Bioorganic & Medicinal Chemistry 17 (2009) 6292–6300

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis, functionalization and photo-Bergman chemistry of enediyne bioconjugates

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ARTICLE INFO

Article history:
Received 2 June 2009
Revised 17 July 2009
Accepted 21 July 2009
Available online 26 July 2009

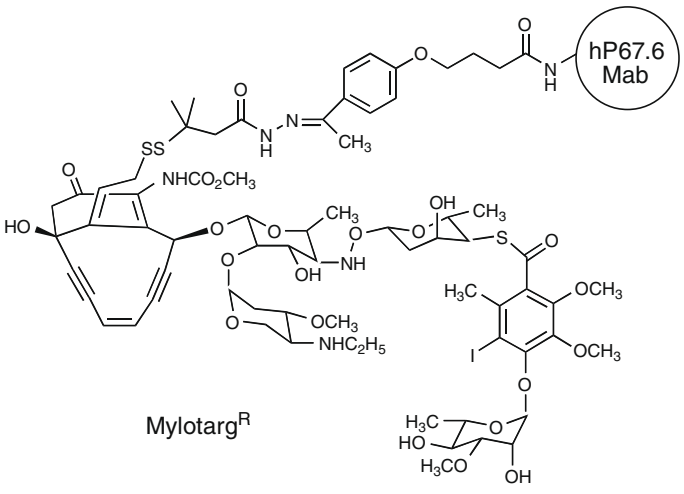
Keywords:
Enediyne
Photo-Bergman
Bioconjugate
Antibody conjugate
Nanoparticles
PDT

ABSTRACT

Methodology is outlined for the chemical synthesis of versatile photo-Bergman enediyne building blocks and their conjugates. Routes to both mono and bis conjugated enediyne templates are detailed together with representative examples of their bioconjugates, nanoconjugates, PEG derivatives and water soluble salts. The immunocompetence of antibody conjugates is retained, and application in the form of reagents for photodynamic therapy (PDT) advanced.

1. Introduction

Isolated from soil bacteria, enediynes are among the most potent groups of antineoplastic agents ever discovered.[1] Introduced in 2000, Mylotarg® an anti-CD33 antibody conjugate of the enediyne calicheamicin, was the first ever Mab-cytotoxin conjugate to be approved by the FDA, and is clinically effective against acute myeloid leukemia (AML).[2] Through complex triggering mechanisms the natural enediyne pharmacophores are converted to cytotoxic cyclic diradical species via a process commonly referred to as the Bergman cycloaromatization, ultimately abstracting hydrogen atoms from surrounding macromolecules leading to DNA damage and cell death.[3] A number of designed enediyne prodrugs have been developed, with a variety of inbuilt triggering mechanisms. Perhaps the most simplistic of all relies on the photo-Bergman reaction, wherein otherwise shelf-stable enediyne substrates are transformed to cytotoxic diradicals on demand through irradiation. The first and perhaps best studied version of this transformation is exemplified by bis-phenyl enediyne **1** which transforms to diyl **2** under UV irradiation, en route to aryl **3** (Scheme 1).[3] The basic photo-Bergman pharmacophore however has limited application in drug design due to the lipophilic nature of the substrates. Accordingly, we and others have become interested in developing efficient routes to substituted photoactivated enediyne templates, and investigating their bioconjugation chemistries. In pursuit of these goals, as documented herein, we have concentrated on (i) preparation of versatile enediyne building blocks (ii) derivatization to form water soluble analogues (iii) coupling to form PEG-ylated derivatives (iv) formation of antibody conjugated chimeras and (v) investigation of the potential to form nanoparticle coupled derivatives.



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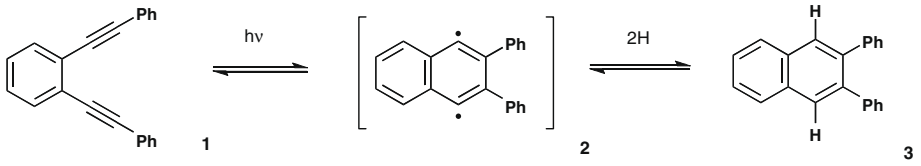
doi:10.1016/j.bmc.2009.07.059



Scheme 1. The basic photo-Bergman cycloaromatization.

1.1. Preparation of building blocks

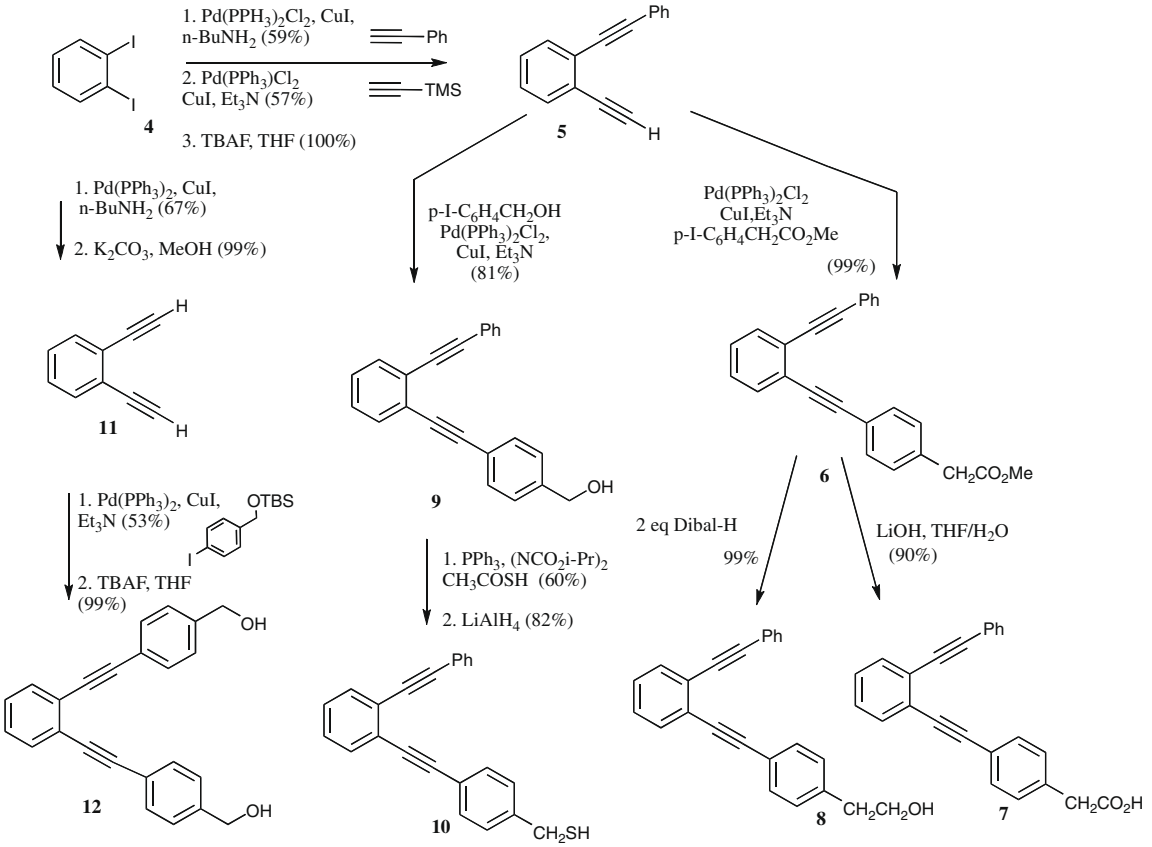
A variety of efficient Pd-mediated routes to differentially substituted aryl enediynes have been developed, and we elected to commence from di-iodobenzene **4**, which can be transformed easily into enediyne template **5** (Scheme 2). Conversion to phenacetic ester **6** allowed formation of either acid **7** or ethanol **8** as needed in excellent yield. Conditions were also developed for direct coupling to form enediyne alcohol **9**, which in turn allowed us to access enediyne thiol **10**. In order to expand the repertoire of the photo-Bergman reaction and fully explore bioconjugation strategies we also executed a route to bis-benzyl alcohol **12**. This merely necessitated formation of parent unsubstituted enediyne **11** followed by double coupling–deprotection to give the desired product in good yield.

1.2. Water soluble analogues

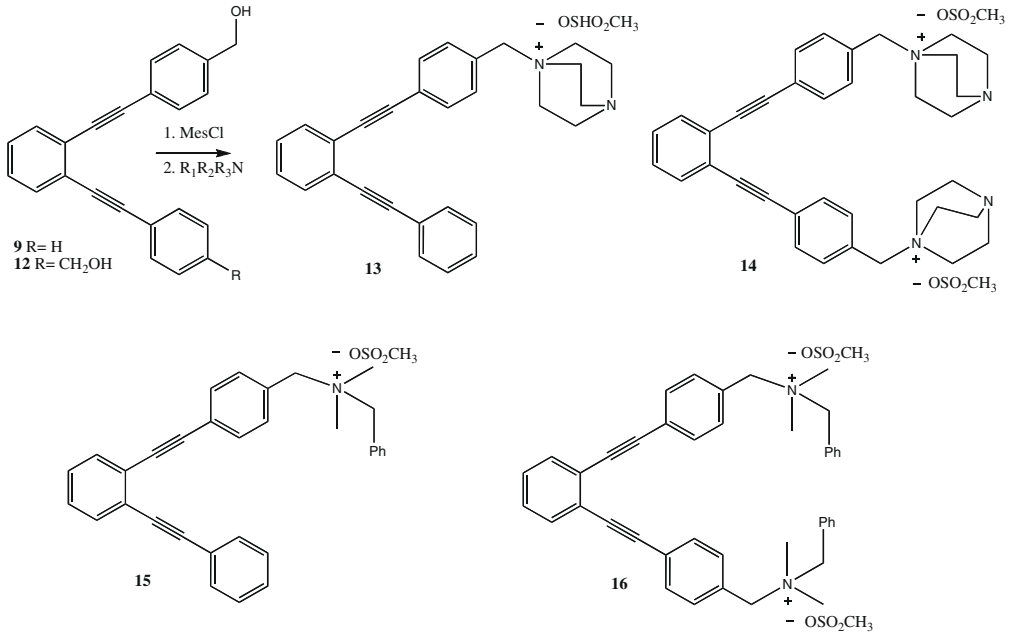
Though carboxylate **7** and diol **12** have some solubility in polar solvents, we wished to also advance formation of more versatile ammonium salts, and investigated transformation of both **9** and **12** via their corresponding mesylate derivatives. Though a variety of amines were screened, the corresponding ammonium salt(s) from DABCO proved the most effective with products **13** and **14** isolated in good yield and essentially stable as crystalline salts (Scheme 3). The salts proved to be freely soluble in polar organic solvents and buffering media, and more importantly also in water.

1.3. Formation of PEG-ylated derivatives

With a variety of mono and bis-functionalized enediyne templates in hand we began to investigate formation of PEG-ylated derivatives. PEG-ylation is now a mainstream strategy in drug development, enhancing the properties of lipophilic molecules and in the case of targeting of solid tumours, providing enhanced circulatory and localizing benefit via the RES mechanism.[4] Conversion of **9** into the PEG-ylated ester **17** was effective using a PEG diacid under EDCI coupling (Scheme 4). Attempts to effect the same coupling on diol **12** met with difficulty, invariably leading to inseparable mixtures of mono and bis esters **18** and **19**. Remedy was found by conversion to the bis succinate **20**, allowing efficient production of the bis-PEG-ylated ester **21** through carbodiimide coupling.



Scheme 2. Synthesis of photoenediyne building blocks.



Scheme 3. Improved water solubility through benzylammonium enediyne salts.

1.4. Antibody conjugates

With PEG ester chemistry established, we turned our attention to preparation of monoclonal antibody conjugates. Direct coupling of PEG carboxylate **17** with the S-NHS ester of an available cardiac specific antibody (2G4) gave derivative **22** (Scheme 5).[5] Alternatively, coupling of template **7** via linker **23** followed by reductive amination, allowed preparation of congener **24**.[6] In both cases immunochemical analysis confirmed activity of the derivatized products by ELISA.[7]

1.5. Photo-Bergman chemistry

The enediynes, whether in parent, ammonium salt, PEG-ylated, or bioconjugated form underwent photo-Bergman cyclization to give the corresponding product arenes **26** via presumed diyl intermediates **25** (Scheme 6). Optimal conditions involve irradiation through a low pressure Hg lamp (450 W) using isopropanol as hydrogen atom donor. Conversion to the cycloaromatized species is conveniently monitored utilizing UV/vis spectroscopy based on the characteristic increase in wavelength associated with aromatization. NMR and MS confirmed the identity of products, in the case of PEG-ylated derivatives and bioconjugates using MALDI-MS.

To assess DNA cleaving ability of the photo-Bergman substrates, irradiation of **17** in the presence of bacteriophage (ΦX174 RfI) DNA was conducted. Correcting for control photodegradation, **17** largely induced concentration dependent conversion to type II DNA (24%), with the onset of minimal conversion to type III based on random single-stranded cutting events. This moderate level of degradation presumably reflects affinity of the enediyne warhead itself for DNA as has been observed in other cases,[8] however targeting entities are known to improve affinity.[9] The study confirms however that in PEG-ylated form, the enediynes are still able to effectively form the cytotoxic diyl radicals, supporting the design approach.

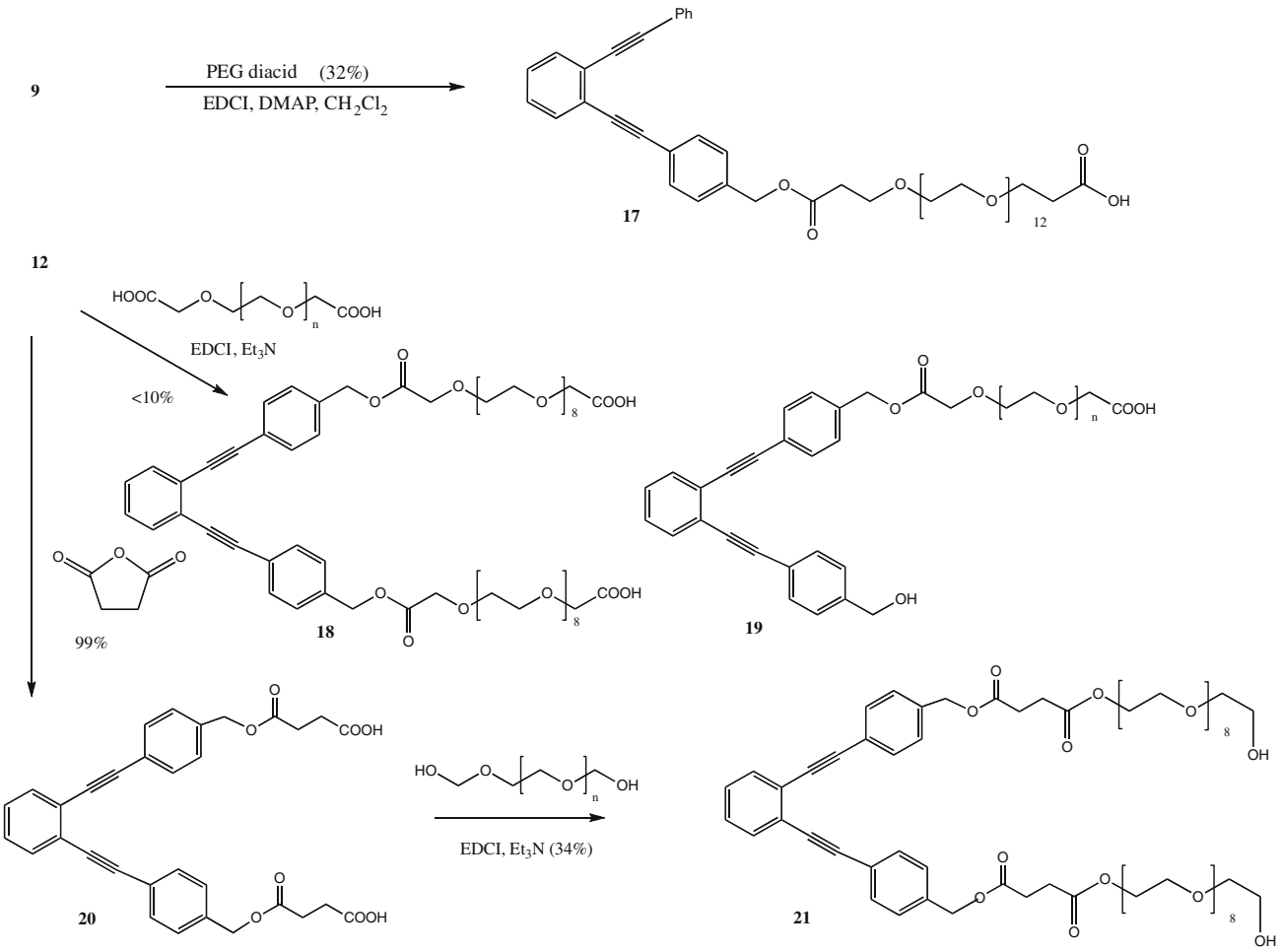
1.6. Future applications—Nanoparticle conjugates

Functionalized Au nanoparticle derivatives are becoming staples of investigational medicinal chemistry,[10] and we sought to demonstrate coupling of an enediyne building block. Reductive coupling of enediyne **10** with $HAuCl_4$ gave nanoconjugate **27** (Scheme 7).[11] The morphology of the Au derivatives was assessed through TEM analysis (Fig. 1). The nanoparticles showed an average core size of approx. 5.0 nm, anticipated based on a benzyl mercapto Au nanoparticle prepared as a standard. TEM images were re-taken after a period of one month to verify the shelf-stability of the nanoparticles. NMR proved to be an excellent means to assess conversion, with conjugated and non-conjugated versions showing characteristic spectra.

In summary, readily available linear (aryl) enediynes can be prepared and efficiently functionalized allowing conversion to a variety of thermally stable derivatives. The inherent hydrophobicity of the enediyne cores can be addressed through PEG-ylation and derivatization as ammonium salts. In all cases the enediynes undergo photo-induced cycloaromatization to yield arene products. Bioconjugation in the form of monoclonal antibody derivatives is achieved either through amide or ester linked PEG's and immunocompetence is retained. Finally, Au nanoparticle enediyne conjugates can be prepared via a two-phase method. The methodology described herein is flexible and efficient, and we expect application in bioorganic, medicinal and materials photochemistry. The DNA cleavage capacity of these photoactivated systems has been confirmed, lending the possibility for the design of targeted applications in a number of areas.

2. Experimental procedures

NMR spectra were obtained either on a Varian Mercury 300 (300 MHz) or a Varian Inova 500 (500 MHz) spectrometer. Mass spectra were obtained on a Hewlett Packard 6890 Plus GC with 5973 Mass Selective Detector and Agilent HP Ultra 1, 25 m × 0.2 mm column with a 0.11 μm film thickness. The electron impact ionization source was run at 70 eV. MALDI was conducted on an Applied Biosystems 4800 MALDI-TOF/TOF. High resolution TEM analyses were performed on JEOL, JEM 1010 and the images captured using an Ex41B 4MP bottom mount CCD camera system (Advanced Microscopy Techniques). Thin layer chromatography (TLC) was performed using Silica Gel 60 F524 precoated plates (Scientific Adsorbants Inc.) Preparative thin layer chromatography was carried with Silica Gel GF (Analtech, Inc.). Flash chro-

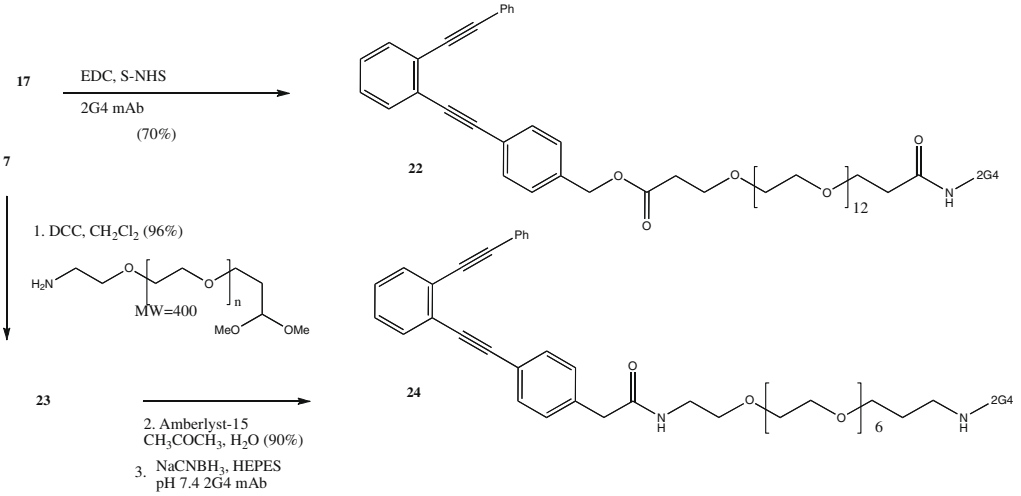


Scheme 4. Optimized route to PEG-ylated photo-enediynes.

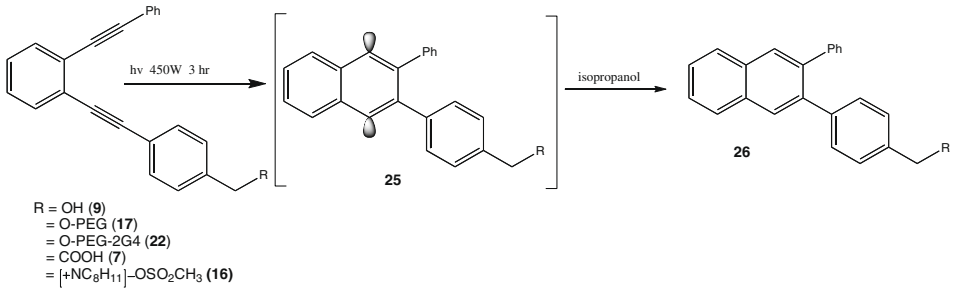
matography was performed using Silica Gel 60 (230–400 mesh, Whatman Inc.). All reactions were carried out under anhydrous conditions, under inert atmosphere (nitrogen or argon) with dry, freshly distilled solvents and flame dried glassware unless otherwise noted. All PEG reagents were purchased from Laysan Bio, Inc.

2.1. 1-Iodo-2-(ethynylphenyl)benzene

To a solution of 1,2-diiodobenzene (**4**) (10 g, 30.03 mmol) in diethylether (30 mL) was added dichloro(bistriphenylphosphine)palladium (0.85 g, 1.21 mmol). The solution was degassed



Scheme 5. Antibody conjugation of the photo-enediynes.



Scheme 6. Photo-Bergman cyclization of enediynes.

(N_2) for 45 min. In a second flask copper iodide (0.49 g, 2.55 mmol) and *n*-butyl amine (1.78 g, 24.3 mmol) were added to a degassed solution of phenyl acetylene (0.62 g, 6.0 mmol) in ether (20 mL). The diiodobenzene solution was cannulated into the alkyne solution and the resulting mixture stirred for 12 h at 25 °C and then saturated ammonium chloride (20 mL) added. Ethyl acetate (20 mL) was added to the biphasic mixture, and the organic layer washed with water (20 mL) and the combined aqueous layers were extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$) and concentrated in vacuo. The crude solid was subjected to flash chromatography (hexanes) to yield the title compound (1.08 g, 59%) as a white solid (mp 62–65 °C); R_f 0.36 (hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): 7.87 (dd, 1H, J = 3.9, 6.3 Hz), 7.61 (m, 2H), 7.5 (dd, 1H, J = 4.5, 7.2 Hz), 7.35 (m, 4H), 7.03 (dd, 1H, J = 3.6, 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 138.7, 132.7, 132.6, 129.5, 129.3, 128.7, 128.5, 127.8, 122.0, 101.2, 93.0, 91.6; HRMS (CI), m/z $C_{14}H_9I$ $(M+H)^+$: calcd 304.9828, obsd 304.9828.

2.2. 1-(Trimethylsilylethynyl)-2-(ethynylphenyl)benzene

To a degassed solution of 1-iodo-2-(ethynylphenyl)benzene (1.08 g, 3.55 mmol) in triethylamine (10 mL) was added dichloro(bistriphenylphosphine)palladium (0.1 g, 0.14 mmol) and copper iodide (0.055 g, 0.29 mmol). The solution was degassed and trimethylsilylacetylene (0.46 g, 4.67 mmol) added. The solution was again degassed and then stirred for 12 h at 25 °C. The solution was quenched with saturated ammonium chloride (10 mL). Ethyl acetate (20 mL) was added to the biphasic mixture. The organic layer was washed with water (20 mL) and the combined aqueous layers were extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$) and concentrated in vacuo. The reside was subjected to column chromatography (1:99, ethylacetate/hexanes) to yield the title compound as a yellow oil (0.55 g, 57%); R_f: 0.25 (hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): 7.56 (m, 2H), 7.50 (m, 2H), 7.32 (m, 3H), 7.23 (m, 2H), 0.26 (9H); ^{13}C NMR (75 MHz, $CDCl_3$): 132.5 131.9, 128.6, 128.5, 128, 0.0; HRMS (CI), m/z $C_{19}H_{18}Si$ $(M+H)^+$: calcd 275.1256, obsd 275.1256.

2.3. 1-(Ethynylphenyl)-2-ethynylbezene (5)

A solution of 1-(trimethylsilylethynyl)-2-(ethynylphenyl)benzene (0.73 g, 2.66 mmol) in tetrahydrofuran (20 mL) was cooled to 0 °C. Tetrabutylammonium fluoride (2.79 g, 10.7 mmol, 1.0 M in THF) was added dropwise. The reaction mixture was stirred for 2.5 h at 0 °C. Water (15 mL) was added slowly to quench the reaction. The biphasic mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$) and the solution filtered through a short plug of silica gel and concentrated in vacuo to yield the title compound as a yellow oil (0.53 g, 100%). R_f: 0.27 (hexanes); ^{1}H NMR (300 MHz, $CDCl_3$): 7.59–7.52 (m, 4H), 7.36–7.25 (m, 5H), 3.36 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 132.8, 132.0, 128.6, 128.1, 127.9, 126.6, 124.9, 123.4, 93.78, 88.1, 88.3; HRMS (CI), m/z $C_{16}H_{10}$ $(M+H)^+$: calcd 203.0863, obsd 203.0861.

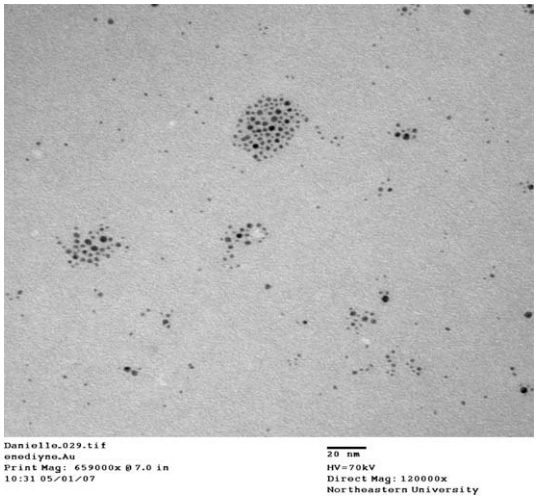
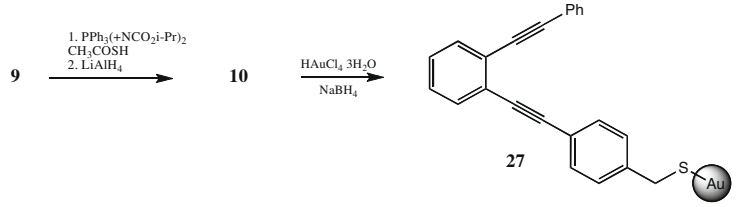


Figure 1. TEM image of Au enediynenanoparticles **27**.

2.4. (4-Iodo-phenyl)-acetic acid methyl ester

A 25 mL round bottom flask was charged with a mixture of fluoroboric acid (1.0 mmol, 310 μL of 48% in H_2O) and 4-iodophenylacetic acid (0.262 g, 1.0 mmol) in dichloromethane (15 mL). The mixture was cooled to 0 °C and stirred vigorously while trimethylsilyldiazomethane (0.56 mL, 1.8 M in hexanes, 1.0 mmol) was added dropwise over 5 min. With continued vigorous stirring at



Scheme 7. Synthesis of Au nanoparticles stabilized by thiolated enediyne.

0 °C, three additional portions of trimethylsilyldiazomethane (0.28 mL, 0.5 mmol), (0.14 mL, 0.25 mmol) and (0.14 mL, 0.25 mmol) were added dropwise at intervals of 20 min then mixture was stirred at 0 °C for an additional 30 min. Water (30 mL) was added slowly, then the mixture was extracted with dichloromethane (10 mL), the organic layer washed with water (10 mL), brine (10 mL), then dried ($MgSO_4$) and concentrated in vacuo. The crude residue was purified by flash chromatography (ethyl acetate/hexanes, 15:85), to yield the title compound as a pale yellow oil (0.252 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.68 (d, J = 8.7 Hz, 2H), 7.06 (d, J = 8.7 Hz, 2H), 3.72 (s, 3H), 3.59 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 171.6, 137.9, 133.8, 131.5, 92.9, 52.4, 40.8; HRMS (EI), m/z $C_9H_9IO_2$ $(M+H)^+$: calcd 276.9725, obsd 276.0001.

2.5. 4-(2-Phenylethynyl-phenylethynyl)-phenyl acetic acid methyl ester (6)

To a degassed solution of 1-(ethynylphenyl)-2-ethynylbenzene (0.28 g, 1.4 mmol) in triethylamine (20 mL) was added (4-iodophenyl)acetic acid methyl ester (0.46 g, 1.68 mmol), dichloro-(bistriphenylphosphine)palladium (0.04 g, 0.05 mmol), and copper iodide (0.02 g, 0.12 mmol). The solution was degassed again (45 min N_2 purge) and then stirred at 25 °C for 5 h. Saturated ammonium chloride (20 mL) was added, followed by dichloromethane (20 mL). The organic layer was washed with water (20 mL) and the combined aqueous layers were extracted with dichloromethane (20 mL). The combined organic layers were washed with brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (85:15, hexanes/ethyl acetate) to yield the title compound as a yellow oil (0.44 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.57–7.25 (m, 13H), 3.71 (s, 3H), 3.64 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 171.8, 134.5, 132.4, 132.1, 131.9, 129.6, 128.7, 128.6, 128.2, 127.7, 126.1, 123.5, 122.4, 93.8, 93.6, 88.7, 88.5, 52.4, 41.3; HRMS (EI), m/z $(M+H)^+$: $C_{25}H_{18}O_2$ calcd 351.1385, obsd 350.1099.

2.6. 2-(4-((2-(Phenyethynyl)phenyl)ethynyl)phenyl)acetic acid (7)

Lithium hydroxide monohydrate (0.97 g, 2.48 mmol) was added to a solution of 4-(2-phenylethynyl-phenylethynyl)-phenyl acetic acid methyl ester (0.087 g, 0.248 mmol) in THF/water 5:4 (13 mL). The resulting mixture was stirred for 24 h at 25 °C then the solution was condensed in vacuo. The reside was diluted with dichloromethane (5 mL), washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (20:80, ethyl acetate/hexanes) to afford the title compound as a colourless oil (0.075 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.62–7.45 (m, 6H), 7.40–7.20 (m, 7H), 3.68 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 150.7, 132.7, 132.1, 132.0, 131.9, 129.7, 128.7, 128.6, 128.2, 126.1, 126.0, 123.5, 122.7, 93.8, 93.4, 88.8, 88.5, 41.2; HRMS (ESI), m/z $C_{24}H_{16}O_2$ $(M+H)^+$: calcd 337.1299, obsd 337.2001.

2.7. 2-(4-((2-(Phenylethynyl)phenyl)ethynyl)phenyl)ethanol (8)

Diisobutyl aluminium hydride (DIBAL-H) in toluene (1 M, 1.778 mmol, 3 mL) was added to a solution of 4-(2-phenylethynyl-phenylethynyl)-phenyl acetic acid methyl ester (0.623 g, 1.778 mmol) in THF (13 mL) at −78 °C. The mixture was stirred for 30 min then an additional equivalent of DIBAL-H added. Methanol (5 mL), then water (0.5 mL) was added, then the solution was filtered. The filtrate was extracted with dichloromethane (3 × 5 mL), washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (20:80, ethyl acetate/hexanes) to afford the title compound (0.57 g, 99%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.59–7.12 (m, 13H) 3.88 (t, J = 6.0 Hz, 2H), 2.9 (t, J = 6.0 Hz, 2H), 2.1 (br, s, OH); ^{13}C NMR (75 MHz, $CDCl_3$): 139.4, 132.1, 131.9, 129.3, 128.6, 127.8, 126.1, 123.5, 121.7, 93.7, 88.5, 88.4, 63.6, 39.3; HRMS (ESI), m/z $C_{24}H_{18}O$ $(M+H)^+$: calcd 323.1435, obsd 322.2074.

2.8. 4-(2-Phenylethynyl-phenylethynyl)-benzyl alcohol (9)

To a degassed solution of 1-(ethynylphenyl)-2-ethynylbenzene (0.53 g, 2.62 mmol) in triethylamine (35 mL) was added 4-iodobenzyl alcohol (0.737 g, 3.15 mmol), dichloro(bis triphenylphosphine)palladium (0.074 g, 0.11 mmol) and copper iodide (0.04 g, 0.21 mmol). The solution was degassed again (45 min N_2 purge) and then stirred at room temperature for 4 h. Saturated ammonium chloride (30 mL) was added followed by ethyl acetate (30 mL). The organic layer was washed with water (30 mL) and the combined aqueous layers extracted with ethyl acetate (2 × 40 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash chromatography (70:30, hexanes/ethyl acetate) to yield the title compound (0.65 g, 81%) as a tan solid. (mp 95–97 °C); ^{1}H NMR (300 MHz, $CDCl_3$): 7.59–7.25 (m, 13H), 4.71 (s, 2H), 1.75 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): 141.4, 132.1, 132.0, 131.9, 128.7, 128.6, 128.3, 127.1, 126.1, 123.5, 122.8, 93.8, 93.7, 88.6, 88.5, 65.2; HRMS (CI), m/z $C_{23}H_{16}O$ $(M+H)^+$: calcd 309.1281, obsd 309.1280.

2.9. 4-(2-Phenylethynyl-phenylethynyl)-benzyl thioacetate

Diisopropylazodicarboxylate (0.21 mL, 1.06 mmol) was added dropwise to a solution of triphenylphospine (0.26 g, 1.05 mmol) in THF (2.5 mL) at 0 °C. The solution was stirred for 30 min at 0 °C then a solution of (4-((2-phenylethynyl) phenyl)ethynyl)phenyl)methanol (0.16 g, 0.05 mmol) and thiolacetic acid (0.08 g, 1.05 mmol) in THF (2 mL) added dropwise. The mixture was stirred for 1 h at 0 °C then warmed to 25 °C and stirred for an additional 1 h. The solution was extracted with dichloromethane (10 mL) and the organic layer washed with saturated sodium bicarbonate (4 × 2 mL). The solution was concentrated in vacuo to approx. ¼ original volume then diluted with an equal volume of a 1:1 hexanes/ether mixture. The solution was filtered through a pad of Celite and dried ($MgSO_4$). The solution was again, diluted with an equal volume of hexanes/ether and then filtered over a pad of silica gel and concentrated in vacuo. The reside was subjected to flash chromatography (100% dichloromethane) to yield the title compound as a yellow oil (0.11 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.58–7.25 (m, 13H), 4.12 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): 194.9, 138.1, 132.3, 131.9, 131.6, 128.9, 128.4, 128.0, 125.8, 123.3, 122.3, 93.6, 93.3, 88.6, 88.2, 33.3, 30.3. HRMS (CI), m/z $C_{25}H_{18}OS$ $(M+H)^+$: calcd 367.1156, obsd 367.0902.

2.10. 4-(2-Phenylethynyl-phenylethynyl)-benzyl thiol (10)

A solution of lithium aluminium hydride (0.013 g, 0.34 mmol, 1 M) in ether (0.6 mL) was added dropwise to a solution of 4-(2-phenylethynyl-phenylethynyl)-benzyl thioacetate (0.11 g, 0.3 mmol) in ether (1.5 mL) at 0 °C. The mixture was stirred at 0 °C for 20 min, then warmed to 25 °C and stirred for an additional 30 min. The mixture was cooled to 0 °C and treated with hydrochloric acid (3 N, ~1.5 mL) until all solid dissolved. The solution was diluted sequentially with ether (2 mL), water (0.2 mL), sodium hydroxide (0.02 mL, 15% in water) then water (0.006 mL). The mixture was warmed to room temperature, stirred for 15 min then extracted with ether (2 × 2 mL). The combined organic layers were washed with brine

(5 mL), dried ($MgSO_4$) and concentrated in vacuo to yield the title compound as a yellow oil (0.8 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.57–7.19 (m, 13H), 3.75 (d, 2H, J = 7.8 Hz), 1.77 (t, SH J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 146.8, 138.1, 132.2, 131.9, 128.7, 128.2, 127.3, 125.4, 123.5, 122.3, 93.6, 93.2, 88.6, 88.4, 29.9. HRMS (CI), m/z $C_{23}H_{16}S$ $(M+H)^+$: calcd 324.10, obsd 324.20.

2.11. 1,2-Bis((trimethylsilyl)ethynyl)benzene

$Pd(PPh)_2Cl_2$. (0.032 g, 3 mol %) and CuI (0.014 g, 5 mol %) were added to a degassed (Ar 30 min) solution of 1,2-diiodobenzene (0.500 g, 198 μL, 1.516 mmol) in triethylamine (5 mL). The solution was degassed (Ar, 30 min) then TMS–acetylene (535 μL, 2.5 equiv) added and the mixture stirred for 16 h at 25 °C. Saturated aqueous NH_4Cl (5 mL) was added followed by ethyl acetate (10 mL). The layers were separated and the organic layer was washed with water (2 × 10 mL). The aqueous layers were back extracted with ethyl acetate (2 × 10 mL) and the combined organic extracts washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography to afford the title compound (0.272 g, 66.4%) as a yellow oil.[12] TLC (hexanes/ethyl acetate = 9:1): R_f 0.53; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45 (dd, J = 6.3 Hz, 3.3 Hz, 2H), 7.19–7.23 (m, 2H), 0.27 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 132.24, 127.99, 125.75, 103.23, 98.36, 0.00.

2.12. 1,2-Diethynylbenzene (11)

Potassium carbonate (0.542 g) was added to a solution of 1,2-bis((trimethylsilyl) ethynyl)benzene (0.212 g, 0.784 mmol) in methanol/THF (1:1, 80 mL). The resulting solution was stirred at 25 °C (Ar atmosphere) for 30 min then diluted with ether (300 mL). The mixture was washed with saturated ammonium chloride (4 × 150 mL), water (4 × 150 mL) then dried ($MgSO_4$) and condensed in vacuo to afford the title compound (0.098 g, 99%) as a clear oil.[13] TLC (hexanes/ethyl acetate = 9:1): R_f 0.42; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50 (dd, J = 6.3 Hz, 2H), 7.24–7.31 (m, 2H), 3.33 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 132.87, 128.76, 125.30, 82.09, 81.49.

2.13. 1,2-Bis((4-((*tert*-butyldimethylsilyloxy)methyl)phenyl)-ethynyl)benzene

$Pd(PPh)_2Cl_2$. (0.0445 g, 8 mol %) and CuI (0.1208 g, 8 mol %) were added to a degassed (Ar, 30 min) solution of 1,2-diethynylbenzene (0.100 g, 0.793 mmol) and 4-((*tert*butyldimethylsilyloxy)methyl)-phenyl iodide[14] (0.607 g, 1.75 mmol) in triethylamine (25 mL). The resulting solution was stirred at 25 °C (Ar atmosphere) for 5 h then diluted with saturated ammonium chloride (5 mL) and ethyl acetate (300 mL). The organic layer was washed with brine (100 mL), dried ($MgSO_4$) and condensed in vacuo. The residue was purified by flash column chromatography (19:1 hexanes/ethyl acetate) to afford the title compound (0.240 g, 53%) as a yellow solid. Mp = 109–111 °C; TLC (hexanes/ethyl acetate = 19:1): R_f 0.35; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48–7.60 (m, 4H), 7.21–7.36 (m, 8H), 4.76 (s, 4H), 0.95 (s, 18H), 0.11 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.66, 147.16, 136.96, 136.83, 133.12, 131.21, 131.16, 127.04, 99.00, 93.30, 69.93, 31.20, 23.63, 0.0; HRMS (CI), m/z $C_{36}H_{46}O_2Si_2$ $(M+H)^+$ calcd 567.3116, obsd 567.3115.

2.14. (4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))-dimethanol (12)

Tetrabutylammonium fluoride (815 μL, 0.815 mmol, 1 M in THF) was added to a solution of 1,2-bis((4-((*tert*-butyldimethylsilyloxy)methyl)phenyl)ethynyl)benzene in tetrahydrofuran at 0 °C (Ar atmosphere) and stirred for 30 min. Water (20 mL) was added. The solution was condensed in vacuo, diluted with ethyl acetate (300 mL) and the organic layer washed with water (200 mL) and brine (100 mL), dried ($MgSO_4$) and condensed in vacuo. The residue was purified by flash chromatography (2:1 ethyl acetate/hexanes) to afford the title compound (0.133 g, 98%) as a white solid. Mp = 146–147 °C; TLC (hexanes/ethyl acetate = 1:1): R_f 0.18; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54–7.59 (m, 6H), 7.34–7.38 (m, 4H), 7.30–7.33 (m, 2H), 4.73 (s, 4H), 1.4–1.9 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 141.32, 131.97, 131.91, 128.19, 127.00, 125.93, 122.66, 93.57, 88.52, 65.11; HRMS $C_{24}H_{18}O_2$ (CI), m/z $(M)^+$: calcd 338.1307, obsd 338.1307.

2.15. Standard procedure for photo-cyclization of enediynes

A solution of enediyne (5 mg, 0.005% in HPLC grade isopropanol) is added to a 14 mL quartz UV cuvette and degassed (Ar, 30 min). In a photochemical box, a 0.01 M potassium chromate solution filter is placed between the lamp and the solution, and irradiation initiated using a 450 W Hanovia low-pressure Hg lamp. The reaction is stirred for up to 3 h (N_2 atmosphere) and monitored by both UV–vis and mass spectrometry to determine the rate and extent of formation of cylcloaromatized product. Representative λ_{max} **16** = 275 nm (**26** = 305 nm); **22** = 308 nm (**26** = 364 nm). Fluorescence quantum yields based on analysis of **9/26** were consistent with those reported for **1**.[15]

2.16. Photoinduced DNA cleavage

ΦX174 (RfI) DNA was irradiated (Hanovia 450 W medium pressure Hg lamp) in TRIS–HCl at pH 8.5 with DNA (50 μM in base pairs) as control or with **17** at 10^{-6} M for 3 h at 37 °C. Crude mixtures were subjected to gel electrophoresis (10% agarose), stained with ethidium bromide, then subjected to scanning densitometry. Under these conditions up to 20–25% of RfII DNA is produced (corrected for 3–5% produced on photoirradiation in absence of substrate) together with 2–5% RfIII form, derived from random degradation of RfII form. Irradiation in the presence of cycloaromatized substrates or incubation in the presence of enediynes resulted in no increase in DNA degradation over controls.

2.17. 1-(4-((2-(Phenylethynyl)phenyl)ethynyl)benzyl)-4-aza-1-azoniabicyclo[2.2.2]octane methanesulfonate (salt) (13)

Triethylamine (41 μL, 3 equiv) and methanesulfonyl chloride (15 μL, 2 equiv) was added to a solution of 4-(2-phenylethynyl-phenylethynyl)-benzyl alcohol in dichloromethane (4 mL) at 0 °C (Ar atmosphere) then stirred at 25 °C for 1 h. The resulting solution was diluted with ethyl acetate (25 mL) and washed with citric acid (5%, 30 mL), sodium bicarbonate (5%, 30 mL) and brine (30 mL), dried ($MgSO_4$) and condensed in vacuo. This residue was added dropwise to a solution of 1,4-diazabicyclo[2.2.2]octane (DABCO) (0.0086 g, 0.7666 mmol) in acetonitrile (2 mL) at 0 °C. The resulting solution was stirred at 0 °C for 30 min, warmed to 25 °C and condensed in vacuo. The residue was recrystallized from hexanes, methanol and ethyl acetate to afford the title compound 0.036 g, 94%) as a yellow solid. Mp 60–61 °C; ^{1}H NMR (500 MHz, $CDCl_3$): 7.51–7.57 (m, 8H), 7.34–7.39 (m, 2H), 7.24–7.33 (m, 3H), 4.89 (s, 2H), δ 3.59 (t, J = 7.5 Hz, 6H), 3.13 (t, J = 7.5 Hz, 6H), 2.84 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): δ 135.54, 134.20, 134.10, 134.02, 133.53, 130.69, 128.94, 128.01, 127.94, 127.14, 125.24, 95.39, 93.90, 92.08, 89.65, 69.60, 54.40, 46.98, 40.28; HRMS $C_{29}H_{27}N_2^+$ (EI), m/z $(M)^+$: calcd 403.2169, obsd 403.2174.

2.18. 1,1′-(4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methylene)bis(4-aza-1-azoniabicyclo[2.2.2]octane) methanesulfonate (14)

Triethylamine (22.2 μL, 6 equiv) and methanesulfonyl chloride (12.42 μL, 6 equiv) was added to a solution of (4,4′-(1,2-phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))dimethanol (0.009 g, 0.0266 mmol) in dichloromethane (4 mL) at 0 °C (Ar atmosphere) then stirred at 25 °C for 1 h. The resulting solution was diluted with ethyl acetate (15 mL) and washed with citric acid (5%, 20 mL), sodium bicarbonate (5%, 20 mL) and brine (20 mL), dried ($MgSO_4$) and condensed in vacuo. The residue was added dropwise to a solution of 1,4-diazabicyclo[2.2.2]octane (DABCO) (0.006 g, 0.2 equiv) in acetonitrile (2 mL) at 0 °C. The resulting solution was stirred at 25 °C for 90 min then condensed in vacuo. The residue was recrystallized from hexanes, methanol and ethyl acetate to afford the title compound (0.0176 g, 92%) as a yellow solid. Mp 133–135 °C; ^{1}H NMR (300 MHz, $CDCl_3$): 7.44–7.51 (m, 4H), 7.28–7.37 (m, 6H), 7.01–7.08 (m, 2H), 4.85 (s, 4H),3.58 (t, 12H, J = 7.2 Hz), δ 3.14 (t, 12H, J = 7.2 Hz), 2.81 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): 134.90, 134.85, 133.43, 130.20, 128.38, 127.12, 126.52, 93.28, 91.11, 68.86, 53.71, 46.29, 39.74; HRMS $C_{36}H_{40}N_2^+$ (TOF MS ES+), m/z $(M+H)^{2+}$: calcd 264.1627, obsd 264.1614.

2.19. *N*-Benzyl-*N*,*N*-dimethyl-1-(4-((2-(phenylethynyl)phenyl)-ethynyl)phenyl)methanaminium chloride (15)

Triethylamine (21.7 μL, 3 equiv) and methanesulfonyl chloride (12.1 μL, 3 equiv) was added to a solution of 4-(2-Phenylethynyl-phenylethynyl)-benzyl alcohol (0.016 g, 0.0519 mmol) in dichloromethane (4 mL) at 0 °C (Ar atmosphere) then stirred at 25 °C for 1 h. The resulting solution was diluted with ethyl acetate (30 mL) and washed with citric acid (5%, 30 mL), sodium bicarbonate (5%, 30 mL) and brine (30 mL), dried ($MgSO_4$) and condensed in vacuo . Acetonitrile (1 mL) was added and the solution was added dropwise to a solution of *N*,*N*′-dimethylbenzylamine (7.8 μL, 1 equiv) in acetonitrile (1.5 mL) at 25 °C. The resulting solution was stirred at 60 °C for 4 h then condensed in vacuo. The residue was dissolved in ethyl acetate (10 mL) and the solution was washed with brine (10 × 10 mL). The brine washings were back extracted with dichloromethane (10 × 10 mL) then the combined organic layers were dried ($MgSO_4$) and condensed in vacuo to afford the title compound (0.020 g, 83.6%) as a white solid. Mp 112–115 °C; ^{1}H NMR (500 MHz, CD_3OD): 7.68 (d, J = 8.5 Hz, 2H) 7.50–7.64 (m, 11H), 7.33–7.45 (m, 5H), 4.63 (s, 2H), 4.61 (s, 2H), δ 2.95 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 134.74, 134.50, 133.38, 133.16, 133.12, 132.72, 132.19, 130.58, 130.10, 130.00, 129.84, 129.67, 129.12, 128.87, 127.36, 127.33, 126.44, 124.54, 94.71, 93.21, 91.48, 88.94, 70.04, 69.43, 55.65; HRMS $C_{32}H_{28}$N+ (TOF MS ES+), m/z $(M)^+$: calcd 426.2222, obsd 426.2210.

2.20. *N*,*N*′-(4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methylene)bis(*N*,*N*-dimethyl-1-phenylmethanaminium) methanesulfonate (16)

Triethylamine (16 μL, 6 equiv) and methanesulfonyl chloride (9 μL, 6 equiv) was added to a solution of (4,4′-(1,2-phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))dimethanol (0.007 g, 0.02069 mmol) in dichloromethane (2 mL) at 0 °C (Ar atmosphere) then stirred at 25 °C for 1 h. The resulting solution was diluted with ethyl acetate (20 mL) and washed with citric acid (5%, 30 mL), sodium bicarbonate (5%, 30 mL) and brine (30 mL), dried ($MgSO_4$) and condensed in vacuo. This residue was diluted with acetonitrile (1 mL) and added dropwise to a solution of *N*,*N*′-dimethylbenzylamine (6.1 μL, 2 equiv) in acetonitrile (1.5 mL) at 0 °C. The resulting solution was stirred at 60 °C for 3 h then condensed in vacuo and diluted with 49:1 hexanes/methanol (10 mL). After vigorous stirring the methanol layer was removed, the hexanes layer was warmed to 25 °C and methanol (1 mL) added. This process was repeated three times and the methanol extracts were combined, dried ($MgSO_4$), and condensed in vacuo to afford the title compound (0.008 g, 52%) as a white solid. Mp 55–57 °C; ^{1}H NMR (500 MHz, CD_3OD): δ 7.41–7.77 (m, 22H), 4.66 (s, 4H), 4.64 (s, 4H), 2.98 (s, 12H), 2.72 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 134.82, 134.50, 133.40, 132.20, 132.09, 130.58, 130.19, 129.27, 128.88, 127.22, 126.55, 93.35, 91.20, 70.02, 69.42, 43.09, 39.67; HRMS $C_{42}H_{42}N_2^+$ (TOF MS ES+), m/z $(M)^{2+}$: calcd 287.1674, obsd 287.1667.

2.21. 4-(2-Phenylethynyl-phenylethynyl)-benzyle ester polyethylene glycol carboxylic acid (17)

(4-((2-Phenylethynyl)phenyl)ethynyl)phenyl)methanol (0.081 g, 0.26 mmol) was added to a solution of polyethyleneglycol (PEG) diacid (690.8 g/mol, 0.40 g, 0.58 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.11 g, 0.58 mmol) and 4-dimethylaminopyridine (0.01 g, 0.08 mmol) in dichloromethane (8 mL). The solution was stirred at 25 °C for 12 h, then washed with brine (20 mL), dried ($MgSO_4$), and condensed in vacuo. The reside was purified by flash chromatography (dichloromethane/methanol (1:1) to yield the title compound (0.09 g, 32%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.58–7.26 (m, 13 H), 5.15 (s, 2H), 3.8–3.38 (m, PEG, 53H), 2.7 (t, 2H, J = 6.3 Hz), 2.59 (t, 2H, J = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 174.1, 171.55, 138.8, 136.4, 132.0, 131.9, 128.7, 128.6, 128.3, 127.2, 126.1, 125.9, 123.5, 121.8, 93.9, 93.4, 89.0, 88.5, 70.7, 67.0, 66.8, 66.0, 35.34, 32.9, 32.1, 29.9; HRMS $C_{53}H_{72}O_{17}$ (MALDI) $(M+Na)^+$ m/z calcd 1003.90, obsd 1003.30.

2.22. 4,4′-(4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methylene)bis(oxy)bis(4-oxobutanoic acid) (20)

(4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))dimethanol (0.0165 g, 0.0488 mmol) was added to a solution of 4-dimethylaminopyridine (0.006 g, 1 equiv) and succinic anhydride (0.0059 g, 1.2 equiv) in dichloromethane (3 mL). The mixture was stirred at 25 °C (Ar atmosphere) for 12 h. The solution was diluted with dichloromethane (10 mL) and washed with aqueous sodium carbonate (5%, 25 mL). The dichloromethane layer was removed and the aqueous layer acidified by dropwise addition of concentrated hydrochloric acid. This solution was back extracted with ethyl acetate (3 × 25 mL), washed with brine (25 mL), dried ($MgSO_4$), and condensed in vacuo to afford the title compound (0.0205 g, 99%) as a white solid. Mp 126–127 °C; TLC (dichloromethane/methanol/aqueous ammonium hydroxide = 80:19:1): R_f 0.22; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.53–7.60 (m, 6H), 7.29–7.35 (m, 6H), 5.17 (s, 4H), 2.64–2.75 (m, 8H); ^{13}C NMR (75 MHz, CD_3OD): δ 176.16, 173.98, 138.32, 132.82, 132.66, 129.47, 129.22, 126.95, 124.16, 94.15, 89.36, 66.85, 30.05, 29.83; HRMS $C_{32}H_{26}O_8$ (TOF MS ES+), m/z $(M+Na)^+$: calcd 561.1536, obsd 561.1525.

2.23. 4,4′-(4,4′-(1,2-Phenylenebis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methyleneoxy-polyethyelene glycol alcohol) (21)

4,4′-(4,4′-(1,2-Phenylenebis(ethyne-1,2-diyl))bis(4,1-phenylene))-bis(methylene)bis(oxy) bis(4-oxobutanoic acid) (0.004 g, 0.00742 mmol) was added to a solution of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDCI) (0.0031 g, 2.2 equiv) and 4-dimethylaminopyridine (DMAP) (0.0020 g, 2.2 equiv) in dichloromethane (1.5 mL) at 25 °C (Ar atmosphere). The mixture was refluxed for 4 h then cooled to 25 °C and 400M_n polyethylene glycol (5.8 μL, 2.2 equiv) was added. The solution was stirred for 48 h at 25 °C, then diluted

with dichloromethane (10 mL), washed with brine (2 × 10 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude residue was purified via preparative thin layer chromatography (90:9:1 CH_2Cl_2/MeOH/aq NH_4OH) to yield the title compound (3.7 mg, 39%) as a yellow oil. TLC (dichloromethane/methanol/aqueous ammonium hydroxide = 80:19:1): R_f 0.31; HRMS $C_{66}H_{90}O_{24}$ (MALDI): m/z $(M+2Na)^{2+}$ calcd1285.43982, obsd 1287.44434.

2.24. Enediyne antibody conjugate (22)

A solution of the 2G4 antibody (0.0075 g, 1.5 mL water), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.040 g, 0.21 mmol) and S-NHS (0.045 g, 0.21 mmol) was added to a solution of **21** in phosphate buffered saline (0.5 mL, pH 7.4).

The mixture was stirred for 5 h at 25 °C then purified via dialysis using a spectrum dialysis bag (250 kDa) against distilled water and stirred at 25 °C for 12 h. The mixture was kept at −80 °C for 2 h followed by freeze-drying (under vacuum at −48 °C for 24 h) to solidify the purified title compound as a white solid (0.020 g, 70%). ELISA testing was performed using unconjugated 2G4 antibody as a control, using absorbance profile to confirm conjugation of the antibody to the PEG-ylated enediyne.

2.25. *N*-(3,3-Dimethoxy-propoxy)-PEG 4-(2-phenylethynyl-phenyletyhyl)-phenyl acetamide (23)

N,*N*′-Dicyclohexylcarbodiimide (0.019 mg, 0.090 mmol) was added to a solution of 4-(2-phenyletheynyl-phenylethynyl)-phenyl acetic acid (0.033 g, 0.090 mmol) in dichloromethane (15 mL). The solution was stirred for 10 min then 400M_n PEG amine (0.048 g, 0.09 mmol) was added and the solution was stirred for 12 h at 25 °C. The solution was concentrated in vacuo and the residue was subjected to column chromatography (alumina, 100% acetone) to yield the title compound (0.0668 g, 96%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): 7.56–7.20 (m, 13H), 4.51 (t, J = 6.3 Hz, 1H), 3.67–3.38 (m, 16 × CH_2), 3.33 (s, 6H), 1.88 (q, J = 6.3 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 170.68, 135.81, 132.24, 132.05, 132.01, 131.85, 129.64, 128.24, 126.03, 125.96, 123.48, 122.36, 102.44, 93.81, 93.55, 88.78, 88.49, 70.75, 70.50, 69.92, 67.39, 53.27, 43.78, 39.67, 33.23; HRMS $C_{43}H_{55}NO_{10}$ (ESI), m/z $(M+Na)^+$: calcd 768.3721, obsd 768.2378.

2.26. Enediyne antibody conjugate (24)

Deprotection of the **23** was achieved by treatment of an acetone/water (1:1) solution of the propionaldehyde dimethylacetal O-PEG derivative (0.20 g, 0.13 mmol) with Amberlyst 15 ion-exchange resin (1.24 g). The reaction mixture was stirred for 24 h at 25 °C. The mixture was filtered over a glass plug and concentrated in vacuo. The crude oil was crystallized from a 1:1 dichloromethane/ether mixture (−78 °C) to yield *N*-(3-acetaldehyde)-PEG 4-(2-phenylethynyl-phenyletyhyl)-phenyl acetamide (0.208 g, 100%) as clear oil; ^{1}H NMR (300 MHz, $CDCl_3$): 9.78 (s, 1H), 7.75–7.20 (m, 13 H), 4.0–3.54 (m, 26H), 3.48–3.38 (m, 4H), 2.71–2.61 (m, 2H); HRMS $C_{41}H_{49}NO_9$ (ESI), m/z $(M+CH_3OH+H)^+$: calcd 732.3232, obsd 732.4016.

HEPES buffer (500 μL) were added to a solution of 2G4 (0.001 g, 0.395 mL) and *N*-(3-acetaldehyde)-PEG 4-(2-phenylethynyl-phenyletyhyl)-phenyl acetamide (0.002136 g, 0.00265 mmol) in dimethylformamide (100 μL). Sodium cyanoborohydride (2.513 g, 40 mmol) was added and the mixture was stirred at 4 °C for 14 days. The mixture was transferred to the MACROSEP 50 K OMEGA (PALL Life Science) resin with phosphate buffered saline (1 mL, pH 7.4) and then centrifuged for 30 min (5000 g, −4 °C). An additional portion of phosphate buffered saline (1 mL) was added and the mixture centrifuged for an additional 60 min. The concentrate (0.7 mL) was collected and analyzed by UV–vis scan in the 200–600 nm range.

2.27. 4-(2-Phenylethynyl-phenylethynyl)-benzyl thiol gold nanoparticle conjugate (27)

A solution of gold(III) chloride trihydrate (0.100 g, 0.25 mmol) in water (10 mL) was stirred for 10 min. To this was added solution of tetraoctylammonium bromide (0.5 g, 0.91 mmol) in toluene (30 mL) and the combined solution was stirred for 30 min. (4-((2-(Phenylethynyl)phenyl)ethynyl)phenyl)methanethiol (0.1 g, 0.31 mmol) was added and the mixture stirred for an additional 20 min. An aqueous solution of sodium borohydride (0.126 g, 3.33 mmol, 0.37 M) was added dropwise. The solution was stirred for 2 h at 25 °C then the organic phase was washed with water (3 × 20 mL) and concentrated in vacuo to approx. 5 mL. The solution was diluted with ethanol (25 mL) and allowed to stand overnight at −20 °C. The precipitated nanoparticles were collected by vacuum filtration and washed sequentially with water (5 × 20 mL), ethanol (5 × 20 mL), acetonitrile (5 × 20 mL), and acetone (5 × 20 mL), to yield the title compound (0.1 g) as a black powder. The gold nanoparticles were characterized by high resolution transmission electron microscopy (TEM). TEM samples were prepared by spreading one drop of a 1 mg/mL clustered toluene solution onto a standard carbon-coated Formvar film on a copper grid (200 mesh). Average core diameter was found to be 5.0 nm.

Acknowledgements

We thank the NIH (RO1GM57123) for financial support of this work, and Amit Kale and Dmitriy Mongayt for performing ELISA assays.

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