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Novel desosamine-modified 14- and 15-membered macrolides without antibacterial activity

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ABSTRACT

Novel modifications of the desosamine sugar of 14- and 15-membered antibacterial macrolides, in which the desosamine was fused with N-substituted-1,3-oxazolidin-2-ones, were developed in order to completely suppress antibacterial activity and make them promising agents for other biological targets. The synthesis of such bicyclic desosamine derivatives, especially 1,3-oxazolidin-2-one formation, was optimized and conducted under mild conditions without a need for protection/deprotection steps for other functional groups. A focused series of novel desosamine-modified macrolide derivatives was prepared and their antibacterial activities tested. It was shown that these macrolide derivatives do not possess any residual antibacterial activity.

In recent years, the immunomodulatory properties of macrolide antibiotics were noted, revealing their very promising potential for the treatment of various diseases not connected to bacterial action.[1] For any of these novel biological applications, however, the antibacterial activity of macrolides is an undesired property due to the possibility of inducing bacterial resistance. Indeed, it has been shown that the reduction of antibacterial activity of certain macrolides through chemical modification does not necessarily diminish their immunomodulatory potential.[2] The reduction of a macrolide's antibacterial activity can be achieved in a few ways, from simple removal of the cladinose sugar,[3] through modifications of the aglycon ring including contraction,[4] to more complex modifications of the desosamine sugar.[5] However, these modifications substantially alter the macrolide structures and their physico-chemical properties. For this reason, new modifications that provide complete suppression of antibacterial activity with minor alterations of macrolide structures and/or physico-chemical properties would be of great significance. Our goal was to modify the desosamine sugar of 14- and 15-membered antibacterial macrolides by annelation of 1,3-oxazolidin-2-ones to the 2′,3′-positions of desosamine. Because the desosamine sugar is directly involved in the binding of macrolides to ribosomes through a network of hydrogen bonds and ionic interactions, such a simple modification should diminish the antibacterial activity of macrolide antibiotics.[5a,5b] Moreover, subsequent introduction of various substituents via N-alkylations of the 1,3-oxazolidin-2-one ring could completely suppress antibacterial activity but also fine tune the macrolide physico-chemical properties. These macrolides would be evaluated as potential agents for other biological targets.

The annelation of a *N*-methyl substituted 1,3-oxazolidin-2-one ring to desosamine has been achieved previously but only together with simultaneous formation of an 11,12-cyclic carbonate moiety on the macrolactone ring.[5a,5b,6] These modifications diminished antibacterial activity but also caused a dramatic decrease in solubility in common organic solvents. We envisioned that the removal of both methyl groups from the 3′-*N*,*N*-dimethylamino group of clarithromycin 9a-lactam (1), clarithromycin (2), and azithromycin (3), would provide vicinal 2′,3′-amino alcohols that could be bridged via 1,3-oxazolidin-2-one formation without altering the macrolactone ring. The nitrogen of the 1,3-oxazolidin-2-one ring would be then used as a position for the attachment of various alkyl substituents through N-alkylations performed in a parallel synthesis manner.

As we reported previously,[7] removal of the first methyl from the 3′-*N*,*N*-dimethylamino group of desosamine is a fairly well known process that can be achieved using several reagents,[8] that is sodium acetate/iodine combination,[9] diethylazodicarboxylate (DEAD),[10] *N*-iodo succinimide (NIS),[11] and benzoylchloroformate.[12] In our hands the sodium acetate/iodine method proved effective for the preparation of 3′-*N*-demethyl-6-*O*-methyl-9a-aza-9a-homoerythromycin A (4) and 3′-*N*-demethyl-6-*O*-methylerythromycin A

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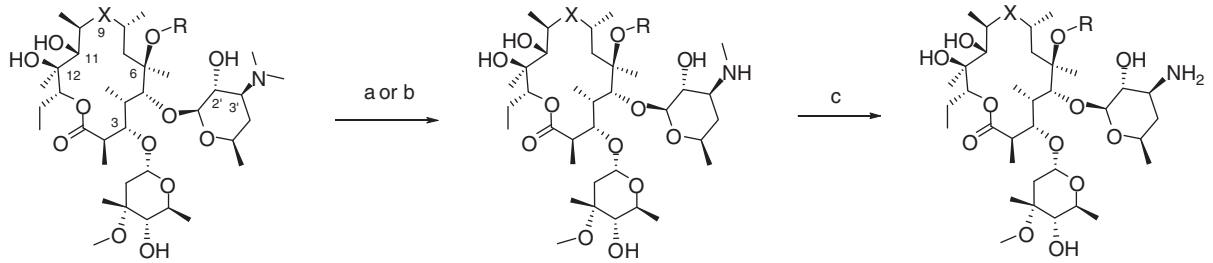


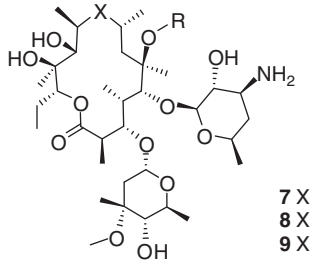
1 X = -NH-C(=O)-; R = CH_3
2 X = -C(=O)-; R = CH_3
3 X = -N(-CH_3)-CH_2-; R = H

4 X = -NH-C(=O)-; R = CH_3
5 X = -C(=O)-; R = CH_3
6 X = -N(-CH_3)-CH_2-; R = H

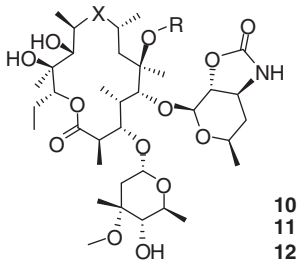
7 X = -NH-C(=O)-; R = CH_3
8 X = -C(=O)-; R = CH_3
9 X = -N(-CH_3)-CH_2-; R=H

Scheme 1. Reagents and conditions: (a) Demethylation of 1 and 2: sodium acetate trihydrate, iodine, methanol, 500 W halogen lamp irradiation, 4–5 h; (b) demethylation of 3: N-iodosuccinimide, acetonitrile, 1.5 h; (c) sodium methoxide, iodine, methanol, tris(hydroxymethyl)aminomethane (for 4–5), 0 °C to rt.

Table 1
Cyclization of 2′,3′-vicinal amino alcohols 7–9 to 1,3-oxazolidin-2-one derivatives 10–12



7 X = -NH-C(=O)-; R = CH_3
8 X = -C(=O)-; R = CH_3
9 X = -N(-CH_3)-CH_2-; R = H

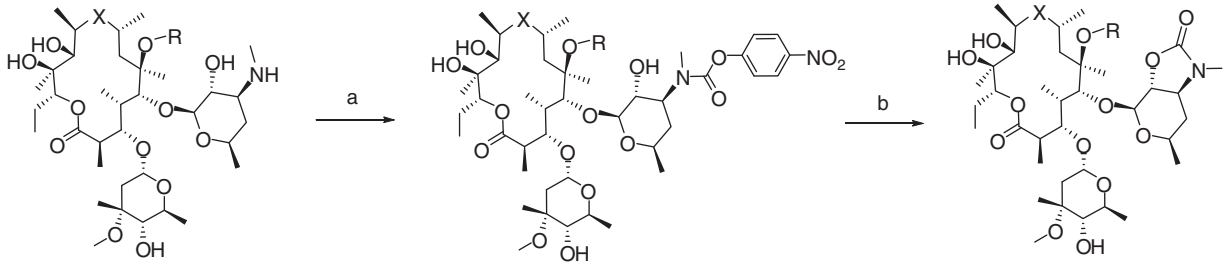


10 X = -NH-C(=O)-; R = CH_3
11 X = -C(=O)-; R = CH_3
12 X = -N(-CH_3)-CH_2-; R = H

No.	Scaffold	Conditions	Product	Yield (%)
1	**9**	(1) Benzoyl chloroformate, DCM; (2) NaH, DMF	**12**	16
2	**7**	Triphosgene, pyridine	**10**	22
3	**8**		**11**	60
4	**9**		**12**	22
5	**9**	(1) 4-Nitrophenyl chloroformate, DCM; (2) pH 12	**12**	61
6	**7**	4-Nitrophenyl chloroformate, TEA, DCM	**10**	63
7	**8**		**11**	81
8	**9**		**12**	54

(5) while for the preparation of 3′-*N*-demethyl-9-deoxo-9a-methyl-9a-aza-9a-homoerythromycin A (6) the optimal method was found to use *N*-iodosuccinimide (Scheme 1). All three 3′-N-demethylated compounds 4–6 can be easily purified by column chromatography using a $CH_2Cl_2/CH_3OH/NH_4OH$ (90:9:1.5) mixture as an eluent.

Because the demethylation of secondary amines is more difficult in comparison to tertiary amines, removal of the second methyl group from 3′-*N*-demethylated compounds 4–6 was achieved using similar conditions, but this reaction has to be driven in the presence of a stronger base such as sodium methoxide. Interestingly, the sodium methoxide/iodine procedure afforded complete removal of the second methyl group only from 6. For an unknown reason, the same procedure provided incomplete demethylations (around 50%) of 4 and 5, which could not be improved by using larger excess of sodium methoxide. The degree of second demethylation of 5 and 7 was somewhat enhanced by addition of tris(hydroxymethyl)aminomethane[13] into the reaction mixture. The degree of demethylation of 5 was increased to 80%, while the degree of demethylation of 4 was a little lower (70%).[14] An obvious idea to perform di-demethylation of 1–3 directly to 7–9 in one step in the presence



4 X = -NH-C(=O)-; R=CH_3
5 X = -C(=O)-; R = CH_3
6 X = -N(-CH_3)-CH_2-; R = H

13 X = -NH-C(=O)-; R = CH_3
14 X = -C(=O)-; R = CH_3
15 X = -N(-CH_3)-CH_2-; R = H

16 X = -NH-C(=O)-; R = CH_3
17 X = -C(=O)-; R = CH_3
18 X = -N(-CH_3)-CH_2-; R = H

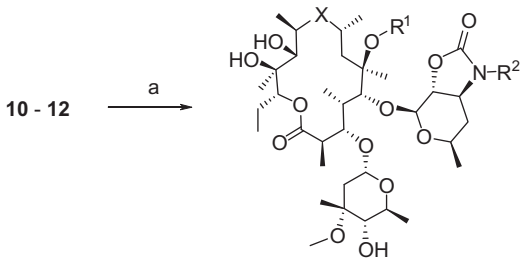
Scheme 2. Reagents and conditions: (a) 4-Nitrophenyl chloroformate, triethylamine, dichloromethane; (b) sodium hydride, *N,N* dimethylformamide.

of sodium methoxide/iodine combination was unachievable due to considerable macrolide degradation.

A few methods for the cyclization of 2′,3′-vicinal amino alcohols of 3′-*N,N*-didemethylated compounds 7–9 to 1,3-oxazolidin-2-one derivatives 10–12 were explored without protection/deprotection steps of other functional groups. A two-step reaction of 9 with benzoyl chloroformate and subsequent cyclization in basic conditions[15] proved unsuccessful, affording a low yield of the desired 1,3-oxazolidin-2-one derivative 12 (<20%, Table 1, entry 1). The cyclizations of 7 and 9 using 0.9 equiv of bis(trichloromethyl) carbonate (triphosgene)[16] in pyridine afforded somewhat better yields of compounds 10 and 12, respectively (Table 1, entries 2 and 4). Interestingly, the same reaction of the clarithromycin derivative 8 afforded the desired product 11 in much better yield of 60% (Table 1, entry 3). It seems that small differences of the macrolactone rings in 7–9 have a profound impact on the reactivity of the amino alcohol functionality of the desosamine sugar. The excess of triphosgene in these reactions caused parallel formation of 11,12-cyclic carbonates. Finally, a third method that involves modified 4-nitrophenyl chloroformate procedure[6] proved optimal for the synthesis of desired 2′,3′-fused 1,3-oxazolidin-2-ones 10–12 in good yields. The reaction of 9 with 4-nitrophenyl chloroformate without the presence of any base affords the 3′-(4-nitrophenyl)carbamate intermediate that readily cyclized into 1,3-oxazolidin-2-one 12 during the extractive work-up when the pH was increased to 12 (Table 1, entry 5). However, when the same reaction conditions were applied to compounds 7 and 8, which do not possess a tertiary amine as a part of the macrolactone ring, substantial cleavage of the cladinose sugar was observed.

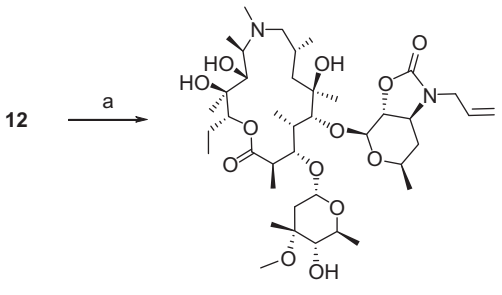
Therefore, addition of a base, that is triethylamine, to the reaction mixture was necessary to inhibit cladinose cleavage. At the same time, since 3′-(4-nitrophenyl)carbamate readily cyclized in basic conditions, the addition of an excess of triethylamine induced relatively fast cyclization of intermediary-formed 3′-(4-nitrophenyl)carbamates of 7 and 8, as well as 9, to 1,3-oxazolidin-2-ones 10, 11, and 12, respectively. Further optimization of the reaction conditions provided a novel one-step synthesis of 2′,3′-fused 1,3-oxazolidin-2-ones 10–12 from corresponding 3′-*N,N*-didemethylated macrolides 7–9 via a 3′-(4-nitrophenyl)carbamate intermediate (Table 1, entries 6 to 8).[17]

The same cyclization protocol using 4-nitrophenyl chloroformate and triethylamine was also applied to 3′-*N*-methyl compounds 4–6 (Scheme 2) in order to prepare 3-methyl-1, 3-oxazolidin-2-one derivatives 16–18. Interestingly, spontaneous cyclization of 3′-(4-nitrophenyl)methylcarbamates 13–15 to 3-methyl-1,3-oxazolidin-2-ones 16–18 in basic conditions was not observed either during the reaction or during work-up extraction at pH 12. Even prolonged stirring at pH 12 did not provide the cyclized product. However, cyclization of intermediates 13–15 to



	X	R^1	R^2	Yield
19		CH_3		64%
20		CH_3		59%
21		CH_3		51%
22		CH_3		42%
23		CH_3		63%
24		H		41%
25		H		44%
26		H		39%
27		H		52%

Scheme 3. Reagents and conditions: (a) R^2-iodide, tetrabutylammonium fluoride, cesium carbonate, *N,N*-dimethylformamide, rt.



27

Scheme 4. Reagents and conditions: (a) Allyl *tert*-butyl carbonate, tris(dibenzylideneacetone)dipalladium, di(diphenylphosphino)butane, 80 °C.

Table 2
Antibacterial activities of macrolides **1–27**

Strain	MIC (μg/mL)											
	1	2	3	4	5	6	7	8	9	10–12	**16–18**	**19–27**
S. aureus	32	0.25	1	>64	4	16	>64	>64	>64	>64	>64	>64
S. pneumoniae	1	<0.125	<0.125	32	<0.25	2	>64	2	4	>64	>64	>64
S. pyogenes	4	<0.125	<0.125	64	<0.25	2	>64	2	32	>64	>64	>64
M. catarrhalis	0.5	<0.125	<0.125	16	2	2	>64	16	8	>64	>64	>64
H. influenzae	16	8	2	>64	>64	>64	>64	>64	64	>64	>64	>64

the desired 2′,3′-fused 3-methyl-1,3-oxazolidin-2-ones 16–18 can be readily achieved using sodium hydride (see Supplementary data for reaction conditions).

In order to attach other alkyl substituents to the 1,3-oxazolidin-2-one ring of 10–12, N-alkylations using alkyl iodides were explored. Simple alkylation reactions of compound 10 using a few chosen alkyl iodides in the presence of a base such as diisopropylethylamine or sodium hydride proved unsuccessful. Either no alkylation was observed or, when sodium hydride was used, considerable degradation of macrolides occurred after prolonged reaction times. Successful N-alkylations of 10–12 were achieved in the presence of tetrabutylammonium iodide and cesium carbonate[18] to afford a series of novel 3′-N-alkylated macrolides 19–27 in good yields after chromatographic purifications (Scheme 3).[19] N-allylation of 1,3-oxazolidin-2-one 12 was accomplished using palladium-catalyzed allylation with allyl *tert*-butyl carbonate (Scheme 4) to afford 3-allyl-3-oxazolidin-2-one 27 in a good yield (52%). Structures of all new macrolides were confirmed by combining NMR (1D and 2D) and MS data (see Supplementary data). In general, no solubility problems were detected.

The antibacterial activities of novel macrolides having N-substituted 1,3-oxazolidin-2-ones fused to the desosamine sugar were assessed on a panel of five common respiratory pathogens and compared with parent macrolides 1–3, as well as with the 3'-N-demethylated compounds 4–6 and 3′-*N*,*N*-didemethylated compounds 7–9 (Table 2.).[20] The removal of one methyl group from 3′-N-position (4–6) already slightly diminishes antibacterial activity. Removal of the second methyl group (7–9) further reduces antibacterial activity but not completely for 2 and 3. However, formation of 1,3-oxazolidin-2-ones 10–12, as well as N-substituted-1,3-oxazolidin-2-ones (16–27) completely abolishes antibacterial activity of parent macrolides 1–3.

In conclusion, a simple method for the synthesis of novel 14- and 15-membered macrolide derivatives having N-substituted-1,3-oxazolidin-2-ones fused to the desosamine sugar have been envisioned and developed. The method is suitable for large scale synthesis of prospective drug candidates. Since these desosamine-modified macrolides completely suppress the antibacterial activity of the parent antibacterial macrolides, their evaluation as potential agents for other biological targets is currently ongoing.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.03.076.

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19. *General procedure for the alkylation of 10–12:* To a solution of the corresponding 3′-N,N-didemethylated macrolides 4–6 in *N*,*N*-dimethylformamide (c = 0.06 g/mL) under nitrogen atmosphere, cesium carbonate (1.5 equiv) and tetrabutylammonium iodide (1.5 equiv) were added. After stirring for 30 min at rt, corresponding alkyl iodide or benzyl bromide (1.5 equiv) was added and stirring continued for 5 h. The reaction mixture was diluted with ethyl acetate, washed with saturated aq $NaHCO_3$ solution, and dried over Na_2SO_4. The solvent was evaporated and products purified by column chromatography (eluent $CH_2Cl_2/CH_3OH/NH_4OH$ (90:2.5:0.25)).
20. Minimum inhibitory concentrations (MICs) were determined for all new compounds on a panel of macrolide susceptible Gram-positive (*Staphylococcus aureus* ATCC13709, *Streptococcus pneumoniae* ATCC149619, *Streptococcus pyogeneses* ATCC700294) and Gram-negative (*Haemophilus influenzae* ATCC49247, *Morexella catarrhalis* ATCC23246) bacterial strains MIC values were determined using microdillution method as recommended by CLSI[21] in appropriate media (Mueller–Hinton broth (MHB) for *S. aureus* and *M. catarrhalis*, MHB supplemented with 5% horse serum for streptococci, and Hemophilus test medium for *H. influenzae*) media. Tested compounds were dissolved in DMF (5 mg/mL) and double diluted in media to give concentration ranges from 64 to 0.125 μg/mL. MICs were determined after 24 h incubation at 37 °C.
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