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# Clarithromycin–adenine and related conjugates

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**Abstract**—Macrolide–nucleoside and macrolide–nucleobase conjugates (chimeras) have been synthesised by linking erythromycin A oxime derivatives and clarithromycin C11,C12-oxazolidinone derivatives with 3′-amino-3′-deoxythymidine or adenine through different spacers; clarithromycin–adenine conjugate **16a** is the most active species against *Micrococcus luteus*.
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Erythromycin-related macrolides are among the safest and most effective antibiotics for the treatment of respiratory infections. Although erythromycin A (EA, **1a**) is unstable in the acidic media of the stomach (owing to the well-known formation of hemiketals involving C9) and causes gastrointestinal side effects, semi-synthetic derivatives such as clarithromycin (**1b**) and roxithromycin (**1c**), a derivative of EA oxime (**1d**), overcome most of these problems.[1] However, the development of resistance to these antibiotics is on the rise. Although certain modifications of the macrolide backbone confer activity against macrolide-resistant pathogens,[2] better antibiotics are still needed.[3] Preparation of conjugates and hybrid compounds (or chimeras) is one of the newest strategies that are currently being investigated.[4] Since macrolide antibiotics inhibit bacterial protein synthesis by selectively binding to ribosomal RNA,[1b,5] we felt that conjugation of a macrolide with a nucleoside or nucleotide could enhance the affinity for the ribosome by hydrogen bonding and/or electrostatic interactions. Third-generation macrolide antibiotics such as telithromycin[6] (Fig. 1) or ABT-773[7] already use a similar strategy, incorporating heteroaromatic rings capable of further interactions with the ribosome; these rings appear to be crucial for activity against macrolide–lincosamide–streptogramin B ($MSL_B$) resistant strains.

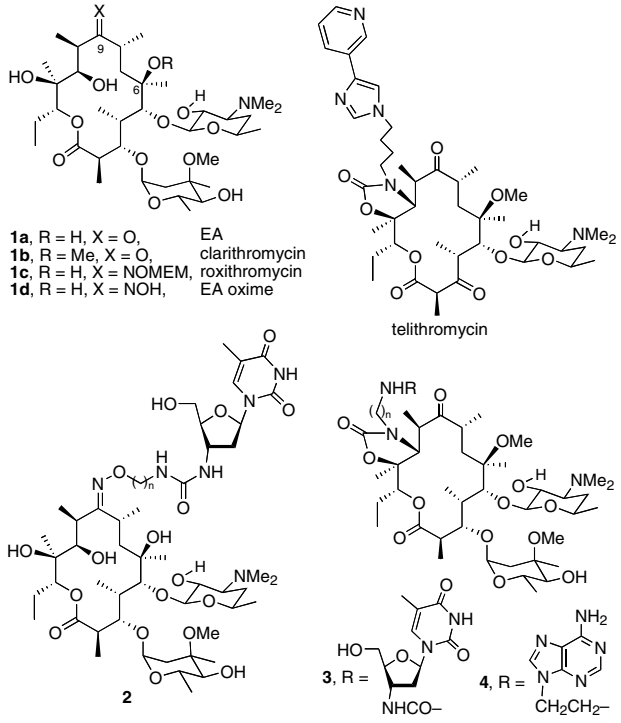


**Figure 1.**

We report here the synthesis of compounds **2**–**4**. In the series **2a**–**c**, the erythromycin backbone has been linked through an oxime ether to a nucleoside;[8] as a first approach, a thymidine was chosen. In clarithromycin derivatives, the link between the antibiotic and

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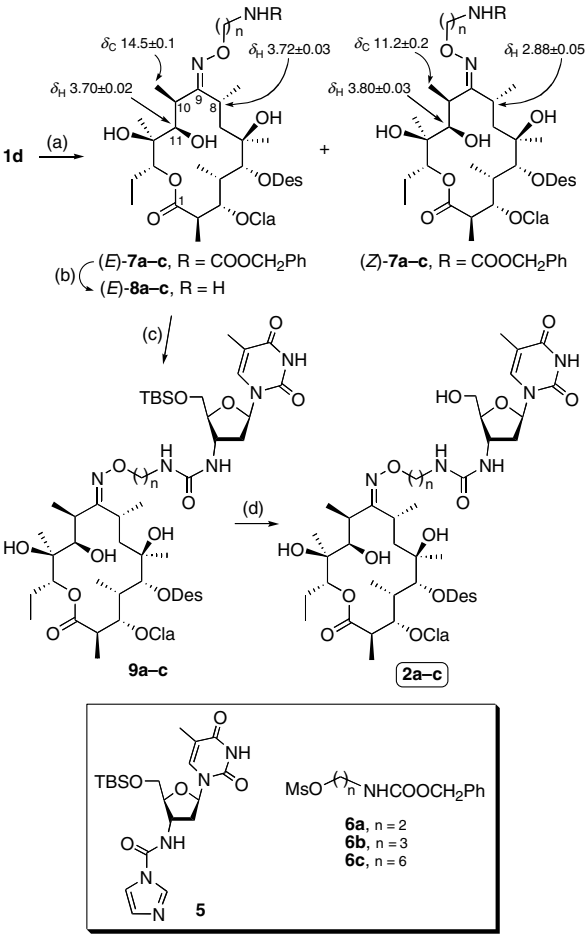
nucleoside (see **3a**–**c**) and between the antibiotic and adenine (see **4a**,**b**) has been established through a cyclic carbamate, or oxazolidinone, at positions C11,C12.[9,10]

For the preparation of **2** (Scheme 1), we envisaged the coupling of the thymidine derivative **5** prepared[11] from AZT with a suitably alkylated erythromycin oxime. Mesylates **6a**–**c** with different chain lengths were chosen as alkylating agents. Alkylation of the oxime **1d** was attempted under a variety of conditions (e.g., with MeO-Na in DMF and with $Na_2CO_3$ in EtOH) but no oxime ether could be isolated. However, when the reaction was performed with $K_2CO_3$ as the base and acetone as the solvent, the desired oximes (*E*)-**7a**–**c** were obtained in good yields. The corresponding (*Z*)-oximes, arising from the partial isomerisation of EA (*E*)-oxime in the basic reaction medium,[12] were also isolated.

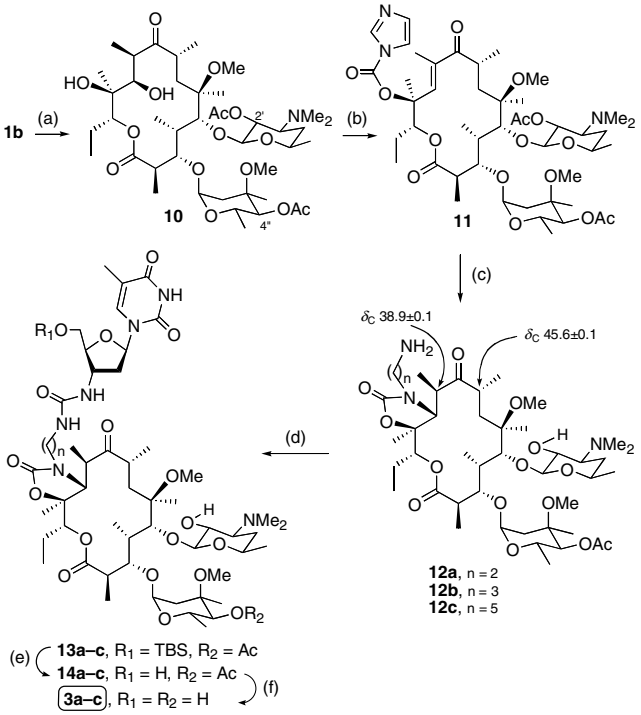
The *E* or *Z* configuration of **7a**–**c** was determined by careful comparison of their $^1H$ and $^{13}C$ NMR spectra with those of (*E*)- and (*Z*)-oximes of **1d**[12,13] and **1b**.[14] The proton H11 appears at a lower field for the *Z*-oxime ethers due to the deshielding effect of the alcoxyimino group. The same effect causes H8 to be deshielded in the *E* compounds, in relation to the *Z* ones (Scheme 1). Furthermore, the $^{13}C$ NMR chemical shift for 10-Me is characteristic: it appears at ca. 15 ppm in the (*E*)-oxime ethers, and at ca. 11 ppm for the (*Z*)-oxime ethers. This is most probably due to a shielding effect caused by the steric interaction between the alcoxyimino group and 10-Me.[12,15]

Removal of the benzyloxycarbonyl group of the major compounds, (*E*)-**7a**–**c**, by catalytic hydrogenation afforded the free amines **8a**–**c** in quantitative yield. These amines were coupled with thymidine derivative **5** in $CH_2Cl_2$–DMF to yield protected chimeras **9a**–**c**. Removal of the TBS group was accomplished by treatment with HF–pyridine in THF as the solvent to give the desired **2a**–**c**.

Chimeras **3a**–**c** were prepared by coupling amines **12a**–**c** with thymidine **5**, as shown in Scheme 2. The clarithromycin 2′,4″-diacetate, **10**, was converted to 12-*O*-imidazolylcarbonyl macrolide **11** by treatment with an excess of 1,1′-carbonyldiimidazole and NaH in DMF–THF. This transformation proceeds through a 11,12-cyclic carbonate intermediate.[9a] The 11,12-carbamate group (oxazolidinone ring) was introduced by treating crude **11** with the corresponding diamines followed by deprotection of the 2′-acetate. Only the natural 10*R* epimer was formed. For **12a**–**c**, the stereochemistry at C10 was determined by NMR spectroscopy. Previous studies showed that the $^{13}C$ signal for C10 is diagnostic:[9a] it




**Scheme 1.** Reagents and conditions: (a) **6a**–**c**, $K_2CO_3$, acetone, reflux, 24 h, 50–65% of (*E*)-oxime plus 15–25% of (*Z*)-oxime; (b) $H_2$, Pd/C, MeOH, rt, 1.5–4 h, 94–99%; (c) **5**, $CH_2Cl_2$, DMF, rt, 2–3 h, 72–80%; (d) HF–pyridine, THF, 0–4 °C, 14–24 h, 76–84%.




**Scheme 2.** Reagents and conditions: (a) $Ac_2O$, $Et_3N$, DMAP, $CH_2Cl_2$, rt, 17 h, 99%; (b) $Im_2CO$, NaH, DMF, THF, rt, 4 h; (c) $H_2N(CH_2)_nNH_2$ ($n = 2$, 3, 5), $CH_3CN$, rt, 18 h, then MeOH, reflux, 4 h, 73–90% (overall, for the three last steps); (d) **5**, DMF, $CH_2Cl_2$, rt, 4 h, 80–86%; (e) HF–pyridine, THF, 0–4 °C, 18 h, 92–97%; (f) $NH_4OH$, MeOH, rt, 4 d, 77–91%.

appears at ca. 37 ppm in the natural isomer, whereas it is shifted to ca. 52 ppm for the unnatural 10*S* epimer.

Coupling of amines **12a–c** with **5** took place smoothly in $CH_2Cl_2$–DMF. Removal of the TBS group (to convert **13a–c** into **14a–c**, respectively), followed by treatment with aqueous ammonia to cleave the 4″-OAc groups, furnished the desired compounds **3a–c**.

Compounds **2a–c**, **12a–c**, **13a–c**, **14a–c** and **3a–c** were tested against *Micrococcus luteus* ATCC 9341 by the standard agar dilution method.[16] All compounds showed weak antibacterial activity (Table 1). The most active ones were amines **12a–c**, although with potencies below 40% of that of the reference antibacterial agent (EA, **1a**). Under identical conditions the value that we have determined for clarithromycin, **1b**, is 122% and that for azithromycin is 110%. Long-chain spacers ($n = 6$ for the case of **2c** and its precursors, $n = 5$ for the case of **3c** and its precursors) did not afford any advantage. All these side chains seem to be too bulky. Perhaps they also lack basic centres that could be required for the interaction with the nucleotides in the peptidyl-transferase tunnel.[5]

Thus, we set out to synthesise macrolide–adenine chimeras **4a**,**b**, in which the link between the two components is through an amine moiety. They were prepared by reductive amination of amines **12a**,**b** with the hydrate of aldehyde **15**,[17] followed by hydrolysis of the OAc group (Scheme 3).

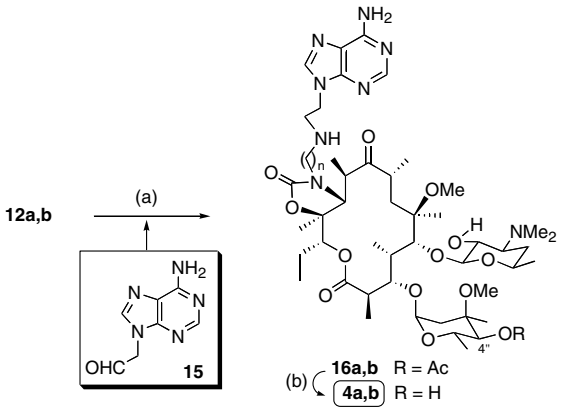
Compounds **4a**,**b** and their precursors **16a**,**b** were tested against *M. luteus* ATCC 9341, as above indicated.[16] The values for their biological activity are also shown in Table 1. Among all the samples tested so far, 4″-*O*-acetyl derivative **16a** showed the highest potency (Table 1, entry 10), although it is still lower than desired. A spacer with an additional $CH_2$ (from $n = 2$–3) is detrimental; a HB donor at 4″ (4″-OH) is also detrimental (entry 11) with regard to its 4″-OAc derivative (entry 10).

The antimycobacterial activities of compounds **2a–c** and **3a–c** were checked against *Mycobacterium tuberculosis* $H_{37}R_v$.[18] Inhibition percentages around 73–83% were

**Table 1.** Percentage of potency against *Micrococcus luteus*, in relation to EA, **1a**

| Entry | Compound | Percentage of activity of each series (%) | | |
|---|---|---|---|---|
| | | **a** | **b** | **c** |
| 1 | (*E*)-**7** | 30.6 | —[a] | —[a] |
| 2 | (*Z*)-**7** | 31.4 | —[a] | —[a] |
| 3 | (*E*)-**8** | 24.8 | —[a] | —[a] |
| 4 | (*E*)-**9** | 7.4 | 1.3 | 3.4 |
| 5 | **2** | 7.8 | 8.4 | 5.5 |
| 6 | **12** | 37.5 | 36.2 | 17.2 |
| 7 | **13** | 4.1 | 6.4 | 2.2 |
| 8 | **14** | 1.6 | 1.7 | 3.7 |
| 9 | **3** | 1.3 | 1.7 | 2.5 |
| 10 | **16** | 49.8 | 29.5 | — |
| 11 | **4** | 33.0 | 7.3 | — |

[a] Not determined.




**Scheme 3.** Reagents and conditions: (a) $NaBH_3CN$, $CH_3CN$–$H_2O$, pH 4–5, rt, 24 h, 42–50%; (b) $NH_4OH$, MeOH, rt, 3 d, 84–90%.

noted. Samples of **16a**,**b**, **4a** and **4b** have also been submitted to evaluation;[18] results will be reported in due course.

In summary, syntheses of erythromycin–thymidine chimeras **2a–c** and of clarithromycin–thymidine chimeras **3a–c** are disclosed. Clarithromycin–adenine conjugates **4a** and **4b** are also reported and characterised for the first time. The biological studies are preliminary but indicate some detrimental factors to be avoided if the discovery of new leads is pursued. Further biological tests as well as virtual screenings (by computational docking into the known ribosome structure of series of alternative conjugates) are underway.

## Acknowledgements

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## Supplementary data

Spectral data for compounds **2a–c**, **3a–c** and **4a**,**b** can be found in the Supplementary data associated with this article in the online version, at doi:doi:10.1016/j.tetlet.2006.01.079.

## References and notes

1. (a) Sunazuka, T.; Omura, S.; Iwasaki, S.; Omura, S. In *Macrolide Antibiotics: Chemistry, Biology and Practice*, 2nd ed.; Omura, S., Ed.; Academic Press, Elsevier: Orlando, 2002; pp 99–180; (b) Katz, L.; Ashley, G. W. *Chem. Rev.* **2005**, *105*, 499–527.
2. Ma, Z.; Clark, R. F.; Brazzale, R.; Wang, S.; Rupp, M. J.; Li, L.; Griesgaber, G.; Zhang, S.; Yong, H.; Tam Pham, L.; Nemoto, P. A.; Chu, D. T. W.; Plattner, J. J.; Zhang, X.; Zhong, P.; Cao, Z.; Nilius, A. M.; Shortridge, V. D.;

Flamm, R.; Mitten, M.; Meulbroek, J.; Ewing, P.; Alder, J.; Or, Y. S. *J. Med. Chem.* **2001**, *44*, 4137–4156.
3. (a) Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853–3874; (b) Walsh, C.; Wright, G. *Chem. Rev.* **2005**, *105*, 391–393.
4. Reviews: (a) Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996–4028; (b) Mehta, G.; Singh, V. *Chem. Soc. Rev.* **2002**, *31*, 324–334.
5. Schlünzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814–821.
6. (a) Agouridas, C.; Denis, A.; Auger, J.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. *J. Med. Chem.* **1998**, *41*, 4080–4100; (b) Denis, A.; Agouridas, C.; Auger, J.-M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Martret, O. L.; Loyau, V.; Tessot, N.; Pejac, J.-M.; Perron, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3075–3080; (c) Berisio, R.; Harms, J.; Schluenzen, F.; Zarivach, R.; Hansen, H. A. S.; Fucini, P.; Yonath, A. *J. Bacteriol.* **2003**, *185*, 4276–4279.
7. Or, Y. S.; Clark, R. F.; Wang, S.; Chu, D. T. W.; Nilius, A. M.; Flamm, R. K.; Mitten, M.; Ewing, P.; Alder, J.; Ma, Z. *J. Med. Chem.* **2000**, *43*, 1045–1049.
8. For reports of 9-oxime derivatives with good antibacterial activity, see: (a) Chantot, J. F.; Bryskier, A.; Gasc, J. C. *J. Antibiot.* **1986**, *39*, 660–668; (b) Denis, A.; Pejac, J.-M.; Bretin, F.; Bonnefoy, A. *Bioorg. Med. Chem.* **2003**, *11*, 2389–2394; (c) Kawashima, Y.; Yamada, Y.; Asaka, T.; Misawa, Y.; Kashimura, M.; Morimoto, S.; Ono, T.; Nagate, T.; Hatayama, K. *Chem. Pharm. Bull.* **1994**, *42*, 1088–1096; (d) Akemi, N.; Narita, K.; Ohmoto, S.; Takahashi, Y.; Yoshizumi, S.; Yoshida, T.; Kada, N.; Okezaki, E.; Kato, H. *Chem. Pharm. Bull.* **2001**, *49*, 1120–1127.
9. This ring is known to enhance antibacterial activity against resistant pathogens, by increasing the conformational rigidity of the macrolactone. See: (a) Baker, W. R.; Clark, J. D.; Stephens, R. L.; Kim, K. H. *J. Org. Chem.* **1988**, *53*, 2340–2345; (b) Fernandes, P. B.; Baker, W. R.; Freiberg, L. A.; Hardy, D. J.; MacDonald, E. J. *Antimicrob. Agents Chemother.* **1989**, *33*, 78–81.
10. For ketolides with heteroaromatic appendages (including two examples with adenine) linked via a thioether, see: Hunziker, D.; Wyss, P.-C.; Angehrn, P.; Mueller, A.; Marty, H.-P.; Halm, R.; Kellenberger, L.; Bitsch, V.; Biringer, G.; Arnold, W.; Stämpfli, A.; Schmitt-Hoffmann, A.; Cousot, D. *Bioorg. Med. Chem.* **2004**, *12*, 3503–3519.
11. Costa, A. M.; Vilarrasa, J. *Tetrahedron Lett.* **2000**, *41*, 3371–3375.
12. Wilkening, R. R.; Ratcliffe, R. W.; Doss, G. A.; Bartizal, K. F.; Graham, A. C.; Herbert, C. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1287–1292.
13. (a) Esteban, J.; Costa, A. M.; Urpí, F.; Vilarrasa, J. *Tetrahedron Lett.* **2004**, *45*, 5563–5567; (b) J. Esteban, Master Thesis, Universitat de Barcelona, 2002; (c) Wilkening, R. R. EP 0,503,932 A1, 1992.
14. Spectral data of clarithromycin (*E*)-oxime: $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ 0.84 (t, $J = 7.4$, 3H, Me15), 0.99 (d, $J = 6.8$, 3H, 8-Me), 1.08 (d, $J = 8.0$, 3H, 4-Me), 1.13 (s, 3H, 12-Me), 1.14 (d, $J = 8.0$, 3H, 10-Me), 1.20 (d, $J = 6.8$, 3H, 2-Me), 1.23 (d, $J = 6.0$, 3H, 5′-Me), 1.25 (s, 3H, 3″-Me), 1.31 (d, $J = 6.4$, 3H, 5″-Me), 1.48 (s, 3H, 6-Me), 1.48–1.61 (m, 4H, H7a+H7b+H14a+H2″a), 1.67 (m, 1H, H4′b), 1.89–1.98 (m, 2H, H4+H14b), 2.30 (s, 6H, $NMe_2$), 2.37 (d, $J = 15.2$, 1H, H2″b), 2.44 (m, 1H, H3′), 2.58 (q, $J = 7.2$, 1H, H10), 2.89 (dq, $J = 9.2$, $J = 7.2$, 1H, H2), 3.03 (m, 1H, H4″), 3.11 (s, 3H, 3″-OMe), 3.21 (dd, $J = 10.0$, 7.2, 1H, H2′), 3.33 (s, 3H, 6-OMe), 3.48 (m, 1H, H5′), 3.66 (d, $J = 7.2$, 1H, H5), 3.74–3.80 (m, 2H, H3+H8), 3.75 (s, 1H, H11), 4.03 (dq, $J = 9.2$, 6.4, 1H, H5″), 4.44 (d, $J = 7.2$, 1H, H1′), 4.94 (d, $J = 4.4$, 1H, H1″), 5.11 (dd, $J = 11.2$, 2.0, 1H, H13); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ 9.1 (4-Me), 10.6 (C15), 14.9 (10-Me), 16.0 (2-Me), 16.1 (12-Me), 18.6 (5″-Me), 18.7 (8-Me), 20.0 (6-Me), 21.2, 21.5, 21.5 (C14, 5′ -Me, 3″-Me), 25.4 (C8), 28.8 (C4′), 32.9 (C10), 34.9 (C2″), 37.4 (C7), 39.1 (C4), 40.3 ($NMe_2$), 45.1 (C2), 49.5 (3″-OMe), 51.2 (6-OMe), 65.5 (C3′), 65.7 (C5″), 68.6 (C5′), 70.2 (C11), 71.1 (C2′), 72.7 (C3″), 74.1 (C12), 76.9 (C13), 78.0 (C4″), 78.5 (C6), 78.8 (C3), 80.5 (C5), 96.1 (C1″), 102.8 (C1′), 170.9 (C9), 175.7 (C1). The assignments indicated throughout have been established or corroborated by 2D NMR experiments (HSQC and COSY).
(*Z*)-Oxime: $^1$H NMR ($CDCl_3$, 500 MHz): $\delta$ 0.80 (t, $J = 7.3$, 3H, Me15), 1.0–1.4 (m, 28H, H4′a+2-Me+4-Me+6-Me+8-Me+10-Me+12-Me+5′-Me+3″-Me+5″-Me), 1.40–1.50 (m, 2H, H7a+H14a), 1.51–1.57 (m, 2H, H7b+H2″a), 1.64 (m, 1H, H4′b), 1.86–1.95 (m, 2H, H4+H14b), 2.27 (s, 6H, $NMe_2$), 2.33 (d, $J = 15.0$, 1H, H2″b), 2.43 (ddd, $J = 12.5$, 10.5, 3.3, 1H, H3′), 2.55 (m, 1H, H10), 2.73 (m, 1H, H8), 2.84 (dq, $J = 9.3$, 7.3, 1H, H2), 2.98 (d, $J = 9.0$, 1H, H4″), 3.06 (s, 3H, 3″-OMe), 3.18 (dd, $J = 10.3$, 7.3, 1H, H2′), 3.33 (s, 3H, 6-OMe), 3.44 (m, 1H, H5′), 3.58 (d, $J = 7.5$, 1H, H5), 3.73 (d, $J = 9.5$, 1H, H3), 3.93 (br s, 1H, H11), 3.98 (dq, $J = 9.3$, 6.3, 1H, H5″), 4.39 (d, $J = 7.0$, 1H, H1′), 4.89 (d, $J = 4.5$, 1H, H1″), 5.04 (dd, $J = 11.0$, 2.0, 1H, H13); $^{13}$C NMR ($CDCl_3$, 75.4 MHz): $\delta$ 9.1 (4-Me), 10.6 (C15), 11.6 (10-Me), 15.9, 16.6 (2-Me, 12-Me), 18.6 (5″-Me), 19.8, 19.9 (8-Me, 6-Me), 21.3, 21.5, 21.5 (C14, 6-Me, 3″-Me), 28.9 (C4′), 34.2 (C10), 34.9 (C2″), 36.1 (C8), 37.4 (C7), 39.1 (C4), 40.2 ($NMe_2$), 45.2 (C2), 49.4 (3″-OMe), 50.2 (6-OMe), 65.4 (C3′), 65.6 (C5″), 68.6 (C5′), 70.5 (C11), 71.1 (C2′), 72.7 (C3″), 74.8 (C12), 76.6 (C13), 78.0 (C4″), 78.6 (C6), 78.9 (C3), 80.5 (C5), 96.1 (C1″), 102.8 (C1′), 167.1 (C9), 175.9 (C1).
15. McGill, J. M.; Johnson, R. *Magn. Res. Chem.* **1993**, *31*, 273–277.
16. The assays were performed in accordance with the Code of Federal Regulations guidelines: CFR Title 21, Part 436105.
17. Xu, Z.-Q.; Qiu, Y.-L.; Chokekijchai, S.; Mitsuya, H.; Zemlicka, J. *J. Med. Chem.* **1995**, *38*, 875–882.
18. Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF, Alabama, USA), cf. http://www.taacf.org.