Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 934–937
Bioorganic & Medicinal Chemistry Letters

Photoactivated enediynes as targeted antitumoral agents: Efficient routes to antibody and gold nanoparticle conjugates

Danielle Falcone, Jane Li, Amit Kale and Graham B. Jones*

Bioorganic and Medicinal Chemistry Laboratories, Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA

Received 29 October 2007; revised 18 December 2007; accepted 19 December 2007
Available online 7 January 2008

Dedicated to Professor Elias J. Corey on the occasion of his 80th birthday.

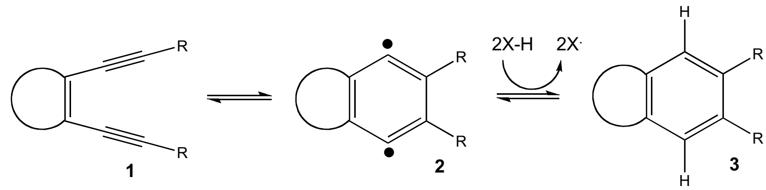
Abstract—Efficient syntheses of a series of functionalized aryl enediynes have been developed. The building blocks were used to effect conjugation to carrier PEG templates which allowed subsequent coupling to a cardiac targeted monoclonal antibody. Immunocompetence of the enediyne-Mab conjugates was demonstrated by ELISA, and both parent enediynes and bioconjugates underwent successful photo-Bergman cyclization. Finally, surface modified (Au) nanoparticle conjugates were prepared and size confirmed by TEM analysis. Application as long-circulating photoactivated prodrugs is anticipated.

The enediynes are a class of potent antitumoral agents isolated from soil bacteria.[1] Over 20 of this class are now known, two of which have entered clinical evaluation. One of these (Mylotarg®) represents the first ever monoclonal antibody–cytotoxin conjugate to be approved by the FDA and is currently used for treatment of acute myeloid leukemia (AML).[2] The natural enediynes use a variety of elaborate triggering mechanisms which activate the pharmacophore **1** via a cascade process ultimately involving Bergman cycloaromatization, to produce cytotoxic diyl radicals **2**, which abstract H atoms from cellular and nuclear macromolecules en route to arenes **3** (Scheme 1).[3] There has been considerable interest in the preparation of designed enediynes, including the potential for photoactivated prodrugs. The latter process, commonly referred to as the photo-Bergman cyclization, has been studied in some depth,[4] and parameters affecting photoconversion delineated.[5] In order to extend the versatility of the enediynes as controlled cytotoxins, we became interested in the possibility of assembling photo-Bergman precursors that could be routinely derivatized, specifically for bioconjugation chemistry.

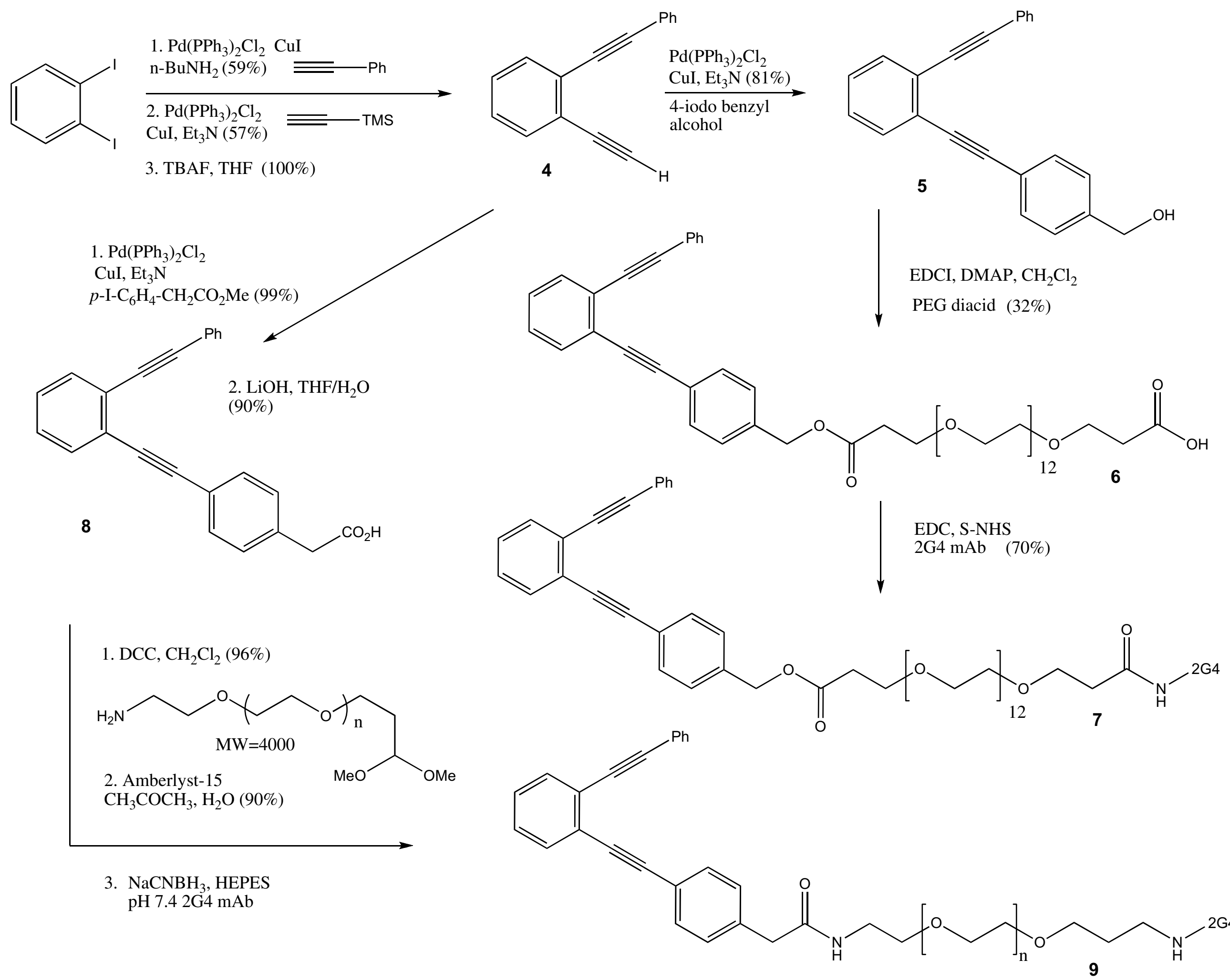
Accordingly, a differentially substituted aryl enediyne **4** was firstly prepared from 1,2-di-iodobenzene via sequential coupling–deprotection (Scheme 2).[6] Palladium mediated coupling with 4-iodobenzyl alcohol then gave enediyne **5**, reactions readily scaleable through multigram level. The viability of **5** as a photo-Bergman substrate was confirmed (vide infra) as was its shelf-life, fidelity preserved at room temperature over a period of weeks. With this building block in hand we elected to demonstrate versatility via formation of bioconjugates under established coupling methods. We firstly investigated preparation of a C-16 PEG conjugate **6**. PEGylation has become a commonly used strategy for the delivery of various lipophilic drug candidates.[7] Enhanced circulatory capacity coupled with reported affinity for specific tumor neovasculature suggest such conjugates hold promise in a number of clinical applications.[7] Conjugate **6** was formed in good yield via carbodiimide coupling of the PEG diacid, and displayed marked solubility in buffered organic and aqueous solutions. One of the expected properties of the conjugate (**6**) was facile derivatization to form three component drug-linker-antibody conjugates, and this was exemplified by coupling to an available cardio-myosin targeting antibody (2-G4) viz. **7** via NHS coupling.[8] The immunocompetence and fidelity of the enediyne bioconjugate **7** was confirmed by ELISA (Fig. 1). To demonstrate versatility of the enediyne building blocks we also developed a three component approach involving reductive

Keywords: Enediyne; Photo-Bergman; Bioconjugation; Antibody conjugate; Nanoparticles.
* Corresponding author. Tel.: +1 617 373 8619; fax: +1 617 373 8795; e-mail: gr.jones@neu.edu

0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2007.12.045



Scheme 1. The Bergman cycloaromatization of 3-hexene-1,5-diynes.



Scheme 2. Preparation, PEGylation, and bioconjugation of aryl enediyne photo-prodrugs.

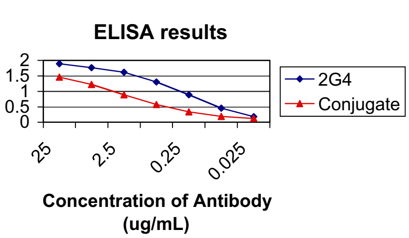
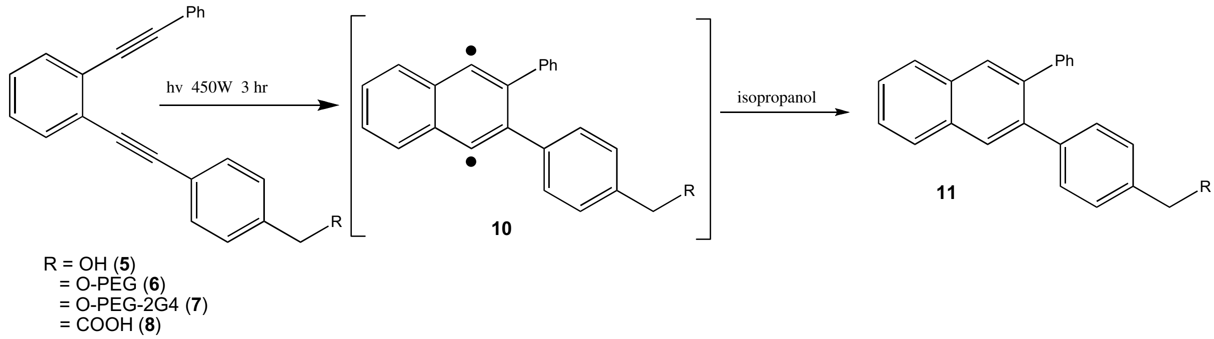


Figure 1. Relative immunoaffinity (**7**).

amination. Specifically **4** was coupled to a phenylacetic ester, and the (revealed) carboxylate **8** amidated with a differentially substituted (α-amino-ω-acetal) PEG with MW of approx 4000.[9] The carboxaldehyde was liberated using Amberlyst resin, then reductive amination of the product in the presence of the 2G4 mAb produced bioconjugate **9** in >50% yield (based on recovered aldehyde). Identical strategy was successfully used to prepare the bioconjugate of the related mAb 2G5 and immunocompetency confirmed by ELISA.[8]

With the parent enediynes and two bioconjugates in hand we were able to assess the impact of derivatization on photo-Bergman cyclization. Gratifyingly, simple irradiation of either **5**, **6**, **7**, **8** or **9** resulted in smooth



Scheme 3. Photo-Bergman cyclization of enediyne core and bioconjugates.

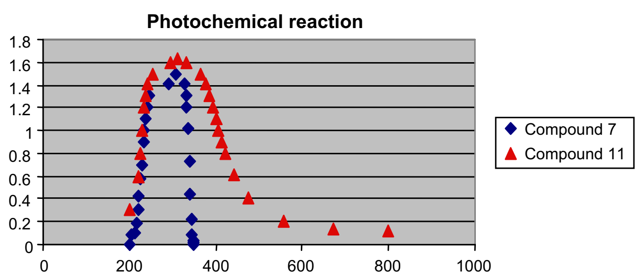


Figure 2. UV/vis data for photolysis [**7** (♦) and **11** (Δ), R = 2G4] (*y* axis = absorbance *x* axis = wavelength (nm)).

conversion to cyclized adducts **11** in buffered media, presumably via the intermediate diyl radicals **10** (Scheme 3), adducts confirmed by LC–MS analysis (unoptimized yields 18–35%). Conveniently UV–vis spectroscopy proved an effective means to monitor reaction progress, which in the case of **7** was complete within 3 h of irradiation as evidenced by consumption of enediyne (Fig. 2). Preliminary experiments involving incubation of **6** in the presence of duplex σX174 DNA confirmed photocleaving ability at μM concentrations.[10,11] The observation that both immunocompetence and photo-cyclization capacity is retained in compound **7** suggests that numerous applications in the design of targeted chemotherapeutic agents and biochemical reagents may be attainable.

To further demonstrate the versatility of building block **5** we opted to pursue development of a surface modified (Au) nanoparticle conjugate. Au nanoparticles have received significant attention due to their clinical tolerance and ease of derivatization using standard thiolate coupling chemistry.[12]

Mitsunobu coupling of **5** with thioacetic acid gave **12** without incident which underwent reduction to **13**, allowing for the Au derivative **14** to be produced via the Brust two-phase method (Scheme 4).[13] Compound **14** was evaluated using transmission electron microscopy, which revealed a particle size range of 3–5 nm (Fig. 3), and fidelity of the conjugate was further supported by NMR spectroscopy. Preliminary analysis suggests **14** to be viable in photo-Bergman cycloaromatization chemistry,[14] and we are currently investigating the potential for mixed thiol adducts (including thiol linked PEG-mAb conjugates), where the degree of enediyne loading can be modulated.[15]

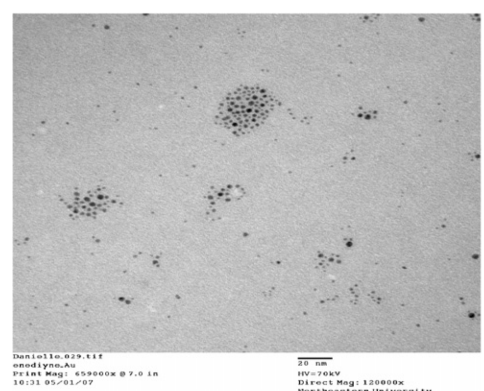
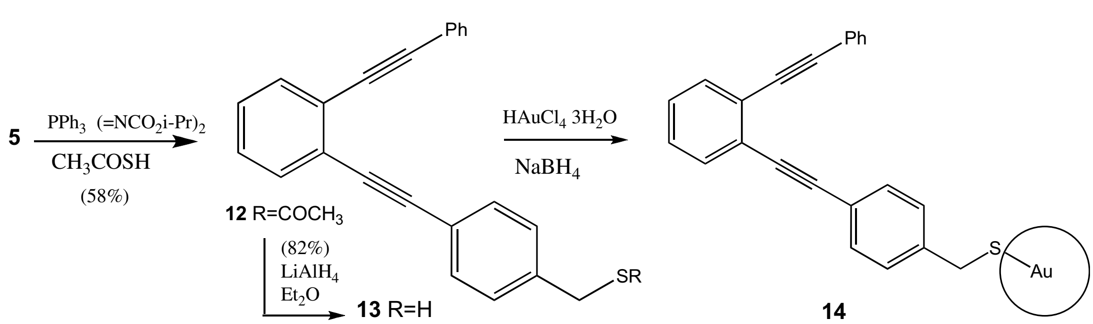


Figure 3. TEM image of gold nanoparticles **14** (*y* axis = absorbance *x* axis = wavelength (nm)).



Scheme 4. Preparation of Au modified nanoparticle–enediyne conjugate.

In summary, simple readily available linear [aryl] enediynes can be prepared and converted to versatile and thermally stable bioconjugates. Subsequent photoactivation of the systems is equally effective in parent and (bio)conjugated state, thus allowing the development of targeted cytotoxins and biochemical reagents. The scope and in vitro applications of this technology will be reported in due course.

Acknowledgments

We thank the NIH (RO1GM57123) and PRF (33920-AC1) for financial support of this work and Dmitriy Mongayt for performing ELISAs on **7** and **9**.

References and notes

1. Xi, Z.; Goldberg, I. H. DNA damaging enediyne compounds. In *Comprehensive Natural Products Chemistry*; Barton, D. H. R., Nakanishi, K., Eds.; Pergamon: Oxford, 1999; 7, p 553.
2. Hamann, P. R.; Hinman, L. M.; Beyer, C. F.; Lindh, D.; Upeslacis, J.; Flowers, D. A.; Bernstein, I. *Bioconjug. Chem.* **2002**, *13*, 40.
3. Jones, G. B.; Fouad, F. S. *Curr. Pharm. Des.* **2002**, *8*, 2415.
4. Turro, N. J.; Evenzahav, A.; Nicolaou, K. C. *Tetrahedron Lett.* **1994**, *35*, 8089.
5. (a) Funk, R. L.; Young, E. R. R.; Williams, R. M.; Flanagan, M. F.; Cecil, T. L. *J. Am. Chem. Soc.* **1996**, *118*, 3291; (b) Jones, G. B.; Wright, J. W.; Plourde, G.; Purohit, A. D.; Wyatt, J. K.; Hynd, G.; Fouad, F. *J. Am. Chem. Soc.* **2000**, *122*, 9872; (c) Choy, N.; Blanko, B.; Wen, J.; Krishnan, A.; Russell, K. C. *Org. Lett.* **2000**, *2*, 3761; (d) Alabugin, I. V.; Kovalenko, S. V. *J. Am. Chem. Soc.* **2002**, *124*, 9052.
6. All new compounds were characterized by appropriate spectroscopic and analytical methods.
7. For overview see Torchilin, V. P. *CHEMTECH* **1999**, *29*, 27.
8. Torchilin, V. P.; Lukyanov, A. N.; Gao, Z.; Papahadjopoulos-Sternberg, B. *Proc. Nat. Acad. Sci. U.S.A.* **2003**, *100*, 6039.
9. Li, J.; Crasto, C. F.; Weinberg, J.; Amiji, M.; Shenoy, D.; Sridhar, S.; Bubley, G.; Jones, G. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5558.
10. σX174 RfI DNA (50 μM in base pairs) was irradiated (Hanovia 450W medium pressure Hg lamp) in Tris–HCl at pH 8.5 as control or with **6** at 10^{-6} M for 3 h at 37 °C. Crude mixtures were subjected to gel electrophoresis (10% agarose), stained with ethidium bromide, and then subjected to scanning densitometry. Correcting for control photodegradation, **6** largely induced conversion to type II DNA (24%), with the onset of minimal conversion to type III based on random single-stranded cutting events. Full details, including competition experiments with DNA binders, will be reported in due course.
11. In addition to DNA damage induced by *p*-benzyne diradicals, the possibility for damage via a C1–C5 cyclization pathway and through photoinduced electron transfer also exists for enediynes of this class. See: Breiner, B.; Schlatterer, J. C.; Kovalenko, S. V.; Greenbaum, N. L.; Alabugin, I. V. *Angew. Chem. Int. Ed.* **2006**, *45*, 3666.
12. (a) Jackson, A. M.; Hu, Y.; Silva, P. J.; Stellacci, F. *J. Am. Chem. Soc.* **2006**, *128*, 11135; (b) Love, J. C.; Estroff, L. A.; Kriebel, J. K.; Nuzzo, R. G.; Whitesides, G. M. *Chem. Rev.* **2005**, *105*, 1103.
13. Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D.; Whyman, R. *J. Chem. Soc. Chem. Commun.* **1994**, 801.
14. Photoirradiation (3 h, 37 °C) followed by oxidative cleavage [Huang, J.; Hemminger, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 3342] confirmed formation of arene photo-Bergman adducts by GC–MS.
15. Chen, S.; Murray, R. W. *Langmuir* **1999**, *15*, 682.