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Synthesis and photophysical properties of novel cyclonucleosides—substituent effects on fluorescence emission

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ABSTRACT

An intramolecular [3+2]-cycloaddition between an azide and a disubstituted alkyne afforded a number of novel adenosine-derived cyclonucleosides, which exhibited fluorescence in the visible range. The synthesis and photophysical properties of these potential fluorescent probes are described.

1. Introduction

Fluorescent nucleotides have found a plethora of applications in life science including their use in signal transduction research of G-proteins and kinases, as probes for nucleoside-utilising proteins or for labelling of DNA through incorporation by polymerases.[1]

We have previously reported the preparation of some novel fluorescent adenosine-based nucleosides ('fluorosides') possessing a bridging 1,2,3-triazole moiety between the purine base and the ribose.[2] These nucleosides can be regarded as fluorescent derivatives of 8-methanocyclo-adenosine derivative **1**, a micromolar inhibitor of *Brugia malayi* (a tropical nematode parasite) asparaginyl tRNA synthetase,[3] and could be useful to explore the structure, dynamics and recognition of ligands by the asparaginyl tRNA synthetase (Fig. 1).

It is well established that the substituents in a fluorescent organic compound significantly influence its absorption and emission maxima, and quantum yields. Studies of the substituent effects on fluorophores have been conducted on a variety of different structures, including coumarin,[4] naphthalene,[5] BODIPY,[6] thioxanthone,[7] imidazol[1,2-*a*]pyridine[8] and pyrimidopyrimidoindole,[9] and showed that their fluorescence properties could be successfully modulated by the introduction of appropriate substituents.

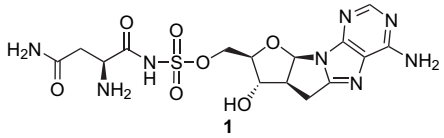
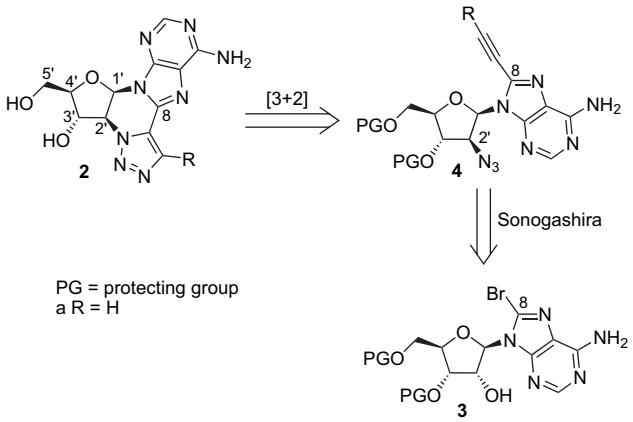


Figure 1.

In this paper, we report the complete synthesis and photophysical properties of a number of fluorosides.[10] We also analysed the relationship between their fluorescence properties and their substituents.

In our previous communication,[2] we reported the synthesis of a small number of compounds of structure **2** as well as the fluorescence properties of compound **2a** (Scheme 1). In this paper, we



Scheme 1. Retrosynthesis of compound **2**.

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report the synthesis of a number of analogues of **2**, with variation of the substituent at the 4-position of the 1,2,3-triazole moiety. The effect of such substitutions on the fluorescence properties of the resulting nucleosides is also investigated.

The general synthetic strategy (Scheme 1) was in essence the same as that previously utilised by us. A Sonogashira reaction[11] between a suitably protected 8-bromoadenosine derivative **3** and various terminal alkynes installed the 8-alkynyl group of cyclisation precursor **4**.[2,12] The alkynes were chosen to give products with electron-withdrawing and electron-donating substituents on the final 1,2,3-triazole ring. This ring was to be formed by a thermal Huisgen [3+2]-cycloaddition between the disubstituted alkyne and the 2′-azide.

2. Results and discussion

2.1. Synthesis

8-Bromoadenosine was synthesised using a literature procedure.[13] Following a modified literature procedure,[14] simultaneous protection of its 3′- and 5′-hydroxyl groups was achieved with $TIPDSCl_2$ (1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane) affording known compound **5**. This aryl bromide was the substrate for the Sonogashira reaction, which was used to install the 8-alkynyl substituents.

The Sonogashira reactions of **5** were performed using $Pd(PPh_3)_2Cl_2$ as the palladium source. Attempts to perform the cross-coupling at room temperature led to rather low conversion of **5** due to competing homocoupling of the alkyne. In addition, the cross-coupled products **6** and starting material **5** effectively co-eluted on flash chromatography, leading to poor recovery of pure product. Performing the cross-coupling using microwave heating (120 °C, 12.5 min) with 4 equiv of the alkyne, 10 mol % $Pd(PPh_3)_2Cl_2$, 20 mol % CuI and 4 equiv of triethylamine in THF led to complete conversion of **5** to the cross-coupled products **6b**–**l**. The coupling proceeded smoothly in all cases, providing the desired coupled products in good yields (73–88%) after flash chromatography. The coupling of **5** with *tert*-butyl-ethynyl-dimethylsilane proceeded smoothly at room temperature. Propiolate derivative **6m** was prepared indirectly using 3,3,3-triethoxypropyne (prepared by sodium hydroxide-mediated hydrolysis[15] of trimethyl-triethoxyprop-1-ynyl-silane[16]) as the coupling partner to give compound **6l**. Use of ethyl propiolate as the coupling partner gave none of the desired cross-coupled product with **5**, instead addition of the 2′-hydroxy to the triple bond occurred in a conjugate fashion, affording an α,β-unsaturated ester. 3,3,3-Triethoxypropyne proved to be less reactive in the Sonogashira reaction than any of the other alkynes used, with 20 min of microwave heating to 120 °C required to force the reaction to completion. An attempt to hydrolyse the orthoester moiety of **6l** using an acidic ion-exchange resin[17] (Dowex 50-WX2-400) in benzene gave a mixture of products by TLC. The use of trifluoroacetic acid in a 1:1 mixture of ethanol and THF afforded the desired carboxylic ester **6m** in good yield (86%).

Triflation of the 2′-hydroxyl group of compounds **6** was performed with trifluoromethanesulfonyl chloride and DMAP in dichloromethane. Triflates **7b** and **7k** were used directly in the subsequent nucleophilic substitution reaction, without recording their crude NMR spectra. The crude 2′-*O*-triflates **7**, despite being stable to column chromatography, were sufficiently pure to be used without purification. Attempted triflation of propiolate **6m** gave a very messy reaction, with none of the desired 2′-*O*-trilfate obtained after careful chromatography of the complex crude mixture. Attempted 2′-O-triflation of compounds **6h** and **6j** failed to give the desired products, due the presence of unprotected amino and hydroxyl groups, respectively, which competed with the 2′-hydroxyl group.

Introduction of the 2′-azide was achieved by nucleophilic displacement of the 2′-*O*-triflate of compounds **7**. As reported in our initial communication, this displacement occurred at room temperature for R=TMS due to initial cleavage of the TMS group followed by nucleophilic displacement of the 2′-*O*-triflate. The 2′-azide **8** could not be isolated as it underwent in situ [3+2]-cycloaddition with the alkyne to give the desired 1,2,3-triazole. For alkyne substituents other than R=H, the initial nucleophilic substitution is significantly more difficult due to steric hindrance about the 2′-position. The azide anion must approach the 2′-position of **7** from the same side of the sugar ring as the purine base. Bulky substituents on the alkyne shield this face of the sugar ring, thus slowing the rate of nucleophilic displacement. For R=TBDMS (**7k**), a 30% yield of the cyclised product (**10k**) was obtained after 120 h at room temperature.

The displacement and subsequent cyclisation were more satisfactorily performed by using 10 equiv of sodium azide and using microwave heating (150 °C for 15 min). However, these conditions were largely incompatible with the silyl protecting group. In addition to the 3′,5′-*O*-TIPDS-protected cycloaddition products, some cleavage of the TIPDS protecting group occurred, with mono-cleavage at both the 3′- and 5′-positions being observed as well as complete TIPDS cleavage to give the highly polar deprotected nucleosides **2**. These complex crude reaction mixtures were treated with ammonium fluoride in methanol to completely remove the silyl groups.[18] The highly polar deprotected products were difficult to isolate, so the crude deprotection mixture was treated with acetic anhydride in pyridine to acetylate the 3′- and 5′-hydroxyl functionalities affording diacetylated nucleosides **9**. These acetate-protected compounds could be isolated and purified by column chromatography, affording pure compounds **9** in modest yield (17–34%) calculated from the crude triflates **7** (Scheme 2).

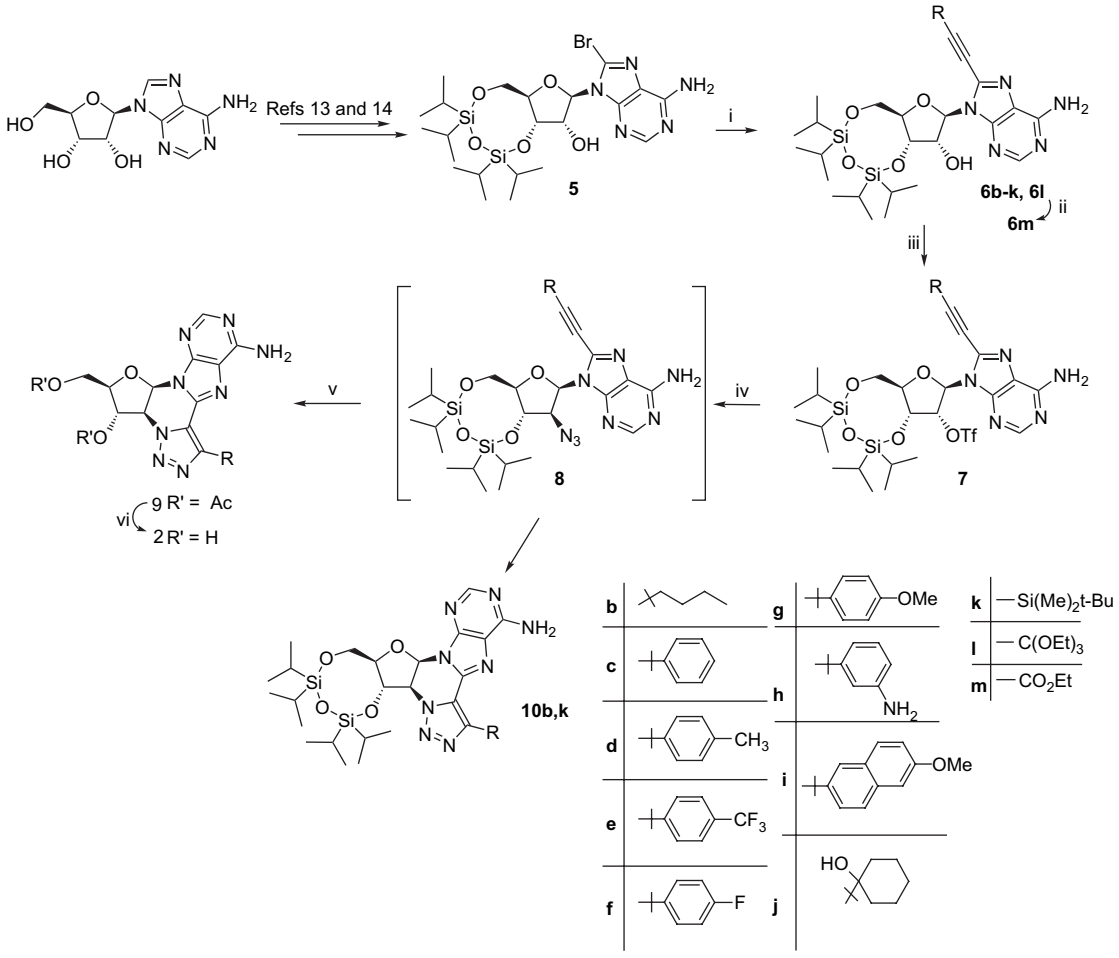
The 3′- and 5′-*O*-acetyl esters were cleaved by treating **9** with ammonia in methanol at room temperature overnight. Concentration under reduced pressure and drying under vacuum afforded the analytically pure deprotected nucleosides **2**.

2.2. Photophysical properties

The 3′,5′-di-*O*-acetyl derivatives **9** were used to measure the UV absorption and fluorescence emission spectra, due to the insolubility of the fully deprotected nucleosides **2**. The 3′,5′-*O*-TIPDS protected nucleoside **10b** was also sufficiently soluble in organic solvents to allow analysis of its photophysical characteristics. As they are electronically isolated from each other, it was assumed that the nature of the 3′- and 5′-*O* protecting groups had no effect on the absorption or emission wavelengths of the chromophore.

The compounds showed, in general, two absorption bands: a higher energy transition centred between approximately 240–260 nm (not shown in Table 1 or Fig. 2) and a higher energy transition with maxima in the range 308–338 nm. With respect to the lower energy transition, the nature of the substituent R had an effect on the UV absorption maxima of compounds **9** and **10**. A substituent, which cannot participate in π-conjugation (R=*n*-butyl, **10b**) had a minimal effect on λ_{max} compared to the parent compound (R=H, λ_{max}=307 nm[2]). A π-conjugating substituent, e.g., a phenyl ring (R=Ph, **9c**) shifted λ_{max} to a longer wavelength. The presence of electron-donating or electron-withdrawing substituents in the 4-position of the phenyl ring had only a small effect on λ_{max}, showing that the extent of conjugation in the chromophore plays a larger role in determining λ_{max}. This is further borne out by the observation that the presence of a substituted naphthyl group (**9i**) gave the highest value for λ_{max} (338 nm).

Excitation at the lower energy band led to fluorescence emission with maxima in the range 378–455 nm (i.e., in the visible range), with the value of λ_{em} dependant on the triazole substituent, R. A



Scheme 2. (i) RC≡CH, $Pd(PPh_3)_2Cl_2$, NEt_3, THF, 120 °C, microwave, 12 min; (ii) TFA, 75% aqueous EtOH, rt, 1.5 h, 86% (for **6l**); (iii) trifluoromethanesulfonyl chloride, DMAP, CH_2Cl_2, 0 °C, 1 h; (iv) NaN_3, DMF, 150 °C, microwave, 15 min then rt, see Section 4 for reaction times; (v) (a) NH_4F, MeOH, reflux, 5 h then rt, overnight, (b) acetic anhydride, pyridine, rt, overnight; (vi) NH_3, MeOH, rt, overnight.

non-conjugating alkyl substituent (R=*n*-butyl, **10b**) gave both the lowest λ_{em} and the lowest fluorescence intensity. With respect to the phenyl-substituted triazoles (**9c**–**e**,**g**), the effect of the *p*-substituent on the Stokes shift was unpredictable, with electron-withdrawing (–CF_3, **9e**) and electron-donating (–OCH_3, **9g**) substituents giving almost identical Stokes shifts in methanol. It appears that the extent of conjugation in the chromophore plays a greater role in determining the Stokes shift (i.e., a greater degree of conjugation led to a longer λ_{em}). However, it must be pointed out that alkyl-substituted triazole **10b** exhibited a greater Stokes shift than either **9c** (R=Ph) or **9d** (R=4-Me–Ph), making such generalisations of apparently limited utility.

The influence of hydrogen bonding on the fluorescence properties of compounds **9** was investigated by measuring the UV and fluorescence spectra of the compounds in methanol (hydrogen bonding possible) and acetonitrile (non-hydrogen bonding solvent). The absorption maxima were largely insensitive to solvent changes, with the same values obtained for λ_{max} in either methanol or acetonitrile. However, in each case, as can be seen from Table 1, λ_{em} was shifted to a shorter wavelength when the emission spectra

Table 1
Photophysical properties of compounds **9c**,**d**,**e**,**g**,**i** and **10b**

Compound	λ_{max}[a] (nm)	λ_{em} (MeOH) (nm)	Stokes shift (MeOH) (nm)	λ_{em} (MeCN) (nm)	Stokes shift (MeCN) (nm)
10b, R=*n*-Bu	308	385	77	378	70
9c, R=Ph	324	395	71	387	63
9d, R=4-Me–Ph	326	390	64	383	57
9e, R=4-CF_3–Ph	326	415	89	395	69
9g, R=4-OMe–Ph	332	419	87	415	83
9i, R=4-OMe–naphth-6-yl	338	455	117	449	111

[a] Conditions for absorption and emission spectra: 5×10^{-6} M in methanol or acetonitrile. Identical values were obtained for λ_{max} in both solvents.

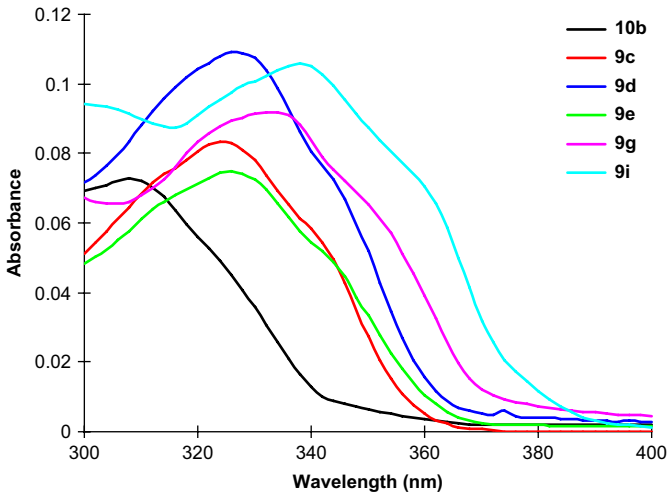


Figure 2. UV absorbance spectra (5×10^{-6} M) of compounds **9c**,**d**,**e**,**g**,**i** and **10b** in methanol.

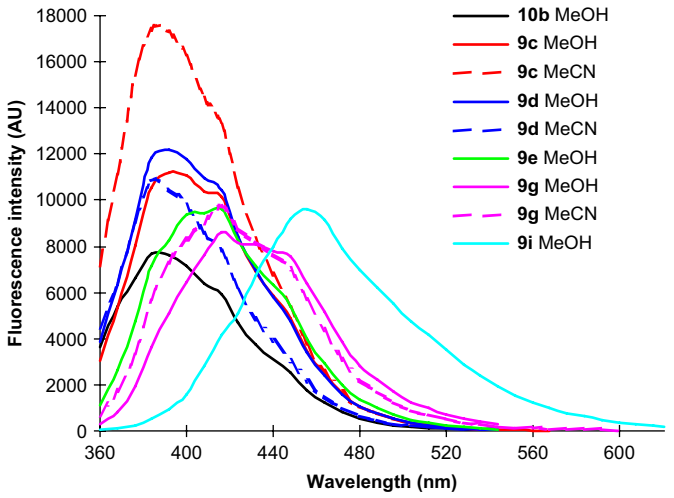


Figure 3. Fluorescence emission spectra (5×10^{-6} M) of compounds **9c**,**d**,**e**,**g**,**i** and **10b** in methanol (smooth lines) and acetonitrile (dotted lines). For each compound $\lambda_{ex}=\lambda_{max}$ (see Table 1).

were recorded in acetonitrile compared to the values obtained in methanol. In other words, a larger Stokes shift is observed when the compounds are analysed in a solvent that is a hydrogen bond donor. Compound **9e** (R=4-CF_3–Ph) exhibited an anomalously large difference in Stokes shift on changing from methanol to acetonitrile, with a difference of 20 nm between the two solvents (the median difference in Stokes shift exhibited by the other compounds being 7 nm).

Fluorescence emission intensity was also affected by the solvent, but not in a predictable way, with **9c** and **9g** emitting more intense fluorescence in acetonitrile, while the opposite was true for compound **9d** (Fig. 3).

3. Conclusion

We have synthesised a series of adenosine-based nucleosides possessing a bridging 1,5-disubstituted 1,2,3-triazole moiety between the purine base and the ribose. The 4-substituent of the 1,2,3-triazole ring was easily varied, through the use of various terminal alkynes in a Sonogashira cross-coupling reaction. Bulky substituents on the alkynes slowed down both the nucleophilic displacement of the 2′-*O*-triflates as well as the cycloaddition reaction. The final compounds of general structure **2** and **10** were found to be fluorescent with emission centred at around 400 nm and with a Stokes shift of the order of 57–111 nm. Analysis of the solvatochromic behaviour of these nucleosides showed that λ_{em} was shifted to a shorter wavelength when the emission was recorded in acetonitrile compared with methanol. Fluorescence emission intensity was also affected by the solvent, but not in a predictable way.

4. Experimental

4.1. General

Solvents were dried using standard procedures. ^{1}H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectra recorded at 100 MHz in the solvents specified using a Jeol ECX spectrometer. Spectra were referenced to residual non-deuterated solvent (^{1}H NMR: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.55 ppm and ^{13}C NMR: $CDCl_3$ 77.2 ppm, DMSO-d_6 39.5 ppm). Mass spectra were run using the FAB (*p*-nitrobenzyl alcohol) method. IR spectra were recorded as KBr disks. Fluorescence and UV spectra were obtained using a Molecular Devices SpectraMax M2 plate reader. Flash chromatography was performed using Merck Geduran Si 60 (0.063–0.200 mm) silica gel. Thin layer chromatography (TLC) was performed using Merck Silica Gel 60 F_{254} aluminium-backed plates and visualised using UV light (254 and/or 366 nm) and potassium permanganate dip. The solvents used to determine R_f values are the same as used for chromatographic purification, unless otherwise stated. Microwave heating was performed using a Biotage Initiator microwave, with a fixed hold time at the desired reaction temperature (wattage was automatically varied to maintain the desired temperature). Nucleoside numbering is used throughout.

4.2. General procedure (A) for Sonogashira reactions of 3′,5′-*O*-TIPDS-8-bromoadenosine 5 using microwave heating

A solution of 3′,5′-*O*-TIPDS-8-bromoadenosine **5** (500 mg, 0.85 mmol), $Pd(PPh_3)_2Cl_2$ (60 mg, 0.085 mmol), CuI (32 mg, 0.17 mmol) and triethylamine (344 mg, 3.40 mmol) in THF (10 mL) was placed in a sealed 25 mL microwave vial. The appropriate terminal alkyne (4 equiv) was then added and the mixture heated to 120 °C for 12.5 min (fixed hold time on). The solvents were removed under reduced pressure and the residue dissolved in $CHCl_3$ (100 mL) and the solution washed with 1 M aqueous HCl (50 mL), water (50 mL) and brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure affording the crude product, which was purified by column chromatography (19:1 CH_2Cl_2/MeOH).

4.2.1. 3′,5′-O-TIPDS-8-(hex-1-ynyl)-adenosine ***6b***

Yield: 366 mg (73%), pale grey foam. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −40.4 (*c* 0.1, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3416, 3178, 2239 (C≡C), 1646, 1599, 1464, 1329, 1298, 1137, 1088, 1037, 885, 694. δ_H (400 MHz, $CDCl_3$) 8.22 (1H, s), 6.13 (1H, s), 5.93 (2H, br s), 5.58–5.53 (1H, m), 4.81 (1H, d, *J*=5.9 Hz), 4.05–3.99 (3H, m), 3.36 (1H, br s), 2.51 (2H, t, *J*=7.3 Hz), 1.65 (2H, quin, *J*=7.3 Hz), 1.48 (2H, sext, *J*=7.3 Hz), 1.26–1.00 (28H, m), 0.94 (3H, t, *J*=7.3 Hz); δ_C (100 MHz, $CDCl_3$) 155.2, 153.3, 148.9, 135.6, 119.6, 99.1, 89.9, 82.3, 74.7, 72.1, 69.5, 62.9, 30.0, 22.2, 19.3, 17.54, 17.48, 17.44, 17.38, 17.3, 17.2, 17.12, 17.09, 13.6, 13.3, 13.2, 12.8, 12.7; MS (FAB) *m*/*z* (%)=590 (68), 216 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{28}H_{47}N_5O_5Si_2$: 590.3193, found: 590.3189. Anal. Calcd for $C_{28}H_{47}N_5O_5Si_2$: C, 57.01; H, 8.03; N, 11.87. Found: C, 56.89; H, 7.97; N, 11.81.

4.2.2. 3′,5′-O-TIPDS-8-(phenylethynyl)-adenosine ***6c***

Yield: 385 mg (74%), yellow glass. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −46.0 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3417, 3171, 2218 (C≡C), 1653, 1598, 1465, 1328, 1299, 1136, 1090, 1036, 885, 756, 691. δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s), 7.66–7.63 (2H, m), 7.47–7.36 (3H, m), 6.24 (1H, s), 5.68 (2H, br s), 5.56 (1H, t, *J*=6.1 Hz), 4.87–4.84 (1H, m), 4.09–4.01 (3H, m), 3.31 (1H, br s), 1.19–1.02 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.5, 153.6, 149.0, 135.1, 132.3, 130.1, 128.6, 120.5, 120.0, 96.2, 90.1, 82.5, 77.6, 74.6, 72.1, 62.9, 17.6, 17.5, 17.44, 17.39, 17.3, 17.2, 17.14, 17.12, 17.09, 13.3, 13.2, 12.78, 12.75. Anal. Calcd for $C_{30}H_{43}N_5O_5Si_2$: C, 59.08; H, 7.11; N, 11.48. Found: C, 58.94; H, 7.05; N, 11.41.

4.2.3. 3′,5′-O-TIPDS-8-(p-tolylethynyl)-adenosine ***6d***

Yield: 456 mg (86%), yellow solid. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −47.1 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3414, 3172, 2216 (C≡C), 1646, 1598, 1464, 1329, 1298, 1134, 1089, 1036, 885, 698. δ_H (400 MHz, $CDCl_3$) 8.19 (1H, s), 7.46 (2H, d, *J*=8.1 Hz), 7.13 (2H, d, *J*=8.1 Hz), 6.58 (2H, br s), 6.22 (1H, s), 5.53 (1H, t, *J*=5.9 Hz), 4.84 (1H, d, *J*=5.9 Hz), 4.09–3.99 (3H, m), 3.57 (1H, br s), 2.35 (3H, s), 1.18–0.97 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.5, 153.5, 149.0, 140.6, 135.3, 132.2, 129.4, 119.9, 117.4, 96.6, 90.0, 82.4, 77.4, 74.6, 72.2, 63.0, 21.8, 17.6, 17.50, 17.46, 17.4, 17.3, 17.2, 17.12, 17.09, 13.3, 13.2, 12.78, 12.76. MS (FAB) *m*/*z* (%)=624 (36), 250 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{31}H_{45}N_5O_5Si_2$: 624.3037, found: 624.3030.

4.2.4. 3′,5′-O-TIPDS-8-(p-trifluoromethylphenylethynyl)-adenosine **6e**

Yield: 461 mg (80%), yellow foam. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −48.4 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3330, 3178, 2226 (C≡C), 1646, 1465, 1323, 1297, 1132, 1090, 1067, 1037, 885, 843, 696. δ_H (400 MHz, $CDCl_3$) 8.26 (1H, s), 7.77 (2H, d, *J*=8.0 Hz), 7.67 (2H, d, *J*=8.0 Hz), 6.21 (1H, s), 5.62 (2H, br s), 5.57 (1H, t, *J*=6.3 Hz), 4.88–4.86 (1H, m), 4.08–4.02 (3H, m), 3.31 (1H, br s), 1.24–1.03 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.5, 153.9, 149.1, 134.5, 132.6, 131.7 (q, *J*=33 Hz), 128.6, 125.6 (q, *J*=4 Hz), 124.2, 123.7 (q, *J*=251 Hz), 94.3, 90.1, 82.5, 79.6, 74.6, 72.0, 62.8, 17.53, 17.45, 17.43, 17.36, 17.3, 17.2, 17.11, 17.07, 13.3, 13.2, 12.78, 12.75; MS (FAB) *m*/*z* (%)=678 (52), 304 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{31}H_{42}F_3N_5O_5Si_2$: 678.2754, found: 678.2752. Anal. Calcd for $C_{31}H_{42}F_3N_5O_5Si_2$: C, 54.93; H, 6.25; N, 10.33. Found: C, 55.08; H, 6.21; N, 10.30.

4.2.5. 3′,5′-O-TIPDS-8-(p-fluorophenylethynyl)-adenosine **6f**

Yield: 402 mg (76%), yellow foam. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −38.4 (*c* 0.1, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3414, 3172, 2220 (C≡C), 1645, 1598, 1465, 1329, 1297, 1130, 1091, 1037, 885, 837, 695. δ_H (400 MHz, $CDCl_3$) 8.24 (1H, s), 7.65 (2H, ddt, *J*=9.5, 5.1, 2.6 Hz), 7.14–7.08 (2H, m), 6.21 (1H, d, *J*=1.1 Hz), 5.61 (2H, br s), 5.57 (1H, t, *J*=6.2 Hz), 4.87–4.84 (1H, m), 4.08–4.00 (3H, m), 3.31 (1H, d, *J*=1.1 Hz), 1.22–1.02 (28H, m); δ_C (100 MHz, $CDCl_3$) 164.9, 162.4, 155.4, 153.7, 149.1, 135.0, 134.5 (d, *J*=9 Hz), 120.2 (d, *J*=210 Hz), 116.7 (d, *J*=3 Hz), 116.1 (d, *J*=22 Hz), 95.1, 90.1, 82.5, 74.7, 72.1, 62.9, 17.54, 17.48, 17.44, 17.38, 17.3, 17.2, 1712, 17.09, 13.3, 13.2, 12.78, 12.76; MS (FAB) *m*/*z* (%)=628 (45), 254 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{30}H_{42}FN_5O_5Si_2$: 628.2786, found: 628.2779. Anal. Calcd for $C_{30}H_{42}FN_5O_5Si_2$: C, 57.39; H, 6.74; N, 11.15. Found: C, 57.28; H, 6.67; N, 11.08.

4.2.6. 3′,5′-O-TIPDS-8-(p-methoxyphenylethynyl)-adenosine **6g**

Yield: 476 mg (88%), yellow solid. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −38.7 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3321, 3174, 2214 (C≡C), 1645, 1602, 1465, 1329, 1296, 1252, 1135, 1090, 1035, 885, 832, 694. δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s), 7.60 (2H, dt, *J*=9.3, 2.3 Hz), 6.92 (2H, dt, *J*=9.3, 2.3 Hz), 6.24 (1H, d, *J*=1.1 Hz), 5.60 (2H, br s), 5.56 (1H, t, *J*=6.1 Hz), 4.86–4.83 (1H, m), 4.09–4.01 (3H, m), 3.86 (3H, s), 3.30 (1H, d, *J*=1.1 Hz), 1.19–1.02 (28H, m); δ_C (100 MHz, $CDCl_3$) 161.0, 155.4, 153.4, 149.0, 135.5, 134.0, 120.0, 114.3, 112.4, 96.7, 90.0, 82.4, 76.7, 74.7, 72.2, 63.0, 55.4, 17.6, 17.49, 17.45, 17.4, 17.3, 17.14, 17.12, 17.09, 13.3, 13.2, 12.77, 12.75; MS (FAB) *m*/*z* (%)=640 (52), 266 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{31}H_{45}N_5O_6Si_2$: 640.2986, found: 640.2989. Anal. Calcd for $C_{31}H_{45}N_5O_6Si_2$: C, 58.19; H, 7.09; N, 10.94. Found: C, 58.28; H, 7.04; N, 10.86.

4.2.7. 3′,5′-O-TIPDS-8-(m-aminophenylethynyl)-adenosine **6h**

Yield: 389 mg (73%), yellow solid. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −40.2 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3373, 3172, 2216 (C≡C), 1638, 1599, 1576, 1464, 1329, 1299, 1130, 1089, 1036, 885, 692. δ_H (400 MHz, $CDCl_3$) 8.21 (1H, s), 7.14 (1H, t, *J*=7.7 Hz), 7.00 (1H, d, *J*=7.7 Hz), 6.89 (1H, s), 6.71 (1H, d, *J*=7.7 Hz), 6.22 (1H, s), 6.07 (2H, br s), 5.54 (1H, t, *J*=6.0 Hz), 4.86 (1H, d, *J*=6.0 Hz), 4.12–3.99 (3H, m), 3.78 (2H, br s), 3.43 (1H, br s), 1.19–1.01 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.3, 153.6, 149.1, 146.6, 135.4, 129.6, 122.7, 121.1, 120.1, 118.1, 117.1, 96.7, 90.0, 82.5, 77.0, 62.9, 17.6, 17.49, 17.45, 17.4, 17.3, 17.14, 17.1, 13.3, 13.2, 12.79, 12.76; MS (FAB) *m*/*z* (%)=625 (28), 251 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{30}H_{44}N_6O_5Si_2$: 625.2990, found: 625.2988. Anal. Calcd for $C_{30}H_{44}N_6O_5Si_2$: C, 57.66; H, 7.10; N, 13.45. Found: C, 57.83; H, 7.06; N, 13.37.

4.2.8. 3′,5′-O-TIPDS-8-(2-methoxynaphthyl-6-ethynyl)-adenosine **6i**

Yield: 440 mg (76%), pale brown powder. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −34.7 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3446, 2212 (C≡C), 1636, 1602, 1468, 1330, 1297, 1135, 1089, 1035, 886, 859, 693. δ_H (400 MHz, $CDCl_3$) 8.24 (1H, s), 8.12 (1H, s), 7.76 (1H, d, *J*=5.0 Hz), 7.74 (1H, d, *J*=5.0 Hz), 7.62 (1H, dd, *J*=8.9, 1.6 Hz), 7.20 (1H, dd, *J*=8.9, 2.4 Hz), 7.14 (1H, d, *J*=2.4 Hz), 6.30 (1H, d, *J*=1.1 Hz), 5.63 (2H, br s), 5.56 (1H, t, *J*=6.0 Hz), 4.89–4.87 (1H, m), 4.09–4.05 (3H, m), 3.95 (3H, s), 3.33 (1H, br s), 1.17–1.02 (28H, m); δ_C (100 MHz, $CDCl_3$) 159.1, 155.4, 153.5, 149.1, 135.4, 135.1, 132.9, 132.0, 129.7, 128.7, 128.2, 127.2, 120.1, 119.9, 115.2, 105.9, 97.1, 90.1, 82.5, 74.7, 72.2, 63.0, 55.5, 17.6, 17.49, 17.45, 17.4, 17.30, 17.27, 17.14, 17.11, 13.3, 13.2, 17.79, 17.77; MS (FAB) *m*/*z* (%)=690 (44), 316 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{35}H_{47}N_5O_6Si_2$: 690.3143, found: 690.3129. Anal. Calcd for $C_{35}H_{47}N_5O_6Si_2$: C, 60.93; H, 6.87; N, 10.15. Found: C, 60.93; H, 6.76; N, 10.06.

4.2.9. 3′,5′-O-TIPDS-8-(cycloxexan-1-ol-1-ethynyl)-adenosine **6j**

Yield: 427 mg (80%), grey foam. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −36.5 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3378, 3210, 2234 (C≡C), 1643, 1598, 1464, 1330, 1136, 1081, 1037, 885, 862, 697. δ_H (400 MHz, $CDCl_3$) 8.19 (1H, s), 6.11 (1H, s), 5.91 (2H, br s), 5.50 (1H, t, *J*=6.2 Hz), 4.83 (1H, d, *J*=6.2 Hz), 4.20 (1H, br s), 4.08–3.97 (3H, m), 3.33 (1H, s), 2.14–2.01 (2H, m), 1.82–1.49 (6H, m), 1.38–0.90 (30H, m); δ_C (100 MHz, $CDCl_3$) 155.3, 153.6, 148.9, 134.5, 119.3, 101.6, 90.0, 82.4, 74.5, 72.4, 72.0, 68.8, 62.8, 39.8, 39.6, 39.3, 25.1, 23.1, 17.54, 17.46, 17.42, 17.38, 17.25, 17.10, 17.06, 13.3, 13.2, 12.76, 12.73; MS (FAB) *m*/*z* (%)=632 (52), 258 (100); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{30}H_{49}N_5O_6Si_2$: 632.3200, found: 632.3292. Anal. Calcd for $C_{30}H_{49}N_5O_6Si_2$: C, 57.02; H, 7.82; N, 11.08. Found: C, 57.12; H, 7.75; N, 10.88.

4.2.10. 3′,5′-O-TIPDS-8-([tert-butyldimethylsilyl]ethynyl)-adenosine **6k**

This compound was prepared by stirring overnight at room temperature, using the same amounts of reagents as for the microwave method. Yield: 239 mg (43%), yellow foam. R_f 0.6 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −36.6 (*c* 0.07, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3414, 3177, 2167 (C≡C), 1644, 1596, 1466, 1327, 1135, 1091, 1037, 885, 826, 695. δ_H (400 MHz, $CDCl_3$) 8.21 (1H, s), 6.15 (1H, d, *J*=1.1 Hz), 5.74 (2H, br s), 5.50 (1H, t, *J*=6.4 Hz), 4.83 (1H, d, *J*=6.4 Hz), 4.09–4.00 (3H, m), 3.29 (1H, d, *J*=1.5 Hz), 1.17–0.99 (37H, m), 0.25 (6H, s); δ_C (100 MHz, $CDCl_3$) 155.3, 153.8, 140.0, 134.9, 119.8, 103.0, 92.7, 90.1, 82.5, 74.5, 72.2, 63.0, 26.2, 17.6, 17.5, 17.44, 17.39, 17.3, 17.12, 17.09, 16.8, 13.3, 13.2, 12.79, 12.75, −4.9; HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{30}H_{53}N_5O_5Si_3$: 648.3432, found: 648.3471. Anal. Calcd for $C_{30}H_{53}N_5O_5Si_3$: C, 55.6; H, 8.2; N, 10.8. Found: C, 55.5; H, 8.3; N, 10.4.

4.2.11. 3′,5′-O-TIPDS-8-(triethoxyprop-1-ynyl)-adenosine **6l**

This reaction was heated in the microwave to 150 °C for 20 min (fixed hold time on) to force the reaction to completion. Yield: 121 mg (47%), brown glass. R_f 0.4 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −41.3 (*c* 0.07, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3444, 1646, 1464, 1131, 1088, 1037, 884, 697. δ_H (400 MHz, $CDCl_3$) 8.21 (1H, s), 6.09 (1H, d, *J*=1.5 Hz), 5.95 (2H, br s), 5.46 (1H, t, *J*=5.9 Hz), 4.86 (1H, dt, *J*=5.9, 1.5 Hz), 4.06–3.99 (3H, m), 3.79 (6H, q, *J*=7.2 Hz), 3.26 (1H, d, *J*=1.5 Hz), 1.28 (9H, t, *J*=7.2 Hz), 1.15–1.00 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.5, 154.0, 149.1, 133.6, 120.0, 109.2, 90.6, 90.0, 82.5, 77.3, 74.4, 72.0, 62.8, 59.6, 17.5, 17.44, 17.42, 17.38, 17.3, 17.11, 17.07, 14.9, 13.3, 13.2, 12.77, 12.74. HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{31}H_{53}N_5O_8Si_2$: 680.3511, found: 680.3541.

4.2.12. 3′,5′-O-TIPDS-8-(carboethoxyethynyl)-adenosine **6m**

A solution of 3′,5′-*O*-TIPDS-8-(triethoxyprop-1-ynyl)-adenosine **6l** (122 mg, 0.16 mmol) and trifluoroacetic acid (2 drops) in 75% aqueous ethanol (20 mL) and THF (5 mL) was stirred at room temperature for 1.5 h. The mixture was concentrated to dryness and the residue azeotroped with ethanol (3×10 mL) to remove

water. The dried residue was purified by column chromatography (19:1 $CHCl_2$/MeOH) affording 80 mg (86%) **6m** as a pale brown foam. R_f 0.5 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −36.9 (*c* 0.09, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3447, 2230 (C≡C), 1717 (C═O), 1638, 1460, 1325, 1248, 1136, 1088, 1037, 884, 697. δ_H (400 MHz, $CDCl_3$) 8.25 (1H, s), 6.17 (2H, br s), 6.10 (1H, d, *J*=1.5 Hz), 5.44 (1H, t, *J*=6.0 Hz), 4.87 (1H, dt, *J*=6.0, 1.5 Hz), 4.34 (2H, q, *J*=7.2 Hz), 4.07–3.98 (3H, m), 3.36 (1H, d, *J*=1.5 Hz), 1.14–1.02 (28H, m); δ_C (100 MHz, $CDCl_3$) 155.9, 154.8, 152.5, 149.2, 132.0, 128.5, 89.9, 85.5, 82.7, 74.4, 73.1, 71.8, 63.0, 62.6, 17.53, 17.45, 17.44, 17.37, 17.3, 17.11, 17.06, 14.1, 13.3, 13.2, 12.78, 12.74; MS (FAB) *m*/*z* (%)=606 (30), 232 (100); HRMS-FAB: *m*/*z* [M+H^+] calcd for $C_{27}H_{43}N_5O_7Si_2$: 606.2779, found: 606.2780.

4.3. General procedure (B) for 2′-O-triflation of 3′,5′-*O*-TIPDS-8-alkynyladenosines 6

Trifluoromethanesulfonyl chloride (1.2 equiv) was added at 0 °C to a solution of the 3′,5′-*O*-TIPDS-8-alkynyladenosine **6** (1 equiv) and DMAP (3 equiv) in dry CH_2Cl_2 (to give a 0.1 M concentration of the secondary alcohol) and the resulting mixture stirred at 0 °C for 1 h. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with 1% aqueous acetic acid (75 mL). The aqueous layer was extracted with $CHCl_3$ (100 mL) and the combined organic extracts washed with saturated aqueous $NaHCO_3$ (75 mL) and brine (75 mL), dried over $MgSO_4$ and concentrated under vacuum, affording the crude product **7**, which was used in subsequent reactions without further purification.

4.3.1. 2′-O-Triflyl-3′,5′-O-TIPDS-8-(phenylethynyl)-adenosine ***7c***

768 mg (1.26 mmol) of 3′,5′-*O*-TIPDS-8-(phenylethynyl)-adenosine **6c** afforded 935 mg crude **7c** as a pale yellow solid. δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s), 7.64–7.62 (2H, m), 7.48–7.41 (3H, m), 6.36 (1H, s), 5.93 (1H, d, *J*=5.1 Hz), 5.71 (1H, dd, *J*=8.8, 5.1 Hz), 4.17–4.12 (1H, m), 4.08–4.03 (2H, m), 1.19–1.02 (28H, m).

4.3.2. 2′-O-Triflyl-3′,5′-O-TIPDS-8-(p-tolylethynyl)-adenosine ***7d***

415 mg (0.67 mmol) of 3′,5′-*O*-TIPDS-8-(*p*-tolylethynyl)-adenosine **6d** afforded 506 mg crude **7d** as a yellow foam. δ_H (400 MHz, $CDCl_3$) 8.22 (1H, s), 7.51 (2H, d, *J*=8.1 Hz), 7.23 (2H, d, *J*=8.1 Hz), 6.36 (1H, s), 5.89 (1H, d, *J*=5.6 Hz), 5.67 (1H, dd, *J*=8.6, 5.6 Hz), 4.17–4.11 (1H, m), 4.07–4.02 (2H, m), 2.41 (3H, s), 1.19–0.99 (28H, m).

4.3.3. 2′-O-Triflyl-3′,5′-O-TIPDS-8-(p-trifluoromethylphenylethynyl)-adenosine ***7e***

186 mg (0.27 mmol) of 3′,5′-*O*-TIPDS-8-(*p*-trifluoromethylphenylethynyl)-adenosine **6e** afforded 200 mg crude **7e** as a pale yellow solid. δ_H (400 MHz, $CDCl_3$) 8.25 (1H, s), 7.75 (2H, d, *J*=8.4 Hz), 7.70 (2H, d, *J*=8.4 Hz), 6.34 (1H, s), 5.94 (1H, d, *J*=5.2 Hz), 5.71 (1H, dd, *J*=9.4, 5.2 Hz), 4.18–4.03 (3H, m), 1.19–1.03 (28H, m).

4.3.4. 2′-O-Triflyl-3′,5′-O-TIPDS-8-(2-methoxynaphthyl-6-ethynyl)-adenosine ***7g***

218 mg (0.32 mmol) of 3′,5′-*O*-TIPDS-8-(*2*-methoxynaphthyl-6-ethynyl)-adenosine **6g** afforded 241 mg crude **7g** as a pale yellow solid. δ_H (400 MHz, $CDCl_3$) 8.23 (1H, s), 8.09 (1H, s), 7.77–7.74 (2H, m), 7.58 (1H, dd, *J*=7.0, 1.0 Hz), 7.23–7.20 (1H, m), 7.14 (1H, d, *J*=2.6 Hz), 6.42 (1H, s), 5.94 (1H, d, *J*=5.4 Hz), 5.72 (1H, d, *J*=9.4, 5.4 Hz), 4.16–4.03 (3H, m), 3.95 (3H, s), 1.19–1.02 (28H, m).

4.3.5. 2′-O-Triflyl-3′,5′-O-TIPDS-8-([tert-butyldimethylsilyl]ethynyl)-adenosine ***7k***

203 mg (0.31 mmol) of 3′,5′-*O*-TIPDS-8-([*tert*-butyldimethylsilyl]ethynyl)-adenosine **6k** afforded 232 mg crude **7k** as a colourless glass. δ_H (400 MHz, $CDCl_3$) 8.21 (1H, s), 6.26 (1H, s), 5.94 (1H, d, *J*=5.2 Hz), 5.69 (1H, dd, *J*=9.3, 5.2 Hz), 5.65 (2H, br s), 4.12 (1H, dd, *J*=13.2, 2.9 Hz), 4.05–4.01 (2H, m), 1.21–1.03 (28H, m), 1.02 (9H, s), 0.25 (6H, s).

4.3.6. 3′,5′-O-TIPDS protected n-butyl-substituted triazole ***10b***

3′,5′-*O*-TIPDS-8-(hex-1-ynyl)-adenosine **6b** (250 mg, 0.42 mmol) was 2′-*O*-triflated according to general procedure B, affording crude **7b** as a pale yellow solid (212 mg). A mixture of this crude **7b** and sodium azide (189 mg, 2.90 mmol) in DMF (5 mL) in a sealed microwave vial was heated to 150 °C in the microwave for 15 min (fixed hold time on) and the resulting solution stirred at room temperature for a further 72 h affording a pale brown suspension. The solvents were removed under reduced pressure and the residue coevaporated with toluene (3×25 mL). The solid residue was partitioned between water (50 mL) and $CHCl_3$ (3×50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (19:1 $CHCl_2$/MeOH) afforded 43 mg (24%) **10b** as a colourless glass. R_f 0.52 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −55.0 (*c* 0.05, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3445, 1652, 1594, 1462, 1055, 885, 693. δ_H (400 MHz, $CDCl_3$) 8.42 (1H, s), 6.66 (1H, d, *J*=4.5 Hz), 5.84 (2H, br s), 5.45 (1H, dd, *J*=4.2, 1.9 Hz), 5.11 (1H, dd, *J*=4.5, 2.0 Hz), 4.22 (1H, dt, *J*=9.6, 4.0 Hz), 3.99 (1H, dd, *J*=11.4, 4.0 Hz), 3.45 (1H, dd, *J*=11.4, 9.6 Hz), 3.15 (2H, t, *J*=7.6 Hz), 1.86 (2H, quin, *J*=7.6 Hz), 1.45 (2H, sext, *J*=7.6 Hz), 1.16–0.91 (31H, m); δ_C (100 MHz, $CDCl_3$) 155.5, 154.2, 149.9, 147.6, 137.6, 121.6, 120.6, 85.8, 79.4, 78.6, 65.9, 63.7, 30.7, 25.4, 22.4, 17.6, 17.43, 17.38, 17.3, 17.2, 17.1, 17.0, 14.0, 13.53, 13.45, 13.2, 13.0, 12.5; MS (FAB) *m*/*z* (%)=615 (100); HRMS-FAB: *m*/*z* [M+H^+] calcd for $C_{28}H_{46}N_8O_4Si_2$: 615.3259, found: 615.3245.

4.3.7. 3′,5′-O-TIPDS protected tert-butyldimethylsilyl-substituted triazole ***10k***

3′,5′-*O*-TIPDS-8-([*tert*-butyldimethylsilyl]ethynyl)-adenosine **6k** (203 mg, 0.31 mmol) was 2′-*O*-triflated according to general procedure B, affording crude **7k** as a colourless glass (232 mg). A mixture this crude **7k** and sodium azide (98 mg, 1.50 mmol) in DMF (5 mL) was stirred at room temperature for 21 h affording a pale brown suspension. The solvents were removed under reduced pressure and the residue coevaporated with toluene (3×25 mL). The solid residue was partitioned between water (50 mL) and $CHCl_3$ (3×50 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (19:1 $CHCl_2$/MeOH) afforded 60 mg (30%) **10k** as pale yellow solid. R_f 0.65 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ −87.6 (*c* 0.1, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3386, 1637, 1576, 1465, 1146, 1115, 1039, 886, 841, 780, 702. δ_H (400 MHz, $CDCl_3$) 8.42 (1H, s), 6.67 (1H, d, *J*=4.7 Hz), 5.88 (2H, br s), 5.49 (1H, dd, *J*=4.2, 2.0 Hz), 5.16 (1H, dd, *J*=4.7, 2.0 Hz), 4.24 (1H, dt, *J*=9.3, 4.2 Hz), 3.98 (1H, dd, *J*=11.4, 4.2 Hz), 3.42 (1H, dd, *J*=11.4, 9.3 Hz), 1.17–0.96 (37H, m), 0.55 (3H, s), 0.54 (3H, s); δ_C (100 MHz, $CDCl_3$) 155.7, 154.2, 149.9, 144.4, 137.6, 130.7, 120.5, 85.8, 79.2, 78.8, 65.9, 63.7, 26.8, 17.9, 17.6, 17.44, 17.37, 17.31, 17.27, 17.2, 17.1, 17.04, 16.95, 13.52, 13.48, 13.2, 13.0, 12.5, −5.4, −5.6; HRMS-FAB: *m*/*z* [M+H^+] calcd for $C_{30}H_{53}N_8O_4Si_3$: 674.3576, found: 674.3502.

4.4. General procedure (C) for the preparation of 3′,5′-di-*O*-acetyl triazoles 9

A mixture of a 2′-*O*-triflyl-3′,5′-*O*-TIPDS-8-alkynyl-adenosine (1 equiv) and sodium azide (10 equiv) in DMF (5 mL) in a sealed vial was heated to 150 °C in a microwave for 15 min (fixed hold time on) and the resulting solution stirred at room temperature for 120 h affording a pale brown suspension. The solvent was removed under reduced pressure and the oily residue coevaporated with toluene (3×25 mL). The solid residue was partitioned between water (50 mL) and $CHCl_3$ (3×50 mL), and the combined organic extracts washed with brine, dried over $MgSO_4$ and concentrated under

reduced pressure. The solid residue was dissolved in methanol (20 mL) and ammonium fluoride (10 equiv, calculated with respect to the starting triflate) was added. The mixture was refluxed for 5 h and then stirred at room temperature overnight. The solvent was removed under reduced pressure and the solid residue taken up in pyridine (5 mL) and acetic anhydride (1 mL) was added. The reaction was stirred overnight at room temperature. The volatiles were removed under reduced pressure, the oily residue coevaporated with toluene (3×25 mL) and then purified by column chromatography (19:1 $CHCl_2$/MeOH).

4.4.1. 3′,5′-Di-O-acetyl phenyl triazole ***9c***

549 mg (0.74 mmol) of 2′-*O*-Triflyl-3′,5′-*O*-TIPDS-8-(phenylethynyl)-adenosine **7c** afforded 95 mg (27%) **9c** as a yellow solid. R_f 0.44 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +102.6 (*c* 0.05, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 8.54–8.51 (2H, m), 8.38 (1H, s), 7.50–7.45 (2H, m), 7.43–7.38 (1H, m), 6.79 (1H, d, *J*=4.0 Hz), 6.33 (2H, br s), 6.29 (1H, d, *J*=2.9 Hz), 5.24 (1H, d, *J*=4.0 Hz), 4.39 (1H, q, *J*=2.9 Hz), 4.12 (1H, dd, *J*=12.3, 2.9 Hz), 4.09 (1H, dd, *J*=12.3, 2.9 Hz), 2.24 (3H, s), 1.61 (3H, s); δ_C (100 MHz, $CDCl_3$) 170.3, 169.7, 155.8, 154.2, 149.9, 146.0, 137.1, 129.6, 129.0, 128.7, 128.2, 121.3, 120.1, 83.1, 79.6, 77.7, 63.0, 62.6, 20.8, 20.3; UV (MeOH) λ_{max} (ε)=246 (19,000), 324 (16,800); MS (FAB) *m*/*z* (%)=477 (33); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{22}H_{20}N_8O_5$: 477.1635, found: 477.1630. Anal. Calcd for $C_{22}H_{20}N_8O_5$: C, 55.46; H, 4.23; N, 23.52. Found: C, 55.36; H, 4.14; N, 23.41.

4.4.2. 3′,5′-Di-O-acetyl 4-methylphenyl triazole ***9d***

396 mg (0.61 mmol) of 2′-*O*-Triflyl-3′,5′-*O*-TIPDS-8-(4-methylphenylethynyl)-adenosine **7d** afforded 65 mg (22%) **9d** as a yellow solid. R_f 0.46 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +114.8 (*c* 0.05, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 8.35 (2H, d, *J*=8.0 Hz), 8.34 (1H, s), 7.21 (2H, d, *J*=8.0 Hz), 6.76 (1H, d, *J*=4.2 Hz), 6.55 (2H, br s), 6.26 (1H, d, *J*=2.6 Hz), 5.23 (1H, d, *J*=4.2 Hz), 4.38 (1H, q, *J*=2.6 Hz), 4.10 (1H, dd, *J*=12.1, 2.6 Hz), 4.07 (1H, dd, *J*=12.1, 2.6 Hz), 2.34 (3H, s), 2.23 (3H, s), 1.58 (3H, s); δ_C (100 MHz, $CDCl_3$) 170.3, 169.7, 155.8, 153.9, 149.7, 146.1, 139.7, 137.1, 129.4, 128.0, 126.1, 120.9, 120.0, 83.1, 79.6, 77.7, 62.9, 62.6, 21.5, 20.8, 20.2; UV (MeOH) λ_{max} (ε)=252 (29,560), 326 (21,800); MS (FAB) *m*/*z* (%)=491 (77); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{23}H_{22}N_8O_5$: 491.1791, found: 491.1773. Anal. Calcd for $C_{23}H_{22}N_8O_5$: C, 56.32; H, 4.52; N, 22.8. Found: C, 56.19; H, 4.60; N, 22.72.

4.4.3. 3′,5′-Di-O-acetyl 4-trifluoromethylphenyl triazole ***9e***

200 mg (0.25 mmol) of 2′-*O*-Triflyl-3′,5′-*O*-TIPDS-8-(4-trifluoromethylphenylethynyl)-adenosine **7e** afforded 46 mg (34%) **9e** as a yellow solid. R_f 0.38 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +112.6 (*c* 0.05, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3448, 1745, 1641, 1583, 1327, 1227, 1164, 1118, 1067, 850. δ_H (400 MHz, $CDCl_3$) 8.76 (2H, d, *J*=8.4 Hz), 8.45 (1H, s), 7.76 (2H, d, *J*=8.4 Hz), 6.84 (1H, d, *J*=4.2 Hz), 6.31 (1H, d, *J*=2.6 Hz), 6.08 (2H, br s), 5.28 (1H, d, *J*=4.2 Hz), 4.42 (1H, q, *J*=3.0 Hz), 4.14 (1H, dd, *J*=12.5, 3.0 Hz), 4.09 (1H, dd, *J*=12.5, 3.0 Hz), 2.25 (3H, s), 1.61 (3H, s); δ_C (100 MHz, $CDCl_3$) 170.2, 169.6, 155.8, 154.9, 150.0, 144.5, 136.6, 132.6, 131.2 (q, *J*=32 Hz), 128.4, 126.9 (q, *J*=286 Hz), 125.6 (q, *J*=4 Hz), 122.2, 120.1, 83.1, 79.5, 77.6, 63.1, 62.6, 20.8, 20.2; UV (MeOH) λ_{max} (ε)=254 (27,400), 326 (14,980); MS (FAB) *m*/*z* (%)=545 (48); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{23}H_{19}F_3N_8O_5$: 545.1509, found: 545.1509.

4.4.4. 3′,5′-Di-O-acetyl 4-methoxyphenyl triazole ***9g***

2′-O-Triflation of 3′,5′-*O*-TIPDS-8-(*p*-methoxyphenylethynyl)-adenosine **6g** using general triflation procedure A afforded 566 mg crude 2′-*O*-triflyl-3′,5′-*O*-TIPDS-8-(4-methoxyphenylethynyl)-adenosine **7g**. This afforded 63 mg (17%) compound **9g** as a yellow solid using general procedure B. R_f 0.48 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +88.5 (*c* 0.06, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3444, 1746, 1638, 1248, 1225. δ_H (400 MHz, $CDCl_3$) 8.54 (2H, dt, *J*=9.5, 2.4 Hz), 8.42 (1H, s), 7.02 (2H, dt, *J*=9.5, 2.4 Hz), 6.80 (1H, d, *J*=4.2 Hz), 6.30 (1H, d, *J*=2.2 Hz), 5.96 (2H, br s), 5.24 (1H, d, *J*=4.2 Hz), 4.41–4.38 (1H, m), 4.13 (1H, dd, *J*=12.1, 3.1 Hz), 4.10 (1H, dd, *J*=12.1, 2.7 Hz), 3.86 (3H, s), 2.25 (3H, s), 1.62 (3H, s); δ_C (100 MHz, $CDCl_3$) 170.3, 169.7, 160.6, 155.8, 154.4, 149.9, 145.9, 137.2, 129.6, 121.5, 120.5, 120.0, 114.0, 83.1, 79.6, 77.7, 62.9, 62.6, 55.4, 20.8, 20.2; UV (MeOH) λ_{max} (ε)=258 (21,840), 333 (18,280); MS (FAB) *m*/*z* (%)=507 (65); HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{23}H_{22}N_8O_6$: 507.1741, found: 507.1731.

4.4.5. 3′,5′-Di-O-acetyl 4-methoxynaphth-6-yl triazole ***9i***

422 mg (0.59 mmol) of 2′-*O*-Triflyl-3′,5′-*O*-TIPDS-8-(2-methoxynaphthyl-6-ethynyl)-adenosine **7i** afforded 99 mg (30%) **9i** as a yellow glass. R_f 0.49 (9:1 $CHCl_2$/MeOH); $[\alpha]_D^{20}$ +144.6 (*c* 0.05, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 1746, 1637, 1220. δ_H (400 MHz, $CDCl_3$) 9.00 (1H, s), 8.46 (1H, dd, *J*=8.7, 1.9 Hz), 8.39 (1H, s), 7.73 (2H, dd, *J*=8.7, 2.9 Hz), 7.10 (1H, dd, *J*=8.7, 2.5 Hz), 7.05 (1H, d, *J*=1.9 Hz), 6.78 (1H, d, *J*=4.2 Hz), 6.29 (1H, d, *J*=2.2 Hz), 6.13 (2H, br s), 5.24 (1H, d, *J*=4.2 Hz), 4.40 (1H, q, *J*=2.8 Hz), 4.15–4.07 (2H, m), 3.89 (3H, s), 2.25 (3H, s), 1.59 (3H, s); δ_C (100 MHz, $CDCl_3$) 170.3, 169.7, 158.5, 155.7, 154.5, 149.9, 146.2, 137.0, 135.1, 130.2, 128.7, 127.8, 127.1, 125.8, 124.2, 121.2, 120.1, 119.3, 105.8, 83.1, 79.6, 77.8, 63.0, 62.6, 55.4, 20.3, 20.8; UV (MeOH) λ_{max} (ε)=240 (52,340), 338 (21,160). Anal. Calcd for $C_{27}H_{24}N_8O_6$: C, 58.27; H, 4.35; N, 20.13. Found: C, 58.20; H, 4.43; N, 20.08.

4.5. General procedure (D) for deprotection of 3′,5′-di-*O*-acetates

The 3′,5′-di-*O*-acetyl compounds were dissolved in 7 N ammonia in methanol (5 mL) and the mixture stirred at room temperature for 20 h (after approximately 10 min, the solutions became cloudy). The solvents were removed under reduced pressure and the residue dried under vacuum for 20 h affording the pure product without the need for chromatographic purification.

4.5.1. 4-Trifluoromethylphenyl triazole ***2e***

44 mg (0.08 mmol) of 3′,5′-Di-*O*-acetyl 4-trifluoromethylphenyl triazole **9e** afforded 36 mg (97%) **2e** as a white solid. $[\alpha]_D^{20}$ +216.4 (*c* 0.03, DMSO); ν_{max} (KBr)/cm^{-1} 3449, 3343, 1641, 1332. δ_H (400 MHz, DMSO-d_6) 9.13 (2H, d, *J*=8.0 Hz), 8.31 (1H, s), 7.96 (2H, d, *J*=8.0 Hz), 7.77 (2H, br s), 6.64 (1H, d, *J*=4.8 Hz), 6.45 (1H, d, *J*=5.1 Hz), 5.34 (1H, d, *J*=4.8 Hz), 5.14 (1H, dd, *J*=5.1, 3.2 Hz), 4.74 (1H, t, *J*=5.5 Hz), 4.05–4.02 (1H, m), 3.17–3.08 (2H, m); δ_C (100 MHz, DMSO-d_6) 156.7, 155.0, 149.3, 149.9, 142.8, 136.4, 134.0, 129.1, 128.8, 126.3, 123.2, 123.1, 119.4, 87.4, 79.8, 76.3, 60.2; HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{19}H_{16}F_3N_8O_3$: 462.1376, found: 462.2230.

4.5.2. 4-Methylphenyl triazole ***2d***

55 mg (0.11 mmol) of 3′,5′-Di-*O*-acetyl 4-methylphenyl triazole **9d** afforded 45 mg (100%) **2d** as a pale yellow solid. $[\alpha]_D^{20}$ +267.7 (*c* 0.03, DMSO); ν_{max} (KBr)/cm^{-1} 3447, 3335, 1641. δ_H (400 MHz, DMSO-d_6) 8.70 (2H, d, *J*=8.1 Hz), 8.29 (1H, s), 7.66 (2H, br s), 7.40 (2H, d, *J*=8.1 Hz), 6.62 (1H, d, *J*=4.4 Hz), 6.42 (1H, d, *J*=5.2 Hz), 5.28 (1H, d, *J*=4.4 Hz), 5.13 (1H, dd, *J*=5.2, 2.7 Hz), 4.77 (1H, t, *J*=5.5 Hz), 4.02 (1H, td, *J*=5.2, 2.7 Hz), 3.12–3.04 (2H, m), 2.40 (3H, s); δ_C (100 MHz, DMSO-d_6) 156.0, 154.1, 149.3, 143.9, 138.3, 136.3, 129.4, 127.7, 126.7, 121.1, 118.8, 86.8, 79.2, 75.6, 65.0, 60.7, 21.1; HRMS-FAB: *m*/*z* [M+H$^+$] calcd for $C_{19}H_{19}N_8O_3$: 408.1659, found: 408.1489.

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References and notes

1. *Fluorescence Spectroscopy, Imaging and Probes: New Tools in Chemical, Physical and Life Sciences*; Kraayenhof, R., Visser, A. J. W. G., Gerritsen, H. C., Eds.; Springer: Berlin, 2002.
2. O'Mahony, G.; Ehrman, E.; Grøtli, M. *Tetrahedron Lett.* **2005**, *46*, 6745–6748.
3. Sukuru, S. C. K.; Crepin, T.; Milev, Y.; Marsh, L. C.; Hill, J. B.; Anderson, R. J.; Morris, J. C.; Rohatgi, A.; O'Mahony, G.; Grøtli, M.; Danel, F.; Page, M. G. P.; Haertlein, M.; Cusack, S.; Kron, M. A.; Kuhn, L. A. *J. Comput.-Aided Mol. Des.* **2006**, *20*, 159–178.
4. Takechi, H.; Oda, Y.; Nishizono, N.; Oda, K.; Machida, M. *Chem. Pharm. Bull.* **2000**, *48*, 1702–1710.
5. Kosower, E. M. *Acc. Chem. Res.* **1982**, *15*, 259–266.
6. Boyer, J. H.; Haag, A. M.; Sathyamoorthi, G.; Soong, M. L.; Thangaraj, K.; Pavlopoulos, T. G. *Heteroat. Chem.* **1993**, *4*, 39–49.
7. Neumann, M. G.; Gehlen, M. H.; Encinas, M. V.; Allen, N. S.; Corrales, T.; Peinado, C.; Catalina, F. *J. Chem. Soc., Faraday Trans.* **1997**, *93*, 1517–1521.
8. Tomoda, H.; Hirano, T.; Saito, S.; Mutai, T.; Araki, K. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1327–1334.
9. Mizuta, M.; Seio, K.; Miyata, K.; Sekine, M. *J. Org. Chem.* **2007**, *72*, 5046–5055.
10. For a recent Tetrahedron Symposium-in-Print see: Fluorescent nucleoside analogues: synthesis, properties and applications. *Tetrahedron*; Tor, Y., Ed.; 2007; 63, pp 3415–3614.
11. Chinchilla, R.; Najera, C. *Chem. Rev.* **2007**, *107*, 874–922.
12. Flasche, W.; Cismas, C.; Herrmann, A.; Liebscher, J. *Synthesis* **2004**, 2335–2341.
13. Ikehara, M.; Kaneko, M.; Okano, R. *Tetrahedron* **1970**, *26*, 5675–5682.
14. Maruyama, T.; Kozai, S.; Manabe, T.; Yazima, Y.; Satoh, Y.; Takaku, H. *Nucleosides Nucleotides* **1999**, *18*, 2433–2442.
15. Eaborn, C.; Walton, D. R. M. *J. Organomet. Chem.* **1965**, *4*, 217–228.
16. Baldwin, J. E.; Pritchard, G. J.; Rathmell, R. E. *Synth. Commun.* **2000**, *30*, 3833–3847.
17. For the orthoester hydrolysis with Amberlyst 15 see: Adamo, M. F. A.; Adlington, R. M.; Baldwin, J. E.; Pritchard, G. J.; Rathmell, R. E. *Tetrahedron* **2003**, *59*, 2197–2205.
18. Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177–1180.