Efficient Synthesis of 9- and 13-Oxo Leucomycin Derivatives Using Hypervalent Iodine Reagents in Solution and on Solid Support

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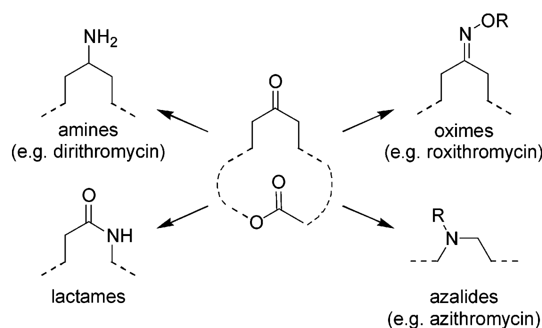
An efficient procedure for the highly selective oxidation of leucomycines to the corresponding 16-membered 9- and 13-oxo macrolides using hypervalent iodinates is presented. Both the Dess−Martin periodinane (DMP) and polymer-bound 2-iodoxybenzoic acid (IBX) show clear advantages over the previously employed manganese dioxide. Key intermediates for a variety of further chemical derivatization methods (**2a**, **2b**) are obtained in very good yields without the requirement of protecting groups.

In recent years, a dramatic increase of antibiotic resistance has been observed, which urges the rapid development of novel antibacterial therapeutics. As a complementation of metabolic engineering strategies,[1] the chemical derivatization of macrolides is a powerful approach to complex polyketide antibiotics, which may defeat resistance mechanisms.[2,3] In particular, keto-derivatives of 14- and 16-membered macrolides have emerged as key intermediates for the synthesis of a large variety of biologically active natural product derivatives (Scheme 1).[4−6] Important examples are represented by amines (e.g., dirithromycin[7]), oximes, such as roxithromycin,[8] amides,[6] and azalides, such as azithromycin[9,10] (Scheme 1). However, many more carbonyl reactions are conceivable, and yet analogues of 16-membered macrolides, such as the leucomycines, are only relatively little explored.[11]

To date, the limiting factor in the synthesis of leucomycin analogues is the availability of 9-keto derivatives. While the native 9-hydroxy macrolides can be readily obtained by large-scale fermentation of the microbial producer strains, the selective oxidation of the allylic alcohol represents a critical bottleneck. Yet in all reported protocols the well-known oxidant for allylic alcohols, manganese(IV) oxide,[12−15] has been employed. Unfortunately, this reagent provided the desired products only in unsatisfactory yields (~20%), as in the oxidation of the 9-hydroxy group of leucomycin A3.[16−18] We frequently observed a complete lack of turnover and the formation of undefined side products using alternative substrates with activated manganese(IV) oxide. To overcome these limitations, we were motivated to establish a more efficient and versatile reagent that provides the desired ketones in high yield, preferably without formation of unwanted side products and without the need of protective groups.

The model substrates used in this study, forocidines **1a**−**c**, were obtained by acidic cleavage of the mycarose moiety of the leucomycin complex. These 16-membered macrolides were isolated in multigram amounts from a large-scale fermentation broth of *Streptomyces hygroscopicus.*[19,20] Among various oxidizing agents, we found the Dess−Martin periodinane (DMP), a well-known mild oxidant of primary and secondary alcohols, [21,22] to be ideally suited for the oxidation of **1a**. HPLC monitoring revealed

Scheme 1. Examples for the Synthesis of Macrolide Derivatives from Oxo Macrolides



that the allylic alcohols were smoothly transformed into the desired ketones. The 9-oxo product (**2a**) was obtained in excellent yield without formation of side products. Best results were obtained employing DMP in a modified protocol (method 1).[23] According to Meyer and Schreiber the presence of a small amount of water, preferably added as a dilute solution in CH_2Cl_2, generates an activated Dess−Martin periodinane species that decomposes more rapidly and accelerates the reaction.[23] With this method, **1a** was readily converted and 9-oxoforocidin (**2a**) was obtained in 61% isolated yield (compared to <20% using activated manganese dioxide).

In addition to the 9-oxo derivative **1a**, we were also able to efficiently synthesize the corresponding 13-oxo derivative (**2b**) from 13α-isoforocidin (**1b**) and 13*β*-isoforocidin (**1c**) using DMP (Scheme 2). The slightly lower yield of **2b** can be rationalized by a sterically disfavored reaction compared to the oxidation of the 13α-isomer. The 9- and 13-oxo forocidines were purified by normal- and reverse-phase chromatography, and their structures were fully elucidated by MS, IR, and 1D- and 2D-NMR. To the best of our knowledge, the 13-oxo macrolide **2b** represents a new member of the leucomycin family. Standard assays reveal that **2b** has an antimicrobial profile similar to the parent forocidines. Careful analyses of the reaction mixtures clearly demonstrated that the allylic hydroxy groups were exclusively oxidized, while the aldehyde and aliphatic alcohol residues were not affected. In fact, DMP may serve as an additional means for structure determination since obscurities in hydroxy group positions could be easily resolved.

To facilitate the synthetic protocol and workup, e.g., for parallel synthesis, we investigated the alternative use of

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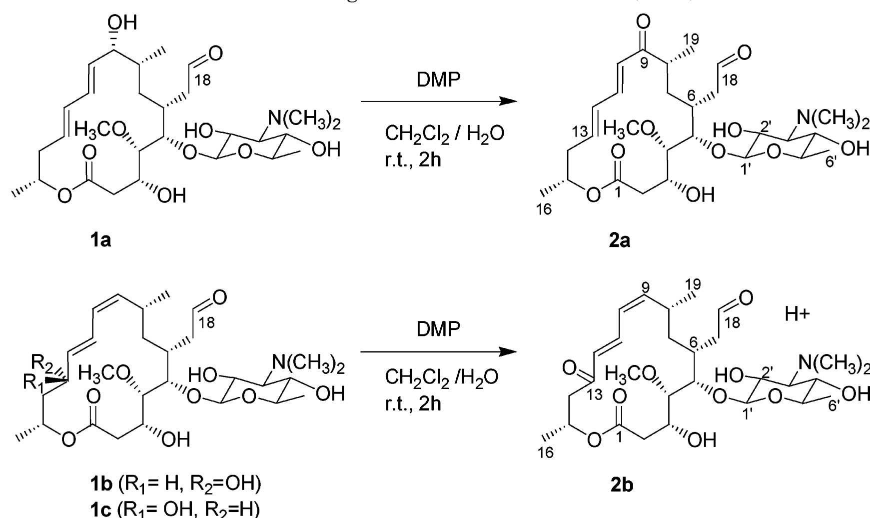
Scheme 2. Selective Oxidation of Forocidines **1a**−**c** Using Dess−Martin Periodinane (DMP)



polymer-bound 2-iodoxybenzoic acid (IBX, Figure 1) for forocidin oxidation. IBX has previously been used for the

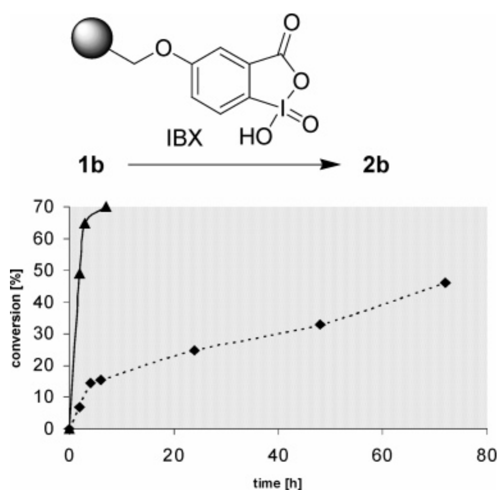


Figure 1. Relative product/educt ratio as monitored by HPLC: (▲) DMP; (◆) IBX-PS.

oxidation of alcohols and aldehydes to carboxylic acids[24,25] and ketones.[26] The leucomycin derivative **1b** was selectively oxidized with IBX, giving the unsaturated ketone **2b**, albeit with longer reaction times and lower turnover (see Figure 1). The reduced reaction rate, which may be attributed to steric hindrance of both reactant and oxidant, could not be significantly compensated with increased reaction temperature. Although the efficiency of DMP as oxidant in solution is clearly superior to the corresponding periodinane supported on polystyrene, longer reaction rates and lower yields may be taken into account if simple reconditioning and the possibility of reagent recycling are favored. Still, IBX-PS provides markedly higher yields of the oxo compounds than manganese dioxide.

In conclusion, we have demonstrated a new and highly efficient approach to 9- and 13-keto leucomycin derivatives using hypervalent iodine reagents. Since 9-keto leucomycines represent valuable key intermediates in the synthesis of biologically active macrolide derivatives, a crucial step in the protocol could be significantly improved (up to 61% isolated yield compared to <20% using manganese dioxide). With both DMP and IBX, allylic 9- and 13-OH groups of the highly functionalized macrolides **1a**−**c** were selectively oxidized in good to very good yields without the need of protecting groups. In addition, **2b** is the first example of a 13-keto forocidine, which may provide the starting point to other novel semisynthetic macrolide antibiotics.

Table 1. Use of Polystyrene-Bound Periodinane IBX Compared to DMP and MnO_2

entry	educt	product	DMP (%)	IBX-PS (%)	MnO_2 (%)
1	**1a**	**2a**	61	32	< 20
2	**1b**	**2b**	36	25	< 10
3	**1c**	**2b**	24	<20	<10

Experimental Section

General Experimental Procedures. Solvents were dried and distilled under nitrogen before use employing standard drying agents. Dess−Martin periodinane was purchased from Lancaster, and IBX-polystyrene from Novabiochem. Normal-phase chromatography was performed using silica gel M60 (Macherey-Nagel, 0.043−0.063 mm/230−400 mesh) with a $CHCl_3$/MeOH gradient 9:1 (v/v) containing 1% aqueous NH_3 as eluent. For high-pressure liquid chromatography (reverse-phase) Phenomenex columns (Synergi 10u, Max-RP 80A, 10 μm, 250 mm × 4.6 mm, and 250 mm × 30 mm, respectively) and a gradient of ACN/H_2O containing 0.05% diethylamine were used. NMR spectra (in $CDCl_3$) were measured on Bruker Avance DRX 300 and DRX 500 instruments. Chemical shifts are reported in ppm. ESIMS were recorded on a VG Quattro Fisons instrument, and HRESI was recorded on a Finnigan MAT 95XL sector field mass spectrometer equipped with direct inlet system (IE 70 eV). IR spectra were measured using an FTIR Satellite FTIR Mattson spectrometer (Chicago, IL).

Synthesis of Forocidines 1a−**c**. Leucomycin base (turimycin, 10 g, batch number 181 from *S. hygroscopicus*, see ref 11) was dissolved in 0.3 M HCl (150 mL). The resulting yellow solution was kept for 20 h at ambient temperature. After carefully increasing the pH to 3−4 with aqueous NaOH (20%, w/v) the solution was extracted 10 times with $CHCl_3$ (50 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated, affording 1.62 g of an oily residue of acyl mycaroses. The weakly acidic aqueous phase was adjusted to pH 8−9 with NaOH (20%, w/v) and extracted eight times with $CHCl_3$ (50 mL). The combined organic solutions were dried over Na_2SO_4 and evaporated under reduced pressure, affording 1.96 g of forocidines as a solid yellowish foam. Purification of the components (**1a**−**c**) was carried out as reported previously.[20]

Method 1. Dess−Martin periodinane (101.8 mg, 0.24 mmol) was added to a solution of forocidin (**1a**, **1b**, or **1c**) (111.4 mg, 0.2 mmol) in CH_2Cl_2 (3 mL). After stirring for 30 min at room

temperature a solution of H_2O (10 μL) in CH_2Cl_2 (10 mL) was added slowly via a dropping funnel. The pale yellow solution was stirred for 2−3 h until formation of a noticeable precipitate, then concentrated under reduced pressure, and the residue was taken up in ether (30 mL). It was washed with 10% $Na_2S_2O_3$/saturated aqueous $NaHCO_3$ (v/v 1:1, 15 mL), followed by H_2O (10 mL) and brine (10 mL). The combined aqueous layers were extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were dried with Na_2SO_4 and concentrated. Reverse-phase (acetonitrile (0.05% diethylamine/H_2O) or normal-phase chromatography ($CHCl_3$/CH_3OH, v/v 9:1, 1% NH_3) provided the ketone as a pale yellow solid.

Method 2. IBX polystyrene (96 mg, 0.1 mmol, 2 equiv) was swollen by stirring in CH_2Cl_2 (4 mL) for 15 min. Then a solution of forocidin (**1a**, **1b**, or **1c**) (40 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction was monitored by analytical HPLC. After removing the resin by filtration, the filtrate was evaporated and the resulting substance was purified by preparative HPLC.

Compound 2a: IR (film) ν_{max} ~3400 (−OH), 2979−2792 (−CH, −OCH_3), 1716 (m, −CHO), 1679 (C=C), 1632 (C=O), 1591, 1453, 1376/1359, 1252, 1166 (m, C−O−C), 1058, 1003 (s), 835 (m, −CH) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.15 (3H, d, J = 6.7 Hz, H-19), 1.20 (1H, d, J = 6.1 Hz, H-6′), 1.28 (3H, d, J = 6.3 Hz, H-16), 1.49 (1H, m, H-7a), 1.61 (1H, m, H-7b), 1.84 (1H, m, H-6), 2.14 (1H, m, H-14a), 2.21 (1H, d, J = 15.8 Hz, H-2a), 2.29 (1H, dd, J = 10 Hz, H-3′), 2.4 (1H, nr, H-17a), 2.44 (6H, s, $N(CH_3)_2$), 2.45 (1H, m, H-14b), ~2.5 (1H, nr, H-8), 2.7 (1H, nr, H-2b), 2.73 (1H, m, H-17b), 3.00 (1H, dd, J = 9.4, 9.2 Hz, H-4′), 3.09 (1H, d, J = 9.3 Hz, H-4), 3.23 (1H, m, H-5′), 3.41 (1H, dd, J = 10.0, 7.6 Hz, H-2′), 3.51 (3H, s, H-20), 3.75 (1H, d, J = 10.7 Hz, H-3), 4.02 (1H, d, J = 9.2 Hz, H-5), 4.39 (1H, d, J = 7.3 Hz, H-1′), 5.17 (1H, m, H-15), 6.1 (2H, m, H-12, H-13), 6.27 (1H, d, J = 15.1 Hz, H-10), 7.21 (1H, m, H-11), 9.64 (1H, s, H-18) (nr = not resolved); ^{13}C NMR ($CDCl_3$, 80 MHz) δ 17.4 (CH_3, C-19), 17.8 (CH_3, C-6′), 20.2 (CH_3, C-16), 32.0 (CH, C-6), 32.4 (CH_2, C-7), 38.1 (CH_2, C-2), 41.5 (CH_2, C-14), 41.6 (CH_3, $N(CH_3)_2$), 44.7 (CH, C-8), 46.0 (CH_2, C-17), 61.8 (CH_3, C-20), 67.7 (CH, C-3), 68.8 (CH, C-15), 70.1 (CH, C-3′), 70.8 (CH, C-4′), 71.0 (CH, C-2′), 73.4 (CH, C-5′), 79.4 (CH, C-5), 85.3 (CH, C-4), 104.5 (CH, C-1′), 122.4 (CH, C-10), 131.7 (CH, C-12), 141.7 (CH, C-13), 143.2 (CH, C-11), 173.4 (C, C-1), 202.4 (C, C-9), 202.5 (CH, C-18) (NMR shifts nearly identical with carimbose B[27]); MS(ESI+) m/z 556 $(M + H)^+$, 588 $(555 + MeOH)^+$, 610 $(555 + MeOH + Na)^+$; MS(ESI−) m/z 554 $(M - H)^-$; HREIMS m/z 555.3019 (calcd for $C_{28}H_{45}NO_{10}$, 555.3043).

Compound 2b: IR (film) ν_{max} ~3400 (b, −OH), 2973−2795 (−CH, −OCH_3), 1719 (m, −CHO), 1627 (m, C=O), 1590 (w), 1451, 1377/1357, 1279, 1166 (m, C−O−C), 1059, 1002 (s), 835 (m, −CH) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.05 (3H, d, J = 6.6 Hz, H-19), 1.23 (1H, d, J = 6.1 Hz, H-6′), ~1.3 (2H, nr, H-7a, H-7b), 1.34 (3H, d, J = 6.4 Hz, H-16), 2.02 (1H, m, H-6), 2.14 (1H, m, H-8), 2.25 (1H, dd, J = 14.3 Hz, H-2a), 2.34 (1H, dd, J = 15.1, 4.4 Hz, H-3′), 2.35 (1H, nr, H-17a), 2.48 (1H, nr, H-14a), 2.50 (6H, s, $N(CH_3)_2$), 2.68 (1H, dd, J = 14.5, 11.3 Hz, H-2b), 3.0 (1H, nr, H-4), 3.04 (1H, nr, H-4′), 3.05 (2H, nr, H-14b, H-17b), 3.25 (1H, m, H-5′), 3.40 (1H, dd, J = 10.3, 7.4 Hz, H-2′), 3.52 (3H, s, H-20), 3.89 (1H, d, J = 11.0 Hz, H-3), 3.91 (1H, d, J = 9.0 Hz, H-5), 4.39 (1H, d, J = 7.4 Hz, H-1′), 5.2 (1H, m, H-15), 5.95 (1H, dd, J = 15.3, 9.26 Hz, H-9), 6.05 (1H, d, J = 15.8 Hz, H-12), 6.15 (1H, dd, J = 15.3, 10.6 Hz, H-10), 7.05 (1H, dd, J = 15.8, 10.6 Hz, H-11), 9.73 (1H, s, H-18) (nr = not resolved); ^{13}C NMR ($CDCl_3$, 80 MHz) δ 17.7 (CH_3, C-6′), 20.7 (CH_3, C-19), 21.5 (CH_3, C-16), 30.6 (CH, C-6), 35.5 (CH, C-8), 37.2 (CH_2, C-7), 39.5 (CH_2, C-2), 41.6 (CH_3, $N(CH_3)_2$), 44.5 (CH_2, C-14), 46.2 (CH_2, C-17), 61.9 (CH_3, C-20), 67.6 (CH, C-3), 70.0 (CH, C-15), 70.3 (CH, C-3′), 70.7 (CH, C-4′), 70.9 (CH, C-2′), 73.2 (CH, C-5′), 79.9 (CH, C-5), 85.8 (CH, C-4), 104.0 (CH, C-1′), 129.2 (CH, C-10), 130.8 (CH, C-12), 144.8 (CH, C-11), 150.7 (CH, C-9), 171.4 (C, C-1), 199.2 (C, C-13), 202.7 (CH, C-18); MS(ESI+) m/z 556 $(M + H)^+$, 588 $(555 + MeOH)^+$, 610 $(555 + MeOH + Na)^+$; MS(ESI−) m/z 554 $(M - H)^-$; MS (HRESI) m/z 556.3109 (M + H, calcd for $C_{28}H_{46}NO_{10}$, 556.3122).

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