Stereoselective synthesis of β,γ-unsaturated amino acids

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Abstract - Two conceptually different approaches to the synthesis of β,γ-unsaturated α-amino acids are described. α-Alkenylglycines of type 11 are obtained by Vitamin B_{12} or cobester catalyzed reductive eliminations of 5-chloromethyl substituted oxazolines 10. An efficient synthesis of E- and Z-β-alkenylglycines of type 7 is achieved by a highly stereoselective amidoalkylation reaction of the corresponding E- and Z-alkenylsilanes with the in situ generated acyliminium ion 33.

INTRODUCTION

Pyridoxal 5'-phosphate is an essential coenzyme for the great majority of enzymes catalyzing some chemical change at the α-, β-, or γ-carbons of the common α-amino acids.

Scheme 1

Pyridoxal 5'-
Phosphate
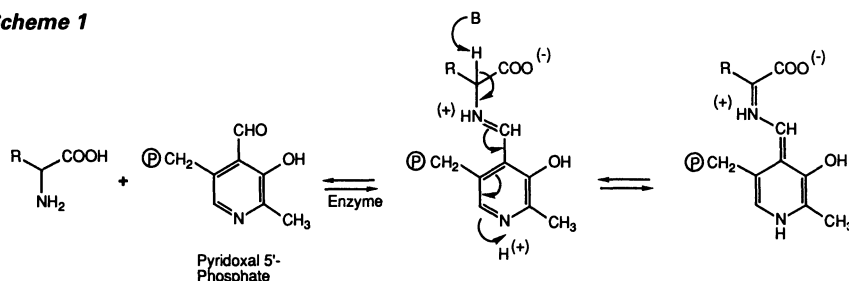
All these pyridoxal phosphate dependent enzymes function via initial imine formation between the amino acid and the cofactor, followed by a transimination step which is caused either by proton abstraction at the α-carbon or by decarboxylation.

Rando was the first to demonstrate that β,γ-unsaturated α-amino acids may act as "suicide" substrates for these enzymes (ref. 1). "Suicide" substrates are irreversible enzyme inhibitors possessing latent reactive groupings which are specifically unmasked by the action of the target enzyme (ref. 2). Upon activation a chemical reaction between the inhibitor and the enzyme occurs resulting in irreversible inactivation of the enzyme.

Active "suicide" inhibitors of this type are represented by the naturally occurring vinylglycine (ref. 3, 4) and rhizobitoxin (ref. 5) as well as the synthetic E-2-methyl-3,4-didehydroglutamic acid (ref. 6) and Z-propenylglycine (ref. 7).
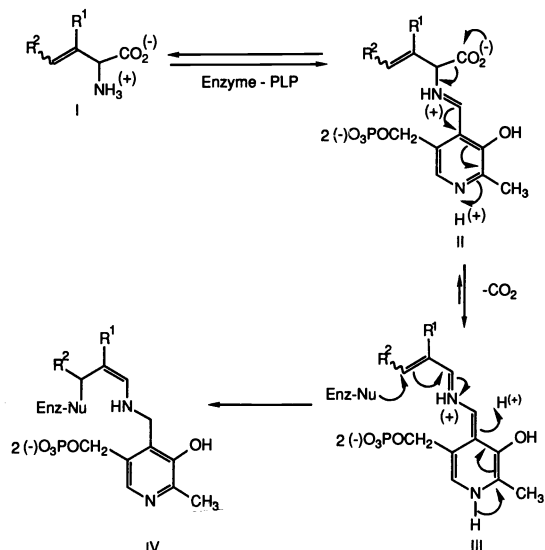
A possible mechanism of inactivation is outlined in scheme 2.

By analogy with the natural enzymic reaction, the β,γ-unsaturated amino acid (I) reacts with pyridoxal 5'-phosphate at the active site yielding the aldimine (II). However, instead of leading to transamination, proton abstraction (or decarboxylation) at the α-carbon unmasks the potent Michael acceptor (III). Reaction of this electrophilic intermediate with an active site nucleophile then leads to covalent binding and irreversible inactivation of the enzyme.

Because of the obvious interest in this class of inhibitors various methods for their preparation exist (ref. 8). However most of them are neither generally applicable nor are they stereoselective. This communication describes two conceptually different approaches to the synthesis of α- and β-substituted alkenylglycines of type 11 and 7.

Scheme 2

Scheme 3

a) $R^1 = H$, $R^2 = CH_3$ b) $R^1 = H$, $R^2 = C_2H_5$ c) $R^1 = R^2 = CH_3$
d) $R^1 = C_2H_5$, $R^2 = H$ e) $R^1 = H$, $R^2 = C_6H_5$

OXAZOLINE ROUTE

A few years ago a new synthetic method leading to this class of compounds was developed by Heinzer and Bellus at our Central Research Laboratories (ref. 9). In contrast to other existing methods it allows the regiocontrolled introduction of the double bond (scheme 3).

Starting with ethyl isocyanoacetate (**1**) and the α-chlorocarbonyl compounds **2**, the desired amino acids **5** can be obtained in overall yields ranging from 20-30%.

In the first step oxazolines of type **3** are formed in a cuprous oxide catalyzed reaction of the isonitrile **1** with the readily available chlorocarbonyl component **2** (ref. 10). In a zinc induced reductive elimination step, **3** is ring opened to the β,γ-unsaturated N-formylamino acid ester **4** thereby introducing the double bond precisely into the desired β,γ-position. Hydrolysis of **4** with 6N hydrochloric acid affords the corresponding amino acid hydrochloride, which can be converted to the free amino acid **5** by treatment with propylene oxide.

It was found that the β-substituted vinylglycine derivatives **7** can be obtained stereoselectively as the thermodynamically more stable E-isomer whereas oxazolines **8** give rise to diastereomeric mixtures of E- and Z-**9** (scheme 4).

Due to the lower reactivity of the chloromethyl-oxazolines **10** the corresponding α-alkenylglycine derivatives **11** were usually obtained in somewhat lower yields accompanied by the α,β-unsaturated isomer **12**.
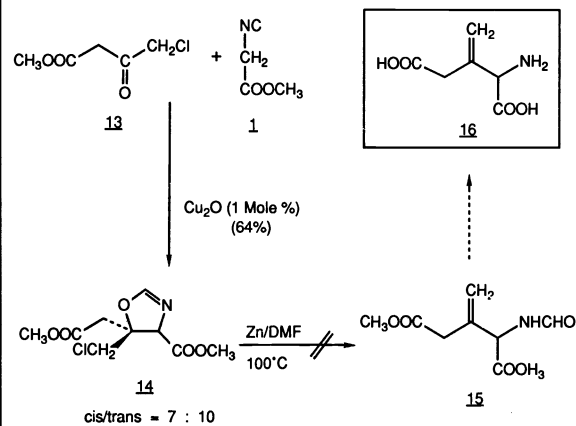
Scheme 4

R, R^1 = Alkyl
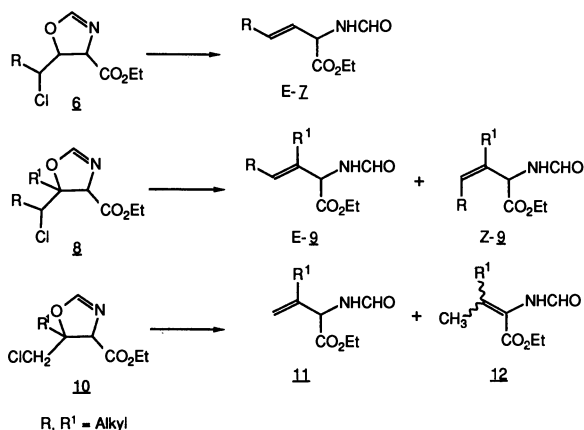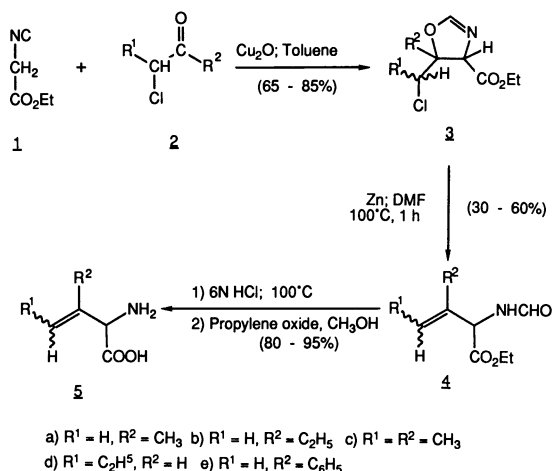
Scheme 5

In order to explore the scope of this method we focused our attention on the synthesis of β-methylene-DL-glutamic acid 16. With the close resemblance of 16 to glutamic acid itself, it seemed likely that this compound might act as a potent and selective inhibitor of those pyridoxal phosphate dependent enzymes which use glutamate as a natural substrate. β-Methylene glutamic acid, which has not previously been synthesized, contains a terminal double bond which is doubly activated by the two allylic carboxyl groups and is therefore very susceptible towards isomerization.

The desired oxazoline derivative 14 could be synthesized in 64% yield as a cis/trans diastereomeric mixture. However all attempts to form the unsaturated amino acid ester 15 by reductive elimination with zinc or with low valent chromium and titanium reagents failed (scheme 5).

In light of these difficulties we turned our attention to Vitamin B_{12} and derivatives thereof (scheme 6) which have been reported to catalyze the reductive elimination of a variety of β-haloethyl protecting groups under extremely mild conditions (ref. 11).

Scheme 6
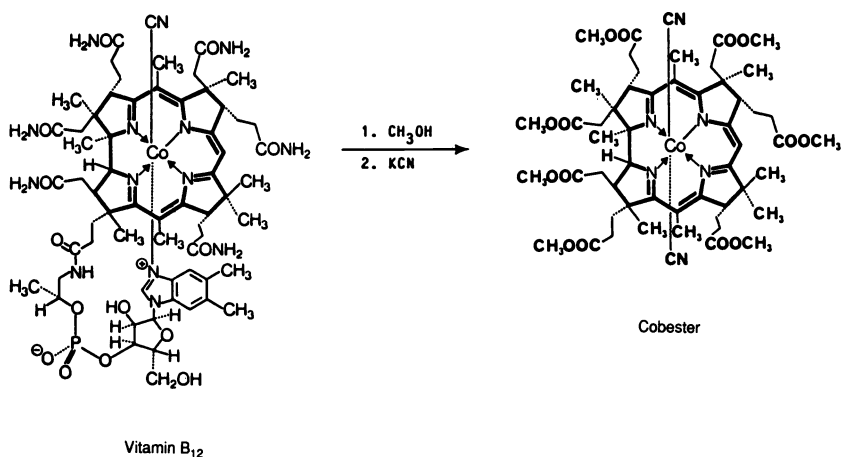
Vitamin B_{12} is a nontoxic, cobalt containing natural product, which is produced industrially, based on a fermentation process. It is surprising that only a few applications of the use of Vitamin B_{12} as a catalyst in organic synthesis have been reported thus far (ref. 12). One drawback may be the very low solubility of Vitamin B_{12} in many common organic solvents. However, this problem can be easily overcome (ref. 13) by conversion to the much more lipophilic dicyanocobyrinic acid heptamethylester (cobester).

Indeed when Vitamin B_{12} or cobester were used as catalysts the reductive elimination reaction proceeded under very mild conditions as indicated in scheme 7.

Treatment of a diastereomeric mixture of the oxazoline 14 with zinc in the presence of a catalytic amount of Vitamin B_{12} afforded the unsaturated amino acid derivative 15 at room temperature. The fairly modest yields of 15 are mainly due to loss of material during chromatographic separation from the isomers 17 and 18 which were also formed during the reaction.

Scheme 7
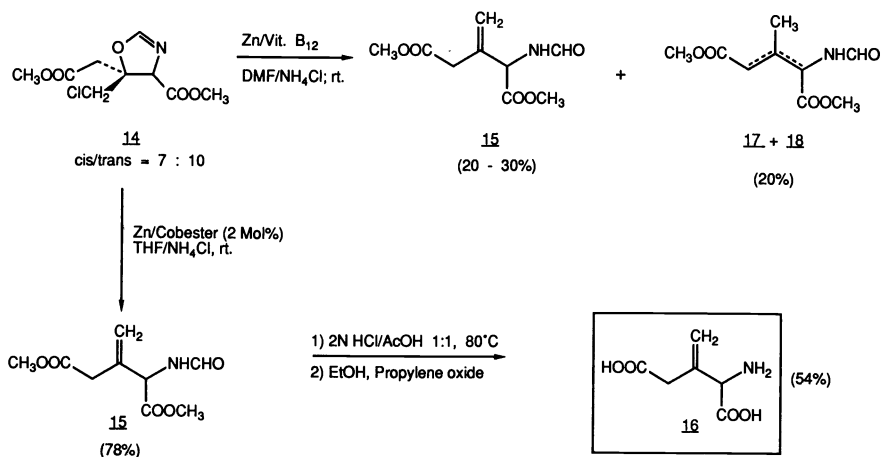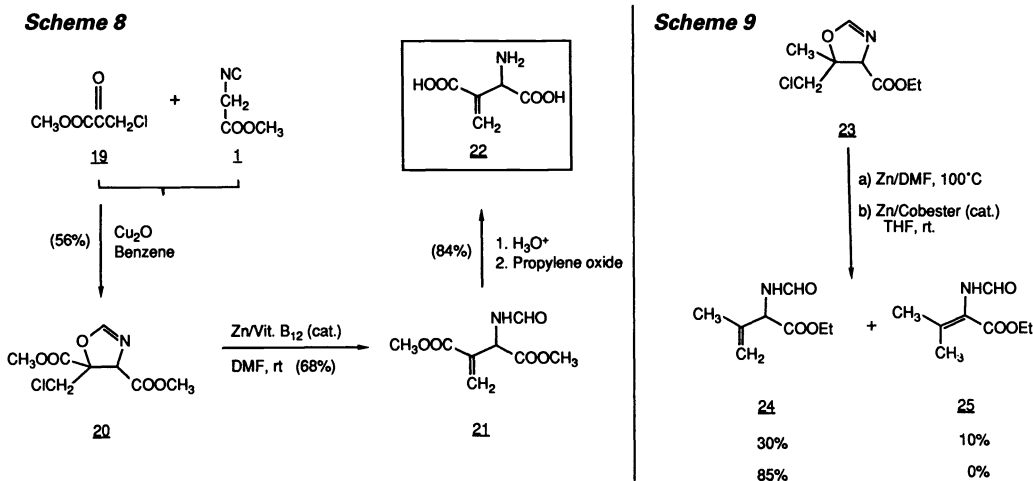
It is known that in such reductive eliminations the catalytically active species is not Vitamin B_{12} itself, but the corresponding reduced cobalt(I)-complex. We have found that this cobalt(I)-complex can be conveniently generated in situ under the reaction conditions employed and that this reaction can simply be monitored by the change in color from red to green. It should be mentioned that the outcome of the reductive elimination is critically dependent on the amount of ammonium chloride used. In the absence of ammonium chloride no reduction of Vitamin B_{12} takes place. If the amount exceeds 20 mole% however, over-reduction of 15 is observed.

A much cleaner elimination process resulted, if the reaction was performed in THF and in the presence of a catalytic amount of cobester. Under these conditions 15 was obtained exclusively and could be isolated in 78% yield. The hydrolysis of 15 could be achieved under carefully controlled conditions affording the free amino acid 16 in 54% yield.

Analogously β-methylene-DL-aspartic acid (22), which has been reported to be a specific suicide inhibitor of glutamate-aspartate transaminase (ref. 14), can be prepared in a three step synthesis and with excellent overall yield, starting from the readily available methyl chloropyruvate 19 (scheme 8).
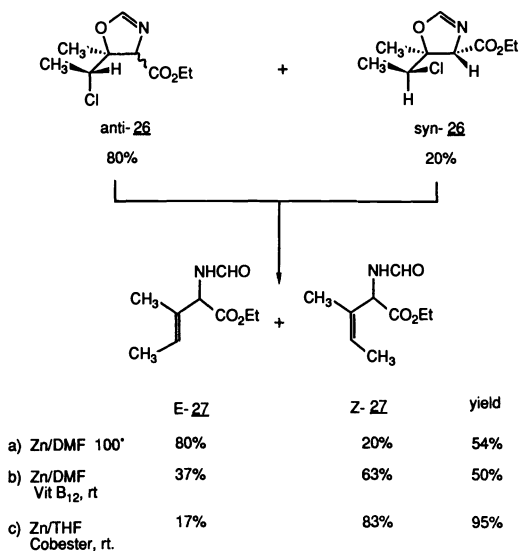
Scheme 8

Scheme 9

It is noteworthy that in the reductive elimination step Vitamin B_{12} proved to be superior to cobester. The latter, although reducing 20 very quickly, led to considerable overreduction of the acrylic ester portion in 21. In a control experiment run without catalyst no reaction occurred. Higher temperatures led to complete destruction of the starting material.

We were able to demonstrate that the Vitamin B_{12} or cobester catalyzed procedure can be successfully applied to the preparation of the amino acids summarized in scheme 3. In all cases the reaction temperature of the reductive elimination step could be reduced from 100°C to room temperature. The products were obtained in very high yields and free of any α,β-unsaturated isomers. A striking example is presented in scheme 9. Whereas the uncatalyzed reduction of 23 afforded a mixture of the desired product 24 and the corresponding α,β-unsaturated isomer 25 in only 30% and 10% yield respectively, the cobester catalyzed reaction led exclusively to 24 which was isolated in 85% yield.
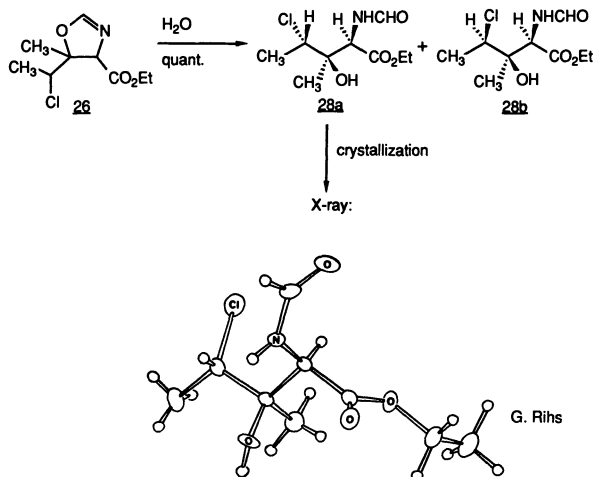
During these investigations we observed that the stereochemical course of the Vitamin B_{12} or cobester catalyzed reductive elimination is complementary to the uncatalyzed reaction (scheme 10).

Treatment of a diastereomeric mixture of the oxazoline 26 consisting of 80% of the anti-isomers 26a and 20% of the syn-isomer 26b with zinc in DMF at 100°C afforded an 80:20 mixture of E- and Z-27 in 54% yield, comparable with the syn/anti ratio of the starting material. By repeating the reaction in the presence of a catalytic amount of Vitamin B_{12} at room temperature, we were surprised to obtain a 37:63 mixture of E/Z-27 (50%), with the Z-isomer being formed predominantly. An even higher preference for the Z-isomer was observed with the cobester/THF system. Under these conditions the reductive elimination proceeded with excellent yields and with essentially reversed stereochemistry, as compared to the uncatalyzed reaction. As the stereochemical assignment of the diastereomers 26 has been rigorously established, both by ^{13}C-NMR spectroscopic analysis (cis/trans relationship of the substituents at the oxazoline ring) and by single crystal X-ray analysis of the major isomer of the hydrolysis product 28 (scheme 10a), we can conclude that the overall stereochemical course of the reductive elimination is anti in the absence of catalyst, but predominantly syn in the presence of a catalytic amount of Vitamin B_{12} or cobester. The same stereochemical outcome has independently been observed by Scheffold in a mechanistic study of the Vitamin B_{12} catalyzed reductive elimination of threo- and erythro-vicinal halohydrins (ref. 12a, 15).

Scheme 10

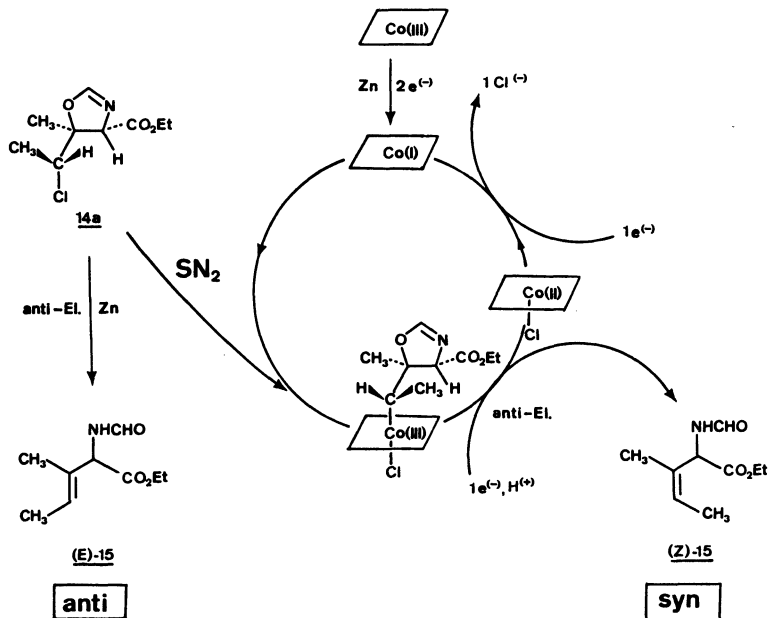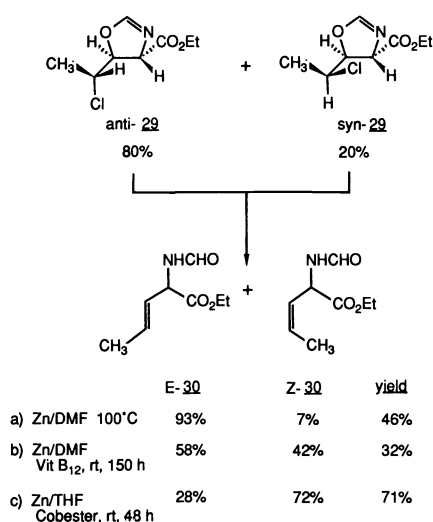|	E-27	Z-27	yield
a) Zn/DMF 100°	80%	20%	54%
b) Zn/DMF Vit B$_{12}$, rt	37%	63%	50%
c) Zn/THF Cobester, rt.	17%	83%	95%

Scheme 10a

A possible mechanism is summarized in scheme 11. In the thermodynamically favored conformation of the anti-isomer 26a the oxygen and chlorine atom are antiperiplanar to each other and therefore ideally oriented for a zinc induced anti-elimination. In order to enter the catalytic cycle, both Vitamin B$_{12}$ and cobester have to be first reduced to the corresponding cobalt(I)-species. Cobalt(I)-complexes, which are known to be super nucleophiles (ref. 16), react rapidly with alkylhalides to form cobalt(III)-alkyl derivatives. Although the stereochemical course of such substitution reactions is not uniform (ref. 17), we favor a mechanism by which the cobalt(I)-complex undergoes the oxidative addition in a SN$_2$-type manner with inversion of configuration at the carbon atom. Alkyl cobalt(III)-complexes with potential leaving groups in β-positions are usually stable. However, a one electron reduction at the cobalt atom destabilizes the cobalt-carbon bond leading to spontaneous β-elimination. The resulting cobalt(II)-complex is easily reduced by zinc, thereby entering the next catalytic cycle. If one assumes that the β-elimination of the cobalt(II)-complex again proceeds with anti-stereochemistry, then the overall process must be syn as a consequence of consecutive SN$_2$- and anti-elimination reaction.

Stimulated by these findings we became interested in evaluating whether this method would also apply to the stereoselective synthesis of E- and Z-β-alkenylglycines. Towards this end, we elected to study E- and Z-propenylglycine. It should be pointed out that the Z-isomer has been reported by Walsh to be an irreversible inhibitor of microbial methionine γ-lyase, while the E-isomer is inactive and completely metabolized by this enzyme (ref. 7).
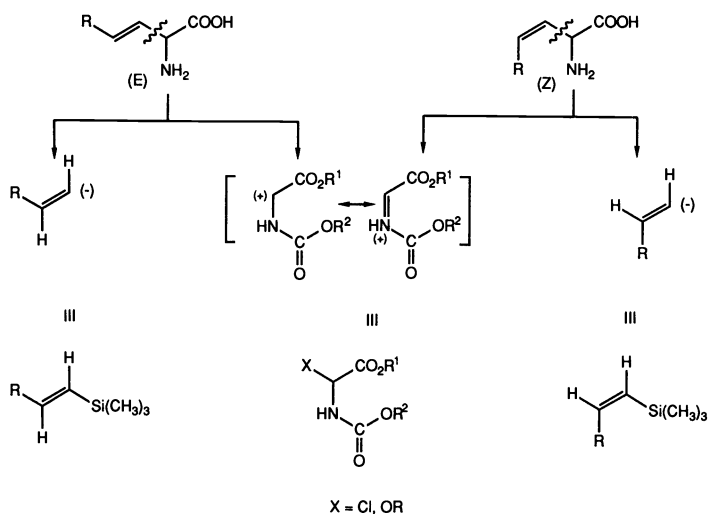
Scheme 11

Scheme 12

anti- 29   syn- 29
80%   20%

		E- 30	Z- 30	yield
a)	Zn/DMF 100°C	93%	7%	46%
b)	Zn/DMF Vit B$_{12}$, rt, 150 h	58%	42%	32%
c)	Zn/THF Cobester, rt, 48 h	28%	72%	71%

Scheme 13

X = Cl, OR

As can be seen from scheme 12 the uncatalyzed reductive elimination of the oxazoline 29 (anti:syn = 80:20) proceeded with a strong preference for the E-isomer 30. Again the cobester/THF system led not only to a considerable improvement of the yield but also to a remarkably high proportion of the thermodynamically less stable Z-isomer 30. The same trend could also be observed with the Vitamin B$_{12}$/DMF system, although it was much less pronounced. We believe that these differences in selectivity are not caused by the catalysts themselves but rather are due to solvent effects.

AMIDOALKYLATION ROUTE

Despite the encouraging results obtained in the cobester catalyzed reductive elimination reaction, the preparation of the Z-β-alkenylglycines still required a cumbersome chromatographic separation from the minor E-isomer. A conceptually different approach to this type of compounds is shown in scheme 13.

We anticipated that the E-configured amino acid should be obtained by connecting a stereospecifically generated E-vinyl anion equivalent with an acyliminium ion. In an analogous fashion a Z-vinyl anion equivalent should produce the corresponding Z-amino acid.

Vinylsilanes are very versatile synthons for vinylanions and they have been shown to react with a number of electrophiles with retention of the double bond configuration (ref. 18). Vinylsilanes can be prepared stereoselectively by a variety of methods.

We expected that the reaction of vinylsilanes with the in situ generated acyliminum ion would lead directly to the corresponding unsaturated amino acid derivatives.

Scheme 14 summarizes the results of the amidoalkylation of various β-silylstyrenes.

Scheme 14
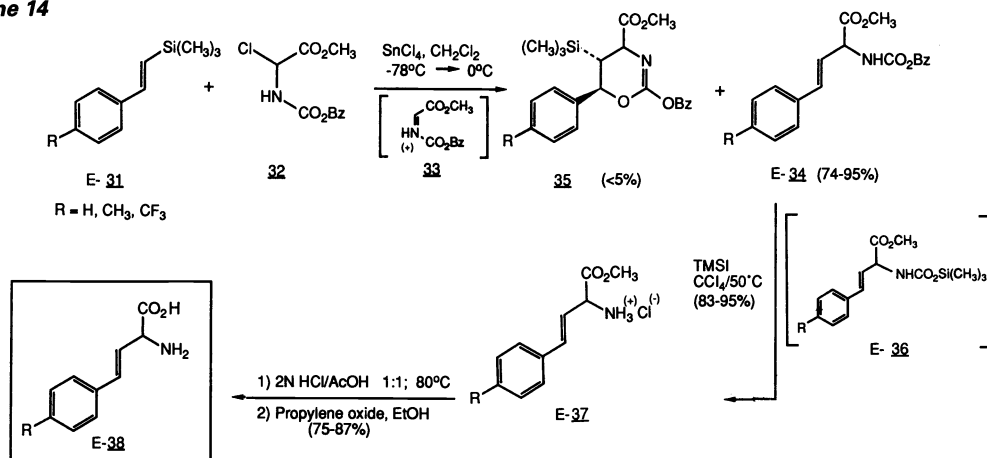
Treatment of a solution of the E-silylstyrene **31** and the chloroglycine derivative **32** with tin tetrachloride at low temperature afforded the desired amidoalkylation product E-**34** in very high yield with complete retention of the double bond configuration. Only traces of the [4+2]-cycloaddition product **35** were detected which is remarkable considering that this reaction pathway is usually the major one observed in the reaction between acyliminium ions and olefins (ref. 19). A systematic variation of the Lewis acid revealed that tin tetrachloride is the reagent of choice for the in situ generation of the reactive acyliminium ion species **33**. Titanium tetrachloride on the other hand gave no **34** but led to considerable desilylation of the starting material. For the conversion of E-**34** to the free amino acid the appropriate choice of the N-protecting group turned out to be crucial. Protecting groups which had to be removed under basic, strongly acidic or reducing conditions were unsuitable. The carbobenzoxy moiety was found to be a viable protecting group which could be cleaved with trimethylsilyl iodide under mild conditions without affecting the ester function. The intermediate silylcarbamate E-**36**, detectable by ^{1}H-NMR-spectroscopic analysis, was not isolated, but directly converted to the amino ester hydrochloride E-**37** followed by hydrolysis to the free amino acid E-**38**. Encouraged by these results we turned our attention to the thermodynamically less stable Z-isomer (scheme 15).

Scheme 15
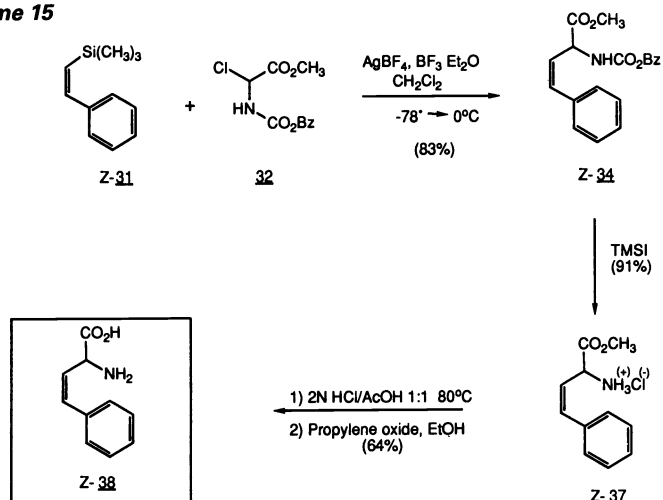
To our surprise, Z-**31** underwent no reaction whatsoever under the conditions described above. We believe that the decreased reactivity of Z-**31** is due to the fact that the most stable conformation of Z-**31** is one in which the phenyl ring is not in the same plane as the styrene double bond. In order to achieve amidoalkylation the reactive acyliminium ion had to be generated irreversibly at low temperature by the action of a stoichiometric amount of silver tetrafluoroborate on **32**. By this modification Z-**34** was obtained in 83% yield and excellent stereoselectivity (>95%). Deprotection and hydrolysis to the free amino acid was achieved in good overall yield under the reaction conditions described above.

We have found that the amidoalkylation reaction may be equally well applied to the stereo-selective synthesis of β-alkenylglycines (scheme 16).

Scheme 16

Vinylsilane	Amidoalkylation Product	Yield [%]	Stereoselectivity	Amino Acid	Yield [%]
E-**39**	E-**40**	64	>95	E-**41**	66
Z-**39**	Z-**40**	75	>95	Z-**41**	52
Z-**42**	Z-**43**	72	>95	Z-**44**	45

E-hexenylsilane <u>39</u> reacted with <u>32</u> to give the E-amino acid derivative E-<u>40</u> in 64% yield while Z-<u>39</u> lead to the corresponding Z-isomer <u>40</u> in 75% yield and very high stereo-selectivity (>95%). Likewise, the amidoalkylation product Z-<u>43</u> was obtained from Z-<u>42</u> with a very high degree of stereoselectivity and was easily transformed under the standard conditions to the Z-propenylglycine <u>44</u> mentioned earlier.

Preliminary results seem to indicate that this reaction may not be extended to alkenyl-silanes which are more highly substituted at the silicon bearing atom. Despite these restrictions, the amidoalkylation approach offers a simple and stereoselective entry to the otherwise difficult to obtain E- and Z-β-alkenylglycines. Studies which concentrate on an asymmetric version of this reaction are currently under investigation.

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