European Journal of Medicinal Chemistry 45 (2010) 5943–5949

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and antibacterial activity of C-12 pyrazolinyl spiro ketolides

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ARTICLE INFO

Article history:
Received 12 May 2010
Received in revised form
27 September 2010
Accepted 28 September 2010
Available online 7 October 2010

Dedicated to Professor Henry N.C. Wong on the occasion of his 60th birthday

Keywords:
Ketolide
Anti-bacterial activity
Pyrazolinyl

ABSTRACT

A series of C-12 pyrazolinyl spiro ketolide derivatives were designed and synthesized. The C-12 modifications involved replacing the natural C-12 methyl group in clarithromycin core with different pyrazolinyl spiros via chemical synthesis. Potential anti-bacterial activities against both erythromycin-susceptible and erythromycin-resistant bacteria were reported.

1. Introduction

The first macrolide antibiotic erythromycin A has been widely used as an agent to treat upper and lower respiratory tract infections for about six decades [1]. Due to its chemical instability and gastro-intestinal side effects [2], erythromycin A had been gradually modified to produce the second-generation macrolides such as clarithromycin, azithromycin and roxithromycin in the 1990s [3,4]. Recently the growing emergence of bacterial resistance, however, has compromised the utility of macrolides [5]. The discovery of ketolides, in which the C-3 cladinosyl is modified as a keto group, significantly improved the activities against macrolide-resistant bacteria [6]. Two of the most efficient ketolides are telithromycin and cethromycin (ABT-773) [7,8] (Fig. 1). In our previous work [9], C-12 pyrazolinyl intermediate was firstly synthesized from clarithromycin according to the bioisostere principle by replacing C-11,12 cyclic carbamate of telithromycin with C-12 pyrazolinyl ring. In this paper, we report herein on the preparation of a novel C-12 pyrazolinyl spiro macrolide core, conversion of the core to ketolide derivatives, and in *vitro* anti-bacterial activities of the resulting ketolides.

2. Results and discussion

The purpose of this paper was to synthesize some novel ketolides bearing different pyrazolinyl moieties via [2 + 3] cycloaddition on C-12,21 double bond. The synthetic routes for accessing such C-12 modified macrolides and ketolides were outlined in Schemes 1–3. The key step of this strategy was the formation of a C-12,21 double bond, which offered a site for reacting with diazo compounds.

Intermediate **5** was prepared by methods described previously [9]. Briefly, clarithromycin was used as a starting material to react with benzoic anhydride in the presence of triethylamine and 4-dimenthylaminopyridine (DMAP) to give C-2′, 4″ dibenzoyl ester macrolide. Subsequent reduction of C-9 carbonyl group using $NaBH_4$ led to C-9 hydroxyl macrolide **1**. Treatment of compound **1** with 2,2-dimethoxypropane and pyridinium p-toluenesulfonate (PPTS) for protection of C-9 and C-11 hydroxyl groups, generated C-9, C-11 dimethylketal macrolide. Then C-12 hydroxyl group of this C-9,11 dimethylketal macrolide was eliminated by $SOCl_2$ to form a C-12,21 exocyclic alkenyl macrolide **2** [10]. In the next two steps, C-3 cladinose was hydrolyzed and C-9,11 dihydroxyl groups were converted to dimethylketal group again. Under these reaction conditions [11], oxidation of C-3 hydroxyl of compound **4** afforded a C-12,21 exocyclic alkenyl ketolide derivative **5**.

C-12 pyrazolinyl spiro ketolide derivatives **6** were then synthesized via [2 + 3] cycloaddition [12] of compound **5** with series of

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doi:10.1016/j.ejmech.2010.09.060

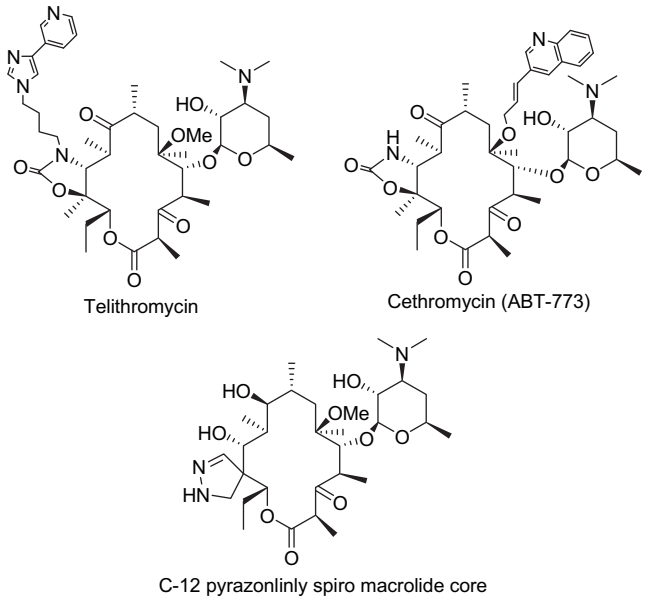
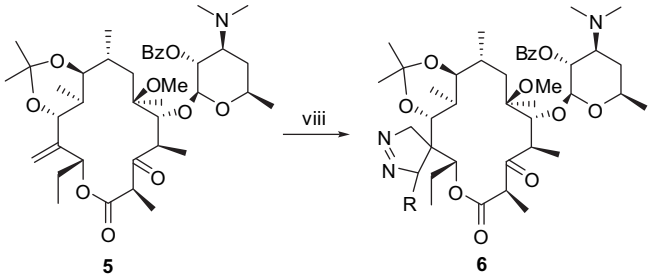


Fig. 1. Structures of novel ketolides.

diazo compounds. The mechanism of the formation of pyrazolines on C-12,21 double bond could be illustrated as Scheme 4 [13], and it accorded with the frontier molecular orbital (FMO) theory. According to this concept, cycloadditions of simple diazoalkanes and diazoacetic esters are highest occupied molecular orbital (HOMO, dipole)–lowest unoccupied molecular orbital (LUMO, dipolarophile) controlled. Two reaction conditions were used in this step: The newly prepared diazomethane [14] and diazoethane were dissolved in ether, and the reactions proceeded in room temperature; The newly prepared ethyl diazoacetate and methyl diazoacetate [15], due to their lower reaction activities, were dissolved in 1,2-dichloroethane, so that the reactions could proceed at a higher temperature (84 °C). Dark condition was a very important factor for this kind of reaction, for the diazo compounds would decompose to

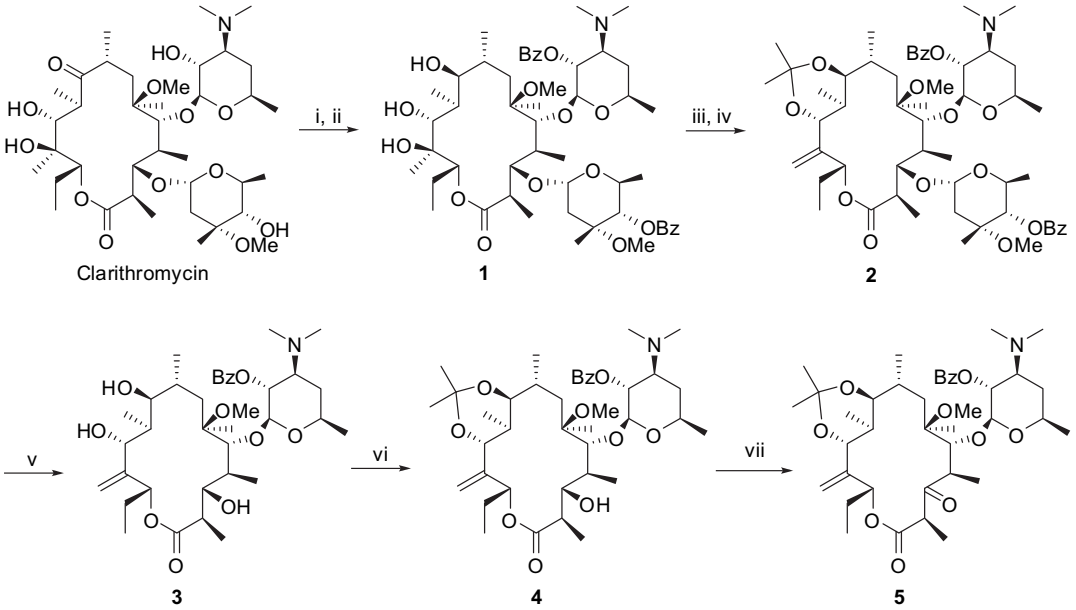


Scheme 2. Reagents and conditions: (viii) $RCHN_2$, ether, 0–25 °C or 1,2-dichloroethane, 84 °C, yields range from 69.7% to 98.2%.

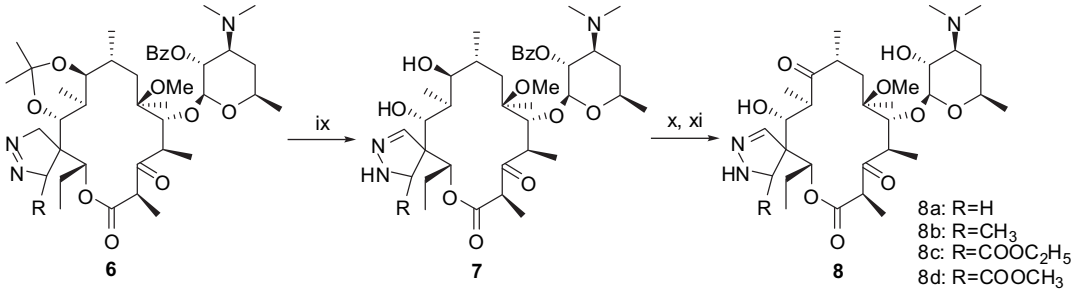
carbene in the light, which led to the generation of many byproducts. Unfortunately, compound **6** was then identified as a pair of stereoisomers which could not be separated by general gel column chromatography. This means that there is no stereoselectivity in these cycloadditions. The diazo compounds could attack to the C-12,21 double bond from both *anti* and *syn* faces.

Removal of C-9,11 hydroxyl protecting group gave compound **7**. In this step, N=N of the pyrazolinyl rearranged to C=N in the presence of a catalytic amount of PPTS. C-9 hydroxyl group of compound **7** was selectively oxidized by using Dess-Martin periodinane [16] to form C-9 carbonyl C-12 pyrazolinyl spiro ketolide derivatives, and successive deprotection of the C-2′ benzoyl group [17] yielded final compounds **8a-d**.

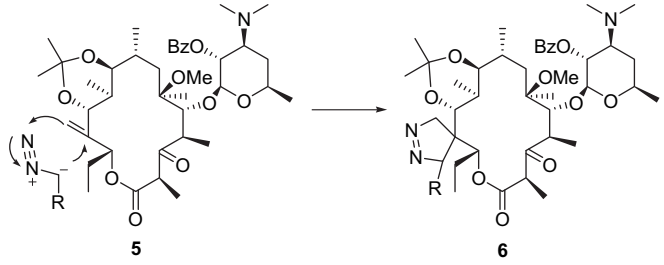
The anti-bacterial activities of the final compounds in *vitro* were assessed against both erythromycin-susceptible and erythromycin-resistant bacteria, with particular emphasis on respiratory pathogens. Broth microdilution MIC (Lowest concentration of compound inhibiting visible growth) determinations were performed according to procedures established by the national committee for Clinical Laboratory Standards [18]. Results are presented in Table 1 for twelve selected bacterial strains, including erythromycin-susceptible *Staphylococcus. aureus* ATCC 29213, *S. aureus* ATCC 6538p, *S. aureus* ATCC 26001 *Staphylococcus pneumonia* 2860, *Haemophilus. influenza* 5096 and erythromycin-resistant *S. aureus* 5776, *S. aureus* 5677, *S. aureus* AD-08, *S. pneumonia* 1210, *S. pneumonia* 5158, *S. pneumonia*



Scheme 1. Reagents and conditions: (i) $(Bz)_2O$, DMAP, Et_3N, EtOAc; (ii) $NaBH_4$, THF; (iii) 2,2-dimethoxypropane, PPTS, acetone; (iv) $SOCl_2$, Et_3N, EtOAc, 0 °C; (v) diluted HCl, acetonitrile/H_2O; (vi) 2,2-dimethoxypropane, PPTS, acetone; (vii) Dess-Martin periodinane, CH_2Cl_2, r.t.; 40.7% for seven steps.



Scheme 3. Reagents and conditions: (ix) PPTS, acetonitrile/H_2O; (x) Dess-Martin periodinane, CH_2Cl_2, 0 °C; (xi) CH_3OH, reflux, yields range from 32.9% to 38.3% for two steps. Overall yields range from 12.0% to 16.0%.



Scheme 4. Illustration of the formation of pyrazolines on C-12,21 double bond reacted with diazo compounds.

673, *H. influenza* 2412. Twofold differences in the MIC value are within the error of the method.

Table 1 summarizes the anti-bacterial activities of the target compounds. All C-12 pyrazolinyl spiro analogs exhibited distinct activities against *S. aureus* erythromycin-susceptible strains and improved activities against *S. aureus* erythromycin-resistant strains, and with almost comparative bioactivities against *S. pneumonia* and *H. influenza* strains compared with erythromycin A and clarithromycin. In general, among the C-12 pyrazolinyl spiro derivatives, compounds (**8c** and **8d**) with ester substituents possessed better anti-bacterial activities than those of compounds (**8a** and **8b**) with alkyl substituents. For *S. aureus* ATCC 29213 and *S. aureus* ATCC 6538p strains, both compounds **8c** and **8d** exhibited slightly better activities than **8a** and **8b** with the MIC value of 0.12 μg/mL. Compound **8d** was found to be more potent than other compounds against *S. aureus* 26001. All of the designed molecules showed significantly increased inhibitory profiles than erythromycin A and clarithromycin against erythromycin-resistant *S. aureus* 5676 and *S. aureus* 5677 strains. Compounds **8c** and **8d** displayed slightly improved potencies than erythromycin A and clarithromycin against erythromycin-resistant *S. pneumonia* 5158 and *S. pneumonia* 673 strains. Compounds **8a-d** were found to be less potential than erythromycin A and clarithromycin in both erythromycin-resistant *S. pneumonia* 1210 as well as *H. influenza* 2412 and erythromycin-susceptible *S. pneumonia* 2860 strains. All of the compounds exhibited the same MIC values as erythromycin A and clarithromycin towards erythromycin-susceptible *H. influenza* 5096 strain.

3. Conclusion

A series of C-12 pyrazolinyl spiro ketolides were designed and synthesized for the first time. Compounds with various alkane and ester groups at pyrazolinyl spiros were investigated for their anti-bacterial activities against both erythromycin-susceptible and erythromycin-resistant bacteria, with particular emphasis on respiratory pathogens. All derivatives were found to have better anti-bacterial activities than erythromycin A and clarithromycin against *S. aureus* strains, and with almost equivalent bioactivities against *S. pneumonia* and *H. influenza* strains. Among the C-12 pyrazolinyl spiro ketolides, compounds (**8c** and **8d**) with ester substituents displayed better anti-bacterial activities than those of compounds (**8a** and **8b**) with alkyl substituents. This suggests that ketolides possessing spiro C-12 pyrazolinyl moiety may have presented improved anti-bacterial properties. The result can be useful to aid the designing of new ketolides with better anti-bacterial activities.

4. Experimental

All reagents and solvents were reagent grade. Further purification and drying by standard methods were employed when necessary. Clarithromycin and its derivatives were pre-dried azeotropically from benzene. CH_2Cl_2 and EtOAc were distilled from CaH_2. All organic solvents were evaporated under reduced pressure with a rotary evaporator. The plates used for thin-layer chromatography (TLC) were Qingdao-haiyang silica gel GF254 (0.1 mm thickness) precoated on glass plates, and they were visualized

Table 1
MIC (μg/mL) for C-12 pyrazolinyl spiro ketolides.

Compound	Erythromycin A	Clarithromycin	8a	8b	8c	8d
S. aureus ATCC 29213	0.5	0.25	0.25	0.5	0.12	0.12
S. aureus ATCC 6538p	0.5	0.5	0.25	0.25	0.12	0.12
S. aureus 26001	≤ 0.063	≤0.063	0.12	0.12	0.12	0.03
S. aureus 5676	>1024	>1024	4	8	8	4
S. aureus 5677	>1024	>1024	8	8	8	4
S. aureus AD-08	>1024	>1024	512	512	512	256
S. pneumonia 1210	>32	>16	>64	>64	>64	>64
S. pneumonia 2860	0.25	0.125	0.5	0.5	0.5	0.5
S. pneumonia 5158	>32	>16	>64	>64	8	8
S. pneumonia 673	>32	>16	>64	>64	8	8
H. influenza 2412	4	8	>8	>8	>8	>8
H. influenza 5096	0.06	0.06	0.06	0.06	0.06	0.06

under both long (365 nm) and short (254 nm) UV light. Column chromatography was performed using Qingdao-haiyang silica gel (200–300 mesh). NMR spectra were recorded on Bruker-AV400 MHz spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR). All NMR measurements were carried out at 300 K in deuterated chloroform solution unless otherwise stated. Chemical shifts are reported as parts per million (ppm) in d unit in the scale relative to the resonance of $CDCl_3$ (7.26 ppm in the ^{1}H, 77.00 ppm for the central line of the triplet in the ^{13}C modes, respectively). Coupling constants (J) are reported in Hz. Splitting patterns are described by using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{1}H NMR data is reported in this order: chemical shift; multiplicity; coupling constant(s), number of proton. Mass spectra (ERMS) were obtained with a Thermofinnigan MAT95XL spectrometer. Relevant data were tabulated as m/z. High-resolution mass spectra (HRMS) were obtained on a Agilent 6410 TOF LC/MSD in ESI^+ or ESI^- mode.

Compound **2** was prepared as the method of literature [10].

4.1. (2S,3R,4S,6R)-4-(dimethylamino)-2-((3R,4S,5S,6R,7R,9R,10S,11S,12R,14R)-14-ethyl-4,10,12-trihydroxy-7-methoxy-3,5,7,9,11-pentamethyl-13-methylene-2-oxooxacyclotetradecan-6-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**3**)

To a solution of **2** (39.2 g, 40 mmol) in acetonitrile (145.6 mL) was added HCl (3.3 mol/L, 145.6 mL) dropwise at room temperature. The solution was then stirred at room temperature for 24 h. The reaction mixture was diluted by CH_2Cl_2 (400 mL), washed with saturated $NaHCO_3$ (2 × 120 mL). The organic layer was separated and the aqueous layer was extracted by CH_2Cl_2 (2 × 200 mL). The combined organic layer was washed with brine (120 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel column (4:1 petroleum ether-acetone with 1% Et_3N) to give **3** (23.2 g, 85.2%) as a white foam. ^{1}H NMR ($CDCl_3$, ppm) δ 0.60 (d, J = 7.2 Hz, 3H), 0.84 (t, J = 7.3 Hz, 3H), 0.90 (d, J = 7.3 Hz, 3H), 0.96 (d, J = 7.1 Hz, 3H), 1.13 (s, 1H), 1.18 (d, J = 6.6 Hz, 3H), 1.30–1.32 (m, 6H), 1.33–1.90 (m, 10H), 2.01 (s, 1H), 2.26 (s, 6H), 3.03 (s, 1H), 3.22 (s, 3H), 3.30–3.34 (m, 1H), 3.47 (dd, J = 9.8, 7.2 Hz, 1H), 3.57–3.61 (m, 1H), 3.89 (d, J = 2.6 Hz, 1H), 4.4 (s, 1H), 4.78 (d, J = 7.6 Hz, 1H), 5.0 (m, 1H), 5.06 (m, 1H), 5.16 (s, 1H), 5.30 (s, 1H), 5.61 (t, J = 7.2 Hz, 1H), 7.44 (t, J = 7.6 Hz, 2H), 7.56 (t, J = 7.3 Hz, 1H), 8.02 (d, J = 8.4 Hz, 2H). MS (ESI): 679.1 (MH^+).

4.2. (2S,3R,4S,6R)-4-(dimethylamino)-2-((1R,3R,6R,7S,8S,9R,10R,12R,13S,17S)-3-ethyl-7-hydroxy-10-methoxy-6,8,10,12,15,15,17-heptamethyl-2-methylene-5-oxo-4,14,16-trioxabicyclo[11.3.1]heptadecan-9-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**4**)

To a solution of **3** (20.4 g, 30 mmol) in acetone (400 mL) was added 2,2-dimethoxypropane (90 mL, 730 mmol) and PPTS (25.5 g, 101.1 mmol) at room temperature. The solution was stirred and heated at 60 °C for 18 h. Upon cooling, the solution was added Et_3N (25.5 mL 176.7 mmol) and stirred for another 1 h. The solution was concentrated in vacuo and the residue was dissolved in CH_2Cl_2 (390 mL), washed with 10% KH_2PO_4 (2 × 210 mL). The aqueous layer was back extracted with EtOAc (2 × 70 mL). The combined organic layer was washed with brine (135 mL), dried over Na_2SO_4 and concentrated. The residue was purified on a silica gel (4:1 petroleum ether-acetone with 1% Et_3N) to give **4** (19.5 g, 91.3%) as a white foam. ^{1}H NMR ($CDCl_3$, ppm) δ 0.75 (m, 9H), 0.87 (d, J = 6.9 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.19–1.31 (m, 14H), 1.35–1.63 (m, 4H), 1.74–1.82 (m, 2H), 1.84–1.95 (m, 2H), 2.26 (s, 6H), 2.32–2.39 (m, 1H), 2.94 (d, J = 4.1 Hz, 1H), 2.96 (d, J = 4.4 Hz, 1H), 3.19 (s, 3H), 3.61 (m, 1H), 3.68 (dd, J = 3.7, 10.0 Hz, 1H), 3.75 (d, J = 4.4 Hz, 1H), 4.38 (d, J = 1.8 Hz, 1H), 4.74 (d, J = 7.7 Hz, 1H), 4.95–5.07 (m, 2H), 5.26 (s, 1H), 5.32 (s, 1H), 7.42 (t, J = 7.8 Hz, 2H), 7.51 (t, J = 6.2 Hz, 1H), 8.03 (d, J = 7.1 Hz, 2H). MS (ESI): 719.0 (MH^+).

4.3. (2S,3R,4S,6R)-4-(dimethylamino)-2-((1R,3R,6R,8R,9R,10R,12R,13S,17S)-3-ethyl-10-methoxy-6,8,10,12,15,15,17-heptamethyl-2-methylene-5,7-dioxo-4,14,16-trioxabicyclo[11.3.1]heptadecan-9-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**5**)

To a solution of **4** (14.4 g, 20 mmol) in CH_2Cl_2 (110 mL) at room temperature was added Dess-Martin periodinane (12.7 g, 30 mmol). The solution was stirred for 1 h and then was diluted with EtOAc (380 mL). After washing with 1:1 saturated $NaHCO_3$/10% $Na_2S_2O_3$ (120 mL), the aqueous layer was extracted with EtOAc (2 × 110 mL) and the combined organic layer was washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel (3:1 petroleum ether-acetone with 1% Et_3N) to give **5** (13.6 g, 95.6%) as a white foam. ^{1}H NMR ($CDCl_3$, ppm) δ 0.84–0.87 (m, 7H), 0.92 (d, J = 7.2 Hz, 3H), 1.04 (d, J = 6.8 Hz, 3H), 1.11–1.16 (dd, J = 19.6, 7.2 Hz, 1H), 1.27 (d, J = 1.6 Hz, 3H), 1.28 (d, J = 2.8 Hz, 3H), 1.31 (s, 3H), 1.32 (s, 3H), 1.36 (s, 3H), 1.41–1.47 (m, 2H), 1.74–1.87 (m, 4H), 2.04–2.09 (m, 1H), 2.26 (s, 6H), 2.74–2.87 (m, 2H), 3.01 (s, 3H), 3.48–3.55 (m, 1H), 3.56–3.62 (m, 1H), 4.11–4.15 (m, 1H), 4.18 (d, J = 2.4 Hz, 1H), 4.52 (d, J = 7.2 Hz, 1H), 4.93 (t, J = 13.6 Hz, 1H), 5.01–5.05 (m, 1H), 5.33 (s, 1H), 5.46 (s, 1H), 7.42 (t, J = 7.4 Hz, 2H), 7.54 (t, J = 7.4 Hz, 1H), 8.03 (d, J = 7.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 9.5, 14.0, 14.9, 16.3, 19.9, 20.5, 21.1, 23.5, 23.9, 26.7, 31.6, 33.8, 34.6, 40.3, 48.5, 49.9, 50.8, 63.4, 68.7, 71.3, 75.8, 77.9, 80.0, 80.4, 99.8, 101.9, 114.3, 127.8, 129.3, 130.2, 132.2, 141.7, 164.8, 168.6, 203.8. MS (ESI): 716.8 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{40}H_{62}NO_{10}$: 716.4368, Found: 716.4315.

4.4. (2S,3R,4S,6R)-4-(dimethylamino)-2-((1R,3R,6R,8R,9R,10R,12R,13S,17S)-3-ethyl-10-methoxy-6,8,10,12,15,15,17-heptamethyl-5,7-dioxo-3′,5′-dihydro-4,14,16-trioxaspiro[bicyclo[11.3.1]heptadecane-2,4′-pyrazole]-9-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**6a**)

Newly prepared CH_2N_2-ether solution (30 mL, CH_2N_2 15 mmol approx) was added to **5** (4.29 g, 6 mmol) in a tinfoil wrapped three-neck flask at 0 °C. The solution was stirred for 5 h and then warmed to 25 °C. After stirring for 44 h, the solution was dried over Na_2SO_4, filtered, and concentrated. The residue was purified on a silica gel (8:1 petroleum ether-acetone with 1% Et_3N) to give **6a** (4.08 g, 89.0%) as a white foam. ^{1}H NMR ($CDCl_3$, ppm) δ 0.81 (t, J = 7.5 Hz, 3H), 0.85–0.88 (m, 2H), 0.96 (d, J = 7.2 Hz, 3H), 1.01 (d, J = 6.6 Hz, 3H), 1.10–1.11 (m, 4H), 1.14 (s, 3H), 1.19 (s, 3H), 1.26–1.30 (m, 4H), 1.32 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.41–1.51 (m, 3H), 1.76–1.80 (m, 2H), 1.88–1.94 (m, 1H), 3.55–3.60 (m, 1H), 4.09 (d, J = 6.8 Hz, 1H), 4.41–4.62 (m, 4H), 5.00–5.05 (m, 1H), 5.37–5.40 (m, 1H), 7.44 (t, J = 8.3 Hz, 2H), 7.56 (t, J = 7.3 Hz, 1H), 8.03 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 9.6, 11.1, 15.5, 17.8, 19.0, 20.1, 21.6, 23.9, 24.7, 27.0, 32.1, 32.6, 35.6, 40.2, 41.2, 44.9, 50.7, 64.1, 69.4, 70.8, 72.5, 75.3, 75.7, 79.6, 80.7, 81.1, 83.4, 100.5, 101.2, 102.1, 128.6, 130.3, 131.0, 133.1, 165.9, 175.7. MS (ESI): 758.8 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{41}H_{63}N_3O_{10}$: 757.4513, Found: 757.4504.

4.5. (2S,3R,4S,6R)-4-(dimethylamino)-2-((1R,3R,6R,8R,9R,10R,12R,13S,17S)-3-ethyl-10-methoxy-3′,6,8,10,12,15,15,17-octamethyl-5,7-dioxo-3′,5′-dihydro-4,14,16-trioxaspiro[bicyclo[11.3.1]heptadecane-2,4′-pyrazole]-9-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**6b**)

Following the synthesis of compound **6a** using compound **5** (4.29 g, 6 mmol) and newly prepared diazoethane-ether solution (120 mL, CH_3CHN_2 60 mmol approx) yielded compound **6b** (4.53 g,

98.2%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.76 (t, J = 4.9 Hz, 3H), 0.82–1.16 (m, 10H), 1.19 (s, 3H), 1.25 (s, 6H), 1.27–1.53 (m, 15H), 1.70–1.85 (m, 3H), 2.13 (s, 3H), 2.26 (s, 6H), 2.71–2.75 (m, 1H), 2.82–2.87 (m, 1H), 2.90 (s, 3H), 3.44–3.49 (m, 1H), 3.54–3.59 (m, 1H), 4.05–4.09 (m, 1H), 4.47–4.59 (m, 2H), 5.01 (t, J = 7.4 Hz, 1H), 7.42(t, J = 8.3 Hz, 2H), 7.54 (t, J = 7.3 Hz, 1H), 8.00 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 10.6, 14.4, 17.8, 18.0, 18.5, 20.9, 21.6, 23.3, 24.0, 26.6, 29.6, 30.5, 32.4, 34.1, 35.2, 40.6, 49.3, 50.2, 50.9, 63.7, 69.0, 69.9, 75.2, 78.0, 80.4, 81.2, 88.8, 100.4, 102.0, 102.2, 128.2, 129.7, 130.4, 132.7, 165.1, 168.8, 203.7. MS (ESI): 772.9 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{42}H_{66}N_3O_{10}$: 772.4743, Found: 772.4705.

*4.6. (1R,3R,6R,8R,9R,10R,12R,13S,17S)-ethyl-9-((2S,3R,4S,6R)-3-(benzoyloxy)-4-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yloxy)-3-ethyl-10-methoxy-6,8,10,12,15,15,17-heptamethyl-5,7-dioxo-3′,5′-dihydro-4,14,16-trioxaspiro[bicyclo[11.3.1]heptadecane-2,4′-pyrazole]-3′-carboxylate (**6c**)*

Newly prepared ethyl diazoacetate-1,2- dichloroethane solution (18 mL, ethyl diazoacetate 10 mmol approx) was added to compound **5** (0.72 g, 1.01 mmol) in a tinfoil wrapped three-neck flask. Then the solution was heated at 84 °C for 25 h. The solution was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel (8:1 petroleum ether-acetone with 1% Et_3N) to give compound **6c** (0.53 g, 64%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.77–0.81 (m, 1H), 0.82–0.85 (m, 1H), 0.85–0.92 (m, 6H) 0.94 (d, J = 8.2 Hz, 2H), 0.97 (d, J = 7.0 Hz, 3H), 1.01–1.07 (m, 4H), 1.25 (s, 3H), 1.28 (t, J = 5.5 Hz, 9H), 1.33 (s, 6H),1.34–1.37 (m, 4H), 1.93 (s, 1H), 2.30 (s, 6H), 2.65–2.74 (m, 1H), 2.89 (s, 3H), 2.96–3.13 (m, 4H), 3.52 (dd, J = 13, 2, 6.0 Hz, 1H), 3.58–3.73 (m, 2H), 4.13 (d, J = 8.5 Hz, 1H), 4.29 (dd, J = 14.5, 7.5 Hz, 2H), 4.58–4.67 (m, 2H), 7.45 (t, J = 8.3 Hz, 2H), 7.58 (t, J = 7.3 Hz, 1H), 8.05 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 9.8, 10.7, 14.0, 14.2, 15.2, 16.3, 18.8, 19.1, 20.5, 22.6, 24.7, 26.4, 29.2, 29.5, 29.6, 31.8, 39.5, 41.5, 44.2, 47.1, 49.5, 51.8, 63.4, 68.5, 70.3, 70.9, 77.7, 78.2, 79.6, 92.1, 100.8, 114.5, 128.5, 129.9, 130.7, 133.7, 145.3, 163.5, 164.9, 169.1, 204.0. MS (ESI): 831.0(MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{44}H_{68}N_3O_{12}$: 830.4798, Found: 830.4790.

*4.7. (1R,3R,6R,8R,9R,10R,12R,13S,17S)-methyl-9-((2S,3R,4S,6R)-3-(benzoyloxy)-4-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yloxy)-3-ethyl-10-methoxy-6,8,10,12,15,15,17-heptamethyl-5,7-dioxo-3′,5′-dihydro-4,14,16-trioxaspiro[bicyclo[11.3.1]heptadecane-2,4′-pyrazole]-3′-carboxylate (**6d**)*

Following the synthesis of compound **6c** using compound **5** and newly prepared methyl diazoacetate-1,2- dichloroethane solution (18 mL, methyl diazoacetate 10 mmol approx) yielded compound **6d** (1.64 g, 69.7%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.74 (t, J = 7.5 Hz, 3H), 0.85 (d, J = 7.0 Hz, 3H), 0.98 (t, J = 6.7 Hz, 4H), 1.05 (d, J = 6.6 Hz, 4H), 1.10 (s, 3H), 1.15 (s, 3H), 1.25 (m, 11H), 1.44 (m, 3H), 1.80 (m, 3H), 2.13 (s, 1H), 2.24 (s, 6H), 2.71 (t, J = 8.3 Hz, 1H), 2.83 (m, 2H), 2.89 (s, 3H), 3.46 (q, J = 7.0 Hz, 1H), 3.57 (m, 1H), 4.05 (d, J = 9.9 Hz, 1H), 4.51 (m, 3H), 5.01 (m, 1H), 5.36 (m, 1H), 7.41 (t, J = 8.3 Hz, 2H), 7.53 (t, J = 7.3 Hz, 1H), 7.99 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 11.2, 13.6, 14.0, 16.9, 17.1, 19.1, 20.3, 20.8, 23.4, 23.6, 27.3, 29.5, 30.2, 32.5, 33.7, 34.0, 35.2, 41.3, 48.8, 50.0, 51.0, 52.0, 63.7, 65.5, 69.6, 71.4, 71.9, 77.9, 78.0, 80.1, 100.8, 101.4, 128.4, 129.7, 130.8, 133.1, 163.1, 165.1, 169.0, 204.2. MS (ESI): 816.9 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{43}H_{66}N_3O_{12}$: 816.4641, Found: 816.4641.

*4.8. (2S,3R,4S,6R)-4-(dimethylamino)-2-((6R,9R,11R,12R,13R,15R,16S,17S,18R)-6-ethyl-16,18-dihydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10-dioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-1-en-12-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**7a**)*

To a solution of **6a** (1.14 g, 1.50 mmol) in 2:1 acetonitrile/H_2O (15 mL) was added PPTS (1.89 g, 8.34 mmol), and the reaction was stirred for 20 h at 82 °C. Upon cooling, the solution was diluted with EtOAc (30 mL), washed with saturated $NaHCO_3$ (2 × 70 mL). The aqueous layer was extracted with EtOAc (2 × 30 mL), and the combined organic layer was washed with brine (70 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified on a silica gel (3:1 petroleum ether-acetone with 1% Et_3N) to give compound **7a** (0.96 g, 90%) as a white foam. ^{1}H NMR ($CDCl_3$, ppm) δ 0.82 (t, J = 7.2 Hz, 3H), 0.93 (d, J = 7.2 Hz, 3H), 0.97–1.02 (m, 5H), 1.06–1.1.08 (m, 1H), 1.22–1.31 (m, 9H), 1.34 (s, 2H), 1.38–1.1.48 (m, 2H), 1.75–1.84 (m, 2H), 1.92–1.97 (m,1H), 2.07–2.10 (m,1H), 2.17 (s, 1H), 2.27 (s, 6H), 2.43 (q, J = 1.4 Hz, 1H), 2.88–2.90 (m, 1H), 2.95 (s, 3H), 2.99–3.04 (m, 1H), 3.25–3.26 (m, 1H), 3.62–3.74 (m, 3H), 4.41 (d, J = 6.8 Hz, 1H), 4.55 (d, J = 7.6 Hz, 1H), 4.94 (d, J = 9.6 Hz, 1H), 5.02–5.06 (m, 1H), 5.50 (dd, J = 7.8, 2.6 Hz, 1H), 6.07 (s, 1H), 6.56 (s, 1H), 7.46 (t, J = 8.3 Hz, 2H), 7.58 (t, J = 7.3 Hz, 1H), 8.03 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 10.4, 13.2, 14.8, 15.6, 20.1, 20.7, 21.0, 22.9, 31.4, 32.3, 33.9, 35.5, 37.2, 40.7, 45.4, 50.2, 51.2, 63.6, 69.3, 69.5, 70.2, 76.8, 78.6, 79.3, 82.6, 101.4, 128.3, 128.5, 129.7, 130.5, 132.9, 141.0, 165.1, 170.0, 205.0. MS (ESI): 718.7 (MH^+). HRMS (ESI) m/z (MH^-). Calcd for $C_{38}H_{58}N_3O_{10}$: 716.4128 Found: 716.4090.

*4.9. (2S,3R,4S,6R)-4-(dimethylamino)-2-((6R,9R,11R,12R,13R,15R,16S,17S,18R)-6-ethyl-16,18-dihydroxy-13-methoxy-4,9,11,13,15,17-hexamethyl-8,10-dioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-1-en-12-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**7b**)*

Following the synthesis of compound **7a** using compound **6b** (2.28 g, 3.01 mmol) yielded compound **7b** (2.16 g, 83.2%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.82 (t, J = 7.2 Hz, 3H), 0.99 (d, J = 6.2 Hz, 2H), 1.07 (d, J = 6.7 Hz, 3H), 1.20–1.24 (m, 11H), 1.36 (s, 3H), 1.41–1.49 (m, 3H), 1.69–1.78 (m, 3H), 1.91–2.07 (m, 3H), 2.26 (s, 6H), 2.46 (s, 1H), 2.79 (d, J = 10.4 Hz, 1H), 2.96 (s, 3H), 2.81–3.05 (m, 2H), 3.22 (s, 1H), 3.60–3.75 (m, 2H), 3.79 (s, 1H), 3.86–4.05 (m, 1H), 4.36 (d, J = 7.0 Hz, 1H), 4.60 (d, J = 7.4 Hz, 1H), 4.95 (s, 1H), 5.04 (t, J = 8.3 Hz, 1H), 5.45 (d, J = 10.7 Hz, 1H), 6.10 (s, 1H), 6.47 (s, 1H), 7.44 (t, J = 8.2 Hz, 2H),7.61 (t, J = 7.3 Hz, 1H), 8.03 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) 13.2, 14.8, 15.3, 16.2, 16.9, 18.5, 19.6, 20.0, 21.4, 26.7, 29.2, 30.8, 33.6, 35.9, 37.9, 40.1, 42.5, 49.8, 50.3, 61.7, 68.9, 70.8, 74.4, 79.3, 80.5, 82.6, 85.1, 103.7, 127.3, 128.7, 128.8, 131.9, 150.2, 164.8, 168.9, 204.8. MS (ESI): 732.9 (MH^+). HRMS (ESI) m/z (MH^-). Calcd for $C_{39}H_{60}N_3O_{10}$: 730.4284, Found: 730.4249.

*4.10. (6R,9R,11R,12R,13R,15R,16S,17S,18R)-ethyl-12-((2S,3R,4S,6R)-3-(benzoyloxy)-4-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yloxy)-6-ethyl-16,18-dihydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10-dioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-3-ene-1-carboxylate (**7c**)*

Following the synthesis of compound **7a** using compound **6c** (1.0 g, 1.20 mmol) yielded compound **7c** (0.82 g, 85.6%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.80 (t, J = 4.9 Hz, 3H), 0.84–0.87 (m, 2H), 0.91 (d, J = 7.2 Hz, 3H), 0.96 (dd, J = 13.5, 7.1 Hz, 5H), 1.28 (s, 3H), 1.30 (t, J = 2.7 Hz, 6H), 1.33–1.36 (m, 7H), 1.39–1.48 (m, 2H), 1.79–1.85 (m, 3H), 2.27 (s, 6H), 2.81–2.89 (m, 1H), 2.95 (s, 3H), 3.00 (t, J = 7.2 Hz, 1H), 3.27–3.32 (m, 1H), 3.62–3.68 (m, 1H), 3.73 (q, J = 6.7 Hz, 1H), 3.80 (s, 1H), 4.26–4.31 (m, 2H), 4.44 (d, J = 7.0 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 5.03 (dd, J = 10.4, 7.6 Hz, 1H), 5.32 (d, J = 10.2 Hz, 1H), 5.49 (dd, J = 11.0, 2.6 Hz, 1H), 6.86 (s, 1H), 7.45 (t,

J = 8.3 Hz, 2H), 7.55 (t, J = 7.3 Hz, 1H), 8.04 (d, J = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 10.2, 13.8, 14.3, 14.8, 15.8, 19.8, 20.7, 21.1, 22.6, 30.4, 32.5, 33.6, 34.2, 34.8, 40.8, 45.8, 49.9, 51.2, 61.1, 63.6, 69.3, 70.5, 72.1, 73.2, 75.5, 79.7, 82.6, 101.4, 128.4, 129.7, 130.4, 132.9, 138.8, 163.0, 165.2, 169.6, 205.1. MS (ESI): 791.0 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{41}H_{64}N_3O_{12}$: 790.4485, Found: 790.4482.

*4.11. (6R,9R,11R,12R,13R,15R,16S,17S,18R)-methyl-12-((2S,3R,4S,6R)-3-(benzoyloxy)-4-(dimethylamino)-6-methyltetrahydro-2H-pyran-2-yloxy)-6-ethyl-16,18-dihydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10-dioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-3-ene-1-carboxylate (**7d**)*

Following the synthesis of compound **7a** using compound **6d** (1.44 g, 1.76 mmol) yielded compound **7d** (1.12 g, 81.9%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.74 (t, J = 4.7 Hz, 3H), 0.76–0.81 (m, 2H), 0.83 (d, J = 7.2 Hz, 3H), 0.89 (dd, J = 13.3, 6.9 Hz, 5H), 1.15 (t, J = 2.6 Hz, 2H), 1.17–1.39 (m, 12H), 1.72–1.75 (m, 3H), 2.06–2.11 (m, 1H), 2.26 (s, 6H), 2.75–2.84 (m, 2H), 2.88 (s, 3H), 3.22–3.25 (m, 1H), 3.58–3.73 (m, 3H), 3.76 (s, 3H), 4.04 (s, 1H), 4.35 (d, J = 6.8 Hz, 1H), 4.47 (d, J = 7.5 Hz, 1H), 4.97 (dd, J = 10.4, 7.5 Hz, 1H), 5.29 (d, J = 9.8 Hz, 1H), 5.42 (dd, J = 10.8, 2.4 Hz, 1H), 6.86 (s, 1H), 7.39 (t, J = 8.2 Hz, 2H), 7.51 (t, J = 7.2 Hz, 1H), 7.96 (d, J = 6.7 Hz, 2H). ^{13}C NMR ($CDCl_3$, ppm) δ 10.2, 13.8, 14.8, 15.8, 19.8, 20.7, 21.0, 22.6, 31.6, 32.5, 33.7, 34.2, 35.1, 40.8, 45.8, 50.0, 51.1, 52.0, 63.6, 69.3, 70.5, 71.9, 73.3, 75.5, 77.3, 79.7, 82.6, 101.5, 128.4, 129.7, 130.4, 132.9, 138.4, 163.7, 165.1, 169.6, 205.0. MS (ESI): 777.0 (MH^+). HRMS (ESI) m/z (MH^-). Calcd for $C_{40}H_{60}N_3O_{12}$: 774.4182, Found: 774.4147.

*4.12. (2S,3R,4S,6R)-4-(dimethylamino)-2-((6R,9R,11R,12R,13R,15R,17R,18R)-6-ethyl-18-hydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10,16-trioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-1-en-12-yloxy)-6-methyltetrahydro-2H-pyran-3-yl benzoate (**8a**)*

To a solution of **7a** (0.54 g, 0.75 mmol) in CH_2Cl_2 (8 mL) at −5 °C was added Dess-Martin periodinane (0.38 g, 0.90 mmol). The solution was stirred for 20 h, and more Dess-Martin periodinane (22 mg, 0.04 mmol) was added. After stirring for 8 h, the solution was diluted with EtOAc (170 mL) and washed with 1:1 saturated $NaHCO_3$/10% $Na_2S_2O_3$ (2 × 35 mL). The combined aqueous layer was back extracted with EtOAc (4 × 35 mL) and the combined organic layer were then washed with brine (35 mL), dried over Na_2SO_4, filtered, and concentrated. To the crude material was added CH_3OH (2 mL) and the solution was stirred for 15 h at 65 °C. Upon concentration, the residue was purified on a silica gel (3:1 petroleum ether-acetone with 1% Et_3N) to give compound **8a** (0.20 g, 42.5%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) 0.78 (t, J = 7.4 Hz, 3H), 1.13 (d, J = 7.6 Hz, 3H), 1.18–1.26 (m, 11H), 1.29 (d, J = 6.9 Hz, 3H), 1.35 (s, 3H), 1.58–1.67 (m, 3H), 1.77 (s, 3H), 2.01–2.09 (m, 1H), 2.12 (s, 1H), 2.31–2.36 (m, 1H), 2.41–2.47 (m, 1H), 2.64 (d, J = 18.8 Hz, 1H), 2.81 (s, 3H), 2.86–3.00 (m, 2H), 3.13–3.18 (m, 2H), 3.12–3.21 (m, 2H), 3.40–3.57 (m, 2H), 4.01 (d, J = 10.2 Hz, 1H), 4.26 (d, J = 7.3 Hz, 1H), 5.02 (dd, J = 11.2, 2.1 Hz, 1H), 5.88 (s, 1H). ($CDCl_3$) δ. ^{13}C NMR ($CDCl_3$, ppm) δ 7.8, 10.1, 10.4, 11.8, 13.5, 19.2, 20.0, 20.3, 29.2, 30.7, 38.1, 39.4, 40.2, 40.8, 42.3, 42.9, 49.3, 49.9, 50.4, 62.0, 68.9, 69.9, 70.8, 79.1, 81.2, 101.3, 148.9, 162.4, 201.8, 202.7. MS (ESI): 612.9 (MH^+). HRMS (ESI) m/z (MH^-). Calcd for $C_{31}H_{52}N_3O_9$: 610.3849, Found: 610.3844.

*4.13. (6R,9R,11R,12R,13R,15R,17R,18R)-12-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-6-ethyl-18-hydroxy-13-methoxy-4,9,11,13,15,17-hexamethyl-7-oxa-2,3-diazaspiro[4.13]octadeC-1-ene-8,10,16-trione (**8b**)*

Following the synthesis of compound **8a** using compound **7b** (0.73 g, 1.01 mmol) yielded compound **8b** (0.25 g, 39.6%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.76 (t, J = 7.7 Hz, 3H), 0.79–0.88 (m, 2H), 0.91 (d, J = 7.2 Hz, 3H), 0.94–1.00 (m, 2H), 1.11 (t, J = 6.9 Hz, 1H), 1.15 (d, J = 7.5 Hz, 3H), 1.22 (d, J = 6.9 Hz, 3H), 1.26–1.33 (m, 5H), 1.37–1.45 (m, 4H), 1.53–1.66 (m, 2H), 1.67 (s, 3H), 1.79–1.82 (m, 1H), 2.01–2.12 (m, 1H), 2.28 (s, 6H), 2.62–2.65 (m, 1H), 2.68 (s, 1H), 2.85 (s, 3H), 2.87–3.04 (m, 2H), 3.15 (t, J = 8.0 Hz, 1H), 3.44 (q, J = 6.9 Hz, 1H), 3.54–3.61 (m, 1H), 4.06 (d, J = 10.1 Hz, 1H), 4.50 (d, J = 7.6 Hz, 1H), 5.02–5.08 (m, 1H), 6.90 (s, 1H). ^{13}C NMR ($CDCl_3$, ppm) δ 8.1, 10.4, 11.2, 11.7, 15.2, 18.6, 19.0, 20.7, 20.8, 21.9, 29.1, 30.4, 31.7, 39.4, 41.1, 43.0, 50.6, 50.9, 52.6, 65.2, 66.7, 70.3, 71.2, 73.0, 78.7, 83.9, 116.8, 149.1, 159.7, 204.6, 206.1. MS (ESI): 626.9 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{32}H_{56}N_3O_9$: 626.3972, Found: 626.4007.

*4.14. (6R,9R,11R,12R,13R,15R,17R,18R)-ethyl-12-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-6-ethyl-18-hydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10,16-trioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-3-ene-1-carboxylate (**8c**)*

Following the synthesis of compound **8a** using compound **7c** (0.79 g, 1.00 mmol) yielded compound **8c** (0.33 g, 48.1%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.83 (t, J = 7.5 Hz, 3H), 0.84–0.86 (m, 2H), 0.88 (d, J = 7.2 Hz, 3H), 1.18 (d, J = 7.5 Hz, 4H), 1.20–1.25 (m, 10H), 1.34 (t, J = 7.0 Hz, 5H), 1.40 (s, 3H), 1.64–1.70 (m, 2H), 1.84 (s, 2H), 2.27 (s, 6H), 2.36–2.46 (m, 2H), 2.82 (s, 3H), 2.88–2.94 (m, 2H), 3.17–3.27 (m, 3H), 3.57 (q, J = 6.9 Hz, 2H), 3.68 (s, 1H), 4.05 (d, J = 10.2 Hz, 1H), 4.32 (t, J = 7.5 Hz, 1H), 5.08–5.11 (m, 1H), 6.95 (s, 1H). ^{13}C NMR ($CDCl_3$, ppm) δ 10.1, 10.2, 14.6, 15.4, 18.3, 18.8, 20.0, 21.2, 22.4, 27.6, 28.2, 29.7, 39.0, 40.3, 42.5, 47.3, 47.7, 50.9, 51.1, 65.8, 69.3, 69.6, 70.3, 74.7, 78.8, 79.8, 85.7, 104.0, 154.9, 168.5, 169.4, 203.9, 217.4. MS (ESI): 684.9 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{34}H_{58}N_3O_{11}$: 684.4027 Found: 684.4010.

*4.15. (6R,9R,11R,12R,13R,15R,17R,18R)-methyl-12-((2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyltetrahydro-2H-pyran-2-yloxy)-6-ethyl-18-hydroxy-13-methoxy-9,11,13,15,17-pentamethyl-8,10,16-trioxo-7-oxa-2,3-diazaspiro[4.13]octadeC-3-ene-1-carboxylate (**8d**)*

Following the synthesis of compound **8a** using compound **7d** (0.78 g, 1.03 mmol) yielded compound **8d** (0.30 g, 44.7%) as a white solid. ^{1}H NMR ($CDCl_3$, ppm) δ 0.83–0.91 (m, 4H), 0.95 (d, J = 7.0 Hz, 2H), 1.14 (t, J = 5.75 Hz, 3H), 1.23–1.27 (m, 3H), 1.30–1.37 (m, 3H), 1.40 (d, J = 8.5 Hz, 2H), 1.61 (d, J = 13.5 Hz, 2H), 1.66–1.68 (m, 2H), 1.76 (s, 3H), 1.87–1.93 (m, 2H), 2.06–2.14 (m, 2H), 2.27 (s, 6H), 2.43–2.49 (m, 1H), 2.69 (s, 1H), 2.77 (s, 3H), 2.85–2.89 (m, 2H), 2.98–3.01 (m, 2H), 3.18–3.21 (m, 1H), 3.51–3.58 (m, 2H), 3.84 (s, 3H), 4.00 (d, J = 10.5 Hz, 1H), 4.31 (t, J = 9.0 Hz, 2H), 4.80 (d, J = 11.5 Hz, 1H), 4.95 (dd. J = 10.2, 3.0 Hz, 1H), 6.97 (s, 1H). ^{13}C NMR ($CDCl_3$, ppm) δ 9.9, 11.4, 14.8, 18.1, 19.6, 21.1, 25.2, 27.2, 29.7, 38.2, 40.2, 42.1, 43.9, 45.3, 47.6, 49.7, 51.2, 52.3, 65.9, 71.3, 74.7, 78.2, 81.1, 83.5, 88.1, 104.6, 151.6, 162.8, 168.2, 203.3, 218.5. MS (ESI): 670.8 (MH^+). HRMS (ESI) m/z (MH^+). Calcd for $C_{33}H_{56}N_3O_{11}$: 670.3909, Found: 670.3927.

Acknowledgement

This work was financially supported by the Science Foundation of Guangdong Province (06025150).

Appendix. Supplementary data

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.ejmech.2010.09.060.

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