

Improved Preparation of *N*-(Phenylisopropylloxycarbonyl) Amino Acids from the Corresponding Fluoroformate

HENRY FRANZÉN and ULF RAGNARSSON

Institute of Biochemistry, Biomedical Center, University of Uppsala, Box 576, S-751 23 Uppsala, Sweden

2-Phenylisopropyl fluoroformate has been prepared from the corresponding alcohol and carbonyl chloride fluoride. Although rather labile even at 0 °C, this new derivative seems an attractive intermediate for the preparation of *N*-(2-phenylisopropylloxycarbonyl) amino acids for peptide synthesis. This is demonstrated for a number of different amino acids.

Carbonyl chloride fluoride was used already several years ago to prepare *t*-butyloxycarbonyl fluoride, *p*-methoxybenzyloxycarbonyl fluoride and furfuryloxycarbonyl fluoride. In contrast to the corresponding chlorides, these reagents were stable enough to be useful for the preparation of *N*-protected amino acids.^{1,2} With the advent of a convenient laboratory method for conversion of trichlorofluoromethane to carbonyl chloride fluoride,³ fluoroformates of a variety of alcohols became as easily available as the corresponding, but occasionally less stable, chloroformates. An economical procedure for the synthesis of *t*-butyloxycarbonyl fluoride based on the carbonyl chloride fluoride preparation just mentioned was recently published.⁴

Another type of amino protecting group, which is characterized by extreme sensitivity to acid, has gained in importance during the last decade. The earliest example and still the most widely used protecting group of this type is 2-(*p*-biphenyl)isopropylloxycarbonyl,⁵ better known as Bpoc. As an alternative to Bpoc we recently introduced the corresponding 2-phenylisopropylloxycarbonyl (Ppoc) amino acids.⁶ Since carbonyl chloride fluoride was not available to us at that time, we then used the corresponding unsubstituted phenyl ester derivative

for activation purposes. Further experience with this protecting group has now prompted us to try the more direct method of introduction *via* the fluoroformate.

Bpoc-amino acids have already been prepared from the corresponding fluoroformate.⁷

EXPERIMENTAL

All amino acids were obtained either from Ajinomoto Co. Inc., Tokyo or Tanabe Seiyaku Co., Osaka, Japan. Except for glycine they were all of the L-configuration. Melting points were taken in open capillary tubes and were corrected. To establish the purity of prepared Ppoc-amino acids, thin-layer chromatography was performed on precoated silica plates (Merck DC-Fertigplatten Kieselgel 60F₂₅₄). Optical rotations were recorded with a Perkin-Elmer, model 141, polarimeter.

2-Phenylisopropyl fluoroformate (Ppoc-fluoride). A 1 l 3-necked round-bottomed flask equipped with a magnetic stirrer, a pressure-equalizing dropping funnel with condenser (cold finger), a thermometer and a double-surface reflux condenser (Davis) was connected (with glass tubing) *via* the top of the condenser to a wash-bottle containing conc. sulfuric acid and further to a 2 l 3-necked round-bottomed flask. The latter was equipped with a paddle stirrer, a wide gas-inlet tube and a reflux condenser for cooling with alcohol and dry ice (−78 °C). The first flask was loaded with 375 g of sulfuric acid containing 65 % SO₂, the dropping funnel with 172 g of trichlorofluoromethane and the second flask with 116 g (0.85 mol) of 2-phenylisopropanol and 132 ml (0.90 mol) of triethylamine in 1.1 l of dry ether. The Davis condenser and the ether solution were cooled at −20 °C during the experiment. The trichlorofluoromethane was added dropwise during 2 h at a temperature of 30–34 °C. After another 2 h at −20 °C, the excess carbonyl chloride fluoride was removed with a water aspirator under cooling at −20 °C and

Table 1. Preparation of Ppoc-amino acids from Ppoc-fluoride.

Ppoc-amino acid/ DCHA-salt	pH	Yield/%	M.p./°C	$[\alpha]_D^{25}/^\circ$ (c 1, CH ₃ OH)
Ala/DCHA-salt	9.5	83	114.5–115.5	–3.9
Arg(NO ₂)/DCHA-salt ^a	9.2	70	105 (decomp.)	+4.0
Gly	9.2	78	73–74	–
Leu ^b	9.5	56	112 (decomp.)	–21.0
Lys(Z)/DCHA-salt	9.4	80	89–91	+12.7
Met	9.4	75	76–77.5	–16.7
Phe/DCHA-salt	9.4	91	151–152.5	+37.6
Pro	9.5	87	105.5–106.5	–34.8

^a Anal. C₂₈H₄₄N₆O₆·H₂O: C, H, N. ^b Reaction performed at 20 °C.

the precipitate was filtered off and washed with cold ether. The solution was concentrated on a rotary evaporator at a bath temperature of 0 °C, leaving a colourless residue (250–350 g) suitable for conversion of amino acids to Ppoc derivatives. The content of Ppoc-fluoride, as determined below, corresponded to a 69–73 % conversion, calculated on the alcohol. When the fluoride was not used immediately after preparation and assay it was always stored at –80 °C (see below).

Determination of content of Ppoc-fluoride. This was essentially done as for benzyloxycarbonyl chloride.⁸ A 5 g aliquot of the reagent solution was added under stirring to 25 ml of conc. aqueous ammonia, initially at –20 °C. After 1 h the reaction mixture was cooled again to –20 °C before the precipitate was collected and carefully washed on the filter with water. After drying to constant weight, up to 2.5 g of product with m.p. 99–101 °C was obtained. Recrystallization from ethyl acetate–light petroleum did not change the melting point. Anal. C₁₀H₁₃NO₂: C, H, N.

Stability tests on Ppoc-fluoride. The stability of the fluoride was investigated at –80, –20 and 0 °C using the gravimetric procedure just mentioned. As indicated in Fig. 1, the compound is completely stable for several months at –80 °C but is not stable enough at –20 °C.

General procedure for the preparation of Ppoc-amino acids from Ppoc-fluoride. This procedure is essentially that described by Schnabel *et al.*² Amino acid (0.10 mol) was dissolved or suspended in 40 ml of 50 % dioxane–water at 0 °C. Sodium hydroxide (4 M) was added automatically with the aid of a pH-stat (see Table 1) together with 0.11 mol of properly cooled Ppoc-fluoride in small portions during 1 h. After evaporation of most of the dioxane at room temperature, 100 ml of water were added. Following extraction with 2 × 50 ml of ether, the solution was acidified to pH 4 (pH-meter) with saturated KHSO₄-solution and was carefully extracted with ethyl acetate. The com-

bined extracts were washed once with a small quantity of water before drying over anhydrous MgSO₄. Upon evaporation of the solvent solid Ppoc-amino acids were occasionally obtained directly, but sometimes only after addition of light petroleum. When no solid product could be obtained, the oil was dissolved in ether and a small excess of dicyclohexylamine was added to give the corresponding salt.

The results obtained with individual amino acids are summarized in Table 1.

RESULTS AND DISCUSSION

Owing to the thermolability of Ppoc-fluoride, as illustrated in Fig. 1, no further purification was attempted. A sample kept at 0 °C had decomposed completely in less than 3 days. The stability may differ slightly from batch to batch.

In early experiments a lower reaction temperature of –40 °C and a smaller excess of trichlorofluoromethane and fuming sulfuric acid were used according to the description of Wackerle and Ugi.⁴ However, this yielded only about

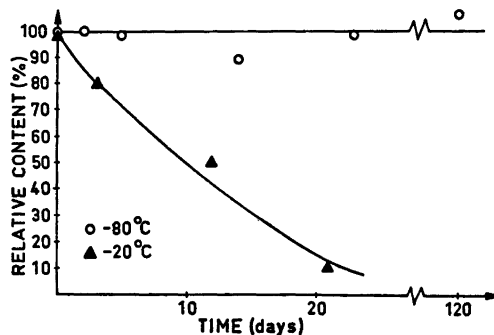


Fig. 1. Stability of Ppoc-fluoride.

50 % conversion of the alcohol. Water-cooling of the Davis condenser proved inadequate. Too much trichlorofluoromethane escaped reaction, especially when the reaction temperature reached the upper limit. We also consider it essential to establish a constant carbonyl chloride fluoride flow through the system to avoid clogging the gas inlet tube. The quantity of ether used should be close to the minimum required to allow efficient stirring and absorption of carbonyl chloride fluoride.

Initially other procedures for conversion of Ppoc-fluoride to Ppoc-amino acids were also tried, such as reaction in DMSO and DMF in the presence of 1,1,3,3-tetramethylguanidine.⁹ In the case of proline a 62 % yield of Ppoc-Pro was obtained, but leucine did not give any Ppoc-Leu at all. Further experiments were, therefore, abandoned. We interpret this to mean that Ppoc-fluoride is too unstable and decomposes before leucine goes into solution. Therefore, we turned to aqueous dioxane as solvent. Apart from the conditions described under Experimental, a few experiments were also carried out without access to pH-control. Even in these cases nearly quantitative yields of Ppoc-Pro were obtained.

The recent successful synthesis of apamin¹⁰ in our laboratory with the exclusive use of Ppoc-amino acids provided the impetus to improve our earlier method for their preparation. Our present experience and results seem to indicate that Ppoc-fluoride, on the average, gives higher yields of Ppoc-amino acids than does 2-phenylisopropyl phenyl carbonate and the work-up procedure is somewhat simpler. Ppoc-fluoride is inexpensive and relatively uncomplicated to prepare, especially if carbonyl chloride fluoride is commercially available, and compares well with the phenyl carbonate except with respect to its stability on storage.

Acknowledgements. We are grateful to the Swedish Natural Science Research Council (Project No. K 3020-100), the Swedish National Board for Technical Development (Project No. 78-7038) and Bachem Inc., Torrance, U.S.A. for financial support.

REFERENCES

1. Schnabel, E., Herzog, H., Hoffmann, P., Klauke, E. and Ugi, I. In Bricas, E., Ed., *Peptides 1968*, Proc. Ninth European Pep-

tide Symposium, Orsay, France 1968; North Holland Publishing Company, Amsterdam 1968, p. 91.

2. Schnabel, E., Herzog, H., Hoffmann, P., Klauke, E. and Ugi, I. *Justus Liebigs Ann. Chem.* 716 (1968) 175.
3. Siegemund, G. *Angew. Chem.* 85 (1973) 982.
4. Wackerle, L. and Ugi, I. *Synthesis* (1975) 598.
5. Sieber, P. and Iselin, B. *Helv. Chim. Acta* 51 (1968) 622.
6. Sandberg, B. E. B. and Ragnarsson, U. *Int. J. Pept. Protein Res.* 6 (1974) 111; 7 (1975) 503.
7. Schnabel, E., Schmidt, G. and Klauke, E. *Justus Liebigs Ann. Chem.* 743 (1971) 69.
8. Carter, H. E., Frank, R. L. and Johnston, H. W. *Org. Synth.* 23 (1943) 13.
9. Ragnarsson, U., Karlsson, S., Sandberg, B. E. and Larsson, L.-E. *Org. Synth.* 53 (1973) 25.
10. Sandberg, B. E. B. and Ragnarsson, U. *Int. J. Pept. Protein Res.* 11 (1978) 238.

Received June 12, 1979.