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Novel *N*-demethylation of ketolide: application to the solution phase parallel synthesis of *N*-desosaminyl-substituted ketolides using ion exchange resins

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Abstract—A series of *N*-desosaminyl-substituted ketolides was synthesized in parallel by reductive amination of various aldehydes by a *N*-desmethyl-ketolide scaffold followed by a solid phase extraction protocol using ion exchange resins. In addition, we have demonstrated that diethyl azodicarboxylate (DEAD) can be used efficiently as a *N*-demethylating reagent to access to *N*-desmethyl-ketolides. © 2002 Elsevier Science Ltd. All rights reserved.

Macrolides, including erythromycin, are an old and well-known family of oral antibiotics. Their spectrum of activity covers most relevant bacterial species responsible for upper and lower respiratory tract infections.[1] However, the extensive clinical application of macrolide antibiotics has resulted in an increasing emergence of macrolide MLS_B resistance in respiratory pathogens such as *S. pneumoniae*.[2] The ketolides,[3] such as telithromycin[3b] or ABT-733[3c] are a new class of semisynthetic erythromycin derivatives, in which the C-3 L-cladinose is replaced by a 3-keto function, have recently been generated to address the problem of erythromycin resistance. They are very active against erythromycin-resistant and penicillin resistant *S. pneumoniae*, and the most advanced compound of this new class, telithromycin[3b] has demonstrated clinical efficacy in human respiratory tract infections.[4] In addition to their antibacterial activities, several non-antibacterial properties of macrolides such as anti-inflammatory, immunomodulatory and prokinetic were also reported during the last decades.[5] Particularly, substitutions at the 3′-*N* nitrogen of the desosamine sugar have generated interesting compounds displaying gastrointestinal activities,[6] LHRH antagonism[7] and inhibition of IL8 release.[8] Although, such activities have not yet been reported for ketolide compounds, their functional diversity makes this new class attractive for the parallel synthesis of libraries of diverse compounds endowed with various biological properties. Further to the widely used solid phase organic synthesis methods, several alternative strategies have been recently developed, especially those using solid supported reagents or scavengers.[9] These techniques allow the classical solution phase reactions to be easily worked-up by solid phase

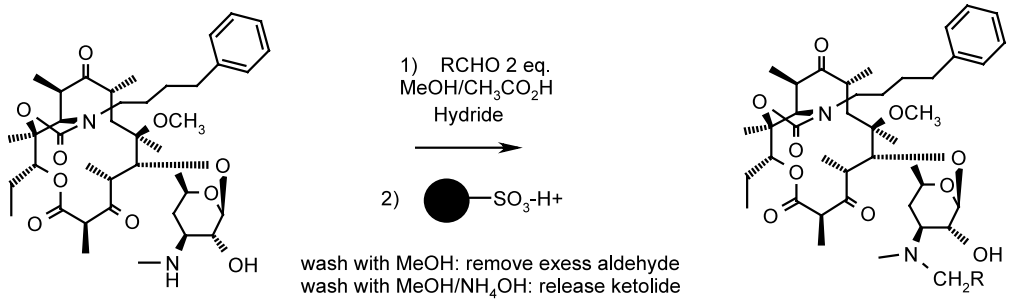

Figure 1. Reductive amination of *N*-desmethyl ketolide followed by solid phase extraction with acidic resin.

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extraction. These procedures can be easily automated and amenable to a parallel format. The desired substance is separated from the reaction mixture (or conversely, the associated impurities) by selective interaction with a functionalized polymer. Careful choice of reagents is necessary to insure that the desired product can be separated from excess reagents or starting material. The basic properties of amino groups have been frequently exploited to develop solid phase extraction purification procedures using ion exchange chromatography.[10]

Based on this strategy, we have developed a very simple parallel synthetic method for generating 3′-*N* substituted ketolides using liquid phase reductive amination of aldehydes and a solid phase extraction protocol (Fig. 1). The targeted library was designed to introduce diversity at the tertiary nitrogen of the desosamine sugar of the known ketolide **I**.[3a] Therefore, we needed first to achieve the synthesis of the mono-*N*-desmethyl analogue **II** (Fig. 2). Although several *N*-demethylation methods are available in the macrolide field, they are not applicable in each case because of many sensitive functionalities. For instance iodine can demethylate cleanly erythromycin,[6b] in contrast this reagent does not work with a ketolide and was shown recently to be replaced by NIS.[11] On the other hand, we have experienced some trouble (poor yields or side products depending on the series macrolide or ketolide) with the classical method using an alkyl chloroformate.[12] Therefore we were interested in the development of an alternative method which can provide smoothly the *N*-desmethyl ketolide **I** as well as other analogues. We found that the desmethylation procedure using diethyl azodicarboxylate (DEAD) described by Smissman[13] could be successfully employed with ketolides in a one-step procedure. This reaction, although seldom used, proceeds very efficiently with the ketolides and offers a new alternative to the previous methods. Typically 1.1 equiv. of DEAD was reacted with **I** in acetone at room temperature for 24 h and the resulting adduct was then hydrolyzed in a refluxing 1/1 MeOH/saturated $NH_4^+Cl^-$ mixture to give the *N*-demethylated ketolide **II** in 82% yield.[14] The expected demethylated product was obtained in good yield with no major side product isolated. Having the demethylated starting material in hand, we were next interested in setting up the reductive amination protocol. The most common methods to carry out reductive amination of aldehydes use a hydride reducing agent such as $NaBH_3CN$, $NaBH(OAc)_3$ or a borane–pyridine complex (BAP). In the context of combinatorial and parallel synthetic methods (solid phase synthesis, solid supported reagent and solid phase extraction) these three reagents have been used successfully.[10b,15] Thus, we decided to test these hydrides to optimize the reaction of **II** with 4-hydroxy-3-chloro-benzaldehyde as a model (Table 1) and to further extend the appropriate conditions to a set of various aldehydes. The reactions were all carried out in methanol and monitored by both TLC and HPLC. $NaBH(OAc)_3$ appeared to be the less reactive hydride, converting only 70% of the *N*-desmethyl ketolide. Increasing the amount of aldehyde or acetic acid did not improve the conversion rate. The classical Borch reduction conditions were more efficient (100% conversion rate), however, the reaction time 72–48 h was quite long. Finally, reacting **II** with BAP reagent and 5 equiv.

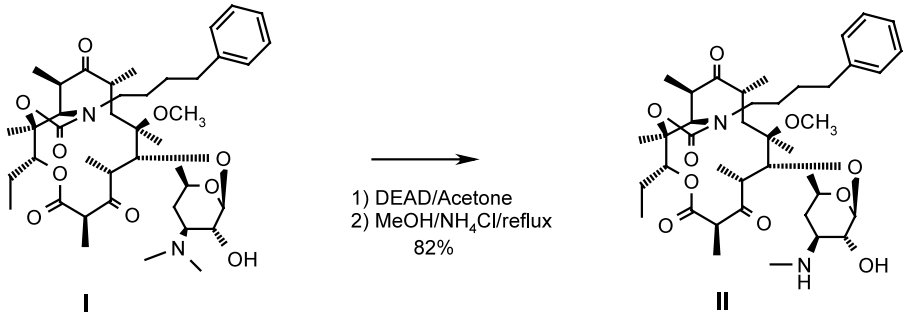


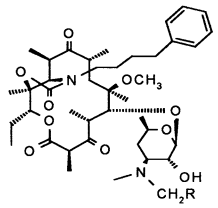
Figure 2. *N*-Demethylation of ketolide using DEAD.

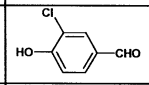
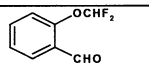
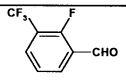
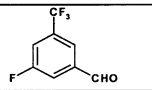
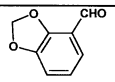
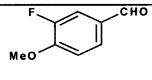
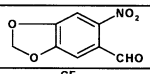
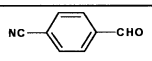
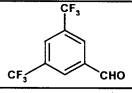
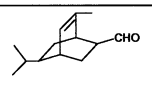
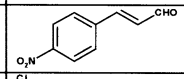
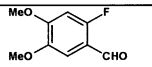
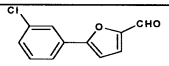
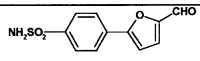
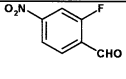
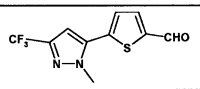
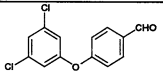
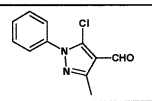
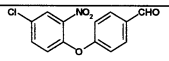
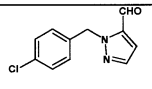
Table 1. Optimization of reductive amination of **II** with 4-hydroxy-4-chloro-benzaldehyde

Aldehyde (equiv.)	Hydride (2 equiv.)	CH_3CO_2H (equiv.)	% Conversion[a]	Time (h)
1.2	$NaBH(OAc)_3$	0	<70	>24
1.2	$NaBH(OAc)_3$	3	<70	>24
2	$NaBH(OAc)_3$	3	<70	>24
2	$NaBH_3CN$	3	100	72
3	$NaBH_3CN$	3	100	48
2	BAP	0	100	48
2	BAP	2	100	24
5	BAP	2	100	8

[a] Determined by C-18 reverse phase (Waters 4.6×254 mm Symetry®) HPLC, monitored at 210 nm, eluting with CH_3CN/H_2O/trifluoroacetic acid (400 mg/l).

Table 2. Synthesis of *N*-desosaminyl substituted ketolide library



Entry	Aldehyde RCHO and entry	Yield %	Purity* %	MS $(M+H)^+$	Entry	Aldehyde RCHO and entry	Yield %	Purity* %	MS $(M+H)^+$
1		70	95	871.5	11		74	96	887.6
2		83	98	907.5	12		86	98	907.5
3		70	93	865.5	13		72	95	869.5
4		99	95	910.6	14		82	95	846.6
5		90	97	957.6	15		82	99	907.8
6		90	96	892.6	16		80	95	899.6
7		82	98	922.7	17		89	92	966.7
8		88	95	884.5	18		74	96	975.7
9		79	96	981.7	19		99	94	935.7
9		72	95	992.7	20		80	(44)**	935.7

* % purity determined by C-18 reverse phase (Waters 4.6 x 254 mm Symetry®) HPLC, monitored at 210 nm, eluting with CH_3CN/H_2O/trifluoroacetic acid 400mg/l) ; ** Yield after chromatography over silica eluting with ethyl acetate/methanol/triethylamine 85/10/5.

of aldehyde gave a 100% conversion of **II** into the expected ketolide[16] in 8 h. Next, the solid phase extraction protocol was set up using the strongly acidic Dowex® 50W×8 or Amberlyst® 15 resins in various amounts. The reaction was diluted with additional methanol and acetic acid and then poured over the resin in a solid phase extraction cartridge to load the compound. The mixture was then filtered off and the resin was washed once with MeOH to eliminate the remaining aldehyde. Finally the compound was released by addition of a mixture of MeOH and triethylamine and filtration. The Amberlyst® 15 resin turned out to give the most efficient and reproducible purification results in contrast to the Dowex® 50W×8 resin which gave only a partial loading of the final compound and no release at all. We observed that 26 equiv. of resin were optimal to obtain a complete loading and release. These conditions were thus selected to synthesize a small library of 20 compounds.[16] Among the 20 aldehydes used, 19 gave the desired pure compound with high yields (Table 1). Only one aldehyde (entry 20) was in fact retained by the resin and hence released at the same time as the final compound; this was likely to happen because of the basic nitrogen of the pyrrazol ring (the aldehyde in excess was later eliminated by classical chromatography). In contrast, two other pyrrazoles reacted quite well likely because of a lower pK_a due to the electron withdrawing groups CF_3 or chlorine (entries 18 and 19). Overall, the reaction gave the expected ketolide in good yields and with a high purity. All the final compounds were characterized by HPLC, mass spectrometry and NMR.

In conclusion, we have demonstrated that a complex hemisynthetic molecule such as a ketolide can be easily modified and purified by ion exchange resins in a parallel process to generate small libraries of analogues. Furthermore, we have developed a new simple and efficient methodology to *N*-demethylate a ketolide scaffold. The biological activities of these new compounds will be reported elsewhere.

Acknowledgements

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14. Typical *N*-demethylation procedure: To a solution of **I** (14 g, 18.8 mmol) in 260 ml of acetone, was added in 1 h 30 min 3.6 g (20.7 mmol) of diethyl azodicarboxylate (DEAD) diluted in 20 ml of acetone. The reaction was stirred at room temperature for 24 h and evaporated to dryness. The brown residue was diluted in 40 ml of MeOH and 40 ml of saturated $NH_4^+Cl^-$ and refluxed for 1 h. After evaporation to dryness, the residue was taken up with 80 ml of water and the pH was adjusted to 8 with aqueous ammonium hydroxide and finally extracted with 3×150 ml of ethyl acetate. Drying over $MgSO_4$ and evaporation of the solvent afforded 16 g of crude product. The residue was purified by column chromatography over silica eluting with a 85/10/5 ethyl acetate/MeOH/triethylamine mixture to afford 11.3 g (82%) of **II** as a white foam. *Spectral data for* ***II***: EI-MS = 731^+ (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H) **C$\underline{H}_3$CH$_2$**, 1.02 (d, 3H) **10-CH$_3$**, 1.16 (d, 3H) **8-CH$_3$**, 1.23 (d, 3H) **5′-Me**, 1.27 (d, 3H) **4-CH$_3$**, 1.33 (s, 3H) **6-CH$_3$**, 1.38 (d, 3H) **2-CH$_3$**, 1.47 (s, 3H) **12-CH$_3$**, 1.66 (m, 4H) **C$\underline{H}_2$–C$\underline{H}_2$**, 2.43 (s, 3H) **NC$\underline{H}_3$**, 2.48 (m, 1H) **H′$_3$**, 2.60 (m, 1H) **H$_8$**, 2.62 (t, 2H) **CH$_2$Φ**, and 2.62 (s, 3H) **6-OCH$_3$**, 3.08 (dq, 1H) **H$_4$**, 3.11 (q, 1H) **H$_{10}$**, 3.59 (s, 1H) **H$_{11}$** and (m, 1H) **H′$_5$**, 3.64 (m, 2H) **C$\underline{H}_2$NCO**, 3.86 (q, 1H) H_2, 4.27 (d, 1H) **H$_5$**, 4.29 (d, 1H) **H′$_1$**, 4.97 (dd, 1H) **H$_{13}$**, 7.1–7.25 (m, 5H) phenyl. Anal. calcd (%) for $C_{40}H_{62}N_2O_{10}$: C, 65.73; H, 8.55; N, 3.83. Found: C, 65.4; H, 8.7; N, 3.7%.
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16. Typical reductive amination experiment: A solution of **II** (0.1 g, 0.14 mmol) and 4-chloro-3-hydroxy-benzaldehyde (0.11 g, 0.7 mmol) in 3.5 ml of methanol and glacial acetic acid (17 μl, 0.28 mmol) was stirred for 30 min at room temperature. The borane–pyridine complex (0.28 μl, 0.28 mmol) was added and the reaction was stirred for 8 h. The reaction was diluted with 8 ml of a 9/1 MeOH/AcOH mixture and poured over 2 ml (26 equiv.) of Amberlyst 15® in a 20 ml solid phase extraction cartridge fitted with the appropriate polyethylene frit (Supelco). The cartridge was shaken for 30 min, filtered off, the resin being finally washed with 15 ml of MeOH. Then 8 ml of a 88/12 MeOH/triethylamine mixture were added and the cartridge was shaken for 30 min. The resin was filtered off over a 45 μ polypropylene filter (Millex) and rinsed once with 10 ml of MeOH. The solution was then evaporated to give 85 mg (70% yield) of compound **1**. *Spectral data for compound* ***1*** (Table 2, entry 1): MS = 871.5^+ (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.86 (t, 3H) **C$\underline{H}_3$CH$_2$**, 1.01 (d, 3H) **10-CH$_3$**, 1.16 (d, 3H) **8-CH$_3$**, 1.27 (d, 3H) **5′-Me**, 1.31 (d, 3H) **4-CH$_3$**, 1.34 (s, 3H) **6-CH$_3$**, 1.38 (d, 3H) **2-CH$_3$**, 1.47 (s, 3H) **12-CH$_3$**, 1.65 (m, 4H) **C$\underline{H}_2$–C$\underline{H}_2$**, 2.17 (s, 3H) **NC$\underline{H}_3$**, 2.60 (m, 1H) **H$_8$** and (t, 2H) **CH$_2$Φ**, 2.62 (s, 3H) **6-OCH$_3$**, 3.12 (m, 1H) **H$_4$** and (m, 1H) **H$_{10}$**, 3.30 (m, 1H) **H′$_3$**, 3.59 (s, 1H) **H$_{11}$**, 3.30–3.62 (m, 2H) **ΦC$\underline{H}_2$N**, 3.65 (m, 2H) **C$\underline{H}_2$NCO**, 3.86 (q, 1H) H_2, 4.24 (d, 1H) **H$_5$**, 4.30 (d, 1H) **H′$_1$**, 4.97 (dd, 1H) **H$_{13}$**, 5.59 (m, 1H) **Φ**OH, 7.1–7.30 (m, 6H) aromatics, 6.96 (d, 1H) and 7.06 (dd) aromatics. Anal. calcd (%) for $C_{47}H_{67}N_2O_{11}Cl$: C, 64.77; H, 7.75; N, 3.21; Cl, 4.07. Found: C, 63.9; H, 7.6; N, 3.2; Cl, 4.1%.