# DRUG DISCOVERY INTERFACE

# Stability Studies of Oxazolidine-Based Compounds Using 1H NMR Spectroscopy

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*Received 23 February 2009; revised 24 December 2009; accepted 18 January 2010*

*Published online 12 March 2010 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22108*

**ABSTRACT:** A series of oxazolidine-based compounds with a variety of substituents in positions 2 and 3 was synthesized and their stability studied. Ring opened intermediates formed on addition of limiting amounts of $D_2O$ to oxazolidine solutions, as observed by NMR. As the hydrolysis reactions proceeded, a series of novel dimeric β-amino alcohol compounds formed via an internal reaction between ephedrine and the ring opened intermediates. 2-Phenyl substituted oxazolidine compounds containing electron withdrawing nitro substituents were more rapidly hydrolyzed than the unsubstituted derivative and methoxy substituted compounds, with the nitro substituents appearing to stabilize the ring opened intermediates. Two oxazolidine derivatives, with a methyl and proton at position 2, were found to be more stable to oxazolidine hydrolysis than the 2-phenyl substituted compounds. Oxazolidines incorporating phenyl substituents at position 3 were synthesized and found to be less stable than those incorporating a methyl substituent at position 3. These fundamental structure–activity relationships may be useful when choosing oxazolidine derivatives as synthetic intermediates and as prodrugs for the delivery of compounds containing either β-amino alcohol or aldehyde components. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3362–3371, 2010

**Keywords:** NMR spectroscopy; prodrugs; stabilization; stability; synthesis; structure

## INTRODUCTION

Oxazolidines **1** (Fig. 1) have been synthesized for a variety of applications, including bacteriocides, fungicides, herbicides,[1] and mosquito repellents.[2] Several members of this class have been applied as chiral derivatizing or resolving agents,[3] and used as chiral auxiliaries to effect asymmetric inductions in a variety of reactions.[4–6] Ephedrine **2** and pseudoephedrine **3** react quantitatively with aldehydes to yield chiral oxazolidine derivatives with high diastereomeric purity.[7] Oxazolidines are used widely as optical inductors due to their easy hydrolysis.[8]

There have been a number of reports in which the labile nature of the oxazolidine system has been exploited, including its use for protection of the β-amino alcohol group. It has also been utilized in the synthesis of dipeptide compounds as transition state mimics for proteolytic enzymes.[9] Oxazolidines can be used as labile transient intermediates for synthesis, with an ability to direct stereochemical preference. Previous studies have also assessed the usefulness of oxazolidines as a drug delivery system[10–12] and have shown that oxazolidines undergo facile hydrolysis in the pH range 1–11 at 37°C. It was found that by variation of substitution on the 2-aryl ring of the oxazolidine derivatives it was possible to control oxazolidine hydrolysis. The reaction rates in neutral and basic solutions were found to decrease with both increasing steric effects of the substituents derived from the carbonyl component, and increasing basicity of the oxazolidines.[12] Therefore, it would seem likely that upon administration of many of the biologically active oxazolidines previously described, hydrolysis of the unstable ring system may occur, resulting in the release of the β-amino alcohol derivative and the aldehyde or

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Journal of Pharmaceutical Sciences, Vol. 99, 3362–3371 (2010)

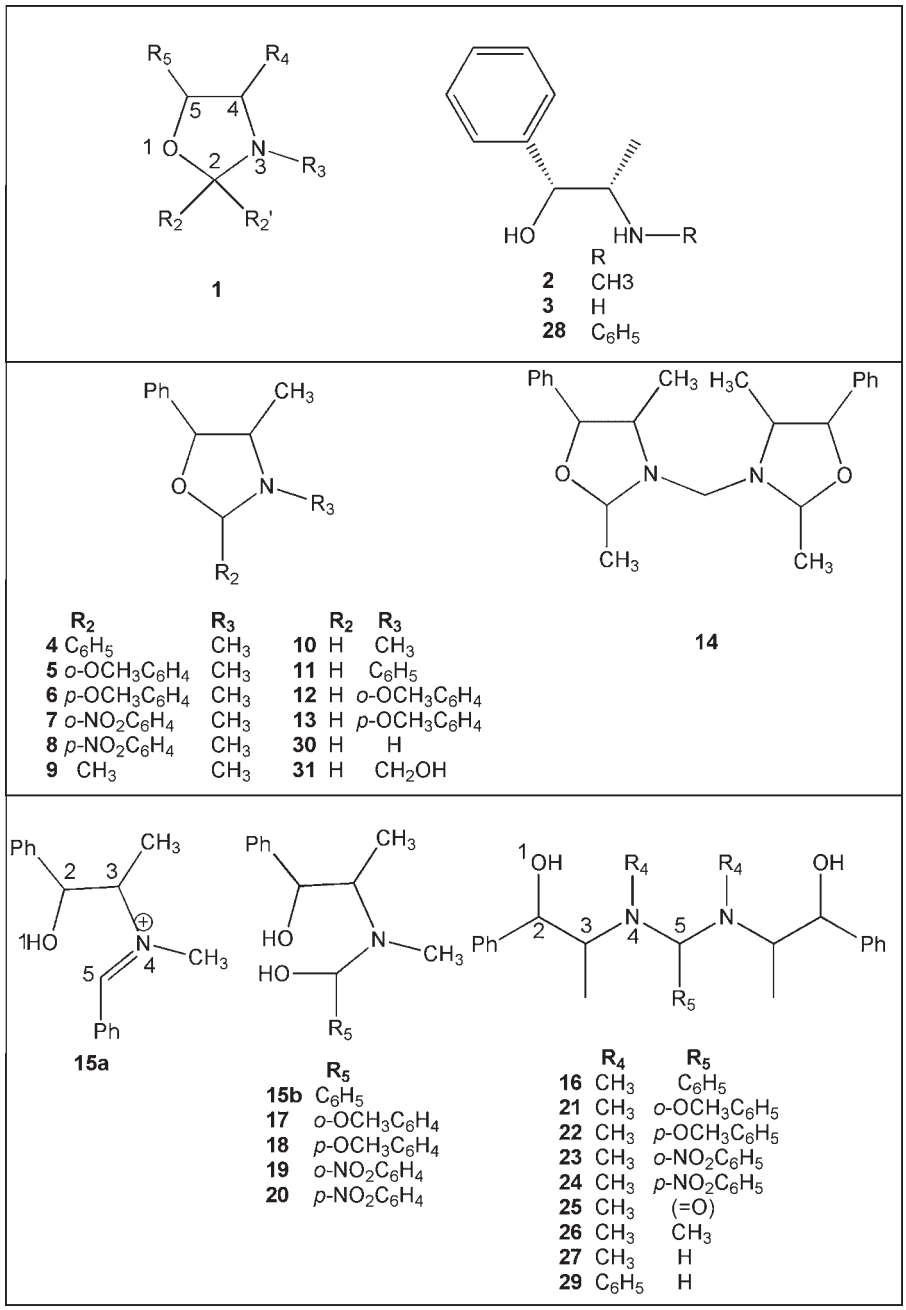


**Figure 1.** Structures of oxazolidines and derivatives.

ketone compound. In the absence of evidence for modes of action for oxazolidines themselves, it is possible that the activity of these compounds may be due to the hydrolysis products.

There are numerous drugs containing a β-amino alcohol moiety, including sympathomimetic drugs such as ephedrine, salbutamol, terbutaline, and ethambutol, as well as β-adrenergic blocking agents; for example, propanolol and alprenolol. Drugs of the sympathomimetic class have difficulty transporting across cell membranes to their site of actions due to p$K_a$ values >8 and low lipophilicity. Furthermore, drugs of this type cannot be administered dermally because, in the pH range compatible with skin (pH 3–8), they largely exist as cations and cannot be efficiently absorbed. Several of these drugs also go through pronounced first-pass metabolism; for example, in the gastrointestinal tract, phenylephrine, isoprenaline, and terbutaline undergo significant first-pass metabolism and thus the ability to administer these drugs orally is restricted.

Oxazolidines appear to be suitable prodrugs for molecules containing the β-amino alcohol moiety. Oxazolidines are much weaker bases (p$K_a$ 6–7) than

the parent β-amino alcohols, giving the cyclic systems higher lipophilicity at physiological pH.[13] This increased lipophilicity may become advantageous when β-amino alcohol type drug delivery problems are due to low lipophilicity, as is the case for dermal absorption.[13]

There are a number of biologically important molecules that incorporate a carbonyl group; for example, steroids. Moreover, side effects due to higher than desired *in vivo* levels of steroids resulting from topical application, may be avoided by using an oxazolidine prodrug that hydrolyzes slowly to allow controlled release of the parent drug. In considering oxazolidines as prodrug candidates for carbonyl containing substances, their weakly basic character may be advantageous in that the transformation of such substances into oxazolidines introduces a readily ionizable moiety, which in turn changes the solubility characteristics of the parent compound.

The aim of this study was to examine the stability of a series of compounds incorporating substituents at positions 2 and 3 of the oxazolidine ring, as summarized in Figure 1. Compounds **4–8** explore the influence of aromatic substitutions at position 2; compound **9** explores alkyl substituents at both positions 2 and 3; compounds **10–13** explore the influence of varying aromatic substitutions at position 3; and the dimeric oxazolidine **14** was known from earlier work[14] and was studied to assess the reactivity of the *N*,*N*'-methylene bridge. $^1$H NMR spectroscopy was used to follow hydrolysis, as it has the advantage of providing information on the chemical structure of intermediates as well as their relative concentrations during the reactions. These studies should further enhance the understanding of factors that affect oxazolidine hydrolysis and may be useful for selecting promoieties for the delivery of β-amino alcohols or carbonyl containing compounds.

## EXPERIMENTAL

### NMR Methods

$^1$H NMR data were recorded on a Bruker AM-300 NMR spectrometer operating at 300.13 MHz. $^1$H NMR spectra were recorded in $d_6(CH_3)_2CO$ and $d_4CH_3OH$ for **4–8** and in $d_4CH_3OH$ for **9–13**. The choice of solvent was determined by the solubility of the oxazolidine compounds and the need to avoid an overlap of the NMR signals of the compounds and those of the solvent. Chemical shifts are expressed in ppm relative to tetramethylsilane. Conditions for Fourier transform measurements were spectral width 4500 Hz, pulse width 5.0 μs, acquisition time 2.0 s, repetition time 2.0 s, number of data points 16,384 and number of transients 32. A solution of each oxazolidine compound ($1.75 \times 10^{-5}$ mol of the oxazolidines in 500 μL deuterated solvent) was prepared and 700 μL of $D_2O$ was added. Spectra were recorded at various intervals for up to several weeks. Oxazolidine hydrolysis was monitored by the relative intensities of peaks corresponding to the oxazolidine and the decomposition products.

A solution of the oxazolidine compounds (0.035 M) in the appropriate solvent ($1.75 \times 10^{-5}$ mol of the oxazolidines **4–8** in deuterated solvent, 500 μL) was prepared and 700 μL of $D_2O$ was added. Most oxazolidine hydrolysis occurred in the first few hours, so spectra were recorded frequently over this period. Further spectra were recorded after several days and again after several weeks.

### Synthetic Procedures

*3,4S-Dimethyl-2S,5R-diphenyl-oxazolidine* **4**.[15–20] (1R,2S)-(−)-2-Methylamino-1-phenyl-1-propanol (2.06 g, 12.5 mmol) and freshly distilled benzaldehyde (1.32 g, 12.5 mmol) were dissolved in anhydrous toluene (30 mL). Anhydrous sodium sulphate (500 mg) was added and the solution was heated to reflux for 8 h. The solution was then filtered hot and the toluene evaporated under reduced pressure to yield a white solid, which was recrystallized from ethanol to give 2.68 g (85%) of **4** as fine white crystals. A similar approach was used for compounds **5–9**.

*3,4S-Dimethyl-5R-phenyloxazolidine* **10**.[14,16,20–23] A stirred solution of paraformaldehyde (750 mg, 25 mmol) and potassium hydroxide (10 mg) in super dry methanol (40 mL) was treated with (1R, 2S)-(−)-ephedrine (2.08 g, 12.5 mmol). Sodium sulphate (500 mg) was added and the suspension was heated to reflux for 6 h. The drying agent was filtered off, the methanol evaporated under reduced pressure and the resulting crude oil purified by distillation to afford 1.88 g (85%) of compound **10** as a clear oil.

*3,5R-Diphenyl-4S-methyloxazolidine* **11**. A catalytic amount of potassium hydroxide was added to a suspension of formaldehyde (50 mg, 1.67 mmol) in anhydrous methanol (5 mL). When the formaldehyde had dissolved, the solution was treated with (1R,2S)-2-phenylamino-1-phenyl-1-propanol (93 mg, 0.41 mmol) and refluxed for 4 h. The methanol evaporated under reduced pressure and the resulting residue was filtered through a plug of silica to remove decomposition products. Purification was by radial chromatography of the dark yellow residue eluting with chloroform/petroleum ether. A similar approach was used for **12** and **13**.

Melting points were determined on a Mettler FP2 melting point apparatus and are uncorrected. Infrared spectra were recorded on a Hitachi 270-30 grating spectrophotometer. Mass spectra were recorded on a Jeol JMX DX-300 double-focusing instrument at 70 eV. Fast atom bombardment (FAB) mass spectra were recorded on the same instrument with a Jeol-

 DOI 10.1002/jps

FABMS source and argon as incident particle. Flash chromatography was performed on Merck Kieselgel 60 $F_{254}$ (230–400 mesh) silica. Preparative thin layer chromatography was performed on glass plates coated with 2 and 4 mm layers of silica gel (Merck Kieselgel 60 $F_{254}$). Analytical thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 $F_{254}$ precoated aluminium backed plates. Radial chromatography was performed using a Model 7924T Harrison Research Chromatotron instrument on Merck Kieselgel 60 $PF_{254}$. Elemental analysis was carried out by the Australian Microanalytical Service, AMDEL, Melbourne, and the Chemical and Micro Analytical Service, Pty. Ltd, Melbourne.

LC/MS was performed by injection of samples (5 μL) onto a Phenomenex Luna C8(2) column (50 mm × 4.6 mm, 5 μm, 100 Å). Samples were eluted at 0.5 mL/min using a gradient of 5% solvent B (80% MeOH): 95% solvent D (water/0.1% formic acid) to 100% solvent B over 4 min. Solvent delivery was achieved using an Agilent 1200 Series HPLC pump system (Agilent Technologies, Santa Clara, CA). Multimode electrospray mass spectra were acquired in positive ion mode on an Agilent 6120 Quadrupole mass spectrometer (Agilent Technologies). Scan data were acquired at a fragmentor voltage of 130 V over a mass range of *m*/*z* 100–1000. Instrument control and data analysis was performed using Agilent MSD ChemStation Rev. B.04.01 software (Agilent Technologies).

## RESULTS AND DISCUSSION

The primary aim was to use $^1$H NMR spectroscopy to obtain information on the factors affecting oxazolidine ring stability. The main interest centered on the effects of substituents at positions 2 and 3 of the oxazolidine ring. (−)-Ephedrine **2** and (−)-norephedrine **3** were chosen as the β-amino alcohol precursors for several reasons. Firstly, **2** and related compounds are important as sympathomimetic drugs; secondly, **2** and **3** were readily available and the chemistry involved in oxazolidine formation, using these starting materials, is straightforward; and finally, ephedrine-derived oxazolidines have relatively simple $^1$H NMR spectra. As oxazolidine hydrolysis occurs, the appearance of extra peaks in the spectra resulting from oxazolidine degradation products should be easy to monitor and identify. A diverse range of oxazolidine derivatives incorporating substituents of differing steric and electronic nature at positions 2 and 3 was synthesized, as shown in Figure 1. The first series of compounds had varying aromatic substituents at position 2, namely compounds **4–8**. The phenyl-substituted derivatives incorporated both methoxy and nitro functions at either positions 2″ or 4″. A comparison of compounds **4–8** allowed examination of the electronic effects of the phenyl substituent at position 2 on oxazolidine ring stability. The influence of the placement of functional groups at positions 2″ and 4″ was also observed.

The stability studies on the 2-phenyl substituted compounds **4–8** indicated that, under the experimental conditions used, oxazolidine hydrolysis did not go to completion. In previous experiments under different conditions,[24] conversion of the oxazolidine to the corresponding β-amino alcohol and aldehyde component occurred quickly. In the current study, the addition of a limited amount of water to the oxazolidine compounds in an organic solvent solution resulted in slower oxazolidine hydrolysis.

An example of the trends observed in $^1$H NMR spectra following addition of $D_2O$ to the 2-phenyl oxazolidine/$d_6$-acetone solutions for the hydrolysis of **4** is shown in Figure 2. However, the $^1$H NMR spectra of the major intermediate are not consistent with the formation of a cationic Schiff base **15a**[16] that was proposed based on absorption spectroscopy studies. A model cationic Schiff base system characterized by NMR indicated an imine proton shift of $\delta$ 9–10 ppm and $NCH_3$ shift of around $\delta$ 4 ppm[25] whereas the observed major intermediate had shifts of $\sim\delta$ 5 and $\delta$ 2 ppm, respectively. The observed chemical shifts are instead consistent with a ring opened intermediate **15b** that will be in equilibrium with **15a**. Rate determining hydrolysis of the intermediates then occurs to give the final hydrolysis product **2** and benzaldehyde. Subsequently, the dimeric species, **16**, may be formed via reaction of **15a** with the hydrolysis product **2**, as illustrated in Figure 3. LC/MS analysis supported the interpretation of the dimer, with a peak at 459.1 Da, which corresponds in mass to a potassium adduct of the dimer (418.26 Da).

To investigate substituent effects on the reaction kinetics the experiment was repeated in $d_4CH_3OH$ for the five oxazolidine compounds **4–8**, and intermediates were observed immediately following addition of $D_2O$. Tables 1 and 2 show $^1$H NMR parameters for the parent oxazolidines **4–8** and the corresponding hydrolysis products **15b** and **17–20** in $d_6(CH_3)_2CO$/$D_2O$ and $d_4CH_3OH$/$D_2O$. Figure 4 shows the degree of intermediate formation as a function of time for **4–8**. The least stable oxazolidine derivatives are the nitro-substituted oxazolidines **7** and **8**. Under the conditions examined, the *p*-nitrophenyl substituted derivative **8** underwent 89% decomposition to form the corresponding intermediate, whereas the *o*-nitrophenyl derivative **7** experienced 62% decomposition. This result was expected since the nitro groups draw electron density from carbon C-2, encouraging electron donation from the oxazolidine nitrogen to form the imine bond with resultant ring-opening. The unsubstituted oxazolidine derivative **4** underwent 34% oxazolidine decomposition to

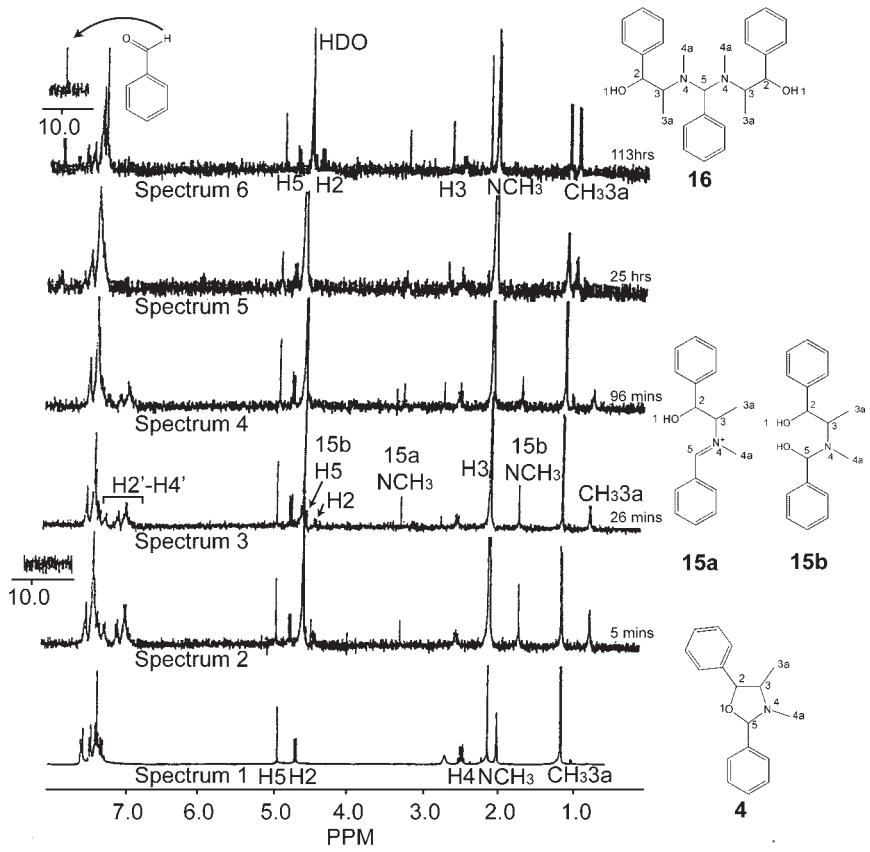


**Figure 2.** Spectra used to monitor the hydrolysis of **4** following addition of $D_2O$ to an oxazolidine/$d_6(CH_3)_2CO$ solution. Signal assignments for **4** are shown in spectrum 1, the ring opened and cationic Schiff base intermediates **15a** and **15b** in spectrum 3 and the dimeric species **16** and benzaldehyde in spectrum 6.

form the corresponding intermediate, whereas the *p*-methoxyphenyl substituted derivative **6** underwent 15% decomposition. Surprisingly, the *o*-methoxyphenyl substituted compound **5** experienced 40% hydrolysis to form the intermediate **17**. The greater reactivity of **5**, compared with **4** and **6**, may be attributed to intramolecular catalysis by the *ortho*-situated methoxy group. A similar enhanced reactivity was observed for the related oxazolidine, 2*S*-(*o*-hydroxyphenyl)-3,4*S*-dimethyl-5*R*-phenyloxazolidine relative to the unsubstituted 2-phenyloxazolidine **4**.[24]

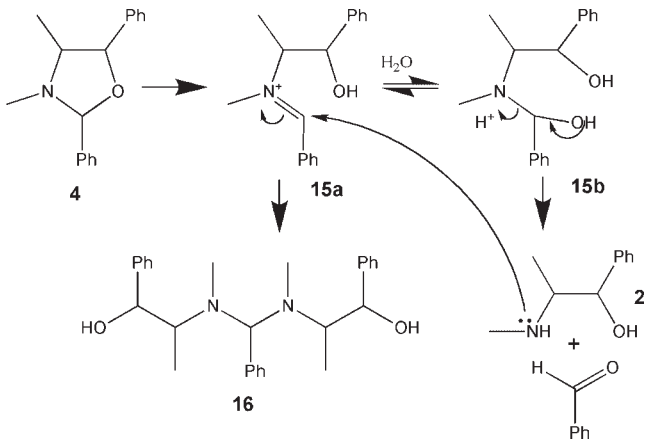


**Figure 3.** Mechanism for formation of cationic Schiff base intermediate, ring hydrolysis intermediate and dimer product.

The intermediates observed in the hydrolysis of oxazolidines **4**–**8** persisted for varying lengths of time, depending on the substituent in the aromatic group attached to the imine bond. However, in all cases the intermediates eventually underwent further reaction to form a second compound. To investigate the identity of the second compound, ephedrine (**2**) was added to the solution from an experiment carried out on **4** in $d_6(CH_3)_2CO/D_2O$ after incubation for 4 days. Examination of spectrum 6 in Figure 2 showed that a peak for the aldehydic proton of benzaldehyde was present at $\delta$ 9.63 ppm at $T = 113$ h. Clearly, oxazolidine hydrolysis must be progressing to release ephedrine and benzaldehyde. However, "spiking" of the reaction mixture with ephedrine at this time showed that no ephedrine was present in the reaction mixture. Evidently, any ephedrine formed undergoes further reaction to form the identified product.

 DOI 10.1002/jps

**Table 1.** $^1$H NMR Spectral Data for Oxazolidines **4–8**, in $d_6(CH_3)_2CO/D_2O$ (A) and $d_4CH_3OH/D_2O$ (B)

| | Chemical Shift (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **4** | | **5** | | **6** | | **7** | | **8** | |
| Proton | A | B | A | B | A | B | A | B | A | B |
| **$CH_3$-4α, d** | 1.07 (5.7)[a] | 1.19 (6.0) | 1.05 (6.0) | 1.22 (6.1) | 1.06 (5.9) | 1.16 (6.0) | 1.07 (6.1) | 1.19 (6.0) | 1.07 (6.0) | 1.19 (5.8) |
| **$NCH_3$, s** | 2.48 | 2.20 | 2.43 | 2.66 | 2.44 | 2.65 | 2.49 | 2.73 | 2.57 | 2.24 |
| **H-4 dq** | 2.48 | 2.72 | 2.43 | 2.66 | 2.44 | 2.65 | 2.49 | 2.73 | 2.57 | 2.7 |
| **H-5, d** | 4.68 (8.5) | 4.84 (9.4) | 4.61 (8.8) | 4.78 (8.8) | 4.64 (8.8) | 4.73 (8.6) | 4.59 (8.7) | 4.60 (9.0) | 4.74 (8.8) | 4.87 (9.0) |
| **H-2, s** | 4.87 | 5.01 | 5.36 | 5.54 | 4.81 | 4.99 | 5.36 | 5.57 | 5.16 | 5.19 |
| **$OCH_3$, s** | | 3.72 | 3.87 | 3.70 | 3.85 | | | | | |
| Arom Protons, m | 7.2–7.5 | 7.3–7.5 | 6.8–7.5 | 7.0–7.7 | 6.7–7.3 | 7.0–7.5 | 7.1–7.6 | 7.1–7.7 | 7.3–8.2 | 7.3–8.3 |

[a]Coupling constants in Hz in parentheses.

Examination of the $^1$H NMR spectra resulting from addition of $D_2O$ to the $d_4CH_3OH$ and $d_6(CH_3)_2CO$/oxazolidine solutions after several days suggested that ephedrine **2** was reacting with the cationic Schiff base intermediate **15a** to form the dimeric species **16**, as outlined in Figure 3. Spectrum 6 in Figure 2 shows that most of the signals corresponding to the dimeric intermediate are identifiable. A doublet for the $CH_3$-3α protons at $\delta$ 0.95 ppm and a singlet for the $NCH_3$ protons at $\delta$ 2.14 ppm integrate to six protons. The H-3 doublet of quartets of **16** at $\delta$ 2.58 (which overlaps the corresponding proton H-4 of the parent oxazolidine **4** at $\delta$ 2.48 ppm) and the doublet for H-2 of **16** at $\delta$ 4.34 ppm both integrate to two protons. The bridging methine proton, designated H-5, was assigned as a singlet at $\delta$ 4.92 ppm and the corresponding proton, H-2 of the parent oxazolidine **4**, as a singlet at $\delta$ 4.87 ppm. The aromatic signals integrate correctly to the required number of protons corresponding to the parent oxazolidine **4**, the β-amino alcohol dimer **16** and benzaldehyde. Interesting to note is the benzaldehyde signal at $\delta$ 9.63 ppm, which integrates in a ratio of 1:3 with the methyl protons of the second formed product, consistent with the formation of a β-amino alcohol dimer. Detailed 1H NMR data for the proposed β-amino alcohol dimers **16** and **21–24** resulting from the hydrolysis of the oxazolidines **4–8** are given in Table 3.

Figure 4 also shows that the nitro-substituted intermediates from **7** and **8** are the most stable and can be detected for up to 5 days. Figure 5 shows the complementary formation of the β-amino alcohol dimers, showing that the methoxy substituted analogs dimerize most rapidly. Thus, it seems that under the conditions examined, the nitro substituents stabilize the intermediates or render them less reactive to ephedrine, and thus less likely to undergo dimerization. The enhanced electron density at C-5 of the nitro-substituted intermediates relative to the methoxy analogs probably contributes to a diminished rate of nucleophilic attack upon this position in the intermediate compounds.

Under the experimental conditions used here, the dimeric compounds were stable and present for several weeks. However, in most cases, spectra recorded after 4 weeks showed the gradual appearance of peaks corresponding to ephedrine, though only in small amounts (<5%). Although control over the extent of initial oxazolidine hydrolysis

**Table 2.** 1H NMR Spectral Data for the Intermediates **15** and **17–20,** in $d_6(CH_3)_2CO/D2O$ (A) and $d_4CH_3OH/D_2O$ (B)

| | Chemical Shift (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **15** | | **17** | | **18** | | **19** | | **20** | |
| Proton | A | B | A | B | A | B | A | B | A | B |
| $CH_3$-3α, d | 0.7 (5.7)[a] | 0.62 (5.7) | 0.71 (6.1) | 0.67 (6.3) | 0.74 (6.7) | 0.69 (6.6) | 0.68 (6.1) | 0.64 (6.0) | 0.65 (6.1) | 0.66 (6.1) |
| $NCH_3$, s | 1.64 | 1.56 | 1.72 | 1.72 | 1.67 | 1.62 | 1.78 | 1.73 | 1.64 | 1.62 |
| H-3 dq | 2.07 | 1.97 | 2.07 | 2.06 | 2.07 | 1.98 | — | 2.04 | 2.04 | 1.95 |
| H-2, d | 4.38 (8.3) | 4.33 (8.8) | 4.30 (9.7) | 4.33 (8.6) | 4.37 (8.5) | 4.36 (9.8) | 4.22 (10.5) | 4.2 (9.8) | 4.3 (8.9) | 4.31 (9.0) |
| C<u>H</u>=N, s | 4.49 | 4.47 | 5.25 | 5.31 | 4.47 | 4.47 | 5.22 | 5.25 | 4.48 | 4.49 |
| O<u>C</u>H$_3$, s | — | — | 3.24 | 3.13 | 3.22 | 3.09 | — | — | — | — |
| Arom Protons, m | 6.8–7.3 | 6.78–7.16 | 6.95–7.5 | 6.32–7.6 | 6.52–7.8 | 6.5–7.7 | 6.8–7.8 | 6.7–7.7 | 6.9–7.2 | 6.8–7.6 |

[a]Coupling constants in Hz in parentheses.

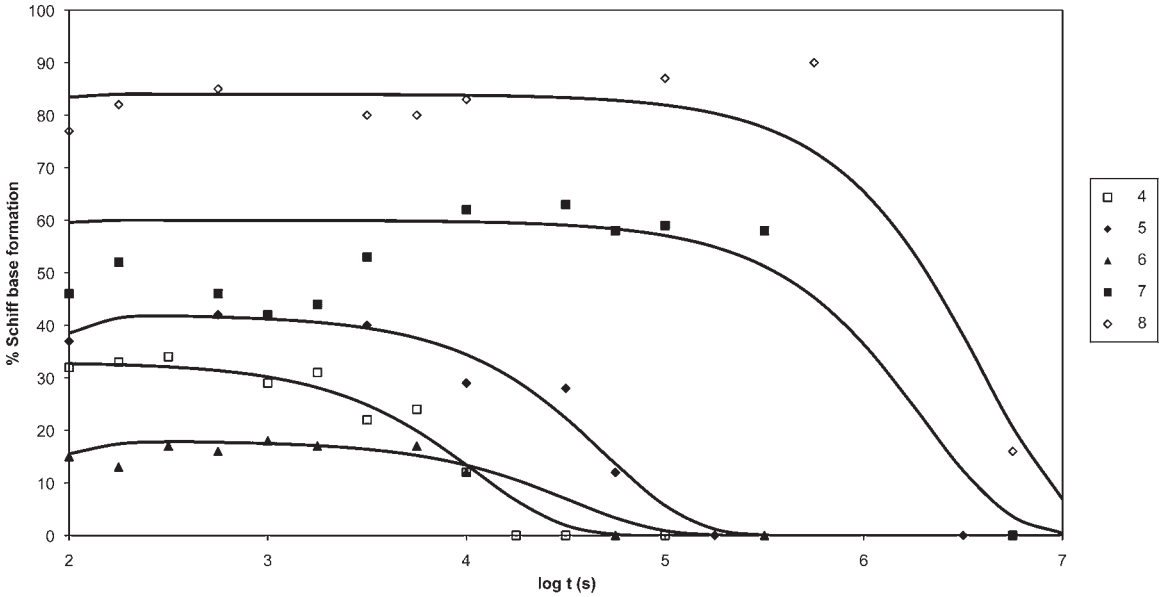


**Figure 4.** Plot of percentage formation of the hydrolysis intermediates against time following hydrolysis of compounds **4–8**. Times are in units of seconds and are represented on a log scale. Composite exponential functions were used to fit the data (i.e., $I = I_0\left(\frac{k_1}{k_2-k_1}\right)(e^{-k_1 t} - e^{-k_2 t})$ where $I$ is the observed intensity at time $t$, $I_0$ is the initial intensity (arbitrary units) and $k_1$ and $k_2$ are rate constants ($s^{-1}$) for the formation and degradation, respectively, of the intermediate). The values of $k_1$ and $k_2$ for the various intermediates are as follows: **4**: $k_1 = 0.21\,s^{-1}$, $k_2 = 9.0 \times 10^{-5}\,s^{-1}$; **5**: $k_1 = 0.025\,s^{-1}$, $k_2 = 2.0 \times 10^{-5}\,s^{-1}$; **6**: $k_1 = 0.02\,s^{-1}$, $k_2 = 3.0 \times 10^{-5}\,s^{-1}$; **7**: $k_1 = 0.05\,s^{-1}$, $k_2 = 5.0 \times 10^{-7}\,s^{-1}$; **8**: $k_1 = 0.05\,s^{-1}$, $k_2 = 2.5 \times 10^{-7}\,s^{-1}$.

can be exerted by substituent variation, eventually all 2-phenyloxazolidine derivatives almost completely hydrolyzed to give mainly the dimeric product with small amounts of the oxazolidine starting material.

The formation of the dimeric compounds reported here is consistent with the previous report of a structurally related dimer **14** incorporating oxazolidine rings instead of the β-amino alcohol groups.[24,26] None of the proposed β-amino alcohol dimer derivatives **21–24** have been previously reported. However, related compounds of the general structure **25**, differing only in the incorporation of a carbonyl function at the bridging carbon C-5, have been synthesized.[27] The existence of these structurally related compounds seems to indicate that the formation of the proposed dimeric compounds is possible and that they should be reasonably stable. The additional reactivity of **5** can be attributed to direct catalytic involvement of the *o*-methoxy group.

Under the experimental conditions used here, the dimeric compounds were stable and present for several weeks. However, in most cases, spectra

**Table 3.** 1H NMR Spectral Data for the β-Amino Alcohol Dimers **16**, and **21–24**, in d6(CH3)2CO/D2O (A) and d4CH3OH/D2O (B)

| | Chemical Shift (ppm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **16** | | **21** | | **22** | | **23** | | **24** | |
| Proton | A | B | A | B | A | B | A | B | A | B |
| CH3-3α, d | 0.95 (6.1)[a] | 0.82 (6.5) | 0.95 (6.2) | 0.85 (6.5) | 0.95 (6.1) | 0.81 (6.2) | 0.96 (6.6) | 1.0 (6.7) | 0.95 (6.7) | 1.1 (6.8) |
| $NCH_3$, s | 2.14 | 2.44 | 2.14 | 2.48 | 2.14 | 2.42 | 2.14 | 2.64 | 2.14 | 2.24 |
| H-3 dq | 2.58 | 2.93 | 2.50 | 3.0 | 2.49 | 2.92 | 2.66 | 3.27 | 2.66 | 3.13 |
| H-2, d | 4.34 (9.2) | 4.42 (9.0) | 4.35 (9.2) | 4.42 (8.0) | 4.35 (9.0) | 4.41 (8.8) | 4.45 (9.4) | 4.96 (9.2) | 4.35 (9.0) | 4.52 (8.8) |
| H-5, s | 4.92 | 5.01 | 4.29 | 4.87 | 4.96 | 4.67 | 5.44 | 5.57 | 5.04 | 5.01 |
| $OCH_3$, s | | | 3.69 | 3.94 | 3.78 | 3.91 | | | | |
| Arom Protons, m | 7.0–7.81 | 7.0–7.8 | 6.8–7.6 | 6.9–7.8 | 6.86–7.9 | 7.0–7.6 | 6.8–7.7 | 6.7–7.7 | 7.3–8.2 | 7.3–8.3 |

[a]Coupling constants in Hz in parentheses.

 DOI 10.1002/jps

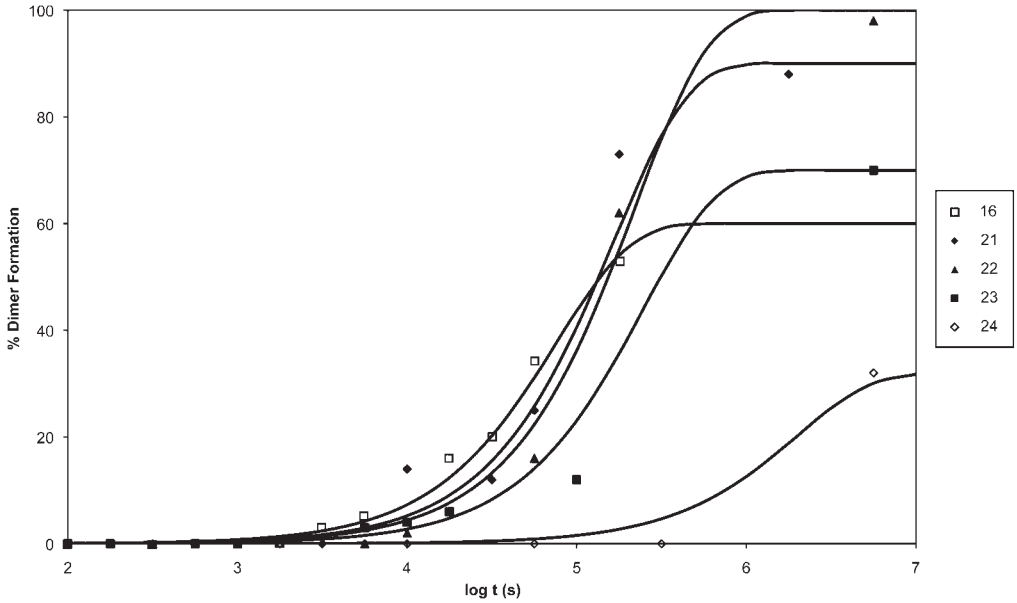


**Figure 5.** Plot of percentage formation of the β-amino alcohol dimers **16** and **21–24** against time. Times are in units of seconds and are represented on a log scale. An exponential function was used to fit the data (i.e., $I = I_0 e^{-k_1 t}$ where $I$ is the observed intensity at time $t$, $I_0$ is the initial intensity (arbitrary units) and $k_1$ is the rate constant ($s^{-1}$) for the formation of the dimer). The values of $k_1$ for the various dimers are as follows: **16**: $k_1 = 1.3 \times 10^{-5}\,s^{-1}$; **21**: $k_1 = 6.0 \times 10^{-6}\,s^{-1}$; **22**: $k_1 = 4.5 \times 10^{-6}\,s^{-1}$; **23**: $k_1 = 4.0 \times 10^{-6}\,s^{-1}$; **24**: $k_1 = 5.0 \times 10^{-7}\,s^{-1}$.

recorded after 4 weeks showed the gradual appearance of peaks corresponding to ephedrine, though only in small amounts (<5%). Although control over the extent of initial oxazolidine hydrolysis can be exerted by substituent variation, eventually all 2-phenyloxazolidine derivatives almost completely hydrolyzed to give mainly the dimeric product with small amounts of the oxazolidine starting material.

To compare the effect of alkyl substituents at position 2 relative to aryl or unsubstituted derivatives, two further oxazolidines **9** and **10** were examined. An experiment was performed using similar conditions to those used to study the 2-phenyl substituted oxazolidines **4–8**. Compounds **9** and **10** were found to be more stable to hydrolysis than the 2-phenyl substituted oxazolidines **4–8**. Only one major decomposition product was evident in both cases. Minor amounts (<5%) of unidentified decomposition products were also evident in the $^1$H NMR spectra. Spiking the reaction mixtures with ephedrine confirmed that the newly formed peaks did not correspond to ephedrine. The intermediates resulting from the hydrolysis of the oxazolidines **9** and **10** were not stable and underwent rapid hydrolysis to form the dimeric compounds **26** and **27**. The intermediates were not detected in the $^1$H NMR spectra, which is not surprising given that the imine bonds would be relatively unstable without a phenyl substituent. Given that the intermediates **15** and **17–20** were shown to be unstable compared with the dimeric compounds **16** and **21–24**, respectively, it is reasonable to assume that the intermediate formed from the hydrolysis of the oxazolidines **9** and **10** coincides with related dimeric products. Table 4 shows $^1$H NMR parameters for the parent oxazolidines **9** and **10** and Table 5 shows the $^1$H NMR data for the corresponding β-amino alcohol dimers **26** and **27**.

Three oxazolidine derivatives **11–13**, which incorporate 2 or 4-substituted phenyl substituents at position 3, were tested to compare the effect of an aryl function relative to an alkyl substituent on the

**Table 4.** 1H NMR Data for Oxazolidine Derivatives **9** and **10** in $d_4CH_3OH/D_2O$

| | Chemical Shift (ppm) | | | Coupling (Hz) | |
|---|---|---|---|---|---|
| Proton | **9** | **10** | Multiplicity | **9** | **10** |
| $CH_3$-4α | 1.12 | 1.15 | d | 6.1 | 6.3 |
| $NCH_3$ | 2.28 | 2.38 | s | — | — |
| H-4 | 2.57 | 2.66- | dq | 6.1, 9.0 | 6.2, 8.7 |
| H-5 | 4.55 | 4.54 | d | 9.0 | 8.6 |
| H-2 | 4.36 | — | q | 5.3 | — |
| $CH_3$-2α | 1.37 | — | d | 5.3 | — |
| H-$2_B$ | — | 4.36 | d | — | 4.0 |
| H-$2_A$ | — | 4.7 | d | — | 3.9 |
| Aromatic Protons | 7.35-7.45 | 7.35-7.45 | m | — | — |

**Table 5.** $^1$H NMR Spectral Data for the β-Amino Alcohol Dimers **26** and **27** in $d_4CH_3OH/D_2O$

| | Chemical Shift (ppm) | | | Coupling (Hz) | |
|---|---|---|---|---|---|
| Proton | **26** | **27** | Multiplicity | **26** | **27** |
| $CH_3$-3α | 0.89 | 0.84 | d | 6.6 | 6.6 |
| $NCH_3$ | 2.49 | 2.44 | s | — | — |
| H-3 | 3.08 | 2.98 | dq | 6.4, 8.7 | 6.4, 8.8 |
| H-2 | 4.5 | 4.46 | d | 8.6 | 8.6 |
| H-5 | 3.32 | — | q | 5.3 | — |
| $CH_3$-5α | 1.17 | — | d | 7.0 | — |
| CH2 | — | 2.86 | s | — | — |
| Aromatic Protons | 7.35-7.45 | 7.35-7.45 | m | — | — |

oxazolidine nitrogen. Several experiments were attempted but the progress of the hydrolysis reactions could not be monitored until solutions became clear enough for reasonable $^1$H NMR spectra to be recorded; in a number of cases this took several weeks. For example, 700 μL of $D_2O$ was added to a 0.04 M solution of **11** in $d_4CH_3OH$ and the $^1$H NMR spectrum recorded after 48 days indicated that 70% oxazolidine decomposition had occurred. Two decomposition products were detected, as defined by two new doublets at $\delta$ 1.04 and 1.16 ppm assigned to either the β-amino alcohol **28** or the β-amino alcohol dimer **29**. The doublet for the $CH_3$-4α protons of the parent oxazolidine derivative **11** appears at $\delta$ 0.89 ppm.

NMR stability studies of the *o*- and *p*-methoxy substituted derivatives **12** and **13** were hampered by solubility problems and no accurate measure of oxazolidine hydrolysis could be determined. Future work on the stability of these compounds will require the use of alternative solvent systems.

Previously we studied the stability of the dimeric oxazolidine derivative **14** obtained from the condensation of **3** and formaldehyde.[14] An attempt was made to synthesize 4-methyl-5-phenyloxazolidine **30** by the condensation of **3** with formaldehyde, as previously reported.[27] The reaction appeared to proceed smoothly and yielded a single product, but on examination of $^{13}$C and $^1$H NMR spectra it became apparent that the product was not **30** as expected, but the dimeric species **14** (structure shown in Fig. 1). Further investigation revealed that a dimeric species would be expected if a molar excess of formaldehyde[26,28] was used in the reaction, compared with the single molar equivalent for production of the monomer. We previously reported[14] that dimer **14** is stable in chloroform; however, in methanol it spontaneously hydrolyzes to yield two compounds, the oxazolidine **30** and the *N*-hydroxymethyl oxazolidine derivative **31**, which, on addition of water, break down at substantially different rates; **30** is essentially stable whereas **31** breaks down rapidly.

The *N*,*N*′-methylene bridge of **14** is very reactive and prone to hydrolysis. Previous studies[29,30] have utilized the unstable and reactive nature of this compound in the synthesis of a number of *N*-substituted norephedrine derivatives by reacting the oxazolidine dimer **14** with various acetate derivatives. It appears that the basicity of the oxazolidine nitrogen is the driving force causing the preferential hydrolysis of **31**. The oxazolidine nitrogen of **31** is more basic than the secondary amine **30**, firstly because it is a tertiary nitrogen and secondly because the hydroxymethyl group is an electron donating substituent, driving electron density toward the nitrogen. These factors assist in the oxazolidine nitrogen of **31** by donating electron density to form the cationic imine intermediate.

In summary, a number of oxazolidine-based compounds derived from ephedrine and various aldehyde compounds were synthesized and their stability studied using $^1$H NMR spectroscopy. Oxazolidine hydrolysis did not yield ephedrine and the aldehyde component as expected, but on addition of $D_2O$ to $d_4CH_3OH$ or $d_6(CH_3)_2CO$/oxazolidine solutions the formation of intermediate ring opened and cationic Schiff base derivatives was detected. As the hydrolysis reactions proceeded a series of novel dimeric β-amino alcohol compounds formed via a reaction between ephedrine and the cationic Schiff base intermediate. The relative stability of the oxazolidine compounds was examined using $^1$H NMR spectroscopy. The resultant structure–reactivity relationships may be useful when deciding on oxazolidine derivatives as prodrugs for the delivery of compounds containing either a β-amino alcohol and or aldehyde component.

## ACKNOWLEDGMENTS

We thank Dr. Shane Simonsen and Dr. David Wilson (University of Queensland) for valuable assistance in the revision of the manuscript. D.J.C. is supported by a National Health and Medical Research Council Fellowship.

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