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A structure–activity relationship of non-peptide macrocyclic histone deacetylase inhibitors and their anti-proliferative and anti-inflammatory activities

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ABSTRACT

Inhibition of the enzymatic activity of histone deacetylase (HDAC) is a promising therapeutic strategy for cancer treatment and several distinct small molecule histone deacetylase inhibitors (HDACi) have been reported. We have previously identified a new class of non-peptide macrocyclic HDACi derived from 14- and 15-membered macrolide skeletons. In these HDACi, the macrocyclic ring is linked to the zinc chelating hydroxamate moiety through a *para*-substituted aryl-triazole cap group. To further delineate the depth of the SAR of this class of HDACi, we have synthesized series of analogous compounds and investigated the influence of various substitution patterns on their HDAC inhibitory, anti-proliferative and anti-inflammatory activities. We identified compounds **25b** and **38f** with robust anti-proliferative activities and compound **26f** (IC_{50} 47.2 nM) with superior anti-inflammatory (IC_{50} 88 nM) activity relative to SAHA.

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, ERα, p53, HSP90, NF-YA, and GATA-1.[3] Histone deacetylase inhibitors (HDACi) have been shown to cause growth arrest, differentiation, and apoptosis in a variety of cancer cell lines.[4] To date, several classes of small molecule HDAC inhibitors—fitting a three-motif pharmacophoric model, namely, a zinc binding group (ZBG), a hydrophobic linker, and a recognition cap group[5]—have been reported. Morever, many HDACi have been approved for hematological malignancies (Fig. 1a). The approved HDACi are suberoylanilide hydroxamic acid (SAHA)[6,7] and FK228[8] 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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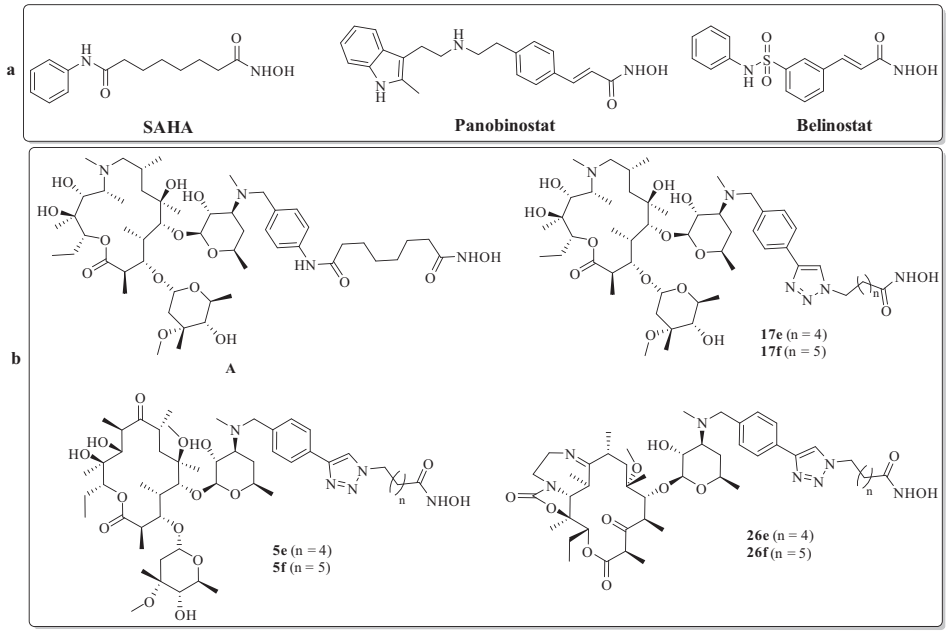


Figure 1. Representative HDACi: (a) Approved hydroxamic acid based HDACi, (b) selected examples of non-peptide (**5e–f**, **17e–f**, **26e–f**) macrocyclic HDACi.

moiety, by its bioisostere, triazole unit, increased the HDAC inhibitory potency of matched compounds by almost 8-fold [**17f** (HDAC1/2 IC_{50} 13.9 nM vs **A** (HDAC1/2 IC_{50} 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-group[19] afforded the final compounds **5a–d**, **8a–f**, **15a–b**, **16a–b**, and **17a–d** (Scheme 1).

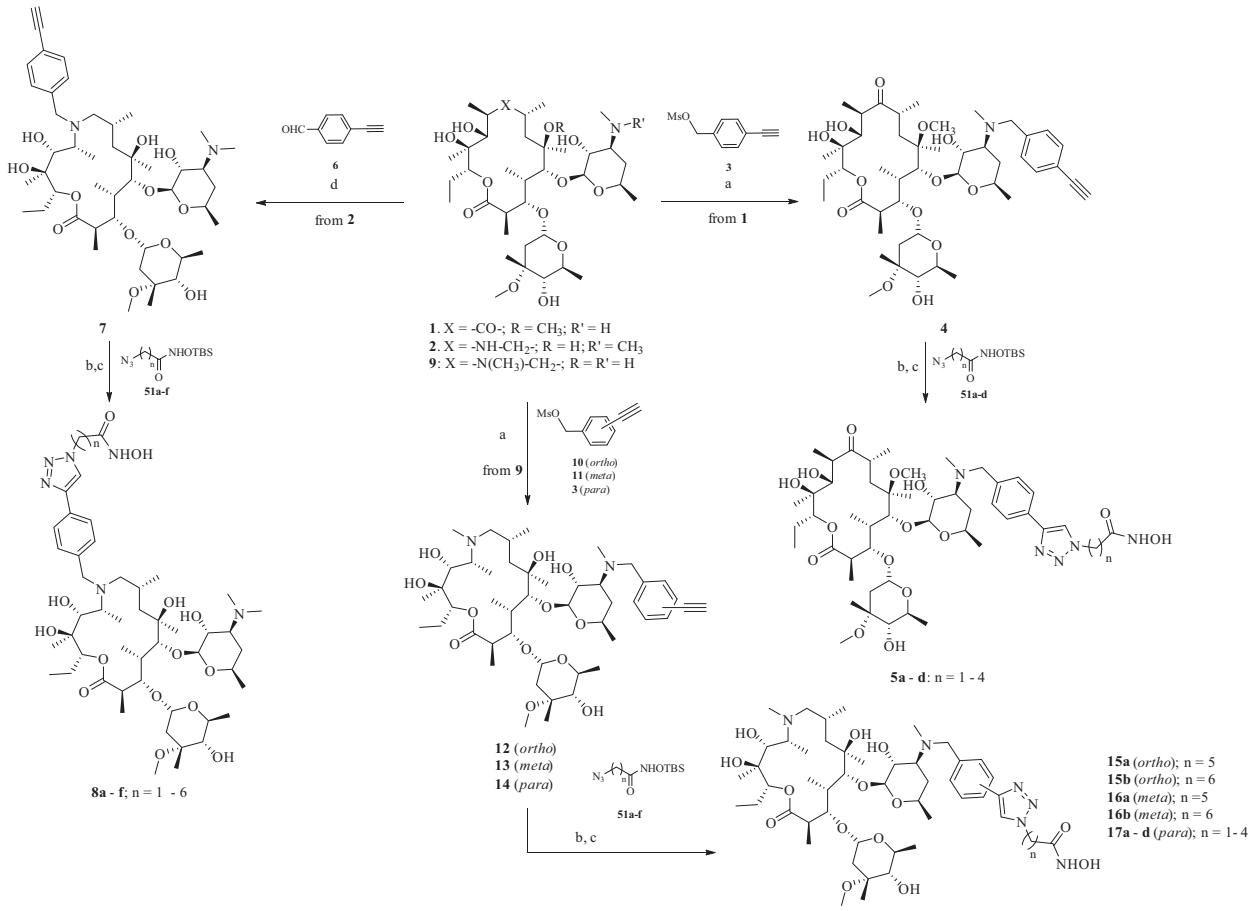
The triketolide derived hydroxamic acid compounds were synthesized in two steps starting from previously reported intermediate 3′-desmethyltricyclic ketolide **18**.[15,20a] Reductive amination reactions between **18** and 2-ethynylbenzaldehyde **19**, 3-ethynylbenzaldehyde **20**, 4-ethynylbenzaldehyde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

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

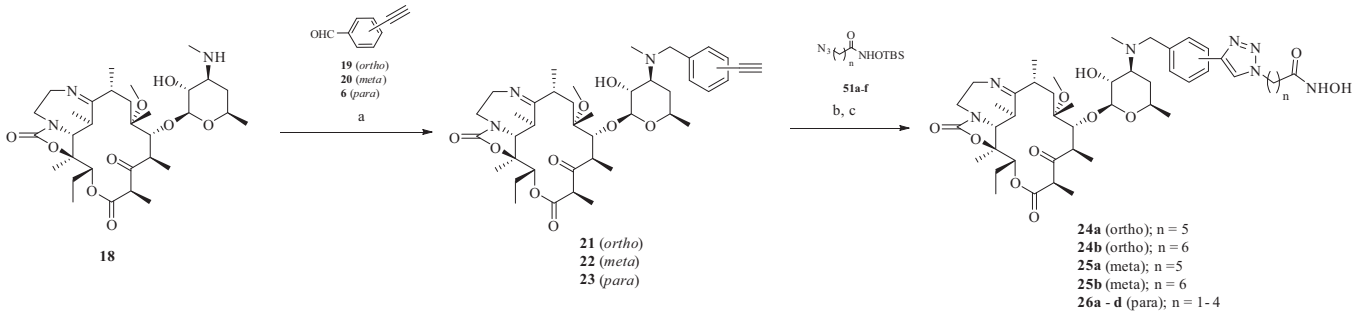
We also synthesized macrocyclic-nonpeptide-hydroxamic acids with dimethylamino methyl group placed in the *C*4″ position of cladinose sugar (Scheme 4), a substitution which may impact extra acid-stability to the cladinose sugar glycosidic bond.[20c] Also, we were interested in probing if this small change 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** (IC_{50} 713 nM,



Scheme 1. Reagents and conditions: (a) Hünig's base, DMSO, 85 °C, 3 h; (b) CuI (15 mol %), Hünig's base, THF–DMSO (1:1), 40 °C, 12 h; (c) CsF, MeOH, rt, 30 min; (d) $NaBH_3CN$, AcOH, DMF, 70 °C, 7 h.

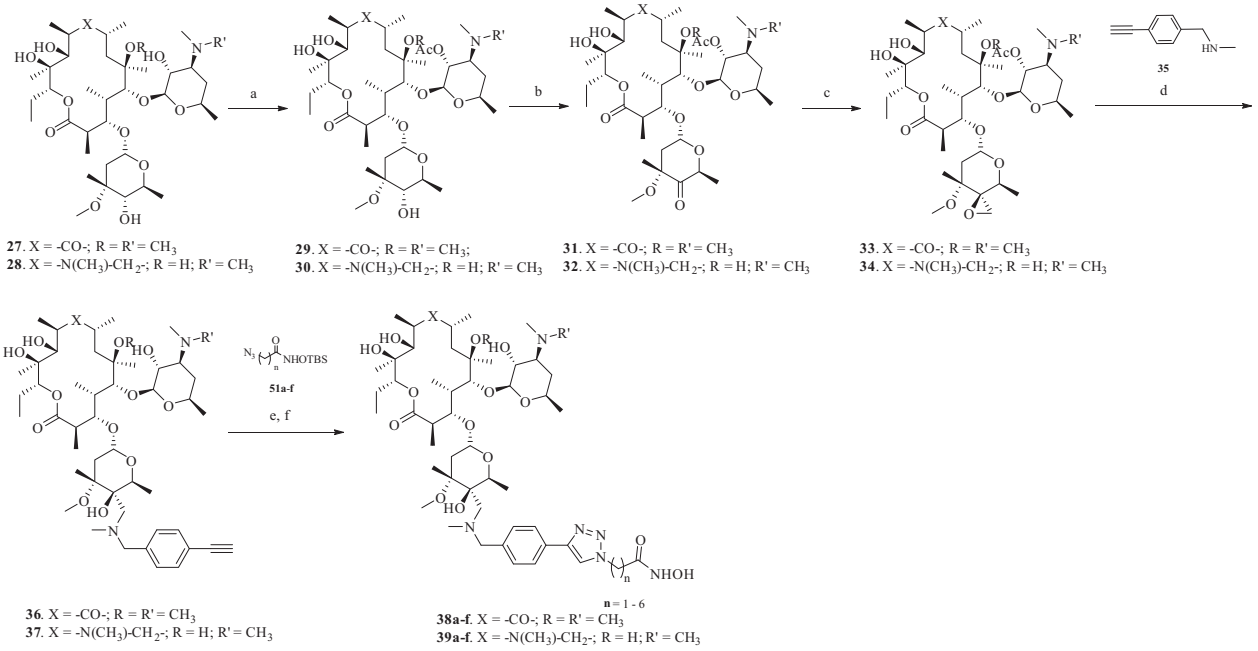


Scheme 2. Reagents and conditions: (a) borane–pyridine complex, AcOH, MeOH, rt, 9 h. (b) CuI (15 mol %), Hünig's base, THF–DMSO (1:1), 40 °C, 12 h; (c) CsF, MeOH, rt, 30 min.

Table 1), **16a** and **17f** (IC_{50}s 604 nM and 314 nM, respectively, Table 2), and **25b** (IC_{50} 310 nM, Table 4).

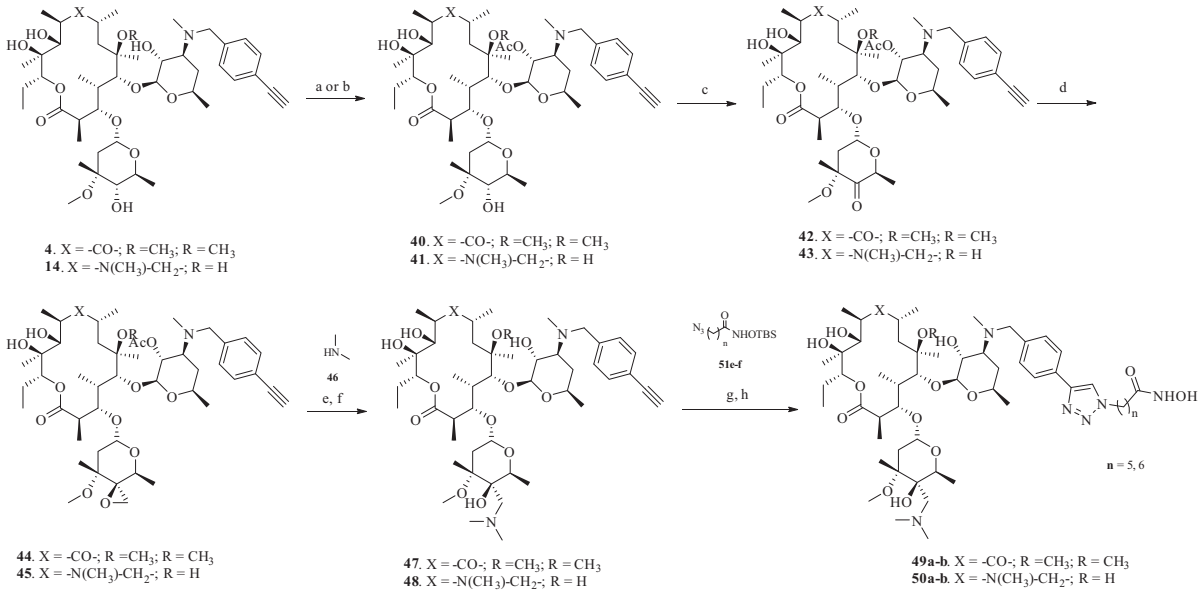
Analysis of the HDACs 1 and 6 inhibitory effects of these compounds revealed an interesting trend that is largely dependent on the macrolide template, the substitution pattern on the aryl cap group and the length of the methylene spacer-group separating the triazolyl group from the hydroxamate moiety. 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}



27. X = -CO-; R = R' = CH_3
28. X = -N(CH_3)-CH_2-; R = H; R' = CH_3
29. X = -CO-; R = R' = CH_3;
30. X = -N(CH_3)-CH_2-; R = H; R' = CH_3
31. X = -CO-; R = R' = CH_3
32. X = -N(CH_3)-CH_2-; R = H; R' = CH_3
33. X = -CO-; R = R' = CH_3
34. X = -N(CH_3)-CH_2-; R = H; R' = CH_3
36. X = -CO-; R = R' = CH_3
37. X = -N(CH_3)-CH_2-; R = H; R' = CH_3
38a-f. X = -CO-; R = R' = CH_3
39a-f. X = -N(CH_3)-CH_2-; R = H; R' = CH_3

Scheme 3. Reagents and conditions: (a) CH_2Cl_2, Ac_2O, rt, 3 h; (b) NCS, DMS, TEA, CH_2Cl_2, −15 °C, 4.5 h; (c) $(CH_3)_3SO^+I^-$, 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.



4. X = -CO-; R =CH_3; R = CH_3
14. X = -N(CH_3)-CH_2-; R = H
40. X = -CO-; R =CH_3; R = CH_3
41. X = -N(CH_3)-CH_2-; R = H
42. X = -CO-; R =CH_3; R = CH_3
43. X = -N(CH_3)-CH_2-; R = H
44. X = -CO-; R =CH_3; R = CH_3
45. X = -N(CH_3)-CH_2-; R = H
47. X = -CO-; R =CH_3; R = CH_3
48. X = -N(CH_3)-CH_2-; R = H
49a-b. X = -CO-; R =CH_3; R = CH_3
50a-b. X = -N(CH_3)-CH_2-; R = H

Scheme 4. Reagents and conditions: (a) for **40**: acetic anhydride, CH_2Cl_2, rt, 3 h; (b) for **41**: acetic anhydride, CH_2Cl_2, 40 °C, 48 h; (c) NCS, DMS, TEA, CH_2Cl_2, −15 °C, 6 h; (d) $(CH_3)_3SO^+I^-$, NaH, DMSO, THF, rt, 4 h; (e) KI, MeOH, 60 °C, 6 h; (f) MeOH, 90 °C, 3 days; (g) CuI (15 mol %), Hünig's base, THF, rt, 12 h; (h) CsF, MeOH, rt, 2 h.

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 (IC_{50} 361 nM) inhibitory potency compared to the analogous desosamine modified compound **5c** (IC_{50} 70.9 nM) and was completely inactive against HDAC1 (27% at 10 μM for **38c** vs 9.91 μM IC_{50} 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** (IC_{50} 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** (IC_{50} 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 1
HDAC1, HDAC6, and HDAC8 inhibition activities (IC_{50} in nM) of clarithromycin derived hydroxamic acid compounds

Compound	*n*	HDAC1	HDAC6	HDAC8
5a	1	>10 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**, azithromycin- and triketolide-derived analogs having *ortho*- and *meta*-substitution patterns at the aryltriazolyl cap group. For the azithromycin series, the *ortho*-substituted compounds **15a**–**b**, and *meta*-substituted compound **16b** have attenuated anti-HDAC activities relative to the corresponding para-substituted compounds **17e**–**f**. The *meta*-substituted, five methylene-linked compound **16a** is slightly more potent than the corresponding 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 *meta*-substituted compounds **24a**–**b** and **25a**–**b** are mostly less active against the corresponding para-substituted compounds **26e**–**f** with the *ortho*-substituted compounds **24a**–**b** devoid of HDAC1 inhibition activity. An exception within this series is compound **25b** which is approximately 2- and 5-fold more potent than the analogous **26e** against HDAC6 and HDAC8 respectively (Table 4).

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 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 (IC_{50} ≈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 2
HDAC1, HDAC6, and HDAC8 inhibition activities (IC_{50} in nM) of azithromycin derived hydroxamic acid compounds

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

Table 3
HDAC1, HDAC6, and HDAC8 Inhibition Activities (IC_{50} in nM) of 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} values in μM)[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-κB inhibition) of selected HDAC inhibitors

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

* I_{max} (%) at 1 μM.

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 HDACi[25] 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 anti-inflammatory activities in BEAS-2B cell infected with nontypeable Haemophilus influenza (NTHi) using NF-κB luciferase assay[31] NTHi is a Gram-negative bacterium which causes infection in the human respiratory tract.[32,33] Upon infection by NTHi, transcriptional regulator, NF-κB, in human epithelial cell is strongly activated by translocating from cytoplasm to nucleus and consequently up-regulating certain pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. To pre-screen these compounds for their effect on NF-κB activity, we treated them with NTHi infected BEAS-2B cells at 1 μM. We observed that compounds lacking or those with weak anti-HDAC activities did not suppress NF-κB activation while those compounds with potent anti-HDAC activities suppressed NF-κB 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-κB 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-κB activation with IC_{50} ranging from low to high nanomolar. The only exception is **25a**, which despite suppressing NF-κB activation at 1 μM, showed no dose-dependent effect. Based on IC_{50}, 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 μM, these compounds suppressed NF-κB 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-1α, IL-1β mRNA expression relative to SAHA in BEAS-2B cells (Fig. 2). Collectively, these data further suggest that the suppression of NF-κB activation induced by these compounds is derived from their HDAC inhibition activities.

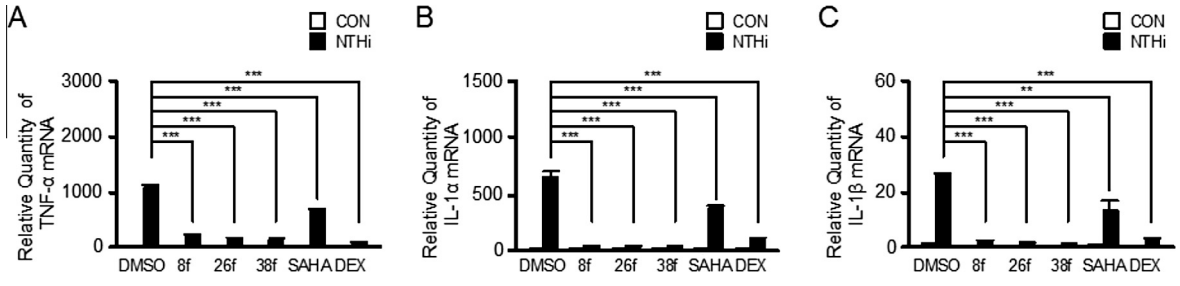


Figure 2. HDACi suppress NTHi-induced expression of cytokines. BEAS-2B cells were pretreated with HDAC inhibitor (**8f, 26f, 38f**; 1 μM) or SAHA (1 μM) or DEX (0.1 μM) for 2 h followed by 1.5 h stimulation with NTHi, and cytokines mRNA (TNF-α, IL-1α and IL-1β) expressions were analyzed. Data are mean ± STD (n = 3); $^{**}p$ <0.01, $^{***}p$ <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 F_{254}) 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. ^{1}H NMR Spectra were recorded in parts per million (ppm) relative to the residual peaks of $CHCl_3$ (7.24 ppm) in $CDCl_3$ or CHD_2OD (4.78 ppm) in CD_3OD or DMSO-d_5 (2.49 ppm) in DMSO-d_6. ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm) or CD_3OD 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 $NaHCO_3$ solution (5 mL), water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, 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. ^{1}H NMR ($CDCl_3$, 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%). ^{1}H NMR ($CDCl_3$, 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%). ^{1}H NMR ($CDCl_3$, 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%). ^{1}H NMR ($CDCl_3$, 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%). ^{1}H NMR ($CDCl_3$, 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%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{13}H_{20}O_2N_4Si$ [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 CuI (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 NH_4Cl/NH_4OH 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 Na_2SO_4 and concentrated in vacuo. Crude obtained was purified by preparative chromatography (Silica gel, 12:1:0.1 DCM–MeOH–NH_4OH) to give target compound **5a** as light yellow solid (0.068 g, 40%). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}CNMR (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 $C_{48}H_{78}N_5O_{15}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.25 (s, 1H), 7.78 (d, J = 8.1 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 5.13 (dd, J = 11.1, 2.1 Hz, 1H), 4.74 (t, J = 6.5 Hz, 2H), 4.51 (d, J = 7.2 Hz, 1H), 4.11 (dd, J = 9.4, 6.3 Hz, 1H), 3.93 (d, J = 13.2 Hz, 1H), 3.79–3.69 (m, 5H), 3.68–3.62 (m, 1H), 3.38–3.32 (m, 2H), 3.30 (dt, J = 3.3, 1.6 Hz, 3H), 3.18 (s, 3H), 3.11 (d, J = 7.8 Hz, 1H), 3.03 (d, J = 7.3 Hz, 4H), 3.01 (d, J = 9.5 Hz, 2H), 2.94–2.84 (m, 2H), 2.77 (t, J = 6.3 Hz, 2H), 2.64–2.53 (m, 1H), 2.40 (s, 1H), 2.34 (s, 4H), 1.96–1.78 (m, 5H), 1.67 (d, J = 13.0 Hz, 1H), 1.60–1.47 (m, 2H), 1.39 (d, J = 14.0 Hz, 4H), 1.25 (q, J = 7.8 Hz, 5H), 1.21 (d, J = 6.0 Hz, 7H), 1.18–1.08 (m, 17H), 0.85 (t, J = 7.4 Hz, 3H). ^{13}C 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 $C_{49}H_{79}N_5O_{15}Na$ [M+Na$^+$]: 1000.5454, found 1000.5479.

5.1.9. (Clarithromycin-3′-(*N*-(4-triazolylbenzyl)))-*N*-hydroxybutanamide (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%). ^{1}H NMR (500 MHz, $CDCl_3$) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{50}H_{83}N_5O_{15}$ [M+H$^+$]: 992.5807, found 992.5839.

5.1.10. (Clarithromycin-3′-(*N*-(4-triazolylbenzyl)))-*N*-hydroxypentanamide (5d)

Reaction of 3′-*N*(desmethyl)-3′-*N*-(4-ethynylbenzyl)clarithromycin **4** (0.15 g, 0.18 mmol) with 5-azido-*N*-((*tert*-butyldimethylsilyl)oxy)pentanamide **51d** (0.072 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%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.86 (s, 1H), 7.75 (s, 2H), 7.35 (s, 2H), 5.03 (dd, J = 11.1, 1.6 Hz, 1H), 4.87 (d, J = 3.7 Hz, 1H), 4.41 (t, J = 9.4 Hz, 1H), 4.35 (s, 2H), 3.99–3.89 (m, 2H), 3.83 (d, J = 10.5 Hz, 1H), 3.78–3.68 (m, 2H), 3.62 (d, J = 6.9 Hz, 1H), 3.54–3.41 (m, 2H), 3.38–3.29 (m, 1H), 3.17 (d, J = 20.3 Hz, 1H), 3.12 (s, 3H), 3.04–2.91 (m, 5H), 2.91–2.81 (m, 1H), 2.64 (s, 1H), 2.56 (dd, J = 10.3, 7.4 Hz, 1H), 2.24 (d, J = 40.5 Hz, 6H), 1.88 (dt, J = 26.6, 13.4 Hz, 4H), 1.85–1.73 (m, 2H), 1.71–1.56 (m, 3H), 1.56–1.41 (m, 2H), 1.42–1.33 (m, 3H), 1.33–1.27 (m, 2H), 1.30–1.18 (m, 8H), 1.16 (d, J = 7.0 Hz, 3H), 1.13–1.00 (m, 15H), 0.82 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 175.8, 170.3, 147.3, 129.6, 125.7, 120.1, 102.6, 95.9, 80.9, 78.3, 74.2, 72.5, 70.7, 69.0, 68.4, 65.6, 63.8, 57.6, 50.5, 49.8, 49.3, 45.9, 45.1, 45.0, 39.2, 39.0, 37.2, 36.8, 34.7, 31.7, 29.8, 29.6, 29.3, 22.1, 21.4, 21.2, 20.9, 19.8, 18.6, 17.9, 15.9, 12.2, 10.5, 9.1, 8.5. HRMS (ESI) m/z Calcd for $C_{51}H_{83}N_5O_{15}Na$ [M+Na$^+$]: 1028.5783, found 1028.5795.

5.1.11. (N^{10}-(4-Ethynylbenzyl))azithromycin (7)

N^{10}-Desmethylazithromycin **2** (2.72 g, 3.55 mmol) and 4-ethynylbenzaldehyde **6**[39] (2.31 g, 17.75 mmol) were dissolved in anhydrous DMF (40 mL). Acetic acid (2.0 mL, 35.50 mmol) was added and the solution was stirred for 30 min and then sodium cyanoborohydride (465 mg, 7.10 mmol) was added to the reaction mixture. The mixture was then stirred at 70 °C for 7 h after which it was cooled and the pH of the solution was raised to 8 by adding saturated aqueous $NaHCO_3$ solution. The crude mixture was diluted into CH_2Cl_2 and was washed water, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by column chromatography (Silica gel, 5:1:1 EtoAc/hexane/triethylamine) to afford the title compound **7** as white solid (603 mg, 20%). ^{1}H 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 C_{46} H_{78} O_{12} N_2 [M+2H$^+$]: 425.2772, found 425.2762.

5.1.12. (Azithromycin(-N^{10}-(4-triazolylbenzyl)))-*N*-hydroxyacetamide (8a)

In an oven dried round bottomed flask charged with a magnetic stirring bar, (N^{10}-(4-ethynylbenzyl))azithromycin **7** (120 mg,

0.141 mmol) and 2-azido-*N*-((*tert*-butyldimethylsilyl)oxy)acetamide **51a** (73 mg, 0.254 mmol) were placed under argon. To the mixture were added 2 mL of degassed1:1 mixture of THF/DMSO, CuI (4 mg, 0.02 mmol) and *N*,*N*′-diisopropylethylamine (20 μL, 0.09 mmol) in succession. The mixture was heated at 45 °C for 12 h. The reaction was cooled to room temperature and diluted with 50 mL of ethyl acetate. Organic layer was washed with 4:1 mixture of satd. NH_4Cl soln.–NH_4OH soln. (2 × 10 mL), water (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was subjected to next reaction without further purification.

The crude was dissolved in methanol (2 mL) and caesium fluoride (43 mg, 0.282 mmol) was added and the resulting reaction was stirred at room temperature for 2 h. Reaction was quenched by adding water and was extracted with ethyl acetate (100 mL) and the organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 CH_2Cl_2–MeOH–concd NH_4OH soln.) to give **8a** as a white solid (35 mg, 25%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.25 (s, 1H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 4.90 (m, 2H), 4.55 (d, *J* = 6.1 HZ, 1H), 4.04 (m, 4H), 3.69 (m, 3H), 3.51 (m, 3H), 3.37 (m, 1H), 3.08 (td, *J* = 16.2, and 8.9 Hz, 2H), 2.97 (m, 4H); 2.72 (m, 1H), 2.85 (m, 3H), 2.50 (s, 6H), 2.34 (t, *J* = 12.6 Hz, 1H), 2.06 (m, 2H), 1.78 (m, 5H), 1.64 (m, 5H), 1.47 (m, 3H), 1.18 (m, 17H), 0.81 (t, *J* = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 178.6, 148.8, 131.7, 126.8, 123.8, 103.5, 97.6, 80.8, 79.2, 77.0, 76.5, 74.6, 73.8, 72.3, 71.6, 71.4, 68.5, 67.0, 66.3, 62.4, 50.1, 47.8, 46.5, 40.2, 38.1, 36.3, 35.4, 32.9, 31.4, 29.8, 21.8, 20.4, 19.1, 14.8, 14.6, 14.4, 13.5, 11.7, 10.6, 9.6. HRMS (ESI) *m*+2/2*z* Calcd for $C_{48}H_{82}N_6O_{14}$ [M+2H$^+$]: 483.2939, found 483.2933.

5.1.13. (Azithromycin-(*N*^{10}-(4-triazolylbenzyl)))-*N*-hydroxypropanamide (8b)

Reaction of (*N*^{10}-(4-ethynylbenzyl))azithromycin **7** (85 mg, 0.10 mmol) and 3-azido-*N*-((*tert*-butyldimethylsilyl)oxy)propanamide **51b** (34 mg, 0.14 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8b** as white solid (38 mg, 40%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.21 (s, 1H), 7.71 (d, *J* = 7.4 Hz, 2H), 7.44 (d, *J* = 7.5 Hz, 2H), 4.91 (m, 2H), 4.56 (d, *J* = 6.2 Hz, 1H), 4.12 (m, 1H), 3.99 (m, 2H), 3.74 (m, 3H), 3.51 (m, 2H), 3.18 (m, 5H), 3.07 (m, 4H), 2.96 (m, 2H); 2.82 (m, 1H), 2.62 (s, 6H), 2.36 (d, J = 15.1 Hz, 2H), 2.00 (dd, J = 22.1 and 17.7 Hz, 1H), 1.86 (m, 5H), 1.68 (m, 5H), 1.52 (m, 3H), 1.32 (ddd, J = 17.7, 10.9, and 4.0 Hz, 3H), 1.14 (m, 17H), 1.03 (m, 7H), 0.78 (t, *J* = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 178.6, 169.5, 148.5, 133.7, 132.5, 132.1, 130.0, 126.9, 123.0, 97.6, 80.9, 79.0, 76.5, 74.6, 73.8, 71.2, 69.2, 68.5, 67.1, 66.6, 47.8, 40.3, 39.9, 36.2, 35.4, 31.7, 31.2, 30.1, 30.6, 30.2, 29.5, 25.1, 24.2, 22.7, 21.8, 21.7, 19.2, 14.8, 14.5, 11.6, 11,5, 10.4, 9.4. HRMS (ESI) *m*/*z* Calcd for $C_{49}H_{83}N_6O_{14}$ [M+H$^+$]: 979.5962, found 979.5967.

5.1.14. (Azithromycin-(*N*^{10}-(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%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.25 (s, 1H), 7.70 (d, *J* = 7.7 Hz, 2H), 7.41 (d, *J* = 7.5 Hz, 2H), 4.88 (m, 2H), 4.55 (d, *J* = 6.1 Hz, 2H), 4.39 (s, 3H), 4.06 (m, 4H), 3.69 (m, 4H), 3.49 (m, 2H), 3.01 (m, 10H), 2.80 (m, 2H), 2.56 (s, 6H), 2.36 (dt, J = 47.8 and 19.1 Hz, 3H), 2.03 (m, 7H), 1.84 (m, 7H), 1.62 (m, 3H), 1.47 (m, 3H), 1.28 (m, 8H), 1.15 (m, 17H), 1.01 (m, 7H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 178.6, 148.8, 131.8, 126.8, 122.5, 103.3, 97.6, 80.8, 79.1, 76.5, 74.6, 71.4, 69.2, 68.6, 67.1, 66.5, 50.8, 50.1, 47.8, 46.5, 40.3, 36.2, 35.4, 31.4, 30.3, 27.4, 25.1, 24.1, 22.9, 22.7, 22.3, 21.8, 21.7, 19.1, 14.9, 14.5, 11.7, 11.5, 10.5, 9.5. HRMS (ESI) *m*/*z* Calcd for $C_{50}H_{85}N_6O_{14}$ [M+H$^+$]: 993.6118, found 993.6125.

5.1.15. (Azithromycin-(*N*^{10}-(4-triazolylbenzyl)))-*N*-hydroxypentanamide (8d)

Reaction of (*N*^{10}-(4-ethynylbenzyl))azithromycin **7** (131 mg, 0.15 mmol) and 5-azido-*N*-((*tert*-butyldimethylsilyl)oxy)pentanamide **51d** (44 mg, 0.28 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8d** as white solid (60 mg, 40%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.24 (s, 1H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.41 (d, *J* = 7.6 Hz, 2H), 4.92 (d, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 4.3 Hz, 1H), 4.55 (d, *J* = 6.3 Hz, 1H), 4.37 (t, *J* = 6.6 Hz, 2H), 4.08 (ddd, *J* = 23.2, 20.9, 10.7 Hz, 2H), 3.70 (dd, *J* = 29.4, 21.5 Hz, 3H), 3.54 (dd, *J* = 20.2, 11.4 Hz, 2H), 3.27 (s, 4H), 3.10 (dt, *J* = 14.4, 7.0 Hz, 1H), 2.96 (dd, *J* = 17.4, 9.9 Hz, 5H), 2.87 (d, *J* = 11.2 Hz, 1H), 2.79 (m, 1H), 2.49 (s, 7H), 2.36 (d, *J* = 15.1 Hz, 1H), 2.18 (s, 1H), 2.05 (m, 4H), 1.88 (d, *J* = 6.5 Hz, 3H), 1.79 (m, 4H), 1.67 (s, 2H), 1.57 (m, 4H), 1.48 (m, 2H), 1.31 (m, 6H), 1.20 (d, *J* = 5.8 Hz, 6H), 1.16 (m, 10H), 1.09 (m, 6H), 0.80 (m, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.6, 172.4, 169.5, 148.6, 133.8, 132.6, 131.6, 130.0, 126.3, 122.4, 103.6, 97.6, 80.8, 79.2, 76.8, 76.5, 74.6, 73.8, 71.8, 69.3, 68.9, 67.1, 66.2, 51.2, 50.1, 49.8, 49.78, 49.7, 49.6, 49.5, 49.4, 49.3, 49.2, 49.0, 48.8, 48.6, 47.8, 46.5, 40.3, 36.3, 35.4, 33.1, 31.8, 31.5, 30.9, 30.8, 30.3, 29.9, 25.1, 24.2, 23.8, 23.1, 22.7, 22.4, 21.8, 21.8, 20.4, 19.1, 16.1, 14.9, 14.6, 11.8, 11.6, 10.6, 9.8, 9.3. HRMS (ESI) *m*/*z* Calcd for $C_{51}H_{87}N_6O_{14}$ [M+H$^+$]: 1007.6275, found 1007.6278.

5.1.16. (Azithromycin-(*N*^{10}-(4-triazolylbenzyl)))-*N*-hydroxyhexanamide (8e)

Reaction of (*N*^{10}-(4-ethynylbenzyl))azithromycin **7** (155 mg, 0.18 mmol) and 6-azido-*N*-((*tert*-butyldimethylsilyl)oxy)hexanamide **51e** (94 mg, 0.33 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **8a**, gave **8e** as white solid (70 mg, 38%). ^{1}H NMR (500 MHz, MeOD) δ (ppm) 8.23 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 4.92 (dd, *J* = 10.2, 2.3 Hz, 1H), 4.88 (d, *J* = 4.7 Hz, 1H), 4.52 (d, *J* = 7.1 Hz, 1H), 4.37 (t, *J* = 7.0 Hz, 2H), 4.10 (m, 2H), 3.65 (q, *J* = 13.5 Hz, 3H), 3.56 (m, 2H), 3.49 (m, 1H), 3.29 (s, 3H), 3.22 (m, 6H), 2.98 (m, 2H), 2.91 (m, 1H), 2.80 (dd, *J* = 19.0, 12.8 Hz, 3H), 2.33 (m, 9H), 2.15 (m, 1H), 2.02 (t, *J* = 7.3 Hz, 3H), 1.90 (m, 4H), 1.71 (dd, *J* = 40.3, 26.2 Hz, 3H), 1.60 (dt, *J* = 15.1, 7.5 Hz, 4H), 1.53 (dt, *J* = 11.8, 6.9 Hz, 3H), 1.30 (ddd, *J* = 15.6, 12.5, 10.2 Hz, 6H), 1.22 (m, 8H), 1.15 (dd, *J* = 16.9, 8.2 Hz, 12H), 1.07 (m, 12H), 0.96 (m, 2H), 0.82 (tt, *J* = 24.9, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.6, 172.7, 148.9, 131.3, 126.64, 122.2, 104.0, 97.6, 80.7, 79.3, 76.5, 74.5, 72.5, 69.2, 67.0, 65.7, 57.6, 51.4, 50.1, 49.8, 49.7, 49.6, 49.6, 49.4, 49.4, 49.4, 49.4, 49.3, 49.3, 49.2, 49.2, 49.2, 49.2, 49.2, 49.2, 49.1, 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-(*N*^{10}-(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%). ^{1}H NMR (500 MHz, MeOD) δ (ppm) 8.22 (s, 1H), 7.66 (t, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 4.92 (m, 1H), 4.87 (d, *J* = 4.7 Hz, 1H), 4.50 (d, *J* = 7.0 Hz, 1H), 4.35 (t, *J* = 7.1 Hz, 2H), 4.09 (m, 2H), 3.65 (t, *J* = 13.9 Hz, 3H), 3.55 (m, 1H), 3.28 (s, 3H), 3.21 (m, 5H), 2.97 (dd, *J* = 9.3, 5.0 Hz, 1H), 2.81

(m, 4H), 2.32 (m, 7H), 2.12 (m, 1H), 2.01 (m, 3H), 1.88 (dd, J = 13.9, 6.9 Hz, 3H), 1.72 (dd, J = 43.3, 24.2 Hz, 4H), 1.53 (m, 4H), 1.32 (m, 7H), 1.21 (t, J = 6.4 Hz, 5H), 1.15 (m, 10H), 1.08 (dd, J = 15.7, 7.4 Hz, 9H), 0.79 (m, 6H). ^{13}C NMR (126 MHz, MeOD) δ (ppm) 178.4, 172.8, 148.7, 131.2, 126.4, 122.0, 103.8, 97.4, 80.5, 79.1, 76.3, 74.3, 72.3, 69.1, 66.9, 65.5, 57.4, 51.3, 49.9, 49.6, 49.4, 49.4, 49.3, 49.2, 49.1, 48.9, 48.8, 48.6, 48.4, 46.4, 40.5, 36.1, 33.5, 31.7, 31.0, 29.3, 27.0, 26.4, 23.0, 22.2, 21.7, 21.6, 18.9, 17.2, 17.1, 11.6, 10.6. HRMS (ESI) m/z Calcd for $C_{53}H_{91}N_6O_{14}$ [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 Et_2O (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%). ^{1}H NMR ($CDCl_3$, 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 (15 mg, 0.065 mmol), and triphenylphosphine (28 mg, 0.108 mmol) in anhydrous triethylamine (5 mL) was heated to reflux under argon. After 15 min of reflux, a clear yellow solution resulted and a white precipitate began to form. The reaction was stopped after 4 h of refluxing, cooled, and filtered. The orange-brown filtrate was mixed with 50 ml of saturated $NaHCO_3$ and extracted with dichloromethane (3 × 50 mL). The organic fractions were combined, dried over anhydrous Na_2SO_4, 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 K_2CO_3 (100 mg) in anhydrous methanol (10 mL) under argon for 3 h at room temperature. Saturated $NaHCO_3$ (50 mL) was added to the reaction mixture and extracted with dichloromethane (3 × 50 mL). The organic fractions were combined, dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (Silica gel, CH_2Cl_2–Hexane 2:3) afforded the target compound **20** as a brown-white solid (430 mg, 62%). ^{1}H NMR ($CDCl_3$, 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 $NaHCO_3$ (50 mL), water (50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude was subjected to next reaction without further purification.

The crude was dissolved in anhydrous methanol (10 mL) and was treated with K_2CO_3 (100 mg) under argon at room temperature for 3 h. Saturated $NaHCO_3$ (50 mL) was added to the reaction mixture and extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were combined, dried over Na_2SO_4, 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). ^{1}H NMR ($CDCl_3$, 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 $NaHCO_3$ solution (10 mL) and the organic phase was separated. The aqueous phase was extracted twice with dichloromethane (100 mL). The combined organic layer was washed with water (20 mL), brine (20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude mesylated product **10** was used in the next step without further purification.

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 $NaHCO_3$ (3 × 50 mL) and saturated brine (50 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (Silica gel, 12:1:0.1 to 4:1:0.1 CH_2Cl_2–Acetone–Et_3N) to give **12** as a brown-white solid (600 mg, 44%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 178.8, 141.3, 133.2, 129.6, 128.9, 127.1, 122.2, 102.9, 94.5, 83.8, 82.1, 81.4, 78.2, 77.9, 76.8, 74.2, 73.8, 73.6, 72.8, 70.5, 70.1, 68.6, 65.5, 63.4, 56.5, 49.2, 45.2, 42.2, 42.1, 36.3, 36.1, 34.7, 29.5, 27.5, 26.7, 21.9, 21.5, 21.4, 21.2, 18.2, 16.2, 14.7, 11.2, 9.0, 7.4. HRMS (MALDI) m/z Calcd for $C_{46}H_{77}N_2O_{12}$ [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%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 178.5, 141.5, 131.6, 130.2, 129.9, 127.3, 122.5, 102.8, 94.7, 83.8, 83.5, 78.1, 76.8, 74.3, 74.0, 73.5, 72.8, 70.8, 70.7, 70.0, 68.5, 65.5, 62.2, 57.4, 49.3, 45.1, 42.3, 41.7, 36.8, 36.4, 34.7, 30.0, 27.4, 26.7, 22.0, 21.6, 21.3, 21.2, 18.2, 16.2, 14.9, 11.2, 9.2, 7.5. HRMS (MALDI) m/z Calcd for $C_{46}H_{77}N_2O_{12}$ [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 NH_4-OH/saturated NH_4Cl (40 mL) and extracted with 20% MeOH/CH_2Cl_2 (3 × 20 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude was subjected to the next reaction without further purification.

The crude was dissolved in anhydrous methanol (2 mL) and treated with caesium fluoride (20 mg, 0.12 mmol) at room temperature for 2 h. Water was added to the reaction and the mixture was extracted with ethyl acetate (2 × 50 mL). The organic fraction was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by preparative chromatography (Silica gel, 12:1:0.1 CH_2Cl_2/MeOH/concd NH_4OH) to give **15a** as a brown-white solid (40 mg, 67%). ^1H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{52}H_{89}N_6O_{14}$ [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 ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 178.4, 171.4, 146.4, 136.2, 131.1, 130.1, 128.6, 127.8, 122.6, 102.6, 94.6, 83.7, 78.5, 78.4, 78.1, 76.7, 73.9, 73.8, 73.3, 72.7, 69.3, 68.2, 66.2, 62.7, 61.0, 56.0, 51.3, 50.1, 49.2, 45.3, 42.8, 35.7, 29.7, 28.6, 28.0, 27.0, 26.7, 26.4, 25.8, 25.3, 25.0, 21.8, 21.4, 21.3, 18.1, 17.7, 16.8, 14.4, 11.5, 8.9, 6.5. HRMS (MALDI) m/z Calcd for $C_{53}H_{91}N_6O_{14}$ [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 ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{52}H_{89}N_6O_{14}$ [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)heptanamide **51f** (0.035 g, 0.118 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **15a**, afforded **16b** as a brown-white solid (28 mg, 46%). ^1H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{53}H_{91}N_6O_{14}$ [M+H$^+$]: 1035.6588, found 1035.6714.

5.1.28. (Azithromycin-3′-(*N*-(4-triazolylbenzyl)))-*N*-hydroxyacetamide (17a)

(3′-*N*-(4-Ethynylbenzyl))azithromycin **14** (0.10 g, 0.12 mmol) and 2-Azido-*N*-((*tert*-butyldimethyl silyl)oxy)ethanamide **51a** (0.05 g, 0.22 mmol) were dissolved in degassed anhydrous THF (5 mL) . CuI (0.01 g, 0.06 mmol) and Hünig's base (0.04 mL, 0.24 mmol) were added to the reaction and the resulting reaction was stirred at room temperature for another 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 NH_4Cl solution /NH_4OH solution, water (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, 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 Na_2SO_4, filtered and concentrated in vacuo. . The crude was purified by preparative chromatography (Silica gel, 5:1:1 EtOAc/MeOH/NH_4OH) to give the title compound **17a** as light yellow solid (0.045 g, 40% yield). ^1H NMR (400 MHz, CD_3OD) δ (ppm) 8.47 (d, J = 2.4 Hz, 1H), 7.96 (d, J = 7.0 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 5.13 (d, J = 4.6 Hz, 2H), 4.66 (d, J = 7.2 Hz, 1H), 4.31–4.20 (m, 2H), 4.15 (d, J = 12.9 Hz, 1H), 3.86 (d, J = 12.7 Hz, 2H), 3.76–3.70 (m, 2H), 3.52 (d, J = 6.9 Hz, 2H), 3.43 (dd, J = 3.2, 1.6 Hz, 3H), 3.29 (s, 3H), 3.20–3.12 (m, 2H), 3.07 (s, 1H), 3.01–2.97 (m, 1H), 2.88 (s, 3H), 2.55 (s, 3H), 2.30 (d, J = 14.2 Hz, 2H), 2.09–2.01 (m, 28H), 1.69 (dd, J = 15.2, 5.1 Hz, 2H), 1.56 (s, 3H), 1.44 (s, 3H), 1.32 (s, 3H), 1.17 (s, 3H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) 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 $C_{48}H_{82}N_6O_{14}$ [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, CD_3OD) δ (ppm) 8.39 (s, 1H), 7.93 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.3 Hz, 2H), 5.14 (d, J = 4.5 Hz, 1H), 4.88 (s, 3H), 4.67 (d, J = 7.3 Hz, 1H), 4.23 (d, 2H), 4.15 (d, J = 13.2 Hz, 1H), 3.86 (d, J = 13.3 Hz, 2H), 3.77–3.71 (m, 2H), 3.62–3.52 (m, 2H), 3.46–3.41 (m, 3H), 3.30 (s, 3H), 3.16 (d, J = 9.5 Hz, 2H), 2.91 (s, 3H), 2.55 (s, 3H), 2.32 (s, 1H), 2.15–2.01 (m, 24H), 1.70 (d, J = 10.6 Hz, 2H), 1.57 (s, 3H),

1.48 (d, J = 6.9 Hz, 2H), 1.43 (s, 3H), 1.38–1.33 (m, 2H), 1.29 (s, 3H), 1.20 (s, 3H), 1.16 (s, 3H), 1.03 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 178.3, 175.0, 147.1, 138.1, 129.6, 125.4, 102.5, 95.4, 83.0, 78.4, 77.8, 76.5, 73.9, 73.2, 72.3, 70.7, 70.0, 69.8, 65.4, 64.7, 60.8, 57.1, 5.54, 51.2, 48.2, 48.0, 47.9, 47.2, 46.1, 45.7, 45.3, 45.2, 41.9, 41.1, 36.1, 35.6, 31.7, 29.4, 29.1, 25.5, 22.7, 22.4, 20.6, 16.1, 13.2, 12.9, 10.1, 8.5, 6.9. HRMS (ESI) m/z Calcd for $C_{49}H_{83}N_6O_{14}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.21 (s, 1H), 7.66 (d, J = 7.5 Hz, 2H), 7.33 (d, J = 6.2 Hz, 2H), 4.35 (d, J = 7.1 Hz, 2H), 3.97–3.88 (m, 1H), 3.66–3.55 (m, 1H), 3.42 (s, 3H), 3.26 (d, J = 10.4, 7.0 Hz, 3H), 3.13–3.11 (m, 2H), 2.96 (s, 3H), 2.92–2.78 (m, 1H), 2.66 (dd, J = 12.4, 7.4 Hz, 3H), 2.59 (s, 3H), 2.29 (s, 3H), 2.18 (d, J = 15.0 Hz, 2H), 2.00 (dd, J = 20.2, 12.5 Hz, 2H), 1.81 (d, J = 7.6 Hz, 2H), 1.75 (s, 24H), 1.39 (dd, J = 15.2, 9.7 Hz, 2H), 1.26 (s, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 3.8 Hz, 3H), 1.06–1.00 (m, 1H), 0.96 (s, 3H), 0.88 (d, J = 7.4 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.71 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.2, 172.2, 149.5, 131.7, 131.6, 127.5, 123.1, 104.7, 97.6, 85.2, 80.6, 80.1, 78.8, 76.7, 76.1, 75.6, 75.1, 73.1, 70.1, 65.4, 59.4, 51.5, 50.8, 50.5, 50.2, 50.1, 49.5, 47.6, 44.1, 38.2, 37.7, 36.7, 32.8, 31.6, 31.5, 31.3, 31.2 28.1, 27.8, 22.9, 22.9, 22.8, 22.4, 19.8, 18.2, 16.5, 12.3, 10.6, 9.0. HRMS (ESI) m/z Calcd for $C_{50}H_{85}N_6O_{14}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.28 (s, 1H), 7.73 (d, J = 7.7 Hz, 2H), 7.40 (d, J = 7.9 Hz, 2H), 4.47 (d, J = 7.2 Hz, 1H), 4.41 (s, 2H), 4.12 (d, J = 17.0 Hz, 2H), 3.80 (d, J = 13.0 Hz, 1H), 3.68 (s, 1H), 3.64–3.50 (m, 4H), 3.35–3.24 (m, 2H), 3.13 (s, 3H), 3.00–2.91 (m, 2H), 2.76 (dd, J = 7.5, 4.4 Hz, 2H), 2.65 (d, J = 11.8 Hz, 1H), 2.39 (s, 3H), 2.32 (d, J = 15.0 Hz, 2H), 2.24 (s, 3H), 2.15–2.09 (m, 3H), 1.98–1.86 (m, 22H), 1.31 (s, 3H), 1.23 (d, J = 5.9 Hz, 5H), 1.18–1.09 (m, 7H), 1.06 (s, 3H), 1.01 (d, J = 7.5 Hz, 4H), 0.91 (s, 2H), 0.84 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.2, 149.5, 141.7, 131.6, 131.4, 127.4, 122.9, 104.8, 97.3, 85.3, 80.5, 80.2, 78.9, 76.3, 76.2, 76.1, 75.1, 73.3, 70.0, 67.3, 65.3, 59.6, 51.8, 50.8, 50.3, 50.1, 50.0, 49.8, 49.5, 49.3, 47.5, 44.3, 43.9, 38.2, 37.6, 36.8, 32.8, 31.5, 28.5, 28.3, 24.4, 23.1, 22.9, 22.5, 19.8, 18.2, 16.5,12.4, 10.7, 8.6. HRMS (ESI) m/z Calcd for $C_{51}H_{87}N_6O_{14}$ [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 μL, 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 $NaHCO_3$ solution (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, concentrated in vacuo and purified by preparative chromatography (Silica gel, 12:1 CH_2Cl_2/MeOH) to afford compound **21** as a brown solid (210 mg, 58%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{41}H_{60}N_3O_9$ [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%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.15 (m, 2H), 7.06 (m, 2H), 4.72 (d, J = 8.0 Hz, 1H), 4.08 (d, J = 7.2 Hz, 1H), 3.99 (d, J = 8.4 Hz, 1H), 3.53 (m, 4H), 3.76 (m, 1H), 3.20 (m, 3H), 2.78 (m, 2H), 2.78 (m, 2H), 2.44 (m, 4H), 1.93 (s, 3H), 1.34 (m, 28H), 0.82 (d, J = 7.2 Hz, 3H), 0.62 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 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 $C_{41}H_{60}N_3O_9$ [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 NH_4OH soln./saturated aqueous NH_4Cl soln. (2 × 4 mL), water (10 mL), and brine (10 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was subjected to next reaction without further purification.

The crude was dissolved in 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 Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by preparative chromatography (Silica gel, 12:1:0.1 CH_2Cl_2/MeOH/concd NH_4OH) to give **24a** as a brown-white solid (30 mg, 55%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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*-hydroxyheptanamide (24b)

Reaction of (3′-*N*-(2-ethynylbenzyl))tricyclic Ketolide **21** (0.05 g, 0.067 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl)oxy) heptanamide **51f** (0.04 g, 0.134 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **24b** as a brown-white solid (35 mg, 56%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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*-hydroxyhexanamide (25a)

Reaction of (3′-*N*-(3-ethynylbenzyl))tricyclic Ketolide **22** (0.04 g, 0.05 mmol) and 6-azido-*N*-((*tert*-butyldimethylsilyl)oxy) hexanamide **51e** (0.03 g, 0.11 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **25a** as a brown-white solid (32 mg, 64%). ^{1}H NMR ($CDCl_3$, 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). ^{13}C NMR ($CDCl_3$, 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 $C_{47}H_{72}N_7O_{11}$ [M+H$^+$]: 910.5284, found 910.5278.

5.1.37. (Tricyclic Ketolide-3′-(*N*-(3-triazolylbenzyl)))-*N*-hydroxyheptanamide (25b)

Reaction of (3′-*N*-(3-ethynylbenzyl))tricyclic Ketolide **22** (0.05 g, 0.067 mmol) and 7-azido-*N*-((*tert*-butyldimethylsilyl)oxy) heptanamide **51f** (0.04 g, 0.134 mmol) followed by TBS deprotection with caesium fluoride as described for the synthesis of **24a**, gave **25b** as a brown-white solid (34 mg, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.74 (s, 1H), 7.71 (s, 1H),. 7.63 (d, J = 7.6 Hz, 1H), 7.30 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 4.89 (d, J = 8.4 Hz, 1H), 4.32 (m, 2H), 4.25 (d, J = 7.2 Hz, 1H), 4.17 (d, J = 8.4 Hz, 1H), 3.92 (m, 1H), 3.71 (m, 4H), 3.20 (m, 5H), 2.64 (m, 5H), 2.15 (s, 3H), 1.71 (m, 36H), 0.99 (d, J = 7.2 Hz, 3H), 0.79 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 204.3, 181.9, 170.5, 169.6, 156.1, 147.6, 139.6, 130.7, 129.0, 128.6, 126.0, 124.7, 119.9, 103.8, 81.6, 78.9, 78.5, 76.5, 70.4, 69.5, 65.4, 59.9, 57.6, 51.2, 50.1, 49.4, 49.1, 47.9, 42.3, 38.6, 36.9, 36.4, 29.8, 29.7, 29.5, 27.9, 25.6, 24.9, 22.1, 21.0, 19.6, 19.1, 16.2, 14.4, 14.2, 12.9, 10.9, 10.4. HRMS (MALDI) m/z Calcd for $C_{48}H_{74}N_7O_{11}$ [M+H$^+$]: 924.5441, found 924.5455.

5.1.38. (Tricyclic Ketolide-3′-(*N*-(4-triazolylbenzyl)))-*N*-hydroxyacetamide (26a)

In an oven dried round bottomed flask charged with a magnetic stirring bar, (3′-*N*-(4-ethynylbenzyl))tricyclic ketolide **23**[15] (0.165 g, 0.224 mmol) and 2-azido-*N*-((*tert*-butyldimethylsilyl) oxy)acetamide **51a** (0.09 g, 0.40 mmol) were placed under argon. To the mixture were added 2 mL of degassed1:1 mixture of THF and DMSO, CuI (6.4 mg, 0.03 mmol) and *N*,*N*′-diisopropylethylamine (30 μL, 0.15 mmol) in succession. The mixture was heated at 45 °C for 12 h. The reaction mixture was cooled to room temperature and diluted with 50 mL of ethyl acetate. Organic layer was washed with 4:1 mixture of satd. NH_4Cl soln. and NH_4OH soln. (2 × 10 mL), water (10 mL), and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was used for the next reaction without further purification.

The crude was dissolved in methanol (3 mL) and to that solution caesium fluoride (68 mg, 0.442 mmol) was added and the resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by adding water and extracted with ethyl acetate (100 mL) and the organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by preparative chromatography (Silica gel, 10:1:0.1 CH_2Cl_2/MeOH/concd NH_4OH soln.) to give **26a** as a white solid (76 mg, 40%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.24 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 5.00 (s, 2H), 4.18 (m, 2H), 3.81 (dd, J = 44.1, 13.9 Hz, 2H), 3.69 (m, 2H), 3.50 (m, 4H), 3.26 (m, 1H), 3.09 (p, J = 7.5 Hz, 1H), 2.85(m, 2H); 2.72 (m, 1H), 2.58 (m, 5H), 2.21 (d, J = 15.7 Hz, 3H), 1.72 (m, 2H), 1.60 (m, 2H), 1.52 (m, 1H), 1.44 (m, 3H), 1.19 (m, 17 H), 1.01 (t, J = 13.0 Hz, 3H), 0.77 (t, J = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 184.6, 170.0, 163.4, 156.6, 147.3, 139.0, 129.4, 125.3, 122.3, 103.6, 82.2, 78.5, 77.7, 76.2, 70.7, 69.0, 57.3, 50.7, 49.8, 48.6, 48.3, 42.1, 41.9, 38.1, 36.4, 36.0, 30.9, 21.8, 20.1, 18.9, 17.8, 15.1, 13.5, 11.7, 9.8, 9.6. HRMS (ESI) m/z Calcd for $C_{43}H_{64}N_7O_{11}$ [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%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.13 (s, 1H), 7.64 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 4.65 (t, J = 6.2 Hz, 2H), 4.19 (dd, J = 16.2, and 7.7 Hz, 2H), 3.87 (m, 1H), 3.72 (m, 3H), 3.57 (m, 1H), 3.53 (m, 1H), 3.48 (m, 2H), 3.27 (m, 1H), 3.09 (m, 1H), 2.88 (dd, J = 14.3, and 10.1 Hz, 2H), 2.69 (t, J = 10.8 Hz, 3H), 2.60 (m, 4H), 2.18 (d, J = 14.1 Hz, 3H), 1.74 (m, 2H), 1.62 (t, J = 7.6 Hz, 2H), 1.54 (m, 1H), 1.47 (m, 3H), 1.21 (m, 17H), 1.03 (t, J = 12.4 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 196.6, 176.1, 161.6, 159.1, 148.2, 138.7, 130.9, 120.8, 116.8, 112.8, 95.2, 73.7, 70.0, 69.2, 67.8, 62.3, 60.6, 55.6, 51.7, 48.9, 42.3, 40.2, 39.8, 37.5, 33.7, 33.5, 29.6, 27.9, 27.5, 22.5, 13.4, 11.6, 10.5, 9.4. HRMS (ESI) m/z Calcd for $C_{44}H_{66}N_7O_{11}$ [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%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm) 8.23 (s, 1H), 7.69 (d, J = 7.9 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 4.41 (t, J = 6.7 Hz, 2H), 4.19 (dd, J = 15.6, and 7.7 Hz, 2H), 3.88 (m, 2H), 3.73 (q, J = 13.3 Hz, 3H), 3.59 (m, 1H), 3.50 (dd, J = 17.4 and 9.4 Hz, 2H), 3.28 (dd, J = 10.0 and 7.6 Hz, 1H), 3.09 (m, 1H), 2.89 (dd, J = 14.6 and 10.4 Hz, 2H), 2.69 (dd, J = 15.5, and 6.7 Hz, 1H), 2.60 (m, 4H), 2.17 (m, 5H), 2.08 (m, 2H), 1.75 (m, 2H), 1.62 (m, 1H), 1.55 (m, 1H), 1.48 (s, 3H), 1.22 (m, 17H), 1.02 (d, J = 6.9 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 206.6, 186.1, 171.6, 158.2, 149.0, 141.0, 130.8, 126.8, 122.4, 105.2, 83.8, 80.1, 79.2, 77.9, 72.3, 70.7, 65.6, 61.7, 59.0, 52.4,

51.9, 50.8, 50.2, 49.9, 48.0, 39.7, 37.9, 37.5, 32.6, 30.5, 27.4, 26.2, 23.4, 21.6, 20.5, 19.4, 16.6, 15.1, 13.2, 11.3, 11.1, 9.5. HRMS (ESI) *m*/*z* Calcd for $C_{45}H_{68}N_7O_{11}$ [M+H$^+$]: 882.4971, found 882.4971.

5.1.41. Tricyclic Ketolide-3′-(*N*-(4-triazolylbenzyl)))-*N*-hydroxypentanamide (26d)

Reaction of (3′-*N*-(4-ethynylbenzyl))tricyclic ketolide **23** (0.114 g, 0.150 mmol) and 5-azido-*N*-((*tert*-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%). ^{1}H NMR (500 MHz, MeOH-d_4) δ (ppm)8.23 (s, 1H), 7.70 (d, *J* = 7.9 Hz, 2H), 7.37 (d, *J* = 7.9 Hz, 2H), 4.38 (d, *J* = 6.8 Hz, 2H), 4.20 (dd, *J* = 17.7, and 7.7 Hz, 2H), 3.94 (q, *J* = 6.7 Hz, 1H), 3.88 (d, *J* = 14.6 Hz, 1H), 3.81 (d, *J* = 13.3 Hz, 1H), 3.71 (m, 2H), 3.58 (dd, *J* = 20.0, and 11.2 Hz, 2H), 3.50 (m, 1H), 3.30 (dd, *J* = 9.9, and 7.6 Hz, 1H), 3.11 (m, 1H), 2.89 (dd, J = 14.4, and 10.4 Hz, 2H), 2.74 (m. 1H), 2.60 (m, 3H), 2.23 (s, 3H), 2.07 (t, *J* = 7.1 Hz, 2H), 1.88 (m, 2H), 1.77 (m, 2H), 1.57 (m, 5H), 1.48 (s, 3H), 1.24 (m, 17H), 1.02 (d, *J* = 6.9 Hz, 3H), 0.79 (t, *J* = 7.4 Hz, 3H). ^{13}C (125 MHz, MeOH-d_4) δ (ppm) 206.6, 186.2, 172.4, 171.6, 158.2, 148.8, 131.0, 126.9, 122.4, 105.2, 83.8, 80.0, 79.2, 77.9, 72.2, 70.6, 65.6, 61.7, 58.8, 52.4, 51.2, 50.2, 49.9, 39.7, 37.9, 37.6, 33.1, 32.5, 30.8, 23.8, 23.4, 21.6, 20.5, 19.4, 16.6, 15.1, 13.3, 11.4, 11.1. HRMS (ESI) *m*/*z* Calcd for $C_{46}H_{70}N_7O_{11}$ [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 quenched by adding saturated aqueous $NaHCO_3$ solution (50 mL), the organic layer was separated. The aqueous layer was extracted with DCM (2 × 25 mL) and the combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude compound **31** (6.35 g) was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (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). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 221.0, 210.6, 175.6, 170.4, 99.6, 96.4, 80.0, 78.7, 78.1, 78.0, 76.7, 76.0, 74.2, 72.9, 72.7, 69.8, 69.2, 67.5, 61.7, 51.2, 50.2, 49.9, 45.1, 44.4, 39.0, 38.4, 38.1, 37.9, 37.6, 37.2, 30.8, 30.4, 29.5, 28.3, 26.5, 21.3, 21.3, 20.9, 20.5, 20.4, 20.0, 19.5, 18.9, 17.8, 16.2, 16.1, 15.5, 14.7, 13.0, 12.2, 10.4, 8.9. HRMS (MALDI) *m*/*z* Calcd for $C_{40}H_{70}NO_{14}$ [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 trimethyloxosulfonium iodide (3.90 g, 17.7 mmol) was added over a period of 5 min. When hydrogen evolution ceased and a clear solution obtained, a solution of compound **31** (6.35 g, 8.06 mmol) in anhydrous THF (10 mL) was added over a period of 10 min and left to stir for 2 h. Once TLC showed 100% conversion, THF was removed under vacuum and ethyl acetate was added to the remaining solution. The solution was washed severally with H_2O to remove DMSO. Organic layer was dried with $NaSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography using EtOAc/Acetone (5:1) to give compound **33** as a white solid (2.43 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) 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, *J* = 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). ^{13}C NMR (101 MHz, $CDCL_3$) δ (ppm) 221.1, 175.3, 170.0, 100.1, 96.4, 96.3, 80.3, 78.9, 78.3, 78.2, 76.6, 76.1, 74.2, 73.7, 71.7, 69.1, 67.8, 64.3, 63.2, 60.4, 60.2, 50.4, 49.6, 46.6, 45.3, 44.8, 40.7, 38.6, 38.1, 37.2, 36.0, 30.8, 29.2, 21.6, 21.3, 21.0, 20.2, 19.7, 18.2, 18.0, 16.1, 15.9, 14.9, 14.2, 14.2, 12.4, 10.5, 9.1. HRMS (MALDI) *m*/*z* Calcd for $C_{41}H_{77}NO_{14}$ [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/NH_4-OH (2:1:0.6:0.1) to give compound **36** as a light yellow solid (2.41 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.33 (d, *J* = 8.1 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 4.98–4.86 (m, 2H), 4.35–4.28 (m, 1H), 4.06 (q, *J* = 5.9 Hz, 1H), 3.66 (d, *J* = 11.1 Hz, 2H), 3.57 (d, *J* = 7.0 Hz, 2H), 3.34 (s, 1H), 3.20 (s, 3H), 3.09 (dd, *J* = 10.0, 7.3 Hz, 2H), 3.02 (d, *J* = 10.1 Hz, 1H), 2.97 (d, *J* = 6.6 Hz, 1H), 2.94 (s, 2H), 2.91–2.85 (m, 2H), 2.81–2.72 (m, 1H), 2.47 (d, *J* = 7.4 Hz, 1H), 2.34 (dd, *J* = 15.6, 6.7 Hz, 1H), 2.24 (s, 1H), 2.20 (s, 6H), 2.10 (s, 3H), 2.04 (d, *J* = 3.6 Hz, 1H), 2.00 (s, 1H), 1.96 (s, 1H), 1.93–1.87 (m, 1H), 1.84–1.70 (m, 3H), 1.57 (dd, *J* = 19.0, 11.8 Hz, 2H), 1.41–1.31 (m, 1H), 1.30 (d, *J* = 11.1 Hz, 3H), 1.15–1.07 (m, 10H), 1.05 (s, 4H), 0.97 (dd, *J* = 17.3, 7.0 Hz, 10H), 0.71 (dd, *J* = 13.8, 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 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 [$C_{49}H_{81}N_2O_{13}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{51}H_{86}N_6O_{15}$ [M+H$^+$]: 1021.6073, found 1021.6095.

5.1.46. (Clarithromycin-4″(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxypropanamide (38b)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin **36** (0.15 g,0.17 mmol) with 3-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)propanamide **51b** (0.061 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38b** as a light yellow solid (0.07 g, 47%). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 175.7, 146.9, 137.8, 129.2, 125.5, 121.0, 102.2, 96.2, 80.9, 80.9, 78.7, 78.4, 78.1, 77.9, 76.4, 75.9, 75.4, 74.1, 71.2, 70.9, 70.5, 68.8, 67.8, 67.3, 65.1, 62.6, 56.3, 50.8, 50.5, 50.5, 49.4, 46.0, 45.0, 44.7, 44.6, 43.1, 40.2, 39.1, 38.8, 37.0, 31.2, 29.9, 29.5, 25.4, 21.3, 20.8, 20.1, 19.8, 19.5, 18.9, 18.6, 18.1, 17.8, 15.8, 15.2, 14.7, 14.5, 12.9, 12.1, 11.3, 10.4, 10.3, 10.3, 9.3, 9.2. HRMS (MALDI) m/z Calcd for $C_{52}H_{87}N_6O_{15}$ [M+H$^+$]: 1035.6270, found 1035.6210.

5.1.47. (Clarithromycin-4″(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxybutanamide (38c)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin **36** (0.15 g, 0.17 mmol) with 4-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)butanamide **51c** (0.064 g, 0.245 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38c** as a light yellow solid (0.060 g, 40%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.32 (s, 1H), 7.77 (d, J = 7.7 Hz, 2H), 7.36 (d, J = 7.9 Hz, 2H), 5.09 (d, J = 9.2 Hz, 1H), 4.46 (s, 2H), 4.39 (d, J = 7.2 Hz, 1H), 4.19 (d, J = 6.4 Hz, 1H), 3.82–3.61 (m, 5H), 3.48 (s, 1H), 3.29–3.21 (m, 4H), 3.05 (t, J = 4.8 Hz, 2H), 3.00 (d, J = 9.9 Hz, 3H), 2.92–2.82 (m, 1H), 2.67 (s, 1H), 2.54 (s, 1H), 2.41 (s, 5H), 2.30 (s, 1H), 2.26–2.18 (m, 6H), 2.11 (t, J = 7.4 Hz, 3H), 1.95–1.79 (m, 4H), 1.75 (d, J = 13.0 Hz, 1H), 1.62 (d, J = 14.6 Hz, 1H), 1.52–1.40 (m, 1H), 1.36 (s, 3H), 1.18 (dt, J = 11.1, 7.0 Hz, 15H), 1.13–1.02 (m, 11H), 0.81 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 176.1, 171.4, 147.5, 138.4, 129.8, 129.5, 125.9, 120.8, 102.7, 102.4, 96.9, 96.6, 81.2, 81.2, 80.3, 79.1, 78.8, 78.5, 78.2, 76.3, 76.2, 75.7, 74.4, 71.5, 71.1, 70.8, 70.1, 69.2, 68.3, 68.1, 67.9, 67.7, 65.6, 63.1, 60.6, 56.9, 53.6, 51.2, 50.8, 49.7, 49.5, 45.4, 45.1, 44.9, 43.4, 40.5, 39.4, 39.1, 37.4, 31.6, 29.8, 29.5, 26.2, 21.7, 21.2, 21.1, 20.4, 20.1, 19.9, 19.3, 19.0, 18.5, 18.2, 16.1, 15.6, 15.0, 14.9, 14.3, 13.2, 12.5, 11.7, 10.8, 9.6, 9.5. HRMS (MALDI) m/z Calcd for $C_{53}H_{89}N_6O_{15}$ [M+H$^+$]1049.6386, found 1049.6376.

5.1.48. (Clarithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxypentanamide (38d)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin **36** (0.15 g, 0.17 mmol) with 5-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)pentanamide **51d** (0.068 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38d** as a light yellow solid (0.067 g, 44%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.30 (s, 1H), 7.76 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 5.12–5.03 (m, 1H), 4.47–4.36 (m, 3H), 4.19 (d, J = 6.4 Hz, 1H), 3.81–3.59 (m, 5H), 3.48 (s, 1H), 3.29–3.18 (m, 4H), 3.04 (dd, J = 10.3, 4.5 Hz, 2H), 3.00 (d, J = 9.6 Hz, 4H), 2.92–2.82 (m, 1H), 2.66 (s, 1H), 2.55 (d, J = 7.3 Hz, 1H), 2.40 (s, 6H), 2.30 (s, 1H), 2.23 (s, 4H), 2.16 (d, J = 9.6 Hz, 1H), 2.15–2.06 (m, 4H), 1.86 (ddd, J = 20.9, 14.7, 7.6 Hz, 6H), 1.75 (d, J = 13.2 Hz, 1H), 1.62 (d, J = 12.2 Hz, 3H), 1.50–1.41 (m, 1H), 1.35 (s, 3H), 1.26–1.12 (m, 16H), 1.09 (td, J = 12.8, 7.9 Hz, 11H), 0.81 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 176.5, 147.8, 138.7, 130.3, 129.9, 126.3, 121.0, 103.1, 97.0, 81.6, 79.5, 79.2, 78.9, 78.7, 76.7, 74.9, 71.5, 71.2, 69.6, 68.7, 68.1, 66.0, 63.6, 57.3, 51.6, 51.2, 50.4, 50.1, 45.8, 45.5, 43.8, 40.9, 39.9, 39.5, 37.8, 32.0, 30.2, 29.9, 22.7, 22.2, 21.5, 20.8, 20.3, 19.4, 18.9, 18.6, 16.5, 15.9, 15.4, 15.3, 13.6, 12.9, 12.1, 11.2, 11.1, 10.0, 9.9. HRMS (MALDI) m/z Calcd for $C_{54}H_{91}N_6O_{15}$ [M+H$^+$]: 1063.6542, found 1063.6588.

5.1.49. (Clarithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxyhexanamide (38e)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin **36** (0.135 g, 0.149 mmol) with 6-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)hexanamide **51e** (0.077 g, 0.268 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38e** as a light yellow solid (0.053 g, 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.86 (s, 1H), 7.77 (d, J = 7.4 Hz, 2H), 7.31 (d, J = 7.8 Hz, 2H), 5.06–4.95 (m, 2H), 4.43–4.30 (m, 2H), 4.19–4.11 (m, 1H), 3.81–3.71 (m, 2H), 3.65 (dd, J = 13.3, 6.4 Hz, 2H), 3.48–3.39 (m, 1H), 3.29 (s, 3H), 3.21 (dd, J = 12.4, 6.3 Hz, 3H), 3.06 (d, J = 6.5 Hz, 1H), 3.05–2.93 (m, 4H), 2.92–2.82 (m, 1H), 2.58–2.45 (m, 2H), 2.31 (dd, J = 28.6, 7.4 Hz, 6H), 2.20 (d, J = 14.3 Hz, 3H), 2.11 (dd, J = 35.3, 9.4 Hz, 5H), 2.00–1.83 (m, 5H), 1.75–1.57 (m, 6H), 1.57–1.49 (m, 2H), 1.41–1.27 (m, 7H), 1.28–1.11 (m, 16H), 1.13–1.01 (m, 11H), 0.90–0.85 (m, 1H), 0.85–0.74 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 176.0, 147.4, 138.4, 129.8, 129.4, 125.8, 120.1, 102.7, 96.6, 96.5, 81.1, 81.0, 78.4, 78.2, 77.3, 77.1, 76.8, 76.6, 76.2, 76.1, 76.0, 74.3, 71.1, 70.7, 69.1, 68.3, 67.6, 65.4, 63.1, 57.0, 51.2, 51.0, 50.7, 50.1, 49.6, 45.3, 45.0, 43.2, 40.3, 39.4, 39.0, 37.2, 31.5, 29.8, 29.7, 29.3, 28.5, 26.2, 25.6, 24.9, 24.5, 21.7, 21.0, 19.7, 18.9, 18.1, 16.0, 16.0, 15.4, 14.9, 14.8, 13.1, 12.3, 11.5, 10.6, 9.3. HRMS (MALDI) m/z Calcd for $C_{55}H_{93}N_6O_{15}$ [M+H$^+$]: 1077.6699, found 1077.6692.

5.1.50. (Clarithromycin-4″-(methylamino)-*N*(methyl)-4-benzyltriazolyl))-*N*-hydroxyheptanamide (38f)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) clarithromycin **36** (0.15 g, 0.17 mmol) with 7-Azido-*N*-((*tert*-butyldimethylsilyl)oxy)heptanamide **51f** (0.075 g, 0.249 mmol) followed by TBS removal with caesium fluoride as described for the synthesis of compound **5a**, gave **38f** as a light yellow solid (0.060 g, 40%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.34 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 5.16–5.08 (m, 1H), 4.96 (d, J = 5.2 Hz, 1H), 4.45 (t, J = 6.5 Hz, 3H), 4.22 (d, J = 6.4 Hz, 1H), 3.85–3.64 (m, 5H), 3.52 (s, 1H), 3.33–3.23 (m, 9H), 3.12–2.98 (m, 5H), 2.90 (dd, J = 19.0, 11.7 Hz, 1H), 2.77 (s, 1H), 2.58 (s, 1H), 2.49 (s, 6H), 2.35 (s, 1H), 2.31–2.23 (m, 4H), 2.21 (s, 1H), 2.18–2.12 (m, 3H), 2.12–2.04 (m, 4H), 1.99–1.85 (m, 6H), 1.83 (d, J = 13.2 Hz, 2H), 1.69–1.53 (m, 7H), 1.44–1.31 (m, 12H),

1.28–1.18 (m, 15H), 1.12 (dt, J = 11.4, 4.9 Hz, 12H), 0.90 (s, 1H), 0.85 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 176.2, 147.6, 138.4, 130.1, 129.7, 126.1, 120.3, 102.8, 96.8, 81.4, 78.6, 78.4, 74.6, 71.3, 71.0, 69.3, 68.4, 67.9, 65.7, 63.3, 57.1, 51.6, 51.0, 50.4, 49.9, 45.5, 45.2, 43.5, 40.7, 39.6, 39.2, 37.5, 31.8, 30.1, 30.0, 28.9, 28.8, 28.2, 26.6, 26.0, 25.6, 25.3, 21.9, 21.3, 20.6, 20.0, 19.5, 19.1, 18.3, 16.3, 15.7, 15.2, 15.0, 12.6, 10.9, 9.7. HRMS (MALDI) m/z Calcd for $C_{56}H_{95}N_6O_{15}$ [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 $NaHCO_3$ 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 Na_2SO_4, filtered and concentrated in vacuo to give the target compound as a white solid (2.50 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 5.25 (dd, J = 1.6, 0.9 Hz, 1H), 4.93 (s, 3H), 4.74–4.67 (m, 2H), 4.59 (d, J = 10.2 Hz, 2H), 4.51 (d, J = 7.5 Hz, 2H), 4.18–4.13 (m, 2H), 4.01–3.93 (m, 2H), 3.59 (s, 2H), 3.54 (d, J = 6.3 Hz, 2H), 3.30 (s, 3H), 3.23 (s, 2H), 2.96 (d, J = 9.0 Hz, 2H), 2.79–2.71 (m, 2H), 2.68–2.52 (m, 4H), 2.40 (d, J = 11.8 Hz, 2H), 2.28 (d, J = 13.8 Hz, 4H), 2.04–2.01 (m, 1H), 1.98 (d, J = 2.3 Hz, 3H), 1.94–1.85 (m, 3H), 1.64 (d, J = 13.7 Hz, 3H), 1.53 (dd, J = 15.3, 4.9 Hz, 2H), 1.28–1.23 (m, 7H), 1.20 (d, J = 6.4 Hz, 3H), 1.16 (d, J = 6.0 Hz, 3H), 1.11 (d, J = 8.3 Hz, 3H), 1.09 (s, 3H), 1.03 (d, J = 6.7 Hz, 3H), 0.99 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{40}H_{76}N_2O_{13}$ [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 $NaHCO_3$ solution and the organic layer was separated. The aqueous layer was extracted twice with DCM (2 × 50 mL). The combined organic layer was washed with water 50 mL), brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by column chromatography (Silica gel, 12:1:0.5 DCM/MeOH/NH_4OH) to yield the product (2.49 g 90%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ^{1}H NMR (400 MHz, $CDCl_3$), 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 (×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/NH_4OH) 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. ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 $C_{41}H_{74}N_2O_{13}$ [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 Na_2SO_4 and concentrated in vacuo. The residue obtained was purified by column chromatography to give compound **35** as a yellow liquid (0.15 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) 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). ^{13}C NMR (101 MHz, $CDCl_3$) 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 Na_2SO_4, filtered and concentrated in vacuo. The crude was purified by column chromatography column (Silica gel, 4:3:2:0.1 Hexane/EtOAc/MeOH/NH_4OH) to give compound **37** as light yellow solid (2.0 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.43 (d, J = 8.2 Hz, 2H), 7.21(d, 2H), 5.04 (d, J = 4.6 Hz, 1H), 4.62 (d, J = 8.6 Hz, 1H), 4.44 (d, J = 6.0 Hz, 1H), 4.24–4.15 (m, 3H), 3.67 (d, J = 6.4 Hz, 2H), 3.63 (s, 3H), 3.46 (s, 3H), 3.26 (d, J = 21.0 Hz, 6H), 3.05 (s, 1H), 2.95 (d, J = 14.7 Hz, 3H), 2.81 (d, J = 7.1 Hz, 3H), 2.66 (s, 3H), 2.44 (d, J = 10.1 Hz, 2H), 2.31 (d, J = 10.5 Hz, 9H), 2.18 (s, 3H), 2.15 (d, J = 0.5 Hz, 1H), 2.02

(t, J = 11.7 Hz, 3H), 1.92 (dd, J = 15.0, 5.0 Hz, 2H), 1.29 (s, 3H), 1.21 (t, J = 6.9 Hz, 10H), 1.14 (s, 3H), 1.12–1.02 (m, 10H), 0.88 (t, J = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 178.6, 139.4, 132.2, 128.8, 121.2, 102.8, 95.0, 94.8, 83.8, 83.3, 76.8, 74.2, 73.5, 70.9, 70.1, 68.1, 67.2, 67.2, 65.7, 63.2, 62.6, 62.5, 56.9, 49.6, 49.5, 45.4, 45.2, 43.2, 42.3, 42.2, 41.1, 40.9, 40.5, 40.4, 36.2, 31.3, 29.0, 27.5, 26.7, 22.7, 21.9, 21.3, 18.8, 16.2, 15.0, 14.6, 11.2, 9.2, 7.2. HRMS (ESI) *m + 2/2z* Calcd for $C_{49}H_{85}N_3O_{12}$ [M+2H$^+$]: 453.8061, found 453.8055.

5.1.56. (Azithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxyacetamide (39a)

(4″-(Methylamino)-*N*(methyl)(4-ethynylbenzyl))azithromycin **37** (0.14 g, 0.16 mmol) and 2-Azido-*N*-((tert-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 quenched by adding a solution of 4:1 saturated NH_4Cl/NH_4OH (30 mL) and extracted with 20% $MeOH/CH_2Cl_2$ (3 × 30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (Silica gel, 5:1:1 EtOAc/$MeOH/NH_4OH$) to give the product (0.131 g, 80% yield) as light yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.33 (s, 1H), 7.79 (d, J = 6.5 Hz, 2H), 7.37 (d, J = 7.1 Hz, 2H), 5.46 (s, 1H), 5.07 (d, J = 4.6 Hz, 3H), 4.44 (d, J = 6.9 Hz, 1H), 4.32–4.18 (m, 3H), 3.69 (s, 2H), 3.64–3.56 (m, 3H), 3.48 (d, J = 25.5 Hz, 2H), 3.34 (s, 3H), 3.32–3.21 (m, 3H), 3.09–2.93 (m, 3H), 2.85–2.64 (m, 3H), 2.41 (t, J = 20.9 Hz, 12H), 2.33–2.20 (m, 3H), 2.19–2.09 (m, 3H), 2.00 (dd, J = 25.3, 18.4 Hz, 3H), 1.95–1.83 (m, 3H), 1.82–1.70 (m, 3H), 1.52–1.39 (m, 3H), 1.32 (s, 3H), 1.24 (d, J = 6.3 Hz, 3H), 1.22–1.12 (m, 13H), 1.08 (s, 3H), 1.03 (d, J = 7.2 Hz, 3H), 0.96–0.82 (m, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 177.3, 149.4, 140.9, 131.9, 131.6, 127.7, 124.6, 105.0, 102.0, 97.7, 85.6, 80.3, 78.9, 78.3, 76.3, 76.0, 73.6, 73.2, 73.0, 71.0, 69.8, 69.5, 67.0, 65.5, 65.0, 59.0, 51.0, 47.6, 45.0, 44.5, 44.1, 41.4, 37.7, 33.9, 33.2, 32.6, 31.6, 31.4, 28.6, 28.2, 24.6, 23.2, 23.0, 20.2, 18.2, 16.5, 15.3, 12.3, 10.7, 8.8. HRMS (ESI) m + 2/2z 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-benzyltriazolyl))-*N*-hydroxypropanamide (39b)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.20 g, 0.218 mmol) and 3-azido-*N*-((*tert*-butyldimethylsilyl)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%). ^{1}H NMR (500 MHz, CD_3OD) δ (ppm) 8.26 (d, J = 10.7 Hz, 1H), 7.78 (d, J = 7.6 Hz, 2H), 7.39 (d, J = 7.8 Hz, 2H), 5.48 (s, 1H), 5.13–5.07 (m, 2H), 4.74 (s, 3H), 4.46 (d, J = 7.2 Hz, 2H), 4.32–4.22 (m, 1H), 3.69 (d, J = 18.3 Hz, 3H), 3.67–3.57 (m, 1H), 3.57–3.48 (m, 2H), 3.38 (d, J = 13.2 Hz, 3H), 3.33–3.25 (m, 1H), 3.02 (dd, J = 23.0, 16.2 Hz, 3H), 2.86–2.75 (m, 3H), 2.74–2.65 (m, 3H), 2.44 (s, 3H), 2.31–2.19 (m, 3H), 2.19–2.10 (m, 2H), 2.09–1.97 (m, 3H), 1.94–1.87 (m, 9H), 1.87–1.82 (m, 2H), 1.81–1.72 (m, 3H), 1.53–1.40 (m, 3H), 1.35 (d, J = 11.2 Hz, 3H), 1.27 (t, J = 11.9 Hz, 13H), 1.24–1.19 (m, 3H), 1.16 (d, J = 6.7 Hz, 3H), 1.10 (s, 3H), 1.05 (d, J = 7.5 Hz, 3H), 0.96–0.86 (s, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.4, 177.1, 169.8, 149.2, 140.8, 131.8, 131.3, 127.5, 123.5, 104.8, 97.3, 85.4, 80.3, 78.9, 78.3, 76.2, 76.0, 74.5, 73.4, 72.9, 70.9, 69.9, 69.2, 66.8, 65.6, 65.0, 63.0, 58.8, 57.0, 55.8, 50.9, 48.1, 47.5, 44.8, 43.9, 41.3, 37.7, 33.9, 32.7, 31.6, 28.3, 24.5, 23.3, 22.8, 21.2, 20.1, 18.2, 16.8, 15.2, 12.3, 10.8, 8.8. HRMS (ESI) *m/z* Calcd for $C_{52}H_{90}N_7O_{14}$ [M+H$^+$]: 1036.6540, found 1036.6550.

5.1.58. (Azithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxybutanamide (39c)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.20 g, 0.18 mmol) and 4-azido-*N*-((*tert*-butyldimethylsilyl)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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.30 (s, 1H), 7.76 (d, J = 7.2 Hz, 2H), 7.34 (d, J = 7.7 Hz, 2H), 5.44 (s, 1H), 5.06 (d, J = 4.7 Hz, 1H), 4.51–4.35 (m, 3H), 4.30–4.19 (m, 2H), 3.60 (dd, J = 23.4, 16.5 Hz, 3H), 3.45 (d, J = 25.7 Hz, 1H), 3.32 (s, 3H), 3.27–3.16 (m, 3H), 2.99 (d, J = 14.7 Hz, 1H), 2.84–2.69 (m, 2H), 2.63–2.43 (m, 2H), 2.30 (d, J = 14.5 Hz, 10H), 2.24–2.04 (m, 15H), 1.95 (dd, J = 13.7, 6.5 Hz, 3H), 1.91–1.77 (m,3H), 1.77–1.64 (m, 3H), 1.49–1.30 (m, 3H), 1.27 (s, 3H), 1.21 (d, J = 6.0 Hz, 6H), 1.16 (d, J = 6.1 Hz, 11H), 1.03 (dd, J = 19.7, 6.7 Hz, 7H), 0.85 (dd, J = 14.1, 7.0 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.7, 177.2, 149.4, 141.0, 131.9, 131.6, 127.7, 123.7, 104.9, 97.6, 85.5, 80.4, 78.8, 78.4, 76.4, 76.2, 74.5, 73.6, 73.1, 70.9, 69.9, 69.4, 66.9, 65.7, 64.9, 57.2, 55.6, 51.1, 48.2, 47.5, 44.9, 44.3, 43.9, 41.5, 37.7, 34.0, 33.6, 33.4, 32.8, 31.7, 31.4, 28.5, 28.2, 24.6, 23.2, 22.7, 20.3, 18.2, 16.7, 15.3, 12.5, 10.8, 8.7. HRMS (ESI) *m+2/2z* 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-benzyltriazolyl))-*N*-hydroxypentanamide (39d)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.14 g, 0.15 mmol) and 5-azido-*N*-((*tert*-butyldimethylsilyl)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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.72 (s, 1H), 7.19 (d, J = 7.9 Hz, 2H), 6.77 (d, J = 8.0 Hz, 2H), 4.47 (d, J = 5.0 Hz, 1H), 3.85 (d, J = 6.7 Hz, 3H), 3.63 (dd, J = 17.6, 4.5 Hz, 2H), 3.09 (s, 2H), 3.04–2.97 (m, 3H), 2.75 (s, 3H), 2.68 (dt, J = 3.3, 1.6 Hz, 2H), 2.43 (d, J = 14.3 Hz, 2H), 2.19 (dd, J = 7.4, 4.6 Hz, 2H), 2.12 (d, J = 11.6 Hz, 1H), 1.89 (d, J = 18.4 Hz, 15H), 1.64 (d, J = 7.1 Hz, 5H), 1.59–1.46 (m, 6H), 1.37 (dd, J = 16.4, 7.9 Hz, 7H), 1.15 (t, J = 7.3 Hz, 1H), 1.03 (s, 3H), 0.85 (td, J = 14.5, 7.5 Hz, 3H), 0.73 (s, 3H), 0.60 (ddd, J = 18.8, 13.2, 6.4 Hz, 15H), 0.49 (s, 3H), 0.43 (d, J = 7.3 Hz, 3H), 0.32 (d, J = 6.8 Hz, 3H), 0.27 (t, J = 7.4 Hz, 3H) .^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.2, 176.9, 172.9, 149.1, 140.6, 131.6, 127.3, 122.8, 104.7, 97.1, 85.3, 80.1, 78.6, 78.0, 76.1, 75.8, 74.3, 73.1, 71.1, 69.7, 66.6, 64.6, 64.5, 62.8, 58.7, 56.9, 55.5, 52.7, 51.6, 50.8, 47.3, 44.5, 44.1, 43.9, 41.4, 37.3, 33.6, 33.0, 32.7, 31.3, 30.0, 28.6, 26.7, 24.2, 23.1, 22.7, 20.0, 17.9, 16.4, 16.1, 15.0, 12.2, 10.6, 8.2. HRMS (ESI) *m/z* Calcd for $C_{54}H_{94}N_7O_{14}$ [M+H$^+$]: 1064.6853, found 1064.6854.

5.1.60. (Azithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxyhexanamide (39e)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.09 g, 0.10 mmol) and 6-azido-*N*-((*tert*-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%). ^{1}H NMR (400 MHz, CD_3OD) 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). ^{13}C NMR (126 MHz, CD_3-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 $C_{55}H_{97}N_7O_{14}$ [M+2H$^+$]: 539.8541, found 539.8539.

5.1.61. (Azithromycin-4″-(methylamino)-*N*(methyl)(4-benzyltriazolyl))-*N*-hydroxyheptanamide (39f)

Reaction of (4″-(methylamino)-*N*(methyl)(4-ethynylbenzyl)) azithromycin **37** (0.16 g, 0.18 mmol) and 7-azido-*N*-((*tert*-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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.71 (s, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 6.77 (d, *J* = 8.1 Hz, 2H), 4.49 (d, *J* = 5.0 Hz, 1H), 3.83 (dd, *J* = 10.2, 7.1 Hz, 3H), 3.72–3.63 (m, 2H), 3.50–3.40 (m, 1H), 3.11 (d, *J* = 16.2 Hz, 1H), 3.02 (d, *J* = 7.0 Hz, 1H), 2.99–2.95 (m, 1H), 2.90 (dd, *J* = 17.7, 14.2 Hz, 1H), 2.75 (s, 3H), 2.71–2.62 (m, 3H), 2.43 (d, *J* = 14.7 Hz, 1H), 2.24 (d, *J* = 7.0 Hz, 1H), 2.18 (dd, *J* = 7.5, 4.6 Hz, 1H), 2.00 (t, *J* = 14.8 Hz, 1H), 1.75 (d, *J* = 10.6 Hz, 9H), 1.70–1.58 (m, 6H), 1.55–1.44 (m, 3H), 1.44–1.34 (m, 3H), 1.33–1.30 (m, 3H), 1.31–1.25 (m, 3H), 1.19–1.10 (m, 2H), 1.04 (d, *J* = 7.4 Hz, 3H), 0.76 (dd, *J* = 20.3, 12.8 Hz, 3H), 0.70 (s, 3H), 0.68–0.61 (m, 6H), 0.60 (dt, *J* = 12.7, 4.4 Hz, 12H), 0.53–0.46 (m, 10H), 0.43 (d, *J* = 7.5 Hz, 5H), 0.34–0.24 (m, 3H). ^{13}C NMR (126 MHz, CD_3-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*+2/2*z* 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 $NaHCO_3$, brine and H_2O, the organic layer was dried with anhydrous Na_2SO_4 and concentrated in vacuo to give compound **40** (128 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.2 Hz, 2H), 7.19 (d, *J* = 8.1 Hz, 2H), 5.01 (dd, *J* = 11.1, 2.2 Hz, 1H), 4.87 (d, *J* = 4.8 Hz, 1H), 4.74 (dd, *J* = 10.6, 7.4 Hz, 1H), 4.48 (d, *J* = 7.4 Hz, 1H), 3.94 (s, 1H), 3.86 (dt, *J* = 12.1, 6.1 Hz, 1H), 3.66 (t, *J* = 11.1 Hz, 2H), 3.63 (s, 1H), 3.52 (d, *J* = 6.5 Hz, 1H), 3.46 (s, 1H), 3.40 (dd, *J* = 11.3, 6.7 Hz, 1H), 3.18 (s, 1H), 3.07 (s, 3H), 3.02 (d, *J* = 6.2 Hz, 1H), 2.99–2.91 (m, 5H), 2.80 (dt, *J* = 14.2, 7.2 Hz, 1H), 2.73 (d, *J* = 4.5 Hz, 1H), 2.59–2.47 (m, 2H), 2.29–2.24 (m, 1H), 2.20–2.15 (m, 9H), 2.06–2.01 (m, 4H), 1.93–1.76 (m, 3H), 1.71 (t, *J* = 12.8 Hz, 1H), 1.62 (d, *J* = 11.6 Hz, 1H), 1.57–1.47 (m, 3H), 1.42 (ddd, *J* = 13.2, 8.3, 3.1 Hz, 1H), 1.37 (t, *J* = 5.2 Hz, 1H), 1.36–1.28 (m, 4H), 1.25–1.17 (m, 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 $C_{48}H_{76}NO_{14}$ [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 $NaHCO_3$ (50 mL), water (50 mL), and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The solid crude product **41** (1.1 g, 95%) was sufficiently pure to be used for the next reaction without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.38 (d, *J* = 8.1 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 5.06–5.00 (m, 1H), 4.94 (dd, *J* = 10.6, 2.3 Hz, 1H), 4.86 (dd, *J* = 10.5, 7.6 Hz, 1H), 4.79 (d, *J* = 4.4 Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.13 (t, *J* = 5.6 Hz, 1H), 3.97 (dq, *J* = 12.5, 6.2 Hz, 1H), 3.64 (d, *J* = 13.8 Hz, 1H), 3.57–3.44 (m, 2H), 3.40 (d, *J* = 4.3 Hz, 1H), 3.29 (dd, *J* = 12.3, 4.8 Hz, 1H), 3.20 (s, 3H), 3.02–2.94 (m, 2H), 2.74 (td, *J* = 12.2, 4.2 Hz, 1H), 2.67–2.58 (m, 1H), 2.53 (q, *J* = 7.2 Hz, 2H), 2.28 (d, *J* = 14.9 Hz, 2H), 2.19 (s, 3H), 2.14 (d, *J* = 6.8 Hz, 3H), 2.10 (d, *J* = 7.4 Hz, 3H), 2.04 (d, *J* = 5.4 Hz, 3H), 1.99–1.89 (m, 1H), 1.82–1.70 (m, 3H), 1.68–1.34 (m, 3H), 1.28–1.17 (m, 13H), 1.13 (d, *J* = 7.1 Hz, 6H), 1.04–0.91 (m, 10H), 0.89–0.80 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 176.5, 175.5, 171.0, 170.0, 140.8, 132.0, 128.4, 128.1, 128.0, 120.7, 101.4, 96.5, 84.3, 83.7, 80.6, 79.8, 77.9, 76.4, 75.3, 74.1, 72.9, 71.2, 69.0, 65.6, 61.9, 61.1, 58.2, 49.4, 48.9, 45.5, 42.9, 41.4, 36.9, 35.1, 34.4, 31.0, 29.6, 27.1, 25.2, 23.6, 21.9, 21.6, 20.6, 17.9, 14.1, 11.7, 11.1, 8.3. HRMS (ESI) *m+2/2z* 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 °C for 10 min then dimethyl sulfide (0.020 mL, 0.26 mmol) was added drop wise to form a white turbid solution. After stirring for 20 min, a DCM (5 mL) solution of compound **40** (0.128 g, 0.144 mmol) was added over a period of 30 min and the resulting suspension was stirred at −15*** °C for 30 min. Subsequently, TEA (0.030 mL, 0.23 mmol) was added and the suspension cleared up within a min. Stirring continued at −10 °C for 2 h and the reaction was quenched with saturated $NaHCO_3$ (10 mL). The organic layer extracted with DCM (50 mL), dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Residue was purified by preparative chromatography (Silica gel, Hexane/EtOAc/MeOH (3:2:0.05) to give compound **31** as a yellow solid (0.055 g, 45%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.32 (s, 1H), 7.77 (d, *J* = 7.7 Hz, 2H), 7.36 (d, *J* = 7.9 Hz, 2H), 5.09 (d, *J* = 9.2 Hz, 1H), 4.96–4.91 (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). ^{13}C 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 $C_{48}H_{74}NO_{14}$ [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 $NaHCO_3$ 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 Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by column chromatography (Silica gel, 12:1:0.5 DCM/MeOH/NH_4OH) to give the title compound **43** (1.79 g, 93% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.37 (d, J = 8.1 Hz, 2H), 7.17 (d, J = 7.3 Hz, 2H), 5.18–5.04 (m, 1H), 5.05–4.73 (m, 2H), 4.65 (d, J = 9.7 Hz, 1H), 4.48–4.34 (m, 1H), 4.20 (d, J = 4.3 Hz, 1H), 4.16–4.06 (m, 1H), 3.95 (dd, J = 15.2, 8.5 Hz, 1H), 3.72–3.57 (m, 2H), 3.56–3.37 (m, 3H), 3.31 (dd, J = 16.4, 9.8 Hz, 1H), 3.23 (t, J = 5.2 Hz, 1H), 3.19 (s, 1H), 3.07 (d, J = 6.2 Hz, 1H), 3.05–2.91 (m, 2H), 2.86–2.55 (m, 2H), 2.49 (d, J = 10.1 Hz, 1H), 2.27 (dd, J = 11.5, 6.2 Hz, 3H), 2.20 (d, J = 5.6 Hz, 3H), 2.17–2.13 (m, 2H), 2.11 (d, J = 5.3 Hz, 2H), 2.08–1.95 (m, 5H), 1.83–1.70 (m, 2H), 1.66–1.46 (m, 2H), 1.44–1.34 (m, 3H), 1.34–1.16 (m, 14H), 1.13 (dd, J = 6.8, 4.2 Hz, 4H), 1.10–1.01 (m, 4H), 1.00–0.91 (m, 3H), 0.90–0.73 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) 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%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.40 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.1 Hz, 2H), 4.88 (d, J = 3.5 Hz, 1H), 4.84–4.73 (m, 2H), 4.56 (d, J = 7.5 Hz, 1H), 3.98–3.91 (m, 1H), 3.69 (s, 1H), 3.67–3.61 (m, 2H), 3.49 (d, J = 14.0 Hz, 2H), 3.34 (s, 1H), 3.06 (s, 3H), 3.04 (s, 2H), 2.90 (d, J = 4.1 Hz, 1H), 2.81–2.67 (m, 3H), 2.64 (d, J = 4.1 Hz, 1H), 2.49 (dt, J = 21.9, 7.4 Hz, 1H), 2.38 (dt, J = 9.9, 4.9 Hz, 1H), 2.35–2.25 (m, 2H), 2.21 (s, 3H), 2.18 (s, 1H), 2.15 (s, 1H), 2.08 (s, 3H), 1.83 (d, J = 4.8 Hz, 1H), 1.81–1.71 (m, 2H), 1.43–1.32 (m, 3H), 1.30–1.22 (m, 9H), 1.19 (t, J = 5.8 Hz, 7H), 1.12 (t, J = 9.0 Hz, 4H), 1.04–0.97 (m, 10H), 0.92 (d, J = 6.5 Hz, 3H), 0.88 (dd, J = 10.4, 4.7 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 216.1, 178.8, 171.3, 169.8, 140.7, 131.9, 128.4, 120.6, 99.7, 96.0, 83.7, 79.7, 78.7, 73.8, 71.5, 68.2, 63.1, 61.5, 60.4, 58.2, 50.3, 49.4, 46.7, 41.6, 41.1, 38.3, 37.0, 36.4, 35.7, 33.4, 31.3, 29.7, 24.6, 21.3, 21.1, 20.2, 19.2, 17.9, 14.3, 14.1, 10.9, 10.7, 7.8. HRMS (ESI) m/z Calcd for $C_{49}H_{76}NO_{14}$ [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/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 Na_2SO_4 and concentrated to give light yellow solid product (1.63 g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.37 (d, J = 8.0 Hz, 2H), 7.18 (d, J = 6.7 Hz, 2H), 5.26 (s, 1H), 5.06 (d, J = 10.0 Hz, 1H), 4.98 (d, J = 8.5 Hz, 1H), 4.95–4.76 (m, 2H), 4.72 (dd, J = 12.6, 6.2 Hz, 1H), 4.62 (t, J = 7.4 Hz, 1H), 4.50 (s, 1H), 4.44 (d, J = 7.6 Hz, 1H), 4.09 (t, J = 7.3 Hz, 1H), 4.03 (dd, J = 12.6, 5.9 Hz, 1H), 3.70–3.60 (m, 1H), 3.60–3.52 (m, 1H), 3.51–3.42 (m, 1H), 3.34 (d, J = 19.2 Hz, 1H), 3.28–3.14 (m, 2H), 3.09 (d, J = 7.6 Hz, 1H), 3.01 (d, J = 4.1 Hz, 1H), 2.91 (d, J = 4.3 Hz, 1H), 2.82–2.61 (m, 3H), 2.61–2.52 (m, 1H), 2.35–2.25 (m, 1H), 2.24–2.11 (m, 6H), 2.10–2.00 (m, 4H), 1.95–1.84 (m, 2H), 1.83–1.64 (m, 4H), 1.56 (ddd, J = 20.9, 13.1, 6.4 Hz, 2H), 1.49–1.32 (m, 3H), 1.30–1.14 (m, 10H), 1.12 (d, J = 8.3 Hz, 2H), 1.06 (s, 3H), 1.03 (d, J = 4.9 Hz, 3H), 1.03–0.97 (m, 4H), 0.94 (dd, J = 15.2, 8.7 Hz, 3H), 0.91–0.75 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ (ppm) 182.2, 175.8, 173.7, 169.7, 145.2, 142.9, 140.2, 132.2, 131.9, 128.6, 128.1, 120.9, 106.6, 105.0, 103.7, 97.4, 83.7, 78.3, 75.5, 74.3, 73.7, 72.5, 71.0, 70.6, 69.9, 69.3, 65.5, 65.3, 61.7, 60.9, 59.3, 49.9, 49.4, 42.7, 40.4, 39.7, 37.0, 31.9, 29.2, 22.9, 21.5, 19.3, 17.9, 15.2, 14.1, 11.7, 11.0, 10.3, 8.8. HRMS (ESI) m/z Calcd for $C_{49}H_{79}N_2O_{13}$ [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%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.43 (d, J = 8.2 Hz, 3H), 7.26–7.20 (m, 2H), 5.04 (dd, J = 11.0, 2.2 Hz, 1H), 4.98 (d, J = 5.0 Hz, 1H), 4.37 (d, J = 7.2 Hz, 1H), 4.07–4.00 (m, 1H), 3.94 (s, 1H), 3.79 (s, 1H), 3.75 (s, 2H), 3.73–3.69 (m, 2H), 3.66 (dd, J = 8.5, 5.8 Hz, 1H), 3.62 (dd, J = 6.6, 3.6 Hz, 1H), 3.61–3.57 (m, 1H), 3.50–3.42 (m, 2H), 3.39 (dd, J = 12.3, 7.7 Hz, 3H), 3.29 (dd, J = 10.2, 7.2 Hz, 1H), 3.21 (d, J = 3.9 Hz, 1H), 3.16 (d, J = 3.7 Hz, 2H), 3.09 (s, 3H), 3.06 (t, J = 4.5 Hz, 4H), 3.05–3.01 (m, 4H), 2.99 (d, J = 8.0 Hz, 1H), 2.91–2.80 (m, 1H), 2.66 (s, 1H), 2.62 (s, 1H), 2.52 (dd, J = 15.6, 7.6 Hz, 2H), 2.36 (s, 9H), 2.23 (d, J = 4.3 Hz, 4H), 2.18–2.15 (m, 1H), 2.07 (s, 1H), 2.03 (s, 2H), 1.99 (d, J = 6.8 Hz, 1H), 1.90 (dt, J = 30.0, 10.5 Hz, 6H), 1.64–1.52 (m, 2H), 1.49–1.42 (m, 1H), 1.41–1.33 (m, 6H), 1.31–1.22 (m, 10H), 1.22–1.17 (m, 5H), 1.15–1.01 (m, 25H), 0.91 (dt, J = 14.0, 5.6 Hz, 3H), 0.87–0.78 (m, 5H). ^{13}C NMR (126 MHz, $CDCl_3$) 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 $C_{49}H_{82}N_2O_{13}$ [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 NH_4OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.38 (t, J = 5.6 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 4.98 (d, J = 4.7 Hz, 1H), 4.80 (dd, J = 10.7, 7.5 Hz, 1H), 4.61 (dd, J = 16.8, 8.0 Hz, 1H), 4.53–4.47 (m, 1H), 4.32 (dd, J = 11.6, 6.2 Hz, 1H), 4.08 (t, J = 7.2 Hz, 1H), 4.02 (q, J = 6.4 Hz,

1H), 3.70 (dt, J = 9.6, 5.5 Hz, 1H), 3.67–3.59 (m, 2H), 3.54 (d, J = 6.2 Hz, 1H), 3.49 (s, 1H), 3.41 (ddd, J = 22.7, 11.0, 6.6 Hz, 2H), 3.31 (d, J = 7.3 Hz, 1H), 3.20–3.12 (m, 1H), 3.11–2.98 (m, 3H), 2.92 (d, J = 3.3 Hz, 1H), 2.85 (d, J = 2.4 Hz, 1H), 2.78 (dd, J = 17.2, 10.1 Hz, 1H), 2.73 (s, 1H), 2.65 (dd, J = 14.3, 7.6 Hz, 2H), 2.57 (d, J = 9.5 Hz, 1H), 2.45 (dt, J = 23.1, 10.2 Hz, 1H), 2.31 (d, J = 11.6 Hz, 7H), 2.24–2.16 (m, 3H), 2.13 (dd, J = 11.4, 4.5 Hz, 1H), 2.10–2.01 (m, 4H), 2.00–1.78 (m, 6H), 1.68 (dd, J = 23.3, 12.9 Hz, 2H), 1.23 (qd, J = 15.5, 7.1 Hz, 17H), 1.13 (dd, J = 9.7, 4.5 Hz, 3H), 1.09–0.98 (m, 8H), 0.86 (ddd, J = 11.8, 10.2, 6.2 Hz, 3H). ^{13}C 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 $C_{51}H_{86}N_3O_{13}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 8.36 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 5.12 (d, J = 10.8 Hz, 1H), 4.45 (t, J = 6.9 Hz, 3H), 4.31 (d, J = 7.2 Hz, 1H), 4.09 (d, J = 6.5 Hz, 1H), 3.90 (d, J = 13.4 Hz, 1H), 3.72 (d, J = 13.5 Hz, 2H), 3.59 (d, J = 12.8 Hz, 2H), 3.20 (dd, J = 19.5, 9.0 Hz, 3H), 3.11 (d, J = 15.2 Hz, 2H), 3.00 (d, J = 8.4 Hz, 6H), 2.89 (s, 1H), 2.65 (s, 1H), 2.59 (s, 3H), 2.42 (s, 6H), 2.34 (s, 4H), 2.28 (s, 1H), 2.21 (s, 1H), 2.10 (t, J = 7.4 Hz, 4H), 1.96 (dd, J = 15.3, 7.4 Hz, 5H), 1.82 (s, 7H), 1.73–1.60 (m, 5H), 1.44–1.33 (m, 9H), 1.27 (d, J = 10.6 Hz, 7H), 1.22 (dd, J = 12.4, 6.3 Hz, 7H), 1.21–1.16 (m, 6H), 1.17–1.09 (m, 17H), 1.06 (d, J = 7.5 Hz, 4H), 0.91 (d, J = 10.9 Hz, 5H), 0.84 (dd, J = 9.5, 5.1 Hz, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm) 175.7, 147.5, 129.7, 125.8, 102.9, 96.8, 94.9, 78.3, 76.1, 74.3, 71.3, 70.9, 69.0, 68.3, 67.5, 51.3, 50.7, 50.7, 50.1, 50.5, 49.4, 47.2, 45.3, 45.3, 44.6, 39.4, 37.5, 36.8, 33.7, 31.9, 29.7, 28.6, 23.2, 22.7, 21.6, 21.3, 19.7, 18.1, 16.0, 15.9, 15.1, 14.1, 12.3, 10.6, 9.2, 7.8. HRMS (ESI) m/z Calcd for $C_{55}H_{93}N_6O_{15}$ [M+H$^+$] 1077.6693, found 1077.6692.

5.1.71. (Clarithromycin-(4′-*N*-(4-benzyltriazolyl))-4″-(*N*,*N*-dimethylaminomethyl))-*N*-hydroxyheptanamide (49b)

Reaction of compound **47** (0.024 g, 0.022 mmol) with 7-Azido-*N*-((*tert*-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%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.37 (s, 1H), 7.82 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 5.14–5.07 (m, 1H), 4.45 (t, J = 7.0 Hz, 2H), 4.30 (d, J = 7.2 Hz, 1H), 4.09 (d, J = 6.5 Hz, 1H), 3.92 (d, J = 13.0 Hz, 1H), 3.72 (d, J = 12.3 Hz, 2H), 3.64–3.57 (m, 2H), 3.34 (d, J = 2.4 Hz, 2H), 3.09 (s, 1H), 3.04–2.97 (m, 7H), 2.89 (s, 2H), 2.59 (s, 3H), 2.49 (s, 7H), 2.36 (s, 3H), 2.28 (s, 1H), 2.15 (s, 1H), 2.11–2.03 (m, 5H), 1.92 (dd, J = 13.5, 6.7 Hz, 6H), 1.83 (d, J = 10.2 Hz, 4H), 1.61 (d, J = 7.5 Hz, 6H), 1.42–1.33 (m, 12H), 1.28 (s, 6H), 1.23 (d, J = 6.0 Hz, 5H), 1.19 (d, J = 7.2 Hz, 4H), 1.17–1.08 (m, 16H), 1.05 (d, J = 7.5 Hz, 4H), 0.97 (s, 1H), 0.91 (d, J = 11.7 Hz, 4H), 0.84 (t, J = 7.4 Hz, 4H). ^{13}C NMR (101 MHz, cd$_3$od) 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 $C_{56}H_{97}N_6O_{15}$ [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 NH_4Cl/NH_4OH (30 mL) was added to the reaction mixture and extracted with 20% MeOH/CH_2Cl_2 (3 × 30 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative chromatography (Silica gel, 5:1:1 EtOAc/MeOH/NH_4OH) to give the product (0.16 g, 80%) as light yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.74 (s, 1H), 7.20 (d, J = 7.8 Hz, 2H), 6.83 (d, J = 7.9 Hz, 2H), 3.83 (s, 2H), 3.74 (d, J = 7.3 Hz, 1H), 3.60–3.46 (m, 2H), 3.26 (d, J = 13.0 Hz, 1H), 2.99–2.89 (m, 3H), 2.78 (s, 1H), 2.75–2.67 (m, 3H), 2.37 (d, J = 3.6 Hz, 3H), 2.23–2.10 (m, 2H), 1.96 (t, J = 14.2 Hz, 3H), 1.80–1.64 (m, 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). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.5, 178.8, 177.3, 173.7, 172.8, 149.4, 141.7, 131.8, 127.5, 122.8, 105.1, 102.9, 97.3, 86.5, 80.7, 78.8, 77.9, 76.3, 75.9, 73.7, 72.8, 71.4, 70.0, 69.2, 64.7, 63.2, 61.6, 60.1, 55.7, 53.1, 52.1, 50.9, 48.5, 47.3, 44.5, 44.1, 43.9, 38.3, 37.6, 32.9, 32.4, 32.3, 32.2, 31.9, 31.7, 30.2, 28.4, 27.9, 23.1, 22.7, 19.7, 18.1, 16.4, 12.4, 10.7, 8.4. HRMS (ESI) m+2/2z Calcd for $C_{55}H_{97}N_7O_{14}$ [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%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm) 7.46 (s, 1H), 6.92 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 8.0 Hz, 2H), 3.55 (d, J = 6.2 Hz, 2H), 3.47 (d, J = 7.2 Hz, 1H), 3.33–3.17 (m, 2H), 2.99 (d, J = 12.9 Hz, 1H), 2.76 (d, J = 13.2 Hz, 1H), 2.67 (dd, J = 14.5, 9.8 Hz, 3H), 2.51 (s, 1H), 2.43 (ddd, J = 6.2, 4.9, 2.3 Hz, 2H), 2.26 (t, J = 15.8 Hz, 1H), 2.09 (d, J = 5.7 Hz, 3H), 1.98–1.83 (m, 2H), 1.69 (dd, J = 14.5, 9.6 Hz, 2H), 1.55–1.37 (m, 13H), 1.35–1.23 (m, 2H), 1.22 (d, J = 13.4 Hz, 3H), 1.16–1.01 (m, 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). ^{13}C NMR (126 MHz, CD_3OD) δ (ppm) 180.4, 180.1, 177.3, 173.5, 172.7, 149.5, 141.7, 141.2, 131.8, 131.4, 127.4, 122.8, 105.1, 102.8, 97.3, 86.1, 80.3, 78.9, 76.5, 75.7, 73.7, 72.8, 71.5, 70.1, 69.2, 64.6, 63.5, 61.7, 60.1, 55.7, 53.2, 52.1, 51.0, 48.5, 47.6, 47.4, 44.1, 43.8, 38.5, 38.0, 37.6, 32.1, 31.5, 29.0, 28.8, 27.7, 26.8, 23.1, 22.4, 20.0, 18.2, 16.4, 16.0, 12.6, 10.8, 8.5. HRMS (ESI) m+2/2z Calcd for $C_{56}H_{99}N_7O_{14}$ [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 μM, 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 $MgCl_2$, and 0.1 mg/mL BSA) in 96-well microtiter plates (60 min, 37 °C). Solution-phase deacetylation reactions were quenched with trichostatin A (TSA) and transferred to SAMDI plates to immobilize the substrate components. SAMDI plates were composed of an array of self-assembled monolayers (SAMs) presenting maleimide in standard 384-well format for high-throughput handling capability. Following immobilization, plates were washed to remove buffer constituents, enzyme, inhibitor, and any unbound substrate and analyzed by MALDI mass spectrometry using automated protocols. Deacetylation yields in each triplicate sample were determined from the integrated peak intensities of the molecular ions for the substrate and the deacetylated product ion by taking the ratio of the former over the sum of both. Yields were plotted with respect to inhibitor concentration and fitted to obtain IC_{50} values for each isoform−inhibitor pair.

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

HDAC1, HDAC6, and HDAC2 were purchased from BPS Biosciences. The catalytic domain of HDAC8 was expressed as previously reported.[21e] Briefly, an amplicon was prepared by PCR with the following primers: forward (5′−3′) TATTCTCGAGGA-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-κB activity was measured by luciferase assay. BEAS-2B cells were transfected with NF-κB 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. IC_{50} was determined by treating the cell with a serial dose of the drug followed by luciferase assay. % inhibition was calculated using the following equation; % 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× ~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 semiquantitative RT-PCR analysis, PCR amplifications were performed with PrimeSTAR Max polymerase (Takara) by following the manufacturer's instruction. The primer for TNF-α, IL-1β were described previousery.[40,41] IL-1α: 5′-*CGAGCCAATGATCAGTACCTC*-3′ and 3′-*CACCCATATATTTCACTG* -5′.

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Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR spectral and NF-κB activity in NTHi infected BEAS-2B cells treated with 1 μM 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.

References and notes

1. Marks, P. A. *Oncogene* **2007**, *26*, 1351.
2. Patil, V.; Guerrant, W.; Chen, P. C.; Gryder, B.; Benicewicz, D. B.; Khan, S. I.; Tekwani, B. L.; Oyelere, A. K. *Bioorg. Med. Chem.* **2010**, *18*, 415.
3. (a) Jenuwein, T. *Science* **2001**, *293*, 1074; (b) Johnstone, R. W. *Nat. Rev. Drug Disc.* **2002**, *1*, 287; (c) Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Disc.* **2006**, *5*, 769; (d) Somech, R.; Izraeli, S. A.; Simon, J. . *Cancer Treat. Rev.* **2004**, *30*, 461; (e) Boyes, J.; Byfield, P.; Nakatani, Y.; Ogryzko, V. *Nature* **1998**, *396*, 594; (f) Gu, W.; Roeder, R. G. *Cell* **1997**, *90*, 595; (g) Jin, S.; Scotto, K. W. *Mol. Cell. Biol.* **1998**, *18*, 4377; (h) Yang, X. J.; Seto, E. *Nat. Rev. Mol. Cell. Biol.* **2008**, *9*, 206; (i) Vigushin, D. M.; Coombes, R. C. *Curr. Cancer Drug Targets* **2004**, *4*, 205; (j) Choudhary, C.; Kumar, C.; Gnad, F.; Nielsen, M. L.; Rehman, M.; Walther, T. C.; Olsen, J. V.; Mann, M. *Science* **2009**, *325*, 834.
4. Kelly, W. K.; O'Connor, O. A.; Marks, P. A. *Exp. Opin. Invest. Drugs* **2002**, *11*, 1695.
5. Miller, T. A.; Witter, D. J.; Belvedere, S. *J. Med. Chem.* **2003**, *46*, 5097.
6. Molecule of the month. Vorinostat. *Drug News & Persp.* **2006**, **19**, 352.
7. Grant, S.; Easley, C.; Kirkpatrick, P. *Nat. Rev. Drug Disc.* **2007**, *6*, 21.
8. Campas-Moya, C. *Drugs Today* **2009**, *45*, 787.
9. RajKumar, S. V. *Lancet Oncol.* **2014**, *15*, 1178.
10. Guha, M. *Nat. Rev. Drug Disc.* **2015**, *14*, 225.
11. Rashidi, A.; Cashen, A. *Future Oncol.* **2015**, *11*, 1659.
12. Yurek-George, A.; Cecil, A. R.; Mo, A. H.; Wen, S.; Rogers, H.; Habens, F.; Maeda, S.; Yoshida, M.; Packham, G.; Ganesan, A. *J. Med. Chem.* **2007**, *50*, 5720.
13. Mwakwari, S. C.; Patil, V.; Guerrant, W.; Oyelere, A. K. *Curr. Top. Med. Chem.* **2010**, *10*, 1423.
14. Oyelere, A. K.; Chen, P. C.; Guerrant, W.; Mwakwari, S. C.; Hood, R.; Zhang, Y.; Fan, Y. *J. Med. Chem.* **2009**, *52*, 456.
15. Mwakwari, S. C.; Guerrant, W.; Patil, V.; Khan, S. I.; Tekwani, B. L.; Gurard-Levin, Z. A.; Mrksich, M.; Oyelere, A. K. *J. Med. Chem.* **2010**, *53*, 6100.
16. Jakopovic, I. P.; Krajacic, M. B.; Skugor, M. M.; Stimac, V.; Pesic, D.; Vujasinovic, I.; Alihodzic, S.; Cipcic Paljetak, H.; Kragol, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3527.
17. Lee, Y.; Choi, J. Y.; Fu, H.; Harvey, C.; Ravindran, S.; Roush, W. R.; Boothroyd, J. C.; Khosla, C. *J. Med. Chem.* **2011**, *54*, 2792.

18. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem.* **2002**, *41*, 2596; (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
19. Cosner, C. C.; Helquist, P. *Org. Lett.* **2011**, *13*, 3564.
20. (a) Denis, A.; Renou, C. *Tetrahedron Lett.* **2002**, *43*, 4171; (b) Mercep, M.; Mesic, M.; Tomaskovic, L.; Markovic, S.; Makaruha, O.; Poljak, V. US 2004014685.; (c) Miljkovic, M. *Carbohydrates: Synthesis, Mechanisms, and Stereoelectronic Effects*; Springer: New York, 2009; (d) Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010**, *31*, 455.
21. (a) Min, D. H.; Yeo, W. S.; Mrksich, M. *Anal. Chem.* **2004**, *76*, 3923; (b) Gurard-Levin, Z. A.; Mrksich, M. *Biochemistry* **2008**, *47*, 6242; (c) Mrksich, M. *ACS Nano* **2008**, *2*, 7; (d) Gurard-Levin, Z. A.; Kim, J.; Mrksich, M. *ChemBioChem* **2009**, *10*, 2159; (e) Gurard-Levin, Z. A.; Kilian, K. A.; Kim, J.; Bahr, K.; Mrksich, M. *ACS Chem. Biol.* **2010**, *5*, 863.
22. Vaguliene, N.; Zemaitis, M.; Lavinskiene, S., et al. *BMC Immunol.* **2013**, *14*, 36.
23. Azad, N.; Rojanasakul, Y.; Vallyathan, V. *J. Toxicol. Environ. Health* **2008**, *11*, 1.
24. Lu, H.; Ouyang, W.; Huang, C. *Mol. Cancer Res.* **2006**, *4*, 221.
25. Tang, J.; Yan, H.; Zhuang, S. *Clin. Sci.* **2013**, *124*, 651.
26. (a) Shi, Z. J.; Ouyang, D. Y.; Zhu, J. S.; Xu, L. H.; He, X. H. *Int. J. Immunopharmacol.* **2012**, *12*, 580; (b) Wang, D.; Zhao, M.; Chen, G.; Cheng, X.; Han, X.; Lin, S.; Zhang, X.; Yu, X. *Apoptosis* **2013**, *18*, 1348.
27. (a) Yoshida, M.; Kijima, M.; Akita, M.; Beppu, T. *J. Biol. Chem.* **1990**, *265*, 17174; (b) Furumai, R.; Ito, A.; Ogawa, K.; Maeda, S.; Saito, A.; Nishino, N.; Horinouchi, S.; Yoshida, M. *Cancer Sci.* **2011**, *102*, 1081.
28. Tong, X.; Yin, L.; Giardina, C. *Biochem. Biophy. Res. Commun.* **2004**, *317*, 463.
29. Choo, Q. Y.; Ho, P. C.; Tanaka, Y.; Lin, H. S. *Molecules* **2013**, *18*, 14085.
30. (a) Blanchard, F.; Chipoy, C. *Drug Discovery Today* **2005**, *10*, 197; (b) Adcock, I. M.; British, J. *Pharmacology* **2007**, *150*, 829.
31. Ishinaga, H.; Jono, H.; Lim, J. H.; Kweon, S. M.; Xu, H.; Ha, U. H.; Xu, H.; Koga, T.; Yan, C.; Feng, X. H.; Chen, L. F.; Li, J. D. *EMBO J.* **2007**, *26*, 1150.
32. (a) Moghaddam, S. J.; Clement, C. G.; De la Garza, M. M.; Zou, X.; Travis, E. L.; Young, H. W.; Evans, C. M.; Tuvim, M. J.; Dickey, B. F. *Am. J. Respir. Cell Mol. Biol.* **2008**, *38*, 629; (b) Poolman, J. T.; Bakaletz, L.; Cripps, A.; Denoel, P. A.; Forsgren, A.; Kyd, J.; Lobet, Y. *Vaccine* **2000**, *19*, S108.
33. (a) Loukides, S.; Bartziokas, K.; Vestbo, J.; Singh, D. *Curr. Drug Targets* **2013**, *14*, 235; (b) Chung, K. F.; Adcock, I. M. *Eur. Respir. J.* **2008**, *31*, 1334.
34. Zhu, D.; Xu, Y.; Liu, Y.; Chen, X.; Zhao, Z.; Lei, P. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6274.
35. Althuon, D.; Rönicke, F.; Fürniss, D.; Quan, J.; Wellhöfer, I.; Jung, N.; Schepers, U.; Bräse, S. *Org. Biomol. Chem.* **2015**, *13*, 4226.
36. Miller, M. J.; Zhao, G.; Vakulenko, S.; Franzblau, S.; Mollmann, U. *Org. Biomol. Chem.* **2006**, *4*, 4178.
37. Yang, C.; Mi, J.; Feng, Y.; Ngo, L.; Gao, T.; Yan, L.; Zheng, Y. G. *J. Am. Chem. Soc.* **2013**, *135*, 7791.
38. Gryder, B. E.; Akbashev, M. J.; Rood, M. K.; Raftery, E. D.; Meyers, W. M.; Dillard, P.; Khan, S.; Oyelere, A. K. *ACS Chem. Biol.* **2013**, *8*, 2550.
39. Kim, K. T.; Kim, B. H. *Chem. Commun.* **2013**, 1717.
40. Miyata, M.; Lee, Ji-. Y.; Miyata, S.; Wang, W. Y.; Xu, H.; Kai, H.; Kobayashi, K. S.; Flavell, R. A.; Li, J.-D. *Nat. Commun.* **2015**, *6*, 6062.
41. Miyata, S.; Miyata, M.; Lee, B.-C.; Xu, H.; Kai, H.; Yan, C.; Li, J.-D. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, E1800.