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2-Phenyl Isopropyl Esters as Carboxyl Terminus Protecting Groups in the Fast Synthesis of Peptide Fragments

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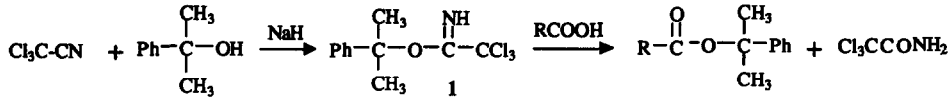
Abstract. *The esters of Fmoc aminoacids derived from 2-phenylisopropanol have been prepared by using 2-phenylisopropyltrichloroacetimidate 1. They prevent diketopiperazine formation during amino deprotection of dipeptides and can be cleaved in the presence of Boc and O-Bu^t. They are thus well suited for the protection of C-terminus when a Fmoc strategy is used to build up peptide fragments.*

The introduction of the base-sensitive fluorenemethoxycarbonyl (Fmoc) group[1] in peptide synthesis has led to great improvements in both classical and solid phase peptide synthesis.[2] The Fmoc Amino Acid Chloride Solution Technique (FAACST)[3] using acyl chlorides allows expedient syntheses of small peptides. The major limitation of this methodology is the impossibility to prepare the acyl chloride of Fmoc amino acids bearing some acid-sensitive protecting groups on the side chain. This drawback has been circumvented by using pentafluorophenyl esters whenever trifunctional amino acids bearing protecting groups derived from t-butanol were used[4] or more conveniently by using non-activated Fmoc amino acids and PyBOP®, as the carboxyl activating reagent as recently reported.[5] This approach could be used to prepare longer peptide fragments provided that the carboxyl terminus is protected by a group labile under conditions where Boc, O-Bu^t and Fmoc groups are stable. In the course of our studies concerning the tetrapeptide NAcSDKP[6], a stem cell proliferation inhibitor, we needed a set of protecting groups in order to be able to elongate the tetrapeptide either towards the C- or the N-terminus. Several esters were eligible for protection of the C-terminus. Some phenacyl esters[7] have already been reported in the literature as producing diketopiperazines at the dipeptide stage, especially when Pro was the C-terminal amino acid. We prepared the dipeptides Fmoc-Lys(Boc)-ProOR where R = Cam[8], Bg[9], Phe[10] which turned out to give a very easy cyclization during Fmoc deprotection of the dipeptide. The use of the ester derived from 2-phenylisopropanol was also explored. This group though closely resembling the O-t-butyl one, is more sensitive towards acid on account of its phenyl group. It can also be looked upon as a substituted benzyl ester. This group, first reported by Blotny and Taschner[11], has never been used since then as a carboxyl protecting group in peptide synthesis although it can be cleaved selectively in the presence of a t-butyl ester.[12] The preparation of 2-phenylisopropylesters according to the procedure used to prepare t-butylesters (DCCI, DMAP, 2-phenylisopropanol)[13] was unsuccessful. Another method described[14] for the preparation of t-butyl esters, using the imidate derived from the condensation of t-butanol with trichloroacetonitrile, was investigated to prepare 2-phenylisopropylesters. 2-Phenylisopropyltrichloroacetimidate 1 has been prepared by reacting 2-phenylisopropanol with trichloroacetonitrile in the presence of NaH (0.1 equiv.).[15]



The reaction of **1** (2 equiv.) with Fmoc-Pro **2** according to ref. 14, with BF_3-etherate as a catalyst, was not successful. Only in the absence of BF_3-etherate did the reaction succeed, giving the Fmoc-ProOPp ester in good yield (82%).

General Procedure

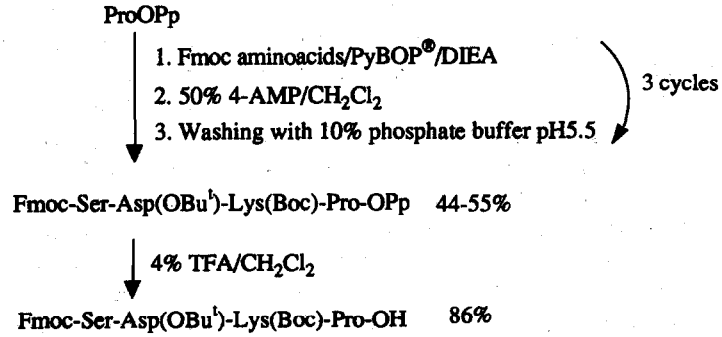
To a solution of Fmoc aminoacid (0.25 mmole) in 1.5 ml CH_2Cl_2 was added a solution of the imidate **1** (2 equiv.) in cyclohexane.[16] After stirring overnight at room temperature, the precipitate of trichloroacetamide was filtered off and the filtrate was evaporated to dryness. Flash chromatography of the residue yielded the pure ester.

The esters, Fmoc-Asp(OBu^t)OPp **5**,[17] and Fmoc-Lys(BocOPp **6**, have been prepared from the protected aminoacids Fmoc-Asp(OBu^t) **3** and Fmoc-Lys(Boc) **4** respectively in excellent yields (90% and 98%).

In order to evaluate the chemoselectivity of the esterification reaction, the ester of Z-Ser **7** has been prepared. Tetrahydrofuran was used as a cosolvent to achieve the solubilization of the protected amino acid. The reaction proceeded quite nicely as in the previous examples giving the Z-SerOPp ester in 67% yield along with the dialkylated product Z-Ser(Pp)OPp (13% yield).

The conditions for selective cleavage of the ester group have been studied with compounds **5** and **6** to check the stability of the OBu^t and Boc groups. Deprotection of **5** and **6** with 2% TFA/CH_2Cl_2 at room temperature was complete after one hour with 2% TFA/CH_2Cl_2. It yielded respectively **3** and **4** and no cleavage of Boc or OBu^t group was observed by TLC. A catalytic amount of BF_3-etherate in CH_2Cl_2 was sufficient to cleave the ester within minutes but the selectivity as regard to Boc and OBu^t groups was mediocre. 80% acetic acid slowly hydrolyzed the ester.

The dipeptide Fmoc-Lys(Boc)-ProOPp has also been prepared from Fmoc-Lys(Boc) and 2-phenylisopropyl prolinate.[18] Deprotection of Fmoc under classical conditions (20% piperidine in CH_2Cl_2) yielded the deprotected dipeptide ester and no diketopiperazine could be found after the 30 min. reaction.



Scheme 1

Finally, the synthesis of the tetrapeptide Fmoc-Ser-Asp(OBu^t)-Lys(Boc)-ProOPp was carried out by using the procedure recommended by Hoeg-Jansen *et al.*[5] starting from ProOPp (Scheme 1). The fully protected tetrapeptide synthesized within a day has been purified on a silica gel column (44-55% overall yield from ProOPp). The acidolysis of the C-terminal ester was performed with 4% TFA/CH_2Cl_2 for 15 min. at room temperature. The higher concentration of TFA allowed completion of the reaction within a shorter time and no deprotection of Boc or OBu^t was detected under these conditions. The resulting fragment was purified on a silica gel column (86% yield).[19]

The esters of Fmoc aminoacids derived from 2-phenylisopropanol have been prepared in excellent yields under very mild conditions by using the 2-phenylisopropyltrichloroacetimidate. The usefulness of these esters as C-terminus protecting groups has been demonstrated by the synthesis of the tetrapeptide Fmoc-Ser-Asp(OBu^t)-Lys(Boc)-ProOPp. Selective deprotection of the C-terminus ester has been achieved by mild acidolysis yielding a peptide fragment available for further elongation through its C-terminus.

References and Notes

Abbreviations : OPp was proposed as the abbreviation for the 2-phenylisopropyl ester in accordance with the one (Ppoc) proposed for the corresponding urethane by Sandberg and Ragnarsson : *Int. J. Peptide Protein Res.*, **1974**, *6*, 111-119. PyBOP®, benzotriazolyloxytris(pyrrolidino) phosphonium hexafluorophosphate; Boc : t-butyloxycarbonyl; OBu^t : t-butyl ester; 4-AMP : 4-aminomethylpiperidine; DIEA : diisopropylethylamine; TFA : trifluoroacetic acid; Cam : carboxamidomethyl, Bg : N-Benzydrylglycolamide.

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16. Imidate **1** was prepared according to ref. 15 (86% crude yield). **1** was stored as a solution in cyclohexane (1 ml/mmole) and used crude for the esterification reaction. ^{1}H-NMR spectroscopy showed the presence of about 5% 2-phenylisopropanol which was easily removed during chromatography of the ester.

^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.9 (s, 6H, 2 x CH_3), 7.2 - 7.5 (m, 5H, aromatic protons), 8.2 (br s, 1H, NH). ^{13}C-NMR (62.5 MHz, $CDCl_3$) δ 28.2 (2 x CH_3), 85.1, 92.7, 123.3, 127.2, 128.6, 145.1, 159.4.

17. Fmoc-Asp(OBu^t)OPp : ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.75 (s, 3H), 1.85 (s, 3H), 2.75-3.0 (dq, 2H, J = 4.5, 16.9 Hz), 4.2 (t, 1H, J = 7 Hz), 4.35 (m, 2H), 4.55 (m, 1H), 5.82 (bd, 1H, J = 8.5 Hz), 7.15-7.80 (m, 13H).
^{13}C-NMR (62.5 MHz, $CDCl_3$) δ 28.0, 28.1, 28.6, 37.8, 47.2, 51.1, 67.2, 81.7, 83.6, 120.0, 124.3, 125.2, 127.1, 127.2, 127.7, 128.3, 141.3, 143.8, 143.9, 145.1, 156.0, 169.4, 170.2.

18. ProOPp was prepared from Fmoc-ProOPp by the classical method (20% piperidine, CH_2Cl_2) in 70-86% yield. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 1.8-2.3 (m, 10H), 2.6-3.2 (m, 3H), 3.75 (q, 1H, J= 8.3 Hz), 7.3 (m, 5H).
^{13}C-NMR (62.5 MHz, $CDCl_3$) δ 25.4, 28.3, 28.7, 30.3, 46.9, 60.3, 82.2, 124.2, 127.1, 128.3, 145.5, 173.8.

19. Fmoc-Ser-Asp(OBu^t)-Lys(Boc)Pro-OH. FAB-MS MNa^+ 846; MK^+ 862.

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