

Reversal of Regioselectivity and Enhancement of Rates of Nitrile Oxide Cycloadditions through Transient Attachment of Dipolarophiles to Cyclodextrins

Lorna Barr,^[a] Stephen F. Lincoln,^[b] and Christopher J. Easton*^[a]

Abstract: The reactions of nitrile oxides with monosubstituted dipolarophiles, such as propiolamide, typically afford proportionally 80% or more of the 3,5-disubstituted cycloadducts. By contrast, the reactions of 6^A-deoxy-6^A-propynamido- β -cyclodextrin with 4-*tert*-butylbenzoxirane and 4-phenylbenzoxirane afford >90% and approximately 85% of the corresponding 3,4-disubstituted isoxazoles, respectively. As well as reversing the regioselectivity, the cyclodextrin increases the rates of these cycloadditions. The extent of the acceleration is up to more than three orders of magnitude for the production of the cycloadd-

uct preferred by the cyclodextrin, but even the rate of reaction to give the less favored regioisomer is increased. With 6^A-deoxy-6^A-propynamido- β -cyclodextrin, the cycloadducts are not easily separated from the cyclodextrin, as the amide bond is not readily cleaved. In comparison, the regioselectivity of the cycloadditions of 4-*tert*-butylbenzoxirane with acrylic acid, methacrylic acid, and crotonic acid is also altered by formation of the corre-

sponding cyclodextrin esters, by factors of 500, >10, and >100, respectively. The rates of cycloaddition are also increased by up to 475 times, and in these cases the products of cycloaddition are readily released from the cyclodextrin through ester hydrolysis. Incorporating these processes into a reaction cycle, acylation of β -cyclodextrin with *p*-nitrophenyl acrylate and subsequent treatment first with 4-*tert*-butylbenzoxirane and then with base, the latter to catalyze ester hydrolysis and regenerate the β -cyclodextrin, affords proportionally fivefold more of the 3,4-disubstituted isoxazoline than is produced directly from acrylic acid.

Keywords: cycloaddition • cyclodextrins • inclusion compounds • molecular reactors • regioselectivity

Introduction

Enzymes are nature's catalysts, and they act by binding substrates and exploiting functional groups in their active sites to facilitate reactions of the bound species. The development of artificial enzymes based on macromolecular hosts containing appropriate functional groups is a dynamic area of research.^[1–4] The aim is not only to mimic enzymes but also to produce new catalysts or molecular reactors to affect the outcomes of different classes of chemical processes. As examples, systems have been constructed to accelerate^[5–13]

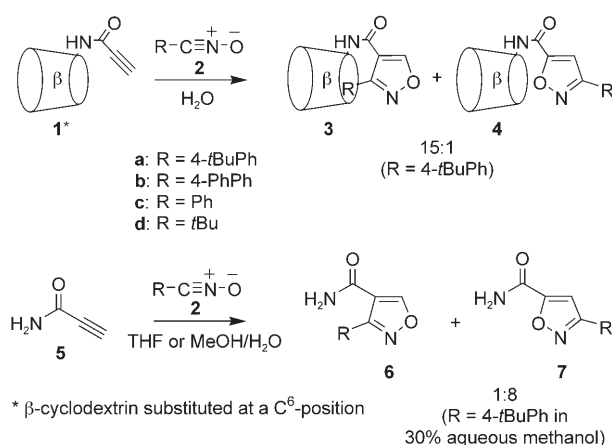
and catalyze reactions,^[14–35] as well as to control their regio-^[5,14,30,36] and stereochemistry.^[6,7,26,27,29,32,34,35,37–39] In one case, a metalloporphyrin-derived supramolecular assembly has been reported to simultaneously complex manganese-porphyrin oxidation catalysts and substrates to be oxidized.^[15] Complexation of the catalysts was found to increase their turnover numbers by up to 100-fold. Sanders and co-workers have also exploited metalloporphyrin cyclic oligomers as artificial enzymes, to accelerate Diels–Alder reactions by several orders of magnitude^[6–10] and manipulate the ratio of formation of *endo* and *exo* cycloadducts.^[6,7] Although large rate accelerations were observed, substrate turnover was negligible because the reaction products had a strong affinity for the host species. This is a common limitation with artificial enzymes, and achieving turnover continues to be one of the major challenges for developing such systems.^[40]

Cyclodextrins have been used extensively as molecular scaffolds^[41–46] for the construction of artificial enzymes and molecular reactors. Derivatives incorporating a wide range of functional groups have been prepared and used to affect transformations of molecules encapsulated within the modi-

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fied hosts, including allylations,^[47–49] Diels–Alder cycloadditions,^[50–52] oxidations,^[53,54] hydrolyses,^[55–61] enolizations,^[62,63] reductions,^[64,65] aldol condensations,^[66] indigoid dye syntheses,^[67,68] aromatic substitutions,^[69–71] and aliphatic hydroxylations.^[72–77] Their scope is indicated by the activity of a ditelluride β -cyclodextrin dimer as a glutathione peroxidase mimic, which catalyzes the reduction of cumene peroxide by an aryl thiol 200000 times more effectively than diphenyl diselenide.^[64] We have used cyclodextrins to reverse the regioselectivity of nitrile oxide cycloadditions (Scheme 1), thus



Scheme 1. Effect of β -cyclodextrin on the regioselectivity of reactions of propiolamide (**5**) with nitrile oxides.

changing the ratio of formation of the regioisomeric cycloadducts by factors of up to 120; from 1:8 for the isoxazoles **6a** and **7a** to 15:1 for their analogous cyclodextrin derivatives **3a** and **4a**.^[78] We have now also studied the effect of the cyclodextrins on the rates of these cycloadditions and found that they are accelerated by up to more than three orders of magnitude. The cycloadducts are not easily separated from the cyclodextrin and there is no turnover with this system, as the amides linking the cycloadducts to the cyclodextrin resist hydrolysis. To avoid these limitations, we have investigated a variety of alternative approaches for transiently linking dipolarophiles to the cyclodextrin. We now report that with acrylates the cyclodextrin changes the ratio of formation of the cycloadducts by up to at least 500-fold, and accelerates the rates of cycloaddition by up to 475 times. Furthermore, the products are readily released from the cyclodextrin through ester hydrolysis. Consequently, the cyclodextrin can be exploited in a cyclic manner.

Results and Discussion

The reactions of 4-*tert*-butylbenzotrile oxide (**2a**), 4-phenylbenzotrile oxide (**2b**), and benzotrile oxide (**2c**) with 6^A-deoxy-6^A-propylamido- β -cyclodextrin (**1**) in aqueous solution proceeded as reported previously^[78] to give the cycloadducts **3a–c** and **4a–c**. A similar reaction of *tert*-butylnitrile

oxide (**2d**) afforded only the isoxazole **4d**, within the limits of detection based on analysis of the crude product using ¹H NMR spectroscopy. Earlier, the reaction of the cyclodextrin derivative **1** with the nitrile oxide **2a** was compared with that of methyl propiolate but, as a closer comparison, propiolamide (**5**) was used in the present study. It reacted with the nitrile oxides **2a–d** in THF to give the regioisomeric pairs of products **6a–d** and **7a–d**. The ratios of formation of the isoxazoles **3a–d:4a–d** and **6a–d:7a–d** are summarized in Table 1. The ratio of formation of the *tert*-butylphenyl-substituted isoxazoles **6a** and **7a** was also investigated in 30% aqueous methanol, although this solvent was unsuitable for preparative work due to the low solubility of the hydroximinoyl chlorides that were used for the in situ preparation of the nitrile oxides **2a–d**. Water was used as the solvent in the above and other experiments involving cyclodextrins, as organic solvents are known to adversely affect the formation of cyclodextrin inclusion complexes.^[44] Comparative experiments that did not involve cyclodextrins required organic solvents to dissolve the substrates, but in those cases similar results were obtained in THF and aqueous methanol solutions, the latter including various water/methanol ratios, indicating that the solvent has little effect on these processes.

Table 1. Ratios of formation of the isoxazoles **3a–d:4a–d** and **6a–d:7a–d**.

Nitrile oxide	Isoxazole ratio	
	3:4	6:7
2a	15:1 ^[a]	1:4 ^[b] 1:8 ^[c]
2b	5:1 ^[a]	1:6 ^[b]
2c	1:2 ^[a]	1:5 ^[b]
2d	< 1:100 ^[a,d]	1:30 ^[b]

[a] In aqueous solution at 25°C. [b] In THF at 25°C. [c] In 30% aqueous methanol at 25°C. [d] None of the isoxazole **3d** was produced, within the limits of detection using ¹H NMR spectroscopy.

The reactions of the nitrile oxides **2a–d** with propiolamide (**5**) gave proportionally 80% or more of the 3,5-disubstituted isoxazoles **7a–d**. By contrast, with the added cyclodextrin moiety, the alkyne **1** reacts with the nitrile oxide **2a** to afford more than 90% of the 3,4-disubstituted isoxazole **3a**, the opposite regioisomer. This reversal of regioselectivity is also pronounced, but less markedly so, with the nitrile oxide **2b**, which gives approximately 85% of the 3,4-disubstituted isoxazole **3b**. The effect of the cyclodextrin on these reactions is consistent with inclusion of the nitrile oxides **2a** and **2b** within the annulus of the macrocycle **1**, to control the alignment for cycloaddition. There is much less effect on the reactions of the nitrile oxides **2c** and **2d**, presumably because these species do not readily form cyclodextrin inclusion complexes.

The effect of the cyclodextrin on the rates of the cycloadditions was examined in a series of competitive experiments, involving treatment of each of the nitrile oxides **2a–c** generally with a mixture of a 20-fold excess of propiolamide (**5**) and a twofold excess of the cyclodextrin derivative **1**, at var-

ious absolute concentrations in aqueous solution. As the cyclodextrin favors the formation of 3,4-disubstituted isoxazoles, the reactions of the dipoles **2a–c** were analyzed by ¹H NMR spectroscopy for the ratios of formation of the cycloadducts **3a–c** and **6a–c**. These values were adjusted to allow for the relative concentrations of the dipolarophiles **1** and **5** in order to obtain values for the rate enhancements induced by the cyclodextrin substituent. The results of these experiments are shown in Table 2. With the nitrile oxide **2d**, none of the isoxazole **3d** was detected, so it was not possible to measure the ratios of formation of the isoxazoles **3d** and **6d**.

Table 2. Results of competitive reactions of the nitrile oxides **2a–c** with the alkynes **1** and **5**.^[a]

Expt.	Reactant concentration [mM]					Product ratio 3:6	Rate enhancement ^[b]
	Dipolarophile 1	5	2a	Dipole 2b	2c		
1	50	500	25			5:1	50
2	10	100	5			60:1	600
3	2	20	1			>100:1 ^[c]	>1000
4	2	100	1			30:1	1500
5	2	20		1		53:1	530
6	2	20			1	2.5:1	25

[a] In aqueous solution at 25 °C. [b] Rate enhancement induced by the cyclodextrin moiety of the alkyne **1**, as calculated from the product ratio by allowing for the relative concentrations of the dipolarophiles **1** and **5**. [c] None of the isoxazole **6a** was produced, within the limits of detection using ¹H NMR spectroscopy.

Under the conditions used, the cyclodextrin of the alkyne **1** increases the rate of formation of the 3,4-disubstituted isoxazoles **3a–c** versus **6a–c** by up to 1500 times. The magnitude of the rate enhancement depends on the concentrations of the reagents, being larger in more dilute solutions (Table 2, experiments 1–4). This finding is consistent with the reactions of the cyclodextrin derivative **1** involving formation of inclusion complexes with the nitrile oxides **2a–c** prior to cycloaddition. They therefore show less dependence on reagent concentration than the reactions of propiolamide (**5**), which are presumably second-order processes that depend directly on the concentrations of both the nitrile oxides **2a–c** and the dipolarophile **5**. The nature of the nitrile oxides **2a–c** also affects the extent of the rate increase (experiments 3, 5, and 6), which is greatest for 4-*tert*-butylbenzonitrile oxide (**2a**) and 4-phenylbenzonitrile oxide (**2b**). This corresponds with the larger effect of the cyclodextrin on the reversal of regioselectivity of the cycloadditions of these two species, and is likewise consistent with the expectation that they form more thermodynamically stable cyclodextrin inclusion complexes.

It is not practical to determine the relative stabilities of the inclusion complexes formed between the cyclodextrin derivative **1** and the nitrile oxides **2a–c**, given that the contributing species react with each other. Instead, these were assessed through competitive experiments, involving treatment of the cyclodextrin derivative **1** with an excess of mixtures of the nitrile oxides **2a–c** in 5% aqueous methanol (Table 3). The selectivity of formation of the isoxazoles **3a** > **3b** > **3c** illustrates the order of reactivity of the nitrile oxides

2a > **2b** > **2c**, which corresponds with the extents of the reversal of regioselectivity (Table 1) and rate enhancements (Table 2) seen in their reactions with the alkynes **1** and **5**, and is likely to parallel the association constants of the corresponding inclusion complexes.

In principle, there are alternative explanations for the results illustrated in Tables 2 and 3. The reactions of the nitrile oxides **2a–c** with propiolamide (**5**) could be affected by the presence of the cyclodextrin derivative **1** or vice versa. For example, inclusion of **2a–c** in the annulus of **1** could retard their reactions with **5**, rather than enhancing their rates of reaction with the former dipolarophile. To assess this possibility,

the kinetics of the reactions of the nitrile oxide **2a** (1 mM) with either **5** (2 mM) or **1** (2 mM) to give the isoxazoles **6a** and **7a** or **3a** and **4a** were investigated by HPLC. The reactions of the cyclodextrin **1** were studied in water at 25 °C, while 30% aqueous methanol was used with **5** to maintain the nitrile oxide **2a** and its hydroximinoyl chloride precursor in solution in the absence of a cyclodextrin. Under these conditions, the reactions are pseudo-

first-order with respect to the nitrile oxide **2a** as the limiting reagent, particularly in the initial phases. The pseudo-first-order rate constants determined by using this approach are $>9.3 \times 10^{-3}$, $>6.3 \times 10^{-4}$, 5.0×10^{-6} , and $3.8 \times 10^{-5} \text{ s}^{-1}$ for the formation of the isoxazoles **3a**, **4a**, **6a**, and **7a**, respectively.

It follows that the reaction of the cyclodextrin derivative **1** to give the 3,4-disubstituted isoxazole **3a** is at least 1860 times faster than that of the alkyne **5**, which lacks the cyclodextrin substituent, to give the corresponding cycloadduct **6a**. This result is in good agreement with that of experiment 4 in Table 2, carried out under similar conditions, and confirms that the templating effect of the cyclodextrin moiety corresponds to rate enhancement. Furthermore, even the reaction to give the 3,5-disubstituted isoxazole **4a**, which is the less favored of the cycloadditions for the cyclodextrin derivative **1**, is still more than 15 times faster than that of propiolamide (**5**) to give the corresponding regioiso-

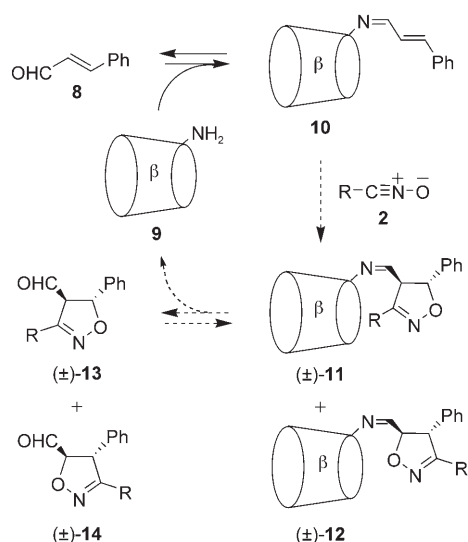
Table 3. Results of competitive reactions of the cyclodextrin derivative **1** with the nitrile oxides **2a–c**.^[a]

Expt.	Reactant concentration [μM]				Product ratio 3a:3b/3a:3c	Selectivity ^[b]
	1	2a	2b	2c		
7	25	50	100	–	40:1	80:1 (2a:2b)
8	25	50	–	100	>100:1 ^[c]	>200:1 (2a:2c)
9	25	50	–	500	75:1	750:1 (2a:2c)

[a] In 5% aqueous methanol at 25 °C. [b] Selectivity for reaction of the nitrile oxide **2a**, as calculated from the product ratio by allowing for the relative concentrations of the nitrile oxides **2a–c**. [c] None of the isoxazole **3c** was produced, within the limits of detection using ¹H NMR spectroscopy.

meric isoxazole **7a**. Presumably, the rate accelerations are entropic in origin, and result from the preorganization of the nitrile oxide **2a** and the dipolarophile **1** within the host-guest complex. However, a more detailed analysis of the basis of the effects would require a more thorough kinetic analysis at various temperatures and, even then, it would probably be difficult to distinguish the enthalpic and entropic contributions to the cycloaddition from those of the complexation.

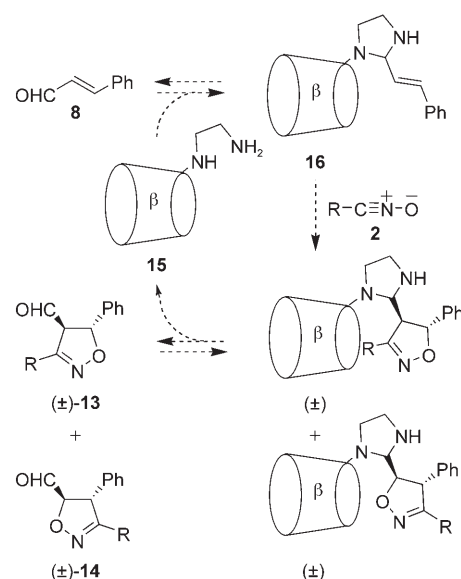
The reactions described above are of limited synthetic utility, as the amide bond attaching the cyclodextrin in the isoxazoles **3a-d** and **4a-d** is not readily cleaved to release the heterocycles. Therefore, a more transient link was required. Several alternatives were considered. Initially the concept of using imine tethers was explored by investigating reactions of the Schiff base 6^A-(1-aza-4-phenylbuta-1,3-dienyl)-6^A-deoxy-β-cyclodextrin (**10**), generated from 6^A-amino-6^A-deoxy-β-cyclodextrin (**9**)^[79] and cinnamaldehyde (**8**, Scheme 2). It was anticipated that the imine **10** would



Scheme 2. Proposed temporary attachment of a dipolarophile to a cyclodextrin as an imine to affect cycloadditions.

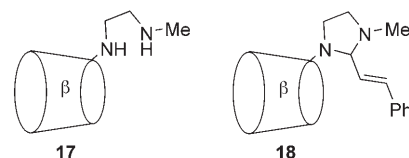
form reversibly and that, with the cyclodextrin attached, it might be more reactive than the free aldehyde **8**. Then hydrolysis of the imines **11** and **12** would release the cycloadducts **13** and **14** and regenerate the aminocyclodextrin **9**. Instead, in the event, the nitrile oxides **2a** and **2c** reacted with the α,β-unsaturated imine **10** by cycloaddition to the imine functionality rather than the alkene moiety. Analogous regioselectivity has been observed in reactions of nitrile oxides with the Schiff base of cinnamaldehyde (**8**) and aniline.^[80] Consequently, the cycloaddition destroys the imine and the cycloadducts are not released, as required for turnover of the cyclodextrin **9**.

A second hypothesis was to use a diazacyclopentane moiety as the transient tether between the dipolarophile and the cyclodextrin (Scheme 3). Accordingly, it was antici-



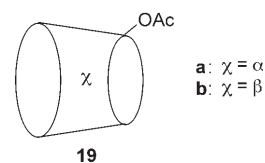
Scheme 3. Planned transient bonding of a dipolarophile to a cyclodextrin via a diazacyclopentane to affect cycloadditions.

ated that reaction of cinnamaldehyde (**8**) with 6^A-(2-aminoethylamino)-6^A-deoxy-β-cyclodextrin (**15**)^[81] would give the diazacyclopentane **16**. Subsequent cycloaddition followed by hydrolysis would then produce the free cycloadducts **13** and **14** and regenerate the cyclodextrin **15**. Model studies with cinnamaldehyde (**8**), 1,2-diaminoethane, and *N,N*-dimethyl-1,2-diaminoethane established that it was necessary to use the secondary diamine 6^A-(2-methylaminoethylamino)-6^A-deoxy-β-cyclodextrin (**17**) to generate the diazacyclopentane



18, otherwise the reaction stopped at the intermediate α,β-unsaturated imine. Even so, the diazacyclopentane **18** was found to be in equilibrium with the amine **17** and the aldehyde **8**, and cinnamaldehyde (**8**) is many orders of magnitude more reactive than the diazacyclopentane **18** toward nitrile oxide cycloaddition. Thus the diazacyclopentane unit was also shown to be unsuitable for temporarily attaching dipolarophiles to cyclodextrins to manipulate cycloadditions.

The third alternative approach, which was therefore considered, was to use cyclodextrin esters. In earlier studies,^[82] we had observed that the cyclodextrin esters **19a** and **19b**, prepared by acylation of α- and β-cyclodextrin, readily undergo hydrolysis at 37°C in 0.1 M car-

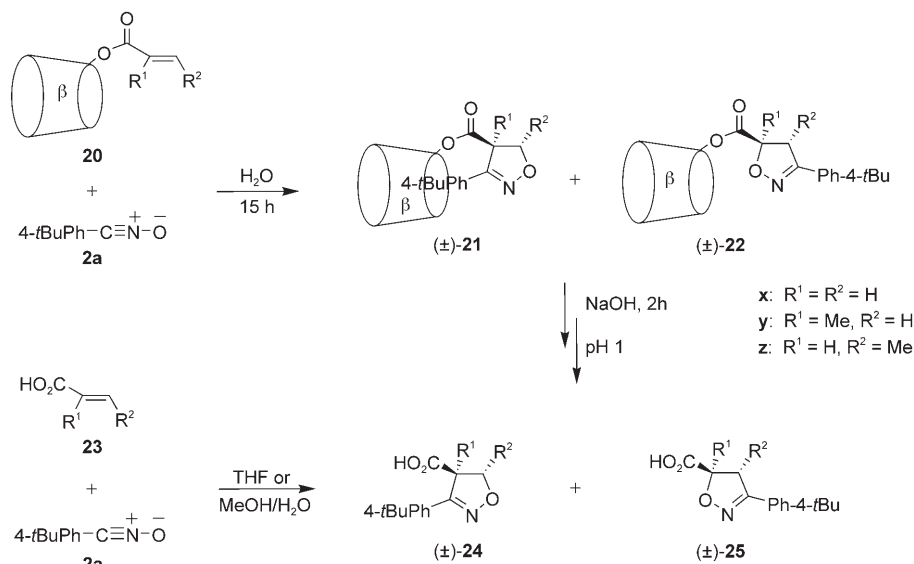


bonate buffer, with half-lives of 9.4, 3.6, 1.2, and 0.54, and 9.6, 3.4, 1.2, and 0.48 h, at pH 9.5, 10.0, 10.5, and 11.0, respectively. Thus it seemed plausible that acylation of a cyclodextrin with a dipolarophile, followed by accelerated cycloaddition of a nitrile oxide to the cyclodextrin derivative and then hydrolysis of the product esters, would produce regioisomeric cycloadducts in ratios affected by the temporary association with the cyclodextrin. In addition, the cyclodextrin would be regenerated for further reaction.

Key to this proposal is that the dipolarophile when attached to a cyclodextrin must react differently than when free. To investigate this, the cyclodextrin esters **20x–z** were prepared through reaction of 6^A-*O*-toluenesulfonyl- β -cyclodextrin^[83] with the cesium salts of the appropriate acids **23x–z**. Attempts to obtain the corresponding propiolate using this method resulted only in the formation of β -cyclodextrin. The esters **20x–z** were each treated with 4-*tert*-butylbenzoxazole nitrile oxide (**2a**) in aqueous solution at 25 °C, to give mixtures of the cycloadducts **21x–z** and **22x–z** (Scheme 4). Without purification, these adducts were hydrolyzed by stirring with 0.5 M aqueous sodium hydroxide to afford, after

zoles in the ratio 1:25, which is similar to the ratio of 1:20 for the cycloadducts **24x** and **25x** produced using acrylic acid (**23x**). Therefore, the differences in the ratios of the isoxazolines **24x–z** and **25x–z** produced either directly from the acids **23x–z** or via the cyclodextrin derivatives **20x–z** are not simply accounted for as being due to esterification of the dipolarophiles. Instead they are consistent with inclusion of the nitrile oxide **2a** within the annulus of the cyclodextrins **20x–z** affecting the regioselectivity of the cycloadditions, as described above for the propiolamide **1**. The effect of the cyclodextrin moiety of the ester **20x** changes the ratios of production of the regioisomers **24x** and **25x** by a factor of at least 500, to proportionally more than 95% of the 3,4-disubstituted isoxazoline **24x**, from more than 95% of the other regioisomer **25x** with acrylic acid (**23x**). This reversal of selectivity is of greater magnitude than that seen in the reactions of the nitrile oxide **2a** with the propiolamides **1** and **5**. The cyclodextrin group of the esters **23y** and **23z** also changes the ratios of formation of the regioisomers **24y** and **25y**, and **24z** and **25z**, by factors of at least 10 and 100, respectively.

As well as reversing the regioselectivity, the cyclodextrin increases the rate of reaction of the nitrile oxide **2a** with the ester **20x**. From a competitive experiment using 4-*tert*-butylbenzoxazole nitrile oxide (**2a**; 1 mM), the cyclodextrin acrylate **20x** (2 mM), and acrylic acid (**23x**; 20 mM), and after treatment to allow for ester hydrolysis, the cycloadducts **24x** and **25x** were isolated in a ratio of 2:1. As the cycloadducts **24x** and **25x** are derived primarily from the dipolarophiles **20x** and **23x**, respectively, and allowing for the relative concentrations of the dipolarophiles **20x** and **23x** used, it follows that the former is approximately 20 times more reactive under these conditions. Taking into account the ability



Scheme 4. Reactions of the acids **23x–z** and the corresponding cyclodextrin derivatives **20x–z** with the nitrile oxide **2a**.

acidification, the corresponding acids **24x–z** and **25x–z**. The acids **23x–z** also reacted directly with the nitrile oxide **2a** to give the cycloadducts **24x–z** and **25x–z**. The ratios of formation of the isoxazolines **24x–z** and **25x–z** are shown in Table 4.

The ratios of the isoxazolines **24x–z** and **25x–z** formed directly from the acids **23x–z** depend on the nature of the dipolarophiles. The pattern is as expected^[84] for reactions of nitrile oxides with acrylic acid (**23x**) and the steric effects of methyl substituents in the cases of the acids **23y** and **23z**. In THF, the reaction of the nitrile oxide **2a** with methyl acrylate gave the corresponding 3,4- and 3,5-disubstituted isoxa-

Table 4. Ratios of formation of the isoxazolines **24x–z** and **25x–z**.

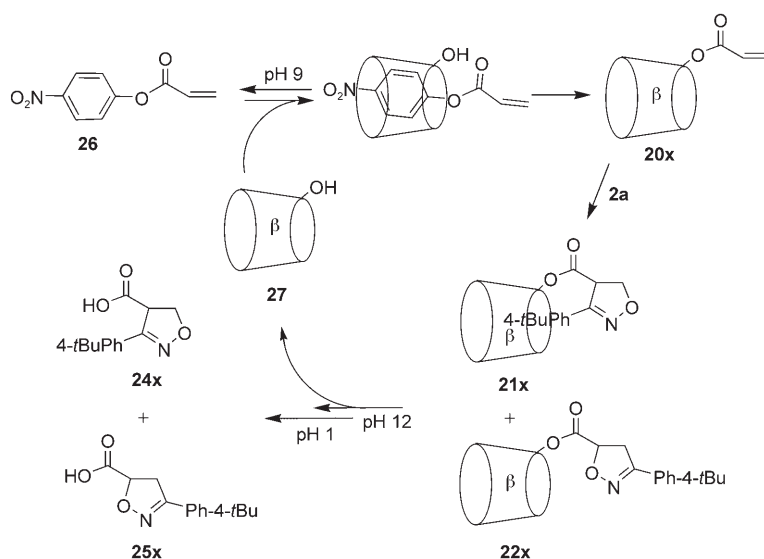
	Dipolarophile	
	20	23
24x:25x	20:1 ^[a]	1:20 ^[b] 1:25 ^[c]
24y:25y	1:10 ^[a]	< 1:100 ^[b,d]
24z:25z	> 100:1 ^[a,e]	1:1 ^[b]

[a] In aqueous solution at 25 °C. [b] In THF at 25 °C. [c] In 50% aqueous methanol at 25 °C. [d] None of the isoxazoline **24y** was produced, within the limits of detection using ¹H NMR spectroscopy. [e] None of the isoxazoline **25z** was produced, within the limits of detection using ¹H NMR spectroscopy.

of the cyclodextrin to increase the proportion of the 3,4-disubstituted isoxazole **24x** formed to 95% from less than 4% in its production directly from acrylic acid (**23x**), this corresponds to a 475-fold rate enhancement for the reaction to give the regioisomer favored by the cyclodextrin.

The above reactions demonstrate that cyclodextrin esters can be used to reverse the regioselectivity of nitrile oxide cycloadditions and that the products are readily released from the cyclodextrin through ester hydrolysis, so the cyclodextrin could, in principle, be recycled. We also attempted to incorporate these features more directly into a reaction cycle, albeit with only modest success (Scheme 5). β -Cyclodextrin (**27**; 2.7 mM) was treated with *p*-nitrophenyl acrylate

Presumably the effectiveness of this reaction cycle to alter the regioselectivity of the cycloadditions is limited by competing reactions of the acrylate **26** and the *meta* isomer, as well as the product of their hydrolysis, acrylic acid (**23x**), with the nitrile oxide **2a**. In any event, the reactions of the esters **20x–z** demonstrate that dipolarophiles can be esterified with β -cyclodextrin (**27**) before nitrile oxide cycloadditions followed by base-catalyzed hydrolyses to produce cycloadducts free of the cyclodextrin. This effectively reverses the regioselectivity and substantially enhances the rates of the cycloadditions, thus providing ready access to 4-substituted isoxazolines with various potential applications in chemistry^[84,86,87] and pharmacy.^[88–90]



Scheme 5. Temporary acylation of β -cyclodextrin (**27**) to affect cycloaddition of the nitrile oxide **2a**.

(**26**; 0.45 mM) in phosphate buffer (0.05 M, pH 9.0) at 25 °C for 3 h, under which conditions it was anticipated, based on the work of Bender et al.^[55,56] and Kassara et al.,^[85] that transesterification of a cyclodextrin primary hydroxyl group would occur to give the ester **20x**. 4-*tert*-Butylbenzohydroximinoyl chloride (0.31 mM) was then added as the precursor of the nitrile oxide **2a**, and the mixture was left to stand for 16 h for cycloaddition to occur. It was then adjusted to pH 12.0 and left for 2 h to allow hydrolysis of the cycloadducts **21x** and **22x** and regeneration of the β -cyclodextrin (**27**), before acidification gave the acids **24x** and **25x** in the ratio 1:3. This compares with a ratio of 1:20 from acrylic acid (**23x**), showing that although the major product is still the 3,5-disubstituted isoxazoline **25x**, the proportion of the 3,4-disubstituted regioisomer **24x** is increased approximately fivefold (from <5 to 25%). By following a similar protocol but using *m*-nitrophenyl acrylate instead of the *para* isomer **26**, the acids **24x** and **25x** were obtained in a ratio of 1:2, although in this case the reactions are likely to involve temporary acylation of a cyclodextrin secondary hydroxyl group.^[55,56,85]

Experimental Section

General: NMR spectra were recorded on a Varian Gemini 300 spectrometer operating at 300 (¹H) and 75 MHz (¹³C) or on a Varian Inova 500 spectrometer operating at 500 (¹H) and 125 MHz (¹³C). Low (EI) and high (HRMS) resolution electron impact mass spectra were recorded on a Micromass VG AutoSpec M mass spectrometer operating with an ionization potential of 70 eV and a source potential of 8 kV. Low-resolution electrospray ionization (ESI) mass spectra were recorded on a Micromass VG Quattro II mass spectrometer. The Australian National University Micro-analytical Service performed the elemental analyses. Melting points (m.p.) were determined on a Kofler hot-stage melting point apparatus under a Reichert microscope and are uncorrected. Chromatography on silica

refers to the use of Merck silica gel 60 (40–63 μ m) with analytical grade solvents, driven by a positive pressure of nitrogen.

β -Cyclodextrin (**27**) was obtained from Nihon Shokuhin Kako (Japan) in 99.1% purity. It was recrystallized from water and dried under vacuum over P₂O₅ to constant weight before use. The nitrile oxides **2a–d** and their precursor hydroximinoyl chlorides, and the cyclodextrin derivatives **1**, **3a–c**, and **4a–c**, were prepared as described in previous studies.^[78]

***N*-(6^A-Deoxy- β -cyclodextrin-6^A-yl)-3-*tert*-butylisoxazole-5-carboxamide (**4d**):** A solution of 6^A-deoxy-6^A-propynamido- β -cyclodextrin (**1**)^[78] (24 mg, 20 μ mol) and 4-*tert*-butylhydroximinoyl chloride (8 mg, 80 μ mol) in methanol (0.1 mL) and water (1.5 mL) was stirred at 25 °C for 1 h. Triethylamine (8 mg, 80 μ mol) was added and the mixture was stirred for a further 15 h. The solution was then diluted with water/ethanol (1:4, v/v, 10 mL) before being washed with ethyl acetate (3 \times 10 mL) and concentrated in vacuo. The residual cream-colored solid was recrystallized from water to give **4d** as a colorless solid (22 mg, 85%). ¹H NMR (300 MHz, [D₆]DMSO): δ = 8.60 (m, 1H), 7.11 (s, 1H), 5.91–5.73 (m, 14H), 4.90–4.80 (m, 7H), 4.53–4.39 (m, 6H), 3.80–3.30 (m, 42H), 1.31 ppm (s, 9H); ¹³C NMR (125 MHz, [D₆]DMSO): δ = 172.2, 163.0, 156.0, 104.4, 102.0, 101.5, 84.2, 81.5–81.1, 73.0–72.0, 69.6, 59.9–59.3, 56.0, 31.9, 29.1 ppm; MS (ESI): *m/z* (%): 1323 (8) [M+K]⁺, 1307 (35) [M+Na]⁺, 1285 (21) [M+H]⁺, 662 (100); HRMS *m/z*: calcd for C₅₀H₈₁N₂O₃₆: 1285.4569 [M+H]⁺; found: 1285.4548; elemental analysis calcd (%) for C₅₀H₈₀N₂O₃₆·8H₂O: C 42.00, H 6.77, N 1.97; found: C 41.61, H 6.25, N 1.70.

General procedure for reactions of propiolamide (5) with the nitrile oxides 2a–d: Triethylamine (1.1 mol. equiv) in THF (0.2 mL) was added over approximately 1 h to propiolamide (5; 0.25–0.43 mmol) and the appropriate hydroximinoyl chloride (1.0 mol. equiv) in THF (5 mL). The mixture was stirred at 25 °C for 20 h, then concentrated under reduced pressure. The residue was analyzed by ¹H NMR spectroscopy to determine the ratio of formation of the cycloadducts 6a–d and 7a–d, before being dissolved in dichloromethane. This solution was washed with water (2 × 10 mL), then dried (MgSO₄) and concentrated under reduced pressure.

3-(4-*tert*-Butylphenyl)isoxazole-4-carboxamide (6a) and 3-(4-*tert*-butylphenyl)isoxazole-5-carboxamide (7a): By using the general procedure, 6a and 7a were produced in a ratio of 1:4. Chromatography of the mixture on silica, with elution by 0–5% methanol in dichloromethane, afforded a mixture of the two cycloadducts 6a and 7a in the same ratio and as a colorless solid (81%). Recrystallization of the mixture from dichloromethane afforded the isoxazole 7a. M.p. 239–241 °C; ¹H NMR (500 MHz, [D₆]DMSO): δ = 8.41 (brs, 1H), 8.03 (brs, 1H), 7.84 (d, *J* = 8.5 Hz, 2H), 7.56 (m, 3H), 1.34 ppm (s, 9H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 164.3, 162.4, 157.2, 153.4, 126.5, 126.1, 125.1, 104.7, 34.6, 30.9 ppm; MS (EI): *m/z* (%): 244 (41) [*M*]⁺, 229 (100), 201 (21), 91 (16), 77 (11); elemental analysis calcd (%) for C₁₄H₁₆N₂O₂: C 68.83, H 6.60, N 11.47; found: C 68.93, H 6.75, N 11.38. In addition to the resonances for the isoxazole 7a, the NMR spectra of the mixture showed signals characteristic of the regioisomer 6a; ¹H NMR (500 MHz, [D₆]DMSO): δ = 9.33 (s, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.51 ppm (d, *J* = 8.5 Hz, 2H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 115.8 ppm. When the reaction was repeated in water/methanol (3:7, v/v) instead of THF, and under much more dilute conditions, the isoxazoles 6a and 7a were produced in the ratio 1:8.

3-(4-Biphenyl)isoxazole-4-carboxamide (6b) and 3-(4-biphenyl)isoxazole-5-carboxamide (7b): By using the general procedure, 6b and 7b were produced in a ratio of 1:6 as a colorless solid (75%). Washing the mixture with ether afforded the isoxazole 7b as a colorless solid. M.p. 270–275 °C (decomp); ¹H NMR (300 MHz, [D₆]DMSO): δ = 8.43 (brs, 1H), 8.05 (brs, 1H), 8.03 (d, *J* = 8.4 Hz, 2H), 7.87 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.69 (s, 1H), 7.53 (t, *J* = 7.5 Hz, 2H), 7.45 ppm (d, *J* = 7.5 Hz, 1H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 164.5, 162.2, 157.1, 142.1, 139.1, 129.1, 128.1, 127.4, 127.3, 126.9, 126.8, 104.8 ppm; MS (EI): *m/z* (%): 264 (100) [*M*]⁺, 220 (85), 121 (82), 69 (58); HRMS *m/z*: calcd for C₁₆H₁₂N₂O₂: 264.0899; found: 264.0896. In addition to the resonances for the isoxazole 7b, the NMR spectra of the mixture showed signals characteristic of the regioisomer 6b; ¹H NMR (300 MHz, [D₆]DMSO): δ = 9.38 ppm (s, 1H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 115.9 ppm.

3-Phenylisoxazole-4-carboxamide (6c) and 3-phenylisoxazole-5-carboxamide (7c): By using the general procedure, 6c and 7c were produced in a ratio of 1:5. Chromatography of the mixture on silica, with elution by 0–5% methanol in dichloromethane, afforded a mixture of the two cycloadducts 6c and 7c in the same ratio as a colorless solid (57%). Recrystallization of the mixture from dichloromethane afforded the isoxazole 7c. M.p. 214–217 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 8.44 (brs, 1H), 8.06 (brs, 1H), 7.92 (m, 2H), 7.61 (s, 1H), 7.55 ppm (m, 3H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 164.5, 162.6, 157.1, 130.6, 129.3, 127.9, 126.7, 104.8 ppm; MS (EI): *m/z* (%): 188 (74) [*M*]⁺, 144 (100), 116 (47), 77 (73); HRMS *m/z*: calcd for C₁₀H₈N₂O₂: 188.0586; found: 188.0584. In addition to the resonances for the isoxazole 7c, the NMR spectra of the mixture showed signals characteristic of the regioisomer 6c; ¹H NMR (300 MHz, [D₆]DMSO): δ = 9.35 ppm (s, 1H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 115.9 ppm.

3-*tert*-Butylisoxazole-4-carboxamide (6d) and 3-*tert*-butylisoxazole-5-carboxamide (7d): By using the general procedure, 6d and 7d were produced in a ratio of 1:30. Chromatography of the mixture on silica, with elution by 0–5% methanol in dichloromethane, afforded a mixture of the two cycloadducts 6d and 7d in the same ratio and as a colorless solid (60%). Recrystallization of the mixture from hexanes afforded the isoxazole 7d. M.p. 137–140 °C; ¹H NMR (300 MHz, [D₆]DMSO): δ = 8.25 (brs, 1H), 7.90 (brs, 1H), 7.10 (s, 1H), 1.30 ppm (s, 9H); ¹³C NMR (75 MHz, [D₆]DMSO): δ = 172.3, 163.4, 157.4, 104.7, 31.9, 29.1 ppm; MS (EI): *m/z* (%): 168 (25) [*M*]⁺, 153 (100), 68 (79); HRMS *m/z*: calcd for C₈H₁₂N₂O₂:

168.0899; found: 168.0902; elemental analysis calcd (%) for C₈H₁₂N₂O₂: C 57.13, H 7.19, N 16.66; found: C 57.06, H 7.22, N 16.49. In addition to the resonances for the isoxazole 7d, the ¹H NMR spectrum of the mixture showed signals characteristic of the regioisomer 6d; ¹H NMR (300 MHz, [D₆]DMSO): δ = 9.15 (s, 1H), 1.38 ppm (s, 9H).

Determination of pseudo-first-order rate constants for reactions of 4-*tert*-butylbenzoxazole (2a) with propiolamide (5) and 6^A-deoxy-6^A-propylamido-β-cyclodextrin (1): A solution of the amide 5 (2 mM), 4-*tert*-butylbenzohydroximinoyl chloride (1 mM), and triethylamine (1 mM) in 30% aqueous methanol (8 mL) maintained at 25 °C was analyzed by HPLC (Alltima C₁₈ 4.6 × 250 mm column, with elution at 0.9 mL min⁻¹ using acetonitrile/water 70:30) at 15–30 min intervals over a period of 24 h. The increase in the areas of the peaks due to the cycloadducts 6a and 7a, which had retention times of 5.2 and 6.5 min, respectively, was monitored. The logarithmic plot of the data showed a linear correlation with *R*² > 0.998 data, from which the pseudo-first-order rate constants for the formation of the isoxazoles 6a and 7a were calculated to be 5.0 × 10⁻⁶ and 3.8 × 10⁻⁵ s⁻¹, respectively. In a similar manner, a solution of the cyclodextrin derivative 1 (2 mM), 4-*tert*-butylbenzohydroximinoyl chloride (1 mM), and triethylamine (1 mM) in water was analyzed by HPLC, except that the column was eluted with acetonitrile/water 15:85, and under these conditions the cycloadducts 3a and 4a had retention times of 4.1 and 9.6 min, respectively. Formation of the cycloadducts 3a and 4a was complete within 2–3 min, on which basis the half-life for the overall cycloadditions was determined to be < 1 min. Allowing for the ratio of formation of the cycloadducts 3a and 4a, the pseudo-first-order rate constants for their formation were therefore calculated to be > 9.3 × 10⁻³ and > 6.3 × 10⁻⁴ s⁻¹, respectively.

General procedure for preparation of the cyclodextrin esters 20x–z: Aqueous solutions of cesium hydroxide (50%, 0.39 mL, 2.3 mmol) were added dropwise to either acrylic acid (23x), methacrylic acid (23y), or crotonic acid (23z; 2.3 mmol), and the mixtures were stirred at 25 °C for 0.5 h before they were concentrated under reduced pressure. The resultant colorless solids were each suspended in dry DMF (3 mL) under a nitrogen atmosphere and 6^A-*O*-toluenesulfonyl-β-cyclodextrin (0.3 g, 0.23 mmol) was added. The mixtures were heated at 90 °C for 24 h in the first two cases and at 50 °C for 72 h in the third. The solutions were then cooled to 25 °C, and ethanol (40 mL) and diethyl ether (20 mL) were added. The precipitates that formed were separated by filtration and washed with ethanol (2 × 20 mL) and diethyl ether (20 mL). Chromatography of the residues on Sephadex G15 with elution by water afforded the esters 20x–z as colorless solids.

6^A-*O*-Acryloyl-β-cyclodextrin (20x): Yield: 0.21 g, 76%; m.p. 287–289 °C (decomp); ¹H NMR (300 MHz, D₂O): δ = 6.46 (d, *J* = 17.0 Hz, 1H), 6.24 (dd, *J* = 10.5, 17.0 Hz, 1H), 6.02 (d, *J* = 10.5 Hz, 1H), 5.07 (m, 7H), 4.62 (m, 1H), 4.37 (dd, *J* = 5.0, 9.0 Hz, 1H), 4.13 (m, 1H), 3.98–3.80 (m, 26H), 3.72–3.55 ppm (m, 13H); ¹³C NMR (75 MHz, D₂O): δ = 170.8, 135.7, 130.0, 104.7, 83.9, 75.9–74.4, 72.2, 66.8, 63.1 ppm; MS (ESI): *m/z* (%): 1211 (85) [*M*+Na]⁺, 1189 (100) [*M*+H]⁺; elemental analysis calcd (%) for C₄₂H₇₂O₃₆·6H₂O: C 41.67, H 6.53; found: C 41.49, H 6.57.

6^A-*O*-Methacryloyl-β-cyclodextrin (20y): Yield: 80 mg, 29%; m.p. 246–249 °C (decomp); ¹H NMR (300 MHz, D₂O): δ = 6.18 (apparent s, 1H), 5.79 (apparent s, 1H), 5.09 (m, 7H), 4.65 (m, 1H), 4.37 (dd, *J* = 6.0, 12.9 Hz, 1H), 4.15 (m, 1H), 4.01–3.81 (m, 26H), 3.72–3.58 (m, 13H), 1.96 ppm (s, 3H); ¹³C NMR (75 MHz, D₂O): δ = 172.0, 138.5, 130.1, 104.7, 84.0, 74.6–75.9, 72.4, 67.0, 63.1, 20.3 ppm; MS (ESI): *m/z* (%): 1225 (44) [*M*+Na]⁺, 1203 (100) [*M*+H]⁺; elemental analysis calcd (%) for C₄₆H₇₄O₃₆·3H₂O: C 43.95, H 6.41; found: C 44.00, H 6.35.

6^A-*O*-Crotonyl-β-cyclodextrin (20z): Yield: 98 mg, 35%; m.p. 245–250 °C (decomp); ¹H NMR (300 MHz, D₂O): δ = 7.11 (dq, *J* = 7.0, 15.6 Hz, 1H), 5.98 (d, *J* = 15.6 Hz, 1H), 5.08 (m, 7H), 4.59 (m, 1H), 4.33 (dd, *J* = 5.6, 12.2 Hz, 1H), 4.13 (m, 1H), 4.01–3.80 (m, 26H), 3.73–3.57 (m, 13H), 1.92 ppm (d, *J* = 7.0 Hz, 3H); ¹³C NMR (75 MHz, D₂O): δ = 171.2, 151.3, 123.7, 104.7, 83.9, 75.9, 74.9–74.4, 72.3, 66.4, 63.1, 20.4 ppm; MS (ESI): *m/z* (%): 1225 (100) [*M*+Na]⁺; elemental analysis calcd (%) for C₄₆H₇₄O₃₆·6H₂O: C 42.14, H 6.61; found: C 42.30, H 6.38.

General procedure for reactions of the esters 20x–z with 4-*tert*-butylbenzoxazole (2a): Solutions of the esters 20x–z (0.054–0.084 mmol) in

water (5.5 mL) were treated with 4-*tert*-butylbenzohydroximinoyl chloride (1.1 mol equiv dissolved in 0.1 mL ethanol), and the mixtures were stirred at 25°C for 1 h before triethylamine (1.2 mol equiv) was added over a period of 1 h. The mixtures were then stirred for 15 h before being diluted with water/ethanol (1:4, v/v, 20 mL), washed with ethyl acetate (3×30 mL), and concentrated under reduced pressure. The residues were dissolved in aqueous sodium hydroxide (0.5 M, 10 mL) and the solutions were stirred at 25°C for 2 h before being acidified with HCl and extracted with ethyl acetate (3×30 mL). The extracts were dried (MgSO₄) and concentrated under reduced pressure, and the resulting residues were analyzed by ¹H NMR spectroscopy to determine the ratios of the cycloadducts **24x-z** and **25x-z**.

3-(4-*tert*-Butylphenyl)-2-isoxazoline-4-carboxylic acid (24x): By using the general procedure, the acids **24x** and **25x** were obtained as a 20:1 mixture. The acid **25x** was identified by comparison with an authentic sample, obtained as described below. Chromatography of the mixture on silica, with elution by 0–2% methanol in dichloromethane containing 1% acetic acid gave **24x** as a colorless solid (43%). M.p. 116–119°C; ¹H NMR (300 MHz, CDCl₃): δ = 7.70 (d, *J* = 8.7 Hz, 2H), 7.43 (d, *J* = 8.7 Hz, 2H), 4.86 (dd, *J* = 5.8, 8.7 Hz, 1H), 4.63 (dd, *J* = 8.7, 10.8 Hz, 1H), 4.51 (dd, *J* = 5.8, 10.8 Hz, 1H), 1.33 ppm (s, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 174.8, 154.8, 153.9, 126.9, 125.8, 125.2, 77.2, 73.4, 34.9, 31.1 ppm; MS (EI): *m/z* (%): 247 (33) [M]⁺, 232 (100); HRMS *m/z*: calcd for C₁₄H₁₇NO₃: 247.1208; found: 247.1205.

3-(4-*tert*-Butylphenyl)-4-methyl-2-isoxazoline-4-carboxylic acid (24y): By using the general procedure, the acids **24y** and **25y** were obtained as a 1:10 mixture (55%). The acid **25y** was identified by comparison with an authentic sample, obtained as described below. In addition to the resonances for the acid **25y**, the ¹H NMR spectrum of the mixture showed signals characteristic of the regioisomer **24y**. ¹H NMR (300 MHz, CDCl₃): δ = 7.62 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 4.84 (d, *J* = 7.8 Hz, 1H), 4.37 (d, *J* = 7.8 Hz, 1H), 1.66 (s, 3H), 1.32 ppm (s, 9H).

3-(4-*tert*-Butylphenyl)-5-methyl-2-isoxazoline-4-carboxylic acid (24z): The crude product obtained by using the general procedure comprised of only the acid **24z** and none of the regioisomer **25z**, as determined by comparison with an authentic sample, obtained as described below. Chromatography of the crude product on silica, with elution by 0–2% methanol in dichloromethane containing 1% acetic acid gave the isoxazoline **24z** as a colorless solid (42%). M.p. 113–115°C; ¹H NMR (300 MHz, CDCl₃): δ = 7.65 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 5.11 (m, 1H), 4.07 (d, *J* = 5.7 Hz, 1H), 1.44 (d, *J* = 6.3 Hz, 3H), 1.32 ppm (s, 9H); ¹³C NMR (125 MHz, CDCl₃): δ = 172.9, 153.6, 153.5, 126.7, 125.8, 125.7, 82.1, 59.8, 34.8, 31.1, 20.8 ppm; MS (EI): *m/z* (%): 261 (43) [M]⁺, 246 (100), 202 (17), 160 (14), 57 (7); elemental analysis calcd (%) for C₁₅H₁₉NO₃: C 68.94, H 7.33, N 5.36; found: C 68.70, H 7.43, N 5.25.

General procedure for reactions of the acids 23x-z with 4-*tert*-butylbenzotriazole (2a): Solutions of the acids **23x-z** (1.29 mmol) and 4-*tert*-butylbenzohydroximinoyl chloride (1.42 mmol) in THF (15 mL) were treated with triethylamine (1.55 mmol) in THF (1 mL) over a period of 20 h, then they were concentrated under reduced pressure. The residues were analyzed by ¹H NMR spectroscopy to determine the ratios of formation of the cycloadducts **24x-z** and **25x-z**, before they were dissolved in dichloromethane (20 mL). These solutions were washed with water (2×50 mL) containing 1% acetic acid, dried (MgSO₄), and concentrated under reduced pressure.

3-(4-*tert*-Butylphenyl)-2-isoxazoline-5-carboxylic acid (25x): By using the general procedure, the acids **24x** and **25x** were obtained as a 1:20 mixture. The acid **24x** was identified by comparison with an authentic sample, obtained as described above. Chromatography of the crude product mixture on silica, with elution by 0–2% methanol in dichloromethane containing 1% acetic acid, afforded **25x** as a colorless solid (46%). M.p. 109–112°C; ¹H NMR (300 MHz, CDCl₃): δ = 7.61 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 5.20 (t, *J* = 8.6 Hz, 1H), 3.70 (m, 2H), 1.33 ppm (s, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 172.1, 156.9, 154.7, 126.9, 125.9, 124.9, 77.2, 39.6, 35.0, 31.1 ppm; MS (EI): *m/z* (%): 247 (59) [M]⁺, 232 (95), 202 (100), 175 (42), 160 (71); elemental analysis calcd (%) for C₁₄H₁₇NO₃: C 67.51, H 6.96, N 5.62; found: C 67.47, H 6.94, N 5.47. When the reaction was repeated in water/methanol (1:1, v/v) instead of

THF, and under much more dilute conditions, the isoxazoles **24x** and **25x** were produced in the ratio 1:25.

3-(4-*tert*-Butylphenyl)-5-methyl-2-isoxazoline-5-carboxylic acid (25y): The crude product obtained by using the general procedure comprised only the acid **25y** and none of the regioisomer **24y**, as determined by comparison with an authentic sample, obtained as described above. Chromatography of the crude product on silica, with elution by 0–2% methanol in dichloromethane containing 1% acetic acid gave the isoxazoline **25y** as a colorless solid (38%). M.p. 163–166°C partial, 195–199°C; ¹H NMR (300 MHz, CDCl₃): δ = 7.58 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 8.9 Hz, 2H), 3.88 (d, *J* = 17.1 Hz, 1H), 3.28 (d, *J* = 17.1 Hz, 1H), 1.76 (s, 3H), 1.32 ppm (s, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 175.2, 157.2, 154.3, 126.7, 125.8, 125.5, 85.8, 45.2, 34.9, 31.1, 23.21 ppm; MS (EI): *m/z* (%): 261 (43) [M]⁺, 246 (56), 216 (76), 202 (54), 174 (85), 160 (62), 144 (100), 116 (68); elemental analysis calcd (%) for C₁₅H₁₉NO₃: C 68.94, H 7.33, N 5.36; found: C 68.75, H 7.27, N 5.22.

3-(4-*tert*-Butylphenyl)-4-methyl-2-isoxazoline-5-carboxylic acid (25z): By using the general procedure, the acids **24z** and **25z** were obtained as a 1:1 mixture. The acid **24z** was identified by comparison with an authentic sample, obtained as described above. Chromatography of the mixture on silica, with elution by 0–2% methanol in dichloromethane containing 1% acetic acid gave **25z** as a colorless solid (10%). M.p. 132–136°C; ¹H NMR (300 MHz, CDCl₃): δ = 7.62 (d, *J* = 8.7 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 4.79 (d, *J* = 3.9 Hz, 1H), 4.06 (dq, *J* = 3.9, 7.2 Hz, 1H), 1.45 (d, *J* = 7.2 Hz, 3H), 1.33 ppm (s, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 172.3, 160.9, 154.1, 127.2, 125.9, 124.4, 84.3, 77.2, 34.9, 31.1, 18.3 ppm; MS (EI): *m/z* (%): 261 (30) [M]⁺, 246 (82), 216 (21), 144 (42), 84 (95), 66 (100), 57 (59); elemental analysis calcd (%) for C₁₅H₁₉NO₃: C 68.94, H 7.33, N 5.36; found: C 68.72, H 7.06, N 5.26.

Methyl 3-(4-*tert*-butylphenyl)-2-isoxazoline-5-carboxylate and methyl 3-(4-*tert*-butylphenyl)-2-isoxazoline-4-carboxylate: Methyl acrylate (65 mg, 0.76 mmol) and 4-*tert*-butylbenzohydroximinoyl chloride (40 mg, 0.19 mmol) were dissolved in THF (5 mL). The mixture was stirred at 25°C and triethylamine (21 mg, 0.21 mmol) dissolved in THF (1 mL) was added over a period of 20 h. The solution was then concentrated under reduced pressure and the residue was analyzed by ¹H NMR spectroscopy. This showed the presence of the title 3,5- and 3,4-disubstituted isoxazolines in the ratio 25:1. The crude product was dissolved in dichloromethane (10 mL) and the solution was washed with water (3×15 mL), dried (MgSO₄), and concentrated under reduced pressure. Chromatography of the residue on silica, with elution by 0–10% ethyl acetate in hexanes gave methyl 3-(4-*tert*-butylphenyl)-2-isoxazoline-5-carboxylate as a colorless oil (26 mg, 53%). ¹H NMR (300 MHz, CDCl₃): δ = 7.61 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 5.18 (dd, *J* = 10.2, 7.8 Hz, 1H), 3.81 (s, 3H), 3.64 (m, 2H), 1.33 ppm (s, 9H); ¹³C NMR (75 MHz, CDCl₃): δ = 170.8, 155.9, 154.0, 126.7, 125.6, 77.8, 52.8, 39.1, 34.9, 31.1 ppm; MS (EI): *m/z* (%): 261 (72) [M]⁺, 246 (100), 202 (91), 91 (32); HRMS *m/z*: calcd for C₁₅H₁₉NO₃: 261.1365; found: 261.1363. In addition to the resonances for the 3,5-disubstituted isoxazoline, the ¹H NMR spectrum of the mixture showed signals characteristic of the 3,4-disubstituted regioisomer: ¹H NMR (300 MHz, CDCl₃): δ = 4.82 (dd, *J* = 6.0, 8.8 Hz, 1H), 4.63 (dd, *J* = 8.8, 11.4 Hz, 1H), 4.51 ppm (dd, *J* = 6.0, 11.4 Hz, 1H).

***p*-Nitrophenyl acrylate (26):** 1,3-Dicyclohexylcarbodiimide (1.03 g, 5.0 mmol) was added to a solution of *p*-nitrophenol (556 mg, 4.0 mmol) and acrylic acid (**23x**; 0.27 mL, 4.0 mmol) in freshly distilled dichloromethane (20 mL). 4-(Dimethylamino)pyridine (50 mg) was then added, and the mixture was stirred at 25°C for 18 h before being filtered. The filtrate was concentrated under reduced pressure. The residue was chromatographed on silica, with elution by 5–25% ethyl acetate in hexanes, to give the ester **26** as a cream-colored solid (417 mg, 54%). M.p. 63–65°C (lit.^[91] 61–64°C); ¹H NMR (300 MHz, CDCl₃): δ = 8.30 (d, *J* = 9.3 Hz, 2H), 7.34 (d, *J* = 9.3 Hz, 2H), 6.67 (d, *J* = 17.1 Hz, 1H), 6.34 (dd, *J* = 10.5, 17.1 Hz, 1H), 6.11 ppm (dd, *J* = 10.5 Hz, 1H); MS (EI): *m/z* (%): 193 (20) [M]⁺, 55 (100).

***m*-Nitrophenyl acrylate:** The title compound was obtained by using the procedure described above for the preparation of the *para* isomer, except that *m*-nitrophenol was used as the starting alcohol. The ester was isolated as a cream-colored solid (465 mg, 60%). M.p. 35–36°C (lit.^[91] 34–

35°C); $^1\text{H NMR}$ (300 MHz, CDCl_3): δ = 8.10 (m, 2H), 7.55 (m, 2H), 6.67 (d, J = 17.4 Hz, 1H), 6.34 (dd, J = 10.5, 17.4 Hz, 1H), 6.11 ppm (d, J = 10.5 Hz, 1H); MS (EI): m/z (%): 193 (42) $[M]^+$, 139 (100), 93 (68).

Sequential reactions of *p*-nitrophenyl acrylate (26) and the meta isomer with β -cyclodextrin (27), 4-*tert*-butylbenzoxynitrile oxide (2a), and base to give the isoxazolines 24x and 25x: Solutions of either *p*-nitrophenyl acrylate (26) or the meta isomer (8.6 mg, 45 μmol) in methanol (0.5 mL) were stirred with β -cyclodextrin (27; 0.27 mmol) in phosphate buffer (0.05 M, pH 9.0, 100 mL) for 3 and 1.3 h, respectively. 4-*tert*-Butylbenzoxynitrile oxide (6.6 mg, 31 μmol) in methanol (0.5 mL) was added and the mixtures were stirred at 25°C for 16 h, then they were adjusted to pH 12.0 with dilute aqueous NaOH. After a further 2 h, the solutions were acidified and extracted with ethyl acetate (3 \times 50 mL). The organic extracts were dried (MgSO_4) and concentrated under reduced pressure, and the residues were analyzed by $^1\text{H NMR}$ spectroscopy to determine the ratios of formation of the cycloadducts 24x and 25x, by comparison with authentic samples obtained as described above.

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