# Control of the Regioselectivity of N-Nucleophile Addition to N-Carbonyl Protected Dehydroalanines: a Model for the Ammonia-lyase Enzymes

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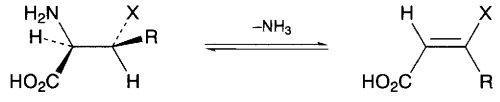
The regioselectivity of the addition of *N*-nucleophiles to *N*-carbonyl protected dehydroalanine derivatives, β-conjugate addition *vs.* α-imine capture, can be controlled completely by varying the *N*-protecting group, or, the carboxy protecting group thereby providing a model for the chemistry of dehydroalanine residues within enzymes.

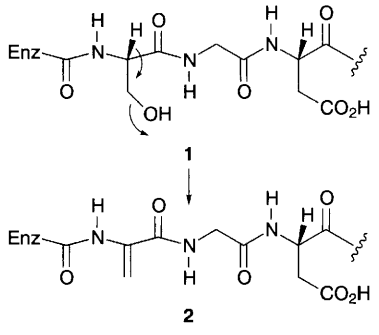
The ammonia-lyase enzymes, methylaspartase,[1,2] phenylalanine ammonia-lyase[3] and histidine ammonia-lyase[3] catalyse the reversible elimination of ammonia from their respective amino acids to give the corresponding conjugated acids, Scheme 1.

These enzymes are believed to operate *via* mechanisms which require the formation of a dehydroalanine residue at the active site of the enzyme. For methylaspartase (EC 4.3.1.2) the dehydroalanine moiety appears to be generated by the self-catalysed dehydration of a serine residue (Ser-173) within the polypeptide **1**, Scheme 2. Furthermore, it is emerging that for enzymes which operate *via* the intermediacy of a dehydroalanine residue, there is a conserved nucleic acid deduced sequence motif, -Ser-Gly-Asp-,[4,5] where the serine residue becomes dehydrated.



R = Me, X = $CO_2H$; Methylaspartase
R = H, X = Ph; Phenylalanine ammonia-lyase
R = H, X = 4-Imidazole; Histidine ammonia-lyase

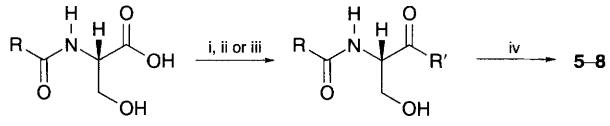
**Scheme 1**



**Scheme 2**

In the past it has been largely assumed that the dehydroalanine residues within these enzymes would undergo conjugate addition with the amino groups of amino acid substrates to give substituted 2,3-diaminopropionic acid residues prior to the elimination of ammonia from the substrate, (Scheme 3, path A).[3,6] Here we show that such reactions are feasible. We also show that an alternative mechanism, in which the dehydroalanine moiety behaves as an enamine and gives a conjugated imine which can then react with amines (Scheme 3, path B), should not be discounted as a mechanism of importance in enzymic deamination reactions.

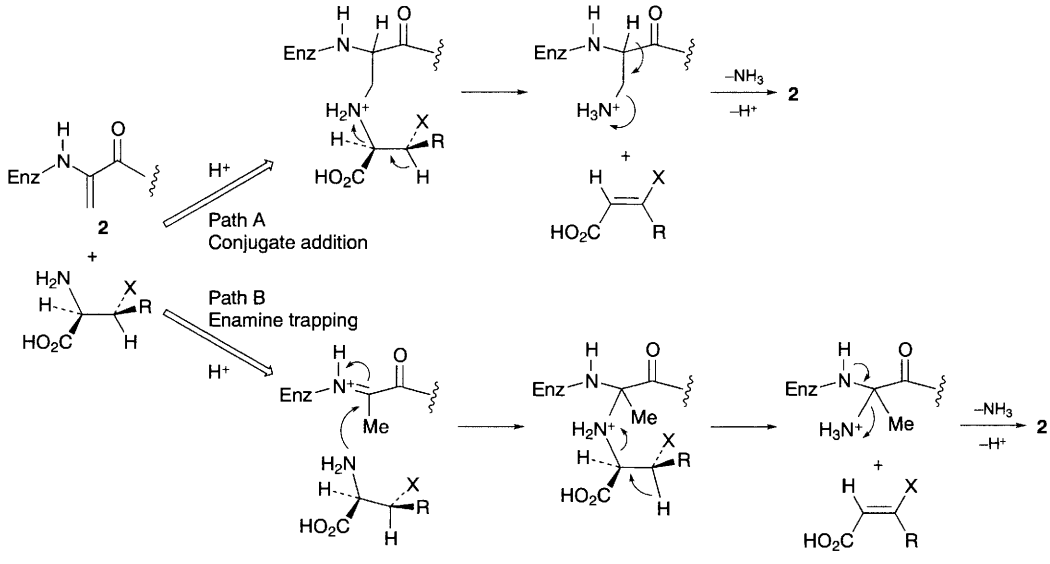
*N*-Alkyloxycarbonyldehydroalanine methyl esters and ethylamides, compounds **5–8** were prepared from *N*-alkyloxycarbonyl serine esters and ethylamides **4** through treatment with diisopropylcarbodiimide and copper(I) chloride,[7] Scheme 4. The serine esters (**4**, R = Bu$^t$O or BnO, R′ = OMe) were prepared by reacting the appropriate N-protected (2*S*)-serine free acid **3** with diazomethane and the ethylamides (**4**, R = Bu$^t$O or BnO, R′ = HNEt) were prepared by first activating the free acid **3** as the mixed isobutylcarbonic anhydride and then treating with ethylamine. *N*-Acetyldehydroalanine was ob-



**3** R = Bu$^t$O, BnO

**4** R = Bu$^t$O, BnO
R′ = HNEt, OMe

**Scheme 4** *Reagents*: i, *N*-methylmorpholine, *tert*-butylchloroformate; ii, $H_2NEt$, THF; iii, $CH_2N_2$, $Et_2O$; iv, 1,3-diisopropylcarbodiimide, Cu$^t$Cl, $CH_2Cl_2$



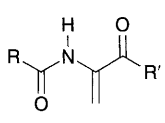
**Scheme 3**

tained commercially and was converted to the required methyl ester **9** using one equivalent of diazomethane. The *N*-acetyldehydroalanine ethylamide **10** was prepared using a modification of the method of Harada and Takasaki.[8] All compounds and intermediates showed the expected spectral and analytical properties.

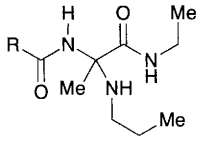
Treatment of either of the *N*-alkyloxydehydroalanine ethylamides (**5** and **6**) with propylamine in methanol at room temperature gave exclusively the diaminals (**11** and **12**). Note that in the reaction of compound **6** the carbobenzoxy group was hydrolysed to give the free amine. These diaminal products presumably arise through the protonation of the respective terminal methylene moieties, to give conjugated imines or iminium ions, which are then attacked by the nucleophile at $C^{\alpha}$, see Scheme 3 path B. Note that the new methyl group in the product **11** showed complete deuterium incorporation when reactions were performed in [$^2H_4$]methanol, as judged by $^1H$- *and* $^{13}C$-*NMR* spectroscopy but the olefinic protons in the unreacted starting material remained unexchanged. This observation indicates that the reaction of the nucleophile with the nascent imine is faster than the rate of its tautomerisation back to starting material.

On the other hand, treatment of either of the methyl esters (**7** and **8**) with propylamine under identical conditions gave only the conjugate addition products, the 1,2-diaminopropionic ester derivatives (**13** and **14**). Thus, it appears that the replacement of an O atom in the esters (**7** and **8**) by an N atom in the amides (**5** and **6**) can sufficiently deactivate the *N*-alkyloxycarbonylaminoacrylyl system to conjugate addition such that it behaves as an eneamine.

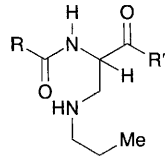
Treatment of *N*-acetyldehydroalanine methyl ester **9** with propylamine in methanol under similar conditions gave the conjugate addition product **15**, as expected. However, the ethylamide **10** also gave a conjugate addition product, compound **16**. This result is in complete contrast to that obtained for the urethanes **5** and **6** and indicates that the N-protecting group can also profoundly influence the regioselectivity of the reaction.



| | R | R′ |
|---|---|---|
| **5** | Bu$^t$O | HNEt |
| **6** | BnO | HNEt |
| **7** | Bu$^t$O | OMe |
| **8** | BnO | OMe |
| **9** | Me | OMe |
| **10** | Me | HNEt |



| | R |
|---|---|
| **11** | Bu$^t$O |
| **12** | BnO |



| | R | R′ |
|---|---|---|
| **13** | Bu$^t$O | OMe |
| **14** | BnO | OMe |
| **15** | Me | OMe |
| **16** | Me | HNEt |

**Table 1** Time required to consume 25% of the dehydroalanine

| Compound | *t*/h | Product |
|---|---|---|
| **5** | >150 | **11** |
| **10** | 134 | **16** |
| **7** | 12.5 | **13** |
| **8** | 7.5 | **14** |
| **9** | 1.5 | **15** |

The reaction rates of propylamine with the dehydroalanine derivatives (**5** and **7–10**) at a concentration of 0.06 mmol dm$^{-3}$ also varied markedly depending on the carboxy group protection present. Each of the compounds (**5** and **7–10**) were incubated with propylamine at 30 °C in [$^2H_4$]methanol and the extent of the reaction was monitored directly by $^1H$ NMR spectroscopy. The time required to consume 25% of the dehydroalanine compound is shown in Table 1. The ethylamide **5** reacted very slowly to give the diaminal **11**, after 150 hours only 8% of the dehydroalanine had been consumed.

Taken together, the results of this study indicate that there is a fine balance between the α- and β-addition of N-nucleophiles to the dehydroalanine system. It appears that the loss of amide resonance in the C-1 carboxamide moiety on changing and N atom for an O atom promotes β-addition whilst a reduction in the delocalisation of the lone pair of the α-amino group on changing from acyl to urethane protection promotes α addition. Since torsion about the amide bonds flanking the dehydroalanine residue in enzymes could be easily controlled to adjust the electronic properties of the putative Michael acceptor, either of the two mechanisms depicted in Scheme 3 could operate. The fact that the sterically hindered amine dimethylamine is accepted as a nucleophile for the amination of fumaric acid by methylaspartase[9] argues in favour of the conjugate addition mechanism, path A, Scheme 3. Interestingly, the dimethyl ester of the natural substrate (2*S*,3*S*)-3-methylaspartic acid reacts smoothly with the *N*-acetyldehydroalanine methyl ester **9** at 30 °C to give the conjugate addition product.

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