*https://doi.org/10.31925/farmacia.2022.4.6* **ORIGINAL ARTICLE**

# HYDROPHOBICALLY MODIFIED DEXTRAN ESTERS AS POTENTIAL EXTERNAL BIOCIDES

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*Manuscript received: February 2022*

**Abstract**

Amphiphilic dextran-based esters were synthesized by coupling several disubstituted 1,2,3-triazole derivatives on the polysaccharide. The polymers were characterized by elemental analysis and spectroscopy measurements: X-ray photoelectron (XPS), Fourier transform infrared (FTIR), carbon-13 nuclear magnetic resonance ($^{13}$C NMR). Triazole derivatives and their corresponding dextran esters showed a moderate to good antimicrobial activity, the antifungal activity being stronger than the antibacterial one. Halogenated polymers proved to be potential external biocides due to their both bactericidal and fungicidal activity.

**Rezumat**

Esterii amfifili bazați pe dextrani au fost sintetizați prin cuplarea pe polizaharida a mai multor derivați disubstituiți ai 1,2,3-triazolului. Polimerii au fost caracterizați prin analiza elementală și măsurători spectroscopice (de fotoelectroni cu raze X - XPS, în infraroșu cu transformată Fourier - FTIR, rezonanță magnetică nucleară carbon-13 - $^{13}$C RMN). Derivații de triazol și esterii dextranului corespunzători au prezentat o activitate antimicrobiană moderată spre bună, activitatea antifungică fiind mai puternică decât cea antibacteriană. Polimerii halogenați s-au dovedit a fi produse potențiale biocide externe datorită activității lor bactericide și fungicide.

**Keywords**: dextran esters, 1,2,3-triazole derivatives, antimicrobial activity

## Introduction

The number of deaths, caused by infections due to bacteria, viruses and fungi, is continuously growing. The elimination of microorganisms is difficult due to their fast and facile mutation [34, 39, 53, 57]. Therefore, it is essential to create new antimicrobial agents for treating the increasing resistance of microorganisms. Antimicrobial polymers represent a class of therapeutics which combated the microbial infections. In contrast with low-molecular-weight antimicrobial agents, the polymeric ones have the benefit that they are non-volatile and chemically stable, do not penetrate by skin, have lower toxicity and long-term activity [30, 59]. Based on the type of polymeric system, antimicrobial polymers can be classified [7]: (1) biocidal polymers, with intrinsic antimicrobial activity; (2) polymeric biocides, which have biocide molecules attached on the polymer backbone; and (3) biocide-releasing polymers, having biocide molecules included in a polymeric matrix.

1,2,3-triazoles and their derivatives, synthesized in the last decades by "click chemistry" [1, 48, 55], have various pharmacological activities, such as: anticancer [14, 15, 24], anti-HIV [25, 37, 64], antiviral [9, 19, 32], antimycobacterial [3, 4, 20, 42], antifungal [2, 13, 29], antidiabetic [5, 18, 49], antitubercular [41, 46, 50], antimalarial [8, 10, 43], anti-inflammatory [38, 45]. These N-heterocyclic derivatives linked to carbohydrates have captured broad attention, due to their increased solubility and biological activity [17, 28, 54]. Thus, new antimicrobial products based on 1,2,3-triazole derivatives conjugated with monosaccharides (D-glucose, D-galactose, D-mannose, D-fructose [6] or D-galactose, D-glucose, D-xylose [16]) or polysaccharides (starch [52], lignocellulosic fibres [31]) were synthesised and evaluated concerning their antimicrobial activity.

Recently, we synthesized new hydrophobically modified esters based on dextran, and we preliminary tested some of the obtained polymers against bacterial and yeast strains [51]. In the present work, a more in-depth study was made for establishing the correlation

between chemical structure and antimicrobial activity for a series of amphiphilic dextran esters. The polymers were synthesized by an one-pot procedure, consisting on the reaction between the polysaccharide and different 1,4-disubstituted-1,2,3-triazole-4-carboxylates *in situ* previously activated with N,N'-carbonyldiimidazole (CDI). The obtained polymers were characterized by elemental analysis, XPS, FTIR and NMR spectroscopy. Also, antibacterial and antifungal activity for 1,2,3-triazole-4-carboxylic acid derivatives and their corresponding polymers was tested against Gram-positive, Gram-negative bacteria and a pathogenic yeast by using disk diffusion method and broth microdilution method.

## Materials and Methods

*Materials*

Dextran from *Leuconostoc* spp (Mr = 40,000 Da), N,N'-carbonyldiimidazole and pyrene were bought from Sigma-Aldrich and employed as received. Dimethylsulfoxide was dried on molecular sieves.

The microbial strains were achieved from the Culture Collection of the Department of Microbiology, Faculty of Pharmacy, "Gr. T. Popa" University of Medicine and Pharmacy, Iași, Romania.

*Synthesis of dextran esters*

Triazole carboxylates (***1a-f***) synthesis was previously described in detail [51] and implied two steps: "click" reaction between ethyl propiolate and organic azides in the presence of copper(II) sulphate pentahydrate and sodium L-ascorbate, followed by the alkaline hydrolysis (30% KOH aqueous solution) of obtained ethyl esters.

The synthesis of polysaccharide esters (***2a-f***) was carried out by esterification of dextran with derivatives (***1a-f***) activated *in situ* with CDI (Figure 1), using the procedure presented in our previous work [51], with some modifications.

Preparation of dextran ester **2a** is given as an example. 0.86 g (3.72 mmol) **1a**, solved in 8 mL of dry DMSO, reacted with 0.6 g (3.72 mmol) CDI under magnetic stirring, at r.t, for 8 h. Then, 0.15 g (0.93 mmol) dextran was added and the mixture was allowed to react under stirring, at 80°C, for 24 h. The product was separated by precipitation in 0.1 M aqueous solution of sodium bicarbonate for removing triazole derivative, and after it was filtered and washed several times with deionized water. Finally, dextran ester was dried at r.t. under vacuum. In the following, the atoms are numbered as shown in Figure 1. Dextran esters **2e** and **2f** contain a supplementary carbon atom, namely $C^{13}$, attributed to methyl (product **2e**) or methoxy group (product **2f**) which are bound in para position of the benzene ring.

*Methods*

*Preparation of polymeric self-aggregates*

Polymeric self-associates were acquired by solving dextran ester in DMSO (0.1 g polymer/10 mL solvent) followed by the drop-wise addition of DMSO polymeric solution to the twice its volume of Millipore water under magnetic stirring. The mixture was introduced into a dialysis bag (12 kDa cut-off) and dialyzed against Millipore water. The obtained colloidal solution was used by itself or after dilution for fluorometric analysis.

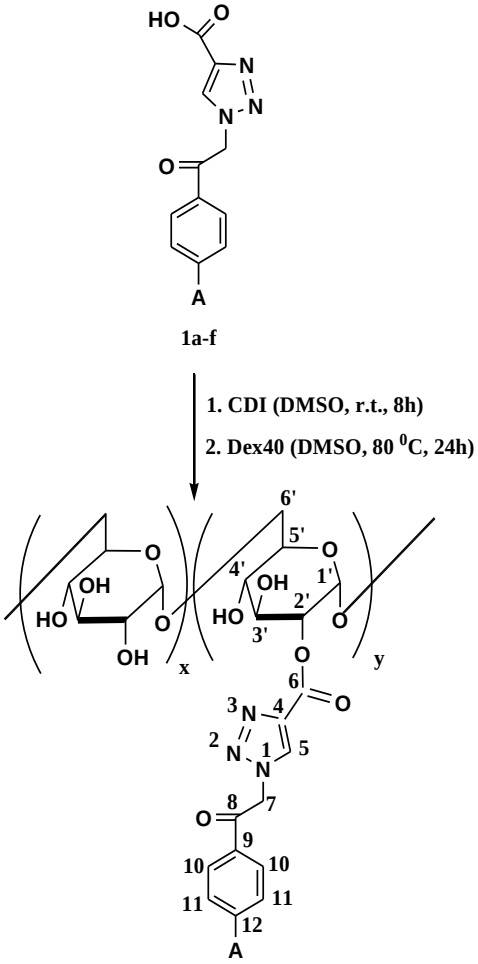


**Figure 1.**
Synthetic route for polymers **2a-f**
(A = H(**a**), F(**b**), Cl(**c**), Br(**d**), $CH_3$(**e**), $OCH_3$(**f**))

*Chemical structure characterization*

Elemental analysis (N%) was realized by using CHNS 2400 II Perkin Elmer analyser. XPS measurements were accomplished on a KRATOS Axis Nova (Kratos Analytical, Manchester, UK) which employed a monochromated Al Kα source with 20 mA current and 15 kV voltage (300 W) and base pressure of $10^{-9}$ to $10^{-8}$ Torr in the sample chamber. The XPS survey spectra for the polymers were obtained with a resolution of 1 eV and a pass energy of 160 eV. The high resolution spectra for all the elements found from the survey spectra were achieved using a pass energy of 20 eV and a step size of 0.1 eV. Vision Processing (Version 2.2.10) software was employed for background subtraction (Linear-type), peak integration, fitting and

quantitative chemical analysis. All binding energies were referenced to the C1s peak at 285 eV. For XPS analysis, the powder polymers were put in a stream of nitrogen to eliminate non-adhered materials, and degassed for two hours. After, the samples were placed onto the sample stage by an aluminium powder support. FTIR spectra were registered with a Bruker Vertex 70 spectrophotometer on KBr pellets. $^{13}C$ NMR spectra were achieved in DMSO-$d_6$ with a Bruker Advance DRX 400C spectrometer (100 MHz), using tetramethyl silane (TMS) as internal reference compound.

*Fluorescence experiments*

The critical aggregation concentrations, *CAC*, of polymeric self-aggregates were obtained using pyrene as fluorescence probe. Polymeric concentrations, ranging from 1.3 mg/mL to 0.0007 mg/mL, were achieved by successive dilution of acquired self-associates in aqueous solution as explained in *Preparation of polymeric self-aggregates*. A known aliquot of pyrene solution in methanol ($7.0 \cdot 10^{-4}$ M) was put into a set of vials, succeeded by solvent vaporization. Thereafter, aqueous polymeric solutions of known concentration were inserted into the vials and the mixtures were preserved at r.t. for 24 h for system equilibration. The final concentration of the chromophore in vials was $7.0 \cdot 10^{-7}$ M. Steady-state fluorescence emission spectra were achieved with a LS 55 PerkinElmer fluorescence spectrometer, using an excitation wavelength of 337 nm, the excitation and emission slits widths being set at 5 and 2 nm, respectively. The emission spectra of the fluorophore, recorded between 350 and 550 nm, allowed the calculation of the polarity parameter ($I_3/I_1$) by knowing pyrene fluorescence intensities at 372 nm ($I_1$) and 383 nm ($I_3$).

*Antimicrobial activity*

*Disc-diffusion method*

Polymeric antimicrobial activity was examined using Gram positive bacterium (*Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and a pathogenic yeast (*Candida albicans* ATCC 90028). The antimicrobial susceptibility tests were assessed using disk diffusion methods [11, 12]. Mueller Hinton agar (Oxoid) and Mueller-Hinton agar Fungi (Biolab) were inoculated with the suspensions of the tested microorganisms. Sterile stainless-steel cylinders (5 mm internal diameter; 10 mm height) were placed on the agar surface in Petri plates. Then, 100 µL of the evaluated products (**1a-f** and **2a-f**, as 1 wt% DMSO solutions) were adjoined into cylinders. The plates were kept 10 minutes at r.t. to obtain a homogeneous distribution of the compound in the medium and after, incubated at 35°C for 24 hrs. As reference antimicrobial drugs were used commercial available discs containing Ciprofloxacin (5 µg/disk), Fluconazole (25 µg/disk) and Voriconazole (1 µg/disc). After incubation, the diameters of inhibition zones were measured in mm, including disc size.

*Broth microdilution method*

Some polymers were evaluated for the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC). Serial double dilutions of each extract were inoculated in cation-adjusted Mueller-Hinton broth (Oxoid) (CAMHB), with equal volumes of bacterial/ yeast suspension (106 CFU/mL). MIC was the lowest concentration of extract where complete inhibition of visible growth was observed after 24 h of incubation at 37°C (for antibacterial test) and 24°C (for anti-fungal test). All the wells, showing complete inhibition of microorganisms' growth, were subcultured and incubated overnight at 37°C (for antibacterial test) and 24°C (for antifungal test). The highest dilution of extracts, which afforded the killing of 99.9% of tested microorganisms, was recorded as MBC/MFC. MIC and MBC/MFC of ciprofloxacin/fluconazole towards bacterial/yeast strains were also assessed.

## Results and Discussion

*Synthesis and chemical structure characterization*

The polysaccharide esters, **2a-f**, were synthesized using a one-pot procedure (Figure 1). Triazole carboxylates, **1a-f**, reacted with the coupling agent (CDI) to provide the activated compounds (acylimidazoles) and two by-products: $CO_2$ and imidazole (Im). Further, the acylimidazolide reacted *in situ* with dextran to obtain dextran ester.

The structural characterization of the polymers was performed by elemental analysis, XPS, FTIR and $^{13}C$ NMR spectroscopy. Elemental analysis and X-ray photoelectron spectroscopy proved the effective binding of triazole derivatives on dextran skeleton and enabled to obtain polymeric degree of substitution which values were higher than those previously reported [51] due to the higher molar ratio between the polysaccharide and 1,2,3-triazole derivative. DS were calculated by taking into account the nitrogen percent determined by elemental analysis. X-ray photoelectron spectroscopy was used only to prove esters formation and not for the evaluation of DS values because it is a surface analysis technique. XPS afforded the determination of the binding energies of all polymeric bonds and their relative intensity (%) (Table I).

The survey spectra and deconvoluted C1s, O1s and N1s spectra for both native dextran and one of the polymers, **2a**, used as an example, were showed in Figure 2. Thus, a distinct peak corresponding to the orbital of N (1s, 397 eV) (Figure 2a) was present in the wide spectrum of compound **2a**, due to the occurrence of 1,2,3-triazole derivative moiety in polymeric chemical structure, while no peak from N1s could be seen for pure dextran. Peaks from C (1s, 284.44 eV) and O (1s, 528.75 eV), O (2s, 23.31 eV), O (KLL, 974.76 eV) were found in both survey spectra of neat poly-saccharide and dextran ester. The C1s spectrum of

pristine dextran (Figure 2b) revealed a main peak at 286.5 eV which could be assigned to C–O bond.
Two small peaks at 285 eV and 287.8 eV could also be observed and were attributed to the C-C/C-H and C-O-C bonds which occurred in neat polysaccharide structure. The O1s spectrum of pure polysaccharide (Figure 2c) showed two peaks. The main one, at 532.7 eV, could be ascribed to the O–C bond. The second one, at 533.1eV, was smaller and it confirmed the existence of C-O-C bond in native dextran. In contrast to dextran XPS spectrum, two new and small peaks for the C 1s signal appeared in the spectrum of ester **2a** at binding energies (BEs) of 285.9 and 289.2 eV indicating C-N and O-C=O bonds formation (Figure 2d).

**Table I**

Binding energies (eV), relative intensity (%) and their assignment for dextran esters

| Polymer code | C 1s | | O 1s | | N 1s | |
|---|---|---|---|---|---|---|
| | BE (eV) | Assignment | BE (eV) | Assignment | BE (eV) | Assignment |
| **2a** | 285 (32.47%) | C-C/C-H | 532.4 (42.96%) | O-C | 400.3 (36.07%) | N-N |
| | 285.9 (7.09%) | C-N | | | | |
| | 286.5 (43.63%) | C-O | 533 (49.82%) | C-O-C | 401 (35.67%) | N-C |
| | 287.8 (12.95%) | C=O/ C-O-C | | | | |
| | 289.2 (3.86%) | O-C=O | 533.8 (7.22%) | O=C | 402 (28.26%) | N=N |
| **2b** | 285 (20.33%) | C-C/C-H | 532.3 (40.54%) | O-C | 400 (42.49%) | N-N |
| | 285.7 (6.45%) | C-N | | | | |
| | 286.3 (36.68%) | C-O | 532.8 (38.82%) | C-O-C | 401 (34.06%) | N-C |
| | 287.5 (23.20%) | C=O/ C-O-C | | | | |
| | 288.5 (9.19%) | C-F | | | | |
| | 289.5 (4.15%) | O-C=O | 534.4 (20.64%) | O=C | 401.9 (23.45%) | N=N |
| **2c** | 285 (34.64%) | C-C/C-H | 531.7 (19.69%) | O-C | 399.8 (32.43%) | N-N |
| | 285.6 (6.18%) | C-N | | | | |
| | 286.3 (42.42%) | C-O/C-Cl | 532.5 (64.51%) | C-O-C | 400.5 (34.39%) | N-C |
| | 287.7 (12.76%) | C=O/ C-O-C | | | | |
| | 289.1 (4%) | O-C=O | 533.5 (15.8%) | O=C | 401.7 (33.18%) | N=N |
| **2d** | 285 (23.27%) | C-C/C-H | 532.2 (40.5%) | O-C | 400.2 (43.53%) | N-N |
| | 285.8 (5%) | C-N | | | | |
| | 286.5 (35.73%) | C-O/C-Br | 533.2 (48.9%) | C-O-C | 401.4 (33.12%) | N-C |
| | 287.9 (27.23%) | C=O/ C-O-C | | | | |
| | 289.4 (8.77%) | O-C=O | 534.3 (10.6%) | O=C | 402.5 (23.35%) | N=N |
| **2e** | 285 (33.68%) | C-C/C-H | 531.5 (12.2%) | O-C | 400.1 (49.87%) | N-N |
| | 285.7 (6.08%) | C-N | | | | |
| | 286.5 (44.63%) | C-O | 532.6 (65.5%) | C-O-C | 401.3 (29.59%) | N-C |
| | 287.8 (12.52%) | C=O/ C-O-C | | | | |
| | 289.2 (3.09%) | O-C=O | 533.5 (22.3%) | O=C | 402 (20.54%) | N=N |
| **2f** | 285 (23.26%) | C-C/C-H | 531.9 (19.36%) | O-C | 399.9 (38.16%) | N-N |
| | 285.7 (6.3%) | C-N | | | | |
| | 286.5 (52.69%) | C-O | 532.8 (64.33%) | C-O-C | 401 (33.98%) | N-C |
| | 287.7 (13.67%) | C=O/ C-O-C | | | | |
| | 289.2 (4.08%) | O-C=O | 533.7 (16.31%) | O=C | 401.8 (27.86%) | N=N |

The O 1s spectrum of polysaccharide-based polymer showed a new broad peak at 533.8 eV pointing out the formation of C=O bond (Figure 2e). The N 1s spectrum of product **2a** revealed three intensive peaks at BEs of 400.3, 401 and 402 eV relative to N-N, N-C and N=N bonds formation, respectively (Figure 2f).
DS values, expressed in moles of 1,2,3-triazolic group *per* 100 glucopyranosidic units ($U_{Gl}$) were determined with equation (**1)** by considering the nitrogen content provided by elemental analysis (Table II).

$$DS = \frac{162 \times \%N}{(100 \times 3 \times 14) - [\%N \times (M_S - 1)]} \times 100, \text{ mol/100 } U_{Gl} \quad (\mathbf{1}),$$

where, %N is the nitrogen percent determined by elemental analysis; 14 is elemental mass for N and 162 is molar mass of glucopyranoside unit of dextran; $M_S$ is the molecular weight of N-heterocyclic pendant group (-CO-Tr-$CH_2$-CO-$C_6H_4$-**A**) (Tr =1,2,3-triazole) (**A**=H, F, Cl, Br, $CH_3$, $OCH_3$).
Polymers were achieved by nucleophilic acyl substitution between dextran and acyl compound obtained by the reaction between N-heterocycle derivatives and the coupling agent. Moderate DS values of the polymers could be explained by steric hindrance exerted between two bulky groups (-Tr-$CH_2$-CO-$C_6H_4$-**A** and –Im) bound to the carbonyl group of acylimidazolide in the first step of nucleophilic substitution followed in the second stage by elimination of imidazole with the formation of dextran esters. Degree of substitution values increased with the augmentation of triazole derivatives solubility in the reaction medium [47], as follows: $DS_{Dex\text{-}T\text{-}CH3} < DS_{Dex\text{-}T\text{-}H} < DS_{Dex\text{-}T\text{-}Br} < DS_{Dex\text{-}T\text{-}Cl} < DS_{Dex\text{-}T\text{-}F} < DS_{Dex\text{-}T\text{-}OCH3}$.

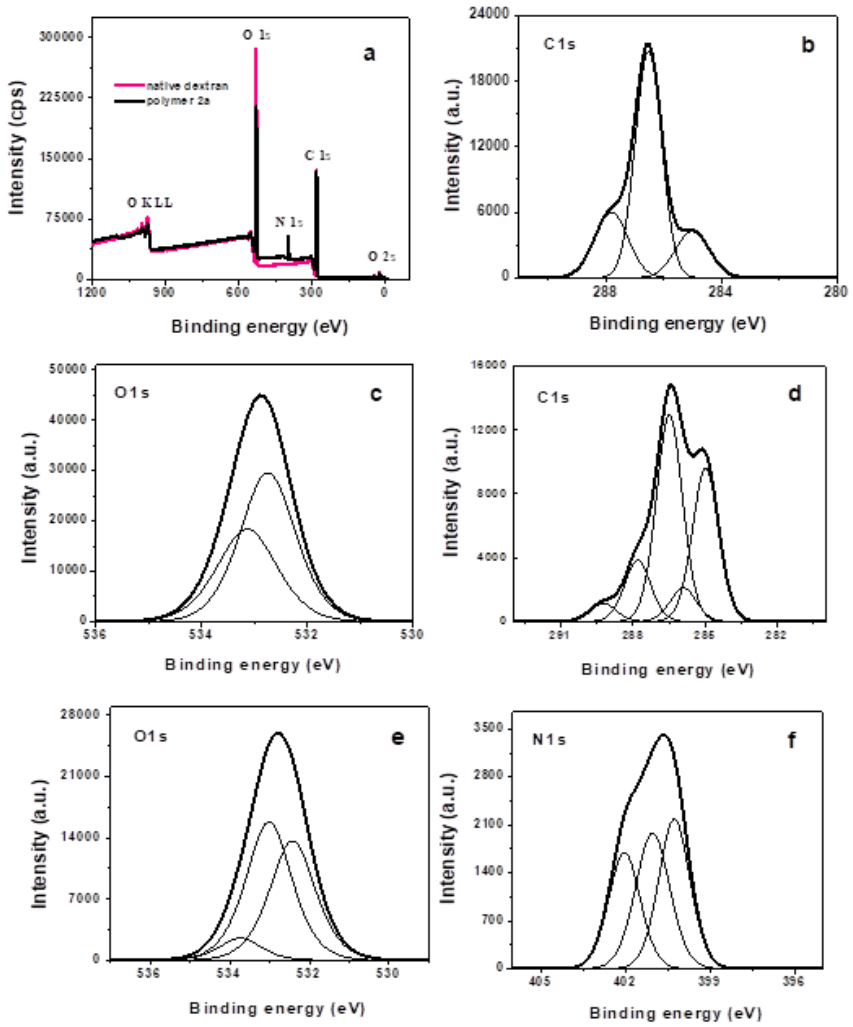


**Figure 2.**
The survey spectra for pristine dextran and polymer **2a** and deconvoluted profiles for pure dextran: (**b**) C 1s, (**c**) O 1s and polymer **2a:** (**d**) C 1s, (**e**) O 1s, (**f**) N 1s

**Table II**
Characteristics of polymers **2a-f**

| Polymer code | Substitution degree (mol/100 $U_{GI}$) [a] | Weight content of hydrophobic groups (wt %) | CAC (mg/mL) |
|---|---|---|---|
| **2f** | 87 | 58.4 | 0.020 |
| **2b** | 82 | 54.1 | 0.038 |
| **2c** | 77 | 55.4 | 0.027 |
| **2d** | 71 | 60.3 | 0.016 |
| **2a** | 67 | 47.0 | 0.058 |
| **2e** | 65 | 49.3 | 0.046 |

FTIR and NMR were also used for the characterization of dextran-based polymers, the spectral data of the polymers **2a-f** being collected in Table III.

FTIR spectroscopy proved the successful attachment of 1,2,3-triazolic moieties on polysaccharide skeleton by the comparison of FTIR spectrum (Figure 3c) of one of the polymers, **2a,** with those of its corresponding disubstituted triazole, **1a** (Figure 3b) and native dextran (Figure 3a).

So, a new signal in the range 1732 - 1735 $cm^{-1}$, attributed to the $C=O_{ester}$, appeared in FTIR spectrum of dextran ester while the signal for $C=O_{acid}$ (1682 - 1698 $cm^{-1}$ [51]) disappeared. Also, in FTIR spectrum of the polymer **2a** could be seen mutual stretching adsorption bands with those that occurred in FTIR spectra of pure dextran and triazole derivative **1a**. Thus, common signals with neat dextran were those for $O\text{-}H_{alcohol}$ (3415 - 3428 $cm^{-1}$) and C-H (2924 - 2935 $cm^{-1}$) while mutual adsorption bands with 1,2,3-triazole derivative were those for $C=C_{triazole}$ (1574 - 1598 $cm^{-1}$) and $C=O_{ketone}$ (1689 - 1705 $cm^{-1}$).

**Table III**

FTIR and $^{13}C$ NMR spectral data of dextran polymers

| Spectral analysis | Bound/Carbon atom | Polymer code | | | | | |
|---|---|---|---|---|---|---|---|
| | | **2a** | **2b** | **2c** | **2d** | **2e** | **2f** |
| FTIR ν ($cm^{-1}$) | O-H | 3415 | 3425 | 3415 | 3428 | 3424 | 3418 |
| | C-H | 2932 | 2924 | 2935 | 2933 | 2925 | 2929 |
| | $C=O_{ester}$ | 1735 | 1734 | 1734 | 1732 | 1735 | 1735 |
| | $C=O_{ketone}$ | 1702 | 1703 | 1705 | 1704 | 1697 | 1689 |
| | $C=C_{triazole}$ | 1597 | 1598 | 1585 | 1587 | 1582 | 1574 |
| | C-F | - | 1161 | - | - | - | - |
| | C-Cl | - | - | 1095 | - | - | - |
| | C-Br | - | - | - | 1071 | - | - |
| | $C_{(arom)}$-O-$C_{(aliph)}$ | - | - | - | - | - | 1047 |
| $^{13}C$ NMR δ (ppm) | $C^{7}$ | 56.3 | 56.3 | 56.2 | 56.2 | 56.3 | 56.2 |
| | $C^{6'}$ | 65.2 | 65.5 | 65.3 | 65.1 | 65.8 | 65.4 |
| | $C^{2'}$-$C^{5'}$ | 67.1-76.6 | 67.2-76.6 | 67.1-76.4 | 66.2-76.6 | 67.1-76.6 | 67.2-73.5 |
| | $C^{2''}$ | 76.3 | 76.7 | 76.5 | 76.6 | 76.4 | 76.3 |
| | $C^{1''}$ | 95.6 | 95.5 | 95.2 | 95.3 | 95.6 | 95.6 |
| | $C^{1'}$ | 98.7 | 98.3 | 98.3 | 98.4 | 98.1 | 98.4 |
| | $C^{11}$ | 128.1 | 116.2 | 128.7 | 132.4 | 129.7 | 128.2 |
| | $C^{10}$ | 129.6 | 131.6 | 129.8 | 130.2 | 128.4 | 130.3 |
| | $C^{5}$ | 130.9 | 130.6 | 130.9 | 130.9 | 130.8 | 130.7 |
| | $C^{12}$ | 134.8 | 164.8 | 133.5 | 128.7 | 131.5 | 159.3 |
| | $C^{4}$ | 137.8 | 138.7 | 133.7 | 138.5 | 138.6 | 138.5 |
| | $C^{9}$ | 141.3 | 144.6 | 140.5 | 140.8 | 144.8 | 140.7 |
| | $C^{6}$ | 159.6 | 160.4 | 159.8 | 159.7 | 160.2 | 160.2 |
| | $C^{8}$ | 191.8 | 190.2 | 191.2 | 191.3 | 191.3 | 189.7 |
| | $C^{13}$ | - | - | - | - | 21.3 | 58.7 |

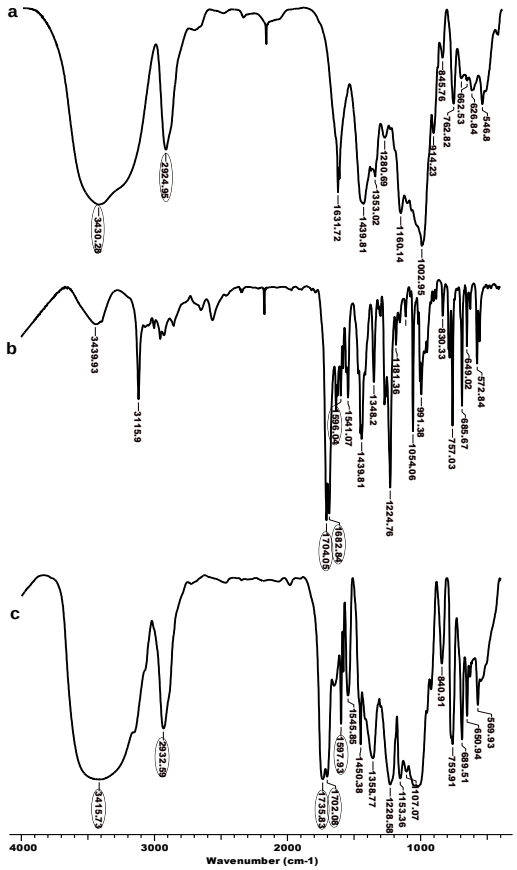


**Figure 3.**

FTIR spectra of native dextran (**a**), triazole derivative **1a** (**b**) and polymer **2a** (**c**)

$^{13}C$ NMR spectroscopy was used for the characterization of dextran-based polymers because of a limited separation of polymeric peaks in $^{1}H$ NMR spectra. $^{13}C$ NMR spectrum of polymer **2a** (Figure 4c) was compared with those of its corresponding 1,2,3-triazole-4-carboxylic acid 1a (Figure 4b) and pristine dextran (Figure 4a). $^{13}C$ NMR spectra revealed that the attachment of 1,2,3-triazole group on dextran backbone happened preferentially at the hydroxyl group bound to the second carbon of the polysaccharide ring by the presence of the signal corresponding to substituted second carbon of the polysaccharide ring ($C^{2''}$) at 76.6 ppm whilst the picks for substituted $C^{3'}$ or $C^{4'}$ could not be found. Additionally, the occurrence in $^{13}C$ NMR spectrum of dextran ester **2a** of the signals at 95.37 and 98.27 ppm showed that the substitution reaction at the hydroxyl group bound to $C^{2'}$ was incomplete. The pick from 95.37 ppm corresponded to the first carbon of glucopyranosidic unit which was adjacent to substituted second carbon ($C^{1''}$) while the pick from 98.27 ppm belonging to the first carbon next to unsubstituted second carbon ($C^{1'}$).

The signals, belonging to carbon atoms of 1,2,3-triazole moiety, could be located in $^{13}C$ NMR spectra of compounds **1a** and **2a** at: 56.3 ($C^{7}$,), 128.1 ($C^{11}$) 129.6 ($C^{10}$), 130.9 ($C^{5}$), 134.8 ($C^{12}$), 137.8 ($C^{4}$), 141.3 ($C^{9}$), 159.6 ($C^{6}$) and 191.8 ppm ($C^{8}$). Also, the picks corresponding to carbon atoms of dextran unit could be seen in $^{13}C$ NMR spectra of native polysaccharide

(Figure 4a) and dextran ester (Figure 4c) at: 66 ($C^{6'}$), 70.0 ($C^{4'}$), 70.4 ($C^{5'}$), 71.8 ($C^{2'}$) and 73.3 ppm ($C^{3'}$).

*Critical aggregation concentration of polymeric self-aggregates*

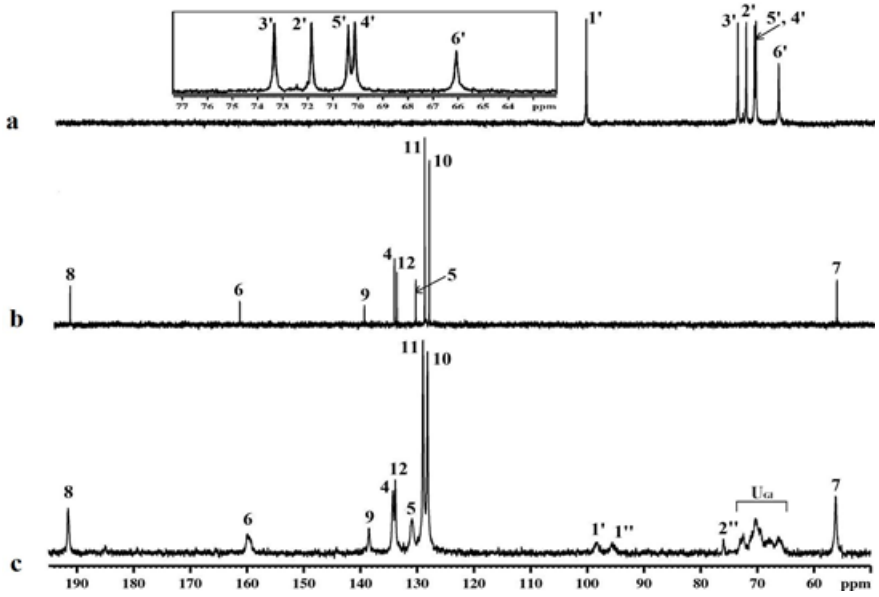


**Figure 4.**
$^{13}$C-NMR spectra of pure dextran (**a**), triazole derivative **1a** (**b**) and polymer **2a** (**c**)

Hydrophobically-modified polymers **2a-f** could self-assemble in aqueous solution and their capacity to associate was studied by fluorometry using pyrene as fluorescence probe. It is known that pyrene polarity parameter ($I_3/I_1$), which is ratio of intensity of its third (382 nm) ($I_3$) to first (373 nm) ($I_1$) vibronic peaks, is a measure of the local polarity of the chromophore microenvironment. The interception of two straight lines afforded the determination of polymeric critical aggregation concentrations (Figure 5) and these values depended on the weight content of hydrophobic 1,2,3-triazole moieties (wt%).

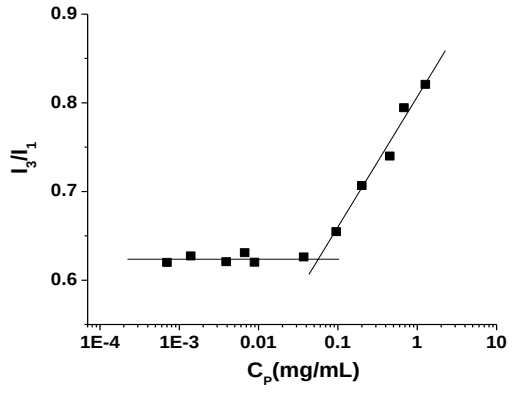


**Figure 5.**
Variation of pyrene polarity parameter with the polymer concentration for dextran ester **2a**

CAC values increased with the decrease of wt (%) (Table II). Critical aggregation concentrations of compounds **2a-f** were in agreement to those related to other amphiphilic dextran-based polymers with the same molar mass of the polysaccharide and having as side chains different lipophilic moieties, such as: tocopherol succinate [26], stearic acid [62] or retinal [61].

Figure 5, representing the variation of polarity parameter *versus* the concentration of dextran ester **2a**, showed almost unchanged values of $I_3/I_1$ at low polymer concentrations while $I_3/I_1$ values increased with the augmentation of polysaccharide ester concentrations. This behaviour is due to the progressive embedding of pyrene molecules in polymeric hydrophobic micro-domains which were obtained as a result of the self-organization of amphiphilic polymer in aqueous medium.

*Antimicrobial activity*

The antimicrobial activity of substituted 1,2,3-triazole-4-carboxylic acids, **1a-f**, and their corresponding dextran-based polymers, **2a-f**, were assessed against four different strains which are frequently connected with infections: a Gram-positive bacterium (*Staphylococcus aureus* ATCC 25923), two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and a pathogenic yeast (*Candida albicans* ATCC 90028). Antimicrobial activity was qualitatively estimated by using antimicrobial susceptibility test [27] which afforded the evaluation of the inhibition zone diameter, the results being collected in Table IV**.**

As positive control for antimicrobial activity was used ciprofloxacin and for antifungal activity, fluconazole and voriconazole. The examined compounds **1a-f** and

**2a-f** showed antimicrobial activity against both Gram-positive bacterium and pathogenic yeast. Conversely, all the products did not reveal any Gram-negative bacteria growth inhibition in the employed concentration range. That is in accordance with earlier studies which indicated that Gram-positive bacteria are more receptive to small or macromolecular products than Gram-negative bacteria due to their mesh-like peptidoglycan layer, located outside the plasma membrane, which can be easily traversed by different biocides [21, 22]. Instead, in the case of Gram-negative bacteria, the peptidoglycan layer is sandwiched between an inner cell membrane and an outer membrane which make extremely difficult the passive diffusion of the compounds [21, 22]. This behaviour is similar to other antimicrobial small molecules or polymers which contained 1,2,3-triazole moieties in their chemical structure [16, 63]. It is known that 1,2,3-triazoles are antimicrobial agents *per se*, because they can inhibit synthesis of the cell membrane, cell wall, and nucleic acids of bacteria [35].

The antimicrobial action is generally caused by ionic and hydrophobic interactions between the biocide and the outer phospholipid membrane of the microorganism [40]. The ionic attractions were exerted between the partial positive charge of N-heterocycle-containing products, induced by electron-withdrawing effect of carboxylic group linked directly to C4 of 1,2,3-triazolic ring, and negatively charged outer cellular membrane of bacteria or fungi while the hydrophobic forces were established between their lipophilic segments. As a result of both ionic and hydrophobic forces, several defects in the membrane integrity with loss of cytoplasmic constituents were determined, causing the death of the microorganism [23, 36].

**Table IV**

Antibacterial and antifungal activities of the compounds **1a-f** and **2a-f**

| Polymer sample | Diameter of inhibition zones (mm) | | | |
|---|---|---|---|---|
| | ***S. aureus ATCC 25923*** | ***E. coli ATCC 25922*** | ***P. aeruginosa ATCC 27853*** | ***C. albicans ATCC 90028*** |
| **1a** | 11.0 ± 0.00 | 0 | 0 | 16.0 ± 0.00 |
| **1b** | 15.1 ± 0.05 | 0 | 0 | 17.1 ± 0.05 |
| **1c** | 14.0 ± 0.00 | 0 | 0 | 18.0 ± 0.00 |
| **1d** | 14.0 ± 0.00 | 0 | 0 | 16.0 ± 0.00 |
| **1e** | 12.1 ± 0.05 | 0 | 0 | 15.3 ± 0.57 |
| **1f** | 13.1 ± 0.05 | 0 | 0 | 15.0 ± 0.00 |
| **2a** | 14.0 ± 0.00 | 0 | 0 | 17.0 ± 0.00 |
| **2b** | 20.0 ± 0.00 | 0 | 0 | 20.1 ± 0.05 |
| **2c** | 18.0 ± 0.00 | 0 | 0 | 18.1 ± 0.05 |
| **2d** | 17.0 ± 0.00 | 0 | 0 | 17.8 ± 0.00 |
| **2e** | 13.7 ± 0.57 | 0 | 0 | 16.0 ± 0.05 |
| **2f** | 14.6 ± 0.05 | 0 | 0 | 15.0 ± 0.05 |
| Ciprofloxacin (5 µg/disc) | 32.7 ± 0.00 | 32.1 ± 0.05 | 32.0 ± 0.00 | NT* |
| Fluconazole (25 µg/disc) | NT* | NT* | NT* | 30.0 ± 0.00 |
| Voriconazole (1 µg/disc) | NT* | NT* | NT* | 31.5 ± 0.50 |

NT* = not tested

Moderate to good antibacterial results were observed for all tested compounds, the products being less active than specific agents used as positive controls (Table IV). The best results were obtained for products having halogens (F, Cl, Br) in para position of the benzene ring whether the compounds were low-molecular-weight or polymeric ones. Their enhanced antimicrobial activity could be explained by the existence of a supplementary partial positive charge on benzene ring, induced by the presence of electron-withdrawing substituents (F, Cl, Br) bound to the aromatic ring. The improvement of the antimicrobial activity has also been observed for other polymers which contained halogen atoms in their chemical structure [33, 60]. The stronger electron-withdrawing capacity of the substituent (F > Cl > Br), the enhanced the antibacterial activity. The antifungal activity was generally higher than the antibacterial one for each product and the occurrence of strong electron-withdrawing substituents enhanced the antifungal properties as in the case of antibacterial activity (Table IV).

Antimicrobial activity was greater for polymers compared to their corresponding 1,2,3-triazole derivatives due to the occurrence of an increased local concentration of N-heterocyle groups which augmented the ionic and hydrophobic interactions between the polymer and lipid bilayer of bacteria or fungi membrane. The same antimicrobial behaviour was observed for hydrophobically modified acrylate-based polymers and their corresponding monomers [58]. Polymers, having the best qualitative antimicrobial results, were further evaluated by using broth microdilution method which provided the minimum inhibitory concentrations (MIC), minimal bactericidal concentration (MBC) and the minimal fungicidal concentration (MFC) (Table V).

The self-aggregation process of substrate is an important factor which influenced the antimicrobial activity by changing the membrane wholeness of the micro-

organisms. MIC values were higher than CAC values which denoted those above-mentioned polymers were active only in their self-assembled form for both antibacterial and antifungal activity.

**Table V**

MIC and MBC/MFC values of tested polymers

| Compound | *S. aureus ATCC 25923* | | *C. albicans ATCC 90028* | |
|---|---|---|---|---|
| | **MIC (mg/mL)** | **MBC (mg/mL)** | **MIC (mg/mL)** | **MFC (mg/mL)** |
| **2b** | 2.00 | 4.00 | 0.312 | 0.65 |
| **2c** | 2.50 | 5.00 | 0.625 | 1.75 |
| **2d** | 2.5 | > 5.00 | 1.25 | 2.85 |
| Ciprofloxacin | 1[a] | 2[a] | NT* | NT* |
| Fluconazole | NT* | NT* | 8[a] | 16[a] |

[a] Values are expressed in μg/mL; NT* = not tested

This behaviour could also be seen for other macromolecular amphiphilic compounds for which the antimicrobial activity became effective when the concentration was above CAC [44, 58]. The capacity of self-aggregation of dextran esters (Table II) correlated with their antimicrobial activity (Table V). Thus, the increase of self-association ability of the polymers determined a decrease of their bactericidal and fungicidal properties. MIC values of dextran amphiphilic esters were of the same order of magnitude with values reported for other non-ionic carbohydrate-derived containing 1,2,3-triazole moieties. Thus, for 4-sulfanilamido substitued-1,2,3-triazoles conjugated with monosaccharides were recorded MIC values between 0.08 - 5 mg/mL against *S. aureus* and 0.31 - 5 mg/mL against *C. albicans* [6] while for other 1,2,3-triazoles glycoside, MIC values were between 5 - 10 mg/mL against *S. aureus* and *C. albicans* [16].

An antimicrobial agent is considered to be bactericidal and/or fungicidal if the ratios MBC/MIC and/or MFC/MIC ≤ 4 [56]. The tested polymers (Table V) proved to have both bactericidal and fungicidal activity, as their ratios satisfied above-mentioned condition.

## Conclusions

Hydrophobically modified dextran esters having 1,2,3-triazole derivatives as pendant groups were obtained by a one-pot reaction. Structural characteristics of the polymers were revealed by elemental analysis, XPS, FTIR and $^{13}C$ NMR spectroscopy. Antimicrobial susceptibility tests showed that the polymers having electron-withdrawing substituents in their structure revealed the best antimicrobial activity while broth microdilution method proved bactericidal and fungicidal properties for this type of polysaccharide esters. The comparison between CAC and MIC values, proved the correlation between self-organizing capacity of dextran esters and their antimicrobial activity.

## Conflict of interest

The authors declare no conflict of interest.

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