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THE USE OF PENICILLIN ACYLASE FOR SELECTIVE N-TERMINAL DEPROTECTION IN PEPTIDE SYNTHESIS

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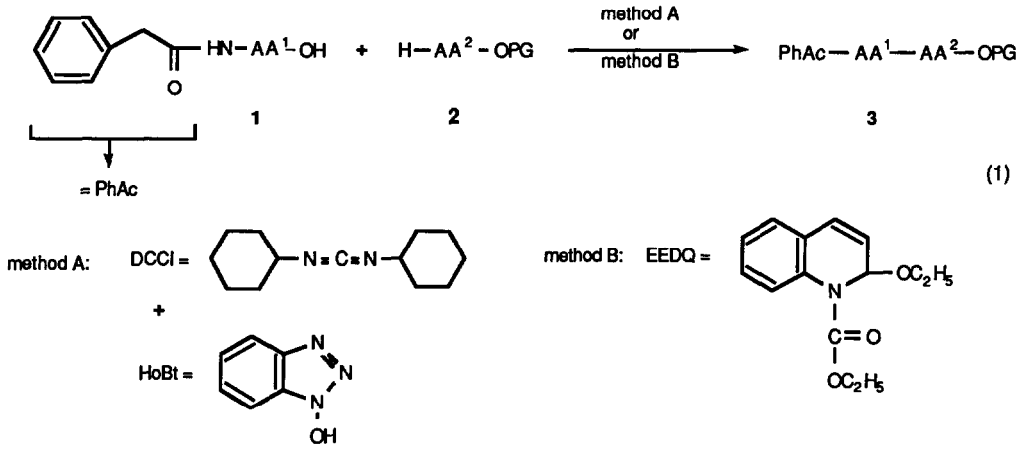
Summary. Penicillin acylase from E. coli (EC 3.5.1.11) accepts a broad range of N-phenylacetyl-dipeptide esters as substrates. The enzyme hydrolyses the N-terminal protecting group selectively at room temp. and pH=8.1 without affecting the peptide- or the ester-bonds. Alternatively methyl-, benzyl-, tert-butyl and allyl esters can be cleaved chemically leaving the phenylacetamido moiety intact.

The development of new protecting groups for peptide synthesis remains to be a challenge.[1] In many cases the selective deprotection of functional groups under nearly neutral conditions and in the presence of structures being sensitive to acids, bases and reduction has to be accomplished. Enzymes often operate under extremely mild conditions and may combine a highly selective mode of action with a broad substrate specificity. Therefore they could prove as valuable tools in achieving the abovementioned aim. Penicillin acylase from E. coli (EC 3.5.1.11) selectively hydrolyses phenylacetamides[2] and has been used, for instance, in the construction of aspartame derivatives.[3] Very recently its utilization in a process for the continuous production of dipeptides, based exclusively on enzymatic transformations has been presented.[4] The appearance of these reports prompted to describe in this paper the application of penicillin acylase for the selective liberation of the amino function in classical peptide synthesis in solution.

N-Phenylacetyl amino acids[5] (PhAc-AA-OH) can be coupled to various amino acid esters at 0°C in good yields using either the modified carbodiimide procedure[6] (method A) or EEDQ[7] (method B) as the condensing agent (scheme (1); table 1.). In general the EEDQ-method is superior since it delivers purer products with better yields. For PhAc-Phe-Leu-OBzl **3e** the extent of racemization in the coupling reaction was found to be 6.5% for method A and 5.9% for method B by HPLC (column 250/4 mm: lichrospher 100 Si, 5µm; solvent system: hexane/i-propanol 96/4; diode array detection).

The phenylacetamide moiety is not affected during the cleavage of the methyl[8]-, benzyl[8]-, tert-butyl[8] and allyl esters[9], i.e. it is stable to acids, bases and during hydrogenolysis (scheme (2)).

On the other hand, as is demonstrated in table 1., even in the presence of organic cosolvents like methanol or N-methyl-2-pyrrolidinone (NMP), penicillin acylase accepts a broad range of protected dipeptides as substrates. It tolerates variations in the N- and the C-terminal amino acid as well as in the ester groups. The hydrolysis rates decline with increasing steric demand of the N-terminal amino acid (compare for instance entries **3g-3m**). Especially N-phenylacetyl-



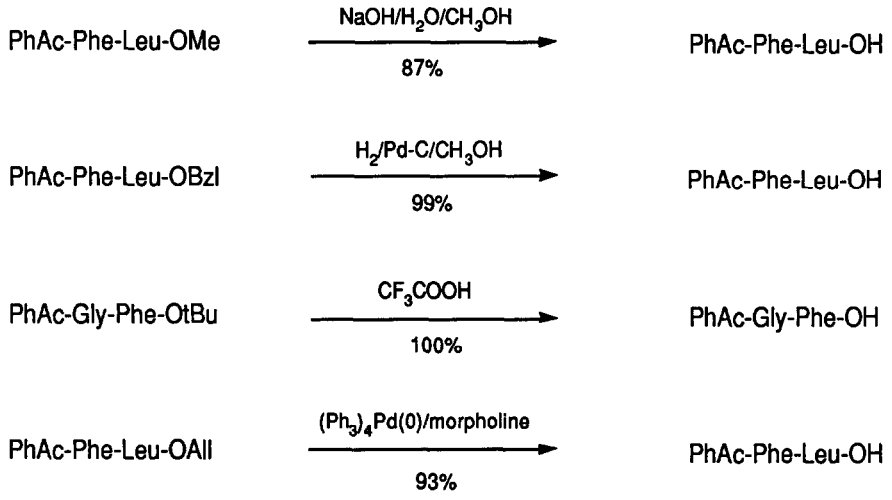
PG = methyl (Me)[8]; benzyl (Bzl)[8]; allyl (All)[9]; tert-butyl (tBu)[8].

Table 1.: Synthesis of the protected dipeptides **3** and enzymatic hydrolysis of the phenylacetyl moiety.

Entry	AA1	AA2	PG	Yield [%] method A	Yield [%] method B	Vol% NMP	pH	Initial Rate of Hydrolysis
3a	Gly	Val	Me	74	-	40	7.5	113
3b	Phe	Leu	Me	15	85	40	7.5	0.1
3c	Gly	Ser	Bzl	61	-	30	7.5	108
3d	Ala	Val	Bzl	-	96	30	7.5	1.2
3e	Phe	Leu	Bzl	29	87	40	7.5	0.15
3f	Gly	Leu	All	87	-	28	7.5	71
3g	Ile	Ala	All	-	78	40	7.5	0.3
3h	Phe	Leu	All	65	-	40	7.5	0.1
3i	Gly	Phe	tBu	-	98	25	8.1	21
3j	Ala	Ala	tBu	-	72	17	8.1	7.5
3k	Ser	Leu	tBu	-	78	20	8.1	0.4
3l	Thr	Ala	tBu	-	69	20	8.1	6.5
3m	Phe	Ala	tBu	-	73	40	8.1	0.1
	benzylpenicillin					-	8.1	100

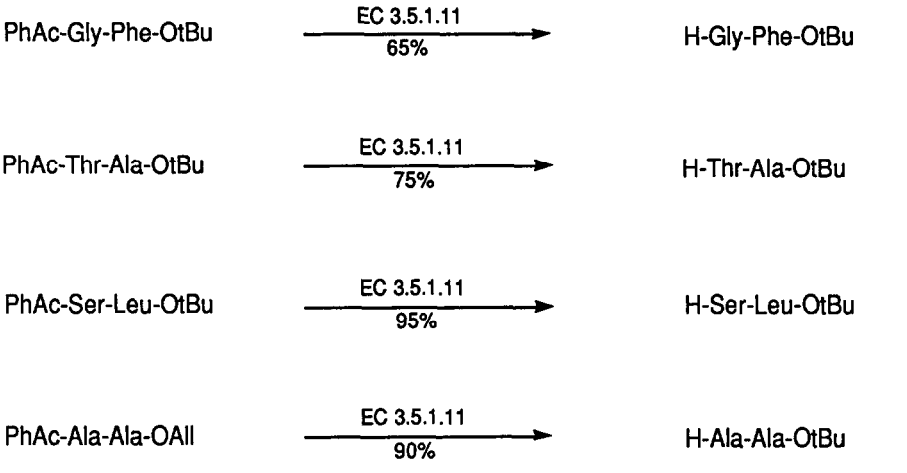
AA= amino acid; PG = protecting group; NMP = N-methyl-2-pyrrolidinone.

phenylalanine peptides are attacked only very slowly. However in the light of the recent finding that PhAc-Tyr-Ala-OH is accepted by the enzyme as a substrate[4], the low reaction rates observed for the dipeptides **3b, 3e, 3h** and **3m** could be due to limited solubility of the substrates under the reaction conditions. They also may indicate inhibition of the enzyme by the phenylalanyl structure.



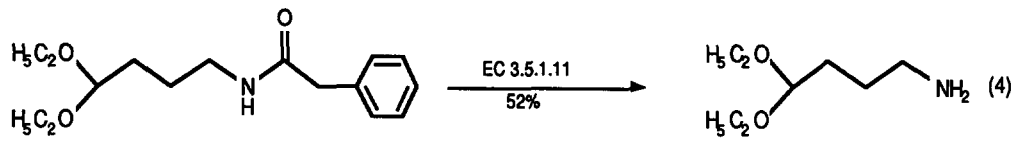
(2)

For the preparative synthesis of N-terminally deblocked dipeptide esters the tert-butyl derivatives are suited best since they are not prone to diketopiperazine formation. Thus at room temperature and pH=8.1 the partially deprotected dipeptide derivatives are obtained in good yields (scheme (3)).[10] Neither the peptide bonds nor the ester functions are attacked during the enzyme-mediated reactions. This is in accordance with earlier observations[3,4].



(3)

The enzymatic reaction can be carried out even in the presence of more sensitive functionalities like for instance the very acid-labile acetalic structure present in **4** (scheme (4)).



These results and observations that penicillin acylase also hydrolyzes the phenylacetamides of ß-amino alcohols[11], aminonitriles[12] and 1-phenyl- and 1-(naphthyl)ethyl amines[13] suggest that this enzymatic deprotection technique may find numerous applications.

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10) General procedure: 1.5 mMol of totally protected dipeptide are dissolved in a mixture of 60ml 0.01m sodium phosphate buffer and 20ml of N-methyl-2-pyrrolidinone or in a mixture of 56ml buffer and 24ml of methanol at pH=8.1 and 200 units penicillin acylase (immobilized on Eupergit C[14]) are added. Until the reaction has reached an equilibrium (ca. 20-30h), the pH is kept constant by titration with 0.1n NaOH. The enzyme is centrifuged off and after evaporation of the solvents the remaining residue is taken up in 20ml of water. The pH is adjusted to 4 and after extraction with methylenechloride to 12. The aqueous phase is then extracted five times with methylenechloride and the combined organic layers are dried over magnesium sulfate. After removal of the solvent in vacuo the dipeptide esters remain as colorless residues. They are characterized by elemental analysis and ^{1}H-NMR.
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