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Synthesis and Antibacterial Activity of Novel 6-*O*-Substituted Erythromycin A Derivatives

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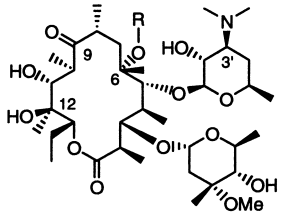
Abstract—A series of novel 6-*O*-substituted erythromycin A derivatives has been synthesized. Good in vitro antibacterial activity has been demonstrated for analogues incorporating a variety of structural features. The methodology disclosed is expected to find application in the design of future macrolide antibiotics that target the prevalent bacterial resistance problem. © 2000 Elsevier Science Ltd. All rights reserved.

Macrolide antibiotics are a preferred drug class for the safe and effective treatment of respiratory tract infections. Erythromycin A (**1**), for example, has enjoyed widespread clinical use for more than 40 years. This 14-membered macrolide, however, undergoes decomposition within the acidic medium of the stomach. This results in poor oral bioavailability and undesired gastrointestinal side effects.[1] One approach to overcome these liabilities is to improve the acid stability of the macrolide through modification of functional groups involved in the degradation process. It is known that the degradative pathway involves interactions between the hydroxyl groups at C-6 and C-12 with the carbonyl at C-9.[2,3] A C-6 hydroxyl blocking strategy has been successfully employed in the development of second generation macrolides such as the 6-*O*-methyl derivative of erythromycin A, clarithromycin (**2**).[4] Clarithromycin exhibits markedly improved acid stability relative to erythromycin A resulting in better bioavailability and pharmacokinetics, increased gastrointestinal tolerance, and a superior spectrum of antibacterial activity.[1] 6-*O*-Substituted macrolides, therefore, constitute a rational approach to compounds with enhanced biological profiles.



(1) Erythromycin A R=H
(2) Clarithromycin R=CH_3

Despite intense interest, previously reported modifications of the sterically hindered C-6 hydroxyl position of erythromycin A have been limited to methylation products.[5] Attempts to install higher alkyl groups have been accompanied by dramatic losses in selectivity for the C-6 hydroxyl.[3] We have recently developed a methodology for the selective introduction of a variety of substituents at the C-6 position. This communication will highlight the synthesis and in vitro antibacterial activity of a series of novel 6-*O*-substituted erythromycin A derivatives with particular focus on the synthetic versatility of the methodology as well as the potential for application to newer macrolide classes[6] which target the rapidly emerging problem of bacterial resistance.[7]

Our general 6-*O*-alkylation strategy was adapted from the manufacturing process used for the production of

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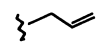
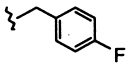
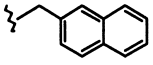
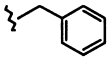
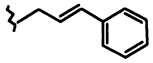
clarithromycin.[8] By utilizing suitably protected erythromycin A (**3**),[8] a core group of electrophiles was selectively attached to the C-6 hydroxyl as outlined in Scheme 1 and Table 1. Although not detailed in this account, less reactive electrophiles did not alkylate. Alkylation of compound **3** with allylic, propargylic, and benzylic bromides in the presence of potassium hydroxide resulted in incomplete conversion of the C-6 hydroxy starting material. We surmised that an incompatability between the hydroxide base and the reactive alkyl halides was responsible for the low yields. A survey of alternative bases applied to the allylation of **3** revealed potassium *tert*-butoxide to be optimal. Overall yields for **4b** were improved by more than 20% when potassium hydroxide was replaced by the hindered alkoxide. A more systematic study of 6-*O*-alkylation with potassium *tert*-butoxide is in progress. Following the alkylation step, removal of the trimethylsilyl and cyclohexylketal protecting groups under acidic conditions provided the 9-oxime-6-*O*-alkylerythromycin A products. Subsequent deoximation using either sodium bisulfite or sodium nitrite[9] afforded the desired 6-*O*-alkylerythromycin A targets (**4b–g**).

Allyl compound **4b** was envisioned as a versatile synthetic intermediate that could be further modified in order to explore structure–activity relationships (SAR) of the series. Specific targets pursued were governed by attempts to define the scope of our chemistry approach and by a desire to assess the biological tolerance for structural variation at the C-6 position. The strategy is described in Scheme 2. Heck arylation[10] of the allylic olefin provided a series of conjugated derivatives exemplified by entries **4h** and **4i**. Reduction of the terminal double bond by catalytic hydrogenation yielded the 6-*O*-propyl analogue (**4a**). Selective oxidation of the allyl side chain employing Wacker conditions[11] delivered methyl ketone **4j** in modest yield. Ozonolysis of **4b** to the corresponding aldehyde was accompanied by competing oxidation of the dimethylamino nitrogen at the 3′ position of the desosamine sugar. However, subsequent selective reduction of the *N*-oxide with triphenylphosphine afforded the desired aldehyde (**4k**) in good yield. Similarly, epoxidation of **4b** with *m*-chloroperbenzoic acid produced the *N*-oxide of the desired compound requiring treatment with sodium bisulfite to liberate the target epoxide (**4m**) as a mixture of diastereomers (~1:1). These *N*-oxidation products could be efficiently suppressed by the initial addition of a stoichiometric amount of acid to the reaction mixture.

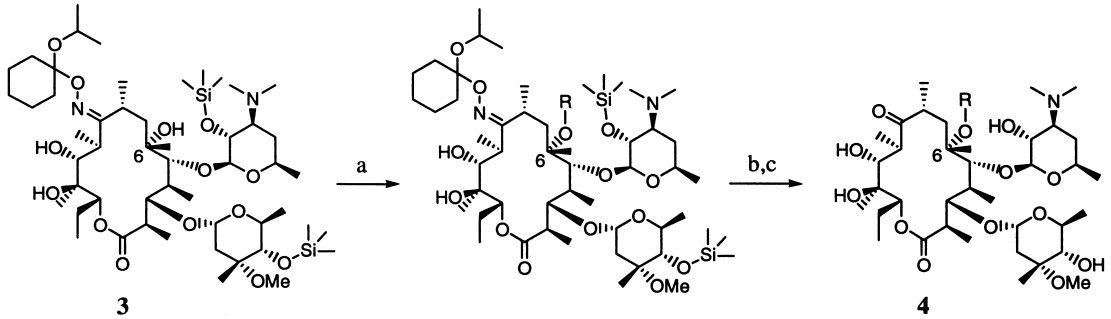
Table 1. Scope of 6-*O*-alkylation of **3**

Entry	R	Yield[a]	Entry	R	Yield[a]
4b		33%[b]	**4e**		21%
4c		51%	**4f**		10%
4d		19%	**4g**		24%

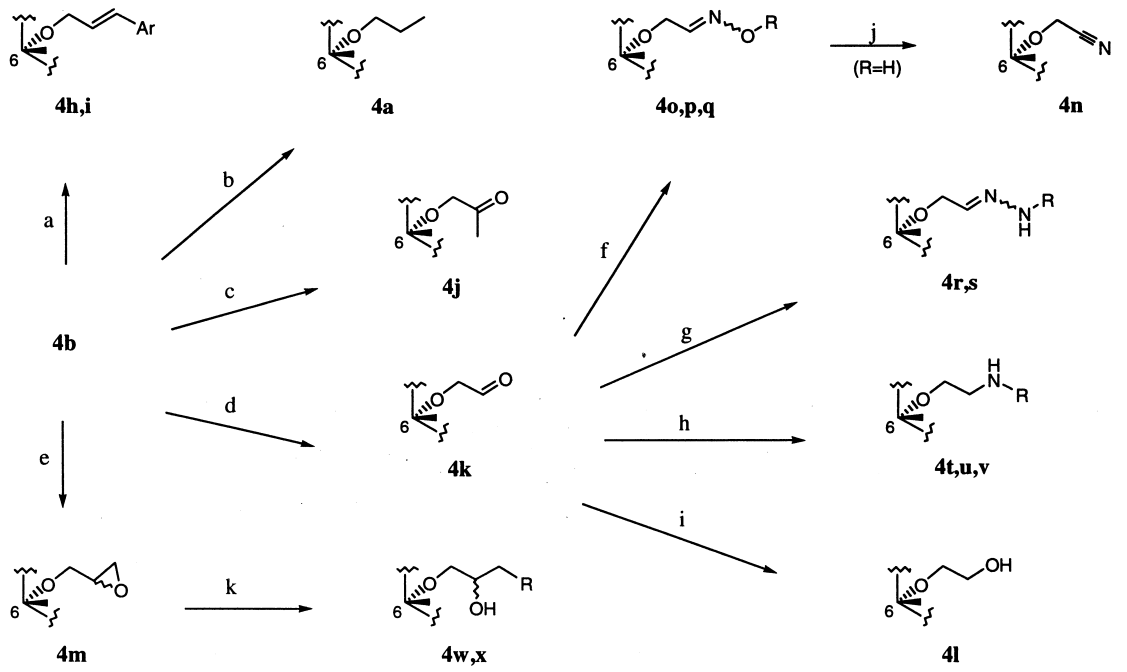
[a]Purified yield over three steps.
[b]KOBu^t employed as the base in Scheme 1, step a.

Aldehyde **4k** and epoxide **4m** were further modified to generate analogues encompassing a broader range of physicochemical properties. A series of oximes (**4o–q**) and hydrazones (**4r**,**s**) was prepared via the condensation of hydroxylamines and hydrazines, respectively, with **4k**. In each instance, the products were isolated as mixtures of *E:Z* isomers about the carbon–nitrogen double bond. The parent oxime (**4o**) was transformed to the corresponding nitrile (**4n**) by carbodiimide-mediated dehydration in the presence of cuprous chloride. Alcohol and amine products were also readily derived from **4k**. Reductive amination of the parent aldehyde provided a series of amine targets such as compounds **4t–v**. Alcohol **4l** was obtained by the reduction of **4k** in the presence of *L*-Selectride at low temperature. Treatment of epoxide **4m** with selected amine nucleophiles at elevated temperatures yielded β-substituted alcohol derivatives represented by entries **4w** and **4x**. Sulfur, oxygen and carbon nucleophiles have also been successfully employed and will be discussed in a future report.

These analogues were screened for in vitro antibacterial activity against representative erythromycin-susceptible and erythromycin-resistant staphylococci, streptococci and pneumococci (Table 2). Minimum inhibitory concentrations (MICs) were determined using standard



Scheme 1. (a) R-Br, KOH, DMSO-THF, 0 °C; (b) HOAc, Ch_3CN-H_2O; (c) (i) $NaNO_2$ (5 equiv), HCl (5 equiv), EtOH-H_2O, 70 °C, 2 h, or (ii) $NaHSO_3$ (4 equiv), HCO_2H (2.5 equiv), EtOH-H_2O, 80 °C, 2 h.



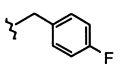
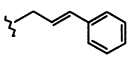
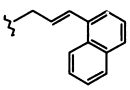
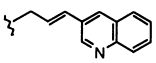
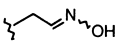
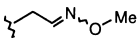
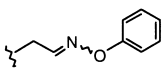
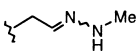
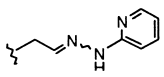
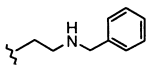
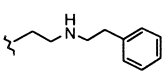
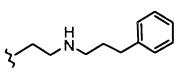
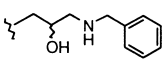
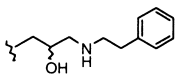
Scheme 2. (a) Ar-Br or Ar-I, $Pd(OAc)_2$, P(o-tolyl)$_3$, Et_3N, CH_3CN, 80 °C, 73–84%; (b) H_2 (1 atm), 10% Pd-C, CH_3OH, 58%; (c) $PdCl_2CuCl_2$, O_2, H_2O, DMF, 36%; (d) (i) O_3, CH_2Cl_2, −78 °C then Me_2S, 0 °C, (ii) PPh_3, THF, 60 °C, 61% (two steps); (e) *m*-CPBA, Ch_2Cl_2 then $NaHSO_3$, 54%; (f) HCl H_2N-OR, NMM, CH_3OH, 70–94%; (g) NH_2NHR, $MgSO_4$, THF, 59–66%; (h) (i) R-NH_2, molecular sieves, CH_2Cl_2, (ii) $NaBH_3CN$ or H_2 (1 atm), 10% Pd-C, AcOH, CH_3OH, 43–69%; (i) $LiB[CH(CH_3)C_2H_5]_3H$, THF, −78 °C, 37%; (j) 1,3-diisopropylcarbodiimide, CuCl, THF, 50%; (k) R-NH_2, $CHCl_3$ or DMF, 60–90 °C, 37–50%.

agar dilution methods.[12] Resistant isolates possessing the inducible or constitutive type of MLS (macrolide–streptagramin–lincosamide) resistance,[13] as well as those mediated by macrolide efflux mechanisms,[13b,14] were included in the assay. *Haemophilus influenzae*, another of the prevalent respiratory tract pathogens, was also examined.

In general, 6-*O*-substituted side chains larger than methyl were well tolerated when appropriately functionalized. Although the 6-*O*-propyl entry (**4a**) was less active than the corresponding 6-*O*-methyl parent (**2**), variations in the nature and length of the side chain in **4a** rendered derivatives with overall antibacterial activities comparable to the reference macrolides (**1**, **2**). In particular, the introduction of multiple bonds (**4c**), heteroatoms (**4l**, **4n**), or conjugated aromatic systems (**4g**, **4i**) maintained activities over much of the bacterial spectrum. Furthermore, those analogues containing aromatic substituents tethered to the macrolide by a hydrocarbon linker showed enhanced activity against both efflux and constitutively resistant *S. pyogenes* and *S. pneumoniae*. Among this group, the fluorobenzyl (**4e**) and naphthylallyl (**4h**) entries were particularly effective, each exhibiting greater than 16-fold improved activity against both *S. pyogenes* 930 and *S. pneumoniae* 5737. Those derivatives possessing both an aromatic group and one or more heteroatoms in the side chain spacer also exhibited very good antibacterial activity. In fact, phenyloxime **4q**, phenethylamine **4u**, phenylpropylamine **4v**, and phenethylaminoalcohol **4x** were the optimal compounds in this study, each possessing either enhanced or retained activity across the tested panel. In addition, **4u** and **4v** were the only agents examined which demonstrated improved activity against *H. influenzae* DILL and *S. aureus* 6538P, respectively. Similarly, **4q** and **4x** showed unique activity enhancements against susceptible streptococci when compared to other entries.

In summary, the synthesis of a novel series of 6-*O*-substituted erythromycin A derivatives has been described. The synthetic approach has been demonstrated to be sufficiently flexible to provide access to a variety of C-6 side chain structures that were heretofore inaccessible. Furthermore, SAR studies confirm that the 6-*O* position displays a high tolerance for structural variation with regard to both size and nature of substituents. Several analogues have been identified that feature in vitro antibacterial activities comparable to those for clarithromycin across a spectrum of representative pathogens. Those side chains containing aromatic groups produced the most potent antibacterial effects including marked activity increases against resistant organisms. Ultimately, the ability to modify this key position in the macrolide affords new opportunities to exploit the SAR of other macrolide classes currently showing promise in overcoming drug resistance.[15]

Table 2. In vitro antibacterial activity of 6-*O*-substituted erythromycin A derivatives **4**[a]

Entry	R	Minimum inhibitory concentration (μg/mL)								
		S. aureus		*S. pyogenes*			*S. pneumoniae*			*H. influenzae*
		6538P	A5177	EES61	PIU 2548	930	ATCC6303	5649	5737	DILL
1	Erythromycin A	0.2	6.2	0.03	32	>128	0.06	16	>128	4
2	Clarithromycin	0.2	6.2	0.03	32	>128	0.06	16	>128	8
4a		3.1	25	0.05	64	>128	—	—	—	32
4b		0.78	12.5	—	64	>128	—	—	—	32
4c		0.2	12.5	0.03	—	>128	0.06	16	>128	16
4d		1.56	12.5	0.06	16	>128	—	16	64	—
4e		3.1	12.5	1	8	8	1	8	8	64
4g		0.39	6.2	0.03	16	32	0.06	4	32	4
4h		0.78	12.5	0.06	8	8	0.25	16	8	4
4i		0.2	3.1	0.06	4	64	0.06	4	128	8
4j		3.1	—	—	128	>128	—	—	—	—
4k		0.78	25	0.06	128	>128	0.5	64	>128	8
4l		0.39	6.2	0.125	64	>64	0.125	32	>64	8
4m		25	25	4	>128	>128	—	—	—	>128
4n		0.2	—	0.03	32	>128	0.06	16	>128	8
4o		0.2	—	0.015	64	—	0.06	32	>128	16
4p		0.78	25	0.125	64	>128	0.125	32	>128	16
4q		0.2	3.1	0.008	32	128	0.008	16	128	4
4r		0.78	25	0.06	128	>128	0.125	64	>128	8
4s		0.78	25	0.125	128	>128	0.25	64	>128	8
4t		0.78	12.5	0.25	64	>128	0.125	32	>128	8
4u		0.2	12.5	0.015	8	64	0.03	4	64	2
4v		0.05	3.1	0.03	32	>128	0.03	16	>128	4
4w		0.39	6.2	0.125	—	—	0.125	64	>128	8
4x		0.2	6.2	0.03	16	>64	0.008	32	32	4

[a]*S. aureus* 6538P, *S. pyogenes* EES61 and *S. pneumoniae* ATCC6303: erythromycin susceptible; *S. pyogenes* 2548 and *S. pneumoniae* 5649: macrolide efflux resistance; *H. influenzae* DILL: ampicillin-resistant; S. *aureus* A5177: inducible MLS resistance; *S. pyogenes* 930 and *S. pneumoniae* 5979: constitutive MLS resistance.

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15. An early example of the successful application of this methodology has recently appeared: Ma, Z.; Clark, R. F.; Wang, S.; Nilius, A.; Flamm, R. K.; Or, Y. Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 26–29 September 1999, Abstract No. 2133.