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Regioselective Synthesis of Bifunctional Macrolides for Probing Ribosomal Binding

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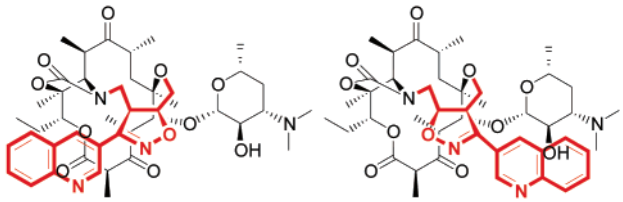
ABSTRACT



Bifunctional macrolides projecting an anchor group to the right or left spatial position of the lactone ring were synthesized. The regioselectivity of the key [3 + 2] cycloaddition process was controlled by the remote cladinose group attached to the C-3 position. These conformationally constrained molecules were employed as molecular probes to study the ribosomal binding sites of bifunctional macrolide antibiotics.

Macrolides, exemplified by erythromycin A, are important antibiotics that have been widely used for the treatment of bacterial infections.[1] They exert potent antibacterial activity by selectively binding to the bacterial ribosomes and inhibiting the protein synthesis process.[2] However, bacterial resistance to macrolide antibiotics has become increasingly prevalent over the past decade.[3] One of the major resistant mechanisms involves dimethylation of a specific adenine group in the macrolide binding site, resulting in a significant loss of the binding affinity.[4] Recently this type of resistance has been effectively addressed by the introduction of ketolides, a new family of clinically important macrolides that are characterized by having a carbonyl group at the C-3 position. Ketolides with an aryl group properly attached have shown potent activity against erythromycin-resistant bacteria. The aryl group is believed to form an additional interaction with the ribosome, compensating for the free energy loss due to the adenine dimethylation.[1b] ABT-773[5] and RU-004[6] are two of the most significant compounds resulting from the application of this anchor group strategy (Figure 1).

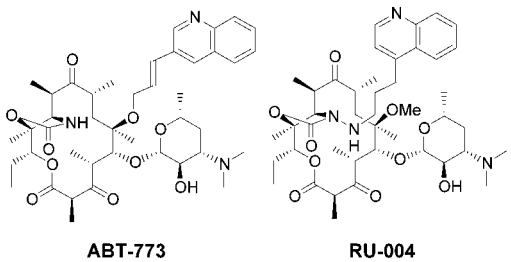


Figure 1. Structures of ABT-773 and RU-004.

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(1) For recent reviews, see: (a) Zhanel, G. G.; Dueck, M.; Hoban, D. J.; Vercaigne, L. M.; Embil, J. M.; Gin, A. S.; Karlowsky, J. A. *Drugs* **2001**, *61*, 443−498. (b) Ma, Z.; Nemoto, P. *Curr. Med. Chem.−Anti-Infect. Agents* **2002**, *1*, in press.

(2) Vester, B.; Douthwaite, A. *Antimicrob. Agents Chemother*. **2001**, *45*, 1−12.

(3) Doern, G. V.; Heilmann, K. P.; Huynh, H. K.; Rhomberg, P. R.; Coffman, S. L.; Brueggemann, A. B. *Antimicrob. Agents Chemother*. **2001**, *45*, 1721−1729.

(4) Nakajima, Y. *J. Infect. Chemother*. **1999**, *5*, 61−74.

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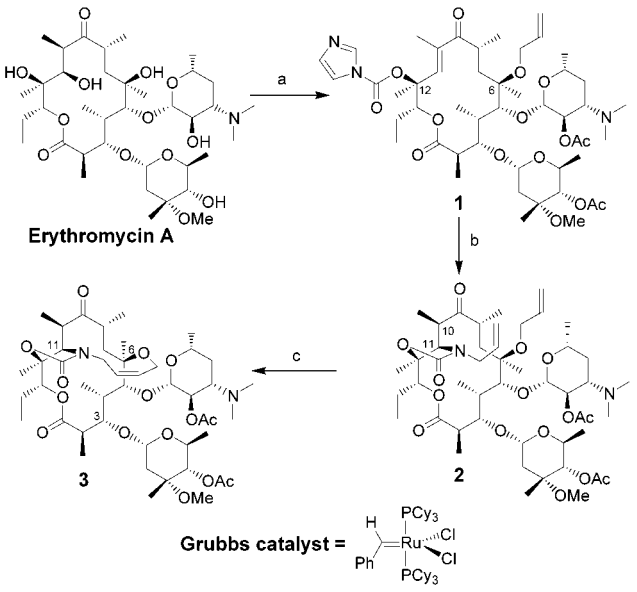
The quinolyl group, in both ABT-773 and RU-004, is tethered to the macrolide skeleton through a flexible linker, which allows the anchor group to move freely and occupy various spatial positions. Some recent studies showed that the aryl group in RU-004 protected the chemical modification of a specific adenine base in the Domain II region of the ribosomal RNA, indicating that the aryl group might interact with this region of the bacterial ribosome.[7] It is fundamentally important to understand how the anchor group interacts with the ribosome in order to design more potent macrolides that overcome bacterial resistance. We report herein the synthesis of two conformationally constrained macrolides that deliver an anchor group to the defined spatial regions and utilization of these molecules as probes for the anchor group binding site.

Treatment of 6-*O*-allyl-12-*O*-acylimidazolide **1**[5] with allylamine provided the 11,12-cyclic carbamate **2** in a single step in an excellent yield (Scheme 1). The reaction was

Scheme 1. Synthesis of 6,11-(*cis*-2-Butenylene) Bridged Macrolide Intermediate[a]



[a] Reagents and conditions: (a) ref 5; (b) allylamine, CH_3CN, H_2O, rt, 48 h, 90%; (c) Grubbs catalyst, CH_2Cl_2, rt, 24 h, 97%.

stereoselective, generating two chiral centers at the C-10 and C-11 positions, both having the natural configurations of erythromycin.[8] Ring-closing metathesis[9] of **2**, catalyzed by Grubbs catalyst, provided the 6,11-*cis*-butenylene bridged compound **3** in 97% yield.[10] The chirality of the C-10 and C-11 centers and the *cis*-configuration of the 2-butenylene bridge were confirmed by the X-ray single-crystal structure of a sequential compound of **3** (Figure 2).

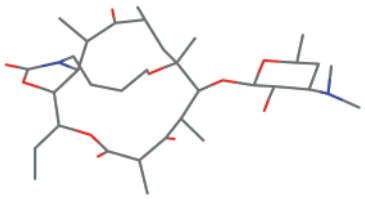
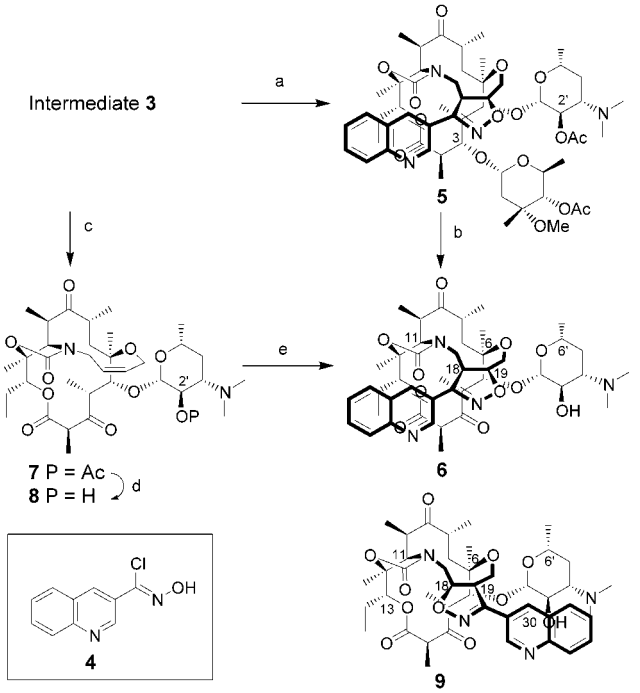


Figure 2. X-ray single-crystal structure of 6,11-butenylene bridged macrolide **8**.

1,3-Dipolar cycloaddition of **3** and **4** (which generated 3-quinolyl nitrile oxide in situ) produced a single compound **5** both regioselectively and stereoselectively in 71% yield (Scheme 2).[11] We believe that the *cis*-butenylene-bridged macrolide **3** adopts a conformation similar to that shown in Figure 2. This conformation only allows the nitrile oxide to approach from the top face of the molecule, providing

Scheme 2. Synthesis of Conformationally Constrained Bifunctional Macrolides[a]



[a] Reagents and conditions: (a) **4**, Et_3N, THF, rt, 48 h, 71%; (b) (i) 2 N HCl, EtOH, rt, 24 h, 56%; (ii) Dess−Martin periodinane, CH_2Cl_2, rt, 2 h, 53%; (iii) MeOH, rt, 20 h, 80%; (c) (i) 2 N HCl, EtOH, rt, 24 h, 83%; 9ii) $NCS/Me_2S/Et_3N$, CH_2Cl_2, −10 °C to rt, quant; (d) MeOH, reflux, 4 h, 87%; (e) (i) **4**, Et_3N, THF, rt, 24 h, 58% (two regioisomers in 2:1 ratio); (ii) MeOH, rt, 20 h, 92% (**6**:**9** in 2:1 ratio).

(5) Ma, Z.; Clark, R. F.; Brazzale, A.; Wang, S.; Rupp, M. J.; Li, L.; Griesgraber, G.; Zhang, S.; Yong, H.; Phan, L. T.; 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.

(6) Agouridas, C.; Denis, A.; 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. *J. Med. Chem.* **1998**, *41*, 4080−4100.

(7) Douthwaite, S.; Hansen, L. H.; Mauvais, P. *Mol. Microbiol.* **2000**, *36*, 183−193.

isoxazoline adduct **5** in a stereoselective fashion.[12] Hydrolysis of the C-3 cladinose group from **5** gave the corresponding 3-OH compound, which was further converted to the C-3 carbonyl product under Dess−Martin conditions. Finally, the 2′-OH protecting group was removed with methanol[13] to give the anchor-left isomer **6** in good yield.

Molecular modeling of **3**[14] suggests that the C-3 cladinose group is placed above the macrolide ring and blocks one of the two pathways for the incoming nitrile oxide, leading to anchor-left isomer **5** as the single product (Figure 3). We

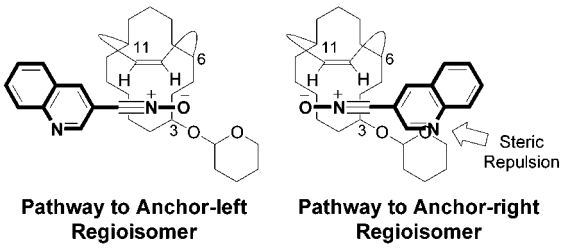


Figure 3. Two potential pathways for the dipolar addition of **3** and nitrile oxide generated from **4**.

rationalized that removing the cladinose group prior to the [3 + 2] cycloaddition should eliminate this remote steric effect and allow the formation of the anchor-right isomer. Thus, acid hydrolysis of the cladinose from **3** followed by oxidation of the remaining hydroxyl group under Corey−Kim conditions[15] provided the C-3 carbonyl compound **7** in excellent yield. Deprotection of **7** in refluxing MeOH provided compound **8**, whose structure was confirmed by X-ray crystallography (Figure 2). Dipolar addition of **7** with the nitrile oxide generated in situ from **4**[11] provided a mixture of two regioisomers, which leads to compounds **6** and **9** in a 2:1 ratio upon removal of the 2′-OH acetyl protecting group.

The structures of **6** and **9** as regioisomers were confirmed respectively by various homo and hetero 2D NMR experiments, especially the proton-carbon long-range correlation experiment. The dipolar interaction between H-13 and H-18/H-19 is observed in both compounds **6** and **9**, confirming that the whole group between the C-6 and C-11 overlays on top of the macrolide ring. The extra NOE observed in compound **9** between the H-5 and one of the quinolyl protons (H-30) indicates that compound **9** has an anchor-right configuration. This configuration places the quinolyl group in the proximity of cladinose, resulting in a significant upfield shift for the C-6′ methyl protons (observed at 0.31 ppm for **9** as compared to 1.23 ppm for **6**) due to the strong shielding effect of the aromatic ring.

The antibacterial activities of **6**, **8**, and **9**, as determined by using the agar dilution method, are reported as minimum inhibitory concentration (MIC) against four representative Gram-positive organisms (Table 1).[5] *Streptococcus Pyogenes*

Table 1. Antibacterial Activity of Conformationally Constrained Bifunctional Macrolides

	MIC (μg/mL)				
organism[a]	Ery[b]	**8**	**6**	**9**	ABT[c]
Spy EES61	0.06	0.25	0.25	2	0.004
Spy 930	>128	>128	64	>128	1
Spn 6303	0.12	0.25	0.25	2	0.004
Spn 5737	>128	>128	32	>128	0.25

[a] *Spy* represents *Streptococcus pyogenes* and *Spn* represents *Streptococcus pneumoniae*. [b] Ery represents erythromycin A. [c] ABT represents ABT-773.

EES 61 and *Streptococcus pneumoniae* ATCC 6303 are erythromycin-susceptible strains, while *Streptococcus Pyogenes* 930 and *Streptococcus pneumoniae* 5737 are erythromycin-resistant strains with the macrolide binding site methylated.

The anchor-left and the anchor-right isomers **6** and **9** differ significantly in potency (Table 1). Compound **6** is about 10-fold more potent than **9** against the susceptible strains as well as the resistant strains with methylated binding sites. The data are consistent with the notion that the quinolyl group in compound **6** interacts with a secondary binding site on bacterial ribosomes, while the quinolyl group in **9** exerts a detrimental steric effect to the binding. The anchoring effect of **6** is more profound against methylated ribosomes where the primary binding has been compromised.

Detailed knowledge of the anchor-ribosome interaction is critical for the rational design of a new generation of macrolides that overcome antibiotic resistance. Compounds **6** and **9** are both relatively rigid molecules that confine the quinolyl group to a limited spatial region (Figure 4). It appears that the quinolyl group in compound **6** is positioned such that a secondary interaction with the ribosome is allowed, but such an interaction does not exist in structure **9**. This work suggests that the secondary binding site for the bifunctional macrolides is located on the left side of the macrolide ring above the C-13 carbon. It is interesting to note that ABT-773 adopts a fold-in conformation in its crystal form where the quinolyl group is positioned on top of the

(8) For a similar intramolecular Michael addition reaction and its stereoselectivity, see ref 5 and: Baker, W. R.; Clark, J. D.; Stephens, R. L.; Kim, K. H. *J. Org. Chem.* **1988**, *53*, 2340−2345.

(9) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 5426−5427.

(10) Conditions: A solution of **2** (2.50 g, 2.71 mmol) and Grubbs catalyst (250 mg, 0.30 mmol) in 500 mL of CH_2Cl_2 was stirred at room temperature under N_2 for 24 h. Product was purified by recrystalization from an acetone/hexane mixture.

(11) Conditions: Et_3N (0.834 mL, 6.00 mmol) was added to a solution of **3** (908 mg, 1.00 mmol) and **4** (412 mg, 2.00 mmol) in THF (40 mL) at room temperature over 1 h period. The mixture was stirred at room temperature for 48 h. Product was purified by chromatography eluting with 1:3 acetone/hexane.

(12) The regio- and stereochemistry of **5** was confirmed by converting **5** to **6**, which was characterized by various 2D NMR experiments.

(13) Hydrolysis of the 2′-OH protecting group was carried out under very mild conditions, apparently as a result of a neighboring group effect exerted by the 3′-NMe_2 group.

(14) The molecular model of **3** was created based on the crystal structure of clarithromycin where the cladinose group occupies a spacial position above the macrolide ring. See ref 5 for crystal structure of clarithromycin.

(15) Corey, E. J.; Kim, C. U. *J. Am. Chem. Soc.* **1972**, *94*, 7586−7587.

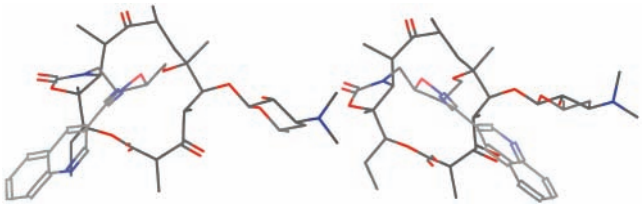


Figure 4. Minimized conformations of anchor-left and anchor-right regioisomers.

C-13 carbon.[16] The quinolyl group in RU-004 occupies a similar spatial position both in crystal and in solution forms.[17]

The atomic-resolution crystal structure of bacterial ribosomes has been recently determined, which represents one of the most significant developments in the area of macrolide research.[18] Cocrystal structures of the 50S ribosomal subunit isolated from the eubacterium *Deinococcus radiodurants* complexed with erythromycin and two other nonbifunctional macrolides has also become available.[19] These cocrystal structures revealed some detailed interactions of a nonbifunctional macrolide with Domain V of the ribosomal RNA. If the lactone portion of **6** is overlayed with that of ribosome-bound erythromycin, the quinolyl group of **6** is projected to a new site in domain II of ribosomal RNA, indicating that the potential binding site for the anchor group is located in this region of the ribosome. The same interaction in Domain II has also been suggested by ribosome footprinting experiments. Bifunctional macrolides, such as ABT-773 and RU-004, block the chemical modification of the same binding site in Domain II, while erythromycin and other nonbifunctional macrolides have no protective effect in this region.[7]

The molecules prepared for the current study are highly rigid, and that may actually prohibit the formation of an effective secondary interaction with the ribosome. This possibility is strongly supported by the fact that compound **6** is less active than both ABT-773 and RU-004, where the quinolyl group moves freely and allows the formation of a suitable conformation for effective binding. Therefore, future work will be directed at the design and synthesis of less rigid molecules that allow the anchor group to move in a broader spatial area and at conformationally constrained molecules that deliver the anchor group to a more effective spatial position.

Acknowledgment. The authors thank Prof. Peter Beak for many helpful suggestions during this research, and Ms. Mai-Ha Bui and Ms. Niru Soni for determination of antibacterial activity.

Supporting Information Available: Characterization data for compounds **6** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) See ref 5 and: 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.

(17) See ref 6 and: Bertho, G.; Gharbi-Benarous, J.; Delaforge, M.; Lang, C.; Parent, A.; Girault, J.-P. *J. Med. Chem.* **1998**, *41*, 3373−3386.

(18) (a) Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905−920. (b) Nissen, P.; Hansen, J.; Ban, N.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 920−929.

(19) Schlunzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814−821.