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Synthesis and Photophysical Characterisation of Fluorescent 8-(1*H*-1,2,3-Triazol-4-yl)adenosine Derivatives

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A series of 8-(1*H*-1,2,3-triazol-4-yl)-substituted adenosine derivatives have been synthesised by using Sonogashira cross-coupling and click chemistry. The use of click chemistry enables an easy access to different substituents in the 4-position of the triazole ring. The modified nucleosides show high absorptivities due to a single strongly allowed electronic transition and, for some of the derivatives, high quantum yields in organic as well as in water solution making them promising as fluorescent probes in nucleic acid contexts. Furthermore, the different substituents of the 1,2,3-triazole makes the wavelength of emission tunable without changing the absorption properties substantially.

Introduction

The field of fluorescent nucleic acid base analogues has expanded significantly in recent years. There have been reported a number of fluorescent nucleosides with an artificial nucleobase or modified nucleobase,[1] which have been used to study the structures of nucleic acids[2,3] and their biological functions such as replication, transcription, recombination and repair.[4,5] Several fluorescent pyrimidine analogues have been reported, e.g. the tricyclic cytosine analogues 1,3-diaza-2-oxophenothiazine (tC)[6,7] and its oxo analogue tC^O,[8] which have high fluorescence quantum yields even after incorporation into single- and double-stranded DNA. Furthermore, the phenylpyrrolocytosine is designed to engage guanine with an additional hydrogen bond[9] as well as the furan-modified pyrimidines that have been shown to detect abasic sites[3] and binding of ligands to RNA.[5] However, most of the fluorescent nucleobase analogues are purine derivatives. Among them, 2-aminopurine (2-AP) is the most frequently used.[10] 2-AP has a high quantum yield (Φ = 0.68) at a physiological pH and is selectively excited by light of lower energy than the natural nucleic acid bases. Other commercially available fluorescent purine analogues are the pteridines, 3-methylisoxanthopterin (3MI),[11] and 6-methylisoxanthopterine (6MI).[12]

Adenosine have previously been functionalized in the 8-position resulting in nucleoside analogues with fluorescent properties.[13,14] Our approach to modified fluorescent nucleosides involves the functionalisation of adenosine in the 8-position with differently substituted triazole rings by using a 1,4-regioselective copper-catalyzed azide/alkyne cycloaddition (CuAAC).[15] The 1,3-dipolar cycloaddition, often referred to as click chemistry, have been used in various applications, e.g. organic synthesis, drug discovery, and chemical biology[16] and has successfully been exploited in conjugation of various molecules to oligonucleotides.[17] The use of click chemistry allows the 4-substituent of the 1,2,3-triazole ring to be easily varied, through the use of different azides, which enables tuning of the electronic and optical properties of the nucleoside analogues.

In this paper, we present the synthesis and photophysical characterisation of a series of 8-(1*H*-1,2,3-triazol-4-yl)adenosine analogues showing promising photophysical properties such as high fluorescence quantum yields (up to 64%), high absorptivities (up to 24000 $M^{-1}cm^{-1}$), and, thus, high brightness (absorptivity × quantum yield) as well as tunable emission wavelengths.

Results and Discussion

Synthesis

Starting from commercially available adenosine (**1**), 8-bromoadenosine (**2**) was easily obtained by using bromine in a sodium acetate buffer as described previously (Scheme 1).[18] The hydroxy groups in the 3′- and 5′-position of compound **2** were protected with a tetraisopropyldi-

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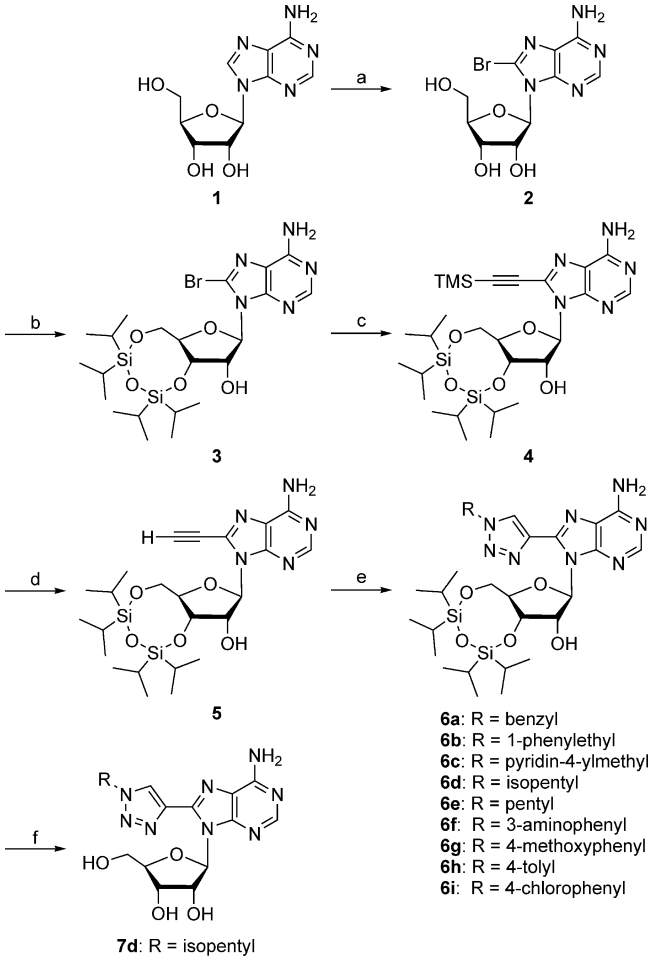
silyl group (TIPDS) making compound **3** much more soluble in organic solvents.[19] The TMS-protected alkyne was installed by using a Sonogashira coupling protocol, and compound **4** was obtained in good yields (80%).[14]



Scheme 1. Reagents and conditions: (a) Br_2/NaOAc buffer, 83%; (b) tetraisopropyldisiloxane dichloride, pyridine, 65%; (c) TMS/acetylene, $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, THF, 50 °C, 50 min, 80%; (d) NH_3 (aq. 25%)/EtOAc (1.5:1, v/v), room temp., 14 h, 81%; (e) general procedure A–C, see Exp. Sect.; (f) TBAF (1 M in THF; 2 equiv.), THF, room temp., overnight, 99%.

Deprotection of the TMS group was performed under basic conditions (NH_3 in ethyl acetate) to yield the terminal alkyne **5**. The 1,2,3-triazole ring was synthesised by using a copper-catalyzed [3+2]-cycloaddition between the alkyne **5** and the corresponding azide. This reaction allows the 4-substituent of the 1,2,3-triazole ring to be easily varied, through the use of various azides. Compounds **6a**–**6c** were prepared by a copper-catalyzed [3+2]-cycloaddition according to the general method A. The reaction was carried out as a two-step one-pot reaction in which compound **5** was treated with the preformed crude triflic azide (obtained from NaN_3 and triflic anhydride in dichloromethane/water)[20] in the presence of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) and sodium ascorbate by using microwave-assisted heating at 60 °C for 5 min. The copper present in the crude azide products was sufficient to drive the cycloaddition reactions to completion, and products **6a**–**6c** were obtained in high yields (73–99%).

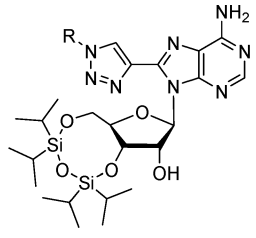
Compounds **6d** and **6e** were prepared according to the general method B, in which compound **5** was treated with the crude azide (preformed from NaN_3 and the corresponding alkyl bromide in water) by using $CuSO_4$/sodium ascorbate and TBTA in DMSO.

The azides required for preparation **6f**–**6i** were synthesized by a proline-promoted CuI-catalyzed coupling reaction in DMSO (general method C).[21] Compound **5**, CuI and TBTA were added directly to the reaction mixture containing the crude azide and allowed to react under microwave-assisted heating at 60 °C for 5 min. This two-step one-pot procedure generated **6f**–**6i** in moderate yields (51–61%). Nucleoside derivative **6d** was deprotected, in order to investigate the photochemical properties for the adenine derivatives in water. Compound **6d** was treated with 1 M TBAF in THF overnight leading to the water-soluble compound **7d** in 98% yield after column chromatography.

Photophysical Characterisation

Molar absorptivities and quantum yields for the nucleoside derivatives (**6a**–**6i**) were determined in THF. All derivatives show similar absorption properties; the molar extinction coefficient varies between 16000 and 24000 $M^{-1}cm^{-1}$, and the absorption maximum varies between 289 and 296 nm (Table 1). Furthermore, the nucleosides with aliphatic substituents (**6a**–**6e**) show a very similar absorption envelope (Figure 1). This similarity is not very surprising, because the conjugated π-system of these compounds ends at the triazole ring, and the non-conjugated part has a very small influence on the spectroscopic properties. The absorption envelope for these compounds (**6a**–**6e**) is more structured and slightly blueshifted compared to the derivatives

Table 1. Photophysical characterisation of absorption and fluorescence properties of compounds **6a**–**6i** in THF.



Compound	R	Abs_{max} [nm]	Em_{max} [nm]	ε [$M^{-1}cm^{-1}$]	Φ_F
6a	benzyl	290	344	16000	0.64
6b	1-phenylethyl	289	342	21000	0.63
6c	pyridin-4-ylmethyl	289	346	16000	0.49
6d	isopentyl	289	343	18000	0.62
6e	pentyl	289	342	17000	0.62
6f	3-aminophenyl	296	402	24000	0.38
6g	4-methoxyphenyl	294	370	23000	0.03
6h	4-tolyl	294	368	21000	0.05
6i	4-chlorophenyl	294	396	21000	0.05

with aromatic substituents (**6g**–**6i**). The fluorescence properties show a larger deviation between the aliphatic and aromatic nucleosides. The derivatives with aliphatic substituents (**6a**–**6e**) have very similar properties with an emission maximum around 344 nm. In addition, they exhibit high quantum yields (0.49–0.64), structured emissions, and comparably small Stokes' shifts (53–57 nm). The emission maximum for the nucleosides with the aromatic substituents (**6f**–**6i**) are redshifted (25–55 nm) compared to the aliphatic derivatives (**6a**–**6e**). The aromatically substituted nucleosides (**6g**–**6i**) show low quantum yields (0.03–0.05) and large Stokes' shift (74–102 nm). In contrast, nucleoside **6f** exhibits a high fluorescence quantum yield (0.38) despite the conjugation between the aromatic substituent and the triazole ring. Furthermore, it shows the strongest absorption (24000 $M^{-1}cm^{-1}$) and largest Stokes shift (106 nm). The tuneability in emission wavelength is very interesting and is potentially useful in fluorescence resonance energy transfer experiments, where the emission wavelength should energetically overlap the absorption of the acceptor. In fact, the energy of the emission of the nucleosides matches the energy of the absorption of our previously characterized base analogues tC/tC^{O},[7,8] and thus, constitutes promising nucleic acid FRET donor candidates.

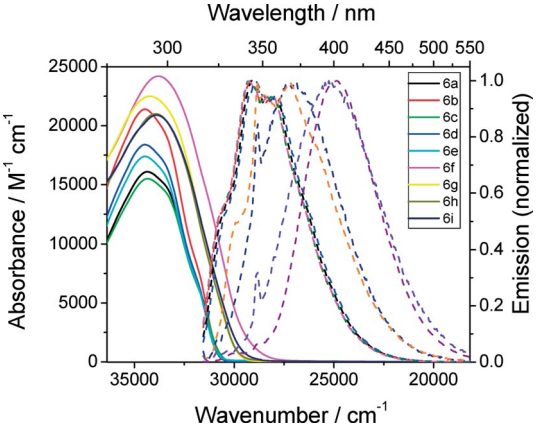


Figure 1. Absorption (solid line) and emission (dashed line) of nucleoside **6a**–**6i** at room termperature in THF. The sharp peak at 347 nm for the weakly fluorescent derivatives is due to Raman scattering.

Because hydrogen bonding often has a strong influence on the fluorescence quantum yield, one of the more promising derivatives **6d** was deprotected to yield **7d** (Scheme 1), and the photophysical properties of **7d** were measured in water. The absorption maximum (282 nm) is slightly blueshifted (7 nm) in water compared to that in THF, and the vibronic structure disappears (Figure 2). Furthermore, the emission maximum (354 nm) is slightly redshifted, and the vibronic structure is lost.

The redshift of the emission is commonly observed in highly polar and hydrogen-bonding solvents and is usually explained by a larger stabilisation of the excited state compared to the ground state. Importantly, the high fluorescence quantum yield is retained in water (Φ = 0.50), which makes compound **7d** a very promising fluorescent base analogue. In fact, the brightness value as a monomer (not within a base stack) is calculated to be 9000 $M^{-1}cm^{-1}$, which is more than twice the value of 2-aminopurine under similar conditions.[10,22]

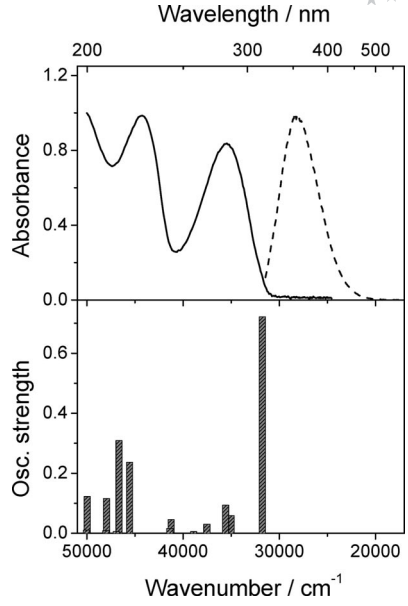


Figure 2. Absorption (solid line) and emission (dashed line) spectra of compound **7d** in water (top). Calculated oscillator strengths and energies for the singlet–singlet electronic transitions below 50000 cm^{-1} (bottom).

Theoretical Evaluation

In order to gain further insights on the potential energy surface of the adenine–triazole bond, a series of density funtional theory (DFT) calculations were performed giving a conformational analysis of the triazole ring and the adenine ring of the model compound 9-methyl-8-(1*H*-1,2,3-triazol-4-yl)adenine. A relaxed energy scan of the torsional angle (ω) at the B3LYP/6-31G(d) level shows that the two rings are coplanar in the global minimum having the methyl groups in an *anti* arrangement (ω = 180°, conformer A, Figure 3). Rotation of the triazole ring leads to a local minimum, in which the methyl groups are positioned *syn* to each other, but in this conformer the two rings are not coplanar (ω = 22°) due to the steric repulsion between the triazole ring and the adenine ring (conformer B, Figure 3).

The global and local minima (conformer A and B) and the transition state (TS_{ROT}) for the rotation between the two minima was optimised at the B3LYP/6-311G(d,p) level of theory.[23] The calculation shows that the global minimum is 6.7 kcal mol^{-1} (conformer A, Figure 4) more stable than the local minimum (conformation B), and the transition state for the rotation is located 7.5 kcal mol^{-1} above the global minimum. This suggests that the main conformer populated in the ground state is conformer A. The global minimum is furthermore shallow enough to allow a quite broad distribution of torsional angles in the ground state.

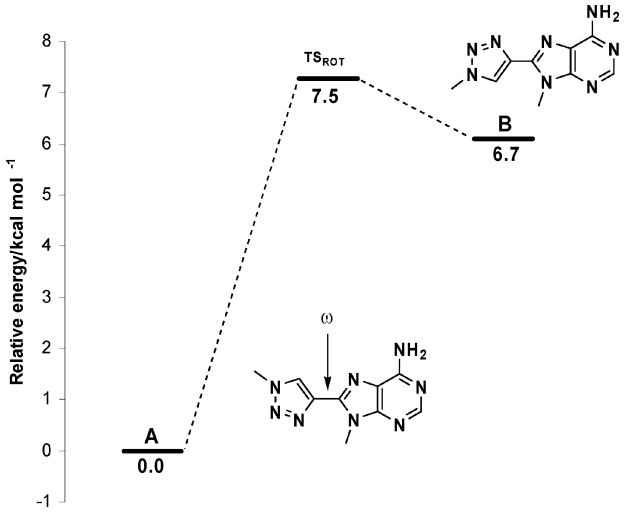


Figure 3. Potential energy surface and electrostatic potential of the model compound 9-methyl-8-(triazol-4-yl)adenine. Energies are calculated at the B3LYP/6-311G(d,p) level of theory.

Molecular orbital calculations of electronic transitions were made for the DFT-optimized model compound. The calculated electronic transitions agree very well with the experimentally observed spectrum, as is usually the case for the INDO-based methods applied on "simple" heteroaromatic compounds.[24] The lowest singlet electronic transition ($S_0 \rightarrow S_1$) for the model with co-planar adenine and triazole rings (conformer A) is calculated to be at 31700 cm^{-1} (315 nm), which is slightly lower in energy than observed.

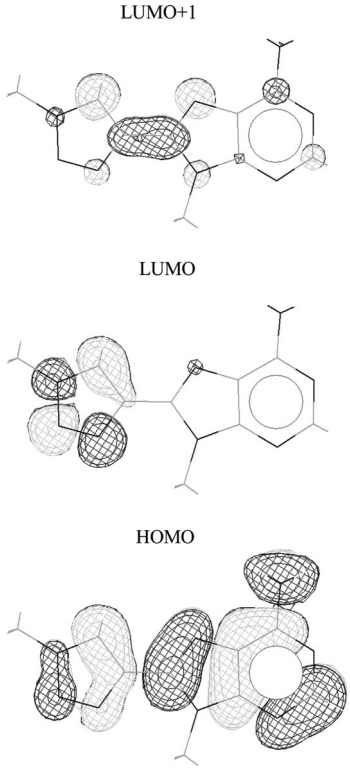


Figure 4. Frontier orbitals for the model compound 9-methyl-8-(1*H*-1,2,3-triazol-4-yl)adenine.

This electronic tranistion is dominated by the HOMO → LUMO+1 configuration (see Supporting Information). Furthermore, this transition is quite strong (f = 0.7) and isolated from other strong electronic transitions (Table S1). It is thus reasonable to assign the lowest absorption band of the studied compounds to this single, strongly allowed electronic transition. By looking at the dominating configurations for this transition it is also possible to predict that the formal single bond between the adenine and triazol moieties in the ground state is gaining bond order in the excited state (see orbital diagrams, Figure 4) and, thus, a planarized excited state would result. Furthermore, the effect on the electronic transitions by twisting the molecular planes were investigated. The lowest electronic transition of the model compound blueshifts upon twisting the dihedral angle, and this might explain why the absorption spectrum is slightly broader than the emission and also why the calculated transition energy (for the co-planar compound) is slightly lower than experimentally observed.

Finally, the effect of substitution on the triazol ring was investigated by replacing the methyl group in the model compound by either a conjugated (4-tolyl) or a non-conjugated (benzyl) substituent. Very marginal redshifts along with slightly increased oscillator strengths were observed (not shown) in accordance with the experimental observations.

Conclusions

A series of adenine derivatives have been synthesised by using Sonogashira cross coupling and click chemistry. The click chemistry provides a means of easy tuning of the fluorescence properties of the nucleosides. The wavelength of the emission can be varied up to 60 nm by using different azide derivatives, without changing the absorption properties substantially. Most importantly, the nucleosides show high absorptivities due to a single strongly allowed electronic transition and, for one of the derivatives, high fluorescence quantum yields in organic as well as in water solution.

The target compounds have an intact hydrogen-bonding pattern ensuring specific interactions with thymine, which together with excellent photophysical properties make the modified nucleosides promising as fluorescent probes in nucleic acids. We are currently developing the synthesis of the deoxyadenosine analogues, which are to be incorporated into DNA and photophysically characterised as base analogues.

Experimental Section

General: All reagents and solvents were of analysis or synthesis grade. ^1H and ^{13}C NMR spectra were recorded with a JEOL JNM-EX 400-spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ or [D_6]DMSO. Chemical shifts are reported in ppm with the solvent residual peak as internal standard ($CHCl_3$: δ_H= 7.26 ppm; $CDCl_3$: δ_C= 77.0 ppm; [D_6]DMSO: δ_H= 2.50 ppm, δ_C =

39.52 ppm). IR spectra were recorded with a Perkin–Elmer 16PC FT-IR spectrometer. The reactions were monitored by thin-layer chromatography (TLC), on silica-plated aluminium sheets (Silica gel 60 F_{254}, E. Merck) and detecting spots by UV light (254 and 365 nm). Flash chromatography was performed on Merck Silica gel 60 (0.040–0.063 mm). Microwave heating was performed with a Biotage Initiator microwave apparatus, with a fixed hold time at the desired reaction temperature (wattage was automatically varied to maintain the desired temperature). High-resolution mass spectrometric data (nanospray FT-ICR-MS) were obtained from BioAnser, Sahlgrenska Science Park, Gothenburg, Sweden.

Method A. General Procedure for the Synthesis of 6a–6c: NaN_3 (6.6 equiv.) was dissolved in water and dichloromethane (0.03 mL/mmol NaN_3, respectively). The solution was cooled to 0 °C, and triflic anhydride (3.3 equiv.) was added dropwise to the cooled solution. The reaction mixture was stirred vigorously at 0 °C for 2 h, and the solution was extracted with dichloromethane. The organic phase was washed with saturated aqueous $NaHCO_3$ to give a crude triflic azide solution. Benzylamine (22 mg, 0.21 mmol), $CuSO_4 \cdot 5H_2O$ (0.02 equiv.) and $NaHCO_3$ (1.1 equiv.) were dissolved in water (0.36 mL/mmol amine), and the crude triflic azide solution was added followed by MeOH until the solution became homogeneous. The reaction mixture was stirred at room temperature for 30 min. Compound **5** (0.1 g, 0.187 mmol), TBTA (0.06 equiv.) and L-ascorbic acid sodium salt (0.11 equiv.) were added, and the reaction mixture was heated in a microwave cavity at 60 °C for 5 min. The solvent was removed under reduced pressure, and care was taken not to let the solid residual run dry. The crude product was purified by column chromatography.

8-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6a): The title compound was synthesised according to the general procedure A. The crude product was purified by column chromatography (toluene/EtOAc, 1:2). Compound **5** (0.1 g, 0.187 mmol) and benzylamine (22.0 mg, 0.205 mmol) gave **6a** (0.126 g, 99%) as a white foam. R_f = 0.25 (toluene/EtOAc, 1:2). ^{1}H NMR ($CDCl_3$): δ = 1.00–1.18 (m, 28 H), 3.39 (br. s, 1 H), 3.99–4.06 (m, 3 H), 5.03–5.04 (m, 1 H), 5.54–5.64 (m, 3 H), 5.84 (br. s, 2 H), 6.94 (d, J = 1.1 Hz, 1 H), 7.32–7.38 (m, 5 H), 8.09 (s, 1 H), 8.19 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.3, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 54.6, 62.7, 71.9, 74.4, 82.3, 90.2, 119.8, 125.1, 128.5, 129.2, 129.4, 133.9, 139.6, 142.6, 150.7, 152.8, 155.3 ppm. IR (KBr): $\tilde{\nu}$ = 695, 884, 1037, 1085, 1131, 1466, 1580, 1641, 2866, 2945 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{31}H_{47}N_8O_5Si_2$ [M + H] 667.3203; found 667.3212.

8-[1-(1-Phenylethyl)-1*H*-1,2,3-triazol-4-yl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6b): The title compound was synthesised according to the general procedure A. The residue was dissolved in EtOAc (40 mL) and washed with brine (2 × 5 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (toluene/EtOAc, 1:1). Compound **5** (0.10 g, 0.19 mmol) and (1-phenylethyl)amine (25 mg, 0.21 mmol) gave **6b** (86.4 mg, 86%) as a white foam. R_f = 0.28 (toluene/EtOAc, 2:3). ^{1}H NMR ($CDCl_3$): δ = 1.00–1.22 (m, 28 H), 3.25 (t, J = 7.3 Hz, 2 H), 3.42 (br. s, 1 H), 3.99–4.05 (m, 3 H), 4.64–4.68 (m, 2 H), 5.06 (d, J = 5.9 Hz, 1 H), 5.57 (t, J = 6.2 Hz, 1 H), 5.99 (br. s, 2 H), 6.78 (s, 1 H), 7.11–7.13 (m, 2 H), 7.21–7.29 (m, 3 H), 7.92 (s, 1 H), 8.19 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.3, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 36.7, 52.0, 62.7, 71.9, 74.3, 82.3, 90.1, 119.8, 125.4, 127.4, 128.8, 129.1, 136.7, 138.7, 142.6, 150.6, 152.8, 155.4 ppm. IR (KBr): $\tilde{\nu}$ = 698, 884, 1036, 1086, 1131, 1466, 1581, 1641, 2866, 2944 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{32}H_{49}N_8O_5Si_2$ [M + H] 681.3359; found 681.3398.

8-[1-(Pyridin-4-ylmethyl)-1*H*-1,2,3-triazol-4-yl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6c): The title compound was synthesised according to the general procedure A. The residue was dissolved in EtOAc (40 mL) and washed with brine (2 × 5 mL). The organic phase was dried ($MgSO_4$), filtered, concentrated and purified by column chromatography (dichloromethane/MeOH, 16:1). Compound **5** (0.10 g, 0.19 mmol) and 4-picolylamine (22 mg, 0.21 mmol) gave **6c** (92 mg, 73%) as a white foam. R_f = 0.21 (dichloromethane/MeOH, 16:1). ^{1}H NMR ($CDCl_3$): δ = 0.99–1.23 (m, 28 H), 3.55 (br. s, 1 H), 3.98–4.06 (m, 3 H), 5.05 (d, J = 5.9 Hz, 1 H), 5.53–5.64 (m, 3 H), 6.16 (br. s, 2 H), 6.93 (s, 1 H), 7.13 (br. s, 2 H), 8.17–8.21 (m, 2 H), 8.59 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.7, 12.8, 13.2, 13.3, 17.1, 17.1, 17.3, 17.4, 17.5, 17.5, 17.5, 53.0, 62.4, 71.7, 74.3, 82.2, 90.2, 119.7, 122.4, 125.5, 139.9, 142.1, 143.0, 150.6, 150.7, 152.9, 155.4 ppm. IR (KBr): $\tilde{\nu}$ = 696, 884, 1038, 1085, 1133, 1466, 1581, 1607, 1643, 2866, 2945 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{30}H_{46}N_9O_5Si_2$ [M + H] 668.3155; found 668.3138.

Method B. General Procedure for the Synthesis of 6d–6e: NaN_3 (23 equiv.) was added to a solution of the appropriate 1-bromoalkyl derivative (10 equiv.) in water (0.53 mL/mmol NaN_3), and the reaction mixture was refluxed for 20 h. The water phase was extracted with dichloromethane (2 × 10 mL), and the combined organic layers were washed with water (10 mL), dried with $MgSO_4$,and the solvent was removed under reduced pressure. The generated azide was added to a solution of compound **5** (1 equiv.), TBTA (0.05 equiv.), L-ascorbic acid (0.1 equiv.), $CuSO_4$ (0.05 equiv.) in DMSO (2.7 mL/mmol adenosine derivative), and the reaction mixture was heated in the microwave cavity at 60 °C for 5 min. The reaction mixture was diluted with water (10 mL), and the water phase was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (2 × 10 mL), dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (toluene/EtOAc, 1:1).

8-(1-Isopentyl-1*H*-1,2,3-triazol-4-yl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6d): The title compound was synthesised according to the general procedure B. The crude product was purified by column chromatography (toluene/EtOAc, 1:1). Compound **5** (100 mg, 0.187 mmol) and 1-bromo-3-methylbutane (283 mg, 1.87 mmol) gave **6d** (106 mg, 87%) as a white foam. R_f = 0.20 (1:1 toluene/EtOAc). ^{1}H NMR ($CDCl_3$): δ = 0.96–0.97 (m, 6 H), 1.01–1.19 (m, 28 H), 1.56–1.67 (m, 1 H), 1.81–1.86 (m, 2 H), 3.43 (s, 1 H), 4.02–4.07 (m, 3 H), 4.43–4.46 (m, 2 H), 5.05 (d, J = 6.2 Hz, 1 H), 5.56–5.59 (m, 1 H), 5.98 (s, 2 H), 6.91 (s, 1 H), 8.16 (s, 1 H), 8.19 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.3, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 22.3, 25.5, 39.0, 49.1, 62.8, 72.0, 74.4, 82.3, 90.2, 119.8, 124.9, 139.1, 142.8, 150.7, 152.8, 155.3 ppm. IR (KBr): $\tilde{\nu}$ = 696, 884, 1037, 1087, 1133, 1298, 1467, 1581, 1642, 2867, 2947 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{29}H_{51}N_8O_5Si_2$ [M + H] 647.3516; found 647.3488.

8-(1-Pentyl-1*H*-1,2,3-triazol-4-yl)-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6e): The title compound was synthesised according to the general procedure B. The crude product was purified by column chromatography (toluene/EtOAc, 1:1). Compound **5** (100 mg, 0.19 mmol) and 1-bromopentane (283 mg, 1.87 mmol) gave **6e** (95 mg, 78%) as a white foam. R_f = 0.25 (toluene/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ = 0.85–0.89 (m, 3 H), 1.00–1.17 (m, 28 H), 1.31–1.36 (m, 4 H), 1.89–1.95 (m, 2 H), 3.55 (s, 1 H), 4.03–4.05 (m, 3 H), 4.36–4.40 (m, 2 H), 5.06 (d, J = 5.9 Hz, 1 H), 5.55–5.58 (m, 1 H), 6.20 (s, 2 H), 6.92 (s, 1 H), 8.16 (s, 2 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.7, 12.8, 13.2, 13.3, 13.9, 17.1, 17.1, 17.1, 17.3, 17.4,

17.5, 17.5, 17.5, 22.1, 28.6, 30.0, 50.7, 62.7, 71.9, 74.3, 82.2, 90.2, 119.7, 125.0, 138.9, 142.7, 150.6, 152.7, 155.4 ppm. IR (KBr): $\tilde{\nu}$ = 696, 884, 1037, 1087, 1134, 1296, 1466, 1580, 1643, 2867, 2946 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{29}H_{51}N_8O_5Si_2$ [M + H] 647.3516; found 647.3490.

Method C. General Procedure for the Synthesis of 6f–6i: NaN_3 (5.3 equiv.) was added to a solution of CuI (0.2 equiv.), L-proline (0.4 equiv.), NaOH (0.4 equiv.) and 4-iodoanisole (48.2 mg, 0.206 mmol) in DMSO (2.5 mL/mmol NaN_3). The reaction mixture was heated in the microwave cavity at 120 °C for 5 min. Compound **4** (50 mg, 0.094 mmol), TBTA (0.05 equiv.) and CuI (0.05 equiv.) were added to the reaction mixture, and the reaction mixture was heated in the microwave cavity at 60 °C for 5 min. The reaction mixture was diluted with water (4 mL/mL DMSO), and the water phase was extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with brine (2 × 5 mL), dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography.

8-[1-(3-Aminophenyl)-1*H*-1,2,3-triazol-4-yl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6f): The title compound was synthesised according to the general procedure C. The crude product was purified by column chromatography (toluene/EtOAc, 1:3). Compound **5** (50 mg, 0.09 mmol) and 3-iodoaniline (45 mg, 0.21 mmol) gave **6f** as a white foam (38 mg, 61%). R_f = 0.18 (toluene/EtOAc, 1:3). ^{1}H NMR ($CDCl_3$): δ = 1.02–1.25 (m, 28 H), 3.36 (s, 1 H), 4.02–4.10 (m, 5 H), 5.09 (d, J = 5.9 Hz, 1 H), 5.57–5.60 (m, 1 H), 5.83 (s, 2 H), 6.73 (d, J = 7.0 Hz, 1 H), 6.96 (s, 1 H), 7.04 (d, J = 7.7 Hz, 1 H), 7.13 (s, 1 H), 7.25–7.29 (m, 1 H), 8.22 (s, 1 H), 8.56 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.4, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 62.7, 71.9, 74.3, 82.3, 90.2, 106.9, 110.0, 115.7, 119.8, 123.2, 130.7, 137.4, 139.4, 142.4, 148.2, 150.7, 152.9, 155.4 ppm. IR (KBr): $\tilde{\nu}$ = 693, 884, 1037, 1087, 1129, 1333, 1465, 1638, 2865, 2944, 3356 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{30}H_{46}N_9O_5Si_2$ [M + H] 668.3155; found 668.3127.

8-[1-(4-Methoxyphenyl)-1*H*-1,2,3-triazol-4-yl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6g): The title compound was synthesised according to the general procedure C. The crude product was purified by column chromatography (toluene/EtOAc, 1:2). Compound **5** (50 mg, 0.09 mmol) and 4-iodoanisole (48 mg, 0.21 mmol) gave **6g** as a white foam (33 mg, 51%). R_f = 0.22 (toluene/EtOAc, 1:2). ^{1}H NMR ($CDCl_3$): δ = 1.04–1.24 (m, 28 H), 3.44 (br. s, 1 H), 3.86 (s, 3 H), 4.01–4.10 (m, 3 H), 5.09 (d, J = 5.9 Hz, 1 H), 5.56–5.60 (m, 1 H), 6.00 (s, 2 H), 6.98 (s, 1 H), 7.02 (d, J = 8.8 Hz, 2 H), 7.66 (d, J = 8.8 Hz, 2 H), 8.20 (s, 1 H), 8.52 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.3, 17.1, 17.2, 17.3, 17.4, 17.5, 17.5, 17.6, 55.8, 62.7, 71.9, 74.4, 82.3, 90.3, 115.0, 119.9, 122.4, 123.3, 129.9, 139.6, 142.4, 150.8, 152.9, 155.4, 160.3 ppm. IR (KBr): $\tilde{\nu}$ = 1035, 1090, 1252, 1519, 1651, 2866, 2943 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{31}H_{47}N_8O_5Si_2$ [M + H] 638.3152; found 638.3124.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-8-[1-(4-tolyl)-1*H*-1,2,3-triazol-4-yl]adenosine (6h): The title compound was synthesised according to the general procedure C. The crude product was purified by column chromatography (toluene/EtOAc, 1:1). Compound **5** (50 mg, 0.09 mmol) and 4-iodotoluene (45 mg, 0.21 mmol) gave **6h** as a white foam (31 mg, 50%). R_f = 0.21 (toluene/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ = 1.05–1.25 (m, 28 H), 2.44 (s, 3 H), 3.35 (br. s, 1 H), 4.02–4.10 (m, 3 H), 5.08 (d, J = 5.9 Hz, 1 H), 5.57–5.61 (m, 1 H), 5.79 (s, 2 H), 6.98 (s, 1 H), 7.34–7.36 (m, 2 H), 7.65–7.67 (m, 2 H), 8.22 (s, 1 H), 8.57 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.4, 17.1, 17.2, 17.4, 17.5, 17.5, 17.6, 17.6, 21.3, 62.8, 72.0, 74.4, 82.4, 90.3, 120.0, 120.8, 123.2, 130.5, 134.3, 139.7, 139.7, 142.5, 150.8, 152.9, 155.3 ppm. IR (KBr): $\tilde{\nu}$ = 697, 884, 1037, 1088, 1129, 1333, 1466, 1639, 2866, 2944 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{31}H_{47}N_8O_5Si_2$ [M + H] 667.3203; found 667.3196.

8-[1-(4-Chlorophenyl)-1*H*-1,2,3-triazol-4-yl]-3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)adenosine (6i): The title compound was synthesised according to the general procedure C. The crude product was purified by column chromatography (toluene/EtOAc, 3:4). Compound **5** (0.10 g, 0.19 mmol) and 1-chloro-4-iodobenzene (98 mg, 0.41 mmol) gave **6i** as a white foam (76 mg, 59%). R_f = 0.29 (toluene/EtOAc, 3:4). ^{1}H NMR ($CDCl_3$): δ = 1.01–1.25 (m, 28 H), 3.37 (br. s, 1 H), 4.02–4.09 (m, 3 H), 5.09 (d, J = 5.9 Hz, 1 H), 5.57–5.60 (m, 1 H), 5.85 (s, 2 H), 6.95 (s, 1 H), 7.53 (d, J = 8.8 Hz, 2 H), 7.74 (d, J = 8.8 Hz, 2 H), 8.22 (s, 1 H), 8.60 (s, 1 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 12.8, 12.8, 13.2, 13.4, 17.1, 17.2, 17.6, 17.5, 17.5, 17.6, 17.6, 62.7, 71.9, 74.4, 82.4, 90.3, 120.0, 122.1, 123.1, 130.3, 135.0, 135.4, 140.1, 142.1, 150.8, 153.0, 155.4 ppm. IR (KBr): $\tilde{\nu}$ = 694, 884, 1036, 1090, 1128, 1332, 1466, 1503, 1580, 1639, 2866, 2944 cm^{-1}. HRMS (FT-ICR-MS): calcd. for $C_{30}H_{44}ClN_8O_5Si_2$ [M + H] 687.2657; found 687.2629.

8-(1-Isopentyl-1*H*-1,2,3-triazol-4-yl)adenosine (7d): TBAF (1 M in THF, 0.56 mL) was added to a solution of compound **6d** (45.0 mg, 0.07 mmol) in dry THF (5 mL), and the reaction mixture was stirred at room temp. overnight. Dowex 50Wx8-400 (0.35 g) was added to the mixture, and the solution was stirred for 20 min. The Dowex was filtered off and washed with pyridine/MeOH/H_2O (3:1:1). The filtrate was co-evaporated with toluene to remove exess of pyridine. Purification by column chromatography ($CHCl_3$/MeOH, 97:3 with 1% AcOH) gave **6** (28 mg, < 99%) as a white powder. ^{1}H NMR ([D$_6$]DMSO): δ = 0.94 (d, J = 6.6 Hz, 6 H), 1.54–1.61 (m, 1 H), 1.80 (dd, J = 7.0, 14.3 Hz, 2 H), 3.53 (dddd, J = 3.3, 3.7, 8.8, 9.2 Hz, 1 H), 3.70 (dt, J = 3.3, 3.7 Hz, 1 H), 3.97 (dd, J = 3.3, 8.9 Hz, 1 H), 4.20 (dt, J = 2.2, 2.6 Hz, 1 H), 4.52 (t, J = 7.3 Hz, 2 H), 5.07 (q, J = 6.6, 12.1 Hz, 1 H), 5.14 (d, J = 4.4 Hz, 1 H), 5.27 (d, J = 6.2 Hz, 1 H), 5.73 (dd, J = 3.7, 9.2 Hz, 1 H), 6.65 (d, J = 6.6 Hz, 1 H), 7.49 (s, 2 H), 8.15 (s, 1 H), 8.69 (s, 1 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 19.2, 22.0, 22.7, 25.0, 38.4, 48.1, 62.2, 70.9, 71.6, 86.3, 89.3, 126.3, 138.1, 142.0, 150.0, 151.3, 154.3 ppm. HRMS (FT-ICR-MS): calcd. for $C_{17}H_{25}N_8O_4$ [M + H] 405.1992; found 405.1986.

Photophysical Measurements: All photophysical measurements were performed in aqueous or THF solutions. Absorption spectra were measured with a Varian Cary 4000 spectrophotometer. The extinction coefficient of the different base analogues was determined by measuring the absorption of samples of known concentration (duplicate measurements). Samples were prepared from small amounts, in duplicate, typically 1 mg of compound dissolved in known volumes of THF. The steady-state fluorescence was measured with a Spex Fluorolog 3 spectrofluorimeter (JY Horiba). The quantum yields (QY) of the different nucleosides were measured relative to quinine sulfate (QY = 0.55)[25] in 0.5 M H_2SO_4 (aq.) with the excitation wavelength of 314 nm.

Calculations: All DFT calculations were performed by using the Jaguar program from Schrödinger Inc.[26] The potential energy surface of the triazole–adenine bond of 9-methyl-8-(1*H*-1,2,3-triazol-4-yl)adenine was investigated by a relaxed energy scan (36 points, 10° increment) at the B3LYP/6-31G(d) level of theory.[23] Structures A and B and transition state TS_{ROT} were further optimised at the 6-311G(d,p) level of theory and verified as either minima or saddle point by a frequency calculation. Singly substituted configurations based on the 10 highest occupied and 10 lowest unoccupied molecular orbitals (200 configurations) were used in the configurational

interactions calculation by applying the ZINDO/S model Hamiltonian as implemented in the HyperChem. 7.5 program package.[27]

Supporting Information (see footnote on the first page of this article): Calculated electronic transitions of the model compound; ^{1}H and ^{13}C NMR spectra of compounds **6a**–**6i**.

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