A Modified Benzhydrylamine as a Handle Reagent for the Solid Phase Synthesis of Peptide Amides Based on the Fluorenylmethoxycarbonyl Method

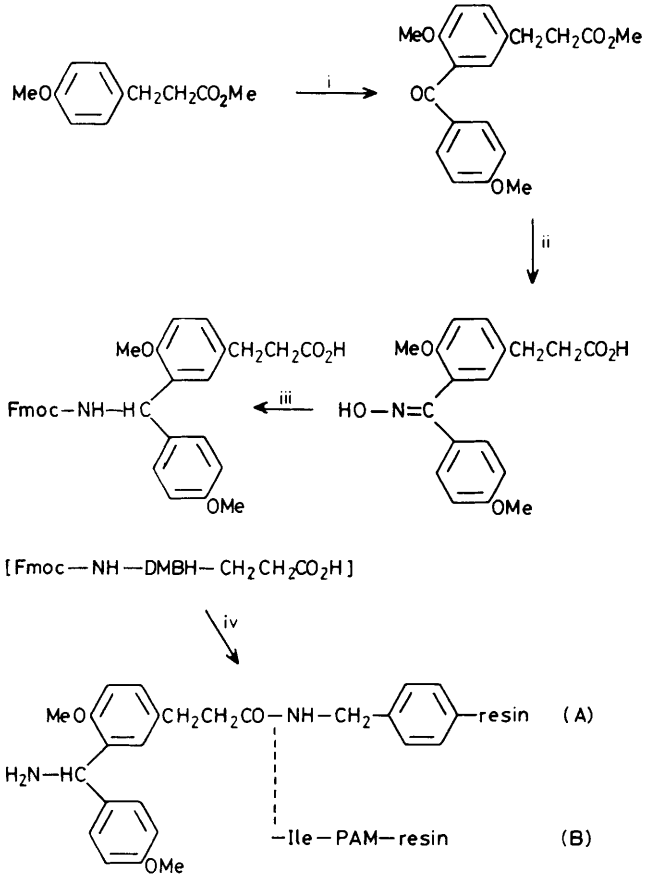
Susumu Funakoshi, Eigoro Murayama, Lili Guo, Nobutaka Fujii, and Haruaki Yajima*

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

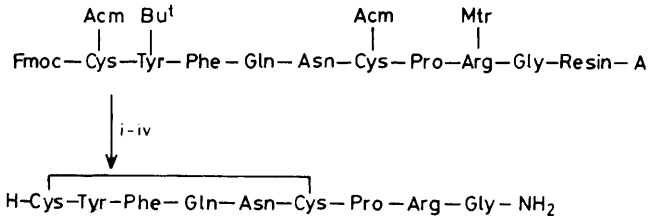
An easily preparable dimethoxybenzhydrylamine derivative, 3-(α-Fmoc-amino-4-methoxybenzyl)-4-methoxyphenyl propionic acid (Fmoc = fluoren-9-ylmethoxycarbonyl) is a useful precursor of the *C*-terminal amide, when applied to Fmoc-based solid phase peptide synthesis; as a cleavage reagent from the resin, thioanisole-mediated trimethylsilyl bromide in trifluoroacetic acid is recommended.

We report that a modified dimethoxybenzhydrylamine, 3-(α-Fmoc-amino-4-methoxybenzyl)-4-methoxyphenylpropionic acid(Fmoc-NH-DMBH-$CH_2CH_2CO_2H$), can be applied to the Fmoc (fluoren-9-ylmethoxycarbonyl, based solid phase synthesis of peptide amides, when acylated onto a polystyrene support.

Recently, the base-labile N^{α}-Fmoc protecting group[1] has been used in automated solid phase peptide synthesis[2] in conjunction with acid-labile side-chain protecting groups, *e.g.* Boc and Bu^t esters, which can be removed in the final step by mild acid treatment, *e.g.* trifluoroacetic acid (TFA). However, the preparation of peptide amides by this Fmoc



Scheme 1. Preparation of the modified dimethoxybenzhydrylamine (DMBH) resin, PAM = 4-(oxymethyl)phenylacetamidomethyl. *Reagents and conditions:* i, *p*-$MeOC_6H_4COCl$, $AlCl_3$; ii, NaOH, NH_2OH; iii, Zn/AcOH, Fmoc-OSu; iv, resin, DCC + HOBt, 20% piperidine/DMF.



Scheme 2. Fmoc based solid phase synthesis of Arg-vasopressin, Acm = acetamidomethyl, Mtr = 4-methoxy-2,3,6-trimethylbenzenesulphonyl. *Reagents and conditions*: i, 20% piperidine/DMF; ii, $(CF_3CO_2)_3Tl$/TFA; iii, 1 M Me_3SiBr–thioanisole/TFA; iv, Gel filtration on Sephadex G-10.

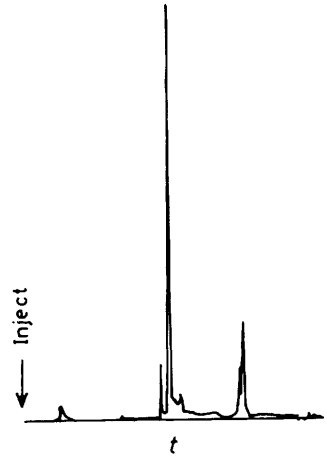


Figure 1. H.p.l.c. of the gel-filtered sample: column: Cosmosil 5C18 (4.6 × 50 mm); buffer: gradient of MeCN (15—30%, 45 min) in 0.1% TFA; flow rate: 1 ml/min; o.d.: 275 nm; retention time: 40.2 min.

strategy seems not to be well established, since suitable precursors of the amide function are required which are preferably more acid-labile than those hitherto employed in Merrifield solid phase synthesis.[3] Several resins based on benzylamine[4] or benzhydrylamine[5] have been introduced, but for practical applications the use of precursors, named handle derivatives, which can be directly introduced onto the commercially available polystyrene resins, is attractive, and a modified benzylamine, 5-[(2′ or 4′)-Fmoc-aminomethyl-3′,5′-dimethoxyphenyl]valeric acid (overall yield 15%, after 7 steps) has been introduced.[6] We modified the benzhydrylamine moiety by introducing two methoxy groups at both sides of the aromatic rings to make the C–N linkage acid-labile and further by adding the propionic acid group as an attachment site onto the polystyrene resin.

Fmoc-NH-DMBH-$CH_2CH_2CO_2H$ was prepared easily by a five-step sequence starting from methyl 3-*p*-methoxyphenylpropionate (Scheme 1, overall yield 68%). The resulting Fmoc-handle reagent was loaded on the aminomethylpolystyrene resin[8] (amino content 0.72 mmol/g) by condensation with dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxybenzotriazole (HOBt)[9] until the resin became negative to the Kaiser test.[10] The Fmoc group attached was removed by treatment with 20% piperidine in dimethylformamide (DMF), before use. Thus, the 2,4′-dimethoxybenzhydrylamine resin anchored with the propionyl linkage (resin A), was readily prepared. In addition, we condensed the Fmoc reagent onto the H-Ile-PAM-resin[11] (Ile content 0.76 mmol/g), then the Fmoc group was removed as described above. In this resin (resin B), Ile served to monitor the progress of reactions, after acid hydrolysis.

The usefulness of these new supports for the solid phase synthesis of peptide amides was demonstrated by several model experiments. Condensation of Fmoc-Phe-OH with the resin (A or B) *via* the pentafluorophenyl ester[12] was complete within 60 min. After Fmoc cleavage, H-Phe-NH_2 was obtained in *ca.* 100% yield in both cases by treatment (25 °C; 60 min) of the resin with TFA in the presence of thioanisole[13] (conc. 1 M). When the treated resin B was hydrolysed with 6 M HCl, no Phe was detected on an amino acid analyser indicating that Phe was completely cleaved from the resin by the above TFA–thioanisole treatment. In this deprotection, the role of thioanisole is significant, since Phe was liberated in only 2% yield from resin B by treatment (25 °C, 60 min) with TFA–CH_2Cl_2 (1 : 1).

By using resin B, tetragastrin[14] was then synthesized. Fmoc-Trp-Met-Asp(OBu^t)-Phe-resin B, prepared manually according to the principle of the Fmoc strategy,[2] was first treated with 20% piperidine in DMF, then with 1 M thioanisole/TFA (28 °C; 60 min). After purification by h.p.l.c., the product with an identical retention time on h.p.l.c. with that of an authentic sample of synthetic tetragastrin (purchased from Peptide Institute Inc., Osaka, Japan) was obtained in 41% yield (cleavage yield 60%, based on Ile in the resin B). Neuromedin B[15] was also synthesized. Fmoc-Gly-Asn-Leu-

Trp-Ala-Thr(Bu^t)-Gly-His(Boc)-Phe-Met-resin B prepared as described above was treated with 20% piperidine in DMF, then with 1 M thioanisole/TFA (28 °C, 60 min). After h.p.l.c. purification, the product was obtained in 21% yield (cleavage yield 43%). In this synthesis, we were able to demonstrate that the peptide could be cleaved from the resin, together with the protecting groups employed, by treatment (0 °C, 60 min) with 1 M trimethylsilyl bromide–thioanisole/TFA[16] more effectively than with thioanisole/TFA (yield 36% after h.p.l.c. purification, cleavage yield 70%).

The useful combination of this handle reagent and the Me_3SiBr/TFA deprotecting reagent for the solid phase synthesis of peptide amides was demonstrated by the synthesis of Arg-vasopressin[17] as an example (Scheme 2). In this synthesis, we were able to establish the disulphide bond on the resin by treatment with thallium trifluoroacetate[18] and deprotect Arg(Mtr)[19] completely with 1 M Me_3SiBr/TFA. Fmoc-Cys(Acm)-Tyr (Bu^t)-Phe-Gln-Asn-Cys (Acm)-Pro-Arg(Mtr)-Gly-resin A, prepared manually as above, was first treated with 20% piperidine, then with thallium trifluoroacetate in TFA (0 °C, 60 min), and finally with 1 M Me_3SiBr–thioanisole/TFA (0 °C, 60 min). H.p.l.c. of the crude product showed a main peak (Figure 1) possessing an identical retention time with that of an authentic sample of Arg-vasopressin (purchased from Peptide Institute Inc.). Its isolation yield by h.p.l.c. was 30%.

The easily preparable handle reagent introduced here may thus be of wide applicability in solid phase synthesis of *C*-terminal peptide amides. The use of Me_3SiBr–thioanisole/TFA as an alternative deprotecting reagent to TFA–thioanisole may lead to the possibility of applying a variety of amino acid derivatives for the Fmoc based solid phase peptide synthesis, since this reagent can cleave various protecting groups currently employed in peptide chemistry under relatively mild conditions.

Received, 12th November 1987; Com. 1652

References

1 L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, 1970, **92**, 5748.
2 A. Dryland and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1986, 125; L. Cameron, M. Meldal, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1987, 270, and references cited therein.
3 P. G. Pietta and G. R. Marshall, *Chem. Commun.*, 1970, 650; R. C. Orlowski, R. Walter, and D. Winkler, *J. Org. Chem.*, 1976, **41**, 3701; G. R. Matsueda and J. M. Stewart, *Peptides*, 1981, **2**, 45; J. P. Tam, *J. Org. Chem.*, 1985, **50**, 5291.
4 P. G. Pietta, P. F. Cavallo, K. Takahashi, and G. R. Marshall, *J. Org. Chem.*, 1974, **39**, 44; D. H. Rich and S. K. Gurwara, *Tetrahedron*, 1975, 301; R. Colombo, *Int. J. Pept. Protein Res.*, 1982, **19**, 71.
5 B. Penke and J. Rivier, *J. Org. Chem.*, 1987, **52**, 1197; R. Colombo, *J. Chem. Soc., Chem. Commun.*, 1981, 1012.
6 F. Albericio and G. Barany, *Int. J. Pept. Protein Res.*, 1987, **30**, 206.
7 G. F. Sigler, W. D. Fuller, N. C. Chaturvedi, M. Goodman, and M. Verlander, *Biopolymers*, 1983, **22**, 2157; P. B. W. Ten Kortenaar, B. G. van Dijk, J. M. Pecters, B. J. Raaben, P. J. H. M. Adams, and G. I. Tesser, *Int. J. Pept. Protein Res.*, 1986, **27**, 398.
8 A. R. Mitchell, S. B. H. Kent, B. W. Erickson, and R. B. Merrifield, *Tetrahedron Lett.*, 1976, 3759.
9 W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
10 E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.
11 A. R. Mitchell, B. W. Erickson, M. N. Ryabtsev, R. S. Hodges, and R. B. Merrifield, *J. Am. Chem. Soc.*, 1976, **98**, 7357.
12 J. Kovacs, L. Kisfaludy, and M. Q. Ceprini, *J. Am. Chem. Soc.*, 1967, **89**, 183.
13 Y. Kiso, K. Ukawa, and T. Akita, *J. Chem. Soc., Chem. Commun.*, 1980, 101.
14 J. C. Anderson, M. A. Barton, P. M. Hardy, G. W. Kenner, J. Preston, and R. C. Sheppard, *J. Chem. Soc.* (*C*), 1967, 108.
15 N. Minamino, K. Kangawa, and H. Matsuo, *Biochem. Biophys. Res. Commun.*, 1983, **114**, 541; M. Okada, S. Kimura, K. Goto, I. Kanazawa, S. Ohashi, M. Shiraki, and E. Munekata, *Peptide Chem. Jpn.*, 1983, **21**, 111.
16 N. Fujii, A. Otaka, N. Sugiyama, M. Hatano, and H. Yajima, *Chem. Pharm. Bull.*, 1987, **35**, 3880.
17 V. du Vigneuad, D. T. Gish, and P. G. Katsoyannis, *J. Am. Chem. Soc.*, 1954, **76**, 4751; R. Acher and J. Chauvet, *Biochim. Biophys. Acta*, 1954, **14**, 421.
18 N. Fujii, A. Otaka, S. Funakoshi, K. Bessho, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1987, 163.
19 M. Fujino, O. Nishimura, M. Wakimasu, and C. Kitada, *J. Chem. Soc., Chem. Commun.*, 1980, 668.