Tetrahedron 67 (2011) 9588–9594

Contents lists available at SciVerse ScienceDirect

Tetrahedron

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

# Synthesis of *N*-propynyl analogues of peptide nucleic acid (PNA) monomers and their use in the click reaction to prepare *N*-functionalized PNAs

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ARTICLE INFO

*Article history:*
Received 11 July 2011
Received in revised form 9 September 2011
Accepted 26 September 2011
Available online 6 October 2011

*Keywords:*
Click chemistry
Dipolar cycloadditions
Peptide nucleic acids
PEG analogues

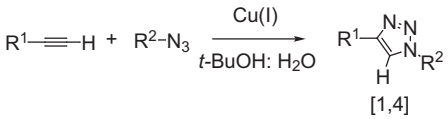
ABSTRACT

Application of the click reaction for coupling a 2-(2-aminoethoxy)ethoxy (AEE) function to thyminyl, cytosinyl and adeninyl peptide nucleic acid (PNA) monomer analogues bearing a *N*-propynyl group, in place of the original *N*-2-aminoethyl moiety, has been explored. The *N*-propynyl PNA analogues were prepared from glycine ethyl ester hydrochloride and the structure of the thyminyl derivative was confirmed by X-ray diffraction. These monomers were employed in click reactions with Boc-protected AEE azide to afford the corresponding triazolyl-linked PNA–AEE conjugates in yields ranging from 64 to 76%. [1,4]-Regiochemistry was verified from a NOESY correlation experiment.



## 1. Introduction

In 2002, Sharpless et al. and Meldal et al. independently reported copper(I)-catalyzed Hüisgen 1,3-dipolar [3+2] cycloadditions involving organic azides and terminal alkynes (Scheme 1).[1,2] In both cases, the corresponding [1,4]-disubstituted 1,2,3-triazole products were formed chemo- and regioselectively in high yields. It is this cycloaddition which has become associated with the term 'click chemistry'.[3] Copper(I)-catalyzed alkyne–azide couplings are reliable, efficient, simple, and extremely versatile; they can be performed in a variety of solvents, even water, and in the presence of many functional groups.[4,5] Given these advantages, it is therefore not surprising that this reaction has found much use in recent years for generating a vast array of compounds for many diverse applications,[6] including those with biological potential.[7,8]




**Scheme 1.** Copper(I)-catalyzed Hüisgen [3+2] cycloaddition.

Previously, we have reported the design and synthesis of adeninyl, cytosinyl and thyminyl peptide nucleic acid (PNA) monomers and a range of thyminyl PNA oligomers bearing either pentacosa-10,12-diynoyl or stearoyl moieties at either the N-or C-termini (**1** and **2**, respectively, Fig. 1).[9,10] PNAs are DNA mimics in which the entire deoxyribose-phosphate backbone has been replaced by a structurally homomorphous uncharged, achiral polyamide backbone composed of *N*-(2-aminoethyl)glycine subunits.[11] An important feature of PNAs is that they bind with higher affinity and sequence specificity to both single-stranded (ss-DNA) and RNA than their natural oligonucleotide counterparts.[12] Monomers **1** and oligomers **2** were subsequently investigated for their abilities to be incorporated into polydiacetylene (PDA)-containing liposomes for possible use as colorimetric nucleic acid biosensors. PDAs are conjugated polymers, which exhibit interesting chromic effects; blue to red transitions can be induced by heat,[13] solvent variations,[14] changes in pH[15,16] and, most notably, upon exposing PDA assemblies incorporating biologically active head groups to their appropriate biomolecular targets.[17]

In order to enhance the aqueous solubilities of both the lipid-functionalized PNA monomers and oligomers and their resulting PDA liposomes, we have recently explored the use of the click reaction to couple a 2-(2-aminoethoxy)ethoxy (AEE) hydrophilic spacer to the N-terminus of PNA (**3**, Fig. 1). Despite the numerous advantages offered by the click reaction, there are relatively few reports in the literature to date involving the application of this copper (I)-catalyzed cycloaddition to the N-functionalization of PNAs. This alkyne–azide coupling has been successfully used to: (i) attach ferrocene moieties to PNA oligomers;[18–21] (ii) prepare peptide–PNA conjugates;[22] (iii) immobilize PNA capture probes onto microarrays;[23] and, (iv) synthesize novel rhenium and

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doi:10.1016/j.tet.2011.09.124

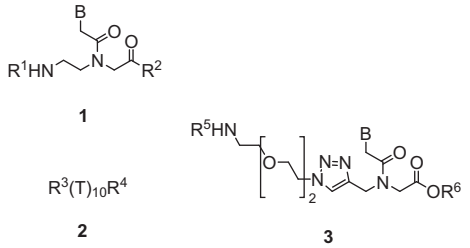


**Fig. 1.** B=Adenin-9-yl; thymin-1-yl; $N^6$-Cbz-adenin-9-yl; $N^6$-Cbz-cytosin-1-yl. $R^1$=H; $CH_3(CH_2)_{11}C_4(CH_2)_8CO$; $CH_3(CH_2)_{16}CO$. $R^2$=OH; $NH(CH_2)_2\{O(CH_2)_2\}_2NHCO(CH_2)_8C_4(CH_2)_{11}CH_3$; $NH(CH_2)_9C_4(CH_2)_{11}CH_3$. T=Thyminyl PNA monomer. $R^3$=$CH_3(CH_2)_{16}CO$; $CH_3(CH_2)_{11}C_4(CH_2)_8CO$; AcAsp. $R^4$=$LysNH_2$; $AspNH_2$; $Lys(CO(CH_2)_8C_4(CH_2)_{11}CH_3)NH_2$; $Lys(CO(CH_2)_{16}CH_3)NH_2$. $R^5$=Boc; pentacosa-10,12-diynoyl; stearoyl. $R^6$=OEt; PNA oligomer.

$^{99m}$technetium tricarbonyl-containing PNA bioconjugates.[24,25] In these examples, the click reactions were facilitated either by derivatisation of the N-terminal PNA residue through coupling of a suitable alkynoic or azido acid spacer or by replacement of the nucleobase of the N-terminal PNA monomer with an alkyne-containing side chain. However, for our studies, we wished to maintain the PNA nucleobase for recognition purposes and we wanted to avoid addition of an alkyl spacer, as it was envisaged that this may further exacerbate the aqueous solubility difficulties already encountered. Therefore, taking these considerations on board, PNA monomer analogues have been designed in which the original *N*-2-aminoethyl moiety has been replaced with a *N*-propynyl group. Thus, here we report the synthesis of the *N*-propynyl analogues of the adeninyl, cytosinyl and thyminyl PNA monomers, preparation of Boc-protected AEE azide and, finally, their use in the click reaction to afford triazolyl-linked PNA–AEE conjugates **3** (Fig. 1, where $R^5$=Boc and $R^6$=OEt).

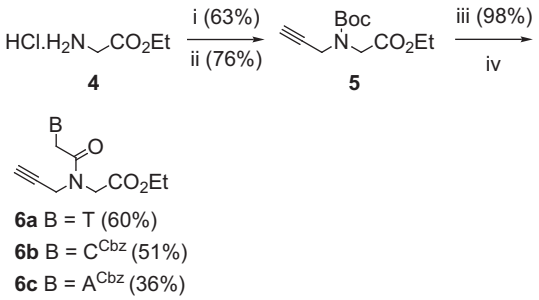
## 2. Results and discussion

### 2.1. *N*-Propynyl PNA monomers

The *N*-propynyl analogues of the thyminyl, cytosinyl and adeninyl PNA monomers (**6a**–**c**, respectively) were successfully prepared from commercially available glycine ethyl ester hydrochloride **4** as outlined in Scheme 2. Thus, the amino function of **4** was first protected with a Boc group using a standard procedure to afford Boc-glycine ethyl ester.[26] Subsequent treatment of a solution of this product in anhydrous DMF with 3-bromoprop-1-yne in the presence of sodium hydride gave the *N*-alkynyl derivative **5**. Finally, following Boc removal, the resulting amino deprotected compound was coupled to thymin-1-ylacetic acid,[27] $N^4$-Cbz-cytosin-1-yl acetic acid[28] and $N^6$-Cbz-adenin-9-ylacetic acid[28] using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate (HBTU) as the activating agent in the presence of an organic base. After work-up and purification by flash chromatography, the desired *N*-propynyl PNA analogues **6a**–**c** were obtained in yields ranging from 35 to 59% over the two steps.

As had been found for the original PNA monomers,[29] the $^1$H and $^{13}$C NMR spectra recorded for *N*-propynyl PNA analogues **6a**–**c** showed the presence of two rotameric forms in solution. However, unlike the original PNA monomers, which exhibited a 70:30 preference for the cis rotamer of the tertiary amide bond over the trans rotamer (where the cis rotamer is defined as the conformer in which the side chain methylene carbonyl is directed towards the C-terminus of the PNA monomer), *N*-propynyl PNA analogues **6a**–**c** showed no such preference with both rotamers present in equivalent amounts.

The structure of the thyminyl derivative **6a** was further confirmed by an X-ray crystallographic study. The ORTEP diagram of **6a**



**Scheme 2.** Boc=$(CH_3)_3COCO$; $A^{Cbz}$=$N^6$-Cbz-adenin-9-yl; $C^{Cbz}$=$N^6$-Cbz-cytosin-1-yl; T=thymin-1-yl. Reagents and conditions: (i) $[(CH_3)_3COCO]_2O$, dioxane, $H_2O$, NaOH (pH ~10.5), 0 °C to rt; (ii) HC≡$CCH_2Br$, NaH, DMF, toluene, 0 °C to rt; (iii) TFA, $CH_2Cl_2$, rt; (iv) $BCH_2CO_2H$,[19,20] HBTU, DIPEA or TEA, DMF, rt.

is shown in Fig. 2. Compound **6a** was found to crystallize with one molecule in the asymmetric unit and in the same monoclinic space group, $P2_1/(1)n$, as the original *N*-Boc-protected thyminyl PNA monomer.[30] Interestingly, although in solution **6a** had been found to exist in two rotameric forms, in the crystal structure only the cis rotamer was present. Packing analysis revealed that the crystal of **6a** had the expected centrosymmetric hydrogen-bonded dimeric structure, which had been observed in the crystal structure of the original thyminyl PNA monomer[30] as well as other PNA analogues.[31] The N(3)–H···O(4) distance was 1.90 Å. The dimers were positioned vertically such that the thyminyl rings were stacked above each other at a distance of 4.90 Å. Further study of the intermolecular interactions showed several close contacts between adjacent molecules of **6a** including one involving alkynyl C(13)–H and side chain carbonyl O(19), with an H···O distance of 2.30 Å.

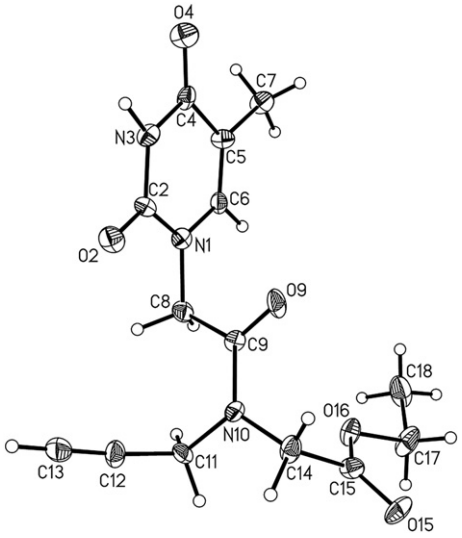
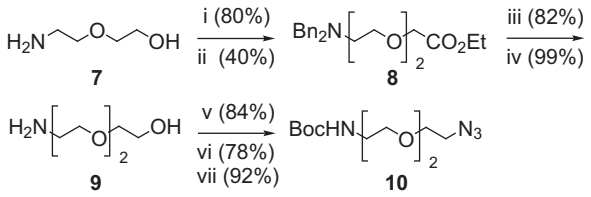



**Fig. 2.** ORTEP diagram of thymin-1-yl derivative **6a**.

### 2.2. Boc-protected AEE azide

The required Boc-protected AEE azide **10** was synthesized from commercially available 2-(2-aminoethoxy)ethanol **7** as shown in Scheme 3. The dibenzyl protection of **7** and subsequent alkylation to afford **8** were accomplished following the procedures reported by Koskinen et al.,[32] except that ethyl bromoacetate was employed in the latter reaction in place of methyl bromoacetate. The next step involved reduction of **8** to give the corresponding alcohol. Although this could be achieved by treatment with $LiAlH_4$, the best yield of the alcohol was obtained when using the $NaBH_4$–methanol system[33] (82% cf. 72% with $LiAlH_4$). The dibenzylamino protecting group was subsequently removed by hydrogenation, using an H-

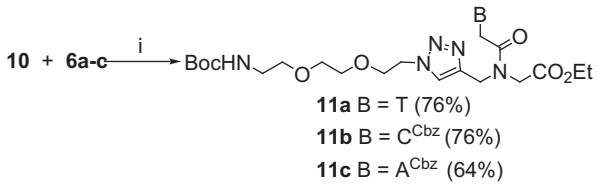
Cube® continuous-flow reactor, to give amino alcohol **9** in a near quantitative yield. After re-protection of the free amino moiety of **9** with a Boc group, the hydroxyl function was successfully converted into the corresponding mesylate by treatment with methanesulfonyl chloride in the presence of triethylamine. Finally, the desired Boc-protected AEE azide **10** was obtained, in a 60% yield over the last three steps, by treatment of a solution of the mesylate in anhydrous DMF with sodium azide at 60 °C.




**Scheme 3.** Reagents and conditions: (i) BnBr, $K_2CO_3$, $H_2O$, rt;[32] (ii) (a) NaH, THF, Δ (b) $BrCH_2CO_2Et$, 0 °C to rt; (iii) (a) $NaBH_4$, THF, Δ (b) MeOH, Δ; (iv) 10% Pd/C, EtOH, H-cube®, 70 bar, 70 °C; (v) $[(CH_3)_3COCO]_2O$, $Et_3N$, MeOH, Δ; (vi) $MeSO_2Cl$, $Et_3N$, $CH_2Cl_2$, 0 °C to rt; (vii) $NaN_3$, DMF, 60 °C.

## 2.3. Click reactions

Click reactions involving the thyminyl, cytosinyl and adeninyl *N*-propynyl PNA analogues **6a**–**c**, respectively, and Boc-protected AEE azide **10** have been explored (Scheme 4). The procedure reported by Sharpless et al.[1] had to be slightly modified as it was noticed that the *N*-propynyl PNA derivatives **6a**–**c** displayed limited solubility in the standard *tert*-butanol/water (1:1, v/v) solvent mixture. This problem was overcome by adding **6a**–**c** to the reaction mixture as a solution in dichloromethane. This modification also simplified work-up as the organic products were easily separated from the copper salts by mere separation of the organic and aqueous phases upon completion of the reaction. Thus, a solution of the required *N*-propynyl PNA analogue **6a**–**c** in dichloromethane was added to a solution of azide **10** in *tert*-butanol/water followed by copper(II) sulfate pentahydrate and a 1 M(aq) solution of sodium ascorbate at rt. After work-up and purification by flash chromatography, the desired click products **11a**–**c** were afforded in yields ranging from 64 to 76%. These reactions have not been optimized.




**Scheme 4.** Reagents and conditions: $CuSO_4 \cdot 5H_2O$, sodium ascorbate, *t*-BuOH/$H_2O$/$CH_2Cl_2$ (1:1:1, v/v/v), rt.

As expected, the $^1H$ and $^{13}C$ NMR spectra recorded for **11a**–**c** revealed the presence of two rotamers. The formation of the triazolyl moiety of **11a**–**c** was verified from the $^1H$ NMR spectra by the disappearance of two triplet peaks between 2.21–2.30 ppm and 2.32–2.48 ppm, corresponding to the terminal alkyne proton in the two rotameric forms of **6a**–**c**, and the appearance of two singlet peaks between 7.71–7.73 ppm and 7.94–8.12 ppm due to the triazolyl proton in the two rotameric forms of **11a**–**c**. The 1,4-regiochemistry of this click reaction was confirmed by a two dimensional NOESY correlation experiment performed on the model triazole compound **12** (Fig. 3), prepared from propynyl intermediate **5** (Scheme 2) and Boc-protected AEE azide **10**. This study clearly showed NOE correlations between the triazolyl proton and both the *N*1- and *C*4-methylene protons (**a** and **b**, respectively, Fig. 3). If the corresponding [1,5]-regioisomer had been produced, the triazolyl proton would have only shown an NOE correlation with the *C*5-methylene protons. Compound **12** was examined here, rather than click products **11a**–**c**, as it is devoid of aromatic protons other than the triazolyl proton which facilitated identification of NOE correlation peaks for this proton.

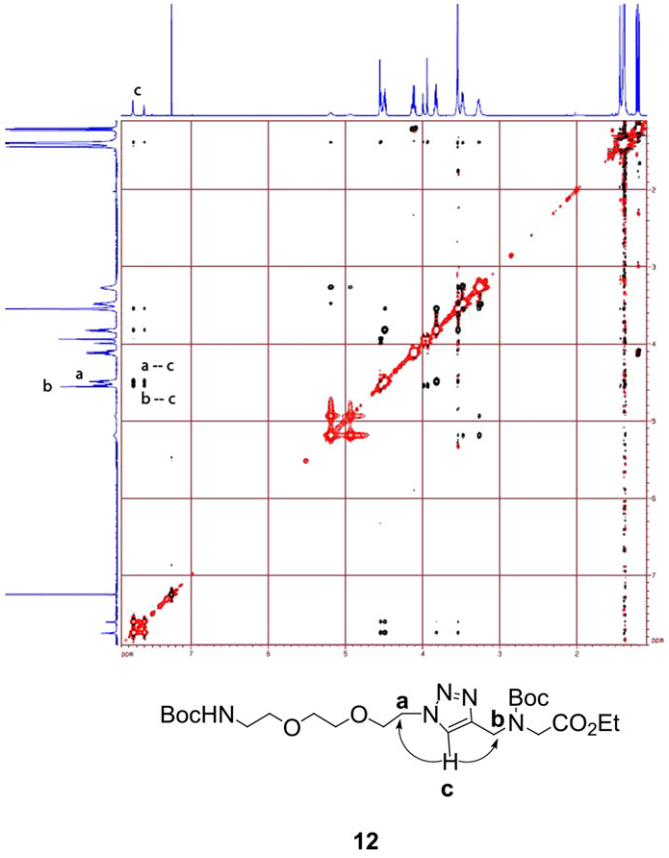



**Fig. 3.** 2D NOESY spectrum recorded for [1,4]-regioisomer of triazole **12** ($CDCl_3$) and key NOE contacts.

## 3. Conclusion

In summary, a viable synthetic route for the preparation of *N*-propynyl analogues of the thyminyl, cytosinyl and adeninyl PNA monomers **6a**–**c**, respectively, has been developed. These monomers have been successfully employed in click reactions with Boc-protected AEE azide **10** to afford the corresponding triazolyl-linked conjugates. Studies are currently underway using these *N*-propynyl PNA monomers for preparing lipid-functionalized PNA oligomers bearing 'clicked' hydrophilic spacers for incorporation into PDA liposomes.

## 4. Experimental section

### 4.1. General

All starting materials were purchased from either Alfa Aesar, Fisher Scientific, Novabiochem or Sigma–Aldrich Chemical companies and were generally used as supplied without further purification. Anhydrous solvents were prepared following standard procedures.[34] Analytical thin layer chromatography (TLC) was performed on aluminium plates pre-coated with Merck Kieselgel 60 $GF_{254}$ (Art. 05554). Products were visualised by UV light and/or by staining the plate with an alkaline $KMnO_4$ solution [$KMnO_4$ (3 g), $K_2CO_3$ (20 g), 5% (w/v) (aq) NaOH (5 mL) and

$H_2O$ (300 mL)] followed by heating. Column chromatography refers to the method of Still et al.[35] and was performed using DAVISIL® silica (60 Å; 35–70 μm) purchased from Fisher (cat. S/0693/60). Melting points were determined using a Stuart Melting Point SMP10 apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1600 FT–IR spectrometer. $^1$H and $^{13}$C NMR spectra were recorded on Bruker AC 200 or DPX 400 spectrometers at 200 MHz ($^1$H) and 50 MHz ($^{13}$C) or at 400 MHz ($^1$H) and 101 MHz ($^{13}$C), respectively. $^1$H and $^{13}$C chemical shifts ($\delta$) are reported in parts per million (ppm) relative to $SiMe_4$, using the $^{13}$C signals or residual proton signals of deuterated solvents as internal standards. Coupling constants ($J$) are reported in hertz (Hz). Low resolution (LRMS) and high resolution (HRMS) mass spectra were recorded at the EPSRC National Mass Spectrometry Service Centre, Swansea, UK. Elemental analyses and X-ray crystallography were carried out by analytical services in Chemistry, School of Engineering & Physical Sciences at Heriot-Watt University.

*4.1.1. N-(tert-Butoxycarbonyl)-N-(propyn-3-yl)glycine ethyl ester (**5**).* A solution of di-*tert*-butyl dicarbonate (5.14 g, 23.5 mmol) in dioxane (7 mL) was added dropwise to a stirred solution of glycine ethyl ester hydrochloride **4** (3.02 g, 21.6 mmol) in water (60 mL) at 0 °C. After being left to stir for a further 15 min at this temperature, the reaction was allowed to warm slowly to rt whilst the pH was maintained at 10.5 by the addition of 2 M(aq) NaOH. After 2 h, the reaction mixture was concentrated in vacuo and the residual paste was dissolved in $CH_2Cl_2$ (50 mL). The resulting organic solution was washed with water (20 mL), the two phases were separated and the aqueous layer was re-extracted with $CH_2Cl_2$ (30 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude product obtained was purified by column chromatography (EtOAc/petroleum ether (40–60 °C), 6:4) to afford *N*-Boc-glycine ethyl ester (2.77 g, 63%) as a yellow liquid; $R_f$ 0.66 (EtOAc/petroleum ether (40–60 °C), 6:4); $\delta_H$ (200 MHz; $CDCl_3$) 1.22 (2H, t, $J$ 7.2, $OCH_2CH_3$), 1.39 (9H, s, $C(CH_3)_3$), 3.83 (2H, d, $J$ 5.6, $NHCH_2CO$), 4.10 (2H, q, $J$ 7.2, $OCH_2CH_3$), 5.04 (1H, br s, NH); $\delta_C$ (50 MHz; $CDCl_3$) 14.0, 28.2, 42.3, 61.1, 79.6, 155.7, 170.3; $m/z$ (CI) 204 ([M+H]$^+$, 20%),165 (90), 104 (100), 58 (10), 44 (16); HRMS (ESI) $m/z$: [M+H]$^+$, found 204.1230. $C_9H_{18}NO_4$ requires 204.1230.

A solution of 3-bromopropyne in toluene (80% w/v, 2.53 mL, 22.7 mmol) was added dropwise to a stirred solution of *N*-Boc-glycine ethyl ester (1.54 g, 7.57 mmol) in anhydrous DMF (5 mL) at rt under $N_2$ atmosphere. The resulting mixture was cooled to 0 °C and NaH (60% disp. in mineral oil, 0.61 g, 15.2 mmol) was added portionwise. Subsequently, the reaction was allowed to warm slowly to rt whereupon it was left to stir for a further 18 h. The reaction was quenched by the careful addition of water (15 mL) and the mixture was concentrated in vacuo. The residual paste was dissolved in EtOAc (30 mL) and the resulting organic solution was washed with water (15 mL). The two phases were separated and the aqueous layer was re-extracted with EtOAc (6×10 mL). The combined organic layers were washed with water (2×10 mL) followed by brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure and the crude residue obtained was purified by column chromatography (EtOAc/petroleum ether (40–60 °C), 1:5) to yield the *title compound* **5** (1.39 g, 76%) as a deep yellow oil; $R_f$ 0.53 (EtOAc/petroleum ether (40–60 °C), 2:8); $\nu_{max}$(film) 3270, 2980, 2934, 1748, 1706 cm$^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers observed) 1.26–1.30 (3H, m, $OCH_2CH_3$), 1.39 and 1.49 (9H, s, $C(CH_3)_3$), 2.22 (1H, t, $J$ 2.5, $CH_2C{\equiv}CH$), 3.89 and 4.09 (2H, s, $NCH_2CO$), 4.13 and 4.19 (2H, s, $NCH_2C{\equiv}CH$), 4.18–4.22 (2H, m, $OCH_2CH_3$); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers observed) 14.1 and 14.2, 28.1 and 28.2, 36.6 and 37.1, 46.9 and 47.5, 61.0, 72.3 and 72.6, 78.7, 80.9 and 81.0, 154.6 and 154.7, 169.7; $m/z$ (CI) 242 ([M+H]$^+$, 15%), 203 (75), 142 (100), 68 (26); HRMS (ESI) $m/z$: [M+H]$^+$, found 242.1387. $C_{12}H_{20}NO_4$ requires 242.1387.

*4.1.2. N-(Thymin-1-ylacetyl)-N-(propyn-3-yl)glycine ethyl ester (**6a**).* TFA (8 mL) was added dropwise to a stirred solution of **5** (1.00 g, 4.14 mmol) in $CH_2Cl_2$ (8 mL) at rt and the mixture was left to stir for 1 h. Subsequently, the solvent was removed under reduced pressure and the residue afforded was co-evaporated with toluene (2×10 mL) followed by $Et_2O$ (2×10 mL) before being dried in a vacuum desiccator over $P_2O_5$. The trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester (1.04 g, 98%) was obtained as a brown oil and was used in the next stage without further purification.

HBTU (1.34 g, 3.53 mmol) followed by DIPEA (2.00 mL, 12.0 mmol) were added to a stirred solution of thymin-1-ylacetic acid[27] (0.68 g, 3.70 mmol) in DMF (10 mL) at rt and the mixture was left to stir for 15 min. Subsequently, a solution of the trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester (8.86 g, 3.36 mmol) in DMF (10 mL) was slowly added to the reaction mixture followed by a further quantity of DIPEA (3.00 mL, 28.0 mmol). The mixture was stirred at rt overnight and then the solvent was removed in vacuo. The residue was dissolved in $CH_2Cl_2$ (30 mL) and the resulting organic solution was washed successively with 1 M(aq) $NaHCO_3$ (3×15 mL), 1 M(aq) $KHSO_4$ (2×15 mL), water (15 mL) and, finally, brine (15 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude orange foam afforded was purified by column chromatography (EtOAc/MeOH, 95:5) to yield the *title compound* **6a** (0.62 g, 60%) as a cream coloured foam; mp 162–163 °C; [Found: C, 54.77; H, 5.53; N, 13.38. $C_{14}H_{17}N_3O_5$ requires C, 54.72; H, 5.58; N, 13.67%]; $R_f$ 0.32 (EtOAc/MeOH, 95:5); $\nu_{max}$(film) 3238, 3165, 2999, 2832, 1726, 1724, 1682, 1664 cm$^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers observed) 1.26 and 1.31 (2H, t, $J$ 7.1, $OCH_2CH_3$), 1.34 (3H, s, thyminyl C(5)-$CH_3$), 2.30 and 2.48 (1H, t, $J$ 2.4, $CH_2C{\equiv}CH$), 4.18 and 4.26 (2H, q, $J$ 7.1, $OCH_2CH_3$), 4.27–4.29 (4H, m, $COCH_2NCH_2C{\equiv}CH$), 4.50 and 4.70 (2H, s, $NCOCH_2N$), 7.01 (1H, s, thyminyl C(6)-H), 9.50 (1H, s, thyminyl N(3)-H); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers observed) 12.2, 14.0 and 14.1, 36.3 and 38.0, 47.7, 48.0 and 48.1, 61.7 and 62.3, 73.8 and 74.6, 76.6 and 77.1, 110.9, 141.1, 151.3, 164.5, 167.1, 168.7 and 168.9; $m/z$ (ESI) 308 ([M+H]$^+$, 100%), 325 (70), 142 (25); HRMS (ESI) $m/z$: [M+H]$^+$, found 308.1240. $C_{14}H_{18}N_3O_5$ requires 308.1241.

*4.1.3. N-[N$^4$-(Benzyoxycarbonyl)cytosin-1-ylacetyl]-N-(propyn-3-yl) glycine ethyl ester (**6b**).* The trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester was prepared as described for **6a**. HBTU (0.36 g, 1.50 mmol) followed by TEA (0.28 mL, 2.00 mmol) were added to a stirred solution of $N^4$-benzyloxycarbonyl cytosin-1-ylacetic acid[28] (0.32 g, 1.10 mmol) in DMF (6 mL) at rt and the mixture was left to stir for 15 min. Subsequently, a solution of the trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester (0.23 g, 1.02 mmol) in DMF (6 mL) was slowly added to the reaction mixture followed by a further quantity of TEA (0.85 mL, 6.10 mmol). The mixture was stirred at rt overnight and then the solvent was removed in vacuo. The residue was dissolved in $CH_2Cl_2$ (10 mL) and the resulting organic solution was washed successively with 1 M(aq) $NaHCO_3$ (3×15 mL), 1 M(aq) $KHSO_4$ (2×15 mL), water (15 mL) and, finally, brine (15 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude orange foam afforded was purified by column chromatography (EtOAc/MeOH, 95:5) to yield the *title compound* **6b** (0.22 g, 51%) as a white foam; mp 130–132 °C; [Found: C, 59.02; H, 5.07; N, 13.19. $C_{21}H_{22}N_4O_6$ requires C, 59.15; H, 5.20; N, 13.14%]; $R_f$ 0.24 (EtOAc); $\nu_{max}$ (KBr) 3476, 3280, 2984, 1744, 1668, 1629, 1558 cm$^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers observed) 1.16 and 1.22 (2H, t, $J$ 7.1, $OCH_2CH_3$), 2.21 and 2.32 (1H, t, $J$ 2.4, $CH_2C{\equiv}CH$),

4.07 and 4.20 (2H, q, *J* 7.1, OC$H_2$CH$_3$), 4.11 and 4.23 (2H, s, NC$H_2$CO), 4.17 and 4.18 (2H, s, NC$H_2$C≡CH), 4.52 and 4.78 (2H, s, NCOC$H_2$N), 5.62 (2H, s, PhC$H_2$O), 6.96–7.02 (1H, m, cytosinyl C(5)–*H*), 7.26–7.32 (5H, m, Ph), 7.38–7.43 (1H, m, cytosinyl C(6)–*H*), 9.24 (1H, br s, NH); $\delta_C$ ($CDCl_3$) (two rotational isomers were observed) 13.8, 36.3 and 38.2, 47.8 and 48.1, 50.8 and 50.9, 62.0 and 62.5, 67.9, 73.9 and 74.7, 76.4 and 76.8, 96.3, 128.4, 128.6, 128.7, 134.9, 149.8, 152.2, 157.3, 163.3, 167.5 and 167.6, 169.4 and 169.5; *m*/*z* (ESI) 427 ([M+H]$^+$, 61%), 319 (3), 85 (100); HRMS (ESI) *m*/*z*: [M+H]$^+$, found 427.1612. $C_{21}H_{23}N_4O_6$ requires 427.1612.

*4.1.4. N-[N$^6$-(Benzyloxycarbonyl)adenin-9-ylacetyl]-N-(propyn-3-yl) glycine ethyl ester (**6c**).* The trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester was prepared as described for **6a**. HBTU (0.40 g, 1.06 mmol) followed by DIPEA (0.19 mL, 1.10 mmol) were added to a stirred solution of $N^6$-(benzyloxycarbonyl)adenin-9-ylacetic acid[28] (0.33 g, 1.01 mmol) in DMF (30 mL) at rt and the mixture was left to stir for 15 min. Subsequently, a solution of the trifluoroacetic acid salt of *N*-(propyn-3-yl)glycine ethyl ester (0.30 g, 1.17 mmol) in DMF (20 mL) was slowly added to the reaction mixture followed by a further quantity of DIPEA (0.34 mL, 1.93 mmol). The mixture was stirred at rt overnight and then the solvent was removed in vacuo. The residue was dissolved in $CH_2Cl_2$ (10 mL) and the resulting organic solution was washed successively with 1 M(aq) $NaHCO_3$ (3×15 mL), 1 M(aq) $KHSO_4$ (2×15 mL), water (15 mL) and, finally, brine (15 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The crude orange foam afforded was purified by column chromatography (EtOAc/MeOH, 9:1) to yield the *title compound* **6c** (0.16 g, 36%) as a white solid; mp 120–122 °C; [Found C, 58.49; H, 4.62; N, 18.86. $C_{22}H_{22}N_6O_5$ requires C, 58.66; H, 4.92; N, 18.66%]; $R_f$ 0.39 (EtOAc/MeOH, 95:5); $\nu_{max}$ (KBr) 3237, 3190, 3125, 3029, 2957, 1763, 1749, 1646, 1610 cm$^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers were observed) 1.24 and 1.32 (2H, t, *J* 7.1, OCH$_2$C$H_3$), 2.30 and 2.48 (1H, t, *J* 2.4, CH$_2$C≡C*H*), 4.18 and 4.28 (2H, q, *J* 7.1, OC$H_2$CH$_3$), 4.25 and 4.38 (2H, s, NC$H_2$CO), 4.31 and 4.32 (2H, s, NC$H_2$C≡CH), 4.98 and 5.17 (2H, s, NCOC$H_2$N), 5.28 (2H, s, PhC$H_2$O), 7.32–7.42 (5H, m, Ph), 8.02 and 8.05 (1H, s, adeninyl C(8)–*H*), 8.71 and 8.74 (1H, s, adeninyl C(2)–*H*), 8.86 (1H, br s, N*H*); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers were observed) 14.0 and 14.1, 36.4 and 38.1, 43.7 and 43.8, 47.5 and 47.9, 61.6 and 62.3, 67.1, 73.9 and 74.7, 76.7 and 77.2, 121.4, 128.4, 128.6, 128.7, 135.4, 143.1 and 143.8, 149.4 and 149.5, 150.9 and 151.0, 151.4 and 151.5, 152.9 and 153.1, 165.8 and 165.9, 167.2; *m*/*z* (ESI) 451 ([M+H]$^+$, 100%), 342 (36); HRMS (ESI) *m*/*z*: [M+H]$^+$, found 451.1725. $C_{22}H_{23}N_6O_5$ requires 451.1724.

*4.1.5. 8-Dibenzylamino-3,6-dioxaoctanoic acid ethyl ester (**8**).* A solution of *N*,*N*-dibenzyl-5-amino-3-oxapentan-1-ol[32] (2.86 g, 10.00 mmol) in anhydrous THF (8 mL) was added to a stirred slurry of NaH (60% disp. in mineral oil, 0.48 g, 12.0 mmol) in anhydrous THF (15 mL) at rt. The mixture was heated at reflux for 2 h. After being cooled to 0 °C, ethyl bromoacetate (1.22 mL, 11.0 mmol) was slowly added dropwise and the mixture was allowed to warm to rt before being left to stir for 17 h. Subsequently, the reaction was quenched by the careful addition of water (5 mL) and THF was removed under reduced pressure. The aqueous solution was extracted with EtOAc (5×20 mL) and the combined organic layers were washed with water (2×10 mL) followed by brine (10 mL). The combined aqueous phases were re-extracted with a further quantity of EtOAc (20 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield a brown liquid. The crude product was purified by column chromatography (EtOAc/petroleum ether (40–60 °C), 4:6) to give the *title compound* **8** (2.16 g, 40%) as a pale yellow oil; $R_f$ 0.38 (EtOAc/petroleum ether (40–60 °C), 4:6); $\nu_{max}$ (film) 3027, 2872, 1754, 1494 cm$^{-1}$; $\delta_H$ (200 MHz; $CDCl_3$) 1.16 (3H, t, *J* 7.1, OCH$_2$C$H_3$), 2.61 (2H, t, *J* 6.2, NC$H_2$CH$_2$O), 3.46–3.52 (6H, m, C$H_2$O(C$H_2$)$_2$O), 3.57 (4H, s, 2× NC$H_2$Ph), 4.03 (2H, s, OC$H_2$CO), 4.10 (2H, q, *J* 7.1, OC$H_2$CH$_3$), 7.12–7.32 (10H, m, 2× Ph); $\delta_C$ (50 MHz; $CDCl_3$) 14.4, 52.8, 59.1, 60.9, 68.9, 70.1, 70.5, 71.1, 126.9, 128.3, 128.9, 139.8, 170.6; *m*/*z* (ESI) 372 ([M+H]$^+$, 100%), 153 (50); HRMS (ESI) *m*/*z*: [M+H]$^+$, found 372.2170. $C_{22}H_{30}NO_4$ requires 372.2169.

*4.1.6. 8-Amino-3,6-dioxaoctan-1-ol (**9**).* Finely powdered $NaBH_4$ (3.86 g, 102 mmol) was added portionwise, over a period of 15 min, to a stirred solution of **8** (5.83 g, 15.7 mmol) in THF (80 mL) at reflux. Subsequently, methanol (80 mL) was carefully added dropwise to the stirred reaction mixture at reflux over another 15 min. The resulting mixture was left to stir at reflux for a further 60 min. After this time, the mixture was allowed to cool to rt before the reaction was quenched by the careful addition of a saturated aqueous solution of $NH_4Cl$ (80 mL). The organic layer was separated and the aqueous phase was extracted with EtOAc (6×80 mL). The combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude oil afforded was purified by column chromatography (EtOAc/petroleum ether (40–60 °C), 2:1) to yield the intermediate, 8-dibenzylamino-3,6-dioxaoctan-1-ol (4.22 g, 82%), as a colourless oil; $R_f$ 0.33 (EtOAc/petroleum ether (40–60 °C), 7:3); $\nu_{max}$ (film) 3436, 3027, 2871, 2871, 1494, 1453 cm$^{-1}$; $\delta_H$ (200 MHz; $CDCl_3$) 2.73 (2H, t, *J* 6.2, NC$H_2$CH$_2$O), 2.90 (1H, br s, O*H*), 3.57–3.69 (m, 10H, C$H_2$O(-C$H_2$)$_2$O(C$H_2$)$_2$OH), 3.72 (4H, s, 2× NC$H_2$Ph), 7.24–7.48 (10H, m, 2× NC$H_2$Ph); $\delta_C$ (50 MHz; $CDCl_3$) 52.8, 59.1, 61.8, 70.1, 70.5, 72.7, 127.0, 128.3, 128.9, 139.8; *m*/*z* (CI) 330 ([M+H]$^+$, 100%), 268 (3), 254 (6), 240 (30), 238 (23), 210 (30); HRMS (ESI) *m*/*z*: [M+H]$^+$, found 330.2068. $C_{20}H_{28}NO_3$ requires 330.2064.

Using a 10% Pd/C cartridge, a 0.05 M solution of 8-dibenzylamino-3,6-dioxaoctan-1-ol (1.00 g, 3.04 mmol) in absolute ethanol (60 mL) was cycled twice through an H-Cube® continuous-flow reactor at a flow rate of 1 mL/min. The applied pressure was set to 70 bar and the temperature to 70 °C. At the end of the second run, the entire reaction mixture was collected and concentrated in vacuo to give the *title compound* **9** (0.45 g, 99%) as a colourless oil; $\nu_{max}$ (film) 3365, 3312, 3302, 2873, 2854, 1599 cm$^{-1}$; $\delta_H$ (200 MHz; $D_2O$) 2.88 (2H, t, *J* 5.3, NH$_2$C$H_2$CH$_2$O), 3.54–3.72 (10H, m, C$H_2$O(C$H_2$)$_2$O(C$H_2$)$_2$OH); $\delta_C$ (50 MHz; $D_2O$) 39.1, 60.0, 68.6, 69.2, 69.3, 71.4.

*4.1.7. 8-tert-Butoxycarbonylamino-3,6-dioxaoct-1-yl azide (**10**).* Di-*tert*-butyl dicarbonate (1.43 g, 6.57 mmol) was added to a stirred solution of **9** (0.48 g, 3.19 mmol) in 10% (v/v) TEA/MeOH (20 mL) at rt. The reaction mixture was heated at reflux for 3 h and then allowed to cool to rt. The solvent was removed in vacuo and the residual oil was purified by column chromatography (EtOAc/MeOH, 95:5) to yield the intermediate, 8-*tert*-butoxycarbonylamino-3,6-dioxaoctan-1-ol (0.67 g, 84%), as a colourless oil; $R_f$ 0.40 (EtOAc/MeOH, 95:5); $\nu_{max}$ (film) 3356, 2976, 1694, 1526 cm$^{-1}$; $\delta_H$ (200 MHz; $CDCl_3$) 1.38 (9H, s, C(C$H_3$)$_3$), 2.98 (1H, br s, O*H*), 3.25 (2H, br dt, NHC$H_2$CH$_2$O), 3.49 (2H, t, *J* 5.3, NHCH$_2$C$H_2$O), 3.54–3.61 (6H, m, O(C$H_2$)$_2$OC$H_2$), 3.68 (2H, br dt, CH$_2$C$H_2$OH), 5.27 (1H, br s, N*H*); $\delta_C$ (50 MHz; $CDCl_3$) 28.7, 39.9, 61.3, 69.9, 70.5, 70.6, 72.3, 79.5, 156.3; *m*/*z* (CI) 250 ([M+H]$^+$, 15%), 150 (100); HRMS (ESI) *m*/*z*: [M+H]$^+$, found 250.1649. $C_{11}H_{24}NO_5$ requires 250.1649.

Methanesulfonyl chloride (0.18 mL, 2.29 mmol) was added slowly dropwise to a stirred solution of 8-*tert*-butoxycarbonylamino-3,6-dioxaoctan-1-ol (0.52 g, 2.08 mmol) and TEA (0.44 mL, 3.12 mmol) in anhydrous $CH_2Cl_2$ (10 mL) at 0 °C under $N_2$ atmosphere. The reaction mixture was allowed to slowly warm to rt before being left to stir overnight. After this time, the reaction was quenched by the addition of ice cool water (10 mL) and the two phases were separated. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (10 mL) followed by brine (10 mL) and dried ($MgSO_4$). The solvent was removed under

reduced pressure and the crude residue obtained was purified by column chromatography (EtOAc/petroleum ether (40–60 °C), 4:6) to give the intermediate, 8-*tert*-butoxycarbonylamino-3,6-dioxaoct-1-yl methanesulfonate (0.53 g, 78%), as a colourless oil: $R_f$ 0.46 (EtOAc/MeOH, 95:5); $\nu_{max}$ (film) 2976, 2935, 2874, 1708, 1521, 1352, 1175 $cm^{-1}$; $\delta_H$ (200 MHz; $CDCl_3$) 1.38 (9H, s, $C(CH_3)_3$), 3.01 (3H, s, $OSO_2CH_3$), 3.24 (2H, br dt, $NHCH_2CH_2O$), 3.47 (2H, t, *J* 5.3, $NHCH_2CH_2O$), 3.58–3.61 (4H, m, $OCH_2CH_2O$), 3.71 (2H, t, *J* 4.6, $OCH_2CH_2OSO_2$), 4.33 (2H, t, *J* 4.6, $OCH_2CH_2OSO_2$), 4.93 (1H, br s, N*H*); $\delta_C$ (50 MHz; $CDCl_3$) 28.6, 37.9, 40.5, 69.2, 69.4, 70.4, 70.5, 70.8, 79.5, 155.8; *m*/*z* (CI) 345 ($[M+NH_3]^+$, 17%), 328 ($[M+H]^+$, 20), 289 (70), 271 (100), 228 (35); HRMS (ESI) *m*/*z*: $[M+H]^+$, found 328.1426. $C_{12}H_{26}NO_7S$ requires 328.1424.

Sodium azide (0.31 g, 4.76 mmol) was added to a stirred solution of 8-*tert*-butoxycarbonylamino-3,6-dioxaoct-1-yl methanesulfonate (0.59 g, 1.81 mmol) in anhydrous DMF (20 mL) at rt under $N_2$ atmosphere. The reaction mixture was gently heated at 60 °C for 4 h. After this time, the reaction was poured onto a mixture of ice/water (1:1) (ca. 20 mL) and the resulting aqueous solution was extracted with $CH_2Cl_2$ (3×20 mL). The organic phase was separated, dried ($MgSO_4$) and the solvent was carefully removed under reduced pressure to give the *title compound* **10** (0.45 g, 92%) as a yellow oil. This was used directly, without further purification, in the click reactions to prepare **11a**–**c** and **12**; $\nu_{max}$ (film) 3360, 2977, 2930, 2870, 2109, 1713 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) 1.42 (9H, s, $C(CH_3)_3$), 3.27–3.30 (2H, m, $NHCH_2CH_2O$), 3.38 (2H, t, *J* 5.3, $OCH_2CH_2N_3$), 3.52 (2H, t, *J* 5.3, $NHCH_2CH_2O$), 3.59–3.64 (4H, m, $OCH_2CH_2O$), 3.65 (2H, t, *J* 5.3, $OCH_2CH_2N_3$), 5.00 (1H, br s, N*H*); $\delta_C$ (101 MHz; $CDCl_3$) 28.4, 40.3, 50.6, 70.0, 70.2, 70.3, 70.5, 79.1, 155.9; *m*/*z* (CI) 292 ($[M+NH_4]^+$, 7%), 275 ($[M+H]^+$, 12), 236 (50), 175 (95), 44 (100); HRMS (ESI) *m*/*z*: $[M+H]^+$, found 275.1715. $C_{11}H_{23}N_4O_4$ requires 275.1714.

*4.1.8. N-[N$^1$-(8-tert-Butoxycarbonylamino-3,6-dioxaoct-1-yl)-1,2,3-triazol-4-yl]methyl-N-(thymin-1-ylacetyl)glycine ethyl ester (**11a**).* A solution of the thyminyl monomer **6a** (0.11 g, 0.34 mmol) in $CH_2Cl_2$ (3 mL) was added to a stirred solution of the azide **10** (0.09 g, 0.33 mmol) in $H_2O$/*t*-BuOH (1:1) (6 mL) at rt. Subsequently, $CuSO_4 \cdot 5H_2O$ (0.01 g, 0.03 mmol) followed by 1 M(aq) sodium ascorbate solution (0.07 mL, 0.07 mmol) were added and the resulting yellow solution was left to stir for 48 h. After this time, the two layers were separated and the organic phase was dried ($MgSO_4$) before being concentrated in vacuo. The crude residue obtained was purified by column chromatography (EtOAc/MeOH, 95:5) to give the *title compound* **11a** (0.14 g, 76%) as a yellow foam; $R_f$=0.12 (EtOAc/MeOH, 95:5); $\nu_{max}$ (film) 3357, 2978, 1743, 1679, 1678, 1514 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers were observed) 1.19 and 1.22 (3H, t, *J* 7.1, $OCH_2CH_3$), 1.42 (9H, s, $C(CH_3)_3$), 1.89 and 1.91 (3H, s, thyminyl C(5)–*H*), 3.28–3.30 (2H, m, $NHCH_2CH_2O$), 3.50–3.54 (2H, m, $NHCH_2CH_2O$), 3.56–3.61 (4H, m, $OCH_2CH_2O$), 3.78–3.85 (2H, m, triazolyl N(1)–$CH_2CH_2O$), 4.14 and 4.28 (2H, s, $NCH_2CO$), 4.16 and 4.18 (2H, q, *J* 7.1, $OCH_2CH_3$), 4.42 and 4.80 (2H, s, $NCOCH_2N$), 4.50 and 4.56 (2H, t, *J* 4.9, triazolyl N(1)–$CH_2CH_2O$), 4.68 and 4.73 (2H, s, triazolyl C(4)–$CH_2N$), 5.17 and 5.23 (1H, br t, N*H*), 7.02 and 7.05 (1H, s, thyminyl C(6)–*H*), 7.73 and 7.94 (1H, s, triazolyl C(5)–*H*), 9.06 and 9.20 (1H, br s, thyminyl N(3)–*H*); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers were observed) 12.3, 15.2, 28.4, 40.1, 42.6 and 43.4, 47.6 and 48.1, 47.8 and 48.9, 50.2 and 50.4, 62.1, 69.3, 69.9, 70.2, 70.4, 79.2 and 79.3, 110.8 and 110.8, 123.7 and 124.3, 140.8 and 140.9, 144.2 and 144.3, 151.1, 156.0, 164.1, 167.1 and 167.2, 168.6 and 168.9; *m*/*z* (ESI) 582 ($[M+H]^+$, 100%), 482 (65); HRMS (ESI) *m*/*z*: $[M+H]^+$, found 582.2881. $C_{25}H_{40}N_7O_9$ requires 582.2882.

*4.1.9. N-[N$^1$-(8-tert-Butoxycarbonylamino-3,6-dioxaoct-1-yl)-1,2,3-triazol-4-yl]methyl-N-[N$^4$-(benzyloxycarbonyl)cytosin-1-ylacetyl] glycine ethyl ester (**11b**).* A solution of the cytosinyl monomer **6b** (0.29 g, 0.69 mmol) in $CH_2Cl_2$ (5 mL) was added to a stirred solution of the azide **10** (0.18 g, 0.68 mmol) in $H_2O$/*t*-BuOH (1:1) (10 mL) at rt. Subsequently, $CuSO_4 \cdot 5H_2O$ (0.04 g, 0.17 mmol) followed by 1 M(aq) sodium ascorbate solution (0.34 mL, 0.34 mmol) were added and the resulting yellow solution was left to stir for 48 h. After this time, the two layers were separated and the organic phase was dried ($MgSO_4$) before being concentrated in vacuo. The crude residue obtained was purified by column chromatography (EtOAc/MeOH, 93:7) to give the *title compound* **11b** (0.32 g, 76%) as an off-white foam; $R_f$ 0.26 (EtOAc/MeOH, 93:7); $\nu_{max}$ (film) 3262, 3143, 2979, 2931, 1745, 1692, 1668, 1625 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers were observed) 1.22 and 1.27 (3H, t, *J* 7.1, $OCH_2CH_3$), 1.42 (9H, s, $C(CH_3)_3$), 3.22–3.31 (2H, m, $NHCH_2CH_2O$), 3.47–3.67 (6H, m, $CH_2O(CH_2)_2O$), 3.79–3.88 (2H, m, triazolyl N(1)–$CH_2CH_2O$), 4.09 (2H, br s, $NCH_2CO$), 4.13 and 4.18 (2H, q, *J* 7.1, $OCH_2CH_3$), 4.39 and 4.88 (2H, s, $NCOCH_2N$), 4.48 and 4.55 (2H, br t, *J* 4.9, triazolyl N(1)–$CH_2CH_2O$), 4.67 and 4.76 (2H, s, triazolyl C(4)–$CH_2N$), 5.19 (2H, s, $PhCH_2O$), 6.67 and 6.99 (1H, br t, N*H*), 7.20–7.65 (7H, m, Ph and cytosinyl C(5)–*H* and C(6)–*H*), 7.71 and 8.12 (1H, s, triazolyl C(5)–*H*); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers were observed) 14.1, 28.3, 40.3, 42.8 and 43.8, 47.5, 49.1, 49.7 and 50.3, 61.4, 67.9, 69.9, 70.2, 70.4, 79.3, 95.2, 123.1, 128.3, 128.4, 128.7, 134.4, 143.2, 149.9, 152.1, 153.0, 155.6, 163.2, 167.1, 168.6; *m*/*z* (ESI) 701 ($[M+H]^+$, 100%), 601(13); HRMS (ESI) *m*/*z*: $[M+H]^+$, found 701.3243. $C_{32}H_{45}N_8O_{10}$ requires 701.3253.

*4.1.10. N-[N$^1$-(8-tert-Butoxycarbonylamino-3,6-dioxaoct-1-yl)-1,2,3-triazol-4-yl]methyl-N-[N$^6$-(benzyloxycarbonyl)adenin-9-ylacetyl]glycine ethyl ester (**11c**).* A solution of the adeninyl monomer **6c** (0.47 g, 1.05 mmol) in $CH_2Cl_2$ (5 mL) was added to a stirred solution of the azide **10** (0.24 g, 0.87 mmol) in $H_2O$/*t*-BuOH (1:1) (10 mL) at rt. Subsequently, $CuSO_4 \cdot 5H_2O$ (0.05 g, 0.22 mmol) followed by 1 M(aq) sodium ascorbate solution (0.44 mL, 0.44 mmol) were added and the resulting yellow solution was left to stir for 48 h. After this time, the two layers were separated and the organic phase was dried ($MgSO_4$) before being concentrated in vacuo. The crude residue obtained was purified by column chromatography (EtOAc/MeOH, 9:1) to give the *title compound* **11c** (0.40 g, 64%) as a white foam; $R_f$ 0.20 (EtOAc/MeOH, 9:1); $\nu_{max}$ (film) 3362, 3248, 3134, 2988, 2936, 2884, 1747, 1706, 1674, 1612 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers were observed) 1.19 and 1.22 (3H, t, *J* 7.1, $OCH_2CH_3$), 1.41 (9H, s, $C(CH_3)_3$), 3.24–3.32 (2H, m, $NHCH_2CH_2O$), 3.45 (2H, t, *J* 4.9, $NHCH_2CH_2O$), 3.51–3.63 (4H, m, $OCH_2CH_2O$), 3.79 and 3.86 (2H, t, *J* 4.9, triazolyl N(1)–$CH_2CH_2O$), 4.13 and 4.24 (2H, q, *J* 7.1, $OCH_2CH_3$), 4.18 and 4.42 (2H, s, $NCH_2CO$), 4.50 and 4.60 (2H, t, *J* 4.9, triazolyl N(1)–$CH_2CH_2O$), 4.71 and 4.84 (2H, s, $NCOCH_2N$), 5.01 and 5.42 (2H, s, triazolyl C(4)–$CH_2N$), 5.10 and 5.22 (1H, br s, N*H*), 5.30 (2H, s, $PhCH_2O$), 7.33–7.45 (5H, m, Ph), 7.73 and 7.97 (1H, s, triazolyl C(5)–*H*), 8.12 (1H, br s, adeninyl C(8)–*H*), 8.72 and 8.76 (1H, s, adeninyl C(2)–*H*), 8.89 (1H, br s, N*H*); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers were observed) 14.1 and 14.2, 28.4, 40.3, 42.8 and 43.8, 43.8 and 44.2, 47.8 and 49.2, 50.2 and 50.5, 61.5 and 62.2, 67.7, 69.1 and 69.2, 70.1, 70.4, 70.6, 79.5, 121.4, 123.6 and 124.5, 128.5, 128.6, 128.7, 135.5, 141.8 and 142.3, 144.1, 149.3, 151.1, 151.4, 152.8, 156.0, 166.3, 168.6 and 168.7; *m*/*z* (ESI) 725 ($[M+H]^+$, 100%), 649 (15); HRMS (ESI) *m*/*z*: $[M+H]^+$, found 725.3358. $C_{33}H_{45}N_{10}O_9$ requires 725.3365.

*4.1.11. N-[N$^1$-(8-tert-Butoxycarbonylamino-3,6-dioxaoct-1-yl)-1,2,3-triazol-4-yl]methyl-N-[tert-butoxycarbonylamino]glycine ethyl ester (**12**).* A solution of the propynyl derivative **5** (0.09 g, 0.36 mmol) in $CH_2Cl_2$ (3 mL) was added to a stirred solution of the azide **10** (0.09 g, 0.33 mmol) in $H_2O$/*t*-BuOH (1:1) (6 mL) at rt. Subsequently, $CuSO_4 \cdot 5H_2O$ (0.01 g, 0.03 mmol) followed by 1 M(aq) sodium ascorbate solution (0.07 mL, 0.07 mmol) were added and the

resulting deep yellow solution was left to stir for 48 h. After this time, the two layers were separated and the organic phase was dried ($MgSO_4$) before being concentrated in vacuo. The crude residue obtained was purified by column chromatography (EtOAc/MeOH, 9:1) to give the *title compound* **12** (0.13 g, 76%) as an off-white solid: $R_f$ 0.40 (EtOAc/MeOH, 9:1); $\nu_{max}$ (film) 3362, 2977, 2933, 1748, 1704, 1514 $cm^{-1}$; $\delta_H$ (400 MHz; $CDCl_3$) (two rotational isomers were observed) 1.22 (3H, t, *J* 7.2, $OCH_2CH_3$), 1.38 (9H, s, $C(CH_3)_3$), 1.40 (9H, s, $C(CH_3)_3$), 3.28 (2H, br dt, $NHCH_2CH_2O$), 3.52 (2H, m, $NHCH_2CH_2O$), 3.56 (4H, m, $OCH_2CH_2O$), 3.82 (2H, m, triazolyl N(1)–$CH_2CH_2O$), 3.95 and 3.99 (2H, s, $NCH_2CO$), 4.13 (2H, q, *J* 7.2, $OCH_2CH_3$), 4.44 (2H, m, triazolyl N(1)–$CH_2CH_2O$), 4.55 and 4.57 (2H, s, triazolyl C(4)–$CH_2N$), 4.92 and 5.20 (1H, br t, N*H*), 7.56 and 7.75 (1H, s, triazolyl C(5)–*H*); $\delta_C$ (101 MHz; $CDCl_3$) (two rotational isomers were observed) 14.0 and 14.1, 28.2, 28.3, 40.3, 42.8 and 43.4, 48.1 and 48.8, 50.1 and 50.2, 60.8 and 60.9, 69.4 and 69.5, 70.0, 70.2, 70., 80.5, 80.6, 123.1 and 123.9, 144.2 and 144.6, 155.2, 155.9, 169.8; *m*/*z* (EI) 515 ($[M]^+$, 20%).

### 4.2. X-ray crystallographic diffraction study of *N*-(thymin-1-ylacetyl)-*N*-(propyn-3-yl)glycine ethyl ester (6a)

The crystals of **6a** were grown at rt using the vapour diffusion method in which the solvent was methanol and the precipitant was diethyl ether. Single crystal X-ray diffraction data were collected at 100 K on a Bruker X8 APEX2 CCD diffractometer[36] using the APEX2 suite of programs.[37] The positions of hydrogen atoms were calculated and constrained to idealised geometry, except for the thyminyl N(3)–H hydrogen atom, which was located from the difference Fourier map and its isotropic displacement parameter was constrained to 1.2×U(eq) of N(3) and the N–H distance restrained to 0.90(2) Å. The crystal was not single and was treated as having three components. The cell was found using CELL_NOW and the intensity data was integrated using SAINT_PLUS followed by scaling and correction by TWINABS. The crystal parameters and structure refinement details for **6a** are given in Table 1. Crystallographic data (excluding structure factors) for this structure have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 829606. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or email: deposit@ccdc.cam.ac.uk).

**Table 1**
Crystallographic data and structure refinement details for compound **6a**

| Parameter | **6a** |
|---|---|
| Empirical formula | $C_{14}H_{17}N_3O_5$ |
| Formula weight | 307.31 |
| Temperature | 100(2) K |
| Wavelength | 0.71073 Å |
| Crystal system | Monoclinic |
| Space group | $P2(1)/n$ |
| Unit cell dimensions | $a$=4.8969(15) Å $\alpha$=90° |
| | $b$=15.069(5) Å $\beta$=96.444(13)° |
| | $c$=20.118(5) Å $\gamma$=90° |
| Volume | 1475.2(8) $Å^3$ |
| *Z* | 4 |
| Density (calculated) | 1.384 $Mg/m^3$ |
| Absorption coefficient | 0.107 $mm^{-1}$ |
| *F*(000) | 648 |
| Crystal size | 0.44×0.20×0.12 $mm^3$ |
| $\theta$ Range for data collection | 1.69–33.12° |
| Index ranges | $-6\le h\le 6$, $0\le k\le 21$, $0\le l\le 28$ |
| Reflections collected | 31,957 |
| Independent reflections | 6683 [$R_{int}$=0.0763] |
| Completeness to $\theta$=25.00° | 89.5% |
| Absorption correction | Semi-empirical from equivalents |
| Max. and min transmission | 0.9873 and 0.6355 |
| Refinement method | Full-matrix least-squares on $F^2$ |
| Data/restraints/parameters | 6683/19/206 |
| Goodness-of-fit on $F^2$ | 0.996 |
| Final *R* indices [$I>2\sigma(I)$] | *R*1=0.0922, *wR*2=0.2518 |
| *R* indices (all data) | *R*1=0.1223, *wR*2=0.2700 |
| Largest diff. peak and hole | 0.395 and −0.518 e $Å^{-3}$ |


## Acknowledgements

The authors thank ScotChem and Heriot-Watt University for financial support (to J.R.), Dr. A.S.F. Boyd for recording NMR spectra, Dr. G.M. Rosair for X-ray crystallography, Mrs. C. Graham for elemental analysis and the EPSRC National Mass Spectrometry Service Centre, Swansea, U.K. for LRMS and HRMS data.


## References and notes


1. Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
2. Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
3. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
4. Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51–68.
5. Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302–1315.
6. Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249–1262.
7. Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278–308.
8. New, K.; Brechbiel, M. W. *Cancer Biother. Radiopharm.* **2009**, *24*, 289–302.
9. Howarth, N. M.; Lindsell, W. E.; Murray, E.; Preston, P. N. *Tetrahedron Lett.* **2003**, *44*, 8089–8092.
10. Howarth, N. M.; Lindsell, W. E.; Murray, E.; Preston, P. N. *Tetrahedron* **2005**, *61*, 8875–8887.
11. Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *54*, 1497–1500.
12. Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796–2823.
13. Chance, R. R.; Patel, G. N.; Witt, J. D. *J. Chem. Phys.* **1979**, *71*, 206–211.
14. (a) Chance, R. R. *Macromolecules* **1980**, *13*, 396–398; (b) Rawiso, M.; Aime, J. P.; Fave, J. L.; Schott, M.; Müller, M. A.; Schmidt, M.; Baumgartl, H.; Wegner, G. *J. Phys.* **1988**, *49*, 861–880.
15. (a) Preziosi, A. F.; Bhattacharjee, H. R.; Patel, G. N. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1980**, *21*, 166–167; (b) Bhattacharjee, H. R.; Preziosi, A. F.; Patel, G. N. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1980**, *21*, 168–169; (c) Bhattacharjee, H. R.; Preziosi, A. F.; Patel, G. N. *J. Chem. Phys.* **1980**, *73*, 1478–1480; (d) Patel, G. N.; Preziosi, A. F.; Bhattacharjee, H. R. *J. Polym. Sci. Pol. Sym.* **1984**, *71*, 247–257.
16. Agh-Atabay, N. M.; Lindsell, W. E.; Preston, P. N.; Tomb, P. J. *Polym. Int.* **1993**, *31*, 367–374.
17. Yoon, B.; Lee, S.; Kim, J.-M. *Chem. Soc. Rev.* **2009**, *38*, 1958–1968.
18. Gasser, G.; Hüsken, N.; Köster, S. D.; Metzler-Nolte, N. *Chem. Commun.* **2008**, 3675–3677.
19. Sosniak, A. M.; Gasser, G.; Metzler-Nolte, N. *Org. Biomol. Chem.* **2009**, *7*, 4992–5000.
20. Hüsken, N.; Gasser, G.; Köster, S. D.; Metzler-Nolte, N. *Bioconjugate Chem.* **2009**, *20*, 1578–1586.
21. Hüsken, N.; Gębala, M.; Schuhmann, W.; Metzler-Nolte, N. *ChemBioChem* **2010**, *11*, 1754–1761.
22. Gogoi, K.; Mane, M. V.; Kunte, S. S.; Kumar, V. A. *Nucleic Acids Res.* **2007**, *35* e139.
23. Lim, S. Y.; Chung, W.-Y.; Lee, H. K.; Park, M. S.; Park, H. G. *Biochem. Biophys. Res. Commun.* **2008**, *376*, 633–636.
24. Gasser, G.; Jäger, K.; Zenker, M.; Bergmann, R.; Steinbach, J.; Stephan, H.; Metzler-Nolte, N. *J. Inorg. Biochem.* **2010**, *104*, 1133–1140.
25. Gasser, G.; Sosniak, A. M.; Leonidova, A.; Braband, H.; Metzler-Nolte, N. *Aust. J. Chem.* **2011**, *64*, 265–272.
26. Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*, 2nd ed.; Springer: Berlin, 1994.
27. Kosynkina, L.; Wang, W.; Liang, T. C. *Tetrahedron Lett.* **1994**, *35*, 5173–5176.
28. Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.* **1994**, *59*, 5767–5773.
29. Chen, S.-M.; Mohan, V.; Kiely, J. S.; Griffith, M. C.; Griffey, R. H. *Tetrahedron Lett.* **1994**, *35*, 5105–5108.
30. Goujon, J. Towards the Development of a Novel Colourimetric Nucleic Acid Biosensor Based on Peptide Nucleic Acid-Functionalised Polydiacetylene Liposomes. Ph.D. Thesis, Heriot-Watt University, U.K, 2009.
31. Sanjayan, G. J.; Pedireddi, V. R.; Ganesh, K. N. *Org. Lett.* **2000**, *2*, 2825–2828.
32. Koskinen, A. M. P.; Valo, T.; Vihavainen, S.; Hakala, J. M. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 573–578.
33. da Costa, J. C. S.; Pais, K. C.; Fernandes, E. L.; de Oliveira, P. S. M.; Mendonça, J. S.; de Souza, M. V. N.; Peralta, M. A.; Vasconcelos, T. R. A. *Arkivoc* **2006**, *i*, 128–133.
34. Procter, G.; Leonard, J.; Lygo, B. *Advanced Practical Organic Chemistry*, 2nd ed.; CRC: Cheltenham, UK, 1994.
35. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
36. Bruker. *APEX2 Version 2.1*; Bruker AXS: Madison, Wisconsin, USA, 2004.
37. Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122.