CLEAVAGE OF 4-PYRIDYLGLYCINE DERIVATIVES BY Ni(II)-PHTHALOCYANINE CATALYSED AUTOXIDATION - MODEL EXPERIMENTS FOR PEPTIDE SEGMENT COUPLING BY FOUR COMPONENT CONDENSATIONS

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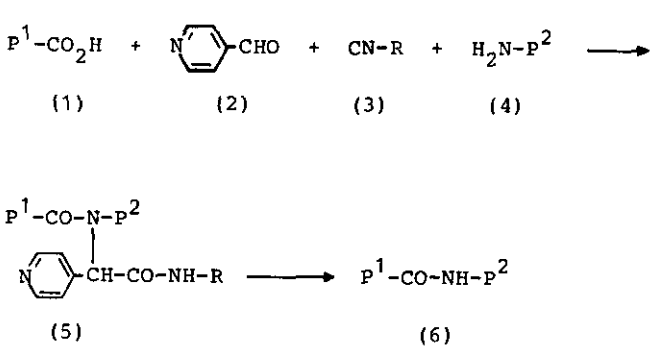
Abstract - The four component condensation of 4-pyridinecarboxaldehyde and aliphatic isonitriles with suitable protected α-amino acid derivatives yields products which can be cleaved by oxygen in the presence of nickel(II)-phthalocyanine into elongated peptide derivatives.

The cleavability of the esters and urethanes of 4-hydroxymethylpyridine[1] stimulated Waki and Meienhofer[2] to study the use of 4-pyridinecarboxaldehyde (2) as an aldehyde component for peptide segment coupling by 4CC[3]. The cleavage (5)→(6) of the 4CC products (5) has been investigated recently[2,3].

The 4-pyridylglycine derivatives (5) are obtained from carboxylic acids e.g. α-amino acid derivatives with a protected amino group (1), 4-pyridinecarboxaldehyde (2), isonitriles (3,R = tert-butyl, cyclohexyl), and primary amines such as carboxyl protected α-amino acid derivatives (4) by 4CC according to Scheme 1.

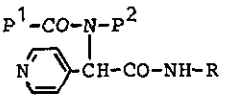
Scheme 1



The cleavage of the condensation products (5) has been already achieved by electroreduction[2], by treatment with acetanhydride in the presence of 4-dimethylaminopyridine[3], and by photolysis[4]. However, none of these model experiments yielded results on which a method for peptide segment coupling could be based.

We have now found that the peptide derivatives (5b-g, see Table 1) are cleaved by autoxidation with pure oxygen[5].

Table 1[+]



(5)

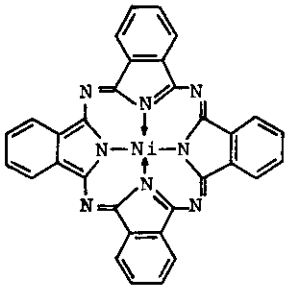
	R	P^1-CO	-N-P^2
a	Bu-t	Ø-CH_2-CO-	-N-CH_2-Ø
b	Bu-t	Z-Val-	-Ala-OEt
c	Bu-t	Z-Val-	-Gly-OMe
d	Hx-c	BOC-Gly-Ala-	-Leu-OEt
e	Hx-c	BOC-Gly-Leu-	-Ala-OEt
f	Hx-c	BOC-Gly-Leu-	-Leu-Gly-OEt
g	Hx-c	BOC-Gly-Leu-	-Ala-Gly-OEt

[+]All abbreviations are conventional, except:

AcOEt	ethylacetate
BOC	$(CH_3)_3C$-O-CO-
Bu-t	tert - butyl
Hx-c	cyclohexyl
MeOH	methanol
Ni(II)Pc	nickel(II)phthalocyanine
OEt	ethylester
OMe	methylester
Z	Ø-CH_2-O-CO-

The model compound (5a), however, undergoes autoxidation very slowly in the absence of radical initiators and catalysts whereas it is rapidly cleaved by oxygen in the presence of dicyclohexylperoxydicarbonate as a radical initiator,

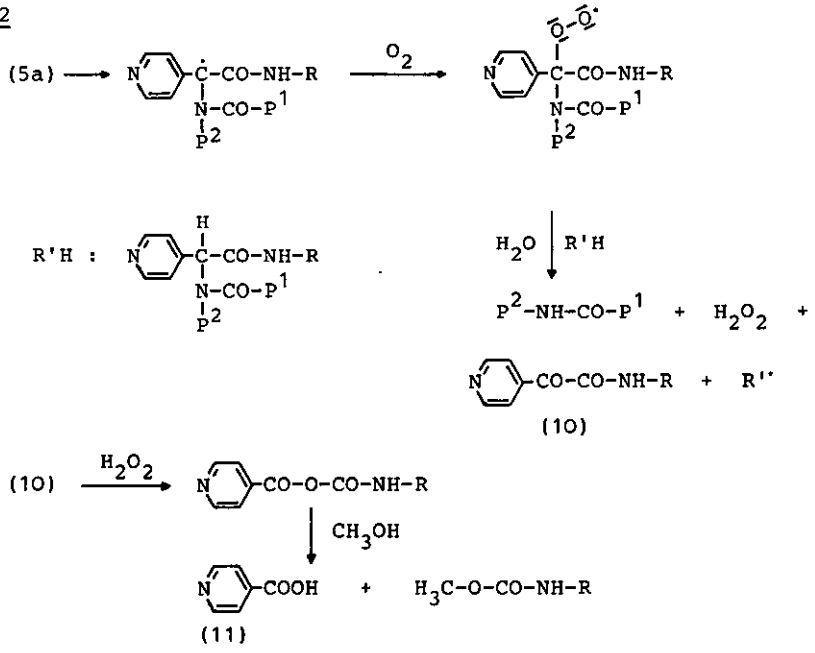
or in the presence of Ni(II)Pc (7).



(7)

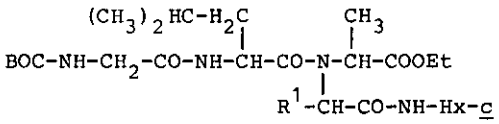
Since the compounds (10) - (11) have been isolated as coproducts of the autoxidation of (5a), we assume that the autoxidation of the 4-pyridylglycine derivatives (5) proceeds by a mechanism which is described by Scheme 2.

Scheme 2



R: Bu-t

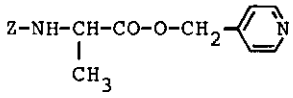
The 4CC products (8) and (9) of 2-pyridinecarboxaldehyde and 3-pyridinecarboxaldehyde are stable against autoxidation by pure oxygen.



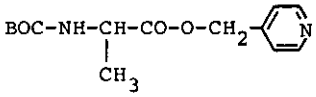
(8) R^1: 

(9) R^1: 

The alanine derivatives (12) and (13)



(12)



(13)

are neither autoxidized by pure oxygen, nor by oxygen in the presence of dicyclohexylperoxydicarbonate, or Ni(II)Pc (7). Thus autoxidation is not generally usable in the cleavage of 4-pyridylmethyl-protected peptide derivatives.

Experimental

Melting points are uncorrected. ^{1}H-nmr-spectra were carried out on a Varian A 60, Varian EM 360 (60 MHz) and on a Bruker WP 200 (200 MHz) spectrometer. The ir-spectra were measured on a Perkin Elmer model 177 and 257. Optical rotation was determined with a Perkin Elmer model 141. TLC (silica gel 60 F_{254}, E. Merck) was mainly carried out with the solvent system CH_2Cl_2/AcOEt/MeOH(10:1.5:1).

Materials

Glycine, L-alanine, L-leucine and L-valine were purchased from Carl Roth KG, H-Gly-OMe·HCl and H-Gly-OEt·HCl were obtained from E. Merck. All other protected α-amino acids were prepared by literature procedures[6]. The three isomeric pyridinecarboxaldehydes were purchased from E. Merck. Benzylamine was redistilled before use. The dicyclohexylperoxydicarbonate (Interox CHPC from Peroxid-Chemie GmbH) was technically pure.

Segment Condensation

N-(α-tert-butylcarbamoyl)-4-pyridylmethyl-phenylaceticacid-N-benzylamide (5a)

Benzylamine (3.24 ml, 30 mM) was added to a stirred solution of 4-pyridinecarboxaldehyde (2.82 ml, 30 mM) in 45 ml of dry methanol containing some molecular sieve (3 Å), followed after 10 min. by tert-butylisocyanide (3.80 ml, 33 mM) and phenylacetic acid (4.08 g, 30 mM).

The reaction was complete after stirring 20h at 20°C. A pale yellow solid had precipitated. The solvent was evaporated and the residue dissolved in CH_2Cl_2. The solution was washed twice with 20 ml of 3% tartaric acid, 7.5% $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The residue was recrystallized from ether to yield colourless needles: 10.72 g (86%), mp 158 - 158.5°C.
Anal. ($C_{26}H_{29}N_3O_2$): calc. C 75.15 H 7.03 N 10.11, found C 75.04 H7.18 N 9.95.
ir (KBr): 3270 (m), 1685 (s), 1630 (vs), 1555 (m). ^{1}H-nmr ($CDCl_3$): 8.45 (2H, m), 7.5 - 6.90 (12H, m), 6.38 (1H, m), 5.87 (1H, m), 4.71 (2H, m), 3.71 (2H, s), 1.26 (9H, s).

N^{α}-Benzyloxycarbonylvalyl-N^{α}-(α-tert-butylcarbamoyl-4-pyridylmethyl)alanine Ethylester (5b)

4-pyridinecarboxaldehyde (0.47 ml, 5 mM) was added to a cooled (0°C), stirred solution of H-Ala-OEt·HCl (0.77 g, 5 mM) and triethylamine (0.73 ml, 5.25 mM) in 10 ml of dry methanol. After 2h tert-butylisocyanide (0.63 ml, 5.5 mM) and Z-Val-OH (1.26 g, 5 mM) were added and the reaction mixture was stirred for 48 h at 20°C. The solvent was evaporated and the oily residue dissolved in AcOEt/MeOH (10:0.5). Washings and work up as described for (5a) provided a crude condensation product which was cleaved without further purification.

N^{α}-Benzyloxycarbonylvalyl-N^{α}-(α-tert-butylcarbamoyl-4-pyridylmethyl)glycine Methylester (5c)

The product was prepared in the same scale and under the same conditions as described for (5b).

N^{α}-tert-butyloxycarbonylglycylalanyl-N^{α}-(α-cyclohexylcarbamoyl-4-pyridylmethyl) leucine Ethylester (5d)

This condensation product and the three other products (5e - 5g, see Table 1), which only differ in the sequence of the α-amino acids, were prepared on a 3 mM scale by use of BOC-protected dipeptide derivatives and amino acid or dipeptide ethylesters together with cyclohexylisocyanide and 4-pyridinecarboxaldehyde according to the procedure described for (5b).

Oxidative Cleavage

Oxidation of (5a) in the presence of Ni(II)Pc

To 1.00 g (2.41 mM) of (5a), dissolved in 10 ml AcOEt/MeOH (10:1), was added an equimolar quantity (1.37 g) of Ni(II)Pc. This reaction mixture was then saturated with pure oxygen and stirred vigorously for 24 h, while oxidation was monitored by TLC. The Ni(II)Pc was filtered off and the solution purified by filtration over a small column filled with charcoal, which was then rinsed thoroughly with methanol. After evaporation of the solvent 0.20 g of the obtained mixture were chromatographed by PTLC, solvent CH_2Cl_2/AcOEt/MeOH (10:1.5:0.8). The isolated phenylaceticacid-N-benzylamide was identified by TLC, ir- and ^{1}H-nmr-spectroscopy.

Analytical data of the isolated compounds (10) and (11):

Isonicotinoyl-tert-butylformamide (10)

Oil, ir (KBr) : 3330 (s), 1665 (s), 1645 (vs), 1525 (m). ^{1}H-nmr ($CDCl_3$) : 8.87 (2H, m), 8.13 (2H, m), 7.12 (1H, m), 1.47 (9H, s). MS (70 eV) : no M^+, m/e 79, 100, 107. 2,4-dinitrophenylhydrazone: yellow plates, decomp. > 220°C. ir(KBr): 3440 (m), 1655 (m), 1610 (s), 1595 (m), 1555 (w), 1490 (vs). MS (20 eV): m/e 386 (M^+), 330, 151, 106.

Isonicotinic acid (11)

White solid, mp 315°C (closed tube). Anal. ($C_6H_5NO_2$): calc. C 58.54 H 4.10 N 11.38, found C 58.17 H4.17 N 11.49. ir(KBr): 3650-3150 (m), 1700 (s). ^{1}H-nmr(CF_3COOH): 9.71 (2H, m), 8.85 (2H, m). MS (70 eV): m/e 123 (M^+), 78.

Oxidation of compounds (5b) to (5g) with pure oxygen

The oxidation of these 4CC products was carried out as described for (5a). No Ni(II)Pc or other radical initiator was used. After the reaction was complete the solvent was evaporated and from each sample 0.20g were chromatographed by PTLC.

Analytical data of the peptide derivatives obtained from (5b) to (5g):

Z-Val-Ala-OEt (from (5b))

Time of cleavage : 3 d. Solvent for chromatography: CH_2Cl_2/AcOEt (2:1), white solid, mp 155-156°C. (CH_2Cl_2/pentane), yield 0.04 g (18%, referred to H-Ala-OEt). Anal. ($C_{18}H_{26}N_2O_5$): calc. C 61.70 H 7.48 N 7.99, found C 61.40 H 7.63 N 7.78.
ir(KBr): 3300 (m), 1735 (m), 1695 (m), 1650 (s), 1540 (m).
^{1}H-nmr($CDCl_3$): 7.36 (5H, s), 6.36 (1H, m), 5.37 (1H, m), 5.12 (2H, s), 4.57 (1H, m), 4.20 (2H, q), 4.06 (1H, m), 2.15 (1H, m), 1.67 (2H, m), 1.43 (2H, d), 1.28 (3H, t), 0.96 (6H, dd). $[\alpha]_D^{20}$ = - 42.9° (c = 0.1, EtOH) (lit.[7] $[\alpha]_D$ = - 40.0° (c = 1.4, EtOH).

Z-Val-Gly-OMe (from (5c))

Time of cleavage: 3 d. Solvent system: CH_2Cl_2/AcOEt (5:4). White solid, mp 161-162°C (lit.[8] 160-162°C), (CH_2Cl_2/AcOEt/pentane), yield 0.02 g (8%, referred to H-Gly-OMe). Anal. ($C_{16}H_{22}N_2O_5$): calc. C 59.62 H 6.88 N 8.69, found C 59.51 H 6.83 N 8.65.
ir(KBr): 3290 (m), 1755 (m), 1690 (m), 1660 (s), 1565 (m), 1540 (m).
^{1}H-nmr($CDCl_3$): 7.20 (5H, s), 6.87 (1H, m), 5.37 (1H, m), 5.03 (2H, s), 3.97 (2H, d), 3.68 (3H, s), 2.07 (1H, o), 0.95 (6H, dd). $[\alpha]_D^{20}$ = - 27.5° (c = 0.1, MeOH) (lit.[8] $[\alpha]_D$ = - 26.7° (c = 3, MeOH).

BOC-Gly-Ala-Leu-OEt (from (5d))

Time of cleavage: 8 d. Solvent system: CH_2Cl_2/AcOEt/MeOH (10:1.5:1). Pale yellow oil, yield 0.09 g (49%, referred to H-Leu-OEt). Anal. ($C_{18}H_{33}N_3O_6$): calc. C 55.80 H 8.58 N 10.84, found C 56.10 H 8.80 N 10.52.
ir($CHCl_3$): 3410 (m), 1720 (s), 1670 (vs), 1490 (m).
^{1}H-nmr($CDCl_3$): 7.28-6.92 (2H, m), 5.7-5.32 (1H, m), 4.91-4.25 (2H, m), 4.20 (2H, q)

3.84 (2H, d), 1.88-1.52 (3H, m), 1.52 (9H, s), 1.52-1.13 (6H, d, t), 0.93 (6H, d). $[\alpha]_D^{20}$ = - 51.1° (c = 0.4, MeOH).

BOC-Gly-Leu-Ala-OEt (from (5e))

Time of cleavage: 8 d. Solvent system: CH_2Cl_2/AcOEt/MeOH (10:1.5:1). White solid, mp 149-150°C, yield 0.08 g (44%, referred to H-Ala-OEt). Anal. ($C_{18}H_{33}N_3O_6$): calc. C 55.80 H 8.58 N 10.84, found C 55.77 H 8.52 N 10.91.
ir(KBr): 3300 (s), 1750 (s), 1705 (m), 1680 (s), 1655 (vs), 1565 (m), 1520 (s).
^{1}H-nmr($CDCl_3$): 7.2 (2H, m), 5.6 (1H, m), 4.79-4.30 (2H, m), 4.19 (2H, q), 3.82 (2H, d), 1.87-1.42 (3H, m), 1.42 (9H, s), 1.42-1.17 (6H, d, t), 0.93 (6H, d). $[\alpha]_D^{20}$ = - 49.5° (c = 0.5, MeOH).

BOC-Gly-Ala-Leu-Gly-OEt (from (5f))

Time of cleavage: 8 d. Solvent system: CH_2Cl_2/AcOEt/MeOH (10:1.5:1). White solid, mp 179-180°C, yield 0.08 g (26%, referred to H-Leu-Gly-OEt). Anal. ($C_{20}H_{36}N_4O_7$): calc. C 54.04 H 8.16 N 12.60, found C 53.43 H 8.10 N 12.88.
ir(KBr): 3280 (s), 1745 (m), 1720 (m), 1685 (s), 1665 (s), 1640 (vs), 1570-1500 (s).
^{1}H-nmr(DMSO-d_6): 8.30 (1H, m), 8.02 (1H, m), 7.91 (1H, m), 7.03 (1H, m), 4.45-4.20 (2H, m), 4.08 (2H, q), 3.82 (2H, m), 3.55 (2H, d), 1.69-1.38 (3H, m), 1.38 (9H, s), 1.32-1.00 (6H, d, t), 0.85 (6H, dd). $[\alpha]_D^{20}$ = -57.6° (c = 0.15, MeOH).

BOC-Gly-Leu-Ala-Gly-OEt (from (5g))

Time of cleavage: 4 d. Solvent system: CH_2Cl_2/AcOEt/MeOH (10:1.5:1). White solid, mp 160-161.5°C, yield 0.07 g (24%, referred to H-Ala-Gly-OEt). Anal. ($C_{20}H_{36}N_4O_7$): calc. C 54.04 H 8.16 N 12.60, found C 53.60 H 8.09 N 12.72.
ir(KBr): 3270 (s), 1730 (m), 1700 (m), 1680 (s), 1630 (vs), 1560-1500 (s).
^{1}H-nmr(DMSO-d_6): 8.25 (1H, m), 8.13 (1H, d), 7.85 (1H, d), 7.0 (1H, m), 4.50-4.20 (2H, m), 4.10 (2H, q), 3.83 (2H, m), 3.56 (2H, d), 1.74-1.38 (3H, m), 1.38 (9H, s), 1.30-1.05 (6H, d, t), 0.85 (6H, t). $[\alpha]_D^{20}$ = - 55.0° (c = 0.15, MeOH).

Benzyloxycarbonylalanyl-4-pyridylmethylester (12)

At 20°C dicyclohexylcarbodiimide (4.13 g, 20 mM) was added to a well stirred suspension of Z-alanine (4.46 g, 20 mM) and 4-pyridinemethanol (1.96 g, 18 mM) in 90 ml CH_2Cl_2. After 20 h the formed dicyclohexylurea was filtered off and washed with CH_2Cl_2. The solvent was then reduced to a volume of about 70 ml which was washed twice with 25 ml of 7.5% $NaHCO_3$ and 15 ml of water. Drying ($MgSO_4$) and evaporating the CH_2Cl_2 yielded a crude product of which 2.00 g were extracted with three portions of 20 ml citric acid (2M). This solution was neutralized by excess solid $NaHCO_3$ and extracted with ethylacetate. Recrystallisation of the product from AcOEt/ether yielded 0.97 g of a white solid, mp 108.5 - 110°C. Anal. ($C_{17}H_{18}N_2O_4$): calc. C 64.96 H 5.77 N 8.91, found C 64.79 H 5.90 N 8.70.
ir (KBr): 3220 (w), 3040 (w), 2990 (w), 2950 (w), 1750 (s), 1710 (vs), 1610 (m), 1570 (s), 1560 (m).
^{1}H-nmr ($CDCl_3$): 8.63 (2H, dd), 7.37 (5H, s), 7.24 (2H, m), 6.00-5.52 (1H, m), 5.19 (2H, s), 5.15 (2H, s), 4.55 (1H, q), 1.44 (3H, d).

tert-butyloxycarbonylalanyl-4-pyridylmethylester (13)

This compound was synthesized and purified as described for (12), extraction was carried out with four 20 ml portions of citric acid (0.7 M). Yield: 1.18g, white crystals (ether/pentane), mp 68-69°C. Anal. ($C_{14}H_{20}N_2O_4$): calc. C 59.99 H 7.19 N 9.99, found C 59.93 H 7.20 N 9.92.
ir(KBr): 3380 (s), 2990 (m), 2940 (w), 1740 (vs), 1685 (vs), 1610 (m), 1515 (vs).
^{1}H-nmr ($CDCl_3$): 8.47 (2H, dd), 7.15 (2H, m), 5.53-5.20(1H, m), 5.12 (2H, s), 4.3 (1H, q), 1.47 (9H, s), 1.38 (3H, d).

Acknowledgements

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