Tetrahedron Vol. 32, pp. 1069 to 1072. Pergamon Press 1976. Printed in Great Britain

SYNTHESIS OF 2-HYDROXYETHYLSULFONYL-METHYL-SUBSTITUTED POLYSTYRENES AND THEIR APPLICATION IN SOLID PHASE PEPTIDE SYNTHESIS

GODEFRIDUS I. TESSER*, JAN T. W. A. R. M. BUIS,
ERIK TH. M. WOLTERS and ELISABETH G. A. M. BOTHÉ-HELMES

Department of Organic Chemistry, Catholic University, Toernooiveld, Nijmegen, The Netherlands

In Memoriam JOSEF RUDINGER

(*Received* 24 *November* 1975; *Accepted for publication* 9 *December* 1975)

Abstract—2-Hydroxyethylsulfonylmethyl-substituted polystyrenes are obtained from Merrifield's chloromethylated, cross-linked copolymer, by treatment with sodium 2-hydroxyethylmercaptide in liquid ammonia, followed by oxidation with m-chloroperbenzoic acid. Boc-amino acids can be esterified with the polymer, using dicyclohexylcarbodiimide in dichloromethane. Further condensations are possible via conventional procedures. Protected peptide derivatives, thus prepared, can be detached from the resin by brief treatment with a base. A high elimination rate was observed when the cleavage was performed with a 0.1 M solution of sodium hydroxide containing methanol and a limited amount of water. In the absence of methanol only trace amounts of the product were liberated.

SEVERAL years ago we presented a short communication[1] dealing with a new type of polymer for solid-phase peptide synthesis, which seemed to have an advantage over the conventional chloromethylated resin originally introduced by Merrifield.[2] The polymer was developed by analogy with the carboxyl protecting group of Hardy *et al.*[3,4] and Stirling,[5] by the introduction of 2-hydroxyethylsulfonyl groups into Merrifield's chloromethylated resin. Therefore, the resin **1** was reacted with 2-hydroxyethylmercaptan and oxidized to the sulfone **2**, and the first *t*-butyloxycarbonylamino acid, Boc-(Abc)[1]-OH(Scheme 1) was introduced by esterification. After deprotection and further elongation of the resulting polymeric amino acid ester **3**, according to the general procedure of Merrifield, the product was removed from the resin by a brief treatment with an alkaline reagent. The reaction, which is considered to proceed via β-elimination, yielded a protected peptide derivative **4**, bearing a free C-terminal group, which may be used in further condensations.

More convenient and useful procedures for the preparation of the resin and its application are now described, following additional experience in ours and other groups.[6,7] In the subsequent paper[8] the utility of the resin will be compared with Merrifield's resin in the preparation of the sensitive ACTH-(5–10)-hexapeptide using an automatic peptidesynthesizer.

Synthesis of cross-linked 2-hydroxyethylsulfonylmethyl polystyrene. Substitution of halogen in chloromethyl polystyrene[2,9] by 2-hydroxyethylthio groups was performed in liquid ammonia, followed by treatment with dimethylformamide. In this way complete replacement was obtained in resins with chlorine contents from 2 to 6%. In the IR spectra peaks at 1120 and 1265 cm^{-1}, caused by the chloromethyl groups, disappeared and new peaks appeared at 1060 (C–O) and 3200–3600 cm^{-1} (OH). Chlorine was not detectable by combustion analysis (<0.2%). For the oxidation of the thioether function to the desired sulfone, several peracids were compared. The reactivity of perbenzoic acid appeared to be rather low, but it oxidized the polymeric sulfide completely during the course of 24 hr. Pertrifluoroacetic acid, highly diluted in dichloromethane, oxidized immediately the thioether functions. However, this preparation (from 100% hydrogen peroxide and trifluoroacetic anhydride, is not without danger, both to the experimenter and to the resin. Excellent results were obtained with m-chloroperbenzoic acid, which oxidized the thioether functions rapidly and completely, giving a colourless product with the correct S/O ratio, and exhibiting new strong peaks at 1120 and 1300 cm^{-1} in its IR spectrum ($-SO_2-$).

Esterification of t-butyloxycarbonylamino acids with the resin alcohol. The introduction of the first amino acyl residue (i.e. t-Boc-Gly-OH) was performed most effectively by the use of dicyclohexylcarbodiimide in dichloromethane. Examination of the product by elemental analysis and amino acid analysis revealed that the incorporation slowed down, regardless of excess reagent, after 4 hr when about 40% of the OH groups were acylated. Free OH groups remaining after the esterification step were blocked by formylation with a mixture of formic acid and acetic anhydride.[10] The effect of this treatment was monitored by IR spectroscopy: narrowing of the broad hydroxyl absorbtion band (3200–3600 cm^{-1}), which was reduced to a small peak at 3400 cm^{-1} (NH). Other *t*-butyloxycarbonylamino acids were introduced in the same way. For the esterification of N-protected asparagine, N,N-dimethylformamide dineopentyl acetal[11] proved to be the most effective reagent.

Cleavage of the peptidyl-resin ester bond. The detachment of a peptide derivative, synthesized on the resin, was extensively investigated using Z-Ala-Phe-Leu-Gly-polymer. This was obtained from the Boc-glycyl resin by three consecutive coupling cycles, using the general method of Merrifield,[2] but with the insertion of a formylation step after termination of each acylation with a Boc-amino acid. The protected tetrapeptide is known[12] as its methyl ester. It constituted a good model for our investigation since the methyl ester is hydrolysed with difficulty by base, thus allowing a discrimination between

hydrolytic cleavage and detachment by β-elimination. Basic salts (sodium azide, sodium cyanide and sodium thiophenolate) or organic bases (pyridine, imidazole, N-alkylmorpholines, N-alkylpiperidines and triethylamine) were ineffective for the detachment of the peptide. Only traces of the peptide were liberated during 20 min with sodium hydroxide (0.1 N in 75% aqueous dioxan). The amount did not substantially increase after a reaction period of several hours.

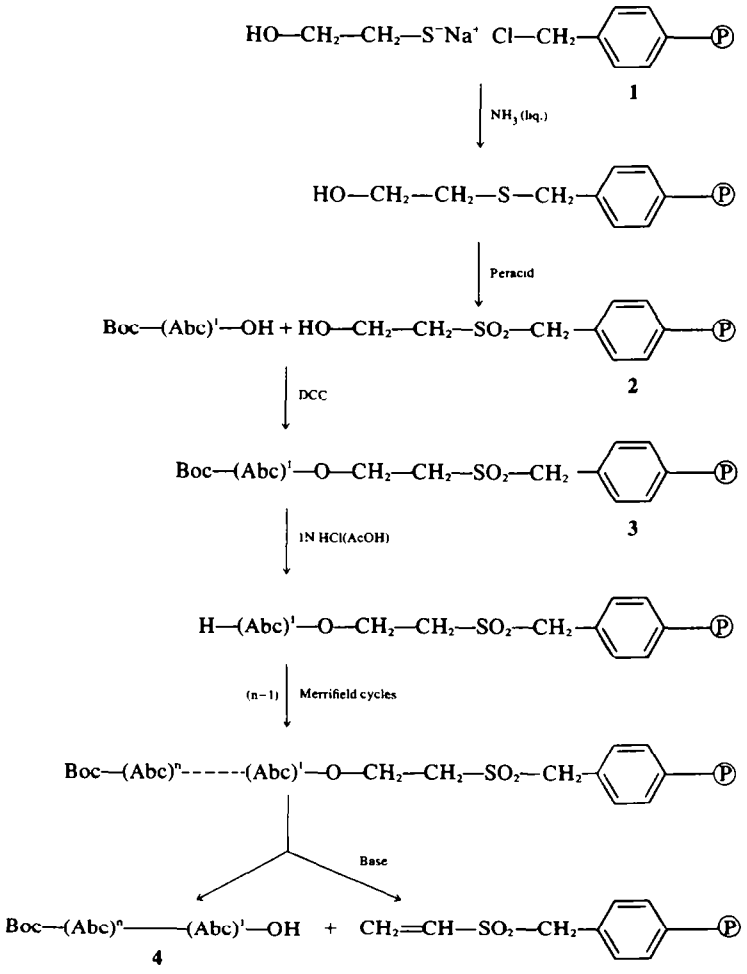
The inadequacy of OH ions to detach the model compound was attributed to a restricted penetration of these ions into the interior of the resin beads. Whereas sodium methoxide (0.1 N in dioxan-methanol, 3:1) induced immediate release of the peptide, albeit partly in the esterified form. We found, however, that a very high acid/ester ratio was obtained if the cleavage was performed with the homogeneous mixture of dioxan, methanol and 4 N aqueous sodium hydroxide (30:9:1). This reagent mixture, which is 0.1 N in respect of base and contains 2.5% water, liberated completely Z-Ala-Phe-Leu-Gly-OH within 45 sec and one recrystallization of the crude product from methanol gave an analytically pure product. Further experiments[8] with the sulfone resin revealed that the acid/ester ratio in the product increases with the length of the peptide chain. β-Elimination is also preferred to methanolysis by vigorous agitation during the cleavage period and by a high base concentration. The use of a strong base during the detachment incurs the risk of secondary reactions (racemization, transpeptidation or degradation) of the product, once it has been liberated and remains in solution. However, detachment periods of 3 min appeared to be permissible in this respect; they restrict secondary reactions to less than 2%. This reaction time was always sufficient for complete removal of a peptide from the sulfone resin.

The method cannot be applied to compounds containing C-terminal asparaginyl residues as aminosuccinimide derivatives will be detached from the carrier on treatment with a base. This reaction may be precluded by introduction of an amide protecting group[13] prior to the esterification step. Detachment of Nps-Asn(Mbh)-OH from the resin could be performed satisfactorily. However, as the Mbh group must be maintained throughout the whole sequence of reactions leading to any desired C-terminal asparagine peptide, which is difficult to achieve,[13] this observation is of limited value.

EXPERIMENTAL

M.ps are uncorrected, IR spectra were measured on a Perkin-Elmer 257 IR spectrophotometer as KBr-disks. TLC of peptide derivatives, liberated from the resin, was performed on precoated silica plates (Merck, F_{254}) using chloroform–methanol (3:1) as the solvent.

2-Hydroxyethylsulfonylmethyl polystyrene. Dry ammonia



Scheme 1.

(500 ml) was condensed into a 1 liter round bottommed 3-necked flask equipped with a stirrer, a guard tube filled with NaOH grains, and a filling tube. 2-Mercaptoethanol (20 ml) was added with stirring giving a ppt. Na was dissolved in the suspension until a blue colour persisted. The colour was discharged with a few drops of 2-mercaptoethanol, and chloromethyl polystyrene (30 g, 6% of chlorine) was suspended in the clear soln. Evaporation of the solvent left a colourless, viscous syrup embedding the polymer. Dimethylformamide (300 ml) was added to dilute the syrup, and the suspension was stirred under N_2 for 12 hr. The slightly yellow suspension was filtered, washed with the same solvent and then successively with methanol, water, acetic acid and methanol. Elemental analysis of the resulting 2-hydroxyethylthiomethyl polystyrene (31.4 g) indicated 5.1% of S and less than 0.2% of Cl.

The product (10 g, 16 m eq. of S) was suspended in dichloromethane (75 ml), and 6.8 g of *m*-chloroperbenzoic acid (85% active, 33.6 mmoles) dissolved in CH_2Cl_2 (40 ml) were added slowly. The temp. rose to the b.p. of the mixture during the oxidation. The suspension was left at room temp. for several hr and then filtered. After washing with dichloromethane and then with EtOH, a sample was subjected to elemental analysis: the O/S ratio was found to be 2.95 (theoretical 3.0).

Benzyloxycarbonyl - L - alanyl - L - phenylalanyl - L - leucyl - glycine 2-ethylsulfonylmethyl polystyrene ester. t-Butyloxycarbonylglycine (2.0 g, 11.1 mmoles) and then dicyclohexylcarbodiimide (2.29 g, 11.1 mmoles) were added to a suspension of 8 g of 2-hydroxyethylsulfonylmethyl polystyrene (0.556 meq. of OH per g of resin) in 50 ml of dichloromethane. The vessel was agitated for 4 hr by axial rotation in a tilted position at room temp. After filtration and successive washing with CH_2Cl_2, AcOH, DMF and EtOH, the remaining resin contained 0.215 meq. of glycyl residues/g of adduct (40% esterification). The resin was suspended in benzene (50 ml) and treated with a 10-fold molar excess of formic/Ac_2O (prepared from Ac_2O and formic acid 20:9 by weight and containing a few drops of pyridine) for 20 hr to block remaining free OH groups. After filtration the resin was washed with CH_2Cl_2, Et_3N-CH_2Cl_2 (1:9, freshly prepared), EtOH and CH_2Cl_2, and dried *in vacuo*. Boc-groups were eliminated by treatment with 1 N HCl in AcOH for 1 hr. The synthesis on the resin was completed by three successive condensations with Boc-Leu, Boc-Phe and Z-Ala. Free amino groups, if present, were blocked after each acylation by formylation. Determination of the Cl content of the resin after cleavage of the Boc-groups revealed that the acylation of glycyl residues was quantitative, that of the leucyl residues was approximately 90%.

Detachment of the tetrapeptide from the resin

Analytical procedure. Resin samples (10 mg, comprising about 1 mg of benzyloxycarbonyltetrapeptide) were weighed out into small test tubes and were treated with various basic reagents (0.1 ml of 0.1 M solns). The base was neutralized after periods ranging from 5 sec to 5 min by addition of 0.1 ml of 0.2 N AcOH in MeOH, and the supernatant soln was subjected directly to TLC. In this way, a mixture of dioxan, MeOH and 4 N NaOH (30:9:1) was selected as the most suitable reagent. It liberates the benzyloxycarbonyltetrapeptide almost completely as the free acid in about 45 sec, without formation of side products. Completeness of the detachment was proven by resuming the process on the same samples, but now for the longest period (5 min) with each sample.

Preparative procedure. The loaded resin (2 g) was suspended in a 5-fold excess of the reagent and stirred vigorously for 1 min. The suspension was poured onto a coarse sintered funnel, the filtrate was collected in a receiver containing an excess of HCl, and the resin was washed with MeOH. Evaporation of the filtrate, followed by extraction of the residue with EtOAc and water and crystallization of the residue gave 150 mg of the pure benzyloxycarbonyltetrapeptide m.p. 188°. (Found: C 62.0; H 6.6; N 10.3. $C_{28}H_{36}N_4O_7$ requires: C 62.20; H 6.71; N 10.36%). Amino acid analysis of the acid hydrolysate Ala, 0.95; Gly 0.98; Leu, 1.00 (reference); Phe 1.02). Treatment of the product with ethereal diazomethane gave the corresponding methyl ester m.p. 196°, $[\alpha]_D^{20} = -52.6°$ ($c = 1$, MeOH), lit.[12] m.p. 186–187°, $[\alpha]_D^{20} = -52.6°$ ($c = 1$, MeOH). The protected ester was also obtained by an independent "classical" method in which a stepwise chain-elongation was carried out starting from H-Gly-OMe. Quantitative ester hydrolysis of the resulting Z-Ala-Phe-Leu-Gly-OMe proved to be difficult.

ADDENDUM

GEORGES TEUTSCH
Centre de Recherches, Roussel, UCLAf, 93-Romainville, France

In an independent investigation, the applicability of the principle of β-elimination towards solid phase peptide synthesis was also examined in our laboratories. The sulfonyl resin was developed along the same line as described above (i.e. by reacting Merrifield's chloromethylated resin with potassium β-hydroxyethylmercaptide in dimethylacetamide and subsequent oxidation with m-chloroperbenzoic acid). Several Boc-amino acids—Asp(OBzl), Cys(Pmb), Gly, Phe, Pro and Thr(Bzl)—were esterified with the resin using dicyclohexylcarbodiimide at ambient temperature or with carbonyldiimidazole in refluxing tetrahydrofuran. The latter method gave incorporations approaching complete substitution (e.g. with Boc-Asp(OBzl), but extensive racemization accompanied the esterification. The dicyclohexylcarbodiimide technique was thus preferred. For detachment the *unsolvated fluoride ion* was used. For this purpose a resin adduct containing Boc-Asp(OBzl) (500 mg, corresponding to 0.38 mmoles) was added to a slurry of anhydrous tetramethylammonium fluoride (400 mg) in dimethylformamide (5 ml), and left for 1.5 h at 38–40°. After evaporation of the filtrate, the residue was dissolved in water and extracted with dichloromethane to give 85 mg (71%) of Boc-Asp(OBzl)-OH. The stability of Boc-amino acids—Ala, Arg(NO_2), Cys(Pmb), Gly, Phe, Pro, Ser(Bzl), Thr(Bzl) and Tyr(Bzl)—towards the reagent (4.5 h and 60°) proved to be excellent, the only exception being His(Dnp) which was extensively decomposed by the reagent, apparently by fission of the imidazole ring. In the application to solid phase syntheses removal of this protective function, prior to final detachment by selective deprotection (e.g. with thiophenol), is thus an important precaution. Boc-His(Tos) lost partially its imidazole protecting group during 2 h at 60°, without further attack of the amino acid.

In conclusion: By its high charge density the unsolvated fluoride ion is a strong base which can induce the desired β-elimination by virtue of its easy access to the interior of the polymer. An advantage of the method is that methanol is unnecessary during the exposure of the resin ester to the base; this excludes concommitant transesterification.

Acknowledgements—The investigation has been carried out under the auspices of the Netherlands Foundation for Chemical

Research (SON) and with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). We are grateful to Dr. M. Blom (DSM, Geleen) for performing the sulfur and oxygen analyses.

REFERENCES

[1]G. I. Tesser and B. W. J. Ellenbroek, *Proceedings of the Eighth European Peptide Symposium*, (Eds. H. C. Beyerman, A. v/d Linde, W. Maassen v/d Brink) p. 124 North Holland, Amsterdam (1967).
[2]R. B. Merrifield, *J. Am. Chem. Soc.* **85**, 2149 (1963).
[3]P. M. Hardy, H. N. Rydon and R. C. Thompson, *Tetrahedron Letters* 2525 (1968).
[4]P. M. Hardy, H. N. Rydon and R. C. Thompson, *J. Chem. Soc. Perkin I*, 5 (1972).
[5]A. W. Miller and C. J. M. Stirling, *Ibid.* 2612 (1968).
[6]G. R. Marshall and R. B. Merrifield, *Biochemical Aspects of Reactions on Solid Supports* (Editor G. R. Stark), p. 111, Academic Press, New York (1971).
[7]G. Teutsch (Uclaf, France), personal communication.
[8]Subsequent paper in this journal.
[9]K. W. Pepper, H. M. Paisley and M. A. Young, *J. Chem. Soc.* 4097 (1953).
[10]A. van Es and W. Stevens, *Rec. Trav. Chim.* **84**, 704 (1955).
[11]J. Schreiber, Ref. [1], p. 107.
[12]J. H. Jones, B. Liberek and G. T. Young, *J. Chem. Soc.* (*C*), 2371 (1967).
[13]W. König and R. Geiger, *Chem. Ber.* **103**, 2041 (1970).