Synthesis of 4-Aza Analogs of Epothilone D

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Abstract: An efficient synthesis of epothilone D analogs of type **1** has been developed, which is based on a highly diastereoselective Evans aldol reaction as one of the key steps. A profound difference in the relative stabilities of TBS-protecting groups on C7-O and C15-O was observed between secondary and primary amide groups at C5-N4. Although modeling studies had suggested analogs of type **1** to assume a conformation similar to the NMR-derived conformation of tubulin-bound epothilone A, compounds **1a**–**d** were found to be significantly less active than the parent compound epothilone D.

Key words: epothilones, total synthesis, aldol reaction, microtubule inhibitors, natural products

Epothilones are natural products that were first isolated from culture extracts of the cellulose-degrading myxobacterium *Sorangium cellulosum*.[2] These substances (Figure 1) are highly potent antiproliferative agents, which inhibit tumor cell growth through the same mechanism of action as the clinical anticancer drug Taxol®, i. e. the stabilization of cellular microtubules and interference with microtubule dynamics.

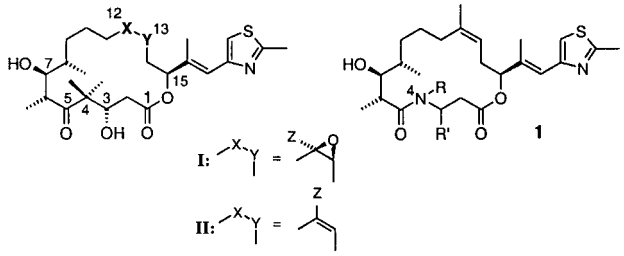

Figure 1 Structures of epothilones A (**I**, Z = H), B (**I**, Z = CH_3), C (**II**, Z = H), and D (**II**, Z = CH_3) and of target structures **1**.

Numerous syntheses of natural epothilones have been reported and a large number of analogs have been prepared for SAR studies.[3] Out of these structures, only few are characterized by the replacement of carbon atoms in the macrocyclic skeleton by heteroatoms and those investigated to date were found to be poorly active.[4] Overall, however, the potential of such modifications remains largely unexplored, which is in spite of the fact that the replacement of carbon by hetero-atoms in complex structures could lead to improved synthetic accessibility and offer the potential to generate large sets of diverse analogs in a rather straightforward manner (e. g. through amide bond formation or reductive amination in the case of nitrogen).

Our laboratory has recently been involved in the determination of the bioactive conformation of epothilone A and one of the characteristic features of the structure is the presence of a *syn*-periplanar conformation about the C4-C5 bond.[5] The same geometry would be enforced in analogs of type **1**, provided that the amide bond between N4 and C5 would be present in a *cis* conformation. At the same time preliminary modeling studies indicate that the presence of a *cis* amide bond in this position should allow replacement of the C1-C4 segment by various types of β-amino acids without causing significant distortions in the bioactive conformation of the C5-O16 segment. Based on these considerations and given the fact that structures of type **1** would lend themselves to an efficient combinatorial chemistry approach employing a single advanced intermediate [i. e. carboxylic acid **10** (**21**), vide infra] we embarked on the synthesis of some prototypic examples of analogs of type **1**.[6]

Our retrosynthetic analysis for these target structures (Scheme 1) was based on a prospective endgame involving amide bond formation between **10** and **11** followed by macrolactonization and deprotection. Carboxylic acid **10** would be the result of a Pd(0)-catalyzed coupling between vinyl iodide **8** and the zincate derived from alkyl iodide **7**. Alkyl iodide **7** in turn would be derived from the aldol product of aldehyde **2**[7] and the propionyl Evans-oxazolidinone **3** (vide infra).

As for other syntheses of epothilones and their analogs, one of the key challenges in the implementation of this synthetic scheme was the generation of the C6-C7 bond in a highly stereoselective manner. In order to establish this bond in the most efficient way possible, we carried out an extensive investigation of the reaction between aldehyde **2** and propionyl oxazolidinone **3** (Scheme 2, Table 1).

As illustrated by the data shown in Table 1, this study resulted in the identification of a set of optimized reaction conditions which provided the desired aldol product **4a** in 90% isolated yield with none of the other isomers being detectable in the crude product by TLC analysis. Compound **4a** was converted into its TBS ether **5** by treatment with TBSOTf and 2,6-lutidine in 91% yield. Hydrogenolytic removal of the benzyl group followed by standard functional group transformation then gave the desired iodide **7** in 81% yield (Scheme 3).

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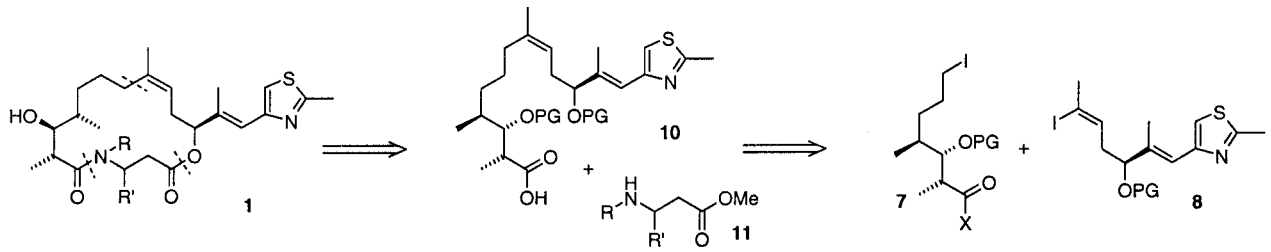
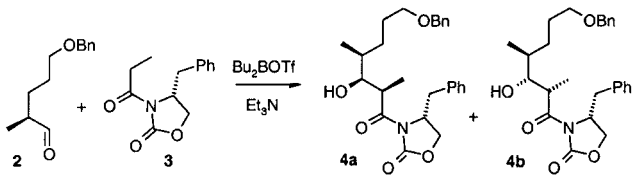
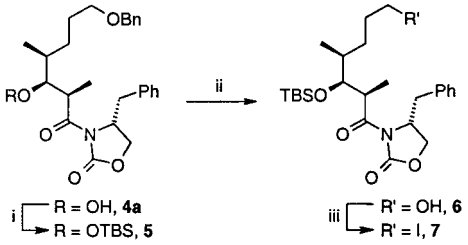


Scheme 1 Retrosynthetic analysis for 4-aza epothilones **1**.



Scheme 2 Aldol reaction between aldehyde **2** and propionyl oxazolidinone **3**.



Scheme 3 *Reaction conditions*: (i) TBSOTf, 2,6-lutidine, 0 °C → r.t., 4 h, **91%**; (ii) H_2, Pd/C (10%), MeOH, r.t., 6 h, **82%**; (iii) a) MeSCl, Et_3N, 0 °C, 30 min; b) NaI, acetone, 50 °C, 3 h, **81%** (two steps).

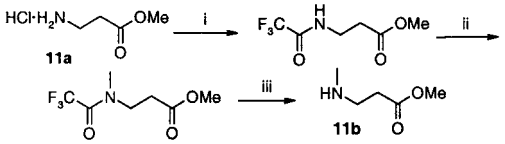
The *syn*/*anti* stereochemistry about the C6-C7/C7-C8 bonds was unequivocally established by X-ray crystallography at the stage of iodide **7**.[8] Thus, contrary to what has been observed for other substrates, the excess of Lewis acid employed in the aldol reaction between **2** and **3** does not lead to preferential formation of the *anti* isomer with respect to the substituents at C6 and C7.[9]

Table 1 Variation of Reaction Conditions in the Aldol Reaction between **2** and **3**: Effects on Selectivity and Yield

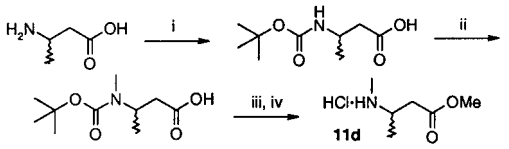
Entry	Reagents (equiv)[a]			Procedure[b]	Ratio **4a**:**4b**	Yield (%)
	3	Bu_2BOTf	Et_3N			
1	0.9	1.2	1.4	A	90:10	38/4
2	0.9	1.2	1.4	B	100:0	43
3	0.9	1.3	1.5	B	100:0	46
4	0.9	1.4	1.6	C	100:0	60
5	1.11	1.5	1.7	C	100:0	78
6	1.3	1.5	1.7	C	100:0	90

[a] All reactions were performed with 1 equiv of aldehyde **2**.
[b] Oxazolidinone **3**, Bu_2BOTf, Et_3N, CH_2Cl_2, 0 °C, 1 h; then addition of **2** at –78 °C and A, B, or C. Procedure A: –78 °C for 15 min, 0 °C for 2 h; procedure B: –78 °C for 1 h, –20 °C for 1 h, 0 °C for 1 h; procedure C: –78 °C for 3 h.

β-Amino acid esters **11b** and **11d** were prepared from commercially available β-glycine methyl ester hydrochloride **11a** and DL-3-aminobutyric acid, respectively, as outlined in Scheme 4 and Scheme 5. Racemic **11c** (Scheme 1, R = H; R′ = CH_3) was obtained through direct acid-catalyzed esterification of DL-3-aminobutyric acid.



Scheme 4 *Reaction conditions*: (i) $(CF_3CO)_2O$, *i*-Pr_2EtN, CH_2Cl_2, 0 °C, 1.5 h, **95%**; (ii) K_2CO_3, MeI, DMF, 0 °C → r.t., 24 h, **91%**; (iii) NH_4OH (2 M), MeOH, r.t., 4 h, **76%**.



Scheme 5 *Reaction conditions*: i) BOC_2O, Et_3N, H_2O–dioxane, r.t., 12 h, **50%**; (ii) NaH, MeI, THF, 0 °C → r.t., 4 h; (iii) MeOH, DCC, DMAP, 0 °C, 2 h, **68%** (two steps); (iv) HCl–dioxane (4 N), r.t., 30 min, **96%**.

Pd(0)-catalyzed coupling[7,10,11] between the zincate derived from alkyl iodide **7** and vinyl iodide **8** provided the protected olefin **9** as a single double bond isomer in a good 71% yield (Scheme 6).

Vinyl iodide **8** was prepared by a modification of the literature procedure,[6] which involves the use of the isolated Wittig salt $[CH_3CH(I)PPh_3]^+I^-$ in the olefination step, rather than preparing this reagent in situ. Although this approach did not lead to significantly improved yields for the olefination reaction, it proved to be highly advantageous upon scale-up and gave more reproducible results. The analogous TES-protected vinyl iodide **19** was obtained from **8** via CSA-catalyzed TBS-removal and reprotection of the free OH group by reaction with TESOTf.

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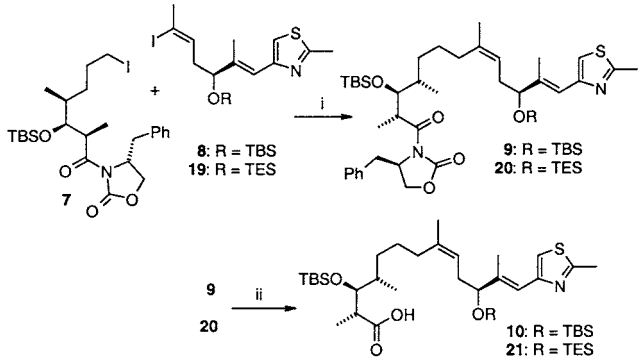
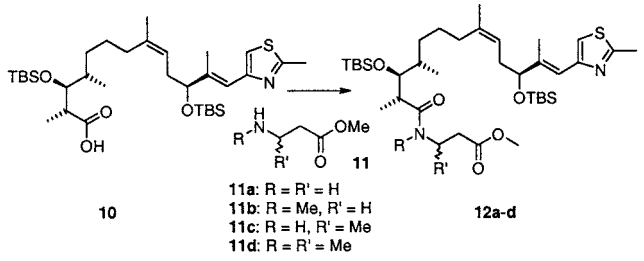


Scheme 6 *Reaction conditions*: (i) a) **7**, Zn-Cu, 1,2-dibromoethane, TMSCl, DMA, TMSOTf; b) $Pd(PPh_3)_4$, **8** (**19**), benzene, 65 °C; **9**: **85%**, **20**: **71%**; (ii) LiOH, H_2O_2, THF–H_2O 4:1, r.t., 3 h; **10**: **78%**; **21**: **70%**.

Cleavage of the chiral auxiliary with LiOOH (Scheme 6) followed by HBTU-mediated coupling[12] of the resulting acid **10** with amino acid esters **11a**–**d** gave the protected *seco* esters **12a**–**d** in good to excellent yields (Scheme 7).

Progression of the synthesis then required the selective removal of the TBS protecting group from the allylic hydroxyl group at position C15 (Figure 2). Numerous examples exist in the literature where this step has been successfully carried out as part of the total synthesis of natural epothilones or different analog structures.[13] For example, Nicolaou et al. have described the selective cleavage of the C15-OTBS ether in tris-TBS protected precursors of epothilone A or B by means of 6 equivalents of TBAF at room temperature.[13] Surprisingly, however, treatment of **12a** with three equivalents of TBAF at 0 °C resulted in the selective cleavage of the C7-OTBS group rather than the desired cleavage at C15-O. The same result was obtained with **12c**, whereas N-methylated derivatives **12b** and **12d** gave the desired C15 alcohols without problems (Figure 2, Table 2).

These observations may be rationalized by neighboring group participation in the case of the secondary amide groups (**12a** and **12c**), which could involve initial transfer of the TBS group to the amide oxygen (through a five-membered transition state) and the formation of a labile TBS-imidate. In order to resolve the selectivity problem in this crucial deprotection step, the TBS group in **8** was replaced by a more labile TES group to produce **19** (vide supra). Coupling of **7** with **19** gave **20** (Scheme 6), which was further transformed into the corresponding C15-OTES derivative **21**. Coupling of **21** with **11a** and **11c** gave the corresponding amides, from which the TES-group on C15-O could be removed selectively with an equimolar mixture of TBAF and HOAc to give the desired *seco*-esters **14a** and **14c** in 86% and 84% yield, respectively.



Scheme 7 *Reaction conditions*: *i*-Pr_2EtN, HBTU, DMF, r.t., 3 h; **12a**: 83%; **12b**: 78%; **12c**: 94%; **12d**: 72%.

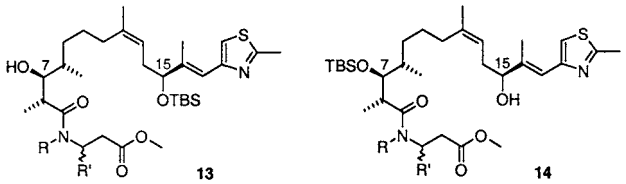
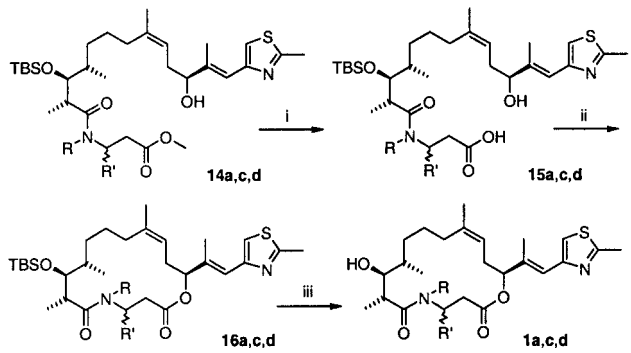


Figure 2 C7-O- and C15-O-deprotected *seco*-esters **13** and **14**.

Table 2 Selective Deprotection of Tris-TBS-Protected *seco*-Acid Methyl Esters **12**

Entry	Ester	TBAF (equiv)	Temp (°C)	Product	Deprotection (position ***C***-O)	Yield (%)
1	**12a**	3	0	**13a**	7	65
2	**12b**	6	r.t.	**14b**	15	65
3	**12c**	3	0	**13c**	7	74
4	**12d**	6	r.t.	**14d**	15	70

Completion of the syntheses through (i) ester saponification, (ii) Yamaguchi macrolactonization (trichlorobenzoyl chloride and DMAP), and (iii) TBS-removal from C7-O with HF·pyridine was then straightforward and gave the desired epothilone analogs **1** in 49–57% yield from **14** (3 steps; Scheme 8).[14]



Scheme 8 *Reaction conditions*: (i) LiOH, THF–H_2O 7:1, r.t., 5 h, 64–90%; (ii) 2,4,6-$Cl_3PhCOCl$, Et_3N, THF, r.t., 30 min, then DMAP, toluene, r.t., 3 h, 74–90%; (iii) HF·pyridine, MeCN, r.t., 6 h, 67–86%.

In the case of **16c** and **16d** isomers at C3 were readily separable by flash column chromatography. The individual diastereoisomers were obtained in 25–30% yield (yields in Scheme 8 refer to the combined yields for the pure diastereoisomers) and were then deprotected separately.

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In conclusion, we have developed an efficient stereoselective route to a new class of aza analogs of epothilones, which is based on a highly diastereoselective aldol reaction as one of the key steps. Biological evaluation of compounds **1** revealed a significant loss in biological potency compared with epothilone D.[15] However, only a limited number of building blocks have been investigated as potential C1-C4 replacements in this pilot study. The methodology developed in the course of this work sets the stage for a more comprehensive exploration of different amino acids, which may yet result in the discovery of potent analogs of epothilones. In addition, intermediates **10** and **21** should also be valuable building blocks for the preparation of other types of epothilone analogs.

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(6) The target structures are analogs of epothilone D (Figure 1), which promotes tubulin polymerization to the same degree as epothilone B. Although less active than epothilone B, epothilone D is also a highly active antiproliferative agent, which inhibits human cancer cell growth in vitro with low nM IC50 values (cf. ref.[3]).

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(14) As the first compound synthesized in this series, **1b** was obtained from **12b** by removal of both TBS-groups with HF·pyridine, ester saponification, and macrolactonization of the fully deprotected seco-acid. This approach gave pure **1b** in only 13% isolated yield (for the cyclization step) after purification by preparative HPLC. Although clearly unsatisfactory, the amount of material obtained through this process was sufficient for complete in vitro biological profiling of **1b** and the compound proved to possess only low biological activity. In light of this result resynthesis of **1b** from **14b** was deemed insensible.

(15) The results of the biological evaluations will be published separately.

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