Carboranyl thymidine analogues for neutron capture therapy

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Neutron capture therapy (NCT) is a binary radio-chemotherapeutic modality for the treatment of cancer. A major focus of NCT-related research is the development of novel tumor-selective agents that serve as the chemical component in NCT. Thymidine analogues substituted with a boron-containing carborane cluster at the N3 position, designated **3CTAs** (3-carboranyl thymidine analogues), constitute one class of these new improved NCT agents. Their chemical, structural and biological properties are discussed in this *Feature Article*.

Introduction

Neutron capture therapy (NCT) is a binary cancer treatment modality, which relies both on a chemical and a radiation component.[1] There are two types of NCT, boron neutron capture therapy (BNCT) and gadolinium neutron capture therapy (GdNCT).[2]

In the case of BNCT, a neutron capture reaction occurs when ^{10}B, a stable isotope, is delivered selectively to tumor cells, and then irradiated with low energy (thermal) neutrons to produce lithium- and helium nuclei (α-particles) (Fig. 1).[1] These high linear energy transfer (high LET) particles can produce a variety of cytocidal effects, including DNA double strand breaks.[3,4] Because of their short ranges (<10 μm), lethal

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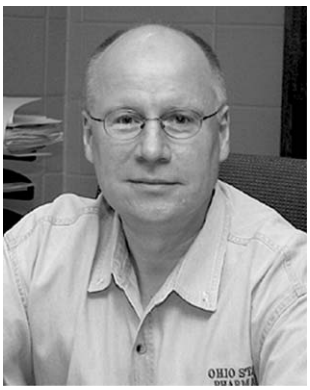

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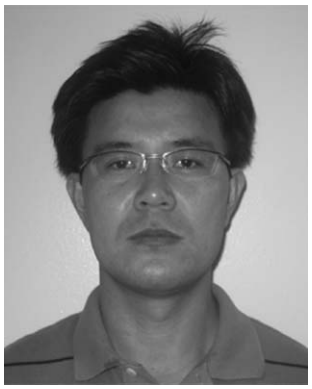

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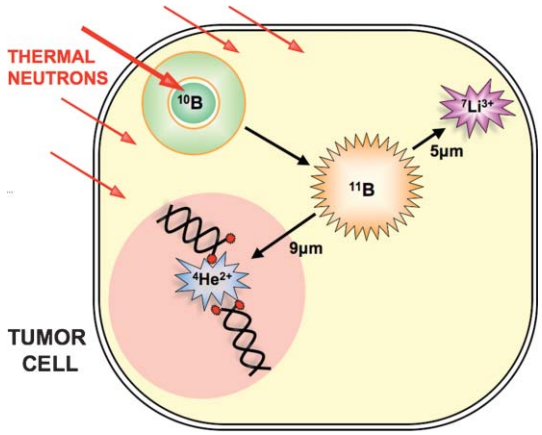

Fig. 1 Basic concept of BNCT. A thermal neutron is captured by ^{10}B producing an excited ^{11}B isotope, which instantaneously undergoes nuclear fission to yield $^{4}He^{2+}$ (α-particle) and recoiling $^{7}Li^{3+}$. Both particles can kill cancer cells, primarily by causing DNA double strand breaks.

damage is largely restricted to the targeted tumor cells (Fig. 1).[5] Boron-10 is 2000 times more likely to capture thermal neutrons than the major atoms in biological tissue (C, H, N, O, P).[5] For successful BNCT, ~20 μg ^{10}B g^{-1} tumor and tumor-to-normal tissue ratios in excess of 3 : 1 to 4 : 1 are required.[1]

Currently, clinical BNCT trials focus on brain tumors, head and neck cancer, metastatic liver cancer, and cutaneous and intracerebral metastatic melanoma.[6] Although considered suboptimal as boron-delivery agents,[1] L-4-dihydroxyborylphenylalanine (BPA) and sodium mercaptoundecahydro-*closo*-dodecaborate (BSH) have been used in these clinical trials.[6] In recent years, the development of novel improved boron-carriers for BNCT has focused on nanoparticles,[7] porphyrin-type macrocycles,[8–11] amino acids,[12] and nucleosides.[13] The chemical, structural and biological properties of thymidine (Thd) analogues, substituted with carboranyl substituents at the N3 position (3-carboranyl Thd analogues or **3CTAs**), will be the subject of this *Feature Article*. Different types of boronated nucleoside- and nucleotide analogues have been discussed in detail in other recent publications.[13–22]

Kinase-mediated trapping (KMT)

A prerequisite for successful BNCT is the selective accumulation of ^{10}B in a tumor. This could be accomplished by targeting specific kinases that are overexpressed in tumor cells with boronated substrates. Phosphorylation by kinases could result in the selective accumulation of the negatively charged metabolites, as shown in Fig. 2 using the example of a Thd analogue substituted at the N3 position with a therapeutic or diagnostic entity. The selective accumulation of cancer therapeutics and diagnostics in tumor cells *via* phosphorylation by intracellular kinases has been referred to as kinase-mediated trapping (KMT).[23] Kinases of low molecular weight substrates seem to be especially suited for such a task. These include, but are not limited to, thymidine kinase 1 (TK1),[24–40] deoxycytidine kinase (dCK),[28,41–52] uridine-cytidine kinase (UCD),[53,54] thymidine monophosphate kinase (TMPK),[55] hexokinase 2 (HK2),[56] and sphingosine kinase 1 (SK1).[57,58]

TK1 and dCK are cytosolic deoxyribonucleoside kinases (dNKs).[59,60] Both enzymes are responsible for the monophosphorylation of various anticancer- and antiviral nucleoside analogues.[61–67] Mono-, di- and triphosphates of these nucleoside analogues can accumulate selectively in millimolar concentrations in cancerous or infected cells.[41–44,68,69] The triphosphates are usually the active metabolites responsible for antiviral/anticancer activity by inhibition of polymerases or reverse transcriptases and termination of DNA chain growth.[61–67] However, they are also responsible for most of the severe toxic side-effects of nucleoside analogue-based cancer chemotherapy by causing damage especially to bone marrow- and intestinal epithelium cells.[65,67]

TK1 may be especially attractive for KMT. In normal cells, TK1 is almost exclusively active in the S-phase.[70,71] However, in some cancer cells, TK1 activity also remained high in other phases of the cell cycle, especially G2 and M.[70–73] TK1 is overexpressed in many different types of malignancies,[24–40] and has become an important prognostic indicator for tumor

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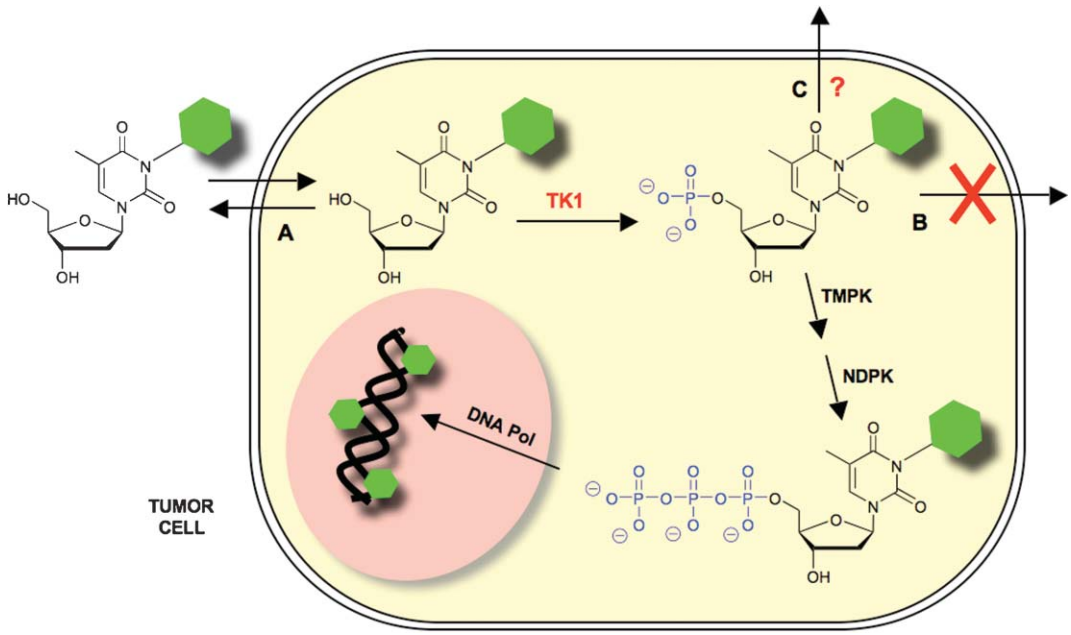


Fig. 2 Basic concept of KMT. A Thd analogue, substituted at the N3 position with a therapeutic or diagnostic entity (●), crosses the membrane of a cancer cell either by passive diffusion or nucleoside transport (**A**). TK1 catalyzes the monophosphorylation of the Thd analogue. Thereby, it causes its intracellular entrapment (**B**) because nucleotides are not substrates of nucleoside transporters and/or because the acquired negative charge makes passive diffusion unlikely. However, multidrug resistance proteins, such as MRP4, MRP5 or MRP8, may facilitate the cellular efflux of the monophosphate (**C**). Further phosphorylation by TMPK and nucleoside diphosphate kinase (NDPK) produces the triphosphate of the Thd analogue, which may be incorporated into DNA by DNA polymerase (DNA pol). *Phosphorylation, and not DNA incorporation, is the underlying mechanism for KMT.*

proliferation and aggressiveness.[74] Aspects of TK1-mediated trapping of 3CTAs, which are the subject of this feature article, will be discussed in a later chapter. In this chapter, we only discuss general features of KMT and several non-boronated agents that operate based on KMT.

The potential positron emission tomography (PET) agent 3′-deoxy-3′-[^{18}F]fluorothymidine (**FLT**)[75–80] (Fig. 3) is a good substrate of TK1[81,82] and it is retained intracellularly primarily in mono- and triphosphate (MP and TP) forms.[83] The latter is not a suitable substrate for DNA polymerase and <1% is incorporated into DNA.[80,83,84] **FLT** may be considered as the prototype of the nucleoside analogues that accumulate intracellularly by TK1-mediated trapping.

Radiolabeled 1-(2′-fluoro-5-methyl-β-arabinofuranosyl)-uracil (**FMAU**) and 1-(2′-fluoro-5-iodo-β-arabinofuranosyl)-uracil (**FIAU**) (Fig. 3) also have been proposed as imaging agents.[85–87] Both agents are not as effectively phosphorylated by TK1 as **FLT**.[82,88] However, their triphosphate forms are incorporated into DNA.[89,90] This makes **FMAU** and **FIAU** representatives of the nucleoside analogues that accumulate intracellularly *via* DNA incorporation. Comparative *in vitro* studies with **FLT**, **FMAU** and **FIAU** in A549 cells indicated that the initial intracellular retention of all three nucleoside analogues depends primarily on TK1 activity and the capacity of TK1 to phosphorylate these nucleoside analogues rather than incorporation of their triphosphates into DNA.[83]

The discovery that 3CTAs are effectively phosphorylated by TK1[91] has stimulated the design, synthesis and evaluation of other Thd analogues having various diagnostic and therapeutic moieties at N3 position (Fig. 3).[23,92–97] Among these agents, **NFT202** [3-(2-fluoroethyl)thymidine] has been studied most extensively, both *in vitro* and *in vivo*.[92–94] The TK1 substrate characteristics and DNA incorporation capacities of **NFT202** were comparable to those of **FLT**.[94] The affinity of **NFT202** for nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transporter was five times higher than that of **FLT** and it was not a substrate for *E. coli* Thd phosphorylase.[94] The *in vitro* cellular uptake of **NFT202** in TK1 (+) and TK1 (−) L-M cells was dependant on TK1 activity.[93] In A549 cells, uptake correlated positively with the fraction of cells in the S-phase.[93] However, uptake of **FLT** in A549 cells was 2–3 times higher.[93] When administered intravenously (i.v.), **FLT**, but not **NFT202**, showed tumor selectivity in C57Bl/6 mice bearing Lewis lung carcinomas (LL/2).[93] Similar observations were made when **FLT** or a Thd analogue, substituted at N3 with a ^{99m}Tc-labeled monoamino-monoamide dithiol (MAMA) ligand, were injected i.v. into radiation-induced fibrosarcoma (RIF) tumor-bearing mice.[96] Interestingly, **FLT** was 12 times more effective in inhibiting Thd phosphorylation by TK1 than **NFT202** (IC_{50} values: 6.7 *vs.* 81 μM)[92] and the ^{99m}Tc–MAMA-Thd complex appeared to be completely devoid of any TK1-inhibitory capacity (IC_{50} values: >500 *vs.* 48.45 μg mL^{-1} for Thd).[96]

Aspland *et al.* synthesized several Thd analogues with either fluorescein, paclitaxel or vinblastine attached to N3 of Thd *via* methylene spacers of varying lengths (Fig. 3).[23] A Thd-fluorescein conjugate accumulated in CEM and K562 cells in correlation with TK1 activity levels in these cells. Various Thd-paclitaxel- and Thd-vinblastine conjugates were phosphorylated by TK1 to a significant extent and they also retained or

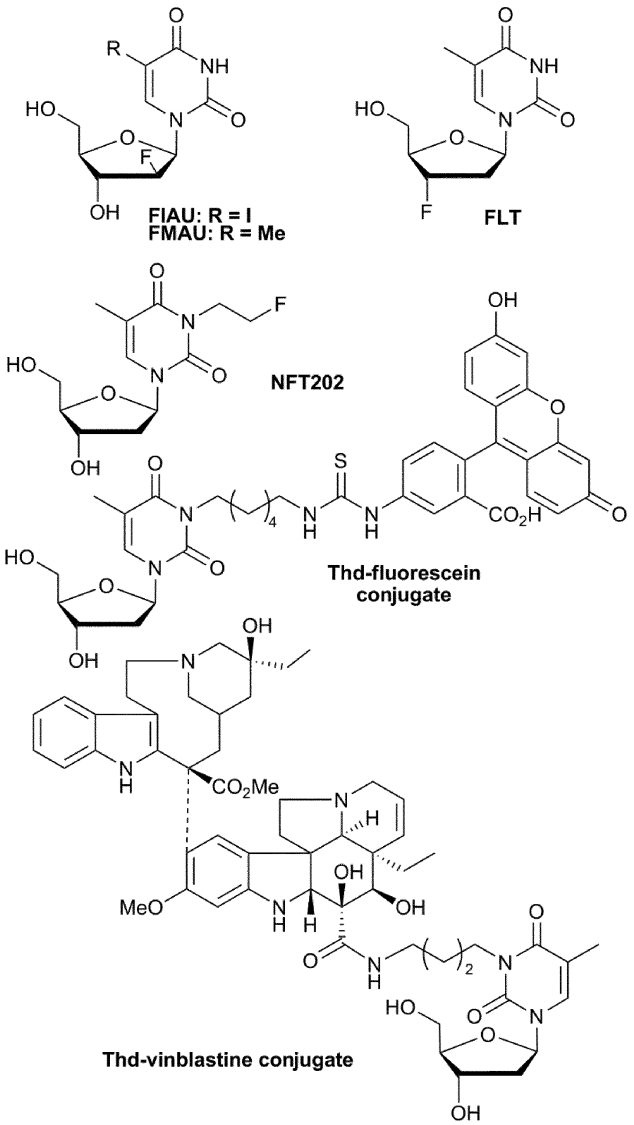


Fig. 3 Non-boronated Thd analogues that are trapped intracellularly by incorporation into DNA (**FIAU**, **FMAU**) or by KMT (**FLT**, **NFT202**, Thd-fluorescein- and Thd-vinblastine conjugates).

even exceeded the microtubule binding affinities of parental paclitaxel and vinblastine. In K562 leukemia cells, EC_{50} values of 10–43 nM were found for the Thd-vinblastine conjugates. However, they were less toxic than vinblastine (EC_{50}: 1 nM). The reduced cytotoxicity was attributed to a decrease in lipophilicity resulting from the hydrophilic Thd moiety, which could impede passive diffusion. It is noteworthy that not all tested Thd-paclitaxel- and Thd-vinblastine conjugates were phosphorylated by TK1. Lack of any TK1 substrate characteristics was also observed for a Thd analogue substituted at N3 with an ^{111}In-labeled 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (DO3A) macrocycle.[95]

TK1-like enzymes

Based on amino acid sequence identities and overall structure, dNKs can be divided in two families. Human TK1 (hTK1) belongs to a group of enzymes referred to as "TK1-like enzymes" or "TK1-like family", only accepting endogenous Thd or 2′-deoxyuridine (dUrd) as substrates, whereas dCK, thymidine kinase 2 (TK2) and deoxyguanosine kinase (dGK) belong to a second group of enzymes with broader substrate specificity, which has been described as the "non-TK1-like family".[60,98–100] The TKs from poxviruses[101,102] and various bacteria[60,81,98–100,103–108] [*Bacillus anthracis* TK (*Ba*TK), *Bacillus cereus* TK (*Bc*TK), *Ureaplasma urelyticum* TK (*Uu*TK), *Clostridium acetobutylicum* TK (*Ca*TK), *Thermotoga maritime* TK (*Tm*TK)] belong to the TK1-like enzymes with 35–65% sequence identity to hTK1. TKs from herpes viruses comprise a third group of dNKs with amino acid sequences and overall structures more closely related to the enzymes of the non-TK1-like family[60,98,109–112] Dual TK- and TMPK activity is a special feature of most herpes viruses TKs.[112–115] Recent crystallographic studies with *Ba*TK and *Bc*TK, having 96% amino acid sequence identity,[99] have shown that depending on the presence or absence of the endogenous substrate, Thd, in the substrate binding pocket, TK1-like enzymes exist in a closed and a more open form (Fig. 4).[99] In the case of *Ba*TK (cyan, Fig. 4), a strong hydrogen bond network exists between backbone amino acids of the so-called "lasso" domain with tyrosine-179 and arginine-157 as well as $C_{3'}$–OH, N_3–H and C_4=O of Thd, thereby causing closure of the lasso domain. In the case of *Bc*TK (yellow, Fig. 4), an exogenous substrate, 2-methyl-2,4-pentanediol (MPD), has displaced Thd thereby causing a breakdown of this hydrogen bond network and, consequently, an opening of the lasso domain. However, MPD could be part of a weaker hydrogen bond network with tyrosine-179 and backbone amino acids of the lasso domain. It is therefore conceivable that in TK1-like enzymes without any substrate, the highly flexible lasso domain may even take a different conformation than in *Bc*TK. This flexibility of the lasso domain may explain specific substrate/inhibitor characteristics of N3-substituted Thd analogues, as will be discussed in a

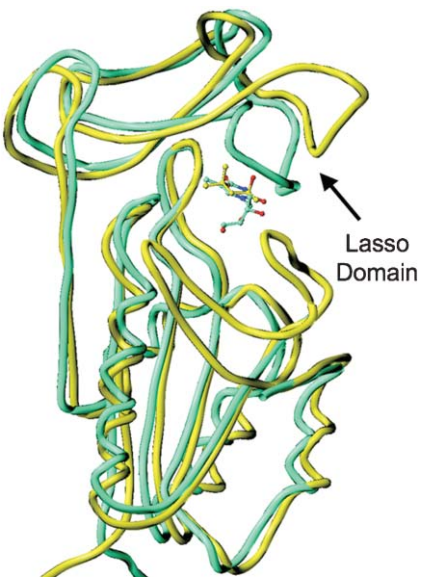


Fig. 4 Overlap of crystal structures of *Ba*TK (cyan, PDB# 2J9R) and *Bc*TK (yellow, PDB# 2JA1).

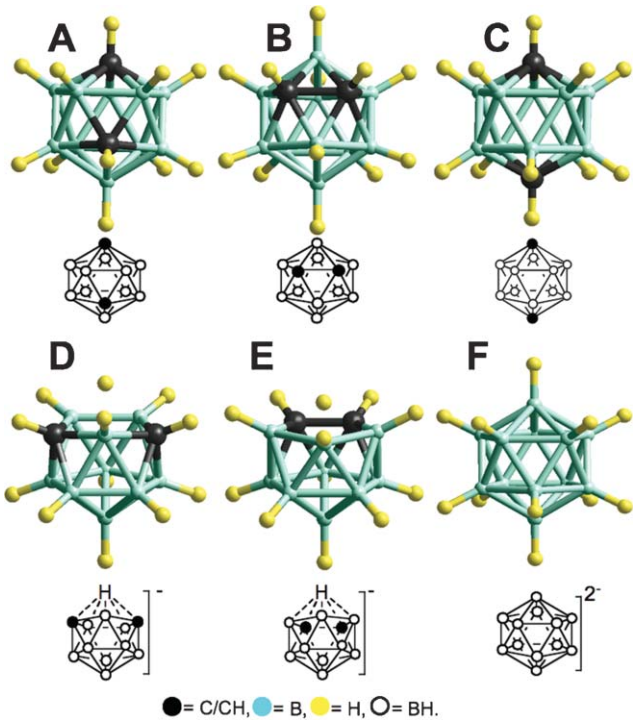


Fig. 5 Boron clusters frequently used in BNCT compound development; *closo-m*-carborane (**A**), *closo-o*-carborane (**B**), *closo-p*-carborane (**C**), *nido-m*-carborane (**D**), *nido-o*-carborane (**E**), dodecahydrododecaborate (2-) (**F**). Structures were optimized at the B3PW91/6-31G* level.

following chapter. Conformational flexibility of proteins, as in the case of TK1-like enzymes, is more and more recognized as a significant problem in computer-aided structure-based drug design.[116]

Boron clusters in BNCT compound development

Carborane cages (Fig. 5) have been the preferred boron moieties in BNCT compound development because of their chemically modifiable nature, structural and physicochemical versatility, high boron content and stability under physiological conditions.[5,117–122] In addition, boron cluster-specific computational drug design techniques have been developed recently.[123–128] Carboranes have also found increasing use as hydrophobic structural units in the conventional design and synthesis of biologically active agents (*e.g.* carboranyl transthyretin amyloidosis inhibitors, carboranyl antifolates, carboranyl cholesterols and carboranyl estrogen- and androgen receptor agonists and antagonists).[128–136] The chemical reactivity of σ-aromatic carboranes is, in part, similar to that of π-aromatic benzene.[127,128,137–143] *Closo*-carborane cages are in dimensions and lipophilicity similar to adamantane.[125,128,144,145] The degradation of the highly lipophilic *closo-m*-carborane to the corresponding water-soluble anionic *nido-m*-carborane is possible with tetrabutylammonium fluoride (TBAF) in refluxing THF,[121,146] however, the equally lipophilic *closo-o*-carborane is even more reactive and can be degraded either by TBAF or by bases, such as piperidine and pyrrolidine, at lower temperatures to the corresponding anionic *nido-o*-carborane.[147] Recently, it was reported that *closo-o*-carboranes substituted with electron-withdrawing groups can be degraded to the corresponding *nido-o*-carboranes in wet DMSO under neutral conditions.[148] *Closo-p*-carborane, on the other hand, is stable under conditions sufficient for *closo-m*- and *closo-o*-carborane degradation[121,146] and can only be degraded under extremely harsh reaction conditions to the corresponding *nido*-form.[149] This difference in cage-degradation conditions could facilitate the elegant modeling of physicochemical properties in compounds containing different types of carboranes (lipophilic *vs.* hydrophilic *vs.* amphiphilic).

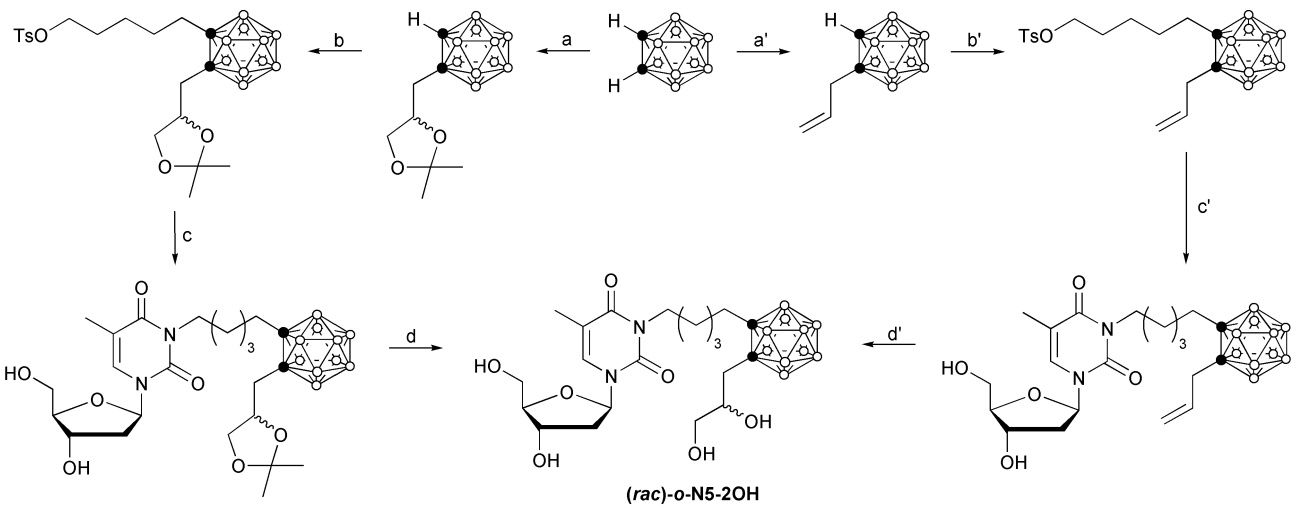
Another boron cluster that has found fairly frequent use as a boron moiety in the design and synthesis of BNCT agents is the very stable two-fold negatively charged dodecahydrododecaborate (2−) [$B_{12}H_{12}^{2-}$] cage (Fig. 5).[118,122] Most recently, it has found application in the design and synthesis of boronated porphyrin-type macrocycles and boronated surrogates of phospholipids and cholesterol for incorporation into liposomes.[8,11,150–152]

It has been reported that the B–I bond in boron clusters may be less susceptible to enzymatic and/or hydrolytic cleavage than the C–I bond.[153] Therefore, radiohalogenated pharmaceuticals containing astatine-211 or radioactive bromine/iodine attached to a boron cluster have attracted considerable attention in recent years.[154–160]

Synthesis of 3CTAs

A 10-step synthesis for the most widely studied 3CTA, designated **(*rac*)-*o*-N5-2OH**† (3-[5-{2-(2,3-dihydroxyprop-1-yl)-*o*-carboran-1-yl}pentan-1-yl]thymidine), was originally reported in 2002 by Al-Madhoun *et al.*[161] This lengthy synthetic route resulted in a mixture of two epimers with naturally-occurring boron isotope distribution and was found impractical for the synthesis of **N5-2OH** in ^{10}B enriched- and/or stereochemically pure form. Therefore, two shorter and more versatile synthetic routes were developed by Byun *et al.* (Scheme 1).[127] The starting material for both synthetic routes was *closo-o*-carborane, which reacted with (*rac*)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate (a) or allyl bromide (a′). Pentane-1,5-diol di-*p*-tosylate was used to link the carborane clusters to Thd (b, b′, c, c′). In order to minimize disubstitution during reaction steps b and b′, the lithium salts of the monosubstituted *o*-carboranes were first dissolved in benzene and then added very slowly to solutions of 1,5-pentanediol di-*p*-ditosylate in benzene at low temperatures. Acidic cleavage of the acetonide protective group (d) or oxidation of the allyl function with osmium tetraoxide (d′) yielded **(*rac*)-*o*-[^{10}B]-N5-2OH** as epimeric mixtures with *R*/*S*-ratio of approximately 1 : 1 and 2 : 3, respectively. The overall yields were 41% (a–d) (S. Narayanasamy, unpublished results) and 15% (a′–d′),[127] respectively. Both synthetic routes were used to synthesize **(*rac*)-*o*-[^{10}B]-N5-2OH** for preclinical neutron irradiation studies. The synthetic sequence involving steps a–d was found

† Throughout the paper, the prefixes (*rac*), (*R*) and (*S*) used in connection with **N5-2OH** isomers refer only to the stereogenic center in the 2,3-dihydroxypropyl side chain of the molecules.



Scheme 1 Synthetic routes to **(*rac*)-*o*-N5-2OH**. *Reagents and conditions*: (a) *n*-BuLi, 2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate, benzene, rt, 14 h; (b) *n*-BuLi, 1,5-pentanediol di-*p*-tosylate, benzene, 5–10 °C, 1 h; (c) Thd, K_2CO_3, DMF–acetone (1 : 1), 50 °C, 48 h; (d) 17% HCl, MeOH. (a′) *n*-BuLi, allyl bromide, THF, rt, 12 h; (b′) *n*-BuLi, 1,5-pentanediol di-*p*-tosylate, benzene, 5–10 °C, 1 h; (c′) Thd, K_2CO_3, DMF–acetone (1 : 1), 35 °C, 96 h; (d′) OsO_4, *N*-methylmorpholine-*N*-oxide, 1,4-dioxane , rt, 6 h.

to be advantageous since it produced higher yields, did not require highly toxic osmium tetraoxide and permitted the synthesis of pure stereoisomers using (*R*)- or (*S*)-2,2-dimethyl-1,3-dioxolane-4-ylmethyl *p*-tosylate as starting materials. **(*R*)-*o*-N5-2OH**, **(*S*)-*o*-N5-2OH**, **(*rac*)-*m*-N5-2OH** and **(*rac*)-*p*-N5-2OH** were synthesized in 15%–20% overall yield utilizing this reaction sequence (Fig. 6).[127]

The dihydroxypropyl group was attached to the second carbon of the *closo*-*o*-carborane cage in **(*rac*)-*o*-N5-2OH** in an attempt to reduce lipophilicity. Nevertheless, **(*rac*)-*o*-N5-2OH** lacks functional groups that are ionizable under physiological conditions and the carborane cluster renders the entire molecule still very lipophilic (log P = 2.09).[162] Consequently, aqueous solutions containing 50–70% DMSO had to be used in biological studies to dissolve this agent.[24,127,162,163] This lack of water-solubility was a major motivation for the design and synthesis of second-generation 3CTAs with improved solubility properties that retained the excellent TK1 substrate characteristics and cell membrane crossing capacity of **(*rac*)-*o*-N5-2OH**. So far, three 3CTAs were identified that seem to fulfill this requirement.

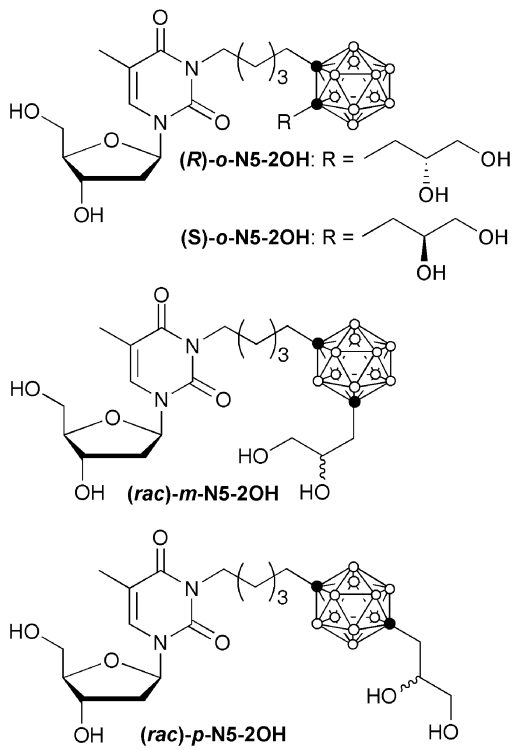
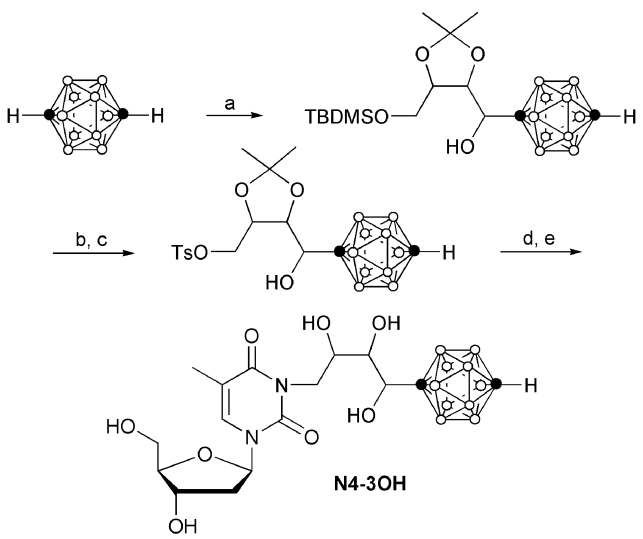


Fig. 6 Stereochemical- and geometrical isomers of **N5-2OH**.

This first agent is **N4-3OH**, which was prepared by adapting the general synthetic strategy applied for the synthesis of **(*rac*)-*o*-N5-2OH** (Scheme 2).[125,164] The mono-lithium salt of

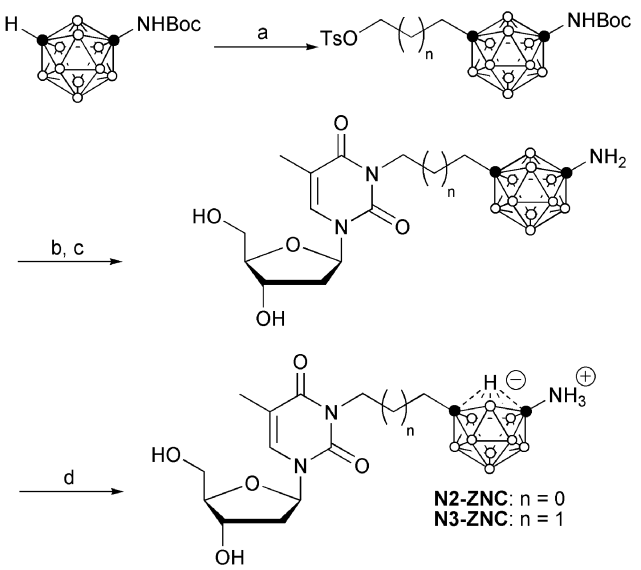


Scheme 2 Synthesis of **N4-3OH**. *Reagents and conditions*: (a) *n*-BuLi, 4-(*tert*-butyldimethylsilyloxy)-2,3-(isopropylidenedioxy)butanal, THF, rt, 12 h; (b) TBAF, THF, −78 °C, 1 h; (c) TsCl, pyridine–CH_2Cl_2 (1 : 6), rt, 12 h; (d) Thd, K_2CO_3, DMF–acetone (1 : 1), 50 °C, 24–48 h; (e) 17% HCl in MeOH, rt, 14 h.

closo-p-carborane was reacted with 4-(*tert*-butyldimethylsilyloxy)-2,3-(isopropylidenedioxy)butanal,[165] which was used to introduce the linker unit between carborane and Thd (step a). In contrast to the syntheses for various **N5-2OH** isomers, a four-carbon spacer was used that also contained three hydroxyl groups. It was hypothesized that the lower molecular weight of **N4-3OH** combined with the additional hydroxyl group would improve water-solubility. Further standard reactions (steps b–e) furnished **N4-3OH** in 12% overall yield. The reaction of *closo-p*-carborane with an aldehyde generated an unprotected hydroxyl group at the carbon atom next to the bulky cluster. This hydroxyl group did not seem to participate/interfere significantly with the following reactions presumably due to steric hindrance by the neighboring carborane.

Another strategy to improve water-solubility of 3CTAs is shown in Scheme 3.[146,166] In this reaction sequence, *tert*-butoxycarbonyl (Boc)-protected amino-*closo-m*-carborane[167] served as the starting material and ethane-1,2-diol di-*p*-tosylate or propane-1,3-diol di-*p*-tosylate were used to insert the linker between the cluster and Thd (steps a and b). The Boc-group was removed with trifluoroacetic acid (TFA) (step c) and the *closo-m*-carborane cage was converted into the corresponding *nido-m*-carborane (step d). The final products, **N2-ZNC** and **N3-ZNC**, were isolated in 10% and 8% overall yield, respectively, as zwitterionic structures.[146,166]

Although they were significantly more hydrophilic than **(*rac*)-*o*-N5-2OH**,[125,146,166] **N2-ZNC**, **N3-ZNC** and **N4-3OH** should still be able to cross cell membranes by passive diffusion because theoretical calculations indicated that their physicochemical parameters were in the range of those of the antiviral nucleoside analogues abacavir, zidovudine (AZT) and emtricitabine, which presumably enter cells largely by passive diffusion.[168–171] Sufficient lipophilicity is a very important criterion for 3CTAs because they may not be substrates of nucleoside transporters located in cellular membranes.[125] Despite improved hydrophilicity, neither of the three second-generation 3CTAs was completely water-soluble and up to 25% DMSO had to be used for their complete dissolution.[146]



Scheme 3 Synthesis of **N2-ZNC** and **N3-ZNC**. *Reagents and conditions*: (a) ethanediol di-*p*-tosylate (or propanediol di-*p*-tosylate), *n*-BuLi, benzene, 10 °C, 0.5–1 h; (b) Thd, K_2CO_3, DMF–acetone (1 : 1), 50 °C, 48 h ; (c) CF_3COOH, CH_2Cl_2, rt, 24 h; (d) TBAF, THF, 70 °C, 1–2 h.

Other types of hydrophilically-enhanced 3CTAs were synthesized containing *e.g.* polyethylene glycol (PEG) groups or simple *nido*-carborane cages (Fig. 7).[143,166] Although all of these analogues were substrates of TK1, their overall substrates characteristics were inferior to **N5-2OH**, **N4-3OH**, **N2-ZNC** and **N3-ZNC**.[13] In the case of 3CTAs with negatively charged *nido*-carborane cages, it also seems unlikely that they can enter cells effectively *via* passive diffusion.

Zwitterionic NH_3^+-*nido-m*-carborane, an interesting new boron moiety for BNCT compound development

Zwitterionic **N3-ZNC** and **N2-ZNC** showed hydrophilicity/lipophilicity properties midway between those of lipophilic *closo*- and hydrophilic *nido*-3CTAs, demonstrating the usefulness of NH_3^+-*nido-m*-carborane as a novel boron moiety in BNCT drug design.[146,166] The unique physicochemical characteristics of zwitterionic NH_3^+-*nido-m*-carborane (Fig. 8) are the result of an intramolecular charge-compensation between the positively charged NH_3^+-group and the negatively charged carborane cage. Interestingly, NH_3^+-*nido-m*-carborane also showed very interesting chemical and spectroscopic properties.[172] The compound reacted with several ketones (*e.g.*, acetophenone, cyclohexanone, methyl ethyl ketone) and aldehydes (*e.g.*, acrolein, acetaldehyde, anisaldehyde, 2,4-dimethoxybenzaldehyde) in anhydrous benzene without addition of any catalyst to generate iminium-type compounds, such as the acetone complex of NH_3^+-*nido-m*-carborane shown in Fig. 8.[172] These iminium *nido-m*-carboranes were stable in anhydrous polar and non-polar solvents but they hydrolyzed rapidly in aqueous milieu to release the free ammonium precursor.[172] Therefore, we do not anticipate that this specific reactivity of NH_3^+-*nido-m*-carborane will negatively impact its use as a boron-carrying moiety in BNCT.

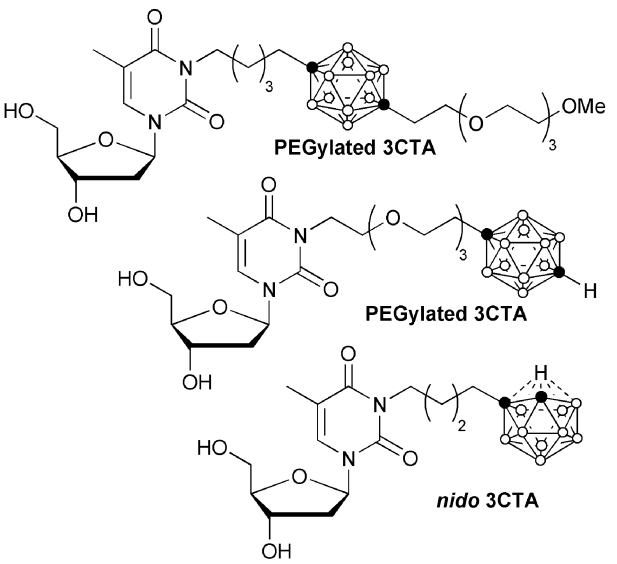


Fig. 7 Miscellaneous 3CTAs.

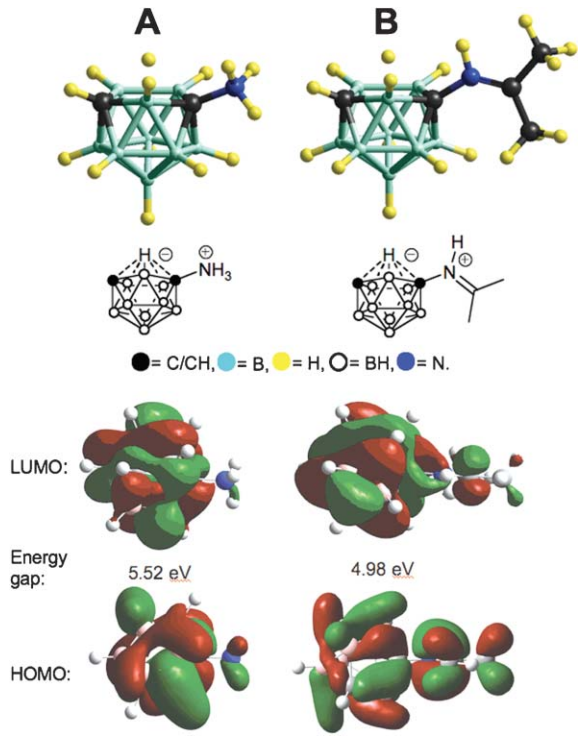


Fig. 8 Structures and HOMOs/LUMOs of NH_3^+-*nido*-*m*-carborane (**A**) and its acetone complex (**B**).

The UV absorption spectrum of the acetone complex of NH_3^+-*nido*-*m*-carborane showed bands at the 220 and 253 nm.[172] Only the 220 nm band is characteristic for the 3D σ-aromatic system of carboranes.[173] Electron delocalization between the 3D σ-aromatic system of the *nido*-*m*-carborane cluster and the non-aromatic iminium group produced a bathochromic shift with the appearance of a strong absorption band at 253 nm.[172]

Theoretical molecular orbital (HOMO/LUMO) calculations of NH_3^+-*nido*-*m*-carborane and its acetone complex at the B3LYP/6-31G* level substantiated the observation of the bathochromic shift (Fig. 8).[172] Complete overlap of alternating-phase orbitals with similar sizes led to the delocalization of electrons between the negatively charged *nido*-*m*-carborane cluster and the positively charged nitrogen in the acetone complex. In contrast, there was no significant overlap of alternating-phase orbitals of the corresponding moieties in NH_3^+-*nido*-*m*-carborane, which is also consistent with the larger energy gap between HOMO and LUMO (5.52 *vs.* 4.98 eV).[172]

In vitro, enzymatic and computational studies with 3CTAs

Overall, uptake and retention of **(*rac*)-*o*-N5-2OH** and other 3CTAs (*e.g.* **N3-ZNC**) in various cell lines (F98 glioma, MRA 27 melanoma, TK1 (+)/TK (−) CEM lymphoblast and TK1 (+)/TK1 (−) L929 fibroblast) correlated with TK1 activities and their *in vitro* toxicities were generally moderate to low.[24,146,162,164] This indicated that the tumor cell selectivity of 3CTAs relies to some extent on KMT, which is further supported by the fact that **(*rac*)-*o*-N5-2OH** was a good substrate of TK1 with a rk_{cat}/K_M value of 35.8% ($rk_{cat}/K_M = k_{cat}/K_M$ relative to that of Thd).[162] For comparison, the rk_{cat}/K_M values of the established TK1 substrates **FLT** and AZT are 7.6 and 43.7%, respectively.[82,125] **(*rac*)-*o*-N5-2OH** was also found to be a good substrate of *Ba*TK.[81] The TK1 substrate characteristics of **(*R*)-*o*-N5-2OH**, **(*S*)-*o*-N5-2OH**, **(*rac*)-*m*-N5-2OH** and **(*rac*)-*p*-N5-2OH** were comparable with that of **(*rac*)-*o*-N5-2OH**[127] while those of **N2-ZNC**[146] and **N4-3OH**[125] appeared to be superior. **(*rac*)-*o*-N5-2OH** and other 3CTAs were not substrates of TK2,[91,143,146,161] *Drosophila melanogaster* (*Dm*)-dNK,[64] dCK,[164] and thymidine phosphorylase (TPase).[162] N5-2OH monophosphate was not a substrate of deoxynucleotidase-1 (dNT-1),[162] whereas other 3CTA monophosphates were effectively dephosphorylated by alkaline phosphatase.[91,174]

Similar to **NFT202** and other N3-substituted Thd analogues,[92,96] **(*rac*)-*o*-N5-2OH** and **N4-3OH** were only weak inhibitors of monophosphorylation of Thd by TK1.[125,162] This phenomenon may be explained by the specific binding pattern of 3CTAs to the active site of TK1-like enzymes, as demonstrated by **(*rac*)-*o*-N5-2OH** docked into the crystal structure of *Bc*TK. As shown in Fig. 9 (generated with FlexX/SYBYL 7.1, Tripos, St. Louis, MO), the open conformation of *Bc*TK accommodates binding of the entire molecule of **(*rac*)-*o*-N5-2OH** in the vicinity of active site with the bulky carboranyl side chain oriented towards the surface of enzyme. Interestingly, it was not possible to dock **(*rac*)-*o*-N5-2OH** anywhere near the active site of the closed crystal structure of *Ba*TK. Therefore, we hypothesize that only open forms of TK1-like enzymes allow effective binding of **(*rac*)-*o*-N5-2OH** and other 3CTAs. Fig. 10 shows the alignment of twenty-one 3CTAs (generated with SYBYL 7.1).[126] These 3CTAs have TK1 phosphorylation rates of 10–72% compared to that of Thd.[126] The large area occupied by the N3-carboranyl

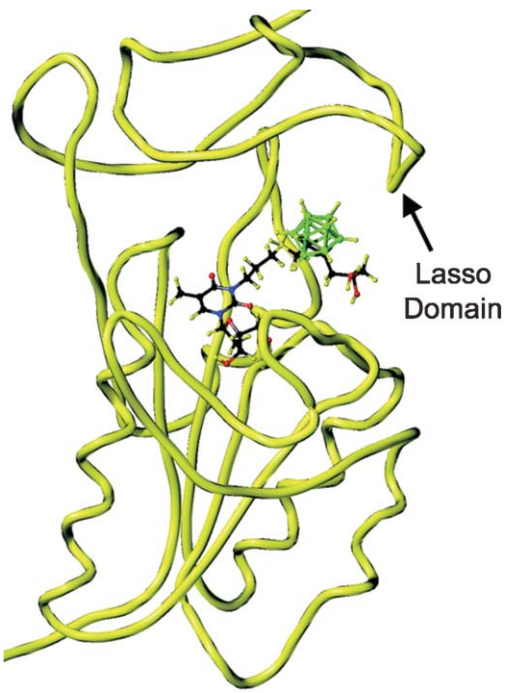


Fig. 9 Docked pose of N5-2OH in the crystal structure of *Bc*TK

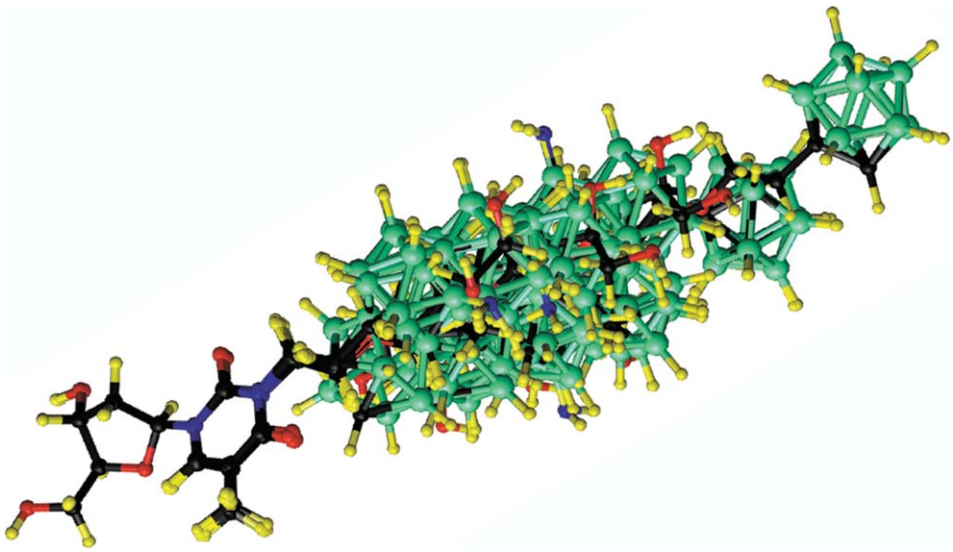


Fig. 10 Alignment of twenty-one 3CTAs.

substituents of these 3CTAs demonstrates impressively how flexible the active sites of TK1-like enzymes must be to accommodate the binding of these structures.

When docked into *Bc*TK, the Thd portion of **(*rac*)-*o*-N5-2OH** did not bind to the same active site region as Thd in *Ba*TK (see Fig. 4 and 9). Similar observations were made when Thd was extracted from *Ba*TK and docked into *Bc*TK. In this case, Thd bound to the same active site region as the Thd portion of docked **(*rac*)-*o*-N5-2OH** with a similar orientation of the thymine base. Therefore, we have developed the following hypothesis for the binding of **(*rac*)-*o*-N5-2OH** to TK1-like enzymes: the initial approach of **(*rac*)-*o*-N5-2OH** (or Thd) to open forms of TK1-like enzymes may be directed to a location different from that of Thd in closed *Ba*TK. As a result of **(*rac*)-*o*-N5-2OH** binding, the lasso domain may close and thus, force the deoxyribose portion of **(*rac*)-*o*-N5-2OH** into an orientation that allows for the effective transfer of the γ-phosphate from ATP either to the 5′- or 3′-hydroxyl group of **(*rac*)-*o*-N5-2OH** while the carborane cluster will be further relocated to the enzyme surface (“induced fit”). However, due to the bulkiness of the carboranyl N3-substituent, complete closure of the active site will not be possible and **(*rac*)-*o*-N5-2OH** continues to be susceptible to competition by Thd, the endogenous substrate of TK1-like enzymes. This could accounts for the weak TK1-inhibitory capacity of 3CTAs.

There is a sensitive balance between the inhibitor and substrate characteristics of endogenous and exogenous nucleoside kinase-activated compounds, as has been convincingly demonstrated by Black, Knecht and collaborators in suicide gene therapy studies with ganciclovir and AZT.[175,176] They showed that both nucleoside analogues must be able to compete favorably with endogenous nucleoside substrates at the substrate-binding sites of their activating kinases, Herpes Simplex Virus thymidine kinase (HSV-TK) and *Dm*-dNK, respectively, to be effective as suicide gene therapy agents in triphosphate form on the DNA/DNA polymerase level.

In vivo biological evaluation of (*rac*)-*o*-N5-2OH

Based on its low *in vitro* toxicity and favorable *in vitro* uptake and retention, *in vivo* studies were initiated to evaluate **(*rac*)-*o*-N5-2OH** as a boron-delivery agent for NCT. Initial biodistribution and therapy studies were carried out in immunologically deficient NIH nude mice bearing subcutaneous (s.c.) implants of TK1 (+) or TK1 (−) L929 tumors.[163] These were produced by s.c. implantation of L929 wild-type cells, which are TK1 positive, or their mutant counterparts, L-M, which are TK1 negative. Initially, **(*rac*)-*o*-N5-2OH** was administered systemically by intraperitoneal (i.p.) injection. However, tumor boron uptake was low and blood and normal tissue values were high. This finding is consistent with the observed lack of tumor accumulation of **NFT202** and a ^{99m}Tc-MAMA-Thd complex following i.v. injection.[93,96] We therefore evaluated direct intratumoral (i.t.) injection as a route of administration. Following i.t. injection of 500 μg (∼100 μg boron) of **(*rac*)-*o*-N5-2OH**, TK1 (+) tumors had a boron concentration that was approximately three times greater than that of TK1 (−) tumors (22.8 *vs.* 8.4 μg g^{-1}) with very low levels in normal tissue.[163] Based on the favorable *in vivo* uptake data, therapy studies were carried out in nude mice bearing s.c. implants of either TK1 (+) or TK1 (−) L929 tumors.[163] Thirteen days following tumor implantation, the animals were transported to the Nuclear Reactor Laboratory at the Massachusetts Institute of Technology (MIT) for BNCT. **(*rac*)-*o*-[^{10}B]N5-2OH** at a concentration of 31.6 mM (50 μg ^{10}B) was solubilized in 70% DMSO and injected i.t., followed by a second injection 2 h later. Animals then were irradiated with a collimated beam of thermal neutrons at the MIT reactor (MITR). The measured absorbed dose rate for the boron-neutron capture reaction was 4.0 cGy min^{-1} per μg of ^{10}B in tissue. Animals bearing TK1 (+) tumors had a 15-fold inhibition in tumor growth, as determined by volume determinations, compared to those bearing TK1 (+) tumors that had not received BNCT (247 mm^3 *vs.* 3603 mm^3). Animals bearing TK1 (−) tumors had a modest reduction in tumor volume, but this was not significantly different from that of

animals bearing TK1 (+) tumor that did not receive **(*rac*)-*o*-N5-2OH**. These studies provided clear evidence for both target selectivity and therapeutic efficacy.

Based on these findings, biodistribution and therapy studies were initiated using syngeneic Fischer rats bearing intracerebral (i.c.) implants of the F98 glioma.[163] This model has been used extensively by us to evaluate the efficacy of BNCT.[177–181] Since systemic administration of **(*rac*)-*o*-N5-2OH** resulted in low tumor boron values, animals received 375 μg of the agent (~75 μg boron), solubilized in 10 μL 50% DMSO, administered directly into the tumor by convection-enhanced delivery (CED). This technique, by which agents are infused directly into the central nervous system, is an innovative method to increase drug uptake and distribution within the brain. It primarily has been used to deliver high molecular weight agents,[182,183] and less frequently for low molecular weight drugs.[184] We have used CED for the administration of boronated bioconjugates of epidermal growth factor (EGF),[185,186] the anti-EGFR monoclonal antibody (MoAb) Cetuximab,[187] and the anti-EGFRvIII MoAb L8A4[188] to rats bearing i.c. F98 gliomas that had been genetically engineered to express either EGFR or EGFRvIII. CED of these agents resulted in high tumor uptake and tumor retention with concomitantly low uptake in the adjacent normal brain tissue. At 2½ h following CED of **(*rac*)-*o*-N5-2OH**, there was an eightfold increase in boron concentrations in the tumor, while in contrast, uptake in the surrounding ipsilateral brain was low (16.2 *vs.* 2.2 μg g^{-1}) and the blood concentration was even lower (<0.5 μg g^{-1}).[24] In contrast, CED of BPA at a concentration equivalent to 75 μg boron resulted in boron concentrations of 24.2 μg g^{-1} (tumor), 14.5 μg g^{-1} (ipsilateral brain) and 3.5 μg g^{-1} (blood).[24] These results demonstrated that the biodistribution of **(*rac*)-*o*-N5-2OH**, following CED, was similar to that of the boronated bioconjugates, but differed markedly from that of BPA. When BPA was administered i.v. (500 mg kg^{-1} body weight), a more favorable tumor/normal brain ratio was found with boron concentrations of 10.7 μg g^{-1} (tumor), 3.8 μg g^{-1} (brain) and 5.2 μg g^{-1} (blood).[187] Nevertheless, BPA appears to have a tendency to accumulate in normal brain even following systemic administration. This has also been observed by Miyatake and coworkers in pharmacokinetic studies with C6 tumor-bearing rats[189,190] and it may have been a limiting factor in BPA-based clinical BNCT.[6,191–195] In theory, the i.v. administration of BPA, combined with CED of **(*rac*)-*o*-N5-2OH**, should increase the tumor boron concentrations while normal brain boron concentrations would only increase modestly.

Based on the favorable *in vivo* uptake data of **(*rac*)-*o*-N5-2OH**, pilot BNCT studies were carried out in F98 glioma bearing rats approximately 2 weeks following i.c. tumor implantation.[163] The amount of 500 μg (~100 μg boron) of **(*rac*)-*o*-[^{10}B]N5-2OH** was administered by CED and the animals were irradiated 24 h later at the MITR. There was a 52% increase in median lifespan (%ILS) of animals that received **(*rac*)-*o*-[^{10}B]N5-2OH** and NCT compared to untreated controls. The corresponding mean survival times (MST) were 38 days and 25 days, respectively, which were comparable to those that we have obtained with BPA using the same tumor model.[178] Further studies are in progress to evaluate **(*rac*)-*o*-[^{10}B]N5-2OH** as a boron delivery agent using the RG2 rat glioma model[196] and promising preliminary data recently have been obtained.

Conclusions

KMT appears to be an effective mechanism for the selective entrapment of anticancer diagnostics and therapeutics in tumors, and, in the case of 3CTAs, has been the biochemical basis for promising preclinical BNCT.

Versatile synthetic routes for the large-scale preparation of **N5-2OH**, the most widely studied 3CTA, in ^{10}B-enriched- and/or stereochemically pure form were developed and three second-generation 3CTAs (**N4-3OH**, **N2-ZNC** and **N3-ZNC**) were identified that have the potential to replace **N5-2OH** as the current lead 3CTA. As "side products" of our program for the development of boronated nucleoside analogues, *closo*-carborane-specific computational drug design techniques were developed,[123–128] zwitterionic NH_3^+-*nido*-*m*-carborane was discovered as a novel boron moiety for NCT compound development,[146,166,172] and the fascinating molecular interactions of **N5-2OH** with TK1-like enzymes were studied.

However, there are several shortcomings and problems associated with the currently available 3CTA technology that demand further exploration: (1) The most promising 3CTAs (**N5-2OH**, **N2-ZNC**, **N3-ZNC**, **N4-3OH**) were not water-soluble and required organic solvents for dissolution. This warrants the development of 3CTA-specifc drug delivery techniques that could be based on lipid-nanotechnology or the design and synthesis of water-soluble third-generation 3CTA prodrugs. (2) The ineffective competition with Thd at the substrate binding site of TK1-like enzymes presumably is another major shortcoming of currently available 3CTAs and other therapeutic and diagnostic N3-substituted thymidine analogues. It could be responsible for their lack of *in vivo* tumor selectivity following systemic administration. It is conceivable that this drawback can only be overcome by producing very high local drug concentrations of these agents, either by i.t. injection or CED, which may restrict their application range to brain tumors. This limitation calls for the design and synthesis of 3CTAs with improved TK1-inhibitory capacity. (3) Almost nothing is known about the *in vitro*- and *in vivo* metabolism of 3CTAs, including their possible incorporation into DNA (Fig. 2). Indeed, it is even unknown if 3CTAs can be phosphorylated beyond the monophosphate level in phosphoryl transfer assays with purified recombinant TMPK and NDPK. Also, very little information is available about the mechanisms of cellular influx and efflux of 3CTAs and their metabolites. Recently, it has been discovered that the presence of the multidrug resistance proteins MRP4, MRP5 and MRP8 in cancer cells is associated with resistance to numerous nucleoside analogues used in anticancer and antiviral therapy.[197–201] Presumably only the monophosphate forms of antiviral and anticancer nucleoside derivatives, but not their di- and triphosphate forms, are substrate of these efflux pumps.[197–200] It is conceivable that 3CTAs-monophosphates or other N3-substituted thymidine analogues are also substrates of MRP4, MRP5 or MRP8 (Fig. 2), which could contribute to their lack of *in vivo* tumor selectivity following

systemic administration. Obtaining the missing information regarding the metabolism and cellular entrance/exit strategies of 3CTAs will be crucial to understand their biological properties (*e.g.* toxicity and therapeutic efficacy)[13,117] and to decide if it is worthwhile to advance 3CTA-based BNCT to a clinical stage.

Acknowledgements

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References

1 R. F. Barth, J. A. Coderre, M. G. H. Vicente and T. E. Blue, *Clin. Cancer Res.*, 2005, **11**, 3987–4002.
2 C. Salt, A. J. Lennox, M. Takagaki, J. A. Maguire and N. S. Hosmane, *Russ. Chem. Bull.*, 2004, **53**, 1871–1888.
3 J.-P. Pouget and S. J. Mather, *Eur. J. Nucl. Med. Imaging*, 2001, **28**, 541–561.
4 E. J. Hall, *Radiobiology for the Radiologist*, Lippincott Williams & Williams, Philadelphia, 5th edn, 2000.
5 A. H. Soloway, W. Tjarks, B. A. Barnum, F.-G. Rong, R. F. Barth, I. M. Codogni and J. G. Wilson, *Chem. Rev.*, 1998, **98**, 1515–1562.
6 R. Barth and F. H. Joensuu, *Radiother. Oncol.*, 2007, **82**, 119–122.
7 G. Wu, R. F. Barth, W. Yang, R. J. Lee, W. Tjarks, M. V. Backer and J. M. Backer, in *Nanotechnology for Cancer Therapy*, ed. M. M. Amiji, CRC Press, Boca Raton, FL, 2007, pp. 77–103.
8 M.-S. Koo, T. Ozawa, R. A. Santos, K. R. Lamborn, A. W. Bollen, D. F. Deen and S. B. Kahl, *J. Med. Chem.*, 2007, **50**, 820–827.
9 M. W. Renner, M. Miura, M. W. Easson and M. G. H. Vicente, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 145–157.
10 M. Ratajski, J. Osterloh and D. Gabel, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 159–166.
11 A. Semioshkin, O. Tsaryova, O. Zhidkova, V. Bregadze and D. Wohrle, *J. Porphyrins Phthalocyanines*, 2006, **10**, 1293–1300.
12 G. W. Kabalka and M.-L. Yao, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 111–125.
13 Y. Byun, S. Narayanasamy, J. Johnsamuel, A. K. Bandyopadhyaya, R. Tiwari, A. S. Al-Madhoun, R. F. Barth, S. Eriksson and W. Tjarks, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 127–144.
14 B. S. Burnham, *Curr. Med. Chem.*, 2005, **12**, 1995–2010.
15 B. R. Shaw, M. Dobrikov, X. Wang, J. Wan, K. He, J.-L. Lin, P. Li, V. Rait, Z. A. Sergueeva and D. Sergueev, *Ann. N. Y. Acad. Sci.*, 2003, **1002**, 12–29.
16 J. S. Summers and B. R. Shaw, *Curr. Med. Chem.*, 2001, **8**, 1147–1155.
17 P. Li, Z. Xu, H. Liu, C. K. Wennefors, M. I. Dobrikov, J. Ludwig and B. R. Shaw, *J. Am. Chem. Soc.*, 2005, **127**, 16782–16783.
18 A. B. Olejniczak, J. Plesek and Z. J. Lesnikowski, *Chem. Eur. J.*, 2006, **13**, 311–318.
19 Z. J. Lesnikowski, E. Paradowska, A. B. Olejniczak, M. Studzinska, P. Seekamp, U. W. Schuessler, D. Gabel, R. F. Schinazi and J. Plesek, *Bioorg. Med. Chem.*, 2005, **13**, 4168–4175.
20 R. F. Schinazi, S. J. Hurwitz, I. Liberman, A. S. Juodawlkis, P. Tharnish, J. Shi, D. C. Liotta, J. A. Coderre and J. Olson, *Clin. Cancer Res.*, 2000, **6**, 725–730.
21 C. Moore, B. I. Hernandez-Santiago, S. J. Hurwitz, C. Tan, C. Wang and R. F. Schinazi, *J. Neuro-Oncol.*, 2005, **74**, 275–280.
22 S. Rossi, R. F. Schinazi and G. Martini, *Biochim. Biophys. Acta*, 2005, **1712**, 81–91.
23 S. E. Aspland, C. Ballatore, R. Castillo, J. Desharnais, T. Eustaquio, P. Goelet, Z. Guo, Q. Li, D. Nelson, C. Sun, A. J. Castellino and M. J. Newman, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5194–5198.
24 R. F. Barth, W. Yang, A. S. Al-Madhoun, J. Johnsamuel, Y. Byun, S. Chandra, D. R. Smith, W. Tjarks and S. Eriksson, *Cancer Res.*, 2004, **64**, 6287–6295.
25 I. Al-Nabulsi, Y. Takamiya, Y. Voloshin, A. Drischilo, R. L. Martuza and T. J. Jorgensen, *Cancer Res.*, 1994, **54**, 5614–5617.
26 L. Persson, J. Boethius, J. S. Goronowitz, C. Källander and L. Lindgren, *J. Neurosurg.*, 1985, **63**, 568–572.
27 L. Persson, S. J. Gronowitz and C. F. R. Källander, *Acta Neurochirugica*, 1986, **80**, 123–127.
28 U. Seitz, M. Wagner, B. Neumaier, E. Wawra, G. Glatting, G. Leder, R. M. Schmid and S. N. Reske, *Eur. J. Nucl. Med. Mol. Imaging*, 2002, **29**, 1174–1181.
29 T. Yusa, Y. Yamaguchi, H. Ohwada, Y. Hayashi, N. Kuroiwa, T. Morita, M. Asanagi, Y. Moriyama and S. Fujimura, *Cancer Res.*, 1988, **48**, 5001–5006.
30 E. K. Walther, N. Dahlmann and H. T. Gorgulla, *Head Neck*, 1993, **15**, 230–235.
31 R. Fujiwaki, K. Hata, K. Nakayama, M. Moriyama, O. Iwanari, H. Katabuchi, H. Okamura, E. Sakai and K. Miyazaki, *Int. J. Cancer*, 2002, **99**, 328–335.
32 K. Y. Look, D. H. Moore, G. P. Sutton, N. Prajda, M. Abonyi and G. Weber, *Anticancer Res.*, 1997, **17**, 2353–2356.
33 X.-L. Sun, H. N. Jayaram, K. Gharehbaghi, Q.-J. Li, X. Xian and A. C. Antony, *Cancer Res.*, 1999, **59**, 940–946.
34 C. Luccioni, M. Muleris, L. Sabatier and B. Dutrillaux, *Mutat. Res.*, 1988, **200**, 55–62.
35 L. Zou, P. G. Zhang, S. Zou, Y. Li and Q. He, *Int. J. Biol. Markers*, 2002, **17**, 135–140.
36 S. Greco, S. Marsigliante, G. Leo and C. Storelli, *Cancer Lett.*, 2000, **160**, 13–19.
37 S. Romain, F. Spyratos, C. Laine-Bidron, C. Bouchet, O. Guirou, P.-M. Martin, J. Oglobine and H. Magdelenat, *Eur. J. Clin. Chem. Clin. Biochem.*, 1995, **33**, 603–608.
38 J. Borovansky, J. Stribrna, M. Elleder and I. Netikova, *Melanoma Res.*, 1994, **4**, 275–279.
39 B. Jacobsson, F. Albertioni and S. Eriksson, *Cancer Lett.*, 2001, **165**, 195–200.
40 Y. Z. Xu and W. Plunkett, *J. Biol. Chem.*, 1993, **268**, 22363–22368.
41 R. Grunewald, J. L. Abbruzzese, P. Tarassoff and W. Plunkett, *Cancer Chemother. Pharmacol.*, 1991, **27**, 258–262.
42 B. A. Chabner, in *Cancer Chemotherapy and Biotherapy*, ed. B. A. Chabner and D. L. Longo, Lippincott-Raven Publishers, Philadelphia, 2nd edn, 1996, pp. 213–233.
43 J. Liliemark, *Clin. Pharmacokinet.*, 1997, **32**, 120–131.
44 W. Plunkett, J. O. Liliemark, T. M. Adams, B. Nowak, E. Estey, H. Kantarjian and M. J. Keating, *Cancer Res.*, 1987, **47**, 3005–3011.
45 S. Madani, J. Baillon, J. Fries, O. Belhadj, A. Bettaieb, M. Ben Hamida and G. Herve, *Eur. J. Cancer Clin. Oncol.*, 1987, **23**, 1485–1490.
46 T. Spasokoukotskaja, E. S. Arner, O. Brosjo, P. Gunven, G. Juliusson, J. Liliemark and S. Eriksson, *Eur. J. Cancer*, 1995, **31A**, 202–208.
47 C. L. van der Wilt, J. R. Kroep, W. J. P. Loves, M. G. Rots, C. J. Van Groeningen, G. J. Kaspers and G. J. Peters, *Eur. J. Cancer*, 2003, **39**, 691–697.
48 E. Giovannetti, V. Mey, R. Danesi, I. Mosca and M. Del Tacca, *Clin. Cancer Res.*, 2004, **10**, 2936–2943.
49 G. Keszler, T. Spasokoukotskaja, Z. Csapo, S. Virga, M. Staub and M. Sasvari-Szekely, *Nucleosides, Nucleotides Nucleic Acids*, 2004, **23**, 1335–1342.
50 G. Keszler, S. Virga, T. Spasokoukotskaja, P. I. Bauer, M. Sasvari-Szekely and M. Staub, *Arch. Biochem. Biophys.*, 2005, **436**, 69–77.
51 G. Keszler, T. Spasokoukotskaja, S. Virga, M. Sasvari-Szekely and M. Staub, *Nucleosides, Nucleotides Nucleic Acids*, 2004, **23**, 1357–1361.
52 Z. Csapo, G. Keszler, G. Safrany, T. Spasokoukotskaja, I. Talianidis, M. Staub and M. Sasvari-Szekely, *Biochem. Pharmacol.*, 2003, **65**, 2031–2039.
53 D. Murata, Y. Endo, T. Obata, K. Sakamoto, Y. Syouji, M. Kadohira, A. Matsuda and T. Sasaki, *Drug Metab. Dispos.*, 2004, **32**, 1178–1182.

54 Y. Shimamoto, K. Koizumi, H. Okabe, H. Kazuno, Y. Murakami, F. Nakagawa, A. Matsuda, T. Sasaki and M. Fukushima, *Jpn. J. Cancer Res.*, 2002, **93**, 825–833.
55 S. H. Huang, A. Tang, B. Drisco, S. Q. Zhang, R. Seeger, C. Li and A. Jong, *DNA Cell Biol.*, 1994, **13**, 461–471.
56 T. A. D. Smith, *Nucl. Med. Commun.*, 1998, **19**, 97–105.
57 B. Ogretmen and Y. A. Hannun, *Nat. Rev. Cancer*, 2004, **4**, 604–616.
58 O. Cuvillier, *Anti-Cancer Drugs*, 2007, **18**, 105–110.
59 E. S. Arner and S. Eriksson, *Pharmacol. Ther.*, 1995, **67**, 155–186.
60 M. P. B. Sandrini and J. Piskur, *Trends Biochem. Sci.*, 2005, **30**, 225–228.
61 E. De Clercq, *J. Clin. Virol.*, 2004, **30**, 115–133.
62 D. Sampath, V. A. Rao and W. Plunkett, *Oncogene*, 2003, **22**, 9063–9074.
63 A. R. Van Rompay, M. Johansson and A. Karlsson, *Pharmacol. Ther.*, 2003, **100**, 119–139.
64 A. S. Al-Madhoun, W. Tjarks and S. Eriksson, *Mini Rev. Med. Chem.*, 2004, **4**, 341–350.
65 C. M. Galmarini, L. Jordheim and C. Dumontet, *Expert Rev. Anticancer Ther.*, 2003, **3**, 717–728.
66 C. M. Galmarini, J. R. Mackey and C. Dumontet, *Leukemia*, 2001, **15**, 875–890.
67 C. M. Galmarini, J. R. Mackey and C. Dumontet, *Lancet Oncol.*, 2002, **3**, 415–424.
68 L. W. Frick, D. J. Nelson, M. H. St. Clair, P. A. Furman and T. A. Krenitsky, *Biochem. Biophys. Res. Commun.*, 1988, **154**, 124–129.
69 A. Fridland, M. C. Connelly and R. Ashmun, *Mol. Pharmacol.*, 1990, **37**, 665–670.
70 M. Hengstschlaeger, M. Knoefler, E. W. Muellner, E. Ogris, E. Wintersberger and E. Wawra, *J. Biol. Chem.*, 1994, **269**, 13836–13842.
71 M. Hengstschlaeger, E. W. Muellner and E. Wawra, *Int. J. Oncol.*, 1994, **4**, 207–210.
72 P. Chiba, T. Tihan, R. Eher, U. Koeller, C. Wallner, R. Goebl and W. Linkesch, *Br. J. Haematol.*, 1989, **71**, 451–455.
73 J. L. Sherley and T. J. Kelly, *J. Biol. Chem.*, 1988, **263**, 8350–8358.
74 K. L. O'Neill, F. Zhang, H. Li, D. G. Fuja and B. K. Murray, *Leukemia*, 2007, **21**, 560–563.
75 N. Lawrentschuk, A. M. T. Poon and A. M. Scott, *Clin. Nucl. Med.*, 2006, **31**, 788–789.
76 T. Saga, H. Kawashima, N. Araki, J. Takahashi, A. Y. Nakashima, T. Higashi, N. Oya, T. Mukai, M. Hojo, N. Hashimoto, T. Manabe, M. Hiraoka and K. Togashi, *Clin. Nucl. Med.*, 2006, **31**, 774–780.
77 M. Muzi, A. M. Spence, F. O'Sullivan, D. A. Mankoff, J. M. Wells, J. R. Grierson, J. M. Link and K. A. Krohn, *J. Nucl. Med.*, 2006, **47**, 1612–1621.
78 A. Buck, K. M. Bommer, S. Stilgenbauer, M. Juweid, G. Glatting, H. Schirrmeister, T. Mattfeldt, D. Tepsic, D. Bunjes, F. Mottaghy, M. B. Krause, J. B. Neumaier, H. Dohner, P. Moller and S. N. Reske, *Cancer Res.*, 2006, **66**, 11055–11061.
79 A. Agool, B. Schot, W. P. Jager and L. E. Vellenga, *J. Nucl. Med.*, 2006, **47**, 1592–1598.
80 J. Toyohara, A. Waki, S. Takamatsu, Y. Yonekura, Y. Magata and Y. Fujibayashi, *Nucl. Med. Biol.*, 2002, **29**, 281–287.
81 C. Carnrot, S. R. Vogel, Y. Byun, L. Wang, W. Tjarks, S. Eriksson and A. J. Phipps, *Biol. Chem.*, 2006, **387**, 1575–1581.
82 B. Munch-Petersen, L. Cloos, G. Tyrsted and S. Eriksson, *J. Biol. Chem.*, 1991, **266**, 9032–9038.
83 J. R. Grierson, J. L. Schwartz, M. Muzi, R. Jordan and K. A. Krohn, *Nucl. Med. Biol.*, 2004, **31**, 829–837.
84 X. B. Kong, Q. Y. Zhu, P. M. Vidal, K. A. Watanabe, B. Polsky, D. Armstrong, M. Ostrander, S. A. Lang, Jr., E. Muchmore and T. C. Chou, *Antimicrob. Agents Chemother.*, 1992, **36**, 808–818.
85 J. R. Bading, A. H. Shahinian, P. Bathija and P. S. Conti, *Nucl. Med. Biol.*, 2000, **27**, 361–368.
86 P. S. Conti, M. M. Alauddin, J. R. Fissekis, B. Schmall and K. A. Watanabe, *Nucl. Med. Biol.*, 1995, **22**, 783–789.
87 H. Wang, P. Oliver, L. Nan, S. Wang, Z. Wang, J. K. Rhie, R. Zhang and D. L. Hill, *Cancer Chemother. Pharmacol.*, 2002, **49**, 419–424.
88 J. Wang and S. Eriksson, *Antimicrob. Agents Chemother.*, 1996, **40**, 1555–1557.
89 J. M. Collins, R. W. Klecker and A. G. Katki, *Clin. Cancer Res.*, 1999, **5**, 1976–1981.
90 W. Lewis, R. R. Meyer, J. F. Simpson, J. M. Colacino and F. W. Perrino, *Biochemistry (Moscow)*, 1994, **33**, 14620–14624.
91 A. J. Lunato, J. Wang, J. E. Woollard, A. K. M. Anisuzzaman, W. Ji, F.-G. Rong, S. Ikeda, A. H. Soloway, S. Eriksson, D. H. Ives, T. E. Blue and W. Tjarks, *J. Med. Chem.*, 1999, **42**, 3378–3389.
92 J. Balzarini, S. Celen, A. Karlsson, T. de Groot, A. Verbruggen and G. Bormans, *Antiviral Chem. Chemother.*, 2006, **17**, 17–23.
93 J. Toyohara, A. Hayashi, A. Gogami and Y. Fujibayashi, *Nucl. Med. Biol.*, 2006, **33**, 765–772.
94 J. Toyohara, A. Hayashi, A. Gogami, M. Hamada, Y. Hamashima, T. Katoh, M. Node and Y. Fujibayashi, *Nucl. Med. Biol.*, 2006, **33**, 751–764.
95 M. Schmid, B. Neumaier, A. T. J. Vogg, K. Wczasek, C. Friesen, F. M. Mottaghy, A. K. Buck and S. N. Reske, *Nucl. Med. Biol.*, 2006, **33**, 359–366.
96 S. Celen, T. de Groot, J. Balzarini, K. Vunckx, C. Terwinghe, P. Vermaelen, L. Van Berckelaer, H. Vanbilloen, J. Nuyts, L. Mortelmans, A. Verbruggen and G. Bormans, *Nucl. Med. Biol.*, 2007, **34**, 283–291.
97 M. M. Alauddin, P. Ghosh and J. G. Gelovani, *J. Labelled Compd. Radiopharm.*, 2006, **49**, 1079–1088.
98 S. Eriksson, B. Munch-Petersen, K. Johansson and H. Eklund, *Cell. Mol. Life Sci.*, 2002, **59**, 1327–1346.
99 U. Kosinska, C. Carnrot, M. P. B. Sandrini, A. R. Clausen, L. Wang, J. Piskur, S. Eriksson and H. Eklund, *FEBS J.*, 2007, **274**, 727–737.
100 M. P. B. Sandrini, A. R. Clausen, B. Munch-Petersen and J. Piskur, *Nucleosides, Nucleotides Nucleic Acids*, 2006, **25**, 1153–1158.
101 K. El Omari, N. Solaroli, A. Karlsson, J. Balzarini and D. K. Stammers, *BMC Struct. Biol.*, 2006, **6** (no pp given).
102 N. Solaroli, M. Johansson, J. Balzarini and A. Karlsson, *Nucleosides, Nucleotides Nucleic Acids*, 2006, **25**, 1189–1192.
103 M. S. Birringer, M. T. Claus, G. Folkers, D. P. Kloer, G. E. Schulz and L. Scapozza, *FEBS Lett.*, 2005, **579**, 1376–1382.
104 C. Carnrot, R. Wehelie, S. Eriksson, G. Boelske and L. Wang, *Mol. Microbiol.*, 2003, **50**, 771–780.
105 U. Kosinska, C. Carnrot, S. Eriksson, L. Wang and H. Eklund, *FEBS J.*, 2005, **272**, 6365–6372.
106 A. P. Kuzin, M. Abashidze, F. Forouhar, S. M. Vorobiev, T. B. Acton, L. C. Ma, R. Xiao, G. T. Montelione, L. Tong and J. F. Hunt, *Northeast Structural Genomics Target Car26*, 2006, PDB code: 1XX6.
107 M. Welin, U. Kosinska, N.-E. Mikkelsen, C. Carnrot, C. Zhu, L. Wang, S. Eriksson, B. Munch-Petersen and H. Eklund, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17970–17975.
108 D. Segura-Pena, S. Lutz, C. Monnerjahn, M. Konrad and A. Lavie, *J. Mol. Biol.*, 2007, **369**, 129–141.
109 C.-C. Wu, T.-Y. Hsu and J.-Y. Chen, *Biochemistry (Moscow)*, 2005, **44**, 4785–4793.
110 C.-C. Wu, M.-C. Chen, Y.-R. Chang, T.-Y. Hsu and J.-Y. Chen, *Biochem. J.*, 2004, **379**, 795–803.
111 L. E. Bird, J. Ren, A. Wright, K. D. Leslie, B. Degreve, J. Balzarini and D. K. Stammers, *J. Biol. Chem.*, 2003, **278**, 24680–24687.
112 A. Gardberg, L. Shuvalova, C. Monnerjahn, M. Konrad and A. Lavie, *Structure*, 2003, **11**, 1265–1277.
113 E. A. Gustafson, A. C. Chillemi, D. R. Sage and J. D. Fingeroth, *Antimicrob. Agents Chemother.*, 1998, **42**, 2923–2931.
114 E. A. Gustafson, R. F. Schinazi and J. D. Fingeroth, *J. Virol.*, 2000, **74**, 684–692.
115 T. Suzutani, L. C. Davies and R. W. Honess, *J. Gen. Virol.*, 1993, **74**, 1011–1016.
116 M. Y. Mizutani, Y. Takamatsu, T. Ichinose, K. Nakamura and A. Itai, *Proteins*, 2006, **63**, 878–891.
117 M. F. Hawthorne and W. Lee Mark, *J. Neuro-Oncol.*, 2003, **62**, 33–45.
118 D. Gabel and Y. Endo, in *Molecular Clusters of the Main Group Elements*, ed. M. Driess and H. Noeth, Wiley-VCH, Weinheim, Germany, 2004, pp. 95–125.
119 W. Tjarks, *J. Organomet. Chem.*, 2000, **614–615**, 37–47.

120 J. F. Valliant, K. J. Guenther, A. S. King, P. Morel, P. Schaffer, O. O. Sogbein and K. A. Stephenson, *Coord. Chem. Rev.*, 2002, **232**, 173–230.
121 M. A. Fox, W. R. Gill, P. L. Herbertson, J. A. H. MacBride, K. Wade and H. M. Colquhoun, *Polyhedron*, 1996, **15**, 565–571.
122 V. I. Bregadze, I. B. Sivaev and S. A. Glazun, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 75–109.
123 J. Johnsamuel, Y. Byun, T. P. Jones, Y. Endo and W. Tjarks, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 3213–3216.
124 J. Johnsamuel, Y. Byun, T. P. Jones, Y. Endo and W. Tjarks, *J. Organomet. Chem.*, 2003, **680**, 223–231.
125 S. Narayanasamy, B. T. S. Thirumamagal, J. Johnsamuel, Y. Byun, A. S. Al-Madhoun, E. Usova, G. Y. Cosquer, J. Yan, A. K. Bandyopadhyaya, R. Tiwari, S. Eriksson and W. Tjarks, *Bioorg. Med. Chem.*, 2006, **14**, 6886–6899.
126 A. K. Bandyopadhyaya, R. Tiwari and W. Tjarks, *Bioorg. Med. Chem.*, 2006, **14**, 6924–6932.
127 Y. Byun, B. T. S. Thirumamagal, W. Yang, S. Eriksson, R. F. Barth and W. Tjarks, *J. Med. Chem.*, 2006, **49**, 5513–5523.
128 B. T. S. Thirumamagal, X. B. Zhao, A. K. Bandyopadhyaya, S. Narayanasamy, J. Johnsamuel, R. Tiwari, D. W. Golightly, V. Patel, B. T. Jehning, M. V. Backer, R. F. Barth, R. J. Lee, J. M. Backer and W. Tjarks, *Bioconjugate Chem.*, 2006, **17**, 1141–1150.
129 T. Ogawa, K. Ohta, T. Yoshimi, H. Yamazaki, T. Suzuki, S. Ohta and Y. Endo, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3943–3946.
130 Y. Endo, *Farumashia*, 2006, **42**, 421–425.
131 T. Goto, K. Ohta, T. Suzuki, S. Ohta and Y. Endo, *Bioorg. Med. Chem.*, 2005, **13**, 6414–6424.
132 Y. Endo, T. Yoshimi, K. Ohta, T. Suzuki and S. Ohta, *J. Med. Chem.*, 2005, **48**, 3941–3944.
133 Y. Endo, K. Ohta, T. Yoshimi and K. Yamaguchi, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2004, **179**, 799–802.
134 S. Fujii, T. Goto, K. Ohta, Y. Hashimoto, T. Suzuki, S. Ohta and Y. Endo, *J. Med. Chem.*, 2005, **48**, 4654–4662.
135 R. L. Julius, O. K. Farha, J. Chiang, L. J. Perry and M. F. Hawthorne, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 4808–4813.
136 R. C. Reynolds, S. R. Campbell, R. G. Fairchild, R. L. Kisliuk, P. L. Micca, S. F. Queener, J. M. Riordan, W. D. Sedwick, W. R. Waud, A. K. W. Leung, R. W. Dixon, W. J. Suling and D. W. Borhani, *J. Med. Chem.*, 2007, **50**, 3283–3289.
137 S. Ghirmai, J. Malmquist, H. Lundquist, V. Tolmachev and S. Sjoeberg, *J. Labelled Compd. Radiopharm.*, 2004, **47**, 557–569.
138 K. J. Winberg, M. Persson, P.-U. Malmstrom, S. Sjoeberg and V. Tolmachev, *Nucl. Med. Biol.*, 2004, **31**, 425–433.
139 K. J. Winberg, E. Mume, V. Tolmachev and S. Sjoeberg, *J. Labelled Compd. Radiopharm.*, 2005, **48**, 195–202.
140 L. Eriksson, V. Tolmachev and S. Sjoeberg, *J. Labelled Compd. Radiopharm.*, 2003, **46**, 623–631.
141 L. Eriksson, K. J. Winberg, R. T. Claro and S. Sjoeberg, *J. Org. Chem.*, 2003, **68**, 3569–3573.
142 I. P. Beletskaya, V. I. Bregadze, V. A. Ivushkin, P. V. Petrovskii, I. B. Sivaev, S. Sjoeberg and G. G. Zhigareva, *J. Organomet. Chem.*, 2004, **689**, 2920–2929.
143 J. Johnsamuel, N. Lakhi, A. S. Al-Madhoun, Y. Byun, J. Yan, S. Eriksson and W. Tjarks, *Bioorg. Med. Chem.*, 2004, **12**, 4769–4781.
144 J. L. Fauchere, D. Kim Quang, P. Y. C. Jow and C. Hansch, *Experientia*, 1980, **36**, 1203–1204.
145 K. Yamamoto and Y. Endo, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2389–2392.
146 Y. Byun, J. Yan, A. S. Al-Madhoun, J. Johnsamuel, W. Yang, R. F. Barth, S. Eriksson and W. Tjarks, *J. Med. Chem.*, 2005, **48**, 1188–1198.
147 W. Tjarks, A. K. M. Anisuzzaman, L. Liu, A. H. Soloway, R. F. Barth, D. J. Perkins and D. M. Adams, *J. Med. Chem.*, 1992, **35**, 1628–1633.
148 C. L. Powell, M. Schulze, S. J. Black, A. S. Thompson and M. D. Threadgill, *Tetrahedron Lett.*, 2007, **48**, 1251–1254.
149 D. C. Busby and M. F. Hawthorne, *Inorg. Chem.*, 1982, **21**, 4101–4103.
150 E. Justus, D. Awad, M. Hohnholt, T. Schaffran, K. Edwards, G. Karlsson, L. Damian and D. Gabel, *Bioconjugate Chem.*, 2007, **18**, 1287–1293.
151 H. Nakamura, M. Ueno, J.-D. Lee, H. S. Ban, E. Justus, P. Fan and D. Gabel, *Tetrahedron Lett.*, 2007, **48**, 3151–3154.
152 J.-D. Lee, M. Ueno, Y. Miyajima and H. Nakamura, *Org. Lett.*, 2007, **9**, 323–326.
153 V. Tolmachev, J. Koziorowski, I. Sivaev, H. Lundqvist, J. Carlsson, A. Orlova, L. Gedda, P. Olsson, S. Sjoeberg and A. Sundin, *Bioconjugate Chem.*, 1999, **10**, 338–345.
154 E. Mume, A. Orlova, P.-U. Malmstroem, H. Lundqvist, S. Sjoeberg and V. Tolmachev, *Nucl. Med. Biol.*, 2005, **32**, 613–622.
155 A. Bruskin, I. Sivaev, M. Persson, H. Lundqvist, J. Carlsson, S. Sjoberg and V. Tolmachev, *Nucl. Med. Biol.*, 2004, **31**, 205–211.
156 A. Sjoestroem, V. Tolmachev, O. Lebeda, J. Koziorowski, J. Carlsson and H. Lundqvist, *J. Radioanal. Nucl. Chem.*, 2003, **256**, 191–197.
157 M. F. Hawthorne and A. Maderna, *Chem. Rev.*, 1999, **99**, 3421–3434.
158 D. S. Wilbur, M.-K. Chyan, D. K. Hamlin, B. B. Kegley, R. Risler, P. M. Pathare, J. Quinn, R. L. Vessella, C. Foulon, M. Zalutsky, T. J. Wedge and M. F. Hawthorne, *Bioconjugate Chem.*, 2004, **15**, 203–223.
159 D. S. Wilbur, M.-K. Chyan, D. K. Hamlin, R. L. Vessella, T. J. Wedge and M. F. Hawthorne, *Bioconjugate Chem.*, 2007, **18**, 1226–1240.
160 A.-C. Steffen, Y. Almqvist, M.-K. Chyan, H. Lundqvist, V. Tolmachev, D. S. Wilbur and J. Carlsson, *Oncol. Rep.*, 2007, **17**, 1141–1147.
161 A. S. Al-Madhoun, J. Johnsamuel, J. Yan, W. Ji, J. Wang, J.-C. Zhuo, A. J. Lunato, J. E. Woollard, A. E. Hawk, G. Y. Cosquer, T. E. Blue, S. Eriksson and W. Tjarks, *J. Med. Chem.*, 2002, **45**, 4018–4028.
162 A. S. Al-Madhoun, J. Johnsamuel, R. F. Barth, W. Tjarks and S. Eriksson, *Cancer Res.*, 2004, **64**, 6280–6286.
163 R. F. Barth, W. Yang, G. Wyu, Y. Byun, W. Tjarks, E. Eriksson, P. J. Binns and K. J. Riley, in *Advances in Neutron Capture Therapy 2006. Proceedings of ICNCT–12*, ed. Y. Nakagawa, T. Kobayashi and H. Fukuda, International Society for Neutron Capture Therapy, Kagawa, Japan, 2006, pp. 109–112.
164 B. T. S. Thirumamagal, J. Johnsamuel, G. Y. Cosquer, Y. Byun, J. Yan, S. Narayanasamy, W. Tjarks, R. F. Barth, A. S. Al-Madhoun and S. Eriksson, *Nucleosides, Nucleotides Nucleic Acids*, 2006, **25**, 861–866.
165 P. Davoli, A. Spaggiari, L. Castagnetti and F. Prati, *Org. Biomol. Chem.*, 2004, **2**, 38–47.
166 Y. Byun, J. Yan, A. S. Al-Madhoun, J. Johnsamuel, W. Yang, R. F. Barth, S. Eriksson and W. Tjarks, *Appl. Radiat. Isot.*, 2004, **61**, 1125–1130.
167 R. A. Kasar, G. M. Knudsen and S. B. Kahl, *Inorg. Chem.*, 1999, **38**, 2936–2940.
168 M. A. Cabrita, S. A. Baldwin, J. D. Young and C. E. Cass, *Biochem. Cell Biol.*, 2002, **80**, 623–638.
169 W. B. Mahony, B. A. Domin, S. M. Daluge and T. P. Zimmerman, *Biochem. Pharmacol.*, 2004, **68**, 1797–1805.
170 M. Hu, *J. Pharm. Sci.*, 1993, **82**, 829–833.
171 C. Flexner, in *Goodman and Gilman's the Pharmacological Basis of therapeutics*, ed. L. L. Brunton, J. S. Lazo and K. L. Parker, McGraw-Hill, New York, 11th edn, 2006, pp. 1273–1314.
172 Y. Byun and W. Tjarks, *Tetrahedron Lett.*, 2006, **47**, 5649–5652.
173 R. N. Grimes, *Carboranes*, Academic Press, London, U.K., 1970.
174 A. S. Al-Madhoun, W. Ji, J. Johnsamuel, J. Yan, N. Lakhi, J. Byun, J. Woollard, A. Hawk, T. Blue, W. Tjarks and S. Eriksson, in *Research and Development in Neutron Capture Therapy*, ed. W. Sauerwein, R. Moss and A. Wittig, Monduzi Editori, Bologna, Italy, 2002, pp. 89–93.
175 W. Knecht, B. Munch-Petersen and J. Piskur, *J. Mol. Biol.*, 2000, **301**, 827–837.
176 M. E. Kokoris and M. E. Black, *Protein Sci.*, 2002, **11**, 2267–2272.
177 R. F. Barth, W. Yang, J. H. Rotaru, M. L. Moeschberger, C. P. Boesel, A. H. Soloway, D. D. Joel, M. M. Nawrocky, K. Ono and J. H. Goodman, *Int. J. Radiat. Oncol. Biol. Phys.*, 2000, **47**, 209–218.

178 R. F. Barth, W. Yang, J. H. Rotaru, M. L. Moeschberger, D. D. Joel, M. M. Nawrocky, J. H. Goodman and A. H. Soloway, *Cancer Res.*, 1997, **57**, 1129–1136.
179 W. Yang, R. F. Barth, J. H. Rotaru, C. P. Boesel, D. A. Wilkie, J. C. Bresnahan, M. Hadjiconstantinou, V. M. Goettl, D. D. Joel and M. M. Nawrocky, *J. Neuro-Oncol.*, 2000, **48**, 179–190.
180 W. Yang, R. F. Barth, R. T. Bartus, J. H. Rotaru, M. L. Moeschberger, A. K. Ferketich, M. M. Nawrocky, J. A. Coderre and J. H. Goodman, *Neurosurgery*, 2000, **47**, 189–197.
181 R. F. Barth, J. C. Grecula, W. Yang, J. H. Rotaru, M. Nawrocky, N. Gupta, B. J. Albertson, A. K. Ferketich, M. L. Moeschberger, J. A. Coderre and E. K. Rofstad, *Int. J. Radiat. Oncol. Biol. Phys.*, 2004, **58**, 267–277.
182 D. A. Reardon, G. Akabani, R. E. Coleman, A. H. Friedman, H. S. Friedman, J. E. Herndon, II, R. E. McLendon, C. N. Pegram, J. M. Provenzale, J. A. Quinn, J. N. Rich, J. J. Vredenburgh, A. Desjardins, S. Guruangan, M. Badruddoja, J. M. Dowell, T. Z. Wong, X.-G. Zhao, M. R. Zalutsky and D. D. Bigner, *J. Clin. Oncol.*, 2006, **24**, 115–122.
183 I. F. Parney, S. Kunward, M. McDermott, M. Berger, M. Prados, S. Cha, D. Croteau, R. K. Puri and S. M. Chang, *J. Neurosurg.*, 2005, **102**, 267–275.
184 J. W. Degen, S. Walbridge, A. O. Vortmeyer, E. H. Oldfield and R. R. Lonser, *J. Neurosurg.*, 2003, **99**, 893–898.
185 R. F. Barth, W. Yang, D. M. Adams, J. H. Rotaru, S. Shukla, M. Sekido, W. Tjarks, R. A. Fenstermaker, M. Ciesielski, M. M. Nawrocky and J. A. Coderre, *Cancer Res.*, 2002, **62**, 3159–3166.
186 W. Yang, R. F. Barth, D. M. Adams, M. J. Ciesielski, R. A. Fenstermaker, S. Shukla, W. Tjarks and M. A. Caligiuri, *Cancer Res.*, 2002, **62**, 6552–6558.
187 G. Wu, W. Yang, R. F. Barth, S. Kawabata, M. Swindall, A. K. Bandyopadhyaya, W. Tjarks, B. Khorsandi, T. E. Blue, A. K. Ferketich, M. Yang, G. A. Christoforidis, T. J. Sferra, P. J. Binns, K. J. Riley, M. J. Ciesielski and R. A. Fenstermaker, *Clin. Cancer Res.*, 2007, **13**, 1260–1268.
188 W. Yang, R. F. Barth, G. Wu, S. Kawabata, T. J. Sferra, A. K. Bandyopadhyaya, W. Tjarks, A. K. Ferketich, M. L. Moeschberger, P. J. Binns, K. J. Riley, J. A. Coderre, M. J. Ciesielski, R. A. Fenstermaker and C. J. Wikstrand, *Clin. Cancer Res.*, 2006, **12**, 3792–3802.
189 K. Yokoyama, S.-I. Miyatake, Y. Kajimoto, S. Kawabata, A. Doi, T. Yoshida, M. Okabe, M. Kirihata, K. Ono and T. Kuroiwa, *Radiat. Res.*, 2007, **167**, 102–109.
190 K. Yokoyama, S.-I. Miyatake, Y. Kajimoto, S. Kawabata, A. Doi, T. Yoshida, T. Asano, M. Kirihata, K. Ono and T. Kuroiwa, *J. Neuro-Oncol.*, 2006, **78**, 227–232.
191 Y. Nakagawa, K. Pooh, T. Kobayashi, T. Kageji, S. Uyama, A. Matsumura and H. Kumada, *J. Neuro-Oncol.*, 2003, **62**, 87–99.
192 A. Z. Diaz, *J. Neuro-Oncol.*, 2003, **62**, 101–109.
193 P. M. Busse, O. K. Harling, M. R. Palmer and W. S. Kiger, *J. Neuro-Oncol.*, 2003, **62**, 111–121.
194 H. Joensuu, L. Kankaanranta, T. Seppala, I. Auterinen, M. Kallio, M. Kulvik, J. Laakso, J. Vahatalo, M. Kortesniemi, P. Kotiluoto, T. Seren, J. Karila, A. Brander, E. Jarviluoma, P. Ryynanen, A. Paetau, I. Ruokonen, H. Minn, M. Tenhunen, J. Jaaskelainen, M. Farkkila and S. Savolainen, *J. Neuro-Oncol.*, 2003, **62**, 123–134.
195 J. A. Coderre and G. M. Morris, *Radiat. Res.*, 1999, **151**, 1–18.
196 R. F. Barth, *J. Neuro-Oncol.*, 1998, **36**, 91–102.
197 P. Borst, J. Balzarini, N. Ono, G. Reid, H. de Vries, P. Wielinga, J. Wijnholds and N. Zelcer, *Antiviral Res.*, 2004, **62**, 1–7.
198 M. Pastor-Anglada, P. Cano-Soldado, M. Molina-Arcas, M. P. Lostao, I. Larrayoz, J. Martinez-Picado and F. J. Casado, *Virus Res.*, 2005, **107**, 151–164.
199 M. Pastor-Anglada, M. Molina-Arcas, F. J. Casado, B. Bellosillo, D. Colomer and J. Gil, *Leukemia*, 2004, **18**, 385–393.
200 Q. Tian, J. Zhang, E. Chan, W. Duan and S. Zhou, *Drug Dev. Res.*, 2005, **64**, 1–18.
201 C. A. Ritter, G. Jedlitschky, H. M. zu Schwabedissen, M. Grube, K. Koeck and H. K. Kroemer, *Drug Metab. Rev.*, 2005, **37**, 253–278.