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## Lobatamide C: Total Synthesis, Stereochemical Assignment, Preparation of Simplified Analogues, and V-ATPase Inhibition Studies

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**Abstract:** The total synthesis and stereochemical assignment of the potent antitumor macrolide lobatamide C, as well as synthesis of simplified lobatamide analogues, is reported. Cu(I)-mediated enamide formation methodology has been developed to prepare the highly unsaturated enamide side chain of the natural product and analogues. A key fragment coupling employs base-mediated esterification of a  $\beta$ -hydroxy acid and a salicylate cyanomethyl ester. Three additional stereoisomers of lobatamide C have been prepared using related synthetic routes. The stereochemistry at C8, C11, and C15 of lobatamide C was assigned by comparison of stereoisomers and X-ray analysis of a crystalline derivative. Synthetic lobatamide C, stereoisomers, and simplified analogues have been evaluated for inhibition of bovine chromaffin granule membrane V-ATPase. The salicylate phenol, enamide NH, and *ortho*-substitution of the salicylate ester have been shown to be important for V-ATPase inhibitory activity.

### Introduction

Biological metabolites are the subject of ongoing investigations in the search for new medicinal leads including anti-infective and anti-cancer agents. Recently, a number of unique antitumor natural products containing a central benzolactone core bearing an unusual enamide side chain have been reported. Members include salicylhalalamides A and B,<sup>1</sup> lobatamides A–F,<sup>2</sup> apicularens A and B,<sup>3</sup> CJ-12,950 and CJ-13,357,<sup>4</sup> and oximidines I and II<sup>5</sup> (Figure 1). The lobatamides, containing a 15-membered ring macrodilactone and a divinylcarbinol moiety, were isolated in 1998 by Boyd et al. from a southwestern Pacific

tunicate.<sup>2</sup> Suzumura et al. also independently isolated YM-75518A–C, identical to lobatamides A–C, and the Z-oxime stereoisomer YM-75518D (Figure 1).<sup>6</sup> The absolute stereochemistry of the C15 divinylcarbinol of YM-75518A was assigned as (*S*) using modified Mosher ester analysis.<sup>6b</sup> However, the configurations of the two remaining stereocenters were not determined and thus required chemical synthesis for full confirmation.

Extensive biological evaluation of lobatamides has been performed against the National Cancer Institute's (NCI) 60 human tumor cell line (mean panel GI<sub>50</sub>'s approximately 1.6 nM).<sup>2</sup> Significantly, biological studies indicate that both salicylhalalamides and lobatamides represent antitumor natural products with a novel mechanism of action.<sup>2</sup> Recently, it has been reported that the salicylate enamide macrolides selectively inhibit vacuolar-type proton ATPases (V-ATPases),<sup>7</sup> ubiquitous proton-translocating pumps of eukaryotic cells.<sup>8</sup> Moreover, it has been found that proton-extruding V-ATPases are expressed

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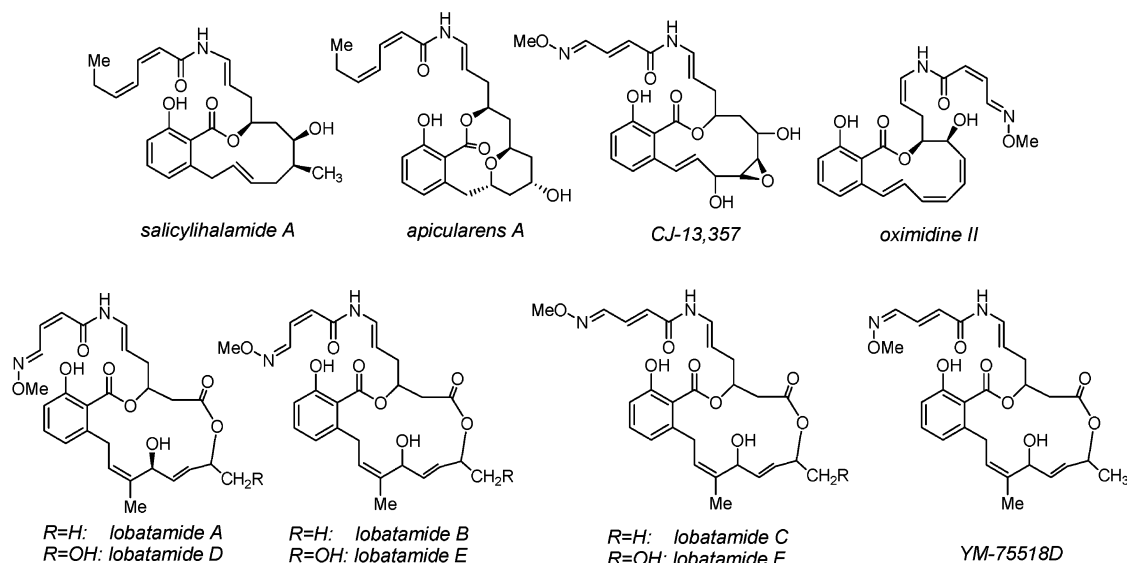


Figure 1. Salicylate enamide natural products.

on the plasma membranes of human tumor cells.<sup>9</sup> Accordingly, these natural products are exciting new targets for chemical synthesis, lead optimization studies, and preparation of designed analogues and molecular probes to further define interactions with the molecular target. The salicylihalamides have been synthesized in several laboratories.<sup>1c–h</sup> De Brabander and co-workers have reported the preparation and structure–function analysis of a number of promising salicylihalamide derivatives.<sup>10</sup> Herein, we report a full account of the total synthesis and stereochemical assignment of lobatamide C,<sup>11</sup> as well as structure–activity studies in the lobatamide series.

### Development of Methodology for Synthesis of the Enamide Side Chain

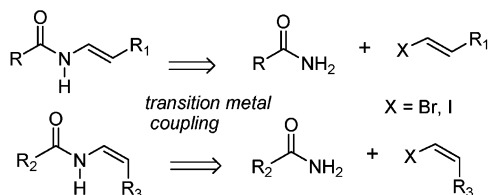
To enable the synthesis of the salicylate enamides as an entire class, we required a general method to synthesize highly unsaturated enamides. In general, enamides have been shown to have ambident reactivity, both electrophilic at the  $\alpha$ -carbon and nucleophilic at the  $\beta$ -carbon. Enamides may be regarded as deactivated enamines and will react with powerful electrophiles such as bromine, peracids, and lead(IV) acetate.<sup>12</sup> They are stable compounds under neutral or basic conditions and with Brønsted acids give rate-determining protonation on carbon, leading to a reactive *N*-acyliminium ion intermediate that may either undergo hydrolysis of the double bond to form carbonyl compounds and amides<sup>13</sup> or react with a range of nucleophiles, including oxygen, sulfur, or  $\pi$ -based nucleophiles.<sup>14</sup>

Enamides have been previously synthesized using a number of methods, including *N*-acylation of imines,<sup>15</sup> elimination of

$\alpha$ -substituted amides,<sup>16</sup> isomerization of *N*-allylamides,<sup>17</sup> palladium(II)-catalyzed amidation of alkenes,<sup>18</sup> direct addition of amides to alkynes,<sup>19</sup> acid-catalyzed condensation of aldehydes and amides,<sup>20</sup> amide Peterson olefination,<sup>21</sup> Horner–Wittig and Wadsworth–Emmons reactions,<sup>20b,22</sup> and *N*-acylation of protected enamines.<sup>1f,23</sup> Other recent enamide formation methods have also been developed, including organometal addition to vinyl isocyanates,<sup>5b,24</sup> oxidative decarboxylation–elimination,<sup>25</sup> Ru-catalyzed chain extension,<sup>26</sup> and rearrangement of *N*-( $\alpha$ -silyl)allyl amides.<sup>27</sup> Some of these methods have been employed in the synthesis of enamide-containing natural products such as lycorine,<sup>28</sup> mycalolide A,<sup>29</sup> chondriamides,<sup>25</sup> crocacin D,<sup>30</sup> TMC-95 A and B,<sup>27</sup> and the salicylihalamides.<sup>1c–f</sup>

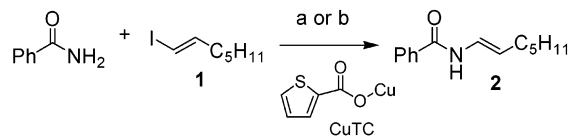
In considering potential new methods for the formation of enamides, we have focused our efforts on transition metal-catalyzed vinylic substitution reactions of vinyl halides and amides, due to the ready availability and stability of the reaction partners and the possibility that such C–N bond constructions may occur in a stereocontrolled manner (Figure 2). It was envisioned that a coupling process could be developed wherein *E* and *Z* vinyl halides could be converted to the corresponding

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**Figure 2.** Stereoselective C–N bond construction for enamide synthesis.

**Scheme 1<sup>a</sup>**



<sup>a</sup> Reagents and conditions: (a) CuI (0.10 equiv), Cs<sub>2</sub>CO<sub>3</sub> (1.2 equiv), PPh<sub>3</sub> (0.20 equiv), NMP, 90 °C, 12 h, 31% (<sup>1</sup>H NMR); (b) CuTC (0.10 equiv), Cs<sub>2</sub>CO<sub>3</sub> (1.2 equiv), NMP, 90 °C, 12 h, 59% (<sup>1</sup>H NMR).

*E* and *Z* enamides. This is significant since many of the existing approaches to prepare enamides are not stereoselective. As an entry to our studies, we were attracted to a study by Ogawa et al. who reported the copper iodide-promoted substitution of vinyl bromides and potassium amides (1 equiv of CuI, HMPA, 130 °C) to afford enamides in low to moderate (38–45%) yields.<sup>31</sup> On the basis of this precedent and related Cu(I)-catalyzed C–N cross coupling reactions,<sup>32</sup> we focused on developing a Cu(I)-catalyzed amidation method that would occur at milder temperatures and would be suitable for the installation of potentially labile enamides on complex substrates.<sup>33</sup>

Using benzamide and (*E*)-1-iodo-1-heptene (**1**)<sup>34</sup> as model substrates, we initially compared Cu(I) phosphine<sup>35</sup> and Cu(I) carboxylate catalysts with Cs<sub>2</sub>CO<sub>3</sub> as base (Scheme 1). We obtained a higher conversion (59%) for enamide **2** using Liebeskind's Cu(I) thiophenecarboxylate (CuTC),<sup>36</sup> which led us to undertake further optimization with this catalyst.

After reaction optimization, optimal conditions for amidations were discovered by employing CuTC (30 mol %), Cs<sub>2</sub>CO<sub>3</sub> as base, and rigorous vacuum purge degassing of the reaction mixture in 1-methyl-2-pyrrolidinone (NMP) prior to heating (90 °C, 12 h). Using these conditions, a number of enamides were prepared as shown in Table 1. Both benzamide and (*E,E*)-2,4-hexadienamide (**3**)<sup>37</sup> participated in vinylic substitution of **1** to afford enamides **2** and **4** in good yields (entries 1 and 2). It should be mentioned that, under the same conditions using an excess of vinyl iodide **1** (2.2 equiv) and Cs<sub>2</sub>CO<sub>3</sub> (2.0 equiv), the coupling reaction with benzamide afforded only enamide **2** (77%) and recovered vinyl iodide **1** (70%) without any evidence of an *N,N*-divinyl amide product.<sup>38</sup> Using (*E*)-β-iodostyrene

(**5**),<sup>39</sup> related transformations provided the desired enamides **6** and **7** (entries 3 and 4). The coupling reaction was also successful for secondary amides. Amidations of **1** and **5** with *N*-methylformamide provided enamides **8** and **9** as a mixture of rotamers in good yields (entries 5 and 6). With 2-pyrrolidinone, enamide **10**<sup>20a</sup> was obtained in 99% yield (entry 7). Amidation of a *Z*-vinyl iodide **11**<sup>40</sup> provided *Z*-enamide **12** in only 23% yield (entry 8) likely due to competitive elimination of the *Z*-vinyl iodide to the corresponding terminal alkyne under the reaction conditions.<sup>41</sup>

To investigate the nature of the halogen substituent, we evaluated (*E*)-1-bromo-1-heptene (**13**)<sup>42</sup> and (*Z*)-(4-bromo-3-butenyl)benzene<sup>43</sup> in coupling reactions with benzamide. However, only trace amounts of enamide products were obtained in these experiments. To compare the reactivities of vinyl iodides and vinyl bromides in the C–N bond formation, we conducted the competition experiment depicted in Scheme 2. Cu-mediated amidation was performed with vinyl bromide **13**, (*E*)-1-iodo-1-pentene (**14**),<sup>34</sup> and benzamide in NMP (90 °C). In this case, a 7:1 ratio of enamides **15** and **2** was detected by HPLC<sup>44</sup> analysis of the crude reaction mixture, which further supports that vinyl bromides are significantly less reactive than vinyl iodides as amidation substrates.<sup>45</sup>

To prepare enamides related to the lobatamides and related salicylate natural products, we next prepared 4-(methoxyimino)-2-butenamides **16** and **17** (Scheme 3). Treatment of 5-hydroxy-2(5*H*)-furanone<sup>46</sup> with aqueous methoxyamine hydrochloride led to the formation of 4-(methoxyimino)-(2*Z*)-butenoic acid **18** (92%).<sup>47</sup> The corresponding 4-(methoxyimino)-(2*Z*)-butenamide **16** was prepared in 88% yield by formation of the mixed anhydride of **18** and subsequent reaction with aqueous ammonia. (2*Z*)-Butenamide **16** could be fully isomerized to (2*E*)-butenamide **17** in 81% yield under acidic conditions.

Next, we conducted model amidation reactions with butenamides **16** and **17** and representative vinyl iodides (Table 2). Treatment of (2*E*)-butenamide **17** with vinyl iodide **1** or **5** using *N,N*-dimethylacetamide (DMA) as solvent afforded unsaturated enamides **19** and **20** in 57% and 52% yield, respectively (entries 1 and 2). However, cross-coupling of (2*Z*)-butenamide **16** with vinyl iodide **1** under similar conditions (90 °C, 12 h) did not afford the desired enamide **21**. In an effort to improve this reaction, 2,2,6,6-tetramethyl-3,5-heptanedione<sup>48</sup> was used as

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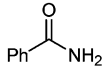
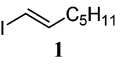
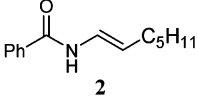
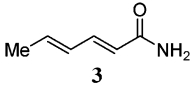
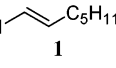
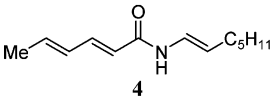
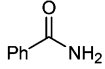

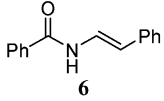
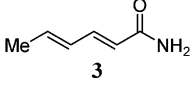

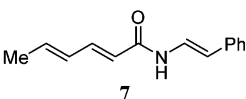
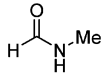
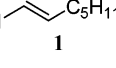
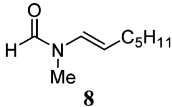
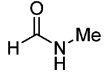

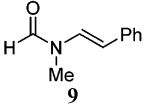

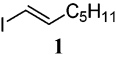
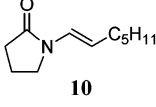
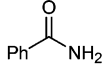

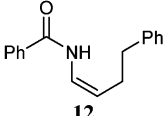
(45) For recent reports concerning the relative reactivity of bromo and iodo substrates in Cu(I)-catalyzed couplings, see: (a) Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, 124, 14844. (b) Zanon, J.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, 125, 2890.

(46) Doerr, I. L.; Willette, R. E. *J. Org. Chem.* **1973**, 38, 3878.

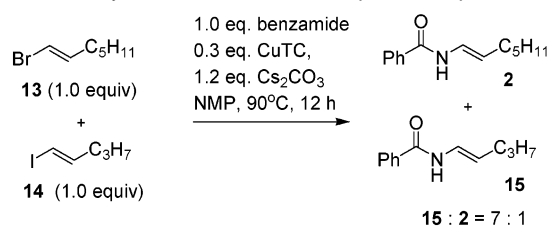
(47) Schroeter, S. H.; Appel, R.; Brammer, R.; Schenck, G. O. *Justus Liebigs Ann. Chem.* **1966**, 697, 42.

(48) Buck, E.; Song, Z. J.; Tschaen, D.; Dormer, P. G.; Volante, R. P.; Reider, P. J. *Org. Lett.* **2002**, 4, 1623.

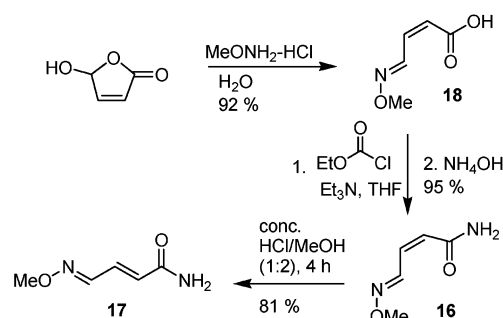
**Table 1.** CuTC-Catalyzed Enamide Coupling of Amides and Vinyl Iodides<sup>a</sup>

entry	amides	vinyl iodide	enamide	yields <sup>b</sup>
1				71
2				69
3				57
4				58
5				75 <sup>c</sup>
6				70 <sup>d</sup>
7				99
8				23 <sup>e</sup>

<sup>a</sup> Performed using 30 mol % of CuTC, 1.0 equiv of vinyl halide, 1.5 equiv of amide, and 1.5 equiv of Cs<sub>2</sub>CO<sub>3</sub>, NMP, 90 °C, 12 h. <sup>b</sup> Isolated yields after silica gel chromatography. <sup>c</sup> 3:1 mixture of rotamers by NMR (400 MHz). <sup>d</sup> 1.3:1 mixture of rotamers by NMR (400 MHz). <sup>e</sup> Reaction performed at 60 °C.

**Scheme 2.** Vinyl Bromide vs Iodide Competition Experiment

ligand to increase the reactivity and turnover of CuTC (Scheme 4). However, the reaction afforded only 20% of enamide **21** along with the unexpected *N,N*-divinyl amide **23** (17%).<sup>38</sup> Finally, enamide **21** was obtained in 52% yield using Rb<sub>2</sub>CO<sub>3</sub> as base<sup>49</sup> and *N,N'*-dimethylethylenediamine (**24**) as ligand<sup>32d,e</sup> (entry 3). Entry 4 illustrates the stereoselective coupling of *Z*-amide **16** and *Z*-vinyl iodide **11**. The reaction provided a modest yield (30%) of the desired *Z*-enamide **22** when only CuTC was used as catalyst. However, when diamine ligand **24**

**Scheme 3**

was added, the yield of enamide **22** was enhanced to 55%. This initial study provided encouragement for the stereospecific construction of the *Z*-enamide side chain of oximidines (cf. Figure 1).

Since diamine ligand **24** remarkably facilitated the production of enamides **21** and **22**, we employed **24** as ligand in couplings to prepare enamides **19** and **2**. However, the yield of **19** (54%) was slightly lower than that solely using CuTC as catalyst (57%), while the yield of **2** was only 40%.<sup>50</sup> These results

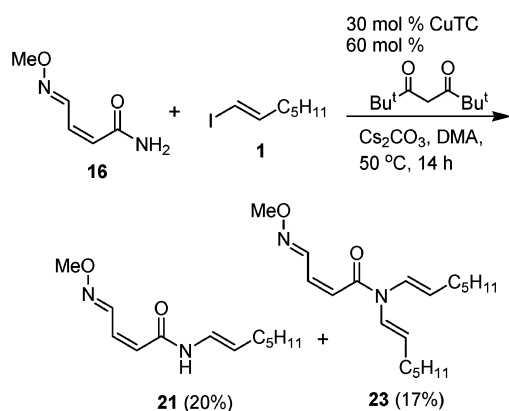
(49) For use of Rb<sub>2</sub>CO<sub>3</sub> as a base in Pd-catalyzed C–N bond formation, see: Watanabe, M.; Nishiyama, M.; Yamamoto, T.; Koie, Y. *Tetrahedron Lett.* **2000**, *41*, 481.



**Table 2.** Cu(I)-Catalyzed Vinyllic Substitution of Butenamides **16** and **17**

entry	amides	vinyl iodide	enamide	yields <sup>a</sup>
1	<b>17</b>	<b>1</b>	<b>19</b>	57 <sup>b</sup>
2	<b>17</b>	<b>5</b>	<b>20</b>	52 <sup>b</sup>
3	<b>16</b>	<b>1</b>	<b>21</b>	52 <sup>c</sup>
4	<b>16</b>	<b>11</b>	<b>22</b>	55 <sup>d</sup>

<sup>a</sup> Isolated yields after silica gel chromatography. <sup>b</sup> Reaction performed with 0.3 equiv of CuTC, 1.5 equiv of **17**, 1.5 equiv of Cs<sub>2</sub>CO<sub>3</sub>, DMA, 90 °C, 12 h. <sup>c</sup> 0.5 equiv of CuTC, 0.5 equiv of **24**, 1.5 equiv of **16**, 1.1 equiv of Rb<sub>2</sub>CO<sub>3</sub>, DMA, 60 °C, 12 h. <sup>d</sup> 0.3 equiv of CuTC, 0.6 equiv of **24**, 1.5 equiv of **16**, 1.1 equiv of Rb<sub>2</sub>CO<sub>3</sub>, DMA, 60 °C, 14.5 h.

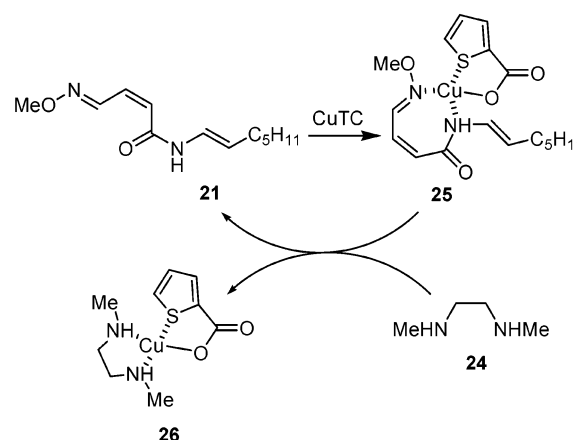
**Scheme 4**

indicate that ligand **24** is most effective when used with (2*Z*)-butenamide **16**. It is conceivable that enamide **21** may chelate with CuTC to form complex **25** (Scheme 5), which may substantially deactivate the catalyst. Addition of diamine **24** may compete with enamide **21** to transform a complex such as **25** to **26**, which may be available for further catalysis.

**First Retrosynthetic Analysis of Lobatamide C**

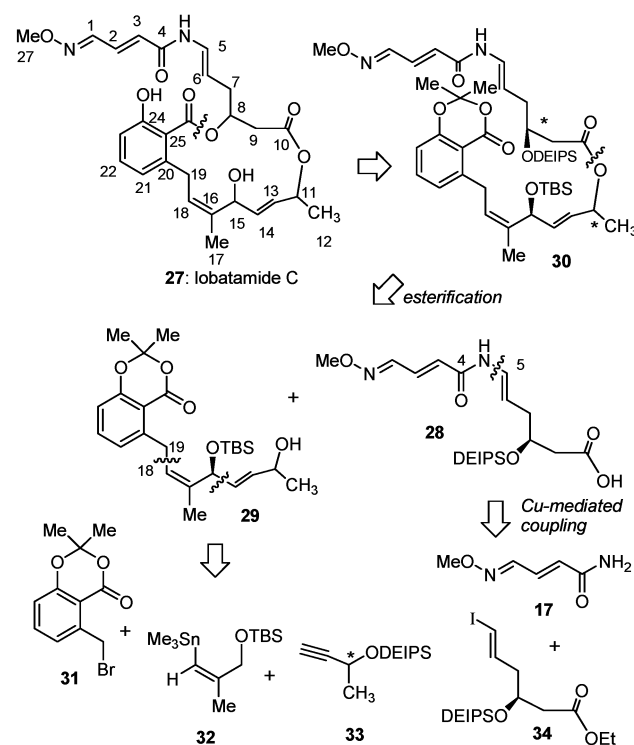
After the establishment of a workable methodology for the synthesis of the enamide side chain, we initiated studies toward the total synthesis of the lobatamides. Our plan for the synthesis of our first target, lobatamide C (**27**), is outlined in Figure 3. In contrast to previous syntheses of the salicylate enamide natural products<sup>1c–g,3c,d</sup> in which the enamide side chain was typically installed at a late stage in the synthesis, our general plan for lobatamide C was to pursue a convergent coupling of fragments with the enamide side chain preinstalled. This synthetic plan was primarily based on our general concern that base-catalyzed

(50) Conditions for preparation of **19**: 30 mol % of CuTC, 60 mol % of **24**, 1.0 equiv of vinyl iodide **1**, 1.5 equiv of amide **17**, 1.1 equiv of Cs<sub>2</sub>CO<sub>3</sub>, DMA, 60 °C, 12 h. For preparation of **2**: 30 mol % of CuTC, 60 mol % of **24**, 1.0 equiv of vinyl iodide **1**, 1.5 equiv of benzamide, 1.5 equiv of Cs<sub>2</sub>CO<sub>3</sub>, NMP, 90 °C, 12 h.

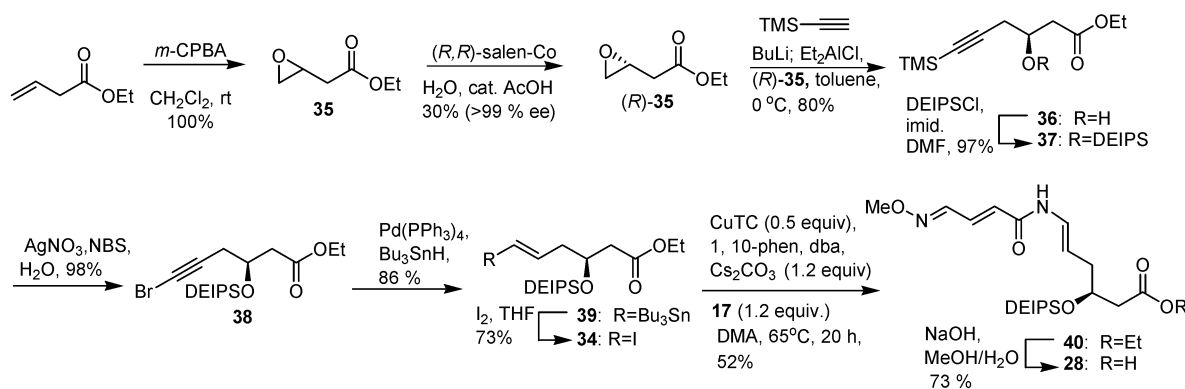
**Scheme 5**

amidation to construct enamides may be difficult on fragile and potentially elimination prone macrodilactone substrates. As we were later to discover, this convergent approach creates synthetic challenges with regard to manipulation and reactivity of enamide-containing intermediates but also adds an element of flexibility for construction of analogues that vary at the salicylate portion.

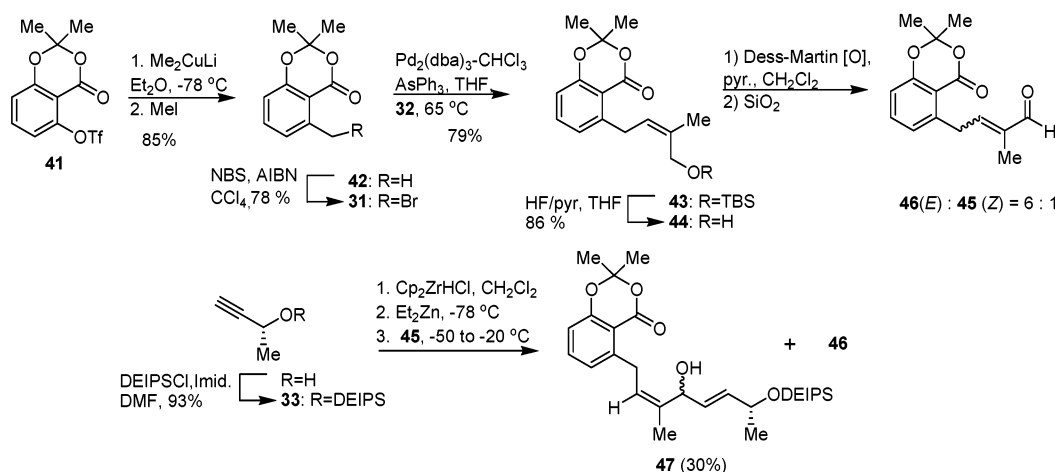
Retrosynthetic analysis of the lobatamide C skeleton reveals two principal fragments: the C1–C10 enamide sector **28** and the C11–C26 salicylate subunit **29** containing a divinylcarbinol moiety and an acetonide protecting group. We planned to prepare the precursor **30** from esterification of subunits **28** and **29**, which could be desilylated and macrocyclized by removal of the 1,3-dioxin-4-one to furnish the salicylate ester under basic<sup>51</sup> or potentially photochemical conditions.<sup>52</sup> Although the configuration of lobatamide C at C8 and C11 was not determined,<sup>2</sup> we first focused on preparation of the 8*S* enantiomer of **30** in light of the stereochemistry of salicylilalamides<sup>1c</sup> and

**Figure 3.** First retrosynthetic analysis for lobatamide C.

Scheme 6



Scheme 7



oximides<sup>5</sup> (cf. Figure 1). The stereochemistry at C8 could easily be altered in the event that this prediction was found to be incorrect. Further disconnection of fragment **29** at the C18–C19 and C14–C15 bonds led to benzyl bromide **31**, Z-vinyl stannane **32**,<sup>53</sup> and nonracemic alkyne **33**. The divinylcarbinol moiety may be obtained by addition of a vinylzinc species (derived from **33** by hydrozirconation and transmetalation),<sup>54</sup> to an enal which will be prepared by Stille coupling<sup>55</sup> of **31** and **32**, desilylation, and alcohol oxidation. It was anticipated that stereocontrol at C15 could be established using chiral ligands in the vinylzinc addition step.<sup>56</sup> Enamide subunit **28** may be prepared by Cu(I)-catalyzed amidation of vinyl iodide **34** with amide **17** according to the C–N bond formation protocols previously described.

### Synthesis of C1–C10 Subunit (**28**)

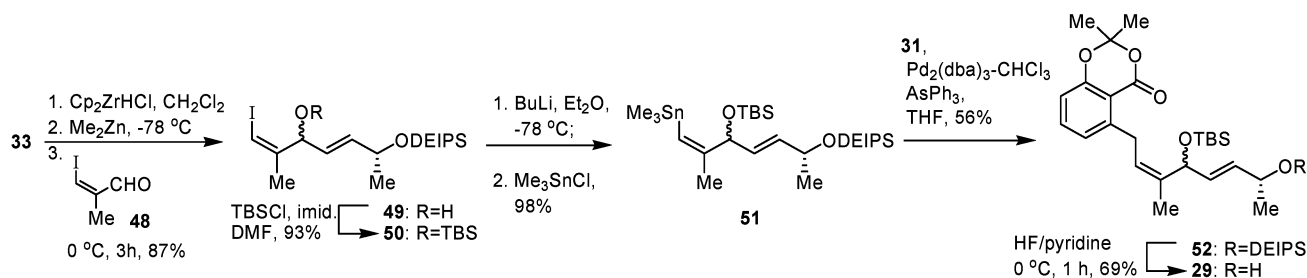
Preparation of a protected form of the C1–C10 enamide fragment **28** is shown in Scheme 6. Ethyl vinyl acetate was transformed in quantitative yield into (±)-ethyl-3,4-epoxybutanoate **35** using *m*-CPBA.<sup>57</sup> Hydrolytic kinetic resolution (HKR) developed by Jacobsen and co-workers<sup>58</sup> was used to

establish the C8 stereocenter and efficiently prepare multigram amounts of (*R*)-**35** (>99% ee). (*R*)-**35** was next employed in epoxide ring-opening reactions in order to prepare a vinyl iodide substrate for enamide synthesis. Addition of the acetylenic alane reagent<sup>59</sup> derived from trimethylsilylacetylene led to facile epoxide opening and afforded **36** in 80% yield. Silyl protection of **36** using chlorodiethylisopropylsilane (DEIPSCI), followed by treatment of the resulting silyl ether **37** with AgNO<sub>3</sub>/NBS/H<sub>2</sub>O,<sup>60</sup> afforded the bromoalkyne intermediate **38** that was converted to the (*E*)-stannylalkene **39** using Pattenden's method<sup>61</sup> (78%, three steps). Iodine exchange of **39** furnished vinyl iodide **34** in 73% yield with full retention of olefin stereochemistry. In contrast, iodination of the desilylated analogue of **39** afforded a 5:1 *E*:*Z* mixture of vinyl iodides, indicating the importance of protecting the secondary alcohol. After considerable experimentation, we found that Cu(I)-mediated vinylic substitution of **34** with the butenamide **17** (CuTC, 1,10-phenanthroline,<sup>32a</sup> dba, Cs<sub>2</sub>CO<sub>3</sub>, DMA) led to a 52% yield of the C1–C10 enamide fragment **40**. Critical to the success and reproducibility of this reaction were the use of a moderate reaction temperature (65

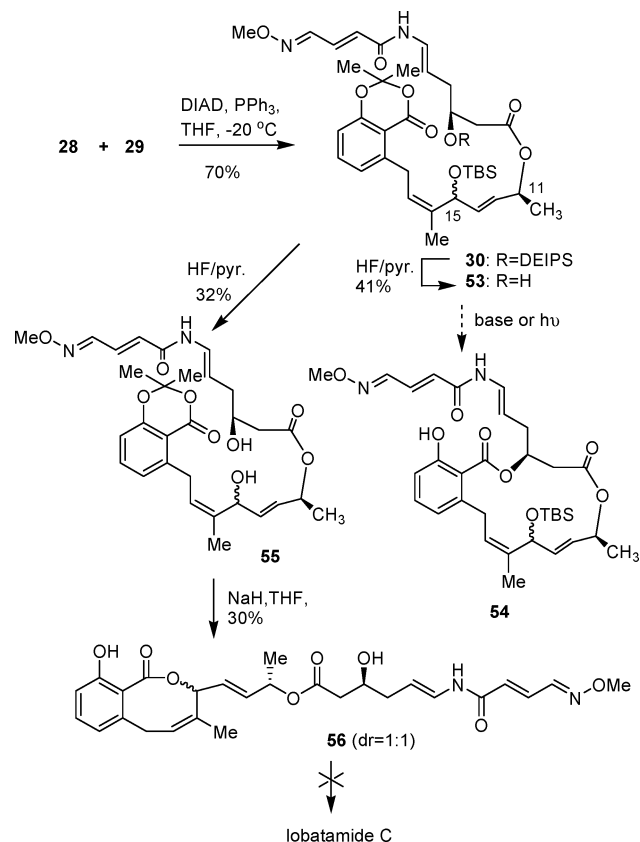
- (51) Bhattacharjee, A.; De Brabander, J. K. *Tetrahedron Lett.* **2000**, *41*, 8069.  
 (52) (a) Chapman, O. L.; McIntosh, C. L. *J. Am. Chem. Soc.* **1970**, *92*, 7001.  
 (b) Liu, R. C.-Y.; Luszyk, J.; McAllister, M. A.; Tidwell, T. T.; Wagner, B. D. *J. Am. Chem. Soc.* **1998**, *120*, 6247.  
 (53) Han, Q.; Wiemer, D. F. *J. Am. Chem. Soc.* **1992**, *114*, 7697.  
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 (55) Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1997**, *50*, 1. For a similar Stille coupling in synthesis of the salicylaldehydes, see ref 1h.  
 (56) For a review on catalytic asymmetric organozinc addition to carbonyls, see: Pu, L.; Yu, H.-B. *Chem. Rev.* **2001**, *101*, 757.  
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 (59) (a) Fried, J.; Sih, J. C.; Lin, C. H.; Dalven, P. *J. Am. Chem. Soc.* **1972**, *94*, 4343. (b) Larcheveque, M.; Petit, Y. *Bull. Chim. Soc. Fr.* **1989**, 130.  
 (60) Tobe, Y.; Nakanishi, H.; Sonoda, M.; Wakabayashi, T.; Achiba, Y. *J. Chem. Soc., Chem. Commun.* **1999**, *17*, 1625. Optimization studies indicate that inclusion of water (2.0 equiv) is important for high yield and reproducibility for the bromination reaction.  
 (61) (a) Boden, C. D. J.; Pattenden, G.; Ye, T. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2417. (b) Claus, E.; Kalesse, M. *Tetrahedron Lett.* **1999**, *40*, 4157.

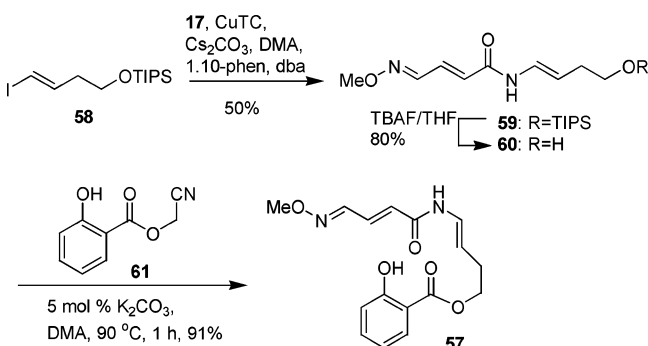
Scheme 8



Scheme 9



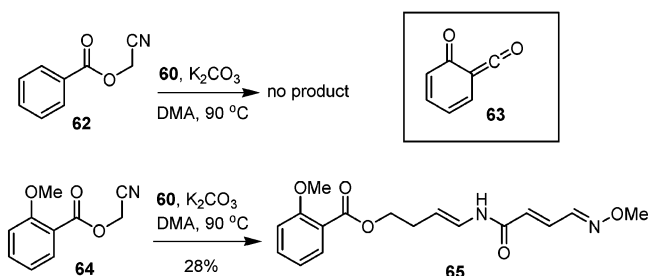
Scheme 10



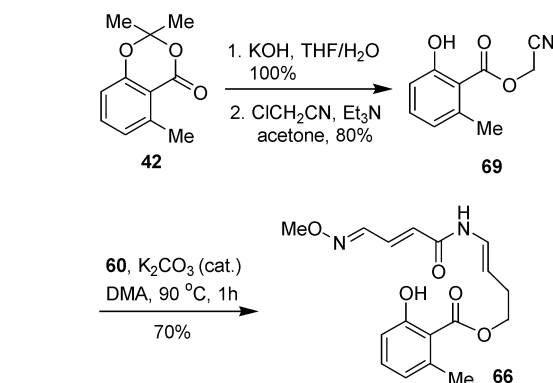
°C) to suppress elimination of the sensitive  $\beta$ -silyloxy ester coupling substrate, the use of 1,10-phenanthroline as supporting ligand for CuTC, and use of high purity cesium carbonate.<sup>62</sup> Synthesis of the target C1–C10 fragment was completed by hydrolysis of **40** to afford the labile enamide acid **28** (73%).

(62) Yin, J.; Buchwald, S. L. *Org. Lett.* **2000**, 2, 1101.

Scheme 11



Scheme 12



### Synthesis of the C11–26 Fragment (29)

Synthesis of the C11–C26 salicylate fragment **29** commenced with preparation of salicylate benzyl bromide **31**, prepared in two steps from the known aryl triflate **41**<sup>63</sup> (Scheme 7). Treatment of **41** with lithium dimethylcuprate<sup>64</sup> and MeI<sup>65</sup> led to the production of 6-methylsalicylate derivative **42** that was brominated<sup>66</sup> to afford benzylic bromide **31** (78%). Coupling of **31** with vinylstannane **32**<sup>53</sup> using the conditions reported by Kamlage<sup>67</sup> ( $\text{Pd}_2\text{dba}_3\text{--CHCl}_3$ ,  $\text{AsPh}_3$ , THF) afforded Z-allylic silyl ether **43** (79%). Silyl deprotection using HF/pyridine provided allylic alcohol **44**, which was oxidized to Z-enal **45** using Dess–Martin periodinane.<sup>68</sup> However, **45** was found to be readily isomerized to E-enal **46** during purification on silica gel (**46**:**45** = 6:1). Hence, crude **45** was used in subsequent coupling reactions. Hydrozirconation of DEIPS-protected (R)-3-buten-2-ol (**33**), followed by transmetalation with  $\text{Et}_2\text{Zn}$  and addition to **45** according to the general procedure of Wipf and

(63) Fürstner, A.; Konetzki, I. *Tetrahedron* **1996**, 52, 15071.

(64) McMurry, J. E.; Mohanraj, S. *Tetrahedron Lett.* **1983**, 24, 2723.

(65) Quenching the reaction with MeI was found to significantly improve the reaction yield, likely due to methylation of an aryl copper side product. Cf. Cohen, T.; Wood, J.; Dietz, A. G., Jr. *Tetrahedron Lett.* **1974**, 15, 3555.

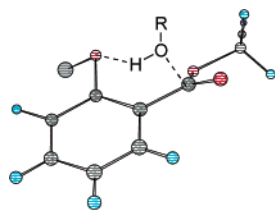
(66) Eicher, T.; Tiefensee, K.; Pick, R. *Synthesis* **1988**, 525.

(67) (a) Kamlage, S.; Sefkow, M.; Peter, M. G. *J. Org. Chem.* **1999**, 64, 2938.

(b) Farina, V.; Krishnan, B. *J. Am. Chem. Soc.* **1991**, 113, 9585.

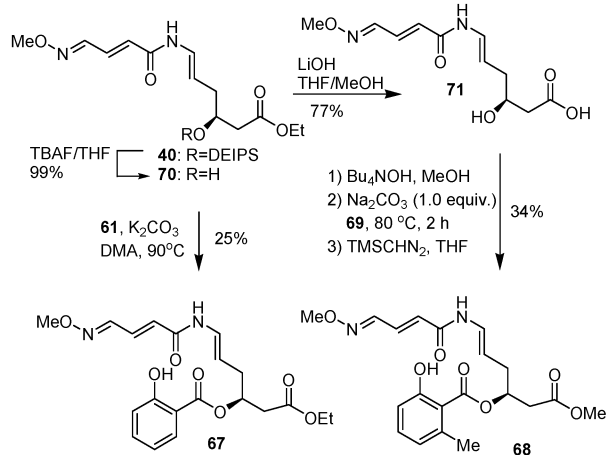
(68) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, 48, 4155.





**Figure 4.** Proposed transesterification using intramolecular general base catalysis.

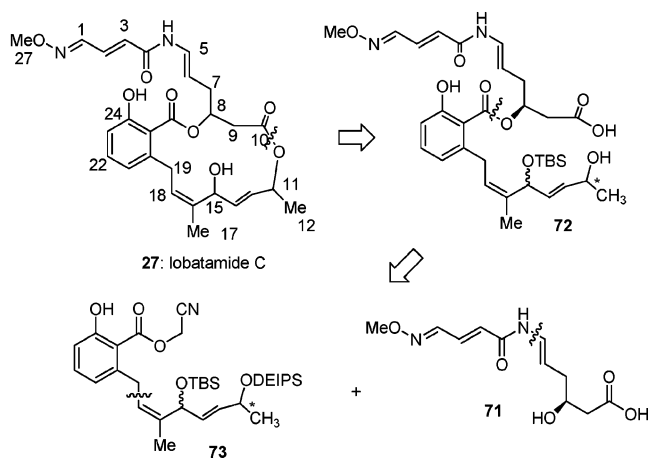
### Scheme 13



co-workers,<sup>54</sup> led to the target divinylcarbinol fragment **47** as a 1:1 mixture of diastereomers (30% yield). Although the *Z*-olefin stereochemistry of **47** was verified using NOE experiments, the presence of significant amounts of isomerized *E*-enal **46** led to rapid alteration of the synthetic sequence and use of configurationally stable *Z*-enal **48**<sup>69</sup> (Scheme 8). In the event, hydrozirconation, zirconocene–zinc transmetalation and addition to enal **48** afforded divinylcarbinol **49** as a 1:1 nonseparable mixture of diastereomers (87%). Compound **49** was further advanced by silylation of the secondary alcohol, followed by lithiation–trimethylstannylation, to afford vinyl stannane **51** which was coupled with benzyl bromide **31** to furnish C11–C26 fragment **52** (56%). Selective deprotection of **52** using HF/pyridine at 0 °C afforded the target alcohol **29** (69%).

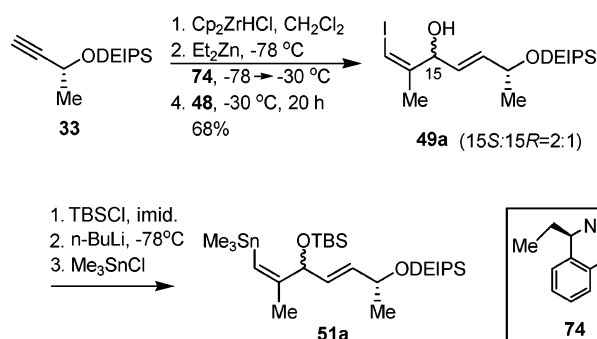
### Fragment Coupling and Attempted Macrocyclization

With fragments **28** and **29** in hand, we attempted their coupling using standard acylation methods. However, both Yamaguchi<sup>70</sup> and Keck<sup>71</sup> esterification conditions did not furnish the desired ester C11-epi-**30**, presumably due to decomposition of the sensitive enamide side chain. However, the esterification was successfully accomplished using Mitsunobu conditions to afford **30** (70%, Scheme 9). Selective deprotection of the C8 DEIPS ether of **30** using HF/pyridine afforded acyclic alcohol **53** (41%). Extensive studies were performed to prepare macrolactone **54** by intramolecular transesterification of **53**. Treatment of **53** under basic conditions (NaH<sup>51</sup> or NaHMDS, THF) or distannoxane transesterification catalysts<sup>72</sup> did not furnish the desired cyclized product **54**. Under these conditions,

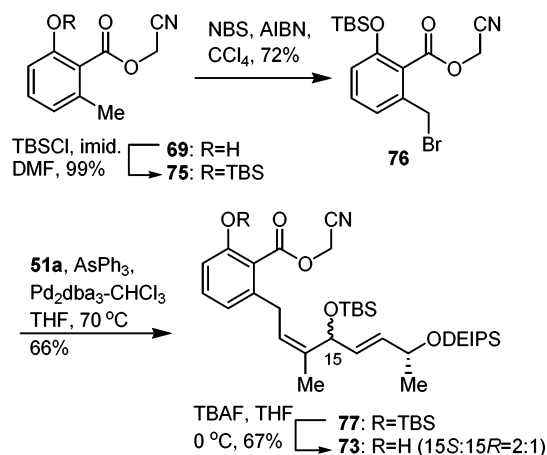


**Figure 5.** Revised retrosynthesis of lobatamide C

### Scheme 14



### Scheme 15



compound **53** was substantially decomposed. Attempted photolysis of **53** (Ace-Hanovia UV lamp 450 W, Pyrex filter, 20 min) in an effort to form the macrolactone by trapping of a keto–ketene intermediate<sup>52</sup> led to complete decomposition of the substrate. Due to a concern that the C15 TBS ether may block macrocyclization, we next prepared compound **55** by removing the TBS ether with HF/pyridine. However, treatment of **55** under basic conditions (NaH, THF) led to the production of the eight-membered lactone enamide **56** (dr 1:1) instead of the desired natural product. Compound **56** is formally an isomer of lobatamide C in which the divinylcarbinol forms a salicylate ester, leaving a pendant C8 hydroxyl. Attempts to rearrange **56** to 15-membered ring lobatamide C (**27**) by transactonization

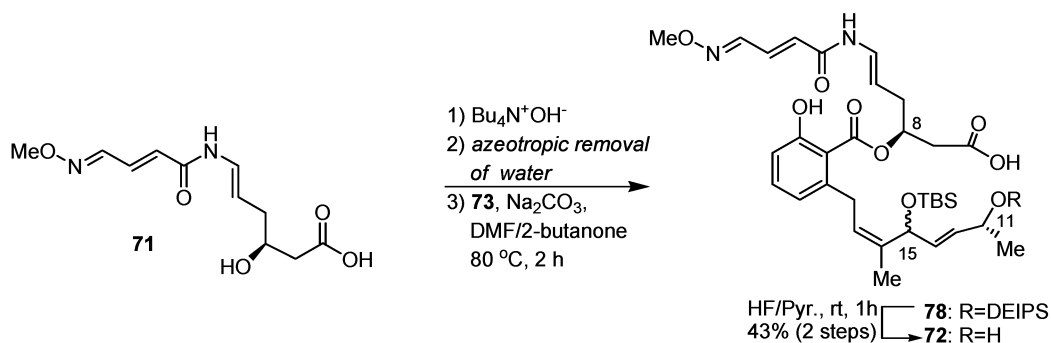
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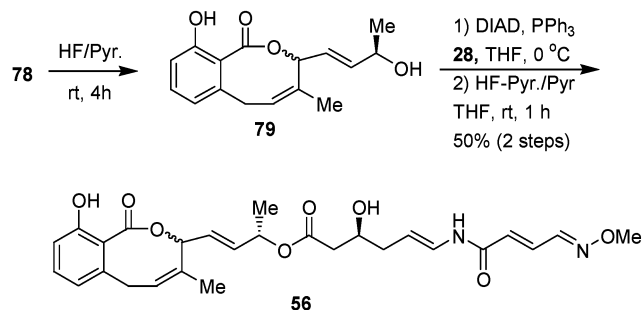
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Scheme 16



Scheme 17



protocols<sup>73</sup> (e.g., catalytic PPTS/ $\text{CH}_2\text{Cl}_2$  or  $\text{Et}_2\text{Zn}/\text{CH}_2\text{Cl}_2$ ) were not successful.

### Development of Methodology for the Construction of Salicylate Esters

In parallel with our total synthesis efforts, we initiated studies toward the synthesis and biological evaluation of simplified analogues of the lobatamides in order to clarify the minimal core structure (pharmacophore) required for V-ATPase inhibition. In initial studies, a series of acyclic analogues were prepared using the C1–C10 enamide fragment. Our first target was simplified salicylate enamide **57** that eliminates the C8 stereogenic center (Scheme 10). TIPS-protected vinyl iodide **58**<sup>74</sup> underwent CuTC-mediated cross-coupling with (2*E*)-butenamide **17** to furnish enamide **59**, which was desilylated using TBAF to afford enamide alcohol **60**. Encouraged by patent literature for esterification of enamides to basic conditions, we first treated enamide alcohol **60** with cyanomethyl ester **61**<sup>75</sup> in the presence of a catalytic amount of  $\text{K}_2\text{CO}_3$  (DMA, 90 °C). Salicylate enamide **57** was rapidly and cleanly produced under these conditions (91%).

As a control experiment, we found that the acylation using benzoyloxyacetonitrile **62** with alcohol **60** under similar reaction conditions did not afford the desired ester product (Scheme 11). We initially suspected that keto–ketene **63** was the active acylating agent.<sup>76</sup> However, trapping experiments with *N,N*-

dimethylcyanamide, ethyl vinyl ether, ethoxyacetylene, and *N*-benzylmaleimide failed to provide the corresponding Diels–Alder adducts.<sup>77</sup> Interestingly, treatment of **60** and methoxy cyanomethyl ester **64** under the same conditions afforded the ester product **65**, albeit in lower chemical yield (28%) and limited conversion (Scheme 11). These results, in conjunction with conformational analysis of the sodium salt of **61**,<sup>78</sup> suggested an alternative mechanistic pathway involving intramolecular general base catalysis. In the conformer shown in Figure 4, the ester carbonyl is out of planarity, which is expected to increase the reactivity of the carbonyl toward transesterification. In addition, the *o*-hydroxyl may be oriented to act as a general base catalyst and direct attack of the alcohol to the  $\pi^*$  of the salicylate carbonyl. This proposed transesterification mechanism is substantiated by literature precedent.<sup>79</sup>

To ultimately apply this transesterification methodology to the construction of the salicylate portion of lobatamide C, we next prepared analogues **66**–**68**. In particular, compounds **66** and **68** contain *o*-salicylate alkyl substituents. Hydrolysis of benzodioxinone **42** with KOH quantitatively provided 6-methylsalicylic acid<sup>80</sup> (Scheme 12), which was converted to cyanomethyl ester **69** (80%).<sup>81</sup> Acylation of enamide alcohol **60** with **69** in the presence of  $\text{K}_2\text{CO}_3$  in DMA afforded analogue **66** (70%). Analogue **67** was prepared from secondary alcohol **70** (prepared by TBAF desilylation of **40**), which exhibited significantly lower reactivity in acylation with cyanomethyl ester **61** (25% yield, Scheme 13). In addition, 15% of the  $\beta$ -elimination product, as well as considerable amounts of recovered **70**, were obtained. Under similar conditions, acylation of **70** with cyanomethyl ester **69** did not afford the desired salicylate product. However, the derived acid **71**, after neutralization with  $\text{Bu}_4\text{NOH}$  and heating with **69** and  $\text{Na}_2\text{CO}_3$  (1.0 equiv) in DMA/2-butanone (80 °C, 2 h), furnished the desired salicylate ester. The tetrabutylammonium salt of **71** both increases the solubility of the enamide alcohol fragment<sup>82</sup> and likely blocks  $\alpha$ -deprotonation/elimination of the  $\beta$ -salicyloxyester product. A number

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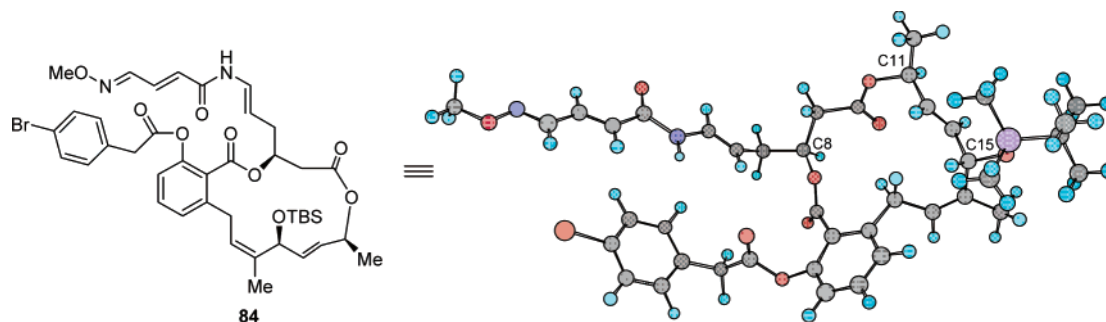


Figure 6. X-ray Crystal Structure of **84**.

0.17, MeOH), TLC  $R_f$  values in three solvent systems, and COMPARE profiles in NCI's 60 tumor cell line.<sup>85</sup>

### Stereochemical Assignment of Lobatamide C (**27a**)

The absolute configuration of **27a** at C15 was determined to be *S* using a modified Mosher's ester analysis according to Suzumura's procedure.<sup>86</sup> In addition, 11*R* diastereomers of lobatamide C (**27c** and **27d**) were prepared independently from (*S*)-3-butyn-2-ol (Scheme 19). TIPS-protected (*S*)-3-butyn-2-ol **80** was converted to divinylcarbinol **81** (dr = 1:1) using hydrosilylation–transmetalation–enal addition. Fortunately, diol **82** (dr = 1:1, obtained by TBAF desilylation of **81**) was resolved by recrystallization in  $\text{CHCl}_3$ . Using this method, diastereomers **82a** and **82b** were obtained in high diastereomeric purity (>95% de).<sup>87</sup> Selective protection of diols **82a** and **82b** with DEIPSCI furnished divinylcarbinols **83a** and **83b**. Lobatamide C diastereomers **27c** and **27d** were synthesized from **83a** and **83b**, respectively, employing the same route. Both HPLC and NMR studies indicated that **27c** and **27d** did not match natural lobatamide C.

Although numerous allylic alcohols have been reported to undergo inversion in Mitsunobu reactions,<sup>88</sup> recent work has documented examples of retention of stereochemistry in Mitsunobu esterifications.<sup>89</sup> To fully confirm the stereochemical assignment of lobatamide C, compound **54a** was derivatized to afford bromophenyl acetate **84** (Figure 6). Single X-ray crystal structure of **84** indicates that the stereochemistry at C11 is *S*, clearly showing that the C11 stereogenic center was inverted in the Mitsunobu macrocyclization. The crystal structure of **84** thus fully confirms the stereochemistry of lobatamide C as 8*S*, 11*S* and 15*S* without ambiguity.

### Synthesis and Biological Evaluation of Simplified Lobatamide Analogues

Although several simplified analogues (**57**, **65**–**68**) were prepared during the fragment coupling studies, we synthesized an additional lobatamide derivative **85** bearing an *N*-methyl en-

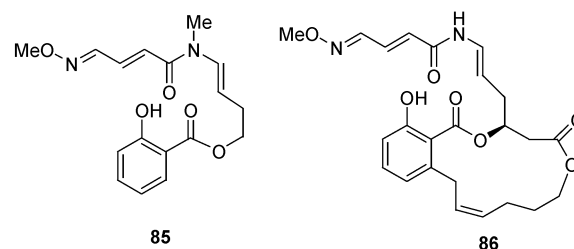


Figure 7.

amide and a macrocyclic analogue **86**, which replaces the fragile divinylcarbinol moiety of lobatamide C with a *Z*-olefin and a simplified three-carbon segment (Figure 7).<sup>90</sup> The preparation of **86** is shown in Scheme 20. Stille coupling of vinyl stannane **87**<sup>91</sup> and benzylic bromide **76** afforded salicylate **88**. Selective desilylation (TBAF) provided cyanomethyl ester **89**, which was coupled with enamide acid **71** to furnish the salicylate **90**. Desilylation (HF–pyridine/pyridine) provided hydroxy acid **91** which underwent Mitsunobu macrolactonization (0.005 M) to afford 14-membered macrolactone analogue **86** (65%).

Synthetic lobatamide C (**27a**), stereochemical isomers (**27b**–**d**), and lobatamide analogues were evaluated for activity against bovine V-ATPase from chromaffin granule membranes. As shown in Table 3, both lobatamide C and its C15 epimer **27b** showed potent inhibition (2.1 and 3.6 nM), while the 11*R* isomers of lobatamide C (**27c,d**) showed lower activity (20 and 21 nM). The eight-membered ring lobatamide C isomer **56** had significantly reduced activity. For simplified lobatamide analogues, compounds **57** were found to inhibit bovine V-ATPase with modest activity. Methylated analogues **65** and **85** had significantly reduced activities against V-ATPase, which indicates the importance of a free phenol and enamide NH. Macrolactone **86** showed good V-ATPase inhibition (1.4  $\mu\text{M}$ ), but not at the nanomolar potency of the lobatamides, which indicates that the ring size and substitution of the macrolactone are critical for potent V-ATPase inhibition. Interestingly, permutation of the *ortho* hydrogen of analogue **57** to a methyl group substantially increased the activity (**66**, 1.6  $\mu\text{M}$ ). 6-Methyl salicylate compound **68** also showed potent inhibition of V-ATPase (0.1  $\mu\text{M}$ ), approximately 180 times more active than compound **67** without a methyl group *ortho* to the carbonyl. Conformational analysis<sup>78</sup> of **67** and **68** (Figure 8) indicates that conformers of *ortho*-H substituted salicylate **67** maintain near planarity of the carbonyl with the aromatic ring. In contrast, in analogue **68** the *o*-methyl substituent forces the carbonyl out-of-plane by approximately 60°, presumably because of steric

(85) Synthetic **27a** and **27b** were evaluated against NCI's 60 tumor cell line. Mean panel  $\text{GI}_{50}$ 's: 3.7 nM (natural lobatamide C), 5.1 nM (**27a**), and 227 nM (**27b**);  $\text{GI}_{50}$  (COMPARE correlation): 1.00 (natural lobatamide C), 0.83 (**27a**), 0.80 (**27b**).

(86) See Supporting Information. We thank Dr. K. Suzumura (Yamanouchi Pharmaceutical Co.) for providing experimental details on the Mosher ester analysis of YM75518A.

(87) The absolute configurations of diols **82a** and **82b** were assigned by HPLC analysis and comparison of optical rotations of diols obtained from compound **49**. See Supporting Information.

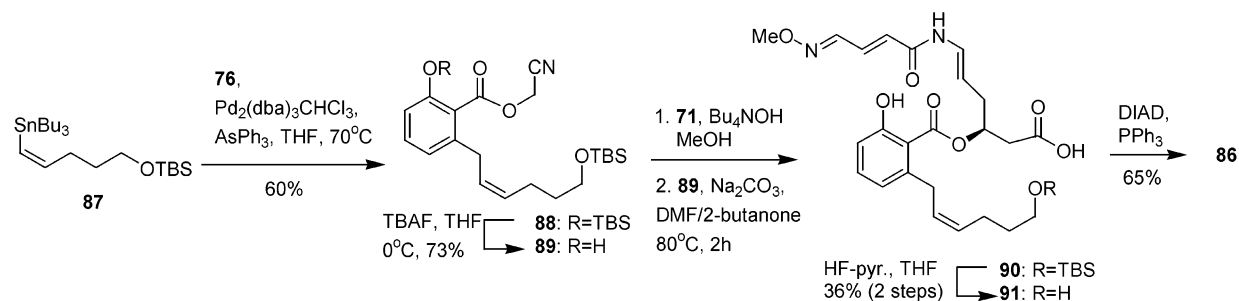
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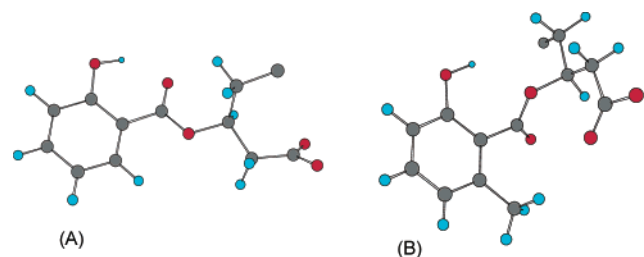
Scheme 20

**Table 3.** Effect of Simplified Analogues of the Lobatamides against Bovine V-ATPase<sup>a</sup>

compd	IC <sub>50</sub> (μM)	compd	IC <sub>50</sub> (μM)
<b>27a</b>	0.0021	<b>66</b>	1.6
<b>27b</b>	0.0036	<b>67</b>	18
<b>27c</b>	0.020	<b>68</b>	0.10
<b>27d</b>	0.021	<b>85</b>	>200
<b>56</b>	<i>b</i>	<b>86</b>	1.4
<b>57</b>	<i>c</i>	<b>92</b>	0.21
<b>65</b>	no effect	<b>93</b>	0.06

<sup>a</sup> ATPase activity determined as described in the Supporting Information using 20 μg of membrane protein and the indicated amount of inhibitor.

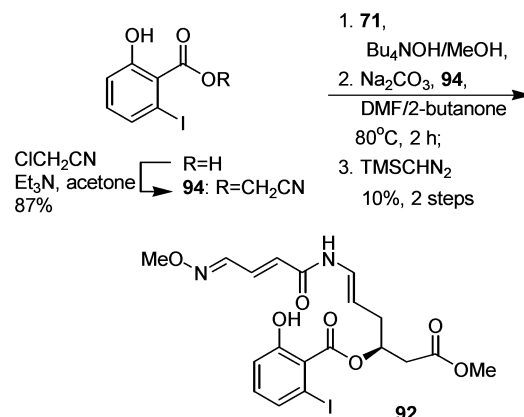
<sup>b</sup> 27% inhibition at 30 μM, higher concentrations not soluble. <sup>c</sup> 25% inhibition at 20 μM, higher concentrations not soluble.

**Figure 8.** Representative minimum energy conformations of **67** (A) and **68** (B) (Chem 3D, enamide side chain omitted for clarity).

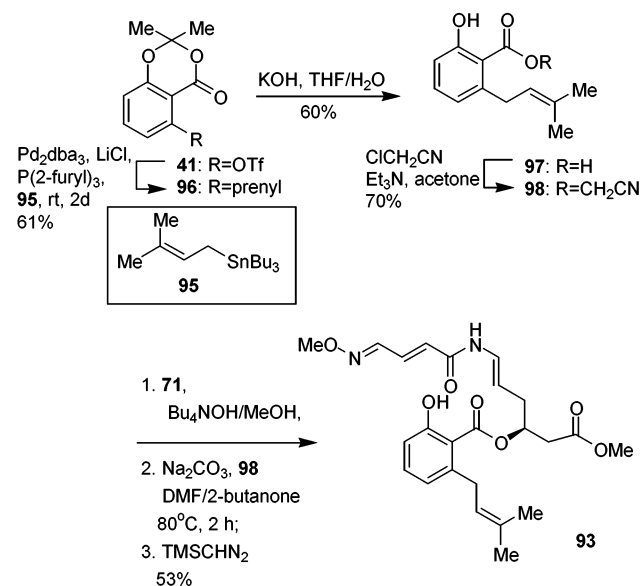
hindrance effects.<sup>92</sup> This steric effect is also seen in the X-ray crystal structure of apicularen A<sup>3b</sup> and compound **84** (salicylate ester carbonyl out of planarity by 80° and 52°, respectively) and may be an important conformation of the salicylate moiety in potent V-ATPase inhibitors.

On the basis of this observation, compound **92** and **93** with sterically demanding substituents (iodine and prenyl) *ortho* to the carbonyl were prepared in order to potentially increase V-ATPase inhibition potency. Na<sub>2</sub>CO<sub>3</sub>-mediated coupling of enamide acid **71** and iodo cyanomethyl ester **94** (prepared from 6-iodosalicylic acid<sup>93</sup>) afforded analogue **92** after methylation (Scheme 21). Preparation of prenylated analogue **93** commenced with triflate **41** (Scheme 22). Stille coupling of **41** and prenyl stannane **95** afforded acetonide **96**, which was hydrolyzed and reprotected as cyanomethyl ester **98**. Analogue **93** was obtained using similar transesterification conditions. Evaluation of **92** and **93** against bovine V-ATPase showed that **92** has lower inhibition activity (210 nM) than analogue **68**, while **93** showed slightly higher activity (60 nM). These results indicate that it is possible to produce relatively potent, acyclic salicylate enamide

Scheme 21



Scheme 22



V-ATPase inhibitors. However, in addition to steric hindrance effects, specific orientation of the functional groups by the macrolactone scaffold may be necessary to achieve low nanomolar inhibition against mammalian V-ATPases.

## Conclusion

We have achieved a highly convergent synthesis of the potent antitumor and V-ATPase inhibitory natural product lobatamide C. For construction of the enamide side chain of lobatamides and related natural products, we have developed a mild and stereoselective Cu(I)-catalyzed vinylic substitution protocol which complements related C–N bond formation methodolo-

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gies.<sup>32</sup> A key step in the total synthesis involves base-mediated esterification of hydroxy enamide and salicylate cyanomethyl fragments. Macrocyclization was achieved using an intramolecular Mitsunobu reaction. The stereochemistry of lobatamide C was assigned as 8*S*,11*S*,15*S* by comparison to that of synthesized diastereoisomers. Preparation and X-ray crystallographic analysis of a lobatamide C derivative fully confirmed the stereochemical assignment. A number of simplified lobatamide analogues have been prepared in an effort to probe structure–activity relationships. Lobatamide C isomers and simplified analogues were evaluated against bovine V-ATPase from chromaffin granule membranes, which showed that the salicylate phenol, enamide NH, and *ortho*-substitution of the salicylate ester are important for V-ATPase inhibition. In addition, a number of nanomolar acyclic salicylate enamides inhibitors of bovine V-ATPase were uncovered in this study. Further studies on the application of Cu(I)-catalyzed vinylic substitution and preparation of other lobatamide derivatives are in progress and will be reported in due course.

**Acknowledgment.** We thank Mr. Scott Magee for initial experiments on the lobatamides, Dr. Michael R. Boyd for providing authentic lobatamide C, Prof. J. Snyder (Boston University) for helpful comments, Dr. Emil Lobkovsky (Cornell University) for X-ray crystallographic analysis, Waters, Inc. for providing HPLC equipment, Rhodia Chirex for providing salen catalysts, and Chemetall GmbH (Boston, MA) for providing Cs<sub>2</sub>CO<sub>3</sub>. Financial support for this study was provided by NIH (GM62842, J.A.P., Jr., and GM28703, GM58903, B.J.B.). C.-T.L. was supported in part by the Boston University Undergraduate Research Opportunities Program and Pfizer Summer Undergraduate Research Fellowship.

**Supporting Information Available:** Experimental procedures and characterization data for all new compounds, including X-ray crystal structure coordinates for **84**. X-ray crystallographic file in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>

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