Natural Products

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# Modular Asymmetric Synthesis of Aigialomycin D, a Kinase-Inhibitory Scaffold**

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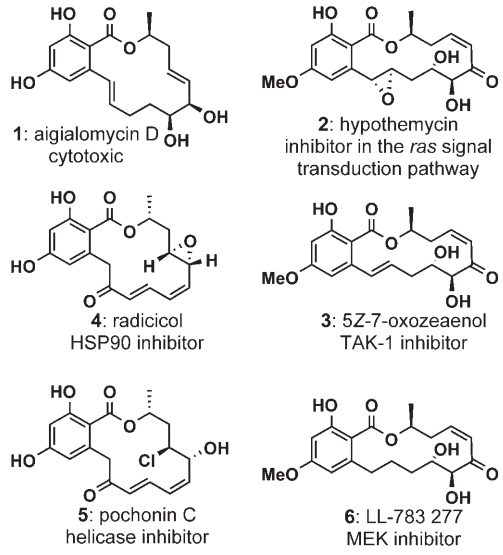
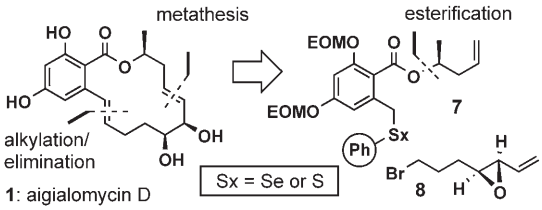
*Dedicated to K. C. Nicolaou on the occasion of his 60th birthday*

Aigialomycin D (**1**) was isolated[1] together with the known hypothemycin[2,3] (**2**) from the mangrove fungus *Aigialus*




*parvus* in Thailand. Both these natural products were found to be low-concentration (μM) inhibitors of the malaria parasite *Plasmodium falciparum* and are also cytotoxic. An elegant synthesis of aigialomycin D was reported by Danishefsky and co-workers utilizing a late-stage Diels–Alder reaction to build the aromatic ring.[4] Our interest in the resorcyclides[5–8] comes from the observation that there are several potent kinase inhibitors amongst this relatively small class of natural products.[9] Indeed, 5*Z*-7-oxozeaenol (**3**) is an inhibitor of TAK-1,[10] structurally related LL-783277 (**6**) is a MEK inhibitor,[11] and hypothemycin has been reported to inhibit the *ras* signaling pathway.[12] Structurally related radicicol (**4**) and pochonin C (**5**), on the other hand, do not inhibit a specific kinase but rather a specific ATPase (HSP90[13] and HSV-helicase,[14] respectively). Despite the lack of structural similarity between radicicol and ATP, radicicol was found to bind to the ATP-binding site of HSP90.[15]

The high potential to find new ATPase or kinase inhibitors amongst this class of natural products[16,17] coupled to the interest in inhibitions of these two enzyme classes from a therapeutic as well as a chemical genomic perspective[18,19] led us to seek a modular diversity-oriented synthesis amenable to preparation of libraries extending beyond the naturally available compounds. The retrosynthetic disconnections are shown in Scheme 1. A key point is the use of the




***Scheme 1.*** Retrosynthetic analysis. EOM = ethoxymethyl.

thio- or selenoether at the benzylic position (see **7**) which should facilitate alkylation chemistry and provide a potential attachment point for immobilization. The macrocycle would be formed by a metathesis reaction, and the diol moiety was anticipated to originate from *trans* epoxide **8**, which can be obtained by Sharpless asymmetric epoxidation. The key intermediate **8** was prepared in four steps from commercially available products (Scheme 2). Thus, cross-metathesis of 5-bromopentene (**9**) with unprotected 1,4-butenediol in the presence of the second-generation Hoveyda–Grubbs catalyst[20] afforded the allylic alcohol **10** in excellent yield and *E*/*Z* ratio (>25:1). This is a convenient alternative to the conventional sequence involving formation of an aldehyde, Horner–Wadsworth–Emmons olefination, and reduction with DIBAL to *trans* allylic alcohols.[21,22] Sharpless epoxidation of allylic alcohol **10** followed by oxidation with $SO_3$·py and Wittig olefination afforded the epoxide **8** in 62% overall yield. The epoxide could be converted into the protected diol **11** by $Sc(OTf)_3$-catalyzed opening of the epoxide[6] followed by protection with an acetonide group.

The aromatic portion **7** was obtained through a three-step sequence starting with Mitsunobu esterification[5,23] of the unprotected benzoic acid **12** followed by protection of both phenol groups (Scheme 3). The selenide was introduced at the benzylic position by deprotonation with LDA followed by addition of diphenyldiselenide (59% yield over three steps). Compound **7** was efficiently alkylated with either bromide **8** or **11** to yield the metathesis precursor **13** and **14**, respectively.

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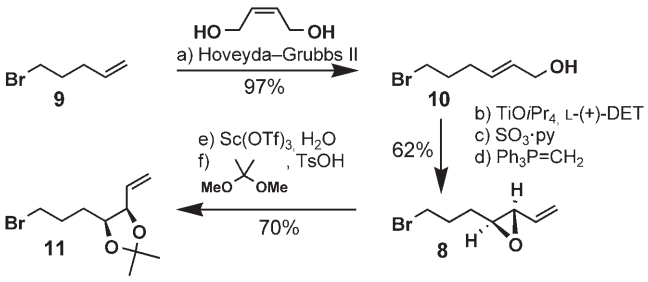
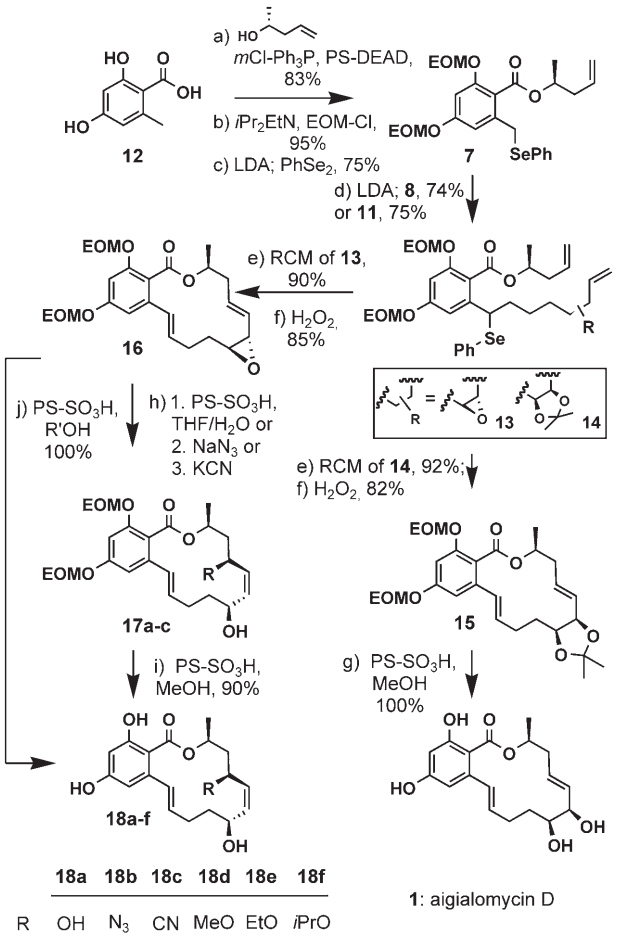


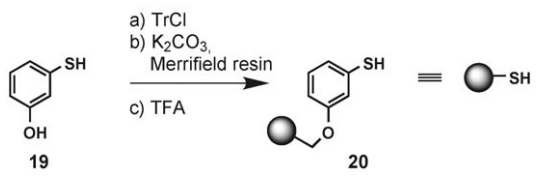

***Scheme 2.*** Synthesis of key intermediates **8** and **11**. a) **9** (1.0 equiv), 2-buten-1,4-diol (2.0 equiv), Hoveyda–Grubbs catalyst II (0.01 equiv), $CH_2Cl_2$, 23 °C, 4 h, 97%; b) L-(+) diethyl tartrate (0.12 equiv), Ti(O*i*Pr)$_4$ (0.1 equiv), *t*BuOOH (1.52 equiv), $CH_2Cl_2$, −40 °C, 30 min; then, **10** (1.0 equiv), −24 °C, 12 h, 85%; c) $SO_3$·py (3.47 equiv), $CH_2Cl_2$/DMSO, 0 °C, 30 min; d) $Ph_3P{=}CH_2$ (1.8 equiv), THF, −10 °C, 10 min, 70% over two steps; e) Sc(OTf)$_3$ (0.2 equiv), THF/$H_2O$ (10:1), 23 °C, 2.5 h, 100%; f) dimethoxypropane (10 equiv), TsOH·$H_2O$ (0.05 equiv), $CH_2Cl_2$, 23 °C, 12 h, 70%. Grubbs–Hoveyda catalyst II = 1,3-(bis-(mesityl)-2-imidazolidinylidene)dichloro-(*o*-isopropoxyphenylmethylene)ruthenium; DET = diethyl tartrate; Tf = trifluoromethanesulfonyl; Ts = *p*-toluenesulfonyl.

Treatment of **14** with second-generation Grubbs catalyst[24,25] under equilibrating conditions[26] (80 °C for 12 h) afforded the macrocycle with $E/Z > 10{:}1$. This macrocycle was then treated with $H_2O_2$ to oxidize and eliminate the selenide, thus affording compound **15** in 77% overall yield. Interestingly, inversing the sequence of reaction (oxidation/elimination of the selenide followed by the metathesis on the triene) gave rise to a significant amount of the undesired six-membered ring product from the cyclization with the benzylic alkene. Global deprotection of the acetonide and EOM groups afforded aigialomycin D (**1**)[27] in a total of 10 steps and 21% overall yield. When the diol was masked as an epoxide, macrocycle **16** was obtained in excellent yield starting from diene **13** through the same sequence (RCM, selenide oxidation/elimination). Interestingly, in this case, the order of reaction could be inversed without observing the competing ring-closure corresponding to the six-membered ring (the geometry of the *trans* epoxide favors macrocyclization). However, Lewis or protic acid ($ScOTf_3$, TsOH, TFA, HFIP) mediated opening of the epoxide failed to yield the desired 1,2-*cis*-diol and led in all cases to an $S_N2'$ opening to afford **17a** or **18a**. Although the epoxide **16** could not be converted into aigialomycin D, this diverging reaction pathway proved to be quite general and could be carried out with different nucleophiles such as $NaN_3$ or KCN to obtain **17b** and **c**, respectively. Deprotection of the EOM ethers (PS-$SO_3H$, MeOH) from **17a–c** or concomitant epoxide opening/deprotection with different alcohols (MeOH, EtOH, *i*PrOH) in the presence of sulfonic acid resin from **16** afforded aigialomycin analogues **18a–f**.

In the interest of streamlining the synthesis, the phenyl selenide was replaced by a polymer-bound thioether. The required thiol resin was prepared in a similar fashion as previously reported[28] from 3-hydroxythiophenol rather than 4-hydroxyphenol to avoid the deactivating effect of a *para*-phenol (Scheme 4). The chemistry leading to aigialomycin was found to be equally efficient on solid phase as in solution.

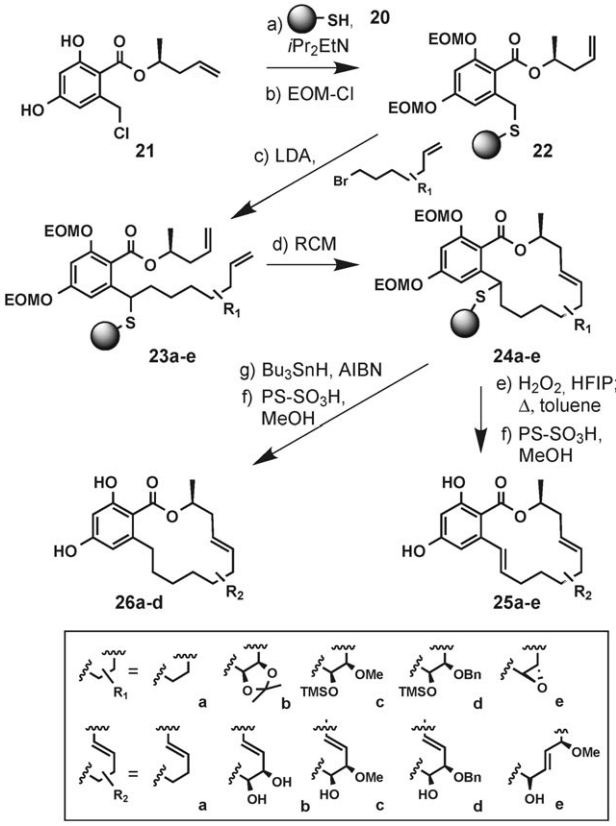



***Scheme 3.*** Total synthesis of aigialomycin D (**1**) and divergent synthetic pathways. a) PS-DEAD (2.5 equiv, 1.3 mmol g$^{-1}$), (*R*)-(+)-penten-2-ol (1.0 equiv), *m*-Cl$Ph_3$P (2.0 equiv), $CH_2Cl_2$, 23 °C, 0.5 h, 83%; b) *i*$Pr_2$EtN (4.0 equiv), EOMCl (4.0 equiv), TBAI (cat.), DMF, 80 °C, 5 h, 95%; c) LDA (2.0 equiv), THF, −78 °C; then $(PhSe)_2$ (0.9 equiv), 2 h, 75%; d) LDA (2.0 equiv), **8** or **11** (1.0 equiv), THF/HMPA (10:1), −78 °C, 20 min, 74% (**8**) and 75% (**11**); e) Grubbs II catalyst (0.05 equiv), toluene, 80 °C, 12 h, 90% (from **13**) and 92% (from **14**); f) $H_2O_2$ (2.0 equiv), THF, 23 °C, 3 h, 82% (**15**) and 85% (**16**); g) PS-$SO_3H$ (9.0 equiv, 3.2 mmol g$^{-1}$), MeOH, 50 °C, 2 h, quant.; h) 1. PS-$SO_3H$ (10.0 equiv, 2.9 mmol g$^{-1}$), THF/$H_2O$ (1:1); 45 °C, 16 h, quant.; or 2. $NaN_3$ (5 equiv), PS-$SO_3H$ (0.1 equiv, 2.9 mmol g$^{-1}$), DMF, 65 °C, 12 h, 83%; or 3. KCN (5 equiv), PS-$SO_3H$ (0.1 equiv, 2.9 mmol g$^{-1}$), DMF, 65 °C, 12 h, 89%; i) PS-$SO_3H$ (10.0 equiv, 2.9 mmol g$^{-1}$), MeOH, 45 °C, 3 h, >90%; j) PS-$SO_3H$ (10.0 equiv, 2.9 mmol g$^{-1}$), R′OH, 45 °C, 3 h, quant. PS = polymer-supported; DEAD = diethyl azodicarboxylate; TBAI = tetrabutylammonium iodide; DMF = *N*,*N*-dimethylformamide; LDA = lithium diisopropylamide; HMPA = hexamethylphosphoramide.




***Scheme 4.*** Conversion of Merrifield resin into a thiophenol resin (**20**). a) TrCl (1.0 equiv), pyridine (1.0 equiv), $CH_2Cl_2$, 23 °C, 12 h, 100%; b) $K_2CO_3$ (2.0 equiv), PS-$CH_2Cl$ (1.0 equiv), DMF, 50 °C, 12 h, 100%; c) TFA/$CH_2Cl_2$/$Et_3SiH$ (9:10:1), 23 °C, 1 h, 100%. Tr = triphenylmethyl; TFA = trifluoroacetic acid.

Unprotected ester **21**[29] was loaded onto a thiophenol resin **20**, and the phenols were protected as EOM ethers (Scheme 5). Alkylation of the polymer-bound thioether with a variety of alkyl bromides yielded the desired metathesis precursors **23a–e**. Interestingly, initial attempts to carry out the ring-closing metathesis under the conditions successfully used in solution failed. However, excellent yields were attained in $CH_2Cl_2$ solvent at 120 °C using microwave irradiation for 75 min. As the catalyst is short-lived at that temperature, it was added in three portions of 6 mol%. Notably, oligomer products were not observed under these conditions (the loading was calculated to be 0.33 mmol $g^{-1}$).




***Scheme 5.*** Diversity-oriented synthesis of aigialomycin D (**1**) and analogues. a) PS-SH (1.0 equiv, 0.6–0.8 mmol $g^{-1}$), **21** (1.1 equiv), *i*$Pr_2$EtN (1.0 equiv), DMF, 60 °C, 12 h, 82% (by mass gain considering 0.8 mmol $g^{-1}$ for PS-SH); b) DBU (4.0 equiv), EOMCl (4.0 equiv), TBAI (cat.), DMF, 23 °C, 12 h, ≈96% (by mass gain considering 0.8 mmol $g^{-1}$ for PS-SH); 80% over two steps (by radical cleavage, AIBN (cat), *n*$Bu_3SnH$ (5.0 equiv), toluene, 150 °C, microwave, 10 min); c) LDA (6.0 equiv), $R^1Br$ (2.0 equiv), THF/HMPA (10:1), −78 °C, 20 min, ≈quant. (by radical cleavage (AIBN (cat.), $Bu_3SnH$ (5.0 equiv), toluene, 150 °C, microwave, 10 min) and by oxidation/elimination ($H_2O_2$ (4.0 equiv), $CH_2Cl_2$/HFIP (1:1), 23 °C, 12 h; then toluene, 80 °C, 3 h); d) Grubbs II catalyst (3×0.06 equiv), $CH_2Cl_2$, 120 °C, microwave, 25 min, 100% (by radical cleavage, AIBN (cat.), $Bu_3SnH$ (5.0 equiv), toluene, 150 °C, microwave, 10 min); e) $H_2O_2$ (4.0 equiv), $CH_2Cl_2$/HFIP (1:1), 23 °C, 12 h; then toluene, 80 °C, 3 h, >90%; f) PS-$SO_3H$ (10.0 equiv, 2.9 mmol $g^{-1}$), MeOH, 45 °C, 3 h, >90%; g) AIBN (cat), $Bu_3SnH$ (5.0 equiv), toluene, 150 °C, microwave, 10 min; >98%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene; TBAI = tetrabutylammonium iodide; AIBN = 2,2′-azobisisobutyronitrile; HFIP = hexafluoropropanol.

The compounds were released from the resin by using both an oxidation ($H_2O_2$, HFIP)[30]/elimination and free-radical cleavages ($Bu_3SnH$, AIBN) followed by global deprotection with sulfonic acid resin to obtain aigialomycin D (**25b**) and its analogues **25a**, **c–e** as well as dihydroaigialomycin and its analogues **26a–d** (**25e** is identical to **18d**).

Aigialomycin D (**1**) and selected analogues obtained by the described chemistry were tested for their activity against a panel of kinases.[31] As shown in Table 1, aigialomycin D was found to inhibit CDK1/cyclin B and CDK5/p25 at 5.7 and 5.8 μM, respectively, as well as GSK-3 at 14 μM but much less PfGSK-3, the *Plasmodium* homologue of GSK-3 (Table 1).[32] Importantly, the acetonide-protected aigialomycin D or compounds with a different relative arrangement of the hydroxy groups and the olefin (**18a** and **b**) showed no activity. As previously reported,[4] we confirmed that aigialomycin D is not an inhibitor of HSP90. These results show that aigialomycin D is not an indiscriminate ATP antagonist. The lack of activity of aigialomycin against HSP90 may be rationalized by the very high energetic penalty of adopting the required conformation to fit in the ATP-binding pocket of HSP90.

***Table 1:*** Biological activity of aigialomycin D (**1**) and selected analogues.

| | IC 50 [μM] | | | |
|---|---|---|---|---|
| Compound/kinase | CDK1 | CDK5 | GSK3 | PfGSK3 |
| **1** | 5.7 | 5.8 | 14 | 100 |
| **1**–acetonide | >100 | 64 | 70 | >100 |
| **18a** | >100 | >100 | >100 | >100 |
| **18b** | >100 | >100 | >100 | >100 |

In conclusion, we have developed a flexible polymer-supported synthesis of aigialomycin D amenable to the preparation of libraries extending beyond the diversity of the analogues accessible from natural sources. The reported chemistry extends the utility of the thioether resin for the solid-phase synthesis of natural-product libraries. It is unlikely that the antimalarial activity of aigialomycin D can be attributed to PfGSK-3 inhibition; however, its cytotoxicity in human cells may be attributed to CDK/GSK-3 inhibition. The fact that aigialomycin D inhibits selected kinases further supports the hypothesis that the resorcyclides constitute a promising scaffold for ATP antagonism/kinase inhibition and may be a valuable class of compounds for chemical genetics.

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