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Bromoacylpolystyrene, a New Type of Polymer Support for Solid Phase Peptide Synthesis

Since the introduction of solid phase peptide synthesis by Merrifield using chloromethylated polystyrene,[1a-e] several modifications of the polymer support have been reported.[2a-f]

Although the polymer carriers in these modifications have respective merits, more convenient one is still desired for easier attachment of protected amino acids to the resin and more convenient cleavage of the finished peptide from the resin as acid, amide or hydrazide, preserving protecting groups on the peptide, if necessary.

We now report a use of bromoacylpolystyrene for the solid phase method, which is a new type of polymer support and may have advantage in the above respects. The use of phenacyl ester group for protection of the carboxyl function in peptide synthesis has been reported.[3a-c]

Partially bromoacetylated polystyrene-divinylbenzene was prepared by stirring styrene–divinylbenzene copolymer (2% DVB, 200—300 mesh beads (Mitsubishi Chemicals Co., Ltd.), 50 g) with bromoacetylbromide (40.4 g) and aluminium chloride (26.7 g) in nitrobenzene (total 300 ml) at room temperature and subsequent washing with 80% methanol, water, dimethylformamide, chloroform and methanol. The resultant polymer (70.75 g) contained 2.24 meq/g of Br as determined by the Volhard method.[1a]

α-Bromopropionyl–polystyrene–divinylbenzene containing 1.55 meq/g of Br was also obtained in a similar manner using methylene chloride as solvent and by washing the product with the same solvent, 50% dioxane, water and methanol.

Esterification of a protected amino acid to the resin was carried out by stirring the resin in ethyl acetate containing an equivalent amount of the protected amino acid and triethylamine at room temperature.

For example, *tert*-butyloxycarbonyl(BOC)-O-benzyltyrosine was esterified to the above bromoacetyl resin in a yield of 1.01 mmole/g and to the bromopropionyl resin in a yield of 0.41 mmol/g (The yields are based on Volhard titration values after removal of BOC group from the protected amino acid-resins, so that, to be exact, they are the yields of H-O-benzyl-Tyr on the resins).

Cleavage of the peptide from the resin was first examined on several BOC- and *tert*-amyloxycarbonyl(AOC)[4]–amino acid–resins as model compounds. BOC-leucine monohydrate (mp 82—85°, $[\alpha]_D^{20}$ −24.9° (c=2.2, AcOH); authentic sample: mp 82—86°, $[\alpha]_D^{23}$ −24.6° (c=2.1, AcOH)) was recovered in 99% yield by stirring the BOC-leucyloxyacetyl resin in an excess of 0.5N NaOH–dioxane (1:2 by vol.) at room temperature. AOC-phenylalanine amide (mp 125—127°, $[\alpha]_D^{20}$ −3.4° (c=0.9, AcOH)) was obtained in 87% yield from the AOC-phenyl-

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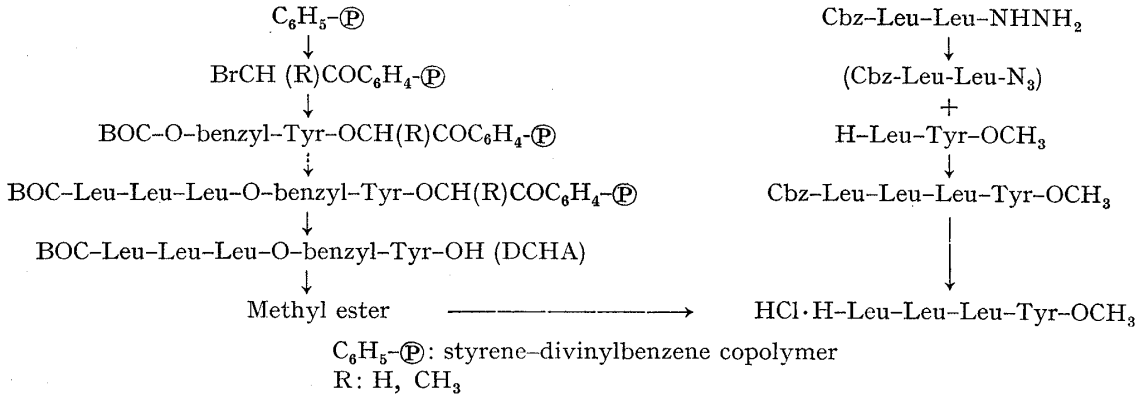
alanyloxyacetyl resin which was treated with NH_3 saturated in methanol. Both L- and D-AOC-phenylalanyloxyacetyl resins gave corresponding hydrazides in 88 and 92% yield respectively when the resins were treated with large excess of hydrazine hydrate in methanol at room temperature. L-Hydrazide (mp 109°, $[\alpha]_D^{20}$ −5.1° (c=1.2, AcOH)) was identical with D-isomer (mp 109°, $[\alpha]_D^{20}$ +5.1° (c=1.3, AcOH)) in all respects except the opposite optical rotations.

Usefulness of the bromoacetyl– and bromopropionyl–resins for peptide synthesis was then examined in the synthesis of a tetrapeptide, leucyl-leucyl-leucyl-tyrosine derivative.



C_6H_5-Ⓟ: styrene–divinylbenzene copolymer
R: H, CH_3

Removal of the BOC group by 1.5 N HCl in acetic acid and the following coupling reaction using threefold excess of each amino acid and dicyclohexylcarbodiimide in methylene chloride were carried out as described by Merrifield.[1a] The resultant tetrapeptide–resin was then cleaved in the same manner as for BOC-leucine described above and the product was purified as dicyclohexylamine (DCHA) salt. Thus, BOC-Leu-Leu-Leu-O-benzyl-Tyr-OH DCHA salt was obtained in 36% yield from the BOC-O-benzyltyrosyloxyacetyl resin and in 13% yield from the BOC-O-benzyltyrosyloxypropionyl resin.

The former product (mp 163—164°, $[\alpha]_D^{26}$ −15.1° (c=0.9, MeOH). *Anal.* Calcd. for $C_{51}H_{81}O_8N_5$: C, 68.65; H, 9.15; N, 7.85. Found: C, 68.71; H, 9.25; N, 7.88) was identified with the latter (mp 161—163°, $[\alpha]_D^{26}$ −14.2° (c=1.2, MeOH))

Methyl ester of the above product (mp 169—170°, $[\alpha]_D^{26}$ −43.5° (c=0.8, MeOH)), prepared in 94% yield by treatment with diazomethane, was converted into H-Leu-Leu-Leu-Tyr-OCH_3 hydrochloride in 90% yield by hydrogenation in the presence of dry HCl in methanol. The product (mp 234° (decomp.), $[\alpha]_D^{25}$ −27.8° (c=0.9, MeOH). *Anal.* Calcd. for $C_{28}H_{47}O_6N_4Cl$: C, 58.88; H, 8.30; N, 9.82; Cl, 6.21. Found: C, 58.68; H, 8.32; N, 9.87; Cl, 6.51) was proved to be identical with the specimen prepared by coupling carbobenzoxy(Cbz)-Leu-Leu hydrazide with H-Leu-Tyr-OCH_3 by the azide method (Cbz-Leu-Leu-Leu-Tyr-OCH_3: 55.6% yield, mp 198—200°, $[\alpha]_D^{25}$ −55.6° (c=1, MeOH)) and following hydrogenation over Pd-charcoal in methanolic hydrogen chloride (33% yield, mp 234° (decomp.), $[\alpha]_D^{25}$ −25.6° (c=1, MeOH)) by mixed mp, IR spectra, TLC and electrophoresis.

Further evaluation of this modification for the solid phase peptide synthesis is in progress in this laboratory.

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