Natural Product Synthesis

Total Synthesis of the Salicylate Enamide Macrolide Oximidine III: Application of Relay Ring-Closing Metathesis**

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Vacuolar-type (H^+)-adenosine triphosphatases (V-ATPases) are important membrane-bound proteins that control the pH level of intracellular compartments in eukaryotic cells. These proteins affect many membrane processes, such as intracellular membrane transport, bone resorption, and tumor metastasis.[1] A number of structurally related salicylate enamide macrolides have recently been disclosed as V-ATPase inhibitors. Unlike other known V-ATPase inhibitors (for example, bafilomycins and concanamycins), salicylate enamide macrolides are selective inhibitors of mammalian V-ATPases.[2] The oximidines[3,4] (Scheme 1) are a growing subclass of the salicylate enamides and contain both macrocyclic triene and diene epoxide moieties. Oximidine III (**3**) from *Pseudomonas sp.* QN05727 was recently identified by Hayakawa et al.[4] Compound **3** is closely related to oximidines I (**1**) and II (**2**)[5] but contains an *E* enamide side chain and lacks a secondary hydroxy group at C14. Interestingly, **3** exists in two conformers at ambient temperature[4] and its activity against transformed 3Y1 cells is three to eightfold higher than that of **1**. To further evaluate the structure–activity relationships (SARs) of the oximidines, we initiated a program to synthesize **3** and the corresponding epoxide and enamide stereoisomers.

Our retrosynthetic analysis for oximidine III is shown in Scheme 1. We planned to utilize late-stage copper(I)-mediated cross-coupling[6] of *E* vinyl iodide (*E*)-**4** with amide **5**[6a] to attach the enamide side chain. Macrocyclization would then rely on ring-closing metathesis (RCM)[7] of precursors **6** and **7**, which may be prepared by base-mediated transesterification[5a,8] of 4*H*-1,3-benzodioxin-4-one **8** and alcohol **9**. Our experience with oximidine II[5a] suggested that the ability

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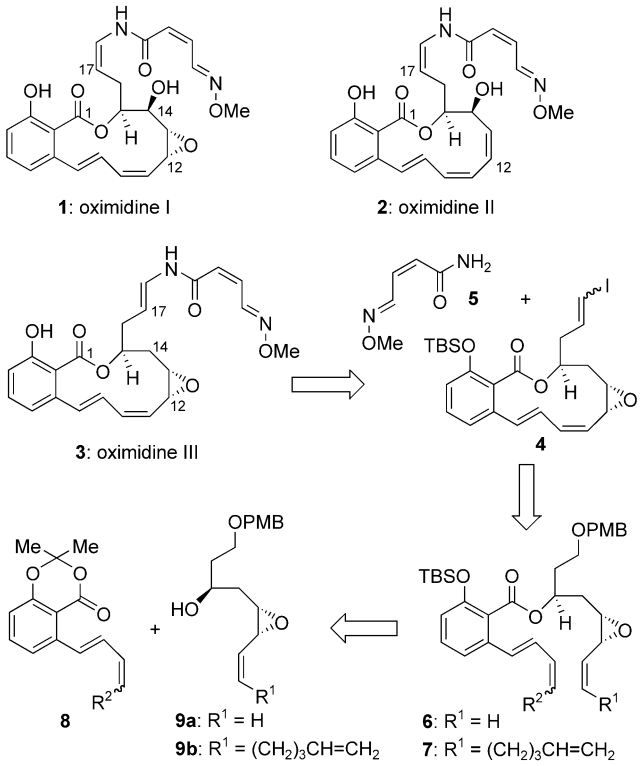


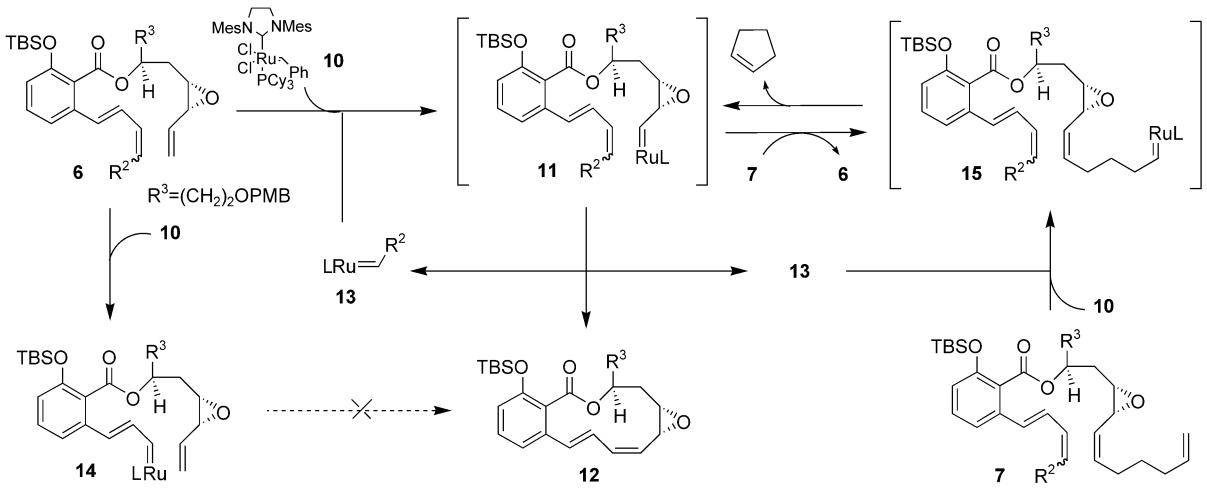
Scheme 1. Chemical structures of the oximidines and retrosynthetic analysis of oximidine III (**3**). TBS = *tert*-butyldimethylsilyl, PMB = *para*-methoxybenzyl, R^2 = Me or *i*Pr (see Table 1).

to perform RCM cleanly may be related to successful initiation of the metathesis reaction. We hoped that the ruthenium catalyst **10** (Scheme 2) would react with the epoxy alkene function of substrate **6** to form intermediate **11**, and that **11** would undergo macrocyclization to afford the desired 12-membered macrolactone **12**. Accordingly, we planned to substitute the terminus of the conjugated diene to avoid formation of stable ruthenium complex **14**. In the event that initiation of the metathesis at the vinyl epoxide site proved difficult,[9] we planned to evaluate an alternative approach to the generation of intermediate **11** involving the introduction of a relay moiety.[10] We hoped that the ruthenium catalyst would react with the least sterically hindered terminal olefin of substrate **7** to provide intermediate **15**. After release of cyclopentene as a by-product, **15** could then be converted into the desired intermediate **11** for RCM macrocyclization.

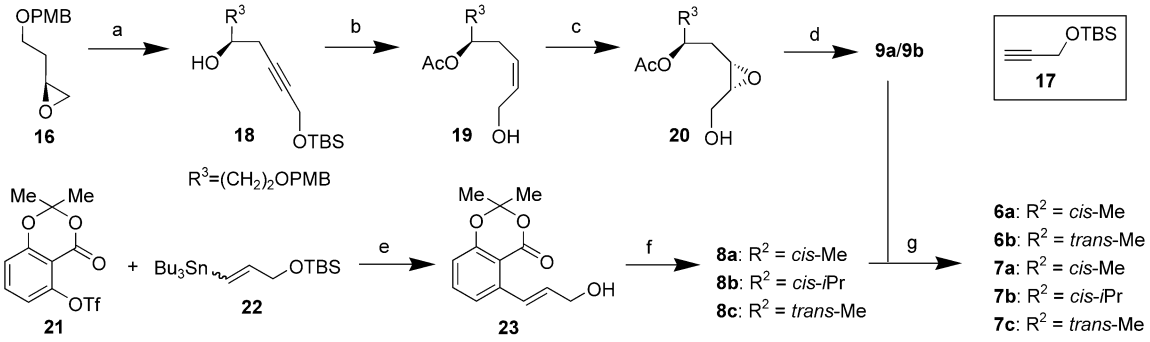
Synthesis of vinyl epoxide fragments **9a**/**9b** was initiated by adding chiral, nonracemic epoxide **16**[11] to an alkynyl lithium reagent derived from **17** to afford alkynol **18** (Scheme 3). Acetylation of **18**, followed by desilylation and Lindlar semihydrogenation, afforded allylic alcohol **19** (85%, 4 steps). Sharpless asymmetric epoxidation[12] oxidized **19** to epoxide **20** (78%, d.r. > 16:1).[13] The primary alcohol was then oxidized by treatment with Dess–Martin periodinane[14] to form an intermediate aldehyde, which was treated with either methylene or 5-hexenylidenetriphenylphosphorane to afford vinyl epoxides **9a**/**9b** after deacetylation.

Preparation of the salicylate fragment began with Stille coupling of triflate **21**[6c] and vinyl stannane **22** (E:Z = 6:1),[15] followed by desilylation with TBAF to form E allylic alcohol **23** (82%, 2 steps). In this transformation the Z alkene isomer was converted into a lactone by-product that was easily separated from the desired product by flash chromatography. Oxidation of **23** with MnO_2 and Wittig olefination gave E,Z dienes **8a**,**b**. Treatment of **9b** with NaHMDS followed by addition of **8a–c**[5a] and in situ silylation afforded tetraenes **7a–c**, respectively. Substrates **6a** (R^2 = *cis*-Me) and **6b** (R^2 = *trans*-Me) were prepared from **8a**, **8c**, and **9a** by employing analogous procedures.

We next evaluated a number of substrates in RCM macrocyclizations (Table 1). Treatment of **6b** with the Grubbs second-generation catalyst **10** (CH_2Cl_2, reflux) afforded a low yield of the desired product **12**, accompanied by significant amounts of oligomers. Substrate **6a** reacted more slowly with catalyst **10** and also afforded a low yield of **12** (entries 1 and 2, Table 1). This result may be explained by the formation of the unreactive alkylidene **14** (Scheme 2). We next examined the relay RCM reactivity of substrates **7a–c**. We found that it was necessary to add the substrate to a solution of ruthenium catalyst in CH_2Cl_2 to prevent formation of by-product **6**

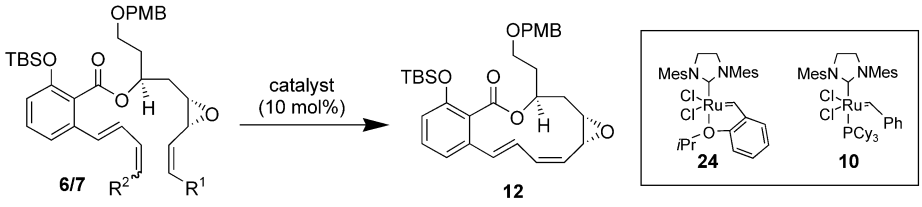


Scheme 2. Analysis of ring-closing metathesis pathways. Mes = trimethylphenyl, L = ligand.



Scheme 3. Synthesis of RCM precursors. a) **17**, *n*BuLi, then **16**, $BF_3{\cdot}OEt_2$, THF, −78–25 °C; b) 1. Ac_2O, pyridine, cat. DMAP, CH_2Cl_2; 2. AcOH/H_2O/THF (3:1:1), 60 °C; 3. Lindlar catalyst, 1 atm H_2, Et_3N, THF, 85 % for 4 steps; c) $Ti(OiPr)_4$, D-DIPT, 4-Å molecular sieves, CH_2Cl_2, −20 °C, 76 %; d) 1. Dess–Martin periodinane, CH_2Cl_2, RT; 2. $Ph_3P^+Me{\cdot}Br^-$, NaHMDS, THF, −10 °C, 62 %; or $Ph_3P^+(CH_2)_4CH{=}CH_2{\cdot}I^-$, NaHMDS, THF, −10 °C, 70 %, *Z*:*E* > 16:1; 3. K_2CO_3, MeOH, RT, 95 %; e) 1. $[Pd_2(dba)_3]$, tri(2-furyl)phosphane, LiCl, DMF, 60 °C; 2. TBAF, THF, RT, 82 % for 2 steps; f) 1. MnO_2, CH_2Cl_2, RT; 2. $(Ph_3PEt){\cdot}Br$ or $(Ph_3PiBu){\cdot}Br$, NaHMDS, −78–25 °C, 60–62 % for 2 steps (for **8c**, see ref. [5a]); g) **9**, NaHMDS, **8**; then TBSCl, imidazole, THF, 0–25 °C, 92–96 %. THF = tetrahydrofuran, DMAP = 4-dimethylaminopyridine, DIPT = diisopropyl tartrate, HMDS = 1,1,1,3,3,3-hexamethyldisilazane, dba = *trans*,*trans*-dibenzylideneacetone, DMF = dimethylformamide, TBAF = tetrabutylammonium fluoride.

Table 1: Evaluation of substrates and conditions for RCM macrocyclization.[a]

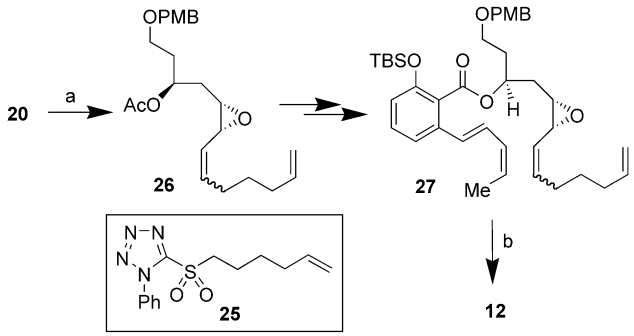


	SM[b]	R^1	R^2	Addition time [min]	Catalyst	Conversion [%][e]	Yield [%][f]
1[c]	**6a**	H	*cis*-Me	–	**10** or **24**	38	15
2[c]	**6b**	H	*trans*-Me	–	**10** or **24**	75	12
3	**7a**	$(CH_2)_3CH{=}CH_2$	*cis*-Me	120	**10**	> 90	45
4	**7b**	$(CH_2)_3CH{=}CH_2$	*cis*-*i*Pr	150	**10**	71	30
5	**7c**	$(CH_2)_3CH{=}CH_2$	*trans*-Me	240	**10**	77	30
6	**7a**	$(CH_2)_3CH{=}CH_2$	*cis*-Me	60	**10**	> 90	58
7	**7a**	$(CH_2)_3CH{=}CH_2$	*cis*-Me	60	**24**	> 90	58
8[d]	**7a**	$(CH_2)_3CH{=}CH_2$	*cis*-Me	30	**24**	> 90	71

[a] Reactions were conducted in refluxing CH_2Cl_2 unless otherwise noted; the final concentration of **6**/**7** was 2.0 mM. [b] SM = starting material. [c] Catalyst added to the starting material in refluxing CH_2Cl_2 and stirred for 2 h. [d] Reaction conducted at 50 °C in 1,2-dichloroethane. [e] Conversions based on recovered starting material. [f] Yields of isolated products; in all cases, only 12-membered cyclic *E*,*Z* diene **12** was isolated.

(Scheme 2).[16] Substrate **7a** was optimal for the RCM-RCM process; significant amounts of the corresponding by-products **6** were obtained when substrates **7b** and **7c** were used, which indicates slow reaction rates for the second RCM (entries 3–5, Table 1). Faster addition of the substrate resulted in reduced decomposition of the product (entry 6, Table 1). The recyclable ruthenium catalyst **24** described by Hoveyda and co-workers[17] showed a similar reactivity to catalyst **10** (entry 7, Table 1) and produced a colorless product. We investigated the relationship between the addition rate and temperature further in an attempt to minimize oligomer formation.[7c] We found that production of oligomers was minimized at a higher temperature than that initially used (entry 8, Table 1). Catalyst **24** was pretreated with 20 mol % *o*-isopropoxystyrene to convert the highly reactive ruthenium–alkylidene species **13** (R^2 = Me, Scheme 2) into the less reactive catalyst **24** during the reaction. A slower reaction rate was observed, but the chemical yield was not improved.

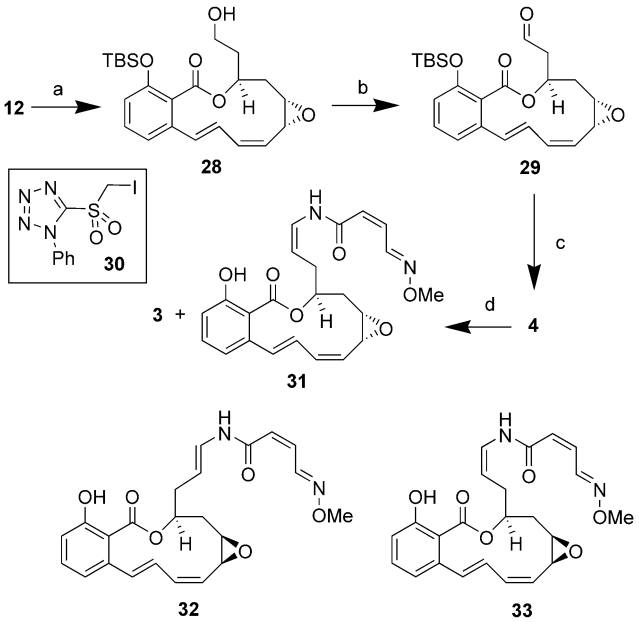
To evaluate the influence of the vinyl epoxide olefin geometry on the relay RCM process we prepared epoxy alkene **26** (*E*:*Z* = 1.3:1, Scheme 4) from **20** and phenyltetrazole sulfone **25**.[18] Compound **26** was converted into RCM precursor **27** in several steps (see Scheme 3). Relay RCM (2.0 mM, DCE, 50 °C) of substrate **27** provided only a 34 % yield of the desired product **12**. The *E* vinyl epoxide stereoisomer of **27** was purified and used in the further study of this transformation. Submission of this *E* isomer of **27** to relay RCM conditions gave neither the desired macrolactone **12** nor by-product **6a**, only oligomeric by-



Scheme 4. Relay RCM with substrate **27**. a) 1. Dess–Martin periodinane, CH_2Cl_2, RT; 2. **25**, NaHMDS, −78–25 °C, 74 % (*E*:*Z* = 1.3:1). b) 10 mol % **24**, DCE, 2.0 mM, 50 °C, 30 min, 34 %. DCE = dichloroethane.

products were formed, which indicates that the *Z* configuration of the epoxy alkene is required for a fast reaction rate in the first RCM step.

To advance **12** to oximidine III, the PMB protecting group was removed by treatment with DDQ and the resulting alcohol **28** was oxidized by PDC to produce aldehyde **29** (Scheme 5). Transformation of **29** into *E* vinyl iodide (*E*)-**4**



Scheme 5. Syntheses of oximidine III (**3**) and its enamide stereoisomer **31**. a) DDQ, CH_2Cl_2, pH 7 buffer, 0–25 °C, 95%; b) PDC, 4-Å molecular sieves, CH_2Cl_2; c) **30**, NaHMDS, THF, −78–25 °C, 62% for 2 steps (*E*:*Z* ≈ 1:1); d) TBAF (1.0 equiv), THF, 0–25 °C; concentrate, then **5**, CuTC, *N*,*N*′-dimethylethylenediamine, K_2CO_3, DMA, 50 °C, 1 h, 45% (*E*:*Z* = 7:1). DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, PDC = pyridinium dichromate, CuTC = copper(I) thiophenecarboxylate, DMA = *N*,*N*-dimethylacetamide.

was problematic because the vinyl epoxide is sensitive to both acidic and reductive[19] conditions. Treatment of **29** with the Seyferth/Gilbert reagent[20] converted the aldehyde into a terminal alkyne. Hydrozirconation of the alkyne, followed by addition of iodine (THF, 0 °C) afforded vinyl iodide (*E*)-**4** in low yield. Direct homologation of aldehyde **29** was achieved by Kociénski–Julia olefination with the novel phenyltetrazole sulfone reagent **30**.[21] This transformation led to an inseparable mixture of vinyl iodides (*E*)-**4** and (*Z*)-**4** (62% yield, *E*:*Z* = 1:1).[22]

We next tested the crucial late-stage Cu^I-mediated vinylic amidation reaction. The TBS protecting group of **4** was first removed by treatment with TBAF to avoid the use of excess base.[23] The deprotected compound, presumed to be the phenolate salt, was submitted directly to amidation by treatment with copper(I) thiophenecarboxylate-*N*,*N*′-dimethylethylenediamine.[5a] The vinyl iodides were consumed quickly when a stoichiometric amount of CuTC was used. Oximidine III (**3**) and the corresponding *Z* enamide stereoisomer **31** were isolated in 45% yield (*E*:*Z* = 7:1) after 1 h (50 °C). Extended reaction times resulted in decomposition of **3** and a higher yield of *Z* enamide **31** (3 h, 21% yield, *E*:*Z* = 1:1).[6c] The synthetic product **3** was confirmed as identical to natural oximidine III by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, $[\alpha]_D^{20}$ measurement, HPLC, and TLC R_f values in three solvent systems.

After successful synthesis of oximidine III (**3**) and its enamide stereoisomer **31**, we also synthesized **32** (the C12-C13 epimer of oximidine III) and its enamide stereoisomer **33** by employing L-DIPT in the epoxidation step. Unlike oximidine III (**3**) and **31**, both **32** and **33** each exist as a single conformer, as shown by ^1H NMR spectroscopy (RT). Oximidines **3** and **31**–**33** were evaluated for activity against bovine V-ATPase and their IC_{50} values (concentration required for 50% inhibition) were found to be 2.2, 65, 4.3, and 65 nM, respectively.[24] These initial SAR data indicate that the C17-C18 olefin geometry has a more substantial effect on V-ATPase inhibition than the C12-C13 epoxide stereochemistry.

In summary, enantioselective total syntheses of the natural product oximidine III (**3**) and stereoisomers **31**–**33** have been developed, which has allowed unambiguous assignment of the relative and absolute stereochemistry of **3**. A relay RCM strategy was employed to facilitate the crucial macrocyclization reaction, and a well-defined substrate possessing two differentially functionalized RCM alkene partners was found to be required for the RCM-RCM process. A novel phenyltetrazole sulfone reagent **30** was developed for homologation of aldehydes to form vinyl iodides under mild conditions. Further synthetic studies on the oximidines and simplified analogues of these compounds will be reported in due course.

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Keywords: antitumor agents · enamides · natural products · ring-closing metathesis · vinyl iodides

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[22] The *E*:*Z* ratio was determined by ^{1}H NMR analysis.
[23] In our synthesis of oximidine II (ref. [5a]), we utilized a silylated phenol substrate treated with base and amide (2 equiv each) in a copper-mediated coupling to effect both silyl ether deprotection and enamide formation. However, use of excess base and amide in the syntheses described herein led to significant decomposition of the sensitive vinyl epoxide moiety.
[24] V-ATPase activity was determined as described in the Supporting Information.

