A New t-Butyl-based Acid-labile Protecting Group for the Guanidine Function of N^{α}-Fluorenylmethoxycarbonyl-arginine

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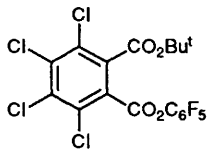
Bis(*o*-t-butoxycarbonyltetrachlorobenzoyl) groups effectively protect the guanidino group of arginine during peptide synthesis; they are cleaved in a two-stage process by treatment with trifluoroacetic acid and then with very dilute acid.

The use of base-labile N^{α}-fluorenylmethoxycarbonyl (Fmoc) amino acids for the temporary protection of α-amino groups is now well established in solid-phase peptide synthesis.[1] For the more permanent protection of amino acid side chains, t-butyl derivatives usually have appropriate base stability and may be cleaved at the end of the synthesis by mild acid treatment. No satisfactory t-butyl-based protecting group for the guanidine side chain of arginine has yet been described. The N^{G}-methoxytrimethylbenzenesulphonyl[2] (Mtr), and the more recent pentamethylchromansulphonyl[3] (Pmc) derivatives have useful acid lability, but both may give significant side reactions when cleaved in the presence of tryptophan residues.[4] There is therefore urgent need for a new, acid-labile guanidine protecting group. We report here studies on a novel t-butyl derivative which also has the special advantage in the arginine series of being cleaved by a mild, two-stage procedure.

Aroyl- or arylsulphonyl-guanidines with an *ortho*-substituent to provide anchimeric assistance should readily undergo acidolysis. Guttman and Pless have referred briefly in a symposium account[5] to the simple N^{α}-benzyloxycarbonyl-N^{G}-*o*-carboxybenzoyl-arginine, but dismissed it as a possible protected derivative because the basicity of the guanidine group was insufficiently reduced by monoacylation. It seemed to us that the reported[5] lability of this derivative to aqueous acid was due to participation by the *o*-carboxy substituent in



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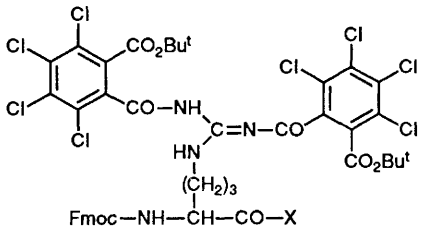

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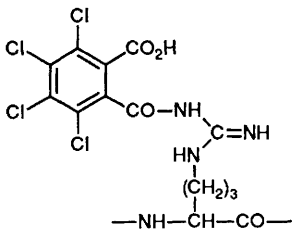

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H—Lys—Asp—Tyr—Ala—Leu—Arg—Phe—Gly—OH

4

H—Ala—Trp—Arg—Glu—OH

5

hydrolysis of the acylguanidine, and that the corresponding t-butyl ester would provide a 'protected protecting group' with acid lability appropriate to the Fmoc-t-butyl peptide synthesis strategy. Our experiences in the cleavage of corresponding *diacyl* N^G,N^G-bis(*o*-t-butoxycarbonylbenzoyl)-arginine derivatives were complex, and no reliable deprotection procedure emerged.[6] However, more recent studies using the bis(2-t-butoxycarbonyl-3,4,5,6-tetrachlorobenzoyl) (Btb) derivative **2**† have given promising results.‡

Tetrachlorophthalic anhydride was converted by way of its mono t-butyl ester into the activated derivative (**1**, Pfp = pentafluorophenyl; Btb-OPfp), m.p. 132–134 °C.[6] This reacted with N^{α}-Fmoc-L-arginine phenacyl ester hydrobromide in the presence of diisopropylethylamine to give the N^G,N^G-bis(*o*-t-butoxycarbonylbenzoyl) derivative **2**, X = OCH_2COPh [FAB MS *m/z* centred at 1199 (M + H^+; calcd. for $C_{53}H_{46}O_{11}N_4Cl_8$, M = 1198)].§ The analogous pentafluorophenyl ester **2**, X = OC_6F_5, Fmoc-Arg(Btb)$_2$-OPfp, had *m/z* 1247 (calc. for $C_{51}H_{39}N_4O_{10}Cl_8F_5$, M = 1246). The activated ester is not indefinitely stable in dimethylformamide (DMF) solution, probably being transformed into the δ-lactam[7] [80% conversion in 1 h (HPLC)], supporting the assignment of the Btb groups to the ω-nitrogen atoms. Nevertheless it reacts smoothly with amino groups in solid-phase synthesis under polar reaction conditions.[1c,8] Repeated coupling will clearly be better than prolonged reaction times in the case of hindered acylations.

Early experiments using Fmoc-Arg-Gly-OH (Fmoc-polyamide solid-phase synthesis)[1c] and Fmoc-Arg-Gly-NH_2 (solution synthesis) established the value of the bis-Btb group for effective protection of the guanidine function and conditions for its cleavage. Fmoc-Arg(Btb)$_2$-Gly-polydimethylacrylamide resin was cleaved by 95% aq. trifluoroacetic acid (TFA) yielding tetrachlorophthalic acid¶ and an

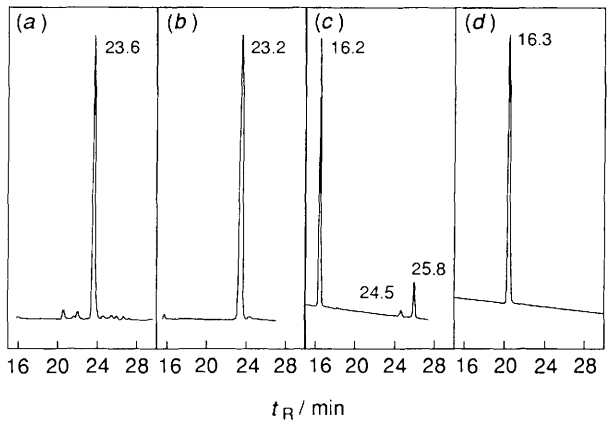

Fig. 1 HPLC elution profiles for (*a*) crude Arg(Ctb)-octapeptide **4**; the main peak contained 93.4% of the total peptide. (*b*) Purified Arg(Ctb)-octapeptide **4**; (*c*) crude **4**; and (*d*) purified **4**. Conditions: Aquapore RP300 column; buffer A contained 0.1% aq TFA, B 90% acetonitrile, 10% A. Gradient of 10–40% B over 25 min. Flow rate 1.5 ml min^{-1}. The effluent was monitored at 230 nm. No significant peaks were present outside the elution range shown.

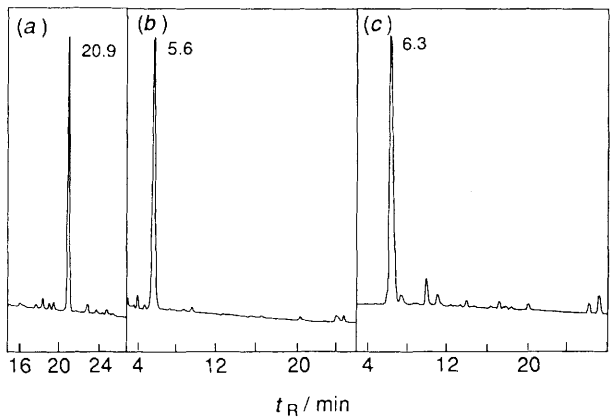

Fig. 2 HPLC profiles for (*a*) crude Arg(Ctb)-tetrapeptide **5**; (*b*) crude tetrapeptide **5** obtained from the foregoing; (*c*) crude **5** prepared from Fmoc-Arg(Pmc)-OPfp. HPLC conditions as in Fig. 1.

† Btb = *o*-t-**b**utoxycarbonyl-**t**etrachloro-**b**enzoyl. The Btb groups have been assigned arbitrarily to the ω-nitrogen atoms.

‡ The *mono*-N^G-(2-isopropoxycarbonyl-3,4,5,6-tetrachlorobenzoyl)-derivative has been mentioned[5] for protection of N^{α}-benzyloxycarbonyl-arginine, but apparently has not found successful application. In our hands, similar reaction conditions using **1** afforded a *mono*-N^G-(2-t-butoxycarbonyl-3,4,5,6-tetrachlorobenzoyl)-arginine *lactam*.[6]

§ The complex multiplet had the theoretical pattern corresponding to the isotopic distribution of *eight* chlorine atoms.

¶ The initial product in the first-stage cleavage is probably tetrachlorophthalic anhydride which in other experiments has been trapped as its benzylamine adduct.

Fmoc-Arg-Gly-OH derivative not identical (HPLC) with authentic Fmoc-dipeptide. Treatment of this with 50% aq. acetic acid (100 °C; 2 h)[5] gave further tetrachlorophthalic acid and Fmoc-Arg-Gly-OH (HPLC); it is therefore a mono-(*o*-carboxytetrachlorobenzoyl) (Ctb) derivative. Fortuitously it was observed that simply keeping the crude cleavage product (which contained residual traces of TFA) overnight in moist acetonitrile also converted it into authentic Fmoc-dipeptide. Other solvents [dimethylformamide, dimethylacetamide (DMA), dioxane, dimethylsulphoxide] containing up to 10% of water were also effective. The best conditions found used freshly distilled DMF or HPLC-grade DMA containing 5% 0.01 mol dm^{-3} aq. HCl for 24 h at room temperature.

The new protecting group was used to synthesise the sequence H-Lys-Asp-Tyr-Ala-Leu-Arg-Phe-Gly-OH **4** from the N-terminal region of the λ-cro repressor protein. Two continuous-flow solid-phase syntheses of this sequence were carried out using respectively bis-Btb and Pmc for arginine protection. Fmoc-amino acid pentafluorophenyl esters were used as acylating species throughout with kieselguhr-supported polydimethylacrylamide supports functionalised with the hydroxymethylphenoxyacetic acid linkage agent and norleucine internal reference amino acid.[1c,8] The octapeptide-resin was cleaved with trifluoroacetic acid containing 5% phenol for 1 h to give, after washing with ether to remove tetrachlorophthalic acid or anhydride, the crude Arg(Ctb)-octapeptide (63%, HPLC in Fig. 1*a*). The crude peptide was purified by preparative HPLC (42% overall yield, HPLC in Fig. 1*b*) and then kept in DMF containing 5% 0.01 mol dm^{-3} aq. HCl for 24 h, the mixture evaporated and the residue washed with ether. The crude free octapeptide (HPLC in Fig. 1*c*) contained no precursor Ctb-peptide. Material eluting at 24.5 min was non-peptidic (tetrachlorophthalic acid?); eluates at 16.2 and 25.8 min both had the correct amino acid composition but the latter amounted to only 2% of the total by amino acid analysis.‖ After further purification, the final octapeptide was obtained in 25% overall yield (HPLC in Fig. 1*d*, found: Asp, 0.99; Gly, 1.00; Ala, 0.99; Leu, 1.00; Tyr, 0.98; Phe, 0.98; Lys, 0.97; Arg, 0.96). In the Arg(Pmc) synthesis, the octapeptide resin was cleaved using a mixture (90 : 5 : 3 : 3) of trifluoroacetic acid ethanedithiol, anisole and thioanisole for 3 h at room temperature (removal of the Pmc group was incomplete after 2 h). The crude peptide (52%) was purified by preparative HPLC to give the product (31% overall yield) with HPLC behaviour identical with the foregoing.

H-Ala-Trp-Arg-Glu-OH **5** was also assembled using bis-Btb and Pmc protecting groups for the single arginine residue. The crude Arg(Ctb) peptide detached from the resin with trifluoroacetic acid–phenol–water (90 : 5 : 5) (HPLC, in Fig. 2*a*) was purified and cleaved again with 0.01 mol dm^{-3} HCl–DMF as above (Fig. 2*b*). In the Arg(Pmc) series, direct cleavage of the peptide resin with a mixture of trifluoroacetic acid, ethanedithiol, anisole and thioanisole (90 : 5 : 3 : 3) gave the HPLC profile of Fig. 2*c*. A slightly inferior result was obtained using the phenol–trifluoroacetic acid mixture above.

We conclude that bis(t-butoxycarbonyltetrachlorobenzoyl)-arginine derivatives show good promise for guanidine protection in Fmoc-t-butyl solid-phase synthesis. Inadequate arginine protection during synthesis is commonly indicated by the formation of ornithine derivatives. None was found during the above experiments. The Btb groups are cleaved in a mild two-stage process in which the highly basic guanidine function is not liberated during the initial detachment from the resin. This significantly facilitates purification of strongly basic peptide sequences and is also advantageous in the lysine series.[9] Acyl transfer to nucleophilic side chains does not seem to be a serious complication during cleavage under the acidic conditions and in the presence of water or other scavengers, though caution is of course required until many more sequences have been studied.

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‖ Presumably it contains a phthaloyl chromophore. The extinction coefficient for the Ctb-octapeptide at 230 nm is about seven times that of the free peptide which contains tyrosine as the only significant chromophore.

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