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Hydrogelation and spontaneous fiber formation of 8-aza-7-deazaadenine nucleoside 'click' conjugates

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ABSTRACT

Nucleoside hydrogels based on benzyl azide 'click' conjugates of 8-aza-7-deaza-2′-deoxyadenosine bearing 7-ethynyl, 7-octa-(1,7-diynyl), and 7-tri-prop-2-ynyl-amine side chains were synthesized (**1**, **3**, **4**). The cycloaddition adduct with the shortest linker (**1**) yields the most powerful hydrogelator forming stable gels at a concentration of 0.3 wt % of **1** in water. One molecule of **1** catches 7500 water molecules. Cycloaddition of the 8-aza-7-deaza-7-azido-2′-deoxyadenosine (**9**) and 3-phenyl-1-propyne (**10**) leads to the isomeric conjugate **2**, with a C–N connectivity between the nucleobase and triazole moiety. This gel is less stable than that of the adduct **1**. Both gels show a similar stability over a wide pH range (4.0–10.0). Xerogels of **1** and **2** studied by scanning electron microscopy (SEM) reveal that both click adducts (**1** and **2**) form long fibers spontaneously.

1. Introduction

Low molecular weight gelators (LMWGs)[1–3] have received attention because of their potential use in tissue engineering,[4] targeted drug delivery,[5] light harvesting,[6] or as nanomaterials.[7–9] In such 'physical' gels small subunits are linked non-covalently, which is different from the more common gels that are based on polymeric gelators. The dynamic balance between hydrophilic and hydrophobic interactions of gelator molecules plays a defining role in the aggregation of molecules in a given solvent. The self-assembly of low molecular weight building blocks, which results from supramolecular entanglements of molecules or chemically cross-linked species into a network, traps liquid in the network leading to a self-supporting gel.[10–12] Such hydrogels are of great importance because of their capability to entrap a large number of water molecules. Hydrogels can form nanotubes or nanofibers, which find extensive applications in material science, diagnostics, medicine and supramolecular chemistry.[4,5,7–9]

Among the monomeric naturally occurring compounds forming gels are amino acids,[13–18] sugars,[19–21] nucleosides[22–26] and other chemical entities.[27–30] Nucleosides themselves seldom offer the possibility of gelation but when these nucleosides are modified at certain positions and/or linked to residues, which can cause a variation in the hydrophilic–hydrophobic forces, gels are formed. Guanine was the first and foremost nucleobase tried and tested for molecular assemblies. In 1904, Bang[31] showed that guanosine and its analogs, mainly guanylic acid (GMP), form gels in aqueous solution. In 1962, Gellert[32] proposed that four guanines are related to each other by the operation of fourfold rotation axis, leading to a planar tetramer arrangement. Later in 1971, Guschlbauer[33] and others[34–36] showed that, not only guanosine but also several of its analogs form gels. Further, it was demonstrated that isoguanosine[37] can also form gels; our laboratory showed that gels of 2′-deoxyisoguanosine formed in 0.5 M NaCl are stable.[38] This gel formation is cation dependent. Most of the hydrogel forming nucleosides were found by serendipity and not by rational design. Nucleosides can easily bind water molecules as they contain a variety of donor and acceptor centers.

Over the years, a number of LMW gelators have been synthesized,[1–3,22–26] and this concept is extended to nucleoside based gelators because of their excellent biocompatibility. Click conjugates of 2′-deoxyuridine, prepared by the Huisgen–Meldal–Sharpless cycloaddition were reported to form stable hydrogels.[24] Here we selected the artificial nucleoside 8-aza-7-deaza-2′-deoxyadenosine, which was decorated with various side chains at the 7-position for gelation studies.[39–41] Triazole moieties with a lipophilic residue were introduced by click conjugation, and the gelation behavior was studied in water. Each derivative is an amphiphile featuring multiple functionalities derived from a combination of lipophilic and

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hydrophilic units (Fig. 1). While nucleoside conjugates **1** and **2** have an almost identical structure, compound **3** represents a molecule with a long linker arm and **4** with a branched side chain decorating the nucleoside with two proximal benzyl and 1,2,3-triazole moieties. With those compounds in hand, the relationship of nucleoside structure with gel formation was studied, xerogels were formed and analyzed by scanning electron microscopy. Equimolar mixtures of isomeric nucleosides (**1** and **2**) behave differently than the pure components. The influence of base modification will be presented along with experiments using nucleoside conjugates with related or complementary nucleobases.

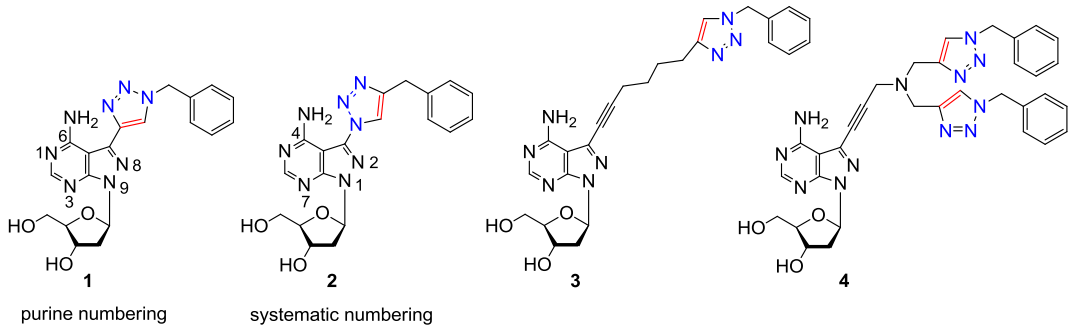


Fig. 1. The benzyltriazole appended nucleosides with variable linker arms and branched side chain used in this study.

2. Results and discussion

2.1. Synthesis and characterization of monomers

The nucleosides bearing short (**7**), long (**11**),[41] and branched (**12**) linker arms were prepared by *Sonogashira* cross coupling reaction of common iodo precursor **5**[39–41] and respective alkynes as shown in Scheme 1. At first, the trimethylsilyl compound **6** was obtained by *Sonogashira* cross coupling from the iodo precursor **5**. Further deprotection of the silyl group with anhydrous K_2CO_3 gave the free nucleoside **7** (85%). Then the [3+2] cycloaddition click reaction[42–45] was performed in THF/H_2O/*t*-BuOH (3:1:1) with benzyl azide **8** to give the click adduct **1** in 88% yield. Its regioisomer **2** was synthesized by reacting iodo-precursor **5** with the alkyne **10** under microwave assistance.[46] Nucleoside azide **9** was formed as intermediate in situ, which was not isolated. This intermediate subsequently undergoes 'click' reaction with 3-phenyl-1-propyne **10**, to yield **2** as the *N*-regioisomer of **1** in 83% yield. Nucleoside **12** bearing a branched side chain, was prepared in 67% yield, which was further clicked with benzyl azide as described above to give the click adduct **4** in 76% yield. The click adduct **3** and its precursor **11** were prepared as reported in literature[41] and characterized.

The closely related regioisomers **1** and **2** can be easily identified by characteristic signals in ^{1}H and ^{13}C NMR spectra. In click adduct **1** the amine protons are found at 8.13 and 9.10 ppm whereas its regioisomer **2** shows the respective protons at 8.19 and 8.36 ppm. In ^{13}C NMR the C5-carbon for click adduct **1** appears at 97.9 ppm, whereas it is shifted to 91.7 ppm in its regioisomer **2** (Supplementary data). Furthermore, it was observed in the ^{1}H NMR spectrum that the protons of the amino groups of compounds **1** and **2** are sharp and clearly separated (Supplementary data, Figs. S8 and S10) while those of the 7-deazapurine conjugate **13** form a broad signal (Fig. S16). From that we conclude that one of the NH_2 protons of compound **1** and **2** is involved in hydrogen bonding with a triazole nitrogen as acceptor site. This is a consequence of the higher acidity of the 6-amino group protons of the 8-aza-7-deazaadenine conjugates **1** and **2** compared to that of the 7-deazapurine analog **13**. An additional factor is the proximity of the triazole ring to one of the amino group protons thereby forming a seven-membered ring system. This assumption is further supported by compound **4**, which does not show a separation of the proton signals (Fig. S14) due to the distant position of the triazole moiety. These phenomena might influence the gelation process.

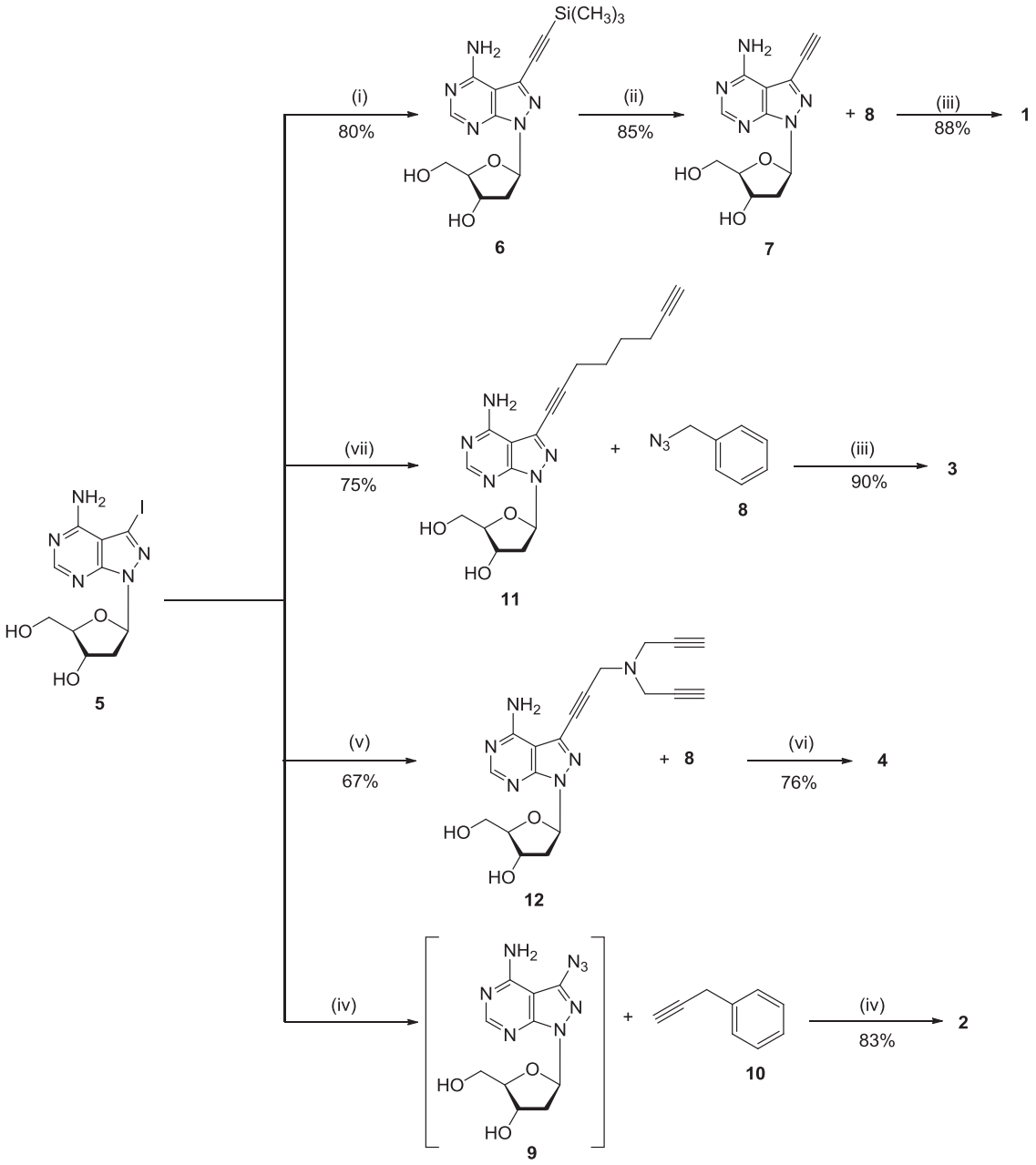
The click conjugation and the nature of the connectivity of triazole system have decisive impact on the pattern of UV/vis spectra. As shown in Fig. 2, when the triazole ring is connected to the base through a C–C bond (**1**), a strong absorption maximum at 254 nm was observed along with a shoulder at 289 nm. But when the triazole is connected to the base through a C–N bond as in case of **2**, the absorption maximum is found at 287 nm with a 265 nm shoulder. So, the electronic properties of the nucleobase will be changed by the connectivity of the triazole system. This might cause changes in the stacking properties of the base moiety.

2.2. Water gelation of nucleoside conjugates

In the above mentioned click adducts, like in conventional amphiphiles, the nucleoside conjugates display a polar head group (sugar moiety), which is responsible for the water solubility and other more or less hydrophobic units (nucleobase, 1,2,3-triazole moiety and benzyl residues) that offer aid to aggregation. In all compounds investigated in this study, the molecules form a glycosylic linkage to a 2′-deoxyribofuranosyl moiety, which is directly connected to the nucleobase; the triazole system is either directly attached (**1** and **2**) or linked by branched or non-branched arms (conjugates **3** and **4**). Regarding base modification, we studied 8-aza-7-deazaadenine derivatives along with conjugates displaying a 7-deazaadenine base (**13**) (Scheme 2); the corresponding uridine compound **14**[24] (Supplementary data) was used in base pairing studies and for comparison.

2.3. Stability and rheology of gels

To study the gelation ability of adducts **1**–**4**, they were suspended in a given volume of water, heated to a clear solution and cooled. Gel formation was established when the gel passes the tube-inversion test.[24] The click adduct **1** forms a robust gel in a given volume of water with a minimum gelation concentration (MGC) of 3 mg/mL, thereby demonstrating its excellent gelation behavior toward water (Fig. 3a). Further increases in the concentration of the click adduct had a further positive effect on gel stability. This has been tested up to a concentration of 11 mg/mL of compound **1**, which leads to an even more stable gel. Next, the gelation behavior of the click adducts bearing long (**3**) and branched (**4**) linker arms was studied. Conjugate **3** did not form a gel at all, neither at low nor at high concentrations (Table 1). Apparently, the increased hydrophobic character arising from the



Scheme 1. Reagents and conditions: (i) Trimethylsilylacetylene, CuI, $[Pd^0[P(Ph_3)_4]$, Et_3N, DMF, rt, 12 h; (ii) anhydrous K_2CO_3, MeOH, rt, 2 h; (iii) benzyl azide, $CuSO_4 \cdot 5H_2O$, Na-ascorbate, THF/H_2O/*t*-BuOH, rt, 12 h; (iv) NaN_3, L-proline, $CuSO_4$, Na-ascorbate, $Na_2CO_3 \cdot 10H_2O$, DMF/H_2O (4:1), MW, 30 min, 130 °C; (v) tripropargylamine, CuI, $[Pd^0[P(Ph_3)_4]$, Et_3N, DMF, rt, 12 h; (vi) $CuSO_4 \cdot 5H_2O$, Na-ascorbate, EtOH/H_2O (1:1), rt, 12 h; (vii) octa-(1,7)-diyne, CuI, $[Pd^0[P(Ph_3)_4]$, Et_3N, DMF, rt, 12 h.

additional methylene groups in the linker units perturbed the balance between the hydrophilic–hydrophobic properties. Then, the branched nucleoside adduct **4** was tested. With an MGC of 2 mg/mL, conjugate **4** forms a colloidal solution, which turns to a clear solution in 24 h. Within 2–3 weeks it forms very fine fibers as shown in Fig. 3d. In contrary, the nucleosides **5**, **7**, **11**, and **12** bearing no clicked side chain did not form a gel.

Next, the gelation ability of compound **2** (Fig. 3b) was compared with conjugate **1** (Fig. 3a). Even though molecules **1** and **2** are of the same size and have an almost identical structure, conjugate **2** behaves differently with regard to gelation than nucleoside **1**. The gel is less stable at a low nucleoside concentration and an increase of the nucleoside concentration did not increase the stability significantly, while conjugate **1** became more stable at higher nucleoside concentration. This indicates that the triazole ring connectivity formed during click reaction plays a decisive role in the gelation process. Then, the gelation ability of conjugate **1** in the presence of conjugate **2** in an equimolar mixture was tested (Fig. 3c). As indicated in Table 1, the gelation ability of adducts **1** and **2** was found to be existing in the mixture of both conjugates. However, while individual conjugates formed stable gels at a concentration of 3 mg/mL, very unstable gels were formed by the mixture, which did not pass the 'tube-inversion' test. The hydrogel behavior in the tube-inversion test is demonstrated in Fig. 3a–c. A T_{gel} of 61 °C was determined for compound **1** (c=3 mg/mL) by a dropping ball experiment as described in literature.[28]

As it is known that hydrogen bonding plays a vital role in the balance of counteracting forces—hydrophilic hydrophobic forces—we wanted to study whether base pairing influences the gel formation.[22,35] For this, the click adduct of benzyl azide and

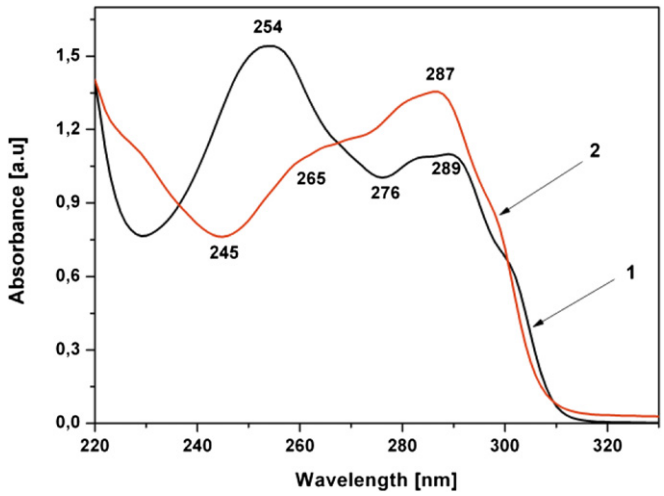
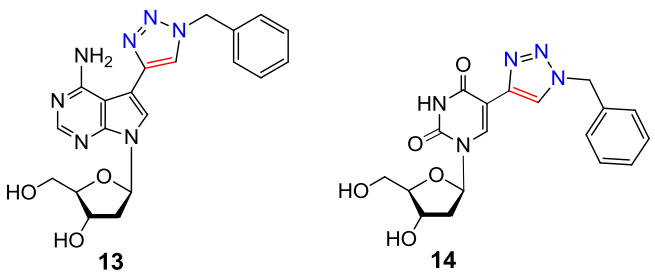


Fig. 2. UV profiles of the click nucleoside conjugates **1** and **2**. The measurements were performed in methanol (0.98 μM).



Scheme 2.

5-ethynyl-2′-deoxyuridine (**14**) was synthesized as reported (see Supplementary data), and its gelation ability was already confirmed.[24] Then, equimolar amounts of compound **1** and the complementary uridine adduct **14** (both dissolved in water) were tested for gelation. The mixture of **1** and **14** resulted into a clear solution, whereas these individual conjugates form stable gels. The results of all gel formation studies are summarized in Table 1.

Changes of pH of the gelating systems have been found to alter the properties of gels.[47–49] This prompted us to investigate the hydrogelation of the nucleoside conjugates at different pH values. Slightly acidic (pH 4.0 and pH 5.0) as well as alkaline conditions (pH 9.0 and 10.0) were chosen, and gel formation of the nucleoside

Table 1
Minimum gelation concentrations (MGCs) of nucleoside click conjugates forming hydrogels[a]

Compd	MGC (mg/mL)	Gel	Compd	MGC (mg/mL)	Gel
1	0.1	u	**1**+**2**	0.1–0.5	u
	0.2	u	**1**+**4**	0.1–0.5	n
	0.3–0.5	s	**2**+**4**	0.3	n
2	0.1	u	**3**	0.1–0.5	n
	0.2	u	**4**	0.1–0.3	f
	0.3–0.5	s			

[a] s: stable gel; u: unstable gel; n: no gel. f: fiber. MGCs provided in parentheses (wt %).

conjugates **1** and **2** was studied. Under all those pH values, no changes in gel stability were observed in case of 'adenine' click conjugate **1**. So, there is no pH response within a pH range of 4–10 in the case of gel **1** (pK_a value of **1** is 3.89). But when the pH dependent gelation property of the already known 'uridine' adduct **14**[24] was compared, destabilization of **14** occurred at higher pH values (pH=10) due to the deprotonation of the nucleobase (pK_a value of **14** is 8.74; see Supplementary data, Fig. S2).

The transition phenomenon of viscous fluid (sol) to an elastic solid (gel), which comprises a 3D-supramolecular network is referred as gelation. In order to determine the viscoelastic properties of gels, rheological experiments were performed. The storage modulus and loss modulus were measured and are expressed as G′ and G″, respectively. For a gel, the elastic modulus G′ must be relatively independent of frequency of deformation, and G′ must be greater than G″.[16] As it is demonstrated in Fig. 4, these two characteristics are observed where G′ and G″ were depicted as a function of frequency.

In Fig. 4a–c, the storage modulus G′ is always higher than the loss modulus G″ for both, compound **1** and its *N*-isomer compound **2**, and even for the mixture of compounds **1** and **2**, when measured at 25 °C. As the temperature was increased, the difference between G′ and G″ decreases and eventually both merges together in case of compound **1** (see Supplementary data) depicting that at higher temperature the transition from gel to sol occurs leading to a destruction of the gel. But the situation is somewhat different in case of the *N*-isomer **2**. The crossover between G′ and G″ exists at 50 Hz at 25 °C, and an additional crossover point appears around 10 Hz at 75 °C (see Supplementary data). This demonstrates that the gel is less stable at such frequencies. On the contrary, the mixture of compounds **1** and **2** does not show the crossover point at 25 °C (Fig. 4c), but the magnitude of both moduli is lower than for the individual compounds. Taken together, the gel formed by

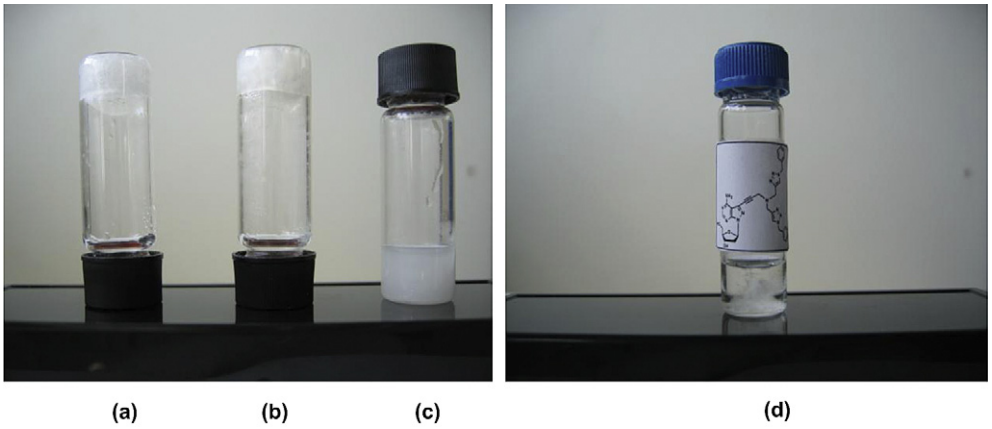


Fig. 3. Tube-inversion test depicting gelation: Gelation test for (a) click adduct **1**. (b) Click adduct **2**. (c) Equimolar mixture of **1** and **2**. (d) Image of branched adduct **4** depicting fiber formation rather than gelation.

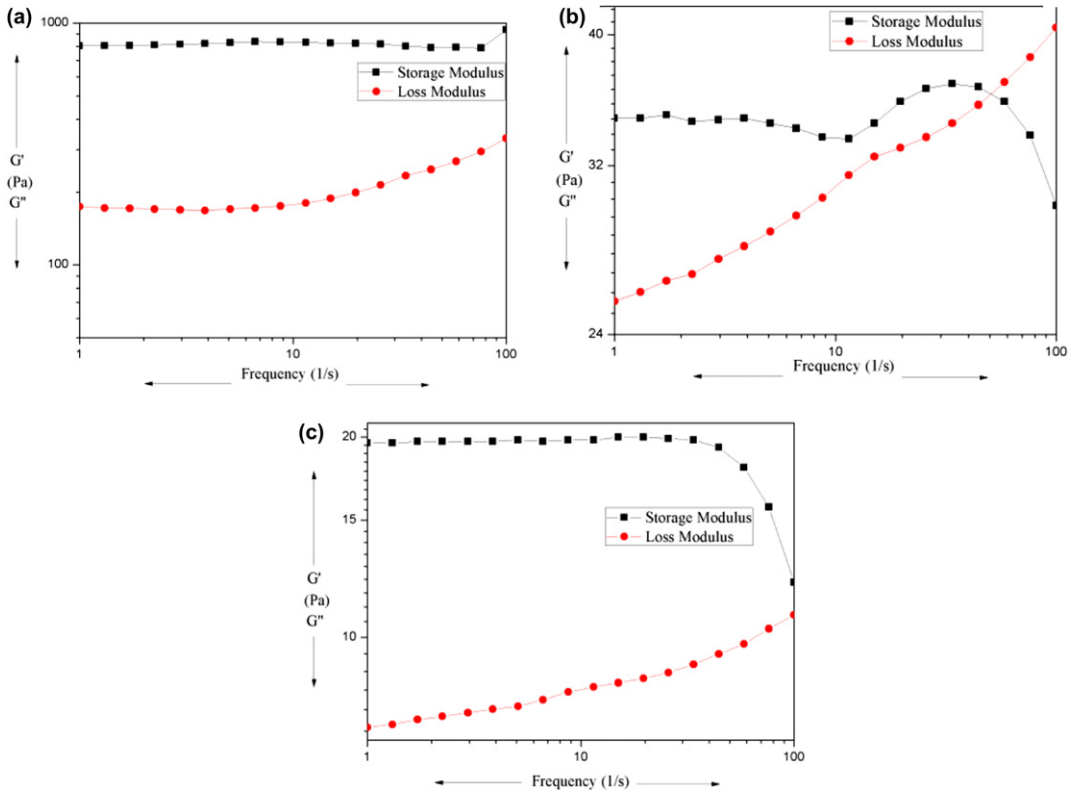


Fig. 4. Evolution of G′ and G″ as a function of the frequency sweep. Measurement of compound (a) **1** at 25 °C; (b) **2** at 25 °C and (c) **1**+**2** at 25 °C.

compound **1** is much stronger than the gel of compound **2** as well as its mixture with **1**.

2.4. Fiber formation of xerogel click conjugates 1 or 2

To get an insight into the microstructures, scanning electron microscopy (SEM) was performed on xerogels (20 days, room temperature dried gels). The figures shown are typical SEM pictures of compounds **1** or **2** at different magnification. Fig. 5a displays the picture with the magnification (9000×) of the conjugate **1**. Careful inspection of Fig. 5a indicates that the fibers are formed spontaneously during gel formation. The fibers of **1** are hollow with small channels inside (nanotubes). These channels can be seen very clearly from high resolution magnification pictures (Fig. 5b). Lower magnification pictures confirm that these fibers are inter-tangling with each other forming supramolecular networks. Compound **2** (Fig. 5d–f) forms also fibers. However, these fibers appear to be solid rather than hollow. For comparison, SEM measurements of 2'-deoxyuridine click conjugate **14** were performed (see Supplementary data, Fig. S1).[24] The structure of the molecular assembly of compounds **1** and **2** within the fibers is unknown. Nevertheless, hydrogen bonding as well as stacking interactions are anticipated to be the main forces holding the molecules together.

2.5. Influence of nucleobase structure, triazole connectivity and linker units on gel assembly and molecular modeling

Molecular forces hold the monomeric gelator molecules together thereby forming a supramolecular assembly. These forces are hydrophobic interactions, stacking interactions, and also hydrogen bonding. This is clearly evidenced by the influence of structural changes of compound **1** forming the most stable hydrogel. Experiments with closely related molecules reveal that the click conjugates respond sensitively to changes on the modification of the triazole moiety and the nucleobase. The short linker armed click adducts such as **1** with a C-linked triazole moiety efficiently gelates in water whereas that with the isomeric *N*-linked triazole (**2**) forms gels of lower stability. Long or branched linkers bearing two triazole moieties within one molecule do not lead to gels at all. So, it seems to be important that the triazole moiety is directly linked to the nucleobase.

Apart from structural changes in the triazole moiety the effect of nucleobase structure on gel formation was studied. For this, a shape mimic of compound **1** was used. Replacement of the pyrazolo[3,4-*d*]pyrimidine skeleton by a pyrrolo[2,3-*d*]pyrimidine moiety with one ring nitrogen less resulted in the adduct **13**, which was synthesized and tested for gelation. This 7-deazaadenine click conjugate **13**, does not form a gel at any given concentration. In order to detect conformational changes on both click conjugates, molecular dynamics simulations were performed using *Hyperchem* 8.0 in the absence of water molecules. The results for compounds **1**, **2**, **3**, and **13** are shown in Fig. 6. From this, it can be concluded that compounds **1**–**3** lock rather similar. Nevertheless, compounds **1** and **2** form a gel while compound **13** does not. As expected the overall structure of conjugate **3** with the long linker looks rather different. It stays to prove why so significant changes in the gelation properties are observed for compounds of so similar molecules (**1** and **13**). Earlier, protecting group experiments performed on amino groups demonstrated that the amide character of the 6-amino group is significantly higher for the 8-aza-7-deazapurines **1** and **2** than for the 7-deazapurine nucleoside **13**. Consequently, the amino groups of **1** and **2** are better proton donors and can participate in hydrogen bonding. The more hydrophobic character of

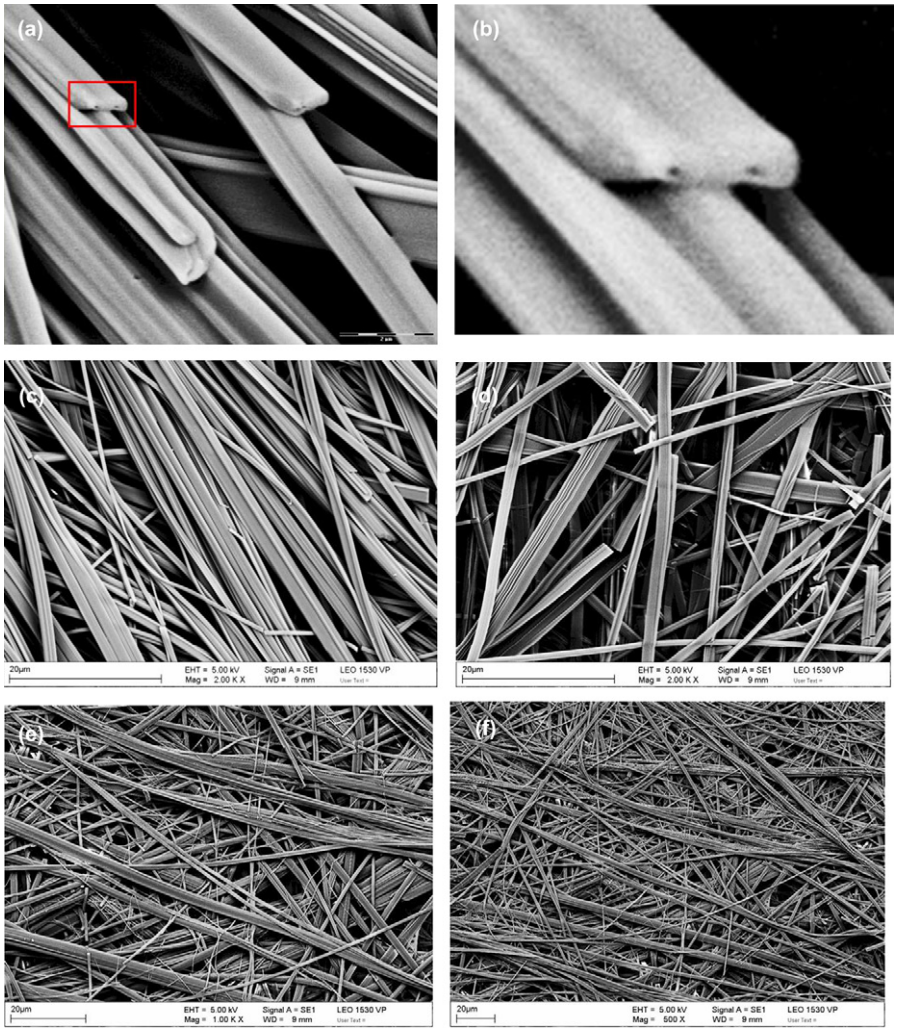


Fig. 5. SEM images of compound **1** with the scale bars 20 μm of (a) compound 1 (b) highlighting the channels of fibers encircled in the image of compound **1**. (c) Compound **1** with different magnification. (d)–(f) Compound **2** with the scale bar 20 μm with different magnifications.

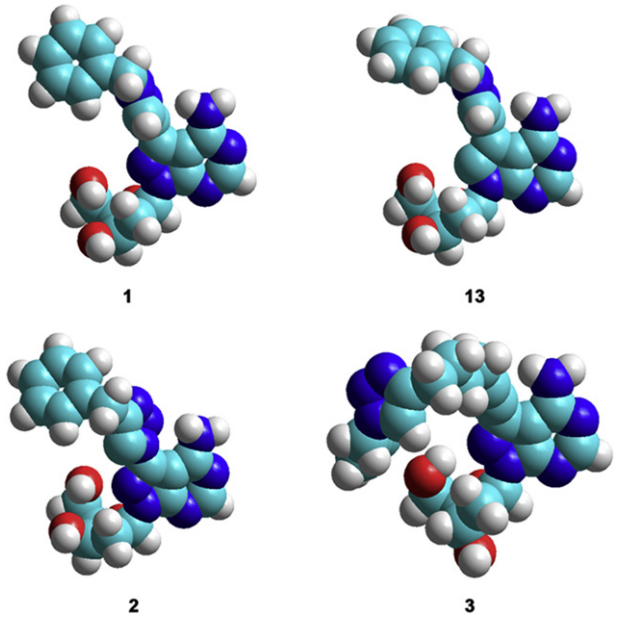


Fig. 6. Molecular dynamics (MD) simulation models of conjugate **1**, conjugate **2**, conjugate **3** and conjugate **13**. The molecules were constructed using *Hyperchem 8.0* and energy minimized using OPLS force field calculations.

the 7-deazaadenine base of **13** together with a lower amino group acidity as well as weaker stacking interactions may account for this behavior.

3. Conclusion

We have demonstrated the ability of 8-aza-7-deazaadenosine 'click' conjugate **1** to form a stable gel at a minimum gel concentration of 3 mg in 1 mL of water. Calculations on individual molecules reveal that one molecule of **1** can catch 7500 water molecules. The hydrogel of the closely related **2** is less stable; both form nanofibers. Equimolar mixtures of **1** and **2** and those containing complementary nucleobases (**1**) and thymine click conjugate **14** behave differently as the pure conjugates and do not form stable gels in mixtures. The pyrrolo[2,3-*d*]pyrimidine shape mimic of **1**, with one nitrogen atom less in the 'purine' moiety, does not form a gel at any given concentration. The rheology of the gel formed by conjugate **1** is entirely different from the rheology of its closely related conjugate **2**, which displays crossover points signifying the gel formed by conjugate **2** is much weaker than that of conjugate **1**. Moreover, the regioisomeric mixture of conjugate **1** and **2** forms a very unstable gel, which cannot pass the tube-inversion test. Both, storage and loss moduli of the mixture are much lesser than for the individual compounds. The click adducts **1** and **2** exhibit good gel

stability over a wide range of pH, making them promising candidates for various purposes, such as targeted drug delivery, wound healing[50] or material science.[51]

4. Experimental section

4.1. General materials and methods

All chemicals were purchased from Acros, Fluka or Sigma–Aldrich (Sigma–Aldrich Chemie GmbH, Deisenhofen, Germany). Solvents were of laboratory grade. Thin-layer chromatography (TLC) was performed on TLC aluminum sheets covered with silica gel 60 F_{254} (0.2 mm). Flash column chromatography (FC): silica gel 60 (VWR, Germany) at 0.4 bar; UV spectra: U-3200 spectrometer (Hitachi, Tokyo, Japan); NMR spectra: Avance-DPX-300 spectrometer (Bruker, Rheinstetten, Germany), at 300 MHz for ^{1}H and 75.48 MHz for ^{13}C; δ in parts per million relative to Me_4Si as internal standard. All the synthesized compounds were characterized by elemental analyses, ^{1}H and ^{13}C NMR spectra. Elemental analyses were performed by Mikroanalytisches Laboratorium Beller (Göttingen, Germany).

4.2. General procedure for gelation

The click adduct (3 mg) was suspended in 1 mL of water in a glass vial. The mixture was heated until it became a clear solution. After complete dissolution, the solution was gradually allowed to cool to room temperature. Then after the sample was subjected to a 'tube-inversion test'.[24] The compound is said to be a gelator, when the sample passes tube-inversion test and was characterized as a gel. When the formed gel flows neither freely like clear solution nor self-supporting, it is called partial gel.

*4.2.1. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)-3-[(trimethylsilanyl) ethynyl]-1H-pyrazolo[3,4-d] pyrimidin-4-amine (**6**).* To a solution of compound **5**[39,40] (0.95 g, 2.51 mmol) in dry DMF (25 mL), CuI (0.096 g, 0.50 mmol), $Pd(PPh_3)_4$ (0.29 g, 0.25 mmol), dry Et_3N (0.51 g, 0.7 mL, 5.05 mmol), and 6 equiv of trimethylsilylacetylene (1.48 g, 2.14 mL, 15.1 mmol) were added. The reaction mixture, which slowly turned to black was stirred under inert atmosphere for 12 h (TLC monitoring). The reaction mixture was evaporated and the oily residue was adsorbed on silica gel and subjected to FC (silica gel, column 15×3 cm, eluted with CH_2Cl_2/MeOH 95:5→90:10) affording one main zone. Evaporation of the solvent gave **6** (0.7 g, 80%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.6. UV λ_{max} (MeOH)/nm 246 (ε/dm^3 mol^{-1} cm^{-1} 107,00), 285 (12,800). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 0.22 (9H, s, 3× CH_3), 2.20–2.28 (1H, m, C2′-H_α), 2.72–2.80 (1H, m, C2′-H_β), 3.48–3.54 (1H, m, C5′–H), 3.78–3.83 (1H, m, C4′–H), 4.38–4.43 (1H, m, C3′–H), 4.76–4.80 (1H, t, J=5.7 Hz, C5′–OH), 5.29–5.31 (1H, d, J=4.5 Hz, C3′–OH), 6.52–6.56 (1H, t, J=6.3 Hz, C1′–H), 8.25 (1H, s, C2–H). Anal. Calcd for $C_{15}H_{21}N_5O_3Si$ (347.44): C, 51.85; H, 6.09; N, 20.16. Found: C, 51.86; H, 5.96; N, 20.01.

*4.2.2. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)-3-ethynyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**7**).* To the solution of compound **6** (0.06 g, 0.17 mmol) in methanol (6 mL), anhydrous K_2CO_3 (0.006 g, 0.04 mmol) was added and stirred at room temperature for 2 h. After completion of the reaction (monitored by TLC), solvent was evaporated and the residue was subjected to FC (silica gel, column 15×3 cm, eluted with CH_2Cl_2/MeOH 94:6→90:10). Evaporation of the solvent afforded compound **7** (0.04 g, 85%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.4. UV λ_{max} (MeOH)/nm 246 (ε/dm^3 mol^{-1} cm^{-1} 10,400), 285 (12,900). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.21–2.29 (1H, m, C2′-H_α), 2.72–2.80 (1H, m, C2′-H_β), 3.37–3.54 (2H, m, C5′–H), 3.80–3.81 (1H, m, C4′–H), 4.40–4.45 (1H, m, C≡CH), 4.68 (1H, m, C3′–H), 4.74–4.78 (1H, t, J=5.7 Hz, C5′–OH), 5.27–5.28 (1H, d, J=4.8 Hz, C3′–OH), 6.52–6.56 (1H, m, C1′–H), 8.24 (1H, s, C2–H). Anal. Calcd for $C_{12}H_{13}N_5O_3$ (275.26): C, 52.36; H, 4.76; N, 25.44. Found: C, 52.33; H, 4.69; N, 25.31.

*4.2.3. 1-[2-Deoxy-β-D-erythro-pentofuranosyl]-3-[(1-benzyl-1′,2′,3′-triazol-4′-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**1**).* To a solution of **7** (0.1 g, 0.36 mmol) and benzyl azide (0.058 g, 54 μL, 0.43 mmol) in THF/H_2O/t-BuOH (3:1:1, 5 mL) was added sodium ascorbate (362 μL, 0.36 mmol) of a freshly prepared 1 M solution in water, followed by the addition of copper(II) sulfate pentahydrate 7.5% in water (312 μL, 0.08 mmol). The emulsion was stirred for 12 h at room temperature and the solution was evaporated and the residue was applied to FC (silica gel, column 10×3 cm, CH_2Cl_2/MeOH 90→10). From the main zone, compound **1** (0.075 g, 88%) was isolated as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.5. UV λ_{max} (MeOH)/nm 254 (ε/dm^3 mol^{-1} cm^{-1} 14,900), 289 (10,600). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.24–2.32 (1H, m, C2′-H_α), 2.79–2.88 (1H, m, C2′-H_β), 3.38–3.46 (1H, m, C5′–H), 3.53–3.64 (1H, m, C5′–H), 3.82–3.87 (1H, m, C4′–H), 4.48–4.49 (1H, d, J=4.2 Hz, C3′–H), 4.79–4.83 (1H, t, J=5.7 Hz, C5′–OH), 5.28–5.29 (1H, d, J=4.5 Hz, C3′–OH), 5.73 (2H, s, CH_2), 6.58–6.62 (1H, t, J=6.3 Hz, C1′–H), 7.33–7.41 (5H, m, Ar–H), 8.13 (1H, br s, NH_a), 8.24 (1H, s, triazole–H), 8.83 (1H, s, C2–H), 9.10 (1H, br s, NH_b). Anal. Calcd for $C_{19}H_{20}N_8O_3$ (404.81): C, 55.88; H, 4.94; N, 27.44. Found: C, 55.98; H, 4.85; N, 27.35.

*4.2.4. 4-Amino-7-(2-deoxy-β-D-erythro-pentofuranosyl)-5-[1-benzyl-1′,2′,3′-triazol-4′-yl]-7H-pyrrolo-[2,3-d]pyrimidine (**13**).* Procedure as described above for compound **1**. 4-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-(ethynyl)-7*H*-pyrrolo[2,3-*d*] pyrimidine[52] **12a**, (0.82 g, 0.3 mmol), benzyl azide (78 μL, 0.36 mmol), sodium ascorbate (0.238 g, 1.2 mmol), and copper(II) sulfate pentahydrate (0.075 g, 0.3 mmol). EtOH/H_2O (1:1, 5 mL). Compound **13** was isolated as a colorless solid. Yield (0.067 g, 55%). TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.7. UV λ_{max} (MeOH)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} 14,400), 275 (10,800). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.15–2.24 (1H, m, C2′-H_α), 2.41–2.45 (1H, m, C2′-H_β), 3.45–3.63 (2H, m, C5′–H), 3.79–3.83 (1H, m, C4′–H), 4.33–4.35 (1H, t, J=6.0 Hz, C3′–H), 5.02 (1H, br s, C5′–OH), 5.28 (1H, br s, C3′–OH), 5.68 (2H, s, CH_2), 6.51–6.55 (1H, dd, J=6.2 Hz, C1′–H), 7.33–7.44 (5H, m, Ar–H), 7.86 (1H, s, C8–H), 8.08 (1H, s, C2–H), 8.511 (1H, s, triazole–H).

*4.2.5. 1-[2-Deoxy-β-D-erythro-pentofuranosyl]-3-[(4-benzyl-1′,2′,3′-triazol-1′-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**2**).* Compound **5** (0.050 g, 0.13 mmol), 3-phenyl-1-propyne **10** (0.026 g, 27 μL, 0.22 mmol), NaN_3 (0.013 g, 0.20 mmol), L-proline (0.004 g, 0.03 mmol, 20 mol %), $Na_2CO_3 \cdot 10H_2O$ (0.012 g, 0.04 mmol, 30 mol %), and sodium ascorbate (0.014 g, 0.07 mmol) were suspended in a DMF/H_2O (4:1, 2.5 mL) mixture in a 2–5 mL microwave vessel. To the stirred mixture, $CuSO_4$ (20 mol %, as a 1 M solution in H_2O) was added and the vessel was sealed and subjected to microwave irradiation at 130 °C for 30 min (ramp time: 45 s, prestirring: 20 s, high). After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature. The solvent was evaporated to dryness and subjected to FC (silica gel, column 10×3 cm, CH_2Cl_2/MeOH 90→10). Isolation of main zone afforded **2** (0.045 g, 83%) as a colorless solid. TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.5. UV λ_{max} (MeOH)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} 7800), 286 (13,800). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.26–2.34 (1H, m, C2′-H_α), 2.77–2.85 (1H, m, C2′-H_β), 3.40–3.43 (1H, m, C5′–H), 3.50–3.56 (1H, m, C5′–H), 3.80–3.85 (1H, m, C4′–H), 4.16 (2H, s, CH_2), 4.47 (1H, br s, C3′–H), 4.73–4.77 (1H, t, J=5.7 Hz, C5′–OH), 5.29–5.31 (1H, d, J=4.2 Hz, C3′–OH), 6.61–6.65 (1H, t, J=6.3 Hz, C1′–H), 7.20–7.25 (1H, m, Ar–H), 7.29–7.36 (4H, m,

Ar–H), 8.20 (1H, br s, NH_a), 8.30 (1H, s, triazole–H), 8.36 (1H, br s, NH_b), 8.72 (1H, s, C2–H). Anal. Calcd for $C_{19}H_{20}N_8O_3$ (404.81): C, 55.88; H, 4.94; N, 27.44. Found: C, 56.05; H, 4.86; N, 27.20.

*4.2.6. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)-3-[di(prop-2-ynl) amino]prop-1-ynyl]-1H-pyrazolo[3,4-d] pyrimidin-4-amine (**12**).* A solution of compound **5** (0.1 g, 0.26 mmol) in dry DMF (5 mL), was treated with CuI (0.01 g, 0.05 mmol), $Pd(PPh_3)_4$ (0.031 g, 0.03 mmol), dry Et_3N (0.053 g, 73 μL, 0.52 mmol) and 6 equiv of tri(prop-2-ynyl)amine (0.35 g, 480 μL, 2.6 mmol). The reaction mixture, which slowly turns to black was stirred under inert atmosphere for 12 h. After the completion of the reaction (TLC monitoring), the reaction mixture was evaporated and the oily residue was adsorbed on silica gel and subjected to FC (silica gel, column 15×3 cm, eluted with 98:2→94:6) affording one main zone. Evaporation of the solvent gave **12** (0.065 g, 67%) as a colorless foam. TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.5. UV λ_{max} (MeOH)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 10,600), 286 (10,000). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.20–2.28 (1H, m, C2′-H_α), 2.74–2.82 (1H, m, C2′-H_β), 3.27 (2H, s, CH_2), 3.48–3.53 (4H, m, CH_2), 3.75 (2H, s, C≡CH), 3.78–3.83 (1H, m, C4′–H), 4.40–4.43 (1H, m, C3′–H), 4.78–4.82 (1H, t, *J*=5.7 Hz, C5′–OH), 5.29–5.30 (1H, d, *J*=4.5 Hz, C3′–OH), 6.52–6.56 (1H, t, *J*=6.3 Hz, C1′–H), 8.24 (1H, s, C2–H). Anal. Calcd for $C_{19}H_{20}N_6O_3$ (380.4): C, 59.99; H, 5.30; N, 22.09. Found: C, 59.94; H, 5.24; N, 21.96.

*4.2.7. 1-[2-Deoxy-β-D-erythro-pentofuranosyl]3-{3-[bis(1-benzyl-1′,2′,3′-triazol-4-ylmethyl)amino]prop-1-ynyl}-1H-pyrazolo[3,4-d] pyrimidin-4-amine (**4**).* Procedure as described above for compound **1**. Compound **12** (0.1 g, 0.27 mmol), benzyl azide (0.084 g, 78 μL, 0.63 mmol), sodium ascorbate (262 μL, 0.26 mmol), and copper(II) sulfate pentahydrate 7.5% in water (226 μL, 0.06 mmol). EtOH/H_2O (1:1, 5 mL). Compound **4** was isolated as a colorless solid. Yield (0.133 g, 76%). TLC (silica gel, CH_2Cl_2/MeOH 9:1). R_f 0.6. UV λ_{max} (MeOH)/nm 255 (ε/dm^3 mol^{-1} cm^{-1} 32,400), 291 (24,900). ^{1}H NMR [DMSO-d_6, 300 MHz]: δ 2.21–2.29 (1H, m, H_α), 2.76–2.85 (1H, m, C2′-H_β), 3.49–3.57 (2H, m, C5′–H), 3.81 (5H, s, C4′-H, 2x CH_2), 4.42–4.45 (1H, m, C3′–H), 4.80–4.84 (1H, t, *J*=5.7 Hz, C5′–OH), 5.29–5.31 (1H, d, *J*=4.5 Hz, C3′–OH), 5.59 (4H, s, CH_2), 6.54–6.58 (1H, t, *J*=6.3 Hz, C1′–H), 7.28–7.39 (10H, m, Ar–H), 8.13 (2H, s, triazole–H), 8.25 (1H, s, C2–H). Anal. Calcd for $C_{33}H_{34}N_{12}O_3$ (646.7): C, 61.29; H, 5.30; N, 25.99. Found: C, 61.57; H, 5.18; N, 25.85.

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Supplementary data

^{13}C NMR chemical shifts and coupling constants of the 8-aza-7-deaza-2′-deoxyadenosine derivatives. SEM images. pK_a profiles of click conjugates. Rheological measurements. ^{1}H NMR and ^{13}C NMR spectra of new compounds (Figs. S4–S17). Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.07.015.

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