Non-Peptide Macrocyclic Histone Deacetylase Inhibitors

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Received September 9, 2008

Inhibition of histone deacetylase inhibitors (HDACi) hold great promise in cancer therapy because of their demonstrated ability to arrest proliferation of nearly all transformed cell types. Of the several structurally distinct small molecule HDACi reported, macrocyclic depsipeptides have the most complex recognition cap-group moieties and present an excellent opportunity for the modulation of the biological activities of HDACi. Unfortunately, the structure−activity relationship (SAR) studies for this class of compounds have been impaired largely because most macrocyclic HDACi known to date comprise complex peptide macrocycles. In addition to retaining the pharmacologically disadvantaged peptidyl backbone, they offer only limited opportunity for side chain modifications. Here, we report the discovery of a new class of macrocyclic HDACi based on the macrolide antibiotics skeletons. SAR studies revealed that these compounds displayed both linker-length and macrolide-type dependent HDAC inhibition activities with IC_{50} in the low nanomolar range. In addition, these non-peptide macrocyclic HDACi are more selective against HDACs 1 and 2 relative to HDAC 8, another class I HDAC isoform, and hence have subclass HDAC isoform selectivity.

Introduction

Inhibition of histone deacetylases (HDACs[a]) has recently been clinically validated as a novel therapeutic strategy for cancer treatment.[1] Because of their demonstrated ability to arrest proliferation of nearly all transformed cell types,[2] HDAC inhibitors (HDACi) hold great promise as agents of choice, either as stand-alone therapeutics or in combination with others, in the fight against the cancer scourge. To date, several structurally distinct small molecule HDACi have been reported including aryl hydroxamates, benzamides, short-chain fatty acids, electrophilic ketones, and macrocyclic peptides (Scheme 1).[3−6] All HDACi so far reported fit a three-motif pharmacophoric model, namely, a zinc-binding group (ZBG), a hydrophobic linker, and a recognition cap group.[3] The X-ray crystal structures of a bacterial HDAC homologue, histone deacetylase-like protein (HDLP) bound to suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA), and recently human HDAC8 and HDAC7 have validated this model.[7,8] Of these HDACi, macrocyclic peptides have the most complex recognition cap-group moieties and present an excellent opportunity for the modulation of the biological activities of HDACi. Although cyclic-peptide HDACi possess potent HDAC inhibition activity (nanomolar range), their broad application in cancer therapy currently remains largely unproven.[3] One promising exception, FK-228 (Scheme 1), is currently in phase II study for the treatment of cutaneous T-cell lymphoma.[9]

The dearth of clinically effective cyclic-peptide HDACi may be in part due to development problems characteristic of large peptides, most especially poor oral bioavailability. In addition to retaining the pharmacologically disadvantaged peptidyl backbone, they offer only limited opportunity for side chain modifications.[10] Identification of non-peptide macrocyclic HDACi will offer a new class of macrocyclic HDACi with potentially more favorable druglike properties. Furthermore, this will aid comprehensive SAR studies and further enhance our understanding of the roles of specific interactions between the enzyme outer rim and inhibitor cap groups in HDACi activity and selectivity. Herein, we report the discovery of a new class of potent, non-peptide macrocyclic HDACi derived from the macrolide macrocyclic ring structures.

Results and Discussion

Macrolides are glycosylated polyketide antibiotics that have been in use for over 50 years for the treatment of respiratory tract infections. Additionally, macrolides have elicited other non-antibiotic effects, including anti-inflammatory and immunomodulatory effects that make them promising candidates for the management of diseases of chronic airway inflammation.[11,12] More recently, macrolides derived from the 6-*O*-methylerythromycin A ring have been reported to serve as nonpeptidic surrogates for the peptide backbone of macrocyclic peptide luteinizing hormone-releasing hormone (LHRH) receptor antagonists.[13] Drawing inspiration from the peptidomimetic property of macrolides, we hypothesize that an appropriate substitution of the cyclic peptide moiety of a prototypical cyclic-peptide HDACi with macrolide skeletons will generate a new class of potent non-peptide macrocyclic HDACi.

To test our hypothesis, we first sought a SAHA−macrolide conjugate that incorporated the 15-membered azalide ring of azithromycin as the macrolide template (Scheme 1). The azithromycin skeleton is an attractive choice because of its excellent pharmacokinetic (PK) profile and ease of chemical transformation of key moieties on the skeleton.[14] Our design approach is to attach the HDAC inhibiting group to a macrolide moiety that is remote from the macrocyclic ring. We anticipate that this will minimize the potential steric clash at the HDAC active site that might result from the introduction of the azithromycin skeleton, a macrocyclic ring not optimized to bind

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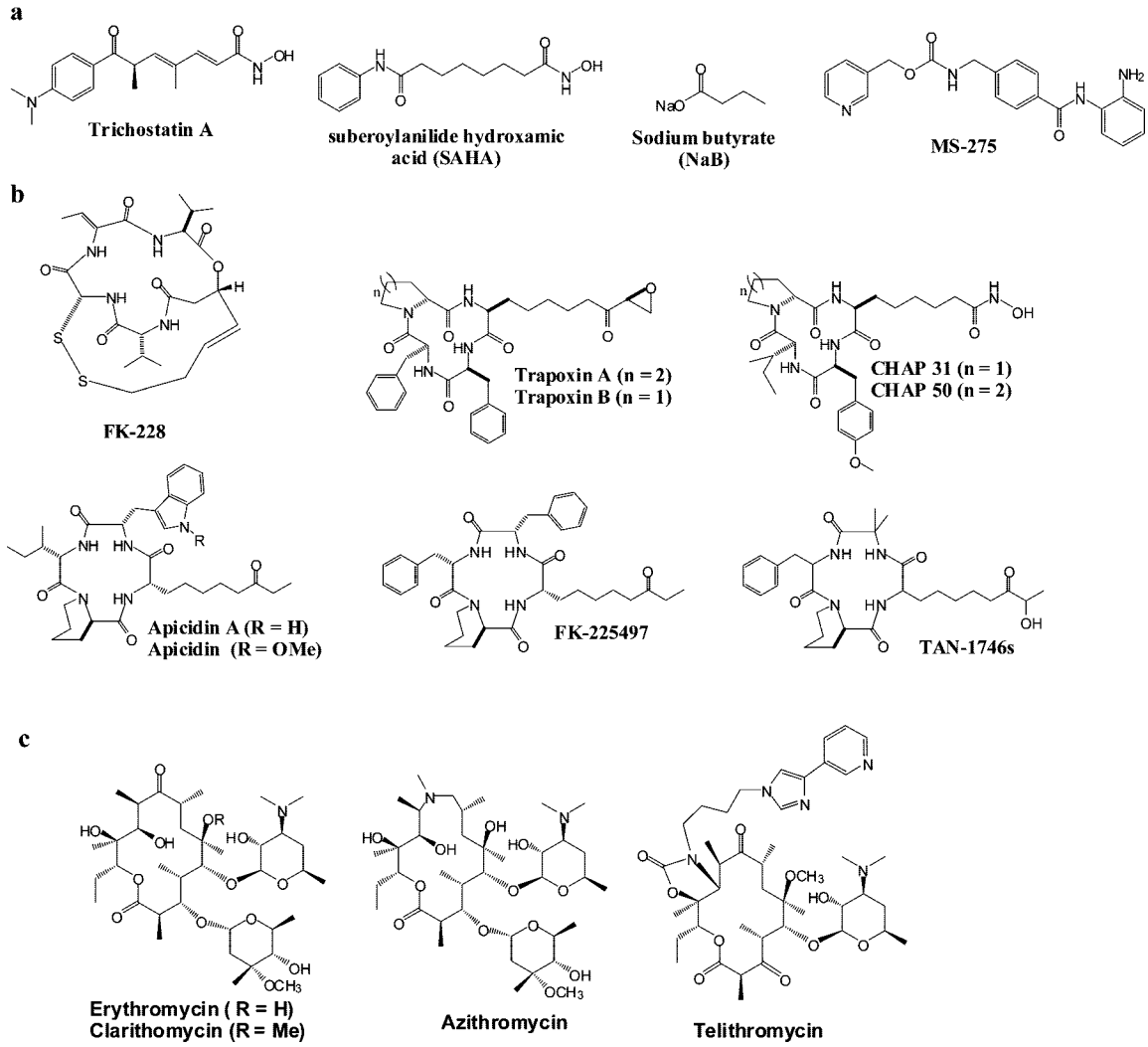
[a] Abbreviations: HDAC, histone deacetylase; HDACi, histone deacetylase inhibitors; HDLP, histone deacetylase-like protein; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A; ZBG, zinc-binding group.

10.1021/jm801128g CCC: $40.75
Published on Web 12/18/2008

Scheme 1. (a) Selected Examples of Acyclic HDAC Inhibitors, (b) Representative Examples of Cyclic-Peptide HDAC Inhibitors, and (c) Representative Examples of Macrolide Antibiotics



the HDAC enzyme. The NMR structure[15] and 3D model revealed that the 3′-tertiary amine of the desosamine sugar and the 4″-OH of the cladinose sugar met our design requirement. We opted for the former group because of the well established facile transformations of the tertiary amine moiety.[13,14] Almost all modifications of this moiety have resulted in the attenuation of the antibacterial activity of this class of compound.[14b] Furthermore, coupling of such desosamine modifications with cladinose sugar removal will result in compounds devoid of antibacterial activity,[16] a property of the parent macrolides undesirable to our goals. Toward this end, we synthesized compound **8**, which incorporates a SAHA-like moiety (Figure 1) into the 3′-tertiary amine of azithromycin, as a prototype molecule. Compound **8** was synthesized from azithromycin **5** and methyl 8-chloro-8-oxooctanoate **1** through a five-step synthetic route as shown in Figure 1. To gain some preliminary insights into the roles of key macrolide moieties in anti-HDAC activity of **8**, we synthesized compound **10**, an analogue of **8** lacking the cladinose sugar. Acid promoted removal of the cladinose sugar according to a published protocol[13] quantitatively yielded compound **9**, which was converted to the hydroxamate **10** using the same protocol for the synthesis of **8** from **7** (Figure 1). We then tested for the HDAC inhibition activity of **8** and **10** using a cell free kit assay[17] and found that both compounds caused a concentration dependent inhibition of HDACs 1 and 2 from HeLa cell nuclear extract (Table 1). In fact, both compounds have identical anti-HDAC activity, with IC_{50} values in the low nanomolar range. Conversely, compound **7**, the methyl ester precursor of **8**, is completely inactive in this assay. This result suggests that the 15-membered azalide ring of the azithromycin macrolide is a suitable non-peptidyl surrogate for the macrocyclic peptide moiety of a typical cyclic-peptide HDACi. Additionally, the binding orientations of these compounds at the HDAC active site may be such that the hydroxamate group is efficiently presented to chelate the active site Zn^{2+} ion while the cladinose moiety is oriented away from the enzyme's outer rim.

We then initiated structure−activity relationship studies on **8** and **10** to optimize the HDAC binding affinity of these compounds. Preliminarily, we focused on the effects of the modification of the linker-cap group connection moiety, macrolide skeleton, and linker length on anti-HDAC activity. We used the AutoDock program[18] and the crystal structure of a histone deacetylase-like protein (HDLP)[7] to guide our structural optimization. In an earlier study, we demonstrated the suitability of a 1,2,3-triazole ring as an alternative linker-cap group connection moiety in SAHA-like HDAC inhibitors.[19] In addition to serving as an alternative connection moiety with a potentially favorable pharmacokinetic properties, the triazole ring is a more synthetically tractable group[20] and has been adopted in the construction of active-site directed chemical probes for profiling HDAC activities in proteomes and live cells.[21] Its incorporation

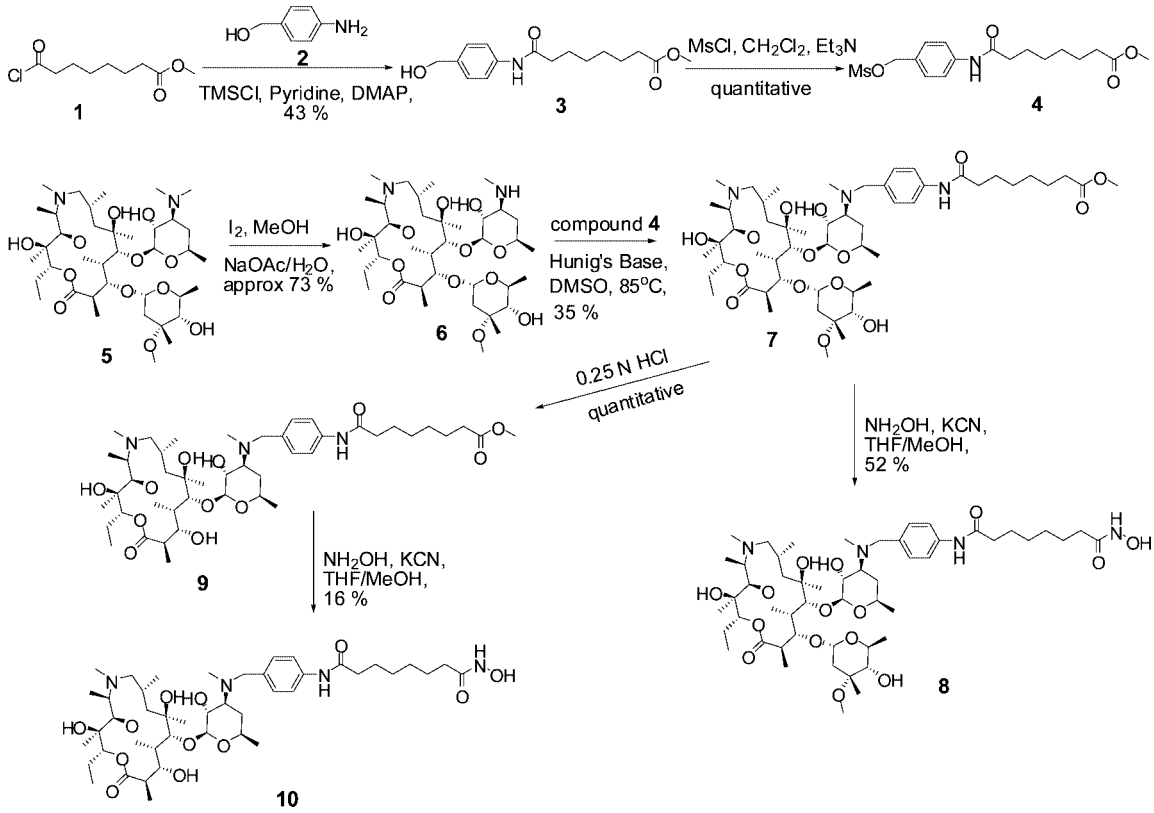


Figure 1. Synthesis of prototypical non-peptide macrocyclic HDACi **8** and **10**.

Table 1. In Vitro HDAC Inhibition (IC_{50}) and Isoform Selectivity of Non-Peptide Macrocyclic HDACi[a]

compd	HDAC 1/2 (nM)	HDAC 8 (nM)	isoform selectivity[b]
7	ND	NT	*c*
8	107.1	6680	62
10	109.8	2320	21
16a	91.6	4730	51
16b	88.8	3740	42
16c	13.9	994	72
16d	10.6	1020	97
16e	58.9	7130	121
16f	72.4	6780	94
16g	145.5	11050	38
16h	226.7	ND	*c*
24a	37.0	3990	108
24b	44.3	4750	107
24c	4.1	1890	462
24d	1.9	1390	743
24e	55.6	5880	106
24f	123.0	4420	36
24g	169.8	10550	56
24h	223.4	ND	*c*
SAHA	65.0	1860	29

[a] IC_{50} values were determined using a cell free kit assay.[17] Each value is obtained from three independent experiments. ND: not determinable. NT: not tested. [b] Calculated by dividing the IC_{50} of HDAC 8 by the IC_{50} of HDAC 1/2. [c] Undeterminable.

into our design could facilitate a facile, high yielding synthesis of the triazole-based SAHA-macrolide conjugates and thus provide solution to the problems of low yields that currently plagued the synthesis of the amide-based compounds **8** and **10** (Figure 1).

Accordingly, we proceeded to test the compatibility of the triazole ring with the anti-HDAC activity of **8** and **10**. We prepared compounds **16a** and **16b**, analogues of **8** and **10**, respectively, having the amide moiety connecting the cap group and the linker region substituted with a 1, 2, 3-triazole ring (Figure 2a). The reaction of 4-ethynylbenzyl mesylate **11** with compound **6** led to the desired desosamine alkylation product **12** in good yields. Copper(I) catalyzed cycloaddition reaction of alkyne **12** with *O*-silyl azidohydroxamate **14**[19,20] gave the desired cycloadduct **15** in good yields. Similarly, the reaction of alkynes **12** and **13** with azido ester **18** gave the desired cycloadducts **19a** and **19b**, respectively. The deprotection of the silyl group of compound **15** is accomplished with TBAF treatment, leading to desired hydroxamate **16a** in good yields. However, difficulties were encountered in separating **16a** from the TBAF reagent. In much the same manner as in the synthesis of **8** or **10** (Figure 1), the conversion of methyl ester **19a** and **19b** to the corresponding hydroxamate is dogged by low yields. Subsequently, we found that a direct Cu(I) catalyzed cycloaddition between unprotected azidohydroxamate **17** and alkyne **12** or **13** in anhydrous, degassed THF under exclusion of oxygen uneventfully gave the desired hydroxamates (Figure 2a).

HDAC inhibition studies revealed that the triazolyl compounds **16a** and **16b** have virtually identical anti-HDAC activity as the amide compounds **8** and **10** (Table 1). This result is in contrast with our observation on simple aliphatic hydroxamates where the introduction of the triazole ring led to an enhancement of anti-HDAC activity.[19] To gain insights on the molecular interactions between these non-peptide macrocyclic HDACi and HDAC active site, we performed molecular docking analysis of **16b** on HDLP, using the AutoDock program, as previously described.[18,19] We chose to use the HDLP structure because it shared conserved active site residues with class I HDACs. Additionally, direct docking experiments using this structure or HDAC1 homology model built from the same HDLP structure have given docking results that are essentially of the same quality and also agreed with experimentally obtained data. Either of these approaches has been extensively used in the literature to interrogate the binding interactions of HDAC inhibitors at the protein active site.[18b,c] The structures we

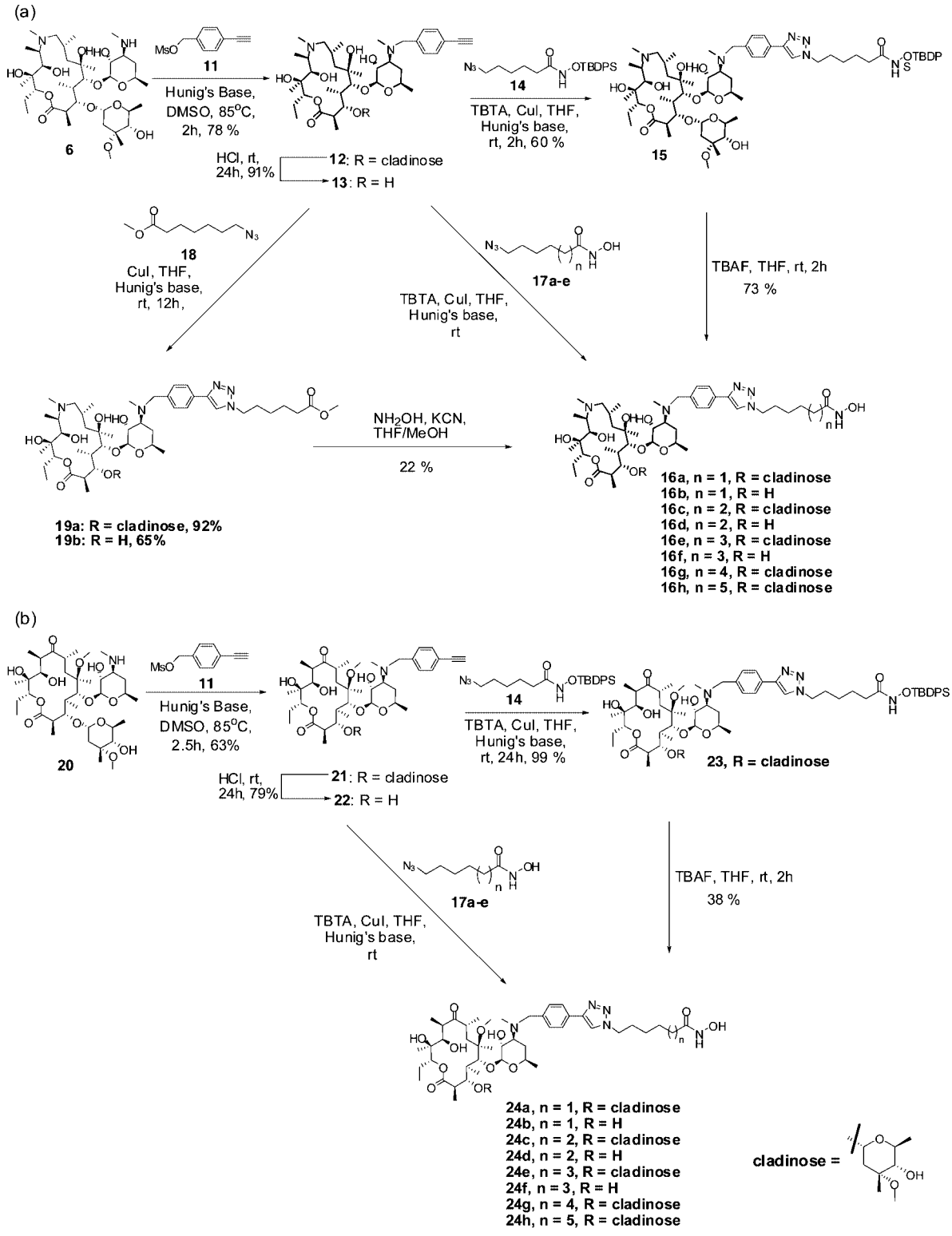


Figure 2. SAR studies on non-peptide macrocyclic HDACi: (a) synthesis of triazole-linked non-peptide macrocyclic HDACi based on azithromycin 15-membered ring; (b) synthesis of triazole-linked nonpeptide macrocyclic HDACi based on clarithromycin 14-membered ring.

obtained from our docking experiments indicated interesting molecular surface complementarities between the macrolide skeleton of **16b** and the HDAC outer rim. Previous investigations have shown that there are four possible binding pockets on the HDLP surface whose interactions with the HDACi cap groups could enhance the inhibitor binding ability.[18b] Compound **16b** adopts a docked structure that placed the macrolide macrocyclic ring in binding pockets 1 and 3 (Figure 3a). In addition, the hydrophobic components of the macrolide ring optimally interact with the hydrophobic residues in pockets 1 and 3 while the hydrophilic hydroxyl groups are oriented away from the pocket's hydrophobic residues. Compared to the structure of SAHA, the hydroxamate moiety of **16b** is farther oriented from the active site Zn^{2+} ion (Figure 3b). On the basis of the preceding observation, we inferred that optimization of the linker region could result in compounds with enhanced HDAC affinity due to a better presentation of the hydroxamate moiety to the catalytic Zn^{2+} ion. Subsequent docking analyses

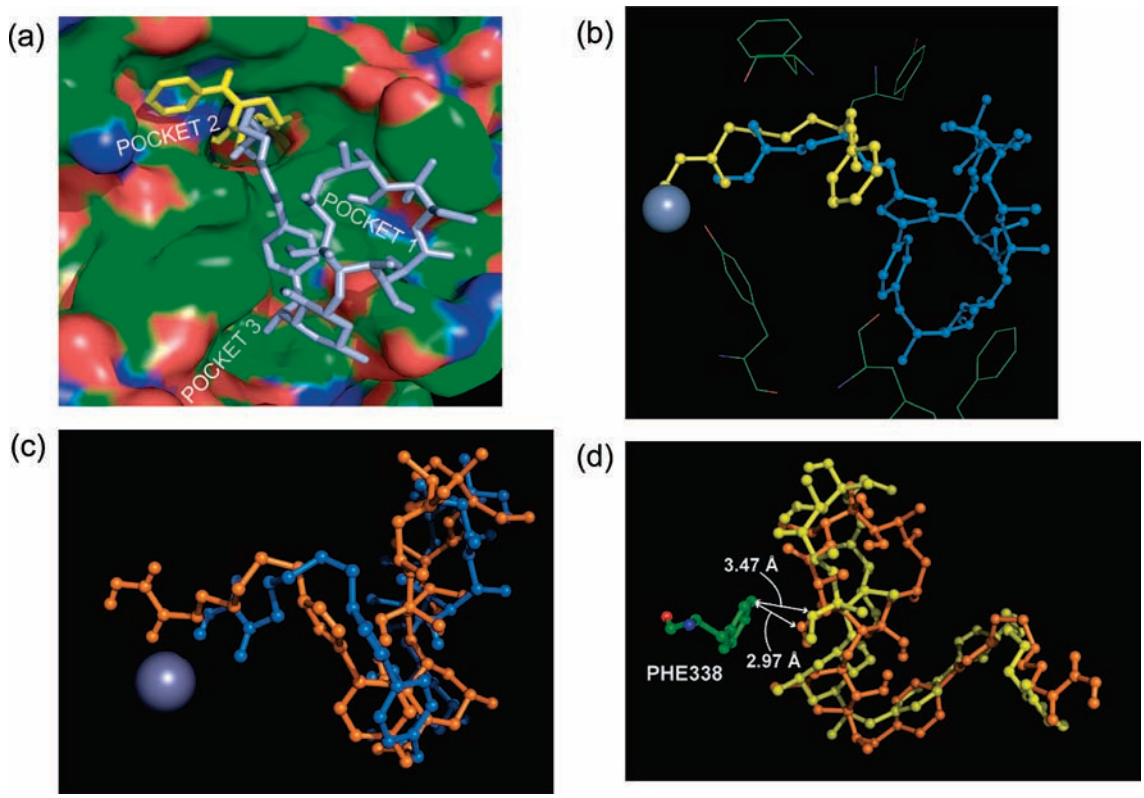


Figure 3. Docked structures of SAHA and 14- and 15-membered macrocyclic HDACi at the active site of HDLP. (a) Superposition of the low energy conformation of **16b** (blue) and SAHA (yellow) revealed the pocket binding preferences of inhibitors at the HDLP surface. Shown are ball and stick models of the orientations of the hydroxamate groups of SAHA and **16b** (b) and of **16b** and **16d** (orange) (c) with respect to the active site Zn^{2+} ion (gray ball). (d) Relative orientation of the macrocyclic rings of **16d** and **24d** (yellow) with respect to Phe 338 at the HDLP surface.

with analogues of **16b** having varied methylene-linker lengths revealed that **16d**, a C_7-linker compound, optimally interacts with the Zn^{2+} ion (Figure 3c). Interestingly, compound **24d**, an analogue of **16d** in which the 15-membered azithromycin ring has been substituted with the 14-membered clarithromycin ring, has a slight preference for the enzyme. A closer analysis of the docked structures of **16d** and **24d** revealed that the C12−C14 region of the larger 15-membered ring is about 0.5 Å closer to the phenyl ring of Phe338 that defines one of the hydrophobic pockets at the enzyme outer rim compared to that of the 14-membered compound (Figure 3d). This might compromise the binding affinity of the 15-membered compounds relative to the 14-membered analogues.

To experimentally test these in silico observations, we synthesized compounds **16c**−**h** and **24a**−**h**, the 15- and 14-membered non-peptide macrocyclic hydroxamates respectively (parts a and b of Figure 2, respectively). Results from HDAC inhibition assay on these compounds revealed HDAC inhibition activities that essentially paralleled the in silico prediction (Table 1). The compounds displayed both linker-length and macrolide-type dependent HDAC inhibition activities. For compounds derived from the same macrolide ring, an increase in the linker length from C_6 to C_7 conferred a better anti-HDAC activity. Further linker length increase did not improve HDAC inhibition activity; in fact such an increase is detrimental to function in some cases. For compounds with C_6 and C_7 linkers, a head-to-head comparison between 14- and 15-membered macrolides revealed that the 14-membered compounds are about 2- to 5-fold better HDACi than their 15-membered counterparts (Table 1; see **16c** and **24c**, for example). However, this preference dissipated with increase in linker length. This is presumably due to a relief of steric clash between the macrocyclic ring and the phenyl ring of Phe338 at the enzyme's outer rim, conferred by the longer linkers.

To obtain evidence for the HDAC isoform selectivity of the macrocyclic HDACi described herein, we tested their HDAC8 inhibition activity. We chose HDAC8 because it is in the same subclass as HDACs 1 and 2, the principal HDACs contained in the HeLa cell nuclear extract used in the assay kit employed in this study. There are very few examples of HDAC inhibitors that are selective for HDAC isoforms within the same class; hence, this choice should permit a quick, yet rigorous assessment of HDAC isoform selectivity of our compounds. Compared to SAHA, all non-peptide macrocyclic hydroxamates tested are more selective for HDAC 1/2. In particular, C_7-linked, 14-membered compounds **24c** and **24d** are several-fold more selective than their 15-membered counterparts (Table 1). Although HDLP and HDAC8 shared similar amino acid sequences and topology at the active site, observation from the analysis of X-ray data, however, revealed significant inhibitor specific changes in the enzyme active site topology of HDAC8.[8b] Our docking analysis operates in the rigid receptor mode, and it is incapable of capturing such crystallographically observed ligand induced conformational changes. Nevertheless, we performed molecular docking analysis of **16b** on the HDAC8 structure reported by Somoza et al.[8b] In contrast to its docked structure on HDLP, the orientation of **16b** that has a chance of making any interaction with the active site Zn^{2+} ion is that which adopted a closed conformation nestled atop of the entrance to the enzyme active site. In this conformation, the linker group wrapped around the macrocyclic ring to orient the hydroxamate moiety toward the Zn^{2+} ion, albeit much farther away to make any stabilizing interaction (see Figure S1, Supporting Information). An alternative lower energy confirmation of **16b** oriented

Table 2. Cell Growth Inhibition Data for Non-Peptide Macrocyclic HDACi[a]

compd	SKMES 1 (μM)	NCI-H69 (μM)	DU-145 (μM)	lung fibroblast (μM)	HMEC (μM)
8	NT	NT	>25	NT	NT
10	NT	NT	<25	NT	NT
16a	1.79	1.92	1.45	>10	>10
16b	1.68	1.77	1.24	>10	>10
16c	2.33	3.45	1.88	>10	>10
16d	2.56	3.01	1.97	>10	>10
16e	4.89	4.56	5.89	>10	>10
16f	4.67	3.99	5.68	>10	>10
16g	7.54	8.45	>10	>10	>10
24a	2.15	2.67	2.98	>10	>10
24b	1.95	1.92	3.29	>10	>10
24c	1.33	1.45	1.12	>10	>10
24d	1.28	1.49	1.05	>10	>10
24e	4.89	5.67	6.97	>10	>10
24f	4.45	5.09	5.78	>10	>10
24g	7.12	7.29	8.14	>10	>10
SAHA	2.42	2.06	2.12	>10	>10

[a] EC_{50} values were determined from trypan blue exclusion data. Each value is obtained from a duplicate of four simultaneous experiments. NT: not tested.

the hydroxamate moiety away from the Zn^{2+} ion. It is therefore possible that the observed isoform selectivity may be due to the inability of the non-peptide macrocyclic hydroxamates herein described to efficiently induce active site conformational changes that facilitate HDAC8 specific inhibitor association with the enzyme active site.[22a] Alternatively, HDAC8 activity has been observed to depend on the sequence of its peptide substrate;[22b] hence, it is also conceivable that the extent of enzyme inhibition, and consequently isoform selectivity, may depend on the substrate used in the inhibition study.

To further test for HDAC isoform selectivity, we investigated the effect of selected macrocyclic HDACi on the deacetylase activity of HDAC6, a representative member of class II HDAC. For this preliminary study, we chose compounds **16c** and **16d**, and **24c** and **24d**, the most potent representative 15- and 14-membered macrocyclic HDACi, respectively (Table 1). Cell free HDAC6 inhibition assay was performed as recommended by the supplier.[17] Unlike SAHA, which equally inhibited HDAC1/2 and HDAC6, we observed that the macrocyclic HDACi still displayed significant preference for HDAC1/2 (see Table S1, Supporting Information). This observation is in agreement with the literature reports that suggest that complex headgroups tend to promote isoform selectivity.[3,22c]

To screen for the whole cell activity of compounds described in this study, we studied their effect on the viability of SK-MES-1 (human NSCLC cell line), NCI-H69 (human SCLC cell line), DU-145 (a human prostate cancer cell line), and non-transformed human primary lung fibroblasts and mammary epithelial cell lines. The nontransformed cell lines were investigated to obtain evidence for compound selective toxicity. Drug concentrations necessary for 50% inhibition of cell viability (EC_{50}) were quantitatively measured using trypan blue exclusion,[23] as previously described.[19] Table 2 shows the EC_{50} values of each compound. The EC_{50} values obtained for SAHA are in close agreement with the reported values under similar experimental conditions.[24] Macrocyclic methyl ester **7**, the precursor to compound **8** (Figure 1), has no effect on cell viability (data not shown). This result may not be unexpected, since compound **7** has no HDAC inhibition activity (Table 1). However, all macrocyclic hydroxamates inhibit the proliferation of all transformed cells studied. Most importantly, compounds **16b**, **24c**, and **24d** are at least twice as potent as SAHA in DU-145 cells (Table 2). Gratifyingly, none of the macrocyclic

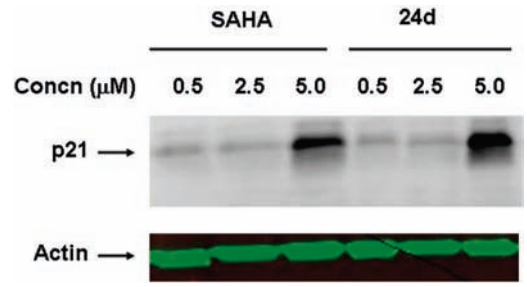


Figure 4. Western blot detection of $p21^{WAF1/CIP1}$ expression levels in SK-MES-1 cells exposed to SAHA and **24d**. Cells are exposed to test agents for 8 h. Compound numbers and concentrations are displayed in the top part. Immunoblotting with anti-actin antibody (in green) is used as a control for equivalent protein loading.

hydroxamates tested shows any growth inhibitory effects on the normal human primary lung fibroblast and mammary epithelial cell lines at concentrations in excess of 10 μM. These data showed that the macrocyclic compounds are selectively toxic to the transformed cells, a trait that tracks with those of many HDACi.

An important biomarker that is primarily associated with intracellular HDAC inhibition is the expression levels of the $p21^{WAF1/CIP1}$ gene. Up-regulation of the $p21^{WAF1/CIP1}$ gene has been generally observed with cellular HDAC inhibition.[25,26] As part of a study aimed at cellular target and mechanistic validation, we investigated the effect of **24d**, a representative macrocyclic HDACi, on the intracellular status of $p21^{WAF1/CIP1}$ protein in SK-MES-1 cells. We used SAHA as a positive control for HDAC inhibition. Cultured cells were exposed to various concentrations of our test agents for 8 h, and the cellular $p21^{WAF1/CIP1}$ expression levels were determined by Western blotting according to literature protocol.[27] We found that both **24d** and SAHA induced a dose dependent increase in $p21^{WAF1/CIP1}$ expression (Figure 4). This result provided additional evidence that the likely mechanism of the antiproliferative activities of the non-peptide macrocyclic hydroxamates described here is through intracellular HDACs inhibition.

Conclusion

We have identified a new class of non-peptide HDACi derived from the macrocyclic skeletons of clinically useful macrolides. These compounds will enable a molecular description of the interaction between the HDAC enzyme's outer rim and the inhibitors' macrocyclic cap groups, thereby further aiding our understanding of the roles of this interaction in inhibitors' binding affinity and possibly HDAC isoform selectivity. In addition, because of the selective tissue distribution that may be conferred by the appended macrolide moiety, some of these HDACi are anticipated to have targeted anticancer activity. Specifically, compounds incorporating azithromycin skeleton could be selectively accumulated in the lungs,[28] thereby possessing lung-selective anticancer activity. The prospect of tissue-specific HDACi delivery is a particularly enticing alternative to isoform selective HDACi and could lead to the identification of new chemotherapeutic agents for use in targeted cancer therapy applications. Efforts are underway in our laboratory to profile the tissue distribution of the new class of HDACi described here.

Experimental Section

Clarithromyin and azithromycin were purchased from Greenfield Chemical and Pfizer, respectively. 4-Ethynylbenzyl alcohol, ethyl 6-bromohexanoate, ethyl 7-bromoheptanoate, 8-bromooctanoic acid,

and methyl 10-bromodecanoate were purchased from Sigma Aldrich. 9-Bromononanoic acid was purchased from Karl Industries Inc. Common reaction solvents were either high performance liquid chromatography (HPLC) grade or American Chemical Society (ACS) grade and used without further purification. HDAC fluorimetric assay kit and recombinant HDACs were procured from BIOMOL International, PA. Analtech silica gel plates (60 F_{254}) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 μm) were used for purification. UV light was used to examine the spots. The 200−400 mesh silica gel was used in column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. ^{1}H NMR spectra were recorded in parts per million (ppm) relative to the peak of $CDCl_3$ (7.24 ppm), CD_3OD (3.31 ppm), DMSO-d_6 (2.49 ppm), or acetone-d_6 (2.04 ppm). ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm), CD_3OD (49.0 ppm), DMSO-d_6 septet (39.7 ppm), or acetone-d_6 (2.04 ppm) and were recorded with complete heterodecoupling. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. 4-Ethynylbenzyl methylsulfonate **11** and azidoalkyl esters were synthesized by adapting literature protocol.[29−31] 6-Azido-*O*-silyl hexahydroxamate **14** was prepared from the corresponding azidocarboxylic acid, *t*-$BuPh_2SiCl$ and NaH, according to the procedure described by Muri et al.[32]

Synthesis of Methyl 8-(4-(Hydroxymethyl)phenylamino)-8-oxooctanoate (3). To a solution of (4-aminophenyl)methanol **2** (0.314 g, 2.500 mmol) in anhydrous pyridine (5 mL) was added chlorotrimethylsilane (0.32 mL, 2.500 mmol) at room temperature, and stirring continued for 2 h. The mixture was cooled in an ice bath to 0 °C, and to the mixture was added methyl 8-chloro-8-oxooctanoate **1** (0.32 mL, 2.200 mmol) and a catalytic amount of DMAP. The mixture was allowed to warm to room temperature, and stirring continued overnight. Water (5 mL) and 1 M TBAF in tetrahydrofuran (THF) (0.25 mL, 0.250 mmol) were added, and stirring continued for an additional 30 min. EtOAc (50 mL) and 1 N HCl (30 mL) were added, the two layers were separated, the organic layer was washed with 1 N HCl (30 mL) and saturated brine (30 mL) and dried over Na_2SO_4. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with EtOAc/hexanes 2:1 to give 275 mg (43%) compound **3** as a yellow-white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.26 (4H, m), 1.47−1.56 (4H, m), 2.26 (4H, m), 3.55 (3H, s), 4.40 (2H, d, J = 5.6 Hz), 5.06 (1H, t, J = 5.6 Hz), 7.19 (2H, d, J = 8.8 Hz), 7.51 (2H, d, J = 8.8 Hz), 9.79 (1H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 24.3, 24.9, 28.2, 28.3, 33.2, 36.3, 51.2, 62.6, 118.8, 126.9, 136.9, 138.0, 171.2, 173.4; HRMS (FAB, thioglycerol) calcd for $[C_{16}H_{23}NO_4 + H]^+$ 294.1705, found 294.1652.

4′-Desmethylazithromycin (6). To a solution of azithromycin (2.000 g, 2.548 mmol) and sodium acetate (1.78 g, 21.500 mmol) in 80% aqueous methanol (30 mL) at 90 °C was added iodine (0.700 g, 2.756 mmol) in three batches within 5 min. The mixture was maintained at pH 8−9 by addition of 1 M NaOH (2 mL, once at 10 min of reaction time), and stirring continued for 3 h. The mixture was poured into cold water containing 5% sodium thiosulfate (80 mL) and extracted with CH_2Cl_2 (2 × 40 mL). The aqueous layer was basified with concentrated NH_4OH and extracted with 10% MeOH in CH_2Cl_2 (3 × 40 mL), and the organic layer was dried over Na_2SO_4. Solvent was evaporated off to give 1.57 g of compound **6** as an off-white solid (>90% purity, TLC, CHC_2Cl_2/MeOH/NH_4OH 12:1:0.1). The crude **6** was used without further purification.

Azithromycinarylalkyl Methyl Ester (7). To a solution of compound **3** (0.175 g, 0.597 mmol) in CH_2Cl_2 (7 mL) and triethylamine (Et_3N) (0.24 mL, 1.800 mmol) was added mesyl chloride (0.10 mL, 1.200 mmol) at 0 °C, and the mixture was allowed to warm to room temperature. Stirring continued for 1 h, during which TLC revealed a quantitative conversion into a higher R_f product. CH_2Cl_2 (40 mL) and saturated sodium bicarbonate (30 mL) were added, and the two layers were separated. The organic layer was washed with sodium bicarbonate (2 × 30 mL), saturated brine (30 mL) and dried over Na_2SO_4. Solvent was evaporated off to give compound **4** as a white solid.

A mixture of 4′-desmethylazithromycin **6** (0.315 g, 0.430 mmol) and crude compound **4** in anhydrous DMSO (7 mL) and Hunig's base (0.7 mL) was stirred at 85 °C for 1.5 h. The mixture was cooled and diluted with EtOAc (60 mL) and washed with saturated $NaHCO_3$ (40 mL) and saturated brine (40 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo and the crude was purified by preparative TLC, eluting with EtOAc/hexanes/Et_3N 3:2:0.1 to give 152 mg (35%) of compound **7** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78−0.82 (7H, m), 0.94−1.03 (11H, m), 1.09−1.32 (22H, m), 1.34−1.58 (5H, m), 1.59− 1.73 (4H, m), 1.77−2.01 (5H, m), 2.12 (3H, s), 2.20−2.28 (8H, m), 2.42−2.53 (2H, m), 2.54−2.71 (3H, m), 2.87−3.01 (2H, m), 3.11 (3H, s), 3.25−3.33 (3H, m), 3.42 (2H, m), 3.54−3.65 (6H, m), 3.97 (1H, m), 4.18 (1H, m), 4.36 (1H, d, J = 6.8 Hz), 4.61 (2H, m), 5.03 (1H, d, J = 4.4 Hz), 7.14 (2H, d, J = 8.4 Hz), 7.42 (2H, d, J = 8.4 Hz), 7.73 (1H, s), 8.97 (1H, bs); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.5, 9.1, 11.3, 14.8, 16.2, 16.9, 18.2, 20.5, 21.2, 21.4, 21.5, 21.9, 24.6, 25.3, 26.7, 27.5, 28.7, 29.6, 33.9, 34.7, 36.2, 36.7, 37.3, 39.1, 41.9, 42.2, 45.1, 48.5, 49.3, 51.4, 57.3, 62.2, 64.3, 65.4, 68.5, 69.9, 70.5, 72.7, 73.5, 73.8, 74.1, 77.7, 77.9, 83.4, 94.4, 102.6, 119.5, 129.0, 134.2, 137.0, 171.0, 173.8, 178.4 MS; HRMS (FAB, mnba) calcd for $[C_{53}H_{91}N_3O_{15} + H]^+$ 1010.6529, found 1010.6450.

Azithromycinarylalkylhydroxamic Acid (8). To a solution of compound **7** (0.050 g, 0.050 mmol) in 1:1 THF/MeOH (2 mL) was added hydroxylamine (50% in H_2O) (0.07 mL, 1.260 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The mixture was partitioned between 5% MeOH in CH_2Cl_2 (30 mL) and saturated sodium bicarbonate (25 mL). The two layers were separated, and the aqueous layer was extracted with 5% MeOH in CH_2Cl_2 (2 × 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na_2SO_4. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH_2Cl_2/MeOH/NH_4OH 10:1:0.1 to give 26 mg (52%) compound **8** as brown-white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.87−0.92 (6H, m), 1.03−1.12 (12H, m), 1.17−1.37 (m), 1.43−1.69 (m), 1.75−1.88 (6H, m), 1.99 (4H, m), 2.08 (3H, m), 2.13−2.19 (3H, m), 2.24−2.41 (13H, m), 2.54 (1H, d, J = 11.2 Hz), 2.75−2.80 (4H, m), 3.00 (1H, d, J = 9.6 Hz), 3.19 (4H, m), 3.47−3.51 (1H, m), 3.60−3.78 (6H, m), 4.14−4.22 (3H, m), 4.50 (1H, d, J = 7.2 Hz), 5.02 (1H, d, J = 4.8 Hz), 7.29 (2H, d, J = 8.0 Hz), 7.49 (2H, d, J = 8.4 Hz); ^{13}C NMR (acetone-d_6, 100 MHz) δ 7.6, 9.7, 11.6, 14.3, 15.3, 17.4, 18.9, 21.7, 21.9, 22.2, 22.5, 26.0, 26.1, 27.3, 27.9, 30.9, 32.6, 33.2, 35.5, 36.5, 36.8, 37.6, 43.0, 43.1, 46.1, 49.6, 58.6, 63.1, 64.1, 64.5, 66.2, 68.9, 70.0, 71.6, 73.3, 73.6, 74.2, 74.9, 75.3, 78.0, 78.9, 84.3, 95.6, 103.7, 119.8, 130.0, 135.0, 139.5, 171.9, 178.8; HRMS (EI) calcd for $[C_{52}H_{90}N_4O_{15} + H]^+$ 1011.6481, found 1011.6580

Desclasinose Azithromycinarylalkyl Hydroxamate (10). A mixture of compound **7** (0.050 g, 0.050 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated, and the aqueous layer was washed with EtOAc (2 × 20 mL), basified with concentrated NH_4OH, and then extracted with 5% MeOH in CH_2Cl_2 (2 × 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over Na_2SO_4. Solvent was evaporated off to give compound **9**, which was used for the next reaction without further purification.

To a solution of compound **9** in 1:1 THF/MeOH (2 mL) was added hydroxylamine (50% in H_2O) (0.05 mL, 0.790 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The mixture was partitioned between 5% MeOH in CH_2Cl_2 (30 mL) and saturated brine (20 mL). The two layers were separated, and the organic layer was dried over Na_2SO_4. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH_2Cl_2/MeOH/NH_4OH 9:1:0.1 to give 7 mg (16%) compound **10** as brown-white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.78 (3H, m), 0.85 (3H, d, J = 7.2 Hz), 0.91 (3H, d, J = 8.0 Hz), 0.99 (3H, s), 1.08−1.14 (10H, m), 1.18−1.79 (20H, m), 1.87 (2H, m), 1.98 (2H, t, J = 7.4 Hz),

2.05−2.13 (2H, m), 2.17 (3H, s), 2.25 (2H, t, J = 7.4 Hz), 2.52−2.65 (4H, m), 2.95 (1H, bs), 3.24 (m), 3.37−3.56 (6H, m), 3.67 (1H, d, J = 13.2 Hz), 4.53 (2H, d, J = 7.6 Hz), 7.19 (2H, d, J = 8.4 Hz), 7.39 (2H, d, J = 8.4 Hz); HRMS (EI) calcd for $[C_{44}H_{76}N_4O_{12} + H]^+$ 853.5538, found 853.5488.

4′-Ethynylbenzylazithromycin (12). To a solution of 4′-desmethylazithromycin **6** (0.940 g, 1.280 mmol) in anhydrous DMSO (15 mL) was added Hunig's base (1.5 mL) and 4-ethynylbenzyl methanesulfonate **11** (0.380 g, 1.800 mmol). The reaction mixture was heated with stirring under argon at 85 °C for 2 h. The mixture was cooled and diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ (3 × 60 mL) and saturated brine (60 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (silica, 12:1:0.05 CH_2Cl_2/MeOH/concentrated NH_4OH) to give 850 mg (78%) of **12** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (6H, m), 0.99 (3H, d, J = 7.6 Hz), 1.05 (7H, m), 1.11−1.32 (19H, m), 1.36−1.55 (2H, m), 1.68−1.77 (2H, m), 1.81−2.07 (4H, m), 2.22 (3H, s), 2.27−2.31 (4H, m), 2.51 (2H, m), 2.65−2.75 (2H, m), 2.87 (1H, bs), 2.98 (1H, t, J = 9.8 Hz), 3.04 (1H, s), 3.10 (3H, s), 3.29−3.34 (2H, m), 3.40−3.47 (2H, m), 3.58 (1H, d, J = 6.8 Hz), 3.65 (1H, s), 3.74 (1H, d, J = 13.2 Hz), 3.99 (1H, m), 4.21 (1H, dd, J = 2 Hz, 4.4 Hz), 4.38 (1H, d, J = 7.2 Hz), 4.65 (1H, dd, J = 2.8 Hz, 10 Hz), 5.06 (1H, d, J = 4.4 Hz), 7.23 (2H, d, J = 8 Hz), 7.41 (2H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.5, 9.2, 11.3, 14.9, 16.3, 18.3, 21.3, 21.4, 21.6, 22.0, 26.8, 27.6, 29.6, 34.7, 36.3, 36.9, 42.0, 42.3, 45.2, 49.2, 57.7, 62.3, 63.7, 65.4, 68.5, 70.0, 70.6, 72.7, 73.5, 73.8, 74.2, 77.1, 77.9, 78.0, 83.4, 83.7, 94.5, 102.6, 120.8, 128.5, 132.0, 139.6, 178.3; HRMS (FAB, mnba) calcd for $[C_{46}H_{76}N_2O_{12} + H]^+$ 849.5476, found 849.5411.

Descladinose-4′-ethynylbenzylazithromycin (13). A solution of 4′-ethynylbenzylazithromycin **12** (0.12 g, 0.14 mmol) in 0.25 N HCl (15 mL) was stirred at room temperature for 20 h and poured into EtOAc (20 mL). The two layers were separated, and the aqueous layer was washed with EtOAc (2 × 20 mL), basified with concentrated NH_4OH, and then extracted with 5% MeOH in CH_2Cl_2 (2 × 30 mL). The combined organic layer was washed with saturated brine (30 mL) and dried over Na_2SO_4. Solvent was evaporated off to give 89 mg (91%) of descladinose compound **12** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.80−1.52 (16H, m), 1.65−1.90 (2H, m), 1.97 (3H, s), 2.20−2.30 (6H, m), 2.42−2.69 (3H, m), 2.77 (1H, s), 2.89 (1H, s), 3.02 (1H, s), 3.18 (1H, s), 3.30−3.37 (3H, m), 3.49−3.63 (6H, m), 3.72 (2H, d, J = 10.6 Hz), 3.84 (1H, s), 3.89 (2H, s), 4.04 (3H, q, J = 14.4, 7.2 Hz), 4.41 (1H, d, J = 7.2 Hz), 4.64 (1H, d, J = 10.8 Hz), 7.15 (2H, d, J = 8.0 Hz), 7.37 (2H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.7, 7.9, 10.9, 14.2, 16.1, 20.9, 21.0, 21.2, 25.8, 26.6, 29.2, 35.9, 36.4, 37.0, 42.0, 44.5, 57.6, 60.3, 62.4, 65.3, 69.8, 70.4, 70.9, 73.0, 74.1, 75.4, 79.4, 83.3, 94.9, 106.4, 120.8, 128.2, 131.9, 139.2, 171.0, 177.2; HRMS (FAB, thioglycerol) calcd for $[C_{38}H_{62}N_2O_9 + H]^+$ 691.4533, found 691.4513.

Azithromycin-*N*-benzyltriazolyl-*O*-silylhexahydroxamate (15). 4′-Ethynylbenzylazithromycin **12** (0.045 g, 0.050 mmol) and 6-azido-*O*-silyl hexahydroxamate **14** (0.060 g, 0.146 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper(I) iodide (0.010 g, 0.050 mmol), Hunig's base (0.5 mL), and tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) (0.016 g, 0.030 mmol) were then added to the reaction mixture, and stirring continued for 2 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (2 × 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) to give 38 mg (60%) of silyl protected compound **15** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82−1.52 (39H, m) 1.71−2.04 (13H, m), 2.21 (3H, s), 2.28 (6H, s), 2.44−2.57 (2H, m), 2.65−2.70 (2H, m), 2.96 (1H, br s), 3.09 (2H, s), 3.31−3.35 (2H, m), 3.40−3.48 (6H, m), 3.56−3.60 (1H, m), 3.76 (1H, d, J = 13.2 Hz), 4.00 (1H, br s), 4.20 (3H, br s), 4.39 (1H, d, J = 6.8 Hz), 4.64 (1H, d, J = 9.2 Hz), 5.09 (1H, br s) 7.29−7.41 (10H, m), 7.64−7.83 (5H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.4, 9.1, 11.3, 14.7, 16.3, 18.2, 21.3, 21.4, 21.5, 22.0, 26.7, 27.5, 29.6, 29.7, 29.9, 34.6, 36.2, 36.9, 42.2, 45.2, 49.3, 49.9, 57.6, 62.4, 64.1, 65.4, 68.5, 69.9, 70.5, 72.7, 73.5, 74.1, 77.7, 77.9, 83.4, 94.3, 102.7, 119.2, 125.5, 127.6, 129.0, 135.5, 147.1, 178.4; HMRS (ESI) calcd for $[C_{68}H_{106}N_6O_{14}Si + H]^+$1259.7609, found 1259.7570.

Methyl Azithromycin-*N*-benzyltriazolylhexanoate (19a). 4′-Ethynylbenzylazithromycin **12** (0.045 g, 0.053 mmol) and azido ester **18** (0.014 g, 0.080 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper(I) iodide (0.010 g, 0.053 mmol) and Hunig's base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 25 mL) and again with saturated NH_4Cl (25 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with hexane/EtOAc/Et_3N 3:2:0.1 to give 50 mg (92%) of **19a** as a white-brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82−0.90 (3H, m), 0.98 (3H, d, J = 7.6 Hz), 1.05−1.52 (24H, m), 1.60−1.74 (6H, m), 1.80−2.06 (9H, m), 2.22−2.37 (9H, m), 2.56 (3H, m), 2.67 (3H, m), 2.95 (2H, t, J = 9.8 Hz), 3.07 (3H, m), 3.29−3.34 (1H, m), 3.46 (3H, m), 3.54 (1H, d, J = 6.8 Hz), 3.61 (3H, s), 3.68 (1H, bs), 3.77 (1H, m), 3.97 (1H, m), 4.18 (1H, m), 4.34−4.38 (3H, m), 4.69 (1H, m), 5.06 (1H, d, J = 4 Hz), 7.32 (2H, d, J = 6.4 Hz), 7.73−7.75 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.7, 9.2, 11.3, 14.2, 14.7, 16.5, 18.2, 21.4, 21.5, 22.2, 24.2, 25.9, 26.6, 27.3, 29.7, 30.0, 33.6, 34.6, 36.4, 36.9, 42.4, 45.3, 45.8, 49.3, 50.0, 51.5, 57.7, 63.9, 65.5, 68.6, 69.4, 70.5, 72.7, 73.8, 74.2, 77.2, 77.6, 78.0, 83.4, 94.4, 102.7, 119.3, 125.5, 129.1, 129.4, 147.2, 173.4, 178.1; HRMS (FAB, mnba) calcd for $[C_{53}H_{89}N_5O_{14} + H]^+$ 1020.6484, found 1020.6430.

Methyl Descladinoseazithromycin-*N*-benzyltriazolylhexanoate (19b). Compound **13** (0.080 g, 0.115 mmol) and azido ester **18** (0.030 g, 0.173 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper(I) iodide (0.010 g, 0.053 mmol) and Hunig's base (0.05 mL) were then added to the reaction mixture, and stirring continued for 12 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 25 mL) and again with saturated NH_4Cl (25 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC, eluting with hexane/EtOAc/Et_3N 3:2:0.1 to give 65 mg (65%) of **19b** as a white-brown solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79−0.86 (6H, m), 1.00−1.07 (6H, m), 1.17−1.26 (m), 1.42−1.51 (m), 1.55−1.72 (6H, m), 1.80−1.94 (6H, m), 2.00−2.05 (2H, m), 2.1 (3H, s), 2.23−2.27 (4H, m), 2.33 (3H, s), 2.47 (1H, d, J = 10.4 Hz), 2.58−2.72 (5H, m), 3.32−3.41 (3H, m), 3.52−3.73 (6H, m), 3.92−4.00 (2H, m), 4.34 (2H, t, J = 7.0 Hz), 4.41 (1H, d, J = 7.6 Hz), 4.69 (1H, d, J = 10.8 Hz), 7.24 (2H, d, J = 8.4 Hz), 7.71 (2H, d, J = 8 Hz), 7.73 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.7, 7.9, 8.7, 10.9, 16.1, 16.1, 20.9, 21.2, 24.2, 25.8, 25.9, 26.6, 29.2, 29.6, 30.0, 33.6, 36.0, 36.3, 37.1, 42.0, 44.5, 45.8, 50.0, 51.5, 57.7, 62.6, 65.1, 69.9, 70.4, 73.1, 74.1, 75.3, 79.4, 94.8, 106.4, 119.3, 125.5, 128.9, 129.6, 138.2, 147.1, 173.4, 177.2. HRMS (EI) calcd for $[C_{45}H_{75}N_5O_{11} + H]^+$ 862.5541, found 862.5566.

Representative Procedure for Conversion of Methyl or Ethyl Ester to Hydroxamic Acid. 6-Azidohexahydroxamic Acid (17a). To a solution of ethyl 6-azidohexanoate (1.00 g, 5.840 mmol) in 1:1 THF (5 mL) and anhydrous methanol (5 mL) were added aqueous hydroxylamine (4 mL, 70.100 mmol) and KCN (0.070 g, 1.170 mmol), and the stirring continued for 24 h. The mixture was diluted with EtOAc (30 mL) and washed with saturated $NaHCO_3$ (2 × 30 mL) and saturated brine (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give 797 mg (80%) of **17a** as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.24−1.30 (2H, m), 1.45−1.53 (4H, m), 1.93 (2H, t, J = 7.2 Hz), 3.29 (2H, t, J = 6.8 Hz), 8.65 (1H, s), 10.3 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.8, 26.1, 28.4, 32.6, 51.1, 171.4; HRMS (ESI) calcd for $[C_6H_{12}N_4O_2 + H]^+$ 173.0947, found 173.0983.

7-Azidoheptahydroxamic Acid (17b). Reaction of ethyl 7-azidoheptanoate (1.00 g, 5.400 mmol) and aqueous hydroxylamine

(4 mL, 70.100 mmol) within 24 h as described for the synthesis of **17a** gave 820 mg (82%) of **17b** as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.23−1.32 (4H, m), 1.45−1.52 (4H, m), 1.92 (2H, t, J = 7.6 Hz), 3.29 (2H, t, J = 7.2 Hz), 8.63 (1H, br s), 10.3 (1H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.1, 26.2, 28.5, 28.6, 32.7, 51.2, 171.6; HRMS (FAB, thioglycerol) calcd for $[C_7H_{14}N_4O_2 + H]^+$ 187.1195, found 187.1163.

8-Azidooctahydroxamic Acid (17c). Reaction of methyl 8-azidooctanoate (0.580 g, 2.910 mmol) and aqueous hydroxylamine (2.49 mL, 37.800 mmol) within 24 h as described for the synthesis of **17a** gave 432 mg (74%) of **17c** as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33−1.38 (6H, m), 1.54−1.63 (4H, m), 2.13 (2H, t, J = 7.6 Hz), 3.24 (2H, t, J = 6.8 Hz), 8.85 (1H, br); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.5, 26.7, 29.0, 29.1, 29.2, 33.1, 51.6, 172.0; HRMS (FAB, thioglycerol) calcd for $[C_8H_{16}N_4O_2 + H]^+$ 201.1351, found 201.1352.

9-Azidononahydroxamic Acid (17d). Reaction of methyl 9-azidononanoate (0.290 g, 1.370 mmol) and aqueous hydroxylamine (1.18 mL, 17.900 mmol) within 24 h as described for the synthesis of **17a** gave 225 mg (77%) of **17d** as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30−1.40 (8H, m), 1.56−1.61 (4H, m), 2.13 (2H, t, J = 7.2 Hz), 3.25 (2H, t, J = 8); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.6, 26.8, 29.0, 29.2, 29.3, 33.1, 51.6, 172.2; HRMS (FAB, thioglycerol) calcd for $[C_9H_{18}N_4O_2 + H]^+$ 215.1508, found 215.1529.

10-Azidodecahydroxamic Acid (17e). Reaction of methyl 10-azidodecanoate (0.300 g, 1.310 mmol) and aqueous hydroxylamine (1.13 mL, 17.100 mmol) within 24 h as described for the synthesis of **17a** gave 254 mg (84%) of **17e** as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.24−1.39 (8H, m), 1.54−1.65 (4H, m), 2.12 (2H, t, J = 7.6 Hz), 3.24 (2H, t, J = 7.2 Hz), 9.0 (1H, br); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.6, 26.9, 29.0, 29.2, 29.3, 29.4, 29.5, 33.2, 51.7, 172.2; HRMS (FAB, thioglycerol) calcd for $[C_{10}H_{20}N_4O_2 + H]^+$ 229.1665, found 229.1666

Azithromycin-*N*-benzyltriazolylhexahydroxamic Acid (16a). Method A. To a solution of compound **19a** (0.040 g, 0.040 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H_2O) (0.03 mL, 0.540 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The mixture was partitioned between 5% MeOH in CH_2Cl_2 (30 mL) and saturated sodium bicarbonate (25 mL). The two layers were separated, and the aqueous layer was extracted with 5% MeOH in CH_2Cl_2 (2 × 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na_2SO_4. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH_2Cl_2/MeOH/NH4OH 10:1:0.1 to give 6.5 mg (16%) of compound **16a** as a brown-white solid.

Method B. 4′-Ethynylbenzylazithromycin **12** (0.100 g, 0.109 mmol) and 6-azidohexahydroxamic acid **17a** (0.081 g, 0.117 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper(I) iodide (0.011 g, 0.070 mmol) and Hunig's base (0.5 mL) were then added to the reaction mixture, and stirring continued for 4 h. The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) to give 71 mg (59%) of **16a** as a brown-white solid.

Method C. To a solution of silyl protected compound **15** (0.025 g, 0.020 mmol) in THF (1 mL) was added 1 M TBAF in THF (0.030 mL, 0.030 mmol), and the mixture was stirred at room temperature for 2 h, during which TLC revealed a near-quantitative conversion to a lower R_f product. The mixture was partitioned between CH_2Cl_2 (30 mL) and saturated NH_4Cl (25 mL). The two layers were separated, and the organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/Et_3N) to give 15 mg (73%) of **16a** as a brown-white solid. ^{1}H NMR (acetone-d_6, 400 MHz) δ 0.83−0.92 (6H, m), 1.02 (3H, d, J = 7.6 Hz), 1.08−1.11 (8H, m), 1.14 (3H, d, J = 7.6 Hz), 1.18 (3H, d, J = 6 Hz), 1.24−1.29 (15H, m), 1.33−1.47 (3H, m), 1.54 (1H, dd, J = 4.8 Hz, 15.2 Hz), 1.60−1.69 (5H, m), 1.80−2.01 (m), 2.06−2.12 (1H, m), 2.18−2.24 (1H, m), 2.26 (3H, s), 2.28−2.31 (1H, m), 2.35−2.41 (4H, m), 2.51 (1H, d, J = 10 Hz), 2.65−2.96 (m), 3.12 (3H, s), 3.22−3.29 (1H, m), 3.47 (1H, m), 3.54−3.69 (6H, m), 3.81 (1H, d, J = 13.2 Hz), 4.11 (1H, m), 4.24 (1H, m), 4.45 (3H, t, J = 7.0 Hz), 4.50 (1H, d, J = 6.8 Hz), 4.75 (1H, d, J = 7.2 Hz), 4.97 (1H, d, J = 5.2 Hz), 7.42 (2H, d, J = 8.0 Hz), 7.84 (2H, d, J = 8.0 Hz), 8.35 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 6.5, 8.7, 11.5, 14.4, 16.7, 17.7, 21.3, 21.6, 21.8, 24.6, 25.7, 26.7, 27.0, 29.2, 29.6, 29.9, 33.0, 34.5, 35.6, 36.7, 41.8, 42.7, 45.3, 49.3, 50.0, 53.4, 57.8, 62.6, 63.4, 65.9, 68.6, 69.3, 70.4, 72.6, 73.3, 73.8, 77.8, 78.3, 78.4, 83.4, 94.5, 102.8, 119.6, 125.7, 129.3, 129.7, 138.6, 147.4, 171.3, 178.4; HMRS (ESI) calcd for $[C_{52}H_{88}N_6O_{14} + H]^+$ 1021.6437, found 1021.6409.

Desclasinose Azithromycin-*N*-benzyltriazolylhexahydroxamic Acid (16b). Method A. To a solution of compound **19b** (0.040 g, 0.050 mmol) in 1:1 THF/MeOH (3 mL) was added hydroxylamine (50% in H_2O) (0.04 mL, 0.540 mmol) and a catalytic amount of KCN. The mixture was stirred at room temperature for 24 h. The mixture was partitioned between 5% MeOH in CH_2Cl_2 (30 mL) and saturated sodium bicarbonate (25 mL). The two layers were separated, and the aqueous layer was extracted with 5% MeOH in CH_2Cl_2 (2 × 20 mL). The combined organic layer was washed with saturated brine (40 mL) and dried over Na_2SO_4. Solvent was evaporated off and the crude was purified by preparative TLC, eluting with CH_2Cl_2/MeOH/NH_4OH 10:1:0.1 to give 9.0 mg (23%) of compound **16b** as brown-white solid.

Method B. Reaction of descladinose compound **13** (0.134 g, 0.188 mmol) and 6-azidohexahydroxamic acid **17a** (0.130 g, 0.755 mmol) within 8 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 73 mg (43%) of **16b** as a brown-white solid. ^{1}H NMR (acetone-d_6, 400 MHz) δ 0.82−0.90 (9H, m), 1.02 (3H, d, J = 7.2 Hz), 1.07 (3H, s), 1.09 (3H, d, J = 6.8 Hz), 1.18−1.23 (m), 1.28 (3H, s), 1.31−1.39 (m), 1.46−1.56 (4H, m), 1.61−1.65 (3H, m), 1.80−1.99 (7H, m), 2.05−2.11 (2H, m), 2.18−2.21 (1H, m), 2.24 (3H, s), 2.25−2.29 (1H, m), 2.35 (3H, s), 4.47 (1H, d, J = 9.2 Hz), 2.61−2.67 (1H, m), 2.70−2.77 (1H, m), 3.30−3.34 (1H, m), 3.41 (1H, m), 3.52−3.65 (5H, m), 3.81 (1H, d, J = 13.2 Hz), 4.44 (2H, t, J = 7.0 Hz), 4.59 (1H, d, J = 7.6 Hz), 4.87 (1H, dd, J = 1.8 Hz, 11.0 Hz), 7.43 (2H, d, J = 8.4 Hz), 7.83 (2H, d, J = 8.4 Hz), 8.34 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.3. 7.9, 10.7, 16.0, 16.2, 20.9, 21.1, 24.4, 25.6, 25.7, 26.5, 29.0, 29.6, 29.7, 35.8, 36.3, 36.8, 42.1, 44.4, 50.0, 57.9, 62.7, 64.0, 69.8, 70.5, 70.8, 73.3, 74.1, 75.0, 79.5, 94.8, 106.5, 120.0, 125.8, 129.2, 129.5, 138.4, 147.3, 170.5, 177.5; HMRS (ESI) calcd for $[C_{44}H_{74}N_6O_{11} + H]^+$ 863.5494, found 863.5544.

Azithromycin-*N*-benzyltriazolylheptahydroxamic Acid (16c). Reaction of 4′-ethynylbenzylazithromycin **12** (0.134 g, 0.158 mmol) and 7-azidoheptahydroxamic acid **17b** (0.125 g, 0.672 mmol) within 4 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 93 mg (56%) of **16c** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81−1.51 (30H, m), 1.54−1.65 (6H, m), 1.70−2.14 (9H, m), 2.20−2.38 (9H, m), 2.46−2.56 (2H, m), 2.60−2.70 (2H, m), 3.00 (3H, s), 3.31 (2H, t, J = 8.8 Hz), 3.38−3.54 (6H, m), 3.60 (1H, s), 3.78 (1H, d, J = 12.8 Hz), 3.98−4.20 (2H, m), 4.36 (3H, d, J = 7.2 Hz), 4.49 (1H, d, J = 7.2 Hz), 5.11 (1H, d, J = 4.0 Hz), 7.32 (2H, d, J = 7.6 Hz), 7.73 (1H, s), 7.75 (2H, d, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 6.6, 8.8, 11.5, 14.4, 16.6, 17.7, 21.3, 21.6, 21.8, 25.1, 26.0, 26.7, 27.1, 28.2, 29.2, 29.6, 30.0, 33.1, 34.5, 35.7, 36.7, 41.8, 42.7, 45.3, 49.3, 50.3, 50.7, 57.9, 62.7, 63.0, 65.8, 68.6, 69.4, 70.4, 72.6, 73.2, 73.8, 77.8, 78.1, 78.2, 83.5, 94.4, 102.8, 119.3, 125.7, 129.4, 129.7, 138.4, 147.4, 171.3, 178.4; HMRS (ESI) calcd for $[C_{53}H_{90}N_6O_{14} + H]^+$ 1035.6587, found 1035.6628.

Descladinose Azithromycin-*N*-benzyltriazolylheptahydroxamic Acid (16d). Reaction of descladinose-4′-ethynylbenzylazithromycin **13** (0.130 g, 0.188 mmol) and 7-azidoheptahydroxamic acid **17b**

(0.130 g, 0.755 mmol) within 8 h, according to the protocols of Method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 78 mg (47%) of **16d** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.66−2.32 (42H, m), 2.47 (2H, d, J = 10.8 Hz), 2.63−2.70 (4H, m), 3.34−3.51 (6H, m), 3.62−3.69 (5H, m), 4.20−4.40 (5H, m), 4.74 (2H, br s), 7.26 (2H, br s), 7.73 (3H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.4, 7.9, 10.7, 16.0, 16.1, 20.9, 21.1, 25.0, 25.7, 26.5, 28.0, 28.9, 29.8, 35.8, 36.3, 36.9, 42.0, 44.4, 50.1, 57.9, 62.7, 63.9, 69.9, 70.4, 70.8, 73.3, 74.1, 75.2, 79.5, 94.9, 106.6, 119.7, 125.7, 129.2, 129.6, 138.4, 147.3, 177.5; HMRS (ESI) calcd for $[C_{45}H_{76}N_6O_{11} + H]^+$ 877.5645, found 877.5665.

Azithromycin-*N*-benzyltriazolyloctahydroxamic Acid (16e). Reaction of 4′-ethynylbenzylazithromycin **12** (0.100 g, 0.120 mmol) and 8-azidooctahydroxamic acid **17c** (0.047 g, 0.240 mmol) within 2.5 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 72 mg (58%) of **16e** as brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (3H, t, J = 4.0 Hz), 0.87−1.22 (18H, m), 1.29 (9H, s), 1.30−2.28 (18H, m), 2.29 (6H, s), 2.30−3.00 (8H, m), 3.10 (3H, s), 3.20−3.79 (9H, m), 3.99−4.03 (1H, m), 4.35−4.40 (3H, m), 4.65 (1H, d, J = 8.0 Hz), 5.11 (1H, d, J = 4.8 Hz), 7.34 (2H, d, J = 8.0 Hz), 7.72 (1H, s), 7.77 (2H, d, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 7.5, 9.8, 11.6, 15.4, 18.2, 19.1, 21.5, 22.1, 22.7, 25.6, 26.3, 26.6, 28.7, 29.0, 29.6, 30.2, 32.0, 32.8, 35.2, 36.4, 37.2, 42.2, 45.3, 49.2, 50.1, 58.3, 63.2, 65.4, 67.7, 70.8, 73.3, 74.2, 77.0, 78.4, 83.4, 102.8, 121.6, 125.6, 129.7, 130.0, 135.0, 147.0, 177.8; HRMS (FAB, thioglycerol) calcd for $[C_{54}H_{92}N_6O_{14} + H]^+$ 1049.6749, found 1049.6648.

Descladinose Azithromycin-*N*-benzyltriazolyloctahydroxamic Acid (16f). Reaction of descladinose-4′-ethynylbenzylazithromycin **13** (0.100 g, 0.144 mmol) and 8-azidooctahydroxamic acid **17c** (0.049 g, 0.246 mmol) within 2.5 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 94 mg (73%) of **16f** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84−0.91 (9H, m), 1.04−1.11 (9H, m), 1.23−1.30 (12H, m), 1.37−2.16(14H, m), 2.2 (3H, s), 2.35 (3H, s), 2.49−2.74 (5H, m), 3.22−3.75 (6H, m), 4.10−4.12 (1H, m), 4.34−4.42 (3H, m), 4.77 (1H, d, J = 12 Hz), 7.30 (2H, d, J = 7.6 Hz), 7.79 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 7.6, 8.2, 11.0, 14.4, 16.2, 16.4, 21.1, 21.4, 25.2, 25.9, 26.1, 26.7, 28.4, 28.8, 28.9, 29.1, 29.9, 30.2, 36.1, 36.6, 42.3, 44.6, 50.5, 51.6, 58.2, 60.6, 63.0, 64.0, 70.1, 70.7, 73.6, 74.4, 75.4, 79.7, 95.1, 106.8, 120.0, 126.0, 129.5, 129.9, 138.6, 147.6, 170.6, 177.8; HRMS (FAB, thioglycerol) calcd for $[C_{46}H_{78}N_6O_{11} + H]^+$ 891.5806, found 891.5776.

Azithromycin-*N*-benzyltriazolylnonahydroxamic Acid (16g). Reaction of 4′-ethynylbenzylazithromycin **12** (0.100 g, 0.120 mmol) and 9-azidononahydroxamic acid **17d** (0.043 g, 0.200 mmol) within 2.5 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 64 mg (51%) of **16g** as brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84−1.30 (30H, m), 1.33−2.26 (20H, m), 2.30 (6H, s), 2.38−2.68 (8H, m), 2.99 (3H, s), 3.32−3.84 (9H, m), 4.03−4.08 (1H, m), 4.35−4.41 (3H, m), 4.53 (1H, d, J = 8.0 Hz), 5.13 (1H, d, J = 4.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.75 (1H, s), 7.78 (2H, d, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 6.9, 9.0, 11.6, 14.7, 16.9, 18.0, 21.6, 21.8, 22.1, 25.6, 26.4, 26.9, 27.3, 28.8, 29.0, 29.1, 29.5, 29.9, 30.4, 34.8, 36.0, 37.0, 42.1, 43.0, 45.6, 49.5, 50.5, 58.1, 63.5, 66.1, 68.8, 70.7, 72.9, 74.1, 78.1, 78.3, 78.5, 83.7, 94.4, 94.7, 103.1, 119.6, 126.0, 129.7, 130.0, 147.6, 178.7; HRMS (ESI) calcd for $[C_{55}H_{94}N_6O_{14} + H]^+$ 1063.6900, found 1063.6861.

Azithromycin-*N*-benzyltriazolyldecahydroxamic Acid (16h). Reaction of 4′-ethynylbenzylazithromycin **12** (0.100 g, 0.120 mmol) and 10-azidodecahydroxamic acid **17e** (0.045 g, 0.20 mmol) within 4.5 h, according to the protocols of method B described for the synthesis of compound **16a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 70 mg (56%) of **16h** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85−1.36 (30H, m), 1.41−2.24 (22H, m), 2.28, 2.36 (6H, s), 2.33−3.10 (8H, m), 3.05 (3H, s), 3.23−3.82 (9H, m), 4.06−4.10 (1H, m), 4.36−4.41 (3H, m), 4.49 (1H, d, J = 8.0 Hz), 5.15 (1H, d, J = 4.0 Hz), 7.34 (2H, d, J = 8 Hz), 7.75 (1H, s), 7.78 (2H, d, J = 8.0 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 6.7, 9.0, 11.7, 14.6, 16.9, 17.9, 21.6, 21.9, 22.0, 25.6, 26.4, 26.8, 27.0, 27.3, 28.8, 29.0, 21.9, 29.3, 29.5, 29.9, 30.3, 33.6, 34.9, 35.8, 37.0, 42.1, 43.0, 45.6, 49.6, 50.6, 51.6, 58.0, 62.8, 63.9, 66.2, 68.9, 69.6, 70.7, 72.9, 73.5, 74.0, 74.1, 78.2, 78.6, 83.6, 94.6, 103.0, 119.6, 126.0, 129.6, 129.9, 138.9, 147.6, 178.6; HRMS (MALDI) calcd for $[C_{56}H_{96}N_6O_{14} + H]^+$ 1077.7057, found 1077.6971.

4′-Desmethylclarithromycin (20). To a solution of clarithromycin (3.320 g, 4.440 mmol) and sodium acetate (3.280 g, 39.900 mmol) in 80% aqueous methanol (50 mL) at 85 °C was added iodine (1.240 g, 4.890 mmol) in three batches within 5 min. The mixture was maintained at pH 8−9 by additions of 1 M NaOH (2 × 3 mL, once at 10 and 45 min of reaction time). Stirring was continued at 85 °C for 3 h, and TLC analysis indicated about 90% consumption of the starting material. A solution of 5% sodium thiosulfate (120 mL) and dichloromethane (80 mL) was added, and the two layers were separated. The aqueous layer was extracted with CH_2Cl_2 (60 mL) and the combined organic layers were washed with saturated brine, dried over Na_2SO_4, and concentrated in vacuo to give 2.4 g of **20**, which was used without further purification.

4′-Ethynylbenzylclarithromycin (21). To a solution of 4′-desmethylclarithromycin **20** (2.400 g, 3.340 mmol) in anhydrous DMSO (30 mL) was added Hunig's base (3 mL) and 4-ethynylbenzyl methanesulfonate **11** (0.920 g, 4.340 mmol). The reaction mixture was then heated with stirring under argon at 85 °C for 2.5 h. The mixture was cooled and diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ (3 × 60 mL) and saturated brine (60 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (silica, gradient 12:1; 10:1; 8:1; CH_2Cl_2/acetone) to give 1.8 g (63%) of **21** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (3H, t, J = 7.2 Hz), 1.03−1.28 (18H, m), 1.37 (3H, s), 1.40−1.55 (3H, m), 1.65−1.90 (6H, m), 2.03 (1H, d, J = 10.0 Hz), 2.22 (3H, s), 2.30 (1H, d, J = 15.2 Hz), 2.40−2.60 (2H, m), 2.80−2.90 (2H, m), 2.94−3.00 (6H, m), 3.04 (1H, s), 3.09 (3H, s), 3.16 (1H, s), 3.24−3.29 (1H, m), 3.38−3.46 (3H, m), 3.59 (1H, d, J = 6.8 Hz), 3.70−3.75 (3H, m), 3.88−3.95 (1H, m), 4.37 (1H, d, J = 7.2 Hz), 4.88 (1H, d, J = 4.4 Hz), 5.02 (1H, dd, J = 11.6, 2.4 Hz), 7.23 (2H, d, J = 12.0 Hz), 7.42 (2H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.2, 10.7, 12.4, 16.1, 18.1, 18.7, 19.9, 21.1, 21.5, 29.3, 32.4, 34.8, 36.9, 37.2, 39.2, 45.0, 45.2, 49.3, 50.6, 53.4, 57.6, 63.3, 65.6, 68.5, 69.0, 70.6, 72.5, 74.2,76.5, 77.8, 78.1, 78.2, 80.8, 95.8, 102.5, 120.9, 128.6, 132.0, 133.5, 139.4, 175.4; HRMS (ESI) calcd for $[C_{46}H_{73}NO_{13} + H]^+$ 848.5155, found 848.5181.

Descladinose-4′-ethynylbenzylclarithromycin (22). To a solution of 4′-ethynylbenzylclarithromycin **21** (0.500 g, mmol) in ethanol (20 mL) was added 1 N HCl (20 mL), and stirring continued for 22 h at room temperature. The reaction mixture was basified with concentrated NH_4OH to about pH 9. The reaction mixture was diluted with distilled water (40 mL) and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with saturated brine (40 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purifed by flash chromatography (silica, 8:1 CH_2Cl_2/acetone) to give 320 mg (79%) of **22** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (3H, t, J = 7.6 Hz), 1.09−1.28 (12H, m), 1.34 (3H, s), 1.40−1.55 (3H, m), 1.70−1.74 (2H, m),1.87−1.94 (3H, m), 2.08−2.15 (6H, m), 2.54−2.66 (2H, m), 2.94−2.98 (3H, m), 3.05 (1H, s), 3.25 (1H, s), 3.31−3.42 (2H, m), 3.48−3.56 (2H, m), 3.66 (2H, d, J = 10.0 Hz), 3.82 (1H, s), 3.90 (1H, s), 4.35 (1H, d, J = 7.6 Hz), 5.14 (1H, dd, J = 10.8, 2.0 Hz), 7.18 (2H, d, J = 8.0 Hz), 7.42 (2H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.4, 10.5, 12.7, 15.3, 16.3, 17.8, 18.8, 21.4, 29.2, 35.9, 36.6, 37.5, 38.7, 44.5, 45.5, 49.6, 57.8, 65.0, 69.7, 70.1, 70.6, 74.1, 77.9, 78.9, 83.3, 88.5, 106.5, 121.0, 128.4, 132.1, 139.1, 174.7; HRMS (ESI) calcd for $[C_{38}H_{59}NO_{10} + H]^+$ 690.4212, found 690.4259.

Clarithromycin-*N*-benzyltriazolyl-*O*-silyl-hexahydroxamate (23). 4′-Ethynylbenzylclarithromycin **21** (0.100 g, 0.118 mmol) and 6-azido-*O*-silyl hexahydroxamate **14** (0.103 g, 0.251 mmol) were dissolved in anhydrous THF (6 mL) and stirred under argon at room temperature. Copper(I) iodide (0.011 g, 0.057 mmol), TBTA (0.060 g, 0.094 mmol), and Hunig's base (0.1 mL) were then added to the reaction mixture, and stirring continued for 24 h. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (silica, 20:1 CH_2Cl_2/MeOH) to give 147 mg (99%) of compound **23** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.03−1.51 (30H, m), 1.67−1.88 (12H, m) 2.10−2.29 (8H, m), 2.53−2.58 (3H, m), 2.82−2.90 (2H, m), 2.93−3.01 (6H, m), 3.10 (3H, s), 3.17 (2H, s), 3.28−3.32 (1H, m), 3.40−3.48 (3H, m), 3.60−3.78 (4H, m), 3.91−3.97 (1H, m), 4.22 (1H, br s), 4.41 (2H, d, J = 8.0 Hz), 4.87 (1H, d, J = 4.8 Hz), 5.03 (1H, d, J = 10.8 Hz), 7.21−7.23 (2H, m), 7.30−7.42 (10H, m), 7.64−7.76 (3H, m); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.1, 10.6, 12.3, 16.0, 18.0, 18.6, 19.8, 21.0, 21.3, 21.5, 26.7, 29.3, 29.6, 29.9, 34.7, 36.8, 37.1, 39.0, 39.2, 45.0, 45.2, 47.0, 49.3, 49.9, 50.5, 53.4, 54.0, 65.5, 68.5, 68.9, 70.6, 72.3, 74.1, 76.4, 77.7, 78.1, 78.2, 80.7, 95.7, 102.5, 119.3, 123.5, 125.4, 127.7, 128.4, 128.8, 129.1, 133.5, 134.4, 135.5, 143.9, 147.0, 175.4; HMRS (ESI) calcd for $[C_{68}H_{103}N_5O_{15}Si + H]^+$1258.7292, found 1258.7313.

Clarithromycin-*N*-benzyltriazolylhexahydroxamic Acid (24a). Method A. 4′-Ethynylbenzylclarithromycin 21 (0.100 g, 0.120 mmol) and 6-azidohexahydroxamic acid **17a** (0.080 g, 0.470 mmol) were dissolved in anhydrous THF (5 mL) and stirred under argon at room temperature. Copper(I) iodide (0.011 g, 0.057 mmol) and Hunig's base (0.5 mL) were added to the reaction mixture, and stirring continued for 2.5 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 1:4 NH_4OH/saturated NH_4Cl (3 × 30 mL) and saturated NH_4Cl (30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) to give 70 mg (58%) of **24a** as a brown-white solid.

Method B. To a solution of compound **23** (0.085 g, 0.067 mmol) in anhydrous THF (1.5 mL) was added 1 M TBAF in THF (0.1 mL, 0.100 mmol). The mixture was stirred under argon for 2 h. The reaction mixture was diluted with 5% MeOH in dichloromethane (10 mL) and washed with saturated NH_4Cl (15 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified on preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/Et_3N). The purified product was dissolved in 5% MeOH in CH_2Cl_2 (10 mL) and washed with distilled water (10 mL) and saturated brine (10 mL) to remove the last trace of associated TBAF. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give 26 mg (38%) of **24a** as a brown-white product. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, t, J = 7.6 Hz), 1.03−1.52 (21H, m), 1.62−1.92 (12H, m) 2.04−2.29 (8H, m), 2.48−2.60 (3H, m), 2.82−2.90 (2H, m), 2.93−2.99 (6H, m), 3.09 (3H, s), 3.19 (2H, s), 3.28−3.33 (1H, m), 3.42−3.46 (3H, m), 3.60 (1H, d, J = 7.6 Hz), 3.70−3.80 (3H, m), 3.90−3.98 (1H, m), 4.37−4.40 (3H, m), 4.87 (1H, d, J = 4.8 Hz), 5.03 (1H, dd, J = 11.6, 2.4 Hz), 7.34 (2H, d, J = 7.6 Hz), 7.77 (2H, d, J = 7.6 Hz), 7.82 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.1, 10.5, 12.2, 15.9, 17.9, 18.5, 19.7, 20.9, 21.2, 21.4, 24.3, 25.5, 29.4, 29.6, 34.7, 36.8, 37.1, 39.0, 39.1, 45.0, 45.1, 49.3, 49.9, 50.5, 53.3, 57.5, 63.6, 65.5, 68.5, 69.0, 70.7, 72.4, 74.2, 77.8, 78.2, 80.9, 95.9, 102.6, 119.8, 125.6, 129.4, 147.4, 175.8; HMRS (ESI) calcd for $[C_{52}H_{85}N_5O_{15} + H]^+$ 1020.6114, found 1020.6121.

Descladinose Clarithromycin-*N*-benzyltriazolylhexahydroxamic Acid (24b). Reaction of descladinose-4′-ethynylbenzylclarithromycin **22** (0.075 g, 0.109 mmol) and 6-azidohexahydroxamic acid **17a** (0.040 g, 0.233 mmol) within 4 h, according to the protocols of method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 47 mg (51%) of **24b** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79 (3H, t, J = 7.2 Hz), 1.08−1.32 (22H, m), 1.39−1.64 (3H, m), 1.71−1.81 (2H, m), 1.82−1.96 (3H, m), 2.04−2.18 (6H, m), 2.51−2.70 (4H, m), 2.92−2.98 (3H, m), 3.18−3.38 (3H, m), 3.45−3.55 (2H, m), 3.60−3.74 (3H, m), 3.81 (3H, s), 3.90 (1H, s), 4.33 (3H, br s), 5.13 (1H, d, J = 10.4 Hz), 7.29 (2H, br s), 7.74 (2H, br s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.6, 10.7, 12.9, 15.5, 16.4, 18.0, 19.0, 21.5, 21.6, 29.5, 29.9, 36.1, 36.7, 37.7, 39.0, 44.7, 45.7, 49.8, 50.3, 58.2, 64.6, 70.0, 70.3, 70.9, 74.4, 78.3, 79.1, 88.5, 106.7, 120.3, 126.1, 129.6, 129.9, 147.7, 175.4; HMRS (ESI) calcd for $[C_{44}H_{71}N_5O_{12} + H]^+$ 862.5172, found 862.5155.

Clarithromycin-*N*-benzyltriazolylheptahydroxamic Acid (24c). Reaction of 4′-ethynylbenzylclarithromycin **21** (0.130 g, 0.153 mmol) and 7-azidoheptahydroxamic acid **17b** (0.105 g, 0.565 mmol) within 2.5 h, according to the protocols of method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 105 mg (67%) of **24c** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (3H, t, J = 8.0 Hz), 1.04−1.52 (21H, m), 1.67−1.92 (14H, m), 2.14−2.29 (8H, m), 2.52−2.60 (3H, m), 2.82−2.90 (2H, m), 2.95−3.00 (6H, m), 3.10 (3H, s), 3.16 (2H, s), 3.27−3.32 (1H, m), 3.41−3.46 (3H, m), 3.59 (1H, d, J = 6.8 Hz), 3.69−3.79 (3H, m), 3.90−3.95 (1H, m), 4.34−4.39 (3H, m), 4.87 (1H, d, J = 4.4 Hz), 5.02 (1H, d, J = 9.2 Hz), 7.33 (2H, d, J = 6.4 Hz), 7.77 (3H, d, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.1, 10.5, 12.2, 15.9, 17.9, 18.5, 19.8, 20.9, 21.2, 21.4, 24.7, 25.5, 27.7, 29.8, 34.7, 36.8, 37.1, 39.1, 45.0, 45.2, 49.3, 50.0, 50.5, 57.6, 63.6, 65.6, 68.6, 69.0, 70.7, 72.4, 74.2, 77.8, 78.3, 80.9, 95.9, 102.7, 119.5, 125.7, 129.4, 147.5, 175.8; HMRS (ESI) calcd for $[C_{53}H_{87}N_5O_{15} + H]^+$ 1034.6271, found 1034.6246.

Descladinose Clarithromycin-*N*-benzyltriazolylheptahydroxamic Acid (24d). Reaction of descladinose-4′-ethynylbenzylclarithromycin **22** (0.075 g, 0.109 mmol) and 7-azidoheptahydroxamic acid **17b** (0.040 g, 0.233 mmol) within 4 h, according to the protocols of method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 80 mg (84%) of **24d** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.78 (3H, t, J = 7.2 Hz), 1.06−1.31 (22H, m), 1.40−1.53 (5H, m), 1.71 (2H, d, J = 11.6 Hz), 1.80−1.91 (3H, m), 2.01−2.20 (6H, m), 2.50−2.65 (4H, m), 2.91−2.97 (3H, m), 3.16 (1H, t, J = 6.4 Hz), 3.26−3.35 (2H, m), 3.42−3.54 (2H, m), 3.64−3.71 (3H, m), 3.80 (1H, br s), 3.90 (1H, br s), 4.30−4.34 (3H, m), 5.12 (1H, dd, J = 11.6, 2.4 Hz), 7.27 (2H, d, J = 8.0 Hz), 7.72 (2H, d, J = 7.2 Hz), 7.80 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.3, 10.3, 12.5, 15.2, 16.1, 17.6, 18.6, 21.1, 21.3, 24.8, 25.1, 25.5, 26.2, 27.8, 28.5, 29.1, 29.6, 29.7, 32.3, 35.8, 36.3, 37.4, 38.6, 44.3, 45.4, 49.5, 50.0, 51.2, 57.8, 64.2, 69.7, 69.9, 70.6, 74.1, 77.9, 78.7, 88.0, 106.3, 119.9, 125.7, 129.3, 129.5, 138.3, 147.3, 175.1; HMRS (ESI) calcd for $[C_{45}H_{73}N_5O_{12} + H]^+$ 876.5329, found 876.5301.

Clarithromycin-*N*-benzyltriazolyloctahydroxamic Acid (24e). Reaction of 4′-ethynylbenzylclarithromycin **21** (0.101 g, 0.120 mmol) and 8-azidooctahydroxamic acid **17c** (0.047 g, 0.240 mmol) within 2.5 h, according to the protocols of method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 92 mg (74%) of **24e** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.04−2.05 (45H, m), 2.22 (3H, s), 2.19−2.82 (m, 7H), 3.00 (3H, s), 3.08 (3H, s), 2.91−3.80 (6H, m), 3.95 (3H, m), 4.38 (3H, m), 4.88 (1H, d, J = 4.4 Hz), 5.04 (1H, dd, J = 10.8, 2.0 Hz), 7.33 (2H, d, J = 7.6 Hz), 7.71 (1H, s), 7.77 (2H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.8, 9.4, 10.8, 12.5, 16.2, 18.2, 18.8, 20.0, 21.2, 21.5, 21.6, 25.1, 26.0, 26.7, 28.2, 28.6, 28.9, 29.9, 30.1, 35.0, 36.9, 37.4, 39.2, 39.4, 45.2, 45.4, 46.1, 49.6, 50.4, 50.8, 51.6, 58.1, 63.6, 65.9, 68.4, 69.3, 70.9, 72.8, 74.4, 78.0, 78.5, 81.2, 96.1, 120.1, 102.6, 126.1, 130.2, 147.4, 176.0; HRMS (ESI) calcd for $[C_{54}H_{90}N_5O_{15} + H]^+$ 1048.6486, found 1048.6427.

Descladinose Clarithromycin-*N*-benzyltriazolyloctahydroxamic Acid (24f). Reaction of descladinose-4′-ethynylbenzylclarithromycin **22** (0.10 g, 0.144 mmol) and 8-azidooctahydroxamic acid **17c** (0.049 g, 0.246 mmol) within 2.5 h, according to the protocols of method

A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 10:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 117 mg (90%) of **24f** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.10−2.09 (37H, m), 2.18 (3H, s), 2.19−2.68 (7H, m), 2.98−3.73 (4H, m), 3.83 (2H, s), 3.93 (1H, m), 4.36 (3H, m), 5.16 (1H, d, J = 8.0 Hz), 7.31 (2H, d, J = 8.0 Hz), 7.77 (2H, d, J = 8.0 Hz), 7.79 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 8.5, 10.6, 12.8, 15.4, 16.4, 17.9, 18.9, 21.4, 21.6, 29.4, 36.1, 36.7, 37.7, 38.9, 44.7, 45.7, 49.8, 58.0, 65.2, 70.0, 70.4, 70.8, 74.4, 76.8, 78.2, 79.2, 83.6, 88.8, 106.9, 121.3, 128.7, 132.5, 139.6, 175.2; HRMS (FAB, thioglycerol) calcd for $[C_{46}H_{76}N_5O_{12} + H]^+$ 890.5490, found 890.5562.

Clarithromycin-*N*-benzyltriazolylnonahydroxamic Acid (24g). Reaction of 4′-ethynylbenzylclarithromycin **21** (0.100 g, 0.120 mmol) and 9-azidononahydroxamic acid **17d** (0.043 g, 0.20 mmol) within 2.5 h, according to the protocols of method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 54 mg (42%) of **24g** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, t, J = 7.2 Hz), 1.04−2.02 (47H, m), 2.24 (3H, s), 2.10−2.97 (7H, m), 3.00, 3.09 (6H, s), 3.20−3.82 (6H, m), 3.88 (3H, m), 4.39 (3H, m), 4.88 (1H, d, J = 4.0 Hz), 5.05 (1H, d, J = 10.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.77 (3H, d, J = 4.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.0, 9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 21.2, 21.5, 21.6, 21.7, 21.8, 26.3, 26.8, 28.7, 28.9, 29.1, 29.9, 30.3, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.4, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.9, 138.7, 147.6, 176.1; HRMS (ESI) calcd for $[C_{55}H_{91}N_5O_{15} + H]^+$ 1062.6584, found 1062.6586.

Clarithromycin-*N*-benzyltriazolyldecahydroxamic Acid (24h). Reaction of 4′-ethynylbenzylclarithromycin **21** (0.10 g, 0.120 mmol) and 10-azidodecahydroxamic acid **17e** (0.045 g, 0.197 mmol) within 2.5 h, according to the protocols of Method A described for the synthesis of compound **24a**, followed by preparative TLC (silica, 12:1:0.1 CH_2Cl_2/MeOH/concentrated NH_4OH) gave 68 mg (53%) of **24h** as a brown-white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) 0.82 (3H, t, J = 7.2 Hz), 1.05−2.12 (49H, m), 2.24 (3H, s), 2.26−2.97 (7H, m), 3.01, 3.10 (6H, s), 3.19−3.80 (6H, m), 3.95 (3H, m), 4.39 (3H, m), 4.89 (1H, d, J = 4.0 Hz), 5.04 (1H, d, J = 8.0 Hz), 7.35 (2H, d, J = 8.0 Hz), 7.76 (1H, s), 7.79 (2H, d, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.3, 10.8, 12.5, 16.1, 18.2, 18.8, 20.0, 21.2, 21.5, 21.7, 25.4, 26.2, 28.9, 29.0, 29.3, 29.6, 29.9, 30.2, 35.0, 37.0, 37.4, 39.3, 39.4, 45.2, 45.4, 49.6, 50.5, 50.8, 51.6, 57.8, 63.8, 65.8, 68.8, 69.2, 70.9, 72.7, 74.5, 76.8, 78.1, 78.5, 81.1, 96.1, 102.9, 119.7, 125.9, 129.6, 129.8, 138.9, 147.6, 176.1; HRMS (ESI) calcd for $[C_{56}H_{93}N_5O_{15} + H]^+$ 1076.6740, found 1076.6667.

HDAC Activity Assay. In vitro HDAC inhibition was assayed using the HDAC Fluorimetric Assay/Drug Discovery Kit as previously described.[17,19] Briefly, 15 μL of HeLa nuclear extract was mixed with 5 μL of 10× compound and 5 μL of assay buffer. Fluorogenic substrate (25 μL) was added, and reaction was allowed to proceed for 15 min at room temperature and then stopped by addition of a developer containing TSA. Fluorescence was monitored after 15 min at excitation and emission wavelengths of 360 and 460 nm, respectively. IC_{50} values were determined using logit plots.

Cell Culture and Viability. SK-MES-1 and NCI-H69 lung cancer cell lines and DU-145 prostate cancer cell line were obtained from ATCC (Manassas, VA) and were maintained in the recommended complete growth mediums. MCF-7 cell line (a generous gift from Dr. Al Merill) was maintained in EMEM containing 10% fetal bovine serum. Human mammary epithelial cells (HMEC) (Lonza Biosciences) were maintained in complete MEGM per product instructions. Human normal lung fibroblasts (a generous gift from Dr. Barker) were maintained in EMEM containing 10% FBS. All cell lines were maintained in a 37 °C environment containing 5% CO_2. All compounds to be tested were dissolved to a concentration of 10 mM in DMSO and stored at −80 °C. For cell viability experiments, cells were passaged 24 h prior to dosing. All compounds were diluted to appropriate concentrations in DMSO and fresh medium such that the final concentration of DMSO was 0.1%. To control wells, only fresh medium was added. Viability was assessed after 72 h by trypan blue staining.

Western Blotting. SK-MES-1 cells were passaged 24 h prior to dosing with test compounds. Compounds were diluted to appropriate concentrations and were added to fresh medium so that the final concentration of DMSO was 0.1%. Cells were dosed with compounds for 8 h. Cells were washed with ice-cold PBS, recovered by centrifugation, and then lysed on ice in RIPA buffer containing protease inhibitors. Lysates were mixed repeatedly by pipetting and centrifuged at 14 000 rpm at 4 °C. Protein concentration was determined by Bradford assay (Bio-Rad). Protein denaturation and electrophoresis were performed as described.[27] Proteins were transferred to a nitrocellulose membrane for 1 h at 4 °C. The membrane was then blocked for 1 h in Odyssey blocking buffer (LI-COR Biosciences). Mouse anti-p21waf1 (Zymed) and rabbit antiactin (Sigma) primary antibodies and IR-labeled secondary antibodies (LI-COR Biosciences) were added as recommended (LI-COR Biosciences), and two-color detection was used to image actin and p21waf1 by incubating with different host animal secondary antibodies. Detection was performed on an Odyssey infrared imaging system (LI-COR Biosciences). Blots were imaged simultaneously in both 700 and 800 nm channels at 169 μm resolution and analyzed on the imager software.

Acknowledgment. We thank Professor Jim Powers for a helpful discussion on the manuscript. We are grateful to Professors Al Merrill and Donald Doyle for the use of their cell culture facilities. We thank Professor Thomas Barker for a generous gift of primary lung fibroblasts. We are indebted to Chiaolong Hsiao for his assistance on molecular docking. This work was financially supported by Georgia Institute of Technology (A.K.O. and Y.F.), by the Blanchard fellowship to A.K.O., by Georgia Cancer Coalition Distinguished Cancer Clinicians and Scientists Program, and by NIH Grant GM085261 (Y.F.). P.C.C. and W.G. are recipients of the GAANN predoctoral fellowship from the Georgia Tech Center for Drug Design, Development and Delivery. R.H. is a Beckman Undergraduate Research Fellow.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectral information, docked structure of **16d**, and isoform selectivity results. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM801128G