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Methods

Soluble Polymers in Organic Synthesis
4. A New Method for Reversible Attachment of C-Terminal Amino Acids to Poly(Ethylene Glycol) Supports

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SUMMARY

The reversible attachment of C-terminal amino acids to poly(ethylene glycol) supports succeeds in high yields by reaction of amino acid derivatives with 4-(2-bromopropionyl)phenylacetic acid N-hydroxysuccinimidester and subsequent coupling to the polymer.

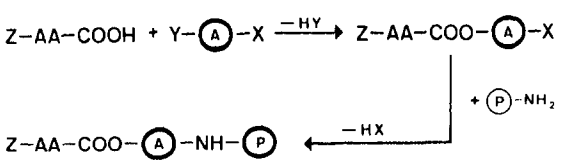
INTRODUCTION

Polyethylene glycols (PEG) have been successfully applied as support in organic synthesis.
In the liquid-phase-method (LPM) of peptide synthesis, PEG combines strategical features of the conventional and solid phase methods, acting as C-terminal solubilizing protecting group (1,2). Up to now, a large number of biological active peptides and peptide fragments as well as model peptides for conformational studies have been synthesized by this procedure (3).
One of the main problems in polymer supported peptide synthesis, which has not been solved yet sufficiently, is the reversible attachment of the growing peptide chain to the polymer. For cleaving off the peptide under mild conditions, so called "handles" or "anchor-groups" with variable chemical stabilities have been introduced (4). The attachment of the C-terminal amino acid to the handle often proceeds in very un-

+ For communication 3 of this series see: Anzinger, H., Mutter, M.; Pol. Bull. 6, 595 (1982)

satisfactory yields, mainly with sterically hindered amino acids. In the past, several procedures have been reported to overcome this problem. In general, the C-terminal amino acid is reacted in a first step with functionalized anchor molecules resulting in an ester-linkage (scheme 1); after isolation and characterization of these low molecular weight compounds the linkage with the polymer support succeeds via amide bond according to scheme 1

SCHEME 1:



AA = amino acid
A = anchor
P = polymer support
Z = protecting group

The overall yield using the outlined principle is rather low in many cases, so that these procedures have not been applied to LPM so far (5-9). In the present communication, we describe a comparatively simple way of synthesis, which allows to attach even sterically hindered amino acid derivatives to polymer supports in high yields. For this purpose, we have selected as an anchor-compound the photolabile 4-(2-bromopropionyl)-phenylacetic acid, an analogue of a compound recently introduced by Tjoeng et al. (10,11).

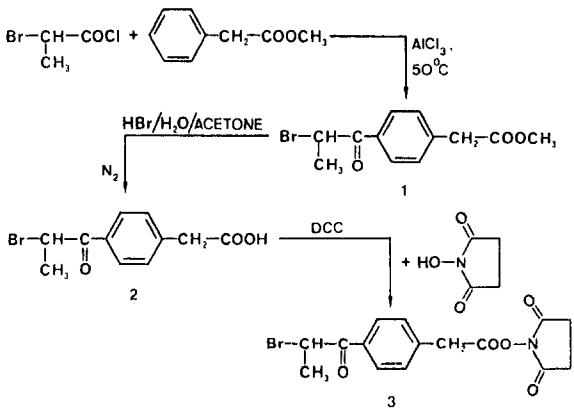
RESULTS AND DISCUSSIONS

In scheme 2 the synthesis of the key-compound 4-(2-bromopropionyl)-phenylacetic acid N-hydroxysuccinimidester 3 is shown.

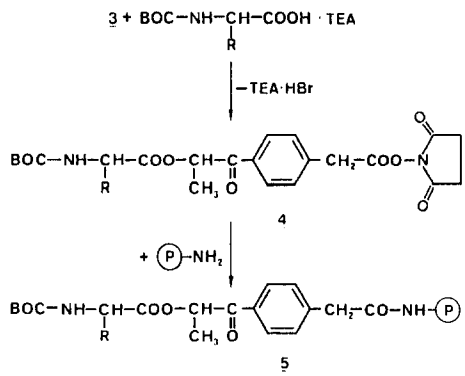
1 was prepared according to Tjoeng et al. (11); in our case, a mixture of the 1,3- and 1,4-substituted isomers was obtained in good overall yield. The ester mixture was saponified under nitrogen with HBr/water/acetone and the 1,4-substituted acid 2 was isolated by fractionated crystallization. In the following step, 2 was esterified with N-hydroxy-succinimide to yield colourless needles of compound 3, which can be stored for many months without decomposing.

The N-hydroxysuccinimideester serves as protection and activation group for the carboxyl function of the phenylacetic acid. Amino acid derivatives (e.g. in the N-BOC-protected form) are esterified with 3 as triethyl-ammonium salts to yield compound 4 (scheme 3). The commonly used dicyclo-hexylammonium salts of amino acids proved to be unsuitable for the synthesis of 4, resulting in its dicyclohexylamide. According to this general

SCHEME 2:



SCHEME 3:



procedure, the amino acid derivatives listed in Table 1 were reacted to 4 in overall yields of ca. 70%, all products were characterized by ^{1}H-NMR, IR and field-desorption mass spectrometry.

Table 1

Amino acid derivative		yield of 4 (%)	(A) Rf-values (B)	
BOC-Ala-O-R	(4a)	70	0.61	0.30
BOC-Gly-O-R	(4b)	66	0.55	0.24
BOC-Tyr(OBzl)-O-R	(4c)	73	0.70	0.36
BOC-Glu(OBzl)-O-R	(4d)	76	0.68	0.33
BOC-Lys(Z)-O-R	(4e)	69	0.57	0.22

Attachment of amino acid-anchor-derivatives (compounds 4) to the polymer support.

The succinimidesters 4 were coupled to the amino derivative of poly(ethylene glycol monomethylether) M-PEG-NH_2, $\bar{M}_n$ = 5000, 0.17 mM NH_2/g. The results are presented in Table 2.

Table 2: R = $-\underset{\underset{CH_3}{|}}{CH}-CO-C_6H_4-CH_2COOSu$ OSu = N-hydroxysuccinimide PS = polystyrene (2% crosslinked)

Amino acid-anchor derivative		Loading (%) to M-PEG-NH_2	PS-CH_2-NH_2
BOC-Gly-O-R	(4b)	./.	90.1
BOC-Ala-O-R	(4a)	99	./.
BOC-Tyr(OBzl)-O-R	(4c)	95.1	./.
BOC-Glu(OBzl)-O-R	(4d)	97.3	95.5
BOC-Lys(Z)-O-R	(4e)	96.3	99

Reaction of 4 with M-PEG-NH_2 under mild conditions (CH_2Cl_2, room temperature, 20-24 h) resulted in very good yields of 5 as determined by amino acid analysis and micro titration. The resulting polymers gave ninhydrin-negative and UV_{254nm}-positive reactions and showed different R_f-values in TLC compared to the unloaded materials. As expected, the IR-spectra showed a significant absorption at $\tilde{\nu}_{CO}$=1660-1710 cm^{-1}. The derivatives 4 can be used in analogous manner for reaction with heterogenous supports. As a typical representative, aminomethyl-polystyrene (2% crosslinked, 2,6 mmol NH_2/g) was loaded with the amino acid derivatives 4b,d,e in yields of >90% (Table 2, last column).

In order to avoid any loss in yield during the isolation of 4, we applied alternatively a direct procedure for the attachment of 4d and 4e to the polymer support: After complete reaction of the N-protected amino acids with 3, the raw mixture, consisting of succinimidester 4 and excess amino acid derivative, was coupled to amino-PEG in comparative yields as listed in Table 2 for the purified amino acid-anchor derivatives. Consequently, the relatively tedious isolation step of the esters 4 can be avoided, rendering the overall procedure outlined in scheme 3 even more effective. Our results show that the attachment of the C-terminal amino acids to polymers via reactive anchor molecules can be performed mainly quantitatively under mild conditions, thus eliminating the danger of unspecific side reactions on the polymer support. The procedure outlined here for a photolabile handle between polymer and peptide can be easily transfered to other anchoring groups, e.g. with acid or base lability.

EXPERIMENTAL SECTION

Abbreviations:

DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DCHA, dicyclohexylamine; DMF, dimethylformamide; PE, petrolether; PS, Merrifield-Resin; TEA, triethylamine; TLC, thin-layer chromatography.

Infrared (IR) spectra were taken with a Beckman Instruments model IR 4220, nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Physics model WH 90 or WP 60 CW, respectively. Amino acid analysis were obtained on a Biotronik Chromatography System LC 6000 E after hydrolysis with 6 N HCl in sealed glass tubes at 110°C for 24 h. Electron impact (EI) mass spectra were recorded on a Varian MAT model CH 7 A,

field desorption (FD) mass spectra on a Varian MAT model 711. Thin-layer (TLC) chromatography was performed with precoated silica gel 60 F_{254} (200 um) plates obtained from Merck, Darmstadt. The following solvent systems were used: (A) ethylacetate-cyclohexane 5:2, (B) ethylacetate-cyclohexane 1:1, (C) methylenechloride-methanol 7:1, (D) acetic acid-n-butanol-water 1:3:1.
L-BOC-Tyr(OBzl)-OH and L-BOC-Glu(OBzl)-OH were obtained from Bachem, chloromethyl-polystyrene (2% crosslinked, 2,3 mmol/g Cl) and 2-bromopropionyl-chloride as well as phenylacetic acid from EGA. All solvents and bulk chemicals were reagent grade. Monofunctional amino-PEG ($\overline{M}$=5000, 0.17 mmol NH_2/g) was prepared according to (14).
L-BOC-Lys(Z)-OH, L-BOC-Gly-OH and L-BOC-Ala-OH were synthesized as described in (12). Aminomethyl-polystyrene was obtained according to (13) with 2.6 mmol NH_2/g. Methyl phenylacetate and methyl [4-(2-bromopropionyl)-phenyl] acetate 1 were prepared according to Tjoeng et al (10,11), 4-(2-bromopropionyl)phenylacetic acid (2) by the procedure of Tjoeng using HBr instead of HCl.

4-(2-Bromopropionyl)phenylacetic Acid N-Hydroxysuccinimidester (3)

4-(2-Bromopropionylphenylacetic acid (4.4 g, 16.3 mmol) and N-hydroxysuccinimide (1.9 g, 16.5 mmol) were dissolved in 50 ml dioxane/ethyl acetate (9:1) and cooled to 5° C. 8.5 ml 2M DCC-solution were added and the whole mixture was stirred at room temperature for 15 h. The DCU was filtered off, washed carefully with dioxane (3x10 ml) and the filtrate was evaporated to dryness at 40°C bath temperature in vacuo. The resulting oil was dissolved in a small amount of 2-propanol and crystallized in the ice-box to 4.2 g (71% yield) colourless needles. mp: 112-113°C; R_f (A) 0.65; EI-MS: m/e 367/369; ^{1}H-NMR ($CDCl_3$) δ 1,832-1,907 (3H,d,-CH_3); 2,79 (4H,s,succ.); 4.0(2H,s,-CH_2-COOH); 5,197-5,415(1H,q,-CH-); 7,414-8,05(4H,m,AA'BB',C_6H_4.) IR (KBr) $\tilde{\nu}_{CO}$: 1810(m), 1783(m), 1738 (s), 1685(s) cm^{-1} Anal. calcd for $C_{15}H_{14}NO_5Br$: C,48.9; H,3.8; N,3.8; Found: C,49.2; H,3.87; N,3.7.

4-[2-(N-BOC-Alanyl)propionyl]phenylacetic Acid N-Hydroxysuccinimidester (4a)

The alanyl-compound is typical. L-BOC-Ala-OH (1.14 g, 6 mmol) in 25 ml dry acetone was added to 3 (0.74 g, 2 mmol) in 25 ml acetone and neutralized exactly under stirring and cooling to 5° C with a 10% TEA-solution. After stirring over night at room temperature no traces of 3 were detectable in TLC (A,B). After filtration the solution was

taken to dryness in vacuo at 40° C, the oily residue was dissolved in 200 ml ethyl acetate and successively washed 3 times with each 150 ml of water, 0.5M citric acid, water, pH 9.5 buffer (0.5M K_2CO_3, 0.5M $NaHCO_3$(2:1)) and water. After drying with anhydrous $MgSO_4$ the solution was taken to dryness and the resulting oil was crystallized from CH_2Cl_2/PE. 0.67 g (70% yield); R_f (A): 0.61; (B) 0.3; FD-MS: m/e 477 $(M+H)^+$.

The compounds 4b-4e (see Table 1) were synthesized in an analogous way.

4-[2-(N-BOC-Alanyl)propionyl]phenylacetamido-PEG (5)

To M-PEG-NH_2 ($\bar{M}$=5000, 0.17 mmol NH_2/g)(0.2 g,0.034 mmol/g) in 2 ml CH_2Cl_2 4a (40,4 mg, 0.085 mmol) were added. After stirring for 24 h at room temperature the polymer was precipitated by slowly addition of dry ether (150 ml) under cooling in the ice-bath. The precipitate was then filtered and washed with ether until no traces of 4a were detected by TLC. The polymer was dried in vacuo to give 180 mg of esterified PEG derivative. Loading with amino acid is shown in Table 2. R_f (C) 0.66, (D) 0.0. IR (KBr): $\tilde{\nu}_{CO}$ (cm^{-1}): 1710-1660.

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