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Rapid access to epothilone analogs via semisynthetic degradation and reconstruction of epothilone D

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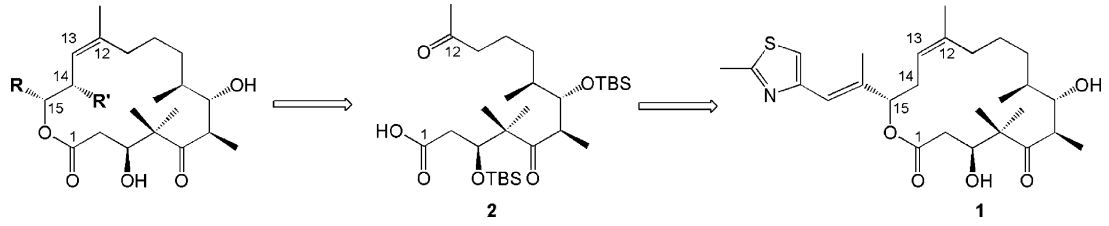
Abstract—A facile and efficient route to epothilone analogs has been developed from the natural product epothilone D (**1**). Degradation of **1** via an oxidative cleavage sequence provides acid intermediate **4** rapidly in six steps. From **4**, a variety of epothilone analogs have been prepared utilizing ring-closing metathesis to reconstruct the trisubstituted-12,13-double bond. Using this approach, we report a number of epothilone analogs with varying C-15 aromatic side chains and C-14 allylic substitutions and their biological activities.

The epothilone class of microtubule stabilizers has attracted considerable attention as novel chemotherapeutic agents.[1] Among the most promising is epothilone D (**1**), which displays superior anti-tumor activity relative to paclitaxel and is currently undergoing human clinical trials.[2] A variety of total syntheses of **1** have been reported.[3,4] Though these approaches are amenable to analog production, they require a substantial synthetic investment to generate key intermediates.

Julien and Shah have developed a heterologous bacterial expression system containing the epothilone D polyketide synthase genes to produce **1** by fermentation.[5] With substantial quantities of **1** available to us by this process, we sought a means by which to generate analogs from **1** via degradation and reconstruction of the macrolactone (Scheme 1). We now report a rapid and efficient degradation approach to intermediates such as **2** and the elaboration of **2** into analogs of **1**.

Our degradation strategy focused on the cleavage of the 12,13-alkene of **1** followed by base promoted β-elimination of the resulting carboalkoxyaldehyde to provide the C1-12 acid **2**. To this end, epothilone D (**1**) was first protected as the bis-TBS ether (Scheme 2). Stoichiometric osmium mediated dihydroxylation provided the 12,13-diol in 75%.[6] Oxidative cleavage of the vicinal diol with $Pb(OAc)_4$ provided the keto aldehyde, which could be isolated if desired. We found that when this crude aldehyde was treated directly with K_2CO_3, the elimination of the aromatic side chain proceeded smoothly to provide acid **2**. Thus, over only three steps, epothilone D **1** was advanced to intermediate **2**.



Scheme 1.

Keywords: Epothilone; Epothilone analogs; Degradation; Semisynthesis; Ring closing metathesis.
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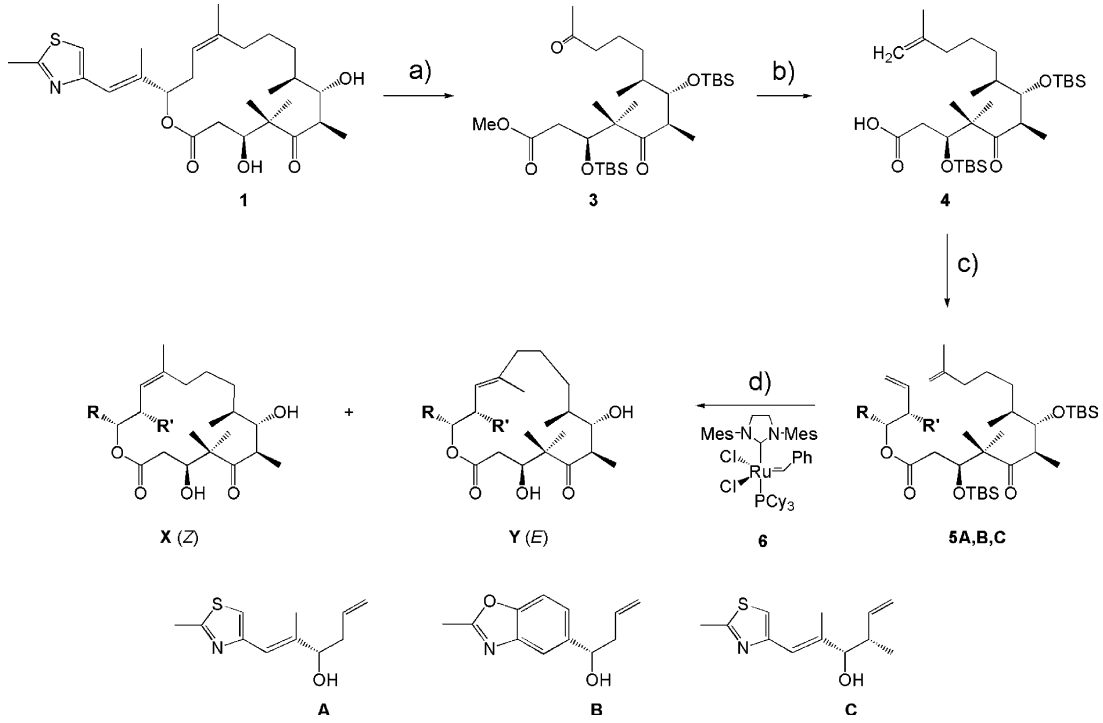


Scheme 2. Conditions and reagents: (a) (1) TBSOTf, NEt_2, CH_2Cl_2, −78 °C, 85%; (2) OsO_4, TMEDA, −78 °C then $NaHSO_3$, 65 °C, 75%; (3) $Pb(OAc)_4$, PhH then K_2CO_3, MeOH; (4) $TMSCHN_2$, MeOH, PhMe, 81% over two steps; (b) (1) Cp_2TiMe_2, PhMe, 80 °C, 74%; (2) LiOH, *i*-PrOH, H_2O, 86%; (c) **A**, **B**, or **C**, EDC, DMAP, CH_2Cl_2, 54–82%; (d) (1) see Table 1; (2) TFA, CH_2Cl_2, 0 °C to rt; or HF–pyridine, THF, 0 °C to rt, 32–59%.

Olefination of the 12-keto group of **2** would provide acid **4**, an ideal intermediate for the reconstitution of the macrolactone with a variety of alkenyl alcohols (Scheme 2). To facilitate purification and the subsequent olefination, the acid was esterified with $TMSCHN_2$ to provide methyl ester **3**. The ketone of **3** was converted to the terminal alkene with dimethyltitanocene in 74% yield.[7] Hydrolysis of the methyl ester provided acid **4** in 86% yield. Acid **4** is available in six steps and 52% overall yield from epothilone D (**1**), demonstrating the efficiency of this route.

A variety of reported total syntheses has relied on ring closing metathesis to establish the trisubstituted-12,13-double bond of epothilone using the Schrock molybdenum catalyst.[8,9] We decided to investigate the versatility of this approach to making epothilone analogs further by employing degradation intermediate **4**. In particular we examined a number of metathesis partners that differ structurally (**A**–**C**, Scheme 2) to probe the generality of this ring closing metathesis using Grubbs second-generation ruthenium catalyst (**6**).[10]

Structure–activity relationships in the epothilone class clearly indicate that changes in the aromatic side chain can afford potent cytotoxins.[4] Thus, side chains **A**–**C** were chosen not only as probes of steric sensitivity of the ring closing metathesis reactions but because of their potential as representatives of active classes of epothilone analogs. Bicyclic aryl side chains such as benzoxazole **B** are known to be excellent surrogates for the naturally occurring vinyl thiazole of epothilone.[11] Furthermore, appropriate substitution at the 14-position of epothilone as in 14-methyl-epothilone D has been shown to promote the population of the active conformation as well as improving esterase stability.[12] To this end, alkenyl alcohols **A**,[13] **B**,[14] and **C**[12] were prepared as previously described. These alcohols were coupled to acid **4** under the agency of EDC and catalytic DMAP in 54–82% yield to provide esters **5A**–**C** (Scheme 2).

Metathesis conditions and reaction outcomes for substrates **5A**–**C** are shown in Table 1. Ring closing metathesis of ester **5A** with Grubbs second-generation ruthenium catalyst (**6**) provided a 1:1 mixture of *E*:*Z* isomers (**X**,**Y**) of **1** in 59% overall yield. Similar results were observed with the benzoxazole ester **5B** demonstrating the permissiveness of **6** to differing side chains. However, in the presence of the additional steric bulk of the allylic methyl of **5C**, no ring closing was observed in refluxing CH_2Cl_2 with 20 mol% **6**. Only under more forcing conditions did we observe ring closing to yield **8** as a 1:1.3 mixture of *E*:*Z* isomers in 35% overall yield. The resulting ring-closed analogs were deprotected with either trifluoroacetic acid or HF·pyridine to provide the final products in 9–18% overall yield from intermediate **4**.[15]

The reconstituted epothilone analogs were assayed against a panel of human tumor cell lines (Table 2). The biological activity of the benzoxazole side chain (**7**) indicates that it is an excellent replacement for the vinyl thiazole. Though some activity is lost upon substitution at the 14-position of epothilone (**8**), the 14-position remains an intriguing position for analogs, particularly as a site to improve in vivo stability. As has been noted,

Table 1. Metathesis conditions and outcomes

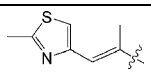
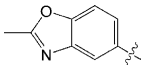
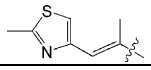
Compound (**X**,**Y**)	R	R′	Substrate concentration (mM)	Catalyst	Solvent	Temperature	Time	Yield (%)	*E*/*Z*
1		–H	1	**6** (20 mol %)	CH_2Cl_2	Reflux	6 h	59	1:1
7		–H	2.9	**6** (20 mol %)	CH_2Cl_2	Reflux	8 h	60	1:1
8		–Me	2	**6** (129 mol %)	Toluene	Reflux	5 days	35	1:1.3

Table 2. Cytotoxicity of epothilone analogs (IC_{50} nM)

Compound	MCF-7	NCI/ADR	A549
1*Z*	17	58	27
1*E*	200	440	290
7*Z*	36	140	49
7*E*	350	~1300	400
8*Z*	47	280	79
8*E*	62	320	160

the *Z* isomers are consistently more potent than the *E* isomer.[3]

In conclusion, we have developed a facile and efficient route to epothilone analogs via a degradation approach to epothilone D (**1**). Acid intermediate **4** can be produced rapidly in six steps from **1**. Employing ring-closing metathesis with Grubbs second-generation ruthenium catalyst to reconstruct the trisubstituted-12,13-double bond, we have prepared a variety of sterically differentiated epothilone analogs. The outcome of the metathesis is dependent on the nature of the substitution at the allylic position. Using this approach, we now have ready access to numerous epothilone analogs with varying C-15 aromatic side chains and C-14 allylic substitutions. Furthermore, this strategy highlights the power of degradation and semisynthesis when natural products are available as building blocks.[16]

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14. **B** was prepared from 2-methylbenzoxazole-5-carbaldehyde in an analogous fashion to **A**.
15. **7E**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.00 (m, 2H), 1.04 (s, 3H), 1.17 (d, 3H, $J = 6.2$ Hz), 1.28 (m, 6H), 1.62 (s, 5H), 1.73 (br, 1H), 1.99 (m, 1H), 2.15 (m, 1H), 2.46 (m, 2H), 2.64 (s, 5H), 3.27 (m, 1H), 3.68 (br, 1H), 4.27 (d, 1H, $J = 9.2$ Hz), 5.13 (br s, 1H), 6.03 (br s, 1H), 7.27 (d, 1H, $J = 8.4$ Hz), 7.44 (d, 1H, $J = 8.0$ Hz), 7.69 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 14.40, 14.50, 15.65, 15.95, 20.00, 20.57, 29.64, 31.61, 35.00, 37.76, 38.95, 39.44, 42.52, 52.76, 72.10, 75.71, 75.91, 110.15, 116.87, 119.66, 122.77, 136.57, 138.74, 150.46, 164.70, 170.40, 220.29; HRMS calcd for $C_{28}H_{39}NO_6$: 485.27774; found: 486.28648 (M+H).
8E: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.00–0.90 (m, 8H), 1.07 (s, 3H), 1.16 (m, 4H), 1.27 (s, 3H), 1.61 (s, 3H), 1.76 (m, 2H), 2.04 (s, 2H), 2.09 (s, 3H), 2.55–2.40 (m, 2H), 2.70 (s, 3H), 2.83 (m, 1H), 3.15 (m, 1H), 3.72 (s, 1H), 4.11 (m, 1H), 4.95 (d, 1H, $J = 8.40$ Hz),), 5.02 (d, 1H, $J = 8.0$ Hz), 6.55 (s, 1H), 6.97 (s, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 13.92, 14.98, 16.14, 17.81, 19.06, 21.33, 24.91, 31.68, 34.94, 38.19, 38.53, 39.03, 42.45, 52.75, 72.24, 75.04, 84.43, 116.36, 122.56, 127.34, 136.12, 170.72, 220.18; HRMS calcd for $C_{28}H_{43}NO_5S$: 505.2862; found: 506.29418 (M+H).
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