DEXTRAN DERIVATIVES

III.* SYNTHESIS OF ESTERS OF DEXTRAN WITH N-SUBSTITUTED AMINO ACIDS BY THE MIXED ANHYDRIDE METHOD AND STUDY OF THE CONDITIONS FOR ELIMINATING THE PROTECTIVE GROUPS

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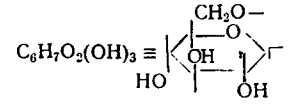
We have previously described the synthesis of O-acetylaminoacyl derivatives (AAD)† of the polysaccharide dextran by the condensation of acyl amino acids with Russian clinical dextran ("polyglucin") with the aid of DCHC [2].

To widen the possibilities of obtaining these derivatives of dextran, we have investigated other methods of forming the ester bond. This paper considers the synthesis of AADs with the aid of the mixed anhydrides of N-protected amino acids with ethyl chloroformate, phosphorus oxychloride, p-toluenesulfonyl chloride, and benzenesulfonyl chloride. In addition, the conditions for eliminating the N-protective groups (Tos, BOC, NPS) from the AADs obtained are given. The condensation of dextran with the N-protected amino acids was performed in the following way:

$$[C_6H_7O_2(OH)_3]_m \xrightarrow{nR'NHRCOOH} [C_6H_7O_2(OH)_{3-n}(OCORNHR')_n]_m \rightarrow$$

(I–VII)

$$\rightarrow [C_6H_7O_2(OH)_{3-n}(OCORNH_2)_n]_m$$



I R=CH_2, R′=Tos
II R=CH_2, R′=NPS
III R=$(CH_2)_5$, R′=Boc
IV R=$CHCH_3$, R′=BOC
V R=$CHCH(CH_3)_2$, R′=NPS
VI R=$CHCH_2C_6H_5$, R′=BOC
VII R=$CHCH_2C_6H_5$, R′=NPS.

The mixed anhydride of the N-protected amino acid obtained in the usual way, in pyridine (for $POCl_3$, TosCl, and $PhSO_2Cl$) or in chloroform containing triethylamine (for EtOCOCl), was added with cooling to a solution of dextran in a mixture of diethyl sulfoxide and pyridine. On the following day the polymer was precipitated with ethanol. The reaction products from low-molecular-weight compounds were purified by gel filtration on Sephadex G-50. Some AADs were synthesized by the carbodiimide method that we have described previously [2]. The substances obtained and their properties are given in Table 1. A positive hydroxamic acid reaction for an ester group was used as proof of the formation of covalent bond between the amino acid and the dextran. The degree of substitution of the dextran by the N-protected amino acid residues was determined from the nitrogen content of the products (Kjeldahl).

It has been reported previously [1] that when nonpolar groupings are present in the N-protected amino acids, the solubility of the corresponding AADs in water depends on the degree of their substitution.

* For Communication II, see [1].

† The following abbreviations have been adopted: DCHC – dicyclohexylcarbodiimide; Tos – tosyl; BOC – tert-butoxycarbonyl; Boc – benzyloxycarbonyl; NPS – o-nitrobenzenesulfenyl; Aen – ω-aminoenanthyl; and DCHA – dicyclohexylamine.

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TABLE 1. Properties of the Derivatives of Dextran and N-Protected Amino Acids

AAD	Condensing agent and base	Amt. (mole)/anhydroglucose unit of the dextran: amino acid	Amt. (mole)/anhydroglucose unit of the dextran: condensing agent	γ^*_{found} in the reaction product	$\gamma_{found}/\gamma_{theor}$, %	Solubility of the reaction product in water	λ_{max}, nm (for the H_2O reaction product)
Tos-Gly-(dextran) (I)	$POCl_3/C_5H_5N$	0,2	0,2	0,76	3,8	+	290
	$TosCl/C_5H_5N$	0,4	0,2	0,47	2,4	+	290
	$DCHC/C_5H_5N$	0,5	1,5	5,7	11,4	+	287
NPS-Gly-(dextran) (II)	Et OCOCl/Et_3N	0,5	0,5	1,39	2,8	+	287, 405
Bco-Gly-(dextran)	$DCHC/C_5H_5N$ [2]	1,0	4,0	11,1	11,1	+	287
	$DCHC/C_5H_5N$ [2]	0,1	0,12	2,8	28,0	+	287
Bco-Aen-(dextran) (III)	$PhSO_2Cl/C_5H_5N$	0,1	0,1	4,08	40,8	+	287
	Et OCOCl/Et_3N	0,5	0,5	3,68	7,4	+	287
	$DCHC/C_5H_5N$ [2]	0,1	0,12	2,2	22,0	+	287
BOC-*L*-Ala-(dextran) (IV)	$DCHC/C_5H_5N$	0,5	1,0	9,82	19,6	+	—
	Et OCOCl/Et_3N	0,5	0,5	0,24	1,9	+	—
NPS-*L*-Val-(dextran) (V)	$DCHC/C_5H_5N$	1,0	1,0	1,42	1,4	+	242, 285, 390
BOC-*L*-Phe-(dextran) (VI)	$DCHC/C_5H_5N$	0,4	1,9	13,7	31,2	slight	287
NPS-*L*-Phe-(dextran) (VII)	$DCHC/C_5H_5N$	0,5	1,0	16,9	33,8	—	390†
Ac-*L*-His-(dextran)	$DCHC/C_5H_5N$ [2]	0,1	0,12	1,05	10,5	+	—

*γ represents the number of substituents per 100 anhydroglucose units of the dextran.

† In dimethyl sulfoxide.

Thus, the AADs (VI) and, particularly, (VII) with γ = 13.7 and 16.9, respectively, are sparingly soluble in water (they dissolve in dimethyl sulfoxide). With lower degrees of substitution of the radical on the nitrogen atom, water-soluble AADs are obtained [for example, (V)].

The macromolecular structure of the dextran is preserved in the AADs, as is confirmed by the gel chromatography of the latter on Sephadex G-75 [1, 2]. None of the methods of forming an ester bond used leads to appreciable degradation of the polysaccharide chains, as is shown by the coincidence of the elution curves of the AADs and the curve for dextran [2]. In some cases [for example, the AADs (III) obtained by different methods], the results of gel chromatography were confirmed by molecular-weight determinations and sedimentation analyses by the method of unestablished equilibrium. No appreciable differences in M_w between the AADs and the initial dextran were found.

To compare the various methods of obtaining AADs it is convenient to use the ratio $\gamma_{found}/\gamma_{theor}$, where the first magnitude is the degree of substitution of the dextran by acylaminoacyl residues found (from the nitrogen content) and the second is that degree of substitution that would have been obtained if the acylation reaction had taken place to the extent of 100%. This ratio reflects the efficiency of the acylating agent at low degrees of substitution, when the groups introduced into the molecule still have no effect on the ease of acylation. It can be seen from Table 1 that the best results were obtained in the acylation of the dextran with DCHC and benzenesulfonyl chloride. In the case of ethyl chloroformate, the reaction depends strongly on the small amount of water present in the dextran; phosphorus oxychloride and tosyl chloride are ineffective as condensing agents. Furthermore, in the mixed anhydride method it is impossible to use an excess of condensing agent as this could lead to the acylation of the polymer by it. Consequently, it may be considered that the carbodiimide method for the O-aminoacylation of dextran has advantages over the mixed anhydride method using benzene sulfonyl chloride.

Various methods were tried for the elimination of the N-protective groups from the AADs synthesized. We have previously described the elimination of the Boc groups for AADs by hydrogenation [2]. However, the use of the method is limited because of the small number of hydrogenolyzable N-protective groups. In addition, hydrogenation is complicated for derivatives of sulfur-containing amino acids. Since for the synthesis of O-aminoacyldextrans containing trifunctional amino acids and also for O-peptidyldextrans we must choose protective groups capable of selective elimination, we investigated the behavior of the AADs under the conditions used in peptide synthesis for the elimination of Tos, BOC, and NPS groups.

TABLE 2. Results of a Comparison of the Methods of Eliminating the N-Protective Groups from the O-Acylaminoacyl Derivatives of Dextran

Starting material	Reaction conditions and medium	Results obtained
Tos-Gly- (dextran)(I) (γ-5,7)	Na + solution of the polymer in liquid NH_3	Complete ammonolysis of the glycine–dextran bond
BOC-*L*-Phe-(dextran) (VI) (γ-13,7)	Anhydrous CF_3COOH; the polymer gradually dissolves	BOC protection eliminated with considerable cleavage of the polymer, accompanied by trifluoroacetylation
BOC-*L*-Phe-(dextran) (VI) (γ-13,7)	50% CF_3COOH; the polymer gradually dissolves	BOC protection eliminated with cleavage of the polymer
BOC-*L*-Phe-(dextran) (VI) (γ-13,7)	Suspension of the polymer in 4 M HCl in anhydrous dioxane	Cleavage of the polymer
BOC-*L*-Ala-(dextran) (IV) (γ-9,82)	Dowex 50×2 cation-exchange resin or SE-Sephadex C-50; aqueous soln. of the polymer	Degree of elimination of the BOC groups insignificant
NPS-*L*-Phe-(dextran) (VII) (γ-16,9)	Suspension of the polymer in in 4 M HCl in dioxane	NPS protection eliminated only from the surface of the granules
NPS-*L*-Phe-(dextran) (VII) (γ-16,9)	Solution of the polymer in 4 M HCl in $PhNO_2$	Cleavage of the polymer
NPS-*L*-Phe-(dextran) (VII) (γ-16,9)	Solution of the polymer and PhSH in a mixture of DMSO and pyridine (1 : 1)	75% of NPS groups split off *
NPS-Gly- (dextran)(II) (γ-1,39)	The same	80% of NPS groups split off
NPS-*L*-Val-(dextran) (V) (γ-1,42)	The same	50% of NPS groups split off
NPS-Gly-(dextran) (II) (γ-1,39)	Aqueous solution of the polymer treated with solutions of $Na_2S_2O_3$ and KI	87.5% of NPS groups split off
NPS-*L*-Val-(dextran) (V) (γ-1,42)	The same	55% of NPS groups split off

* The macromolecular structure of the polymer is retained.

To check the retention of the macromolecular structure, as in the condensation reaction, we used comparative gel chromatography of the starting materials and the reaction products. In those cases in which the AADs after the appropriate treatment were eluted with a volume of water greater than V_1 (the pore volume within the gel granules), it was considered that appreciable degradation of the macromolecule had taken place during the elimination of the protective groups.

The results of the experiments performed on the elimination of the Tos, BOC, and NPS protective groups from the AADs are given in Table 2. Reduction with sodium in liquid ammonia, which is used in peptide chemistry for splitting off not only tosyl but also a number of other protective groups (N-Boc, S, and N^{im}-benzyl groups, etc.), leads in the AADs to the complete ammonolysis of the ester bond between the acyl amino acid and the dextran (apparently, because of the presence of traces of moisture in the AADs); consequently it is not applicable. The acidolytic elimination of BOC and NPS protective groups cannot be used on the AADs, either. In a homogeneous medium the glucosidic bonds of the dextran undergo acidolysis, while in anhydrous trifluoroacetic acid the hydroxy groups of the carbohydrate undergo trifluoroacetylation, in addition. In a heterogeneous medium, the protective groups are split off only from the surface of the particles of the AADs; we were unable to bring about a deeper reaction. In view of the macromolecular nature of BOC-L-Ala-dextran (IV), we performed acidolysis with Dowex 50 × 2 cation-exchange resin or with SE-Sephadex C-50 in a coarse-pored hydrophilic matrix. However, in both cases only about 3% of the amino groups was liberated. Under the same conditions BOC-L-alanine was converted almost completely into L-alanine. Consequently, the cause of the small extent to which the reaction proceeds is the polymeric nature of the AADs.

EXPERIMENTAL

The general procedures (evaporation, drying, gel chromatography, detection of the polysaccharide) have been described in the previous paper [2].

I. Synthesis of the AADs

1. O-(Tos-Glycyl)dextran (I). A. With Tosyl Chloride. To a solution of 4.04 g (17.6 mmoles) of N-Tos-glycine in 40 ml of absolute pyridine was added dropwise 1.68 g (8.8 mmoles) of tosyl chloride in 10 ml of pyridine, and the mixture was stirred at 0°C for 30 min. The resulting solution was poured into an ice-cooled solution of 7.15 g (44.1 mmoles) of dextran in 100 ml of absolute dimethyl sulfoxide and 40 ml of absolute pyridine. This mixture was stirred at 0°C for 2 h and at 20°C for 24 h. The polymer was precipitated with ethanol and, after decantation, it was triturated in ethanol, dried, and subjected to gel filtration on Sephadex G-50. The eluates containing the polymer were concentrated to ≈ 20 ml. The product was again precipitated with ethanol, triturated in ethanol, and dried. Yield 6.09 g. Found, %: N 0.40; $\gamma_{found} = 0.47$ ($\gamma_{theor} = 20$).

B. With Phosphorus Oxychloride. The reaction was performed as in experiment 1A with 7.79 g (48.1 mmoles) of dextran and the products of the interaction of 2.18 g (9.6 mmoles) of N-Tos-glycine and 0.87 ml (9.6 mmoles) of freshly distilled phosphorus oxychloride. The reaction product was purified as described above. Yield 6.35 g. Found, %: N 0.65; $\gamma_{found} = 0.76$ ($\gamma_{theor} = 20$).

C. With DCHC. The reaction was performed as described previously [2] with 4.0 g (24.7 mmoles) of dextran, 7.62 g (37 mmoles) of DCHC, and 2.82 g (12.3 mmoles) of N-Tos-glycine. Yield 3.87 g. Found, %: N 0.46; $\gamma_{found} = 5.70$ ($\gamma_{theor} = 50$).

2. O-(Boc-ω-Aminoenanthyl)dextran (III). A. With Benzenesulfonyl Chloride. Compound (III) was obtained in a similar manner to compound (I) in experiment 1A from 7.56 g (46.7 mmoles) of dextran, dissolved in 60 ml of absolute dimethyl sulfoxide, and 20 ml of absolute pyridine, to which the mixture obtained by the reaction of 1.30 g (4.67 mmoles) of N-Boc-ω-aminoenanthic acid in 35 ml of absolute pyridine containing 0.6 ml (4.67 mmoles) of benzenesulfonyl chloride was added dropwise. The product was purified as described above. Yield 6.85 g. Found, %: N 0.33; $\gamma_{found} = 4.08$ ($\gamma_{theor} = 10$).

B. With Ethyl Chloroformate. To a solution of 4.71 g (16.8 mmoles) of N-Boc-ω-aminoenanthic acid in 70 ml of absolute chloroform was added 2.4 ml (16.8 mmoles) of triethylamine, and then the mixture was cooled to −10°C and, with stirring, 1.53 ml (16 mmoles) of ethyl chloroformate in 10 ml of chloroform was added. Stirring was continued for another 10 min, and then to the solution cooled to −8°C was added 4.86 g (30 mmoles) of dextran in 100 ml of dimethyl sulfoxide and 20 ml of pyridine. The resulting mixture was stirred at 0°C for 1 h and at 20°C for 16 h. The polymer was precipitated with ethanol and was then treated in the usual way. Yield 3.7 g. Found, %: N 0.30; $\gamma_{found} = 3.86$ ($\gamma_{theor} = 50$).

3. O-(NPS-Glycyl)dextran (II). With Ethyl Chloroformate. The reaction was performed in a similar manner to the preparation of (III) (experimental 2B) using 3 g (18.5 mmoles) of dextran, 2.22 g (9.7 mmoles) of N-NPS-glycine, and 0.88 ml (9.2 mmoles) of ethyl chloroformate. Yield 2.43 g. Found %: N 0.12; $\gamma_{found} = 1.39$ ($\gamma_{theor} = 50$).

4. O-(BOC-L-Alanyl)dextran (IV). A. With Ethyl Chloroformate. This was obtained in the same way as (IV) (experiment 2B) from 4 g (24.7 mmoles) of dextran, 2.46 g (13 mmoles) of BOC-L-alanine, and 1.18 ml (12.3 mmoles) of ethyl chloroformate. Yield 3.68 g. Found, %: N 0.08; $\gamma_{found} = 0.94$ ($\gamma_{theor} = 50$).

B. With DCHC. The method of synthesis has been described previously [2]; 5 g (30.9 mmoles of dextran, 2.9 g (15.5 mmoles) of BOC-L-alanine, and 6.35 g (30.9 mmoles) of DCHC were used. Yield 4.18 g. Found, %: N 0.77; $\gamma_{found} = 9.82$ ($\gamma_{theor} = 50$).

5. O-(NPS-L-Valyl)dextran (V). With DCHC. Compound (V) was obtained by the method described in [2] from 2.4 g (14.8 mmoles) of dextran, 2 g (7.4 mmoles) of NPS-L-valine, and 3.06 g (14.8 mmoles) of DCHC. Yield 2.56 g. Found, %: N 0.24; $\gamma_{found} = 1.42$ ($\gamma_{theor} = 100$). Yellow water-soluble powder.

6. O-(BOC-L-Phenylalanyl)dextran (VI). With DCHC. The reaction was performed as before [2] with 3 g (18.6 mmoles) of dextran, 7.21 (35 mmoles) of DCHC, and the BOC-L-phenylalanine from 4 g (8.2 mmoles) of its DCHA salt. After the precipitation of the polymer from the reaction mixture with ethanol, it was washed with absolute ethanol and with hot n-butanol and was reprecipitated with butanol from dimethyl sulfoxide. Yield 2.71 g. Found %: N 0.95; $\gamma_{found} = 13.7$ ($\gamma_{theor} = 48.5$). White powder sparingly soluble in water.

7. O-(NPS-L-Phenylalanyl)dextran (VII). With DCHC. The reaction was performed as described previously [2], using 2.98 g (18.5 mmoles) of dextran, 3.78 g (18.3 mmoles) of DCHC, and 2.92 g (9.25

mmoles) of NPS-L-phenylalanine, and the product was worked up as in the preceding experiment. Yield 2.54 g. Found, %: N 1.18. $\gamma_{found} = 16.9$ ($\gamma_{theor} = 50$). Yellow water-insoluble powder.

II. Removal of the Protective Groups from the AADs

1. Reductive Elimination of the Tosyl Group with Sodium in Liquid Ammonia. To 100 ml of liquid ammonia that had been distilled over sodium was added 0.50 g of Tos-glycyldextran(I) with $\gamma = 5.7$, which had been dried to constant weight, and this was followed by metallic sodium in small portions until the solution had acquired a stable blue color which did not disappear for 3 min. The excess of sodium was decomposed by the addition of solid ammonium chloride. The ammonia was allowed to evaporate (with stirring) and, with cooling, the solid white residue was acidified with dilute hydrochloric acid to pH ≈ 6. The polymer was precipitated from the resulting solution with ethanol and was desalted with Sephadex G-50 twice. Then the polymer obtained was isolated in the usual way. Yield 0.38 g. In an alkaline hydrolyzate of the polymer, an amino-acid analysis showed a small amount of only one ninhydrin-positive substance, which was not glycine.

2. Elimination of the o-Nitrophenylsulfenyl Group. A. With Thiophenol. A solution of 0.8 g of O-(NPS-L-phenylalanyl)dextran (VII) with $\gamma = 16.9$ (0.63 meq of NPS groups) in 20 ml of a 1 : 1 mixture of dimethyl sulfoxide and pyridine was mixed with 3.2 ml (31.6 mmoles) of thiophenol and the mixture was left overnight. The polymer was precipitated from the light yellow solution with ethanol, and, after decantation, the precipitate was triturated in ethanol and was twice reciprecipitated from dimethyl sulfoxide with ethanol. Yield 0.6 g. Yellow water-insoluble substance. A measurement of the optical densities at 390 nm of solutions in dimethyl sulfoxide of the starting material and the reaction product (with a linear relationship between the concentration of NPS groups and the optical density of the solution) showed that 75.5% of the protective groups had been split off. After retreatment of the reaction product with thiophenol under the same conditions, its optical density at 390 nm had remained unchanged.

The reaction with 0.050 g of O-(NPS-glycyl)dextran (II) having $\gamma = 1.39$ was performed similarly. Yield 0.40 g. The faintly yellow product was readily soluble in water. The amount of NPS groups in the polymer was determined spectrophotometrically in water as described above. The NPS groups had been removed to the extent of 79.9%.

The same treatment of O-(NPS-L-valyl)dextran (V) with $\gamma = 1.42$ led to the splitting off of 49.2% of the NPS groups. It can be seen from the nitrogen content of the reaction (found, N %: 0.20) that no valine residues were split off simultaneously with the NPS groups.

B. With Sodium Thiosulfate and Potassium Iodide. A solution of 0.5 g of O-(NPS-glycyl)dextran (II) with $\gamma = 1.39$ in 10 ml of water was treated with 1 ml of 2 M sodium thiosulfate solution and 0.5 ml of 1 M potassium iodide solution. After 20 min, the polymer was precipitated with ethanol and was then worked up in the usual way. This gave a faintly yellow-colored water-soluble substance. Yield 0.37 g. It was found spectrophotometrically (in water at 405 nm) that 87.5% of the NPS groups had been split off.

In a similar treatment of O-(NPS-L-valyl)dextran (V) with $\gamma = 1.42$, 55.9% of the NPS groups were split off.

3. Elimination of the tert-Butyloxycarbonyl Group. A. On SE-Sephadex C-50 in the H^+ Form. O-(BOC-L-alanyl)dextran (IV) with $\gamma = 9.82$ (2 g; 1.15 meq of BOC groups) in 5 ml of water was deposited on a column (3 × 20 cm) containing 360 ml of SE-Sephadex C-50 in the H^+ form swollen in water (24 meq of SO_3^{2-} groups) and left at 20°C for 5 h. During this period of standing, the column was washed periodically with small amounts of water such that the volume of eluate issuing did not exceed V_0 of the column (≈ 100 ml). The column was washed with water until the reaction for carbohydrate (test with sulfuric acid) was negative, and the eluate was concentrated to ≈ 5 ml, acidified with concentrated hydrochloric acid to pH 4, and freed from excess of acid by filtration through Sephadex G-50. The polymer was isolated in the usual way and dried. Yield 1.78 g. From the results of the titration of the hydrochloride groups of the polymer with alkali to phenolphthalein, 3.7% of the total BOC groups had been split off.

B. With Dowex 50 × 2 Cation-Exchange Resin in the H^+ Form. The reaction with O-(BOC-L-alanyl)dextran (IV) was performed as described in the preceding experiment. The proportion of BOC groups eliminated was ≈ 3%.

CONCLUSIONS

1. New methods for the O-aminoacylation of dextran ("polyglucin") with mixed anhydrides of N-protected amino acids with benzenesulfonyl chloride, tosyl chloride, ethyl chloroformate, and phosphorus oxychloride have been proposed.

2. By these methods, and also by the method developed previously using dicyclohexylcarbodiimide, esters of dextran with N-tosylglycine, N-o-nitrophenylsulfenylglycine, N-benzyloxycarbonyl-ω-aminoenanthic acid, N-tert-butoxycarbonyl-L-alanine, N-tert-butoxycarbonyl-L-phenylalanine, N-o-nitrophenylsulfenyl-L-phenylalanine, and N-o-nitrophenylsulfenyl-L-valine have been synthesized.

3. Methods for eliminating the o-nitrophenylsulfenyl group from esters of NPS amino acids with dextran by means of thiophenol or a mixture of $Na_2S_2O_3$ + KI have been recommended.

LITERATURE CITED

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