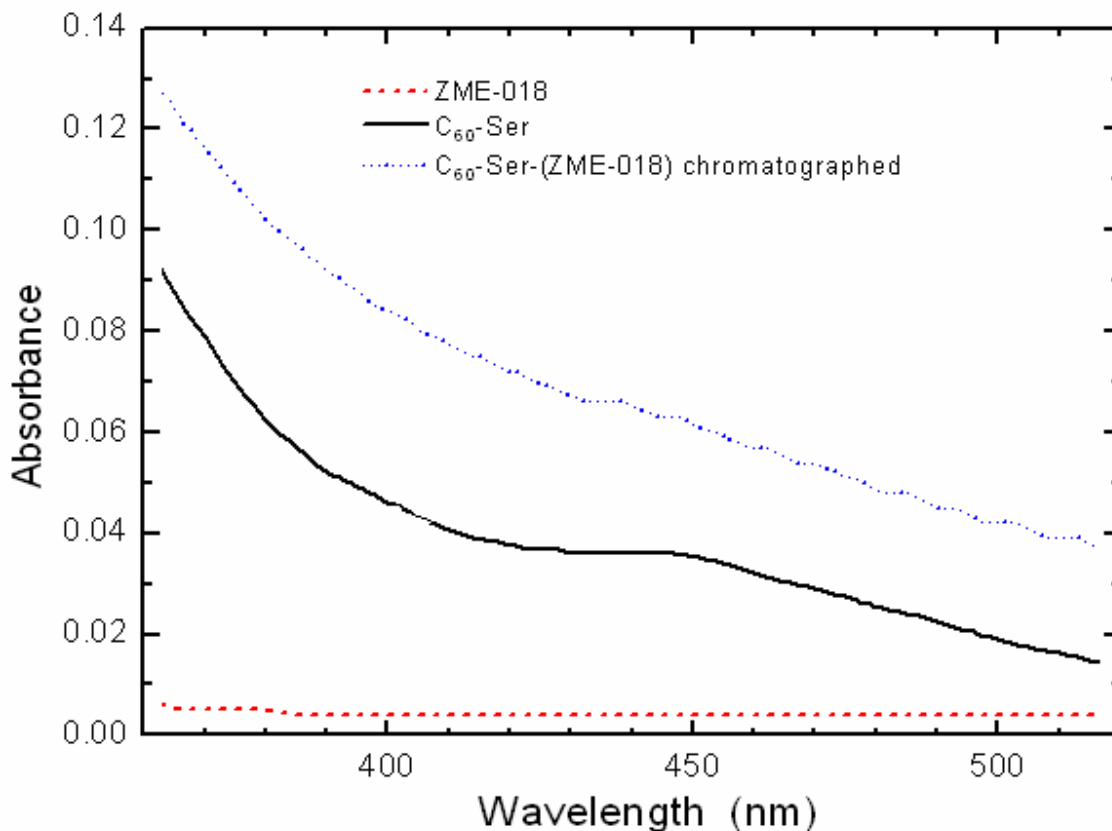


Figure 10. UV-vis absorption spectra of ZME-018, C_{60} -Ser, and their conjugate after two stages of purification ($[C_{60}\text{-Ser}] = 10 \mu\text{M}$).

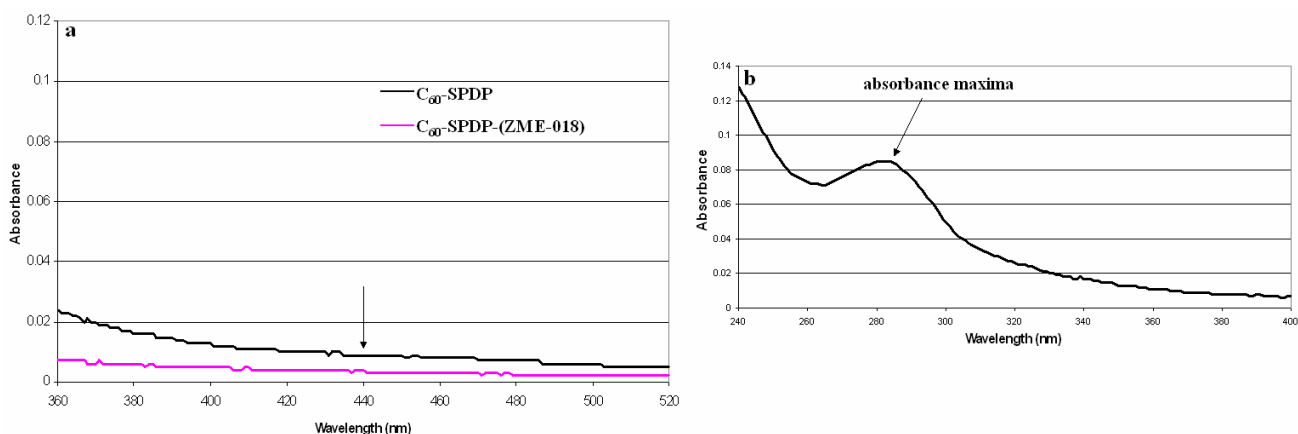


Figure 11: UV-vis absorption spectra of a) C_{60} -SPDP-(ZME-018) at 6 μM and C_{60} -SPDP at 30 μM showing that the intensity at 440 nm is not sufficient for concentration determination in the μM range. b) C_{60} -SPDP absorption maxima at 282 nm at 10 μM .

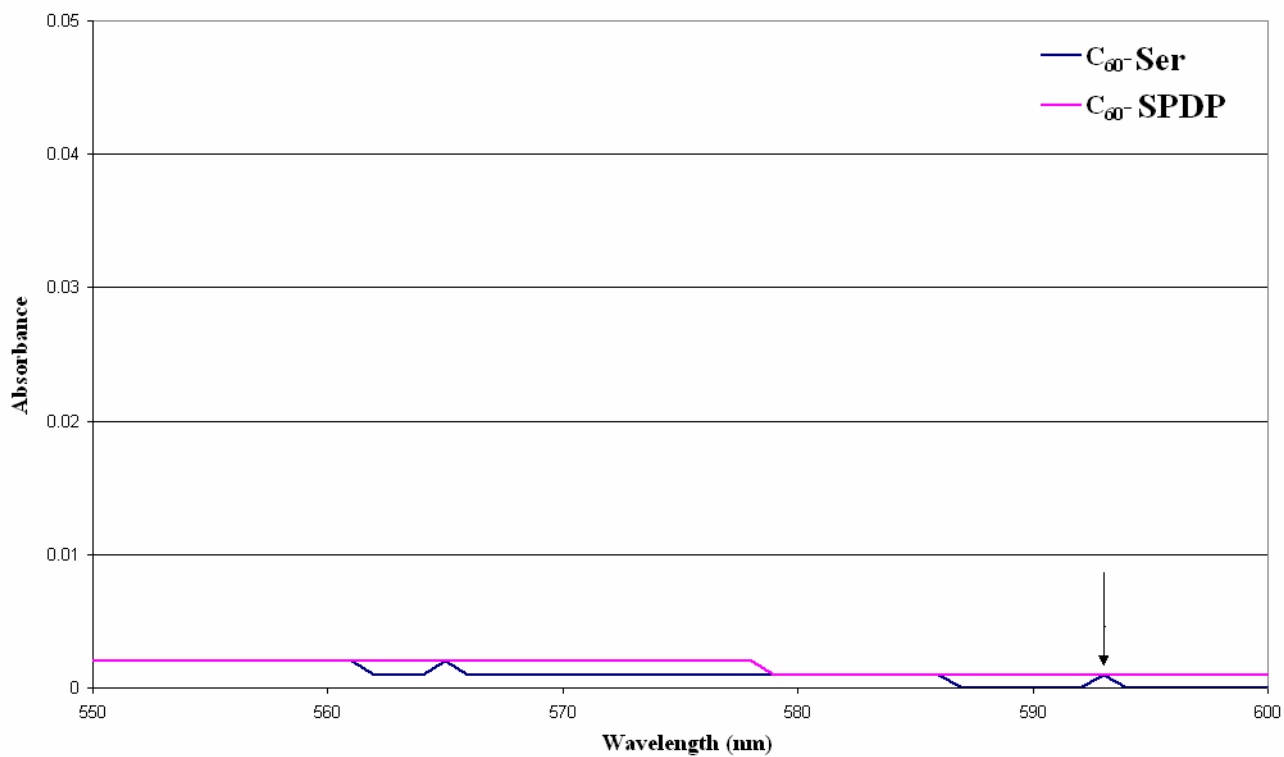


Figure 12. UV-vis absorption spectra of the C₆₀-derivatives showing negligible intensity at 595 nm (the Biorad wavelength) for [C₆₀-Ser] = 15 μM and [C₆₀-SPDP] = 6 μM.

ELISA

ELISA plates were prepared by versene-stripping desired A375m cells from tissue culture flasks with washing twice using DPBS, followed by re-suspension in DPBS to a cell concentration of 1 M/ml. 50 μ of the cell suspension were placed into Falcon 3912 96-well μ l plates, leaving 2 wells without cells for blanks. The plates were dried overnight at 37 °C and stored at 4 °C. 200 μ l of blocking buffer was added to wells for 1 hour at room temperature. The blocking buffer was removed via decanting, with immediate addition of 100 μ l/well standards and unknowns. The plate was incubated at room temperature for 3 hours and solution removed. Each well was washed 3x with a washing buffer for preparation of IgG component detection. Anti-mouse IgG-HRP was diluted 1:1000 in diluting buffer, making 11 ml/plate, which was aliquoted 100 μ l/well and incubated 15 minutes at room temperature. The wells were then washed 3x with washing buffer. 11 μ l of H₂O₂ was added to 11 ml of ABTS and aliquoted 100 μ l/well and incubated for 10 minutes at room temperature. The plate was then read at 405 nm to calculate the 50% inflection point of the concentration curve IC(50) values.

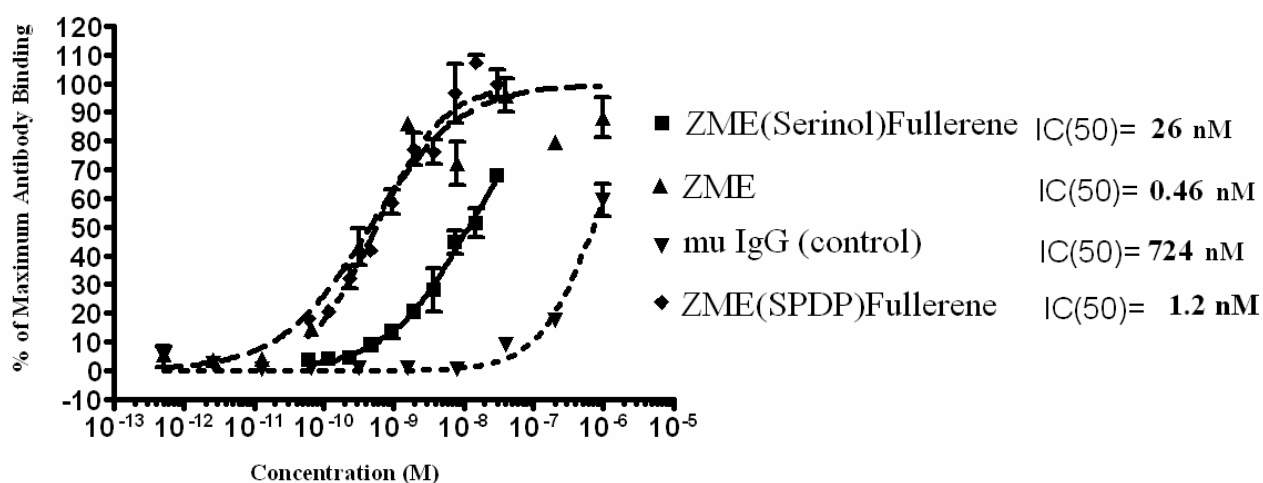


Figure 13. ELISA: A375 Dead Cell: Testing of C₆₀-ZME-018 immunoconjugates.

Biorad Protein Assay

Biorad protein assays were used to determine the concentration of ZME-018 in the immunoconjugates. A standard curve (Figure 14) was made from absorbance values determined at specific concentrations of ZME-018 and then absorbance values of the immunoconjugate samples were determined using the standard curve. The absorbance values were 0.056 for ZME-018 in C₆₀-SPDP-(ZME-108) and 0.049 for ZME-018 in C₆₀-Ser-(ZME-018). The concentration was calculated using a molecular weight of ZME-018 to be 150,000 g/mol.

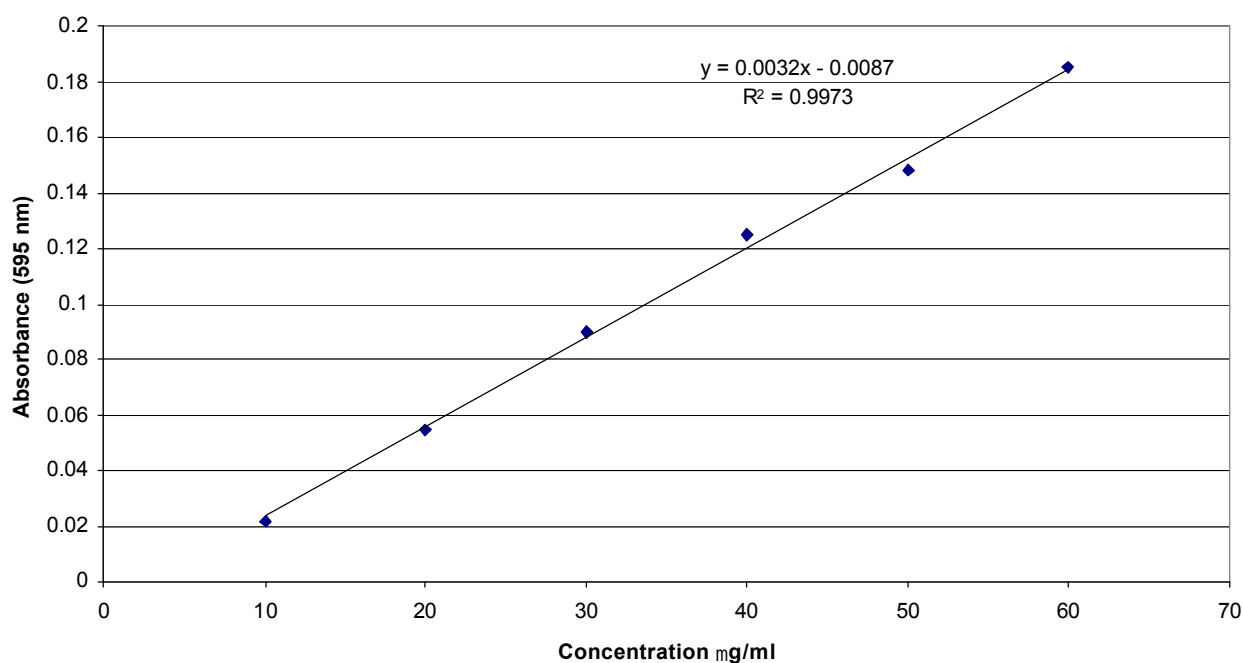


Figure 14. Biorad protein assay standard curve used to determine the ZME-018 concentration in immunoconjugates.