Tetrahedron Letters,Vol.30,No.33,pp 4423-4426,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

2-HYDROXYIMINO-2-PHENYLACETONITRILE ACTIVE ESTERS IN PEPTIDE SYNTHESIS

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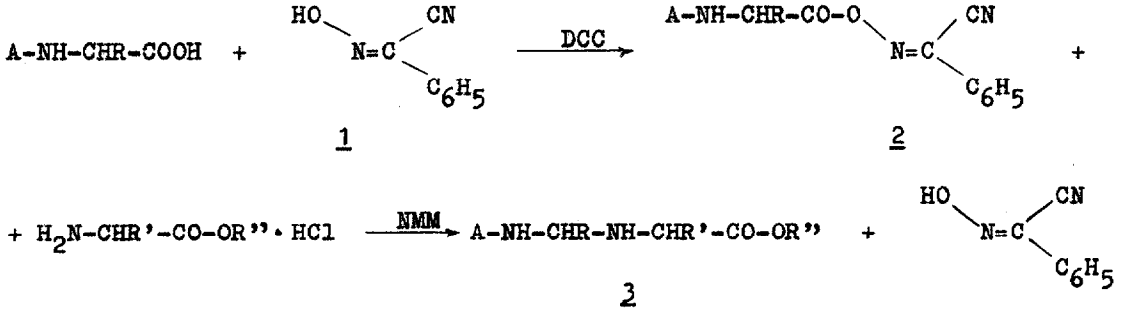
Abstract: A number of 2-hydroxyimino-2-phenylacetonitrile esters of acylamino acids have been synthesized. These compounds react readily with amino acid or peptide esters to elongate the peptide chain.

The elongation of the peptide chain by active esters is a smooth method which can also be applied to peptides containing free carboxyl groups. Active esters of N-urethane protected amino acids with e.g. pentachlorophenol[1] or N-hydroxysuccinimide[2] are prepared with dicyclohexylcarbodiimide (DCC) without recemization as crystaline solids, which can be stored over appreciable periods.

In this communication we present a novel, simple and effective route for the peptide synthesis via 2-hydroxyimino-2-phenylacetonitrile active esters.



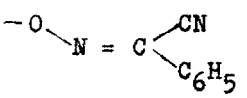
1 2 3

A - urethane protected group
NMM - N-methylmorpholine

2-Hydroxyimino-2-phenylacetonitrile 1 is a stable, easily accessible compound. It was used by Itoh et al[3] for the preparation of the new reagent for tert-butoxycarbonylation and also by Przybylski and Sokołowska[4] as the antiracemization additive in peptide synthesis by the method of mixed anhydrides. We found that 1[5] forms active esters 2 with a number of N-protected amino acids with high yield (Table 1).

Table 1. Active esters 2 prepared

entry	active ester[6]	m.p. °C	$[\alpha]_D^{20}$	yield %
1	Boc-Ala-ON[a]	oil	-26,6[c]	77
2	Boc-Arg(NO_2)-ON	68-70	-10,4[b]	76
3	Boc-Asn-ON	118-120	-2,6[b]	81
4	Boc-Asp(OBzl)-ON	106-107	-19,0[b]	74
5	Boc-Gln-ON	138-139	-21,8[b]	80
6	Boc-Glu(OBzl)-ON	109-111	-18,4[b]	75
7	Boc-Gly-ON	76-77	-	87
8	Boc-Leu-ON	oil	-46,8[c]	78
9	Boc-Lys(Z)-ON	85-87	-20,4[b]	81
10	Boc-Met-ON	95-96	-51,8[b]	79
11	Boc-Pro-ON	46-47	-21,0[b]	91
12	Boc-Phe-ON	80-81	-4,8[b]	75
13	Boc-Ser(OBzl)-ON	oil	-31,4[c]	78
14	Boc-Thr-ON	158-160	-8,7[c]	72
15	Boc-Trp-ON	111-117	+5,8[b]	75
16	Boc-Tyr(OBzl)-ON	122-123	+6,4[b]	85
17	Boc-Val-ON	oil	-13,2[c]	78
18	Z-Asn-ON	133-135	-15,0[b]	82
19	Z-Gln-ON	146-148	-14,2[b]	96
20	Z-Ile-ON	77-78	-43,4[c]	83
21	Z-Phe-ON	129-131	-7,8[b]	88
22	Z-Pro-ON	96-97	-48,0[b]	97
23	Z-Val-ON	88-89	-20,4[b]	75

a. -ON = 

b. Optical rotation in DMF, c = 1.

c. Optical rotation in methanol, c = 1.

N-tert-butoxycarbonyl- and benzyloxycarbonyl- protective groups were used. The following procedure was employed: equimolar amounts of N-protected amino acid, 1 and DCC were stirred in ethyl acetate or dimethylformamide for 1 h at 0°C and for additional 1 h at room temperature. Dicyclohexylurea was filtered off and the solvent evaporated. Crude esters were precipitated with petroleum ether and, if necessary, recrystallized from methanol

The same active esters were obtained when the procedure typical for mixed anhydrides was used: equimolar amounts of N-protected amino acid, NMM and iso-butylchloroformate were stirred in THF at -15°C. After 10 min the solution of equimolar amount of 1 and NMM was added and the stirring continued for 1 h at -15°C and for 1 h at room temperature. The mixture was then treated in the usual manner to give the appropriate active ester.

Aminolysis of the active esters by means of hydrochlorides of amino acid esters was carried out at room temperature in dioxan/water (1:1) solution for 3 h in the presence of equimolar amount of NMM. Dipeptides were either precipitated during the reaction or precipitated afterwards with diluted hydrochloric acid. In the latter case the crude product was dissolved in ethyl acetate and washed with the aqueous sodium bicarbonate solution to remove 1[7]. The yield of pure dipeptides 2 varied from 70 to 90%.

To demonstrate the usefulness of this method the N-terminal pentapeptide of Substance P, Arg-Pro-Lys-Pro-Gln, was synthesized in 26% overall yield refering to the protected peptide (Table 2).

Table 2. Synthesis of N-terminal Substance P pentapeptide.

entry	peptide	yield %	m.p., °C	$[\alpha]_D^a$
1	Boc-Pro-Gln-OBzl	80	116-118	-42
2	Boc-Lys(Z)-Pro-Gln-OBzl	76	127-128	-48
3	Boc-Pro-Lys(Z)-Pro-Gln-OBzl	73	143-145	-28
4	Boc-Arg(NO_2)-Pro-Lys(Z)-Pro-Gln-OBzl[b]	70	162-164	-41,2

a. Optical rotations were taken in methanol, c = 1.

b. Amino acid analysis Arg 0.95, Pro 2, Lys 0.95, Glu 0.92

Acknowledgment: This work was supported by the Polish Academy of Sciences, project 06.03.4.5.

References and Notes

1. Kovacs J., Kisfaludy L., Ceprini M.Q., Johnson R.H., Tetrahedron, 25, 2555 (1969).
2. Anderson G.W., Zimmerman J.E., Callahan F., J.Am.Chem.Soc., 85, 3039, (1963).
3. Itoh M., Hagiwara D., Kamiya T., Bull.Chem.Soc. Japan, 50, 718 (1977).
4. Przybyłski J., Sokołowska B., Polish J.Chem., 58, 455 (1984).
5. More accessible anti isomer of 2-hydroxyimino-2-phenylacetonitrile was used, see Organic Synthesis, 59, 95 (1979).
6. All newly synthesized compounds were characterized by elemental analysis, ^{1}H-NMR and IR spectra. The presence of phenyl group at 7.2-8.1 ppm and nitrile group at 2280 cm^{-1} was characteristic.
7. 1 can be recovered from the alkaline solution by acidifying and recrystallization from water.

(Received in UK 4 July 1989)