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A generally applicable synthesis of amino acid *p*-nitroanilides as synthons

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Introduction

Amino acid *p*-nitroanilides are versatile chromogenic substrates for proteolytic enzymes [1]. Their synthesis is problematic because of the low nucleophilicity of *p*-nitroaniline, since the current coupling methods of peptide synthesis appear inadequate. In an earlier paper we mentioned the applicability of phosphorus oxychloride as condensing agent in the synthesis of protected arginine *p*-nitroanilides [2]. We now describe here the use of this reagent in the synthesis of orthogonally protected *p*-nitroanilides and their transformation into synthons for chromogenic substrates.

Results and Discussion

We found that phosphorus oxychloride [3] is an excellent condensing agent for amines and alcohols (J. Broos, personal communication) of low nucleophilicity (Scheme 1). Virtually all Boc- and Z-protected amino acid *p*-nitroanilides were obtained in high yield (70–90%) in an optically pure form, some being given in Table 1. We also found Boc-Ala-pNA to be excellently stable in 50% piperidine in DMF, which prompted us to synthesize Fmoc-amino acid *p*-nitroanilides, which have not been mentioned in the literature until now (Table 1). The use of Fmoc as α -amino protection allows side chain protections which can be cleaved off under mildly acidic conditions at the end of the synthesis. As an example, Scheme 2 depicts the synthesis of the chromogenic substrate (S2238) used in the determination of thrombin.

Bz-Ile-Glu-Gly-Arg-pNA.HCl (S2222), Bz-Ile-Glu(N-piperidyl)-Gly-Arg-pNA.HCl (S2337), from Boc-Arg-pNA.HCl and H-D-Val-Leu-Lys-pNA.2HCl

Scheme 1. Equimolar amounts of phosphorus oxychloride and carboxylic acid are required in this procedure. X symbolizes an α -amino protective group, X' a side-chain protection and AA stands for an amino acid.

Table 1 Some protected amino acid *p*-nitroanilides synthesized with phosphorus oxychloride as condensing agent

	Y (%)	m.p. (°C)	α_D		Y (%)	m.p. (°C)	α_D
Boc-Glu(OBzl)-pNA	84	foam	-7.9 ^a	Fmoc-Glu(OtBu)-pNA	83	98	-26.8 ^b
Boc-Lys(Z)-pNA	84	foam	-8.1 ^a	Fmoc-Ser(tBu)-pNA	79	foam	-36.2 ^b
Boc-Arg(HCl)-pNA	91	187	-12.8 ^a	Fmoc-Tyr(tBu)-pNA	79	94 (dec.)	+24.5 ^b
Z-Arg(HCl)-pNA	96	174	-10.7 ^a	Fmoc-Arg(HCl)-pNA	88	96 (dec.)	-45.5 ^b
Msc-Arg(HCl)-pNA	65	amorph	-12.4 ^a	Fmoc-Lys(Boc)-pNA	81	116-117	-23.3 ^b
Fmoc-Gly-pNA	89	212-213		Fmoc-Cys(Trt)-pNA	90	foam	-18.8 ^b
Fmoc-Phe-pNA	88	197-199	+13.3 ^b	Fmoc-His(Trt)-pNA	95	foam	-6.1 ^b
Fmoc-Val-pNA	70	189-191	-27.2 ^b	Fmoc-Asn(Trt)-pNA	71	214-215	-25.3 ^b
Fmoc-Met-pNA	79	183	-39.1 ^b				

^a c = 1, MeOH.

^b c = 1, DMF.

(S2251), from Boc-Lys(Z)-pNA or Fmoc-Lys(Boc)-pNA, were synthesized in high yield as substrates for factor X_a and plasmin, respectively, and exhibited the known kinetic data. Our conclusion, that phosphorus oxychloride is a powerful condensing agent in the synthesis of *p*-nitroanilides, is confirmed by these results.

Scheme 2. Synthesis-route of S2238, a chromogenic substrate for thrombin.

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3. Used for the first time as coupling reagent in peptide synthesis by Wieland et al., see: Liebigs Ann. Chem., 599(1956)70. The synthesis of an amino acid *p*-nitroanilide with phosphorus oxychloride was for the first time carried out by one of us (G.I.T.) and recommended by Planta, R.J. and Gruber, M., Biochim. Biophys. Acta.