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A Structure-activity Relationship of Non-peptide Macrocyclic Histone Deacetylase Inhibitors and Their Anti-proliferative and Anti-inflammatory Activities

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Abstract

Inhibition of the enzymatic activity of histone deacetylase (HDAC) is a promising therapeutic strategy for cancer treatment and several distinct small molecule histone deacetylase inhibitors (HDACi) have been reported. We have previously identified a new class of non-peptide macrocyclic HDACi derived from 14- and 15-membered macrolide skeletons. In these HDACi, the macrocyclic ring is linked to the zinc chelating hydroxamate moiety through a *para*-substituted aryl-triazole cap group. To further delineate the depth of the SAR of this class of HDACi, we have synthesized series of analogous compounds and investigated the influence of various substitution patterns on their HDAC inhibitory, anti-proliferative and anti-inflammatory activities. We identified compounds **25b** and **38f** with robust anti-proliferative activities and compound **26f** (IC₅₀ 47.2 nM) with superior anti-inflammatory (IC₅₀ 88 nM) activity relative to SAHA.

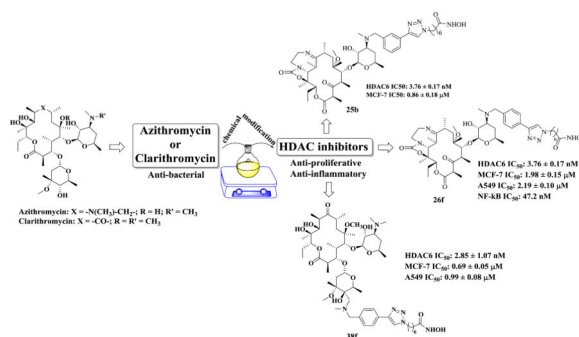
Graphical Abstract

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Supporting Information Available: ¹H NMR, ¹³C NMR spectral and NF-κB activity in NTHi infected BEAS-2B cells treated with 1 μM of tested compounds. This material is available free of charge via the Internet at



Keywords

Histone deacetylase (HDAC); histone deacetylase inhibitors (HDACi); non-peptide macrocyclic HDACi; anti-cancer; structure activity relationship study (SAR); anti-inflammation; NTHi; NF-κB

Introduction

Histone deacetylases (HDACs) are an emerging therapeutic target for cancer and other diseases such as malaria and leishmania.^{1,2} Together with histone acetyltransferases (HATs), they regulate the chromatin structure by controlling the acetylation state of histone proteins as well as non-histone proteins such as tubulin, ERα, p53, HSP90, NF-YA, and GATA-1.³ Histone deacetylase inhibitors (HDACi) have been shown to cause growth arrest, differentiation, and apoptosis in a variety of cancer cell lines.⁴ To date, several classes of small molecule HDAC inhibitors – fitting a three-motif pharmacophoric model, namely, a zinc binding group (ZBG), a hydrophobic linker, and a recognition cap group⁵ – have been reported. Moreover, many HDACi have been approved for hematological malignancies (Fig. 1a). The approved HDACi are suberoylanilide hydroxamic acid (SAHA)^{6,7} and FK228⁸ approved for the treatment of cutaneous T-cell lymphoma, Panobinostat and Chidamide, approved for multiple myeloma,^{9,10} and Belinostat recently granted accelerated approval for peripheral T cell lymphoma.¹¹ Macrocyclic HDACi (Fig. 1b) possess the most complex cap group moieties capable of optimal interactions with amino acid residues near the entrance of the HDAC active site, an attribute that is essential for the modulation of the biological activities of these agents. Although they possess potent HDAC inhibition activity (nanomolar range), the interest in their clinical development has been hampered by the disadvantages associated with the peptide group(s) present in the macrocycles and the difficulty in the synthesis of strained ring frameworks for structure activity relationship (SAR) studies.¹²

In previous studies, we have shown that macrocycles derived from two 14-membered macrolide rings – clarithromycin (**5e** and **5f**) and TE-802 (**26e** and **26f**), and a 15-membered azalide ring – azithromycin (**17e** and **17f**), are excellent mimetics for the peptide backbone of macrocyclic HDACi.^{13,14,15} The replacement of the amide moiety, by its bioisostere, triazole unit, increased the HDAC inhibitory potency of matched compounds by almost 8-fold [**17f** (HDAC1/2 IC₅₀ 13.9 nM vs **A** (HDAC1/2 IC₅₀ 107.1 nM)].¹⁴ Drawing inspiration from the naturally occurring HDACi such as TSA (trichostatin A) we have hitherto studied

only the *para*-substitution pattern of the aryl-triazole cap group of these non-peptide macrocyclic HDACi.^{14,15}

To further understand the depth of the SAR of this class of HDAC inhibitors, we investigated and disclosed herein the consequence of (i) *ortho*-, *meta*-, and *para*-substitution pattern of the triazole cap group; (ii) varied methylene linker lengths; and (iii) point of attachment of aryl-triazole cap group, on their biological activities. We observed that the new compounds reported here possess anti-HDAC, anti-proliferative and anti-inflammatory activities that are highly dependent of the identity of the macrolide, the point of attachment of the HDAC inhibition group and the methylene linker lengths.

Chemistry

The syntheses of the target compounds are achieved following the reaction routes shown in schemes 1–4. The crucial ethynylbenzyl moieties were installed at either the desosamine sugar (azithromycin and clarithromycin) or *N10* position of azithromycin to furnish the requisite alkynyl-macrolides **4**, **7**, **12**, **13** and **14** following the literature procedure.^{14,15,16,17} Subsequently, copper (I) catalyzed azide-alkyne-cycloaddition (AAC)¹⁸ reaction between TBS-protected azidohydroxamates **51a–f** and compounds **4**, **7** and **12–14**, followed by removal of TBS-group¹⁹ afforded the final compounds **5a–d**, **8a–f**, **15a–b**, **16a–b**, and **17a–d** (Scheme 1).

The triketolide derived hydroxamic acid compounds were synthesized in two steps starting from previously reported intermediate 3'-desmethyltricyclic ketolide **18**.^{15,20a} Reductive amination reactions between **18** and 2-ethynylbenzaldehyde **19**, 3-ethynylbenzaldehyde **20**, 4-ethynylbenzaldehyde **6** yielded 3'-(2-ethynylbenzyl)tricyclic ketolide **21**, 3'-(3-ethynylbenzyl)tricyclic ketolide **22**, and 3'-(4-ethynylbenzyl)tricyclic ketolide **23** in 53%, 83%, and 65% respectively. AAC reaction between TBS-protected azidohydroxamates **51a–f** and compounds **21–23** followed by removal of TBS-group afforded the desired compounds **24a–b**, **25a–b**, and **26a–d** (Scheme 2).

Introduction of the ethynylbenzyl moiety to cladinose sugar of clarithromycin **27** and azithromycin **28** was achieved in four steps.^{20b} Acetic anhydride treatment of clarithromycin **27** and azithromycin **28** in dichloromethane gave selective 2'-*O*-acetylclarithromycin **29** and 2'-*O*-acetylazithromycin **30**. Corey-Kim oxidation of **29** and **30** followed by Corey-Chaykovsky epoxidation of intermediates 4''-oxo-2'-*O*-acetylclarithromycin **31** and 4''-oxo-2'-*O*-acetylazithromycin **32** yielded epoxy compounds **33** and **34**. Diastereoselective opening of epoxides **33** and **34** with 4-ethynylbenzyl-*N*-methyamine **35** in methanol, followed by a concomitant acetyl group deprotection, gave key intermediates **36** and **37** respectively. The alkynyl intermediates **36** and **37** were subjected to AAC reaction with various TBS-protected azido hydroxamates **51a–f** followed by removal of TBS-group to give target molecules **38a–f** and **39a–f** in moderate to good yields (scheme 3).

We also synthesized macrocyclic-nonpeptide-hydroxamic acids with dimethylamino methyl group placed in the C4'' position of cladinose sugar (scheme 4), a substitution which may impact extra acid-stability to the cladinose sugar glycosidic bond.^{20c} Also, we were

interested in probing if this small change influenced the HDAC inhibitory and antiproliferative activity profile. Syntheses of the target compounds **49a–b** and **50a–b** were achieved from intermediates **4** and **14** following the same route as described for compounds **38a–f** and **39a–f**. The previously synthesized compounds **5f**, **17f**, and **26f** are included here as controls for each macrolide group.

Results and discussion

All the new and six previously synthesized controls (**5e**, **5f**, **17e**, **17f**, **26e**, **26f**)^{14,15} compounds were tested against recombinant class I (HDAC1 and HDAC8) and class IIb (HDAC6) enzymes to evaluate their anti-HDAC activities. HDAC activity was determined by the label-free mass spectrometry-based SAMDI assay.²¹ As anticipated from previous observations, most of these new analogs were less active against HDAC8 except for compounds **5f** (IC₅₀ 713 nM, Table 1), **16a** and **17f** (IC₅₀s 604 nM and 314 nM, respectively, Table 2), and **25b** (IC₅₀ 310 nM, Table 4).

Analysis of the HDACs 1 and 6 inhibitory effects of these compounds revealed an interesting trend that is largely dependent on the macrolide template, the substitution pattern on the aryl cap group and the length of the methylene spacer-group separating the triazolyl group from the hydroxamate moiety. For compounds in the same macrolide series, an increase in the length of the methylene spacer (C1 – C6) resulted in gradual increase in HDAC1 and HDAC6 inhibitory potencies. In most cases, the maximum change was observed at five methylene spacers and potency plateaued at six methylene spacers. In general, compounds having five or six methylene spacers showed low to mid nanomolar HDAC1 (**5e–f**, **8e–f**, **15a–b**, **16a–b**, **17e–f**, **25a–b**, **26e–f**, **38e–f**, **39e–f**, **49a–b**, **50a–b**) and HDAC6 (**5e–f**, **8e–f**, **15a–b**, **16a–b**, **17e–f**, **25a–b**, **26e–f**, **38e–f**, **39e–f**, **49a–b**, **50a–b**) inhibitory potency (Tables 1–4). Conversely, compounds having one to four methylene spacers were either inactive or very poorly active (**5a–d**, **8a–d**, **17ad**, **26a–d**, **38a–d**, and **39a–d**) (Tables 1–4).

Introduction of the dimethylamino methyl group at cladinose sugar C4 position had a modest effect on HDAC1 inhibitory activities in both clarithromycin (**49a–b**) and azithromycin (**50a–b**) series when compared to their analogs **5e–f** and **17e–f**. However, HDAC6 inhibition was affected substantially in the case of the clarithromycin compounds, with a 5 to 10 fold drop in potency (**49a**: IC₅₀ 32.9 nM; **49b**: IC₅₀ 31.4 nM compared to **5e**: IC₅₀ 3.61 nM, **5f**: IC₅₀ 6.67 nM). Attachment of the aryl-triazolyl cap group to cladinose sugar (**38a–f** and **39a–f**) also influenced HDAC1 and HDAC6 inhibitory activities. In the clarithromycin series, compound **38c**, in which the hydroxamic acid moiety was separated from aryl-triazole cap by three methylene groups, showed a five-fold decrease in HDAC6 (IC₅₀ 361 nM) inhibitory potency compared to the analogous desosamine modified compound **5c** (IC₅₀ 70.9 nM) and was completely inactive against HDAC1 (27% at 10 μ M for **38c** vs 9.91 μ M IC₅₀ value for **5c**). Interestingly for **38f**, in which the hydroxamic acid moiety was separated from the aryl-triazole cap by six methylene groups, the HDAC6 inhibitory activity increased by two fold compared to control compound **5f** (IC₅₀ 2.85 nM vs 6.67 nM, respectively). Moreover, the HDAC1 inhibitory activity of **38f** was eight-fold higher than that of **5f** (IC₅₀ 23.9 nM vs IC₅₀ 207 nM, respectively). In azithromycin series,

the only noticeable change was exhibited by compound **39f** which showed a four-fold loss in HDAC6 inhibition potency, relative to the analogous control compound **17f** (IC₅₀ 31.3 nM vs 7.29 nM, respectively).

Docking studies on compounds **8a–f** (azithromycin-derived analogs with aryl-triazolyl cap group attached to N10 position of azithromycin), using AutoDock Vina as previously reported,^{14,15,20d} predicted that compounds with 4–6 methylene spacers would have HDAC1 and HDAC6 inhibitory activities (supplementary information). HDAC inhibition data presented in Table 3 validated the docking prediction as compound **8f** emerged the best in this series with low nonamolar HDAC1 and single digit nanomolar HDAC6 inhibition activities.

To further delineate the SAR of this class of HDACi, with synthesized compounds **15a–b**, **16a–b**, **24a–b** and **25a–b**, azithromycin- and triketolide-derived analogs having ortho- and meta-substitution patterns at the aryltriazolyl cap group. For the azithromycin series, the ortho-substituted compounds **15a–b**, and meta-substituted compound **16b** have attenuated anti-HDAC activities relative to the corresponding para-substituted compounds **17e–f**. The meta-substituted, five methylene-linked compound **16a** is slightly more potent than the corresponding parasubstituted compound **17e** against HDAC1 while the trend is reversed against HDAC6. However, the two compounds have identical HDAC8 inhibition activities (Table 2). The triketolide-based ortho- and meta-substituted compounds **24a–b** and **25a–b** are mostly less active against the corresponding para-substituted compounds **26e–f** with the ortho-substituted compounds **24a–b** devoid of HDAC1 inhibition activity. An exception within this series is compound **25b** which is approximately 2- and 5-fold more potent than the analogous **26e** against HDAC6 and HDAC8 respectively (Table 4).

Cell growth inhibitory assay

To verify if the anti-HDAC activities of these macrolide-derived hydroxamates translate to antiproliferative activities, we tested representative members of each group against two transformed cell lines – lung (A549) and breast (MCF-7) cancers – and one normal cell line (Vero - monkey kidney epithelial cell). We selected compounds with diverse anti-HDAC activities, ranging from those with weak HDAC inhibition activities to those which potentially inhibit the HDAC isoforms tested. SAHA and the previously disclosed control compounds **5f**, **17f** and **26f** are all cytotoxic to the two cancer cell lines (Table 5). We observed that compounds **8d**, **15b**, **17c** and **24b** are devoid of antiproliferative activities up to the maximum tested concentration. This observation may not be surprising since these compounds either lack or poorly inhibit HDAC1. Surprisingly however, compounds **25a** and **39f**, despite their good anti-HDAC1 activities, are non-cytotoxic against A549 and MCF-7 cells. This result is in contrast to the effect of **16b** which, despite having a similar anti-HDAC1 activity as **25a** and **39f**, is robustly anti-proliferative against the two cancer cell lines. The reason for the lack of whole cell effect of **25a** and **39f** is not obvious from this study. However, the triketolide-derived compound **25b**, an analog of **25a** with a single extra methylene linker but not much different anti-HDAC1 activity, was cytotoxic to both cancer cell lines with a strong preference for the MCF-7 cell (IC₅₀ ≈ 900 nM). All other strongly HDAC inhibiting compounds tested (**8f**, **16b**, **25b**, **38f**, **49b** and **50b**) possess varying degree

of antiproliferative activities against A549 and MCF-7 cell lines. For analogous compounds (same methylene-linker length), the macrolide type and the points of attachment to the macrolide templates are strong determinants of potency. Specifically, the azithromycin-derived desosamine functionalized compound **17f**, despite its weaker anti-HDAC1 activity, is slightly more cytotoxic than the analogous N-10 modified compound **8f** and the cladinose sugar amine functionalized **50b**. However, amine functionalization of the cladinose sugar enhanced the cytotoxicity of the clarithromycin compound **49b** relative to analogous azithromycin **50b**. In fact, the cladinose ring is the optimum point of attachment of the HDAC inhibiting moiety to the clarithromycin template as the resulting HDACi **38f** is the most potent among the compounds tested for antiproliferative activity. In contrast, the analogous azithromycin compound **39f** is inactive (Table 5).

To obtain preliminary information about tumor cell selectivity, we tested these compounds against the non-transformed Vero cell line. The control compound SAHA is not tumor cell selective as it is about 3–5 folds more cytotoxic to the Vero cell compared to the two tumor cell lines. In contrast, the macrolide-derived compounds are either significantly less cytotoxic or equally cytotoxic to the transformed and normal cells tested. Compound **25b** has a selectivity edge over others, being 2.7-fold and 23-fold more selective for A549 and MCF-7 respectively (Table 5).

Anti-inflammatory Activity Assay

Inflammation is a salient factor in cancer, particularly lung cancer and many other chronic lung diseases.^{22,23,24} HDACs have been implicated in the regulation of inflammation and HDACi²⁵ such as SAHA,²⁶ trichostatin A (TSA),²⁷ butyrates,²⁸ and MS-275,²⁹ attenuate cellular inflammation processes through inhibition of NF- κ B activation and or blockage of proinflammatory cytokine release.³⁰ In addition to their antibiotic activities, the macrolide templates (azithromycin, and clarithromycin) for the HDACi described herein have intrinsic antiinflammatory and immunostimulatory activities. To test if these macrolide-derived HDACi preserve these useful properties and additively or synergistically enhance the latent antiinflammatory activity of HDACi, we evaluated their anti-inflammatory activities in BEAS-2B cell infected with nontypeable *Haemophilus influenzae* (NTHi) using NF- κ B luciferase assay³¹ NTHi is a Gram-negative bacterium which causes infection in the human respiratory tract.^{32,33} Upon infection by NTHi, transcriptional regulator, NF- κ B, in human epithelial cell is strongly activated by translocating from cytoplasm to nucleus and consequently up-regulating certain proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α . To pre-screen these compounds for their effect on NF- κ B activity, we treated them with NTHi infected BEAS-2B cells at 1 μ M. We observed that compounds lacking or those with weak anti-HDAC activities did not suppress NF- κ B activation while those compounds with potent anti-HDAC activities suppressed NF- κ B activation to varying degrees which closely correlate with their HDAC inhibition potency (Supporting Information, Figure S1).

We then determined the IC₅₀ values of selected compounds which suppressed NF- κ B activation in the pre-screening. The tested compounds were selected from each of the three macrolide templates reported here and we used SAHA as a positive control. We observed that these compounds suppressed the NTHi-induced NF- κ B activation with IC₅₀ ranging

from low to high nanomolar. The only exception is **25a**, which despite suppressing NF- κ B activation at 1 μ M, showed no dose-dependent effect. Based on IC₅₀, the TE-802-derived **26f** is the most potent among these compounds and it is 2-fold more potent than SAHA (Table 6). In addition to **26f**, compounds **5f**, **8f**, **38f** and **49b** have lower I_{max} value compared to SAHA, implying that at maximum concentration of 1 μ M, these compounds suppressed NF- κ B activity more than SAHA. Interestingly, the starting macrolide templates did not exhibit any anti-inflammatory activity in this assay as their relative percentage luciferase activity was indistinguishable from no drug treatment in presence of NTHi (100%). To further confirm the mechanism of anti-inflammatory activities of this class of macrolide HDACi, we performed Q-PCR analysis to determine the effect of these HDACi on the expression levels of mRNAs of inflammatory cytokines known to be NTHi-inducible.^{40, 41} We observed that representative macrolide-derived HDACi **8f**, **26f** and **38f** more significantly suppressed NTHi-induced TNF- α , IL-1 α , IL-1 β mRNA expression relative to SAHA in BEAS-2B cells (Fig. 2). Collectively, these data further suggest that the suppression of NF- κ B activation induced by these compounds is derived from their HDAC inhibition activities.

Conclusion

We have synthesized diverse series of non-peptide macrocyclic hydroxamic acid based HDAC inhibitors derived from three macrolide skeletons to further explore the SAR of this class of HDACi. Several of these compounds exhibited nanomolar anti-HDAC activity against recombinant HDAC1 and HDAC6 enzymes. Among the new compounds tested for whole cell activity, compounds **16b**, **38f**, and **49b** potently inhibited lung cancer cell line (A549) while **16b**, **25b**, **38f**, and **49b** potently inhibited breast cell line (MCF-7). Unlike SAHA which is much more toxic to the non-transformed Vero cells, these macrolide-derived compounds are either significantly less cytotoxic to the Vero cells or equally cytotoxic to the transformed and normal cells tested with compound **25b** being the most tumor cell line selective. Also, many of these compounds exhibited anti-inflammatory activity in NTHi infected BSAS-2B cells and a lead compound (**26f**) is 2-fold more potent than SAHA.

Experimental

Materials and methods

All commercially available starting materials were used without further purification. Clarithromycin and azithromycin were purchased from Greenfield Chemicals. 2-Ethynylbenzyl alcohol, 3-bromobenzaldehyde, and 4-ethynylbenzyl alcohol were purchased from Sigma-Aldrich. Reaction solvents were either high performance liquid chromatography (HPLC) grade or American Chemical Society (ACS) grade and used without further purification. Analtech silica gel plates (60 F₂₅₄) were used for analytical TLC, and Analtech preparative TLC plates (UV 254, 2000 μ m) were used for purification. UV light and anisaldehyde/iodine stain were used to visualize the spots. 200–400 Mesh silica gel was used in column chromatography. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian-Gemini 400 MHz or Bruker 500 MHz magnetic resonance spectrometer. ¹H

NMR Spectra were recorded in parts per million (ppm) relative to the residual peaks of CHCl₃ (7.24 ppm) in CDCl₃ or CHD₂OD (4.78 ppm) in CD₃OD or DMSO-*d*₅ (2.49 ppm) in DMSO-*d*₆. ¹³C spectra were recorded relative to the central peak of the CDCl₃ triplet (77.0 ppm) or CD₃OD septet (49.3 ppm) or DMSO-*d*₆ septet (39.7 ppm) and were recorded with complete hetero-decoupling. Original 'fid' files were processed using MestReNova LITE (version 5.2.5–5780) program. High-resolution mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta. 3'-Desmethylclarithromycin (**1**), 3'-desmethylazithromycin (**9**), 3'-Desmethyltricyclic ketolide (**18**), 2-ethynylbenzyl methanesulfonate (**10**), 3-ethynylbenzyl methanesulfonate (**11**), 4-ethynylbenzyl methanesulfonate (**3**), 4-ethynylbenzaldehyde (**6**), 3'-*O*-acetylclarithromycin (**29**) were synthesized as we previously reported.^{14,15,34}

2-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)acetamide (51a)—2-Azidoacetic acid **53**³⁵ (540 mg, 5.34 mmol) was dissolved in anhydrous dichloromethane (20 mL). The solution was cooled to 0 °C and then TBTU (2.06 g, 6.41 mmol) was added and the solution was stirred for another 15 min at 0 °C. After that *O*-((*tert*-butyldimethylsilyl) hydroxylamine **59**³⁶ (1.28 g, 6.95 mmol) dissolved in 5 mL of anhydrous dichloromethane containing Hünig's base (2 mL, 10.69 mmol) was added and the resulting reaction mixture was stirred at room temperature for 12 h. Reaction was quenched by adding water (5 mL) and the organic layer was separated. The aqueous layer was extracted twice with dichloromethane (10 mL) and the combined organic layer was washed with saturated aqueous NaHCO₃ solution (5 mL), water (10 mL), brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated in *vacuo*. The crude was purified by column chromatography (Silica gel, 15% ethyl acetate in hexane) to afford the target compound **51a** (438 mg, 35%) as colorless oil. ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.29 (bs, ¹H), 3.97 (s, 2H), 0.97 (s, 9H), 0.16 (s, 6H).

3-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)propanamide (51b)—3-azidopropanoic acid **54**³⁷ (413 mg, 3.59 mmol) and *O*-((*tert*-butyldimethylsilyl) hydroxylamine **59** (1.38 g, 4.31 mmol) were subjected to same reaction condition as described for the synthesis of **51a**, afforded **51b** as colorless oil (351 mg, 40 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.78 (bs, ¹H), 3.62 (t, *J* = 6.9 Hz, 2H), 2.30 (t, *J* = 6.9 HZ, 2H), 0.93 (s, 9H), 0.16 (s, 6H).

4-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)butanamide (51c)—4-azidobutanoic acid **55**³⁸ (539 mg, 4.18 mmol) and *O*-((*tert*-butyldimethylsilyl) hydroxylamine **59** (1.61 g, 5.02 mmol) were subjected to same reaction condition as described for the synthesis of **51a**, afforded **51c** as colorless oil (745 mg, 68 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.79 (bs, ¹H), 3.33 (t, *J* = 6.9 Hz, 2H), 2.17 (t, *J* = 6.9 Hz, 2H), 1.89 (q, *J* = 6.9 Hz and 4.3 Hz, 2H), 0.93 (s, 9H), 0.16 (s, 6H).

5-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)pentanamide (51d)—5-azidopentanoic acid **56**³⁸ (427 mg, 2.98 mmol) and *O*-((*tert*-butyldimethylsilyl) hydroxylamine **59** (877 g, 5.96 mmol) were subjected to same reaction condition as described for the synthesis of **51a**, afforded **51d** as colorless oil (284 mg, 35 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.22 (bs, ¹H), 3.25 (t, *J* = 6.9 Hz, 2H), 2.27 (bs, 2H), 1.63 (m, 4H), 0.91 (s, 9H), 0.16 (s, 6H).

6-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)hexanamide (51e)—6-azidoheptanoic acid **57**³⁸ (1.08 g, 6.87 mmol) and *O*-(*tert*-butyldimethylsilyl) hydroxylamine **59** (2.20 g, 6.87 mmol) were subjected to same reaction condition as described for the synthesis of **51a**, afforded **51f** as colorless oil (1.62 g, 82 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.64 (bs, ¹H), 3.24 (t, *J* = 6.9 Hz, 2H), 2.35 (bs, 2H), 1.61 (dq, *J* = 21.9 and 7.2 Hz, 4H), 1.39 (dd, *J* = 15.1 and 8.0 Hz, 2H), (0.93 (s, 9H), 0.16 (s, 6H).

7-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)heptanamide (51f)—7-azidoheptanoic acid **58**³⁸ (1.01 g, 5.90 mmol) and *O*-(*tert*-butyldimethylsilyl) hydroxylamine **59** (1.64 g, 8.93 mmol) were subjected to same reaction condition as described for the synthesis of **51a**, afforded **51e** as colorless oil (1.28 g, 72 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.03 (bs, ¹H), 3.22 (t, *J* = 6.9 Hz, 2H), 2.22 (bs, 2H), 1.62 (m, 4H), 1.22 (m, 4H), 0.93 (s, 9H), 0.16 (s, 6H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 171.8, 51.5, 33.2, 28.6, 26.6, 25.8, 25.4, 18.4, -5.1. HRMS (ESI) *m/z* Calcd. for C₁₃H₂₀O₂N₄Si [M+H⁺]: 301.2054, found 301.2050.

(Clarithromycin-3'-*N*-(4-triazolylbenzyl))-*N*-hydroxyacetamide (5a)—3'-*N*-(desmethyl)-3'-*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15 g, 0.18 mmol) and 2-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)acetamide **51a** (0.073 g, 0.318 mmol) are dissolved in anhydrous THF and purged with argon for 10 min. DIPEA (0.06ml, 0.35mmol) and CuI (0.017 g, 0.088 mmol) were then added to the mixture and purged further for another 20 minutes. The resulting suspension was stirred at room temperature for 12 h. Reaction was quenched with a solution of 4:1 satd. Aqueous NH₄Cl/NH₄OH and extracted with a mixture of 10% MeOH in DCM. Combined organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude was subjected to next reaction without further purification.

The crude was dissolved in anhydrous methanol (2 mL) alongside caesium fluoride (0.04g, 0.27mmol) and left to stir under Ar until TLC showed complete conversion (1.5 h). Water was added to quench the reaction and the aqueous layer extracted with DCM. Combined organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. Crude obtained was purified by preparative chromatography (Silica gel, 12:1:0.1 DCM-MeOH-NH₄OH) to give target compound **5a** as light yellow solid (0.068 g, 40%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.39 (s, ¹H), 7.84 (d, *J* = 8.1 Hz, 2H), 7.50 (d, *J* = 8.2 Hz, 2H), 5.12 (d, *J* = 3.7 Hz, 2H), 4.53 (d, *J* = 7.2 Hz, ¹H), 4.08 (dq, *J* = 12.6, 6.9 Hz, 3H), 3.80 – 3.68 (m, 5H), 3.42 (d, *J* = 7.5 Hz, ¹H), 3.30 (dt, *J* = 3.3, 1.6 Hz, 4H), 3.17 (s, 3H), 3.15 – 3.07 (m, 2H), 3.04 (s, ¹H), 3.01 (d, *J* = 9.5 Hz, 2H), 2.94 – 2.86 (m, ¹H), 2.59 (d, *J* = 9.0 Hz, ¹H), 2.44 (s, 3H), 2.38 (d, *J* = 15.2 Hz, ¹H), 2.15 (s, ¹H), 2.04 – 2.00 (m, 2H), 1.98 – 1.82 (m, 4H), 1.67 (d, *J* = 13.0 Hz, ¹H), 1.52 (ddd, *J* = 18.2, 13.3, 6.1 Hz, 3H), 1.38 (d, *J* = 17.4 Hz, 4H), 1.24 (dq, *J* = 12.9, 8.0 Hz, 13H), 1.13 (dt, *J* = 12.5, 8.6 Hz, 16H), 0.85 (dd, *J* = 8.4, 6.3 Hz, 3H). ¹³CNMR (126 MHz, CDCl₃) δ (ppm) 175.8, 147.2, 130.0, 125.8, 122.2, 102.5, 96.1, 81.2, 78.5, 78.3, 74.2, 72.7, 70.8, 69.1, 68.2, 65.7, 64.3, 57.7, 50.6, 49.4, 45.1, 39.2, 39.1, 37.3, 36.7, 34.9, 29.7, 22.7, 21.3, 21.0, 19.9, 18.7, 18.0, 16.0, 12.3, 10.6, 9.3. HRMS (ESI) *m/z* Calcd. for C₄₈H₇₈N₅O₁₅ [M+H⁺]: 964.5494, found 964.5541.

(Clarithromycin-3'-*N*-(4-triazolylbenzyl))-*N*-hydroxypropanamide (5b)—Reaction of 3'-*N*-(desmethyl)-3'-*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15 g, 0.18 mmol)

with 3-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)propanamide **51b** (0.065 g, 0.265 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of compound **5a**, gave **5b** as a light yellow solid (0.067 g, 39%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.25 (s, ¹H), 7.78 (d, *J* = 8.1 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 5.13 (dd, *J* = 11.1, 2.1 Hz, ¹H), 4.74 (t, *J* = 6.5 Hz, 2H), 4.51 (d, *J* = 7.2 Hz, ¹H), 4.11 (dd, *J* = 9.4, 6.3 Hz, ¹H), 3.93 (d, *J* = 13.2 Hz, ¹H), 3.79 – 3.69 (m, 5H), 3.68 – 3.62 (m, ¹H), 3.38 – 3.32 (m, 2H), 3.30 (dt, *J* = 3.3, 1.6 Hz, 3H), 3.18 (s, 3H), 3.11 (d, *J* = 7.8 Hz, ¹H), 3.03 (d, *J* = 7.3 Hz, 4H), 3.01 (d, *J* = 9.5 Hz, 2H), 2.94 – 2.84 (m, 2H), 2.77 (t, *J* = 6.3 Hz, 2H), 2.64 – 2.53 (m, ¹H), 2.40 (s, ¹H), 2.34 (s, 4H), 1.96 – 1.78 (m, 5H), 1.67 (d, *J* = 13.0 Hz, ¹H), 1.60 – 1.47 (m, 2H), 1.39 (d, *J* = 14.0 Hz, 4H), 1.25 (q, *J* = 7.8 Hz, 5H), 1.21 (d, *J* = 6.0 Hz, 7H), 1.18 – 1.08 (m, 17H), 0.85 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm) 175.8, 167.1, 147.1, 129.7, 129.4, 127.3, 125.7, 121.3, 102.7, 96.0, 81.1, 78.4, 78.3, 77.8, 76.7, 74.3, 72.6, 70.8, 69.1, 69.0, 68.4, 65.7, 63.8, 57.6, 50.6, 49.4, 46.2, 45.2, 45.1, 39.3, 39.1, 37.3, 36.9, 34.8, 33.1, 29.9, 29.7, 21.4, 21.2, 21.0, 19.8, 18.6, 18.0, 16.0, 12.3, 10.6, 9.2. HRMS (ESI) *m/z* Calcd. for C₄₉H₇₉N₅O₁₅Na [M+Na⁺]: 1000.5454, found 1000.5479.

(Clarithromycin-3'-(*N*-(4-triazolylbenzyl)))-*N*-hydroxybutanamide (5c)—Reaction of 3' -*N*(desmethyl)-3' -*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15g, 0.18mmol) with 4-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)butanamide **51c** (0.082 g, 0.318 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of compound **5a**, gave **5c** as a light yellow solid (0.075 g, 43%). ¹H NMR (500 MHz, CDCl₃) δ (ppm) 7.85 (s, ¹H), 7.74 (d, 2H), 7.34 (d, 2H), 5.03 (d, *J* = 11.2 Hz, ¹H), 4.88 (d, *J* = 4.1 Hz, ¹H), 4.41 (d, *J* = 6.9 Hz, 4H), 4.01 – 3.89 (m, 3H), 3.81 (d, *J* = 10.8 Hz, ¹H), 3.77 – 3.69 (m, 4H), 3.67 – 3.55 (m, 3H), 3.46 (dd, *J* = 8.1, 5.3 Hz, 3H), 3.37 – 3.27 (m, 2H), 3.18 (d, *J* = 13.1 Hz, ¹H), 3.15 (d, *J* = 16.9 Hz, 5H), 3.06 – 2.92 (m, 8H), 2.89 – 2.80 (m, 2H), 2.57 (dd, *J* = 11.0, 6.5 Hz, 3H), 2.35 – 2.13 (m, ¹H), 1.90 (dd, *J* = 14.4, 7.3 Hz, 3H), 1.80 (dd, *J* = 26.9, 14.6 Hz, 3H), 1.68 (d, *J* = 13.9 Hz, 2H), 1.58 – 1.43 (m, 3H), 1.43 – 1.36 (m, 5H), 1.31 – 1.20 (m, 14H), 1.16 (d, *J* = 7.1 Hz, 5H), 1.14 – 0.99 (m, 23H), 0.96 – 0.85 (m, ¹H), 0.87 – 0.77 (m, 5H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm) 176.0, 147.6, 129.8, 125.8, 120.3, 102.8, 96.0, 81.2, 78.4, 78.0, 74.4, 72.7, 70.9, 69.2, 68.7, 65.9, 64.0, 57.7, 50.8, 49.3, 45.4, 39.3, 37.4, 36.9, 35.1, 29.8, 29.7, 21.6, 21.0, 19.7, 18.9, 18.4, 16.0, 12.4, 10.7, 9.3. HRMS (ESI) *m/z* Calcd. for C₅₀H₈₃N₅O₁₅ [M+H⁺]: 992.5807, found 992.5839.

(Clarithromycin-3'-(*N*-(4-triazolylbenzyl)))-*N*-hydroxypentanamide (5d)—Reaction of 3' -*N*(desmethyl)-3' -*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15 g, 0.18 mmol) with 5-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)pentanamide **51d** (0.072g, 0.265mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of compound **5a**, gave **5d** as a light yellow solid (0.068 g, 45%). ¹H NMR (500 MHz, CDCl₃) δ (ppm) 7.86 (s, ¹H), 7.75 (s, 2H), 7.35 (s, 2H), 5.03 (dd, *J* = 11.1, 1.6 Hz, ¹H), 4.87 (d, *J* = 3.7 Hz, ¹H), 4.41 (t, *J* = 9.4 Hz, ¹H), 4.35 (s, 2H), 3.99 – 3.89 (m, 2H), 3.83 (d, *J* = 10.5 Hz, ¹H), 3.78 – 3.68 (m, 2H), 3.62 (d, *J* = 6.9 Hz, ¹H), 3.54 – 3.41 (m, 2H), 3.38 – 3.29 (m, ¹H), 3.17 (d, *J* = 20.3 Hz, ¹H), 3.12 (s, 3H), 3.04 – 2.91 (m, 5H), 2.91 – 2.81 (m, ¹H), 2.64 (s, ¹H), 2.56 (dd, *J* = 10.3, 7.4 Hz, ¹H), 2.24 (d, *J* = 40.5 Hz, 6H), 1.88 (dt, *J* = 26.6, 13.4 Hz, 4H), 1.85 – 1.73 (m, 2H), 1.71 – 1.56 (m, 3H), 1.56 – 1.41 (m, 2H), 1.42 – 1.33 (m, 3H), 1.33 – 1.27 (m, 2H), 1.30 – 1.18 (m, 8H), 1.16 (d, *J* = 7.0 Hz, 3H), 1.13 – 1.00 (m,

15H), 0.82 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 175.8, 170.3, 147.3, 129.6, 125.7, 120.1, 102.6, 95.9, 80.9, 78.3, 74.2, 72.5, 70.7, 69.0, 68.4, 65.6, 63.8, 57.6, 50.5, 49.8, 49.3, 45.9, 45.1, 45.0, 39.2, 39.0, 37.2, 36.8, 34.7, 31.7, 29.8, 29.6, 29.3, 22.1, 21.4, 21.2, 20.9, 19.8, 18.6, 17.9, 15.9, 12.2, 10.5, 9.1, 8.5. HRMS (ESI) m/z Calcd for $\text{C}_{51}\text{H}_{83}\text{N}_5\text{O}_{15}\text{Na}$ [$\text{M}+\text{Na}^+$]: 1028.5783, found 1028.5795.

(N10-(4-Ethynylbenzyl))azithromycin (7)—*N*10-Desmethylazithromycin **2** (2.72 g, 3.55 mmol) and 4-ethynylbenzaldehyde **6**³⁹ (2.31 g, 17.75 mmol) were dissolved in anhydrous DMF (40 mL). Acetic acid (2.0 mL, 35.50 mmol) was added and the solution was stirred for 30 min. and then sodium cyanoborohydride (465 mg, 7.10 mmol) was added to the reaction mixture. The mixture was then stirred at 70 °C for 7 h after which it was cooled and the pH of the solution was raised to 8 by adding saturated aqueous NaHCO_3 solution. The crude mixture was diluted into CH_2Cl_2 and was washed water, dried over anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. The crude was purified by column chromatography (Silica gel, 5:1:1 EtoAc/hexane/triethylamine) to afford the title compound **7** as white solid (603 mg, 20%). ^1H NMR (500 MHz, $\text{MeOH}-d_4$) δ (ppm) 7.41 (s, 4H), 5.01 (m, 2H), 4.60 (d, $J = 6.8$ Hz, ^1H), 4.20 (ddd, $J = 24.8, 13.7, 6.6$ Hz, 2H), 3.92 (d, $J = 10.9$ Hz, ^1H), 3.73 (ddd, $J = 37.9, 14.5, \text{ and } 5.8$ Hz, 4H), 3.45 (m, ^1H), 3.38 (d, $J = 11.2$ Hz, 3H), 3.32 (m, ^1H), 3.07 (dd, $J = 11.5, \text{ and } 7.2$ Hz, ^1H), 2.99 (m, ^1H), 2.88 (dd, $J = 14.7, \text{ and } 8.4$ Hz, 2H), 2.79 (m, ^1H), 2.47 (d, $J = 15.1$ Hz, ^1H), 2.40 (s, 6H), 2.19 (m, ^1H), 2.11 (m, ^1H), 1.96 (d, $J = 21.6$ Hz, ^1H), 1.74 (m, 4H), 1.62 (m, 2H), 1.43 (m, ^1H), 1.32 (m, 4H), 1.27 (m, ^1H), 1.22 (m, 4H), 1.18 (d, $J = 7.4$ Hz, 3H), 1.14 (dd, $J = 8.0, \text{ and } 4.1$ Hz, 3H), 0.94 (m, 3H), 0.89 (t, $J = 7.3$ Hz, 3H). ^{13}C (125 MHz, $\text{MeOH}-d_4$) δ (ppm) 178.7, 132.9, 130.7, 104.2, 97.6, 80.7, 79.4, 78.4, 76.9, 76.5, 74.5, 72.7, 69.4, 67.1, 65.6, 50.1, 46.6, 40.8, 36.3, 31.9, 23.2, 22.3, 21.9, 21.8, 19.1, 11.8, 10.8. HRMS (ESI) $m+2/2z$ Calcd for $\text{C}_{46}\text{H}_{78}\text{O}_{12}\text{N}_2$ [$\text{M}+2\text{H}^+$]: 425.2772, found 425.2762.

(Azithromycin(-N10-(4-triazolybenzyl)))-N-hydroxyacetamide (8a)—In an oven dried round bottomed flask charged with a magnetic stirring bar, (*N*10-(4-ethynylbenzyl))azithromycin **7** (120 mg, 0.141 mmol) and 2-azido-*N*-(*tert*-butyldimethylsilyl) oxy)acetamide **51a** (73 mg, 0.254 mmol) were placed under argon. To the mixture were added 2 mL of degassed 1:1 mixture of THF/DMSO, CuI (4 mg, 0.02 mmol) and *N,N'*-diisopropylethylamine (20 μL , 0.09 mmol) in succession. The mixture was heated at 45 °C for 12 h. The reaction was cooled to room temperature and diluted with 50 mL of ethyl acetate. Organic layer was washed with 4:1 mixture of satd. NH_4Cl soln.- NH_4OH soln. (2 \times 10 mL), water (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude was subjected to next reaction without further purification.

The crude was dissolved in methanol (2 mL) and caesium fluoride (43 mg, 0.282 mmol) was added and the resulting reaction was stirred at room temperature for 2 h. Reaction was quenched by adding water and was extracted with ethyl acetate (100 mL) and the organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 CH_2Cl_2 -MeOH-conc. NH_4OH soln.) to give **8a** as a white solid (35 mg, 25 %). ^1H

NMR (500 MHz, MeOH- d_4) δ (ppm) 8.25 (s, ^1H), 7.69 (d, $J = 7.5$ Hz, 2H), 7.42 (d, $J = 7.5$ Hz, 2H), 4.90 (m, 2H), 4.55 (d, $J = 6.1$ Hz, ^1H), 4.04 (m, 4H), 3.69 (m, 3H), 3.51 (m, 3H), 3.37 (m, ^1H), 3.08 (td, $J = 16.2$, and 8.9 Hz, 2H), 2.97 (m, 4H); 2.72 (m, ^1H), 2.85 (m, 3H), 2.50 (s, 6H), 2.34 (t, $J = 12.6$ Hz, ^1H), 2.06 (m, 2H), 1.78 (m, 5H), 1.64 (m, 5H), 1.47 (m, 3H), 1.18 (m, 17H), 0.81 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 178.6, 148.8, 131.7, 126.8, 123.8, 103.5, 97.6, 80.8, 79.2, 77.0, 76.5, 74.6, 73.8, 72.3, 71.6, 71.4, 68.5, 67.0, 66.3, 62.4, 50.1, 47.8, 46.5, 40.2, 38.1, 36.3, 35.4, 32.9, 31.4, 29.8, 21.8, 20.4, 19.1, 14.8, 14.6, 14.4, 13.5, 11.7, 10.6, 9.6. HRMS (ESI) $m+2/2z$ Calcd for $\text{C}_{48}\text{H}_{82}\text{N}_6\text{O}_{14}$ [$\text{M}+2\text{H}^+$]: 483.2939, found 483.2933.

(Azithromycin-(N10-(4-triazolylbenzyl)))-N-hydroxypropanamide (8b)—Reaction of (N10-(4-ethynylbenzyl)azithromycin **7** (85 mg, 0.10 mmol) and 3-azido-*N*-((*tert*-butyldimethylsilyl) oxy)propanamide **51b** (34 mg, 0.14 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8b** as white solid (38 mg, 40 %). ^1H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.21 (s, ^1H), 7.71 (d, $J = 7.4$ Hz, 2H), 7.44 (d, $J = 7.5$ Hz, 2H), 4.91 (m, 2H), 4.56 (d, $J = 6.2$ Hz, ^1H), 4.12 (m, ^1H), 3.99 (m, 2H), 3.74 (m, 3H), 3.51 (m, 2H), 3.18 (m, 5H), 3.07 (m, 4H), 2.96 (m, 2H); 2.82 (m, ^1H), 2.62 (s, 6H), 2.36 (d, $J = 15.1$ Hz, 2H), 2.00 (dd, $J = 22.1$ and 17.7 Hz, ^1H), 1.86 (m, 5H), 1.68 (m, 5H), 1.52 (m, 3H), 1.32 (ddd, $J = 17.7$, 10.9, and 4.0 Hz, 3H), 1.14 (m, 17H), 1.03 (m, 7H), 0.78 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 178.6, 169.5, 148.5, 133.7, 132.5, 132.1, 130.0, 126.9, 123.0, 97.6, 80.9, 79.0, 76.5, 74.6, 73.8, 71.2, 69.2, 68.5, 67.1, 66.6, 47.8, 40.3, 39.9, 36.2, 35.4, 31.7, 31.2, 30.1, 30.6, 30.2, 29.5, 25.1, 24.2, 22.7, 21.8, 21.7, 19.2, 14.8, 14.5, 11.6, 11.5, 10.4, 9.4. HRMS (ESI) m/z Calcd for $\text{C}_{49}\text{H}_{83}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 979.5962, found 979.5967.

(Azithromycin-(N10-(4-triazolylbenzyl)))-N-hydroxybutanamide (8c)—Reaction of (N10-(4-ethynylbenzyl)azithromycin **7** (93 mg, 0.11 mmol) and 4-azido-*N*-((*tert*-butyldimethylsilyl) oxy)butanamide **51c** (51 mg, 0.20 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8c** as white solid (54 mg, 50 %). ^1H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.25 (s, ^1H), 7.70 (d, $J = 7.7$ Hz, 2H), 7.41 (d, $J = 7.5$ Hz, 2H), 4.88 (m, 2H), 4.55 (d, $J = 6.1$ Hz, 2H), 4.39 (s, 3H), 4.06 (m, 4H), 3.69 (m, 4H), 3.49 (m, 2H), 3.01 (m, 10H), 2.80 (m, 2H), 2.56 (s, 6H), 2.36 (dt, $J = 47.8$ and 19.1 Hz, 3H), 2.03 (m, 7H), 1.84 (m, 7H), 1.62 (m, 3H), 1.47 (m, 3H), 1.28 (m, 8H), 1.15 (m, 17H), 1.01 (m, 7H), 0.80 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 178.6, 148.8, 131.8, 126.8, 122.5, 103.3, 97.6, 80.8, 79.1, 76.5, 74.6, 71.4, 69.2, 68.6, 67.1, 66.5, 50.8, 50.1, 47.8, 46.5, 40.3, 36.2, 35.4, 31.4, 30.3, 27.4, 25.1, 24.1, 22.9, 22.7, 22.3, 21.8, 21.7, 19.1, 14.9, 14.5, 11.7, 11.5, 10.5, 9.5. HRMS (ESI) m/z Calcd. for $\text{C}_{50}\text{H}_{85}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 993.6118, found 993.6125.

(Azithromycin-(N10-(4-triazolylbenzyl)))-N-hydroxypentanamide (8d)—Reaction of (N10-(4-ethynylbenzyl)azithromycin **7** (131 mg, 0.15 mmol) and 5-azido-*N*-((*tert*-butyldimethylsilyl) oxy)pentanamide **51d** (44 mg, 0.28 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8d** as white solid (60 mg, 40 %). ^1H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.24 (s, ^1H), 7.69 (d, $J = 7.6$ Hz, 2H), 7.41 (d, $J = 7.6$ Hz, 2H), 4.92 (d, $J = 10.2$ Hz, ^1H), 4.88 (d, $J = 4.3$ Hz, ^1H), 4.55

(d, $J = 6.3$ Hz, ^1H), 4.37 (t, $J = 6.6$ Hz, 2H), 4.08 (ddd, $J = 23.2, 20.9, 10.7$ Hz, 2H), 3.70 (dd, $J = 29.4, 21.5$ Hz, 3H), 3.54 (dd, $J = 20.2, 11.4$ Hz, 2H), 3.27 (s, 4H), 3.10 (dt, $J = 14.4, 7.0$ Hz, ^1H), 2.96 (dd, $J = 17.4, 9.9$ Hz, 5H), 2.87 (d, $J = 11.2$ Hz, ^1H), 2.79 (m, ^1H), 2.49 (s, 7H), 2.36 (d, $J = 15.1$ Hz, ^1H), 2.18 (s, ^1H), 2.05 (m, 4H), 1.88 (d, $J = 6.5$ Hz, 3H), 1.79 (m, 4H), 1.67 (s, 2H), 1.57 (m, 4H), 1.48 (m, 2H), 1.31 (m, 6H), 1.20 (d, $J = 5.8$ Hz, 6H), 1.16 (m, 10H), 1.09 (m, 6H), 0.80 (m, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.6, 172.4, 169.5, 148.6, 133.8, 132.6, 131.6, 130.0, 126.3, 122.4, 103.6, 97.6, 80.8, 79.2, 76.8, 76.5, 74.6, 73.8, 71.8, 69.3, 68.9, 67.1, 66.2, 51.2, 50.1, 49.8, 49.78, 49.7, 49.6, 49.5, 49.4, 49.3, 49.2, 49.0, 48.8, 48.6, 47.8, 46.5, 40.3, 36.3, 35.4, 33.1, 31.8, 31.5, 30.9, 30.8, 30.3, 29.9, 25.1, 24.2, 23.8, 23.1, 22.7, 22.4, 21.8, 21.8, 20.4, 19.1, 16.1, 14.9, 14.6, 11.8, 11.6, 10.6, 9.8, 9.3. HRMS (ESI) m/z Calcd. for $\text{C}_{51}\text{H}_{87}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 1007.6275, found 1007.6278.

(Azithromycin-(M10-(4-triazolybenzyl))-N-hydroxyhexanamide (8e))—Reaction of (N10-(4-ethynylbenzyl))azithromycin **7** (155 mg, 0.18 mmol) and 6-azido-*N*-((*tert*-butyldimethylsilyl) oxy)hexanamide **51e** (94 mg, 0.33 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8e** as white solid (70 mg, 38 %). ^1H NMR (500 MHz, MeOD) δ (ppm) 8.23 (s, ^1H), 7.68 (d, $J = 8.0$ Hz, 2H), 7.41 (d, $J = 8.0$ Hz, 2H), 4.92 (dd, $J = 10.2, 2.3$ Hz, ^1H), 4.88 (d, $J = 4.7$ Hz, ^1H), 4.52 (d, $J = 7.1$ Hz, ^1H), 4.37 (t, $J = 7.0$ Hz, 2H), 4.10 (m, 2H), 3.65 (q, $J = 13.5$ Hz, 3H), 3.56 (m, 2H), 3.49 (m, ^1H), 3.29 (s, 3H), 3.22 (m, 6H), 2.98 (m, 2H), 2.91 (m, ^1H), 2.80 (dd, $J = 19.0, 12.8$ Hz, 3H), 2.33 (m, 9H), 2.15 (m, ^1H), 2.02 (t, $J = 7.3$ Hz, 3H), 1.90 (m, 4H), 1.71 (dd, $J = 40.3, 26.2$ Hz, 3H), 1.60 (dt, $J = 15.1, 7.5$ Hz, 4H), 1.53 (dt, $J = 11.8, 6.9$ Hz, 3H), 1.30 (ddd, $J = 15.6, 12.5, 10.2$ Hz, 6H), 1.22 (m, 8H), 1.15 (dd, $J = 16.9, 8.2$ Hz, 12H), 1.07 (m, 12H), 0.96 (m, 2H), 0.82 (tt, $J = 24.9, 7.3$ Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.6, 172.7, 148.9, 131.3, 126.64, 122.2, 104.0, 97.6, 80.7, 79.3, 76.5, 74.5, 72.5, 69.2, 67.0, 65.7, 57.6, 51.4, 50.1, 49.8, 49.7, 49.6, 49.6, 49.4, 49.4, 49.4, 49.4, 49.3, 49.3, 49.2, 49.2, 49.2, 49.2, 49.2, 49.2, 49.2, 49.1, 48.9, 48.8, 48.6, 46.5, 40.7, 36.3, 33.6, 31.8, 31.0, 27.0, 26.2, 23.2, 22.3, 21.9, 21.7, 19.1, 17.5, 17.4, 17.2, 11.8, 10.7. HRMS (ESI) m/z Calcd. for $\text{C}_{52}\text{H}_{88}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 1021.6431, found 1021.6444.

(Azithromycin-(M10-(4-triazolybenzyl))-N-hydroxyheptanamide (8f))—Reaction of (N10-(4-ethynylbenzyl))azithromycin **7** (125 mg, 0.15 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl) oxy)heptanamide **51f** (80 mg, 0.27 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8f** as white solid (75 mg, 45 %). ^1H NMR (500 MHz, MeOD) δ (ppm) 8.22 (s, ^1H), 7.66 (t, $J = 8.0$ Hz, 2H), 7.40 (d, $J = 8.0$ Hz, 2H), 4.92 (m, ^1H), 4.87 (d, $J = 4.7$ Hz, ^1H), 4.50 (d, $J = 7.0$ Hz, ^1H), 4.35 (t, $J = 7.1$ Hz, 2H), 4.09 (m, 2H), 3.65 (t, $J = 13.9$ Hz, 3H), 3.55 (m, ^1H), 3.28 (s, 3H), 3.21 (m, 5H), 2.97 (dd, $J = 9.3, 5.0$ Hz, ^1H), 2.81 (m, 4H), 2.32 (m, 7H), 2.12 (m, ^1H), 2.01 (m, 3H), 1.88 (dd, $J = 13.9, 6.9$ Hz, 3H), 1.72 (dd, $J = 43.3, 24.2$ Hz, 4H), 1.53 (m, 4H), 1.32 (m, 7H), 1.21 (t, $J = 6.4$ Hz, 5H), 1.15 (m, 10H), 1.08 (dd, $J = 15.7, 7.4$ Hz, 9H), 0.79 (m, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.4, 172.8, 148.7, 131.2, 126.4, 122.0, 103.8, 97.4, 80.5, 79.1, 76.3, 74.3, 72.3, 69.1, 66.9, 65.5, 57.4, 51.3, 49.9, 49.6, 49.4, 49.4, 49.3, 49.2, 49.1, 48.9, 48.8, 48.6, 48.4, 46.4, 40.5, 36.1, 33.5, 31.7, 31.0, 29.3, 27.0, 26.4, 23.0, 22.2, 21.7, 21.6, 18.9, 17.2, 17.1, 11.6, 10.6. HRMS (ESI) m/z Calcd for $\text{C}_{53}\text{H}_{91}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 1035.6588, found 1035.6591.

2-Ethynylbenzaldehyde (19)—To a solution of (2-ethynylphenyl)methanol **60** (510 mg, 3.82 mmol) in dichloromethane (20 mL) was added pyridinium dichromate (2.87 g, 7.64 mmol) and stirred at room temperature for 5 h. The reaction mixture was quenched with cold Et₂O (100 mL) and the suspension was filtered off and the filtrate was concentrated *in vacuo*. The crude was subjected to column chromatography (Silica gel, 1:2 ethyl acetate-hexane) to give the title compound **19** as a brown-white solid (420 mg, 85 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 10.54 (¹H, s), 7.93 (¹H, m), 7.55 (3H, m), 3.46 (¹H, s).

3-Ethynylbenzaldehyde (20)—A solution of 3-bromobenzaldehyde **61** (1.0 g, 5.40 mmol), ethynyltrimethylsilane (850 mg, 8.6 mmol), palladium (II) acetate (15 mg, 0.065 mmol), and triphenylphosphine (28 mg, 0.108 mmol) in anhydrous triethylamine (5 mL) was heated to reflux under argon. After 15 min of reflux, a clear yellow solution resulted and a white precipitate began to form. The reaction was stopped after 4 h of refluxing, cooled, and filtered. The orange-brown filtrate was mixed with 50 ml of saturated NaHCO₃ and extracted with dichloromethane (3 × 50 mL). The organic fractions were combined, dried over anhydrous Na₂SO₄, and concentrated to yield oil which was purified by silica gel column (EtOAc/Hexane 1:4) to give 0.86 g of silyl intermediate which was subsequently treated with anhydrous K₂CO₃ (100 mg) in anhydrous methanol (10 mL) under argon for 3 h at room temperature. Saturated NaHCO₃ (50 mL) was added to the reaction mixture and extracted with dichloromethane (3 × 50 mL). The organic fractions were combined, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. Purification by column chromatography (Silica gel, CH₂Cl₂-Hexane 2:3) afforded the target compound **20** as a brownwhite solid (430 mg, 62 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 10.00 (¹H, s), 7.99 (¹H, s), 7.87 (¹H, m), 7.74 (¹H, m), 7.50 (¹H, m), 3.16 (¹H, s).

3-Ethynylbenzyl alcohol (52)—Palladium (II) acetate (14 mg, 0.064 mmol) and triphenylphosphine (28 mg, 0.106 mmol) were added to a solution of 3-bromobenzyl alcohol **51** (1.0 g, 5.34 mmol) and ethynyltrimethylsilane (0.84 g, 8.55 mmol) in triethylamine (5 mL). The reaction mixture was subjected to reflux under argon for 4 h. The orange-brown reaction mixture was cooled, diluted with excess ether (200 mL) and filtered. The filtrate was washed saturated NaHCO₃ (50 mL), water (50 mL), brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude was subjected to next reaction without further purification.

The crude was dissolved in anhydrous methanol (10 mL) and was treated with K₂CO₃ (100 mg) under argon at room temperature for 3 h. Saturated NaHCO₃ (50 mL) was added to the reaction mixture and extracted with CH₂Cl₂ (3 × 50 mL). The organic fractions were combined, dried over Na₂SO₄, concentrated *in vacuo* and the crude was purified by column chromatography (Silica gel, 4:0.5 CH₂Cl₂-Acetone) to give the target compound **52** as yellow oil (490 mg; 69 % overall yield). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.31 (4H, m), 4.61 (2H, s), 3.02 (¹H, s).

2-Ethynylbenzyl methanesulfonate (10)—(2-Ethynylphenyl)methanol **60** (400 mg, 3.03 mmol) was dissolved in anhydrous dichloromethane. Triethylamine (0.84 mL, 6.05 mmol) was added at room temperature and the reaction mixture was stirred for 10 min. After

cooling the reaction mixture to $-15\text{ }^{\circ}\text{C}$, methanesulfonyl chloride (0.28 mL, 3.63 mmol) was added to the reaction mixture. After 40 min, the reaction was quenched by adding saturated aqueous NaHCO_3 solution (10 mL) and the organic phase was separated. The aqueous phase was extracted twice with dichloromethane (100 mL). The combined organic layer was washed with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude mesylated product **10** was used in the next step without further purification.

(3'-N-(2-Ethynylbenzyl)azithromycin (12))—To a solution of 3'-desmethylazithromycin **9** (1.2 g, 1.63 mmol) in anhydrous DMSO (8 mL) was added *N,N*-diisopropylethylamine (3 mL) and 2-ethynylbenzyl methanesulfonate **10** (450 mg, 2.12 mmol). The reaction mixture was heated with stirring under argon at $85\text{ }^{\circ}\text{C}$ for 3 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL) and washed with saturated NaHCO_3 ($3 \times 50\text{ mL}$) and saturated brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (Silica gel, 12:1:0.1 to 4:1:0.1 CH_2Cl_2 -Acetone- Et_3N) to give **12** as a brownwhite solid (600 mg, 44 %). ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 7.43 (d, $J = 7.6\text{ Hz}$, ^1H), 7.26 (m, 2H), 7.16 (d, $J = 7.2\text{ Hz}$, ^1H), 5.02 (d, $J = 7.6\text{ Hz}$, ^1H), 4.72 (m, ^1H), 4.62 (d, $J = 9.6\text{ Hz}$, ^1H), 4.35 (d, $J = 7.2\text{ Hz}$, ^1H), 4.14 (m, ^1H), 3.98 (m, ^1H), 3.57 (m, 5H), 3.22 (s, 2H), 2.92 (m, 4H), 2.64 (m, 2H), 2.46 (m, 2H), 2.34 (s, 3H), 2.19 (s, 3H), 2.09 (m, ^1H), 1.83 (m, 6H), 1.42 (m, 3H), 1.04 (m, 30H). ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm) 178.8, 141.3, 133.2, 129.6, 128.9, 127.1, 122.2, 102.9, 94.5, 83.8, 82.1, 81.4, 78.2, 77.9, 76.8, 74.2, 73.8, 73.6, 72.8, 70.5, 70.1, 68.6, 65.5, 63.4, 56.5, 49.2, 45.2, 42.2, 42.1, 36.3, 36.1, 34.7, 29.5, 27.5, 26.7, 21.9, 21.5, 21.4, 21.2, 18.2, 16.2, 14.7, 11.2, 9.0, 7.4. HRMS (MALDI) m/z Calcd for $\text{C}_{46}\text{H}_{77}\text{N}_2\text{O}_{12}$ [$\text{M}+\text{H}^+$]: 849.5471, found 849.5530.

(3'-N-(3-Ethynylbenzyl)azithromycin (13))—Reaction of 3'-desmethylazithromycin **9** (1.42 g, 2.04 mmol) in anhydrous DMSO (8 mL), *N,N'*-diisopropylethylamine (3 mL) and 3-ethynylbenzyl methanesulfonate **11** (560 mg, 2.65 mmol) as described for the synthesis of **12**, afforded compound **13** as a brown-white solid (530 mg, 32 %). ^1H NMR (CDCl_3 , 400 MHz) δ (ppm) 7.41 (m, 2H), 7.22 (m, 2H), 5.14 (d, $J = 6.0\text{ Hz}$, ^1H), 4.70 (d, $J = 8.0\text{ Hz}$, ^1H), 4.43 (d, $J = 7.6\text{ Hz}$, ^1H), 4.25 (m, ^1H), 4.05 (m, ^1H), 3.55 (m, 5H), 3.19 (s, 3H), 3.16 (s, 2H), 3.00 (m, 2H), 2.64 (m, 4H), 2.32 (s, 3H), 2.23 (s, 3H), 1.89 (m, 7H), 1.23 (m, 33H). ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm) 178.5, 141.5, 131.6, 130.2, 129.9, 127.3, 122.5, 102.8, 94.7, 83.8, 83.5, 78.1, 76.8, 74.3, 74.0, 73.5, 72.8, 70.8, 70.7, 70.0, 68.5, 65.5, 62.2, 57.4, 49.3, 45.1, 42.3, 41.7, 36.8, 36.4, 34.7, 30.0, 27.4, 26.7, 22.0, 21.6, 21.3, 21.2, 18.2, 16.2, 14.9, 11.2, 9.2, 7.5. HRMS (MALDI) m/z Calcd. for $\text{C}_{46}\text{H}_{77}\text{N}_2\text{O}_{12}$ [$\text{M}+\text{H}^+$]: 849.5471, found 849.5385.

(Azithromycin-3'-(N-(2-triazolylbenzyl))-N-hydroxyhexanamide (15a))—To a solution of 3'-(2-ethynylbenzyl)azithromycin **12** (50 mg, 0.06 mmol) and copper (I) iodide (8 mg, 0.04 mmol) in anhydrous THF (5 mL) under argon was added *N,N'*-diisopropylethylamine (0.6 mL), and stirred under argon at room temperature for 15 min. 6-azido-*N*-((*tert*-butyldimethylsilyl)oxy)hexanamide **51e** (34 mg, 0.12 mmol) was added to the reaction mixture, and stirring continued for 3 h. The reaction mixture was diluted with

1:4 NH₄OH/saturated NH₄Cl (40 mL) and extracted with 20% MeOH/CH₂Cl₂ (3 × 20 mL). The organic layer was dried over Na₂SO₄ and concentrated *in vacuo*. The crude was subjected to the next reaction without further purification.

The crude was dissolved in anhydrous methanol (2 mL) and treated with caesium fluoride (20 mg, 0.12 mmol) at room temperature for 2 h. Water was added to the reaction and the mixture was extracted with ethyl acetate (2 × 50 mL). The organic fraction was washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude product was purified by preparative chromatography (Silica gel, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) to give **15a** as a brown-white solid (40 mg, 67 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.72 (s, ¹H), 7.50 (m, ¹H), 7.41 (m, ¹H), 7.28 (m, 2H), 5.08 (d, *J* = 4.8 Hz, ¹H), 4.34 (m, 3H), 4.07 (m, ¹H), 3.95 (m, ¹H), 3.40 (m, 8H), 2.85 (m, 4H), 2.61 (m, 2H), 2.45 (m, 2H), 2.28 (s, 5H), 2.13 (s, 3H), 1.92 (m, 8H), 1.59 (m, 8H), 1.18 (m, 24H), 0.84 (m, 6H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 178.4, 171.2, 146.4, 136.0, 131.1, 128.5, 127.6, 122.6, 102.6, 94.6, 83.6, 78.5, 78.4, 78.1, 76.7, 73.9, 73.8, 73.4, 72.7, 70.3, 69.3, 68.3, 66.2, 62.6, 61.0, 56.1, 51.2, 50.1, 49.2, 45.3, 42.8, 35.6, 29.9, 28.5, 27.0, 26.7, 26.2, 25.8, 25.0, 22.7, 21.8, 21.7, 21.4, 21.3, 17.7, 16.8, 14.4, 11.6, 8.9, 6.4. HRMS (MALDI) *m/z* Calcd for C₅₂H₈₉N₆O₁₄ [M+H⁺]: 1021.6431, found 1021.6469.

(Azithromycin-3'-(*N*-(2-triazolylbenzyl)))-*N*-hydroxyheptanamide (**15b**)—

Reaction of 3'-(2-ethynylbenzyl)azithromycin **12** (0.05 g, 0.059 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl) oxy)heptanamide **51f** (0.035 g, 0.118 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **15a**, afforded **15b** as a brown-white solid (41 mg, 68 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.73 (s, ¹H), 7.44 (m, 2H), 7.30 (m, 2H), 5.23 (d, *J* = 4.0 Hz, ¹H), 4.35 (m, 3H), 4.04 (m, ¹H), 3.95 (m, ¹H), 3.41 (m, 8H), 2.83 (m, 4H), 2.62 (m, 2H), 2.46 (m, 2H), 2.25 (m, 8H), 1.92 (m, 8H), 1.56 (m, 10H), 1.19 (m, 24H), 0.84 (m, 6H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 178.4, 171.4, 146.4, 136.2, 131.1, 130.1, 128.6, 127.8, 122.6, 102.6, 94.6, 83.7, 78.5, 78.4, 78.1, 76.7, 73.9, 73.8, 73.3, 72.7, 69.3, 68.2, 66.2, 62.7, 61.0, 56.0, 51.3, 50.1, 49.2, 45.3, 42.8, 35.7, 29.7, 28.6, 28.0, 27.0, 26.7, 26.4, 25.8, 25.3, 25.0, 21.8, 21.4, 21.3, 18.1, 17.7, 16.8, 14.4, 11.5, 8.9, 6.5. HRMS (MALDI) *m/z* Calcd. for C₅₃H₉₁N₆O₁₄ [M+H⁺]: 1035.6588, found 1035.6581.

(Azithromycin-3'-(*N*-(3-triazolylbenzyl)))-*N*-hydroxyhexanamide (**16a**)—

Reaction of 3'-*N*-(3-ethynylbenzyl)azithromycin **13** (0.05 g, 0.059 mmol) and 6-azido-*N*-((*tert*-butyldimethylsilyl) oxy)hexanamide **51e** (0.034 g, 0.12 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **15a**, afforded **16a** as a brown-white solid (28 mg, 47 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.10 (s, ¹H), 7.82 (m, 2H), 7.36 (m, 2H), 5.10 (m, ¹H), 4.37 (m, 3H), 4.03 (m, 2H), 3.41 (m, 8H), 2.78 (m, 4H), 2.28 (m, 16H), 1.89 (m, 4H), 1.40 (m, 32H), 0.91 (m, 6H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 178.2, 171.2, 147.3, 139.0, 130.9, 129.2, 126.8, 125.8, 125.9, 125.2, 120.6, 102.8, 94.7, 83.4, 78.4, 77.8, 76.8, 74.2, 72.8, 72.7, 70.3, 68.4, 66.0, 63.4, 56.0, 54.0, 51.2, 51.1, 50.1, 49.3, 42.2, 36.8, 35.6, 34.6, 32.8, 29.7, 29.3, 28.5, 26.2, 25.5, 24.5, 21.8, 21.6, 21.3, 18.0, 17.7, 16.8, 14.1, 12.0, 11.4, 8.9, 6.9. HRMS (MALDI) *m/z* Calcd. for C₅₂H₈₉N₆O₁₄ [M+H⁺]: 1021.6431, found 1021.6500.

(Azithromycin-3'-(N-(3-triazolylbenzyl)))-N-hydroxyheptanamide (16b)—

Reaction of (3'-N-(3-ethynylbenzyl))azithromycin **13** (0.05 g, 0.059 mmol) and 7-azido-N-((*tert*-butyldimethylsilyl)oxy)heptanamide **51f** (0.035 g, 0.118 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **15a**, afforded **16b** as a brown-white solid (28 mg, 46 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.84 (s, ¹H), 7.74 (m, 2H), 7.34 (m, 2H), 5.15 (d, *J* = 4.0 Hz, ¹H), 4.73 (m, 3H), 4.08 (m, 2H), 3.85 (m, ¹H), 3.39 (m, 7H), 2.64 (m, 4H), 2.26 (m, 16H), 1.85 (m, 4H), 1.40 (m, 34H), 0.92 (m, 6H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 178.3, 171.5, 147.5, 139.0, 130.6, 129.1, 128.9, 126.3, 124.8, 120.1, 102.7, 94.6, 83.3, 78.4, 78.3, 76.7, 74.0, 73.9, 73.3, 72.7, 70.5, 69.2, 68.6, 66.0, 63.6, 62.9, 58.0, 51.3, 50.2, 49.3, 45.4, 42.8, 41.8, 36.9, 35.7, 33.0, 29.9, 29.7, 28.6, 28.5, 26.3, 25.3, 21.8, 21.7, 21.4, 21.3, 17.7, 16.8, 14.5, 11.5, 8.8, 6.6. HRMS (MALDI) *m/z* Calcd. for C₅₃H₉₁N₆O₁₄ [M+H⁺]: 1035.6588, found 1035.6714.

(Azithromycin-3'-(N-(4-triazolylbenzyl)))-N-hydroxyacetamide (17a)—(3'-N-(4-

ethynylbenzyl))azithromycin **14** (0.10 g, 0.12 mmol) and 2-Azido-N-((*tert*butyldimethyl silyl)oxy) ethanamide **51a** (0.05 g, 0.22 mmol) were dissolved in degassed anhydrous THF (5 mL). CuI (0.01 g, 0.06 mmol) and Hünig's base (0.04 mL, 0.24 mmol) were added to the reaction and the resulting reaction was stirred at room temperature for another 12h. Then, the reaction mixture was diluted with excess ethyl acetate (30 mL) and was transferred to a separatory funnel and then the ethyl acetate layer was washed with a solution (20 mL) of 4:1 mixture of saturated aqueous NH₄Cl solution /NH₄OH solution, water (10 mL), brine (10 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude was dissolved in 5 mL of anhydrous methanol and to that the solution caesium fluoride (0.03 g, 0.18 mmol) was added and the reaction was stirred at room temperature for 2 h. Afterward, water (10 mL) and ethyl acetate (30 mL) were added to the reaction and the organic layer was separated and then the organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude was purified by preparative chromatography (Silica gel, 5:1:1 EtOAc/MeOH/NH₄OH) to give the title compound **17a** as light yellow solid (0.045 g, 40% yield). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.47 (d, *J* = 2.4 Hz, ¹H), 7.96 (d, *J* = 7.0 Hz, 2H), 7.62 (d, *J* = 7.4 Hz, 2H), 5.13 (d, *J* = 4.6 Hz, 2H), 4.66 (d, *J* = 7.2 Hz, ¹H), 4.31 – 4.20 (m, 2H), 4.15 (d, *J* = 12.9 Hz, ¹H), 3.86 (d, *J* = 12.7 Hz, 2H), 3.76 – 3.70 (m, 2H), 3.52 (d, *J* = 6.9 Hz, 2H), 3.43 (dd, *J* = 3.2, 1.6 Hz, 3H), 3.29 (s, 3H), 3.20 – 3.12 (m, 2H), 3.07 (s, ¹H), 3.01 – 2.97 (m, ¹H), 2.88 (s, 3H), 2.55 (s, 3H), 2.30 (d, *J* = 14.2 Hz, 2H), 2.09 – 2.01 (m, 28H), 1.69 (dd, *J* = 15.2, 5.1 Hz, 2H), 1.56 (s, 3H), 1.44 (s, 3H), 1.32 (s, 3H), 1.17 (s, 3H), 1.03 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) δ (ppm) 180.7, 177.2, 149.5, 141.0, 140.9, 131.7, 127.5, 124.5, 104.7, 102.3, 97.6, 85.1, 80.1, 78.9, 76.8, 76.7, 76.2, 75.5, 73.2, 70.2, 70.1, 67.5, 66.8, 65.4, 59.3, 52.0, 51.0, 50.1, 49.3, 47.5, 44.1, 43.5, 38.4, 37.7, 36.7, 33.8, 32.7, 31.6, 29.1, 27.7, 24.5, 22.8, 19.8, 18.2, 16.5, 12.3, 10.6, 8.9. HMRS (ESI) *m*+2/*z* Calcd for C₄₈H₈₂N₆O₁₄ [M+2H⁺]: 483.2939, found for 483.2935.

Azithromycin-3'-(N-(4-triazolylbenzyl)))-N-hydroxypropanamide (17b)—

Reaction of (3'-N-(4-ethynylbenzyl))azithromycin **14** (0.18 g, 0.22 mmol) and 3-Azido-N-((*tert*-butyldimethyl silyl)oxy) propanamide **51b** (0.08 g, 0.33 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **17a**, gave **17b** as light

yellow solid (170 mg, 79%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.39 (s, ^1H), 7.93 (d, $J = 8.0$ Hz, 2H), 7.61 (d, $J = 8.3$ Hz, 2H), 5.14 (d, $J = 4.5$ Hz, ^1H), 4.88 (s, 3H), 4.67 (d, $J = 7.3$ Hz, ^1H), 4.23 (d, 2H), 4.15 (d, $J = 13.2$ Hz, ^1H), 3.86 (d, $J = 13.3$ Hz, 2H), 3.77 – 3.71 (m, 2H), 3.62 – 3.52 (m, 2H), 3.46 – 3.41 (m, 3H), 3.30 (s, 3H), 3.16 (d, $J = 9.5$ Hz, 2H), 2.91 (s, 3H), 2.55 (s, 3H), 2.32 (s, ^1H), 2.15 – 2.01 (m, 24H), 1.70 (d, $J = 10.6$ Hz, 2H), 1.57 (s, 3H), 1.48 (d, $J = 6.9$ Hz, 2H), 1.43 (s, 3H), 1.38 – 1.33 (m, 2H), 1.29 (s, 3H), 1.20 (s, 3H), 1.16 (s, 3H), 1.03 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 178.3, 175.0, 147.1, 138.1, 129.6, 125.4, 102.5, 95.4, 83.0, 78.4, 77.8, 76.5, 73.9, 73.2, 72.3, 70.7, 70.0, 69.8, 65.4, 64.7, 60.8, 57.1, 5.54, 51.2, 48.2, 48.0, 47.9, 47.2, 46.1, 45.7, 45.3, 45.2, 41.9, 41.1, 36.1, 35.6, 31.7, 29.4, 29.1, 25.5, 22.7, 22.4, 20.6, 16.1, 13.2, 12.9, 10.1, 8.5, 6.9. HRMS (ESI) m/z Calcd. for $\text{C}_{49}\text{H}_{83}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 979.5962, found for 979.5958.

Azithromycin-3'-(*N*-(4-triazolylbenzyl))-*N*-hydroxybutanamide (17c)—Reaction of (3'-*N*-(4-ethynylbenzyl)azithromycin **14** (0.15 g, 0.17 mmol) and 4-Azido-*N*-((tert-butyl)dimethyl silyloxy) butanamide **51c** (0.08 g, 0.32 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **17a**, gave **17c** as light yellow solid (110 mg, 62%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.21 (s, ^1H), 7.66 (d, $J = 7.5$ Hz, 2H), 7.33 (d, $J = 6.2$ Hz, 2H), 4.35 (d, $J = 7.1$ Hz, 2H), 3.97 – 3.88 (m, ^1H), 3.66 – 3.55 (m, ^1H), 3.42 (s, 3H), 3.26 (d, $J = 10.4$, 7.0 Hz, 3H), 3.13 – 3.11 (m, 2H), 2.96 (s, 3H), 2.92 – 2.78 (m, ^1H), 2.66 (dd, $J = 12.4$, 7.4 Hz, 3H), 2.59 (s, 3H), 2.29 (s, 3H), 2.18 (d, $J = 15.0$ Hz, 2H), 2.00 (dd, $J = 20.2$, 12.5 Hz, 2H), 1.81 (d, $J = 7.6$ Hz, 2H), 1.75 (s, 24H), 1.39 (dd, $J = 15.2$, 9.7 Hz, 2H), 1.26 (s, 3H), 1.16 (d, $J = 6.8$ Hz, 3H), 1.11 (d, $J = 3.8$ Hz, 3H), 1.06 – 1.00 (m, ^1H), 0.96 (s, 3H), 0.88 (d, $J = 7.4$ Hz, 3H), 0.84 (d, $J = 6.8$ Hz, 3H), 0.71 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.2, 172.2, 149.5, 131.7, 131.6, 127.5, 123.1, 104.7, 97.6, 85.2, 80.6, 80.1, 78.8, 76.7, 76.1, 75.6, 75.1, 73.1, 70.1, 65.4, 59.4, 51.5, 50.8, 50.5, 50.2, 50.1, 49.5, 47.6, 44.1, 38.2, 37.7, 36.7, 32.8, 31.6, 31.5, 31.3, 31.2, 28.1, 27.8, 22.9, 22.9, 22.8, 22.4, 19.8, 18.2, 16.5, 12.3, 10.6, 9.0. HRMS (ESI) m/z Calcd for $\text{C}_{50}\text{H}_{85}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 993.6118, found 993.6113.

Azithromycin-3'-(*N*-(4-triazolylbenzyl))-*N*-hydroxypentanamide (17d)—Reaction of (3'-*N*-(4-ethynylbenzyl)azithromycin **14** (0.14 g, 0.16 mmol) and 5-Azido-*N*-((tert-butyl)dimethyl silyloxy) pentanamide **51d** (0.07 g, 0.24 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **17a**, gave **17d** as light yellow solid (110 mg, 66%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.28 (s, ^1H), 7.73 (d, $J = 7.7$ Hz, 2H), 7.40 (d, $J = 7.9$ Hz, 2H), 4.47 (d, $J = 7.2$ Hz, ^1H), 4.41 (s, 2H), 4.12 (d, $J = 17.0$ Hz, 2H), 3.80 (d, $J = 13.0$ Hz, ^1H), 3.68 (s, ^1H), 3.64 – 3.50 (m, 4H), 3.35 – 3.24 (m, 2H), 3.13 (s, 3H), 3.00 – 2.91 (m, 2H), 2.76 (dd, $J = 7.5$, 4.4 Hz, 2H), 2.65 (d, $J = 11.8$ Hz, ^1H), 2.39 (s, 3H), 2.32 (d, $J = 15.0$ Hz, 2H), 2.24 (s, 3H), 2.15 – 2.09 (m, 3H), 1.98 – 1.86 (m, 22H), 1.31 (s, 3H), 1.23 (d, $J = 5.9$ Hz, 5H), 1.18 – 1.09 (m, 7H), 1.06 (s, 3H), 1.01 (d, $J = 7.5$ Hz, 4H), 0.91 (s, 2H), 0.84 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.2, 149.5, 141.7, 131.6, 131.4, 127.4, 122.9, 104.8, 97.3, 85.3, 80.5, 80.2, 78.9, 76.3, 76.2, 76.1, 75.1, 73.3, 70.0, 67.3, 65.3, 59.6, 51.8, 50.8, 50.3, 50.1, 50.0, 49.8, 49.5, 49.3, 47.5, 44.3, 43.9, 38.2, 37.6, 36.8, 32.8, 31.5, 28.5, 28.3, 24.4, 23.1, 22.9, 22.5, 19.8, 18.2, 16.5, 12.4, 10.7, 8.6. HRMS (ESI) m/z Calcd. for $\text{C}_{51}\text{H}_{87}\text{N}_6\text{O}_{14}$ [$\text{M}+\text{H}^+$]: 1007.6275, found 1007.6272.

(3'-N-(2-Ethynylbenzyl))tricyclic Ketolide (21)—A solution of 3'-desmethyltricyclic ketolide **18** (0.30 g, 0.48 mmol) and 2-ethynylbenzaldehyde **19** (0.19 g, 1.45 mmol) in MeOH (5 mL) and acetic acid (55.2 μ L, 0.96 mmol) was stirred for 30 min at room temperature. Borane-pyridine complex (0.12 mL, 0.96 mmol) was added and the reaction stirred was for another 3 h. The reaction was diluted with ethyl acetate (20 mL) and washed with saturated aqueous NaHCO₃ solution (20 mL) and brine (20 mL). The organic layer was dried over Na₂SO₄, concentrated *in vacuo* and purified by preparative chromatography (Silica gel, 12:1 CH₂Cl₂/MeOH) to afford compound **21** as a brown solid (210 mg, 58 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.44 (d, *J* = 7.2 Hz, ¹H), 7.22 (m, 3H), 4.88 (d, *J* = 8.4 Hz, ¹H), 4.22 (d, *J* = 7.2 Hz, ¹H), 4.15 (d, *J* = 8.8 Hz, ¹H), 3.91 (m, ¹H), 3.70 (m, 4H), 3.35 (m, 4H), 2.94 (m, 2H), 2.59 (m, 4H), 2.10 (s, 3H), 1.48 (m, 27H), 0.98 (d, *J* = 7.2 Hz, 3H), 0.79 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 204.2, 181.2, 169.5, 156.0, 141.1, 133.3, 129.3, 128.9, 127.2, 122.1, 104.0, 82.1, 81.6, 81.5, 79.2, 78.4, 76.4, 70.4, 69.5, 65.2, 59.9, 56.7, 51.1, 49.5, 49.1, 48.1, 42.7, 42.3, 38.5, 36.3, 35.8, 29.5, 22.0, 21.2, 19.6, 19.1, 16.4, 14.4, 12.8, 10.8, 10.4. HRMS (MALDI) *m/z* Calcd. for C₄₁H₆₀N₃O₉ [M+H⁺]: 738.4324, found 738.4348.

(3'-N-(3-Ethynylbenzyl))tricyclic Ketolide (22)—Reaction of 3'-desmethyltricyclic ketolide **18** (0.50 g, 0.8 mmol) and 3-ethynylbenzaldehyde **20** (0.21 g, 1.6 mmol) in MeOH (9 mL) and acetic acid (91.4 μ L, 1.6 mmol) for 1 h at room temperature followed by addition of borane-pyridine complex (0.2 mL, 1.6 mmol) within 3 h and then purification as described in the protocol for **21** afforded **22** as a brown solid (490 mg, 83 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.15 (m, 2H), 7.06 (m, 2H), 4.72 (d, *J* = 8.0 Hz, ¹H), 4.08 (d, *J* = 7.2 Hz, ¹H), 3.99 (d, *J* = 8.4 Hz, ¹H), 3.53 (m, 4H), 3.76 (m, ¹H), 3.20 (m, 3H), 2.78 (m, 2H), 2.78 (m, 2H), 2.44 (m, 4H), 1.93 (s, 3H), 1.34 (m, 28H), 0.82 (d, *J* = 7.2 Hz, 3H), 0.62 (t, *J* = 7.6 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 204.2, 181.1, 169.5, 156.0, 139.1, 132.2, 131.0, 129.1, 128.4, 122.2, 103.8, 83.5, 81.5, 79.2, 78.5, 76.5, 70.3, 69.5, 65.6, 59.9, 57.2, 51.1, 49.5, 48.0, 42.8, 42.3, 38.5, 36.8, 36.3, 29.5, 29.2, 22.0, 21.1, 19.6, 19.1, 16.4, 14.3, 12.8, 10.8, 10.4. HRMS (MALDI) *m/z* Calcd. for C₄₁H₆₀N₃O₉ [M+H⁺]: 738.4324, found 738.4329.

(Tricyclic Ketolide-3'-(N-(2-triazolylbenzyl)))-N-hydroxyhexanamide (24a)—To a round bottomed flask containing (3'-N-(2-ethynylbenzyl))tricyclic Ketolide **21** (0.045 g, 0.061 mmol) and copper (I) iodide (10 mg, 0.05 mmol) under argon, was added degassed THF (4 mL) and Hunig's base (0.5 mL). Stirring under argon and at room temperature continued for 15 min after which 6-azido-N-((*tert*-butyldimethylsilyloxy)hexanamide **51e** (35 mg, 0.12 mmol) was added to the reaction mixture, and stirred for an additional 24 h at 40 °C. The reaction mixture was diluted with ethyl acetate (50 mL) and the organic layer was washed with 1:4 NH₄OH soln./saturated aqueous NH₄Cl soln. (2 \times 4 mL), water (10 mL), and brine (10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude was subjected to next reaction without further purification.

The crude was dissolved in anhydrous methanol (2 mL) and caesium fluoride (18 mg, 0.12 mmol) was added and the resulting reaction mixture was stirred at room temperature for 2 h. Reaction was quenched by adding water (10 mL) and was extracted with ethyl acetate (30

mL) and the organic layer was washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude was purified by preparative chromatography (Silica gel, 12:1:0.1 CH₂Cl₂/MeOH/conc. NH₄OH) to give **24a** as a brown-white solid (30 mg, 55 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.78 (s, ¹H), 7.52 (m, ¹H), 7.28 (m, 3H), 4.88 (d, *J* = 8.4 Hz, ¹H), 4.33 (m, 2H), 4.14 (m, 2H), 3.79 (m, 5H), 3.43 (m, ¹H), 3.16 (m, 2H), 2.98 (m, ¹H), 2.87 (m, ¹H), 2.62 (m, 5H), 2.08 (s, 3H), 1.59 (m, 34H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.78 (t, *J* = 7.6 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 204.4, 182.5, 170.3, 169.6, 156.1, 147.0, 136.0, 131.1, 130.5, 129.9, 128.3, 127.9, 122.3, 105.0, 103.5, 81.6, 78.6, 78.5, 76.5, 70.3, 69.3, 63.6, 59.9, 56.9, 51.1, 49.9, 49.2, 49.1, 47.9, 42.3, 38.4, 36.3, 35.3, 29.6, 29.3, 28.5, 25.6, 24.4, 21.1, 22.1, 19.6, 19.0, 16.4, 14.4, 12.9, 10.8, 10.4. HRMS (MALDI) *m/z* Calcd. for C₄₇H₇₂N₇O₁₁ [M+H⁺]: 910.5284, found 910.5245.

(Tricyclic Ketolide-3'-(N-(2-triazolylbenzyl)))-N-hydroxyheptanamide (24b)—

Reaction of (3'-*N*-(2-ethynylbenzyl))tricyclic Ketolide **21** (0.05 g, 0.067 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl)oxy)heptanamide **51f** (0.04 g, 0.134 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **24b** as a brown-white solid (35 mg, 56 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.80 (s, ¹H), 7.51 (m, ¹H), 7.31 (m, 3H), 4.88 (d, *J* = 10.4 Hz, ¹H), 4.34 (m, 2H), 4.20 (d, *J* = 7.2 Hz, ¹H), 4.15 (d, *J* = 8.4 Hz, ¹H), 3.78 (m, 5H), 3.46 (m, ¹H), 3.19 (m, 2H), 3.0 (m, ¹H), 2.88 (m, ¹H), 2.68 (m, 2H), 2.64 (s, 3H), 2.16 (s, 3H), 1.62 (m, 36H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.79 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 204.4, 182.3, 170.4, 169.6, 156.0, 147.0, 130.5, 129.9, 128.4, 122.3, 103.6, 81.6, 78.7, 78.5, 76.5, 70.2, 69.1, 64.1, 63.9, 59.9, 53.7, 51.3, 51.1, 50.1, 49.1, 47.9, 42.3, 42.0, 38.5, 36.4, 35.2, 32.7, 32.2, 29.7, 28.6, 27.7, 26.3, 25.5, 25.2, 24.8, 22.0, 21.1, 19.6, 19.0, 18.0, 16.3, 14.4, 12.9, 12.1, 10.8, 10.4. HRMS (MALDI) *m/z* Calcd. for C₄₈H₇₄N₇O₁₁ [M+H⁺]: 924.5441, found 924.5422.

(Tricyclic Ketolide-3'-(N-(3-triazolylbenzyl)))-N-hydroxyhexanamide (25a)—

Reaction of (3'-*N*-(3-ethynylbenzyl))tricyclic Ketolide **22** (0.04 g, 0.05 mmol) and 6-azido-*N*-((*tert*-butyldimethylsilyl)oxy)hexanamide **51e** (0.03 g, 0.11 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **25a** as a brown-white solid (32 mg, 64 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.86 (s, ¹H), 7.74 (m, 2H), 7.35 (m, ¹H), 7.23 (m, ¹H), 4.94 (d, *J* = 9.6 Hz, ¹H), 4.37 (m, 2H), 4.31 (d, *J* = 6.4 Hz, ¹H), 4.23 (d, *J* = 8.0 Hz, ¹H), 3.98 (m, ¹H), 3.63 (m, 5H), 2.13 (m, 4H), 2.60 (m, 5H), 2.20 (s, 3H), 1.68 (m, 34H), 1.04 (d, *J* = 6.4 Hz, 3H), 0.84 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 204.3, 182.1, 170.3, 169.6, 156.0, 147.5, 139.5, 130.7, 128.4, 126.0, 124.6, 120.2, 103.8, 81.6, 78.9, 78.5, 76.4, 70.3, 69.5, 65.4, 59.9, 57.6, 53.6, 51.1, 50.0, 49.3, 49.1, 47.9, 42.5, 42.3, 42.9, 38.5, 36.8, 29.6, 29.5, 25.6, 22.0, 21.1, 19.6, 19.1, 18.1, 16.2, 14.4, 12.8, 12.2, 10.8, 10.4. HRMS (MALDI) *m/z* Calcd. for C₄₇H₇₂N₇O₁₁ [M+H⁺]: 910.5284, found 910.5278.

(Tricyclic Ketolide-3'-(N-(3-triazolylbenzyl)))-N-hydroxyheptanamide (25b)—

Reaction of (3'-*N*-(3-ethynylbenzyl))tricyclic Ketolide **22** (0.05 g, 0.067 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl)oxy)heptanamide **51f** (0.04 g, 0.134 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **25b** as a brown-white solid (34 mg, 55 %). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.74 (s, ¹H), 7.71

(s, ^1H), 7.63 (d, $J = 7.6$ Hz, ^1H), 7.30 (t, $J = 7.6$ Hz, ^1H), 7.18 (d, $J = 7.6$ Hz, ^1H), 4.89 (d, $J = 8.4$ Hz, ^1H), 4.32 (m, 2H), 4.25 (d, $J = 7.2$ Hz, ^1H), 4.17 (d, $J = 8.4$ Hz, ^1H), 3.92 (m, ^1H), 3.71 (m, 4H), 3.20 (m, 5H), 2.64 (m, 5H), 2.15 (s, 3H), 1.71 (m, 36H), 0.99 (d, $J = 7.2$ Hz, 3H), 0.79 (t, $J = 7.6$ Hz, 3H). ^{13}C NMR (CDCl_3 , 100 MHz) δ (ppm) 204.3, 181.9, 170.5, 169.6, 156.1, 147.6, 139.6, 130.7, 129.0, 128.6, 126.0, 124.7, 119.9, 103.8, 81.6, 78.9, 78.5, 76.5, 70.4, 69.5, 65.4, 59.9, 57.6, 51.2, 50.1, 49.4, 49.1, 47.9, 42.3, 38.6, 36.9, 36.4, 29.8, 29.7, 29.5, 27.9, 25.6, 24.9, 22.1, 21.0, 19.6, 19.1, 16.2, 14.4, 14.2, 12.9, 10.9, 10.4. HRMS (MALDI) m/z Calcd. for $\text{C}_{48}\text{H}_{74}\text{N}_7\text{O}_{11}$ [$\text{M}+\text{H}^+$]: 924.5441, found 924.5455.

(Tricyclic Ketolide-3'-(N-(4-triazolylbenzyl)))-N-hydroxyacetamide (26a)—In an oven dried round bottomed flask charged with a magnetic stirring bar, (3'-N-(4-ethynylbenzyl))tricyclic ketolide **23**¹⁵ (0.165 g, 0.224 mmol) and 2-azido-N-((*tert*-butyldimethylsilyl)oxy)acetamide **51a** (0.09 g, 0.40 mmol) were placed under argon. To the mixture were added 2 mL of degassed 1:1 mixture of THF and DMSO, CuI (6.4 mg, 0.03 mmol) and *N,N'*-diisopropylethylamine (30 μL , 0.15 mmol) in succession. The mixture was heated at 45 °C for 12 h. The reaction mixture was cooled to room temperature and diluted with 50 mL of ethyl acetate. Organic layer was washed with 4:1 mixture of satd. NH_4Cl soln. and NH_4OH soln. (2 \times 10 mL), water (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude was used for the next reaction without further purification.

The crude was dissolved in methanol (3 mL) and to that solution caesium fluoride (68 mg, 0.442 mmol) was added and the resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by adding water and extracted with ethyl acetate (100 mL) and the organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ soln.) to give **26a** as a white solid (76 mg, 40 %). ^1H NMR (500 MHz, $\text{MeOH}-d_4$) δ (ppm) 8.24 (s, ^1H), 7.70 (d, $J = 8.0$ Hz, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 5.00 (s, 2H), 4.18 (m, 2H), 3.81 (dd, $J = 44.1, 13.9$ Hz, 2H), 3.69 (m, 2H), 3.50 (m, 4H), 3.26 (m, ^1H), 3.09 (p, $J = 7.5$ Hz, ^1H), 2.85 (m, 2H), 2.72 (m, ^1H), 2.58 (m, 5H), 2.21 (d, $J = 15.7$ Hz, 3H), 1.72 (m, 2H), 1.60 (m, 2H), 1.52 (m, ^1H), 1.44 (m, 3H), 1.19 (m, 17 H), 1.01 (t, $J = 13.0$ Hz, 3H), 0.77 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, $\text{MeOH}-d_4$) δ (ppm) 184.6, 170.0, 163.4, 156.6, 147.3, 139.0, 129.4, 125.3, 122.3, 103.6, 82.2, 78.5, 77.7, 76.2, 70.7, 69.0, 57.3, 50.7, 49.8, 48.6, 48.3, 42.1, 41.9, 38.1, 36.4, 36.0, 30.9, 21.8, 20.1, 18.9, 17.8, 15.1, 13.5, 11.7, 9.8, 9.6. HRMS (ESI) m/z Calcd. for $\text{C}_{43}\text{H}_{64}\text{N}_7\text{O}_{11}$ [$\text{M}+\text{H}^+$]: 854.4658, found 854.4656.

(Tricyclic Ketolide-3'-(N-(4-triazolylbenzyl)))-N-propanamide (26b)—Reaction of (3'-N-(4-ethynylbenzyl))tricyclic ketolide **23** (0.20 g, 0.271 mmol) and 3-azido-N-((*tert*-butyldimethylsilyl)oxy)propanamide **51b** (0.11 g, 0.406 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **26a**, gave **26b** as white solid (105 mg, 45 %). ^1H NMR (500 MHz, $\text{MeOH}-d_4$) δ (ppm) 8.13 (s, ^1H), 7.64 (d, $J = 8.1$ Hz, 2H), 7.35 (d, $J = 8.1$ Hz, 2H), 4.65 (t, $J = 6.2$ Hz, 2H), 4.19 (dd, $J = 16.2$, and 7.7 Hz, 2H), 3.87 (m, ^1H), 3.72 (m, 3H), 3.57 (m, ^1H), 3.53 (m, ^1H), 3.48 (m, 2H), 3.27 (m, ^1H), 3.09 (m, ^1H), 2.88 (dd, $J = 14.3$, and 10.1 Hz, 2H), 2.69 (t, $J = 10.8$ Hz, 3H), 2.60 (m, 4H),

2.18 (d, $J = 14.1$ Hz, 3H), 1.74 (m, 2H), 1.62 (t, $J = 7.6$ Hz, 2H), 1.54 (m, ^1H), 1.47 (m, 3H), 1.21 (m, 17H), 1.03 (t, $J = 12.4$ Hz, 3H), 0.78 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 196.6, 176.1, 161.6, 159.1, 148.2, 138.7, 130.9, 120.8, 116.8, 112.8, 95.2, 73.7, 70.0, 69.2, 67.8, 62.3, 60.6, 55.6, 51.7, 48.9, 42.3, 40.2, 39.8, 37.5, 33.7, 33.5, 29.6, 27.9, 27.5, 22.5, 13.4, 11.6, 10.5, 9.4. HRMS (ESI) m/z Calcd. for $\text{C}_{44}\text{H}_{66}\text{N}_7\text{O}_{11}$ [$\text{M}+\text{H}^+$]: 868.4815, found 868.4812.

(Tricyclic Ketolide-3'-(N-(4-triazolylbenzyl)))-N-hydroxybutanamide (26c)—

Reaction of (3'-N-(4-ethynylbenzyl))tricyclic ketolide **23** (0.173 g, 0.230 mmol) and 4-azido-N-((*tert*-butyldimethylsilyloxy)butanamide **51c** (0.011 g, 0.420 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **26a**, gave **26c** as white solid (106 mg, 52 %). ^1H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.23 (s, ^1H), 7.69 (d, $J = 7.9$ Hz, 2H), 7.36 (d, $J = 7.9$ Hz, 2H), 4.41 (t, $J = 6.7$ Hz, 2H), 4.19 (dd, $J = 15.6$, and 7.7 Hz, 2H), 3.88 (m, 2H), 3.73 (q, $J = 13.3$ Hz, 3H), 3.59 (m, ^1H), 3.50 (dd, $J = 17.4$ and 9.4 Hz, 2H), 3.28 (dd, $J = 10.0$ and 7.6 Hz, ^1H), 3.09 (m, ^1H), 2.89 (dd, $J = 14.6$ and 10.4 Hz, 2H), 2.69 (dd, $J = 15.5$, and 6.7 Hz, ^1H), 2.60 (m, 4H), 2.17 (m, 5H), 2.08 (m, 2H), 1.75 (m, 2H), 1.62 (m, ^1H), 1.55 (m, ^1H), 1.48 (s, 3H), 1.22 (m, 17H), 1.02 (d, $J = 6.9$ Hz, 3H), 0.79 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 206.6, 186.1, 171.6, 158.2, 149.0, 141.0, 130.8, 126.8, 122.4, 105.2, 83.8, 80.1, 79.2, 77.9, 72.3, 70.7, 65.6, 61.7, 59.0, 52.4, 51.9, 50.8, 50.2, 49.9, 48.0, 39.7, 37.9, 37.5, 32.6, 30.5, 27.4, 26.2, 23.4, 21.6, 20.5, 19.4, 16.6, 15.1, 13.2, 11.3, 11.1, 9.5. HRMS (ESI) m/z Calcd. for $\text{C}_{45}\text{H}_{68}\text{N}_7\text{O}_{11}$ [$\text{M}+\text{H}^+$]: 882.4971, found 882.4971.

Tricyclic Ketolide-3'-(N-(4-triazolylbenzyl)))-N-hydroxypentanamide (26d)—

Reaction of (3'-N-(4-ethynylbenzyl))tricyclic ketolide **23** (0.114 g, 0.150 mmol) and 5-azido-N-((*tert*-butyldimethylsilyloxy)pentanamide **51d** (0.044 g, 0.28 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **26a**, gave **26d** as white solid (67 mg, 50%). ^1H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.23 (s, ^1H), 7.70 (d, $J = 7.9$ Hz, 2H), 7.37 (d, $J = 7.9$ Hz, 2H), 4.38 (d, $J = 6.8$ Hz, 2H), 4.20 (dd, $J = 17.7$, and 7.7 Hz, 2H), 3.94 (q, $J = 6.7$ Hz, ^1H), 3.88 (d, $J = 14.6$ Hz, ^1H), 3.81 (d, $J = 13.3$ Hz, ^1H), 3.71 (m, 2H), 3.58 (dd, $J = 20.0$, and 11.2 Hz, 2H), 3.50 (m, ^1H), 3.30 (dd, $J = 9.9$, and 7.6 Hz, ^1H), 3.11 (m, ^1H), 2.89 (dd, $J = 14.4$, and 10.4 Hz, 2H), 2.74 (m, ^1H), 2.60 (m, 3H), 2.23 (s, 3H), 2.07 (t, $J = 7.1$ Hz, 2H), 1.88 (m, 2H), 1.77 (m, 2H), 1.57 (m, 5H), 1.48 (s, 3H), 1.24 (m, 17H), 1.02 (d, $J = 6.9$ Hz, 3H), 0.79 (t, $J = 7.4$ Hz, 3H). ^{13}C (125 MHz, MeOH- d_4) δ (ppm) 206.6, 186.2, 172.4, 171.6, 158.2, 148.8, 131.0, 126.9, 122.4, 105.2, 83.8, 80.0, 79.2, 77.9, 72.2, 70.6, 65.6, 61.7, 58.8, 52.4, 51.2, 50.2, 49.9, 39.7, 37.9, 37.6, 33.1, 32.5, 30.8, 23.8, 23.4, 21.6, 20.5, 19.4, 16.6, 15.1, 13.3, 11.4, 11.1. HRMS (ESI) m/z Calcd. for $\text{C}_{46}\text{H}_{70}\text{N}_7\text{O}_{11}$ [$\text{M}+\text{H}^+$]: 896.5128, found 896.5121.

4''-Oxo-3'-O-acetylclarithromycin (31)—A solution of *N*-chlorosuccinimide (2.00 g, 14.97 mmol) in anhydrous DCM (30 mL) was stirred at -15 °C for 10 min. Dimethyl sulfide (1.10 mL, 14.97 mmol) was then added drop wise to the solution. After stirring for 20 minutes at the same temperature a DCM solution (10 mL) of acetylated clarithromycin **29** (5.91 g, 7.49 mmol) was added over a period of 30 min to the suspension and the resulting suspension was stirred at -15 °C for another 30 min, afterward TEA (2.09 mL, 14.97 mmol)

was added. The resulting solution was stirred at $-10\text{ }^{\circ}\text{C}$ for another 2 h. The reaction was quenched by adding saturated aqueous NaHCO_3 solution (50 mL), the organic layer was separated. The aqueous layer was extracted with DCM ($2 \times 25\text{ mL}$) and the combined organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The crude compound **31** (6.35 g) was sufficiently pure to be used for the next step without further purification. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) 5.11 – 5.02 (m, ^1H), 4.97 (d, $J = 10.7\text{ Hz}$, ^1H), 4.75 (dd, $J = 10.3, 7.6\text{ Hz}$, ^1H), 4.39 (t, $J = 6.1\text{ Hz}$, ^1H), 4.29 (q, $J = 6.7\text{ Hz}$, ^1H), 3.67 – 3.53 (m, 3H), 3.38 (td, $J = 15.1, 5.0\text{ Hz}$, 2H), 3.30 (d, $J = 1.3\text{ Hz}$, 4H), 3.15 (t, $J = 9.1\text{ Hz}$, 3H), 2.93 – 2.82 (m, 4H), 2.78 (dd, $J = 16.9, 7.3\text{ Hz}$, ^1H), 2.68 (d, $J = 1.3\text{ Hz}$, ^1H), 2.65 (d, $J = 2.7\text{ Hz}$, ^1H), 2.60 (d, $J = 1.2\text{ Hz}$, ^1H), 2.56 (d, $J = 1.3\text{ Hz}$, ^1H), 2.49 – 2.37 (m, 8H), 2.29 – 2.14 (m, 3H), 2.07 – 2.02 (m, 2H), 1.97 (t, $J = 8.3\text{ Hz}$, 3H), 1.94 – 1.73 (m, 15H), 1.60 – 1.47 (m, ^1H), 1.41 – 1.33 (m, 2H), 1.32 – 1.23 (m, 8H), 1.21 (d, $J = 9.9\text{ Hz}$, 3H), 1.12 (dd, $J = 11.2, 6.4\text{ Hz}$, 7H), 1.07 – 0.93 (m, 10H), 0.78 (t, $J = 9.6\text{ Hz}$, 3H), 0.70 (t, $J = 7.2\text{ Hz}$, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) 221.0, 210.6, 175.6, 170.4, 99.6, 96.4, 80.0, 78.7, 78.1, 78.0, 76.7, 76.0, 74.2, 72.9, 72.7, 69.8, 69.2, 67.5, 61.7, 51.2, 50.2, 49.9, 45.1, 44.4, 39.0, 38.4, 38.1, 37.9, 37.6, 37.2, 30.8, 30.4, 29.5, 28.3, 26.5, 21.3, 21.3, 20.9, 20.5, 20.4, 20.0, 19.5, 18.9, 17.8, 16.2, 16.1, 15.5, 14.7, 13.0, 12.2, 10.4, 8.9. HRMS (MALDI) m/z Calcd. for $\text{C}_{40}\text{H}_{70}\text{NO}_{14}$ [$\text{M}+\text{H}^+$]: 788.4791, found 788.4781.

4'-Epoxy-3'-O-acetylclarithromycin (33)—NaH (60% in mineral oil) (0.73 g, 17.7 mmol) was added to an oven dried three-neck round bottom flask and was washed with petroleum ether. The flask was immediately flushed with Ar and 5 mL of anhydrous DMSO added through the septum. The mixture was stirred at room temperature under Ar and trimethylloxosulfonium iodide (3.90 g, 17.7 mmol) was added over a period of 5 min. When hydrogen evolution ceased and a clear solution obtained, a solution of compound **31** (6.35 g, 8.06 mmol) in anhydrous THF (10 mL) was added over a period of 10 min and left to stir for 2 h. Once TLC showed 100% conversion, THF was removed under vacuum and ethyl acetate was added to the remaining solution. The solution was washed severally with H_2O to remove DMSO. Organic layer was dried with NaSO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography using EtOAc: Acetone (5:1) to give compound **33** as a white solid (2.43g, 38%). $^1\text{H NMR}$ (400 MHz, CDCl_3) δ (ppm) 5.06 (dq, $J = 6.2, 4.6\text{ Hz}$, 2H), 4.77 – 4.59 (m, 4H), 3.95 (s, ^1H), 3.76 – 3.66 (m, 3H), 3.55 (s, ^1H), 3.45 (dd, $J = 9.3, 6.0\text{ Hz}$, ^1H), 3.34 – 3.28 (m, 4H), 3.19 (s, ^1H), 3.01 (d, $J = 7.0\text{ Hz}$, ^1H), 3.01 – 2.95 (m, 4H), 2.94 – 2.90 (m, ^1H), 2.86 (dd, $J = 9.9, 7.1\text{ Hz}$, ^1H), 2.69 – 2.63 (m, ^1H), 2.61 (dd, $J = 9.8, 4.4\text{ Hz}$, 2H), 2.53 (dd, $J = 9.6, 7.1\text{ Hz}$, ^1H), 2.34 (s, ^1H), 2.27 – 2.20 (m, 7H), 2.19 – 2.13 (m, 2H), 2.06 – 2.00 (m, 4H), 1.95 – 1.83 (m, 3H), 1.72 – 1.61 (m, 2H), 1.56 (d, $J = 1.6\text{ Hz}$, ^1H), 1.53 (s, ^1H), 1.49 – 1.36 (m, ^1H), 1.33 (d, $J = 7.2\text{ Hz}$, 3H), 1.28 (s, ^1H), 1.26 – 1.12 (m, 1^1H), 1.14 – 0.99 (m, 16H), 0.92 (t, $J = 8.2\text{ Hz}$, 3H), 0.81 (dd, $J = 9.8, 4.9\text{ Hz}$, 3H). $^{13}\text{C NMR}$ (101 MHz, CDCl_3) δ (ppm) 221.1, 175.3, 170.0, 100.1, 96.4, 96.3, 80.3, 78.9, 78.3, 78.2, 76.6, 76.1, 74.2, 73.7, 71.7, 69.1, 67.8, 64.3, 63.2, 60.4, 60.2, 50.4, 49.6, 46.6, 45.3, 44.8, 40.7, 38.6, 38.1, 37.2, 36.0, 30.8, 29.2, 21.6, 21.3, 21.0, 20.2, 19.7, 18.2, 18.0, 16.1, 15.9, 14.9, 14.2, 14.2, 12.4, 10.5, 9.1. HRMS (MALDI) m/z Calcd. for $\text{C}_{41}\text{H}_{77}\text{NO}_{14}$ [$\text{M}+\text{H}^+$]: 802.4947, found 802.4938.

(4''-(Methylamino)-N(methyl)(4-ethynylbenzyl)clarithromycin (36)—1-(4-Ethynylphenyl)-N-methylmethanamine **35** (1.52 g, 10.47 mmol) was added to a solution of compound **33** (2.80 g, 3.49 mmol) in MeOH (20 mL). The solution was heated at 60 °C for 6 h. Excess MeOH was evaporated off and the crude was purified by column chromatography using Hexane:EtOAc:MeOH:NH₄OH (2:1:0.6:0.1) to give compound **36** as a light yellow solid (2.41g, 73%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.33 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 4.98 – 4.86 (m, 2H), 4.35 – 4.28 (m, ¹H), 4.06 (q, *J* = 5.9 Hz, ¹H), 3.66 (d, *J* = 11.1 Hz, 2H), 3.57 (d, *J* = 7.0 Hz, 2H), 3.34 (s, ¹H), 3.20 (s, 3H), 3.09 (dd, *J* = 10.0, 7.3 Hz, 2H), 3.02 (d, *J* = 10.1 Hz, ¹H), 2.97 (d, *J* = 6.6 Hz, ¹H), 2.94 (s, 2H), 2.91 – 2.85 (m, 2H), 2.81 – 2.72 (m, ¹H), 2.47 (d, *J* = 7.4 Hz, ¹H), 2.34 (dd, *J* = 15.6, 6.7 Hz, ¹H), 2.24 (s, ¹H), 2.20 (s, 6H), 2.10 (s, 3H), 2.04 (d, *J* = 3.6 Hz, ¹H), 2.00 (s, ¹H), 1.96 (s, ¹H), 1.93 – 1.87 (m, ¹H), 1.84 – 1.70 (m, 3H), 1.57 (dd, *J* = 19.0, 11.8 Hz, 2H), 1.41 – 1.31 (m, ¹H), 1.30 (d, *J* = 11.1 Hz, 3H), 1.15 – 1.07 (m, 10H), 1.05 (s, 4H), 0.97 (dd, *J* = 17.3, 7.0 Hz, 10H), 0.71 (dd, *J* = 13.8, 6.6 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 220.8, 175.7, 139.1, 132.1, 128.6, 121.2, 102.7, 96.4, 83.2, 80.9, 78.9, 78.0, 76.3, 76.1, 75.9, 74.2, 70.9, 70.6, 68.9, 68.2, 67.4, 65.4, 63.1, 56.9, 50.6, 49.5, 45.2, 44.8, 43.2, 40.2, 39.3, 38.8, 37.1, 31.3, 28.7, 21.7, 20.9, 20.2, 19.6, 19.1, 18.7, 18.0, 15.9, 15.3, 14.8, 14.6, 13.0, 12.2, 11.4, 10.6, 10.5, 9.3, 9.1. HRMS (ESI) *m/z* Calcd. for [C₄₉H₈₁N₂O₁₃ [M+H⁺]: 905.5739, found 905.5693.

(Clarithromycin-(4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxyacetamide (38a)—Reaction of (4''-(Methylamino)-N(methyl)(4-ethynylbenzyl)clarithromycin **36** (0.15 g, 0.17 mmol) with 2-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)acetamide **51a** (0.057 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38a** as a light yellow solid (0.057 g, 38%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.37 (d, *J* = 5.4 Hz, ¹H), 7.82 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 5.50 (d, *J* = 5.4 Hz, ¹H), 5.12 (d, *J* = 14.2 Hz, 2H), 5.00 – 4.95 (m, ¹H), 4.43 (d, *J* = 7.3 Hz, ¹H), 4.22 (d, *J* = 6.4 Hz, ¹H), 3.85 – 3.65 (m, 5H), 3.61 (s, ¹H), 3.50 (s, ¹H), 3.32 – 3.25 (m, 5H), 3.13 – 3.00 (m, 5H), 2.96 – 2.85 (m, ¹H), 2.76 (d, *J* = 10.3 Hz, ¹H), 2.58 (s, ¹H), 2.53 (s, ¹H), 2.48 (s, 5H), 2.35 (s, ¹H), 2.32 – 2.25 (m, 3H), 2.24 (s, ¹H), 2.21 (s, ¹H), 2.16 (d, *J* = 7.6 Hz, ¹H), 2.14 (d, *J* = 5.4 Hz, ¹H), 2.01 (s, ¹H), 1.98 – 1.74 (m, 5H), 1.66 (d, *J* = 12.7 Hz, ¹H), 1.57 – 1.44 (m, ¹H), 1.40 (s, 3H), 1.31 – 1.15 (m, 17H), 1.12 (dt, *J* = 11.4, 4.9 Hz, 10H), 0.85 (t, *J* = 7.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm) 176.0, 147.3, 129.4, 125.8, 122.3, 102.4, 96.5, 81.1, 78.3, 78.2, 74.3, 70.8, 69.1, 68.0, 67.5, 65.4, 62.9, 53.5, 50.7, 49.6, 45.2, 45.0, 43.4, 40.3, 39.1, 37.3, 31.5, 29.7, 21.5, 21.0, 19.8, 18.9, 18.0, 16.0, 15.4, 14.9, 14.8, 13.1, 12.3, 11.5, 10.7, 10.6, 9.3. HRMS (MALDI) *m/z* Calcd. for C₅₁H₈₆N₆O₁₅ [M+H⁺]: 1021.6073, found 1021.6095.

(Clarithromycin-4''(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxypropanamide (38b)—Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl)clarithromycin **36** (0.15 g, 0.17 mmol) with 3-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)propanamide **51b** (0.061 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38b** as a light yellow solid (0.07g, 47%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.22 (s, ¹H), 7.75 (d, *J* =

7.7 Hz, 2H), 7.36 (d, $J = 7.9$ Hz, 2H), 5.13 – 5.06 (m, ^1H), 4.97 – 4.90 (m, ^1H), 4.69 (d, $J = 18.5$ Hz, 2H), 4.40 (d, $J = 7.0$ Hz, ^1H), 4.19 (d, $J = 6.5$ Hz, ^1H), 3.81 – 3.64 (m, 5H), 3.47 (s, ^1H), 3.30 – 3.20 (m, 5H), 3.09 – 3.03 (m, 2H), 3.00 (d, $J = 8.3$ Hz, 3H), 2.94 – 2.82 (m, ^1H), 2.73 (t, $J = 19.8$ Hz, 3H), 2.56 (d, $J = 7.6$ Hz, ^1H), 2.41 (s, 6H), 2.31 (s, ^1H), 2.25 (d, $J = 12.9$ Hz, 4H), 2.18 (s, ^1H), 2.16 – 2.12 (m, ^1H), 2.14 – 2.09 (m, 7H), 1.95 – 1.80 (m, 4H), 1.76 (d, $J = 12.9$ Hz, ^1H), 1.62 (d, $J = 14.9$ Hz, ^1H), 1.53 – 1.41 (m, ^1H), 1.36 (s, 3H), 1.27 – 1.13 (m, 16H), 1.09 (td, $J = 13.0, 8.1$ Hz, ^1H), 0.83 (t, $J = 12.0, 5.5$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 175.7, 146.9, 137.8, 129.2, 125.5, 121.0, 102.2, 96.2, 80.9, 80.9, 78.7, 78.4, 78.1, 77.9, 76.4, 75.9, 75.4, 74.1, 71.2, 70.9, 70.5, 68.8, 67.8, 67.3, 65.1, 62.6, 56.3, 50.8, 50.5, 50.5, 49.4, 46.0, 45.0, 44.7, 44.6, 43.1, 40.2, 39.1, 38.8, 37.0, 31.2, 29.9, 29.5, 25.4, 21.3, 20.8, 20.1, 19.8, 19.5, 18.9, 18.6, 18.1, 17.8, 15.8, 15.2, 14.7, 14.5, 12.9, 12.1, 11.3, 10.4, 10.3, 10.3, 9.3, 9.2. HRMS (MALDI) m/z Calcd. for $\text{C}_{52}\text{H}_{87}\text{N}_6\text{O}_{15}$ [$\text{M}+\text{H}^+$]: 1035.6270, found 1035.6210.

(Clarithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxybutanamide (38c)—Reaction of (4''-(methylamino)-N(methyl)(4-

ethynylbenzyl)clarithromycin **36** (0.15 g, 0.17 mmol) with 4-Azido-N-((*tert*-butyldimethylsilyl)oxy)butanamide **51c** (0.064 g, 0.245 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38c** as a light yellow solid (0.060g, 40%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.32 (s, ^1H), 7.77 (d, $J = 7.7$ Hz, 2H), 7.36 (d, $J = 7.9$ Hz, 2H), 5.09 (d, $J = 9.2$ Hz, ^1H), 4.46 (s, 2H), 4.39 (d, $J = 7.2$ Hz, ^1H), 4.19 (d, $J = 6.4$ Hz, ^1H), 3.82 – 3.61 (m, 5H), 3.48 (s, ^1H), 3.29 – 3.21 (m, 4H), 3.05 (t, $J = 4.8$ Hz, 2H), 3.00 (d, $J = 9.9$ Hz, 3H), 2.92 – 2.82 (m, ^1H), 2.67 (s, ^1H), 2.54 (s, ^1H), 2.41 (s, 5H), 2.30 (s, ^1H), 2.26 – 2.18 (m, 6H), 2.11 (t, $J = 7.4$ Hz, 3H), 1.95 – 1.79 (m, 4H), 1.75 (d, $J = 13.0$ Hz, ^1H), 1.62 (d, $J = 14.6$ Hz, ^1H), 1.52 – 1.40 (m, ^1H), 1.36 (s, 3H), 1.18 (dt, $J = 11.1, 7.0$ Hz, 15H), 1.13 – 1.02 (m, ^1H), 0.81 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 176.1, 171.4, 147.5, 138.4, 129.8, 129.5, 125.9, 120.8, 102.7, 102.4, 96.9, 96.6, 81.2, 81.2, 80.3, 79.1, 78.8, 78.5, 78.2, 76.3, 76.2, 75.7, 74.4, 71.5, 71.1, 70.8, 70.1, 69.2, 68.3, 68.1, 67.9, 67.7, 65.6, 63.1, 60.6, 56.9, 53.6, 51.2, 50.8, 49.7, 49.5, 45.4, 45.1, 44.9, 43.4, 40.5, 39.4, 39.1, 37.4, 31.6, 29.8, 29.5, 26.2, 21.7, 21.2, 21.1, 20.4, 20.1, 19.9, 19.3, 19.0, 18.5, 18.2, 16.1, 15.6, 15.0, 14.9, 14.3, 13.2, 12.5, 11.7, 10.8, 9.6, 9.5. HRMS (MALDI) m/z Calcd. for $\text{C}_{53}\text{H}_{89}\text{N}_6\text{O}_{15}$ [$\text{M}+\text{H}^+$] 1049.6386, found 1049.6376.

(Clarithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxypentanamide (38d)—Reaction of (4''-(methylamino)-N(methyl)(4-

ethynylbenzyl)clarithromycin **36** (0.15 g, 0.17 mmol) with 5-Azido-N-((*tert*-butyldimethylsilyl)oxy)pentanamide **51d** (0.068 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38d** as a light yellow solid (0.067g, 44%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.30 (s, ^1H), 7.76 (d, $J = 8.0$ Hz, 2H), 7.36 (d, $J = 8.1$ Hz, 2H), 5.12 – 5.03 (m, ^1H), 4.47 – 4.36 (m, 3H), 4.19 (d, $J = 6.4$ Hz, ^1H), 3.81 – 3.59 (m, 5H), 3.48 (s, ^1H), 3.29 – 3.18 (m, 4H), 3.04 (dd, $J = 10.3, 4.5$ Hz, 2H), 3.00 (d, $J = 9.6$ Hz, 4H), 2.92 – 2.82 (m, ^1H), 2.66 (s, ^1H), 2.55 (d, $J = 7.3$ Hz, ^1H), 2.40 (s, 6H), 2.30 (s, ^1H), 2.23 (s, 4H), 2.16 (d, $J = 9.6$ Hz, ^1H), 2.15 – 2.06 (m, 4H), 1.86 (ddd, $J = 20.9, 14.7, 7.6$ Hz, 6H), 1.75 (d, $J = 13.2$ Hz, ^1H), 1.62 (d, $J = 12.2$ Hz, 3H), 1.50 –

1.41 (m, ^1H), 1.35 (s, 3H), 1.26 – 1.12 (m, 16H), 1.09 (td, $J = 12.8, 7.9$ Hz, ^1H), 0.81 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 176.5, 147.8, 138.7, 130.3, 129.9, 126.3, 121.0, 103.1, 97.0, 81.6, 79.5, 79.2, 78.9, 78.7, 76.7, 74.9, 71.5, 71.2, 69.6, 68.7, 68.1, 66.0, 63.6, 57.3, 51.6, 51.2, 50.4, 50.1, 45.8, 45.5, 43.8, 40.9, 39.9, 39.5, 37.8, 32.0, 30.2, 29.9, 22.7, 22.2, 21.5, 20.8, 20.3, 19.4, 18.9, 18.6, 16.5, 15.9, 15.4, 15.3, 13.6, 12.9, 12.1, 11.2, 11.1, 10.0, 9.9. HRMS (MALDI) m/z Calcd. for $\text{C}_{54}\text{H}_{91}\text{N}_6\text{O}_{15}$ $[\text{M}+\text{H}^+]$: 1063.6542, found 1063.6588.

(Clarithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxyhexanamide (38e)—Reaction of (4''-(methylamino)-N(methyl)(4-

ethynylbenzyl)clarithromycin **36** (0.135 g, 0.149 mmol) with 6-Azido-N-((*tert*-butyldimethylsilyl)oxy)hexanamide **51e** (0.077 g, 0.268 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38e** as a light yellow solid (0.053g, 35%). ^1H NMR (500 MHz, CDCl_3) δ (ppm) 7.86 (s, ^1H), 7.77 (d, $J = 7.4$ Hz, 2H), 7.31 (d, $J = 7.8$ Hz, 2H), 5.06 – 4.95 (m, 2H), 4.43 – 4.30 (m, 2H), 4.19 – 4.11 (m, ^1H), 3.81 – 3.71 (m, 2H), 3.65 (dd, $J = 13.3, 6.4$ Hz, 2H), 3.48 – 3.39 (m, ^1H), 3.29 (s, 3H), 3.21 (dd, $J = 12.4, 6.3$ Hz, 3H), 3.06 (d, $J = 6.5$ Hz, ^1H), 3.05 – 2.93 (m, 4H), 2.92 – 2.82 (m, ^1H), 2.58 – 2.45 (m, 2H), 2.31 (dd, $J = 28.6, 7.4$ Hz, 6H), 2.20 (d, $J = 14.3$ Hz, 3H), 2.11 (dd, $J = 35.3, 9.4$ Hz, 5H), 2.00 – 1.83 (m, 5H), 1.75 – 1.57 (m, 6H), 1.57 – 1.49 (m, 2H), 1.41 – 1.27 (m, 7H), 1.28 – 1.11 (m, 16H), 1.13 – 1.01 (m, ^1H), 0.90 – 0.85 (m, ^1H), 0.85 – 0.74 (m, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 176.0, 147.4, 138.4, 129.8, 129.4, 125.8, 120.1, 102.7, 96.6, 96.5, 81.1, 81.0, 78.4, 78.2, 77.3, 77.1, 76.8, 76.6, 76.2, 76.1, 76.0, 74.3, 71.1, 70.7, 69.1, 68.3, 67.6, 65.4, 63.1, 57.0, 51.2, 51.0, 50.7, 50.1, 49.6, 45.3, 45.0, 43.2, 40.3, 39.4, 39.0, 37.2, 31.5, 29.8, 29.7, 29.3, 28.5, 26.2, 25.6, 24.9, 24.5, 21.7, 21.0, 19.7, 18.9, 18.1, 16.0, 16.0, 15.4, 14.9, 14.8, 13.1, 12.3, 11.5, 10.6, 9.3. HRMS (MALDI) m/z Calcd. for $\text{C}_{55}\text{H}_{93}\text{N}_6\text{O}_{15}$ $[\text{M}+\text{H}^+]$: 1077.6699, found 1077.6692.

(Clarithromycin-4''-(methylamino)-N(methyl)-4-benzyltriazolyl))-N-hydroxyheptanamide (38f)—Reaction of (4''-(methylamino)-N(methyl)(4-

ethynylbenzyl)clarithromycin **36** (0.15 g, 0.17 mmol) with 7-Azido-N-((*tert*-butyldimethylsilyl)oxy)heptanamide **51f** (0.075 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38f** as a light yellow solid (0.060g, 40%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.34 (s, ^1H), 7.81 (d, $J = 8.2$ Hz, 2H), 7.40 (d, $J = 8.2$ Hz, 2H), 5.16 – 5.08 (m, ^1H), 4.96 (d, $J = 5.2$ Hz, ^1H), 4.45 (t, $J = 6.5$ Hz, 3H), 4.22 (d, $J = 6.4$ Hz, ^1H), 3.85 – 3.64 (m, 5H), 3.52 (s, ^1H), 3.33 – 3.23 (m, 9H), 3.12 – 2.98 (m, 5H), 2.90 (dd, $J = 19.0, 11.7$ Hz, ^1H), 2.77 (s, ^1H), 2.58 (s, ^1H), 2.49 (s, 6H), 2.35 (s, ^1H), 2.31 – 2.23 (m, 4H), 2.21 (s, ^1H), 2.18 – 2.12 (m, 3H), 2.12 – 2.04 (m, 4H), 1.99 – 1.85 (m, 6H), 1.83 (d, $J = 13.2$ Hz, 2H), 1.69 – 1.53 (m, 7H), 1.44 – 1.31 (m, 12H), 1.28 – 1.18 (m, 15H), 1.12 (dt, $J = 11.4, 4.9$ Hz, 12H), 0.90 (s, ^1H), 0.85 (t, $J = 7.3$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 176.2, 147.6, 138.4, 130.1, 129.7, 126.1, 120.3, 102.8, 96.8, 81.4, 78.6, 78.4, 74.6, 71.3, 71.0, 69.3, 68.4, 67.9, 65.7, 63.3, 57.1, 51.6, 51.0, 50.4, 49.9, 45.5, 45.2, 43.5, 40.7, 39.6, 39.2, 37.5, 31.8, 30.1, 30.0, 28.9, 28.8, 28.2, 26.6, 26.0, 25.6, 25.3, 21.9, 21.3, 20.6, 20.0, 19.5, 19.1, 18.3, 16.3, 15.7, 15.2, 15.0, 12.6, 10.9, 9.7. HRMS (MALDI) m/z Calcd. for $\text{C}_{56}\text{H}_{95}\text{N}_6\text{O}_{15}$ $[\text{M}+\text{H}^+]$: 1091.6815, found 1091.6869.

3'-O-Acetylazithromycin (30)—Acetic anhydride (0.8 ml, 8.34 mmol) was added to a solution of azithromycin **28** (2.50 g, 3.34 mmol) in DCM (10 mL) at room temperature. The resulting solution was stirred under Ar for 3 h. The reaction was quenched by adding saturated NaHCO₃ and the organic layer was separated. The aqueous layer was extracted twice with DCM (20 mL) and the combined organic layer was washed with water, brine, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo* to give the target compound as a white solid (2.50 g, 95%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 5.25 (dd, *J* = 1.6, 0.9 Hz, ¹H), 4.93 (s, 3H), 4.74 – 4.67 (m, 2H), 4.59 (d, *J* = 10.2 Hz, 2H), 4.51 (d, *J* = 7.5 Hz, 2H), 4.18 – 4.13 (m, 2H), 4.01 – 3.93 (m, 2H), 3.59 (s, 2H), 3.54 (d, *J* = 6.3 Hz, 2H), 3.30 (s, 3H), 3.23 (s, 2H), 2.96 (d, *J* = 9.0 Hz, 2H), 2.79 – 2.71 (m, 2H), 2.68 – 2.52 (m, 4H), 2.40 (d, *J* = 11.8 Hz, 2H), 2.28 (d, *J* = 13.8 Hz, 4H), 2.04 – 2.01 (m, ¹H), 1.98 (d, *J* = 2.3 Hz, 3H), 1.94 – 1.85 (m, 3H), 1.64 (d, *J* = 13.7 Hz, 3H), 1.53 (dd, *J* = 15.3, 4.9 Hz, 2H), 1.28 – 1.23 (m, 7H), 1.20 (d, *J* = 6.4 Hz, 3H), 1.16 (d, *J* = 6.0 Hz, 3H), 1.11 (d, *J* = 8.3 Hz, 3H), 1.09 (s, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 0.99 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 178.2, 176.2, 169.8, 166.3, 100.6, 95.4, 83.8, 78.7, 78.1, 75.8, 74.9, 74.4, 73.5, 72.6, 71.8, 70.1, 68.2, 65.6, 63.7, 61.9, 49.3, 45.1, 42.0, 40.7, 36.5, 35.1, 30.4, 27.2, 26.3, 22.5, 22.0, 21.6, 21.2, 21.1, 18.5, 16.3, 15.4, 11.3, 9.2, 7.8. HRMS (ESI) *m*+2/*z* Calcd. for C₄₀H₇₆N₂O₁₃ [M+2H⁺]/2: 396.2668, found 396.2656.

4''-Oxo-3'-O-acetylazithromycin (32)—*N*-iodosuccinimide (0.75 g, 5.61 mmol) was dissolved in anhydrous DCM (20 mL) and the solution was cooled to –15 °C. After 10 min, dimethyl sulfide (0.5 ml, 6.32 mmol) was added drop wise. The white suspension was stirred at –15 °C for 20 min, then a DCM solution (5 mL) of compound **30** (2.78 g, 3.51 mmol) was added over 30 min. The resulting suspension was stirred at –15 °C for 30 min, and triethylamine (0.8 ml, 5.6 mmol) was added. The solution became clear in a minute and stirring was continued at –10 °C for 2 h. The reaction was quenched by adding saturated aqueous NaHCO₃ solution and the organic layer was separated. The aqueous layer was extracted twice with DCM (2× 50 mL). The combined organic layer was washed with water (50 mL), brine (50 mL), dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude was purified by column chromatography (Silica gel, 12:1:0.5 DCM: MeOH: NH₄OH) to yield the product (2.49 g 90%) as white solid. ¹H NMR (400 MHz, CDCl₃) δ ¹H NMR (400 MHz, CDCl₃) 6.21 (d, *J* = 41.6 Hz, 2H), 5.27 (t, *J* = 6.6 Hz, ¹H), 5.19 (s, ¹H), 5.05 (t, *J* = 7.0 Hz, ¹H), 5.00 – 4.93 (m, ¹H), 4.93 – 4.87 (m, ¹H), 4.72 – 4.54 (m, ¹H), 4.39 (q, *J* = 6.6 Hz, ¹H), 4.36 – 4.27 (m, ¹H), 4.11 – 4.06 (m, ¹H), 4.05 – 3.99 (m, 5H), 3.50 – 3.41 (m, ¹H), 3.36 (d, *J* = 6.1 Hz, ¹H), 3.29 (s, ¹H), 3.26 (s, 3H), 3.24 – 3.20 (m, 5H), 3.18 – 3.12 (m, ¹H), 2.70 – 2.51 (m, 4H), 2.41 (d, *J* = 12.0 Hz, ¹H), 2.20 (dt, *J* = 16.6, 9.6 Hz, 4H), 2.15 – 2.08 (m, 2H), 2.01 (d, *J* = 4.6 Hz, ¹H), 1.97 (s, 3H), 1.93 (s, ¹H), 1.86 (s, 3H), 1.72 – 1.65 (m, ¹H), 1.65 – 1.57 (m, ¹H), 1.53 (d, *J* = 14.9 Hz, ¹H), 1.47 – 1.38 (m, ¹H), 1.34 – 1.28 (m, 7H), 1.22 (t, *J* = 6.2 Hz, ¹H), 1.18 (d, *J* = 7.3 Hz, ¹H), 1.09 (ddd, *J* = 23.5, 15.0, 8.7 Hz, 3H), 0.97 (d, *J* = 8.6 Hz, 3H), 0.84 (dd, *J* = 6.6, 3.3 Hz, 3H), 0.82 – 0.72 (m, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 211.4, 210.7, 177.7, 176.6, 173.3, 170.0, 169.9, 101.8, 100.4, 97.7, 95.4, 85.1, 82.8, 81.2, 73.8, 72.6, 70.7, 69.2, 68.4, 63.2, 62.3, 60.7, 50.9, 49.8, 45.4, 44.5, 41.4, 40.2, 36.2, 29.3, 26.9, 24.0, 22.6, 20.7, 16.2, 14.5, 11.6, 10.8, 8.5, 7.8. HRMS (ESI) *m*/*z* Calcd. for C₄₀H₇₃N₂O₁₃ [M + 2H⁺]/2: 395.2590, found 395.2587.

4''-Epoxy-3'-O-acetylazithromycin (34)—NaH (0.3 g, 7.18 mmol, 60% w/w) was added to an oven dried three necked round bottom flask and was washed with petroleum ether (×3). The flask was immediately flushed with argon and dry DMSO (6 mL) was introduced through a septum. The mixture was stirred at room temperature under Ar. Over a period of 5 min, trimethylloxosulfonium iodide (1.58 g, 7.18 mmol) was added to the reaction mixture. When hydrogen gas ceased to evolve, the resulting yellow clear solution was treated with a solution of oxidized azithromycin **32** (2.57 g, 3.26 mmol) in anhydrous THF (5 mL) over 10 min, and left to stir for 2 h. TLC (4:3:1:0.1 Hexane: EtOAc: MeOH: NH₄OH) after 2 h showed 100% conversion to the product. THF was removed under vacuum and EtOAc (50 mL) was added to the remaining solution. The solution was washed severely with water to remove DMSO. Organic layer was dried over Na₂SO₄ and concentrated to give the product (2.48 g, 95% yield) as white solid. ¹H NMR (400 MHz, CDCl₃) δ 5.04 (d, *J* = 17.3 Hz, 2H), 4.83 (s, ¹H), 4.68 (d, *J* = 11.2 Hz, 2H), 4.06 (s, ¹H), 3.68 (s, 2H), 3.63 – 3.52 (m, 2H), 3.29 (p, *J* = 16.5 Hz, 7H), 2.86 (s, 2H), 2.72 – 2.39 (m, 9H), 2.11 (dd, *J* = 21.0, 9.5 Hz, 4H), 1.98 (d, *J* = 14.8 Hz, 2H), 1.83 (s, 3H), 1.66 – 1.47 (m, 5H), 1.41 (s, 3H), 1.27 (dt, *J* = 58.5, 7.3 Hz, 14H), 1.06 – 0.98 (m, 9H), 0.96 (s, 3H), 0.90 – 0.73 (m, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 180.6, 167.0, 158.0, 127.9, 121.1, 104.1, 102.5, 95.8, 92.1, 74.2, 73.6, 64.7, 63.5, 60.3, 56.2, 50.0, 49.6, 46.4, 45.4, 43.3, 42.3, 41.2, 40.4, 37.1, 36.7, 31.9, 29.8, 28.4, 26.9, 25.5, 23.6, 22.6, 20.9, 18.3, 16.8, 15.4, 14.1, 13.9, 11.4, 8.9, 7.9. HRMS (ESI) *m/z* Calcd. for C₄₁H₇₄N₂O₁₃ [M + H⁺]: 803.5264, found 803.5262.

1-(4-Ethynylphenyl)-N-methylmethanamine (35)—Methylamine (9.32 mL, 18.63 mmol, 2M in THF) was added to a solution of 4-ethynylbenzyl methanesulfonate (**3**) (0.39 g, 1.86 mmol) in THF (20 mL) and left to stir at 50 °C for 12 h. Methylamine was evaporated off and the residue dissolved in 1M HCl. This was then extracted multiple times with DCM. The aqueous layer was basified with 1M NaOH and extracted with DCM. Organic layer was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The residue obtained was purified by column chromatography to give compound **35** as a yellow liquid (0.15g, 55%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.41 – 7.36 (d, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 3.65 (d, *J* = 9.0 Hz, 2H), 3.03 (d, *J* = 3.2 Hz, ¹H), 2.65 (s, ¹H), 2.34 (s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 140.5, 132.1, 128.1, 120.6, 83.7, 55.3, 35.6.

(4''-(Methylamino)-N(methyl)(4-ethynylbenzyl))azithromycin (37)—Compound **35** (1.057 g, 7.3 mmol) was added to a solution of compound **34** (1.95 g, 2.4 mmol) in MeOH (10 mL). The mixture was stirred under argon at 60 °C for 6 h, after that the solution was cooled to room temperature and diluted with excess ethyl acetate (100 mL). The organic layer was washed with saturated aqueous NaHCO₃ solution (30 mL), water (20 mL), brine (20 mL), dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude was purified by column chromatography column (Silica gel, 4:3:2:0.1 Hexane: EtOAc: MeOH: NH₄OH) to give compound **37** as light yellow solid (2.0 g, 92% yield). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.43 (d, *J* = 8.2 Hz, 2H), 7.21(d, 2H), 5.04 (d, *J* = 4.6 Hz, ¹H), 4.62 (d, *J* = 8.6 Hz, ¹H), 4.44 (d, *J* = 6.0 Hz, ¹H), 4.24 – 4.15 (m, 3H), 3.67 (d, *J* = 6.4 Hz, 2H), 3.63 (s, 3H), 3.46 (s, 3H), 3.26 (d, *J* = 21.0 Hz, 6H), 3.05 (s, ¹H), 2.95 (d, *J* = 14.7 Hz, 3H), 2.81 (d, *J* = 7.1 Hz, 3H), 2.66 (s, 3H), 2.44 (d, *J* = 10.1 Hz, 2H), 2.31 (d, *J* = 10.5 Hz, 9H), 2.18 (s,

3H), 2.15 (d, $J = 0.5$ Hz, ^1H), 2.02 (t, $J = 11.7$ Hz, 3H), 1.92 (dd, $J = 15.0, 5.0$ Hz, 2H), 1.29 (s, 3H), 1.21 (t, $J = 6.9$ Hz, 10H), 1.14 (s, 3H), 1.12 – 1.02 (m, 10H), 0.88 (t, $J = 7.6$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 178.6, 139.4, 132.2, 128.8, 121.2, 102.8, 95.0, 94.8, 83.8, 83.3, 76.8, 74.2, 73.5, 70.9, 70.1, 68.1, 67.2, 67.2, 65.7, 63.2, 62.6, 62.5, 56.9, 49.6, 49.5, 45.4, 45.2, 43.2, 42.3, 42.2, 41.1, 40.9, 40.5, 40.4, 36.2, 31.3, 29.0, 27.5, 26.7, 22.7, 21.9, 21.3, 18.8, 16.2, 15.0, 14.6, 11.2, 9.2, 7.2. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{49}\text{H}_{85}\text{N}_3\text{O}_{12}$ [$\text{M} + 2\text{H}^+$]: 453.8061, found 453.8055.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxyacetamide (39a)—(4''-(Methylamino)-N(methyl)(4-

ethynylbenzyl)azithromycin **37** (0.14 g, 0.16 mmol) and 2-Azido-*N*-((tert-butylidimethylsilyl)oxy) ethaneamide **51a** (0.06 g, 0.23 mmol) were dissolved in anhydrous THF (5 mL) and purged with Ar for 15 min. Copper (I) iodide (0.01 g, 0.08 mmol) and Hunig's base (0.06 mL, 0.31 mmol) were then added to the reaction mixture. The reaction mixture was purged with Ar for additional 15 min and stirring continued for 12 h. Caesium fluoride (0.04 g, 0.24 mmol) and MeOH (5 mL) were added to the mixture to remove TBS protecting group and the reaction continued for an additional 2 h. The reaction was quenched by adding a solution of 4:1 saturated $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ (30 mL) and extracted with 20% MeOH/ CH_2Cl_2 (3×30 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by preparative TLC (Silica gel, 5:1:1 EtOAc: MeOH: NH_4OH) to give the product (0.131 g, 80% yield) as light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.33 (s, ^1H), 7.79 (d, $J = 6.5$ Hz, 2H), 7.37 (d, $J = 7.1$ Hz, 2H), 5.46 (s, ^1H), 5.07 (d, $J = 4.6$ Hz, 3H), 4.44 (d, $J = 6.9$ Hz, ^1H), 4.32 – 4.18 (m, 3H), 3.69 (s, 2H), 3.64 – 3.56 (m, 3H), 3.48 (d, $J = 25.5$ Hz, 2H), 3.34 (s, 3H), 3.32 – 3.21 (m, 3H), 3.09 – 2.93 (m, 3H), 2.85 – 2.64 (m, 3H), 2.41 (t, $J = 20.9$ Hz, 12H), 2.33 – 2.20 (m, 3H), 2.19 – 2.09 (m, 3H), 2.00 (dd, $J = 25.3, 18.4$ Hz, 3H), 1.95 – 1.83 (m, 3H), 1.82 – 1.70 (m, 3H), 1.52 – 1.39 (m, 3H), 1.32 (s, 3H), 1.24 (d, $J = 6.3$ Hz, 3H), 1.22 – 1.12 (m, 13H), 1.08 (s, 3H), 1.03 (d, $J = 7.2$ Hz, 3H), 0.96 – 0.82 (m, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.3, 149.4, 140.9, 131.9, 131.6, 127.7, 124.6, 105.0, 102.0, 97.7, 85.6, 80.3, 78.9, 78.3, 76.3, 76.0, 73.6, 73.2, 73.0, 71.0, 69.8, 69.5, 67.0, 65.5, 65.0, 59.0, 51.0, 47.6, 45.0, 44.5, 44.1, 41.4, 37.7, 33.9, 33.2, 32.6, 31.6, 31.4, 28.6, 28.2, 24.6, 23.2, 23.0, 20.2, 18.2, 16.5, 15.3, 12.3, 10.7, 8.8. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{51}\text{H}_{89}\text{N}_7\text{O}_{14}$ [$\text{M} + 2\text{H}^+$]: 511.8228, found 511.8230.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxypropanamide (39b)—Reaction of (4''-(methylamino)-N(methyl)(4-

ethynylbenzyl)azithromycin **37** (0.20 g, 0.218 mmol) and 3-azido-*N*-((tert-butylidimethylsilyl)oxy)propanamide **51b** (0.08 g, 0.32 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **39a**, gave **39b** as light yellow solid (0.178 g, 79%). ^1H NMR (500 MHz, CD_3OD) δ (ppm) 8.26 (d, $J = 10.7$ Hz, ^1H), 7.78 (d, $J = 7.6$ Hz, 2H), 7.39 (d, $J = 7.8$ Hz, 2H), 5.48 (s, ^1H), 5.13 – 5.07 (m, 2H), 4.74 (s, 3H), 4.46 (d, $J = 7.2$ Hz, 2H), 4.32 – 4.22 (m, ^1H), 3.69 (d, $J = 18.3$ Hz, 3H), 3.67 – 3.57 (m, ^1H), 3.57 – 3.48 (m, 2H), 3.38 (d, $J = 13.2$ Hz, 3H), 3.33 – 3.25 (m, ^1H), 3.02 (dd, $J = 23.0, 16.2$ Hz, 3H), 2.86 – 2.75 (m, 3H), 2.74 – 2.65 (m, 3H), 2.44 (s, 3H), 2.31 – 2.19 (m, 3H), 2.19 – 2.10 (m, 2H), 2.09 – 1.97 (m, 3H), 1.94 – 1.87 (m, 9H), 1.87 – 1.82 (m, 2H), 1.81 – 1.72 (m, 3H), 1.53 – 1.40 (m, 3H), 1.35 (d, $J = 11.2$ Hz, 3H), 1.27 (t, $J = 11.9$ Hz, 13H), 1.24 – 1.19 (m,

3H), 1.16 (d, $J = 6.7$ Hz, 3H), 1.10 (s, 3H), 1.05 (d, $J = 7.5$ Hz, 3H), 0.96 – 0.86 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.4, 177.1, 169.8, 149.2, 140.8, 131.8, 131.3, 127.5, 123.5, 104.8, 97.3, 85.4, 80.3, 78.9, 78.3, 76.2, 76.0, 74.5, 73.4, 72.9, 70.9, 69.9, 69.2, 66.8, 65.6, 65.0, 63.0, 58.8, 57.0, 55.8, 50.9, 48.1, 47.5, 44.8, 43.9, 41.3, 37.7, 33.9, 32.7, 31.6, 28.3, 24.5, 23.3, 22.8, 21.2, 20.1, 18.2, 16.8, 15.2, 12.3, 10.8, 8.8. HRMS (ESI) m/z Calcd. for $\text{C}_{52}\text{H}_{90}\text{N}_7\text{O}_{14}$ $[\text{M}+\text{H}^+]$: 1036.6540, found 1036.6550.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxybutanamide (39c)—

Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl)azithromycin **37** (0.20 g, 0.18 mmol) and 4-azido-N-((tert-butyl)dimethylsilyloxy) butanamide **51c** (0.08 g, 0.31 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **39a**, gave **39c** as light yellow solid (0.173 g, 80%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.30 (s, ^1H), 7.76 (d, $J = 7.2$ Hz, 2H), 7.34 (d, $J = 7.7$ Hz, 2H), 5.44 (s, ^1H), 5.06 (d, $J = 4.7$ Hz, ^1H), 4.51 – 4.35 (m, 3H), 4.30 – 4.19 (m, 2H), 3.60 (dd, $J = 23.4, 16.5$ Hz, 3H), 3.45 (d, $J = 25.7$ Hz, ^1H), 3.32 (s, 3H), 3.27 – 3.16 (m, 3H), 2.99 (d, $J = 14.7$ Hz, ^1H), 2.84 – 2.69 (m, 2H), 2.63 – 2.43 (m, 2H), 2.30 (d, $J = 14.5$ Hz, 10H), 2.24 – 2.04 (m, 15H), 1.95 (dd, $J = 13.7, 6.5$ Hz, 3H), 1.91 – 1.77 (m, 3H), 1.77 – 1.64 (m, 3H), 1.49 – 1.30 (m, 3H), 1.27 (s, 3H), 1.21 (d, $J = 6.0$ Hz, 6H), 1.16 (d, $J = 6.1$ Hz, ^1H), 1.03 (dd, $J = 19.7, 6.7$ Hz, 7H), 0.85 (dd, $J = 14.1, 7.0$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.7, 177.2, 149.4, 141.0, 131.9, 131.6, 127.7, 123.7, 104.9, 97.6, 85.5, 80.4, 78.8, 78.4, 76.4, 76.2, 74.5, 73.6, 73.1, 70.9, 69.9, 69.4, 66.9, 65.7, 64.9, 57.2, 55.6, 51.1, 48.2, 47.5, 44.9, 44.3, 43.9, 41.5, 37.7, 34.0, 33.6, 33.4, 32.8, 31.7, 31.4, 28.5, 28.2, 24.6, 23.2, 22.7, 20.3, 18.2, 16.7, 15.3, 12.5, 10.8, 8.7. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{53}\text{H}_{93}\text{N}_7\text{O}_{14}$ $[\text{M}+2\text{H}^+]$: 525.8385, found 525.8385.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxypentanamide (39d)—

Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl)azithromycin **37** (0.14 g, 0.15 mmol) and 5-azido-N-((tert-butyl)dimethylsilyloxy) pentanamide **51d** (0.06 g, 0.23 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **39a**, gave **39d** as light yellow solid (0.125 g, 75%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 7.72 (s, ^1H), 7.19 (d, $J = 7.9$ Hz, 2H), 6.77 (d, $J = 8.0$ Hz, 2H), 4.47 (d, $J = 5.0$ Hz, ^1H), 3.85 (d, $J = 6.7$ Hz, 3H), 3.63 (dd, $J = 17.6, 4.5$ Hz, 2H), 3.09 (s, 2H), 3.04 – 2.97 (m, 3H), 2.75 (s, 3H), 2.68 (dt, $J = 3.3, 1.6$ Hz, 2H), 2.43 (d, $J = 14.3$ Hz, 2H), 2.19 (dd, $J = 7.4, 4.6$ Hz, 2H), 2.12 (d, $J = 11.6$ Hz, ^1H), 1.89 (d, $J = 18.4$ Hz, 15H), 1.64 (d, $J = 7.1$ Hz, 5H), 1.59 – 1.46 (m, 6H), 1.37 (dd, $J = 16.4, 7.9$ Hz, 7H), 1.15 (t, $J = 7.3$ Hz, ^1H), 1.03 (s, 3H), 0.85 (td, $J = 14.5, 7.5$ Hz, 3H), 0.73 (s, 3H), 0.60 (ddd, $J = 18.8, 13.2, 6.4$ Hz, 15H), 0.49 (s, 3H), 0.43 (d, $J = 7.3$ Hz, 3H), 0.32 (d, $J = 6.8$ Hz, 3H), 0.27 (t, $J = 7.4$ Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.2, 176.9, 172.9, 149.1, 140.6, 131.6, 127.3, 122.8, 104.7, 97.1, 85.3, 80.1, 78.6, 78.0, 76.1, 75.8, 74.3, 73.1, 71.1, 69.7, 66.6, 64.6, 64.5, 62.8, 58.7, 56.9, 55.5, 52.7, 51.6, 50.8, 47.3, 44.5, 44.1, 43.9, 41.4, 37.3, 33.6, 33.0, 32.7, 31.3, 30.0, 28.6, 26.7, 24.2, 23.1, 22.7, 20.0, 17.9, 16.4, 16.1, 15.0, 12.2, 10.6, 8.2. HRMS (ESI) m/z Calcd. for $\text{C}_{54}\text{H}_{94}\text{N}_7\text{O}_{14}$ $[\text{M}+\text{H}^+]$: 1064.6853, found 1064.6854.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxyhexanamide (39e)—

Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl)azithromycin **37** (0.09 g, 0.10 mmol) and 6-azido-N-((tert-butyl)dimethylsilyloxy)hexanamide **51e** (0.04 g, 0.16 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **39a**, gave **39e** as light yellow solid (0.090 g, 80%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.27 (s, ¹H), 7.74 (d, *J* = 8.1 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H), 5.05 (d, *J* = 5.0 Hz, ¹H), 4.78 (dd, *J* = 10.0, 2.3 Hz, 3H), 4.37 (dd, *J* = 15.3, 7.2 Hz, 3H), 4.23 (dd, *J* = 12.4, 5.5 Hz, 2H), 4.00 (dq, *J* = 13.6, 6.9 Hz, ¹H), 3.66 (d, *J* = 17.8 Hz, ¹H), 3.60 – 3.52 (m, 2H), 3.46 (dd, *J* = 9.9, 5.4 Hz, ¹H), 3.29 (d, *J* = 12.7 Hz, 3H), 3.26 – 3.16 (m, 3H), 3.02 – 2.93 (m, ¹H), 2.78 – 2.68 (m, 2H), 2.59 – 2.46 (m, ¹H), 2.27 (d, *J* = 16.0 Hz, 3H), 2.22 – 2.15 (m, 3H), 2.10 (t, *J* = 8.1 Hz, 2H), 2.03 (d, *J* = 16.0 Hz, 3H), 1.97 – 1.91 (m, 5H), 1.91 – 1.86 (m, 3H), 1.83 (d, *J* = 3.9 Hz, 2H), 1.73 – 1.63 (m, 2H), 1.60 – 1.47 (m, 3H), 1.32 (dd, *J* = 21.9, 6.6 Hz, 6H), 1.26 (d, *J* = 5.2 Hz, 3H), 1.23 – 1.17 (m, 6H), 1.17 – 1.08 (m, ¹H), 1.01 (dd, *J* = 17.4, 6.0 Hz, ¹H), 0.89 – 0.79 (m, 3H). ¹³C NMR (126 MHz, CD₃OD) δ (ppm) 180.2, 177.0, 173.3, 149.2, 140.7, 131.8, 131.3, 127.4, 122.9, 104.8, 97.2, 85.4, 80.1, 78.8, 78.3, 76.4, 75.9, 73.3, 73.2, 71.3, 69.9, 69.1, 66.6, 64.9, 58.7, 55.6, 53.0, 52.0, 50.9, 47.5, 44.7, 44.2, 43.9, 41.4, 37.4, 34.2, 33.7, 33.1, 32.6, 31.6, 30.3, 28.5, 28.2, 27.5, 26.6, 24.4, 22.9, 22.7, 20.0, 18.0, 16.4, 16.1, 12.4, 10.6, 8.4. HRMS (ESI) *m*+2/*z* Calcd. for C₅₅H₉₇N₇O₁₄ [M+2H⁺]: 539.8541, found 539.8539.

(Azithromycin-4''-(methylamino)-N(methyl)(4-benzyltriazolyl))-N-hydroxyheptanamide (39f)—

Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl)azithromycin **37** (0.16 g, 0.18 mmol) and 7-azido-N-((tert-butyl)dimethylsilyloxy)heptanamide **51f** (0.08 g, 0.27 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **39a**, gave **39f** as light yellow solid (0.154 g, 80%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 7.71 (s, ¹H), 7.18 (d, *J* = 8.1 Hz, 2H), 6.77 (d, *J* = 8.1 Hz, 2H), 4.49 (d, *J* = 5.0 Hz, ¹H), 3.83 (dd, *J* = 10.2, 7.1 Hz, 3H), 3.72 – 3.63 (m, 2H), 3.50 – 3.40 (m, ¹H), 3.11 (d, *J* = 16.2 Hz, ¹H), 3.02 (d, *J* = 7.0 Hz, ¹H), 2.99 – 2.95 (m, ¹H), 2.90 (dd, *J* = 17.7, 14.2 Hz, ¹H), 2.75 (s, 3H), 2.71 – 2.62 (m, 3H), 2.43 (d, *J* = 14.7 Hz, ¹H), 2.24 (d, *J* = 7.0 Hz, ¹H), 2.18 (dd, *J* = 7.5, 4.6 Hz, ¹H), 2.00 (t, *J* = 14.8 Hz, ¹H), 1.75 (d, *J* = 10.6 Hz, 9H), 1.70 – 1.58 (m, 6H), 1.55 – 1.44 (m, 3H), 1.44 – 1.34 (m, 3H), 1.33 – 1.30 (m, 3H), 1.31 – 1.25 (m, 3H), 1.19 – 1.10 (m, 2H), 1.04 (d, *J* = 7.4 Hz, 3H), 0.76 (dd, *J* = 20.3, 12.8 Hz, 3H), 0.70 (s, 3H), 0.68 – 0.61 (m, 6H), 0.60 (dt, *J* = 12.7, 4.4 Hz, 12H), 0.53 – 0.46 (m, 10H), 0.43 (d, *J* = 7.5 Hz, 5H), 0.34 – 0.24 (m, 3H). ¹³C NMR (126 MHz, CD₃OD) δ (ppm) 180.5, 177.4, 173.6, 149.4, 149.0, 140.7, 132.3, 131.9, 131.5, 127.6, 123.2, 122.9, 105.1, 97.3, 85.6, 80.2, 78.9, 78.2, 76.5, 75.8, 73.5, 71.6, 70.1, 69.3, 66.7, 65.1, 64.7, 58.7, 55.8, 53.3, 52.2, 50.9, 47.7, 44.9, 44.5, 44.3, 41.8, 37.7, 34.4, 33.4, 32.9, 32.0, 31.6, 30.7, 30.3, 28.9, 28.0, 27.4, 23.3, 20.2, 18.4, 16.7, 16.5, 12.6, 10.9, 8.6. HRMS (ESI) *m*+2/*z* Calcd. for C₅₆H₉₉N₇O₁₄ [M+2H⁺]: 546.8620, found 546.8621.

((3'-O-Acetyl)-4'-N-(4-ethynylbenzyl)clarithromycin (40)—

(4'-ethynylbenzyl)clarithromycin (0.15 g, 0.177 mmol) was dissolved in anhydrous DCM (5 mL) and acetic anhydride (0.04 mL, 0.442 mmol) added to the solution. The mixture was left to stir at room temperature under Ar for 3 days after which TLC shows full consumption of the starting material. The reaction was washed with NaHCO₃, brine and H₂O, the organic

layer was dried with anhydrous Na_2SO_4 and concentrated *in vacuo* to give compound **40** (128 mg, 81%). ^1H NMR (400 MHz, CDCl_3) δ 7.38 (d, $J = 8.2$ Hz, 2H), 7.19 (d, $J = 8.1$ Hz, 2H), 5.01 (dd, $J = 11.1, 2.2$ Hz, ^1H), 4.87 (d, $J = 4.8$ Hz, ^1H), 4.74 (dd, $J = 10.6, 7.4$ Hz, ^1H), 4.48 (d, $J = 7.4$ Hz, ^1H), 3.94 (s, ^1H), 3.86 (dt, $J = 12.1, 6.1$ Hz, ^1H), 3.66 (t, $J = 11.1$ Hz, 2H), 3.63 (s, ^1H), 3.52 (d, $J = 6.5$ Hz, ^1H), 3.46 (s, ^1H), 3.40 (dd, $J = 11.3, 6.7$ Hz, ^1H), 3.18 (s, ^1H), 3.07 (s, 3H), 3.02 (d, $J = 6.2$ Hz, ^1H), 2.99 – 2.91 (m, 5H), 2.80 (dt, $J = 14.2, 7.2$ Hz, ^1H), 2.73 (d, $J = 4.5$ Hz, ^1H), 2.59 – 2.47 (m, 2H), 2.29 – 2.24 (m, ^1H), 2.20 – 2.15 (m, 9H), 2.06 – 2.01 (m, 4H), 1.93 – 1.76 (m, 3H), 1.71 (t, $J = 12.8$ Hz, ^1H), 1.62 (d, $J = 11.6$ Hz, ^1H), 1.57 – 1.47 (m, 3H), 1.42 (ddd, $J = 13.2, 8.3, 3.1$ Hz, ^1H), 1.37 (t, $J = 5.2$ Hz, ^1H), 1.36 – 1.28 (m, 4H), 1.25 – 1.17 (m, ^1H), 1.14 (t, $J = 7.6$ Hz, 3H), 1.08 (t, $J = 6.0$ Hz, 8H), 0.85 (t, $J = 7.5$ Hz, 3H), 0.79 (t, $J = 7.4$ Hz, 2H). HRMS (ESI) $m+z$ Calcd. for $\text{C}_{48}\text{H}_{76}\text{NO}_{14}$ [$\text{M} + \text{H}^+$]: 890.5260, found 890.5256.

((3'-O-Acetyl)-4'-N-(4-ethynylbenzyl)azithromycin (41))—To the solution of (4'-ethynylbenzyl)azithromycin **14** (1.00 g, 1.18 mmol) in DCM (10 mL) was added acetic anhydride (0.13 mL, 1.41 mmol). Then the mixture was heated to 40 °C in a pressure tube and stirring continued for 48 h. The reaction mixture was cooled and diluted with DCM (100 mL) and washed with saturated NaHCO_3 (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 , filtered and concentrated *in vacuo*. The solid crude product **41** (1.1 g, 95%) was sufficiently pure to be used for the next reaction without any further purification. ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.38 (d, $J = 8.1$ Hz, 2H), 7.17 (d, $J = 8.1$ Hz, 2H), 5.06 – 5.00 (m, ^1H), 4.94 (dd, $J = 10.6, 2.3$ Hz, ^1H), 4.86 (dd, $J = 10.5, 7.6$ Hz, ^1H), 4.79 (d, $J = 4.4$ Hz, ^1H), 4.45 (d, $J = 7.6$ Hz, ^1H), 4.13 (t, $J = 5.6$ Hz, ^1H), 3.97 (dq, $J = 12.5, 6.2$ Hz, ^1H), 3.64 (d, $J = 13.8$ Hz, ^1H), 3.57 – 3.44 (m, 2H), 3.40 (d, $J = 4.3$ Hz, ^1H), 3.29 (dd, $J = 12.3, 4.8$ Hz, ^1H), 3.20 (s, 3H), 3.02 – 2.94 (m, 2H), 2.74 (td, $J = 12.2, 4.2$ Hz, ^1H), 2.67 – 2.58 (m, ^1H), 2.53 (q, $J = 7.2$ Hz, 2H), 2.28 (d, $J = 14.9$ Hz, 2H), 2.19 (s, 3H), 2.14 (d, $J = 6.8$ Hz, 3H), 2.10 (d, $J = 7.4$ Hz, 3H), 2.04 (d, $J = 5.4$ Hz, 3H), 1.99 – 1.89 (m, ^1H), 1.82 – 1.70 (m, 3H), 1.68 – 1.34 (m, 3H), 1.28 – 1.17 (m, 13H), 1.13 (d, $J = 7.1$ Hz, 6H), 1.04 – 0.91 (m, 10H), 0.89 – 0.80 (m, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) 176.5, 175.5, 171.0, 170.0, 140.8, 132.0, 128.4, 128.1, 128.0, 120.7, 101.4, 96.5, 84.3, 83.7, 80.6, 79.8, 77.9, 76.4, 75.3, 74.1, 72.9, 71.2, 69.0, 65.6, 61.9, 61.1, 58.2, 49.4, 48.9, 45.5, 42.9, 41.4, 36.9, 35.1, 34.4, 31.0, 29.6, 27.1, 25.2, 23.6, 21.9, 21.6, 20.6, 17.9, 14.1, 11.7, 11.1, 8.3. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{48}\text{H}_{80}\text{N}_2\text{O}_{13}$ [$\text{M} + 2\text{H}^+$]: 446.2825, found 446.2810.

((4'-Oxo)-3'-O-acetyl)-4'-N-(4-ethynylbenzyl)clarithromycin (42)—A solution of *N*-chlorosuccinimide (0.034 g, 0.23 mmol) in DCM (5 mL) was stirred at –15 °C for 10 min then dimethyl sulfide (0.020 mL, 0.26 mmol) was added drop wise to form a white turbid solution. After stirring for 20 min, a DCM (5 mL) solution of compound **40** (0.128 g, 0.144 mmol) was added over a period of 30 min and the resulting suspension was stirred at –15 °C for 30 min. Subsequently, TEA (0.030 mL, 0.23 mmol) was added and the suspension cleared up within a min. Stirring continued at –10 °C for 2 h and the reaction was quenched with saturated NaHCO_3 (10 mL). The organic layer extracted with DCM (50 mL), dried with anhydrous Na_2SO_4 , filtered, and concentrated *in vacuo*. Residue was purified by preparative chromatography (Silica gel, Hexane/EtOAc/MeOH (3:2:0.05)) to give compound

31 as a yellow solid (0.055 g, 45%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.32 (s, ¹H), 7.77 (d, *J* = 7.7 Hz, 2H), 7.36 (d, *J* = 7.9 Hz, 2H), 5.09 (d, *J* = 9.2 Hz, ¹H), 4.96 – 4.91 (m, ¹H), 4.46 (s, 2H), 4.39 (d, *J* = 7.2 Hz, ¹H), 4.19 (d, *J* = 6.4 Hz, ¹H), 3.81 – 3.60 (m, 5H), 3.48 (s, ¹H), 3.28 – 3.19 (m, 4H), 3.10 – 2.95 (m, 5H), 2.92 – 2.82 (m, ¹H), 2.67 (s, ¹H), 2.54 (s, ¹H), 2.41 (s, 6H), 2.30 (s, ¹H), 2.27 – 2.16 (m, 7H), 2.13 (dd, *J* = 20.0, 10.6 Hz, 4H), 1.94 – 1.69 (m, 6H), 1.62 (d, *J* = 14.6 Hz, ¹H), 1.50 – 1.41 (m, ¹H), 1.42 – 1.31 (m, 4H), 1.26 – 1.13 (m, 16H), 1.13 – 1.01 (m, 12H), 0.86 – 0.76 (m, 3H). ¹³C NMR (126 MHz, MeOD) δ (ppm) 212.0, 177.2, 172.2, 142.0, 133.0, 129.9, 122.5, 101.8, 97.9, 84.5, 81.5, 80.5, 79.8, 78.5, 78.2, 76.0, 74.2, 73.9, 73.1, 70.7, 69.9, 62.6, 59.1, 51.8, 51.1, 50.9, 46.6, 46.0, 39.8, 39.6, 39.2, 39.1, 39.1, 38.6, 37.4, 32.1, 22.3, 21.8, 21.7, 21.1, 20.9, 20.4, 19.4, 18.5, 17.3, 16.7, 16.3, 15.1, 13.7, 12.8, 11.1, 10.1, 9.9. HRMS (ESI) *m/z* Calcd. for C₄₈H₇₄NO₁₄ [M+H⁺]: 888.5104, found 888.5113.

((4''-Oxo)-3'-O-acetyl)-4'-N-(4-ethynylbenzyl)azithromycin (43)—A solution of *N*-chlorosucciniamide (0.46 g, 3.47 mmol) in DCM (5 mL) was stirred at –15 °C for 10 min. Then DMS (0.30 mL, 3.90 mmol) was added drop wise to form a white solution. After stirring for 20 min a DCM (5 mL) solution of compound **41** (1.93 g, 2.17 mmol) was added over 30 min and the resulting suspension was stirred at –15 °C for 30 min. Subsequently, TEA (0.5 ml, 3.47 mmol) was added and the reaction cleared up within a min. Stirring continued at –10 °C for 2 h and the reaction was quenched with saturated aqueous NaHCO₃ solution (20 mL), extra DCM (20 mL) was added and the organic layer was separated. The aqueous layer was again extracted with DCM (2 × 10 mL) and the combined organic layer was dried over anhydrous Na₂SO₄, filtered, and concentrated *in vacuo*. The crude was purified by column chromatography (Silica gel, 12:1:0.5 DCM: MeOH: NH₄OH) to give the title compound **43** (1.79 g, 93% yield) as white solid. ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.37 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 7.3 Hz, 2H), 5.18 – 5.04 (m, ¹H), 5.05 – 4.73 (m, 2H), 4.65 (d, *J* = 9.7 Hz, ¹H), 4.48 – 4.34 (m, ¹H), 4.20 (d, *J* = 4.3 Hz, ¹H), 4.16 – 4.06 (m, ¹H), 3.95 (dd, *J* = 15.2, 8.5 Hz, ¹H), 3.72 – 3.57 (m, 2H), 3.56 – 3.37 (m, 3H), 3.31 (dd, *J* = 16.4, 9.8 Hz, ¹H), 3.23 (t, *J* = 5.2 Hz, ¹H), 3.19 (s, ¹H), 3.07 (d, *J* = 6.2 Hz, ¹H), 3.05 – 2.91 (m, 2H), 2.86 – 2.55 (m, 2H), 2.49 (d, *J* = 10.1 Hz, ¹H), 2.27 (dd, *J* = 11.5, 6.2 Hz, 3H), 2.20 (d, *J* = 5.6 Hz, 3H), 2.17 – 2.13 (m, 2H), 2.11 (d, *J* = 5.3 Hz, 2H), 2.08 – 1.95 (m, 5H), 1.83 – 1.70 (m, 2H), 1.66 – 1.46 (m, 2H), 1.44 – 1.34 (m, 3H), 1.34 – 1.16 (m, 14H), 1.13 (dd, *J* = 6.8, 4.2 Hz, 4H), 1.10 – 1.01 (m, 4H), 1.00 – 0.91 (m, 3H), 0.90 – 0.73 (m, 3H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm) 211.6, 177.2, 176.5, 170.0, 169.7, 140.6, 132.0, 128.3, 120.6, 100.5, 94.6, 83.6, 75.8, 75.3, 74.3, 73.9, 73.0, 72.4, 72.2, 71.2, 69.8, 69.0, 68.0, 65.6, 63.1, 61.8, 58.3, 51.2, 49.5, 48.9, 45.1, 37.1, 36.9, 35.2, 34.4, 29.5, 23.0, 22.1, 21.5, 21.1, 20.6, 18.1, 15.5, 14.3, 12.3, 11.3, 9.0, 7.7. HRMS (ESI) *m/z* Calcd. for C₄₈H₇₇N₂O₁₃ [M+H⁺]: 889.5420, found 889.5412.

((4''-Epoxy)-3'-O-acetyl)-4'-N-(4-ethynylbenzyl)clarithromycin (44)—Compound **42** (0.40 g, 0.45 mmol) was reacted with NaH (60% dispersion in mineral oil) (0.040 g, 0.99 mmol) and trimethyloxosulfonium iodide (0.218 g, 0.99 mmol) as described for the synthesis of compound **32** to give compound **44** after purification by preparative chromatography (Silica gel, Hexane/EtOAc/EtOH (4:1:0.5), as yellow solid (0.35 g, 77%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.40 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 8.1 Hz,

2H), 4.88 (d, $J = 3.5$ Hz, ^1H), 4.84 – 4.73 (m, 2H), 4.56 (d, $J = 7.5$ Hz, ^1H), 3.98 – 3.91 (m, ^1H), 3.69 (s, ^1H), 3.67 – 3.61 (m, 2H), 3.49 (d, $J = 14.0$ Hz, 2H), 3.34 (s, ^1H), 3.06 (s, 3H), 3.04 (s, 2H), 2.90 (d, $J = 4.1$ Hz, ^1H), 2.81 – 2.67 (m, 3H), 2.64 (d, $J = 4.1$ Hz, ^1H), 2.49 (dt, $J = 21.9, 7.4$ Hz, ^1H), 2.38 (dt, $J = 9.9, 4.9$ Hz, ^1H), 2.35 – 2.25 (m, 2H), 2.21 (s, 3H), 2.18 (s, ^1H), 2.15 (s, ^1H), 2.08 (s, 3H), 1.83 (d, $J = 4.8$ Hz, ^1H), 1.81 – 1.71 (m, 2H), 1.43 – 1.32 (m, 3H), 1.30 – 1.22 (m, 9H), 1.19 (t, $J = 5.8$ Hz, 7H), 1.12 (t, $J = 9.0$ Hz, 4H), 1.04 – 0.97 (m, 10H), 0.92 (d, $J = 6.5$ Hz, 3H), 0.88 (dd, $J = 10.4, 4.7$ Hz, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) 216.1, 178.8, 171.3, 169.8, 140.7, 131.9, 128.4, 120.6, 99.7, 96.0, 83.7, 79.7, 78.7, 73.8, 71.5, 68.2, 63.1, 61.5, 60.4, 58.2, 50.3, 49.4, 46.7, 41.6, 41.1, 38.3, 37.0, 36.4, 35.7, 33.4, 31.3, 29.7, 24.6, 21.3, 21.1, 20.2, 19.2, 17.9, 14.3, 14.1, 10.9, 10.7, 7.8. HRMS (ESI) m/z Calcd. for $\text{C}_{49}\text{H}_{76}\text{NO}_{14}$ $[\text{M}+\text{H}^+]$: 902.5260, found 902.5260.

((4''-Epoxy)-3'-O-acetyl)-4'-N-(4-ethynylbenzyl)azithromycin (45)—NaH (0.2 g, 4.67 mmol, 60% w/w) was added to an oven dried three necked flask and was washed with petroleum ether ($\times 3$). The flask was immediately flushed with Ar and dry DMSO (6 mL) was introduced through a septum. The mixture was stirred at room temperature under Ar. Then trimethyloxosulfonium iodide (1.05 g, 4.67 mmol) was added over a period of 5 min. After hydrogen gas ceased to evolve, the resulting yellow clear solution was treated with a solution of compound **43** (1.88 g, 2.12 mmol) in anhydrous THF (5 mL) over 10 min and stirring continued for 2 h after which TLC (4:1:0.5 Hex, EtOAc: EtOH) showed a near quantitative conversion to a new product. THF was evaporated off, EtOAc (20 mL) was added to the remaining residue and the mixture was washed with water several times to remove DMSO. The organic layer was dried over Na_2SO_4 and concentrated to give light yellow solid product (1.63 g, 85% yield). ^1H NMR (400 MHz, CDCl_3) δ (ppm) 7.37 (d, $J = 8.0$ Hz, 2H), 7.18 (d, $J = 6.7$ Hz, 2H), 5.26 (s, ^1H), 5.06 (d, $J = 10.0$ Hz, ^1H), 4.98 (d, $J = 8.5$ Hz, ^1H), 4.95 – 4.76 (m, 2H), 4.72 (dd, $J = 12.6, 6.2$ Hz, ^1H), 4.62 (t, $J = 7.4$ Hz, ^1H), 4.50 (s, ^1H), 4.44 (d, $J = 7.6$ Hz, ^1H), 4.09 (t, $J = 7.3$ Hz, ^1H), 4.03 (dd, $J = 12.6, 5.9$ Hz, ^1H), 3.70 – 3.60 (m, ^1H), 3.60 – 3.52 (m, ^1H), 3.51 – 3.42 (m, ^1H), 3.34 (d, $J = 19.2$ Hz, ^1H), 3.28 – 3.14 (m, 2H), 3.09 (d, $J = 7.6$ Hz, ^1H), 3.01 (d, $J = 4.1$ Hz, ^1H), 2.91 (d, $J = 4.3$ Hz, ^1H), 2.82 – 2.61 (m, 3H), 2.61 – 2.52 (m, ^1H), 2.35 – 2.25 (m, ^1H), 2.24 – 2.11 (m, 6H), 2.10 – 2.00 (m, 4H), 1.95 – 1.84 (m, 2H), 1.83 – 1.64 (m, 4H), 1.56 (ddd, $J = 20.9, 13.1, 6.4$ Hz, 2H), 1.49 – 1.32 (m, 3H), 1.30 – 1.14 (m, 10H), 1.12 (d, $J = 8.3$ Hz, 2H), 1.06 (s, 3H), 1.03 (d, $J = 4.9$ Hz, 3H), 1.03 – 0.97 (m, 4H), 0.94 (dd, $J = 15.2, 8.7$ Hz, 3H), 0.91 – 0.75 (m, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ (ppm) 182.2, 175.8, 173.7, 169.7, 145.2, 142.9, 140.2, 132.2, 131.9, 128.6, 128.1, 120.9, 106.6, 105.0, 103.7, 97.4, 83.7, 78.3, 75.5, 74.3, 73.7, 72.5, 71.0, 70.6, 69.9, 69.3, 65.5, 65.3, 61.7, 60.9, 59.3, 49.9, 49.4, 42.7, 40.4, 39.7, 37.0, 31.9, 29.2, 22.9, 21.5, 19.3, 17.9, 15.2, 14.1, 11.7, 11.0, 10.3, 8.8. HRMS (ESI) m/z Calcd. for $\text{C}_{49}\text{H}_{79}\text{N}_2\text{O}_{13}$ $[\text{M}+\text{H}^+]$: 903.5577, found 903.5568.

((4''-(*N,N*-Dimethylaminomethyl)-4'-N-(4-ethynylbenzyl)clarithromycin (47)—*N,N*-Dimethylmethanamine **46** (4.22 mL, 8.46 mmol, 2M in THF) was added to a solution of compound **44** (0.10 g, 0.11 mmol) in MeOH (20 mL). The solution was heated at 60 °C for 6 h. MeOH and residual methanamine was evaporated off to give light yellow solid which was again dissolved in MeOH (10 mL) and heated at 90 °C for three days after which TLC showed complete conversion. Excess MeOH was evaporated off to give compound **47** as

yellow solid (0.08 g, 83%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.43 (d, *J* = 8.2 Hz, 3H), 7.26 – 7.20 (m, 2H), 5.04 (dd, *J* = 11.0, 2.2 Hz, ¹H), 4.98 (d, *J* = 5.0 Hz, ¹H), 4.37 (d, *J* = 7.2 Hz, ¹H), 4.07 – 4.00 (m, ¹H), 3.94 (s, ¹H), 3.79 (s, ¹H), 3.75 (s, 2H), 3.73 – 3.69 (m, 2H), 3.66 (dd, *J* = 8.5, 5.8 Hz, ¹H), 3.62 (dd, *J* = 6.6, 3.6 Hz, ¹H), 3.61 – 3.57 (m, ¹H), 3.50 – 3.42 (m, 2H), 3.39 (dd, *J* = 12.3, 7.7 Hz, 3H), 3.29 (dd, *J* = 10.2, 7.2 Hz, ¹H), 3.21 (d, *J* = 3.9 Hz, ¹H), 3.16 (d, *J* = 3.7 Hz, 2H), 3.09 (s, 3H), 3.06 (t, *J* = 4.5 Hz, 4H), 3.05 – 3.01 (m, 4H), 2.99 (d, *J* = 8.0 Hz, ¹H), 2.91 – 2.80 (m, ¹H), 2.66 (s, ¹H), 2.62 (s, ¹H), 2.52 (dd, *J* = 15.6, 7.6 Hz, 2H), 2.36 (s, 9H), 2.23 (d, *J* = 4.3 Hz, 4H), 2.18 – 2.15 (m, ¹H), 2.07 (s, ¹H), 2.03 (s, 2H), 1.99 (d, *J* = 6.8 Hz, ¹H), 1.90 (dt, *J* = 30.0, 10.5 Hz, 6H), 1.64 – 1.52 (m, 2H), 1.49 – 1.42 (m, ¹H), 1.41 – 1.33 (m, 6H), 1.31 – 1.22 (m, 10H), 1.22 – 1.17 (m, 5H), 1.15 – 1.01 (m, 25H), 0.91 (dt, *J* = 14.0, 5.6 Hz, 3H), 0.87 – 0.78 (m, 5H). ¹³C NMR (126 MHz, CDCl₃) δ (ppm) 175.7, 169.9, 167.8, 132.3, 132.0, 130.9, 128.8, 128.5, 121.1, 102.8, 96.4, 83.4, 81.3, 78.3, 78.0, 76.5, 76.0, 74.3, 70.9, 70.3, 69.0, 68.3, 68.2, 67.6, 63.6, 58.0, 57.8, 50.8, 50.6, 49.4, 47.3, 45.3, 44.9, 39.3, 38.8, 38.7, 37.2, 36.9, 31.3, 30.4, 29.7, 28.9, 23.7, 23.0, 21.6, 21.0, 19.8, 18.5, 18.1, 16.0, 16.0, 15.1, 14.1, 12.4, 11.0, 10.6, 9.3. HRMS (ESI) *m*+2/*z* Calcd. for C₄₉H₈₂N₂O₁₃ [*M*+2*H*⁺]: 453.2903 found 453.2892.

((4''-(*N,N*-Dimethylaminomethyl)-4'-*N*-(4-ethynylbenzyl))azithromycin (48)—

Reaction of compound **45** (2.00 g, 2.2 mmol) and *N,N*-dimethylmethyl amine **46** (8.92 mL, 168.3, 2M in THF) mmol) in anhydrous methanol (20 mL) as described for the synthesis of **47**, gave **48** as white solid (2.0 g, 90%) after purification by column chromatography (Silica gel, 20:1:0.1 DCM, MeOH, and NH₄OH). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.38 (t, *J* = 5.6 Hz, 2H), 7.19 (d, *J* = 8.0 Hz, 2H), 4.98 (d, *J* = 4.7 Hz, ¹H), 4.80 (dd, *J* = 10.7, 7.5 Hz, ¹H), 4.61 (dd, *J* = 16.8, 8.0 Hz, ¹H), 4.53 – 4.47 (m, ¹H), 4.32 (dd, *J* = 11.6, 6.2 Hz, ¹H), 4.08 (t, *J* = 7.2 Hz, ¹H), 4.02 (q, *J* = 6.4 Hz, ¹H), 3.70 (dt, *J* = 9.6, 5.5 Hz, ¹H), 3.67 – 3.59 (m, 2H), 3.54 (d, *J* = 6.2 Hz, ¹H), 3.49 (s, ¹H), 3.41 (ddd, *J* = 22.7, 11.0, 6.6 Hz, 2H), 3.31 (d, *J* = 7.3 Hz, ¹H), 3.20 – 3.12 (m, ¹H), 3.11 – 2.98 (m, 3H), 2.92 (d, *J* = 3.3 Hz, ¹H), 2.85 (d, *J* = 2.4 Hz, ¹H), 2.78 (dd, *J* = 17.2, 10.1 Hz, ¹H), 2.73 (s, ¹H), 2.65 (dd, *J* = 14.3, 7.6 Hz, 2H), 2.57 (d, *J* = 9.5 Hz, ¹H), 2.45 (dt, *J* = 23.1, 10.2 Hz, ¹H), 2.31 (d, *J* = 11.6 Hz, 7H), 2.24 – 2.16 (m, 3H), 2.13 (dd, *J* = 11.4, 4.5 Hz, ¹H), 2.10 – 2.01 (m, 4H), 2.00 – 1.78 (m, 6H), 1.68 (dd, *J* = 23.3, 12.9 Hz, 2H), 1.23 (qd, *J* = 15.5, 7.1 Hz, 17H), 1.13 (dd, *J* = 9.7, 4.5 Hz, 3H), 1.09 – 0.98 (m, 8H), 0.86 (ddd, *J* = 11.8, 10.2, 6.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ (ppm) 178.0, 169.7, 140.7, 131.8, 128.3, 120.5, 100.4, 95.6, 84.2, 83.5, 78.8, 76.8, 76.0, 74.9, 74.2, 73.3, 71.4, 70.2, 70.1, 67.5, 67.4, 61.9, 61.3, 58.4, 57.9, 53.6, 49.0, 47.3, 47.2, 45.1, 44.7, 41.9, 41.4, 40.1, 36.8, 36.5, 31.5, 30.6, 30.0, 27.0, 26.5, 21.7, 21.3, 20.9, 18.5, 16.1, 15.7, 14.7, 11.1, 9.38, 7.5. ESI MS *m/z* Calcd. for C₅₁H₈₆N₃O₁₃ [*M*+*H*⁺]: 948.61.

(Clarithromycin-(4'-*N*-(4-benzyltriazolyl))-4''-(*N,N*-dimethylaminomethyl))-*N*-hydroxyhexanamide (49a)—

Reaction of compound **47** (0.05 g, 0.06 mmol) with 6-Azido-*N*-((*tert*-butyldimethylsilyl) oxy)hexanamide **51e** (0.075 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **49a** as light yellow solid (0.02 g, 44%). ¹H NMR (400 MHz, CD₃OD) δ (ppm) 8.36 (s, ¹H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.3 Hz, 2H), 5.12 (d, *J* = 10.8 Hz, ¹H), 4.45 (t, *J* = 6.9 Hz, 3H), 4.31 (d, *J* = 7.2 Hz, ¹H), 4.09 (d, *J* = 6.5 Hz, ¹H), 3.90 (d, *J* = 13.4 Hz, ¹H), 3.72 (d,

$J = 13.5$ Hz, 2H), 3.59 (d, $J = 12.8$ Hz, 2H), 3.20 (dd, $J = 19.5, 9.0$ Hz, 3H), 3.11 (d, $J = 15.2$ Hz, 2H), 3.00 (d, $J = 8.4$ Hz, 6H), 2.89 (s, ^1H), 2.65 (s, ^1H), 2.59 (s, 3H), 2.42 (s, 6H), 2.34 (s, 4H), 2.28 (s, ^1H), 2.21 (s, ^1H), 2.10 (t, $J = 7.4$ Hz, 4H), 1.96 (dd, $J = 15.3, 7.4$ Hz, 5H), 1.82 (s, 7H), 1.73 – 1.60 (m, 5H), 1.44 – 1.33 (m, 9H), 1.27 (d, $J = 10.6$ Hz, 7H), 1.22 (dd, $J = 12.4, 6.3$ Hz, 7H), 1.21 – 1.16 (m, 6H), 1.17 – 1.09 (m, 17H), 1.06 (d, $J = 7.5$ Hz, 4H), 0.91 (d, $J = 10.9$ Hz, 5H), 0.84 (dd, $J = 9.5, 5.1$ Hz, 5H). ^{13}C NMR (101 MHz, CDCl_3) δ (ppm) 175.7, 147.5, 129.7, 125.8, 102.9, 96.8, 94.9, 78.3, 76.1, 74.3, 71.3, 70.9, 69.0, 68.3, 67.5, 51.3, 50.7, 50.7, 50.1, 50.5, 49.4, 47.2, 45.3, 45.3, 44.6, 39.4, 37.5, 36.8, 33.7, 31.9, 29.7, 28.6, 23.2, 22.7, 21.6, 21.3, 19.7, 18.1, 16.0, 15.9, 15.1, 14.1, 12.3, 10.6, 9.2, 7.8. HRMS (ESI) m/z Calcd. for $\text{C}_{55}\text{H}_{93}\text{N}_6\text{O}_{15}$ [$\text{M}+\text{H}^+$] 1077.6693, found 1077.6692.

(Clarithromycin-(4'-N-(4-benzyltriazolyl))-4''-(N,N-dimethylaminomethyl))-N-hydroxyheptanamide (49b)—Reaction of compound **47** (0.024 g, 0.022 mmol) with 7-Azido-N-((tert-butyl)dimethylsilyl)oxy)heptanamide **51f** (0.075 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **49b** as a light yellow solid (0.021 g, 83%). ^1H NMR (400 MHz, CD_3OD) δ 8.37 (s, ^1H), 7.82 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 8.2$ Hz, 2H), 5.14 – 5.07 (m, ^1H), 4.45 (t, $J = 7.0$ Hz, 2H), 4.30 (d, $J = 7.2$ Hz, ^1H), 4.09 (d, $J = 6.5$ Hz, ^1H), 3.92 (d, $J = 13.0$ Hz, ^1H), 3.72 (d, $J = 12.3$ Hz, 2H), 3.64 – 3.57 (m, 2H), 3.34 (d, $J = 2.4$ Hz, 2H), 3.09 (s, ^1H), 3.04 – 2.97 (m, 7H), 2.89 (s, 2H), 2.59 (s, 3H), 2.49 (s, 7H), 2.36 (s, 3H), 2.28 (s, ^1H), 2.15 (s, ^1H), 2.11 – 2.03 (m, 5H), 1.92 (dd, $J = 13.5, 6.7$ Hz, 6H), 1.83 (d, $J = 10.2$ Hz, 4H), 1.61 (d, $J = 7.5$ Hz, 6H), 1.42 – 1.33 (m, 12H), 1.28 (s, 6H), 1.23 (d, $J = 6.0$ Hz, 5H), 1.19 (d, $J = 7.2$ Hz, 4H), 1.17 – 1.08 (m, 16H), 1.05 (d, $J = 7.5$ Hz, 4H), 0.97 (s, ^1H), 0.91 (d, $J = 11.7$ Hz, 4H), 0.84 (t, $J = 7.4$ Hz, 4H). ^{13}C NMR (101 MHz, cd_3od) δ 223.5, 179.1, 149.8, 132.2, 127.7, 123.6, 105.6, 99.1, 83.2, 80.8, 79.1, 77.0, 73.5, 71.6, 70.5, 69.7, 63.3, 60.6, 53.5, 52.2, 51.4, 48.7, 47.8, 47.4, 41.5, 38.1, 33.1, 32.2, 31.5, 30.9, 30.6, 28.2, 27.6, 23.1, 20.0, 19.8, 18.1, 17.7, 16.9, 13.7, 12.2, 11.3 HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{56}\text{H}_{97}\text{N}_6\text{O}_{15}$ [$\text{M}+2\text{H}^+$]: 546.3461, found 546.3469.

(Azithromycin-(4'-N-(4-benzyltriazolyl))-4''-(N,N-dimethylaminomethyl))-N-hydroxyhexanamide (50a)—Compound **48** (0.17 g, 0.18 mmol) and 6-Azido-N-((tert-butyl)dimethyl silyl)oxy) hexanamide **51e** (0.08 g, 0.30 mmol) were dissolved in anhydrous THF (5 mL) and stirred under Ar at room temperature. Copper (I) iodide (0.02 g, 0.09 mmol) and Hunig's base (0.07 mL, 0.37 mmol) were added to the mixture and stirring continued for 12 h. Caesium fluoride (0.04 g, 0.28 mmol) and MeOH (4 mL) were added to the mixture to remove TBS protecting group and stirring continued for an additional 2h. A solution of 4:1 saturated $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ (30 mL) was added to the reaction mixture and extracted with 20% MeOH/ CH_2Cl_2 (3 \times 30 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by preparative chromatography (Silica gel, 5:1:1 EtOAc: MeOH: NH_4OH) to give the product (0.16 g, 80%) as light yellow solid. ^1H NMR (400 MHz, CD_3OD) δ (ppm) 7.74 (s, ^1H), 7.20 (d, $J = 7.8$ Hz, 2H), 6.83 (d, $J = 7.9$ Hz, 2H), 3.83 (s, 2H), 3.74 (d, $J = 7.3$ Hz, ^1H), 3.60 – 3.46 (m, 2H), 3.26 (d, $J = 13.0$ Hz, ^1H), 2.99 – 2.89 (m, 3H), 2.78 (s, ^1H), 2.75 – 2.67 (m, 3H), 2.37 (d, $J = 3.6$ Hz, 3H), 2.23 – 2.10 (m, 2H), 1.96 (t, $J = 14.2$ Hz, 3H), 1.80 – 1.64 (m, ^1H), 1.60 – 1.45 (m, 3H), 1.43 – 1.30 (m, ^1H), 1.28 (s, ^1H), 1.22 (d, $J = 6.6$ Hz, ^1H), 1.13 (dd, $J = 24.4, 9.4$ Hz, 3H),

1.02 (d, $J = 15.3$ Hz, 2H), 0.93 – 0.81 (m, ^1H), 0.74 (dd, $J = 14.2, 7.2$ Hz, 3H), 0.68 (dd, $J = 10.0, 4.1$ Hz, 5H), 0.67 – 0.58 (m, 3H), 0.55 (d, $J = 6.7$ Hz, 6H), 0.48 (d, $J = 4.3$ Hz, 6H), 0.38 (d, $J = 7.5$ Hz, 3H), 0.33 – 0.21 (m, 10H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 178.8, 177.3, 173.7, 172.8, 149.4, 141.7, 131.8, 127.5, 122.8, 105.1, 102.9, 97.3, 86.5, 80.7, 78.8, 77.9, 76.3, 75.9, 73.7, 72.8, 71.4, 70.0, 69.2, 64.7, 63.2, 61.6, 60.1, 55.7, 53.1, 52.1, 50.9, 48.5, 47.3, 44.5, 44.1, 43.9, 38.3, 37.6, 32.9, 32.4, 32.3, 32.2, 31.9, 31.7, 30.2, 28.4, 27.9, 23.1, 22.7, 19.7, 18.1, 16.4, 12.4, 10.7, 8.4. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{55}\text{H}_{97}\text{N}_7\text{O}_{14}$ [$\text{M} + 2\text{H}^+$]: 539.8541, found 539.8549.

(Azithromycin-(4'-N-(4-benzyltriazolyl)-4''-(N,N-dimethylaminomethyl))-N-hydroxyheptanamide (50b)—Reaction of compound **48** (0.08 g, 0.09 mmol) with 7-Azido-*N*-((*tert*-butyldimethylsilyl) oxy)heptanamide **51f** (0.04 g, 0.14 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **50a**, gave **50b** as light yellow solid (0.069 g, 70%). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 7.46 (s, ^1H), 6.92 (d, $J = 8.0$ Hz, 2H), 6.56 (d, $J = 8.0$ Hz, 2H), 3.55 (d, $J = 6.2$ Hz, 2H), 3.47 (d, $J = 7.2$ Hz, ^1H), 3.33 – 3.17 (m, 2H), 2.99 (d, $J = 12.9$ Hz, ^1H), 2.76 (d, $J = 13.2$ Hz, ^1H), 2.67 (dd, $J = 14.5, 9.8$ Hz, 3H), 2.51 (s, ^1H), 2.43 (ddd, $J = 6.2, 4.9, 2.3$ Hz, 2H), 2.26 (t, $J = 15.8$ Hz, ^1H), 2.09 (d, $J = 5.7$ Hz, 3H), 1.98 – 1.83 (m, 2H), 1.69 (dd, $J = 14.5, 9.6$ Hz, 2H), 1.55 – 1.37 (m, 13H), 1.35 – 1.23 (m, 2H), 1.22 (d, $J = 13.4$ Hz, 3H), 1.16 – 1.01 (m, ^1H), 0.87 (t, $J = 11.8$ Hz, 3H), 0.75 (d, $J = 15.1$ Hz, 3H), 0.46 (d, $J = 19.0$ Hz, 6H), 0.44 – 0.40 (m, 3H), 0.35 (dt, $J = 15.7, 9.2$ Hz, 6H), 0.32 – 0.25 (m, 6H), 0.20 (t, $J = 5.5$ Hz, 6H), 0.11 (d, $J = 7.5$ Hz, 3H), 0.07 – 0.06 (m, 6H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.4, 180.1, 177.3, 173.5, 172.7, 149.5, 141.7, 141.2, 131.8, 131.4, 127.4, 122.8, 105.1, 102.8, 97.3, 86.1, 80.3, 78.9, 76.5, 75.7, 73.7, 72.8, 71.5, 70.1, 69.2, 64.6, 63.5, 61.7, 60.1, 55.7, 53.2, 52.1, 51.0, 48.5, 47.6, 47.4, 44.1, 43.8, 38.5, 38.0, 37.6, 32.1, 31.5, 29.0, 28.8, 27.7, 26.8, 23.1, 22.4, 20.0, 18.2, 16.4, 16.0, 12.6, 10.8, 8.5. HRMS (ESI) $m+2/z$ Calcd. for $\text{C}_{56}\text{H}_{99}\text{N}_7\text{O}_{14}$ [$\text{M} + 2\text{H}^+$]: 546.8620, found 546.8611.

In Vitro HDAC Inhibition: SAMDI Assay

The maleimide-presenting SAMs and expression of HDAC8 enzyme were prepared as previously reported.²¹ To obtain IC_{50} values, we incubated isoform-optimized substrates (50 μM , detailed below) with enzyme (250nM, detailed below) and inhibitor (at concentrations ranging from .1 nM to 1.0 mM) in HDAC buffer (25.0 mM Tris-HCl, pH 8.0, 140 mM NaCl, 3.0 mM KCl, 1.0 mM MgCl_2 , and 0.1 mg/mL BSA) in 96-well microtiter plates (60 min, 37 °C). Solution-phase deacetylation reactions were quenched with trichostatin A (TSA) and transferred to SAMDI plates to immobilize the substrate components. SAMDI plates were composed of an array of self-assembled monolayers (SAMs) presenting maleimide in standard 384-well format for high-throughput handling capability. Following immobilization, plates were washed to remove buffer constituents, enzyme, inhibitor, and any unbound substrate and analyzed by MALDI mass spectrometry using automated protocols. Deacetylation yields in each triplicate sample were determined from the integrated peak intensities of the molecular ions for the substrate and the deacetylated product ion by taking the ratio of the former over the sum of both. Yields were plotted with respect to inhibitor concentration and fitted to obtain IC_{50} values for each isoform–inhibitor pair.

Isoform-optimized substrates were prepared by traditional Fmoc solid-phase peptide synthesis (reagents supplied by Anaspec) and purified by semi-preparative HPLC on a reverse-phase C18 column (Waters). The peptide GRK^{ac}FGC was prepared for HDAC1 and HDAC8 experiments, whereas the peptide GRK^{ac}YGC was prepared for HDAC6 experiments.

HDAC1, HDAC6, and HDAC2 were purchased from BPS Biosciences. The catalytic domain of HDAC8 was expressed as previously reported.^{21e} Briefly, an amplicon was prepared by PCR with the following primers: forward (5'–3') TATTCTCGAGGA-CCACATGCTTCA and reverse (5'–3') ATAAGCTAGCATG-GAGGAGCCGGA. A pET21a construct bearing the genetic insert between the NheI and XhoI restriction sites was transformed into *Escherichia coli* BL21(DE3) (Lucigen) and expressed by standard protocols. Following purification by affinity chromatography, the His-tagged enzyme-containing fractions were purified by FPLC (AKTA) on a Superdex size-exclusion column (GE), spin-concentrated, and stored at –80 °C in HDAC buffer with 10% glycerol.

Cell Viability Assay

All cell lines used in this study (A549, MCF-7 and VERO) were maintained in DMEM (Lonza, GA) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA) and 1% *Penicillin-Streptomycin*. Prior to treatment with various drug concentrations and subsequent incubation for 72 h, cells were incubated in a 96 well plate for 24 h. Cell viability was measured using the MTS assay (CellTiter 96 Aqueous One Solution and CellTiter 96 Non-Radioactive Cell Proliferation Assays, Promega, Madison, WI) protocol as described by the manufacturer. For all drugs tested, DMSO concentration was maintained at 0.1%.

Anti-inflammatory Activity Assay

NF- κ B activity was measured by luciferase assay. BEAS-2B cells were transfected with NF- κ B luciferase reporter construct in pGL3 basic vector.³¹ 24 h after transfection, the cells were treated with drugs for 1 h followed by stimulation with NTHi for 5 h. Then cell were lysed with cell lysis buffer (250 mM Tris HCl (pH 7.5), 0.1% Triton-X, 1 mM DTT) and luciferase activity was measured by luciferase assay system (Promega). Relative luciferase activity (RLA) was determined using the following equation; $RLA = \text{luciferase unit of the cells treated with NTHi and drug} / \text{luciferase unit of the cells treated with mock}$. IC_{50} was determined by treating the cell with a serial dose of the drug followed by luciferase assay. % Inhibition was calculated using the following equation; $\% \text{ inhibition} = RLA \text{ of the cells treated with indicated concentration of the drug} / RLA \text{ of the cells treated with mock}$.

Real-Time quantitative RT-PCR analysis

Total RNA was isolated with TRIzol reagent (Life Technologies) by following the manufacturer's instruction. For the reverse transcription reaction, TaqMan reverse transcription reagents (Life Technologies) were used as described previously. For quantitative RT-PCR analysis, PCR amplifications were performed by using SYBR Green Universal Master Mix (Life Technologies). In brief, reactions were performed in triplicate containing 2 \times Universal Master Mix, 1 μ L of template cDNA, 500 nM primers in a final

volume of 12.5 μ L, and they were analyzed in a 96-well optical reaction plate (USA Scientific). Reactions were amplified and quantified by using a StepOnePlus Real-Time PCR System and the manufacturer's corresponding software (StepOnePlus Software v2.3; Life Technologies). The relative quantities of mRNAs were obtained by using the comparative Ct method and were normalized by using human cyclophilin as an endogenous control. For semiquantitative RT-PCR analysis, PCR amplifications were performed with PrimeSTAR Max polymerase (Takara) by following the manufacturer's instruction. The primer for TNF- α , IL-1 β were described previously.^{40,41} IL-1 α : 5'-CGAGCCAATGATCAGTACCTC-3' and 3'-CACCCATATATTTCACTG-5'.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

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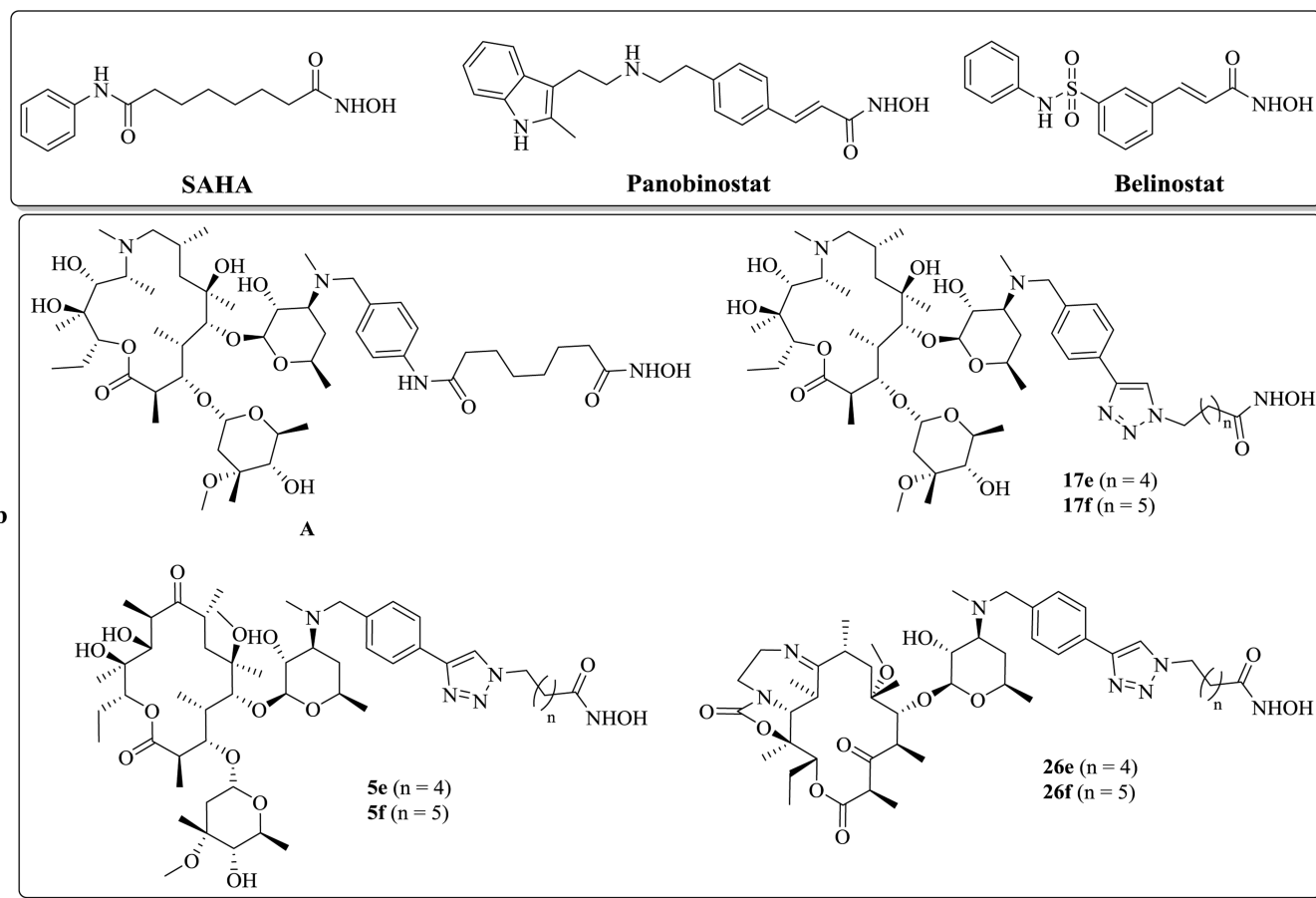


Figure 1. Representative HDACi: (a) Approved hydroxamic acid based HDACi, (b) Selected examples of non-peptide (**5e–f**, **17e–f**, **26e–f**) macrocyclic HDACi.

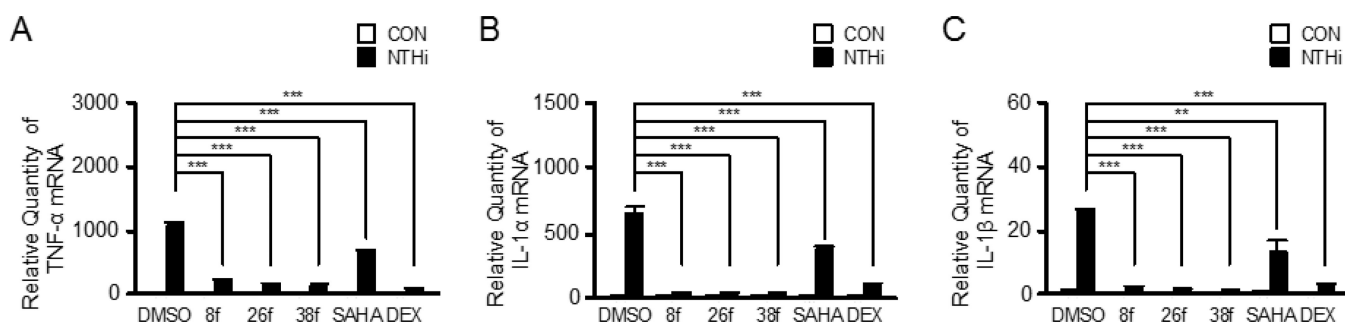
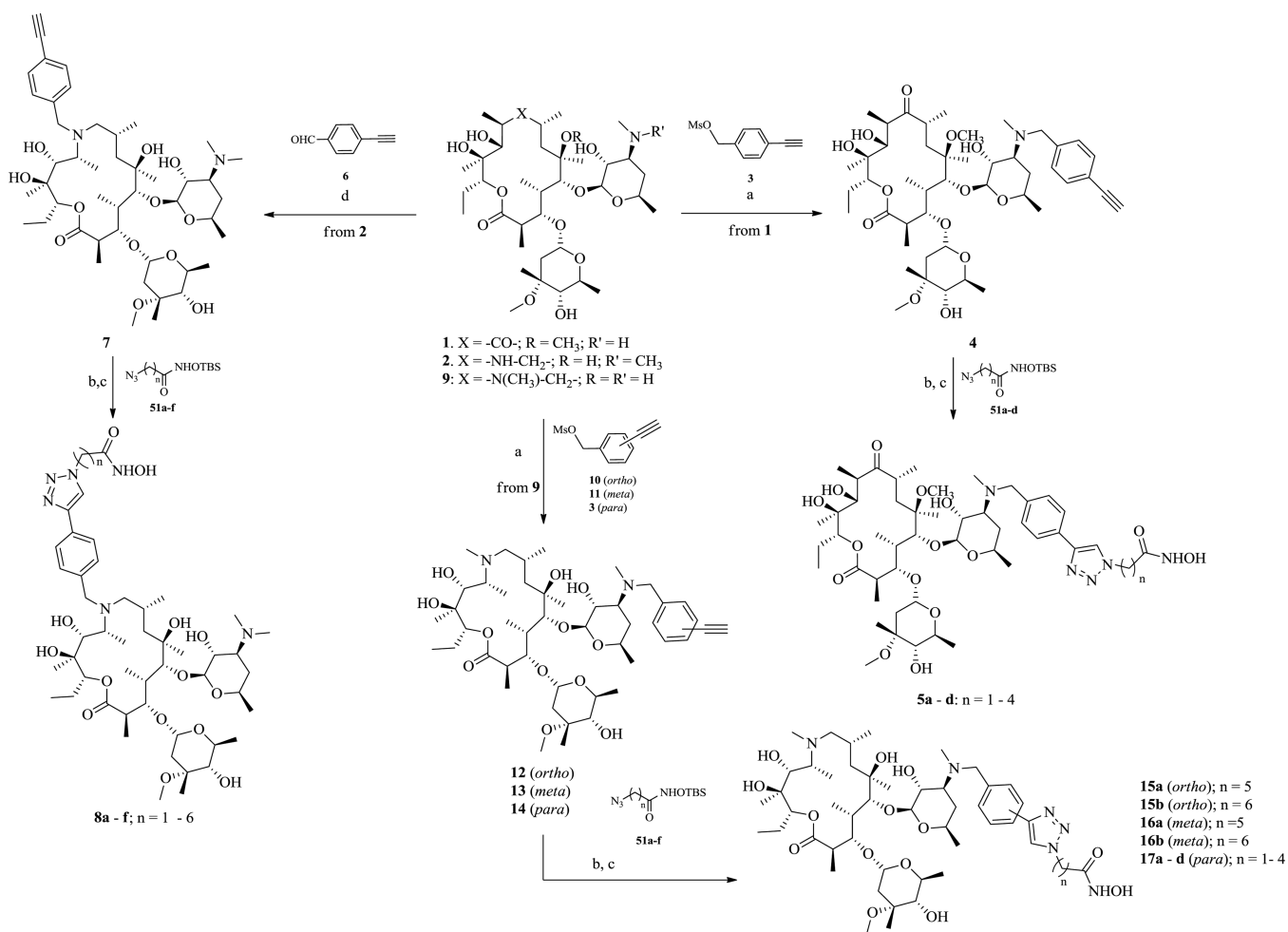
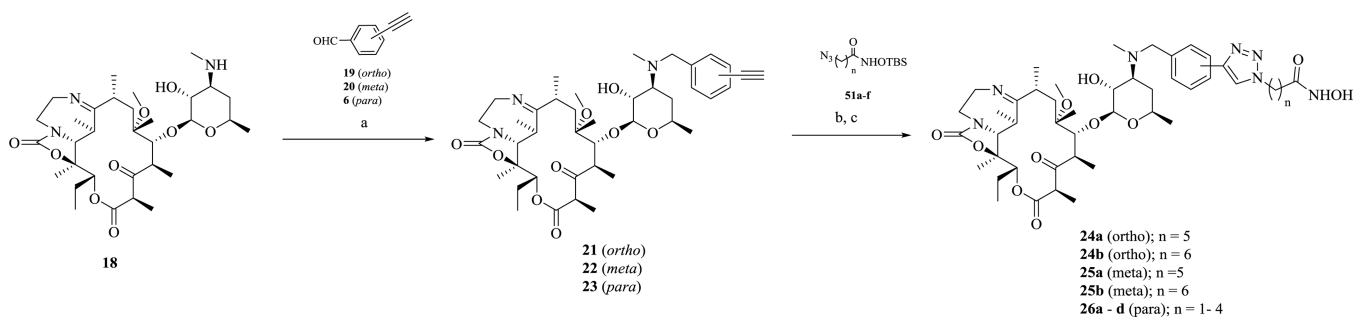


Figure 2.

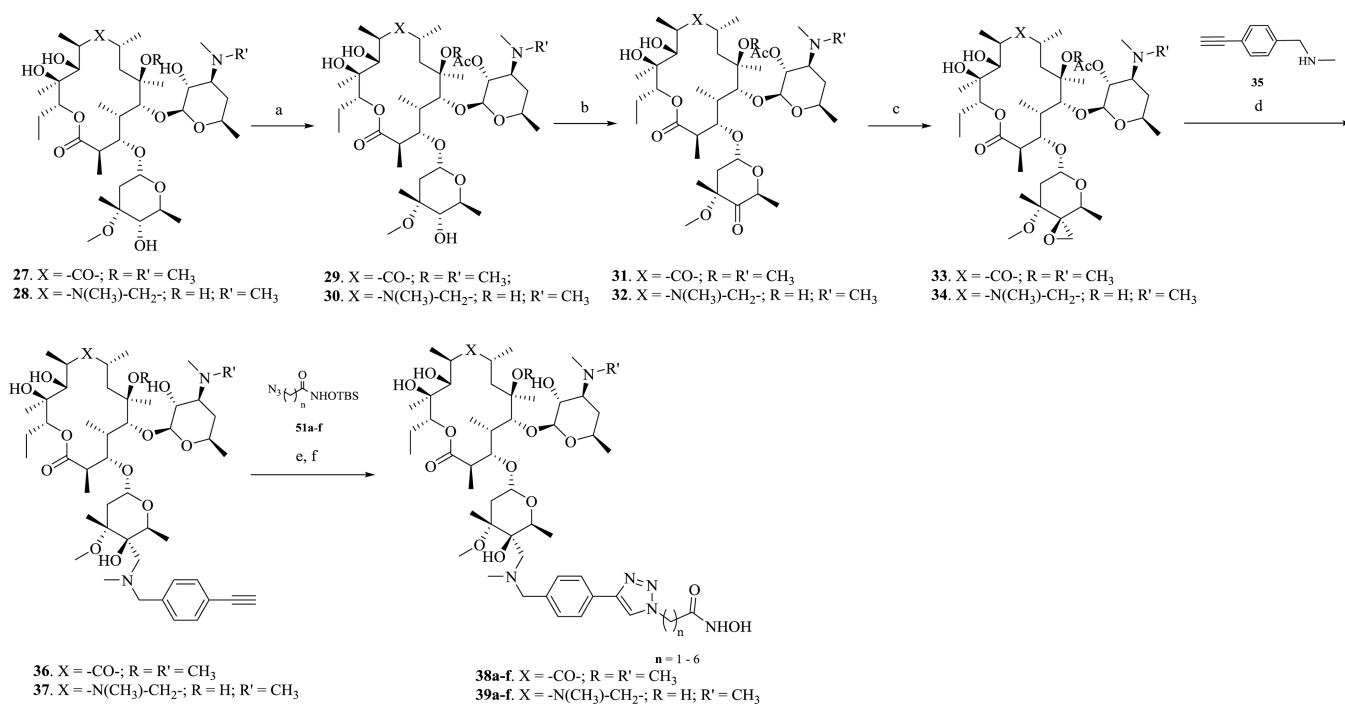
HDACi suppress NTHi-induced expression of Cytokines. BEAS-2B cells were pretreated with HDAC inhibitor (**26f**, **38f**; 1 μ M) or SAHA (1 μ M) or DEX (0.1 μ M) for 2 h followed by 1.5 h stimulation with NTHi, and cytokines mRNA (TNF- α , IL-1 α and IL-1 β) expressions were analyzed. Data are mean \pm *stn*(=3); ***p*<0.01, ****p*<0.005. Data are representative of three independent experiments. CON = BEAS-2B cells treated with PBS control; NTHi = BEAS-2B cells treated with NTHi.

**Scheme 1.**

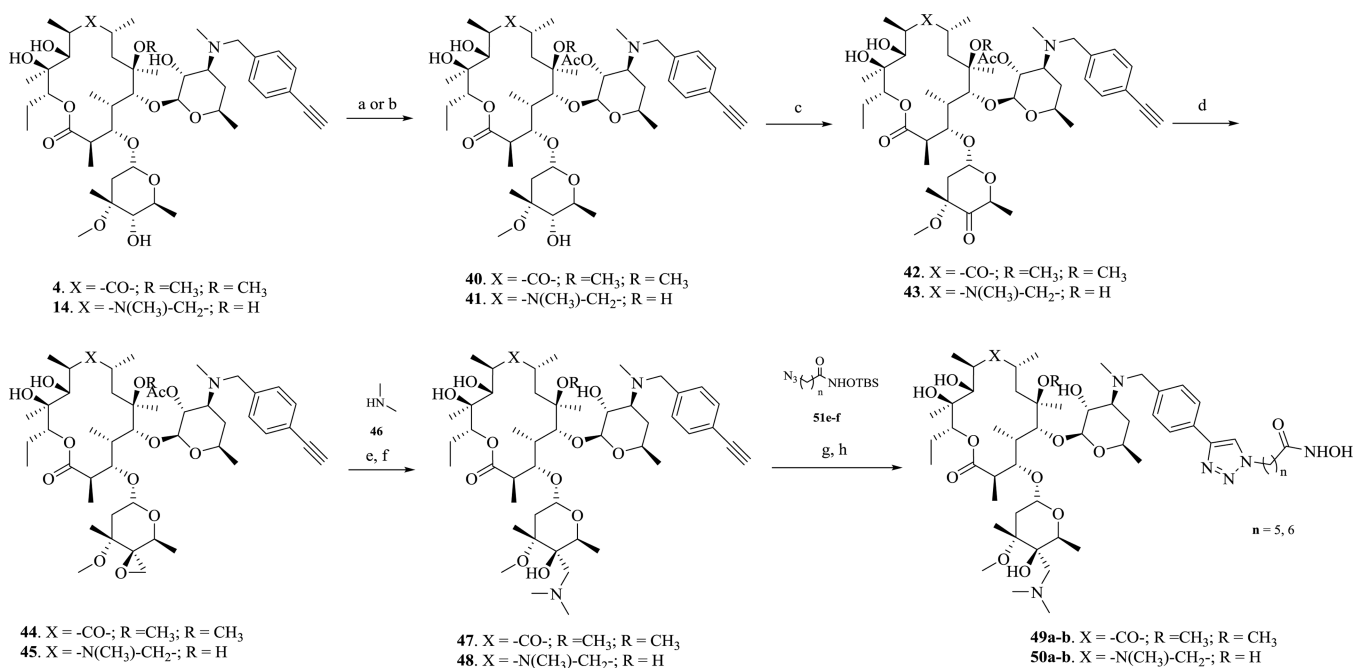
(a) Hünig's base, DMSO, 85 °C, 3 h; (b) CuI (15 mol%), Hünig's base, THF-DMSO (1:1), 40 °C, 12 h; (c) CsF, MeOH, rt, 30 min; (d) NaBH₃CN, AcOH, DMF, 70 °C, 7 h.

**Scheme 2.**

(a) borane-pyridine complex, AcOH, MeOH, rt, 9 h. (b) CuI (15 mol%), Hünig's base, THF-DMSO (1:1), 40 °C, 12 h; (c) CsF, MeOH, rt, 30 min.

**Scheme 3.**

(a) CH₂Cl₂, Ac₂O, rt, 3 h; (b) NCS, DMS, TEA, CH₂Cl₂, -15 °C, 4.5 h; (c) (CH₃)₃SO⁺I⁻, NaH, DMSO, THF, rt, 4 h; (d) KI, MeOH, 60 °C, 6 h; (e) CuI (15 mol%), Hünig's base, THF, rt, 12 h; (f) CsF, MeOH, rt, 2 h.

**Scheme 4.**

(a) for **40**: acetic anhydride, CH₂Cl₂, rt, 3 h; (b) for **41**: acetic anhydride, CH₂Cl₂, 40 °C, 48 h; (c) NCS, DMS, TEA, CH₂Cl₂, -15 °C, 6 h; (d) (CH₃)₃SO⁺I⁻, NaH, DMSO, THF, rt, 4 h; (e) KI, MeOH, 60 °C, 6 h; (f) MeOH, 90 °C, 3 days; (g) CuI (15 mol%), Hünig's base, THF, rt, 12 h; (h) CsF, MeOH, rt, 2 h.

Table 1

HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC_{50} in nM) of clarithromycin derived hydroxamic acid compounds

Compound	n	HDAC1	HDAC6	HDAC8
5a	1	> 10 μ M	> 10 μ M	> 10 μ M
5b	2	> 10 μ M	919 \pm 45	2450 \pm 600
5c	3	9910 \pm 3000	70.9 \pm 12.6	986 \pm 81
5d	4	4870 \pm 500	120 \pm 19	877 \pm 125
5e	5	533 \pm 123	3.61 \pm 1.01	1180 \pm 310
5f	6	207 \pm 84	6.67 \pm 1.23	713 \pm 748
38a	1	> 10 μ M	5350 \pm 190	7120 \pm 2130
38b	2	> 10 μ M	3560 \pm 370	10200 \pm 1600
38c	3	> 10 μ M	361 \pm 67	2530 \pm 880
38d	4	3690 \pm 860	269 \pm 100	1820 \pm 400
38e	5	652 \pm 130	5.89 \pm 2.73	985 \pm 325
38f	6	23.9 \pm 3.3	2.85 \pm 1.07	1840 \pm 460
49a	5	201 \pm 17	32.9 \pm 2.4	3200 \pm 820
49b	6	33.3 \pm 8.1	31.4 \pm 5.5	2210 \pm 300

Table 2

HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC₅₀ in nM) of azithromycin derived hydroxamic acid compounds

Compound	n	HDAC1	HDAC6	HDAC8
15a	5	613 ± 57	178 ± 30	4300 ± 2160
15b	6	923 ± 238	30.9 ± 12.4	1280 ± 400
16a	5	109 ± 12	43.8 ± 1.8	604 ± 161
16b	6	101 ± 15	41.0 ± 4.6	1440 ± 140
17a	1	> 10 μM	5660 ± 320	> 10 μM
17b	2	> 10 μM	2170 ± 200	> 10 μM
17c	3	> 10 μM	106 ± 13	> 10 μM
17d	4	1650 ± 456	145 ± 42	5380 ± 650
17e	5	316 ± 61	14.3 ± .6	644 ± 244
17f	6	68.6 ± 3.3	7.29 ± .56	314 ± 90
39a	1	> 10 μM	> 10 μM	4510 ± 660
39b	2	> 10 μM	2030 ± 300	2230 ± 560
39c	3	> 10 μM	181 ± 24	1180 ± 210
39d	4	531 ± 79	95.3 ± 12.3	709 ± 141
39e	5	203 ± 35	31.9 ± 1.2	786 ± 176
39f	6	50.4 ± 8.4	31.3 ± 1.3	817 ± 308
50a	5	160 ± 66	14.7 ± 1.8	1090 ± 240
50b	6	79.5 ± 54	18.9 ± 3.4	749 ± 98

Table 3

HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC_{50} in nM) of *N10*-modified azithromycin derived hydroxamic acid compounds

Compound	n	HDAC1	HDAC6	HDAC8
8a	1	> 10 μ M	> 10 μ M	> 10 μ M
8b	2	> 10 μ M	1230 \pm 300	> 10 μ M
8c	3	> 10 μ M	620 \pm 110	2770 \pm 580
8d	4	2550 \pm 550	24.5 \pm 7.4	> 10 μ M
8e	5	145 \pm 20	10.3 \pm 3.8	2370 \pm 890
8f	6	16.1 \pm 2.5	4.72 \pm .20	1700 \pm 190

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Table 4

HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC_{50} in nM) of TE-802 derived hydroxamic acid compounds

Compound	n	HDAC1	HDAC6	HDAC8
24a	5	> 10 μ M	248 \pm 20	1190 \pm 300
24b	6	> 10 μ M	75.4 \pm 6.9	2450 \pm 380
25a	5	109 \pm 12	17.5 \pm 2.2	3230 \pm 960
25b	6	54.5 \pm 11.1	3.76 \pm .17	310 \pm 47
26a	1	> 10 μ M	> 10 μ M	2850 \pm 550
26b	2	> 10 μ M	1730 \pm 220	> 10 μ M
26c	3	> 10 μ M	194 \pm 24	2240 \pm 440
26d	4	269 \pm 72	77.2 \pm 4.0	3210 \pm 890
26e	5	73.1 \pm 4.5	8.90 \pm .34	2030 \pm 200
26f	6	20.8 \pm 5.7	6.99 \pm .18	1500 \pm 260

Table 5Anti-proliferative activity of selected HDAC inhibitors (IC₅₀ values in μM)^a

Compound	A549	MCF-7	Vero
5f	2.29 \pm 0.73	2.86 \pm 0.10	4.90 \pm 0.34
8d	NI	NI	NT
8f	8.28 \pm 0.96	6.30 \pm 0.58	6.66 \pm 0.21
15b	NI	NI	NT
16b	4.09 \pm 0.46	2.37 \pm 0.32	8.35 \pm 1.34
17c	NI	NI	NT
17f	2.32 \pm 0.53	4.08 \pm 1.03	5.90 \pm 0.18
24b	NI	NI	NT
25a	NI	NI	NT
25b	7.48 \pm 0.23	0.86 \pm 0.18	>20
26f	2.19 \pm 0.10	1.98 \pm 0.15	NT
38f	0.99 \pm 0.08	0.69 \pm 0.05	1.55 \pm 0.12
39f	NI	NI	NT
49b	3.58 \pm 0.79	1.43 \pm 0.17	2.08 \pm 0.14
50b	6.80 \pm 0.50	5.92 \pm 1.82	5.73 \pm 0.49
SAHA	5.00 \pm 0.24	3.27 \pm 0.05	1.03 \pm 0.09

^aEach value is obtained from a duplicate of three simultaneous experiments.

NI=No inhibition, NT=not tested

Table 6Anti-inflammatory activity (NF- κ B inhibition) of selected HDAC inhibitors

Compound	IC ₅₀ (nM)	*I _{max} %
5e	785	45.1
5f	243	35.9
8f	244	31.4
16b	609	43.3
17f	280	41.4
25a	NA	71
26f	47.2	35.3
38e	785	50.7
38f	197	35.6
39e	368	46.1
49b	260	33.4
50b	575	43.4
SAHA	88	37.4

*I_{max} (%) at 1 μ M.