Pergamon

Tetrahedron Letters 41 (2000) 4287–4290

TETRAHEDRON LETTERS

Design and synthesis of a novel photoaffinity taxoid as a potential probe for the study of paclitaxel–microtubules interactions

Songnian Lin,[a] Kan Fang,[b] Masaru Hashimoto,[b] Koji Nakanishi[b] and Iwao Ojima[a,*]

[a]*Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, USA*
[b]*Department of Chemistry, Columbia University, New York, NY 10027, USA*

Received 17 February 2000; revised 4 April 2000; accepted 5 April 2000

Abstract

A novel and non-radioactive bifunctional photoaffinity probe (BPP) for the study of paclitaxel–microtubules interactions is designed and synthesized. This new BPP-taxoid bears a 3-nitro-5-trifluoromethyldiazirinyl-phenoxyacetyl group at C-3′N for photoaffinity/photocleavage and a biotin subunit at C-7 for affinity chromatography.

Keywords: taxoid; bifunctional probe; photoaffinity label; biotin; diazirine.

Taxol® (paclitaxel) and taxotère® (docetaxel), are extremely important new chemotherapeutic agents for the treatment of various cancers.[1–4] Since the first characterization of paclitaxel in 1971,[5] its complex structure, unique mechanism of action,[6] and potent antineoplastic activity[7,8] have served as the impetus for intensive studies not only in clinical oncology, but also in the wide range of biomedical research arena. Searching for the three-dimensional biologically active structures (binding structures) of paclitaxel has been one of the active directions along this line.[9] Clarifying the binding site of paclitaxel at microtubules will provide a full understanding of paclitaxel–microtubule interactions when paclitaxel binds to microtubules, and develop a rational basis for improving drug design. Although X-ray analysis or electron crystallography may eventually provide this information,[10] photoaffinity labeling and protein sequencing can provide direct information about the structure of the drug binding site, and considerable progress has been made in this area.[11,12] Recently, a new and efficient photoaffinity labeling protocol for ligand/receptor interaction studies has been developed in these laboratories.[13] We describe here the design and synthesis of a novel photoreactive taxoid as the probe for paclitaxel–microtubule interactions applying the new protocol.

* Corresponding author. Tel: 1-631-632-7890; fax: 1-631-632-7942; e-mail: iojima@notes.cc.sunysb.edu

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00627-4

As Fig. 1 shows, a bifuntional photoaffinity probe (BPP) with a photoaffinity label (site A) and a photocleavable moiety (site B) can be used to streamline the frequently tedious photoaffinity labeling process. The new process will include: (i) the BPP-taxoid is bound to the receptor (microtubules) and photolyzed; (ii) the receptor is cleaved enzymatically or chemically; (iii) the crosslinked peptide fragments (with biotin tag) are separated from non-crosslinked ones; (iv) taxoid is detached from the crosslinked peptides by site B photocleavage; (v) the mixture of peptides with nitrophenolic marker is sequenced by tandem MS. Tandem MS should be able to directly sequence the respective peptide fragment at femtomole level without separation of mixture. Moreover, no radioisotope is required.

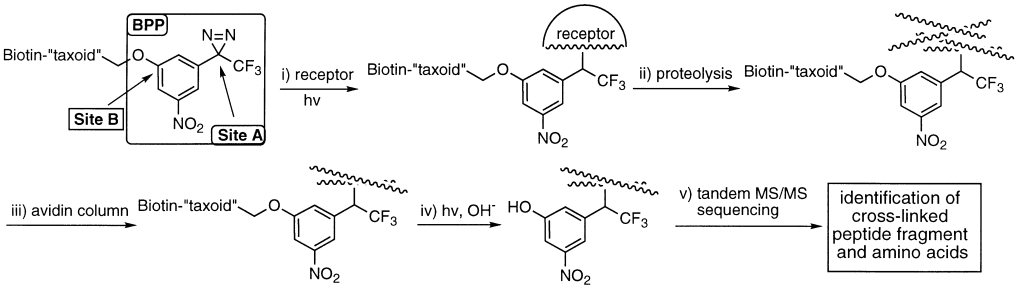


Figure 1. Proposed BPP protocol

In order to assess the efficacy of the proposed BPP protocol, a novel, bifunctional photoaffinity probe of paclitaxel with a biotin tag (**1**, Fig. 2) was designed. The BPP moiety for photoaffinity/photocleavage, 3-nitro-5-trifluoromethyldiazirinyl-phenoxyacetyl group, is connected to the C-3′ position, and the biotin tag for affinity chromatography, is attached to the C-7 position. BPP-taxoid **1** was synthesized through the following three steps: (a) LiHMDS-mediated coupling of modified baccatin III **4** with β-lactam **3**; (b) attachment of biotin tether (**9**) to the C-7 position of taxoid **6** by a succinyl linkage, and (c) connecting the BPP moiety **14** to the C-3′N position of taxoid **12**.

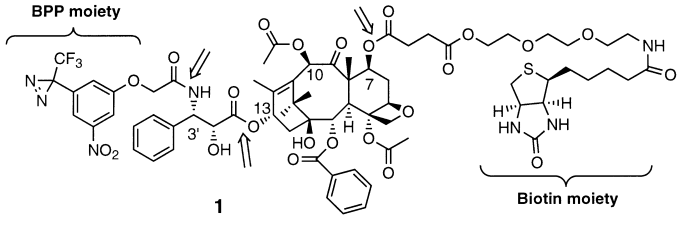
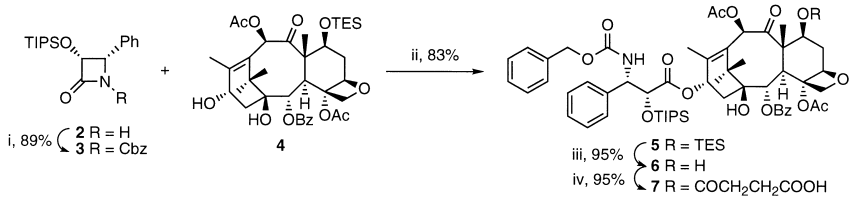


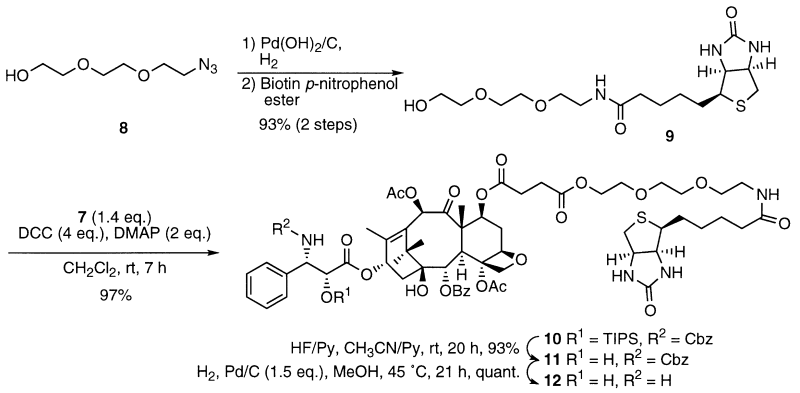
Figure 2. Taxoid photoaffinity labeling probe **1**

(3*R*,4*S*)-3-TIPSO-4-phenyl-β-lactam **5** with 96% ee was prepared in excellent yield through a highly efficient chiral ester enolate–imine cyclocondensation reaction.[14–16] Protection of the *N*-H group with Cbz-Cl gave *N*-Cbz-β-lactam **3**. Taxoid **5** was then synthesized using the ring-opening coupling protocol developed in these laboratories.[1,11,14–18] Thus, the couplings of 7-TES-baccatin (**4**)[19] with β-lactam **3** was carried out to afford taxoid **5**. 7-TES group was selectively removed by 0.1N HCl/EtOH to give 7-OH-taxoid **6**. Treatment of **6** with succinic anhydride in the presence of Et_3N and DMAP afforded taxoid-acid **7** bearing a monosuccinic acid moiety at the C-7 position (Scheme 1).



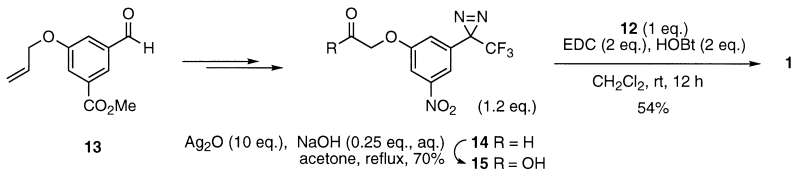
Scheme 1. Synthesis of taxoid-acid **7**. *Reaction conditions*: (i) Cbz-Cl (4 equiv.), Et_3N/DMAP, CH_2Cl_2, reflux, 20 h; (ii) LiHMDS (1.5 equiv.), THF, –40 to –30°C, 30 min; (iii) HCl (0.1N), EtOH, rt, 24 h; (iv) succinic anhydride (10 equiv.), Et_3N (excess), DMAP (6 equiv.), CH_2Cl_2, reflux 5 h

Alcohol **9** was prepared in two steps from 2-[(2-azidoethoxy)ethoxy]ethanol and biotin *p*-nitrophenol ester,[20] which was smoothly coupled with taxoid acid **7** using DCC/DMAP to afford taxoid **10** with a biotin tag. It was found that the Cbz group at the C-3′N position cannot be removed by hydrogenolysis in the presence of the TIPS group at the C-2′ position. Thus, TIPS group was removed first. Hydrogenolysis of the resulting C-2′-OH taxoid **11** on Pd–C afforded taxoid-amine **13**, which is ready for coupling with the BPP moiety (Scheme 2).



Scheme 2. Synthesis of taxoid-amine **12**

BPP-aldehyde **14** was synthesized from 3-allyloxy-5-methyloxycarbonylbenzaldehyde (**13**) using the method reported previously.[13] Oxidation of aldehyde **14** with Ag_2O provided BPP-acid **15**. The coupling of BPP-acid **15** with taxoid-amine **12** using EDC/HOBt afforded desired BPP-taxoid **1**[21] bearing a biotin tag at C-7 (Scheme 3).



Scheme 3. Final synthesis of taxoid probe **1**

In conclusion, we have designed and synthesized a new bifunctional photoaffinity probe of paclitaxel for the study of paclitaxel–microtubules interactions based on a non-radioactive

photoaffinity labeling protocol. The results of photoaffinity experiments with BPP-taxoid probe **1** will be reported in due course.

Acknowledgements

This research was supported by grants from the NIH (NIGMS, to I.O. and AI10187, to K.N.). Generous support from Indena, SpA, Italy is also gratefully acknowledged.

References

1. Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol®: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995; pp. 317–375.
2. Georg, G. I.; Chen, T. T.; Ojima, I.; Vyas, D. M. *Taxane Anticancer Agents: Basic Science and Current Status*; American Chemical Society: Washington DC, 1995.
3. Guénard, D.; Guéritte-Vogelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160–167.
4. *Taxol®: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995.
5. Wani, M. C.; Taylor, H. L.; Wall, M. E.; Coggon, P.; McPhail, A. T. *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
6. Schiff, P. B.; Fant, J.; Horwitz, S. B. *Nature* **1979**, *277*, 665–667.
7. Arbuck, S. G.; Blaylock, B. A. In *Taxol®: Science and Applications*; Suffness, M., Ed.; CRC Press: Boca Raton, 1995; pp. 379–415.
8. Rowinsky, E. K.; Onetto, N.; Canetta, R. M.; Arbuck, S. G. *Semin. Oncol.* **1992**, *19*, 646–662.
9. Ojima, I.; Chakravarty, S.; Inoue, T.; Lin, S.; He, L.; Horwitz, S. B.; Kuduk, S. D.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4256–4261 and references cited therein.
10. Nogales, E.; Wolf, S. G.; Downing, K. H. *Nature* **1998**, *391*, 199–203.
11. Ojima, I.; Kuduk, S. D.; Chakravarty, S. In *Adv. Med. Chem.*; Maryanoff, B. E.; Reitz, A. B., Ed.; JAI Press: Greenwich, CT, 1998; Vol. 4; pp. 69–124 and references cited therein.
12. Rao, S.; He, L.; Chakravarty, S.; Ojima, I.; Orr, G. A.; Horwitz, S. B. *J. Biol. Chem.* **1999**, *274*, 37990–37994.
13. Fang, K.; Hashimoto, M.; Jockusch, S.; Turro, N. J.; Nakanishi, K. *J. Am. Chem. Soc.* **1998**, *120*, 8543–8544.
14. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C.-M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985–7012.
15. Ojima, I.; Lin, S.; Chakravarty, S.; Fenoglio, I.; Park, Y. H.; Sun, C.; Appendino, G.; Pera, P.; Veith, J. M.; Bernacki, R. J. *J. Org. Chem.* **1998**, *63*, 1637–1645.
16. Ojima, I.; Lin, S.; Wang, T. *Curr. Med. Chem.* **1999**, *6*, 927–954.
17. Ojima, I.; Lin, S. *J. Org. Chem.* **1998**, *63*, 224–225.
18. Holton, R. A.; Biediger, R. J.; Boatman, P. D. In *Taxol®: Science and Applications*; Suffness, M., Ed.; CRC Press: New York, 1995; pp. 97–121.
19. Denis, J.-N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 5917–5919.
20. Hashimoto, M.; Liu, Y.; Fang, K.; Li, H. Y.; Campiani, G.; Nakanishi, K. *Bioorg. Med. Chem.* **1999**, *7*, 1181–1194.
21. Mp 112–124°C; $[\alpha]_D^{20}$ –13.5 (*c* 0.52, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.17 (s, 3H), 1.45 (m, 2H), 1.67 (m, 4H), 1.80 (s, 3H), 1.85 (s, 3H), 1.86 (m, 1H), 2.16 (s, 3H), 2.20 (m, 2H), 2.25 (m, 2H), 2.31 (s, 3H), 2.50 (m, 1H), 2.60 (m, 4H), 2.72 (m, 1H), 2.90 (dd, J = 12.5, 5.0 Hz, 1H), 3.15 (m, 1H), 3.46 (m, 2H), 3.57–3.75 (m, 8H), 3.85 (d, J = 6.5 Hz, 1H), 4.16 (d, J = 8.5 Hz, 1H), 4.29 (d, J = 8.5 Hz, 1H), 4.36 (dd, J = 8.0, 5.0 Hz, 1H), 4.41 (m, 1H), 4.57 (dd, J = 7.5, 5.0 Hz, 1H), 4.63 (d, J = 14.5 Hz, 1H), 4.69 (d, J = 14.5 Hz, 1H), 4.74 (d, J = 3.5 Hz, 1H), 4.91 (d, J = 10.0 Hz, 1H), 5.56 (dd, J = 10.0, 7.0 Hz, 1H), 5.61 (dd, J = 8.5, 3.0 Hz, 1H), 5.65 (d, J = 6.5 Hz, 1H), 6.06 (t, J = 9.0 Hz, 1H), 6.16 (s, 1H), 6.81 (bs, 1H), 7.08 (s, 1H), 7.29 (t, J = 7.0 Hz, 1H), 7.37 (t, J = 7.5 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.49 (t, J = 7.0 Hz, 2H), 7.63 (t, J = 7.5 Hz, 1H), 7.68 (s, 1H), 7.86 (t, J = 2.0 Hz, 1H), 8.06 (d, J = 7.5 Hz, 2H), 8.30 (d, J = 9.0 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ –65.51.; HRMS (FAB) *m*/*z* calcd for $C_{70}H_{82}N_7O_{24}F_3S{\cdot}H^+$: 1494.5162. Found: 1494.5177 (Δ = –1.0 ppm).