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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 to SAHA.

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1. 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, ER α , 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. Morever, many HDACi have been approved for hematological malignancies (Fig. 1a). The approved HDACi are

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http://dx.doi.org/10.1016/j.bmc.2015.10.045 0968-0896/© 2015 Elsevier Ltd. All rights reserved. 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.^{13–15} The replacement of the amide

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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.

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.

2. 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 N^{10} position of azithromycin to furnish the requisite alkynyl-macrolides **4**, **7 12**, **13** and **14** following the literature procedure.^{14–17} 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'-Oacetylclarithromycin 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 vields (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.

3. 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,

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Scheme 1. Reagents and conditions: (a) Hünig's base, DMSO, 85 °C, 3 h; (b) Cul (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. Reagents and conditions: (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.

Table 1), **16a** and **17f** (IC_{50} s 604 nM and 314 nM, respectively, Table 2), and **25b** (IC_{50} 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, 17a-d, 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 5–10 fold drop in potency (**49a**: IC_{50} 32.9 nM; **49b**: IC_{50} 31.4 nM compared to **5e**: IC_{50} 3.61 nM, **5f**: IC_{50}

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27. X = -CO-; R = R' = CH₃ 28. X = -N(CH₃)-CH₂-; R = H; R' = CH₃

33. $X = -CO - R = R' = CH_2$ **34**. X = -N(CH₃)-CH₂-; R = H; R' = CH₃



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Scheme 3. Reagents and conditions: (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.

NHOH



Scheme 4. Reagents and conditions: (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) Cul (15 mol %), Hünig's base, THF, rt, 12 h; (h) CsF, MeOH, rt, 2 h.

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** (IC_{50} 23.9 nM vs IC_{50} 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 N^{10} 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

Table I											
HDAC1,	HDAC6,	and	HDAC8	inhibition	activities	(IC ₅₀	in	nM)	of	clarithromyci	in
derived	hvdroxar	nic a	cid comi	oounds							

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

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, azithromycinand 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 metasubstituted, five methylene-linked compound 16a is slightly more potent than the corresponding para-substituted 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 metasubstituted 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).

3.1. Cell growth inhibitory assay

To verify if the anti-HDAC activities of these macrolide-derived hydroxamates translate to anti-proliferative activities, we tested

Table 2

HDAC1, HDAC6, and HDAC8 inhibition activities (IC $_{\rm 50}$ in nM) of azithromycin derived hydroxamic acid compounds

Compound	п	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

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 potently 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 \approx 900 nM). All other strongly HDAC inhibiting compounds tested (8f, 16b, 25b, 38f, **49b** and **50b**) possess varying degree of anti-proliferative 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 anti-proliferative 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

Table 3

HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC_{50} in nM) of N^{10} -modified azithromycin derived hydroxamic acid compounds

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

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

Table 5
Anti-proliferative activity of selected HDAC inhibitors $(IC_{50} \text{ values in } \mu M)^a$

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

^a Each value is obtained from a duplicate of three simultaneous experiments. NI = no inhibition, NT = not tested.

Table 6 Anti-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

 * $I_{\rm max}$ (%) at 1 μ M.

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).

3.2. Anti-inflammatory activity assay

Inflammation is a salient factor in cancer, particularly lung cancer and many other chronic lung diseases.^{22–24} 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-κB activation and or blockage of pro-inflammatory cytokine release.³⁰ In addition to their antibiotic activities, the macrolide templates (azithromycin, and clarithromycin) for the HDACi described herein have intrinsic anti-inflammatory and immunostimulatory activities. To test if these macrolide-derived HDACi preserve these useful properties and additively or synergistically enhance the latent anti-inflammatory activity of HDACi, we evaluated their antiinflammatory activities in BEAS-2B cell infected with nontypeable Haemophilus influenza (NTHi) using NF-κB 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 pro-inflammatory 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 1μ M. We observed that compounds lacking or those with weak anti-HDAC activities did not suppress NF-kB activation while those compounds with potent anti-HDAC activities suppressed NF-KB activation to varying degrees which closely correlate with their HDAC inhibition potency (Supporting information, Fig. S1).

We then determined the IC_{50} values of selected compounds which suppressed NF-kB 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 µM, 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 uM, these compounds suppressed NF-KB 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.



Figure 2. HDACi suppress NTHi-induced expression of cytokines. BEAS-2B cells were pretreated with HDAC inhibitor (**8f, 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 ± STD (*n* = 3); "*p* <0.005. Data are representative of three independent experiments. CON = BEAS-2B cells treated with DMEM control; NTHi = BEAS-2B cells treated with NTHi.

4. 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.

5. Experimental

5.1. 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 µm) 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-d₅ (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}

5.1.1. 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 (br s, 1H), 3.97 (s, 2H), 0.97 (s, 9H), 0.16 (s, 6H).

5.1.2. 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 (br s, 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).

5.1.3. 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 (br s, 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).

5.1.4. 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 (br s, 1H), 3.25 (t, *J* = 6.9 Hz, 2H), 2.27 (br s, 2H), 1.63 (m, 4H), 0.91 (s, 9H), 0.16 (s, 6H).

5.1.5. 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 (br s, 1H), 3.24 (t, *J* = 6.9 Hz, 2H), 2.35 (br s, 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).

5.1.6. 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 (br s, 1H), 3.22 (t, *J* = 6.9 Hz, 2H), 2.22 (br s, 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.

5.1.7. (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 (0.06 ml, 0.35 mmol) and Cul (0.017 g, 0.088 mmol) were then added to the mixture and purged further for another 20 min. 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 Na_2SO_4 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 (0.04 g, 0.27 mmol) 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, $CDCl_3$) 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.

5.1.8. (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, / = 9.4, 6.3 Hz, 1H), 3.93 (d, / = 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, /=3.3, 1.6 Hz, 3H), 3.18 (s, 3H), 3.11 (d, *J* = 7.8 Hz, 1H), 3.03 (d, *I* = 7.3 Hz, 4H), 3.01 (d, *I* = 9.5 Hz, 2H), 2.94–2.84 (m, 2H), 2.77 (t, I = 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, *I* = 14.0 Hz, 4H), 1.25 (q, *I* = 7.8 Hz, 5H), 1.21 (d, *I* = 6.0 Hz, 7H), 1.18–1.08 (m, 17H), 0.85 (t, J = 7.4 Hz, 3H). ¹³C NMR (126 MHz, $CDCl_3$) 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.

5.1.9. (Clarithromycin-3'-(*N*-(4-triazolylbenzyl)))-*N*-hydroxybu-tanamide (5c)

Reaction of 3'-*N*(desmethyl)-3'-*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15 g,0.18 mmol) 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, 11H), 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.

5.1.10. (Clarithromycin-3'-(N-(4-triazolylbenzyl)))-Nhydroxypentanamide (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 (0.072 g, 0.265 mmol) 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, *I* = 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, /=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). ¹³C NMR (126 MHz, CDCl₃) 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 C₅₁H₈₃N₅O₁₅Na [M+Na⁺]: 1028.5783, found 1028.5795.

5.1.11. (N¹⁰-(4-Ethynylbenzyl))azithromycin (7)

 N^{10} -Desmethylazithromycin **2** (2.72 g, 3.55 mmol) and 4ethynylbenzaldehyde 6^{39} (2.31 g, 17.75 mmol) were dissolved in anhvdrous 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 NaHCO₃ solution. The crude mixture was diluted into CH₂Cl₂ and was washed water, dried over anhydrous Na₂SO₄, 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, 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, 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, and 7.2 HZ, 1H), 2.99 (m, 1H), 2.88 (dd, J = 14.7, 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, 11H), 1.22 (m, 4H), 1.18 (d, J = 7.4 Hz, 3H), 1.14 (dd, J = 8.0, and 4.1 Hz, 3H), 0.94 (m, 3H), 0.89 (t, J = 7.3 Hz, 3H). 13 C (125 MHz, 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 C₄₆ H₇₈ O₁₂ N₂ [M+2H⁺]: 425.2772, found 425.2762.

5.1.12. (Azithromycin(-N¹⁰-(4-triazolylbenzyl)))-*N*hydroxyacetamide (8a)

In an oven dried round bottomed flask charged with a magnetic stirring bar, $(N^{10}-(4-\text{ethynylbenzyl}))$ 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 degassed1:1 mixture of THF/ DMSO, Cul (4 mg, 0.02 mmol) and *N*,*N*-diisopropylethylamine (20 μ L, 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. NH₄Cl soln.-NH₄OH soln. (2 × 10 mL), water (10 mL), and brine (10 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 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 guenched by adding water and was extracted with ethyl acetate (100 mL) and the organic laver was washed with brine (10 mL), dried over anhydrous Na₂SO₄, filtered and concentrated in vacuo. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 CH₂Cl₂-MeOH-concd NH₄OH 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, / = 7.5 Hz, 2H), 7.42 (d, / = 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). ¹³C (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 C48H82N6O14 [M +2H⁺]: 483.2939, found 483.2933.

5.1.13. (Azithromycin-(N¹⁰-(4-triazolylbenzyl)))-*N*-hydroxypropanamide (8b)

Reaction of $(N^{10}-(4-\text{ethynylbenzyl}))$ 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). ¹³C (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 C₄₉H₈₃N₆O₁₄ [M+H⁺]: 979.5962, found 979.5967.

5.1.14. (Azithromycin-(N¹⁰-(4-triazolylbenzyl)))-*N*-hydroxybutanamide (8c)

Reaction of (N^{10} -(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). ¹³C (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 $C_{50}H_{85}N_6O_{14}$ [M+H⁺]: 993.6118, found 993.6125.

5.1.15. (Azithromycin-(N¹⁰-(4-triazolylbenzyl)))-*N*-hydroxypentanamide (8d)

Reaction of $(N^{10}-(4-\text{ethynylbenzyl}))$ 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). ¹³C 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 C₅₁H₈₇N₆O₁₄ [M+H⁺]: 1007.6275, found 1007.6278.

5.1.16. (Azithromycin-(N¹⁰-(4-triazolylbenzyl)))-N-hydroxyhexanamide (8e)

Reaction of $(N^{10}-(4-\text{ethynylbenzyl}))$ 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). ¹³C 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, 49.3, 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 $C_{52}H_{88}N_6O_{14}$ [M+H⁺]: 1021.6431, found 1021.6444.

5.1.17. (Azithromycin-(N¹⁰-(4-triazolylbenzyl)))-N-hydroxyheptanamide (8f)

Reaction of (N^{10} -(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). ¹³C 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 $C_{53}H_{91}N_6O_{14}$ [M+H⁺]: 1035.6588, found 1035.6591.

5.1.18. 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).

5.1.19. 3-Ethynylbenzaldehyde (20)

A solution of 3-bromobenzaldehyde 61 (1.0 g, 5.40 mmol), ethynyltrimethylsilane (850 mg, 8.6 mmol), palladium (II) acetate 0.065 mmol), and triphenylphosphine (28 mg, (15 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 orangebrown filtrate was mixed with 50 ml of saturated NaHCO₃ and extracted with dichloromethane (3 \times 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 $(3 \times 50 \text{ mL})$. 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 brown-white 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).

5.1.20. 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 K_2CO_3 (100 mg) under argon at room temperature for 3 h. Saturated NaHCO₃ (50 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (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 CH_2Cl_2 -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).

5.1.21. 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 °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 NaHCO₃ 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 Na₂SO₄, filtered and concentrated in vacuo. The crude mesylated product **10** was used in the next step without further purification.

5.1.22. (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 °C for 3 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL) and washed with saturated NaHCO₃ (3 \times 50 mL) and saturated brine (50 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by column chromatography (Silica gel, 12:1:0.1 to 4:1:0.1 CH₂Cl₂-Acetone-Et₃N) to give 12 as a brown-white solid (600 mg, 44%). ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 7.43 (d, J = 7.6 Hz, 1H), 7.26 (m, 2H), 7.16 (d, J = 7.2 Hz, 1H), 5.02 (d, J = 7.6 Hz, 1H), 4.72 (m, 1H), 4.62 (d, J = 9.6 Hz, 1H), 4.35 (d, J = 7.2 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). ¹³C NMR (CDCl₃, 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 C₄₆H₇₇N₂O₁₂ [M+H⁺]: 849.5471, found 849.5530.

5.1.23. (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 (CDCl₃, 400 MHz) *δ* (ppm) 7.41 (m, 2H), 7.22 (m, 2H), 5.14 (d, *J* = 6.0 Hz, 1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 7.6 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). ¹³C NMR (CDCl₃, 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 C₄₆H₇₇N₂O₁₂ [M +H⁺]: 849.5471, found 849.5385.

5.1.24. (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 NH₄-OH/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 \times 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/ concd 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.

5.1.25. (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 $(CDCl_3, 100 \text{ MHz}) \delta$ (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.

5.1.26. (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.

5.1.27. (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)hep-

tanamide **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.

5.1.28. (Azithromycin-3'-(N-(4-triazolylbenzyl)))-N-hydroxyace-tamide (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 12 h. 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, I = 7.4 Hz, 2H), 5.13 (d, I = 4.6 Hz, 2H), 4.66 (d, I = 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, / = 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+2/2z Calcd for C₄₈H₈₂N₆O₁₄ [M+2H⁺]: 483.2939, found for 483.2935.

5.1.29. 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, CD₃OD) 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). ¹³C NMR (126 MHz, CD₃OD) 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 C₄₉H₈₃N₆O₁₄ [M+H⁺]: 979.5962, found for 979.5958.

5.1.30. 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-butyldimethyl silyl)oxy)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, I = 7.5 Hz, 2H), 7.33 (d, I = 6.2 Hz, 2H), 4.35 (d. / = 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). ¹³C NMR (126 MHz, CD₃OD) 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 $C_{50}H_{85}N_6O_{14}$ [M +H⁺]: 993.6118, found 993.6113.

5.1.31. 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-butyldimethyl silyl)oxy)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, CD₃OD) 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, *I* = 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, *I* = 11.8 Hz, 1H), 2.39 (s, 3H), 2.32 (d, *I* = 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). ¹³C NMR (126 MHz, CD₃-OD) 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 C₅₁H₈₇N₆O₁₄ [M+H⁺]: 1007.6275, found 1007.6272.

5.1.32. (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.

5.1.33. (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 µL, 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, I = 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.

5.1.34. (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*-butyldimethylsilyl)oxy)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. (2×4 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/concd 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 $C_{47}H_{72}N_7O_{11}$ [M+H⁺]: 910.5284, found 910.5245.

5.1.35. (Tricyclic Ketolide-3'-(*N*-(2-triazolylbenzyl)))-*N*-hydrox-yheptanamide (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 $C_{48}H_{74}N_7O_{11}$ [M+H⁺]: 924.5441, found 924.5422.

5.1.36. (Tricyclic ketolide-3'-(*N*-(3-triazolylbenzyl)))-*N*-hydroxy-hexanamide (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.

5.1.37. (Tricyclic Ketolide-3'-(*N*-(3-triazolylbenzyl)))-*N*-hydroxy-heptanamide (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). ¹³C NMR (CDCl₃, 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 $C_{48}H_{74}N_7O_{11}$ [M+H⁺]: 924.5441, found 924.5455.

5.1.38. (Tricyclic Ketolide-3'-(*N*-(4-triazolylbenzyl)))-*N*-hydroxy-acetamide (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 degassed1:1 mixture of THF

and DMSO, CuI (6.4 mg, 0.03 mmol) and *N*,*N*'-diisopropylethylamine (30 μ L, 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. NH₄Cl soln. and NH₄OH soln. (2 × 10 mL), water (10 mL), and brine (10 mL), dried over anhydrous Na₂SO₄, 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 Na₂SO₄, filtered and concentrated in vacuo. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 CH₂Cl₂/MeOH/concd NH₄OH soln.) to give **26a** as a white solid (76 mg, 40%). ¹H NMR (500 MHz, MeOH- d_4) δ (ppm) 8.24 (s, 1H), 7.70 (d, I = 8.0 Hz, 2H), 7.34 (d, I = 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). ¹³C $(125 \text{ MHz}, \text{ MeOH-}d_4) \delta$ (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 C₄₃H₆₄N₇O₁₁ [M+H⁺]: 854.4658, found 854.4656.

5.1.39. (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, MeOH- d_4) δ (ppm) 8.13 (s, 1H), 7.64 (d, I = 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, I = 7.4 Hz, 3H). ¹³C (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 C₄₄H₆₆N₇O₁₁ [M+H⁺]: 868.4815, found 868.4812.

5.1.40. (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*-butyldimethylsilyl) oxy)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). ¹³C (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

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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 C₄₅H₆₈N₇O₁₁ [M+H⁺]: 882.4971, found 882.4971.

5.1.41. Tricyclic Ketolide-3'-(*N*-(4-triazolylbenzyl)))-*N*-hydrox-ypentanamide (26d)

Reaction of (3'-N-(4-ethynylbenzyl))tricyclic ketolide 23 (0.114 g, 0.150 mmol) and 5-azido-N-((tert-butyldimethylsilyl) oxy)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). ¹³C (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 C₄₆H₇₀N₇O₁₁ [M+H⁺]: 896.5128, found 896.5121.

5.1.42. 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 °C for another 2 h. The reaction was guenched by adding saturated aqueous NaHCO₃ 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 Na₂SO₄, 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. ¹H NMR $(400 \text{ MHz}, \text{CDCl}_3) \delta$ (ppm) 5.11–5.02 (m, 1H), 4.97 (d, *J* = 10.7 Hz, 1H), 4.75 (dd, *J* = 10.3, 7.6 Hz, 1H), 4.39 (t, *J* = 6.1 Hz, 1H), 4.29 (q, J = 6.7 Hz, 1H), 3.67–3.53 (m, 3H), 3.38 (td, J = 15.1, 5.0 Hz, 2H), 3.30 (d, J = 1.3 Hz, 4H), 3.15 (t, J = 9.1 Hz, 3H), 2.93–2.82 (m, 4H), 2.78 (dd, J = 16.9, 7.3 Hz, 1H), 2.68 (d, J = 1.3 Hz, 1H), 2.65 (d, J = 2.7 Hz, 1H), 2.60 (d, J = 1.2 Hz, 1H), 2.56 (d, J = 1.3 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 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 Hz, 3H), 1.12 (dd, J = 11.2, 6.4 Hz, 7H), 1.07–0.93 (m, 10H), 0.78 (t, J = 9.6 Hz, 3H), 0.70 (t, J = 7.2 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) 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 C₄₀H₇₀NO₁₄ [M +H⁺]: 788.4791, found 788.4781.

5.1.43. 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 trimethyloxosulfo-

nium 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 H₂O to remove DMSO. Organic layer was dried with NaSO₄ 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 (2.43 g, 38%). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 5.06 (dq, J = 6.2, 4.6 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 Hz, 1H), 3.34–3.28 (m, 4H), 3.19 (s, 1H), 3.01 (d, J = 7.0 Hz, 1H), 3.01–2.95 (m, 4H), 2.94–2.90 (m, 1H), 2.86 (dd, J = 9.9, 7.1 Hz, 1H), 2.69–2.63 (m, 1H), 2.61 (dd, J = 9.8, 4.4 Hz, 2H), 2.53 (dd, J = 9.6, 7.1 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, / = 1.6 Hz, 1H), 1.53 (s, 1H), 1.49– 1.36 (m, 1H), 1.33 (d, J = 7.2 Hz, 3H), 1.28 (s, 1H), 1.26–1.12 (m, 11H), 1.14–0.99 (m, 16H), 0.92 (t, J = 8.2 Hz, 3H), 0.81 (dd, J = 9.8, 4.9 Hz, 3H). ¹³C NMR (101 MHz, CDCL₃) 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 C₄₁H₇₇NO₁₄ [M+H⁺]: 802.4947, found 802.4938.

5.1.44. (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/NH₄-OH (2:1:0.6:0.1) to give compound 36 as a light yellow solid (2.41 g, 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, / = 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.

5.1.45. (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.

5.1.46. (Clarithromycin-4"(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxypropanamide (38b)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin 36 (0.15 g,0.17 mmol) with 3-Azido-N-((tertbutyldimethylsilyl)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 (0.07 g, 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, 11H), 0.83 (t, J = 12.v0, 5.5 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) 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 C₅₂H₈₇N₆O₁₅ [M+H⁺]: 1035.6270, found 1035.6210.

5.1.47. (Clarithromycin-4"(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxybutanamide (38c)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin 36 (0.15 g, 0.17 mmol) with 4-Azido-N-((tertbutyldimethylsilyl)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 (0.060 g, 40%). 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), 4.46 (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, 11H), 0.81 (t, J = 7.2 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) 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 C₅₃H₈₉N₆O₁₅ [M+H⁺]1049.6386, found 1049.6376.

5.1.48. (Clarithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxypentanamide (38d)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin 36 (0.15 g, 0.17 mmol) with 5-Azido-N-((tertbutyldimethylsilyl)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 (0.067 g, 44%). ¹H NMR (400 MHz, CD₃OD) 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, 11H), 0.81 (t, I = 7.3 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) 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 $C_{54}H_{91}N_6O_{15}$ [M+H⁺]: 1063.6542, found 1063.6588.

5.1.49. (Clarithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxyhexanamide (38e)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin 36 (0.135 g, 0.149 mmol) with 6-Azido-N-((tertbutyldimethylsilyl)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 (0.053 g, 35%). ¹H NMR (500 MHz, CDCl₃) 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, *I* = 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, 11H), 0.90-0.85 (m, 1H), 0.85–0.74 (m, 3H). 13 C NMR (126 MHz, CDCl₃) 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 C₅₅H₉₃N₆O₁₅ [M+H⁺]: 1077.6699, found 1077.6692.

5.1.50. (Clarithromycin-4"-(methylamino)-*N*(methyl)-4-benzyl-triazolyl))-*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 (0.060 g, 40%). ¹H NMR (400 MHz, CD₃OD) 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), S. Tapadar et al./Bioorg. Med. Chem. xxx (2015) xxx-xxx

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). ¹³C NMR (126 MHz, CDCl₃) 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 C₅₆H₉₅N₆O₁₅ [M+H⁺]: 1091.6815, found 1091.6869.

5.1.51. 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, I = 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 + 2/2z Calcd for C₄₀H₇₆N₂O₁₃ [M+2H⁺]/2: 396.2668, found 396.2656.

5.1.52. 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 \times 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/2z Calcd for $C_{40}H_{73}N_2O_{13}$ [M+2H⁺]/2: 395.2590, found 395.2587.

5.1.53. 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 $(\times 3)$. 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, trimethyloxosulfonium 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 Na_2SO_4 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.

5.1.54. 1-(4-Ethynylphenyl)-N-methylmethanamine (35)

Methylamine (9.32 mL, 18.63 mmol, 2 M 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 1 M HCl. This was then extracted multiple times with DCM. The aqueous layer was basified with 1 M 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 (0.15 g, 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.

5.1.55. (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 NaHCO $_3$ 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, /=7.1 Hz, 3H), 2.66 (s, 3H), 2.44 (d, /=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). ¹³C NMR (126 MHz, CDCl₃) 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* + 2/2*z* Calcd for C₄₉H₈₅N₃O₁₂ [M+2H⁺]: 453.8061, found 453.8055.

5.1.56. (Azithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxyacetamide (39a)

(4"-(Methylamino)-N(methyl)(4-ethynylbenzyl))azithromycin 37 (0.14 g, 0.16 mmol) and 2-Azido-N-((tert-butyldimethyl silyl) 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 guenched by adding a solution of 4:1 saturated NH₄Cl/NH₄OH (30 mL) and extracted with 20% MeOH/CH₂Cl₂ (3×30 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The crude product was purified by preparative TLC (Silica gel, 5:1:1 EtOAc/ MeOH/NH₄OH) to give the product (0.131 g, 80% yield) as light yellow solid. ¹H NMR (400 MHz, CD₃OD) 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). ¹³C NMR (126 MHz, CD₃OD) 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 + 2/2z Calcd for $C_{51}H_{89}N_7O_{14}$ [M+2H⁺]: 511.8228, found 511.8230.

5.1.57. (Azithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxypropanamide (39b)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin 37 (0.20 g, 0.218 mmol) and 3-azido-N-((tertbutyldimethylsilyl)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, CD₃OD) 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, / = 11.2 Hz, 3H), 1.27 (t, / = 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). ¹³C NMR (126 MHz, CD₃OD) 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 $C_{52}H_{90}N_7O_{14}$ [M+H⁺]: 1036.6540, found 1036.6550.

5.1.58. (Azithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxybutanamide (39c)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin 37 (0.20 g, 0.18 mmol) and 4-azido-N-((tertbutyldimethylsilyl)oxy)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, CD₃OD) 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, 11H), 1.03 (dd, J = 19.7, 6.7 Hz, 7H), 0.85 (dd, J = 14.1, 7.0 Hz, 3H). ¹³C NMR (126 MHz, CD₃OD) 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+2/2z Calcd for $C_{53}H_{93}N_7O_{14}$ [M+2H⁺]: 525.8385, found 525.8385.

5.1.59. (Azithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxypentanamide (39d)

Reaction of (4"-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin 37 (0.14 g, 0.15 mmol) and 5-azido-N-((tertbutyldimethylsilyl)oxy)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, CD₃OD) 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, / = 18.4 Hz, 15H), 1.64 (d, / = 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, / = 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).¹³C 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 C₅₄H₉₄N₇O₁₄ [M+H⁺]: 1064.6853, found 1064.6854.

5.1.60. (Azithromycin-4"-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxyhexanamide (39e)

Reaction of (4''-(methylamino)-N(methyl)(4-ethynylbenzyl))azithromycin **37** (0.09 g, 0.10 mmol) and 6-azido-*N*-((*tert*butyldimethylsilyl)oxy)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, 11H), 1.01 (dd, *J* = 17.4, 6.0 Hz, 11H), 0.89–0.79 (m, 3H). ¹³C NMR (126 MHz, CD₃–OD) 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*+2/2*z* Calcd for C₅₅H₉₇N₇O₁₄ [M+2H⁺]: 539.8541, found 539.8539.

5.1.61. (Azithromycin-4["]-(methylamino)-*N*(methyl)(4-benzyl-triazolyl))-*N*-hydroxyheptanamide (39f)

Reaction of (4"-(methylamino)-N(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.16 g. 0.18 mmol) and 7-azido-*N*-((*tert*butyldimethylsilyl)oxy)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, *I* = 8.1 Hz, 2H), 6.77 (d, *I* = 8.1 Hz, 2H), 4.49 (d, *I* = 5.0 Hz, 1H), 3.83 (dd, / = 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, CD₃-OD) 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+2/2z Calcd for $C_{56}H_{99}N_7O_{14}$ [M+2H⁺]: 546.8620, found 546.8621.

5.1.62. ((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 Na₂SO₄ and concentrated in vacuo to give compound **40** (128 mg, 81%). ¹H NMR (400 MHz, CDCl₃) 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, 11H), 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 C₄₈H₇₆NO₁₄ [M+H⁺]: 890.5260, found 890.5256.

5.1.63. ((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 NaHCO₃ (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na₂SO₄, 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, CDCl₃) 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). ¹³C NMR (101 MHz, CDCl₃) 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+2/2z Calcd for $C_{48}H_{80}N_2O_{13}$ [M+2H⁺]: 446.2825, found 446.2810.

5.1.64. ((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 $-15 \degree$ 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 guenched with saturated NaHCO₃ (10 mL). The organic layer extracted with DCM (50 mL), dried with anhydrous Na₂SO₄, 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.

5.1.65. ((4"-Oxo)-3'-O-acetyl)-4'-*N*-(4-ethynylbenzyl)) azithromycin (43)

A solution of *N*-chlorosuccinimide (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, I = 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 $C_{48}H_{77}N_2O_{13}$ [M+H⁺]: 889.5420, found 889.5412.

5.1.66. ((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, *I* = 3.5 Hz, 1H), 4.84–4.73 (m, 2H), 4.56 (d, *I* = 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, / = 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). ¹³C NMR $(101 \text{ MHz}, \text{ CDCl}_3) \delta$ (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 C₄₉H₇₆NO₁₄ [M+H⁺]: 902.5260, found 902.5260.

5.1.67. ((4"-Epoxy)-3'-O-acetyl)-4'-N-(4-ethynylbenzyl)) azithromycin (45)

NaH (0.2 g, 4.67 mmol, 60%/w) was added to an oven dried three necked flask and was washed with petroleum ether (×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 Na₂SO₄ and concentrated to give light yellow solid product (1.63 g, 85% yield). ¹H NMR (400 MHz, CDCl₃) δ (ppm) 7.37 (d, I = 8.0 Hz, 2H, 7.18 (d, I = 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). ¹³C NMR (126 MHz, CDCl₃) 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 C₄₉H₇₉N₂O₁₃ [M+H+]: 903.5577, found 903.5568.

5.1.68. ((4"-(N,N-Dimethylaminomethyl)-4'-N-(4-ethynylbenzyl))clarithromycin (47)

N,N-Dimethylmethylamine 46 (4.22 mL, 8.46 mmol, 2 M 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 methylamine 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, *I* = 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, /=10.2, 7.2 Hz, 1H), 3.21 (d, /=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, / = 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 + 2/2z Calcd for C₄₉H₈₂N₂O₁₃ [M+2H⁺]: 453.2903 found 453.2892.

5.1.69. ((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, 2 M 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,

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1H), 3.70 (dt, J = 9.6, 5.5 Hz, 1H), 3.67–3.59 (m, 2H), 3.54 (d, *I* = 6.2 Hz, 1H), 3.49 (s, 1H), 3.41 (ddd, *I* = 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, $CDCl_3$) 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.

5.1.70. (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, CDCl₃) 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 C₅₅H₉₃N₆O₁₅ [M+H⁺] 1077.6693, found 1077.6692.

5.1.71. (Clarithromycin-(4'-*N*-(4-benzyltriazolyl))-4"-(*N*,*N*-dime-thylaminomethyl))-*N*-hydroxyheptanamide (49b)

Reaction of compound 47 (0.024 g, 0.022 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 **49b** as a light yellow solid (0.021 g, 83%). ¹H NMR (400 MHz, CD₃OD) 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). ¹³C NMR (101 MHz, cd₃od) 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+2/2z Calcd for C₅₆H₉₇N₆O₁₅ [M+2H⁺]: 546.3461, found 546.3469.

5.1.72. (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-butyldimethyl 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 2 h. A solution of 4:1 saturated NH₄Cl/NH₄OH (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 Na₂SO₄ and concentrated in vacuo. The crude product was purified by preparative chromatography (Silica gel, 5:1:1 EtOAc/MeOH/NH₄OH) to give the product (0.16 g, 80%) as light yellow solid. ¹H NMR (400 MHz, CD₃OD) 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, / = 7.3 Hz, 1H), 3.60–3.46 (m, 2H), 3.26 (d, / = 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, 11H), 1.60-1.45 (m, 3H), 1.43-1.30 (m, 11H), 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). ¹³C NMR (126 MHz, CD₃OD) 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*+2/2*z* Calcd for C₅₅H₉₇N₇O₁₄ [M+2H+]: 539.8541, found 539.8549.

5.1.73. (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, CD₃OD) δ (ppm) 7.46 (s, 1H), 6.92 (d, *I* = 8.0 Hz, 2H), 6.56 (d, *I* = 8.0 Hz, 2H), 3.55 (d, *I* = 6.2 Hz, 2H), 3.47 (d, / = 7.2 Hz, 1H), 3.33–3.17 (m, 2H), 2.99 (d, / = 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, 11H), 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). ¹³C NMR (126 MHz, CD₃OD) 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+2/2z Calcd for C₅₆H₉₉N₇O₁₄ [M+2H⁺]: 546.8620, found 546.8611.

5.2. 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 μ M, detailed below) with enzyme (250 nM, 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 MgCl₂, 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 IC₅₀ 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-CCACA TGCTTCA 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.

5.3. 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%.

5.4. Anti-inflammatory activity assay

NF-KB activity was measured by luciferase assay. BEAS-2B cells were transfected with NF-kB 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 = luciferase unit of the cells treated with NTHi and drug/luciferase unit of the cells treated with mock. IC₅₀ was determined by treating the cell with a serial dose of the drug followed by luciferase assay. % inhibition was calculated using the following equation; % inhibition = RLA of the cells treated with indicated concentration of the drug/RLA of the cells treated with mock.

5.5. 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 $2 \times \sim$ Universal Master Mix, 1 µL of template cDNA, 500 nM primers in a final volume of 12.5 µL, 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 semiguantitative 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 previousery.^{40,41} IL-1 α : 5'-CGAGCCAATGATCAGTACCTC-3' and 3'-CACCCATATATTTCACTG -5'.

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Supplementary data

Supplementary data (¹H NMR, ¹³C NMR spectral and NF-κB activity in NTHi infected BEAS-2B cells treated with 1 μ M of tested compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.10.045.

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