Materials Today Communications 2 (2015) e70–e83

Contents lists available at ScienceDirect

Materials Today Communications

journal homepage: www.elsevier.com/locate/mtcomm

Poly(amide-triazole)s obtained by regioselective, microwave-assisted click polymerization of bio-based monomers

Daniela M. Fidalgo, Adriana A. Kolender, Oscar Varela*

CIHIDECAR-CONICET-UBA, Dpto. Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 1428 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 5 December 2014
Accepted 7 December 2014
Available online 23 December 2014

Keywords:
Biomaterials
Click polymerization
Polytriazole
Carbohydrate

ABSTRACT

The Cu(I)-catalyzed, microwave-assisted click polymerization of bio-based α-azide-ω-alkyne monomers afforded, with high regioselectivity, stereoregular poly(amide-triazole)s. The monomers were prepared starting from D-glucono-1,5-lactone as a renewable resource. The synthetic route involves the selective protection of this sugar lactone with formaldehyde to give a gluconic acid derivative, which was subjected to amidation of the carboxylic acid function with alkynylamines (2-propynyl, 3-butynyl, and 4-pentynylamines) and substitution of the primary hydroxyl group by azide. The regioselective click polymerization of these AB-type alkyne/azide monomers led to a series of linear biosourced poly(amide-triazole)s containing mostly (>95%) 1,4-disubstituted triazole linkages. In contrast, the thermal, metal-free click polymerization led to random distribution of 1,4- and 1,5-disubstituted triazoles in the polymer backbone. The length of the methylene chain linked to the amide of the monomer and the cycloaddition regioselectivity strongly affected the properties of the materials, mainly the T_g values, which were unexpectedly high.

1. Introduction

"Click chemistry" is a synthetic approach used to connect two building blocks in a high yielding and selective process [1]. One of the most reliable examples of a click reaction is the highly efficient and regiospecific copper(I) catalyzed azide-alkyne cycloaddition (CuAAC) [2]. The CuAAC reaction finds expanding applications in diverse disciplines ranging from organic synthesis, combinatorial and medicinal chemistry, chemical biology and material sciences, and comprises modern research areas such as drug discovery [3], bioconjugation [4] and nanoscience [5]. The reaction has been successfully applied in the bio-imaging field, for the labeling of cell surfaces, imaging small molecules inside of living cells or modifying cells with nanomaterials [6]. The CuAAC click reaction is also an established tool for the construction of complex molecular architectures, including polymers [7] and dendrimers [8] and, more recently, for the formation of shape persistent or rigid, preorganized macrocyclic species [9]. Particularly, the click polymerization is a versatile procedure for the synthesis of poly(triazole)s with linear or hyperbranched structures [10]. The "clicking" reaction is increasing exponentially for ligation within polymers and led to highly defined polymeric materials with unique functional properties such as luminescence, chromism, chain helicity, light refractivity, photovoltaic effect, cytocompatibility and biodegradability [9,10].

In the field of carbohydrates, the CuAAC click chemistry has been employed for the synthesis of varied derivatives, for carbohydrate drug discovery and for the development of neoglycopolymers [11]. However, we have found just a few examples on the synthesis of linear polytriazoles based on the CuAAC step growth polymerization of carbohydrate derived alkyne and azide functionalized AABB or AB monomers. Drockenmuller and coworkers have prepared these type of monomers by conversion of the two reactive alcohol groups of 1,4:3,6-dianhydrohexitols, such as isomannide, isosorbide and isoidide, into azide and/or alkyne functions. The polymerization of α-azide-ω-alkyne monomers furnished the corresponding linear AB-polytriazoles [12,13], while the analogous A_2B_2 polymers have been prepared by polymerization of diazide (AA) and dialkyne (BB) comonomers [13,14]. Partially biosourced networks were also obtained by reaction of one of the AB monomers with an A_2B_2 aliphatic cross-linker [13,14]. The group of Galbis and Bueno-Martínez has synthesized a 1,4-diazido-1,4-dideoxythreitol precursor, which reacted with diyne-esters derived from polyethyleneglycol, aliphatic diols and isosorbide, to afford click polyesters with free hydroxyl groups along the polymer chain [15]. Linear poly(amide-triazole)s have also been prepared

* Corresponding author. Tel.: +54 11 4576 3352.
E-mail address: varela@qo.fcen.uba.ar (O. Varela).

http://dx.doi.org/10.1016/j.mtcomm.2014.12.001
2352-4928/

by click polyaddition of AB-type amide monomer prepared from D-glucose [16].

In this article we report the synthesis of self-reacting azide-alkyne monomers from D-glucono-1,5-lactone, a cheap and commercially available derivative of D-glucose. The monomers were designed to have sites, as the amide linkages, for the degradation of the resulting polymers. The key step of the monomer synthesis was the conversion of the carboxylate function of an aldonic acid, derived from gluconolactone, into an ω-alkyne-amide. This strategy allowed us to prepared three monomers, according to the length of the $(CH_2)_n$ chain (n = 1–3) of the alkyne-amine. These monomers were subjected to both the Cu(I)-catalyzed and the metal-free thermal click polymerization to afford poly(amide-triazole)s. Remarkable regioselectivity in favor of the formation of 1,4-triazole linkages was observed for the microwave-assisted CuAAC polymerizations. The presence of the triazole rings and *NH* in the repeating unit of these new polyamides could induce biological activities by association through hydrogen bonding and dipole interactions to biological targets [17]. Furthermore, due to the high nitrogen content of the polymers, they were assayed for metal removal from aqueous solutions, as a potential use for the treatment of effluents.

2. Experimental

2.1. General methods

D-Glucono-1,5-lactone was purchased from Pfanstiehl Laboratories Inc. and used as received. Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 (E. Merck) aluminum-supported plates (layer thickness 0.2 mm). Visualization of the spots was effected by exposure to UV light, by charring with a solution of 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde or with cerium molibdate solution. Column chromatography was performed with Silica Gel 60 (230–400 mesh, E. Merck). Microwave reactions were carried out in an Anton Paar Monowave 300 reactor. Optical rotations were measured with a Perkin–Elmer 343 digital polarimeter at 25 °C and are expressed in $cm^3\ (g\,dm)^{-1}$, concentrations are given in $g\,cm^{-3}$. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AMX 500 instrument (^{1}H: 500 MHz; ^{13}C: 125.7 MHz) or a Bruker AC 200, in $CDCl_3$ solutions (tetramethylsilane as internal standard) unless otherwise indicated. The assignments were assisted by 2D COSY, DEPT, and HSQC techniques. IR spectra (film or KBr pellets) were recorded with a Thermo Scientific Nicolet 6700 Fourier transform infrared (FTIR) spectrometer. Thermogravimetric analysis (TGA) was performed in a Shimadzu TGA-51 instrument; samples were heated at a rate of 10 °C min^{-1}, from 20 to 700 °C. Differential scanning calorimetry (DSC) was conducted with a DSC Q20 TA instrument. Samples of about 2 mg were heated from 20 to 300 °C at a rate of 10 °C min^{-1} (isothermic 5 min), then cooled at 10 °C min^{-1} to −50 °C (isothermic 5 min), then a second heating cycle at 10 °C min^{-1} to 300 °C (isothermic 5 min), and finally cooled at 10 °C min^{-1} to −50 °C. High resolution mass spectrometry (HRMS-ESI) was performed in a Bruker microTOF-Q II instrument. 2,4:3,5-Di-*O*-methylidene-D-gluconic acid (**2**) was obtained from D-glucono-1,5-lactone (**1**) as already described [18]. 4-Pentynylamine hydrochloride was prepared from 4-pentynol, using a modification of the procedures described by Salzberg [19] and Ganem [20].

UV-MALDI-TOF analysis of the polymeric samples was performed using an Ultraflex II TOF/TOF mas spectrometer equipped with a high-performance solid-state laser (λ = 355 nm). The system is operated by the Flexcontrol 2.4 software package (Bruker Daltonics GmbsH). Samples were irradiated with a laser power of 60–95% and measured in the linear positive ion mode. Mass spectra were recorded between 0 and 20 kDa, as the sum of 1000 single laser shots. External calibration was performed with commercial Peptide calibration standard 2 and Protein calibration standard 1 (Bruker), containing angiotensin II ($[M+H]^+$ 1046.54), angiotensin I ($[M+H]^+$ 1296.68), substance P ($[M+H]^+$ 1347.74), bombesin (1619.82), ACTH clip 1–17 ($[M+H]^+$ 2093.09), ACTH clip 18–39 ($[M+H]^+$ 2465.20), somatostatin ($[M+H]^+$ 3147.47), insulin ($[M+H]^+$ 5734.51), ubiquitin I ($[M+H]^+$ 8565.76), cytochrome C ($[M+H]^+$ 12,360.97), myoglobin ($[M+H]^+$ 16,952.30). Sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid) and gentisic acid (2,5-dihydroxybenzoic acid) were used as matrices. Matrices solutions were prepared as saturated solutions in acetonitrile-H_2O containing 0.1% TFA (70: 30). Analyte solutions (1 μg/mL) were freshly prepared in TFA. Samples were loaded onto the sample probe using the mixture method: the analyte stock solution was mixed with the matrix solution in 1:1 vol/vol ratio. A 0.5 μL aliquot of this analyte/matrix solution was deposited onto the stainless steel probe tip and dried at room temperature. Then, an additional portion of 0.5 μL was applied to the dried solid layer on the probe, causing it to redissolve partially, and the solvent was left to dry again. The surface morphology of the poly(amide-triazole)s was analyzed using SEM with a Zeiss Supra 40 instrument with an in-lens secondary detector.

2.2. Monomer synthesis

*6-O-Acetyl-2,4:3,5-Di-O-methylidene-D-gluconic acid (**3**).* To a solution of 2,4:3,5-di-*O*-methylidene-D-gluconic acid [18] (**2**, 0.2 g, 0.91 mmol) in pyridine (2 mL) was added acetic anhydride (2 mL). The mixture was stirred for 2 h and then concentrated under reduced pressure to give compound **3** as a white solid (0.184 g, 77%) The compound was pure enough to be used without further purification in the following reactions. An analytical sample was obtained by column chromatography (EtOAc). mp: 181 °C. $[\alpha]_D^{25}$ = +16.7 (c = 1.0 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.11 (3H, s, COCH_3), 3.70 (1H, s, H-4), 4.15 (1H, s, H-3), 4.17 (1H, t, $J_{5,6a}$ = $J_{5,6b}$ 6.9 Hz, H-5), 4.30 (1H, dd, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.38 (d, $J_{2,3}$ 2.0 Hz), 4.47 (1H, dd, $J_{5,6b}$ 7.1, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.84, 5.29 (2H, 2 d, J 6.6 Hz, OCH_2O), 4.99, 5.03 (2H, 2 d, J 6.4 Hz, OCH_2O); ^{13}C NMR (125,7 MHz, $CDCl_3$, δ): 20.8 (COCH_3), 60.7 (C-6); 67.4 (C-3), 70.7 (C4), 73.6 (C-5), 76.0 (C-2), 88.4, 92.1 (OCH_2O), 170.0 (C-1), 170.8 (COCH_3); HRMS (ESI-Tof, m/z): $[M+Na]^+$, calcd for $[C_{10}H_{14}O_8Na]^+$: 285.0581, found: 285.0592.

*6-O-Acetyl-2,4:3,5-di-O-methylidene-N-(prop-2-yn-1-yl)-D-gluconamide (**4**).* 1-[3-(Dimethyl-amino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI, 0.877 g, 4.57 mmol) was added to a stirred solution of **3** (0.767 g, 2.92 mmol), 1-hydroxybenzotriazole (HOBt, 0.697 g, 4.55 mmol) and diisopropylethylamine (DIPEA, 161 μL, 0.92 mmol) in dichloromethane (DCM, 8.8 mL) at 0 °C. The mixture was stirred for 30 min under a nitrogen atmosphere, and then propargylamine (0.427 mL, 6.66 mmol) was added. The reaction mixture was allowed to warm to room temperature and the stirring was maintained overnight. Monitoring by TLC (DCM-EtOAc 2:1) indicated the formation of a major product (R_f 0.35). After concentration *in vacuo*, the resultant residue was purified by column chromatography (DCM-EtOAC 4:1) to yield the amide **4** (0.622 g, 71%). mp: 163 °C (recryst from EtOAc); $[\alpha]_D^{25}$ = +34.9 (c = 0.7 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.12 (3H, s, COCH_3), 2.26 (1H, t, $J_{1'a,3'}$ = $J_{1'b,3'}$ = 2.6 Hz, H-3′), 3.67 (1H, s, H-4), 4.02 (1H, m, $J_{1'a,3'}$ 2.6, $J_{1'a,NH}$ 4.8, $J_{1'a,1'b}$ 17.6 Hz, H-1′a), 4.18–4.25 (3H, m, H-2, H-3, H-5), 4,26 (1H, m, $J_{1'b,3'}$ 2.6, $J_{1'b,NH}$ 6.1, $J_{1'a,1'b}$ 17.6 Hz, H-1′b), 4.31 (1H, dd, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.50 (1H, dd, $J_{5,6b}$ 7.2, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.84, 5.29 (2H, 2 d, J 6.5 Hz, OCH_2O), 4.98, 5.04 (2H, 2 d, J 6.5 Hz, OCH_2O), 6.78 (1H, m, NH); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 20.8 (COCH_3), 28.8 (C-1′), 60.6

(C-6); 67.5 (C-3), 71.1 (C-4), 71.9 (C-3′), 73.6 (C-5), 77.4 (C-2), 78.7 (C-2′), 88.5, 92.7 (OCH_2O), 166.8 (C-1), 170.4 ($COCH_3$); Anal. Calcd for $C_{13}H_{17}NO_7$: C 52.17, H 5.73, N 4.68, Found: C 52.47, H 5.88, N 4.85; HRMS (ESI-Tof, *m/z*): $[M+Na]^+$, calcd for $[C_{13}H_{17}NO_7Na]^+$: 322.0897 found: 322.0899.

*2,4:3,5-Di-O-methylidene-N-(prop-2-yn-1-yl)-D-gluconamide (**5**).* To a solution of compound **4** (0.344 g, 1.15 mmol) in DCM (14 mL) was added 0.1 M sodium methoxide in MeOH (14 mL). After stirring at room temperature for 2 h, the mixture was neutralized with acetic acid. The insoluble deacetylated product was recovered by centrifugation, and then washed with water and acetone. Compound **4** was isolated as an amorphous solid (0.257 g, 87%). mp: 231 °C (decomp); $[\alpha]_D^{25}$ = +47.1 (*c* = 0.4 in DMSO); ^1H NMR (500 MHz, DMSO-d_6, δ): 3.02 (1H, t, $J_{1'a,3'}$ = $J_{1'b,3'}$ 2.5 Hz, H-3′), 3.67 (1H, dd, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.6 Hz, H-6a), 3.70–3.79 (4H, m, H-4, H-5, H-6b, H-1′a), 3.93 (1H, m, $J_{1'b,3'}$ 2.5, $J_{1'b,NH}$ 6.2, $J_{1'a,1'b}$ 17.2 Hz, H-1′b), 4.09 (1H, s, H-3), 4.26 (1H, d, $J_{2,3}$ 1.9 Hz, H-2), 4.73, 4.90 (2H, 2 d, *J* 6.3 Hz, OCH_2O), 4.81, 5.09 (2H, 2 d, *J* 6.5 Hz, OCH_2O), 6.78 (1H, t, $J_{1'a,NH}$ = $J_{1'b,NH}$ 6.2 Hz, N*H*); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 27.7 (C-1′), 58.5 (C-6), 67.5 (C-3), 70.4 (C-4), 72.4 (C-3′), 76.1 (C-5), 76.9 (C-2), 81.2 (C-2′), 87.6, 91.4 (OCH_2O), 167.1 (C-1); Anal. Calcd for $C_{11}H_{15}NO_6$: C 51.36, H 5.88, N 5.44, Found: C 51.46, H 5.88, N 5.55; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{11}H_{16}NO_6]^+$: 258.0972 found: 258.0970, $[M+Na]^+$, calcd for $[C_{11}H_{15}NO_6Na]^+$: 280.0792 found: 280.0802

*2,4:3,5-Di-O-methylidene-N-(prop-2-yn-1-yl)-6-O-tosyl-D-gluconamide (**6**).* A solution of compound **5** (0.488 g, 0.19 mmol) and tosyl chloride (0.566 g, 2.96 mmol) in pyridine (3.4 mL) was stirred at room temperature for 2 days. After addition of methanol (1 mL), the mixture was concentrated *in vacuo*. The resulting residue was purified by column chromatography (DCM-EtOAc 9:1), to afford **6** as a white solid (0.651 g, 83%). mp: 160 °C (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +33.4 (*c* = 0.6 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 2.23 (1H, t, $J_{1'a,3'}$ = $J_{1'b,3'}$ 2.6 Hz, H-3′), 2.47 (3H, s, CH_3Ph) 3.69 (1H, s, H-4), 3.98 (1H, m, $J_{1'a,3'}$ 2.6, $J_{1'a,NH}$ 6.1, $J_{1'a,1'b}$ 17.6 Hz, H-1′a), 4.08 (1H, s, H-3), 4.13–4.21 (3H, m, $J_{2,3}$ 1.9, $J_{1'b,3'}$ 2.6, $J_{1'b,NH}$ 6.1, $J_{1'a,1'b}$ 17.6 Hz, H-2, H-5, H-1′b), 4.21–4.25 (2H, m, $J_{5,6a}$ 7.3, $J_{5,6b}$ 5.9, $J_{6a,6b}$ 10.6 Hz, H-6a, H-6b), 4.77–4.80, 4.88, 5.24 (4H, 4 d, *J* 6.5 Hz, OCH_2O), 6.72 (1H, m, NH), 7.38 (2H, d, *J* 8.0 Hz, H-aromatic), 7.8 (2H, d, *J* 8.3, H-aromatic); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 21.7 (CH_3Ph), 28.8 (C-1′), 65.8 (C-6), 67.3 (C-3), 70.4 (C-4), 71.9 (C-3′), 72.9 (C-5), 77.2 (C-2), 78.7 (C-2′), 88.7, 92.2 (OCH_2O), 128.0, 130.1, 132.1, 145.6 (C-aromatic), 166.6 (C-1); Anal. Calcd for $C_{18}H_{21}NO_8S$: C 52.55, H 5.14, N 3.40, Found: C 52.25, H 5.09, N 3.28; HRMS (ESI-Tof, *m/z*): $[M+Na]^+$, calcd for $[C_{18}H_{21}NO_8SNa]^+$: 434.0880 found: 434.0864.

*6-Azido-6-desoxy-2,4:3,5-di-O-methylidene-N-(prop-2-yn-1-yl)-D-gluconamide (**7**).* To a solution of compound **6** (0.25 g, 0.61 mmol) in DMF (2.7 mL), was added NaN_3 (0.078 g, 2.1 mmol). The mixture was stirred at 70 °C overnight, and then concentrated *in vacuo*. The residue was resuspended in DCM, filtered and concentrated, to yield compound **7** (0.163 g, 94%) as an amorphous solid. $[\alpha]_D^{25}$ = +89.6 (*c* = 0.5 in DMSO); IR (KBr): ν = 3278 (w, N—H), 2098 (s, N_3), 1666 cm^{-1} (s, C=O); ^1H NMR (500 MHz, DMSO-d_6, δ): 3.02 (1H, t, $J_{1',3'}$ 2.5 Hz, H-3′); 3.41 (1H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 13.3 Hz, H-6a); 3.67 (1H, s, H-4); 3.76 (1H, m, $J_{1'a,3'}$ 2.5, $J_{1'a,NH}$ 5.8, $J_{1'a,1'b}$ 17.3 Hz, H-1′a); 3.91–3.94 (2H, m, H-5, H-1′b); 4.07 (1H, dd, $J_{5,6b}$ 9.8, $J_{6a,6b}$ 13.3 Hz, H-6b); 4.13 (1H, s, H-3); 4.27 (1H, d, $J_{2,3}$ 1.9 Hz, H-2); 4.78–4.80, 4.97, 5.09 (4H, 4 d, *J* 6.3, *J* 6.5 Hz, OCH_2O); 8.13 (1H, t, $J_{1'a,NH}$ = $J_{1'b,NH}$ 5.8 Hz; NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 27.7 (C-1′), 46.6 (C-6), 67.2 (C-3), 70.0 (C-4), 72.5 (C-3′), 74.5 (C-5), 76.7 (C-2), 81.2 (C-2′), 86.4, 91.3 (OCH_2O), 166.9 (CO); Anal. Calcd for $C_{11}H_{14}N_4O_5$: C 46.81, H 5.00, N 19.85, Found: C 46.45, H 4.93, N 19.66; HRMS (ESI-Tof, *m/z*): $[M+Na]^+$, calcd for $[C_{11}H_{14}N_4O_5Na]^+$: 305.0856 found: 305.0860, $[M+K]^+$, calcd for $[C_{11}H_{14}N_4O_5K]^+$: 321.0596 found: 321.0589.

*6-O-Acetyl-2,4:3,5-di-O-methylidene-N-(but-3-yn-1-yl)-D-gluconamide (**8**).* To a stirred solution of **3** (0.200 g, 0.76 mmol), HOBt (0.182 g, 1.18 mmol) and DIPEA (42 μL, 0.24 mmol) in DCM (2.3 mL) cooled to 0 °C, was added EDCI (0.229 g, 1.19 mmol). The mixture was stirred for 30 min under a nitrogen atmosphere, and then 1-amino-3-butyne (0.094 mL, 1.14 mmol) was added. The reaction mixture was allowed to warm to room temperature and the stirring was maintained overnight. TLC (DCM-EtOAc 2:1) indicated the formation of a major product (R_f 0.35). The mixture was concentrated *in vacuo*, and the resultant residue was purified by column chromatography (DCM-EtOAC 9:1) to yield the amide **8** (0.191 g, 80%). mp: 114–115 °C (recrystallized from EtOAc); $[\alpha]_D^{25}$ = +34.9 (*c* = 0.7 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 2.03 (1H, t, $J_{2'a,4'}$ = $J_{2'b,4'}$ 2.6 Hz, H-4′), 2.13 (3H, s, $COCH_3$), 2.39 (1H, m, $J_{2'a,4'}$ 2.6, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ 6.6, $J_{2'a,2'b}$ 16.4 Hz, H-2′a), 2.43 (1H, m, $J_{2'b,4'}$ 2.6, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ 6.6, $J_{2'a,2'b}$ 16.4 Hz, H-2′b), 3.42 (1H, m, $J_{1'a,2'a}$ = $J_{1'a,2'b}$ = $J_{1'a,NH}$ 6.6, $J_{1'a,1'b}$ 13.2 Hz, H-1′a), 3.57 (1H, m, $J_{1'b,2'a}$ = $J_{1'b,2'b}$ = $J_{1'b,NH}$ 6.6, $J_{1'a,1'b}$ 13.2 Hz, H-1′a), 3.67 (1H, s, H-4), 4.18–4.21 (3H, m, H-2, H-3, H-5), 4.31 (1H, dd, $J_{5,6b}$ 5.9, $J_{6a,6b}$ 11.9 Hz, H-1b), 4.51 (1H, dd, $J_{5,6a}$ 7.2, $J_{6a,6b}$ 11.9), 4.84, 5.29 (2H, 2 d, *J* 6.5 Hz, OCH_2O), 4.98, 5.04 (2H, 2 d, *J* 6.5 Hz, OCH_2O), 6.89 (1H, m, NH); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 19.5 (C-2′), 20.9 ($COCH_3$), 37.8 (C-1′), 60.8 (C-6), 67.7 (C-3), 70.3 (C4), 71.2 (C-4′), 73.8 (C-5), 77.6 (C-2), 81.2 (C-3′), 88.7, 92.3 (OCH_2O), 167.3 (CO), 170.6 ($COCH_3$); Anal. Calcd for $C_{14}H_{19}NO_7$: C 53.67, H 6.11, N 4.47 Found: C 53.97, H 6.18, N 4.31; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{14}H_{20}NO_7]^+$: 314.1234 found: 314.1237, $[M+Na]^+$, calcd for $[C_{14}H_{19}NO_7Na]^+$: 336.1054 found: 336.1056.

*6-O-Acetyl-2,4:3,5-di-O-methylidene-N-(pent-4-yn-1-yl)-D-gluconamide (**9**).* To a cooled solution (0 °C) of **3** (0.401 g, 1.53 mmol), HOBt (0.366 g, 2.38 mmol) and DIPEA (0.400 mL, 2.32 mmol) in DCM (6 mL) was added EDCI (0.460 g, 2.40 mmol). The mixture was stirred for 30 min under a nitrogen atmosphere, and then 4-pentyn-1-amine hydrochloride (0.277 g, 2.30 mmol) was added. The reaction mixture was allowed to warm to room temperature overnight. Examination by TLC (DCM-EtOAc 2:1) indicated the formation of a major product (R_f 0.20). After concentration, the resulting residue was purified by column chromatography (DCM-EtOAC 9:1) to yield the amide **9** (0.286 g, 57%). mp: 100 °C (recrystallized from EtOAC); $[\alpha]_D^{25}$ = +27.8 (*c* = 0.6 in $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$, δ): 1.74 (1H, m, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ = $J_{2'a,3'a}$ = $J_{2'a,3'b}$ 6.9, $J_{2'a,2'b}$ 13.8 Hz, H-2′a), 1,79 (1H, m, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ = $J_{2'b,3'a}$ = $J_{2'b,3'b}$ 6.9, $J_{2'a,2'b}$ 13.6 Hz, H-2′b), 2.09 (3H, s, $COCH_3$), 1.98 (1H, t, $J_{3'a,5'}$ = $J_{3'b,5'}$ 2.7 Hz, H-5′), 2.25 (1H, m, $J_{3'a,5'}$ 2.7, $J_{2'a,3'a}$ = $J_{2'b,3'a}$ 6.9, $J_{3'a,3'b}$ 17.0 Hz, H-3′a), 2.26 (1H, m, $J_{3'b,5'}$ 2.7, $J_{2'a,3'b}$ = $J_{2'b,3'b}$ 7.1, $J_{3'a,3'b}$ 17.0 Hz, H-3′b), 3.41 (1H, m, $J_{1'a,2'a}$ = $J_{1'a,2'b}$ = $J_{1'a,NH}$ 6.9, $J_{1'a,1'b}$ 13.5 Hz, H-1′a); 3.49 (1H, m, $J_{1'b,2'a}$ = $J_{1'b,2'b}$ = $J_{1'b,NH}$ 6.9, $J_{1'a,1'b}$ = 13.5 Hz, H-1′b), 3.63 (1H, s, H-4); 4.13 (1H, d, $J_{2,3}$ = 1.9 Hz, H-2); 4.15–4.26 (2H, m, H-3, H-5); 4.28 (1H, dd, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.49 (1H, dd, $J_{5,6b}$ 7.2, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.80, 5.24 (2H, 2 d, *J* 6.5 Hz, OCH_2O); 4.94, 5.02 (2H, 2 d, *J* 6.6 Hz, OCH_2O); 6.66 (1H, m, NH); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 16.0 (C-2′), 20.9 ($COCH_3$), 28.2 (C-3′), 38.2 (C-1′), 60.7 (C-6), 67.8 (C-3), 69.2 (C-5′), 71.2 (C-4), 73.7 (C-5), 77.6 (C-2), 83.3 (C-4′), 88.6, 92.3 (OCH_2O), 167.2 (CO), 170.6 ($COCH_3$); Anal. Calcd for $C_{15}H_{21}NO_7$: C 55.04, H 6.47, N 4.28, Found: C 55.07, H 6.47, N 4.09; HRMS (ESI-Tof, *m/z*): $[M+Na]^+$, calcd for $[C_{15}H_{21}NO_7Na]^+$: 350.1210 found: 350.1211.

*2,4:3,5-Di-O-methylidene-N-(but-3-yn-1-yl)-D-gluconamide (**10**).* To a solution of alkynyl amide **8** (0.250 g, 0.80 mmol) in DCM (9.7 mL) was added 0.1 M sodium methoxide in MeOH (9.7 mL). The mixture was stirred at room temperature for 2 h, then it was neutralized with Dowex 50 (H+) resin, filtered and concentrated *in vacuo* to afford compound **10** (0.200 g, 92%). After recrystallization from EtOAc-hexane it gave mp: 141 °C (decomp); $[\alpha]_D^{25}$ = -4.6 (*c* = 0.4 in DMSO); ^1H NMR (500 MHz, DMSO-d_6, δ): 2.29 (2H, m,

$J_{2',4'}$ 2.7, $J_{1'a,2'}$ = $J_{1'b,2'}$ 7.3 Hz, H-2′), 2.81 (1H, t, $J_{3',4'}$ 2.7 Hz, H-4′), 3.11 (1H, m, $J_{1'a,NH}$ 5.7, $J_{1'a,2'}$ 7.3, $J_{1'a,1'b}$ 12.9 Hz, H-1′a), 3.28–3.33 (1H, m, H-1′b), 3.66–3.75 (4H, m, H-4, H-5, H-6a, H-6b), 3.78 (1H, s, H-4), 4.08 (1H, s, H-3), 4.22 (1H, d, $J_{2,3}$ 1.9 Hz, H-2), 4.73, 4.90 (2H, 2 d, *J* 6.3 Hz, OCH_2O), 4.81, 5.10 (2H, 2 d, *J* 6.3 Hz, OCH_2O), 4.95 (1H, s, OH), 7.79 (1H, t, $J_{1'a,NH}$ = $J_{1'b,NH}$ 6.0 Hz, NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 19.1 (C-2′), 37.9 (C-1′), 58.9 (C-6), 67.9 (C-3), 70.8 (C-4), 72.6 (C-4′), 76.6 (C-5), 77.3 (C-2), 82.4 (C-3′), 88.0, 91.8 (OCH_2O), 167.7 (CO); Anal. Calcd for $C_{12}H_{17}NO_6$: C 53.13, H 6.32, N 5.16, Found: C 52.95, H 6.34, N 5.14; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{12}H_{18}NO_6]^+$: 272.1129 found: 272.1132, $[M+Na]^+$, calcd for $[C_{12}H_{17}NO_6Na]^+$: 294.0948 found: 294.0954

*2,4:3,5-Di-O-methylidene-N-(pent-4-yn-1-yl)-D-gluconamide (**11**).* To a solution of compound **9** (0.154 g, 0.47 mmol) in DCM (5.7 mL) was added 0.1 M sodium methoxide in MeOH (5.7 mL). The mixture was stirred at room temperature for 2 h, and then it was neutralized with Dowex 50 W (H^+) resin, filtered and concentrated to afford compound **11** (0.116 g, 86%). mp: 99 °C (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +24.7 (*c* = 0.4 in DMSO); ^{1}H NMR (500 MHz, DMSO-d_6, δ): 1.57 (1H, m, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ = $J_{2'a,3'a}$ = $J_{2'a,3'b}$ 6.9, $J_{2'a,2'b}$ 13.7 Hz, H-2′a), 1.60 (1H, m, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ = $J_{2'b,3'a}$ = $J_{2'b,3'b}$ 6.9, $J_{2'a,2'b}$ = 13.7 Hz, H-2′b), 2.11 (1H, m, $J_{3',5'}$ 2.3, $J_{2'a,3'}$ = $J_{2'b,3'}$ 6.9 Hz, H-3′), 2.57 (1H, t, $J_{3',5'}$ 2.3 Hz, H-5′), 3.13 (1H, m, $J_{1'a,NH}$ = $J_{1'a,2'a}$ = $J_{1'a,2'b}$ 6.9, $J_{1'a,1'b}$ = 13.7 Hz, H-1′a), 3.16 (1H, m, $J_{1'b,NH}$ = $J_{1'b,2'a}$ = $J_{1'b,2'b}$ 6.9, $J_{1'a,1'b}$ = 13.7 Hz, H-1′a), 3.67–3.77 (4H, m, $J_{5,6a}$ 4.5, $J_{5,6b}$ 6.4, $J_{6a,6b}$ 9.1 Hz, H-4, H-5, H-6a, H-6b), 4.08 (1H, s, H-3), 4,20 (1H, d, $J_{2,3}$ 1.8 Hz, H-2), 4.73, 4.92 (2H, 2 d, *J* 6.3 Hz, OCH_2O), 4.81, 5.10 (2H, 2 d, *J* 6.3 Hz, OCH_2O), 7.73 (1H, m, NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 15.3 (C-3′), 28.1 (C-2′), 37.5 (C-1′), 58.4 (C-6), 67.5 (C-3), 70.4 (C-4), 71.2 (C-5′), 76.1 (C-5), 77.0 (C-2), 84.1 (C-4′), 87.6, 91.3 (OCH_2O), 167.1 (CO); Anal. Calcd for $C_{13}H_{19}NO_6$: C 54.73, H 6.71, N 4.91, Found: C 54.72, H 6.77, N 4.91; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{13}H_{20}NO_6]^+$: 286.1285 found: 286.1286, $[M+Na]^+$, calcd for $[C_{13}H_{19}NO_6Na]^+$: 308.1105 found: 308.1102.

*2,4:3,5-Di-O-methylidene-N-(but-3-yn-1-yl)-6-O-tosyl-D-gluconamide (**12**).* A solution of compound **10** (0.138 g, 0.51 mmol) and tosyl chloride (0.152 g, 0.80 mmol) in pyridine (0.9 mL) was stirred at room temperature overnight. After addition of methanol (1 mL), the mixture was concentrated *in vacuo*. The resulting residue was purified by column chromatography (DCM-EtOAc 2:1) to afford **12** as a white solid (0.199 g, 92%). mp: 126 °C (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +44.0 (*c* = 0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.00 (1H, t, $J_{2'a,4'}$ = $J_{2'b,4'}$ 2.7 Hz, H-4′), 2.37 (1H, m, $J_{2'a,4'}$ 2.7, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ 6.6, $J_{2'a,2'b}$ 16.9 Hz, H-2′a), 2.41 (1H, m, $J_{2'b,4'}$ = 2.7, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ 6.6, $J_{2'a,2'b}$ 16.9 Hz, H-2′b), 2.46 (3H, s, CH_3Ph), 3.38 (1H, m, $J_{1'a,NH}$ = 5.6, $J_{1'a,2'a}$ = $J_{1'a,2'b}$ = 6.8, $J_{1'a,1'b}$ 13.2 Hz, H-1′a), 3.53 (1H, m, $J_{1'b,NH}$ 5.6, $J_{1'b,2'a}$ = $J_{1'b,2'b}$ 6.5, $J_{1'a,1'b}$ 13.2 Hz, H-1′b), 3.68 (1H, s, H-4), 4.07 (1H, s, H-3), 4.11 (1H, d, $J_{2,3}$ 2.0 Hz, H-2), 4.14 (1H, m, $J_{4,5}$ 1.1, $J_{5,6a}$ = $J_{5,6b}$ 6.9 Hz, H-5), 4.23 (1H, dd, $J_{5,6b}$ 6.9, $J_{6a,6b}$ 10.2 Hz, H-6b), 4.26 (1H, dd, $J_{5,6a}$ 7.2, $J_{6a,6b}$ 10.2 Hz, H-6a), 4.77–4.80, 4.87, 5.23 (4H, m, *J* 6.5 Hz, OCH_2O), 6.84 (1H, t, $J_{1'a,NH}$ = $J_{1'b,NH}$ 5.6 Hz, NH), 7.38 (2H, d, *J* 8.4 Hz, H-aromatic), 7.80 (2H, d, *J* 8.4 Hz, H-aromatic); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 19.3 (C-2′), 21.7 (CH_3Ph), 37.7 (C-1′), 65.8 (C-6), 67.4 (C-3), 70.2 (C-4), 70.4 (C-4′), 73.0 (C-5), 77.3 (C-2), 81.0 (C-3′), 88.7, 92.2 (OCH_2O), 128.0, 130.2, 132.1, 145.6 (C-aromatic), 167.0 (CO); Anal. Calcd for $C_{19}H_{23}NO_8S$: C 53.64, H 5.45, N 3.29, Found: C 53.87, H 5.52, N 3.18; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{19}H_{24}NO_8S]^+$: 426.1217 found: 426.1221, $[M+Na]^+$, calcd for $[C_{19}H_{23}NO_8SNa]^+$: 448.1037 found: 448.1039.

*2,4:3,5-Di-O-methylidene-N-(pent-4-yn-1-yl)-6-O-tosyl-D-gluconamide (**13**).* To a solution of compound **11** (0.235 g, 0.85 mmol) in pyridine (1.6 mL) was added tosyl chloride (0.258 g, 1.35 mmol). The solution was stirred at room temperature for 3 days. Then, it was concentrated *in vacuo* and the residue was purified by column chromatography (DCM-EtOAc 9:1), to afford **13** as a white solid (0.33 g, 88%). mp: 110 °C (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +29.1 (*c* = 0.4 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, δ): 1.72 (1H, m, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ = $J_{2'a,3'a}$ = $J_{2'a,3'b}$ 7.0, $J_{2'a,2'b}$ 13.9 Hz, H-2′a), 1.77 (1H, m, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ = $J_{2'b,3'a}$ = $J_{2'b,3'b}$ 7.0, $J_{2'a,2'b}$ 13.9 Hz, H-2′b), 1.96 (1H, t, $J_{3'a,5'}$ = $J_{3'b,5'}$ 2.7 Hz, H-5′), 2.22 (1H, m, $J_{3'a,5'}$ 2.7, $J_{2'a,3'a}$ = $J_{2'b,3'a}$ 7.0, $J_{3'a,3'b}$ 19.9 Hz, H-3′a), 2.25 (1H, m, $J_{3'b,5'}$ 2.7, $J_{2'a,3'b}$ = $J_{2'b,3'b}$ 7.0, $J_{3'a,3'b}$ 19.9 Hz, H-3′a), 2.47 (3H, s, CH_3Ph), 3.39 (1H, m, $J_{1'a,NH}$ = $J_{1'a,2'a}$ = $J_{1'a,2'b}$ 6.8, $J_{1'a,1'b}$ 13.5 Hz, H-1′a), 3.45 (1H, m, $J_{1'b,NH}$ = $J_{1'b,2'a}$ = $J_{1'b,2'b}$ 6.8, $J_{1'a,1'b}$ 13.5 Hz, H-1′b), 3.68 (1H, s, H-4), 4.07 (1H, s, H-3), 4.09 (1H, d, $J_{2,3}$ 2.0 Hz, H-2), 4.15 (1H, m, H-5), 4.23 (1H, dd, $J_{5,6a}$ 5.9, $J_{6a,6b}$ 10.6 Hz, H-6a), 4.26 (1H, dd, $J_{5,6b}$ 7.2, $J_{6a,6b}$ 10.6 Hz, H-6b), 4.77–4.80, 4.88, 5.23 (4H, m, OCH_2O), 6.64 (1H, m, NH), 7.38 (2H, d, *J* 8.6 Hz, H-aromatic), 7.81 (2H, d, *J* 8.3 Hz, H-aromatic); ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 16.0 (C-3′), 21.9 (CH_3Ph), 28.2 (C-2′), 38.2 (C-1′), 65.9 (C-6), 67.5 (C-3), 69.3 (C-5′), 70.5 (C-4), 73.1 (C-5), 77.5 (C-2), 83.3 (C-4′), 88.9, 92.3 (OCH_2O), 128.2, 130.3, 132.2, 145.8 (C-aromatic), 167.1 (CO); Anal. Calcd for $C_{20}H_{25}NO_8S$: C 54.66, H 5.73, N 3.19, Found: C 54.72, H 5.67, N 3.08; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{20}H_{26}NO_8S]^+$: 440.1374 found: 440.1380, $[M+Na]^+$, calcd for $[C_{20}H_{25}NO_8SNa]^+$: 462.1193 found: 462.1207.

*6-Azido-6-deoxy-2,4:3,5-di-O-methylidene-N-(but-3-yn-1-yl)-D-gluconamide (**14**).* To a solution of compound **12** (0.220 g, 0.52 mmol) in DMF (2.3 mL), was added NaN_3 (0.066 g, 1.8 mmol). The mixture was stirred at 70 °C overnight and then it was concentrated *in vacuo*. The residue was resuspended in DCM, filtered and concentrated, to yield compound **14** (0.140 g, 92%) as an amorphous solid. $[\alpha]_D^{25}$ = +38.1 (*c* = 0.3 in DMSO); IR (KBr): ν = 3278 (w, N—H), 2098 (s, N_3), 1666 cm^{-1} (s, C=O); ^{1}H NMR (500 MHz, DMSO-d_6, δ): 2.28 (2H, m, $J_{1'a,2'}$ = $J_{1'b,2'}$ 7.3, $J_{2',4'}$ = 2.6 Hz, H-2′), 2.80 (1H, t, $J_{2',4'}$ 2.6 Hz, H-4′), 3.12 (1H, m, $J_{1'a,NH}$ 6.0, $J_{1'a,2'}$ 7.3, $J_{1'a,1'b}$ 13.0 Hz, H-1′a), 3.30 (1H, m, $J_{1'b,NH}$ 6.0 $J_{1'b,2'}$ 7.3, $J_{1'a,1'b}$ 13.0 Hz, H-1′b), 3.33–3.42 (1H, m, H-6a), 3.66 (1H, s, H-4), 3.91 (1H, dd, $J_{5,6a}$ 5.0, $J_{5,6b}$ 9.9 Hz, H-5), 4.07 (1H, dd, $J_{5,6b}$ 9.9, $J_{6a,6b}$ 13.2 Hz, H-6b), 4.10 (1H, s, H-3), 4.23 (1H, d, $J_{2,3}$ 1.9 Hz, H-2), 4.78–4.79, 4.98, 5.09 (4H, m, OCH_2O), 7.81 (1H, t, $J_{1'a,NH}$ = $J_{1'b,NH}$ 6.0 Hz; NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 19.1 (C-2′), 37.9 (C-1′), 47.1 (C-6), 67.7 (C-3), 70.5 (C-4), 72.6 (C-4′), 75.0 (C-5), 77.2 (C-2), 82.4 (C-3′), 86.8, 91.8 (OCH_2O), 167.6 (CO); Anal. Calcd for $C_{12}H_{16}N_4O_5$: C 48.65, H 5.44, N 18.91, Found: C 48.42, H 5.19, N 18.63; HRMS (ESI-Tof, *m/z*): $[M+Na]^+$, calcd for $[C_{12}H_{16}N_4O_5Na]^+$: 319.1013 found: 319.1008.

*6-Azido-6-deoxy-2,4:3,5-di-O-methylidene-N-(pent-4-yn-1-yl)-D-gluconamide (**15**).* To a solution of compound **13** (0.135 g, 0.31 mmol) in DMF (1.4 mL), was added NaN_3 (0.040 g, 1.1 mmol) and stirred at 70 °C overnight. Upon concentration, the residue was resuspended in DCM, filtered and concentrated, to yield compound **13** (0.095 g, 99%) as a white solid. mp: 101 °C (recryst from DMSO-acetone); $[\alpha]_D^{25}$ = +40.2 (*c* = 0.4 in DMSO); IR (KBr): ν = 3278 (w, N—H), 2098 (s, N_3), 1666 cm^{-1}(f, C=O); ^{1}H NMR (500 MHz, DMSO-d_6, δ): 1.59 (1H, m, $J_{1'a,2'a}$ = $J_{1'b,2'a}$ = $J_{2'a,3}$ 7.2, $J_{2'a,2'b}$ 13.5 Hz, H-2′a), 1.61 (1H, m, $J_{1'a,2'b}$ = $J_{1'b,2'b}$ = $J_{2'b,3}$ 7.2, $J_{2'a,2'b}$ 13.5 Hz, H-2′b), 2.11 (1H, m, $J_{3',5'}$ 2.6, $J_{2'a,3'}$ = $J_{2'b,3'}$ 7.2 Hz, H-3′), 2.75 (1H, t, $J_{3',5'}$ 2.6 Hz, H-5′), 3.13 (1H, m, $J_{1'a,NH}$ = $J_{1'a,2'a}$ = $J_{1'a,2'b}$ 7.2, $J_{1'a,1'b}$ 13.1 Hz, H-1′a), 3.17 (1H, m, $J_{1'b,NH}$ = $J_{1'b,2'a}$ = $J_{1'b,2'b}$ 7.2, $J_{1'a,1'b}$ 13.1 Hz, H-1′b), 3.42 (1H, dd, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 13.2 Hz, H-6a), 3.67 (1H, s, H-4), 3.94 (1H, dd, $J_{5,6a}$ 5.0, $J_{5,6b}$ 9.8 Hz, H-5), 4.09 (1H, dd, $J_{5,6b}$ 9.8, $J_{6a,6b}$ 13.2 Hz, H-6b), 4.11 (1H, s, H-3), 4.22 (1H, d, $J_{2,3}$ 1.8 Hz, H-2), 4.78–4.80, 4.99, 5.10 (4H, m, OCH_2O), 7.75 (1H, m, NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 15.3 (C-3′), 28.1 (C-2′), 37.5 (C-1′), 46.6 (C-6), 67.2 (C-3), 70.1 (C-4), 71.2 (C-5′), 74.5 (C-5), 76.8 (C-2), 84.1 (C-4′), 86.4, 91.3 (OCH_2O), 170.0 (CO); Anal. Calcd for $C_{13}H_{18}NO_5$: C 50.31, H 5.85, N 18.05, Found: C 50.61, H 6.13, N 17.77; HRMS (ESI-Tof, *m/z*): $[M+H]^+$, calcd for $[C_{13}H_{19}N_4O_5]^+$:

311.1350 found: 311.1340, $[M+Na]^+$, calcd for $[C_{13}H_{18}N_4O_5Na]^+$: 333.1169 found: 333.1160

2.3. General procedure for the polymerization

2.3.1. Procedure A: copper-catalyzed reaction

Monomer **7** (0.100 g, 0.35 mmol) was polymerized in DMF or DMSO (concentration: 0.4 M) under Ar atmosphere, in the presence of $Cu(OAc)_2$-ascorbic acid or CuOAc. The mixture was heated either in an oil bath at 100 °C for 18 h or under microwave (Mw) irradiation at 100 °C for 30 min or 2 h. The poly(amide-triazole)s precipitated from the reaction mixture, and were recovered by centrifugation. Then the solid was washed with DMSO, NH_3(c), H_2O and acetone to yield polymer **16**.

Monomers **14** and **15** were polymerized under the optimized conditions described for **7**. To a solution of α-azido-ω-alkynylamide (0.32–0.34 mmol) in DMSO was added 5% CuOAc, under Ar atmosphere, and heated in a microwave reactor at 100 °C for 30 min.

Poly(amide-triazole) ***16***. $[\alpha]_D^{25}$ = +110.2 (*c* = 0.7 in TFA-H_2O 1:1); IR (KBr): ν = 3309 (w, N—H), 1658 (s, C=O), 3070 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 3.79 (1H, s, H-4), 4.10 (1H, m, H-5), 4.20–4.41 (4H, m, H-2, H-3, H-1′a, H-1′b), 4.61–4.63 (1H, m, H-6a), 4.78–4.82 (2H, m, OCH_2O), 5.06–5.11 (3H, m, H-6b, OCH_2O), 7.84 (1H, s, H-3′), 8.15–8.19 (1H, s, NH); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 34.2 (C-1′), 45.9 (C-6), 67.3 (C-3), 69.8 (C-4), 74.5 (C-5), 76.8 (C-2), 86.3, 91.4 (OCH_2O), 123.4 (C-3′), 145.1 (C-2′), 167.2 (CO). Anal. Calcd for $[C_{11}H_{14}N_4O_5\cdot(H_2O)_{0.5}]$: C 45.36, H 5.19, N 19.24, Found: C 45.14, H 5.04, N 18.97

Poly(amide-triazole) ***18***. $[\alpha]_D^{25}$ = +105.2 (*c* = 0.2 in TFA-H_2O 1:1); IR (KBr): ν = 3417 (w, N—H), 1658 (s, C=O), 3085 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 2.78 (2H, m, H-2′), 3.31–3.44 (2H, m, H-1′), 3.77 (1H, s, H-4), 4.21 (1H, m, H-5), 4.26 (2H, s, H-2, H-3), 4.63 (1H, m, H-6a), 4.78 (2H, m, OCH_2O), 5.01–5.09 (3H, m, H-6b, OCH_2O), 7.87 (1H, s, NH), 7.92 (1H, s, H-4′); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 25.4 (C-2′), 38.1 (C-1′), 46.3 (C-6), 67.2 (C-3), 69.9 (C-4), 74.4 (C-5), 76.9 (C-2), 86.4, 91.5 (OCH_2O), 123.4 (C-4′), 144.5 (C-3′), 167.1 (CO). Anal. Calcd for $[C_{12}H_{16}N_4O_5\cdot(H_2O)]$: C 45.86, H 5.77, N 17.83, Found: C 45.79, H 5.77, N 17.59

Poly(amide-triazole) ***20***. $[\alpha]_D^{25}$ = +102.3 (*c* = 0.7 in TFA-H_2O 1:1); IR (KBr): ν = 3294 (w, N—H), 1659 (s, C=O), 3077 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 1.73 (2H, m, H-2′), 2.58 (2H, s, H-3′), 3.15 (2H, m, H-1′), 3.76 (1H, s, H-4), 4.21 (1H, m, H-5), 4.27 (2H, s, H-2, H-3), 4.65 (1H, m, H-6a), 4.76–4.82 (2H, m, OCH_2O), 5.02 (1H, m, H-6b), 5.11 (2H, m, OCH_2O), 7.78 (1H, m, NH), 7.88 (1H, s, H-5′); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 22.4 (C-3′), 28.9 (C-2′), 37.9 (C-1′), 46.2 (C-6), 67.2 (C-3), 69.8 (C-4), 74.4 (C-5), 77.0 (C-2), 86.4, 91.4 (OCH_2O), 122.8 (C-5′), 146.5 (C-4′), 167.0 (CO). Anal. Calcd for $[C_{13}H_{18}N_4O_5\cdot(H_2O)_{0.5}]$ C 48.90, H 6.00, N 17.55, Found: C 48.31, H 6.04, N 17.35.

2.3.2. Procedure B: thermal polymerization in solution

Monomers (0.050–0.100 g, 0.16–0.35 mmol) were polymerized in DMSO (concentration: 0.4 M) under Ar atmosphere at 160 °C for 2 h (MW). The poly(amide-triazole)s were precipitated with acetone from the reaction mixture.

The signals related to the 1,4-substituted triazole moiety had been labeled as *y* while those of the 1,5-substituted triazole as *z*.

Poly(amide-triazole) ***17***. $[\alpha]_D^{25}$ = +90.4 (*c* = 0.5 TFA-H_2O 1:1); IR (KBr): ν = 3309 (w, N—H), 1666 (s, C=O), 3070 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 3.69 (1H, s, H-4), 4.20–4.22 (0.6H, m, H-5*y*), 4.20–4.43 (4.4H, m, H-2, H-3, H-5*z*, H-1′a, H-1′b), 4.61–4.63 (1H, m, H-6a), 4.78–4.82 (2H, m, $O(CH_2)O$), 5.00–5.11 (3H, m, H-6b, OCH_2O), 7.56 (0.4H, s, H-3′*z*), 7.85 (0.6H, s, H-3′*y*), 8.19 (0.6H, s, NH*y*), 8.51 (0.4H, s, NH*z*); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 31.2 (C-3′*z*), 34.2 (C-3′*y*), 44.6 (C-6*z*), 46.2 (C-6*y*), 67.3 (C-3), 69.9 (C-4), 74.2 (C-5*z*), 74.5 (C-5*y*), 76.9 (C-2), 86.3 (OCH_2Oy), 86.7 (OCH_2Oz), 91.4 (OCH_2O), 123.4 (C-3′*y*), 132.9, 135.5 (C-2′*z*, C-3′*z*), 145.1 (C-2′*y*), 167.2 (CO*y*), 167.7 (CO*z*). Anal. Calcd for $[C_{11}H_{14}N_4O_5\cdot(H_2O)]$: C 44.00, H 5.37, N 18.66, Found: C 44.50, H 5.17, N 18.38

Poly(amide-triazole) ***19***. $[\alpha]_D^{25}$ = +84.6 (*c* = 0.2 in TFA-H_2O 1:1); IR (KBr): ν = 3293 (w, N—H), 1658 (s, C=O), 3070 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 2.78 (1.2H, m, H-2′*y*), 2.87–2.81 (0.8H, m, H-2′*z*), 3.31–3.44 (2H, m, H-1′), 3.77 (0.6H, s, H-4*y*), 3.84 (0.4H, s, H-4*z*), 4.19–4.27 (2.6H, m, H-2, H-3*y*, H-5), 4.37 (0.4H, s, H-3*z*), 4.62–4.64 (1H, m, H-6a), 4.74–5.01 (2H, m, OCH_2O), 5.03–5.10 (3H, m, H-6b, OCH_2O), 7.58 (0.4H, s, H-4′*z*), 7.87–7.91 (1.2H, m, H-4′*y*, NH*y*), 8.02 (0.4H, m, NH*z*); ^{13}C NMR (125.7 MHz, DMSO-d_6, δ): 22.5 (C-2′*z*), 25.4 (C-2′*y*), 37.9 (C-1′), 44.4 (C-6*z*), 46.2 (C-6*y*), 67.2 (C-3), 69.8 (C-4*z*), 67.2 (C-4*y*), 72.2 (C-5), 76.9 (C-2), 86.6, 91.4 (OCH_2O), 123.1 (C-4′*y*), 132.1, 135.9 (C-4′*z*, C-3′*z*), 144.3 (C-3′*y*), 167.1 (CO*y*), 167.4 (CO*z*). Anal. Calcd for $[C_{12}H_{16}N_4O_5\cdot(H_2O)_{0.5}]$ C 47.21, H 5.61, N 18.35, Found: C 47.50, H 5.47, N 18.31.

Poly(amide-triazole) ***21***. $[\alpha]_D^{25}$ = +81.4 (*c* = 0.6 in TFA-H_2O 1:1); IR (KBr): ν = 3409 (w, N—H), 1666 (s, C=O), 3077 cm^{-1} (m, C=C); ^1H NMR (500 MHz, DMSO-d_6, δ): 1.74 (2H, m, H-2′), 2.59 (1.2H, m, H-3′*y*), 2.66 (0.8H, m, H-3′*z*), 3.16 (2H, m, H-1′), 3.77 (0.6H, s, H-4*y*), 3.85 (0.4H, s, H-4*z*), 4.20–4.28 (2.2H, m, H-2*y*, H-3*y*, H-5), 4.34–4.35 (0.8H, m, H-2*z*, H-3*z*), 4.59–4.66 (1H, m, H-6a), 4.75–4.83 (2H, m, OCH_2O), 4.93–5.07 (1H, m, H-6b), 5.10–5.12 (2H, m, OCH_2O), 7.58 (0.4H, s, H-5′*z*), 7.78 (0.6H, m, NH*y*), 7.89 (1H, s, NH*z*, H-5′*y*). ^{13}C NMR (125.7 MHz, $CDCl_3$, δ): 19.7 (C-3′*z*), 22.5 (C-3′*y*), 28.3 (C-2′*z*), 29.0 (C-2′*y*), 37.7 (C-1′*z*) 38.0 (C-1′*y*), 44.6 (C-6*z*), 46.4 (C-6*y*), 67.4 (C-3), 70.0 (C-4*y*), 70.2 (C-4*y*), 74.6 (C-5), 77.1 (C-2), 86.6 ($O(CH_2)Oy$), 86.9 ($O(CH_2)Oz$), 91.6 ($O(CH_2)O$), 123.1 (C-5′*z*), 131.5, 138.3 (C-5′*z*, C-4′*z*), 146.5 (C-4′*y*), 167.2 (CO*y*), 167.4 (CO*z*). Anal. Calcd for $[C_{13}H_{18}N_4O_5\cdot(H_2O)_{0.5}]$ C 48.90, H 6.14, N 17.06, Found: C 48.95, H 5.85, N 16.82.

2.3.3. Procedure C: thermal bulk polymerization

Monomers (0.050 g, 0.17 mmol) were polymerized in the bulk under Ar atmosphere at 160 °C for 2 h (oil bath). The poly(amide-triazole)s were purified by dissolution in DMSO and precipitation with acetone.

2.4. Cu and Cr removal from aqueous solution

The removal of copper or chromium ions was assayed for solutions of $CuSO_4$ (50 ppm) and $K_2Cr_2O_7$ (50 ppm) that were prepared in deionized distilled water. Samples were adjusted to pH 2. Atomic absorption data were recorded on a Shimadzu AA6800, upgrade Autosampler Kit ASC-6100.

Batch. Poly(amide-triazole)s **16** and **17** (20 mg) were stirred with a Cu(II) solution (5 mL) at 45 °C for 12 h. A control experiment was carried out by stirring a suspension of the polymer (20 mg) in water (5 mL).

Column. Poly(amide-triazole) **16** was dissolved in TFA (2.0 mL). Silica-gel (0.20 g) was added to the solution, and then the mixture was concentrated *in vacuo* to give the polymer-supported system which was used to test the removal of metal ions by column chromatography.

Cu(II) or Cr(VI) solutions (3.7 mL) were passed through the dry stationary phase and the metal content of the eluent was determined.

A control experiment was carried out subjecting the silica-gel to the same treatment, without including polymeric material. The amount of metal eluted from the column in the control experiment was subtracted from that eluted from the polymer supported column. The concentration of metal (in ppm) was determined using atomic absorption spectrophotometry, after the convenient dilution of the eluates (aliquots of 3.0 mL were diluted to 25 mL).

Cr(VI) solutions were also measured by UV–visible spectrophotometric determination of diphenylcarbazide-Cr complex,

according to the Standard Method 3500-Cr B, for determination of dissolved Cr(VI) [21].

3. Results and discussion

3.1. Polymer synthesis and characterization

A family of homologous poly(amide-triazole)s was prepared, using α-azido-ω-alkynylamides derived from D-glucono-1,5-lactone (**1**) as monomeric units. These monomers have been synthesized from the gluconic acid derivative **2**, obtained by acetalation of **1** with formaldehyde [18]. The model monomer **7** was prepared following the route illustrated in Scheme 1. The alkynyl function was incorporated by amidation of the carboxylic acid group of **2** with propargylamine, while the free hydroxyl group of **2** was easily converted into azide. The monomers were designed to contain an amide function to act as donor and acceptor of hydrogen bond once incorporated into the polymer chain. Moreover, the 1,4-disubstituted 1,2,3-triazole residues, formed during the polymerization reaction, resemble amide bonds, in terms of planarity and dipole moment. It was also considered that the rigid bicyclic system of the condensed 1,3-dioxane rings in **7** would prevent the cyclization of the monomer during the click polymerization.

Prior to the amide formation by reaction of **2** with propargylamine, the free hydroxyl group of **2** was acetylated to give compound **3**. The acetylation at HO-6 was needed in order to prevent autocondensation of the ω-hydroxyacid **2** during activation of the carboxylic group with a condensing reagent. On the other hand, when the direct tosylation at C-6 of compound **2** was attempted, oligomerization was observed by intermolecular esterification. The alkynyl amide **4** was then prepared by condensation of the carboxylic acid **3** with propargylamine promoted by EDCI and HOBt [22]. Removal of the acetyl protecting group by treatment of **4** with sodium methoxide led to the ω-hydroxy amide **5**, which was converted into the sulfonyl derivative **6** by tosylation. Nucleophilic substitution of the tosyl group by azide yielded monomer **7** (overall yield 30% from **1**). The structure of **7** was confirmed by NMR methods assisted by 2D NMR techniques. Thus the ^{1}H NMR spectrum of **7** showed diagnostic signals for the alkyne and NH protons (3.02 and 8.13 ppm) and the doublets of the four acetal protons of two methylidene groups. The signals of the diastereotopic methylene protons vicinal to NH and N_3 were clearly identified by 2D spectra.

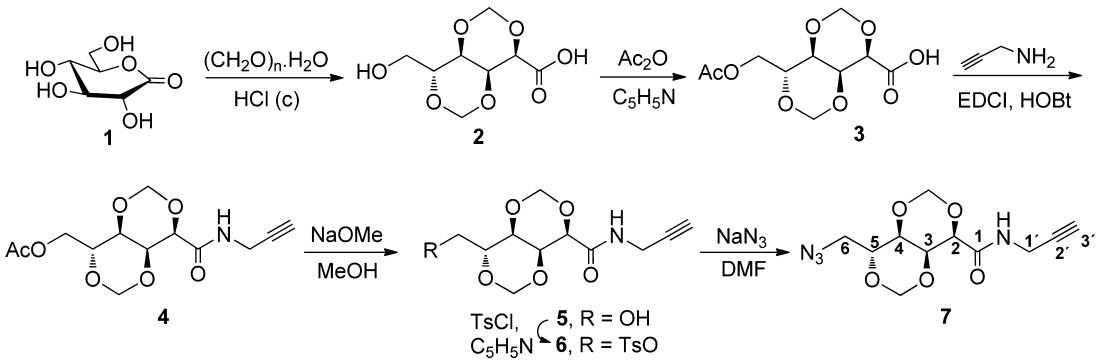
Other two monomeric α-azido-ω-alkynes **14** and **15** have been prepared, following the same synthetic route and starting from the common precursor **3** (Scheme 2). The monomers **7**, **14** and **15** differ in the length of the alkynyl chain linked to the N atom of the amide function. As the 4-pentynylamine, that was required for the synthesis of **15**, was not commercially available, it was prepared by a slight modification of the Gabriel synthesis already reported [19,20].

In order to determine the optimal conditions for the click polymerizations, the reaction of the model α-azido-ω-alkynylamide **7** was conducted using different copper salts (Cu(I), Cu(II)/ascorbate), solvents (DMSO, DMF, bulk), and heating conditions (conventional heating, MW) as described in Table 1. Isolated yields are reported, after purification of the polymer and the washings required for elimination of copper ions. Therefore, although the recovery of polymeric material is almost quantitative, the yield decreased by removal of the oligomers formed in the less efficient polymerizations. This was verified by NMR analysis of the residue obtained after evaporation of the washing liquids. Since the monomer was soluble only in DMSO or DMF, the polymerization solvent was limited to these two. According to the regioselectivity of the polymerization, the click polymers may be represented by structures **16** or **17** (Scheme 3). For the highly regioselective CuAAC reactions, the formation of 1,5-triazole rings are negligible, and the polymer is well represented by structure **16**. In the absence of copper salts the less regioselective cycloaddition leads to 1,4- and 1,5-disubstituted triazoles, which are randomly distributed along the polymeric chain, as in **17**. The molecular weight (M_n) of the polymers (end group analysis) and the regioselectivity of the click reactions were estimated from the NMR spectra, as explained later.

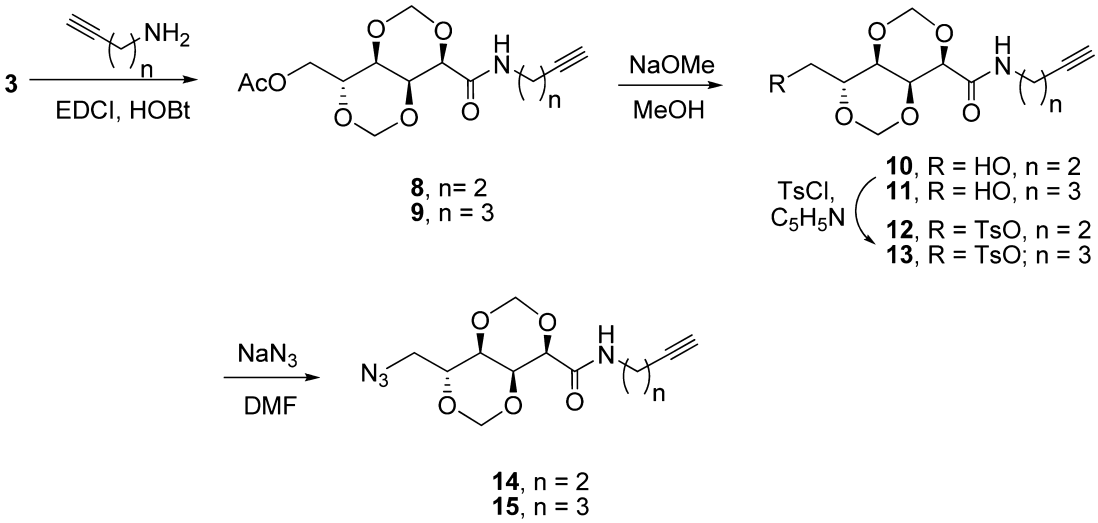
In first instance, the polymerizations were carried out at 100 °C in DMF with Cu(II)/ascorbic acid as catalyst. The product obtained by conventional heating showed low molecular weight and it was recovered in moderate yield, although the reaction was highly regioselective (entry 1). Under the same conditions, microwave heating for 30 min improved the yield and the molecular weight of the polymer, while the regioselectivity was slightly diminished (entry 2). A lowering in the amount of $Cu(OAc)_2$ and ascorbic acid (5 and 10%, respectively), under MW heating for 30 min (entry 3) led to lower molecular weight and yield, which was attributed to an incomplete polymerization. Therefore, the reaction was led to proceed for 2 h (entry 4) with the expected increment in yield and polymerization degree. When DMSO was used as solvent (entry 6) with conventional heating, both the yield and molecular weight increased, probably due to a better solubility of the polymer in this solvent. Similar results were obtained using MW irradiation in DMSO (30 min), although the regioselectivity was smaller (entry 7). However, the use of a double amount of catalyst (*i.e.* 0.10 molar equivalents of Cu(II) and ascorbic acid, entry 8) led to a material that exhibited improved properties in terms of molecular weight, yield, and 1,4-:1,5-disubstituted triazole ratio. A further increment in the concentration of the catalyst had a negative effect in the polymerization process (entry 9). The replacement of $Cu(OAc)_2$/ascorbic acid by CuOAc on the click polymerization was also evaluated in both solvents (entries 5 and 10). The yields were quite similar, but the molecular weight and selectivities were higher in the Cu(I)-catalyzed reaction in DMSO. Under these conditions, the results were comparable to those of entry 8, which required higher amount of Cu(II)/ascorbic acid. As the purification of the polymer was easier when the concentration of copper was smaller and the ascorbic acid was absent, we considered that entry 10 represented the optimized reaction conditions for the copper-assisted click polymerization.

Finally, metal free thermal polymerizations were conducted. The reactions were performed using DMSO as solvent or in the bulk (entries 11 and 12, respectively). The thermal polycondensation in solution required higher temperature and longer reaction time (entry 11). The bulk polymerization was conducted with conventional heating (entry 12), because MW reactions at such a high temperature (180 °C) led to partial decomposition of the monomer. The reaction temperature was selected on the basis of the DSC thermogram observed for monomer **7**, which showed an intense exothermic event centered at 172 °C, attributed to the 1,3-dipolar cycloaddition. In comparison to the CuAAC polymerizations, the thermal ones led to lower yields and the polymers exhibited lower molecular weights. The regioselectivity was also poorer for the thermally obtained poly(amide-triazole). This result is in agreement with the decisive role played by Cu(I) in the control of the regioselectivity, according with the mechanism of the cycloaddition reaction [2a].

The degree of polymerization was preliminarily established by FTIR spectroscopy. The azide group in monomer **7** gave a strong absorption at 2098 cm^{-1} (Fig. 1a). This peak also appears in the FTIR spectra of oligomeric products (Fig. 1b), due to the residual azide terminal groups. The intensity of the peak gradually decreases according to the degree of the polymerization and is absent in the spectra of the polymers of high M_n (Fig. 1c), in agreement with the fact that this material show no terminal end by NMR, as explained later.



Scheme 1. Synthesis of monomer **7**.



Scheme 2. Synthesis of monomers **14** and **15**.

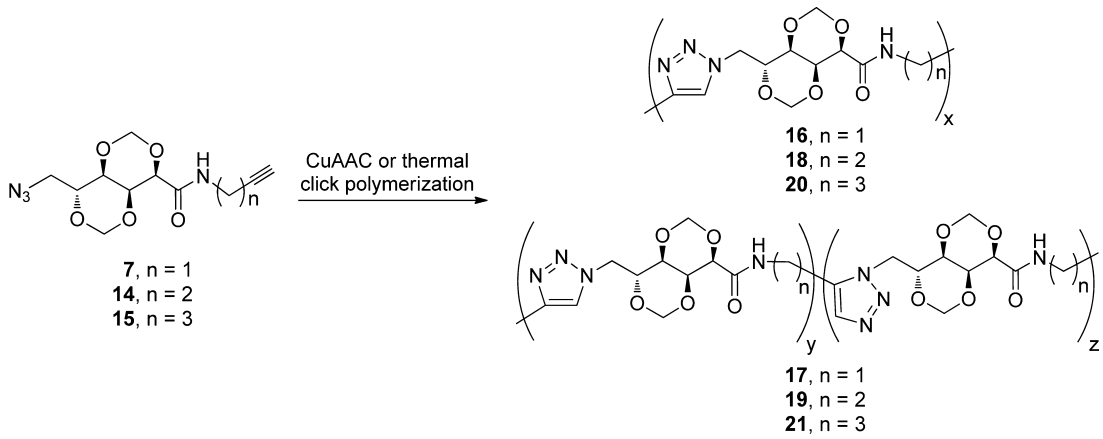
Similar to other azide-alkyne click polymers [13], the poly(amide-triazole)s **16** and **17** were highly insoluble in water and common organic solvents. The insolubility and thermal properties observed for **16** suggest a rather compact packing of the chains for this material. To provide some degree of flexibility to the polymer backbone, the precursor monomers **14** and **15**, which possess a longer alkynyl chain compared with that of **7**, have been synthesized following a route similar to the one that led to **7** (Scheme 2).

Monomers **14** and **15** were subjected to catalytic and thermal polymerizations under the optimized conditions described for compound **7** (Table 1, entry 10). A lower temperature was used for the bulk polymerization since DSC exhibited an endothermal process at 162 °C for both α-azido-ω-alkynylamides **14** and **15**. When Cu(I) salts were added (entries 1 and 4, Table 2), high molecular weights and excellent regioselectivities were observed since no terminal groups or 1,5-disubstituted triazole residues were detected in the NMR spectra. In contrast, thermal polymerizations

Table 1
Click polymerization of monomer **7**.

Entry	Solvent	Cu (mol equiv)	Ascorbic Acid (mol equiv)	Temp (°C)	Time (h)	Yield (%)	M_n	1,4: 1,5 Ratio
1	DMF	0.50[a]	1	100	18	68	5920	100: 0
2	DMF	0.50[a]	1	100[b]	0.5	90	9030	93: 7
3	DMF	0.05[a]	0.10	100[b]	0.5	84	3670	89: 11
4	DMF	0.05[a]	0.10	100[b]	2	92	8180	86: 14
5	DMF	0.05[c]	–	100[b]	0.5	89	6210	96: 4
6	DMSO	0.05[a]	0.10	100	18	97	>17,000[d]	92: 8
7	DMSO	0.05[a]	0.10	100[b]	0.5	92	14,400	80: 20
8	DMSO	0.10[a]	0.20	100[b]	0.5	87	>17,000[d]	98: 2
9	DMSO	0.25[a]	0.50	100[b]	0.5	71	12,140	86: 14
10	DMSO	0.05[c]	–	100[b]	0.5	91	>17,000[d]	95: 5
11	DMSO	–	–	160[b]	2	87	6210	60: 40
12	–[e]	–	–	185	2	78	6770	67: 33

[a] $Cu(OAc)_2$.
[b] Microwave.
[c] CuOAc.
[d] Not determined due to the absence of terminal groups.
[e] Bulk.



Scheme 3. Click polymerization of monomers **7**, **14** and **15**.

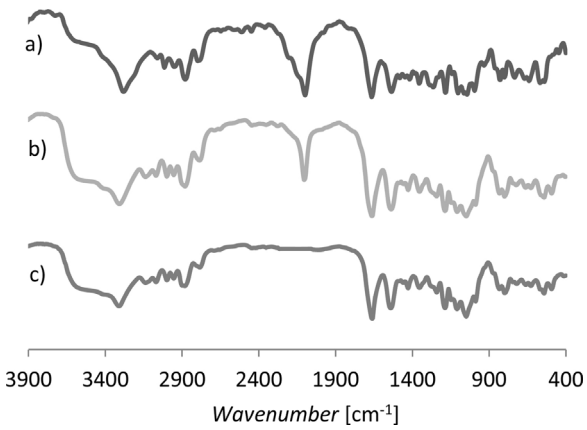


Fig. 1. FTIR spectra of (a) monomer **7**; (b) oligomeric poly(amide-triazole) **16** obtained according to Table 1, entry 3, and (c) poly(amide-triazole) **16** obtained according to Table 1, entry 6.

led to lower molecular weights and poorer selectivities (1,4-:1,5-disubstituted triazole ratio was approximately 1,2:1.0).

The purification of polymers resulting from the CuAAc polymerization is usually difficult, as residual copper can persist even after extensive washing with EDTA solutions [23]. However, as the poly(amide-triazole)s obtained by CuAAC polyaddition were highly insoluble in water, they were washed with an aqueous ammonium hydroxide solution, which produced an efficient removal of the metal ions, as revealed by elemental analysis and by the copper released (<0.10 ppm), determined by atomic absorption, after prolonged extraction of the polymer with water. Additionally, It has been reported that residual amounts of copper induced widening of the proton of the triazole ring and low resolution of the other signals [16]. In contrast, in our case, the ^{1}H NMR spectra of purified CuAAC and thermal click polymers exhibited sharp signals, with a similar width and resolution to those of monomer **7** (Fig. 2). To record the NMR spectra, the highly insoluble polymer samples were treated with trifluoroacetic acid in dichloromethane and, after concentration, were dissolved in DMSO-d_6. It was observed that, after polymerization, all the signals of the monomer backbone were shifted downfield and that a new signal due to the 1,4-triazole proton appeared at 7.84 ppm. The resonances of the methylene protons vicinal to the terminal azide or alkynyl groups for polymers of low molecular weight showed chemical shifts similar to those of the monomer, in the region of 3.4 (H-6a), 4.1 (H-6b) and 3.8 ppm (H-1′a). In order to determine the molecular weight (M_n) by end-group analysis, the integrals of these signals were compared with that one of H-6a (4.6 ppm) from the repeating unit (see Supplementary data). For the M_n determination, the total consumption of the monomer was verified by TLC for all the polymerizations conducted. Moreover, oligomers were removed by washing of the polymer with DMSO. Figure 2c shows the spectrum of polymer **17** obtained from thermal, copper-free polymerization. The signals of the 1,5-disubstituted triazole at 8.51 (amide-NH) and 7.56 (CH) were employed for the quantification of 1,4-:1,5-disubstituted triazole ratio, which showed to be 2:1.

Polymers **16** and **17** were also characterized according to their ^{13}C NMR spectra (Fig. 3). In the spectrum of poly(amide-triazole) **16** (Fig. 3b), the formation of the 1,4-disubstituted ring was evidenced by the resonances at 123.4 and 145.1 ppm that replaced those at 72.5 and 81.2 ppm due to the alkyne carbon of the monomer (Fig. 3a). Most of the signals appeared duplicated in the spectrum of polymer **17**, due to the regioisomerism of the triazole. Thus, in addition to the resonances of 1,4-triazole, the ones of 1,5-disubstituted ring were detected at 132.9 and 135.5 ppm (Fig. 3c).

A similar spectral analysis was performed for poly(amide-triazole)s **18**, **19** and **20**, **21** (see Supplementary data). The determination of the ratio 1,4- and 1,5-disubstituted triazole and the evaluation of the M_n by end group analysis was performed using selected signals of the NMR spectra, as described for **16** and **17**.

Table 2
Click polymerization of monomers **14** and **15**.

Entry	Polytriazole	Solvent	Cu (molar equiv)	Temperature (°C)	Time (h)	Yield (%)	M_n	1,4: 1,5 Ratio
1	**18**	DMSO	0.05[a]	100 (Mw)[b]	0.5	93	>17,000	100: 0
2	**19**	–[c]	–	160	2	97	2370	55: 45
3	**19**	DMSO	–	160 (Mw)[b]	2	76	2960	58: 42
4	**20**	DMSO	0.05[a]	100 (Mw)	2	79	>17,000	100: 0
5	**21**	–[c]	–	160	2	98	4680	58: 42
6	**21**	DMSO	–	160 (Mw)[b]	2	64	2800	57: 43

[a]Cu(OAc).
[b]Microwave.
[c]Bulk.

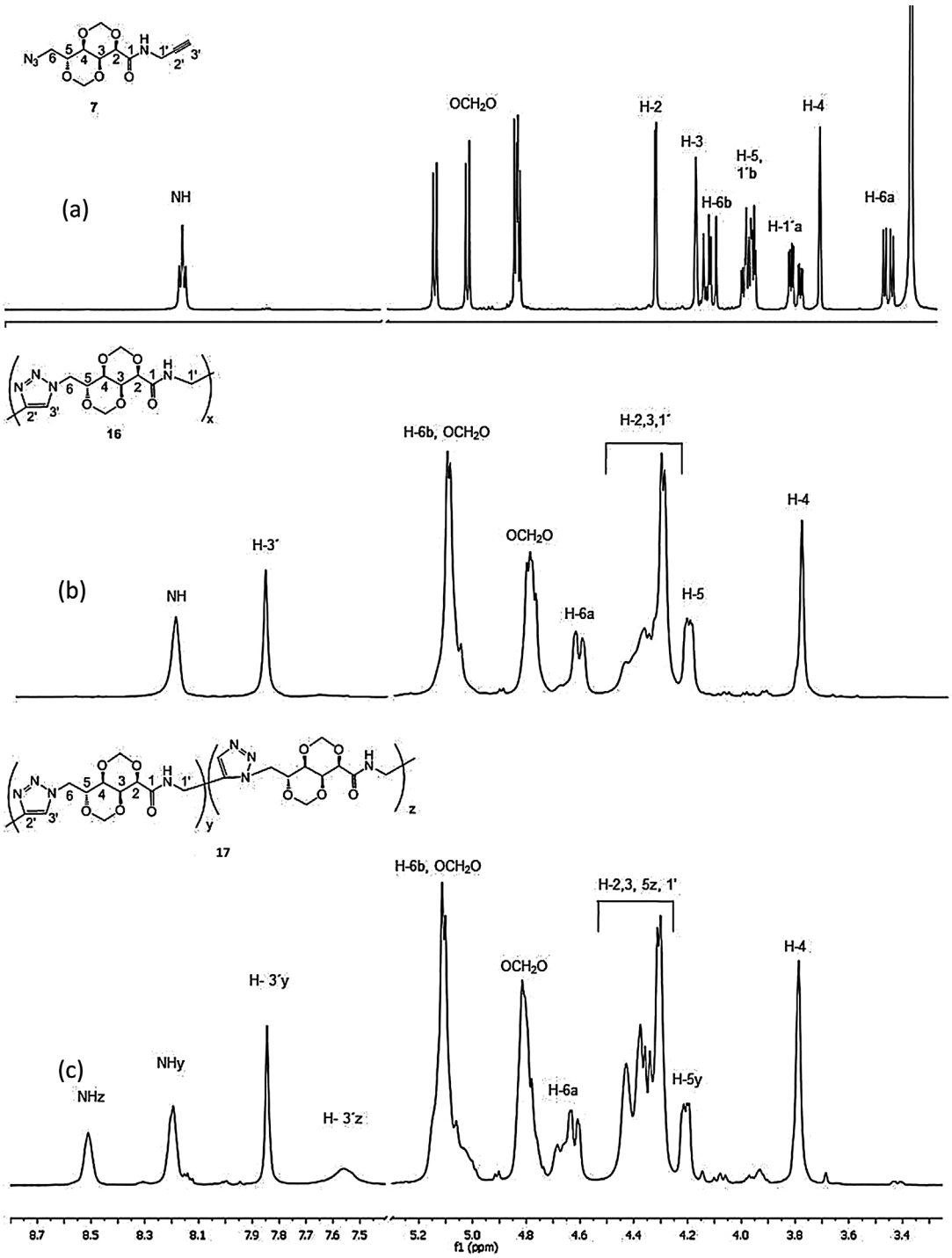


Fig. 2. ^{1}H NMR spectra of: (a) monomer **7**, (b) poly(amide-triazole) **16** obtained by CuAAC polymerization and (c) poly(amide-triazole) **17** obtained by thermal metal-free polymerization.

Additionally, the determination of the molecular weight of the poly(amide-triazole)s by means of UV–MALDI–TOF mass spectrometry (MS) was also attempted. Two matrices were assayed: sinapinic and gentisic acids. Poly(amide-triazole) **16** of low molecular weight (Table 1, entry 5) gave an unimodal distribution of $[M+H]^+$ ions with both matrices (Fig. 4). The spectrum recorded with sinapinic acid showed a pattern of signals in agreement with the expected weights of the intact polymer chains and with the spacing between two consecutive peaks coincident with the mass of the repeating units. The peak of maximum intensity was achieved at *m*/*z* 3386, corresponding to a degree of polymerization of 12 (Fig. 4a). In the spectrum obtained with gentisic acid (Fig. 4b), in addition to the expected $[M+H]^+$ ions of the polymer, a lower intensity distribution was observed and assigned to the adduct of the polymer with the matrix $[M+C_7H_6O_4+H]^+$. Taking into account the *m*/*z* values and the relative intensities of each peak, the low molecular weight polymer **16** showed the following values using sinapinic or gentisic acids as matrix, respectively: M_n = 3337 or 2260, M_w = 3587 and 2859, polydispersity = 1.1 in both cases. The results suggest that gentisic acid is somewhat

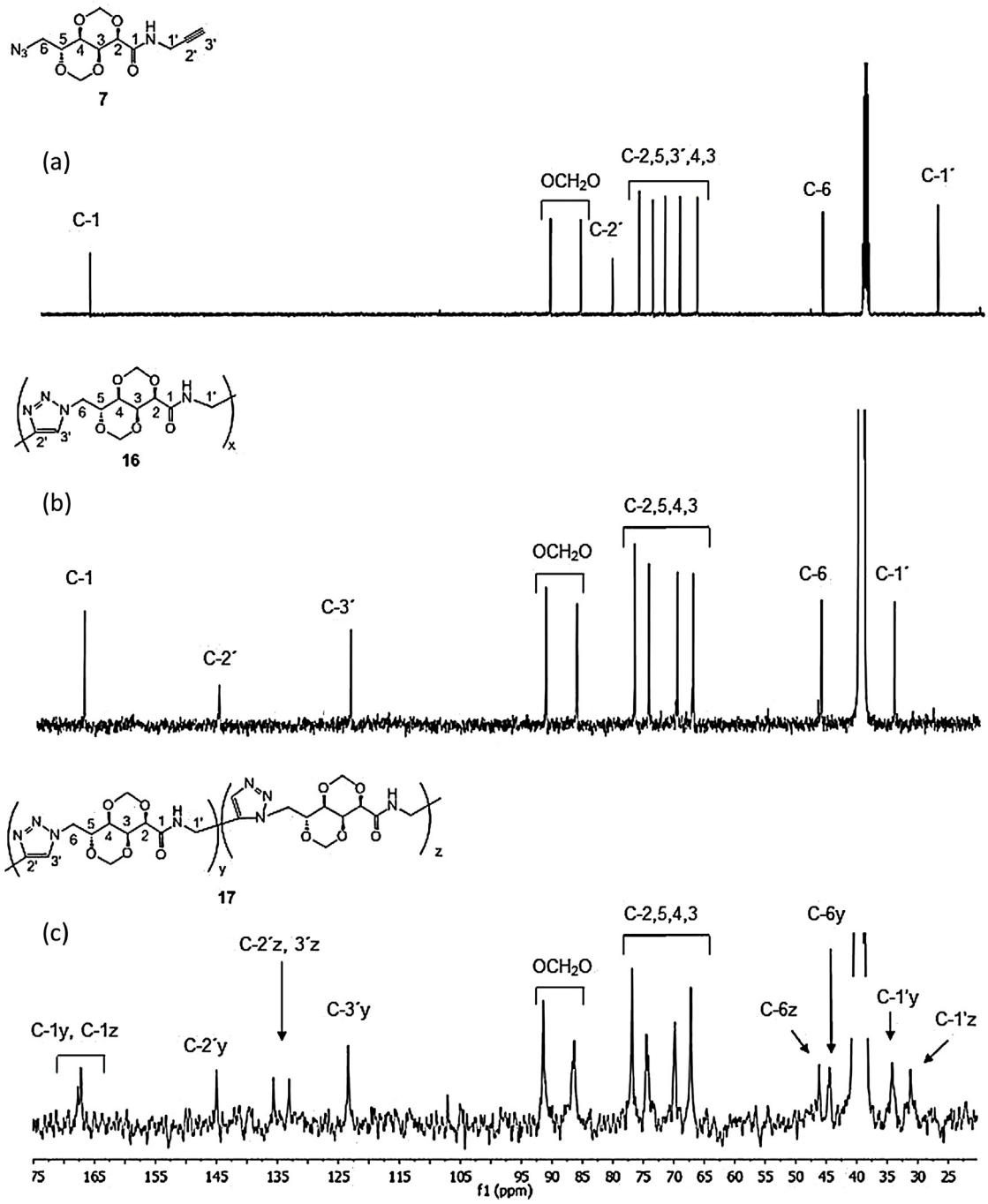


Fig. 3. ^{13}C NMR spectra of: (a) monomer **7**, (b) poly(amide-triazole) **16** obtained by CuAAC polymerization and (c) poly(amide-triazole) **17** obtained by thermal metal-free polymerization.

less efficient for desorption/ionization of the polymers. Furthermore, the M_n values determined by mass spectrometry, using both matrices, are also smaller than those measured using the end-group analysis by NMR, indicating again that the desorption/ionization process is rather difficult for the larger chains of this polymer. The same effect was also observed for polymers having large molecular weights. Thus the material with $M_n > 17{,}000$, according to NMR (Table 1, entry 10), showed no signals in the MALDI spectra recorded with sinapinic acid, even though higher laser intensities were applied. However, peaks were detected in the MALDI spectrum when gentisic acid was employed as matrix (Fig. 4c). The pattern of the spectrum was similar to that of the sample of lower molecular weight, but in this case ions were observed up to m/z 11,849 (Fig. 4c inset). The MALDI MS of poly(amide-triazole)s **18** and **20** were similar and showed the highest m/z values at 13,326 (polymer **18**) and 13,027 (polymer **20**). The mentioned difficulties for desorption/ionization processes of these materials, under the conditions studied, prevent the accurate determination of their molecular weights. In fact, due to the high insolubility of the polymers, we were not able to determine their M_w by GPC. The poly(amide-triazole)s were also insoluble at 70 °C in solvents such as DMF-0.5% LiBr [24] or DMF-TMEDA-0.05% LiBr [25], that have been successfully employed for other relatively insoluble polytriazoles.

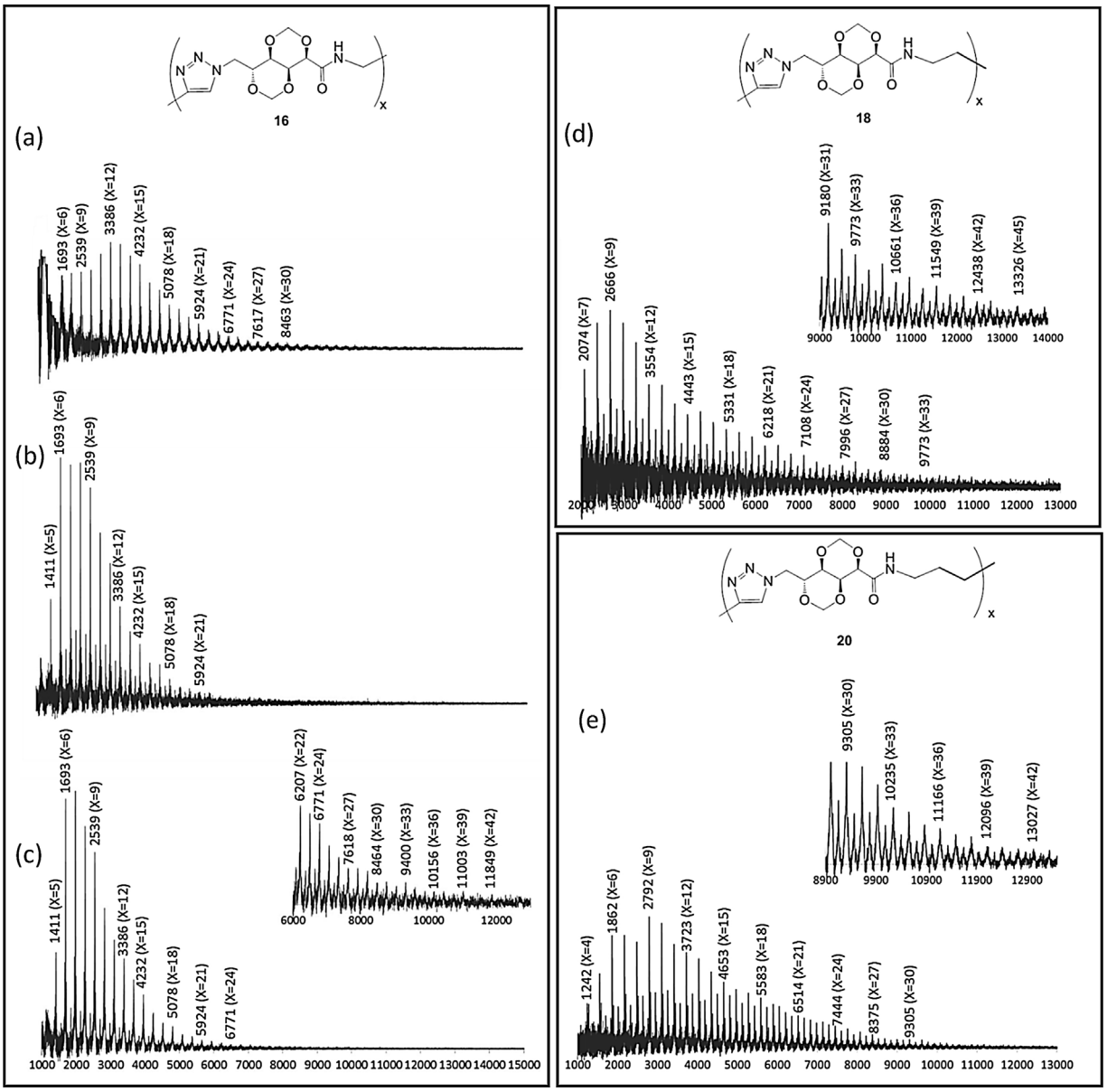


Fig. 4. UV-MALDI-TOF spectra of poly(amide-triazole)s: (a) **16** according to the conditions of Table 1, entry 5, using sinapinic acid as matrix; (b) **16** according to the conditions of Table 1, entry 5, using gentisic acid as matrix; (c) **16** according to the conditions of Table 1, entry 10 (gentisic acid); (d) **18** (gentisic acid); (e) **20** (gentisic acid).

Therefore, we were able to set a lower limit for the M_n values, according to the minimum area of signals detected by NMR.

3.2. Thermal analysis

The thermal stability of α-amido-ω-alkynylamides **7**, **11**, and **15**, as well as those of the poly(amide-triazole)s, was evaluated by TGA under N_2 atmosphere, in the range of 40–700 °C (Fig. 5 and Table 3). The TG curves showed that decomposition took place in two stages. All the poly(amide-triazole)s were stable below 300 °C.

The monomers and polymers were also studied by DSC. The DSC traces for monomers **7**, **14** and **15** exhibited an exothermal process attributed to the click polymerization. The temperature of such a process was centered at 171.7 °C, 162.0 and 161.9 for **7**, **14** and **15**, respectively.

Except for polymer **16**, all the poly(amide-triazole)s exhibited unexpectedly high glass transitions (T_g) at well-defined temperatures (Table 4 and Fig. 6). The same T_g values were measured

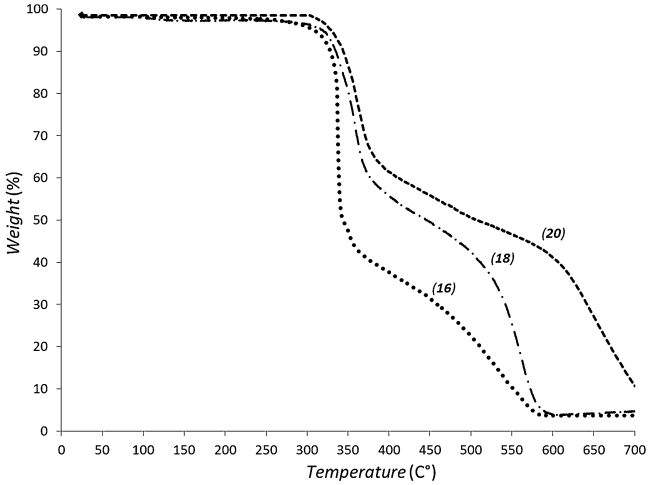


Fig. 5. TG Analyses of poly(amide-triazole)s.

Table 3
Thermal analysis of poly(amide-triazole)s **16**–**21**.

Polymer	Procedure	$T_{10\%}$[a] (°C)	T_{de}[b] (°C)	T_g
16	A	345	337/500	–
17	B/C	347	362/600	243
18	A	339	355/548	222
19	B/C	349	351/564	212
20	A	344	362/662	188
21	B/C	348	337/498	189

[a]Temperature at which a 10% weigth loss is recorded by TGA.
[b]Temperatures of maximum degradation for each degradation stage, determined by TGA.

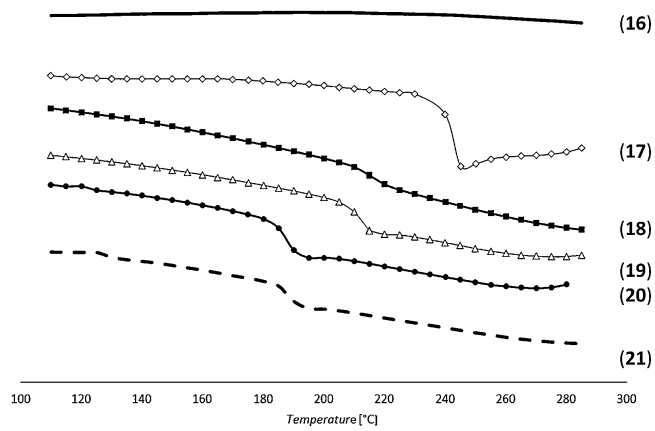


Fig. 6. DSC traces (second heating cycle) of poly(amide-triazole)s **16**–**21**.

for the first and subsequent heating and cooling cycles. As general trend, for polymers having longer alkynyl chains, lower T_g values were measured. This result suggests that T_g values decreased for more flexible polymer chains. For the same repeating unit, higher T_g values were recorded for polymers having mostly 1,4-triazol units, in agreement with a higher stereoregularity, and also associated to larger molecular weights. In the case of polymer **16**, with the less flexible backbone, the T_g was not observed, suggesting that decomposition might take place before the T_g is reached. The high T_g values observed, together with the high insolubility of the poly(amide-triazole)s, suggest that they were arranged as rather compact networks that involve the rigid structures of the two fused 1,3-dioxane rings constituent of the repeating unit, stabilized by intra and intermolecular association of the chains by hydrogen bonding and dipolar interactions of the 1,2,3-triazole and amide groups. In fact, the scanning electron microscopy (SEM) images of poly(amide-triazole)s (Fig. 7) shows a regular morphology with amorphous and extended microstructures formed by aggregation of small particles.

3.3. *Metal removal from aqueous solution by poly(amide-triazole)* ***16***

Taking into account the high nitrogen content and poor solubility in water of the poly(amide-triazole)s, they were thought as potential materials for metal capture in effluents. Therefore, polymer **16** was preliminary tested regarding to the capacity to remove Cu(II) or Cr(VI), as respective cationic or anionic species.

Treatment in batch of the metal solutions with the polymer was unsuccessful, as no metal retention was detected, probably due to the high hydrophobicity of the polymer, which remains in water as compact particles. As an alternative approach, poly(amide-triazole) **16** was dissolved in acid solution and mixed with silica-gel, afterwards the system was concentrated *in vacuo* to give a polymer-supported material. This was used as a stationary phase for column chromatography and the ionic solutions of Cu(II) and Cr(VI) were eluted. In order to test the affinity of silica-gel for the ions, control experiments were carried out subjecting the adsorbent to the same acid treatment, but in the absence of poly(amide-triazole) **16**. To evaluate the efficiency of the polymer for metal removal, different polymer: metal ratios were evaluated, as indicated in Table 4.

The stationary phase was prepared starting from a constant mass of polymer, and the initial concentration of Cu(II) was gradually decreased, in order to achieve higher polymer: Cu(II) ratios (entries 1–3). As expected, for a relative higher triazole content, a higher overall retention was observed, although the retention depends on the initial Cu(II) concentration. For example, in the case of an initial copper concentration of 350 ppm (entry 1), the retention is 50 mg Cu(II)/g polymer. The amount of Cu(II) eluted from the column was determined by atomic absorption spectrophotometry and corrected by subtraction of the amount of metal released in the control experiment, determined by the same method. Similar results have been reported for the in batch removal of Cu(II) from waste water, by a polythioester and a polythioether with 1,2,4-triazole units included in the main chain [26]. The polythioester proved to be more efficient, since it retained 44% of copper ions after 4 h, while the polythioether removed 16% from an initial solution of 20 ppm of Cu(II). Longer times of contact of the polymer with the Cu(II) solutions showed higher retention values.

The removal of Cr(VI) was also preliminarily studied, using the same procedure applied to the removal of Cu(II). The metal contents of the initial, eluted and control solutions were measured by atomic absorption and by colorimetric determination of the diphenylcarbazide-Cr(VI) complex [21] (entries 4–6), giving similar results. When 40: 1 triazole: metal ratio was used, 42% of the ions were retained (entry 6). In an additional experiment, the stationary phase was regenerated with 0.1 M HCl, washed with water and another portion of the chromium solution was passed through (entry 7). Similar retention values were obtained, which indicated that the stationary phase could be recycled.

Table 4
Metal retention by poly(amide-triazole) **16**.

Entry	Metal	Triazole: metal ratio[a]	Metal concentration (ppm)		Metal retention[d]
			Initial	Recovered	(%)
1	Cu[b]	1: 1	350.0	275.4	21
2	Cu[b]	20: 1	53.3	40.0	25
3	Cu[b]	40: 1	32.0	22.0	32
4	Cr[b]	20: 1	61.0	36.8	40
5	Cr[c]	20: 1	50.0	27.9	44
6	Cr[c]	40: 1	25.0	14.6	42
7	Cr[c]	20: 1	50.0	34.2	31

[a]The content of triazole moieties was calculated according to the ratio: mass of polymer/molecular weight of the repeating unit.
[b]Determined by atomic absorption spectrophotometry.
[c]Determined by colorimetry.
[d]The values reported were corrected with respect to the control as explained in the Experimental section.

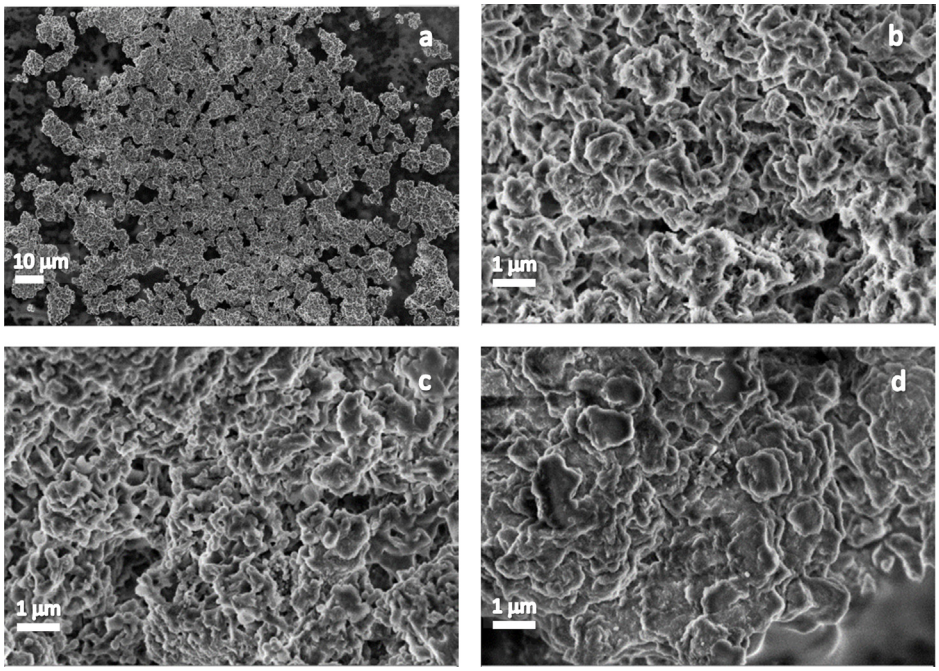


Fig. 7. SEM images of poly(amide-triazole)s: (a) and (b) **16**; (c) **18**; and (d) **20**.

4. Conclusions

Commercially available and inexpensive D-glucono-1,5-lactone, the product of oxidation of glucose, has been employed as a renewable resource in the synthesis of new biosourced poly(amide-triazole)s. The α-azido-ω-alkyne monomers precursors of these polymers have been readily synthesized *via* a straightforward route from 2,4;3,5-di-*O*-methylidene-D-gluconic acid, obtained in one step by formilydenation of gluconolactone. The amide function that appears in the repeating unit of the polymers, comes from the *N*-alkynyl gluconamides monomeric precursors, which also contained an azide group located at the opposite end of the sugar unit. The azide-alkyne AB-type monomers proved to be suitable for the step-growth click polymerization. The click reaction was catalyzed by Cu(I) or, alternatively, was conducted thermally, in the absence of metal catalyst.

The Cu(I)-catalyzed click polymerizations showed to be highly regioselective in favor of 1,4-disubstituted triazole linkages and, under microwave irradiation, polymers of large molecular weights were obtained in short reaction times. In contrast, the metal free click polymerization required longer reaction times or much higher temperatures, and led to the formation of 1,4- and 1,5-disubstituted triazoles, randomly distributed along the polymer chain. The molecular weights were smaller than those of the CuAAC polymers. The poly(amide-triazole)s showed to be amorphous materials, thermally stable up to 350 °C and with a two-step thermal decomposition process, with T_d approximately at 350 and 560 °C. They exhibited unexpectedly high T_g values, which decreased progressively with the increment in the length of the methylene groups within the repeating unit, in agreement with an increased flexibility of the polymer chain. The stereoregular polymers, which possess also higher molecular weights, showed higher T_g values than those produced by the less regioselective thermal polymerization.

The poly(amide-triazole)s were highly insoluble in water and most of the organic solvents (except for TFA-DMSO). They were also stable to acid (0.5 mM TFA, 40 °C, 7 days) or alkaline (0.5 mM NaOH, 40 °C, 7 days) conditions. All these results suggest that the polymer chains are closely packed together to form a complex network stabilized by hydrogen bonding and dipolar interactions. Furthermore, the polymers were assayed as metal-trapping agents, due to their high content in N. The preliminary studies showed an acceptable retention of metals, as Cu(II) and Cr(VI).

Acknowledgments

Support of this work by the University of Buenos Aires (Project 01/W526), the National Research Council of Argentina (CONICET, Project PIP 2012 11220110100370) and the National Agency for Promotion of Science and Technology (ANPCyT, PICT 2012-0717) is gratefully acknowledged. D.M. Fidalgo is a fellow from CONICET. O. Varela and A.A. Kolender are Research Members from CONICET.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mtcomm.2014.12.001.

References

[1] H.C. Kolb, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. 40 (2001) 2004–2021.
[2] (a) V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem. Int. Ed. 41 (2002) 2596–2599;
(b) C.W. Tornøe, C. Christensen, M. Meldal, J. Org. Chem. 67 (2002) 3057–3064.
[3] D. Zeng, B. Zeglis, J.S. Lewis, C.J. Anderson, J. Nucl. Med. 54 (2013) 829–832.
[4] H. Durmaz, A. Sanyal, G. Hizal, U. Tunca, Polym. Chem. 3 (2012) 825–835.
[5] (a) E. Lallana, A. Sousa-Herves, F. Fernández-Trillo, R. Riguera, E. Fernández-Megia, Pharm. Res. 29 (2012) 1–34;
(b) H. He, C. Gao, Curr. Org. Chem. 15 (2011) 3667–3691;
(c) S. Campidelli, Curr. Org. Chem. 15 (2011) 1151–1159.
[6] H. Yu, X. Wang, Curr. Org. Chem. 17 (2013) 594–609.
[7] (a) U. Tunca, J. Polym. Sci. A: Polym. Chem. 52 (2014) 3147–3165;
(b) W.H. Binder, R. Sachsenhofer, Macromol. Rapid Commun. 29 (2008) 952–981;
(c) W.H. Binder, R. Sachsenhofer, Macromol. Rapid Commun. 28 (2007) 15–54;
(d) W.H. Binder, C. Kluger, Curr. Org. Chem. 10 (2006) 1791–1815.
[8] N. El Brahmi, S. El Kazzouli, S. Mignani, M. Bousmina, J.P. Majoral, Tetrahedron 69 (2013) 3103–3133.
[9] D. Pasini, Molecules 18 (2013) 9512–9530.
[10] A. Qin, J.W.Y. Lam, B.Z. Tang, Chem. Soc. Rev. 39 (2010) 2522–2544.
[11] V. Aragão-Leonetti, V.L. Campo, A.S. Gomes, R.A. Field, I. Carvalho, Tetrahedron 66 (2010) 9475–9492.

[12] C. Besset, S. Binauld, M. Ibert, P. Fuertes, J.-P. Pascault, E. Fleury, J. Bernard, E. Drockenmuller, Macromolecules 43 (2010) 17–19.
[13] P.D. Raytchev, C. Besset, E. Fleury, J.-P. Pascault, J. Bernard, E. Drockenmuller, Pure Appl. Chem. 85 (2013) 511–520.
[14] C. Besset, J. Bernard, E. Fleury, J.-P. Pascault, P. Cassagnau, E. Drockenmuller, R. Williams, Macromolecules 43 (2010) 5672–5678.
[15] M. Bueno, I. Molina, J. Galbis, Polym. Degrad. Stabil. 97 (2012) 1662–1670.
[16] I. Molina-Pinilla, M. Bueno-Martínez, K. Hakkou, J.A. Galbis, J. Polym. Sci. A: Polym. Chem. 52 (2014) 629–638.
[17] (a) H.C. Kolb, K.B. Sharpless, Drug Discov. Today 8 (2003) 1128–1137;
(b) N.G. Angelo, P.S. Arora, J. Am. Chem. Soc. 127 (2005) 17134–17135.
[18] R. Marín, S. Muñoz Guerra, J. Appl. Polym. Sci. 114 (2009) 3723–3736.
[19] P.L. Salzberg, J.V. Supniewski, Org. Synth. Coll. 1 (1941) 119, 7 (1927) 8.
[20] J.O. Osby, M.G. Martin, B. Ganem, Tetrahedron Lett. 25 (1984) 2093–2096.
[21] APHA Standard Methods for the Examination of Water and Wastewater, 21st ed., 2005, Method 3500-Cr B.
[22] W. König, R. Geiger, Chem. Ber. 103 (1970) 788–798.
[23] M. Fuchs, W. Goessler, C. Pilger, C.O. Kappe, Adv. Synth. Catal. 352 (2010) 323–328.
[24] S. Hartwig, S. Hecht, Macromolecules 43 (2010) 242–248.
[25] Y. Nagao, A. Takasu, J. Polym. Sci. A: Polym. Chem. 48 (2010) 4207–4218.
[26] A.H.R. Al-Daraji, I.H.R. Tomi, Polym. Plast. Technol. Eng. 51 (2012) 1327–1333.