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Abstract: Human cytosolic thymidine kinase (hTK1) has proven to be a suitable target for the noninvasive imaging of cancer cell proliferation using radiolabeled thymidine analogues such as F-18]3'-fluoro-3'-deoxythymidine (F-18]FLT). A thymidine analogue for single photon emission computed tomography (SPECT), which incorporates the readily available and inexpensive nuclide technetium-99m, would be of considerable practical interest. hTK1 is known to accommodate modification of the structure of the natural substrate thymidine at the positions N3 and C3' and, to a lesser extent, C5. In this work, we used the copper-catalyzed azide-alkyne cycloaddition to synthesize two series of derivatives in which thymidine is functionalized at either the C3' or N3 position with chelating systems suitable for the M(CO)(3) core (M = Tc-99m, Re). The click chemistry approach enabled complexes with different structures and overall charges to he synthesized from a common precursor. Using this strategy, the first organometallic hTK1 substrates in which thymidine is modified at the C3' position were identified. Phosphorylation of the organometallic derivatives was measured relative to thymidine. We have shown that the influence of the overall charge of the derivatives is dependent on the position of functionalization. In the case of the C3'-functionalized derivatives, neutral and anionic substrates were most readily phosphorylated (20-28% of the value for the parent ligand thymidine), whereas for the N3-functionalized derivatives, cationic and neutral complexes were apparently better substrates for the enzyme (14-18%) than anionic derivatives (9%).

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Charge dependent substrate activity of C3′ and N3 functionalized, organometallic technetium and rhenium-labelled thymidine derivatives towards human thymidine kinase 1

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Abstract

Human cytosolic thymidine kinase (hTK1) has proven to be a suitable target for the non-invasive imaging of cancer cell proliferation using radiolabelled thymidine analogues such as [^{18}F]3′-fluoro-3′-deoxythymidine ([^{18}F]FLT). A thymidine analogue for single photon emission computed tomography (SPECT) incorporating the readily available and inexpensive nuclide technetium-99m would be of considerable practical interest. hTK1 is known to accommodate modification of the structure of the natural substrate thymidine at the positions, N3, C3′ and, to a lesser extent, C5. In this work, we used the copper catalyzed azide-alkyne cycloaddition to synthesize two series of derivatives in which thymidine is functionalized at either the C3′ or N3 position with chelating systems suitable for the $M(CO)_3$ core (M = ^{99m}Tc, Re). The click chemistry approach enabled complexes with different structures and overall charges to be synthesized from a common precursor. Using this strategy, the first organometallic hTK1 substrates in which thymidine is modified at the C3′ position were identified. Phosphorylation of the organometallic derivatives was measured relative to thymidine. We have shown that the influence of the overall charge of the derivatives is dependent on the position of functionalization. In the case of the C3′-functionalized derivatives, neutral and anionic substrates were most readily phosphorylated (20-28% of the value for the parent ligand thymidine), whereas for the N3-functionalized derivatives, cationic and neutral complexes were apparently better substrates for the enzyme (14-18%) than anionic derivatives (9%).

Introduction

Human thymidine kinase 1 (hTK1[1]) is a cytosolic enzyme which catalyzes the γ-phosphate transfer from ATP to the 5′-hydroxyl groups of thymidine (dT) and 2′-deoxyuridine (dUrd). Other hTK1 substrates include 3′-azido-3′-deoxythymdine, AZT and 3′-fluoro-3′-deoxythymidine, FLT, where thymidine has been modified at the C3′-position, and the phosphorylation of which is the first step in their activation as anti-HIV prodrugs.(*1, 2*) It is well documented, however, that hTK1 is among the most selective of the nucleoside kinases, and is particularly sensitive to changes in the structure of the natural substrates dT and dUrd.(*3*) Eriksson *et al.* have shown that minor modification of the structure at the 5-position is possible, for example, 5-halo and 5-ethyl substitutions are tolerated, but substitution with bulkier groups such as 5-propenyl or 5-(2-chloroethyl) are not.(*1, 4*) More recently, Tjarks and co-workers have shown that hTK1 is also amenable to modification of thymidine at the N3-position.(*5, 6*) Functionalization with a series of bulky carborane derivatives, tethered from N3 of the pyrimidine base with varying spacer length, does not prevent phosphorylation of the nucleoside analogue. The experimental results of substrate activity investigations have all since been rationalized by the determination of the 3-D structure of hTK1, which confirms that hTK1 has a much smaller binding site than other nucleoside kinases.(*7*)

Unlike the activity of other mammalian deoxyribonucleoside kinases, hTK1 activity is stringently cell cycle regulated.(*3*) Active enzyme is not found in resting cells, but activity increases dramatically by the time cells reach the S-phase, to coincide with DNA synthesis. A number of thymidine analogues, which aim to target the high hTK1 activity in proliferating cells, have been developed. As potential agents for boron neutron capture therapy, thymidine derivatives incorporating *closo-o-* and *nido-m*-carboranes have been extensively investigated.(*5, 6, 8, 9*) Similarly, thymidine derivatives labelled with radionuclides suitable for positron emission tomography (PET) have been developed with the goal of monitoring tumour growth and response to therapy.(*10-12*) However, PET nuclides typically have very short half-lives and their production is expensive, usually requiring an onsite cyclotron. A thymidine analogue labelled with technetium for *in vivo* single photon emission computed tomography (SPECT) would be of considerable interest. Technetium has near perfect decay

[1] Abbreviations: hTK1, human thymidine kinase 1; ATP, adenosine triphosphate; dT, thymidine; dUrd, uridine; AZT, 3′-azido-3′-deoxythymdine; FLT, 3′-fluoro-3′-deoxythymdine; PET, positron emission tomography; SPECT, single photon emission tomography; TBDMS, *tertiary*-butyldimethylsilyl; Boc, *tertiary*-butoxycarbonyl; dTTP, thymidine 5'-triphosphate; hENT1, human equilibrative nucleoside transporter; IdUrd, 5-iododeoxyuridine; FdUrd, 5-fluorodeoxyuridine; Glut1, glucose transporter 1; LAT, large neutral amino acid transporter 1; PBS, phosphate buffered saline; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NADH, nicotinamide adenine dinucleotide hydrate; PK, pyruvate kinase; LDH, lactate dehydrogenase; DTT, dithiothreitol; PEP, phospho(enol)pyruvate.

characteristics for diagnostic applications ($T_{1/2}$ = 6 h, 140 keV γ-radiation), moreover it is readily available at low cost from a ^{99}Mo/^{99m}Tc generator system.

Given the promising reports of the retained substrate activity of N3-functionalized carborane thymidine derivatives,(*5, 6, 9*) the N3 position is appealing for functionalization with a technetium complex. We recently reported the synthesis and *in vitro* evaluation of two series of technetium-labelled thymidine derivatives in which the nucleoside was functionalized at the N3 position, and could qualitatively show that all of the compounds retained activity towards hTK1.(*13, 14*) It has also been reported, that minor structural modifications at the C3′ position do not dramatically lower affinity towards hTK1.(*4*) From the elucidation of the structure of hTK1, Welin *et al.* suggest that tolerance of C3′ modification could be a result of the open nature of the C3′/C4′ environment and the proximity of the C3′ substituent to the surface of the enzyme.(*7*) However, to our knowledge there have not been any systematic investigations into the steric tolerance of hTK1 at this position. Tjarks' group report a small series of compounds in which thymidine has been functionalized at the 3′ position with a carborane.(*15*) Unfortunately the low stability of these compounds prevented their thorough characterization with respect to their activity towards hTK1.

The aim of the current investigation was the synthesis of technetium-labelled thymidine analogues, which have the potential to be used in radiopharmacy as proliferation markers. We chose to investigate the substrate activity of C3′-functionalized thymidine analogues, and to compare these with N3-functionalized derivatives, particularly with regard to their overall charge. We used the "click-to-chelate" approach(*14, 16*) for the parallel synthesis of two series of derivatives which could be labelled with technetium and rhenium (Figure 1). This strategy exploits the remarkable features of the copper catalyzed azide-alkyne cycloaddition(*17, 18*) and enables simultaneous formation of the chelating system and conjugation to a biologically relevant molecule in a single, high-yielding step. In the present study a series of bidentate alkynes (Figure 1, **2**-**6**) were reacted with either the commercial C3′-azido thymidine derivative AZT (C3′dT-N_3) or an N3 azido-thymidine derivative (N3dT-N_3, **1**) to yield two sets of conjugates with efficient tridentate triazole-containing chelators for the M(CO)$_3$ core (M = ^{99m}Tc, Re). This enabled us to identify novel organometallic substrates for hTK1 and qualitatively analyze structure-activity relationships. Preliminary investigations were also carried out into the cell-internalizing ability of representative complexes in two human glioblastoma cell lines.

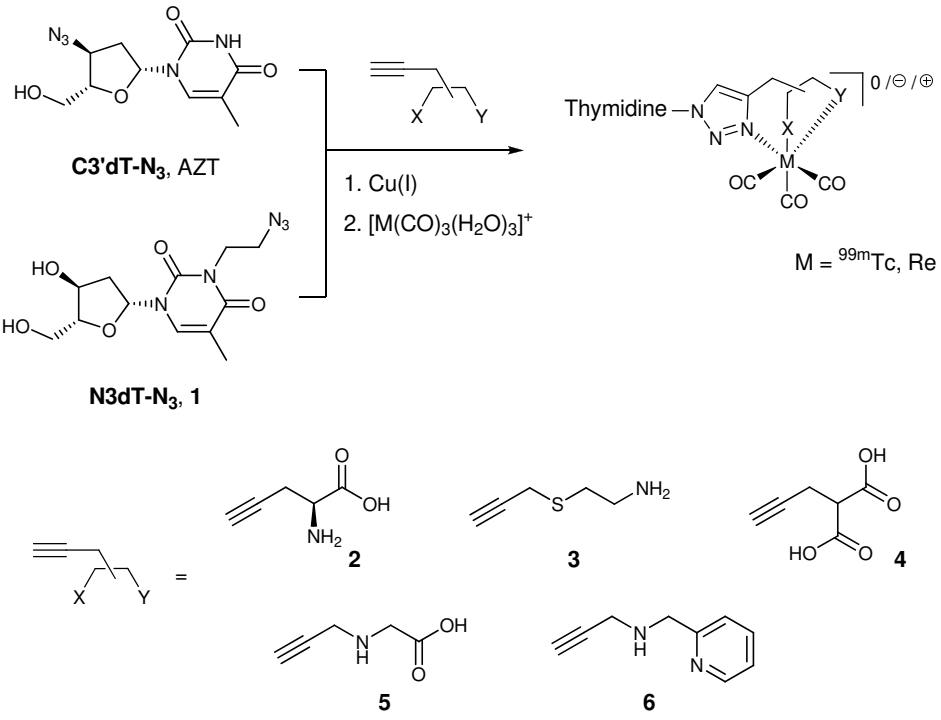


Figure 1. General reaction scheme: alkyne derivatives **2**-**6** are reacted with C3′ and N3 functionalized azido-thymidine derivatives to give conjugates with tridentate chelating systems, which can be labelled with the $M(CO)_3$ core (M = ^{99m}Tc, Re).

Results and discussion

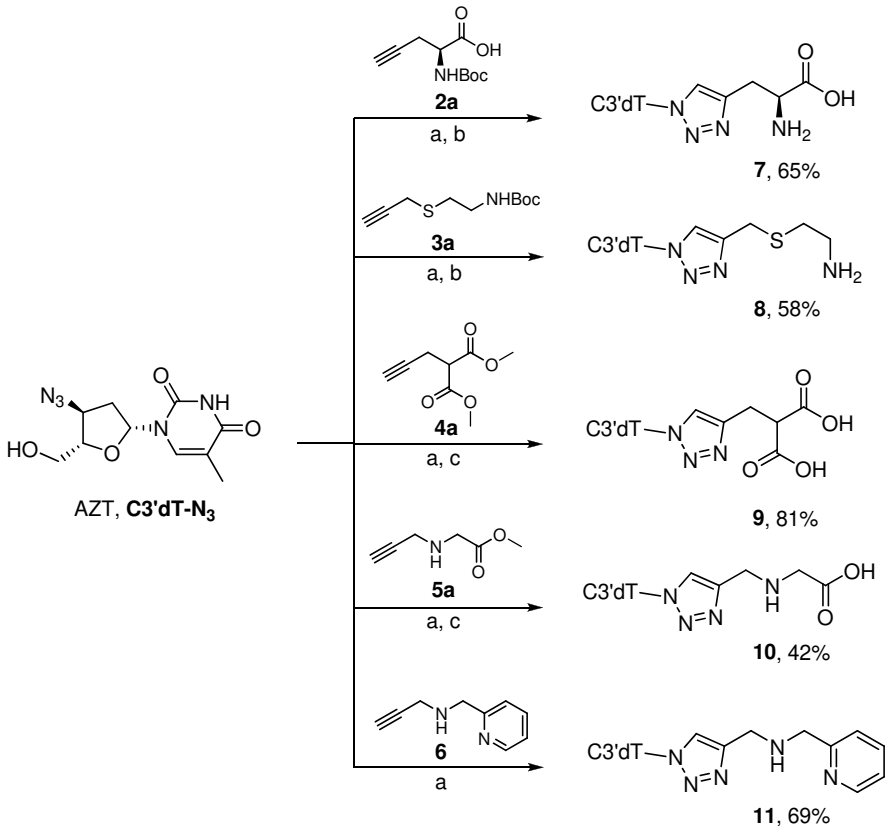
Chemistry

Thymidine can be readily functionalized at the C3′ position using the "click-to-chelate" approach starting from the commercially available azide 3′-azido-3′-deoxythymidine, AZT. Tridentate chelators for the $M(CO)_3$ core were incorporated by reaction of AZT with a suitable alkyne (Scheme 1). Suitable alkynes (Figure 1) were prepared by alkylation of propargylamine with bromoacetate (**5**), alkylation of commercial amines with propargyl bromide (**3** and **6**), or are commercially available (**2** and **4**). Full details of the synthesis of the alkyne components used were recently reported.(*14*) The copper catalyzed azide-alkyne cycloaddition was performed using standard Sharpless conditions: one equivalent of alkyne and one equivalent of azide were stirred at room temperature for 12 hours in a mixture of *t*BuOH and water with catalytic amounts of copper acetate (0.1 equivalents) and sodium ascorbate (0.2 equivalents).(*19*) For ease of purification of the products, alkynes **2**-**5** were substituted with compounds **2a**-**5a**, in which carboxylic acids were protected as methyl esters and primary amines were Boc-protected. However, in previous studies we have shown that for radiolabelling purposes it

is not necessary to use protected substrates, or indeed to purify the ligands prior to radiolabelling.(*14, 16*)

Thymidine derivatives **7** and **8** were prepared from the reactions of AZT with alkynes **2a** and **3a**, respectively. In both cases the Boc-protected triazole products were purified by silica gel chromatography using mixtures of CH_2Cl_2 and MeOH before the Boc protecting groups were removed in a mixture of trifluoroacetic acid (10%) and CH_2Cl_2. Compounds **7** and **8** were purified by solid phase extraction using Sep-Pak® columns. Thymidine derivatives **9** and **10** were prepared from the reactions of AZT with alkynes **4a** and **5a**, respectively. The intermediates were purified by silica gel chromatography before the methyl esters were removed with aqueous NaOH. Compounds **9** and **10** were purified by solid phase extraction using Sep-Pak® columns. Compound **11** was purified by silica gel chromatography after the reaction of AZT and *N*-propargyl-pyridine-2-methylamine, **6**.

Scheme 1. Synthesis of C3′-functionalized thymidine derivatives[a]

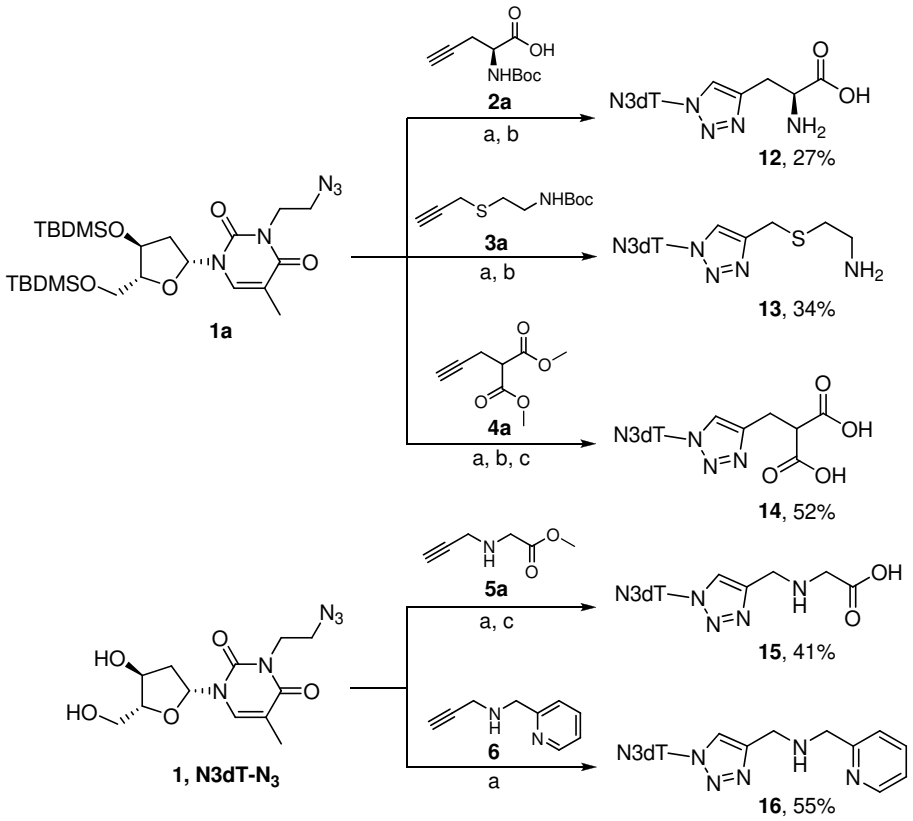


[a] (a) $Cu(OAc)_2.H_2O$, Na(ascorbate), *t*BuOH/H_2O (b) TFA, CH_2Cl_2 (c) NaOH, H_2O, followed by HCl.

The N3-functionalized thymidine derivatives **12**, **13**, **15** and **16** have been reported previously, however, they were only characterized via mass spectrometry.(*14*) For their synthesis, thymidine was functionalized at the N3 position with an azido-ethyl group following the recently reported

procedure.(*14, 20*) In analogous procedures to the synthesis of the C3′-functionalized derivatives described above, tridentate chelators for the $M(CO)_3$ core (M = ^{99m}Tc, Re) were installed at the N3 position by reacting the N3-functionalized azido-thymidine derivative **1** or the *O*-TBDMS protected precursor **1a** with alkynes **2-6** using standard conditions for the click reaction and as outlined in Scheme 2. Thymidine derivatives **12** and **13** were prepared from the reactions of the *O*-TBDMS protected N3-functionalized thymidine precursor, **1a**, and alkynes **2a** and **3a**, respectively. The protected triazole intermediates were purified by silica gel chromatography using mixtures of CH_2Cl_2 and MeOH. In both cases the Boc and TBDMS protecting groups of the products were removed in a single step with HCl in MeOH. Compounds **12** and **13** were purified by solid phase extraction, although compound **12** could also be recrystallized from MeOH. Similarly, derivative **14** was prepared from the reaction of **1a** with alkyne **4a**. The protected intermediate was stirred in HCl/MeOH to remove the TBDMS protecting groups, followed by aqueous NaOH to remove the methyl esters. Thymidine derivative **15** was prepared from the reaction of **1** and alkyne **5a**. The intermediate was purified by silica gel chromatography before the methyl ester was removed with aqueous NaOH. In this case, removal of the TBDMS protecting groups from the azido-thymidine derivative **1a** with NBu_4F proved more efficient before the click reaction, as **1** could more easily be separated from excess NBu_4^+ salts than the more hydrophilic triazole product **15**. Both compounds **14** and **15** were purified by solid phase extraction using Sep-Pak® columns. Compound **16** was purified by silica gel chromatography after the reaction of **1** and *N*-propargyl-pyridine-2-methylamine, **6**.

Scheme 2. Incorporation of tridentate chelators into N3-functionalized thymidine[a]



[a] (a) $Cu(OAc)_2.H_2O$, Na(ascorbate), *t*BuOH/H_2O (b) HCl, MeOH (c) NaOH, H_2O, followed by HCl.

The $Re(CO)_3$ complexes of all of the functionalized thymidine derivatives were prepared from the precursor $[ReBr_3(CO)_3][NEt_4]_2$ and a stoichiometric amount of the corresponding ligand in a mixture of MeOH and water at 50 °C. In each case the quantitative formation of the product was observed by HPLC. The crude products were purified by solid phase extraction using SepPak® columns and a water-MeOH gradient. The IR spectra of all of the rhenium complexes revealed the typical *fac*-$Re(CO)_3$ pattern with significantly blue-shifted, intense CO stretching frequencies (around 2020 cm^{-1} and 1880 cm^{-1}) compared with the starting material $[ReBr_3(CO)_3]^{2-}$ (2000 cm^{-1} and 1868 cm^{-1}). NMR analyses provided evidence that in each case the metal core is site-specifically coordinated to the tridentate metal chelating system introduced at either the C3′ or N3 position, and showed similar features to model complexes which were synthesized with benzyl azide and characterized previously.(*14*) Typically, the proton NMR spectra showed low field shifts of the protons in the chelating system after metal chelation. Independent resonances are observed for each proton in the CH_2 groups of the chelating system; they become distinguishable due to a lack of symmetry in the metal chelate. As was the case with the model complexes, signals for the protons of coordinated primary and secondary amines were also observed in the NMR spectra of the thymidine complexes as a result of the decrease in rate of H/D exchange. A key difference between the thymidine chelates and the analogous model complexes is the appearance of diastereoisomers, which can be distinguished in some cases by NMR and/or HPLC. For the C3′ complexes HPLC analyses revealed two isomeric

products for both [Re(CO)$_3$**8**]Br and [Re(CO)$_3$**10**], but only in the case of [Re(CO)$_3$**11**]Br were two isomers apparent in the ^{1}H NMR spectrum. For the N3-functionalized complexes [Re(CO)$_3$**13/16**]Br, and [Re(CO)$_3$**15**], two diastereoisomers can be distinguished by NMR. The differences in chemical shifts of the two isomers are most pronounced for the complex [Re(CO)$_3$**13**]Br, and HPLC analysis also revealed two product peaks in this case. In analogous model complexes, where a benzyl group substitutes the thymidine residue, isomers were not distinguishable by either HPLC or NMR.(*14*) All of the complexes were readily water soluble, which is a prerequisite for *in vitro* assessment.

Crystals of the complex [Re(CO)$_3$**11**][CF_3CO_2] suitable for x-ray crystallography were prepared by exchange of the bromide anion with the more bulky $CF_3CO_2^-$ anion using the corresponding silver salt. The final crystals were grown by diffusion of diethyl ether into a solution of the complex in methanol. Structural analysis revealed that the counter ion was a mixture of Br^- and $CF_3CO_2^-$ as a result of incomplete precipitation of AgBr. The structure of the complex cation confirmed the expected tridentate, facial coordination of the κN,κN,κN chelator, which has not previously been structurally characterized (Figure 2). The Re-N3 bond length (2.146 (3) Å) is in agreement with the Re-N(triazole) bond length in other Re(CO)$_3$ complexes with triazole containing ligands.(*14*) Similarly the Re-N4 (2.218(4) Å) and Re-N5 (2.181(3) Å) bond lengths are consistent with those found in Re(CO)$_3$ complexes with amine and pyridine containing chelators.(*21-23*) For example, Banerjee *et al.* report the crystallographic characterization of a series of tridentate chelating systems for the M(CO)$_3$ core (M = ^{99m}Tc, Re) which incorporate an aliphatic amine with pyridyl and/or imidazoyl donor groups and are structurally similar to the chelator in compound **11**.(*21*) The Re-N bond lengths in the novel triazole-containing chelating system were consistent with the values reported for the coordinated aliphatic amines (2.23-2.29 Å), pyridyl (2.16-2.18 Å) and imidazoyl (2.14-2.16 Å) groups. Crystallographic data are reported in the supporting information.

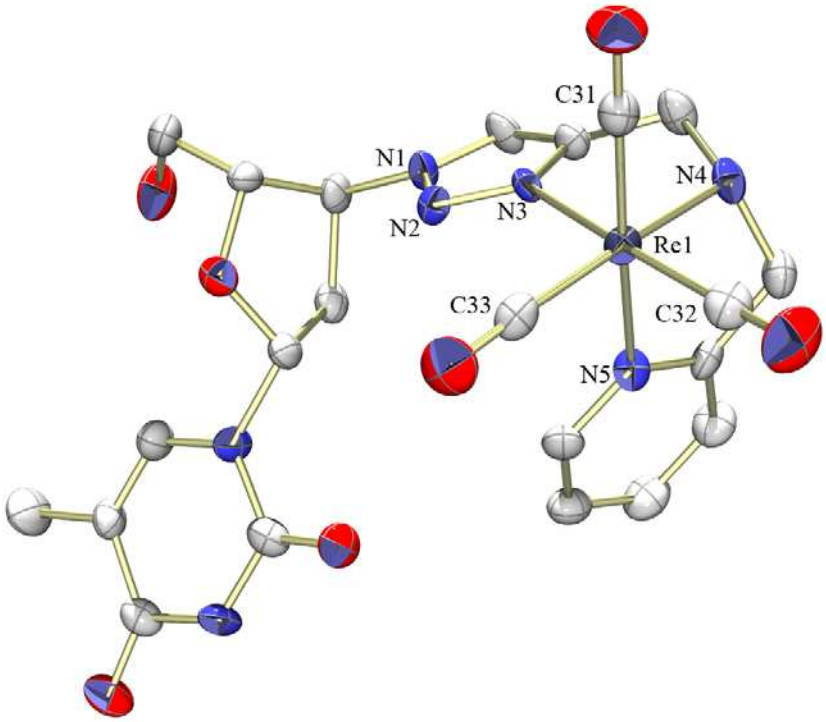


Figure 2. ORTEP-3(*24*) representation of the complex cation $[Re(CO)_3\mathbf{11}]^+$ with thermal ellipsoids shown at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths [Å] and angles [°]: Re(1)-N(3) 2.146(3), Re(1)-N(4) 2.218(4), Re(1)-C(31) 1.915(4), Re(1)-C(32) 1.929(5), Re(1)-N(5) 2.181(3), Re(1)-C(33) 1.900(5), C(33)-Re(1)-C(31) 91.46(18), C(33)-Re(1)-C(32) 88.3(2), C(31)-Re(1)-C(32) 87.17(19), C(32)-Re(1)-N(3) 172.83(16), C(31)-Re(1)-N(5) 171.65(19), C(33)-Re(1)-N(4) 170.51(16).

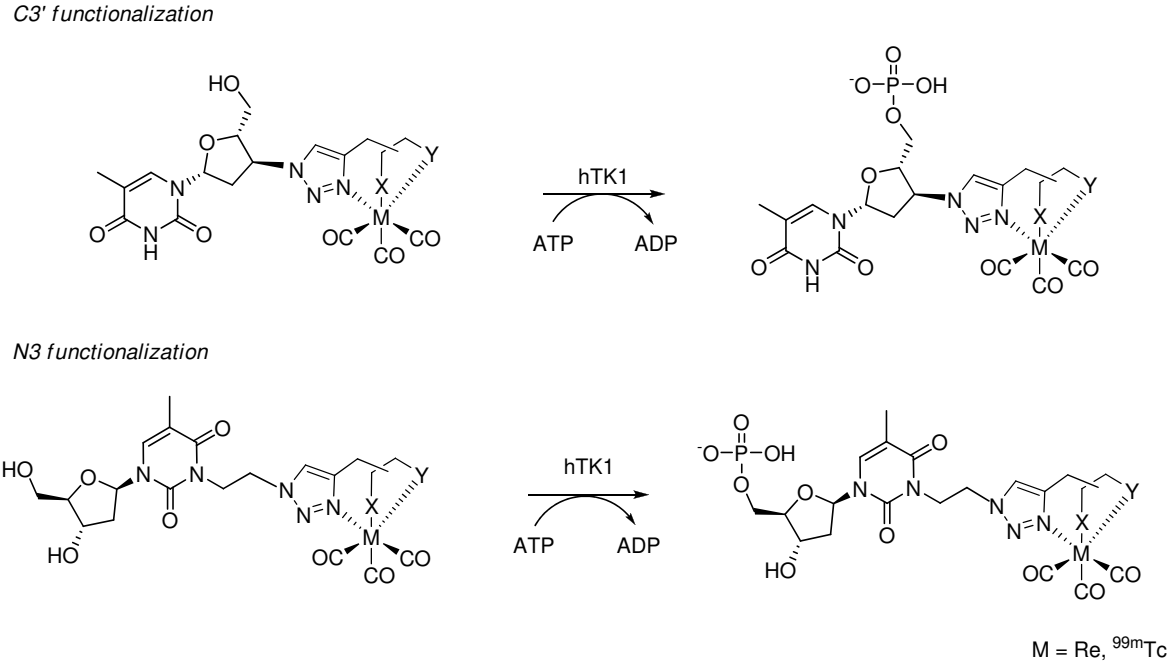
Radioactive technetium-99m complexes were prepared with the ligands **7**, **8**, **10**, **11**, **12**, **13**, **15** and **16**. The complexes were prepared quantitatively by adding the organometallic precursor $[^{99m}Tc(CO)_3(H_2O)_3]^+$(*25*) to a solution of the ligand in physiological phosphate buffer (PBS; pH 7.4) and heating for 45 minutes at 95 °C. In each case a single product was observed. Characterization of the radioactive complexes was accomplished by comparison of the retention times observed in the γ-HPLC trace with those in the UV-trace of the corresponding rhenium complexes. Given the experimental limitations, the retention times matched for all pairs of complexes. For *in vitro* and enzymatic studies ^{99m}Tc complexes were separated from unreacted thymidine derivatives by HPLC.

In vitro evaluation of the organometallic thymidine derivatives

Substrate activity toward human thymidine kinase. We have shown previously that the ^{99m}Tc-labelled complexes $[^{99m}Tc(CO)_3\mathbf{12}]$ and $[^{99m}Tc(CO)_3\mathbf{13}]$ are substrates for hTK1, by incubating a solutions of the complexes ATP in the presence of hTK1. Under the same conditions, substrate activity was not observed with the complexes $[^{99m}Tc(CO)_3\mathbf{15}]^+$ and $[^{99m}Tc(CO)_3\mathbf{16}]^+$.(*14*) In the current investigation, the hTK1 substrate activities of all target rhenium complexes were assessed using a coupled

thymidine kinase-pyruvate kinase-lactate dehydrogenase UV assay (λ = 340 nm) as previously described.(*6, 26*) We were able to show that all of the C3′-functionalized and N3-functionalized rhenium complexes were substrates for hTK1 (Scheme 3). The results with both series of compounds are presented in Table 1. The phosphorylation of thymidine was arbitrarily set to 100%.

Scheme 3. Phosphorylation of C3′ and N3 functionalized ^{99m}Tc/Re(CO)$_3$-labelled thymidine derivatives.



In the first series of complexes the ribose of thymidine is functionalized directly at the C3′ position and there is no spacer between the thymidine and metal chelate. The metal-labelled compounds have varying overall charges depending on the functional groups of the chelating system. The relative rates of phosphorylation ranged from 13-28%. The neutral complex [Re(CO)$_3$**10**] had the highest relative phosphorylation of 27.6 ± 1.9%. There is a clear trend in phosphorylation rate as a function of overall charge. The anionic C3′-functionalized compound (23.1 ± 1.8%) has a comparable rate of phosphorylation to the neutral C3′-functionalized compounds (27.6 ± 1.9% and 20.3 ± 0.8%), whereas the cationic complexes appear to be much less readily phosphorylated (12.5 ± 0.5% and 14.2 ± 0.2% for [Re(CO)$_3$**8**]Br and [Re(CO)$_3$**11**]Br, respectively). This is perhaps surprising given that the structure of hTK1 co-crystallized with the feedback inhibitor thymidine triphosphate (dTTP) shows the proximity and suspected interaction of the C3′ substituent (in the case of dTTP, a hydroxyl group) with a glycine residue and an aspartic acid residue.(*7*) There is, however, an arginine residue in the same region of the active site. Interaction between the C3′ substituent and the cationic side chain of arginine may compensate for the loss of interaction with the C3′ hydroxyl group of thymidine, and thus be an explanation for the greater substrate efficiency of the anionic complex compared to the

cationic complexes. However, at the moment this remains purely speculative and only co-crystallization of the complex with the enzyme would provide further insight into the observed trend.

Table 1. Phosphorylation of organometallic thymidine complexes [$Re(CO)_3$**7**-**16**].

Compound	Position of functionalization	Overall charge	Phosphorylation [%][a]
[$Re(CO)_3$**7**]	C3′	Neutral	20.3 ± 0.8
[$Re(CO)_3$**10**]	C3′	Neutral	27.6 ± 1.9
[$Re(CO)_3$**9**]NEt_4	C3′	Anionic	23.1 ± 1.8
[$Re(CO)_3$**8**]Br	C3′	Cationic	12.5 ± 0.5
[$Re(CO)_3$**11**]Br	C3′	Cationic	14.2 ± 0.2
[$Re(CO)_3$**12**]	N3	Neutral	17.9 ± 0.1
[$Re(CO)_3$**15**]	N3	Neutral	14.1 ± 0.2
[$Re(CO)_3$**14**]NEt_4	N3	Anionic	9.0 ± 0.4
[$Re(CO)_3$**13**]Br	N3	Cationic	10.9 ± 0.4
[$Re(CO)_3$**16**]Br	N3	Cationic	16.8 ± 0.2
Thymidine	-	-	100[a]

[a] The phosphroylation of thymidine was arbitrarily set to 100%.

In the second series of compounds, thymidine was functionalized at the N3 position. As for the C3′-functionalized complexes the overall charges of the derivatives vary, but in all cases there is an ethyl spacer between thymidine and the metal complex. The phosphorylation data show that in general the C3′-functionalized compounds are better substrates for hTK1 than the N3-functionalized compounds. The phosphorylation is relatively low compared to thymidine for all of the complexes (9-18%), although interestingly in this series of compounds the neutral and cationic complexes appear to be better substrates than the anionic complex. This is consistent with results from Tjarks *et al.*, which show that zwitterionic NH_3^+-*nido*-*m*-carborane-substituted thymidine analogues (with a propyl spacer) have high substrate activity.(*27*) However, the variation in the phosphorylation of the two cationic complexes (10.9 ± 0.4% and 16.8 ± 0.2% for [$Re(CO)_3$**13**]Br and [$Re(CO)_3$**16**]Br, respectively), suggests that in addition to overall charge, structural differences in the metal chelate also affect the substrate affinity for hTK1. It is not possible, therefore, to rule out structural differences in addition to overall charge as the reason for the lower substrate affinity of the complex [$Re(CO)_3$**14**]NEt_4, which in contrast to the anionic C3′-functionalized compound, had the lowest phosphorylation rate in the series (9.0 ± 0.4%).

In a second, direct assay the radioactive and non-radioactive complexes [^{99m}Tc/$Re(CO)_3$**X**] (**X** = **7**, **10**, **11**, **15**) were incubated with ATP and recombinant hTK1 only. The enzymatic reactions were

analysed by HPLC (UV-trace at λ = 254 nm for the rhenium complexes, γ-trace in the case of the radioactive Tc-99m complexes). For both the ^{99m}Tc and the Re compounds phosphorylation resulted in a measurable shift in retention time on a C_{18} reversed phase column and the monophosphorylated products [^{99m}Tc/Re(CO)$_3$**X**]-**P** (**X** = **7**, **10**, **11**, **15**) were distinguished clearly from the starting material. In the case of the complex [Re(CO)$_3$**7**], the reaction mixture was purified by HPLC, and the fractions collected were analysed by mass spectroscopy. The fraction corresponding to the starting material showed a mass peak at *m/z* = 651.09, $[C_{18}H_{19}N_6O_9Re]H^+$. The more hydrophilic phosphorylated complex, [Re(CO)$_3$**7**]-**P**, revealed a mass peak at *m/z* = 731.03 $[C_{18}H_{20}N_6O_{12}PRe]H^+$. In time dependent HPLC experiments monitoring the phosphorylation of complex [Re(CO)$_3$**7**], an increase in the concentration of the monophosphorylated species, [Re(CO)$_3$**7**]-**P**, and corresponding decrease in the concentration of the starting complex was observed.

Cell internalization experiments. hTK1 is located in the cytosol, and therefore a potential substrate has to pass through the cell membrane before it can be phosphorylated. If this transport is not achieved actively via nucleoside transporters then it must occur by passive diffusion.(*28*) This has been suggested as the major cell internalization route for the carborane-thymidine derivatives reported by Tjarks *et al*.(*27*) Similarly, AZT is not recognized by the ubiquitously expressed human equilibrative nucleoside transporter type 1 (hENT1) and is transported primarily by passive diffusion.(*29*) By analogy to the N3-functionalized carborane derivatives and AZT, we suspected passive diffusion would be the most likely cell internalization route of our C3′/N3-functionalized complexes.

In a preliminary study of the cell internalizing ability of the organometallic thymidine derivatives, we investigated the internalization of one ^{99m}Tc(CO)$_3$-labelled complex from each series of compounds. We expected the neutral complexes to be the most likely to be internalized based on our previous experience.(*13*) We therefore chose to investigate the neutral complex [^{99m}Tc(CO)$_3$**7**] from the series of C3′-functionalized compounds. To complete a previous series of N3-functionalized thymidine analogues, we chose to investigate the cationic complex [^{99m}Tc(CO)$_3$**16**]$^+$, (internalization of anionic and neutral N3-functionalized thymidine analogues has been observed previously).(*13*) The incorporation of both complexes into two human glioblastoma cell lines was measured and compared with the incorporation of ^{125}I-iododeoxyuridine (^{125}IdUrd), using cell lines and conditions established for the incorporation of ^{125}IdUrd.(*30, 31*) Internalization was measured with and without pretreatment with 5-fluorodeoxyuridine (FdUrd), which is known to increase uptake of 5-iododeoxyuridine by inhibiting endogenous thymidine synthesis and inducing upregulation of nucleoside transporters.(*30, 32-34*) The results for both cell lines, U251 and LN229, and with and without 5-fluorodeoxyuridine pretreatment are presented in Table 2.

Table 2. Internalization into the human glioblastoma cell lines U251 and LN229, with and without pretreatment with 5-fluorodeoxyuridine, as a percentage of the initial activity added.

	U251		LN229	
	Internalization [%]	Pretreated with FdUrd[a] [%]	Internalization [%]	Pretreated with FdUrd[a] [%]
^{125}IdUrd	14.03 ± 1.69	59.29 ± 2.80	8.81 ± 0.43	39.40 ± 1.39
[^{99m}Tc(CO)$_3$**7**]	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
[^{99m}Tc(CO)$_3$**16**]$^+$	0.13 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.15 ± 0.01

[a] Cells were incubated with 1 μM FdUrd for 1 hour before addition of the radioactive dT analogue.

The data show that there was only minor cell-associated radioactivity after incubation of both cell line with [^{99m}Tc(CO)$_3$**7**] and [^{99m}Tc(CO)$_3$**16**]$^+$. This can be rationalized either as a lack of uptake, or as a lack of intracellular retention of the radioactive probes. Assuming that the complexes are stable *in vitro* and are intracellularly phosphorylated, the data suggest that neither of the ^{99m}Tc-labelled derivatives are able to enter the cells. This is further corroborated by the fact that pretreatment with 5-fluorodeoxyuridine (FdUrd), which should stimulate nucleoside transport, had no effect on the amount of radioactivity associated with the cells, further evidence that uptake is not mediated by nucleoside transporters. It is likely that the structural modification at either the C3' or the N3 position is significant enough to prohibit active incorporation mediated by nucleoside transporters. This is in agreement with our previous experience of organometallic thymidine derivates. Although we reported moderate cell uptake of N3-functionalized thymidine derivatives (0.6-4.8%) into SKNMC tumour cells, uptake increased with increasing lipophilicity.(*13*) We assume, therefore, that factors such as hydrophilicity, charge and/or size prevent [^{99m}Tc(CO)$_3$**7**] or [^{99m}Tc(CO)$_3$**16**]$^+$ exhibiting any significant internalization via passive diffusion. An alternative explanation for the negligible accumulation of radioactivity is that either the compounds are not sufficiently effectively phosphorylated, or that they are susceptible to intracellular degradation. It has been shown for a number of N3-functionalized carboranyl thymidine analogues that the compounds cannot compete intracellularly with thymidine and therefore are not effectively phosphorylated.(*35*)

Conclusions

Starting from two azido-functionalized thymidine derivatives, we were able to investigate the influence of the position of the metal complex on the substrate affinity, as well as the influence of charge on the relative phosporylation of two series of novel M(CO)$_3$-labelled thymidine derivatives. The "click-to-chelate" approach was used to synthesize thymidine derivatives functionalized with a

tridentate chelator for the $M(CO)_3$ core (M = ^{99m}Tc, Re) at either the C3′ or N3 position of thymidine. All of the conjugates could be readily labelled with the $M(CO)_3$ precursors to form well-defined organometallic complexes, with differing structures and overall charges. Incubation of the $Re(CO)_3$ derivatives with ATP in the presence of hTK1 led to the formation of monophosphorylated organometallic thymidine derivatives, which were observed by HPLC and unambiguously identified by mass spectroscopy. In this way, we were able to show that all of the compounds tested, which vary in the position of the metal chelate and overall charge, were substrates for hTK1. For each of the complexes the rate of phosphorylation was measured quantitatively relative to thymidine, the natural substrate for hTK1. We could show that for the two sets of compounds investigated the C3′-functionalized thymidine derivatives were on average more readily phosphorylated by hTK1 than the N3-functionalized derivatives. Furthermore, we have shown that the effect of the overall charge of the substrate is dependent on the position of the metal complex. For the C3′-functionalized complexes, the rates of phosphorylation followed the trend neutral ≈ anionic > cationic. In the case of the N3-functionalized derivatives the trend neutral ≈ cationic > anionic was observed for the relative phosphorylation rates of the complexes.

The first cell internalization experiments did not give promising results. Neither of the C3′ or N3 functionalized derivatives tested were either actively or passively transported into cells. It seems unlikely that any of the current set of compounds will be useful as a proliferation marker. The incorporation of, for example, a cell penetrating peptide sequence may improve uptake, however, introduction of an additional targeting group would induce further structural changes that in turn would decrease the substrate activity of compounds which already show significantly lower activity than the natural substrate. As we have shown and noted before in a similar study of technetium-labelled glucose analogues, it is possible to develop ^{99m}Tc-based, small molecular substrates for a given enzyme (such as hexokinase, or in the present case hTK1). However, a critical point during the development of such compounds is their active transport by transporters with further substrate specificity (such as Glut1 or a nucleoside transporter) to their intracellular targets. Although there have been recent reports of organometallic pseudo-amino acids which target the LAT transporter,(*36*) in general it would appear that ^{99m}Tc and metal-based radiopharmaceuticals which target receptors which themselves facilitate internalization show much more promise for the future.

Experimental details

Chemistry

All chemicals were purchased from Sigma-Aldrich or Fluka, Buchs, Switzerland. All chemicals and solvents were of reagent grade and were used without further purification unless otherwise stated. The precursor *fac*-$[Re(CO)_3Br_3][NEt_4]_2$(*37*) was prepared according to the published procedure. $[Na][^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$-generator (Covidien, Petten) with a 0.9% saline solution. Reactions were monitored by HPLC or by thin-layer chromatography (TLC) using precoated silica gel 60 F_{254} aluminium sheets (Merck), and visualized by UV absorption or stained with a solution of ninhydrin in EtOH. Column chromatography was performed using silica gel 60 (Fluka; particle size 0.04-0.063 mm). Analytical HPLC was performed using a Merck-Hitachi L-7000 system equipped with an L-7400 tunable absorption detector and an XBridge™ C-18 reverse phase column (5 µM, 4.6 x 150 mm, Waters). HPLC solvents were either water with 0.1% TFA (solvent A) and MeCN (solvent B), or water (solvent A) and MeOH (solvent B) (for purification of ^{99m}Tc complexes) with a flow rate of 1 mL/min. The gradient was as follows: 0-15 min: gradient from 95% A to 20% A; 15-20 min: gradient from 20% A to 95% A; 20-25 min 95% A. Sep-Pak® columns (Waters) were washed with methanol and water prior to use. Products were eluted using water or a water-methanol gradient. Nuclear magnetic resonance spectra were recorded on a 400 MHz Bruker spectrometer. ^1H and ^{13}C chemical shifts are reported relative to residual solvent peaks or water as a reference. Values of the coupling constant, *J*, are given in Hertz (Hz). The following abbreviations are used for the description of ^1H-NMR spectra: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), broad singlet (bs). The chemical shifts of complex multiplets are given as the range of their occurrence. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR, with a universal ATR sampling accessory. Low resolution mass spectra were recorded with a Micromass Quattro micro™ API LC-ESI using either the negative or positive ionization mode. High resolution mass spectra were recorded with a Bruker FTMS 4.7T BioAPEXII.

^{125}I-IdUrd of high radiochemical purity was prepared from the precursor 5-tri-*n*-butyl-stannyl-2'-deoxyuridine and purified by SepPak® RP-18 cartridges using a modified version of a method originally described by Foulon *et al*.(*38, 39*) Radiochemical purity was repeatedly verified by TLC using polyethyleneimine cellulose F strips (Merck, Darmstadt, Germany) with a mobile phase of 0.2 M ammonium bicarbonate and radioactivity reading on a Tracemaster 20 (Berthold, Bad Wildbad, Germany). In all experiments, ^{125}I-IdUrd represented >98% of overall ^{125}I activity. Contamination with unlabelled IdUrd was avoided in the production of ^{125}I-IdUrd. It was calculated that the standard activity of 5 kBq ^{125}I-IdUrd in 0.5 mL used in the incorporation experiments represented a concentration of 0.2 nM, though this is only an indicative value as it has not been experimentally verified.

3',5'-Di-O-(tert-butyl-dimethyl-silanyl)thymidine. Thymidine (1.00 g, 4.13 mmol) and imidazole (1.18 g, 17.34 mmol) were dissolved in DMF (10 mL) and stirred for 5 minutes at room temperature.

TBDMSCl (1.31 g, 8.67 mmol) was added and the reaction stirred at room temperature and followed by TLC. After 16 hours the reaction mixture was diluted with EtOAc (20 mL), and washed twice with 1 M $NaHCO_3$, and once with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure to give the product as a colourless liquid (1.90 g, 98%). ^{1}H NMR ($CDCl_3$) δ 8.37 (s, 1H); 7.45 (d, *J* = 0.9, 1H); 6.30 (*J* =7.9, 5.8, 1H); 4.38 (m, 1H); 3.91 (m, *J* = 2.5, 2.4, 2.4, 1H); 3.84 (dd, *J* = 11.4, 2.5, 1H); 3.74 (dd, *J* = 11.4, 2.4, 1H); 2.22 (ddd, *J* = 13.1, 5.8, 2.6, 1H); 2.02-1.89 (m, 1H); 0.90 (s, 9H); 0.87 (s, 9H); 0.09 (s, 6H); 0.05 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$) δ 163.52, 149.90, 135.53, 110.64, 87.69, 84.77, 72.21, 62.87, 41.24, 36.36, 31.07, 25.88, 25.68, 12.33, -4.69, -4.89, -5.34, -5.50 ppm. High Res. MS (ESI) *m/z* 471.2706 $[C_{22}H_{42}N_2O_5Si_2]H^+$ (calc. 471.2705).

3-(2-Bromoethyl)-3',5'-di-O-(tert-butyl-dimethyl-silanyl)thymidine. 3',5'-Di-O-(tert-butyl-dimethyl-silanyl)-thymidine (1.90 g, 4.04 mmol) was dissolved in DMF (10 mL). Cs_2CO_3 (4.40 g, 12.39 mmol) was added and the mixture was stirred for 5 minutes at room temperature. Dibromoethane (7.76 g, 41.28 mmol) was added and the reaction stirred at room temperature and followed by TLC. After 2 hours the reaction mixture was diluted with EtOAc (20 mL), and washed twice with 1 M $NaHCO_3$, and once with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography with hexane and EtOAc (20%) to give the pure product as a colorless liquid (2.21 g, 95%). ^{1}H-NMR ($CDCl_3$) δ 7.48 (d, ^4J(H,H)=1.1, 1H); 6.36 (m, *J* =7.9, 5.8, 1H); 4.45-4.29 (m, 3H); 3.95 (m, 1H); 3.87 (dd, *J* =11.4, *J* =2.6, 1H); 3.76 (dd, *J* =11.4, *J* =2.4, 1H); 3.54 (t, *J* =7.2, 2H); 2.30-2.23 (m, 1H); 2.02-1.95 (m, 1H); 1.94 (s, 3H); 0.93 (s, 9H); 0.90 (s, 9H); 0.12 (s, 6H); 0.09 (s, 3H); 0.08 (s, 3H) ppm. ^{13}C-NMR ($CDCl_3$) δ 162.93, 150.48, 133.61, 109.88, 87.68, 85.26, 72.22, 62.85, 42.18, 41.41, 29.49, 27.25, 25.93, 25.73, 13.14, -4.69, -4.87, -5.43, -5.49 ppm. High Res. MS (ESI) *m/z* 577.2124 $[C_{24}H_{45}BrN_2O_5Si_2]H^+$ (calc. 577.2123).

3-(2-Azidoethyl)-3',5'-di-O-(tert-butyl-dimethyl-silanyl)thymidine, 1a. 3-(2-Bromoethyl)-3′,5′-di-O-(tert-butyl-dimethyl-silanyl)thymidine (1.03 g, 1.78 mmol) was dissolved in MeCN (20 mL) and 10 equivalents of NaN_3 (1.16 g 17.8 mmol) were added. The reaction mixture was stirred at 80 °C for 16 hours and followed by TLC. After cooling to room temperature the solvent was removed under reduced pressure and the product extracted into EtOAc. and washed twice with water and once with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced the pressure to give the pure product as a colorless liquid (750 mg, 80%). ^{1}H-NMR ($CDCl_3$) δ 7.48 (d, *J* = 0.9, 1H); 6.36 (m, *J* = 7.9, 5.8, 1H); 4.40 (m, 1H); 4.19 (m, *J* = 6.3, 1.9, 2H); 3.94 (m, *J* = 2.4, 1H); 3.87 (dd, *J* = 11.4, 2.6, 1H); 3.76 (dd, *J* = 11.4, 2.3, 1H); 3.54 (t, *J* = 6.3, 2H); 2.30-2.23 (m, 1H); 2.02-1.95 (m, 1H); 1.93 (s, 3H); 0.92 (s,

9H); 0.89 (s, 9H); 0.19 (s, 6H); 0.08 (s, 3H); 0.07 (s, 3H) ppm. ^{13}C-NMR ($CDCl_3$) δ 163.5, 151.0, 134.0, 110.1, 88.0, 85.7, 72.4, 63.1, 48.6, 41.6, 40.0, 26.1, 25.9, 18.5, 18.1, 13.2, -4.5, -4.7, -5.2, -5.3 ppm. High Res. MS (ESI) *m/z* 562.2851 $[C_{24}H_{45}N_5O_5Si_2]Na^+$ (calc. 562.2851).

3-(2-Azidoethyl)thymidine (N3dT-N_3), 1. 3-(2-Azidoethyl)-3',5'-di-O-(tert-butyl-dimethyl-silanyl)thymidine (900 mg, 1.67 mmol) was dissolved in THF (20 mL). NBu_4F (1.16 g, 3.68 mmol) was added and the reaction was stirred at room temperature and followed by TLC. After two hours the solvent was removed under reduced pressure and a 1:1 mixture of water and EtOAc (40 mL) was added. The solvent was evaporated from the aqueous phase under reduced pressure and the crude product was purified by column chromatography with a mixture of CH_2Cl_2 and methanol (9%) to give the product as a colourless liquid (500 mg, 96%). ^{1}H-NMR (D_2O) δ 7.69 (d, *J* = 1.1, 1H); 6.30 (m, *J* = 6.7, 1H); 4.47 (m, *J* = 6.4, 4.2, 1H); 4.18 (m, *J* = 7.0, 5.4, 2H); 4.04 (m, *J* = 8.7, 3.8, 1H); 3.85 (dd, *J* = 12.5, 5.0, 1H); 3.77 (dd, *J* = 12.5, 3.6, 1H); 3.56 (t, *J* = 5.8, 2H); 2.44-2.32 (m, 2H); 1.92 (d, *J* =0.98, 3H). ^{13}C-NMR ($CDCl_3$) δ 166.03, 152.21, 136.44, 111.16, 87.24, 86.61, 71.01, 61.76, 49.50 (MeOH), 48.90, 40.94, 39.41, 12.88 ppm. High Res. MS (ESI) *m/z* 312.1299 $[C_{12}H_{17}N_5O_5]H^+$ (calc. 312.1302).

Alkynes, ***2****-****6****.* 2-prpargyl glycine, **2**, N-Boc-2-prpargyl glycine, **2a**, and 2-propargyl dimethyl malonate, **4a**, are commercially available. Alkynes **3** and **5** were prepared according to published procedures,(*14*) alkyne **6** was prepared from the reaction of pyridine-2-methylamine (0.53 g, 4.89 mmol) and propargyl bromide (0.58 g, 4.89 mmol) in a solution of triethylamine (1.02 mL, 7.34 mmol) in MeCN (50 mL). The reaction mixture was stirred at 50 °C and followed by TLC. After 4 hours the solvent was removed and the reaction mixture purified by column chromatography with CH_2Cl_2 and MeOH (5%). The product was isolated as a yellow liquid (178 mg, 25%). Full analytical data for compounds **2-6** and **2a-5a** can be found in the supporting information. Data were in agreement with those already published.(*14*)

Copper catalyzed azide-alkyne cyclycloaddition. General procedure A: The alkyne (1-2 equivalents) and azide (1 equivalent) were added to a 1:1 mixture of *t*BuOH and water to form a 0.1 mM solution. 0.1 equivalents of $Cu(OAc)_2.H_2O$ and 0.2 equivalents of sodium ascorbate were added and the mixture stirred at room temperature for 12 hours.

Compound 7. As per general procedure A, with AZT (130 mg, 0.49 mmol) and **2a** (137 mg, 0.64 mmol). The solvent was removed and the residue redissolved in a mixture of CH_2Cl_2 and MeOH (10%). The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (10%) to give the Boc protected triazole product (183 mg, 77%). ^{1}H-NMR (CD_3OD) δ 7.91 (s, 1H), 7.89 (s, 1H), 6.49 (t, *J* = 6.5, 1H), 5.39 (m, 1H), 4.33 (m, 1H), 4.25 (t, *J* = 5.4, 1H), 3.89 (dd, *J* = 12.2, 2.9, 1H), 3.77 (dd, *J* = 12.2, 3.1, 1H), 3.24 (dd, *J* = 14.6, 5.1, 1H), 3.13 (dd, *J* = 14.6, 6.6, 1H),

2.93-2.83 (m, 1H), 2.77-2.64 (m, 1H), 1.91 (s, 3H), 1.41 (s, 9H) ppm. ^{13}C-NMR (CD_3OD) δ 166.29, 152.10, 137.98, 124.35, 111.50, 86.66, 86.40, 80.54, 62.14, 61.07, 38.88, 28.82, 12.51 ppm. High Res. MS (ESI) *m/z* 481.2039 $[C_{20}H_{28}N_6O_8]H^+$ (calc. 481.2041). The intermediate (120 mg, 0.25 mmol) was dissolved in a mixture of CH_2Cl_2 (12.5 mL) and TFA (1.25 mL) and stirred overnight at rt. The solvent was removed under reduced pressure, and the residue redissolved in water (1 mL) and neutralized with 5 M NaOH. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **12** as a colourless oil (81 mg, 85%).^{1}H-NMR ($CDCl_3$) δ ^{1}H NMR (400 MHz, MeOD) δ 8.01 (s, 1H), 7.91 (s, 1H), 6.49 (t, *J* = 6.6, 1H), 5.42 (m, 1H), 4.36 (m, 2H), 3.91 (dd, *J* = 12.1, 3.0, 1H), 3.78 (dd, *J* = 12.1, 3.2, 1H), 3.43 (dd, *J* = 15.7, 5.0, 1H), 3.35 (d, *J* = 15.7, 7.3, 1H), 2.94-2.82 (m, 1H), 2.79-2.65 (m, 1H), 1.91 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 170.88, 166.50, 152.48, 142.79, 138.29, 124.79, 111.94, 86.90, 86.55, 62.44, 61.49, 53.79, 39.18, 27.32, 12.61 ppm. High Res. MS (ESI) *m/z* 381.1515 $[C_{15}H_{20}N_6O_6]H^+$ (calc. 381.1517).

Compound 8. As per general procedure A, with AZT (100 mg, 0.37 mmol) and **3a** (150 mg, 0.70 mmol). The solvent was removed and the residue redissolved in a mixture of CH_2Cl_2 and MeOH (10%). The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (10%) to give the Boc protected triazole product (142 mg, 79%). ^{1}H-NMR (CD_3OD) δ H NMR (400 MHz, MeOD) δ 8.07 (s, 1H), 7.92 (d, *J* = 0.8, 1H), 6.48 (t, *J* = 6.4, 1H), 5.41 (dt, *J* = 8.6, 5.6, 1H), 4.37 (dt, *J* = 5.7, 3.0, 1H), 3.91 (dd, *J* = 12.3, 3.0, 1H), 3.84 (s, 2H), 3.78 (dd, *J* = 12.3, 3.2, 1H), 3.21 (t, *J* = 7.0, 2H), 2.92 (dt, *J* = 12.5, 6.1, 1H), 2.81-2.65 (m, 1H), 2.58 (t, *J* = 7.0, 2H), 1.91 (d, *J* = 0.8, 3H), 1.43 (s, 9H). ppm. ^{13}C-NMR (CD_3OD) δ 166.29, 152.39, 137.97, 111.50, 102.16, 86.67, 86.39, 62.12, 60.79, 38.89, 28.72, 12.50 ppm. High Res. MS (ESI) *m/z* 483.2018 $[C_{20}H_{30}N_6O_6S]H^+$ (calc. 483.2020). The intermediate (130 mg, 0.27 mmol) was dissolved in a mixture of CH_2Cl_2 (13.5 mL) and TFA (1.35 mL) and stirred overnight at rt. The solvent was removed under reduced pressure, and the residue redissolved in water (1 mL) and neutralized with 5 M NaOH. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **13** as a white powder (77 mg, 74%).^{1}H-NMR (CD_3OD) δ 8.08 (s, 1H), 7.90 (d, *J* = 0.8, 1H), 6.49 (t, *J* = 6.5, 1H), 5.42 (dt, *J* = 8.7, 5.2, 1H), 4.44-4.28 (m, 1H), 3.90 (dd, *J* = 12.2, 3.0, 1H), 3.85 (s, 2H), 3.79 (dd, *J* = 12.2, 3.2, 1H), 2.91 (t, *J* = 6.6, 2H), 2.93-2.86 (m, 1H), 2.78-2.72 (m, 1H), 2.69 (t, *J* = 6.6, 2H), 1.89 (d, *J* = 0.8, 3H). ppm. ^{13}C-NMR (CD_3OD) δ 167.49, 153.22, 146.98, 138.33, 124.15, 111.92, 86.84, 86.49, 62.41, 61.39, 50.00, 49.79, 49.58, 49.36, 49.15, 48.94, 48.72, 48.51, 40.92, 39.16, 33.91, 26.14, 12.72 ppm. High Res MS (ESI) *m/z* 383.1498 $[C_{15}H_{22}O_4N_6S]H^+$ (calc. 383.1496).

Compound 9. As per general procedure A, with AZT (70 mg, 0.26 mmol) and **4a** (45 mg, 0.26 mmol). The solvent was removed and the residue redissolved in a mixture of CH_2Cl_2 and MeOH

(10%). The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (5%) to give the ester protected triazole product (109 mg, 95%). ^{1}H-NMR (CD_3OD) δ 7.90 (s, 1H), 7.90 (d, *J* = 1.1, 1H), 6.46 (t, *J* = 6.5, 1H), 5.38 (dt, *J* = 8.7, 5.5, 1H), 4.33 (dt, *J* = 5.9, 3.2, 1H), 3.92-3.85 (m, 2H), 3.75 (dd, *J* = 12.2, 3.2, 1H), 3.71 (s, 6H), 3.28 (d, *J* = 7.6, 2H), 2.88 (ddd, *J* = 13.9, 6.6, 5.6, 1H), 2.72 (ddd, *J* = 14.3, 8.5, 6.3, 1H), 1.91 (d, *J* = 1.1, 3H). ppm. ^{13}C-NMR (CD_3OD) δ 170.76, 166.69, 152.57, 145.56, 138.53, 124.12, 111.75, 86.89, 86.64, 62.42, 61.14, 53.35, 39.28, 25.85, 12.65 ppm. High Res. MS (ESI) *m/z* 438.1622 $[C_{18}H_{23}N_5O_8]H^+$ (calc. 438.1619). The intermediate (100 mg, 0.23 mmol) was dissolved in a mixture of MeOH (1 mL) and 1 M NaOH (1 mL) and stirred for 2 h at rt. The mixture was neutralized by addition of 1 M HCl, before being concentrated under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **14** as a white solid (90 mg, 85%). ^{1}H-NMR (D_2O) δ 7.81 (s, 1H), 7.71 (d, *J* = 1.1, 1H), 6.42 (dd, *J* = 7.0, 6.0, 1H), 5.35 (dt, *J* = 8.7, 6.3, 1H), 4.42 (m, 1H), 3.87 (dd, *J* = 12.8, 3.2, 1H), 3.76 (dd, *J* = 12.8, 4.4, 1H), 3.37 (t, *J* = 7.8, 1H), 3.09 (d, *J* = 7.8, 2H), 2.95 (ddd, *J* = 13.0, 6.9, 1H), 2.79 (ddd, *J* = 13.0, 8.8, 5.7, 1H), 1.88 (d, *J* = 1.1, 3H) ppm. ^{13}C-NMR (D_2O) δ 177.87, 151.46, 146.98, 137.65, 122.60, 111.25, 85.47, 84.36, 60.53, 59.25, 58.31, 36.94, 26.39 ppm. MS (ESI) *m/z* 408.17 $[C_{16}H_{18}N_5O_8]^-$.

Compound 10. As per general procedure A, with AZT (200 mg, 0.74 mmol) and **5a** (142 mg, 1.12 mmol). The solvent was removed and the residue redissolved in a mixture of CH_2Cl_2 and MeOH (10%). The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (10%) to give the ester protected triazole product (140 mg, 48%). ^{1}H-NMR (CD_3OD) δ 8.03 (s, 1H), 7.91 (d, *J* = 0.9, 1H), 6.48 (t, *J* = 6.5, 1H), 5.42 (dt, *J* = 8.6, 5.4, 1H), 4.36 (m, 1H), 3.92 (s, 2H), 3.90 (dd, *J* = 12.2, 3.0, 1H), 3.77 (dd, *J* = 12.2, 3.2, 1H), 3.72 (s, 3H), 3.46 (s, 2H), 2.97-2.83 (m, 1H), 2.80-2.66 (m, 1H), 1.91 (d, *J* = 0.9, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 173.68, 166.61, 152.50, 147.09, 138.39, 124.29, 111.82, 86.85, 86.55, 62.29, 61.18, 52.45, 50.19, 44.39, 39.19, 12.62 ppm. High Res. MS (ESI) *m/z* 395.1674 $[C_{16}H_{22}N_6O_6]H^+$ (calc. 395.1674). The intermediate (100 mg, 0.25 mmol) was dissolved in a mixture of H_2O (2 mL) and 1 M NaOH (0.5 mL) and stirred for 2 h at rt. The mixture was neutralized by addition of 1 M HCl, before being concentrated under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **15** as a white solid (84 mg, 88%). ^{1}H-NMR (D_2O) δ 8.26 (s, 1H), 7.69 (s, 1H), 6.41 (t, *J* = 6.6, 1H), 5.42 (dt, *J* = 8.7, 5.8, 1H), 4.44 (dd, *J* = 9.5, 4.0, 1H), 4.39 (s, 2H), 3.87 (dd, *J* = 12.7, 3.3, 1H), 3.77 (dd, *J* = 12.7, 4.3, 1H), 3.60 (s, 2H), 2.94 (ddd, *J* = 13.0, 6.6, 1H), 2.80 (ddd, *J* = 14.7, 8.7, 6.2, 1H), 1.86 (s, 3H). ppm. ^{13}C-NMR (D_2O) δ 171.62, 167.14, 152.22, 138.98, 138.36, 126.49, 112.13, 86.24, 84.89, 61.21, 60.47, 49.50 (MeOH) 49.12, 41.69, 37.50, 12.15 ppm. High Res. MS (ESI) *m/z* 381.1519 $[C_{15}H_{20}N_6O_6]H^+$ (calc. 381.1517).

Compound 11. As per general procedure A, with AZT (85 mg, 0.32 mmol) and **6** (100 mg, 0.68 mmol). The product was extracted into EtOAc and washed twice with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (5%) to give the pure triazole product as a crystalline yellow solid (92 mg, 69%). ^{1}H-NMR (CD_3OD) δ 8.53 (d, *J* = 4.5, 1H), 7.96 (s, 1H), 7.86-7.77 (m, 2H), 7.48 (d, *J* = 7.8, 1H), 7.36-7.29 (m, 1H), 6.17 (t, *J* = 6.6, 1H), 4.69 (t, *J* = 5.6, 2H), 4.38 (t, *J* = 5.6, 3H), 3.96 (s, 4H), 3.87 (q, *J* = 3.4, 1H), 3.78 (dd, *J* = 12.0, 3.1, 1H), 3.71 (dd, *J* = 12.0, 3.7, 1H), 2.26-2.11 (m, 2H), 1.84 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 166.76, 152.71, 138.36, 124.62, 111.90, 86.86, 86.58, 62.43, 61.46, 39.18, 12.63 ppm. High Res MS (ESI) m/z $[C_{19}H_{23}N_7O_4]H^+$. (calc. 414.1890).

Compound 12. As per general procedure A, with **1a** (487 mg, 0.90 mmol) and **2a** (192 mg, 0.90 mmol). The product was extracted into EtOAc and washed twice with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (5%) to give the Boc-protected triazole product as a colourless oil (335 mg, 49%). ^{1}H-NMR (CD_3OD) δ 7.79 (s, 1H), 7.56 (s, 1H), 6.22 (t, *J* = 6.7, 1H), 4.85 (s, 4H), 4.62 (t, *J* = 5.8, 2H), 4.46 (m, 1H), 4.36 (m, 2H), 4.25 (s, 1H), 3.92 (m, 1H), 3.84 (ddd, *J* = 23.7, 11.4, 3.2, 2H), 3.17 (s, 2H), 2.27-2.21 (m, 1H), 2.18–2.02 (m, 1H), 1.87 (s, 4H), 1.42 (s, 9H), 0.94 (s, 9H), 0.93 (s, 9H), 0.14 (s, 6H), 0.12 (s, 6H) ppm. ^{13}C-NMR (CD_3OD) δ 164.99, 152.00, 135.98, 110.61, 89.33, 87.16, 73.64, 64.16, 42.03, 28.85, 26.55, 26.34, 19.34, 18.92, 13.48, -4.38, -4.55, -5.12, -5.15 ppm. MS (ESI) *m/z* 753.29 $[C_{34}H_{60}N_6O_9Si_2]H^+$. The intermediate (300 mg, 0.40 mmol) was dissolved in MeOH (20 mL). Concentrated HCl (0.4 mL) was added and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure, and the residue redissolved in a mixture of water and EtOAc (1:1, 15 mL). The aqueous phase was neutralized with 1 M NaOH and concentrated under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **7** as a white powder (95 mg, 56%).^{1}H-NMR (D_2O) δ 7.75 (s, 1H), 7.47 (d, *J* = 0.9, 1H), 6.01 (t, *J* = 6.6, 1H), 4.53 (m, 2H), 4.27 (m, *J* = 6.4, 4.4, 1H), 4.19 (t, *J* = 5.4, 2H), 3.85 (m, 2H), 3.67 (dd, *J* = 12.5, 3.5, 1H), 3.58 (dd, *J* = 12.5, 5.1, 1H), 3.18 (dd, *J* = 15.7, 4.9, 1H), 3.09 (dd, *J* = 15.7, 7.3, 1H), 2.26-2.05 (m, 2H), 1.68 (s, 3H).ppm. ^{13}C-NMR (D_2O) δ 173.72, 165.71, 151.81, 142.76, 136.62, 125.91, 110.93, 87.46, 86.68, 70.91, 61.85, 55.14, 49.50 (MeOH), 48.70, 41.81, 39.38, 27.11, 12.70 ppm. High Res. MS (ESI) *m/z* 425.1776 $[C_{17}H_{24}N_6O_7]H^+$ (calc. 425.1779).

Compound 13. As per general procedure A, with **1a** (488 mg, 0.90 mmol) and **3a** (180 mg, 0.84 mmol). The product was extracted into EtOAc and washed twice with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and

evaporated under reduced pressure. The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (5%) to give the Boc-protected triazole product as a colourless oil (240 mg, 38%). ^{1}H-NMR ($CDCl_3$) δ 7.58 (s, 1H), 7.44 (d, *J* = 1.0, 1H), 6.23 (m, 1H), 4.61 (t, *J* = 6.0, 2H), 4.38 (m, 3H), 3.91 (q, *J* = 2.5, 1H), 3.84 (dd, *J* = 11.3, 2.5, 2H), 3.77 (s, 2H), 3.73 (dd, *J* = 11.3, 2.5, 1H), 3.29 (m, 2H), 2.61 (t, *J* = 6.6, 2H), 2.24-2.19 (m, 1H), 1.97-192 (m, 1H), 1.86 (d, *J* = 1.0, 3H), 1.42 (s, 9H), 0.91 (s, 9H), 0.87 (s, 9H), 0.09 (s, 6H), 0.07 (s, 3H), 0.06 (s, 3H).ppm. ^{13}C-NMR ($CDCl_3$) δ 162.96, 150.46, 134.14, 122.15, 109.59, 87.69, 85.28, 71.93, 62.58, 53.51, 47.37, 41.43, 40.39, 39.32, 31.33, 28.11, 25.90, 25.71, 18.39, 17.95, 13.17, -4.64, -4.90, -5.40, -5.49 ppm. High Res. MS (ESI) *m/z* 777.3826 $[C_{34}H_{62}O_7N_6S]Na^+$ (calc. 777.3831). The intermediate (200 mg, 0.27 mmol) was dissolved in MeOH (14 mL). Concentrated HCl (0.28 mL) was added and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure, and the residue redissolved in a mixture of water and EtOAc (1:1, 15 mL). The aqueous phase was neutralized with 1 M NaOH and concentrated under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **8** as a colourless oil (100 mg, 89%).^{1}H-NMR (CD_3OD) δ 7.92 (s, 1H), 7.83 (d, *J* = 0.9, 1H), 6.17 (t, *J* = 6.6, 1H), 4.68 (t, 2H), 4.45-4.29 (m, 3H), 3.89 (m, 1H), 3.79 (dd, *J* = 12.0, 3.1, 1H), 3.76 (s, 2H), 3.72 (dd, *J* = 12.0, 3.7, 1H), 2.78 (t, *J* = 6.6, 2H), 2.59 (t, *J* = 6.6, 2H), 2.27-2.21 (m, 2H), 2.19-2.12 (m, 2H), 1.86 (d, *J* = 0.9, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 165.18, 152.22, 146.90, 136.83, 125.10, 110.57, 89.06, 87.35, 72.07, 62.84, 42.01, 41.59, 41.37, 35.27, 25.94, 13.26 ppm. High Res. MS (ESI) *m/z* 427.1755 $[C_{17}H_{26}O_5N_6S]H^+$ (calc. 427.1758).

Compound 14. As per general procedure A, with **1a** (200 mg, 0.37 mmol) and **4a** (63 mg, 0.37 mmol). The product was extracted into EtOAc and washed twice with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (3%) to give the TBDMS and ester protected triazole product (194 mg, 74%). ^{1}H-NMR (CD_3OD) δ 7.43 (s, 1H), 6.24 (dd, *J* = 7.8, 5.8, 1H), 4.56 (t, *J* = 6.3, 2H), 4.37 (dd, *J* = 7.6, 4.9, 3H), 3.91 (q, *J* = 2.5, 1H), 3.84 (dt, *J* = 5.8, 5.1, 2H), 3.75 (t, *J* = 2.2, 1H), 3.72 (s, 7H), 3.28 (d, *J* = 7.6, 2H), 2.22 (ddd, *J* = 13.2, 5.8, 2.7, 1H), 1.95 (ddd, *J* = 13.2, 7.8, 6.1, 1H), 1.87 (d, *J* = 1.1, 3H), 0.91 (s, 9H), 0.88 (s, 9H), 0.09 (s, 6H), 0.06 (d, *J* = 3.8, 6H) ppm. ^{13}C-NMR (CD_3OD) δ 168.87, 162.94, 150.46, 143.77, 133.89, 122.44, 109.86, 87.69, 85.26, 71.93, 62.86, 52.46, 51.09, 47.38, 41.25, 25.78, 25.69, 24.70, 18.26, 17.69, 12.92, -4.99, -5.79 ppm. High Res. MS (ESI) *m/z* 732.3428 $[C_{32}H_{55}N_5O_9Si_2]Na^+$ (calc. 732.3431). The intermediate (190 mg, 0.27 mmol) was dissolved in MeOH (15 mL). Concentrated HCl (0.3 mL) was added and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure, and the residue redissolved in a mixture of water and EtOAc (1:1, 15 mL). The pH of the aqueous phase was increased to 12 with 1 M NaOH and stirred for a further 2 h at rt. After this time the mixture was neutralized with 1 M HCl

before the solvent was removed under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **9** as a white powder (86 mg, 70%). ^{1}H-NMR (CD_3OD) δ 7.69 (s, 1H), 7.61 (s, 1H), 6.13 (t, *J* = 6.5, 1H), 4.64 (m, 2H), 4.42 (dt, *J* = 6.4, 4.2, 1H), 4.34 (m, 2H), 4.05-3.94 (m, 1H), 3.82 (dd, *J* = 12.5, 3.5, 1H), 3.73 (dd, *J* = 12.5, 5.2, 1H), 3.35 (t, *J* = 7.8, 1H), 3.06 (d, *J* = 7.8, 2H), 2.40-2.17 (m, 2H), 1.84 (s, 3H) ppm. ^{13}C NMR (D_2O) δ 146.94, 136.31, 124.25, 110.63, 87.10, 86.74, 70.80, 61.67, 49.50 (MeOH) 39.35, 12.67. High Res. MS (ESI) *m/z* 476.1378 $[C_{18}H_{23}N_5O_9]Na^+$ (calc. 476.1388).

Compound 15. As per general procedure A, with **1** (144 mg, 0.46 mmol) and **5a** (59 mg, 0.46 mmol). The solvent was removed and the residue redissolved in a mixture of CH_2Cl_2 and MeOH (10%). The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (10%) to give the ester protected triazole product (135 mg, 67%). ^{1}H-NMR (CD_3OD) δ 7.90 (s, 1H), 7.83 (d, *J* = 1.0, 1H), 6.17 (t, *J* = 6.6, 1H), 4.68 (t, *J* = 5.7, 2H), 4.37 (t, *J* = 5.6, 3H), 3.92-3.85 (m, 3H), 3.80 (dd, *J* = 12.0, 3.1, 1H), 3.74-3.70 (dd, *J* = 12.0, 3.8, 1H), 3.72 (s, 3H), 2.24 (ddd, *J* = 13.6, 6.2, 3.9, 1H), 2.16 (ddd, *J* = 11.4, 6.6, 1H), 1.85 (d, *J* = 1.0, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 165.08, 152.25, 136.76, 125.04, 110.37, 88.96, 87.10, 71.92, 62.57, 43.87, 42.08, 41.54, 13.13 ppm. High Res. MS (ESI) *m/z* 439.1933 $[C_{18}H_{26}N_6O_7]H^+$ (calc. 439.1936). The intermediate (112 mg, 0.26 mmol) was dissolved in a mixture of H_2O (2 mL) and 1 M NaOH (0.5 mL) and stirred for 2 h at rt. The mixture was neutralized by addition of 1 M HCl, before being concentrated under reduced pressure. The crude product was purified by solid phase extraction using a Sep-Pak® column. The fractions containing the product were evaporated under reduced pressure to give **10** as a white solid (70 mg, 61%). ^{1}H-NMR (CD_3OD) δ 7.97 (s, 1H), 7.82 (s, 1H), 6.16 (t, *J* = 6.6, 1H), 4.68 (t, *J* = 5.6, 2H), 4.37 (m, 3H), 3.98 (s, 2H), 3.90 (q, *J* = 3.4, 1H), 3.79 (dd, *J* = 12.0, 3.1, 1H), 3.72 (dd, *J* = 12.0, 3.8, 1H), 3.31 (s, 2H), 2.25 (ddd, *J* = 13.5, 6.1, 3.8, 1H), 2.15 (ddd, *J* = 13.5, 6.6, 1H), 1.86 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 176.69, 165.06, 152.03, 145.19, 136.79, 125.45, 110.43, 88.81, 87.25, 71.77, 62.64, 52.00, 43.92, 41.88, 41.34, 13.07 ppm. High Res. MS (ESI) *m/z* 425.1776 $[C_{17}H_{24}N_6O_7]H^+$ (calc. 425.1779).

Compound 16. As per general procedure A, with **1** (280 mg, 0.90 mmol) and **6** (131 mg, 0.88 mmol). The product was extracted into EtOAc and washed twice with saturated NaCl. The aqueous phases were re-extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by column chromatography with a mixture of CH_2Cl_2 and MeOH (5%) to give the pure triazole product as a yellow solid (220 mg, 55%).^{1}H-NMR (CD_3OD) δ 8.53 (d, *J* = 4.5, 1H), 7.96 (s, 1H), 7.86-7.77 (m, 2H), 7.48 (d, *J* = 7.8, 1H), 7.33 (m, 1H), 6.17 (t, *J* = 6.6, 1H), 4.69 (t, *J* = 5.6, 2H), 4.38 (t, *J* = 5.6, 2H), 4.37 (m, 1H), 3.96 (s, 4H), 3.87 (q, *J* = 3.4, 1H), 3.78 (dd, *J* = 12.0, 3.1, 1H), 3.71 (dd, *J* = 12.0, 3.7, 1H), 2.26-2.11 (m,

2H), 1.84 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 165.20, 159.35, 152.25, 150.18, 146.37, 138.93, 136.91, 125.44, 124.40, 124.12, 110.58, 89.06, 87.36, 72.07, 62.84, 53.95, 44.26, 42.13, 41.52, 18.52, 13.22 ppm. High Res. MS (ESI) *m/z* 458.2149 $[C_{21}H_{27}N_7O_5]H^+$ (calc. 458.2146).

Rhenium complexes. Tricarbonyl rhenium complexes were synthesized according to the following general procedure: One equivalent of thymidine containing ligand and one equivalent of $[ReBr_3(CO)_3][NEt_4]_2$were dissolved in a 1:1 mixture of MeOH and water to form a 0.1 M solution. In the reactions with ligands **9** and **14** the pH of the reaction mixture was adjusted to 7 with aqueous NEt_4OH. The reaction was stirred at 50 °C for 2 h and followed by HPLC. The solvents were removed under vacuum and the residue was purified by solid phase extraction using a Sep-Pak® column and a water-MeOH gradient to give the $Re(CO)_3$ complex as a white or off-white powder.

$[Re(CO)_3$**7**]. (41 mg, 59%). ^{1}H-NMR (CD_3OD) δ 8.20 (s, 1H), 7.86 (s, 1H), 6.44 (t, *J* = 6.4, 1H), 5.90 (dd, *J* = 11.0, 5.8, 1H), 5.58-5.44 (m, 1H), 5.18 (d, *J* = 11.4, 1H), 4.45-4.32 (m, 1H), 4.11 (d, *J* = 3.9, 1H), 3.92 (dd, *J* = 12.2, 2.9, 1H), 3.81 (dd, *J* = 12.2, 3.1, 1H), 3.38 (dd, *J* = 16.0, 1H), 3.26 (dd, *J* = 4.2, 1H), 3.02-2.73 (m, 2H), 1.91 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 197.39, 196.67, 184.57, 166.45, 152.22, 144.06, 138.43, 126.48, 111.81, 86.97, 85.89, 62.30, 62.01, 52.78, 38.68, 27.39, 12.19 ppm. High Res MS (ESI) *m/z* 651.0846 $[C_{18}H_{19}N_6O_9Re]H^+$ (calc. 651.0844).

$[Re(CO)_3$**8**]Br. (17 mg, 61%). ^{1}H-NMR (CD_3OD) δ 8.41 (s, 1H), 7.88 (s, 1H), 6.48 (t, *J* = 6.3, 1H), 5.55 (m, 1H), 5.32 (bs, 1H), 4.60 (bs, 1H), 4.53 (d, *J* = 17.2, 1H), 4.56-4.47 (m, 1H), 4.24 (d, *J* = 17.2, 1H), 3.93 (dd, *J* = 12.0, 3.1, 1H), 3.86 (dd, *J* = 12.0, 1H), 3.04-2.96 (m, 1H), 2.95-2.87 (m, 1H), 2.92 (m, 2H), 2.86-2.74 (m, 1H), 2.27 (m, 1H) ppm. ^{13}C-NMR (CD_3OD) δ 166.42, 152.40, 151.52, 138.40, 125.44, 111.65, 87.03, 86.92, 86.23, 85.88, 63.81, 63.66, 62.60, 62.37, 44.65, 39.03, 34.23, 12.61 ppm. High Res. MS (ESI) *m/z* 653.0818 $[C_{18}H_{22}N_6O_7ReS]^+$ (calc. 653.0822).

$[Re(CO)_3$**9**]NEt_4. (30 mg, 45%). ^{1}H-NMR (CD_3OD) δ 8.20 (s, 1H), 7.89 (d, *J* = 1.1, 1H), 6.46 (t, *J* = 6.4, 1H), 5.57-5.47 (m, 1H), 4.43 (dt, *J* = 6.1, 3.2, 1H), 3.95 (dd, *J* = 12.4, 3.0, 1H), 3.85 (dd, *J* = 12.4, 3.3, 1H), 3.61 (t, *J* = 4.7, 1H), 3.44 (d, *J* = 4.7, 2H), 3.37-3.28 (m, 8H), 3.06-2.93 (m, 1H), 2.84 (ddd, *J* = 14.3, 8.7, 5.8, 1H), 1.93 (d, *J* = 1.1, 3H), 1.35-1.27 (m, 12H) ppm. ^{13}C-NMR (CD_3OD) δ 198.32, 179.91, 179.88, 166.42, 152.23, 147.36, 138.48, 126.96, 111.65, 86.92, 86.10, 62.05, 62.03, 53.82, 53.31, 53.28, 53.25, 38.79, 27.02, 12.46, 7.59 ppm. High Res. MS (ESI) *m/z* 680.00629 $[C_{19}H_{19}N_5O_{11}Re]^+$ (calc. 680.0634).

$[Re(CO)_3$**10**]. (58 mg, 83%). ^{1}H-NMR (CD_3OD) δ ^{1}H NMR (400 MHz, MeOD) δ 8.32 (s, 1H), 7.86 (d, *J* = 5.6, 1H), 6.46 (q, *J* = 6.8, 1H), 5.62-5.47 (m, 1H), 4.40 (dd, *J* = 5.2, 3.2, 1H), 4.36-4.22 (m, 2H), 3.88 (dd, 1H), 3.83-3.77 (m, 1H), 3.81 (d, *J* = 17.3, 1H), 3.38 (d, *J* = 17.3, 1H), 3.04-2.93 (m, 1H), 2.85-2.76 (m, 1H), 1.91 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 197.37, 196.71, 183.78, 166.35,

152.20, 149.87, 138.37, 124.04, 111.79, 86.68, 85.88, 62.93, 61.86, 56.29, 53.09, 38.38, 12.50 ppm. High Res MS (ESI) *m/z* 651.0838 $[C_{18}H_{19}N_6O_9Re]H^+$ (calc. 651.0844).

$[Re(CO)_3$**11**$]$Br. (64 mg, 55%). ^{1}H-NMR (CD_3OD) δ ^{1}H NMR (400 MHz, MeOD) δ 8.83 (t, *J* = 5.9, 1H), 8.15 (s, 1H, isomer A), 8.14 (s, 1H, isomer B), 8.00-7.91 (m, 1H), 7.82 (s, 1H), 7.60 (d, *J* = 7.9, 1H), 7.39 (dd, *J* = 13.2, 6.6, 1H), 6.38 (td, *J* = 6.6, 2.2, 1H), 5.40 (dt, *J* = 9.4, 4.9, 1H), 4.78 (d, *J* = 17.0, 1H), 4.69 (d, *J* = 17.0, 1H), 4.52 (d, *J* = 16.5, 1H), 4.40-4.31 (m, 1H, isomer B), 4.36 (d, *J* = 16.5, 1H), 4.17-4.05 (m, 1H, isomer A), 3.84 (dd, *J* = 12.1, 3.3, 1H, isomer B), 3.79 (dd, *J* = 12.2, 3.3, 1H, isomer A), 3.70 (dd, *J* = 12.1, 3.1, 1H, isomer B), 3.60 (dd, *J* = 12.2, 3.2, 1H, isomer A), 2.90-2.80 (m, 1H, isomer A), 2.72 (ddd, *J* = 14.4, 8.3, 6.4, 1H, isomer A), 2.63 (dd, *J* = 14.0, 7.2, 1H, isomer B), 2.48 (ddd, *J* = 14.1, 6.4, 4.8, 1H, isomer B), 1.90 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 166.27, 161.70, 153.16, 151.89, 150.24, 140.91, 137.97, 126.77, 124.37, 124.07, 111.80, 86.69, 86.48, 85.91, 85.65, 63.87, 63.45, 63.15, 62.27, 62.00, 52.77, 39.16, 38.39, 12.19 ppm. High Res MS (ESI) *m/z* 684.1214 $[C_{22}H_{23}N_7O_7Re]^+$ (calc. 684.1212).

$[Re(CO)_3$**12**$]$. (55 mg, 95%). ^{1}H-NMR (CD_3OD) δ 8.08 (s, 1H), 7.85 (s, 1H), 6.20 (t, *J* = 6.6, 1H), 5.91-5.77 (m, 1H), 5.15 (d, *J* = 11.2, 1H), 4.78 (m, 2H), 4.48-4.34 (m, 2H), 4.08 (m, 1H), 3.92 (m, *J* = 3.3, 1H), 3.80 (dd, *J* = 12.0, 3.0, 1H), 3.73 (dd, *J* = 12.0, 3.8, 1H), 3.38 (m, 1H), 3.27 (dd, *J* = 17.6, 4.3, 1H), 2.39 (ddd, *J* = 13.6, 5.9, 3.6, 1H), 2.24-2.18 (m, 1H), 1.89 (s, 3H) ppm. ^{13}C-NMR (DMSO) δ 198.26, 197.44, 196.44, 180.16, 162.49, 150.18, 142.22, 135.08, 126.16, 108.26, 87.38, 84.99, 69.95, 61.07, 50.69, 50.60, 48.57, 48.05, 25.97, 12.84 ppm. High Res MS (ESI) *m/z* 695.1106 $[C_{20}H_{23}N_6O_{10}Re]H^+$ (calc. 695.1107).

$[Re(CO)_3$**13**$]$Br. (20 mg, 74%). ^{1}H-NMR (CD_3OD) δ 8.26 (s, 1H), 8.22 (s, 1H), 7.88 (d, *J* = 0.9, 1H), 7.87 (d, *J* = 0.9, 1H), 6.27-6.22 (m, 1H), 6.21 (t, *J* = 6.5, 1H), 5.43 (bs, 1H), 5.37 (bs, 1H), 4.87-4.79 (m, 2H), 4.79-4.69 (m, 2H), 4.55-4.47 (m, 2H), 4.47-4.42 (m, 2H), 4.43-4.36 (m, 2H), 4.34 (dd, *J* = 7.6, 4.5, 1H), 4.30 (d, *J* = 3.5, 1H), 4.20 (d, *J* = 11.3, 1H), 4.16 (d, *J* = 11.2, 1H), 4.10 (bs, 2H), 3.93 (dd, *J* = 6.6, 3.3, 1H), 3.91 (dd, *J* = 6.7, 3.4, 1H), 3.81 (t, *J* = 2.8, 1H), 3.78 (t, *J* = 2.7, 1H), 3.76-3.72 (m, 1H), 3.72-3.68 (m, 1H), 3.05-2.88 (m, 3H), 2.88-2.76 (m, 2H), 2.76-2.61 (m, 1H), 2.47 (m, 1H), 2.41-2.29 (m, 1H), 2.27-2.19 (m, 4H), 1.88 (s, 3H), 1.87 (s, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 165.44 (165.29), 152.51, 151.23 (150.97), 137.22 (137.04), 126.03 (125.90), 110.87, 89.10, 87.02, 72.50 (72.37), 63.06 (62.99), 51.44 (51.32), 44.90 (44.38), 42.82 (42.65), 41.14 (41.06), 38.92 (38.59), 34.38 (34.27), 13.11 ppm. High Res MS (ESI) *m/z* 697.1086 $[C_{20}H_{26}N_6O_8ReS]^+$ (calc. 697.1084).

$[Re(CO)_3$**14**$]NEt_4$. (67 mg, 65%). ^{1}H-NMR (CD_3OD) δ 8.06 (s, 1H), 7.76 (d, *J* = 1.0, 1H), 6.04 (t, *J* = 6.4, 1H), 4.79-4.68 (m, 2H), 4.51-4.42 (m, 1H), 4.40-4.34 (m, 2H), 3.88 (m, 1H), 3.78 (dd, *J* = 12.1, 3.3, 1H), 3.70 (dd, *J* = 12.1, 4.3, 1H), 3.56 (t, *J* = 4.3, 1H), 3.39 (d, *J* = 4.3, 2H), 3.34-3.25 (m, 8H), 2.28 (ddd, *J* = 13.8, 6.3, 4.0, 1H), 2.14-2.05 (m, 1H), 1.87 (d, *J* = 0.9, 3H), 1.33-1.25 (m, 12H) ppm.

^{13}C-NMR (CD_3OD) δ 164.66, 152.10, 147.03, 137.40, 128.06, 88.54, 87.76, 71.76, 62.64, 53.08, 41.86, 41.31, 12.99, 7.42 ppm. High Res. MS (ESI) *m/z* 721.90 $[C_{21}H_{21}N_5O_{12}Re]^-$ (calc.).

[Re(CO)$_3$**15**]. (96 mg, 84%). ^{1}H-NMR (CD_3OD) δ 8.18 (s, 1H), 7.84 (m, 1H), 7.23-7.12 (m, 1H), 6.26 (m, 1H), 4.81-4.69 (m, 2H), 4.55-4.28 (m, 3H), 3.91 (m, 1H), 3.84-3.68 (m, 3H), 3.35-3.30 (m, 2H), 2.42-2.26 (m, 1H), 2.21 (m, 1H), 1.92-1.84 (m, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 183.90, 165.23, 151.88, 149.46, 136.63, 124.91, 110.45, 88.82, 87.25, 86.96, 71.99, 62.64, 55.73, 53.58, 41.28, 12.98 ppm. High Res MS (ESI) *m/z* 695.1117 $[C_{20}H_{23}N_6O_{10}Re]H^+$ (calc. 695.1107).

[Re(CO)$_3$**16**]Br. (110 mg, 73%). ^{1}H-NMR (CD_3OD) δ 8.77 (t, *J* = 5.0, 1H), 8.01-7.93 (m, 2H), 7.97 (s, 1H), 7.84 (d, 1H), 7.63 (d, *J* = 8.0, 1H), 7.38 (t, *J* = 6.6, 1H), 6.12 (t, *J* = 6.8, 1H), 4.80-4.55 (m, 4H), 4.49 (dd, *J* = 16.7, 8.1, 1H), 4.41 (dt, *J* = 6.1, 3.2, 1H), 4.37-4.21 (m, 3H), 3.99-3.92 (m, 1H), 3.81 (dd, *J* = 12.0, 3.1, 2H), 3.74 (dd, *J* = 12.0, 3.7, 2H), 2.28-2.12 (m, 2H), 1.84 (d, 3H) ppm. ^{13}C-NMR (CD_3OD) δ 164.98 (164.96), 161.85 (161.79), 153.48 (153.46), 152.12 (152.02), 150.64 (150.61), 141.48 (141.45), 136.86, 126.66, 124.57 (124.55), 124.49 (124.43), 110.58 (110.53), 89.10 (89.07), 87.08 (86.92), 72.33 (72.31), 63.96, 62.93 (62.91), 53.13 (53.07), 50.79 (50.56), 41.87 (41.67), 41.27 (41.19), 13.17 ppm. High Res MS (ESI) *m/z* 728.1470 $[C_{24}H_{27}N_7O_8Re]^+$ (calc. 728.1474).

Technetium-99m complexes. The 99m-technetium tricarbonyl complexes of ligands **7**, **8**, **10**, **11**, **12**, **13**, **15** and **16**, were synthesized according to the following general procedure: A solution of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (50 µL; ~500 MBq mL^{-1}) was added to a 10^{-3} M solution of the relevant ligand (50 µL) diluted with PBS (400 µL; pH 7.4) to give a final ligand concentration of 10^{-4} M. The reaction mixtures were heated for 45 min at 95 °C. The radioactive product was separated from unlabelled ligand by HPLC. The complexes were characterized by comparison of their HPLC retention times (γ-trace) with those of their rhenium analogues (UV-trace).

X-ray crystallography

Crystallographic data were collected at 183(2) K on an Oxford Diffraction Xcalibur system with a Ruby detector (Mo K_α radiation, λ = 0.7107 Å) using graphite-monochromated radiation. A suitable crystal was covered with oil (Infineum V8512, formerly known as Paratone N), mounted on top of a glass fiber and immediately transferred to the diffractometer. The program suite CrysAlis^{Pro} was used for data collection, semi-empirical absorption correction and data reduction.(*40*) The structure was solved with direct methods using SIR97(*41*) and refined by full-matrix least-squares methods on F^2 with SHELXL-97.(*42*) The final model was checked with the help of the program Platon.(*43*)

CCDC-743723 contains the supplementary crystallographic data for this paper. This data is presented in the supporting information or can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

***In vitro* analysis**

Phosphorylation transfer assays. Thymidine and thymidine analogues $[Re(CO)_3$**7-16**$]^{0/+/-}$ were assayed at 25 °C for 15 minutes in 200 µL of a mixture containing 155.38 µL water, 10 µL 1 M HEPES buffer (pH 7.5), 0.2 µL 1 M DTT, 0.42 µL 100 mM PEP, 0.5 µL 1 M $MgCl_2$, 7.2 µL 5 mM NADH, 2 µL 100 mM ATP, 0.6 µL pyruvate kinase (1350 U/mL), 0.7 µL lactate dehydrogenase (1420 U/mL), 5 µL hTK1 (~0.5 mg/mL) and 20 µL of a 10 mM solution of thymidine or the thymidine analogue $[Re(CO)_3$**7-16**$]^{0/+/-}$.

For each substrate the linear decrease in UV absorption at 340 nm was measured from 0 to 15 minutes. The gradients of the regression lines between 6 and 12 minutes were compared to thymidine, the phosphorylation of which was assumed to be 100%.

Thymidine analogues $[Re(CO)_3$**X**$]^{0/+}$ (**X** = **7**, **10**, **11, 15**) were also assayed at 37 °C for 1 hour in 200 µL of a mixture containing 112.3 µL water, 10 µL 1 M HEPES buffer (pH 7.5), 0.2 µL 1 M DTT, 0.5 µL 1 M $MgCl_2$, 2 µL 100 mM ATP, 5 µL hTK1 (~0.5 mg/mL), 20 µL of a 10 mM solution of $[Re(CO)_3$**X**$]^{0/+}$ (**X** = **7**, **10**, **11, 15**) and 50 µL of an aqueous solution of $[^{99m}Tc(CO)_3$**X**$]^{0/+}$ (**X** = **7**, **10**, **11, 15**) (~100 kBq). The reaction mixtures were quenched by addition of 800 µL 5 mM EDTA solution and analyzed by HPLC.

Cell lines and culture. Two human glioblastoma cell lines were used: U251 (American Type Culture Collection, ATCC) and LN229 (a gift from Prof. N. de Tribolet, Service of Neurosurgery, University Hospital, Geneva, Switzerland). Cells were cultured at 37 °C and 5% CO_2 in RPMI 1640 with glutamax I (Invitrogen AG, Basel Switzerland), supplemented with 10% heat-inactivated fetal bovine serum (Chemie Brunschwig AG, Basel, Switzerland), penicillin (50 units/ml) and streptomycin (0.05 mg/mL; Life Technologies Inc., Grand Island, NY, USA), which was changed twice a week. Because mycoplasma contamination is a potential source of increased nucleoside turnover and could therefore modulate FdUrd efficacy, the cell lines were regularly tested for four major potential mycoplasma contaminations (Mycoplasma Detection Kit, F. Hoffmann-La Roche Ltd, Basel, Switzerland).

Radioactive dT analogue incorporation assay. 24-well culture dishes were plated with 20,000 cells for LN229 and with 15,000 cells for U251. After 3 days, the exponentially growing cells were

incubated for 2 h at 37 °C and 5% CO_2 with either 5 kBq ^{125}I-IdUrd, 10 kBq [^{99m}Tc(CO)$_3$**7**], or 10 kBq [^{99m}Tc(CO)$_3$**16**]$^+$ in 0.5 mL medium. After this time the supernatant was removed and the cells were washed three times with PBS. The cells were detached with 300 μL of 1 M NaOH. Radioactivity was measured in a gamma counter (Wizard 3" 1480 automatic gamma counter, Wallac Oy, Turku, Finland) and the results expressed as a percentage of the initial activity added. In the FdUrd pre-treatment assay, cells were pre-treated with 1 μM FdUrd and incubated for 1 hour at 37 °C and 5% CO_2. After this time the supernatant was removed and the cells were washed twice with PBS before either 5 kBq ^{125}I-IdUrd, 10 kBq [^{99m}Tc(CO)$_3$**7**], or 10 kBq [^{99m}Tc(CO)$_3$**16**]$^+$ was added in 0.5 mL fresh medium.

Supporting Information Available: NMR and HPLC analysis of target complexes, crystal data and structure refinement for [Re(CO)$_3$11][CF_3CO_2]$_{0.8}$[Br]$_{0.2}$, and further details of the phosphorylation assay. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature cited

(1) Eriksson, S., Kierdaszuk, B., Munch-Petersen, B., Oberg, B., and Johansson, N. G. (1991) Comparison of the substrate specificities of human thymidine kinase-1 and kinase-2 and deoxycytidine kinase toward antiviral and cytostatic nucleoside analogs. *Biochem. Biophys. Res. Commun. 176*, 586-592.

(2) Schinazi, R. F., Mead, J. R., and Feorino, P. M. (1992) Insights into HIV chemotherapy. *AIDS Res. Hum. Retrov. 8*, 963-990.

(3) Eriksson, S., Munch-Petersen, B., Johansson, K., and Eklund, H. (2002) Structure and function of cellular deoxyribonucleoside kinases. *Cell. Mol. Life Sci. 59*, 1327-1346.

(4) Eriksson, S., and Wang, J. H. (1995) Substrate specificities of mitochondrial thymidine kinase and cytosolic deoxycytidine kinase against 5-aryl substituted pyrimidine-2'-deoxyribose analogs. *Nucleosides Nucleotides 14*, 507-510.

(5) Al-Madhoun, A. S., Johnsamuel, J., Yan, J. H., Ji, W. H., Wang, J. H., Zhuo, J. C., Lunato, A. J., Woollard, J. E., Hawk, A. E., Cosquer, G. Y., Blue, T. E., Eriksson, S., and Tjarks, W. (2002) Synthesis of a small library of 3-(carboranylalkyl)thymidines and their biological evaluation as substrates for human thymidine kinases 1 and 2. *J. Med. Chem. 45*, 4018-4028.

(6) Byun, Y., Thirumamagal, B. T. S., Yang, W., Eriksson, S., Barth, R. F., and Tjarks, W. (2006) Preparation and biological evaluation of ^{10}B-enriched 3-[5-{2-(2,3-dihydroxyprop-1-yl)-o-carboran-1-yl}pentan-1-yl]thymidine (N5-2OH), a new boron delivery agent for boron neutron capture therapy of brain tumors. *J. Med. Chem. 49*, 5513-5523.

(7) Welin, M., Kosinska, U., Mikkelsen, N. E., Carnrot, C., Zhu, C. Y., Wang, L. Y., Eriksson, S., Munch-Petersen, B., and Eklund, H. (2004) Structures of thymidine kinase 1 of human and mycoplasmic origin. *Proc. Natl. Acad. Sci. U. S. A. 101*, 17970-17975.

(8) Lunato, A. J., Wang, J. H., Woollard, J. E., Anisuzzaman, A. K. M., Ji, W. H., Rong, F. G., Ikeda, S., Soloway, A. H., Eriksson, S., Ives, D. H., Blue, T. E., and Tjarks, W. (1999) Synthesis of 5-(carboranylalkylmercapto)-2 '-deoxyuridines and 3-(carboranylalkyl)thymidines and their evaluation as substrates for human thymidine kinases 1 and 2. *J. Med. Chem. 42*, 3378-3389.

(9) Johnsamuel, J., Lakhi, N., Al-Madhoun, A. S., Byun, Y., Yan, J. H., Eriksson, S., and Tjarks, W. (2004) Synthesis of ethyleneoxide modified 3-carboranyl thymidine analogues and evaluation of their biochemical, physicochemical, and structural properties. *Bioorg. Med. Chem. 12*, 4769-4781.

(10) Conti, P. S., Alauddin, M. M., Fissekis, J. R., Schmall, B., and Watanabe, K. A. (1995) Synthesis of 2'-fluoro-5-[C-11]-methyl-1-b-D-arabinofuranosyluracil ([C-11]-FMAU) - a potential nucleoside analog for in vivo study of cellular proliferation with PET. *Nucl. Med. Biol. 22*, 783-789.

(11) Shields, A. F., Grierson, J. R., Dohmen, B. M., Machulla, H. J., Stayanoff, J. C., Lawhorn-Crews, J. M., Obradovich, J. E., Muzik, O., and Mangner, T. J. (1998) Imaging proliferation in vivo with [F-18]FLT and positron emission tomography. *Nat. Med. 4*, 1334-1336.

(12) Gardelle, O., Roelcke, U., Vontobel, P., Crompton, N. E. A., Guenther, I., Blauenstein, P., Schubiger, A. P., Blattmann, H., Ryser, J. E., Leenders, K. L., and Kaser-Hotz, B. (2001) [^{76}Br]Bromodeoxyuridine PET in tumor-bearing animals. *Nucl. Med. Biol. 28*, 51-57.

(13) Desbouis, D., Struthers, H., Spiwok, V., Küster, T., and Schibli, R. (2008) Synthesis, in vitro, and in silico evaluation of organometallic technetium and rhenium thymidine complexes with retained substrate activity toward human thymidine kinase type 1. *J. Med. Chem. 51*, 6689-6698.

(14) Struthers, H., Spingler, B., Mindt, T. L., and Schibli, R. (2008) "Click-to-Chelate": Design and incorporation of triazole-containing metal-chelating systems into biomolecules of diagnostic and therapeutic interest. *Chem.--Eur. J. 14*, 6173-6183.

(15) Yan, J. H., Naeslund, C., Al-Madhoun, A. S., Wang, J. H., Ji, W. H., Cosquer, G. Y., Johnsamuel, J., Sjoberg, S., Eriksson, S., and Tjarks, W. (2002) Synthesis and biological evaluation of 3'-carboranyl thymidine analogues. *Bioorg. Med. Chem. Lett. 12*, 2209-2212.

(16) Mindt, T. L., Struthers, H., Brans, L., Anguelov, T., Schweinsberg, C., Maes, V., Tourwe, D., and Schibli, R. (2006) "Click to Chelate": Synthesis and installation of metal chelates into biomolecules in a single step. *J. Am. Chem. Soc. 128*, 15096-15097.

(17) Tornøe, C. W., Christensen, C., and Meldal, M. (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem. 67*, 3057-3064.

(18) Kolb, H. C., and Sharpless, K. B. (2003) The growing impact of click chemistry on drug discovery. *Drug Discovery Today 8*, 1128-1137.

(19) Rostovtsev, V. V., Green, L. G., Fokin, V. V., and Sharpless, K. B. (2002) A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem., Int. Ed. 41*, 2596-2599.

(20) In an evaluation of the "click-to-chelate" approach, the compounds were synthesized using stock solutions (10^{-2} M) of the deprotected analogues of alkynes **2**, **3**, **5** and **6**, compound **1**, copper (II) acetate and sodium ascorbate.

(21) Banerjee, S. R., Levadala, M. K., Lazarova, N., Wei, L., Valliant, J. F., Stephenson, K. A., Babich, J. W., Maresca, K. P., and Zubieta, J. (2002) Bifunctional single amino acid chelates for labeling of biomolecules with the $\{Tc(CO)_3\}^+$ and $\{Re(CO)_3\}^+$ cores. Crystal and molecular structures of $[ReBr(CO)_3(H_2NCH_2C_5H_4N)]$, $[Re(CO)_3\{(C_5H_4NCH_2)_2NH\}]Br$, $[Re(CO)_3\{(C_5H_4NCH_2)_2NCH_2CO_2H\}]Br$, $[Re(CO)_3\{X(Y)NCH_2CO_2CH_2CH_3\}]Br$ (X = Y = 2-pyridylmethyl; X = 2-pyridylmethyl, Y = 2-(1-methylimidazolyl)methyl; X = Y = 2-(1-methylimidazolyl)methyl), $[ReBr(CO)_3\{(C_5H_4NCH_2)NH(CH_2C_4H_3S)\}]$, and $[Re(CO)_3\{(C_5H_4NCH_2)N(CH_2C_4H_3S)(CH_2CO_2)\}]$. *Inorg. Chem. 41*, 6417-6425.

(22) Lazarova, N., Babich, J., Valliant, J., Schaffer, P., James, S., and Zubieta, J. (2005) Thiol- and thioether-based bifunctional chelates for the $\{M(CO)_3\}^+$ core (M = Tc, Re). *Inorg. Chem. 44*, 6763-6770.

(23) Schibli, R., La Bella, R., Alberto, R., Garcia-Garayoa, E., Ortner, K., Abram, U., and Schubiger, P. A. (2000) Influence of the denticity of ligand systems on the in vitro and in vivo behavior of 99mTc(I)-tricarbonyl complexes: a hint for the future functionalization of biomolecules. *Bioconjugate Chem. 11*, 345-351.

(24) Farrugia, L. J. (1997) ORTEP-3 for Windows - a version of ORTEP-III with a Graphical User Interface (GUI). *J. Appl. Crystallogr. 30*, 565.

(25) Alberto, R., Ortner, K., Wheatley, N., Schibli, R., and Schubiger, A. P. (2001) Synthesis and properties of boranocarbonate: A convenient in situ CO source for the aqueous preparation of $[^{99m}Tc(H_2O)_3(CO)_3]^+$. *J. Am. Chem. Soc. 123*, 3135-3136.

(26) Kornberg, A., and Pricer, W. E. (1951) Enzymatic phosphorylation of adenosine and 2,6-diaminopurine riboside. *J. Biol. Chem. 193*, 481-495.

(27) Byun, Y., Yan, J. H., Al-Madhoun, A. S., Johnsamuel, J., Yang, W. L., Barth, R. F., Eriksson, S., and Tjarks, W. (2005) Synthesis and biological evaluation of neutral and zwitterionic 3-carboranyl thymidine analogues for boron neutron capture therapy. *J. Med. Chem. 48*, 1188-1198.

(28) Zhang, J., Visser, F., King, K. M., Baldwin, S. A., Young, J. D., and Cass, C. E. (2007) The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. *Cancer Metastasis Rev. 26*, 85-110.

(29) Yao, S. Y. M., Ng, A. M. L., Sundaram, M., Cass, C. E., Baldwin, S. A., and Young, J. D. (2001) Transport of antiviral 3 '-deoxy-nucleoside drugs by recombinant human and rat equilibrative, nitrobenzylthioinosine (NBMPR)-insensitive (ENT2) nucleoside transporter proteins produced in Xenopus oocytes. *Mol. Membr. Biol. 18*, 161-167.

(30) Dupertuis, Y. M., Vazquez, M., Mach, J. P., De Tribolet, N., Pichard, C., Slosman, D. O., and Buchegger, F. (2001) Fluorodeoxyuridine improves imaging of human glioblastoma xenografts with radiolabeled iododeoxyuridine. *Cancer Res. 61*, 7971-7977.

(31) Perillo-Adamer, F., Delaloye, A. B., Genton, C. S., Schaffland, A. O., Dupertuis, Y. M., and Buchegger, F. (2006) Short fluorodeoxyuridine exposure of different human glioblastoma lines induces high-level accumulation of S-phase cells that avidly incorporate I-125-iododeoxyuridine. *Eur. J. Nucl. Med. Mol. Imaging 33*, 613-620.

(32) Perumal, M., Pillai, R. G., Barthel, H., Leyton, J., Latigo, J. R., Forster, M., Mitchell, F., Jackman, A. L., and Aboagye, E. O. (2006) Redistribution of nucleoside transporters to the cell membrane provides a novel approach for imaging thymidylate synthase inhibition by positron emission tomography. *Cancer Res. 66*, 8558-8564.

(33) Pressacco, J., Mitrovski, B., Erlichman, C., and Hedley, D. W. (1995) Effects of thymidylate synthase inhibition on thymidine kinase activity and nucleoside transporter expression. *Cancer Res. 55*, 1505-1508.

(34) Armstrong, R. D., and Diasio, R. B. (1980) Metabolism and biological activity of 5'-deoxy-5-fluorouridine, a novel fluoropyrimidine. *Cancer Res. 40*, 3333-3338.

(35) Tjarks, W., Tiwari, R., Byun, Y., Narayanasamy, S., and Barth, R. F. (2007) Carboranyl thymidine analogues for neutron capture therapy. *Chem. Commun.*, 4978-4991.

(36) Liu, Y., Pak, J. K., Schmutz, P., Bauwens, M., Mertens, J., Knight, H., and Alberto, R. (2006) Amino acids labeled with $[^{99m}Tc(CO)_3]^+$ and recognized by the L-type amino acid transporter LAT1. *J. Am. Chem. Soc. 128*, 15996-15997.

(37) Alberto, R., Egli, A., Abram, U., Hegetschweiler, K., Gramlich, V., and Schubiger, P. A. (1994) Synthesis and reactivity of $[NEt_4]_2[ReBr_3(CO)_3]$. Formation and structural characterization of the clusters $[NEt_4][Re_3(\mu_3\text{-}OH)(\mu\text{-}OH)_3(CO)_9]$ and $[NEt_4][Re_2(\mu\text{-}OH)_3(CO)_6]$ by alkaline titration. *J. Chem. Soc., Dalton Trans.*, 2815-2820.

(38) Foulon, C. F., Adelstein, S. J., and Kassis, A. I. (1996) Kit formulation for the preparation of radiolabeled iododeoxyuridine by demetallation. *J. Nucl. Med. 37*, S1-S3.

(39) Schaffland, A. O., Delaloye, A. B., Kosinski, M., Dupertuis, Y. M., and Buchegger, F. (2004) The prearation of clinical grade 5-[I-123]iodo-2 '-deoxyuridine and 5-[I-125]iodo-2 '-

deoxyuridine with high in vitro stability and the potential for early proliferation scintigraphy. *Nucl. Med. Commun. 25*, 461-468.

(40) Oxford Diffraction Ltd. (2007) *CrysAlis^{Pro} software system*, Oxford, UK.

(41) Altomare, A., Burla, M. C., Camalli, M., Cascarano, G. L., Giacovazzo, C., Guagliardi, A., Moliterni, A. G. G., Polidori, G., and Spagna, R. (1999) SIR97: a new tool for crystal structure determination and refinement. *J. Appl. Crystallogr. 32*, 115-119.

(42) Sheldrick, G. M. (2008) A short history of SHELX. *Acta Crystallogr., Sect. A: Found. Crystallogr. 64*, 112-122.

(43) Spek, A. L. (2003) Single-crystal structure validation with the program PLATON. *J. Appl. Crystallogr. 36*, 7-13.