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Synthesis, Secondary Structure and Anion Binding of Acyclic Carbohydrate-derived Oligo(amide-triazole)s

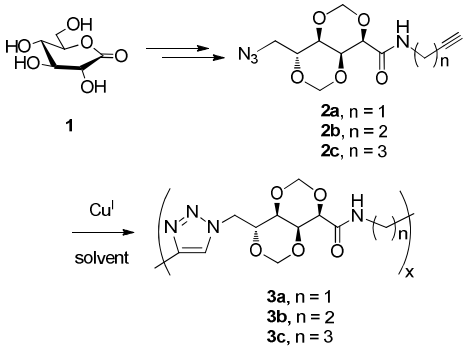
Daniela M. Fidalgo,[a,b] María Eugenia Monge,[c] Oscar Varela,*[a,b] and Adriana A. Kolender*[a,b]

Abstract: A family of linear, carbohydrate-derived oligo(amide-triazole)s has been designed and synthesized. These molecules possess a regular distribution of triazole rings (from one to four) linking the carbohydrate units to give dimer to pentamer derivatives. Their binding to halide anions was qualitatively analyzed by means of NMR spectroscopy and mass spectrometry. All the compounds were able to bind chloride anions, with a stoichiometry that depended on the chain length. The dimer and trimer gave 2:1 host:chloride ratio, while the tetramer and pentamer gave 1:1 complexes. The secondary structure of the oligo(amide-triazole)s was studied using NMR spectroscopy and circular dichroism. These studies showed that the larger host molecules (tetramer and pentamer) adopted a stabilized U-turn and were able to bind just one chloride anion. Only the pentamer displayed a helical conformation, which was slightly distorted in the presence of chloride salts. Interestingly, chloride binding involves not only the triazole-CH but also H atoms from the carbohydrate moieties. These compounds could be applied for chloride sensing by ESI-MS.

Introduction

The copper(I) assisted azide-alkyne cycloaddition (CuAAC), developed by Sharpless and Meldal,[1,2] is a prototype of a click reaction.[3] This reaction leads to the formation of 1,4-disubstituted 1,2,3-triazole rings; while in the ruthenium(II)-catalyzed version of the cycloaddition, 1,5-disubstituted are formed instead.[4] In the absence of a metal catalyst, a mixture of both disubstituted triazole rings are usually obtained.[5] Disubstituted 1,2,3-triazole rings have been considered as non-classical mimetics of peptide linkages in terms of geometry and electronic properties, being the 1,4- or 1,5-disubstituted isomers respective surrogates of *trans* and *cis* peptide bonds.[6-8] Besides, the hydrogen bonding donor capability of the amide N-H could be replaced by the triazole C-H bond.[9,10] The 1*H*-1,2,3-triazoles and their derivatives are attractive molecules due to their unique combination of accessibility and diversity of supramolecular interactions. The ring contains a highly polarized C-H linkage that allows anion complexation by hydrogen bonding, as well as metal *N*- or *C*-coordination with anionic, neutral, or cationic nitrogen donors, carbanionic and mesoionic carbene donors.[11] The groups of Flood,[12,13] Craig,[14] Hecht,[15] Beer,[16] Schubert,[17] and Kubik[18-21] have significantly contributed to the study of 1,2,3-triazoles as anion receptors. Oligo and polytriazoles can adopt a U-turn conformation either mediated by metal cations or anions, or by dipole-dipole interactions. If the two ends of a U-turn structure are very bulky or the number of triazole rings increases, the chain ends would not align on the same plane, but would form a helical structure.[22] Rotational flexible oligotriazole molecules can adopt a reinforced chiral helical conformation upon binding to chloride anion; in this conformation they were able to induce high levels of chirality *via* a close chiral anion-pair complex.[23] Anion receptor chemistry deals with the design of molecules that are able to recognize, respond to, or sense species carrying a negative charge. This area of supramolecular chemistry comprises a wide number or applications, including organocatalysis, separation of anion mixtures in industrial or radioactive waste, and development of anion sensors capable of detecting trace levels of anionic species.[24] Recently it has been reported the application of click prodiginines, soluble in DMSO, as anionophores able to promote transmembrane anion permeation in cells.[25]

In a previous work[26] we have reported the microwave-assisted CuAAC polymerization of bio-based α-azide-ω-alkyne monomers **2**, that afforded stereoregular poly(amide-triazole)s **3** with high regioselectivity (Scheme 1). The monomers were prepared starting from D-glucono-1,5-lactone (**1**), an inexpensive and commercially available derivative of D-glucose, as a renewable resource. The poly(amide-triazole)s were highly insoluble in water and in most organic solvents, except for TFA-DMSO. Moreover, they were stable to acid (0.5 mM TFA, 40 °C, 7 days) or alkaline (0.5 mM NaOH, 40 °C, 7 days) conditions. These results suggested that the polymer chains were closely packed to form a complex network stabilized by hydrogen bonding and dipolar interactions. Due to their high nitrogen content, the polymers were assayed as metal-trapping agents. The preliminary studies showed an acceptable retention of metals, as Cu^{II} and Cr^{VI}.



Scheme 1. Poly(amide-triazole)s obtained from D-glucono-1,5-lactone

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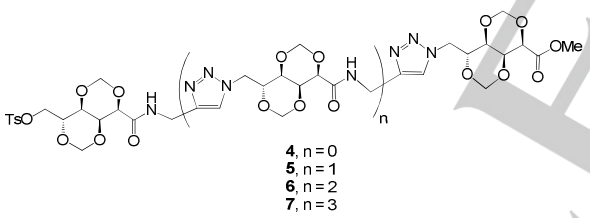
The poly(amide-triazole)s **3a-3c** possessed stereocenters that could induce defined preferred conformations, and the rigid bicyclic system of condensed 1,3-dioxane rings could lead to additional conformational constraints. The triazole rings in the polymer chain should be able to induce a local U-turn with the rigid carbohydrate moieties, in combination with the flexibility provided by the methylene spacers of the repeating units. In fact, some observed NOE contacts corresponding to triazole-CH with amide-NH, H-4 and H-5 (from the carbohydrate residue) suggested that some regions of the polymer could adopt a helical conformation. With the aim of exploring conformational preferences derived from the combination of stereochemical features, amide and triazole interactions, with increasing polymerization degree, herein we report the synthesis of a series of oligo(amide-triazole)s **4**-**7**, derived from D-glucono-1,5-lactone and propargylamine (Scheme 2). Since the adopted conformations of **4**-**7** would influence their interactions with charged species, we have evaluated these oligo(amide-triazole)s as anion receptors, using methodologies based on NMR spectroscopy and mass spectrometry (MS). MS has predominately been used as a qualitative tool in the analysis of complexes, with limitations in obtaining quantitative results. Mathieson *et al.*[27] proposed the application of electrospray ionization (ESI) MS for both the qualitative and quantitative measurement of ion binding, with validation by solution ^{1}H NMR. In the present study we have applied both NMR and MS to determine the binding stoichiometries of oligo(amide-triazole)s **4**-**7**. To the best of our knowledge, this is the first report on anion binding in acyclic carbohydrate-derived triazole compounds.



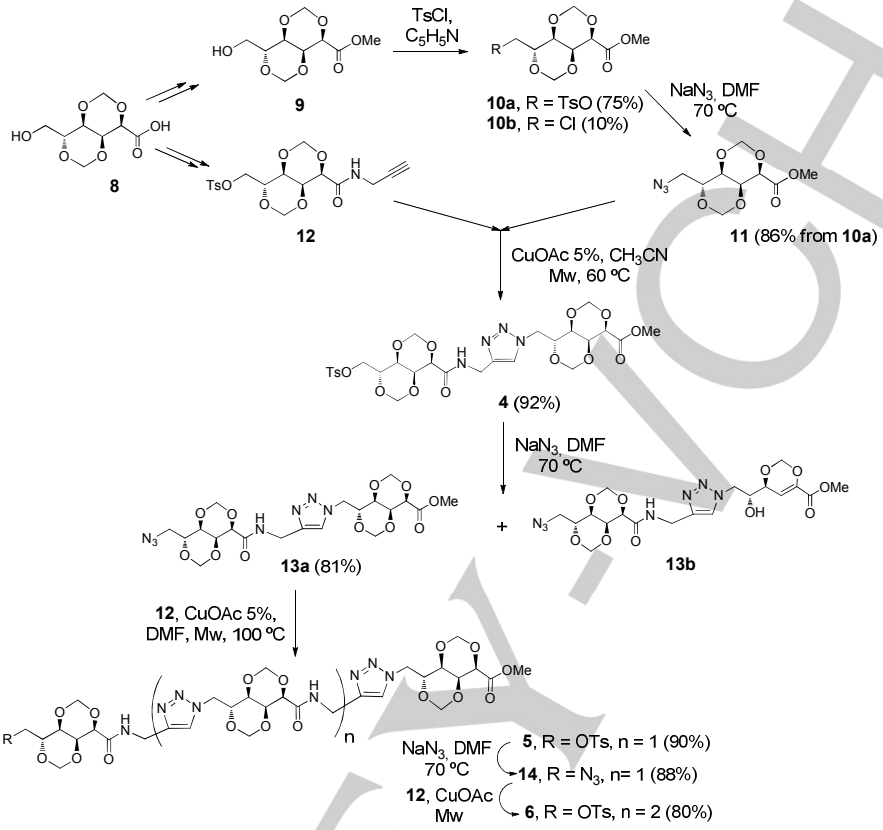
Scheme 2. Oligo(amide-triazole)s synthesized by CuAAC reaction

Results and Discussion

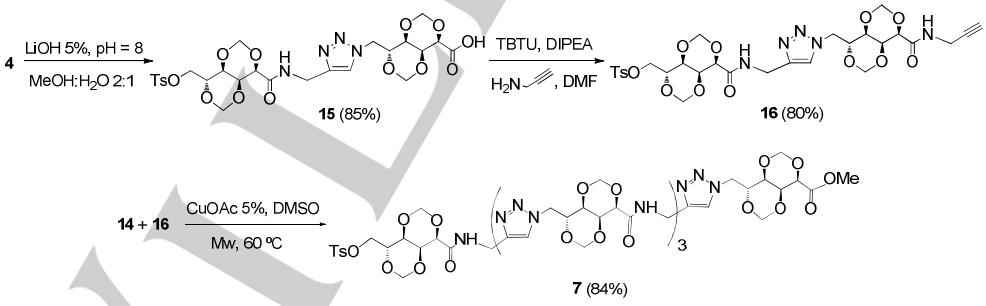
Synthesis

D-Glucono-1,5-lactone was used as the common starting material for the synthesis of methyl ester **9**[28] and amide **12**,[26] *via* the protected gluconic acid **8** (Scheme 3). Treatment of **9** with tosyl chloride gave compound **10a** as main product, together with a minor amount of chlorinated compound **10b**. These compounds were isolated by column chromatography and both of them yielded azide **11**, upon reaction with NaN_3. The overall yield of **11** from ω-hydroxyacid **8** was 55%, and 40% from D-glucono-1,5-lactone.

Microwave-assisted CuAAC was conducted between azide **11** and alkyne **12**, to give the dimer **4** as the first amide-triazole derivative of the series (Scheme 2). Further substitution by azide (DMF, 60 °C, 18 h) gave the expected **13a** (56% yield) together with compound **13b** (22% yield). This by-product was produced by abstraction of the Hα to carbonyl group and elimination of the β-substituent. However, shorter reaction time and higher temperature under microwave (Mw) irradiation improved the yield of the desired product **13a** (81%, after purification). The CuAAC reaction of ω-azide-α-ester **13a** with alkyne **12** yielded trimer **5**, with two 1,4-disubstituted-triazole rings. The subsequent substitution of tosyl group by azide to give compound **14**, was also carried out with conventional heating or under Mw irradiation. In the case of conventional heating, the desired azide **14** was obtained (80% yield) after 24 h, with minor amounts of unsaturated by-product. Although Mw irradiation gave a lower yield (70%), it was preferred since complete conversion was achieved in only 1 h, without formation of the unsaturated derivative. The solubility of the oligo(amide-triazole)s decreased with the chain length and precluded purification by column chromatography or recrystallization. However, compound **14** was obtained in an acceptable degree of purity and was subjected to CuAAC reaction with alkyne **12**, to give tetramer **6**. The low solubility of the oligomers of increasing chain length also forced us to increase the solvent polarity for the CuAAC reaction. For dimer **4**, the cycloaddition was carried out in acetonitrile; while for trimer **5** and tetramer **6**, DMF and DMSO were respectively used. Compound **6** precipitated from the reaction mixture and subsequent substitution of the tosyl group by azide could only be partially achieved (DMSO, 120 °C), to yield a complex mixture of products of difficult separation. Therefore, an alternative synthetic approach was designed to obtain pentamer **7** via the dimer **16** (Scheme 4). Since either free carboxylic acids or azide derivatives proved to be less soluble than tosylated methyl esters, the synthesis of **16** started from methyl ester **4**. Treatment of **4** with LiOH in methanol gave the carboxylic acid **15**, which was converted into the *N*-propargylamide **16**. CuAAC reaction between alkyne **16** and azide **14**, in DMF, led to the pentamer **7**.



Scheme 3. Synthesis of oligo(amide-triazole)s **4**-**6**.



Scheme 4. Synthesis of pentamer **7**.

The structure of the oligomers was confirmed by NMR spectroscopy. All the NMR spectra were recorded in [D_6]DMSO, since it was the only solvent able to dissolve all the compounds. Thus, the ^{1}H NMR spectrum of dimer **4** (Figure 1) exhibited diagnostic amide and triazole resonances at 8.17 and 7.81 ppm, respectively, being the latter overlapped with one of the doublets of the tosyl group. The resonances of both methylene groups linked to the triazole ring were shifted to lower fields, in comparison with those of the methylenes vicinal to the azide or alkyne functions. The methylene protons H-6a, H-6b (linked to the triazole nitrogen) were the most deshielded and appeared as two well separated signals. For dimer **4**, H-4 and H-4' gave resonances at higher field, in the same the region of the methoxyl group.

The general pattern of the spectra remained almost unchanged with the increasing number of repeating units from oligomers **4** to **7**, and they were also similar to that of the poly(amide-triazole) **3a**. The main differences consisted in the increasing complexity of the NH and triazole-CH signals, as well as in the expected increment in the relative integration of the peaks (the integral of the aromatic tosyl signal at 7.50 ppm was assigned as 2H for all the spectra). The integrals of H-3, H-5 and H-6a signals of the terminal (tosyl containing) unit remained practically unchanged from **4** to **7**. These facts validated the estimation of average-number molecular weights of the poly(amide-triazole)s according to the integrals of the signals.[26]

The ^{13}C NMR spectra of dimer **4** (see Supporting Information) exhibited the carbon signals of amide (167.0 ppm), ester (167.8 ppm), and triazole (123.4 ppm and 145.1 ppm, for C-7 and C-8 respectively). Moreover, the general pattern of the ^{13}C NMR spectra of the oligomers were in agreement with those of the poly(amide-triazole) **3a**.[26]

Ion binding studies

The nitrogen-rich 1,2,3-triazole ring possesses a highly polarized C-H linkage which is able to interact with anions by hydrogen bonding.[11] Therefore, we have explored the potential capacity of carbohydrate-derived oligo(amide-triazole)s **4**-**7** as receptors for halide anions (Cl^-, Br^-, and I^-).

Qualitative ion binding studies have been conducted using NMR spectroscopy. In solution, anion binding can be associated to a competition between two solvation spheres for the anion: one provided by the solvent and the other one controlled by a folded cavity lined with triazole-CH and amide-NH protons. The anion sizes are primary determinants of the binding strength by the flexible oligomers. The affinity of the receptors for a given anion is typically well correlated with the downfield shifts of the triazole-CH protons upon binding.[29] On the other hand, a polar and hydrogen bonding acceptor solvent like DMSO could interact with triazole-CH, amide-NH and other potential donors in the host molecule.

To assess the influence of the solvent effect on the NMR experiments we employed dimer **4**, as this was the only member of the series that was soluble in $CDCl_3$. Thus, the ^1H NMR spectra of **4** were recorded in $CDCl_3$ or $[D_6]$DMSO, in the absence or presence of halide anions (See Supporting Information). Upon addition of tetrabutylammonium (TBA) halide salts, the whole pattern of the spectrum recorded in $CDCl_3$ was modified, suggesting an overall conformational change of the receptor. The displacement of the signals was stronger when increasing the electronegativity of the halogen. In general, all signals were shifted upfield, except for triazole-CH that moved downfield. The most pronounced downfield shifting was observed for chloride, and this displacement was taken as an initial proof of anion binding.

When the solvent employed was $[D_6]$DMSO, all the resonances in the 7.0-8.5 ppm range were shifted downfield. As expected, the amide-NH signal underwent the strongest shifting, and moved to a lower field relative to that of triazole-CH, in contrast to the pattern observed in the $CDCl_3$ spectra. Upon addition of TBA chloride, triazole-CH underwent a slight downfield shifting in comparison to pure $[D_6]$DMSO, while amide-NH was shifted upfield. This behavior for the amide-NH resonance could be explained if this proton is involved in an intramolecular NH···O bond with an oxygen from the sugar residue, and such a bond is disrupted by halide or DMSO.[30]

Qualitative studies by NMR spectroscopy were also performed for compounds **6** and **7** with halides. Since these oligo(amide-triazole)s were soluble in the milimolar range only in DMSO, the NMR experiments were carried out in $[D_6]$DMSO, which also dissolved appropriately the tetramethylammonium (TMA) halide salts. The ^1H NMR spectra for **6** and **7** were noticeably changed upon addition of halides, both in the shape and chemical shifts of the amide-NH, triazole-CH (H-7), and H-4, 4' signals (Figure 2). However, the $\Delta\delta$ values were small due to the mentioned H-bonding acceptor capacity of DMSO. Thus, the observed $\Delta\delta$ were averaged values of the chemical shifts of a given proton that interacted either with halide or DMSO.

The spectra of tetramer **6** and pentamer **7** recorded in presence of Cl^-, Br^-, or I^-, indicated that the more electronegative chloride induced the most intense displacement of the triazole protons. These signals were partially overlapped with one doublet of the tosyl group, which remained unchanged in the presence of halide salts. The addition of chloride slightly affected the NH signals, and an unexpected change in chemical shift was registered for the H-4 nuclei that are closer to triazole rings.

The anion binding of oligo(amide-triazole)s to halides was also investigated using ESI MS. Direct infusion electrospray ionization single quadrupole MS (DI-ESI-SQ-MS) experiments were conducted in acetonitrile-water-isopropanol mixtures with analyte levels in the micromolar range, in the presence of potassium halide salts, as TMA salts are inconvenient for this technique.

In the first instance, DI-ESI-SQ-MS experiments were conducted in the positive ion mode. Compound **5** was selected as a proxy of the oligo(amide-triazole)s series to explore the binding capability of the compounds towards cations in the presence of a complexing agent. The mass spectrum of compound **5** exhibited the base peak at *m/z* 973 ($[M+Na]^+$). Additional peaks at *m/z* 991 ($[M+K]^+$), 958 ($[M+K\text{-}MeOH]^+$), 499 ($[M+2Na]^{2+}$), and 491 ($[M+HCHO+2H]^{2+}$) were detected (see Supporting Information). When KCl was added to trimer **5**, the intensity of $[M+K]^+$ ion was highly increased, though the $[M+Na]^+$ ion was still the base peak. The intensities of both $[M+Na]^+$ and $[M+K]^+$ peaks were reduced in one order of magnitude upon addition of 15-crown-5 and the most intense signals were $[M+Na]^+$ and $[M+2Na]^{2+}$. Thus, trimer **5** formed adduct ions with Na^+ and K^+ rather than H^+ despite the electrolytically-induced decrease in solution pH generated in the ESI source.[31] Therefore, crown ether was added to oligomer solutions for anion binding assays, to minimize oligo(amide-triazole) interactions with Na^+ or K^+, and to enhance anion binding.

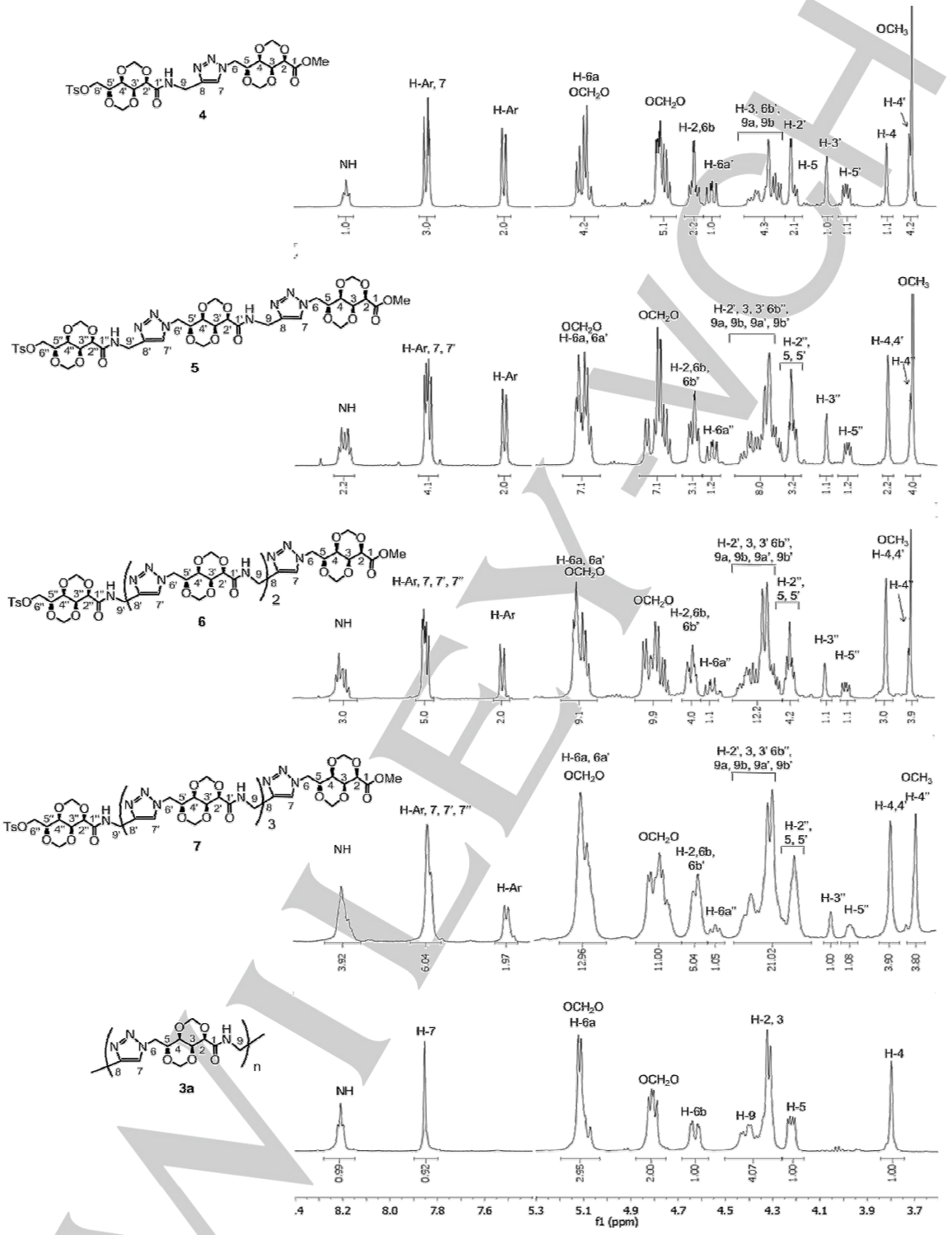


Figure 1. ^{1}H NMR spectra (500 MHz, [D_6]DMSO) of oligo(amide-triazole)s **4-7**, and poli(amide-triazole) **3a**.

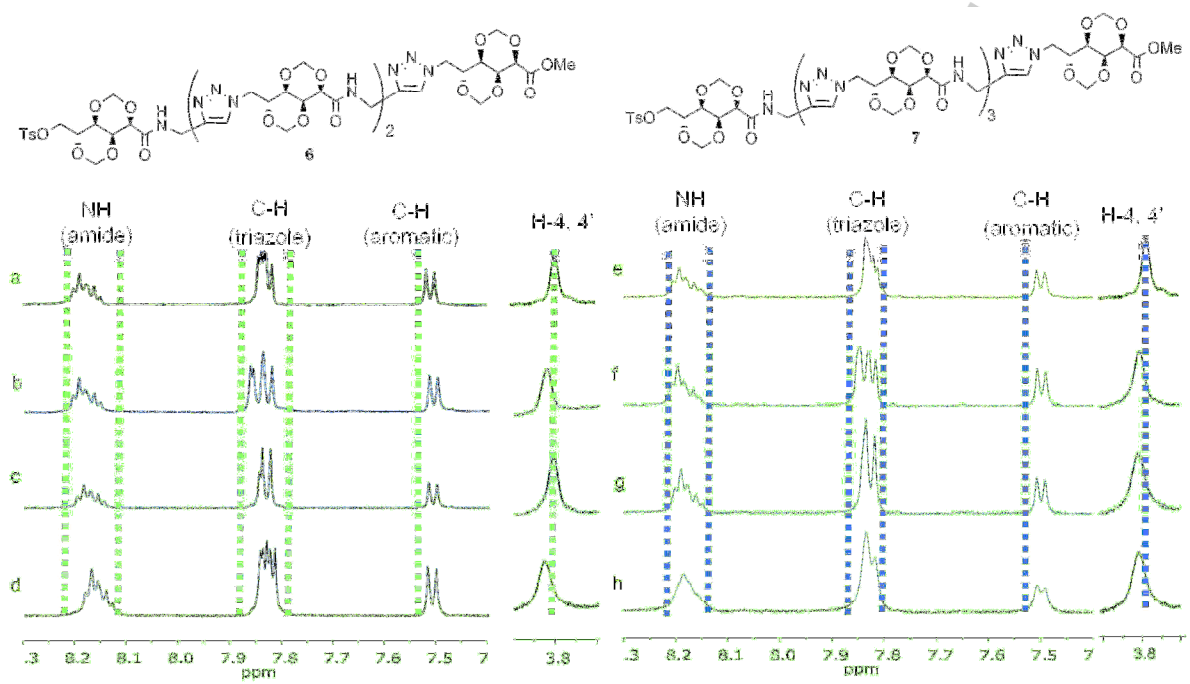


Figure 2. Partial ^{1}H NMR (500 MHz, [D_6]DMSO) spectra of compounds **6** and **7** in the absence or presence of TMA halide salts (60 equiv.): (a) **6** (3 mM); (b) **6** in the presence of TMA Cl; (c) **6** in the presence of TMA Br; (d) **6** in the presence of TMA I; (e) **7** (3 mM); (f) **7** in the presence of TMA Cl; (g) **7** in the presence of TMA Br; (h) **7** in the presence of TMA I.

Qualitative anion binding was then studied by DI-ESI-SQ-MS in the negative ion mode for oligo(amide-triazole)s **6** and **7**, pure or combined with KCl, KBr, or both of them, always in the presence of 15-crown-5, in the molar ratios described in Figure 3. In spite of their similar structure, compounds **6** and **7** showed relevant differences in their ESI MS. The main signals observed in the mass spectrum of pure compound **6** were due to $[M\text{-}H]^-$, $[M+H_2O\text{-}H]^-$, $[M+HCO_2]^-$, and $[M+H_2O+HCO_2]^-$; however, no peaks for due to $[M+Cl]^-$ or $[M+Br]^-$ were observed, suggesting that potential nonspecific binding with halide traces remained under the limit of detection. Nonspecific binding happens when chemical species trapped in the same nanodroplet start to interact as the solvent evaporates.[32,33] When chloride or bromide salts were added, the main ionic species detected corresponded to $[M+Cl]^-$ and $[M+Br]^-$ (*m/z* 1269 and 1314, respectively). Less intense signals, attributed to the loss of formaldehyde from methylidene groups ($[M\text{-}HCHO+Cl]^-$ and $[M\text{-}HCHO+Br]^-$), were also observed.

In the spectrum of pure compound **7**, the $[M+Cl]^-$ (*m/z* 1552) was the base peak, probably due to nonspecific binding to chloride traces and possible contribution of an ESI mechanism[33] different from the typical ion evaporation model (IEM).[34] This distinctive behavior for **7** may be attributed to the particular helical conformation adopted for this compound in solution (see Conformational analysis). Additional ionic species observed in the spectrum corresponded to $[M\text{-}HCHO+OH]^-$ (*m/z* 1502) , $[M\text{-}H]^-$ (*m/z* 1517), $[M+OH]^-$ (*m/z* 1532), and $[M+H_2O+HCO_2]^-$ (*m/z* 1579) ions. Addition of KCl rendered a more intense $[M+Cl]^-$ peak, although the spectrum fingerprint was retained. Upon addition of KBr, a peak for the $[M+Br]^-$ ion was observed, but with an intensity 50% lower than that of the $[M+Cl]^-$ ion.

On the other hand, it is relevant to mention some common features for both oligomers **6** and **7**. Their mass spectra did not exhibit the $[2M+Cl]^-$ or $[2M+Br]^-$ ions, indicating the absence of 2:1 host:chloride stoichiometry, even under favorable conditions for its formation. Remarkably, when both halides were present in equal concentration, the $[M+Cl]^-$ ion highly prevailed, suggesting higher selectivity for Cl^- complexation over Br^-. However, tetramer **6** showed higher selectivity and lower nonspecific binding than pentamer **7** under similar conditions.

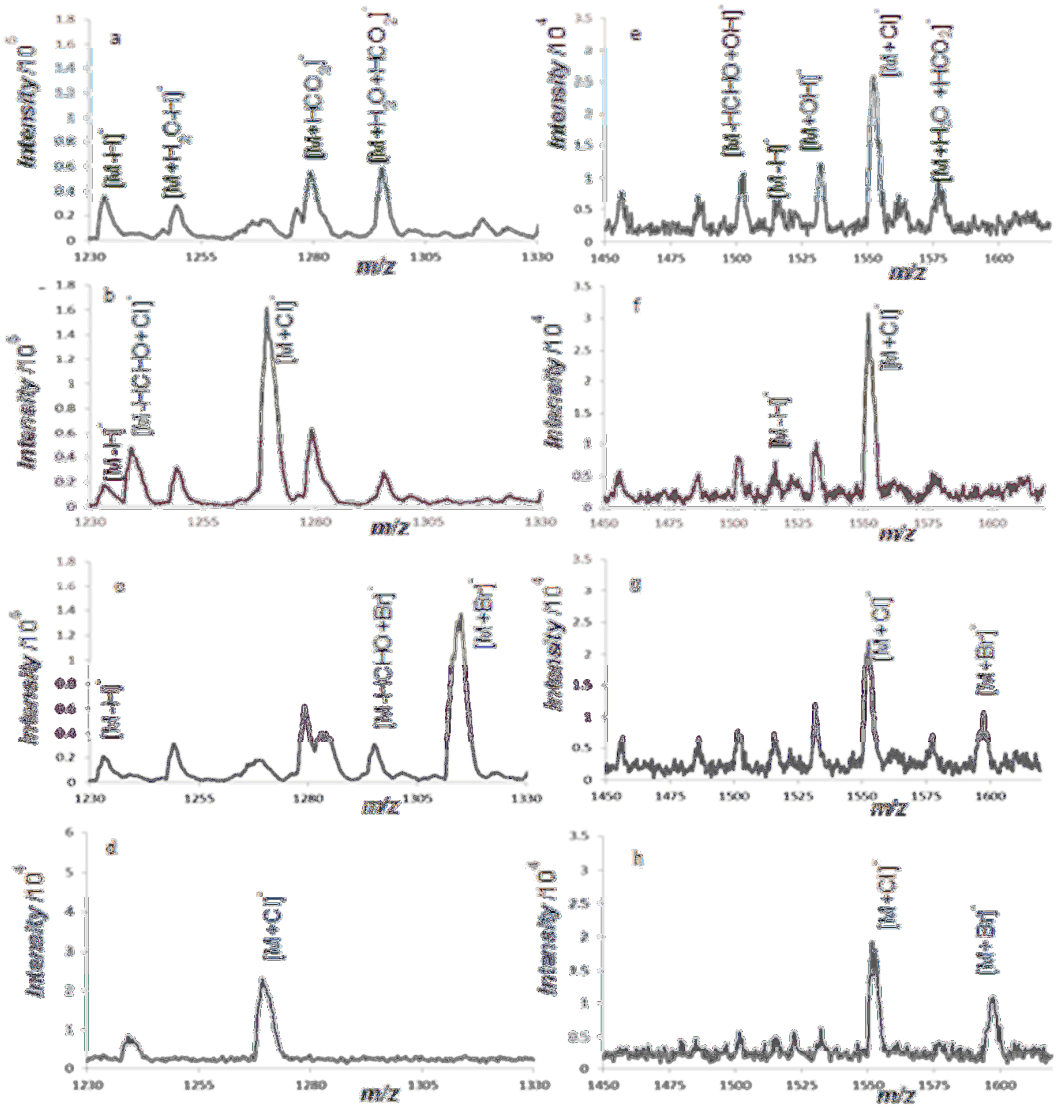


Figure 3. DI-ESI-SQ-MS (negative ion mode) of (a) oligo(amide-triazole) **6** : 15-crown-5 (1:1, 10 μM **6**); (b) **6** : KCl : 15-crown-5 (1:1:1, 10 μM **6**); (c) **6** : KBr : 15-crown-5 (1:1:1, 10 μM **6**); (d) **6** : KCl: KBr : 15-crown-5 (1:10:10:10, 1 μM **6**); (e) **7** : 15-crown-5 (1:1; 5 μM **7**); (f) **7** : KCl : 15-crown-5 (1:1:1; 5 μM **7**); (g) **7** : KBr : 15-crown-5 (1:1:1; 5 μM **7**); (h) **7** : KCl : KBr : 15-crown-5 (1:10:10:10; 1 μM **7**)

Since qualitative analysis indicated high affinity of compounds **6** and **7** to chloride, binding stoichiometries for all the oligo(amide-triazole)s **4**-**7** were determined by the Job´s Method of Continuous Variation for the host (H) and guest (G) compounds.[35] For this purpose we have employed NMR spectroscopy and DI-ESI-SQ-MS. A significant advantage of MS in this approach is that each ionic species generated in the ESI source, analyzed, and detected by the spectrometer provides one specific signal that can be easily identified. In contrast, NMR spectroscopy in the fast exchange time-scale gives a change in chemical shift that is proportional to the ratio of complexed host at equilibrium over the total host concentration, providing valuable information about the nuclei involved in the interaction.

Oligo(amide-triazole)s samples were prepared with concentration ranges adequate for NMR and MS, according to their sensitivity. Oligo(amide-triazole) compounds acted as host (H) and the chloride anion as guest (G). Experimental data obtained from each technique were fitted using a Gaussian function (See Supporting information) and the maximum values were used to obtain the stoichiometry of the H-G complexes.

For Job plots using NMR spectroscopy, the changes in chemical shifts of the triazole protons (H-7) were measured. The triazole-

CH signals were assigned with the assistance of 2D-NMR techniques. For those compounds that had more than one triazole ring (**5**-**7**), individual Job plots were calculated for each proton, and the results were coincident in all cases. The resulting stoichiometries of the oligo(amide-triazole)s-chloride complexes were 2:1 for compounds **4** and **5**, while for tetramer **6** and pentamer **7** the stoichiometry was 1:1.

The MS peaks of thermodynamically preferred species could be accompanied by those from artefacts and from nonspecific complexes. Therefore, we have chosen the Job plots procedure to confirm the stoichiometries of the complexes, rather than the less accurate direct observation of peaks. The DI-ESI-SQ-MS experiments were recorded using oligo(amide-triazole) solutions mixed with equimolar solutions of KCl, also containing crown-ether. The concentration of crown-ether was maintained constant for all the experiments, and the molar fractions of host and guest were varied. In the case of oligomers **4** and **5**, the $[M+Cl]^-$ and $[2M+Cl]^-$ ions were observed and Job plots were calculated for each species, giving a maximum at 0.62 for compound **4** and 0.67 for compound **5** (See Supporting information). Thus, the stoichiometry of these oligo(amide-triazole)s-chloride complexes was 2:1, in agreement with NMR results. For tetramer **6** and pentamer **7**, the maximum was respectively observed at 0.56 and 0.54 molar fraction, in agreement with a 1:1 stoichiometry for the oligo(amide-triazole)-chloride complex. As mentioned above, the main adduct ions in the mass spectra of compounds **6** and **7** were the $[M+Cl]^-$, without any evidence of the presence of higher complexes.

These results show that dimer **4** and trimer **5**, which contain respectively one or two triazole rings, give rise to the 2:1 complexes. Both triazole residues in trimer **5** seem to bind to chloride, since their resonances were shifted in the presence of the anion. However, only one triazole per molecule would bind to chloride, as the rigid nature of the carbohydrate backbone makes the distance unsuitable for the interaction with the other triazole ring of the same molecule. In contrast, higher oligomers **6** and **7** should be flexible enough to bind to two triazole residues in the same molecule. All the triazole rings in compounds **6** and **7** exhibited similar chemical shift displacements, suggesting that all of them are committed with chloride binding. This may be achieved by simultaneous interaction between chloride and two triazoles, or either through sequential interaction with different pairs of rings, in the fast NMR time-scale.

Recent reports have posed objections to the applicability of Job plots.[36,37] However, the procedure may be applied taking into account some recommendations regarding the structure and properties of the receptors, the use of independent procedures of analysis, and the testing of residuals distribution for the performance of the model. In the present work, we have paid careful attention to all those recommendations. Thus, the Job plots were calculated using two independent techniques at different concentration ranges, which led to the same stoichiometry for the oligo(amide-triazole)-chloride complexes.

Conformational analysis

Optical rotations can provide an insight into the conformation, mostly for large molecules.[38] Therefore, the optical rotations of the oligo(amide-triazole)s **4**-**7** have been measured (Table 1) and their values increased with the increasing number of repeating units in the series. Compound **7**, with the longest chain, showed an outstanding large value ($[\alpha]_D^{25}$ = +624.2). For compounds **4**-**6**, higher optical activity values were observed upon TMA chloride addition, relative to the values obtained in the absence of the anion. In contrast, a decrease on the optical activity was observed when TMA chloride was added to a solution of pentamer **7**. These results were consistent with an ordered conformation achieved by pentamer **7**, but which could not be attained by the minor members of the series. This conformation would be distorted but still prevailed after chloride addition. Similar large optical rotations ($[\alpha]_D^{-62}$ = +490) were observed for poly(triphenylmethyl-methacrylates) and were attributed to the helicity of the rigid isotactic sequence of repeating units.[39] Helicenes are examples of small organic molecules with exceptionally high optical activity due to helical conformation.[40]

Table 1. Optical rotations of monomer **2a**, poly(amide-triazole) **3a**, and oligo(amide-triazole)s **4**-**7**, in the presence or absence of TMA chloride.

Compound	$[\alpha]_D^{25}$
2a[a]	+89.6
3a[a]	+162.2
3a: Cl⁻ (1 : 1) [b]	+ 110.6
4[a]	+49.8
4 : Cl⁻ (2 : 1)[a]	+50.6
5[a]	+67.2
5 : Cl⁻ (2 : 1)[a]	+82.0
6[a]	+72.3
6 : Cl⁻ (1 : 1)[a]	+80.0
7[b]	+624.2
7 : Cl⁻ (1 : 1)[b]	+495.6

[a] *c* = 0.2-0.5, DMSO. [b] *c* = 0.02-0.06, DMSO.

The circular dichroism (CD) is also useful for assessing conformations. Therefore, the CD spectra of compounds **4**-**7** were recorded and only compound **7** exhibited CD activity (a maximum at 205 nm and a shoulder at 223 nm; Figure 4). The oligo(amide-triazole) family have the same chromophores, amide and triazole groups with $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions, as well as the same chiral backbones, and they only differ in the number of repeating units. Thus, the CD signal observed for pentamer **7** could be attributed to an ordered conformation that

was not achieved in the shorter oligomers. Furthermore, the observed maxima are consistent with those of a protein α-helix formed from D-amino acid residues, even though in our case the solvent composition prevented the observation of a negative peak below 195 nm. An octameric molecule, constituted by carbohydrate-derived amino acid monomers, with a helical secondary structure, showed a similar CD spectrum.[41] The temperature-dependent CD experiment for compound **7** was measured in the range 0-80 °C. The CD remained almost unchanged up to 60 °C, while at 80 °C the signal intensity at 205 nm was reduced to 28% of the value reached at 0 °C. The CD signal was not recovered upon immediate cooling of the solution. In the presence of chloride salt, the general pattern of the spectra was kept, but it was less intense. The CD signal was more affected with the raise of temperature in the whole range, and at 80 °C the intensity of the band at 205 nm was reduced to 13% of the initial value. Thus, pentamer **7** would attain a helical conformation which is stable until 60 °C. The helix would undergo distortion in the presence of chloride and became less stable with the temperature increase.

Additionally, the folding of oligo(amide-triazole)s **4**-**7** was studied using 2D NOESY experiments. The NOESY spectrum of compound **4** showed just one contact between amide-NH and triazole-CH (H-7). Trimer **5** and tetramer **6** exhibited some additional NOE interactions for triazole-CH (signals of H-7, H-7' or H-7'' are indistinguishable in the NOESY spectra and are labelled as H-7) and NH protons. However, the spectrum of pentamer **7** exhibited new cross-peaks involving methylidene protons with H-7 and amide-NH. Also an enhanced intensity was observed for the cross-peak of H-7 with H-4,H-4' (See Supporting information). These NOE interactions were relevant to explain the conformational preference of the pentamer. The H-4,H-4' nuclei interact with the triazole H-7 included in the same repeating unit, since no cross-peak was observed for H-4'' (Figure 5). Moreover, the contact between triazole-CH and amide-NH is indicative of the proximity of these protons in space. These facts are consistent with a U-conformation centred on the triazole ring, which is also in agreement with the helical conformation of the pentamer. A representation of such a conformation of a model compound analogue of **7**, having the tosyl group replaced by OMe, is shown in Figure 5(b). This conformation is the result of a preliminary molecular modelling using the semiempirical method AM1, and including distance constraints consistent with observed NOE contacts. The NOESY spectrum of polymer **3a** exhibited the same cross-peaks as **7**, with two additional ones (triazole-CH, methylene (H-9) and amide-NH, H-4). These results suggested the presence of ordered (helical) regions inserted in the random coil conformation of the polymer chain.[42] Accordingly, the optical rotation value of the polymer **3a** is smaller than that of **7**, but larger in comparison to those of monomer **2a** and oligomers **4**-**6**.

In the presence of chloride, the NOESY spectrum of pentamer **7** showed an increased intensity of the contact between triazole-CH and H-4,H-4'; while the cross-peaks between methylidene with H-7 and with amide-NH were not detected. Furthermore, amide-NH, triazole-CH and H-4' still remained close in space (NOE contacts observed). These results were in agreement with a small distortion of the helical conformation in the interaction with chloride. Moreover, as the chemical shift of amide-NH remained practically unchanged upon addition of chloride, the NH-Cl^- interaction should be negligible. Probably this NH is involved in the formation of hydrogen bonding with O-2' or O-3', to give 5 or 6-membered rings,[43] respectively**.** It has been previously found that triazole-CH have stronger interactions than amide-NH with halide anions in amidetriazoles.[44]

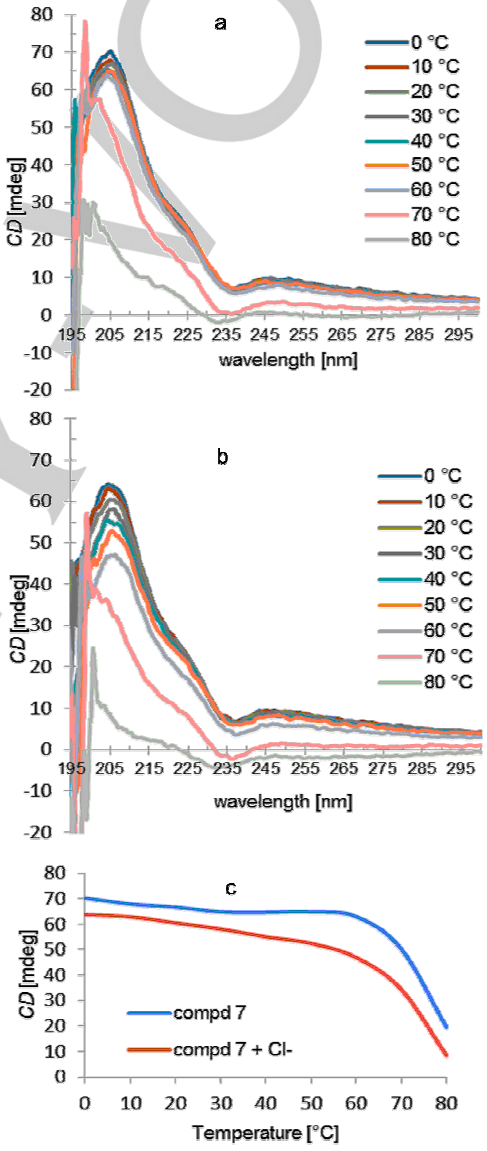


Figure 4. Temperature dependence of CD spectra of compound **7** (10 μM in 2:2:1 CH_3CN-H_2O-*i*PrOH) (a) in the absence or (b) in the presence of KCl (40 equiv.). (c) Temperature variation of CD, measured at 205 nm, in absence or presence of chloride salt.

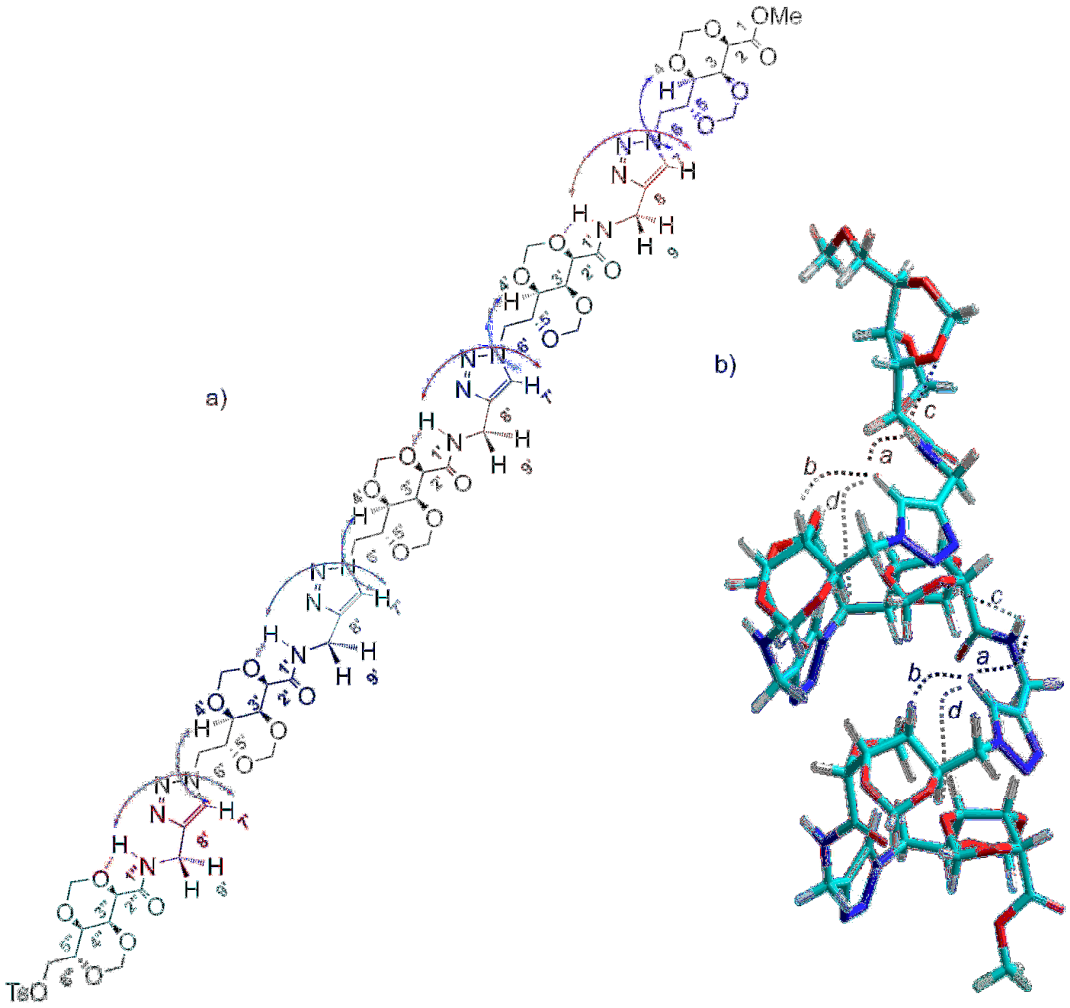


Figure 5. Relevant detected NOE interactions [a] H-7, NH; [b] H-7, H-4 and H-7, H-4'; [c] NH,O-2 hydrogen bond; [d] H-7, H-5 and H-7, H-5': (a) for a linear representation of pentamer **7**; (b) for a schematic helical conformation of a model structure of pentamer **7** (the terminal tosyl group was replaced by OMe)

Conclusions

In summary, we report here the synthesis of a family of linear sugar-derived oligo(amide-triazole)s with chains of an increasing number of carbohydrate and triazole units, from one (dimer) to four (pentamer). These molecules have been designed to act as anion receptors. The binding affinities of the oligomers with halide anions have been studied using NMR spectroscopy and ESI MS. The affinity order was $Cl^- > Br^- > I^-$ for tetramer **6** and pentamer **7**. The binding stoichiometries have been estimated: dimer **4** and trimer **5** formed 2:1 complexes while tetramer **6** and pentamer **7** gave a 1:1 stoichiometry. Anion selectivity is not an intrinsic property of 1,2,3-triazole or amide functional groups, but rather a confluence of conformational and solvation effects.[29] Optical rotation values, NOE contacts and CD experiments were consistent with a helical conformation for the pentameric oligo(amide-triazole) **7**. This ordered conformation was distorted by the presence of chloride, even though remained stable in solution below 60 °C. The active site in the pentamer backbone that interact with chloride involved the triazole-CH, amide-NH, and H-4' from the carbohydrate residue.

These oligo(amide-triazole)s could be applied for chloride sensing by ESI MS. The tetramer **6** should be particularly useful for this purpose, as is more selective to chloride than pentamer **7**, and displays lower nonspecific binding.

Experimental Section

General Experimental Methods. Analytical thin-layer chromatography (TLC) was performed on Silica Gel 60 F254 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 a cerium molibdate solution. Column chromatography was performed with Silica Gel 60 (230–400 mesh). Microwave reactions were carried out in an Anton Paar Monowave 300 reactor in closed vials; the temperature was monitored by an external infrared sensor. Optical rotations were measured at 25 ºC and are expressed as $cm^3\ g^{-1}\ dm^{-1}$. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance II 500 instrument (^{1}H: 500 MHz; ^{13}C: 125.7 MHz). Spectra were referenced to the residual solvent signals.[45] The assignments were assisted by 2D COSY, HMBC, NOESY and HSQC techniques. High resolution mass spectrometry (HRMS) was carried out using electrospray ionization (ESI) either with a Bruker microTOF Q II or with a Waters Xevo G2S QTOF instrument. The QTOF mass spectrometer was operated in negative ion mode with a probe capillary voltage of 2.3 kV, and in the positive ion mode with a capillary voltage of 2.5 kV. The sampling cone voltage was set to 30.0 V. The source and desolvation gas temperatures were set to 120 and 300 ºC, respectively. The nitrogen gas desolvation flow rate was 600 L h^{-1}, and the cone desolvation flow rate was 10 L h^{-1}. The mass spectrometer was calibrated across the range of *m/z* 50-1200 using a 0.5 mM sodium formate solution prepared in 2-propanol/water (90:10 v/v). Data were drift corrected during acquisition using a leucine encephalin (*m/z* 554.2615 for ESI^- and 556.2771 for ESI^+) reference spray infused at 2 µL min^{-1}, every 15 seconds. Data were acquired in the range of *m/z* 50-1200, and the scan time was set to 0.5 s. Circular dichroism experiments were performed with a spectrometer equipped with a temperature controller in quartz cells of 10 mm path length.

2,4:3,5-Di-*O*-methylidene-D-gluconic acid and its methyl ester (**9**) were obtained from D-glucono-1,5-lactone (**1**) as already described.[28]

Tetramethylammonium (TMA) chloride was recrystallized from 2-propanol and dried at 110 ºC under vacuum; TMA bromide and iodide, as well as potassium chloride and bromide, were dried before use as described above.

Synthesis

Methyl 2,4:3,5-di-*O*-methylidene-6-*O*-tosyl-D-gluconate (10a). To a solution of **9**[28] (0.8 g; 3.41 mmol) in pyridine (6 mL) was added tosyl chloride (1.1 g; 5.76 mmol). The mixture was stirred for 20 h at room temperature, until complete consumption of **9**. After concentration at reduced pressure, compound **10a** (0,99 g, 75%) was isolated first as a white crystalline solid by column chromatography (40:60 hexane-EtOAc). m.p. 103 ºC (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +18.3 (*c* = 0.6 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC, TMS): δ = 2.46 (s, 3 H; C*H*$_3$Ph), 3.76 (s, 1 H; H-4), 3.81 (s, 3 H; C*H*$_3$O), 4.05 (s, 1 H; H-3), 4.07-4.12 (m, 1 H; H-5), 4.22-4.28 (m, 2 H; H-6), 4.30 (d, ^3J (H-2,H-3) = 1.8 Hz, 1 H; H-2), 4.76 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 4.86 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O), 4.94 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O), 5.24 (d, ^3J (H,H) = 6.5 Hz, 1 H; OCH_2O), 7.38 (d, ^3J (H,H) = 8.2 Hz, 2 H; H-aromatic), 7.80 (d, ^3J (H,H) = 8.2 Hz, 2 H; H-aromatic).^{13}C NMR (125.7 MHz, $CDCl_3$, 25 ºC, TMS): δ = 52.8 (O*C*H$_3$), 67.0 (C-6), 67.5 (C-3), 70.5 (C-4), 73.2 (C-5), 76.6 (C-2), 89.0, 92.4 (O*C*H$_2$O), 128.1, 130.2, 132.3, 145.7 (C-aromatic), 167.9 (C=O). HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd for $C_{16}H_{21}O_9S$ 389.0913; found: 389.0901.

Next fractions from the column (50:50 hexane-EtOAc) afforded methyl 6-chloro-6-deoxy-2,4:3,5-di-*O*-methylidene-D-gluconate (**10b**), as a white crystalline solid (0.08 g, 10%). m.p. 139-140 ºC (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +37,9 (*c* = 0.2 in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC, TMS): δ = 3.75 (dd, ^3J (H-5,H-6a) = 5.9 Hz, ^3J (H-6a,H-b) = 11.6 Hz, 1 H; H-6a), 3.79 (dd, ^3J (H-5,H-6b) = 8.2 Hz, ^3J (H-6a,H-6b) = 11.6 Hz, 1 H; H-6b), 3.85 (s, 3H; $COCH_3$,), 3.9 (t, ^3J (H-3,H-4) = ^3J (H-4,H-5) = 1.5 Hz, 1 H; H-4), 4.13 (t, ^3J (H-2,H-3) = ^3J (H-3,H-4) = 1.8 Hz, 1 H; H-3), 4.15 (m, ^3J (H-4,H-5) = 2.0 Hz, ^3J (H-5,H-6b) = 5.9 Hz, ^3J (H-5,H-6a) = 8.2 Hz, 1 H; H-5), 4.35 (d, ^3J (H-2,H-3) = 2.0 Hz, 1 H; H-2), 4.81 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O), 4.95 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O), 5.03 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O), 5.30 (d, ^3J (H,H) = 6.1 Hz, 1 H; OCH_2O); ^{13}C NMR (125.7 MHz, $CDCl_3$, 25 ºC, TMS): δ = 40.7 (C-6), 52.9 (O*C*H$_3$), 67.1 (C-3), 71.0 (C-4), 75.7 (C-5), 76.8 (C-2), 88.6, 92.6 (OCH_2O) 167.9 (C=O). HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ calcd for $C_9H_{13}ClO_6Na$ 275.0293; found: 275.0292. $[M + K]^+$, calcd for $C_9H_{13}ClO_6K$: 291.0032; found: 291.0026.

Methyl 6-azido-6-deoxy-2,4:3,5-di-*O*-methylidene-D-gluconate (11). *a*) From 10a. Sodium azide (0.44 g, 6.76 mmol) was added to a solution of **10a** (1.35 g, 3.48 mmol) in DMF (8 mL). The mixture was stirred at 80 ºC for 2 h, and then concentrated at reduced pressure. Excess of NaN_3 was filtered through Celite. Compound **11** was isolated as a white crystalline solid (0,77 g, 86%) by column chromatography (20:80 hexane-EtOAc). m.p. 122 ºC (recryst from EtOAc-hexane); $[\alpha]_D^{25}$ = +23,0 (*c* = 0.5 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, 25 ºC, TMS): δ = 3.54 (dd, ^3J (H-5,H-6a) = 6.5 Hz, ^3J (H-6a,H-6b) = 13.0 Hz, 1 H; H-6a), 3.67 (dd, ^3J (H-5,H-6b) = 6.5 Hz, ^3J (H-6a,H-6b) = 13.0 Hz, 1 H; H-6b), 3.73 (t, ^3J (H-3,H-4) = ^3J (H-4,H-5) = 1.5 Hz, 1 H; H-4), 3.83 (s, 3H, $COCH_3$,), 4.07 (m, ^3J (H-4,H-5) = 1.5 Hz, ^3J (H-5,H-6b) = ^3J (H-5,H-6a) = 6.5 Hz, 1 H; H-5), 4.12 (t, ^3J (H-2,H-3) = ^3J (H-3,H-4) = 1.7 Hz, 1 H; H-3), 4.33 (d, ^3J (H-2,H-3) = 2.0 Hz, 1 H; H-2); 4.78 (d, ^3J (H,H) = 6.6 Hz, 1 H; OCH_2O), 5.00 (d, ^3J (H,H) = 5.8 Hz, 1 H; OCH_2O); 5.05 (d, ^3J (H,H) = 5,8 Hz, 1 H; OCH_2O), 5.27 (d, ^3J (H,H) = 6.6 Hz, 1 H; OCH_2O); ^{13}C NMR (125.7 MHz, $CDCl_3$, 25 ºC, TMS): δ = 49.7 (C-6), 52.7 (O*C*H$_3$), 66.9 (C-3), 71.6 (C-4), 74.7 (C-5), 76.5 (C-2), 88.6, 92.3 (OCH_2O), 167.7 (C=O). HRMS (ESI/Q-TOF) *m/z*: $[M + Na]^+$ calcd for $C_9H_{13}N_3O_6Na$ 282.0702; found: 282.0727.

***b*) From 10b.** To a solution of compound **10b** (0.45 g, 1.78 mmol) in DMF (4 mL) was added NaN_3 (0.22 g, 3.38 mmol). The mixture was stirred at 80 ºC for 2 days, filtered through Celite and the resulting solution was concentrated. Compound **11** (0.30 g, 82%) was isolated by column chromatography (20:80 hexane-EtOAc), and showed identical physical and spectroscopic properties as the compound described above.

Compound 4. To a solution of compounds **11** (0.263 g, 1 mmol) and **12**[26] (0.417 g, 1 mmol) in CH_3CN (5.5 mL), CuOAc (0.006 g, 0.05 mmol) was added. The mixture was subjected to Mw irradiation at 70 ºC for 1 h, under Ar atmosphere. Compound **4** precipitated from the reaction mixture and was recovered by centrifugation. The solid was subsequently washed with 0.16 M EDTA, H_2O and acetone. The product was isolated as a white solid (0.77 g, 86%). m.p. 209 ºC (recryst from DMSO-acetone); $[\alpha]_D^{25}$ = +49.0 (*c* = 0.5 in CH_2Cl_2); ^{1}H NMR (500 MHz, $[D_6]$DMSO, 25 ºC, TMS): δ = 2.43 (s, 3 H; C*H*$_3$Ph), 3,69 (s, 3 H; C*H*$_3$O), 3.70 (s, 1 H; H-4'), 3.80 (s, 1 H; H-4), 3.97 (m, ^3J (H-5',H-6'a) = 4.8 Hz, ^3J (H-5',H-6'b) = 8.5 Hz, 1 H; H-5'), 4.05 (s, 1H, H-3'), 4.19 (m, ^3J (H-5,H-6a) = 4.7 Hz, 1 H; H-5), 4.19 (d, ^3J (H-2',H-3') = 1.7 Hz, 1 H; H-2'), 4.27 (dd, ^3J (H-5',H-6'a) = 4.8 Hz, ^3J (H-6'a,H-6'b) = 11.4 Hz, 1 H; H-6'a), 4.30-4.31 (m, 2H; H-9a, H-3), 4.36 (m, ^3J (H-9b,NH) = 6.0 Hz, ^3J (H-6'a,H-6'b) = 15.2 Hz, 1 H; H-9b), 4.54 (m, ^3J (H-5,H-6'b) = 8.7 Hz, ^3J (H-6'a,H-6'b) = 11.4 Hz, 1 H; H-6'b), 4.61 (m, ^3J (H-2,H-3) = 1.7 Hz, ^3J (H-5,H-6a) = 4.7 Hz, ^3J (H-6a,H-6b) = 14.4 Hz, 2 H; H-2, H-6a), 4.72-4.77 (m, 5 H; O*CH*$_2$O), 5.07-5.11 (m, 4 H; H-6b, O*CH*$_2$O), 7.50 (d, ^3J (H,H) = 8.1 Hz, 2 H; H-aromatic), 7.81 (s, 1 H; H-7), 7.83 (d, ^3J (H,H) = 8.1 Hz, 2 H; H-aromatic), 8.17 (t, ^3J (H-9a,NH) = ^3J (H-9b,NH) = 6.0 Hz, 1 H; NH); ^{13}C NMR (125.7 MHz, $[D_6]$DMSO, 25 ºC, TMS): δ = 21.1 (*C*H$_3$-Ph), 34.1 (C-9), 46.1 (C-6), 51.2 (O*C*H$_3$), 66.2 (C-6'), 67.5 (C-3), 67.5 (C-3'), 69.1 (C-4'), 69.3 (C-4), 73.2 (C-5'), 74.4 (C-5), 75.5 (C-2), 76.7 (C-2´), 123.4 (C-7), 127.8, 130.2, 132.1, 145.2 (C-aromatic), 145.1 (C-8), 167.0 (HN*C*O),

167.8 (CH_3OCO). HRMS (ESI/Q-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{27}H_{34}N_4O_{14}SNa$ 693.1664; found: 693.1684.

Compound 13a. To a solution of compound **4** (0.300 g, 0.45 mmol) in anhydrous DMF (0.76 ml), NaN_3 (0.03 g, 0.46 mmol) was added. The mixture was stirred under N_2 at 60 ºC overnight. After concentration, compound **13a** (0.136 g, 56%) was obtained as a white crystalline solid by column chromatography (80:20 EtOAc-MeOH). m.p. 209-210 ºC (decomp) (recryst from H_2O); $[\alpha]_D^{25}$ = +56.4 (*c* = 0.5 in DMSO); ^{1}H NMR (500 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 3.41 (dd, ^3J(H-5',H-6'a) = 4.8 Hz, ^3J(H-6'a,H-6'b) = 13.2 Hz, 1H; H-6'a), 3.69 (s, 4 H; H-4', CH_3O), 3.80 (s, 1 H; H-4), 3.95 (dd, 1H, ^3J(H-5',H-6'a) = 4.8 Hz, ^3J(H-5',H-6'b) = 9.7 Hz, 1 H; H-5'), 4.09 (dd, 1H, ^3J(H-5',H-6'a) = 9.7 Hz, ^3J(H-6'a,H-6'b) = 13.2 Hz, 1 H; H-6'b), 4.14 (s, 1H; H-3'), 4.19 (dd, ^3J(H-5,H-6a) = 4.7 Hz, ^3J(H-5,H-6b) = 10.4 Hz, 1 H; H-5), 4.28-4.32 (m, ^3J(H-2',H-3') = 1.2 Hz, ^3J(H-7a,NH) = 5.9 Hz , 3 H; H-2', H-3, H-9a), 4.38 (dd, 1H, ^3J(H-9b,NH) = 6.0 Hz, ^3J(H-9a,H-9b) = 15.1 Hz, 1 H; H-9b), 4.60-4.64 (m, ^3J(H-5,H-6a) = 4.6 Hz, ^3J(H-6a,H-6b) = 10.4 Hz, 2 H; H-2, H-6a), 4.77 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 4.80 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 4.84 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 4.80 (d, 1H, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 5.00 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O); 5.05-5.11 (m, 4 H; H-6b, OCH_2O), 7.82 (s, 1 H; H-7), 8.19 (t, ^3J(H-9a,NH) = ^3J(H-9b,NH) = 6.0 Hz, 1 H; NH); ^{13}C NMR (125.7 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 34.1 (C-9), 46.1 (C-6), 46.6 (C-6'), 51.9 (OCH_3), 67.4 (C-3'), 67.5 (C-3), 69.3 (C-4), 70.1 (C-4'), 74.4 (C-5), 74.6 (C-5'), 75.5 (C-2), 76.8 (C-2´), 86.2, 86.4, 91.3 (OCH_2O), 123.3 (C-7), 145.0 (C-8), 167.1 (HNCO), 167.8 (CH_3OCO). HRMS (ESI/Q-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{20}H_{27}N_7O_{11}Na$ 564.1649; found: 564.1661. The byproduct **13b** was isolated as a white solid, from further fractions of the column (90:10 EtOAc-MeOH). Compound **13b** (0.05 g, 22%); m.p. 189-190 ºC (recryst from H_2O). $[\alpha]_D^{25}$ = +12.2 (*c* = 0.5 in DMSO). ^{1}H NMR (500 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 3.42 (dd, ^3J(H-5',H-6'a) = 5.0 Hz, ^3J(H-6'a,H-6'b) = 13.3 Hz, 1 H; H-6'a), 3.69 (s, 1 H; H-4'), 3.73 (s, 3 H; CH_3O), 3.81 (m, ^3J(H-5,H-6b) = 3.0 Hz, ^3J(H-4,H-5) = 7.3 Hz, ^3J(H-5,H-6a) = ^3J(H-5,OH) = 6.4 Hz, 1 H; H-5), 3.95 (m, ^3J(H-5',H-6'b) = 5.0 Hz, ^3J(H-5',H-6'b) = 9.9 Hz, 1 H; H-5'), 4.09 (dd, 1H, ^3J(H-5',H-6'a) = 9.9 Hz, ^3J(H-6'a,H-6'b) = 13.2 Hz, 1 H; H-6'b), 4.15 (s, 1 H; H-3'), 4.27 (dd, ^3J(H-3,H-4) = 2.2 Hz, ^3J(H-4,H-5) = 7.3 Hz, 1 H; H-4), 4.30 (d, 1H, ^3J(H-2',H-3') = 1.7 Hz, 1 H; H-2'), 4.32-4.37 (m, 3 H; H-6a, H-9), 4.51 (dd, ^3J(H-5,H-6b) = 3.0 Hz, ^3J(H-6a,H-6b) = 14.1 Hz, 1 H; H-6b), 4.81 (d, ^3J(H,H) = = 6.4 Hz, 1 H; OCH_2O), 4.83 (d, ^3J(H,H) = 6.5 Hz, 1 H; OCH_2O), 5.00 (d, ^3J(H-,H-) = 6.5 Hz, 1 H; OCH_2O), 5.06 (d, ^3J(H,H) = 5.8 Hz, 1 H; OCH_2O), 5.11 (d, ^3J(H,H) = 6.4 Hz, 1 H; OCH_2O), 5.27 (d, ^3J(H,H) = 5.8 Hz, 1 H; OCH_2O), 5.79 (s, ^3J(H-5,OH) = 6.4, 1 H; OH), 6.26 (d, ^3J(H-3,H-4) = 2.2 Hz, 1 H; H-3), 7.76 (2, 1 H; H-7), 8.17 (t, ^3J(H-9a,NH) = ^3J(H-9b,NH) = 6.1 Hz, 1 H; NH); ^{13}C NMR (125.7 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 34.2 (C-9), 46.6 (C-6'), 52.1, 51.2 (C-6, OCH_3), 67.4 (C-3'), 70.1 (C-4'), 70.9 (C-5), 73.5 (C-4), 74.6 (C-5'), 76.8 (C-2´), 86.4, 89.6, 91.3 (OCH_2O), 112.8 (C-3), 123.8 (C-7), 143.4 (C-2), 144.8 (C-8), 161.3 (CH_3OCO), 167.2 (HNCO). HRMS (ESI/Q-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{19}H_{25}N_7O_{10}Na$: 534.1534; found: 534.1555.

Microwave-assisted synthesis of 13a. To a solution of compound **4** (0.300 g, 0.45 mmol) in anhydrous DMF (0.76 ml), NaN_3 (0.03 g, 0.46 mmol) was added. The mixture was stirred under N_2 and subjected to Mw irradiation at 80 ºC for 5 h, until complete consumption of the starting **4**. After concentration, the residue was subjected to column chromatography (80:20 EtOAc-MeOH) to give compound **13a** (0.19 g, 81%) as a white crystalline solid, which showed identical physical and spectroscopic properties as the compound described above.

Compound 5. To a solution of compounds **12** (0.21 g, 0.51 mmol) and **13a** (0.28 g, 0.51 mmol) in DMF (5 mL) was added CuOAc (0.003 g, 0.024 mmol). The mixture was heated under Mw irradiation at 100 ºC for 30 min (Ar atmosphere). Compound **5** precipitated from the mixture and it was recovered by centrifugation. The solid was sequentially washed with 0.16 M EDTA, H_2O and acetone to give **5** (0.458 g, 94%) as a white solid. m.p. 259 ºC (recryst from DMSO-acetone); $[\alpha]_D^{25}$ = +67.2 (*c* = 0.4 in DMSO); ^{1}H NMR (500 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 2.43 (s, 3 H; CH_3Ph), 3.69 (s, 3 H; CH_3O), 3.70 (s, 1 H; H-4''), 3.80 (s, 2 H; H-4, H-4'), 3.96 (m, ^3J(H-5'',H-6''a) = 4.9 Hz, ^3J(H-5'',H-6''b) = 8.6 Hz, 1 H; H-5''), 4.05 (s, 1 H; H-3''), 4.18-4.21 (m, ^3J(H-5,H-6a) = 4.6 Hz, ^3J(H-5,H-6b) = 10.0 Hz, 3 H; H-5, H-5', H-2'), 4.25-4.42 (m, ^3J(H-5'',H-6''a) = 4.8 Hz, ^3J(H-6''a,H-6''b) = 11.3 Hz, 8 H; H-2', H-3, H-3', H-6''a, H-9, H-9'), 4.54 (m, 1H, ^3J(H-5'',H-6''b) = 8.8 Hz, ^3J(H-6'a,H-6'b) = 11.3 Hz, 1 H; H-6''b), 4.60-4.64 (m, ^3J(H-5,H-6a) = ^3J(H-5',H-6'a) = 4.6 Hz, ^3J(H-6a,H-6b) = ^3J(H-6'a,H-6'b) = 14.4 Hz, 3 H; H-2, H-6a, H-6'a), 4.72-4.82 (m, 7 H; OCH_2O), 5.07-5.11 (m, 7 H; H-6b, H-6'b, OCH_2O), 7.49 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 7.81 (s, 1 H; H-7'), 7.83 (s, 1 H; H-7), 7.83 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 8.17 (t, ^3J(H-9'a,NH') = ^3J(H-9'b,NH') = 6.0 Hz, 1 H; NH''), 8.19 (t, ^3J(H-9a,NH) = ^3J(H-9b,NH) = 6.0 Hz, 1 H; NH); ^{13}C NMR (125.7 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 21.1 (*C*H_3-Ph), 34.1 (C-9, C-9'), 46.1 (C-6, C-6'), 51.9 (OCH_3), 66.2 (C-6''), 67.2, 67.4 (C-3, C-3'), 67.5 (C-3''), 69.1 (C-4''), 69.3; 69.8 (C-4, C-4'), 73.1 (C-5''), 74.4 (C-5, C-5'), 75.5 (C-2), 76.7 (C-2''), 76.8 (C-2'), 86.7 87.2; 91.7 (OCH_2O), 123.3 (C-7, C-7'), 127.8; 130.2; 132.1 (C-aromatic); 144.19 (C-8, C-8'), 145.2 (C-aromatic), 167.0, 167.1 (CONH); 167.8 (*C*O$_2$CH$_3$). HRMS (ESI/Q-TOF) *m/z*: [M + H]$^+$ calcd for $C_{38}H_{49}N_8O_{19}S$ 953.2829; found: 953.2848; [M + Na]$^+$ calcd for $C_{38}H_{48}N_8O_{19}SNa$ 975.2649; found: 975.2669.

Compound 14. To a solution of compound **5** (0.30 g, 0.32 mmol) in anhydrous DMSO (4.3 mL) was added NaN_3 (0.04 g, 0.64 mmol). The reaction mixture was stirred under Ar atmosphere at 100 ºC for 24 h. Compound **14** precipitated from the reaction mixture upon addition of acetone and it was recovered by centrifugation. The solid was sequentially washed with acetone-H_2O (90:10) and acetone. Compound **14** (0.21 g, 80%). m.p. 229 ºC (recryst from DMSO-acetone); $[\alpha]_D^{25}$ +67.0 (*c* = 0.6; DMSO); ^{1}H NMR (500 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 3.42 (dd, ^3J(H-5'',H-6''a) = 4.9 Hz, ^3J(H-6''a,H-6''b) = 13.3 Hz, 1 H; H-6''a), 3.69 (s, 1 H; CH_3O), 3.71 (s, 1 H; H-4''), 3.80 (s, 2 H; H-4, H-4'), 3.95 (dd, ^3J(H-5'',H-6''a) = 4.9 Hz, ^3J(H-5'',H-6''b) = 9.8 Hz, 1 H; H-5''), 4.09 (dd, ^3J(H-5'',H-6''a) = 9.8 Hz, ^3J(H-6''a,H-6''b) = 13.3 Hz, 1 H; H-6''b), 4.15 (s, 1 H; H-3''), 4.19 (m, 2 H; H-5, H-5'), 4.29 (d, ^3J(H-2'',H-3'') = 1.68 Hz, 1 H; H-2''), 4.30 (s, 2 H; H-3, H-3'), 4.32 (d, ^3J(H-2',H-3') = 1.43 Hz, 1 H; H-2'), 4.28-4.40 (m, 4 H; H-9, H-9'), 4.62-4.64 (m, 3 H; H-2, H-6a, H-6'a), 4.76-4.85 (m, 6 H; OCH_2O), 5.00 (d, ^3J(H,H) = 6.6 Hz, 1 H; OCH_2O), 5.06-5.12 (m, 7 H; H-6b, H-6'b, OCH_2O), 7.82, 7.83 (2s, 2 H; H-7, H-7'), 8.19 (m, 2 H; NH, NH'); ^{13}C NMR (125.7 MHz, [D_6]DMSO, 25 ºC, TMS): δ = 34.1 (C-9, C-9'), 46.1 (C-6, C-6'), 46.6 (C-6''), 51.9 (OCH_3), 67.2, 67.3 (C-3, C-3'), 67.4 (C-3''), 69.3, 69.8 (C-4, C-4'), 70.1 (C-4''), 74.4, 74.5 (C-5, C-5'), 74.6 (C-5''), 75.5 (C-2), 76.8 (C-2, C-2'), 86.2, 86.4, 91.3, (OCH_2O), 123.3 (C-7, C-7'), 145.0 (C-8, C-8'), 167.1 (*C*ONH, *C*ONH'), 167.8 (*C*O$_2$CH$_3$). HRMS (ESI/Q-TOF) *m/z* [M + Na]$^+$ calcd for $C_{31}H_{41}N_{11}O_{16}Na$ 846.2630; found: 846.2652.

Microwave-assisted synthesis of 14. To a solution of compound **5** (0.11 g, 0.11 mmol) in dry DMSO (1.5 mL) was added NaN_3 (0.02 g, 0.25 mmol). The mixture was heated under Mw irradiation at 100 ºC for 1 h (Ar atmosphere). Compound **5** (0.07 g, 70%) was isolated by the same procedure described for **13a**.

Compound 6. To a solution of compounds **12** (0.15 g, 0.19 mmol) and **14** (0.08 g, 0.19 mmol) in anhydrous DMSO (5 mL) CuOAc (0.001 g, 0.01 mmol) was added. The mixture was heated under Mw irradiation at 100ºC for 30 min (Ar atmosphere). Upon cooling, compound **6** precipitated off and it was recovered by centrifugation. The solid was sequentially washed with 0.16 M EDTA, H_2O and acetone to give compound **6** (0.17 g, 78%) as a white solid. m.p. 250 ºC (decomp)

(recryst from DMSO-acetone); $[\alpha]_D^{25}$ = +72.3 (*c* = 0.3 in DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 2.08 (s, 3 H; C*H*$_3$Ph), 3.69 (s, 3 H; C*H*$_3$O), 3.70 (s, 1 H; H-4''), 3.79 (s, 3 H; H-4, H-4'), 3.96 (m, ^3J(H-5'',H-6''a) = 4.8 Hz, ^3J(H-5'',H-6''b) = 8.5 Hz, 1 H; H-5''), 4.05 (s, 1 H; H-3''), 4.18-4.22 (m, 4 H; H-5, H-5', H-2'), 4.30 (s, 3 H; H-3, H-3'), 4.32 (s, 2 H; H-2'), 4.25-4.42 (m, 7 H; H-6''a, H-9, H-9'); 4,54 (m, ^3J(H-5'',H-6''b) = 8,5 Hz, ^3J(H-6''a,H-6''b) = 11,4 Hz, 1 H; H-6''b); 4,60-4,64 (m, 4 H; H-2, H-6a, H-6'a); 4.72-4.82 (m, 9 H; OCH$_2$O), 5.05-5.12 (m, 11 H; H-6b, H-6'b, OCH$_2$O), 7.50 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 7.81-7.84 (m, 5 H; H-7, H-7', H-7'',H-aromatic), 8.16 (t, ^3J (H-9''a,NH'') = ^3J (H-9''b,NH'') = 6.0 Hz, 1 H; NH''), 8.19 (t, ^3J(H-9a,NH) = ^3J(H-9b,NH) = 6.0 Hz, 2 H; NH, NH'); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 21.1 (*C*H$_3$-Ph), 34.2 (C-9, C-9', C-9''), 46.1 (C-6, C-6'), 51.9 (OCH$_3$), 66.2 (C-6''), 67.2 (C-3'), 67.4 (C-3), 67.5 (C-3''), 69.1 (C-4''), 69.3 (C-4), 69.8 (C-4'), 73.1 (C-5''), 74.4 (C-5, C-5'), 75.5 (C-2), 76.7 (C-2''), 76.8 (C-2'), 86.2, 86.8, 91.3, 91.4 (OCH$_2$O), 123.4 (C-7, C-7', C-7''), 127.8, 130.2, 132.1 (C-aromatic), 145.0 (C-8, C-8', C-8''), 145.2 (C-aromatic), 167.0, 167.1 (CONH, CONH'), 167.8 (*C*O$_2$CH$_3$). HRMS (ESI/Q-TOF) *m/z*: [M + H]$^+$ calcd for $C_{49}H_{63}N_{12}O_{24}$ 1235.3750; found: 1235.3722.

Compound 15. To a solution of compound **4** (0.37 g, 0.56 mol) in MeOH-H$_2$0 2:1 (12 mL), 5% LiOH in MeOH-H$_2$0 2:1 was added dropwise until reaching pH 8. The solution was stirred at room temperature for 5 h, keeping pH 8. Finally, the solution was acidified to pH 5, upon addition of Dowex 50WX4 (H$^+$). The mixture was filtered and concentrated to give compound **15** (0.31 g, 85%) as a white solid. m.p. 210 °C (decomp) (recryst from H$_2$O-acetone); $[\alpha]_D^{25}$ = +52.3 (*c* = 0.6 in DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 2.43 (s, 3 H; C*H*$_3$Ph), 3.70 (s, 1 H; H-4'), 3.77 (s, 1 H; H-4), 3.96 (m, ^3J(H-5',H-6'a) = 4.8 Hz, ^3J(H-5',H-6'b) = 8.6 Hz, 1 H; H-5'), 4.05 (s, 1 H; H-3'), 4.17-4.20 (m, ^3J(H-2',H-3') = 1.7 Hz, ^3J(H-5,H-6a) = 4.6 Hz, ^3J(H-5,H-6b) = 10.6 Hz, 2 H; H-2', H-5), 4.25-4.28 (m, ^3J(H-5',H-6'a) = 5.9 Hz, ^3J(H-9a,NH) = 5.9 Hz, 3 H; H-3, H-6'a, H-9a), 4.61 (m, ^3J(H-9b,NH) = 5,9 Hz, ^3J(H-9a,H-9b) = 14.6 Hz, 1 H; H-9b), 4.44 (d, ^3J(H-2,H-3) = 1.8 Hz, 1 H; H-2), 4.54 (m, ^3J(H-5',H-6'b) = 8.6 Hz, ^3J(H-6'a,H-6'b) = 11.3 Hz, 1 H; H-6'b), 4.61 (m, ^3J(H-5,H-6a) = 4.6 Hz, ^3J(H-6a,H-6b) = 14.5 Hz, 1 H; H-6a), 4.71-4.77 (m, 5 H; OCH$_2$O), 5.04-5.12 (m, 4 H; H-6b, OCH$_2$O), 7.50 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 7.81 (s, 1 H; H-7), 7.83 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 8.16 (t, 1 H; NH); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 21.1 (*C*H$_3$-Ph), 34.1 (C-9), 46.1 (C-6), 66.2 (C-6'), 67.5 (C-3'), 67.6 (C-3), 69.1 (C-4'), 69.5 (C-4), 73.1 (C-5'), 74.4 (C-5), 75.4 (C-2), 76.7 (C-2'), 86.2, 86.8, 91.3 (OCH$_2$O), 123,3 (C-7), 127.8, 130.2, 132.1 (C-aromatic), 145.0 (C-8), 145.2 (C-aromatic), 167.0 (CONH), 168.7 (CO$_2$H). HRMS (ESI/Q-TOF) *m/z*: [M + H]$^+$ calcd for $C_{26}H_{33}N_4O_{14}S$ 657.1708; found: 657.1681.

Compound 16. To a solution of compound **15** (0.25 g, 0.39 mmol), DIPEA (0.54 mL, 1.10 mmol) and TBTU (0.12 g, 0.39 mmol) in anhydrous DMF (3.2 mL), propargylamine (30 µL, 0.47 mmol) was added. The solution was stirred at room temperature for 24 h and then concentrated. The solid obtained was washed with MeOH and H$_2$O, to give **16** as a white solid (0.21 g, 80%). m.p. 245 °C (recryst from DMSO-acetone); $[\alpha]_D^{25}$ = +68.8 (*c* = 0.5 in CH$_2$Cl$_2$); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 2.43 (s, 3 H; C*H*$_3$Ph), 3.04 (s, 1 H; H-3''), 3.70 (s, 1 H; H-4'), 3.75-3.80 (m, 2 H; H-1''a, H-4), 3.95 (m, 2 H; H-1''b, H-5'), 4.05 (s, 1 H; H-3'), 4.16-4.19 (m, 2 H; H-2', H-5), 4.24-4.28 (m, 5 H; H-2, H-3, H-6'a, H-9), 4.54 (m, ^3J(H-5,H-6'b) = 8.9 Hz, ^3J(H-6'a,H-6'b) = 11.3 Hz, 1 H; H-6'b), 4.62 (m, ^3J(H-5,H-6a) = 4.3 Hz, ^3J(H-6a,H-6b) = 14.0 Hz, 1 H; H-6a), 4,71-4,77 (m, 3 H; OCH$_2$O); 4.81 (d, 1 H; OCH$_2$O); 5.04-5.12 (m, 5 H; H-6b, OCH$_2$O), 7.50 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 7.80 (s, 1 H; H-7), 7.83 (d, ^3J(H,H) = 8.1 Hz, 2 H; H-aromatic), 8.13-8.16 (m, 2 H; NH, NH''); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 21.1 (*C*H$_3$-Ph), 27.7 (C-1''), 34.1 (C-9), 46.1 (C-6), 66.1 (C-6'), 67.1 (C-3), 67.5 (C-3'), 69.1 (C-4'), 69.7 (C-4), 72.5 (C-3''), 73.1 (C-5'), 74.4 (C-5), 76.7 (C-2, C-2'), 81.1 (C-2''), 86.2, 86.8, 91.2, 91.4 (OCH$_2$O), 123.3 (C-7), 127.8, 130.2, 132,1 (C-aromatic), 144.9 (C-8); 145.2 (C-aromatic); 166.9, 167.0 (CONH, CONH'). HRMS (ESI/Q-TOF) *m/z*: [M + H]$^+$ calcd for $C_{29}H_{36}N_5O_{13}S$ 694.2025; found: 694.2043.

Compound 7. To a solution of compounds **14** (0.080 g, 0.09 mmol) and **16** (0.060 g, 0.09 mmol) in dry DMSO (1.1 mL), CuOAc (0.006 g, 0.005 mmol) was added. The mixture was subjected to Mw irradiation at 100 °C for 30 min (Ar atmosphere). Compound **7** precipitated from the mixture upon addition of acetone and it was collected by centrifugation. The solid was sequentially washed with 0.16M EDTA, H$_2$O and acetone, to give compound **7** (0.12 g, 84%) as a white solid. m.p. 253 ºC (decomp) (recryst from DMSO-acetone); $[\alpha]_D^{25}$ +624.2 (*c* 0,06; DMSO); ^{1}H NMR (500 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 2.42 (s, 3 H; C*H*$_3$Ph), 3.69 (s, 4 H; H-4'', C*H*$_3$O), 3.79 (s, 4 H; H-4, H-4'), 3.96 (m, 1 H; H-5''), 4.04 (s, 1 H; H-3''), 4.20 (m, 5 H; H-5, H-5', H-2''), 4.29 (s, 4 H; H-3, H-3'), 4.31 (s, 3 H; H-2'), 4.25-4.41 (m, 9 H; H-6''a, H-9, H-9'), 4.53 (m, 1 H; H-6''b); 4,60-4,62 (m, 5 H; H-2, H-6a, H-6'a), 4.73-4.82 (m, 11 H; OC*H*$_2$O), 5.04-5.11 (m, 13 H; H-6b, H-6'b, OC*H*$_2$O), 7.50 (d, ^3J (H,H) = 8.3 Hz, 2 H; H-aromatic), 7.81-7.83 (m, 6 H; H-7, H-7', H-aromatic), 8.16-8.20 (m, 4 H; NH, NH'); ^{13}C NMR (125.7 MHz, [D$_6$]DMSO, 25 °C, TMS): δ = 21.2 (*C*H$_3$-Ph), 34.2 (C-9, C-9'), 46.1 (C-6, C-6'), 51.9 (O*C*H$_3$), 66.2 (C-6''), 67.2 (C-3'), 67.4 (C-3, C-3''), 69.2 (C-4''), 69.4 (C-4), 69.8 (C-4'), 73.2 (C-5''), 74.4, 74.5 (C-5, C-5'), 75.6 (C-2), 76.7 (C-2''), 76.8 (C-2'), 86.3, 86.8, 91.3, 91.4 (O*C*H$_2$O), 123.4 (C-7, C-7'), 127.8, 130.3, 132.1 (C-aromatic), 145.0 (C-8, C-8'), 145.2 (C-aromatic), 167.1 (HN*C*O, HN*C*O'), 167.9 (CH$_3$O*C*O). HRMS (ESI/Q-TOF) *m/z*: [M + Na]$^+$ calcd for $C_{60}H_{76}N_{16}O_{29}SNa$: 1539.4577; found: 1539.4574.

ESI MS measurements.

Experiments were conducted using a Waters SQD2 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. The instrument was operated in positive ion mode with a probe capillary voltage of 3.5 kV and in negative ion mode with a capillary voltage of 3.0 kV. The sampling cone voltage was set to 45.0 V. The source and desolvation gas temperatures were set to 150 and 350 °C, respectively. The nitrogen gas desolvation flow rate was 550 L h^{-1}, the cone gas flow rate was 10 L h^{-1}. The mass spectrometer was calibrated across the range of *m/z* 20–2023 with a sodium and cesium iodide solution. Data were acquired in scan mode with a scan duration of 0.2 sec and unit resolution. Samples were introduced into the ESI source using direct infusion at a flow rate of 10 µL min^{-1}, and each run was acquired for 2 min. Technical duplicates were acquired in all cases. Data acquisition and processing were carried out using MassLynx, version 4.1 software.

Job plot. Solutions of compounds **4**-**6** (10 µM in 1:1 acetonitrile-water) or compound **7** (5 µM in 3:3:4 acetonitrile-water-*i*PrOH) were prepared. To these solutions, 15-crown-5 was added in the amount required to reach the same concentration as the oligo(amide-triazole). Convenient volumes of host stock solution of oligo(amide-triazole)s **4-6** were mixed with 10 µM KCl solution in 1:1 acetonitrile-water (5 µM KCl in 3:3:4 acetonitrile-water-*i*PrOH for **7**), containing also 15-crown-5. The final volume for each individual experiment was 2 mL. Thus, crown ether and total (host + guest) concentrations were kept constant during the experiments.

NMR measurements. Job plot. Convenient volumes of solutions of oligo(amide-triazole)s **4**-**7** (5 mM in [D$_6$]DMSO) were mixed with 5 mM TMA chloride solutions, in order to have a total 600 µL volume with constant 5 mM (host + guest) concentration, while the molar fraction was varied. The change in the chemical shift of triazole protons was measured, relative to that of the pure compound.

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