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REVIEW

Dedicated to Full Member of the Russian Academy of Sciences V. I. Minkin on his 70th Anniversary

Taxol: Synthesis, Bioactive Conformations, and Structure–Activity Relationships in Its Analogs

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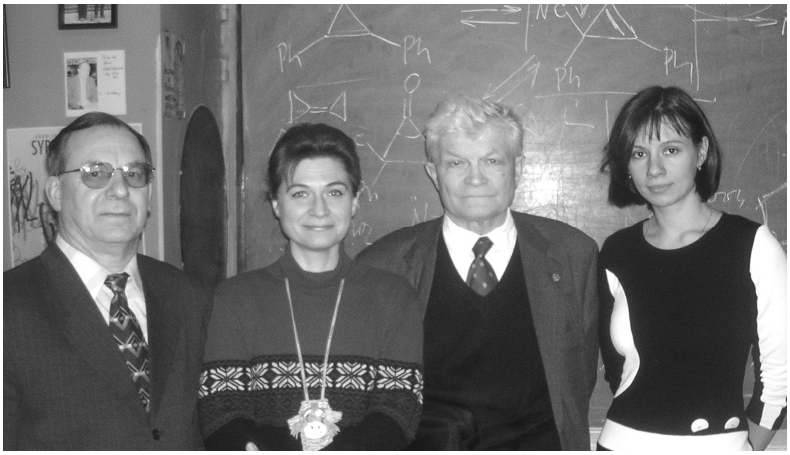
Abstract—The review describes the syntheses and probable biologically active conformations of taxol, a natural antitumor agent, and systematizes published data on the structure–activity relations in the series of taxol analogs.



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***Areas of research interest of the authors*:** organic and medicinal chemistry, computer simulation and quantitative structure–activity relationships in the design of physiologically active compounds.

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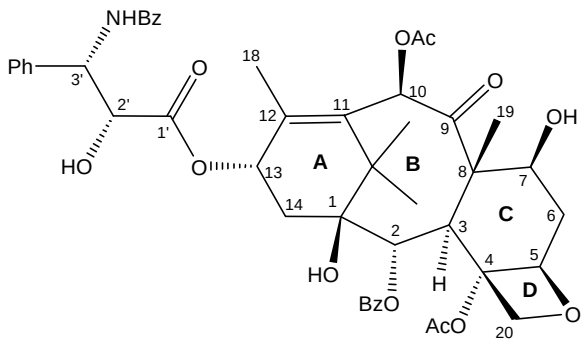
1. INTRODUCTION

Taxol or paclitaxel (**1**) is a diterpene alkaloid isolated from *Taxus brevifolia* bark. This compound and some its analogs occupy an important place among compounds exhibiting antitumor activity [1, 2]. The cellular target of their action is tubulin which is a protein capable of undergoing polymerization to give microtubules. The latter are important structures in cell division and formation of cellular cytoskeleton. Taxol induces uncontrolled polymerization of tubulin and stabilizes microtubules [3], thus preventing fast uncontrolled division of tumor cells. Some other compounds, e.g., *Vinca rosea* alkaloids are also capable of interacting with tubulin; however, unlike taxol, they inhibit the assembly of microtubules.



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In the last 10–15 years, a considerable number of studies were performed on the synthesis of taxol, its chemical modifications, and biological screening of taxol analogs. These studies were aimed at enhancing the antitumor activity and improving the pharmacological profile of the natural compound (mostly via improving the solubility in water). Although most reviews on taxol and its derivatives cover the corresponding pharmacological and medical aspects [4–7], a few articles systematizing relevant chemical information have been published in 1995–2001 [8–11]. However, the recent results of the X-ray diffraction study of tubulin (1998–2001), as well as studies performed in the past 3–4 years on refinement of bioactive conformations of taxol, allowed us to revise some structure–activity relations in the series of taxol and its analogs.

The present review describes the main strategies for the synthesis of taxol, systematizes the available data

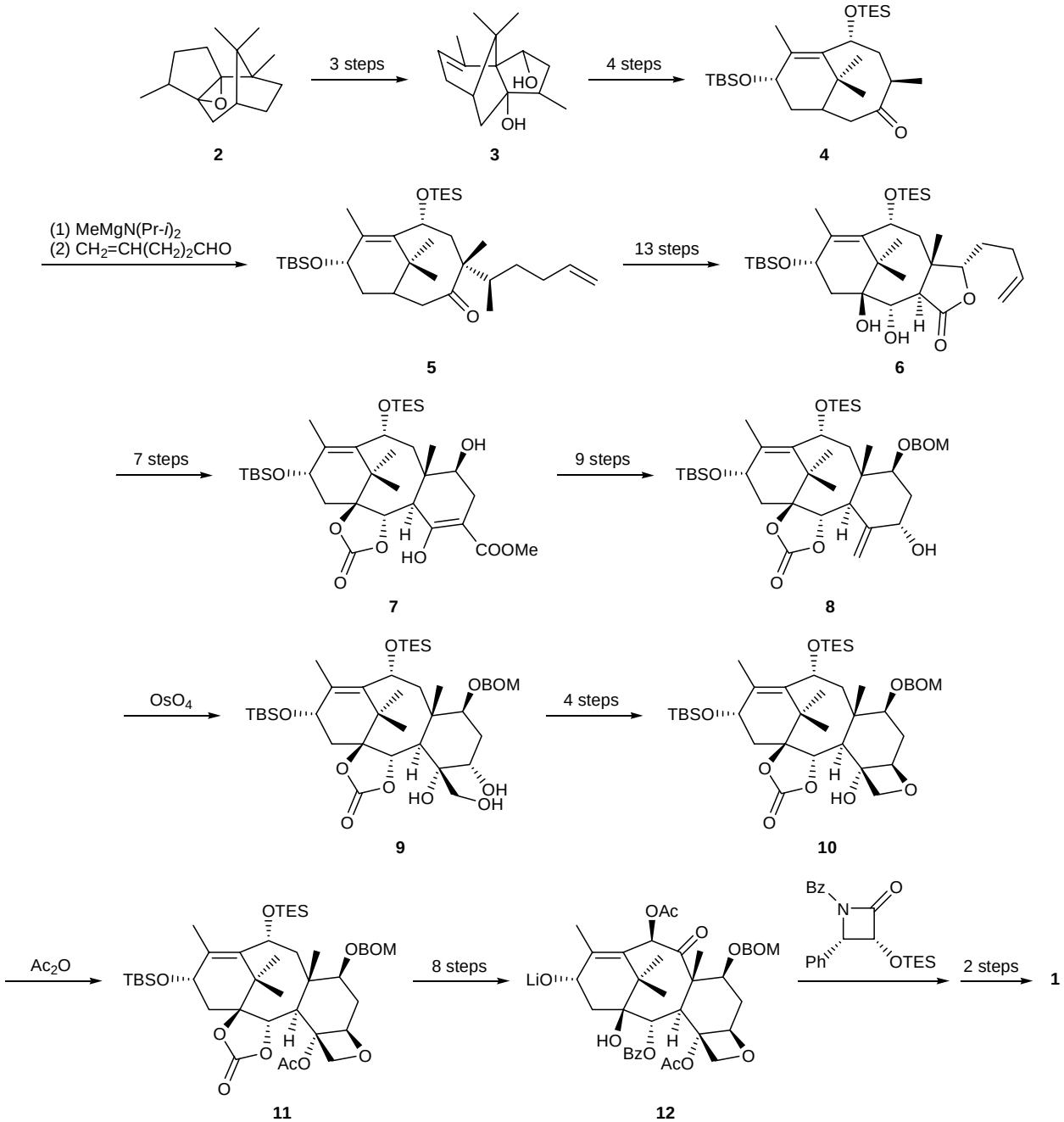
on its biologically active conformations, and analyzes structure–activity relations for analogs of taxol on the basis of both generally accepted views and newest data on its bioactive conformations.

2. SYNTHESES OF TAXOL

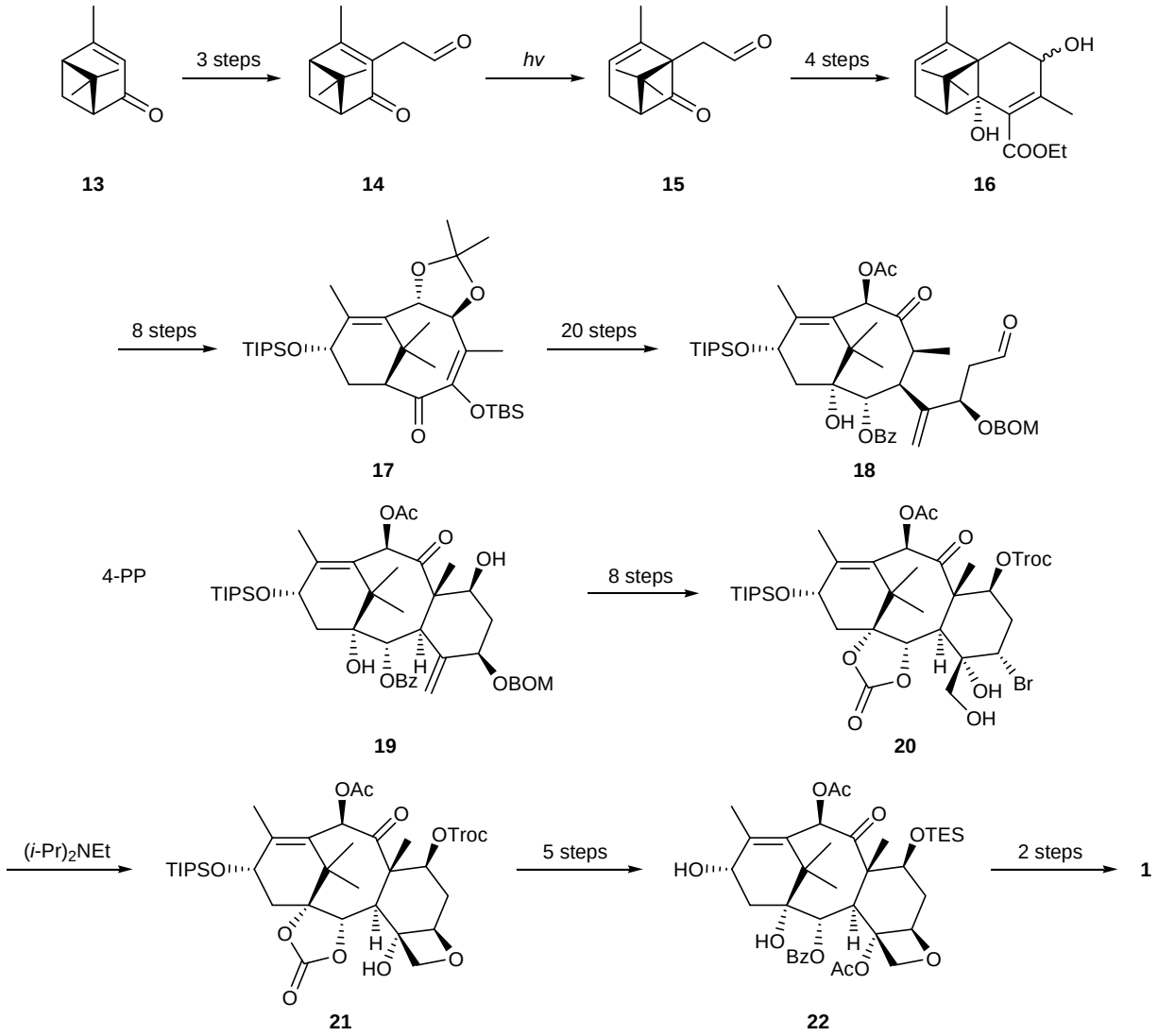
Molecule **1** is a complex polycyclic system having a large number of asymmetric carbon atoms. At present, six versions of the total synthesis of taxol (**1**) are known. Holton [12, 13], Wender [14, 15], and Mukaiyama [16] applied a "linear" approach implying successive building up of the taxol skeleton as shown in Schemes 1–3. It should be noted that different versions for performing particular steps were sometimes proposed by the authors; nevertheless, the general synthetic strategy remained unchanged.

In the Holton synthesis (Scheme 1), the starting compound was natural β-patchoulen (**2**) which was converted into tricyclic diol **3** [12, 17]. Diol **3** was

Scheme 1.



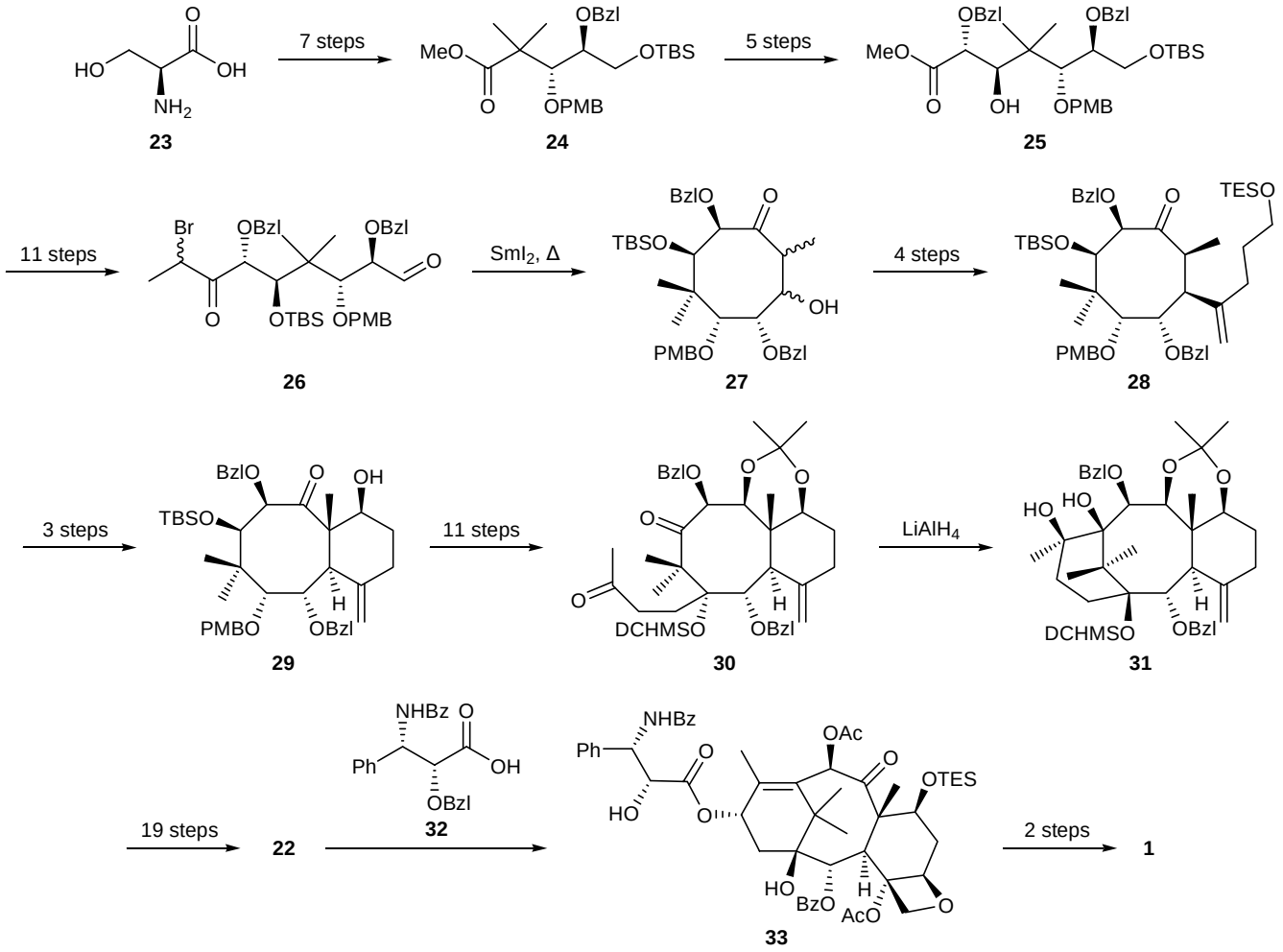
Scheme 2.



transformed into epoxy derivative, and ring **B** (structure **4**) was built up via intramolecular rearrangement. Compound **4** was brought into aldol condensation with 4-pentenal to obtain aldol **5**; a series of redox reactions involving the side chain in **5** gave lactone **6** as one enantiomer. Lactone **6** was then transformed in several steps (including the Dieckmann cyclization) into tricyclic system **7** containing ring **C** [12, 13, 18]. The subsequent reaction sequence was the most complex in the Holton synthesis. It included building up of the acetoxyoxetane fragment. For this purpose, enol ester **7** was converted into allyl alcohol **8** which was oxidized to triol **9** with OsO_4, and cyclization of **9** afforded structure **10** possessing the complete taxane skeleton [13, 19]. Acetylation of the hydroxy group on C^4 (hereinafter, the atom numbering corresponds to that accepted for natural taxol) and stepwise replacement of the protecting groups in **10** by appropriate substituents gave compound **12** [13]. The taxol side chain was attached to **12** via esterification with the corresponding β-lactam. Finally, removal of all protecting groups afforded taxol (**1**) in 4–5% yield, calculated on the initial diol **3** [13].

Wender synthesized taxol (**1**) according to Scheme 2 [14, 15], starting from verbenone (**13**). Compound **13** was converted into aldehyde **14** whose photochemical rearrangement gave compound **15**. The addition of **15** to ethyl propynoate afforded alkynoic acid which was transformed into tricyclic system **16** by treatment with lithium dimethylcuprate [15]. Compound **16** was subjected to a series of redox reactions, protection of the hydroxy groups, and chemoselective epoxidation of the double bond in the bicyclic fragment. The subsequent hydroxy–epoxy fragmentation afforded **AB**

Scheme 3.



ring system **17**. The required substituents were introduced into ring **B** via stereoselective reduction, oxidation, and isomerization, and modification of substituent in the eight-membered ring led to compound **18**. Intramolecular aldol condensation of **18** resulted in **ABC** ring system **19**. Ring **D** was built up in a way similar to the Holton synthesis, by oxidation of the corresponding double bond to diol; however, the hydroxy group on C^5 was preliminarily replaced by bromine (compound **20**). Intramolecular S_N2 substitution of the bromine atom gave compound **21** possessing an oxetane fragment [14, 15, 18, 20–26]. Acylation of the hydroxy group on C^4 and removal of protecting groups afforded compound **22**, and the taxol side chain was attached to **22** as in the Holton synthesis [13–15].

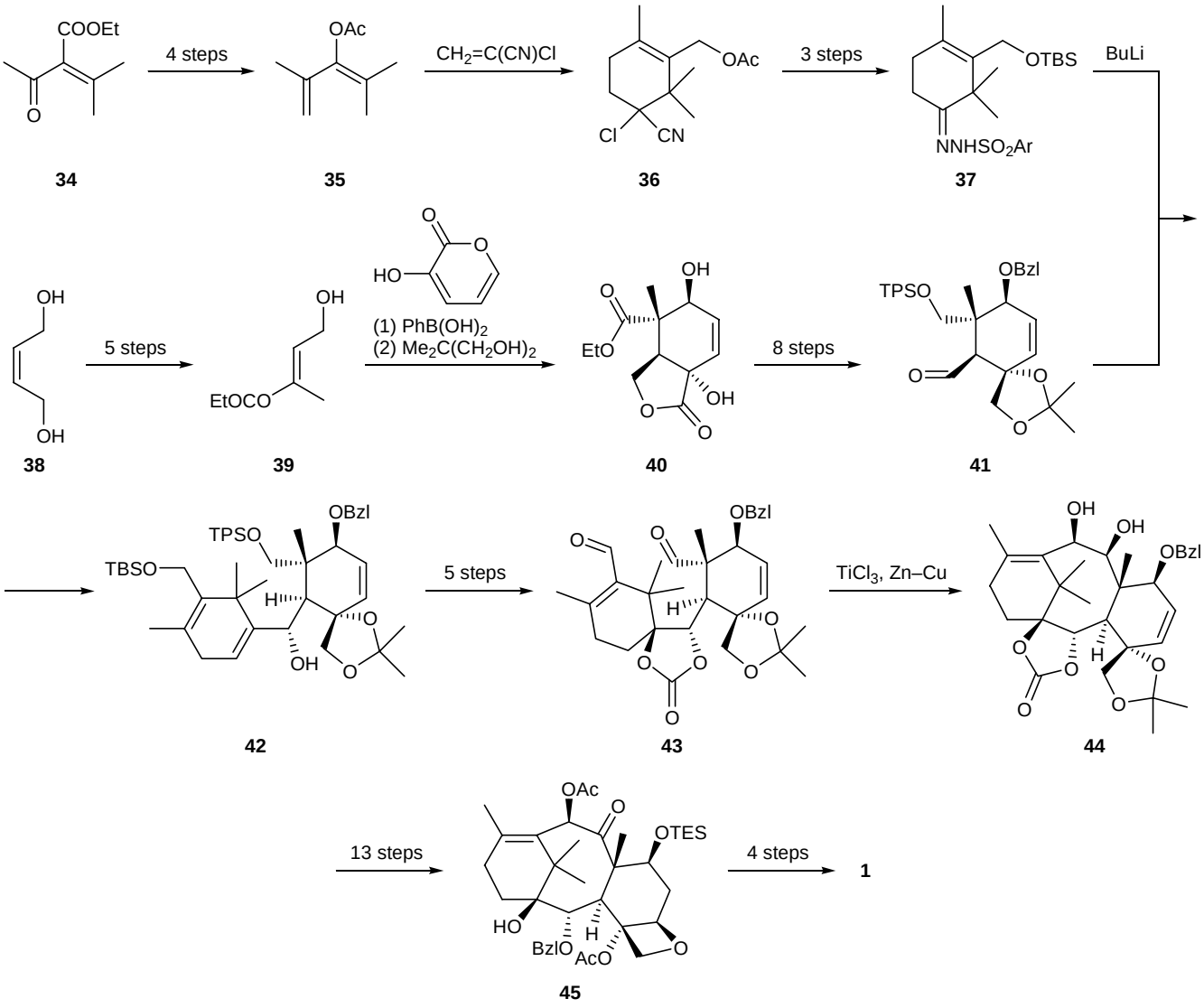
Mukaiyama also used the linear strategy (Scheme 3); however, completely functionalized ring **B** was built up initially from simple starting materials [27–31]. In the first stage, L-serine (**23**) was extended to five- (**24**) and seven-membered carbon chain (**25**) by condensation of intermediate aldehydes with various reactants. After a series of modifications of functional groups and bromination, β-hydroxy ester **25** was converted into keto aldehyde **26**, and cyclization of the latter by the action of SmI_2 gave functionalized ring **B** (structure **27**). Introduction of a double bond into **27**, followed by Michael reaction, afforded β-substituted cyclooctanone **28** which was subjected to intramolecular ring closure. The resulting structure **29** is an analog of the **BC** ring system in taxol. Introduction of an allylmethyl group into the eight-membered ring of **29**, followed by oxygenation of the double bond in the allylmethyl group, gave diketone **30**. Intramolecular cyclization of the latter afforded diol **31** containing completed ring **A**. Modification of functional groups in the tricyclic system and building up ring **D** (as in the Wender synthesis) resulted in formation of compound **22** [16, 32–48] which was brought into reaction with *O*-benzyl-*N*-benzoylphenylisoserine (**32**) in the presence of bis(2-pyridyl) thiocarbonate to

obtain 2'-benzyl-7-triethylsilyltaxol (**33**). Derivative **33** was converted into taxol (**1**) in two steps [16].

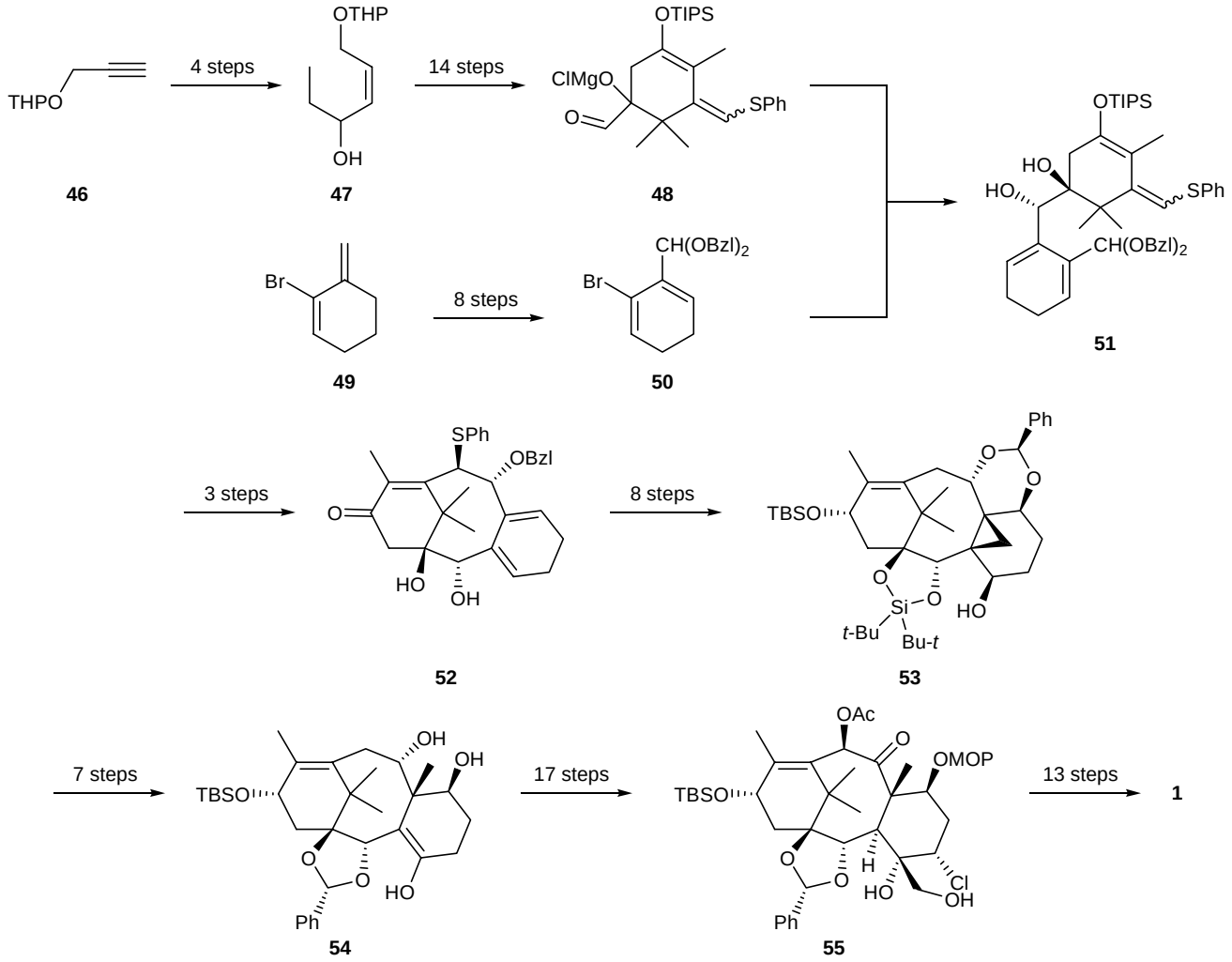
Another three syntheses (Nicolaou [49], Danishefsky [50], and Kuwajima [51]) utilize the convergent approach which implies preliminary assembly of the main molecular fragments and their subsequent junction (Schemes 4–6). According to Nicolaou (Scheme 4), rings **A** (**37**) and **C** (**41**) were built up separately via Diels–Alder reactions from keto ester **34** and 2-butene-1,4-diol (**38**) [52, 53], respectively. Rings **A** and **C** were linked together by the Shapiro reaction; as a result, structure **42** containing elements necessary for building up ring **B** was obtained [49]. Ring **B** was closed via a series of redox reactions leading to dialdehyde **43** and its intramolecular condensation to structure **44** [54–63]. Modification of the two hydroxy groups in ring **C** resulted in formation of ring **D** in a way similar to the Holton synthesis. Removal of protecting groups and introduction of appropriate substituents to C^2 and C^4 led to compound **45** which was oxidized at C^{13} to attach the side chain by the β-lactam technique [49, 64–67].

In the Kuwajima synthesis [51, 68–77] (Scheme 5), synthon **A** was obtained from protected acetylenic alcohol **46** via nucleophilic addition, followed by hydrogenation. Unsaturated alcohol **47** thus formed was subjected to intramolecular ring closure, a series of oxidation reactions, asymmetric dihydroxylation according to Sharpless, and introduction of a phenylsulfanyl group, which afforded aldehyde **48**. Ring **C** was built up on the basis of 2-bromocyclohexenone (**49**) which was converted in 8 steps into cyclohexa-

Scheme 4.



Scheme 5.



diene derivative **50**. The latter was transformed into the corresponding lithium enolate, and nucleophilic addition to aldehyde **48** gave compound **51** with rings **A** and **C** linked together. Cationic cyclization of **51** by the action of $TiCl_2(OPr\text{-}i)_2$ resulted in tricyclic structure **52** having an **ABC** skeleton. Modification of ring **C** included introduction of a methyl group to C^8 through intermediate cyclopropane derivative **53** where the three-membered ring was cleaved by the action of SmI_2. The oxetane fragment in **54** (ring **D**) was built up as in the Wender and Mukaiyama syntheses with the difference that a chlorine atom was introduced instead of bromine to the α-position with respect to the diol fragment (structure **55**). In the final steps, modification of the substituents and addition of the side chain to C^{13} using β-lactam technique were performed [51, 68–77].

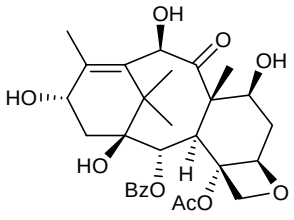
The Danishefsky synthesis (Scheme 6) utilized the **A**+**CD** → **ABCD** scheme, according to which ring **D** was completed before convergence [50]. 2,2,6-Trimethylcyclohexane-1,3-dione (**56**) was converted in several steps into lithium derivative **57** (synthon **A**). The **CD** fragment was obtained from Wieland–Misher ketone (**58**) which was subjected to a redox reaction sequence to obtain triol **59**. Compound **59** was transformed into structure **60** with completed ring **D**. Oxetane derivative **60** was modified by protecting functional groups, oxidized twice (as a result, the second cyclohexane ring was opened), and converted into aldehyde **61**. The **A** and **CD** fragments were linked together via nucleophilic addition of **57** to **61**, followed by removal of the trimethylsilyl protection. The next steps included removal of the acetal protection from tricyclic compound **62** and Wittig reaction of the aldehyde thus formed to obtain derivative **63**. The subsequent intramolecular cyclization according to Heck gave diene **64** with completed ring **B** [50, 78, 79]. Introduction of appropriate substituents

Scheme 6.

led to compound **45** which was converted into taxol (**1**) in a way similar to the Nicolaou synthesis [49, 50].

Apart from the development of total synthetic schemes, attempts were made to synthesize specific structural fragments of the taxol molecule and its analogs. For example, new procedures were proposed for building up ring **A** [80–82], ring **B** [83–85], and **BC** fragment [86] as intermediates in the Mukaiyama synthesis. Shing *et al*. [87] obtained the **CD** fragment in 21 steps starting from (*S*)-(+)-carvone, while Toyota *et al*. [88] synthesized the **AB** fragment from bicyclo-[3.2.1]octane derivative. However, despite much efforts directed toward reducing the number of steps and using accessible reagents, each scheme of the total synthesis of taxol requires tremendous time, the overall yield of the target product being 1–5%.

For commercial purposes, taxol is prepared according to a semisynthetic procedure from natural 10-deacetylbaccatin III (**65**) to which (2*R*,3*S*)-*N*-benzoylphenylisoserine residue (side chain) is attached at a certain step [89]. Likewise, most taxol analogs with a modified side chain are prepared (see below).



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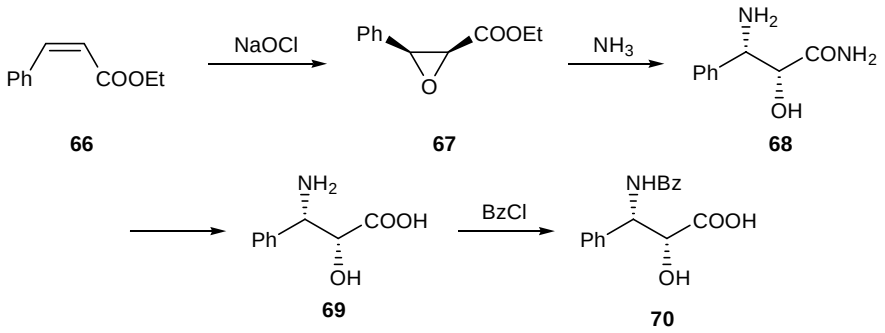
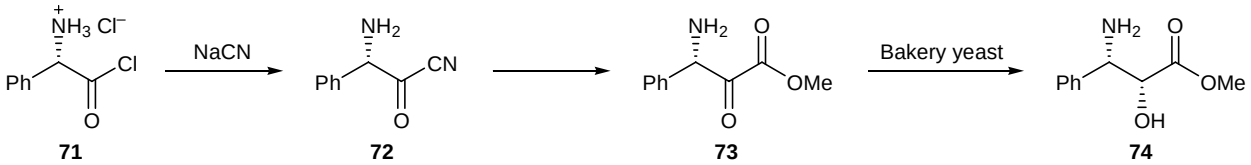
The main procedures for the synthesis of (2*R*,3*S*)-*N*-benzoylphenylisoserine include (1) successive modification of cinnamic acid derivatives [90–93], e.g., as shown in Scheme 7 [94]; (2) reactions leading to extension of the carbon chain in the initial compounds

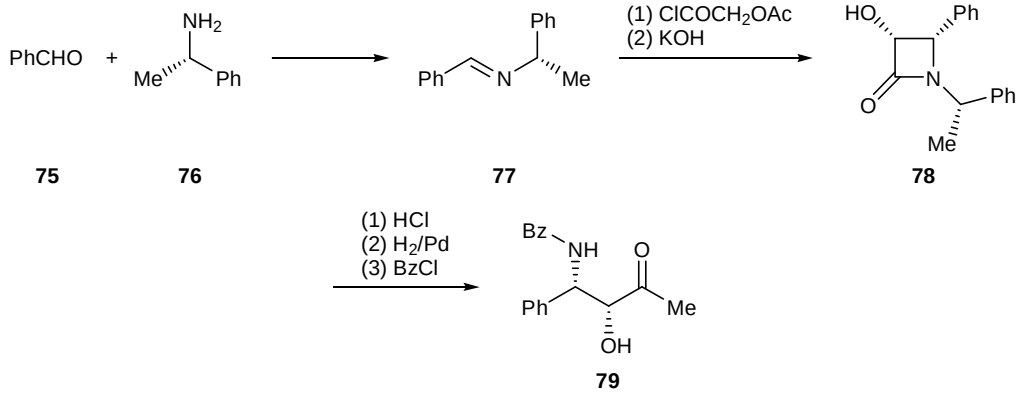
Scheme 7.



Scheme 8.

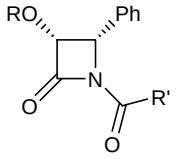


Scheme 9.



[95–97], e.g., according to Scheme 8 [98]; and (3) reactions involving intermediate formation of chiral substituted β-lactams [99–107], e.g., according to Scheme 9 [101, 102].

Development of efficient catalysts and reagents [108–112] and the use of special supports [113] and enzymatic systems [114, 115] made it possible to perform a number of key steps in the synthesis of the side chain with high chemical and enantiomeric yields [116, 117]. The side chain is attached to the 13-hydroxy group in **65** via esterification. Taking into account that conventional esterification with protected (2*R*,3*S*)-*N*-benzoylphenylisoserine and its analogs is characterized by poor yield because of sterically hindered approach to the 13-OH group [118], cyclic protected derivatives are often used for this purpose, e.g., β-lactams **80** [71, 119] (this method is utilized in the commercial synthesis of taxol), oxazolidines **81** [120, 121], dihydrooxazoles **82** [122], and others [11, 117]. Effective methods for the preparation of the corresponding reagents have been developed in the recent years [11, 94, 123].



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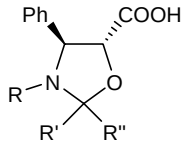

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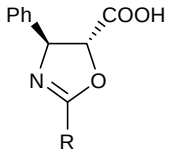

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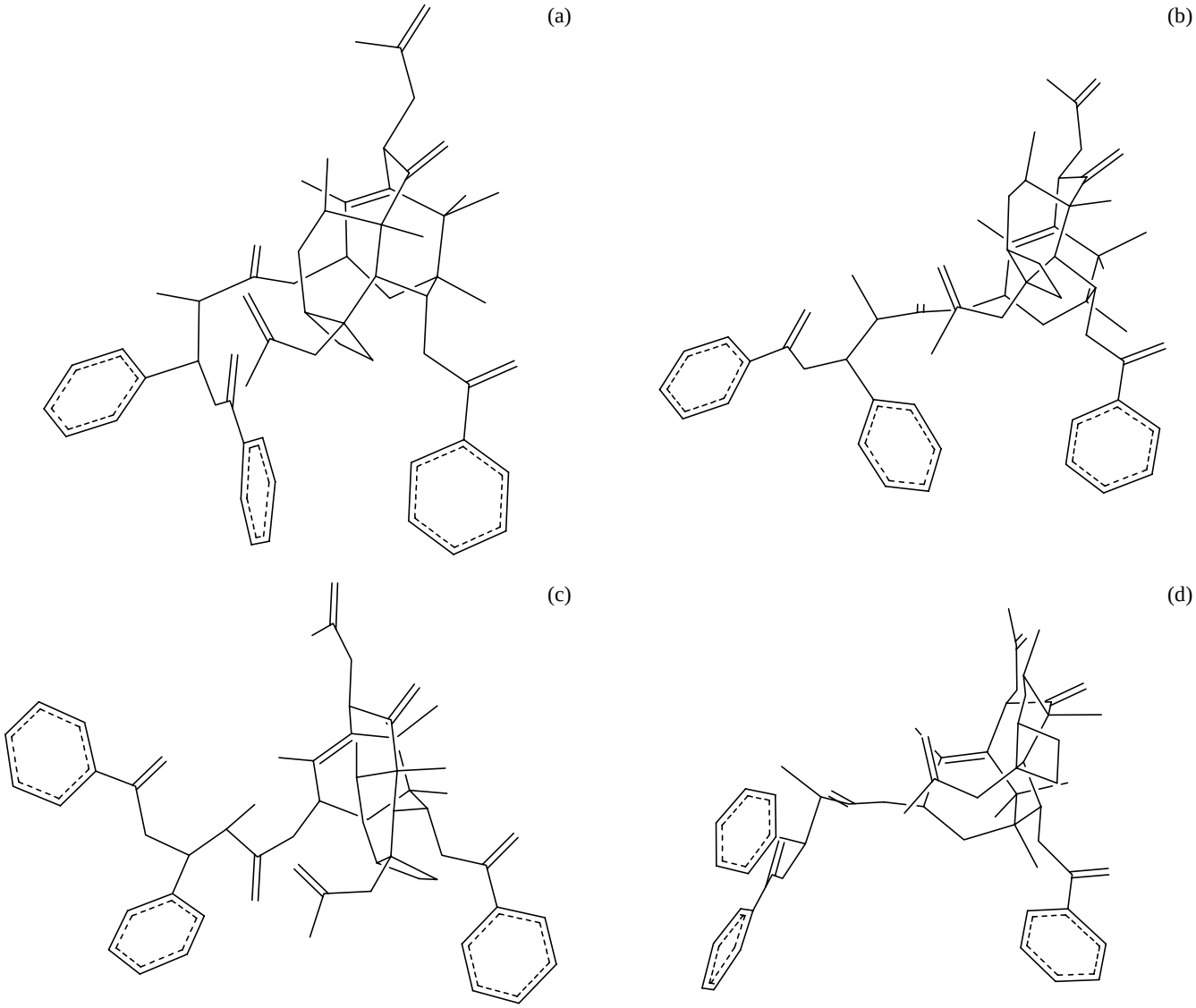
To conclude this section, it should be noted that development of the total syntheses of taxol, including procedures for the preparation of the side chain and its

addition to the taxane skeleton, was very important for elaboration of methods for the synthesis of a wide series of taxol analogs.

3. BIOLOGICALLY ACTIVE CONFORMATIONS OF TAXOL

The antitumor activity of taxol originates from its binding to tubulin. Though taxol also interacts with Bcl-2 protein and induces hyperphosphorylation of the latter [124, 125], it was recently shown that this process is likely to result from complex formation between taxol and tubulin [126]. The site of binding of taxol to tubulin (which is an αβ-dimeric protein) is located on the β-subunit. In outline, the steric structure of the binding site was determined by electronic crystallography [127, 128] and photoaffinity labeling [129, 130]: It is a deep hydrophobic pocket near the protein surface. Nevertheless, up to now there are no common views on the conformation of taxol in the bioactive form. Obviously, the presence of several freely rotating fragments in the four flexible chains attached to the rigid taxane skeleton could give rise to numerous conformations. Attempts to reveal bioactive conformers among them were made by crystallographic and NMR studies.

The first bioactive conformation proposed for taxol and some its analogs was termed *nonpolar* (in some publications this conformation is referred to as *open* which, in our belief, is inappropriate), for it was detected by NMR experiments with compound **1** in



Structures of some taxol conformers: (a) nonpolar, (b) polar, (c) open, and (d) T-shaped (butterfly).

nonpolar solvents. This conformation is characterized by hydrogen bonding between the ester carbonyl group ($C^{1'}$=O), hydroxy group on $C^{2'}$, and side-chain NH group, as well as by hydrophobic interactions between the benzoyl group on C^2 and benzoylamino group on $C^{3'}$ (see figure, a). Here, the 3'-phenyl group is remote from the taxane core [131–135].

However, more recent studies showed that taxol molecule changes its conformation in going to polar solvents. The revealed conformation (which was called *polar* or *hydrophobic collapse*) is characterized by hydrophobic interactions between the benzoyl group on C^2, phenyl on $C^{3'}$, and acetyl on C^4, while the substituent at the amide group (phenyl group in taxol) is not involved in these interactions (see figure, b) [132, 136–138]. Several years later, the first crystallographic study of αβ-tubulin heterodimer (performed with a poor resolution) has been reported [127, 137]; as a result, its steric model has been constructed [the coordinates are now deposited to the Protein Data Bank (PDB)]. The results of a series of studies on docking of taxol into the above model and other works [129, 138, 139] indicate that taxol binds to the biotarget just in the *hydrophobic collapse* conformation.

Nevertheless, the existence of other possible bioactive taxol conformers was presumed in the recent publications. Using the NAMFIS (**N**MR **A**nalysis of **M**olecular **F**lexibility **i**n **S**olution) technique, Snyder *et al*. [140] revealed 8 optimized conformers, including *open* forms (see, e.g., figure, c), which lacked hydrophobic interactions between the benzoyl group on C^2 and any side-chain phenyl group. The authors presumed that in these conformations substituents in the extended taxol side chain interact with the amino acid residues of tubulin rather than with each other.

In 1999, a high-resolution model of the microtubule has been obtained by docking the crystal structure of tubulin into a 20-Å map of the microtubule [130, 138]. Docking of the most probable bioactive taxol conformers into the experimental electron density map of the refined tubulin model showed that in most of the examined conformers, including *polar*, *nonpolar*, and some *open* forms, the side-chain benzene rings appear in the domain possessing almost no electron density [137]. The optimal conformer is characterized by the C^2-benzoyl phenyl ring nearly equidistant from both benzene rings emanating from $C^{3'}$ (see figure, d) [137]. Docking of this conformer (T-taxol or *butterfly* structure) into the refined tubulin model gave rise to a structure in which the imidazole ring of some histidine residue (βHis 229) intervenes between the $C^{3'}$-phenyl and C^2-benzoyl moieties, thus preventing the taxol molecule from hydrophobic collapse. The tubulin–T-taxol model is very consistent with the results of affinity labeling study of β-tubulin; however, this model cannot rationalize some mutations in the tubulin structure in tumors resistant to taxol [137].

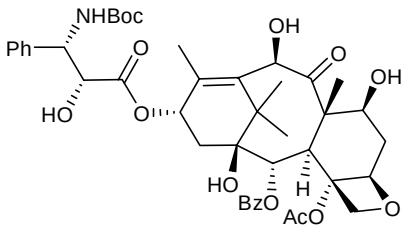
Unambiguous determination of bioactive conformations of taxol still remains a very important problem in the design of taxol analogs. Although some models agree well with the results of structure–activity studies, none of these can explain all the observed relations (see below).

4. STRUCTURE–ACTIVITY RELATIONS IN THE SERIES OF TAXOL ANALOGS

While considering the structure–activity relations, as a measure of the activity we used the ability of a taxol analog to promote polymerization of tubulin with formation of microtubules (or inhibit their depolymerization), for just that parameter is determined by binding to tubulin and the binding efficiency can be predicted by computer simulation. In some cases (for the sake of comparison or because of the lack of relevant data), the cytotoxicity of a compound was used. However, it should be kept in mind that these parameters do not necessarily correlate with each other since the cytotoxicity depends not only on the affinity of a taxol analog for tubulin but also on its ability to penetrate through cell membrane, resistance to metabolic enzymes, and other factors.

4.1. Taxotere

While developing a procedure for the synthesis of taxol from 10-deacetylbaccatin III (**65**), it was found that one of the intermediate products, taxotere (**83**) exhibits a higher activity (by a factor of 1.5–1.7) and a higher cytotoxicity against some tumor cells, as compared to natural taxol [141]. Taxotere is now the only taxol analog which is used in medical practice. Just for that reason, it is also used as a base structure for various modifications.



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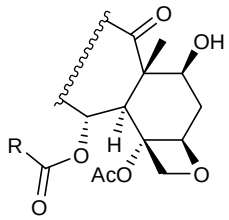
4.2. Variation of Substituents in the Taxane Core

4.2.1. Variations at position *1*. Up to now, synthesis of 1-deoxytaxol has not been reported, though Guo and Paquette [142] noted a three-fold decrease in the cytotoxicity of this compound which was isolated from natural sources as an impurity to taxol. The results of testing of 1-deoxy analogs of taxol with different substituents on C^7 and C^9 (see below) indicated a quite useful (though not very strong) effect of the hydroxy group on C^1 on binding to β-tubulin [11].

4.2.2. Variations at position *2*. Removal of the substituent at C^2 or its migration to the neighboring position (to give 1-benzoyl-2-debenzoyloxytaxol) leads to complete loss of activity [143, 144]. The same result or reduction in the activity is usually obtained by dearomatization of the substituent at the carboxy group. The activity of compounds **84** [R = Me_2C=CH, MeCH=CMe, CH_2=CHCMe_2CH_2, MeCH_2CMe_2CH_2) is lower by about an order of magnitude, while replacement of the benzoyl group by acetyl, valeryl or isovaleryl (**84**, R = Me, Bu, *i*-PrCH_2) results in complete loss of activity [145–148]. These data suggest an important role of phenyl group in the substituent on C^2 for binding to tubulin, which was interpreted by some authors in terms of participation of that group in the formation of *hydrophobic collapse* conformer. According to Gao and Parker [149], this conformation was found for taxol in crystal but not for its inactive analog, 2-debenzoyloxy-2-acetoxytaxol (**84**, R = Me).



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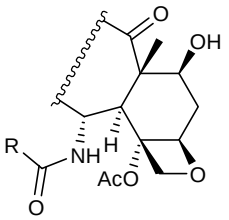


85

However, there are published data demonstrating that the presence of a phenyl group in the C^2-substituent is not a necessary condition for efficient binding to the protein. Replacement of the benzoyl group in taxol and taxotere by cyclohexylcarbonyl (**84**, R = cyclohexyl) almost did not change the activity, while the cytotoxycity toward some tumor cells *in vitro* decreased approximately by an order of magnitude [145, 150, 151]. The fact of conservation of high activity in the cyclohexyl analogs was used as an evidence in favor of the model proposed in [137], where the phenyl ring in the C^2-substituent resides in the hydrophobic area but is not involved in hydrophobic collapse (see above).

Attempted replacement of the benzoyl group in taxol by nonbenzoyl aromatic analogs had a limited success. The activity and cytotoxicity of compounds **84** [R = PhSCH_2CH_2, 2-furyl, 3-pyridinio (*p*-toluenesulfonate), 1-naphthyl, PhCH_2CH_2, PhCH=CH, PhOCH_2] either were considerably lower or disappeared at all. Among the examined heterocyclic analogs, only 3-thenoyl derivative **84** (R = 3-thienyl) showed an activity comparable to that of taxol. Nitrogen-containing analogs **84** (R = 1-methyl-2-pyrrolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl) were less active than taxol by factors of 2.6, 2.8, 4, and 7.3, respectively, and only the first two of these showed an appreciable cytotoxicity [152]. Published data on furan derivatives **84** (R = 2-furyl, 3-furyl) and thiophene analog **84** (R = 2-thienyl) are contradictory [152–154]; however, in either case these compounds turned out to be less active than taxol.

Much greater success was achieved by varying substituents in the C^2-benzoyl phenyl ring [153–157]. Here, the following interesting relations were found. Introduction of small *meta*-substituents often resulted in an appreciable increase (3-OMe, 3-N_3, 3-Cl, 3-CN) or small decrease (by a factor of 1.1–2.7; 3-OEt, 3-SMe, 3-CF_3, 3-NO_2, 3-Me) of the activity and cytotoxicity relative to the natural compound; the 3-F substituent did not change these parameters. Analogs with large *meta*-substituents (3-OPr, 3-OPr-*i*, 3-OCH_2Ph, 3-OPh) almost failed to bind to tubulin. It should be noted that, apart from the size of *meta*-substituent, other factors (probably, electronic) may be important. Some analogs having relatively small groups, such as 3-OH and 3-COMe, are weakly active, while halogen-substituted derivatives exhibit a stronger (3-Br) or equal (3-F) tubulin-polymerizing ability and cytotoxicity [153, 155]. A fairly strong activity was found for analogs with two *meta*-substituents: it was slightly greater than that of taxol for 3,5-$(N_3)_2$, 3,5-F_2, and 3,5-Cl_2 derivatives and lower by a factor of 3–4 for 3,5-$(MeO)_2$- and 3,5-$(O_2N)_2$-substituted analogs.

Among derivatives with a substituent in the *ortho*-position of the benzene ring at C^2, only the 2-azido analog showed an activity comparable to taxol; the other compounds were less (2-Me, 2-OMe) or much less active (2-F, 2-Cl). Simultaneous introduction of both *ortho*- and *meta*-substituents improved the polymerizing ability: 2,3-F_2, 2,5-F_2, and 2,5-$(MeO)_2$ analogs were more active than taxol; however,

2,3-$(MeO)_2$ derivative turned out to be almost inactive. The cytotoxicity of the two 2,5-disubstituted compounds was higher than that of analogous 2,3-disubstituted derivatives [153, 155].

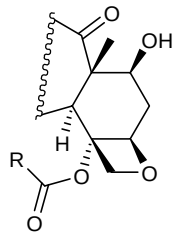
In all cases, *para*-substituted analogs (4-MeO, 4-$PhCH_2O$, 4-F, 4-Cl, 4-CN, 4-HO, 4-N_3, 4-Me, 4-O_2N) either were characterized by considerably lower activity and cytotoxicity or were completely inactive [153–155]. Moreover, introduction of a *para*-substituent into very effective analogs with *meta*- and *ortho*-substituents impaired their binding to tubulin: 2,4,5-F_3, 3,4,5-$(MeO)_3$, 3,4-F_2, and 3,4,5-F_3 derivatives were less active than taxol; an exception was 3,4-Cl_2 analog whose activity remained higher than that of the natural compound.

The fact that *meta*-substitution of the C^2-benzene ring enhances the activity [129] was treated as an evidence in favor of the model implying binding of taxol as *hydrophobic collapse* and related conformations. In this model, the nitrogen atom in 2-(*m*-azidobenzoyl)taxol resides in the vicinity of the His229 imidazole ring, in keeping with the experimental data [129]. However, increase in the activity upon *meta*-substitution and its reduction on introduction of *para*-substituents into the C^2-benzoyl fragment are also consistent with the tubulin–T-shaped taxol model [137] in which the hydrophobic subunit incorporating this fragment is bounded by the three sides of the ring but is open in the vicinity of one of the *meta*-substituents.

A number of 2-amido analogs **85** of taxotere have recently been synthesized. Replacement of the ester moiety on C^2 by benzamido (**85**, R = Ph) or benzyloxycarbamoyl group (**85**, R = $PhCH_2O$) leads to a 15–30-fold reduction in cytotoxicity [158, 159]. As in the taxol series (see above), introduction of a *para*-substituent into the C^2-benzene ring (**85**, R = *p*-$MeOC_6H_4$, *p*-ClC_6H_4, *p*-$O_2NC_6H_4$) results in almost complete loss of cytotoxicity, whereas *meta*-substitution (**85**, R = *m*-$MeOC_6H_4$, *m*-ClC_6H_4) enhances the cytotoxicity approximately twofold as compared to unsubstituted derivative **85** (R = Ph); in any case, the cytotoxicity of substituted analogs is lower by an order of magnitude than that of taxol and taxotere [158].

A conclusion can be drawn that all fragments of the benzoyloxy group on C^2 are important for binding to tubulin and that *meta*-substitution of the aromatic ring therein often gives rise to highly active analogs.

4.2.3. Variations at position *4*. 4-Deacetyltaxol [160] and 4-deacetoxytaxol [161] turned out to be less active than taxol by 2–3 orders of magnitude; this means that the carbonyl group is important for binding to tubulin [162, 163]. Replacement of the acetyl group at C^4 by β-alanine or glutaric acid residue also afforded compounds possessing no cytotoxic properties (the activity was not measured) [164]. Until present, a fairly wide series of analogs containing various ester groups at the 4-position has been examined. Most of these exhibit an activity at the same level as taxol: the activity is slightly lower (by a factor of 1.04–3.4) for compounds **86** with R = FCH_2, Et, Bu, C_5H_{11}, and cyclopentyl and slightly higher (by a factor of 1.5–4) for R = CH_2=CH, *i*-Pr, CCl_3, MeCH=CH, Pr, CH_2=CMe, cyclobutyl, and cyclopropyl. Analog **86** (R = cyclopropyl) showed the maximal activity and cytotoxicity in the given series [162] and was twice as active as its acyclic analog **86** (R = *i*-Pr), indicating a considerable role of even "insignificant" hydrophobic interactions in the binding to tubulin. Compounds having bulky aromatic groups (**86**, R = *p*-FC_6H_4, Ph) were much less active than taxol (by two orders of magnitude and more).



86

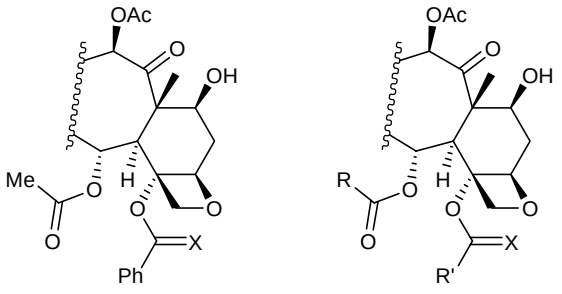
The above data suggest that the methyl group in the 4-acetoxy moiety may be replaced by longer alkyl chains without appreciable loss in activity [162]. This is consistent with the tubulin–T-shaped taxol model in which the C^4-methyl group is located above a deep hydrophobic cleft formed by ten hydrophobic amino acid residues [137].

Introduction of carbonate or carboxamide moieties into position *4* of taxol molecule either increases (**86**, R = PrO, MeO, EtO, BuNH; by a factor of 1.5–2.4) or slightly reduces the activity (**86**, R = 1-aziridinyl; by a factor of 2.8) [162, 163, 165]. Obviously, electronic properties of the terminal group in the substituent on C^4 constitute an important factor, for compound **86** (R = 1-aziridinyl) is less active than its alicyclic analog **86** (R = cyclopropyl) by an order of magnitude. On the other hand, the activity of the aziridine derivative can be enhanced fivefold via slight modification of the taxol side chain, namely by replacement of the phenyl group on $C^{3'}$ by 2-furyl [166], though similar modification of the other C^4-analogs almost does not

affect their activity (or increases it to an insignificant extent) [162] (see also side-chain analogs).

4-Imidazolylcarbonyloxy analog **86** (R = imidazolyl) is almost inactive. Most probably, this is associated with the electron-acceptor character of the imidazole group rather than with its size (cf. **86**, R = cyclopentyl), which weakens interaction between the carbonyl group and the corresponding amino acid residue in the protein. It should be noted that compound **86** (R = MeO) is now under clinical trials [164].

4.2.4. Simultaneous variations of substituents in positions *2* and *4*. 2-Acetoxy-4-benzoyloxytaxol (isotaxol, **87**, X = O) exhibits neither activity nor cytotoxicity, in keeping with the relations observed upon separate variation of each substituent [167]. Taking into account the high activity of taxol analogs with methoxy- or cyclopropylcarbonyloxy group on C^4 and *meta*-substituted benzoyloxy group on C^2 (see above), Chordia *et al*. [165] made an interesting attempt to combine these modifications in a single molecule. Methoxycarbonyl analogs **88** (R' = MeO, R = *m*-MeO-C_6H_4, *m*-MeC_6H_4, *m*-ClC_6H_4) turned out to be more active than taxol. The cytotoxicity of these compounds, as well as of those with R' = OMe, R = *m*-$N_3C_6H_4$ or R' = Me_2C=CH, was equal to or slightly lower (by a factor of no more than 3) than that of the natural substance. Methylsulfanyl(thiocarbonyl) analogs **89** (R = *m*-N-$_3C_6H_4$, *m*-ClC_6H_4Cl, *m*-$MeOC_6H_4$) are less active than the corresponding methoxycarbonyl derivatives approximately by an order of magnitude, presumably due to a larger size of the C^4-substituent. 2,4-Modified analogs with a bulky substituent on C^4 (**88**, R = R' = *m*-ClC_6H_4; R' = *t*-BuO, R = *m*-$MeOC_6H_4$) are almost inactive [165]. These data support the above assumptions concerning the size of substituent on C^4.



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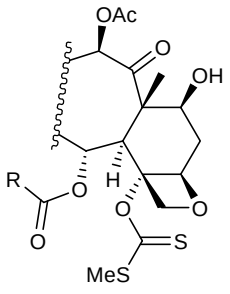

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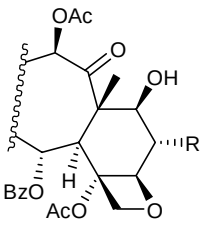
The most interesting results were obtained for a series of 4-cyclopropylcarbonyloxy derivatives **88** (R' = cyclopropyl, R = *m*-$N_3C_6H_4$, *m*-ClC_6H_4, etc.); many of these compounds showed a fairly distinct additivity in the contributions of each substituent (at C^2 and C^4) to the activity. As compared to taxol, the activity of the C^2-*m*-$N_3C_6H_4$ analog was higher by a factor of 3, and of C^4-derivative **86** (R = cyclopropyl), by a factor of 4; the activity of "combined" C^2,C^4-analog **88** (R' = cyclopropyl, R = *m*-$N_3C_6H_4$) was higher by a factor of 12. Despite the strong tubulin-polymerizing ability of the examined 4-cyclopropylcarbonyl derivatives (the most active was compound **88** with R' = cyclopropyl and R = *m*-ClC_6H_4; its activity exceeded that of taxol by a factor of 16), their cytotoxicity was comparable with that of taxol [165].

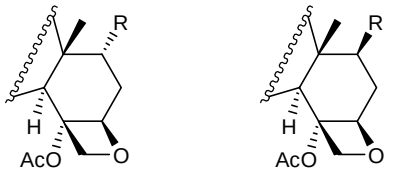
4.2.5. Variations at position *6*. The main metabolic reaction of taxol in humans is hydroxylation of the C^6 carbon atom to give 6α-hydroxytaxol (**90**, R = OH) which is 30 times less active than the parent compound [168]. Other substituents were attached to C^6 with the goal of preventing this reaction and improving pharmacokinetic parameters of the resulting drug. Replacement of hydrogen on C^6 in taxol by a halogen atom (**90**, R = F, Cl, Br) did not lead to an appreciable change in activity and cytotoxicity, but metabolism of these derivatives *in vivo* was blocked to some extent (no 6α-hydroxy metabolites were detected in experiments with a fraction of liver cells capable of hydroxylating taxol) [169]. The cytotoxicity of 6-fluoro-10-acetyltaxotere was higher than those of taxol and 10-acetyltaxotere by an order of magnitude, and the time of its action *in vivo* was longer [169].



90

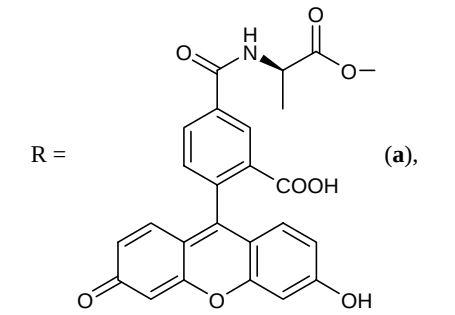
4.2.6. Variations at position *7*. 7-Deoxytaxol is only slightly less active than taxol, though its cyto-

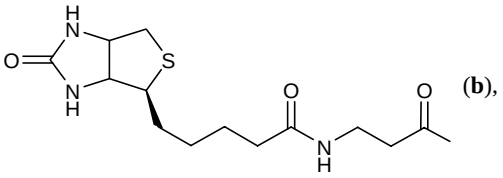
toxicity is the same (or somewhat higher, depending on the type of cells). This suggests an insignificant contribution of the 7-hydroxy group to complex formation with tubulin [170–173]. The activity and cytotoxicity of 7,10-dideoxy-10-acetyltaxotere are approximately equal to those of taxotere [173]. The 7-hydroxy group can be removed, epimerized, or esterified without considerable loss in activity. For example, the tubulin-polymerizing ability of C^7-*epi*-taxol (**91**, R = OH) is approximately twice as low as that of taxol, but the cytotoxicity is twice as high [173, 174].



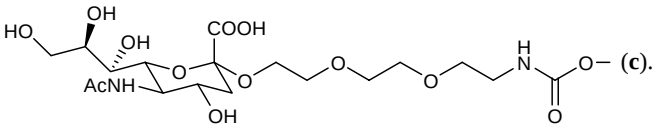
91 **92**

R = (**a**),





(**b**),



(**c**).

Up to now, a large number of derivatives having an ester moiety on C^7 have been examined. Most esters derived from substituted and unsubstituted aliphatic carboxylic and sulfonic acids showed an activity comparable to that of taxol (**92**, R = *i*-BuCOO, *cyclo*-C_5H_9COO, $NCCH_2COO$, $ClCH_2COO$, $AcOCH_2COO$, $MeOCH_2COO$, $HOCO(CH_2)_3COO$, $ClCH_2OCOO$, EtOCOO) or slightly higher (**92**, R = AcO, BuCOO, $MeSCH_2COO$, $Boc(CH_2)_3COO$, $MeSO_2O$) [147, 173, 175–177]. Exceptions were compounds **92** with R = ICH_2COO, *cyclo*-C_3H_5COO, and *t*-BuCOO, which were less active by factors of 3.3, 22, and 8.3, respectively, as well as inactive tridecanoic acid derivative **92** (R = $C_{12}H_{25}COO$) [175]. The lack of activity in the latter is consistent with the data of [178] according to which taxotere derivatives **92** with R = $Me(CH_2)_nCOO$ ($n < 7$) retain a good and almost similar ability to inhibit depolimerization of microtubules (this ability is weaker than that of taxotere by a factor of ~4, and of taxol, by a factor of 2). Extension of the carbon chain to $n \geq 9$ gives inactive and noncytotoxic compounds. It is known that some analogs with nonalkyl chains [**92**, R = $NH_2(CH_2)_3O(CH_2)_2O(CH_2)_2O(CH_2)_3NHCO(CH_2)_2COO$, $NH_2(CH_2)_3NH(CH_2)_4NHCO(CH_2)_2COO$], despite their considerable length, are capable of inhibiting depolymerization of microtubules, though to a lesser extent than the parent compound (**83**). Taking these data into account, Guenard *et al*. [178] presumed that raising the polarity of the C^7-substituent should enhance the activity; however, the results obtained for the $Me(CH_2)_nCOO$ series (n = 1–7) do not support this assumption (the opposite views on the effect of lipophilicity of the 7-substituent on the activity will be discussed below).

Esterification of the 7-OH group with small amino acids either does not change (**92**, R = BuNHCOO) or slightly reduces the activity [**92**, R = $HOCO(CH_2)_3NHCOO$, $Me_2N(CH_2)_2NHCOO$], but the cytotoxicity of the resulting derivatives sharply decreases (by 2–4 orders of magnitude). The data for the given series [cf. **92**, R = $HOCO(CH_2)_3COO$, BuCOO] confirm the assumption made by some authors that reduction in the polarity of the C^7-substituent improves the activity and especially the cytotoxicity [179].

Acylation of the hydroxy group on C^7 with unsaturated acids (**92**, R = MeCH=CHCOO, *trans*-ClCH=CHCOOH) reduces the activity by a factor of 5–6, while derivatives of aromatic (including heterocyclic) acids are less active by a factor of 3–6 [**92**, R = BzO, 5-$O_2NC_6H_4COO$, PhC≡CCOO, *trans*-PhCH=CHCOO] or 8–60 (**92**, R = *p*-MeC_6H_4COO, *p*-ClC_6H_4COO, *p*-$MeOC_6H_4COO$, 3,5-$Cl_2C_6H_3COO$, 1-$C_{10}H_7COO$, 2-methyl-3-pyridylcarbonyloxy, 2-furoyloxy, 5-nitro-2-furoyloxy, 1-methyl-2-pyrrolylcarbonyloxy, 2,3-dichloro-5-pyridylcarbonyloxy); compounds **92** with R = $Ph(CH_2)_2COO$, 4-$N_3C_6H_4COO$, 3-N_3-C_6H_4COO, 3-N_3-5-$O_2NC_6H_3COO$, 3-$Me_2NC_6H_4COO$, and *m*-(*i*-Pr)C_6H_4COO are inactive. Exceptions are thiophene and aminobenzoyl analogs **92** (R = 3-thenoyloxy, *p*-$H_2NC_6H_4COO$, *m*-$H_2NC_6H_4COO$) whose activity is only 1.5–1.7 times lower than that of taxol. It should be emphasized that many C^7-esters derived from aromatic acids possess no cytotoxic properties [147, 175, 177, 178].

Apart from C^7-esters, a number of ethers **92** (R = MeO, MOMO, MEMO, $MeCH_2OCH_2O$) were synthesized (in some ethers, the phenyl group on $C^{3'}$ was replaced by 2-furyl) [180]. The activity of almost all these compounds was equal to that of taxol or somewhat higher. Analogs containing a heteroatom other than oxygen at C^7, including sulfur derivatives, have been reported [173, 181, 182]. The activity of 7-*epi*-SH analog **91** (R = SH) was twice as low, while less polar *S*-methyl derivative **91** (R = SMe) exceeded its diastereoisomer **92** (R = SMe) in tubulin-polymerizing ability by a factor of 18 [182]. Methylsulfanylmethoxy analogs **91** and **92** ($R = MeSCH_2O$) were less active than taxol by factors of 1.9 and 1.1, respectively, and the latter showed a fairly high cytotoxicity against tumor cells resistant to taxol; this compound is now under clinical trials [180, 182]. The above findings, as well as the data for compounds **91** (R = OH, F), indicate that the activity of analogs having small 7-*epi*-substituents is comparable to the activity of taxol. However, introduction of bulky 7-*epi*-substituents (**91**, $R = Me_3SiO$, $PhSiMe_2O$) leads to complete loss of activity. It should be noted that 7-*epi*-triethylsilyloxy derivative **91** is less active than taxol only by a factor of 2 [173].

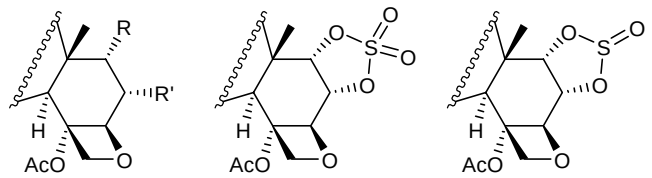
Thus, although the substituent in position *7* does not affect binding to the protein to an appreciable extent and can be replaced by other groups with strongly different structure, there are some limitations concerning its size, especially for 7-*epi* derivatives. Among compounds like **92**, active analogs containing bulky aromatic substituents were found, but the substituent therein was linked to C^7 through a bridging moiety; the attachment through an oxygen atom or ester moiety generally leads to reduction in the activity (see above). For example, taxol and taxotere analogs **92** [$R = PhCH_2CH(NH_2)COO$] and **92a** exhibit almost the same activity as the parent compounds [176, 183]. It should be noted that, according to the NMR data, compound **92a** in DMSO–H_2O (3:7) adopts a *hydrophobic collapse* conformation where rotation of the fluorescein fragment is not restricted [183].

The size of the substituent on C^7 can affect not only the activity but also the character of binding to tubulin [184–186]. For example, [^{3}H]-7-*O*-(*p*-Benzoyloxydihydrocinnamoyl) derivative **92** [$R = p\text{-}BzOC_6H_4\text{-}(CH^3H)_2COO$] stabilizes the microtubules but does not promote polymerization of tubulin. Presumably, this compound is capable of binding to small tubulin oligomers, but the presence of a bulky substituent on C^7 prevents further addition of tubulin heterodimers. Using photoaffinity labeling method, Rao *et al*. [129] found that the above compound binds to Arg284 in the M loop which is involved in the lateral interactions between protofilaments. Docking of that structure into the tubulin model proposed in [137] showed that, in complete agreement with the experimental data, the terminal benzoyl group in the C^7-substituent is located in close proximity to Arg284 and that the entire substituent can distort the natural conformation of the M loop [137].

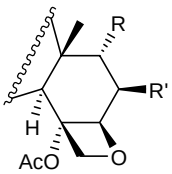
A week sensitivity to variation of the substituent on C^7 in taxol was utilized in the preparation of prodrugs [187, 188], addition of fluorescent (e.g., **92b**) [175, 189–191] and luminescent labels [192], synthesis of water-soluble analogs, etc. [193–196]. For example, C^7-polyethylene glycol derivative (7-PEG-taxol; the polyethylene glycol chain was attached through a carbamate bridge) is readily soluble in water, the cytotoxic properties remaining unchanged [193]. Another water-soluble analog **92c** was obtained by conjugation of taxol with sialic acid through a 2-[2-(2-aminoethoxy)ethoxy]ethanol chain. The intrinsic activity of **92c** is lower than that of taxol by a factor of 4, but it acts *in vivo* as a prodrug which undergoes hydrolysis to taxol by the action of neuraminidase [194].

4.2.7. Simultaneous variations of substituents in positions *6* and *7*. 6,7-Dehydrotaxol possessing a double bond is 1.3 times more active and only slightly (by a factor of 1.2) less cytotoxic than taxol [173]. As shown in [197], 6α-hydroxy-7-deoxytaxol and its diastereoisomer are almost equal to taxol in the activity, but the cytotoxicity of both isomers is lower.

Several **7**-*epi* analogs of taxol with simultaneous variation of the 6-substituent have been reported [198, 199, 172]. The activity of 7-*epi* derivatives **93** with a bulky group on C^6 (R = OH, R' = BzO, *o*-MeC_6H_4-COO, *cyclo*-C_3H_5COO) is lower by a factor of 40 and more, and the cytotoxicity is lower approximately by an order of magnitude [172, 199]. Presumably, the larger size of cyclic sulfate **94a** as compared to analogous cyclic sulfite **94b** is responsible for the lack of activity of the former; on the other hand, this may be due to greater polarity of the C^7-substituent (see



93 **94a** **94b**



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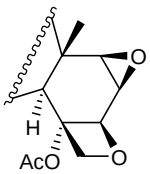
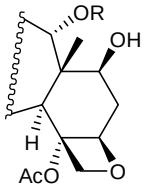


96

above) [198]. The only analog that showed a higher cytotoxicity than taxol (by a factor of 2–3) was azido derivative **95** (R = OH, R' = N_3); amine **95** (R = OH, R' = NH_2) was less cytotoxic than taxol by an order of magnitude. The cytotoxicity of the other derivatives was either similar to that of taxol (**96**) or somewhat (by a factor of no more than 3) lower (**93**, R = R' = OH; R = OH, R' = OAc) [172].

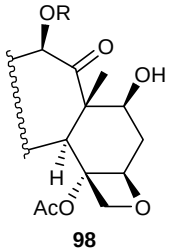
4.2.8. Variations at position *9*. 9-Deoxotaxol is characterized by a similar cytotoxicity to taxol [186]. 9-Deoxo-9-hydroxytaxol (**97**, R= H) and 9-deoxo-9-hydroxytaxotere almost do not differ in the activity from the corresponding parent compounds [179, 200], though their cytotoxicity with respect to some tumor cells is lower on the average by a factor of 3–8. On the other hand, 9-deoxo-9-hydroxy analogs are more stable and better soluble in water than 9-oxo compounds [179]. Methylation of the hydroxy group in 9-deoxo-9-hydroxytaxol(or taxotere) gives compounds **97** (R = Me) with almost the same activity and cytotoxicity as those intrinsic to taxol and taxotere, respectively [179]. Thus the 9-oxo group does not play an important role in binding to tubulin. It should be noted that removal of the acetyl group from position *4* in (9*R*)-9-deoxo-9-hydroxytaxol resulted in almost complete loss in activity, in keeping with the above data indicating the importance of the C^4-substituent [179].



97

4.2.9. Variations at position *10*. The cytotoxicity of 10-deacetoxytaxol is similar to that of taxol; the same applies to analogous taxotere derivative [201, 202]. Removal of the 10-acetyl group from taxol molecule (**98**, R = H) does not change the activity to an appreciable extent, while epimerization at C^{10} improves the activity: 10-*epi*-taxol and 10-*epi*-deacetyltaxol are approximately 1.5–2 times more active and cytotoxic than the natural compound [203]. Oxidation of the 10-hydroxy group in taxotere to ketone moiety gives an analog with almost the same cytotoxicity as that of taxol but somewhat lower as compared to taxotere [204].

Numerous taxol analogs were obtained by modification of the *O*-acetyl group on C^{10}; however, data on the activity of a number of these analogs were not given. In most cases, replacement of that group by other acyl or carbonate moieties with quite different electronic and steric parameters (**98**, R = EtCO, PrCO, *cyclo*-$C_6H_{11}CO$, MeCH=CHCO, Me_2NCO, *cyclo*-C_3H_5CO, MeOCO, *p*-$MeOC_6H_4CO$, $PhOCH_2CO$; **98a**) does not induce considerable changes in cytotoxicity: it varies by a factor of 2–3 in both directions. Exceptions are derivatives of higher alkanoic acids: in this homologous series, the cytotoxicity decreases with extension of the carbon chain. Compound **98** (R = C_3H_7CO) is approximately equal to taxol in this respect; in going to R = BuCO and $C_5H_{11}CO$, the cytotoxicity decreases 1.5-fold, to R = $C_7H_{15}CO$, 5-fold, and to R = $C_{13}H_{27}CO$, 20-fold, while **98** (R = $C_{11}H_{23}CO$) exhibits no cytotoxicity at all. Benzoyl, phenylacetyl, and phenylpropionyl derivatives **98** (R = Bz, $PhCH_2CO$, $PhCH_2CH_2CO$) are characterized by almost similar cytotoxicities. Unsaturated compound **98** (R = PhCH=CHCO) is approximately 2 times less active than its saturated analog **98** (R = $PhCH_2CH_2CO$). In the series of amino and nitro derivatives **98** (R = *o*-$H_2NC_6H_4CO$, *o*-$H_2NC_6H_4CH_2CO$ and R = *o*-$O_2NC_6H_4CO$, *o*-$O_2NC_6H_4CH_2CO$), the latter were approximately twice as cytotoxic as the former. The tubulin-polymerizing ability of ether **98** (R = Me) is approximately equal to that of taxol, while its cytotoxicity is lower by a factor of 6; structurally related 10-spiroepoxy derivative **99** is superior to taxol in both parameters [205].



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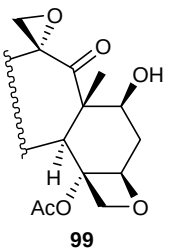


99

98a, R = morpholinocarbonyl; **98b**, R = morpholinoethyl; **98c**, R = thiomorpholinoethyl.

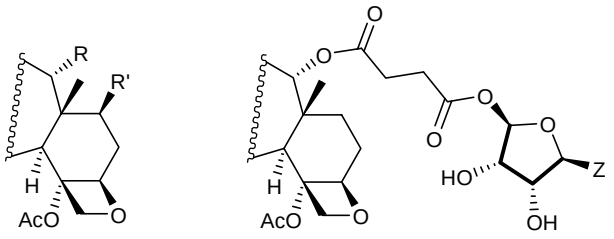
Analogous relations were observed for taxotere analogs obtained by replacement of the 10-hydroxy group by acetoxy, methoxy, methoxycarbonyloxy,

benzoyloxy, and *p*-phenylbenzoyloxy; no appreciable differences in the activity and cytotoxicity of these compounds were found (in the latter case, the ability to inhibit depolymerization of tubulin was measured; it decreased 5-fold) [147, 169, 178, 206–208]. Replacement of the hydroxy group by bulkier substituents, such as *trans*-PhCH=CHCO, did not reduce the activity, but the cytotoxicity decreased by more than an order of magnitude [178]. The cytotoxicity of taxotere derivatives with $Me(CH_2)_nCOO$ groups (n = 1–7) on C^{10}, as well as in the taxol series, slightly decreases as the alkyl chain becomes longer, but their activity remains at the same level which is 3–4 times lower than the activity of taxotere. Further extension of the alkyl chain ($n \geq 9$) gives inactive and noncytotoxic analogs (as in the series of C^7-modified derivatives, see above) [178].

The obtained data indicate that the acetyl group on C^{10} in taxol molecule does not affect its interaction with tubulin to an appreciable extent. Therefore, this group, as well as C^7-substituent, is sometimes modified to improve pharmacokinetic properties and attach fluorescent labels. For example, Soga and co-workers [209, 210] synthesized a series of 10-*O*-aminoethyl taxol derivatives **98** with the goal of improving their solubility in water and enhancing the stability *in vivo*. Among these, compounds **98b** and **98c** showed cytotoxicity comparable to or exceeding that of taxol, while **98c** methanesulfonate was readily soluble in water. Baloglu *et al*. [211] prepared 10-deacetyl-10-(*m*-aminobenzoyl)- and 10-deacetyl-10-[7-(diethylamino)-2-oxochromene-3-carbonyl]taxol and showed that the addition of fluorophoric fragments almost does not affect the tubulin-polymerizing ability.

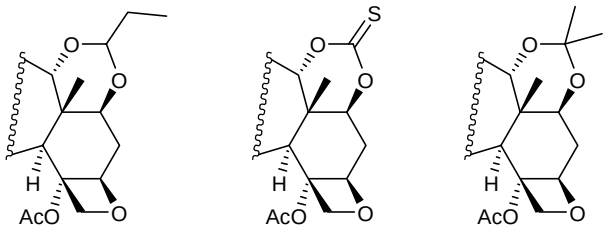
Despite a weak effect of the C^{10}-substituent on the activity, C^{10}-modified analogs were extensively studied in the recent years, for the cytotoxicity of taxol derivatives against tumor cells resistant to it was shown to depend on that substituent [212, 213]. A series of 63 taxol analogs with modified substituents on C^{10} were obtained by combinatorial synthesis [213]; most of these were slightly less active than taxol, and their cytotoxicity against "standard" tumor cells was appreciably lower. However, approximately a half of these derivatives were found to exhibit a higher (maximally, by an order of magnitude) cytotoxicity with respect to tumor cells resistant to taxol. Even more striking results were reported by Ojima *et al*. [212, 214] who examined variations of the C^{10}-substituent in taxol analogs having an alkyl or alkenyl group at $C^{3'}$ (the latter modification almost does not change the activity; see below). Compounds having, e.g., EtCO, *cyclo*-C_3H_5CO, and Me_2NCO groups on C^{10} were by two orders of magnitude more cytotoxic than the parent compound against tumor cells resistant to taxol. Taking into account enhanced activity of taxol derivatives with *meta*-substituted benzene ring at C^2, a number of 3',2,10-modified taxol analogs were synthesized. Some of these, e.g., with an EtCO group at C^{10} and methoxy group in the benzene ring at C^2 were equally cytotoxic with respect to both common and taxol-resistant tumor cells: their cytotoxicity against the latter was higher by 2–3 orders of magnitude, as compared to taxol [214].

4.2.10. Simultaneous variations of substituents in positions *7* and *9*. 7-Deoxy-9-deoxotaxol is approximately similar to taxol in cytotoxicity [186]. Klein *et al*. [179] studied a series of 9-deoxo-9-hydroxy taxol and taxotere analogs with various substituents in position *7*. Most of these, in particular **100a** [R = OH, R' = $HOCH_2CH(OH)CH_2O$, $AcO(CH_2)_2O$, $Et_2N(CH_2)_2O$, $HO(CH_2)_2O$, $CH_2=CHCH_2O$], **101**, and **102**, were less active (by a factor of 1.5–3) than the parent structures, while compounds **103** and **100a** (R = R' = AcO; R = HO, R' = MeO) showed a slightly stronger activity. However, only analogs **100a** (R = OH, R' = MeO, $CH_2=CHCH_2O$) were characterized by comparable or even somewhat higher cytotoxicity than the initial compounds [215]. In the other cases, the cytotoxicity was lower by 1–3 orders of magnitude. A conclusion can be drawn that protection of the hydroxy groups on C^7 or C^9 gives more active analogs



100a **100b**

100b, Z = 5-bromo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl.



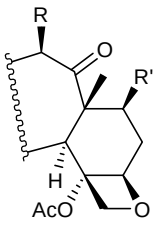
101 **102** **103**

and that the presence of hydroxy or amino groups in the same fragment produces the reverse effect (an opposite viewpoint was reported for a series of 7,10-analogs, see below).

Cheng *et al*. [216] recently synthesized 7-deoxytaxol analogs in which the taxol fragment was combined with a modified nucleoside moiety possessing an antiviral activity, e.g., **100b** (Z = 5-bromo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl). Although almost all these compounds were completely inactive, they showed a quite high cytotoxicity; presumably, they act as prodrugs due to the presence of a readily hydrolyzable moiety at C^9.

4.2.11. Simultaneous variations of substituents in positions *7* and *10*. 10-Deacetyl-7-*epi*-taxol is as active as the natural compound. According to the results of X-ray diffraction study on a single crystal grown from a nonaqueous medium (ethyl acetate), this derivative in crystal has a *hydrophobic collapse* conformation [149].

The activity of 7-deoxy-10-acetyltaxotere is approximately similar to that of taxotere [217]. In the series of other 7,10-taxotere analogs **104** (R = R' = $Me(CH_2)_nCOO$), derivative with n = 5 was inactive; the activity of compounds **104** with n = 1, 2, and 3 successively decreases, in contrast to the corresponding monosubstituted derivatives (see above). On the other hand, structurally related compounds having a carboxy group [**104**, R = R' = $HOCO(CH_2)_nCOO$, n = 2, 3, 7] are approximately twice as active as their methyl analogs [176, 178]. These data gave grounds to presume that alkyl substituents in 7,10-modified analogs reduce the activity and that polar groups retain it [178].



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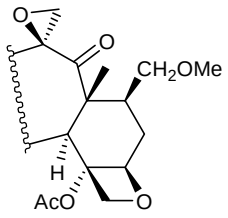

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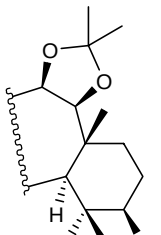
As noted above, some C^7- and C^{10}-monosubstituted taxanes exhibited enhanced cytotoxicity against taxol-resistant tumor cells. An analogous pattern was observed for some 7,10-disubstituted derivatives, e.g., compound **105**; its activity slightly exceeded that of taxol [205]. These data are likely to be responsible for increased interest in the synthesis of 7,10-modified taxol analogs. Jagtap *et al*. [218] used both classical methods and solid-phase combinatorial synthesis to obtain about 30 various 7,10-analogs with acyl substituents containing heterocyclic (e.g., piperazine, morpholine, piperidine, etc. fragments), unsaturated, aromatic, and alkyl groups. However, the activity of most of these derivatives was lower than that of taxol.

4.2.12. Simultaneous variations of substituents in positions *9* and *10*. The relations observed upon separate variation of substituents at C^9 and C^{10} are also valid for simultaneous variation of substituents at both these positions. Reduction of the C^9=O group in combination with hydrolysis and/or epimerization, as well as with oxidation of the acetoxy group at C^{10}, does not lead to appreciable change in activity [179, 205].

4.2.13. Simultaneous variations of substituents in positions *7, 9,* and *10*. 10-Deacetoxy-7-deoxy-9-deoxo analog having no substituents in positions *7*, *9*, and *10* is less cytotoxic than taxol by an order of magnitude [186], indicating once more that substituents at these positions do not play an important role.

Ishiyama *et al*. [219, 220] synthesized 7-deoxy-9,10-acetal derivatives of taxotere (with insignificant side-chain variations), which showed a high cytotoxicity, e.g., compound **106**. The same authors introduced a morpholino group into the C^{10}-substituent and obtained a water-soluble analog possessing its own activity (i.e., not acting as a prodrug) [209, 210]. The results of these two studies led the authors to create analogs **107** in which a morpholino group was attached to the 9,10-acetal moiety. Compounds **107** were more cytotoxic than taxol and taxotere and better soluble in water [221].



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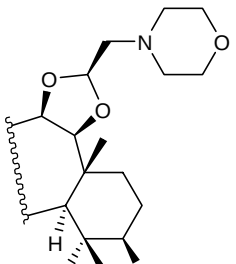

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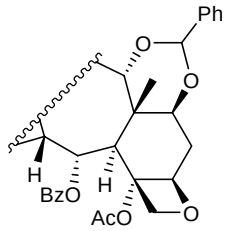
4.2.14. Simultaneous variations of substituents in positions *1, 7,* and *9*. Some 1-deoxytaxol and 1-deoxytaxotere analogs with various substituents at C^7 and C^9 (**100a**, RR' = Me_2C; R = R' = OH; R = MeO, R' = OH, etc.) are less active than the parent compounds by a factor of no more than 3 [11]. Compound **108** with

a bulky aromatic substituent at C^7 and C^9 showed no activity. 1-Deoxy analog **109** (R = R' = Ac) of taxol derivative **100a** (R = R' = AcO; this derivative is somewhat more active than taxol; see above) was less active than taxol by an order of magnitude. Thus removal of the C^1-hydroxy group leads to a small but appreciable reduction in the activity and cytotoxicity. This conclusion is confirmed by the fact that 1-deoxy-9-deoxo-9-hydroxytaxotere is less active than taxol by a factor of 3 [11, 142, 157].



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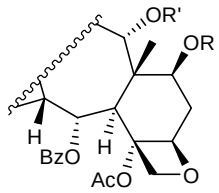

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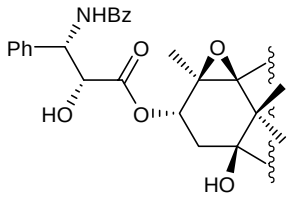
4.2.15. Variations at positions *11*, *12*, and *13*. There are only a few published data on taxol derivatives with modified substituents at positions *11*, *12*, and *13*. Analog **110** with an epoxy group instead of the C^{11}–C^{12} double bond is slightly more active but less cytotoxic than taxol [222]. Migration of the double bond from C^{11}–C^{12} to C^{12}–C^{13} gives isotaxol (**111**) whose cytotoxicity is slightly higher than that of taxol [223].



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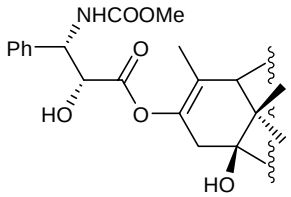

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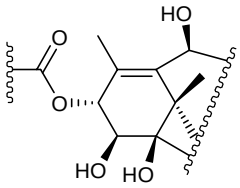
4.2.16. Variations at position *14*. Introduction of a β-hydroxy group into position *14* of the taxotere molecule (compound **112**) almost does not change the activity and cytotoxicity. 1,14-*O*-Isopropylidene derivative **113** is less active than the parent compound by



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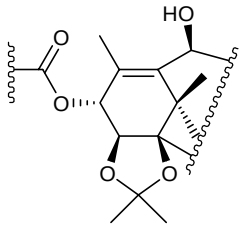

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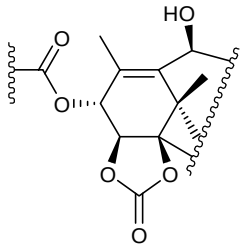
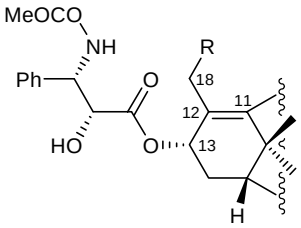


114

a factor of 4 and less cytotoxic by an order of magnitude [224]. The most successful was modification of taxotere through introduction of a carbonate moiety to C^{14}/C^1: the activity and cytotoxicity of compound **114** were equal to those of taxotere. The corresponding taxol analog was less active by a factor of 3 and less cytotoxic by a factor of 10–20 than the natural compound. An analog of **114** with insignificantly modified side chain is now under clinical trials [225, 226].

4.2.17. Variations at position *18*. Uoto *et al*. [227] described several taxol analogs **115** (R = Me, N_3, AcO, CN) with substituted methyl group on C^{12}. All these were less cytotoxic than taxol, the maximal cytotoxicity being observed for **115** (R = Me).



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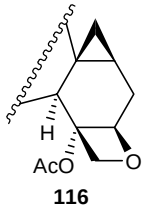
4.2.18. Variations at position *19*. 7,19-Cyclopropanotaxol (**116**) is approximately two times less active and cytotoxic than the natural compound [173]. Analogous derivative of 10-acetyl-9-deoxohydrotaxotere showed approximately the same cytotoxicity as the parent compound (2–10 times higher than that of taxol) [228].



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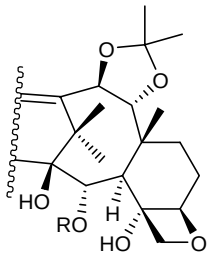
4.2.19. Variations of more than three substituents in the taxane core. Wiegerinck *et al*. [229] synthesized six 7-deoxy-4-deacetyltaxol analogs with structural variations at C^1, C^2, C^9, and C^{10} (**117**, R = Bz

or 2-pyranyl; **118**, R = H or Bz; **119**, R = Ph, R' = H or R = R' = Me). The authors' intention was to find out the effect of replacement of the benzoyl group at C^2 by other groups, e.g., as in benzylidene acetal **119** (R = Ph, R' = H) where both acetal oxygen atoms occupy approximately the same steric positions as the oxygen atoms in the benzoyl group. However, the resulting compounds were inactive as might be expected taking into account at least the lack of acetyl group at C^4.



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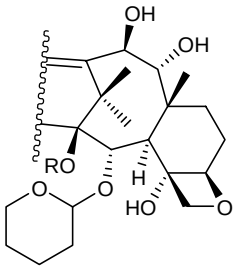

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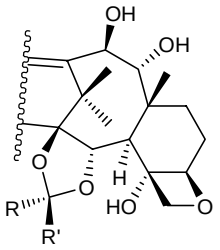

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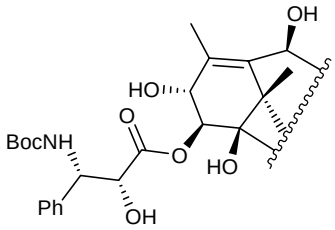
To conclude this section, let us emphasize that general structure–activity relationships in the series of taxol and taxotere analogs obtained by variation of substituents in the taxane core indicate strong dependence of their tubulin-polymerizing ability on the substituents in positions *2* and *4* and weak dependence on those in positions *7*, *9*, and *10*. According to the results of computer simulation [137], the taxol fragment including substituents on C^7, C^9, and C^{10} does not participate in binding to tubulin and is oriented in the opposite direction with respect to the protein surface.

4.3. Modifications of the Side Chain and Simultaneous Modifications of Substituents in the Taxane Core and in the Side Chain

The results of numerous structure–activity studies on taxol analogs indicated a strong effect of the C^{13}-side chain on both the ability to induce uncontrolled polymerization of tubulin and the cytotoxicity. Baccatin (**65**) having a hydroxy group at C^{13} is inactive and almost noncytotoxic [141, 157]. Migration of the side chain to C^{14} (compound **120**) [224], as well as introduction of additional methylene units [230] or replacement by cinnamic or crotonic acid residues, either considerably reduces or eliminates the activity [176]. Configuration of the side chain is a very important factor for binding to tubulin. (2'*S*,3'*R*)-, (2'*R*,3'*R*)-, and (2'*S*,3'*S*)-Isomers of taxol and taxotere are less active than the natural compounds by factors of 1.3–4.5 and 3.6–60, respectively. Analogous relations are typical for some their derivatives [141, 131].



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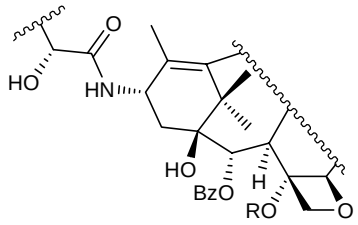
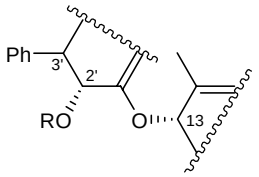
Replacement of the ester moiety in the taxol side chain by amide group gives almost inactive and noncytotoxic analogs **121** (R = H, Ac, MeOCO) [163]. This result should be expected for the 4-hydroxy derivative, while it is somewhat surprising for the other analogs, taking into account that taxol analog with a methoxycarbonyloxy group on C^4 is quite active (see above); presumably, the reason is that replacement of the ester group by amide is accompanied by change in the side chain conformation.



121

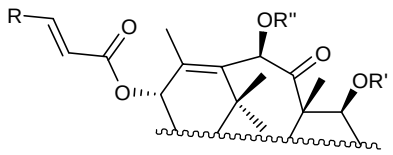
Each of the substituents on $C^{2'}$, $C^{3'}$, and $C^{3'}N$ contributes much to the activity. As shown in [141] for a series of analogs obtained by successive removal of the above substituents, their contributions are additive. For example, in going from taxotere to 2'-deoxy, 3'-de-*tert*-butoxyamino, and 2'-deoxy-3'-de-*tert*-butoxyamino analogs, the ability to inhibit depolymerization of tubulin microtubules decreases by factors of 4.1, 4.5, and 17, respectively. The activity of an analog having no substituents on $C^{2'}$ and $C^{3'}$ is lower by a factor of 41 [141]. The data for 2'-deoxytaxoter indicate that the presence of a hydroxy group on $C^{2'}$ is important for an analog to be highly active.

Earlier structure–activity studies on taxol analogs [131, 231] showed that the tubulin-polymerizing ability of 2'-acetoxy derivatives of taxol and taxotere (**122**, R = Ac) is weaker by an order of magnitude than that of the parent compounds. This cannot be attributed to the size of the acetyl group [232], for the activity of 2'-deoxy analogs also decreases (see above). Likewise, the concept implying that the hydroxy group anchors a conformation necessary for hydrogen bonding with the $C^{1'}$=O carbonyl seems to be unsuccessful (acetylation of the $C^{2'}$-hydroxy group prevents formation of such hydrogen bond) [141]. NMR study of 2'-*O*-acetyl derivative **122** (R = Ac) in polar and nonpolar solvents showed that the side-chain therein has the same conformation as in taxol [233]. It should be noted that *hydrophobic collapse* conformation was not detected for inactive 2'-carbamoyloxytaxol [which is structurally related to **122** (R = Ac)] in crystal [149]. Using a 2'-*O*-acetyl analog of taxol with a fluorescent label at C^{7} it was shown that the 2'-hydroxy group forms a hydrogen bond with the amide carbonyl group of Arg369 [137, 177]. Therefore, the reduced activity of 2'-deoxy and 2'-*O*-acetyl derivatives results from the lack of that interaction. Lee *et al*. [234] developed a procedure for the synthesis of the *syn* and *anti* isomers of *S*-acetyl-*N*-benzoyl-3-phenylisocysteine with a view to obtain 2'-SH analogs of taxol; study of the latter should provide new information on the interaction between the 2'-substituent and Arg369, for the SH group is more acidic than OH.



122

Up to now, there have been reported no $C^{2'}$-modified analogs equal to taxol or taxotere in tubulin-polymerizing ability. The activity of 2'-deoxy derivatives **123** (R = Ph, R' = R" = H; R = Me, R' = H, R" = Ac) is lower by a factor of 20–100, while compounds **123** (R = Ph, R' = R" = CCl_3CH_2COO; R = Ph, R' = CCl_3CH_2COO, R" = Ac) are inactive at all [176]. (2'*R*,3'*S*)-Derivatives of taxol, 10-acetyltaxol, and taxotere, in which the substituents at $C^{2'}$ and $C^{3'}$ are transposed (**124**, R = Bz or Boc) and related compound **124** (R = Ts), as well as the corresponding (2'*S*,3'*R*)-enantiomers **125** (R = Bz or Boc), are less active than taxol by 1–2 orders of magnitude [176]. Analogous results are obtained by removal of the 2'-hydroxy group with simultaneous modification of the 3'-substituent (structure **126**) [235] or incorporation of the 2'- and 3'-substituents into an oxazolidinone ring (compound **127** is almost inactive) [236]. A weak activity of analogs with modified 2'-OH group was recently predicted on the basis of structure–activity correlation for C^{13}-analogs of taxol, which was simulated by quantum-chemical calculations [237].



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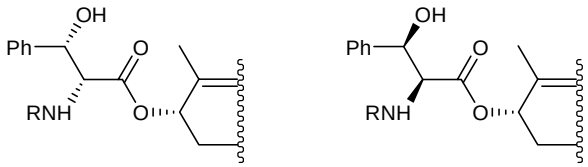
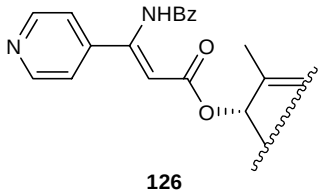


124 **125**



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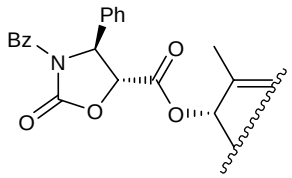
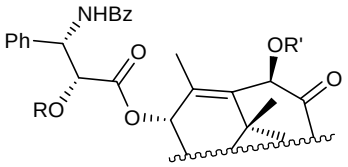


127

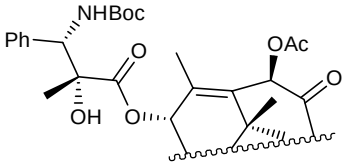
An important note on the cytotoxicity of 2'-modified taxol analogs should be made. The cytotoxicity of 2'-deoxy, 2'-methoxy, and 2'-fluoro derivatives is lower than that of taxol by a factor of 70–200 [238]. On the other hand, if the $C^{2'}$-substituent is readily hydrolyzable by the action of cellular enzymes, gradual release of the cytotoxic natural material can occur *in vivo*. Therefore, inactive analog **122** (R = Ac) is similar to taxol in cytotoxicity, i.e., this compound behaves as a prodrug. Obviously, just the hydrolysis of the ester moiety at $C^{2'}$ is responsible for the high cytotoxicity of the other 2'-ester analogs of 10-deacetyl- and 7-deoxy-10-deacetyltaxol, e.g., **128** (R = R' = PrCO, BzlCO, *o*-$O_2NC_6H_4CO$, *o*-$O_2NC_6H_4CH_2CO$, *p*-$MeOC_6H_4CO$); in other words, these compounds are also prodrugs. However, some 7-deoxy-10-deacetyltaxol analogs **128** [R = R' = $Me(CH_2)_4CO$, R = R' = $Me(CH_2)_6CO$, R = R' = PhCH=CHCO] are noncyto-

toxic; presumably, their hydrolysis is more difficult [207]. The design of prodrugs via replacement of the 2'-hydroxy group in taxol was the subject of extensive studies [157, 169, 183, 207, 232, 236, 239–242].



128

Among other $C^{2'}$-modified analogs, compound **129** having an additional methyl group in that position should be noted. This derivative is more active and cytotoxic than taxol by a factor of 1.5. A probable reason is restriction of free rotation about the $C^{2'}$–$C^{3'}$ bond or an additional interaction between the 2'-methyl group and the protein [243–245].



129

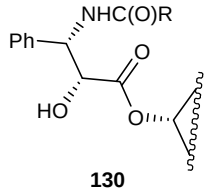
Derivatives formed by modifications at the $C^{3'}$-position constitute one of the largest groups of taxol and taxotere analogs. Phenyl groups at $C^{3'}$ and $C^{3'}N$ can be replaced by substituted aromatic, heterocyclic, and aliphatic moieties, which often leads to active analogs [246–248]. Introduction of a methoxy or hydroxy group into the *para*-position of the benzene ring on $C^{3'}$ increases the activity by a factor of 2 and 1.5, respectively, whereas *p*-F and *p*-Cl substituents reduce the activity 1.1- and 2-fold, respectively. Dichloro-substituted analog with chlorine atoms in the *para*- and *meta*-positions is less active by a factor of 7 [249, 250]. The data on the activity of the *para*-methyl derivative are contradictory: according to [250], such compound is several times more active than taxol, while Jenkins [249] reported that its activity is lower by a factor of 2.4. Analogous relations were observed upon variation of substituent in the 3'-benzoyl ring. Introduction of a chlorine atom and trifluoromethyl, methyl, and sulfinyl groups reduced the activity by factors of 2, 6, 1.1, and 5.5, respectively, while methoxy-substituted analog was twice as active as taxol [176, 250].

The activity of taxol analogs obtained by replacement of the phenyl group on $C^{3'}$ by pyridine and furan heterorings was comparable to that of taxol (it was higher by a factor of 1.1–2), while the cytotoxicity depended on the position of the heteroatom. For example, 2-furyl derivatives was approximately by an order of magnitude more cytotoxic than 3-furyl isomer, while the cytotoxicity of 2- and 4-pyridyl analogs exceeded that of the 3-pyridyl derivative by a factor of 25–30 [235, 249]. 10-Acetyltaxotere analog containing a 2-furyl group on $C^{3'}$ is 5 times more active and 2 times more cytotoxic than taxol [235]. Analogous replacement of the benzene ring in the 3'-benzoylamino group resulted in reduced activity of 2- and 4-pyridyl derivatives (by factors of 3.5 and 2, respectively) and slightly enhanced activity (by a factor of 1.5–2) of 3-pyridyl and furyl analogs. In this series, the maximal activity and cytotoxicity were found for the 3-furyl analog (approximately twice as high as that of taxol), while the cytotoxicity of all pyridine analogs was considerably lower than that of taxol [235].

The presence of an aromatic group at $C^{3'}$ is not a necessary condition for successful binding to tubulin (for instance, 3'*N*-debenzoyl-*N*-butanoyl- and 3'*N*-debenzoyl-*N*-propanoyl-10-deacetyltaxols were detected in small amounts in leaves of some yew species [251]). Taxotere derivatives having a branched alkyl group on $C^{3'}$, e.g., *tert*-butyl, isobutyl, or isobutenyl, instead of phenyl are more active than taxol by a factor of 1.5–3, the most active being the *tert*-butyl analog. Further extension of the carbon chain to neopentyl leads to a slight decrease in activity [129, 252, 253]. Similar variations of the substituent at $C^{3'}N$ resulted in the reverse relation: replacement of the benzoyl group by pivaloyl, isovaleryl, and 3-methyl-2-butenoyl gave analogs with a lower activity (by a factor of 1.5–2.6), while the neopentyl derivative was more active than taxol by a factor of 1.5 [204, 247].

Roh *et al*. [254–256] studied the cytotoxicity of a series of taxol analogs with various 3'-*N*-acyl substituents, in particular, with alicyclic ones. The authors showed that the optimal substituent is a nonpolar group consisting of 3–6 carbon atoms. The cytotoxicity of compound **130** (R = cyclopentyl) is comparable with that of taxol, the cyclohexyl derivative is less cytotoxic by a factor of 5, and in going to those having unbranched hexyl, heptyl, octyl, and longer alkyl radicals, the cytotoxicity decreases by 2–3 orders of magnitude. The presence of a double or triple bond conjugated with the amide carbonyl group, as well as β-substitution at the multiple bond, gives a positive effect. For example, compounds **130** (R = 1-cyclopentenyl and 1-cyclohexenyl) are approximately by

an order of magnitude more cytotoxic than their saturated analogs [254], derivatives **130** with R = isopropenyl and R= MeCH=C(Me) are less cytotoxic than taxol by a factor of 5–200 and 1.1–5, respectively, and the cytotoxicity of **130** (R = *trans*-1-propenyl, isobutenyl) exceeds that of the natural compound by 1–3 orders of magnitude [255, 256].



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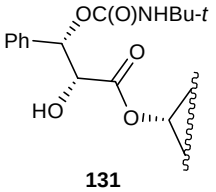

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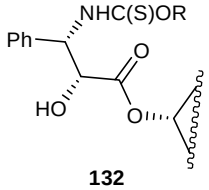

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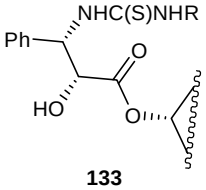
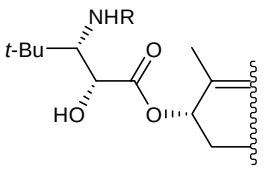


133

Replacement of the phenyl group at $C^{3'}N$ by *tert*-butoxy gave highly active and cytotoxic analogs, 10-acetyltaxotere and taxotere, thus arousing interest to derivatives with O-alkyl substituents. (9*R*)-9-Deoxo-9-hydroxytaxol analogs **130** (R = EtO, *i*-PrO, *tert*-pentyloxy, *t*-BuO, neopentoxy, adamantyloxy) are comparable to taxol in the activity [215]. Bioisosteric replacements of the NHCOO fragment at $C^{3'}$ by urea, isomeric carbamate, thiocarbamate, and thiourea moieties were reported. Taxol and (9*R*)-9-deoxo-9-hydroxytaxol analogs **130** (R = *t*-BuNH) and 3'-*tert*-butylcarbamoyloxy derivative **131** showed a comparable activity with the natural compound [215, 257, 258]. The tubulin-polymerizing ability of thiocarbamate derivatives **132** (R = Bu, *t*-Bu) was stronger by a factor of 2–3, while their analogs with a furyl group on $C^{3'}$ instead of phenyl were more active by a factor of 5–10 [257, 259]. On the other hand, the activity of thiourea derivatives **133** (R = Bu, *t*-Bu) was 20 times lower than that of taxol, and only replacement of the 3'-phenyl group by furyl made their activity approaching the activity of taxol. More than 20-fold difference in the activities of **130** (R = *t*-BuNH) and **133** (R = *t*-Bu) indicates an important role of the carbamoyl group on $C^{3'}$ in binding to tubulin [257].

As follows from the aforesaid, active derivatives of taxol and taxotere can be obtained via various modifications of substituents at $C^{3'}$ and/or $C^{3'}N$. In addition, various labels (e.g., photoaffinity labels) can be introduced via modifications at $C^{3'}N$. It is now believed that the activity of the modified derivatives is determined mainly by the size of substituents attached to $C^{3'}$ and/or $C^{3'}N$ [246]. Apart from the above compounds with bulky substituents at those positions, isotaxotere and its analogs **134** [R = Bz, cyclopropylcarbonyl, cyclobutyl, cyclopentylcarbonyl, 2-thenoyl, 3-(2-thienyl)-acryloyl, 2-thienylacetyl, 1-methyl-2-pyrrolylcarbonyl] and other derivatives of (9*R*)-9-deoxo-9-hydroxytaxol [**135,** R = Ph, R' = Bz; R = Ph, R' = Boc; R = Et, R' = Boc; R = (*S*)-$HOCH_2CH(OH)$, R' = Boc) and taxotere [**135**, R = Ph, R' = $HOCO(CH_2)_3CO$; R = 2-furyl, R' = $Me(CH)_4CO$; R = 2-furyl, R' = $Me(CH)_8CO$; R = PhCH=CH, R' = Boc] were reported. All these compounds were characterized by a fairly high activity which differed from that of taxol by a factor of no more than 2.7 (in both directions) [176, 215, 235, 247, 248, 250, 253]. On the other hand, introduction of small groups (e.g., OH, H, or NH_2) to $C^{3'}$ and/or $C^{3'}N$ in taxol, taxotere, and (9*R*)-9-deoxo-9-hydroxytaxol often gives rise to considerably less active analogs **135** (R = Ph, R' = H; R = Ph, R' = Ac) and **136** (R = Ph, R' = OH; R = Me, R' = OH; R = Ph, R' = H). However, there are exceptions from the general tendency. For example, the activity of analogs **135** (R = Ph, R' = AcCO; R = $PhOCH_2$, R' = Bz; R = naphthyl, R' = Bz; R = $MeCOCH_2$, R' = Boc) is lower than that of taxol by a factor of 3.5–10, while compound **135** (R = Me, R' = Boc) exhibits a comparable activity [247, 250]. Contradictory data were reported for **136** (R = Ph, R' = OH): according to [176], it is three times less active than taxol, whereas according to [258], it is inactive at all. Interestingly, replacement of the phenyl group on $C^{3'}$ in taxotere by small CF_3 or CHF_2 group does not affect the cytotoxicity against standard tumor cells (the activity of these derivatives was not estimated) but increases it 2–3-fold with respect to taxol-resistant tumors; moreover, the cytotoxicity can be enhanced by a factor of 10–15 via simultaneous modification of the C^{10}-substituent (by introduction of Me_2NCO, EtCO, or *t*-$BuCH_2CO$ group; cf. variations at position *10*) [156].



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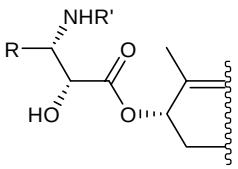

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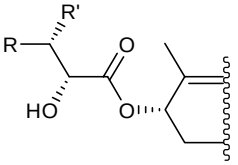
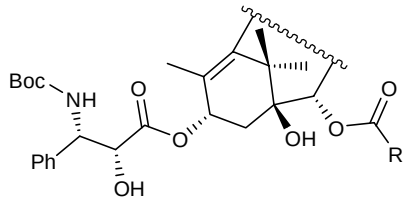


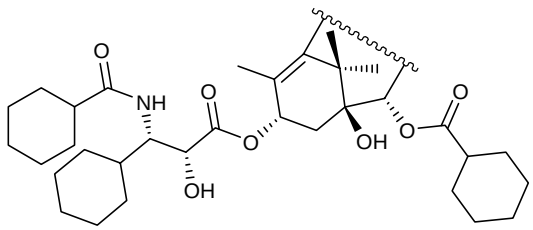
136

Some taxol analogs with a modified side chain attract a specific interest; some information on the bioactive conformation of taxol can be obtained from the data on their activity (see, e.g., structure **129**). For this purpose, substituents important for realization of the *hydrophobic collapse* conformation are subjected to modifications. The activity of taxotere and 10-acetyltaxotere analogs **137** having a cyclohexyl group on $C^{3'}$ (R = cyclohexyl, R' = BocNH) and those with cyclohexyl groups on $C^{3'}$ and C^{2} is lower by a factor of 1.2 and 3, respectively, than that of the parent compound [129]. This means that the activity of the biscyclohexyl analog, as compared with the corresponding monocyclohexyl derivatives (see above), decreases to a much stronger extent than it might be expected assuming that the substituent contributions are additive. It was believed that the cyclohexyl groups in the disubstituted analog (**137**, R = cyclohexyl) are located close to each other, thus inducing undesirable distortion of the *hydrophobic collapse* conformation [129]. Analogous derivative **137** with two isobutenyl groups at $C^{3'}$ and C^{2} (R = Me_2C=CH, nonataxel) at high concentrations promotes polymerization of tubulin to the same extent as does taxol [151, 260]. According to the NMR studies [261], the orientation of the substituents at $C^{3'}$ and C^{2} in nonataxel differs only slightly from that intrinsic to taxol in the *hydrophobic collapse* conformation.



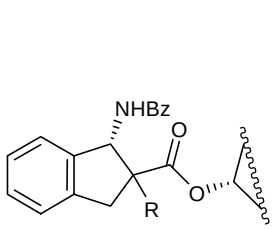
137

However, the high activity of compound **137** (R = cyclohexyl) and analogs with one cyclohexyl group in the side chain does not contradict the tubulin–T-shaped taxol model in which each of the rings is located in the hydrophobic domain and is not involved in the collapse [137]. Furthermore, introduction of the third cyclohexyl group gives compound **138** which is twice as active as taxol [150].

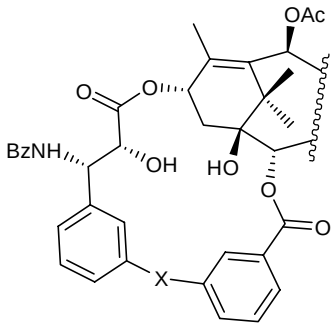


138

Another approach to determination of bioactive taxol conformations via modification of the side chain includes building up of "conformationally constrained" analogs. Compounds **139a** and **139b** in which rotation about the $C^{2'}$–$C^{3'}$ and $C^{3'}$–C_{arom} bonds is restricted by the presence of a short bridge between $C^{2'}$ and *ortho*-carbon atom of the 3'-phenyl ring are less active than taxol by a factor of 4 and 42, respectively; a similar relation was observed for analogous taxotere derivatives [262, 263]. The fairly high activity of **139a** was interpreted in [263] as an evidence in favor of a more open (than *hydrophobic collapse*) taxol conformation upon interaction with tubulin, i.e., T-conformation [262, 136]. The low activity of compound **139b** might be expected taking into account the *S* configuration of the hydroxy group on $C^{2'}$ (see above).



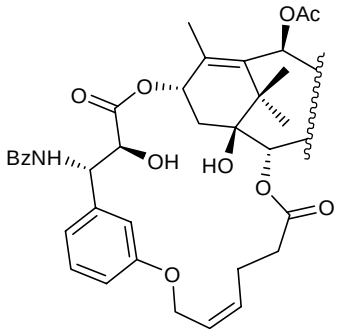
139a, **139b**



140

139, R = (*R*)-OH (**a**), (*S*)-OH (**b**).

Boge *et al*. [264] synthesized macrocyclic conformationally constrained taxol analogs with the $C^{3'}$-phenyl and C^{2}-benzoyloxy groups connected through a two-atom tether to mimic the *hydrophobic collapse* taxol conformation. However, compounds **140** with an ester (X = COO), ethylene (X = CH_2CH_2), and alkene tethers (X = *cis*- and *trans*-CH=CH) did not show activity in a tubulin assembly assay. These data were treated in [265] as arguing against the concept of taxol binding to tubulin in the *hydrophobic collapse* conformation, and the authors synthesized analogs **141** and **142** which simulate T-conformation of taxol. Here, the $C^{3'}$-phenyl group is located at a distance of 9–10 Å from the benzoyl phenyl group on C^{2} and is very close to the acetyl methyl group at C^{4}. The activity of **141** and **142** was lower than that of taxol by factors of 10 and 30, respectively. According to the results of computer simulation, these data may be interpreted in terms of steric hindrances created by the tether in the



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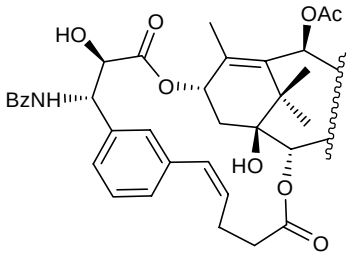

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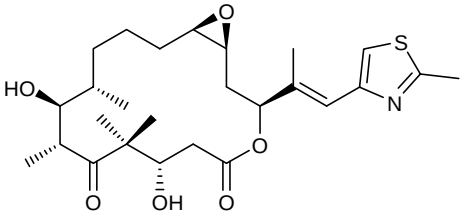

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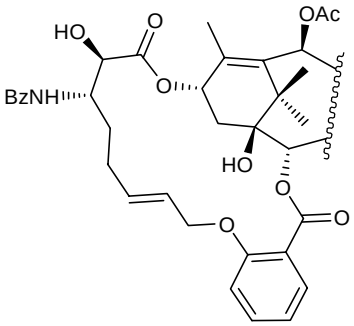

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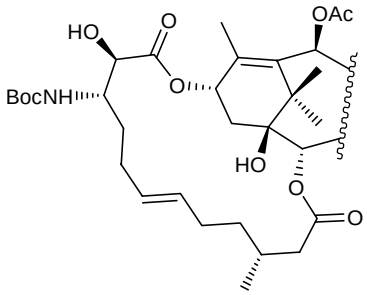

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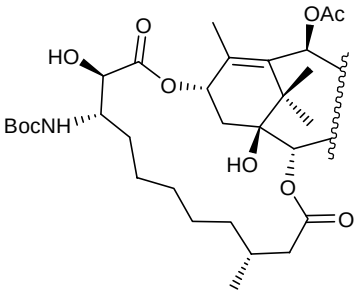


146

vicinity of the taxol binding site on a microtubule, which contains Ala233 and Phe272 amino acid residues.

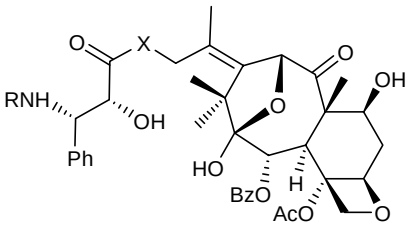
It should be noted that the above examples of modification of the taxol side chain, despite their importance for understanding the mechanism of taxol binding to the protein, do not provide unambiguous conclusion on its bioactive conformation. Therefore, several conformations may be presumed for taxol to fit the binding site in tubulin.

Ojima *et al*. [260, 261] recently prepared constructs including both taxol fragment and fragments of other compounds (e.g., epothilone A, **143**) with a similar mechanism of action and assumingly the same binding site in tubulin. Superposition of structure **143** and taxol molecule shows some similarity mainly in the taxol side-chain region. Taking this into account, macrocyclic compounds were synthesized, in which the taxol C^2 and $C^{3'}$ atoms were linked through a long bridge containing structural fragments of antitumor agent **143**. Compounds **144**–**146** showed a good tubulin-polymerizing ability which was weaker than that of taxol by a factor of 3–5; however, the cytotoxicity of these derivatives was lower by more than two orders of magnitude.

To conclude this section, let us emphasize the following. Although numerous structure–activity studies on taxol analogs indicated that the side chain on C^{13} is important for binding to tubulin, He *et al*. [266] recently advanced a hypothesis according to which introduction of an appropriate *meta*-substituent into the benzoyloxy group on C^2 should enhance binding to tubulin so much that the side chain can be removed without loss in activity. This assumption was based on the fact that 2-*m*-azido baccatin III exhibited a high cytotoxicity. Molecular modeling studies done with the C-2 benzoyl ring of taxol indicated that it fits into a pocket formed by His229 and Asp226 on β-tubulin and that the 2-*m*-azido, in contrast to the 2-*p*-azido substituent, is capable of enhancing the interaction between the benzoyl group and the side chain of Asp226. Interestingly, in the tubulin–T-taxol model [137], the carboxylate moiety of Asp226 appears exactly above the central cationic nitrogen atom of the azido group in 2-*m*-azido baccatin. If the fact that the C^{13} side chain is not an absolute requirement for biological activity in a taxane molecule will be confirmed by further experiments (e.g., by studying binding of 2-*m*-azido baccatin III to tubulin, etc.,), development of a new generation of taxol derivatives would be enabled.

4.4. Skeletal Analogs of Taxol

Some taxol analogs in which one or several rings of the tetracyclic system were modified, as well as those having radically different structures, were reported.



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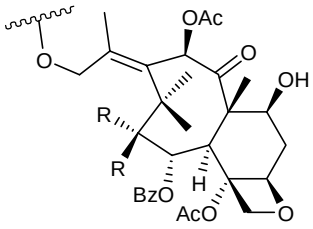

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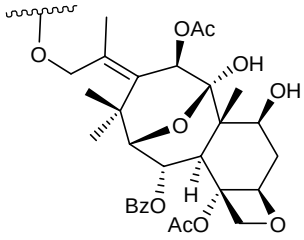

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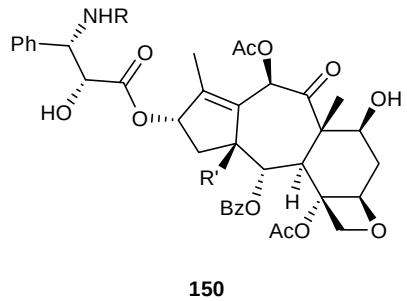
4.4.1. Modifications of rings A, B, C, and D. Nor-*seco*-taxoids **147** (X = O, R = Bz, Boc) with opened ring **A** and some variations in ring **B** were less cytotoxic than taxol by a factor of 20–40 [267], while their analogs **147** with an amide group in the side chain (X = NH, R = Bz, Boc) showed no cytotoxic properties [147]. The latter result is consistent with the data obtained for amides **121**. Compounds **147** (X = O) possess some cytotoxicity due to their ability to adopt a conformation similar to *hydrophobic collapse*; on the other hand, the considerable decrease in cytotoxicity indicates the importance of ring **A** [267]. Appendino *et al*. [268] examined the effect of the 1,10-oxygen tether on the activity, taking into account that it restricts conformational mobility of ring **B**. It was found that taxol and 3'-isobutyl-10-acetyltaxotere derivatives **148** (R = OH, R' = H; RR' = O) and **149** either did not inhibit proliferation of tumor cells at all or their cytotoxicity was lower by 2–3 orders of magnitude as compared to taxol and by a factor of 10–20 as compared to **147** (X = O, R = Bz) [267, 268]. Thus the 1,10-oxygen tether is significant, for it is likely to partially mimic the **A** ring [267].



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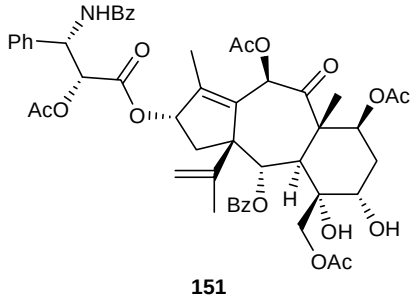
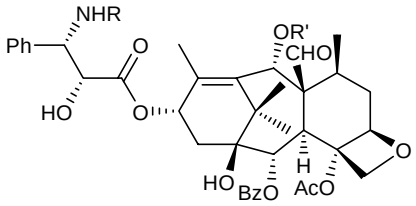


151

Some taxol analogs having a contracted ring **A** were reported (here, ring **B** is also contracted). Compounds **150** [R = Boc, R' = $Me_2C(OH)$; R = Bz, R' = CH_2=C(Me)] were less active than taxol by factors of 1.5 and 3, respectively [26, 269]. Taking into account that the polycyclic skeleton in **150** differs considerably from the taxane structure, these results were somewhat surprising, and they demonstrated the possibility for essential skeletal modifications of rings **A** and **B**. On the other hand, such modifications can strongly impair the cytotoxicity of nor-**A**-taxol derivatives. For example, compound **150** [R = Bz, R' = CH_2=C(Me)] is almost noncytotoxic [26]. Opening of ring **D** in **150** [R = Bz, R' = CH_2=C(Me)] with simultaneous acylation of the side-chain hydroxy group gave noncytotoxic analog **151**; the activity of the latter was not evaluated since 2'-*O*-acyl derivatives usually behave as prodrugs [26].

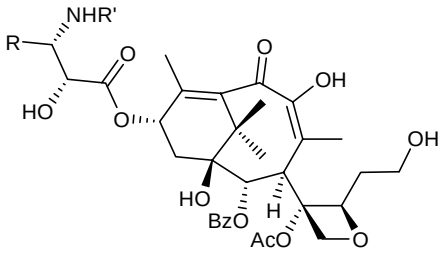
Taxol and taxotere analogs **152** (R = Bz, R' = Ac; R = Boc, R' = H) with contracted ring **B** and modified substituent on C^7 retain an appreciable cytotoxicity (which is lower by approximately an order of magnitude than that of the parent compounds) [228]. Presumably, contraction of ring **B** does not affect the molecular conformation of **152** to an appreciable extent and the role of the substituent on C^7 is also not very significant.



152

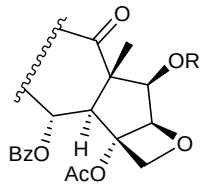
Taxol and taxotere analogs **153** with opened ring **C** were studied in [154]. Their cytotoxicity was found to depend on the side-chain substituent, in contrast to compounds **148** with opened ring **A**. Though the cytotoxicity of **153** [R = Ph, R' = Bz, Boc; R = *i*-Bu, R' = $Me(CH_2)_4CO$] was lower by 1–2 orders of magnitude

than that of taxol, compound **153** (R = *i*-Bu, R' = Boc) showed the same tubulin-polymerizing ability as its analog with closed ring **C** (10-dehydrotaxotere, see above) [204]. The cytotoxicity of **153** (R = *i*-Bu, R' = Boc) was approximately equal to that of taxol. These data suggest that analogs with topologically and stereochemically less complex terpenoid fragment can retain taxol-like activity provided that appropriate substituents are present in the side chain.



153

C-Nor-taxol analogs **154** (R = Ac, H) are less active than taxol by factors of 47 and 8.3 and less cytotoxic by factors of 7 and 2, respectively [270]. Analog **155** with opened ring **D** is inactive and almost noncytotoxic, presumably because of the lack of oxetane ring [199, 270].



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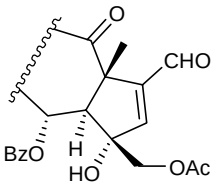
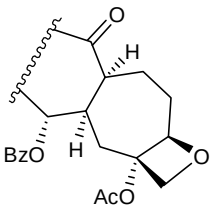


155

19-Nor-7β,8β-methylene taxoid **156** having expanded ring **C** was absolutely inactive and noncytotoxic, despite similarity between its conformation and that of taxotere [271]. The same applies to compound **157** with an additional heteroring fused to rings **B**, **C**, and **D** and analog **158** with modified rings **C** and **D** [270–272]. A conclusion can be drawn that variation of the size and conformation of ring **C** and also of the



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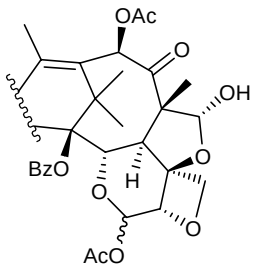

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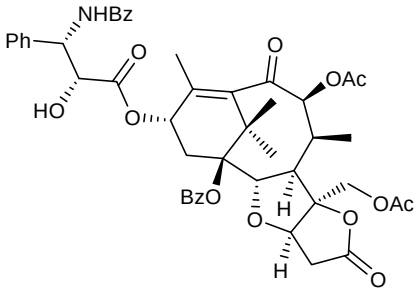
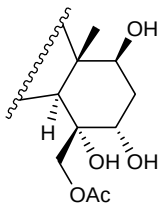


158

conformation of ring **D** produces a negative effect on the activity.

Most studies on modifications of the taxol skeleton were aimed at elucidating the role of the oxetane ring. Compound **159** with opened ring **D** and related analog **160** (R = H) were almost inactive and noncytotoxic [26, 273]. No activity was revealed for derivatives **151** and **155**, as well as for **D**-seco analogs **161** (R = Ph, *m*-ClC_6H_4, *p*-ClC_6H_4) having an amide moiety on C^2, though the corresponding derivatives with intact ring **D** showed some cytotoxic properties (see above) [158].



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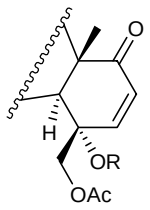

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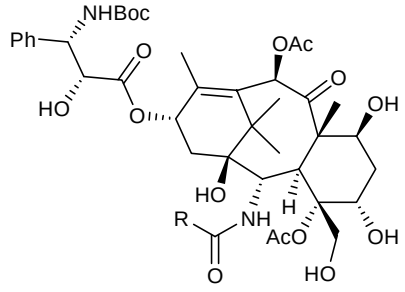
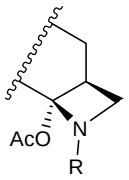


161

A considerable reduction in activity is produced by replacement of the oxygen atom in the oxetane ring by other heteroatoms. Taxotere analog **162** (R = H) is less active than taxotere by an order of magnitude [274]. Very low activity and cytotoxicity relative to taxol were found for thietane analogs of 4-deacetoxy-4-methoxycarbonyloxytaxol, taxotere, and 7-deoxy-10-acetyltaxotere (**163**, R = Ac, CO_2Me) [275, 276]. Insofar as azetidine, thietane, and oxetane fragments are characterized by similar parameters and often are bioisosters, the observed decrease in activity was inter-

preted in terms of a specific interaction between the oxygen atom in ring **D** and tubulin, which does not occur with compounds **162** and **163** [274].



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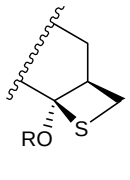

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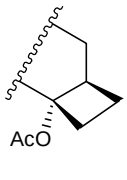
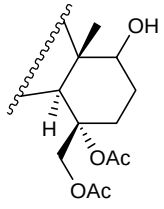


164

The above data indicate an important role of ring **D**. In most studies, the presence of oxetane fragment in a taxol analog is considered to be a necessary condition for binding to tubulin: the oxygen atom therein acts as hydrogen bond acceptor with respect to Thr276, and the oxetane ring endows the taxane skeleton with some conformational rigidity [11]. In particular, the low activity of C^6-nor analogs **154** was explained by the fact that contraction of ring **C** from six- to five-membered makes the oxetane ring more distant from Thr276 thus reducing the efficiency of the $O^{21}\cdots$HO-Thr interaction [137]. However, a different viewpoint has recently been published. Wang *et al*. [277] used a minireceptor model of the taxol binding site on tubulin and showed that the oxetane ring is not necessary to ensure tubulin-polymerizing ability. The authors predicted a series of potentially active analogs having no oxetane ring, e.g., 10-acetyltaxotere derivative **164**. In fact, compound **164** was subsequently synthesized, and it turned out to be approximately equal to taxol in tubulin-polymerizing ability [277, 278]. In terms of the proposed model, the reason for the absence of activity of compounds **159** and **160** (R = H) is the lack of appropriate functionality corresponding to the C^4-acetoxy group in taxol. On the other hand, predictions of a fairly high activity for two C^7-*epi*-taxol analogs **165** (containing an acetyl group in the required position) and of a low activity of compounds **160** (R = Ac) and **166** were unsuccessful: the four **D**-seco-taxoids were inactive and noncytotoxic [272]. Thus the proposed model is to be corrected, mainly as regards steric effects in the vicinity of the oxetane ring. Most probably, this fragment of the pharmacophore is quite sensitive to steric factor, as follows from the lack of activity in analogs **162** with clearly bulky substituents (R = Bzl) [274] and sharply reduced activity of thietane analogs **163** [272, 274–277].



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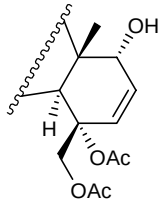
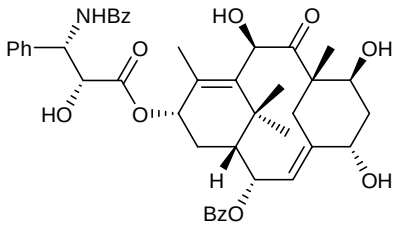


166

4.4.2. Other structural analogs. Soto *et al*. [279] synthesized taxol analog **167** possessing an unusual tricyclo[9.3.1.1]hexadecane skeleton. Although the experimental conformation of this compound was similar to *hydrophobic collapse*, its cytotoxicity was lower by a factor of 30–3000 than that of taxol. Probably, the reason is the absence of other functional groups necessary for effective binding to tubulin, namely C^4-*O*-acetyl group and oxetane ring or related substituents [279]. Taxol analog **168** in which the **C** and **D** rings are replaced by benzene ring showed a considerable cytotoxicity against some tumor cells, while its diastereoisomer was almost noncytotoxic [280]. However, no experimental proofs for binding of compound **168** at the taxol binding site on tubulin have been obtained [277].



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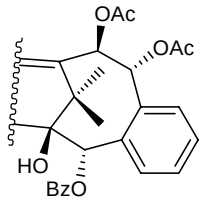

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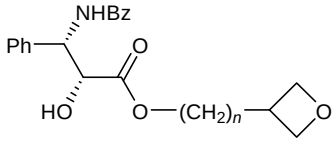
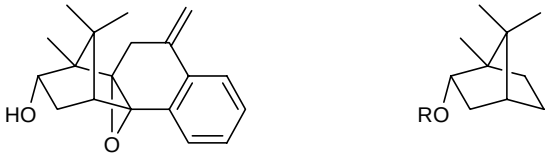


169

In the recent publications [281–283], attempts to create so-called "simplified" taxol analogs were described. Assuming that the role of the taxane skeleton is to hold the side chain and the oxetane ring at a certain distance from each other, Fuju *et al*. [281] synthesized a series of "extremely simplified" taxol analogs **169** (R = Bz, Boc; n = 2–7); the distance between the above fragments in the analog with n = 5 was the closest to that in taxol molecule. However, none of the prepared derivatives showed taxol-like activity.

During the synthesis of the tricyclic taxol skeleton according to Wender, Klar *et al*. [282] isolated

rearrangement product **170** instead of polycyclic epoxy intermediate. An analog of **170** with the taxotere (rather than taxol) side chain attached at the hydroxy group exhibited a weak but appreciable tubulin-polymerizing ability. Structure–activity studies performed on derivatives of this compound showed that removal of the **B**–**C** ring system (**171**, R = taxotere side chain), opening of the epoxy fragment (**172**, R = taxotere side chain, R' = H, OH, MeO), aromatization or contraction of the **B** ring, as well as hydroxylation of the styrene double bond or its hydrogenation to afford β-methyl isomer, lead to complete loss of activity. On the other hand, the α-methyl isomer of the latter compound and some its analogs with modified substituents at $C^{3'}$ (e.g., **173**) were more active than taxol though almost non-cytotoxic [282]. It should be emphasized that the data of [282] give no grounds to believe with certainty that the examined borneol esters bind to the same site on β-tubulin as does taxol.



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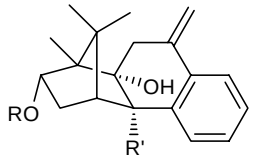

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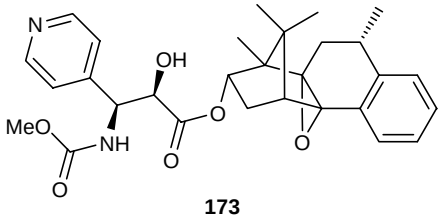

173

To conclude this section, let us note that studies on the design of simplified taxol analogs on the basis of *N*-benzoylphenylisoserine esters derived from alcohols of the adamantane and bicyclo[3.3.1]nonane [284] series have been initiated at the Moscow State University.

5. CONCLUSION

Thus, up to now various structural modifications of the taxol molecule have been performed with the goal of obtaining more active analogs. On the other hand, the number of these analogs is not very large, despite considerable advances in the field of computer simulation of taxol–tubulin models, docking of potential ligands, and structure–activity correlations [129, 137, 237, 246, 285, 286]. Presumably, further progress in the search for active taxol analogs will be determined by success in the elucidation of bioactive conformations of the natural molecule and in the prediction of tubulin-polymerizing ability and cytotoxicity of taxol analogs with account taken of their lipophilicity, resistance to metabolic enzymes, and other parameters. Solution of these problems should make it possible to maximally simplify the structure of tubulin ligands, thus enabling accessible synthesis of a potential antitumor agent.

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