Designed Epothilones: Combinatorial Synthesis, Tubulin Assembly Properties, and Cytotoxic Action against Taxol-Resistant Tumor Cells**

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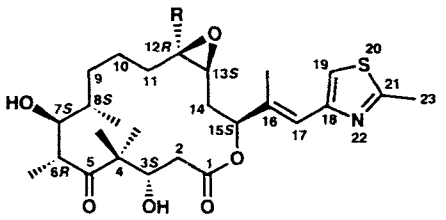
The isolation of epothilones A (**1**) and B (**2**) from the myxobacterium *Sorangium cellulosum* strain 90 by Höfle et al.[1] and the recognition of their cytotoxic action against tumor cells prompted intense research activities in chemistry and biology. In 1995 Bollag et al.[2] reported the Taxol (paclitaxel)-like mechanism of action of these compounds through induction of tubulin assembly and microtubule stabilization.[3] The recognition of their unique action against Taxol-resistant tumor cell lines[4] added to their novelty and potential importance in cancer chemotherapy. In 1996 Höfle et al. published the complete elucidation of the stereochemistry as determined by spectroscopic and X-ray crystallographic techniques.[5] Soon thereafter, a flurry of reports announced several approaches[6] to, and total syntheses of, epothilones A (**1**)[7-11,13] and B (**2**).[10,12,13] In addition to the natural products themselves, several precursors and analogues have been synthesized[7-17] and biologically investigated.[7,10,12,14,17] Notable amongst these are the 12,13-desoxy analogues (lacking the epoxide oxygen) first reported by Danishefsky et al.[7,12] and independently by ourselves,[8,10,11,13] Schinzer et al.,[9] and Höfle et al.[14] Herein we report on a) the solid-phase synthesis of several epothilone A analogues based on a combinatorial approach, b) the tubulin assembly properties of an extensive library of compounds, and c) the cytotoxic actions against breast and ovarian carcinoma cells (including a number of Taxol-resistant tumor cell lines) of a selected number of these designed epothilones. The results provide comprehensive information on structure–activity relationships of epothilones and set the foundation for their further development.



1: R = H : epothilone A
2: R = Me: epothilone B

The structures of epothilones are amenable to modification by changing the configuration of certain stereocenters, the geometry of the double bonds, the size of the rings, and the nature of their substituents. Our synthetic strategies towards these molecules were, therefore, designed on the premise of modifying these elements so as to reach optimum molecular diversity and obtain a maximum number of library members. Table 1 includes the structures of an epothilone library obtained by solution[6a,8,10,11,13,15,16,28] and solid-phase[10,28] combinatorial methods. Biological screening of these compounds was expected to lead to the establishment of sufficient structure–activity relationships to allow the next phase of the program, the design, synthesis, and identification of potential drug candidates, to proceed along a narrower track.

The strategy for the construction of a library of epothilone A analogues was based on our previously reported solid-phase synthesis[10] of epothilone A and Radiofrequency Encoded Combinatorial (REC) chemistry.[18] Scheme 1 summarizes the synthesis of a library of 12,13-desoxyepothilones A from the three key fragments generically denoted as **A**, **B**, and **C**.[19] Thus, SMART Microreactors[20] (SMART = single or multiple addressable radiofrequency tag) containing Merrifield resin were smoothly converted to Microreactors **I** by chain extension and phosphonium salt formation. Phosphonium salt resin **I** was then sorted according to the radiofrequency tag and treated with sodium hexamethyldisilazide (NaHMDS) to generate the corresponding ylides which were treated with the aldehydes **A**. The SMART Microreactors **II** were pooled for washing and subsequent deprotection and oxidation to obtain the polymer-bound aldehydes **III**. Further sorting and treatment with the dianion of the ketoacids **B** provided the polymer-bound carboxylic acids **IV** as a mixture of diastereoisomers. Resorting and esterification with alcohols **C** afforded dienes **V**. The SMART Microreactors were separately treated with $[RuCl_2(=CHPh)(PCy_3)_2]$ catalyst to simultaneously effect cyclization by olefin metathesis[6a,7a,8a,9-11,15,21,22] and cleavage of the products, leading to products as mixtures of four 12,13-desoxyepothilones A (**VII**, **VIII**, **IX**, **X**). Each mixture was identified and subjected to preparative thin-layer chromatography to provide pure compounds, which were individually deprotected by treatment with TFA in dichloromethane and then epoxidized accordingly.[6a,7b,8-11,13,15-17]

The epothilone library (Table 1) was screened for induction of tubulin assembly with 5 μM compound at 37 °C.[2] Previously tested compounds (see references in Table 1) were reevaluated for comparative purposes. Most analogues were subjected to more detailed investigation in cytotoxicity assays with human ovarian and breast cancer cells, including Taxol-resistant lines,[23] and a quantitative tubulin assembly assay that differentiates between potent taxoid compounds (Table 2).[24] It soon became apparent that compounds with assembly values below 40% in the screen yielded high EC_{50} values in the quantitative

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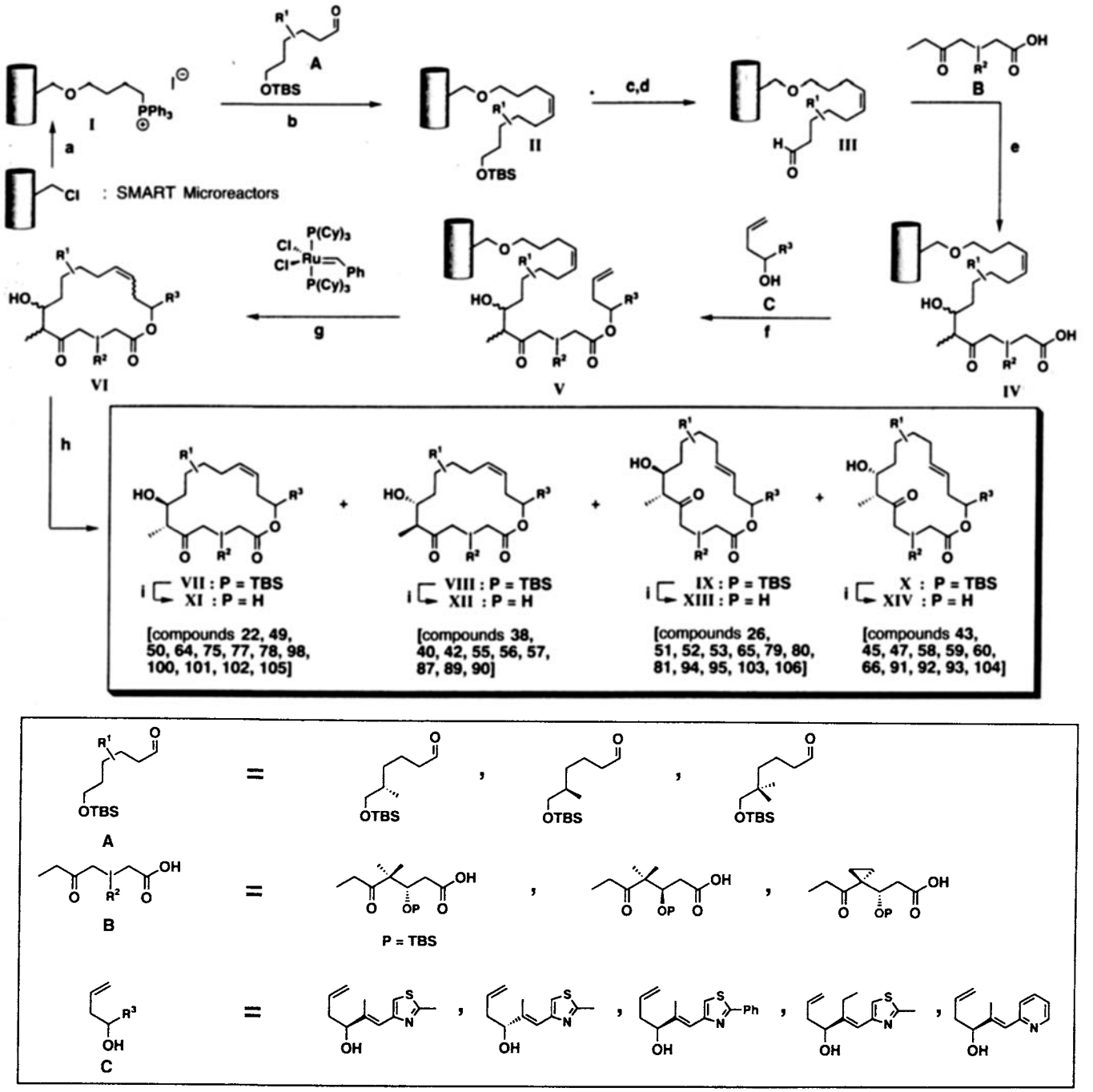

Scheme 1. Solid-phase synthesis of epothilone A analogues by a combinatorial approach. a) 1. 1,4-butanediol (5.0 equiv), NaH (5.0 equiv), nBu$_4$NI (0.1 equiv), DMF, 25 °C, 12 h; 2. Ph_3P (4.0 equiv), I_2 (4.0 equiv), imidazole (4.0 equiv), CH_2Cl_2, 25 °C, 3 h; 3. Ph_3P (10 equiv), 90 °C, 12 h; b) 1. sort SMART Microreactors (with an Accutag-100 apparatus); 2. NaHMDS (3.0 equiv), THF:DMSO (1:1), 25 °C, 12 h; 3. **A** (2.0 equiv), THF, 0 °C, 3 h (≈75% from chloromethyl polystyrene loading based on recovered aldehyde upon ozonolysis); 4. pool; c) 0.2 M HCl in THF, 25 °C, 12 h; d) $(COCl)_2$ (4.0 equiv), DMSO (8.0 equiv), Et_3N (12.5 equiv), −78 → 25 °C (≥95% for two steps, the reactions were monitored by IR analysis of polymer-bound material and by TLC analysis of the products obtained by ozonolysis); e) 1. sort; 2. **B** (2.0 equiv), LDA (2.2 equiv), THF, −78 → −40 °C, 1 h; then add resulting enolate to the resin suspended in a $ZnCl_2$ (2.0 equiv) solution in THF, −78 → −40 °C, 2.0 h (≥90%, based on recovered aldehyde upon ozonolysis); 3. pool; f) 1. sort; 2. **C** (5.0 equiv), DCC (5.0 equiv), 4-DMAP (5.0 equiv), 25 °C, 15 h (≥85% yield as determined by recovered heterocycle fragments obtained upon treatment with NaOMe); g) 1. separation of individual SMART Microreactors; 2. $[RuCl_2(=CHPh)(PCy_3)_2]$ (0.20 equiv), CH_2Cl_2, 25 °C, 48 h; h) automated HPLC (SiO_2, EtOAc/hexane, or C_{18}, H_2O/THF), or preparative thin-layer chromatography, EtOAc/hexanes. The stereochemisty at C-6 and C-7 as well as the geometry of the olefin was tentatively assigned by ^{1}H NMR spectroscopy; i) 20% TFA in CH_2Cl_2 (v/v), 25 °C, 2–12 h. The purity of individual compounds was established by ^{1}H NMR spectroscopy and HPLC. P = protecting group; TBS = *tert*-butyldimethylsilyl; DMSO = dimethyl sulfoxide; TFA = trifluoroacetic acid; LDA = lithium diisopropylamide; DCC = *N,N'*-dicyclohexylcarbodiimide; 4-DMAP = 4-dimethylaminopyridine.

assay and had little inhibitory effect on cell growth (only positive results shown in Table 2).

The glutamate assay was devised to test the hypothesis that taxoids more active than Taxol in tubulin assembly would also be more cytotoxic,[24] and this was validated with over fifty analogues (Hamel et al., unpublished results).[33] With the epothilones, however, the quantitative assay was less successful. A low glutamate concentration resulted in a high false negative rate in predicting cytotoxicity (data not shown), while higher glutamate concentrations (e.g. 0.7 M, Table 2) were comparable

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Table 1. Structures and tubulin polymerization properties of epothilone analogues. Footnotes [a]–[i] are on page 2101.

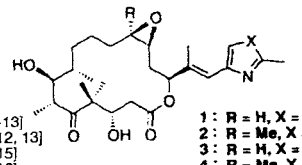
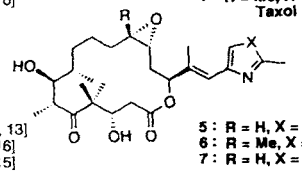
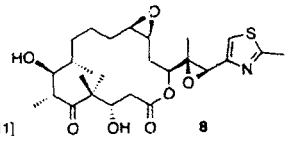
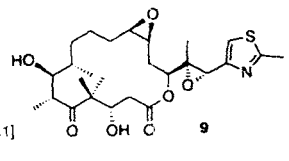
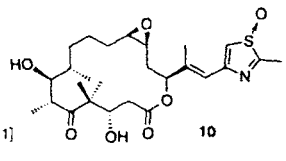
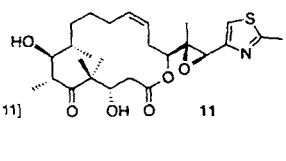
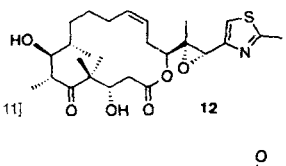
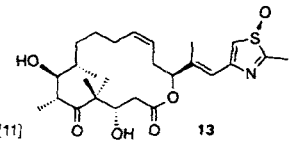
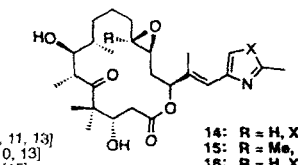
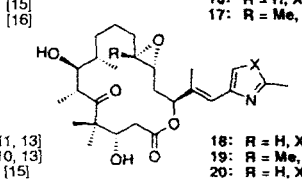
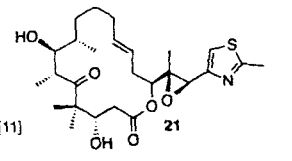
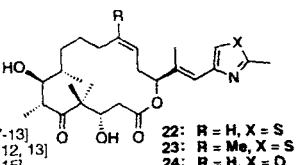
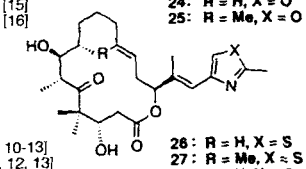
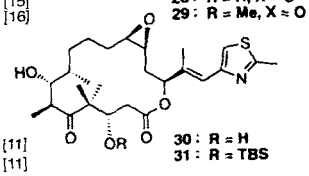
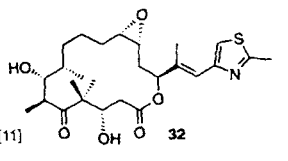
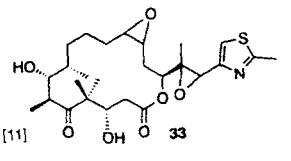
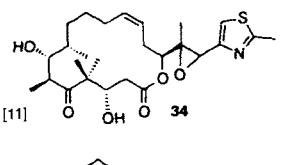
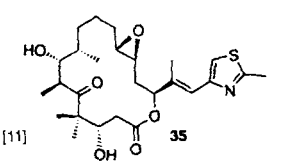
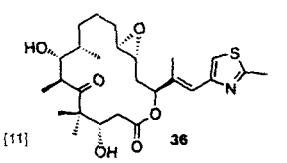
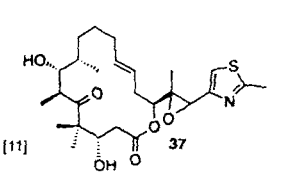
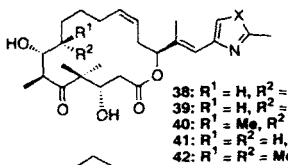
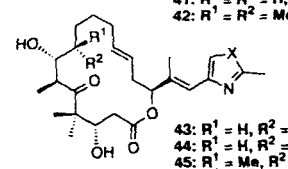
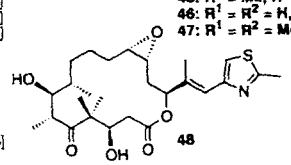
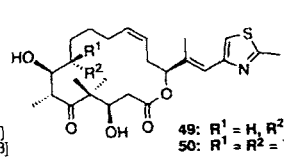
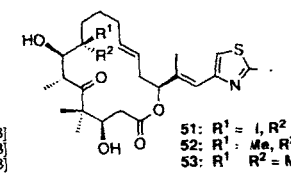
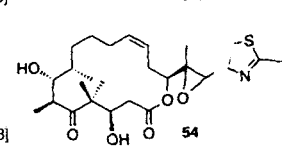
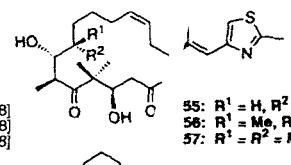
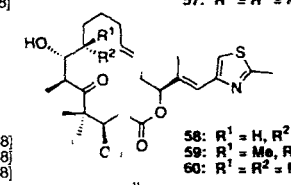
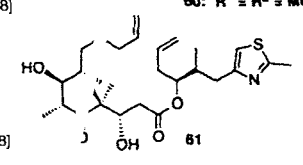
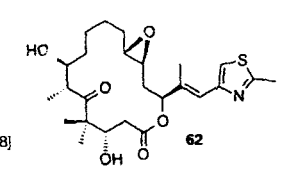
Ref.	Structure	Tubulin polymerization [%][a]
[7-13] [10, 12, 13] [15] [16]		1: R = H, X = S 76 [b] 2: R = Me, X = S 98 [b] 3: R = H, X = O 58 4: R = Me, X = O 93 Taxol 50
[11, 13] [16] [15]		5: R = H, X = S 17 6: R = Me, X = O 71 7: R = H, X = O 24
[11]		8 16
[11]		9 14
[11]		10 26
[8, 11]		11 20
[8, 11]		12 9
[11]		13 22
[10, 11, 13] [10, 13] [15] [16]		14: R = H, X = S 92 15: R = Me, X = S 84 [c] 16: R = H, X = O 64 17: R = Me, X = O 95
[11, 13] [10, 13] [15]		18: R = H, X = S 17 19: R = Me, X = S 63 20: R = H, X = O 46
[11]		21 12
[7-13] [10, 12, 13] [15] [16]		22: R = H, X = S 72 [d] 23: R = Me, X = S 94 [d] 24: R = H, X = O 75 25: R = Me, X = O 93
[7, 10-13] [10, 12, 13] [15] [16]		26: R = H, X = S 76 [e] 27: R = Me, X ≈ S 84 [d] 28: R = H, X = O 43 29: R = Me, X ≈ O 54
[11] [11]		30: R = H 25 31: R = TBS 6
[11]		32 20
[11]		33 7
[11]		34 23
[11]		35 19
[11]		36 16
[11]		37 22
[11] [15] [28] [28] [28]		38: R¹ = H, R² = Me, X = S 13 39: R¹ = H, R² = Me, X = O 18 40: R¹ = Me, R² = H, X = S 5 41: R¹ = R² = H, X = S 23 42: R¹ = R² = Me, X = S 5
[11] [15] [28] [28] [28]		43: R¹ = H, R² = Me, X = S 13 44: R¹ = H, R² = Me, X = O 20 45: R¹ = Me, R² = H, X = S 11 46: R¹ = R² = H, X = S 21 47: R¹ = R² = Me, X = S 4
[28]		48 25
[7] [28]		49: R¹ = H, R² = Me 16 [f] 50: R¹ = R² = [illegible] e 3
[28] [28] [28]		51: R¹ = [illegible] I, R² = Me 21 52: R¹ = [illegible] Me, R² = H 3 53: R¹ = R² = Me 4
[28]		54 7
[28] [28] [28]		55: R¹ = H, R² = Me 23 56: R¹ = Me, R² = H 5 57: R¹ = R² = Me 1
[28] [28] [28]		58: R¹ = H, R² = Me 24 59: R¹ = Me, R² = H 7 60: R¹ = R² = Me 5
[28]		61 18
[28]		62 21

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Table 1 (cont.)

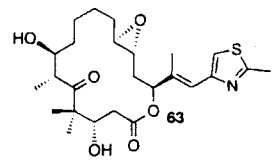
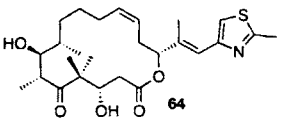
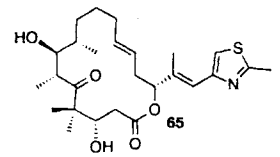
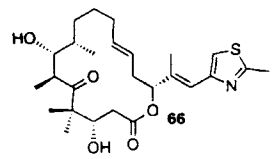
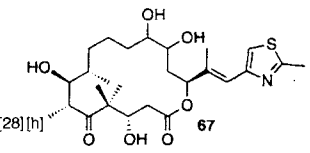
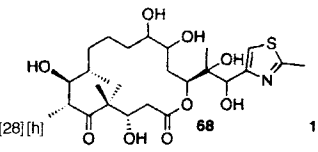
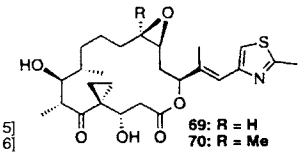
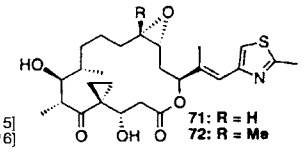
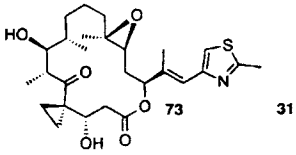
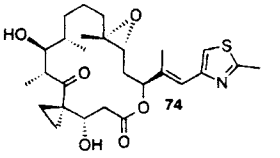
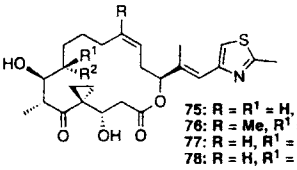
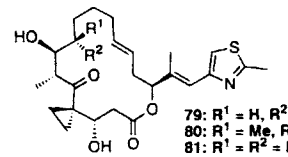
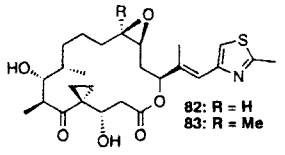
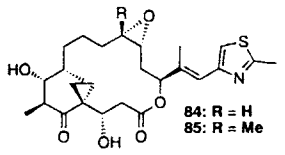
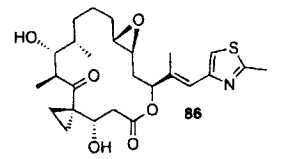
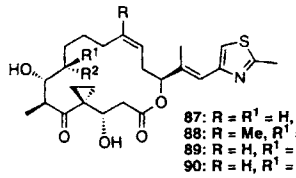
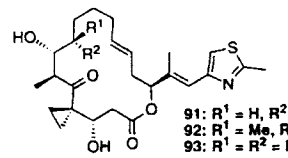
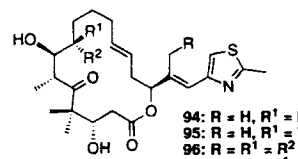
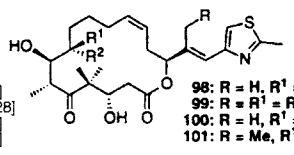
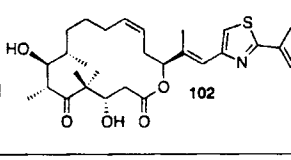
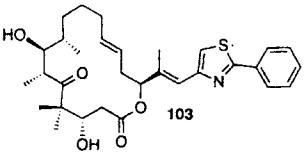
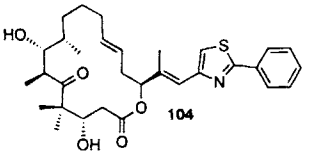
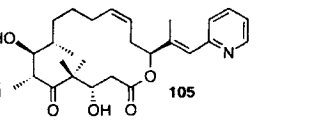
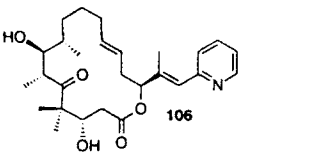
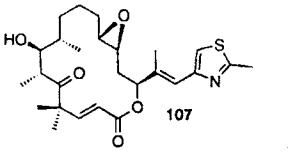
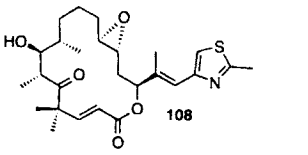
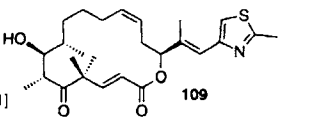
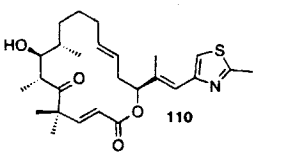
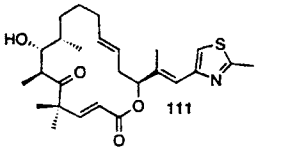
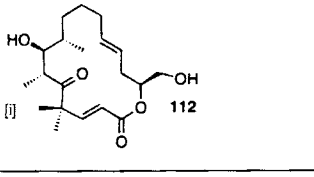
Ref.	Structure	Tubulin polymerization [%][a]
[28]	 **63**	27
[28]	 **64**	10
[28]	 **65**	10
[28]	 **66**	15
[28][h]	 **67**	12
[28][h]	 **68**	13
[15] [16]	 **69: R = H** **70: R = Me**	20 19
[15] [16]	 **71: R = H** **72: R = Me**	5 6
[16]	 **73**	31
[16]	 **74**	18
[15] [16] [28] [28]	 **75: R = R¹ = H, R² = Me** **76: R = Me, R¹ = H, R² = Me** **77: R = H, R¹ = Me, R² = H** **78: R = H, R¹ = R² = Me**	17 22 5 4
[15] [28] [28]	 **79: R¹ = H, R² = Me** **80: R¹ = Me, R² = H** **81: R¹ = R² = Me**	25 9 5
[15] [16]	 **82: R = H** **83: R = Me**	10 27
[15] [16]	 **84: R = H** **85: R = Me**	9 18
[15]	 **86**	3
[15] [16] [28] [28]	 **87: R = R¹ = H, R² = Me** **88: R = Me, R¹ = H, R² = Me** **89: R = H, R¹ = Me, R² = H** **90: R = H, R¹ = R² = Me**	13 6 1 5
[15] [16] [28]	 **91: R¹ = H, R² = Me** **92: R¹ = Me, R² = H** **93: R¹ = R² = Me**	18 5 4
[28] [28] [28] [28]	 **94: R = H, R¹ = Me, R² = H** **95: R = H, R¹ = R² = Me** **96: R = R¹ = R² = H** **97: R = Me, R¹ = H, R² = Me**	11 3 24 48
[28] [17, 28] [28] [28]	 **98: R = H, R¹ = Me, R² = H** **99: R = R¹ = R² = H** **100: R = H, R¹ = R² = Me** **101: R = Me, R¹ = H, R² = Me**	11 23 5[g] 34
[28]	 **102**	25
[11]	 **103**	16
[28]	 **104**	12
[28]	 **105**	51
[28]	 **106**	61
[11]	 **107**	46
[11]	 **108**	28
[11]	 **109**	27
[11]	 **110**	58
[11]	 **111**	20
[28] [i]	 **112**	17

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Table 2. Biological properties of selected epothilone analogues.

Cmpd.	Induction of tubulin polymerization: Screening-assay[a] polymer formation[%]	quantitative glutamate assay[b] EC_{50}[μM]	Inhibition of carcinoma cell growth: Ovarian[d] parental 1A9	Ovarian[d] β-tubulin mutations 1A9PTX10 IC_{50}[nM][relative resistance][f]	Ovarian[d] β-tubulin mutations 1A9PTX22 IC_{50}[nM][relative resistance][f]	Breast[e] MCF7
Taxol	50	4.7	1.4	32 [23]	38 [27]	4.2
1	76	4.6	2.2	20 [9.1]	5.9 [2.7]	5.1
2	98	3.4	0.13	1.0 [7.7]	0.31 [2.4]	1.0
3	58	5.3	3.0	25 [8.3]	8.0 [2.7]	6.1
4	93	–	0.2	1.1 [5.5]	0.9 [4.5]	–
6	71	6.1	1.5	11 [7.3]	3.0 [2.0]	6.2
14	92	6.2	2.0	18 [9.0]	3.0 [1.5]	5.4
15	84	5.6	1.0	8.5 [8.5]	1.0 [1.0]	1.8
16	64	7.8	3.5	32 [9.1]	9.5 [2.7]	>100
19	63	13	6.0	30 [5.0]	6.5 [1.1]	14
20	46	8.1	4.8	34 [7.1]	9.0 [1.9]	5.7
22	72	8.3	32	>100	100	38
23	94	3.9	6.5	23 [3.5]	9.0 [1.4]	9.3
24	75	6.1	68	>100	90	74
25	93	3.3	8.0	30 [3.8]	12 [1.5]	>100
26	76	9.8	60	>100	100	>100
27	84	7.5	61	>100	85	75
28	43	13	>100	–	–	>100
29	54	6.0	32	>100	>100	68
101	34	17	>100	–	–	>100
105	51	7.6	32	>100	70	57
106	61	11	82	>100	>100	78
107	46	–	28	>100	50	–

[a] From Table 1. [b] Assay performed as described in ref. [24]; reaction mixtures contained 10 μM purified tubulin [29], 0.7 M monosodium glutamate, 5% DMSO and drug; incubation was for 20 min at room temperature and reaction mixtures were centrifuged at 14000 rpm; supernatant protein concentration was measured and the EC_{50} value is defined as the drug concentration resulting in a 50% reduction in supernatant protein relative to control values; each EC_{50} value shown is an average (standard deviations ≤20%) obtained in two to four independent assays. [c] Cell growth was evaluated by measurement of increase in cellular protein [30]. [d] The parental ovarian cell line, derived as a clone of line A2780 [31], was used to generate Taxol-resistant cell lines by incubating the cells with increasing concentrations of Taxol with verapamil [23]; the cells were grown in the presence of drug for 96 h; values shown in the table were single determinations, except for those of Taxol, **1** and **2** (average of six determinations each); the values for **1** and **2** are averages of data obtained with both synthetic and natural samples (generously provided to E. H. by Merck Research Laboratories), which did not differ significantly. [e] The MCF7 cells were obtained from the National Cancer Institute drug screening program [32]; cells were grown in the presence of drug for 48 h; each value represents an average of two determinations. [f] Relative resistance is defined as the IC_{50} value obtained for the β-tubulin mutant line divided by that obtained for the parental cell line.

to the screening assay in identifying cytotoxic analogues. If "significant" cytotoxicity is defined as an IC_{50} value below 10 nM, we identified nine analogues with activity against the breast and ovarian lines (**3**, **6**, **14**–**16**, **19**, **20**, **23**, and **25**). With the screening assay, there were no false negatives, but there were seven false positives (agents with limited cytotoxicity yielding >40% polymerization) among examined compounds. With the glutamate assay, the same results were obtained. The nine cytotoxic analogues had EC_{50} values of 3.3–13 μM, but an additional nine agents had EC_{50} values of 6.0–17 μM.

Two Taxol-resistant lines were generated from the 1A9 ovarian cells, and resistance resulted from mutations in the M40 gene, which codes for a highly expressed β_1 isotype in the parental and resistant cell lines.[23] The altered amino acids were residue 270 in the 1A9PTX10 line (Phe → Val) and 364 in 1A9PTX22 (Ala → Thr). This agreed with other observations that the Taxol binding site is on β-tubulin.[25–27] In preliminary results[10] we had reported with **1** and several analogues that 1A9PTX22 cells retained nearly complete sensitivity to epothilones, while 1A9PTX10 cells remained partially resistant to the drugs. These findings have been confirmed (Table 2). The relative resistance observed with 1A9PTX22 cells was 27-fold with Taxol and 1.0–2.7-fold with the eleven cytotoxic epothilones. With 1A9PTX10 cells, relative resistance was 23-fold with Taxol and 3.5–9.1-fold with the epothilones. The Taxol and epothilone binding sites could overlap, since **1** and **2** are competitive inhibitors of Taxol binding to tubulin polymer.[4] If one assumes that Phe270 and Ala364 interact directly with Taxol, the results with the resistant cells suggest that Phe270 is more important than Ala364 in the interaction of epothilones at the Taxol binding site.

The data in Tables 1 and 2, together with previously reported results,[1, 2, 7, 10, 12, 17] revealed important information regarding structure–activity relationships for in vitro tubulin polymerization and in vitro cytotoxicity, and lead to several conclusions. The importance of the macrocycle was confirmed by the lack of significant tubulin polymerization activity of the open-chain olefin metathesis precursor **61**. This conclusion was also reached by Höfle et al.[14] with a degradation product of epothilone A. Inversion of the configuration at C3 resulted in reduced tubulin polymerization potency. Interestingly, however, α,β-unsaturated lactones (e.g. **107** and **110**) retained significant tubulin assembly properties (Table 1) suggesting a conformational, rather than a direct binding effect for this hydroxyl group. Neither **107** nor **110**, however, exhibited significant cytotoxicity indicating an additional role for the 3-OH group. Substitution of the 4-*gem*-dimethyl with a 4,4-ethano moiety (e.g. **69** and **70**) resulted in loss of tubulin polymerization activity in all cases, pointing to the crucial importance of a proper conformation of epothilones for biological activity. Apparently the partial sp^2 character and the accompanied widening of the C3-C4-C5 angle introduced intolerable conformational changes within the macrocycle for effective interaction with tubulin. Another clear requirement for tubulin polymerization activity was the (6*R*,7*S*) configuration as revealed by the failure of all (6*S*,7*R*) stereoisomers to induce tubulin polymerization at significant concentrations (e.g. **30**, **47**, **54**–**60**, **66**, **82**–**93**, **104**, **111**, Table 1). Interestingly, there was a notable decrease in interaction with tubulin upon inversion of

Footnote to Table 1:

[a] Tubulin polymerization was determined by the filtration-colorimetric assay following the procedure of Bollag et al.[2]; purified tubulin [29] (1 mg/mL) was incubated at 37 °C for 30 min in the presence of each compound (5 μM) in MEM buffer [(100 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.75, 1 mM ethylene glycol bis(β-aminoethyl ether). *N*,*N*,*N*′,*N*′-tetraacetic acid, and 1 mM $MgCl_2$]; the mixture was then filtered to remove unpolymerized tubulin by using a 96-well Millipore Multiscreen Durapore hydrophilic 0.22 μm pore size filtration plate; the collected polymerized tubulin was stained with amido black solution and quantified by measuring absorbance of the dyed solution on a Molecular Devices Microplate Reader; the [%] polymerization was calculated relative to the absorbance produced by incubation with 0.5 M GTP + 10% glycerol in MEM buffer (presumed to cause 100% tubulin polymerization); these values represent the average of three experiments. [b] Refs. [1, 2, 10, 12, 17]. [c] Ref. [10]. [d] Refs. [10, 12, 17]. [e] Refs. [7, 12]. [f] Ref. [7]. [g] Ref. [17]. [h] Compounds **67** and **68** were obtained by reaction of compound **22** with OsO_4-NMO. [i] Compound **112** was prepared by desilylation of the corresponding primary *tert*-butyldiphenylsilyl ether (Ref. [11]).

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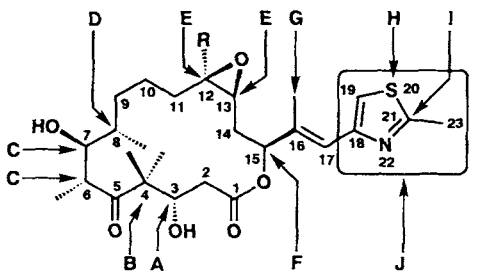
the C8 methyl group (e.g. **98** vs **22**), introduction of a *gem*-dimethyl group at C8 (**100** vs **22** and **95** vs **26**), and removal of the C8 methyl group (e.g. **99** vs **22** and **96** vs **26** and **62** vs **14**). The latter observation was also made independently by Danishefsky et al.[17]

The importance of the natural (12*R*,13*S*) configuration for the epoxide was demonstrated by the general trend of the unnatural (12*S*,13*R*) epoxides to exhibit lower activities in inducing tubulin assembly (e.g. **5** vs **1**, **6** vs **4**, **7** vs **3**; although compound **6** had activities similar to those of epothilone A (**1**), but not B (**2**)). Most interestingly, both the *cis* and *trans* olefins corresponding to epothilones A and B were active in the tubulin assembly assays, and the activities of the *cis* olefins were comparable to those of the natural substances. Similar observations were independently made by Danishefsky et al.[7b, 12, 17] and Höfle et al.[14] However, we found that the *cis* and especially the *trans* olefins were significantly less cytotoxic than the naturally occurring epoxides (**22** and **26** vs **1**, **23** and **27** vs **2**). Moreover, both the α- and β-epoxides derived from the 12,13*E*-olefinic precursors exhibited considerable ability to induce tubulin assembly and inhibit cell growth (**14**, **16**, and **20** vs **1**, **15**, and **19** vs **2**; in fact, compound **15** appears to be one of the most cytotoxic analogues from those shown in Table 2).

The C12 methyl group consistently bestowed higher potency to all epothilones studied as compared to the C12 *des*-methyl counterparts (e.g. **2** vs **1** and **4** vs **3**), with the exception of compounds **14** and **15** where comparable results were obtained. Inversion of configuration at C15 led to loss of ability to induce tubulin polymerization (**64** vs **22**, **65** vs **26**). Replacement of the C16 methyl group with an ethyl group also reduced activity in the tubulin assay (**101** vs **22**, **97** vs **26**) suggesting that the methyl group may play a role in maintaining the planar conformation[5] of the side chain. The inactivity of the C16,C17 epoxides (**8**, **9**, **11**, and **12**) further supports this conclusion. The epothilone pharmacophore tolerated some heterocycle modifications. Thus, a number of oxazole derivatives exhibited activity comparable to the corresponding thiazoles (e.g. **3** vs **1**, **4** vs **2**, **16** vs **14**, **24** vs **22**, **25** vs **23**, **20** vs **18**). Furthermore, replacement of the thiazole with a 2-pyridyl moiety led only to a slight decrease in activity (e.g. **105** vs **22**, **106** vs **26**) in the tubulin assays, whereas substitution of the C23-methyl with a phenyl group yielded inactive compounds (**102**, **103**). Scheme 2 summarizes graphically the structure–activity relationships within the epothilone family of compounds as derived from these and previous studies.[1, 2, 7, 10, 12, 17]

The reported work demonstrates the power of interfacing combinatorial chemistry with chemical biology as facilitated by solid-phase synthesis, REC chemistry, and modern biological assays. Furthermore, this research should facilitate the process of drug discovery and development in the area of cancer chemotherapy.



Scheme 2. Epothilone structure–activity relationships (tubulin binding assay): A) (3*S*) configuration important; B) 4,4-ethano group not tolerated; C) (6*R*,7*S*) configuration crucial; D) (8*S*) configuration important, 8,8-dimethyl group not tolerated; E) epoxide not essential for tubulin polymerization activity, but may be important for cytotoxicity; epoxide configuration may be important; R group important; both olefin geometries tolerated; F) (15*S*) configuration important; G) bulkier group reduces activity; H) oxygen substitution tolerated; I) substitution important; J) heterocycle important.

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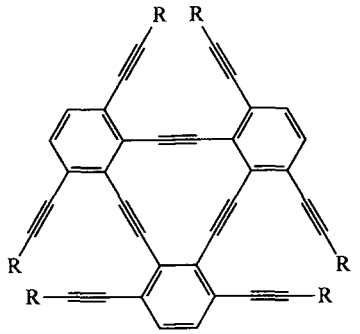
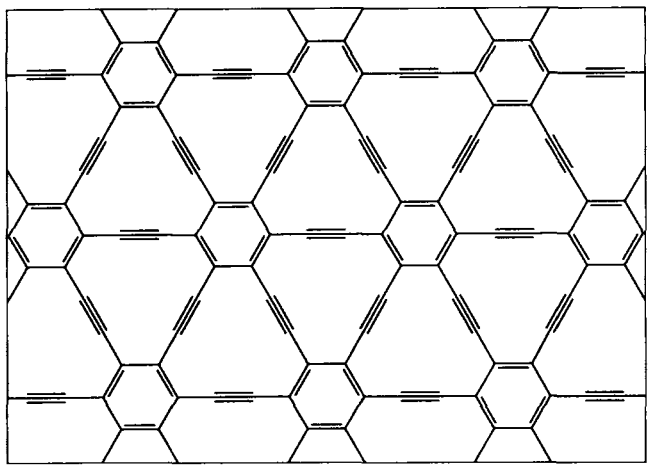
5,6,11,12,17,18-Hexadehydro-1,4,7,10,13,16-hexaethynyltribenzo[*a*,*e*,*i*]cyclododecene: Synthesis and CpCo-Catalyzed Cycloisomerization to the First Superdelocalized Oligophenylenes**

Christian Eickmeier, Heiko Junga, Adam J. Matzger, Frank Scherhag, Moonsub Shim, and K. Peter C. Vollhardt*

The title molecule ("hexaethynyltribenzocyclyne") **1a** is of great interest as a subunit of graphyne **2**,[1] a partially carbomeric[2] graphitic carbon allotrope,[3] as an extended π framework for ligating transition metals with unusual properties,[4] and as a precursor to antikekulene **3** by means of threefold CpCo-catalyzed cycloisomerization.[5] Compound **3** constitutes a much theoretically scrutinized[6] member of the as yet unknown circular phenylenes.[7]



1a, R = H
1b, R = $Si(CH_3)_2[C(CH_3)_2CH(CH_3)_2]$
1c, R = Pr
1d, R = $CH_2C_6H_{11}$



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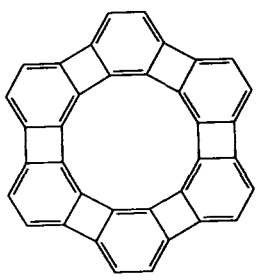
Compound **3** is intriguing in its juxtaposition to kekulene **4**,[8] which has the same number of rings but inner and outer π perimeters with a $4n+2$ electron count, whereas in **3**, the corresponding circuits are of the $4n$ type. However, in **4**, the annulenoid resonance forms suffer from the disruption of all benzenoid circuits and appear to be negligible contributors, as borne out by theory[6c, e, f, 9] and experiment.[8] In contrast, the nonannulenoid resonance alternatives to that depicted in **3** are all expected to be considerably destabilized by cyclobutadienoid antiaromaticity, an underlying feature of all phenylenes.[7] Thus, **3** might be a better candidate than **4** for probing the phenomenon of superdelocalization, although the notion of the



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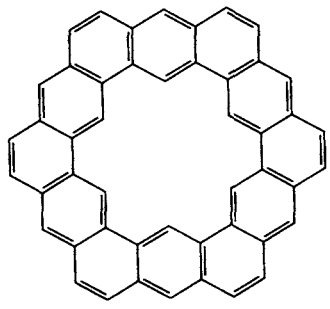

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