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C-3'-N-ACYL ANALOGS OF 9(R)-DIHYDROTAXOL: SYNTHESIS AND STRUCTURE ACTIVITY RELATIONSHIPS

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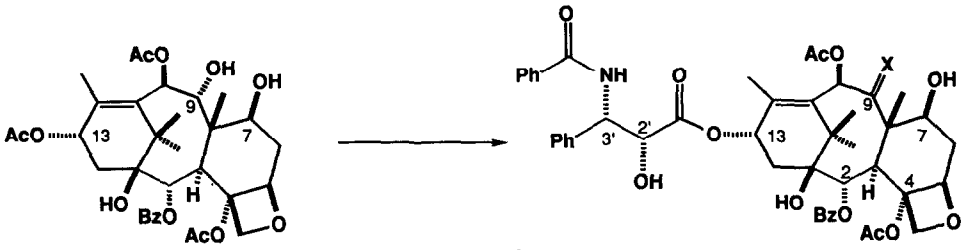
Abstract: C-3'-N-Acyl analogs of 9(R)-dihydrotaxol were synthesized from 7-triethylsilyl-9(R)-dihydrobaccatin III and the corresponding (3R,4S)-N-acyl-3-(1-ethoxyethoxy)-4-phenylazetidin-2-ones. The analogs were tested in a microtubule assembly assay, and in an *in vitro* cytotoxicity assay. The highest activities observed were for the alkylcarbamate substitutions.

The isolation and characterization of 13-acetyl-9(R)-dihydrobaccatin III[1] (**1**) offers the potential for a unique entry into the field of Taxol® antitumor agents. This potential was actualized first with the synthesis of 9(R)-dihydrotaxol (**2**) which exhibited *in vitro* activity comparable to that of Taxol® (**3**).[2] Therefore, having demonstrated that the 9(R)-hydroxyl group is a tolerated modification in the diterpenoid portion of the molecule, studies on the effects of side chain modifications on this new structure were intiated.

Scheme 1



1

2. X = H, α - OH 9(R) - Dihydrotaxol
3. X = O Taxol

The phenylisoserinate side chain at the C-13 position of Taxol® has been established as an essential component for *in vitro* cytotoxic activity by the promotion and stabilization of microtubule assembly. Studies involving systematic deletion and substitution analogs of the side chain have clearly established the natural regiochemical and stereochemical configuration (2'R,3'S) of the C-2' hydroxyl group and a C-3' acylated amine as being optimal.[3] As a result, synthetic modifications have been focused on the C-3' nitrogen and on the C-3' phenyl group.[4] Substitutions on and for the C-3' phenyl ring have been reported, and the full scope of modifications of this position and the consequences on *in vitro* activity are now beginning to be described.[5] Substitutions for the C-3' phenyl ring of the 9(R)-dihydrotaxol have been reported and will be the subject of another paper.[6]

One of the more promising reported changes in the side chain of Taxol® is the replacement of the 3'-N-benzoyl group with a *tert*-butoxycarbonyl (t-BOC) group. A substantial increase in the tubulin activity and *in vitro* cytotoxicity was observed for Taxotère®, the 10-deacetyl-3'-N-t-BOC derivative of Taxol®.[7] The structural divergence of the t-BOC group from the benzoyl group suggests a great potential for modification of this substituent of the side chain. Although one might infer from the clinical status of Taxotère® that the t-BOC substitution represents an optimum substitution of the C-3' nitrogen in the Taxol® series, the published data on the SAR leading to the t-BOC modification is fragmentary. We now report our results on the synthesis and SAR of some C-3'-N-acyl derivatives of 9(R)-dihydrotaxol.

In general, the synthesis (Scheme 2) of the 3'-N-acyl derivatives proceeded by the reaction of the oxyanion of 7-triethylsilyl-9(R)-dihydrobaccatin III (**5**) with the respective (3R,4S)-N-acyl-3-(1-ethoxyethoxy)-4-phenylazetidin-2-ones (**6**) to afford compounds **7a-o**.[8] Subsequent deprotection of **7** at the C-2',7 positions produced the analogs **8a-o**. Compound **4** was obtained by extending the previously reported[2] ability of n-butyllithium to effect regioselective deacylation of the C-13 acetate on a protected derivative of **1**. In fact, the reaction of compound **1** itself with methyllithium in THF at -78°C also effected regioselective deacylation to afford **4**. Subsequently, the C-7 hydroxyl was triethylsilylated to produce **5** which served as a suitably protected intermediate for regioselective acylation exclusively at C-13. The reaction conditions and the unoptimized yields for the coupling reactions and deprotections are listed in Table 1.

Scheme 2

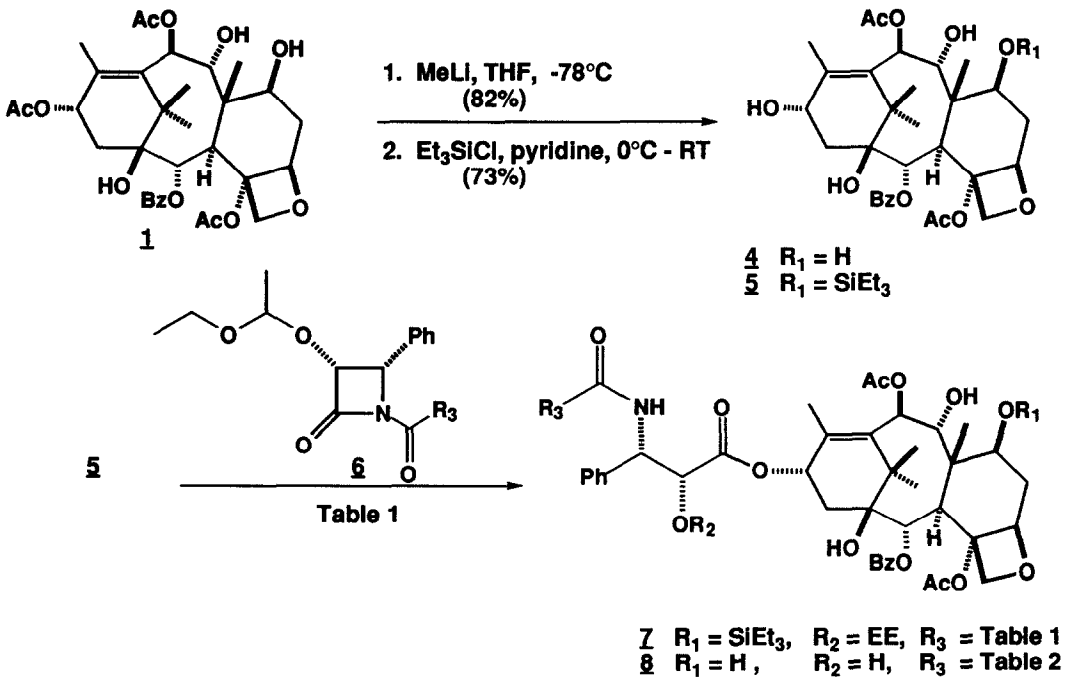


Table 1. Acylation and deprotection conditions for compounds **7** and **8**

	R_3 =	Base	Yield (%) Acylation	Deprotection Conditions	Yield(%) Deprotection
7a	p-CH_3phenyl	NaH	79	C[a]	**8a** 70
7b	p-BnOphenyl	NaH	28	D	**8b** 87
7c	2-furyl	LiHMDS	50	C	**8c** 58
7d	methyl	NaH	48	C	**8d** 38
7e	t-butyl	NaH	43	C	**8e** 81
7f	*tert*-butyl-CH_2	NaH	88	A	**8f** 45
7g	-----	---	----	D	**8g** 83[b]
7h	*tert*-butyl-O	NaH	89	B	**8h** 84
7i	*tert*-amyl-O	NaH	85	C	**8i** 82
7j	isopropyl-O	NaH	85	A	**8j** 79
7k	neopentyl-O	NaH	77	C	**8k** 58
7l	adamantyl-O	NaH	87	A	**8l** 88
7m	isobutyl-O	NaH	85	A	**8m** 80
7n	ethyl-O	NaH	50	C	**8n** 83
7o	benzyl-O	NaH	47	C	**8o** 33

[a] A = 1) HF, Et_3N, MeOH; 2) AcOH B = 1) HF, Et_3N, MeOH; 2) 1% HCl, EtOH
C = 1) 1% HCl, EtOH D = 1) HF, Et_3N, MeOH; 2) AcOH; 3) H_2, Pd

[b] Compound **8g** was synthesized from **7o** using method D followed by t-butylisocyanate, DMAP. The yield shown is for the acylation of the primary amine to give **8g**.

The synthetic compounds were evaluated in a microtubule assembly assay[9] and in *in vitro* cytotoxicity assays against human and mouse tumor cell lines[10] as shown in Table 2. When compared to the reference standard Taxol® (**3**) 9(R)-Dihydrotaxol (**2**), showed very good tubulin activity but slightly diminshed *in vitro* cytoxicity. Compounds **8a-c** show the tubulin activity to be maintained with substitutions on the para position of the C-3' benzamide. Even with replacement of the phenyl by a 2-furyl group tubulin activity and cytotoxicity is retained. The relative lack of cytotoxicity of **8b** stands in contrast to its tubulin activity and the cytotoxicity of **8a** and **8c**. This observation could be attributed to an inability of **8b** to penetrate cellular membranes due to the polar phenol group. Replacing the phenyl ring with a methyl (**8d**) results in complete loss of activity. Activity was restored by the addition of "sufficient" and/or "properly placed" lipophilicity as in **8e-f**. As was observed in the Taxol® series, the alkylcarbamate substitutions (**8h-n**) in general provide a substantial increase in *in vitro* cytotoxicity with the t-butoxycarbonyl derivative **8h** presenting the optimum activity. The relationship of *in vitro* cytotoxicity to *in vivo* potency for this series of compounds remains to be determined.

Table 2

Compound		Tumor Cell[a] Cytotoxicity IC_{50} (μg/mL)				Tubulin
	R_3=	A549	HT-29	B16F10	P388	ED_{50}/ED_{50}taxol
3	Taxol	0.0034	0.0024	0.0041	0.046	1.0
2	9(R)-Dihyrotaxol	0.019	0.0080	0.025	0.053	0.76
8a	p-CH_3phenyl	0.043	0.012	0.025	0.063	0.75
8b	p-HOphenyl	>0.1	0.067	>0.1	>0.1	1.07
8c	2-furyl	0.0072	0.0041	0.012	0.034	0.73
8d	methyl	2.1	0.27	1.1	2.02	3.17
8e	t-butyl	0.11	<0.01	0.035	0.083	2.36
8f	*tert*-butyl-CH_2	0.027	0.009	0.0056	0.052	1.03
8g	*tert*-butyl-NH	0.014	0.0056	0.0046	0.017	1.06
8h	*tert*-butyl-O	0.0003	0.00016	0.0004	0.0025	0.87
8i	*tert*-amyl-O	0.001	0.00067	0.0003	0.0046	0.73
8j	isopropyl-O	0.0012	0.0008	0.0022	0.0092	0.75
8k	neopentyl-O	0.00065	0.0015	0.0074	0.019	0.82
8l	adamantyl-O	0.0043	0.0028	0.0048	0.01	1.54
8m	isobutyl-O	0.0043	0.0023	0.0069	0.028	1.03
8n	ethyl-O	0.0057	0.005	0.018	0.043	0.82
8o	benzyl-O	0.031	0.012	0.044	0.057	---

[a] A549- human lung carcinoma; HT29- human colon adenocarcinoma: B16F10- mouse melanoma; P388- mouse leukemia

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