Pediatric Erythromycins: a Comparison of the Properties of Erythromycins A and B 2′-Ethyl Succinates

Abdolreza Hassanzadeh,[†] Peter A. Gorry,[‡] Gareth A. Morris,[‡] and Jill Barber*[,†]

Schools of Pharmacy and Pharmaceutical Sciences and Chemistry, University of Manchester, Oxford Road, Manchester, U.K. M13 9PL

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The antibiotic erythromycin A is generally administered to children as a suspension of the pro-drug erythromycin A 2′-ethyl succinate. The success of the pro-drug depends on (a) elimination of the unacceptably bitter taste of free erythromycin, (b) its stability against stomach acid, and (c) its smooth (base-catalyzed) hydrolysis in the body to yield active erythromycin. We have investigated the rates and pathways of acid-catalyzed degradation and base-catalyzed hydrolysis of the 2′-ethyl succinates of erythromycins A and B. Esterification does not protect the drugs against acid-catalyzed degradation in solution; however, erythromycin B 2′-ethyl succinate is much more stable than the corresponding erythromycin A ester, degrading nearly 40 times more slowly. The rates of base-catalyzed hydrolysis in conditions mimicking the blood stream are similar for the two pro-drugs. We conclude that erythromycin B 2′-ethyl succinate is an attractive prospect as a pediatric erythromycin pro-drug.

Introduction

It is over 50 years since erythromycin was isolated from a strain of *Saccharopolyspora erythraea*[1] (formerly known as *Streptomyces erythreus*[2]). The drug, which is predominantly erythromycin A (**1**), has a spectrum of activity similar to that of penicillin and is often used in the treatment of penicillin-sensitive patients.[3] It is superior to penicillin in the treatment of deep-seated infections such as tuberculosis. Although safe and effective, erythromycin is not without disadvantages, notably its extreme acid-sensitivity,[4] the vile taste of the free base,[5,6] and its tendency to cause gastrointestinal disturbances.[6] Soon after the introduction of erythromycin into clinical practice, attempts were made to overcome these problems. Esterification of the 2′-position of erythromycin proved to be facile[7] and lead to compounds that were essentially taste-free and somewhat resistant to acid.[8,9] These 2′-esters were found to exhibit very little antibacterial activity and are, therefore, pro-drugs, requiring activation by hydrolysis to the parent drug.[10,11] A wide variety of erythromycin esters have been prepared and studied,[12,13] but very few have entered the clinic.[14]

Erythromycin B (**2**) is a biosynthetic precursor of erythromycin A (**1**) and a minor component (<5%) of commercial erythromycin. Although it has in vitro antibacterial activity similar to that of erythromycin A,[15] it has not found a place as an independent drug, despite the fact that it is relatively stable to acid.[4] Erythromycin A (**1**) degrades in acid by the mechanism shown in Scheme 1.[16,17] The major degradation product is anhydroerythromycin A (**3**). Erythromycin B, which lacks a 12-OH group is unable to cyclize in this way and degrades only slowly in acid, by loss of the cladinose sugar (Scheme 1). In this respect, it resembles the clinically useful derivatives clarithromycin and azithromycin, which lack a 6-OH and a 9-keto function, respectively. Not surprisingly, erythromycin B exhibits pharmacokinetic parameters superior to those of erythromycin A, following oral administration to experimental animals.[18]

The market-leading pediatric erythromycin is erythromycin A 2′-ethyl succinate (**4**). As with other 2′-esters of erythromycin, this compound is essentially taste-free. The 2′-esters of erythromycin A are also reported to show increased acid stability relative to that of the parent compound,[8,9] although Steffansen and Bundgaard have demonstrated very similar acid-sensitivities in the pH range 4−6.[12] Compound **4** is undoubtedly effective in the treatment of infections in children, but there is surprisingly little published data on its stability at acidic pH, such as in the stomach. Under conditions resembling those in the medicine bottle, **4** undergoes slow hydrolysis to the vile-tasting erythromycin A. The taste is intolerable to some children, and they are unable to swallow the drug. We have recently quantified the rates of hydrolysis at 4 °C at pH 6 and pH 8; under these conditions[19] the extents of hydrolysis to erythromycin A in a 21 day period were found to be 26 and 30%, respectively.

In the present study, we have compared the acid-catalyzed degradation of erythromycins A and B 2′-ethyl succinates and the rates of hydrolysis of the two pro-drugs to their parent erythromycins. The intention was to determine whether erythromycin B 2′-ethyl succinate (**8**) would be worthy of further investigation as a pediatric medicine.

Results

NMR spectroscopy was chosen as a means of investigating the degradations of the 2′-ethyl succinates of erythromycins A and B. This method is particularly useful for the identification of unexpected products.

Erythromycin A (Ery A, **1**) is known to degrade in acid to anhydroerythromycin A (AEA, **3**) with erythromycin A enol ether (EAEE, **5**) formed as an intermediate[16,17] (Scheme 1A). Erythromycin B (Ery B, **2**), however, degrades in acid much more slowly, by loss of the cladinose sugar to give 5-*O*-desosaminylerythronolide B (**6**), with erythromycin B enol ether (EBEE, **7**) as an intermediate (Scheme 1B). Likely degradation products of erythromycin A 2′-ethyl succinate (EAES, **4**), therefore, include Ery A (**1**), AEA (**3**), and EAEE (**5**) as well as anhydroerythromycin A 2′-ethyl succinate (AEAES, **9**) and erythromycin A enol ether 2′-ethyl succinate (**10**). Likely degradation products of erythromycin B 2′-ethyl succinate (**8**)

* Corresponding author. Tel: 44-161-275-2369. Fax: 44-161-275-2396. E-mail: jill.barber@manchester.ac.uk.
[†] School of Pharmacy and Pharmaceutical Sciences.
[‡] School of Chemistry.

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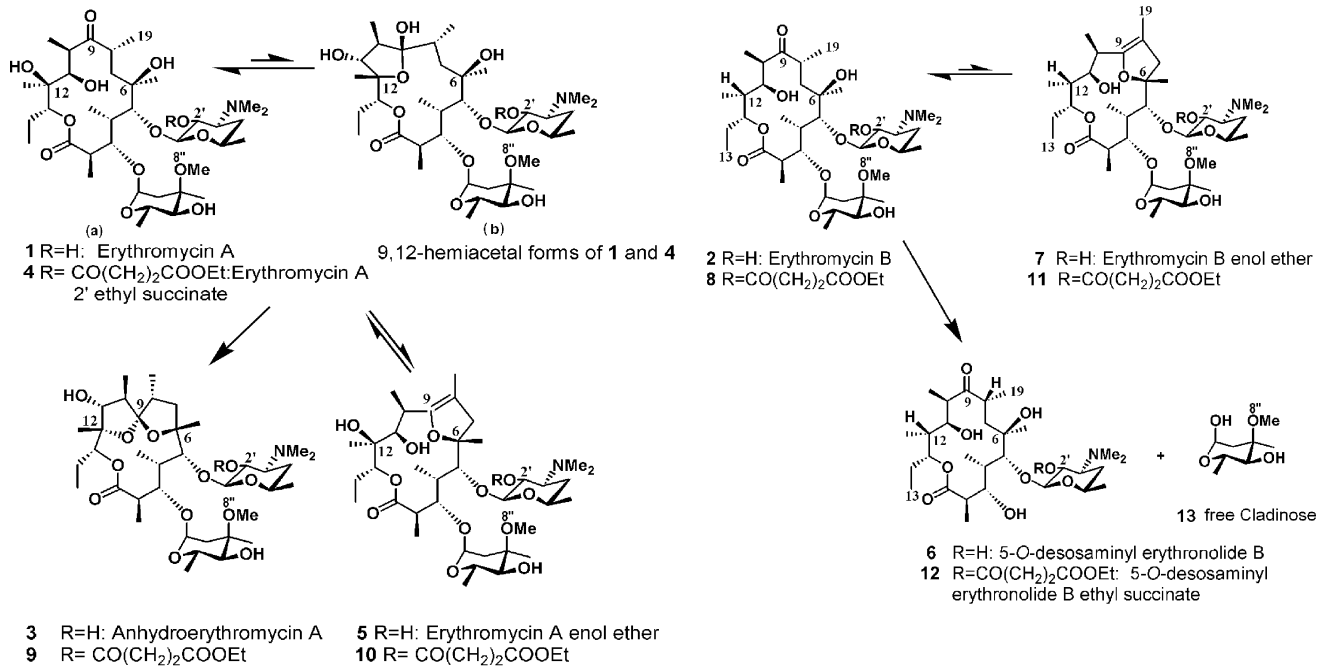
Scheme 1. Acid-Catalyzed Degradation of Erythromycin A (A) and Acid-Catalyzed Degradation of Erythromycin B (B)



include erythromycin B enol ether 2′-ethyl succinate (**11**) and 5-*O*-desosaminyl erythronolide B 2′-ethyl succinate (**12**). Preliminary experiments suggested that it would not be possible to identify transient intermediates from their NMR data without authentic material available for comparison. This was particularly true for EAES (**4**), which, like the parent erythromycin A (**1**), exists in aqueous solution as an equilibrium mixture of 9-ketone and 12,9-hemiacetal isomers[20−22] (Scheme 1A). We, therefore, set out to prepare compounds **3**, **5**, and **7**−**11**. Compound **12** would be expected to form as an end product of the degradation of **8** and be readily identifiable. Compounds **1** and **4** are available commercially; **2** was a gift from Abbott Laboratories (Dr. Thomas Paulus); **6** was prepared by Dr. Nizam Mordi.[4]

Preparation of Potential Intermediates in the Degradation of Erythromycin 2′-Ethyl Succinates. Anhydroerythromycin A (**3**) and erythromycin A enol ether (**5**) were each prepared in one step from **1** by the literature methods.[4,13] Erythromycin B enol ether (**7**) was prepared from Ery B (**2**) by adapting the method for the preparation of **5**. AEAES (**9**) was prepared from EAES (**4**) by adapting the synthesis of **3**. Erythromycin B 2′-ethyl succinate (EBES, **8**) was prepared from **2**, erythromycin A enol ether ethyl succinate (EAEEES, **10**) from **5**, and erythromycin B enol ether ethyl succinate (EBEEES, **11**) from **7** by treatment with ethyl succinyl chloride in the presence of sodium bicarbonate. Thus, **3**, **5**, **7**, and **9** were prepared in a single step from readily available starting materials, and the other compounds were prepared in two steps. Compound **9** is a new compound and was fully characterized as described in Supporting Information.

Acid-Catalyzed Degradations of Erythromycin A, Erythromycin B, and Erythromycins A and B Ethyl Succinate. Gastric emptying time is normally in the region of 20−30 min, with acidity persisting in the upper part of the small intestine. Gastric pH is normally in the range 1−3. For a compound to survive oral administration, a half-life of an hour or more at pH 2 and 37 °C is, therefore, desirable. Erythromycin is a very well-tolerated drug; therefore, large doses may be administered to help compensate for degradation in the stomach. Furthermore, formulations containing large amounts of sugar may be used to facilitate absorption. Esterification of erythromycin A at the 2′ position is a strategy that has been used to improve the acid stability of the drug as well as rendering it taste-free. The question addressed here is the extent to which esterification stabilizes erythromycin A.

Each compound (Erythromycins A and B, **1** and **2**, and their ethyl succinates **4** and **8**) was dissolved in buffer (based on D_2O) at various pH in the region of apparent pH 2−4.5, and monitored at 37 °C by ^{1}H NMR spectroscopy over periods of up to 5 h. At low and high pH, each compound either degraded completely before a measurement could be made or was stable for several hours, respectively. With the experimental protocol used, half-lives could only be measured with reasonable accuracy over a range of 1 to 2 pH units.

A typical time course for erythromycin A (apparent pH 3.0) is shown in Figure 1. It is clear from the later spectra that AEA (**3**) is the final product of acid-catalyzed degradation of Ery A (**1**). Compound **5**, EAEE, forms early in the time course and persists much longer than **1**. The half-life of **1** was determined by comparing the integrals of the H-2″ doublets δ 2.50 (**1a**) and δ 2.46 (**1b**) with the doublets at δ 2.41, due to **3**,[23] and δ 2.56, due to **5**. The choice of a one-proton doublet for this analysis was dictated by the necessity to distinguish four compounds (**1a**, **1b**, **3**, and **5**) in the erythromycin A series. The (normally preferred) 8″-methyl resonances overlapped one another as well as the H-2 of **3** and H-10 of **1**; the well-dispersed anomeric hydrogen signals were too close to the residual water peak to be integrated accurately. The presence of **5** was inferred from the characteristic methyl singlet at δ 1.59, due to H_3-19,[24] and quantified by analysis of the signals due to H-2″. Figure 2A shows these results represented graphically. All of the meaningful rate data are summarized in Table 1.

For erythromycin B (**2**), similar half-lives were observed under much more acidic conditions, apparent pH 2.0 (see Table 1). In this case, the product of the degradation is 5-*O*-desosaminyl erythronolide B (**6**), deuterated at C-8. The signals

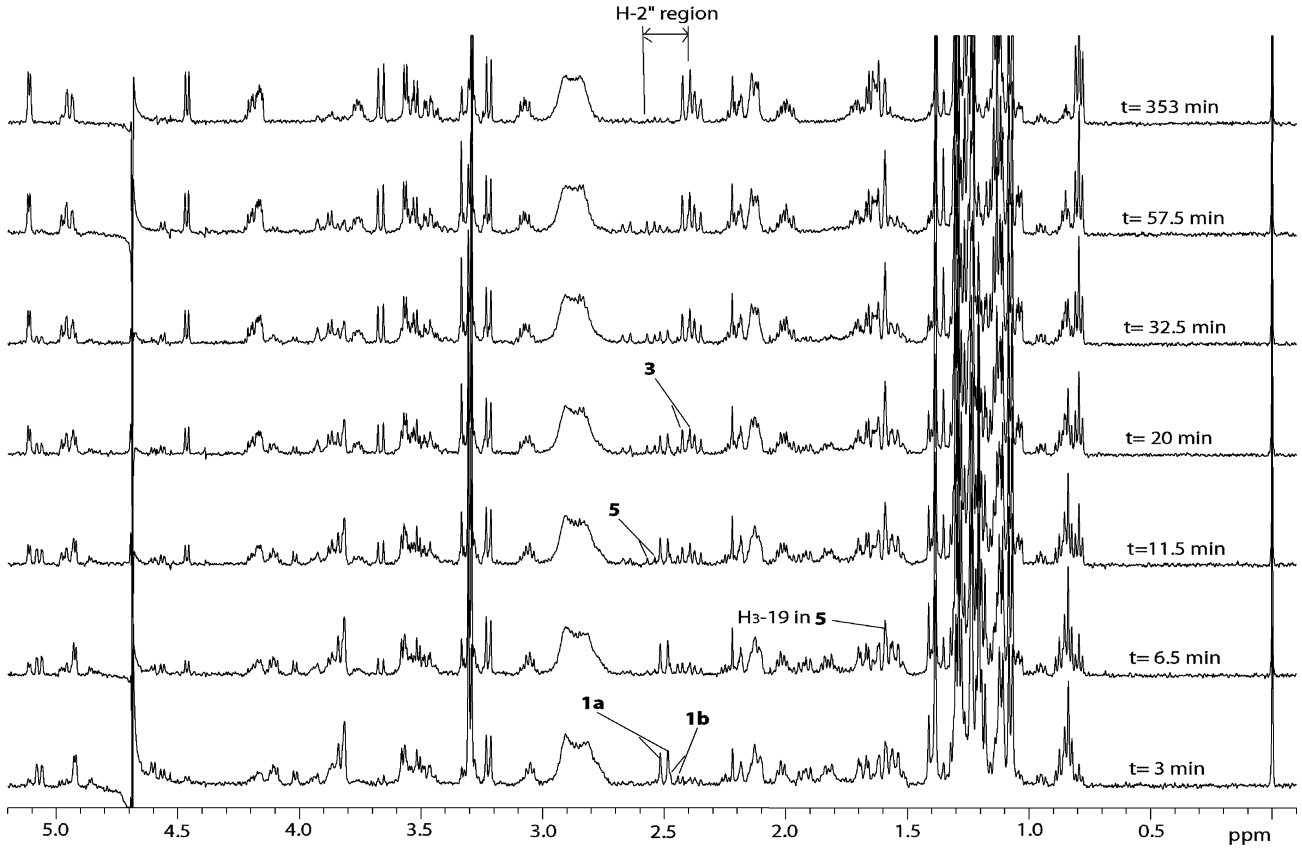


Figure 1. Acid-catalyzed degradation of erythromycin A (**1**) in deuterated sodium phosphate buffer (0.2 M) at apparent pH 3.0, 37 °C, monitored by 500 MHz NMR spectroscopy. The H-2″ region has been marked. The characteristic H_3-19 of **5** is also shown.

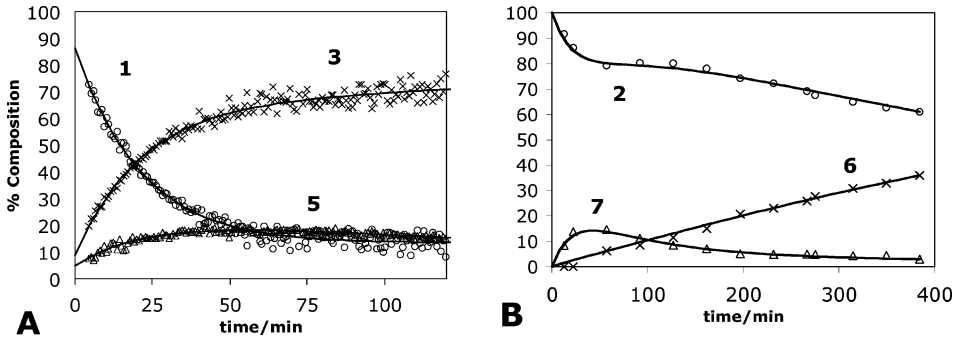


Figure 2. (A) The degradation of erythromycin A (**1**) in deuterated phosphate buffer (0.2 M) at apparent pH 3, 37 °C. Both anhydroerythromycin A (**3**) and erythromycin A enol ether (**5**) accumulate during the time course. (B) The degradation of erythromycin B (**2**) in deuterated phosphate buffer (0.2 M) at apparent pH 2.5, 37 °C. Erythromycin B enol ether (**7**) accumulates and then disappears, distorting the first-order kinetics of erythromycin B degradation. In both graphs, the solid lines are fits to the general kinetic model described in the text.

used to monitor the formation of **6**, the disappearance of **2**, and the accumulation and disappearance of EBEE (**7**) were those due to the characteristic OCH_3-8″ singlet. These appeared with minimal signal overlap in the region δ 3.24−3.33. Monitoring of the 8″-methoxy groups signals is preferred when there is no signal overlap; these signals are strong, narrow, and have short T_1 values (all 0.7−1 s). In these experiments, a phosphate buffer was used, and the degradation was slower than that observed by Mordi et al.[4] using Britton−Robinson buffer. Britton−Robinson buffer is much more effective as a buffering agent at low pH, but the presence of boron means that it is chemically rather further from physiological conditions than phosphate. The overall pattern of degradation was, however, very similar (Figure 2B). The erythromycin B enol ether concentration peaked at about 15% of the total erythromycin species and then decayed rapidly. This apparent deviation from simple first-order kinetics is due to the perturbation of the equilibrium between **2** and **7** by incorporation of deuterium into position 8. The ^{1}H NMR spectrum shows that position 8 of **6** is almost completely deuterated (Figure 7 of Supporting Information).

A typical time course for erythromycin A ethyl succinate (EAES, **4**) is shown in Figure 3, and the results are plotted in Figure 5A. Here, signals corresponding to OCH_3-8″ were monitored. Peaks for erythromycin A 9-ketone ethyl succinate (**4a**), 12,9-hemiacetal (**4b**), erythromycin A enol ether ethyl succinate (EAEEES, **10**), and anhydroerythromycin A ethyl succinate (AEAES, **9**) were detected at δ 3.34, 3.32, 3.36, and 3.29 ppm, respectively.[22]

For erythromycin B ethyl succinate (EBES, **8**), the 9-ketone was detected at δ 3.34, its enol ether (EBEEES, **11**) at δ 3.36, and the free cladinose sugar (**13**, two anomers) at δ 3.24 and δ 3.29 ppm, these signals corresponding to the 8″-methoxy groups. The time course for the degradation of erythromycin B ethyl

Table 1. Experimental Half-Lives and Degradation Rate Constants (k_{ac}) for Erythromycin A (**1**), Erythromycin B (**2**), Erythromycin A 2′-Ethyl Succinate (**4**) and Erythromycin B 2′-Ethyl Succinate (**8**) in Deuterated Sodium Phosphate Buffer (0.2 M) at Apparent pH 2−4.5, 37 °C[a]

	pH 2		pH 2.5		pH 3	
compd	$t_{1/2}$/min	$k_{ac}/10^{-3}$ min^{-1}	$t_{1/2}$ /min	$k_{ac}/10^{-3}$ min^{-1}	$t_{1/2}$/min	$k_{ac}/10^{-3}$ min^{-1}
1					17.9 ± 0.7	31.4 ± 1.3
2	203 ± 3	3.5 ± 0.1	553 ± 13	1.3 ± 0.05	1670 ± 40	0.4 ± 0.01
4	2.0 ± 0.05	290 ± 7			17.8 ± 0.3	31.9 ± 0.6
8	79 ± 1	8.6 ± 0.1	206 ± 2	3.4 ± 0.1	650 ± 10	1.1 ± 0.02

	pH 3.5		pH 4		pH 4.5	
compd	$t_{1/2}$/min	$k_{ac}/10^{-3}$ min^{-1}	$t_{1/2}$/min	$k_{ac}/10^{-3}$ min^{-1}	$t_{1/2}$/min	$k_{ac}/10^{-3}$ min^{-1}
1	48 ± 10	10.7 ± 2.3	148 ± 24	4.2 ± 0.7		
2						
4	68 ± 3	8.7 ± 0.4	179 ± 5	3.0 ± 0.1	215 ± 6	2.3 ± 0.1
8						

[a] Half-lives were determined by fitting the concentrations of erythromycin, enol ether and product to the general kinetic scheme described and then solving the fitted erythromycin decay function to find the time at which the concentration falls to 50%; the rate constants k_{ac} were found directly from the fitting process. In each case, the error limits quoted are twice the standard error estimated in the fitting of k_{ac}.

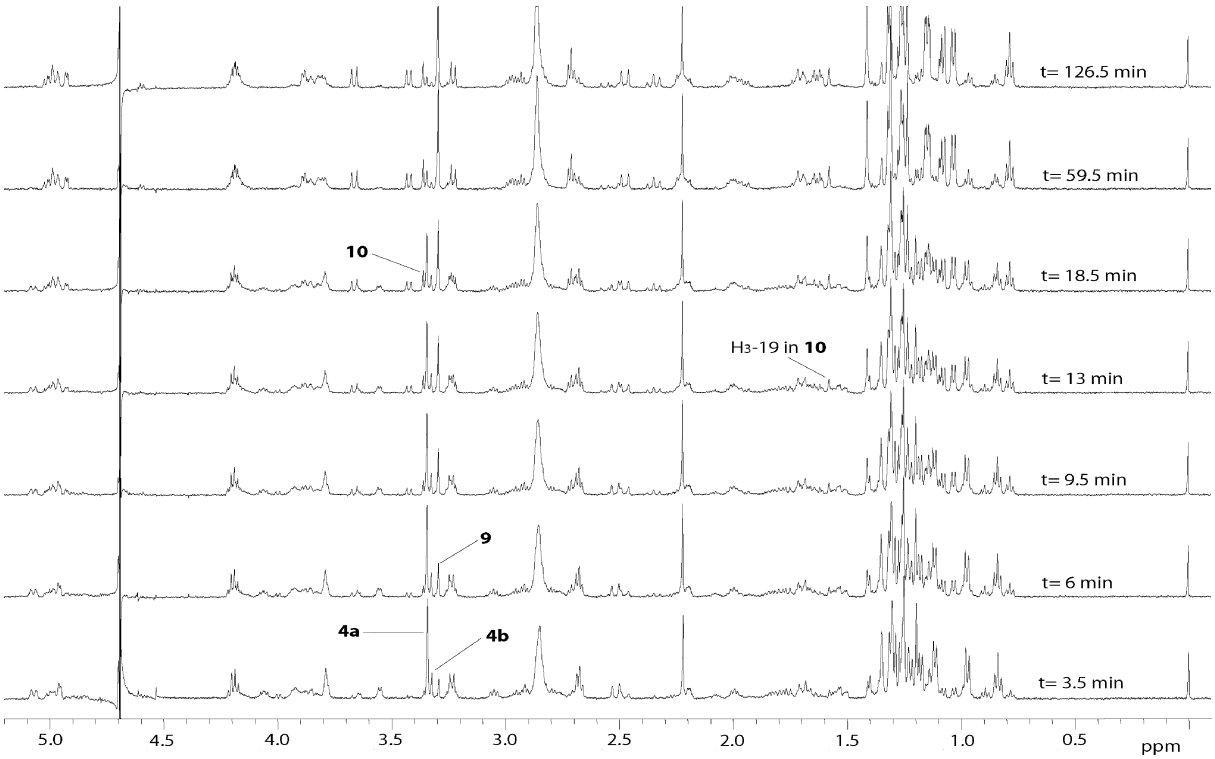


Figure 3. Acid-catalyzed degradation of erythromycin A 2′-ethyl succinate (**4**) in deuterated sodium phosphate buffer (0.2 M) at apparent pH 3.0, 37 °C, monitored by 500 MHz NMR spectroscopy. The H_3-8″ region has been marked. The characteristic H_3-19 of **5** is also shown.

succinate at pH 2.0 is shown in Figure 4, and the results are summarized in Figure 5B and in Table 1. Figure 5B shows that the concentration of EBEEES (**11**) peaks before the first measurement was made and then falls steadily. At all stages, the enol ether concentration is below that seen in the degradation of the parent Ery B (**2**). The degradation of **8**, though very slow compared with the degradation of Ery A (**1**) and EAES (**4**), is actually faster than the degradation of Ery B (**2**), under the same conditions, by a factor of about 3.

Kinetic Analysis. The half-lives for the different erythromycin analogues and derivatives were determined by smoothing the experimental data using a nonlinear least squares global optimization to fit the data to a suitably general kinetic model. The process used allowed multiple datasets to be combined where appropriate, and included the known effect of deuteration on the erythromycin−enol ether interconversion. The solution of the kinetic scheme and the global optimization were performed in Mathematica 5.1 on an Apple Macintosh G5 computer.

We first define a sum of squares function for the fit to a single species, *m*, in a single kinetic run,

$$SSc_m = \sum_{i=1}^{n_{data}} [d_i^m - \text{model}^m(t_i^m)]^2$$

where d_i represents the concentration of the species for this run and $\text{model}^m(t_i)$ the predicted model concentration for species *m*. We then sum over all species in all datasets.

$$SS = \sum_{m=1}^{n_{curves}} SSc_m = \sum_{m=1}^{n_{curves}} \sum_{i=1}^{n_{data}} [d_i^m - \text{model}^m(t_i^m)]^2$$

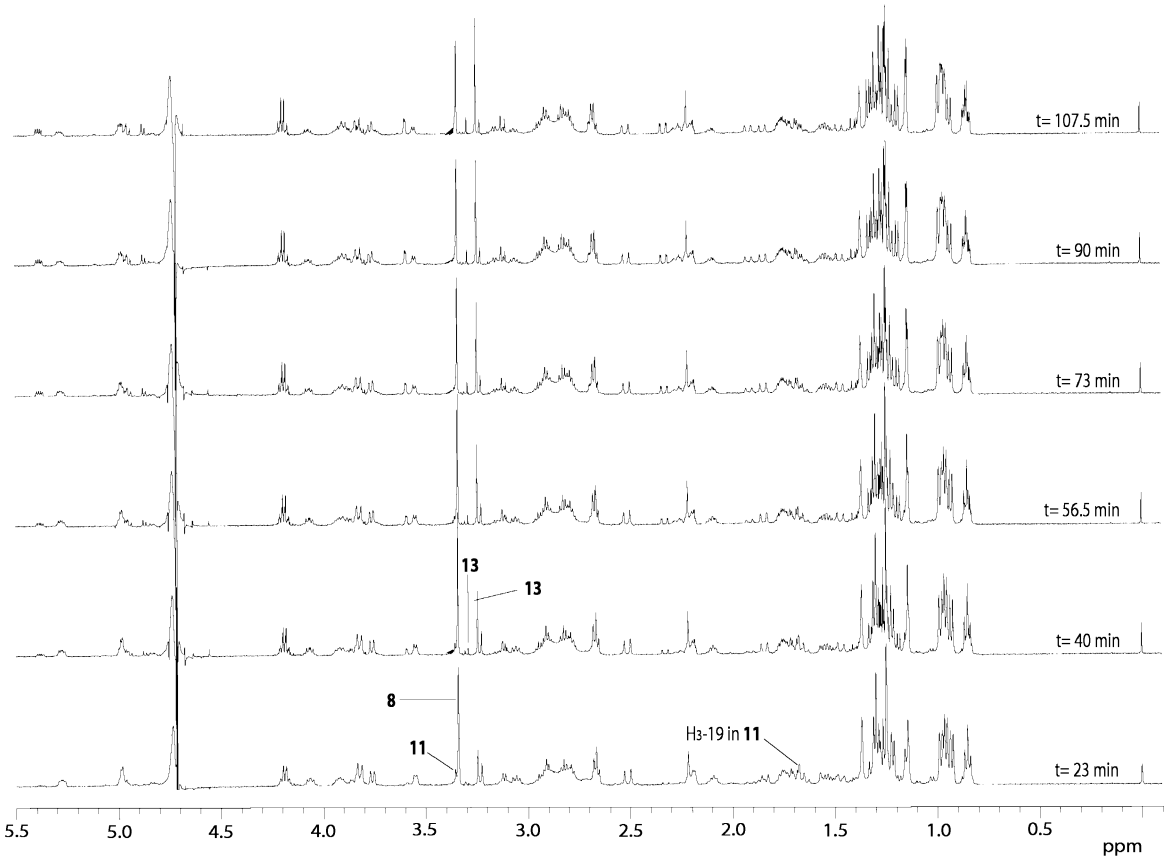


Figure 4. Acid-catalyzed degradation of erythromycin B 2′-ethyl succinate (**8**) in deuterated sodium phosphate buffer (0.2 M) at apparent pH 2.0, 37 °C, monitored by 500 MHz NMR spectroscopy. The H_3-8″ region has been marked. The characteristic H_3-19 of **11** is also shown.

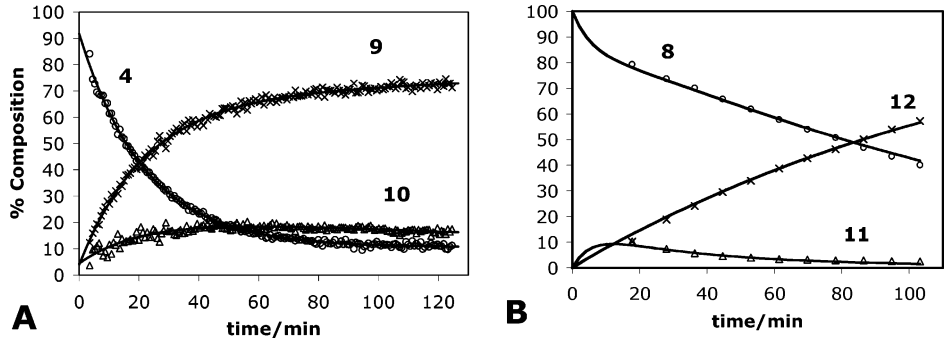


Figure 5. (A) The degradation of erythromycin A 2′-ethyl succinate (**4**) in deuterated phosphate buffer (0.2 M) at apparent pH 3.0, 37 °C. The degradation approximately follows first-order kinetics with both anhydroerythromycin A 2′-ethyl succinate (**9**) and erythromycin A enol ether 2′-ethyl succinate (**10**) accumulating during the reaction. The solid lines are fits to the general kinetic model described in the text. (B) The degradation time course of erythromycin B 2′-ethyl succinate (**8**) in deuterated phosphate buffer (0.2 M) at apparent pH 2.0, 37 °C. The consequences of the kinetic isotope effect are clearly visible in the fitted lines at short times. The final products are 5-*O*-desosaminylerythronolide B ethyl succinate and free cladinose.

This SS function is then minimized to obtain the best fit across all datasets, using the Levenberg−Marquardt algorithm in the FindFit function of Mathematica.

Parameter Error Estimation. The mean square error for the fits is given by the following equation.

$$MSE = \frac{1}{n_p - n_k}\sum_{m=1}^{n_{curves}n_{data}}\sum_{i=1}[d_i^m - \mathrm{model}^m(t_i^m)]^2$$

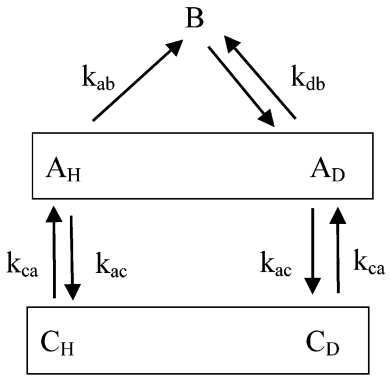
where n_p is the total number of points and n_k the number of parameters being fitted. The standard errors (SE) associated with the model parameters are estimated from the curvature matrix at the solution minimum. The curvature matrix, α, is an $n_k \times n_k$ matrix, with elements calculated from the following equation.

$$\alpha_{p,q} = \sum_{m=1}^{n_{curves}} \alpha c_{p,q} = \sum_{m=1}^{n_{curves}n_{data}}\sum_{i=1}\left(\frac{\partial \mathrm{model}^m(t_i)}{\partial k_p}\right)\left(\frac{\partial \mathrm{model}^m(t_i)}{\partial k_q}\right)$$

The SE associated with a particular parameter is obtained from the MSE matrix, *V*, which is calculated from the inverse of the curvature matrix. The diagonal elements of *V* provide the estimate of the SE for the fitting parameters, k_{mod} (the off diagonal elements allow calculation of the parameter correlation coefficients). Parameter k_i has a 95% confidence interval of

$$k_i = k_{model} \pm 2\sqrt{V_{i,i}} \text{ where } V = \alpha^{-1}MSE$$

Scheme 2. The Kinetic Model Including the Deuterium Isotope Effect[a]



A Erythromycin A (**1**) ketone/hemiacetal
Erythromycin A ES (**4**) ketone/hemiacetal
Erythromycin B (**2**)
Erythromycin B ES (**8**)

B Erythromycin A enol ether (**5**)
Erythromycin A enol ether ES (**10**)
Erythromycin B enol ether (**7**)
Erythromycin B enol ether ES (**11**)

C Anhydroerythromycin A (**3**)
Anhydroerythromycin A ES (**9**)
5-desosaminyl erythronolide B (**6**)
5-desosaminyl erythronolide B ES (**12**)

[a] Species A, B, and C are identified for each system, where D and H labels refer to the atom at position C-8 (ES = 2′-ethyl succinate).

Kinetic Model. The choice of kinetic model is not straightforward. The accepted kinetic scheme for erythromycin A (Ery A, **1**) degradation has the ketone/hemiacetal (**1**) and enol ether (EAEE, **5**) in equilibrium, with an irreversible step to the anhydroerythromycin A (AEA, **3**) from Ery A.[16,17] At long times, this must lead to 100% AEA (**3**) with zero Ery A and EAEE. The current data suggest strongly that at long times and low pH the dehydration step may be reversible, leading to equilibration of the three species and non-zero concentrations of Ery A and EAEE at longer times. Although the current evidence for this revision of the mechanism is not definitive and further work is in progress, the incorporation of the rehydradation step proved essential to the accurate fitting of the low pH experimental data for several of the species under study. An additional complication arises from the incorporation of deuterium from the solvent D_2O at C-8 by the reverse reactions **5** → **1** (and the corresponding reactions **10** → **4**, **7** → **2**, and **11** → **8**) as noted in the earlier study of erythromycin B degradation.[4] Because the dehydration of erythromycins to enol ethers requires the breaking of the C−H bond at C-8, there is a substantial primary kinetic isotope effect on the reverse reactions. Initially, **1** rapidly forms **5** but the reverse reaction forms 8-*d*-erythromycin A (**1$_D$**), which dehydrates more slowly than **1**. Species **1** and **1$_D$** both dehydrate at the same rate to AEA (**3**). Scheme 2 shows the kinetic model adopted throughout the analysis. It is closely related to that used previously for erythromycin B degradation but with the inclusion for erythromycin A and its ethyl succinate of the rehydration step from AEA (**3**) to Ery A(**1**) (rate constant k_{ca}), and it reverts to the usual model[16,17] in H_2O if $k_{ca} = 0$. The analytical form of the kinetic model was obtained by the Laplace transform method, allowing the optimization of the rate constants and initial concentrations described above to be carried out very rapidly.

The pH dependence of the rate constant for erythromycin degradation k_{ac}, summarized in Figure 6, is largely consistent with straightforward acid catalysis (general or specific), as shown by the tendency of the experimental data points to follow a line of unit slope. The data for the experimental half-lives show a very similar trend but are complicated slightly by the reversibility of the dehydration step and the existence of the deuterium isotope effect.

Base-Catalyzed Hydrolysis of Erythromycins A and B 2′-Ethyl Succinates. 2′-Esterified derivatives of erythromycin are antimicrobially inactive, or at least much less active than the parent drug.[10,11] Accordingly, the hydrolytic conversion in the plasma to free erythromycin is vital for antibacterial action. The neighboring amino group [$N(Me)_2$] at C-3′ facilitates esterification at position 2′-OH and is also involved in the hydrolysis reaction, which is believed to proceed nonenzymically in the body.[12] Several detailed mechanisms[25−27] addressing the effect of the neighboring amino group on the rate of ester hydrolysis have been proposed, with general base catalysis involving an attack by water being regarded as the most likely mechanism.[25]

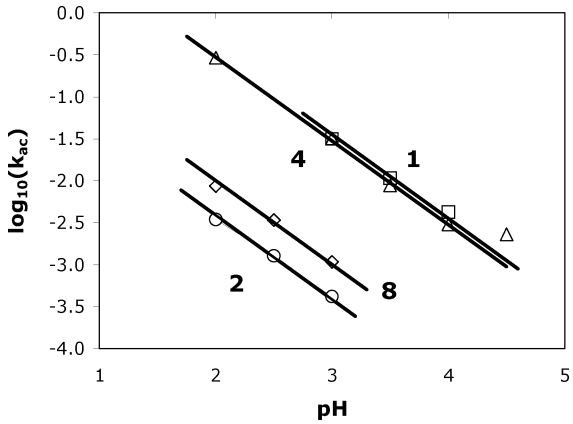


Figure 6. The pH dependence of the degradation rate constants k_{ac} of erythromycin A (**1**, □), erythromycin B (**2**, ○), erythromycin A ethyl succinate (**4**, △) and erythromycin B ethyl succinate (**8**, ◇), with lines of negative unit slope.

The relative stability of erythromycin B ethyl succinate (EBES, **8**) to acid compared with that of erythromycin A ethyl succinate (EAES, **4**) is very encouraging. However, we cannot propose EBES (**8**) as a pediatric erythromycin unless its hydrolysis characteristics in base are also favorable. Ideally for an erythromycin ester to behave as a pro-drug, it must hydrolyze to erythromycin rapidly at 37 °C in the blood but remain stable in the bottle at 4 °C and even at 25 °C (refrigerator and room temperatures). The hydrolysis rates of EAES (**4**) and EBES (**8**) at 25 °C and 37 °C at apparent pH 7 were now determined by NMR spectroscopy. Time courses were measured as previously described (see acid-catalyzed degradation). Figure 7 shows the hydrolysis profile at 37 °C; the corresponding graphs for 25 °C are shown in Supporting Information. Here signals corresponding to OCH_3-8″ were monitored.

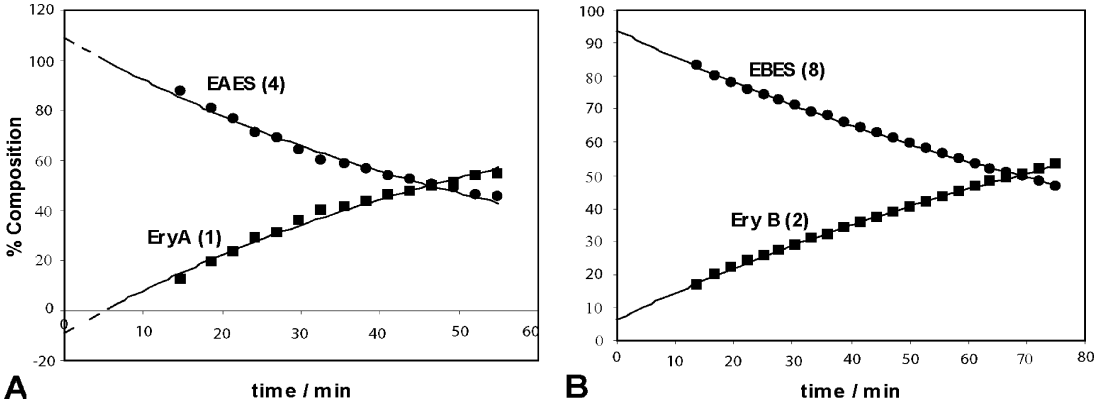


Figure 7. Hydrolysis of ethyl succinates of erythomycins A and B to erythromycins A and B at 37 °C, apparent pH 7. Best fit to first order kinetics shown.

Table 2. Half-Lives and Depletion Rates Constants for Erythromycin A 2′-Ethyl Succinate (**4**) and Erythromycin B 2′-Ethyl Succinate (**8**) in Deuterated Sodium Phosphate Buffer (0.05 M) at Apparent pH 7 at 25 and 37 °C[a]

	25 °C		37 °C	
compd	$t_{1/2}$/min	$k/10^{-3}$ min^{-1}	$t_{1/2}$/min	$k/10^{-3}$ min^{-1}
4	193 ± 1	3.59 ± 0.02	41 ± 1.5	16.9 ± 0.6
8	241.7 ± 0.7	2.87 ± 0.01	75.7 ± 0.6	9.15 ± 0.07

[a] Data were analyzed assuming first order kinetics.

It can be seen that the hydrolysis rates for EAES (**4**) and EBES (**8**) were similar at the two temperatures, with the hydrolysis of EAES proceeding slightly faster than that of EBES. This means that the production of erythromycin B from its ethyl succinate ester is likely to proceed at an acceptable rate in vivo, delivering the antibacterial erythromycin free base. All these data agree reasonably well with the expected first-order kinetics, with no confounding reactions; the results are summarized in Table 2. It is possible to use the data in Table 2 to extract an average activation energy (for erythromycins A and B ethyl succinate) of around 87 kJ mol^{-1} for conversion to the corresponding free base. However, given the small temperature range and small dataset involved, the value is subject to considerable uncertainty.

Discussion

Esterification of the 2′ position in erythromycin A is designed to yield taste-free derivatives that are protected from stomach acid. In the case of erythromycin A ethyl succinate (**4**), the taste-masking strategy works fairly well. However, hydrolysis of the ester in the medicine bottle proceeds at a measurable rate, yielding the vile-tasting erythromycin A. The protection against acid afforded by the ester is, under the experimental conditions used here, undetectable; the degradation of erythromycin A ethyl succinate proceeds at the same rate as that of erythromycin A itself. Any protection from acid in the body can only be afforded by the relatively poor aqueous solubility of the ester, making it less accessible to hydrolysis when administered in large doses.

Erythromycin B is inherently much more stable to acid because it lacks the 12-OH group required for the formation of anhydroerythromycin A (**3**). The half-lives of erythromycin B at various acidic pH were 110−200 times greater than those of erythromycin A. Again, esterification with an ethyl succinyl group provided no additional protection against acid; on the contrary, EBES (**8**) actually degraded somewhat faster (2.5−4 times) than Ery B (**2**) under the conditions studied here.

Erythromycin A, erythromycin B, and their ethyl succinates all equilibrate with the corresponding enol ether derivatives in acidic solution.[4,17,28,29] The position of equilibrium, however, is much more favorable in erythromycin B and its derivatives. Our results (both here and previously reported[4]) show that for erythromycin B the equilibrium lies on the side of the erythromycin ketone. This is still more emphatically the case when the erythromycin is deuterated at C-8. However, when erythromycin A is treated with acid, its enol ether accumulates (any deuterium in the buffer notwithstanding) and after some time is in large excess over erythromycin A. Esterification of erythromycins A and B makes little difference to the position of equilibrium. This result is of great potential importance. Erythromycins and their derivatives have potent gut motilide activity, and this activity is especially associated with enol ether derivatives.[30,31] We can see that enol ether formation is suppressed in erythromycin B and its derivatives, most especially when they are deuterated at C-8, and we would expect these compounds to exhibit reduced gut motilide activity, relative to that of erythromycin A.

It is also clear from our data that the best literature model for the degradation of erythromycin A in acid[16] is actually incomplete. Our data fit this model reasonably well in the early stages of a degradation profile, but at low pH, the conversion of erythromycin A to its anhydride is incomplete; the reaction appears to be reversible. Further work, with the aim of more closely defining the model for erythromycin degradation, is in progress.

Erythromycin 2′-esters themselves show very little antibacterial activity. This experimental result has been well known for many decades, but quite recently, its origins have been illuminated by crystallographic data,[32] which indicate the importance of a hydrogen bond from the 2′-OH group to N1 and N6 of A2041Dr (A2058 Ec) on the bacterial ribosome. It is, thus, important for antibacterial activity that erythromycin esters be hydrolyzed at a reasonable rate in the body. It is almost equally important that hydrolysis be suppressed in the medicine bottle. Hydrolysis yields free erythromycin, which makes the drug intolerable to some children. Our results suggest that erythromycin B ethyl succinate shows fairly similar hydrolysis characteristics to erythromycin A ethyl succinate, but at apparent pH 7 and both 25 and 37 °C, its hydrolysis is somewhat slower (24% and 43%, respectively). We have already reported[19] the hydrolysis characteristics at refrigerator temperature (4 °C) and shown that although erythromycin B 2′-ethyl succinate is more susceptible to hydrolysis at pH 6, it is more stable at pH 8.

It is interesting to note that the kinetics of erythromycin ester hydrolysis appear to be very sensitive to buffer composition and/or to small changes in temperature and pH. Our results are as far as possible internally consistent; experiments at any

particular pH were carried out using the same batch of buffer and, where possible, on the same day. Large variability is, however, seen in the literature.[11,12,33] The bioavailability of erythromycin after administration of its ethyl succinate is likewise reported to be rather variable,[11,12,33] and it is at least possible that this has as much to do with the sensitivity of the chemistry of the drug as with pharmacogenomics.

Overall, these data suggest that erythromycin B esters may be superior to erythromycin A esters in pediatric use. In our previous work, we discussed the possibility of making completely taste-free proprodrugs of erythromycin B.[19] The work described here demonstrates that erythromycin B 2′-ethyl succinate has other advantages (which will be shared by the proprodrugs) over the market leader, erythromycin A 2′-ethyl succinate. Pre-clinical and clinical trials are required to determine whether these chemical advantages prove to be decisive in the clinic.

Experimental Section

General Procedures. All chemicals were purchased from Sigma Aldrich unless otherwise stated. ^{1}H NMR spectra were acquired using a Varian Unity 500 spectrometer operating at 500 MHz or a Bruker AVANCE 300 specrometer operating at 300 MHz. Electrospray-ionization mass spectra (ESI-MS) were acquired on a Micromass Platform mass spectrometer, and the data were analyzed using the program PLATFORM with a Masslynx data system. The sample (10 μL) was injected using a Hewlett-Packard auto-sampler, and the machine was operated at a cone value of 30 eV at 80 °C. For identification purposes, all samples (0.2 mg) were prepared in acetonitrile (1 mL). Water was used as the solvent for running the samples.

Chemical Syntheses. Compounds **1** and **4** are available commercially; **2** was a gift from Abbott Laboratories.

Synthesis of Erythromycin A Enol Ether (5). Erythromycin A enol ether was prepared by a published procedure,[24] and its structure was confirmed by NMR spectroscopy. Yield 68%, mp 133−138 °C (lit. 135−140 °C).[24]

Synthesis of Erythromycin B Enol Ether (7). Erythromycin B enol ether was prepared by a published procedure,[24] and its structure was confirmed by NMR spectroscopy. Yield 70%, mp 128−130 °C (lit. 126−128 °C).[34]

Synthesis of Erythromycin B 2′-Ethyl Succinate (8). Erythromycin B 2′-ethyl succinate was prepared by an adaptation of the published procedure for the preparation of erythromycin A ethyl succinate.[35] Details are given in the Supporting Information. Its structure was confirmed by NMR spectroscopy. Yield 85%, mp 166−168 °C (lit. 167−168 °C).[36]

Synthesis of Erythromycin A Enol Ether 2′-Ethyl Succinate (10). Erythromycin A enol ether 2′-ethyl succinate was prepared as previously described,[19] and its structure was confirmed by NMR spectroscopy. Yield 72%, mp 111−113 °C (lit. 114−116 °C).[19]

Synthesis of Erythromycin B Enol Ether 2′-Ethyl Succinate (11). Erythromycin B 2′-enol ether ethyl succinate was prepared as previously described,[19] and its structure was confirmed by NMR spectroscopy. Yield 60%, mp 132−134 °C (lit. 132−134 °C).[19]

Synthesis of Anhydroerythromycin A (3). Anhydroerythromycin A was prepared by a minor adaptation of the procedure used by Stephens and Conine.[13] Yield 81%, mp 140−149 °C (lit. 142−150 °C).[37]

Synthesis of Anhydroerythromycin A 2′-Ethyl succinate (9). Anhydroerythromycin A 2′-ethyl succinate was prepared by an adaptation of the published procedure for the preparation of 2′-esters of erythromycin.[35] To a solution of anhydroerythromycin A (1 g) in acetone (10 mL, previously dried over anhydrous sodium sulfate) dried sodium bicarbonate (0.533 g) was added. To the reaction mixture, a solution of ethyl succinyl chloride (0.23 mL) previously dissolved in dried acetone (2 mL) was added dropwise. The reaction was stirred overnight at room temperature. The volume of the reaction mixture was then reduced to 5 mL in vacuo. Sodium phosphate buffer (0.2 M, pH 6.5, approx 15 mL) was added to a final pH of above 6, and the mixture was stirred for 10 min. The white residue was filtered off and dissolved in chloroform (30 mL). The organic layer was washed with water (30 mL), dried over anhydrous sodium sulfate and reduced to dryness in vacuo. Compound **9** was recrystallized from a small volume of dichloromethane−hexane, with difficulty (0.77 g, 65%). Its structure was confirmed by NMR spectroscopy. Mp 92−102 °C (from dichloromethane-hexane); *m/z* (Electrospray) 844 $[M + H]^+$; Anal. ($C_{43}H_{73}NO_{15}$) C, H, N.

Acid-Catalyzed Degradation Studies. A stock solution (1 mg) of **1**, **2**, **4**, or **8** in d_6-acetone (approximately 100 mg mL^{-1}) was added to deuterated sodium phosphate buffer (0.2 M) at apparent pH 2, 2.5, 3, 3.5, 4, or 4.5, containing 1 mM TSP as a reference standard. Erythromycins generally have poor dissolution properties in water, and d_6-acetone is effective in accelerating dissolution, which is essential in time course experiments. Any change in chemical shifts caused by the acetone are negligible. An array of 1D-^{1}H spectra was acquired at 37 °C. Each spectrum was recorded with a spinning sample, using the PRESAT pulse sequence with a recycle time of 6 s. Typically, 64 scans per spectrum were collected; spectra were acquired every 6 min, and a total of 12 spectra were acquired per time course. All these parameters were adjusted depending upon the rate of reaction. Two degradations, those shown in Figures 2A and 5A, were carried out at higher time resolution, using 8 scans per spectrum and a slightly shorter recycle time of 5.2 s. All degradation studies were carried out at 37 °C. TSP, at a concentration of 1 mM, was used as a reference standard. The spectra were recorded with a spinning sample, using the PRESAT pulse sequence with a preaquisition saturation delay of 2 s, a 90° pulse width of 15 μs, and a spectral width of 6000 Hz. The spectra were processed with reference deconvolution using the FIDDLE (free induction decay deconvolution for lineshape enhancement) algorithm[38] for line shape correction with a Gaussian time constant of 0.22 s, using the TSP signal as the reference, followed by cubic spline baseline correction. The FIDDLE algorithm compares the experimental time-domain signal of a reference with that predicted by theory, multiplying the raw experimental data by the complex ratio of the two signals to produce a corrected free induction decay (FID).

Base-Catalyzed Hydrolysis Studies. Compounds **4** and **8** dissolved in d_6-acetone were added to deuterated sodium phosphate buffer (0.05 M) at apparent pH 7 to give a total concentration of 2 mM, and the time of addition was recorded. Time courses were acquired as described above at 25 and 37 °C.

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Supporting Information Available: Spectral data and synthetic methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Buss, A. D.; Waigh, R. D. Natural Products as Leads for New Pharmaceuticals. In *Burger's Medicinal Chemistry and Drug Discovery*, 5th ed.; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1995; Vol. 1, pp 1009−1012.

(2) McGuire, J. M.; Bunch, R. L.; Anderson, R. C.; Boaz, H. E.; Flynn, E. H.; Powell, H. M.; Smith, J. W. Ilotycin, a new antibiotic. *Antibiot. Chemother*. 1952, *2*, 281−283.

(3) Gregory, D.; Zeleznik, D.; Harnisch, J. P.; Counts, G. W. Activity of penicillin, erythromycin and cephalosporins against isolates of *Corynebacterium diphtheriae* from cutaneous lesions. *J. Antimicrob. Chemother*. 1979, *5*, 479−481.

(4) Mordi, M. N.; Pelta, M. D.; Boote, V.; Morris, G. A.; Barber, J. Acid-catalyzed degradation of clarithromycin and erythromycin B: a comparative study using NMR spectroscopy. *J. Med. Chem*. 2000, *43*, 467−474.

(5) Graham, E. M. Erythromycin. *Obstet. Gynecol. Clin. North Am.* 1992, *19*, 539−549.

(6) Barber, J.; Mordi, M. N. Erythromycin B, and derivatives thereof, as antibiotic agents for the treatment of microbial infections. *PCT Int. Appl*. 2000, 45.
(7) Jones, P. H.; Perun, T. J.; Rowley, E. K.; Baker, E. J. Chemical modification of erythromycin antibiotics. 3. Synthesis of 4″- and 11-esters of erythromycin A and B. *J. Med Chem.* 1972, *15*, 631−634.
(8) Tsuji, K.; Goetz, J. F. Elevated column temperature for the high-performance liquid chromatographic determination of erythromycin and erythromycin ethylsuccinate. *J. Chromatogr*. 1978, *157*, 185−196.
(9) Kirst, H. A. Semi-synthetic derivatives of erythromycin. *Prog. Med. Chem.* 1993, *30*, 57−87.
(10) Sinkula, A. A. Chemical modification of erythromycin: synthesis and preliminary bioactivity of selected amides and esters. *J. Pharm. Sci*. 1974, *63*, 842−848.
(11) Tardrew, P. L.; Mao, J. C. H.; Kenney, D. Antibacterial activity of 2′-esters of erythromycin. *Appl. Microbiol.* 1969, *18*, 159−65.
(12) Steffansen, B.; Bundgaard, H. Erythromycin prodrugs: kinetics of hydrolysis of erythromycin and various erythromycin 2′-esters in aqueous solution and human plasma. *Int. J. Pharm*. 1989, *56*, 159−168.
(13) Stephens, V. C.; Conine J. W. Esters of erythromycin. III. Esters of low-molecular-weight aliphatic acids. *Antibiotics Ann*. 1959, *1958−1959*, 346−353.
(14) *British Pharmacopoeia*; Stationery Office: London, 2002; Vol. 1, p 676.
(15) Grundy, W. E.; Alford, E. F.; Rickher, C. J.; Bower, R. R.; Rdzok, E. J.; Sylvester, J. C. A microbiological comparison of erythromycin and erythromycin B. *Antibiot. Chemother.* 1955, *5*, 212−217.
(16) Vinckier, C.; Hauchecorne, R.; Cachet, Th.; Van den Mooter, G.; Hoogmartens, J. A new mechanism for the decomposition of erythromycin A in acidic aqueous solution. *Int. J. Pharm.* 1989, *55*, 67−76.
(17) Cachet, Th.; Van den Mooter, G.; Hauchecorne, R.; Vinckier, C.; Hoogmartens, J. Decomposition kinetics of erythromycin A in acidic aqueous solutions. *Int. J. Pharm*. 1989, *55*, 59−65.
(18) Mordi, M. N.; Boote, V.; Hodgkiss, R.; Mistry, A.; Barber, J. Comparative pharmacokinetic study of erythromycin A, erythromycin B and clarithromycin using HPLC and electrospray mass spectrometry, unpublished data.
(19) Bhadra, P. K.; Morris, G. A.; Barber, J. Design, synthesis and evaluation of stable and taste-free erythromycin proprodrugs. *J. Med. Chem.* 2005, *48*, 3878−3884.
(20) Barber, J.; Gyi, J. I.; Lian, L.; Morris, G. A.; Pye, D. A.; Sutherland J. K. The structure of erythromycin A in [$^2H_6$] DMSO and buffered D_2O: full assignments of the ^1H and ^{13}C NMR spectra. *J. Chem. Soc., Perkin Trans. 2* 1991, *10*, 1489−1494.
(21) Everett, J. R.; Hunt, E.; Tyler, J. W. Ketone-hemiacetal tautomerism in erythromycin A in nonaqueous solutions. An NMR spectroscopic study. *J. Chem. Soc., Perkin Trans. 2* 1991, *10*, 1481−1487.
(22) Moatamedolroaya Fini, M. The Design, Synthesis and Evaluation of Macrolide Pro-drugs. Ph.D. thesis, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, U.K., 2001.
(23) Alam, P.; Buxton, P. C.; Embrey, K. J.; Parkinson, J. A.; Barber, J. Assignment of the ^1H and ^{13}C NMR spectra of anhydroerythromycin A in organic and aqueous solutions. *Magn Reson. Chem.* 1996, *34*, 837−839.
(24) Alam, P.; Buxton, P. C.; Parkinson, J. A.; Barber, J. Structural studies on erythromycin A enol ether: full assignments of the ^1H and ^{13}C NMR spectra. *J. Chem. Soc., Perkin Trans. 2*, 1995, 1163−1167.
(25) Chu, S.-H.; Mautner, H. G. Analogs of neuroeffectors. V. Neighboring-group effect in the reaction of esters. The hydrolysis and aminolysis of benzoylcholine, benzoylthiolcholine, benzoylselenolcholine, and of their dimethylamino analogs. *J. Org. Chem*. 1966, *31*, 308−312.
(26) Zaslowsky, J. A.; Fisher, E. The hydrolysis of β-diethylaminoethyl acetate, *J. Phys. Chem*. 1962, *67*, 959−961.
(27) Bruice, P. Y.; Mautner, H. G. Intramolecular catalysis and the involvement of tetrahedral intermediate partitioning in the hydrolysis of benzoylcholine, benzoylthionocholine, and their dimethylamino analogs. *J. Am. Chem. Soc*. 1972, *95*, 1582−1586.
(28) Kim, Y.-H.; Heinze, T. M.; Beger, R.; Pothuluri, J. V.; Cerniglia, C. E. A kinetic study on the degradation of erythromycin A in aqueous solution. *Int. J. Pharm.* 2004, *271*, 63−76.
(29) Barber, J. A. Preparation of antibiotic derivatives of erythromycin macrolides as antibacterial agents. *PCT Int. Appl.* 2004, 32.
(30) Sunazuka, T.; Tsuzuki, K.; Marui, S.; Toyoda, H.; Omura, S.; Inatomi, N.; Itoh, Z. Motilides, macrolides with gastrointestinal motor stimulating activity. II. Quaternary N-substituted derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal and 9,9-dihydroerythromycin A 6,9-epoxide. *Chem. Pharm. Bull.* 1989, *37*, 2701−2709.
(31) Tsuzuki, K.; Sunazuka, T.; Marui, S.; Toyoda, H.; Omura, S.; Inatomi, N.; Itoh Z Motilides, macrolides with gastrointestinal motor stimulating activity. I. O-substituted and tertiary N-substituted derivatives of 8,9-anhydroerythromycin A 6,9-hemiacetal. *Chem. Pharm. Bull.* 1989, *37*, 2687−2700.
(32) Schlunzen, F.; Zarivach, R.; Harms, J.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* 2001, *413*, 814−821.
(33) Taskinen, J.; Ottoila, P. Hydrolysis of 2′-esters of erythromycin. *J. Antimicrob. Chem.* 1988, *21*, 1−8.
(34) Krowicki, K.; Zamojski, A. J. Chemical modification of erythromycins. IV. 8-Hydroxyerythromycin B. *J. Antibiot.* 1973, *26*, 587−592.
(35) Clark, R. K.; Varner E. L. New esters of erythromycin. *Antibiot. Chemother.* 1957, *7*, 487−489.
(36) Bhadra, P. K. Erythromycin B: An Old Drug for the New Millennium. Ph.D. thesis, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, U.K., 2003.
(37) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V., Jr.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. Erythromycin. X. Structure of erythromycin. *J. Am. Chem. Soc.* 1957, *79*, 6062−6070.
(38) Morris, G. A.; Barjat, H.; Horne, T. J. Reference deconvolution methods. *Prog. Nucl. Magn. Reson. Spectrosc.* 1997, *31*, 197−257.

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