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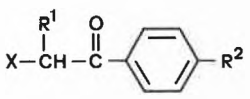
A New Phenacyl-Type Handle for Polymer Supported Peptide Synthesis**

Dieter Bellof and Manfred Mutter*

Abstract: A new phenacyl-type handle, [4-(2-bromopropionyl)phenoxy]acetic acid (PPOA, **4**) is introduced for polymer supported peptide synthesis. The new anchoring system is easily accessible for synthesis, and PPOA-anchored peptides are readily split off by nucleophiles and photolytic reaction.

In polymer supported synthesis so-called handles serve as reversible anchoring linkages between e.g. peptide chain and polymer support[1-3]. In order to guarantee high flexibility in the tactical considerations of peptide synthesis, a large variety of anchoring systems must be available. Despite the considerable progress in this area there is still a lack in systems which are labile to nucleophilic attack. Phenacyl-type handles turned out to be very versatile anchoring groups, being completely stable to acid conditions but readily cleavable by a number of nucleophiles[4-10].

For conventional peptide synthesis, the following types of phenacyl systems proved to be useful as carboxyl protecting groups[11]:



I: $R^1 = CH_3$, $R^2 = H$

II: $R^1 = H$, $R^2 = OCH_3$

Most notably, the introduction of a *p*-methoxy substituent resulted in increased lability of the corresponding phenacyl ester towards photolytic cleavage. In polymer supported synthesis, however, the use of type I systems[12] with $R^2 = CH_2-COOH$ (Ia) is hampered by its difficult synthetic accessibility[13], whereas systems of type II have not been used so far.

In order to combine the advantageous features of I and II, we describe in this communication the synthesis of a new phenacyl-type system [4-(2-bromopropionyl)phenoxy]acetic acid (PPOA, **4**) and its application in polymer supported synthesis.

PPOA (**4**) was prepared according to Scheme 1.

Methyl phenoxyacetate **2** from phenoxyacetic acid (**1**) is reacted with 2-bromopropionyl chloride under standard Friedel-Crafts conditions yielding **3** as a crystalline product. After hydrolysis of the ester, the 1,4-disubstituted key compound **4** is obtained in an overall yield of 55%. In contrary to the synthesis of Ia[12] no undesired side reactions such as halogen exchange or isomer formation were observed in the preparation of **4**. Most notably, the bromo-derivative proved to be superior to the corresponding chloro-analogue, due to its higher reactivity in the succeeding esterification step.

The attachement of **4** to aminomethylated polystyrene resins is achieved by the standard dicyclohexylcarbodiimide

* *Correspondence:* Prof. Dr. M. Mutter
Institut für Organische Chemie
Universität Basel
St. Johanns-Ring 19, CH-4056 Basel

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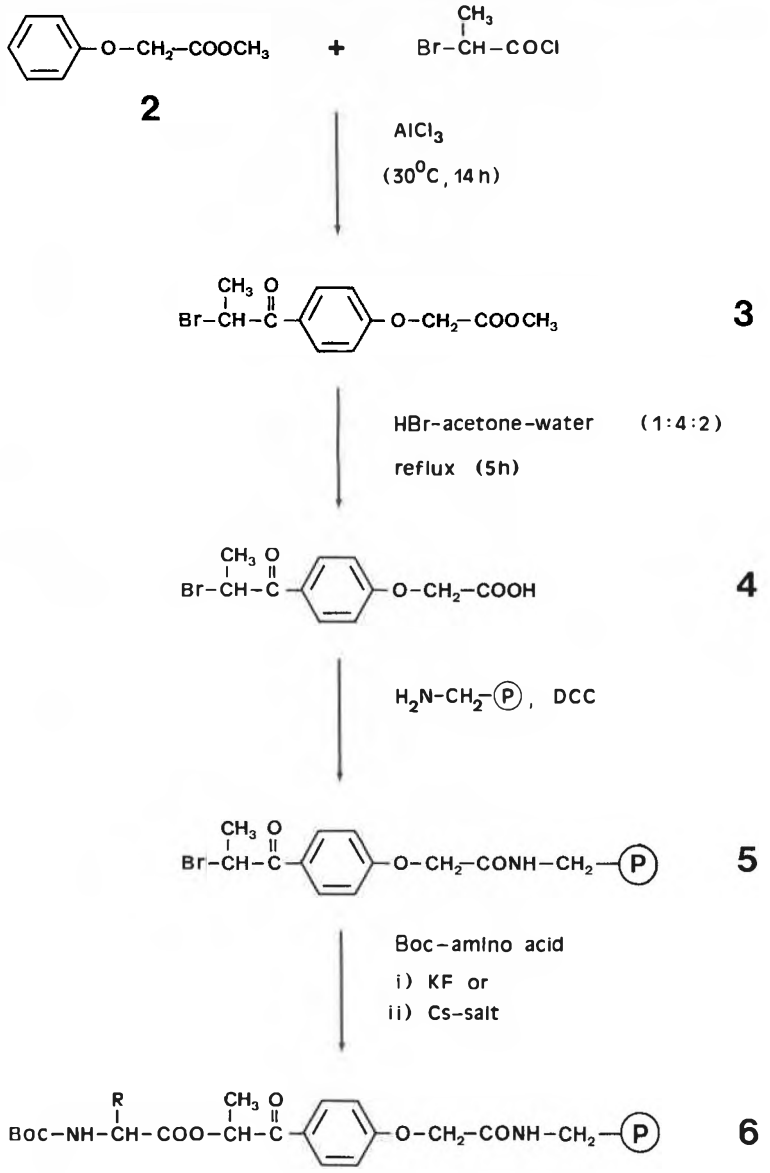
Scheme 1



procedure in one single step to yield **5** quantitatively. The results of the esterification of the C-terminal amino acid derivative to **5** via the KF-method[14] or the cesium salt[9, 15] are summarized in Table 1.

The new anchoring group proved to be completely stable to anhydrous acids: By treatment of the resins **6a–d** with 100% CF_3CO_2H, 1.2 N HCl in HOAc, and 33% HBr in HOAc no cleavage could be observed during six hours. Consequently, the PPOA anchor is suitable for stepwise peptide synthesis using acid labile temporary N^{α}-protecting groups such as BOC, DDZ, NPS or BPOC. On the other hand, the amino acid-PPOA-ester bond is very susceptible to nucleophiles as documented in Table 2. For example, **6a–c** as well as the pentapeptide BOC-Tyr-(Bzl)-Gly-Gly-Phe-Leu-OH could be released in high yields by treatment with alkali in aqueous dioxane and ii) benzyltrimethylammonium hydroxide (Triton B) in methanol. Methyl esters resulted from treatment of the resins **6b, c** with triethylamine in MeOH/dioxane with small amounts of NaOH. By reacting with hydrazine hydrate in methanol, the corresponding hydrazides were obtained in yields of about 90%.

Ammonolyses with ammonia in ethanol were less satisfactory, the yields approaching about 60% after 20–24 hours reaction time.

A most interesting feature of the new anchoring system concerns its increased feasibility towards photolytic cleavage compared to phenacyl groups of type I: When resins **6a–d** are suspended in ethanol, dimethylformamide or dioxane, respectively, and irradiated at wavelengths of 350 nm, the cleavage of the amino acid derivatives from the support proceeds to yields up to 80% within 10 hours (Fig. 1). The higher access to photolytic reactions of the PPOA-system can be expected from the bathochromic shift due to the methoxy-substitution[11].

The new anchoring system was used for the synthesis of Leu^5-enkephaline (Scheme 2). The peptide was built up both by the standard stepwise procedure[3] as well as by coupling the C-terminal tripeptide BOC-Gly-Phe-Leu-OH to the PPOA-resin **5** in order to avoid potential side reactions in the first two steps of synthesis[9]. The protected pentapeptide **8** was cleaved off the support by photolysis. The final target peptide **10** could be obtained in 71% yield as colourless crystals which proved to be pure according to the usual analytical criteria. As expected, the overall yield of the stepwise prepared pentapeptide was lower by ca. 14% due to oxazinone and dioxopiperazine formation.

In summary, the new phenacyl anchoring system exhibits distinct advantages compared to the known phenacyl-type groups with respect to its synthetic accessi-

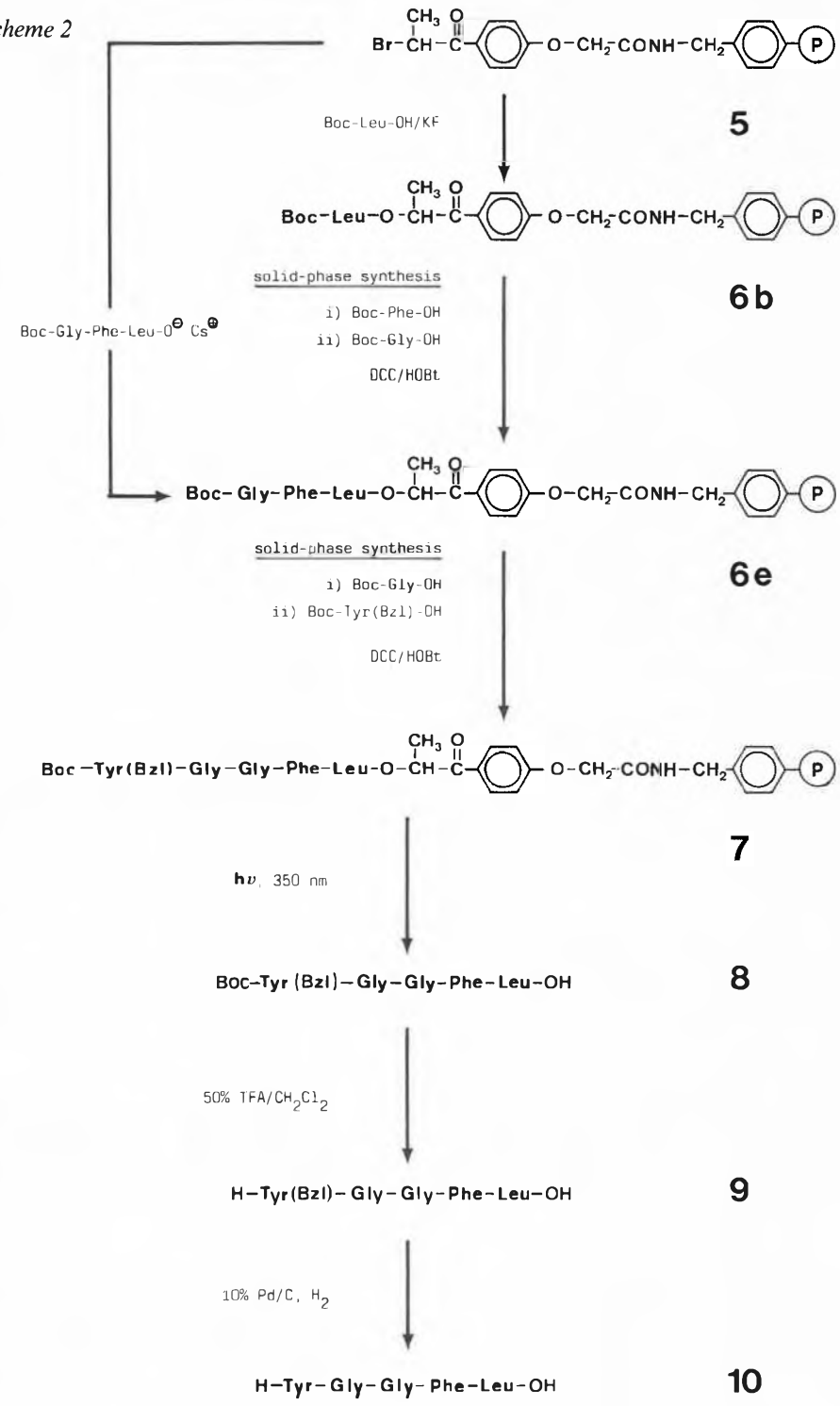
Table 1. Attachment of protected amino acids and peptides to resin **5**.

compound	amino acid derivative	esterification method	time (h)	temperature (°C)	substitution mmol/g of Ⓟ	yield (%)
6a	BOC-Gly-OH	KF/DMF	20	50	1.14	≈ 100
6b	BOC-Leu-OH	KF/DMF	48	50	0.90	83
6c	BOC-Tyr(Bzl)-OH	KF/DMF	48	50	0.71	75
6d	BPOC-Leu-OH	cesium salt	40	40	0.86	92
6e	BOC-Gly-Phe-Leu-OH	cesium salt	72	40	0.85	96

Table 2. Cleavage of protected amino acids and peptides from resins **6 a–e** by nucleophiles.

aminoacyl residue	reagent	time (h)	product	yield (%)
BOC-Gly	0.5 N NaOH in dioxane/water (2:1)	0.5	BOC-Gly-OH	≈ 100
BOC-Leu	0.5 N Et_3N in methanol/dioxane (1:1) + 1 vol. % 1 N NaOH	1.0	BOC-Leu-OCH_3	88
BOC-Tyr(Bzl)	as above	1.0	BOC-Tyr(Bzl)-OCH_3	73
BOC-Gly	10% Triton B/methanol	0.5	BOC-Gly-OH	≈ 100
BOC-Gly	2.5% hydrazine/methanol	2.0	BOC-Gly-$NHNH_2$	93
BOC-Leu	as above	2.0	BOC-Leu-$NHNH_2$	90
BOC-Tyr(Bzl)	as above	2.0	BOC-Tyr(Bzl)-$NHNH_2$	87
BOC-Gly	NH_3/ethanol	20	BOC-Gly-NH_2	60
BOC-Tyr(Bzl)-Gly-Gly-Phe-Leu	10% Triton B/methanol	6	BOC-Tyr(Bzl)-Gly-Gly-Phe-Leu-OH	99
	2.5% hydrazine/ethanol	2	BOC-Tyr(Bzl)-Gly-Gly-Phe-Leu-$NHNH_2$	83

Scheme 2



bility and complements the spectra of attachment modes in polymer-supported peptide syntheses by a useful variant.

Experimental Part

Methyl phenoxyacetate (**2**): Phenoxyacetic acid (250 g, 1.65 mol) was dissolved in a mixture of MeOH (200 g, 6.25 mol) and conc. sulfuric acid (30 g, 0.31 mol) and refluxed for 4 h. After standing overnight, the solution was concentrated under reduced pressure, the residue was poured on 200 g of ice. After extraction with ether (3 × 80 mL), the combined organic layers were washed with water, saturated $NaHCO_3$ solution, and water again (3 × 100 mL each) and dried with $MgSO_4$. The solvent was evaporated and the crude product was destilled in vacuo: 231 g (84%), *b.p.* 127°C/13 mm Hg; $n_D^{20} = 1.5155$.

Methyl [4-(2-Bromopropionyl)phenoxy]acetate (**3**): To $AlCl_3$ (290 g, 2.2 mol) in 400 mL of 1,2-dichloroethane 2-bromopropionyl chloride (181 g, 1.05 mol) was added dropwise under stirring and cooling to −10°C, followed by **2** (165 g, 1.0 mol). The temperature raised and was maintained at 30°C. The reaction mixture was stirred until the HCl evolution had ceased and was allowed to stand overnight. The next day, the solution was hydrolyzed with 700 g of ice, and the aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The combined organic phases were washed with water and saturated $NaHCO_3$ (3 × 100 mL each). After drying with $MgSO_4$, the ester **3** crystallized from the concentrated solvent and was recrystallized from $CHCl_3$/petroleum ether: 217 g (72%), *m.p.* 58°C; ^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.86 (d, 3H, CH_3, J = 5.7 Hz), 3.79 (s, 3H, OCH_3), 4.71 (s, 2H, OCH_2–CO), 5.26 (q, 1H, CH, J = 5.7 Hz), 6.9–8.05 (AA'BB', arom. H); MS: 300, 302 (31%, M^+), 193 (100%, M − $CH(CH_3)$ − Br).

[4-(2-Bromopropionyl)phenoxy]acetic acid (**4**): A solution of **3** (40 g, 0.13 mol) in 150 mL of acetone/conc. HBr/H_2O (4:1:2) was refluxed for 5 h. The main part of the acetone was evaporated and the aqueous residue was extracted with ether (3 × 70 mL). The combined organic layers were washed with water (3 × 50 mL) and carefully extracted with saturated $NaHCO_3$ solution (5 × 50 mL). The alkaline solutions were once washed with ether (100 mL) and acidified with 6M HCl to pH 2 to obtain the crude acid as a white solid. After filtration and washing with water the product was dried and recrystallized from $CHCl_3$: 33 g (87%), *m.p.* 136°C; ^{1}H-NMR ($CDCl_3$/DMSO-d_6 (4:1), 90 MHz): δ = 1.85 (d, 3H, CH_3, J = 5.7 Hz), 4.68 (s, 2H, OCH_2–CO), 5.34 (q, 1H, CH, J = 5.7 Hz), 6.93–8.04 (AA'BB', arom. H); MS: 286, 288 (29%, M^+), 179 (100%, M − $CH(CH_3)$ − Br).

[4-(2-Bromopropionyl)phenoxyacetylaminomethyl]-poly(styrene-co-2%-divinylbenzene) resin (**5**): Aminomethyl-poly(styrene-co-2%-divinylbenzene) beads[16] (1.6 g, 1.69 mmol NH_2/g of resin) were suspended in 20 mL of CH_2Cl_2. After 30 min, **5** (4.65 g, 16.2 mmol) was added, followed by 2M DCC in CH_2Cl_2 (4.05 mL, 8.1 mmol). Coupling was quantitative after shaking for 2 h at room temperature as indicated by a Kaiser-test. The resin was filtered, washed with CH_2Cl_2, DMF, MeOH, and CH_2Cl_2 (3 × 30 mL), and dried under high vacuum: 2.1 g, elemental analysis: Br 10.4% (1.29 mmol Br/g of resin).

Esterification of the N-protected amino acids to resin **5**:

a) KF method. General procedure: To 1 g of resin, suspended in 20 mL of DMF, the protected amino acid derivative was added (5 mmol), followed by powderized anhydrous KF (0.58 g, 10 mmol). After reaction under conditions given in Table 1, the resin was filtered, washed with DMF, DMF/water 1:1, water, MeOH, and CH_2Cl_2 (3 × 30 mL), and dried in vacuo. Yields were calculated from amino acid analysis and microtitration of the corresponding hydrochloride after deprotection with 1.2M HCl/HOAc.

b) Cesium salt procedure: The protected amino acid/peptide derivative (5 mmol) was dissolved in 60 mL of EtOH/water 5:1 and neutralized (pH-meter) with a 10% Cs_2CO_3 solution. The solvent was evapo-

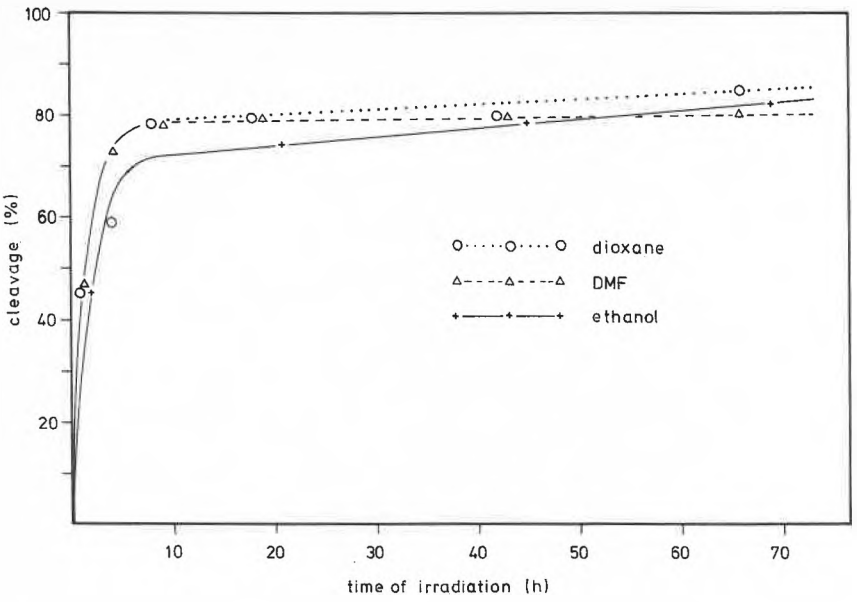


Fig. 1. The rate of photolytic cleavage of the anchoring bond in BOC-Gly derivatives of PPOA-resin **6a** *in various solvents (DMF = dimethylformamide).*

rated in vacuo at 40°C, the resulting residue was repeatedly dissolved in 30 mL of DMF, and evaporated to dryness to remove last traces of water. The cesium salts were obtained as white powders or glassy solids. For coupling to the resin, they were dissolved in 50 mL of DMF, resin **6** (2 g), preswollen in DMF, was added and the mixture was reacted under conditions given in Table 1. Finally, the resin was filtered and worked up as described for the KF method.

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[1] R.B. Merrifield, *J. Am. Chem. Soc. 85* (1963) 2149.
[2] M. Mutter, E. Bayer, in E. Gross, J. Meienhofer: *The Peptides: Analysis, Synthesis, and Biology*, Vol. *II*, Academic Press, New York (1980), p. 285.
[3] G. Barany, R.B. Merrifield, in E. Gross, J. Meienhofer: *The Peptides: Analysis, Synthesis, and Biology*, Vol. *II*, Academic Press, New York (1980), p. 42.
[4] T. Mizoguchi, K. Shigezane, N. Takamura, *Chem. Pharm. Bull. 17* (1969) 411.
[5] S.S. Wang, *J. Org. Chem. 41* (1976) 3258.
[6] J.C. Sheehan, G. Doyle Daves Jr., *J. Org. Chem. 29* (1964) 2006.
[7] J.P. Tam, W.F. Cunningham-Rundles, B.W. Erickson, R.B. Merrifield, *Tetrahedron Lett. 18* (1977) 4001.
[8] F. Weygand, in: *Peptides 1968, Proc. 9th Eur. Pept. Symp. Orsay*, North Holland, Amsterdam (1968), p. 183.
[9] C. Birr, M. Wengert-Müller, A. Buku, in M. Goodman, J. Meienhofer: *Peptides: Proc. 5th Am. Pept. Symp.*, Wiley, New York (1975), p. 510.
[10] F.S. Tjoeng, J.P. Tam, R.B. Merrifield, *Int. J. Pept. Protein Res. 14* (1979) 262.
[11] J.C. Sheehan, K. Umezawa, *J. Org. Chem. 38* (1973) 3771.
[12] F.S. Tjoeng, G.A. Heavner, *J. Org. Chem. 48* (1983) 355.
[13] D. Bellof, M. Mutter, *Polym. Bull. 11* (1984) 49.
[14] K. Horiki, K. Igano, K. Inouye, *Chem. Lett.* (1978) 165.
[15] B.F. Gisin, *Helv. Chim. Acta 56* (1973) 1476.
[16] J.T. Sparrow, *J. Org. Chem. 41* (1976) 1350.