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Hybrids of macrolides and nucleobases or nucleosides

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Abstract

A few examples of hybrids/conjugates/chimeras of erythromycin A derivatives and nucleobases (uracil and thymine) or thymidine-derived nucleosides are reported. Linkers and reaction conditions have been investigated to avoid the degradation of the macrolide moiety (glycoside hydrolysis, ring cleavage, dehydration, etc.).

Keywords: antibiotics; macrolides; uracils; uridines.

The need for more potent and selective therapeutic agents and the fight against new antibiotic- or antiviral-resistant strains are always forcing pharmaceutical companies to search for novel types of bioactive molecular structures. Coupling of two or more different natural products, aimed at obtaining 'unnatural' molecules (hybrids, chimeras or conjugates) with a possible cooperative effect, is an approach which has been investigated by many research groups, often with success.[1,2] In this connection, the recognition ability of nucleobases has been used to design hybrids with a great variety of molecules such as porphyrins,[3] β-lactam antibiotics,[4] steroids,[5] or peptides;[6] some of these conjugates have found applications in fields such as molecular recognition, inhibition of gene expression, or anti-cancer and anti-virus therapies.

Taking into account the remarkable usefulness, nowadays, of the marketed erythromycin A-derived macrolides, viz. azithromycin, clarithromycin, and roxithromycin,[7] we started a project aimed at preparing hybrids of erythromycins and nucleosides.[8] In this preliminary communication, syntheses and features of compounds **1**–**4** are reported.

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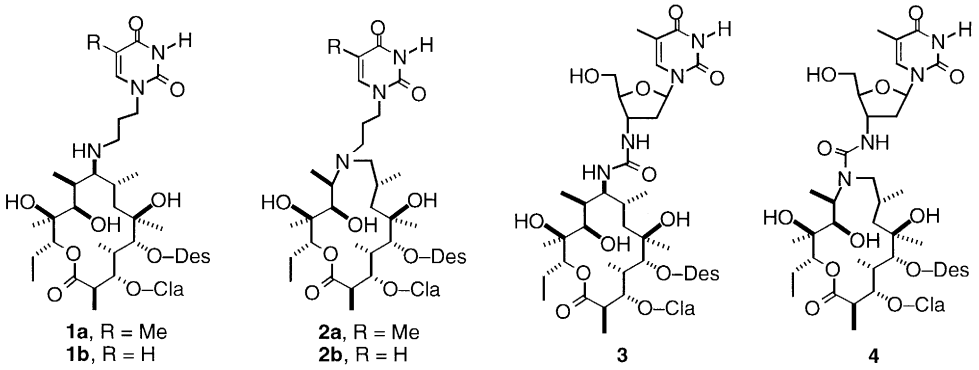
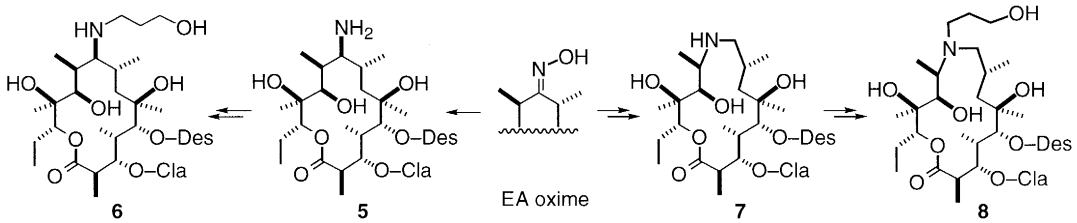


Owing to the well-known instability of erythromycin derivatives in acidic media (formation of internal hemiacetals or acetals if the CO group at C9 has not been modified, quick hydrolysis of the L-cladinose moiety followed by a slower release of D-desosamine, translactonisation, etc.) and to basic media (ring opening, retro-aldol reaction, etc.),[9] the essential point was to link the macrolide and nucleoside substructures by means of protocols that avoided deprotection steps or workups involving strong acidic or basic treatments.

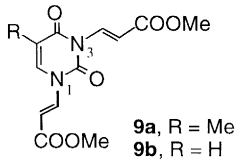
Macrolide components of hybrids **1** and **3**: Reduction of erythromycin A oxime (prepared from erythromycin A, EA, in 83% yield by using a very large excess of $HONH_3^+Cl^-$ in pyridine, at rt for 30 h) with $TiCl_3$ and $NaBH_3CN$, according to the procedure described by Leeds and Kirst,[10] gave (9*S*)-erythromycylamine A (**5**) in 65% yield. Treatment with acrolein (propenal) followed by in situ reduction with $NaBH_4$, as reported by Ryden et al.,[11] afforded (9*S*)-9-*N*-(3-hydroxypropyl)erythromycylamine (**6**) in 46% yield.

Macrolide components of hybrids **2** and **4**: In two synthetic steps from EA oxime (Beckmann-like rearrangement followed by reduction), according to the procedure of Djokic et al.,[12] we prepared in 45% overall yield 9a-aza-9-deoxo-9a-homoerythromycin A (**7**), the synthetic precursor of the antibiotic azitromycin (*N*-Me-**7**). Conversion of **7** into its *N*-3-hydroxypropyl derivative (**8**) was first tried by reaction with acrolein and reduction in situ, as in the previous case, without success, even when using a large excess of acrolein or by heating. On the other hand, reaction of **7** with methyl acrylate (methyl propenoate), used as the solvent, at 60°C for 30 h gave 84% of the conjugate (*hetero*-Michael) adduct. Reduction of the ester group with an excess of DIBALH gave only trace amounts of **8**. The best results were obtained with $LiAlH_4$ (3–4 mmol per mmol of substrate) in THF at 0°C for 1 h (ca. 65% of **8** and 10% of **7**, arising from a retro-addition in the basic medium, with 6–10% of the ester recovered); longer reaction times or larger amounts of $LiAlH_4$ were detrimental, with the appearance of more polar byproducts.

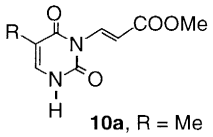


Protected nucleobases as components of hybrids **1** and **2**: We decided to use thymine and uracil protected at N3 with Mocvinyl groups,[13] because of their easy spectroscopic characterization and because this protecting group can be removed afterwards under very controlled conditions. Reaction of these nucleobases (10 mmol) with methyl propiolate (methyl propynoate, 22 mmol) and DMAP (5 mmol),

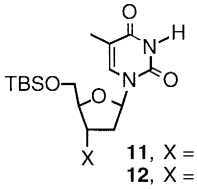
in CH_3CN (80 mL) at rt for 2 h, gave the bis-Mocvinyl derivatives **9a** and **9b**, respectively, in almost quantitative yields. To remove the group at N1, as pyrrolidine[13] turned out to be too reactive and unselective, controlled amounts of morpholine (2 mmol per mmol of **9a**) or of morpholine (1 mmol) plus DBU (0.1 mmol) were employed under dilute conditions (0.01–0.05 M in CH_3CN) at rt; thus, **10a**[14] and **10b** were isolated in ca. 60% yields by column chromatography, **9a** and **9b** being recovered in 20–25%.



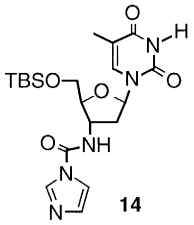
9a, R = Me
9b, R = H



10a, R = Me
10b, R = H



11, X = N_3
12, X = NH_2
13, X = NCO



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Thymidine derivatives as components of hybrids **3** and **4**: At the beginning we decided to study AZT-derived substrates as nucleoside components. Starting from 5′-*O*-*tert*-butyldimethylsilyl-AZT (**11**), it was envisaged that either the corresponding amine (**12**), isocyanate (**13**), or a synthetic equivalent (**14**) might be appropriate. Isocyanate **13** could not be prepared from amine **12** (the symmetrical urea was the major product); reaction of **11** with Me_3P/THF in the presence of an excess of CO_2 succeeded, but **13** was too unstable to be purified and stored. On the other hand, reaction of amine **12** with *N*,*N*′-carbonyldiimidazole in CH_2Cl_2 at 0°C gave **14** in 91% yield; this compound is stable and easy to purify.

Coupling of components: The Mitsunobu reaction between **6** and **10a** (2 equiv), in dioxan at rt for 1 h, afforded the desired Mocvinyl-protected **1a** in 87% yield.[15] It was supposed that secondary hydroxy groups of L-cladinose and D-desosamine moieties would not significantly compete with the primary alcohol of **6**, as found. Deprotection to **1a** was accomplished in 83% isolated yield with 4 equiv. of pyrrolidine in CH_3CN, at rt for 24 h.[16] Similarly, **6** and **10b** gave **1b**, **8** and **10a** yielded **2a**, and **8** and **10b** gave **2b**.

Coupling of **6** with **14**, and that of **8** with **14**, which would give carbamate-linked hybrids, did not proceed at rt, while in refluxing CH_3CN complex mixtures were formed.[17] On the other hand, coupling of amine **5** with **14** in CH_2Cl_2–DMF, at rt for 2 h, gave the urea in 80% yield, the TBS ether of which was cleaved with HF/pyridine in THF at 0°C to afford hybrid **3** in 75% isolated yield. Moreover, coupling of amine **7** with **14**, in CH_3CN–DMF at 50°C for 2 h, afforded, also after deprotection with HF/pyridine, the desired urea **4**,[18] in 50% overall yield.

Concluding remarks: The antibiotic activity of **1**–**4** has been screened against *Bacillus subtilis* ATCC6633, with a negative outcome (only between 4 and 13% of the azithromycin activity). Compounds **1a**,**b** and **2a**,**b** were checked for their anti-mycobacterial activity (at TAACF, Birmingham, AL), showing 80–83% of inhibition against *Mycobacterium tuberculosis* $H_{37}Rv$, and as antitumorals (at NCI, Bethesda), but none was active enough to pass to the next step. Compound **4** showed no activity against the HIV-1 (NL4-3) ‘wild-type’ strain, nor against the HIV-1 AZT-resistant strain. Other samples (final products and reaction intermediates) are under evaluation.

In summary, we have found suitable reaction conditions to link nucleobases and nucleosides to erythromycin A derivatives, without causing undesired reactions in the macrolide substructures. Although the preliminary results reported here are not exciting as far as the bioactivity is concerned, they are important from a synthetic point of view, as the feasibility of different routes is now established. Syntheses of alternative series of macrolide–nucleoside chimeras with longer linkers are in course in our lab, with the hope that some of these structures exhibit a dual-action behaviour, taking into account the features of their parent systems.

Acknowledgements

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14. Spectral data of 3-[(*E*)-2-(methoxycarbonyl)vinyl]thymine (**10a**): ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.80 (d, *J*=1.0, 3H, C6-Me), 3.69 (s, 3H, COOMe), 6.95 (d, *J*=14.7, 1H, C*H*COOMe), 7.41 (dq, *J*=5.1, *J*=1.2, 1H, H6), 8.18 (d, *J*=14.6, 1H, C*H*=CHCOOMe), 11.29 (d, *J*=5.1, 1H, NH); ^{13}C NMR (DMSO-d_6, 50.3 MHz): δ 12.6 (CH_3), 51.8 (CH_3), 107.4 (C), 110.7 (CH), 135.2 (CH), 137.6 (CH), 150.4 (CO), 163.2 (CO), 167.5 (CO); IR (KBr) ν 1750, 1715, 1650; CIMS *m/z* 228 $[M+NH_4]^+$.
15. To a suspension of **6** (71 mg, 0.090 mmol), Ph_3P (50 mg, 0.19 mmol), and **10a** (38 mg, 0.18 mmol) in dioxan (1–2 mL) was added dropwise DEAD (28 μL, 31 mg, 0.18 mmol) in 1 mL of dioxan. After stirring for 1 h, the solvent was evaporated and the products were separated by flash chromatography (from 95:5 CH_2Cl_2:MeOH to 95:5:2 CH_2Cl_2:MeOH:conc. ammonia) to give (9*S*)-9-*N*-{3-[3-(*E*)-2-(methoxycarbonyl)vinylthymin-1-yl]propyl}erythromycylamine A (76 mg, 87%).

16. Pyrrolidine (13 μL, 11 mg, 0.16 mmol) was added to a solution of Mocvinyl-protected **1a** (39 mg, 0.04 mmol) in CH_3CN (0.4 mL). After stirring for 24 h, the solvent was removed and the residue was taken with CH_2Cl_2 and water. The aqueous layer was acidified to pH 5.0, the layers were separated, and the aqueous phase was rinsed with CH_2Cl_2. Afterwards, the aqueous solution was basified to pH 9.0 by addition of 2 M NaOH and was extracted three times with CH_2Cl_2. These organic extracts were collected and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent in vacuo afforded chromatographically and spectroscopically pure (9*S*)-9-*N*-[3-(thymin-1-yl)propyl]erythromycylamine A, **1a** (30 mg, 83%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J*=7.5, 3H, Me-15), 1.01 (d, *J*=6.5, 3H, Me-8), 1.10 (d, *J*=6.5, 3H, Me-4), 1.11 (s, 3H, Me-12), 1.16 (d, *J*=7.0, 3H, Me-10), 1.20 (d, *J*=7.0, 3H, Me-2), 1.22 (d, *J*=6.0, 3H, Me-5′), 1.24 (s, 3H, Me-6 or Me-3′′), 1.26 (s, 3H, Me-3′′ or Me-6), 1.32 (d, *J*=6.5, 3H, Me-5′′), 1.46–1.58 (m, 2H, H14a, H7b), 1.58 (dd, *J*=15.3, *J*=5.1, 1H, H2′′a), 1.67 (ddd, *J*=12.6, *J*=3.8, *J*=2.1, 1H, H4′b), 1.92 (d, *J*=1.0, 3H, Me-thym.), 1.87–1.99 (m, 4H, H4, H14b, $CH_2CH_2CH_2$), 2.10–2.27 (m, 4H, H8, H9, H10, 4′′-OH), 2.29 (s, 6H, NMe_2), 2.38 (d, *J*=15.0, 1H, H2′′b), 2.44–2.55 (m, 2H, H3′, $NHCH_aH_b$), 2.71–2.79 (m, 1H, $NHCH_aH_b$), 2.89 (dq, *J*=7.9, *J*=7.0, 1H, H2), 3.03 (t, *J*=8.7, 1H, H4′′), 3.25 (dd, *J*=10.3, *J*=7.1, 1H, H2′), 3.32 (s, 3H, MeO-3′′), 3.50 (dqd, *J*=10.6, *J*=5.7, *J*=1.8, 1H, H5′), 3.58 (d, *J*=7.5, 1H, H5), 3.67–3.83 (m, 2H, CH_2-thym.), 3.85 (br s, 1H, H11), 4.05 (dq, *J*=9.1, *J*=6.3, 1H, H5′′), 4.20 (d, *J*=7.5, 1H, H3), 4.46 (d, *J*=7.5, 1H, H1′), 4.68 (dd, *J*=10.2, *J*=2.3, 1H, H13), 4.97 (d, *J*=4.5, 1H, H1′′), 7.11 (q, *J*=1.5, 1H, H6-thym.); ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 9.3 (CH_3), 11.1 (CH_3), 12.3 (CH_3), 15.2 (CH_3), 16.6 (CH_3), 16.8 (CH_3), 18.5 (CH_3), 21.4 (CH_3), 21.5 (CH_2), 21.5 (CH_3), 21.7 (CH_3), 26.6 (CH_3), 28.6 (CH_2), 29.3 (CH_2), 29.6 (CH), 31.2 (CH), 35.0 (CH_2), 36.5 (CH_2), 39.9 (CH), 40.2 (CH_3), 44.9 (CH), 45.9 (CH_2), 46.2 (CH_2), 49.4 (CH_3), 65.4 (CH), 65.5 (CH), 69.0 (CH), 70.4 (CH), 70.9 (CH), 71.1 (CH), 72.6 (C), 74.0 (C), 76.1 (C), 77.9 (CH), 78.1 (CH), 79.6 (CH), 83.8 (CH), 96.2 (CH), 103.2 (CH), 110.7 (C), 140.4 (CH), 150.8 (CO), 164.1 (CO), 177.7 (CO); HRFABMS, calcd for $C_{45}H_{81}N_4O_{14}$: 901.5749 [M+1]; found: 901.5726.
17. Including the desired carbamate (FABMS 1176.7 [M+1]) but as a minor product. Reaction of **6** with crude isocyanate **13** gave similar mixtures.
18. Spectral data of 9a-aza-9a-[3′-deoxythymidin-3′-yl)aminocarbonyl]-9-deoxo-9a-homoerythromycin A (**4**): ^{1}H NMR (CD_3OD, 500 MHz) δ 0.90 (t, *J*=7.3, 3H, Me-15), 1.00–1.02 (m, 6H, Me-8, Me-4), 1.19–1.32 (m, 23H, H4′a, H7a, Me-2, Me-6, Me-10, Me-12, Me-5′, Me-3′′, Me-5′′), 1.42–1.54 (m, 2H, H14a, H7b), 1.58 (dd, *J*=15.0, *J*=5.0, 1H, H2′′a), 1.79–1.86 (m, 2H, H4, H4′b), 1.85 (dqd, *J*=14.3, *J*=7.8, *J*=2.3, 1H, H14b), 1.89 (d, *J*=1.0, 3H, Me-thym.), 2.21 (br s, 1H, H8), 2.29–2.39 (m, 2H, H2′-thym., H2′′-thym.), 2.42 (d, *J*=16.0, 1H, H2′′b), 2.44 (br s, 6H, NMe_2), 2.80 (quint, *J*=7.6, 1H, H2), 2.92 (t, *J*=9.9, 1H, H3′), 3.04 (d, *J*=9.5, 1H, H4′′), 3.12 (br s, 1H, H9b or H10), 3.32 (s, 3H, MeO-3′′), 3.49 (d, *J*=7.0, 1H, H5), 3.59 (br s, 1H, H11), 3.71 (dq, *J*=9.4, *J*=5.9, 1H, H5′), 3.81 (dd, *J*=12.5, *J*=3.5, 1H, H5′-thym.), 3.86 (dd, *J*=12.0, *J*=2.5, 1H, H5′′-thym.), 4.00 (br s, 1H, H4′-thym.), 4.08 (d, *J*=8.5, 1H, H3), 4.14 (dq, *J* =9.5, *J*=6.2, 1H, H5′′), 4.29 (q, *J*=6.3, 1H, H3′-thym.), 4.51 (d, *J*=7.0, 1H, H1′), 4.90 (d, *J*=5.0, 1H, H1′′), 5.14 (d, *J*=8.5, 1H, H13), 6.29 (t, *J*=6.0, 1H, H1′-thym.), 7.88 (br s, 1H, H6-thym.); ^{13}C NMR (CD_3OD, 75.4 MHz): δ 10.3 (CH_3), 11.8 (CH_3), 12.5 (CH_3), 13.4 (CH_3), 16.4 (CH_3), 18.7 (CH_3), 19.1 (CH_3), 20.8 (CH_3), 21.6 (CH_3), 21.8 (CH_3), 23.4 (CH_2), 29.0 (CH), 30.8 (CH_2), 31.7 (CH_2), 36.0 (CH_2), 39.1 (CH_2), 40.4 (CH_3), 41.9 (CH), 46.7 (CH), 50.0 (CH_3), 52.5 (CH), 53.6 (CH), 63.0 (CH), 63.0 (CH_2), 65.6 (CH), 66.7 (CH), 68.9 (CH), 72.2 (CH), 74.3 (C), 76.1 (C), 76.4 (CH), 77.4 (C), 77.7 (CH), 79.2 (CH), 80.8 (CH), 85.9 (CH), 87.1 (CH), 87.2 (CH), 97.5 (CH), 104.3 (CH), 111.7 (C), 138.1 (CH), 153.2 (C), 160.8 (CO), 166.4 (CO), 178.1 (CO); HRFABMS, calcd for $C_{48}H_{85}N_5O_{17}$: 1003.5940 [M+1]; found: 1003.5900, calcd for $C_{48}H_{84}N_5O_{17}$: 1002.5862 [M]; found: 1002.5859.