Pergamon

Tetrahedron Letters 41 (2000) 9015–9019

TETRAHEDRON LETTERS

Direct, efficient and selective tritiations of paclitaxel and photoaffinity taxoids

Arthur Y. L. Shu* and J. Richard Heys

Radiochemistry Section, Department of Synthetic Chemistry, SmithKline Beecham Pharmaceuticals, PO Box 1539, King of Prussia, PA 19406, USA

Received 12 September 2000; revised 20 September 2000; accepted 21 September 2000

Abstract

Radiolabeled paclitaxel and three photophore-bearing derivatives were prepared by organoiridium-mediated direct tritium exchange under both catalytic and stoichiometric conditions. The resulting tritiated taxoids had specific activities ranging from 53 to 195 Ci/mmol. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: organoiridum-mediated tritium exchange; paclitaxel; photoaffinity taxoids; Crabtree's catalyst.

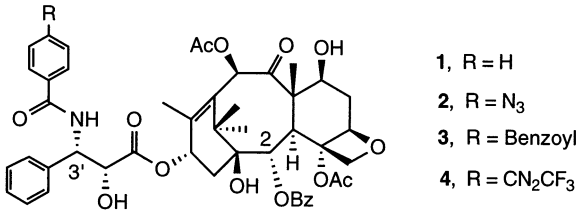
Azidophenyl, benzoylphenyl and diazirenyl groups are among the most common examples of photophores used in photoaffinity labeling; used in tagged form, usually with a radiolabel, they are powerful tools for the identification of cognate macromolecules.[1] Although the incorporation of photophores into ligands of interest can be achieved by straightforward chemical means,[2] preparations of their radiolabeled counterparts, especially in high specific activities, can be challenging.

Our laboratory has been engaged in labeling aromatic molecules using organoiridium-mediated exchange in the presence of isotopic hydrogen gas.[3] The advantage of this method is that it allows the direct utilization of substrates already available, without resorting to the separate and sometimes elaborate synthesis of precursors, such as unsaturated or multi-halogenated derivatives. In addition, high levels of isotopic content can often be realized even when only a *limited* amount of deuterium or tritium gas is utilized. This methodology requires the aromatic substrate to possess a functionality capable of coordinating with the iridium center, which directs the substrate's *ortho* hydrogens to undergo exchange through a metallacycle intermediate. Our aim was to extend this approach to labeling photophore substructures of complex ligands. We selected paclitaxel and several photoreactive derivatives as test platforms for testing the organoiridium-mediated direct tritium exchange.

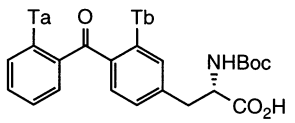
* Corresponding author. Tel: (610)270-4373; fax: (610)270-4110; e-mail: art_shu-1@sbphrd.com

0040-4039/00/$ - see front matter © 2000 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(00)01652-X

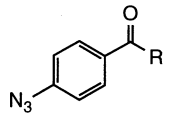
The mechanism of action of paclitaxel (**1**)[4] was in part elucidated with the use of radiolabeled photoaffinity derivatives **2** and **3**, with azido and benzoyl groups attached to the *para* position of the sidechain C-3′ benzamide, respectively;[5] other paclitaxel derived photoaffinity analogs in tritiated forms have since emerged.[6] These were prepared either by multistep sequences involving handling of radioactive intermediates or separate syntheses of unsaturated precursors prior to tritiation. The iridium-mediated isotopic exchange could lend itself to tritiating these analogs directly, providing the stabilities and directing abilities of various photoactivating groups could be calibrated, and the complex core structure of paclitaxel could remain intact under the exchange conditions.



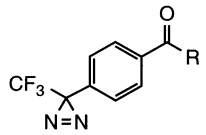
1, R = H
2, R = N_3
3, R = Benzoyl
4, R = CN_2CF_3



5



7a, R = $N(CH_2CH_2)_2$
7b, R = $ON(COCH_2)_2$



8a, R = $N(CH_2CH_2)_2$
8b, R = $ON(COCH_2)_2$

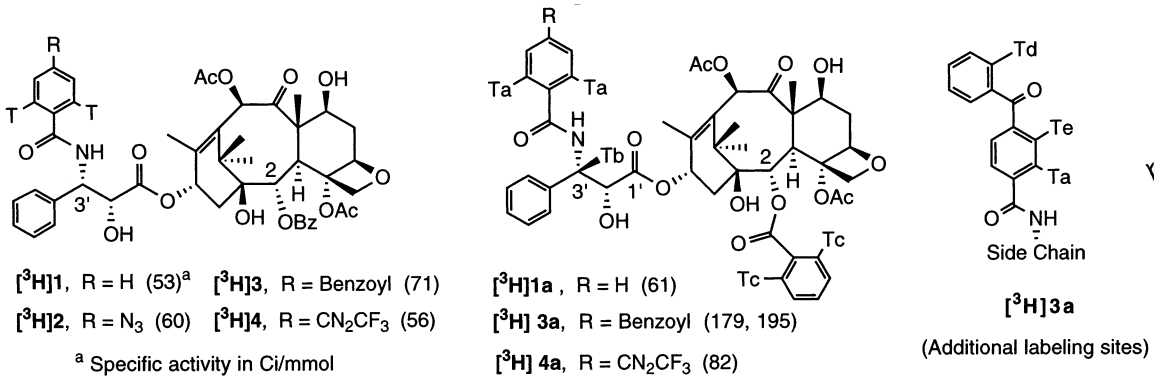
Our previous experience with benzophenones indicated that the benzoylphenyl moiety is stable to the exchange process and is a good directing group.[7] In a model study, treatment of a solution of *N*-boc-4-benzoyl-L-phenylalanine (**5**) in CH_2Cl_2 with $[(COD)Ir(PPh_3)_2]BF_4$ (**6**, 0.3 equiv.) and 1.8 Ci of carrier-free T_2 (specific activity 29 Ci/milliatom T) for 18 h resulted in [^{3}**H**]**5** with a specific activity of 46 Ci/mmol and tritium distribution ratio at Ta:Tb of 1:1.5.[8]

The azido group was unstable to the exchange conditions and devoid of directing ability. However, in the presence of a catalytic amount of iridium complex and a strong directing group such as an amide, exchange adjacent to the latter function can be successful. For example, although the activated ester **7b** was not labeled under a variety of conditions, exposure of pyrrolidine 4-azidobenzamide (**7a**) to 0.2 equiv. of **6** and an excess of D_2 for 18 h afforded 63% of **7a** with the positions *ortho* to the amide fully deuterated.

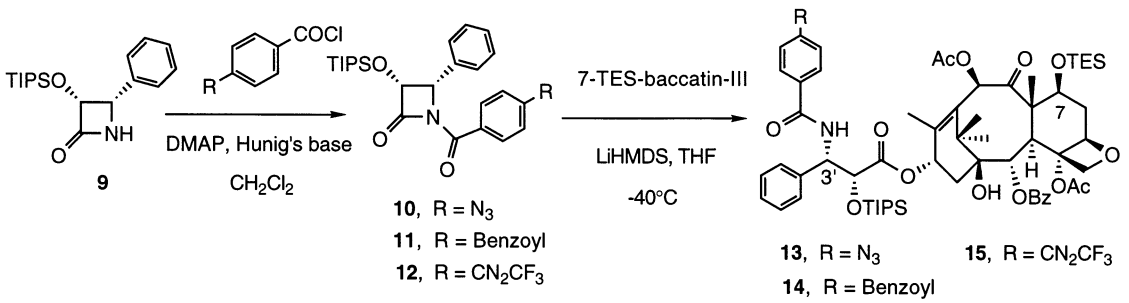
Likewise, the diazirenyl group was compatible with the exchange conditions, but had no directing ability. When pyrrolidine 4-diazirenylbenzamide (**8a**) was exposed to either stoichiometric or catalytic amounts (0.2 equiv.) of complex **6** and an excess of D_2, the exchanged product was recovered in high yields. Only the positions *ortho* to the aminocarbonyl function were fully deuterated. Similar to **7b**, succinyl 4-diazirenylbenzoate (**8b**) failed to incorporate any deuterium.

With some experimental parameters defined for these three photoactivating groups, we turned our attention to the feasibility of performing direct isotopic exchange on paclitaxel. When **1** (2 mg) was treated with complex **6** (0.3 equiv.) in the presence of 4 Ci of carrier-free T_2 for 18 h, the purified [^{3}**H**]**1** had specific activity of 53 Ci/mmol with tritiation occurring *exclusively* at the *ortho* positions of the sidechain C-3′ benzamide.[9] Various ways of making [^{3}**H**]**1** have previously

been reported,[10] but none provided such a high level of label incorporation in a single step. In agreement with the results from our model studies described above and our previous findings,[11] the sidechain C-3′ benzamide, being a stronger Lewis base than the C-2 benzoate, was the sole directing group under these catalytic conditions. Increasing the iridium complex loading to 1.5 equiv. and using Crabtree's catalyst[12] resulted in further tritiation of paclitaxel [^{3}H]**1a** at the *ortho* positions (Tc) in the C-2 benzoate ring as well as sidechain C-3′ benzylic site (Tb) in the ratio of 78:7:15 for Ta:Tb:Tc, respectively. The specific activity was raised to 61 Ci/mmol.



To access photophore-bearing derivatives **2**–**4**, we chose Ojima's chiral β-lactam semisynthetic approach.[13] (–)-(3*R*,4*S*)-β-lactam **9** was acylated with the corresponding *para* substituted benzoyl chlorides to 1-acyl-β-lactams **10**–**12**. These β-lactams, having the C-3 hydroxyl protected as the TIPS ether, were coupled directly with 7-TES-baccatin-III using LiHMDS in THF to provide doubly silylated taxoids **13**–**15**.[14] Desilylation of **13**–**15** in the presence of HF/pyridine in a mixture of pyridine and acetonitrile gave the desired substrates **2**–**4** (Scheme 1).



Scheme 1.

Each of the photoaffinity analogs **2**–**4** was subjected to the catalytic organoiridium-mediated tritium exchange conditions described above for parent paclitaxel, except for replacement of complex **6** with Crabtree's catalyst. The resulting products all had tritium incorporated at the *ortho* positions of the sidechain C-3′ benzamide. Additional tritium was observed in [^{3}H]**3** (23% of total) at the combined sites Td and Te that are *ortho* to the benzophenone carbonyl, reflecting its intermediate directing ability between the C-3′ benzamide and the C-2 benzoate. The specific activities of [^{3}H]**2**–**4** ranged from 56 to 71 Ci/mmol.

Taxoids **3** and **4**, having photophores that are compatible with the exchange conditions, were additionally exposed to high-loading iridium complex tritiation (3 and 1.5 equiv., respectively).

The resulting exchanged products exhibited similar labeling patterns as [^{3}H]**1a**. Diazirene-bearing analog [^{3}H]**4a** was labeled at the sites Ta:Tb:Tc in the ratio of 67.8:3.5:28.7 with a specific activity of 82 Ci/mmol. The isotopic distribution for [^{3}H]**3a** was more complex. Substantial tritium buildup occurred at the benzophenone–carbonyl directed sites Td and Te, resulting in the ratio of 28:3:13:30:26 for Ta:Tb:Tc:Td:Te. When the amount of iridium complex was elevated further to 5 equiv., a noticeable increase of tritium content took place proportionally at sites Tb and Tc,[15] with a ratio of 26(Ta):4(Tb):18(Tc):27(Td):25(Te). The specific activity was raised from 179 to 195 Ci/mmol.

In summary, organoiridium-mediated direct isotope exchange is a powerful methodology for labeling compounds with complex structures regioselectively and to high specific activity, as demonstrated in the one-step tritiations of paclitaxel and related photoaffinity analogs.

Acknowledgements

We thank S. H. Levinson, A. L. Freyer and F. G. Vogt for ^{3}H NMR measurements, L. B. Killmer for MS measurements, and C. R. Newsome for chiral HPLC assays on β-lactam intermediates.

References

1. Bayley, H. *Photogenerated Reagents in Biochemistry and Molecular Biology*; Elsevier: Amsterdam, 1983.
2. Fleming, S. A. *Tetrahedron* **1995**, *51*, 12479–12520.
3. Shu, A. Y. L.; Saunders, D.; Levinson, S. H.; Landvatter, S. W.; Mahoney, A.; Senderoff, S. G.; Mack, J. F.; Heys, J. R. *J. Lab. Comp. Radiopharm.* **1999**, *42*, 797–807.
4. Ojima, I.; Kuduk, S. D.; Chakravarty, S. *Adv. Med. Chem.* **1999**, *4*, 69–124.
5. Swindell, C. S.; Heerding, J. M.; Krauss, N. E.; Horwitz, S. B.; Rao, S. *J. Med. Chem.* **1994**, *37*, 1446–1449.
6. (a) Rimoldi, J. M.; Kingston, D. G. I.; Chaudhary, A. G.; Samaranayake, G.; Grover, S.; Hamel, E. *J. Nat. Prod.* **1993**, *56*, 1313–1330. (b) Dasgupta, D.; Park, H.; Harriman, G. C. B.; Georg, G. I.; Himes, R. H. *J. Med. Chem.* **1994**, *37*, 2976–2980. (c) Ojima, I.; Duclos, O.; Dorman, G.; Simonot, B.; Prestwich, G. D. *J. Med. Chem.* **1995**, *38*, 3891–3894. (d) Rao, S.; Orr, G. A.; Chaudhary, A. G.; Kingston, D. G. I.; Horwitz, D. B. *J. Biol. Chem.* **1995**, *270*, 20235–20238. (e) Wu, Q.; Bounaud, P.-Y.; Kuduk, S. D.; Yang, C.-P. H.; Ojima, I.; Horwitz, S. B.; Orr, G. A. *Biochemistry* **1998**, *37*, 11272–11279. (f) Ojima, I.; Bounaud, P.-Y.; Ahern, D. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1189–1194.
7. Heys, J. R.; Shu, A. Y. L.; Nice, L. E. In *Synthesis and Applications of Isotopically Labelled Compounds*; Allen, J.; Voges, R., Eds.; Wiley: West Sussex, 1995; pp. 175–180.
8. This tritiated compound [^{3}H]**5** was made previously via a multistep sequence. Dorman, G.; Olszewski, J. D.; Prestwich, G. D.; Hong, Y.; Ahern, D. G. *J. Org. Chem.* **1995**, *60*, 2292–2297.
9. General tritiation procedure: paclitaxel or derivatives (2 mg) and iridium complex (0.3 or 1.5 equiv.) in 1 mL of CH_2Cl_2 were exposed to 3.5–4 Ci of carrier-free tritium gas in a stainless steel manifold. The mixtures were stirred for 18 h (low catalyst loading) or 8 h (high catalyst loading). After removal of excess tritium gas, CH_2Cl_2, and tritium labiles (by static vacuum distillation with repeated additions of MeOH), the crude products (91–334 mCi) were purified by semi-preparative HPLC on octadecyl stationary phase eluted with water/MeCN/MeOH. The resulting products (yields: 40–116 mCi) had radiochemical purities of 96–98% by HPLC-radiodetection. Specific activities were determined by MS in chemical ionization (negative ion) mode using NH_3 as the reagent gas. ^{3}H NMR were measured in $CDCl_3$ to determine location of label. Assignments were made by independent 2D experiments (Vogt, F. G. et al., manuscript in preparation) and in reference to Kingston, D. G. I.; Molinero, A. A.; Rimoldi, J. M. In *The Taxane Diterpenoids* (*Progress in the Chemistry of Natural Products*); Herz, W.; Kirby, G. W.; Moore, R. E.; Steglich, W.; Tamm, C., Eds.; Springer-Verlag: Wien, New York, 1993; Vol. 61, pp. 37–39.

10. (a) Parness, J.; Horwitz, S. B. *J. Cell. Biol*. **1981**, *91*, 479–487. (b) Chenu, J.; Takoudju, M.; Wright, M.; Senilh, V.; Guenard, D. *J. Lab. Comp. Radiopharm*. **1987**, *24*, 1245–1255. (c) Taylor, G. F.; Thornton, S. S.; Tallent, C. R.; Kepler, J. A. *J. Lab. Comp. Radiopharm*. **1993**, *33*, 501–515.
11. Heys, J. R. *J. Chem. Soc., Chem. Commun*. **1992**, 680–681.
12. Since the activities of $[(COD)Ir(PPh_3)_2]BF_4$ and Crabtree's catalyst[16] ($[(COD)Ir(py)PCy_3]PF_6$) for catalysis of hydrogen isotope exchange with the present substrates were similar, the latter complex (available commercially) was used.
13. Ojima, I.; Habus, I.; Zhao, M.; Zucco, M.; Park, Y. H.; Sun, C. M.; Brigaud, T. *Tetrahedron* **1992**, *48*, 6985–7012.
14. Ojima, I.; Kuduk, S. D.; Slater, J. C.; Gimi, R. H.; Sun, C. M. *Tetrahedron* **1996**, *52*, 209–224.
15. This similarity between sites Tb and Tc suggested the unusual exchange at the former position might also be directed by an ester, possibly the sidechain C-1′ ester group. Also noticeable was the trend that tritium content at site Tb decreased against the electron-withdrawing power of the *para*-substituents at the C-3′ benzamide.
16. Suggs, J. W.; Cox, S. D.; Crabtree, R. H.; Quirk, J. M. *Tetrahedron Lett*. **1981**, *22*, 303–306.