

# Isothiocyanates. XLVI.

## Preparation of macromolecular polyisothiocyanates

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Received 3 March 1975

Accepted for publication 7 May 1975

Fifteen new polyisothiocyanates were prepared. Those of the aliphatic type (*i*) were obtained by the reaction of cellulose with 5-isothiocyanato-1-pentyl isocyanate or by the reaction of aminoethylcellulose with thiophosgene. The reactions of arylenedi- and triisothiocyanates, respectively, with aminoethylcellulose or the reaction of *N*-4-(4'-aminodiphenyl)amide of carboxymethylcellulose with 1,4-phenylene diisothiocyanate gave the aromatic polyisothiocyanates (*ii*). Treatment of aminoethylcellulose with alkyl, aryl diisothiocyanates yielded polyisothiocyanates of a mixed type (*iii*). The prepared derivatives had the same physical and hydrodynamical properties as the original celluloses. They were characterized by infrared spectra and by the total binding capacity expressed in equiv. —NCS/g carrier on the basis of their ability to react with 2-mercaptoethanol (the reaction with the —SH group). The suitability of the prepared substances for immobilization of enzymes or a reversible binding of low molecular —SH compounds with purpose of their isolation from diluted aqueous solutions is discussed.

Был сделан синтез 15 новых полиизотиоцианатов: *i*) алифатического типа — реакцией целлюлозы с 1-изоцианато-5-изотиоцианатопентаном или реакцией аминоэтилцеллюлозы с тиофосгеном; *ii*) ароматического типа — реакцией ариленди- и арилтриизотиоцианатов с аминоэтилцеллюлозой или реакцией *N*-4-(4'-аминодифенил)амида карбоксиметилцеллюлозы с 1,4-диизотиоцианатобензолом; *iii*) смешанного типа — реакцией алифатическоароматических диизотиоцианатов с аминоэтилцеллюлозой. Синтезированные производные имеют те же самые физические и гидродинамические свойства как исходные целлюлозы. Эти вещества были характеризованы с помощью ИК спектров и общей емкости определенной количеством экв. —NCS/г наполнителя, исходя из способности реагировать с 2-меркаптоэтанолом (реакцией с —SH группой). В работе обсуждается также возможность использования синтезированных веществ для фиксации ферментов или для обратимого связывания низкомолекулярных —SH соединений, с целью их выделения из разбавленных водных растворов.

Polymers containing a reactive functional group can be applied in the preparation of immunoadsorbents, immobilized enzymes, in affinity chromatography, in the chemistry of

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proteins [1], and in organic synthesis [2]. One group of such polymers is represented by macromolecular polyisothiocyanates. The so far described derivatives were prepared by a treatment of thiophosgene with primary amines where the  $\text{NH}_2\text{—R—}$  group was attached to porous glass [3], polystyrene [4, 5], dextrans [6, 7], polyacrylates [8], polyacrylamides [9—11], polyethylene [12], and copolymers of styrene and acrylate [13]. It is not possible to predict the behaviour of an analogous functional group bound to a macromolecular skeleton on the basis of the known facts, e.g. from the reactions of low molecular isothiocyanates [14, 15]. Therefore, the preparation of new macromolecular polyisothiocyanates as well as the elaboration of analytical methods suitable for characterization of their binding properties is a current problem. The aim of this work is to contribute to the solution of this problem.

## Experimental

### *Chemicals and instruments*

The used di- and triisothiocyanates were prepared at the Department of Organic Chemistry, Faculty of Natural Sciences, P. J. Šafárik University, Košice. 5-Isothiocyanato-1-pentyl isocyanate was purchased from the Institute of Macromolecular Chemistry, Freiburg University (West Germany). Commercial aminoethylcellulose AE-41 Whatman (1.0 mequiv./g), carboxymethylcellulose CM 11 Whatman (0.6 mequiv./g), and powdery cellulose Whatman (standard grade) were used as carriers. Cross-linked polyethylenimine (32% N), polyethylenimine-starch (38% N), and deacetylated chitin were obtained from the Institute of Chemistry, Slovak Academy of Sciences, Bratislava. Thiophosgene and organic solvents were distilled before being used. [ $^{35}\text{S}$ ] 4-bromophenyl isothiocyanate (3.4 Ci/mol) was prepared by an isotope exchange reaction with elementary sulfur  $^{35}\text{S}$  according to [16]. The elementary sulfur  $^{35}\text{S}$  (252 Ci/mol) was obtained from the Institute for Research, Production, and Uses of Radioisotopes, Prague. The incorporated radioactivity in the washed samples collected on filters of 30 mm diameter was counted in a Methane flow counter, FH 41 T, Frieseke und Hoepfner, GmbH (West Germany). The i.r. spectra of the synthesized compounds were measured on a double-beam UR-20 (Zeiss, Jena) spectrophotometer using KBr technique. Kinetic measurements at 412 nm were carried out on a VSU-1 (Zeiss, Jena) spectrophotometer at  $25 \pm 0.2^\circ\text{C}$ . pH Measurements of buffers accurate to  $\pm 0.01$  pH unit were made with an OP-205 Radelkis (Hungary) pH meter.

### *Macromolecular polyisothiocyanates*

#### *Preparation of XIII*

To aminoethylcellulose (10 g), 10% sodium hydrogen carbonate (100 ml) and thiophosgene (5 ml) in chloroform (15 ml) were added. After 1 and 2 hrs excess chloroform was added under vigorous stirring at laboratory temperature and the polyisothiocyanate of cellulose was repeatedly washed with chloroform, diluted acetic acid, acetone, and dried under vacuum.

#### *Preparation of I—XII*

Di- and triisothiocyanates (1 g) were added to aminoethylcellulose (5 g) swollen in dimethyl sulfoxide (50 ml) and the mixture was heated to  $60^\circ\text{C}$  under continuous stirring. After 4 hrs the suspension was separated, washed repeatedly, and dried as mentioned above.

#### *Preparation of XIV*

To benzidine (1 g) suspended in water, carboxymethylcellulose (10 g) and tetrahydrofuran solution of *N,N'*-dicyclohexylcarbodiimide were added. After 18 hrs reaction at  $25^\circ\text{C}$  under stirring the suspension

was filtered, then washed with acetone, diluted acetic acid, diluted sodium hydroxide, water and dried at reduced pressure. To the obtained *N*-4-(4'-aminodiphenyl)amide of carboxymethylcellulose, 1,4-phenylene diisothiocyanate (2 g) in dimethyl sulfoxide (100 ml) was added and the reaction mixture was maintained under stirring at 70°C for 5 hrs. Then the suspension was sucked, washed with acetone, and dried under reduced pressure.

#### Preparation of XV

Cellulose (10 g) in anhydrous pyridine (100 ml) and 5-isothiocyanato-1-pentyl isocyanate (4–5 g) were stirred for 4 hrs at 100°C, sucked, and washed repeatedly by suspending in excess ethanol. The reaction mixture was further treated as mentioned in the preparation of XIII. The characteristics of the prepared substances are given in Tables 4 and 5.

#### Determination of the binding capacity for low molecular — SH compounds

To the polymer samples (150 mg) in centrifuge tubes, 13 mM solution of 2-mercaptoethanol (2 ml) in buffer of pH 9 was added and the samples were stirred at 25°C. After centrifugation, 30  $\mu$ l of the supernatant was withdrawn and transferred into 0.1 M TRIS-HCl buffer of pH 7.2 (2.07 ml) containing 20 mM EDTA and 5,5'-dithiobis(2-nitrobenzoic acid) (445  $\mu$ M) [17]. After 5 minutes methanol (7.9 ml) was added and the absorbance was measured at 412 nm. The amount of 2-mercaptoethanol bound chemically to macromolecular polyisothiocyanate was calculated from the decrease of absorbance in comparison with the control sample and after correction to the decrease of concentration by a spontaneous oxidation and adsorption on the corresponding cellulose carrier without —NCS groups.

### Results and discussion

The aliphatic primary amino group attached to the suitable macromolecular skeleton of carbohydrate nature or other insoluble matrix is a suitable initial material for the preparation of macromolecular polyisothiocyanates owing to its readiness to enter series of

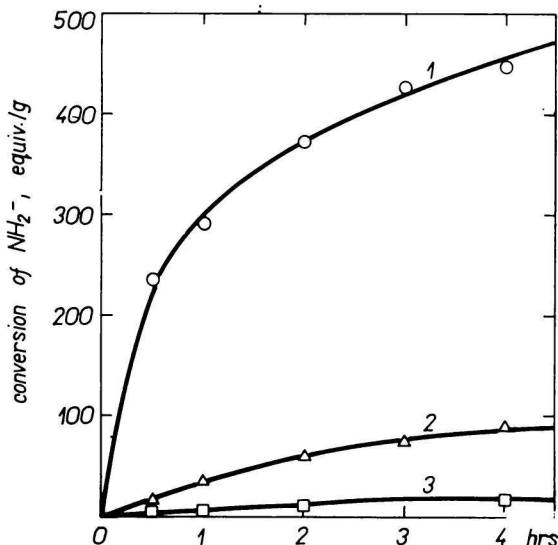


Fig. 1. Reaction kinetics of macromolecular polyamines with [<sup>35</sup>S] 4-bromophenyl isothiocyanate (3.4 Ci/mol) in pyridine at 50 °C. The initial concentration of the isothiocyanate was 7 mM and that of the polymer 5 mg/ml.

1. Deacetylated chitin;
2. polyethylenimine-starch;
3. cross-linked polyethylenimine.

Table 1

Capacity of aminoethylcellulose and their derivatives  
Cel—O—R

| R                      | Method of preparation  | $\mu\text{mol}^a$<br>—NH <sub>2</sub> /g | % —NH <sub>2</sub> |
|------------------------|--|--|--------------------|
| Aminoethyl             | Unmodified   | 960                                      | 100                |
| Isothiocyanatoethyl    | Reaction of AE-cellulose <sup>b</sup><br>with CSCI <sub>2</sub>              | 79                                       | 8                  |
| 3-Isothiocyanatophenyl | Reaction of AE-cellulose <sup>c</sup><br>with 1,3-phenylene diisothiocyanate | 37                                       | 38                 |

a) Determined colorimetrically at 570 nm 20 min after the reaction with ninhydrin at 100°C in the system of 10% pyridine : water = 3 : 2.

b) The reaction with a twofold molar excess of thiophosgene in the system of water : chloroform : NaHCO<sub>3</sub> = 9 : 1.5 : 1 for 4 hrs at 20 °C under stirring.

c) The reaction with a twofold molar excess of 1,3-phenylene diisothiocyanate in the system of borate buffer pH 10: pyridine = 1 : 2 for 1 hr at 50°C under stirring.

Table 2

The effect of temperature on the amount of —NCS groups attached to the polymer (substance I, Table 4) in the reaction of 1,4-phenylene diisothiocyanate with aminoethylcellulose in various reaction systems over 3 hrs

| Solvent            | % of the bound 2-mercaptoethanol |      |      |
|--------------------|----------------------------------|------|------|
|                    | 20°C                             | 40°C | 60°C |
| DMSO               | 0—5                              | 30   | 42   |
| EtOH               | 0—2                              | 24   | 38   |
| TRAB—acetone = 1 1 | 0—2                              | 7    | 12   |

reactions. As seen from the results presented in Table 1, most of the free amino groups of such polyamine can be substituted by thiophosgene or diisothiocyanate.

The preliminary observations on the reaction of macromolecular polyamines with isothiocyanates ([<sup>35</sup>S] 4-bromophenyl isothiocyanate) (Fig. 1) showed that the reaction in such a heterogeneous system proceeded at a much lower rate than expected on the basis of our previous knowledge on reactivity of isothiocyanates with the primary amino group of low molecular amines in solutions [14, 15]. This finding is important for establishing optimal conditions for preparation of polyisothiocyanates. This was proved also in the experiments with diisothiocyanates where the amount of —NCS groups bound to the macromolecular skeleton (equiv. —NCS/g carrier determined from the ability to bind 2-mercaptoethanol) served as a criterion of the required reaction time. The prolonged reaction time and the

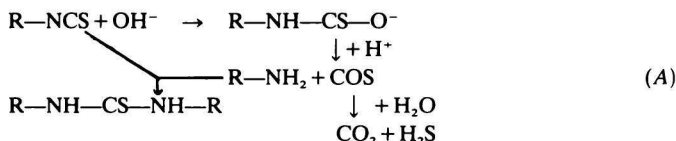
Table 3

Reaction kinetics of aminoethylcellulose with 1,4-phenylene diisothiocyanate in dimethyl sulfoxide and ethanol at 60°C

| Time<br>hrs | DMSO                       |                                      | EtOH                       |                                      |
|-------------|----------------------------|--------------------------------------|----------------------------|--------------------------------------|
|             | Conversion<br>of 2-ME<br>% | $\mu\text{equiv. -NCS/g}$<br>polymer | Conversion<br>of 2-ME<br>% | $\mu\text{equiv. -NCS/g}$<br>polymer |
| 0.1         | 28                         | 24                                   | 30                         | 26                                   |
| 1           | 30                         | 26                                   | 33                         | 28                                   |
| 2           | 51                         | 39                                   | 35                         | 29                                   |
| 5           | 58                         | 43                                   | 47                         | 36                                   |
| 8           | 58                         | 43                                   | 28                         | 24                                   |
| 24          | 51                         | 39                                   | 20                         | 17                                   |

increased temperature involved side reactions leading to inactivation of the formed  $-\text{NCS}$  groups (Tables 2 and 3).

Another factor decisive for the reaction rate was a suitable reaction medium. When water was used as a medium, optimal pH could be maintained because the  $-\text{NH}_2$  group is capable to react with the isothiocyanate only in the unprotonated form. Since the  $\text{p}K_a$  value for the primary aliphatic amino group lies in the region of  $\text{pH} > 10$ , the pH should not fall below this value. On the other hand, at this pH, the concurrent reaction of the  $-\text{NCS}$  group with the  $\text{OH}^-$  ions begins to give the corresponding monothiocarbamates. This leads to undesirable decrease of the  $-\text{NCS}$  group content either in the used diisothiocyanate or in the formed macromolecular polyisothiocyanate (A).



Some organic solvents create a more advantageous medium for binding of diisothiocyanate to macromolecular amine. From numerous solvents studied, dimethyl sulfoxide, methanol, and the mixture of triethylamine buffer (pH 10) and acetone 1:1 (Table 2) were shown to be most advantageous. Pyridine, dimethylformamide, acetone, and dioxan proved to be less suitable.

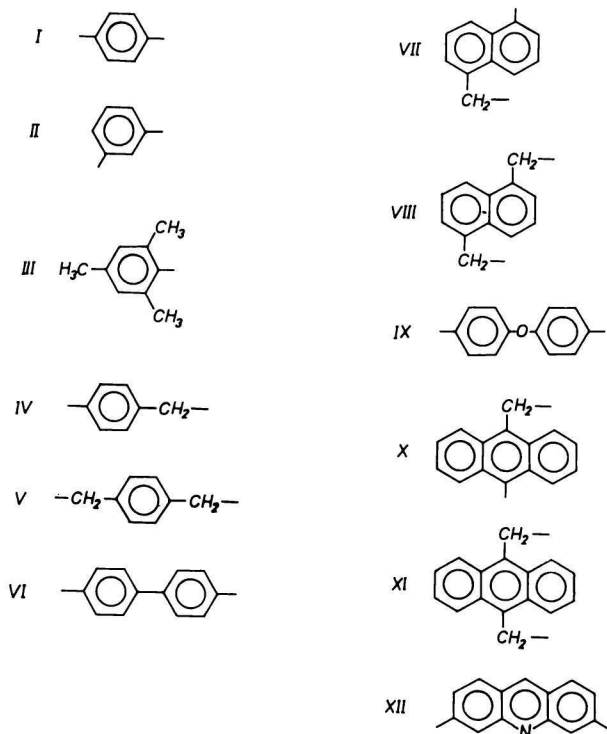
The ratio of diisothiocyanate to the total concentration of amino groups of the used polymer in the reaction mixture was found to be very important in the synthesis of the macromolecular polyisothiocyanates. In the case of 1,4-phenylene diisothiocyanate and aminoethylcellulose it was shown that the suitable molar ratio of  $(\text{NCS}):(\text{NH}_2)$  was 2:1. The decreased amount of isothiocyanate decreased the yield of polyisothiocyanate. On the other hand, neither an essentially increased reaction rate nor a practical improvement of the binding capacity of the prepared polyisothiocyanate was achieved by increasing the excess of the isothiocyanate (followed up to 1:5).

Table 4

Characterization of macromolecular polyisothiocyanates  
 $\text{Cel-O-(CH}_2)_2\text{-NH-CS-R-NCS}$

| Compound*   | Elemental analysis |     |     |      |     | 2-Mercaptoethanol<br>μequiv. -NCS/g |
|-------------|--------------------|-----|-----|------|-----|-------------------------------------|
|             | % C                | % H | % N | % O  | % S |                                     |
| <i>I</i>    | 40.6               | 5.8 | 2.0 | 38.7 | 3.5 | 55                                  |
| <i>II</i>   | 40.5               | 5.9 | 2.2 | 36.7 | 3.5 | 40                                  |
| <i>III</i>  | 41.3               | 5.5 | 4.1 | 35.1 | 9.9 | 37                                  |
| <i>IV</i>   | 43.0               | 6.1 | 2.2 | 36.8 | 3.6 | 23                                  |
| <i>V</i>    | 41.6               | 6.1 | 2.2 | 36.7 | 3.2 | 30                                  |
| <i>VI</i>   | 42.8               | 5.9 | 2.5 | 35.0 | 3.8 | 21                                  |
| <i>VII</i>  | 41.7               | 6.0 | 2.3 | 35.8 | 4.3 | 52                                  |
| <i>VIII</i> | 42.4               | 6.2 | 2.3 | 38.4 | 3.1 | 32                                  |
| <i>IX</i>   | 42.4               | 5.8 | 2.5 | 39.1 | 3.8 | 21                                  |
| <i>X</i>    | 41.7               | 6.5 | 1.5 | 36.0 | 1.1 | 29                                  |
| <i>XI</i>   | 43.1               | 6.1 | 2.2 | 36.7 | 3.7 | 23                                  |
| <i>XII</i>  | 41.5               | 6.0 | 2.6 | 35.7 | 1.0 | 25                                  |



\* The substituent R in compounds *I*—*XII* is as follows:



The method recommended for the preparation of low molecular isothiocyanates was employed in thiophosgenation of the macromolecular polyamine. Examination of the reaction kinetics showed that the sulfur content in the final product determined by elemental analysis did not change practically after the reaction time prolonged above 1 hr (at 25°C). The binding capacity of the prepared polyisothiocyanate for the reaction with amino and thiol compounds did not improve either.

Table 5

Characterization of macromolecular polyisothiocyanates  
Cel—O—R

| No.  | R   | Elemental analysis |     |     |      |     | 2-Mercapto-<br>ethanol<br>μequiv. -NCS/g |
|------|---|--------------------|-----|-----|------|-----|--|
|      |   | % C                | % H | % N | % O  | % S |  |
| XIII | CH <sub>2</sub> CH <sub>2</sub> NCS   | 40.1               | 5.9 | 1.4 | 37.4 | 2.6 | 155                                      |
| XIV  | CH <sub>2</sub> CONH—  |                    |     |     |      |     |  |
|      | —NH—CS—NH—  —NCS       | 38.5               | 5.7 | 0.9 | 39.3 | 6.5 | 27                                       |
| XV   | CONH(CH <sub>2</sub> ) <sub>5</sub> —NCS  | 41.3               | 5.9 | 2.8 | 47.0 | 3.1 | 65                                       |

The etherification degree of the hydroxyl groups in cellulose is deciding for the preparation of the aromatic polyamines from carboxymethylcellulose. When derivatives with a high content of the carboxymethyl groups having the tendency to dissolve in water solutions were used to obtain diphenylamide, gel formation was observed. This was a result of a mutual cross-linking by the reaction of —COOH groups and the bifunctional benzidine. Therefore in further experiments carboxymethylcelluloses with less —COOH groups were used which still had the physical and hydrodynamical properties similar to those of celluloses. The same law as mentioned in the reaction of diisothiocyanates with polyamines holds for the reaction of diisothiocyanates with *N*-4-(4'-aminodiphenyl)amide of carboxymethylcellulose resulting in macromolecular polyisothiocyanate.

When starting from cellulose and isothiocyanatoalkyl isocyanate, it is deciding to remove water completely from cellulose and from the solvent used. In the presence of water, aminoalkyl isothiocyanate is formed. The amine then reacts with both the —NCO group of the reagent and the —NCS group which causes the insufficient yields.

All the hydroxyl groups of the glucose residues in the cellulose chain can react with the —NCO group. However, for macromolecular polyisothiocyanates suitable for column packings and other two-phase systems capable to immobilize enzymes, only a certain degree of substitution is desirable. Carbanilides of cellulose with a high substitution degree of the hydroxyl groups lose the physical properties of the used cellulose [18, 19].

The list of the macromolecular polyisothiocyanates prepared by the reaction of di- and triisothiocyanates with aminoethylcellulose, their elemental composition, and binding capacity established by the reaction with 2-mercaptoethanol are presented in Table 4. The analogous characterization of macromolecular polyisothiocyanates prepared from cellulose, carboxymethylcellulose, and aminoethylcellulose is given in Table 5. The i.r. spectra of the prepared derivatives revealed the characteristic absorption band of the —NCS group at  $2100 \pm 50 \text{ cm}^{-1}$  and the one belonging to —NH—CS—NH— and —O—CO—NH— groups,

respectively, in the region 1600—1700  $\text{cm}^{-1}$ . The R—NCS group was bound through these groups to the polysaccharide skeleton [20].

The results presented indicate that the used methods make the preparation of macromolecular polyisothiocyanates with different nature of —NCS group bound to the cellulose skeleton through alkyl, aryl, and aryl alkyl chains possible. It makes also possible to choose suitable systems for reversible and irreversible covalent binding of low and high molecular biomolecules through their amino and SH groups. The results of the study of the binding capacity of macromolecular polyisothiocyanates can be applied in binding of low molecular thioles. On the other hand, the experimentally proved and theoretically motivated possibility of releasing isothiocyanates from esters of dithiocarbamic acids [21] showed another way of using the polyisothiocyanates, namely, for binding the thiol compounds from very diluted solutions as well as for their release. Releasing can be important in regeneration of polymers and in isolation of the biochemically important SH compounds.

Immobilization of lactate dehydrogenase, alcohol dehydrogenase, and glutamate dehydrogenase on the prepared macromolecular polyisothiocyanates is the subject of our previous work [22]. The so far obtained knowledge points to the fact that the specific activity of the immobilized enzymes is affected to a high degree by the type of used macromolecular polyisothiocyanate, by its specific binding capacity, *i.e.* the amount of the —NCS groups per weight unit of the polymer, and by the total concentration of the bound enzyme.

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