Short Communications

Monitoring of Solid-phase Peptide Synthesis by Potentiometric Titration of Samples

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Potentiometric titration with perchloric acid on the whole batch of resin has been used for monitoring solid-phase peptide synthesis.^{1–5} However, titration on withdrawn samples is in some cases to be considered as advantageous, as the main portion of resin bound product is not subjected to the washing procedure preceding the titration or to the perchloric acid titration itself. Also in large scale preparations titration of the entire amount of resin bound peptide may be disadvantageous. A prerequisite for quantitation of the results, however, is that throughout the synthesis it is possible to account for withdrawn material. In laboratory scale syntheses this can be done by weighing, and as example is presented the monitoring of the chain elongation in the synthesis of the cyclic decapeptide antamanide.

Experimental. Synthetic procedure.* Boc-Phe-O-resin (0.46 mmol Phe/g) was prepared by esterification of the tetramethylammonium salt of Boc-Phe to a chloromethylated (0.81 mmol Cl/g) polystyrene, crosslinked with 2 % divinyl benzene, Bio Beads S-X2, 200–400 mesh from BIO-RAD.⁶ Boc-Phe-Pro-Pro-Phe-Phe-Val-Pro-Pro-Ala-Phe-O-resin was synthesized using 25 g of Boc-Phe-O-resin. Each coupling was performed with a threefold excess of Boc-amino acid and DCC and allowed to proceed for 17–19 h. After coupling the whole amount of resin was dried, weighed and an aliquot withdrawn. Boc-groups were cleaved with 1 N HCl/acetic acid.

Titration. Potentiometric titrations were carried out on samples of approx. 1 g in acetic acid/dichloromethane (1:1 v/v), using a Radiometer titration equipment. Due to the increasing proportion peptide/resin, 0.5 to 0.3 meq was titrated. The titrant was 0.0296 N perchloric acid in acetic acid. The accuracy of the titration was 0.475 ± 0.0015 meq/g, i.e. ± 0.3 %,

an improvement compared to earlier experiments,^{3,4} in which the entire amount of resin bound product was titrated.

Amino acid analysis. 10–15 mg of the samples withdrawn after each coupling step was hydrolyzed for 4 h with 6 N hydrochloric acid–propionic acid 1:1 v/v at 130 °C in sealed ampoules. The cyclic product was hydrolyzed for 24 h with 6 N hydrochloric acid at 110 °C in a sealed ampoule. A Beckman model 121 amino acid analyzer was used for analyzing the hydrolysates.

Cleavage and cyclization. The end product was cleaved from the resin with HBr in trifluoroacetic acid (5 × 20 min). The crude peptide was subjected to high voltage electrophoresis at pH 2 on Whatman No. 1 and to thin-layer chromatography in chloroform–methanol–acetic acid 18:1:1, 2-butanol–formic acid–water 75:15:10, 2-butanol–aqueous ammonia 85:15, and 1-butanol–pyridine–acetic acid–water 30:20:24:6. In all systems a high degree of purity of the product was indicated. Cyclization was performed according to Ref. 7 on the crude peptide. The peptide (6.9 g, 5.5 mmol) was dissolved in dimethylformamide (150 ml), *N*-hydroxysuccinimide (2.76 g, 24.0 mmol) added and the mixture diluted with dichloromethane (1.35 l). The solution was cooled to 0 °C and DCC (2.7 g, 13.1 mmol) in dichloromethane (50 ml) and triethylamine (1 ml, 7.1 mmol) were added. The reaction mixture was stirred at 0 °C for 1.5 h and allowed to attain room temperature and left for 70 h. The solvents were evaporated and the product dissolved in tetrahydrofuran. The precipitated dicyclohexylurea was filtered off and the filtrate concentrated to an oil. 1.7 g of antamanide (27 %) was isolated by chromatography on aluminium oxide and silica gel. M.p. 170–172 °C, Ref. 7, 170–174 °C. Amino acid analysis in $\mu\text{mol/mg}$: Phe 3.53, Val 0.846, Pro 3.60, Ala 0.844. Theoretical, respectively: 3.38, 0.845, 3.38, and 0.845. $[\alpha]_{\text{D}}^{20} - 163^\circ$ (c, 1, methanol), Ref. 7, -148° . The product was homogeneous by thin-layer chromatography. Molecular weight determined by mass spectrometry was 1146.

Results. The course of the chain elongation is demonstrated in Fig. 1. The values are corrected for withdrawal of the samples. As the chain elongation proceeds, an increase in the titration values of the Boc-protected product is seen to occur after step 5, due to *N*-alkylation.⁸ Furthermore, a slight decrease (8 %)

* Abbreviations: Boc = *tert*-butoxycarbonyl; DCC = *N,N'*-dicyclohexylcarbodiimide.

Fig. 1. Titration values obtained during the chain elongation in the synthesis of antamanide carried out from the C-terminal Phe (left) to the N-terminal Phe (right). The ordinate values are the numbers of meq calculated per g of Boc-Phe-O-resin. Filled circles before, and open circles after Boc-group cleavage.

Table 1. Amino acid content of the ten samples from the synthesis of antamanide as determined by titration and amino acid analysis. Values shown in $\mu\text{mol}/\text{mg}$.

Sample No.	Phe Titr.	A ^a	Ala Titr.	A	Pro Titr.	A	Val Titr.	A
1	0.47	0.46	—	—	—	—	—	—
2	0.46	0.44	0.46	0.44	—	—	—	—
3	0.44	0.42	0.44	0.42	0.43	0.42	—	—
4	0.42	0.41	0.42	0.41	0.80	0.81	—	—
5	0.41	0.39	0.41	0.39	0.78	0.78	0.37	0.39
6	0.73	0.74	0.38	0.37	0.74	0.74	0.35	0.37
7	1.01	1.05	0.36	0.35	0.69	0.70	0.33	0.35
8	1.01	1.02	0.36	0.34	1.00	1.02	0.33	0.34
9	0.97	0.97	0.34	0.33	1.24	1.31	0.31	0.33
10	1.21	1.26	0.33	0.32	1.20	1.26	0.30	0.31

^a A = Amino acid analysis.

is demonstrated in the titration values after deprotection, most likely due to cleavage of peptide from the resin. The evident irregularities by the titration of the proline residues coupled as Nos. 4 and 9 cannot be explained, but the subsequent coupling yields correspond to the first obtained titration value. The yield in single steps and the overall yield are to be calculated by subtracting the respective values obtained by titration of the Boc-protected product from the deprotected.⁵ Thus an overall yield of 89 % was achieved.

In Table 1 is presented the content of the amino acid residues calculated from the titration values in the single steps of chain elongation, and determined by amino acid analysis. A good agreement is shown to exist between

the two methods of determination of the amino acid content.

Discussion. The results obtained demonstrate that a quantitative determination of the yields in the single steps during the synthesis of a peptide can be performed by titration on withdrawn samples. A prerequisite is, however, that the proportion between the amount of the sample and the total amount can be determined with sufficient accuracy. Regarding the calculation of the content of the amino acids in a sample, the reliability of the results is somewhat dependent on the course of the synthesis. Thus, if much peptide is lost during the synthesis, this must be taken into consideration by the calculation of the content of the amino acid residues preceding the N-terminal

amino acid in the sample. If a decrease during the synthesis is due to blocking, the titration results will show the actual content of the amino acids. In the present experiment it has not been possible to explain the reason for the gradual slight decrease of the titration values during the synthesis.

In the synthesis of still larger amounts of resin bound peptides, determination of the proportion of the sample to the entire amount by weighing is not possible in practice. In such cases an internal standard has to be used for determination of the amount of derivatized polystyrene in the actual sample, allowing the results to be calculated per weight unit of the polymer. Whether a radioactive labelling of the methylene group in the benzyl ester linkage insures a sufficient accuracy will have to be evaluated by experiments.

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Resolution and Absolute Configuration of 1-Ethyl-1-methyl-2-propynylamine

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The enantiomers of some *N*-(4-*tert*-amino-1-methyl-2-butyryl)-substituted succinimides and 2-pyrrolidones show strong stereospecificity in blocking the motor effects of the muscarinic agent oxotremorine, *N*-(4-pyrrolidino-2-butyryl)-2-pyrrolidone. The (*R*)-(+)-isomers are about twice as active as their corresponding racemates in this respect while the (*S*)-(–)-isomers are practically inactive.^{1,2} In order to investigate the influence of further alkyl substitution on stereospecificity we decided to prepare optical isomers of compounds containing both a methyl and an ethyl group in the 1-position of the butynyl chain. For this purpose we needed the enantiomers of 1-ethyl-1-methyl-2-propynylamine (*1*) as starting material and this communication deals with the resolution of this amine and the determination of its absolute configuration.

The amine was resolved into its (+)- and (–)-enantiomers by fractional crystallization of its (+)- and (–)-hydrogen tartrates, respectively. In order to estimate the optical purity of the enantiomers we used ¹H NMR spectroscopic analyses of the diastereomeric amides formed when optically impure amine is acylated with optically pure (–)-*O*-methylmandelyl chloride.^{3,4}

The absolute configuration of the amine was established by transforming its benzoyl derivative to the corresponding isovaline derivative.

The benzoyl derivative obtained from the (+)-enantiomer of the amine yielded upon oxidation with potassium permanganate the benzoyl derivative of (+)-isovaline. Thus (+)-*1* and (+)-isovaline must be configurationally identical. The latter compound has been correlated into the natural amino acid series (*L*- or *S*-configuration) both by chemical⁴ and enzymatic methods.⁵ Dextrorotatory *1* can therefore be assigned the *S* configuration.

Experimental. Melting points were determined in a metal block using open capillary tubes and calibrated Anschütz thermometers. Microanalyses were carried out at the Microanalytical Laboratory, Royal Agricultural College, Uppsala. IR spectra were run on a Perkin-Elmer 157 G spectrophotometer and ¹H NMR spectra