# Synthesis of 9-deoxo-9a-aza-9a-homoerythromycin A 11,12-Hydrogen Borate and Azithromycin 11,12-Hydrogen Borate. A New Procedure to Obtain Azithromycin Dihydrate

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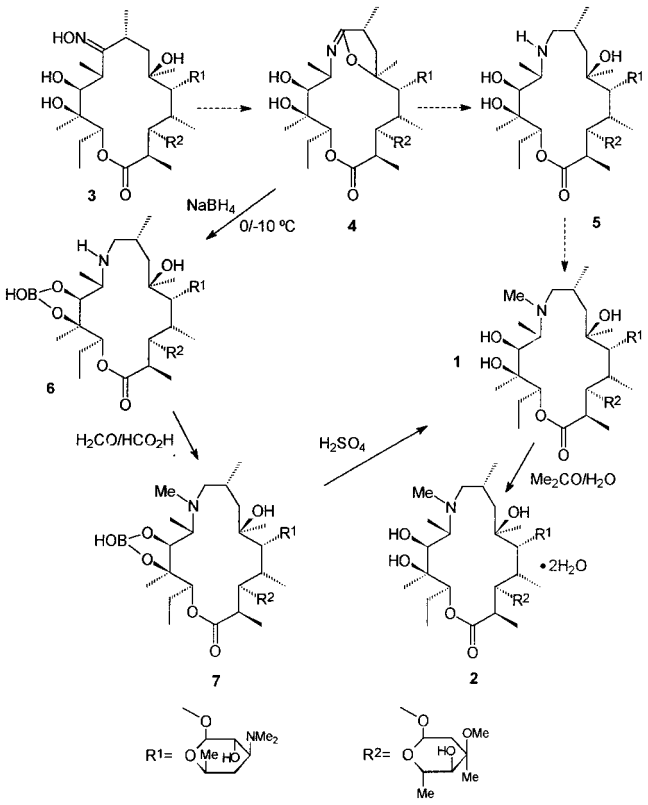
## Introduction

Azithromycin **1** is an azalide[1] antibiotic derived from erythromycin A with improved biological and pharmacodynamic properties over the parent compound.[2] The structural modification consists of the insertion of an *N*-methyl group between the carbon atoms C9 and C10 of the lactone ring of erythromycin A, affording a 15-membered aza macrolide. It has been shown that their antibacterial activity begins by binding to bacterial ribosome[3] and that in the bound state both compounds adopt a similar conformation of the macrocyclic ring.[4] The synthesis of azithromycin[5] is based on the Beckmann rearrangement of erythromycin A oxime **3** to yield the imino ether **4**.[5d] This is reduced to 9-deoxo-9a-aza-9a-homoerythromycin A (**5**), which is finally converted in azithromycin[5e,f] **1** by reductive methylation of the amine nitrogen with formaldehyde and formic acid (Scheme 1).

Three methods have been reported to achieve the reduction of imino ether **4**: (i) catalytic hydrogenation[5d,f] over $PtO_2$; (ii) electrochemically, in an electrolytical cell with a current of 1−2 A/dm$^2$ under an inert atmosphere;[6] and (iii) through the use of sodium borohydride in methanol.[5d] The first two methods are of limited value for the large scale production of azithromycin due to the use of relatively expensive catalysts and equipment to carry out the electrolysis. Alternatively, the reduction with sodium borohydride under the reaction conditions described (temperature range of 4−25 °C and aqueous work up at pH 2.5) has some negative effects on the yield and quality of azaerithromycin **5**. Thus, it is known that treatment of azithromycin with mineral acids hydrolyzes the cladinose and desosamyne sugars, affording the aglycon macrocycle.[5d] The hydrolysis is particularly easy for the cladinose ring and the desomaynilazithromycin is a known contaminant of azithromycin. On the other hand, we have found that azithromycin **1** obtained by this route was always contaminated with a minor compound, **6** (TLC and HPLC control[7a]), not characterized in the literature, and this byproduct is also *N*-methylated, yielding **7** in the last step of the synthesis outlined in Scheme 1, so that the contamination is transferred to the final antibiotic. Careful column chromatography allowed us to isolate both contaminants, and here we report their synthesis and structural identification. Furthermore, intermediates **6** and **7** allowed us to devise a new route to obtain pure azithromycin dihydrate[7b] (**2**) in excellent yields.

**Scheme 1**




## Results and Discussion

The procedure[8] is as follows: 9-deoxo-9a-aza-9a-homoerythromycin A 11,12-hydrogen borate (**6**) is quantitatively obtained by reduction of imino ether **4** with sodium borohydride in methanol at temperatures between 0 and −10 °C, and the aqueous workup is performed in the absence of mineral acids. Compound **6** is converted into azithromycin 11,12-hydrogenborate (**7**) by reductive methylation with formaldehyde and formic acid under refluxing chloroform. Hydrolysis of **7** keeping the pH value at 2 affords azithromycin in its hygroscopic form,

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i.e. monohydrate, which is transformed in the final step to therapeutic agent azithromycin dihydrate[7b] **2** by recrystallization in a mixture of acetone−water (Scheme 1).

The structural assignment of compounds **6** and **7** is based on their MS and NMR spectral data. The FABMS spectrum of compound **6** shows the molecular ion at *m/z* 761, which corresponds to an increase of 26 units related to the molecular weight of **5** ($C_{37}H_{70}N_2O_{12}$) due to the molecular fragment BOH present in the new compound **6** ($C_{37}H_{69}BN_2O_{13}$). The calculated isotopic pattern correlates well with the molecular formula proposed (Supporting Information). Moreover, the $^{11}$B NMR spectrum of **6** shows a broad signal at 10.0 ppm ($W_{1/2}$ 198 Hz), in the expected range for a boron nucleus bonded to three oxygen atoms with a trigonal geometry.[9]

The positions of binding of the boron atom to the molecular framework was determined by identifying the carbon atoms whose chemical shift was affected by the proximity of the boron moiety related to the non-boron-containing product.[10] The assignment of the $^{13}$C spectra was based mainly on the study of the 2D heteronuclear multiple-quantum coherence (HMQC)[11] and heteronuclear multiple-bond coherence (HMBC)[12] spectra (Supporting Information). The strategy of this work was to establish in the HMBC spectrum the connectivity of a known carbon, e.g. the lactone carbon of the macrolide ring, with protons separated by two or three bonds[13] and then to found out in the HMQC spectrum the direct response ($^1J_{CH}$) of these protons with the corresponding carbon nuclei. A full analysis will be published elsewhere.[14] Significantly, the largest $^{13}$C chemical shifts differences between **5** and **6** were observed for C11 and C12. In the boron derivative, C11 is deshielded by 7.68 ppm and C12 by 3.83 ppm relative to the azaerhytromycin A **5** and both signals were slightly broadened. The chemical shift variations for all other carbons were lower than 1.6 ppm. Furthermore, this limiting value corresponded to C10, which may be also affected by the proximity of the boron substituent. Similar $\beta$-deshielding effects have been found in the azaerythromycin A 11,12-cyclic carbonate[5c] and erythromycin A 11,12-methylene acetal.[15] Consequently, these results clearly established the link of the boron atom in **6** to the 11,12-hydroxy groups of the aglycon moiety of **5**.

The structural determination of compound **7** was achieved in an analogous way. The FABMS spectrum of azaerythromycin derivative **7** showed the molecular ion at 775, *m/z* and again the difference of 26 units with the molecular weight of **1** (MW 749) is accounted for by the formation of its hydrogen borate. The $^{11}$B NMR spectrum yields a broad singlet at 10.0 ppm ($W_{1/2}$ 159 Hz). Here, the NMR data have to be compared with those of azithromycin (**1**). The $^1$H and $^{13}$C NMR spectra of **1** have been previously assigned in $CDCl_3$[16] as well as in buffered $D_2O$ and DMSO-$d_6$.[17] As expected, the carbon atoms C11 and C12 in **7** are deshielded related to those of azithromycin (**1**) by 6.6 and 3.5 ppm, respectively. This fact allowed us to identify the link of the boron atom in **7** to the oxygens O11 and O12 of the macrocycle.

The formation of the hydrogen borate **6** is not too surprising if one considers the ability of boron to act as a protective group of 1,2-diols[18] and the favorable arrangement[19] of the hydroxy groups OH11 and OH12 in the conformation of azithromycin in chloroform.[20]

Compound **7** is a new boron-containing azalide and therefore a potential antibiotic. Consequently, its in vitro microbiological activity was screened against Gram-positive and Gram-negative organisms (Supporting Information). Unfortunately, in all cases tested the activity of **7** was significantly lower than that of azithromycin. The best results were obtained against *Bacillus* sp. (inhibitory minimal concentration (IMC) 50 $\mu$g/mL), which corresponds to a 125-fold decrease in activity *vs* that of azithomycin.

## Experimental Section

**General.** Imino ether **4** was prepared according to literature methods.[5d] Fast atom bombardment (FAB) mass spectra were obtained in a Finnigan Mat (Mat95) spectrometer. $^1$H-, $^{13}$C-, and $^{11}$B-NMR spectra were measured in a Bruker AMX-400 at 400, 100.61, and 128.38 MHz, in $CDCl_3$ solutions. NMR chemical shifts are expressed in ppm upfield from TMS ($^1$H and $^{13}$C) and pure $BF_3{\cdot}OEt_2$ ($^{11}$B). 2D HMQC and HMBC spectra were acquired and processed using standard Bruker software. Antibiograms to determine the in vitro biological activity of compound **7** were performed following standard protocols.

**Azaerithromycin Hydrogenoborate (6)**. To a stirred solution of imino ether **4** (89 g, 121 mmol) in methanol (450 mL) was added sodium borohydride (35 g, 921 mmol) over a period of 4 h at a temperature below −5 °C. The mixture was stirred during two additional hours under the same reaction conditions and then it was left at room temperature for 20 h. Solvent evaporation affords a crude product which was redisolved in a mixture of $CH_2Cl_2-H_2O$. The organic layer was separated and dried over anhydrous sodium sulfate. Filtration and solvent evaporation yielded 85 g (92.4%) of azaerithromycin hydrogenoborate **6**.

**6:** $^1$H NMR $\delta$ 5.15 (d, 1H), 4.89 (dd, 1H), 4.59 (dd, 1H), 4.40 (d, 1H), 4.11 (dq, 1H), 3.61 (d, 1H), 3.48 (ddq, 1H), 3.34 (s, 3H), 3.27 (d, 1H), 3.22 (dd, 1H), 3.02 (d, 1H), 3.00 (dd, 1H), 2.72 (dq, 1H), 2.42 (ddd, 1H), 2.33 (d, 1H), 2.28 (s, 6H), 2.25 (dq, 1H), 1.94 (ddq, 1H), 1.86 (ddq, 1H), 1.71 (m, 2H), 1.65 (d, 1H), 1.65 (dd, 1H), 1.57 (dd, 1H), 1.45 (ddq, 1H), 1.39 (dd, 1H), 1.36 (d, 3H), 1.27 (s, 3H), 1.25 (s, 3H), 1.24 (m, 1H), 1.22 (d, 3H), 1.18 (d, 3H), 1.16 (d, 3H), 1.05 (s, 3H), 1.02 (d, 3H), 0.91 (d, 3H), 0.88 (t, 3H); $^{13}$C NMR $\delta$ 180.12, 103.01, 94.47, 82.99, 80.60, 79.66, 78.14, 77.45, 73.58, 72.86, 70.77, 68.78, 65.85, 65.68, 58.65, 57.12, 49.51, 45.54, 42.44, 41.68, 40.34, 34.55, 29.46, 28.74, 27.38, 21.82, 21.53, 21.36, 21.32, 18.20, 15.43, 14.70, 14.45, 11.53, 8.90; $^{11}$B NMR $\delta$ 10.0; FABMS *m/z* 761 $[M]^+$.

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**Azithromycin Hydrogenoborate (7)**. To a solution of azaerithromycin hydrogenoborate (**6**) (50 g, 65.7 mmol) in chloroform (500 mL) was added a mixture of formic acid (5.5 mL, mmol) and 35% aqueous formaldehyde (11.75 mL, mmol). The reaction was refluxed for 14 h and then cooled to room temperature. Water was added (500 mL) and the mixture was then acidified to pH <4 with mineral acid. The organic layer was discarded. Conventional workup of the aqueous layer with methylene dichloride (350 mL) at pH >9 gave a crude product, which when washed with diethyl ether yielded the pure title compound **7** (29 g, 57%).

**7:** $^1$H NMR $\delta$ 10.31 (s, 1H), 5.23 (d, 1H), 4.91 (dd, 1H), 4.53 (dd, 1H), 4.41 (d, 1H), 4.11 (dq, 1H), 3.58 (d, 1H), 3.54 (bs, 1H), 3.49 (ddq, 1H), 3.35 (s, 3H), 3.23 (dd, 1H), 2.99 (dd, 1H), 2.72 (dq, 1H), 2.46 (ddd, 1H), 2.46 (d, 1H), 2.36 (dq, 1H), 2.31 (s, 3H), 2.30 (d, 1H), 2.29 (s, 6H), 2.15 (d, 1H), 1.98 (m, 1H), 1.97 (m, 1H), 1.95 (ddq, 1H), 1.70 (d, 1H), 1.66 (ddd, 1H), 1.57 (dd, 1H), 1.48 (ddq, 1H), 1.37 (d, 3H), 1.30 (s, 3H), 1.29 (m, 1H), 1.26 (ddd, 1H), 1.25 (s, 3H), 1.23 (d, 3H), 1.18 (d, 3H), 1.13 (d, 3H), 1.07 (s, 3H), 1.02 (d, 3H), 0.87 (d, 3H), 0.75 (t, 3H); $^{13}$C NMR $\delta$ 180.16, 103.10, 94.26, 83.37, 80.40, 78.34, 78.26, 78.07, 77.33, 73.52, 72.86, 70.74, 69.05, 68.83, 65.92, 65.57, 64.31, 49.55, 45.56, 42.52, 41.75, 40.37, 35.85, 34.58, 28.75, 27.69, 26.44, 21.98, 21.60, 21.37, 21.36, 18.45, 15.59, 14.65, 11.01, 8.75, 7.09; $^{11}$B NMR $\delta$ 10.0; FABMS m/z 775 $[M]^+$.

**Azithromycin (1).** A solution of azithromycin hydrogenoborate (**7**) (22 g, 28.4 mmol) in a mixture of acetonitrile (250 mL) and water (125 mL) was acidified to pH 2 and stirred for 30 min at a temperature of 15 °C. Then, a mixture of $CH_2Cl_2$–$H_2O$ was added and the pH increased to 9. The organic layer was filtered over Zeolite and evaporated to dryness. The crude product was dissolved in ethanol and precipitated by addition of water. The solid was filtered off and dried under vacuum at 40 °C affording 15 g (70.5%) of azithromicyn (**1**). The compound thus obtained matched the USP standard[7a] for azithromycin.

**Azithromycin Dihydrate (2).** To a solution of azithromicyn (**1**) (51 g, 68.1 mmol) in acetone was added water over 30 min. The solution was stirred for 24 h at room temperature. The solid formed was filtered off and dried under vacuum at 40 °C, affording 45 g (84.2%) of pure azithromycin dihydrate according to the USP standard.[7a]

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**Supporting Information Available:** Copies of the FABMS, $^1$H- $^{13}$C-, DEPT, HMQC, and HMBC spectra of compounds **6** and **7** and a table showing the results of the biological screening of **7** (15 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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