

Effect of β -cyclodextrin on the extraction of isoxazolines from aqueous ethanol into chloroform

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Dedicated to Oswald S. Tee on the occasion of his 60th birthday, and in recognition of his many contributions to chemistry

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Abstract

Regioisomeric pairs of isoxazolines were prepared through reaction of nitrile oxides with ethyl trans-cinnamate. The effect of β -cyclodextrin on the solubility of these compounds in aqueous ethanol and their extraction into chloroform has been examined. Somewhat anomalously, the compound which is least soluble in the aqueous ethanol is least efficiently extracted into the chloroform, presumably because it is most extensively complexed by the cyclodextrin. In one case, extraction of a 1:1 mixture of a regioisomeric pair of isoxazolines resulted in the separation of ca. 50% of each of the compounds in ca. 90% purity, from a single partitioning.

Keywords: Isoxazolines, extraction, β -cyclodextrin, nitrile oxides, ethyl trans-cinnmate

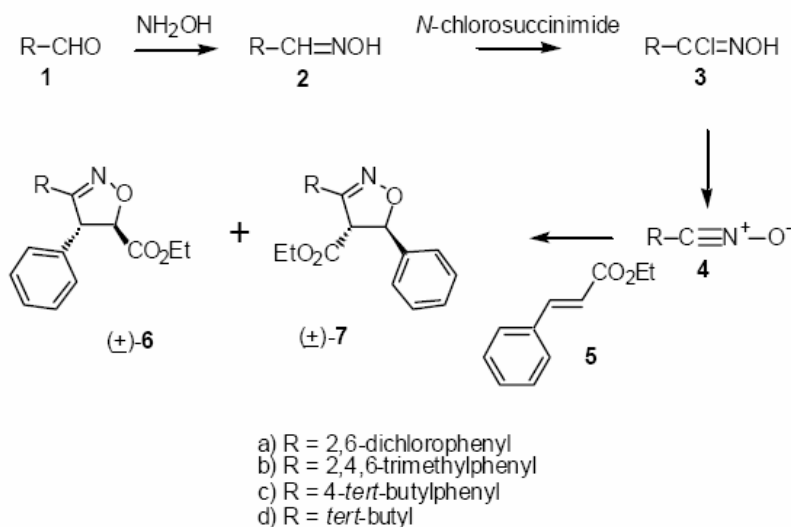
Introduction

In a series of articles published in the early 1990s, Rama Rao *et al.*¹ reported that baker's yeast catalysed 1,3-dipolar cycloaddition reactions of nitrile oxides with cinnamates, vinylpyridines, acrylates and vinylcarbazoles, and that β -cyclodextrin affected the regioselectivity and stereoselectivity of some of these processes. Our preliminary investigations in this area² showed that neither yeast nor β -cyclodextrin significantly affected the cycloadditions, but β -cyclodextrin did have some effect on the isolation of the cycloadducts from the product mixtures. The effects could be duplicated in simple extraction procedures. Accordingly, when a 1:1 mixture of the isoxazolines **6a** and **7a**, or **6b** and **7b**, in ethanol–water (1:4, v/v) containing β -cyclodextrin (0.05 mol dm⁻³) was extracted with organic solvents, samples enriched in either **6a** or **6b** were

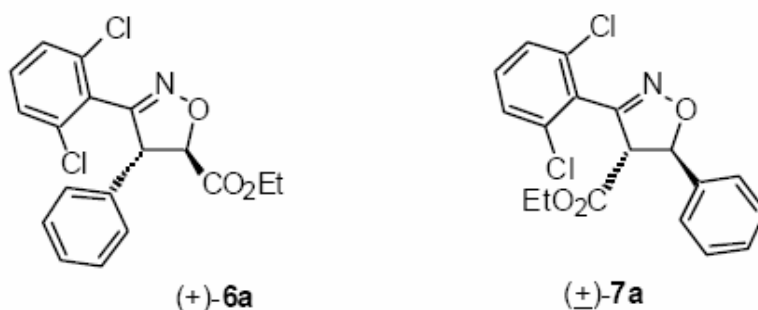
obtained. No enrichment was observed in the absence of a cyclodextrin. To examine these effects in more detail, we have now included the isoxazolines **6c,d** and **7c,d** in our studies. These were chosen because compounds containing the *tert*-butylphenyl moiety are known to form very stable cyclodextrin inclusion complexes, while a *tert*-butyl group associates with β -cyclodextrin only weakly.^{3,4} We have studied the effect of β -cyclodextrin on the extraction of the isoxazolines **6a-d** and **7a-d** from aqueous ethanol into chloroform, and correlated the trends seen in these experiments with the solubility of the isoxazolines **6a-d** and **7a-d** in aqueous ethanol, in the presence and absence of the cyclodextrin.

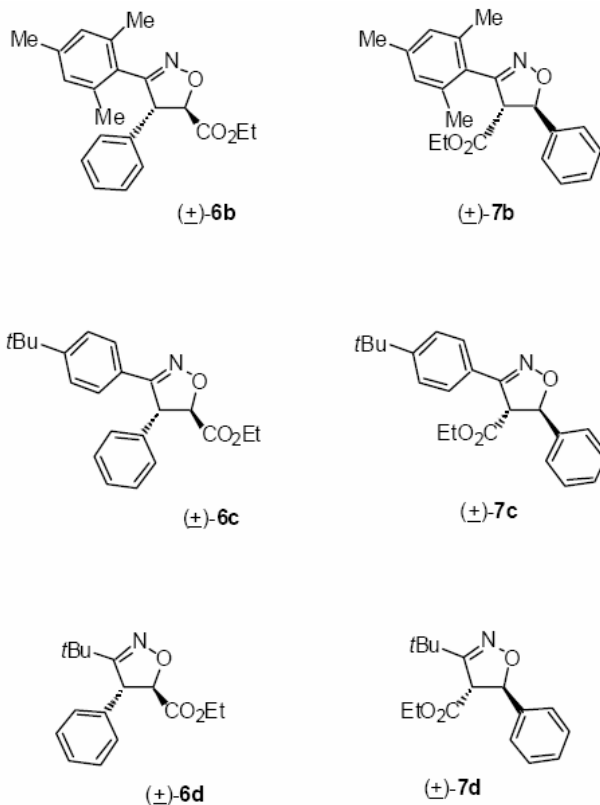
Results and Discussion

The isoxazolines **6a-d** and **7a-d** were prepared as shown in Scheme 1.⁵ The aldehydes **1a-d** were treated with hydroxylamine to give the corresponding oximes **2a-d**, which reacted with *N*-chlorosuccinimide to give the hydroximinoyl chlorides **3a-d**, respectively. The nitrile oxides **4a-d** were prepared *in situ* from the corresponding hydroximinoyl chlorides **3a-d**, through treatment with triethylamine, and they reacted with ethyl *trans*-cinnamate **5** to give the cycloadducts **6a-d** and **7a-d**, respectively, which were separated using flash column chromatography.



Scheme 1





The regioisomers **6a–d** and **7a–d** were distinguished on the basis of their ^1H NMR spectra. As reported previously for similar compounds,⁶ the resonances for the C-4 and C-5 protons of the 5-ethoxycarbonyl-4-phenylisoxazolines **6a–d** are closer in chemical shift ($\Delta\delta < 0.5$ ppm) and show smaller coupling constants ($J < 5.5$ Hz) than those observed for the corresponding 4-ethoxycarbonyl-5-phenylisoxazolines **7a–d** ($\Delta\delta > 1.5$ ppm, $J > 6.0$ Hz). The structure of the isoxazoline **7a** was confirmed through X-ray crystallographic analysis.⁷ Polar effects generally dominate the regioselectivity in reactions of nitrile oxides with 1,2-disubstituted dipolarophiles⁵ and, as expected on this basis, the 4-ethoxycarbonyl-5-phenylisoxazolines **7a**, **7c** and **7d** formed in preference to their regioisomers **6a**, **6c** and **6d**, through selective reaction of the electron-rich oxygen of the nitrile oxides **4a**, **4c** and **4d** at the electron-deficient carbon of the dipolarophile **5**.⁵ By contrast, the isoxazolines **6b** and **7b** formed in approximately equal quantities, presumably as a result of the steric bulk of the mesityl group of the nitrile oxide **4b**, increasing the tendency of the oxygen of the dipole **4b** to add to the least hindered carbon of the dipolarophile **5**.⁵

Solutions of the regioisomeric pairs of the isoxazolines **6a–d** and **7a–d** in ethanol/water (1:4, v/v) containing β -cyclodextrin (0.05 mol dm^{-3}) were extracted with chloroform, and the results are shown in Table 1. The isoxazolines **6d** and **7d** were extracted effectively and without selectivity. By comparison, with the isoxazolines **6a,b** and **7a,b**, a modest selectivity for phase transfer of **6a** over **7a**, and of **6b** over **7b** was observed, while the experiments with **6c** and **7c** showed a more substantial selectivity and the phase transfer of **7c** was significantly retarded. In practical terms it was possible to obtain approximately 50% of each of the isoxazolines **6c** and **7c** in approximately 90% purity, from a single partitioning of a 1:1 mixture.

Table 1. Percentage recovery of the isoxazolines **6a–d** and **7a–d** following extraction into chloroform from ethanol/water (1:4, v/v) solutions containing β -cyclodextrin (0.05 mol dm^{-3}) and equimolar mixtures of pairs of the regioisomers ($0.005 \text{ mol dm}^{-3}$)

Isoxazoline	Number of extractions			
	1	2	3	4
6a	60	85	>95	
7a	45	80	>95	
6b	85	>95		
7b	40	80	>95	
6c	45	80	95	>95
7c	5	20	40	55*
6d	75	95	>95	
7d	75	95	>95	

*>95% recovered through further extraction with ethyl acetate

To explore the origin of these effects, the solubility of the isoxazolines **6a–d** and **7a–d** in ethanol/water (1:4, v/v) was measured, in the presence and absence of β -cyclodextrin (0.05 mol dm^{-3}), and the results are shown in Table 2. At this concentration β -cyclodextrin either increased or had no effect on the solubility of each of the isoxazolines **6a**, **6c**, **6d** and **7a–d** in the aqueous ethanol, while lower concentrations of β -cyclodextrin resulted in similar but proportionately reduced effects. When β -cyclodextrin was added to a solution of the isoxazoline **6b**, the concentration of the isoxazoline **6b** decreased, presumably due to precipitation of the β -cyclodextrin-isoxazoline **6b** complex. Consequently it was not practical to quantify the effect of β -cyclodextrin on the solubility of the isoxazoline **6b**.

Table 2. Solubility of the isoxazolines **6a–d** and **7a–d** in ethanol/water (1:4, v/v) at $30 \text{ }^\circ\text{C}$, in the presence and absence of β -cyclodextrin (0.05 mol dm^{-3})

Isoxazoline	Solubility (mol dm^{-3})	
	No cyclodextrin	With β -cyclodextrin
6a	1.8×10^{-5}	1.8×10^{-5}
7a	1.1×10^{-5}	1.0×10^{-4}
6b	1.7×10^{-5}	–*
7b	2.8×10^{-6}	5.2×10^{-5}
6c	4.9×10^{-6}	1.0×10^{-4}
7c	9.4×10^{-9}	1.1×10^{-5}
6d	2.5×10^{-4}	1.7×10^{-3}
7d	6.7×10^{-4}	4.1×10^{-3}

*Not determined – see text for explanation

The results shown in Tables 1 and 2 are anomalous in that the isoxazoline **7c** which is the least soluble in the aqueous ethanol is least effectively extracted into chloroform, while compounds **6d** and **7d** which are the most soluble in the aqueous ethanol are among those which most readily transfer. Presumably the cause of this anomaly is the cyclodextrin. The effect of the cyclodextrin on the solubility of the isoxazolines **6a**, **6c**, **6d** and **7a–d** indicates the extent to which they are complexed by the cyclodextrin. The data in Table 2 show that of the isoxazolines **6c** and **7c**, the latter is much less soluble in the aqueous ethanol and much more extensively complexed by the cyclodextrin. Consequently, at any time there will be less present uncomplexed in solution and available for extraction into chloroform. It appears that the complexed isoxazolines are not extracted and that dissociation of the isoxazolines from the cyclodextrin complexes is sufficiently slow to retard the extraction process. The selectivity of phase transfer is less with the dichlorophenylisoxazolines **6a** and **7a**, and negligible with the *tert*-butylisoxazolines **6d** and **7d**, decreasing as the solubility in the aqueous ethanol increases and the tendency to be complexed by the cyclodextrin decreases. The partitioning of the isoxazolines **6b** and **7b** is a particular case, where it seems likely that chloroform dissolves the isoxazoline **6b** from the precipitated cyclodextrin-isoxazoline **6b** complex.

Experimental Section

General Procedures. Melting points were determined on a Kofler hot-stage apparatus under a Reichert microscope and are uncorrected. ¹H NMR spectra were recorded on either a Bruker ACP-300, a Varian Gemini 300 or a Varian Mercury 300 spectrometer, as dilute solutions in CDCl₃. Electron impact mass spectra were recorded on either a VG Micromass 7070F or an AEI MS-30 spectrometer, operating at an ionisation potential of 70 eV. Elemental analyses were performed by either the Microanalytical Laboratory, Research School of Chemistry, Australian National University, or Chemical and Microanalytical Services Pty. Ltd., Melbourne, Australia. Chromatography was performed using Merck–Keisegel 60 (230–400 mesh ASTM). The aldehydes **1a–d** and ethyl *trans*-cinnamate **5** were purchased from Aldrich Chemical Co.

General procedure for preparation of the oximes **2a–d** from the corresponding aldehydes **1a–d**

Hydroxylamine hydrochloride (0.45 g, 6.5 mmol) was added to a solution of the aldehyde (5.7 mmol) in water (1.5 mL), ethanol (2 mL) and ice (2.5 mL). Aqueous sodium hydroxide (50%, 1.43 mL) was then added and the mixture was stirred for 1.5 h, then it was washed with ether (1 × 10 mL), acidified (concd HCl) and extracted with ether (2 × 10 mL). The combined extracts were dried (MgSO₄) and concentrated under reduced pressure. The resulting solid was recrystallised from aqueous ethanol.

2,6-Dichlorobenzaldoxime (2a). Reaction of 2,6-dichlorobenzaldehyde **1a** (1.00 g, 5.7 mmol) afforded the title compound **2a** as small white needle-like crystals (0.68 g, 63%); mp 148.8–

149.3 °C; $^1\text{H NMR}$ δ 7.2–7.4 (3H, m, Ar-H), 8.39 (1H, bs, Ar-CH=NOH), 8.80 (1H, s, Ar-CH=NOH). 2,4,6-Trimethylbenzaloxime **2b**. Reaction of 2,4,6-trimethylbenzaldehyde **1b** (2.00 g, 13.5 mmol) afforded the title compound **2b** as small colorless trapezoid crystals (0.61 g, 28%); mp 124–125 °C (lit.⁸ 125–127 °C); $^1\text{H NMR}$ δ 2.30 (3H, s, CH₃), 2.39 [6H, s, (CH₃)₂], 6.90 (2H, s, Ar-H), 8.43 (1H, s, Ar-CH=NOH), 8.90 (1H, bs, CH=NOH).

4-tert-Butylbenzaloxime (2c). Reaction of 4-*tert*-butylbenzaldehyde **1c** (7.76 g, 47.8 mmol) afforded the title compound **2c** as colorless needle-like crystals (4.37 g, 52%); mp 104–106 °C; $^1\text{H NMR}$ δ 1.32 [9H, s, (CH₃)₃], 7.40 (2H, d, J = 6.5 Hz, Ar-H), 7.52 (2H, d, J = 6.5 Hz, Ar-H), 8.14 (1H, bs, CH=NOH), 8.48 (1H, s, Ar-CH=NOH).

Pivaldoxime (2d). Reaction of pivaldehyde **1d** (2.00 g, 23.2 mmol) afforded the title compound **2d** as a pale yellow oil (2.15 g, 92%). $^1\text{H NMR}$ δ 1.11 [9H, s, (CH₃)₃], 8.24 (1H, bs, CH=NOH), 8.48 (1H, s, CH=NOH).

General procedure for preparation of the hydroximinoyl chlorides 3a–d from the corresponding oximes 2a–d

N-Chlorosuccinimide (3.51 g, 26.3 mmol) was added to a stirred solution of the oxime (26.3 mmol) in *N,N*-dimethylformamide (22 mL). The reaction was initially slightly endothermic but soon became exothermic and was kept at 25–35 °C in an ice bath. When the exothermic reaction ceased the solution was poured into ice/water (100 mL) and the mixture was extracted with ether (2 × 50 mL). The combined extracts were washed with water (3 × 50 mL), dried (MgSO₄) and concentrated under reduced pressure. The resulting solid was recrystallised from ether/*n*-hexane.

2,6-Dichlorobenzohydroximinoyl chloride (3a). Reaction of **2a** (5.00 g, 26.3 mmol) afforded the title compound **3a** as large rectangular colorless crystals (4.60 g, 78%); mp 89–91 °C; $^1\text{H NMR}$ δ 7.31–7.41 (3H, m, Ar-H), 8.41 [1H, s, Ar-C(Cl)=NOH].

2,4,6-Trimethylbenzohydroximinoyl chloride (3b). Reaction of **2b** (0.61 g, 3.73 mmol) afforded the title compound **3b** as a pale yellow powder (0.62 g, 84%); mp 49–63 °C; $^1\text{H NMR}$ δ 2.28 [6H, s, (CH₃)₂], 2.41 (3H, s, CH₃), 6.86 (2H, s, Ar-H), 8.31 [1H, s, C(Cl)=NOH].

4-tert-Butylbenzohydroximinoyl chloride (3c). Reaction of **2c** (1.00 g, 5.6 mmol) afforded the title compound **3c** as a pale yellow powder (0.92 g, 77%); mp 69–73 °C; $^1\text{H NMR}$ δ 1.32 [9H, s, (CH₃)₃], 7.43 (2H, d, J = 6.5 Hz, Ar-H), 7.76 (2H, d, J = 6.5 Hz, Ar-H), 8.49 (1H, s, Ar-CH=NOH).

Pivalohydroximinoyl chloride (3d). Reaction of **2d** (1.00 g, 9.9 mmol) afforded the title compound **3d** as a pale yellow oil (0.73 g, 54%); $^1\text{H NMR}$ δ 1.25 [9H, s, (CH₃)₃], 8.44 [1H, s, C(Cl)=NOH].

General procedure for preparation of the isoxazolines 6a–d and 7a–d from the corresponding hydroximinoyl chlorides 3a–d via the nitrile oxides 4a–d

A solution of triethylamine (3.2 ml, 21.5 mmol) and ethyl *trans*-cinnamate **5** (4.77 g, 27.1 mmol) in dry THF (60 mL) was added dropwise with stirring to the hydroximinoyl chloride

(16.3 mmol) in dry THF (30 mL). The mixture was stirred at room T for 1 h, heated to reflux for a further 3 h, then cooled and concentrated under reduced pressure. The residue was taken up in chloroform, and the solution was washed with water (3 × 50 mL), dried (MgSO₄) and concentrated under reduced pressure. Analysis of the residue using ¹H NMR spectroscopy was carried out to determine the ratio of the two isoxazolines present in the product mixture. They were separated by flash chromatography on silica, eluting with ethyl acetate/*n*-hexane (1:5) and each was then recrystallised from ethyl acetate/*n*-hexane.

***trans*-3-(2,6-Dichlorophenyl)-4,5-dihydro-4-phenyl-5-isoxazolecarboxylic acid ethyl ester 6a and *trans*-3-(2,6-dichlorophenyl)-4,5-dihydro-5-phenyl-4-isoxazolecarboxylic acid ethyl ester (7a).** Reaction of **3a** (3.67 g, 16.3 mmol) afforded a mixture of the title compounds **6a** and **7a** in the ratio 15:85.

6a. (0.48 g, 8%); mp 86–87 °C; ¹H NMR δ 1.34 (3H, t, *J* = 7 Hz, CH₃-CH₂-), 4.32 (2H, q, *J* = 7 Hz, CH₃-CH₂-), 5.24 (1H, d, *J* = 5.5 Hz, C5-H), 5.28 (1H, d, *J* = 5.5 Hz, C4H), 7.22–7.46 (8H, m, Ar-H); Anal. Calcd for C₁₈H₁₅Cl₂NO₃: C, 59.36; H, 4.15; N, 3.85. Found: C, 59.39; H, 4.40; N 3.87.

7a. (2.42 g, 41%); mp 85–87 °C; ¹H NMR δ 1.00 (3H, t, *J* = 7 Hz, CH₃-CH₂-), 4.08 (2H, m, CH₃-CH₂-), 4.57 (1H, d, *J* = 9 Hz, C4-H), 6.24 (1H, d, *J* = 9 Hz, C5-H), 7.29–7.49 (8H, m, Ar-H); MS *m/z*: 367, 365, 363 (M⁺, 2%, 8%, 12%), 192 (100), 148, 146 (11, 37). Anal. Calcd for C₁₈H₁₅Cl₂NO₃: C, 59.36; H, 4.15; N, 3.85. Found: C, 59.59; H, 4.20; N, 3.98. The structure was confirmed by X-ray crystallographic analysis⁷.

***trans*-4,5-Dihydro-4-phenyl-3-(2,4,6-trimethylphenyl)-5-isoxazolecarboxylic acid ethyl ester 6b and *trans*-4,5-dihydro-5-phenyl-3-(2,4,6-trimethylphenyl)-4-isoxazolecarboxylic acid ethyl ester (7b).** Reaction of **3b** (6.00 g, 30.3 mmol) afforded a mixture of the title compounds **6b** and **7b** in the ratio 50:50.

6b. (2.99 g, 29%); mp 83–85 °C; ¹H NMR δ 1.35 (3H, t, *J* = 7 Hz, CH₃-CH₂-), 1.98 (3H, s, Ar-CH₃), 2.25 [6H, s, Ar-(CH₃)₂], 4.33 (2H, q, *J* = 7 Hz, CH₃-CH₂-), 4.82 (1H, d, *J* = 4 Hz, C5-H), 5.33 (1H, d, *J* = 4 Hz, C4-H), 7.14–7.28 (7H, m, Ar-H); MS *m/z*: 337 (M⁺, 7%), 264 (M⁺-CO₂CH₂CH₃, 100), 161 (70). Anal. Calcd for C₂₁H₂₃NO₃: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.35; H, 6.66; N, 3.74.

7b. (5.02 g, 49%); mp 85–87 °C; ¹H NMR δ 0.93 (3H, t, *J* = 7 Hz, CH₃-CH₂-), 2.22 [6H, s, Ar-(CH₃)₂], 2.28 (3H, s, Ar-CH₃), 4.00 (2H, m, CH₃-CH₂-), 4.37 (1H, d, *J* = 9.5 Hz, C4-H), 6.11 (1H, d, *J* = 9.5 Hz, C5-H), 7.36–7.47 (7H, m, Ar-H); MS *m/z*: 337 (M⁺, 100%), 192 (58), 186 (67), 104 (96). Anal. Calcd for C₂₁H₂₃NO₃: C, 74.75; H, 6.87; N, 4.15. Found: C, 74.88; H, 6.90; N, 4.08.

***trans*-3-(4-*tert*-Butylphenyl)-4,5-dihydro-4-phenyl-5-isoxazolecarboxylic acid ethyl ester 6c and *trans*-3-(4-*tert*-butylphenyl)-4,5-dihydro-5-phenyl-4-isoxazolecarboxylic acid ethyl ester 7c.** Reaction of **3c** (1.00 g, 4.7 mmol) afforded a mixture of the title compounds **6c** and **7c** in the ratio 27:73.

6c. (0.18 g, 11%); mp 94–96 °C; ¹H NMR δ 1.26 [9H, s, Ar-(CH₃)₃] 1.30 (3H, t, *J* = 7 Hz, CH₃-CH₂-), 4.27 (2H, q, *J* = 7 Hz, CH₃-CH₂-), 4.93 (1H, d, *J* = 4 Hz, C5-H), 5.01 (1H, d, *J* = 4 Hz,

C4-H), 7.26–7.35 (7H, m, Ar-H), 7.56 (2H, d, $J = 8.5$ Hz, Ar-H); MS m/z : 351 (M^+ , 32%), 278 (100), 114 (30), 91 (44). Anal. Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 75.41; H, 6.92; N, 3.97.

7c (0.78 g, 47%); mp 77–79 °C; 1H NMR δ 1.24 (3H, t, $J = 7$ Hz, CH_3-CH_2-), 1.32 [9H, s, Ar-(CH_3)₃], 4.24 (2H, q, $J = 7$ Hz, CH_3-CH_2-), 4.42 (1H, d, $J = 6$ Hz, C4-H), 5.96 (1H, d, $J = 6$ Hz, C5-H), 7.35–7.39 (7H, m, Ar-H), 7.67 (2H, d, $J = 8.5$ Hz, Ar-H); MS m/z : 351 (M^+ , 46%), 336 (M^+-CH_3 , 39), 192 (48), 160 (36), 146 (58), 105 (100), 77 (43). Anal. Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 75.28; H, 7.45; N, 3.85.

trans-3-tert-Butyl-4,5-dihydro-4-phenyl-5-isoxazolecarboxylic acid ethyl ester 6d and trans-3-tert-butyl-4,5-dihydro-5-phenyl-4-isoxazolecarboxylic acid ethyl ester 7d. Reaction of **3d** (0.88 g, 6.49 mmol) afforded a mixture of the title compounds **6d** and **7d** in the ratio 26:74.

6d (0.15 g, 8%); viscous yellow oil; 1H NMR δ 1.06 [9H, s, (CH_3)₃-], 1.25 (3H, t, $J = 7$ Hz, CH_3-CH_2-), 4.27 (2H, q, $J = 7$ Hz, CH_3-CH_2-), 4.55 (1H, d, $J = 3$ Hz, C5-H), 4.77 (1H, d, $J = 3$ Hz, C4-H), 7.26–7.39 (5H, m, Ar-H); MS m/z : 202 ($M^+-CO_2CH_2CH_3$, 25%), 57 [(CH_3)₃, 100]. Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.70; H, 7.61; N, 4.85.

7d (0.64 g, 36%); mp 45–46 °C; 1H NMR δ 1.24 [9H, s, (CH_3)₃] 1.33 (3H, t, $J = 7$ Hz, CH_3-CH_2-), 3.99 (1H, d, $J = 6.5$ Hz, C5-H), 4.29 (2H, q, $J = 7$ Hz, CH_3-CH_2-), 5.76 (1H, d, $J = 6.5$ Hz, C4-H), 7.28–7.41 (5H, m, Ar-H); MS m/z : 202 ($M^+-CO_2CH_2CH_3$, 15%), 192 (68), 146 (44), 105 (100), 57 [(CH_3)₃, 74]. Anal. Calcd for $C_{16}H_{21}NO_3$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.66; H, 7.72; N, 5.01.

General extraction procedure

A solution of an approximately equimolar mixture of a regioisomeric pair of the isoxazolines **6a–d** (0.5 mmol) and **7a–d** (0.5 mmol) in ethanol/water (1:4, v/v, 100 mL) containing β -cyclodextrin (5 mmol) was shaken vigorously with chloroform (100 mL) for 5 s, then the organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was weighed and analysed using 1H NMR spectroscopy. The results are shown in Table 1.

Determination of the solubility of the isoxazolines **6a–d** and **7a–d**

A solution of an approximately equimolar mixture of a regioisomeric pair of the isoxazolines **6a–d** (0.5 mmol) and **7a–d** (0.5 mmol) in ethanol (12.5 mL) was added dropwise and with vigorous stirring to a mixture of ethanol (12.5 mL) and water (75 mL), equilibrated at 30 °C. The resultant suspension was left to stand at 30 °C for 24 h, then it was filtered twice through 0.2 μm syringe filters (Sartorius Ministart[®]) and once through a 0.02 μm syringe filter (Whatman Anotop 25 Plus[®]). The filtrate was extracted with chloroform (3 \times 50 mL) and the combined extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dissolved in acetonitrile (50 μL) and analysed by HPLC [Waters Symmetry[®] C18 3.5 μm (75 mm \times 4.6 mm) column, eluting with acetonitrile/water (7:3, v/v)], using one of the other isoxazolines **6a–d** and **7a–d** as a standard. A similar procedure was used to determine solubility in the presence of β -cyclodextrin (5 mmol), which was added to the aqueous ethanol before adding the solution of the

isoxazolines **6a-d** (1.0 mmol) and **7a-d** (1.0 mmol) in ethanol. However, in these experiments the filtrates were analysed directly by HPLC, without extraction into chloroform. The results are shown in Table 2.

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