Synthesis and DNA-binding affinity studies of glycosylated intercalators designed as functional mimics of the anthracycline antibiotics†

Wei Shi,[a] Robert S. Coleman[b] and Todd L. Lowary*[a]

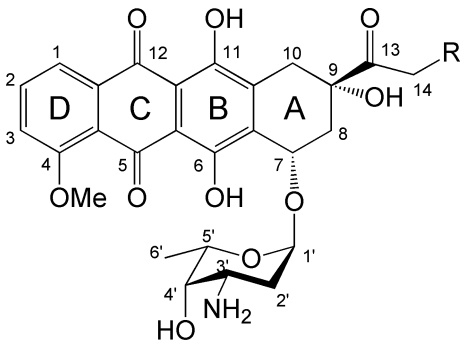
Received 8th May 2009, Accepted 12th June 2009
First published as an Advance Article on the web 17th July 2009
DOI: 10.1039/b909153j

Anthracycline antibiotics such as daunomycin (Dauno) and doxorubicin (Dox) are well-known clinically used cancer chemotherapeutics, which, among other mechanisms, bind to DNA, thereby triggering a cascade of biological responses leading to cell death. However, anthracyclines are cardiotoxic, and drug resistance develops rapidly, thus limiting their clinical use. We report here the synthesis and DNA-binding affinity of a novel class of functional anthracycline mimetics consisting of an aromatic moiety linked to a carbohydrate (**1–12**). In the targets, the aromatic core consists of a 2-phenylbenzo[*b*]furan-3-yl, 2-phenylbenzo[*b*]thiophen-3-yl, 1-tosyl-2-phenylindol-3-yl, or 2-phenylindol-3-yl group that is bound to one of three aminosugars (daunosamine, acosamine, or 4-amino-2,3,4,6-tetradeoxy-α-L-hexopyranoside) *via* a propargyl linker. The DNA binding affinity of these twelve compounds has been evaluated by using both direct and indirect fluorescence measurements. Compared to Dauno and Dox, the DNA binding affinity of these analogues is weaker. However, both aromatic and aminosugar motifs are critical to DNA binding, with more influence coming from the structural features of the aromatic portion.

Introduction

Deoxyribonucleic acid, DNA, is the most fundamental building block of life. Any error within the total DNA content of a cell may affect its proper functioning, thus potentially leading to cell death. By taking advantage of the uncontrolled proliferation of cancer cells, drugs that bind to DNA and inhibit DNA-processing enzymes have long been recognized as effective cancer chemotherapeutic agents.[1] There are two classes of DNA-binding drugs: covalent and non-covalent. The latter group can be further divided into two categories: intercalating agents and minor groove binding agents.[2]

Anthracycline antibiotics are an important class of DNA-intercalating drugs, and rank among the most effective anticancer agents.[3] Among the most well-known anthracycline antibiotics are the naturally occurring compounds daunorubicin (Dauno) and doxorubicin (Dox) (Chart 1).[4,5] Following the discovery of the pharmaceutical potential of Dauno and Dox, much research has been directed towards identifying analogues with better clinical efficacy. To date, hundreds, if not thousands, of analogues of these two natural products have been synthesized.[6] However, only a few have been approved for cancer treatment: idarubicin, valrubicin, epirubicin, pirarubicin, and aclarubicin.[7]



Daunorubicin: R = H
Doxorubicin: R = OH

Chart 1 Structures of daunorubicin (Dauno) and doxorubicin (Dox). The anthraquinone domain is composed of rings B, C, and D.

The mechanism by which the anthracylines act has been suggested to be concentration dependent.[8] At the typical concentrations present after the administration of these compounds, DNA binding is considered to be a key event that triggers cell death.[8] Crystal structures of daunorubicin with DNA have established that these species bind to DNA through intercalation of the anthraquinone moiety between adjacent nucleoside base pairs, while the carbohydrate residue and the saturated cyclohexane ring (ring A) occupy the minor groove.[9]

Although anthracycline antibiotics are widely used in cancer therapy, two major problems exist: drug resistance,[10] and irreversible cardiotoxicity.[11] Although drug resistance could be alleviated by the identification of new anthracycline analogues,[6] the cardiotoxicity, which is associated with the anthraquinone

[a]*Alberta Ingenuity Centre for Carbohydrate Science and Department of Chemistry, The University of Alberta, Gunning-Lemieux Chemistry Centre, Edmonton, AB T6G 2G2, Canada. E-mail: tlowary@ualberta.ca*
[b]*Department of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA*

† Electronic supplementary information (ESI) available: Synthesis of compounds **15–16**, **18–27**, **29**, **30**, **33–37**, **40** and **42–47**, ^{1}H and ^{13}C NMR spectra for new compounds or known compounds synthesized by a new approach (**1–12**, **16**, **20**, **21**, **24–27**, **29**, **30**, **33–37**, **40** and **42–47**), typical HPLC conditions for the purity analysis (examples of **1** and **5**), and one sample figure from the FID(EtBr) asays of compounds **1–4**. See DOI: 10.1039/b909153j

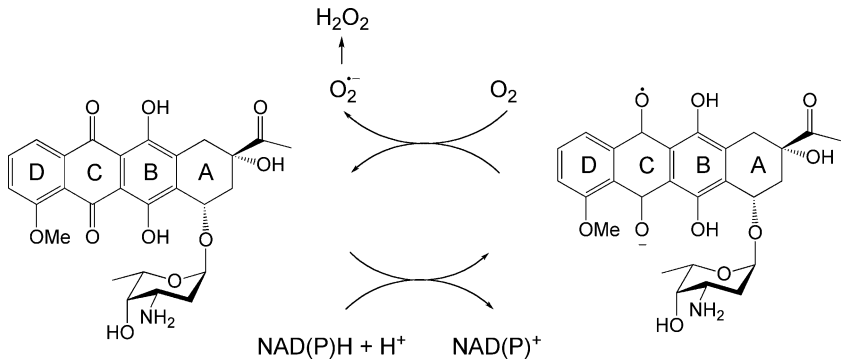


Chart 2 Generation of reactive oxygen species from daunorubicin.

core, remains a significant problem. The mechanism underlying anthracycline-induced cardiotoxicity is complex, but it is believed that the major cause is the single-electron reduction of ring C (Chart 2) leading to the generation of reactive oxygen species that damage the heart.[8,12] There is no specific treatment for anthracycline-related cardiotoxicity. Thus, new strategies that afford a good therapeutic response with minimal cardiotoxicity are of interest.[8]

Among the strategies used for preparing synthetic anthracycline analogs, a diversity-oriented synthesis approach is popular.[6] In this approach a limited number of functional groups at certain loci are modified without changing the basic structural framework of the drug. An alternate strategy, function-oriented synthesis,[13] is based on the preparation of analogs in which the function of each structural moiety in a given molecule are mimicked. In targeting analogs of Dox and Duano for synthesis, we employed this second approach. Based on available crystal structures of anthracycline–DNA complexes, we designed compounds including two structural components: a DNA intercalating moiety and a minor-groove binding moiety.

Because of the vast number of carbohydrate-modified anthracyclines that have been synthesized,[6] our efforts were focused on novel aglycones. Thus, we chose to replace the tetracyclic anthracycline core with relatively simple intercalating moieties. This design feature has two advantages. First, these aromatic structures are more accessible than complex anthracycline aglycones.[14] Moreover, avoiding cardiotoxicity with compounds containing an anthraquinone or anthraquinone-like portion would be unlikely.[8] It should be noted that analogs of Dauno and Dox containing a truncated anthraquinone core have been prepared;[12,15] however, the cytotoxicity of these simplified analogues is lower than the clinically-used drugs.[12]

Earlier studies by Denny, *et al.* showed that aromatic molecules such as 2-phenylquinolines possess antitumor activity due to their ability to bind to DNA *via* "minimal intercalation."[16] It was hypothesized that the use of minimal intercalators would increase the effective drug concentration at a remote tumor site because the loss of the drug due to strong DNA binding during delivery is decreased.[16] More recent work has shown that conjugation of a carbohydrate moiety to simple intercalators *via* a linker functionality improves the binding affinity of these compounds for DNA.[17] Mindful of these considerations, we envisioned conjugating carbohydrates to a flat, aromatic intercalating system through a simple linker (Chart 3).

The propargyl group was selected as the linker because the hydroxyl group and the terminal alkyne can be used to connect carbohydrates and intercalators in a straightforward manner by glycosylation and Sonogashira coupling, respectively. In addition, the alkyne can be further modified to provide additional analogs through, for example, hydrogenation to an alkane or the formation of triazoles through an azide–alkyne [2 + 3] cycloaddition.[18]

As a starting point, we chose to incorporate aminosugars found in the clinically used anthracycline drugs (daunosamine in daunorubicin and doxorubicin; acosamine in epirubicin) as the minor-groove binding carbohydrate domain (**1–4**, and **5–8**, respectively, Chart 3). In addition, because 4-amino-2,3,4,6-tetradeoxy-α-L-hexopyranosides are found in anthracycline analogues that exhibit unique biological properties,[19] we selected 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (4-*N*-TDTH, **9–12**) as the third carbohydrate moiety.

Results and discussion

Design of synthetic route

We envisioned two strategies that could be used to synthesize targets **1–12**. The first (Scheme 1, route A) would involve the initial coupling of propargyl alcohol with iodinated aromatic moieties *via* Sonogashira coupling and subsequent reaction with an appropriate glycosyl donor. The second strategy (Scheme 1, route B) would be to glycosylate propargyl alcohol first, followed by Sonogashira coupling of the terminal alkyne in the product with iodinated precursors of aromatic intercalators. Both of these routes were investigated, but route B is the preferred method because of its higher degree of convergency (see below).

Synthesis of iodinated aromatic moieties

Starting from 2-iodophenol and 2-(methylthio)aniline, 3-iodo-2-phenyl[*b*]benzofuran (**13**)[20] and 3-iodo-2-phenyl[*b*]benzothiophene (**14**)[21] (Scheme 1), were synthesized in high yields based on literature-reported synthetic methodologies. The third required aromatic precursor was 3-iodo-2-phenylindole (**16**), which has been previously synthesized from 2-phenylindole upon treatment with iodine and potassium hydroxide.[22] In our hands, although **16** could be detected in the reaction mixture by TLC, following column chromatography only starting material (2-phenylindole) was recovered. The same result was obtained using *N*-iodosuccinimide

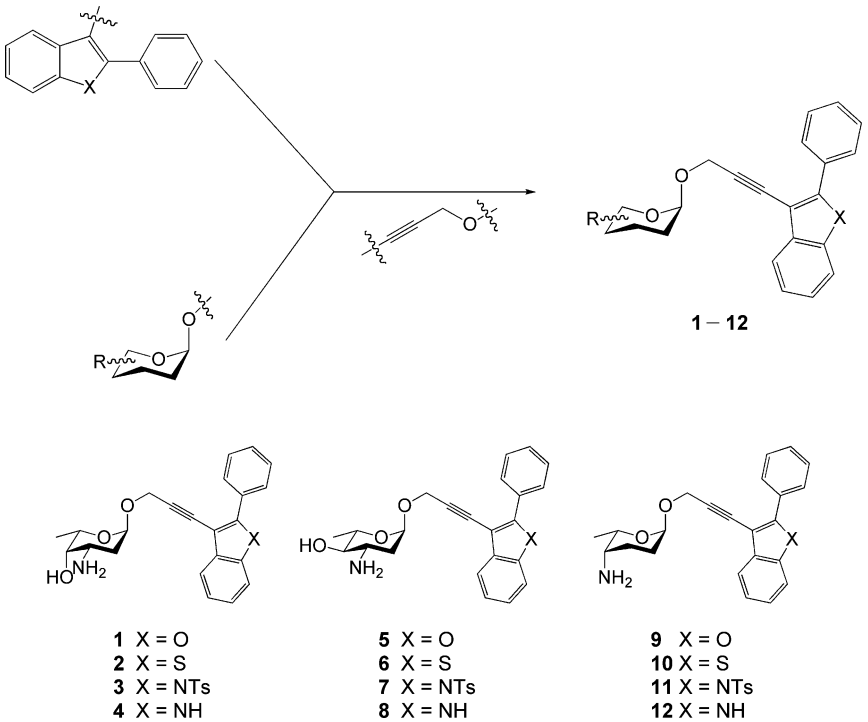
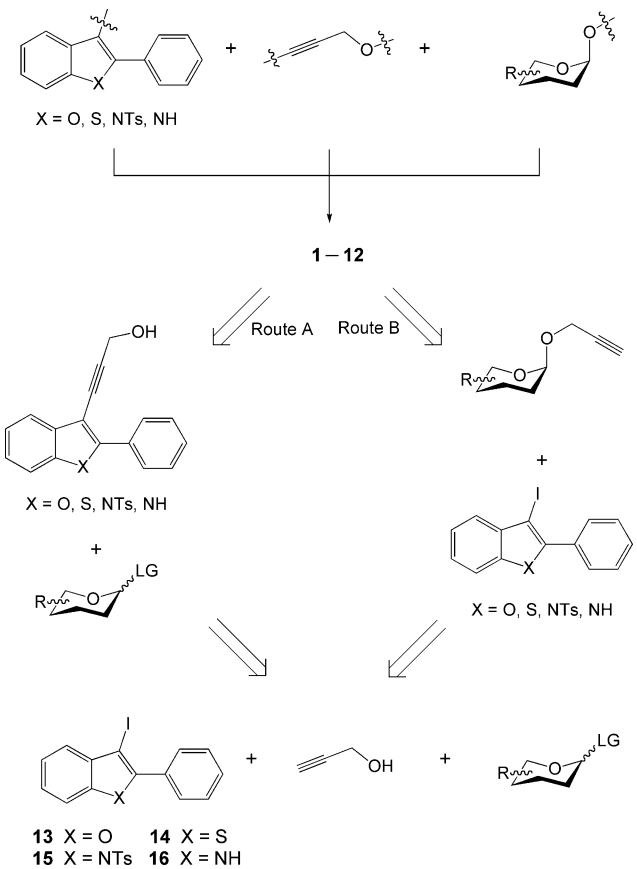


Chart 3 Designed intercalator and carbohydrate conjugate system.



Scheme 1 Retrosynthetic analysis of **1–12**.

(NIS). Thus, another route to **16** was investigated (Scheme 2). Starting from 2-iodoaniline (**17**), Sonogashira coupling with phenylacetylene was followed by tosylation of the nitrogen to give **19** in 91% yield over the two steps.[23] This compound was cyclized upon treatment with iodine and potassium carbonate in acetonitrile to afford the protected indole derivative **15**.[24] Removal of tosylate protecting group was achieved in 86% yield by heating a solution of **16** at 60–70 °C in THF in the presence of tetra-*n*-butylammonium fluoride. In the absence of iodine or NIS, compound **16** can be purified by column chromatography and is sufficiently stable for storage.

Synthesis of aromatic intercalator control compounds

To identify the contribution made by each structural component (the aromatic intercalating moiety, carbohydrate, and the propargyl linker), it was necessary to synthesize the appropriate control compounds consisting solely of these structural domains. Thus, compounds containing only the intercalating moiety, or those that contain both intercalating moieties and the propargyl linker, were synthesized (**20**–**27**, Chart 4).

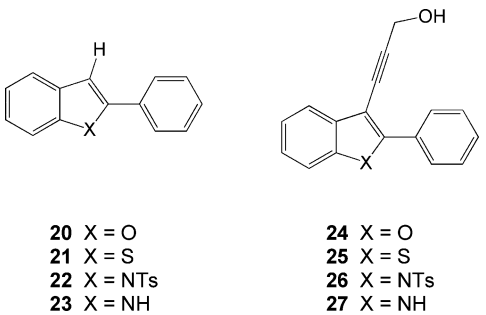
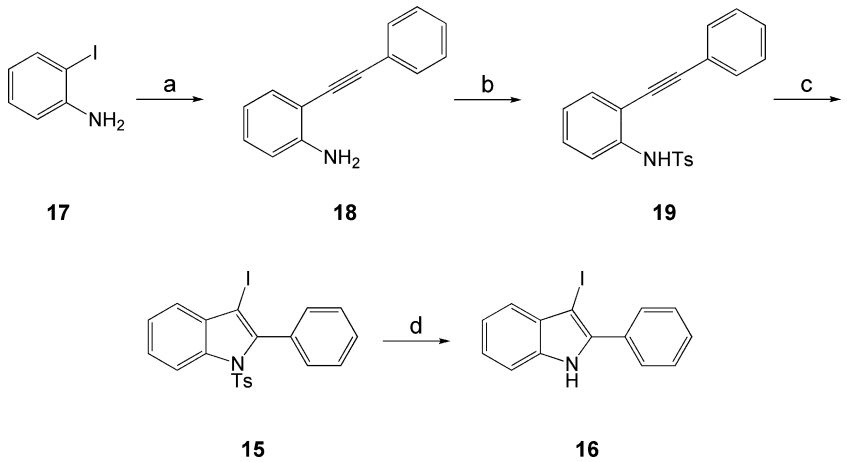


Chart 4 Structures of control compounds containing aromatic domains.

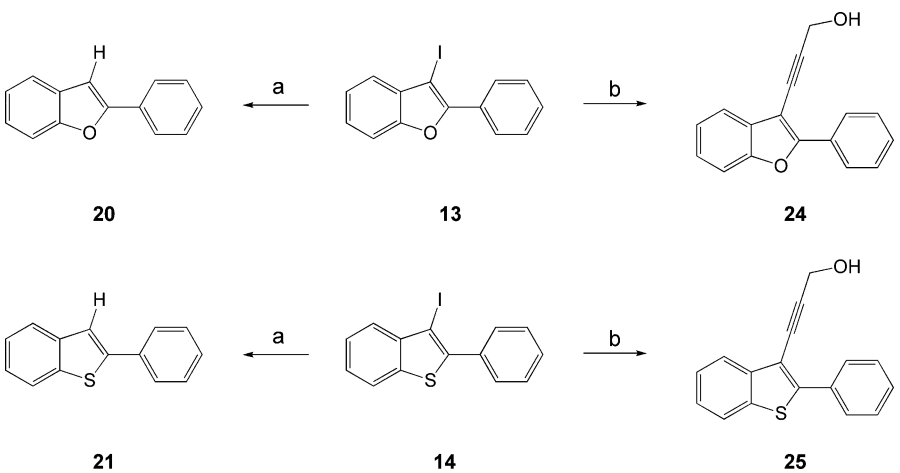


Scheme 2 Reagents and conditions: (a) Phenylacetylene, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 95%; (b) TsCl, Pyridine, THF, 96%; (c) I_2, K_2CO_3, CH_3CN, 98%; (d) *n*-Bu_4NF, THF, 60–70 °C, 86%.

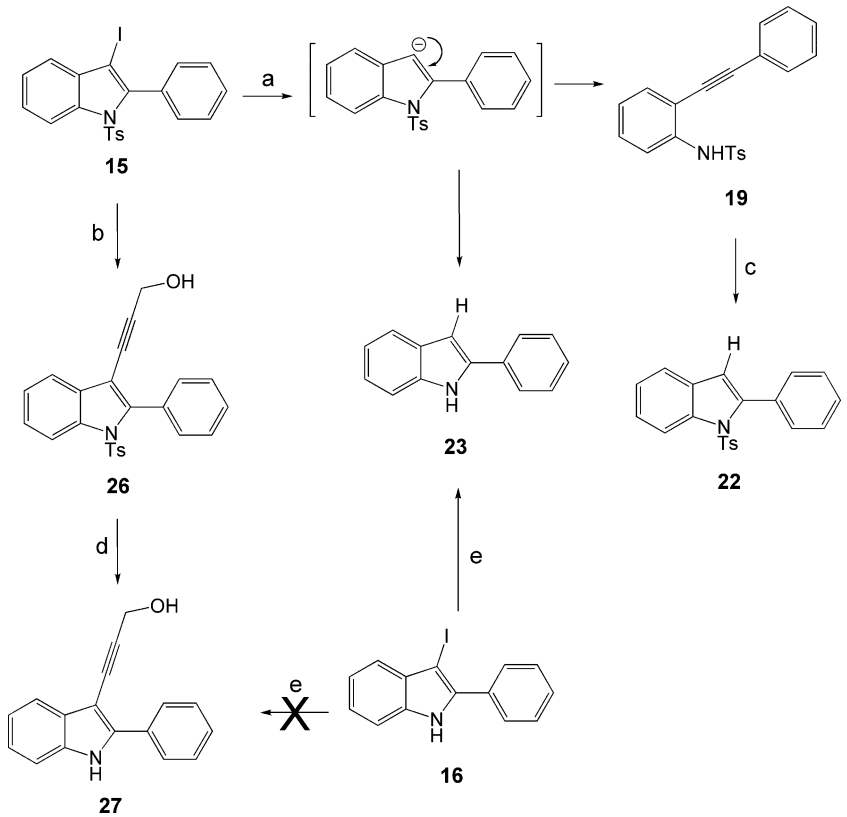
The preparation of **20**, **21**, **24** and **25** is shown in Scheme 3. 2-Phenyl[*b*]benzofuran (**20**) was synthesized from 3-iodo-2-phenyl[*b*]benzofuran (**13**) in 86% yield by lithium–halogen exchange, followed by protonation with ammonium chloride.[25,26] Similar treatment of the corresponding benzothiophene derivative **14**, gave **21** in a comparable (85%) yield. Compounds **24** and **25** were synthesized from **13** and **14**, respectively, in high yields using standard Sonogashira coupling with propargyl alcohol (Scheme 3).

Use of the same general approaches for preparing **22**, **23**, **26** and **27**, led to problems (Scheme 4). For example, treatment of **15** with *n*-butyllithium yielded none of the desired 1-tosyl-2-phenylindole (**22**). Instead, the major product, isolated in 74% yield, was 2-phenylindole (**23**); a minor product was alkyne **19**, which was produced in 20% yield. The latter compound is undoubtedly formed by β-elimination of the organolithium species derived from **15**, while the strongly basic conditions of the reaction appear to promote the deprotection of the tosyl group, thus leading to **23**. Therefore, while this approach gave, unexpectedly, one the target compounds (**23**), an alternative approach was needed for access to 1-tosyl-2-phenylindole, **22**. Fortunately, it was possible to synthesize **22** in modest 52% yield by heating a 1,2-dichloroethane solution of **19** at reflux with 20 mol% copper (II) triflate.[23b]

Difficulties were also encountered in the coupling of **15** to propargyl alcohol. The conditions used in the preparation of **24** and **25** resulted in no reaction; only unreacted **15** was isolated. However, when the reaction mixture was heated at 100 °C in a microwave reactor, the desired product **26** was formed, albeit in modest (52%) yield. Also unexpected was that attempted coupling of 3-iodo-2-phenylindole (**16**) with propargyl alcohol afforded none of **27**, but instead 2-phenylindole (**23**) was isolated as the only product, in 77% yield. Protection of indole nitrogen thus appears necessary to favor Sonogashira coupling over protodemetallation of the organopalladium species derived from **16**. Compound **27** could, however, be synthesized in 45% yield from **26** by treatment with tetra-*n*-butylammonium fluoride as described above for the synthesis of **16** from **15** (Scheme 2). It was also found that compound **27** slowly decomposed during column purification, even using base-deactivated silica gel at the adsorbent.



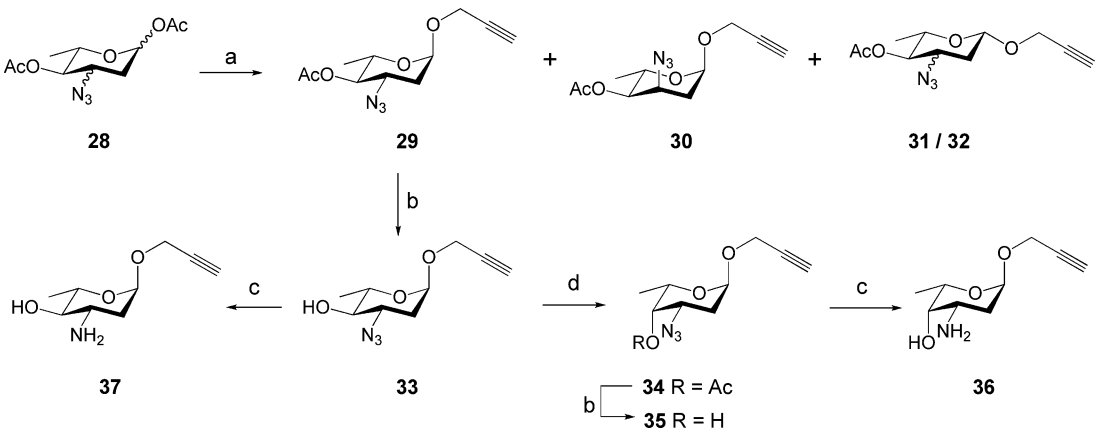
Scheme 3 Reagents and conditions: (a) 1. *n*-BuLi, THF, –78 °C; 2. aq. NH_4Cl, 86% for **20**, 85% for **21**; (b) Propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 73% for **24**, 81% for **25**.



Scheme 4 Reagents and conditions: (a) 1. *n*-BuLi, THF, −78 °C; 2. aq. NH_4Cl, 20% for **19**, 74% for **23**; (b) Propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, piperidine, 100 °C (microwave), 2 h, 52%; (c) $Cu(OTf)_2$, 1,2-dichloroethane, reflux, 52%; (d) *n*-Bu_4NF, THF, 60–70 °C, 45%; (e) Propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, piperidine, 77% for **23**.

Synthesis of propargyl daunosamine and acosamine glycosides

Having a route in place to the aromatic domains, we turned our attention to the preparation of the carbohydrate moieties. The required building blocks, **36** and **37**, were synthesized from a previously reported precursor, **28** (Scheme 5).[27] Compound **28**, prepared as a mixture of four stereoisomers, was converted to propargyl glycosides **29–32** in 84% combined yield. The two α stereoisomers, **29** and **30**, could be obtained as pure compounds by column chromatography in 55% and 5% yield, respectively. However, the two β stereoisomers, **31** and **32**, were inseparable and were obtained as a mixture in 24% yield. Assignment of the stereochemistry at C-1 and C-3 in **29–32** was straightforward based on the multiplicity of the resonances arising from H-1 and H-3. For both α-anomers **29** and **30**, no large coupling was seen for the resonance of the equatorial H-1 with $^3J_{1,2a}$ ~ 3–4 Hz and $^3J_{1,2e}$ ~ 1–2 Hz (a = axial, e = equatorial). For the axially disposed H-3 in **29**, its resonance in the ^{1}H NMR spectrum appeared as a doublet of



Scheme 5 Reagents and conditions: (a) Propargyl alcohol, $BF_3{\cdot}Et_2O$, 84% for **29–32**; (b) K_2CO_3, methanol, 99% for **33**, 94% for **35**; (c) PPh_3, H_2O, THF, 60 °C, 97% for **37**, 89% for **36**; (d) 1. Tf_2O, pyridine; 2. *n*-Bu_4NOAc, CH_3CN, 86%.

doublet of doublets with $^3J_{2a,3}$ ~ 12.4 Hz, $^3J_{3,4}$ ~ 9.8 Hz, and $^3J_{2e,3}$ ~ 5.0 Hz. On the other hand, the resonance of the equatorially oriented H-3 in **30** appeared as an apparent quartet because the coupling constants for $^3J_{3,4}$, $^3J_{2a,3}$, and $^3J_{2e,3}$ are almost identical (~ 3.6 Hz) (see ESI† for details). Although the synthesis of the desired product, **29**, *via* this route is not high yielding, the preparation of **28** is simple and efficient, and the conversion of **28** to **29** proceeds in reasonable yield.

Glycoside **29** could then be efficiently converted into **33** upon reaction with K_2CO_3 in methanol. The reduction of the azide group in **33** using triphenylphosphine–water afforded the propargyl acosaminoside, **37**, in 96% overall yield from **29**. The daunosamine isomer of **37**, glycoside **36**, was obtained from **33** by inversion of the stereochemistry at C-4 through formation of the triflate ester, and displacement with acetate. Deacetylation, followed by the reduction of the azide under the conditions used for the synthesis of **37**, afforded the propargyl glycoside of daunosamine, **36**. Inversion of stereochemistry at C-4 in **33** could also be accomplished *via* a two-step approach involving triflate ester formation and reaction with sodium nitrite or tetra-*n*-butylammonium nitrite.[28] However, the three-step process in Scheme 5 gave a better yield (81%) than the two-step process (45%).

Synthesis of 2-propargyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (4-*N*-TDTH)

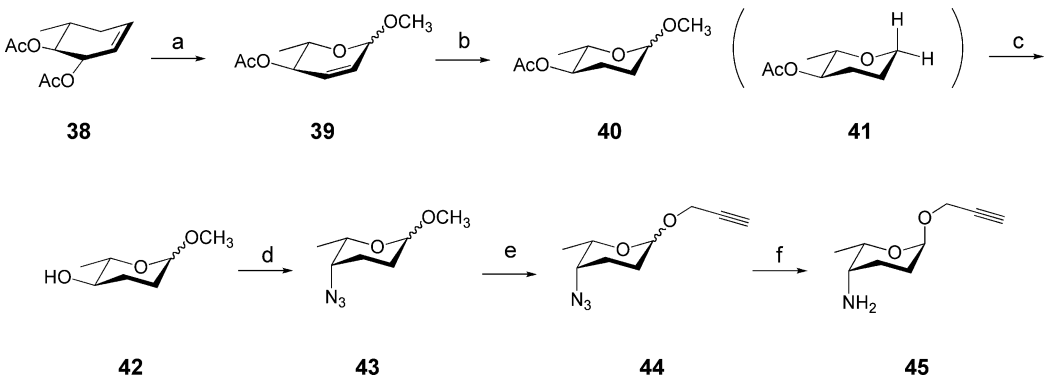
The preparation of the propargyl 4-*N*-TDTH glycoside started from glycal **38** (Scheme 6). A Ferrier I-rearrangement[29] of this alkene in the presence of $SnCl_4$ and methanol afforded a 90% yield of compound **39** as a mixture of α and β glycosides in an approximately 9:1 ratio.[30] The reduction of the alkene **39** to alkane **40** was surprisingly difficult. When 5% palladium on charcoal was used as the hydrogenation catalyst, we observed a significant amount of byproduct **41** in which the anomeric methoxy group was replaced with a hydrogen atom. Although the formation of this byproduct could be avoided by using Wilkinson's catalyst[31] or using diimide reagents[32] as means of hydrogenation, the reaction rate was significantly slower (3–5 days). Finally, it was found that by using ethyl acetate as a solvent, 10% palladium on charcoal gave the highest yield of **40**, with a slight contamination of the byproduct **41**.

With **40** in hand, the deprotection of the acetyl group with potassium carbonate in methanol afforded **42** in 91% yield. Introduction of the azido group with inverted stereochemistry was achieved by mesylate ester formation followed by displacement with sodium azide, affording compound **43** in 76% overall yield. Subsequently, the glycosylation reaction was performed before the reduction of the azido group, which facilitates anomer separation. Glycoside **44**α was then subjected to a Staudinger reaction, which afforded the desired product **45** in 85% yield.

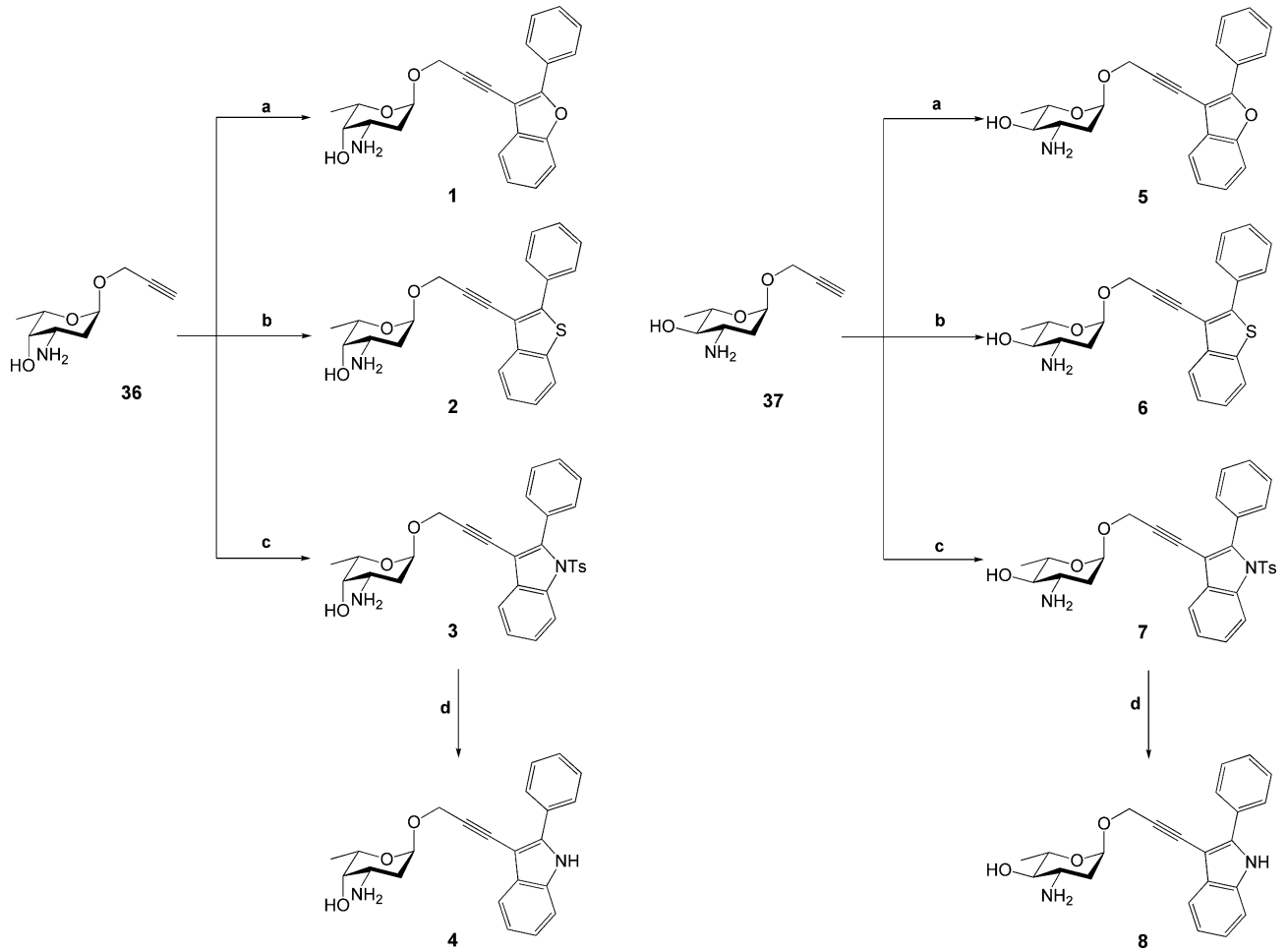
Synthesis of amino analogues 1–8: Coupling of propargyl daunosamine and acosamine glycosides with iodinated aromatics

As mentioned previously, we envisioned two possible synthetic routes for the preparation of final compounds **1–8** (Scheme 1). However, there are two drawbacks associated with route A. First, because **28** is a mixture of four inseparable stereoisomers, it cannot be used as a donor directly, unless the stereoisomeric products would be separable after coupling. Second, the use of route A would require the synthesis of phenylindole derivatives **26** and **27**, which, as outlined in Scheme 4, was difficult. Therefore route B was adopted for the synthesis of **1–8**.

As shown in Scheme 7, coupling of benzofuran **13** with the terminal alkynes proceeds efficiently, requiring only 5 mol% of palladium catalyst. Slightly increased amounts of catalyst (7 mol%) were needed to achieve a comparable transformation for benzothiophene **14**. Because the reaction time for benzothiophene **14** was longer than that for benzofuran **13**, the load of copper(I) co-catalyst was decreased to avoid homocoupling of the alkyne, a common by-product in Sonogashira couplings.[33] The reaction was most difficult with indole **15**, which required 10 mol% of the catalyst and less copper(I) iodide, compared to the reaction with **13**. The use of the amine solvents most commonly employed in Sonagashira couplings (*e.g.*, triethylamine) led to poor product yields. After screening a number of solvents, piperidine was found to give the best results, affording the products in 61–74% yields. Although *N*-tosylated indoles **3** and **7** were not initially among the desired structures, because they were easily available along the synthetic pathway, their DNA-binding abilities were evaluated. Finally, the protected indoles **4** and **8** were synthesized in 89 and 91% yield, respectively, by reaction of **3** and **7** with tetra-*n*-butylammonium fluoride.



Scheme 6 Reagents and conditions: (a) $SnCl_4$, MeOH, 81% for **39**α and 9% for**39**β; (b) H_2, 10% Pd/C, ethyl acetate, 90%; (c) K_2CO_3, methanol, 91%; (d) 1). MsCl, Et_3N, 0 °C; 2) NaN_3, DMF, 110 °C, 76% over two steps; (e) Propargyl alcohol, $BF_3{\cdot}Et_2O$, –40 °C, 69% for **44**α and 20% for **44**β; (e) PPh_3, H_2O, THF, 60 °C, 85%.



Scheme 7 Reagents and conditions: (a) **13**, 5% $Pd(PPh_3)_2Cl_2$, 10% CuI, 74% for **1**, 77% for **5**; (b) **14**, 7% $Pd(PPh_3)_2Cl_2$, 5% CuI, 67% for **2**, 71% for **6**; (c) **15**, 10% $Pd(PPh_3)_2Cl_2$, 5% CuI, 61% for **3**, 74% for **7**; (d) *n*-Bu_4NF, 89% for **4**, 94% for **8**.

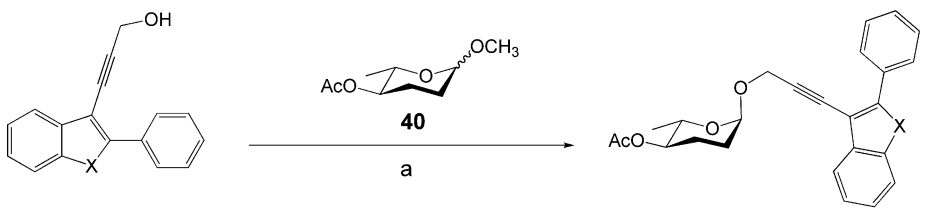
Synthesis of amino analogues 9–12

Unlike **28**, which has four stereoisomers, compound **40** could be obtained in the isomeric form at the anomeric position only (Scheme 6). Therefore, we carried out trial reactions for coupling **24** and **25** with methyl glycoside **40** under the catalysis of several different Lewis acids. Boron trifluoride diethyl etherate gave the best results (Scheme 8); however, the yields were generally low, less than 50%.

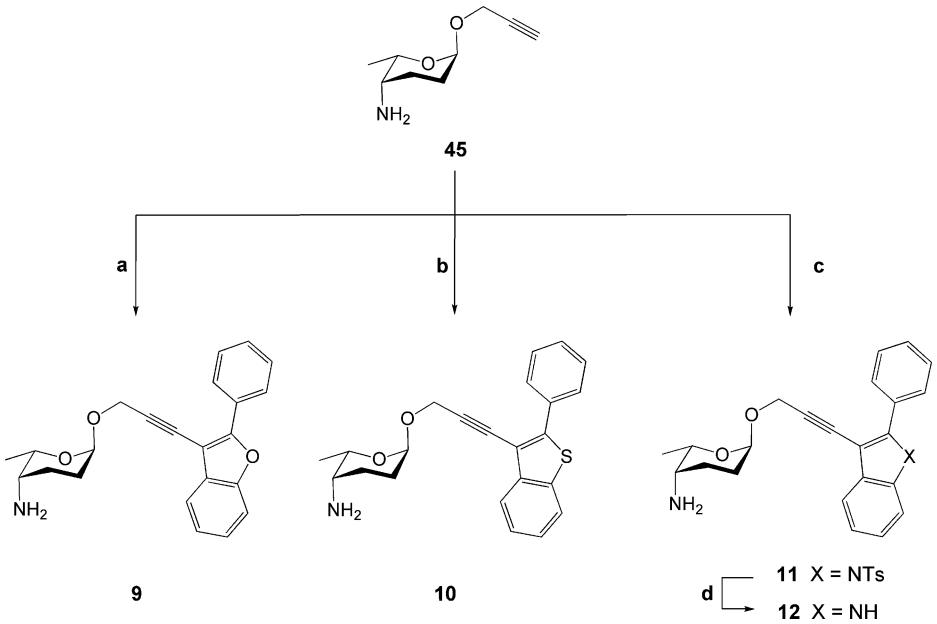
Therefore, the preparation of **9–12** adopted the same route as for the synthesis of **1–8**, in which propargyl glycosides were synthesized first, followed by Sonogashira coupling with aromatic iodides (Scheme 9). This reaction worked well with **13** and **14**; however, we were unable to couple the propargyl amino glycoside **45** with the tosyl protected indole iodide **15** with acceptable efficiency. The yield of **11** was less than 5% under the optimal conditions used for the synthesis of **3** and **7**. The application of microwave heating slightly improve the yield to around 15–20%, but the decomposition of the catalyst under these conditions impeded further improvement.

Fluorescence DNA binding studies

With sufficient quantities of **1–12** available, their DNA binding ability was examined. Due to the significance of DNA binding in drug design, a number of approaches have been developed to



Scheme 8 Reagents and conditions: (a) alcohol, $BF_3{\cdot}Et_2O$, –40→0 °C, 49% for **46** and 43% for **47**.



Scheme 9 Reagents and conditions: (a) **13**, 5% $Pd(PPh_3)_2Cl_2$, 10% CuI, 85%; (b) **14**, 7% $Pd(PPh_3)_2Cl_2$, 5% CuI, 76%; (c) **15**, 10% $Pd(PPh_3)_2Cl_2$, 5% CuI, 100 °C (microwave), 2 h, 17%; (d) *n*-Bu_4NF, 75%.

probe the interactions between DNA and small organic ligands, including, calorimetry,[34] NMR spectroscopy,[35] viscometry,[36] and fluorescence.[37] Due to the relative low water solubility of our analogues, we were interested in sensitive assays that could measure DNA binding affinity at concentrations in the micromolar range. Therefore, fluorescence measurements were deemed most appropriate.

The change in fluorescence of a compound upon binding to DNA can be achieved in two different ways: direct and indirect. The direct assay measures the changes in the fluorescence of the ligand of interest after the addition of that ligand into a DNA solution.[36a,37a–d] The indirect method, the fluorescent intercalator displacement (FID) assay, involves a second fluorescent molecule that is a standard DNA intercalator such as ethidium bromide (EtBr).[37d–f] Instead of monitoring the fluorescence of the ligand being tested, the fluorescence of the standard intercalator is monitored before and after the addition of the ligand.

Direct fluorescence measurements

A one point direct fluorescence titration is an efficient way to detect the binding of a fluorescent ligand to DNA.[36a,37a,b] Figure 1 shows the change of the emission curves of compounds **1–4** at 50 μM upon the addition of sonicated herring sperm DNA (hsDNA) at 20 μg/mL. After the addition of DNA, a fluorescence quenching effect of different amplitudes was observed for all four compounds (Figure 1–B). Similar results were also obtained for compounds **5–12**.

Although it is well known that for EtBr, the intensity of its fluorescence increases upon the addition of DNA,[37a] fluorescence quenching is also common due to either spectral overlap with other fluorophores in the system being tested, or electron transfer from the activated ligand to guanine nucleotides.[38] Due to the lack of spectral overlap between the excited ligand and DNA nucleotides, the only other fluorophores in our system, we believe that fluorescence quenching is most likely due to electron transfer between guanine nucleotides and the photoactivated state of compounds **1–12**. The changes in the emission curves illustrated in Figure 1 demonstrated the ability of **1–12** to bind to DNA, but no more information could be obtained, given the different flourophores in the compounds. To evaluate the DNA binding affinity between different compounds, the FID assay was used.

Fluorescent intercalator displacement (FID) assay

Although the single point direct titration cannot definitively tell the differences of the binding affinity of different ligands, the single point FID titration is a well-accepted method for ranking DNA binding affinity of different ligands using EtBr as a 'ruler'.[39] Multipoint titration, on the other hand, is extensively used to semi-quantitate DNA binding affinity by calculating relative associate constants.[40]

From single point FID titration, the percentage of remaining EtBr could be calculated and was used to determine the relative affinity of **1–12** for DNA (Table 1). Based on the data, we can rank the binding affinity of **1–4** as **3** > **1** > **2** > **4**. When obtaining an overall ranking for **1–12**, some interesting trends emerged. The ranking appears to be determined mainly by the aromatic moiety, and the affinity decreases in the order of *N*-tosyl protected indole > benzo[*b*]furan > benzo[*b*]thiophene ≥ free indole. The carbohydrate moiety, on the other hand, has less influence on the binding affinity. It is of interest to note that although there is a bulky substituent present in the *N*-tosyl protected indole core, it demonstrated the strongest binding. We propose that results from the positioning of the *N*-tosyl group in the minor or major groove, therefore providing additional interactions with the groove walls.

To elucidate possible contribution from each structural moiety (intercalator, minor groove binder, and the linker) to the overall binding affinity of the molecule, we assayed compounds

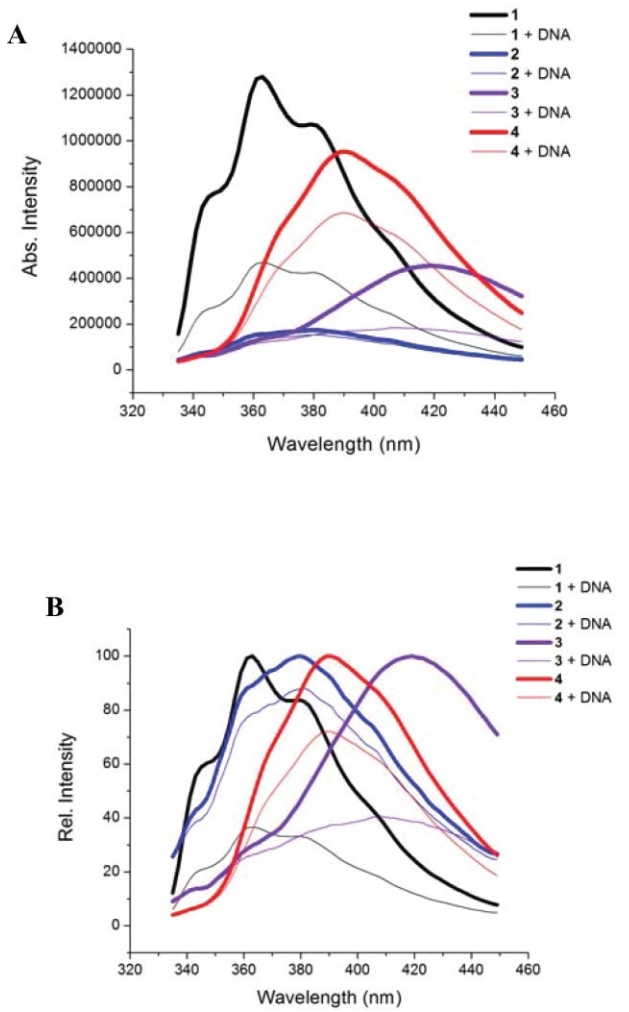


Fig. 1 Emission spectra of **1–4** in the absence and presence of DNA: 50 μM of **1** (black), **2** (blue), **3** (purple), and **4** (red) in the absence (bold line) and presence (thin line) of 20 μg/mL of hsDNA showing (A) absolute intensity and (B) normalized intensity based on the emission maxima from A. Emission (*y*-axis) was measured as a function of emission wavelength (*x*-axis) upon exciting each compound at its excitation maximum.

20–23 (containing only the aromatic intercalating moiety), **24–26** (composed of the intercalating moiety and the propargyl linker), and **36**, **37** and **45** (possessing the carbohydrate moiety and the linker). None showed detectable binding. Thus, there is necessarily a cooperative effect between these three components to achieve the observed binding affinity. Based on the proposed binding model from simulation studies for anthracycline drugs,[41] it is possible that the carbohydrate moiety introduces the molecule to DNA to form a minor groove-bound state. After this, a binding equilibrium is established by the interaction between DNA and the aromatic moiety.

By multipoint titration, the relative binding constant for a ligand can be calculated based on the equation described in the experimental section.[40] Based on the data provided in Table 1, the DNA binding affinity of **3** and **7** is ~20-fold less than EtBr. For compound **11**, which exhibited the highest relative DNA binding affinity, an FID titration was carried out (data not shown). After linear fitting, the concentration of **11** at which there is 50% loss of EtBr fluorescence is about 75 μM; therefore, the DNA binding affinity of **11** is approximately 15-fold less than EtBr. Because Dauno and Dox have similar DNA binding affinity compared to EtBr,[42] the DNA binding affinity of our analogues is ~15-fold weaker compared to these two clinical anthracycline drugs, a respectable binding affinity given the simplicity of our systems.

Table 1 Percentage of remaining EtBr in FID assays of **1–12**, **20–26**, **36**, **37**, and **45**

Compound	Structural domains	% Remaining EtBr
1	Intercalator-linker-carbohydrate	62.0 ± 1.9
2		67.5 ± 2.1
3		47.9 ± 2.7
4		76.7 ± 0.7
5		50.5 ± 4.1
6		73.6 ± 1.1
7		49.7 ± 4.9
8		76.5 ± 2.0
9		68.5 ± 1.9
10		79.9 ± 1.0
11		38.6 ± 1.9
12		78.4 ± 1.9
20	Intercalator	98.6 ± 3.3
21		96.5 ± 4.0
22		100.5 ± 2.5
23		96.0 ± 2.2
24	Intercalator-linker	107.9 ± 0.6
25		95.0 ± 2.7
26		110.7 ± 1.3
36	Carbohydrate-linker	100.0 ± 3.9
37		90.5 ± 1.0
45		93.3 ± 1.6

Conclusions

A panel of novel daunosamine and acosamine-containing glycoconjugates (**1–12**) have been synthesized. These compounds were designed as functional mimics of natural anthracycline antibiotics, and contain three functional subunits: a planar aromatic system as the intercalating moiety, a carbohydrate as the minor/major groove binding moiety, and a propargyl spacer that links these two groups. The DNA binding affinity of all compounds has been evaluated and confirmed using direct and indirect fluorescent assays. Although the aromatic moiety has been identified as the key structural feature affecting the DNA binding affinity, an aminosugar appendage is also indispensible, but structural changes in this group appear to have a weak effect on binding. Further studies of the binding mode and more biological evaluation are currently in progress.

Experimental section

Chemistry

Reactions were carried out in oven dried glassware. All reagents were purchased from commercial sources and were used without further purification unless noted. Before use, reaction solvents were purified by successive passage through columns of alumina and copper in a PURESOLV-400 System from Innovative Technology Inc. under argon atmosphere. Unless stated otherwise,

all reactions were carried out under a positive pressure of argon and were monitored by TLC on silica gel G-25 UV_{254} (0.25 mm, Macherey-Nagel). Spots were detected under UV light and/or by charring with 10% H_2SO_4 in ethanol, or in acidified ethanolic anisaldehyde/vanillin. Solvents were evaporated under reduced pressure and below 40 °C (water bath). Column chromatography was performed on silica gel 60 (40–60 μM). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). Iatrobeads refers to a beaded silica gel 6RS-8060, which is manufactured by Iatron Laboratories (Tokyo). Optical rotations were measured at 22 ± 2 °C. Melting points are uncorrected. ^{1}H NMR spectra were recorded on VARIAN INOVA-NMR spectrometers at 400, 500 or 600 MHz, and chemical shifts are referenced to either TMS (0.0, $CDCl_3$) or CD_2HOD (4.78, CD_3OD). ^{13}C NMR spectra were recorded at 100 or 125 MHz, and ^{13}C chemical shifts are referenced to $CDCl_3$ (77.23 ppm, CD_3Cl_3) or CD_3OD (49.00 ppm, CD_3OD). ^{1}H data are reported as though they were first order. The errors between the coupling constants for two coupled protons were less than 0.5 Hz, and the average number was reported. Assignment of NMR resonances was done based on ^{1}H–^{1}H COSY, HMQC, and in some cases HMBC experiments. In the interpretation of the NMR data for methylene protons on the carbohydrate ring, 'a' and 'e' refer to axial and equatorial orientation, respectively. In the cases where no clear assignment of these hydrogens could be made based on all NMR data, assignments were made taking into consideration the anisotropy effect of a ring σ bond, which results in equatorial hydrogens resonating more downfield than axial hydrogens.[43] Electrospray mass spectra were recorded on samples suspended in mixtures of THF with CH_3OH and added NaCl. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter at the sodium D line (589 nm). Optical rotations are in units of deg mL(dm·g)$^{-1}$. IR spectra were recorded on the Nicolet Magna 750 FTIR spectrometer. The reported purity values were obtained with a Varian HPLC system, using an evaporative light scattering detector (ELSD) 2000ES from Alltech, and a Varian Microsorb-MV 100–5 C18 column. The eluant consisted of acetonitrile and water, the ratio of which depends on the compound. For all basic amino compounds, 0.1% of trifluoroacetic acid was added to facilitate elution and avoid aggregation. When the purity derived from HPLC analysis is greater than 99%, it is reported as > 99%.

General procedure for the preparation of compounds 1–3, 5–7 and 9–10 *via* Sonogashira coupling

To a degassed solution of sugar alkyne (1.3 mmol), iodinated aromatic compound (1.0 mmol), and $PdCl_2(PPh_3)_2$ (see Scheme 2.8/2.9 for amount) in piperidine (6 mL) was added CuI (see Scheme 2.8/2.9 for amount). The reaction mixture was stirred under Ar at room temperature and followed by TLC. Once complete, the reaction was quenched by the addition of a satd aqueous solution of NH_4Cl (10 mL). The aqueous solution was extracted with Et_2O (3 × 10 mL) and the combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography.

Microwave procedure for the preparation of compound 11

To a solution of piperidine (0.75 mL), DMF (0.25 mL), $PdCl_2(PPh_3)_2$ (11 mg, 20 mol%), **15** (38 mg, 0.079 mmol), and propargyl glycoside **45** (22 mg, 0.13 mmol) was added CuI (1.5 mg, 10 mol%). The mixture was stirred at 100 °C in a microwave reactor for 2 h, the reaction was then quenched by the addition of a satd aqueous NH_4Cl solution, and the resulting solution was extracted with EtOAc. The organic fractions were dried (Na_2SO_4), filtered, and concentrated under vacuum to yield the crude product, which was purified by column chromatography.

General procedure for the preparation of compounds 4, 8, and 12

To a solution of *N*-tosyl protected indole **3**, **7**, or **11** (31 mg, 0.058 mmol) in THF (3 mL) was added tetra-*n*-butylammonium fluoride (TBAF) solution in THF (1.0 M, 0.35 mL, 0.35 mmol) at room temperature, and the mixture was stirred for 24 h. A satd aqueous solution of $NaHCO_3$ (30 mL) was then added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to a residue that was purified by column chromatography.

3-(2-Phenyl-benzo[*b*]furan-3-yl)-prop-2-ynyl 3-amino- 2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (1). Pure **1** was obtained by column chromatography on Iatrobeads (1% Et_3N in 7:1 CH_2Cl_2–CH_3OH) in 74% yield: white foamy solid, R_f 0.39 (1% Et_3N in 7:1 CH_2Cl_2–CH_3OH); IR ν 3319 (N–H), 3048 (O–H), 2010 (C≡C) cm^{-1}; $[\alpha]^{23}_D$–98.9 (*c* 0.3, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 8.22–8.26 (m, 2H, Ar), 7.60–7.63 (m, 1H, Ar), 7.48–7.56 (m, 3H, Ar), 7.41–7.45 (m, 1H, Ar), 7.35–7.39 (m, 1H, Ar), 7.29–7.33 (m, 1H, Ar), 5.27 (br d, 1H, $J_{1,2a}$ = 3.3 Hz, H-1), 4.65 (s, 2H, OC$\underline{H}_2$C≡C), 4.05 (br q, 1H, $J_{5,6}$ = 6.5 Hz, H-5), 3.63–3.69 (m, 2H, H-3, H-4), 2.08 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 12.7 Hz, $J_{1,2a}$ = 3.3 Hz, H-2a), 1.91 (ddd, 1H, $J_{2a,2e}$ = 12.7 Hz, $J_{2e,3}$ = 4.5 Hz, $J_{1,2e}$ = 1.0 Hz, H-2e), 1.22 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 158.0 (Ar), 154.8 (Ar), 131.1 (Ar), 130.9 (Ar), 130.6 (Ar), 129.9 (2, Ar), 126.9 (2, Ar), 126.8 (Ar), 124.8 (Ar), 121.0 (Ar), 112.2 (Ar), 99.4 (Ar), 96.6 (C-1), 94.0 (≡C), 78.5 (≡C), 68.0 (C-4), 67.8 (C-5), 56.2 (OCH_2), 48.6 (C-3), 29.4 (C-2), 16.9 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_4$: 378.1700. Found: 378.1702. Purity: >99%.

3-(2-Phenyl-benzo[*b*]thiophene-3-yl)-prop-2-ynyl 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (2). Pure **2** was obtained by column chromatography (1% Et_3N in 9:1 CH_2Cl_2–CH_3OH) in 67% yield: solid yellow paste, R_f 0.43 (1% Et_3N in 7:1 CH_2Cl_2–CH_3OH); IR ν 3361 (N–H), 3013 (O–H), 2010 (C≡C) cm^{-1}; $[\alpha]^{23}_D$–86.8 (*c* 0.3, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD, δ_H) 7.94–7.97 (m, 2H, Ar), 7.84–7.89 (m, 2H, Ar), 7.38–7.50 (m, 5H, Ar), 5.21 (br d, 1H, $J_{1,2a}$ = 3.7 Hz, H-1), 4.60 (ABq, 1H, J = 16.2 Hz, OC$\underline{H}_2$C≡C), 4.58 (ABq, 1H, J = 16.2 Hz, OC$\underline{H}_2$C≡C), 3.98 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.62–3.68 (m, 2H, H-3, H-4), 2.05 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 12.7 Hz, $J_{1,2a}$ = 3.7 Hz, H-2a), 1.76 (br dd, 1H, $J_{2a,2e}$ = 12.7 Hz, $J_{2e,3}$ = 4.6 Hz, H-2e), 1.18 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 147.9 (Ar), 142.3 (Ar), 138.8 (Ar), 134.9 (Ar), 130.1 (Ar), 129.8 (2, Ar), 129.4 (2, Ar), 126.6 (Ar), 126.2 (Ar), 124.0 (Ar), 123.2 (Ar), 114.1 (Ar), 97.5 (C-1), 92.0 (≡C), 81.0 (≡C), 72.1 (C-5), 68.6 (C-4), 55.7 (OCH_2), 47.7 (C-3), 33.6 (C-2), 17.3 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_3S$: 394.1471. Found: 394.1470. Purity: > 99%.

3-(1-Tosyl-2-phenylindol-3-yl)-prop-2-ynyl 3-amino- 2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside (3). Pure **3** was obtained by column chromatography (1% Et_3N in 10:1 CH_2Cl_2–CH_3OH)

in 61% yield: solid off-white paste, R_f 0.45 (1% Et_3N in 7:1 CH_2Cl_2–CH_3OH); IR: ν 3358 (br, N–H, O–H), 2221 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–73.9 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 8.22–8.26 (m, 1H, Ar), 7.38–7.52 (m, 7H, Ar), 7.29–7.33 (m, 1H, Ar), 7.20–7.24 (m, 2H, Ar), 7.08–7.12 (m, 2H, Ar), 4.97 (br d, 1H, $J_{1,2a}$ = 3.7 Hz, H-1), 4.35 (ABq, 1H, J = 16.2 Hz, $OCH_2C{\equiv}C$), 4.32 (ABq, 1H, J = 16.2 Hz, $OCH_2C{\equiv}C$), 3.82 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.61 (br s, 1H, H-4), 3.53 (ddd, 1H, $J_{2a,3}$ = 12.8 Hz, $J_{2e,3}$ = 4.7 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 2.23 (s, 3H, $PhCH_3$), 1.96 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 12.8 Hz, $J_{1,2a}$ = 3.7 Hz, H-2a), 1.76 (br dd, 1H, $J_{2a,2e}$ = 12.8 Hz, $J_{2e,3}$ = 4.7 Hz, H-2e), 1.18 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 147.0 (Ar), 145.3 (Ar), 138.3 (Ar), 135.6 (Ar), 132.3 (2, Ar), 132.0 (Ar), 131.9 (Ar), 130.6 (2, Ar), 130.3 (Ar), 128.6 (2, Ar), 127.9 (2, Ar), 127.0 (Ar), 126.1 (Ar), 120.9 (Ar), 117.6 (Ar), 108.6 (Ar), 96.2 (C-1), 91.7 (≡C), 78.7 (≡C), 67.8 (C-4/C-5), 67.6 (C-5/C-4), 55.8 (OCH_2), 48.6 (C-3), 29.3 (C-2), 21.6 ($PhCH_3$), 16.9 (C-6). HRMS (ESI) calcd for (M + H) $C_{30}H_{31}N_2O_5S$: 531.1948. Found: 531.1945. Purity: > 99%.

3-(2-Phenylindol-3-yl)-prop-2-ynyl 3-amino-2,3,6- trideoxy-α-L-*lyxo*-hexopyranoside (4). Pure **4** was obtained by column chromatography on Iatrobeads (1% Et_3N in 7:1 CH_2Cl_2–CH_3OH) in 89% yield: white semi-solid, R_f 0.29 (1% Et_3N in 6:1 CH_2Cl_2–CH_3OH); IR: ν 3283 (br, N–H, O–H), 2217 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–104.1 (*c* 0.4, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 8.06–8.09 (m, 2H, Ar), 7.58–7.61 (m, 1H, Ar), 7.44–7.49 (m, 2H, Ar), 7.33–7.41 (m, 2H, Ar), 7.15–7.19 (m, 1H, Ar), 7.07–7.11 (m, 1H, Ar), 5.20 (br d, 1H, $J_{1,2a}$ = 2.9 Hz, H-1), 4.56 (ABq, 1H, J = 15.9 Hz, $OCH_2C{\equiv}C$), 4.54 (ABq, 1H, J = 15.9 Hz, $OCH_2C{\equiv}C$), 3.98 (br q, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 3.45 (br s, 1H, H-4), 3.10–3.18 (m, 1H, H-3), 1.72–1.83 (m, 2H, H-2a, H-2e), 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 141.4 (Ar), 137.3 (Ar), 133.3 (Ar), 131.7 (Ar), 129.7 (2, Ar), 129.2 (Ar), 127.7 (2, Ar), 124.0 (Ar), 121.4 (Ar), 120.3 (Ar), 112.4 (Ar), 97.1 (C-1), 95.1 (Ar), 89.8 (≡C), 82.2 (≡C), 71.9 (C-4), 68.5 (C-5), 56.1 (OCH_2), 47.8 (C-3), 33.4 (C-2), 17.3 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{25}N_2O_3$: 377.1860. Found: 377.1859. Purity: >99%.

3-(2-Phenyl-benzo[*b*]furan-3-yl)-prop-2-ynyl-3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (5). Pure **5** was obtained by column chromatography on Iatrobeads (7:1 CH_2Cl_2–CH_3OH) in 77% yield: colorless syrup, R_f 0.27 (7:1 CH_2Cl_2–CH_3OH); IR ν 3328 (N–H), 3276 (N–H), 3058 (O–H), 2221.7 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–89.5 (*c* 2.4, CH_2Cl_2); ^1H NMR (400 MHz, CD_3OD, δ_H) 8.20–8.24 (m, 2H, Ar), 7.58–7.61 (m, 1H, Ar), 7.45–7.52 (m, 3H, Ar), 7.38–7.42 (m, 1H, Ar), 7.32–7.36 (m, 1H, Ar), 7.27–7.31 (m, 1H, Ar), 5.15 (br d, 1H, $J_{1,2a}$ = 3.6 Hz, H-1), 4.60 (s, 2H, $OCH_2C{\equiv}C$), 3.71 (dq, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.02–3.12 (m, 1H, H-3), 2.89 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 2.08 (br dd, 1H, $J_{2a,2e}$ = 13.1 Hz, $J_{2e,3}$ = 3.7 Hz, H-2e), 1.56 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 13.1 Hz, $J_{1,2a}$ = 3.6 Hz, H-2a), 1.25 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 157.9 (Ar), 154.8 (Ar), 131.1 (Ar), 131.0 (Ar), 130.6 (Ar), 129.8 (2, Ar), 126.9 (2, Ar), 126.8 (Ar), 124.7 (Ar), 121.1 (Ar), 112.2 (Ar), 99.5 (Ar), 97.0 (C-1), 94.3 (≡C), 79.1 (C-4), 78.3 (≡C), 70.1 (C-5), 55.8 (OCH_2), 50.5 (C-3), 38.2 (C-2), 18.2 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_4$: 378.1700. Found: 378.1703. Purity: > 99%.

3-(2-Phenyl-benzo[*b*]thiophene-3-yl)-prop-2-ynyl 3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (6). Pure **6** was obtained by column chromatography on Iatrobeads (7:1 CH_2Cl_2–CH_3OH) in 77% yield: yellow oil, R_f 0.27 (7:1 CH_2Cl_2–CH_3OH); IR: ν 3311 (N–H), 3265 (N–H), 3056 (O–H), 2218 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–88.3 (*c* 0.2, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 7.88–7.93 (m, 2H, Ar), 7.82–7.85 (m, 1H, Ar), 7.76–7.80 (m, 1H, Ar), 7.32–7.45 (m, 5H, Ar), 5.09 (br d, 1H, $J_{1,2a}$ = 3.6 Hz, H-1), 4.52 (s, 2H, $OCH_2C{\equiv}C$), 3.67 (dq, 1H, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 6.2 Hz, H-5), 3.03–3.11 (m, 1H, H-3), 2.90 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4), 2.04 (br dd, 1H, $J_{2a,2e}$ = 13.1 Hz, $J_{2e,3}$ = 3.8 Hz, H-2e), 1.62 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 13.1 Hz, $J_{1,2a}$ = 3.6 Hz, H-2a), 1.23 (d, 3H, $J_{5,6}$ = 6.2 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 148.0 (Ar), 142.3 (Ar), 138.8 (Ar), 134.8 (Ar), 130.1 (Ar), 129.8 (2, Ar), 129.3 (2, Ar), 126.6 (Ar), 126.2 (Ar), 124.0 (Ar), 123.2 (Ar), 114.0 (Ar), 96.6 (C-1), 91.8 (≡C), 81.2 (≡C), 78.4 (C-4), 70.1 (C-5), 55.6 (OCH_2), 50.6 (C-3), 37.6 (C-2), 18.2 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_3S$: 394.1471. Found: 394.1469. Purity: > 99%.

3-(1-Tosyl-2-phenylindol-3-yl)-prop-2-ynyl 3-amino- 2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (7). Pure **7** was obtained by column chromatography on Iatrobeads (7:1 CH_2Cl_2–CH_3OH) in 77% yield: yellow oil, R_f 0.27 (7:1 CH_2Cl_2–CH_3OH); IR: ν 3128 (br, N–H, O–H), 2228 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–64.0 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 8.24–8.26 (m, 1H, Ar), 7.39–7.52 (m, 7H, Ar), 7.31–7.34 (m, 1H, Ar), 7.23–7.25 (m, 2H, Ar), 7.11–7.14 (m, 2H, Ar), 4.91 (br d, 1H, $J_{1,2a}$ = 3.6 Hz, H-1), 4.35 (s, 2H, $OCH_2C{\equiv}C$), 3.60 (dq, 1H, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.24–3.31 (m, 1H, H-3), 3.10 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 2.27 (s, 3H, $PhCH_3$), 2.07 (br dd, 1H, $J_{2a,2e}$ = 12.8 Hz, $J_{2e,3}$ = 3.7 Hz, H-2e), 1.76 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 12.8 Hz, $J_{1,2a}$ = 3.6 Hz, H-2a), 1.18 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 147.0 (Ar), 145.4 (Ar), 138.3 (Ar), 135.7 (Ar), 132.3 (2, Ar), 132.0 (Ar), 131.9 (Ar), 130.6 (2, Ar), 130.3 (Ar), 128.5 (2, Ar), 127.9 (2, Ar), 127.0 (Ar), 126.0 (Ar), 120.9 (Ar), 117.6 (Ar), 108.6 (Ar), 95.6 (C-1), 91.4 (≡C), 78.8 (≡C), 74.7 (C-4), 70.1 (C-5), 55.6 (OCH_2), 49.6 (C-3), 35.2 (C-2), 21.5 ($PhCH_3$), 17.9 (C-6). HRMS (ESI) calcd for (M + H) $C_{30}H_{31}N_2O_5S$: 531.1948. Found: 531.1946. Purity: > 99%.

3-(2-Phenylindol-3-yl)-prop-2-ynyl 3-amino-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (8). Pure **8** was obtained by column chromatography on Iatrobeads (7:1 CH_2Cl_2–CH_3OH) in 94% yield: yellow syrup, R_f 0.17 (5:1 CH_2Cl_2–CH_3OH); ν 3286 (br, N–H, O–H), 2218 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–81.9 (*c* 0.3, CH_3OH); ^1H NMR (400 MHz, CD_3OD, δ_H) 8.08–8.10 (m, 2H, Ar), 7.58–7.61 (m, 1H, Ar), 7.45–7.48 (m, 2H, Ar), 7.38–7.41 (m, 1H, Ar), 7.34–7.37 (m, 1H, Ar), 7.15–7.19 (m, 1H, Ar), 7.08–7.11 (m, 1H, Ar), 5.17 (br d, 1H, $J_{1,2a}$ = 3.7 Hz, H-1), 4.56 (s, 2H, $OCH_2C{\equiv}C$), 3.70 (dq, 1H, $J_{4,5}$ = 9.3 Hz, $J_{5,6}$ = 6.3 Hz, H-5), 3.00–3.10 (m, 1H, H-3), 2.87 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 2.05 (br dd, 1H, $J_{2a,2e}$ = 13.1 Hz, $J_{2e,3}$ = 3.9 Hz, H-2e), 1.62 (ddd, 1H, $J_{2a,2e} = J_{2a,3}$ = 13.1 Hz, $J_{1,2a}$ = 3.7 Hz, H-2a), 1.25 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD, δ_C) 141.5 (Ar), 137.3 (Ar), 133.2 (Ar), 131.7 (Ar), 129.7 (2, Ar), 129.2 (Ar), 127.7 (2, Ar), 124.0 (Ar), 121.4 (Ar), 120.3 (Ar), 112.4 (Ar), 96.5 (C-1), 95.0 (Ar), 89.7 (≡C), 82.3 (≡C), 79.0 (C-4), 70.0 (C-5), 56.0 (OCH_2), 50.5 (C-3), 38.0 (C-2), 18.2 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{25}N_2O_3$: 377.1860. Found: 377.1858. Purity: 97.7%.

3-(2-Phenyl-benzo[*b*]furan-3-yl)-prop-2-ynyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (9). Pure **9** was obtained by column chromatography (1:1 hexanes–EtOAc→10:1 CH_2Cl_2–CH_3OH) in 85% yield: yellowish syrup, R_f 0.28 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3383 (N–H), 2222 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–93.7 (*c* 1.9, CH_3OH); ^1H NMR (500 MHz, CD_3OD, δ_H) 8.17–8.22 (m, 2H, Ar), 7.56–7.60 (m, 1H, Ar), 7.43–7.50 (m, 3H, Ar), 7.36–7.40 (m, 1H, Ar), 7.25–7.34 (m, 2H, Ar), 5.09 (br d, 1H, $J_{1,2a} = 4.0$ Hz, H-1), 4.58 (s, 2H, $OCH_2C{\equiv}C$), 4.07 (dq, 1H, $J_{5,6} = 6.6$ Hz, $J_{4,5} = 1.5$ Hz, H-5), 2.68 (br s, 1H, H-4), 2.09 (dddd, 1H, $J_{3a,3e} = 13.8$ Hz, $J_{2a,3a} = 13.8$ Hz, $J_{3a,4} = 4.0$ Hz, $J_{2e,3a} = 4.0$ Hz, H-3a), 1.97 (dddd, 1H, $J_{2a,2e} = 13.8$ Hz, $J_{2a,3a} = 13.8$ Hz, $J_{1,2a} = 4.0$ Hz, $J_{2a,3e} = 4.0$ Hz, H-2a), 1.58–1.64 (m, 1H, H-3e), 1.51–1.57 (m, 1H, H-2e), 1.08 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 157.8 (Ar), 154.8 (Ar), 131.1 (Ar), 131.0 (Ar), 130.5 (Ar), 129.8 (2, Ar), 126.9 (2, Ar), 126.7 (Ar), 124.7 (Ar), 121.1 (Ar), 112.2 (Ar), 99.6 (Ar), 97.3 (C-1), 94.6 (≡C), 78.2 (≡C), 67.8 (C-5), 55.7 (OCH_2), 49.4 (C-4), 26.8 (C-2), 24.2 (C-3), 17.8 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_3$: 362.1751. Found: 362.1753. Purity: > 99%.

3-(2-Phenyl-benzo[*b*]thiophen-3-yl)-prop-2-ynyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (10). Pure **10** was obtained by column chromatography (1:1 hexanes–EtOAc → 10:1 CH_2Cl_2–CH_3OH) in 76% yield: yellow oil, R_f 0.25 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3379 (N–H), 2216 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–97.2 (*c* 2.4, CH_2Cl_2); ^1H NMR (500 MHz, CD_3OD, δ_H) 7.91–7.95 (m, 2H, Ar), 7.83–7.87 (m, 1H, Ar), 7.77–7.81 (m, 1H, Ar), 7.33–7.45 (m, 5H, Ar), 5.03 (br d, 1H, $J_{1,2a} = 4.0$ Hz, H-1), 4.57 (s, 2H, $OCH_2C{\equiv}C$), 4.06 (dq, 1H, $J_{5,6} = 6.6$ Hz, $J_{4,5} = 1.7$ Hz, H-5), 2.64 (br s, 1H, H-4), 2.04 (dddd, 1H, $J_{3a,3e} = 13.9$ Hz, $J_{2a,3a} = 13.9$ Hz, $J_{3a,4} = 4.0$ Hz, $J_{2e,3a} = 4.0$ Hz, H-3a), 1.93 (dddd, 1H, $J_{2a,2e} = 13.9$ Hz, $J_{2a,3a} = 13.9$ Hz, $J_{1,2a} = 4.0$ Hz, $J_{2a,3e} = 4.0$ Hz, H-2a), 1.56–1.62 (m, 1H, H-3e), 1.47–1.53 (m, 1H, H-2e), 1.14 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 147.8 (Ar), 142.3 (Ar), 138.8 (Ar), 134.9 (Ar), 130.1 (Ar), 129.8 (2, Ar), 129.4 (2, Ar), 126.5 (Ar), 126.2 (Ar), 124.1 (Ar), 123.2 (Ar), 114.1 (Ar), 97.2 (C-1), 92.2 (≡C), 81.0 (≡C), 67.9 (C-5), 55.6 (OCH_2), 49.6 (C-4), 26.8 (C-3), 24.2 (C-2), 17.8 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{24}NO_2S$: 378.1522. Found: 378.1520. Purity: > 99%.

3-(1-Tosyl-2-phenylindol-3-yl)-prop-2-ynyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (11). Pure **11** was obtained by column chromatography (1:1, hexanes–EtOAc → 10:1, CH_2Cl_2–CH_3OH) in 19% yield: yellowish oil, R_f 0.20 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3374 (N–H), 2227 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–72.7 (*c* 0.2, CH_2Cl_2); ^1H NMR (500 MHz, CD_3OD, δ_H) 8.23–8.26 (m, 1H, Ar), 7.48–7.53 (m, 3H, Ar), 7.38–7.47 (m, 4H, Ar), 7.30–7.36 (m, 1H, Ar), 7.22–7.25 (m, 2H, Ar), 7.10–7.14 (d, 2H, $J = 8.0$ Hz, Ar), 4.83 (1H, H-1, overlap with CD_3OH signal), 4.35 (ABq, 1H, $J = 16.3$ Hz, $OCH_2C{\equiv}C$), 4.32 (ABq, 1H, $J = 16.3$ Hz, $OCH_2C{\equiv}C$), 3.90 (dq, 1H, $J_{5,6} = 6.6$ Hz, $J_{4,5} = 1.6$ Hz, H-5), 2.66 (br s, 1H, H-4), 2.26 (s, 3H, $PhCH_3$), 1.98 (dddd, 1H, $J_{3a,3e} = 14.0$ Hz, $J_{2a,3a} = 14.0$ Hz, $J_{3a,4} = 4.2$ Hz, $J_{2e,3a} = 4.2$ Hz, H-3a), 1.93 (dddd, 1H, $J_{2a,2e} = 14.0$ Hz, $J_{2a,3a} = 14.0$ Hz, $J_{1,2a} = 4.2$ Hz, $J_{2a,3e} = 4.2$ Hz, H-2a), 1.54–1.60 (m, 1H, H-3e), 1.38–1.44 (m, 1H, H-2e), 0.99 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 146.9 (Ar), 145.2 (Ar), 138.4 (Ar), 135.6 (Ar), 132.3 (2, Ar), 132.0(3) (Ar), 131.9(9) (Ar), 130.6 (2, Ar), 130.2 (Ar), 128.5 (2, Ar), 127.9 (2, Ar), 127.0 (Ar), 126.0 (Ar), 120.9 (Ar), 117.6 (Ar), 108.8 (Ar), 97.1 (C-1), 92.2 (≡C), 78.3 (≡C), 67.6 (C-5), 55.4 (OCH_2), 49.4 (C-4), 26.5 (C-3), 24.1 (C-2), 21.5 ($PhCH_3$), 17.7 (C-6). HRMS (ESI) calcd for (M + H) $C_{30}H_{31}N_2O_4S$: 515.1999. Found: 515.2000. Purity: > 99%.

3-(2-Phenylindol-3-yl)-prop-2-ynyl 4-amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (12). Pure **12** was obtained by column chromatography (10:1, CH_2Cl_2–CH_3OH) in 75% yield: yellowish oil, R_f 0.18 (10:1 CH_2Cl_2–CH_3OH); IR: ν 3356 (N–H), 2218 (C≡C) cm^{-1}; $[\alpha]^{23}_D$)–92.9 (*c* 0.9, CH_2Cl_2); ^1H NMR (500 MHz, CD_3OD, δ_H) 8.06–8.10 (m, 2H, Ar), 7.58–7.62 (m, 1H, Ar), 7.43–7.48 (m, 2H, Ar), 7.38–7.41 (m, 1H, Ar), 7.32–7.37 (m, 1H, Ar), 7.14–7.18 (m, 1H, Ar), 7.07–7.12 (m, 1H, Ar), 5.12 (br d, 1H, $J_{1,2a} = 3.9$ Hz, H-1), 4.57 (s, 2H, $OCH_2C{\equiv}C$), 4.06 (br q, 1H, $J_{5,6} = 6.6$ Hz, H-5), 2.68 (br s, 1H, H-4), 2.09 (dddd, 1H, $J_{3a,3e} = 13.8$ Hz, $J_{2a,3a} = 13.8$ Hz, $J_{3a,4} = 3.9$ Hz, $J_{2e,3a} = 3.9$ Hz, H-3a), 1.93 (dddd, 1H, $J_{2a,2e} = 13.8$ Hz, $J_{2a,3a} = 13.8$ Hz, $J_{1,2a} = 3.9$ Hz, $J_{2a,3e} = 3.9$ Hz, H-2a), 1.58–1.64 (m, 1H, H-3e), 1.50–1.56 (m, 1H, H-2e), 1.09 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6); ^{13}C NMR (125 MHz, CD_3OD, δ_C) 141.4 (Ar), 137.3 (Ar), 133.3 (Ar), 129.7 (Ar), 129.7 (2, Ar), 129.2 (Ar), 127.7 (2, Ar), 124.0 (Ar), 121.4 (Ar), 120.3 (Ar), 112.4 (Ar), 96.9 (C-1), 95.1 (Ar), 89.9 (≡C), 82.1 (≡C), 67.7 (C-5), 56.0 (OCH_2), 49.4 (C-4), 26.8 (C-3), 24.3 (C-2), 17.8 (C-6). HRMS (ESI) calcd for (M + H) $C_{23}H_{25}N_2O_2$: 361.1911. Found: 369.1910. Purity: > 99%.

DNA binding fluorescence assay

The fluorescence was measured according to the literature reported procedure.[36a,37,40] Sheared herring sperm DNA (10 mg/mL in 10 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA, pH 7.5) was purchased from Promega and diluted with BPE (bis-phosphate EDTA) buffer to 2 mg/mL as the stock solution. BPE buffer consists of 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, pH 7.0 in Milli-Q water. Molecular-biological grade DMSO and ethidium bromide (EtBr) were purchased from Sigma-Aldrich. A stock solution of EtBr (1 mM) was prepared in BPE buffer. The test analogues were dissolved in DMSO to make 10 mM stock solutions and further diluted with DMSO, as needed, to the desired concentration prior to the assay. Fluorescence was measured on a PTI (Photon Technology International) MP1 fluorescence system.

Direct fluorescence measurement

To a fluorescence microcell was added 99 μL of a solution of a test compound (50 μM) in BPE buffer containing less than 1% DMSO, and its emission curve was measured from 335 nm to 450 nm at an excitation wavelength of 320 nm at ambient temperature. To the above ligand solution was added 1 μL of a 2 mg/mL stock solution of herring sperm DNA (hsDNA) in BPE buffer. After gentle vortexing and a short-time equilibration (~ 1 min), the emission scan was performed under the same set of parameters until the error between the maxima of two continuous emission curves was < 10%. The fluorescence intensity at the emission maximum before the addition of DNA was set as 100% to normalize the intensity obtained after the addition of the nucleic acid. Under the above assay conditions, no fluorescence was observed for any of the reagents, except for the test compounds. Each compound

was assayed in duplicate, and the values shown in the table reflect an average of this data.

Single-point fluorescent intercalator displacement (FID) assay

Three individual fluorescence measurements were required for this assay. First, to a fluorescence microcell was added 98.5 μL of an ethidium bromide (EtBr) solution in BPE buffer (5 μM), and its emission curve was measured from 540 nm to 700 nm at an excitation wavelength of 520 nm. Second, to the above EtBr solution was added 1 μL of a 2 mg/mL stock solution of hsDNA in BPE buffer. After mixing and a short-time equilibration (~ 10 sec), the emission scan was performed under the same set of parameters until the error between the maxima of two continuous emission curves was < 10%. The intensity difference at the emission maximum from the two previous measurements was set as 100% for normalization. Finally, 0.5 μL of a 10 mM stock solution of a test compound in DMSO was added to the mixture in the cell. After mixing and equilibration (~ 3 min), the emission scan was performed until steady measurments were obtained. The normalized intensity difference at the emission maximum between the first measurement and this measurement was used to represent the percentage of remaining EtBr. Alternatively, the normalized intensity difference between the second and third measurements was used to represent the percentage of displaced EtBr. Under the above assay conditions, no fluorescence was observed for all the reagents and their combinations except for EtBr. Each compound was assayed in triplicate, and the values shown in the table reflect an average of this data.

Multiple-point fluorescent intercalator displacement (FID) assay

The assay is nearly identical to the above single-point assay. The major difference is that in step 3, 0.2 μL aliquots of a 10 mM stock solution of a test compound in DMSO was added to the fluorescing solution in the cell, and the fluorescence was measured after each addition until a 50% reduction of fluorescence occurred, corresponding to a 50% displacement of EtBr by the test compound. The apparent binding constant of the test ligand was then calculated from the equation:

$$K_{\text{Ligand}} = K_{\text{EtBr}} \times \frac{[\text{EtBr}]}{[\text{Ligand}]_{50\%}}$$

where $[\text{Ligand}]_{50\%}$ is the concentration of ligand that gives a 50% reduction of fluorescence, [EtBr] is the concentration of EtBr, and K_{EtBr} is the binding constant for ethidium bromide.

Acknowledgements

This work was supported by the University of Alberta, the Natural Sciences and Engineering Research Council of Canada (NSERC), the Alberta Ingenuity Centre for Carbohydrate Science (TLL), and by grant R01 CA65875 from the National Cancer Institute (to RSC). WS was the recipient of a Studentship from the Alberta Ingenuity Fund.

References

1 S. Neidle and D. E. Thurston, *Nat. Rev. Cancer*, 2005, **5**, 285–296.
2 J. J. Welchi, F. J. Rauscher III and T. A. Beermanill, *J. Biol. Chem.*, 1994, **269**, 31051–31058.
3 (*a*) R. B. Weiss, *Semin. Oncol.*, 1992, **19**, 670–86; (*b*) F. M. Arcamone, in *Anticancer Agents from Natural Products*, ed. G. M. Cragg, D. G. I. Kingston and D. J. Newman, CRC Press: Boca Raton, FL, 2005, Ch. 16, pp. 299–320.
4 (*a*) A. Di Marco, M. Gaetani, L. Dorigotti, M. Soldati and O. Bellini, *Nature*, 1964, **201**, 706–707; (*b*) F. Cassinelli and P. Orezzi, *G. Microbiol.*, 1963, **11**, 167–174.
5 F. Arcamone, G. Cassinelli, G. Fantini, A. Grein, P. Orezzi, C. Pol and C. Spalla, *Biotech. Bioeng.*, 1969, **11**, 1101–1110.
6 Recent examples(*a*) F. Guano, P. Pourquier, S. Tinelli, M. Binaschi, M. Bigioni, F. Animati, S. Manzani, F. Zunino, G. Kohlhagen, Y. Pommier and G. Capranico, *Mol. Pharmacol.*, 1999, **56**, 77–84; (*b*) F. Zunino, G. Pratesi and P. Perego, *Biochem. Pharmacol.*, 2001, **61**, 933–938; (*c*) G. Zhang, L. Fang, L. Zhu, J. E. Aimiuwu, J. Shen, H. Cheng, M. T. Muller, G. E. Lee, D. Sun and P. G. Wang, *J. Med. Chem.*, 2005, **48**, 5269–5278; (*d*) J. Portugal, D. J. Cashman, J. O. Trent, N. Ferrer-Miralles, T. Przewloka, I. Fokt, W. Priebe and J. B. Chaires, *J. Med. Chem.*, 2005, **48**, 8209–8219; (*e*) L. Zhu, X. Cao, W. Chen, G. Zhang, D. Sun and P. G. Wang, *Bioorg. Med. Chem.*, 2005, **13**, 6381–6387; (*f*) D. Horton and A. Khare, *Carbohydr. Res.*, 2006, **341**, 2631–2640.
7 (*a*) F. Arcamone, L. Bernardi, P. Giardino, B. Patelli, A. Di Marco, A. M. Casazza, G. Pratesi and P. Reggiani, *Cancer Treat. Rep.*, 1976, **60**, 829–834; (*b*) M. Israel, E. J. Modest and E. Frei, *Cancer Res.*, 1975, **35**, 1365–1368; (*c*) A. J. Coukell and D. Faulds, *Drugs*, 1997, **53**, 453–482; (*d*) H. Umezawa, Y. Takahashi, M. Kinoshita, H. Naganawa, T. Masuda, M. Ishizuka, K. Tatsuta and T. Takeuchi, *J. Antibiot.*, 1979, **32**, 1082–1084; (*e*) T. Oki, Y. Matsuzawa, A. Yoshimoto, K. Numata and I. Kitamura, *J. Antibiot.*, 1975, **28**, 830–834; (*f*) S. Hori, M. Shirai, S. Hirano, T. Oki, T. Inui, S. Tsukagoshi, M. Ishizuka, T. Takeuchi and H. Umezawa, *Gann.*, 1977, **68**, 685–690.
8 (*a*) G. Minotti, P. Menna, E. Salatorelli, G. Cairo and L. Gianni, *Pharmacol. Rev.*, 2004, **56**, 185–229; (*b*) D. A. Gewirtz, *Biochem. Pharm.*, 1999, **57**, 727–741.
9 (*a*) X. Qu, C. Wan, H. C. Becker, D. Zhong and A. H. Zewail, *Proc. Natl. Acad. Sci. U.S.A.*, 2001, **98**, 14212–14217; (*b*) A. H. J. Wang, G. Ughetto, G. J. Quigley and A. Rich, *Biochemistry*, 1987, **26**, 1152–1163.
10 C. Kimchi-Sarfaty, J. J. Gribar and M. M. Gottesman, *Mol. Pharmacol.*, 2002, **62**, 1–6.
11 (*a*) P. K. Singal, T. Li, D. Kumar, I. Danelisen and N. Iliskovic, *Mol. Cell. Biochem.*, 2000, **207**, 77–86; (*b*) R. Ng, N. Better and M. D. Green, *Semin. Oncol.*, 2006, **33**, 2–14.
12 E. Fan, W. Shi and T. L. Lowary, *J. Org. Chem.*, 2007, **72**, 2917–2928.
13 S. Hanessian, *ChemMedChem*, 2006, **12**, 1300–1330.
14 (*a*) Y. Tamura, M. Sasho, S. Akai, H. Kishimoto, J. Sekihachi and Y. Kita, *Tetrahedron Lett.*, 1986, **27**, 195–198; (*b*) O. Achmatowicz and B. Szechner, *J. Org. Chem.*, 2003, **68**, 2398–2404; (*c*) Y. S. Rho, J. Park, G. Kim, H. Kim, H. Sin, P. W. Suh and D. J. Yoo, *Syn. Comm.*, 2004, **9**, 1703–1722.
15 (*a*) I. Dyong and R. Hermann, *Tetrahedron Lett.*, 1981, **22**, 4225–4226; (*b*) I. Dyong and R. Hermann, *Liebigs Ann. Chem.*, 1986, 545–550; (*c*) *Eur. Pat.*, 475 473, 1992.
16 (*a*) G. J. Atwell, C. D. Bos, B. C. Baguley and W. A. Denny, *J. Med. Chem.*, 1988, **31**, 1048–1052; (*b*) G. J. Atwell, B. C. Baguley and W. A. Denny, *J. Med. Chem.*, 1989, **32**, 396–401.
17 (*a*) A. Kumasaki, T. Goto, S. Nakamura, T. Ihara and M. Takagi, *Chem. Lett.*, **2001**, 370–371; (*b*) K. Toshima, R. Takano, Y. Maeda, M. Suzuki, A. Asai and S. Matsumura, *Angew. Chem. Intl Ed.*, 1999, **28**, 3733–3735; (*c*) K. Toshima, *J. Syn. Org. Chem. Jpn.*, 2005, **63**, 325–338.
18 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem. Int. Ed.*, 2001, **40**, 2004–2021.
19 (*a*) M. Binaschi, R. Farinosi, M. E. Borgnetto and G. Capranico, *Cancer Res.*, 2000, **60**, 3770–3776; (*b*) *US Pat.*, 5 593 970 A, 1997.
20 (*a*) Y. Kondo, F. Shiga, N. Murata, T. Sakamoto and H. Yamanaka, *Tetrahedron*, 1994, **50**, 11803–11812; (*b*) J. R. Labrosse, C. Poncet, P. Lhoste and D. Sinou, *Tetrahedron Asymm.*, 1999, **10**, 1069–1078; (*c*) A. Arcadi, S. Cacchi, G. Fabrizi, F. Marinelli and L. Moro, *Synlett*, 1999, 1432–1434.
21 (*a*) R. C. Larock and L. W. Harrison, *J. Am. Chem. Soc.*, 1984, **106**, 4218–27; (*b*) D. Yue and R. C. Larock, *J. Org. Chem.*, 2002, **67**, 1905–9.
22 V. Bocchi and G. Palla, *Synthesis*, 1982, 1096–7.
23 (*a*) C. Koradin, W. Dohle, A. L. Rodriguez, B. Schmid and P. Knochel, *Tetrahedron*, 2003, **59**, 1571–87; (*b*) K. Hiroya, S. Itoh and T. Sakamoto, *J. Org. Chem.*, 2004, **69**, 1126–36.

24 M. Amjad and D. W. Knight, *Tetrahedron Lett.*, 2004, **45**, 539–41.
25 (*a*) W. Dai and K. W. Lai, *Tetrahedron Lett.*, 2002, **43**, 9377–80; (*b*) S. N. Aslam, P. C. Stevenson, S. J. Phythian, N. C. Veitch and D. V. Hall, *Tetrahedron*, 2006, **62**, 4214–26.
26 B. Hill, M. De Vleeschauwer, K. Houde and M. Belley, *Synlett*, 1998, 407–10. One-pot coupling-cyclization synthesis of 2-phenyl[*b*]benzofuran (**20**), starting from 2-iodophenol and phenylacetylene under typical Sonogashira reaction conditions, has been reported. However, the formation of the homocoupling product of phenylacetylene (1,4-diphenylbutadiyne) made the column purification difficult. Even when phenylacetylene was used as the limiting reagent, and the initiation of cyclization was delayed by heating the reaction mixture at a later time, there was still a small amount of homocoupling product, and the yield also decreased.
27 B. Abbaci, J. Florent and C. Monneret, *Bull. Soc. Chim. Fr.*, 1989, **5**, 667–72.
28 R. Albert, K. Dax, R. W. Link and A. E. Stütz, *Carbohydr. Res.*, 1983, **118**, C5–C6.
29 R. J. Ferrier, *J. Chem. Soc. Perkin Trans 1*, 1979, 1455.
30 B. Renneberg, Y. Li, H. Laatsch and H. Fiebig, *Carbohydr. Res.*, 2000, **329**, 861–872.
31 J. A. Osborn, F. H. Jardine, J. F. Young and G. Wilkinson, *J. Chem. Soc. A.*, 1966, 1711–1732.
32 (*a*) W. Adam and H. J. Eggelte, *Angew. Chem. Int. Ed.*, 1977, **16**, 713; (*b*) A. G. Meyers, B. Zheng and M. Movassaghi, *J. Org. Chem.*, 1997, **62**, 7507.
33 A. Elangovan, Y. H. Wang and T. I. Ho, *Org. Lett.*, 2003, **5**, 1841–1844.
34 (*a*) I. Haq and J. Ladbury, *J. Mol. Recognit.*, 2000, **13**, 188–197; (*b*) I. Haq, T. C. Jenkins, B. Z. Chowdhry, J. Ren and J. B. Chaires, *Methods Enzymol.*, 2000, **323**, 373–405; (*c*) I. Haq, *Arch. Biochem. Biophys.*, 2002, **403**, 1–15; (*d*) F. Leng, J. B. Chaires and M. J. Waring, *Nucleic Acids Res.*, 2003, **31**, 6191–6297; (*e*) J. Lah and G. Vesnaver, *Biochemistry*, 2000, **39**, 9317–9326.
35 (*a*) J. Bunkenborg, C. Behrens and J. P. Jacobsen, *Bioconjugate Chem.*, 2002, **13**, 927–936; (*b*) T. Nishimura, T. Okobira, A. M. Kelly, N. Shimada, Y. Takeda and K. Sakurai, *Biochemistry*, 2007, **46**, 8156–8163; (*c*) S. D. Micco, C. Bassarello, G. Bifulco, R. Riccio and L. Gomez-Paloma, *Angew. Chem. Int. Ed.*, 2006, **45**, 224–228.
36 (*a*) D. Suh and J. B. Chaires, *Bioorg, Med. Chem.*, 1995, **3**, 723–728; (*b*) M. M. Murr, M. T. Harting, V. Guelev, J. Ren, J. B. Chaires and B. L. Iverson, *Bioorg. Med. Chem.*, 2001, **9**, 1141–1148.
37 (*a*) R. S. Coleman, R. J. Perez, C. H. Burk and A. Navarro, *J. Am. Chem. Soc.*, 2002, **124**, 13008–13017; (*b*) D. Suh, Y. K. Oh and J. B. Chaires, *Process Biochem.*, 2001, **37**, 521–525; (*c*) K. M. Hyun, S. D. Choi, S. Lee and S. K. Kim, *Biochem. Biophys. Acta*, 1997, **1334**, 321–326; (*d*) D. L. Boger, B. E. Fink, S. R. Brunette, W. C. Tse and M. P. Hedrick, *J. Am. Chem. Soc.*, 2001, **123**, 5878–5891; (*e*) D. L. Boger and W. C. Tse, *Bioorg. Med. Chem.*, 2001, **9**, 2511–2518; (*f*) D. Bartulewicz, K. Bielawski and A. Bielawska, *Arch. Pharm. Pharm. Med. Chem.*, 2002, **9**, 422–426.
38 (*a*) H. Li, X. Peng and F. Seeia, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 6031–6034; (*b*) M. T. Milano, G. G. Hu, L. D. Williams and W. A. Bernhard, *Radiat. Res.*, 1998, **150**, 101–114.
39 M. A. Lewis and E. C. Long, *Bioorg. Med. Chem.*, 2006, **14**, 3481–3490.
40 (*a*) A. R. Morgan, J. S. Lee, D. E. Pulleyblank, N. L. Murray and D. H. Evans, *Nucleic Acids Res.*, 1979, **7**, 547–569; (*b*) D. L. Boger, B. J. Invergo, R. S. Coleman, H. Zarrinmayeh, P. A. Kitos, S. C. Thompson, T. Leong and L. W. McLaughlin, *Chem.-Biol. Interact.*, 1990, **73**, 29–52; (*c*) B. C. Baguley, W. A. Denny, G. J. Atwell and B. F. Cain, *J. Med. Chem.*, 1981, **24**, 170–177; (*d*) B. C. Baguley and E. M. Falkenhaug, *Nucleic Acids Res.*, 1978, **5**, 161–171; (*e*) M. Lee, A. L. Rhodes, M. D. Wyatt, M. D'Incalci, S. Forrow and J. A. Hartley, *J. Med. Chem.*, 1993, **36**, 863–870.
41 A. Mukherjee, R. Lavery, B. Bagchi and T. T. Hynes, *J. Am. Chem. Soc.*, 2008, **130**, 9747–9755.
42 J. B. Chaires, *Arch. Biochem. Biophys.*, 2006, **453**, 26–31.
43 R. M. Silverstein and F. X. Webster, *Spectrometric identification of organic compounds*, John Wiley & Sons, Inc., New York, 6th edn., 1998, Ch. 4, pp. 144-217.

