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Novel tethers in ketolide antibiotics

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Abstract—Novel macrolide antibiotics which contain a methylene unit between two nitrogen atoms of carbamate groups or between two nitrogen atoms of one carbamate and one urea group were synthesized using the Curtius rearrangement. Such linkers were shown to be stable under physiological conditions, and the resulting ketolides show potent in vitro and in vivo activity against macrolide-resistant respiratory pathogens. The SAR of various heterocycles and linkers was established.

Ketolides are the latest series of macrolide antibiotics developed to counter macrolide-resistant pathogens of respiratory diseases.[1] Ketolides in general have excellent activities against many pathogens of respiratory diseases but especially against macrolide-resistant *Streptococcus pneumoniae*. Telithromycin (**1**) is only ketolide thus far commercialized.[2] Ketolide molecules contain a ketone group at C3, a cyclic carbamate group at C11 and C12, and a heterocycle attached with a tether to the macrolide ring either at C11 nitrogen or C6 oxygen as in the case of cethromycin.[3] It was established earlier that the length of the tether is critical for the antibacterial activity, and a four-carbon alkyl chain appeared to be optimal when the tether is attached at the C11 nitrogen.[2,4] In addition to straight alkyl chains, amine-, hydrazine-, amide-, olefin-, and ether-containing linkers have been reported.[4,5] In pursuing ketolides of improved properties, we envisioned linkers such as those in **2** and **3** (Fig. 1). There was no disclosure of these types of ketolides in the literature at the outset of this project, but some methylene dicarbamate units are known in chemical literature.[6] Although such units in drugs are rare, we rationalized that such a moiety might possess reasonable stability since it is not easily protonated toward hydrolysis. In these linkers there would be five atoms between the heterocycle and the C11 nitrogen, but we speculated that the rigidity of the carbamoyl or urea group might

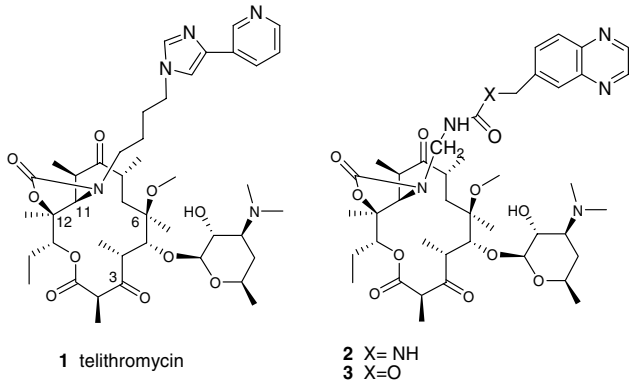

Figure 1. Structures of telithromycin and target molecules.

impart beneficial effect on their antibacterial activity. It was further hypothesized that such linkers could be prepared by using the Curtius rearrangement as shown in Scheme 1. Other approaches of constructing methylene dicarbamate units would have been difficult involving complex and somewhat sensitive macrolide antibiotics.[6]

Scheme 2 indicates the actual synthesis that starts with a well-established intermediate (**8**) in ketolide chemistry.[4] Compound **8** was allowed to react with the *tert*-butyl ester of glycine which added to the imidazole carbonyl group and subsequently cyclized at the C11 position. Then the cladinose was hydrolyzed to give alcohol **9** in a 78% yield for the two steps. The C3 alcohol was oxidized using the Corey–Kim reagent[7] to give compound **10** in a 95% yield. The *tert*-butyl ester was hydrolyzed

Keywords: Ketolide; Telithromycin; Curtius rearrangement; Dicarbamoyl methylene; Antibacterial; Linker; *Streptococcus pneumoniae*; *Streptococcus pyogenes*; *Haemophillus influenzae*.
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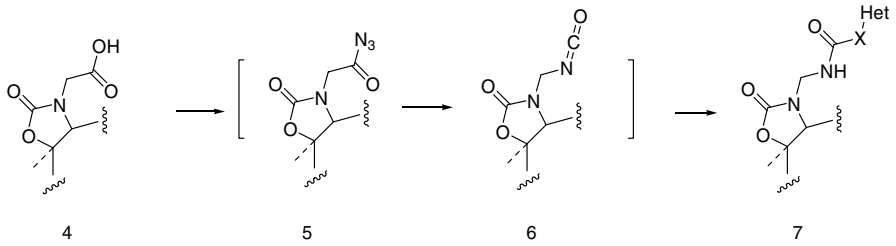
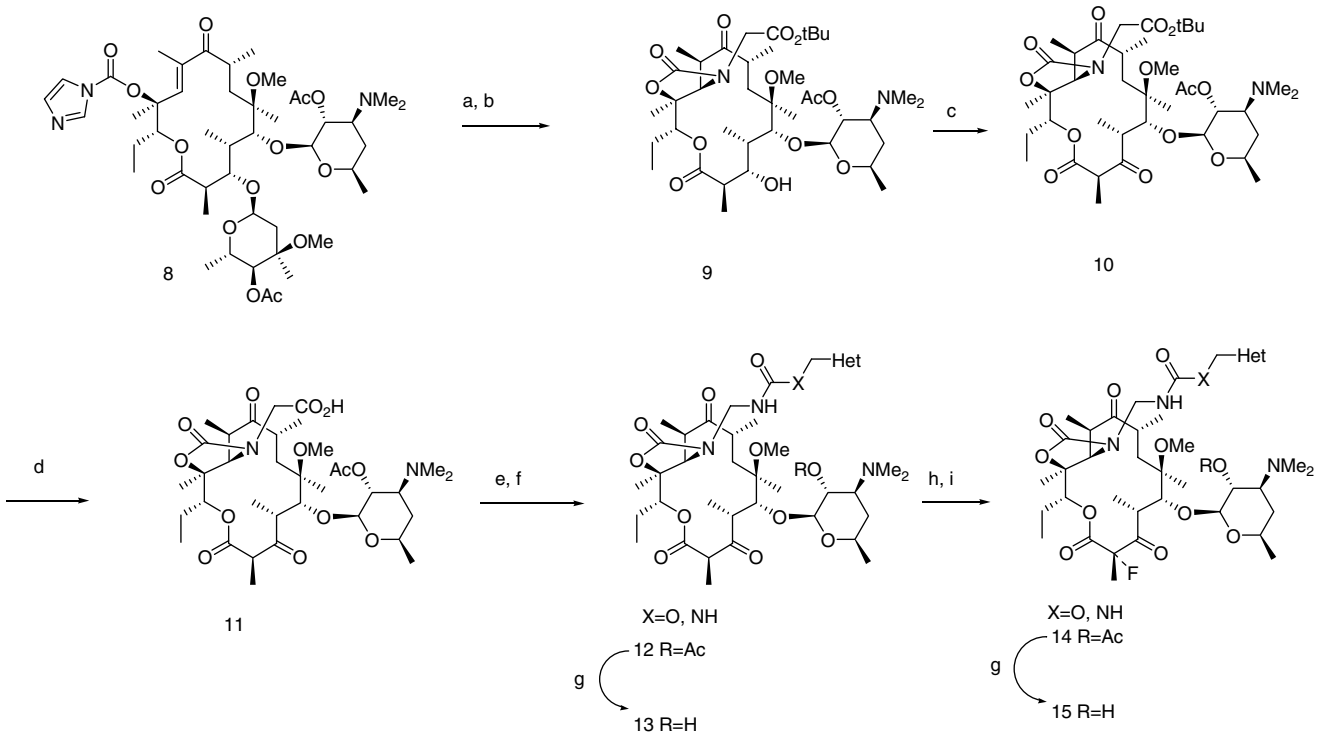


Scheme 1. Design of new linkers.



Scheme 2. Reagents and conditions: (a) $NH_2CH_2CO_2{}^tBu$, DBU, AcCN, 60 °C 2.5 h and then rt overnight; (b) 2 N HCl, ethanol, 38 °C, 3 h, 78% over two steps; (c) NCS, Me_2S, CH_2Cl_2, −10 °C, 95%; (d) Aq H_3PO_4 (85%), 90% yield; (e) $(PhO)_2P(O)N_3$, TEA, toluene, 70 °C; (f) HX–CH_2–Het, X = O or NH, 60–70% for two steps; (g) MeOH, 50 °C, 90%; (h) KHMDS, DMF, −65 °C; (i) Selectfluor™, 70%.

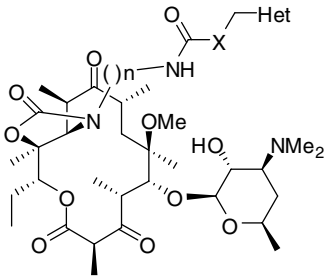
using 85% phosphoric acid[8] and the resulting free acid (**11**) was allowed to react with the Shioiri reagent[9] to affect the Curtius rearrangement. The crude isocyanate intermediate was allowed to react with either a heterocycle-containing alcohol or amine to give a carbamate or a urea product, respectively. The C2′ acetate was subsequently cleaved using methanol to give the final product (**13**). Fluorination at the C2 position was accomplished by first treating **12** with excess potassium hexamethyl disilazane (KHMDS) and then Selectfluor,[10] and the C2′ acetate was removed by methanol as before to give compound **15**.

Since there was little information on the stability of the methylene dicarbamate linkers, the stability of these molecules was also tested. Thus compounds **13e** and **15a** were dissolved in a simulated gastric fluid at pH 1.2 or phosphate buffer solutions at pH 3.6, 6.6, or 7.5. Their stability was followed by HPLC and there was less than 3% variation in the peak area after 24 h at room temperature, indicating reasonable stability.

The analogues prepared here had activity against macrolide-sensitive *Staphylococcus aureus*, *S. pneumoniae*, and *Streptococcus pyogenes*. For clarity, only data against macrolide-resistant organisms are shown in the tables. All minimum inhibitory concentration (MIC) determinations were carried out using NCCLS guidelines.[11] In Tables 1 and 2, average MIC's are given in μg/mL. There are two major mechanisms of resistance, one involving modification of the macrolide-target, the ribosome (*erm* (B)-encoded), and another involving increased efflux of the macrolide (*mef*-encoded). Among the test organisms, *S. pyogenes* 1079 (*erm*), *S. pneumoniae* 1095 (*erm*) and 1175 (*mef*) are erythromycin-resistant strains by the mechanisms indicated in the parentheses.

One methylene-unit linkers greatly enhance the in vitro antibacterial activity compared with two methylene-unit linkers as shown in Table 1. For example, compounds **16**[12] and **13a** share the same heterocycle and the same carbamate functionality, but compound **13a** has much improved potency against four organisms in the table. The

Table 1. Comparison of chain length (MIC, μg/mL)



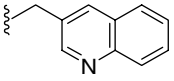
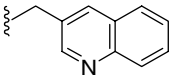
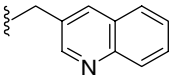
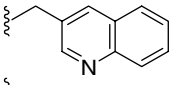
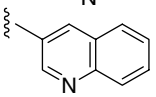
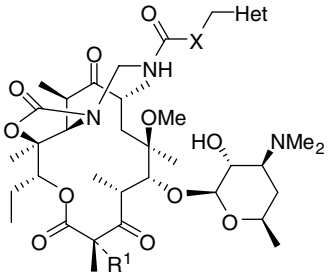
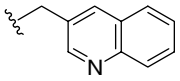
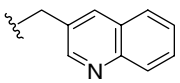
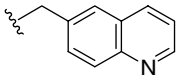
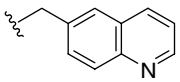
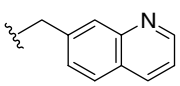
Compound	–CH_2–Het	*n*	X	*S. pne* 1095	*S. pne* 1175	*S. pyo* 1079	*H. inf* 1325
16		2	O	>64.0	0.125	>64.0	32
13a		1	O	<0.063	<0.063	64	8
17		2	NH	>64.0	0.5	>64.0	16
13b		1	NH	<0.063	0.5	16	4
13c	 directly linked to X	1	NH	0.25	<0.063	>64.0	16
Telithromycin 1				<0.063	0.378	18	3.9

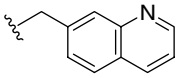
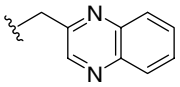
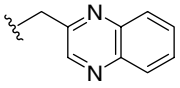
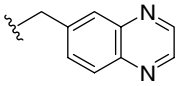
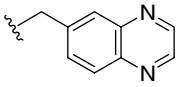
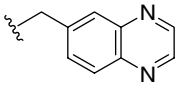
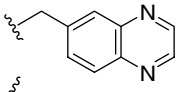
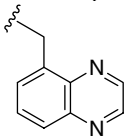
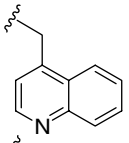
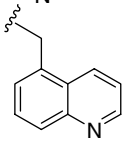
Table 2. Comparison of carbamates versus ureas (MIC, μg/mL)



Compound	–CH_2–Het	X	R^1	*S. pne* 1095	*S. pne* 1175	*S. pyo* 1079	*H. inf* 1325
13a		O	H	<0.063	<0.063	64.0	8.0
13b		N	H	<0.063	0.500	16.0	4.0
13d		O	H	0.250	<0.063	8.0	2.0
13e		N	H	0.250	0.125	4.0	1.0
13f		O	H	<0.063	<0.063	64.0	4.0

(*continued on next page*)

Table 2 (*continued*)

Compound	–CH_2–Het	X	R^1	*S. pne* 1095	*S. pne* 1175	*S. pyo* 1079	*H. inf* 1325
13g		N	H	<0.063	0.500	64.0	4.0
13h		O	H	0.250	0.250	>64.0	8.0
13i		N	H	<0.063	0.250	>64.0	4.0
13j		O	H	<0.063	<0.063	16.0	2.0
13k		N	H	<0.063	0.250	2.00	2.0
15a		O	F	<0.063	<0.063	1.0	2.0
15b		N	F	<0.063	<0.063	1.0	1.0
13l		O	H	<0.063	<0.063	22.6	4
13m		O	H	0.5	<0.063	64.0	8
13n		O	H	0.125	0.125	16	32

same conclusion can apply to the urea series (compounds **17** and **13b**). If the heterocycle is directly attached to the urea nitrogen, it reduces the activity against *erm*-containing *S. pneumoniae* and *S. pyogenes*, but retains the activity against *mef*-containing *S. pneumoniae* (see compound **13c**). Table 2 lists in vitro activities of quinoline and quinoxaline carbamates and ureas. Within each pair of a carbamate and urea analogues, the carbamate analogue appears to be more potent against *mef*-containing *S. pneumoniae* (**13a** vs **13b**, **13d** vs **13e**). On the other hand, urea derivatives are slightly more potent against *erm*-containing *S. pyogenes* (**13a** vs **13b**, **13j** vs **13k**).

Fluorine substitution at C2 improves the activity against *erm*-containing *S. pyogenes* especially in the case of carbamate analogues (**13j** vs **15a**). In the case of urea analogues, the effect is not as profound but it improves activity against *mef*-containing *S. pneumoniae* (**13k** vs **15b**). In terms of the site of attachment within the heterocycle, positions 3 and 7 appear to be optimal among the quinoline carbamates for the *S. pneumoniae* activity, whereas for *S. pyogenes* and *Haemophillus influenzae* activity, position 6 appears to be preferred (see **13a**, **13d**, **13f**, **13m**, and **13n**). Similar trends can be seen for quinoline urea derivatives. For quinoxaline analogues, attachment at position 6 appears to be optimal. Comparison of compound **15a**[13] with telithromycin indicates that the former is more potent against *erm*-containing *S. pyogenes* and *H. influenzae*.

In the murine acute systemic infection model, mice are challenged with a lethal intraperitoneal inoculum of the macrolide-resistant *S. pneumoniae* strain 1095, and treated with compound at 30 min and 4 h post-infection. Animals are monitored for survival for 4 days and the PD_{50}[14] is determined. Compound **15a** was active in this model with a PD_{50} of 71.2 mg/kg (telithromycin's PD_{50} was also 71.5 mg/kg in this experiment). This compound was also evaluated in the gerbil model of otitis media against *H. influenzae* 1100. In this model, gerbils were challenged via an intrabulla inoculation and treated three times a day for two days beginning 18 hours

post-challenge; on day four after challenge, gerbils were euthanized and middle ear fluids collected and bacteria contained therein were enumerated. In this model, compound **15a** had an ED_{50}[15] of 39.5 mg/kg, compared with telithromycin's ED_{50} of 27.5 mg/kg. In a murine soft tissue infection model, wherein bacteria (macrolide-resistant *S. pyogenes* 1068) were inoculated into the thigh tissue and compound was given at 30 min and four hours post-challenge, and bacterial burden determined 24 h after challenge, compound **15a** reduced bacterial burden by 2.3 log 10 at 100 mg/kg; telithromycin showed no bacterial reduction in this experiment. The compounds were dosed orally in all three experiments.

In conclusion, potent novel ketolides were discovered that contain methylene dicarbamate or methylene carbamate-urea linkers. An efficient synthesis of these ketolides was established utilizing the Curtius rearrangement. Such tethers appear to be stable under physiological conditions, and may be useful in other drug molecules. It is of interest that the Curtius rearrangement can be carried out in a complex and somewhat sensitive molecule such as ketolides. In contrast to the generally held notion that four-atom linkers are optimal regardless of the heterocycles, these five-atom linkers gave ketolides that are equally potent or slightly more potent than telithromycin.

Acknowledgments

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11. National Committee for Clinical Laboratory Standards, 2000, NCCLS Document M7-A5, Vol. 20, No. 2, Wayne, PA.
12. Compounds **16** and **17** were prepared in the same way as compounds **13** except starting with a β-alanine adduct.
13. Representative spectroscopic data of **15a**; ^{1}H NMR (400 MHz, methanol-d_4) δ ppm 0.85 (t, J = 7.05 Hz, 3H) 0.98 (d, J = 6.64 Hz, 3H) 1.09–1.32 (m, 13H) 1.46 (br s, 1H) 1.52 (s, 3H) 1.58 (br s, 1H) 1.65 (s, 2H) 1.71 (s, 2H) 1.97 (br s, 2H) 2.52 (s, 3H) 2.64 (d, J = 11.20 Hz, 1H) 2.77 (s, 4H) 3.20 (d, J = 6.64 Hz, 1H) 3.37–3.56 (m, 4H) 3.67 (br s, 1H) 4.06 (d, J = 9.54 Hz, 1H) 4.37 (d, J = 6.64 Hz, 1H) 4.77 (br s, 1H) 4.95 (br s, 1H) 5.12 (br s, 1H) 5.30 (d, J = 12.03 Hz, 2H) 7.04 (br s, 1H) 7.92 (s, 1H) 8.04 (br s, 1H) 8.10 (d, J = 8.29 Hz, 1H) 8.42 (s, 1H) 8.86 (d, J = 3.73 Hz, 2H); MS 846 (M+1).
14. Protective dose; dose that protects 50% of infected, treated animals.
15. Efficacious dose; the dose that cleared 50% of infected, treated animals.