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J. Nat. Prod., **1993**, 56 (8), 1313-1330• DOI: 10.1021/np50098a016 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 8, pp. 1313-1330, August 1993

MODIFIED TAXOLS, 9.[1] SYNTHESIS AND BIOLOGICAL EVALUATION OF 7-SUBSTITUTED PHOTOAFFINITY ANALOGUES OF TAXOL[2]

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ABSTRACT.—The 7-substituted taxol analogues **7**, **19**, **27**, and **32** have been prepared as potential photoaffinity-labeled derivatives for studies of the nature of the binding site of taxol on polymerized tubulin. The analogue **32** has been prepared in both deuterium- and tritium-labeled versions. Tubulin-assembly studies were carried out with these compounds, and it was found that they showed some but not all of the properties of taxol. We conclude that these specific taxol analogues labeled at the 7 position are not ideal derivatives for photoaffinity labeling studies.

The novel diterpenoid taxol [**1**] (1) is now well established as a clinically active anticancer agent, showing excellent activity against ovarian (2) and breast cancer (3). Both its chemistry (4) and clinical activity (5) have recently been reviewed. Taxol acts as an antimitotic agent, blocking cell replication in HeLa cells and fibroblast cells (6). It does this by promoting the assembly of microtubules from tubulin. The microtubules so formed are abnormal, being stable to depolymerization by calcium ion, cold, dilution, or microtubule disassembling drugs (7). It is thus believed that the ability of taxol to promote the assembly of tubulin is responsible for its anticancer activity.

The process by which taxol promotes the assembly of tubulin into microtubules is only partially understood. It is known that taxol binds to assembled microtubules reversibly, with a stoichiometry that is approximately one molecule of taxol per tubulin dimer (7). The binding site is also known to be on the polymerized tubulin rather than on the individual tubulins or tubulin dimers (7,8). The actual binding site for taxol on polymerized tubulin is, however, unknown, although it is known that the site does not overlap with the binding sites of other tubulin-binding compounds such as colchicine, podophyllotoxin, and vinblastine (9).

Because of the clinical importance of taxol, and also because of the difficulty of obtaining it from *Taxus brevifolia* or by partial synthesis from baccatin III, the development of improved analogues is a matter of urgent concern. One approach to this need is by understanding the nature of the taxol binding site on polymerized tubulin. If this can be successfully accomplished in sufficient detail to allow a three-dimensional map of the binding site to be developed, then it might prove possible to design specific taxol analogues that would fit the binding site and thus show significant biological activity.

Present knowledge of the taxol binding site on polymerized tubulin is severely limited by the fact that it has not so far proved possible to obtain an X-ray structure of tubulin or microtubules. Microtubule structure at 18 Å resolution has been determined

[1]For Part 8, see Samaranayake et al. (47).

[2]This manuscript is dedicated to the memory of Professor Edward Leete.

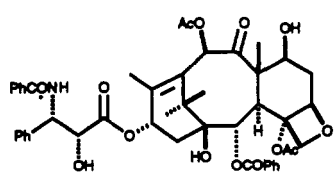
through X-ray fiber diffraction (10), but the resolution of this study is inadequate for detailed analysis of the binding sites.

In the absence of a direct physical method such as X-ray diffraction, the most viable approach to definition of the taxol binding site becomes that of photoaffinity labeling. Photoaffinity labeling has previously been applied to analysis of the interactions of tubulin with vinblastine (11), GTP (12–15), colchicine (16–20), and ATP and GTP derivatives (21–23). One study of direct photoaffinity labeling of tubulin by taxol has appeared (24), which indicated selective binding of taxol to β-tubulin. Since in this study taxol did not carry a specific photoaffinity label, the specific nature of the binding of irradiated drug to tubulin is unknown, and it may be partially random. A short communication describing the synthesis of a C-7 substituted azidonitrobenzoic acid analogue of taxol has also appeared (25). An approach using specifically labeled active taxol analogues should yield more detailed information about points of taxol-tubulin contact in the binding site(s) if reactive peptide sequences can be identified.

In the light of the preceding discussion, we elected to investigate the synthesis and tubulin binding characteristics of taxol carrying specific photoaffinity labels. In this paper we report the synthesis and biological activity of taxol with various photoaffinity labels at the 7 position.

RESULTS AND DISCUSSON

CHEMISTRY.—In the preparation of taxol analogues bearing photoreactive labels, two questions needed to be addressed; selection of the photoreactive label itself and the



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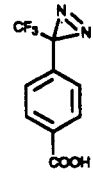

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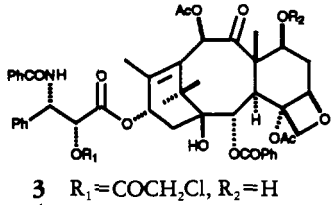
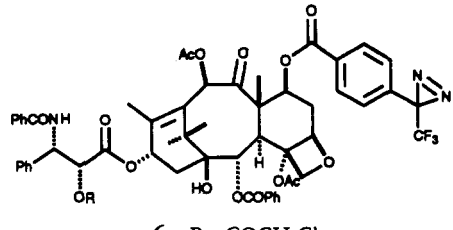


3 R_1=$COCH_2Cl$, R_2=H
4 R_1=$COCH_2Cl$, R_2=COPh
5 R_1=H, R_2=COPh



6 R=$COCH_2Cl$
7 R=H

selection of its site of attachment to taxol. The initial photoreactive label used by Chowdhry and Westheimer (26) was the α-diazo-β,β,β-trifluoropropionyl group, but this type of label has a major disadvantage in that it does not absorb light above 260 nm. Since proteins absorb strongly below 260 nm, it is almost impossible to get enough light into the drug-protein complex to enable photolysis to occur. Many workers have used aryl azides in an attempt to overcome this problem, and several examples cited earlier involved the use of aryl azides. However, aryl nitrenes generated from azides are less reactive than carbenes generated from the Westheimer regent, and C-H bond insertion contributes little to the overall labeling process (27,28).

Fortunately, a solution to this problem has emerged (29,30), describing use of the diazirine group as a photolabile carbene-generating reagent. The synthesis of 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid [**2**] has been described (31), and use of the diazirine photolabel gave excellent labeling of membrane proteins (32). Although the carbene insertion products from diazirine photolabels can undergo elimination reactions leading to loss of the labeled moiety (33), their use under controlled conditions promises to provide the best labeling of tubulin. Thus, we selected the aryl diazirine group as our photoreactive group.

The second question to be addressed is the position of attachment of the photolabel to taxol. In earlier work, we showed that taxol analogues carrying acetyl groups at C-7 retain most of the activity of taxol (34), and we thus elected to use the 7 position for attachment of our photoaffinity labels.

In order to test the effect of introducing an aroyl group at C-7, we first prepared 7-benzoyltaxol [**5**] by benzoylation of 2′-chloroacetyltaxol [**3**] to give 7-benzoyl-2′-chloroacetyltaxol [**4**], followed by deprotection with MeOH Si gel. The position of the benzoyl group in **5** was confirmed by a downfield shift of the C-7 proton to 5.72 ppm from 4.40 ppm in taxol (Table 1). The activity of **5** was determined in an assay similar to that first described by Lataste *et al.* (35). Briefly, microtubule protein was allowed to polymerize at 38° in the presence of GTP and various concentrations of drug, and the rate of depolymerization was determined after rapid cooling to 0°. In this initial assay the concentration of 7-benzoyltaxol required to reduce the depolymerization rate by 50% was twice that of taxol itself (IC_{50} value). Thus, 7-aroyl substituted taxol derivatives appeared to be promising as valid taxol analogues.

The photoaffinity-labeled taxol analogue 7-[4-(1-azi-2,2,2-trifluoroethyl)]benzoyltaxol [**7**] was prepared by coupling 2′-chloroacetyltaxol [**3**] to the azibenzoic acid **2** with dicyclohexylcarbodiimide and 4-pyrrolidinopyridine (DCC/PP), and deprotection of the resulting intermediate **6**. This analogue was about 30% as active as taxol in reducing the disassembly rate of polymerized microtubule protein.

Although **7** did stabilize microtubules, its synthesis did not lend itself to the preparation of isotopically labeled derivatives. Such a compound would greatly facilitate the isolation and sequencing of peptides and identification of reactive amino acid residues, which is required to define the taxol binding site. We thus elected to investigate the synthesis of additional photoreactive analogues that could be labeled conveniently with carbon-14 or tritium. The general approach adopted was to prepare a photoaffinity label consisting of a diazirinyl phenoxyacetic acid, with the expectation that the radiolabel could be readily incorporated into the acetate portion of the molecule.

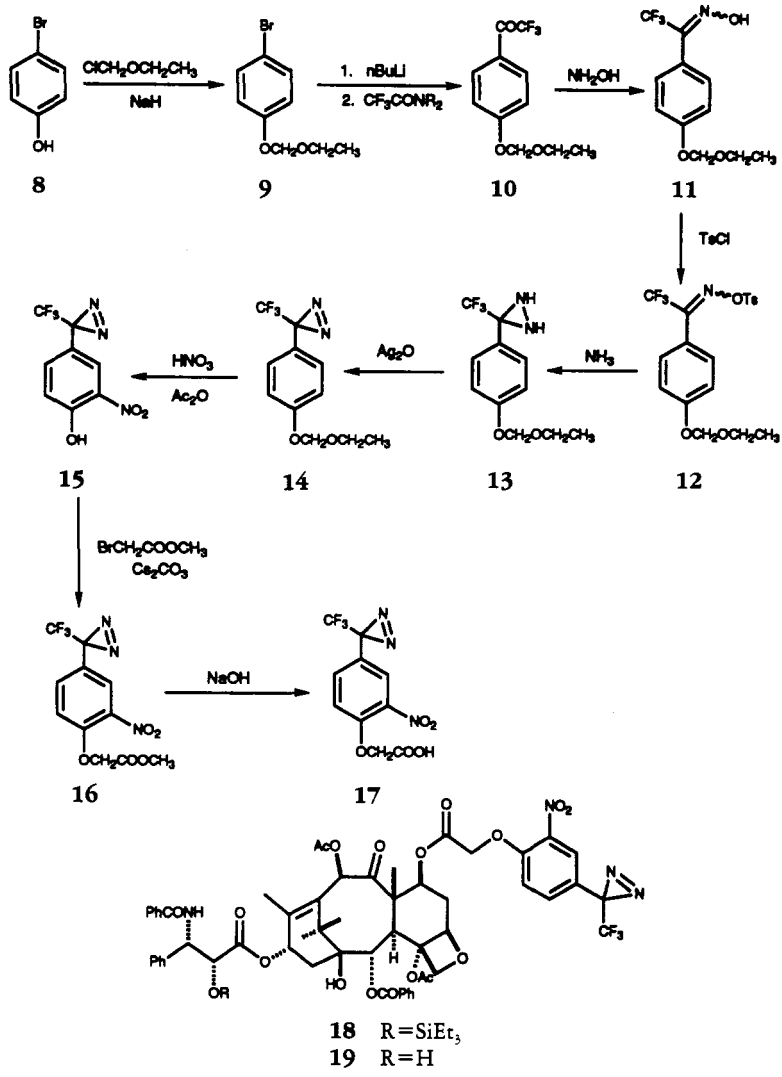
The first photoreactive compound that we prepared was 2-nitro-4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl] phenoxyacetic acid [**17**], prepared from *p*-bromophenol [**8**] via intermediates **9**–**16** by a route similar to that outlined by Kanaoka and his collaborators (36) (see Experimental section). The synthesis proceeded in fair overall yield, but the key alkylation step **15** ↦**16** could only be accomplished in good yield when an excess of methyl bromoacetate was used. Since our objective was to prepare

TABLE 1. ^{1}H-nmr Spectra of Taxol Analogues.[a,b]

Proton	Compound					
	1	5	7	19	27	32
H-2	5.67 (d, 7.1)	5.72 (d, 7.0)	5.73 (d, 6.8)	5.66 (d, 6.9)	5.66 (d, 6.8)	5.66 (d, 6.8)
H-3	3.79 (d, 7.0)	4.00 (d, 7.0)	4.00 (d, 6.8)	3.89 (d, 6.8)	3.92 (d, 6.8)	3.91 (d, 6.8)
H-5	4.94 (br d, 9.0)	5.00 (br, d, 8.8)	4.98 (br d, 8.5)	4.92 (br d, 8.7)	4.94 (br d, 8.4)	4.94 (br d, 8.5)
H-6α	2.54 (m)	2.77 (m)	2.76 (m)	2.67 (m)	2.65 (m)	2.65 (m)
H-6β	1.88 (m)	1.86 (m)	1.82 (m)	1.80 (m)	1.85 (m)	1.85 (m)
H-7	4.40 (m)	5.72 (m)	5.71 (m)	5.65 (m)	5.64 (m)	5.64 (m)
H-10	6.27 (s)	6.32 (s)	6.32 (s)	6.15 (s)	6.20 (s)	6.18 (s)
H-13	6.23 (br t)	6.17 (br t)	6.19 (br t)	6.17 (br t)	6.20 (br t)	6.18 (br t)
H-14α	2.35 (m)	2.32 (m)	2.35 (m)	2.36 (m)	2.35 (m)	2.35 (m)
H-14β	2.28 (m)	2.25 (m)	2.29 (m)	2.31 (m)	2.22 (m)	2.25 (m)
Me-16	1.14 (s)	1.17 (s)	1.18 (s)	1.10 (s)	1.11 (s)	1.16 (s)
Me-17	1.24 (s)	1.17 (s)	1.20 (s)	1.20 (s)	1.21 (s)	1.22 (s)
Me-18	1.79 (br s)	1.94 (s)	1.85 (s)	1.72 (s)	1.73 (s)	1.81 (s)
Me-19	1.68 (s)	1.94 (s)	1.92 (s)	1.75 (s)	1.73 (s)	1.81 (s)
H-20α	4.30 (d, 8.4)	4.31 (d, 8.4)	4.35 (d, 8.4)	4.30 (d, 8.5)	4.31 (d, 8.5)	4.33 (d, 8.5)
H-20β	4.19 (d, 8.5)	4.18 (d, 8.4)	4.23 (d, 8.4)	4.18 (d, 8.5)	4.17 (d, 8.5)	4.19 (d, 8.5)
H-2′	4.78 (dd, 5.4, 2.7)	4.80 (dd,4.9, 2.4)	4.81 (dd, 4.8, 2.5)	4.79 (dd, 5.0, 2.4)	4.80 (dd, 4.9, 2.3)	4.80 (dd, 4.8, 2.3)
H-3′	5.78 (dd, 8.9, 2.8)	5.80 (dd, 9.0, 2.4)	5.81 (dd, 9.0, 2.5)	5.80 (dd, 8.9, 2.4)	5.80 (dd, 8.9, 2.3)	5.80 (dd, 9.0, 2.3)
3′-NH	7.01 (d, 8.9)	7.02 (d, 9.0)	7.04 (d, 9.0)	7.07 (d, 8.9)	7.05 (d, 8.9)	7.05 (d, 9.0)
OBz (ortho)	8.13 (dd, 8.4, 1.3)	8.12 (dd, 8.5, 1.3)	8.13 (dd, 8.5, 1.4)	8.10 (dd, 8.5, 1.4)	8.10 (dd, 8.5, 1.3)	8.10 (dd, 8.6, 1.4)
NBz (ortho)	7.74 (dd, 8.3, 1.2)	7.75 (dd, 8.5, 1.3)	7.75 (dd, 8.2, 1.5)	7.75 (dd, 8.5, 1.5)	7.76 (dd, 8.5, 1.4)	7.77 (dd, 8.6, 1.3)
2′-OH	3.61 (d, 5.4)	3.60 (d, 4.9)	3.60 (d, 4.8)	3.70 (d, 5.0)	3.60 (d, 4.9)	3.59 (4.8)
4-OAc	2.38 (s)	2.40 (s)	2.40 (s)	2.36 (s)	2.37 (s)	2.37 (s)
10-OAc	2.23 (s)	2.14 (s)	2.00 (s)	2.19 (s)	2.17 (s)	2.18 (s)
7-CH_2OAr	—	—	—	4.90 (d, 16.25)	4.83 (d, 16.0)	4.88 (d, 16.2)
				4.75 (d, 16.25)	4.52 (d, 16.0)	4.52 (d, 16.2)
7-ArH	—	7.92 (d, 8.8)	7.93 (d, 8.7)	7.70 (d, 2.3)	6.95 (d, 8.8)	7.35 (dd, 8.8, 8.5)
			7.48 (d, 8.7)	7.32 (d, 8.9)	7.15 (d, 8.8)	6.98 (ddd, 8.8, 2.5, 0.7)
						6.84 (dd, 8.5, 0.7)
						6.75 (br s)

[a]Measured in $CDCl_3$. Chemical shifts (δ) are expressed in ppm from TMS and coupling constants (*J*) in Hz. Multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad.

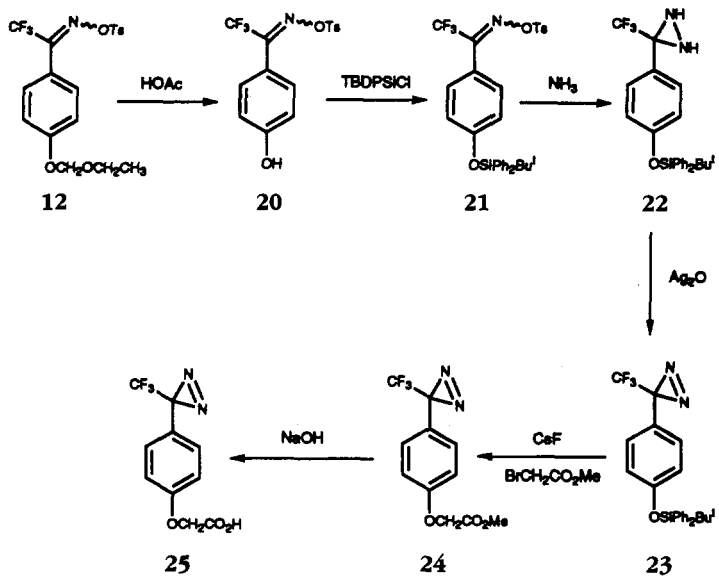
[b]Aryl protons occur in all spectra in the range 7.2–7.6 ppm.



[^{14}C]-labeled acid **17** by using [^{14}C]-labeled methyl bromoacetate, this pathway was not useful. Nevertheless, the taxol derivative **19** was prepared via the protected derivative **18**, and we confirmed that **19** stabilized tubulin polymer (see below).

A second label investigated was 4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxyacetic acid [**25**], which could be prepared from the intermediate **12** of the previous reaction pathway. The *O*-alkylated diazirine [**24**] was prepared by reaction of the substituted *t*-butyldiphenylsilyl phenol [**23**] with CsF in the presence of methylbromoacetate. The taxol analogue **27** was then prepared by acylation of 2′-triethylsilyl taxol with **25** in the presence of DCC/DMAP to afford the protected taxol **26**, followed by deprotection. Compound **27** also stabilized tubulin polymer (see below).

Although the in situ desilylation/*O*-alkylation reaction **23**↦**24** worked reasonably well on a larger scale, the reaction proved troublesome when we attempted to carry out the reaction on a small scale for the preparation of radiolabeled material. We thus turned to the meta analogue of **25**, 3-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenoxyacetic acid



[**30**]. The compound was prepared as described by Baldwin and co-workers (37,38) from the phenol **28** via the ester **29**, except that an isopropyl ether protecting group **39** was used in place of the methoxymethyl ether of the literature method in the synthesis of the phenol **28**. Its structure was confirmed unambiguously by X-ray crystallography (Figure 1); this is only the third report of an X-ray structure of a free diazirine ring (40). The taxol derivative **32** was then prepared via the 2′-protected derivative **31** in the usual way. Like **19** and **27**, **32** stabilized tubulin polymer (see below), and this biological activity and the relative ease of synthesis indicated that **32** should be the reagent of choice for initial studies of photoaffinity labeling of polymerized tubulin. The alkylation step to form ester **29** from phenol **28** proceeded in 90% yield in the presence of cesium carbonate and 1.1 equivalents of methyl bromoacetate, and this reaction could thus readily be used to prepare [^{14}C]-labeled material from [^{14}C]-labeled methyl bromoacetate. Alternatively, the acidic α-protons of **29** or **30** should allow the preparation of [^{3}H]-labeled derivatives by deprotonation and reprotonation.

Deprotonation of the ester **29** or the acid **30** proved more difficult than expected. Thus, although phenoxyacetic acid can readily be deprotonated to form the dilithium salt, both in our hands and in those of others (41), this reaction initially failed when applied to the acid **30**. Treatment of the ester **29** with lithium diisopropylamide (LDA) alone also failed to give useful deuterium incorporation after a $^{2}H_2O$ quench. However, treatment of the ester **29** with LDA (1.1 equiv) at −70°, followed by a $^{2}H_2O$/HMPA quench, yielded the α-monodeuterated acid in 50% yield with 85% label incorporation. The extent of incorporation for both the deuterated version of acid **30** and the coupled product **34** was confirmed by ^{1}H-nmr spectroscopy. The [^{3}H]-labeled taxol analogue **36** was prepared by treatment of the ester **29** with LDA followed by a $^{3}H_2O$/HMPA quench, then a coupling of the resulting acid to the 7 position of taxol in the usual way, followed by deprotection. When $^{3}H_2O$ with a specific activity of 90 Ci/mol was used, the specific activity of the resulting taxol derivative **36** was only 2.55 Ci/mol, indicating that a significant isotope effect occurred during the quench reaction.

BIOCHEMISTRY.—Because nonspecific reactions can be extensive when photoaffinity labeling of proteins is performed [see Safa *et al.* (11) for discussion], we decided to examine the interactions of the taxol analogues with purified tubulin as opposed to the

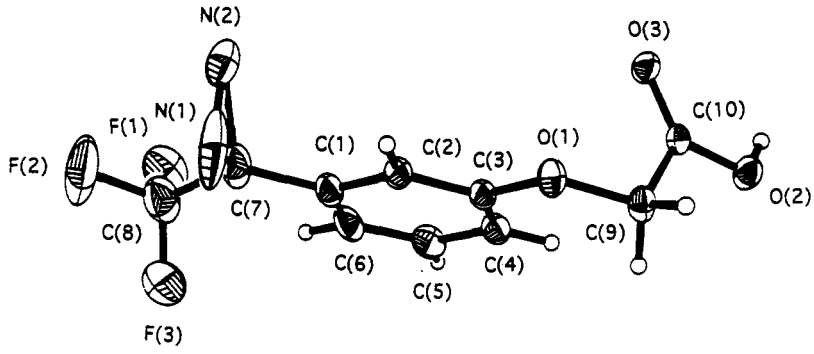
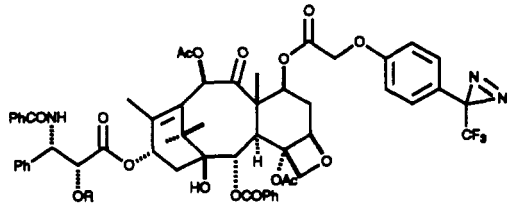


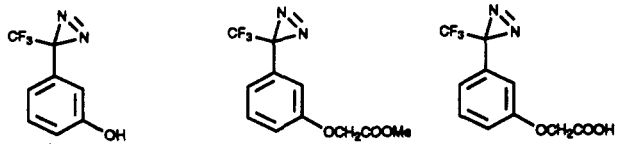
FIGURE 1. Crystal structure of diazirine **30**.

microtubule protein (microtubules obtained from brain tissue and containing microtubule-associated proteins in addition to tubulin) used in our initial studies. This will eliminate the possibility of nonspecific interactions with nontubulin components of the microtubule.

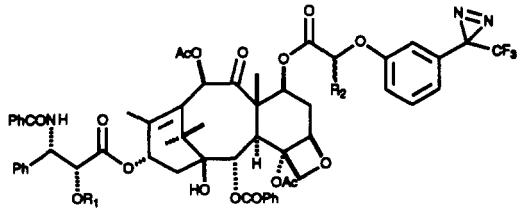
Use of purified tubulin presents us with two major alternatives for examining the interactions of photoreactive taxol analogue with the protein [for greater detail, see Hamel *et al.* (42)]. These are illustrated in Figure 2 with 10 μM tubulin ± 10 μM taxol. Curves 1 (no taxol) and 2 (+ taxol) compare reaction mixtures in whith 1.0 M glutamate



26 R=$SiEt_3$
27 R=H



28 **29** **30**



31 R_1=$SiEt_3$, R_2=H
32 R_1=R_2=H
33 R_1=$SiEt_3$, R_2=D
34 R_1=H, R_2=D
35 R_1=$SiEt_3$, R_2=T
36 R_1=H, R_2=T

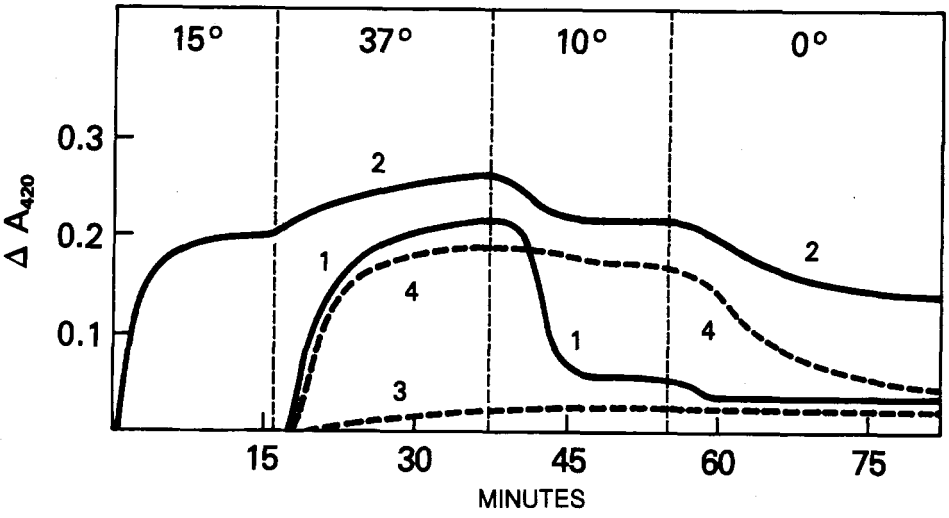


FIGURE 2. Taxol-induced polymerization of purified tubulin under polymerizing (1.0 M glutamate) and nonpolymerizing (0.1 M 4-morpholine-ethanesulfonate) conditions. Curves 1 and 2: 1.0 M monosodium glutamate (2 M stock solution adjusted to pH 6.6 with HCl), 10 μM (1.0 mg/ml) tubulin, 4% (v/v) DMSO, 50 μM GTP, and 10 μM taxol (curve 2 only). Curves 3 and 4: 0.1 M 4-morpholine-ethanesulfonate (1 M stock solution adjusted to pH 6.9 with NaOH), 10 μM (1.0 mg/ml) tubulin, 4% (v/v) DMSO, 100 μM GTP, 0.5 mM $MgCl_2$, and 10 μM taxol (curve 4 only). Baselines were established with cuvette contents at 0°. At time zero the electronic temperature controller was set at 15°. Additional temperatures were set at the times indicated by the dashed lines.

replaces microbule-associated proteins as an inducer of polymerization (43). In the absence of taxol no reaction occurred at 15°, while an extensive reaction occurred at 37°. With taxol, extensive polymerization occurred at the lower temperature. In the absence of taxol the polymer was cold labile, with about 80% of the total depolymerization reaction occurring at 10°. With taxol, less than 50% depolymerization occurred, with the bulk of depolymerization occurring at 0° rather than 10°. Alternatively, one could use a low ionic strength reaction condition, such as 0.1 M 4-morpholine-ethanesulfonate-0.5 mM $MgCl_2$ (pH 6.9), represented by curves 3 (no taxol) and 4 (+ taxol). Without taxol there was essentially no polymerization reaction. With taxol, polymerization occurred at 37° but not at 15°. With taxol there was little depolymerization at 10° and extensive, relatively slow depolymerization at 0°. In this reaction condition microtubule-associated proteins would induce a reaction similar to that represented by curve 1, and addition of taxol + microtubule-associated proteins would yield a reaction similar to that represented by curve 2 [cf. Hamel *et al.* (42)].

Since depolymerization effects at low temperatures seemed to be the best method for quantitative comparison of its analogues with taxol (35), the glutamate system was chosen for initial evaluation. IC_{50} values for the concentration of drug which reduced the rate of depolymerization by 50% were obtained for taxol and compounds **5**, **7**, **19**, **27**, and **32**. The values obtained are presented in Table 2, and typical experiments with taxol and compound **32** are presented in Figure 3A (taxol) and 3B (**32**), with reaction mixtures below (curve 2) and above (curve 3) the IC_{50} compared to a control reaction (curve 1). Under this reaction condition an IC_{50} value of 0.42 μM was obtained for taxol, and none of the analogues was as active as taxol itself. The IC_{50} value obtained for **5** was 0.65 μM. The IC_{50} values for the potential photoreactive compounds ranged from 1.6 μM for **7** to 3.1 μM for **27**. Nevertheless, substoichiometric IC_{50} values (i.e., lower than the tubulin

TABLE 2. IC_{50} Values of Taxol and Analogues in Inhibiting Tubulin Polymer Depolymerization.[a]

Compound	IC_{50} (μM)±SD
Taxol [**1**]	0.42±0.09
5	0.65±0.03
7	1.6±0.3
19	1.9±0.3
27	3.1±0.6
32	2.8±0.3

[a]Reaction mixtures (0.3 ml) contained 1.0 mg/ml (10 μM) purified tubulin, 1.0 M monosodium glutamate (2 M stock solution adjusted to pH 6.6 with HCl), 50 μM GTP, 4% (v/v) DMSO, and varying concentrations of taxol or taxol analogue. Polymerization was initiated by a temperature jump from 0 to 37° (the temperature jump took approximately 75 sec), and the temperature was maintained at 37° for about 20 min. Depolymerization was initiated by a reverse jump to 0° (which took about 6 min to complete), and the maximum rate of depolymerization (as measured by reduction in A_{350} or A_{420}) was determined. IC_{50} values were determined by interpolation. At least three independent determinations were performed with each compound.

concentration of 10 μM) were obtained with all compounds, which indicated they might have promise as photolabels. Note that with compound **32**, concentrations near the IC_{50} value for polymerization had relatively little effect on polymerization (Figure 3B). This was also observed with taxol (Figure 3A) and with the other analogues described here (data not presented).

With the successful preparation of compound **36**, the radiolabeled version of **32**, we began to investigate its incorporation into tubulin polymer. Our initial strategy was to use the low ionic strength reaction condition to minimize the presence of polymer not containing taxol analogue. We were unable, however, to induce polymerization with concentrations of **36** as high as 50 μM. This led us to examine the effects of **32**, as well as compounds **5**, **7**, **19**, and **27**, in greater detail. As shown in Figure 4, with the exception of compound **5**, these agents at 40 μM had little effect on tubulin polymerization in 1.0 M glutamate, and they all failed to induce polymerization in 0.1 M 4-morpholineethane sulfonate (data not presented). With compound **5**, a slight reaction occurred at 10°, and a brisk polymerization occurred when the temperature was increased to 37°, with turbidity greatly exceeding that of the drug-free control and that observed with 10 μM taxol (cf. Figure 2, curve 2). With 10 μM compound **5**, turbidity development and polymer stability was nearly as great as that observed with 40 μM drug (data not presented). These compounds displayed variable effects on depolymerization, and the greatest stabilization occurred with compound **5** (equivalent to that obtained with taxol); the least occurred with compound **27**.

The reduced stabilization that occurred with 40 μM concentrations of compounds **7**, **19**, **27**, and **32** was puzzling in view of the relatively low IC_{50} values for the rate of depolymerization obtained with these analogues. Compound **32** was studied over a range of concentrations, and extent of stabilization, as measured by retention of turbidity, was highly variable. Maximum, although incomplete, stabilization occurred at concentrations near stoichiometric with the tubulin concentration. This would indicate that there is one specific taxol binding site per tubulin molecule in the polymer (7).

Since compound **32/36** failed to induce polymerization of tubulin at low ionic strength but did stabilize the glutamate polymer, the interaction of the radiolabeled **36**

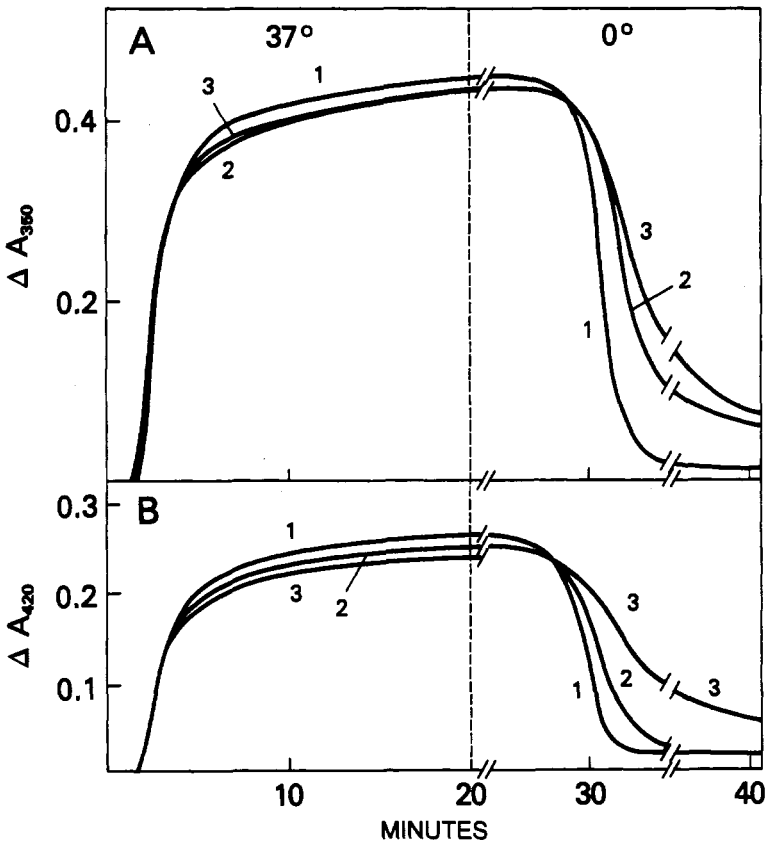


FIGURE 3. Determination of IC_{50} values for inhibition of depolymerization. Reaction conditions are described in detail in legend of Table 1. A. Taxol. Concentrations as follows: curve 1, none; curve 2, 0.25 μM; curve 3, 0.5 μM. B. Compound **32**. Concentrations as follows: curve 1, none; curve 2, 1.0 μM; curve 3, 3.0 μM. At 20 min, as indicated by the first interruption in the abscissa, the chart speed was changed from 30 cm/h to 60 cm/h, and the reaction temperature was reduced to 0° (see Table 1 legend). At approximately 35 min, as indicated by the second interruption in the abscissa, the chart speed was changed back to 30 cm/h.

with tubulin in 1.0 M glutamate was investigated. Polymer formed in the presence of **36** was harvested by centrifugation, with and without a preincubation with taxol and with and without a period of illumination of the reaction mixture at 302 nm. These pellets were dissolved in guanidine hydrochloride to denature the tubulin. Total protein and radiolabel recovered were quantitated, and a portion of each sample was examined by micro-gel filtration chromatography (44) to determine the amount of **36** bound to the denatured protein, which should be a measure of the extent of covalent reaction between tubulin and ligand. These studies are summarized in Table 3. In brief, superstoichiometric amounts of **36** appeared to form a covalent bond with tubulin. However, taxol failed to reduce the extent either of incorporation into pellet or of covalent bond formation, indicating that these reactons were largely nonspecific. In addition, illumination failed to enhance the extent of the covalent reaction, indicating that our photolabel may be too reactive, with a reaction occurring in the low level of ambient light required to perform these experiments.

In summary, we have examined five derivatives of taxol esterified at C-7 (compounds **5**, **7**, **19**, **27**, and **32**). With the exception of compound **5**, these are potential

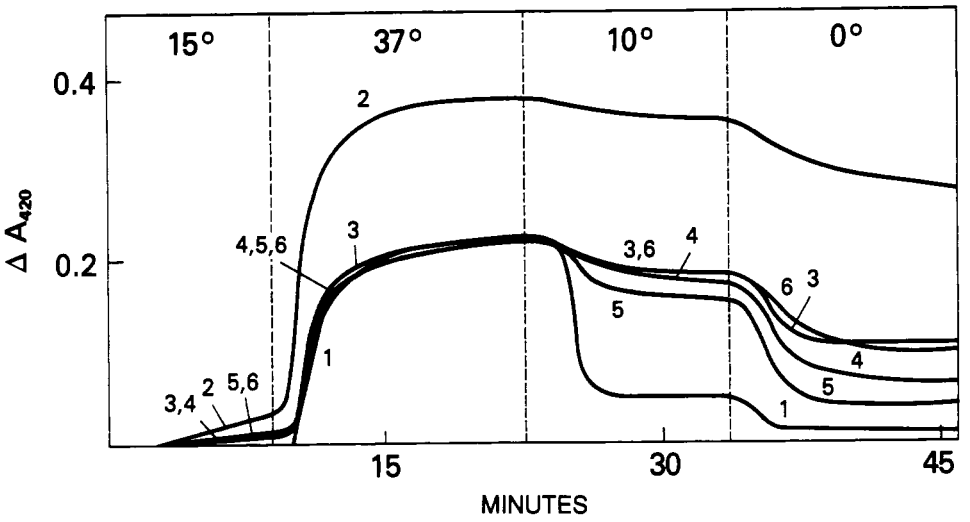


FIGURE 4. Effects of compounds **5**, **7**, **19**, **27**, and **32** on tubulin polymerization and depolymerization. Reaction conditions were as described in the legend of Figure 1 (glutamate system). Compounds were added to the reaction mixtures as follows: curve 1, none; curve 2, 40 μM compound **5**; curve 3, 40 μM compound **7**; curve 4, 40 μM compound **19**; curve 5, 40 μM compound **27**; curve 6, 40 μM compound **32**.

photoreactive agents, and three of them (compounds **19**, **27**, and **32**) can be prepared in radiolabeled forms. The tritiated version of compound **32** appears to be highly reactive with tubulin, probably in low levels of ambient light, but specificity of the covalent reaction could not be demonstrated. An unanticipated finding from our studies was that the taxol analogues we prepared had some, but not all of the properties of taxol. They were all deficient at inducing tubulin polymerization, both at low ionic strength at 37° and in glutamate at a reduced temperature (15°). If polymerization occurred, however, as at 37° in glutamate, all five derivatives stabilized the polymer that had formed. This stabilization occurred at low analogue concentrations and was particularly evident at intermediate temperatures (i.e., 10° as opposed to 0°). The stabilization that occurred with compound **5** was essentially indistinguishable from that observed with taxol. These observations indicate that compound **5** and additional analogues modified at position C-7 merit careful evaluation for potential differences from taxol in antitumor spectrum and/or toxicity.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All moisture-sensitive reactions were performed in oven-dried glassware under a positive pressure of argon. THF and Et_2O were distilled from Na/benzophenone ketyl. CH_2Cl_2 and MeCN were distilled from P_2O_5 and CaH_2, respectively. Mp's were determined on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-nmr spectra were obtained on a Bruker WP-270 spectrometer operating at 270 MHz, and ^{13}C-nmr spectra were obtained on a Varian Unity 400 spectrometer operating at 100.57 MHz. All nmr spectra were recorded in $CDCl_3$ with TMS as an internal standard. Ir data were obtained on a Perkin-Elmer 283B infrared spectrophotometer. Analytical tlc was performed on Si gel 60 F_{254} plates (E. Merck), 0.2-mm layer. Preparative tlc was performed on Si gel GF plate (20×20 cm), 500 or 1000 μm thick (Analtech). Taxol derivatives were visulalized on tlc plates by spraying with a vanillin/H_2SO_4 solution and then heating.

Radioactivity measurements were performed by liquid scintillation counting with a Beckman LS3800 liquid scintillation system. ^3H_2O was purchased from ICN Radiochemicals.

The phrase "worked up by standard methods" refers to diluting the reaction with an excess of organic solvent (i.e., CH_2Cl_2, EtOAc, Et_2O), successive washing with H_2O and brine, drying over Na_2SO_4, and evaporating the solvent in vacuo, unless otherwise noted.

TABLE 3. Evaluation of Compound **36** as a Photoreactive Analogue of Taxol.[a]

	mol compound **36**/mol tubulin (±SD)			
	Low ambient light[b]		Uv illumination[c]	
	Incorporated into polymer[d]	Covalently bound to tubulin[e]	Incorporated into polymer[d]	Covalently bound to tubulin[e]
No taxol[f]	5.1±0.3	3.7±0.2	5.2±0.05	2.7±0.6
Added taxol[f]	5.1±0.3	3.9±0.1	4.8±0.1	3.4±0.3

[a]Each reaction mixture (1.0 ml final volume; all concentrations in terms of final volume) contained 0.5 mg/ml (5 μM) tubulin, 1.0 M monosodium glutamate (pH of 2.0 M stock solution adjusted to 6.6 with HCl), 0.5 mM $MgCl_2$, 100 μM GTP, and, if indicated, 50 μM taxol in 0.975 ml volume. Reaction mixtures were prepared and maintained with minimal light in the room ("low ambient light"). After a 15 min incubation at 37°, compound **36** was added in 25 μl to a final concentration of 25 μM. Incubation was continued at 37° for 15 min. Half the reaction mixtures were left in low ambient light at room temperature for 15 min; the other half were illuminated at 302 nm for 15 min. All reaction mixtures were centrifuged for 10 min at 45,000 rpm in a Beckman Ti70 rotor. The supernatants were aspirated and discarded, and the pellets were washed twice with a solution containing 1.0 M monosodium glutamate (pH 6.6) and 0.5 mM $MgCl_2$. The pellets were each dissolved in 0.5 ml of 3.0 M guanidine hydrochloride. This solution was used to determine the amount of protein and quantity of compound **36** in polymer and to determine the amount of compound **36** covalently linked to tubulin. Each data point was performed in triplicate with the average values and standard deviations (SD) presented.

[b]The reaction mixtures were prepared with as little room illumination as possible. A UVX photometer (manufactured by UVP, Inc. of San Gabriel, CA) with a 365 nm sensor measured an ambient intensity of less than 0.3 $\mu W/cm^2$.

[c]The samples were illuminated at 302 nm for 15 min with a model xx-15 lamp (manufactured by UVP, Inc.). The light source was at a distance of 3.5 cm from the surface of the reaction mixtures. Illumination was performed in a microtiter plate, with each well containing 0.25 ml. The UVX photometer with a 310 nm sensor measured a light intensity of 4.6 mW/cm^2.

[d]The amounts of radiolabel and protein in the polymer pellets were quantitated on aliquots of the guanidine hydrochloride solutions derived from the pellets.

[e]Aliquots of the pellet solutions were placed on syringe columns of Sephadex G 50 (superfine) equilibrated with 3.0 M guanidine hydrochloride and processed by low speed centrifugation as described previously (43). Protein and radiolabel in the filtrates were determined. It is assumed that radiolabel that remains associated with tubulin following denaturation, and gel filtration represents compound **36** which has reacted covalently with the protein.

[f]Taxol was present, as indicated, in the initial incubation prior to addition of compound **36** in an attempt to minimize the interaction of the analogue with tubulin.

Pure taxol was obtained by purification of a crude mixture of taxol and cephalomannine by the published method (45); the mixture was a gift from the National Cancer Institute.

2'-Chloroacetyltaxol [**3**].—Taxol [**1**] (1.117 g, 1.31 mmol) was dissolved in CH_2Cl_2 (2.0 ml) and pyridine (0.40 ml), and the solution was cooled to 0°. Choroacetic anhydride (262 mg, 1.2 equiv) dissolved in CH_2Cl_2 (5 ml) was added to the solution over 30 min. After 65 min the reaction was worked up by standard methods. Purification by Si gel flash cc (50% EtOAc/50 hexanes) yielded **3** (1.19 g, 98%): mp 210–212°; ir (KBr) 1750, 1660 cm^{-1}; fabms *m/z* (rel. int) $[MH]^+$ 930 (100), 931 (56). 932 (33); ^1H nmr 5.50 (1H, d, J=3.0, H-2'), 6.00 (1H, dd, J=8.0, 3.0, H-3'), 4.20 (2H, d, J=3.0, 2'-$OCOCH_2Cl$).

2'-Chloroacetyl-7-benzoyltaxol [**4**].—2'-Chloroacetyltaxol [**3**] (30 mg, 0.032 mmol) was dissolved in CH_2Cl_2 (0.5 ml) and pyridine (10 μl). Benzoyl chloride (5 ml) was added to the solution which was stirred at room temperature for 1 h. The solvent was then removed in vacuo, and residual traces of pyridine were evaporated with *n*-heptane. The residue was purified by preparative tlc with 50% EtOAc/50% hexanes and recrystallized as platelets from hexane/CH_2Cl_2 (19 mg, 52%): mp 140–144°. In this procedure, the hydrolysis of excess benzoyl chloride is largely prevented. If aqueous workup is used, the separation of benzoic acid from the product is very difficult. ^1H nmr 5.60 (1H, d, J=3, H-2'), 4.18 (2H, d, J =3.0, 2'-$OCOCH_2Cl$), 6.05 (1H, dd, J=7.0, 3.0, H-3'), 5.75 (1H, m, H-7), 7.92 [2H, m, 7-OBz (ortho)].

7-Benzoyltaxol [**5**].—A solution of **4** (19 mg, 0.020 mmol) in MeOH-CH_2Cl_2 (9:1) was treated with 50 mg of Si gel (GF_{50}). After stirring for 24 h under argon at room temperature the solvent was evaporated, the residue was dissolved in 5% MeOH in $CHCl_3$, and the solution was filtered through a cotton plug. The residue obtained after evaporation of the solvent was purified by Si gel preparative tlc (50% EtOAc/50% hexanes). Compound **5** was obtained as a white solid (8.2 mg, 47%): mp 168–172°; fabms *m/z* (rel. int.)

$[MH]^+$ 958 (100), 899 (80), 673 (10), 880 (20), 613 (40), 553 (100), 369 (100), 185 (100); ^{1}H nmr see Table 1.

7-Azibenzoyltaxol [**7**].—2′-Chloroacetyltaxol [**3**] (200 mg, 0.215 mmol), DCC (150 mg, 3.3 eq), and a catalytic amount of 4-pyrrolidinopyridine (1 mg) were dissolved in CH_2Cl_2 (3 ml). A solution of azibenzoic acid [**2**] (3 equiv) in CH_2Cl_2 (0.3 ml) was added to the above solution and stirred at room temperature. After 1 h the solution was washed with 5% aqueous HOAc and then H_2O (30 ml×2). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The product was successively purified on Sephadex LH-20 and Si gel columns and recrystallized from CH_2Cl_2/hexane to yield homogenous material (12 mg, 5%): mp 205° (dec); ir ($CHCl_3$) 1732, 1660 cm^{-1}; uv λ max (MeOH) nm (ε) 350 (1050), 280 (5960), 231 (43000); fabms *m/z* (rel. int.) $[MH]^+$ 1066 (72), 1047 (5), 966 (18), 981 (32), 953 (100); ^{1}H nmr see Table 1.

1-Bromo-4-ethoxymethoxybenzene [**9**].—*p*-Bromophenol [**8**] (15.0 g, 86.7 mmol, 1 equiv) in 40 ml of dimethyformamide (DMF) was added dropwise to a slurry of 2.29 g (95.3 mmol, 1.1 equiv) of dry NaH in 100 ml of DMF at 15°. This was stirred for 2 h at room temperature, and 9.0 g of chloromethylethyl ether (95.3 mmol, 1.1 equiv) was added dropwise to the solution. After stirring for 12 h, the mixture was worked up by standard methods, and the crude product was distilled to give **9** as a clear liquid (16.8 g, 84%): bp 107°/3.5 mm Hg; ir (NaCl) 2950, 2860, 1580, 1475, 1220, 1145, 1090, 1070, 980, 820 cm^{-1}; ^{1}H nmr 1.21 (3H, t, J=7.0, Me), 3.70 (2H, q, J=7.0, OCH_2CH_3), 5.18 (2H, s, OCH_2O), 6.92 (2H, d, J=9.0, ArH), 7.37 (2H, d, J=9.0, ArH); eims *m/z* (rel. int.) $[M^+]$ 232, 230 (10), 174 (20), 59 (100); hreims *m/z* $[M]^+$ 229.9938 ($C_9H_{11}BrO_2$ requires 229.9942).

1-(4-Ethoxymethoxyphenyl)-2,2,2-trifluoroethanone [**10**].—A 250-ml round-bottomed flask was charged with 150 ml of Et_2O and 10.0 g of protected bromophenol [**9**] (43.3 mmol, 1.0 equiv). The flask was cooled to −40°, and 19.0 ml of 2.5 M *n*-butyl lithium (47.6 mmol, 1.1 equiv) was added dropwise. The mixture was stirred for 2 h. A solution containing 8.62 g (47.6 mmol, 1.1 equiv) of trifluoroacetylpiperidine [synthesized by reacting 1.2 equiv of piperidine in Et_2O with 1 equiv of trifluoroacetic anhydride in the presence of 1 equiv of triethylamine, washing with 0.1 N aqueous HCl and distillation, in vacuo; bp 63–64°/4.5 mm Hg (31)] in 40 ml of Et_2O was added dropwise at −40°. After 4 h at this temperature the cooling bath was removed, and the mixture was hydrolyzed with 25 ml of saturated aqueous NH_4Cl and worked up by standard methods. The crude product could be carried through the next step without purification or could be distilled to give **10** as a clear liquid (7.1 g, 66%): bp 116–117°/6.0 mm Hg; ir (NaCl) 2960, 2880, 1700, 1590, 1310, 1240, 1140, 970, 930, 840, 760, 730 cm^{-1}; ^{1}H nmr 1.22 (3H, t, J=7.0 Me), 3.73 (2H, q, J=7.0, OCH_2CH_3), 5.32 (2H, s, OCH_2O), 7.15 (2H, d, J=9.0, ArH), 8.05 (2H, d, J=9.0 ArH); ^{13}C nmr 14.92 (Me), 64.90 (OCH_2CH_3), 92.92 (OCH_2O), 116.50 (ArC-H), 117.0 (CF_3, $J_{C\text{-}F}$=291.7 Hz), 123.64 (ArC-$COCF_3$), 132.53 (ArC-H), 163.32 (ArC-OR), 179.25 (CO); eims *m/z* (rel. int.) $[M]^+$ 248 (5), 121 (35), 59 (100); hreims *m/z* $[M]^+$ 248.0668 ($C_{11}H_{11}F_3O_3$ requires 248.0660).

1-(4-Ethoxymethoxyphenyl)-2,2,2-trifluoroethanone oxime [**11**].—Hydroxylamine hydrochloride (4.90 g, 70.6 mmol, 2.5 equiv) was added to 7.0 g of ketone **10** (28.2 mmol, 1.0 equiv) dissolved in 50 ml of pyridine and 25 ml of absolute EtOH. This was refluxed for 6 h, followed by removal of the solvent in vacuo. The residue was dissolved in Et_2O, and the solution was washed successively with 0.1 N HCl, H_2O, and brine. The Et_2O layer was dried over Na_2SO_4 and concentrated to yield a crude liquid which was purified by Si gel flash chromatography (5% EtOAc/95% CH_2Cl_2). The product was obtained as a thick pale yellow liquid (6.1 g, 82%); ir (NaCl) 3349, 3070, 2951, 2360, 1677, 1607, 1513, 1444, 1242, 1153, 1081, 1003, 923, 838 cm^{-1}; ^{1}H nmr 1.23 (3H, t, J=7.0 Me), 3.74 (2H, q, J=7.0, OCH_2CH_3), 5.26 (2H, s, OCH_2O)7.13 (2H, d, J=9, ArH), 7.54 (2H, d, J=9, ArH), 9.87 and 10.00 (*syn*- and *anti*-OH); eims *m/z* (rel. int.) $[M]^+$ 263 (10), 233 (2), 218 (4), 59 (100); hreims *m/z* $[M]^+$ 263.0772 ($C_{11}H_{12}F_3NO_3$ requires 263.0769).

O-(p-*Toluenesulfonyl*)-*1-(4-ethoxymethoxyphenyl)-2,2,2-trifluoroethanone oxime* [**12**].—Compound **11** (6.1 g, 23.2 mmol) dissolved in 40 ml of pyridine was treated with 6.6 of *p*-toluenesulfonyl chloride (34.8 mmol, 1.5 equiv), and the mixture was refluxed for 3 h. The pyridine was removed in vacuo, and the reaction worked up by standard methods. Si gel flash chromatography (30% CH_2Cl_2/70% hexanes) afforded **12** as a white solid (7.5 g, 77%): mp 39–41°; ^{1}H nmr 1.23 (3H, t, J=7.2, CH_2CH_3), 2.47 (3H, s, ArMe), 3.73 (2H, q, J=7.2, OCH_2CH_3), 5.26 (2H, s, OCH_2O). 7.10 (2H, d, J=9.0, ArH), 7.38 (4H, m, ArH), 7.89 (2H, d, J=8.4, ArH); eims *m/z* (rel. int.) $[M^+]$ 417 (1), 248 (25), 155 (80), 91 (100), 65 (30), 59 (100), hreims *m/z* $[M]^+$ 417.0858 ($C_{18}H_{18}F_3NO_5S$ requires 417.0857).

3-(4-Ethoxymethoxyphenyl)-3-(trifluoromethyl)diaziridine [**13**].—A 500-ml three-necked round-bottomed flask was equipped with a dry ice condenser and a gas inlet. Tosyl oxime **12** (6.1 g), dissolved in 100 ml of Et_2O, was added to the flask and cooled to −78°. Approximately 75 ml of anydrous NH_3 was condensed into the flask, and the solution was stirred for 1 h at −78°. The cooling bath was removed, the gas inlet was replaced with a drying tube to prevent pressure buildup, and the condenser was maintained at −78°. The solution was stirred at ambient temperature while the NH_3 refluxed for 2 h. The condenser was removed

and the NH_3 was allowed to evaporate. The remaining residue was dissolved in Et_2O and worked up by standard methods. Si gel flash chromatography (1% EtOAc/99% $CHCl_3$) yielded the product as a clear oil (3.7 g, 96%): ^{1}H nmr 1.21 (3H, t, J=7.1 Me), 2.16 (1H, br d, NH), 2.74 (1H, br d, NH), 3.71 (2H, q, J=7.1, OCH_2CH_3), 5.23 (2H, s, OCH_2O), 7.07 (2H, d, J=8.4, ArH), 7.53 (2H, d, J=8.4, ArH); eims *m/z* (rel. int.) [M^+] 262 (12), 261 (40), 217 (15), 183 (10), 59 (100); hreims *m/z* [M]$^+$ 262.0933 ($C_{11}H_{13}F_3N_2O_2$ requires 262.0929).

3-(4-Ethoxymethoxyphenyl)-3-(trifluoromethyl)-3H-diazirine [**14**].—Ag_2O (13.1 g, 56.5 mmol, 4.0 equiv) was prepared by dropwise addition of 4.5 g of NaOH (112.9 mmol, 8.0 equiv) in 50 ml of H_2O to a boiling solution of 19.2 g of $AgNO_3$ (112.9 mmol, 8.0 equiv) in 150 ml of H_2O. The precipitated Ag_2O was filtered, washed successively with H_2O, Me_2CO, and Et_2O, and used immediately. It was added in the dark to 3.7 g of diaziridine **13** (14.1 mmol, 1 equiv) in 50 ml of Et_2O. The mixture was stirred at room temperature for 1 h and filtered through Celite. The Et_2O was removed by evaporation in vacuo, and purification by Si gel flash chromatography (30% CH_2Cl_2/70% hexanes) afforded **14** as a yellow liquid (3.6 g, 98%); ^{1}H nmr 1.20 (3H, t, J=7.1, Me), 3.70 (2H, d, J=7.1, OCH_2CH_3), 5.22 (2H, s, OCH_2O), 7.05 (2H, d, J=9.0, ArH), 7.14 (2H, d, J=9.0, ArH).

3-(4-Hydroxy-3-nitrophenyl)-3-(trifluoromethyl)-3H-diazirine [**15**].—HNO_3 (2.78 g, 70% w/w, 30.8 mmol, 4.0 equiv) was added to 15.7 g of Ac_2O (154.0 mmol, 20 equiv) at 15°. After 15 min at 15° the temperature was reduced to −30°, and 2.0 g of diazirine **14** (7.70 mmol, 1 equiv) was added in the dark. No starting material was detected after 2.5 h by tlc. The flask was warmed to 0°, and 25 ml of H_2O was slowly added. Workup by standard methods followed by Si gel flash chromatography (40% CH_2Cl_2/60% hexanes) yielded nitrophenol **15** as a bright yellow oil, which solidified below 0° (800 mg, 42%): ir (NaCl) 3220, 1610, 1530, 1480, 1410, 1310, 1150, 980, 820 cm^{-1}; ^{2}H nmr 7.23 (1H, d, J=8.9, ArH), 7.51 (1*H*, dd, J=8.9, 2.2, ArH), 7.95 (1H, d, J=2.2, ArH), 10.50 (1H, s, OH); cims *m/z* (rel. int.) [MH]$^+$ 248 (25), 232 (5), 218 (10), 190 (15), 85 (100), 81 (80).

Methyl 2-nitro-4-{3-(trifluoromethyl)-3H-diazirin-3-yl}phenoxyacetate [**16**].—To 500 mg of nitrophenol **15** (2.02 mmol, 1 equiv) was added 2.48 g of methyl bromoacetate (16.2 mmol, 10 equiv) and 2.5 ml of MeCN. Solid Cs_2CO_3 (2.63 g, 8.08 mmol, 4.0 equiv) was added at room temperature in the dark. After 1 h the mixture was diluted with Et_2O. H_2O was added, and the mixture was acidified with 2 N aqueous HCl. Workup by standard methods followed by Si gel flash chromatography [hexanes-$CHCl_3$-EtOAc (11:4.5:1)] yielded a pale yellow oil (200 mg, 40%): ^{1}H nmr 3.80 (3H, s, -Me), 4.85 (2H, s, OCH_2), 7.09 (1H, d, J=8.9, ArH), 7.41 (1H, dd, J=8.9, 2.4, ArH), 7.71 (1H, d, J=2.4, ArH).

2-Nitro-4-{3-(trifluoromethyl)-3H-diazirin-3-yl}phenoxyacetic acid [**17**].—Ester **16** (200 mg) dissolved in 10 ml of THF was reacted with 1.2 ml of 2 N aqueous NaOH at room temperature in the dark for 1 h. The solution was diluted with EtOAc and washed with H_2O. The basic aqueous layer was acidified into fresh EtOAc with 1 N HCl to pH=2, and the aqueous layer was washed several times with EtOAc to extract the carboxylic acid. The organic layers were combined, washed successively with H_2O and brine, dried over Na_2SO_4, and concentrated to yield **17** as a pale yellow solid (180 mg, 94%): mp 128–130°; ^{1}H nmr 4.86 (2H, s, OCH_2O), 7.05 (1H, d, J=8.9, ArH), 7.44 (1H, dd, J=8.9, 2.4, ArH), 7.74 (1H, d, J=2.4, ArH); ^{13}C nmr 66.38 (CH_2), 117.16 (ArC-H), 120.79, 121.96, 124.66 (ArC-H), 124.81, 132.90 (ArC-H), 141.33, 153.01, 168.66; cims *m/z* (rel. int.) [MH]$^+$ 306 (100), 278 (40), 260 (60) 246 (50), 232 (65), 203 (70), 175 (50), 103 (40), 85 (50); hrcims *m/z* [MH]$^+$ 306.0338 ($C_{10}H_7F_3N_3O_5$ requires 306.0334).

2′-Triethylsilyltaxol.—To a solution of 50 mg of taxol (0.059 mmol, 1 equiv) in 2.0 ml of dry CH_2Cl_2 was added 10 mg of imidazole (0.147 mmol, 2.5 equiv) and 25 μl of triethylsilylchloride at room temperature and the mixture stirred for 30 min. Workup by standard methods followed by Si gel preparative tlc (40% EtOAc/60% hexanes) yielded 2′-triethylsilyltaxol (52 mg, 92%): mp 157–159°; ^{1}H nmr 0.47 (6H, m, $SiCH_2CH_3$). 0.82 (9H, t, J=8.0, $SiCH_2CH_3$), 4.69 (1H, d, J=2, H-2′), 5.71 (1H, dd, J=9.2, H-3′); fabms [MNa]$^+$ 991 (50), 990 (100), 968 (10).

2′-Triethylsilyl-7-{{2-nitro-4-{3-(trifluoromethyl)-3H-daizirin-3-yl}}phenoxy} acetyl taxol [**18**].—2′-Triethylsilyl taxol (25.0 mg, 0.029 mmol, 1 equiv) was dissolved in 1.0 ml of CH_2Cl_2. Acid **17** (26 mg, 0.088 mmol, 3 equiv) was added at room temperature, followed by 18.0 mg of dicyclohexylcarbodiimide (0.088 mmol, 3 equiv) and a catalytic amount of 4-pyrrolidinopyridine. The mixture was stirred in the dark for 1 h. The solvent was evaporated, and the crude residue was purified by Si gel preparative tlc (35% EtOAc/65% hexanes) to yield the coupled product (22 mg, 68%) along with an inseparable impurity. ^{1}H nmr 0.48 (6H, m, $SiCH_2CH_3$), 0.83 (9H, t, J=8.0, $SiCH_2CH_3$), 4.75 and 5.00 (ABq, J=16.30, $PhOCH_2CO_2R$), 5.65 (1H, m, H-7), 7.25–7.75 (3H, hidden under Ph peaks).

7-{2-Nitro-4-{3-(trifluoromethyl)-3H-diazirin-3-yl}phenoxyacetyl taxol [**19**].—Compound **18** (22 mg) was added to 1.0 ml of a 5% HCl/MeOH solution at room temperature and stirred for 10 min. Workup by standard methods followed by Si gel preparative tlc (45% EtOAc/55% hexanes) yielded **19** (15 mg, 75%):

mp 212–218°; ^{1}H nmr see Table 1; fabms *m/z* (rel. int.) $[M]^+$ 1141 (1), 240 (10), 105 (100); hrfabms *m/z* $[MH]^+$ 1141.3490 ($C_{57}H_{56}F_3N_4O_{18}$ requires 1141.3542).

O-(p-*Toluenesulfonyl*)-*1*-(*4-hydroxyphenyl*)-*2,2,2-trifluoroethanone oxime* [**20**].—Protected tosyl oxime **12** (3.2 g) was added to a mixture of 10 ml of THF, 4.0 ml of iPrOH, and 5.0 ml of 8 N aqueous HCl. After stirring at room temperature for 22 h, the solution was worked up by standard methods. Si gel flash chromatography (20% EtOAc/80% hexanes) afforded **20** as a white solid (2.31 g, 84%): mp 104–106°; ^{1}H nmr 2.47 (3H, s, ArMe), 5.9 (1H, s, OH), 6.90 (2H, d, J=9.0, ArH), 7.39 (4H, m, ArH), 7.89 (2H, d, J=9.0, ArH); cims *m/z* (rel. int.) $[MH]^+$ 360 (65), 188 (100), 155 (80), 91 (30); hrcims *m/z* $[MH]^+$ 360.0530 ($C_{15}H_{13}F_3NO_4S$ requires 360.0517).

O-(p-*Toluenesulfonyl*)-*1*-(*4-t-butyldiphenylsiloxy-phenyl*)-*2,2,2-trifluoroethanone oxime* [**21**].—Phenol **20** (2.3 g, 6.4 mmol, 1 equiv) was dissolved in 15 ml of DMF. Imidazole (653 mg, 9.6 mmol, 1.5 equiv) was added at room temperature, followed by addition of 2.64 g of *t*-butyldiphenylsilylchloride (9.6 mmol, 1.5 equiv). The solution was stirred for 5 h and worked up by standard methods. Si gel flash chromatography (30% CH_2Cl_2/70% hexanes) yielded the silyl-protected tosyl oxime as a clear oil (3.44 g, 90%): ^{1}H nmr 1.10 (9H, s, *t*-Bu), 2.45 (3H, s, Me), 6.80 (2H, d, J=9.0 ArH), 7.23–7.87 (16H, m, ArH); eims *m/z* (rel. int.) $[M]^+$ 597 (2), 540 (100), 370 (70), 353 (40), 300 (20), 91 (20).

3-(4-t-Butyldiphenylsiloxyphenyl)-3-(trifluoromethyl)-diaziridine [**22**].—The protected tosyl oxime **21** (3.4 g) dissolved in 50 ml of Et_2O was added to a 250-ml round-bottomed flask equipped with a dry ice condenser and gas inlet. The flask and condenser apparatus were cooled to −78°, and 30 ml of anhydrous NH_3 was admitted through the condenser. This was stirred for 1 h at −78°, and the cooling bath was removed. A drying tube was connected to the inlet, and the NH_3 was refluxed at room temperature for 2 h. The condenser was removed, and the NH_3 was evaporated. Workup by standard methods followed by Si gel flash chromatography (1% EtOAc/99% CH_2Cl_2) afforded diaziridine **22** (712 mg, 28%) along with 1.5 g of *O*-desilylated product: ^{1}H nmr 1.10 (9H, s, *t*-Bu), 2.05 (1H, br d, NH), 2.62 (1H, br d, NH), 6.75 (2H, d, J=8.7, ArH), 7.32–7.72 (12H, m, ArH); eims *m/z* (rel. int.) $[M]^+$ 442 (10), 385 (100), 300 (15), 222 (10), 57 (10).

*3-(4-t-Butyldiphenylsilyloxyphenyl)-3-(trifluoromethyl)-3*H-*diazirine* [**23**].—Freshly prepared Ag_2O (1.50 g, 6.45 mmol, 4.0 equiv) was added in the dark to 710 mg of diaziridine **22** (1.61 mmol, 1 equiv) in 10 ml of Et_2O. The mixture was stirred at room temperature for 1 h and filtered through Celite. Evaporation of Et_2O yielded the product as a pale yellow liquid (680 mg, 96%): ^{1}H nmr 1.08 (9H, s, *t*-Bu), 6.74 (2H, d, J=9.0, ArH), 6.92 (2H, d, J=9.0, ArH), 7.33–7.43 (6H, m, ArH), 7.66–7.70 (4H, m, ArH); fabms *m/z* (rel. int.) $[M]^+$ 440 (6), 412 (65), 383 (10), 307 (11), 197 (44), 135 (100); hrfabms *m/z* $[M]^+$ 440.1513 ($C_{24}H_{23}F_3N_2OSi$ requires 440.1531).

*Methyl 4-{3-(trifluoromethyl)-3*H-*diazirin-3-yl}phenoxyacetate* [**24**].—To 700 mg of diazirine **23** (1.59 mmol, 1 equiv) and 25 ml of dry MeCN was added 1.46 g of methyl bromoacetate (9.55 mmol, 6 equiv), followed by addition of 1.4 g of CsF (9.55 mmol, 6 equiv). The mixture was stirred in the dark at room temperature for 4 h. Workup by standard methods followed by Si gel preparative tlc [hexanes-$CHCl_3$-EtOAc (11:4.5:1.0)] yielded **24** as a clear liquid (196 mg, 46%): ^{1}H nmr 3.80 (3H, s, Me), 4.64 (2H, s, OCH_2), 6.90 (2H, d, J=9.0, ArH), 7.17 (2H, d, J=9.0, ArH).

*4-{3-(Trifluoromethyl)-3*H-*diazirin-3-yl}phenoxyacetic acid* [**25**].—Aqueous 2N NaOH (0.4 ml) was added to a solution of **24** (190 mg) in 10 ml of THF. After stirring in the dark for 1 h at room temperature, the solution was diluted with EtOAc and H_2O. The basic aqueous layer was separated, acidified into fresh EtOAc with 1 N HCl, and washed several times with EtOAc. The organic layers containing the carboxylic acid were combined, dried over Na_2SO_4, and concentrated to yield **25** as a white solid (170 mg, 94%): mp 92–94°; ^{1}H nmr 4.68 (2H, s, OCH_2), 6.90 (2H, d, J=9.0, ArH), 7.17 (2H, d, J=9.0, ArH); cims *m/z* (rel. int.) $[MH]^+$ 261 (50), 233 (90), 232 (100), 215 (30), 175 (40), 145 (40); hrcims *m/z* $[M-28]^+$ 232.0338 ($C_{10}H_7F_3O_3$ requires 232.0347).

*2'-Triethylsilyl-7-{4-{3-(trifluoromethyl)-3*H-*diazirin-3-yl}phenoxyacetyl} taxol* [**26**].—Treatment of 2'-triethylsilyltaxol with acid **25** as previously described in the synthesis of **18** yielded the derivative **26** as a pale yellow amorphous solid (68%): ^{1}H nmr 0.47 (6H, m, $SiCH_2CH_3$), 0.82 (9H, t, J=8.0, $SiCH_2CH_3$), 4.52 and 4.82 (ABq, J=16.30, $PhOCH_2CO_2R$), 5.70 (1H, m, H-7), 6.95 (2H, d, J=9.0, ArH), 7.12 (2H, d, J=9.0, ArH).

*7-{4-{-(Trifluoromethyl)-3*H-*diazirin-3-yl}phenoxyacetyl} taxol* [**27**].—Deprotection of **26** with 5% HCl/MeOH as described for **18** yielded **27** as a pale yellow amorphous solid (73%): ^{1}H nmr see Table 1; fabms *m/z* (rel. int.) $[M]^+$ 1096 (40), 1069 (15), 1018 (15), 812 (45), 753 (100).

*2'-Triethylsilyl-7-{3-{3-(trifluoromethyl)-3*H-*diazirin-3-yl}phenoxyacetyl} taxol* [**31**].—To 20 mg of carboxylic acid [**30**] and 25 mg of 2'-triethylsilyltaxol in 1.0 ml of CH_2Cl_2 was added 16.0 mg of

dicyclohexylcarbodiimide and a catalytic amount of DMAP. This was stirred at room temperature for 30 min. The CH_2Cl_2 was evaporated, and the residue purified by Si gel cc (30% EtOAc/70% hexanes) to yield **31** (27 mg, 86%): mp 118–123°; ^{1}H nmr 0.48 (6H, m, $SiCH_2CH_3$), 0.83 (9H, t, J=8.0, $SiCH_2CH_3$), 4.51 and 4.86 (ABq, J=16.20, $PhOCH_2CO_2R$), 5.62 (1H, m, H-7), 6.74 (1H, br s, ArH), 6.85 (1H, br dd, ArH), 6.95 (1H, dd, J=2.5, 8.4); fabms *m/z* (rel. int.) $[MH]^+$ 1211 (1), 752 (21), 400 (30), 355 (50), 215 (100).

*7-{3-{3-(Trifluoromethyl)-3*H*-diazirin-3-yl}phenoxyacetyl} taxol* [**32**].—Compound **31** (27 mg) was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 min. The solution was diluted with CH_2Cl_2, washed with H_2O and brine, dried over Na_2SO_4, and concentrated to yield the crude product. Purification by Si gel cc yielded **32** as a white solid (19 mg, 78%): mp 190–192°; ^{1}H nmr see Table 1; fabms *m/z* (rel. int.) $[M]^+$ 1096 (40), 812 (45), 753 (100).

*Deuterated analogue of {3-(trifluoromethyl)-3*H*-diazirin-3-yl}phenoxyacetic acid.*—A 50-ml flame-dried round-bottomed flask under argon was charged with 5 ml of THF and 250 mg (0.9125 mmol, 1.0 equiv) of ester **29**. This was cooled to −78°, and 2.03 ml (1.00 mmol, 1.1 equiv) of freshly prepared 0.5 M lithium diisopropylamine was added dropwise to the ester over a period of 2 min. After stirring for 15 min at −78°, a solution of ^2H_2O (0.05 ml) in 0.0625 ml of hexamethylphosphoramide (HMPA) was added. The cooling bath was removed and the reaction stirred at ambient temperature for 1 h. The solution was then diluted with EtOAc, followed by H_2O. The solution was transferred to a separatory funnel, and the EtOAc layer was removed. The aqueous layer was washed with EtOAc twice and then acidified into fresh EtOAc with 1.0 N HCl. The organic layer was washed successively with H_2O and brine and dried over Na_2SO_4. Concentration in vacuo yielded the deuterated form of carboxylic acid **30** (118 mg, 50%): mp 75–78°; ^{1}H nmr analysis confirmed H-D exchange (ca. 85%) by integration at 4.65 ppm with respect to aromatic protons.

*2′-Triethylsilyl-7-{{α-^{2}H}-{3-(trifluoromethyl)-3*H*-diazirin-3-yl}-phenoxyacetyl} taxol* [**33**].—To 20 mg of the ^{2}H analogue of carboxylic acid **30** and 24.7 mg of 2′-triethylsilyltaxol in 1.0 ml of dry CH_2Cl_2 was added 16.0 mg of dicyclohexylcarbodiimide and a catalytic amount of DMAP. This was stirred at room temperature for 30 min. The CH_2Cl_2 was evaporated, and the residue purified by Si gel cc (30% EtOAc/70% hexanes) to yield **33** along with an inseparable impurity (24 mg, 80%).

*2′-Triethylsilyl-7-{{α-^{2}H}-{3-(trifluoromethyl)-3*H*-diazirin-3-yl}-phenoxyacetyl} taxol* [**34**].—Compound **33** (24 mg) was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 min. The solution was diluted with CH_2Cl_2, washed successively with H_2O and brine, dried over Na_2SO_4, and concentrated to yield the crude product. Purification by Si gel cc yielded the deuterated product **34** as a white solid (17 mg, 76%). ^{1}H nmr analysis revealed approximately 85% deuterium incorporation by integration under the methylene proton peaks. The diastereotopic methylene protons which appear as an AB quartet in **32** collapsed to 2 singlets in **34**. ^{1}H nmr 4.51 and 4.86 (ABq, J=16.20, $PhOCH_2CO_2R$), 4.50 and 4.85 (singlets), 5.62 (1H, m, H-7), 6.74 (1H, br s, ArH), 6.85 (1H, br dd, ArH), 6.95 (1H, dd, J=2.5, 8.4).

GENERAL METHOD FOR TRITIUM EXCHANGE REACTIONS. PREPARATION OF [α-^{3}H]-[3-(TRIFLUOROMETHYL)-3*H*-DIAZIRIN-3-YL]PHENOXYACETIC ACID.—A 100-ml flame-dried round-bottomed flask under argon was charged with 20 ml of THF and 1.0 g (3.65 mmol, 1.0 equiv) of ester **29**. This was cooled to −78°, and 8.15 ml (4.00 mmol, 1.1 equiv) of freshly prepared 0.5 M lithium diisopropylamine was added dropwise to the ester over a period of 2 min. After stirring for 15 min at −78°, a solution of ^3H_2O (0.20 ml, 1.0 Ci, 90 mCi/mmol) in 0.25 ml of hexamethylphosphoramide (HMPA) was added. The cooling bath was removed and the reaction stirred at ambient temperature for 1 h. The solution was then diluted with EtOAc, followed by H_2O. The solution was transferred to a separatory funnel, and the ETOAc layer was removed. The aqueous layer was washed with EtOAc twice and then acidified into fresh EtOAc with 1.0 N HCl. The organic layer was washed successively with H_2O and brine and dried over Na_2SO_4. Concentration in vacuo yielded the tritiated form of carboxylic acid **31** (600 mg, 63%), specific activity=5.4 mCi/mmol.

*2′-Triethylsilyl-7-{{α-^{3}H}-{3-(trifluoromethyl)-3*H*-diazirin-3-yl}-phenoxyacetyl} taxol* [**35**].—To 20 mg of ^{3}H-c arboxylic acid [**30**] and 24.7 mg of 2′-triethylsilyltaxol in 1.0 ml of dry CH_2Cl_2 was added 16.0 mg of dicyclohexylcarbodiimide and a catalytic amount of DMAP. This was stirred at room temperature for 30 min. The CH_2Cl_2 was evaporated, and the residue was purified by Si gel cc (30% EtOAc/70% hexanes) to yield **35** (26 mg, 84%), specific activity=3.8 mCi/mmol.

*7-{{α-^{3}H}-{3-(trifluoromethyl)-3*H*-diazirin-3-yl}phenoxyacetyl} taxol* [**36**].—Compound **35** (26 mg) was dissolved in 1.0 ml of 5% HCl in MeOH and stirred at room temperature for 15 min. The solution was diluted with CH_2Cl_2, washed successively with H_2O and brine, dried over Na_2SO_4, and concentrated to yield the crude product. Purification by Si gel cc yielded the tritiated product **36** as a white solid (18 mg, 78%), specific activity=2.55 mCi/mmol. The chromatographic profile matched exactly with that of non-tritiated compound **32**.

BIOCHEMICAL METHODOLOGY.—Purified bovine brain tubulin wa prepared as described previously

(46). Tubulin polymerization was followed by turbidimetry at either 350 or 420 nm in Gilford model 250 spectrophotometers equipped with electronic temperature controllers. Further details are described with the individual experiments.

ACKNOWLEDGMENTS

This work was supported by grant number CA48974 from the National Cancer Institute. We thank Professor J.S. Merola for the X-ray structure of compound **30**. Mass spectra were obtained by Mr. Kim Harich (Virginia Polytechnic Institute and State University) and the Midwest Center for Mass Spectrometry with partial support by the National Science Foundation, Biology Division (Grant No. DIR 9017262). We thank Dr. Kenneth Snader and the National Cancer Institute for the gift of a taxol/cephalomannine mixture.

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Received 28 December 1992