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Synthesis of Photoactivatable Acyclic Analogues of the Lobatamides

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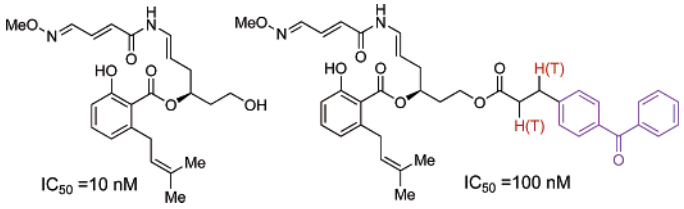

The lobatamides and related salicylate enamide natural products are potent mammalian V-ATPase inhibitors. To probe details of binding of the lobatamides to mammalian V-ATPase, three photoactivatable analogues bearing benzophenone photoaffinity labels have been prepared. The analogues were designed on the basis of a simplified acyclic analogue **2**. Late-stage installation of the enamide side chain and tandem deallylation/amidation were employed in synthetic routes to these derivatives. Simplified analogue **2** showed strong inhibition against bovine clathrin-coated vesicle V-ATPase (10 nM). Analogues **3**−**5** were also evaluated for inhibition of bovine V-ATPase in order to select a suitable candidate for future photoaffinity labeling studies.

Introduction

The salicylate enamide natural products, including the lobatamides[1] and salicylihalamides,[2] are potent antitumor compounds and mammalian vacuolar type proton ATPase (V-ATPase) inhibitors.[3] V-ATPases are essential proton-translocating pumps of eukaryotic cells and play important roles in many processes including receptor-mediated endocytosis, acid secretion, bone degradation, and control of cytoplasmic pH.[4] The V-ATPases are composed of two distinct domains: the cytoplasmic V_1 domain responsible for binding and hydrolysis of ATP and a membrane-embedded V_0 domain responsible for proton translocation across the membrane. For mammalian V-ATPase, the V_0 domain contains subunits *a*, *c*, *c*″, *d*, and Ac45.[5] Unlike other potent V-ATPase inhibitors such as the bafilomycins and concanamycins which inhibit both yeast and mammalian V-ATPases and have been shown to bind to subunit *c* of the V_0 domain,[6,7] the salicylate enamide natural products selectively inhibit mammalian V-ATPases.[3] Although extensive synthetic studies have been performed on these natural products,[8] studies regarding determination of the binding site of these compounds on V-ATPase are limited at this stage. A recent publication by Xie and co-workers disclosed that the salicylate enamide natural product salicylihalamide A binds to the V_0 domain of bovine V-ATPase.[9] However, the particular subunit of the V_0 domain for binding remains to be determined. In addition, recent studies by Huss et al.[6] indicated that salicylihalamide A does not compete with concanamycin A for binding to subunit *c* of the V_0 domain.

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(1) McKee, T. C.; Galinis, D. L.; Pannell, L. K.; Cardellina, J. H., II; Laasko, J.; Ireland, C. M.; Murray, L.; Capon, R. J.; Boyd, M. R. *J. Org. Chem.* **1998**, *63*, 7805.

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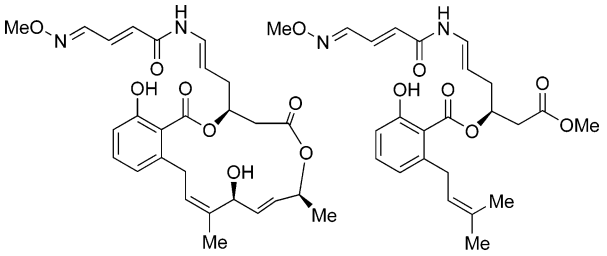
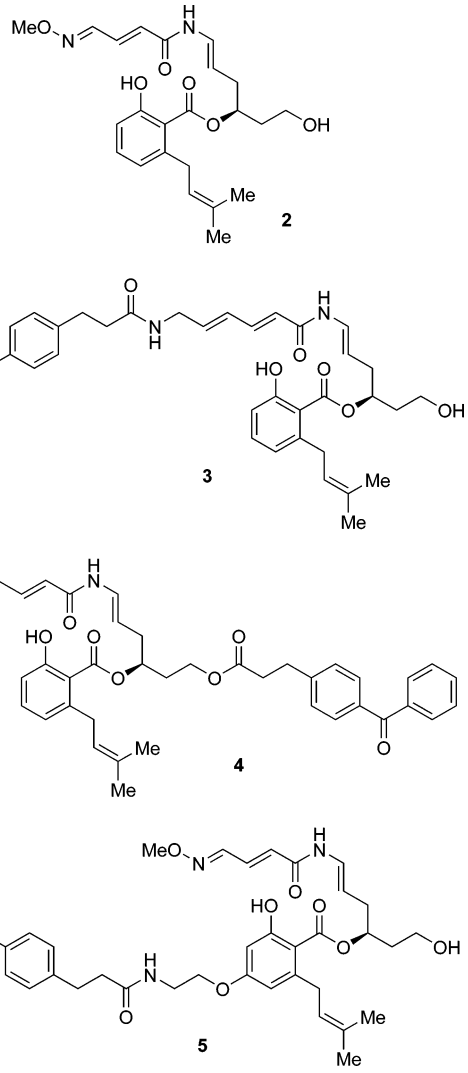


Since photoaffinity reagents derived from natural products and drugs have become powerful tools in exploring the binding sites of target proteins,[10] we have initiated studies to prepare photoactivatable analogues of the lobatamides in order to identify their binding subunits on V-ATPase. In our previous synthesis of lobatamide C and simplified analogues,[11] acyclic analogue **1** was uncovered as a potent inhibitor against bovine chromaffin granule membrane V-ATPase ($IC_{50} = 60$ nM). We thus selected three photoactivatable analogues **3**−**5** as synthetic targets. These three probe reagents were designed on the basis of simplified analogue **2** in which the base-sensitive β-hydroxy ester moiety of analogue **1** was altered to a 2-hydroxyethyl unit. This modification provided an opportunity to install the enamide side chain at a late stage using our methodology for Cu(I)-catalyzed amidation of vinyl iodides.[12] In addition, the primary alcohol could also be utilized as a site of attachment for photoactive groups. The benzophenone moiety was initially employed as a photoactive group due to its easy availability, mild conditions required for photoactivation (350−360 nm), and propensity for selective C−H abstraction.[13] To obtain information on the influence of the site of probe attachment with regard to the V-ATPase inhibition of these analogues, the benzophenone moiety was positioned in different locations of analogue **2**, i.e., the enamide side chain, primary alcohol, and the salicylate ring.[14] After evaluation of nonradiolabeled probes for mammalian V-ATPase inhibition, radiolabeled versions incorporating tritium may be prepared for use in photoaffinity studies.[15] Herein, we report the synthesis of lobatamide probe derivatives **2**−**5** and preliminary evaluation of these compounds as bovine V-ATPase inhibitors.



Results and Discussion

Retrosynthesis of analogue **3** (Figure 1) led to *N*-allyloxycarbonyl (alloc)-protected salicylate enamide **6** and *N*-succinimidyl *p*-benzoyldihydrocinnamate **7**, the latter developed by Prestwich and co-workers.[15a] The *N*-alloc group was chosen due to its stability to basic conditions, and the mild deprotection conditions[16] compatible with the acid-sensitive enamide fragment.[17] Cleavage of the enamide bond of protected derivative **6** affords amide **8** and vinyl iodide **9**, the latter a substrate for CuTC-catalyzed amidation.[11c,12] In contrast to our studies toward the total synthesis of lobatamide C[11a,c] in which the enamide moiety was installed at relatively early stage, construction of the salicylate bond before the enamide synthesis would provide divergent access to analogues **2**−**4** from vinyl iodide **9**. Further disconnection

(10) (a) Fleming, S. A. *Tetrahedron*, **1995**, *46*, 12479. (b) Dormán, G. *Top. Curr. Chem.* **2000**, *211*, 169. (c) Hatanaka, Y.; Sadakane, Y. *Curr. Top. Med. Chem.* **2002**, *2*, 271.

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(12) Shen, R.; Porco, J. A., Jr. *Org. Lett.* **2000**, *2*, 1333. For additional reports concerning Cu-catalyzed synthesis of enamides, see: (b) Lam, P. Y. S.; Vincent, G.; Bonne, D.; Clark, C. G. *Tetrahedron Lett.* **2003**, *44*, 4927. (c) Jiang, L.; Job, G. E.; Klapars, A.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 3667. (d) Pan, X.; Cai, Q.; Ma, D. *Org. Lett.* **2004**, *6*, 1809.

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(15) (a) Olszewski, J. D.; Dorman, G.; Elliott, J. T.; Hong, Y.; Ahern, D. G.; Prestwich, G. D. *Bioconjugate Chem.* **1995**, *6*, 395. For photoaffinity studies employing a tritium-labeled benzophenone moiety, see: (b) Ojima, I.; Duclos, O.; Dormán, G.; Simonot, B.; Prestwich, G. D. *J. Med. Chem.* **1995**, *38*, 8, 3891. (c) Andrus, M. B.; Turner, T. M.; Sauna, Z. E.; Ambudkar, S. V. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2275.

(16) Guibe, F. *Tetrahedron* **1998**, *54*, 2967.

(17) Csizmadia, V. M.; Koshy, K. M.; Lau, K. C. M.; McClelland, R. A.; Nowlan, V. J.; Tidwell, T. T. *J. Am. Chem. Soc.* **1979**, *101*, 974.

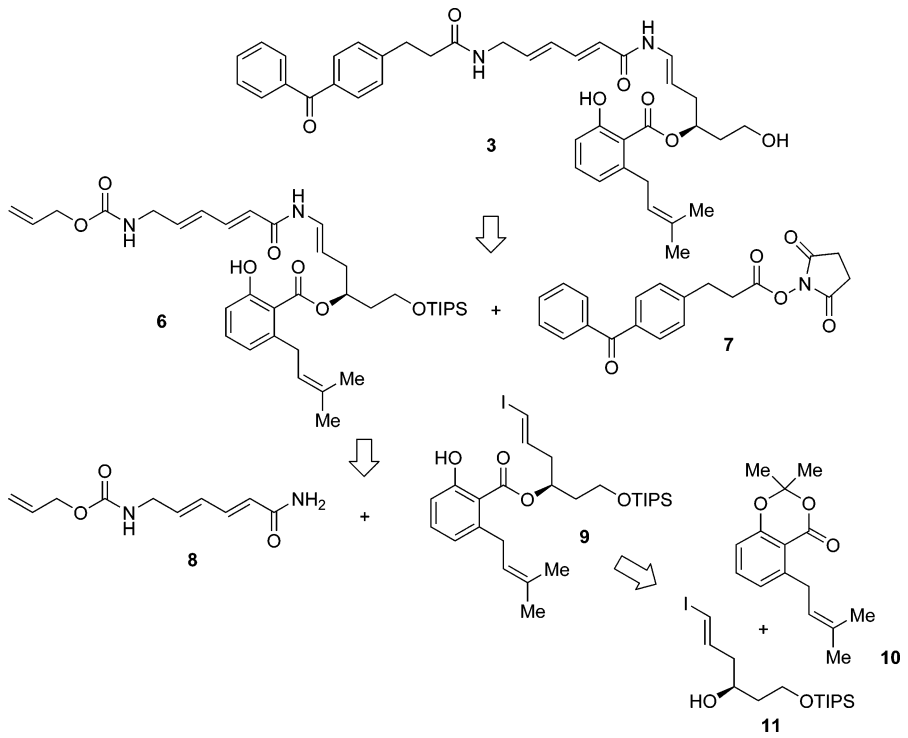
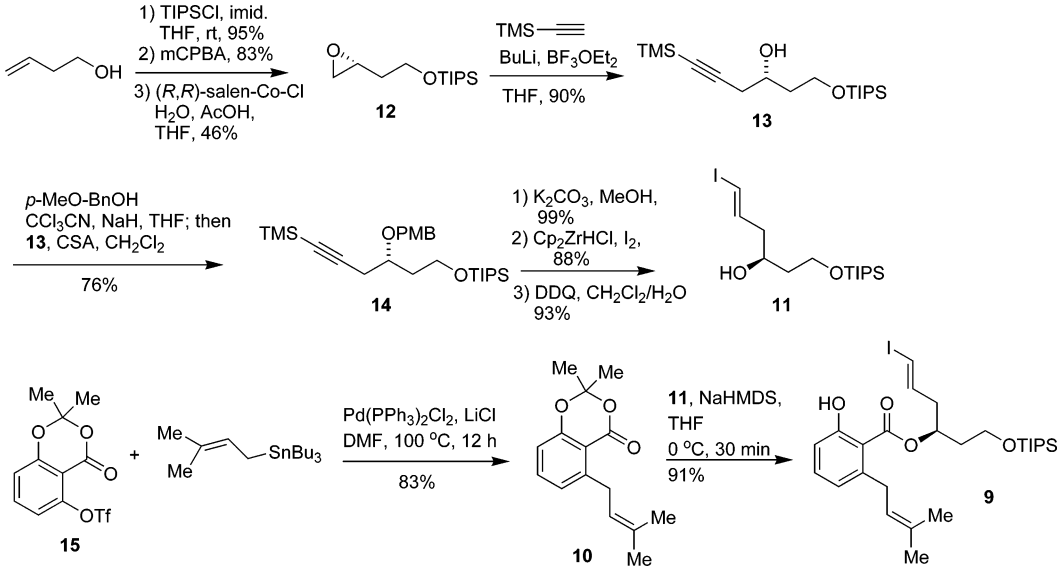


FIGURE 1. Retrosynthetic analysis of photoactivatable analogue **3**.

SCHEME 1. Synthesis of Vinyl Iodide 9



of **9** employs base-mediated ring-opening[18] of benzo[1,3]-dioxin-4-one **10** with alcohol **11**.

Synthesis of Analogue 3. Synthesis of alcohol **11** commenced with chiral epoxide **12**,[19] which was prepared in good yield and high enantiopurity (>99% ee) from 3-buten-1-ol by silyl protection, epoxidation, and hydrolytic kinetic resolution[20] (Scheme 1). $BF_3{\cdot}Et_2O$-mediated epoxide opening with the lithium acetylide derived from trimethylsilylacetylene and subsequent protection of the secondary alcohol **13** with *p*-methoxybenzyl trichloroacetimidate using camphorsulfonic acid as catalyst[21] provided compound **14**, which was further converted to vinyl iodide **11** by desilylation, hydrozirconation/iodination, and deprotection of the *p*-methoxybenzyl ether. Benzo[1,3]dioxin-4-one **10** was obtained by Stille coupling of aryltriflate **15**[22] and tributylprenylstannane using

(18) (a) Bhattacharjee, A.; De Brabander, J. K. *Tetrahedron Lett.* **2000**, *41*, 8069. (b) Wang, X.; Porco, J. A., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 6040.

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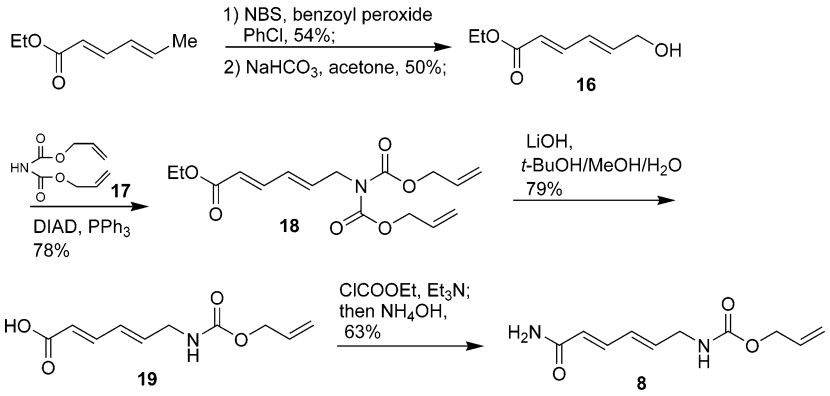
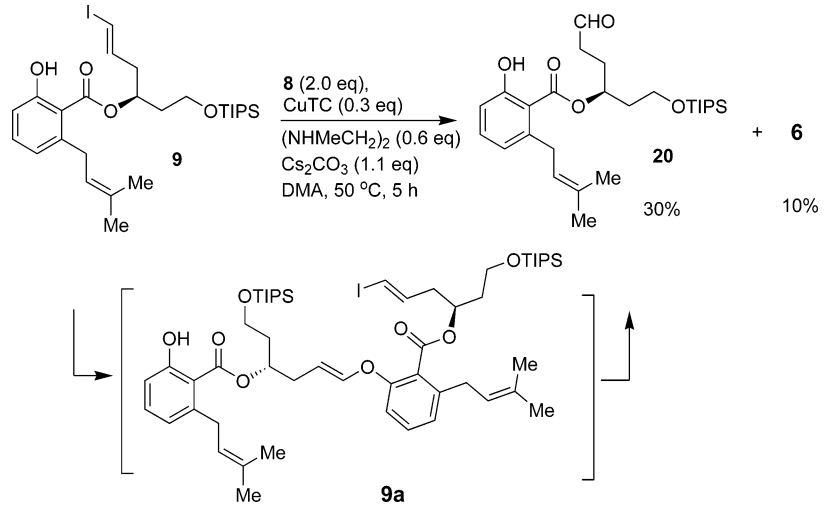
SCHEME 2. Synthesis of Dienoic Acid Amide 8



SCHEME 3. Enamide Coupling of 8 and 9



Engler's protocol.[23] NaHMDS-mediated ring-opening[18] of **10** with secondary alcohol **11** furnished vinyl iodide **9** (91%).

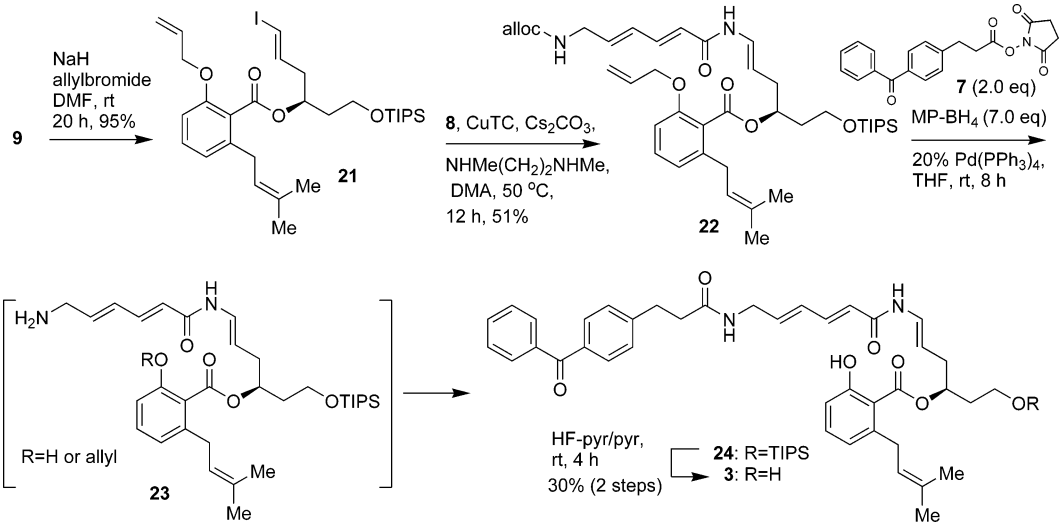
The synthesis of dienoic acid amide **8** was initiated from allylic alcohol **16**, prepared from ethyl sorbate by a bromination/hydrolysis sequence following the protocols of Durrant[24] and Closa[25] (Scheme 2). Mitsunobu coupling of **16** and diallyl imidodicarbonate **17**[26] smoothly provided the desired bis(*N*-alloc)-protected allylic amine **18**, which was carefully hydrolyzed (aq LiOH, *t*-BuOH/MeOH) to dienoic acid **19**. In this transformation, one of the *N*-alloc groups was simultaneously removed. Compound **8** was prepared using mixed anhydride formation of dienoic acid **19** followed by quenching with aqueous ammonium hydroxide.

Initial attempts at Cu(I)-catalyzed amidation of vinyl iodide **9** with dienoic acid amide **8** were found to be problematic due to likely interference by the free phenol-OH. We found that aldehyde **20** was consistently the major product of attempted amidation reactions with only 10% of the desired enamide product **6** obtained (Scheme 3). Aldehyde **20** is likely derived from hydrolysis of vinyl ether intermediate **9a** which may be formed by intermolecular Cu(I)-mediated Ullman coupling of **9**.[27] After considerable evaluation of different bases (K_2CO_3, Rb_2CO_3), supporting ligands (1,10-phenanthroline),[11a] and solvents (THF)[12c] in the coupling reaction, no satisfactory results were obtained. Protection of the phenol-OH with either a triisopropylsilyl (TIPS) ether or 2-nitrobenzyl group[28] did not further improve the yield of the desired salicylate enamide **6**.

However, we were delighted to find that when the phenol-OH of compound **9** was protected as an *O*-allyl ether (Scheme 4), amidation of vinyl iodide **21** using CuTC and *N,N′*-dimethylethylenediamine[12c,29] proceeded cleanly to afford enamide **22** in 51% yield. Although *O*-allyl ethers and *N*-alloc group react differently under Pd(0)-catalyzed deallylation conditions, it was anticipated that, by choice of the appropriate allyl scavenger, both the *N*-alloc and the *O*-allyl groups could be removed in a one-pot operation.[30] Application of $Pd(PPh_3)_2Cl_2$/HSnBu$_3$,[31] $Pd(OAc)_2$/HSiMe$_2$tBu/Et$_3$N,[32] and $Pd(PPh_3)_4$/dimedone[33] to enamides **6** and **22** either caused severe decomposition or led to recovery of starting material.

(23) Engler, T. A.; Reddy, J. P.; Combrink, K. D.; Velde, D. V. *J. Org. Chem.* **1990**, *55*, 1248.
(24) Durrant, G.; Green, R. H.; Lambeth, P. F.; Lester, M. G.; Taylor, N. R. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2211.
(25) Closa, M.; De March, P.; Figueredo, M.; Font, J.; Soria, A. *Tetrahedron* **1997**, *53*, 16803.
(26) (a) Arnould, J. C.; Landier, F.; Pasquet, M. J. *Tetrahedron Lett.* **1992**, *33*, 7133. For a Mitsunobu reaction using **17**, see (b) Kanno, O.; Kawamoto, I. *Tetrahedron* **2000**, *56*, 5639.
(27) For a recent review of Cu-mediated Ullman condensation, see: Ley, S. V.; Thomas, A. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 5400.
(28) Amit, B.; Hazum, E.; Fridkin, M.; Patchornik, A. *Int. J. Pept. Protein Res.* **1977**, *9*, 91.
(29) Klapars, A.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2002**, *124*, 7421.

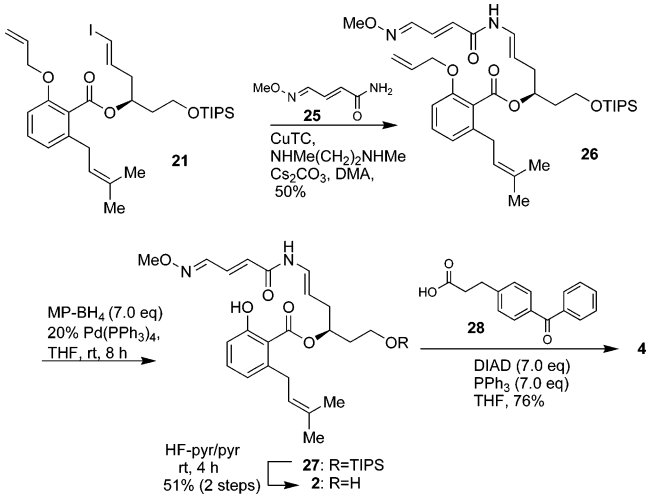
SCHEME 4. Synthesis of Analogue 3



Considering the labile amine intermediate **23**, a tandem deallylation/amidation employing polymer-supported allyl scavengers would be ideal for the transformation to the final target.[30a,b] Using $Pd(PPh_3)_4$ as catalyst, polymer-supported *N*-hydroxyphthalimide,[34] 1-hydroxybenzotriazole-6-sulfonamidomethyl polystyrene (PS-HOBt),[35] and macroporous triethylammonium methylpolystyrene borohydride ($MP\text{-}BH_4$)[36] were evaluated for in situ generation of free amine **23** and tandem amide formation with activated ester **7**. Both PS-*N*-hydroxyphthalimide and PS-HOBt were not effective in this process, while PS-HOBt was found to remove the *N*-alloc group without removal of the *O*-allyl ether. However, treatment of enamide **22** with $MP\text{-}BH_4$ (7.0 equiv), $Pd(PPh_3)_4$ (0.2 equiv) and activated ester **7** (2.0 equiv) in THF smoothly provided the desired benzophenone-attached amide **24**, which was rapidly transformed to analogue **3** by desilylation with HF−pyridine/pyridine (30%, two steps) (Scheme 4).

Syntheses of Analogues 2 and 4. CuTC/*N,N*′-dimethylethylenediamine-catalyzed amidation of *O*-allyl-protected salicylate **21** with butenamide **25**[11c] afforded enamide **26** (Scheme 5), an intermediate required for preparation of analogues **2** and **4**. While unprotected

SCHEME 5. Synthesis of Analogues 2 and 4



salicylate **9** was used instead of **21**, enamide **27** was obtained in only 16% yield. Removal of the *O*-allyl group of **26** using $Pd(PPh_3)_4/MP\text{-}BH_4$, followed by deprotection of the TIPS ether using HF−pyridine/pyridine, provided analogue **2** (51%). Since the acid-sensitive enamide side chain was not stable to other esterification conditions,[11c] analogue **4** was obtained employing a Mitsunobu protocol from analogue **2** and 3-(4-benzoylphenyl)propionic acid **28**.[15a,37]

Synthesis of Analogue 5. Synthesis of analogue **5** was performed on the basis of a similar strategy employed for analogue **3**. Since the benzophenone fragment was attached to the aromatic ring of analogue **5**, preparation of substituted benzo[1,3]dioxin-4-one **35** was required (Scheme 6). Therefore, Mitsunobu coupling of commercial available compound **29** and monosilylated ethylene glycol **30**[38] smoothly afforded aryl ether **31**,[39] which was subsequently transformed to aryl triflate **32**.

(30) For simultaneous reductive deprotection of both *O*-allyl and *N*-alloc groups using $Pd(PPh_3)_4/LiBH_4$, followed by reaction with activated esters to prepare amides, see: (a) Beugelmans, R.; Neuville, L.; Bios-Choussy, M.; Chastanet, J.; Zhu, J. *Tetrahedron Lett.* **1995**, *36*, 3129. (b) Bios-Choussy, M.; Beugelmans, R.; Bouillon, J.-P.; Zhu, J. *Tetrahedron Lett.* **1995**, *36*, 4781. For simultaneous deprotection of *O*-allyl and *N*-alloc groups using $Pd(PPh_3)_4$/morpholine, see: (c) Pelish, H. E.; Westwood, N. J.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, 123, 6740.

(31) Dangles, O.; Guibe, F.; Balavoine, G. *J. Org. Chem.* **1987**, *52*, 4984.

(32) Sakaitani, M.; Ohfune, Y. *J. Org. Chem.* **1990**, *55*, 870.

(33) Kunz, H.; Unverzagt, C. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 436.

(34) Han, C.; Shen, R.; Su, S.; Porco, J. A., Jr. *Org. Lett.* **2004**, *6*, 27.

(35) For further information on PS-HOBT, see: http://www.argotech.com.

(36) For alloc deprotection using a polymer-supported Pd(0) catalyst and a resin-bound borohydride, see: Bhattacharyya, S.; Vo, Lanchi.; Gooding, O. W.; Labadie, J. W. Abstracts of Papers. *227th ACS National Meeting*; Anaheim, CA, March 2004; American Chemical Society: Washington, DC, 2004; ORGN-101. For information on $MP\text{-}BH_4$, see: http://www.argotech.com/products/lead/resins/solutions/mp_borohydride.html.

(37) De Kort, M.; Luijendijk, J.; Van der Marel, G. A.; Van Boom, J. H. *Eur. J. Org. Chem.* **2000**, 3085.

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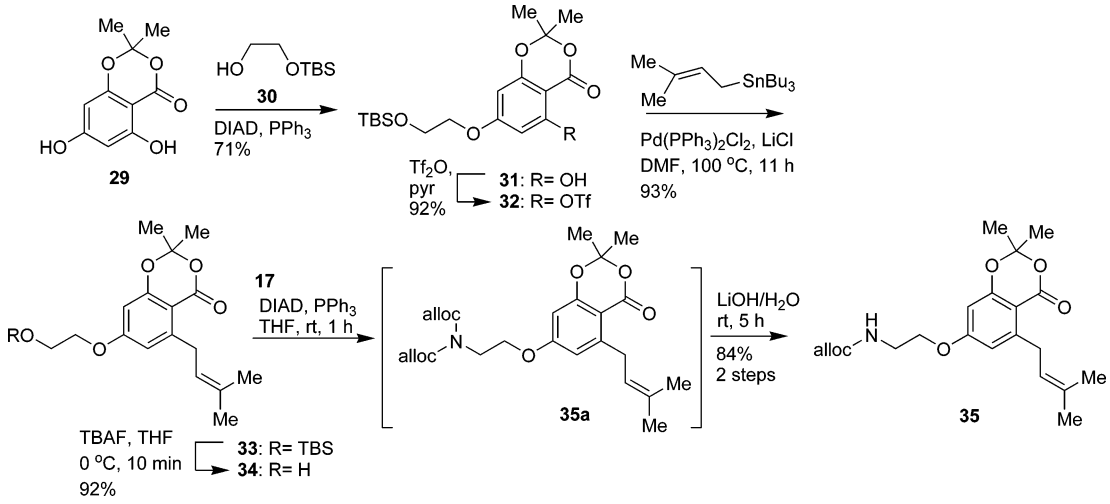
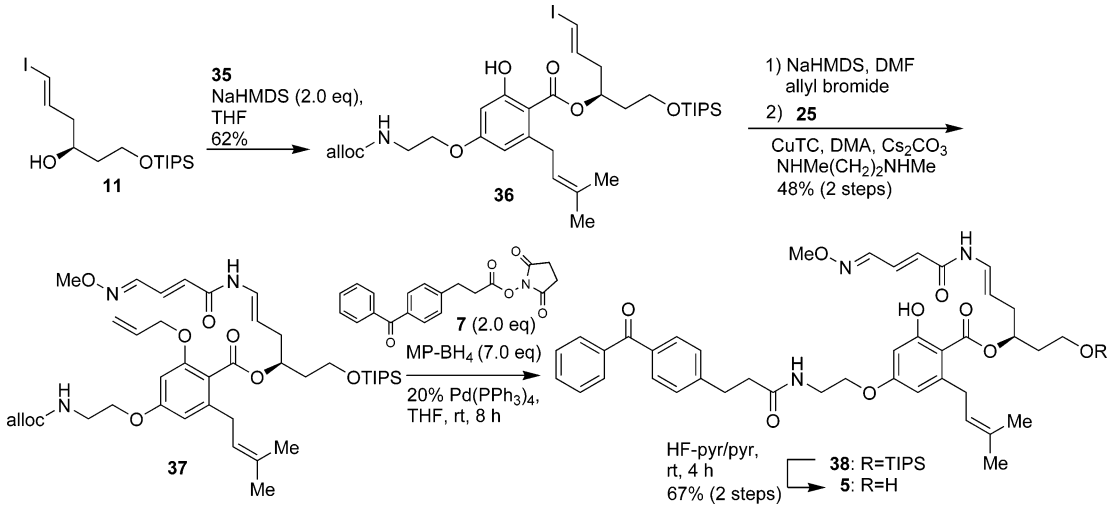
(39) Cf. Takahashi, S.; Kamisuki, S.; Mizushina, Y.; Sakaguchi, K.; Sugawara, F.; Nakata, T. *Tetrahedron Lett.* **2003**, *44*, 1875.

SCHEME 6. Synthesis of Compound 35



SCHEME 7. Synthesis of Analogue 5



Stille coupling of tributylprenyl stannane and aryltriflate **32** afforded prenyl benzo[1,3]dioxin-4-one **33** in 93% yield.[23] Since Pd(0) was used in the Stille reaction, it was necessary to install the *N*-alloc-protected amine at a later stage. Desilylation of **33** with TBAF, followed by Mitsunobu coupling of alcohol **34** and diallyl imidodicarbonate **17**,[26] furnished the desired product **35a**, which was converted to compound **35** by in situ removal one of the *N*-alloc groups using aqueous LiOH (84%).

Treatment of compound **35a** and alcohol **11** under basic conditions (1.1 equiv of NaHMDS) provided **35** as the major product instead of the desired desired salicylate ester indicating the necessity for removal of the additional *N*-alloc group of **35a** prior to transesterification of the benzo[1,3]dioxin-4-one moiety. Accordingly, treatment of benzo[1,3]dioxin-4-one **35** and alcohol **11** with 2.0 equiv NaHMDS afforded vinyl iodide **36** (Scheme 7). Protection of the phenol of **36** with allyl bromide followed by Cu(I)-catalyzed enamide formation furnished the desired enamide **37** in 48% yield (two steps). The tandem deallylation/amidation process was performed similarly as reported for analogue **3**. Final deprotection of the TIPS ether on the primary alcohol using HF-pyridine/pyridine provided analogue **5** in good yield (67%, two steps).

TABLE 1. V-ATPase Inhibition of Analogues 2–5[a]

compd	IC_{50}
lobatamide C	2 nM
2	10 nM
3	4 μM
4	100 nM
5	>10 μM

[a] Inhibition of bovine clathrin-coated vesicle V-ATPase. See the Supporting Information for experimental details.

V-ATPase Inhibition of Analogues 2–5. Analogues **2**–**5** were next evaluated for inhibition of bovine clathrin-coated vesicle V-ATPase (Table 1). Simplified analogue **2** showed high potency for V-ATPase inhibition (10 nM) in line with the potency of the natural product lobatamide C (2 nM). Analogue **3** was found to be much less active than the parent compound **2** (4 μM). Analogue **4** bearing the benzophenone probe as an ester linkage was also determined to be a weaker V-ATPase inhibitor than compound **2** (100 nM). However, analogue **5** exhibited negligible V-ATPase inhibition activity compared to other analogues, which indicated that the 4-alkoxy subsititution of the salicylate to afford a resorcylate ring was not well tolerated in the V-ATPase binding site. Analogue **4** effectively retained useful levels of V-ATPase inhibition

which bodes well for further use of this probe reagent and related derivatives as photoaffinity reagents.

Conclusion

In an effort to explore the binding site of lobatamides on mammalian V-ATPase, photoactivatable analogues **3**−**5** based on the simplified lobatamide analogue **2** were prepared. In initial studies, a benzophenone moiety was employed as a photoactive group and attached to different positions of parent analogue **2**. The synthesis of analogues included late stage installation of enamides from amidation of vinyl iodides bearing allyl ether-protected salicylate esters. An efficient tandem deallylation/amidation process was used for final deprotection and photoaffinity probe attachment. The synthetic route also permits installation of other photoactive groups in addition to the benzophenone moiety or other reporting/modifying groups (e.g., biotin) as required for particular applications. Finally, analogues **2**−**5** have been tested for inhibition of bovine clathrin-coated vesicle V-ATPase. Compound **2** showed the highest potency of inhibition of all analogues tested and afforded the most potent simplified acyclic lobatamide analogue[11c] identified to date. Photoactivatable analogue **4** retained much of the inhibition activity of parent analogue **2** and was superior to analogues **3** and **5**. Further studies employing analogue **4** and related compounds in photoaffinity labeling of mammalian V-ATPase are currently in progress and will be reported in due course.

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Supporting Information Available: Experimental procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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