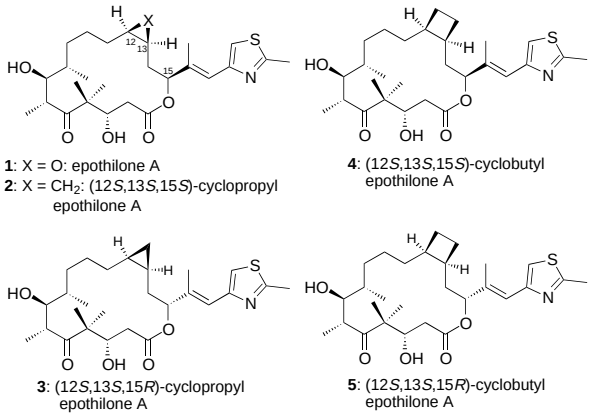
Synthesis and Biological Evaluation of 12,13-Cyclopropyl and 12,13-Cyclobutyl Epothilones**

K. C. Nicolaou,*[a] Kenji Namoto,[a] Jim Li,[a] Andreas Ritzén,[a] Trond Ulven,[a] Mitsuru Shoji,[a] Dan Zaharevitz,[b] Rick Gussio,[b] Dan L. Sackett,[c] Rita D. Ward,[d] Anne Hensler,[e] Tito Fojo,[e] and Paraskevi Giannakakou[e]

KEYWORDS:

antitumor agents · epothilones · natural products · structure–activity relationships · synthesis design

With some members in clinical trials, the epothilones command attention as potential anticancer agents of considerable promise. In addition to several naturally occurring substances, an impressive array of epothilone analogues has been constructed and biologically evaluated.[1, 2] We have previously reported the "partial" construction of two 12,13-cyclopropyl epothilone A analogues.[3] Their structures recently came under closer scrutiny by us and by a Bristol–Myers Squibb (BMS) group, whose independent synthesis of one of these compounds led to a revision of its structure.[4] Here we wish to report i) an unambiguous chemical synthesis of *cis*-(12*S*,13*S*)-cyclopropyl epothilone A (**2**) and its 15*R* epimer **3**; ii) the correct structure of the previously synthesized cyclopropyl epothilones and a mechanistic rationale for their unexpected formation; iii) the chemical synthesis of *cis*-(12*S*,13*S*)-cyclobutyl epothilone A (**4**) and its 15*R* epimer **5**; and iv) the molecular modeling and biological evaluation of the newly synthesized compounds. Remarkably, compounds **2** and **4** exhibit potencies comparable



to those of epothilone A (**1**) in cytotoxicity studies, supporting the notion that the overall shape of the epothilone scaffold is a most significant feature in these structures for biological activity, as opposed to the epoxide oxygen atom whose presence appears to be less relevant, at least for in vitro biological activity.

To probe the effect of the epoxide oxygen atom of epothilone on the molecule's ability to exert its biological action, the cyclopropyl and cyclobutyl analogues **2** and **4** were designed and targeted for chemical synthesis. Their 15*R*-epimeric counterparts (compounds **3** and **5**) were expected to arise concurrently from a similar synthetic sequence and their biological evaluation would shed further light on the existing structure–activity relationships (SARs) within the epothilone class. Molecular modeling[4, 5] revealed almost identical conformations for epothilone A (**1**) and its analogues **2** and **4**, but quite different shapes for the 15*R* isomers **3** and **5**. The unambiguous total synthesis of **2** was also expected to elucidate the uncertainty regarding the structures of the previously synthesized[3] cyclopropyl epothilones.

The synthetic plan toward the designed analogues was patterned after our macrolactonization approach[2f,6] to epothilone A with one new stratagem regarding the coupling of two of the fragments. Specifically, a Nozaki–Kishi coupling reaction[7] was envisioned for the union of the C7–C15 segment [aldehydes **12** (see Scheme 1) and **34** (see Scheme 4)] with the heterocyclic side chain fragment [vinyl iodide **16** (see Scheme 2)]. Significantly, this strategy would also ensure access to both C15 epimers but could, if so desired, be modified to deliver only one of the two by using an oxidation–asymmetric reduction protocol.

[a] *Prof. Dr. K. C. Nicolaou, K. Namoto, J. Li, Dr. A. Ritzén, Dr. T. Ulven, Dr. M. Shoji*
Department of Chemistry and
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-2469
and
Department of Chemistry and Biochemistry
University of California San Diego
9500 Gilman Drive, La Jolla, CA 92093 (USA)
E-mail: kcn@scripps.edu

[b] *Dr. D. Zaharevitz, Dr. R. Gussio*
Target Structure-Based Drug Discovery Group, Information Technology Branch, Developmental Therapeutics Program
National Cancer Institute
National Institutes of Health, Frederick, MD 21702 (USA)

[c] *Dr. D. L. Sackett*
Laboratory of Integrative and Medical Biophysics
National Institute of Child Health and Human Development
National Institutes of Health, Bethesda, MD 20892 (USA)

[d] *Dr. R. D. Ward*
Laboratory of Neurobiology
National Institute of Neurologic Diseases and Stroke
National Institutes of Health, Bethesda, MD 20892 (USA)

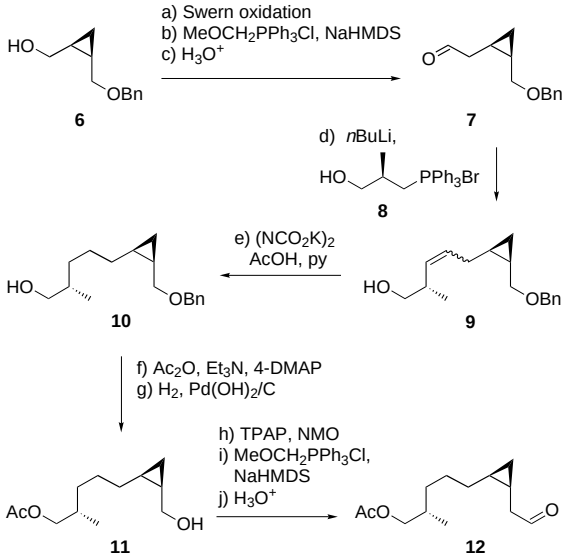
[e] *A. Hensler, Dr. T. Fojo, Dr. P. Giannakakou*
Medicine Branch
National Cancer Institute
National Institutes of Health, Bethesda, MD 20892 (USA)

[**] *Editorial note: The IUPAC names of compounds **2**–**5** are as follows: **2**: (12S,13S,15S)-12,13-methanoepothilone A; **3**: (12S,13S,15R)-12,13-methanoepothilone A; **4**: (12S,13S,15S)-12,13-ethanoepothilone A; **5**: (12S,13S,15R)-12,13-ethanoepothilone A. However, the authors feel that the names used in this Short Communication, and in ref. [3], are more suggestive of the respective structures.*

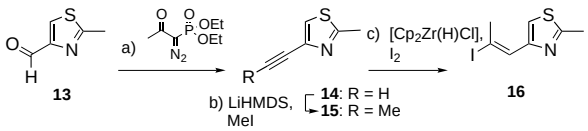
Supporting information for this article is available on the WWW under http://www.chembiochem.com or from the author.

The projected synthesis of the cyclopropyl epothilones required building blocks **12** (see Scheme 1), **16** (see Scheme 2), and **21** (see Scheme 3). While **21** was available from our previous work,[6] **12** and **16** were synthesized in this study as follows. Aldehyde **12** was derived in optically active form from the enantiomerically enriched cyclopropane **6**[8] (Scheme 1). Thus, Swern oxidation of **6** was followed by a Wittig reaction and acidic hydrolysis to afford the homologated aldehyde **7** in 85% overall yield. A second Wittig reaction employing commercially available phosphonium salt **8** led to a mixture of *cis* and *trans* olefins **9** (ca. 20:1, 78%) which was reduced with diimide (generated in situ) to the saturated alcohol **10** (94%). Acetylation of the free hydroxy group in **10** (100%) followed by hydrogenolysis of the benzyl ether led to alcohol **11** (78%) which was oxidized to the corresponding aldehyde (89%) with TPAP/NMO. This two-step reaction was necessary because direct hydrogenation of **9** with a variety of transition metal catalysts produced compounds in which the cyclopropyl ring was opened. The latter compound was homologated to the desired aldehyde **12** by the two-step procedure described above for the generation of **7** (Wittig reaction followed by acidic hydrolysis) in 50% overall yield. The vinyl iodide **16** was constructed from aldehyde **13**[2f,6] by a sequence involving i) acetylene formation (96%); ii) methylation (95%); and iii) hydrozirconation – iodination (40%) (Scheme 2).[9]

Intermediates **12** and **16** were coupled by the Nozaki – Kishi procedure employing $CrCl_2/NiCl_2$,[7] furnishing a diastereomeric mixture of alcohols **17** (ca. 1:1 ratio, 72%) (Scheme 3). This mixture was taken through the sequence until chromatographic separation of the two isomers became feasible upon Yamaguchi macrolactonization (see below).[6] Silylation of **17** furnished silyl ether **18** (100%) which was deacetylated (DIBAL, 99%) and oxidized (DMP, 96%) to afford aldehyde **20** via hydroxy compound **19**. The coupling of aldehyde **20** with ethyl ketone **21** following the previously developed stereoselective aldol protocol[10c] proceeded smoothly and furnished aldol **22**, whose silylation led to the tetrasilyl ether **23** (82% overall yield). Selective removal of the primary silyl group from **23** with HF · py gave alcohol **24** (81%) whose sequential oxidation with DMP and $NaClO_2$ resulted in the formation of carboxylic acid **25**. Selective desilylation at C15 (TBAF) generated hydroxy acid **26** (68% for three steps). Yamaguchi macrolactonization[10c] of **26** led to a mixture of lactones **27** and **28** (ca. 1:1, 82%), which were separated chromatographically. Finally, deprotection of **27** and **28** with TFA completed the synthesis of **2** (91%) and **3** (90%) whose identities were secured by spectroscopic studies and comparisons of their spectra with those of authentic samples of epothilones **1** and **2**.[11] As indicated in Scheme 3, the lack of



Scheme 1. *Synthesis of aldehyde **12**. a) $(COCl)_2$ (1.5 equiv), DMSO (2.0 equiv), Et_3N (5.0 equiv), CH_2Cl_2, −78→0 °C, 1 h, 95%; b) $MeOCH_2PPh_3Cl$ (1.5 equiv), NaHMDS (1.5 equiv), THF, −78→0 °C, 1 h; c) HCl (cat.), acetone/H_2O (9:1, v:v), 50 °C, 1 h, 90% (for 2 steps); d) **8** (1.3 equiv), n*BuLi (1.9 equiv),THF, −78 °C, 1 h, 78% (*cis*:*trans* ca. 20:1); e) $(NCO_2K)_2$ (20 equiv), HOAc (40 equiv), pyridine, 25 °C, 48 h, 94%; f) Ac_2O (1.1 equiv), Et_3N (2.5 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2, 0 °C, 30 min, 100%; g) 20 wt% $Pd(OH)_2/C$ (cat.), H_2 (1 atm), EtOAc/EtOH (1:1, v:v), 2 h, 25 °C, 78%; h) TPAP (0.05 equiv), NMO (1.5 equiv), MS (4 Å), CH_2Cl_2, 25 °C, 1 h, 89%; i) NaHMDS (1.1 equiv), 0 °C, 5 min, THF, then $MeOCH_2PPh_3Cl$ (1.3 equiv), 0 °C, 5 min, 67%; j) HCl (cat.), acetone/H_2O (9:1, v:v), 50 °C, 2 h, 87%. Bn = benzyl, 4-DMAP = 4-dimethylaminopyridine, NaHMDS = sodium hexamethyldisilazide, NMO = N-methylmorpholine N-oxide, py = pyridine, TPAP = tetra-n-propylammonium perruthenate.*

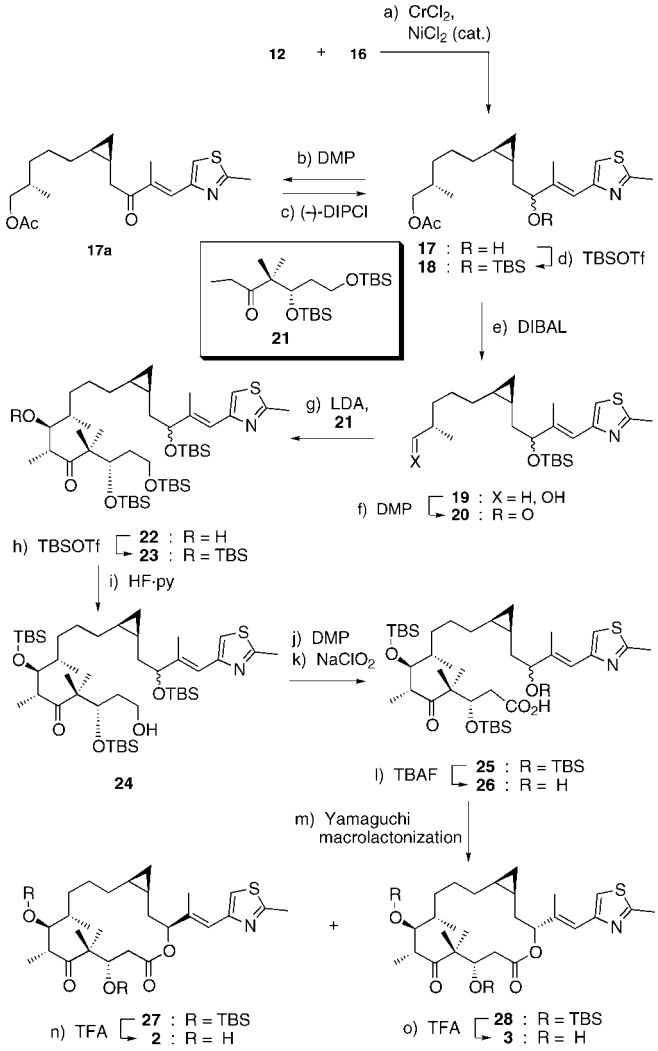


Scheme 2. *Synthesis of the C16 – C22 fragment **16**. a) Phosphonate (1.2 equiv), NaOMe (1.2 equiv), MeOH, −40→0 °C, 30 min, 96%; b) LiHMDS (2.5 equiv), MeI (7.0 equiv), THF, −78→0 °C, 8 h, 95%; c) $[Cp_2Zr(H)Cl]$ (1.1 equiv), THF, 45 °C, 1 h, then I_2 (1.5 equiv), 0 °C, 15 min, 40%. Cp = cyclopentadienyl, LiHMDS = lithium hexamethyldisilazide.*

Editorial Advisory Board Member:[*]
K. C. Nicolaou*,*
born in Cyprus and educated in England and the US, is currently Chairman of the Department of Chemistry at The Scripps Research Institute, where he holds the Darlene Shiley Chair in Chemistry and the Aline W. and L. S. Skaggs Professorship in Chemical Biology, as well as Professor of Chemistry at the University of California, San Diego. His impact on chemistry, biology, and medicine flows from his works in organic synthesis, described in over 500 publications and 65 patents, and his dedication to chemical education as evidenced by his training of over 300 graduate students and postdoctoral fellows. His recent book titled "Classics in Total Synthesis", which he co-authored with Erik J. Sorensen, is used around the world as a teaching tool and source of inspiration for students and practitioners of organic synthesis.

[*] *Members of the Editorial Advisory Board will be introduced to the readers with their first manuscript.*

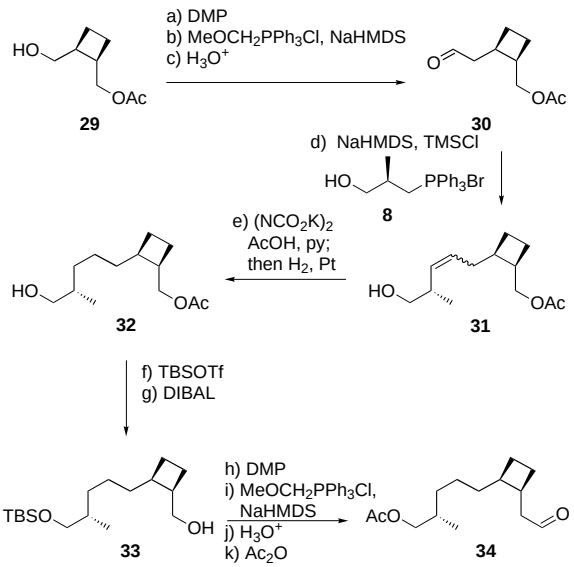
  1439-4227/01/02/01 $ 17.50+.50/0 CHEMBIOCHEM **2001**, No. 1



Scheme 3. *Total synthesis of cyclopropyl epothilone analogues **2** and **3**. a) **12** (1.0 equiv), **16** (2.0 equiv), $CrCl_2$ (10.0 equiv), $NiCl_2$ (cat.), DMSO, 25 °C, 7 h, 72% (ca. 1:1 ratio of C15 epimers); b) DMP (1.2 equiv), CH_2Cl_2, 0 → 25 °C, 30 min, 96%; c) (−)-DIPCl (3.0 equiv), Et_2O, −15 → 25 °C, 23 h, 79% (R:S = 1:24); d) TBSOTf (1.1 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78 °C, 30 min, 100%; e) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 99%; f) DMP (1.2 equiv), CH_2Cl_2, 0 → 25 °C, 30 min, 96%; g) LDA (2.45 equiv) in THF, −78 °C, then **21** (2.4 equiv) in THF, −78 °C, 1 h; then −40 °C, 0.5 h; then **20** (1.0 equiv) in THF, −78 °C, 4 min; h) TBSOTf (2.0 equiv), 2,6-lutidine (5.0 equiv), CH_2Cl_2, 0 °C, 2 h, 82% (for 2 steps); i) HF·py (20% (v/v) in pyridine), THF, 0 °C, 4 h, 81%; j) DMP (1.2 equiv), $NaHCO_3$ (2.5 equiv), CH_2Cl_2, 0 → 25 °C, 30 min; k) $NaClO_2$ (2.5 equiv), 2-methyl-2-butene (75.0 equiv), NaH_2PO_4 (2.0 equiv), tBuOH/H_2O (5:1, v:v), 25 °C, 20 min; l) TBAF (6.0 equiv), THF, 0 → 25 °C, 15 h, 68% (for 3 steps); m) 2,4,6-trichlorobenzoyl chloride (2.4 equiv), Et_3N (6.0 equiv), THF, 0 °C, 1.5 h; then add to a solution of 4-DMAP (2.2 equiv) in THF, 75 °C, 2.5 h, 82% (**27**:**28** = 1:1); n) 20% TFA in CH_2Cl_2, 0 °C, 2 h, 91%; o) 20% TFA in CH_2Cl_2, 0 °C, 14 h, 90%. DMP = Dess–Martin periodinane, DIBAL = diisobutylaluminum hydride, DIPCl = B-chlorodiisopinocampheylborane, LDA = lithium diisopropylamide, TBS = tert-butyldimethylsilyl, TBAF = tetra-n-butylammonium fluoride, Tf = trifluoromethanesulfonyl, TFA = trifluoroacetic acid.*

selectivity in the Kishi–Nozaki coupling of **12** and **16** was "corrected" by oxidation with DMP followed by reduction with (−)-DIPCl, furnishing the 15*S* isomer of **17** in high diastereoselectivity (ca. 24:1).

In addition to fragments **16** and **21**, the planned synthesis of the cyclobutyl epothilones **4** and **5** dictated the availability of

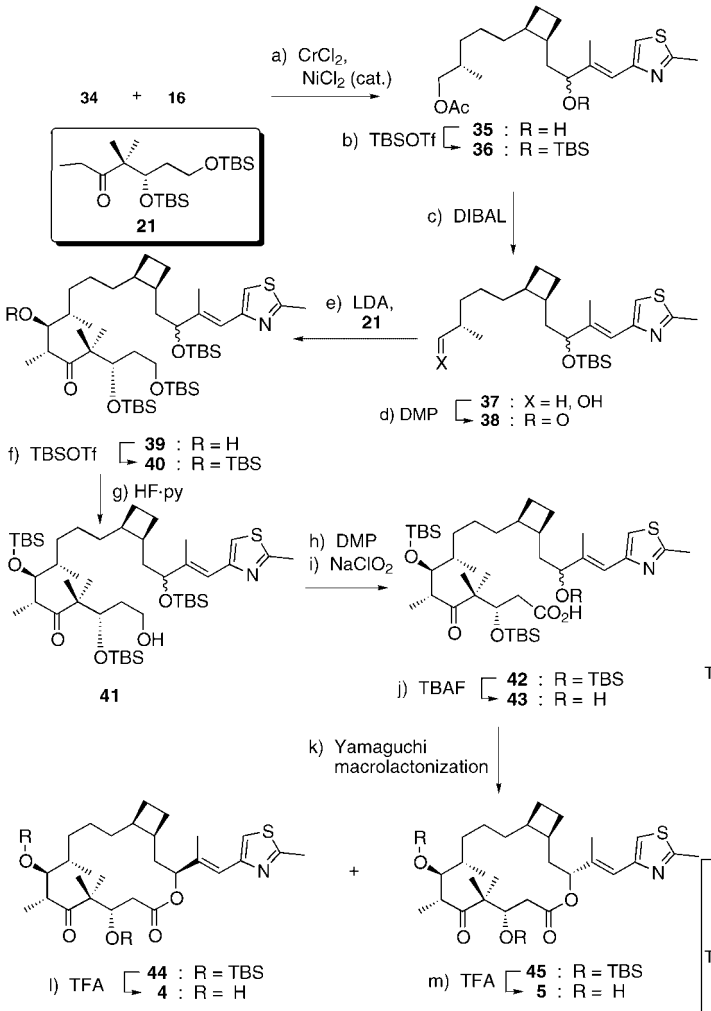


Scheme 4. *Synthesis of aldehyde **34**. a) DMP, CH_2Cl_2, 25 °C, 3 h, 96%; b) $MeOCH_2PPh_3Cl$ (1.5 equiv), NaHMDS (1.5 equiv), THF, −78 °C, 1 h; c) HCl (cat.), acetone/H_2O (9:1, v:v), 50 °C, 1 h, 87% (for 2 steps); d) **8** (1.5 equiv), NaHMDS (2.9 equiv), TMSCl (1.5 equiv), THF, −78 °C, 1 h, 82% (*cis:trans *ca. 20:1); e) $(NCO_2K)_2$ (20.0 equiv), HOAc (40.0 equiv), pyridine, 25 °C, 24 h; then H_2, Pt, MeOH, 25 °C, 82%; f) TBSOTf (1.0 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78 °C, 30 min, 100%; g) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 99%; h) DMP (1.2 equiv), $NaHCO_3$ (2.0 equiv), CH_2Cl_2, 25 °C, 3 h, 94%; i) NaHMDS (1.4 equiv), $MeOCH_2PPh_3Cl$ (1.5 equiv), THF, −78 °C; j) HCl (cat.), acetone/H_2O (9:1, v:v), 50 °C, 1 h, 90% (for 2 steps); k) Ac_2O (1.1 equiv), Et_3N (2.5 equiv), 4-DMAP (cat.), CH_2Cl_2, 0 °C, 20 min, 67%. TMSCl = trimethylsilyl chloride.*

key building block **34**. Its construction from the readily available derivative **29**[12] proceeded as outlined in Scheme 4, the sequence being almost identical to that for aldehyde **12** (see Scheme 1) except for the standard adaptations that were necessary to accommodate the acetate group that replaced the benzyl group in the starting material. Thus, coupling of the three fragments (**34**, **16** and **21**) proceeded as summarized in Scheme 5 via intermediates **35**–**45** and furnished the targeted epothilones **4** and **5** in good overall yield and in optically active form.

While the spectra of the newly synthesized **2** were identical to those of the BMS compound,[4, 11] they differed from those of our previously synthesized cyclopropyl epothilone.[3] This result together with further analysis of the ^{1}H NMR spectra (see Figure 1) of these compounds led to a revision of the original structures to (12*R*,13*R*)-cyclopropyl epothilone and (12*S*,13*R*)-cyclopropyl epothilone (**2b** and **2a**, respectively, in Scheme 6) which are consistent with the proposed mechanistic rationale for their formation as shown in Scheme 6. Thus, contrary to expectation, and presumably because of the neighboring-group effect exerted by the lactone carbonyl group, overall retention of configuration in these reactions is observed, leading to 12*R*,13*R*-cyclopropyl (**2b**) and 12*S*,13*R*-cyclopropyl epothilone (**2a**) rather than the originally assigned structures.[11]

The biological activities of the synthesized epothilones were initially evaluated through cytotoxicity studies by utilizing a set of ovarian carcinoma cell lines, including a parental cell line (1A9) and three drug-resistant cell lines, namely the paclitaxel-resistant cell lines[13] 1A9/PTX10 and 1A9/PTX22 and the epothilone-



Scheme 5. *Total synthesis of cyclobutyl epothilone analogues **4** and **5**. a) **34** (1.0 equiv), **16** (2.0 equiv), $CrCl_2$ (10.0 equiv), $NiCl_2$ (cat.), DMSO, 25 °C, 7 h, 89%; b) TBSOTf (1.1 equiv), 2,6-lutidine (2.5 equiv), CH_2Cl_2, −78 °C, 30 min, 100%; c) DIBAL (2.0 equiv), CH_2Cl_2, −78 °C, 99%; d) DMP (1.2 equiv), CH_2Cl_2, 0→25 °C, 30 min; e) LDA (2.45 equiv) in THF, −78 °C, then **21** (2.4 equiv) in THF, −78 °C, 1 h; then −40 °C, 0.5 h; then **38** (1.0 equiv) in THF, −78 °C, 4 min, 67% (for 2 steps); f) TBSOTf (2.0 equiv), 2,6-lutidine (5.0 equiv), CH_2Cl_2, 0 °C, 2 h; g) HF·py (20% (v/v) in pyridine), THF, 0 °C, 4 h, 86% (for 2 steps); h) DMP (1.2 equiv), $NaHCO_3$ (2.5 equiv), CH_2Cl_2, 0→25 °C, 30 min; i) $NaClO_2$ (2.5 equiv), 2-methyl-2-butene (75.0 equiv), NaH_2PO_4 (2.0 equiv), tBuOH/H_2O (5:1, v:v), 25 °C, 20 min, 93% (for 2 steps); j) TBAF (6.0 equiv), THF, 0→25 °C, 15 h, 54%; k) 2,4,6-trichlorobenzoyl chloride (2.4 equiv), Et_3N (6.0 equiv), THF, 0 °C, 1.5 h; then add to a solution of 4-DMAP (2.2 equiv) in THF, 75 °C, 2.5 h, 67% (**44**:**45** = 3:4); l) 20% TFA in CH_2Cl_2, 25 °C, 1 h, 61%; m) 20% TFA in CH_2Cl_2, 25 °C, 8 h, 60%.*

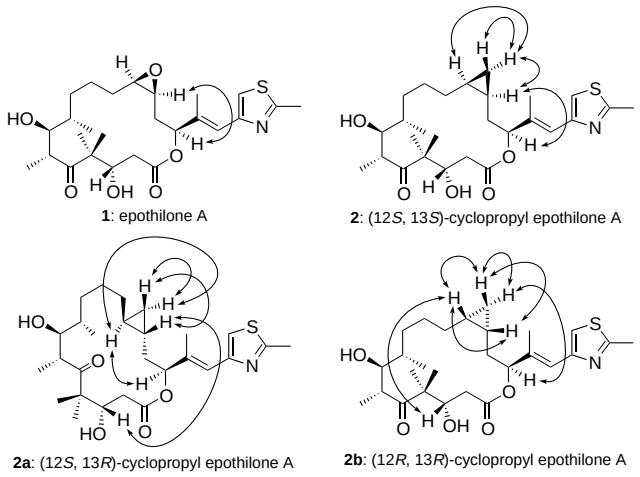
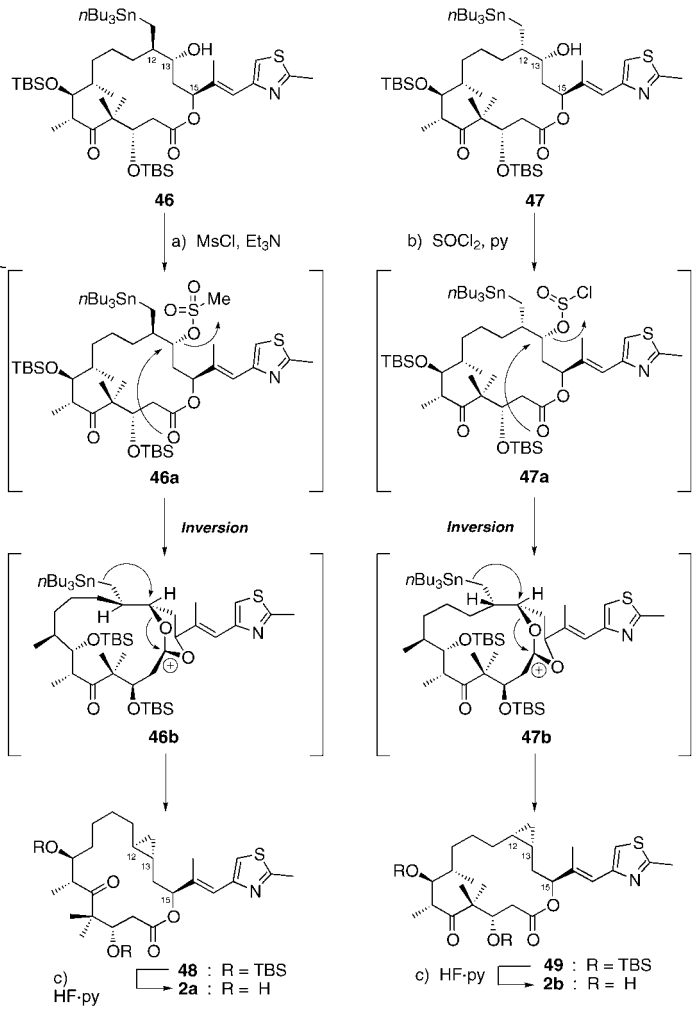


Figure 1. *Stereochemical assignment of 12,13-cyclopropyl epothilones A based on NMR spectroscopy (NOEs).*



Scheme 6. *Proposed mechanism for the unusual formation of 12,13-cyclopropyl epothilones with retention of configuration. The W-shaped transition states required for the previously proposed S_N2-type mechanism force the tin moieties into the macrocycle and are therefore unfavorable. Instead, carbonyl-facilitated carbocation formation is followed by elimination of tin to form the cyclopropane ring with overall retention of configuration at C13. Hence, structures **46**, **47**, **2a** and **2b** should replace **11**, **10**, **4** and **3**, respectively, in ref. [3].*

resistant cell line[14] 1A9/A8. These resistant cell lines harbor distinct acquired β-tubulin mutations which affect drug – tubulin interaction and result in impaired taxane- and epothilone-driven tubulin polymerization. The results of these biological investigations are summarized in Table 1 and reveal some important information for the structure – activity relationships of these compounds. In agreement with the BMS study,[4] we found that the cyclopropyl epothilone A **2** inhibits the 1A9 cell growth more potently than the parent compound epothilone A (**1**). Thus, in the parental 1A9 cells the IC_{50} value for compound **2** is 0.25 nM compared with 2 nM for epothilone A (**1**) and 0.15 nM for

Table 1. *Cytotoxicity [nM] of epothilones **2**, **3**, **4**, and **5** against selected cell lines.*[a]

Compound	Cell line						
	1A9	PTX10 (β270)		PTX22 (β364)		A8 (β274)	
	IC_{50}	IC_{50}	RR	IC_{50}	RR	IC_{50}	RR
paclitaxel (taxol)	1.25	40	32	50	40	8	6
epothilone A **1**	2	11	5.5	3	1.5	68	34
epothilone B	0.15	0.3	3	0.2	2	6	60
CP-epothilone **2**	0.25	9	36	2	8	32	128
CP-epothilone **3**	225	> 300	N/A	> 300	N/A	> 300	N/A
CB-epothilone **4**	3	95	32	45	15	100	33
CB-epothilone **5**	180	> 300	N/A	> 300	N/A	> 300	N/A

[a] IC_{50} values are given in nM. Relative resistance (RR) is calculated as an IC_{50} value for each resistant subline divided by that for the parental cell line (1A9). CB = cyclobutyl, CP = cyclopropyl, N/A = not applicable.

epothilone B. The (15*S*)-cyclobutyl epothilone A **4** retains good activity but is not as potent as compound **2**. It is noteworthy that the 15*R* isomers of both compounds (**3** and **5**) are inactive at low concentrations against the epothilone-sensitive parental 1A9 cells (see Table 1). Molecular modeling had predicted that the 15*R* isomers would not fit properly into the taxol-binding site of tubulin (Figure 2). It is noteworthy that both compounds **2** and **4** display a similar cytotoxicity profile against the β-tubulin mutants compared to epothilone A (**1**). In other words, both compounds **2** and **4** lose activity against the clones PTX10 (β270) and A8 (β274) suggesting that residues 270 and 274 are important for the binding of these compounds to tubulin.

The cytotoxicity analysis was completed by in vitro tubulin polymerization experiments, the results of which are shown in Figure 3 and Table 2. In general, there is good agreement between the in vitro tubulin polymerization potency and the cytotoxicity profile of the compounds tested. Compound **2** shows superior in vitro potency to natural epothilone A (**1**) both by the percentage of tubulin polymerization and by the kinetic analysis, suggesting that this compound is able to nucleate microtubules (which is the first step in the tubulin polymerization reaction) faster than the parent compound **1**. Despite the cytotoxicity results, the cyclobutyl compound **4** also displays superior in vitro tubulin polymerization activity to epothilone A (**1**), albeit to a lesser extent than compound **2**. Finally, tubulin polymerization products of these compounds were examined by electron microscopy. Pictures from these experiments are shown in Figure 4, and while they confirm the formation of closed and open microtubules in all cases, they also point to some structural differences between taxol, the parent epothilone A (**1**), and its analogues **2** and **4**.

In conclusion, we have constructed two 12,13-cyclopropyl (15*S* and 15*R*) and two 12,13-cyclobutyl (15*S* and15*R*) epothilone analogues by total synthesis and evaluated their biological activities. While the 15*S* compounds exhibited potent tubulin polymerization activity and cytotoxicity against tumor cells, the 15*R* isomers were devoid of such actions. This re-enhanced the view that while the oxygen atom at the C12–C13 site is not necessary for biological activity, the proper configuration at C15 is absolutely essential for it.

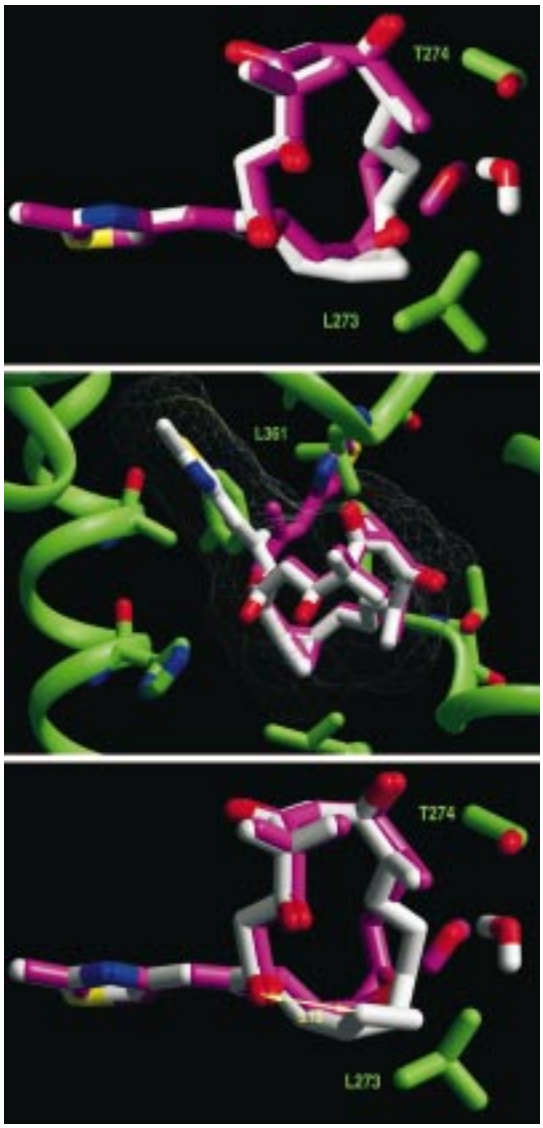


Figure 2. *Molecular modeling of epothilone A (**1**), 12,13-cyclopropyl epothilone (**2**), and 12,13-cyclobutyl epothilone (**4**) within their tubulin binding site. Top: Superposition of epothilone A (**1**, carbon atoms in magenta) hydrogen-bonded to a water molecule (hydrogen atoms in magenta) and 12,13-cyclopropyl epothilone A (**2**, carbon atoms in white). Binding of **2** displaces that water molecule (→ second water molecule with the hydrogen atoms shown in white). The flexible carbon atoms 9 – 14 of **2** enable a closer, more favorable hydrophobic interaction between the cyclopropane moiety and the depicted side chain of Leu 273. The displaced water molecule can still maintain its hydrogen bond to the backbone of Thr 274 (side chain is shown). Middle: 12,13-Cyclopropyl epothilone A (**2**, carbon atoms in white) shown with its molecular surface (three-dimensional grid) in the taxol-binding site of tubulin. Upon inversion of the chiral center at C15 (superimposed compound **3**, carbon atoms shown in magenta), the epothilone side chain sterically clashes with Leu 361 so that this analogue cannot be accommodated in the binding site. Bottom: Superposition of epothilone A (**1**, carbon atoms in magenta) hydrogen-bonded to a water molecule (hydrogen atoms in magenta) and 12,13-cyclobutyl epothilone A (**4**, carbon atoms in white). Binding of **4** displaces that water molecule (→ second water molecule with the hydrogen atoms shown in white). In a similar manner to the 12,13-cyclopropyl analogue, the flexible carbon atoms 9 – 14 of **4** also enable a closer, more favorable hydrophobic interaction between the cyclopropane moiety and the side chain of Leu 273. However, in order to satisfy the steric and pharmacophoric requirements imposed by the taxol-binding site, the bulkier cyclobutane derivative must adopt a less preferred conformation that is unfavorably close (3.13 Å) to the carbonyl group at position 1. (For molecular modeling methods, see ref. [14].)*

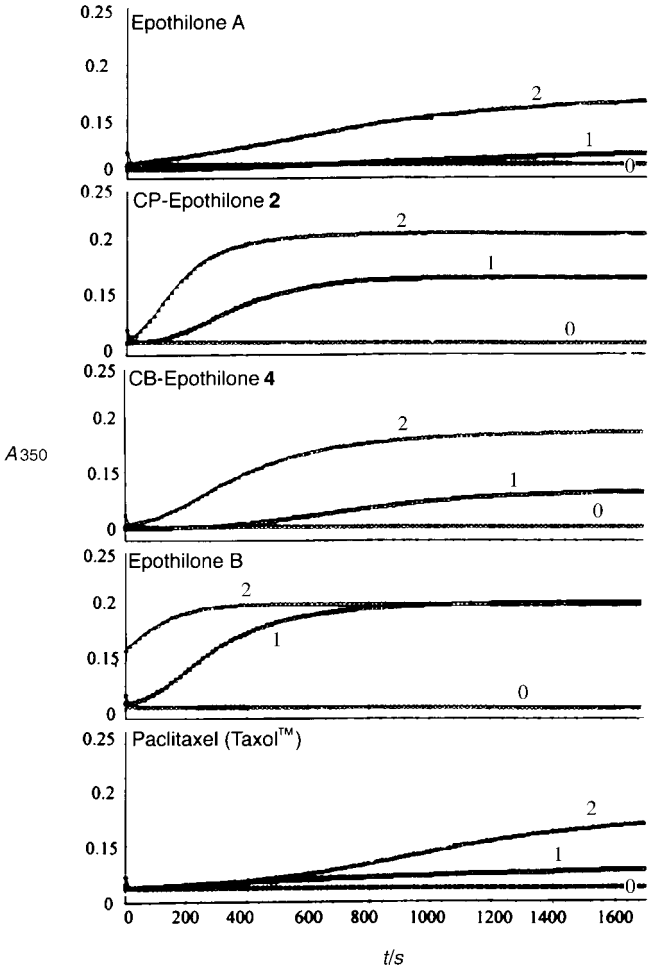


Figure 3. *Comparison of the abilities of epothilone A (**1**), (15S)-cyclopropyl epothilone A (CP-epothilone, **2**), (15S)-cyclobutyl epothilone A (CB-epothilone, **4**) to induce tubulin polymerization, in the presence of 0.05 mM of GTP and in the absence of microtubule-associated proteins (MAPs). Polymerization assays with epothilone B and paclitaxel (taxol) are included for comparison. In each assay, 7.5 μM of rat brain tubulin was mixed with 3 μM (curve 1) or 10 μM (curve 2) of each compound, and polymerization at 30 °C was followed for 30 min. The optical density at 350 nm (A_{350}) was then recorded at 15-s intervals for 30 min. Curves 0 are included as controls, showing tubulin polymerization reactions in the absence of the respective compound.*

Table 2. *Percentage and kinetics of tubulin polymerization by epothilones **2** and **4**.*

Compound	Tubulin polymerization [%][a]		Time to $A_{350}=0.15$ [min][b]	
	3 μM	10 μM	3 μM	10 μM
paclitaxel (taxol)	38	88	>30	18.5
epothilone A	33	87	>30	14.5
epothilone B	96	98	<1	4.0
CP-epothilone **2**	73	97	7.3	2.7
CB-epothilone **4**	46	91	>30	5.5

[a] The percentage of tubulin polymerization induced by each drug was calculated by pelleting microtubules at the end of each polymerization reaction, separating the supernatant (containing the nonpolymerized tubulin) from the pellet (containing the polymerized tubulin) and calculating the percentage of polymerized tubulin by the relative amount of protein in each of the two fractions (pellet and supernatant). Values in the table show the percentage of tubulin polymerization for both drug concentrations used. [b] Time to $A_{350}=0.15$ shows how fast each drug was able to induce tubulin polymerization (see Figure 4).

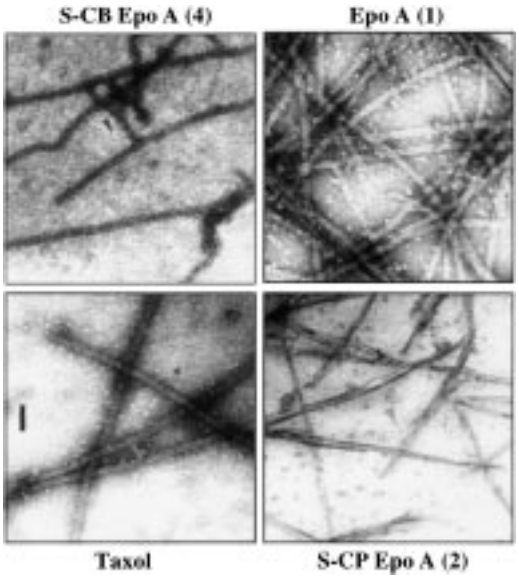


Figure 4. *Electron microscopy photographs of tubulin polymerization products induced by epothilone A (Epo A, **1**), (15S)-CP-epothilone A (S-CP Epo A, **2**), (15S)-CB-epothilone A (S-CB Epo A, **4**), and paclitaxel (Taxol). Samples were removed from each polymerization reaction, spread on Formvar-coated, carbon-coated copper grids, stained with 1% uranyl acetate, and analyzed with a JEOL 1200 CX electron microscope. Bar length=100 nm.*

Experimental Section

Characteristic analytical data of selected compounds:

Cyclopropyl epothilone 2: $R_f=0.33$ (silica gel, ethyl acetate/*n*-hexane, 1:1); $[\alpha]_D^{25}=-52.4$ ($c=0.4$ in CH_2Cl_2); IR (film): $\tilde{\nu}_{max}=2932$, 1728, 1689, 1505, 1457, 1371, 1256, 1187, 1152, 1077, 1037, 1009, 979, 883, 734, 668 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): $\delta=6.94$ (s, 1H), 6.54 (s, 1H), 5.27 (dd, $J=9.2$, 3.9 Hz, 1H), 4.08 (m, 1H), 3.85 (m, 1H), 3.74 (br. s, 1H), 3.20 (dq, $J=7.0$, 4.4 Hz, 1H), 2.69 (s, 3H), 2.62 (br. s, 1H), 2.53 (dd, $J=14.9$, 9.2 Hz, 1H), 2.48 (dd, $J=14.9$, 3.5 Hz), 2.08–2.05 (m, 1H), 2.07 (s, 3H), 1.74–1.67 (m, 1H), 1.57–1.42 (m, 5H), 1.36 (s, 3H), 1.32–1.26 (m, 1H), 1.19–1.11 (m, 1H), 1.17 (d, $J=7.0$ Hz, 3H), 1.16 (s, 3H), 1.00 (d, $J=7.0$ Hz, 3H), 0.74–0.68 (m, 2H), 0.65–0.61 (m, 1H), −0.30 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): $\delta=220.7$, 171.1, 164.9, 152.1, 138.9, 119.9, 115.9, 81.3, 73.5, 73.3, 52.3, 43.0, 39.2, 36.3, 32.2, 31.4, 29.4, 27.1, 22.5, 20.8, 19.1, 17.4, 17.0, 15.2, 13.9, 13.4, 10.0; HR-MS (MALDI-FT-MS): *m/z*: calcd for $C_{27}H_{41}NO_5SNa^+$ [$M+Na^+$]: 514.2598, found: 514.2581.

Cyclobutyl epothilone 4: $R_f=0.22$ (silica gel, ethyl acetate/*n*-hexane, 1:2); $[\alpha]_D^{25}=-57.1$ ($c=0.4$ in CH_2Cl_2); IR (film): $\tilde{\nu}_{max}=2931$, 1789, 1685, 1508, 1458, 1378, 1257, 1185, 1149, 1046, 976, 879, 734, 662, 550 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta=6.93$ (s, 1H), 6.53 (s, 1H), 5.12 (dd, $J=10.4$, 2.4 Hz, 1H), 4.30 (dd, $J=10.4$, 2.4 Hz, 1H), 3.74 (m, 1H), 3.37 (br. s, 1H), 3.26 (dq, $J=7.0$, 1.9 Hz, 1H), 3.18 (br. s, 1H), 2.69 (s, 3H), 2.46 (dd, $J=14.7$, 10.6 Hz, 1H), 2.42–2.34 (m, 1H), 2.35 (dd, $J=14.7$, 2.6 Hz, 1H), 2.21–2.13 (m, 1H), 2.06–2.01 (m, 1H), 2.04 (s, 3H), 2.01–1.93 (m, 2H), 1.86–1.75 (m, 2H), 1.72–1.55 (m, 3H), 1.52–1.37 (m, 2H), 1.35 (s, 3H), 1.33–1.24 (m, 3H), 1.12 (d, $J=6.6$ Hz, 3H), 1.09 (s, 3H), 1.02 (d, $J=7.0$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=221.0$, 170.5, 165.0, 152.1, 139.5, 119.1, 115.6, 80.4, 72.0, 71.9, 53.7, 41.1, 39.7, 38.6, 36.6, 35.5, 34.3, 31.6, 30.5, 26.1, 24.9, 23.9, 22.0, 19.1, 18.1, 17.2, 15.5, 11.5; HR-MS (MALDI-FT-MS): *m/z*: calcd for $C_{28}H_{44}NO_5S^+$ [$M+H^+$]: 506.2935, found: 506.2940.

  1439-4227/01/02/01 $ 17.50+.50/0 CHEMBIOCHEM **2001**, No. 1

We thank Drs. D. H. Huang, G. Siuzdak, and C. N. C. Boddy for NMR spectroscopic, mass spectrometric, and computer modeling assistance, respectively. Financial support for this work was provided by The Skaggs Institute for Chemical Biology, the National Institutes of Health (USA), and CaPCURE, with fellowships from the Naito Foundation, Japan (to M.S.), the Fulbright Scholar Program, the Swedish Institute, and the Bengt Lundquist Minnesfond (all to A.R.), and grants from Abbott, Amgen, ArrayBiopharma, Boehringer-Ingelheim, Glaxo, Hoffmann-La Roche, DuPont, Merck, Novartis, Pfizer, and Schering Plough.

[1] For a review see: K. C. Nicolaou, F. Roschangar, D. Vourloumis, *Angew. Chem.* **1998**, *110*, 2120 – 2153; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014 – 2045.

[2] a) A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976 – 2978; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801 – 2803; b) D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775 – 777; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757 – 759; c) D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073 – 10092; d) D.-S. Su, A. Balog, D. Meng, P. Bertinato, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 2178 – 2181; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2093 – 2096; e) Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170 – 173; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166 – 168; f) K. C. Nicolaou, F. Sarabia, S. Ninkovic, Z. Yang, *Angew. Chem.* **1997**, *109*, 539 – 540; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 525 – 527; g) K. C. Nicolaou, N. Winssinger, J. Pastor, S. Ninkovic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *387*, 268 – 272; h) D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109*, 523 – 524; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 543 – 544; i) S. May, P. A. Grieco, *Chem. Commun.* **1998**, 1597 – 1598; j) J. D. White, K. F. Sundermann, R. G. Carter, *Org. Lett.* **1999**, *1*, 1431 – 1434; k) S. C. Sinha, J. Sun, G. Miller, C. F. Barbas III, R. A. Lerner, *Org. Lett.* **1999**, *1*, 1623 – 1626; l) D. Sawada, M. Shibasaki, *Angew. Chem.* **2000**, *112*, 215 – 219; *Angew. Chem. Int. Ed.* **2000**, *39*, 209 – 212; m) H. J. Martin, M. Drescher, J. Mulzer, *Angew. Chem.* **2000**, *112*, 591 – 593; *Angew. Chem. Int. Ed.* **2000**, *39*, 581 – 583; n) B. Zhu, J. S. Panek, *Org. Lett.* **2000**, *2*, 2575 – 2578.

[3] K. C. Nicolaou, M. R. V. Finlay, S. Ninkovic, N. P. King, Y. He, T. Li, F. Sarabia, D. Vourloumis, *Chem. Biol.* **1998**, *5*, 365 – 372.

[4] J. Johnson, S.-H. Kim, M. Bifano, J. DiMarco, C. Fairchild, J. Gougoutas, F. Lee, B. Long, J. Tokarski, G. Vite, *Org. Lett.* **2000**, *2*, 1537 – 1540.

[5] The difference between the present molecular modeling study and that previously reported[3] is due to the fact that in the earlier study the X-ray-deduced structure of epothilone A was used as opposed to the computationally derived minimum-energy structure employed here and in ref. [4].

[6] K. C. Nicolaou, S. Ninkovic, F. Sarabia, D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay, Z. Yang, *J. Am. Chem. Soc.* **1997**, *119*, 7974 – 7991.

[7] a) K. Takai, T. Kitamura, T. Kuroda, T. Hiyama, H. Nozaki, *Tetrahedron Lett.* **1983**, *24*, 5281 – 5284; b) H. Jin, J. Uenishi, W. J. Christ, Y. Kishi, *J. Am. Chem. Soc.* **1986**, *108*, 5644 – 5646.

[8] A. B. Charette, H. Juteau, H. Lebel, C. Molinaro, *J. Am. Chem. Soc.* **1998**, *120*, 11943 – 11952.

[9] a) J. S. Panek, T. Hu, *J. Org. Chem.* **1997**, *62*, 4912 – 4913; b) D. W. Hart, J. Schwartz, *J. Am. Chem. Soc.* **1974**, *96*, 8115 – 8116.

[10] a) J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989 – 1993; b) J. Mulzer, P. A. Mareski, J. Bushmann, P. Luger, *Synthesis* **1992**, 215 – 228; c) K. C. Nicolaou, D. Hepworth, N. P. King, M. R. V. Finlay, R. Scarpelli, M. M. A. Pereira, B. Bollbuck, A. Bigot, B. Werschkun, N. Winssinger, *Chem. Eur. J.* **2000**, *6*, 2783 – 2800.

[11] We thank Dr. Gregory Vite for kindly providing us with the NMR spectra of an authentic sample of **2** and for pointing out to us the possible involvement of the lactone oxygen atom in the tin-mediated cyclopropane formation of our original synthesis of **2a** and **2b**.

[12] a) K. Laumen, M. Schneider, *Tetrahedron Lett.* **1985**, *26*, 2073 – 2076; b) W. Kasel, P. G. Hultin, J. B. Jones, *J. Chem. Soc. Chem. Commun.* **1985**, 1563 – 1564.

[13] P. Giannakakou, D. L. Sackett, Y.-K. Kang, Z. Zhan, J. T. M. Buters, T. Fojo, M. S. Poruchynsky, *J. Biol. Chem.* **1997**, *272*, 17118 – 17125.

[14] P. Giannakakou, R. Gussio, E. Nogales, K. H. Downing, D. Zaharevitz, B. Bollbuck, G. Poy, D. Sackett, K. C. Nicolaou, T. Fojo, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2904 – 2909.

Received: September 25, 2000 [Z148]

Heterogeneous Assembly of Complementary Peptide Pairs into Amyloid Fibrils with $\alpha - \beta$ Structural Transition

Yuta Takahashi, Akihiko Ueno, and Hisakazu Mihara*[a]

KEYWORDS:

aggregation · amyloid fibrils · electrostatic interactions · peptides · protein structures

Intermolecular self-assembly of a large number of polypeptide chains into macromolecular constructs occurs widely in biological systems. One of such macromolecular self-assemblages of great interest is the amyloid fibril.[1] The amyloid fibril is a misfolded and undesirable state for proteins as biomolecules, since it has been proposed to be a causative agent for a variety of fatal diseases known as amyloid diseases, such as Alzheimer's disease and prion diseases.[2] However, it is considered that the fibril has a highly ordered quaternary structure, in which numerous β-stranded polypeptide chains align regularly,[1] and thus this kind of fibril has the potential to be engineered into proteinaceous materials.[3–6] Amyloid fibril primarily comprises a single polypeptide species, that is, it is a homogeneous self-assemblage. Here we report the heterogeneous assembly of designed peptides into amyloid fibrils accompanied by a drastic secondary structural transition from an α helix to a β sheet. The heterogeneous assembly into fibrils is accomplished by complementary electrostatic interactions between pairs of peptide species, each of which is not able to self-assemble.

The design of peptides that could heterogeneously assemble into amyloid fibrils commenced by engineering our de novo

[a] *Prof. Dr. H. Mihara,[+] Y. Takahashi, Prof. Dr. A. Ueno*
Department of Bioengineering
Graduate School of Bioscience and Biotechnology
Tokyo Institute of Technology
Yokohama 226-8501 (Japan)
Fax: (+81) 45-924-5833
E-mail: hmihara@bio.titech.ac.jp

[+] *Further address:*
Form and Function, PRESTO
Japan Science and Technology Corporation
Yokohama 226-8501 (Japan)

Supporting information for this article is available on the WWW under http://www.chembiochem.com or from the author.